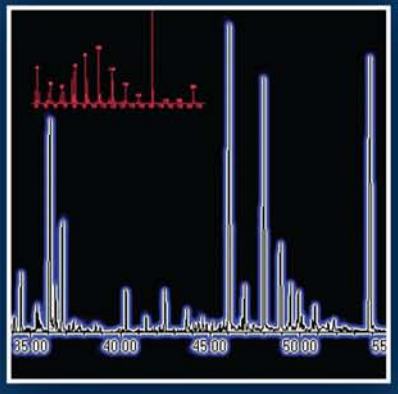
Handbook of ESSENTIAL OILS

Science, Technology, and Applications



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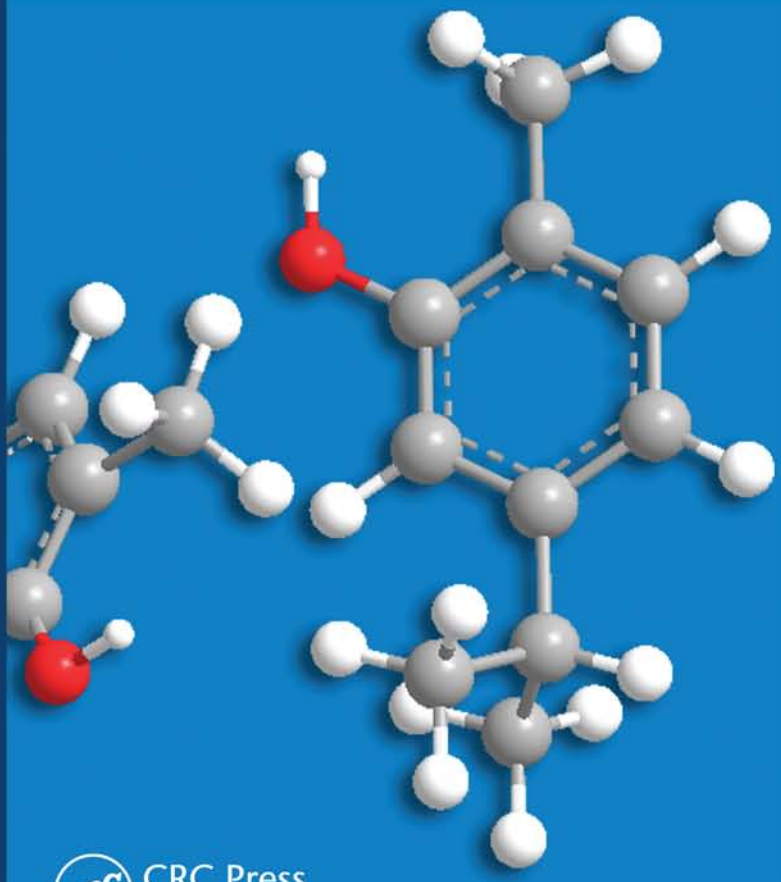

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Editors

K. Hüsnü Can Başer was born on July 15, 1949 in Çankırı, Turkey. He graduated from the Eskisehir I.T.I.A. School of Pharmacy with diploma number 1 in 1972 and became a research assistant in the pharmacognosy department of the same school. He did his PhD in pharmacognosy between 1974 and 1978 at Chelsea College of the University of London.

Upon returning home, he worked as a lecturer in pharmacognosy at the school he had earlier graduated, and served as director of Eskisehir I.T.I.A. School of Chemical Engineering between 1978 and 1980. He was promoted to associate professorship in pharmacognosy in 1981.

He served as dean of the faculty of pharmacy at Anadolu University (1993–2001), vice-dean of the faculty of pharmacy (1982–1993), head of the department of professional pharmaceutical sciences (1982–1993), head of the pharmacognosy section (1982–present), member of the University Board and Senate (1982–2001; 2007), and director of the Medicinal and Aromatic Plant and Drug Research Centre (TBAM) (1980–2002) in Anadolu University.

During 1984–1994, he was appointed as the national project coordinator of Phase I and Phase II of the UNDP/UNIDO projects of the government of Turkey titled "Production of Pharmaceutical Materials from Medicinal and Aromatic Plants," through which TBAM had been strengthened.

He was promoted to full professorship in pharmacognosy in 1987. His major areas of research include essential oils, alkaloids, and biological, chemical, pharmacological, technological, and biological activity research into natural products. He is the 1995 Recipient of the Distinguished Service Medal of IFEAT (International Federation of Essential Oils and Aroma Trades) based in London, United Kingdom and the 2005 recipient of "Science Award" (Health Sciences) of the Scientific and Technological Research Council of Turkey (TUBITAK). He has published 537 papers in international refereed journals (378 in SCI journals), 105 papers in Turkish journals, and 134 papers in conference proceedings.

For more information: http://www.khcbaser.com

Gerhard Buchbauer was born in 1943 in Vienna, Austria. He studied pharmacy at the University of Vienna, from where he received his master's degree (Mag.pharm.) in May 1966. In September 1966, he assumed the duties of university assistant at the Institute of Pharmaceutical Chemistry and received his doctorate (PhD) in pharmacy and philosophy in October 1971 with a thesis on synthetic fragrance compounds. Further scientific education was practised as post doc in the team of Professor C.H. Eugster at the Institute of Organic Chemistry, University of Zurich (1977–1978), followed by the habilitation (post doctoral lecture qualification) in Pharmaceutical Chemistry with the inaugural dissertation entitled "Synthesis of Analogies of Drugs and Fragrance Compounds with Contributions to Structure-Activity-Relationships" (1979) and appointment to the permanent staff of the University of Vienna, and head of the first department of the Institute of Pharmaceutical Chemistry. In November 1991, he was appointed as a full

professor of Pharmaceutical Chemistry, University of Vienna; in 2002, he was elected as head of this institute. He retired in October 2008. He is married since 1973 and was a father of a son since 1974.

Among others, he is still a member of the permanent scientific committee of ISEO, a member of the scientific committee of Forum Cosmeticum (1990, 1996, 2002, and 2008), a member of editorial boards (e.g., *Journal of Essential Oil Research, The International Journal of Essential Oil Therapeutics, Scientia Pharmaceutica*, etc.), assistant editor of *Flavour and Fragrance Journal*, regional editor of *Eurocosmetics*, a member of many scientific societies, for example, *Society of Austrian Chemists*, head of its working group "Food Chemistry, Cosmetics, and Tensides" (2000–2004), *Austrian Pharmaceutical Society*, *Austrian Phytochemical Society*, vicehead of *Austrian Society of Scientific Aromatherapy*, and so on, technical advisor of IFEAT (1992–2008), and organizer of the 27th ISEO (September 2006, in Vienna) together with Professor Dr. Ch. Franz.

Based on the sound interdisciplinary education of pharmacists, it was possible to establish almost completely neglected area of fragrance and flavor chemistry as a new research discipline within the pharmaceutical sciences. Our research team is the only one that conducts fragrance research in its entirety and covers synthesis, computer-aided fragrance design, analysis, and pharmaceutical/medicinal aspects. Because of our efforts, it is possible to show and to prove that these small molecules possess more properties than merely emitting a good odor. Now, this "Viennese Centre of Flavour research" has gained a worldwide scientific reputation documented by more than 400 scientific publications, about 100 invited lectures, and about 200 contributions to symposia, meetings, and congresses, as short lectures and poster presentations.

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1 Introduction

K. Hüsnü Can Başer and Gerhard Buchbauer

Essential oils (EOs) are very interesting natural plant products and among other qualities they possess various biological properties. The term "biological" comprises all activities that these mixtures of volatile compounds (mainly mono- and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) exert on humans, animals, and other plants. This book intends to make the reader acquainted with all aspects of EOs and their constituent aromachemicals ranging from chemistry, pharmacology, biological activity, production, and trade to uses, and regulatory aspects. After an overview of research and development activities on EOs with a historical perspective (Chapter 2), Chapter 3 "Sources of Essential Oils" gives an expert insight into vast sources of EOs. The chapter also touches upon agronomic aspects of EO-bearing plants. Traditional and modern production techniques of EOs are illustrated and discussed in Chapter 4. It is followed by two important chapters "Chemistry of Essential Oils" (Chapter 5) and "Analysis of Essential Oils" (Chapter 6) illustrating chemical diversity of EOs, and analytical techniques employed for the analyses of these highly complex mixtures of volatiles.

They are followed by a cluster of articles on the biological properties of EOs, starting with "The Toxicology and Safety of Essential Oils: A Constituent-Based Approach" (Chapter 7). On account of the complexity of these natural products, the toxicological or biochemical testing of an EO will always be the sum of its constituents which either act in a synergistic or in an antagonistic way with one another. Therefore, the chemical characterization of the EO is very important for the understanding of its biological properties. The constituents of these natural mixtures upon being absorbed into the blood stream of humans or animals get metabolized and eliminated. This metabolic biotransformation leads mostly in two steps to products of high water solubility which enables the organism to get rid of these "xenobiotics" by renal elimination. This mechanism is thoroughly explained in Chapter 8, "Metabolism of Terpenoids in Animal Models and Humans." In Chapter 9, "Biological Activities of Essential Oils," "uncommon" biological activities of EOs are reviewed, such as anticancer properties, antinociceptive effects, antiviral activities, antiphlogistic properties, penetration enhancement activities, and antioxidative effects. The psychoactive, particularly stimulating, and sedative effects of fragrances as well as behavioral activities, elucidated, for example, by neurophysiological methods, are the topics of Chapter 10 ("Effects of Essential Oils in the Central Nervous Systemd"), Section 10.2. Here, the emphasis is put on the central nervous system and on psychopharmacology whereas Chapter 10, Section 10.1 mainly deals with reactions of the autonomic nervous system upon contact with EOs and/or their main constituents. The phytotherapeutic uses of EOs is another overview about scientific papers in peer-reviewed journals over the last 30 years, so to say the medical use of these natural plant products excluding aromatherapeutical treatments and single case studies (Chapter 11, "Phytotherapeutic Uses of Essential Oils"). Another contribution only deals with antimicrobial activities of those EOs that are monographed in the European Pharmacopoeia. In Chapter 12, "*In Vitro* Antimicrobial Activities of Essential Oils Monographed in the European Pharmacopoeia 6th Edition," more than 81 tables show the importance of these valuable properties

of EOs. Aromatherapy with EOs covers the other side of the "classical" medical uses. "Aromatherapy with Essential Oils" (Chapter 13), is written by Maria Lis-Balchin, a known expert in this field and far from esoteric quackery. It completes the series of contributions dealing with the biological properties of EO regarded from various sides and standpoints.

Chapters 14 and 15 by the world-renown experts Y. Asakawa and Y. Noma are concise treatises on the biotransformations of EO constituents. Enzymes in microorganisms and tissues metabolize EO constituents in similar ways by adding mainly oxygen function to molecules to render them water soluble to facilitate their metabolism. This is also seen as a means of detoxification for these organisms. Many interesting and valuable novel chemicals are biosynthesized by this way. These products are also considered as natural since the substrates are natural.

Encapsulation is a technique widely utilized in pharmaceutical, chemical, food, and feed industries to render EOs more manageable in formulations. Classical and modern encapsulation techniques are explained in detail in Chapter 17, "Encapsulation and Other Programmed Release techniques for EOs and Volatile Terpenes."

EOs and aromachemicals are low-volume high-value products used in perfumery, cosmetics, feed, food, beverages, and pharmaceutical industries. Industrial uses of EOs are covered in an informative chapter from a historical perspective.

"Aroma-Vital Cuisine" (Chapter 18) looks at the possibility to utilize EOs in the kitchen, where the pleasure of eating, the sensuality, and the enjoyment of lunching and dining of mostly processed food are stressed. Here, rather the holistic point of view and not too scientific way of understanding EOs is the topic, simply to show that these volatile natural plant products can add a lot of well-feeling to their users.

EOs are not only appealing to humans but also to animals. Applications of EOs as feed additives and for treating diseases in pets and farm animals are illustrated in Chapter 19, "Essential Oils Used in Veterinary Medicine," that comprises a rare collection of information in this subject.

The EO industry is highly complex and fragmented and the trade of EOs is rather conservative and highly specialized. EOs are produced and utilized in industrialized as well as in developing countries worldwide. Their trade situation in the world is summarized in "Trade of Essential Oils" (Chapter 20), authored by a world-renown expert Hugo Bovill.

Storage and transport of EOs are crucial issues since they are highly sensitive to heat, moisture, and oxygen. Therefore, special precautions and strict regulations apply for their handling in storage and transport. "Storage and Transport of Essential Oils" (Chapter 21) will give the reader necessary guidelines to tackle this problem.

Finally, the regulatory affairs of EOs are dealt with in Chapter 22 in order to give a better insight to those interested in legislative aspects. "Recent EU Legislation on Flavors and Fragrances and Its Impact on Essential Oils" comprises the most up-to-date regulations and legislative procedures applied on EOs in the European Union.

This book is hoped to satisfy the needs of EO producers, traders, and users as well as researchers, academicians, and legislators who will find the most current information given by selected experts under one cover.

2 History and Sources of Essential Oil Research

Karl-Heinz Kubeczka

CONTENTS

2.1 FIRST SYSTEMATIC INVESTIGATIONS

The first systematic investigations of constituents from essential oils may be attributed to the French chemist M. J. Dumas (1800–1884) who analyzed some hydrocarbons and oxygen as well as sulfur- and nitrogen-containing constituents. He published his results in 1833. The French researcher

M. Berthelot (1859) characterized several natural substances and their rearrangement products by optical rotation. However, the most important investigations have been performed by O. Wallach, an assistant of Kekule. He realized that several terpenes described under different names according to their botanical sources were often, in fact, chemically identical. He, therefore, tried to isolate the individual oil constituents and to study their basic properties. He employed together with his highly qualified coworkers Hesse, Gildemeister, Betram, Walbaum, Wienhaus, and others fractional distillation to separate essential oils and performed reactions with inorganic reagents to characterize the obtained individual fractions. The reagents he used were hydrochloric acid, oxides of nitrogen, bromine, and nitrosyl chloride—which was used for the first time by W. A. Tilden (1875)—by which frequently crystalline products have been obtained.

At that time, hydrocarbons occurring in essential oils with the molecular formula $C_{10}H_{16}$ were known, which had been named by Kekule *terpenes* because of their occurrence in turpentine oil. Constituents with the molecular formulas $C_{10}H_{16}O$ and $C_{10}H_{18}O$ were also known at that time under the generic name camphor and were obviously related to terpenes. The prototype of this group was camphor itself, which was known since antiquity. In 1891, Wallach characterized the terpenes pinene, camphene, limonene, dipentene, phellandrene, terpinolene, fenchene, and sylvestrene, which has later been recognized to be an artifact.

During 1884–1914, Wallach wrote about 180 articles that are summarized in his book *Terpene und Campher* (Wallach, 1914) compiling all the knowledge on terpenes at that time and already in 1887 he suggested that the terpenes must be constructed from isoprene units. In 1910, he was honored with the Nobel Prize for Chemistry "in recognition of his outstanding research in organic chemistry and especially in the field of alicyclic compounds."

In addition to Wallach, the German chemist A. von Baeyer, who also had been trained in Kekule's laboratory, was one of the first chemists to become convinced of the achievements of structural chemistry and who developed and applied it to all of his work covering a broad scope of organic chemistry. Since 1893, he devoted considerable work to the structures and properties of cyclic terpenes (von Bayer, 1901). Besides his contributions to several dyes, the investigations of polyacetylenes, and so on, his contributions to theoretical chemistry including the strain theory of triple bonds and small carbon cycles have to be mentioned. In 1905, he was awarded the Nobel Prize for Chemistry "in recognition of his contributions to the development of Organic Chemistry and Industrial Chemistry, by his work on organic dyes and hydroaromatic compounds." The frequently occurring acyclic monoterpenes geraniol, linalool, citral, and so on have been investigated by F. W. Semmler and the Russian chemist G. Wagner (1899), who recognized the importance of rearrangements for the elucidation of chemical constitution, especially the carbon-to-carbon migration of alkyl, aryl, or hydride ions, a type of reaction that was later generalized by H. Meerwein (1914) as Wagner–Meerwein rearrangement.

More recent investigations of J. Read, W. Hückel, H. Schmidt, W. Treibs, and V. Prelog were mainly devoted to disentangle the stereochemical structures of menthols, carvomenthols, borneols, fenchols, and pinocampheols, as well as the related ketones (*cf.* Gildemeister and Hoffmann, 1956).

A significant improvement in structure elucidation was the application of dehydrogenation of sesqui- and diterpenes with sulfur and later with selenium to give aromatic compounds as a major method, and the application of the isoprene rule to terpene chemistry, which have been very efficiently used by L. Ruzicka (1953) in Zurich, Switzerland. In 1939, he was honored in recognition of his outstanding investigations with the Nobel Prize in chemistry for his work on "polymethylenes and higher terpenes."

The structure of the frequently occurring bicyclic sesquiterpene ß-caryophyllene was for many years a matter of doubt. After numerous investigations W. Treibs (1952) has been able to isolate the crystalline caryophyllene epoxide from the autoxidation products of clove oil and F. Šorm et al. (1950) suggested caryophyllene to have a 4- and 9-membered ring on bases of infrared (IR) investigations. This suggestion was later confirmed by the English chemist D. H. R. Barton (Barton and Lindsay, 1951), who was awarded the Nobel Prize in Chemistry in 1969.

The application of ultraviolet (UV) spectroscopy in the elucidation of the structure of terpenes and other natural products was extensively used by R. B. Woodward in the early forties of the last century. On the basis of his large collection of empirical data, he developed a series of rules (later called the Woodward rules), which could be applied to finding out the structures of new natural substances by correlations between the position of UV maximum absorption and the substitution pattern of a diene or an α,β-unsaturated ketone (Woodward, 1941). He was awarded the Nobel Prize in Chemistry in 1965. However, it was not until the introduction of chromatographic separation methods and nuclear magnetic resonance (NMR) spectroscopy into organic chemistry, that a lot of further structures of terpenes were elucidated. The almost exponential growth in our knowledge in that field and other essential oil constituents is essentially due to the considerable advances in analytical methods in the course of the last half century.

2.2 RESEARCH DURING THE LAST HALF CENTURY

2.2.1 Essential Oil Preparation Techniques

2.2.1.1 Industrial Processes

The vast majority of essential oils are produced from plant material in which they occur by different kinds of distillation or by cold pressing in the case of the peel oils from citrus fruits.

In water- or hydrodistillation, the chopped plant material is submerged and in direct contact with boiling water. In steam distillation, the steam is produced in a boiler separate of the still and blown through a pipe into the bottom of the still, where the plant material rests on a perforated tray or in a basket for quick removal after exhaustive extraction. In addition to the aforementioned distillation at atmospheric pressure, high-pressure steam distillation is most often applied in European and American field stills and the applied increased temperature significantly reduces the time of distillation. The high-pressure steam-type distillation is often applied for peppermint, spearmint, lavandin, and the like. The condensed distillate, consisting of a mixture of water and oil, is usually separated in a so-called Florentine flask, a glass jar, or more recently in a receptacle made of stainless steel with one outlet near the base and another near the top. There the distillate separates into two layers from which the oil and the water can be separately withdrawn. Generally, the process of steam distillation is the most widely accepted method for the production of essential oils on a large scale.

Expression or cold pressing is a process in which the oil glands within the peels of citrus fruits are mechanically crushed to release their content. There are several different processes used for the isolation of citrus oils; however, there are four major currently used processes. Those are Pellatrice and Sfumatrice—most often used in Italy—and the Brown Peel Shaver as well as the FMC extractor, which are used predominantly in North and South America. For more details see for example Lawrence 1995. All these processes lead to products that are not entirely volatile because they may contain coumarins, plant pigments, and so on; however, they are nevertheless acknowledged as essential oils by the International Organization for Standardization (ISO), the different pharmacopoeias, and so on.

In contrast, extracts obtained by solvent extraction with different organic solvents, with liquid carbon dioxide or by supercritical fluid extraction (SFE) may not be considered as true essential oils; however, they possess most often aroma profiles that are almost identical to the raw material from which they have been extracted. They are therefore often used in the flavor and fragrance industry and in addition in food industry, if the chosen solvents are acceptable for food and do not leave any harmful residue in food products.

2.2.1.2 Laboratory-Scale Techniques

The following techniques are used mainly for trapping small amounts of volatiles from aromatic plants in research laboratories and partly for determination of the essential oil content in plant material. The most often used device is the circulatory distillation apparatus, basing on the

publication of Clevenger in 1928 and which has later found various modifications. One of those modified apparatus described by Cocking and Middleton (1935) has been introduced in the European Pharmacopoeia and several other pharmacopoeias. This device consists of a heated round-bottom flask into which the chopped plant material and water are placed and which is connected to a vertical condenser and a graduated tube, for the volumetric determination of the oil. At the bottom of the tube a three-way valve permits to direct the water back to the flask, since it is a continuous closed-circuit distillation device, and at the end of the distillation process to separate the essential oil from the water phase for further investigations. The length of distillation depends on the plant material to be investigated; however, it is usually fixed to 3–4 h. For the volumetric determination of the essential oil content in plants according to most of the pharmacopoeias, a certain amount of xylene—usually 0.5 mL—has to be placed over the water before running distillation to separate even small droplets of essential oil during distillation from the water. The volume of essential oil can be determined in the graduated tube after subtracting the volume of the applied xylene.

Improved constructions with regard to the cooling system of the above-mentioned distillation apparatus have been published by Stahl (1953) and Sprecher (1963), and in publications of Kaiser et al. (1951) and Mechler et al. (1977), various apparatus used for the determination of essential oils in plant material are discussed and depicted.

A further improvement was the development of a simultaneous distillation–solvent extraction device by Likens and Nickerson in 1964 (*cf.* Nickerson and Likens, 1966). The device permits continuous concentration of volatiles during hydrodistillation in one step using a closed-circuit distillation system. The water distillate is continuously extracted with a small amount of an organic and water-immiscible solvent. Although there are two versions described, one for high-density and one for low-density solvents, the high-density solvent version using dichloromethane is mostly applied in essential oil research. It has found numerous applications and several modified versions including different microdistillation devices have been described (e.g., Bicchi, 1987; Chaintreau, 2001).

A sample preparation technique basing on Soxhlet extraction in a pressurized container using liquid carbon dioxide as extractant has been published by Jennings (1979). This device produces solvent-free extracts especially suitable for high-resolution gas chromatography (HRGC). As a less time-consuming alternative, the application of microwave-assisted extraction has been proposed by several researchers, for example by Craveiro et al. (1989), using a round-bottom flask containing the fresh plant material. This flask was placed into a microwave oven and passed by a flow of air. The oven was heated for 5 min and the obtained mixture of water and oil collected in a small and cooled flask. After extraction with dichloromethane the solution was submitted to gas chromatography-mass spectrometry (GC-MS) analysis. The obtained analytical results have been compared with the results obtained by conventional distillation and exhibited no qualitative differences; however, the percentages of the individual components varied significantly. A different approach yielding solvent-free extracts from aromatic herbs by means of microwave heating has been presented by Lucchesi et al. (2004). The potential of the applied technique has been compared with conventional hydrodistillation showing substantially higher amounts of oxygenated compounds at the expense of monoterpene hydrocarbons.

2.2.1.3 Microsampling Techniques

2.2.1.3.1 Microdistillation

Preparation of very small amounts of essential oils may be necessary if only very small amounts of plant material are available, and can be fundamental in chemotaxonomic investigations and control analysis but also for medicinal and spice plant breeding. In the past, numerous attempts have been made to minimize conventional distillation devices. As an example, the modified Marcusson device may be quoted (Bicchi et al., 1983) by which 0.2–3 g plant material suspended in 50 mL water can be distilled and collected in 100 μL analytical grade pentane or hexane. The analytical results proved to be identical with those obtained by conventional distillation.

Microversions of the distillation–extraction apparatus, described by Likens and Nickerson, have also been developed as well for high-density solvents (Godefroot et al., 1981), as for low-density solvents (Godefroot et al., 1982). The main advantage of these techniques is that no further enrichment by evaporation is required for subsequent gas chromatographic investigation.

A different approach has been presented by Gießelmann et al. (1993) and Kubeczka et al. (1995). By means of a new developed microhydrodistillation device the volatile constituents of very small amounts of plant material have been separated. The microscale hydrodistillation of the sample is performed using a 20 mL crimp-cap glass vial with a Teflon®-lined rubber septum contaning 10 mL water and 200–250 mg of the material to be investigated. This vial, which is placed in a heating block, is connected with a cooled receiver vial by a 0.32 mm I.D. fused silica capillary. By temperature-programmed heating of the sample vial, the water and the volatile constituents are vaporized and passed through the capillary into the cooled receiver vial. There, the volatiles as well as water are condensed and the essential oil collected in pentane for further analysis. The received analytical results have been compared to results from identical samples obtained by conventional hydrodistillation showing a good correlation of the qualitative and quantitative composition. Further applications with the commercially available Eppendorf MicroDistiller® have been published in several papers, for example, by Briechle et al. (1997) and Baser et al. (2001).

A simple device for rapid extraction of volatiles from natural plant drugs and the direct transfer of these substances to the starting point of a thin-layer chromatographic plate has been described by Stahl (1969a) and in his subsequent publications. A small amount of the sample (ca. 100 mg) is introduced into a glass cartridge with a conical tip together with 100 mg silica gel, containing 20% of water, and heated rapidly in a heating block for a short time at a preset temperature. The tip of the glass tube projects ca. 1 mm from the furnace and points to the starting point of the thin-layer plate, which is positioned 1 mm in front of the tip. Before introducing the glass tube it is sealed with a silicone rubber membrane. This simple technique has proven useful for many years in numerous investigations, especially in quality control, identification of plant drugs, and rapid screening of chemical races. In addition to the aforementioned microhydrodistillaion with the so-called TAS procedure (T = thermomicro and transfer; A = application; S = substance), several further applications, for example, in structure elucidation of isolated natural compounds such as zinc dust distillation, sulfur and selenium dehydrogenation, and catalytic dehydrogenation with palladium have been described in the microgram range (Stahl, 1976).

2.2.1.3.2 Direct Sampling from Secretory Structures

The investigation of the essential oils by direct sampling from secretory glands is of fundamental importance in studying the true essential oil composition of aromatic plants, since the usual applied techniques such as hydrodistillation and extraction are known to produce in some cases several artifacts. Therefore only direct sampling from secretory cavities and glandular trichomes and poperly performed successive analysis may furnish reliable results. One of the first investigations with a kind of direct sampling has been performed by Hefendehl (1966), who isolated the glandular hairs from the surfaces of *Mentha piperita* and *Mentha aquatica* leaves by means of a thin film of polyvinyl alcohol, which was removed after drying and extracted with diethyl ether. The composition of this product was in good agreement with the essential oils obtained by hydrodistillation. In contrast to these results, Malingré et al. (1969) observed some qualitative differences in course of their study on *Mentha aquatica* leaves after isolation of the essential oil from individual glandular hairs by means of a micromanipulator and a stereomicroscope. In the same year Amelunxen et al. (1969) published results on *Mentha piperita*, who separately isolated glandular hairs and glandular trichomes with glass capillaries. They found identical qualitative composition of the oil in both types of hairs, but differing concentrations of the individual components. Further studies have been performed by Henderson et al. (1970) on Pogostemon cablin leaves and by Fischer et al. (1987) on *Majorana hortensis* leaves. In the latter study, significant differences regarding the oil composition of the hydrodistilled oil and the oil extracted by means of glass capillaries from the trichomes

was observed. Their final conclusion was that the analysis of the respective essential oil is mainly an analysis of artifacts, formed during distillation, and the gas chromatographic analysis. Even if the investgations are performed very carefully and the successive GC has been performed by cold on-column injection to avoid thermal stress in the injection port, significant differences of the GC pattern of directly sampled oils versus the microdistilled samples have been observed in several cases (Bicchi et al., 1985).

2.2.1.3.3 Headspace Techniques

Headspace (HS) analysis has become one of the very frequently used sampling techniques in the investigation of aromatic plants, fragrances, and spices. It is a means of separating the volatiles from a liquid or solid prior to gas chromatographic analysis and is preferably used for samples that cannot be directly injected into a gas chromatograph. The applied techniques are usually classified according to the different sampling principles in static HS analysis and dynamic HS analysis.

2.2.1.3.3.1 Static HS Methods In static HS analysis, the liquid or solid sample is placed into a vial, which is heated to a predetermined temperature after sealing. After the sample has reached equilibrium with its vapor (in equilibrium, the distribution of the analytes between the two phases depends on their partition coefficients at the preselected temperature, the time, and the pressure), an aliquot of the vapor phase can be withdrawn with a gas-tight syringe and subjected to gas chromatographic analysis. A simple method for the HS investigation of herbs and spices was described by Chialva et al. (1982), using a blender equipped with a special gas-tight valve. After grinding the herb and until thermodynamic equilibrium is reached, the HS sample can be withdrawn through the valve and injected into a gas chromatograph. Eight of the obtained capillary gas chromatograms are depicted in the paper of Chialva and compared with those of the respective essential oils exhibiting significant higher amounts of the more volatile oil constituents. However, one of the major problems with static HS analyses is the need for sample enrichment with regard to trace components. Therefore a concentration step such as cryogenic trapping, liquid absorption, or adsorption on a suitable solid has to be inserted for volatiles occurring only in small amounts. A versatile and often-used technique in the last decade is solid-phase microextraction (SPME) for sampling volatiles, which will be discussed in more detail in a separate paragraph. Since different other trapping procedures are a fundamental prerequisite for dynamic HS methods, they will be considered below. A comprehensive treatment of the theoretical basis of static HS analysis including numerous applications has been published by Kolb et al. (1997, 2006).

2.2.1.3.3.2 Dynamic HS Methods The sensitivity of HS analysis can be improved considerably by stripping the volatiles from the material to be investigated with a stream of purified air or inert gas and trapping the released compounds. However, care has to be taken if grinded plant material has to be investigated, since disruption of tissues may initiate enzymatic reactions that may lead to formation of volatile artifacts. After stripping the plant material with gas in a closed vessel, the released volatile compounds are passed through a trap to collect and enrich the sample. This must be done because sample injection of fairly large sample volumes results in band broadening causing peak distortion and poor resolution. The following three techniques are advisable for collecting the highly diluted volatile sample according to Schaefer (1981) and Schreier (1984) with numerous references.

Cryogenic trapping can be achieved by passing the gas containing the stripped volatiles through a cooled vessel or a capillary in which the volatile compounds are condensed (Kolb et al., 1986). The most convenient way for trapping the volatiles is to utilize part of the capillary column as a cryogenic trap. A simple device for cryofocusing of HS volatiles by using the first part of capillary column as a cryogenic trap has been shown in the aforementioned reference inclusive of a discussion of the theoretical background of cryogenic trapping. A similar on-column cold trapping device, suitable for extended period vapor sampling, has been published by Jennings (1981).

A different approach can be used if large volumes of stripped volatiles have to be trapped using collection in organic liquid phases. In this case, the volatiles distribute between the gas and the liquid and efficient collection will be achieved, if the distribution factor K is favorable for solving the stripped compounds in the liquid. A serious drawback, however, is the necessity to concentrate the obtained solution prior to GC with the risk to loose highly volatile compounds. This can be overcome if a short-packed GC column is used containing a solid support coated with a suitable liquid. Novak et al. (1965) have used Celite coated with 30% silicone elastomer E-301 and the absorbed compounds were introduced into a gas chromatograph after thermal desorption. Coating with 15% silicone rubber SE 30 has been successfully used by Kubeczka (1967) with a similar device and the application of a wall-coated tubing with methylsilicone oil SF 96 has been described by Teranishi et al. (1972). A different technique has been used by Bergström et al. (1973, 1980). They trapped the scent of flowers on Chromosorb® W coated with 10% silicon high vacuum grease and filled a small portion of the sorbent containing the volatiles into a precolumn, which was placed in the splitless injection port of a gas chromatograph. There the volatiles were desorbed under heating and flushed onto the GC column. In 1987, Bichi et al. applied up to 50 cm pieces of thick-film fused silica capillaries coated with a 15 µm dimethylsilicone film for trapping the volatiles in the atmosphere surrounding living plants. The plants under investigation were placed in a glass bell into which the trapping capillary was introduced through a rubber septum while the other end of the capillary has been connected to pocket sampler. In order to trap even volatile monoterpene hydrocarbons, a capillary length of at least 50 cm and sample volume of maximum 100 mL has to be applied to avoid loss of components through breakthrough. The trapped compounds have been subsequently on-line thermally desorbed, cold trapped, and analyzed. Finally, a type of *enfleurage* and especially designed for field experiments has been described by Joulain (1987) to trap the scents of freshly picked flowers. Around 100 g flowers were spread on the grid of a specially designed stainless steel device and passed by a stream of ambient air, supplied by an unheated portable air drier. The stripped volatiles are trapped on a layer of purified fat placed above the grid. After 2 h, the fat was collected and the volatiles recovered in the laboratory by means of vacuum distillation at low temperature.

With a third often applied procedure the stripped volatiles from the HS of plant material and especially from flowers are passed through a tube filled with a solid adsorbent on which the volatile compounds are adsorbed. Common adsorbents most often used in investigations of plant volatiles are above all charcoal and different types of synthetic porous polymers. Activated charcoal is an adsorbent with a high adsorption capacity, thermal and chemical stability, and which is not deactivated by water, an important feature, if freshly collected plant material has to be investigated. The adsorbed volatiles can easily be recovered by elution with small amounts (10–50 µL) of carbon disulfide avoiding further concentration of the sample prior to GC analysis. The occasionally observed incomplete recovery of sample components after solvent extraction and artifact formation after thermal desorption have been largely solved by application of small amounts of special type of activated charcoal as described by Grob et al. (1976). Numerous applications have been described using this special type of activated charcoal, for example, by Kaiser (1993) in a great number of field experiments on the scent of orchids. In addition to charcoal the following synthetic porous polymers have been applied to collect volatile compounds from the HS from flowers and different other plant materials according to Schaefer (1981): Tenax® GC, different Porapak® types (e.g., Porapak® P, Q, R, and T) as well as several Chromosorb® types belonging to the 100 series. More recent developed adsorbents are the carbonaceous adsorbents such as Ambersorb®, Carboxene®, and Carbopak® and their adsorbent properties lie between activated charcoal and the porous polymers. Especially the porous polymers have to be washed repeatedly, for example, with diethyl ether and conditioned before use in a stream of oxygen-free nitrogen at 200–280°C, depending on the sort of adsorbent. The trapped components can be recovered either by thermal desorption or by solvent elution and the recoveries can be different depending on the applied adsorbent (Cole, 1980). Another very important criterion for the selection of a suitable adsorbent for collecting HS samples is the breakthrough volume limiting the amount of gas passing through the trap.

A comprehensive review concerning HS gas chromatographic analysis of medicinal and aromatic plants and flowers with 137 references, covering the period from 1982 to 1988 has been published by Bicchi and Joulain in 1990, thoroughly describing and explaining the different methodological approaches and applications. Among other things most of the important contributions of the Finnish research group of Hiltunen and coworkers on the HS of medicinal plants and the optimization of the HS parameters have been cited in the mentioned review.

2.2.1.3.4 Solid-Phase Microextraction

SPME is an easy-to-handle sampling technique, initially developed for the determination of volatile organic compounds in environmental samples (Arthur et al., 1990), and has gained, in the last years, acceptance in numerous fields and has been applied to the analysis of a wide range of analytes in various matrices. Sample preparation is based on sorption of analytes from a sample onto a coated fused silica fiber which is mounted in a modified GC syringe. After introducing the coated fiber into a liquid or gaseous sample, the compounds to be analyzed are enriched according to their distribution coefficients and can be subsequently thermally desorbed from the coating after introducing the fiber into the hot injector of a gas chromatograph. The commercially available SPME device (Supelco Inc.) consists of a 1 cm length fused silica fiber of ca. 100 μm diameter coated on the outer surface with a stationary phase fixed to a stainless steel plunger and a holder that looks like a modified microliter syringe. The fiber can be drawn into the syringe needle to prevent damage. To use the device, the needle is pierced through the septum that seals the sample vial. Then, the plunger is depressed lowering the coated fiber into the liquid sample or the HS above the sample. After sorption of the sample, which takes some minutes, the fiber has to be drawn back into the needle and withdrawn from the sample vial. By the same procedure the fiber can be introduced into the gas chromatograph injector where the adsorbed substances are thermally desorbed and flushed by the carrier gas into the capillary GC column.

SPME fibers can be coated with polymer liquid (e.g., polydimethylsiloxane, PDMS) or a mixed solid and liquid coating (e.g., Carboxen®/PDMS). The selectivity and capacity of the fiber coating can be adjusted by changing the phase type or thickness of the coating on the fiber according to the properties of the compounds to be analyzed. Commercially available are coatings of 7, 30, and 100 μm of PDMS, an 85 μm polyacrylate, and several mixed coatings for different polar components. The influence of fiber coatings on the recovery of plant volatiles was thoroughly investigated by Bicchi et al. (2000). Details concerning the theory of SPME, technology, its application, and specific topics have been described by Pawliszyn (1997) and references cited therein. A number of different applications of SPME in the field of essential oil analysis have been presented by Kubeczka (1997a). An overview on publications of the period 2000–2005 with regard to HS-SPME has been recently published by Belliardo et al. (2006) covering the analysis of volatiles from aromatic and medicinal plants, selection of the most effective fibers and sampling conditions, and discussing its advantages and limitations. The most comprehensive collection of references with regard to the different application of SPME can be obtained from Supelco on CD.

2.2.1.3.5 Stir Bar Sorptive Extraction and Headspace Sorptive Extraction

Despite the indisputable simplicity and rapidity of SPME, its applicability is limited by the small amount of sorbent on the needle (<0.5 μL) and consequently SPME has no real opportunity to realize quantitative extraction. Parameters governing recovery of analytes from a sample are partitioning constants and the phase ratio between the sorbent and liquid or gaseous sample. Therefore, basing on theoretical considerations, a procedure for sorptive enrichment with the sensitivity of packed PDMS beds (Baltussen et al., 1997) has been developed for the extraction of aqueous samples using modified PDMS-coated stir bars (Baltussen et al., 1999).

The stir bars were incorporated into a narrow glass tube coated with a PDMS layer of 1 mm (corresponding to 55 μL for a 10 mm length) applicable to small sample volumes. Such stir bars are commercially available under name "Twister" (Gerstel, Germany). After certain stirring time the

stir bar has to be removed, introduced into a glass tube, and transferred to thermal desorption instrument. After desorption and cryofocusing within a cooled programmed temperature vaporization (PTV) injector, the volatiles were transferred onto the analytical GC column. Comparison of SPME and the above-mentioned stir bar sorptive extraction (SBSE) technique using identical phases for both techniques exhibited striking differences in the recoveries, which has been attributed to ca. 100 times higher phase ratio in SBSE than in SPME. A comprehensive treatment of SBSE, discussion of the principle, the extraction procedure, and numerous applications was recently been published by David and Sandra (2007).

A further approach for sorptive enrichment of volatiles from the HS of aqueous or solid samples has been described by Tienpont et al. (2000), referred to as headspace sorptive extraction (HSSE). This technique implies the sorption of volatiles into PDMS that is chemically bound on the surface of a glass rod support. The device consists of a ca. 5 cm length glass rod of 2 mm diameter and at the last centimeter of 1 mm diameter. This last part is covered with PDMS chemically bound to the glass surface. HS bars with 30, 50, and 100 mg PDMS are commercially available from Gerstel GmbH, Mühlheim, Germany. After thermal conditioning at 300°C for 2 h, the glass bar was introduced into the HS of a closed 20 mL HS vial containing the sample to be investigated. After sampling for 45 min, the bar was put into a glass tube for thermal desorption, which was performed with a TDS-2 thermodesorption unit (Gerstel). After desorption and cryofocusing within a PTV injector, the volatiles were transferred onto the analytical GC column. As a result, HSSE exceeded largely the sensitivity attainable with SPME. Several examples referring to the application of HSSE in HS analysis of aromatic and medicinal plants inclusive of details of the sampling procedure were described by Bicchi et al. (2000).

2.2.2 Chromatographic Separation Techniques

In the course of the last half century, a great number of techniques have been developed and applied to the analysis of essential oils. A part of them has been replaced nowadays by either more effective or easier-to-handle techniques, while other methods maintained their significance and have been permanently improved. Before going into detail, the analytical facilities in the sixties of the last century should be considered briefly. The methods available for the analysis of essential oils have been at that time (Table 2.1) thin-layer chromatography (TLC), various types of liquid column chromatography (LC), and already gas liquid chromatography (GC). In addition, several spectroscopic techniques such as UV and IR spectroscopy, MS, and ^{1}H-NMR spectroscopy have been available.

TABLE 2.1
Techniques Applied to the Analysis of Essential Oils

Chromatographic Techniques			
Including Two- and Multidimensional Techniques			
TLC	GC	LC	HPLC
CCC	SFC		
Spectroscopic and Spectrometric Techniques			
UV	IR	MS	1H-NMR
13C-NMR	NIR	Raman	
Hyphenated Techniques			
GC-MS	GC-UV	HPLC-GC	SFE-GC
GC-FTIR	GC-AES	HPLC-MS	SFC-GC
GC-FTIR-MS	GC-IRMS	HPLC-NMR	

In the following years, several additional techniques were developed and applied to essential oils analysis, including: high-performance liquid chromatography (HPLC); different kinds of countercurrent chromatography (CCC); supercritical fluid chromatography (SFC); including multidimensional coupling techniques, C-13 NMR, near infrared (NIR), and Raman spectroscopy; and a multitude of so-called hyphenated techniques, which means on-line couplings of chromatographic separation devices to spectrometers, yielding valuable structural information of the individual separated components made their identification feasible.

2.2.2.1 Thin-Layer Chromatography

TLC was one of the first chromatographic techniques and has been used for many years for the analysis of essential oils. This method provided valuable information compared to simple measurements of chemical and physical values and has therefore been adopted as a standard laboratory method for characterization of essential oils in numerous pharmacopoeias. Fundamentals of TLC have been described by Geiss (1987) and in a comprehensive handbook by Stahl (1969b), in which numerous applications and examples on investigations of secondary plant metabolites inclusive of essential oils are given. More recently, the third edition of the handbook of TLC from Shema and Fried (2003) appeared. Further approaches in TLC have been development of high-performance TLC (Kaiser, 1976), and the application of forced flow techniques such as overpressured layer chromatography (OPLC) and rotation planar chromatography (RPC) described by Tyihák et al. (1979) and Nyiredy (2003).

In spite of its indisputable simplicity and rapidity, this technique is now largely obsolete for analyzing such complex mixtures like essential oils, due to its low resolution. However, for the rapid investigation of the essential oil pattern of chemical races or the differentiation of individual plant species, this method can still be successfully applied (Gaedcke and Steinhoff, 2000). In addition, silver nitrate and silver perchlorate impregnated layers have been used for the separation of olefinic compounds, especially sesquiterpene hydrocarbons (Prasad et al., 1947), and more recently for the isolation of individual sesquiterpenes (Saritas, 2000).

2.2.2.2 Gas Chromatography

However, the separation capability of GC exceeded all the other separation techniques, even if only packed columns have been used. The exiting evolution of this technique in the past can be impressively demonstrated with four examples of the gas chromatographic separation of the essential oil from rue (Kubeczka, 1981a), a medicinal and aromatic plant. This oil was separated by S. Bruno in 1961 into eight constituents and represented one of the first gas chromatographic analyses of that essential oil. Only a few years later in 1964 separation of the same oil has been improved using a Perkin Elmer (PE) gas chromatograph equipped with a 2 m packed column and a thermal conductivity detector (TCD) operated under isothermal conditions yielding 20 separated constituents. A further improvement of the separation of the rue oil was obtained after the introduction of temperature programming of the column oven, yielding approximately 80 constituents. The last significant improvements were a result of the development of high-resolution capillary columns and the sensitive flame ionization detector (FID). By means of a 50 m glass capillary with 0.25 mm I.D., the rue oil could be separated into approximately 150 constituents, in 1981. However, the problems associated with the fragility of the glass capillaries and their cumbersome installation lessened the acknowledgment of this column types, despite their outstanding quality. This has changed since flexible fused silica capillaries became commercially available, which are nearly unbreakable in normal usage. In addition, by different cross-linking technologies, the problems associated with wall coating, especially with polar phases, have been overcome, so that all important types of stationary phases used in conventional GC have been commercially available. The most often used stationary phases for the analysis of essential oils have been, and are still today, the polar phases Carbowax 20M (DB-Wax, Supelcowax-10, HP-20M, Innowax, etc.), 14% cyanopropylphenyl–86% methyl polysiloxane (DB-1701, SPB-1701, HP-1701, OV-1701, etc.), and the nonpolar phases PDMS (DB-1, SPB-1, HP-1 and HP-1ms, CPSil-5 CB, OV-1, etc.), and 5% phenyl methyl polysiloxane

(DB-5, SPB-5, HP-5, CPSil-8 CB, OV-5, SE-54, etc.). Besides different column diameters of 0.53, 0.32, 0.25, 0.10, and 0.05 mm I.D., a variety of film thicknesses can be purchased. Increasing column diameter and film thickness of stationary phase increases the sample capacity at the expense of separation efficiency. However, sample capacity has become important, particularly in trace analysis and with some hyphenated techniques such as gas chromatography-Fourier transform infrared (GC-FTIR), in which a higher sample capacity is necessary when compared to GC-MS. On the other hand, the application of a narrow bore column with 100 μm I.D. and a film coating of 0.2 μm have been shown to be highly efficient and theoretical plate numbers of approximately 250,000 were received with a 25 m capillary (Lancas et al., 1988). The most common detector in GC is the FID because of its high sensitivity toward organic compounds. The universal applicable TCD is nowadays used only for fixed-gas detection because of its very low sensitivity as compared to FID, and cannot be used in capillary GC. Nitrogen-containing compounds can be selectively detected with the aid of the selective nitrogen–phosphorus detector (NPD), and chlorinated compounds by the selective and very sensitive electron-capture detector (ECD), which is often used in the analysis of pesticides. Oxygen-containing compounds have been selectively detected with special O-FID analyzer even in very complex samples, which was primarily employed to the analysis of oxygenated compounds in gasoline, utilized as fuel-blending agents (Schneider et al., 1982). The oxygen selectivity of the FID is obtained by two on-line postcolumn reactions: First a cracking reaction forming carbon monoxide, which is reduced in a second reactor yielding equimolar quantities of methane, which can be sensitively detected by the FID. Since in total each oxygen atom is converted to one molecule methane, the FID response is proportional to the amount of oxygen in the respective molecule. Application of the O-FID to the analysis of essential oils has been presented by Kubeczka (1991). However, conventional GC using fused silica capillaries with different stationary phases, including chiral phases, and the sensitive FID, is up to now the prime technique for the analysis of essential oils.

2.2.2.2.1 Fast and Ultrafast GC

Due to the demand for faster GC separations in routine work in the field of GC of essential oils, the development of fast and ultrafast GC seems worthy to be mentioned. The various approaches for fast GC have been reviewed in 1999 (Cramers et al., 1999). The most effective way to speed up GC separation without loosing separation efficiency is to use shorter columns with narrow inner diameter and thinner coatings, higher carrier gas flow rates, and accelerated temperature ramps. In Figure 2.1 the conventional and fast GC separation of lime oil is shown, indicating virtually the same separation efficiency in the fast GC and a reduction in time from approximately 60 to 13 min (Mondello et al., 2000).

An ultrafast GC separation of the essential oil from lime with an outstanding reduction of time was recently achieved (Mondello et al., 2004) using a 5 m capillary with 50 μm I.D. and a film thickness of 0.05 μm operated with a high carrier gas velocity of 120 cm/min and an accelerated three-stage temperature programme. The analysis of the essential oil was obtained in approximately 90 sec, which equates to a speed gain of approximately 33 times in comparison with the conventional GC separation. However, such a separation cannot be performed with conventional GC instruments. In addition, the mass spectrometric identification of the separated components could only be achieved by coupling GC to a time-flight mass spectrometer. In Table 2.2 the separation parameters of conventional, fast, and ultrafast GC separation are given, indicating clearly the relatively low requirements for fast GC, while ultrafast separations can only be realized with modern GC instruments and need a significant higher employment.

2.2.2.2.2 Chiral GC

Besides fast and ultrafast GC separations, one of the most important developments in GC has been the introduction of enantioselective capillary columns in the past with high separation efficiency, so that a great number of chiral substances including many essential oil constituents could be separated and identified. The different approaches of gas chromatographic separation of chiral compounds are briefly

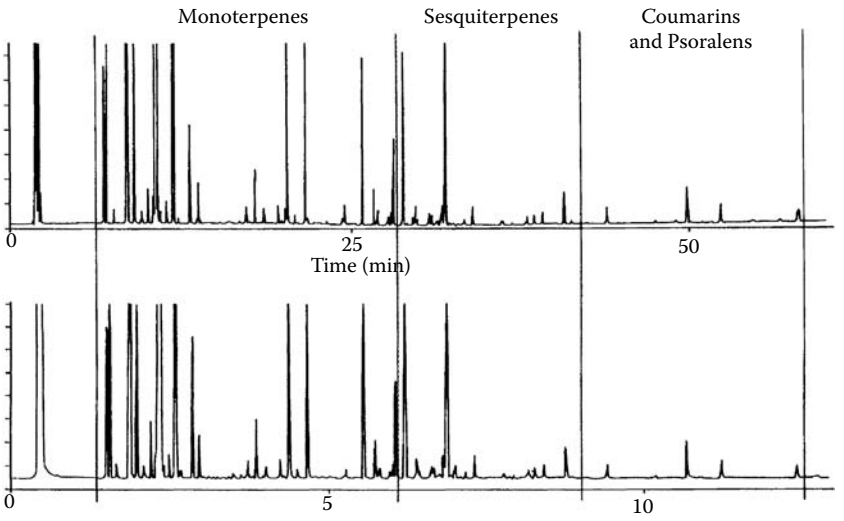

FIGURE 2.1 Comparison of conventional and fast-GC separation of lime oil. (From Mondello, L., et al., 2000. *LC.GC Europe*, 13: 495–502. With permission.)

summarized in Table 2.3. In the mid-1960s, Gil-Av published results with chiral diamide stationary phases for gas chromatographic separation of chiral compounds, which interacted with the analytes by hydrogen bonding forces (Gil-Av et al., 1965). The ability to separate enantiomers using these phases was therefore limited to substrates with hydrogen bonding donor or acceptor functions.

Diastereomeric association between chiral molecules and chiral transition metal complexes was first described by Schurig in 1977. Since hydrogen bonding interaction is not essential for chiral recognition in such a system, a number of compounds could be separated, but this method was limited by the nonsufficient thermal stability of the applied metal complexes.

In 1988 König, as well as Schurig, described the use of cyclodextrin derivatives that act enantioselectively by host–guest interaction by partial intrusion of enantiomers into the cyclodextrin cavity. They are cyclic α-(1–4)-bounded glucose oligomers with 6-, 7-, or 8-glucose units, which can be prepared by enzymatic degradation of starch with specific cyclodextrin-glucanosyltransferases from different bacterial strains, yielding α-, β-, and γ-cyclodextrins and are commercially available. Due to the significant lower reactivity of the 3-hydroxygroups of cyclodextrins, this position can be selectively acylated after alkylation of the 2- and 6-positions (Figure 2.2), yielding several nonpolar cyclodextrin derivatives, which are liquid or waxy at room temperature and which proved very useful for gas chromatographic applications.

TABLE 2.2
Conditions of Conventional, Fast-, and Ultrafast GC

	Conventional GC	Fast GC	Ultrafast GC
Column	30 m	10 m	10–15 m
	0.25 mm I.D.	0.1 mm I.D.	0.1 mm I.D.
	0.25 µm film	0.1 µm film	0.1 µm film
Temperature program	50–350°C	50–350°C	45–325°C
	3°C/min	14°C/min	45–200°C/min
Carrier gas	H_2	H_2	H_2
	u = 36 cm/s	u = 57 cm/s	u = 120 cm/s
Sampling frequency	10 Hz	20–50 Hz	50–250 Hz

TABLE 2.3
Different Approaches of Enantioselective GC

1. Chiral diamide stationary phases (Gil-Av, 1965)
   Hydrogen bonding interaction
2. Chiral transition metal complexation (Schurig, 1977)
   Complexation gas chromatography
3. Cyclodextrin derivatives (König, Schurig, 1988)
   Host–guest interaction, inclusion gas chromatography

König and coworkers reported their first results in 1988 with per-*O*-pentylated and selectively 3-*O*-acylated-2,6-di-*O*-pentylated α-, β-, and γ-cyclodextrins, which are highly stable, soluble in nonpolar solvents, and which possess a high enantioselectivity toward many chiral compounds. In the following years a number of further cyclodextrin derivatives have been synthesized and tested by several groups, allowing the separation of a wide range of chiral compounds, especially due to the improved thermal stability (Table 2.4). With the application of 2,3-pentyl-6-methyl-β- and -γ-cyclodextrin as stationary phases, all monoterpene hydrocarbons commonly occurring in essential oils could be separated (König et al., 1992). The reason for application of two different columns with complementary properties was that on one column not all enantiomers were satisfactorily resolved. Thus, the simultaneous use of these two columns provided a maximum of information and reliability in peak assignment.

After successful application of enantioselective GC to the analysis of enantiomeric composition of monoterpenoids in many essential oils (e.g., Werkhoff et al., 1993; Bicchi et al., 1995; and references cited therein), the studies have been extended to the sesquiterpene fraction. Standard mixtures of known enantiomeric composition were prepared by isolation of individual enantiomers from numerous essential oils by preparative GC and by preparative enantioselective GC. A gas chromatographic separation of a series of isolated or prepared sesquiterpene hydrocarbon enantiomers, showing the separation of 12 commonly occurring sesquiterpene hydrocarbons on a 2,6-methyl-3-pentyl-β-cyclodextrin capillary column has been presented by König et al. (1995). Further investigations on sesquiterpenes have been published by König et al. (1994). However, due to the complexity of the sesquiterpene pattern in many essential oils, it is often impossible to perform directly an enantioselective analysis by coinjection with standard samples on a capillary column with a chiral stationary phase alone. Therefore, in many cases two-dimensional GC had to be performed.

2.2.2.2.3 Two-Dimensional Gas Chromatography

After preseparation of the oil on a nonchiral stationary phase, the peaks of interest have to be transferred to a second capillary column coated with a chiral phase, a technique usually referred to as "heart cutting." In the simplest case, two GC capillaries with different selectivities are serially connected and the portion of unresolved components from the effluent of the first column is directed into a second column, for example, a capillary with a chiral coating. The basic arrangement used in two-dimensional gas chromatography (GC-GC) is shown in Figure 2.3. By means of a valve, the individual fractions of interest eluting from the first column are directed to the second, chiral column, while the rest of the sample may be discarded. With this heart-cutting technique many

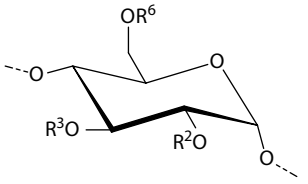

FIGURE 2.2 α-Glucose unit of a cyclodextrin.

TABLE 2.4
Important Cyclodextrin Derivatives

Research Group	Year	Cyclodextrin Derivative
Schurig and Novotny	1988	Per-*O*-methyl-β-CD
König et al.	1988a	Per-*O*-pentyl-(α,β,γ)-CD
König et al.	1988c	3-*O*-acetyl-2,6,-di-*O*-pentyl-(α,β,γ)-CD
König et al.	1989	3-*O*-butyryl-2,6-di-*O*-pentyl-(β,γ)-CD
König et al.	1990	6-*O*-methyl-2,3-di-*O*-pentyl-γ-CD
Köng et al.	1992	2,6-Di-*O*-methyl-3-*O*-pentyl-(β,γ)-CD
Dietrich et al.	1992	2,3-Di-*O*-acetyl-6-*O*-*tert*-butyl-dimethysilyl-β-CD
Dietrich et al.	1992a	2,3-Di-*O*-methyl-6-*O*-*tert*-butyl-dimethylsilyl-(β,γ)-CD
Bicchi et al.	1996	2,3-Di-*O*-ethyl-6-*O*-*tert*-butyl-dimethylsilyl-(β,γ)-CD
Takahisa and Engel	2005	2,3-Di-*O*-methoxymethyl-6-*O*-*tert*-butyl-dimethylsilyl-β-CD
Takahisa and Engel	2005a	2,3-Di-*O*-methoxymethyl-6-*O*-*tert*-butyl-dimethylsilyl-γ-CD

separations of chiral oil constituents have been performed in the past. As an example, the investigation of the chiral sesquiterpene hydrocarbon germacrene D shall be mentioned (Kubeczka, 1996), which was found to be a main constituent of the essential oil from the flowering herb from Solidago canadensis. The enantioselective investigation of the germacrene-D fraction from a GC run using a nonchiral DB-Wax capillary transferred to a 2,6-methyl-3-pentyl-β-cyclodextrin capillary exhibited the presence of both enantiomers. This is worthy to be mentioned, since in most of other germacrene D containing higher plants nearly exclusively the (–)-enantiomer can be found.

The previously mentioned two-dimensional GC design, however, in which a valve is used to direct the portion of desired effluent from the first into the second column, has obviously several shortcomings: The sample comes into contact with the metal surface of the valve body, the pressure drop of both connected columns may be significant and the use of only one-column oven does not permit to adjust the temperature for both columns properly. Therefore, one of the best approaches

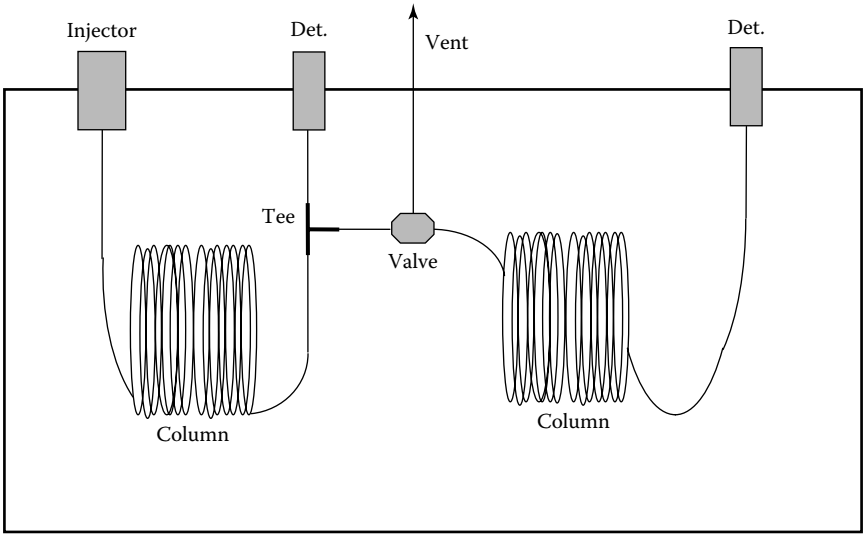

FIGURE 2.3 Basic arrangement used in two-dimensional GC.

to overcome these limitations has been realized by a commercially available two-column oven instrument using a Deans-type pressure balancing interface between the two columns called a "live-T-connection" (Figure 2.4) providing considerable flexibility (Hener, 1990). By means of that instrument the enantiomeric composition of several essential oils has been investigated very successfully. As an example, the investigation of the essential oil from Lavandula angustifolia shall be mentioned (Kreis et al., 1992) showing the simultaneous stereoanalysis of a mixture of chiral compounds, which can be found in lavender oils, using the column combination Carbowax 20M as the precolumn and 2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin as the main column. All the unresolved enantiomeric pairs from the precolumn could be well separated after transferring them to the chiral main column in a single run. As a result, it was found that most of the characteristic and genuine chiral constituents of lavender oil exhibit a high enantiomeric purity.

A different and inexpensive approach for transferring individual GC peaks onto a second column has been presented by Kubeczka (1997a), using an SPME device. The highly diluted organic vapor of a fraction eluting from a GC capillary in the carrier gas flow has been absorbed on a coated SPME fiber and introduced onto a second capillary. As could be demonstrated, no modification of the gas chromatograph had to be performed to realize that approach. The eluting fractions were sampled after shutting the valves of the air, of hydrogen and the make-up gas if applied. In order to minimize the volume of the detector to avoid dilution of the eluting fraction and to direct the gas flow to the fiber surface, a capillary glass tubing of 1.5 mm I.D. was inserted into the FID and fixed and tightened by an O-ring (Figure 2.5). At the beginning of peak elution, controlled only by time, a 100 μm PDMS fiber was introduced into the mounted glass capillary tubing and withdrawn at the end of peak elution. Afterward, the fiber within the needle was introduced into the injector of a second capillary column with a chiral stationary phase. Two examples concerning the investigation of bergamot oil have been shown. At first, the analysis of an authentic sample of bergamot oil, containing chiral linalool and the respective chiral actetate is carried out. Both components were cut separately and transferred to an enantioselective cyclodextrin Lipodex® E capillary. The chromatograms clearly have shown that the authentic bergamot oil contains nearly exclusively the (–)-enantiomers of linalool and linalyl acetate, while the respective (+)-enantiomers could only be detected as traces. In contrast to the authentic sample, a commercial sample of bergamot oil, which was analyzed under the same

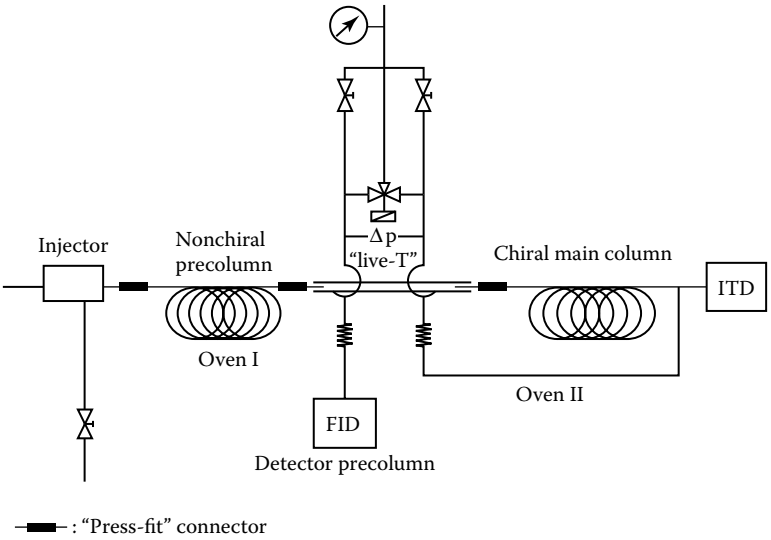

FIGURE 2.4 Scheme of enantioselective multidimensional GC with "live-T" column switching. (From Hener, U. 1990. Chirale Aromastoffe—Beiträge zur Struktur, Wirkung und Analytik. Dissertation, Goethe-University of Frankfurt/Main, Germany. With permission.)

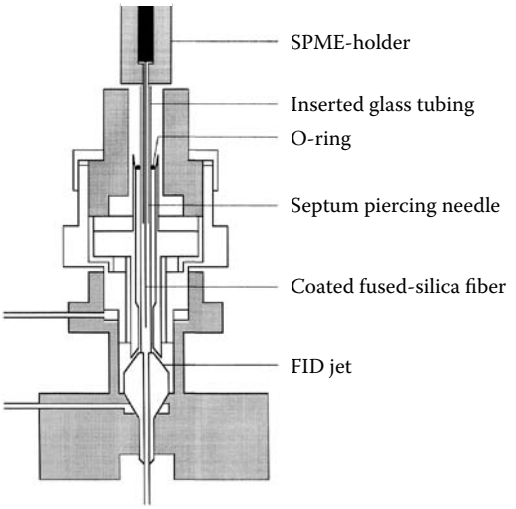

FIGURE 2.5 Cross section of an FID of an HP 5890 gas chromatograph with an inserted SPME fiber. (From Kubeczka, K.-H. 1997b. *Essential Oil Symposium Proceedings*, p. 145. With permission.)

conditions, exhibited the presence of significant amounts of both enantiomers of linalool and linalyl acetate indicating a falsification by admixing the respective racemic alcohol and ester.

2.2.2.2.4 Comprehensive Multidimensional Gas Chromatography

One of the most powerful separation techniques that has been recently applied to the investigation of essential oils is the so-called comprehensive multidimensional gas chromatography (GC × GC). This technique is a true multidimensional gas chromatography (MDGC) since it combines two directly coupled columns and importantly is able to subject the entire sample to simultaneous two-column separation. Using that technique, the need to select heart cuts, as used in conventional MDGC, is no longer required. Since components now are retained in two different columns, the net capacity is the product of the capacities of the two applied columns increasing considerably the resolution of the total system. Details regarding that technique will be given in the chapter of Luigi Mondello.

2.2.2.3 Liquid Column Chromatography

The different types of LC have been mostly used in preparative or semipreparative scale for preseparation of essential oils or for isolation of individual oil constituents for structure elucidation with spectroscopic methods and were rarely used at that time as an analytical separation tool alone, because GC plays a central role in the study of essential oils.

2.2.2.3.1 Preseparation of Essential Oils

A different approach besides two-dimensional GC, which has often been used in the past to overcome peak overlapping in a single GC run of an essential oil has been preseparation of the oil with LC. The most common method of fractionation is the separation of hydrocarbons from the oxygenated terpenoids according to Miller et al. (1952), using silica gel as an adsorbent. After elution of the nonpolar components from the column with pentane or hexane, the more polar oxygen-containing constituents are eluted in order of increasing polarity after applying more and more polar eluents.

A very simple and standardized fractionation in terms of speed and simplicity has been published by Kubeczka (1973) using dry-column chromatography. The procedure, which has been proved useful in numerous experiments for prefractionation of an essential oil, allows a preseparation into five fractions of increasing polarity. The preseparation of an essential oil into oxygenated constituents, monoterpene hydrocarbons, and sesquiterpene hydrocarbons, which is—depending on

the oil composition—sometimes of higher practical use, can be performed successfully using reversed-phase RP-18 HPLC (Schwanbeck et al., 1982). The HPLC was operated on a semipreparative scale by stepwise elution with methanol–water 82.5:17.5 (solvent A) and pure methanol (solvent B). The elution order of the investigated oil was according to decreasing polarity of the components and within the group of hydrocarbons to increasing molecular weight. Fraction 1 contained all oxygenated mono- and sesquiterpenoids, fraction 2 the monoterpene hydrocarbons, and fraction 3—eluted with pure methanol—the sesquiterpene hydrocarbons. A further alternative to the mentioned separation techniques is flash chromatography, initially developed by Still et al. (1978), and which has often been used as a rapid form of preparative LC based on a gas- or air pressure-driven short column chromatography. This technique, optimized for rapid separation of quantities typically in the range of 0.5–2.0 g uses dry-packed silica gel in an appropriate column. The separation of the sample generally takes only 5–10 min and can be performed with inexpensive laboratory equipment. However, impurities and active sites on dried silica gel were found to be responsible for isomerization of a number of oil constituents. After deactivation of the dried silica gel by adding 5% water, isomerization processes could be avoided (Scheffer et al., 1976). A different approach using HPLC on silica gel and isocratic elution with a ternary solvent system for the separation of essential oils has been published by Chamblee et al. (1985). In contrast to the aforementioned commonly used off-line pretreatment of a sample, the coupling of two or more chromatographic systems in an on-line mode offers advantages of ease of automation and usually of a shorter analysis time.

2.2.2.3.2 High-Performance Liquid Column Chromatography

The good separations obtained by GC have delayed the application of HPLC to the analysis of essential oils; however, HPLC analysis offers some advantages, if GC analysis of thermolabile compounds is difficult to achieve. Restricting factors for application of HPLC for analyses of terpenoids are the limitations inherent in the commonly available detectors and the relatively small range of k' values of liquid chromatographic systems. Since temperature is an important factor that controls k' values, separation of terpene hydrocarbons was performed at –15°C using a silica gel column and *n*-pentane as a mobile phase. Monitoring has been achieved with UV detection at 220 nm. Under these conditions, mixtures of commonly occurring mono- and sesquiterpene hydrocarbons could be well separated (Schwanbeck et al., 1979; Kubeczka, 1981b). However, the silica gel had to be deactivated by adding 4.8% water prior to separation to avoid irreversible adsorption or alteration of the sample. The investigation of different essential oils by HPLC already has been described in the seventies of the last century (e.g., Komae et al., 1975; Ross, 1976; Wulf et al., 1978; McKone, 1979; Scott and Kucera, 1979). In the last publication, the authors have used a rather long microbore packed column, which had several hundred thousand theoretical plates. Besides relatively expensive equipment, the HPLC chromatogram of an essential oil, separated on such a column could only be obtained at the expense of long analysis time. The mentioned separation needed about 20 h and may be only of little value in practical applications.

More recent papers with regard to HPLC separation of essential oils were published, for example, by Debrunner et al. (1995), Bos et al. (1996), Frérot et al. (2004), and applications using silver ion-impregnated sorbents have been presented by Pettei et al. (1977), Morita et al. (1983), Friedel et al. (1987), and van Beek et al. (1994). The literature on the use and theory of silver complexation chromatography has been reviewed by van Beek et al. (1995). HPLC has also been used to separate thermally labile terpenoids at low temperature by Beyer et al. (1986), showing the temperature dependence of the separation efficiency. The investigation of an essential oil fraction from Cistus ladanifer using RP-18 reversed-phase HPLC at ambient temperature and an acetonitrile-water gradient was published by Strack et al. (1980). Comparison of the obtained HPLC chromatogram with the respective GC run exhibits a relatively good HPLC separation in the range of sesqui- and diterpenes, while the monoterpenes exhibited, as expected, a significant better resolution by GC. The enantiomeric separation of sesquiterpenes by HPLC with a chiral stationary phase has recently been shown by Nishii et al. (1997), using a Chiralcel® OD column.

2.2.2.4 Supercritical Fluid Chromatography

Supercritical fluids are highly compressed gases above their critical temperature and critical pressure point, representing a hybrid state between a liquid and a gas and which have physical properties intermediate between liquid and gas phases. The diffusion coefficient of a fluid is about two orders of magnitude larger and the viscosity is two orders of magnitude lower than the corresponding properties of a liquid. On the other hand, a supercritical fluid has a significant higher density than a gas. The commonly used carbon dioxide as a mobile phase, however, exhibits a low polarity (comparable to pentane or hexane), limiting the solubility of polar compounds, a problem that has been solved by adding small amounts of polar solvents, for example, methanol or ethanol, to increase mobile-phase polarity, thus permitting separations of more polar compounds (Chester et al., 1986). A further strength of SFC lies in the variety of detection systems that can be applied. The intermediate features of SFC between GC and LC can be profitable when used in a variety of detection systems, which can be classified in *LC*- and *GC-like* detectors. In the first case, measurement takes place directly in the supercritical medium or in the liquid phase, whereas GC-like detection proceeds after a decompression stage.

Capillary SFC using carbon dioxide as mobile phase and a FID as detector has been applied to the analysis of several essential oils and seemed to give more reliable quantification than GC, especially for oxygenated compounds. However, the separation efficiency of GC for monoterpene hydrocarbons was, as expected, better than that of SFC. Manninen et al. (1990) published a comparison of a capillary GC versus a chromatogram obtained by capillary SFC from a linalool–methylchavicol basil oil chemotype exhibiting a fairly good separation by SFC.

2.2.2.5 Countercurrent Chromatography

CCC is according to Conway (1989) a form of liquid–liquid partition chromatography, in which centrifugal or gravitational forces are employed to maintain one liquid phase in a coil or train of chambers stationary, while a stream of a second, immiscible phase is passed through the system in contact with the stationary liquid phase. Retention of the individual components of the sample to be analyzed depends only on their partition coefficients and the volume ratio of the two applied liquid phases. Since there is no porous support, adsorption and catalytic effects encountered with solid supports are avoided.

2.2.2.5.1 Droplet Countercurrent Chromatography (DCCC)

One form of CCC, which has been sporadically applied to separate essential oils into fractions or in the ideal case into individual pure components, is DCCC. The device, which has been developed by Tanimura et al. (1970), consists of 300–600 glass tubes, which are connected to each other in series with Teflon® tubing and filled with a stationary liquid. Separation is achieved by passing droplets of the mobile phase through the columns, thus distributing mixture components at different ratios leading to their separation. With the development of a water-free solvent system, separation of essential oils could be achieved (Becker et al., 1981, 1982). Along with the separation of essential oils, the method allows the concentration of minor components, since relatively large samples can be separated in one analytical run (Kubeczka, 1985).

2.2.2.5.2 Rotation Locular Countercurrent Chromatography (RLCC)

The RLCC apparatus (Rikakikai Co., Tokyo, Japan) consists of 16 concentrically arranged and serially connected glass tubes. These tubes are divided by Teflon® disks with a small hole in the center, thus creating small compartments or locules. After filling the tubes with the stationary liquid, the tubes are inclined to a 30° angle from horizontal. In the ascending mode the lighter mobile phase is applied to the bottom of the first tube by a constant flow pump, displacing the stationary phase as its volume attains the level of the hole in the disk. The mobile phase passes through this hole and enters into the next compartment, where the process continues until the mobile phase emerges from the uppermost locule. Finally, the two phases fill approximately half

of each compartment. The dissolved essential oil subsequently introduced is subjected to a multistage partitioning process that leads to separation of the individual components. Whereas gravity contributes to the phase separation; rotation of the column assembly (60–80 rpm) produces circular stirring of the two liquids to promote partition. If the descending mode is selected for separation, the heavier mobile phase is applied at the top of each column by switching a valve. An overview on applications of RLCC in natural products isolation inclusive of a detailed description of the device and the selection of appropriate solvent systems has been presented by Snyder et al. (1984).

Comparing RLCC to the aforementioned DCCC, one can particularly stress the superior flexibility of RLCC. While DCCC requires under all circumstances a two-phase system able to form droplets in the stationary phase, the choice of solvent systems with RLCC is nearly free. So the limitations of DCCC, when analyzing lipophilic samples, do not apply to RLCC. The separation of a mixture of terpenes has been presented by Kubeczka (1985). A different method, the high-speed centrifugal countercurrent chromatography (HSCCC) developed by Ito and coworkers in the mid of the sixties of the last century (Ito et al., 1966), has been applied to separate a variety of nonvolatile natural compounds; however, separation of volatiles has, strange to say, until now not seriously been evaluated.

2.2.3 Hyphenated Techniques

2.2.3.1 Gas Chromatography-Mass Spectrometry

The advantage on-line coupling of a chromatographic device to a spectrometer is that complex mixtures can be analyzed in detail by spectral interpretation of the separated individual components. The coupling of a gas chromatograph with a mass spectrometer is the most often used and a well-established technique for the analysis of essential oils, due to the development of easy-to-handle powerful systems concerning sensitivity, data acquisition and processing, and above all their relatively low cost. The very first application of a GC-MS coupling for the identification of essential oil constituents using a capillary column was already published by Buttery et al. (1963). In those times, mass spectra have been traced on UV recording paper with a five-element galvanometer and their evaluation was a considerable cumbersome task.

This has changed after the introduction of computerized mass digitizers yielding the mass numbers and the relative mass intensities. The different kinds of GC-MS couplings available at the end of the seventies of the last century have been described in detail by ten Noever de Brauw (1979). In addition, different types of mass spectrometers have been applied in GC-MS investigations such as magnetic sector instruments, quadrupole mass spectrometers, ion-trap analyzers (e.g., ion-trap detector, ITD), and time-of-flight mass spectrometers, which are the fastest MS analyzers and therefore used for very fast GC-MS systems (e.g., in comprehensive multidimensional GC-MS). Surprisingly, a time-of-flight mass spectrometer was used in the very first description of a GC-MS investigation of an essential oil mentioned before. From the listed spectrometers, the magnetic sector and quadrupole instruments can also be used for selective ion monitoring (SIM), to improve sensitivity for the analysis of target compounds and for discrimination of overlapping GC peaks.

The great majority of today's GC-MS applications utilize one-dimensional capillary GC with quadrupole MS detection and electron ionization. Nevertheless, there are substantial numbers of applications using different types of mass spectrometers and ionization techniques. The proliferation of GC-MS applications is also a result of commercially available easy-to-handle dedicated mass spectral libraries (e.g., NIST/EPA/NIH 2005; WILEY Registry 2006; MassFinder 2007; and diverse printed versions such as Jennings and Shibamoto, 1980; Joulain and König, 1998; and Adams, 1989, 1995, 2007 inclusive of retention indices) providing identification of the separated compounds. However, this type of identification has the potential of producing some unreliable results, if no additional information is used, since some compounds, for example, the sesquiterpene

hydrocarbons α-cuprenene and β-himachalene, exhibit identical fragmentation pattern and only very small differences of their retention index values. This example demonstrates impressively that even a good library match and the additional use of retention data may lead in some cases to questionable results, and therefore require additional analytical data, for example, from NMR measurements.

2.2.3.1.1 Gas Chromatography-Chemical Ionization-Mass Spectrometry and Gas Chromatography-Tandem Mass Spectrometry

Although GC-electron impact (EI)-MS is a very useful tool for the analysis of essential oils, this technique can sometimes be not selective enough and requires more sophisticated techniques such as gas chromatography-chemical ionization-mass spectrometry (GC-CI-MS) and gas chromatography-tandem mass spectrometry (GC-MS-MS). The application of CI-MS using different reactant gases is particularly useful, since many terpene alcohols and esters fail to show a molecular ion. The use of OH^- as a reactant ion in negative CI-MS appeared to be an ideal solution to this problem. This technique yielded highly stable quasi-molecular-ions M–H, which are often the only ions in the obtained spectra of the above-mentioned compounds. As an example, the EI and CI spectra of isobornyl isovalerate—a constituent of valerian oil—shall be quoted (Bos et al., 1982). The respective EI mass spectrum shows only a very small molecular ion at 238. Therefore, the chemical ionization spectra of isobornyl acetate were performed exhibiting with isobutene as a reactant gas a $[C_{10}H_{17}]^+$-cation and in the negative CI-mode with OH^- as a reactant gas two signals with the masses 101, the isovalerate anion, and 237 the quasi-molecular-ion $[M–H]^-$. Considering all these obtained data, the correct structure of the oil constituent could be deduced. The application of isobutane and ammonia as reactant gases has been presented by Schultze et al. (1992), who investigated sesquiterpene hydrocarbons by GC-CI-MS. Fundamental aspects of chemical ionization MS have been reviewed by Bruins (1987), discussing the different reactant gases applied in positive and negative ion chemical ionization and their applications in essential oil analysis.

The utilization of GC-MS-MS to the analysis of a complex mixture will be shown in Figure 2.6. In the investigated vetiver oil (Cazaussus et al., 1988), one constituent, the nor-sesquiterpene ketone khusimone, has been identified by using GC-MS-MS in the collision-activated-dissociation mode. The molecular ion at *m/z* 204 exhibited a lot of daughter ions, but only one of them gave a daughter

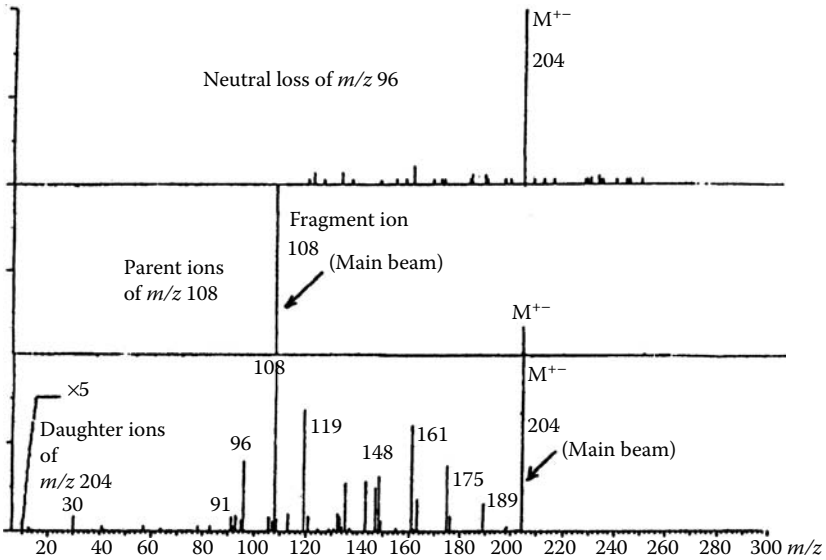

FIGURE 2.6 GC-EIMS-MS of khusimone of vetiver oil. (From Cazaussus, A., et al., 1988. *Chromatographia*, 25: 865–869. With permission.)

ion at *m/z* 108, a fragment rarely occurring in sesquiterpene derivatives so that the presence of khusimone could be undoubtedly identified.

2.2.3.2 High-Resolution Gas Chromatography-Fourier Transform Infrared Spectroscopy

A further hyphenated technique, providing valuable analytical information is the on-line coupling of a gas chromatograph with a FTIR spectrometer. The capability of IR spectroscopy to provide discrimination between isomers makes the coupling of a gas chromatograph to an FTIR spectrometer suited as a complementary method to GC/MS for the analysis of complex mixtures like essential oils. The GC/FTIR device consists basically of a capillary gas chromatograph and an FTIR spectrometer including a dedicated computer and ancillary equipment. As each GC peak elutes from the GC column, it enters a heated IR measuring cell, the so-called light pipe, usually a gold-plated glass tube with IR transparent windows. There the spectrum is measured as an interferogram from which the familiar absorbance spectrum can be calculated by computerized Fourier transformation. After passing the light pipe, the effluent is directed back into the FID of the gas chromatograph. More detailed information on the experimental setup was given by Herres et al. (1986) and Herres (1987).

In the latter publication, for example, the vapor-phase IR spectra of all the four isomers of pulegol and dihydrocarveol are shown, which have been extracted from a GC/FTIR run. These examples convincingly demonstrate the capability of distinguishing geometrical isomers with the aid of vapor-phase IR spectra, which cannot be achieved by their mass spectra. A broad application of GC-FTIR in the analysis of essential oils, however, is limited by the lack of sufficient vapor-phase spectra of uncommon compounds, which are needed for reference use, since the spectra of isolated molecules in the vapor phase can be significantly different from the corresponding condensed-phase spectra.

A different approach has been published by Reedy et al. in 1985, using a cryogenically freezing of the GC effluent admixed with an inert gas (usually argon) onto a rotating disk maintained at liquid He temperature to form a solid matrix trace. After the separation, reflection absorption spectra can be obtained from the deposited solid trace. A further technique published by Bourne et al. (1990) is the subambient trapping, whereby the GC effluent is cryogenically frozen onto a moving IR transparent window of zinc selenide (ZnSe). An advantage of the latter technique is that the unlike larger libraries of conventional IR spectra can be searched in contrast to the limited number of vapor-phase spectra and those obtained by matrix isolation. A further advantage of both cryogenic techniques is the significant higher sensitivity, which exceeds the detection limits of a light pipe instrument by approximately two orders of magnitude.

Comparing GC/FTIR and GC/MS, advantages and limitations of each technique become visible. The strength of IR lies—as discussed before—in distinguishing isomers, whereas identification of homologues can only be performed successfully by MS. The logical and most sophisticated way to overcome these limitations has been the development of a combined GC/FTIR/MS instrument, whereby simultaneously IR and mass spectra can be obtained.

2.2.3.3 Gas Chromatography-Ultraviolet Spectroscopy

The instrumental coupling of gas chromatograph with a rapid scanning UV spectrometer has been presented by Kubeczka et al. (1989). In this study, a UV-VIS diode-array spectrometer (Zeiss, Oberkochen, FRG) with an array of 512 diodes was used, which provided continuous monitoring in the range of 200–620 nm. By interfacing the spectrometer via fiber optics to a heated flow cell, which was connected by short heated capillaries to the GC column effluent, interferences of chromatographic resolution could be minimized. With the aid of this device, several terpene hydrocarbons have been investigated. In addition to displaying individual UV spectra, the available software rendered the analyst to define and to display individual window traces, three-dimensional plots and contour plots, which are valuable tools for discovering and deconvoluting gas chromatographic unresolved peaks.

2.2.3.4 Gas Chromatography-Atomic Emission Spectroscopy

A device for the coupling of capillary gas chromatography with atomic emission spectroscopy (GC-AES) has been presented by Wylie et al. (1989). By means of this coupling 23 elements of a compound including all elements of organic substances separated by GC could be selectively detected providing the analyst not only with valuable information on the elemental composition of the individual components of a mixture, but also with the percentages of the elemental composition. The device incorporates a microwave-induced helium plasma at the outlet of the column coupled to an optical emission spectrometer. From the 15 most commonly occurring elements in organic compounds up to eight could be detected and measured simultaneously, for example, C, O, N, and S, which are of importance with respect to the analysis of essential oils. The examples given in the literature (e.g., Wylie et al., 1989; Bicchi et al., 1992; David et al., 1992; Jirovetz et al., 1992; Schultze, 1993) indicate that the GC-AES coupling can provide the analyst with additional valuable information, which are to some extent complementary to the date obtained by GC-MS and GC-FTIR, making the respective library searches more reliable and more certain.

However, the combined techniques GC-UV and GC-AES have not gained much importance in the field of essential oil research, since UV-spectra offer only low information and the coupling of a GC-AES, yielding the exact elemental composition of a component, can to some extent be obtained by precise mass measurement. Nevertheless, the on-line coupling GC-AES is still today efficiently used in environmental investigations.

2.2.3.5 Gas Chromatography-Isotope Ratio Mass Spectrometry

In addition to enantioselective capillary gas chromatography the on-line coupling of gas chromatography with isotope-ratio mass spectrometry (GC-IRMS) is an important technique in authentication of food flavors and essential oil constituents. The on-line combustion of effluents from capillary gas chromatographic separations to determine the isotopic compositions of individual components from complex mixtures was demonstrated by Matthews et al. (1978). On the basis of this work, the on-line interfacing of capillary GC with IRMS was later improved. With the commercially available GC-combustion IRMS device (GC-C-IRMS) measurements of the ratios of the stable isotopes $^{13}C/^{12}C$ have been accessible and respective investigations have been reported in several papers (e.g., Carle et al., 1990; Bernreuther et al., 1990; Braunsdorf et al., 1992, 1993; Frank et al., 1995; Mosandl et al., 1997). A further improvement was the development of the GC-pyrolysis-IRMS (GC-P-IRMS) making measurements of $^{18}O/^{16}O$ ratios and later $^{2}H/^{1}H$ ratios feasible (Juchelka et al., 1998; Ruff et al., 2000; Hör et al., 2001; Mosandl, 2004). Thus, the GC-P-IRMS device (Figure 2.7) appears today as one of the most sophisticated instruments for the appraisal of the genuineness of natural mixtures.

2.2.3.6 High-Performance Liquid Chromatography-Gas Chromatography

The on-line coupling of an HPLC device to a capillary gas chromatograph offers a number of advantages, above all higher column chromatographic efficiency, simple and rapid method development, simple cleanup of samples from complex matrices, and effective enrichment of the components of interest; additionally, the entire analytical procedure can easily be automated, thus increasing accuracy and reproducibility. The commercially available HPLC-GC coupling consists of an HPLC device that is connected with a capillary gas chromatograph via an interface allowing the transfer of HPLC fractions. Two different types of interfaces have been often used: The on-column interface is a modification of the on-column injector for GC; it is particularly suited for the transfer of fairly small fraction containing volatile constituents (Dugo et al., 1994; Mondello et al., 1994a, b, 1995). The second interface uses a sample loop and allows to transfer large sample volumes (up to 1 mL) containing components with limited volatilities. Figure 2.8 gives a schematic view of such an LC-GC instrument. In the shown position of the six-port valve, the desired fraction of the HPLC effluent is stored in the sample loop, while the carrier gas is passed through the GC columns. After switching the valve, the content of the sample loop is driven by the carrier gas into the large volume injector, vaporized and enters the precolumns, where the sample components are retained and most

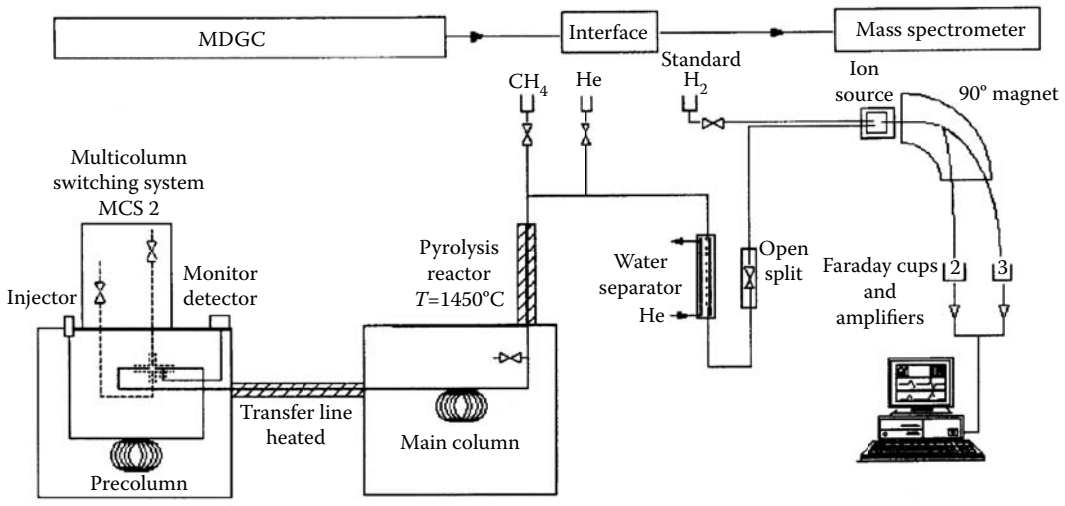

FIGURE 2.7 Scheme of an MDGC-C/P-IRMS device. (From Sewenig, S., et al., 2005. *J. Agric. Chem*., 53: 838–844. With permission.)

of the solvent vapor can be removed through the solvent vapor exit (SVE). After closing this valve and increasing the GC-oven temperature, the sample components are volatilized and separated in the main column reaching the detector. The main drawback of this technique, however, may be the loss of highly volatile compounds that are vented together with the solvent. As an example of an HPLC-GC investigation, the preseparation of lemon oil with gradient elution into four fractions is quoted (Munari et al., 1990). The respective gas chromatograms of the individual fractions exhibit good separation into hydrocarbons, esters, carbonyls, and alcohols, facilitating gas chromatographic separation and identification. Due to automation of all analytical steps involved, the manual

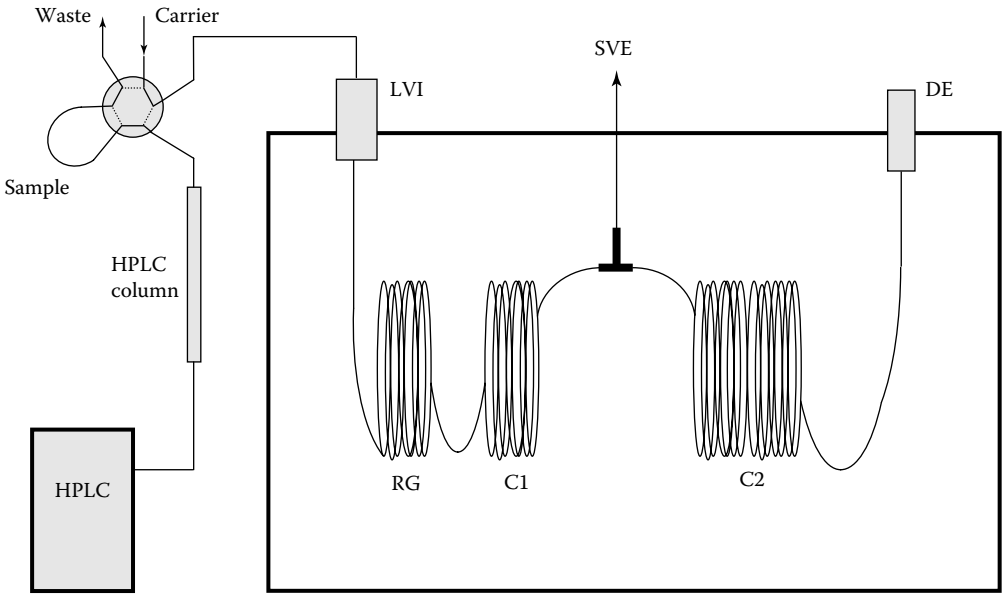

FIGURE 2.8 Basic arrangement of an HPLC-GC device with a sample loop interface. RG: retention gap; C1: retaining column; C2: analytical column; LVI: large volume injector; and SVE: solvent vapor exit.

operations are significantly reduced and very good reproducibility was obtained. In three excellent review articles, the different kinds of HPLC-GC couplings are discussed in detail, describing their advantages and limitations with numerous references cited therein (Mondello et al., 1996, 1999; Dugo et al., 2003).

2.2.3.7 HPLC-MS, HPLC-NMR Spectroscopy

The on-line couplings of HPLC with MS and NMR spectroscopy are further important techniques combining high-performance separation with structurally informative spectroscopic techniques, but they are mainly applied to nonvolatile mixtures and shall not be discussed in more detail here, although they are very useful for investigating plant extracts.

Some details concerning the different ionization techniques used in HPLC-MS have been presented among other things by Dugo et al. (2005).

2.2.3.8 Supercritical Fluid Extraction-Gas Chromatography

Although SFE is not a chromatographic technique, separation of mixtures can be obtained during the extraction process by varying the physical properties such as temperature and pressure to obtain fractions of different composition. Detailed reviews on the physical background of SFE and its application to natural products analysis inclusive of numerous applications have been published by Modey et al. (1995), and more recently by Pourmortazavi et al. (2007). The different types of couplings (off-line and on-line) have been presented by several authors. Houben et al. (1990) described an on-line coupling of SFE with capillary GC using a programmed temperature vaporizer as an interface. Similar approaches have been used by Blanch et al. (1994) in their investigations of rosemary leaves and by Ibanez et al. (1997) studying Spanish raspberries. In both the last two papers an off-line procedure was applied. A different device has been used by Hartonen et al. (1992) in a study of the essential oil of *Thymus vulgaris* using a cooled stainless steel capillary for trapping the volatiles connected via a six-port valve to the extraction vessel and the GC column. After sampling of the volatiles within the trap they have been quickly vaporized and flushed into the GC column by switching the walve. The recoveries of thyme components by SFE-GC were compared with those obtained from hydrodistilled thyme oil by GC exhibiting a good agreement. The SFE-GC analyses of several flavor and fragrance compounds of natural products by transferring the extracted compounds from a small SFE cell directly into a GC capillary has already been presented by Hawthorne et al. (1988). By inserting the extraction cell outlet restrictor (a 20 μm I.D. capillary) into the GC column through a standard on-column injection port, the volatiles were transferred and focused within the column at 40°C, followed by rapid heating to 70°C (30°C/min) and successive usual temperature programming. The suitability of that approach has been demonstrated with a variety of samples including rosemary, thyme, cinnamon, spruce needles, orange peel, and cedar wood. In a review article from Greibrokk, published in 1995, numerous applications of SFE connected on-line with gas chromatography and other techniques, the different instruments, and interfaces have been discussed, including the main parameters responsible for the quality of the obtained analytical results. In addition, the instrumental setups for SFE-LC and SFE-SFC couplings are given.

2.2.3.9 Supercritical Fluid Chromatography-Gas Chromatography

On-line coupling of SFC with gas chromatography has sporadically been used for the investigation of volatiles from aromatic herbs and spices. The requirements for instrumentation regarding the pumps, the restrictors, and the detectors are similar to those of SFE-GC. Additional parts of the device are the separation column and the injector, to introduce the sample into the mobile phase and successively into the column. The most common injector type in SFC is the high-pressure valve injector, similar to those used in HPLC. With this valve, the sample is loaded at ambient pressure into a sample loop of defined size and can be swept into the column after switching the valve to the injection position. The separation columns used in SFC may be either packed or open tubular

columns with their respective advantages and disadvantages. The latter mentioned open tubular columns for SFC can be compared with the respective GC columns; however, they must have smaller internal diameter. With regard to the detectors used in SFC, the FID is the most common applied detector, presuming that no organic modifiers have been admixed to the mobile phase. In that case, for example, a UV detector with a high-pressure flow cell has to be taken into consideration.

In a paper, presented by Yamauchi et al. (1990), cold-pressed lemon-peel oil has been separated by semipreparative SFC into three fractions, namely hydrocarbons, aldehydes and alcohols, and esters together with other oil constituents. The obtained fractions were afterward analyzed by capillary GC. SFC has also often been combined with SFE prior to chromatographic separation in plant volatile oil analysis, since in both techniques the same solvents are used, facilitating an on-line coupling. SFE and on-line-coupled SFC have been applied to the analysis of turmeric, the rhizomes of *Curcuma longa* L., using modified carbon dioxide as the extractant, yielding fractionation of turmerones curcuminoids in a single run (Sanagi et al., 1993). A multidimensional SFC-GC system was developed by Yarita et al. (1994) to separate on-line the constituents of citrus essential oils by stepwise pressure programming. The eluting fractions were introduced into a split/splitless injector of a gas chromatograph and analyzed after cryofocusing prior to GC separation. An SFC-GC investigation of cloudberry seed oil extracted with supercritical carbon dioxide was described by Manninen et al. (1997), in which SFC was mainly used for the separation of the volatile constituents from the low-boiling compounds, such as triacylglycerols. The volatiles were collected in a trap column and refocused before being separated by GC. Finally, an on-line technique shall be mentioned by which the compounds eluting from the SFC column can be completely transferred to GC, but also for selective or multistep heart-cutting of various sample peaks as they elute from the SFC column (Levy et al., 2005).

2.2.3.10 Couplings of SFC-MS and SFC-FTIR Spectroscopy

Both coupling techniques such as SFC-MS and SFC-FTIR have nearly exclusively been used for the investigation of low-volatile more polar compounds. Arpino published in 1990 a comprehensive article on the different coupling techniques in SFC-MS, which have been presented up to 1990 including 247 references. A short overview of applications using SFC combined with benchtop mass spectrometers was published by Ramsey and Raynor (1996). However, the only paper concerning the application of SFC-MS in essential oil research was published by Blum et al. (1997). With the aid of a newly developed interface and an injection technique using a retention gap, investigations of thyme extracts have been successfully performed.

The application of SFC-FTIR spectroscopy for the analysis of volatile compounds has also rarely been reported. One publication found in the literature refers to the characterization of varietal differences in essential oil components of hops (Auerbach et al., 2000). In that paper, the IR spectra of the main constituents were taken as films deposited on AgCl disks and compared with spectra obtained after chromatographic separation in a flow cell with IR transparent windows, exhibiting a good correlation.

2.2.4 Identification of Multicomponent Samples without Previous Separation

In addition to chromatographic separation techniques including hyphenated techniques, several spectroscopic techniques have been applied to investigate the composition of essential oils without previous separation.

2.2.4.1 UV Spectroscopy

UV spectroscopy has only little significance for the direct analysis of essential oils due to the inability to provide uniform information on individual oil components. However, for testing the presence of furano-coumarins in various citrus oils, which can cause photodermatosis when applied

externally, UV spectroscopy is the method of choice. The presence of those components can be easily determined due to their characteristic UV absorption. In the European Pharmacopoeia for example, quality assessment of lemon oil, which has to be produced by cold pressing, is therefore performed by UV spectroscopy in order to exclude cheaper distilled oils.

2.2.4.2 IR Spectroscopy

Several attempts have also been made to obtain information about the composition of essential oils using IR spectroscopy. One of the first comprehensive investigations of essential oils was published by Bellanato and Hidalgo (1971) in the book *Infrared Analysis of Essential Oils* in which the IR spectra of approximately 200 essential oils and additionally of more than 50 pure reference components have been presented. However, the main disadvantage of this method is the low sensitivity and selectivity of the method in the case of mixtures with a large number of components and secondly the unsolvable problem when attempting to quantitatively measure individual component concentrations.

New approaches to analyze essential oils by vibrational spectroscopy using attenuated reflection (ATR) IR spectroscopy and NIR-FT-Raman spectroscopy have recently been published by Baranska et al. (2005) and numerous papers cited therein. The main components of an essential oil can be identified by both spectroscopic techniques using the spectra of pure oil constituents as references. The spectroscopic analysis is based on characteristic key bands of the individual constituents and made it, for example, possible to discriminate the oil profiles of several eucalyptus species. As can be taken from this paper, valuable information can be obtained as a result of the combined application of ATR-IR and NIR-FT-Raman spectroscopy. Based on reference GC measurements, valuable calibration equations have been developed for numerous essential oil plants and related essential oils in order to quantify the amount of individual oil constituents applying different suitable chemometric algorithms. Main advantages of those techniques are their ability to control the quality of essential oils very fast and easily and above all, to quantify and analyze the main constituents of essential oils *in situ*, that means in living plant tissues without any isolation process, since both techniques are not destructive.

2.2.4.3 Mass Spectrometry

MS and proton NMR spectroscopy have mainly been used for structure elucidation of isolated compounds. However, there are some reports on mass spectrometric analyses of essential oils. One example has been presented by Grützmacher (1982). The depicted mass spectrum (Figure 2.9) of an essential oil exhibits some characteristic molecular ions of terpenoids with masses at *m/z* 136, 148, 152, and 154. By the application of a double focusing mass spectrometer and special techniques analyzing the decay products of metastable ions, the components anethole, fenchone, borneol, and cineole could be identified, while the assignment of the mass 136 proved to be problematic.

A different approach has been used by Schultze et al. (1986), investigating secondary metabolites in dried plant material by direct mass spectrometric measurement. The small samples (0.1–2 mg, depending on the kind of plant drug) were directly introduced into a mass spectrometer by means of a heatable direct probe. By heating the solid sample, stored in a small glass crucible, various substances are released depending on the applied temperature, and subsequently their mass spectra can be taken. With the aid of this technique, numerous medicinal plant drugs have been investigated and their main vaporizable components could be identified.

2.2.4.4 ^{13}C-NMR Spectroscopy

^{13}C-NMR spectroscopy is generally used for the elucidation of molecular structures of isolated chemical species. The application of ^{13}C-NMR spectroscopy to the investigation of complex mixtures is relatively rare. However, the application of ^{13}C-NMR spectroscopy to the analysis of

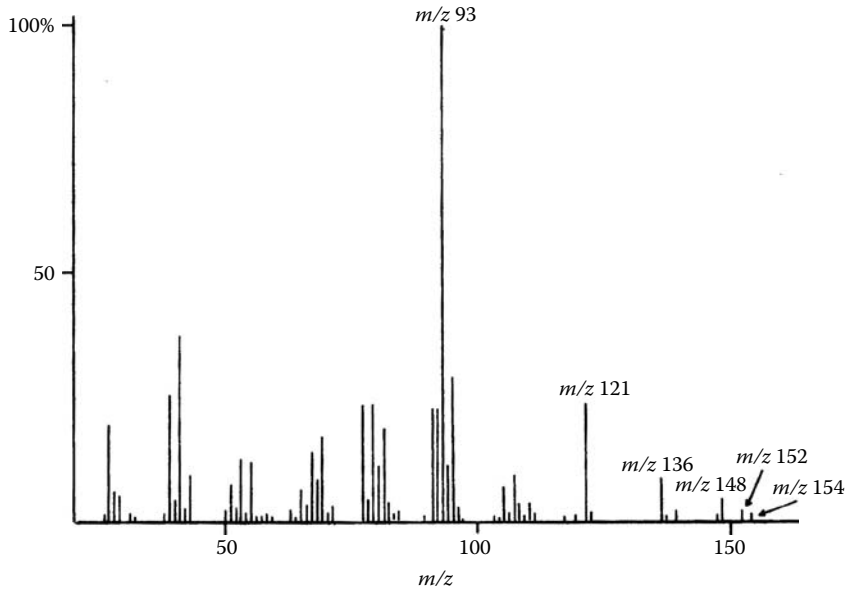

FIGURE 2.9 EI-mass spectrum of an essential oil. (From Grützmacher, H.F. 1982. In *Ätherische Öle Analytik*, *Physiologie, Zusammensetzung*, K.H. Kubeczka (ed.), pp. 1–24. Stuttgart, New York: Georg Thieme Verlag. With permission.)

essential oils and similar complex mixtures offers particular advantages, as have been shown in the past (Formaček and Kubeczka, 1979, 1982; Kubeczka, 2002), to confirm analytical results obtained by GC-MS and for solving certain problems encountered with nonvolatile mixture components or thermally unstable compounds, since analysis is performed at ambient temperature.

The qualitative analysis of an essential oil is based on comparison of the oils spectrum, using broadband decoupling, with spectra of pure oil constituents which should be recorded under identical conditions regarding solvent, temperature, and so on to ensure that differences in the chemical shifts for individual ^{13}C-NMR lines of the mixture and of the reference substance are negligible. As an example, the identification of the main constituent of celery oil is shown (Figure 2.10). This constituent can be easily identified as limonene by the corresponding reference spectrum. Minor constituents give rise to less intensive signals that can be recognized after a vertical expansion of the spectrum. For recognition of those signals also a horizontal expansion of the spectrum is advantageous.

The sensitivity of the ^{13}C-NMR technique is limited by diverse factors such as rotational sidebands, ^{13}C-^{13}C-couplings, and so on, and at least by the accumulation time. For practical use, the concentration of 0.1% of a component in the entire mixture has to be seen as an interpretable limit. A very pretentious investigation has been presented by Kubeczka (1989). In the investigated essential oil, consisting of more than 80 constituents, approximately 1200 signals were counted after a horizontal and vertical expansion in the obtained broadband decoupled ^{13}C-NMR spectrum, which reflects impressively the complex composition of that oil. However, the analysis of such a complex mixture is made difficult by the immense density of individual lines, especially in the aliphatic region of the spectrum, making the assignments of lines to individual components ambiguous. Besides, qualitative analysis quantification of the individual sample components is accessible as described by Formaček and Kubeczka (1982a). After elimination of the ^{13}C-NMR signals of nonprotonated nuclei and calculation of average signal intensity per carbon atom as a measurement characteristic, it has been possible to obtain satisfactory results as shown by comparison with gas chromatographic analyses.

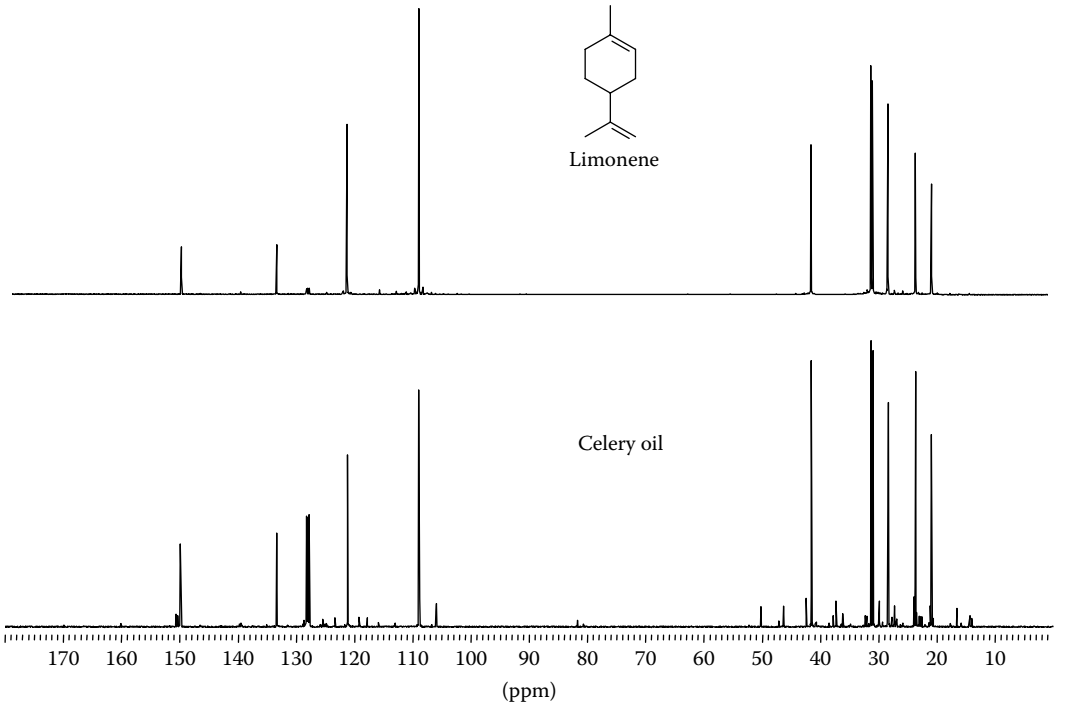

FIGURE 2.10 Identification of limonene in celery oil by ^{13}C-NMR spectroscopy.

During the last years, a number of articles have been published by Casanova and coworkers (e.g., Bradesi et al. (1996) and references cited therein). In addition, papers dealing with computer-aided identification of individual components of essential oils after ^{13}C-NMR measurements (e.g., Tomi et al., 1995), and investigations of chiral oil constituents by means of a chiral lanthanide shift reagent by carbon-13 NMR spectroscopy have been published (Ristorcelli et al., 1997).

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3 Sources of Essential Oils

Chlodwig Franz and Johannes Novak

CONTENTS

3.1 "ESSENTIAL OIL-BEARING PLANTS": ATTEMPT OF A DEFINITION

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin. The respective main compounds are mainly derived from three biosynthetic pathways only, the mevalonate pathway leading to sesquiterpenes, the methyl-erithrytol-pathway leading to mono- and diterpenes, and the shikimic acid pathway *en route* to phenylpropenes. Nevertheless, there are an almost uncountable number of single substances and a tremendous variation in the composition of essential oils. Many of these volatile substances have diverse ecological functions. They can act as internal messengers, as defensive substances against herbivores or as volatiles

directing not only natural enemies to these herbivores but also attracting pollinating insects to their host (Harrewijn et al., 2001).

All plants possess principally the ability to produce volatile compounds, quite often, however, only in traces. "Essential oil plants" in particular are those plant species delivering an essential oil of commercial interest. Two principal circumstances determine a plant to be used as an essential oil plant:

a. A unique blend of volatiles like the flower scents in rose (*Rosa* spp.), jasmine (*Jasminum sambac*), or tuberose (*Polyanthes tuberosa*). Such flowers produce and immediately emit the volatiles by the epidermal layers of their petals (Bergougnoux et al., 2007). Therefore the yield is even in intensive smelling flowers very low, and besides distillation special techniques, as an example, enfleurage has to be applied to recover the volatile fragrance compounds.
b. Secretion and accumulation of volatiles in specialized anatomical structures. This leads to higher concentrations of the essential oil in the plant. Such anatomical storage structures for essential oils can be secretory idioblasts (secretory cells), cavities/ducts, or glandular trichomes (Fahn, 1979, 1988; colorfully documented by Svoboda et al., 2000).

Secretory idioblasts are individual cells producing an essential oil in large quantities and retaining the oil within the cell like the essential oil idioblasts in the roots of *Vetiveria zizanioides* which occurs within the cortical layer and close to the endodermis (Bertea and Camusso, 2002). Similar structures containing essential oils are also formed in many flowers, for example, *Rosa* sp., *Viola* sp., or *Jasminum* sp.

Cavities or ducts consist of extracellular storage space that originate either from schizogeny (created by the dissolution of the middle lamella between the duct initials and formation of an intercellular space) or by lysogeny (programmed death and dissolution of cells). In both cases, the peripheral cells are becoming epithelial cells highly active in synthesis and secretion of their products into the extracellular cavities (Pickard, 2008). Schizogenic oil ducts are characteristic for the Apiaceae family, for example, *Carum carvi*, *Foeniculum vulgare*, or *Cuminum cyminum*, but also for Hypericaceae or Pinaceae. Lysogenic cavities are found in Rutaceae (*Citrus* sp., *Ruta graveolens*), Myrtaceae (e.g., *Syzygium aromaticum*), and others.

Secreting trichomes (glandular trichomes) can be divided into two main categories: peltate and capitate trichomes. Peltate glands consist of a basal epidermal cell, a neck-stalk cell and a secreting head of 4–16 cells with a large subcuticular space on the apex in which the secretion product is accumulated. The capitate trichomes possess only 1–4 secreting cells with only a small subcuticular space (Werker, 1993; Maleci Bini and Giuliani, 2006). Such structures are typical for Lamiaceae (the mint family), but also for *Pelargonium* sp.

The monoterpene biosynthesis in different species of Lamiaceae, for example, sage (*Salvia officinalis*) and peppermint (*Mentha* × *piperita*), is restricted to a brief period early in leaf development (Croteau et al., 1981; Gershenzon et al., 2000). The monoterpene biosynthesis in peppermint reaches a maximum in 15-day-old leaves, only very low rates were observed in leaves younger than 12 days or older than 20 days. The monoterpene content of the peppermint leaves increased rapidly up to day 21, then leveled off, and kept stable for the remainder of the leaf life (Gershenzon et al., 2000).

The composition of the essential oil often changes between different plant parts. Phytochemical polymorphism is often the case between different plant organs. In *Origanum vulgare* ssp. *hirtum*, a polymorphism within a plant could even be detected on a much lower level, namely between different oil glands of a leaf (Johnson et al., 2004). This form of polymorphism seems to be not frequently occurring, differences in the composition between oil glands is more often related to the age of the oil glands (Grassi et al., 2004; Johnson et al., 2004; Novak et al., 2006a; Schmiderer et al., 2008).

Such polymorphisms can also be found quite frequently when comparing the essential oil composition of individual plants of a distinct species (intraspecific variation, "chemotypes") and is based on the plants genetical background.

The differences in the complex composition of two essential oils of one kind may sometimes be difficult to assign to specific chemotypes or to differences arising in the consequence of the reactions of the plants to specific environmental conditions, for example, to different growing locations. In general, the differences due to genetical differences are much bigger than by different environmental conditions. However, many intraspecific polymorphisms are probably not yet detected or have been described only recently even for widely used essential oil crops like sage (Novak et al., 2006b).

3.2 PHYTOCHEMICAL VARIATION

3.2.1 Chemotaxonomy

The ability to accumulate essential oils is not omnipresent in plants but scattered throughout the plant kingdom; in many cases, however, very frequent within—or a typical character of—certain plant families. From the taxonomical and systematic point of view, not the production of essential oils is the distinctive feature since this is a quite heterogeneous group of substances, but either the type of secretory containers (trichomes, oil glands, lysogenic cavities, or schizogenic oil ducts) or the biosynthetically specific group of substances, for example, mono- or sesquiterpenes, phenylpropenes, and so on; the more a substance is deduced in the biosynthetic pathway the more specific it is for certain taxa: monoterpenes are typical for the genus *Mentha*, but menthol is characteristic for *Mentha piperita* and *Mentha arvensis* ssp. *piperascens* only; sesquiterpenes are common in the *Achillea–millefolium* complex, but only *Achillea roseo-alba* (2×) and *Achillea collina* (4×) are able to produce matricine as precursor of (the artifact) chamazulene (Vetter et al., 1997). On the other hand, the phenylpropenoid eugenol, typical for cloves (*Syzygium aromaticum*, Myrtaceae) can also be found in large amounts in that distant species, for example, cinnamon (*Cinnamomum zeylanicum*, Lauraceae) or basil (*Ocimum basilicum*, Lamiaceae); as sources for anethole are known aniseed (*Pimpinella anisum*) and fennel (*F. vulgare*) both Apiaceae, but also star anise (*Illicium verum*, Illiciaceae), *Clausena anisata* (Rutaceae), *Croton zetneri* (Euphorbiaceae), or *Tagetes lucida* (Asteraceae). Finally, eucalyptol (1,8-cineole)—named after its occurrence in *Eucalyptus* sp. (Myrtaceae)—may also be a main compound of the essential oil of galangal (*Alpinia officinarum*, Zingiberaceae), bay laurel (*Laurus nobilis*, Lauraceae), Japan pepper (*Zanthoxylum piperitum*, Rutaceae), and a number of plants of the mint family, for example, sage (*S. officinalis*, *Salvia fruticosa*, *Salvia lavandulifolia*), rosemary (*Rosmarinus officinalis*), and mints (*Mentha* sp.). Taking the above facts into consideration, chemotaxonomically relevant are (therefore) common or distinct pathways, typical fingerprints, and either main compounds or very specific even minor or trace substances [e.g., δ-3-carene to separate *Citrus grandis* from other *Citrus* sp. (Gonzalez et al., 2002)].

The plant families comprising species that yield a majority of the most economically important essential oils are not restricted to one specialized taxonomic group but are distributed among all plant classes: gymnosperms, for example, the families Cupressaceae (cedarwood, cedar leaf, juniper oil, etc.) and Pinaceae (pine and fir oils, etc.), as well as angiosperms, and among them within Magnoliopsida, Rosopsida, and Liliopsida. The most important families of dicots are Apiaceae (e.g., fennel, coriander, and other aromatic seed/root oils), Asteraceae or Compositae (chamomile, wormwood, tarragon oil, etc.), Geraniaceae (geranium oil), Illiciaceae (star anise oil), Lamiaceae (mint, patchouli, lavender, oregano, and many other herb oils), Lauraceae (litsea, camphor, cinnamon, sassafras oil, etc.), Myristicaceae (nutmeg and mace), Myrtaceae (myrtle, cloves, and allspice), Oleaceae (jasmine oil), Rosaceae (rose oil), and Santalaceae (sandalwood oil). In monocots (Liliopsida), it is substantially restricted to Acoraceae (calamus), Poaceae (vetiver and aromatic grass oils), and Zingiberaceae (e.g., ginger and cardamom).

Apart from the phytochemical group of substances typical for a taxon, the chemical outfit depends, furthermore, on the specific genotype, the stage of plant development—also influenced by environmental factors—and the plant part (see Section 3.2.1). Considering all these influences chemotaxonomic statements and conclusions have to be based on comparable material, grown and harvested under comparable circumstances.

3.2.2 Inter- and Intraspecific Variation

Knowledge on biochemical systematics and the inheritance of phytochemical characters depends on extensive investigations of taxa (particularly species) and populations on single-plant basis, respectively, and several examples of genera show that the taxa do indeed display different patterns.

3.2.2.1 Lamiaceae (Labiatae) and Verbenaceae

The presumably largest genus among the Lamiaceae is **sage** (*Salvia* L). consisting of about 900 species widely distributed in the temperate, subtropical, and tropical regions all over the world with major centers of diversity in the Mediterranean, in Central Asia, the Altiplano from Mexico throughout Central and South America, and in southern Africa. Almost 400 species are used in traditional and modern medicine, as aromatic herbs or ornamentals worldwide; among them are *S. officinalis*, *S. fruticosa*, *Salvia sclarea*, *Salvia divinorum*, *Salvia miltiorrhiza*, and *Salvia pomifera* to name a few. Many applications are based on nonvolatile compounds, for example, diterpenes and polyphenolic acids. Regarding the essential oil, there are a vast number of mono- and sesquiterpenes found in sage but, in contrast to, for example, *Ocimum* sp. and *Perilla* sp. (also Lamiaceae), no phenylpropenes were detected.

To understand species-specific differences within this genus, the Mediterranean *S. officinalis* complex (*S. officinalis*, *S. fruticosa*, and *S. lavandulifolia*) will be confronted with the *Salvia stenophylla* species complex (*S. stenophylla*, *Salvia repens*, and *Salvia runcinata*) indigenous to South Africa: In the *S. officinalis* group usually α- and β-thujones, 1,8-cineole, camphor, and in some cases linalool, β-pinene, limonene, or *cis*-sabinyl acetate are the prevailing substances, whereas in the *S. stenophylla* complex quite often sesquiterpenes, for example, caryophyllene or α-bisabolol, are main compounds.

Based on taxonomical studies of *Salvia* spp. (Hedge, 1992; Skoula et al., 2000; Reales et al., 2004) and a recent survey concerning the chemotaxonomy of *S. stenophylla* and its allies (Viljoen et al., 2006), Figure 3.1 shows the up-to-now-identified chemotypes within these taxa. Comparing the data of different publications the picture is, however, not as clear as demonstrated by six *S. officinalis* origins in Figure 3.2 (Chalchat et al., 1998; Asllani, 2000). This might be due to the prevailing chemotype in a population, the variation between single plants, the time of sample collection, and the sample size. This is exemplarily shown by one *S. officinalis* population where the individuals varied in α-thujone, from 9% to 72%, β-thujone, from 2% to 24%; 1,8-cineole, from 4% to 18%; and camphor from 1% to 25%. The variation over three years and five harvests of one clone only ranged as follows: α-thujone 35–72%, β-thujone 1–7%, 1,8-cineole 8–15%, and camphor 1–18% (Bezzi, 1994; Bazina et al., 2002). But also all other (minor) compounds of the essential oil showed respective intraspecific variability (see, e.g., Giannouli and Kintzios, 2000).

S. fruticosa was principally understood to contain 1,8-cineole as main compound but at best traces of thujones, as confirmed by Putievsky et al. (1986) and Kanias et al. (1998). In a comparative study of several origins, Máthé et al. (1996) identified, however, a population with atypically high β-thujone similar to *S. officinalis*. Doubts on if this origin could be true *S. fruticosa* or a spontaneous hybrid of both species were resolved by extensive investigations on the phytochemical and genetic diversity of *S. fruticosa* in Crete (Karousou et al., 1998; Skoula et al., 1999). There it was shown that all wild populations in western Crete consist of 1,8-cineole chemotypes only whereas in the eastern part of the island essential oils with up to 30% thujones, mainly β-thujone, could be observed. In Central Crete, finally, mixed populations were found. A cluster analysis based on

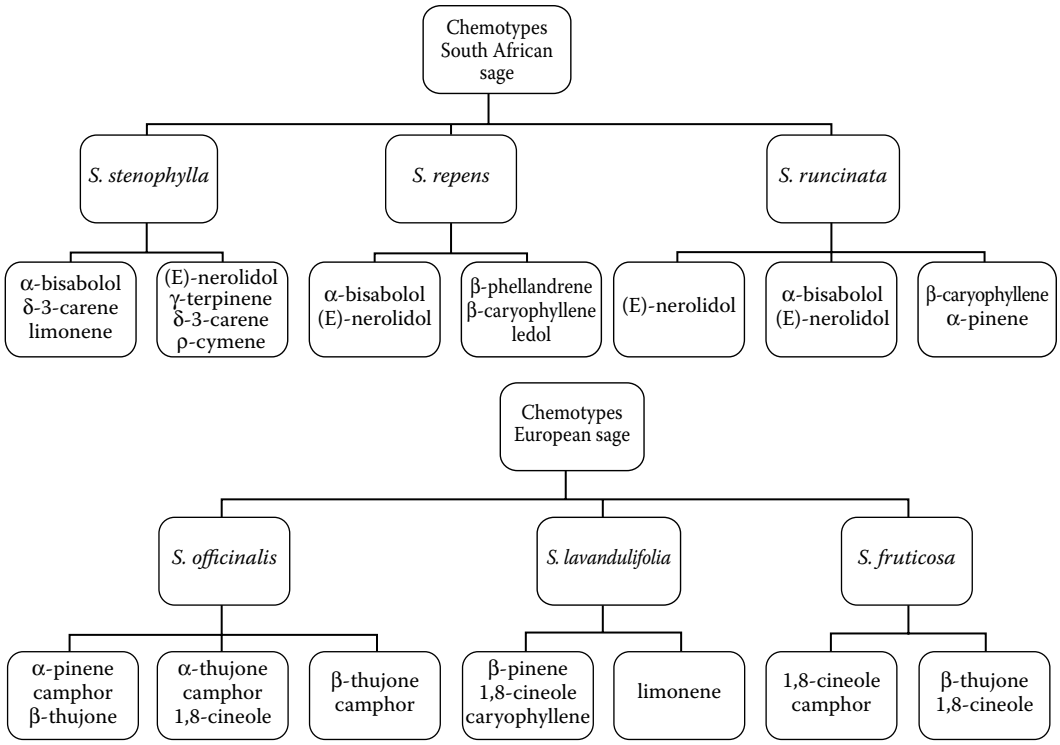

FIGURE 3.1 Chemotypes of some South African and European *Salvia* species.

random amplification of polymorphic DNA (RAPD) patterns confirmed the genetic differences between the West- and East-Crete populations of *S. fruticosa* (Skoula et al., 1999).

A rather interesting example of diversity is **oregano**, which counts to the commercially most valued spices worldwide. More than 60 plant species are used under this common name showing similar flavor profiles characterized mainly by cymyl-compounds, for example, carvacrol and thymol. With few exemptions the majority of oregano species belong to the Lamiaceae and Verbenaceae families with the main genera *Origanum* and *Lippia* (Table 3.1). In 1989, almost all of the estimated 15,000 ton/yr dried oregano originated from wild collection; today, some 7000 ha of *Origanum onites* are cultivated in Turkey alone (Baser, 2002), *O. onites* as well as other *Origanum* species are cultivated in Greece, Israel, Italy, Morocco, and other countries.

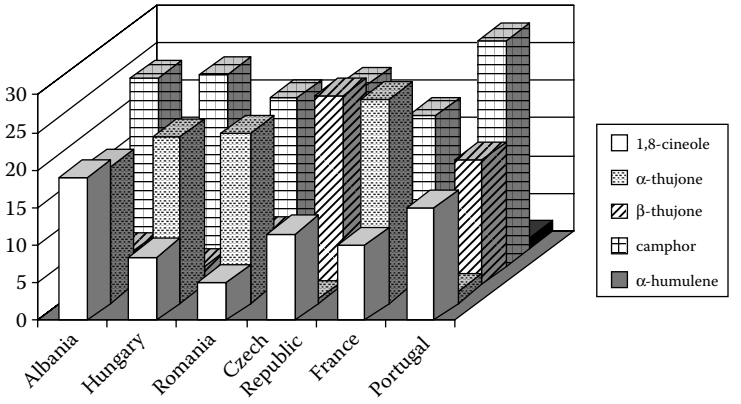

FIGURE 3.2 Composition of the essential oil of six *Salvia officinalis* origins.

TABLE 3.1
Species Used Commercially in the World as Oregano

Family/Species	Commercial Name/s Found in Literature
Labiatae	
Calamintha potosina Schaf.	Oregano de la sierra, oregano, origanum
Coleus amboinicus Lour. (syn. *C. aromaticus* Benth)	Oregano, oregano brujo, oregano de Cartagena, oregano de Espana, oregano Frances
Coleus aromaticus Benth.	Oregano de Espana, oregano, *Origanum*
Hedeoma floribunda Standl.	Oregano, *Origanum*
Hedeoma incona Torr.	Oregano
Hedeoma patens Jones	Oregano, *Origanum*
Hyptis albida HBK.	Oregano, *Origanum*
Hyptis americana (Aubl.) Urb. (*H. gonocephala* Gris.)	Oregano
Hyptis capitata Jacq.	Oregano, *Origanum*
Hyptis pectinata Poit.	Oregano, *Origanum*
Hyptis suaveolens (L.) Poit.	Oregano, oregano cimarron, *Origanum*
Monarda austromontana Epling	Oregano, *Origanum*
Ocimum basilicum L.	Oregano, *Origanum*
Origanum compactum Benth. (syn. *O. glandulosum* Salzm, ex Benth.)	Oregano, *Origanum*
Origanum dictamnus L. (*Majorana dictamnus* L.)	Oregano, *Origanum*
Origanum elongatum (Bonent) Emberger et Maire	Oregano, *Origanum*
Origanum floribundum Munby (*O. cinereum* Noe)	Oregano, *Origanum*
Origanum grosii Pau et Font Quer ex letswaart	Oregano, *Origanum*
Origanum majorana L.	Oregano
Origanum microphyllum (Benth) Vogel	Oregano, *Origanum*
Origanum onites L. (syn. *O. smyrneum* L.)	*Turkish oregano, oregano, *Origanum*
Origanum scabrum Boiss et Heldr. (syn. *O. pulchrum* Boiss et Heldr.)	Oregano, *Origanum*
Origanum syriacum L. var. *syriacum* (syn. *O. maru* L.)	Oregano, *Origanum*
Origanum vulgare L. subsp. *gracile* (Koch) letswaart (syn. *O. gracile* Koch, *O. tyttanthum* Gontscharov)	Oregano, *Origanum*
Origanum vulgare ssp. *hirtum* (Link) letswaart (syn. *O. hirtum* Link)	Oregano, *Origanum*
Origanum vulgare ssp. *virens* (Hoffmanns et Link) letswaart (syn. *O. virens* Hoffmanns et Link)	Oregano, *Origanum*, oregano verde
Origanum vulgare ssp. *viride* (Boiss.) Hayek (syn. *O. viride*) Halacsy (syn. *O. heracleoticum* L.)	*Greek oregano, oregano, *Origanum*
Origanum vulgare L. subsp. *vulgare* (syn. *Thymus origanum* (L.) Kuntze)	Oregano, *Origanum*
Origanum vulgare L.	Oregano, orenga, Oregano de Espana
Poliomintha longiflora Gray	Oregano
Salvia sp.	Oregano
Satureja thymbra L.	Oregano cabruno, oregano, *Origanum*
Thymus capitatus (L.) Hoffmanns et Link (syn. *Coridothymus capitatus* (L.) Rchb.f.)	*Spanish oregano, oregano, *Origanum*
Verbenaceae	
Lantana citrosa (Small) Modenke	Oregano xiu, oregano, *Origanum*
Lantana glandulosissima Hayek	Oregano xiu, oregano silvestre, oregano, *Origanum*
Lantana hirsuta Mart. et Gall.	Oreganillo del monte, oregano, *Origanum*
Lantana involucrata L.	Oregano, *Origanum*
Lantana purpurea (Jacq.) Benth. & Hook. (syn. *Lippia purpurea* Jacq.)	Oregano, *Origanum*
Lantana trifolia L.	Oregano, *Origanum*

continued

TABLE 3.1 (continued)
Species Used Commercially in the World as Oregano

Family/Species	Commercial Name/s Found in Literature
Lantana velutina Mart.&Gal.	Oregano xiu, oregano, *Origanum*
Lippia myriocephala Schlecht.&Cham.	Oreganillo
Lippia affinis Schau.	Oregano
Lippia alba (Mill) N.E. Br. (syn. *L. involucrata* L.)	Oregano, *Origanum*
Lippia berlandieri Schau.	Oregano
Lippia cordiostegia Benth.	Oreganillo, oregano montes, oregano, *Origanum*
Lippia formosa T.S.Brandeg.	Oregano, *Origanum*
Lippia geisseana (R.A.Phil.) Soler.	Oregano, *Origanum*
Lippia graveolens HBK	*Mexican oregano, oregano cimarron, oregano
Lippia helleri Britton	Oregano del pais, oregano, *Origanum*
Lippia micromera Schau.	Oregano del pais, oregano, *Origanum*
Lippia micromera var. *helleri* (Britton) Moldenke	Oregano
Lippia origanoides HBK	Oregano, origano del pais
Lippia palmeri var. *spicata* Rose	Oregano
Lippia palmeri Wats.	Oregano, *Origanum*
Lippia umbellata Cav.	Oreganillo, oregano montes, oregano, *Origanum*
Lippia velutina Mart. et Galeotti	Oregano, *Origanum*
Rubiaceae	
Borreria sp.	Oreganos, oregano, *Origanum*
Scrophulariaceae	
Limnophila stolonifera (Blanco) Merr.	Oregano, *Origanum*
Apiaceae	
Eryngium foetidum L.	Oregano de Cartagena, oregano, *Origanum*
Asteraceae	
Coleosanthus veronicaefolius HBK	Oregano del cerro, oregano del monte, oregano del campo
Eupatorium macrophyllum L. (syn. *Hebeclinium macrophyllum* DC.)	Oregano, *Origanum*

*Oregano species with economic importance according to Lawrence (1984).

In comparison with sage, the genus *Origanum* is much smaller and consists of 43 species and 18 hybrids according to the actual classification (Skoula and Harborne, 2002) with main distribution areas around the Mediterranean. Some subspecies of *O. vulgare* only are also found in the temperate and arid zones of Eurasia up to China. Nevertheless, the genus is characterized by a large morphological and phytochemical diversity (Kokkini, 1996; Baser, 2002; Skoula and Harborne, 2002).

The occurrence of several chemotypes is reported, for example, for commercially used *Origanum* species, from Turkey (Baser, 2002). In *O. onites*, two chemotypes are described, a carvacrol type and a linalool type. Additionally, a "mixed type" with both basic types mixed may occur. In Turkey, two chemotypes of *Origanum majorana* are known, one contains *cis*-sabinene hydrate as chemotypical lead compound and is used as marjoram in cooking ("marjoramy"), while the other one contains carvacrol in high amounts and is used to distil "oregano oil" in a commercial scale. Variability of chemotypes continues also within the "marjoramy" *O. majorana*. Novak et al. (2002) detected in cultivated marjoram accessions additionally to *cis*-sabinene hydrate the occurrence of polymorphism of *cis*-sabinene hydrate acetate. Since this chemotype did not influence the sensorial impression much, this chemotype was not eliminated in breeding, while an "off-flavor" chemotype would have been certainly eliminated in its cultivation history. In natural populations of *O. majorana* from Cyprus besides the "classical" *cis*-sabinene hydrate type, a chemotype with α-terpineol as

main compound was also detected (Novak et al., 2008). The two extreme "off-flavor" chemotypes in *O. majorana*, carvacrol-, and α-terpineol-chemotype are not to be found anywhere in cultivated marjoram, demonstrating one of the advantages of cultivation in delivering homogeneous qualities.

The second "oregano" of commercial value—mainly used in the Americas—is "*Mexican oregano*" (*Lippia graveolens* HBK., Verbenaceae) endemic to California, Mexico, and throughout Central America (Fischer, 1998). Due to wild harvesting, only the few published data show essential oil contents largely ranging from 0.3% to 3.6%. The total number of up-to-now-identified essential oil compounds comprises almost 70 with the main constituents thymol (3.1–80.6%), carvacrol (0.5–71.2%), 1,8-cineole (0.1–14%), and *p*-cymene (2.7–28.0%), followed by, for example, myrcene, γ-terpinene, and the sesquiterpene caryophyllene (Lawrence, 1984, Dominguez et al., 1989, Uribe-Hernández et al., 1992, Fischer et al., 1996; Vernin, 2001).

In a comprehensive investigation of wild populations of *L. graveolens* collected from the hilly regions of Guatemala, three different essential oil chemotypes could be identified, a thymol, a carvacrol, and an absolutely irregular type (Fischer et al., 1996). Within the thymol type, contents of up to 85% thymol in the essential oil could be obtained and only traces of carvacrol. The irregular type has shown a very uncommon composition where no compound exceeds 10% of the oil, and also phenylpropenes, for example, eugenol and methyl eugenol, were present (Fischer et al., 1996; Fischer, 1998). In Table 3.2, a comparison of recent data is given including *Lippia alba* commonly called "oregano" or "oregano del monte" although carvacrol and thymol are absent from the essential oil of this species. In Guatemala, two different chemotypes were found within *L. alba:* a myrcenone and a citral type (Fischer et al., 2004). Besides it, a linalool, a carvone, a camphor—1,8-cineole, and a limonene–piperitone chemotype have been described (Dellacassa et al., 1990; Pino et al., 1997; Frighetto et al., 1998; Senatore and Rigano, 2001).

Chemical diversity is of special interest if on genus or species level both terpenes as well as phenylpropenes can be found in the essential oil. Most Lamiaceae preferentially accumulate mono- (and sesqui-)terpenes in their volatile oils but some genera produce oils also rich in phenylpropenes, among these *Ocimum* sp. and *Perilla* sp.

The genus *Ocimum* comprises over 60 species, of which *Ocimum gratissimum* and *O. basilicum* are of high economic value. Biogenetic studies on the inheritance of *Ocimum* oil constituents were reported by Khosla et al. (1989) and an *O. gratissimum* strain named "Clocimum" containing 65% of eugenol in its oil was described by Bradu et al. (1989). A number of different chemotypes of basil (*O. basilicum)* has been identified and classified (Vernin, 1984; Marotti et al., 1996) containing up to 80% linalool, up to 21.5% 1,8-cineole, 0.3–33.0% eugenol, and also the presumably toxic compounds methyl chavicol (estragole) and methyl eugenol in concentrations close to 50% (Elementi et al., 2006; Macchia et al., 2006).

Perilla frutescens can be classified in several chemotypes as well according to the main monoterpene components perillaldehyde, elsholtziaketone, or perillaketones, and on the other side phenylpropanoid types containing myristicin, dillapiole, or elemicin (Koezuka et al., 1986). A comprehensive presentation on the chemotypes and the inheritance of the mentioned compounds was given by this author in Hay and Waterman (1993). In the referred last two examples not only the sensorial but also the toxicological properties of the essential oil compounds are decisive for the (further) commercial use of the respective species' biodiversity.

Although the Labiatae family plays an outstanding role as regards the chemical polymorphism of essential oils, also in other essential oil containing plant families and genera a comparable phytochemical diversity can be observed.

3.2.2.2 Asteraceae (Compositae)

Only a limited number of genera of the Asteraceae are known as essential oil plants, among them *Tagetes*, *Achillea*, and *Matricaria*. The genus *Tagetes* comprises actually 55 species, all of them endemic to the American continents with the center of biodiversity between 30° northern and 30° southern latitude. One of the species largely used by the indigenous population is

TABLE 3.2
Main Essential Oil Compounds of *Lippia graveolens* and *L. alba* According to Recent Data

	L. graveolens					*L. alba*			
	Fischer et al. (1996) Guatemala			Senatore et al. (2001)	Vernin et al. (2001)	Fischer (1998) Guatemala		Senatore et al. (2001)	Lorenzo (2001)
Compound	**Thymol-Type**	**Carvacrol-Type**	**Irregular Type**	**Guatemala**	**El-Salvador**	**Myrcenone-Type**	**Cineole-Type**	**Guatemala**	**Uruguay**
Myrcene	1.3	1.9	2.7	1.1	t	6.5	1.7	0.2	0.8
p-Cymene	2.7	6.9	2.8	5.5	2.1	t	t	0.7	n.d.
1.8-Cineole	0.1	0.6	5.0	2.1	t	t	**22.8**	**14.2**	1.3
Limonene	0.2	0.3	1.5	0.8	t	1.0	3.2	**43.6**	2.9
Linalool	0.7	1.4	3.8	0.3	t	4.0	2.4	1.2	**55.3**
Myrcenon	n.d.	n.d.	n.d.	n.d.	n.d.	**54.6**	3.2	n.d.	n.d.
Piperitone	n.d.	n.d.	n.d.	n.d.	n.d.	t	t	**30.6**	n.d.
Thymol	**80.6**	**19.9**	6.8	**31.6**	7.3	n.d.	n.d.	n.d.	n.d.
Carvacrol	1.3	**45.2**	1.1	0.8	**71.2**	n.d.	n.d.	n.d.	n.d.
β-Caryophyllene	2.8	3.5	8.7	4.6	9.2	2.6	1.2	1.0	9.0
α-Humulene	1.9	2.3	5.7	3.0	5.0	0.7	t	0.6	0.9
Caryophyll.-ox.	0.3	0.8	3.3	4.8	t	1.8	3.0	1.1	0.6
Z-Dihydrocarvon/ *Z*-Ocimenone	n.d.	n.d.	n.d.	n.d.	n.d.	13.1	0.6	0.1	0.8
E-Dihydrocarvon	n.d.	n.d.	n.d.	n.d.	n.d.	4.9	n.d.	t	1.2

n.d. = Not detectable; t = traces; main compounds in bold.

TABLE 3.3
Main Compounds of the Essential Oil of Selected *Tagetes lucida* Types (in % of dm)

Substance	Anethole Type (2)	Estragole Type (8)	Methyleugenol Type (7)	Nerolidol Type (5)	Mixed Type
Linalool	0.26	0.69	1.01	Tr.	3.68
Estragole	11.57	**78.02**	8.68	3.23	**24.28**
Anethole	**73.56**	0.75	0.52	Tr.	**30.17**
Methyleugenol	1.75	5.50	**79.80**	17.76	**17.09**
β-Caryophyllene	0.45	1.66	0.45	2.39	0.88
Germacrene D	2.43	2.89	1.90	Tr.	5.41
Methylisoeugenol	1.42	2.78	2.00	Tr.	3.88
Nerolidol	0.35	0.32	0.31	**40.52**	1.24
Spathulenol	0.10	0.16	0.12	Tr.	0.23
Carophyllene oxide	0.05	0.27	0.45	10.34	0.53

Location of origin in Guatemala: (2) Cabrican/Quetzaltenango, (5) La Fuente/Jalapa, (7) Joyabaj/El Quiche, (8) Sipacapa/S.Marcos, Mixed Type: Taltimiche/San Marcos.
Main compounds in bold.

"pericon" (*T. lucida* Cav.), widely distributed over the highlands of Mexico and Central America (Stanley and Steyermark, 1976). In contrast to almost all other *Tagetes* species characterized by the content of tagetones, this species contains phenylpropenes and terpenes. A detailed study on its diversity in Guatemala resulted in the identification of several eco- and chemotypes (Table 3.3): anethol, methyl chavicol (estragol), methyl eugenol, and one sesquiterpene type producing higher amounts of nerolidol (Bicchi et al., 1997; Goehler, 2006). The distribution of the three main phenylpropenes in six populations is illustrated in Figure 3.3. In comparison with the plant materials investigated by Ciccio (2004) and Marotti et al. (2004) containing oils with 90–95% estragol, only the germplasm collection of Guatemaltekan provenances (Goehler, 2006) allows to select individuals with high anethol but low to very low estragol or methyleugenol content—or with interestingly high nerolidol content, as mentioned above.

The genus *Achillea* is widely distributed over the northern hemisphere and consists of approximately 120 species, of which the *Achillea millefolium* aggregate (yarrow) represents a polyploid complex of allogamous perennials (Saukel and Länger, 1992; Vetter and Franz, 1996). The different taxa of the recent classification (*minor species* and *subspecies*) are morphologically and chemically to a certain extent distinct and only the diploid taxa *Achillea asplenifolia* and *A. roseo-alba* as well

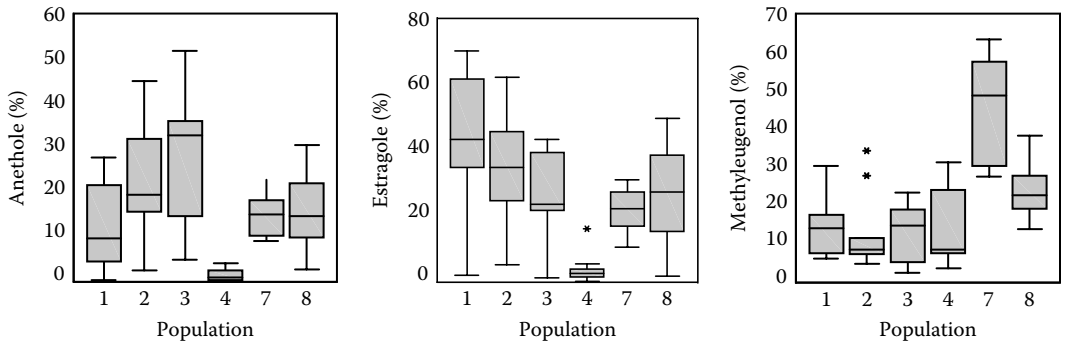

FIGURE 3.3 Variability of anethole, methyl eugenol, and methyl chavicol (estragole) in the essential oil of six *Tagetes lucida*—populations from Guatemala.

TABLE 3.4
Taxa within the *Achillea-Millefolium*-Group (Yarrow)

Taxon	Ploidy Level	Main Compounds
A. *setacea* W. et K.	2×	Rupicoline
A. *aspleniifolia* Vent.	2× (4×)	**7,8-Guajanolide**
		Artabsin-derivatives
		3-Oxa-Guajanolide
A. *roseo-alba* Ehrend.	2×	Artabsin-derivatives 3-Oxaguajanolide
		Matricinderivatives
A. *collina* Becker	4×	Artabsin-derivatives 3-Oxaguajanolide
		Matricinderivatives
		Matricarinderivatives
A. *pratensis* Saukel u. Länger	4×	Eudesmanolides
A. *distans* ssp. Distans W. et K.	6×	Longipinenones
A. *distans* ssp. Styriaca	4×	
A. *tanacetifolia (stricta)* W. et K.	6×	
A *mill*. ssp. sudetica	6×	Guajanolidperoxide
A. *mill*. ssp. Mill. L.	6×	
A. *pannonica* Scheele	8× (6×)	Germacrene
		Guajanolidperoxide

Source: Franz et al., Unpublished.

as the tetraploid *A. collina* and *Achillea ceretanica* are characterized by proazulens, for example, achillicin, whereas the other taxa, especially 6× and 8× contain eudesmanolides, longipinenes, germacranolides, and/or guajanolid peroxides, (Table 3.4). The intraspecific variation in the proazulene content ranged from traces up to 80%, other essential oil components of the azulenogenic species are, for example, α- and β-pinene, borneol, camphor, sabinene, or caryophyllene (Kastner et al., 1992). The frequency distribution of proazulene individuals among two populations is shown in Figure 3.4.

Crossing experiments resulted in proazulene being a recessive character of di- and tetraploid *Achillea* sp. (Vetter et al., 1997) similar to chamomile (Franz, 1993a,b). Finally, according to

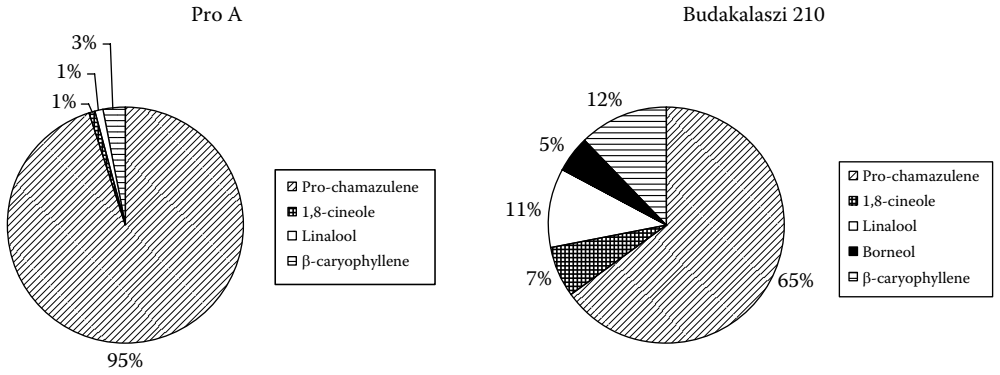

FIGURE 3.4 Frequency distribution of proazulene individuals among two *Achillea* sp. populations.

Steinlesberger et al. (2002) also a plant-to-plant variation in the enantiomers of, for example, α- and β-pinene as well as sabinene exists in yarrow oils, which makes it even more complicated to use phytochemical characters for taxonomical purposes.

Differences in the essential oil content and composition of chamomile flowers (*Matricaria recutita*) have long been recognized due to the fact that the distilled oil is either dark blue, green, or yellow, depending on the prochamazulene content (matricin as prochamazulene in chamomile is transformed to the blue-colored artifact chamazulene during the distillation process). Recognizing also the great pharmacological potential of the bisabolols, a classification into the chemotypes (–)-α-bisabolol, (–)-α-bisabololoxide A, (–)-α-bisabololoxide B, (–)-α-bisabolonoxide (A), and (pro)chamazulene was made by Franz (1982, 1989a). Examining the geographical distribution revealed a regional differentiation, where an α-bisabolol—(pro) chamazulene population was identified on the Iberian peninsula, mixed populations containing chamazulene, bisabolol, and bisabololoxides A/B are most frequent in Central Europe, and prochamazulene—free bisabolonoxide populations are indigenous to southeast Europe and minor Asia. In the meantime, Wogiatzi et al. (1999) have shown for Greece and Taviani et al. (2002) for Italy a higher diversity of chamomile including α-bisabolol types. This classification of populations and chemotypes was extended by analyzing populations at the level of individual plants (Schröder, 1990) resulting in the respective frequency distributions (Figure 3.5).

In addition, the range of essential oil components in the chemotypes of one Central European population is shown in Table 3.5 (Franz, 2000).

Data on inter- and intraspecific variation of essential oils are countless and recent reviews are known for a number of genera published, for example, in the series "Medicinal and Aromatic Plants—Industrial Profiles" (Harwood publications, Taylor & Francis and CRC, Press, respectively).

The generally observed quantitative and qualitative variation in essential oils draws the attention *i.a.* to appropriate random sampling for getting valid information on the chemical profile of a species or population. As concerns quantitative variations of a certain pattern or substance, Figure 3.6 shows

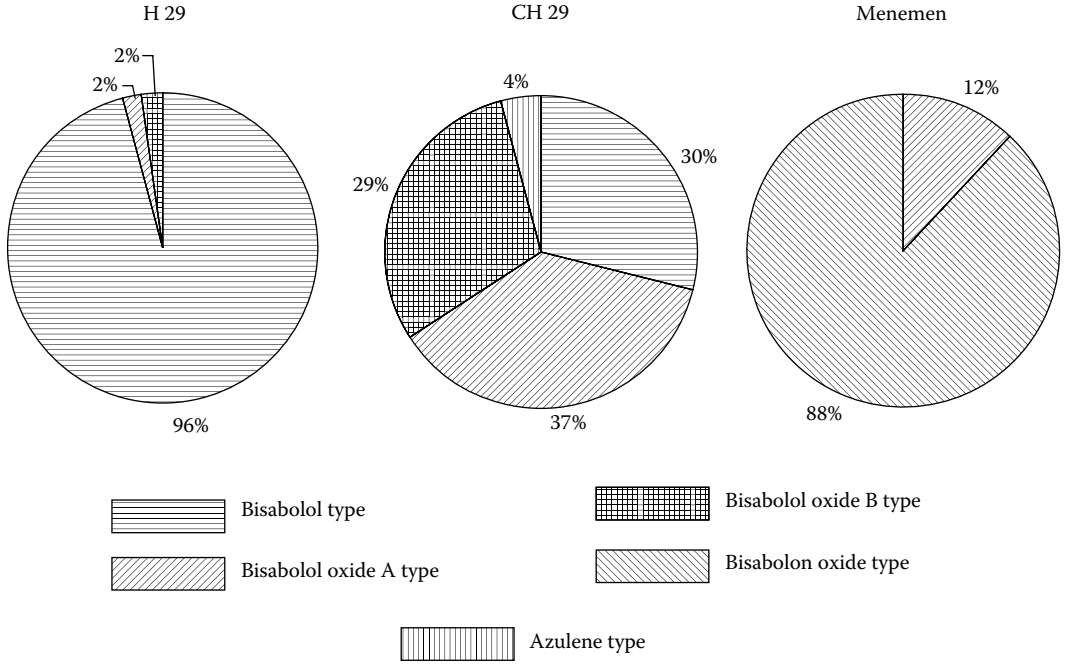

FIGURE 3.5 Frequency distribution of chemotypes in three varieties/populatios of chamomile [*Matricaria recutita* (L.) Rauschert].

TABLE 3.5
Grouping within a European Spontaneous Chamomile, Figures in % of Terpenoids in the Essential Oil of the Flower Heads

	Chamazulen	α-Bisabolol	α-B.-Oxide A	α-B.-Oxide B
		α-Bisabolol-Type		
Range	2.5–35.2	58.8–92.1	n.d.–1.0	n.d.–3.2
Mean	23.2	**68.8**	n.d.	n.d.
		α-Bisabololoxide A-Type		
Range	6.6–31.2	0.5–12.3	31.7–66.7	1.9–22.4
Mean	21.3	2.1	**53.9**	11.8
		α-Bisabololoxide B-Type		
Range	7.6–24.2	0.8–6.5	1.6–4.8	61.6–80.5
Mean	16.8	2.0	2.6	**72.2**
		Chamazulene-Type		
Range	76.3–79.2	5.8–8.3	n.d.–0.8	n.d.–2.6
Mean	**77.8**	7.1	n.d.	n.d.

Source: Franz, Ch., 2000. Biodiversity and random sampling in essential oil plants. Lecture 31st ISEO, Hamburg.

Main compounds in bold.

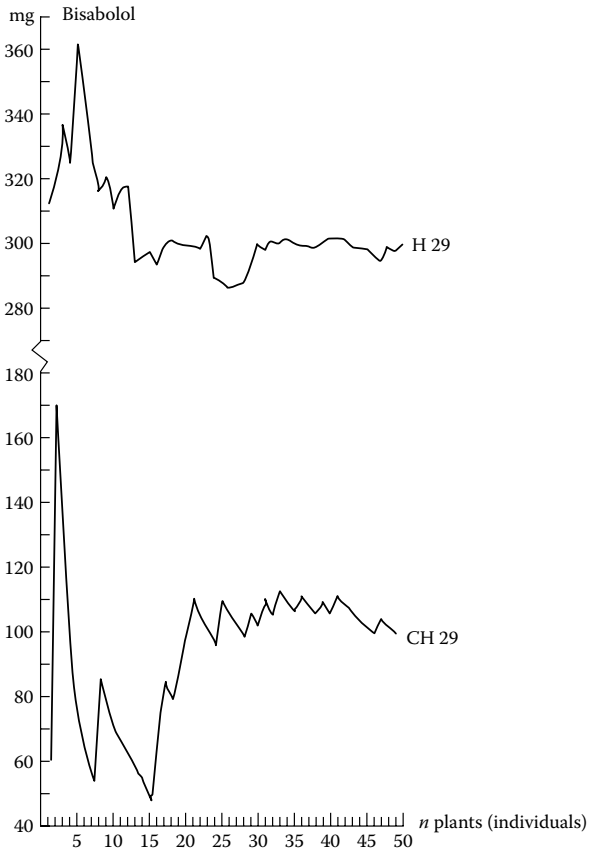

FIGURE 3.6 (–)-α-Bisabolol-content (mg/100 g crude drug) in two chamomile (*Matricaria recutita*) populations: mean value in dependence of the number of individuals used for sampling.

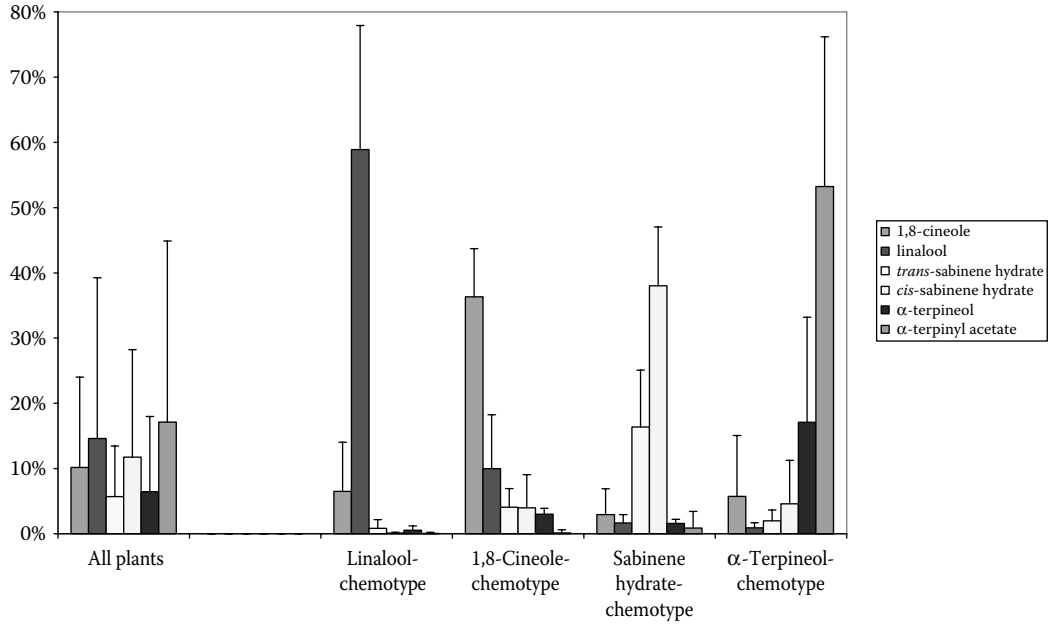

FIGURE 3.7 Mean values of the principal essential compounds of a *Thymus vulgaris* population (left) in comparison to the mean values of the chemotypes within the same population.

exemplarily the bisabolol content of two chamomile populations depending on the number of individual plants used for sampling. At small numbers, the mean value oscillates strongly and only after at least 15–20 individuals the range of variation becomes acceptable. Quite different appears the situation at qualitative differences, that is, "either-or-variations" within populations or taxa, for example, carvacrol/thymol, α-/β-thujone/1,8-cineole/camphor, or monoterpenes/phenylpropenes. Any random sample may give nonspecific information only on the principal chemical profile of the respective population provided that the sample is representative. This depends on the number of chemotypes, their inheritance, and frequency distribution within the population, and generally speaking no less than 50 individuals are needed for that purpose, as it can be derived from the comparison of chemotypes in a *Thymus vulgaris* population (Figure 3.7).

The overall high variation in essential oil compositions can be explained by the fact that quite different products might be generated by small changes in the synthase sequences only. On the other hand, different synthases may be able to produce the same substance in systematically distant taxa. The different origin of such substances can be identified by, for example, the ^{12}C:^{13}C ratio (Mosandl, 1993). "Hence, a simple quantitative analysis of the essential oil composition is not necessarily appropriate for estimating genetic proximity even in closely related taxa" (Bazina et al., 2002).

3.3 IDENTIFICATION OF SOURCE MATERIALS

As illustrated by the previous paragraph, one of the crucial points of using plants as sources for essential oils is their heterogeneity. A first prerequisite for reproducible compositions is therefore an unambiguous botanical identification and characterization of the starting material. The first approach is the classical taxonomical identification of plant materials based on macro- and micromorphological features of the plant. The identification is followed by phytochemical analysis that may contribute to species identification as well as to the determination of the quality of the essential oil. This approach is now complemented by DNA-based identification.

DNA is a long polymer of nucleotides, the building units. One of four possible nitrogenous bases is part of each nucleotide and the sequence of the bases on the polymer strand is characteristic for

each living individual. Some regions of the DNA, however, are conserved on the species or family level and can be used to study the relationship of taxa (Taberlet et al., 1991; Wolfe and Liston, 1998). DNA sequences conserved within a taxon but different between taxa can therefore be used to identify a taxon ("DNA-barcoding") (Hebert et al., 2003a; Kress et al., 2005). A DNA-barcoding consortium was founded in 2004 with the ambitious goal to build a barcode library for all eukaryotic life in the next 20 years (Ratnasingham and Hebert, 2007). New sequencing technologies (454, Solexa, SOLiD) enable a fast and representative analysis, but will be applied due to their high costs in the moment only in the next phase of DNA-barcoding (Frezal and Leblois, 2008). DNA-barcoding of animals has already become a routine task. DNA-barcoding of plants, however, are still not trivial and a scientific challenge (Pennisi, 2007).

Besides sequence information-based approaches, multilocus DNA methods [RAPD, amplified fragment length polymorphism (AFLP), etc.] are complementing in resolving complicated taxa and can become a barcode for the identification of populations and cultivars (Weising et al., 2005). With multilocus DNA methods, it is furthermore possible to tag a specific feature of a plant of which the genetic basis is still unknown. This approach is called molecular markers (in *sensu strictu*) because they mark the occurrence of a specific trait like a chemotype or flower color. The gene regions visualized, for example, on an agarose gel is not the specific gene responsible for a trait but is located on the genome in the vicinity of this gene and therefore co-occurs with the trait and is absent when the trait is absent. An example for such an inexpensive and fast polymerase chain reaction (PCR)-system was developed by Bradbury et al. (2005) to distinguish fragrant from nonfragrant rice cultivars. If markers would be developed for chemotypes in essential oil plants, species identification by DNA and the determination of a chemotype could be performed in one step.

Molecular biological methods to identify species are nowadays routinely used in feed- and foodstuffs to identify microbes, animals, and plants. Especially the discussion about traceability of genetically modified organisms (GMO's) throughout the complete chain ("from the living organism to the super-market") has sped up research in this area (Auer, 2003; Miraglia et al., 2004). One advantage of molecular biological methods is the possibility to be used in a number of processed materials like fatty oil (Pafundo et al., 2005) or even solvent extracts (Novak et al., 2007). The presence of minor amounts of DNA in an essential oil cannot be excluded *a priori* although distillation as separation technique would suggest the absence of DNA. However, small plant or DNA fragments could distill over or the essential oil could come in contact with plant material after distillation.

3.4 GENETIC AND PROTEIN ENGINEERING

Genetic engineering is defined as the direct manipulation of the genes of organisms by laboratory techniques, not to be confused with the indirect manipulation of genes in traditional (plant) breeding. *Transgenic or genetically modified organisms (GMOs)* are organisms (bacteria, plants, etc.) that have been engineered with single or multiple genes (either from the same species or from a different species), using contemporary molecular biology techniques. These are organisms with improved characteristics, in plants, for example, with resistance or tolerance to biotic or abiotic stresses such as insects, disease, drought, salinity, and temperature. Another important goal in improving agricultural production conditions is to facilitate weed control by transformed plants resistant to broadband herbicides like glufosinate. Peppermint has been successfully transformed with the introduction of the bar gene, which encodes phosphinothricin acetyltransferase, an enzyme inactivating glufosinate-ammonium or the ammonium salt of glufosinate, phosphinothricin making the plant insensitive to the systemic, broad-spectrum herbicide Roundup ("*Roundup Ready* mint") (Li et al., 2001).

A first step in genetic engineering is the development and optimization of transformation (gene transfer) protocols for the target species. Such optimized protocols exist for essential oil plants such as lavandin (*Lavandula* × *intermedia*; Dronne et al., 1999), spike lavender (*Lavandula latifolia*; Nebauer et al., 2000), and peppermint (*Mentha* × *piperita*; Diemer et al., 1998; Niu et al., 2000).

TABLE 3.6
Essential Oil Composition and Yield of Transgenic Peppermint Transformed with Genes Involved in Monoterpene Biosynthesis

Gene	Method	Limonene	Mentho-Furan	Pulegone	Menthone	Menthol	Oil Yield [lb/acre]
WT	—	1.7	4.3	2.1	20.5	44.5	97.8
dxr	overexpress	1.6	3.6	1.8	19.6	45.6	137.9
Mfs	antisense	1.7	1.2	0.4	22.7	45.2	109.7
l-3-h	cosuppress	74.7	0.4	0.1	4.1	3.0	99.6

Source: Wildung, M.R. and R.B. Croteau, 2005. *Transgenic Res*., 14: 365–372.
dxr = Deoxyxylulose phosphate reductoisomerase; l-3-h = limonene-3-hydroxylase; mfs = menthofuran synthase; WT = wild type.

In spike lavender, an additional copy of the 1-deoxy-D-xylulose-5-phosphate synthase gene (DXS), the first enzymatic step in the methylerythritol phosphate (MEP) pathway leading to the precursors of monoterpenes, from *Arabidopsis thaliana* was introduced and led to an increase of the essential oil of the leaves of up to 360% and of the essential oil of flowers of up to 74% (Munoz-Bertomeu et al., 2006).

In peppermint, many different steps to alter essential oil yield and composition were already targeted (reviewed by Wildung and Croteau 2005; Table 3.6). The overexpression of deoxyxylulose phosphate reductoisomerase (DXR), the second step in the MEP-pathway, increased the essential oil yield by approximately 40% tested under field conditions (Mahmoud and Croteau, 2001). The overexpression of geranyl diphosphate synthase (GPPS) leads to a similar increase of the essential oil. Menthofuran, an undesired compound, was downregulated by an antisense method (a method to influence or block the activity of a specific gene). Overexpression of the menthofuran antisense RNA was responsible for an improved oil quality by reducing both menthofuran and pulegone in one transformation step (Mahmoud and Croteau, 2003). The ability to produce a peppermint oil with a new composition was demonstrated by Mahmoud et al. (2004) by upregulating limonene by cosuppression of limonene-3-hydroxylase, the enzyme responsible for the transformation of (−)-limonene to (−)-*trans*-isopiperitenol *en route* to menthol.

Protein engineering is the application of scientific methods (mathematical and laboratory methods) to develop useful or valuable proteins. There are two general strategies for protein engineering, random mutagenesis, and rational design. In rational design, detailed knowledge of the structure and function of the protein is necessary to make desired changes by site-directed mutagenesis, a technique already well developed. An impressive example of the rational design of monoterpene synthases was given by Kampranis et al. (2007) who converted a 1,8-cineole synthase from *S. fruticosa* into a synthase producing sabinene, the precursor of α- and β-thujone with a minimum number of substitutions. They went also a step further and converted this monoterpene synthase into a sesquiterpene synthase by substituting a single amino acid that enlarged the cavity of the active site enough to accommodate the larger precursor of the sesquiterpenes, farnesyl pyrophosphate (FPP).

3.5 RESOURCES OF ESSENTIAL OILS: WILD COLLECTION OR CULTIVATION OF PLANTS

The raw materials for producing essential oil are resourced either from collecting them in nature ("wild collection") or from cultivating the plants (Table 3.7).

TABLE 3.7
Important Essential Oil-Bearing Plants—Common and Botanical Names Incl. Family, Plant Parts Used, Raw Material Origin, and Trade Quantities of the Essential Oil

Trade Name	Species	Plant Family	Used Plant Part(s)	Wild Collection/ Cultivation	Trade Quantities[a]
Ambrette seed	*Hibiscus abelmoschus* L.	Malvaceae	Seed	Cult	LQ
Amyris	*Amyris balsamifera* L.	Rutaceae	Wood	Wild	LQ
Angelica root	*Angelica archangelica* L.	Apiaceae	Root	Cult	LQ
Anise seed	*Pimpinella anisum* L.	Apiaceae	Fruit	Cult	LQ
Armoise	*Artemisia herba-alba* Asso	Asteraceae	Herb	Cult/wild	LQ
Asafoetida	*Ferula assa-foetida* L.	Apiaceae	Resin	Wild	LQ
Basil	*Ocimum basilicum* L.	Lamiaceae	Herb	Cult	LQ
Bay	*Pimenta racemosa* Moore	Myrtaceae	Leaf	Cult	LQ
Bergamot	*Citrus aurantium* L. subsp. *bergamia* (Risso et Poit.) Engl.	Rutaceae	Fruit peel	Cult	MQ
Birch tar	*Betula pendula* Roth. (syn. *Betula verrucosa* Erhart. *Betula alba* sensu H.J.Coste. non L.)	Betulaceae	Bark/wood	Wild	LQ
Buchu leaf	*Agathosma betulina* (Bergius) Pillans. *A. crenulata* (L.) Pillans	Rutaceae	Leaf	Wild	LQ
Cade	*Juniperus oxycedrus* L.	Cupressaceae	Wood	Wild	LQ
Cajuput	*Melaleuca leucandendron* L.	Myrtaceae	Leaf	Wild	LQ
Calamus	*Acorus calamus* L.	Araceae	Rhizome	Cult/wild	LQ
Camphor	*Cinnamomum camphora* L. (Sieb.)	Lauraceae	Wood	Cult	LQ
Cananga	*Cananga odorata* Hook. f. et Thoms.	Annonaceae	Flower	Wild	LQ
Caraway	*Carum carvi* L.	Apiaceae	Seed	Cult	LQ
Cardamom	*Elettaria cardamomum* (L.) Maton	Zingiberaceae	Seed	Cult	LQ
Carrot seed	*Daucus carota* L.	Apiaceae	Seed	Cult	LQ
Cascarilla	*Croton eluteria* (L.) W.Wright	Euphorbiaceae	Bark	Wild	LQ
Cedarwood, Chinese	*Cupressus funebris* Endl.	Cupressaceae	Wood	Wild	MQ
Cedarwood, Texas	*Juniperus mexicana* Schiede	Cupressaceae	Wood	Wild	MQ
Cedarwood, Virginia	*Juniperus virginiana* L.	Cupressaceae	Wood	Wild	MQ
Celery seed	*Apium graveolens* L.	Apiaceae	Seed	Cult	LQ
Chamomile	*Matricaria recutita* L.	Asteraceae	Flower	Cult	LQ
Chamomile, Roman	*Anthemis nobilis* L.	Asteraceae	Flower	Cult	LQ
Chenopodium	*Chenopodium ambrosioides* (L.) Gray	Chenopodiaceae	Seed	Cult	LQ
Cinnamon bark, Ceylon	*Cinnamomum zeylanicum* Nees	Lauraceae	Bark	Cult	LQ
Cinnamon bark, Chinese	*Cinnamomum cassia* Blume	Lauraceae	Bark	Cult	LQ
Cinnamon leaf	*Cinnamomum zeylanicum* Nees	Lauraceae	Leaf	Cult	LQ

continued

TABLE 3.7 (continued)
Important Essential Oil-Bearing Plants—Common and Botanical Names Incl. Family, Plant Parts Used, Raw Material Origin, and Trade Quantities of the Essential Oil

Trade Name	Species	Plant Family	Used Plant Part(s)	Wild Collection/ Cultivation	Trade Quantities[a]
Citronella, Ceylon	*Cymbopogon nardus* (L.) W. Wats.	Poaceae	Leaf	Cult	HQ
Citronella, Java	*Cymbopogon winterianus* Jowitt.	Poaceae	Leaf	Cult	HQ
Clary sage	*Salvia sclarea* L.	Lamiaceae	Flowering herb	Cult	MQ
Clove buds	*Syzygium aromaticum* (L.) Merill et L.M. Perry	Myrtaceae	Leaf/bud	Cult	LQ
Clove leaf	*Syzygium aromaticum* (L.) Merill et L.M. Perry	Myrtaceae	Leaf	Cult	HQ
Coriander	*Coriandrum sativum* L.	Apiaceae	Fruit	Cult	LQ
Cornmint	*Mentha canadensis* L. (syn. *M. arvensis* L. f. *piperascens* Malinv. ex Holmes; *M. arvensis* L. var. *glabrata. M. haplocalyx* Briq.; *M. sachalinensis* (Briq.) Kudo)	Lamiaceae	Leaf	Cult	HQ
Cumin	*Cuminum cyminum* L.	Apiaceae	Fruit	Cult	LQ
Cypress	*Cupressus sempervirens* L.	Cupressaeae	Leaf/twig	Wild	LQ
Davana	*Artemisia pallens* Wall.	Asteraceae	Flowering herb	Cult	LQ
Dill	*Anethum graveolens* L.	Apiaceae	Herb/fruit	Cult	LQ
Dill, India	*Anethum sowa* Roxb.	Apiaceae	Fruit	Cult	LQ
Elemi	*Canarium luzonicum* Miq.	Burseraceae	Resin	Wild	LQ
Eucalyptus	*Eucalyptus globulus* Labill.	Myrtaceae	Leaf	Cult/wild	HQ
Eucalyptus, lemon-scented	*Eucalyptus citriodora* Hook.	Myrtaceae	Leaf	Cult/wild	HQ
Fennel bitter	*Foeniculum vulgare* Mill. subsp. *vulgare* var. *vulgare*	Apiaceae	Fruit	Cult	LQ
Fennel sweet	*Foeniculum vulgare* Mill. subsp. *vulgare* var. *dulce*	Apiaceae	Fruit	Cult	LQ
Fir needle, Canadian	*Abies balsamea* Mill.	Pinaceae	Leaf/twig	Wild	LQ
Fir needle, Siberian	*Abies sibirica* Ledeb.	Pinaceae	Leaf/twig	Wild	LQ
Gaiac	*Guaiacum officinale* L.	Zygophyllaceae	Resin	Wild	LQ
Galbanum	*Ferula galbaniflua* Boiss. *F. rubricaulis* Boiss.	Apiaceae	Resin	Wild	LQ
Garlic	*Allium sativum* L.	Alliaceae	Bulb	Cult	LQ
Geranium	*Pelargonium* spp.	Geraniaceae	Leaf	Cult	MQ
Ginger	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome	Cult	LQ
Gingergrass	*Cymbopogon martinii* (Roxb.) H. Wats var. *sofia* Burk	Poaceae	Leaf	Cult/wild	
Grapefruit	*Citrus* × *paradisi* Macfad.	Rutaceae	Fruit peel	Cult	LQ
Guaiacwood	*Bulnesia sarmienti* L.	Zygophyllaceae	Wood	Wild	MQ
Gurjum	*Dipterocarpus* spp.	Dipterocarpaceae	Resin	Wild	LQ
Hop	*Humulus lupulus* L.	Cannabaceae	Flower	Cult	LQ
Hyssop	*Hyssopus officinalis* L.	Lamiaceae	Leaf	Cult	LQ

continued

TABLE 3.7 (continued)
Important Essential Oil-Bearing Plants—Common and Botanical Names Incl. Family, Plant Parts Used, Raw Material Origin, and Trade Quantities of the Essential Oil

Trade Name	Species	Plant Family	Used Plant Part(s)	Wild Collection/ Cultivation	Trade Quantities[a]
Juniper berry	*Juniperus communis* L.	Cupressaceae	Fruit	Wild	LQ
Laurel leaf	*Laurus nobilis* L.	Lauraceae	Leaf	Cult/wild	LQ
Lavandin	*Lavandula angustifolia* Mill. × *L. latifolia* Medik.	Lamiaceae	Leaf	Cult	HQ
Lavender	*Lavandula angustifolia* Miller	Lamiaceae	Leaf	Cult	MQ
Lavender, Spike	*Lavandula latifolia* Medik.	Lamiaceae	Flower	Cult	LQ
Lemon	*Citrus limon* (L.) Burman fil.	Rutaceae	Fruit peel	Cult	HQ
Lemongrass, Indian	*Cymbopogon flexuosus* (Nees ex Steud.) H. Wats.	Poaceae	Leaf	Cult	LQ
Lemongrass, West Indian	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Leaf	Cult	LQ
Lime distilled	*Citrus aurantiifolia* (Christm. et Panz.) Swingle	Rutaceae	Fruit	Cult	HQ
Litsea cubeba	*Litsea cubeba* C.H. Persoon	Lauraceae	Fruit/leaf	Cult	MQ
Lovage root	*Levisticum officinale* Koch	Apiaceae	Root	Cult	LQ
Mandarin	*Citrus reticulata* Blanco	Rutaceae	Fruit peel	Cult	MQ
Marjoram	*Origanum majorana* L.	Lamiaceae	Herb	Cult	LQ
Mugwort common	*Artemisia vulgaris* L.	Asteraceae	Herb	Cult/wild	LQ
Mugwort, Roman	*Artemisia pontica* L.	Asteraceae	Herb	Cult/wild	LQ
Myrtle	*Myrtus communis* L.	Myrtaceae	Leaf	Cult/wild	LQ
Neroli	*Citrus aurantium* L. subsp. *aurantium*	Rutaceae	Flower	Cult	LQ
Niaouli	*Melaleuca viridiflora*	Myrtaceae	Leaf	Cult/wild	LQ
Nutmeg	*Myristica fragrans* Houtt.	Myristicaceae	Seed	Cult	LQ
Onion	*Allium cepa* L.	Alliaceae	Bulb	Cult	LQ
Orange	*Citrus sinensis* (L.) Osbeck	Rutaceae	Fruit peel	Cult	HQ
Orange bitter	*Citrus aurantium* L.	Rutaceae	Fruit peel	Cult	LQ
Oregano	*Origanum* spp.. *Thymbra spicata* L.. *Coridothymus capitatus* Rechb. fil.. *Satureja* spp. *Lippia graveolens*	Lamiaceae	Herb	Cult/wild	LQ
Palmarosa	*Cymbopogon martinii* (Roxb.) H. Wats var. *motia* Burk	Poaceae	Leaf	Cult	LQ
Parsley seed	*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill	Apiaceae	Fruit	Cult	LQ
Patchouli	*Pogostemon cablin* (Blanco) Benth.	Lamiaceae	Leaf	Cult	HQ
Pennyroyal	*Mentha pulegium* L.	Lamiaceae	Herb	Cult	LQ
Pepper	*Piper nigrum* L.	Piperaceae	Fruit	Cult	LQ
Peppermint	*Mentha* x *piperita* L.	Lamiaceae	Leaf	Cult	HQ
Petitgrain	*Citrus aurantium* L. subsp. *aurantium*	Rutaceae	Leaf	Cult	LQ
Pimento leaf	*Pimenta dioica* (L.) Merr.	Myrtaceae	Fruit	Cult	LQ
Pine needle	*Pinus silvestris* L.. *P. nigra* Arnold	Pinaceae	Leaf/twig	Wild	LQ
Pine needle, Dwarf	*Pinus mugo* Turra	Pinaceae	Leaf/twig	Wild	LQ
Pine silvestris	*Pinus silvestris* L.	Pinaceae	Leaf/twig	Wild	LQ

continued

TABLE 3.7 (continued)
Important Essential Oil-Bearing Plants—Common and Botanical Names Incl. Family, Plant Parts Used, Raw Material Origin, and Trade Quantities of the Essential Oil

Trade Name	Species	Plant Family	Used Plant Part(s)	Wild Collection/ Cultivation	Trade Quantities[a]
Pine white	*Pinus palustris* Mill.	Pinaceae	Leaf/twig	Wild	LQ
Rose	*Rosa* x *damascena* Miller	Rosaceae	Flower	Cult	LQ
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Feaf	Cult/wild	LQ
Rosewood	*Aniba rosaeodora* Ducke	Lauraceae	Wood	Wild	LQ
Rue	*Ruta graveolens* L.	Rutaceae	Herb	Cult	LQ
Sage, Dalmatian	*Salvia officinalis* L.	Lamiaceae	Herb	Cult/wild	LQ
Sage, Spanish	*Salvia lavandulifolia* L.	Lamiaceae	Leaf	Cult	LQ
Sage, three lobed (Greek. Turkish)	*Salvia fruticosa* Mill. (syn. *S. triloba* L.)	Lamiaceae	Herb	Cult/wild	LQ
Sandalwood, East Indian	*Santalum album* L.	Santalaceae	Wood	Wild	MQ
Sassafras, Brazilian (Ocotea cymbarum oil)	*Ocotea odorifera* (Vell.) Rohwer [*Ocotea pretiosa* (Nees) Mez.]	Lauraceae	Wood	Wild	HQ
Sassafras, Chinese	*Sassafras albidum* (Nutt.) Nees.	Lauraceae	Root bark	Wild	HQ
Savory	*Satureja hortensis* L.. *Satureja montana* L.	Lamiaceae	Leaf	Cult/wild	LQ
Spearmint, Native	*Mentha spicata* L.	Lamiaceae	Leaf	Cult	MQ
Spearmint, Scotch	*Mentha gracilis* Sole	Lamiaceae	Leaf	Cult	HQ
Star anise	*Illicium verum* Hook fil.	Illiciaceae	Fruit	Cult	MQ
Styrax	*Styrax officinalis* L.	Styracaceae	Resin	Wild	LQ
Tansy	*Tanacetum vulgare* L.	Asteraceae	Flowering herb	Cult/wild	LQ
Tarragon	*Artemisia dracunculus* L.	Asteraceae	Herb	Cult	LQ
Tea tree	*Melaleuca* spp.	Myrtaceae	Leaf	Cult	LQ
Thyme	*Thymus vulgaris* L.. *T. zygis* Loefl. ex L.	Lamiaceae	Herb	Cult	LQ
Valerian	*Valeriana officinalis* L.	Valerianaceae	Root	Cult	LQ
Vetiver	*Vetiveria zizanoides* (L.) Nash	Poaceae	Root	Cult	MQ
Wintergreen	*Gaultheria procumbens* L.	Ericaceae	Leaf	Wild	LQ
Wormwood	*Artemisia absinthium* L.	Asteraceae	Herb	Cult/wild	LQ
Ylang Ylang	*Cananga odorata* Hook. f. et Thoms.	Annonaceae	Flower	Cult	MQ

[a] HQ = High quantities (>1000 t/a); MQ = medium quantities (100–1000 t/a); LQ = low quantities (<100 t/a).

3.5.1 Wild Collection and Sustainability

Since prehistoric times mankind has gathered wild plants for different purposes, among them are aromatic, essential oil-bearing species used as culinary herbs, spices, flavoring agents, and fragrances. With increasing demand of standardized, homogeneous raw material in the industrial societies more and more wild species have been domesticated and systematically cultivated. Nevertheless, a high number of species is still collected from the wild due to the fact that

- Many plants and plant products are used for the subsistence of the rural population.
- Small quantities of the respective species are requested at the market only which make a systematic cultivation not profitable.

- Some species are difficult to cultivate (slow growth rate and requirement of a special microclimate).
- Market uncertainties or political circumstances do not allow investing in long-term cultivation.
- The market is in favor of "ecological" or "natural" labeled wild collected material.

Especially—but not only—in developing countries, parts of the rural population depend economically on gathering high-value plant material. Less than two decades ago, almost all oregano (crude drug as well as essential oil) worldwide came from wild collection (Padulosi, 1996) and even this well-known group of species (*Origanum* sp. and *Lippia* sp.) were counted under "neglected and underutilized crops."

Yarrow (*Achillea millefolium s.l.*), arnica, and even chamomile originate still partly from wild collection in Central and Eastern Europe, and despite several attempts to cultivate spikenard (*Valeriana celtica*), a tiny European mountain plant with a high content of patchouli alcohol, this species is still wild gathered in Austria and Italy (Novak et al., 1998, 2000).

To regulate the sustainable use of biodiversity by avoiding overharvesting, genetic erosion, and habitat loss, international organizations such as IUCN (International Union for Conservation of Nature), WWF/TRAFFIC, and World Health Organization (WHO) have launched together the Convention on Biological Diversity (CBD, 2001), the Global Strategy for Plant Conservation (CBD, 2002), and the Guidelines for the Sustainable Use of Biodiversity (CBD, 2004). These principles and recommendations address primarily the national and international policy level, but provide also the herbal industry and the collectors with specific guidance on sustainable sourcing practices (Leaman, 2006). A standard for sustainable collection and use of medicinal and aromatic plants [the international standard on sustainable wild collection of medicinal and aromatic plants (ISSC-MAP)] was issued first in 2004 and its principles will be shown at the end of this chapter. This standard certifies wild-crafted plant material insofar as conservation and sustainability are concerned. Phytochemical quality cannot, however, be derived from it which is the reason for domestication and systematic cultivation of economically important essential oil plants.

3.5.2 Domestication and Systematic Cultivation

This offers a number of advantages over wild harvest for the production of essential oils:

- Avoidance of admixtures and adulterations by reliable botanical identification.
- Better control of the harvested volumes.
- Selection of genotypes with desirable traits, especially quality.
- Controlled influence on the history of the plant material and on postharvest handling.

On the other side, it needs arable land and investments in starting material, maintenance, and harvest techniques. On the basis of a number of successful introductions of new crops a scheme and strategy of domestication was developed by this author (Table 3.8).

Recent examples of successful domestication of essential oil-bearing plants are *oregano* (Ceylan et al., 1994; Kitiki 1996; Putievsky et al., 1996), *Lippia* sp. (Fischer, 1998), *Hyptis suaveolens* (Grassi, 2003), and *T. lucida* (Goehler, 2006). Domesticating a new species starts with studies at the natural habitat. The most important steps are the exact botanical identification and the detailed description of the growing site. National Herbaria are in general helpful in this stage. In the course of collecting seeds and plant material, a first phytochemical screening will be necessary to recognize chemotypes (Fischer et al., 1996; Goehler et al., 1997). The phytosanitary of wild populations should also be observed so as to be informed in advance on specific pests and diseases. The flower heads of wild *Arnica montana*, for instance, are often damaged by the larvae of *Tephritis arnicae* (Fritzsche et al., 2007).

TABLE 3.8
Domestication Strategy for Plants of the Spontaneous Flora

1. *Studies at the natural habitat*: botany, soil, climate, growing type, natural distribution and propagation, natural enemies, pests and diseases	→ GPS to exactly localize the place
2. *Collection of the wild grown plants and seeds*: establishment of a germplasm collection, *ex situ* conservation, phytochemical investigation (screening)	
3. *Plant propagation*: vegetatively or by seeds, plantlet cultivation; (biotechnol.: *in vitro* propagation)	→ Biotechnol./*in vitro*
4. *Genetic improvement*: variability, selection, breeding; phytochemical investigation, biotechnology (*in vitro* techniques)	→ Biotechnol./*in vitro*
5. *Cultivation treatments*: growing site, fertilization, crop maintenance, cultivation techniques	
6. *Phytosanitary problems*: pests, diseases	→ Biotechnol./*in vitro*
7. *Duration of the cultivation*: harvest, postharvest handling, phytochemical control of the crop produced	→ Technical processes, solar energy (new techniques)
8. *Economic evaluation and calculation*	→ New techniques

Source: Modified from Franz, Ch., 1993c. *Plant Res. Dev.*, 37: 101–111; Franz, Ch., 1993d. *Proc. 12th Int. Congr. of Essential Oils, Fragrances and Flavours*, Vienna, pp. 27–44.

The first phase of domestication results in a germplasm collection. In the next step, the appropriate propagation method has to be developed, which might be derived partly from observations at the natural habitat: while studying wild populations of *T. lucida* in Guatemala we found, besides appropriate seed set, also runners, which could be used for vegetative propagation of selected plants (Goehler et al., 1997). Wherever possible, propagation by seeds and direct sowing is however preferred due to economic reasons.

The appropriate cultivation method depends on the plant type—annual or perennial, herb, vine, or tree—and on the agroecosystem into which the respective species should be introduced. In contrast to large-scale field production of herbal plants in temperate and Mediterranean zones, small-scale sustainable agroforesty and mixed cropping systems adapted to the environment have the preference in tropical regions (Schippmann et al., 2006). Parallel to the cultivation trials dealing with all topics from plant nutrition and maintenance to harvesting and postharvest handling, the evaluation of the genetic resources and the genetic improvement of the plant material must be started to avoid developing of a detailed cultivation scheme with an undesired chemotype.

3.5.3 Factors Influencing the Production and Quality of Essential Oil-Bearing Plants

Since plant material is the product of a predominantly biological process, prerequisite of its productivity is the knowledge on the factors influencing it, of which the most important ones are

1. The already discussed intraspecific chemical polymorphism, derived from it the biosynthesis and inheritance of the chemical features, and as consequence selection and breeding of new cultivars.
2. The intraindividual variation between the plant parts and depending on the developmental stages ("morpho- and ontogenetic variation").
3. The modification due to environmental conditions including infection pressure and immissions.
4. Human influences by cultivation measures, for example, fertilizing, water supply, or pest management.

3.5.3.1 Genetic Variation and Plant Breeding

Phenotypic variation in essential oils was detected very early because of their striking sensorial properties. Due to the high chemical diversity, a continuous selection of the desired chemotypes leads to rather homogenous and reproducible populations, as this is the case with the landraces and common varieties. But Murray and Reitsema (1954) stated already that "a plant breeding program requires a basic knowledge of the inheritance of at least the major essential oil compounds." Such genetic studies have been performed over the last 50 years with a number of species especially of the mint family (e.g., *Ocimum* sp.: Sobti et al., 1978; *Thymus vulgaris*: Vernet, 1976; Gouyon and Vernet, 1982; *Perilla frutescens*: Koezuka et al., 1986; *Mentha* sp.: Croteau, 1991), of the Asteraceae/Compositae (*Matricaria recutita*: Horn et al., 1988; Massoud and Franz, 1990), the genus (*Eucalyptus*: Brophy and Southwell, 2002; Doran, 2002), or the *Vetiveria zizanioides* (Akhila and Rani, 2002).

The results achieved by inheritance studies have been partly applied in targeted breeding as shown exemplarily in Table 3.9. Apart from the essential oil content and composition there are also other targets to be observed when breeding essential oil plants, as particular morphological characters ensuring high and stable yields of the respective plant part, resistances to pest and diseases as well as abiotic stress, low nutritional requirements to save production costs, appropriate homogeneity, and suitability for technological processes at harvest and postharvest, especially readiness for distillation (Pank, 2007). In general, the following breeding methods are commonly used (Franz, 1999).

3.5.3.1.1 Selection by Exploiting the Natural Variability

Since many essential oil-bearing species are in the transitional phase from wild plants to systematic cultivation, appropriate breeding progress can be achieved by simple selection. Wild collections or accessions of germplasm collections are the basis, and good results were obtained, for example, with *Origanum* sp. (Putievsky et al., 1997) in limited time and at low expenses.

Individual plants showing the desired phenotype will be selected and either generatively or vegetatively propagated (individual selection), or positive or negative mass selection techniques can be applied. Selection is traditionally the most common method of genetic improvement and the majority of varieties and cultivars of essential oil crops have this background. Due to the fact, however, that almost all of the respective plant species are allogamous, a recurrent selection is necessary to maintain the varietal traits, and this has especially to be considered if other varieties or wild populations of the same species are nearby and uncontrolled cross pollination may occur.

The efficacy of selection has been shown by examples of many species, for instance, of the Lamiaceae family, starting from "Mitcham" peppermint and derived varieties (Lawrence, 2007), basil (Elementi et al., 2006), sage (Bezzi, 1994; Bernáth, 2000) to thyme (Rey, 1993). It is a well-known method also in the breeding of caraway (Pank et al., 1996) and fennel (Desmarest, 1992) as well as of tropical and subtropical species such as palmarosa grass (Kulkarni, 1990), tea tree (Taylor, 1996), and eucalyptus (Doran, 2002). At perennial herbs, shrubs, and trees clone breeding, that is, the vegetative propagation of selected high-performance individual plants, is the method of choice, especially in sterile or not type-true hybrids, for example, peppermint (*Mentha* × *piperita*) or lavandin (*Lavandula* × *hybrida*). But this method is often applied also at sage (Bazina et al., 2002), rosemary (Mulas et al., 2002), lemongrass (Kulkarni and Ramesh, 1992), pepper, cinnamon, and nutmeg (Nair, 1982), and many other species.

3.5.3.1.2 Breeding with Extended Variability (Combination Breeding)

If different desired characters are located in different individuals/genotypes of the same or a closely related crossable species, crossings are made followed by selection of the respective combination products. Artificial crossings are performed by transferring the paternal pollen to the stigma of the female (emasculated) or male sterile maternal flower. In the segregating progenies individuals with

TABLE 3.9
Some Registered Cultivars of Essential Oil Plant

Species	Cultivar/Variety	Country	Year of Registration	Breeding Method	Specific Characters
Achillea collina	SPAK	CH	1994	Crossing	High in proazulene
Angelica archangelica	VS 2	FR	1996	Recurrent pedigree	Essential oil index of roots: 180
Foeniculum vulgare	Fönicia	HU	1998	Selection	High anethole
Lavandula officinalis	Rapido	FR	1999	Polycross	High essential oil, high linalyl acetate
Levisticum officinale	Amor	PL	2000	Selection	High essential oil
Matricaria recutita	Mabamille	DE	1995	Tetraploid	High α-bisabolol
	Ciclo-1	IT	2000	Line breeding	High chamazulene
	Lutea	SK	1995	Tetraploid	High α-bisabolol
Melissa officinalis	Ildikó	HU	1998	Selection	High essential oil, Citral A + B, linalool
	Landor	CH	1994	Selection	High essential oil
	Lemona	DE	2001	Selection	High essential oil, citral
Mentha piperita	Todd's Mitcham	USA	1972	Mutation	Wilt resistant
	Kubanskaja	RUS	1980ies	Crossing and polyploid	High essential oil, high menthol
Mentha spicata	MSH-20	DK	2000	Recurrent pedigree	High menthol, good flavor
Ocimum basilicum	Greco	IT	2000	Synthetic	Flavor
	Perri	ISR	1999	Cross-breeding	Fusarium Resistant
	Cardinal	ISR	2000	Cross-breeding	
Origanum syriacum	Senköy	TR	1992	Selection	5% essential oil, 60% carvacrol
	Carmeli	ISR	1999	Selection	Carvacrol
	Tavor	ISR	1999	Selection	Thymol
Origanum onites		GR	2000	Selfing	Carvacrol
Origanum hirtum		GR	2000	Selfing	Carvacrol
	Vulkan	DE	2002	Crossing	Carvacrol
	Carva	CH	2002	Crossing	Carvacrol
	Darpman	TR	1992	Selection	2.5% essential oil, 55% carvacrol
Origanum majorana	Erfo	DE	1997	Crossing	High essential oil,
(Majorana hortensis)	Tetrata	DE	1999	Ployploid	Cis-sabinene-hydrate
	G 1	FR	1998	Polycross	
Salvia officinalis	Moran	ISR	1998	Crossing	Herb yield
	Syn 1	IT	2004	Synthetic	α-Thujone
Thymus vulgaris	Varico	CH	1994	Selection	Thymol/carvacrol
	T-16	DK	2000	Recurrent pedigree	Thymol
	Virginia	ISR	2000	Selection	Herb yield

the desired combination will be selected and bred to constancy, as exemplarily described for fennel and marjoram by Pank (2002).

Hybrid breeding—common in large-scale agricultural crops, for example, maize—was introduced into essential oil plants over the last decade only. The advantage of hybrids on the one side is that the F_1 generation exceeds the parent lines in performance due to hybrid vigor and uniformity ("heterosis effect") and on the other side it protects the plant breeder by segregating of the F_2 and following generations in heterogeneous low-value populations. But it needs as precondition

separate (inbred) parent lines of which one has to be male sterile and one male fertile with good combining ability.

In addition, a male fertile "maintainer" line is needed to maintain the mother line. Few examples of F_1 hybrid breeding are known especially at Lamiaceae since male sterile individuals are found frequently in these species (Rey, 1994; Novak et al., 2002; Langbehn et al., 2002; Pank et al., 2002).

Synthetic varieties are based on several (more than two) well-combining parental lines or clones which are grown together in a polycross scheme with open pollination for seed production. The uniformity and performance is not as high as at F_1 hybrids but the method is simpler and cheaper and the seed quality acceptable for crop production until the second or third generation. Synthetic cultivars are known for chamomile (Franz et al., 1985), arnica (Daniel and Bomme, 1991), marjoram (Franz and Novak, 1997), sage (Aiello et al., 2001), or caraway (Pank et al., 2007).

3.5.3.1.3 Breeding with Artificially Generated New Variability

Induced mutations by application of mutagenic chemicals or ionizing radiation open the possibility to find new trait expressions. Although quite often applied, such experiments are confronted with the disadvantages of undirected and incalculable results, and achieving a desired mutation is often like searching for a needle in a haystack. Nevertheless, remarkable achievements are several colchicine-induced polyploid varieties of peppermint (Murray, 1969; Lawrence, 2007), chamomile (Czabajska et al., 1978; Franz et al., 1983; Repčak et al., 1992), and lavender (Slavova et al., 2004).

Further possibilities to obtain mutants are studies of the somaclonal variation of *in vitro* cultures since abiotic stress in cell and tissue cultures induces also mutagenesis. Finally, genetic engineering opens new fields and potentialities to generate new variability and to introduce new traits by gene transfer. Except research on biosynthetic pathways of interesting essential oil compounds genetic engineering, GMO's and transgenic cultivars are until now without practical significance in essential oil crops and also not (yet) accepted by the consumer.

As regards the different traits, besides morphological, technological, and yield characteristics as well as quantity and composition of the essential oil, also stress resistance and resistance to pests and diseases are highly relevant targets in breeding of essential oil plants. Well known in this respect are breeding efforts against mint rust (*Puccinia menthae*) and wilt (*Verticillium dahliae*) resulting in the peppermint varieties "Multimentha," "Prilukskaja," or "Todd's Mitcham" (Murray and Todd, 1972; Pank, 2007; Lawrence, 2007), the development of *Fusarium*-wilt and *Peronospora* resistant cultivars of basil (Dudai, 2006; Minuto et al., 2006), or resistance breeding against *Septoria petroselini* in parsley and related species (Marthe and Scholze, 1996). An overview on this topic is given by Gabler (2002).

3.5.3.2 Plant Breeding and Intellectual Property Rights

Essential oil plants are biological, cultural, and technological resources. They can be found in nature gathered from the wild or developed through domestication and plant breeding. As long as the plant material is wild collected and traditionally used, it is part of the cultural heritage without any individual intellectual property and therefore not possible to protect, for example, by patents. Even finding a new plant or substance is a discovery in the "natural nature" and not an invention since a technical teaching is missing. Intellectual property, however, can be granted to new applications that involve an inventive step. Which consequences can be drawn from these facts for the development of novel essential oil plants and new selections or cultivars?

Selection and genetic improvement of aromatic plants and essential oil crops is not only time consuming but also rather expensive due to the necessity of comprehensive phytochemical and possibly molecular biological investigations. In addition, with few exceptions (e.g., mints, lavender and lavandin, parsley but also Cymbopogon sp., black pepper, or cloves) the acreage per species is rather limited in comparison with conventional agricultural and horticultural crops. And finally, there are several "fashion crops" with market uncertainties concerning their longevity or half-life period,

respectively. The generally unfavorable cost: benefit ratio to be taken into consideration makes essential oil plant breeding economically risky and there is no incentive for plant breeders unless a sufficiently strong plant intellectual property right (IPR) exists. Questioning "which protection, which property right for which variety?" offers two options (Franz, 2001).

3.5.3.2.1 Plant Variety Protection

By conventional methods bred plant groupings that collectively are distinct from other known varieties and are uniform and stable following repeated reproduction can be protected by way of plant breeder's rights. Basis is the International Convention for the Protection of New Varieties initially issued by UPOV (Union for the Protection of New Varieties of Plants) in 1961 and changed in 1991. A plant breeder's right is a legal title granting its holder the exclusive right to produce reproductive material of his plant variety for commercial purposes and to sell this material within a particular territory for up to 30 years (trees and shrubs) or 25 years (all other plants). A further precondition is the "commercial novelty," that is, it must not have been sold commercially prior to the filing date. Distinctness, uniformity, and stability (DUS) refer to morphological (leaf shape, flower color, etc.) or physiological (winter hardiness, disease resistance, etc.), but not phytochemical characteristics, for example, essential oil content or composition. Such "value for cultivation and use (VCU) characteristics" will not be examined and are therefore not protected by plant breeder's rights (Franz, 2001; Llewelyn, 2002; Van Overwalle, 2006).

3.5.3.2.2 Patent Protection (Plant Patents)

Generally speaking, patentable are inventions (not discoveries!) that are novel, involve an innovative step, and are susceptible to industrial application, including agriculture. Plant varieties or essentially biological processes for the production of plants are explicitly excluded from patenting. But other groupings of plants that fall neither under the term "variety" nor under "natural nature" are possible to be protected by patents. This is especially important for plant groupings with novel phytochemical composition or novel application combined with an inventive step, for example, genetical modification, a technologically new production method or a novel type of isolation (product by process protection).

Especially for wild plants and essentially allogamous plants not fulfilling DUS for cultivated varieties (cultivars) and plants where the phytochemical characteristics are more important than the morphological ones, plant patents offer an interesting alternative to plant variety protection (PVP) (Table 3.10).

TABLE 3.10
Advantages and Disadvantages of PVP versus Patent Protection of Specialist Minor Crops (Medicinal and Aromatic Plants)

PVP	Patent
Beginning of protection: registration date	Beginning of protection: application date
Restricted to "varieties"	"Varieties" not patentable, but any other grouping of plants
Requirements: DUS = distinctness, uniformity, stability	Requirements: novelty, inventive step, industrial applicability (=NIA)
Free choice of characters to be used for DUS by PVO (Plant Variety Office)	Repeatability obligatory, product by process option
Phenotypical. Mainly morphological characters (phytochemicals of minor importance)	"Essentially biological process" not patentable
Value for cultivation and use characteristics (VCU) not protected	"Natural nature" not patentable Claims (e.g., phytochemical characters) depend on applicant Phytochemical characters and use/application (VCU) patentable

In conformity with the UPOV Convention of 1991 (http://www.upov.int/en/publications/conventions/1991/content.htm)

- A strong plant IPR is requested.
- Chemical markers (e.g., secondary plant products) must be accepted as protectable characteristics.
- Strong depending rights for essentially derived varieties are needed since it is easy to plagiarize such crops.
- "Double protection" would be very useful (i.e., free decision by the breeder if PVR or patent protection is applied).
- But also researchers exemption and breeders privilege with fair access to genotypes for further development is necessary.

Strong protection does not hinder usage and development; it depends on a fair arrangement only (Le Buanec, 2001).

3.5.3.3 Intraindividual Variation between Plant Parts and Depending on the Developmental Stage (*Morpho-* and *Ontogenetic Variation*)

The formation of essential oils depends on the tissue differentiation (secretory cells and excretion cavities, as discussed above in the introduction to this chapter) and on the ontogenetic phase of the respective plant. The knowledge on these facts is necessary to harvest the correct plant parts at the right time.

Regarding the *differences between plant parts*, it is known from cinnamon (*Cinnamomum zeylanicum*) that the root-, stem-, and leaf oils differ significantly (Wijesekera et al., 1974): only the stem bark contains an essential oil with up to 70% cinnamaldehyde, whereas the oil of the root bark consists mainly of camphor and linalool, and the leaves produce oils with eugenol as main compound. In contrast to it, eugenol forms with 70–90% the main compound in stem, leaf, and bud oils of cloves (*Syzygium aromaticum*) (Lawrence, 1978). This was recently confirmed by Srivastava et al. (2005) for clove oils from India and Madagascar, stating in addition that eugenyl acetate was found in buds up to 8% but in leaves between traces and 1.6% only. The second main substance in leaves as well as buds is β-caryophyllene with up to 20% of the essential oil. In *Aframomum giganteum* (Zingiberaceae), the rhizome essential oil consists of β-caryophyllene, its oxide, and derivatives mainly, whereas in the leaf oil terpentine-4-ol and pinocarvone form the principal components (Agnaniet et al., 2004).

Essential oils of the Rutaceae family, especially citrus oils, are widely used as flavors and fragrances depending on the plant part and species: in lime leaves neral/geranial and nerol/geraniol are prevailing, whereas grapefruit leaf oil consists of sabinene and β-ocimene mainly. The peel of grapefruit contains almost limonene only and some myrcene, but lime peel oil shows a composition of β-pinene, γ-terpinene, and limonene (Gancel et al., 2002). In *Phellodendron* sp., Lis et al. (2004, 2006) found that in flower and fruit oils limonene and myrcene are dominating; in leaf oils, in contrast, α-farnesene, β-elemol, or β-ocimene, are prevailing.

Differences in the essential oil composition between the plant parts of many Umbelliferae (Apiaceae) have exhaustively been studied by the group of Kubeczka, summarized by Kubeczka et al. (1982) and Kubeczka (1997). For instance, the comparison of the essential fruit oil of aniseed (*Pimpinella anisum*) with the oils of the herb and the root revealed significant differences (Kubeczka et al., 1986). Contrary to the fruit oil consisting of almost *trans*-anethole only (95%), the essential oil of the herb contains besides anethole, considerable amounts of sesquiterpene hydrocarbons, for example, germacrene D, β-bisabolene, and α-zingiberene. Also pseudoisoeugenyl-2-methylbutyrate and epoxi-pseudoisoeugenyl-2-methylbutyrate together form almost 20% main compounds of the herb oil, but only 8.5% in the root and 1% in the fruit oil. The root essential oil is characterized by

a high content of β-bisabolene, geijerene, and pregeijerene and contains only small amounts of *trans*-anethole (3.5%). Recently, Velasco-Neguerela et al. (2002) investigated the essential oil composition in the different plant parts of *Pimpinella cumbrae* from Canary Islands and found in all above-ground parts α-bisabolol as main compound besides of δ-3-carene, limonene, and others, whereas the root oil contains mainly isokessane, geijerene, isogeijerene, dihydroagarofuran, and proazulenes—the latter is also found in *Pimpinella nigra* (Kubeczka et al., 1986). Pseudoisoeugenyl esters, known as chemosystematic characters of the genus *Pimpinella*, have been detected in small concentrations in all organs except leaves.

Finally, Kurowska and Galazka (2006) compared the seed oils of root and leaf parsley cultivars marketed in Poland. Root parsley seeds contained an essential oil with high concentrations of apiole and some lower percentages of myristicin. In leaf parsley seeds, in contrast, the content of myristicin was in general higher than apiole, and a clear differentiation between flat leaved cultivars showing still higher concentrations of apiole and curled cultivars with only traces of apiole could be observed. Allyltetramethoxybenzene as the third marker was found in leaf parsley seeds up to 12.8%, in root parsley seeds, however, in traces only. Much earlier, Franz and Glasl (1976) had published already similar results on parsley seed oils comparing them with the essential oil composition of the other plant parts (Figure 3.8). Leaf oils gave almost the same fingerprint than the seeds with high myristicin in curled leaves, some apiole in flat leaves, and higher apiole concentrations than myristicin in the leaves of root varieties. In all root samples, however, apiole dominated largely over myristicin. It is therefore possible to identify the parsley type by analyzing a small seed sample.

As shown already by Figueiredo et al. (1997), in the major number of essential oil-bearing species the oil composition differs significantly between the plant parts, but there are also plant species—as mentioned before, for example, cloves—which form a rather similar oil composition in each plant organ. Detailed knowledge in this matter is needed to decide, for instance how exact the separation of plant parts has to be performed before further processing (e.g., distillation) or use.

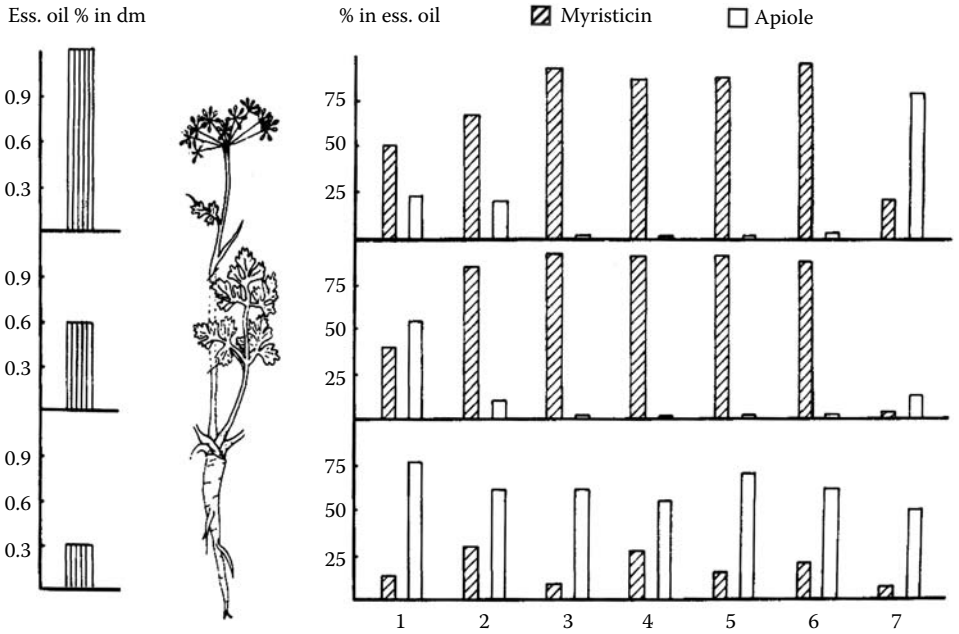

FIGURE 3.8 Differences in the essential oil of fruits, leaves and roots of parsley cultivars (*Petroselinum crispum* (Mill.) Nyman); (left: ess. oil content, right: content of myristicin and apiole in the ess. oil. 1,2: flat leaved cv's, 3–7 curled leaves cv's, 7 root parsley).

Another topic to be taken into consideration is the *developmental stage* of the plant and the plant organs, since the formation of essential oils is phase dependent. In most cases, there is a significant increase of the essential oil production throughout the whole vegetative development.

And especially in the generative phase between flower bud formation and full flowering, or until fruit or seed setting, remarkable changes in the oil yield and compositions can be observed. Obviously, a strong correlation is given between formation of secretory structures (oil glands, ducts, etc.) and essential oil biosynthesis, and different maturation stages, are associated with, for example, higher rates of cyclization or increase of oxygenated compounds (Figueiredo et al., 1997).

Investigations on the ontogenesis of fennel (*Foeniculum vulgare* Mill.) revealed that the best time for picking fennel seeds is the phase of full ripeness due to the fact that the anethole content increases from <50% in unripe seeds to over 80% in full maturity (Marotti et al., 1994). In dill weed (*Anethum graveolens* L.) the content on essential oil rises from 0.1% only in young sprouts to more than 1% in herb with milk ripe umbels (Gora et al., 2002). In the herb, oil α-phellandrene prevails until the beginning of flowering with up to 50%, followed by dill ether, *p*-cymene, and limonene. The oil from green as well as ripe umbels contains, on the other hand, mainly (*S*)-carvone and (*R*)-limonene. The flavor of dill oil changes therefore dramatically, which has to be considered when determining the harvest time for distillation.

Among Compositae (Asteraceae) there are not as many results concerning ontogeny due to the fact that in general the flowers or flowering parts of the plants are harvested, for example, chamomile (*Matricaria recutita*), yarrow (*Achillea millefolium s.l.*), immortelle (*Helichrysum italicum*), or wormwood (*Artemisia* sp.) and therefore the short period between the beginning of flowering and the decay of the flowers is of interest only. In chamomile (*Matricaria recutita*), the flower buds show a relatively high content on essential oil between 0.8% and 1.0%, but the oil yield in this stage is rather low. From the beginning of flowering, the oil content increases until full flowering (all disc florets open) and decreases again with decay of the flower heads. At full bloom there is also the peak of (pro)chamazulene, whereas farnesene and α-bisabolol decrease from the beginning of flowering and the bisabololoxides rise (Franz et al., 1978). This was confirmed by Repčak et al. (1980). The essential oil of *Tagetes minuta* L. at different development stages was investigated by Worku and Bertoldi (1996). Before flower bud formation the oil content was 0.45% only, but it culminated with 1.34% at the immature seed stage. During this period *cis*-ocimene increased from 7.2% to 37.5% and *cis*-ocimenone declined from almost 40–13.1%. Little variations could be observed at *cis*- and *trans*-tagetone only. Similar results have been reported also by Chalchat et al. (1995).

Also for *Lippia* sp. (Verbenaceae) some results are known concerning development stages (Fischer, 1998, Coronel, 2006). The oil content in the aerial parts increases from young buds (<1.0%) to fully blooming (almost 2.0%). But although quantitative variations could be observed for most components of the essential oils, the qualitative composition appeared to be constant throughout the growing season.

A particular situation is given with eucalypts as they develop up to five distinct types of leaves during their lifetime, each corresponding to a certain ontogenetic stage with changing oil concentrations and compositions (Doran, 2002). Usually the oil content increases from young to matured, nonlignified leaves, and is thereafter declining until leaf lignification. Almost the same curve is valid also for the 1,8-cineole concentration in the oil. But comparing the relatively extensive literature on this topic, one may conclude that the concentration at various stages of leaf maturity is determined by a complex pattern of quantitative change in individual or groups of substances, some remaining constant, some increasing, and some decreasing. Tsiri et al. (2003) investigated the volatiles of the leaves of *Eucalyptus camaldulensis* over the course of a year in Greece and found a seasonal variation of the oil concentration with a peak during summer and lowest yields during winter. The constituent with highest concentration was 1,8-cineole (25.3–44.2%) regardless the time of harvest. The great variation of all oil compounds showed however no clear tendency, neither seasonal nor regarding leaf age or leaf position. Doran (2002) concluded therefore that genotypic differences outweigh any seasonal or environmental effects in eucalypts.

There is an extensive literature on ontogenesis and seasonal variation of Labiatae essential oils. Especially for this plant family, great differences are reported on the essential oil content and composition of young and mature leaves and the flowers may in addition influence the oil quality significantly. Usually, young leaves show higher essential oil contents per area unit compared to old leaves. But the highest oil yield is reached at the flowering period, which is the reason that most of the oils are produced from flowering plants. According to Werker et al. (1993) young basil (*O. basilicum*) leaves contained 0.55% essential oil while the content of mature leaves was only 0.13%. The same is also valid to a smaller extent for *O. sanctum*, where the essential oil decreases from young (0.54%) to senescing leaves (0.38%) (Dey and Choudhuri, 1983). Testing a number of basil cultivars mainly of the linalool chemotype, Macchia et al. (2006) found that only some of the cultivars produce methyl eugenol up to 8% in the vegetative stage. Linalool as main compound is increasing from the vegetative (10–50%) to the flowering (20–60%), and postflowering phase (25–80%), whereas the second important substance eugenol reaches its peak at the beginning of flowering (5–35%). According to the cultivars, different harvest dates are therefore recommended. In *O. sanctum*, the content of eugenol (60.3–52.2%) as well as of methyl eugenol (6.6–2.0%) is decreasing from young to senescent leaves and at the same time β-caryophyllene increases from 20.8% to 30.2% (Dey and Choudhuri, 1983).

As regards oregano (*O. vulgare* ssp. *hirtum*), the early season preponderance of *p*-cymene over carvacrol was reversed as the season progressed and this pattern could also be observed at any time within the plant, from the latest leaves produced (low in cymene) to the earliest (high in cymene) (Johnson et al., 2004; Figure 3.9). Already Kokkini et al. (1996) had shown that oregano contains a higher proportion of *p*-cymene to carvacrol (or thymol) in spring and autumn, whereas carvacrol/thymol prevails in the summer. This is explained by Dudai et al. (1992) as photoperiodic reaction: short days with high *p*-cymene, long days with low *p*-cymene production. But only young plants are capable of making this switch, whereas in older leaves the already produced and stored oil remains almost unchanged (Johnson et al., 2004).

Presumably the most studied essential oil plant is peppermint (*Mentha* × *piperita* L.). Already in the 1950s Lemli (1955) stated that the proportion of menthol to menthone in peppermint leaves changes in the course of the development toward higher menthol contents. Lawrence (2007) has just recently shown that from immature plants via mature to senescent plants the content of menthol increases (34.8–39.9–48.2%) and correspondingly the menthone content decreases dramatically (26.8–17.4–4.7%). At the same time, also an increase of menthyl acetate from 8.5% to 23.3% of the oil could be observed. At full flowering, the peppermint herb oil contains only 36.8% menthol but 21.8% menthone, 7.7% menthofuran, and almost 3% pulegone due to the fact that the flower oils are richer in

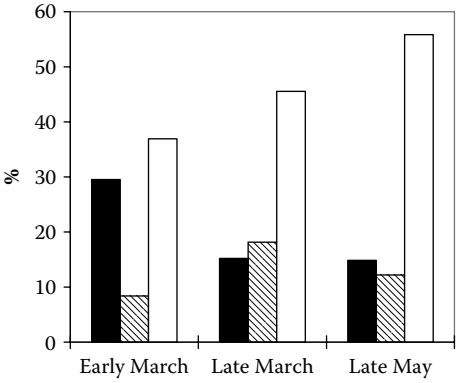

FIGURE 3.9 Average percentages and concentrations of *p*-cymene. γ-terpinene and carvacrol at the different sampling dates of *Origanum vulgare ssp. hirtum*. Solid bars: *p*-cymene; diagonally hatched bars: γ-terpinene; open bars: carvacrol.

menthone and pulegone and contain a high amount of menthofuran (Hefendehl, 1962). Corresponding differences have been found between young leaves rich in menthone and old leaves with high menthol and menthyl acetate content (Hoeltzel, 1964; Franz, 1972). The developmental stage depends, however, to a large extent from the environmental conditions, especially the day length.

3.5.3.4 Environmental Influences

Essential oil formation in the plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply. Tropical species follow in their vegetation cycle the dry and rainy season; species of the temperate zones react more on day length, the more distant from the equator their natural distribution area is located.

Peppermint as typical long day plant needs a minimum day length (hours of day light) to switch from the vegetative to the generative phase. This is followed by a change in the essential oil composition from menthone to menthol and menthyl acetate (Hoeltzel, 1964). Franz (1981) tested six peppermint clones at Munich/Germany and at the same time also at Izmir/Turkey. At the development stage "beginning of flowering," all clones contained at the more northern site much more menthol than on the Mediterranean location, which was explained by a maximum day length in Munich of 16 h 45 min, but in Izmir of 14 h 50 min only. Comparable day length reactions have been mentioned already for oregano (Kokkini et al., 1996; Dudai et al., 1992). Also marjoram (*O. majorana* L.) was influenced not only in flower formation by day length, but also in oil composition (Circella et al., 1995). At long day treatment the essential oil contained more *cis*-sabinene hydrate. Terpinene-4-ol prevailed under short day conditions.

Franz et al. (1986) performed ecological experiments with chamomile, growing vegetatively propagated plants at three different sites, in South Finland, Middle Europe, and West Turkey. As regards the oil content, a correlation between flower formation, flowering period, and essential oil synthesis could be observed: the shorter the flowering phase, the less was the time available for oil formation, and thus the lower was the oil content. The composition of the essential oil, on the other hand, showed no qualitative change due to ecological or climatic factors confirming that chemotypes keep their typical pattern. In addition, Massoud and Franz (1990) investigated the genotype–environment interaction of a chamazulene–bisabolol chemotype. The frequency distributions of the essential oil content as well as the content on chamazulene and α-bisabolol have shown that the highest oil- and bisabolol content was reached in Egypt while under German climatic conditions chamazulene was higher. Similar results have been obtained by Letchamo and Marquard (1993). The relatively high heritability coefficients calculated for some essential oil components—informing whether a character is more influenced by genetic or other factors—confirm that the potential to produce a certain chemical pattern is genetically coded, but the gene expression will be induced or repressed by environmental factors also (Franz, 1993b,d).

Other environmental factors, for instance soil properties, water stress, or temperature, are mainly influencing the productivity of the respective plant species and by this means the oil yield also, but have little effect on the essential oil formation and composition only (Figueiredo et al., 1997; Salamon, 2007).

3.5.3.5 Cultivation Measures, Contaminations, and Harvesting

Essential oil-bearing plants comprise annual, biennial, or perennial herbs, shrubs, and trees, cultivated either in tropical or subtropical areas, in Mediterranean regions, in temperate, or even in arid zones. Surveys in this respect are given, for instance, by Chatterjee (2002) for India, by Carruba et al. (2002) for Mediterranean environments, and by Galambosi and Dragland (2002) for Nordic countries. Nevertheless, some examples should refer to some specific items.

The *cultivation method*—if direct sowing or transplanting—and the timing influence the crop development and by that way also the quality of the product, as mentioned above. Vegetative propagation, necessary for peppermint due to its genetic background as interpecific hybrid, common in *Cymbopogon* sp. and useful to control the ratio between male and female trees in nutmeg (*Myristica*

fragrans), results in homogeneous plant populations and fields. A disadvantage could be the easier dispersion of pests and diseases, as known for "yellow rot" of lavandin (*Lavandula* × *hybrida*) (Fritzsche et al., 2007). Clonal propagation can be performed by leaf or stem cuttings (Goehler et al., 1997; Nicola et al., 2006; El-Keltawi and Abdel-Rahman, 2006) or *in vitro* (e.g., Figueiredo et al., 1997; Mendes and Romano, 1997), the latter method especially for mother plant propagation due to the high costs. *In vitro* essential oil production received increased attention in physiological experiments, but has up to now no practical significance.

As regards *plant nutrition and fertilizing*, a numerous publications have shown its importance for plant growth, development, and biomass yield. The essential oil yield, obviously, depends on the plant biomass; the oil percentage is partly influenced by the plant vigor and metabolic activity. Optimal fertilizing and water supply results in better growth and oil content, for example, in marjoram, oregano, basil, or coriander (Menary, 1994), but also in delay of maturity, which causes quite often "immature" flavors.

Franz (1972) investigated the influence of nitrogen and potassium on the essential oil formation of peppermint. He could show that higher nitrogen supply increased the biomass but retarded the plant development until flowering, whereas higher potassium supply forced the maturity. With increasing nitrogen, a higher oil percentage was observed with lower menthol and higher menthone content; potassium supply resulted in less oil with more menthol and menthyl acetate. Comparable results with *Rosmarinus officinalis* have been obtained by Martinetti et al. (2006), and Omidbaigi and Arjmandi (2002) have shown for *Thymus vulgaris* that nitrogen and phosphorus fertilization had significant effect on the herb yield and essential oil content, but did not change the thymol percentage. Also Java citronella (*Cymbopogon winterianus* Jowitt.) responded to nitrogen supply with higher herb and oil yields, but no influence on the geraniol content could be found (Munsi and Mukherjee, 1986).

Extensive pot experiments with chamomile (*Matricaria recutita*) have also shown that high nitrogen and phosphorus nutrition levels resulted in a slightly increased essential oil content of the anthodia, but raising the potassium doses had a respective negative effect (Franz et al., 1983). With nitrogen the flower formation was in delay and lasted longer; with more potassium the flowering phase was reduced, which obviously influenced the period available for essential oil production. This was confirmed by respective ^{14}C-acetate labeling experiments (Franz, 1981).

Almost no effect has been observed on the composition of the essential oil. Also a number of similar pot or field trials came to the same result, as summarized by Salamon (2007).

Salinity and salt stress get an increasing importance in agriculture especially in subtropical and Mediterranean areas. Some essential oil plants, for example, *Artemisia* sp. and *Matricaria recutita* (chamomile) are relatively salt tolerant. Also thyme (*Thymus vulgaris*) showed a good tolerance to irrigation water salinity up to 2000 ppm, but exceeding concentrations caused severe damages (Massoud et al., 2002). Higher salinity reduced also the oil content, and an increase of *p*-cymene was observed. Recently, Aziz et al. (2008) investigated the influence of salt stress on growth and essential oil in several mint species. In all three mints, salinity reduced the growth severely from 1.5 g/L onward; in peppermint, the menthone content raised and menthol went down to <1.0%, in apple mint, linalool and neryl acetate decreased while myrcene, linalyl acetate, and linalyl propionate increased.

Further problems to be taken into consideration in plant production are *contaminations* with heavy metals, damages caused by pests and diseases, and *residues* of plant protection products. The most important toxic heavy metals Cd, Hg, Pb, and Zn, but also Cu, Ni, and Mn may influence the plant growth severely and by that way also the essential oil, as they may act as cofactors in the plant enzyme system. But as contaminants, they remain in the plant residue after distillation (Zheljazkov et al., 1996, 1997). Some plant species, for example, yarrow and chamomile accumulate heavy metals to a greater extent. This is, however, problematic for using the crude drug or for deposition of distillation wastes mainly. The same is valid for the microbial contamination of the

plant material. More important in the production of essential oils are pests and diseases that cause damages to the plant material and sometimes alterations in the biosynthesis; but little is known in this respect.

In contrast to organic production, where no use of pesticides is permitted, a small number of insecticides, fungicides, and herbicides are approved for conventional herb production. The number, however, is very restricted (end of 2008 several active substances lost registration at least in Europe), and limits for residues can be found in national law and international regulations, for example, the European Pharmacopoeia. For essential oils, mainly the lipophilic substances are of relevance since they can be enriched over the limits in the oil.

Harvesting and the first steps of *postharvest handling* are the last part of the production chain of starting materials for essential oils. The harvest date is determined by the development stage or maturity of the plant or plant part, Harvesting techniques should keep the quality by avoiding adulterations, admixtures with undesired plant parts, or contaminations, which could cause "off-flavor" in the final product. There are many technical aids at disposal, from simple devices to large-scale harvesters, which will be considered carefully in Chapter 4. From the quality point of view, raising the temperature by fermentation should in general be avoided (except, in vanilla), and during the drying process further contamination with soil, dust, insects, or molds has to be avoided.

Quality and safety of essential oil-bearing plants as raw materials for pharmaceutical products, flavors, and fragrances are of highest priority from the consumer point of view. To meet the respective demands, standards and safety as well as quality assurance measures are needed to ensure that the plants are produced with care, so that negative impacts during wild collection, cultivation, processing, and storage can be limited. To overcome these problems and to guarantee a steady, affordable and sustainable supply of essential oil plants of good quality (Figure 3.10), in recent years guidelines for good agricultural practices (GAP) and standards for Sustainable Wild Collection (ISSC) have been established at the national and international level.

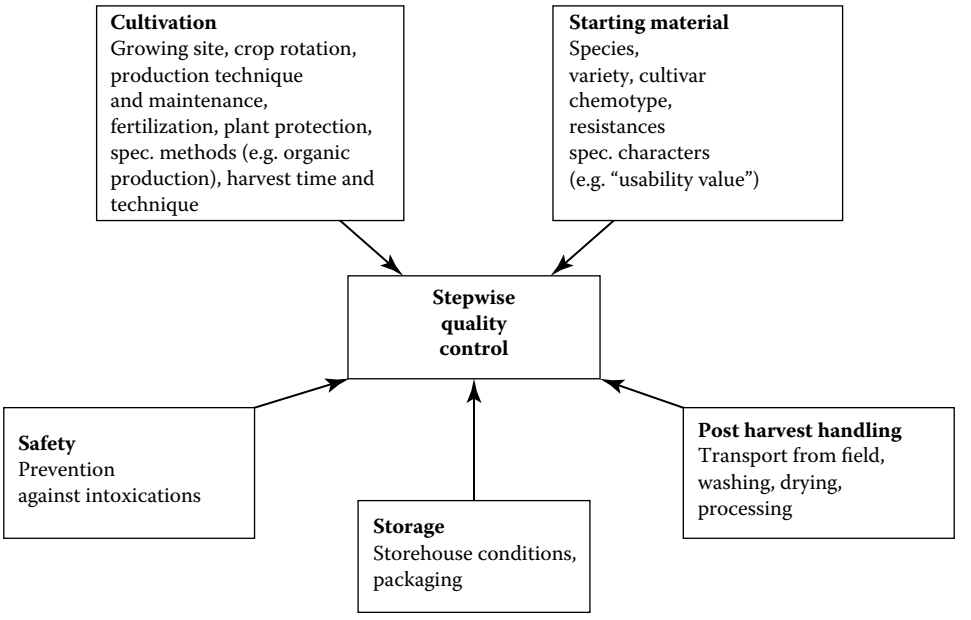

FIGURE 3.10 Main items of "good agricultural practices (GAP)" for medicinal and aromatic plants.

3.6 INTERNATIONAL STANDARDS FOR WILD COLLECTION AND CULTIVATION

3.6.1 GA(C)P: Guidelines for Good Agricultural (and Collection) Practice of Medicinal and Aromatic Plants

First initiatives for the elaboration of such guidelines trace back to a roundtable discussion in Angers, France in 1983, and intensified at an International Symposium in Novi Sad 1988 (Franz, 1989b). A first comprehensive paper was published by Pank et al. (1991) and in 1998 the European Herb Growers Association (EHGA/EUROPAM) released the first version (Máthé and Franz, 1999). The actual version can be downloaded from http://www.europam.net.

In the following it was adopted and slightly modified by the European Agency for the Evaluation of Medicinal Products (EMEA), and finally as Guidelines on good agricultural and collection practices (GACP) by the WHO in 2003.

All these guidelines follow almost the same concept dealing with the following topics:

- Identification and authentication of the plant material, especially botanical identity and deposition of specimens.
- Seeds and other propagation material, respecting the specific standards and certifications.
- Cultivation, including site selection, climate, soil, fertilization, irrigation, crop maintenance, and plant protection with special regard to contaminations and residues.
- Harvest, with specific attention to harvest time and conditions, equipment, damage, contaminations with (toxic) weeds and soil, transport, possible contact with any animals, and cleaning of all equipment and containers.
- Primary processing, that is, washing, drying, distilling; cleanness of the buildings; according to the actual legal situation these processing steps including distillation—if performed by the farmer—is still part of GA(C)P; in all other cases, it is subjected to GMP (good manufacturing practice).
- Packaging and labeling, including suitability of the material.
- Storage and transportation, especially storage conditions, protection against pests and animals, fumigation, and transport facilities.
- Equipment: material, design, construction, easy to clean.
- Personnel and facilities, with special regard to education, hygiene, protection against allergens and other toxic compounds, welfare.

In the case of wild collection the standard for sustainable collection should be applied (see Section 3.6.2).

A very important topic is finally the *documentation* of all steps and measurements to be able to trace back the starting material, the exact location of the field, any treatment with agrochemicals, and the special circumstances during the cultivation period. Quality assurance is only possible if the traceability is given and the personnel is educated appropriately. Certification and auditing of the production of essential oil-bearing plants is not yet obligatory, but recommended and often requested by the customer.

3.6.2 ISSC-MAP: The International Standard on Sustainable Wild Collection of Medicinal and Aromatic Plants

ISSC-MAP is a joint initiative of the German Bundesamt für Naturschutz (BfN), WWF/TRAFFIC Germany, IUCN Canada, and IUCN Medicinal Plant Specialist Group (MPSG). ISSC-MAP intends to ensure the long-term survival of MAP populations in their habitats by setting principles and criteria for the management of MAP wild collection (Leaman, 2006; Medicinal Plant Specialist Group,

2007). The standard is not intended to address product storage, transport, and processing, or any issues of products, topics covered by the WHO Guidelines on GACP for Medicinal Plants (WHO, 2003). ISSC-MAP includes legal and ethical requirements (legitimacy, customary rights, and transparency), resource assessment, management planning and monitoring, responsible collection, and collection, area practices and responsible business practices. One of the strengths of this standard is that resource management not only includes target MAP resources and their habitats but also social, cultural, and economic issues.

3.6.3 FairWild

The FairWild standard (http://www.fairwild.org) was initiated by the Swiss Import Promotion Organization (SIPPO) and combines principles of FairTrade (Fairtrade Labelling Organizations International, FLO), international labor standards (International Labour Organization, ILO), and sustainability (ISSC-MAP).

3.7 CONCLUSION

This chapter has shown that a number of items concerning the plant raw material have to be taken into consideration when producing essential oils. A quality management has to be established tracing back to the authenticity of the starting material and ensuring that all known influences on the quality are taken into account and documented in an appropriate way. This is necessary to meet the increasing requirements of international standards and regulations. The review also shows that a high number of data and information exist, but sometimes without expected relevance due to the fact that the repeatability of the results is not given by a weak experimental design, an incorrect description of the plant material used, or an inappropriate sampling. On the other side, this opens the chance for many more research work in the field of essential oil-bearing plants.

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4 Production of Essential Oils

Erich Schmidt

CONTENTS

4.1 INTRODUCTION

4.1.1 General Remarks

Essential oils have become an integral part of everyday life. They are used in a great variety of ways: as food flavorings, as feed additives, as flavoring agents by the cigarette industry, and in the compounding of cosmetics and perfumes. Furthermore, they are used in air fresheners and deodorizers as well as in all branches of medicine such as in pharmacy, balneology, massage, and homeopathy. A more specialized area will be in the fields of aromatherapy and aromachology. In recent years, the importance of essential oils as biocides and insect repellents has led to a more detailed study of their antimicrobial potential. Essential oils are also good natural sources of substances with commercial potential as starting materials for chemical synthesis.

Essential oils have been known to mankind for hundreds of years, even millenniums. Long before the fragrances themselves were used, the important action of the oils as remedies was recognized. Without the medical care as we enjoy in our time, self-healing was the only option to combat parasites or the suffering of the human body. Later on essential oils were used in the preparation of early cosmetics, powders, and soaps. As the industrial production of synthetic chemicals started and

increased during the nineteenth century, the production of essential oils also increased owing to their importance to our way of life.

The quantities of essential oils produced around the world vary widely. The annual output of some essential oils exceeds 35,000 tons while that of others may reach only a few kilograms. Some production figures, in metric tons, based on the year 2004 are shown in Table 4.1.

Equally wide variations also occur in the monetary value of different essential oils. Prices range from $1.80/kg for orange oil to $120,000.00/kg for orris oil. The total annual value of the world market is of the order of several billions of USD. A large, but variable, labor force is involved in the production of essential oils. While in some cases, harvesting and oil production will require just a few workers, other cases will require manual harvesting and may require multiple working steps. Essential oil production from either wild-growing or from cultivated plants is possible almost anywhere, excluding the world's coldest, permanently snow-covered regions. It is estimated that the global number of plant species is of the order of 300,000. About 10% of these contain essential oils and could be used as a source for their production. All continents possess their own characteristic flora with many odor-producing species. Occasionally, these plants may be confined to a particular geographical zone such as *Santalum album* to India and Timor in Indonesia, *Pinus mugo* to the European Alps, or *Abies sibirica* to the CIS [Commonwealth of Independent States (former Russia)]. For many countries, mainly in Africa and Asia, essential oil production is their main source of exports. Essential oil export figures for Indonesia, Sri Lanka, Vietnam, and even India are very high.

Main producer countries are found in every continent. In Europe, the center of production is situated in the countries bordering the Mediterranean Sea: Italy, Spain, Portugal, France, Croatia, Albania, and Greece, as well as middle-eastern Israel, all of which produce essential oils in industrial quantities. Among Central European countries, Bulgaria, Romania, Hungary, and Ukraine should be mentioned. The huge Russian Federation spread over much of eastern Europe and northern Asia has not only nearly endless resources of wild-growing plants but also large areas of cultivated land. The Asian continent with its diversity of climates appears to be the most important

TABLE 4.1
Production Figures of Important Essential Oils (2008)

Essential Oil	Production in Metric Tons (2008)	Main Production Countries
Orange oils	51000	USA, Brasil, Argentina
Cornmint oil	32000	India, China, Argentina
Lemon oils	9200	Argentina, Italy, Spain
Eucalyptus oils	4000	China, India, Australia, South Africa
Peppermint oil	3300	India, USA, China
Clove leaf oil	1800	Indonesia, Madagascar
Citronella oil	1800	China, Sri Lanka
Spearmint oils	1800	USA, China
Cedarwood oils	1650	USA, China
Litsea cubeba oil	1200	China
Patchouli oil	1200	Indonesia, India
Lavandin oil Grosso	1100	France
Corymbia Citriodora	1000	China, Brazil, India, Vietnam

Source: Perfumer & Flavorist, 2009. A preliminary report on the world production of some selected essential oils and countries, Vol. 34, January 2009.

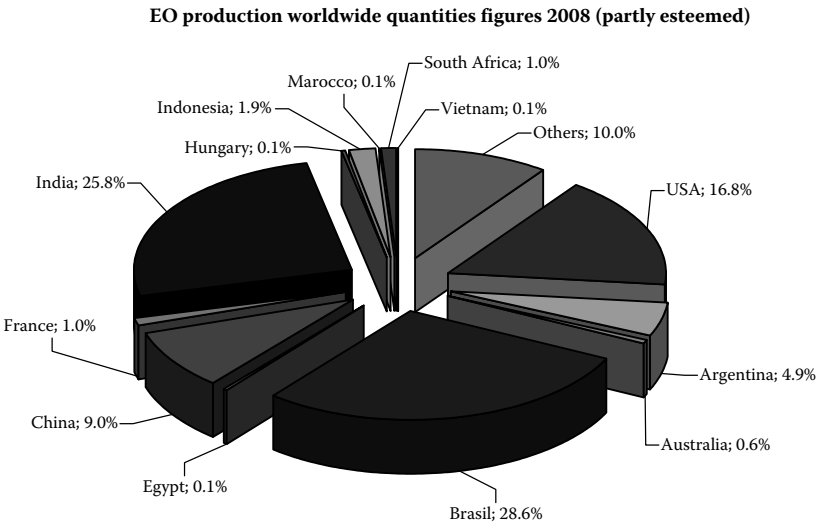

FIGURE 4.1 Production countries and essential oil production worldwide (2008). (Adapted from Perfumer & Flavorist, 2009. A preliminary report on the world production of some selected essential oils and countries, Vol. 34, January 2009.)

producer of essential oils. China and India play a major role followed by Indonesia, Sri Lanka, and Vietnam. Many unique and unusual essential oils originate from the huge Australian continent and from neighboring New Zealand and New Caledonia. Major essential oil-producing countries in Africa include Morocco, Tunisia, Egypt, and Algeria with the Ivory Coast, South Africa, Ghana, Kenya, Tanzania, Uganda, and Ethiopia playing a minor role. The important spice-producing islands of Madagascar, the Comoros, Mayotte, and Réunion are situated along the eastern coast of the African continent. The American continent is also one of the biggest essential oil producers. The United States, Canada, and Mexico possess a wealth of natural aromatic plant material. In South America, essential oils are produced in Brazil, Argentina, Paraguay, Uruguay, Guatemala, and the island of Haiti. Apart from the above-mentioned major essential oil-producing countries there are many more, somewhat less important ones, such as Germany, Taiwan, Japan, Jamaica, and the Philippines. Figure 4.1 shows production countries and essential oil production worldwide (2008).

Cultivation of aromatic plants shifted during the last two centuries. From 1850 to 1950, the centers of commercial cultivation of essential oil plants have been the Provence in France, Italy, Spain, and Portugal. With the increase of labor costs, this shifted to the Mediterranean regions of North Africa. As manual harvesting proved too expensive for European conditions, and following improvements in the design of harvesting machinery, only those crops that lend themselves to mechanical harvesting continued to be grown in Europe. By the early 1990s, even North Africa proved too expensive and the centers of cultivation moved to China and India. At the present time, manual-handling methods are tending to become too costly even in China and, thus India remains as today's center for the cultivation of fragrant plant crops.

4.1.2 Definition and History

Not all odorous extracts of essential oil-bearing plants comply with the International Standards Organization (ISO) definition of an "Essential Oil." An essential oil as defined by the ISO in document ISO 9235.2—aromatic natural raw materials—vocabulary is as follows.

"Product obtained from vegetable raw material—either by distillation with water or steam or—from the epicarp of *Citrus* fruits by a mechanical process, or—by dry distillation" (ISO/DIS 9235.2,

1997, p. 2). Steam distillation can be carried out with or without added water in a still. By contrast, dry distillation of plant material is carried out without the addition of any water or steam to the still (ISO 9235, 1997). Note 2 in Section 3.1.1 of ISO/DIS 9235.2 is of importance. It states that "Essential oils may undergo physical treatments (e.g., re-distillation, aeration) which do not involve significant changes in their composition" (ISO/DIS 9235.2, 1997, p. 2).

An alternative definition of essential oils, established by Professor Dr. Gerhard Buchbauer of the Institute of Pharmaceutical Chemistry, University of Vienna, includes the following suggestion: "Essential oils are more or less volatile substances with more or less odorous impact, produced either by steam distillation or dry distillation or by means of a mechanical treatment from one single species" (25th International Symposium on Essential Oils, 1994). This appears to suggest that mixing several different plant species within the production process is not allowed. As an example, the addition of lavandin plants to lavender plants will yield a natural essential oil but not a natural lavender essential oil. Likewise, wild-growing varieties of *Thymus* will not result in a thyme oil as different chemotypes will totally change the composition of the oil. It follows that blending of different chemotypes of the same botanical species is inadmissible as it will change the chemical composition and properties of the final product. However, in view of the global acceptance of some specific essential oils there will be exceptions. For example, Oil of Geranium ISO/DIS 4730, is obtained from *Pelargonium* × ssp., for example, from hybrids of uncertain parentage rather than from a single botanical species (ISO/DIS 4731, 2005). It is a well established and important article of commerce and may, thus, be considered to be an acceptable exception. In reality it is impossible to define "one single species" as many essential oils being found on the market come from different plant species. Even in ISO drafts it is confirmed that various plants are allowed. There are several examples like rosewood oils, distilled from *Aniba rosaedora* and *Aniba parviflora*, two different plant species. The same happens with the oil of gum turpentine from China, where mainly *Pinus massoniana* will be used, beside other *Pinus* species. Eucalyptus provides another example: Oils produced in Portugal have been produced from hybrids such as *Eucalyptus globulus subsp. globulus* × *Eucalyptus globulus subsp. bicostata* and *Eucalyptus globulus subsp. globulus* × *Eucalyptus globulus subsp. Eucalyptus globulus subsp. pseudoglobulus.* These subspecies were observed from various botanists as separate species. The Chinese eucalyptus oils coming from the Sichuan province are derived from *Cinnamomum longipaniculatum*. Oil of Melaleuca (*terpinen-4-ol type*) is produced from *Melaleuca alternifolia* and in smaller amounts also from *Melaleuca linariifolia* and *Melaleuca dissitiflora*. For the future, this definition must be discussed on the level of ISO rules.

Products obtained by other extraction methods, such as solvent extracts, including supercritical carbon dioxide extracts, concretes or pomades, and absolutes as well as resinoids, and oleoresins are *not essential oils* as they do not comply with the earlier mentioned definition. Likewise, products obtained by enzymic treatment of plant material do not meet the requirements of the definition of an essential oil. There exists, though, at least one exception that ought to be mentioned. The well-known "essential oil" of wine yeast, an important flavor and fragrance ingredient, is derived from a microorganism and not from a plant.

In many instances, the commercial terms used to describe perfumery products as essential oils are either wrong or misleading. So-called "artificial essential oils," "nature-identical essential oils," "reconstructed essential oils," and in some cases even "essential oils complying with the constants of pharmacopoeias" are merely synthetic mixtures of perfumery ingredients and have nothing to do with pure and natural essential oils.

Opinions differ as to the historical origins of essential oil production. According to some, China has been the cradle of hydrodistillation while others point to the Indus Culture (Levey, 1959; Zahn, 1979). On the other hand, some reports also credit the Arabs as being the inventors of distillation. Some literature reports suggest that the earliest practical apparatus for water distillation has been dated from the Indus Culture of some 5000 years ago. However, no written documents have been found to substantiate these claims (Levey, 1955; Zahn, 1979). The earliest documented records of a method and apparatus of what appears to be a kind of distillation procedure were published by Levy

from the High Culture of Mesopotamia (Levey, 1959b). He described a kind of cooking pot from Tepe Gaure in northeastern Mesopotamia, which differed from the design of cooking pots of that period. It was made of brown clay, 53 cm in diameter and 48 cm high. Its special feature was a channel between the raised edges. The total volume of the pot was 37 L and that of the channel was 2.1 L. As the pot was only half-filled when in use the process appears to represent a true distillation. While the Arabs appear to be, apart from the existence of the pot discovered in Mesopotamia, the inventors of hydrodistillation, we ought to go back 3000 years B.C.

The archaeological museum of Texila in Pakistan has on exhibit a kind of distillation apparatus made of burnt clay. At first sight, it really has the appearance of a typical distillation apparatus but it is more likely that at that time it was used for the purification of water (Rovesti, 1977). Apart from that the assembly resembles an eighteenth century distillation plant (Figure 4.2). It was again Levy who demonstrated the importance of the distillation culture. Fire was known to be of greatest importance. Initial heating, the intensity of the heat, and its maintenance at a constant level right down to the cooling process were known to be important parameters. The creative ability to produce natural odors points to the fact that the art of distillation was a serious science in ancient Mesopotamia. While the art of distillation had been undergoing improvements right up to the eighth century, it was never mentioned in connection with essential oils, merely with its usefulness for alchemical or medicinal purposes ("Liber servitorius" of Albukasis). In brief, concentration and purification of alcohol appeared to be its main reason for being in existence, its "raison d'être" (Koll and Kowalczyk, 1957).

The Mesopotamian art of distillation had been revived in ancient Egypt as well as being expanded by the expression of citrus oils. The ancient Egyptians improved these processes largely because of their uses in embalming. They also extracted, in addition to myrrh and storax, the exudates of certain East African coastal species of *Boswellia*, none of which are of course essential oils. The thirteenth century Arabian writer Ad-Dimaschki also provided a description of the distillation process, adding descriptions of the production of distilled rose water as well as of the earliest improved cooling systems. It should be understood that the products of these practices were not essential oils in the present accepted sense but merely fragrant distilled water extracts exhibiting the odor of the plant used.

The next important step in the transfer of the practice of distillation to the Occident, from ancient Egypt to the northern hemisphere, was triggered by the crusades of the Middle Ages from the twelfth century onward. Hieronymus Brunschwyk listed in his treatise "The true art to distil" about

FIGURE 4.2 Reconstruction of the distillation plant from Harappa.

25 essential oils produced at that time. Once again one should treat the expression "essential oils" with caution; it would be more accurate to refer to them as "fragrant alcohols" or "aromatic waters." Improvements in the design of equipment led to an enrichment in the diversity of essential oils derived from starting materials such as cinnamon, sandalwood, and also sage and rosemary (Gildemeister and Hoffmann, 1931).

The first evidence capable to discriminate between volatile oils and odorous fatty oils was provided in the sixteenth century. The availability of printed books facilitated "scientists" seeking guidance on the distillation of essential oils. While knowledge of the science of essential oils did not increase during the seventeenth century, the eighteenth century brought about only small progress in the design of equipment and in refinements of the techniques used. The beginning of the nineteenth century brought about progresses in chemistry, including wet analysis, and restarted again, chiefly in France, in an increased development of hydrodistillation methods. Notwithstanding the "industrial" production of lavender already in progress since the mid-eighteenth century, the real breakthrough occurred at the beginning of the following century. While until then the distillation plant was walled in, now the first moveable apparatus appeared. The "Alambique Vial Gattefossé" was easy to transport and placed near the fields. It resulted in improved product quality and reduced the length of transport. These stills were fired with wood or dried plant material. The first swiveling still pots had also been developed which facilitated the emptying of the still residues. These early stills had a capacity of about 50–100 kg of plant material. Later on their capacity increased to 1000–1200 kg. At the same time, cooling methods were also improved. These improvements spread all over the northern hemisphere to Bulgaria, Turkey, Italy, Spain, Portugal, and even to northern Africa. The final chapter in the history of distillation of plant material came about with the invention of the "alembic à bain-marie," technically speaking a double-walled distillation plant. Steam was not only passed through the biomass, but was also used to heat the wall of the still. This new method improved the speed of the distillation as well as the quality of the top notes of the essential oils thus produced.

The history of the expression of essential oils from the epicarp of citrus fruits is not nearly as interesting as that of hydrodistillation. This can be attributed to the fact that these expressed fragrance concentrates were more readily available in antiquity as expression could be effected by implements made of wood or stone. The chief requirement for this method was manpower and that was available in unlimited amount. The growth of the industry led to the invention of new, and to the improvement of existing machinery, but this topic will be dealt with later on.

4.1.3 Production

Before dealing with the basic principles of essential oil production it is important to be aware of the fact that the essential oil we have in our bottles or drums is not necessarily identical with what is present in the plant. It is wishful thinking, apart for some rare exceptions, to consider an "essential oil" to be the "soul" of the plant and thus an exact replica of what is present in the plant. Only expressed oils that have not come into contact with the fruit juice and that have been protected from aerial oxidation may meet the conditions of a true plant essential oil. The chemical composition of distilled essential oils is not the same as that of the contents of the oil cells present in the plant or with the odor of the plants growing in their natural environment. Headspace technology, a unique method allowing the capture of the volatile constituents of oil cells and thus providing additional information about the plant, has made it possible to detect the volatile components of the plant's "aura." One of the best examples is rose oil. A nonprofessional individual examining pure and natural rose oil on a plotter, even in dilution, will not recognize its plant source. The alteration caused by hydrodistillation is remarkable as plant material in contact with steam undergoes many chemical changes. Hot steam contains more energy than, for example, the surface of the still. Human skin that has come into contact with hot steam suffers tremendous injuries while short contact with a metal surface at 100°C results merely in a short burning sensation. Hot steam will decompose many

aldehydes and esters may be formed from acids generated during the vaporization of certain essential oil components. Some water soluble molecules may be lost by solution in the still water, thus altering the fragrance profile of the oil.

Why do so many plants produce essential oils? Certainly neither to regale our nose with pleasant fragrances of rose or lavender, nor to heighten the taste (as taste is mostly related to odor) of ginger, basil, pepper, thyme, or oregano in our food! Nor to cure diseases of the human body or influence human behavior! Most essential oils contain compounds possessing antimicrobial properties, active against viruses, bacteria, and fungi. Often, different parts of the same plant, such as leaves, roots, flowers, and so on may contain volatile oils of different chemical composition. Even the height of a plant may play a role. For example, the volatile oil obtained from the gum of the trunk of *Pinus pinaster* at a height of 2 m will contain mainly pinenes and significant car-3-ene, while oil obtained from the gum collected at a height of 4 m will contain very little or no car-3-ene. The reason for this may be protection from deer that browse the bark during the winter months. Some essential oils may act not only as insect repellents but even prevent their reproduction. In many cases, it has been shown that plants attract insects that in turn assist in pollinating the plant. It has also been shown that some plants communicate through the agency of their essential oils. Sometimes essential oils are considered to be simply metabolic waste products! This may be so in the case of eucalypts as the oil cells present in the mature leaves of *Eucalyptus* species are completely isolated and embedded deeply within the leaf structure. In some cases essential oils act as germination inhibitors thus reducing competition by other plants (Porter, 2001).

Essential oil yields vary widely and are difficult to predict. The highest oil yields are usually associated with balsams and similar resinous plant exudations, such as gurjun, copaiba, elemi, and Peru balsam, where they can reach 30–70%. Clove buds and nutmeg can yield between 15% and 17% of essential oil while other examples worthy of mention are cardamom (about 8%), patchouli (3.5%) and fennel, star anise, caraway seed, and cumin seed (1–9%). Much lower oil yields are obtained with juniper berries, where 75 kg of berries are required to produce 1 kg of oil, sage (about 0.15%), and other leaf oils such as geranium (also about 0.15%). 700 kg of rose petals will yield 1 kg of oil and 1000 kg of bitter orange flowers are required for the production of also just 1 kg of oil. The yields of expressed fruit peel oils, such as bergamot, orange, and lemon vary from 0.2% to about 0.5%.

A number of important agronomic factors have to be considered before embarking on the production of essential oils, such as climate, soil type, influence of drought and water stress and stresses caused by insects and microorganisms, propagation (seed or clones), and cultivation practices. Other important factors include precise knowledge on which part of the biomass is to be used, location of the oil cells within the plant, timing of harvest, method of harvesting, storage, and preparation of the biomass prior to essential oil extraction.

4.1.4 Climate

The most important variables include temperature, number of hours of sunshine, and frequency and magnitude of precipitations. Temperature has a profound effect on the yield and quality of the essential oils, as the following example of lavender will show. The last years in the Provence, too cold at the beginning of growth, were followed by very hot weather and a lack of water. As a result yields decreased by one-third. The relationship between temperature and humidity is an additional important parameter. Humidity coupled with elevated temperatures produces conditions favorable to the proliferation of insect parasites and, most importantly, microorganisms. This sometimes causes plants to increase the production of essential oil for their own protection. Letchamo have studied the relationship between temperature and concentration of daylight on the yield of essential oil and found that the quality of the oil was not influenced (Letchamo et al., 1994). Herbs and spices usually require greater amounts of sunlight. The duration of sunshine in the main areas of herb and spice

cultivation, such as the regions bordering the Mediterranean Sea, usually exceeds 8 h/day. In India, Indonesia and many parts of China this is well in excess of this figure and two or even three crops per year can be achieved. Protection against cooling and heavy winds may be required. Windbreaks provided by rows of trees or bushes and even stone walls are particularly common in southern Europe. In China the *Litsea cubeba* tree is used for the same purpose. In colder countries, the winter snow cover will protect perennials from frost damage. Short periods of frost with temperatures below –10°C will not be too detrimental to plant survival. However, long exposure to heavy frost at very low subzero temperatures will result in permanent damage to the plant ensuing from a lack of water supply.

4.1.5 Soil Quality and Soil Preparation

Every friend of a good wine is aware of the influence of the soil on the grapes and finally on the quality of the wine. The same applies to essential oil-bearing plants. Some crops, such as lavender, thyme, oregano, and clary sage require meager but lime-rich soils. The Jura Chalk of the Haute Provence is destined to produce a good growth of lavender and is the very reason for the good quality and interesting top note of its oils by comparison with lavender oils of Bulgarian origin growing on different soil types (Meunier, 1985). Soil pH affects significantly oil yield and oil quality. Figueiredo et al. found that the pH value "strongly influences the solubility of certain elements in the soil. Iron, zinc, copper and manganese are less soluble in alkaline than in acidic soils because they precipitate as hydroxides at high pH values" (Figueiredo et al., 2005). It is essential that farmers determine the limits of the elemental profile of the soil. Furthermore, the spacing of plantings should ensure adequate supply with essential trace elements and nutrients. Selection of the optimum site coupled with a suitable climate plays an important role as they will provide a guarantee for optimum crop and essential oil quality.

4.1.6 Water Stress and Drought

It is well known to every gardener that lack of water, as well as too much water, can influence the growth of plants and even kill them. The tolerance of the biomass to soil moisture should be determined in order to identify the most appropriate site for the growing of the desired plant. Since fungal growth is caused by excess water, most plants require well-drained soils to prevent their roots from rotting and the plant from being damaged, thus adversely affecting essential oil production. Lack of water, for example, dryness, exerts a similar deleterious influence. Flowers are smaller than normal and yields drop. Extreme drought can kill the whole plant as its foliage dries closing down its entire metabolism.

4.1.7 Insect Stress and Microorganisms

Plants are living organisms capable of interacting with neighbor plants and warning them of any incipient danger from insect attack. These warning signals are the result of rapid changes occurring in their essential oil composition, which are then transferred to their neighbors who in turn transmit this information on to their neighbors forcing them to change their oil composition as well. In this way, the insect will come into contact with a chemically modified plant material, which may not suit its feeding habits thus obliging it to leave and look elsewhere. Microorganisms can also significantly change the essential oil composition as shown in the case of elderflower fragrance. Headspace gas chromatography coupled with mass spectroscopy (GC/MS) has shown that linalool, the main constituent of elderflowers, was transformed by a fungus present in the leaves, into linalool oxide. The larvae of Cécidomye (*Thomasissiana lavandula*) damage the lavender plant with a concomitant reduction of oil quality. Mycoplasmose and the fungus *Armillaria mellex* can affect the whole plantation and totally spoil the quality of the oil.

4.1.8 Location of Oil Cells

As already mentioned, the cells containing essential oils can be situated in various parts of the plant. Two different types of essential oil cells are known, superficial cells, for example, glandular hairs located on the surface of the plant, common in many herbs such as oregano, mint, lavender, and so on, and cells embedded in plant tissue, occurring as isolated cells containing the secretions (as in citrus fruit and eucalyptus leaves), or as layers of cells surrounding intercellular space (canals or secretory cavities), for example, resin canals of pine. Professor Dr. Johannes Novak (Institute of Applied Botany, Veterinary University, Vienna) has shown impressive pictures and pointed out that the chemical composition of essential oils contained in neighboring cells (oil glands) could be variable but that the typical composition of a particular essential oil was largely due to the averaging of the enormous number of individual cells present in the plant (Novak, 2005). It has been noted in a publication entitled "Physiological Aspects of Essential Oil Production" that individual oil glands do not always secrete the same type of compound and that the process of secretion can be different (Kamatou et al., 2006). Different approaches to distillation are dictated by the location of the oil glands. Preparation of the biomass to be distilled, temperature, and steam pressure affect the quality of the oil produced.

4.1.9 Types of Biomass Used

Essential oils can occur in many different parts of the plant. They can be present in flowers (rose, lavender, magnolia, bitter orange, and blue chamomile) and leaves (cinnamon, patchouli, petitgrain, clove, perilla, and laurel); sometimes the whole aerial part of the plant is distilled (*Melissa officinalis*, basil, thyme, rosemary, marjoram, verbena, and peppermint). The so-called fruit oils are often extracted from seed, which forms part of the fruit, such as caraway, coriander, cardamom, pepper, dill, and pimento. Citrus oils are extracted from the epicarp of species of *Citrus*, such as lemon, lime, bergamot, grapefruit, bitter orange as well as sweet orange, mandarine, clementine, and tangerine. Fruit or perhaps more correctly berry oils are obtained from juniper and *Schinus* species. The well-known bark oils are obtained from birch, cascarilla, cassia, cinnamon, and massoia. Oil of mace is obtained from the aril, a fleshy cover of the seed of nutmeg (*Myristica fragrans*). Flower buds are used for the production of clove oil. Wood and bark exudations yield an important group of essential oils such as galbanum, incense, myrrh, mastix, and storax, to name but a few. The needles of conifers (leaves) are a source of an important group of essential oils derived from species of *Abies*, *Pinus*, and so on. Wood oils are derived mostly from species of *Santalum* (sandalwood), cedar, amyris, cade, rosewood, agarwood, and guaiac. Finally, roots and rhizomes are the source of oils of orris, valerian, calamus, and angelica.

What happens when the plant is cut? Does it immediately start to die as happens in animals and humans? The water content of a plant ranges from 50% to over 80%. The cutting of a plant interrupts its supply of water and minerals. Its life-sustaining processes slow down and finally stop altogether. The production of enzymes stops, auto-oxidative processes start, including an increase in bacterial activity leading to rotting and molding. Color and organoleptic properties, such as fragrance, will also change usually to their detriment. As a consequence of this, unless controlled drying or preparation is acceptable options, treatment of the biomass has to be prompt.

4.1.10 Timing of the Harvest

The timing of the harvest of the herbal crop is one of the most important factors affecting the quality of the essential oil. It is a well-documented fact that the chemical composition changes throughout the life of the plant. Occasionally, it can be a matter of days during which the quality of the essential oil reaches its optimum. Knowledge of the precise time of the onset of flowering often has a great influence on the composition of the oil. The chemical changes occurring during the entire

life cycle of Vietnamese *Artemisia vulgaris* have shown that 1,8-cineole and β-pinene contents before flowering were below 10% and 1.2%, respectively, whereas at the end of flowering they reached values above 24% and 10.4% (Nguyen Thi Phuong Thao et al., 2004). These are very large variations indeed occurring during the plant's short life span. In the case of the lavender life cycle, the ester value of the oil is the quality-determining factor. It varies within a wide range and influences the value of the oil. As a rule of thumb, it is held that its maximum value is reached at a time when about two-third of the lavender flowers have opened and thus, that harvesting should commence. In the past, growers knew exactly when to harvest the biomass. These days the use of a combination of microdistillation and gas chromatography (GC) techniques enables rapid testing of the quality of the oil and thus the determination of the optimum time for harvesting to start. Oil yields may in some cases be influenced by the time of harvesting. One of the best examples is rose oil. The petals should be collected in the morning between 6 a.m. and 9 a.m. With rising day temperatures the oil yield will diminish. In the case of oil glands embedded within the leaf structure, such as in the case of eucalypts and pines, oil yield and oil quality are largely unaffected by the time of harvesting.

4.1.11 Agricultural Crop Establishment

The first step is, in most cases, selection of plant seed which suits best the requirements of the product looked for. Preparation of seedbeds, growing from seed, growing and transplanting of seedlings, and so on should follow well-established agricultural practices. The spacing of rows has to be considered (Dey, 2007). For example, dill prefers wider row spacing than anise, coriander, or caraway (Novak, 2005). The time required before a crop can be obtained depends on the species used and can be very variable. Citronella and lemongrass may take 7–9 months from the time of planting before the first crop can be harvested while lavender and lavandin require up to three years. The most economical way to extract an essential oil is to transport the harvested biomass directly to the distillery. For some plants, this is the only practical option. *Melissa officinalis* ("lemon balm") is very prone to drying out and thus to loss of oil yield. Some harvested plant material may require special treatment of the biomass before oil extraction, for example, grinding or chipping, breaking or cutting up into smaller fragments, and sometimes just drying. In some cases, fermentation of the biomass should precede oil extraction. Water contained within the plant material has been classified by Yanive and Palevitch as chemically, physicochemically, and mechanically bound water (Yanive and Palevitch, 1982). According to these authors only the mechanically bound water, which is located on the surface and the capillaries of plants, can be reduced. Drying can be achieved simply by spreading the biomass on the ground where wind movement effects the drying process. Drying can also be carried out by the use of appropriate drying equipment. Drying, too, can affect the quality of the essential oil. Until the middle of the 1980s cut lavender and lavandin have been dried in the field, (Figure 4.3) a process requiring about three days. The resulting oils exhibited the typical fine, floral odor; however, oil yields were inferior to yields obtained with fresh material. Compared with the present day procedure with container harvesting and immediate processing (the so-called "vert-broyé") this quality of the oil is greener, harsher, and requires some time to harmonize. However, yields are better, and one step in the production process has been eliminated. Clary sage is a good example demonstrating the difference between oils distilled from fresh plant material on the one hand and dried plant material on the other. The chemical differences are clearly shown in Table 4.2. Apart from herbal biomass, fruits and seed may also have to be dried before distillation. These include pepper, coriander, cloves, and pimento berries, as well as certain roots such as vetiver, calamus, lovage, and orris. Clary sage is harvested at the beginning of summer but distilled only at the end of the harvesting season.

Seeds and fruits of the families Apiaceae, Piperaceae, and Myristicaceae usually require grinding up prior to steam distillation. In many cases, the seed has to be dried before comminution takes place. Celery, coriander, dill, ambrette, fennel, and anise belong to the Apiaceae. All varieties of pepper belong to the Piperaceae while nutmeg belongs to the Myristicaceae. The finer the material

FIGURE 4.3 **(See color insert following page 468.)** Lavender drying on the field.

is ground, the better will be the oil yield and, owing to shorter distillation times, also the quality of the oil. In order to reduce losses of volatiles by evaporation during the comminution of the seed or fruit, the grinding can also be carried out under water, preferably in a closed apparatus. Heartwood samples, such as those of *Santalum album*, *Santalum spicatum*, and *Santalum austrocaledonicum*

TABLE 4.2
Differences in the Composition of the Essential Oil of Clary Sage Manufactured Fresh and Dried

Component	"Vert Broyee" (%)	Traditional (%)
Myrcene	0.9–1.0	0.9–1.1
Limonene	0.2–0.4	0.3–0.5
Ocimene cis	0.3–0.5	0.4–0.6
Ocimene trans	0.5–0.7	0.8–1.0
Copaene alpha	0.5–0.7	1.4–1.6
Linalool	13.0–24.0	6.5–13.5
Linalyl acetate	56.0–70.5	62.0–78.0
Caryophyllene beta	1.5–1.8	2.5–3.0
Terpineol alpha	1.0–5.0	Max. 2.1
Neryl acetate	0.6–0.8	0.7–1.0
Germacrene d	1.1–7.5	1.5–12
Geranyl acetate	1.4–1.7	2.2–2.5
Geraniol	1.4–1.7	1.2–1.5
Sclareol	0.4–1.8	0.6–2.8

Minor changes
Middle changes
Big changes

have to be reduced to a very fine powder prior to steam distillation in order to achieve complete recovery of the essential oil. In some cases, coarse chipping of the wood is adequate for efficient essential oil extraction. This includes cedarwood, amyris, rosewood, birch, guaiac, linaloe, cade, cabreuva, and the like.

Plant material containing small branches as well as foliage, which includes pine needles, has to be coarsely chopped up prior to steam distillation. Examples of such material are juniper branches, *Melaleuca alternifolia*, *Corymbia citriodora*, *Pinus mugo*, *Pinus pinaster*, *Pinus sylvestris*, *Pinus nigra*, *Abies alba*, *Abies sibirica*, and *Abies grandis*, as well as mint and peppermint. Present day mechanized harvesting methods automatically effect the chopping up of the biomass. This also reduces the volume of the biomass, thus increasing the quantity of material that can be packed into the still and making the process more economical.

It appears that the time when the seed is sown influences both oil yield as well as essential oil composition, for example, whether it is sown in spring or autumn. Important factors affecting production of plant material are application of fertilizers, herbicides, and pesticides as well as the availability and kind of pollination agents. In many essential oil producing countries, no artificial nitrogen, potassium, or phosphorus fertilizers are used. Instead, both in Europe, as well as overseas, the biomass left over after steam distillation is spread in the fields as an organic fertilizer. Court et al. reported, from the field tests conducted with peppermint, that an increase in fertilizer affects plant oil yield. However, higher doses did not result in further increases in oil yield or in changes in the oil composition (Court et al., 1993). Herbicides and pesticides do not appear to influence either oil yield or oil composition. The accumulation of pesticide residues in essential oils has a negative influence on their quality and on their uses. The yield and quality of essential oils are also influenced by the timing and type of pollinating agent. If the flower is ready for pollination the intensity of its fragrance and the amount of volatiles present are at their maximum. If on the other hand the weather is too cold at the time of flowering, pollination will be adversely affected and transformation to fruit is unlikely to take place. Such an occurrence has a very significant effect on the plant's metabolism and finally on its essential oil. Grapevine cultivators use the following trick to attract pollinators to their vines. A rose flower placed at the end of each grapevine row attracts pollinators who then also pollinate the unattractive flowers of the grapevine.

4.1.12 Propagation from Seed and Clones

Plants can be grown from seed or propagated asexually by cloning. Lavender plants raised from seed are kept for one year in pots before transplanting into the field. It then takes another three years before the plantation yields enough flowers for commercial harvesting and steam distillation. Plants of any species raised from seed will exhibit wide genetic variations among the progeny, as exist between the members of any species propagated by sexual means, for example, humans. In the case of lavender (*Lavandula angustifolia*), the composition of the essential oil from individual plants varies from plant to plant or more precisely, from one genotype to the another. Improvement of the crop by selective breeding of those genotypes that yield the most desirable oil is a very slow process requiring years to accomplish. Charles Denny, who initiated the Tasmanian lavender industry in 1921, selected within 11 years 487 genotypes from a source of 2500 genotypes of *L. angustifolia* for closer examination, narrowing them down to just 13 strains exhibiting large yields of superior oil. Finally, four of these genotypes were grown on a large scale and mixed together in what is called "comunelles." The quality of the oils produced was fairly constant from year to year, both in their physicochemical properties as well as in their olfactory characteristics (Denny, 1995, private information to the author).

Cloning is the preferred method for the replication of plants having particular, usually commercially desirable, characteristics. Clones are obtained from buds or cuttings of the same individual and the essential oils, for example, obtained from them are the same, or very similar to those of the parent. Cloning procedures are well established but may vary in their detail among different species. One important advantage of clones is that commercial harvesting may be possible after a shorter

time as compared with plantations grown from seed. One risk does exist though. If the mother plant is diseased all clones will also be affected and the plantation would have to be destroyed.

No field of agriculture requires such a detailed and comprehensive knowledge of botany and soil science, as well as of breeding and propagation methods, harvesting methods, and so on as that of the cultivation of essential oil-bearing plants. The importance of this is evident from the very large amount of scientific research carried out in this field by universities as well as by industry.

4.1.13 Commercial Essential Oil Extraction Methods

There are three methods in use. Expression is probably the oldest of these and is used almost exclusively for the production of Citrus oils. The second method, hydrodistillation or steam distillation, is the most commonly used one of the three methods, while dry distillation is used only rarely in some very special cases.

4.1.14 Expression

Cold expression, for example, expression at ambient temperature without the involvement of extraneous heat, was practiced long before humans discovered the process of distillation, probably because the necessary tools for it were readily available. Stones or wooden tools were well suited to breaking the oil cells and freeing their fragrant contents. This method was used almost exclusively for the production of *Citrus* peel oils. *Citrus* and the allied genus *Fortunella* belong to the large family Rutaceae. *Citrus* fruits used for the production of the oils are shown in Table 4.3. *Citrus* fruit cultivation is widely spread all over the world with a suitable climate. Oils with the largest production include orange, lemon, grapefruit, and mandarin. Taking world lemon production as an example, the most important lemon-growing areas in

TABLE 4.3
Important Essential Oil Production from Plants of the Rutaceae Family

Botanical Term	Expressed	Distilled	Used Plant Parts
Citrus aurantifolia (Christm.) Swingle	Lime oil	Lime oil distilled	Pericarp; fruit juice or crushed fruits
Citrus aurantium L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso	Bitter orange oil	Neroli oil, Bitter orange petitgrain oil	Flower, pericarp, leaf, and twigs with sometimes little green fruits
Citrus bergamia (Risso et Poit.), *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler	Bergamot oil	Bergamot petitgrain oil	Pericarp, leaf, and twigs with sometimes little green fruits
Citrus hystrix DC., syn. *Citrus torosa* Blanco	Kaffir lime oil, Combava	Kaffir leaves oil	Pericarp, leaves
Citrus latifolia Tanaka	Lime oil Persian type		Pericarp
Citrus limon (L.) *Burm. f.*	Lemon oil	Lemon petitgrain oil	Flower, pericarp, leaf, and twigs with sometimes little green fruits
Citrus reticulate Blanco syn. *Citrus nobilis* Andrews	Mandarin oil	Mandarin petitgrain oil	Flower, pericarp, leaf, and twigs with sometimes little green fruits
Citrus sinensis (L.) Osbeck, *Citrus djalonis* A. Chevalier	Sweet orange oil		Pericarp
Citrus × *paradisi* Macfad.	Grapefruit oil		Pericarp

Europe are situated in Italy and Spain with Cyprus and Greece being of much lesser importance. Nearly 90% of all lemon fruit produced originates from Sicily where the exceptionally favorable climate enables an almost around the year production. There is a winter crop from September to April, a spring crop from February to May, and a summer crop from May to September. The Spanish harvest calendar is very similar. Other production areas in the northern hemisphere are in the United States, particularly in Florida, Arizona, and California, and in Mexico. In the southern hemisphere, large-scale lemon producers are Argentina, Uruguay, and Brazil. Lemon production is also being developed in South Africa, Ivory Coast, and Australia. China promises to become a huge producer of lemon in the future.

The reason for extracting citrus oils from fruit peel using mechanical methods is the relative thermal instability of the aldehydes contained in them. Fatty, for example, aliphatic, aldehydes such as heptanal, octanal, nonanal, decanal, and dodecanal are readily oxidized by atmospheric oxygen, which gives rise to the formation of malodorous carboxylic acids. Likewise, terpenoid aldehydes such as neral, geranial, citronellal, and perilla aldehyde as well as the α- and β-sinensals are sensitive to oxidation. Hydrodistillation of citrus fruit yields poor quality oils owing to chemical reactions that can be attributed to heat and acid-initiated degradation of some of the unstable fruit volatiles. Furthermore, some of the terpenic hydrocarbons and esters contained in the peel oils are also sensitive to heat and oxygen. One exception to this does exist. Lime oil of commerce can be either cold pressed or steam distilled. The chemical composition of these two types of oil as well as their odors differs significantly from each other. The expressed citrus peel is normally treated with hot steam in order to recover any essential oil still left over in it. The products of this process, consisting mainly of limonene, are used in the solvent industry. The remaining peel and fruit flesh pulp is used as cattle feed.

The oil cells of citrus fruit are situated just under the surface in the epicarp, also called flavedo, in the colored area of the fruit. Figure 4.4 is a cross section of the different parts of the fruit also showing the juice cells present in the fruit. An essential oil is also present in the juice cells. However, the amount of oil present in the juice cells is very much smaller than the amount present in the flavedo; also their composition differs from each other.

Until the beginning of the twentieth century, industrial production of cold-pressed citrus oils was carried out manually. One has to visualize huge halls with hundreds of workers, men and women, seated on small chairs handling the fruit. First of all, the fruit had to be washed and cut into two halves. The pulp was then removed from the fruit using a sharp-edged spoon, called the "rastrello" and after the peel was soaked in warm water. The fruit peel was now manually turned inside out so that the epicarp was on the inside, squeezed by hand to break the oil glands and the oil soaked up with a sponge. The peel was now turned inside out once again and wiped with the sponge and the

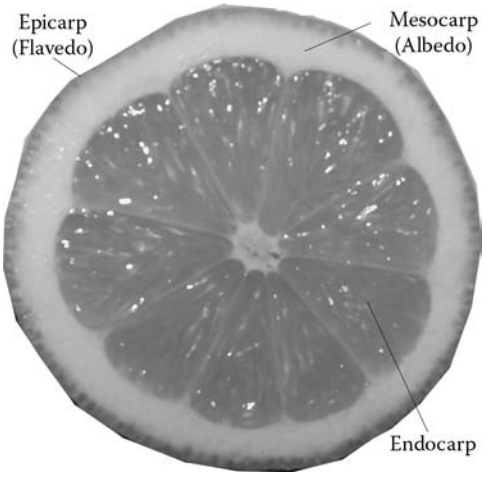

FIGURE 4.4 **(See color insert following page 468.)** Parts of a citrus fruit.

sponge squeezed into a terracotta bowl, the "concolina." After decantation, the oil was collected in metal containers. This was an extremely laborious process characterized by substantial oil losses. A later improvement of the fruit peel expression process was the "scodella" method. The apparatus was a metallic hemisphere lined inside with small spikes, with a tube attached at its center. The fruit placed inside the hemisphere was rotated while being squeezed against the spikes thus breaking the oil cells. The oil emulsion, containing some of the wax coating the fruit, flowed into the central tube was collected, and the oil was subsequently separated by centrifugation.

Neither of these methods, even when used simultaneously, was able to satisfy the increased demand for fruit peel oils at the start of the industrial era. The quantity of fruit processed could be increased but the extraction methods were time wasting and the oil yields too low. With the advent of the twentieth century the first industrial machinery was developed. Today the only systems of significance in use for the industrial production of peel oils can be classified into four categories: "sfumatrici" machines and "speciale sfumatrici," "Pellatrici" machines, "FMC whole fruit process," and "Brown oil extractors (BOEs)" (Arnodou, 1991).

It is important to be aware of the fact that the individual oil glands within the epicarp are not connected to neighboring glands. The cell walls of these oil glands are very tough and it is believed that the oil they contain is either a metabolic waste product or a substance protecting the plant from being browsed by animals.

The machines used in the "sfumatrici" methods consist in principle of two parts, a fixed part and a moveable part. The fruit is cut into two and the flesh is removed. In order to extract the oil, the citrus peel is gently squeezed, by moving it around between the two parts of the device, and rinsing off the squeezed-out oil with a jet of water. The oil readily separates from the liquid on standing and is collected by decantation. Since the epicarp may contain organic acids (citric acid, etc.), it is occasionally soaked in lime solution in order to neutralize the acids present. Greater concentrations of acid could alter the quality of the oil. Degradation of aldehydes is also an important consideration. In the "special sfumatrici" method, the peel is soaked in the lime solution for 24 h before pressing. By means of a metallic chain drawn by horizontal rollers with ribbed forms, the technical process is finished. The oils obtained by these methods may have to be "wintered," for example, refrigerated in order to freeze out the peel waxes that are then filtered off.

In the "Pellatrici" method the peel oil is removed during the first step and the fruit juice in a second step (Figure 4.5). In the first step, the fruit is fed through a slowly turning Archimedean screw-type valve. The screw is covered with numerous spikes that will bruise the oil cells in the epicarp and initiate the flow of oil. The oil is, once again, removed by means of a jet of water. The fruit

FIGURE 4.5 **(See color insert following page 468.)** "Pellatrici method." The spiked Archimedes screw with lemons, washed with water.

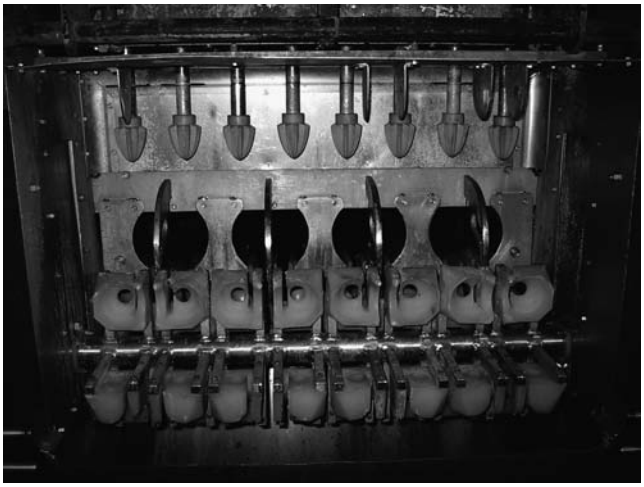

FIGURE 4.6 **(See color insert following page 468.)** "Brown" process. A battery of eight juice squeezers waiting for fruits.

is finally carried to a fast-rotating, spiked, roller carpet where the remaining oil cells, located deeper within the epicarp, are bruised and their oil content recovered, thus resulting in maximum oil yield. The process involves centrifugation, filtration, and "wintering" as previously mentioned.

The "Brown process" (Reeve, 2005) is used mainly in the United States and in South America, but less in Europe. The BOE (Figure 4.6) is somewhat similar to the machinery of the "Pellatrici" method. A device at the front end controls the quantity of fruit entering the machine. The machine itself consists of numerous pairs of spiked rollers turning in the same direction, as well as moving horizontally, thus reaching all oil cells. The spiked rollers as well as the fruit are submerged in water for easy transport. Any residual water and oil adhering to the fruit are removed by a special system of rollers and added to the oil emulsion generated on the first set of rollers. Any solid particles are then removed by passing it through a fine sieve. The emulsion is then centrifuged and the aqueous phase recycled. The BOE is manufactured in V4A steel to avoid contact with iron.

The most frequently used type of extractor is the food machinery corporation (FMC)-in-Line. It is assumed that in the United States more than 50% of extractors are of the FMC type (Figure 4.7). Other large producer countries, such as Brazil and Argentina use exclusively FMC extractors. The

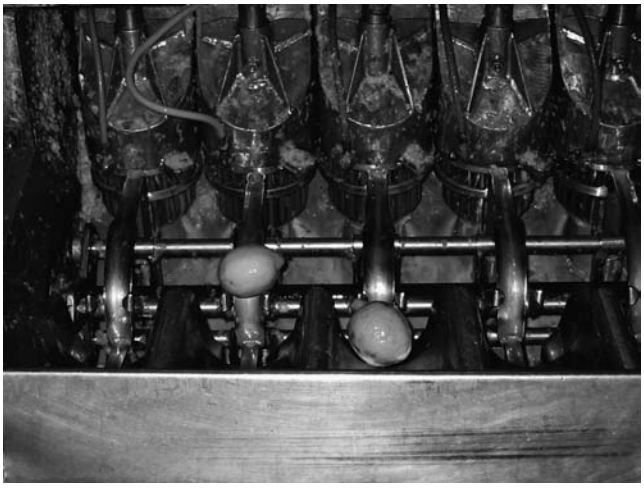

FIGURE 4.7 **(See color insert following page 468.)** FMC extractor.

reason for this is the design of the machinery, as fruit juice and oil are produced in one step without the two coming into contact with each other. The process requires prior grading of the fruit as the cups used in this process are designed for different sizes of fruit. An optimum fruit size is important as bigger fruit would be over squeezed and some essential oil carried over into the juice making it bitter. On the other hand if the fruit were too small the yield of juice would be reduced. Different frame sizes allow treating 3, 5, or 8 fruits at the same time. This technique was revolutionary in its concept and works as follows: the fruit is carried to, and placed into, a fixed cup. Another cup, bearing a mirror image relationship to the fixed cup, is positioned exactly above it. Both cups are built of intermeshing jaws. The moveable cup is lowered toward the fixed cup thus enclosing the fruit. At the same time, a circular knife cuts a hole into the bottom of the fruit. When pressure is applied to the fruit the expressed juice will exit through the cut hole on to a mesh screen and be transported to the juice manifold while at the same time the oil is squeezed out of the surface of the peel. As before the oil is collected using a jet of water. The oil–water emulsion is then separated by centrifugation.

An examination of the developments in the design of citrus fruit processing machinery shows quite clearly that the quality of the juice was more important than the quality of the oil, the only exception being oil of bergamot. Nevertheless, oil quality improved during the last decades and complies with the requirements of ISO Standards. The expressed pulp of the more valuable kind fruit is very often treated with high-pressure steam to recover additional amounts of colorless oils of variable composition. The kinds of fruit treated in this manner are bergamot, lemon, and mandarin.

4.1.15 Steam Distillation

Steam or water distillation is unquestionably the most frequently used method for the extraction of essential oil from plants. The already earlier mentioned history of steam distillation and the long-standing interest of mankind in extracting the fragrant and useful volatile constituents of plants testify to this. Distillation plants of varying design abound all over the world. While in some developing countries traditional and sometimes rather primitive methods are still being used (Figure 4.8), the essential oils produced are often of high quality. Industrialized countries employ technologically more evolved and complex equipment, computer aided with in-process analysis of the final product. Both of these very different ways of commercial essential oil production provide excellent quality oils. One depends on skill and experience, the other on superior technology and expensive equipment. It should be borne in mind that advice by an expert on distillation is a prerequisite for the production of superior quality oils. The term "distillation" is derived from the Latin "distillare" which means "trickling down." In its simplest form distillation is defined as "evaporation and subsequent condensation of a liquid." All liquids evaporate to a greater or lesser degree, even at room temperature. This is due to thermally induced molecular movements within the liquid resulting in some of the molecules being ejected into the airspace above them (diffusing into the air). As the temperature is increased these movements increase as well, resulting in more molecules being ejected, for example, in increased evaporation. The definition of an essential oil, ISO 9235, item 3.1.1 is: "… product obtained from vegetable raw material—either by distillation with water or steam" and in item 3.1.2: "… obtained with or without added water in the still" (ISO/DIS 9235.2, 1997, p. 2). This means that even "cooking" in the presence of water represents a method suitable for the production of essential oils. The release of the essential oil present in the oil glands (cells) of a plant is due to the bursting of the oil cell walls caused by the increased pressure of the heat-induced expansion of the oil cell contents. The steam flow acts as the carrier of the essential oil molecules. The basic principle of either water or steam distillation is a limit value of a liquid–liquid–vapor system. The theory of hydrodistillation is the following. Two nonmiscible liquids (in our case water and essential oil) A and B form two separate phases. The total vapor pressure of that system is equal to the sum of the partial vapor pressures of the two pure liquids:

$$\rho = \rho_A + \rho_B \quad (\rho \text{ is the total vapor pressure of the system}).$$

FIGURE 4.8 Bush distillation device, opened.

With complete nonmiscibility of both liquids, ρ is independent of the composition of the liquid phase. The boiling temperature of the mixture (T_M) lies below the boiling temperatures (T_A and T_B) of liquids A and B. The proportionality between the quantity of each component and the pressure in the vapor phase is given in the formula

$$\frac{N_{oil}}{N_{water}} = \frac{P_{oil}}{P_{water}}$$

where N_{oil} stands for the number of moles of the oil in the vapor phase and N_{water} the number of moles of water in the vapor phase. It is nearly impossible to calculate the proportions as an essential oil is a multicomponent mixture of variable composition.

The simplest method of essential oil extraction is by means of hydrodistillation, for example, by immersion of the biomass in boiling water. The plant material soaks up water during the boiling process and the oil contained in the oil cells diffuses through the cell walls by means of osmosis. Once the oil has diffused out of the oil cells, it is vaporized and carried away by the stream of steam. The volatility of the oil constituents is not influenced by the rate of vaporization but does depend on the degree of their solubility in water. As a result, the more water-soluble essential components will distil over before the more volatile but less water-soluble ones. The usefulness of hydrodiffusion can be demonstrated by reference to rose oil. It is well known that occasionally some of the essential oil constituents are not present as such in the plant but are artifacts of the extraction process. They can be products of either enzymic splitting or chemical degradation, occurring during the steam distillation, of high-molecular-weight and thus nonvolatile compounds present in the plants. These compounds are often glycosides. The main constituents of rose oil, citronellol, geraniol, and nerol are products of a fermentation that takes place during the water-distillation process.

Hydrolysis of esters to alcohols and acids can occur during steam distillation. This can have serious implications in the case of ester-rich oils and special precautions have to be taken to prevent or at least to limit the extent of ester degradation. The most important examples of this are lavender or lavandin oils rich in linalyl acetate and cardamom oil rich in α-terpinyl acetate. Chamazulene, a blue bicyclic sesquiterpene, present in the steam-distilled oil of German chamomile, *Chamomilla recutita* (L.) Rauschert, flower heads is an artifact resulting from matricin by a complex series of chemical reactions: dehydrogenation, dehydration, and ester hydrolysis. As chamazulene is not a particularly stable compound, the deep blue color of the oil can change to green and even yellow on aging.

The design of a water/steam distillation plant at its simplest, sometimes called "false bottom apparatus," is as follows: a still pot (a mild steel drum or similar vessel) is fitted with a perforated metal plate or grate, fixed above the intended level of the water, and a lid with a goose neck outlet. The lid has to be equipped with a gasket or a water seal to prevent steam leaks. The steam outlet is attached to a condenser, for example, a serpentine placed in a drum containing cold water. An oil collector (Florentine flask) placed at the bottom end of the serpentine separates the oil from the distilled water (Figure 4.9). The whole assembly is fixed on a brick fireplace. A separate water inlet is often provided to compensate for water used up during the process. The biomass is placed inside the still pot above the perforated metal plate and sufficient biomass should be used to completely fill the still pot. The fuel used is firewood. This kind of distillation plant was extensively used at the end of the nineteenth century, mainly for field distillations. A disadvantage of this system was that in some cases excessive heat imparted a burnt smell to the oils. Furthermore, when the water level in the still dropped too much the, plant material could get scorched. Till today there is a necessity to clean the distillation vessel after two cycles with water to avoid burning notes in the essential oil. In any case, the quality of oils obtained in this type of apparatus was very variable and varied with each distillation. A huge improvement to this process was the introduction of steam generated externally. The early steam generators were very large and unwieldy and the distillation plant could no longer be transported in the field. The biomass had now to be transported to the distillation plant, unlike with the original type of distillation plant. Originally, the generator was fuelled with dry,

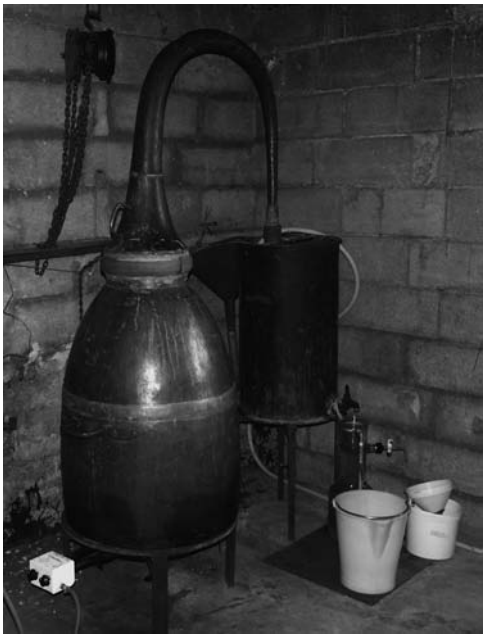

FIGURE 4.9 Old distillation apparatus modernized by electric heating.

extracted biomass. Today gas or fuel oil is used. The delivery of steam can be carried out in various ways. Most commonly, the steam is led directly into the still through its bottom. Overheating is thus avoided and the biomass is heated rapidly. It also allows regulation of steam quantity and pressure and reduces distillation time and improves oil quality. In another method, the steam is injected in a spiraling motion. This method is more effective as the steam comes into contact with a greater surface of the biomass. The velocity of steam throughput and the duration of the distillation depend on the nature of the biomass. It can vary from 100 kg/h in the case of seed and fruits to 400 kg/h for clary sage. The duration of the distillation can vary from about 20 min for Lavender flowers (Denny, 1995, personal communication) to 700 min for dried Angelica root. The values quoted are for a 4 m^3 still pot (Omigbaigi, 2005). Specialists on distillation found as formula that distillation can be stopped when the ratio of oil to water coming from condenser will achieve 1:40. In all cases of hydrodistillation, the distillation water is recovered and reused for steam generation. In a cohobation, the aqueous phase of the distillate is continuously reintroduced into the still pot. In this method, any essential oil constituents emulsified or dissolved in the water are captured, thus increasing total oil yield. There is one important exception: In the case of rose oil the distillation water is collected and redistilled *separately* in a second step. The "floral water" contains increased amounts of β-phenylethyl alcohol, up to 15%, whereas its maximum permissible content in rose oil is 3%. The reason for this is its significant solubility in water, ca. 2%.

The distillation of rose oil is an art in itself as not only quality but quantity as well play an important role. It takes two distillation cycles to produce between 200 and 280 g of rose oil. Jean-François Arnodou describes its manufacture as follows (Arnodou, 1991): The still pot is loaded with 400 kg of rose petals and 1600 L of water. The contents are heated until they boil and steam-distilled. Approximately, the quantity of flowers used is then distilled. That action will last about 2–3 h. Specially designed condensers are required in order to obtain a good quality. The condensing system comprises a tubular condenser followed by a second cooler to allow the oil to separate. The oil is collected in Florentine-type oil separators. About 300 L of the oil-saturated still waters are then redistilled in a separate still in order to recover most of the oil contained in them. Both oils are mixed together and constitute the rose oil of commerce. BIOLANDES described in 1991 the whole process, which uses a microprocessor to manage parameters such as pressure and temperature, regulated by servo-controlled pneumatic valves.

A modern distillation plant consists of the biomass container (still pot), a cooling system (condenser), an oil separator, and a high-capacity steam generator. The kettle (still pot) looks like a cylindrical vertical storage tank with steam pipes located at the bottom of the still. Perforated sieve-like plates are often used to separate the plant charge and prevent compaction, thus allowing the steam unimpeded access to the biomass. The outlet for the oil-laden steam is usually incorporated into the design of the usually hemispherical, hinged still pot lid. The steam is then passed through the cooling system, either a plate heat exchanger or a surface heat exchanger, such as a cold-water condenser. The usually liquid condensate is separated into essential oil and distillation water in an appropriate oil separator such as a Florentine flask. The distillation water may, in some cases, be redistilled and any essential oil recovered dried and stored. Figure 4.10 shows a cross section of such a still.

The following illustrations show different parts of an essential oil production plant. Figure 4.11 shows a battery of four production units in the factory. Each still has a capacity of 3000–5000 L. Owing to their large size, the upper half of the stills is on the level as shown while the lower half is situated on the lower level. Figure 4.12 shows open stills and displays the steam/oil vapor outlets on the underside of the lids leading to the cooling units. On the right side of the illustration, one can see the perforated plate used to prevent clumping of the biomass. Several such perforated plates, up to 12, depending on the type of biomass, are used to prevent clumping. Spacers on the central upright control the optimum distance between these plates for improved steam penetration. Figure 4.13 shows the unloading of the still. Unloading is much faster than the loading process where the biomass is compacted either manually or by means of tractor wheel (Figure 4.14). This type of loading is called "open mouth" loading. Figure 4.15 shows the cooling unit. The cold water enters the tank equipped with a coil condenser. The cooling water is recycled so that no water is wasted. The two main types of industrially used condensers

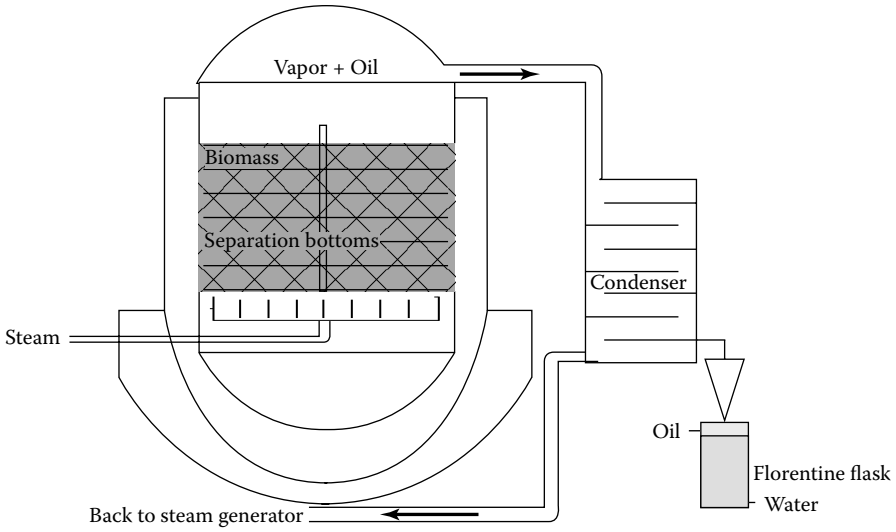

FIGURE 4.10 Cross section of a hydrodistillation plant.

are the following. The earliest was the coil condenser that consisted of a coiled tube fixed in an open vessel of cold water with cold water entering the tank from the bottom and leaving at the top. The oil-rich steam is passed through the coils of the condenser from the top end. The second type of condenser is the pipe bundle condenser where the steam is passed through several vertical tubes immersed in a cold water tank. The tubes have on the inside walls horizontal protuberances that slow down the rate of the steam flow and thus result in more effective cooling. Figure 4.16 shows the inside of a Florentine flask where the oil is separated from the water. Most essential oils are lighter than water and thus float on top of the water. Some essential oils have a specific gravity >1, for example, they are heavier than water thus collecting at the bottom of the collection vessel. A modified design of the Florentine flask for such oils is shown in Figure 4.17. Figure 4.18 shows oil in the presence of turbid distillation water. The liquid phase is contaminated with biomass matter and the oil has to be filtered. The capacity of the still pot depends on the biomass. Weights vary from 150 to 650 kg/m^3. Wilted and dried plants are much lighter than seeds and fruits or dried roots that can be very heavy.

FIGURE 4.11 Battery of four distillation units.

FIGURE 4.12 Open kettle with opening for vapor and oil.

A very special case is the production of the essential oils of Ylang-Ylang from the fresh flowers of *Cananga odorata* (Lam.) Hook. f. et Thomson forma *genuina*. The flowers are water distilled, stopped, and restarted again. In this manner, a total of four fractions is obtained. The chemical composition of the first fraction is characterized by a high concentration of *p*-cresol methylether, methyl benzoate, benzylacetate, linalool, and *E*-cinnamyl acetate. The second fraction contains less f those volatiles but an increased amount of geraniol, geranyl acetate, and β-caryophyllene. The third fraction contains higher boiling substances such as germacrene-D, (*E,E*)-α-farnesene, (*E,E*)-farnesole, benzyl benzoate, (*E,E*)-farnesyl acetate, and benzyl salicylate. Of course, smaller quantities of the lower boiling

FIGURE 4.13 Unloading a kettle.

FIGURE 4.14 Loading a kettle and pressing by concreted tractor wheel.

components are also present. This kind of fractionation has been practiced for a long time. At the same time, the whole oil, obtained by a single distillation is available as "Ylang complete." This serves as an example of the importance the duration of the distillation can have on the quality of the oil.

Raw materials occurring in the form of hard grains have to be comminuted, for example, ground up before water distillation. This is carried out in the presence of water, such as in a wet-grinding turbine, and the water is used later during the distillation. The stills themselves are equipped with blade stirrers ensuring thorough mixing and particularly dislodging oil particles or biomass articles sticking to the walls of the still, the consequence of which can be burning and burnt notes. Dry grinding is likely to result in a significant loss of volatiles. Pepper, coriander, cardamom, celery seed, and angelica seed as well as roots, cumin, caraway, and many other seeds and fruit are treated in this manner. The process used in all these cases is called "turbo distillation." The ratio oil/condensate is

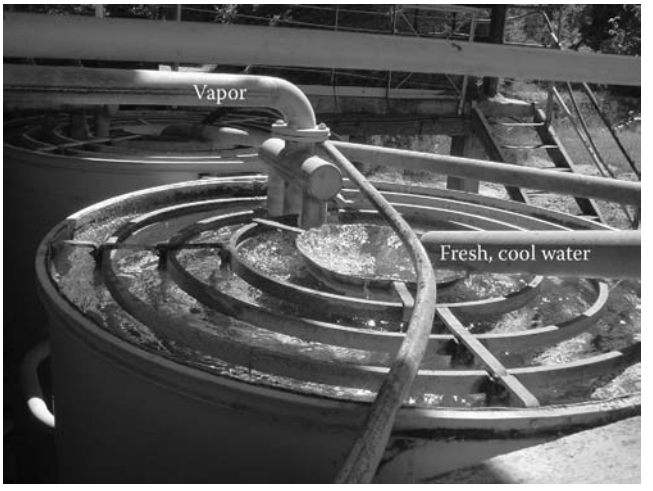

FIGURE 4.15 Cooling unit.

FIGURE 4.16 Inner part of a Florentine flask.

very low when this method is used and it is for that reason that turbo distillation uses a fractionating column to enrich the volatiles. This also assists in preventing small particles of biomass passing into the condenser and contaminating the oil. As in many other distillation and rectification units, cold traps are installed to capture any very volatile oil constituents that may be present. This water-distillation procedure is also used for gums such as myrrh, olibanum, opopanax, and benzoin.

Orris roots are also extracted by water distillation. However, in this case, the distillation has to be carried out under conditions of slightly elevated pressure. This is achieved by means of a reflux column filled with Raschig rings. This is important as the desired constituents, the irones, exhibit very high boiling points. It is noteworthy that in this case there is no cooling of the vapors, as not only the irones but also the long-chain hydrocarbons will immediately be transported to the top of the column. Figure 4.19 shows a Florentine flask with the condensed oil/water emerging at a temperature of nearly 98°C. Orris oil or orris butter (note that the term orris "concrete" is incorrect, as the process is not a solvent extraction) is one of the few essential oils that are, at least partly, solid at room temperature. Depending on its *trans*-anethole content rectified star anise oil is another example of this nature.

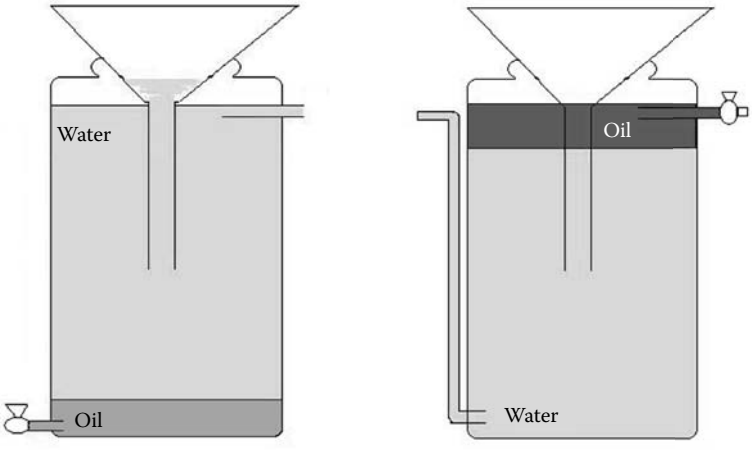

FIGURE 4.17 Two varieties of Florentine flasks.

FIGURE 4.18 **(See color insert following page 468.)** Oil and muddy water in the Florentine flask.

A relatively new technique that saves time in loading and unloading of the biomass is the "on-site" or "container" distillation. The technique is very simple as the container that is used to pick up the biomass and transport it to the distillery serves itself as the still pot. The first plant crops treated in this way were peppermint and mint, clary sage, lavandin grosso, *L. angustifolia*, *Eucalyptus polybractea*, and tea tree. In its simplest form, the mobile still assembly is composed of the following components: A tractor is coupled to an agricultural harvester that cuts the plant material and delivers it directly into the still pot (or vat) via a chute. The still pot (vat) is permanently fixed onto a trailer that is coupled to the harvester. Once the still pot is completely filled, it is towed by the tractor into the factory where it is uncoupled and attached to the steam supply and condenser and

FIGURE 4.19 Orris distillation, Florentine flask at nearly 98°C.

distillation commences. Presupposition for a proper working of the container as vat is a perfect insulation. Every loss of steam and heat will guide to worse quality and diminished quantity. Lids will have to be placed properly and fixed by clamps. The tractor and harvester are attached to an empty still pot and the process is repeated. The design, shape, and size of the still pot as well as the type of agricultural harvester depend on the type of plant crop, the size of the plantation, the terrain, and so on. The extracted biomass can be used as mulch or, after drying, as fuel for the steam boiler. The unloading is automated using metal chains running over the tubes with steam valves. This method requires less manpower and thus reduces labor costs. Loading and unloading costs are minimal. It lends itself best to fresh biomass, lavender and lavandin, mallee eucalypts, tea tree, and so on. It may not be as useful for the harvesting and distillation of mint and peppermint as these crops have to be wilted before oil extraction. Figures 4.20 through 4.22 show the harvesting of mallee eucalyptus, containers in processing, and the whole site of container distillation. Figure 4.23 gives a view into the interior of a container.

Another interesting distillation method has been developed by the LBP Freising, Bavaria, Germany. The plant consists of two tubes, each 2 m long and 25 cm in diameter, open at the top. The tubes are attached vertically to a central axis that can be rotated. One tube is connected, hydraulically or mechanically, to the steam generator and on top to a condenser. During the distillation of the contents of the tube, which may take 25–40 min depending on the biomass, the other tube can be loaded. When the distillation of the first tube has been completed the tubes are rotated around the axis and distillation of the second tube commenced. The first distilled tube can now be emptied and reloaded. The only disadvantage of this type of apparatus is the small size. Only 8.5–21 kg of biomass can be treated. This system has been developed for farmers intending to produce small quantities of essential oil. The apparatus is transportable on a truck and will work satisfactorily provided a supply of power is available.

Most commercially utilized essential oil distillation methods, excepting the mobile still on-site methods, suffer from high labor costs. Apart from harvesting the biomass, 3–4 laborers will be required to load and unload the distillation pots, regulating steam pressure and temperature, and so on. The loading and distribution of the biomass in the distillation vessel may not be homogeneous. This will adversely affect the steam flow through the biomass by channeling, for example, the steam passing through less compacted areas and thus not reaching other more compacted areas. This will result in lower oil yields and perhaps even alter the composition of the oil. In times of high energy

FIGURE 4.20 Harvesting blue mallee with distillation container.

FIGURE 4.21 A battery of containers to be distilled, one opened to show the biomass.

costs the need for consequent recovery has to be considered. Given the demand for greater quantities of essential oils, the question is how to achieve it and at the same time improve the quality of the oils. For this, several considerations have to be taken into account. The first is how to process large quantities of biomass in a given time? Manpower has to be decreased as it still is the most important factor affecting costs. The biomass as a whole has to be treated uniformly to ensure higher oil yields and more constant and thus better oil composition. How can energy costs and water requirements be reduced in an ecologically acceptable way? The answer to this was the development of continuous distillation during the last years of the twentieth century. Until then all distillation processes were discontinuous. Stills had to be loaded, the distillation stopped, and stills unloaded. The idea was to develop a process where the steam production was continuous with permanent unchanged parameters. This was achieved by the introduction of an endless screw that fed the plant material slowly into the still pot from the top and removed the exhausted plant material from the bottom at the same speed. The plant material moves against the flow of dry steam entering the still from the bottom. In this fashion, all of the biomass comes into contact with the steam ensuring optimum essential oil extraction.

FIGURE 4.22 Distillation plant with container technique.

FIGURE 4.23 View inside the distillation container, showing the steam tubes and the metallic grid.

The earliest of these methods is known as the "Padova System." It consists of a still pot 6 m high and about 1.6 m in diameter (Arnodou, 1991). Its total volume is about 8 m^3. The feeding of the still with the plant biomass as well as its subsequent removal is a continuous process. The plant material is delivered via a feed hopper situated at the top of the still. Before entering the still, it is compressed and cut by a rotating knife to ensure a more uniform size. Finally, a horizontal moving cone regulates the quantity of biomass entering the still. The biomass that enters the still moves in the opposite direction to that of the steam. The steam saturated with essential oil vapors is then passed into the cooling system. The exhausted plant material is simultaneously removed by means of an Archimedes screw. This type of plant was originally designed for the distillation of wine residues. A different system is provided by the distillation chimie fine (DCF) aroma process continuous distillation. Once again the plant material is delivered via a hopper to several interconnected tubes. These tubes are slightly inclined and connected to each other. The biomass is carried slowly through the tubes, by means of a worm screw, in a downward direction. Steam is injected at the end of the last tube and is directed upward in the opposite direction to that of the movement of the plant material. The essential oil-laden steam is deflected near the point of entrance of the biomass, into the condenser. The exhausted plant material is unloaded by another worm screw located near the point of the steam entrance to the system.

Texarome, a big producer of cedarwood oil and related products holds a patent on another continuous distillation system. In contrast to other systems, the biomass is conveyed pneumatically within the system. It is a novel system spiked with new technology of that time. Texan cedarwood oil is produced from the whole tree, branches, roots, and stumps. Cedarwood used in Virginia uses exclusively branches, stumps, saw dust, and other waste for oil production; wood is used mainly for furniture making. The wood is passed through a chipper and then through a hammer mill. The dust is collected by means of a cyclone. Any coarse dust is reground to the desired size. The dust is now carried via a plug feeder to the first contactor where superheated steam in reverse flow

exhausts it in a first step and following that in a similar second step at the next contactor. The steam and oil vapors are carried into a condenser. The liquid distillate is then separated in Florentine flasks. This process does all transport entirely by pneumatic means. The recycling of cooling water and the use of the dried plant matter as a fuel contribute to environmental requirements (Arnodou, 1991). In the 1990s, the BIOLANDES Company designed its own system of continuous distillation. The reason for this was BIOLANDES' engagement in the forests of south-western France. Between Bordeaux and Biarritz exists the most important area of pine trees (*Pinus pinaster* Sol.) supplying the paper industry. Twigs and needles have been burnt or left to rot to assist with reforestation with new trees. These needles contain a fine essential oil very similar to that of the dwarf pine oil (*Pinus mugo* Turra.). Compared to other needle oils dwarf pine oil is very expensive and greatly appreciated. The oil was produced by a discontinuous distillation but as demand rose, new and improved methods were required. First of all, the collection of the branches had to be improved. A tractor equipped with a crude grinder and a ground wood storage box follows the wood and branch cutters and transports it to the nearby distillation unit where the biomass is exhausted via a continuous distillation process. In contrast to the earlier described methods the BIOLANDES continuous distillation process operates somewhat differently. The plant material is carried by mechanical means from the storage to the fine cutter and via an Archimedes screw to the top of the distillation pot. The plant material is now compressed by another vertical screw and transported into a chamber which is then hermetically closed on its back but opening at the front. Biomass is falling down allowing the countercurrent passage of hot steam through it. The steam is supplied through numerous nozzles. Endless screws at the bottom of the still continuously dispose of the exhausted biomass. Oil-laden steam is channeled from the top of the still into condenser and then the oil separator.

It is well known that clary sage yields an essential oil on hydrodistillation. However, a very important component of this oil, sclareole, is usually recovered in only very small quantity when this method is used, the reason for this being its very high boiling point. Sclareole can be recovered in very high yield and quality by extraction with volatile solvents. Consequently, BIOLANDES has incorporated an extraction step in its system (Figure 4.24). Any waste biomass, whether of extracted or nonextracted material, is used for energy production or, mostly, for composting. The energy recovery management distinguishes this system from all other earlier described processes. In all of the latter,

FIGURE 4.24 Scheme of the BIOLANDES continuous production unit. A: biomass; B: distillation vat; C: condenser; D: Florentine flask; E: extraction unit; F: solvent recovery; G: exhausted biomass.

large amounts of cold water are required to condense the essential oil-laden steam. This results in significant wastage of water as well as in latent energy losses. The BIOLANDES system recovers this latent heat. Hot water from the condenser is carried into an aerodynamic radiator. Air used as the transfer gas takes up the energy of the hot water, cooling it down, so that it can be recycled to the condenser. The hot air is then used to dry about one-third of the biomass waste that is used as an energy source for steam and even electricity production. In other words, this system is energetically self-sufficient. Furthermore, since it is fully automated, it results in constant quality products. A unit comprising two stills of 7.5 m^3 capacity can treat per hour 3 ton of pine needles, 1.5 ton of juniper branches, and 0.25 ton of cistus branches (Arnodou, 1991). The advantages are once again short processing time of large amounts of biomass, reduced labor costs, and near complete energy sufficiency. All operations are automated and water consumption is reduced to a minimum. The system can also operate under slight pressure thus improving the recovery of higher boiling oil constituents.

The following is a controversial method for essential oil extraction by comparison with classical hydrodistillation methods. In this method, the steam enters the distillation chamber from the top passes through the biomass in the still pot (e.g., the distillation chamber) and percolates into the condenser located below it. Separation of the oil from the aqueous phase occurs in a battery of Florentine flasks. It is claimed that this method is very gentle and thus suitable for the treatment of sensitive plants. The biomass is held in the still chamber (e.g., still pot) on a grid that allows easy disposal of the spent plant matter at the completion of the distillation. The whole apparatus is relatively small, distillation times are reduced, and there is less chance of the oil being overheated. It appears that this method is fairly costly and thus likely to be used only for very high-priced biomass.

Recently, microwave-assisted hydrodistillation methods have been developed, so far mainly in the laboratory or only for small-scale projects. Glass vessels filled with biomass, mainly herbs and fruits or seeds, are heated by microwave power. By controlling the temperature at the center of the vessel, dry heat conditions are established at about 100°C. As the plant material contains enough water, the volatiles are evaporated together with the steam solely generated by the microwave heat and can be collected in a suitably designed condenser/cooling system. In this case, changes in the composition of the oil will be less pronounced than in oil obtained by conventional hydrodistillation. This method has attracted interest owing to the mild heat to which the plant matter is exposed. Kosar reported improvements in the quality of microwave extracted fennel oil due to increases in the yields of its oxygenated components (Kosar et al., 2007).

Very different products can result from the dry distillation of plant matter. ISO Standard 9235 specifies in Section 3.1.4 that products of dry distillation, for example, "… obtained by distillation without added water or steam" are in fact essential oils (ISO/DIS 9235.2, 1997, p. 2). Dry distillation involves heating in the absence of aerial oxygen, normally in a closed vessel, preventing combustion. The plant material is thus decomposed to new chemical substances. Birch tar from the wood exsudate of *Betula pendula* Roth. and cade oil from the wood of *Juniperus oxycedrus* L. are manufactured in this way. Both oils contain phenols, some of which are recognized carcinogens. For this reason, the production of these two oils is no longer of any commercial importance, though very highly rectified and almost phenol-free cade oils do exist.

Some essential oils require rectification. This involves redistillation of the crude oil in order to remove certain undesirable impurities, such as very small amounts of constituents of very low volatility, carried over during the steam or water distillation (such as high-molecular-weight phenols, leaf wax components, etc.) as well as small amounts of very volatile compounds exhibiting an undesirable odor, and thus affecting the top note of the oils, such as sulfur compounds (dimethyl sulfide present in crude peppermint oil), isovaleric aldehyde (present in *E. globulus* oil), and certain nitrogenous compounds (low-boiling amines, etc.). In some cases, rectification can also be used to enrich the essential oil in a particular component such as 1,8-cineole in low-grade eucalyptus oil. Rectification is usually carried out by redistillation under vacuum to avoid overheating and thus

partial decomposition of the oil's constituents. It can also be carried out by steaming. Commonly rectified oils include eucalyptus, clove, mint, turpentine, peppermint, and patchouli. In the case of patchouli and clove oils, rectification improves their, often unacceptably, dark color.

Fractionation of essential oils on a commercial scale is carried out in order to isolate fractions containing a particular compound in very major proportions and occasionally even individual essential oil constituents in a pure state. In order to achieve the required separation, fractionations are conducted under reduced pressure (e.g., under vacuum) to prevent thermal decomposition of the oil constituents, using efficient fractionating columns. A number of different types of fractionating columns are known but the one most commonly used in laboratory stills or small commercial stills is a glass or stainless steel column filled with Raschig rings. Raschig rings are short, narrow diameter, rings made of glass, or any other chemically inert material. Examples of compounds produced on a commercial scale are citral (a mixture of geranial and neral) from *Litsea cubeba*, 1,8-cineole from eucalyptus oil (mainly *Eucalyptus polybractea* and other cineole-rich species) as well as from *Cinnamomum camphora* oil, eugenol from clove leaf oil, α-pinene from turpentine, citronellal from citronella oil, linalool from Ho-oil, geraniol from palmarosa oil, and so on. A small-scale high vacuum plant used for citral production is shown in Figure 4.25. The reflux ratio, for example, the amount of distillate collected and the amount of distillate returned to the still, controls equilibrium conditions of the vapors near the top of the fractionation column, which are essential for good separation of the oil constituents.

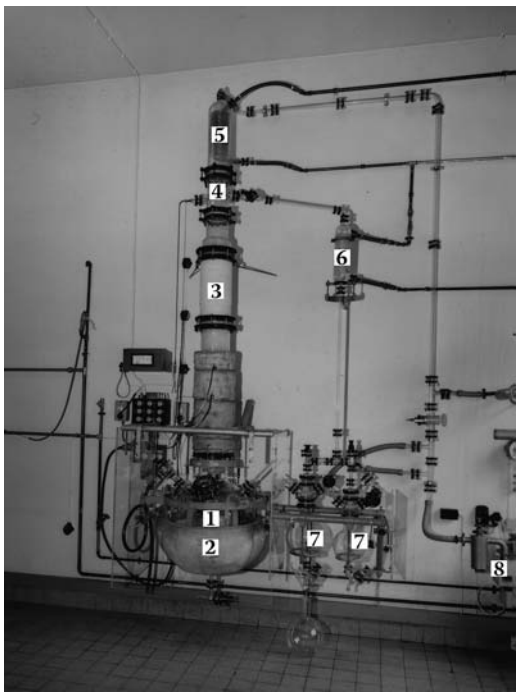

FIGURE 4.25 High vacuum rectification plant in small scale. The distillation assembly is composed of a distillation vessel (1) of glass, placed in an electric heating collar (2). The vessel is surmounted by a jacketed fractionation column (3), packed with glass spirals or Raschig rings, of such a height as to achieve maximum efficiency (e.g., have the maximum number of theoretical plates capable of being achieved for this type of apparatus). The reflux ratio is automatically regulated by a device (4), which also includes the head condenser (5), a glass tube leads the product to another condenser (6), from there to the both receivers (7). The vacuum pump unit is placed on the right (8).

Apart from employing fractional distillation, with or without the application of a vacuum, some essential oil constituents are also obtained on a commercial scale by freezing out from the essential oil, followed by centrifugation at below freezing point of the desired product. Examples are menthol from *Mentha* species (this is usually further purified by recrystallization from a suitable solvent, *trans*-anethole from anise oil, star anise oil, and particularly fennel seed oil and 1,8-cineole from cineole-rich eucalyptus oils.

Most essential oils are complex mixtures of terpenic and sesquiterpenic hydrocarbons, and their oxygenated terpenoid and sesquiterpenoid derivatives (alcohols, aldehydes, ketones, esters, and occasionally carboxylic acids), as well as aromatic (benzenoid) compounds such as phenols, phenolic ethers, and aromatic esters. So-called "terpene-less" and "sesquiterpene-less essential oils are commonly used in the flavor industry. Many terpenes are bitter in taste and many, particularly the terpenic hydrocarbons, are poorly soluble or even completely insoluble in water–ethanol mixtures. Since the hydrocarbons rarely contribute anything of importance to their flavoring properties, their removal is a commercial necessity. They are removed by the so-called "washing process," a method used mostly for the treatment of citrus oils. This process takes advantage of the different polarities of individual essential oil constituents. The essential oil is added to a carefully selected solvent (usually a water–ethanol solution) and the mixture partitioned by prolonged stirring. This removes some of the more polar oil constituents into the water–ethanol phase (e.g., the solvent phase). Since a single partitioning step is not sufficient to effect complete separation, the whole process has to be repeated several times. The water–ethanol fractions are combined and the solvent removed. The residue contains now very much reduced amounts of hydrocarbons but has been greatly enriched in the desired polar oxygenated flavor constituents, aldehydes such as octanal, nonanal, decanal, hexenal, geranial, and neral; alcohols such as nerol, geraniol, and terpinen-4-ol; oxides such as 1,8-cineole and 1,4-cineole; as well as esters and sometimes carboxylic acids. Apart from water–ethanol mixtures, hexane or light petroleum fractions (sometimes called "petroleum ether") have sometimes also been added as they will enhance the separation process. However, these are highly flammable liquids and care has to be taken in their use.

"Folded" or "concentrated" oils are citrus oils from which some of the undesirable components (usually limonene) have been removed by high vacuum distillation. In order to avoid thermal degradation of the oil, temperatures have to be kept as low as possible. Occasionally, a solvent is used as a "towing" agent to keep the temperature low.

Another, more complex, method for the concentration of citrus oils is a chromatographic separation using packed columns. This method allows a complete elimination of the unwanted hydrocarbons. This method, invented by Erich Ziegler, uses columns packed with either silica or aluminum oxide. The oil is introduced onto the column and the hydrocarbons eluted by means of a suitable nonpolar solvent of very low boiling point. The desirable polar citrus oil components are then washed out using a polar solvent (Ziegler, 1982).

Yet another valuable flavor product of citrus fruits is the "essence oil." The favored method for the transport of citrus juice is in the form of a frozen juice concentrate. The fruit juice is partly dehydrated by distilling off under vacuum the greater part of the water and frozen. Distilling off the water results in significant losses of the desirable volatiles responsible for the aroma of the fruit. These volatiles are captured in several cold traps and constitute the "aqueous essence" or "essence oil" that has the typical fruity and fresh fragrance, but slightly less aldehydic than that of the oil. This oil is used to enhance the flavor of the reconstituted juice obtained by thawing and dilution with water of the frozen concentrate.

Producing essential oils today is, from a marketing point of view, a complex matter. As in the field of other finished products, the requirements of the buyer or producer of the consumer product must be fulfilled. The evaluation of commercial aspects of essential oil production is not an easy task and requires careful consideration. There is no sense in producing oils in oversupply. Areas of short supply, depending on climatic or political circumstances should be identified and acted upon. As in other industries global trends are an important tool and should continually be monitored.

For example, which are the essential oils that cannot be replaced by synthetic substitutes such as patchouli oil or blue chamomile oils? A solution to this problem can lie in the breeding of suitable plants. For example, a producer of a new kind "pastis," the traditional aperitif of France, wants to introduce a new flavor with a rosy note in the fennel component of the flavor. This will require the study and identification of oil constituents with "rosy" notes and help biologists to create new botanical varieties by genetic crossing, for example, by genetic manipulation, of suitable target plant species. Any new lines will first be tested in the laboratory and then in field trials. Test distillations will be carried out and the chemical composition of the oils determined. Agronomists and farmers will be involved in all agricultural aspects of the projects: soil research and harvesting techniques. Variability of all physicochemical aspects of the new strains will be evaluated. At this point, the new types of essential oils will be presented to the client. If the client is satisfied with the quality of the oils, the first larger plantations shall be established and consumer market research will be initiated. If everything has gone to plan, that is all technical problems have been successfully resolved and the finished product has met with the approval of the consumers, large-scale production can begin. This example describes the current way of satisfying customer demands.

Global demand for essential oils is on the increase. This also generates some serious problems for which immediate solutions may not easily be found. The first problem is the higher demand for certain essential oils by some of the world's very major producers of cosmetics. They sometimes contract oil quantities that can be of the order of 70% of world production. This will not only raise the price but also restrict consumer access to certain products. From this arises another problem. Our market is to some extent a market of copycats. How can one formulate the fragrance of a competitor's product without having access to the particular essential oil used by him, particularly as this oil may have other functions than just being a fragrance, such as, for example, certain physiological effects on both the body as well as the mind? Lavender oil from *L. angustifolia* is a calming agent as well as possessing anti-inflammatory activity. No similar or equivalent natural essential oil capable of replacing it is known. Another problem affecting the large global players is ensuring the continuing availability of raw material of the required quality needed to satisfy market demand. This is clearly an almost impossible demand as nobody can assure that climatic conditions required for optimum growth of a particular essential oil crop will remain unchanged. Another problem may be the farmer himself. Sometimes it may be financially more worthwhile for the farmer to cultivate other than essential oil plant crops. All these factors may have some detrimental effects on the availability of essential oils. Man's responsibility for the continued health of the environment may also be one of the reasons for the disappearance of an essential oil from the market. Sandalwood (species of *Santalum*, but mainly Indian *Santalum album*) requires in some cases up to 100 years to regenerate to a point where they are large enough to be harvested. This and their uses in religious ceremonies have resulted in significant shortages of Indian oil. Owing to the large monetary value of Indian sandalwood oil, indiscriminate cutting of the wood has just about entirely eliminated it from native forests in Timor (Indonesia). Sandalwood oils of other origins are available, *Santalum spicatum* from western Australia, and *Santalum austrocaledonicum* from New Caledonia and Vanuatu. However, their wood oils differ somewhat in odor as well as in chemical composition from genuine Indian oils.

Some essential oils are disappearing from the market owing to the hazardous components they contain and are, therefore, banned from most applications in cosmetics and detergents. These oil components, all of which are labeled as being carcinogenic, include safrole, asarone, methyleugenol, and elemicin. Plant diseases are another reason for essential oil shortages as they, too, can be affected by a multitude of diseases, some cancerous, which can completely destroy the total crop. For example, French lavender is known to suffer from a condition whereby a particular protein causes a decrease in the growth of the lavender plants. This process could only be slowed down by cultivation at higher altitudes. In the middle of the twentieth century lavender has been cultivated in the Rhône valley at an altitude of 120 m. Today lavender is growing only at altitudes around 800 m. The growing shoots of lavender plants are attacked by various pests, in particular the larvae of

Cécidomye (*Thomasissiana lavandula*) which, if unchecked, will defoliate the plants and kill them. Some microorganisms such as *Mycoplasma* and a fungus *Armillaria mellex* can cause serious damage to plantations. At the present time, the use of herbicides and pesticides is an unavoidable necessity. Wild-growing plants are equally prone to attack by insect pests and plant diseases.

The progression from wild-growing plants to essential oil production is an environmental problem. In some developing countries, damage to the natural balance can be traced back to overexploitation of wild-growing plants. Some of these plants are protected worldwide and their collection, processing, and illegal trading are punishable by law. In some Asian countries, such as in Vietnam, collection from the wild is state controlled and limited to quantities of biomass accruing from natural regeneration.

The state of technical development of the production in the developing countries is very variable and depends largely on the geographical zone they are located in. Areas of particular relevance are Asia, Africa, South America, and eastern Europe. As a rule, the poorer the country, the more traditional and less technologically sophisticated equipment is used. Generally, standards of the distillation apparatus are those of the 1980s. At that time, the distillation equipment was provided and installed by foreign aid programs with European and American know-how. Most of these units are still in existence and, owing to repairs and improvements by local people, in good working order. Occasionally, primitive equipment has been locally developed, particularly when the state did not provide any financial assistance. Initially, all mastery and expertise of distillation techniques came from Europe, mainly from France. Later on, that knowledge was acquired and transferred to their countries by local people who had studied in Europe. They are no longer dependent on foreign know-how and able to produce oils of constant quality. Conventional hydrodistillation is still the main essential oil extraction method used, one exception being hydrodiffusion often used in Central America, mainly Guatemala and El Salvador, and Brazil in South America. The construction of the equipment is carried out in the country itself and makes the producer independent from higher-priced imports. Steam is generated by oil-burning generators only in the vicinity of cities. In country areas, wood or dried spent biomass is used. As in all other essential oil-producing countries, the distillation plants are close to the cultivation areas. Wild-growing plants are collected, provided the infrastructure exists for their transport to the distillation plant. For certain specific products permanent fixed distillation plants are used. A forward leap in the technology will be only possible if sufficient investment funds became available in the future. Essential oil quantities produced in those countries are not small and important specialities such as citral-rich ginger oil from Ecuador play a role on the world market. It should be a compulsory requirement that developing countries treat their wild-growing plant resources with the utmost care. Harvesting has to be controlled to avoid their disappearance from the natural environment and quantities taken adjusted to the ability of the environment to spontaneously regenerate. On the other hand, cultivation will have to be handled with equal care. The avoidance of monoculture will prevent leaching the soil of its nutrients and guard the environment from possible insect propagation. Balanced agricultural practices will lead to a healthy environment and superior quality plants for the production of essential oils.

The following are some pertinent remarks on the now prevailing views of "green culture" and "organically" grown plants for essential oil production. It is unjustified to suggest that such products are of better quality or greater activity. Comparisons of chemical analyses of "bio-oils," for example, oils from "organically" grown plants, and commercially produced oils show absolutely no differences, qualitative or quantitative, between them. While the concept of pesticide- and fertilizer-free agriculture is desirable and should be supported, the huge worldwide consumption of essential oils could never be satisfied by bio-oils.

Finally, some remarks as to the concept of honesty are attached to the production of natural essential oils. During the last 30 years or so, adulteration of essential oils could be found every day. During the early days, cheap fatty oils (e.g., peanut oil) were used to cut essential oils. Such adulterations were easily revealed by means of placing a drop of the oil on filter paper and allowing it to evaporate (Karg, 1981). While an unadulterated essential oil will evaporate completely or at worst

leave only a trace of nonvolatile residue, a greasy patch indicates the presence of a fatty adulterant. As synthetic components of essential oils became available around the turn of the twentieth century some lavender and lavandin oils have been adulterated by the addition of synthetic linalool and linalyl acetate to the stills before commencing the distillation of the plant material. With the advent of improved analytical methods, such as GC and GC/MS, techniques of adulterating essential oil were also refined. Lavender oil can again serve as an example. Oils distilled from mixtures of lavender and lavandin flowers mimicked the properties of genuine good-quality lavender oils. However, with the introduction of chiral GC techniques, such adulterations were easily identified and the genuineness of the oils guaranteed. This also allowed the verification of the enantiomeric distribution of monoterpenes, monoterpenoid alcohols, and esters present in essential oils. Nuclear magnetic resonance (NMR) is probably one of the best, but also one of the most expensive, methods available for the authentication of naturalness and will be cost effective only with large batch quantities or in the case of very expensive oils. In the future, two-dimensional GC (GC/GC) will provide the next step for the control of naturalness of essential oils.

Another important aspect is the correct botanical source of the essential oil. This can perhaps best be discussed with reference to eucalyptus oil of the 1,8-cineole type. Originally, before commercial eucalyptus oil production commenced in Australia, eucalyptus oil was distilled mainly from *E. globulus* Labill. trees introduced into Europe [mainly Portugal and Spain (ISO Standard 770)]. It should be noted that this species exists in several subspecies: *E. globulus* subsp. *bicostata* (Maiden, Blakely, & J. Simm.) Kirkpatr., *E. globulus* Labill. subsp. *globulus*., *E. globulus* subsp. *pseudoglobulus* (Naudin ex Maiden) Kirkpatr., and *E. globulus* subsp. *maidenii* (F. Muell.) Kirkpatr. It has been shown that the European oils were in fact mixed oils of some of these subspecies and of their hybrids (report by H. H. G. McKern of ISO/TC 54 meeting held in Portugal in 1966). The European Pharmacopoeia Monograph 0390 defines eucalyptus oil as the oil obtained from *E. globulus* Labill., *Eucalyptus fruticetorum* F. von Mueller Syn. *Eucalyptus polybractea* R. T. Baker (this is the correct botanical name), *Eucalyptus smithii* R. T. Baker, and other species of *Eucalyptus* rich in 1,8-cineole. The Council of Europe's book Plants in cosmetics, Vol. 1, page 127 confuses the matter even further. It entitles the monograph as *E. globulus* Labill. et al. species, for example, includes any number of unnamed *Eucalyptus* species. The Pharmacopoeia of the Peoples Republic of China (English Version, Vol. 1) 1997 goes even further defining eucalyptus oil as the oil obtained from *E. globulus* Labill. and *Cinnamomum camphora* as well as from other plants of those two families. ISO Standard 3065—Oil of Australian eucalyptus, 80–85% cineole content, simply mentions that the oil is distilled from the appropriate species. The foregoing passage simply shows that Eucalyptus oil does not necessarily have to be distilled from a single species of *Eucalyptus*, for example, *E. globulus*, although suggesting that it is admissible to include 1,8-cineole-rich *Cinnamomum* oils is incorrect and unrealistic. This kind of problem is not unusual or unique. For example, the so-called English lavender oil, considered by many to derive from *L. angustifolia*, is really, in the majority of cases, the hybrid lavandin (Denny, 1995, personal communication).

Another pertinent point is how much twig and leaf material can be used in juniper berry oil? In Indonesia, it is common practice to space individual layers of patchouli leaves in the distillation vessel with twigs of the gurjun tree. Gurjun balsam present in the twigs contains an essential oil that contaminates the patchouli oil. Can this be considered to constitute an adulteration or simply a tool required for the production of the oil?

4.1.16 Concluding Remarks

As mentioned at the beginning, essential oils do have a future. In spite of regulatory limitations, dangerous substance regulations, and dermatological concerns as well as problems with pricing the world production of essentials oil will increase. Essential oils are used in a very large variety of fields. They are an integral constituent of fragrances used in perfumes and cosmetics of all kinds, skin softeners to shower gels and body lotions, and even to "aromatherapy horse care massage oils." They are widely

used in the ever-expanding areas of aromatherapy or, better, aromachology. Very large quantities of natural essential oils are used by the food and flavor industries for the flavoring of smallgoods, fast foods, ice creams, beverages, both alcoholic as well as nonalcoholic soft drinks, and so on. Their medicinal properties have been known for many years and even centuries. Some possess antibacterial or antifungal activity while others may assist with the digestion of food. However, as they are multicomponent mixtures of somewhat variable composition, the medicinal use of whole oils has contracted somewhat, the reason being that single essential oil constituents were easier to test for effectiveness and eventual side effects. Despite all that, the use of essential oils is still "number one" on the natural healing scene. With rising health care and medicine costs, self-medication is on the increase and with it a corresponding increase in the consumption of essential oils. Parallel to this, the increase in various esoteric movements is giving rise to further demands for pure natural essential oils.

In the field of agriculture, attempts are being made at the identification of ecologically more friendly natural biocides, including essential oils, to replace synthetic pesticides and herbicides. Essential oils are also used to improve the appetite of farm animals, leading to more rapid increases in body weight as well as to improved digestion.

Finally, some very cheap essential oils or oil components such as limonene, 1,8-cineole, and the pinenes are useful as industrial solvents while phellandrene-rich eucalyptus oil fractions are marketed as industrial perfumes for detergents and the like.

In conclusion, a "golden future" can be predicted for that useful natural product: the "Essential Oil"!

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5 Chemistry of Essential Oils

Charles Sell

CONTENTS

5.1 INTRODUCTION

The term "essential oil" is a contraction of the original "quintessential oil." This stems from the Aristotelian idea that matter is composed of four elements, namely, fire, air, earth, and water. The fifth element, or quintessence, was then considered to be spirit or life force. Distillation and evaporation were thought to be processes of removing the spirit from the plant and this is also reflected in our language since the term "spirits" is used to describe distilled alcoholic beverages such as brandy, whiskey, and eau de vie. The last of these again shows reference to the concept of removing the life force from the plant. Nowadays, of course, we know that, far from being spirit, essential oils are physical in nature and composed of complex mixtures of chemicals. One thing that we do see from the ancient concepts is that the chemical components of essential oils must be volatile since they are removed by distillation. In order to have boiling points low enough to enable distillation, and atmospheric pressure steam distillation in particular, the essential oil components need to have molecular weights below 300 Daltons (molecular mass relative to hydrogen = 1) and are usually fairly hydrophobic. Within these constraints, nature has provided an amazingly rich and diverse range of chemicals (Hay and Waterman, 1993; Lawrence, 1985) but there are patterns of molecular structure that give clues to how the molecules were constructed. These synthetic pathways have now been confirmed by experiment and will serve to provide a structure for the contents of this chapter.

5.2 BASIC BIOSYNTHETIC PATHWAYS

The chemicals produced by nature can be classified into two main groups. The primary metabolites are those that are universal across the plant and animal family and constitute the basic building blocks of life. The four subgroups of primary metabolites are proteins, carbohydrates, nucleic acids, and lipids. These families of chemicals contribute little to essential oils although some essential oil components are degradation products of one of these groups, lipids being the most significant. The

secondary metabolites are those that occur in some species and not others and they are usually classified into terpenoids, shikimates, polyketides, and alkaloids. The most important as far as essential oils are concerned are the terpenoids and the shikimates are the second. There are a number of polyketides of importance in essential oils but very few alkaloids. Terpenoids, shikimates, and polyketides will therefore be the main focus of this chapter.

The general scheme of biosynthetic reactions (Bu'Lock, 1965; Mann et al., 1994) is shown in Figure 5.1. Through photosynthesis, green plants convert carbon dioxide and water into glucose. Cleavage of glucose produces phosphoenolpyruvate (**1**), which is a key building block for the shikimate family of natural products. Decarboxylation of phosphoenolpyruvate gives the two-carbon unit of acetate and this is esterified with coenzyme-A to give acetyl CoA (**2**). Self-condensation of this species leads to the polyketides and lipids. Acetyl CoA is also a starting point for synthesis of mevalonic acid (**3**), which is the key starting material for the terpenoids. In all of these reactions, and indeed all the natural chemistry described in this chapter, Nature uses the same reactions that chemists do (Sell, 2003). However, nature's reactions tend to be faster and more selective because of the catalysts it uses. These catalysts are called enzymes and they are globular proteins in which an active site holds the reacting species together. This molecular organization in the active site lowers the activation energy of the reaction and directs its stereochemical course (Lehninger, 1993; Matthews and van Holde, 1990).

Many enzymes need cofactors as reagents or energy providers. Coenzyme-A has already been mentioned above. It is a thiol and is used to form thioesters with carboxylic acids. This has two effects on the acid in question. Firstly, the thiolate anion is a better leaving group than alkoxide and so the carbonyl carbon of the thioester is reactive toward nucleophiles. Secondly, the thioester group increases the acidity of the protons adjacent to the carbonyl group and therefore promotes the formation of the corresponding carbanions. In biosynthesis, a key role of adenosine triphosphate (ATP) is to make phosphate esters of alcohols (phosphorylation). One of the phosphate groups of ATP is added to the alcohol to give the corresponding phosphate ester and adenosine diphosphate (ADP). Another group of cofactors of importance to biosynthesis includes pairs such as NADP/NADPH, TPN/TPNH, and DPN/DPNH. These cofactors contain an *N*-alkylated pyridine ring. In each pair, one form comprises an *N*-alkylated pyridinium salt and the other the corresponding *N*-alkyl-1,4-dihydropyridine. The two forms in each pair are interconverted by gain or loss of a hydride anion and therefore constitute redox reagents. In all of the cofactors mentioned here, the reactive part of

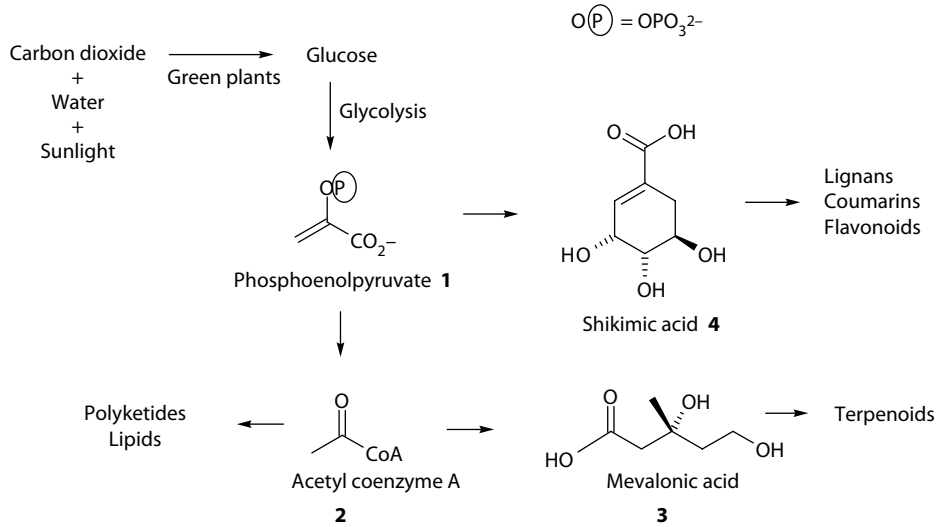


FIGURE 5.1 General pattern of biosynthesis of secondary metabolites.

the molecule is only a small part of the whole. However, the bulk of the molecule has an important role in molecular recognition. The cofactor docks into the active site of the enzyme through recognition and this holds the cofactor in the optimum spatial configuration relative to the substrate.

5.3 POLYKETIDES AND LIPIDS

The simplest biosynthetic pathway to appreciate is that of the polyketides and lipids (Bu'Lock, 1965; Mann et al., 1994). The key reaction sequence is shown in Figure 5.2. Acetyl CoA (**2**) is carboxylated to give malonyl CoA (**5**) and the anion of this attacks the CoA ester of a fatty acid. Obviously, the fatty acid could be acetic acid, making this a second molecule of acetyl CoA. After decarboxylation, the product is a β-ketoester with a backbone that is two-carbon atoms longer than the first fatty acid. Since this is the route by which fatty acids are produced, it explains why fatty acids are mostly even numbered. If the process is repeated with this new acid as the feedstock, it can be seen that various poly-oxo-acids can be built up, each of which will have a carbonyl group on every alternate carbon atom, hence the name polyketides. Alternatively, the ketone function can be reduced to the corresponding alcohol, and then eliminated, and the double bond hydrogenated. This sequence of reactions gives a higher homologue of the starting fatty acid, containing two more carbon atoms in the chain. Long chain fatty acids, whether saturated or unsaturated, are the basis of the lipids.

There are three main paths by which components of essential oils and other natural extracts are formed in this family of metabolites: condensation reactions of polyketides, degradation of lipids, and cyclization of arachidonic acid.

Figure 5.3 shows how condensation of polyketides can lead to phenolic rings. Intramolecular aldol condensation of the tri-keto-octanoic acid and subsequent enolization leads to orsellinic acid (**6**). Polyketide phenols can be distinguished from the phenolic systems of the shikimates by the fact that the former usually retain evidence of oxygenation on alternate carbon atoms, either as acids, ketones, phenols, or as one end of a double bond. The most important natural products containing polyketide phenols are the extracts of oakmoss and treemoss (*Evernia prunastrii*). The most significant in odor terms is methyl 3-methylorsellinate (**7**) and ethyl everninate (**8**), which is usually also present in reasonable quantity. Atranol (**9**) and chloratranol (**10**) are minor components but they are skin sensitizers and so limit the usefulness of oakmoss and treemoss extracts, unless they are removed from them. Dimeric esters of orsellinic and everninic acids and analogues also exist in mosses. They are known as depsides and hydrolysis yields the monomers, thus increasing the odor of the sample. However, some depsides, such as atranorin (**11**), are allergens and thus contribute to safety issues with the extracts.

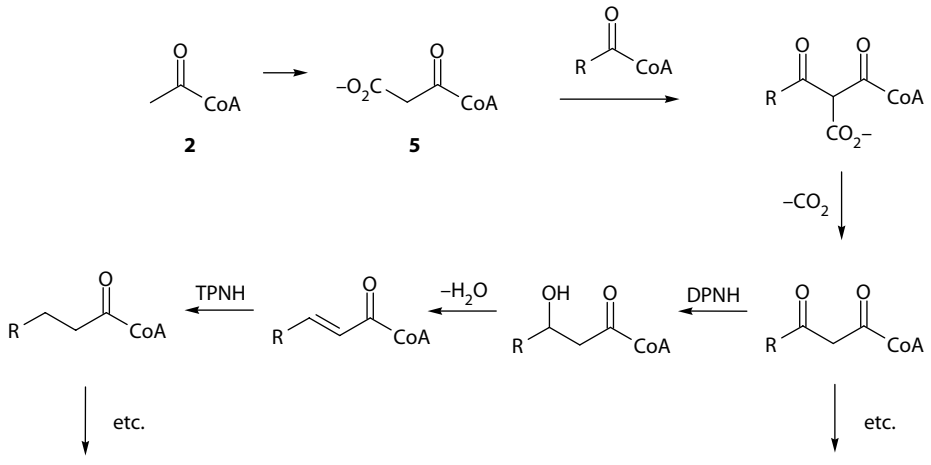


FIGURE 5.2 Polyketide and lipid biosynthesis.

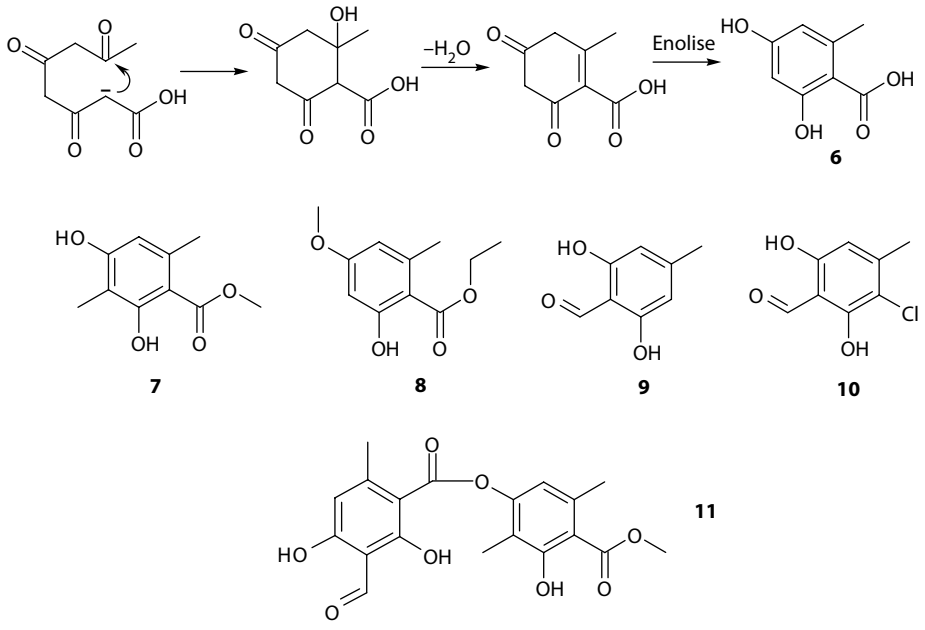


FIGURE 5.3 Polyketide biosynthesis and oakmoss components.

The major metabolic route for fatty acids involves β-oxidation and cleavage giving acetate and a fatty acid with two carbon atoms less than the starting acid, that is, the reverse of the biosynthesis reaction. However, other oxidation routes also exist and these give rise to new metabolites that were not on the biosynthetic pathway. For example, Figure 5.4 shows how allylic oxidation of a dienoic acid and subsequent cleavage can lead to the formation of an aldehyde.

Allylic oxidation followed by lactonization rather than cleavage can, obviously, lead to lactones. Reduction of the acid function to the corresponding alcohols or aldehydes is also possible as are hydrogenation and elimination reactions. Thus a wide variety of aliphatic entities are made available. Some examples are shown in Figure 5.5 to illustrate the diversity that exists. The hydrocarbon (*E,Z*)-1,3,5-undecatriene (**12**) is an important contributor to the odor of galbanum. Simple aliphatic alcohols and ethers are found, the occurrence of 1-octanol (**13**) in olibanum and methyl hexyl ether (**14**) in lavender being examples. Aldehydes are often found as significant odor components of oils, for example, decanal (**15**) in orange oil and (*E*)-4-decenal (**16**) caraway and cardamom. The ketone 2-nonanone (**17**) that occurs in rue and hexyl propionate (**18**), a component of lavender, is just one of a plethora of esters that are found. The isomeric lactones γ-decalactone (**19**) and δ-decalactone (**20**) are found in osmanthus (Essentials Oils Database, n.d.). Acetylenes also occur as essential oil components, often as polyacetylenes such as methyl deca-2-en-4,6,8-triynoate (**21**), which is a component of *Artemisia vulgaris.*

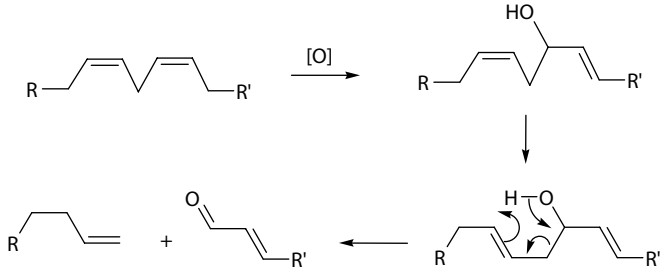


FIGURE 5.4 Fragmentation of polyunsaturated fats to give aldehydes.

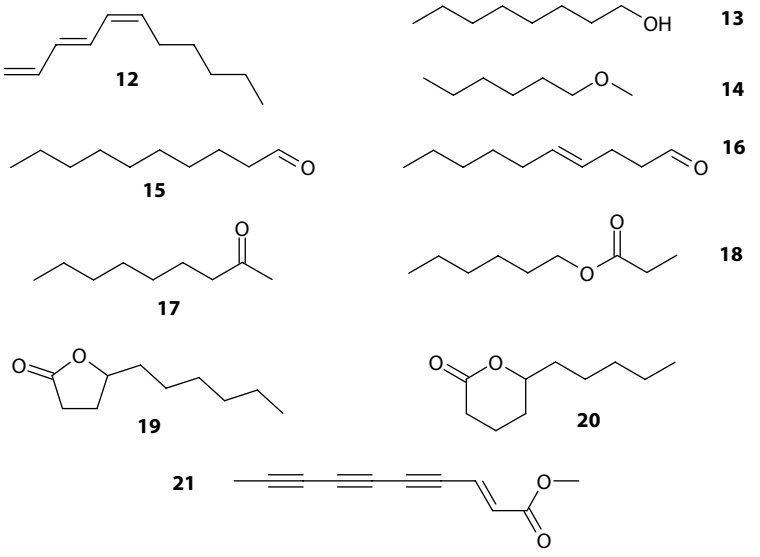


FIGURE 5.5 Some lipid-derived components of essential oils.

Arachidonic acid (**22**) is a polyunsaturated fatty acid that plays a special role as a synthetic intermediate in plants and animals (Mann et al., 1994). As shown in Figure 5.6, allylic oxidation at the 11th carbon of the chain leads to the hydroperoxide (**23**). Further oxidation (at the 15th carbon) with two concomitant cyclization reactions gives the cyclic peroxide (**24**). This is a key intermediate for the biosynthesis of prostaglandins such as 6-ketoprostaglandin $F_{1\alpha}$ (**25**) and also for methyl jasmonate

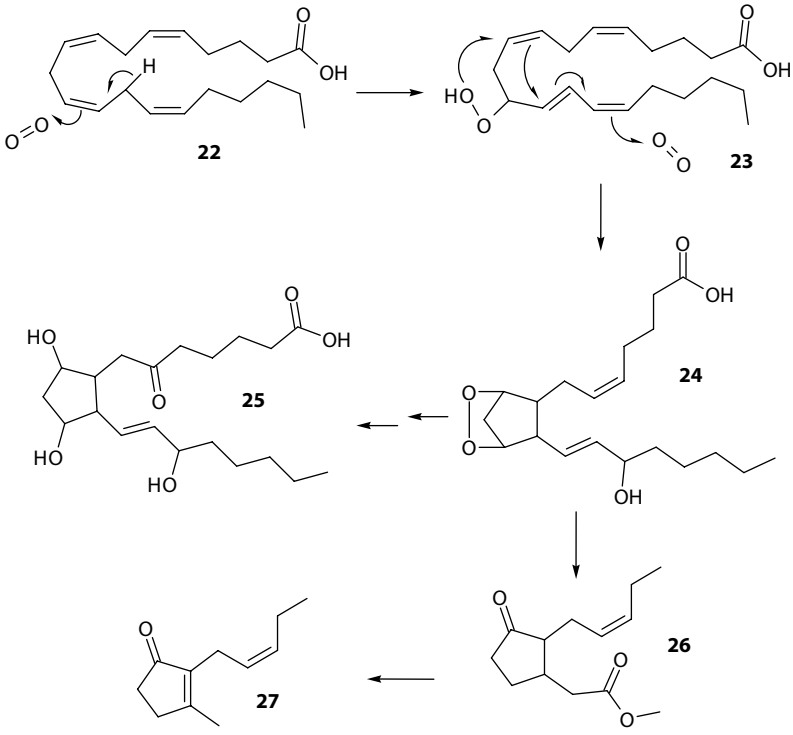


FIGURE 5.6 Biosynthesis of prostaglandins and jasmines.

(**26**). The latter is the methyl ester of jasmonic acid, a plant hormone, and is a significant odor component of jasmine, as is jasmone (**27**), a product of degradation of jasmonic acid.

5.4 SHIKIMIC ACID DERIVATIVES

Shikimic acid (**4**) is a key synthetic intermediate for plants since it is the key precursor for both the flavonoids and lignin (Bu'Lock, 1965; Mann et al., 1994). The flavonoids are important to plants as antioxidants, colors, protective agents against ultraviolet light, and the like, and lignin is a key component of the structural materials of plants, especially woody tissues. Shikimic acid is synthesized from phosphoenol pyruvate (**1**) and erythrose-4 phosphate (**28**), as shown in Figure 5.7, and thus its biosynthesis starts from the carbohydrate pathway. Its derivatives can usually be recognized by the characteristic shikimate pattern of a six-membered ring with either a one- or three-carbon substituent on position 1 and oxygenation in the third, and/or fourth, and/or fifth positions. However, the oxygen atoms of the final products are not those of the starting shikimate since these are lost initially and then replaced.

Figure 5.8 shows some of the biosynthetic intermediates stemming from shikimic acid (**4**) and which are of importance in terms of generating materials volatile enough to be essential oil components. Elimination of one of the ring alcohols and reaction with phosphoenol pyruvate (**1**) gives chorismic acid (**29**) that can undergo an oxy-Cope reaction to give prephenic acid. (**30**) Decarboxylation and elimination of the ring alcohol now gives the phenylpropionic acid skeleton. Amination and reduction of the ketone function gives the essential amino acid phenylalanine (**31**) whereas reduction and elimination leads to cinnamic acid. (**32**) Ring hydroxylation of the latter gives the isomeric *o*- and *p*-coumaric acids, (**33**) and (**34**), respectively. Further hydroxylation gives caffeic acid (**35**) and methylation of this gives ferulic acid. (**36**) Oxidation of the methyl ether of the latter and subsequent cyclization gives methylenecaffeic acid (**37**). In shikimate biosynthesis, it is often possible to arrive at a given product by different sequences of the same reactions and the exact route used will depend on the genetic make-up of the plant.

Aromatization of shikimic acid, without addition of the three additional carbon atoms from phosphoenolpyruvate, gives benzoic acid derivatives. Benzoic acid itself occurs in some oils and its esters are widespread. For example, methyl benzoate is found in tuberose, ylang ylang, and various lilies. Even more common are benzyl alcohol, benzaldehyde, and their derivatives (Arctander, 1960;

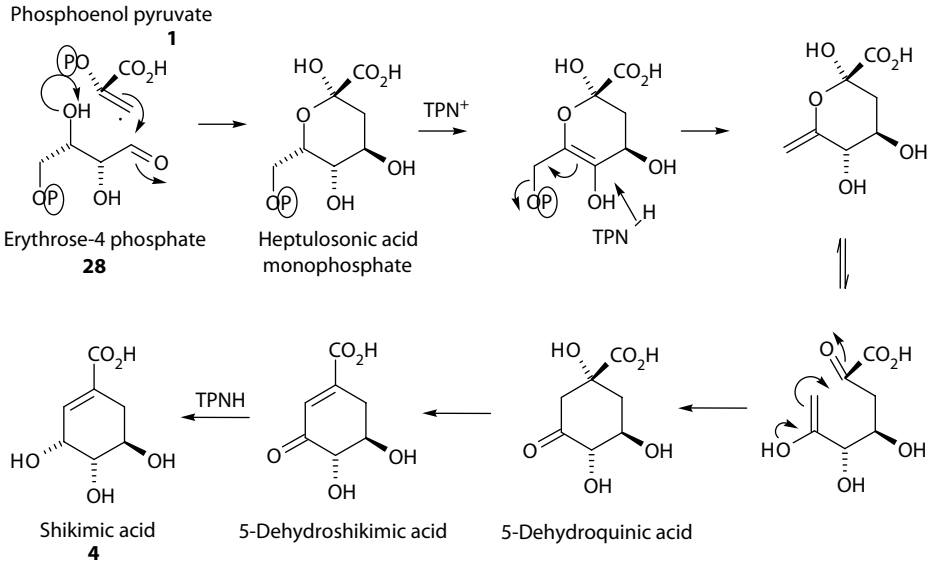


FIGURE 5.7 Biosynthesis of shikimic acid.

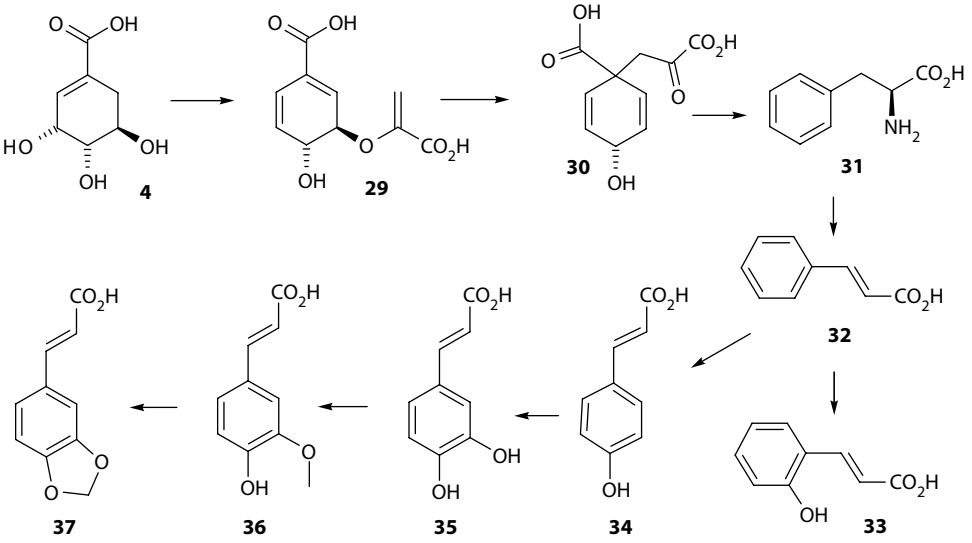


FIGURE 5.8 Key intermediates from shikimic acid.

Essential Oils Database, n.d.; Gildemeister and Hoffmann, 1956; Günther, 1948). Benzyl alcohol occurs in muguet, jasmine, and narcissus, for example, and its acetate is the major component of jasmine oils. The richest sources of benzaldehyde are almond and apricot kernels but it is also found in a wide range of flowers, including lilac, and other oils such as cassia and cinnamon. Hydroxylation or amination of benzoic acid leads to further series of natural products and some of the most significant, in terms of odors of essential oils, are shown in Figure 5.9. *o*-Hydroxybenzoic acid is known as salicylic acid (**38**) and both it and its esters are widely distributed in nature. For instance, methyl salicylate (**39**) is the major component (about 90% of the volatiles) of wintergreen and makes a significant contribution to the scents of tuberose and ylang ylang although only present at about 10% in the former and less than 1% in the latter. *o*-Aminobenzoic acid is known as anthranilic acid (**40**). Its methyl ester (**41**) has a very powerful odor and is found in such oils as genet, bitter orange flower,

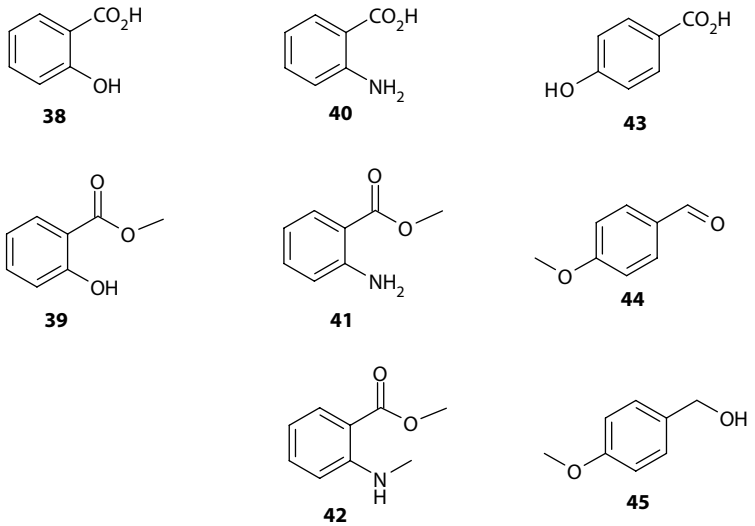


FIGURE 5.9 Hydroxy- and aminobenzoic acid derivatives.

tuberose, and jasmine. Dimethyl anthranilate (**42**), in which both the nitrogen and acid functions have been methylated, occurs at low levels in citrus oils. *p*-Hydroxybenzoic acid has been found in vanilla and orris but much more common is the methyl ester of the corresponding aldehyde, commonly known as anisaldehyde (**44**). As the name suggests, the latter one is an important component of anise and it is also found in oils such as lilac and the smoke of agar wood. The corresponding alcohol, anisyl alcohol (**45**), and its esters are also widespread components of essential oils.

Indole (**46**) and 2-phenylethanol (**47**) are both shikimate derivatives. Indole is particularly associated with jasmine. It usually occurs in jasmine absolute at a level of about 3–5% and makes a very significant odor contribution to it. However, it does occur in many other essential oils as well. 2-Phenylethanol occurs widely in plants and is especially important for rose where it usually accounts for one-third to three-quarters of the oil. The structures of both are shown in Figure 5.10.

Figure 5.10 also shows some of the commonest cinnamic acid-derived essential oil components. Cinnamic acid (**32**) itself has been found in, for example, cassia and styrax but its esters, particularly the methyl ester, are more frequently encountered. The corresponding aldehyde, cinnamaldehyde (**48**), is a key component of cinnamon and cassia and also occurs in some other oils. Cinnamyl alcohol (**49**) and its esters are more widely distributed, occurring in narcissus, lilac, and a variety of other oils. Lactonization of *o*-coumaric acid (**33**) gives coumarin (**50**). This is found in new mown hay to which it gives the characteristic odor. It is also important in the odor profile of lavender and related species and occurs in a number of other oils. Bergapten (**51**) is a more highly oxygenated and substituted coumarin. The commonest source is bergamot oil but it also occurs in other sources, such as lime and parsley. It is phototoxic and consequently constitutes a safety issue for oils containing it.

Oxygenation in the *p*-position of cinnamic acid followed by methylation of the phenol and reduction of the acid to alcohol with subsequent elimination of the alcohol gives estragole (also known as methylchavicol (**52**) and anethole (**53**). Estragole is found in a variety of oils, mostly herb oils such as basil, tarragon, chervil, fennel, clary sage, anise, and rosemary. Anethole occurs in both the (*E*)- and (*Z*)-forms, the more thermodynamically stable (*E*)-isomer (shown in Figure 5.10) is the commoner, the (*Z*)-isomer is the more toxic of the two. Anethole is found in spices and herbs such as anise, fennel, lemon balm, coriander, and basil and also in flower oils such as ylang ylang and lavender.

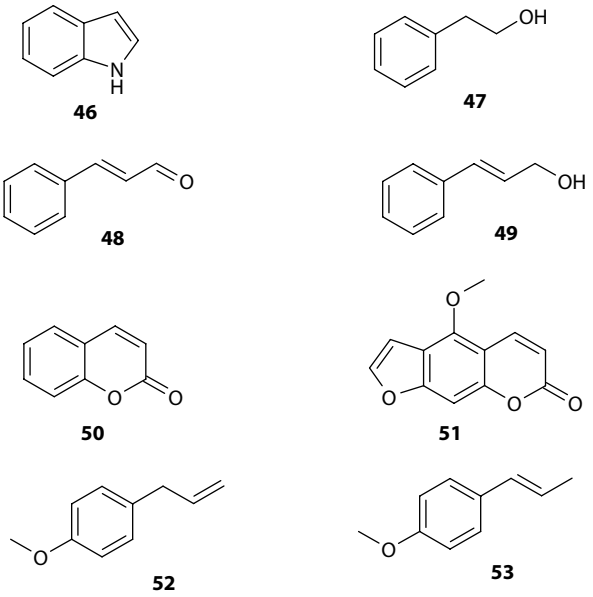


FIGURE 5.10 Some shikimate essential oil components.

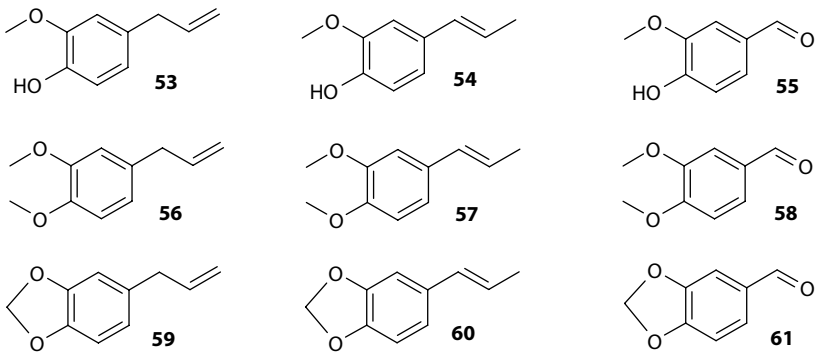


FIGURE 5.11 Ferulic acid derivatives.

Reduction of the side chain of ferulic acid (**36**) leads to an important family of essential oil components, shown in Figure 5.11. The key material is eugenol (**53**), which is widespread in its occurrence. It is found in spices such as clove, cinnamon, and allspice, herbs such as bay and basil, and in flower oils including rose, jasmine, and carnation. Isoeugenol (**54**) is found in basil, cassia, clove, nutmeg, and ylang ylang. Oxidative cleavage of the side chain of shikimates to give benzaldehyde derivatives is common and often significant, as it is in this case, where the product is vanillin (**55**). Vanillin is the key odor component of vanilla and is therefore of considerable commercial importance. It also occurs in other sources such as jasmine, cabreuva, and the smoke of agar wood. The methyl ether of eugenol, methyleugenol (**56**), is very widespread in nature, which, since it is the subject of some toxicological safety issues, creates difficulties for the essential oils business. The oils of some Melaleuca species contain up to 98% methyleugenol and it is found in a wide range of species including pimento, bay, tarragon, basil, and rose. The isomer, methylisoeugenol (**57**), occurs as both (*E*)- and (*Z*)-isomers, the former being slightly commoner. Typical sources include calamus, citronella, and some narcissus species. Oxidative cleavage of the side chain in this set of substances produces veratraldehyde (**58**), a relatively rare natural product. Formation of the methylenedioxy ring, via methylenecaffeic acid (**37**), gives safrole (**59**), the major component of sassafras oil. The toxicity of safrole has led to a ban on the use of sassafras oil by the perfumery industry. Isosafrole (**60**) is found relatively infrequently in nature. The corresponding benzaldehyde derivative, heliotropin (**61**), also known as piperonal, is the major component of heliotrope.

5.5 TERPENOIDS

The terpenoids are, by far, the most important group of natural products as far as essential oils are concerned. Some authors, particularly in older literature, refer to them as terpenes but this term is nowadays restricted to the monoterpenoid hydrocarbons. They are defined as substances composed of isoprene (2-methylbutadiene) units. Isoprene (**62**) is not often found in essential oils and is not actually an intermediate in biosynthesis, but the 2-methylbutane skeleton is easily discernable in terpenoids. Figure 5.12 shows the structures of some terpenoids. In the case of geraniol (**63**), one end of one isoprene unit is joined to the end of another making a linear structure (2,6-dimethyloctane). In guaiol (**64**), there are three isoprene units joined together to make a molecule with two rings. It is easy to envisage how the three units were first joined together into a chain and then formation of bonds from one point in the chain to another produced the two rings. Similarly, two isoprene units were used to form the bicyclic structure of α-pinene (**65**).

The direction of coupling of isoprene units is almost always in one direction, the so-called head-to-tail coupling. This is shown in Figure 5.13. The branched end of the chain is referred to as the head of the molecule and the other as the tail.

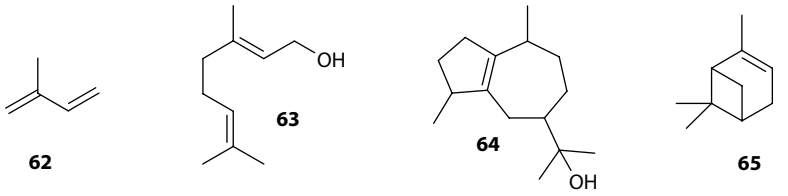


FIGURE 5.12 Isoprene units in some common terpenoids.

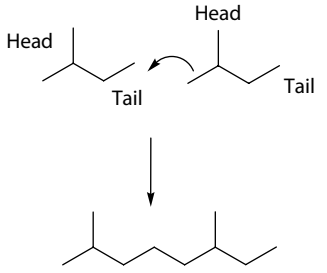


FIGURE 5.13 Head-to-tail coupling of two isoprene units.

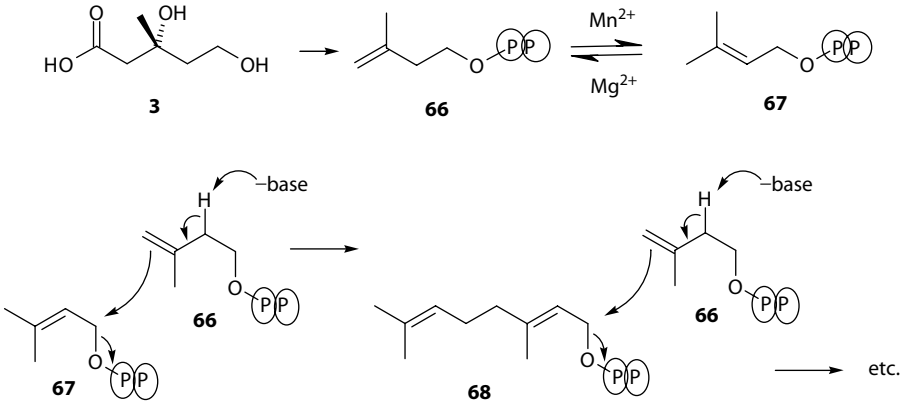


FIGURE 5.14 Coupling of C5 units in terpenoid biosynthesis.

This pattern of coupling is explained by the biosynthesis of terpenoids (Bu'Lock, 1965; Croteau, 1987; Mann et al., 1994). The key intermediate is mevalonic acid (**3**), which is made from three molecules of acetyl CoA (**2**). Phosphorylation of mevalonic acid followed by elimination of the tertiary alcohol and concomitant decarboxylation of the adjacent acid group gives isopentenyl pyrophosphate (**66**). This can be isomerized to give prenyl pyrophosphate (**67**). Coupling of these two 5-carbon units gives a 10-carbon unit, geranyl pyrophosphate (**68**), as shown in Figure 5.14 and further additions of isopentenyl pyrophosphate (**66**) lead to 15-, 20-, 25-, and so on carbon units.

It is clear from the mechanism shown in Figure 5.14 that terpenoid structures will always contain a multiple of five carbon atoms when they are first formed. The first terpenoids to be studied contained 10 carbon atoms per molecule and were called monoterpenoids. This nomenclature has remained and so those with five carbon atoms are known as hemiterpenoids, those with 15, sesquiterpenoids, and those with 20, diterpenoids, and so on. In general, only the hemiterpenoids, monoterpenoids, and sesquiterpenoids are sufficiently volatile to be components of essential oils. Degradation products of higher terpenoids do occur in essential oils, so they will be included in this chapter.

5.5.1 Hemiterpenoids

Many alcohols, aldehydes, and esters, with a 2-methylbutane skeleton, occur as minor components in essential oils. Not surprisingly, in view of the biosynthesis, the commonest oxidation pattern is that of prenol, that is, 3-methylbut-2-ene-1-ol. For example, the acetate of this alcohol occurs in ylang ylang and a number of other oils. However, oxidation has been observed at all positions. Esters such as prenyl acetate give fruity top notes to oils containing them and the corresponding thioesters contribute to the characteristic odor of galbanum.

5.5.2 Monoterpenoids

Geranyl pyrophosphate (**68**) is the precursor for the monoterpenoids. Heterolysis of its carbon–oxygen bond gives the geranyl carbocation (**69**). In natural systems, this and other carbocations discussed in this chapter do not exist as free ions but rather as incipient carbocations held in enzyme active sites and essentially prompted into cation reactions by the approach of a suitable reagent. For the sake of simplicity, they will be referred to here as carbocations. The reactions are described in chemical terms but all are under enzymic control and the enzymes present in any given plant will determine the terpenoids it will produce. Thus essential oil composition can give information about the genetic make-up of the plant. A selection of some of the key biosynthetic routes to monoterpenoids (Devon and Scott, 1972) is shown in Figure 5.15.

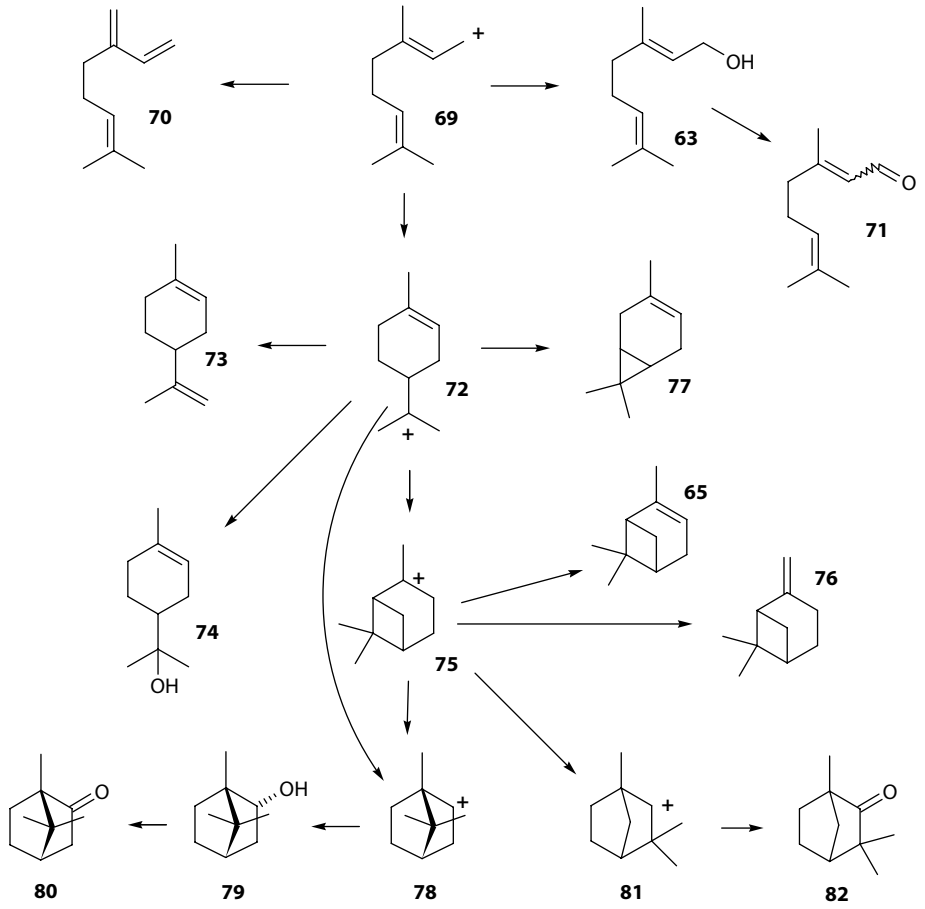


FIGURE 5.15 Formation of monoterpenoid skeletons.

Reaction of the geranyl carbocation with water gives geraniol (**63**) that can subsequently be oxidized to citral (**71**). Loss of a proton from (**69**) gives myrcene (**70**) and this can be isomerized to other acyclic hydrocarbons. An intramolecular electrophilic addition reaction of (**69**) gives the monocyclic carbocation (**72**) that can eliminate a proton to give limonene (**73**) or add water to give α-terpineol (**74**). A second intramolecular addition gives the pinyl carbocation (**75**) that can lose a proton to give either α-pinene (**65**) or β-pinene (**76**). The pinyl carbocation (**75**) is also reachable directly from the menthyl carbocation (**72**). Carene (**77**), another bicyclic material, can be produced through similar reactions. Wagner–Meerwein rearrangement of the pinyl carbocation (**75**) gives the bornyl carbocation (**78**). Addition of water to this gives borneol (**79**) and this can be oxidized to camphor (**80**). An alternative Wagner–Meerwein rearrangement of (**75**) gives the fenchyl skeleton (**81**) from which fenchone (**82**) is derived.

Some of the more commonly encountered monoterpenoid hydrocarbons (Arctander, 1960; Essential Oils Database, n.d.; Gildemeister and Hoffmann, 1956; Günther, 1948; Sell, 2007) are shown in Figure 5.16. Many of these can be formed by dehydration of alcohols and so their presence in essential oils could be as artifacts arising from the extraction process. Similarly, *p*-cymene (**83**) is one of the most stable materials of this class and can be formed from many of the others by appropriate cyclization and/or isomerization and/or oxidation reactions and so its presence in any essential oil could be as an artifact.

Myrcene (**70**) is very widespread in nature. Some sources, such as hops, contain high levels and it is found in most of the common herbs and spices. All isomers of α-ocimene (**84**), β-ocimene (**85**), and allo-ocimene (**86**) are found in essential oils, the isomers of β-ocimene (**85**) being the most frequently encountered. Limonene (**73**) is present in many essential oils but the major occurrence is in the citrus oils that contain levels up to 90%. These oils contain the dextrorotatory (*R*)-enantiomer, and its antipode is much less common. Both α-phellandrene (**87**) and β-phellandrene (**88**) occur widely in essential oils. For example, (–)-α-phellandrene is found in *Eucalyptus dives* and (*S*)-(–)-β-phellandrene in the lodgepole pine, *Pinus contorta*. *p*-Cymene (**83**) has been identified in many essential oils and plant extracts and thyme and oregano oils are particularly rich in it. α-Pinene (**65**),

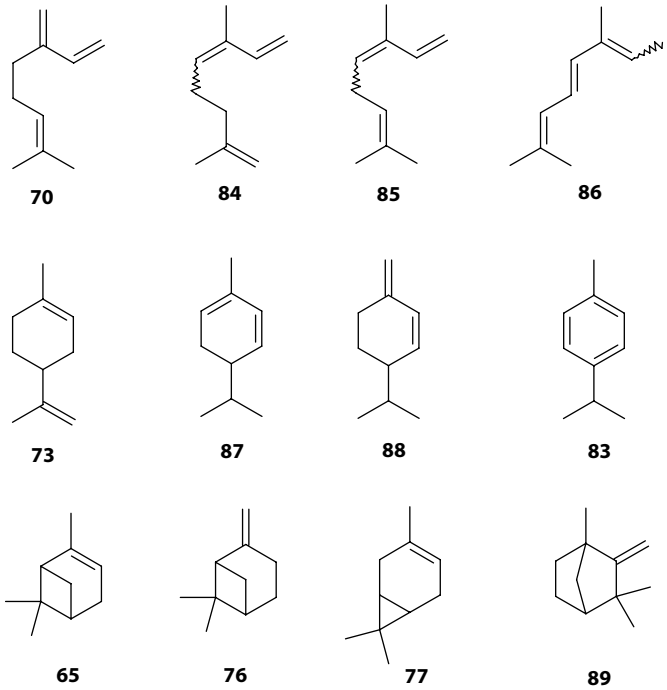


FIGURE 5.16 Some of the more common terpenoid hydrocarbons.

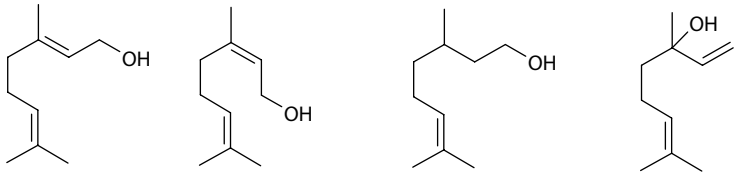
β-pinene (**76**), and 3-carene (**77**) are all major constituents of turpentine from a wide range of pines, spruces, and firs. The pinenes are often found in other oils, 3-carene less so. Like the pinenes, camphene (**89**) is widespread in nature.

Simple hydrolysis of geranyl pyrophosphate gives geraniol, (*E*)-3,7-dimethylocta-2,6-dienol (**63**). This is often accompanied in nature by its geometric isomer, nerol (**90**). Synthetic material is usually a mixture of the two isomers and when interconversion is possible, the equilibrium mixture comprises about 60% geraniol (**63**) and 40% nerol (**90**). The name geraniol is often used to describe a mixture of geraniol and nerol. When specifying the geometry of these alcohols it is better to use the modern (*E*)/(*Z*) nomenclature as the terms *cis* and *trans* are somewhat ambiguous in this case and earlier literature is not consistent in their use. Both isomers occur in a wide range of essential oils, geraniol (**63**) being particularly widespread. The oil of *Monarda fistulosa* contains over 90% geraniol (**63**) and the level in palmarosa is over 80%. Geranium contains about 50% and citronella and lemongrass each contain about 30%. The richest natural sources of nerol include rose, palmarosa, citronella, and davana although its level in these is usually only in the 10–15% range. Citronella and related species are used commercially as sources of geraniol but the price is much higher than that of synthetic material. Citronellol (**91**) is a dihydrogeraniol and occurs widely in nature in both enantiomeric forms. Rose, geranium, and citronella are the oils with the highest levels of citronellol. Geraniol, nerol, and citronellol, together with 2-phenylethanol, are known as the rose alcohols because of their occurrence in rose oils and also because they are the key materials responsible for the rose odor character. Esters (the acetates in particular) of all these alcohols are also commonly encountered in essential oils (Figure 5.17).

Allylic hydrolysis of geranyl pyrophosphate produces linalool (**92**). Like geraniol, linalool occurs widely in nature. The richest source is Ho leaf, the oil of which can contain well over 90% linalool. Other rich sources include linaloe, rosewood, coriander, freesia, and honeysuckle. Its acetate is also frequently encountered and is a significant contributor to the odors of lavender and citrus leaf oils.

Figure 5.18 shows a selection of cyclic monoterpenoid alcohols. α-Terpineol (**74**) is found in many essential oils as is its acetate. The isomeric terpinen-4-ol (**93**) is an important component of Ti tree oil but its acetate, surprisingly, is more widely occurring, being found in herbs such as marjoram and rosemary. *l*-Menthol (**94**) is found in various mints and is responsible for the cooling effect of oils containing it. There are eight stereoisomers of the menthol structure, *l*-menthol is the commonest in nature and also has the strongest cooling effect. The cooling effect makes menthol and mint oils valuable commodities, the two most important sources being cornmint (*Mentha arvensis*) and peppermint (Mentha piperita). Isopulegol (**95**) occurs in some species including *Eucalyptus citriodora* and citronella. Borneol (*endo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol) (**79**) and esters thereof, particularly the acetate, occur in many essential oils. Isoborneol (*exo*-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol) (**96**) is less common; however, isoborneol and its esters are found in quite a number of oils. Thymol (**97**), being a phenol, possesses antimicrobial properties, and oils, such as thyme and basil, which find appropriate use in herbal remedies. It is also found in various *Ocimum* and *Monarda* species.

Three monoterpenoid ethers are shown in Figure 5.19. 1,8-Cineole (**98**), more commonly referred to simply as cineole, comprises up to 95% of the oil of *Eucalyptus globulus* and about 40–50% of



63 **90** **91** **92**

FIGURE 5.17 Key acyclic monoterpenoid alcohols.

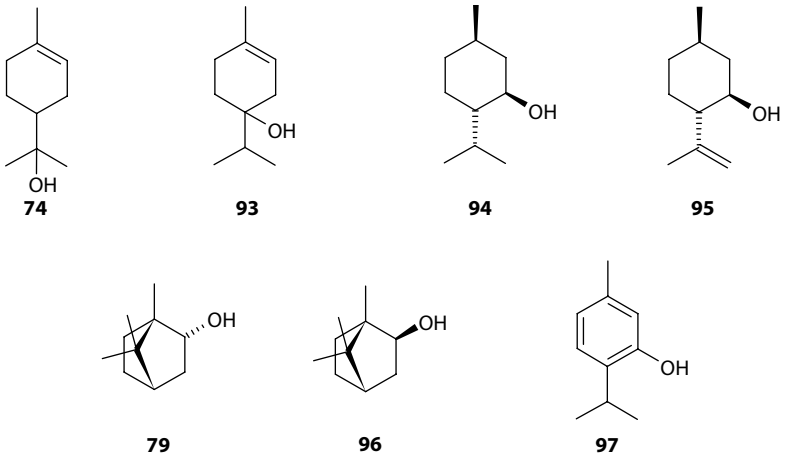


FIGURE 5.18 Some cyclic monoterpenoid alcohol.

Cajeput oil. It also can be found in an extensive range of other oils and often as a major component. It has antibacterial and decongestant properties and consequently, eucalyptus oil is used in various paramedical applications. Menthofuran (**99**) occurs in mint oils and contributes to the odor of peppermint. It is also found in several other oils. Rose oxide is found predominantly in rose and geranium oils. There are four isomers, the commonest being the laevorotatory enantiomer of *cis*-rose oxide (**100**). This is also the isomer with the lowest odor threshold of the four.

The two most significant monoterpene aldehydes are citral (**71**) and its dihydro analogue citronellal (**103**), both of which are shown in Figure 5.20. The word citral is used to describe a mixture of the two geometric isomers geranial (**101**) and neral (**102**) without specifying their relative proportions. Citral occurs widely in nature, both isomers usually being present, the ratio between them usually being in the 40:60 to 60:40 range. Lemongrass contains 70–90% citral and the fruit of *Litsea cubeba* contains about 60–75%. Citral also occurs in *Eucalyptus staigeriana*, lemon balm, ginger, basil, rose, and citrus species. It is responsible for the characteristic smell of lemons although lemon oil usually contains only a few percent of it. Citronellal (**103**) also occurs widely in essential oils. *Eucalyptus citriodora* contains up to 85% citronellal and significant amounts are also found in some chemotypes of *Litsea cubeba*, citronella Swangi leaf oil, and *Backhousia citriodora*. Campholenic aldehyde (**104**) occurs in a limited range of species such as olibanum, styrax, and some eucalypts. Material produced from α-pinene (**65**) is important as an intermediate for synthesis.

Figure 5.21 shows some of the commoner monoterpenoid ketones found in essential oils. Both enantiomers of carvone are found in nature, the (*R*)-(–)- (usually referred to as *l*-carvone) (**105**) being the commoner. This enantiomer provides the characteristic odor of spearmint (*Mentha cardiaca, Mentha gracilis, Mentha spicata*, and *Mentha viridis*), the oil of which usually contains 55–75% of *l*-carvone. The (*S*)-(+)-enantiomer (**106**) is found in caraway at levels of 30–65% and

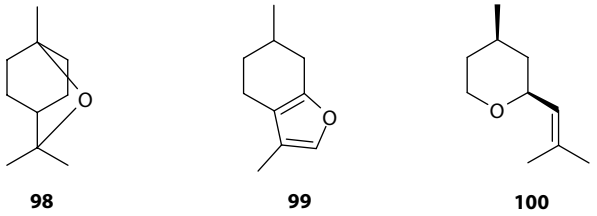


FIGURE 5.19 Some monoterpenoid ethers.

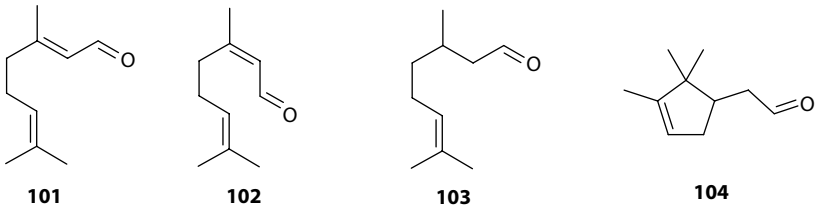


FIGURE 5.20 Some monoterpenoid aldehydes.

in dill at 50–75%. Menthone is fairly common in essential oils particularly in the mints, pennyroyal, and sages but lower levels are also found in oils such as rose and geranium. The *l*-isomer is commoner than the *d*-isomer. Isomenthone is the *cis*-isomer and the two interconvert readily by epimerization. The equilibrium mixture comprises about 70% menthone and 30% isomenthone. The direction of rotation of plane-polarized light reverses on epimerization and therefore *l*-menthone (**107**) gives *d*-isomenthone (**108**). (+)-Pulegone (**109**) accounts for about 75% of the oil of pennyroyal and is also found in a variety of other oils. (–)-Piperitone (**110**) also occurs in a variety of oils, the richest source being *Eucalyptus dives*. Both pulegone and piperitone have strong minty odors. Camphor (**80**) occurs in many essential oils and in both enantiomeric forms. The richest source is the oil of camphor wood but it is also an important contributor to the odor of lavender and of herbs such as sage and rosemary. Fenchone (**82**) occurs widely, for example, in cedar leaf and lavender. Its laevorotatory enantiomer is an important contributor to the odor of fennel.

5.5.3 Sesquiterpenoids

By definition, sesquiterpenoids contain 15 carbon atoms. This results in their having lower volatilities and hence higher boiling points than monoterpenoids. Therefore, fewer of them (in percentage terms) contribute to the odor of essential oils but those that do often have low-odor thresholds and contribute significantly as end notes. They are also important as fixatives for more volatile components.

Just as geraniol (**63**) is the precursor for all the monoterpenoids, farnesol (**111**) is the precursor for all the sesquiterpenoids. Its pyrophosphate is synthesized in nature by the addition of isopentenyl pyrophosphate (**66**) to geranyl pyrophosphate (**68**) as shown in Figure 5.14 and hydrolysis of that gives farnesol. Incipient heterolysis of the carbon–oxygen bond of the phosphate gives the nascent

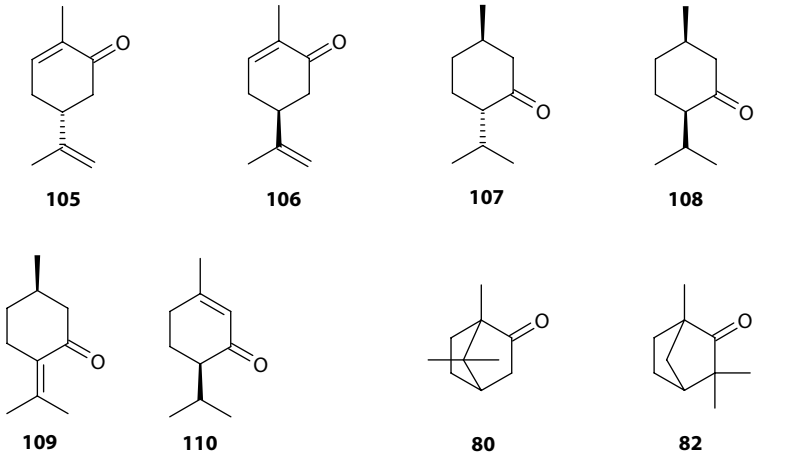


FIGURE 5.21 Some monoterpenoid ketones.

farnesyl carbocation (**112**) and this leads to the other sesquiterpenoids, just as the geranyl carbocation does to monoterpenoids. Starting from farnesyl pyrophosphate, the variety of possible cyclic structures is much greater than that from geranyl pyrophosphate because there are now three double bonds in the molecule. Similarly, there is also a greater scope for further structural variation resulting from rearrangements, oxidations, degradation, and so on (Devon and Scott, 1972). The geometry of the double bond in position 2 of farnesol is important in terms of determining the pathway used for subsequent cyclization reactions and so these are best discussed in two blocks.

Figure 5.22 shows a tiny fraction of the biosynthetic pathways derived from (*Z,E*)-farnesyl pyrophosphate. Direct hydrolysis leads to acyclic sesquiterpenoids such as farnesol (**111**) and nerolidol (**113**). However, capture of the carbocation (**112**) by the double bond at position 6 gives a cyclic structure that of the bisabolane skeleton (**114**) and quenching of this with water gives bisabolol (**115**). A hydrogen shift in (**114**) leads to the isomeric carbocation (**116**) that still retains the bisabolane skeleton. Further cyclizations and rearrangements take the molecule through various skeletons, including those of the acorane (**117**) and cedrane (**118**) families, to the khusane family, illustrated by khusimol (**119**) in the figure. Obviously, a wide variety of materials can be generated along this route, an example being cedrol (**120**) formed by reaction of cation (**118**) with water. The bisabolyl carbocation (**114**) can also cyclize to the other double bonds in the molecule leading to, *inter alia*, the campherenane skeleton (**121**) and hence α-santalol (**122**) and β-santalol (**123**), or, via the cuparane (**124**) and chamigrane (**125**) skeletons, to compounds such as thujopsene (**126**). The carbocation function in

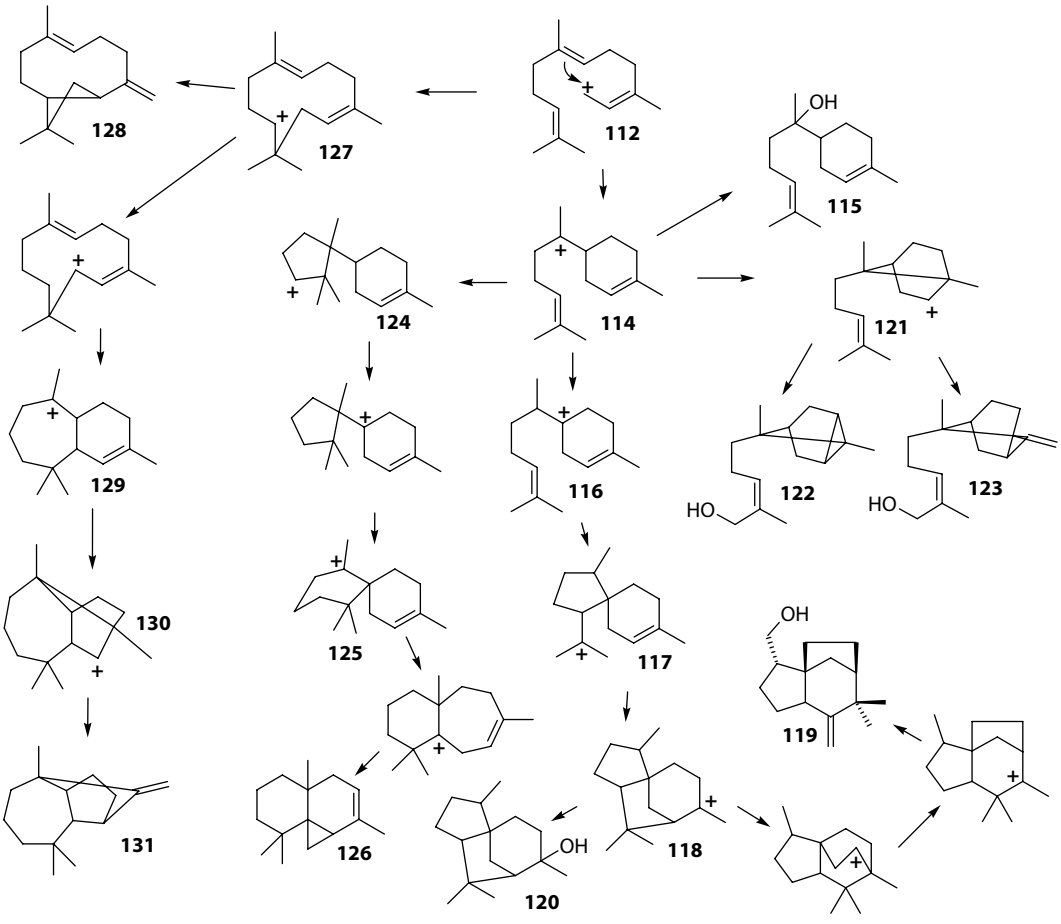


FIGURE 5.22 Some biosynthetic pathways from (*Z,E*)-farnesol.

(**112**) can also add to the double bond at the far end of the chain to give the *cis*-humulane skeleton (**127**). This species can cyclize back to the double bond at carbon 2 before losing a proton, thus giving caryophyllene (**128**). Another alternative is for a series of hydrogen shifts, cyclizations, and rearrangements to lead it through the himachalane (**129**) and longibornane (**130**) skeletons to longifolene (**131**).

Figure 5.23 shows a few of the many possibilities for biosynthesis of sesquiterpenoids from (*E*,*E*)-farnesyl pyrophosphate. Cyclization of the cation (**132**) to C-11, followed by loss of a proton gives all *trans*- or α-humulene (**133**), whereas cyclization to the other end of the same double bond gives a carbocation (**134**) with the germacrane skeleton. This is an intermediate in the biosynthesis of odorous sesquiterpenes such as nootkatone (**135**) and α-vetivone (**137**). β-Vetivone (**137**) is synthesized through a route that also produces various alcohols, for example, (**138**) and (**139**), and an ether (**140**) that has the eudesmane skeleton. Rearrangement of the germacrane carbocation (**134**) leads to a carbocation (**141**) with the guaiane skeleton and this is an intermediate in the synthesis of guaiol (**142**). Carbocation (**141**) is also an intermediate in the biosynthesis of the α-patchoulane (**143**) and β-patchoulane (**144**) skeletons and of patchouli alcohol (**145**).

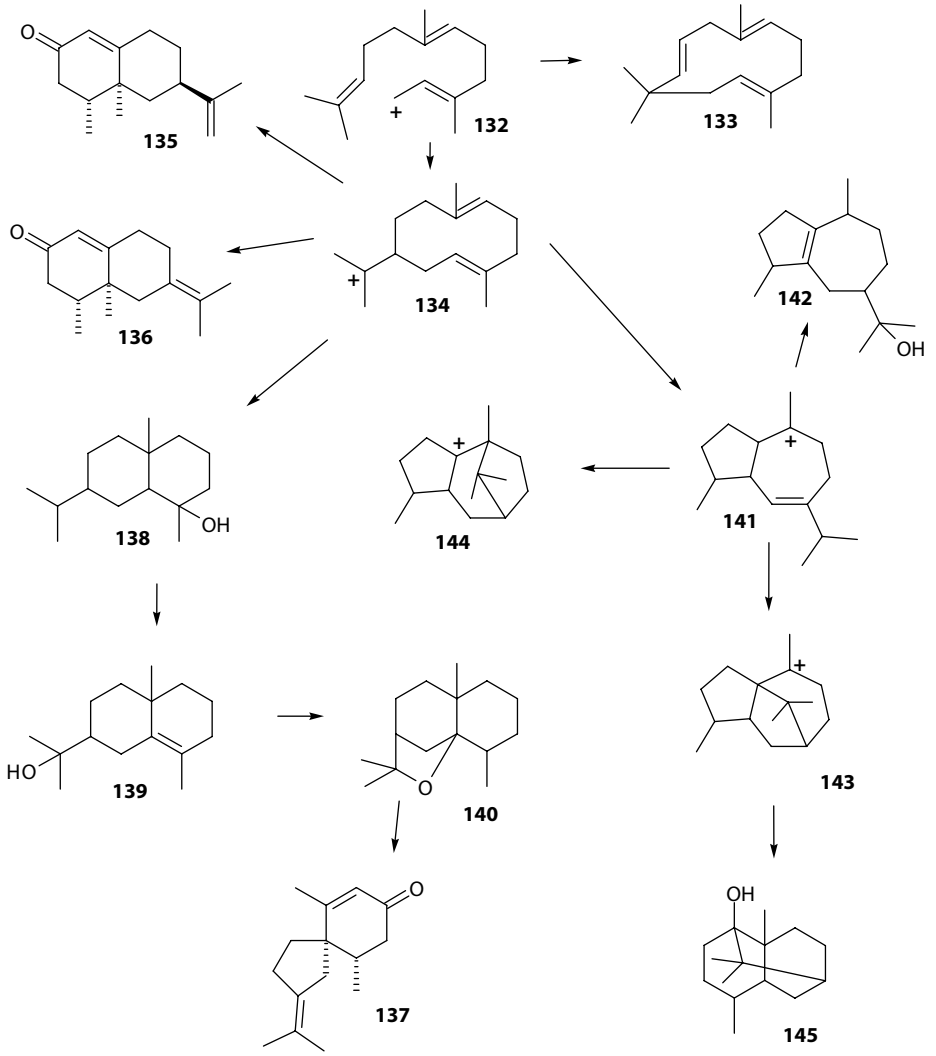


FIGURE 5.23 Some biosynthetic pathways from (*E*,*E*)-farnesol.

All four isomers of farnesol (**111**) are found in nature and all have odors in the muguet and linden direction. The commonest is the (*E,E*)-isomer that occurs in, among others, cabreuva and ambrette seed while the (*Z,E*)-isomer has been found in jasmine and ylang, the (*E,Z*)-isomer in cabreuva, rose, and neroli, and the (*Z,Z*)-isomer in rose. Nerolidol (**113**) is the allylic isomer of farnesol and exists in four isomeric forms, namely, two enantiomers each of two geometric isomers. The (*E*)-isomer has been found in cabreuva, niaouli, and neroli oils among others and the (*Z*)-isomer in neroli, jasmine, ho leaf, and so on. Figure 5.24 shows the structures of farnesol and nerolidol with all of the double bonds in the *trans*-configuration.

α-Bisabolol (**115**) is the simplest of the cyclic sesquiterpenoid alcohols. If farnesol is the sesquiterpenoid equivalent of geraniol and nerolidol of linalool, then α-bisabolol is the equivalent of α-terpineol. It has two chiral centers and therefore exists in four stereoisomeric forms, all of which occur in nature. The richest natural source is *Myoporum crassifolium* Forst., a shrub from New Caledonia, but α-bisabolol can be found in many other species including chamomile, lavender, and rosemary. It has a faint floral odor and anti-inflammatory properties and is responsible, at least in part, for the related medicinal properties of chamomile oil.

The santalols (**122**) and (**123**) have more complex structures and are the principal components of sandalwood oil. Cedrol (**120**) is another complex alcohol but it is more widely occurring in nature than the santalols. It is found in a wide range of species, the most significant being trees of the *Juniperus, Cupressus*, and *Thuja* families. Cedrene (**146**) occurs alongside cedrol in cedarwood oils. Cedrol is dehydrated to cedrene in the presence of acid and so the latter can be an artifact of the former and the ratio of the two will often depend on the method of isolation. Thujopsene (**126**) also occurs in cedarwood oils, usually at a similar level to that of cedrol/cedrene, and it is found in various other oils also. Caryophyllene (**128**) and α-humulene (the all *trans* isomer) (**133**) are widespread in nature, cloves being the best-known source of the former and hops of the latter. The ring systems of these two materials are very strained making them quite reactive chemically and caryophyllene, extracted from clove oil as a by-product of eugenol production, is used as the starting material in the synthesis of several fragrance ingredients. Longifolene (**131**) also possesses a strained ring system. It is a component of Indian turpentine and is therefore readily available as a feedstock for fragrance ingredient manufacture.

Guaicwood oil is the richest source of guaiol (**142**) and the isomeric bulnesol (**147**) but both are found in other oils, particularly guaiol that occurs in a wide variety of plants. Dehydration and dehydrogenation of these give guaiazulene (**148**), which is used as an anti-inflammatory agent. Guaiazulene is also accessible from α-gurjunene (**149**), the major component of gurjun balsam. Guaiazulene is blue in color as is the related olefin chamazulene (**150**). The latter occurs in a variety of oils but it is particularly important in chamomile to which it imparts the distinctive blue tint (Figure 5.25).

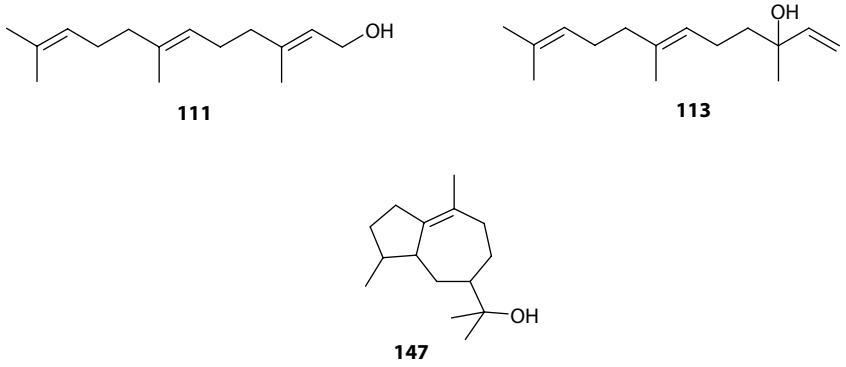


FIGURE 5.24 Some sesquiterpenoid alcohols.

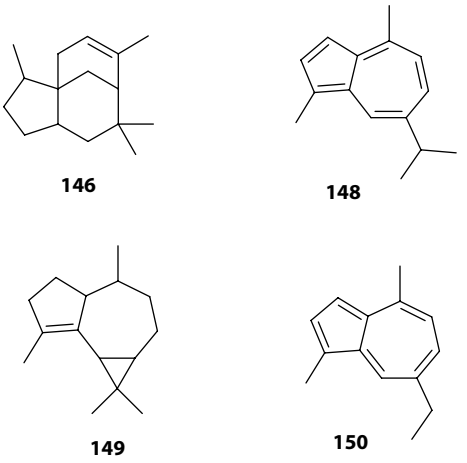


FIGURE 5.25 Some sesquiterpenoid hydrocarbons.

Vetiver and patchouli are two oils of great importance in perfumery (Williams, 1996, 2004). Both contain complex mixtures of sesquiterpenoids, mostly with complex polycyclic structures (Sell, 2003). The major components of vetiver oil are α-vetivone (**136**), β-vetivone (**137**), and khusimol (**119**), but the most important components as far as odor is concerned are minor constituents such as khusimone (**151**), zizanal (**152**), and methyl zizanoate (**153**). Nootkatone (**154**) is an isomer of α-vetivone and is an important odor component of grapefruit. Patchouli alcohol (**145**) is the major constituent of patchouli oil but, as is the case also with vetiver, minor components are more important for the odor profile. These include nor-patchoulenol (**155**) and nor-tetrapatchoulol (**156**) (Figure 5.26).

The molecules of chamazulene (**150**), khusimone (**151**), nor-patchoulenol (**155**), and nor-tetrapatchoulol (**156**) each contain only 14 carbon atoms in place of the normal 15 of sesquiterpenoids. They are all degradation products of sesquiterpenoids. Degradation, either by enzymic action or from environmental chemical processes, can be an important factor in generating essential oil components. Carotenoids are a family of tetraterpenoids characterized by having a tail-to-tail fusion between two diterpenoid fragments. In the case of β-carotene (**157**), both ends of the chain have been cyclized to form cyclohexane rings. Degradation of the central part of the chain leads to a number of fragments that are found in essential oils and the two major families of such are the ionones and damascones. Both have the same carbon skeleton but in the ionones (Leffingwell & Associates, n.d.;

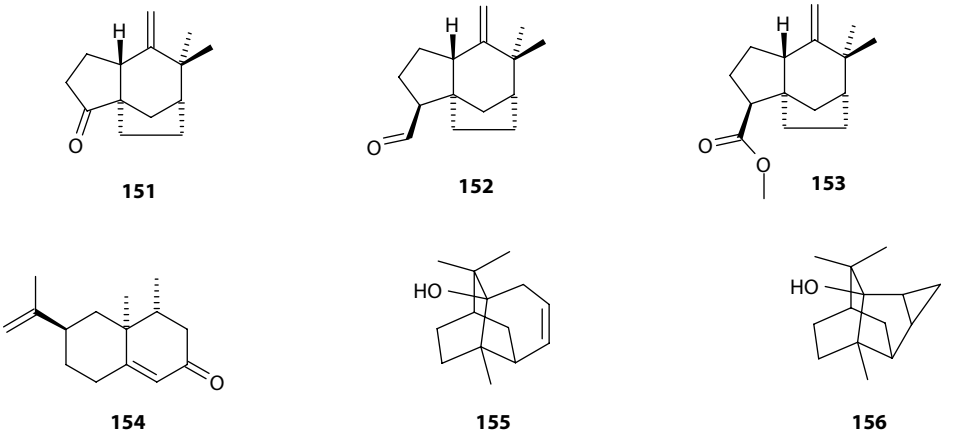


FIGURE 5.26 Components of vetiver, patchouli, and grapefruit.

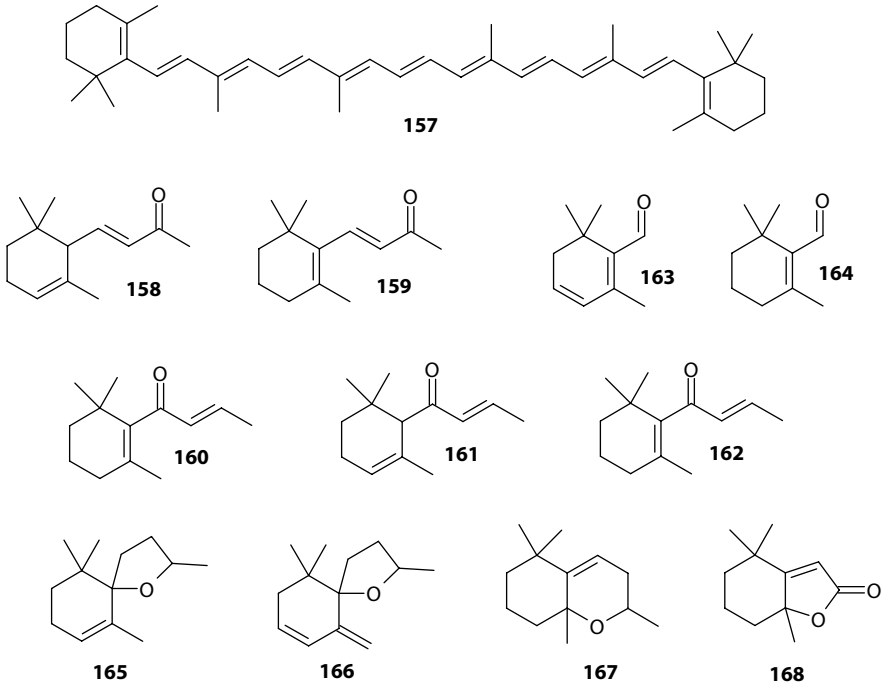


FIGURE 5.27 Carotenoid degradation products.

Sell, 2003) the site of oxygenation is three carbon atoms away from the ring, in damascones oxygenation is found at the chain carbon next to the ring (Figure 5.27).

The ionones occur naturally in a wide variety of flowers, fruits, and leaves, and are materials of major importance in perfumery (Arctander, 1960; Essential Oils Database, n.d.; Gildemeister and Hoffmann, 1956; Günther, 1948; Sell, 2007). About 57% of the volatile components of violet flowers are α- (**158**) and β-ionones (**159**) and both isomers occur widely in nature. The damascones are also found in a wide range of plants. They usually occur at a very low level but their very intense odors mean that they still make a significant contribution to the odors of oils containing them. The first to be isolated and characterized was β-damascenone (**160**), which was found at a level of 0.05% in the oil of the Damask rose. Both β-damascenone (**160**) and the α- (**161**) and β-isomers (**162**) have since been found in many different essential oils and extracts. In the cases of safranal (**163**) and cyclocitral (**165**), the side chain is degraded even further leaving only one of its carbon atoms attached to the cyclohexane ring. About 70% of the volatile component of saffron is safranal and it makes a significant contribution to its odor. Other volatile carotenoid degradation products that occur in essential oils and contribute to their odors include the theaspiranes (**165**), vitispiranes (**166**), edulans (**167**), and dihydroactindiolide (**168**).

The similarity in structure between the ionones and the irones might lead to the belief that the latter are also carotenoid derived. However, this is not the case as the irones are formed by degradation of the triterpenoid iripallidal (**169**), which occurs in the rhizomes of the iris. The three isomers, α- (**170**), β- (**171**), and γ-irone (**172**), are all found in iris and the first two in a limited number of other species (Figure 5.28).

5.6 SYNTHESIS OF ESSENTIAL OIL COMPONENTS

It would be impossible, in a volume of this size, to review all of the reported syntheses of essential oil components and so the following discussion will concentrate on some of the more commercially important synthetic routes to selected key substances. In the vast majority of cases, there is a balance between routes using plant extracts as feedstocks and those using petrochemicals. For some

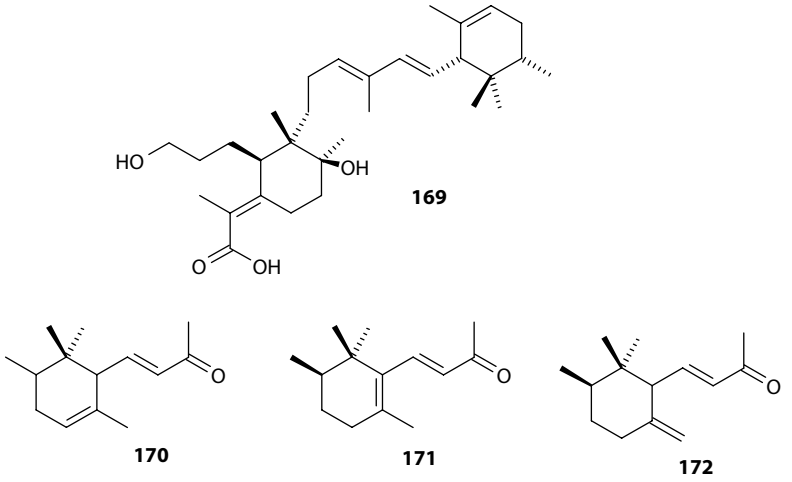


FIGURE 5.28 Iripallidal and the irones.

materials, plant-derived and petrochemical-derived equivalents might exist in economic competition while for others, one source is more competitive. The balance will vary over time and the market will respond accordingly. Sustainability of production routes is a complex issue and easy assumptions might be totally incorrect. Production and extraction of plant-derived feedstocks often requires considerable expenditure of energy in fertilizer production, harvesting, and processing and so it is quite possible that production of a material derived from a plant source would use more mineral oil than the equivalent derived from petrochemical feedstocks.

Figure 5.29 shows some of the plant-derived feedstocks used in the synthesis of lipids and polyketides (Sell, 2006). Rapeseed oil provides erucic acid (**173**) that can be ozonolyzed to give brassylic acid (**174**) and heptanal (**175**), both useful building blocks. The latter can also be obtained, together with undecylenic acid (**176**), by pyrolysis of ricinoleic acid (**177**) that is available from Castor oil. Treatment of undecylenic acid (**176**) with acid leads to movement of the double bond along the chain and eventual cyclization to give γ-undecalactone (**178**), which has been found in narcissus oils. Aldol condensation of heptanal (**175**) with cyclopentanone, followed by Bayer–Villiger oxidation, gives δ-dodecalactone (**179**), identified in the headspace of tuberose. Such aldol reactions, followed by appropriate further conversions, are important in the commercial production of analogues of methyl jasmonate (**26**) and jasmone (**27**).

Ethylene provides a good example of a petrochemical feedstock for the synthesis of lipids and polyketides. It can be oligomerized to provide a variety of alkenes into which functionalization can be introduced by hydration, oxidation, hydroformylation, and so on. Of course, telomerization can be used to provide functionalized materials directly.

Eugenol (**53**) (e.g., clove oil) and safrole (**59**) (e.g., sassafras) are good examples of plant-derived feedstocks that are used in the synthesis of other shikimates. Methylation of eugenol produces methyleugenol (**56**) and this can be isomerized using acid or metal catalysts to give methylisoeugenol (**57**). Similarly, isomerization of eugenol gives isoeugenol (**54**) and oxidative cleavage of this, for example, by ozonolysis gives vanillin (**55**). This last sequence of reactions, when applied to safrole gives isosafrole (**60**) and heliotropin (**61**). All of these conversions are shown in Figure 5.30.

Production of shikimates from petrochemicals for commercial use mostly involves straightforward chemistry (Arctander, 1969; Bauer and Panten, 2006; Däniker, 1987; Sell, 2006). Nowadays the major starting materials are benzene (**180**) and toluene (**181**), which are both available in bulk from petroleum fractions. Alkylation of benzene with propylene gives cumene (**182**), the hydroperoxide of which fragments to give phenol (**183**) and acetone. Phenol itself is an important molecular building block and further oxidation gives catechol (**184**). Syntheses using these last two materials will be discussed below. Alkylation of benzene with ethylene gives ethylbenzene, which is converted to styrene (**185**) via autoxidation, reduction, and elimination in a process known as styrene

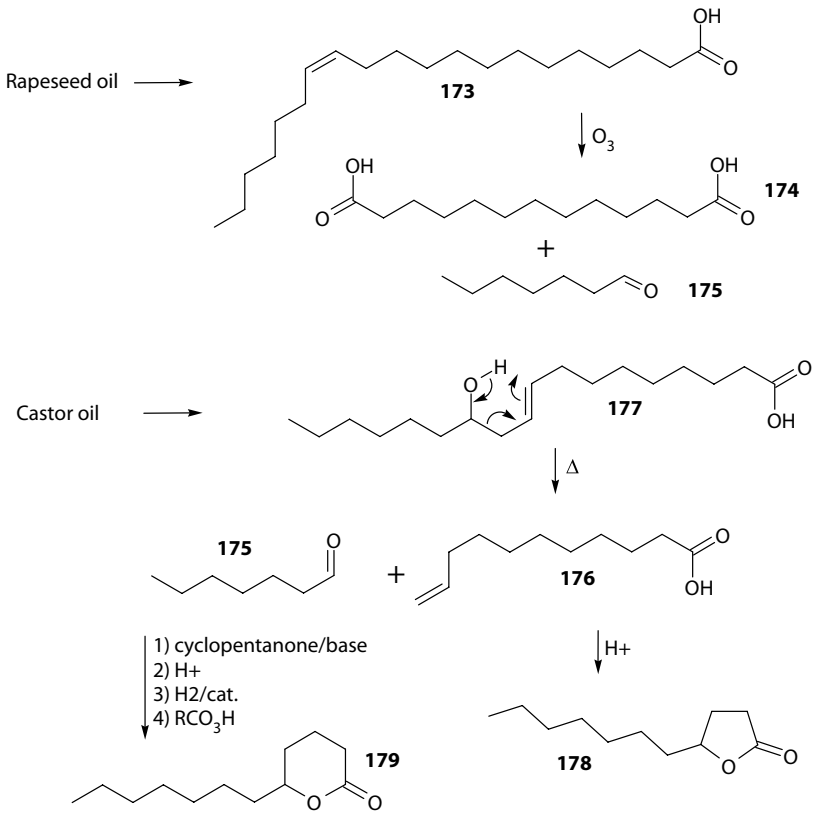


FIGURE 5.29 Some natural feedstocks for synthesis of lipids and polyketides.

monomer/propylene oxide (SMPO) process. The epoxide (**186**) of styrene serves as an intermediate for 2-phenylethanol (**47**) and phenylacetaldehyde (**187**), both of which occur widely in essential oils. 2-Phenylethanol is also available directly from benzene by Lewis acid catalyzed addition of ethylene oxide and as a by-product of the SMPO process. Currently, the volume available from the SMPO process provides most of the requirement. All of these processes are illustrated in Figure 5.31.

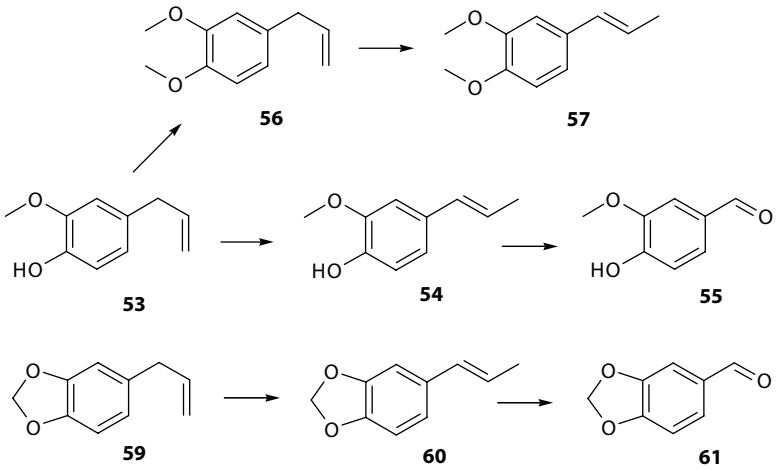


FIGURE 5.30 Shikimates from eugenol and safrole.

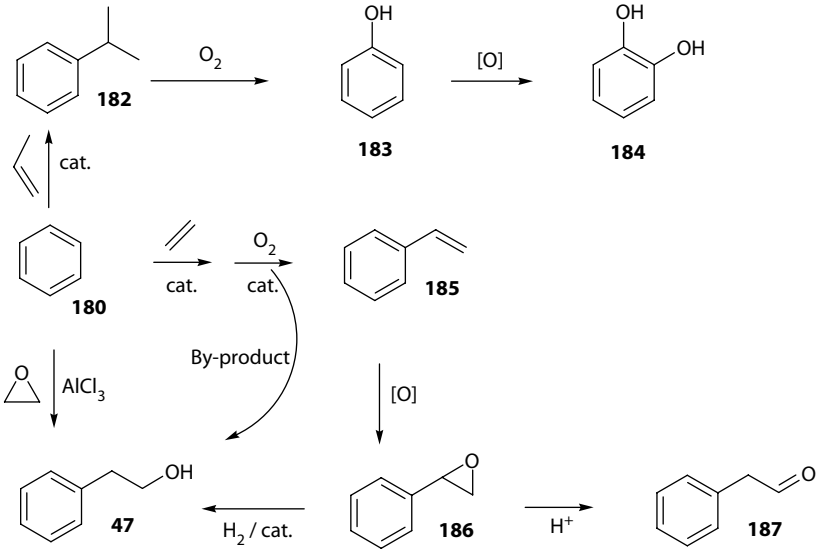


FIGURE 5.31 Benzene as a feedstock for shikimates.

Phenol (**183**) and related materials, such as guaiacol (**188**), were once isolated from coal tar but the bulk of their supply is currently produced from benzene via cumene as shown in Figure 5.31. The use of these intermediates to produce shikimates is shown in Figure 5.32. In principle, anethole (**53**) and estragole (methylchavicol) (**52**) are available from phenol, but in practice, the demand is met by extraction from turpentine. Carboxylation of phenol gives salicylic acid (**38**) and hence serves as a source for the various salicylate esters. Formylation of phenol by formaldehyde, in the presence of a suitable catalyst, has now replaced the Reimer–Tiemann reaction as a route to hydroxybenzaldehydes. The initial products are saligenin (**189**) and *p*-hydroxybenzyl alcohol (**190**), which can be oxidized to salicylaldehyde (**191**) and *p*-hydroxybenzaldehyde (**192**), respectively. Condensation of salicylaldehyde with acetic acid/acetic anhydride gives coumarin (**50**) and *O*-alkylation of *p*-hydroxybenzaldehyde gives anisaldehyde (**44**). As mentioned earlier, oxidation of phenol provides a route to catechol (**184**) and guaiacol (**188**). The latter is a precursor for vanillin, and catechol also provides a route to heliotropin (**61**) via methylenedioxy benzene (**193**).

Oxidation of toluene (**181**) with air or oxygen in the presence of a catalyst gives benzyl alcohol (**194**), benzaldehyde (**195**), or benzoic acid (**196**) depending on the chemistry employed. The demand for benzoic acid far exceeds that for the other two oxidation products and so such processes are usually designed to produce mostly benzoic acid with benzaldehyde as a minor product. For the fragrance industry, benzoic acid is the precursor for the various benzoates of interest while benzaldehyde, through aldol-type chemistry, serves as the key intermediate for cinnamate esters (such as methyl cinnamate (**197**)) and cinnamaldehyde (**48**). Reduction of the latter gives cinnamyl alcohol (**49**) and hence, through esterification, provides routes to all of the cinnamyl esters. Chlorination of toluene under radical conditions gives benzyl chloride (**198**). Hydrolysis of the chloride gives benzyl alcohol (**194**), which can, in principle, be esterified to give the various benzyl esters (**199**) of interest. However, these are more easily accessible directly from the chloride by reaction with the sodium salt of the corresponding carboxylic acid. All of these conversions are shown in Figure 5.33.

Methyl anthranilate (**41**) is synthesized from either naphthalene (**200**) or *o*-xylene (**201**) as shown in Figure 5.34. Oxidation of either starting material produces phthalic acid (**202**). Conversion of this diacid to its imide, followed by the Hoffmann reaction, gives anthranilic acid and the methyl ester can then be obtained by reaction with methanol.

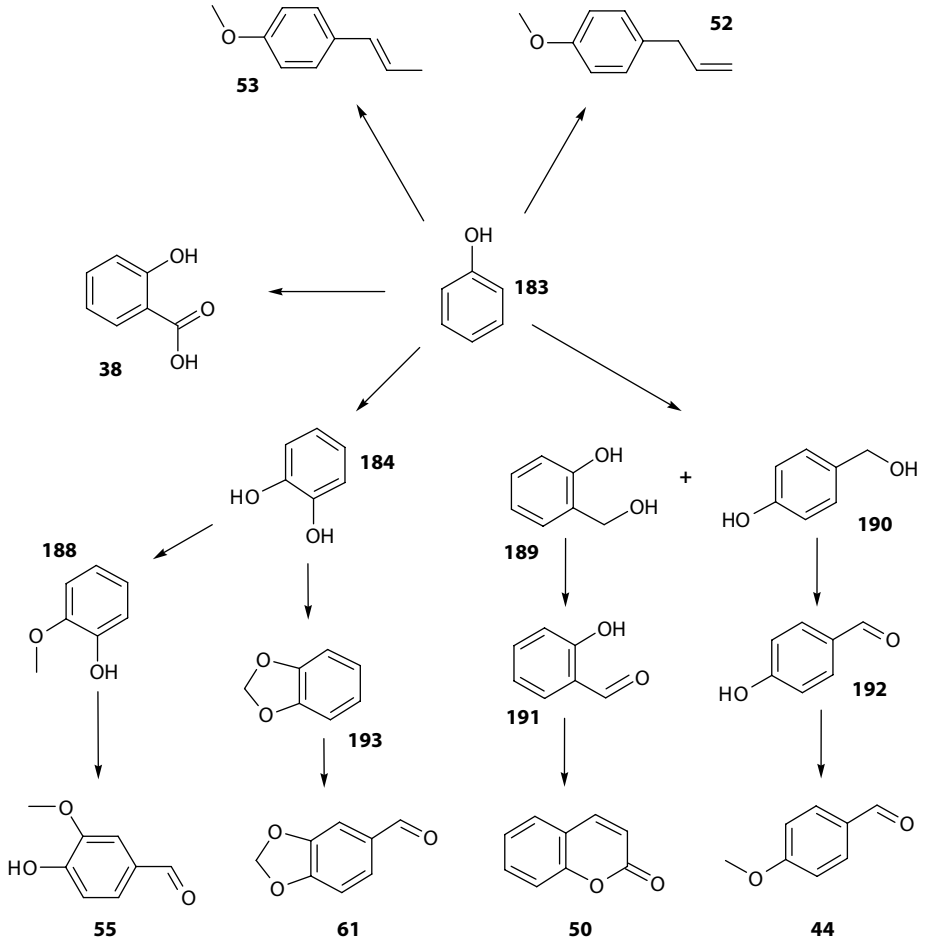


FIGURE 5.32 Synthesis of shikimates from phenol.

In volume terms, the terpenoids represent the largest group of natural and nature identical fragrance ingredients (Däniker, 1987; Sell, 2007). The key materials are the rose alcohols [geraniol (**63**)/nerol (**90**), linalool (**23**), and citronellol (**91**)], citronellal (**103**), and citral (**71**). Interconversion of these key intermediates is readily achieved by standard functional group manipulation. Materials in this family serve as starting points for the synthesis of a wide range of perfumery materials including esters of the rose alcohols. The ionones are prepared from citral by aldol condensation followed by cyclization of the intermediate ψ-ionones.

The sources of the above key substances fall into three main categories: natural extracts, turpentine, and petrochemicals. The balance depends on economics and also on the product in question. For example, while about 10% of geraniol is sourced from natural extracts, it is only about 1% in the case of linalool. Natural grades of geraniol are obtained from the oils of citronella, geranium, and palmarosa (including the variants jamrosa and dhanrosa). Citronella is also used as a source of citronellal. Ho, rosewood, and linaloe were used as sources of linalool but conservation and economic factors have reduced these sources of supply very considerably. Similarly, citral was once extracted from *Litsea cubeba* but over-harvesting has resulted in loss of that source.

Various other natural extracts are used as feedstocks for the production of terpenoids as shown in Figure 5.35. Two of the most significant ones are clary sage and the citrus oils (obtained as by-products of the fruit juice industry). After distillation of the oil from clary sage, sclareol (**203**) is extracted from the residue and this serves as a starting material for naphthofuran (**204**), known

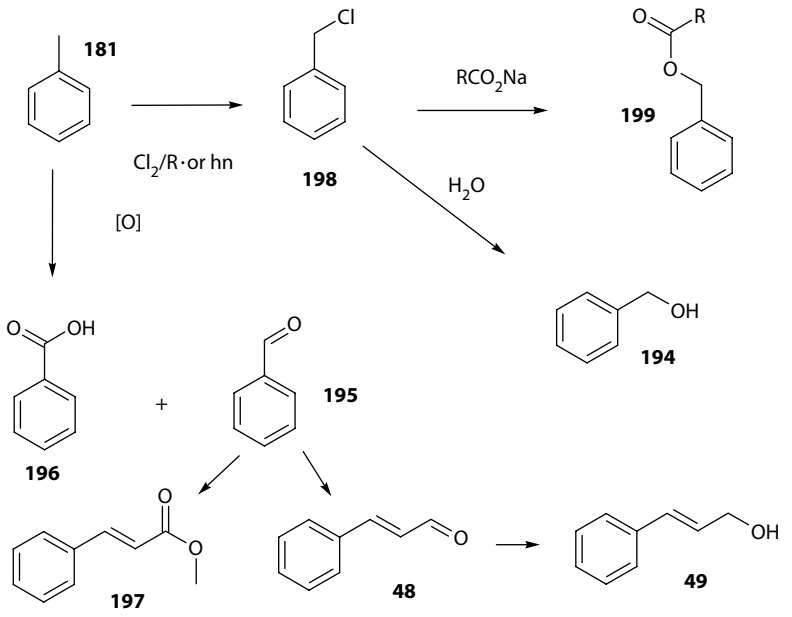


FIGURE 5.33 Shikimates from toluene.

under trade names such as Ambrofix, Ambrox, and Ambroxan. The conversion is shown in Figure 5.35. Initially, sclareol is oxidized to sclareolide (**205**). This was once effected using oxidants such as permanganate and dichromate but nowadays, the largest commercial process uses a biotechnological oxidation. Sclareolide is then reduced using lithium aluminum hydride, borane, or similar reagents and the resulting diol is cyclized to the naphthofuran. *d*-Limonene (**73**) and valencene (**206**) are both extracted from citrus oils. Reaction of *d*-limonene with nitrosyl choride gives an adduct that is rearranged to the oxime of *l*-carvone and subsequent hydrolysis produces the free ketone (**105**). Selective oxidation of valencene gives nootkatone (**135**).

Turpentine is obtained by tapping of pine trees and this product is known as gum turpentine. However, a much larger commercial source is the so-called crude sulfate turpentine (CST), which is obtained as a by-product of the Kraft paper process. The major components of turpentine are the two pinenes with α-pinene (**65**) predominating. Turpentine also serves as a source of *p*-cymene (**83**) and, as mentioned above, the shikimate anethole (**53**) (Zinkel and Russell, 1989).

Figure 5.36 shows some of the major products manufactured from α-pinene (**65**) (Sell, 2003, 2007). Acid-catalyzed hydration of α-pinene gives α-terpineol (**74**), which is the highest tonnage

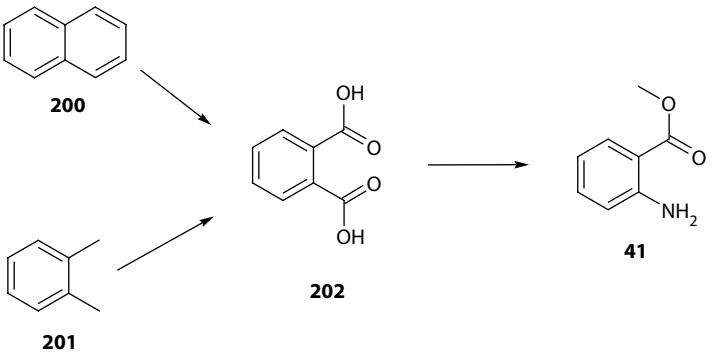


FIGURE 5.34 Synthesis of methyl anthranilate.

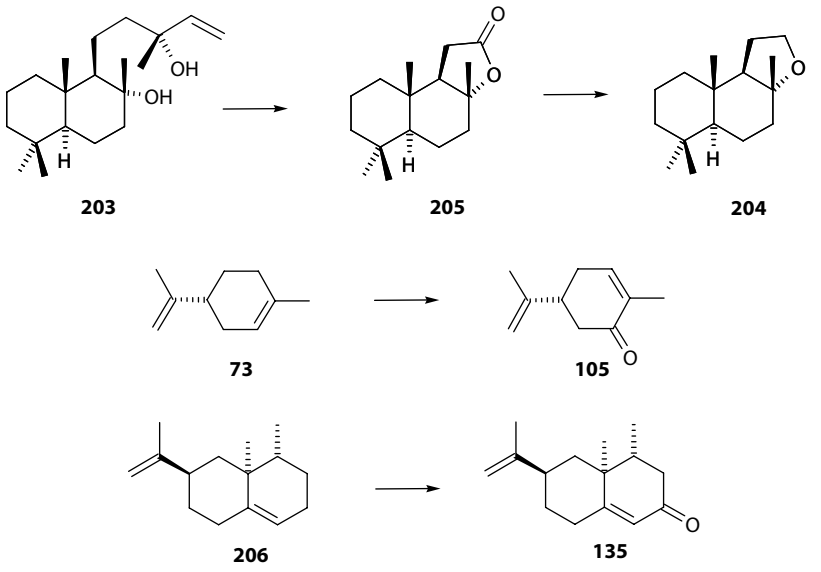


FIGURE 5.35 Partial synthesis of terpenoids from natural extracts.

material of all those described here. Acid-catalyzed rearrangement of α-pinene gives camphene (**89**) and this, in turn, serves as a starting material for production of camphor (**80**). Hydrogenation of α-pinene gives pinane (**207**), which is oxidized to pinanol (**208**) using air as the oxidant. Pyrolysis of pinanol produces linalool (**23**) and this can be rearranged to geraniol (**63**). Hydrogenation of geraniol gives citronellol (**91**) whereas oxidation leads to citral (**71**). The major use of citral is not as

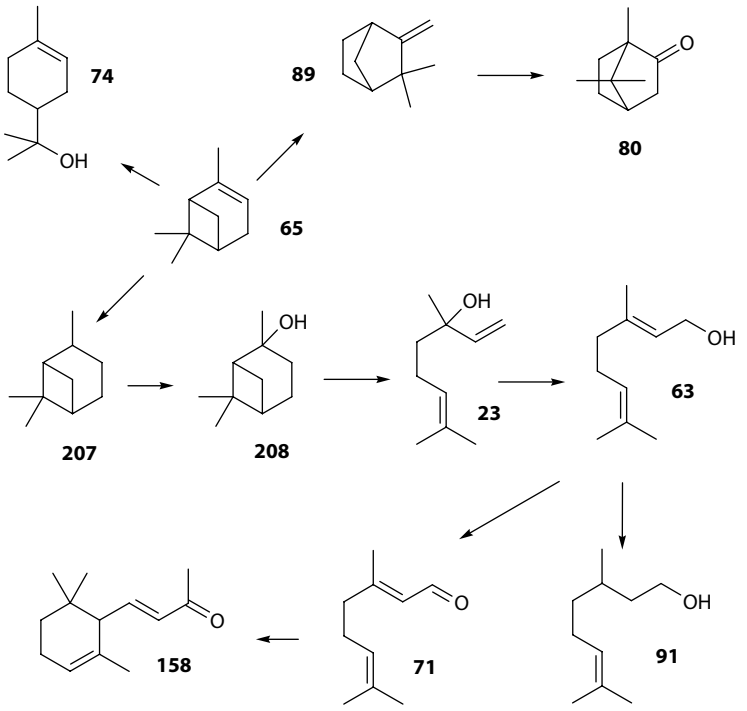


FIGURE 5.36 Products from α-pinene.

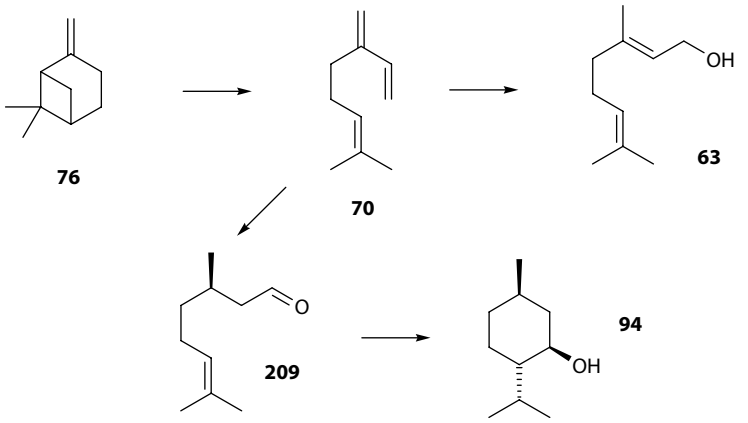


FIGURE 5.37 Products from β-pinene.

a material in its own right, but as a starting material for production of ionones, such as α-ionone (**158**) and vitamins A, E, and K.

Some of the major products manufactured from β-pinene (**76**) are shown in Figure 5.37. Pyrolysis of β-pinene gives myrcene (**70**) and this can be "hydrated" (not in one step but in a multistage process) to give geraniol (**63**). The downstream products from geraniol are then the same as those described in the preceding paragraph and shown in Figure 5.36. Myrcene is also a starting point for *d*-citronellal (**209**), which is one of the major feedstocks for the production of *l*-menthol (**94**) as will be described below.

Currently, there are two major routes to terpenoids that use petrochemical starting materials (Sell, 2003, 2007). The first to be developed is an improved version of a synthetic scheme demonstrated by Arens and van Dorp in 1948. The basic concept is to use two molecules of acetylene (**210**) and two of acetone (**211**) to build the structure of citral (**71**). The route, as it is currently practised, is shown in Figure 5.38. Addition of acetylene (**210**) to acetone (**211**) in the presence of base gives methylbutynol (**212**), which is hydrogenated, under Lindlar conditions, to methylbutenol (**213**). The second equivalent of acetone is introduced as the methyl ether of its enol form, that is, methoxypropene (**214**). This adds to methylbutenol and the resultant adduct undergoes a Claisen rearrangement

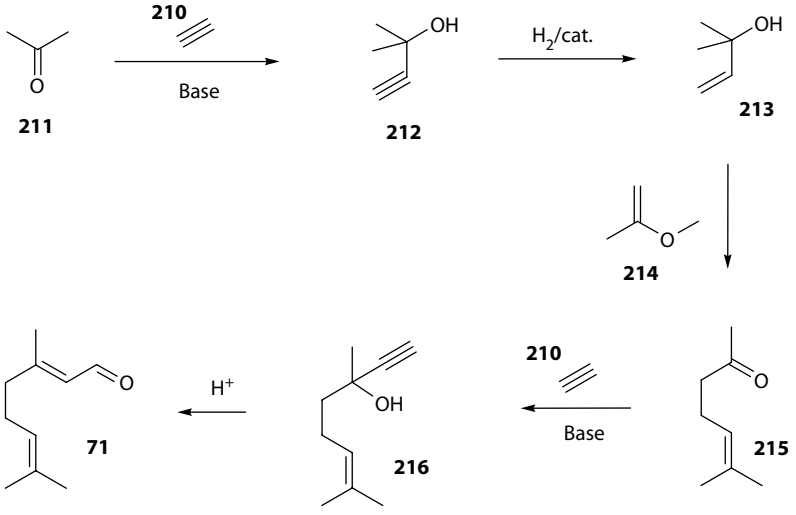


FIGURE 5.38 Citral from acetylene and acetone.

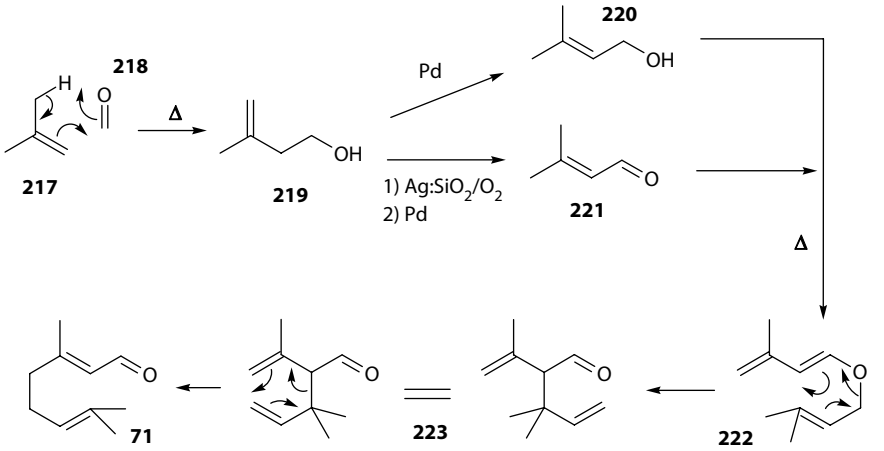


FIGURE 5.39 Citral from isobutylene and acetone.

to give methylheptenone (**215**). Base catalyzed addition of the second acetylene to this gives dehydrolinalool (**216**), which can be rearranged under acidic conditions to give citral (**71**). Hydrogenation of dehydrolinalool under Lindlar conditions gives linalool (**23**) and thus opens up all the routes to other terpenoids as described above and illustrated in Figure 5.36.

The other major route to citral is shown in Figure 5.39. This starts from isobutene (**217**) and formaldehyde (**218**). The ene reaction between these produces isoprenol (**219**). Isomerization of isoprenol over a palladium catalyst gives prenol (**220**) and aerial oxidation over a silver catalyst gives prenal (senecioaldehyde) (**221**). When heated together, these two add together to form the enol ether (**222**), which then undergoes a Claisen rearrangement to give the aldehyde (**223**). This latter molecule is perfectly set up (after rotation around the central bond) for a Cope rearrangement to give citral (**71**). Development chemists have always striven to produce economic processes with the highest overall yield possible thus minimizing the volume of waste and hence environmental impact. This synthesis is a very good example of the fruits of such work. The reaction scheme uses no reagents, other than oxygen, employs efficient catalysts, and produces only one by-product, water, which is environmentally benign.

The synthesis of *l*-menthol (**94**) provides an interesting example of different routes operating in economic balance. The three production routes in current use are shown in Figure 5.40. The oldest

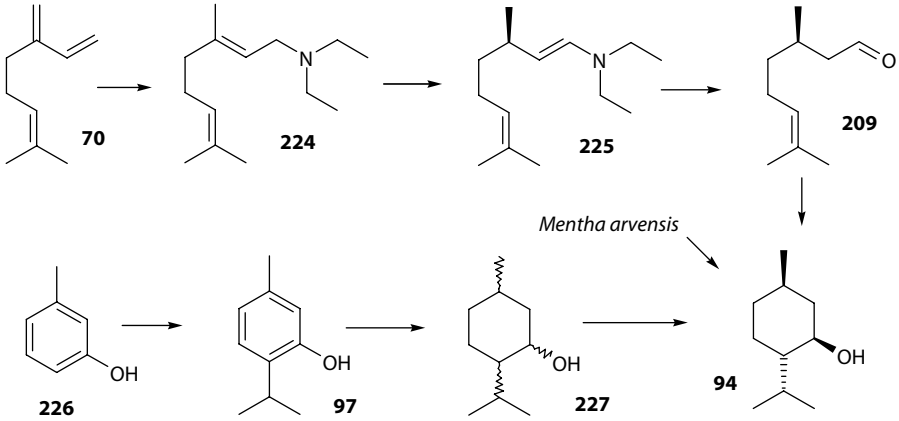


FIGURE 5.40 Competing routes to *l*-menthol.

and simplest route is extraction from plants of the *Mentha* genus and *Mentha arvensis* (cornmint) in particular. This is achieved by freezing the oil to force the *l*-menthol to crystallize out. Diethylamine can be added to myrcene (**70**) in the presence of base and rearrangement of the resultant allyl amine (**224**) using the optically active catalyst ruthenium (*S*)-BINAP perchlorate gives the homochiral enamine (**225**). This can then be hydrolyzed to *d*-citronellol (**209**). The chiral center in this molecule ensures that, on acid catalyzed cyclization, the two new stereocentres formed possess the correct stereochemistry for conversion, by hydrogenation, to give *l*-menthol as the final product. Starting from the petrochemically sourced *m*-cresol (**226**), propenylation gives thymol (**97**), which can be hydrogenated to give a mixture of all eight stereoisomers of menthol (**227**). Fractional distillation of this mixture gives racemic menthol. Resolution was originally carried out by fractional crystallization, but recent advances include methods for the enzymic resolution of the racemate to give *l*-menthol.

Estimation of the long-term sustainability of each of these routes is complex and the final outcome is far from certain. In terms of renewability of feedstocks, *m*-cresol might appear to be at a disadvantage against mint or turpentine. However, as the world's population increases, use of agricultural land will come under pressure for food production, hence increasing pressure on mint cultivation and turpentine, hence, myrcene is a by-product of paper manufacture and is therefore vulnerable to trends in paper recycling and "the paperless office." In terms of energy consumption, and hence current dependence on petrochemicals, the picture is also not as clear as might be imagined. Harvesting and processing of mint requires energy and, if the crop is grown in the same field over time, fertilizer is required and this is produced by the very energy-intensive Haber process. The energy required to turn trees in a forest into pulp at a sawmill is also significant and so turpentine supply will also be affected by energy prices. No doubt, the skills of process chemists will be of increasing importance as we strive to make the best use of natural resources and minimize energy consumption (Baser and Demirci, 2007).

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6 Analysis of Essential Oils

Barbara d'Acampora Zellner, Paola Dugo, Giovanni Dugo, and Luigi Mondello

CONTENTS

6.1 INTRODUCTION

The production of essential oils was industrialized in the first half of the nineteenth century, due to an increased demand for these matrices as perfume and flavor ingredients [1]. As a consequence the need to perform their systematic investigation also became unprecedented. It is interesting to point out that in the second edition of Parry's monograph, published in 1908, about 90 essential oils were listed, and very little was known about their composition [2]. Further important contributions to the essential oil research field were made by Semmler [3], Gildemeister and Hoffmann [4], Finnemore [5], and Guenther [6]. Obviously, it is unfeasible to cite all the researchers involved in the progress of essential oil analysis.

As widely acknowledged, the composition of essential oils is mainly represented by mono- and sesquiterpene hydrocarbons and their oxygenated (hydroxyl and carbonyl) derivatives, along with aliphatic aldehydes, alcohols, and esters. Terpenes can be considered as the most structurally varied class of plant natural products, derived from the repetitive fusion of branched five-carbon units (isoprene units) [7]. In this respect, analytical methods applied in the characterization of essential oils have to account for a great number of molecular species. Moreover, it is also of great importance to highlight that an essential oil chemical profile is closely related to the extraction procedure employed and, hence, the choice of an appropriate extraction method becomes crucial. On the basis

of the properties of the plant material, the following extraction techniques can be applied: steam distillation (SD), possibly followed by rectification and fractionation, solvent extraction (SE), fractionation of solvent extracts, maceration, expression (cold pressing of citrus peels), *enfleurage*, supercritical fluid extraction (SFE), pressurized-fluid extraction, simultaneous distillation–extraction (SDE), Soxhlet extraction, microwave-assisted hydrodistillation (MAHD), dynamic (DHS) and static (SHS) headspace (HS) techniques, solvent-assisted flavor evaporation (SAFE), solid-phase microextraction (SPME), and direct thermal desorption (DTD), among others.

Apart from the great interest in performing systematic studies on essential oils, there is also the necessity to trace adulterations, mainly in economically important essential oils. As can be observed with almost all commercially available products, market changes occur rapidly, affecting individual plants, or industrial processes. In general, market competition, along with the limited interest of consumers with regard to essential oil quality, may induce producers to adulterate their commodities by the addition of products of lower value. Different types of adulterations can be encountered: (a) the simple addition of natural and/or synthetic compounds, with the aim of generating an oil characterized by specific quality values, such as density, optical rotation, residue percentage, ester value, and so on or (b) refined sophistications in the reconstitution and counterfeiting of commercially valuable oils. In the latter case, natural and/or synthetic compounds are added to enhance the market value of an oil, attempting to maintain the qualitative, or even quantitative, composition of natural essential oils, and making adulteration detection a troublesome task. Consequently, the exploitation of modern analytical methodologies, such as gas chromatography (GC) and related hyphenated techniques, is practically unavoidable.

As a consequence of diffused illegal practice in the production of essential oils, there has been an enhanced request for legal standards of commercial purity, while essential oils were included as herbal drugs in pharmacopoeias [8–12], and also in a compendium denominated as Martindale: the complete drug reference (formerly named as Martindale's: The Extra Pharmacopoeia) [13]. In view of the need for standardized methodologies, these pharmacopoeias commonly include the descriptions of several tests, processes, and apparatus. In addition, various international standard regulations have been introduced in which the characteristics of specific essential oils are described, and the botanical source and physicochemical requirements are reported. Such standardized information was created to facilitate the assessment of quality; for example, ISO 3761 (1997) specifies that for Brazilian rosewood essential oil (*Aniba rosaeodora* Ducke) an alcohol content in the 84–93% range, determined as linalool, is required [14]. Moreover, guidelines for the analysis of essential oils are also available; for example, for the measurement of the refractive index (ISO 280, 1198) and optical rotation (ISO 592, 1998), as also for GC analysis using capillary columns [ISO chromatography (ISO 8432, 1987)] [15]. The French Standards Association (Association Française de Normalisation—AFNOR) also develops norms and standard methods dedicated to the essential oil research field, with the aim of assessing quality in relation to specific physical, organoleptic, chemical, and chromatographic characteristics [16].

The present contribution provides an overview on the classical and modern analytical techniques commonly applied to characterize essential oils. Modern techniques will be focused on chromatographic analyses, including theoretical aspects and applications.

6.2 CLASSICAL ANALYTICAL TECHNIQUES

The thorough study of essential oils is based on the relationship between their physical and chemical properties, and is completed by the assessment of organoleptic qualities. The earliest analytical methods applied in the investigation of an essential oil were commonly focused on quality aspects, concerning mainly two properties, namely identity and purity [17].

The following techniques are commonly applied to assess an essential oil physical properties [6,17]: specific gravity (SG), which is the most frequently reported physicochemical property, and is a special case of relative density, $[\rho]^{T(^{\circ}C)}$, defined as the ratio of the densities of a given oil and of

water when both are at identical temperatures. The attained value is characteristic for each essential oil and commonly ranges between 0.696 and 1.118 at 15°C [4]. In cases in which the determinations were made at different temperatures, conversion factors can be used to normalize data.

The measurement of optical rotation, $[\alpha]_D^{20}$, either dextrorotatory or laevorotatory, is also widely recognized. Optical activity is determined by using a polarimeter, with the angle of rotation depending on a series of parameters, such as oil nature, the length of the column through which the light passes, the applied wavelength, and the temperature. The degree and direction of rotation are of great importance for purity assessments, since they are related to the structures and the concentration of chiral molecules in the sample. Each optically active substance has its own specific rotation, as defined in Biot's law:

$$[\alpha]_\lambda^T = \frac{\alpha_\lambda^T}{c \cdot l},$$

where α is the optical rotation at a temperature T expressed in °C, l is the optical path length in dm, λ is the wavelength, and c is the concentration in g/100 mL. It is worthy of note that a standard 100 mm tube is commonly used; in cases in which darker or lighter colored oils are analyzed, longer or shorter tubes are used, respectively, and the rotation should be extrapolated for a 100-mm-long tube. Moreover, prior to the measurement, the essential oil should be dried out with anhydrous sodium sulfate and filtered.

The determination of the refractive index, $[\eta]_D^{20}$, also represents a characteristic physical constant of an oil, usually ranging from 1.450 to 1.590. This index is represented by the ratio of the sine of the angle of incidence (i) to the sine of the angle of refraction (e) of a beam of light passing from a less dense to a denser medium, such as from air to the essential oil:

$$\frac{\sin i}{\sin e} = \frac{N}{n},$$

where N and n are, respectively, the indices of the more and the less dense medium. The Abbé-type refractometer, equipped with a monochromatic sodium light source, is recommended for routine essential oil analysis; the instrument is calibrated through the analysis of distilled water at 20°C, producing a refractive index of 1.3330. In cases in which the measurement is performed at a temperature above or below 20°C, a correction factor per degree must be added or subtracted, respectively [18].

A further procedure that can be applied for the purity assessment of essential oils is based on water solubility; the test, which reveals the presence of polar substances, such as alcohols, glycols and their esters, and glycerin acetates, is carried out as follows: the oil is added to a saturated solution of sodium chloride, which after homogenization is divided into two phases; the volume of the oil, which is the organic phase, should remain unaltered; volume reduction indicates the presence of water-soluble substances. On the other hand, the solubility, or immiscibility, of an essential oil in ethanol reveals much on its quality. Considering that essential oils are slightly soluble in water and are miscible with ethanol, it is simple to determine the number of volumes of water-diluted ethanol required for the complete solubility of one volume of oil; the analysis is carried out at 20°C, if the oil is liquid at this temperature. It must be emphasized that oils rich in oxygenated compounds are more readily soluble in dilute ethanol than those richer in hydrocarbons. Moreover, aged or improperly stored oils frequently present decreased solubility [6].

The investigation on the solubility of essential oils in other media is also widely accepted, such as the evaluation of the presence of water by means of a simple procedure: the addition of a volume of essential oil to an equal volume of carbon disulfide or chloroform; in case the oil is rich in oxygenated constituents it may contain dissolved water, generating turbidity. A further solubility test,

in which the oil is dissolved in an aqueous solution of potassium hydroxide, is applied to oils containing molecules with phenolic groups; finally, the incomplete dissolution of oils rich in aldehydes in a dilute bisulfite solution may denote the presence of impurities.

The estimation of melting and congealing points, as well as the boiling range of essential oils, is also of great importance for identity and purity assessments. Melting point evaluations are a valuable modality to control essential oil purity, since a large number of molecules generally comprised in essential oils melt within a range of 0.5°C or, in the case of decomposition, over a narrow temperature range. On the other hand, the determination of the congealing point is usually applied in cases where the essential oil consists mainly of one molecule, such as the oil of cloves that contains about 90% of eugenol. In the latter case, such a test enables the evaluation of the percentage amount of the abundant compound. At congealing point, crystallization occurs accompanied by heat liberation, leading to a rapid increase in temperature which is then stabilized at the so-called congealing point. A further purity evaluation method is represented by the boiling range determination, through which the percentage of oil that distils below a certain temperature or within a temperature range is investigated.

An additional test usually performed in essential oil analysis is the evaporation residue, in which the percentage of the oil that is not released at 100°C is determined. In the specific case of citrus oils, this test enables purity assessment, since a lower amount of residue in an expressed oil may indicate an addition of distilled volatile components to the oil; an increased residue amount reveals the possible presence of terpenes with higher molecular weights, through the addition of single compounds (or other essential oils), or of heavier oils, such as rosin oil, cheaper citrus oils, or by directly using the citrus oil residue. An example consists of the addition of lime oil to sophisticate lemon oils. In oxidized or polymerized oils the presence of less volatile compounds is common; in this case, a simple test may be carried out by applying a drop of oil on a piece of filter paper; if a transparent spot persists for a period of over 24 h, the oil is most probably degradated. Furthermore, the residue can be subjected to acid and saponification number analyses; for instance, the addition of rosin oil would increase the acid number since this oil, differently from other volatile oils, is characterized by the presence of complex acids. By definition, the acid number is the number of milligrams of potassium hydroxide required to neutralize the free acids contained in 1 g of an oil. This number is preserved in cases in which the essential oil has been carefully dried and stored in dark and airtight recipients. As commonly observed, the acid number increases along the aging process of an oil; oxidation of aldehydes and hydrolysis of esters trigger the increase of the acid number.

Classical methodologies have been also widely applied to assess essential oil chemical properties [6,17], such as the determination of the presences of halogenated hydrocarbons and of heavy metals. The former investigation is exploited to reveal the presence of halogenated compounds, commonly added to the oils for adulteration purposes. Several tests have been developed for halogen detection, with the Beilstein method [19] the one most reported. In practice, a copper wire is cleaned and heated in a Bunsen burner flame to form a coating of copper (II) oxide. It is then dipped in the sample to be tested and once again heated in the flame. A positive test is indicated by a green flame caused by the formation of a copper halide. Attention is to be paid to positive or inconclusive results, since they may be induced by trace amounts of organic acids, nitrogen-containing compounds [6], or salts [20]. An alternative to the Beilstein method is the sodium fusion test, in which the oil is first mineralized, and in the case halogenated hydrocarbons are present, a residue of sodium halide is formed, which is soluble in nitric acid, and precipitates as the respective silver halide by the addition of a small amount of silver nitrate solution [17]. With regard to the detection of heavy metals, several tests are described to investigate and ensure the absence especially of copper and lead. One method is based on the extraction of the essential oil with a diluted hydrochloric acid solution, followed by the formation of an aqueous phase to which a buffered thioacetamide solution is added. The latter reagent leads to the formation of sulfite ions that are used in the detection of heavy metals.

The determination of esters derived from phthalic acid is also of great interest for the toxicity evaluation of an essential oil. Considering that esters commonly contained in essential oils are derived from monobasic acids, at first, saponification is carried out through the addition of an ethanolic potassium hydroxide solution. The formed potassium phthalate, which is not soluble in ethanol, generates a crystalline precipitate [17].

The use of qualitative information alone is not sufficient to correctly characterize an essential oil, and quantitative data are of extreme importance. Classical methods are generally focused on chemical groups and the assessment of quantitative information through titration is widely applied, for example, for the acidimetric determination of saponified terpene esters. Saponification can be performed with heat, and in the case readily saponified esters are to be investigated, in the cold, and afterward the alkali excess is titrated with aqueous hydrochloric acid; thereafter the ester number can be calculated. A further test is the determination of terpene alcohols by acetylating with acetic anhydride; part of the acetic anhydride is consumed in the reaction and can be quantified through titration of acetic acid with sodium hydroxide. The percentage of alcohol can then be calculated. The latter method is applied when the alcoholic constituents of an essential oil are not well known; in case these are established, the oil is saponified and the ester number of the acetylated oil is calculated and used to estimate the free alcohol content.

Other chemical classes worthy of mention are aldehydes and ketones that may be investigated through different tests. The bisulfite method is recommended for essential oils rich in aldehydic compounds, as lemongrass, bitter almond, and cassia, while the neutral sulfite test is more suitable for ketone-rich oils, as spearmint, caraway, and dill oils. For essential oils presenting small amounts of aldehydes and ketones, the hydroxylamine method, or its modification, the Stillman–Reed method are the most indicated ones [20]. In the latter case, the aldehyde and ketone contents are determined through the addition of a neutralized hydroxylamine hydrochloride solution, and subsequent titration with standardized acid (the Stillman–Reed method) [21]; in the former analytical procedure, the aldehyde and ketone content is established through the addition of a hydroxylamine hydrochloride solution, followed by neutralization with the reaction products, that is, alkali of the hydrochloric acid. These methods may be applied in the determination of citral in citrus oils and carvone in caraway oil. With regard to the determination of phenols, such as eugenol in clove oil or thymol and carvacrol in thyme oil, the test is commonly made through the addition of potassium hydroxide solutions, forming water-soluble salts. It has to be pointed out that besides phenols, other constituents are soluble in alkali solutions and in water [6,20].

Essential oils are also often analyzed by means of chromatographic methods. In general, the principle of chromatography is based on the distribution of the constituents to be separated between two immiscible phases; one of these is a stationary bed (a stationary phase) with a large surface area, while the other is a mobile phase that percolates through the stationary bed in a definite direction [22]. Planar chromatography may be referred to as a classical method for essential oil analysis, being well represented by thin-layer chromatography (TLC) and paper chromatography (PC). In both techniques, the stationary phase is distributed as a thin layer on a flat support, in PC being self-supporting, while in TLC coated on a glass, plastic, or metal surface; the mobile phase is allowed to ascend through the layer by capillary forces. TLC is a fast and inexpensive method for identifying substances and testing the purity of compounds, being widely used as a preliminary technique providing valuable information for subsequent analyses [23]. Separations in TLC involve the distribution of one or a mixture of substances between a stationary phase and a mobile phase. The stationary phase is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate. The mobile phase is a developing solvent that travels up the stationary phase, carrying the samples with it. Components of the samples will separate on the stationary phase according to their stationary phase–mobile phase affinities [24]. In practice, a small quantity of the sample is applied near one edge of the plate and its position is marked with a pencil. The plate is then positioned in a developing chamber with one end immersed in the developing solvent, the mobile phase, avoiding the direct contact of the sample with the solvent. When the mobile phase reaches about two-third of the plate length, the

plate is removed, dried, the solvent front is traced, and the separated components are located. In some cases the spots are directly visible, but in others they must be visualized by using methods applicable to almost all organic samples, such as the use of a solution of iodine or sulfuric acid, both of which react with organic compounds yielding dark products. The use of an ultraviolet (UV) lamp is also advisable, especially if a substance that aids in the visualization of compounds is incorporated into the plate, as is the case of many commercially available TLC plates. Data interpretation is made through the calculation of the ratio of fronts (R_f) value for each spot, which is defined as

$$R_f = \frac{Z_S}{Z_{St}},$$

where Z_S is the distance from the starting point to the center of a specific spot, and Z_{St} is the distance from the starting point to the solvent front [24,25]. A concise review on TLC has been made by Sherma [26].

The R_f value is characteristic for any given compound on the same stationary phase using the identical mobile phase. Hence, known R_f values can be compared to those of unknown substances to aid in their identification [24]. On the other hand, separations in PC involve the same principles as those in TLC, differing in the use of a high-quality filter paper as the stationary phase instead of a thin adsorbent layer, by the increased time requirements and poorer resolution. It is worthy to highlight that TLC has largely replaced PC in contemporary laboratory practice [22].

As is well known, essential oils can be characterized by their organoleptic properties, an assessment that involves human subjects as measuring tools. These procedures present an immediate problem, linked to the innate variability between individuals, not only as a result of their previous experiences and expectations, but also to their sensitivity [27]. In this respect, individuals are selected and screened for specific anosmia, as proposed by Friedrich et al. [28]. In the case no insensitivities are found, the panelists are introduced to two sensorial properties, quality, and intensity. Odor quality is described according to the odor families, while intensity is measured through the rating of a sensation based on an intensity interval scale. The assessment of an essential oil odor can be performed through its addition to filter paper strips and subsequent evaluation by the panelists. Considering that each volatile compound is characterized by a different volatility, the evaluation of the paper strip in different periods of time enables the classification of the odors in top, middle, and bottom notes [29]. In addition, the olfactive assessment during the determination of the evaporation residue is also of significance, since by-notes of low-boiling adulterants or contaminants may be detected as the oil vaporizes, and the odor of the final hot residue can reveal the addition of high-boiling compounds. Olfactive analyses are also valuable after the determination of phenols in essential oils, by studying the nonphenolic portion [6].

It is noteworthy that the use of the earliest analytical techniques for the systematic study of essential oils, such as SG, relative density, optical activity, and refractive index, or melting, congealing, and boiling points determinations, are generally applied for the assessment of pure compounds, and may be extended to evaluate essential oils composed of a major compound. Classical methods cannot be used as stand-alone methods and need to be combined with modern analytical techniques, especially GC, for the assessment of essential oil genuineness.

6.3 MODERN ANALYTICAL TECHNIQUES

Most of the methods applied in the analysis of essential oils rely on chromatographic procedures, which enable component separation and identification. However, additional confirmatory evidence is required for reliable identification, avoiding equivocated characterizations.

In the early stages of research in the essential oil field, attention was devoted to the development of methods in order to acquire deeper knowledge on the profiles of volatiles; however, this analytical

task was made troublesome due to the complexity of these real-world samples. Over the last decades, the aforementioned research area has benefited from the improvements in instrumental analytical chemistry, especially in the chromatographic area, and, nowadays, the number of known constituents has drastically increased.

The primary objective in any chromatographic separation is always the complete resolution of the compounds of interest, in the minimum time. To achieve this task the most suitable analytical column (dimension and stationary phase type) has to be used, and adequate chromatographic parameters must be applied to limit peak enlargement phenomena. A good knowledge of chromatographic theory is, indeed, of great support for the method optimization process, as well as for the development of innovative techniques.

In gas chromatographic analysis, the compounds to be analyzed are vaporized and eluted by the mobile gas phase, the carrier gas, through the column. The analytes are separated on the basis of their relative vapor pressures and affinities for the stationary bed. On the other hand, in liquid chromatographic analysis, the compounds are eluted by a liquid mobile phase consisting of a solvent or a mixture of solvents, the composition of which may vary during the analysis (gradient elution), and are separated according to their affinities for the stationary bed. In general, the volatile fraction of an essential oil is analyzed by GC, while the nonvolatile by liquid chromatography (LC).

At the outlet of the chromatography column, the analytes emerge separated in time. The analytes are then detected and a signal is recorded generating a chromatogram, which is a signal versus time graphic, and ideally with peaks presenting a Gaussian distribution-curve shape. The peak area and height are a function of the amount of solute present and its width is a function of band spreading in the column [30], while retention time can be related to the solute's identity. Hence, the information contained in the chromatogram can be used for qualitative and quantitative analysis.

6.3.1 Use of GC and Linear Retention Indices in Essential Oils Analysis

The analysis of essential oils by means of GC began in the 1950s, when professor Liberti [31] started analyzing citrus essential oils only a few years after James and Martin first described gas–liquid chromatography (GLC), commonly referred to as GC [32], a milestone in the evolution of instrumental chromatographic methods.

After its introduction, GC developed at a phenomenal rate, growing from a simple research novelty to a highly sophisticated instrument. Moreover, the current-day requirements for high resolution and trace analysis are satisfied by modern column technology. In particular, inert, thermostable, and efficient open-tubular columns are available, along with associated selective detectors and injection methods, which allow on-column injection of liquid and thermally labile samples. The development of robust fused-silica columns, characterized by superior performances to that of glass columns, brings open-tubular GC columns within the scope of almost every analytical laboratory.

At present, essential oil GC analyses are more frequently performed on capillary columns, which, after their introduction, rapidly replaced packed GC columns. In general, packed columns support larger sample size ranges, from 10 to 20 μL, and thus the dynamic range of the analysis can be enhanced. Trace-level components can be easily separated and quantified without preliminary fractionation or concentration. On the other hand, the use of packed columns leads to lower resolution due to the higher pressure drop per unit length. Packed columns need to be operated at higher column flow rates, since their low permeability requires high pressures to significantly improve resolution [33]. It is worthy of note that since the introduction of fused-silica capillary columns considerable progress has been made in column technology, a great number of papers regarding GC applications on essential oils have been published.

The choice of the capillary column in an essential oil GC analysis is of great importance for the overall characterization of the matrix; the stationary phase chemical nature and film thickness, as well as the column length and internal diameter, are to be considered. In general, essential oil GC analyses are carried out on 25–50 m columns, with 0.20–0.32 mm internal diameters, and 0.25 μm

stationary phase film thickness. It must be noted that the degree of separation of two components on two distinct stationary phases can be drastically different. As is well known, nonpolar columns produce boiling-point separations, while on polar stationary phases compounds are resolved according to their polarity. Considering that essential oil components, such as terpenes and their oxygenated derivatives, frequently present similar boiling points, these elute in a narrow retention time range on a nonpolar column. In order to overcome this limit, the analytical method can be modified by applying a slower oven temperature rate to widen the elution range of the oil or by using a polar stationary phase, as oxygenated compounds are more retained than hydrocarbons. However, choosing different stationary phases may provide little improvement as resolution can be improved for a series of compounds but new coelutions can also be generated.

Considering gas chromatographic analyses using flame ionization detector (FID), thermal conductivity detector (TCD), or other detectors which do not provide structural information of the analyzed molecules and retention data, more precisely retention indices, are used as the primary criterion for peak assignment. The retention index system was based on the fact that each analyte is referenced in terms of its position between the two *n*-paraffins that bracket its retention time. Furthermore, the index calculation is based on a linear interpolation of the carbon chain length of these bracketing paraffins. The most thoroughly studied, diffused, and accepted retention index calculation methods are based on the logarithmic-based equation developed by Kováts in 1958 [34], for isothermal conditions, and on the equation propounded by van den Dool and Kratz in 1963 [35], which does not use the logarithmic form and is used in the case of temperature-programming conditions. Values calculated using the latter approach are commonly denominated in literature as retention index (I), linear retention index (LRI), or programmed-temperature retention index (PTRI or I^T), while the ones derived from the former equation are usually referred to as Kováts index (KI).

In general, retention index systems are based on the incremental structure–retention relationship, namely, that any regular increase in a series of chemical structures should provide a regular increase in the corresponding retention times. This means that the retention index concept is not restricted to the use of *n*-alkanes as standards. In practice, any homologous series presenting a linear relationship between the adjusted retention time, being logarithmic based or not, and the carbon number can be used.

In the characterization of volatiles, the most commonly applied reference series is *n*-alkanes. However, the latter commonly present fluctuant behavior on polar stationary phases. In consideration of the fact that retention index values are correlated to retention mechanisms, alternative standard series of intermediate polarity have been introduced, such as 2-alkanones, alkyl ethers, alkyl halides, alkyl acetates, and alkanoic acid methyl esters [22]. Shibamoto [36] suggested the use of polar compounds series, such as ethyl esters, as an alternative. The most feasible choice, when analyzing volatiles, is to apply reference series as *n*-alkanes, fatty acid ethyl esters (FAEEs), or fatty acid methyl esters (FAMEs), employed according to the stationary phase to be used.

Additionally, it is highly advisable to use two analytical columns coated with stationary phases of distinct polarities to obtain two retention index values and enhance confidence in assignments [37–39]. Identifications made on a single column can only be accepted if used in combination with spectroscopic detection systems. When *n*-alkanes are used, it is accepted that the reproducibility of retention indices between different laboratories are comprised within an acceptable range of ±5 units for methyl silicone stationary phases, and ±10 units for polyethylene glycol phases. A further aspect of great importance, which is frequently overseen, is the analytical reproducibility of retention indexes. Moreover, it is worthwhile to highlight that in practice it was found that the use of an initial isothermal hold in the GC oven temperature program does not provide additional resolution [40].

6.3.2 Gas Chromatography-Mass Spectrometry

Mass spectrometry (MS) can be defined as the study of systems through the formation of gaseous ions, with or without fragmentation, which are then characterized by their mass-to-charge ratios (*m/z*)

and relative abundances [41]. The analyte may be ionized thermally, by an electric field or by impacting energetic electrons, ions, or photons.

During the past decade, there has been a tremendous growth in popularity of mass spectrometers as a tool for both, routine analytical experiments and fundamental research. This is due to a number of features including relatively low cost, simplicity of design and extremely fast data acquisition rates. Although the sample is destroyed by the mass spectrometer, the technique is very sensitive and only low amounts of material are used in the analysis.

In addition, the potential of combined gas chromatography-mass spectrometry (GC-MS) for determining volatile compounds, contained in very complex flavor and fragrance samples, is well known. The subsequent introduction of powerful data acquisition and processing systems, including automated library search techniques, ensured that the information content of the large quantities of data generated by GC-MS instruments was fully exploited. The most frequent and simple identification method in GC-MS consists of the comparison of the acquired unknown mass spectra with those contained in a reference MS library.

A mass spectrometer produces an enormous amount of data, especially in combination with chromatographic sample inlets [42]. Over the years, many approaches for analysis of GC-MS data have been proposed using various algorithms, many of which are quite sophisticated, in efforts to detect, identify, and quantify all of the chromatographic peaks. Library search algorithms are commonly provided with mass spectrometer data systems with the purpose to assist in the identification of unknown compounds [43].

However, as is well known, compounds such as isomers, when analyzed by means of GC-MS, can be incorrectly identified; a drawback which is often observed in essential oil analysis. As is widely acknowledged, the composition of essential oils is mainly represented by terpenes, which generate very similar mass spectra; hence, a favorable match factor is not sufficient for identification and peak assignment becomes a difficult, if not impracticable, task (Figure 6.1). In order to increase the reliability of the analytical results and to address the qualitative determination of compositions of complex samples by GC-MS, retention indices can be an effective tool. The use of retention indices in conjunction with the structural information provided by GC-MS is widely accepted, and routinely used to confirm the identity of compounds. Besides, retention indices when incorporated to MS libraries can be applied as a filter, thus shortening the search routine for matching results, and enhancing the credibility of MS identification [44].

According to D. Joulain and W. A. König [45], provided data contained in mass spectral libraries have been recorded using authentic samples, it can be observed that the mass spectrum of a given sesquiterpene is usually sufficient to ensure its identification when associated with its retention index obtained on methyl silicone stationary phases. Indeed, for the aforecited class of compounds, there would be no need to use a polyethylene glycol phase, which could even lead to misinterpretations caused by possible changes in the retention behavior of sesquiterpene hydrocarbons as a result of column aging or deterioration. Moreover, according to the authors, attention should be paid to the retention index and the mass spectrum registration of each individual sesquiterpene, since many compounds with rather similar mass spectra elute in a narrow range; more than 160 compounds can elute within 100 retention index units on a methyl silicone-based column, for example, 1400–1500.

6.3.3 Fast GC for Essential Oil Analysis

Nowadays in daily routine work, apart from increased analytical sensitivity, demands are also made on the efficiency in terms of speed of the laboratory equipment. Regarding the rapidity of analysis, two aspects need to be considered: (i) the costs in terms of time required, for example, as is the case in quality control analysis, and (ii) the efficiency of the utilized analytical equipment.

When compared to conventional GC, the primary objective of fast GC is to maintain sufficient resolving power in a shorter time, by using adequate columns and instrumentation in combination

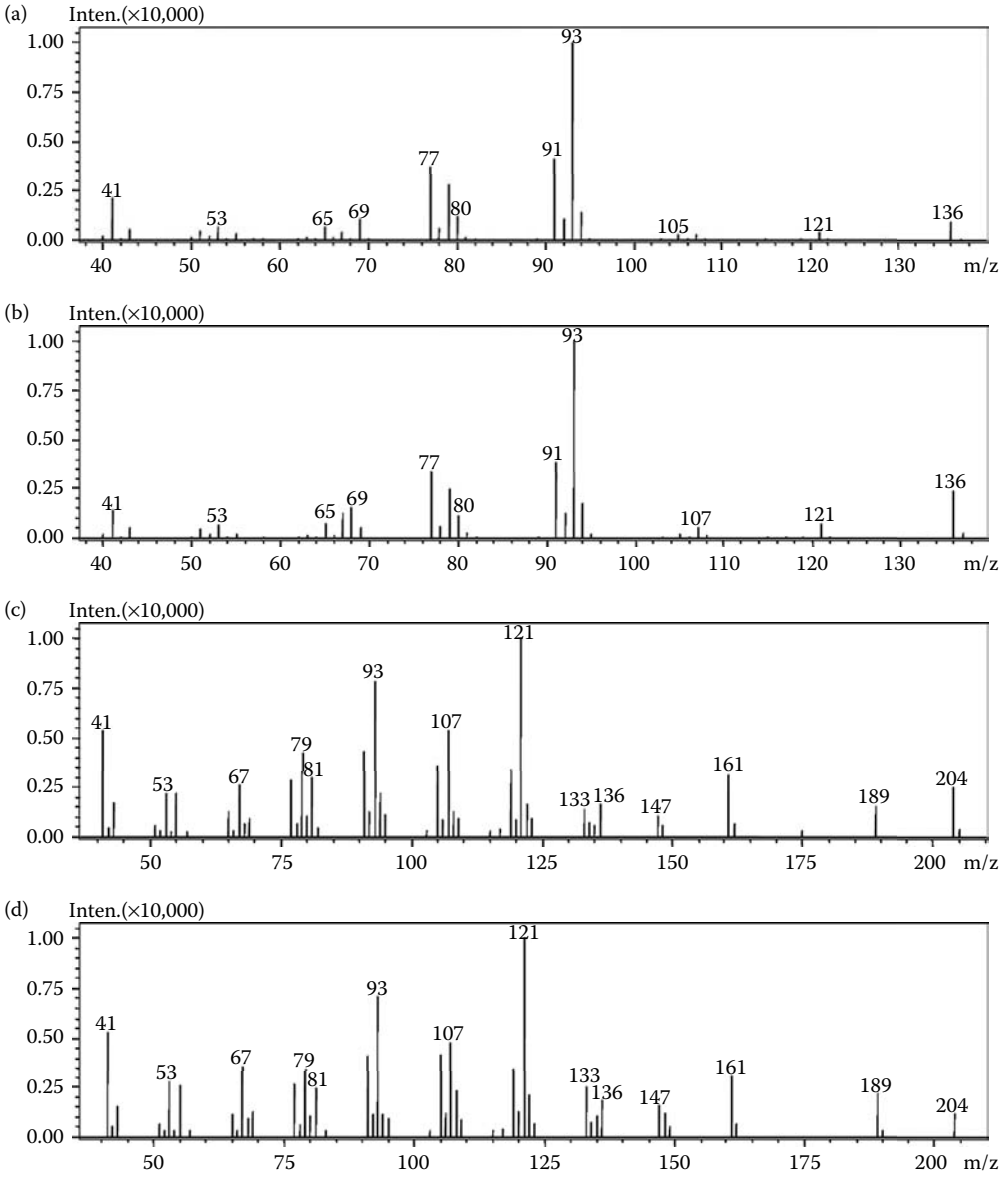

FIGURE 6.1 Representation of the similarity between mass spectra of monoterpenes: sabinene (a) and β-phellandrene (b); and sesquiterpenes: bicyclogermacrene (c) and germacrene B (d).

with optimized run conditions to provide 3–10 times faster analysis times [46–48]. The technique can be accomplished by manipulating a number of analysis parameters, such as column length, column I.D., stationary phase, film thickness, carrier gas, linear velocity, oven temperature, and ramp rate. Fast GC is typically performed using short, 0.10 or 0.18 mm I.D. capillary columns with hydrogen carrier gas and rapid oven temperature ramp rates. In general, capillary gas chromatographic analysis may be divided into three groups, based solely on column internal diameter types; namely, as conventional GC when 0.25 mm I.D. columns are applied, fast GC using 0.10–0.18 mm I.D. columns, and ultrafast GC for columns with an I.D. of 0.05 mm or less. In addition, GC analyses times between 3 and 12 min can be defined as "fast," between 1 and 3 min as "very fast," and below 1 min as "ultrafast." Fast GC requires instrumentation provided with high split ratio injection

systems because of low sample column capacities, increased inlet pressures, rapid oven heating rates, and fast electronics for detection and data collection [49].

The application of two methods, conventional (30 m × 0.25 mm I.D., 0.25 μm d_f column) and fast (10 m × 0.10 mm I.D., 0.10 μm d_f column), on five different citrus essential oils (bergamot, mandarin, lemon, bitter oranges, and sweet oranges) has been reported [49]. The fast method allowed the separation of almost the same compounds as the conventional analysis, while quantitative data showed good reproducibility. The effectiveness of the fast GC method, through the use of narrow-bore columns, was demonstrated. An ultrafast GC lime essential oil analysis was also performed on a 5 m × 50 μm capillary column with 0.05 μm stationary phase film thickness [50]. The total analysis time of this volatile essential oil was less than 90 s; a chromatogram is presented in Figure 6.2.

Another technique, ultrafast module-GC (UFM-GC) with direct resistively heated narrow-bore columns, has been applied to the routine analysis of four essential oils of differing complexities; chamomile, peppermint, rosemary, and sage [51]. All essential oils were analyzed by conventional GC with columns of different lengths; namely, 5 and 25 m, with a 0.25 mm I.D., and by fast GC and UFM-GC with narrow-bore columns (5 m × 0.1 mm I.D.). Column performances were evaluated and compared through the Grob test, separation numbers, and peak capacities. UFM-GC was successful in the qualitative and quantitative analysis of essential oils of different compositions with analysis times between 40 s and 2 min versus 20–60 min required by conventional GC. UFM-GC allows to drastically reduce the analysis time, although the very high column heating rates may lead to changes in selectivity compared to conventional GC, and that are more marked than those of classical fast GC. In a further work the same researchers [52] stated that in UFM-GC experiments the appropriate flow choice can compensate, in part, the loss of separation capability due to the heating rate increase.

Besides the numerous fast GC application on citrus essential oils, other oils have also been subjected to analysis, such as rose oil by means of ultrafast GC [53] and very fast GC [54], both using narrow-bore columns. Rosemary and chamomile oils have been investigated by means of fast GC on two short conventional columns of distinct polarity (5 m × 0.25 mm I.D.) [55]. The latter

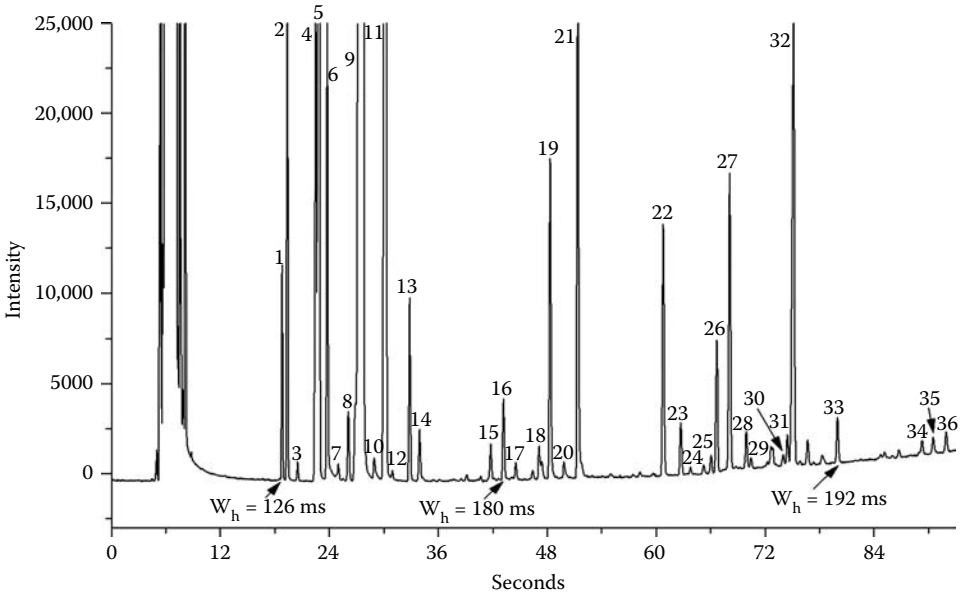

FIGURE 6.2 Fast GC analysis of a lime essential oil on a 5 m × 5 mm (0.05 μm film thickness) capillary column, applying fast temperature programming. The peak widths of three components are marked to provide an illustration of the high efficiency of the column, even under extreme operating conditions (for peak identification see on Ref. [50]). (From Mondello, L. et al., 2004. *J. Sep. Sci*., 27: 699–702. With permission.)

oil has also been analyzed through fast HS-SPME-GC on a narrow-bore column [56]. Fast and very fast GC analyses on narrow-bore columns have also been carried out on patchouli and peppermint oils [57].

6.3.4 Gas Chromatography-Olfactometry for the Assessment of Odor-Active Components of Essential Oils

The discriminatory capacity of the mammalian olfactory system is such that thousands of volatile chemicals are perceived as having distinct odors. It is accepted that the sensation of odor is triggered by highly complex mixtures of volatile molecules, mostly hydrophobic, and usually occurring in trace-level concentrations (ppm or ppb). These volatiles interact with odorant receptors of the olfactive epithelium located in the nasal cavity. Once the receptor is activated, a cascade of events is triggered to transform the chemical-structural information contained in the odorous stimulus into a membrane potential [58,59], which is projected to the olfactory bulb, and then transported to higher regions of the brain [60] where the translation occurs.

It is known that only a small portion of the large number of volatiles occurring in a fragrant matrix contributes to its overall perceived odor [61,62]. Further, these molecules do not contribute equally to the overall flavor profile of a sample; hence, a large GC peak area, generated by a chemical detector does not necessarily correspond to high odor intensities, due to differences in intensity/concentration relationships.

The description of a gas chromatograph modified for the sniffing of its effluent to determine volatile odor activity was first published in 1964 by Fuller et al. [63]. In general, gas chromatograhy-olfactometry (GC-O) is carried out on a standard GC that has been equipped with a sniffing port, also denominated olfactometry port or transfer line, in substitution of, or in addition to, the conventional detector. When a flame FID or a mass spectrometer is also used, the analytical column effluent is split and transferred to the conventional detector and to the human nose. GC-O was a breakthrough in analytical aroma research, enabling the differentiation of a multitude of volatiles, previously separated by GC, in odor-active and non-odor-active, related to their existing concentrations in the matrix under investigation. Moreover, it is a unique analytical technique that associates the resolution power of capillary GC with the selectivity and sensitivity of the human nose.

GC-O systems are often used in addition to either a FID or a mass spectrometer. With regard to detectors, splitting column flow between the olfactory port and a mass spectral detector provides simultaneous identification of odor-active compounds. Another variation is to use an in-line, non-destructive detector such as a TCD [64] or a photoionization detector (PID) [65]. Especially when working with GC-O systems equipped with detectors that do not provide structural information, retention indexes are commonly associated to odor description supporting peak assignment.

Over the last decades, GC-O has been extensively used in essential oil analysis in combination with sophisticated olfactometric methods; the latter were developed to collect and process GC-O data, and hence, to estimate the sensory contribution of a single odor-active compound. The odor-active compounds of essential oils extracted from citrus fruits (*Citrus* sp.), such as orange, lime, and lemon, were among the first character impact compounds identified by flavor chemists [66].

GC-O methods are commonly classified in four categories: dilution, time-intensity, detection frequency, and posterior intensity methods. Dilution analysis, the most applied method, is based on successive dilutions of an aroma extract until no odor is perceived by the panelists. This procedure, usually performed by a reduced number of assessors is mainly represented by CHARM (combined hedonic aroma response method) [67], developed by Acree and coworkers, and AEDA (aroma extraction dilution analysis), first presented by Ullrich and Grosch [68]. The former method has been applied to the investigation of two sweet orange oils from different varieties, one Florida Valencia and the other Brazilian Pera [69]. The intensities and qualities of their odor-active components were assessed. CHARM results indicated for both the oils that the most odor-active compounds are associated with the polar fraction compounds: straight chain aldehydes (C_8–C_{14}), β-sinensal, and linalool presented the major CHARM responses. On the other hand, AEDA has

been used to investigate the odor-active compounds responsible for the characteristic odors of juzu oil (*Citrus junos* Sieb. ex Tanaka) [70] and dadai (*Citrus aurantium* L. var. *cyathifera* Y. Tanaka) [71] cold-pressed essential oils.

Time-intensity methods, such as OSME (Greek word for odor), are based on the immediate recording of the intensity as a function of time by moving the cursor of a variable resistor [72]. An interesting application of the time-intensity approach was demonstrated for cold-pressed grapefruit oil [73], in which 38 odor-active compounds were detected and, among these, 22 were considered as aroma impact compounds. A comparison between the grapefruit oil GC chromatogram and the corresponding time-intensity aromagram for that sample is shown in Figure 6.3.

A further approach, the detection frequency method [74,75], uses the number of evaluators detecting an odor-active compound in the GC effluent as a measure of its intensity. This GC-O method is performed with a panel composed of numerous and untrained evaluators; 8–10 assessors are a good agreement between low variation of the results and analysis time. It must be added that the results attained are not based on real intensities and are limited by the scale of measurement. An application of the detection frequency method was reported for the evaluation of leaf- and wood-derived essential oils of Brazilian rosewood (*Aniba rosaeodora* Ducke) essential oils by means of enantioselective-GC-olfactometry (Es-GC-O) analyses [76].

Another GC-O technique, the posterior intensity method [77], proposes the measurement of a compound odor intensity, and its posterior scoring on a previously determined scale. This posterior registration of the perceived intensity may cause a considerable variance between assessors. The attained results may generally be well correlated with detection frequency method results, and to a lesser extent, with dilution methods. In the above-mentioned research performed on the essential oils of Brazilian rosewood, this method was also used to give complementary information on the intensity of the linalool enantiomers [76].

Other GC-O applications are also reported in literature using the so-called peak-to-odor impression correlation, the method in which the olfactive quality of an odor-active compound perceived by a panelist is described. The odor-active compounds of the essential oils of black pepper

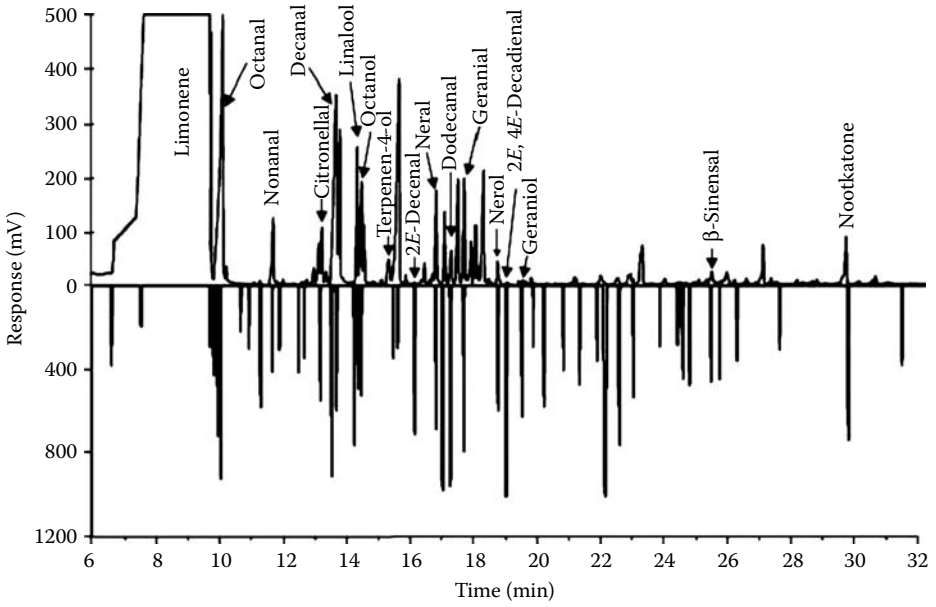

FIGURE 6.3 GC-FID chromatogram with some components identified by means of MS (top) and a time-intensity aromagram of grapefruit oil (bottom). The separation was performed on a polyethylene glycol column (30 m × 0.32 mm I.D., 0.25 µm film thickness). (From Lin, J. and R.L. Rouseff, 2001. *Flavour Fragr. J.*, 16: 457–463. With permission.)

(*Piper nigrum*) and Ashanti pepper (*Piper guineense*) were assessed applying the aforecited correlation method [78]. The odor profile of the essential oils of leaves and flowers of *Hyptis pectinata* (L.) Poit. was also investigated by using the peak-to-odor impression correlation [79].

The choice of the GC-O method is of extreme importance for the correct characterization of a matrix, since the application of different methods to an identical real sample can distinctly select and rank the odor-active compounds according to their odor potency and/or intensity. Commonly, detection frequency and posterior intensity methods result in similar odor intensity/concentration relationships, while dilution analysis investigate and attribute odor potencies.

6.3.5 Gas Chromatographic Enantiomer Characterization of Essential Oils

Capillary GC is currently the method of choice for enantiomer analysis of essential oils and enantioselective-GC (Es-GC) has become an essential tool for stereochemical analysis mainly after the introduction of cyclodextrin (CD) derivatives as chiral stationary phases (CSPs) in 1983 by Sybilska and Koscielski, at the University of Warsaw, for packed columns [80], and applied to capillary columns in the same decade [81,82]. Moreover, Nowotny et al. first proposed diluting CD derivatives in moderately polar polysiloxane (OV-1701) phases to provide them with good chromatographic properties and a wider range of operative temperatures [83].

The advantage on the application of Es-GC lies mainly in its high separation efficiency and sensitivity, simple detection, unusually high precision and reproducibility, as also the need for a small amount of sample. Moreover, its main use is related with the characterization of the enantiomeric composition and the determination of the enantiomeric excess (ee) and/or ratio (ER) of chiral research chemicals, intermediates, metabolites, flavors and fragrances, drugs, pesticides, fungicides, herbicides, pheromones, and so on. Information on ee or ER is of great importance to characterize natural flavor and fragrance materials, such as essential oils, since the obtained values are useful tools, or even "fingerprints," for the determination of their quality, applied extraction technique, geographic origin, biogenesis, and also authenticity [84].

A great number of essential oils have already been investigated by means of Es-GC using distinct CSPs; unfortunately, a universal chiral selector with widespread potential for enantiomer separation is not available, and thus effective optical separation of all chiral compounds present in a matrix may be unachievable on a single chiral column. In 1997, Bicchi et al. [85] reported the use of columns that addressed particular chiral separations, noting that certain CSPs preferentially resolved certain enantiomers. Thus, a 2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-CD on polymethylphenylsiloxane (PS086) phase allowed the characterization of lavender and citrus oils containing linalyl oxides, linalool, linalyl acetate, borneol, bornyl acetate, α-terpineol, and *cis*- and *trans*-nerolidol. On the other hand, peppermint oil was better analyzed by using a 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-CD on PS086 phase, and especially for α- and β-pinene, limonene, menthone, isomenthone, menthol, isomenthol, pulegone, and methyl acetate. König [86] performed an exhaustive investigation of the stereochemical correlations of terpenoids, concluding that when using a heptakis (6-*O*-methyl-2,3-di-*O*-penthyl)-β-CD and octakis (6-*O*-methyl-2,3-di-*O*-penthyl)-γ-CD in polysiloxane, the presence of both enantiomers of a single compound is common for monoterpenes, less common for sesquiterpenes, and never observed for diterpenes.

Substantial improvements in chiral separations have been extensively published in the field of chromatography. At present, over 100 stationary phases with immobilized chiral selectors are available [22], presenting increased stability and extended lifetime. It can be affirmed that enantioselective chromatography has now reached a high degree of sophistication. To better characterize an essential oil, it is advisable to perform Es-GC analysis on at least two, or better three, columns coated with different CD derivatives. This procedure enables the separation of more than 85% of the racemates that commonly occur in these matrices [87], while the reversal of enantiomer elution order can take place in several cases. The analyst must be aware of some practical aspects prior to an Es-GC analysis: as is well accepted, variations in linear velocity can affect the separation of

enantiomeric pairs; resolution (R_S) can be improved by optimizing the gas linear velocity, a factor of high importance in cases of difficult enantiomer separation. Satisfactory resolution requires $R_S \geq 1$, and baseline resolution is obtained when $R_S \geq 1.5$ [88]. Resolution can be further improved by applying slow temperature ramp rates (1–2°C/min is frequently suggested). Moreover, according to the CSP used, the initial GC oven temperature can affect peak width; initial temperatures of 35–40°C are recommended for the most column types. Furthermore, attention should be devoted to the column sample capacity, which varies with different compounds; overloading results in broad tailing peaks and reduced enantiomeric resolution. The troublesome separation and identification of enantiomers due to the fact that each chiral molecule splits into two chromatographic signals, for each existing stereochemical center is also worthy of note. As a consequence, the increase in complexity of certain regions of the chromatogram may lead to imprecise ee and/or ER values. In terms of retention time repeatability, and also reproducibility, it can be affirmed that good results are being achieved with commercially available chiral columns.

The retention index calculation of optically active compounds can be considered as a troublesome issue due to complex inclusion complexation retention mechanisms on CD stationary phases; if a homologous series, such as the *n*-alkanes, is used, the hydrocarbons randomly occupy positions in the chiral cavities. As a consequence, *n*-alkanes can be considered as unsuitable for retention index determinations. Nevertheless, other reference series can be employed on CD stationary phases, such as linear chain FAMEs and FAEEs. However, retention indices are seldom reported for optically active compounds, and publications refer to retention times rather than indices.

The innovations in Es-GC analysis have not only concerned the development and applications of distinct CSPs, but also the development of distinct enantioselective analytical techniques, such as Es-GC-mass spectrometry (Es-GC/MS), Es-GC-O, enantioselective multidimensional gas chromatography (Es-MDGC), Es-MDGC/MS, Es-GC hyphenated to isotopic ratio mass spectrometry (Es-GC/IRMS), Es-MDGC/IRMS, and so on.

It is obvious that an enantioselective separation in combination with MS detection presents the additional advantage of qualitative information. Notwithstanding, a difficulty often encountered is that related to peak assignment, due to the similar fragmentation pattern of isomers. The reliability of Es-GC/MS results can be increased by using an effective tool, namely retention indices. It can be assumed that in the enantioselective recognition of optically active isomers in essential oils, mass spectra can be exploited to locate the two enantiomers in the chromatogram, and the LRI when possible, enables their identification [89]. In addition, the well-known property of odor activity recognized for several isomers can be assessed by means of Es-GC/MS/O and can represent an outstanding tool for precise enantiomer characterization (Figure 6.4).

As demonstrated by Mosandl and his group [90], Es-GC-O is a valid tool for the simultaneous stereodifferentiation and olfactive evaluation of the volatile optically active components present in essential oils. It is worthwhile to point out that the preponderance of one of the enantiomers, defined by the enantiomeric excess, results in a characteristic aroma [91], and is of great importance for the olfactive characterization of the sample.

6.3.6 LC and Liquid Chromatography Hyphenated to MS in the Analysis of Essential Oils

Some natural complex matrices do not need sample preparation prior to GC analysis, for example, essential oils. The latter generally contain only volatile components, since their preparation is performed by SD. Citrus oils, extracted by cold-pressing machines, are an exception, containing more than 200 volatile and nonvolatile components. The volatile fraction represents 90–99% of the entire oil, and is represented by mono- and sesquiterpene hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols, and esters; the nonvolatile fraction, constituting 1–10% of the oil, is represented mainly by hydrocarbons, fatty acids, sterols, carotenoids, waxes, and oxygen heterocyclic compounds (coumarins, psoralens, and polymethoxylated flavones—PMFs) [92].

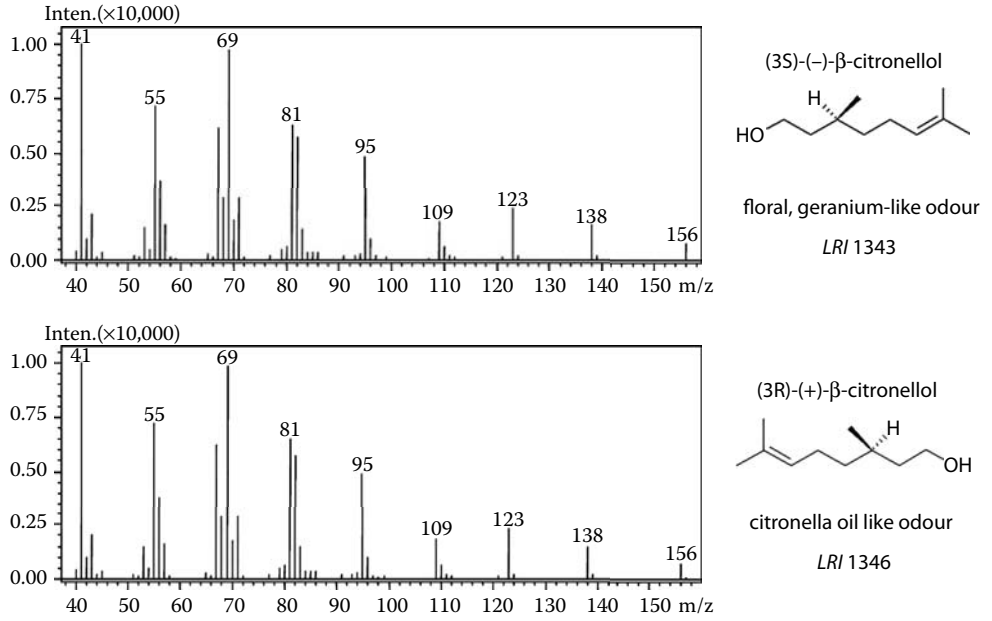

FIGURE 6.4 Representation of the mass spectra similarity of β-citronellol enantiomers.

In some specific cases, the information attained by means of GC is not sufficient to characterize a citrus essential oil, and the analysis of the nonvolatile fraction can be required. Oxygen heterocyclic compounds, which are a distinct class of flavonoids, can have an important role in the identification of a cold-pressed oil and in the control of both quality and authenticity [92–95]. The analysis of these compounds is usually performed by means of LC, also referred to as high-performance liquid chromatography (HPLC), in normal (NP-HPLC) or reversed-phase (RP-HPLC) applications. The former method, commonly used when the analytes of interest are slightly polar, separates analytes based on polarity by using a polar stationary phase and a nonpolar mobile phase. The degree of adsorption on the polar stationary phase increases on the basis of analyte polarity, and the extension of this interaction has a great influence on the elution time. In general, the interaction strength is related to the nature of the analyte functional groups and to steric factors. On the other hand, RP-HPLC is based on the use of a nonpolar stationary phase and an aqueous, moderately polar mobile phase. Retention times are therefore shorter for polar molecules, which elute more readily. Moreover, retention times are increased by the addition of a polar solvent to the mobile phase, and decreased by the addition of a more hydrophobic solvent.

The on-line coupling of two columns, namely, a μ-Porasil (30 cm × 3.9 mm I.D., with 10 μm particle size; Waters Corporation; Milford, USA) and a Zorbax silica (25 cm × 4.6 mm I.D., with 7 μm particle size; Phenomenex, Bologna, Italy), in the NP-HPLC analysis of bitter orange essential oils with UV detection has been reported. A large number of cold-pressed Italian and Spanish, commercial and laboratory-made oils, as also mixtures of bitter orange with sweet orange, lemon, lime, and grapefruit oils were analyzed [93]. A total of four coumarins [osthol (1), meranzin (5), isomeranzin (6), and meranzin hydrate (14)], three psoralens [bergapten (2), epoxybergamottin (3), and epoxybergamottin hydrate (13)], and four PMFs [tangeretin (8), heptamethoxyflavone (9), nobiletin (10), and tetra-*O*-methylscutellarein (11)] were identified. In addition, further three unidentified coumarins (peaks 4, 7, and 12) were detected. The bracketed numbers refer to those in Figure 6.5. In general, Italian essential oils exhibited a higher content of oxygen heterocyclic compounds than the Spanish oils. The use of NP and RP-HPLC with microbore columns and UV detection has also been reported for lemon and bergamot [96], bitter orange and grapefruit [97] essential oils. Orange and

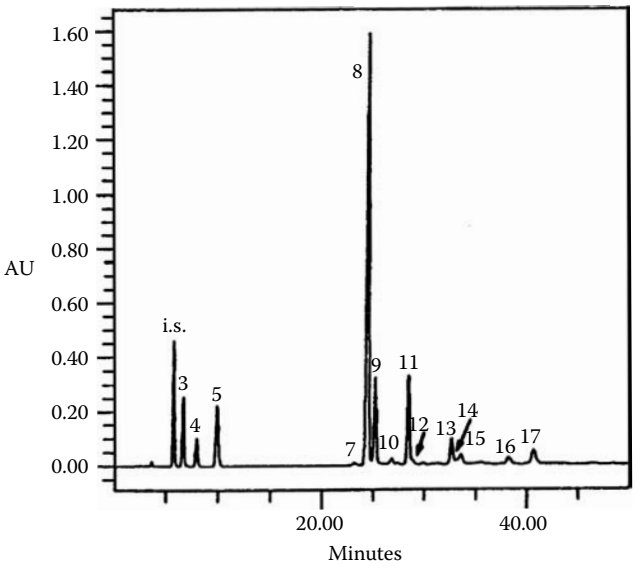

FIGURE 6.5 HPLC chromatogram of Italian genuine bitter orange oil. For peak identification, refer to the text (i.s.—internal standard). (From Dugo, P. et al., 1996. *J. Agric. Food Chem*., 44: 544–549. With permission.)

mandarin essential oils have also been analyzed by NP and RP-HPLC, but with UV and spectrofluorimetric detection in series [98]. For the identification of chromatographic peaks of all the aforementioned oils, a preparative HPLC was used; the purified fractions were then analyzed by GC-MS and HPLC/MS.

The oxygen heterocyclic compounds present in the nonvolatile residue of citrus essential oils has also been extensively investigated by means of high-performance liquid chromatography/atmospheric pressure ionization mass spectrometry (HPLC/API/MS) [99]. The mass spectra obtained at different voltages of the "sample cone" have been used to build a library. Citrus essential oils have been analyzed with this system, using an optimized NP-HPLC method, and the mass spectra were compared with those of the laboratory-constructed library. This approach allowed the rapid identification and characterization of oxygen heterocyclic compounds of citrus oils, the detection of some minor components for the first time in some oils, and also the detection of authenticity and possible adulteration.

Apart from citrus oils, other essential oils have also been analyzed by means of LC, such as the blackcurrant bud essential oil [100]. The latter was fractionated into hydrocarbons and oxygenated compounds and the two fractions were submitted to RP-HPLC analysis. Volatile carbonyls consist of some of the most important compounds for the blackcurrant flavor and, hence, were analyzed in detail. The carbonyls were converted into 2,4-dinitrophenylhydrazones and the mixture of 2,4-dinitrophenylhydrazones was separated into derivatives of keto acids and monocarbonyl and dicarbonyl compounds. Each fraction was submitted to chromatographic investigation.

6.3.7 Multidimensional Gas Chromatographic Techniques

In spite of the considerable instrumental advances made, the detection of all the constituents of an essential oil is an extremely difficult task. For example, GC chromatograms relative to complex mixtures are characterized frequently by several overlapping compounds: Well-known examples are octanal and α-phellandrene, as well as limonene and 1,8-cineol, on 5% diphenyl–95% dimethylpolysiloxane stationary phases, while unsufficient resolution is observed between citronellol and nerol or geraniol and linalyl acetate. On the other hand, the overlapping of monoterpene alcohols

and esters with sesquiterpene hydrocarbons is frequently reported on polyethylene glycol stationary phases [101]. Hence, the direct identification of a component in such mixtures can be a cumbersome challenge. The use of multidimensional gas chromatography (MDGC) can be of great help in complex sample analysis. In MDGC, key fractions of a sample are selected from the first column and reinjected onto a second one, where ideally, they should be fully resolved. The instrumentation usually involves the use of a switching valve arrangement and two chromatographic columns of differing polarities, but generally of identical dimensions. Furthermore, when heart-cut operations are not carried out, the primary column elutes normally in the first dimension (^{1}D) GC system, while heart-cut fractions are chromatographically resolved on the secondary column [102]. The capillaries employed can be operated in either a single or two distinct GC ovens, with both GC systems commonly equipped with detectors.

The use of MDGC has also been described in a wide range of enantioselective gas chromatographic applications [103], involving the use of chiral selectors as the stationary phase for the determination of ee and/or ER, as well as for adulteration assessments.

A fully automated, multidimensional, double-oven GC-GC system has been presented by Mondello et al. The system is based on the use of mechanical valves that allow the automatic multiple transfers of different fractions from the precolumn to the analytical one, and the analysis of all transferred fractions during the same gas chromatographic run. A system of pneumatic valves emitted pressure variations in order to maintain constant retention times in the precolumn, even after numerous transfers. In addition, when the system was not applied in the multidimensional configuration, the two gas chromatographs could be operated independently. The system has been used for the determination of the enantiomeric distribution of monoterpene hydrocarbons and monoterpene alcohols in the essential oils of lime [104], mandarin [105], and lemon [106]. In Figure 6.6, the analysis of the latter oil is outlined to illustrate the technique: the chromatogram of the lemon oil obtained on an SE-52 column with the system in stand-by position is shown in Figure 6.6a, while the one attained with the system in the cut position is illustrated in Figure 6.6b. Figure 6.6c shows the chiral separation of the fractions transferred to the main analytical column. Well-resolved peaks of components present in large amounts, as also of the minor compounds, were attained through the partial transfer of the major concentration components.

MDGC is a useful approach for the fractionation of compounds of particular interest in a specific sample, one of its major applicational areas is chiral analysis, using a conventional column as ^{1}D and a CSP capillary in the second dimension (^{2}D). Es-MDGC analysis has been used for the direct enantioselective evaluation of limonene in *Rutaceae* and *Gramineae* essential oils [107]. It is noteworthy that (4R)-(+)-limonene of high ee values were found in *Rutaceae* oils, as bergamot, orange, mandarin, lemon, or lime oils, while the (4S)-(−)-isomer was present in higher amounts in the *Gramineae* oils, such as citronella or lemongrass. The ratios of α-pinene and β-pinene enantiomers were also taken into consideration.

The use of Es-MDGC using a primary polyethylene glycol stationary phase, and a secondary heptakis (2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsylil)-β-cyclodextrin, has been applied to rose oils. The technique proved to be highly efficient for the assessment of origin and quality control of economically important rose oil, using (3S)-(−)-citronellol, (2S,4R)-(−)-*cis*- and *trans*-rose oxides as markers [108]. Buchu leaf oil has also been assessed through Es-MDGC, and (1S)-menthone, isomenthone, (1S)-pulegone, (1S)-thiols, and (1S)-thiolacetates as enantiopure sulfur compounds [109]. A further application was reported on mint and peppermint essential oils, which contain (1R)-configured monoterpenoids [110]. Es-MDGC equipped with a 5% diphenyl–95% dimethylpolysiloxane and a 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl-β-CD, as the ^{1}D and ^{2}D, respectively, enabled the simultaneous stereodifferentiation of menthone, neomenthol, isomenthone, menthol, neoisomenthol, and menthylacetate.

The technique has also been successfully applied to the authenticity assessment of various commercially available rosemary oils [111]. The ER of α-pinene, camphene, β-pinene, limonene, borneol, terpinen-4-ol, α-terpineol, linalool, and camphor were measured; moreover, (1S)-(−)-borneol of high enantiomeric purity (higher than 90%) has been defined as a reliable indicator of

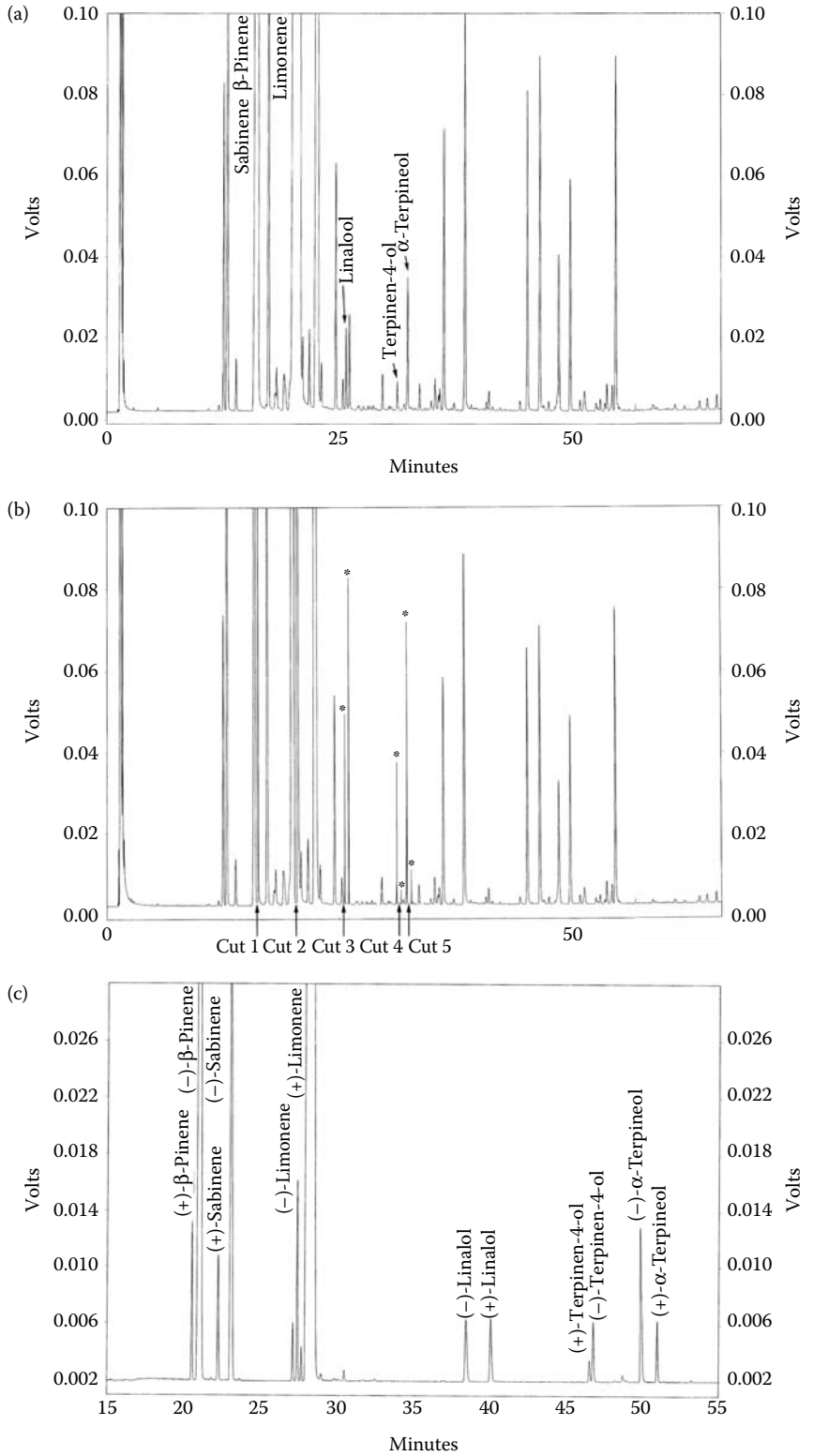

FIGURE 6.6 GC chromatogram of cold-pressed lemon oil obtained on an SE-52 column (a), GC chromatogram of cold-pressed lemon oil obtained on an SE-52 column with five heart-cuts (b), and GC-GC chiral chromatogram of the transferred components (c). (From Mondello, L. et al., 1999. *J. High Res. Chromatogr.*, 22: 350–356. With permission.)

genuine rosemary oils. A recently created high-performance MDGC system has been recently used in this specific field of essential oil research [111]. A conventional method and a fast MDGC method were developed and applied to the enantioselective analysis of rosemary oil. Prior to "heart-cutting," a "stand-by" analysis was carried out in order to define the retention time cutting windows of the chiral components to be reanalyzed in the ^{2}D; retention time windows of eight peaks were defined. The eight peaks (camphene, β-pinene, sabinene, limonene, camphor, isoborneol, borneol, terpinen-4-ol, and α-terpineol) were then cut and transferred. It must be added that, in some cases, only a portion of the entire peak, that is, limonene, was diverted onto the secondary column. The fast MDGC method was applied on a twin set of 0.1 mm I.D. microbore columns with the objective of reproducing the conventional analytical result in a much shorter time (Figure 6.7). The overall run-time requested for the conventional analysis was of 43 min, while it was of 8.7 min for the fast MDGC application, with a speed gain of nearly a factor of five.

MDGC heart-cutting methods, using valve and valveless flow switching interfaces, extend the separation power of capillary GC, although the ^{2}D analysis can only be restricted to a few regions

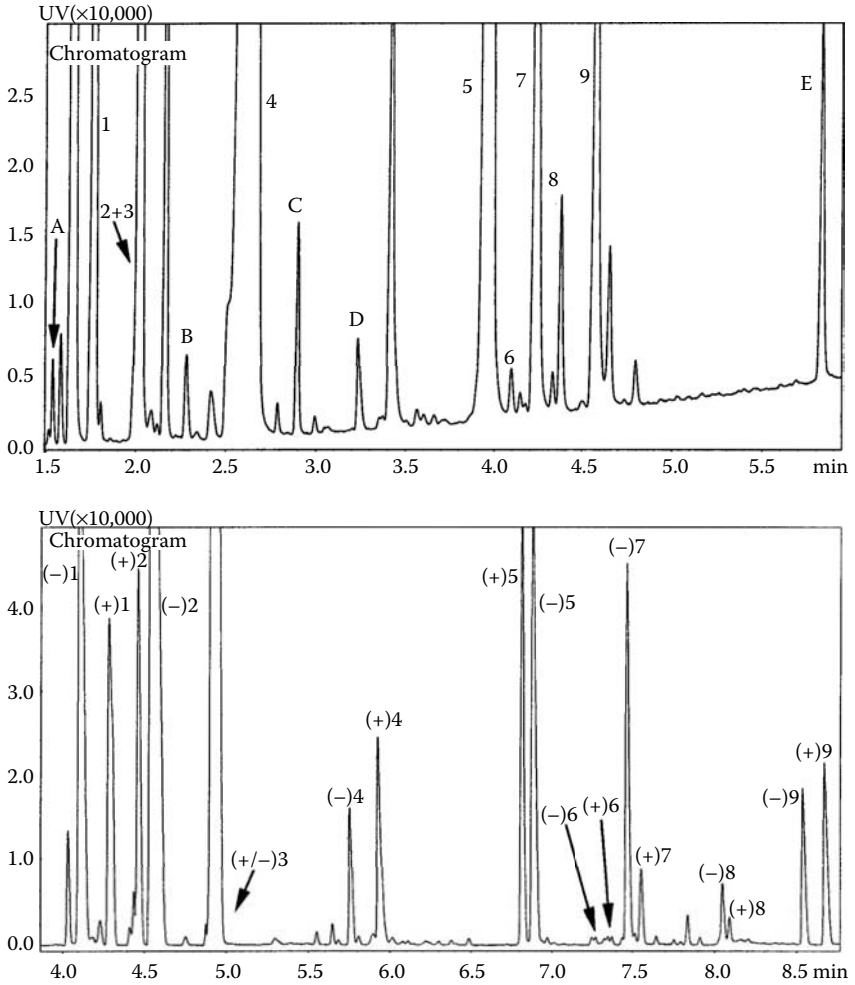

FIGURE 6.7 A 4.5 min chromatogram expansion relative to the fast MDGC rosemary analysis with the transfer system in stand-by position (top); tricyclene (peak A), α-phellandrene (peak B), unknown (peak C), α-terpinolene (peak D), and bornyl acetate (peak E). A 5 min ^{2}D chromatogram expansion relative to the fast MDGC rosemary oil application (bottom); the peak numbers refer to camphene (1), β-pinene (2), sabinene (3), limonene (4), camphor (5), isoborneol (6), borneol (7), terpinen-4-ol (8), and α-terpineol (9). (From Mondello, L. et al., 2006. *J. Chromatogr.* A, 1105: 11–16. With permission.)

of the chromatogram. In order to attain a complete two-dimensional characterization of a sample, a comprehensive approach, such as comprehensive two-dimensional gas chromatography (GC × GC), has to be used.

GC × GC produces an orthogonal two-column separation, with the complete sample transfer achieved by means of a modulator; the latter entraps, refocuses, and releases fractions of the GC effluent from the ^{1}D, onto the ^{2}D column, in a continuous mode; this method enables an accurate screening of complex matrices, offering very high resolution and enhanced detection sensitivity [112,113]. The two columns must possess different separation mechanism, commonly a low polarity or nonpolar column is used in the ^{1}D, and a polar column is used as the fast ^{2}D column. Moreover, a two-dimensional separation can be defined as comprehensive if other two conditions are respected [114,115], namely, equal percentages (either 100% or less) of all sample components pass through both columns and eventually reach the detector; and the resolution obtained in the ^{1}D is essentially maintained.

One of the first applications of GC × GC to essential oils was performed by Dimandja et al. [116], who investigated the separation of peppermint (*Mentha piperita*) and spearmint (*Mentha spicata*) essential oil components. The latter oil is mainly characterized by four major components, that is, carvone, menthol, limonene, and menthone, while the former is mainly represented by menthol, menthone, menthylacetate, and eucalyptol. Both essential oils were considered as being of moderate complexity, containing <100 sample components. The GC × GC system used in this research was composed of a GC equipped with a thermal modulation unit (Zoex Corporation, Lincoln, Nebraska, USA), illustrated in Figure 6.8. The thermal modulation cycle is a three-step process, involving sample accumulation, focusing, and acceleration stages. The column set used was composed of a nonpolar column (1 m × 100 µm I.D., 3.5 µm d_f) in the ^{1}D and one of intermediate polarity (2 m × 100 µm I.D., 0.5 µm d_f) in the ^{2}D, connected by a press fit. A two- to threefold increase in separation power was obtained for the GC × GC analyses of both mint oils; peppermint essential oil was found to contain 89 identifiable peaks by GC × GC compared to 30 peaks in GC-MS, while in the spearmint oil, 68 peaks were detected by GC × GC and 28 by means of GC-MS. The simple alignment of the ^{2}D retention times of the investigated oils revealed that both have 52 components in common, as opposed to 18 matches by monodimensional GC.

GC × GC analyses of essential oils of high complexity have also been carried out, such as of vetiver oil (*Vetiver zizanoide*s) [117]. The work was performed on a gas chromatographic system (6890GC, Agilent Technologies, Santa Clara, USA) retrofitted to a longitudinal modulated cryogenic system (LMCS, Chromatography Concepts, Doncaster, Australia). In Figure 6.9, a schematic diagram of the

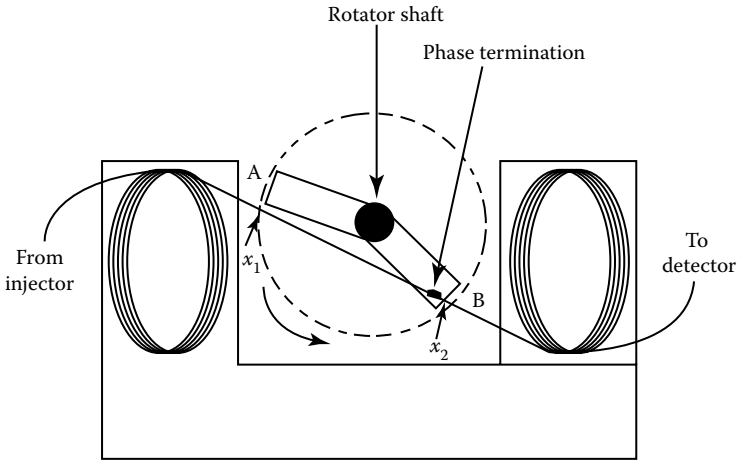

FIGURE 6.8 Top-view scheme of the thermal modulator. The arrows marked as x_1 and x_2 indicate the length of the modulator tube. The rotating heater is in position A prior to each modulation cycle. Its counterclockwise movement (from position A to B) over the modulator tube produces the thermal modulation. (From Dimandja, J.M.D. et al., 2000. *J. High Res.Chromatogr.*, 23: 208–214. With permission.)

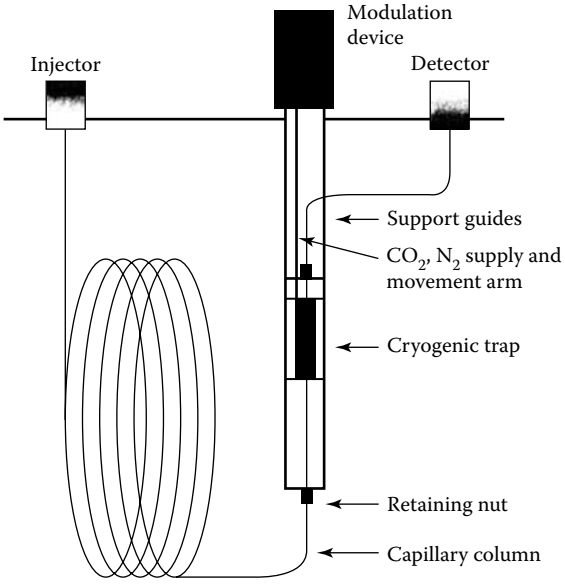

FIGURE 6.9 Scheme of the LMCS located inside a GC oven. (From Marriott, P. et al., 2000. *Flavour Fragr. J.*, 15: 225–239. With permission.)

cryotrap is presented; the columns are anchored with retaining nuts to the support frame so that the trap can move up and down along the column, its movement is controlled by a stepper motor. Liquid cryogen is supplied to the inlet of the trap, passing through a narrow restrictor and expanding to cool the body of the trap. A secondary, small flow of nitrogen passing through the center of the body prevents the build up of ice which would otherwise freeze the column to the trap [118]. Analysis were performed on a 5% phenyl–95% dimethylpolysiloxane ^{1}D column (25 m × 0.25 mm I.D., 0.25 µm d_f) connected to a 50% phenyl–50% dimethylpolysiloxane ^{2}D column (0.8 m × 0.1 mm I.D., 0.1 µm d_f), applying a modulation frequency of 4 s cycle. About 200 compounds could be detected by means of GC × GC analysis. The authors reported that the GC-MS analysis of vetiver oil, with peak deconvolution, would still not have been sufficient for the identification of coeluting substances.

French lavender (*Lavandula angustifolia*) and tea tree (*Melaleuca alternifolia*), essential oils were also submitted to GC × GC analyses using a nonpolar (5% phenyl–95% dimethylpolysiloxane)–polar (polyethylene glycol) column set [119]. The work, developed using an LMCS, enabled the determination of elution patterns within the ^{2}D space useful for the correlation of component retention behavior with their chemical and structural properties. The GC × GC approach provides higher sensitivity, greater peak resolution, and capacity, as well as an essential oil fingerprint pattern. The enhanced peak capacity and sensitivity of GC × GC in the tea tree oil application can be observed in the conventional GC and untransformed GC × GC chromatograms illustrated in Figure 6.10.

Lavender essential oil has been further investigated by means of GC × GC retrofitted with an LMCS and hyphenated to time-of-flight mass spectrometry (GC × GC/TOFMS), thus generating a three-dimensional analytical approach [120]. The authors outlined that the vacuum effect on the ^{2}D column in GC × GC/TOFMS may generate differing retention times with respect to an equivalent analysis performed on a GC × GC system at ambient pressure conditions. Lavender essential oil was further investigated through the comparison of GC-MS and GC × GC analyses [121], as illustrated in Figure 6.11. At least 203 components were counted in the ^{2}D separation space, which was characterized by a well-defined monoterpene hydrocarbon region, and a sesquiterpene hydrocarbon area. The oxygenated derivatives of both these groups generally elute closely after the main group in the ^{1}D, but due to their wide range of component polarities these are found to spread throughout a broader region of the ^{2}D plane. According to the authors, by using GC × GC the detection of subtle

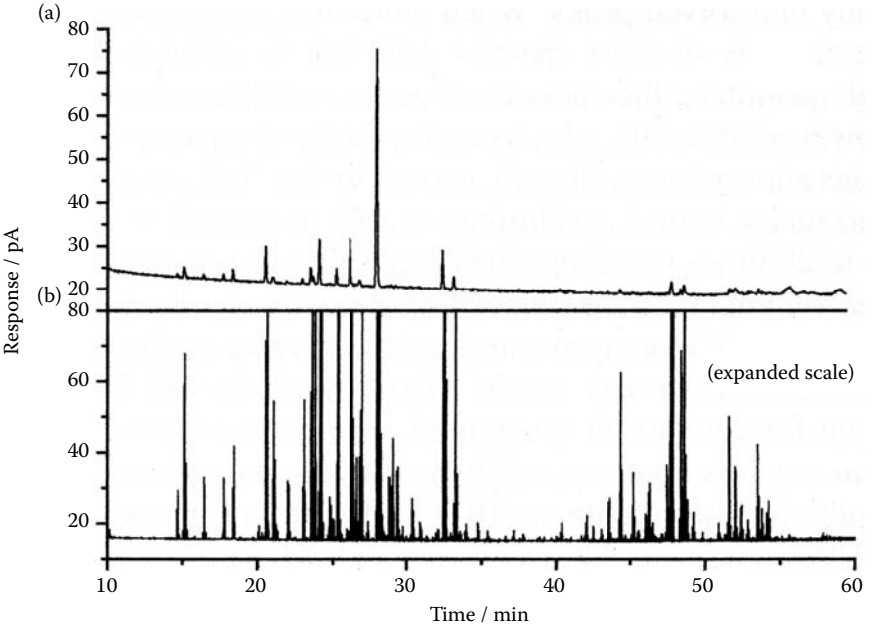

FIGURE 6.10 Comparison of monodimensional GC and pulsed GC × GC result for tea tree oil; both chromatograms are shown at identical sensitivity. (From Shellie, R. et al., 2000. *J. High Resolution Chromatogr.*, 23: 554–560. With permission.)

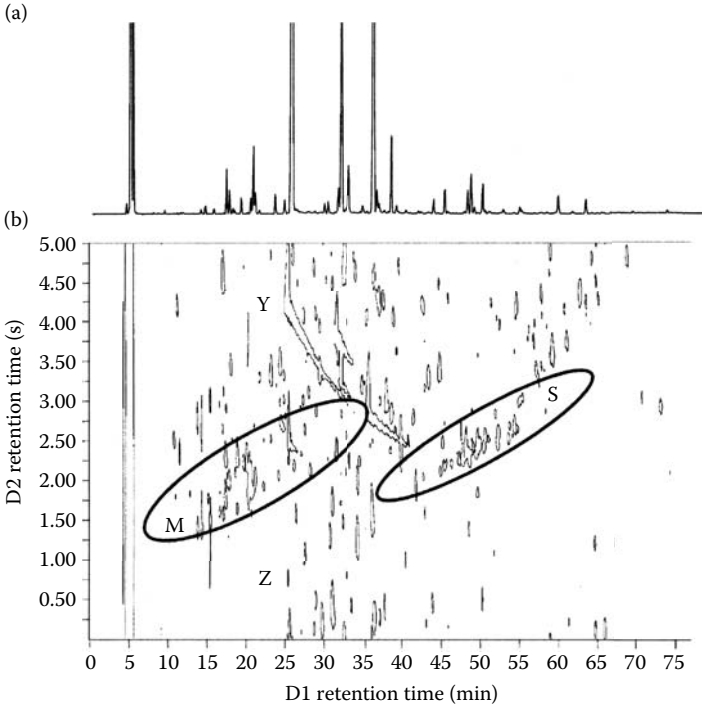

FIGURE 6.11 Reconstructed gas chromatographic trace for a lavender essential oil (a), and the two-dimensional separation space for the GC × GC analysis of the same sample (b). The minor component Z overlaps completely from the major component Y in the ^{1}D; M: monoterpene hydrocarbons, S: sesquiterpene hydrocarbons. (From Shellie, R. et al., 2002. *J. Chromatogr. A*, 970: 225–234. With permission.)

differences in the analyses of lavender essential oils from different cultivars should be simplified, since it could be based on a ^{2}D pictorial representation of the volatile components.

Further relevant essential oil investigations have been performed: the early works using FID, while the more recent ones using, preferably, a TOFMS as detector. Among the essential oils previously studied by means of GC × GC are peppermint [122] and Australian sandalwood [123], with the latter also analyzed through GC × GC/TOFMS in the same work. Essential oils derived from *Thymbra spicata* [124], *Pistacia vera* [125], hop [126], *Teucrium chamaedrys* [127], *Rosa damascena* [128], coriander [129], and *Artemisia annua* [130], as well as tobacco [131], have also been subjected to GC × GC/TOFMS analyses. The references cited herein represent only a fraction of the studies performed by means of GC × GC on essential oils.

6.3.8 Multidimensional Liquid Chromatographic Techniques

HPLC has acquired a role of great importance in food analysis, as demonstrated by the wide variety of applications reported. Single LC column chromatographic processes have been widely applied for sample profile elucidation, providing satisfactory degrees of resolving power; however, whenever highly complex samples require analysis, a monodimensional HPLC system can prove to be inadequate. Moreover, peak overlapping may occur even in the case of relatively simple samples, containing components with similar properties.

The basic principles of MDGC are also valid for multidimensional LC (MDLC). The most common use of MDLC separation is the pretreatment of a complex matrix in an off-line mode. The off-line approach is very easy, but presents several disadvantages: it is time-consuming, operationally intensive, difficult to automate, and to reproduce. Moreover, sample contamination or formation of artifacts can occur. On the other hand, on-line MDLC, though requiring specific interfaces, offers the advantages of ease of automation and greater reproducibility in a shorter analysis time. In the on-line heart-cutting system, the two columns are connected by means of an interface, usually a switching valve, which allows the transfer of fractions of the first column effluent onto the second column.

In contrast to comprehensive gas chromatography (GC × GC), the number of comprehensive liquid chromatography (LC × LC) applications reported in literature are much less. It can be affirmed that LC × LC presents a greater flexibility when compared to GC × GC since the mobile phase composition can be adjusted in order to obtain enhanced resolution [132]. Comprehensive HPLC systems, developed, and applied to the analysis of food matrixes, have employed the combination of either NP × RP or RP × RP separation modes. However, it is worthy of note that the two separation mechanisms exploited should be as orthogonal as possible, so that no or little correlation exists between the retention of compounds in both dimensions.

A typical comprehensive two-dimensional HPLC separation is attained through the connection of two columns by means of an interface (usually a high-pressure switching valve), which entraps specific quantities of ^{1}D eluate, and directs it onto a secondary column. This means that the first column effluent is divided into "cuts" that are transferred continuously to the ^{2}D by the interface. The type of interface depends on the methods used, although multiport valve arrangements have been the most frequently employed.

Various comprehensive HPLC systems have been developed and proven to be effective for the separation of complex sample components, and in the resolution of a number of practical problems. In fact, the very different selectivities of the various LC modes enable the analysis of complex mixtures with minimal sample preparation. However, comprehensive HPLC techniques are complicated by the operational aspects of switching effectively from one operation step to another, by data acquisition and interpretation issues. Therefore careful method optimization and several related practical aspects should be considered.

In the most common approach, a microbore LC column in the first and a conventional column in the ^{2}D are used. In this case, an 8-, 10-, or 12-port valve equipped with two sample loops (or trapping columns) is used as an interface. A further approach foresees the use of a conventional

LC column in the first and two conventional columns in the ^{2}D. One or two valves that allow transfers from the first column to two parallel secondary columns (without the use of storage loops) are used as interface.

One of the best examples of the application of comprehensive NPLC × RPLC in essential oil analysis is represented by the analysis of oxygen heterocyclic components in cold-pressed lemon oil, by using a normal phase with a microbore silica column in the ^{1}D and a monolithic C18 column in the ^{2}D with a 10-port switching valve as interface [133]. In Figure 6.12, an NPLC × RPLC separation of the oxygen heterocyclic fraction of a lemon oil sample is presented. Oxygen heterocyclic components (coumarins, psoralens, and PMFs) represent the main part of the nonvolatile fraction of cold-pressed citrus oils. Their structures and substituents have an important role in the characterization of these oils. Positive peak identification of these compounds was obtained by both the relative location of the peaks in the two-dimensional plane, which varied in relation to their chemical structure, and by characteristic UV spectra. In a later experiment, a similar setup was used for a citrus oil extract composed of lemon and orange oil [134]. The main difference with respect to the earlier published work [133] was the employment of a bonded-phase (diol) column in the ^{1}D. Under optimized LC conditions, the high degree of orthogonality between the NP and RP systems tested, resulted in increased ^{2}D peak capacity.

A novel approach for the analysis of carotenoids, pigments mainly distributed in plant-derived foods, especially in orange and mandarin essential oils, has been recently developed by Dugo et al. [135,136]. In terms of structures, food carotenoids are polyene hydrocarbons, characterized by a C_{40} skeleton that derives from eight isoprene units. They present an extended conjugated double bond (DB) system that is responsible for the yellow, orange, or red colors in plants and are notable for their wide distribution, structural diversity, and various functions. Carotenoids are usually classified in two main groups: hydrocarbon carotenoids, known as carotenes (e.g., β-carotene and lycopene), and oxygenated carotenoids, known as xanthophylls (e.g., β-cryptoxanthin and lutein). The elucidation of carotenoid patterns is particularly challenging, because of the complex composition of carotenoids in natural matrices, their great structural diversity, and their extreme instability. An innovative comprehensive dual-gradient elution HPLC system was employed using an NPLC × RPLC setup, composed of silica and C18 columns in the ^{1}D and ^{2}D, respectively. Free carotenoids in orange essential oil and juice (after saponification), were identified by combining the two-dimensional retention data

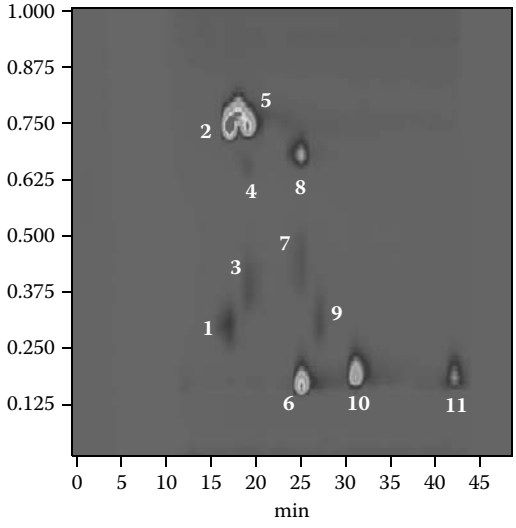

FIGURE 6.12 Comprehensive NP (adsorption)-LC × RP-LC separation of the oxygen heterocyclic fraction of a lemon oil sample (for peak identification see Ref. [133]). (From Dugo, P. et al., 2004. *Anal. Chem.*, 73: 2525–2530. With permission.)

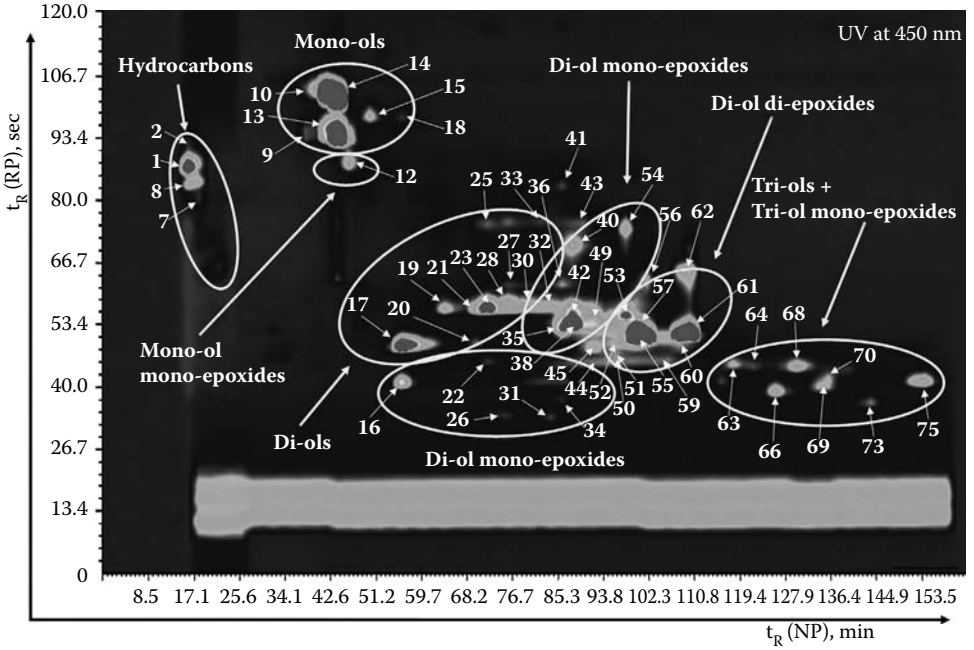

FIGURE 6.13 Contour plot of the comprehensive HPLC analyses of carotenoids present in sweet orange essential oil with peaks and compound classes indicated (for peak identification see Ref. 136). (From Dugo, P. et al., 2006. *Anal. Chem*., 78: 7743–7750. With permission.)

with UV-visible spectra [136] obtained by using a photodiode array detection (DAD) detector (Figure 6.13). A recent study of the carotenoid fraction of a saponified mandarin oil has been performed by means of comprehensive LC, in which a ^{1}D microbore silica column was applied for the determination of free carotenoids, and a cyanopropyl column for the separation of esters; a monolithic column was used in the ^{2}D [135]. Detection was performed by connecting a DAD system in parallel with an MS detection system operated in the atmospheric pressure chemical ionization (APCI) positive-ion mode. Thus, the identification of free carotenoid and carotenoid esters was carried out by combining the information provided by the DAD and MS systems, and the peak positions in the two-dimensional chromatograms.

6.3.9 On-Line Coupled Liquid Chromatography-Gas Chromatography (LC-GC)

The analysis of very complex mixtures is often troublesome due to the variety of chemical classes to which the samples components belong to, and to their wide range of concentrations. As such, several compounds cannot be resolved by monodimensional GC. In this respect, less complex and more homogeneous mixtures can be attained by the fractionation of the matrix by means of LC prior to GC separation. The multidimensional LC-GC approach combines the selectivity of the LC separation with the high efficiency and sensitivity of GC separation, enabling the separation of compounds with similar physicochemical properties in samples characterized by a great number of chemical classes.

For the highly volatile components, commonly present in essential oils, the most adequate transfer technique is partially concurrent eluent evaporation [137]. In the latter technique, proposed by Grob, a retention gap is installed, followed by a few meters of precolumn and the analytical capillary GC column, both with identical stationary phase, for the separation of the LC fractionated components. A vapor exit is placed between the precolumn and the analytical column, allowing partial evaporation of the solvent. Hence, column and detector overloading are avoided. This transfer technique can be applied to the analysis of GC components with a boiling point of at least 50°C higher than the solvent.

The composition of citrus essential oils has been greatly exploited by means of LC-GC, and the development of new methods for the study of single classes of components has been well reported. The aldehyde composition in sweet orange oil has been investigated [138], as also industrial citrus oil mono- and sesquiterpene hydrocarbons [139], and the enantiomeric distribution of monoterpene alcohols in lemon, mandarin, sweet orange, and bitter orange oils [140,141].

The hyphenation of LC-GC systems to mass spectrometric detectors has also been reported for the analyses of neroli [142], bitter and sweet oranges, lemon, and petitgrain mandarin oils [143]. It has to be highlighted that the preliminary LC separation, which reduces mutual component interference, greatly simplifies MS identification.

6.4 GENERAL CONSIDERATIONS ON ESSENTIAL OIL ANALYSIS

As evidenced by the numerous techniques described in the present contribution, chromatography, especially GC, has evolved into the dominant method for essential oil analysis. This is to be expected because the complexity of the samples must be unraveled by some type of separation, before the sample constituents can be measured and characterized; in this respect, GC provides the greatest resolving power for most of these volatile mixtures.

In the past, a vast number of investigations have been carried out on essential oils, and many of these natural ingredients have been investigated following the introduction of GC-MS, which marked a real turning point in the study of volatile molecules. Es-GC also represented a landmark in the detection of adulterations, and in the cases where the latter technique could fail, gas chromatography correlated to isotope ratio mass spectrometry (GC-IRMS) by means of a combustion interface has proved to be a valuable method to evaluate the genuineness of natural product components. In addition, the introduction of GC-O was a breakthrough in analytical aroma research, enabling the differentiation of a multitude of volatiles in odor-active and non-odor-active, according to their existing concentrations in a matrix. The investigation of the nonvolatile fraction of essential oils, by means of LC and its related hyphenated techniques, contributed greatly toward the progress of the knowledge on essential oils. Many extraction techniques have also been developed, boosting the attained results. Moreover, the continuous demand for new synthetic compounds reproducing the sensations elicited by natural flavors triggered analytical investigations toward the attainment of information on scarcely known properties of well-known matrices.

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7 Safety Evaluation of Essential Oils: A Constituent-Based Approach

Timothy B. Adams and Sean V. Taylor

CONTENTS

As a practical matter, the analytical requirements for the quantification and identification of chemical constituents are based on exposure to the essential oil from food and/or flavor use. With increased exposure there is a requirement for lower detection limits and therefore identification of a greater number of constituents. The flexibility of the guide is reflected in the fact that high intake of major congeneric groups of low toxicologic concern will be evaluated along with low intake of minor congeneric groups of significant toxicological concern. The guide also provides a comprehensive evaluation of all congeneric groups and constituents that account for the majority of the composition

of the essential oil. The overall objective of the guide is to organize and prioritize the chemical constituents of an essential oil such that no significant risk associated with the intake of essential oil goes unevaluated. The guide is, however, not intended to be a rigid checklist and requires that expert judgment be applied to ensure that each essential oil is exhaustively evaluated.

7.1 INTRODUCTION

Based on their action on the human senses, plants and essential oils and extracts derived from them have functioned as sources of food, preservatives, medicines, symbolic articles in religious and social ceremonies, and remedies to modify behavior. In many cases, essential oils and extracts gained widespread acceptance as multifunctional agents due to their strong stimulation of the human gustatory (taste) and olfactory (smell) senses. Cinnamon oil exhibits a pleasing warm spicy aftertaste, characteristic spicy aroma, and preservative properties that made it attractive as a food flavoring and fragrance. Four millennia ago, cinnamon oil was the principal ingredient of a holy ointment mentioned in Exodus 32:22–26. Because of its perceived preservative properties, cinnamon and cinnamon oil were sought by Egyptians for embalming. According to Dioscorides (Dioscorides, first century AD), cinnamon was a breath freshener, would aid in digestion, counteract the bites of venomous beasts, reduce inflammation of the intestines and the kidneys, and act as a diuretic. Applied to the face, it was purported to remove undesirable spots. It is no wonder that in 1000 BC cinnamon was more expensive than gold.

Based on histories of use of selected plants and plant products that strongly impact the senses, it is not unexpected that society would bestow powers to heal, cure diseases, and spur desirable emotions in the effort to improve the human condition, often with only a limited understanding or acknowledgment of the toxic effects associated with high doses of these plant products. The "natural" origin of these products and their long history of use by humans have, in part, mitigated concerns as to whether these products work or are safe under conditions of intended use (Arctander, 1969). The adverse effects resulting from the human use of pennyroyal oil as an abortifacient or wild germander as a weight control agent are reminders that no substances is safe independent of considerations of dose. In the absence of information concerning efficacy and safety, recommendations for the quantity and quality of natural product to be consumed as a medicine remain ambiguous. However, when the intended use is as a flavor or fragrance that is subject to governmental regulation, effective and safe levels of use are defined by fundamental biological limits and careful risk assessment.

Flavors and fragrances are complex mixtures that act directly on the gustatory and olfactory receptors in the mouth and nose leading to taste and aroma responses, respectively. Saturation of these receptors by the individual chemicals within the flavors and fragrances occurs at very low levels in animals. Hence, with few exceptions the effects of flavors and fragrances are self-limiting. The evolution of the human diet is tightly tied to the function of these receptors. Taste and aroma not only determine what we eat but often allow us to evaluate the quality of food and, in some cases, identify unwanted contaminants. The principle of self-limitation taken together with the long history of use of essential oils in food argues that these substances are safe under intended conditions of use. In the United States, the conclusion by the U.S. Food and Drug Administration (21 CFR Sec. 182.10, 182.20, 482.40, and 182.50) that these oils are "generally recognized as safe" (GRAS) for their intended use was based, in large part, on these two considerations. In Europe and Asia, the presumption of "safe under conditions of use" has been bestowed on essential oils based on similar considerations.

For other intended uses such as dietary supplements or direct food additives, a traditional toxicology approach has been used to demonstrate the safety of essential oils. This relies on performing toxicity tests on laboratory animals, assessing intake and intended use, and determining adequate margins of safety between daily intake by humans and toxic levels resulting from animal studies. Given the constantly changing marketplace and the consumer demand for new and interesting

products, however, many new intended uses for natural products are required. The resources necessary to test all natural products for each intended use are simply not economical. For essential oils that are complex mixtures of chemicals, the traditional approach is effective only when specifications for the composition and purity are clearly defined and adequate quality controls are in place for the continued commercial use of the oil. In the absence of such specifications, the results of toxicity testing apply specifically and only to the article tested. Recent safety evaluation approaches (Schilter et al., 2003) suggest that a multifaceted decision-tree approach can be applied to prioritize natural products and the extent of data required to demonstrate safety under conditions of use. The latter approach offers many advantages, both economic as well as scientific, over more traditional approaches. Nevertheless, various levels of resource-intensive toxicity testing of an essential oil are required in this approach.

7.2 CONSTITUENT-BASED EVALUATION OF AN ESSENTIAL OIL

Because essential oils are mixtures of volatile organic substances of known chemical structure that react, either singly or in combination, with biomolecules (proteins, etc.) to produce biological responses, it should be possible to relate the intake of high doses of these substances to observed toxicity. No attempt has yet been made to evaluate the safety of a natural product based on its chemical composition and the variability of that composition for the intended use. The chemical constitution of a natural product is fundamental to understanding the product's intended use and factors affecting its safety. Recent advances in analytical methodology have made intensive investigation of the chemical composition of a natural product economically feasible and even routine. High-throughput instrumentation necessary to perform extensive qualitative and quantitative analysis of complex chemical mixtures and to evaluate the variation in the composition of the mixture is now a reality. In fact, analytical tools needed to chemically characterize these complex mixtures are becoming more cost effective, while the cost of traditional toxicology is becoming more cost intensive. Based on the wealth of existing chemical and biological data on the constituents of essential oils and similar data on essential oils themselves, it is possible to validate a constituent-based safety evaluation of an essential oil.

As noted above, it is scientifically valid to evaluate the safety of a natural mixture based on its chemical composition. Fundamentally, it is the interaction between one or more molecules in the natural product and macromolecules (proteins, enzymes, etc.) that yield the biological response, regardless of whether it is a desired functional effect such as a pleasing taste, or a potential toxic effect such as liver necrosis. Many of the advertised beneficial properties of ephedra are based on the presence of the central nervous system stimulant ephedrine. So too, the gustatory and olfactory properties of coriander oil are, in part, based on the binding of the linalool, benzyl benzoate, and other molecules to the appropriate receptors. It is these molecular interactions of chemical constituents that ultimately determine conditions of use.

7.3 SCOPE OF ESSENTIAL OILS USED IN FOOD

7.3.1 Plant Sources

Essential oils, as products of distillation, are mixtures of mainly low-molecular-weight chemical substances. Sources of essential oils include components (e.g., pulp, bark, peel, leaf, berry, and blossom) of fruits, vegetables, spices, and other plants. Essential oils are prepared from foods and nonfood sources. Many of the approximately 100 essential oils used as flavoring substances in food are derived directly from food (i.e., lemon oil, basil oil, and cardamom oil); far fewer are extracts from plants not normally consumed as food (e.g., cedar leaf oil or balsam fir oil).

Whereas an essential oil is typically obtained by steam distillation of the plant or plant part, an oleoresin is produced by extraction of the same with an appropriate organic solvent. The volatile

constituents of the plant isolated in the essential oil are primarily responsible for aroma and taste of the plant. Hence, borneol, bornyl acetate, camphor, and other volatile constituents in rosemary oil can provide a flavor intensity as potent as the mass of dried rosemary used to produce the oil. A few exceptions include cayenne pepper, black pepper, ginger, paprika, and sesame seeds that contain key nonvolatile flavor constituents (e.g., gingerol and zingerone in ginger). These nonvolatile constituents are often higher-molecular-weight hydrophilic substances that would be lost in the preparation of an essential oil but are present in the fixed oil of an oleoresin. For economic reasons, crude essential oils are often produced via distillation at the source of the plant raw material and subsequently further processed at modern flavor facilities. The methods of preparation of essential oils are reviewed in Chapter 4.

7.3.2 Processing of Essential Oils for Flavor Functions

Because essential oils are a product of nature, environmental and genetic factors will impact the chemical composition of the plant. Factors such as species and subspecies, geographical location, harvest time, plant part used, and method of isolation all affect the chemical composition of the crude material separated from the plant. The variability of the composition of the crude essential oil as isolated from nature has been the subject of much research and development since plant and oil yields are major economic factors in crop production.

However, the crude essential oil that arrives at the flavor processing plant is not normally used as such. The crude oil is often subjected to a number of processes that are intended to increase purity and to produce a product with the intended flavor characteristics. Some essential oils may be distilled and cooled to remove natural waxes and improve clarity, while others are distilled more than once (i.e., rectified) to remove undesirable fractions or to increase the relative content of certain chemical constituents. Some oils are dry or vacuum distilled. Normally, at some point during processing, the essential oil is evaluated for its technical function as a flavor. This evaluation typically involves analysis [normally by gas chromatography (GC) or liquid chromatography] of the composition of the essential oil for chemical constituents that are markers for the desired technical flavor effect. For an essential oil such as cardamom oil, levels of target constituents such as terpinyl acetate, 1,8-cineole, and limonene are markers for technical viability as a flavoring substance. Based on this initial assessment, the crude essential oil may be blended with other sources of the same oil or chemical constituents isolated from the oil to reach target ranges for key constituent markers that reflect flavor function. The mixture may then be further rectified by distillation. Each step of the process is driven by flavor function. Therefore, the chemical composition of product to be marketed may be significantly different from that of the crude oil. Also, the chemical composition of the processed essential oil is more consistent than that of the crude batches of oil isolated from various plant harvests. The range of concentrations for individual constituents and for groups of structurally related constituents in an essential oil are dictated, in large part, by the requirement that target levels of flavor-marker constituents must be maintained.

7.3.3 Chemical Composition and Congeneric Groups

In addition to the key chemical markers for the technical flavor effect, an essential oil found on the market will normally contain many other chemical constituents, some having little or no flavor function. However, the chemical constituents of essential oils are not infinite in structural variation. Because they are derived from higher plants, these constituents are formed via one of four or five major biosynthetic pathways: lipoxygenase oxidation of lipids, shikimic acid, isoprenoid (terpenoid), and photosynthetic pathways. In ripening vegetables, lipoxygenases oxidize polyunsaturated fatty acids, eventually yielding low-molecular-weight aldehydes (2-hexenal), alcohols (2,6-nonadienol), and esters, many exhibiting flavoring properties. Plant amino acids phenylalanine and tyrosine are formed via the shikimic acid pathway and can subsequently be deaminated,

oxidized, and reduced to yield important aromatic substances such as cinnamaldehyde and eugenol. The vast majority of constituents detected in commercially viable essential oils are terpenes [e.g., hydrocarbons (limonene), alcohols (menthol), aldehydes (citral), ketones (carvone), acids, and esters (geranyl acetate)] that are formed via the isoprene pathway (Roe and Field, 1965). Since all of these pathways operate in plants, albeit to different extents depending on the species, season, and growth environment, many of the same chemical constituents are present in a wide variety of essential oils.

A consequence of having a limited number of plant biosynthetic pathways is that structural variation of chemical constituents in an essential oil is limited. Essential oils typically contain 5–10 distinct chemical classes or congeneric groups. Some congeneric groups, such as aliphatic terpene hydrocarbons, contain upwards of 100 chemically identified constituents. In some essential oils, a single constituent (e.g., citral in lemongrass oil) or congeneric group of constituents (e.g., hydroxyallylbenzene derivatives; eugenol, eugenyl acetate, etc., in clove bud oil) comprise the majority of the mass of the essential oil. In others, no single congeneric group predominates. For instance, although eight congeneric groups comprise >98% of the composition of oil of *mentha piperita* (peppermint oil), >95% of the oil is accounted for by three chemical groups—(1) terpene aliphatic and aromatic hydrocarbons, (2) terpene alicyclic secondary alcohols, ketones, and related esters, and (3) terpene 2-isopropylidene substituted cyclohexanone derivatives and related substances.

The formation and members of a congeneric group are chosen based on a combination of structural features and known biochemical fate. Substances with a common carbon-skeletal structure and functional groups that participate in common pathways of metabolism are assigned to the same congeneric group. For instance, menthyl acetate hydrolyzes prior to absorption yield menthol, which is absorbed and is inconvertible with menthone in fluid compartments (e.g., the blood). Menthol is either conjugated with glucuronic acid and excreted in the urine or undergoes further hydroxylation mainly at C8 to yield a diol that is also excreted, either free or conjugated. Despite the fact that menthyl acetate is an ester, menthol is an alcohol, menthone a ketone, and 3,8-menthanediol a diol; they are structurally and metabolically related (Figure 7.1). Therefore are members of the same congeneric group.

In the case of *Mentha piperita*, the three principal congeneric groups listed above have different metabolic options and possess different organ-specific toxic potential. The congeneric group of terpene aliphatic and aromatic hydrocarbons is represented mainly by limonene and myrcene. The second and most predominant congeneric group is the alicyclic secondary alcohols, ketones, and related esters that include *d*-menthol, menthone, isomenthone, and menthyl acetate. Although the

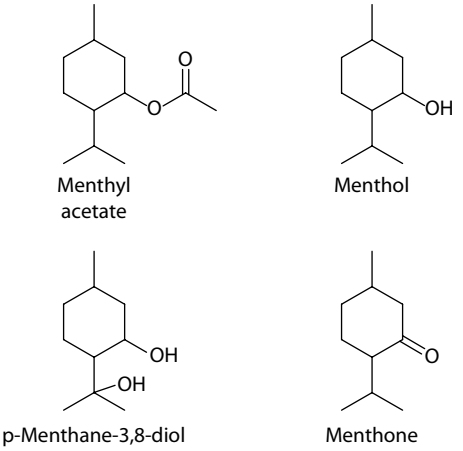

FIGURE 7.1 Congeneric groups are formed by members sharing common structural and metabolic features, such as the group of 2-isopropylidene substituted cyclohexanone derivatives and related substances.

third congeneric group contains alicyclic ketones similar in structure to menthone, it is metabolically quite different in that it contains an exocyclic isopropylidene substituent that undergoes hydroxylation principally at the C9 position, followed by ring closure and dehydration to yield a heteroaromatic furan ring of increased toxic potential. In the absence of a C4–C8 double bond, neither menthone nor isomenthone can participate in this intoxication pathway. Hence they are assigned to a different congeneric group.

The presence of a limited number of congeneric groups in an essential oil is critical to the organization of constituents and subsequent safety evaluation of the oil itself. Members of each congeneric group exhibit common structural features and participate in common pathways of pharmacokinetics and metabolism and exhibit similar toxicologic potential. If the mass of the essential oil (>95%) can be adequately characterized chemically and constituents assigned to well-defined congeneric groups, the safety evaluation of the essential oil can be reduced to (1) a safety evaluation of each of the congeneric groups comprising the essential oil; and (2) a "sum of the parts" evaluation of the all congeneric groups to account for any chemical or biological interactions between congeneric groups in the essential oil under conditions of intended use. Validation of such an approach lies in the stepwise comparison of the dose and toxic effects for each key congeneric group with similar equivalent doses and toxic effects exhibited by the entire essential oil.

Potential interactions between congeneric groups can, to some extent, be analyzed by an in-depth comparison of the biochemical and toxicologic properties of different congeneric groups in the essential oil. For some representative essential oils that have been the subject of toxicology studies, a comparison of data for the congeneric groups in the essential oil with data on the essential oil itself (congeneric groups together) is a basis for analyzing the presence or absence of interactions. Therefore, the impact of interaction between congeneric groups is minimal if the levels of and endpoints for toxicity of congeneric groups (e.g., tertiary terpene alcohols) are similar to those of the essential oil (e.g., coriander oil).

Since composition plays such a critical role in the evaluation, analytical identification requirements are also critical to the evaluation. Complete chemical characterization of the essential oil may be difficult or economically unfeasible based on the small volume of essential oil used as a flavor ingredient. In these few cases, mainly for low-volume essential oils, the unknown fraction may be appreciable and a large number of chemical constituents will not be identified. However, if the intake of the essential oil is low or significantly less than its intake from consumption of food (e.g., thyme) from which the essential oil is derived (e.g., thyme oil), there should be no significant concern for safety under conditions of intended use. For those cases in which chemical characterization of the essential oil is limited but the volume of intake is more significant, it may be necessary to perform additional analytical work to decrease the number of unidentified constituents or, in other cases, to perform selected toxicity studies on the essential oil itself. A principal goal of the safety evaluation of essential oils is that no congeneric groups that have significant human intakes should go unevaluated.

7.3.4 Chemical Assay Requirements and Chemical Description of Essential Oil

The safety evaluation of an essential oil involves specifying the biological origin, physical and chemical properties, and any other relevant identifying characteristics. An essential oil produced under good manufacturing practices (GMP) should be of an appropriate purity (quality), and chemical characterization should be complete enough to guarantee a sufficient basis for a thorough safety evaluation of the essential oil under conditions of intended use. Because the evaluation is based primarily on the actual chemical composition of the essential oil, full specifications used in a safety evaluation will necessarily include not only information on the origin of the essential oil (commercial botanical sources, geographical sources, plant parts used, degree of maturity, and methods of isolation) and physical properties (specific gravity, refractive index, optical rotation, solubility, etc.), but also chemical assays for a range of essential oils currently in commerce.

7.3.4.1 Intake of the Essential Oil

Based on current analytical methodology, it is possible to identify literally hundreds of constituents in an essential oil and quantify the constituents to part per million levels. But is this necessary or desirable? From a practical point of view, the level of analysis for constituents should be directly related to the level of exposure to the essential oil. The requirements to identify and quantify constituents for use of 2,000,000 kg of peppermint oil annually should be far greater than that for use of 2000 kg of coriander oil or 50 kg of myrrh oil annually. Also, there is a level at which exposure to each constituent is so low that there is no significant risk associated with intake of that substance. A conservative no-significant-risk-level of 1.5 μg/d (0.0015 mg/d or 0.000025 mg/kg/d) has been adopted by regulatory authorities as a level at which the human cancer risk is below one in one million (FDA, 2005). Therefore, if consumption of an essential oil results in an intake of a constituent that is <1.5 μg/d, there should be no requirement to identify and quantify that constituent.

Determining the level to which the constituents of an essential oil should be identified depends on estimates of intake of food or flavor additives. These estimates are traditionally calculated using a "volume-based" or a "menu-census" approach. A volume-based approach assumes that the total annual volume of use of a substance reported by an industry is distributed over a portion of the population consuming that substance. A menu-census approach is based on the concentration of the substance (essential oil) added to each flavor, the amount of flavor added to each food category, the portion of food consumed daily, and the total of all exposures across all food types. Although the latter is quite accurate for food additives consumed at higher levels in a wide variety of food such as food emulsifiers, the former method provides an efficient and conservative approximation of intake, if a fraction of the total population is assumed to consume all of the substance.

For the World Health Organization (WHO) and the U.S. Food and Drug Administration (U.S. FDA), intake is calculated using a method known as the *per capita* intake (PCI × 10) method (Rulis et al., 1984; Woods and Doull, 1991). The PCI × 10 method assumes that only 10% of the population consumes the total annual reported volume of use of a flavor ingredient. This approximation provides a practical and cost-effective approach to the estimation of intake for flavoring substances. The annual volumes of flavoring agents are relatively easy to obtain by industry-wide surveys, which can be performed on a regular basis to account for changes in food trends and flavor consumption. The most recent poundage survey of U.S. flavor producers was collected in 2005 and published by the Flavor and Extract Manufacturers (FEMA) in 2007 (Adams et al., 2007). Similar surveys were conducted in recent years in Europe (EFFA, 2005) and Japan (JFFMA, 2002).

Calculation of intake using the PCI × 10 method has been shown to result in conservative estimates of intake and this is appropriate for safety evaluation. Over the last three decades, two comprehensive studies of flavor intake have been undertaken. One involved a detailed dietary analysis (DDA) of a panel of 12,000 consumers who recorded all foods that they consumed over a 14-day period, and the flavoring ingredients in each food were estimated by experienced flavorists to estimate intake of each flavoring substance (Hall, 1976; Hall and Ford, 1999). The other study utilized a robust full stochastic model (FSM) to estimate intake of flavoring ingredients by typical consumers in the United Kingdom (Lambe et al., 2002). The results of the data-intensive DDA method and the model-based FSM support the use of PCI data as a conservative estimate of intake.

With regard to essential oils, the PCI × 10 method provides overestimates of intake for oils that are widely distributed in food. The large annual volume of use reported for essential oils such as orange oil, lemon oil, and peppermint oil indicates widespread use in a large variety of foods resulting in consumption of these oils by significantly more than 10% of the population. Citrus flavor is pervasive in a multitude of foods and beverages. Therefore, for selected high-volume essential oils, a simple PCI rather than a per capita × 10 intake may be more appropriate. However, the intake of the congeneric groups and the group of unidentified constituents for these high-volume oils is still estimated by the PCI × 10 method.

7.3.4.2 Analytical Limits on Constituent Identification

As described above, the analytical requirements for detection and identification of the constituents of an essential oil are set by the intake of the oil and by the conservative assumption that constituents with intakes <1.5 μg/d will not need to be identified. For instance, if the annual volume of use of coriander oil in the USA is 10,000 kg, then the estimated daily PCI of the oil is

$$\frac{10{,}000\ \text{kg/yr} \times 10^9\ \mu\text{g/kg}}{365\ \text{d/yr} \times 28{,}000{,}000\ \text{persons}} = 978\ \mu\text{g coriander oil/person/d.}$$

Based on the intake of coriander oil (978 μg/d), any constituent present at >0.15% would need to be chemically characterized and quantified:

$$\frac{1.5\ \mu\text{g/d}}{978\ \mu\text{g/d}} \times 100 = 0.15\%.$$

For the vast majority of essential oils, meeting these characterization requirements does not require exotic analytical techniques and the identification of the constituents is of a routine nature. However, what would the requirements be for very high-volume essential oils, such as orange oil, cold-pressed oil (567,000 kg), or peppermint oil (1,229,000 kg)? In these cases, a practical limit must be applied and can be justified based on the concept that the intake of these oils is widespread and far exceeds the 10% assumption of PCI × 10. Based on current analytical capabilities, 0.10% or 0.05% could be used as a reasonable limit of detection, with the lower level used for an essential oil that is known or suspected to contain constituents of higher toxic potential (e.g., methyl eugenol in basil).

7.3.4.3 Intake of Congeneric Groups

Once the analytical limits for identification of constituents have been met, it is key to evaluate the intake of each congeneric group from consumption of the essential oil. A range of concentration of each congeneric group is determined from multiple analyses of different lots of the essential oil used in flavorings. The intake of each congeneric group is determined from mean concentrations (%) of constituents recorded for each congeneric group. For instance, for peppermint oil the alicyclic secondary alcohol/ketone/related ester group may contain (–)-menthol, (–)-menthone, (–)-menthyl acetate, and isomenthone in mean concentrations of 43.0%, 20.3%, 4.4%, and 0.40%, respectively, with that congeneric group accounting for 68.1% of the oil. It should be emphasized that although members in a congeneric group may vary among the different lots of oil, the variation in concentration of congeneric groups in the oil is relatively small.

Routinely, the daily PCI of the essential oil derived from the annual volumes is reported in industry surveys (NAS, 1965, 1970, 1975, 1982, 1987; Lucas et al., 1999, 2005; EFFA, 2005; JFFMA, 2002). If a conservative estimate of intake of the essential oil is made using a volume-based approach such that a defined group of constituents and congeneric groups are set for each essential oil, target constituents can be monitored in an ongoing quality control program and the composition of the essential oil can become one of the key specifications linking the product that is distributed in the marketplace to the chemically based safety evaluation.

Limited specifications for the chemical composition of some essential oils to be used as food flavorings are currently listed in the Food Chemicals Codex (FCC, 2008). For instance, the chemical assay for cinnamon oil is given as "not less than 80%, by volume, as total aldehydes." Any specification developed related to this safety evaluation procedure should be consistent with already published specifications including FCC and ISO standards. However, based on chemical

analyses for the commercially available oil, the chemical specification or assay can and should be expanded to

1. Specify the mean of concentrations for congeneric groups with confidence limits that constitute a sufficient number of commercial lots constituting the vast majority of the oil.
2. Identify key constituents of intake >1.5 μg/d in these groups that can be used to efficiently monitor the quality of the oil placed into commerce over time.
3. Provide information on trace constituents that may be of a safety concern.

For example, given its most recent reported annual volume (649 kg), it is anticipated that a chemical specification for lemongrass oil would include (1) >97.6% of the composition chemically identified; (2) not more than 92% aliphatic terpene primary alcohols, aldehydes, acids, and related esters, typically measured as citral; and (3) not more than 15% aliphatic terpene hydrocarbons, typically measured as myrcene. The principal goal of a chemical specification is to provide sufficient chemical characterization to ensure safety of the essential oil from use as a flavoring. From an industry standpoint, the specification should be sufficiently descriptive as to allow timely quality control monitoring for constituents that are responsible for the technical flavor function. These constituents should also be representative of the major congeneric group or groups in the essential oil. Also, monitored constituents should include those that may be of a safety concern at sufficiently high levels of intake of the essential oil (e.g., pulegone). The scope of a specification should be sufficient to ensure safety in use, but not impose an unnecessary burden on industry to perform ongoing analyses for constituents unrelated to the safety or flavor of the essential oil.

7.4 SAFETY CONSIDERATIONS FOR ESSENTIAL OILS, CONSTITUENTS, AND CONGENERIC GROUPS

7.4.1 Essential Oils

7.4.1.1 Safety of Essential Oils: Relationship to Food

The close relationship of natural flavor complexes to food itself has made it difficult to evaluate the safety and regulate the use of essential oils. In the United States, the Federal Food Drug and Cosmetic Act (FFDCA) recognizes that a different, lower standard of safety must apply to naturally occurring substances in food than applies to the same ingredient intentionally added to food. For a substance occurring naturally in food, the Act applies a realistic standard that the substance must "… not ordinarily render it [the food] injurious to health" (21 CFR 172.30). For added substances, a much higher standard applies. The food is considered to be adulterated if the added substance "… may render it [the food] injurious to health" (21 CFR 172.20). Essential oils used as flavoring substances occupy an intermediate position in that they are composed of naturally occurring substances, many of which are intentionally added to food as individual chemical substances. Because they are considered neither a direct food additive nor a food itself, no current standard can be easily applied to the safety evaluation of essential oils.

The evaluation of the safety of essential oils that have a documented history of use in foods starts with the presumption that they are safe based on their long history of use over a wide range of human exposures without known adverse effects. With a high degree of confidence one may presume that essential oils derived from food are likely to be safe. Annual surveys of the use of flavoring substances in the United States (Lucas et al., 1999, 2005; NAS, 1965, 1970, 1975, 1981, 1987; 21 CFR 172.510) in part, document the history of use of many essential oils. Conversely, confidence in the presumption of safety decreases for natural complexes that exhibit a significant change in the pattern of use or when novel natural complexes with unique flavor properties enter the food supply. Recent consumer trends that have changed the typical consumer diet have also changed the exposure levels to essential oils in a variety of ways. As one example, changes in the use of cinnamon oil in low-fat cinnamon pastries would alter intake for a specialized population of eaters. Secondly, increased

international trade has coupled with a reduction in cultural cuisine barriers, leading to the introduction of novel plants and plant extracts from previously remote geographical locations. Osmanthus absolute (FEMA No. 3750) and Jambu oleoresin (FEMA No. 3783) are examples of natural complexes recently used as flavoring substances that are derived from plants not indigenous to the United States and not commonly consumed as part of a Western diet. Furthermore, the consumption of some essential oils may not occur solely from intake as flavoring substances; rather, they may be regularly consumed as dietary supplements with advertised functional benefits. These impacts have brought renewed interest in the safety evaluation of essential oils. Although the safety evaluation of essential oil must still rely heavily on knowledge of the history of use, a flexible science-based approach would allow for rigorous safety evaluation of different uses for the same essential oil.

7.4.2 Safety of Constituents and Congeneric Groups in Essential Oils

Of the many naturally occurring constituents so far identified in plants, there are none that pose, or reasonably might be expected to pose a significant risk to human health at current low levels of intake when used as flavoring substances. When consumed in higher quantities, normally for other functions, some plants do indeed exhibit toxicity. Historically, humans have used plants as poisons (e.g., hemlock) and many of the intended medicinal uses of plants (pennyroyal oil as an abortifacient) have produced undesirable toxic side effects. High levels of exposure to selected constituents in the plant or essential oil (i.e., pulegone in pennyroyal oil) have been associated with the observed toxicity. However, with regard to flavor use, experience through long-term use and the predominant self-limiting impact of flavorings on our senses have restricted the amount of a plant or plant part that we use in or on food.

Extensive scientific data on the most commonly occurring major constituents in essential oils have not revealed any results that would give rise to safety concerns at low levels of exposure. Chronic studies have been performed on more 30 major chemical constituents (menthol, carvone, limonene, citral, cinnamaldehyde, benzaldehyde, benzyl acetate, 2-ethyl-1-hexanol, methyl anthranilate, geranyl acetate, furfural, eugneol, isoeugenol, etc.) found in many essential oils. The majority of these studies were hazard determinations that were sponsored by the National Toxicology Program (NTP) and they were normally performed at dose levels many orders of magnitude greater than the daily intakes of these constituents from consumption of the essential oil. Even at these high intake levels, the majority of the constituents show no carcinogenic potential (Smith et al., 2005a). In addition to dose/exposure, for some flavor ingredients the carcinogenic potential that was assessed in the study is related to several additional factors including the mode of administration, species and sex of the animal model, and target organ specificity. In the vast majority of studies, the carcinogenic effect occurs through a nongenotoxic mechanism in which tumors form secondary to preexisting high-dose, chronic organ toxicity, typically to the liver or kidneys. Selected subgroups of structurally related substances (e.g., aldehydes and terpene hydrocarbons) are associated with a single-target organ and tumor type in a specific species and sex of rodent (i.e., male rat kidney tumors secondary to α-2u-globulin neoplasms with limonene in male rats) or using a single mode of administration (i.e., forestomach tumors that arise due to high doses of benzaldehyde and hexadienal given by gavage).

Given their long history of use, it is unlikely that there are essential oils consumed by humans that contain constituents not yet studied that are weak nongenotoxic carcinogens at chronic high-dose levels. Even if there are such cases, because of the relatively low intake (Adams et al., 2005) as constituents of essential oils, these yet-to-be-discovered constituents would be many orders of magnitude less potent than similar levels of aflatoxins (found in peanut butter), the polycyclic heterocyclic amines (found in cooked foods), or the polynuclear aromatic hydrocarbons (also found in cooked foods). There is nothing to suggest that the major biosynthetic pathways available to higher plants are capable of producing substances such that low levels of exposure to the substance would result in a high level of toxicity or carcinogenicity. Thus, while the minor constituents should be considered, particularly in those plant families and genera known to contain constituents of concern, there is less need for caution than when dealing with xenobiotics, or with substances from origins other than those considered here.

The toxic and carcinogenic potentials exhibited by constituent chemicals in essential oils can largely be equated with the toxic potential of the congeneric group to which that chemical belongs. A comparison of the oral toxicity data (JECFA, 2004) for limonene, myrcene, pinene, and other members of the congeneric group of terpene hydrocarbons show similar low levels of toxicity with the same high-dose target organ endpoint (kidney). Likewise, dietary toxicity and carcinogenicity data (JECFA, 2001) for cinnamyl alcohol, cinnamaldehyde, cinnamyl acetate, and other members of the congeneric group of 3-phenyl-1-propanol derivatives show similar toxic and carcinogenic endpoints. The safety data for the congeneric chemical groups that are found in vast majority of essential oils have been reviewed (Adams et al., 1996, 1997, 1998, 2004; Newberne et al., 1999; Smith et al., 2002a, 2002b; JECFA, 1997–2004). Available data for different representative members in each of these congeneric groups support the conclusion that the toxic and carcinogenic potential of individual constituents adequately represent similar potentials for the corresponding congeneric group.

The second key factor in the determination of safety is the level of intake of the congeneric group from consumption of the essential oil. Intake of the congeneric group will, in turn, depend on the variability of the chemical composition of the essential oil in the marketplace and on the conditions of use. As discussed earlier, chemical analysis of the different batches of oil obtained from the same and different manufacturers will produce a range of concentrations for individual constituents in each congeneric group of the essential oil. The mean concentration values (%) for constituents are then summed for all members of the congeneric group. The total % determined for the congeneric group is multiplied by the estimated daily intake (PCI $\times$ 10) of the essential oil to provide a conservative estimate of exposure to each congeneric group from consumption of the essential oil.

In some essential oils, the intake of one constituent, and therefore, one congeneric group, may account for essentially all of the oil (e.g., linalool in coriander oil, citral in lemongrass oil, and benzaldehyde in bitter almond oil). In other oils, exposure to a variety of congeneric groups over a broad concentration range may occur. As noted earlier, cardamom oil is an example of such an essential oil. Ultimately, it is the relative intake and the toxic potential of each congeneric group that is the basis of the congeneric group-based safety evaluation. The combination of relative intake and toxic potential will prioritize congeneric groups for the safety evaluation. Hypothetically, a congeneric group of increased toxic potential that accounts for only 5% of the essential oil may be prioritized higher than a congeneric group of lower toxic potential accounting for 95%.

The following guide and examples therein are intended to more fully illustrate the principles described above that are involved in the safety evaluation of essential oils. Fermentation products, process flavors, substances derived from fungi, microorganisms, or animals; and direct food additives are explicitly excluded. The guide is designed specifically for application to approximately 100 essential oils that are currently in use as flavoring substances, and for any new essential oils that are anticipated to be marketed as flavoring substances. The guide is a tool to organize and prioritize the chemical constituents and congeneric groups in an essential oil in such a way as to allow a detailed analysis of their chemical and biological properties. This analysis as well as consideration of other relevant scientific data provides the basis for a safety evaluation of the essential oil under conditions of intended use. Validation of the approach is provided, in large part, by a detailed comparison of the doses and toxic effects exhibited by constituents of the congeneric group with the equivalent doses and effects provided by the essential oil.

7.5 THE GUIDE AND EXAMPLE FOR THE SAFETY EVALUATION OF ESSENTIAL OILS

7.5.1 Introduction

The guide does not employ criteria commonly used for the safety evaluation of individual chemical substances. Instead, it is a procedure involving a comprehensive evaluation of the chemical and biological properties of the constituents and congeneric groups of an essential oil. Constituents in the oil that are of known structure are organized into congeneric groups that exhibit similar

metabolic and toxicologic properties. The congeneric groups are further classified according to levels (Structural Classes I, II, and III) of toxicologic concern using a decision-tree approach (Cramer et al., 1978; Munro et al., 1996). Based on intake data for the essential oil and constituent concentrations, the congeneric groups are prioritized according to intake and toxicity potential. The procedure ultimately focuses on those congeneric groups that due to their structural features and intake may pose some significant risk from the consumption of the essential oil. Key elements used to evaluate congeneric groups include exposure, structural analogy, metabolism, and toxicology, which include toxicity, carcinogenicity, and genotoxic potential (Adams et al., 1996, 1997, 1998, 2004; Woods and Doull, 1991; Oser and Ford, 1991; Oser and Hall, 1977; Newberne et al., 1999; Smith et al., 2002a, 2002b). Throughout the analysis of these data, it is essential that professional judgment and expertise be applied to complete the safety evaluation of the essential oil. As an example of how a typical evaluation process for an essential oil is carried out according to this guide, the safety evaluation for flavor use of cornmint oil (*Mentha arvensis*) is outlined in Section 7.5.3.2.1.

7.5.2 Elements of the Guide for the Safety Evaluation of the Essential Oil

7.5.2.1 Introduction

In Step 1 of the guide, the evaluation procedure estimates intake based on industry survey data for each essential oil. It then organizes the chemically identified constituents that have an intake >1.5 μg/d into congeneric groups that participate in common pathways of metabolism and exhibit similar toxic potential. In Steps 2 and 3, each identified chemical constituent is broadly classified according to toxic potential (Cramer et al., 1978) and then assigned to a congeneric group of structurally related substances that exhibit similar pathways of metabolism and toxicologic potential.

Before the formal evaluation begins, it is necessary to specify the data (e.g., botanical, physical, and chemical) required to completely describe the product being evaluated. In order to effectively evaluate an essential oil, attempted complete analyses must be available for the product intended for the marketplace from a number of flavor manufacturers. Additional quality control data are useful, as they demonstrate consistency in the chemical composition of the product being marketed. A Technical Information paper drafted for the particular essential oil under consideration organizes and prioritizes these data for efficient sequential evaluation of the essential oil.

In Steps 8 and 9, the safety of the essential oil is evaluated in the context of all congeneric groups and any other related data (e.g., data on the essential oil itself or for an essential oil of similar composition). The procedure organizes the extensive database of information on the essential oil constituents in order to efficiently evaluate the safety of the essential oil under conditions of use. It is important to stress, however, that the guide is not intended to be nor in practice operates as a rigid checklist. Each essential oil that undergoes evaluation is different, and different data will be available for each. The overriding objective of the guide and subsequent evaluation is to ensure that no significant portion of the essential oil should go unevaluated.

7.5.2.2 Prioritization of Essential Oil According to Presence in Food

In Step 1, essential oils are prioritized according to their presence or absence as components of commonly consumed foods. Many essential oils are isolated from plants that are commonly consumed as a food. Little or no safety concerns should exist for the intentional addition of the essential oil to the diet, particularly if intake of the oil from consumption of traditional foods (garlic) substantially exceeds intake as an intentionally added flavoring substance (garlic oil). In many ways, the first step applies the concept of "long history of safe use" to essential oils. That is, if exposure to the essential oil occurs predominantly from consumption of a normal diet a conclusion of safety is straightforward. Step 1 of the guide clearly places essential oils that are consumed as part of a traditional diet on a lower level of concern than those oils derived from plants that are either not part of the traditional diet or whose intake is not predominantly from the diet. The first step also mitigates

the need to perform comprehensive chemical analysis for essential oils in those cases where intake is low and occurs predominantly from consumption of food. An estimate of the intake of the essential oil is based on the most recent poundage available from flavor industry surveys and the assumption that the essential oil is consumed by only 10% of the population for an oil having a survey volume <50,000 kg/yr and 100% of the population for an oil having a survey volume >50,000 kg/yr. In addition, the detection limit for constituents is determined based on the daily PCI of the essential oil.

7.5.2.2.1 Cornmint Oil

To illustrate the type of data considered in Step 1, consider cornmint oil. Cornmint oil is produced by the steam distillation of the flowering herb of *Mentha arvensis*. The crude oil contains upwards of 70% (−)-menthol, some of which is isolated by crystallization at low temperature. The resulting dementholized oil is cornmint oil. Although produced mainly in Brazil during the 1970s and 1980s, cornmint oil is now produced predominantly in China and India. Cornmint has a more stringent taste compared to that of peppermint oil, *Mentha piperita*, but can be efficiently produced and is used as a more cost-effective substitute. Cornmint oil isolated from various crops undergoes subsequent "clean up," further distillation, and blending to produce the finished commercial oil. Although there may be significant variability in the concentrations of individual constituents in different samples of crude essential oil, there is far less variability in the concentration of constituents and congeneric groups in the finished commercial oil. The volume of cornmint oil reported in the most recent U.S. poundage survey is 327,494 kg/yr (Gavin et al., 2008), which is approximately 25% of the potential market of peppermint oil. Because cornmint oil is a high-volume essential oil, it is highly likely that the entire population consumes the annual reported volume, and therefore the daily PCI is calculated based on 100% of the population (280,000,000). This results in a daily PCI of approximately 3.2 mg/person/d (0.0533 mg/kg bw/d) of cornmint oil.

$$\frac{327{,}494\ \text{kg/yr} \times 10^9\ \mu\text{g/kg}}{365\ \text{days/yr} \times 280 \times 10^6\ \text{persons}} = 3204\ \mu\text{g/person/d}.$$

Based on the intake of cornmint oil (3204 μg/d), any constituent present at >0.047% would need to be chemically characterized and quantified:

$$\frac{1.5\ \mu\text{g/d}}{3204\ \mu\text{g/d}} \times 100 = 0.047\%.$$

7.5.2.3 Organization of Chemical Data: Congeneric Groups and Classes of Toxicity

In Step 2, constituents are assigned to one of three structural classes (I, II, or III) based on toxic potential (Cramer et al., 1978). Class I substances contain structural features that suggest a low order of oral toxicity. Class II substances are clearly less innocuous than Class I substances, but do not contain structural features that provide a positive indication of toxicity. Class III substances contain structural features (e.g., an epoxide functional group, unsubstituted heteroaromatic derivatives) that permit no strong presumption of safety, and in some cases may even suggest significant toxicity. For instance, the simple aliphatic hydrocarbon, limonene, is assigned to Structural Class I while elemicin, which is an allyl-substituted benzene derivative with a reactive benzylic/allylic position, is assigned to Class III. Likewise, chemically unidentified constituents of the essential oil are automatically placed in Structural Class III, since no presumption of safety can be made.

The toxic potential of each of the three structural classes has been quantified (Munro et al., 1996). An extensive toxicity database has been compiled for substances in each structural class. The database covers a wide range of chemical structures, including food additives, naturally occurring substances, pesticides, drugs, antioxidants, industrial chemicals, flavors, and fragrances. Conservative no-observable-effect-levels (fifth percentile NOELs) have been determined for each class. These fifth percentile NOELs for each structural class are converted to human exposure thresholds levels by applying a 100-fold safety factor and correcting for mean bodyweight (60/100). The human exposure threshold levels are referred to as thresholds of toxicological concern (TTC). With regard to flavoring substances, the TTC are even more conservative, given that the vast majority of NOELs for flavoring substances are above the 90th percentile. These conservative TTC have since been adopted by the WHO and Commission of the European Communities for use in the evaluation of chemically identified flavoring agents by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) (JECFA, 1997; EC, 1999).

Step 3 is a key step in the guide. It organizes the chemical constituents into congeneric groups that exhibit common chemical and biological properties. Based on the well-recognized biochemical pathways operating in plants, essentially all of the volatile constituents found in essential oils, extracts, and oleoresins belong to well-recognized congeneric groups. Recent reports (Maarse et al., 1992, 1994, 2000; Nijssen et al., 2003) of the identification of new naturally occurring constituents indicate that newly identified substances fall into existing congeneric groups. The Expert Panel, JECFA, and the European Communities (EC) have acknowledged that individual chemical substances can be evaluated in the context of their respective congeneric group (Smith et al., 2005; JECFA, 1997; EC, 1999). The congeneric group approach provides the basis for understanding the relationship between the biochemical fate of members of a chemical group and their toxicologic potential. Within this framework, the objective is to continuously build a more complete understanding of the absorption, distribution, metabolism, and excretion of members of the congeneric group and their potential to cause systemic toxicity. Within the guidelines, the structural class of each congeneric group is assigned based on the highest structural class of any member of the group. Therefore, if an essential oil contained a group of furanone derivatives that were variously assigned to structural classes II and III, then in the evaluation of the oil the congeneric group would, in a conservative manner, be assigned to Class III.

The types and numbers of congeneric groups in a safety evaluation program are, by no means, static. As new scientific data and information become available, some congeneric groups are combined while others are subdivided. This has been the case for the group of alicyclic secondary alcohols and ketones that were the subject of a comprehensive scientific literature review (SLR) in 1975 (FEMA, 1975). Over the last two decades, experimental data have become available indicating that a few members of this group exhibit biochemical fate and toxicologic potential inconsistent with that for other members of the same group. These inconsistencies, almost without exception, arise at high-dose levels that are irrelevant to the safety evaluation of low levels of exposure to flavor use of the substance. However, given the importance of the congeneric group approach in the safety assessment program, it is critical to resolve these inconsistencies. Additional metabolic and toxicologic studies may be required to distinguish the factors that determine these differences. Often the effect of dose and a unique structural feature results in utilization of a metabolic activation pathway not utilized by other members of a congeneric group. Currently, evaluating bodies including JECFA, EFSA, and the FEMA Expert Panel have classified flavoring substances into the same congeneric groups for the purpose of safety evaluation.

Step 4 incorporates the available analytical data for constituents in the essential oil into the congeneric group approach. First, the percentages of the individual constituents that comprise each congeneric group are summed. The highest determined percentages for each constituent are used to calculate a total amount for each congeneric group, since this provides the highest possible amount of each congeneric group within the oil. Based on that high percentage and the estimated daily PCI of the essential oil, the daily PCI of the congeneric group from the essential oil is estimated.

In Steps 5, 6, and 7, each congeneric group in the essential oil is evaluated for safety in use. In Step 5, an evaluation of the metabolism and disposition is performed to determine, under current conditions of intake, whether the group of congeneric constituents is metabolized by well-established detoxication pathways to yield innocuous products. That is, such pathways exist for the congeneric group of constituents in an essential oil and safety concerns will arise only if intake of the congeneric group is sufficient to saturate these pathways potentially leading to toxicity. If a significant intoxication pathway exists (e.g., pulegone), this should be reflected in a higher decision-tree class and lower TTC threshold. At Step 6 of the procedure, the intake of the congeneric group relative to the respective TTC for one of the three structural classes (1800 μg/d for Class I; 540 μg/d for Class II; 90 μg/d for Class III; see Table 7.1) is evaluated. If the intake of the congeneric group is less than the threshold for the respective structural class, the intake of the congeneric group presents no significant safety concerns. The group passes the first phase of the evaluation and is then referred to Step 8, the step in which the safety of the congeneric group is evaluated in the context of all congeneric groups in the essential oil.

If, at Step 5, no sufficient metabolic data exist to establish safe excretion of the product, or if activation pathways have been identified for a particular congeneric group, then the group moves to Step 7 and toxicity data are required to establish safe use under current conditions of intake. There are examples where low levels of xenobiotic substances can be metabolized to reactive substances. In the event that reactive metabolites are formed at low levels of intake of naturally occurring substances, a detailed analysis of dose-dependent toxicity data must be performed. Also, if the intake of the congeneric group is greater than the human exposure threshold (suggesting metabolic saturation may occur), then toxicity data are also required. If, at Step 7, a database of relevant toxicological data for a representative member or members of the congeneric group indicates that a sufficient margin of safety exists for the intake of the congeneric group, the members of that congeneric group are concluded to be safe under conditions of use of the essential oil. The congeneric group then moves to Step 8.

TABLE 7.1
Structural Class Definitions and Their Human Intake Thresholds

Class	Description	Fifth Percentile NOEL (mg/kg/d)	Human Exposure Threshold (TTC)[a] (μg/d)
I	Structure and related data suggest a low order of toxicity. If combined with low human exposure, they should enjoy an extremely low priority for investigation. The criteria for adequate evidence of safety would also be minimal. Greater exposures would require proportionately higher priority for more exhaustive study	3.0	1800
II	Intermediate substances. They are less clearly innocuous than those of Class I, but do not offer the basis either of the positive indication of toxicity or of the lack of knowledge characteristic of those in Class III	0.91	540
III	Permit no strong initial presumptions of safety, or that may even suggest significant toxicity. They thus deserve the highest priority for investigation. Particularly, when per capita intake is high of a significant subsection of the population has a high intake, the implied hazard would then require the most extensive evidence for safety-in-use	0.15	90

[a] The human exposure threshold was calculated by multiplying the fifth percentile NOEL by 60 (assuming an individual weighs 60 kg) and dividing by a safety factor of 100.

In the event that insufficient data are available to evaluate a congeneric group at Step 7, or the currently available data result in margins of safety that are not sufficient, the essential oil cannot be further evaluated by this guide and must be set aside for further considerations.

Use of the guide requires scientific judgment at each step of the sequence. For instance, if a congeneric group that accounted for 20% of a high-volume essential oil was previously evaluated and found to be safe under intended conditions of use, the same congeneric group found at less than 2% of a low-volume essential oil does not need to be further evaluated.

Step 8 considers additivity or synergistic interactions between individual substances and between the different congeneric groups in the essential oil. As for all other toxicological concerns, the level of exposure to congeneric groups is relevant to whether additive or synergistic effects present a significant health hazard. The vast majority of essential oils are used in food in extremely low concentrations, which therefore results in very low intake levels of the different congeneric groups within that oil. Moreover, major representative constituents of each congeneric group have been tested individually and pose no toxicological threat even at dose levels that are orders of magnitude greater than normal levels of intake of essential oils from use in traditional foods. Based on the results of toxicity studies both on major constituents of different congeneric groups in the essential oil and on the essential oil itself, it can be concluded that the toxic potential of these major constituents is representative of that of the oil itself, indicating the likely absence of additivity and synergistic interaction. In general, the margin of safety is so wide and the possibility of additivity or synergistic interaction so remote that combined exposure to the different congeneric groups and the unknowns are considered of no health concern, even if expert judgment cannot fully rule out additivity or synergism. However, case-by-case considerations are appropriate. Where possible combined effects might be considered to have toxicological relevance, additional data may be needed for an adequate safety evaluation of the essential oil.

Additivity of toxicologic effect or synergistic interaction is a conservative default assumption that may be applied whenever the available metabolic data do not clearly suggest otherwise. The extensive database of metabolic information on congeneric groups (JECFA, 1997–2004) that are found in essential oils suggests that the potential for additive effects and synergistic interactions among congeneric groups in essential oils is extremely low. Although additivity of effect is the approach recommended by National Academy of Sciences (NAS)/National Research Council (NRC) committees (NRC, 1988, 1994) and regulatory agencies (EPA, 1988), the Presidential Commission of Risk Assessment and Risk Management recommended (Presidential Commission, 1996, p. 68) that "For risk assessments involving multiple chemical exposures at low concentrations, without information on mechanisms, risks should be added. If the chemicals act through separate mechanisms, their attendant risks should not be added but should be considered separately." Thus, the risks of chemicals that act through different mechanisms, that act on different target systems, or that are toxicologically dissimilar in some other way should be considered to be independent of each other. The congeneric groups in essential oils are therefore considered separately.

Further, the majority of individual constituents that comprise essential oils are themselves used as flavoring substances that pose no toxicological threat at doses that are magnitudes greater than their level of intake from the essential oil. Rulis (1987) reported that "The overwhelming majority of additives present a high likelihood of having safety assurance margins in excess of 10^5." He points out that this is particularly true for additives used in the USA at less than 100,000 lb/yr. Because more than 90% of all flavoring ingredients are used at <10,000 lb/yr (Hall and Oser, 1968), this alone implies intakes commonly many orders of magnitude below the no-effect level. Nonadditivity thus can often be assumed. As is customary in the evaluation of any substance, high-end data for exposure (consumption) are used, and multiple other conservatisms are employed to guard against underestimation of possible risk. All of these apply to complex mixtures as well as to individual substances.

7.5.2.3.1 Cornmint Oil Congeneric Groups

In cornmint oil, the principal congeneric group is composed of terpene alicyclic secondary alcohols, ketones, and related esters, as represented by the presence of (–)-menthol, (–)-menthone,

(+)-isomenthone, (–)-menthyl acetate, and other related substances. Samples of triple-distilled commercial cornmint oil may contain up to 95% of this congeneric group. The biochemical and biological fate of this group of substances has been previously reviewed (Adams et al., 1996; JECFA, 1999). Key data on metabolism, toxicity, and carcinogenicity are cited below in order to complete the evaluation. Although constituents in this group are effectively detoxicated via conjugation of the corresponding alcohol or ω-oxidation followed by conjugation and excretion (Yamaguchi et al., 1994; Madyastha and Srivatsan, 1988; Williams, 1940), the intake of the congeneric group (3044 μg/person/d or 3.04 mg/person/d; see Table 7.2) is higher than the exposure threshold of 540 μg/person/d or 0.540 mg/person/d for Structural Class II. Therefore, toxicity data are required for this congeneric group. In both short- and long-term studies (Madsen et al., 1986; JEFCA, 2000a), menthol, menthone, and other members of the group exhibit no-observable-adverse-effect-levels (NOAELs) at least 1000 times the daily PCI ("eaters only") (3.04 mg/person/d or 0.05 mg/kg bw/d) of this congeneric group resulting from intake of the essential oil. For members of this group, numerous *in vitro* and *in vivo* genotoxicity assays are consistently negative (Heck et al., 1989; Sasaki et al., 1989; Muller, 1993; Florin et al., 1980; Rivedal et al., 2000; Zamith et al., 1993; NTP Draft, 2003). Therefore, the intake of this congeneric group from consumption of *Mentha arvensis* is not a safety concern.

Although it is a constituent of cornmint oil and is also a terpene alicyclic ketone structurally related to the above congeneric group, pulegone exhibits a unique structure (i.e., 2-isopropylidenecyclohexanone) that participates in a well-recognized intoxication pathway (Figure 7.2) (McClanahan et al., 1989; Thomassen et al., 1992; Adams et al., 1996; Chen et al., 2001) that leads to the formation of menthofuran. This metabolite subsequently oxidizes and the ring opens to yield a highly reactive 2-ene-1,4-dicarbonyl intermediate that reacts readily with proteins resulting in hepatotoxicity at intake levels at least two orders of magnitude less than no observable effect levels for structurally related alicyclic ketones and secondary alcohols (menthone, carvone, and menthol). Therefore, pulegone and its metabolite (menthofuran), which account for <2% of commercial cornmint oil, are considered separately in the guide. In this case, the daily PCI of 64 μg/person/d (1.07 μg/kg bw/d) does not exceed the 90 μg/d threshold for Class III. However, a 90-day study on pulegone (NTP, 2002) showed a NOAEL (9.375 mg/kg bw/d) that is approximately 8700 times the intake of pulegone and its metabolites as constituents of cornmint oil. Also, in a 28-day study with peppermint oil (*Mentha piperita*) containing approximately 4% pulegone and menthofuran, a NOAEL of 200 mg/kg bw/d for male rats and a NOAEL of 400 mg/kg bw/d for female rats were established that corresponds to a NOAEL of 8 mg/kg bw/d for pulegone and menthofuran (Serota, 1990). In a 90-day study with a mixture of *Mentha piperita* and *Mentha arvensis* oils (Splindler and Madsen, 1992; Smith et al., 1996), a NOAEL of 100 mg/kg bw/d was established that corresponds to a NOAEL of 4 mg/kg bw/d for pulegone and menthofuran.

The only other congeneric group that accounts for >2% of the composition of cornmint oil is a congeneric group of terpene hydrocarbons [(+) and (–)-pinene, (+) limonene, etc.]. Although these may contribute up to 8% of the oil, upon multiple redistillations during processing the hydrocarbon content can be significantly reduced (<3%) in the finished commercial oil. Using the 8% figure to determine a conservative estimate of intake, the intake of terpene hydrocarbons is 256 μg/person/d (4.27 μg/kg bw/d). This group is predominantly metabolized by cytochrome P450-catalyzed hydroxylation, conjugation, and excretion (Ishida et al., 1981; Madyastha and Srivatsan, 1987; Crowell et al., 1994; Poon et al., 1996; Vigushin et al., 1998; Miyazawa et al., 2002). The daily PCI of 256 μg/person/d is less than the exposure threshold (1800 μg/person/d) for Structural Class I. Although no additional data would be required to complete the evaluation of this group, NOAELs (300 mg/kg bw/d) from long-term studies (NTP, 1990) on principal members of this group are orders of magnitude greater than the daily PCI ("eaters only") of terpene hydrocarbons (0.025 mg/kg bw/d). Therefore, all congeneric groups in cornmint oil are considered safe for use when consumed in cornmint oil.

TABLE 7.2
Safety Evaluation of Cornmint Oil, *Mentha arvensis*[a]

Steps 2 and 3: Constituent Identification/Congeneric Grouping/Structural Class Assignment		Step 4: Oil Composition by Congeneric Group, Resulting Intake		Steps 5,6,7,8: Metabolism/Toxicology Data/Evaluation			
Congeneric Group Assignment	**Structural Class (TTC) (µg/Person/d)**	**High % From Multiple Commercial Samples**	**Intake (µg/ Person/d)**	**Metabolism Pathways**	**Intake of Congeneric Group or Total of Unidentified Constituents Group < TTC for Class?**	**Relevant Toxicity Data if Intake of Group >TTC**	**Additive Interactions Among Congeneric Groups?**
Secondary alicyclic saturated and unsaturated alcohol/ketone/ ketal/ester (e.g., menthol, menthone, isomenthone, menthyl acetate)	II (540)	95	3044	1. Glucuronic acid conjugation of the alcohol followed by excretion in the urine	No, 3044 µg/person/d >540 µg/ person/d	NOEL of 600,000 µg/ kg bw/d for menthol (103-week dietary study in mice) (NCI, 1979)	None anticipated
				2. ω-Oxidation of the side chain substituents to yield various polyols and hydroxy acids and excreted as glucuronic acid conjugates		NOEL of 400,000 µg/kg bw/d for menthone (28-day gavage study in rats) (Madsen et al., 1986)	
Aliphatic terpene hydrocarbon (e.g., limonene, pinene)	I (1800)	8	256	1. ω-Oxidation to yield polar hydroxy and carboxy metabolites excreted as glucuronic acid conjugates	Yes, 256 µg /person/d, <1800 µg/ person/d	Not required	

2-Isopropylidene cyclohexanone and metabolites (e.g., pulegone)	III (90)	2	64	1. Reduction to yield menthone or isomenthone, followed by hydroxylation of ring or side chain positions and then conjugation with glucuronic acid 2. Conjugation with glutathione in a Michael-type addition leading to mercapturic acid conjugates that are excreted or further hydroxylated and excreted 3. Hydroxylation catalyzed by cytochrome P-450 to yield a series of ring- and side chain-hydroxylated pulegone metabolites, one of which is a reactive 2-ene-1,4-dicarbonyl derivative. This intermediate is known to form protein adducts leading to enhanced toxicity (Austin et al., 1988)	Yes, 64 µg/person/d <90 µg/person/d	Not required. But NOAEL of 9375 µg/kg bw/d for pulegone (90-day gavage study in rats) (NTP, 2002)

Note: In Steps 2 and 3, individual constituents and their assignment to structural classes are not shown.

[a] Based on daily PCI of 3204 µg/person/d for cornmint oil, as determined in Step 1.

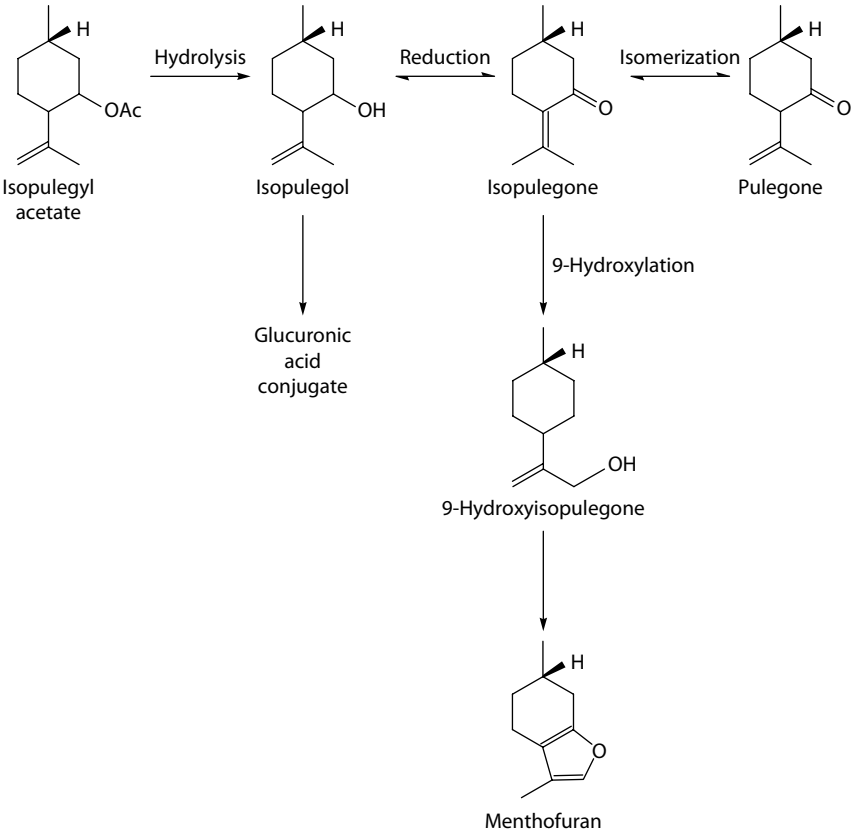

FIGURE 7.2 Metabolism of isopulegone, pulegone, and isopulegyl acetate.

Finally, the essential oil itself is evaluated in the context of the combined intake of all congeneric groups and any other related data in Step 8. Interestingly, members of the terpene alicyclic secondary alcohols, ketones, and related esters, multiple members of the monoterpene hydrocarbons, and peppermint oil itself show a common nephrotoxic effect recognized as α-2u-globulin nephropathy. The microscopic evidence of histopathology of the kidneys for male rats in the mint oil study is consistent with the presence of α-2u-globulin nephropathy. In addition, a standard immunoassay for detecting the presence of α-2u-globulin was performed on kidney sections from male and female rats in the mint oil study (Serota, 1990). Results of the assay confirmed the presence of α-2u-globulin nephropathy in male rats (Swenberg and Schoonhoven, 2002). This effect is found only in males rats and is not relevant to the human health assessment of cornmint oil. Other toxic interactions between congeneric groups are expected to be minimal given that the NOELs for the congeneric groups and those for finished mint oils are on the same order of magnitude.

Based on the above assessment and the application of the scientific judgment, cornmint oil is concluded to be "GRAS" under conditions of intended use as a flavoring substance. Given the criteria used in the evaluation, recommended specifications should include the following chemical assay:

1. Less than 95% alicyclic secondary alcohols, ketones, and related esters, typically measured as (−)-menthol.
2. Less than 2% 2-isopropylidenecyclohexanones and their metabolites, measured as (−)-pulegone.
3. Less than 10% monoterpene hydrocarbons, typically measured as limonene.

7.6 SUMMARY

The safety evaluation of an essential oil is performed in the context of all available data for congeneric groups of identified constituents and the group of unidentified constituents, data on the essential oil or a related essential oil, and any potential interactions that may occur in the essential oil when consumed as a flavoring substance.

The guide provides a chemically based approach to the safety evaluation of an essential oil. The approach depends on a thorough quantitative analysis of the chemical constituents in the essential oil intended for commerce. The chemical constituents are then assigned to well-defined congeneric groups that are established based on extensive biochemical and toxicologic information, and this is evaluated in the context of intake of the congeneric group resulting from consumption of the essential oil. The intake of unidentified constituents considers the consumption of the essential oil as a food, a highly conservative toxicologic threshold, and toxicity data on the essential oil or an essential oil of similar chemical composition. The flexibility of the guide is reflected in the fact that high intake of major congeneric groups of low toxicologic concern will be evaluated along with low intake of minor congeneric groups of significant toxicological concern (i.e., higher structural class). The guide also provides a comprehensive evaluation of all congeneric groups and constituents that account for the majority of the composition of the essential oil. The overall objective of the guide is to organize and prioritize the chemical constituents of an essential oil in order that no reasonably possible significant risk associated with the intake of essential oil goes unevaluated.

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8 Metabolism of Terpenoids in Animal Models and Humans

Walter Jäger

CONTENTS

8.1 INTRODUCTION

Terpenoids are main constituents of plant-derived essential oils. Because of their pleasant odor or flavor they are widely used in the food, fragrance, and pharmaceutical industry. Furthermore, in traditional medicine, terpenoids are also well known for their analeptic, antibacterial, antifungal,

antitumor, and sedative activities. Although large amounts are used in the industry, the knowledge about their biotransformation in humans is still scarce. Yet, metabolism of terpenoids can lead to the formation of new biotransformation products with unique structures and often different flavor and biological activities compared to the parent compounds.

All terpenoids easily enter the human body by oral absorption, penetration through the skin, or inhalation very often leading to measurable blood concentrations. A number of different enzymes, however, readily metabolize these compounds to more water-soluble molecules. Although nearly every tissue has the ability to metabolize drugs, the liver is the most important organ of drug biotransformation. In general, metabolic biotransformation occurs at two major categories called Phase I and Phase II reactions (Spatzenegger and Jäger, 1995). Phase I concerns mostly cytochrome P450 (CYP)-mediated oxidation as well as reduction and hydrolysis. Phase II is a further step where a Phase I product is completely transformed to high water solubility. This is done by attaching already highly water-soluble endogenous entities such as sugars (glucuronic acids) or salts (sulfates) to the Phase I intermediate and forming a Phase II final product. It is not always necessary for a compound to undergo both Phases I and II; indeed for many terpenoids one or the other is enough to eliminate these volatile plant constituents.

In the following concise review, special emphasis will be put on metabolism of selected mono- and sesquiterpenoids not only in animal and *in vitro* models but also in humans.

8.2 METABOLISM OF MONOTERPENES

8.2.1 Camphene

Camphene is found in many plants at high concentrations, especially in the essential oil of the leaves and flowers of *Thymus vulgaris*. Based on expectorant, spasmolytic, and antimicrobial properties, camphene-containing remedies are successfully used in the treatment against cough and infections of the respiratory tract. The far greatest amount of camphene, however, is used in the liquor industry (Wichtel, 2002). Data about the *in vivo* metabolism of camphene are scarce as there is only one publication demonstrating various biotransformation products in the urine of rabbits after its oral administration. As shown in Figure 8.1, camphene is metabolized into two diasteromeric glycols (camphene-2,10-glycols). Their formation obviously involves two isomeric epoxide intermediates, which are hydrated by epoxide hydrolase. Further metabolites, namely 6-hydroxycamphene, 7-hydroxycamphene, 3-hydroxytricyclene, and 10-hydroxytricyclene, were apparently formed through the nonclassical cation intermediate (shown in brackets), structures of which were identified by IR, UV NMR, mass spectrometry, and chemical degradation (Ishida et al., 1979). So far, there are no studies available about the biotransformation of camphene in human liver microsomes or in human subjects.

8.2.2 Camphor

Camphor, a bicyclic monoterpene, is extracted from the woods of *Cinnamomum camphora*, a tree located in Southeast Asia and North America. Furthermore, it is also one of the major constituents of the essential oil of common sage (*Salvia officinalis*). Solid camphor forms white, fatty crystals with intensive camphoraceous odor and is used commercially as a moth repellent and preservative in pharmaceuticals and cosmetics (Wichtel, 2002). In dogs, rabbits, and rats, camphor is extensively metabolized whereas the major hydroxylation products of D- and L-camphor were 5-*endo*-and 5-*exo*-hydroxycamphor. A small amount was also identified as 3-*endo*-hydroxycamphor (Figure 8.2). Both 3- and 5-bornane groups can be further reduced to 2,5-bornanedione. Minor biotransformation steps also involve the reduction of camphor to borneol and isoborneol. Interestingly, all hydroxylated camphor metabolites are further conjugated in a Phase II reaction with glucuronic acid

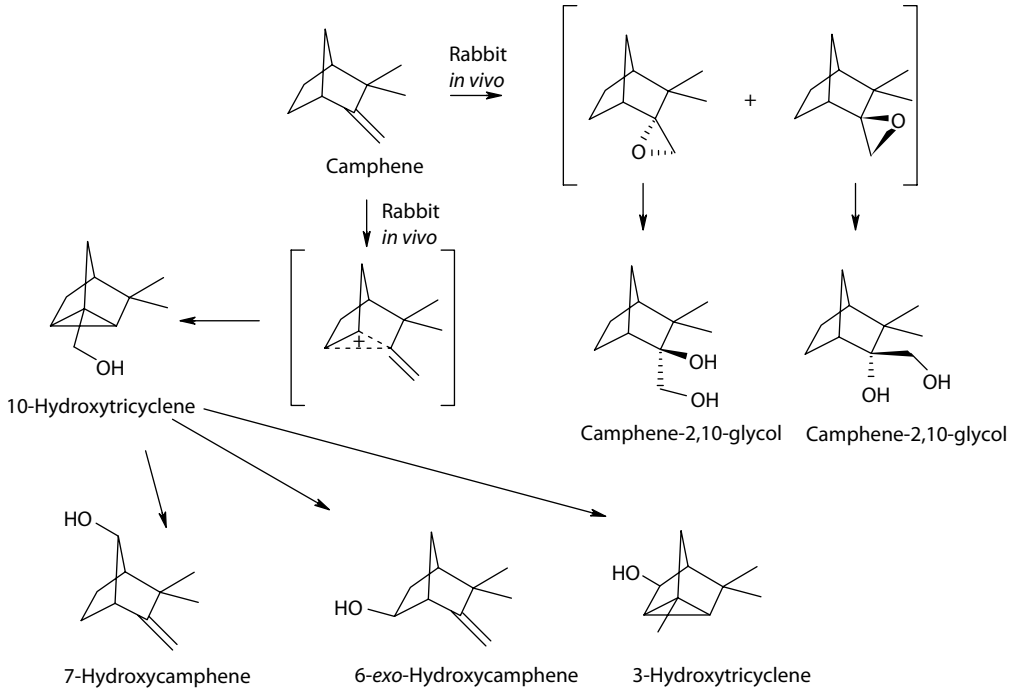

FIGURE 8.1 Urinary excretion of camphene metabolites in rabbits. (Adapted from Ishida, T. et al., 1979. *J. Pharm. Sci*., 68: 928–930; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

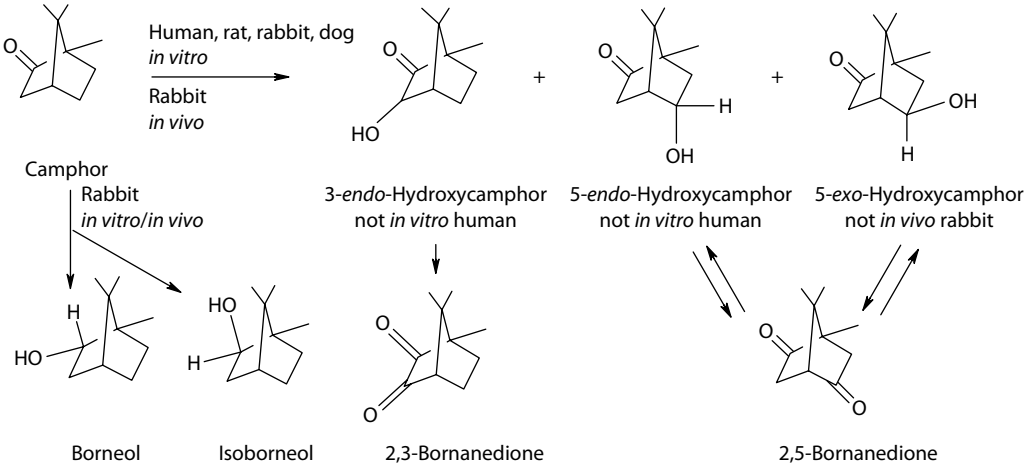

FIGURE 8.2 Metabolisms of camphor in dogs, rabbits, and rats. (Adapted from Leibmann, K.C. and E. Ortiz, 1972. *Drug Metab. Dispos*., 1: 543–551; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria; Gyoubu, K. and M. Miyazawa, 2007. *Biol. Pharm. Bull*., 30: 230–233.)

(Leibmann and Ortiz, 1972; Gyoubu and Miyazawa, 2007). Based on animal data, camphor should be also extensively metabolized in humans. Indeed, a very recent study using human liver microsomes could also show hydroxylation to 5-*exo*-hydroxycamphor. The formation of other metabolites namely borneol, isoborneol, and various glucuronides are therefore also suggested in the blood and urine of human volunteers after oral administration of camphor.

8.2.3 Carvacrol

Carvacrol is a monoterpenic alcohol which is found in high concentrations (3–5%) in the essential oil of *Thymus vulgaris*, a plant widely distributed in Middle and South Europe. Carvacrol is reportedly used as a flavor additive in a number of foods and beverages (Wichtel, 2002). Screening the literature for its metabolism, only one study could be found investigating biotransformation product in rat urine after oral application. In rat, only small amounts of unchanged carvacrol were excreted after 24 h. Based on the sample preparation protocol using β-glucuronidase and sulfatase before GC analysis, carvacrol might also be excreted as their glucuronide and sulfate, respectively. An interesting feature of this study is the demonstration that both of the aliphatic groups present undergo extensive metabolism. Noteworthy is also the fact that aromatic hydroxylation to 2,3-dihydroxy-*p*-cymene is only a minor important pathway for carvacrol. Further oxidation of 2-hydroxymethyl-5-(1-methylethyl)-phenol may also take place leading to the monocarboxylic acid metabolite, whose chemical structure was identified as 2-hydroxymethyl-4-(1-methyl)benzoic acid (Austgulen et al., 1987) (Figure 8.3). There currently are not any studies available in the literature about carvacrol biotransformation in humans.

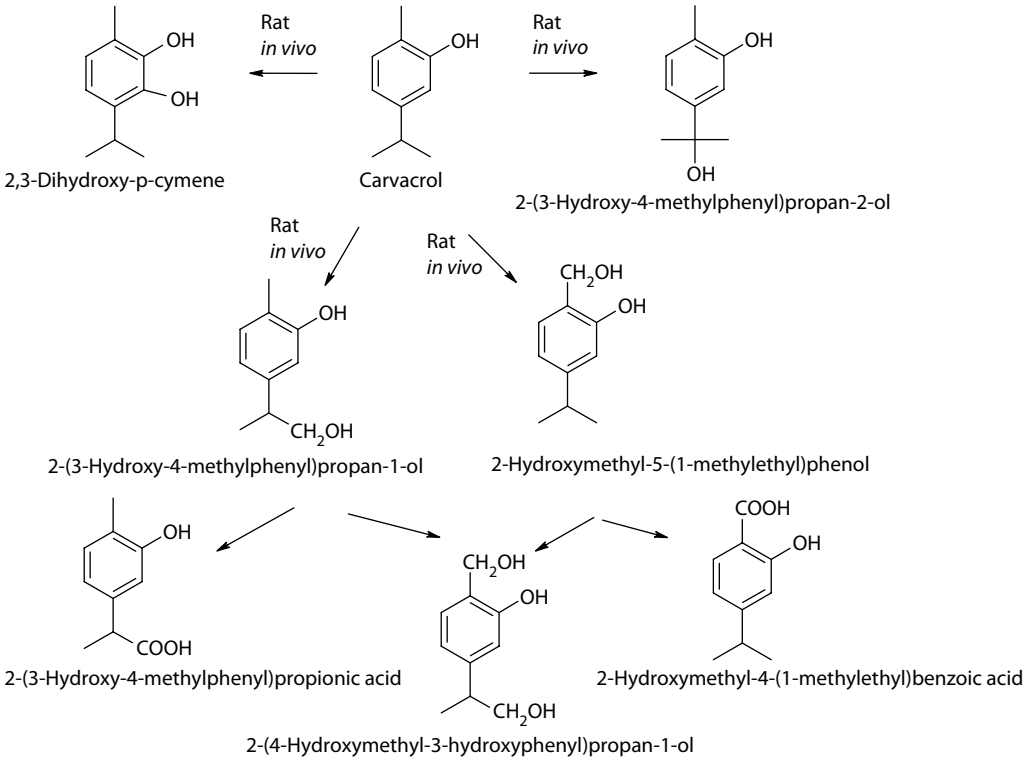

FIGURE 8.3 Metabolism and urinary excretion of carvacrol in rats. (Adapted from Austgulen, L.T. et al., 1987. *Pharmacol. Toxicol*., 61: 98–102; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

8.2.4 Carvone

The (*R*)-(−)- and (*S*)-(+)-enantiomers of monoterpene ketone carvone are found in various plants. While (*S*)-(+)-carvone is the main constituent of the essential oil of caraway (*Carum carvi*), the oil of spearmint leaves (*Mentha spicata* var. *crispa*) contains about 50% of (*R*)-(−)-carvone besides other terpenes (Wichtel, 2002). Both enantiomers are not only different in odor and taste, but they also have different use in the food, fragrance, and pharmaceutical industries. Because of minty odor and taste, large amounts of (*R*)-(−)-carvone are frequently added to toothpastes, mouth washes, and chewing gums. (*S*)-(+)-carvone possesses the typical caraway aroma and is therefore mainly used as a taste enhancer in the food and fragrance industry. Due to its spasmolytic effect, (*S*)-(+)-carvone is also used as stomachic and carminative in many pharmaceutical formulations. Furthermore, in combination with other essential oils, (*S*)-(+)- and (*R*)-(−)-carvone are applied in aromatherapy massage treatments for nervous tension and several skin disorders (Jäger et al., 2001). After separate topical applications of (*R*)-(−)- and (*S*)-(+)-carvone, both enantiomers are rapidly absorbed resulting in significantly higher maximal plasma concentrations (C_{max}) and areas under the blood concentrations time curves (AUC) for (*S*)-(+)- compared to (*R*)-(−)-carvone (88.0 versus 23.9 ng/mL plasma and 5420 versus 1611 ng/mL min, respectively). As demonstrated in Figure 8.4, analysis of control- and ß-glucuronidase pretreated urine samples only revealed stereoselective metabolism of (*R*)-(−)-carvone but not of (*S*)-(+)-carvone to (4*R*,6*S*)-(−)-carveol and (4*R*,6*S*)-(−)-carveol glucuronide indicating that stereoselectivity in Phases I and II metabolism has significant effects on (*R*)-(−)- and (*S*)-(+)-carvone pharmacokinetics (Jäger et al., 2000) (Figure 8.4).

The metabolites in plasma for both enantiomers in plasma were below detection limit. Contrary to the study of Jäger et al. (2000), however, a recent study of Engel could not demonstrate any differences in the formation of metabolites after peroral application of (*R*)-(−)- and (*S*)-(+)-carvone (1 mg/kg body weight) to human volunteers. This may be due to the separation of biotransformation products on a nonchiral gas chromatography column. As shown in Figure 8.5, besides carveol, also dihydrocarveol, carvonic acid (possibly via 10-hydroxycarvone formation), dihydrocarvonic acid, and uroterpenolone could be identified in the urine samples (Engel, 2001).

8.2.5 1,4-Cineole

1,4-Cineole, a monoterpene cyclic ether, is known to be a major flavor constituent of lime (*Citrus aurantiifolia*) and *Eucalyptus polybractea* has been used for many years as a fragrance and flavoring agent (Miyazawa et al., 2001a). Although there are no *in vivo* data about the metabolism of 1,4-cineole in humans, recent *in vitro* and *in vivo* animal studies demonstrated extensive biotransformation of this monoterpene strongly suggesting biotransformation in the human body too. After oral application to rabbits, four neutral and one acidic metabolite could be isolated from urine namely 9-hydroxy-1,4-cineole, 3,8-dihydroxy-1,4-cineole, 8,9-dihydroxy-1,4-cineole, 1,4-cineole-8-en-9-ol, and 1,4-cineole-9-carboxylic acid (Asakawa et al., 1988). Using rat and human liver

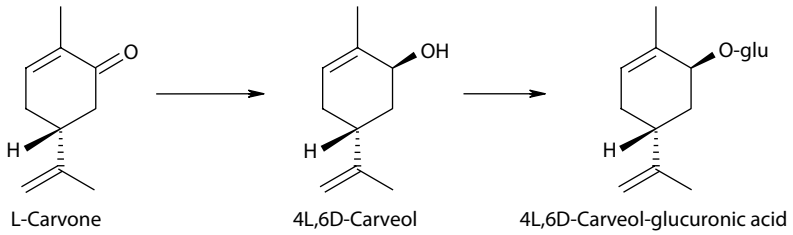

FIGURE 8.4 Metabolic pathway of (*R*)-(−)-carvone in healthy subjects. (Adapted from Jäger, W. et al., 2000. *J. Pharm. Pharmacol*., 52: 191–197; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.).

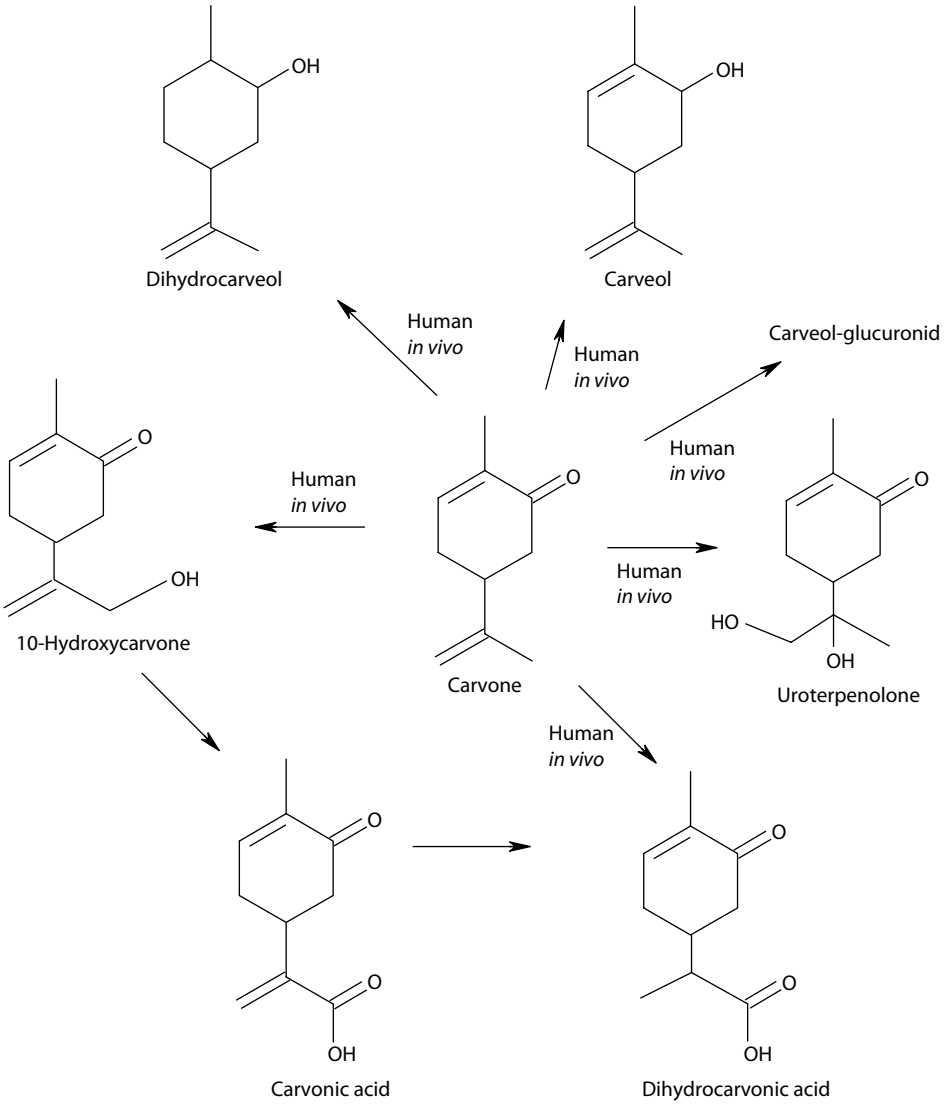

FIGURE 8.5 Proposed metabolic pathway of (*R*)-(−)- and (*S*)-(+)-carvone in healthy volunteers. (Adapted from Engel, W., 2001. *J. Agric. Food Chem*., 49: 4069–4075; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

microsomes, however, only 1,4-cineole 2 hydroxylation could be observed indicating species-related differences in 1,4-cineole metabolism (Miyazawa et al., 2001a) (Figure 8.6).

8.2.6 1,8-Cineole

1,8-Cineole, a monoterpene cyclic ether which is also named eucalyptol, is widely distributed in plants and is found in high concentrations in the essential oil of *Eucalyptus polybractea*. It is extensively used in cosmetics, for cough treatment, muscular pain, neurosis, rheumatism, asthma, and urinary stones (Wichtel, 2002). Using rat liver microsomes, 1,8-cineole is predominantly converted to 3-hydroxy-1,8-cineole, followed by 2- and then 9-hydroxycineole (Miyazawa et al., 2001b). As seen in Figure 8.7, in human liver microsomes, however, only the 2-hydroxy- and 3-hydroxy products

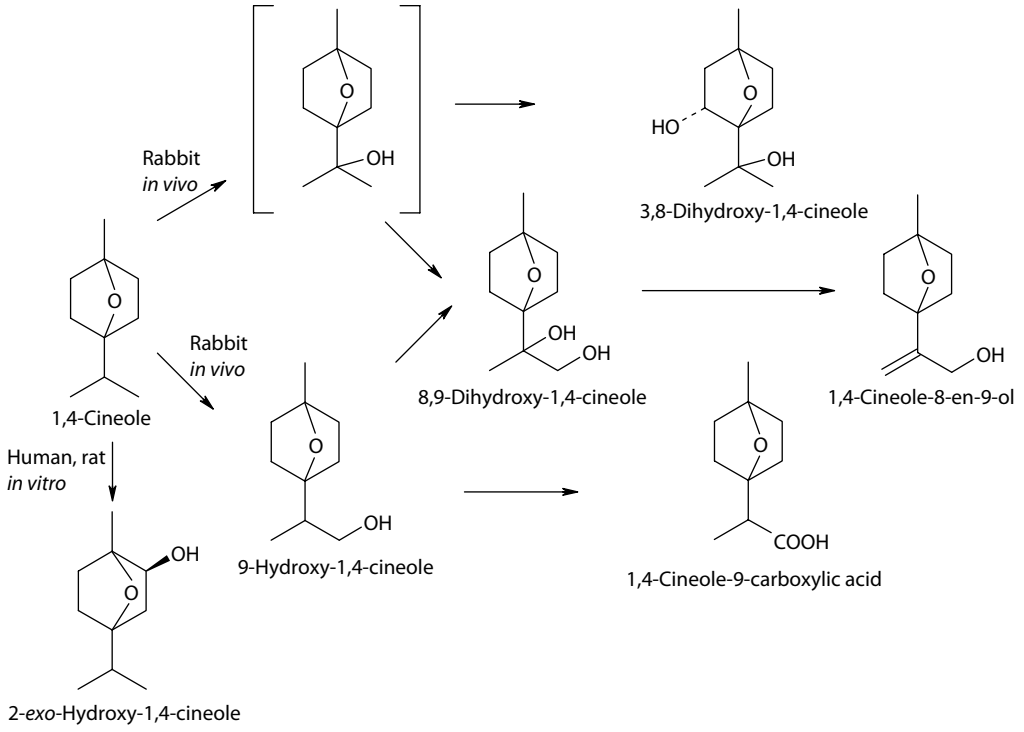

FIGURE 8.6 Proposed metabolism of 1,4-cineole in rabbits and in rat and human liver microsomes. (Adapted from Asakawa, Y., M. Toyota, and T. Ishida, 1988. *Xenobiotica*, 18: 1129–1134; Miyazawa, M. et al., 2001a. *Xenobiotica*, 31: 713–723; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

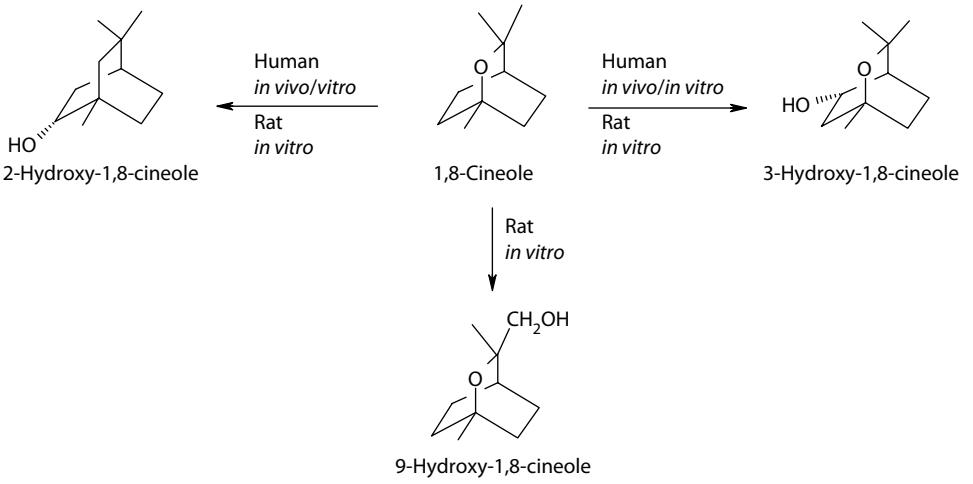

FIGURE 8.7 Proposed metabolism of 1,8-cineole *in vitro* (rat and human liver microsomes) and *in vivo* (rabbits and humans). (Adapted from Miyazawa, M. et al., 2001b. *Drug Metab. Dispos*., 29: 200–205; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

catalyzed by the isoenzmye CYP3A4 were seen (Miyazawa et al., 2001b). Both metabolites could also be identified in the urine of three human volunteers after oral administration of a cold medication containing 1,8-cineole (Miyazawa et al., 2001b). Both metabolites can therefore be used as urinary markers for the intake of 1,8-cineole in humans.

8.2.7 Citral

Both natural and synthetic citral are composed of an isomeric mixture of geranial (*E*-3,7-dimethyl-2,6-octadienal) and neral (*Z*-3,7-dimethyl-2,6-octadienal). In the isomeric mixtures, geranial is usually the predominant isomer. It occurs naturally in essential oils of citrus fruits (i.e., up to 5% in lemon oil) and in a variety of herbs and plants such as *Melissa officinalis*, lemongrass (70–80%), and eucalyptus (Wichtel, 2002). Because of its intense lemon aroma and flavor, citral has been used extensively in the food, cosmetic, and detergent industries since the early 1900s (Boyer and Petersen, 1991). Studies in rats have shown that citral is rapidly metabolized to several acids and a biliary glucuronide and excreted, with urine (48–63%) as the major route of elimination of citral, followed by expired air (8–17%), and feces (7–16%). As demonstrated in Figure 8.8, seven urinary metabolites were isolated and identified: 3-hydroxy-3,7-dimethyl-6-octenedioic acid,

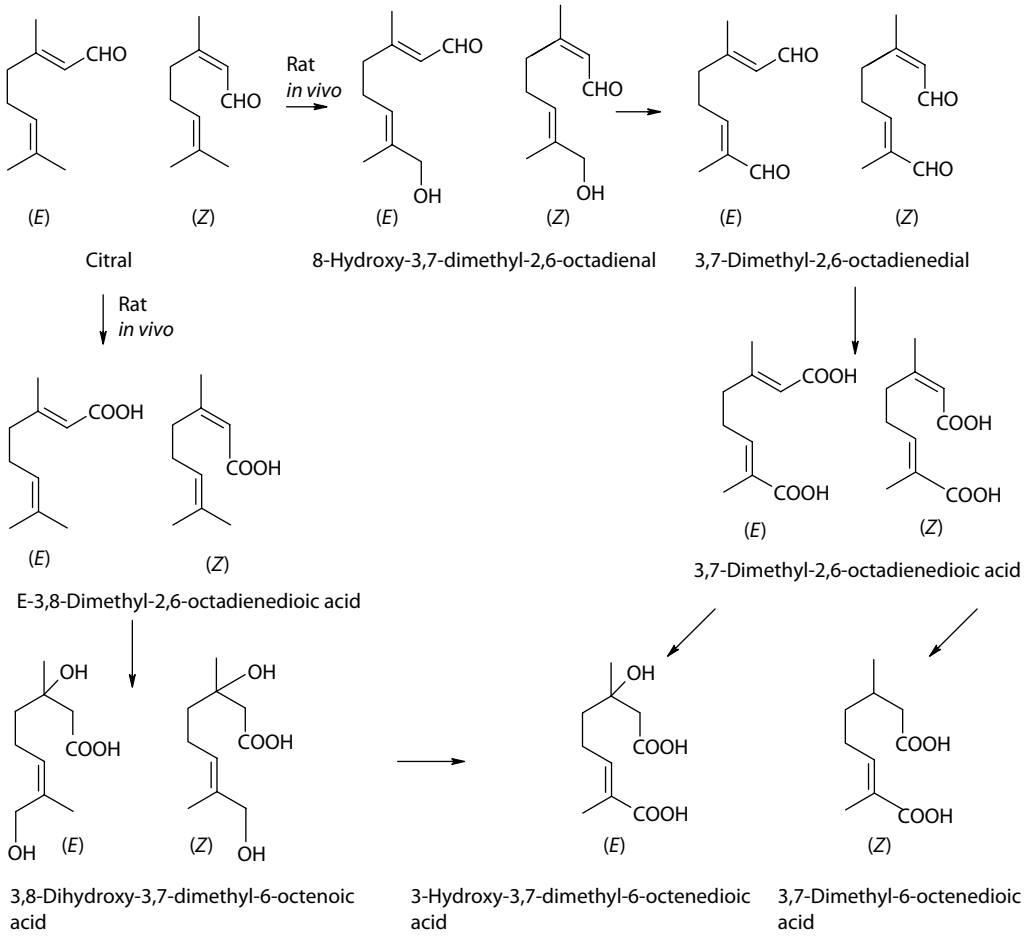

FIGURE 8.8 Proposed metabolism of citral in rats. (Adapted from Diliberto, J.J. et al., 1990. *Drug Metab. Dispos.*, 18: 886–875; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

3,8-dihydroxy-3,7-dimethyl-6-octenedioic acid 3,9-dihydroxy-3,7-dimethyl-6-octenedioic acid, *E*- and *Z*-3,7-dimethyl-2,6-octenedioic acid, 3,7-dimethyl-6-octenedioic acid, and *E*-3,7-dimethyl-2,6-octenedioic acid (Diliberto et al., 1990). There currently are not any *in vitro* or *in vivo* data available about the metabolism of citral in humans. However, based on the rat study mentioned above, extensive biotransformation of citral is highly suggested.

8.2.8 Citronellal

Citronellal is a monocyclic monoterpene with highest concentrations in the essential oil of *Melissa officinalis* (1–20%). Although citronellal is not commonly used in food and flavor industry, it is a main constituent in many pharmaceutical preparations as a mild sedative or stomachicum (Wichtel, 2002). Although extensively used in patients, data about its metabolism are scarce. Only one study described biotransformation of citronellal in rabbits. Ishida et al. could isolate three neutral metabolites of (+)-citronellal in the urine of rabbits namely (−)-*trans*-menthane-3,8-diol, (+)-*cis* menthane-3,8-diol, and (−)-isopulegol (Figure 8.9). An additional acidic metabolite (6*E*)-3, 7-dimethyl-6-octene-1,8-dioic acid was formed as the result of regioselective oxidation of the aldehyde and dimethyl allyl groups (Ishida et al., 1989). Based on animal data, metabolism of citronellal is also expected in humans.

8.2.9 Fenchone

Fenchone, a bicyclic monoterpene, is widely distributed in plants with highest concentrations in the essential oil of *Foeniculum vulgare*. Fenchone has camphoraceous fragrance and is used as a food flavor and in perfumes (Wichtel, 2002). A recent study (Miyazawa and Gyoubu, 2006) investigated the biotransformation of fenchone in human liver microsomes demonstrating the formation of 6-*exo*-hydroxyfenchone, 6-*endo*-hydroxyfenchone, and 10-hydroxyfenchone (Figure 8.10). There currently are not any data about metabolism of this compound in human volunteers. However, based on the

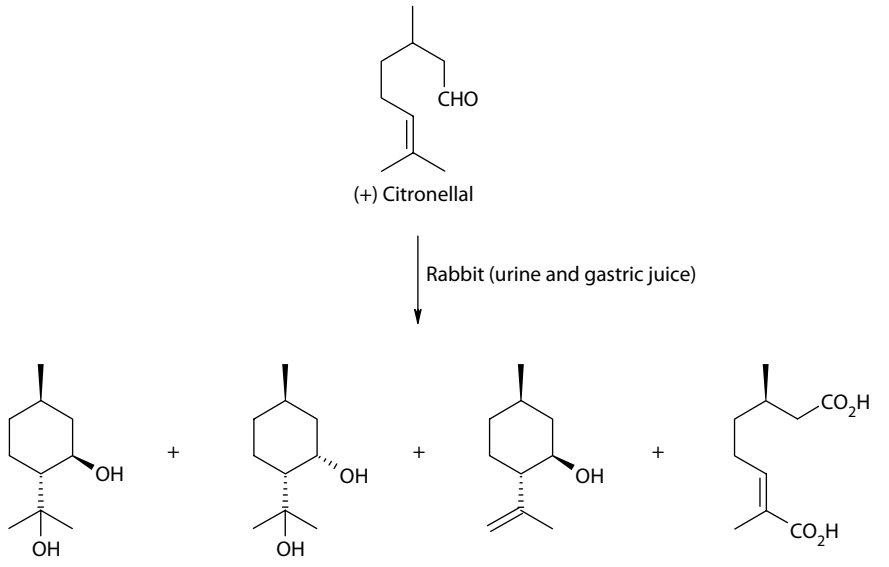

FIGURE 8.9 Proposed metabolism of citronellal in rabbits. (Adapted from Ishida, T. et al., 1989. *Xenobiotica*, 19: 843–855; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

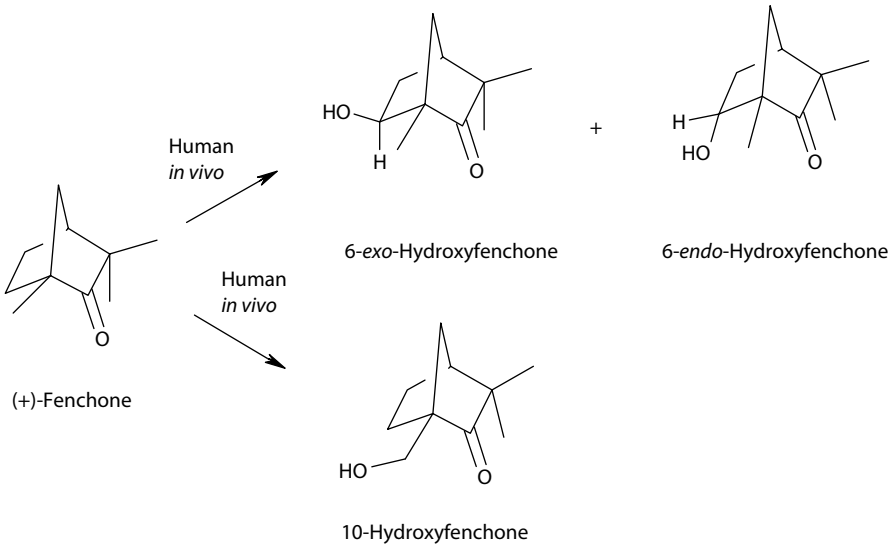

FIGURE 8.10 Proposed metabolism of fenchone in human liver microsomes. (Adapted from Miyazawa, M. and K. Gyoubu, 2006. *Biol. Pharm. Bull*., 29: 2354–2358; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

in vitro experiments using human liver microsomes, identical biotransformation products should also be found in blood or urine samples of humans after dietary intake of fenchone-containing products.

8.2.10 Geraniol

Geraniol is a monoterpene alcohol with pronounced concentrations in the essential oil of *Cymbopogon winteranus* Jowitt (12–25%). It is also found in small quantities in rose, palmarosa, citronella, geranium, lemon, and many other essential oils. It has a rose-like odor and is commonly used in perfumes and in the flavor industry (Chadha and Madyastha, 1984). In a study of Chadha and Madyastha, several metabolites could be identified in rat urine after oral administration (Chadha and Madyastha, 1984). Geraniol can be either metabolized to 8-hydroxygeraniol and via 8-carboxygeraniol to 3,7-dimethyl-2,6-octenedioic acid (Hildebrandt's acid) or directly oxidized to geranic acid and 3-hydroxycitronelic acid (Figure 8.11). Formation of 8-hydroxygeraniol and 8-carboxygeraniol are due to selective oxidation of the C-8 in geraniol. The 8-hydroxylation of geraniol also occurs in higher plants where it is the first step in the biosynthesis of indole alkaloids.

8.2.11 Limonene

The monocyclic monoterpene (+)- and (−)-limonene enantiomers have been shown to be present in orange peel (*Citrus aurantium* L. sp. *aurantium*) and other plants and are extensively used as fragrances in household products and components of artificial essential oils. The (+)-limonene isomeric form is more abundantly present in plants than the racemic mixture and the (−)-limonene isomeric form (Wichtel, 2002). It has previously been shown that (+) limonene has chemopreventive activities in experimental animal models including rats and mice (Crowell et al., 1992). Because of the greater importance of (+)-limonene in the food and fragrance industry, only its metabolism and not that of (−)-limonene is described below. Several research groups have successfully described the biotransformation of (+)-limonene *in vitro* (rat and human liver microsomes) and *in vivo* (rat, mice, guinea

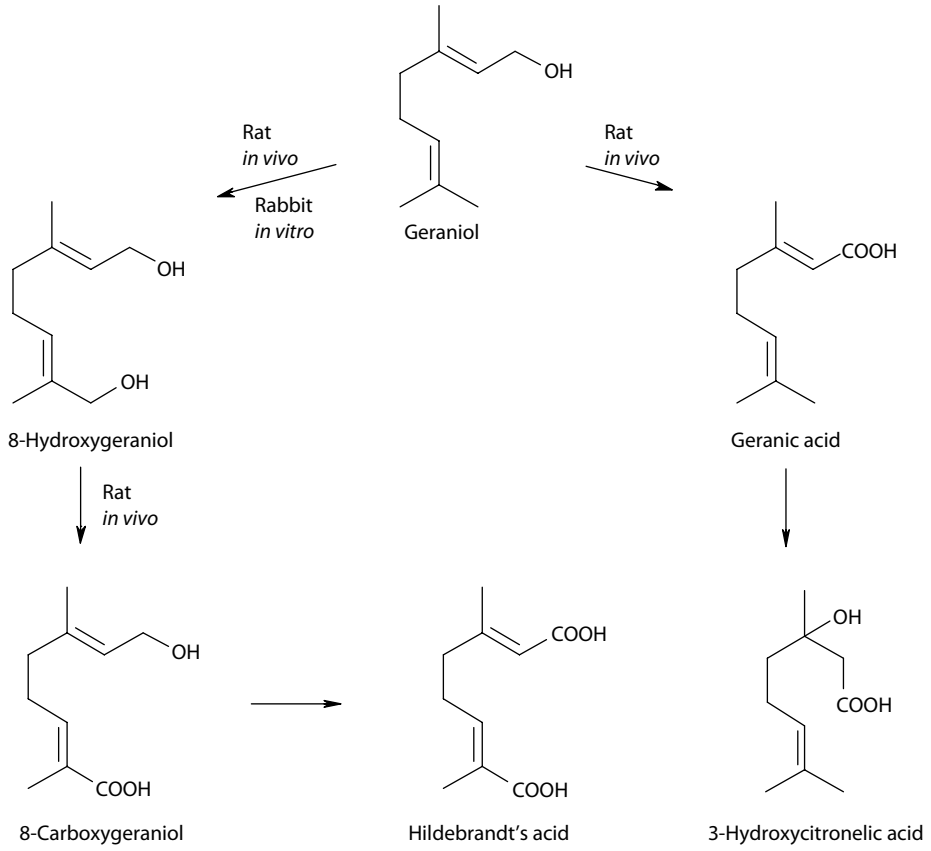

FIGURE 8.11 Proposed metabolism of geraniol in rats. (Adapted from Chadha, A. and M.K. Madyastha, 1984. *Xenobiotica*, 14: 365–374; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

pigs, dogs, rabbits, human volunteers, and patients). As shown in Figure 8.12, (+)-limonene is extensively biotransformed to several metabolites whereas in humans the main biotransformation products are perillyl alcohol; perillic acid; *p*-mentha-1,8-dien-carboxylic acid (an isomer of perillic acid); *cis*-dihydroperillic acid; *trans*-dihydroperillic acid; limonene; 1,2-diol; limonene-10-ol; limonene-8,9-diol; several glucuronides of perillic acid; dihydroperillic acid; and limonene-10-ol (Crowell et al., 1992; Miyazawa et al., 2002; Shimada et al., 2002).

8.2.12 Linalool

Linalool can be obtained naturally by fractional distillation and subsequent rectification from oils of the Cajenne rosewood, Brazil rosewood, Mexican linaloe, and coriander seed. The far highest concentration of linalool is found in the essential oil of *Ocimum basilicum* (up to 75%). Pure linalool possesses a fresh, clean, mild, light floral odor with a slight citrus impression and is used in large quantities in soap and detergent products (Wichtel, 2002). Although linalool is used in large quantities in the fragrance industry, there are no data available about its biotransformation in humans. In rat, however, linalool is metabolized by cytochrome P450 (CYP) isoenzymes to dihydrolinalool and tetrahydrolinalool and to 8-hydroxylinalool, which is further oxidized to 8-carboxylinalool (Figure 8.13). CYP-derived metabolites are then converted to glucuronide conjugates (Chadha and Madyastha, 1984).

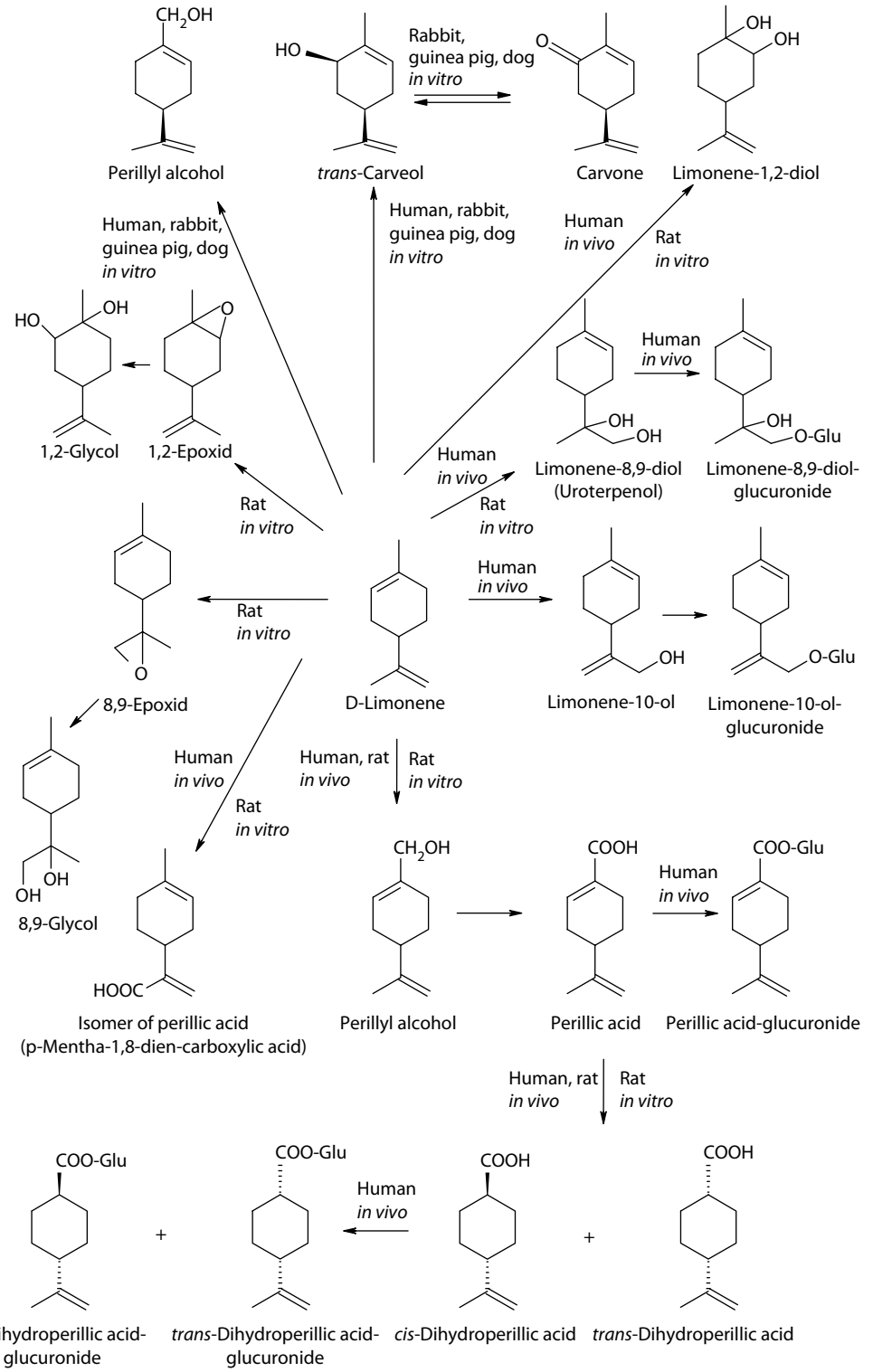

FIGURE 8.12 Proposed metabolism of (+)-limonene in rats, rabbits, guinea pigs, dogs, and humans. (Adapted from Crowell, P.L. et al., 1992. *Cancer Chemother. Pharmacol*., 31: 205–212; Miyazawa, M. et al., 2002. *Drug Metab. Dispos*., 30: 602–607; Shimada, T. et al., 2002. *Drug Metab. Pharmacokinetics*, 17: 507–515; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

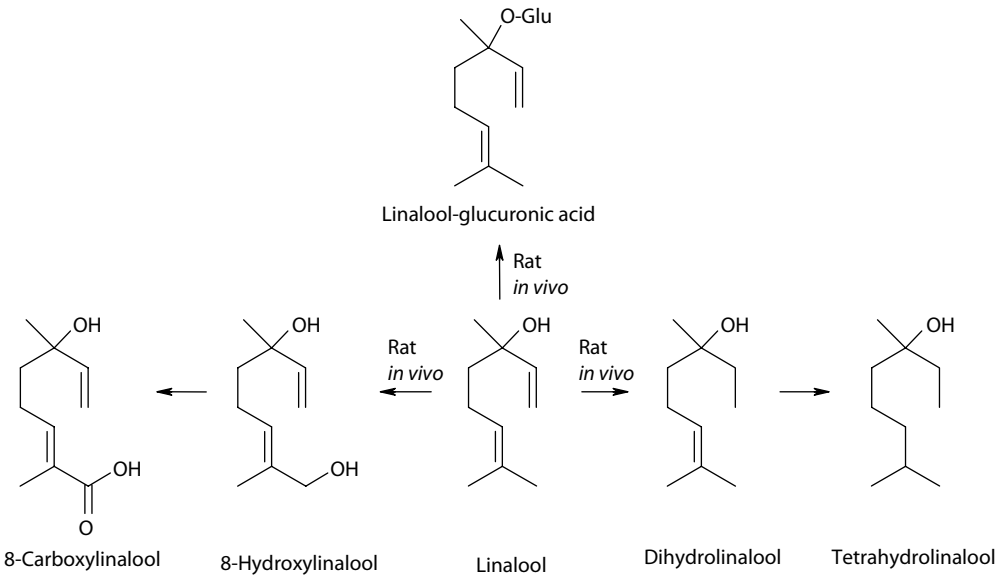

FIGURE 8.13 Proposed metabolism of linalool in rats. (Adapted from Chadha, A. and M.K. Madyastha, 1984. *Xenobiotica*, 14: 365–374; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

8.2.13 Linalyl Acetate

Linalyl acetate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, and toilet soaps, as well as in noncosmetic products such as household cleaners and detergents.

Linalyl acetate can be found in many plants, however, it is in the highest concentration in the essential oil of *Citrus aurantium* spp. *aurantium* (Wichtel, 2002). As an ester, linalyl acetate is hydrolyzed *in vivo* by carboxylesterases or esterases to linalool (Figure 8.14), which is then further metabolized to numerous oxidized biotransformation products (see metabolism of linalool) (Bickers et al., 2003).

8.2.14 Menthol

Menthol is a major component of various mint oils. The plant oil, often referred to as peppermint oil (from *Mentha piperita*) or cornmint oil (from *Mentha arvensis*), is readily extracted from the plant by steam distillation (Wichtel, 2002). It has a pleasant typical minty odor and taste, and is widely

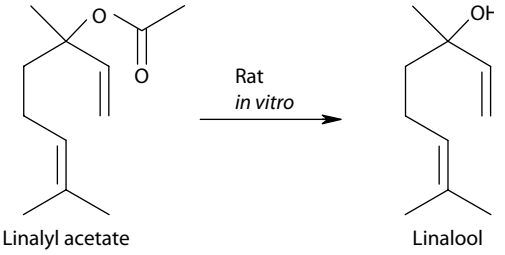

FIGURE 8.14 Proposed metabolism of linalyl acetate in rats. (Adapted from Bickers, D. et al., 2003. *Food Chem. Toxicol.*, 41: 919–942; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

used to flavor foods and oral pharmaceutical preparations ranging from common cold medications to toothpastes (Mühlbauer et al., 2003). After oral administration to human volunteers, menthol is rapidly metabolized and only menthol glucuronide could be measured in plasma or urine. Interestingly, unconjugated menthol was only detected after a transdermal application. In rats, however, hydroxylation at the C-7 methyl group and at C-8 and C-9 of the isopropyl moiety form a series a mono- and dihydroxymenthols and carboxylic acids, some of which are excreted in part as glucuronic acid conjugates. Additional metabolites are mono- and or dihydroxylated menthol derivatives (Figure 8.15). Similar to humans, the main metabolite in rats was again menthol glucuronide (Madyastha and Srivatsan, 1988b; Gelal et al., 1999; Spichinger et al., 2004).

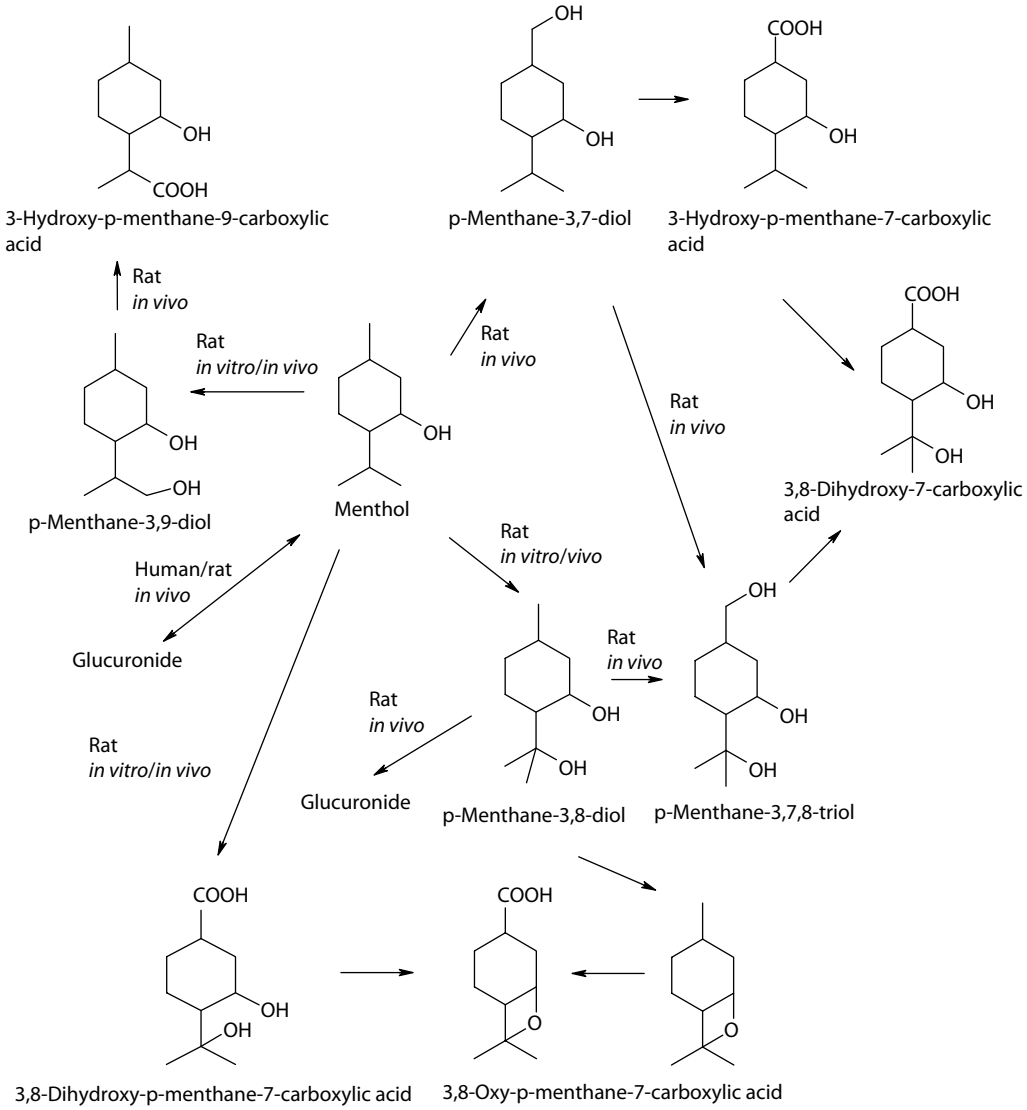

FIGURE 8.15 Proposed metabolism of menthol in rats and humans. (Adapted from Madyastha, K.M. and V. Srivatsan, 1988a. *Environ. Contam. Toxicol.*, 41: 17–25; Gelal, A. et al., 1999. *Clin. Pharmacol. Ther.*, 66: 128–235; Spichinger, M. et al., 2004. *J. Chromatogr. B*, 799: 111–117; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

8.2.15 Myrcene

Myrcene is the major constituent of the essential oil of hop (*Humulus lupulus*), which is used in the manufacture of alcoholic beverages. Hop is also frequently used in many pharmaceutical preparations as a mild sedative in the treatment of insomnia (Wichtel, 2002). After oral application, Ishida and coworkers could identify several metabolites in the urine of rabbits whereby the formation of the two glycols may be due to the hydration of the corresponding epoxides formed as intermediates (Ishida et al., 1981). The formation of uroterpenol may proceed through limonene, which was clearly derived from myrcene in the acidic conditions of rabbit stomachs (Figure 8.16).

8.2.16 Pinene

There are two structural isoforms found in nature: α- and β-pinene. As the name suggests, both isoforms are important constituents of pine resin. Interestingly, α-pinene is more common in European pines, whereas β-pinene is more common in North America. They are also found in the resin of many

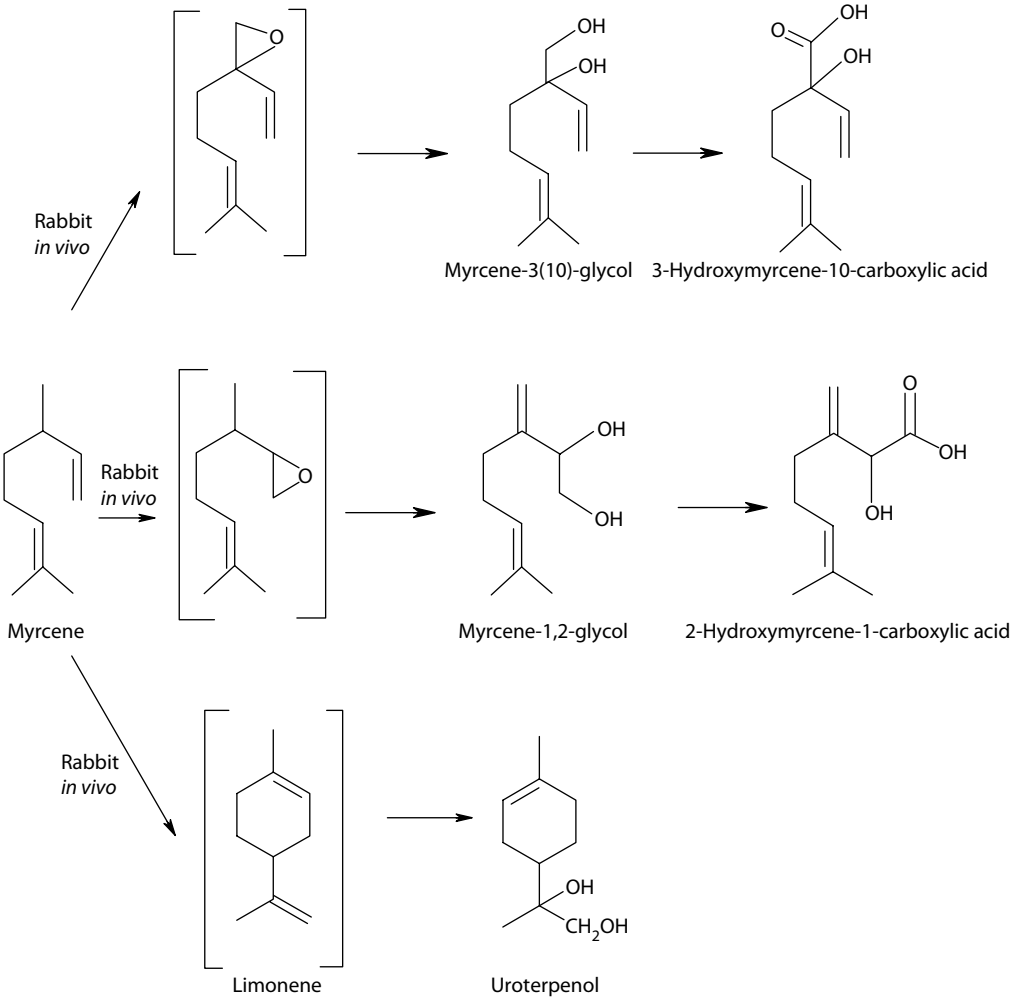

FIGURE 8.16 Proposed metabolism of myrcene in rabbits. (Adapted from Ishida, T. et al., 1981. *J. Pharm. Sci*., 70: 406–415; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

other conifers, and more widely in many plants. One of the highest concentrations of α- and β-pinene is in the essential oil of the fruit of juniper (*Juniperis communis*) with a total content of over 80% of these isoforms. Furthermore, α-pinene is also found in the essential oil of rosemary (*Rosmarinus officinalis*) and the racemic mixture in eucalyptus oil. In the industry, α- and β-pinene are used in the production of alcoholic beverages like gin (Wichtel, 2002). As shown in Figure 8.17, metabolism of

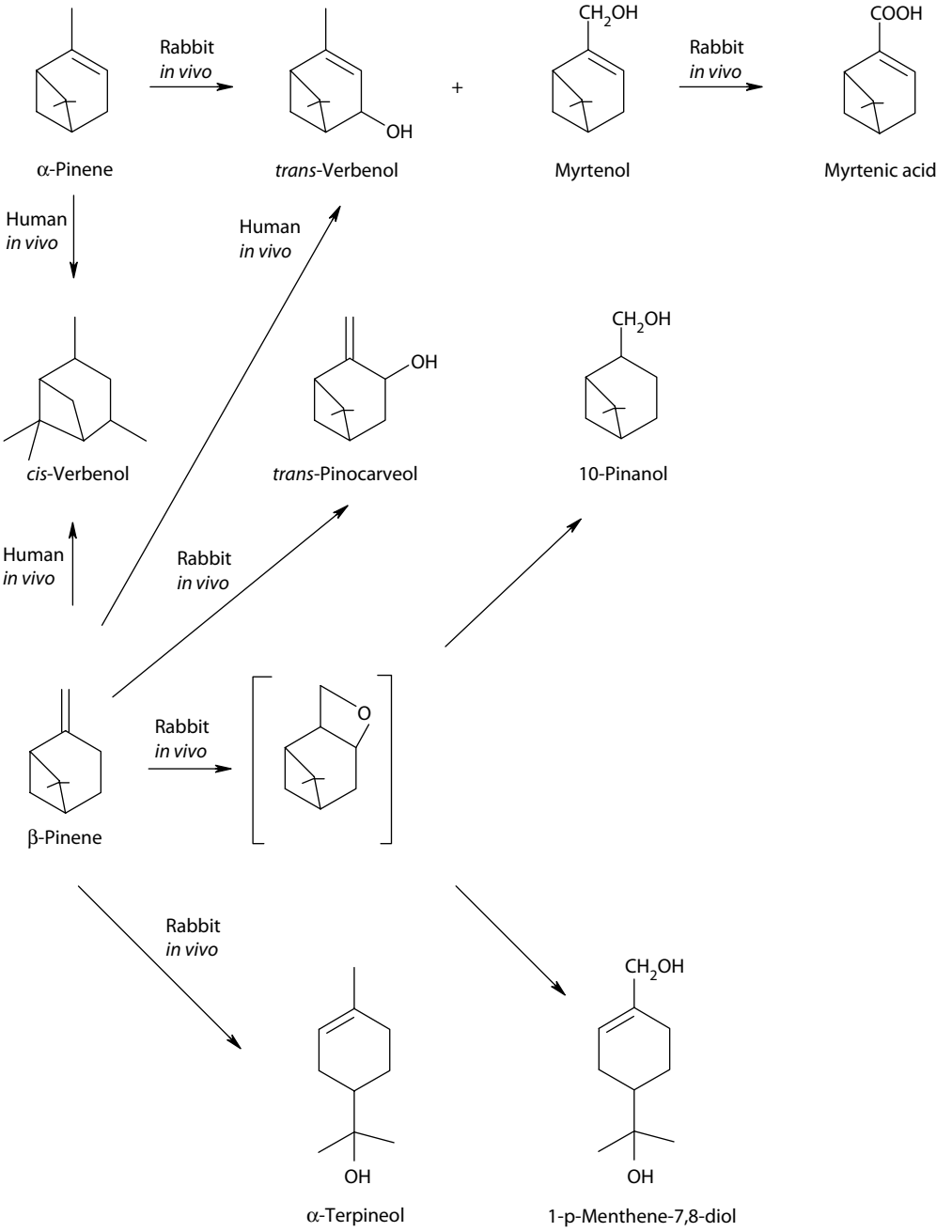

FIGURE 8.17 Proposed metabolism of α- and β-pinene in rabbits and humans. (Adapted from Ishida, T. et al., 1981. *J. Pharm. Sci*., 70: 406–415; Eriksson, K. and J.O. Levin, 1996. *J. Chromatogr. B*, 677: 85–98; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

α- and β-pinene in humans leads to the formation of *trans*- and *cis*-verbenol, respectively. Recent data analyzing the human urine after occupational exposure of sawing fumes also suggest that *cis*- and *trans*-verbenol are being further hydroxylated to diols. The main urinary metabolite of α-pinene in rabbits is *trans*-verbenol; the minor biotransformation products are myrtenol and myrtenic acid. The main urinary metabolites of β-pinene, in rabbits, however, is *cis*-verbenol indicating stereoselective hydroxylation (Ishida et al., 1981; Eriksson and Levin, 1996).

8.2.17 Pulegone

(*R*)-(+)-Pulegone is a monoterpene ketone present in essential oils from many mint species. Two mints, *Hedeoma pulegoides* and *Mentha pulegium*, both commonly called pennyroyal, contain essential oils, which are chiefly, pulegone (Madyastha and Raj, 1992). (*S*)-(−) Pulegone is found rarely in essential oil. Pennyroyal oil has been used as a flavoring agent in food and beverages, as well as a component in fragrance products and flea repellents. Pennyroyal herb has also been used for the purpose of inducing menstruation and abortion. In higher doses, however, penny royal oil may have resulted in central nervous system toxicity, gastritis, hepatic and renal failure, pulmonary toxicity, and death. The content of pulegone was found to be greater than 80% of the terpenes in pennyroyal oils that were obtained from health food stores, and was found to be both hepatotoxic and pneumotoxic in mice (Engel, 2003). Based on the observed toxicity in animal models and humans, several studies were performed in order to investigate the metabolism of (*R*)-(+)-pulegone. At nontoxic concentrations, pulegone is oxidized selectively at the 10-position, forming 10-hydroxypulegone. Alternatively, it may be reduced to menthone, which has been detected in trace levels in urine samples. It might be possible that pulegone is also reduced at the carbonyl group first; however, no trace of pulegol was found in the urine samples. Consequently, pulegol is either reduced very efficiently to menthol or rearranged to 3-*p*-menthen-8-ol (Engel, 2003) (Figure 8.18).

8.2.18 α-Terpineol

α-Terpineol, a monocyclic monoterpene tertiary alcohol, which has been isolated from a variety of α-terpineol, was found in the essential sources such as cajuput oil, pine oil, and petit grain oil. But the far highest concentration (up to 30%) of α-terpineol was found in the essential oil of the tee tree (*Melaleuca alternifolia*). Based on its pleasant odor similar to lilac, α-terpineol is widely used in the manufacture of perfumes, cosmetics, soaps, and antiseptic agents (Madyastha and Srivatsan, 1988a; Wichtel, 2002). After oral administration to rats (600 mg/kg body weight), α-terpineol is metabolized to *p*-menthane-1,2,8-triol probably formed from the epoxide intermediate. Notably, allylic methyl oxidation and the reduction of the 1,2-double bound are the major routes for the biotransformation of α-terpineol in rat (Figure 8.19). Although allylic oxidation of C-1 methyl seems to be the major pathway, the alcohol *p*-ment-1-ene-7,8-diol could not be isolated from the urine samples. Probably, this compound is accumulated and is readily further oxidized to oleuropeic acid (Madyastha and Srivatsan, 1988a).

8.2.19 α- and β-Thujone

α-Thujone and β-thujone are bicyclic monoterpenes that differ in the stereochemistry of the C-4 methyl group. The isomer ratio depends on the plant source, with high content of α-thujone in cedar leaf oil and β-thujone in wormwood oil. They are also common constituents in herbal medicines, essential oils, foods, flavorings, and beverages. α-Thujone is perhaps best known as the active ingredient of the alcoholic beverage absinthe, which was a very popular European drink in the 1800s (Wichtel, 2002). *In vivo* rat and mouse models conformed to the *in vitro* data using rat, mice, and human liver microsomes where α- and β-thujone are extensively metabolized to six hydroxythujones and three dehydrothujones (Figure 8.20). The dehydro derivatives are possibly formed by

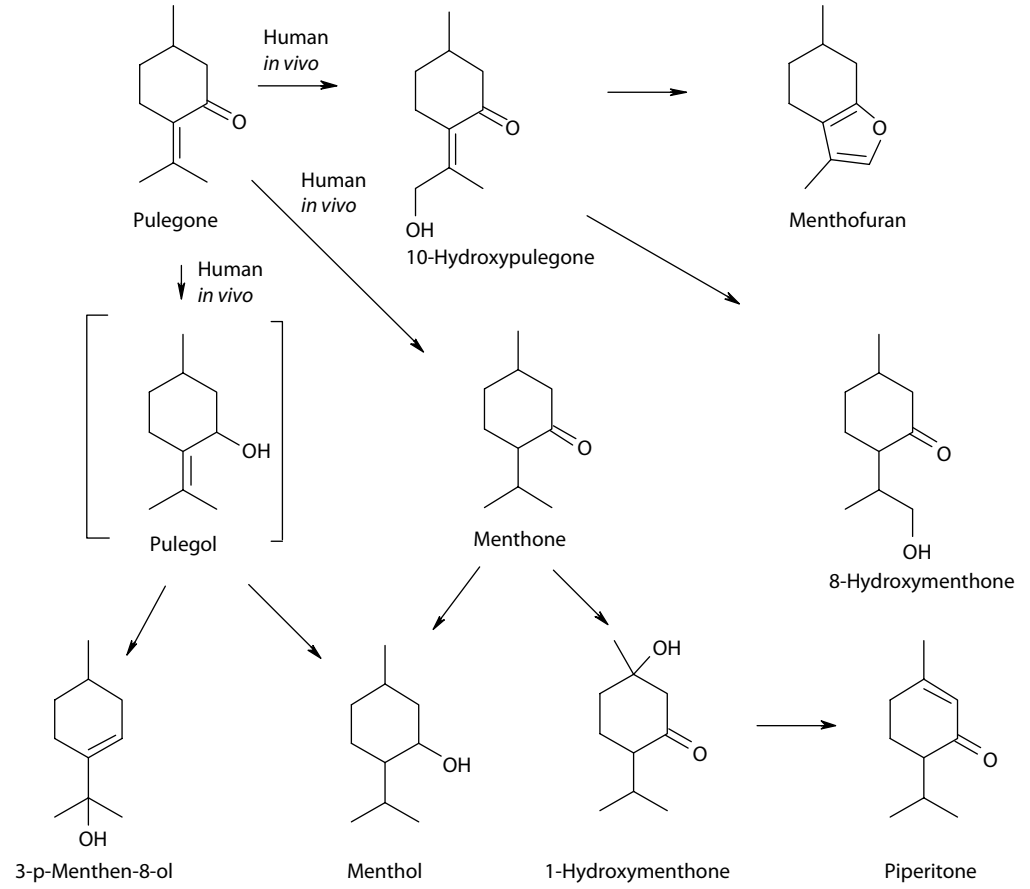

FIGURE 8.18 Proposed metabolism of pulegone in humans. (Adapted from Engel, W., 2003. *J. Agric. Food Chem*., 51: 6589–6597; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

hydroxylation of methyl substituents at position 8 or 10 followed by dehydration. In Phase II reactions, several metabolites are further conjugated with glucuronic acid (Ishida et al., 1989; Höld et al., 2000, 2001). Based on the *in vitro* and *in vivo* data, biotransformation should also be pronounced in humans after the intake of α- and β-thujone.

8.2.20 Thymol

Natural sources of thymol are the bee balms (*Monarda fistulosa* and *Monarda didyma*). Larger quantities of thymol exist in the essential oil of thyme (*Thymus vulgaris*) with concentrations up to 70%. Thymol is the primary ingredient in modern commercial mouthwash formulae as it demonstrates antiseptic properties. This may explain the use of thyme in herbal medicine to treat mouth and throat infections (Wichtel, 2002). It is noteworthy that thymol is also used as flavor additive in a number of foods and beverages. Although after oral application to rats, large quantities were excreted unchanged or as their glucuronide and sulfate conjugates; extensive oxidation of the methyl and isopropyl groups also occurred (Austgulen et al., 1987). This resulted in the formation of derivatives of benzyl alcohol and 2-phenylpropanol and their corresponding carboxylic acids. Ring hydroxylation was only a minor reaction (Figure 8.21).

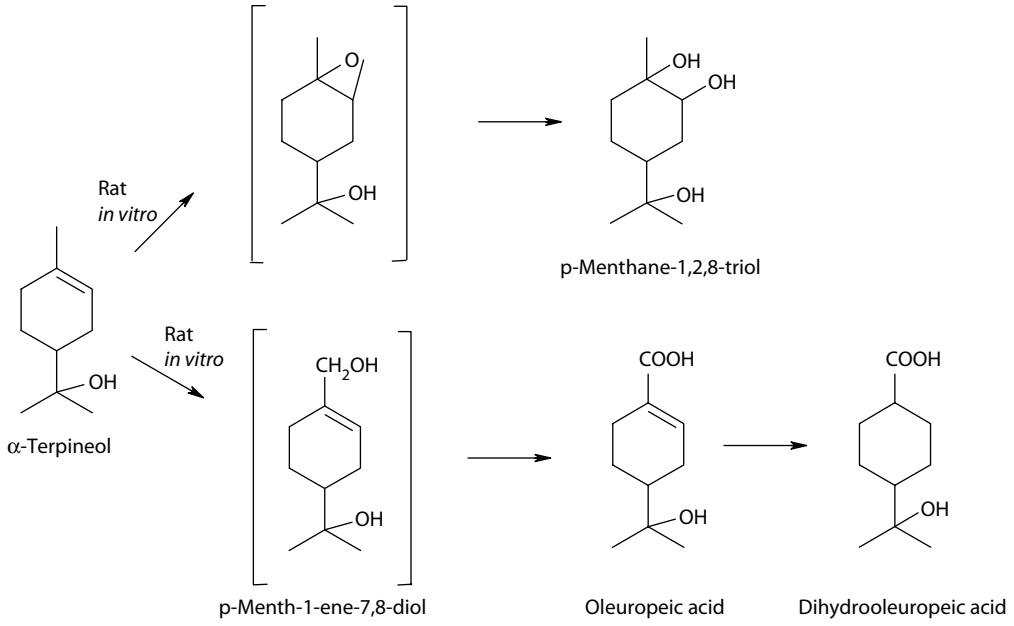

FIGURE 8.19 Proposed metabolism of α-terpineol in rats. (Adapted from Madyastha, K.M. and V. Srivatsan, 1988a. *Environ. Contam. Toxicol.*, 41: 17–25; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

8.3 METABOLISM OF SESQUITERPENES

8.3.1 Caryophyllene

(–)-β-Caryophyllene is the main sesquiterpene of hops and is being used as a cosmetic additive in soaps and fragrances (Wichtel, 2002). Storage of fresh hops under aerobic conditions leads to rapid oxidation. The main product of this reaction (–)-caryophyllene-5,6-oxide markedly affects the quality of beer. In herbal medicine, (–)-β-caryophyllene is also responsible for the mild sedative properties of hops. Furthermore, it also demonstrates cytotoxicity against breast cancer cells *in vitro* (Asakawa et al., 1986; DeBarber et al., 2004). The biotransformation of (–)-β-caryophyllene in rabbits yielded the main metabolite [10*S*-(–)-14-hydroxycaryophyllene-5,6-oxide] and the minor biotransformation product cayrophyllene-5,6-oxide-2,12-diol. The formation of the minor metabolites is easily explained via the diepoxide intermediate. Thus, it is suggested that regioselective hydroxylation of the epoxide occurred (Asakawa et al., 1981, 1986). Whether (–)-β-caryophyllene also shows the same metabolic pathway in human is not known yet. However, based on *in vivo* data from rabbits, extensive biotransformation in humans is highly suggested after oral administration (Figure 8.22).

8.3.2 Farnesol

Farnesol is a natural organic compound, which is a sesquiterpene alcohol present in many essential oil such as citronella, neroli, cyclamen, lemon grass, rose, and musk. Interestingly, it is also produced in humans where it acts on numerous nuclear receptors and has received considerable attention due to its apparent anticancer properties. It is used in perfumery to emphasize the odors of sweet floral perfumes (DeBarber et al., 2004). *In vitro* studies using recombinant drug metabolizing

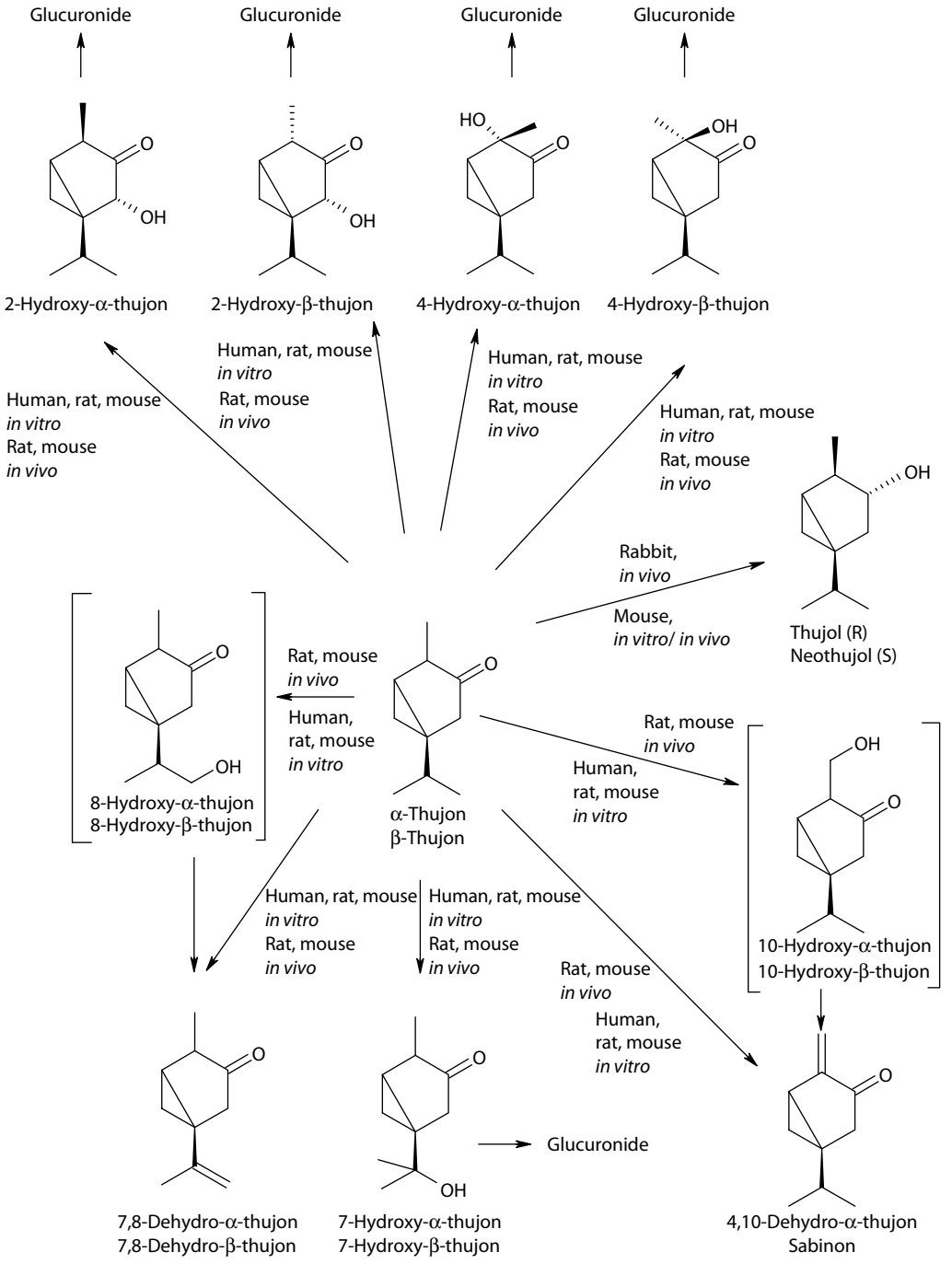

FIGURE 8.20 Proposed *in vitro* and *in vivo* metabolism of α- and β-thujone in rats. (Adapted from Ishida, T. et al., 1989. *Xenobiotica*, 19: 843–855; Höld, K.M. et al., 2000. *Environ. Chem. Toxicol*., 97: 3826–3831; Höld, K.M. et al., 2001. *Chem. Res. Toxicol*., 14: 589–595; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

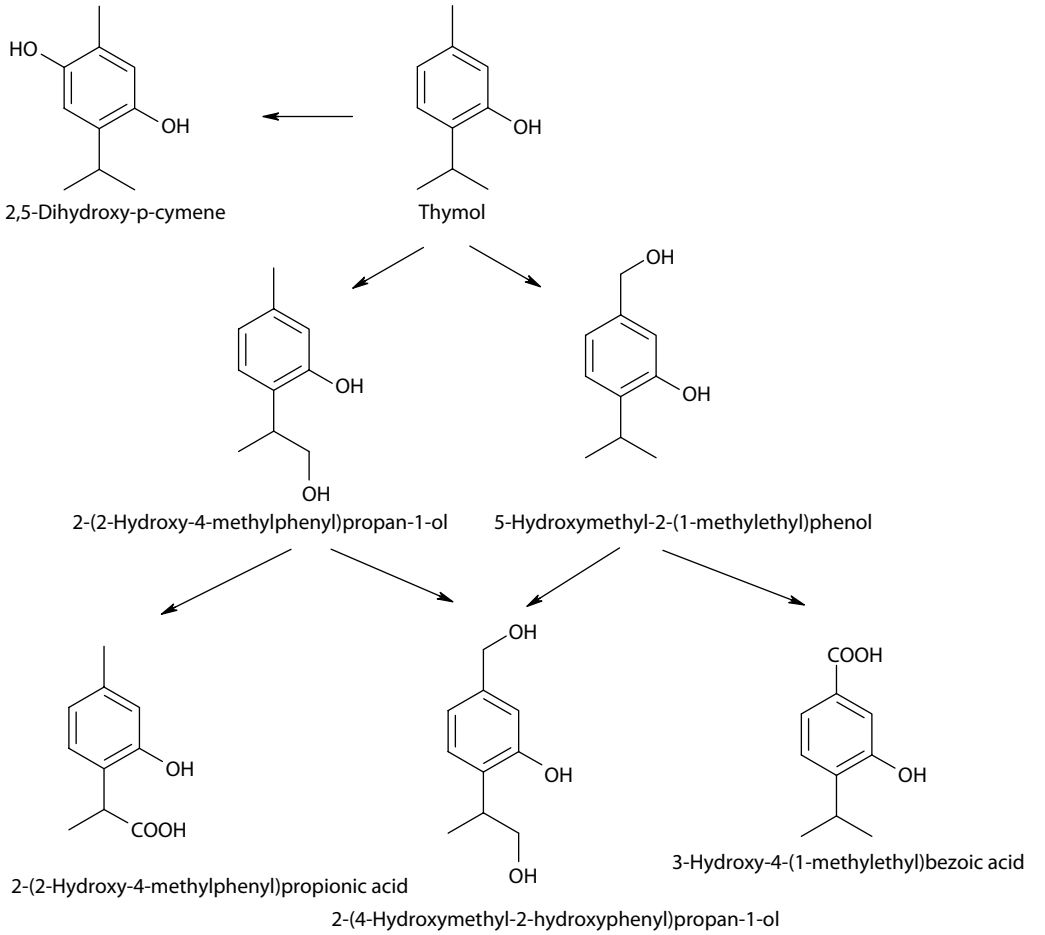

FIGURE 8.21 Proposed metabolism of thymol in rats. (Adapted from Austgulen, L.T. et al., 1987. *Pharmacol. Toxicol*., 61: 98–102; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

enzymes and human liver microsomes have shown that cytochrome P450 isoenzymes participate in the metabolism of farnesol to 12-hydroxyfarnesol (Figure 8.23). Subsequently, farnesol and 12-hydroxyfarnesol are glucuronidated to farnesyl glucuronide and 12-hydroxyfarnesyl glucuronide, respecively (DeBarber et al., 2004; Staines et al., 2004).

8.3.3 Longifolene

Longifolene is the common chemical name of a naturally occurring, oily liquid hydrocarbon, which is found primarily in certain pine resins especially in that of *Pinus longifolia*, a tree that gives longifolene its name. Besides pines, longifolene is also a main constituent of clove (Wichtel, 2002). Based on its pleasant odor, longifolene is used in the food industry. In rabbits, longifolene is metabolized as follows: attack on the *exo*-methylene group from the *endo*-face to form its epoxide followed by isomerization of the epoxide to a stable *endo*-aldehyde. Then rapid cytochrome P450-catalyzed hydroxylation of this *endo*-aldehyde occurs (Asakawa et al., 1986) (Figure 8.24).

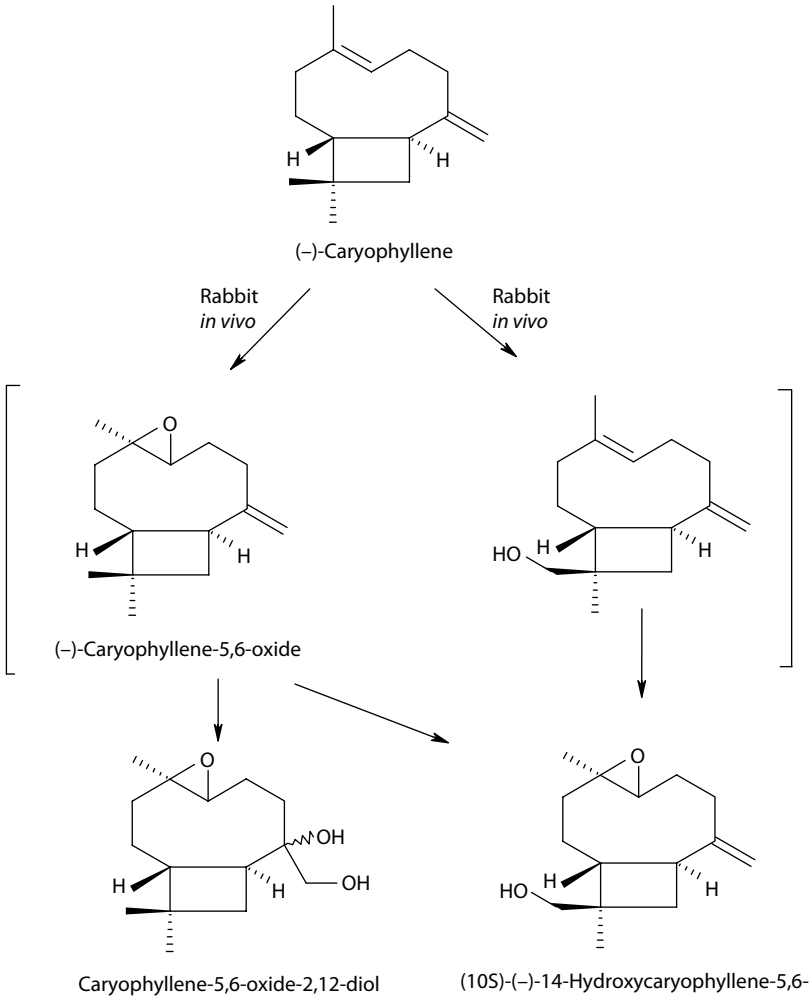

FIGURE 8.22 Proposed metabolism of β-caryophyllene in rabbits. (Adapted from Asakawa, Y. et al., 1981. *J. Pharm. Sci*., 70: 710–711; Asakawa, Y. et al., 1986. *Xenobiotica*, 16: 753–767; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

8.3.4 Patchouli Alcohol

Patchouli alcohol is the major active ingredient and the most odor-intensive component of patchouli oil, the volatile oil of *Pogostemon cablin* and *Pogostemon patchouli*. Patchouli oil is widely used in the cosmetic and oral hygiene industries to scent perfumes and flavor toothpaste (Bang et al., 1975). Modern research has also demonstrated various pharmacological activities of this oil including antiemetic, antibacterial, and antifungal properties. In rabbits and dogs, patchouli alcohol is hydroxylated at the C-15 yielding a diol that is subsequently oxidized to a hydroxyl acid. After decarboxylation and oxidation, the 3,4-unsaturated norpatchoulen-1-ol is formed, which also has a characteristic odor (Figure 8.25). All these urinary metabolites are also found as glucuronides, explaining their excellent water solubility (Bang et al., 1975; Ishida, 2005).

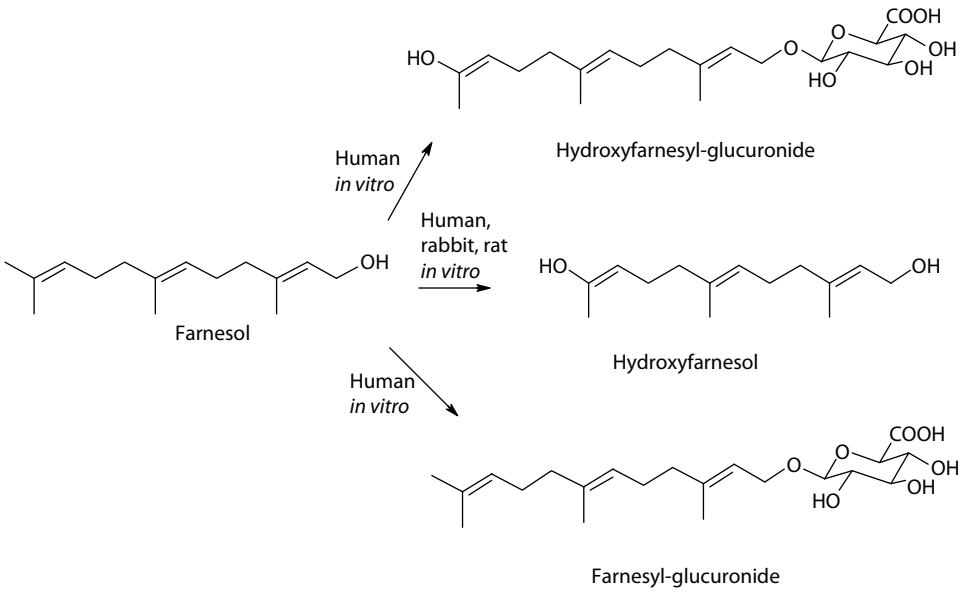

FIGURE 8.23 Proposed metabolism of farnesol in human liver microsomes. (Adapted from DeBarber, A.E. et al., 2004. *Biochim. Biophys. Acta*, 1682: 18–27; Staines, A.G. et al., 2004. *Biochem. J.*, 384: 637–645; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

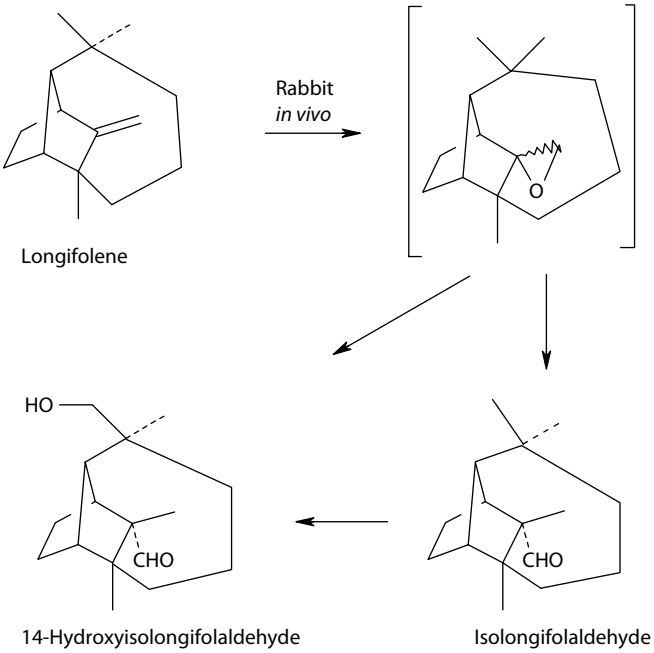

FIGURE 8.24 Proposed metabolism of (+)-longifolene in rabbits. (Adapted from Asakawa, Y. et al., 1986. *Xenobiotica*, 16: 753–767; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

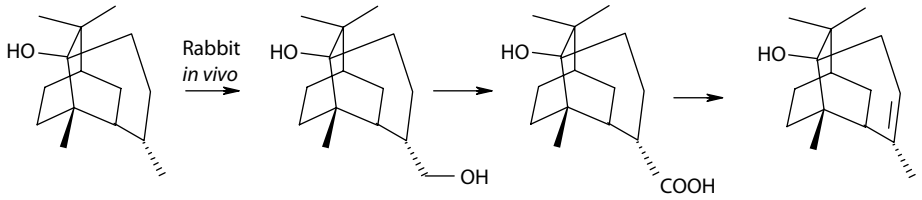

FIGURE 8.25 Proposed metabolism of patchouli alcohol in rabbits and dogs. (Adapted from Bang, L. et al., 1975. *Tetrahedron Lett*. 26: 2211–2214; Ishida, T., 2005. *Chem. Biodivers*., 2: 569–590; Jahrmann, R., 2007. Metabolismus von Monterpenen und Sesquiterpenen in Mensch und Säugetier: Bedeutung für die pharmazeutische Praxis. MPharm. diploma thesis, University of Vienna, Austria.)

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9 Biological Activities of Essential Oils

Gerhard Buchbauer

CONTENTS

9.1 INTRODUCTION

The term "biological" in this context comprises all properties, from for example "abdomen" to "zymase," which any natural product may possess. And these can be attributed to the whole animated nature, to all living organisms, namely plants, animals, and especially humans. However, only the effects of essential oils (EOs) on human beings is the topic of the present chapter. Excluded therefore, are all "botanical" activities, for example, plant care and interplant communication such as the prevention of germination of seeds of a potentially rivalry plant by emitting an EO, or the "cry for help" of a plant when it is attacked by pests and the "victim" volatilizes a fragrance which itself attracts enemies of these varmints. Neither are covered animal messengers, so-called pheromones, as well as EOs as veterinary therapeutics in animal care and feed. All these properties go beyond the scope and frame of this treatise and would rather fill a separate volume.

Therefore, the subject matter of this chapter is the therapeutic uses of EOs and/or single fragrance compounds in human medicine and care. However, even this field seems to be too extensive, so that cosmetic uses and repellents are excluded, too. Since a chapter on the pharmacological properties of EOs is already contained in this volume (see Chapter 10), only those pharmacological activities

which are of secondary (but not less important!) interest to the traditional pharmacologist will be discussed in this chapter. In particular, these are properties which do not directly aim at the central or autonomic nervous systems and for which the molecular mechanisms are only of minor relevance, for example, antioxidative effects, anticancer properties, penetration enhancing activities, and so on.

In general, the prominent literature databases have been searched and the literature mainly back to the year 2000 is discussed in this chapter. Readers interested in earlier studies are referred to three reviews by the author, which were published some years ago (Buchbauer, 2002, 2004, 2007). However, where it seemed necessary also earlier investigations have been included here and topics are discussed shortly which were not dealt with in these earlier compilations. Nevertheless, in a few cases some overlapping with related chapters may occur in the present book.

9.1.1 Anticancer Properties*

A very promising field of treatment with EOs is their application against tumors. Especially since the 1990s the anticancer properties of EOs and/or their main constituents and/or metabolites have gained more and more interest, inasmuch as such a "natural" therapy is accepted all over the world by the patients. One of the most prominent compounds in that sense is either *d*-limonene, the main constituent of the EO of sweet orange peel oil (*Citrus sinensis*, Rutaceae) as well as of other citrus fruit peel oils, or perillyl alcohol, the most important metabolite of this monoterpene hydrocarbon. Perillyl alcohol has been developed as a clinical candidate at the National Cancer Institute because of its greater potency than limonene, which may enable potentially effective systemic concentrations of the active principles to be achieved at considerably lower doses (Phillips et al., 1995). Perillyl alcohol is effective as an inhibitor of farnesyl transferase. In the early developmental stages of mouse lung carcinogenesis the *ras*-protein undergoes a series of modifications, and farnesylation at the cysteine is one of these, which leads to the anchoring of *ras*-p-21-gen to the plasma membrane in its biologically active state. Perillyl alcohol administered to test mice showed a 22% reduction in tumor incidence and a 58% reduction in tumor multiplicity (Lantry et al., 1997). Perillyl alcohol reduced the growth of hamster pancreatic tumors (>50% of the controls), or even led to a complete regression (16%). Thus, perillyl alcohol may be an effective chemotherapeutic agent for human pancreatic cancer (Löw-Baselli et al., 2000; Stark et al., 1995). Perillyl alcohol also inhibited significantly the incidence (percentage of animals with tumors) and multiplicity (tumor/animals) of invasive adenocarcinomas of the colon and exhibited increased apoptosis of the tumor cells. Scientists from the Purdue University report that the rate of apoptosis is over sixfold higher in perillyl alcohol-treated pancreatic adenocarcinoma cells than in untreated cells, and that the effect of perillyl alcohol on pancreatic tumor cells is significantly greater than its effect on nonmalignant pancreatic ductal cells (Stayrock et al., 1997). Moreover, this monoterpene alcohol-induced increase in apoptosis in all of the pancreatic tumor cells is associated with a 2–8-fold increase in the expression of a proapoptotic protein which preferentially stimulates the apoptosis in malignant cells. Perillyl alcohol is also effective in reducing liver tumor growth. Two weeks after diethyl nitrosamine exposure was discontinued, the animals were divided into perillyl alcohol-treated and untreated groups. The mean liver tumor weight for the perillyl alcohol-treated rats of perillyl alcohol treatment was 10-fold less than that for the untreated animals (Mills et al., 1995). A newer study found that this monoterpene alcohol potentially attenuates ferric-nitrilo-acetate-induced oxidative damage and tumor promotional events (Jahangir et al., 2007). Monoterpenes such as *d*-limonene and perillyl alcohol, as well as other terpene alcohols, such as geraniol, carveol, farnesol, nerolidol, β-citronellol, linalool, and menthol, showed inhibitory activities on induced neoplasia of the large bowl and duodenum. Nerolidol, especially, has an impact on the protein prenylation and is able to reduce the adenomas in rats fed with these compounds to an extent of about 82% compared to the

* Adorjan, M., 2007. Part of her master thesis, University of Vienna.

controls (Wattenberg, 1991). Geraniol prevents the growth of cultured tumor cells, especially those of rat hepatomas and melanomas (Yu et al., 1995). Dietary geraniol increased the 50% survival time of mice significantly and even 20% of the animals remained free of tumors when fed a geraniol-containing diet 14 days before an intraperitoneal transfer of the tumor cells (Shoff et al., 1991). Similar studies indicate that the colon tumors of animals fed with perillyl alcohol exhibited increased apoptosis as compared to those fed the control diet (Reddy et al., 1997). Therefore, consumption of diets containing fruits and vegetables rich in monoterpenes, such as *d*-limonene, reduces the risk of developing cancer of the colon, mammary gland, liver, pancreas, and lung (Crowell, 1999).

In the following, the anticancer activity of some EOs published since 2000 up to now will be discussed. In these papers several different cell lines were used to determine the anticancer activity of the EOs tested: A-549 (human lung carcinoma cell line), B16F10 (mouse cell line), CO25 (N-*ras* transformed mouse myoblast cell line), DLD-1 (human colon adenocarcinoma cell line), Hep-2 (human laryngeal cancer cell line), HL-60 (human promyelocytic leukemia cell line), J774 (mouse monocytic cell line), K562 (human erythroleucemic cell line), nuclear-factor-κ-B (human mouth epidermal carcinoma cell line), M14 WT (human melanoma cell line), Neuro-2a (mouse neuroblastoma cell line), P388 (murine leukemia cell line), SP2/0 (mouse plasmocytoma cell line), and then Caco-2, K562, MCF-7, PC-3, M4BEU, ACHN, Bel-7402, Hep G2, HeLa, and CT-26 (different human cancer cell lines).

El Tantawy et al. (2000) investigated the EO of *Senecio mikanioides* O. (cape ivy, Asteraceae), grown in Egypt. The main components of the oil of the plant's aerial parts were α-pinene (23%) and β-myrcene (11.3%), whereas dehydroaromadendrene (31.8%) and camphene (19.7%) were the major compounds in the underground organs, analyzed by gas chromatography/mass spectrometry (GC-MS). Both EOs had a potent cytotoxic activity against the growth of certain human cell lines *in vitro*. Another study dealt with the EO of *Nigella sativa* (black cumin, Ranunculaceae) seeds and its main constituent, thymoquinone (Badary et al., 2000). This substance was tested against fibrosarcomas induced by 20-methylcholanthrene (MC) in Swiss albino mice *in vivo* and *in vitro*. The mice got 0.01% thymoquinone in drinking water 1 week before and thereafter MC treatment. At the end of the experiment there was a significant inhibition of MC-induced fibrosarcoma compared to MC alone (tumor incidence 43%, less MC-induced mortality). In comparison to the control group, in the liver of MC-induced tumor-bearing mice a reduction in hepatic lipid peroxides, an increase in glutathione content and enzyme activities of glutathione S-transferase (GST) and quinone transferase (QT) are observed. Furthermore, the *in vitro* tests showed an inhibition of the survival of the tumor cells. These data indicate that thymoquinone could be a powerful chemopreventive agent against MC-induced fibrosarcoma tumors, probably because of its interference with DNA synthesis. Some years later, Ali and Blunden (2003) published a review about the seeds of black cumin, which is used in folk medicine. The EO and its major constituent thymoquinone were found to have antineoplastic activity and to be protective against nephrotoxicity and hepatotoxicity induced by diseases or chemicals.

The anticancerogenic effect of the EO of the *Melissa officinalis* L. (Lamiaceae) was investigated by Allahverdiyev et al. (2001) using cell cultures of Hep-2 cells derived from human laryngeal cancer. The activity of the EO was examined by morphologic changes and by flow cytometry, compared to methotrexate (MTX, an antagonist of folic acid) and etoposid, a partial synthetically obtainable glycoside of podophyllotoxin. The EO was able to terminate the cells of the G1 and S phases, whereas MTX was active in the S and G2 phases and etoposid in the G1 phase. Their findings showed that the essential balm oil possesses anticancerogenic effects because MTX blocks the transfer of one-carbon fragments by its affinity to dihydrofolic acid reductase, which leads to an obstruction of the nucleic acid synthesis. De Sousa et al. (2004) found in an *in vitro* assay that the EO of this plant was very effective against various human cancer cell lines (A549, MCF-7, Caco-2, HL-60, K562) and mouse cell lines (B16F10).

Legault et al. (2003) performed a study about the antitumor activity of balsam fir oil (*Abies balsamea*, Pinaceae) using the tumor cell lines MCF-7, PC-3, A-549, DLD-1, M4BEU, and CT-26.

Balsam fir oil was active against all tumor cell lines with GI_{50} values ranging from 0.76 to 1.7 mg/mL. After GC-MS analysis among the monoterpenes found (about 96%) α-humulene proved itself to be responsible for cytotoxicity (GI_{50} 55 μM). Both the EO and α-humulene induced a dose- and time-dependent decrease in cellular glutathione (GSH) content and an increase in reactive oxygen species (ROS) production.

Zeytinoglu et al. (2003) studied the effects of carvacrol, one of the main compounds in the EO of oregano (obtained from the Lamiaceae *Origanum onites* L.) on the DNA synthesis of N-*ras*-transformed mouse myoblast cells CO25. This monoterpenic phenol was able to inhibit the DNA synthesis in the growth medium and *ras*-activating medium, which contained dexamethason. The authors concluded that carvacrol may find application in cancer therapy because of its growth inhibition of myoblast cells even after activation of mutated N-*ras*-oncogene. Also, Ipek et al. (2003) investigated the thymol-isomer carvacrol using the *in vitro* sister-chromatid-exchange (SCE) assay on human peripheral blood lymphocytes. The inhibitory effect of carvacrol was checked in the presence of mitomycin C (MMC) in the same assay. The formation of SCE was not increased by any dose of carvacrol, while it decreased the rate of SCE induced by MMC. These findings demonstrate that carvacrol shows a significant antigenotoxic activity in mammalian cells, indicating its usage as an antigenotoxic agent.

The fresh leaf the EO of the Moraceae *Streblus asper* Lour. comprising the major compounds phytol (45.1%), α-farnesene (6.4%), *trans*-farnesyl acetate (5.8%), caryophyllene (4.9%), and *trans*,*trans*-α-farnesene (2.0%) was tested against mouse lymphocytic leukemia cells (P388) and showed a significant anticancer activity (ED_{50} 30 μg/mL) (Phutdhawong et al., 2004). Also another EO, this time from the fruits of the Anonaceae *Xylopia aethiopica* (Ethiopian pepper), a plant grown in Nigeria, showed in a concentration of 5 mg/mL a cytotoxic effect in the carcinoma cell line (Hep-2) (Asekun and Adeniyi, 2004). Last but not least, also terpinen-4-ol, the major component of the tea tree oil (TTO) (*Melaleuca alternifolia*, Myrtaceae), was investigated by Calcabrini et al. (2004) as to its anticancer effects in human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamycin-resistant cells. TTO as well as terpinen-4-ol were able to impair the growth of human M14 melanoma cells, whereupon the effect was stronger on their resistant variants, which express high levels of P-glycoprotein in the plasma membrane, overcoming resistance to caspase-dependent apoptosis exerted by P-glycoprotein-positive tumor cells.

Some other EOs from "prominent" plants were investigated if they could be used in cancer therapy. One of these plants is the Asteraceae *Chrysanthemum boreale* Makino whose EO was studied on the apoptosis of KB cells by Cha et al. (2005). Different cytotoxic effects hallmarking apoptosis (DNA fragmentation, apoptotic body formation, and sub-G1 DNA content) proceeded dose dependently. The caspase-3 activity was induced rapidly and transiently by treatment with an apoptosis-inducing concentration of the EO. Eugenol isolated from clove oil (*Eugenia caryophyllata*, Myrtaceae) was investigated by Yoo et al. (2005) using human promyelocytic leukemia cells (HL-60) and might be a potent agent in cancer therapy. After treatment with eugenol the HL-60 cells showed hallmarks of apoptosis such as DNA fragmentation and formation of DNA ladders in agarose gel electrophoresis. Apoptotic cell death was induced via generation of ROS, inducing a mitochondrial permeability transition, reducing antiapoptotic protein bcl-2 level and inducing cytochrome C release to the cytosol. In traditional medicine very prominent is the bog myrtle *Myrica gale* L. (Myricaceae), a native plant in Canada as well as in Scotland. GC-MS analysis of the leaf EO revealed 53 components with myrcene, limonene, α-phellandrene, and β-caryophyllene as the major compounds. In the 60-min fraction of this oil the caryophyllene oxide content was higher (9.9%) than in the 30-min fraction (3.5%). The anticancer activity of these extracts was tested in human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line DLD-1. The 60-min fraction showed a higher anticancer activity against both cell types than the 30-min fraction. The higher cell growth inhibition induced by the 60-min fraction could be caused by the accumulation of sesquiterpenes (Sylvestre et al., 2005).

Some Thai medicinal plants were checked for their antiproliferative activity on human mouth epidermal carcinoma (KB) and murine leukemia (P388) cell lines by Manosroi et al. (2006) using the MTX assay. From 17 Thai plants the Myrtaceae *Psidium guajava* L. (common guava) leaf oil showed the highest antiproliferative activity in KB cell line, which is 4.37 times more potent than vincristine, a well-known mitosis inhibitor. In P388 cells the Lamiaceae *Ocimum basilicum* L. (basil) oil had the highest effect, which is 12.7 times less potent than the thymin antagonist 5-fluorouracil.

In another study, the EO of the leaves of the Euphorbiaceae *Croton flavens* L. (yellow balsam) from Guadeloupe, a native plant from the Caribbean area, was analyzed by Sylvestre et al. (2006) and as main components viridiflorene (12.2%), germacrone (5.3%), (*E*)-γ-bisabolene (5.3%), and β-caryophyllene (4.9%) ascertained. The EO was found to be active against human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line DLD-1. Three of the 47 components of the EO, namely α-cadinol, β-elemene, and α-humulene, showed also a cytotoxic activity against tumor cell lines. Yu et al. (2007) tested the EO of the rhizome of the Aristolochiaceae *Aristolochia mollissima* for its cytotoxicity on four human cancer cell lines (ACHN, Bel-7402, Hep G2, HeLa). The rhizome oil possessed a significantly greater cytotoxic effect on these cell lines than the oil from the aerial plant.

The success of chemotherapeutic agents is often hindered by the development of drug resistance, with multidrug-resistant phenotypes reported in a number of tumors. In a recent study of an Italian research team, the effects of the monoterpene alcohol linalool on the growth of two human breast adenocarcinoma cell lines were investigated, both as a single agent and in combination with doxorubicin. Linalool inhibited only moderately cell proliferation; however, in subtoxic concentrations potentiates doxorubicin-induced cytotoxicity and proapoptotic effects in both cell lines, MCF7 WT and MCF7 AdrR. The results of the Italian author group suggest that linalool improves the therapeutic index in the management of breast cancer, especially multidrug resistance (MDR) tumors (Ravizza et al., 2008).

The EO of *Cyperus rotundus* (Cyperaceae) contains cyperene, α-cyperone, isolongifolen-5-one, rotundene, and cyperorotundene as principal constituents. An *in vitro* cytotoxicity assay indicated that this oil was very effective against L1210 leukemia cells, which correlates with significantly increased apoptotic DNA fragmentation (Kilani et al., 2008).

Finally, Yan et al. (2008) reported on the cytotoxic activity of the EO and extracts of *Lynderia strychnifolia* (Lauraceae), a plant which is widely used in traditional Chinese medicine. Three human cancer cell lines (A549, HeLa, and Hep G2) were examined by *in vitro* assays. The strongest cytotoxicity on the cancer cells showed the leaf oil with 50% inhibitory concentration (IC_{50}) values ranging between 22 and 24 μg/mL after 24 h of treatment. The EO of the leaves and also of the roots exhibited greater cytotoxicity than ethanol extracts.

9.1.2 Antinociceptive Effects*

Although it has been mentioned in the introduction that in this book the comprehensive term "biological properties" does not include activities affecting the central and the peripheral nervous system, nevertheless for this subchapter a small exception had to be made, because the antinociceptive system belongs to the central nervous system. The function of antinociception is to aggravate the forwarding of pain impulses, which alleviates the sensation of pain. It is assumed that the so-called nociceptors are nerve endings responsible for nociception. They are sensory receptors that send signals, which cause the perception of pain in response to a potentially damaging stimulus. When the nociceptors are activated, they can trigger a reflex. Due to this system it can be explained why pains in a stress situation (e.g., caused by an injury after a traffic accident) are not noted in the first instance, but later after the decay of the tension. To test this pain-relieving capacity,

* Adorjan, M., 2007. Part of her master thesis, University of Vienna.

experimentally generated pains in animal experiments were performed (Hunnius, 2007). The animal experiments that were mostly used are as follows:

1. *Formalin test:* A small volume of formalin is injected in the hind paw of a rat or a mouse and the pain-related behavior (paw lifting, paw licking) is observed. There are two phases of the test. In the first phase (10 min) the immediate reaction, that reflects the activation of peripheral nociceptors, is measured. The second phase (60 min) reflects a spinal hypersensibilization (Pharmacon, 2007).
2. *Acetic acid-induced writhing test:* Acetic acid is administered intraperitoneally to the test animals and the number of writhings is registered.
3. *Tail-flick test:* The tail is irritated by a thermal stimulus and the movement of the tail is monitored.
4. *Hot-plate test:* The test animal is put on a heated surface ("a hot plate") and the thermal pain reflexes are recorded.
5. *Carrageenin edema test:* The test animals get carrageenin injected and the volume of the paw is measured.
6. *Dextran edema test:* The test animals get dextran injected and the paw volume is measured.
7. *PBQ-induced abdominal constriction test:* The test animals get injected intraperitoneally a solution of *p*-benzoquinone and the number of abdominal contractions is recorded.

The antinociceptive effects of *N. sativa* (black cumin, Ranunculaceae) oil and its major component thymoquinone were investigated by Abdel-Fattah et al. (2000). After oral administration of doses ranging from 50 to 400 mg/kg the nociceptive response was suppressed in the hot-plate test, tail-pinch test, acetic acid-induced writhing test, and in the early phase of formalin test. There was also an inhibition of nociceptive response in the late phase of the formalin test after systemic administration and i.p. injection of thymoquinone. The s.c. injection of naloxone (1 mg/kg) significantly blocked the antinociceptive effect of *N. sativa* oil and thymoquinone in the early phase of the formalin test. In *N. sativa* oil- and thymoquinone-tolerant mice the antinociceptive effect of morphine was significantly reduced, but not vice versa. These findings indicate that *N. sativa* oil as well as thymoquinone induce an antinociceptive effect by means of an indirect activation of μ1- and μ-opioid receptor subtypes.

The EO from the leaves of *Cymbopogon citratus* (lemongrass, Poaceae) showed in the acetic acid-induced writhing test a strong inhibition dose dependently. Also in the formalin test the EO could cause an inhibition especially in the second phase of the test (100% at 200 mg/kg i.p.). Otherwise the opioid antagonist naloxone reversed the central antinociception, suggesting that the EO of *Cymbopogon citratus* plays a role in central and peripheral levels (Viana et al., 2000).

Abdon et al. (2002) studied the antinociceptive effect of the EO of *Croton nepetaefolius* Baill. (Euphorbiaceae), an aromatic plant distributed in the northeast of Brazil and used in folk medicine as sedative, orexigen, and antispasmodic medicine, on male Swiss mice using the acetic acid-induced writhings, the hot-plate test and the formalin test. The EO was administered orally. Writhings were reduced effectively at the highest dose tested. In hot-plate test latency was assessed at all times of observation. In formalin test a significant reduction of paw licking could be noticed in the second phase of the test at 100 mg/kg and in both phases at 300 mg/kg. The analgesic effect of morphine was reversed significantly by pretreatment with naloxone in both phases in the formalin test.

The antinociceptive effect of *Satureja hortensis* L. (summer savory, Lamiaceae) extracts and EO, a medicinal plant used in Iranian folk medicine as stomachic, muscle, and bone pain deliver was assessed by Hajhashemi et al. (2002). The hydroalcoholic extract as well as the polyphenolic fraction and EO of the aerial parts of the herb were screened for their antinociceptive activity in the light tail-flick test, as well as in the formalin and also in the acetic acid-induced writhing test. While

there was no significant result in the light tail-flick test the EO decreased the number of writhings induced by acetic acid compared to the control at the highest doses given. In the formalin test the hydroalcoholic extract, then the polyphenolic fraction and the EO showed an antinociceptive activity, which could not be reversed by pretreatment with naloxone or caffeine. These findings demonstrate that this effect caused by *Satureja hortensis* L. is not mediated by opioidergic or adenosine receptors.

Another *Satureja* species of the Lamiaceae family, namely *Satureja thymbra* L., was investigated by Karabay-Yavasoglu et al. (2006). The antinociceptive activity of the EO was assessed in mice by the formalin test and in rats by the light tail-flick test and the hot-plate test. An antinociceptive effect could only be detected in the hot-plate test during the early phase and the late phase. In the tail-flick test the EO did not produce any significant antinociceptive effect. Nevertheless the authors concluded that the EO of *Satureja thymbra* may have an analgesic activity in mice and rats. A screening of the leaf EO of the Lauraceae *Laurus nobilis* L. (sweet bay) for antinociception in mice and rats was made by Sayyah et al. (2003). They reported a significant analgesic effect in tail flick and formalin tests, which was comparable to reference analgesics such as morphine and piroxicam.

In another study the antinociceptive effects of *Teucrium polium* L., a wild-growing Iranian plant belonging to the Lamiaceae, were investigated by Abdollahi et al. (2003). The total extract and the EO significantly inhibited pain-related behavior in the acetic acid-induced writhing test compared to the control.

The hydroalcoholic extract, the polyphenolic fraction, and the EO of the Lamiaceae *Zataria multiflora* Boiss. (Zataria), a plant used in traditional medicine for pain therapy and several gastrointestinal diseases, were checked by Jaffary et al. (2004) using writhing, tail flick, and formalin test in mice and rats. As main components in the EO linalool, linalyl acetate, and *p*-cymene could be detected. In the writhing test the EO and the hydroalcoholic extract were able to decrease the pain reflexes significantly ($p < .05$, $n = 6$). Both EO and hydroalcoholic extract were effective in tail-flick test ($p < .05$, $p < .01$, $n = 6$), whereas oral administration did not show any effect, which indicates an inactivation or extensively metabolization in liver or in gastrointestinal sections (see Figure 9.1). Furthermore, antinociception was indicated in both phases of formalin test ($p < .01$, $n = 6$). Due to the overall activity in these tests scientists concluded that *Zataria multiflora* has a clear central and peripheral antinociceptive activity.

The antinociceptive effects of *Lavandula hybrida* Reverchon "Grosso" (Lamiaceae) EO and its main components linalool and linalyl acetate were examined by Barocelli et al. (2004). The number of acetic acid-induced writhings was significantly decreased after oral administration of 100 mg/kg or

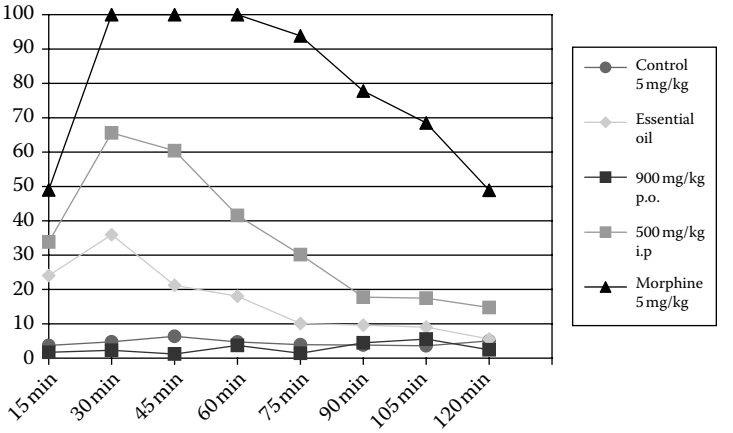

FIGURE 9.1 Antinociceptive activity of *Zataria multiflora* in tail-flick test in rats.

inhalation of lavender EO for 60 min. After pretreatment with naloxone, atropine, and mecamylamine the postinhalative analgesia in hot-plate test was suppressed, which indicates an involvement both of the opioidergic and of the cholinergic system.

In another study the involvement of adenosine A1 and A2A receptors in (–)-linalool-induced antinociception was found by Peana et al. (2006b). The authors also mentioned that they have already shown the antinociceptive effect of (–)-linalool in recent studies in different animal models. The antinociceptive and antihyperalgesic effects were ascribed to the stimulation of opioidergic, cholinergic, and dopaminergic systems as well as to the interaction with K-channels, the local anesthetic activity, the negative modulation of glutamate transmission, and the blockade of *N*-methyl-D-aspartic acid (NMDA) receptors (Peana et al., 2006a). In the present study the authors used 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective A1 receptor antagonist, and 3,7-dimethyl-1-propargylxanthine (DMPX), a selective A2A receptor antagonist, to measure the depression of the antinociceptive effect of (–)-linalool in the hot-plate test in mice. The decrease of the antinociceptive effect of linalool was significantly for both DPCPX (0.1 mg/kg i.p.) and DMPX (0.1 mg/kg i.p.) at the highest doses tested. These results indicate that the antinociceptive effects of (–)-linalool, the natural occurring enantiomer in the EOs of lavender, are, at least partially, mediated by adenosine A1 and A2A. The authors also examined the role of nitric oxide (NO) and prostaglandin E2 (PGE2) using lipopolysaccharide (LPS)-induced responses in macrophage cell line J774.A1. The nitrite accumulation in the culture medium was significantly inhibited after exposure of LPS-stimulated cells to (–)-linalool, whereas the LPS-stimulated increase of inducible nitric oxide synthetase (iNOS) expression was not inhibited at all. On the other hand, (–)-linalool had no effect on the release of PGE2 and on the increase of inducible cyclooxygenase-2 (COX-2) expression. These findings demonstrate that the reduction of NO production is, at least partially, responsible for the molecular mechanism of (–)-linalool antinociceptive effect, supposably through cholinergic and glutamatergic activities. Coming back to an earlier study of this author group (Peana et al., 2003), the influence of opioidergic and cholinergic systems in (–)-linalool-induced antinociception was examined. In acid-induced writhing test a significant reduction of writhings could be shown at doses ranging from 25 to 75 mg/kg. Whereupon the effect was completely inverted by naloxone, an opioid receptor antagonist, and by atropine, an unselective muscarinic receptor antagonist. In hot-plate test only the dose of 100 mg/kg was of significance. Moreover (–)-linalool showed a dose-dependent increase of motility effects, which excludes the participation of any sedative effect. The conclusion of this study is that opioidergic and cholinergic system play an important role in (–)-linalool-induced antinociception.

In a recent published study, the contribution of the glutamergic system in the antinociception elicited by (–)-linalool in mice was investigated (Batista et al., 2008). This monoterpene alcohol administered intraperitoneally, or orally, or intrathecally inhibited dose dependently glutamate-induced nociception in mice. Furthermore, (–)-linalool reduced significantly the biting response caused by intrathecal injection of glutamate when this alcohol was given i.p. This antinociception is possible due to mechanisms operated by ionotropic glutamate receptors, namely AMPA, NMDA, and kainite.

In a further study De Araujo et al. (2005) screened the EO of *Alpinia zerumbet* (Pers.) Burtt. et Smith (shell ginger, Zingiberaceae), an aromatic plant native to the tropical and subtropical regions of the world and used in folk medicine for various diseases, including hypertension. In the acetic acid-induced writhing test the oral administration was effective, in the hot-plate test the EO increased the remedy time and also paw licking could be reduced significantly in the second phase of formalin test at 100 mg/kg. At 300 mg/kg a decrease was noticed in both phases of the test. After pretreatment with naloxone i.p. the analgesia was reversed significantly, completely for the first phase and partially for the second phase of the test. Therefore, also this EO shows a dose-dependent antinociceptive effect, which supposably includes the participation of opiate receptors.

Lino et al. (2005) used the EO of *Ocimum micranthum* Willd. (Lamiaceae) from Northeastern Brazil to study its antinociceptive activity in the hot plate and in the acetic acid-induced writhing test. Upon administration of low doses the EO could inhibit the number of writhings up to 79%. The antinociceptive effect was not influenced by pretreatment with naloxone. In the formalin test paw-licking time decreased to 61%. Also in this case the pretreatment with naloxone could not reverse the antinociceptive effect, confirming that there is no involvement of the opioid system. However, an involvement of the NO system was suggested because of the reverse of the antinociception by L-arginine in the second phase of the formalin test.

Santos et al. (2005) tested the antinociceptive activity of leaf EO of the Euphorbiaceae *Croton sonderianus* in mice using chemical and thermal methods. After i.p. injection of acetic acid, formalin, and capsaicin the EO could provoke an inhibition of nociception. On the other hand, there was no evidence for effectivity against thermal nociception in the hot-plate test; however, the acetic acid-induced writhing and the capsaicin-induced hind-paw licking could be reduced more effectively. The antinociceptive effect in both capsaicin and formalin test was significantly antagonized by glibenclamide. These findings indicate that glibenclamide-sensitive $KATP^+$-channels are involved in the antinociceptive effect of *Croton sonderianus* EO.

The same author studied also the antinociceptive properties in animal experiments after oral administration of 1,8-cineole, a terpenoid oxide in many EOs. By pretreatment of mice with naloxone the antinociceptive effect of this bicyclic ether was not inverted in the formalin test (Santos et al., 2000).

Methyleugenol, a prominent fragrance substance because of its allergenic potential, was isolated from *Asiasari radix* (Aristolochiaceae) and its antinociceptive effect on formalin-induced hyperalgesia in mice investigated by Yano et al. (2006). The oral administration of methyleugenol suppressed the duration of licking and biting in the second phase of the test in the same way as diclofenac, a nonsteroidal anti-inflammatory drug. Furthermore, the substance could decrease pain-related behaviors induced by intrathecal injection of NMDA, whereas diclofenac did not influence this behavior. All these antinociceptive effects of methyleugenol were depressed by bicuculline, a γ-aminobutyric acid (A) antagonist, whereas COX-1 and -2 activity was not affected. The conclusion of this study was that methyleugenol is a potent inhibitor of NMDA-receptor-mediated hyperalgesia via GABA(A) receptors.

Iscan et al. (2006) analyzed the EO of the Asteraceae *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. ssp. *aleppica* by GC and GC-MS and found as main component in both oils 1,8-cineole (32.5% and 26.1%). For testing the antinociceptive effect, male Swiss albino mice were used for the *p*-benzoquinone-induced abdominal constriction test. The number of abdominal contractions (writhing moments) was counted for 15 min, whereupon the antinociceptive activity was illustrated as percentage change from writhing controls. As reference drug Aspirin® at doses of 100 and 200 mg/kg was used. The EO of *Achillea aleppica* ssp. *aleppica*, which was also rich in bisabolol and its derivates, could induce a significant antinociception by reducing the number of writhes. In comparison with acetylsalicylic acid the active component of *Achillea aleppica* ssp. *aleppica* was not as potent as the drug. There are a number of isolated components from both EOs, which cause an antinociceptive effect. In particular, (–)-linalool has been closely investigated. The molecular mechanisms of the antinociceptive effect are different. They can be mediated by adenosine A1 and A2A or NMDA receptors, or the reduction of the NO production can play an important role. Also some can be glibenclamid-sensitive $KATP^+$-channel dependent or influenced by the opioidergic or cholinergic system.

Finally, also the antinociceptive and anti-inflammatory effects of the EO from *Eremanthus erythropappus* (Asteraceae) leaves were reported by a Brazilian author group. The EO proved to be significantly antinociceptive in the acetic acid-induced writhing test in mice, as well in the formalin test, and also in both phases of the paw-licking test, and in the hot-plate test. The exudate volume after intrapleural injection of carrageenan was significantly reduced as well as the leukocyte mobilization by administration of this oil 4 h before the start of the study (Sousa et al., 2008).

9.1.3 Antiviral Activities*

Besides the manifold, well-documented, and, in human and veterinary medicine, very often used antimicrobial and antifungal properties of nearly all EOs (see Chapter 12), this group of natural compounds also possesses distinct antiviral properties. Viruses are submicroscopic particles (ranging from 20 to 300 nm) that can infect cells of a biological organism. They replicate themselves only by infecting a host cell and cannot reproduce on their own. Unlike living organisms, viruses do not respond to changes in their environment (Hunnius, 2007). EOs are able to suppress the viruses in different ways. They can inhibit their replication or they can prevent their spread from cell to cell. In the following the antiviral activity of some EOs against different viruses such as Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2), pseudorabies virus (PrV), influenza virus A3, Junin virus (JUNV), and dengue virus type 2 (DEN-2) will be discussed. In 1999, Benencia et al. (1999) published their results on the antiviral activity of sandalwood oil (*Santalum album*, Santalaceae) against Herpes simplex virus type 1 and type 2. The authors found that the EO inhibited the replication of the viruses. HSV-1 was more influenced than HSV-2 dose dependently.

De Logu et al. (2000) investigated the inactivation of HSV-1 and HSV-2 and the prevention of cell-to-cell virus spread by the EO of the Asteraceae *Santolina insularis.* The plaque-reduction assay showed an IC_{50} values of 0.88 µg/mL for HSV-1 and 0.7 µg/mL for HSV-2, respectively, whereas another test on Vero cells showed a cytotoxic concentration (CC_{50}) of 112 µg/mL, which leads to a CC_{50}/IC_{50} ratio of 127 for HSV-1 and 160 for HSV-2. These findings indicate that the antiviral effect of the EO was caused by direct virucidal effects. There was no antiviral activity detected in a postattachment assay. Due to attachment assays it was shown that virus adsorption was not reduced. Additionally, the reduction of plaque formation assay indicated that the EO reduced cell-to-cell transmission of both HSV-1 and HSV-2.

Another study was made on the antiviral activity of Australian TTO and eucalyptus oil against Herpes simplex virus in cell culture by Schnitzler et al. (2001). The authors used a standard neutral red dye uptake assay to evaluate the cytotoxic effects of TTO and eucalyptus oil and found a moderate toxicity for RC-37 cells of both oils approaching 50% (TC_{50}) at very low concentrations. In the plaque-reduction assay an IC_{50} for plaque formation of 0.0009% HSV-1 and 0.0008% HSV-2 for TTO and of 0.009% (HSV-1) and 0.008% (HSV-2) for eucalyptus oil was determined. In a viral suspension test a very strong virucidal activity against HSV-1 and HSV-2 could be shown. TTO reduced plaque formation by 98.2% for HSV-1 and by 93.0% for HSV-2 at noncytotoxic concentrations, respectively, whereas eucalyptus oil reduced virus titers by 57.9% for HSV-1 and 75.4% for HSV-2 at noncytotoxic concentrations. Additionally, the authors investigated the mode of antiviral action of both EOs. After pretreatment of the virus prior to adsorption plaque formation was clearly inhibited. The findings show that both oils affect the virus before or during adsorption, but not after penetration into the host cell. The authors suggest the application of both oils as antiviral agents in recurrent herpes infections, although the active components are yet unknown.

Farag et al. (2004) examined the chemical and biological properties of the EOs of different *Melaleuca* species (teatree, TTO, Myrtaceae). The authors used the EOs of the fresh leaves of *Melaleuca ericifolia*, *Melaleuca leucadendron* (weepin teatree), *Melaleuca armillaris*, and *Melaleuca styphelioides*. Methyl eugenol (96.8%) was the main compound of the EO of *Melaleuca ericifolia*, whereas 1,8-cineole (64.3%) was the major constituent of *Melaleuca leucadendron*. The EO of *Melaleuca armillaris* was rich in 1,8-cineole (33.9%) and of terpinen-4-ol (18.8%). The main constituents of *Melaleuca styphelioides* were caryophyllene oxide (43.8%) followed by (–)spathulenol (9.6%). The highest virucidal effect of the EOs against HSV-1 in African green monkey kidney cells (Vero) by plaque reduction was caused by the volatile oil of *Melaleuca armillaris* (up to 99%), followed by that of *Melaleuca leucadendron* (92%) and *Melaleuca ericifolia* (91.5%).

* Adorjan, M., 2007. Part of her master thesis, University of Vienna.

Primo et al. (2001) examined *in vitro* the antiviral activity of the EO from the Lamiaceae *Minthostachys verticillata* (Griseb.) Epling against HSV-1 and PrV using the viral plaque-reduction assay. The EO influences HSV-1 and PrV multiplication, an activity which is attributed to the main constituents of the EO, namely menthone (39.5%) and especially pulegone (44.6%). The therapeutic index values attained 10.0 and 9.5 for HSV-1 and PrV, respectively.

In a chicken embryo hemagglutination valence-reduction test, Yan et al. (2002) investigated the inhibition of influenza virus A3 by the Lamiaceae *Mosla chinensis* EO. The authors assessed the activity of the EO for treatment against pneumonia in experiments with mice and found that the cytopathic effect (CPE) caused by influenza virus A3 was reduced in Vero cells by this EO. The hemagglutination valence was reduced from 1:1280 to 1:20 and 1:160 at concentrations of 500, 250, and 50 mg/mL in 9-day-old chicken, respectively. At a dosage of 100 µg/g/d, the therapeutic treatment of mice against pneumonia was successful.

The virucidal effect of the EO of the Lamiaceae *Mentha piperita* against HSV-1 and HSV-2 was tested *in vitro* on RC-37 cells using a plaque-reduction assay (Schuhmacher et al., 2003). The EO showed high virucidal effects against HSV-1 and HSV-2. At concentrations that did not produce a cytotoxic effect plaque formation was significantly inhibited by 82% for HSV-1 and 92% for HSV-2, respectively. A reduction of more than 90% for both herpes viruses could be achieved at higher concentrations of peppermint oil. The authors also demonstrated that the antiviral effect depended on time. After 3 h of incubation of HSV with the EO an antiviral activity of about 99% was shown. To investigate the mechanism of antiviral action, peppermint oil was added at different times to the cells or viruses during infection. When HSV was pretreated with the EO before adsorption, both HSV types were significantly reduced. These findings demonstrate that *Mentha piperita* oil influences the virus before adsorption, but not after penetration into the host cell. The EO also reduces plaque formation of an acyclovir-resistant strain of HSV-1 significantly by 99%. This oil might be useful for topical application as a virucidal agent in recurrent infection, considering its lipophilic properties, which enables it to penetrate the skin.

Another study as to the inhibiory effect of some EOs on HSV-1 replication *in vitro* was carried out by Minami et al. (2003). The best results were achieved by lemongrass, which inhibited the viral replication completely even at a concentration of 0.1%.

Furthermore, Garcia et al. (2003) studied the virucidal activity against HSV-1, JUNV, and DEN-2 of eight different EOs obtained from plants of San Luis Province, Argentinia. The EOs of *Lippia junelliana* and *Lippia turbinata* (Verbenaceae) exhibited the highest virucidal effect against JUNV at virucidal concentrations (VC_{50}) values from 14 to 20 ppm, whereas the EOs of *Aloysia gratissima* (whitebrush, Verbenaceae), *Heterotheca latifolia* (camphorweed, Asteraceae), and *Tessaria absinthioides* (tessaria, Asteraceae) reduced JUNV from 52 to 90 ppm. The virucidal activity depended on time and temperature. The EOs of *Aloysia gratissima*, *Artemisia douglasiana* (mugwort, Asteraceae), *Eupatorium patens* (Asteraceae), and *Tessaria absinthioides* inhibited HSV-1 in the range of 65–125 ppm. A discernible effect on DEN-2 infectivity could only be produced by *Artemisia douglasiana* and *Eupatorium patens* with VC_{50} values of 60 and 150 ppm, respectively.

The antiviral activity of the EO of the Lamiaceae *Melissa officinalis* L. against HSV-2 was examined by Allahverdiyev et al. (2004). The effect of the essential oil on HSV-2 replication in Hep-2 cells was tested in five different concentrations (25, 50, 100, 150, and 200 µg/mL). Up to a concentration of 100 µg/mL *Melissa officinalis* oil did not cause any toxic effect to Hep-2 cells, but it was slightly toxic at concentrations over 100 µg/mL. At nontoxic concentrations the replication of HSV-2 was reduced. Recently, Schnitzler et al. (2008) confirmed these findings: The lipophilic nature of the EO of lemon balm helps to affect the virus before adsorption thus exerting a direct antiviral effect. After the penetration of the herpes virus into the host cell there was no affection recorded anymore.

Yang et al. (2005) studied the anti-influenza virus activities of the volatile oil from the roots of the Asclepiadaceae *Cynanchum stauntonii* and found that the volatile oil caused an antiviral effect against influenza virus *in vitro* and also in *in vivo* experiments and was able to prevent the number of deaths induced by the virus in a dose-dependent manner.

Another study was carried out on the liposomal incorporation of *Artemisia arborescens* L. (powis castle, Asteraceae) EO and its *in vitro* antiviral activity by Sinico et al. (2005). The antiviral effect was tested against HSV-1 by a quantitative tetrazolium-based colorimetric method. The authors found that the EO can be incorporated in good amounts in vesicular dispersions and that these vesicle dispersions were stable for at least 6 months. During this period neither oil leakage nor vesicle size alteration occurred and even after a year of storage oil retention was still good, but vesicle fusion was present. The best antiviral results were observed when vesicles were made with P90H (=hydrogenated (P90H) soy phosphatidyl-choline).

An evaluation of antiviral properties of various EOs from South American plants was carried out by Duschatzky et al. (2005). The authors assessed the cytotoxicity and *in vitro* inhibitory activity of the EOs against HSV-1, DENV-2, and JUNV by a virucidal test. The best results were observed with the EOs of *Heterothalamus alienus* (Asteraceae) and *Buddleja cordobensis* (Scrophulariaceae) against JUNV, with virucidal concentration 50% (VC_{50}) values of 44.2 and 39.0 ppm and therapeutic indices (cytotoxicity to virucidal action ratio) of 3.3 and 4.0, respectively. The oils caused the inhibitory effect interacting directly with the virions.

Reichling et al. (2005) investigated the virucidal activity of a β-triketone-rich EO of the Myrtaceae *Leptospermum scoparium* (manuka oil) against HSV-1 and HSV-2 *in vitro* on RC-37 cells (monkey kidney cells) using a plaque-reduction assay. The addition of the oil to the cells or viruses at different times during the infection cycle made it possible to determine the mode of the antiviral action. After pretreatment with manuka oil 1 h before cell infection both virus types were significantly inhibited. At concentrations that were not cytotoxic, the plaque formation reduction reached levels of 99.5% for HSV-1 and 98.9% for HSV-2. The IC_{50} of the EO for virus plaque formation was 0.0001% V/V (=0.96 μg/mL) for HSV-1 and 0.00006% V/V (=0.58 μg/mL) for HSV-2. When the host cells were pretreated before viral infection, plaque formation could not be influenced. After the virus penetrated the host cells only the replication of HSV-1 particle was significantly reduced to about 41% by manuka oil.

A phytochemical analysis and *in vitro* evaluation of the biological activity against HSV-1 of *Cedrus libani* A. Rich. (cedar of libanon, Pinaceae) was made by Loizzo et al. (2008a). The authors identified the active constituents for the *in vitro* antiviral activity against HSV-1 and evaluated the cytotoxic effects in Vero cells. The IC_{50} values of cones and leaves extract were 0.50 and 0.66 mg/ mL, respectively, without provoking a cytotoxic effect, whereas the EO showed a comparable activity with an IC_{50} value of 0.44 mg/mL. In another study the author group found that the EO of *Laurus nobilis* (Lauraceae) and *Thuja orientalis* (Cupressaceae) were very effective against SARS-coronavirus (IC_{50}: 120 ± 1.2 μg/mL, resp. 130 ± 0.4 μg/mL) and against Herpes simplex virus type 1 (IC_{50}: 60 + 0.5 μg/mL, resp. > 1000 μg/mL) (Loizzo et al., 2008b).

Ryabchenko et al. (2007, 2008) presented a study that dealt with antitumor, antiviral, and cytotoxic effects of some single fragrance compounds. The antiviral properties were investigated in an *in vitro* plaque formation test in 3T6 cells against mouse polyoma virus. Natural and synthetic nerolidol showed the highest inhibitory activity, followed by *trans*,*trans*-farnesol and longifolene.

9.1.4 Antiphlogistic Activity*

Processes by which the body reacts to injuries or infections are called inflammations. There are several inflammatory mediators such as the tumor necrosis factor-α (TNF-α); interleukin (IL)-1β, IL-8, IL-10; and the PGE2. In the following the inhibitory effects of some EOs on the expression of these inflammatory mediators and on other reasons for inflammations will be shown. Shinde et al. (1999) performed studies on the anti-inflammatory and analgesic activity of the Pinaceae *Cedrus deodara* (Roxb.) Loud. (deodar cedar, Pinaceae) wood oil. They examined the volatile oil obtained by steam distillation of the wood of this *Cedrus* species for its anti-inflammatory and analgesic

* Adorjan, M., 2007. Part of her master thesis, University of Vienna.

effect at doses of 50 and 100 mg/kg body weight and observed a significant inhibition of carrageenin-induced rat paw edema. At doses of 100 mg/kg body weight both exudative–proliferative and chronic phases of inflammation in adjuvant arthritic rats were reduced. In acetic acid-induced writhing and also in hot-plate test both tested doses exhibited an analgesic effect in mice.

The anti-inflammatory-related activity of EOs from the leaves and resin of species of *Protium* (Burseraceae), which are commonly used in folk medicine, was evaluated by Siani et al. (1999). The resin oil contains mainly monoterpenes and phenylpropanoids: α-terpinolene (22%), *p*-cymene (11%), *p*-cimen-8-ol (11%), limonene (5%), and dillapiol (16%), whereas the leaves dominantly comprise sesquiterpenes. The authors tested the resin of *Protium heptaphyllum* (PHP) and the leaves of *Protium strumosum* (PS), *Protium grandifolium* (PG), *Protium lewellyni* (PL), and *Protium hebetatum* (PHT) for their anti-inflammatory effect using a mouse pleurisy model induced by zymosan and LPS. In addition, they screened the plants for their NO production from stimulated macrophages and for the proliferation of neoplastic cell lines: Neuro-2a (mouse neuroblastoma), SP2/0 (mouse plasmocytoma), and J774 (mouse monocytic cell line). After administration of 100 mg/kg p.o. 1 h before stimulation with zymosan, an inhibition of protein extravasation could be observed with the oils from PHP, PS, and PL, whereas total or different leukocyte counts could not be reduced. Also the neutrophilic accumulation could be decreased by the oils from PG, PL, and PHT, while PHP and especially PL lead to a reduction of LPS-induced eosinophilic accumulation in mouse pleural cavity. PHT also showed the ability to inhibit the mononuclear accumulation.

The NO production from stimulated mouse macrophages could be changed by *in vitro* treatment with the EOs. A reduction of the LPS-induced NO production of 74% was achieved by PHP and of 46% by PS. On the contrary, PL caused an increase of 49% in NO production. Concerning the cell-line proliferation, Neuro-2a was affected in the range of 60–100%, SP2/0 of 65–95%, and J774 of 70–90%. As to the suggestion of the authors, these EOs could be used as efficient pharmacological tools.

Another study was made on the anti-inflammatory effect in rodents of the EO of the Euphorbiaceae *Croton cajucara* Benth. (Sacaca, Euphorbiaceae) by Bighetti et al. (1999). At a dose of 100 mg/kg the EO exerted an anti-inflammatory effect in animal models of acute (carrageenin-induced paw edema in mice) and chronic (cotton pellet granuloma) inflammation. Compared with the negative control a dose-dependent reduction of carrageenin-induced edema was achieved. This EO also reduced chronic inflammation by 38%, whereas diclofenac only achieved an inhibition of 36%. The migration of neutrophils into the peritoneal cavity could not be inhibited by the EO. The anti-inflammatory effect seemed to be related to the inhibition of COX.

Santos et al. (2000) investigated the anti-inflammatory activity of 1,8-cineole, a terpenoid oxide present in many plant EOs. Inflammation could be reduced in some animal models, that is, paw edema induced by carrageenin and cotton pellet-induced granuloma. This effect was caused at an oral dose range of 100–400 mg/kg. The authors suggest a potentially beneficial use in therapy as an anti-inflammatory and analgesic agent.

The anti-inflammatory effect of the EO of the Myrtaceae *Melaleuca alternifolia* (TTO) was evaluated by Hart et al. (2000). The authors tested the ability of TTO to inhibit the production of inflammatory mediators such as the TNF-α, IL-1β, IL-8, IL-10, and the PGE2 by LPS-activated human peripheral blood monocytes. A toxic effect on monocytes was achieved at a concentration of 0.016% vol/vol by TTO emulsified by sonication in a glass tube into culture medium containing 10% fetal calf serum (FCS). In addition, a significant suppression of LPS-induced production of TNF-α, IL-1β and IL-10 (by approximately 50%), and PGE2 (by approximately 30%) after 40 h could be observed with the water-soluble components of TTO at a concentration equivalent to 0.125%. The main constituents of this EO were terpinen-4-ol (42%), α-terpineol (3%), and 1,8-cineole (2%). When tested individually, only terpinen-4-ol inhibited the production of the inflammatory mediators after 40 h.

The mechanisms involved in the anti-inflammatory action of inhaled TTO in mice were investigated by Golab et al. (2007). The authors used sexually mature, 6–8-week-old, C57BI10 × CBA/H

(F1) male mice and divided them into two groups. One group was injected i.p. with zymosan to induce peritoneal inflammation and the other simultaneously with antalarmin, a CRH-1 receptor antagonist, to block hypothalamic–pituitary–adrenal (HPA) axis function. After 24 h of injection the mice were killed by CO_2 asphyxia, and the peritoneal leukocytes (PTLs) isolated and counted. Additionally, the levels of ROS and COX activity were detected in PTLs by fluorometric and colorimetric assays, respectively. The result was that TTO inhalation led to a strong anti-inflammatory effect on the immune system stimulated by zymosan injection, whereas PTL number, ROS level, and COX activity in mice without inflammation were not affected. The HPA axis was shown to play an important role in the anti-inflammatory effect of TTO and antalarmin was observed to abolish the influence of inhaled TTO on PTL number and their ROS expression in mice with experimental peritonitis. In mice without inflammation these parameters were not affected.

A further study was made on the anti-inflammatory activity of linalool and linalyl acetate constituents of many EOs by Peana et al. (2002). The authors evaluated the anti-inflammatory effect of (–)-linalool, that is, the naturally occurring enantiomer, and its racemate form, present in various amounts in distilled or extracted EOs. Due to the fact that in linalool-containing oils there is also linalyl acetate present, this monoterpene ester was also tested for its anti-inflammatory activity. Both the pure enantiomer and its racemate caused a reduction of edema after systemic administration in carrageenin-induced rat paw edema test. Better results could be observed with the pure enantiomer, which elicited a delayed and more prolonged effect at a dose of 25 mg/kg, whereas the efficiency of the racemate form lasted only for 1 h after carrageenin administration. At higher doses, there were no differences between the (–) enantiomer and the racemate and there could be achieved no increase of the effect with increasing the dose. Equimolar doses of linalyl acetate on local edema did not provoke the same effect as the corresponding alcohol. These results demonstrate a typical prodrug behavior of linaly acetate.

Salasia et al. (2002) examined the anti-inflammatory effect of cinnamyl tiglate contained in the volatile oil of kunyit (*Curcuma domestica* Val.; Zingiberaceae) on carrageenin-induced inflammation in Wistar albino rats (*Rattus norvegicus*). Cinnamyl tiglate was found in the second fraction of the volatile oil at a concentration of 63.6%. After induction of an inflammation in rats by injection of 1% carrageenin and administration of cinnamyl tiglate orally at various doses (control group treated with aspirin) and the EO a plethysmograph measured the degree of inflammation: At a dose of 17.6% of the volatile oil of kunyit/kg body weight the highest anti-inflammatory effect could be observed ($p \leq 0.01$), followed by the effect of a dose of 4.4%/kg body weight ($p \leq 0.05$). At lower doses the inflammation could not be reduced ($p \geq 0.05$).

The *in vitro* anti-inflammatory activity of the EO from the Caryophyllaceae *Ligularia fischeri* var. *spiciformis* (ligularia) in murine macrophage RAW 264.7 cells was evaluated by Kim et al. (2002). They examined the effects of the EOs isolated from various plants on LPS-induced release of NO, PGE2, and TNF-α by the macrophage RAW 264.7 cells. The EO of *Ligularia fischeri* var. *spiciformis* achieved the best results among the tested oils inhibiting significantly the LPS-induced generation of NO, PGE2, and TNF-α in RAW 264.7 cells. Additionally, the EO reduced the expression of iNOS and COX-2 enzyme in a dose-dependent manner. Therefore, the mechanism of the anti-inflammatory effect of this EO is the suppression of the release of iNOS, COX-2 expression, and TNF-α.

The *in vitro* anti-inflammatory activity of the EO of the Asteraceae *Chrysanthemum sibiricum* in murine macrophage RAW 264.7 cells was evaluated by Lee et al. (2003). The aim was to study the effect not only on the formation of NO, PGE2, and TNF-α, but also on iNOS and COX-2 in LPS-induced murine macrophage RAW 264.7 cells. The EO had a similar effect on both enzymes and the inhibitory effects were concentration dependent. Additionally, the volatile oil also furnished a reduction of the formation of TNF-α.

An evaluation of the anti-inflammatory activity of the EOs from the Asteraceae *Porophyllum ruderale* (PR) (yerba porosa) and *Conyza bonariensis* (CB) (asthmaweed) in a mouse model of pleurisy induced by zymosan and LPS was made by Souza et al. (2003). The activity of the main compounds of each oil, β-mycrene (in PR), and limonene (in CB) in the LPS-induced pleurisy

model as well as the immunoregulatory activity was examined by measurement of the inhibition of NO and production of the cytokines, γ-interferon, and IL-4. After oral administration of the oils, a reduction of the LPS-induced inflammation including cell migration could be observed. A similar effect could be provoked with the use of limonene alone. Pure β-mycrene and limonene were also able to reduce the production of NO at not cytotoxic doses. In addition, β-myrcene and limonene also inhibited significantly γ-interferon.

The anti-inflammatory effect of the leaf EO of the Lauraceae *Laurus nobilis* Linn. (sweet bay) in mice and rats was investigated by Sayyah et al. (2003). A dose-dependent anti-inflammatory effect could be observed in the formalin-induced edema test, which could be compared with the effect of nonsteroid anti-inflammatory drugs such as piroxicam.

Silva et al. (2003) examined the anti-inflammatory effect of the EO of three species of the Myrtaceae *Eucalyptus citriodora* (EC, lemon eucalyptus), *Eucalyptus tereticornis* (ET, forest red gum), and *Eucalyptus globulus* (EG, blue gum eucalyptus), from which many species are used in Brazilian folk medicine to treat various diseases such as cold, flu, fever, and bronchial infections. An inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan and vascular permeability induced by carrageenan and histamine could be observed. But there were no consistent results obtained for parameters as activity and dose–response relationship, which demonstrates the complex nature of the oil and the assays used. However, these findings provide support for the traditional use of *Eucalyptus* in Brazilian folk medicine and further investigations should be made in order to develop possibly new classes of anti-inflammatory drugs from components of the EOs of the *Eucalyptus* species.

The anti-inflammatory activity of the EOs of *Ocimum gratissimum* (African brasil, Lamiaceae), *Eucalyptus citriodora* (lemon eucalyptus, Myrtaceae), and *Cymbopogon giganteus* (Poaceae) was evaluated by Sahouo et al. (2003). The authors tested the inhibitory effect of the three plants *in vitro* on soybean lipoxygenase L-1 and COX function of prostaglandin H synthetase. The two enzymes play an important role in the production of inflammatory mediators. The EO of *Eucalyptus citriodora* evidently suppressed L-1 with an IC_{50} value of 72 μg/mL. Both enzymes were inhibited by only one EO that of *Ocimum gratissimum* with IC_{50} values of 125 μg/mL for COX function of PGHS and 144 μg/mL for L-1, respectively, whereas the oils of *Eucalyptus citriodora* and *Cymbopogon giganteus* did not affect the COX.

Lourens et al. (2004) evaluated the *in vitro* biological activity and chemical composition of the EOs of four indigenous South African *Helichrysum* species (Asteraceae), such as *Helichrysum dasyanthum*, *Helichrysum felinum* (strawberry everlasting), *Helichrysum excisum*, and *Helichrysum petiolare* (licorice plant). An interesting anti-inflammatory effect could be observed in the lipooxygenase-5 assay at doses between 25 and 32 μg/mL. Analysis of the chemical composition showed that the EOs comprise mainly monoterpenes such as α-pinene, 1,8-cineole, and *p*-cymene, only the oil of *Helichrysum felinum* was dominated by sesquiterpenes in low concentrations with β-caryophyllene as main compound on top.

The composition and *in vitro* anti-inflammatory activity of the EO of South African *Vitex* species (Verbenaceae), such as *Vitex pooara* (waterberg poora-berry), *Vitex rehmanni* (pipe-stem tree), *Vitex obovata* ssp. *obovata* (hairy fingerleaf), *V. obovata* ssp. *wilmsii*, and *Vitex zeyheri* (silver pipe-stem tree), were analyzed by Nyiligira et al. (2004). After determination of the composition of the EOs by GC-MS their *in vitro* anti-inflammatory activity was investigated in a 5-lipoxygenase assay. All EOs effectively suppressed 5-lipoxygenase, which plays an important role in the inflammatory cascade. The best results were achieved by *V. pooara* with an IC_{50} value of 25 ppm. The use of the EO data matrix presents chemotaxonomic evidence, which supports infrageneric placement of *V. pooara* in subgenus *Vitex*, whereas the other four species are placed in subgenus *Holmskiodiopsis*.

Ganapaty et al. (2004) examined the composition and anti-inflammatory activity of the Geraniaceae *Pelargonium graveolens* (rose geranium) EO. Citronellol, geranyl acetate, geraniol, citronellyl formate, and linalool were identified in the leaf oil by GC-MS. A significant anti-inflammatory effect could be observed in the carrageenan-induced rat paw edema test.

Another study was made on the *in vitro* anti-inflammatory activity of paeonol from the EO of the Paeoniaceae *Paeonia moutan* (tree peony) and its derivate methylpaeonol by Park et al. (2005). The authors isolated paeonol (2-hydroxy-5-methoxyacetophenone) by silica gel column chromatography and methylated it by dimethylsulfate to yield methylpaeonol (2,5-di-*O*-methylacetophenone). A suppression of the NO formation in LPS-induced macrophage RAW 264.7 cells was observed with both compounds in nitrite assay. Additionally, a reduction of iNOS-synthase and COX-2 formation was achieved in the Western blotting assay. These findings demonstrate that paeonol is partly responsible for the anti-inflammatory effect of *Paeonia moutan* and that synthesized derivates are promising candidates for new anti-inflammatory agents.

The anti-inflammatory effect of the EO from the leaves of indigenous *Cinnamomum osmophloeum* Kaneh. (camphor tree, Lauraceae) was studied by Chao et al. (2005). Twenty-one components, among which the monoterpenes 1,8-cineole (17%) and santolina triene (14.2%) and the sesquiterpenes spathulenol (15.7%) and caryophyllene oxide (11.2%), were analyzed as main constituents. In the anti-inflammatory assay it was found that the EO exerted a high capacity to suppress pro-IL-1β protein expression induced by LPS-treated J774A.1 murine macrophage at dosages of 60 μg/mL. Additionally, IL-1β and IL-6 production was reduced at the same dose. The TNF-α production could not be influenced by this dose of the EO.

A further study was carried out on the anti-inflammatory activity of the EO from *Casaeria sylvestris* Sw. (wild coffee, Flacourtiaceae) by Esteves et al. (2005). The EO having a total yield of 2.5% showed a LD_{50} of 1100 mg/kg in mouse. Its composition was analyzed by GC and mainly sesquiterpenes, such as caryophyllene, thujopsene, α-humulene, β-acoradiene, germacrene-D, bicyclogermacrene, calamenene, germacrene B, spathulenol, and globulol identified as main compounds. After oral administration of this EO to rats, a reduction by 36% in carrageenan-induced edema was achieved in the rat assay ($p < 0.05$, Student's *t*-test). In rat paw edema dextran-induced and vascular permeability assay using histamine, no significant result could be observed. Additionally, the writhing test using acetic acid demonstrated an inhibition of writhes with the EO by 58% and with indomethacin by 56%.

Ramos et al. (2006) investigated the anti-inflammatory activity of EOs from five different Myrtaceae species, *Eugenia brasiliensis* (grumichama), *Eugenia involucrate*, *Eugenia jambolana*, *Psidium guajava* (guava), and *Psidium widgrenianum*. The oils were obtained by steam distillation and analyzed by GC-MS and the correlation of retention indexes. In *Eugenia brasiliensis*, *Eugenia involucrate*, and *Psidium guajava* mainly sesquiterpenes could be identified, whereas monoterpenes dominated in *Psidium widgrenianum* and *Eugenia jambolana*. Afterwards the volatile compounds in zymosan and LPS-induced inflammatory models were tested. In zymosan-induced pleurisy, no reduction of leukocyte accumulation or protein leakage could be observed after p.o. administration of up to 100 mg/kg. *Eugenia jambolana* suppressed the total leukocyte (up to 56%) and eosinophil (up to 74%) migration in LPS-induced pleurisy, but the response did not correlate with the dose. *Psidium widgrenianum* only inhibited eosinophil migration (up to 70%) and both *Eugenia jambolana* and *Psidium widgrenianum* were also tested *in vitro* for their inhibitory effect on the production of NO. A potent suppression was achieved by *Eugenia jambolana* (up to 100%) in a dose-dependent manner, whereas only a moderate effect could be observed with *Psidium widgrenianum* (51%) test at concentrations below cytotoxic activity (25 μg/well).

The anti-inflammatory activity of the Myrtaceae *Eugenia caryophyllata* (clove) EO was evaluated in an animal model by Ozturk et al. (2005). The chemical composition of this EO by GC yielded β-caryophyllene (44.7%), eugenol (44.2%), α-humulene (3.5%), eugenyl acetate (1.3%), and α-copaene (1.0%) as main constituents. Then the volumes of the right hind paws of rats were measured with a plethysmometer in eight groups: physiological serum, ethylalcohol, indomethacin (3 mg/kg), etodolac (50 mg/kg), cardamom (0.05 ml/kg), EC-I (0.025 ml/kg), EC-II (0.050 ml/kg), EC-III (0.100 ml/kg), and EC-IV (0.200 ml/kg). Afterwards the drugs were injected i.p. and γ-carrageenan s.c. into the plantar regions and the difference of the volumes determined after 3 h. A reduction of the inflammation by 95.7% could be observed with indomethacin, to a lesser extent

by the other seven groups (Table 9.1). The result of the study was that the EO of *Eugenia caryophyllata* exerted a remarkable anti-inflammatory effect.

Alitonou et al. (2006) investigated the EO of Poaceae *Cymbopogon giganteus*, widely used in traditional medicine against several diseases, from Benin for its potential use as an anti-inflammatory agent. The analysis of this EO furnished *trans-p*-1(7),8-menthadien-2-ol (22.3%), *cis-p*-1-(7),8-menthadien-2-ol (19.9%), *trans-p*-2,8-menthadien-1-ol (14.3%), and *cis-p*-2,8-menthadien-1-ol (10.1%) as main components. Additionally, it was found that the leaf EO suppressed the 5-lipoxygenase *in vitro*. Moreover, the antiradical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (as to this property see Section 9.1.6).

A further study was carried out on the anti-inflammatory effect of the EO of Iranian black cumin seeds (BCS) (*N. sativa* L.; Ranunculaceae) by Hajhashemi et al. (2004). *p*-Cymene (37.3%) and thymoquinone (13.7%) were found to be the main compounds. For the detection of the anti-inflammatory activity, carrageenan-induced paw edema test in rats was used and also the croton oil-induced ear edema in mice. After oral administration of this EO at various doses no significant anti-inflammatory effect could be observed in the carrageenan test, whereas i.p. injection of the same doses significantly reduced carrageenan-induced paw edema. At doses of 10 and 20 μL/ear, BCS-EO also caused a reduction of a croton oil-induced edema. An anti-inflammatory effect could be observed after both systemic and local administration and thymoquinone seemed to play an important role in this pharmacological effect.

The downregulation of the leukotriene biosynthesis by thymoquinone, the active compound of the EO of the Ranunculaceae *N. sativa*, and its influence on airway inflammation in a mouse model was examined by El Gazzar et al. (2006). Bronchial asthma is often caused by chronic airway inflammation and leukotrienes are potent inflammatory mediators. Their levels arose in the air passages when an allergen challenge has been going on. The authors sensitize mice and challenged them with ovalbumin (OVA) antigen, which led to an increase of leukotrien B4 and C4, Th2 cytokines, and eosinophils in bronchoalveolar lavage (BAL) fluid. Additionally, lung tissue eosinophilia and nose goblet cells were remarkably elevated. After administration of thymoquinone before OVA challenge, 5-lipoxygenase expression by lung cells was suppressed and therefore the levels of LTB4 and LTC4 were reduced. A reduction of Th2 cytokines and BAL fluid and lung tissue eosinophilia, all parameters of airway inflammation, could also be observed. These findings demonstrate the anti-inflammatory activity of thymoquinone in experimental asthma.

Also El Mezayen et al. (2006) studied the effect of thymoquinone, the major constituent of the EO of *N. sativa* seeds, on COX expression and prostaglandin production in a mouse model of allergic airway inflammation. Prostaglandins play an important role in modulating the inflammatory responses in a number of conditions, including allergic airway inflammation. They are formed through arachidonic acid metabolism by COX-1 and -2 in response to various stimuli. The authors sensitized mice and challenged them through the air passage with OVA, which caused a significant

TABLE 9.1
Reduction of Inflammation by the EO of *Eugenia caryophyllata* (EC), Indomethacin, and Etodolac in Animal Model

	Reduction of Inflammation (%)
Indomethacin (3 mg/kg)	95.7
Etodolac (50 mg/kg)	43.4
EC-I (0.025 mL/kg)	46.5
EC-II (0.050 mL/kg)	90.2
EC-III (0.100 mL/kg)	66.9
EC-IV (0.200 mL/kg)	82.8

elevation of the PGD2 and PGE2 expression in the airways. Additionally, inflammatory nose cells and Th2 cytokine levels in the BAL fluid, lung airway eosinophilia, and goblet cell hyperplasia were raised and the COX-2-protein expression in the lung was induced. After i.p. injection of thymoquinone for 5 days before the first OVA challenge a significant decrease in Th2 cytokines, lung eosinophilia, and goblet cell hyperplasia could be observed, which was caused by the suppression of COX-2 protein expression and a reduction of the PGD2 production. Thymoquinone also slightly inhibited the COX-1 expression and the production of PGE2. This time the results demonstrated the anti-inflammatory effect of thymoquinone during the allergic response in the lung caused by the suppression of PGD2 synthesis and Th2-driven immune response.

The effect of thymoquinone from the volatile oil of black cumin on rheumatoid arthritis in rat models was investigated by Tekeoglu et al. (2006). Arthritis was induced in rats by Freund's incomplete adjuvant and the rats were divided into five groups: controls 0.9% NaCl ($n = 7$), 2.5 mg/kg thymoquinone ($n = 7$), 5 mg/kg thymoquinone ($n = 7$), Bacilli Chalmette Guerin (BCG) 6 × 105 CFU ($n = 7$), and MTX 0.3 mg/kg ($n = 7$). The level of inflammation was characterized by radiological and visual signs on the claw and by TNF-α and IL-1β expression and the results of the different groups were compared. Thymoquinone reduced adjuvant-induced arthritis in rats, which was confirmed clinically and radiologically.

Biochemical and histopathological evidences for beneficial effects of the EO of the Lamiaceae *Satureja khuzestanica* Jamzad, an endemic Iranian plant, on the mouse model of inflammatory bowel diseases were found by Ghazanfari et al. (2006). The EO was tested on the experimental mouse model of inflammatory bowel disease, which is acetic acid-induced colitis and used prednisolone as control. For best results, also biochemical, macro- and microscopic examinations of the colon were performed. In acetic acid-treated mice a significant increase of lipid peroxidation could be observed compared to the control group, which was significantly restored by treatment with the EO and prednisolone. The EO decreased the lipid peroxidation up to 42.8% dose dependently, whereas prednisolone caused a decrease of 33.3%. Also a significant increase of the myeloperoxidase activity could be observed compared to the control group in acetic acid-treated mice, which was also significantly restored by treatment with the EO and prednisolone. The EO lowered the myeloperoxidase activity by 25% and 50% on average, whereas that of the control group was decreased by 53%. In addition, the EO- and prednisolone-treated groups exhibited significantly lower score values of macro- and microscopic characters after comparison to the acetic acid-treated group. These findings demonstrate that the beneficial effect of *Satureja khuzestanica* Jamzad EO could be compared to that of prednisolone. The antioxidant, antimicrobial, anti-inflammatory, and antispasmodic properties of the EO may be responsible for the protection of animals against experimentally induced inflammatory bowel diseases.

The biological activity and the composition of the EOs of 17 indigenous *Agathosma* (Rutaceae) species were examined by Viljoen et al. (2006a) in order to validate their traditional use. The analysis of the EOs furnished 322 different components. The anti-inflammatory activity was detected with the 5-lipoxygenase assay and all oils inhibited inflammations *in vitro* with *Agathosma collina* achieving the best results (IC_{50} value of about 25 μg/mL). These results show that the EOs of the different *Agathosma* species strongly suppress the 5-lipoxgenase.

The chemical composition and biological activity of the EOs of four related *Salvia* species (Lamiaceae) also indigenous to South Africa was evaluated by Kamatou et al. (2006). The authors isolated the EOs from fresh aerial parts by hydrodistillation and analyzed the chemical composition by GC-MS. The differences between the different species were rather quantitative. Forty-three components were identified accounting for 78% of *Salvia africana-caerulea*, 78% of *Salvia africana-lutea*, 96% of *Salvia chamelaeagnea*, and 81% of *Salvia lanceolata* total EO. *Salvia africana-caerulea* and *Salvia lanceolata* mainly contained oxygenated sesquiterpenes (59% and 48%, respectively), whereas *Salvia chamelaeagnea* was dominated by oxygen-containing monoterpenes (43%) and *Salvia africana-lutea* by monoterpene hydrocarbons (36%). The anti-inflammatory effect was tested with the 5-lipoxygenase method.

Viljoen et al. (2006b) studied the chemical composition and *in vitro* biological activities of seven Namibian species of *Eriocephalus* L. (Asteraceae). The EOs of *Eriocephalus ericoides* ssp. *ericoides* (samples 1 and 2), *Eriocephalus merxmuelleri*, *Eriocephalus scariosus*, *Eriocephalus dinteri*, *Eriocephalus luederitzianus*, *Eriocephalus klinghardtensis*, and *Eriocephalus pinnatus* were analyzed by GC-MS. *Eriocephalus ericoides* ssp. *ericoides* (sample 1), *Eriocephalus merxmuelleri*, and *Eriocephalus scariosus* contained high levels of 1,8-cineole and camphor and *Eriocephalus scariosus* was also rich in santolina alcohol (14.8%). Most camphor was found in *Eriocephalus dinteri* (38.4%), whereas the major compound of *Eriocephalus ericoides* ssp. *ericoides* (sample 2) was linalool (10.4%). The composition of *Eriocephalus luederitzianus* and *Eriocephalus klinghardtensis* was similar, both containing high levels of α-pinene, β-pinene, *p*-cymene, and γ-terpinene. *Eriocephalus luederitzianus* additionally was rich in α-longipinene (10.3%) and β-caryophyllene (13.3%). *Eriocephalus pinnatus* was different from the other taxa containing mainly isoamyl 2-methylbutyrate (7.9%) and isoamyl valerate (6.5%). The anti-inflammatory activity was evaluated using the 5-lipoxygenase enzyme and *Eriocephalus dinteri* achieved best results (IC_{50}: 35 μg/mL).

Wang and Zhu (2006) studied the anti-inflammatory effect of ginger oil (*Zingiber officinale*, Zingiberaceae) with the model of mouse auricle edema induced by xylene and rat paw edema induced by egg white for acute inflammation and the granuloma hyperplasia model in mouse caused by filter paper for chronic inflammation. Additionally, the influence of ginger oil on delayed-type hypersensitivity (DHT) induced by 2,4-dinitrochlorobenzene (DNCB) in mice was observed. The authors found that ginger oil significantly inhibited both mouse auricle edema and rat paw edema and it also reduced the mouse granuloma hyperplasia and DHT.

The anti-inflammatory activity of *Carlina acanthifolia* (acanthus-leaved thistle, Asteraceae) root EO was evaluated by Dordevic et al. (2007). In traditional medicine the root of the plant is used for the treatment of a variety of diseases concerning stomach and skin. The anti-inflammatory activity was tested in the carrageenan-induced rat paw edema assay and the oil inhibited the edema in all applied concentrations. The effect could be compared to indomethacin, which was used as control.

Another study was made on the anti-inflammatory effect of the EO and the active compounds of the Boraginaceae *Cordia verbenacea* (black sage) by Passos et al. (2007). It was found that the carrageenan-induced rat paw edema, the myeloperoxidase activity, and the mouse edema elicited by carrageenan, bradykinin, substance P, histamine, and the platelet-activating factor could be inhibited after systemic (p.o.) administration of 300–600 mg/kg EO. It also suppressed carrageenan-evoked exudation, the neutrophil influx to the rat pleura and the neutrophil migration into carrageenan-stimulated mouse air pouches. Additionally, a reduction of edema caused by *Apis mellifera* venomous OVA in sensitized rats and OVA-evoked allergic pleurisy could be observed. TNF-α was significantly inhibited in carrageenan-treated rat paws by the EO, whereas the IL-1β expression was not influenced. No affection was caused of neither the PGE2 formation after intrapleural injection of carrageenan, nor of the COX-1 or COX-2 activities *in vitro*. Both sesquiterpenes, α-humulene and *trans*-caryophyllene (50 mg/kg p.o.) obtained from the EO, lead to a remarkable reduction of the carrageenan-induced mice paw edema. All in all, this study demonstrated the anti-inflammatory effect of the EO of *Cordia verbenacea* and its active compounds. The possible mechanism of this effect might be caused by the interaction with the TNF-α production. The authors suggest that *Cordia verbenacea* EO could represent new therapeutic options for the treatment of inflammatory diseases.

The topical anti-inflammatory effect of the leaf EO of the Verbenaceae *Lippia sidoides* Cham. was studied by Monteiro et al. (2007). In northwestern Brazil, the plant is widely used in the social medicine program "Live Pharmacies" as a general antiseptic because of its strong activity against many microorganisms. After topical application of 1 and 10 mg/ear, in 45.9% and 35.3%, a significant reduction ($p < 0.05$) of the acute ear edema induced by 12-tetradecanoylphorbol 13-acetate (TPA) could be observed.

The anti-inflammatory effects of the EO from *Eremanthus erythropappus* leaves (Asteraceae) have already been discussed in the chapter dealing with antinociception (Sousa et al., 2008). An interesting study concerning the healing of *Helicobacter pylori*-associated gastritis by the

volatile oil of "*Amomum*" (several *Amomum* species, which are used in similar manner to cardamon (*Elettaria cardamomum*), Zingiberaceae) was published recently by a Chinese author group. The effects of this EO on the expressions of mastocarcinoma-related peptide and platelet-activating factor was assessed and its potential mechanism discussed. The mechanism of this volatile oil for its antigastritis activity could be the influence on the decrease of the expression of the platelet-activating factor and thus regulating the hydrophobicity of the gastric membrane. About 88% of the patients with a proven *Helicobacter pylori* infection were treated and showed a higher healing rate compared to the control group having received a traditional "Western" tertiary medicinal treatment (Huang et al., 2008).

9.1.5 Penetration Enhancement*

A number of EOs are able to improve the penetration of various drugs through living membranes, for example, the skin. The improvement of the penetration can be achieved by the interaction of the EOs with liquid crystals of skin lipids. In the following, the penetration enhancing effect of some EOs will be discussed. For determination of the penetration enhancing effect different experimental setups were used: Valia–Chien horizontal diffusion cells, Keshary–Chien diffusion cells, and Franz diffusion cells. Furthermore, the scientists using polarizing microscopy, differential scanning calorimetry (DSC), x-ray diffraction, and high performance liquid chromatography (HPLC) to detect the penetration through the skin.

The interaction of eucalyptus oil with liquid crystals of skin lipids was proved by Abdullah et al. (1999) using polarizing microscopy, DSC, and x-ray diffraction. Crystal 1 (matrix 1) consisted of five fatty acids of stratum corneum, crystal 2 (matrix 2) consisted of cholesterol together with five fatty acids. Dispersion and swelling of the lamellar structure were observed after application of small amounts of eucalyptus oil, whereas large amounts resulted in their breakage and disappearance. The EO did not promote the formation of any other structures. This interaction seems to be the explanation for the increase of permeation of drugs through stratum corneum in the presence of eucalyptus oil and similar penetration enhancers.

Li et al. (2001) investigated the effects of eucalyptus oil on percutaneous penetration and absorption of a clobetasol propionate cream using vertical diffusion cells. The *in vitro* penetration of the cream containing 0.05% clobetasol propionate through mouse abdominal skin was detected at 2, 4, 6, 8, 10, and 24 h (cumulative amount Q, μg/g) and at steady state (J, μg/cm²/h). The quantity of clobetasol propionate within the whole stria of skin after 24 h (D, μg/g) was measured too. Eucalyptus oil was able to increase Q and J, whereas D was not influenced in that way, which indicates that eucalyptus oil would increase clobetasol propionate percutaneous absorption and cause unwanted side effects.

Cinnamon oil, eugenia oil, and galangal oil have been studied for their potency as percutaneous penetration enhancers for benzoic acid (Shen et al., 2001). Valia–Chien horizontal diffusion cell and HPLC were used to detect benzoic acid penetration through skin.

Skin penetration of benzoic acid was significantly enhanced by all three volatile oils. In combination with ethanol and propylene glycol the amount of benzoic acid was increased, but the permeability coefficients were decreased. In conclusion, cinnamon oil, eugenia oil, and galangal oil might be used as percutaneous penetration enhancers for benzoic acid.

Monti et al. (2002) tried to examine the effects of six terpene-containing EOs on permeation of estradiol through hairless mouse skin. Therefore, *in vitro* tests with cajeput, cardamom, melissa, myrte, niaouli, and orange oil (all 10% wt/wt concentration in propylene glycol) have been carried out. Niaouli oil was found as the best permeation promoter for estradiol. Tests with its single main components 1,8-cineole, α-pinene, α-terpineol, and *d*-limonene (all 10% wt/wt concentration in propylene glycol) showed that the whole niaouli oil was a better activity promoter than the single compounds. These data demonstrate complex terpene mixtures to be potent transdermal penetration enhancers for moderately lipophilic drugs like estradiol.

* Adorjan, M., 2007. Part of her master thesis, University of Vienna.

Different terpene-containing EOs have been investigated for their enhancing effect in the percutaneous absorption of trazodone hydrochloride through mouse epidermis (Das et al., 2006). Fennel oil, eucalyptus oil, citronella oil, and mentha oil were applied on the skin membrane in the transdermal device, as a pretreatment or both using Keshary–Chien diffusion cells and constantly stirring saline phosphate buffer of pH 7.4 at 37 ± 1°C as receptor phase. Pretreatment of the skin with EOs increased the flux values of trazodone hydrochloride compared with the values obtained when the same EOs were included in the transdermal devices. The percutaneous penetration flux was increased with skin permeation by 10% EOs in the following order: fennel oil > eucalyptus oil > citronella oil > mentha oil. The quantity of trazodone hydrochloride kept in the skin was very similar for all EOs and much higher than in control group.

An *in vitro* study about Australian TTO was made by Reichling et al. (2006). The aim of the study was to investigate the penetration enhancement of terpinen-4-ol, the main compound of TTO, using Franz diffusion cells with heat seperated human epidermis and infinite dosing conditions. The three semisolid preparations with 5% TTO showed the following flux values: semisolid oil-in-water (O/W) emulsion (0.067 μL/cm²/h) > white petrolatum (0.051 μL/cm²/h) > ambiphilic cream (0.022 μL/cm²/h). Because of the lower content of terpinen-4-ol, the flux values were significantly reduced compared to native TTO (0.26 μL/cm²/h). The papp values for native TTO ($1.62 \pm 0.12 \times 10^{-7}$ cm/s) and ambiphilic cream were comparable ($2.74 \pm 0.06 \times 10^{-7}$ cm/s), whereas with white petrolatum ($6.36 \pm 0.21 \times 10^{-7}$ cm/s) and semisolid O/W emulsion ($8.41 \pm 0.15 \times 10^{-7}$ cm/s) higher values indicated a penetration enhancement. Between permeation and liberation there was no relationship observed (Table 9.2). The stratum corneum absorption and retention of linalool and terpinen-4-ol was investigated by Cal and Krzyzaniak (2006). Both monoterpenes were applied to eight human subjects as oily solution or as carbomeric hydrogel. The stratum corneum absorption after application of carbomeric hydrogel was better than that achieved with an oily solution. Two mechanisms of elimination from the stratum corneum were observed: evaporation from the outer layer and drainage of the stratum corneum reservoir via penetration into dermis. The retention of the monoterpenes in the stratum corneum during the elimination phase was a steady state between 6 and 13 μg/cm².

Also linalool alone, one of the most prominent monoterpene alcohols, is used in many dermal preparations as penetration enhancer. In a series of *in vitro* studies it was shown that linalool enhanced its own penetration (Cal and Sznitowska, 2003) as well as the absorption of other therapeutics, such as haloperidol (Vaddi et al., 2002a, 2002b), metoperidol (Komuru et al., 1999), propanolol hydrochloride (Kunta et al., 1997), and transcutol (Ceschel et al., 2000). Cal (2005) showed in another *in vitro* study the influence of linalool on the absorption and elimination kinetics and was able to prove that this monoterpene alcohol furnished the highest absorption ratio compared to an oily solution or an O/W emulsion.

Wang, L.H. et al. (2008) reported on enhancer effects on human skin penetration of aminophylline from cream formulations and investigated for this report four EOs, namely rosemary, ylang, lilac, and peppermint oil compared with three plant oils, the liquid wax jojoba oil, and the fatty oils

TABLE 9.2
Flux Values and Papp Values of the Three Semisolid Preparations of Australian TTO 5%

	Flux Values (μL/cm²/h)	Papp Values (cm/s)
Native TTO	0.26	$1.62 \pm 0.12 \times 10^{-7}$
Ambiphilic cream	0.022	$2.74 \pm 0.06 \times 10^{-7}$
Semisolid O/W emulsion	0.067	$8.41 \pm 0.15 \times 10^{-7}$
White petrolatum	0.051	$6.36 \pm 0.21 \times 10^{-7}$

of corn germs and olives. In this study the EOs were less effective in their penetration enhancement than the three plant oils. The effect of penetration enhancers on permeation kinetics of nitrendipine through two different skin models was evaluated by Mittal et al. (2008) and also this author group found that the used EOs (thyme oil, palmarosa oil, petit grain oil, and basil oil) were inferior in their penetration enhancement effects to oleic acid but superior to a lot of other common permeation enhancers, such as sodium lauryl sulfate, myristic acid, lauric acid, Tween 80, or Span 80. In contrast to these two reports Jain et al. (2008) found that basil oil is a promising penetration enhancer for improved drug delivery of labetolol. The effect of clove oil on the transdermal delivery of ibuprofen in the rabbit *in vitro* and *in vivo* methods was investigated by Shen et al. (2007). The *in vitro* results indicated a significant penetration enhancement effect of the clove oil whereas the *in vivo* results showed a weaker enhancement. The good transdermal delivery of ibuprofen from the essential clove oil could be attributed to the principal constituents eugenol and acetyl eugenol.

9.1.6 Antioxidative Properties*

Free radicals are aggressive, unstable, and highly reactive atoms or compounds because of their single electron. They attack other molecules to reach a steady stage, thereby changing their properties and making disorders inside possible. Free radicals result from products from different metabolic activities. A large number occur because of smog, nitrogen oxides, ozone, cigarette smoke, and toxic heavy metal. Also chemicals such as organic solvents, halogenated hydrocarbons, pesticides, and cytostatic drugs cause a high number of free radicals. If a lot of energy is built or has to be supplied, such as sporty high-performance, extreme endurance sports, sunbathing, solarium, exposure, x-rays, UV radiation, pyrexia, or infections, they are also massively produced (Schehl and Schroth, 2004). Preferred for attack are nucleic acids of the DNA and RNA, proteins, and especially polyunsaturated fatty acids of the membrane lipids. To protect the body's own structure from damages, all aerobe living cells use enzymatic and nonenzymatic mechanisms. Scavangers are able to yield electrons and so they dispose free radicals. Also, enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase are very important in protection mechanisms (Eckert et al., 2006). Oxidative and antioxidative processes should keep the balance. If the balance is in benefit for the oxidative processes, it is called "oxidative stress."

9.1.6.1 Reactive Oxygen Species

Basically, oxygen radicals (Table 9.3) are built by one-electron reduction in the context of autoxidation of cell-mediated compounds. The superoxide radical arises from autoxidation of hydroquinone, flavine, hemoglobin, glutathione, and other mercaptans and also from UV light, x-rays, or gamma rays. Two-electron reduction yields hydrogen peroxide. The very reactive hydroxyl radical is built by three-electron reduction, which is mostly catalyzed by metal ion (Löffler and Petrides, 1998). A lot of other oxygen radicals are accumulated by secondary reactions because they damage the biomolecules, ROS participate in different diseases, mainly cancer, aging, diabetes mellitus, and atherosclerosis.

9.1.6.2 Antioxidants

Antioxidants are substances that are able to protect organisms from oxidative stress. A distinction is drawn between three types of antioxidants: enzymatic antioxidants, nonenzymatic antioxidants, and repair enzymes.

Well-known naturally occurring antioxidants are vitamin C (ascorbic acid), which are contained in many citrus fruits, or on the other hand members of vitamin E family, which appear for example in nuts and sunflower seeds. Also β-carotene and lycopine, which also belong to the family of carotenoides, are further examples of natural antioxidants. On the other hand, there are many synthetic

* Scheurecker, M., 2007. Part of her master thesis, University of Vienna.

TABLE 9.3
Reactive Oxygen Species

Species	Name
$O_2^{-\bullet}$	Superoxide radical
$HO_2^{\bullet}$	Perhydroxyl
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
$RO^{\bullet}$	R-oxyl radical
$ROO^{\bullet}$	R-dioxyl radical

substances such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and the water-soluble vitamin E derivative troxol (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) noted for their antioxidative activity. Different studies suspect the synthetic antioxidants to cause different diseases, so there has been much interest in the antioxidative activity of naturally occurring substances (Salehi et al., 2005).

To the group of enzymatic antioxidants belongs the manganese- or zinc-containing SOD, the selenium-containing glutathione peroxidase, and the iron-containing catalase. Their capacity is addicted to adequate trace elements and minerals (Schehl et al., 2004). Nonenzymatic antioxidants have to be admitted with food or substitution, for example, α-tocopherol, L-ascorbic acid, β-carotene, and secondary ingredients of plants. Repair enzymes delete damaged molecules and substitute them.

9.1.7 Test Methods

9.1.7.1 Free Radical Scavenging Assay

This spectrophotometric assay uses the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Yadegarinia et al., 2006). The model of scavenging stable DPPH-free radicals can be used to evaluate the antioxidative activities in a relatively short time (Conforti et al., 2006). The samples are able to reduce the stable free DPPH radical to 1,1-diphenyl-2-picrylhydrazyl that is yellow colored. The hydrogen or electron donation abilities of the samples are measured by means of the decrease of the absorbance resulting in a color change from purple to yellow (Gutierrez et al., 2006). Another procedure can be applied for an EO. A dilution of the EO in toluene is applied onto a thin-layer chromatography (TLC) plate and toluene-ethyl-acetate is used as a developer (Sökmen et al., 2004a). The plates are sprayed with 0.4 mM DPPH in methanol. The active compounds were detected as yellow spots on a purple background. Only those compounds, which changed the color within 30 min, are taken as a positive result.

9.1.7.2 β-Carotene Bleaching Test

The lipid peroxidation inhibitory activities of EOs are assessed by the β-carotene bleaching tests (Yadegarinia et al., 2006). In this method, the ability to minimize the coupled oxidation of β-carotene and linoleic acid is measured with a photospectrometer. The reaction with radicals shows a change in this orange color. The β-carotene bleaching test shows better results than the DPPH assay because it is more specialized in lipophilic compounds. The test is important in the food industry because the test medium is an emulsion, which is near to the situation in food, therefore allowable alternatives to synthetic antioxidants can be found. An only qualitative assertion uses the TLC procedure. A sample of the EOs is applied onto a TLC plate and is sprayed with β-carotene and linoleic acid. Afterwards, the plate is abandoned to the daylight for 45 min. Zones with constant yellow colors show an antioxidative activity of the component (Guerrini et al., 2006).

9.1.7.3 Deoxyribose Assay

This assay is used for determining the scavenging activity on the hydroxyl radical. The pure EO is applied in different concentrations (Dordević et al., 2006). The competition between deoxyribose and the sample about hydroxyl radicals that are engendered by an Fe^{3+}/EDTA/H_2O_2 system is measured. The radicals were formed to attack the deoxyribose and they are detected by their ability to degrade 2-deoxy-2-ribose into fragments. These degradation products generate with 2-thiobarbituric acid (TBA) at a low pH and upon heating pink chromogens. The TBA-reactive substances could be determined spectrophotometrically at 532 nm. So the damage of 2-deoxy-2-ribose by the radicals is detected with the aid of the TBA assay.

9.1.7.4 TBA Test

This assay is basically used to appoint the lipid oxidation. It is an older test for receiving the oxidation status of fats spectrophotometrically. Thereby the aldehydes, which are generated by the autoxidation of unsaturated fatty acids, are converted into red or yellow colorimeters with TBA. But it is also used for the determination of the potency of antioxidants with thiobarbituric acid reactive substances (TBARS). Thereby the antioxidant activity is measured by the inhibition of the lipid oxidation. It concerns the spectrophotometric detection of malonic aldehyde, one of the secondary lipid peroxidation products, which generates a pink pigment with TBA (Ruberto et al., 2000; Varda-Ünlü et al., 2003).

9.1.7.5 Xanthine–Xanthine Oxidase Assay

Superoxide radicals are produced by a xanthine–xanthine oxidase system. Xanthine is able to generate $O_2^{\bullet}$ and H_2O_2 by using xanthine oxidase as a substrate. The superoxide radicals are able to reduce the yellow colored nitro triazolium blue (NTB) to the blue formazan, which is used for monitoring the reaction. The superoxide anions are measured spectrophotometrically. This test method was developed to explore the reaction between antioxidants and $O_2^{\bullet}$. So the inhibition of the superoxide reductase is a mark for the ability of antioxidative activity.

9.1.7.6 Linoleic Acid Assay

This test system was developed to determine the ability of substances to inhibit the generation of hydroxy peroxides at the early stages of the oxidation of linoleic acid, as well as for its inhibitory potential after the formation of secondary oxidized products such as aldehydes, ketones, or hydrocarbons (Jirovetz et al., 2006). Either the oxidation of linoleic acid is monitored by measuring the values of conjugated dienes or TBARS spectrophotometrically or hydroperoxy-octadeca-dienoic acid isomers (HOPES) generated during the oxidation are measured (Marongiu et al., 2004).

The biological features of *Mentha piperita* L. (peppermint, Lamiaceae) oil and *Myrtus communis* L. (myrtle, Myrtaceae) oil from Iran were studied by Yadegarinia et al. (2006). One of the main constituents of the EO of *Myrtus communis* is 1,8-cineole (also called eucalyptol), which showed the most powerful DPPH radical scavenging activity. Myrtle oil contains about 18% 1,8-cineole and showed a scavenging activity of 3.5% upon reduction of the DPPH radical to the neutral DPPH-H form. Better results yielded the β-carotene bleaching test. Also the EO of *Mentha aquatica* (water mint) contains up to 14% 1,8-cineole and proved itself in the DPPH assay to be an acceptably radical scavenger and the most active compound in the oil. With *Mentha communis* oil comprising 21.5% limonene, an antioxidative activity was assessed with about 43% in contrast to the reference oil of *Thymus porlock* (Lamiaceae) having an efficiency of 77% (Mimica-Dukic et al., 2003). Also α-terpinene, a constituent of the EO of *Mentha piperita* (about 20%), showed in both test procedures a scavenging activity lower than that of the standard found with the EO of *Thymus porlock*, but similar to the efficiency of the synthetic antioxidant trolox (23.5% versus 28.3%). Another main compound of this oil is β-caryophyllene with about 8%. *Mentha piperita* oil attained a scavenging power of 23.5% and is also able to inhibit the lipid oxidation, which was determined in the β-carotene bleaching test. The results of the standard and the EO were correlated with the results from the

DPHH assay. The peppermint oil shows a lipid peroxidation inhibition of 50% compared with 77% of the standard.

Sesuvium portulacastrum (sea purslane, Ficoidaceae) was collected in the northern, western, and central part of Zimbabwe to determine the chemical activity of the essential leaf oils, which showed besides an antibacterial and antifungal also a significant antioxidative activity (Magwa et al., 2006). Sea purslane is used by the traditional healers in Southern Africa to treat various infections and kidney problems. The secondary metabolites from this plant species have a great potential as substitutes for synthetic raw materials in food, perfumery, cosmetic, and pharmaceutical industries. Thus, the composition and the biological activities of the EO of this Ficoidaceae were studied. One of the major chemical compounds is 1,8-cineole (6.8%). The antioxidative testing was carried out by a modified β-carotene bleaching test. The background of these test methods is that β-carotene is a yellow antioxidant, which becomes colorless when it encounters light or oxygen. With the attendance of another antioxidant, which is not so sensitive to light or oxygen, the yellow color of the β-carotene could continue for some time. Retention of the yellow areas around the test compounds shows an antioxidative activity. In comparison to the positive control (ascorbic acid) with a diameter of 27 mm, the EO showed a 15.9-mm zone of color retention. Mainly responsible to the antioxidative activity besides this bicyclic ether is the content of 2-β-pinene (13.6%), α-pinene (14%), limonene (6.4%), and ocimene, α-terpinolene, and camphene, which also belong to the group of main compounds of the EO of *Sesuvium portulacastrum*.

Thymus (thyme, Lamiaceae) is a very big genus, to which more than 300 evergreen species belong. All of them are well-known aromatic and medical plants and also the oil of different species are used against various diseases. So, many species of this genus were tested for their biological activities inclusive the antioxidative properties of the EOs and their constituents. The EOs of *Thymus caespititius* (Azoricus thyme, Lamiaceae), *Thymus camphoratus* (camphor thyme), and *Thymus mastichina* (mastic thyme) show in different investigations an antioxidative activity, which is comparable to the action of α-tocopherol, a well-known naturally occurring antioxidant (Miguel et al., 2003). One reason for this capacity could be that the EO of all three species contains a high concentration of 1,8-cineole. Because species of the genus *Thymus* (thyme) are widely used as medicinal plants and spices, the antioxidative activity of the EO of *Thymus pectinatus* comprising up to 16% γ-terpinene was determined by multifarious test systems. To detect the hydroxyl scavenging activity a deoxyribose assay was used, a DPPH assay was arranged, and the inhibition of lipid peroxidation formation by a TBA assay was measured. Fifty percent of the free DPPH radicals were scavenged by the EO, which is a stronger antioxidative activity than that of the used standards (BHT, curcumin, and ascorbic acid). Furthermore, the EO showed an inhibitory effect in the deoxyribose assay, and comparable results emanated from the TBA assay, where the oil had an IC_{50} value similar to the activity of BHT. Finally, also the EO of *Thymus zygis* (sauce thyme) which contains among other monoterpenes *p*-cymene showed a dose-dependent antioxidative activity (Dorman and Deans, 2004). Furthermore, the above described species, *Thymus pectinatus*, is characterized by a very high content of the phenolic compound thymol. Therefore, two fractions of this EO, characterized by a high content of thymol (95.5% and 80.7%, respectively), were studied using four different test methods to assess their antioxidative activity. Fifty percent of the free radicals in the test with DPPH were scavenged by 0.36 ± 0.10 μg/mL of the EO. This is a stronger inhibition as the controls afford. Then the deoxyribose assay was used to identify the inhibition of the degradation of hydroxyl radicals by the EO and its main compounds. The investigation showed that thymol has an IC_{50} value of 0.90 ± 0.05 μg/mL and the EO an IC_{50} value of 1.40 ± 0.03 μg/mL. To prove the inhibition of the lipid peroxidation, also the TBA method was used and afforded an IC_{50} value of 9.50 ± 0.02 μg/mL for the EO. Also in this case the EO showed a better protection than the control substances BHT and curcumin, so to say the two major compounds, thymol and carvacrol, show a very strong antioxidative activity. Then the EO of another thyme species with a thymol content of about 31%, namely *Thymus eigii*, was studied by the DPPH and the β-carotene bleaching test (Tepe et al., 2004). For the rapid screening DPPH on TLC was applied. Compendiously after the plate had been sprayed

three spots appeared, which were identified as thymol, carvacrol, and α-terpineol. Thus, an antioxidative activity of this EO was established as well. Also in the volatile extract of *Thymus vulgaris* (common thyme), thymol dominates with 72%. In a test system, where the inhibition of hexane oxidation is used to determine the antioxidative activity, thymol shows one of the strongest activities. In a concentration of 10 μg/mL, the capacity to decrease the oxidation is equal to the activity of BHT and α-tocopherol. These two, well-known, antioxidants inhibit the hexane oxidation up to 89% and 99% in a concentration of 5 μg/mL over 30 days. Also carvacrol, an isomer of thymol and existing in a concentration of about 6% in this oil, is able to hinder the oxidation of hexane by 95–99% at 5 μg/mL over a period of 30 days. This is comparable with the activity of BHT and α-tocopherol (Randonic et al., 2003; Sacchetti et al., 2004; Faleiro et al., 2005). These findings could be confirmed by a very recent study of Chizzola et al. (2008). The antioxidative activity depends on the concentration of the phenolic constituents thymol and/or carvacrol.

Thymol as well as carvacrol protects low-density lipoprotein (LDL) from oxidation (Pearson et al., 1997), and this antioxidative activity is dependent on the concentration: below 1.25 μM no effect was detected, whereas at a concentration between 2.5 and 5.0 μM thymol offers a very strong antioxidative capacity. Finally, the EOs of 15 different wild grown *Thymus* species show the ability to delay lard becoming rancid, because of the inhibition of lipid oxidation. The value of generated peroxides was measured to detect the antioxidative activity. Therefore, variable concentrations of the EO were added to lard and each mixture stored at 60°C. In regular time intervals samples were taken and the peroxide amount was determined. As standards BHT, BHA, and thymol were used. A "thymol- and a thymol/carvacrol group" were able to keep the peroxide value low for a period of 7 days. The EOs of *Thymus serpyllus* (creeping thyme) and *Thymus spathulifolius* comprise high contents of carvacrol (58% and 30% respectively) and exhibit both an antioxidative activity near to BHT. Another species is *Thymus capitata* (Corido thyme), with an amount of 79% carvacrol, and shows an antioxidative activity assessed by the TBA assay in variable concentrations. There is no difference at the concentration of 1000 mg/L between the capacity of the oil and the control BHT, but the antioxidative properties are better than BHA and α-tocopherol. In a micellar model system, where the decrease of just generated conjugated dienes is used to determine the antioxidative ability, *Thymus capitata* oil showed an antioxidative index of 96% and proved a good protective activity in the primary lipid oxidation. The oil is better effective against lipid oxidation and in higher concentrations better than BHA and α-tocopherol. The addition of a radical inducer reduced the antioxidative activity of the EO. Carvacrol is capable to prevent up to 96% the primary step of lipid oxidation. Also *Thymus pectinatus* EO contains carvacrol as one of the main compound and shows a strong antioxidative activity. Fifty percent inhibition of lipid peroxidation (detected by the TBA assay) was attained at a concentration of 5.2 μg/mL carvacrol (Vardar-Ünlü et al., 2003; Miguel et al., 2003; Hazzit et al., 2006).

The EO of *Ziziphora clinopodioides* ssp. *rigida* (blue mint bush) was isolated by hydrodistillation of the dried aerial parts, which was collected during the anthesis. The main compounds are thymol and 1,8-cineole with a content of 8% and 2.7%, respectively. Different extracts were tested by the DPPH assay to determine the antioxidative activity and showed that the free radical scavenging activity of the menthol extract was superior to all other extracts. Polar extracts exhibited stronger antioxidant activity than nonpolar extracts (Salehi et al., 2005).

Many species of the genus *Artemisia* (wormwood, Asteraceae) are used as spices, for alcoholic drinks and also in the folk and traditional medicine. The chemical compounds and the antioxidative activity of the EOs isolated from the aerial parts of *Artemisia absinthium* (vermouth), *Artemisia santonicum* (sea wormwood), and *Artemisia spicigera* (sluggish wormwood) were investigated (Kordali et al., 2005). The analysis of the EO of *Artemisia santonicum* and *Artemisia spicigera* showed two main components, namely 1,8-cineole and camphor. In addition, it is noticed that the EO of these two species contain no thujone derivates in contrast to *Artemisia absinthium*. Earlier studies have also shown that 1,8-cineole and camphor are main components of the EO of some *Artemisia* species. The antioxidative activity of the EO of *Artemisia santonicum* and

Artemisia spicigera was analyzed by the thiocyanate method and a high antioxidative activity was found. With the thiocyanate test and the DPPH radical scavenging assay, the high antioxidative activity of *Artemisia santonicum* EO is ensured. On account of their adequate antioxidative activity, *Artemisia santonicum* and *Artemisia spicigera* could possibly be used in the liqueur-making industry, because they do not include thujone derivates. The antioxidative activity of the EO from another wormwood species, namely *Artemisia molinieri* (mugwort), was investigated by chemoluminescence. The main compounds of this EO are α-terpinene with 36.4% and then 1,4-cineole (<17%), germacrene D (up to 15%), *p*-cymene, and ascaridol. In the used test method the EO or α-tocopherol, the reference compound, are added to a solution of AAPH (=2,2-azobis (2-amidinopropane)-dihydrochloride) and luminol. Chemoluminescence intensities of both blank and assay were monitored and the percentage of inhibition was calculated. The EO showed a high antioxidative activity that is similar to the activity of α-tocopherol (Masotti et al., 2003). Also *Artemisia abyssinica* and *Artemisia afra* (African wormwood)—due to the content of α-terpineol—showed an inhibition in the deoxyribose assay (Burits et al., 2001). A Canadian author team investigated seven wild collected *Artemisia* species from Western Canada, determined the composition of the EOs from their aerial parts, and found—interestingly—that these oils exerted only weak antioxidant activities in the β-carotene/linoleate model and DPPH test (Lopes-Lutz et al., 2008).

The GC-MS analysis of the EO of the herbal parts of *Achillea biebersteinii* Afan (yarrow, Asteraceae), which were collected during the anthesis, furnished a high number of oxygenated monoterpenes, such as piperitone (34.9%), 1,8-cineole (13.0%), and camphor (8.8%) as the main compounds. The antioxidative activity has been assessed by four different methods: free radical scavenging assay with DPPH, hydroxyl radical scavenging with the deoxyribose assay, inhibition of the lipid peroxidation with the TBA test, and inhibition of the superoxide radicals xanthine–xanthine assay. Curcumin, ascorbic acid, and BHT were taken as positive control. Difficulties arising from the water-insoluble parts of the extracts render the spectroscopic measuring negatively. On this account, only the water-soluble parts of the extract could be determined for antioxidative action. The results of the tests showed that the EO possesses a better capacity in the donation of the hydrogen atoms or electrons than curcumin and BHT, but there was no difference in the action to ascorbic acid (Sökmen et al., 2004). Also hydroxyl radical scavenging and the lipid peroxidation inhibition were more effective than curcumin. Due to these results the EO of *Achillea biebersteinii* could be a valuable raw material for natural antioxidant additives. Also the EO of *Achillea millefolium* ssp. *millefolium* (common yarrow) in which 1,8-cineole and α-terpineol are major constituents shows a strong antioxidative activity. This was assessed by the deoxyribose assay, the xanthine–xanthine assay, and the DPPH test (Candan et al., 2003). Also the inhibition of the lipid peroxide generation was measured. The results of these tests were compared with the antioxidative activity of three well-known and often used antioxidants, namely BHT, curcumin, and ascorbic acid. On account of the scavenging activity for the stable free radical DPPH with an IC_{50} value of 1.56 μg/ml, this oil showed a better antioxidative capacity than the just aforementioned well-known antioxidants. Other *Achillea* species wild samples of *Achillea ligustica* (Ligurian yarrow), collected in different parts of Sardinia, are also characterized by the content of 1,8-cineole as one of the main compounds of the EO with an average value of about 2.9%. In the DPPH assay the EO shows an interesting antioxidative activity that can be used for applying such oils as nutraceutical products or as additives in food industry (Tuberoso et al., 2005).

Helichrysum (strawflower, Asteraceae) species are traditionally used for the treatment of wounds, infections, and respiratory conditions (Lourens et al., 2004). In the inflammatory process free radicals adsorb on the phagocyte cells, so the antioxidative activity of the extract and the EOs of different *Helichrysum* species were examined. Therefore, the plant material of *Helichrysum dasyanthum*, *Helichrysum excisum*, and *Helichrysum petiolare* were collected and the EO obtained by hydrodistillation. The GC-MS analysis showed that 1,8-cineole (20–34%), *p*-cymene (6–10%), and α-pinene (3–17%) are principal components of the EO. The EOs of all three different species show an antioxidative activity in the DPPH assay, but it has to be noticed that the extract, which was obtained by a cold

extraction, had a better activity in the assay than the EOs. These test scores are an important argument for the use of some plants of this genus in the traditional medicine (Grassmann et al., 2000).

Also the EO of the fresh fruits by *Xylopia aethiopica* (Meleguetta pepper, Negro pepper, and African pepper tree, Anonaceae) is characterized by the occurrence of 1,8-cineole and β-pinene. But also EOs from the leaves (containing about 17% β-pinene and even 24.5% germacrene D), barks, stems, and roots are known and examined for their composition and antioxidative activities. The antioxidative activity of the EO from different parts of the plant was determined by the free radical scavenging assay with DPPH and the xanthine–xanthine assay. All tested samples were found to interact with the stable free radical DPPH in a time-dependent manner (Karioti et al., 2004). The highest radical scavenging in the DPPH assay was measured with the EO of the fresh fruits (comprising about 9% germacrene D) with an interaction of 85.6%. Generally, all the EOs of the different parts possess the ability to scavenge free radicals. Also the xanthine–xanthine assay showed that the capability to reduce the superoxide radical is given.

Plants of the genus *Melaleuca* (Tea tree, Myrtaceae) are rich in volatile oils. On this account, different species were investigated for their biological activity as well as for their antioxidative capacity. A GC-MS analysis revealed that 1,8-cineole is the major compound of the EO extracted from *Melaleuca armillaris* (33.9%). For the study 50 male albino rats were treated differently. Besides the control group the rats received multiple doses of the EO 3 times a week for 1 month. To evaluate the antioxidative activity the following estimations were carried out: SOD, vitamin C, catalase, glutathione, and lipid peroxides (Farag et al., 2004). An alternation of the antioxidant status induced by CCl_4, as free radical inducer, before and after the administration of the EO was studied as well. The EO of *Melaleuca armillaris* increased the value of vitamin E and vitamin C. The same effect was given by the level of SOD and lipid peroxide compared to the control group. Only the level of catalase was decreased by the treatment of *Melaleuca* oil. TTO from *Melaleuca alternifolia* (Australian tea tree) is known for its wide spectrum of biological activities, therefore the antioxidative properties of TTO was assessed by various methods, such as the DPPH radical scavenging or the hexanal/hexanoic acid assay (Hyun-Jin et al., 2004). The crude EOs, as well as the most active fractions (fractions 5 and 6 after silica gel open column chromatography and C_{18}-HPLC), were used for the investigation. The three major compounds in these fractions were γ-terpinene (20.6%), α-terpinene (9.6%), and α-terpineol. A correlation between the scavenging activity and the concentration was emerged in the DPPH assay. At a concentration of 10 mM, the activity of α-terpinene with more than 80% was near to the scavenging capacity of BHT. Eighty-two percent activity was determined for a concentration of 180 mM which is also close to the synthetic antioxidant (85%). It was shown that α-terpinene had the strongest scavenging effect in this test system compared to the other two compounds. The second test system, which was performed, was the hexanal/hexanoic acid assay. In lower concentrations (30 and 90 mM) α-terpinene exhibited a strong inhibitory activity at first but it decreased extremely fast. The inhibitory effect increased with the arising concentration. α-Terpinene (180 mM) as well as γ-terpinene had a blocking action of 65% over the 30 days. It should be noticed that the antioxidative activity of BHT was stronger compared to any isolated compound of the TTO at lower concentrations.

Vitamin E is a natural antioxidant, which occurs in the plasma red cells and tissues, disarms the free radicals, and anticipates the peroxidation of polyunsaturated fatty acids and phospholipids. Also vitamin C is one of the naturally occurring antioxidants, which actually increase the efficiency of vitamin E to avoid the lipid peroxidation. SOD protects the cells of hydrogen peroxide anion free radicals, furnishes the decrease of the catalase which decomposes H_2O_2, thus yielding a reduction of the oxidative process. This research shows that the EO of *Melaleuca armillaris*, which has 1,8-cineole as the major chemical compound, can be used as a suppressor for free radicals and is able to avoid damages caused by oxidative stress generated by chemical or physical factors.

Myrtol standardized and eucalyptus oil, which both contain about 70–80% 1,8-cineole, were examined in a Fenton system which is a very sensitive indicator for ROS such as α-keto-γ-methiolbutyric acid (KMB) or 1-aminocylopropane-1-carboxylic acid (ACC). OH radicals are generated by

the reaction between hydrogen peroxide and Fe^{2+} (Grassmann et al., 2000). The KMB is transformed into ethene, carbon dioxide, formiate, and dimethyldisulfide. The ethene can be detected in very low quantities by GC. Both oils prevent KMB before destruction and so no ethene can be detected.

The EOs of citrus fruits (Rutaceae)—obtained upon pressing the peels—are also called "agrumen oils" and are characterized normally by a high content of the monoterpene hydrocarbon *d*-limonene which even after boiling the fruits remains in the peel in a substantial quantity. So, citrus fruits are a very interesting source for the occurrence of antioxidants. Twenty-six citrus fruits and their flavor compounds were investigated for an antioxidative activity by the thiocyanate test. The EO of *Citrus sinesis* Osbeck var. Sanguinea Tanaka form a *Tarocco* (Tarocco orange) which contains as major component limonene (84.5%) shows a very high antioxidative activity of about more than 90%. Also the oil of *Citrus aurantium* Linn. var. *cyathifera* Y. Tanaka (Dadai) exerts an antioxidative activity up to 19% but it was slightly weaker than that of the standard troxol, a water-soluble vitamin E derivative. Also all the citrus EOs show an antioxidative activity against linoleic acid peroxidation (Song et al., 2001). The scavenging activity of the authentic compounds was evaluated in the DPPH test and the activity from limonene was assessed from 8.8% to 16.5%, whereas γ-terpinene showed that in this test a noticeable radical scavenging effect for γ-terpinene was 84.7%, which is 3.5 times stronger as that of trolox. The results of the thiocyanate test reveal that the EOs and their flavor components can be used in the food industry to protect aliments of oxidation and to avoid lipid peroxidation. Correlating results are afforded by another investigation of 24 different citrus species and their authentic compounds. γ-Terpinene showed also in the linoleic acid assay a very strong antioxidative activity, which manifests itself in a better peroxide value than trolox. The EOs obtained from *Citrus yuko* Hort. ex Tanaka (Yuko) and *Citrus limon* Brum. f. cv. *Eureka* (Lisbon lemon) contain an appreciable content of γ-terpinene with 18.6% and 8.8%, respectively. Both citrus species offered a strong antioxidative activity of more than 90% due to the high amount of γ-terpinene in the oils (Choi et al., 2000). Ao et al. (2008) studied whether EOs from Rutaceae can effectively scavenge singlet oxygen upon irradiation, using electron spin resonance and found that the investigated 12 oils (eight of them obtained by conventional expression, four by steam distillation) enhanced singlet oxygen production, whereby the content of limonene is made responsible for this result. However, two expressed oils and three oils obtained by steam distillation showed singlet oxygen scavening activity.

The EO of *Cuminum cyminum* (cumin, Apiaceae) consists of 21.5% limonene which is able to abate the concentration of the DPPH free radical, although its efficiency is a bit lower than that of trolox. Since the EO is also able to decrease the lipid peroxidation, a β-carotene bleaching test was arranged. This assay furnished better results than the DPPH free radical scavenging test. The possibility for that could be the higher specificity of this test method for lipophilic compounds. α-Pinene is another major constituent of this oil and contributes as well to the antioxidative activity. The implication of these investigations is the fact that the EO of cumin is competent enough to neutralize free radicals and to protect unsaturated fatty acids of oxidation (Gachkar et al., 2007).

Limonene is also one of the major compounds of the EO of *Ocotea bofo* Kunth (Lauraceae, "anis de arbol" in Ecuador, "moena rosa" in northern Peru or "pau de quiabo" in Brazil), (~5.0%), which was tested for its antioxidative activity by three different methods. The DPPH assay furnished a scavenging activity that was higher than that of the synthetic reference trolox, but lower than the activity of the natural and commercial EO reference *Thymus vulgaris*, whereas the β-carotene bleaching test showed that the EO is comparable to standards in the inhibition of oxidation. Another method was carried out to assess the antioxidative activity using a specific test kit of photochemistry. A light emission curve was recorded over 130 s, using inhibition as the parameter to evaluate the antioxidant potential (Guerrini et al., 2006). The activity was calculated by the integral under the curve and showed that *Ocotea bofo* EO here has a comparable scavenging activity to trolox. Besides, also the aromatic monoterpene *p*-cymene (about 5% in the EO) contributes to the antioxidative activity (Table 9.4).

TABLE 9.4
Antioxidative Activity of *Ocotea bofo* EO Performed by DPPH and β-Carotene Bleaching Assays

	Inhibition %		
Sample	**DPPH**	**β-Carotene Bleaching Test**	**Photochemiluminescence (mmol of trolox/L)**
Ocotea bofo EO	64.23 ± 0.03	75.82 ± 0.04	3.14 ± 0.02
Thymus vulgaris EO	75.64 ± 0.04	90.94 ± 0.05	0.34 ± 0.06
BHA	84.35 ± 0.04	86.74 ± 0.04	
A-tocopherol			4.28 ± 0.5
Trolox	94.44 ± 0.05	84.60 ± 0.04	3.94 ± 0.06

Salvia (belonging to the family of Lamiaceae) EO is used in folk medicine all over the world and many studies were carried out to assess its constitution and biological activity. Three different species were collected in the southern part of Africa: *Salvia stenophylla* (blue mountain sage), *Salvia repens* (creeping sage), and *Salvia runcinata* (African sage) (Kamatou et al., 2005). Limonene is one of the major compounds of the EO from *Salvia repens* with 9.8%. Interestingly, in the other two species this monoterpene was absent. The antioxidant behavior of the EO and the phenolic composition of *Rosmarinus officinalis* (Lamiaceae) and *Salvia fruticosa* M., both collected in an island of the Ionian Sea (Greece) were investigated. The principal component of the EO was 1,8-cineole and flavonoids that of the methanolic extracts. The phenolic content correlates with the antioxidant activity (Papageorgiou et al., 2008).

To determine the radical scavenging capacity, a modified DPPH test was used where the test tubes were analyzed with HPLC. Different concentrations were plated out in a 96-well plate with control wells containing dimethyl sulfoxide (DMSO). The decolorization was investigated by measuring the absorbance at 560 nm. Vitamin C provided as a positive control. The LC_{50} value of *Salvia repens* EO (comprising about 22% β-caryophyllene) rests with more than 100.0 μg/mL or *Salvia multicaulis* which showed an IC_{50} value of 17.8 μg/mL, two examples for a low scavenging activity (Erdemoglu et al., 2006). But the reason for this result could be that in this method a stable free radical is used while in other investigations unstable radicals were applied and there a better antioxidative activity was adopted.

The EO of *Crithmum maritimum* (=*Cachrys maritima*, Apiaceae, rock samphire) comprises limonene and γ-terpinene with an amount of 22.3% and 22.9%, respectively, as the major components. Two different test methods (TBA assay and a micellar model system where the antioxidative activity in different stages of the oxidative process of the lipid matrix was monitored) were used. Both assays explain the very high activity of this EO. In the TBA assay BHT and α-tocopherol were used as positive standards and the oil showed a better capacity than those substances. Comparable results were obtained by the micellar method system where the EO acts as a protector of the oxidation of linoleic acid and inhibits the formation of conjugated dienes (Ruberto et al., 2000). The modification of LDL by an oxidative process for instance can lead to atherosclerosis. Natural antioxidants such as β-carotene, ascorbic acid, α-tocopherol, EOs, and so on are able to protect LDL against this oxidative modification. γ-Terpinene proved itself to be the strongest inhibitor of all used authentic compounds for the formation of TBARS in the Cu^{2+}-induced lipid oxidation system (Grassmann et al., 2003). So, the addition of γ-terpinene to food can possibly stop the oxidative modification of LDL and reduce the atherosclerosis risk.

α-Pinene and α-terpineol are two of the main compounds of the EO from *Juniperus procera* (African juniper, Cupressaceae). The oil was studied for its antioxidative activity, because the aerial parts of this plant are used in traditional medicine against different diseases and ailments, for example,

ulcers, headaches, stomach disorders, intestinal worms, rheumatic pains, liver diseases, as an emmenagogue, and to heal wounds. To determine the antioxidative activity *in vitro* test methods were used: DPPH assay, deoxyribose assay, and the assay for nonenzymatic lipid peroxidation. The EO showed an IC_{50} value of 14.9 µL/mL in the DPPH assay, a 50% inhibition in the deoxyribose assay at 0.4 µL/mL, and an IC_{50} value of 0.20 µL/mL by inhibition of the lipid peroxidation. On the other hand, pure α-pinene showed an IC_{50} value of 0.78 µL/mL and 50% of the lipid peroxidation were inhibited by 0.51 µL/mL. To determine the inhibition of nonenzymatic lipid peroxidation bovine liposomes, $FeCl_3$ and ascorbic acid were used. The generated aldehydes form pink compounds with TBA and were detected by measuring the absorbance at 532 nm.

Eleven different EOs were investigated for their antioxidative activities. The oils extracted from *Eucalyptus globulus* (*Eucalyptus*, Myrtaceae), *Pinus radiata* (Monterey Pine, Pinaceae), *Piper crassinervium* (Piperaceae), and *Psidium guajava* (Guayava, Myrtaceae) contain 20%, 21.9%, 10%, and 29.5% α-pinene, respectively. Three different test methods were used to evaluate the antioxidative activities of these EOs: DPPH assay, β-carotene bleaching test, and photoluminescence. In the DPPH assay the EO from *Piper crassinervium* expressed an activity of 43.0 ± 0.30%, which was lower than that of the reference *Thymus vulgaris* EO, but comparable with the activity of trolox, whereas the other oils were almost ineffective. In the β-carotene bleaching test similar data were obtained. The EOs of *Eucalyptus globulus* and *Piper crassinervium* showed results between 66% and 49% inhibition. The photoluminescence test method is very rapid on the photo-induced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide (Sacchetti et al., 2005). With this method only the EO of *Piper crassinervium* showed an antioxidative activity, whereas the other oils were almost ineffective.

One of the main compounds of the eucalyptus oil besides 1,8-cineole are the monoterpene hydrocarbons α-pinene (10–12%), *p*-cymene, and α-terpinene, and the monoterpene alcohol linalool. This oil is used to treat diseases of the respiratory tract in which ROS play an important role, so the antioxidative activity of eucalyptus oil was of interest. The results obtained by assessing this activity were compared with those of myrtle standardized oil of *Myrtus communis* (Myrtaceae), which is also used to combat infections of the respiratory tract. The antioxidative activity was determined by the Fenton test where the $OH^{\bullet}$-radicals are built from H_2O_2, whereas Fe^{2+} acts as the electron donator. The ROS reacts with the sensitive indicators KMB and ACC, which release a measurable signal (Grassmann et al., 2000). After an incubation time of 30 min at 37ºC, the generated ethene can be quantified by GC, because KMB dissociates into ethene, carbon dioxide, formiate, and dimethylsulfide. Both, the eucalyptus oil and the myrtle standardized oil were able to inhibit the generation of ethene in dependence on the concentration of the oils. In an *in vitro* system the EO of *Eucalyptus globulus* was investigated for its property to inhibit the autoxidation of linoleic acid and compared with the activity of BHT and showed very good protection for linoleic acid with an IC_{50} value of 7 µg. In another test the inhibition of the nonenzymatic lipid peroxidation was investigated. A solution of bovine brain extract and various concentrations of linalool were submitted to a TBA assay: Linalool caused a 50% inhibition at a concentration of 0.67 µL/mL.

The EO, obtained from the leaves of *Origanum syriacum* L. (Syrian oregano, Lamiaceae), is a very popular Arab spice which is used as a herbal (traditional) medicament, flavor, fragrance, and for aromatherapy in form of bath, massage, steam inhalation, and vaporization (Alma et al., 2003), was investigated with regard to the antioxidative activity of its chemical compounds, for example, γ-terpinene, *p*-cymene, and β-caryophyllene (about 28%, 16%, and 13% respectively) using the thiocyanate method, DPPH radical scavenging activity, and the reducing power. The latter property was determined in a concentration from 100 to 500 mg/L and compared with the reducing power of ascorbic acid. The EO was able to increase the absorbance but the reducing power is lower than that of the used standard. It should be noticed that the power of the EO increased with a higher concentration. Also in the DPPH assay the standard BHT showed a better radical scavenging than the EO. In addition, 500 mg/L EO scavenged only 17% DPPH. In comparison to that, BHT showed an activity of 82% in a concentration of 100 mg/L, again dependent on the concentration. The thiocyanate

test was carried out with concentrations of 20, 40, and 60 mg/L of the EO. Also here the activity increased with a higher concentration. The antioxidative activity was comparable to that of BHT. Even at low concentrations (20 mg/L) a high antioxidative activity was found. At least also the reducing power of the EO using potassium ferricyanide was assessed by measuring the absorbance at 700 nm; however, the EO attained only a reducing power of 0.77, compared with that of ascorbic acid with a value of 0.96. Because an increasing absorbance means a stronger reducing power, it was shown that in this case only a low reducing capacity could be recorded, as in the method with DPPH. The EO of *Origanum floribundum* comprises only 8.4% thymol, but about 30% of carvacrol. This oil was able to decrease the generation of TBARS in the TBA assay just as well as BHT, BHA, and α-tocopherol, in the absence and presence of a radical inducer. The DPPH radical activity of oregano EO in a menthol extract exists, because it is able to reduce the stable free radical DPPH with values of IC_{50} ranging from 378 to 826 mg/L, however inferior to the capacity of BHA (Hazzit et al., 2006). Similar results also furnished the carvacrol-rich oil from *Origanum glandulosum* (Algerian oregano) and from *Origanum acutidens* (hops oregano). This EO was tested with the radical scavenging method using DPPH and the β-carotene/linoleic assay. In both test systems the oil exhibits an antioxidative activity. As shown in other investigations, the activity in the β-carotene/linoleic assay was higher than that in the DPPH assay and is comparable with the ability of BHT, which was used as a positive control.

Also the species oregano (*Origanum vulgare*) is characterized by a high content of thymol (33%) and carvacrol, as was shown by a GC-MS analysis of (oregano) EO. The property to inhibit the generation of malonic aldehyde in the first stage of lipid oxidation is determined by TBA test method. And the micellar model system is used to measure the decrease of conjugated dienes formed by linoleic acid spectrophotometrically at 234 nm. In a concentration of 640–800 mg/L the EO shows a protective activity, which is superior to the standard substances BHA and α-tocopherol but equal to BHT. When the amount of the EO is increased to 1000 mg/L, the antioxidative capacity is better than that of BHT (Faleiro et al., 2005). The meat of poultry is very sensitive for oxidative deterioration because the meat is rich in polyunsaturated fatty acids. Turkeys are more sensitive than chicken because they cannot store α-tocopherol in their tissues to the same extent. A study was carried out if a diet with the EO of *Origanum vulgare* ssp. *hirtum* (oregano) is able to degrade the susceptibility to lipid oxidation. Thirty 10-week-old female turkeys were divided into five groups. In the control group the animals were fed with a standard diet. The other turkeys were also fed with the same bush but containing different concentrations of oregano EO and oregano herbs: 5 g oregano herb/kg, 10 g oregano herb/kg, 100 mg oregano EO, and 200 mg EO. After 4 weeks all the turkeys were slaughtered and worked up. The breasts were tight, minced, and stored at 4°C over 9 days. At days 0, 3, 6, and 9 the concentration of malonic aldehyde was measured spectrophotometrically using the TBA assay. At each stage the control group showed the highest content of malonic aldehyde. On the third day the amount of malonic aldehyde increased in every group. The group with 100 mg EO addition showed a lower concentration than the control group but a higher one as the group with 200 mg EO. On the sixth day the content of malonic aldehyde also increased and similar to the previous sample the group with 200 mg EO had the lowest content. Thus, it is shown that a diet with oregano EO can delay the deterioration induced by lipid oxidation (Botsoglou et al., 2003; Florou-Paneri et al., 2005). Responsible for this result is carvacrol, which is one of the main compounds of the oil and exerts also in other studies a strong antioxidative activity. In conclusion, these results prove that the EO of *Origanum vulgare* possesses protective properties in the first and second step of lipid peroxidation and can be used to replace synthetic antioxidants in the food industry or other areas. Another species, namely *Origanum majorana* L. from Albania, showed in the DPPH test a better antiradical activity than the phenolic compound thymol. This EO exhibited a scavenging effect on the hydroxyl radical OH as well and finally was also capable of antioxidant activity in a linoleic acid emulsion system where at a concentration of 0.05% it inhibited conjugated dienes formation by 50% and the generation of linoleic acid secondary oxidized products by about 80% (Schmidt et al., 2008).

One of the main compounds with about 23% found in the volatile oil from *Trachyspermum ammi* (ajwain, ajowan, omum, Apiaceae), which is a very popular aromatic plant in India and used for flavoring food as well as in the Ayurvedic medicine, is γ-terpinene. Another main compound of the ajowan oil is *p*-cymene (about 31%), which contributes to the antispamodic and carminative properties as well. To determine the antioxidative activity of the EO a wide range of test methods were used. To simulate the different stages of lipid peroxidation three different test methods were exerted: the determination of peroxide values, a TBA assay, and the linoleic acid assay. The antioxidative activity of the oil and of an acetone extract was compared to that of BHT, BHA, and of a control: a sample with crude linseed oil. It could be shown that the oil was a better inhibitor than the synthetic antioxidants (Singh et al., 2004). Due to the fact that all results of the different test methods correlate, it can be concluded that at a concentration of 200 ppm the inhibitor activity can be put into the following order: acetone extract > EO > BHA > BHT > control.

Terpinolene was identified as one of the main compounds (about 6%) in the EO from *Curcuma longa* (turmeric, Zingiberaceae). In the course of an investigation of 11 different EOs also turmeric oil was determined for its antioxidative activity. The EO of *Curcuma longa* showed a noteworthy scavenging activity of about 62%, an antioxidative activity twice of that of trolox but only marginally lower than that of reference oil from *Thymus vulgaris*. Also the β-carotene bleaching test furnished comparable results, namely an inhibition activity of 72% versus 91% of *Thymus vulgaris* oil and 87% of BHA. This prevention of oxidation was also shown by the photoluminescence test method that is based on the photo-induced autoxidation inhibition of luminol by antioxidants mediated from the radical anion superoxide (Sacchetti et al., 2004). The EO of *Curcuma longa* showed a noticeable inhibition activity of about 28 mmol trolox/L.

The protective effect of terpinolene with reference to the lipoproteins of human blood and compared to that of the well-known antioxidative substances, such as α-tocopherol and β-carotene, was investigated. The oxidative modification of LDL, which was obtained from the blood of healthy volunteers, can be detected with the use of the increasing absorbance at 234 nm. An elongation of the time until rapid extinction (lag-phase) exhibits an antioxidative activity. The longer the lag-phase lasts, the better is the antioxidative capacity. Similar to other test systems with other EOs, the antioxidative capacity is dependent on the concentration. In that case a higher concentration of terpinolene means that the LDL particles are better loaded with terpinolene molecules. The result of this investigation proved that the protective and thus the antioxidative activity of terpinolene is only a bit weaker than that of the most common antioxidant α-tocopherol.

In the course of the determination of the antioxidative activity of the EOs obtained from 34 different citrus species and the main compounds of the oils, among them terpinolene, were investigated for their radical scavenging activity in a DPPH test system. Terpinolene offers a scavenging power of 87%, which was much higher than the antioxidative activity of the standard trolox. Terpinolene also showed a relative lipid peroxidation rate of 18%, which is an indication of a superior antioxidative activity, because the relative lipid peroxidation rate offered by the well-known and often used antioxidant α-tocopherol was about 30%.

The leaves, flowers, and stems of *Satureja hortensis* (summer savory, Lamiaceae), a common plant widely spread in Turkey, are used as tea or as addition to foods on account of the aroma and the flavor. As a medical plant it is known for its antispasmodic, antidiarrheal, antioxidant, sedative, and antimicrobial properties. Also this EO was investigated for its antioxidative properties. The GC-MS analysis showed that besides 9% *p*-cymene, carvacrol and thymol are the main compounds of about 22 constituents of the oil. They occur at a ratio of approximately 1:1, which is representative for the genus *Satureja*, namely 29% of thymol and 27% of carvacrol. In a linoleic acid test system the EO showed an inhibition activity of 95%, this is an indicator for a strong antioxidative activity because the control BHT attained an inhibition of 96% (Güllüce et al., 2003). Thymol is one of the main components of the EO from *Satureja montana* L., ssp. *montana* (savory) and also one of the glycosidically bound volatile aglycones that were found. The EO with 45% thymol shows a very strong antioxidative capacity that was a bit lower than the standards, BHT, and α-tocopherol. The

activity of the isolated glycosides is similar to that of the EO. Identification of the volatile aglycone shows a value of 2.5% thymol (Randonic et al., 2003). To evaluate the antioxidative properties of ingredients, EO and glycosides, the β-carotene bleaching test was chosen. The DPPH assay yields a weaker result than the β-carotene bleaching test, in which the EO had an efficiency of inhibition about 95%. Compared to the inhibition from BHT of 96%, this result shows a high antioxidative activity of the EO, due to the high content of thymol and carvacrol.

Since *Nigella sativa* (black cumin, devil in the bush, fennel flower, Ranunculaceae) seeds are used for the treatment of inflammations it was reasonable to investigate the ability of the volatile oil to act as a radical scavenger (Burits and Bucar, 2000). Experiments have shown that *Nigella* oil and the main compounds are able to inhibit in liposomes the nonenzymatic lipid peroxidation. Besides carvacrol (6–12%) thymoquinone, *p*-cymene is one of the major constituents of the oil (7–15%). In the free radical DPPH test the EO showed to be a very weak scavenger for radicals (IC_{50} value: 460.0 μg/ml). Totally contrary results were furnished by the TBA assay: the volatile oil of *Nigella* seeds exhibited a very strong inhibition capacity, namely at a concentration of 0.0011 μg/ml already 50% of the lipid peroxidation could be stopped.

As to the next oil a scientific confusion must be mentioned: the common name *black caraway* belongs to the seeds of *Nigella sativa* (Ranunculaceae) and not to the Apiaceae plant *Carum*. Therefore, *Carum nigrum* is a wrong botanical name, even if the seeds of *N. sativa* are black and resemble to the seeds of *Carum carvi*. The confusion that has been created by the authors of the articles (Singh et al., 2006) by using possibly local justified terms, but nevertheless wrong botanical names still persists.

The EO of *Carum nigrum* (black caraway) comprising, for example, thymol (~19%), β-caryophyllene (~8%), and germacrene D (~21%), and its oleoresin were able to scavenge free radicals in the DPPH assay with an effect of 41–71% and 50–80%, respectively. This can be compared with the efficiency of BHT and BHA. However, this activity could only be observed using a high concentration of the EO and oleoresin not in lower ones. A good antioxidative activity was assessed also by the other tests: the linoleic acid assay exhibited that the EO and oleoresin are able to decrease the rate of peroxide during the incubation time. The deoxyribose assay proved that both EO and oleoresin are able to prevent the formation of hydrogen peroxides dependently of the concentration. Also the chelating effect with iron was screened and the absorbance of the mixture determined at 485 nm (Singh et al., 2006). A mixture of this EO, the oleoresin, and crude mustard oil were studied to assess the generated peroxides as well as the TBA value was measured in order to determine secondary products of the oxidation. This mixture showed a clear antioxidative activity, better than the control, and in the linoleic system were also able to keep the amount of the peroxides, formed by the oxidation of linoleic acid, very low in comparison to the standard antioxidants BHA and BHT. Finally, the rather moderate chelating capacity could—nevertheless—be beneficial for the food industry because ferrous ions are the most effective pro-oxidants in food systems (Singh et al., 2006).

The EO of *Monarda citriodora* var. *citriodora* (lemon bee balm, Lamiaceae) contains about 10% *p*-cymene. The oil was determined for its antioxidative activity in two different *in vitro* test systems. In one the oxidation of lipids was induced by Fe^{2+} and in the other assay AAPH was used. Oxygen in the presence of iron(II) generates superoxide anion radicals. In the aqueous phase AAPH undergoes steady-state decomposition into carbon-centered free radicals (Dorman and Deans, 2004). The oil of *Monarda citriodora* was active oil at concentrations of 50 and 100 ppm. In the test system where the radicals were built from AAPH, the oil of *Monarda citriodora* showed a concentration-dependent pattern of antioxidative activity. *Monarda citriodora* showed a better activity at 10 ppm than the EO of *Thymus zygis* and an equal inhibition power like *Origanum vulgare* but the activity was lower than that of the standards BHT and BHA. The EO obtained from the stem with leaves and the flowers of *Monarda didyma* L. (golden balm or honey balm, Lamiaceae) contains *p*-cymene, 10.5% in the stem with leaves and 9.7% in the flowers. The EO was studied by two different test methods to determine its antioxidative activity. The EO showed a good free radical scavenging

activity in the DPPH assay. Also the properties to inhibit the lipid peroxidation in the 5-lipogenase test system furnished a strong inhibition that is similar to BHT.

In some Mediterranean countries *Thymbra spicata* (spiked thyme, Lamiaceae) is applied as spice for different meals and as herbal tea. Two of the main compounds of the EO from this plant are carvacrol (86%) and thymol (4%). The investigation of different natural antioxidants becomes more and more interesting in order to attain more safety in the food industry. The lipid oxidation may be one of the reasons for different changes in the quality of meat and thus the addition of *Thymus spicata* EO has a positive influence on its quality. As material of examination served the Turkish meal sucuk, which is prepared of lamb, lamb tail fat, beef, salt, sugar, clean dry garlic spices, and olive oil. During ripening on the days 2, 4, 6, 8, 10, 13, and 15, a sample was taken and different parameters were determined, among them was also TBARS. They were detected spectrophotometrically by measuring the absorbance at 538 nm. During the first 8 days the TBARS increased from 0.18 to 1.14 mg/kg. The addition of *Thymus spicata* EO reduced the value of TBARS more than BHT. This result shows that the EO from *Thymus spicata* exerts a safety effect on the quality of the meat and thus can be used as a natural antioxidant in the food industry. The EO of *Thymbra capitata* (conehead thyme, Lamiaceae) collected in Portugal contains 68% carvacrol. Both the oil and carvacrol were tested for their antioxidative activity together with sunflower oil via the assessment of liberated iodine and its titration with sodium thiosulfate solution in the presence of starch as an indicator (Miguel et al., 2003). The antioxidative capacity was determined by the peroxide value of the samples, which were taken continuously during a period when the test solution was stored at 60°C. The EO and carvacrol exhibit approximately the same antioxidative activity during a period of 36 days.

It is known that free radicals induce deterioration of food because they start the chain reaction of the oxidation of polyunsaturated fatty acids. *Zataria multiflora* (Zataria, Lamiaceae) is used for flavoring yoghurt and as a medical plant, and therefore aroused the interest to study also the biological activities of its EO. Using the ammonium thiocyanate method this oil exhibits a strong antioxidative activity, as could be shown in an experiment. To determine the inhibition of oxidation, ammonium thiocyanate and ferrous chloride were added and the absorbance was measured spectrophotometrically at 500 nm. BHT was used for the positive control. Thymol with about 38% the main compound of this EO shows a very strong capacity to avoid the lipid peroxidation up to 80%, which is close to the inhibition of BHT (97.8%). The high content of thymol, carvacrol (38%), and γ-terpinene is the reason for the excellent antioxidative activity. In the DPPH assay the results were less convincing. The EO of *Zataria multiflora* is more liable to prevent lipid peroxidation than scavenging free radicals. The value of conjugated dienes is also decreased by the oil.

Linalool is one of the main compounds (up to 15%) of the EO obtained from *Rosmarinus officinalis* L. (rosemary, Lamiaceae). The EO was tested using two different methods: radical scavenging with the DPPH assay and the β-carotene bleaching test. The oil was able to reduce the stable free radical DPPH and showed a slightly weaker scavenging activity than the standard trolox. But the efficiency of rosemary oil was clearly weaker than that of the reference oil *Thymus porlock*. By the β-carotene bleaching test it could be shown that this EO has the ability to prevent the lipid peroxidation with a capacity close to the used standards.

The genus *Ocimum* contains various species and the EOs are used as an appendage in food, cosmetics, and toiletries. *Ocimum basilicum* (sweet basil, Lamiaceae) is used fresh or dried as a food spice nearly all over the world. The antioxidative activities of different *Ocimum* species were studied in order to assess the potential to substitute synthetic antioxidants. Linalool and eugenol (~12%) are the main compounds in the diverse oils. In the HPLC-based xanthine–xanthine assay, the EO of *Ocimum basilicum* var. *purpurascens* (dark opal basil) contains linalool, eugenol, and β-caryophyllene as main compounds and shows a very strong antioxidative capacity with an IC_{50} value of 1.84 μL (Salles-Trevisan et al., 2006). Linalool as a pure substance yielded the same test results. In the DPPH assay linalool showed a bit weaker activity than in the xanthine–xanthine test

method. *Ocimum micranthum* (least basil) is original in the South and Central American tropics and is used in these territories as a culinary and medical plant. The EO comprises eugenol (up to 51%) as main compound. In the DPPH assay the EO was able to scavenge about 77% of the free DPPH radicals, which is 3 times stronger than that of trolox. The efficiency was also better than those of *Ocimum basilicum* (basil) commercial EO and only slightly weaker than those of *Thymus vulgaris* (thyme) commercial EO. These two EOs were used as a standard because of their known antioxidative activity (Sacchetti et al., 2004). The β-carotene bleaching test proved that the EO inhibited the lipid peroxidation up to 93% after an incubation time of 60 min. The antioxidative activity determined in this test is better than the activity of BHA. In a special test using the photochemoluminescence method the EO of *Ocimum micranthum* attained a 10 times better antioxidative activity as *Ocimum basilicum* and *Thymus vulgaris* commercial EO. These data are of significance because the results of this assay correlate easily with the therapeutic, nutritional, and cosmetic potential of a given antioxidant and the capability to quench $O_2^{\bullet-}$ is useful to describe the related capacity to counteract ROS-induced damages to the body (Sacchetti et al., 2004). Furthermore, the antioxidative activities of the EOs obtained from *Thymus vulgaris* and *Ocimum basilicum* were determined by the aldehyde/carboxylic acid assay. Various amounts of the EOs were added to a dichloromethane solution of hexanal-containing undecane as a GC internal standard and the antioxidant-standard substances BHT and α-tocopherol (Lee et al., 2005). After 5 days the concentration of hexane was determined. All samples showed good antioxidative properties as well as pure eugenol furnishing an inhibition of the hexanal oxidation by 32%.

Linalool (~12%), limonene (~18%), and α-terpineol (~2%) are the main volatile compounds in an infusion prepared of the green leaves from *Illex paraguariensis* (mate, Aquifoliaceae). In order to determine the protective activity against the oxidation of lipids, the infusion was submitted to the ferric thiocyanate test. It could be seen that the infusion of green mate shows similar antioxidative activity as the synthetic antioxidant BHT (Bastos-Markowizc et al.,2006).

The leaves and barks of *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* (cinnamon, Lauraceae) are widely used as spice, flavoring agent in foods, and in various applications in medicine (Schmidt et al., 2006). The leaf oil is very rich in eugenol (up to 75%). To investigate the antioxidative activity five different methods were used: scavenging effect on DPPH, detection of the hydroxyl radicals by deoxyribose assay, evaluation of the antioxidant activity in the linoleic acid model system, determination of conjugated dienes formation, and determination of the TBARS. In the DPPH radical scavenging assay the EO showed an inhibition of 94% at a concentration of 8.0 μg/mL. To reach a radical scavenging activity of 89% a concentration of 20.0 μg/mL was necessary, which is comparable to the efficiency of the standard compounds BHT or BHA. The EO also exhibits a very strong inhibition of the hydroxyl radicals in the deoxyribose assay. The oil prevented 90% at 0.1 μg/mL and eugenol causes a blocking of 71% at the same concentration. Quercetin, which was used in this method as a positive control, showed a weaker efficiency. In a modified deoxyribose assay, in which $FeCl_3$ is added to the sample, both the EO and eugenol showed an antioxidative activity. The EO and eugenol were able to chelate the Fe^{3+} ions and so the degradation of deoxyribose was prevented. To assess the oxidation of linoleic acid the determination of the conjugated diene content and TBARS were used. Cinnamon EO is able to inhibit the generation of conjugated dienes. In a concentration of 0.01% the formation of conjugated dienes is avoided (up to 57%) similar to the efficiency of BHT (~59%). On the day 5 of linoleic acid storage, malonic aldehyde was detected with TBARS. The cinnamon oil showed an inhibitory action of 76% at a concentration of 0.01% oil compared with the 76% of BHT at the same concentration.

Another Lauraceae is *Laurus nobilis* (laurel). The EO obtained from the leaves of wild grown shrubs is characterized by a very high content of eugenol. The biological activities, especially the antioxidative properties, of the extract were studied in different *in vitro* test methods. The scavenging capacity in the DPPH assay yielded an IC_{50} value of 0.113 mg/mL. Also the β-carotene bleaching test of the nonpolar fractions was able to protect the lipids from oxidation. After an incubation

time of 60 min an IC_{50} value of 1 µg/mL was calculated. The last applied method to determine the antioxidative activity used liposomes obtained from bovine brain extract. This test offers a high antioxidative activity with an IC_{50} value of 115.0 µg/mL. The high content of eugenol renders it possible to use *Laurus nobilis* leaf extract as a natural antioxidant. Similar results of the antioxidative power were found using the FRAP assay and the DPPH assay of the EO obtained from *Laurus nobilis* leaves collected in Dalmatia (Conforti et al., 2003). In the first test system the change in absorbance at 593 nm owing to the formation of blue-colored Fe^{III} tripyridyltriazine from a colorless Fe^{III} form by the action of electron-donating antioxidants was measured. The sample was incubated at 37ºC and over a defined period the ferric reducing antioxidant power (FRAP) values were determined. Using the DPPH assay the EO of *Laurus nobilis* exhibited an antioxidative activity of nearly 90% at a concentration of 20 g/L, which is near to the activity of BHT with 91%.

Many aromatic herbs are used as an aroma additive in foodstuffs and in fat-containing food systems to prevent or delay some chemical deteriorations occurring during the storage (Politeo et al., 2006). For the investigation of the antioxidative activities of the EO of *Syzygium aromaticum* (cloves, Myrtaceae), three variable tests methods were employed: TBA assay, radical scavenging with DPPH, and the determination of FRAP. In all three assays it was found that the cloves EO—probably on account of its high content of eugenol (up to 91%)—shows a noticeable antioxidative activity. The determination of the FRAP assay measures the aforementioned change in absorbance at 593 nm owing to the formation of blue-colored Fe^{III} tripyridyltriazine (Politeo et al., 2006). In the radical scavenging test with DPPH an inhibition capacity of 91% at 5.0 µg/mL was found for the EO. Pure eugenol, BHT, and BHA needed a concentration of 20 µg/mL to arrive at the same result. In the deoxyribose assay the EO furnished a hydroxyl radical scavenging of about 94% at 0.2 µg/mL. The inhibitory effect of eugenol was 91% at a concentration of 0.6 µg/ml. Quercetin, the positive control, showed an inhibition of 77.8% at 20.0 µg/mL. The capture of $OH^{\bullet}$ by cloves oil is attributed to the hydrogen-donating ability of the phenolic compound eugenol, which is found in a high concentration in the EO (Jirovetz et al., 2006). In a linoleic acid model system the inhibition of generated hydroxy peroxides in the early stages of the oxidation of linoleic acid and the secondary oxidized products were detected by two different indicators: The adoption of conjugated dienes and TBARS. At a concentration of 0.005% the cloves oil outbid the activity of BHT. The capacity of the EO was about 74% compared to 59% achieved by BHT at 0.001%. The same results were obtained by the determination of TBARS. Here the EO's activity was also equal to BHT.

Melissa officinalis L. (lemon balm, Lamiaceae) is a well-known herb and is also used as a medicinal plant for the treatment of different diseases such as headache, gastrointestinal disorders, nervousness, and rheumatism. The EO is well known for its antibacterial and antifungal properties, so it was investigated for its antioxidative activity too (Mimica-Dukic et al., 2004). The analyses of the chemical composition of the EO showed that β-caryophyllene is one of the main compounds (~4.6%) besides geranial, neral, citronellal, and linalool. The free radical scavenging capacity was determined by the DPPH assay, the protection of lipid peroxidation was investigated with the TBA assay, and the scavenging activity of the oil for hydroxyl radicals was measured with the deoxyribose assay, also a rapid screening for the scavenging compounds was made. Lemon balm scavenged in the DPPH test 50% of the radicals at a concentration of 7.58 µg/mL compared with the standard BHT, which attained an IC_{50} value of 5.37 µg/mL. At a concentration of 2.13 µg/mL the highest inhibition (about 60%) of generated hydroxyl radicals in the deoxyribose assay was found compared with BHT as a positive control (~19%). Strong antioxidative activity of the EO was also determined in the TBA test system, where the inhibition of the lipid peroxidation was determined. Sixty-seven percent inhibition was caused by 2.13 µg/mL EO, in comparison with 37% of BHT. A rapid screening for the scavenging capacity with DPPH on a TLC plate exhibited that caryophyllene is one of the most active compounds.

Croton urucurana (Euphorbiaceae) is known as "dragon's blood" and is used in traditional medicine because of its wound and ulcer healing, antidiarrheic, anticancer, anti-inflammatory, antioxidant, and antirheumatic properties. The antioxidative fractions of the EO obtained were determined with

a rapid screening using the DPPH assay on TLC. The main compound of the most active fraction was α-bisabolol with 38.3%. The isolated fraction exhibited a 50% radical scavenging activity at a concentration of 1.05 mg/mL. This is a lower antioxidative activity than that of BHT. The EO of *Croton urucurana* showed an activity in the DPPH assay with an IC_{50} value of 3.21 mg/mL (Simionatto et al., 2007).

The EO of *Elionurus elegans* Kunth. (African pasture grass, Poaceae) was investigated for its biological activities. GC/MS analyses showed that the oil contains α-bisabolol (1.6% in the roots and 1.2% in the aerial parts). None of the other compounds exhibited in individual test systems antioxidative activities with the exception of limonene and, which were available only in low amounts. The antioxidant activity of the EO was tested with the chemiluminescence method using a luminometer, where the chemiluminescence intensity of the reaction mixture containing the EO or a standard (α-tocopherol), AAPH, and luminol was measured. The IC_{50} value for the EO obtained from the aerial parts amounted to 30% and the 50% inhibition rate of the roots EO to 46%.

Teucrium marum (mint plant, Lamiaceae) is used in the traditional medicine for its antibacterial, anti-inflammatory, and antipyretic activities. The antioxidative activity of the EO, which contains about 15% β-bisabolene, was investigated in three different test systems. The inhibition of lipid peroxide formation and of superoxide radicals and the radical scavenging activity with DPPH were tested. The oil exhibited a scavenging power in the DPPH assay (IC_{50} value 13.13 μg/mL), which is similar to the well-known antioxidants BHT, ascorbic acid, and trolox. Using the xanthine–xanthine assay, in which the inhibition of superoxide radicals is tested, the EO showed a better scavenging activity for the superoxide radicals than BHT (IC_{50} value 0.161 μg/mL against 2.35 g/mL); however, the efficiency of ascorbic acid and trolox was higher with IC_{50} values of 0.007 and 0.006 μg/mL, respectively. The inhibition of lipid peroxidation was determined with the 5-lipidoxygenase test, where the formation of the hydroxy peroxides was measured spectrophotometrically at 235 nm. The activities of the EO and trolox were similar with IC_{50} values of 12.48 and 11.88 μg/mL, respectively. The inhibition power was better than ascorbic acid with an IC_{50} value of 18.63 μg/mL. The antioxidative activity of BHT was higher than the activity of the EO with an IC_{50} value of 3.86 μg/mL (Ricci et al., 2005).

In the course of the investigation of the antioxidative activity of EOs obtained from three different citrus species (Rutaceae), β-bisabolene was also determined for its antioxidative property. The LDL oxidation was measured spectrophotometrically at 234 nm by the formation of TBARS. The results were expressed as nmol malonic dialdehyde/mg of protein. In the test system, β-bisabolene inhibited only TBARS formation from the AAPH-induced oxidation of LDL (Takahashi et al., 2003).

Several naturally occurring EOs containing for example carvacrol, anethole, perillaldehyde, cinnamaldehyde, linalool, and *p*-cymene were investigated for their effectiveness in their antioxidant activities and simultaneously also as to their ability in reducing a decay in fruit tissues. The tested EOs show positive effects on enhancing anthocyanins and antioxidative activity of fruits (Wang, C.Y. et al., 2008). In another study, the EO from black currant buds (*Ribes nigrum* L., Grossulariaceae) was analyzed by GC-MS and GC/-O and was tested for its radical scavenging activity, which—and this was the outcome of the present study—varied within a broad range, for example, from 43% to 79% in the DPPH reaction system (Dvaranauskaite et al., 2008). The antioxidant and antimicrobial properties of the rhizome EO of four different *Hedychium* species (Zingiberaceae) were investigated by Joshi et al. (2008). The rhizome EOs from all *Hedychium* species tested exhibited moderate to good Fe^{2+} chelating activity, whereas especially *Hedychium spicatum* also showed a complete different DPPH radical scavenging profile than the samples from the other species. Finally, a very strong superoxide anion scavenging and an excellent DPPH-scavenging activity besides a strong hypolipidemic property possessed a methanol fractionate of the mountain celery seed EO (*Cryptotaenia japonica* Hassk, Apiaceae). The principal constituents of this fraction after a successive gel column adsorption were γ-selinene, 2-methylpropanal, and (*Z*)-9-octadecenamide (Cheng et al., 2008).

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10 Effects of Essential Oils in the Central Nervous System

CONTENTS

10.1 CENTRAL NERVOUS SYSTEM EFFECTS OF ESSENTIAL OILS IN HUMANS

Eva Heuberger

10.1.1 Introduction

A number of attempts have been made to unravel the effects of natural essential oils (EOs) and fragrances on the human central nervous system (CNS). Among these attempts two major lines of research have been followed to identify psychoactive, particularly stimulating and sedative, effects of fragrances. On the one hand, researchers have investigated the influence of EOs and fragrances on brain potentials, which are indicative of the arousal state of the human organism by means of

neurophysiological methods. On the other hand, behavioral studies have elucidated the effects of EOs and fragrances on basic and higher cognitive functions, such as alertness and attention, learning and memory, or problem solving. The scope of the following section, although not claiming to be complete, is to give an overview about the current knowledge in these fields. Much of the research reviewed has been carried out in healthy populations and only recently investigators have started to focus on clinical aspects of the administration of fragrances and EOs. However, since this topic is covered in another chapter of this volume, it is omitted here in the interest of space.

Olfaction differs from other senses in several ways. First, in humans and many other mammals, the information received by peripheral olfactory receptor cells is mainly processed in brain areas located ipsilaterally to the stimulated side of the body, whereas in the other sensory systems, it is transferred to the contralateral hemisphere. Second, in contrast to the other sensory systems, olfactory information reaches a number of cortical areas without being relayed in the thalamus (Kandel et al., 1991; Zilles and Rehkämpfer, 1998; Wiesmann et al., 2001) (Figure 10.1). Owing to this missing thalamic control as well as to the fact that the olfactory system presents anatomical connections and overlaps with brain areas involved in emotional processing, such as the amygdala, hippocampus, and prefrontal cortex of the limbic system (Reiman et al., 1997; Davidson and Irwin, 1999; Phan et al., 2002; Bermpohl et al., 2006), the effects of odorants on the organisms are supposedly exerted not only via pharmacological but also via psychological mechanisms. In humans and probably also in other mammals, psychological factors may be based on certain stimulus features, such

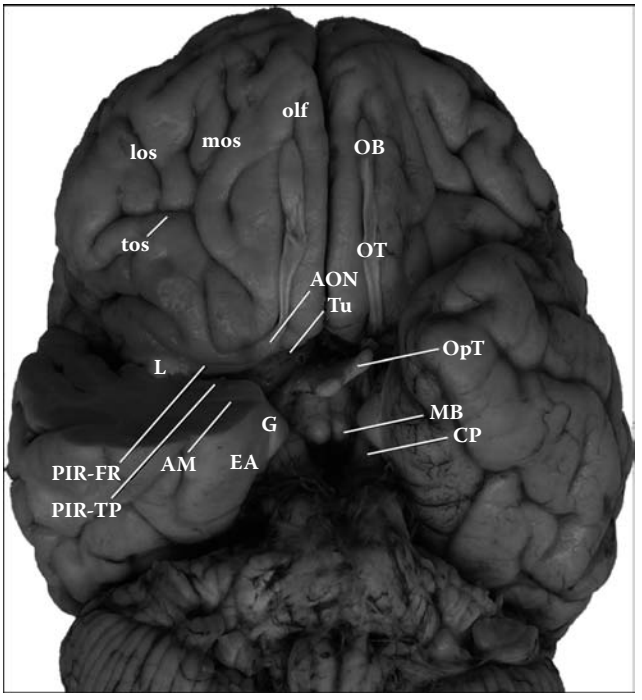

FIGURE 10.1 Macroscopic view of the human ventral forebrain and medial temporal lobes, depicting the olfactory tract, its primary projections, and surrounding nonolfactory structures. The right medial temporal lobe has been resected horizontally through the mid-portion of the amygdala (AM) to expose the olfactory cortex. AON, anterior olfactory nucleus; CP, cerebral peduncle; EA, entorhinal area; G, gyrus ambiens; L, limen insula; los, lateral olfactory sulcus; MB, mammillary body; mos, medial olfactory sulcus; olf, olfactory sulcus; PIR-FR, frontal piriform cortex; OB, olfactory bulb; OpT, optic tract; OT, olfactory tract; tos, transverse olfactory sulcus; Tu, olfactory tubercle; PIR-TP, temporal piriform cortex. Figure prepared with the help of Dr. Eileen H. Bigio, Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois. (From Gottfried, J.A. and D.A. Zald, 2005. *Brain Res. Rev.*, 50: 287–304. With permission.)

as odor valence (Baron and Thomley, 1994), on semantic cues, for example, memories and experiences associated with a particular odor, as well as on placebo effects related to the expectation of certain effects (Jellinek, 1997). None of the latter mechanisms is substance, that is, odorant, specific but their effectiveness depends on cognitive mediation and control.

Many odorants stimulate not only the olfactory system via the first cranial nerve (N. olfactorius) but also the trigeminal system via the fifth cranial nerve (N. trigeminus), which enervates the nasal mucosa. The trigeminal system is part of the body's somatosensory system and mediates mechanical and temperature-related sensations, such as itching and burning or warmth and cooling sensations. Trigeminal information reaches the brain via the trigeminal ganglion and the ventral posterior nucleus of the thalamus. The primary cortical projection area of the somatosensory system is the contralateral postcentral gyrus of the parietal lobe (Zilles and Rehkämpfer, 1998). The reticular formation in the brain stem, which is part of the reticular activating system (RAS) (Figure 10.2), receives collaterals from the trigeminal system. Thus, trigeminal stimuli have direct effects on arousal. Utilizing this direct connection, highly potent trigeminal stimulants, such as ammonia and menthol, have been used in the past in smelling salts to awaken people who fainted.

It has been shown in experimental animals that, due to their lipophilic properties, fragrances not only penetrate the skin (Hotchkiss, 1998) but also the blood–brain barrier (Buchbauer et al., 1993). Also, odorants have been found to bind to several types of brain receptors (Aoshima and Hamamoto, 1999; Elisabetsky et al., 1999; Okugawa et al., 2000), and it has been suggested

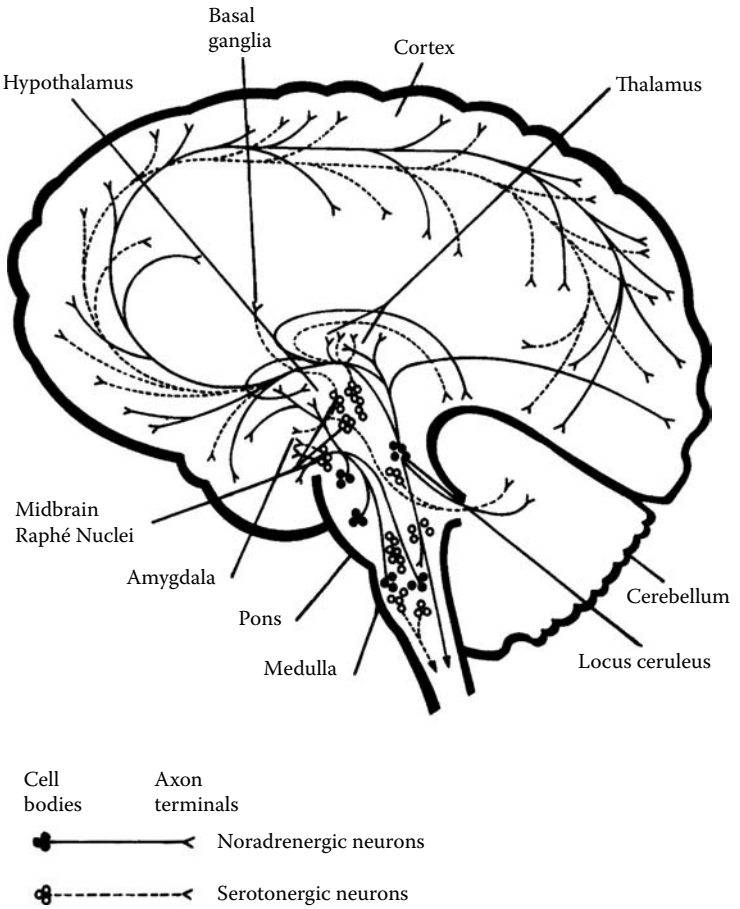

FIGURE 10.2 Schematic of the RAS with noradrenergic and serotonergic connections. (From Grilly, D.M., 2002. *Drugs & Human Behavior.* Boston: Allyn & Bacon. With permission.)

that these odorant–receptor interactions are responsible for psychoactive effects of fragrances in experimental animals. With regard to these findings, it is important to note that Heuberger et al. (2001) have observed differential effects of fragrances as a function of chirality. It seems likely that such differences in effectiveness are related to enantiomeric selectivity of receptor proteins. However, the question whether effects of fragrances on human arousal and cognition rely on a similar psychopharmacological mechanism remains to be answered.

10.1.2 Activation and Arousal: Definition and Neuroanatomical Considerations

Activation, or arousal, refers to the ability of an organism to adapt to internal and external challenges (Schandry, 1989). Activation is an elementary process that serves in the preparation for overt activity. Nevertheless, it does not necessarily result in overt behavior (Duffy, 1972). Activation varies in degree and can be described along a continuum from deep sleep to overexcitement. Early theoretical accounts of activation have emphasized physiological responses as the sole measurable correlate of arousal. Current models, however, consider physiological, cognitive, and emotional activity as observable consequences of activation processes. It has been shown that arousal processes within each of these three systems, that is, physiological, cognitive, and emotional, can occur to varying degrees so that the response of one system need not be correlated linearly to that of the other systems (Baltissen and Heimann, 1995).

It has long been established that the RAS, which comprises the reticular formation with its sensory afferents and widespread hypothalamic, thalamic, and cortical projections, plays a crucial role in the control of both phasic and tonic activation processes (Becker-Carus, 1981; Schandry, 1989). Pribram and McGuinness (1975) distinguish three separate but interacting neural networks in the control of activation (Figure 10.3). The arousal network involves amygdalar and related frontal cortical structures and regulates phasic physiological responses to novel incoming information. The activation network centers on the basal ganglia of the forebrain and controls the tonic physiological readiness to respond. Finally, the effort network, which comprises hippocampal circuits, coordinates the arousal and activation networks. Noradrenergic projections from the locus ceruleus, which is located within the dorsal wall of the rostral pons, are particularly important in the regulation of

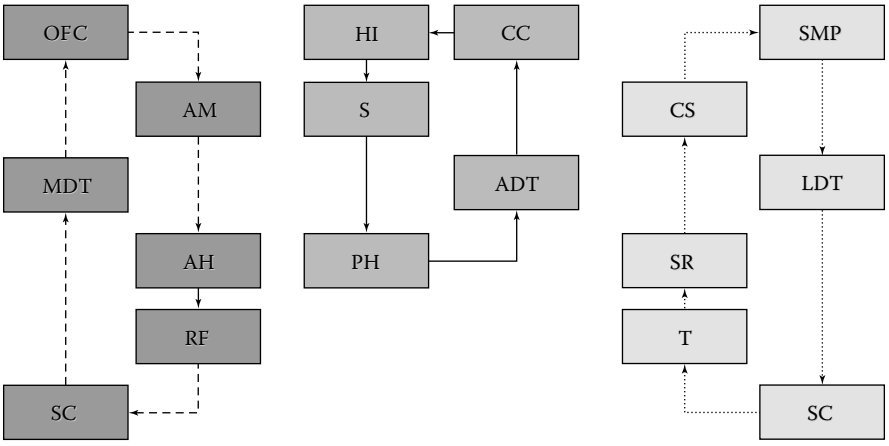

FIGURE 10.3 Control of activation processes. OFC, orbitofrontal cortex; AM, amygdala; MDT, medial dorsal thalamus; AH, anterior hypothalamus; RF, reticular formation; SC, spinal cord; HI, hippocampus; CC, cingulate cortex; S, septum; ADT, anterior dorsal thalamus; PH, posterior hypothalamus; SMP, sensory-motor projections; CS, corpus striatum; LDT, lateral dorsal thalamus; SR, subthalamic regions; T, tectum. Left, structures of the arousal network; middle, structures of the effort network; right, structures of the activation network. (Adapted from Pribram, K. H. and D. McGuinness, 1975. *Psychol. Rev.*, 82(2): 116–149.)

circadian alertness, the sleep–wake rhythm, and the sustenance of alertness (alerting) (Pedersen et al., 1998; Aston-Jones et al., 2001). On the other hand, tonic alertness seems to be dependent on cholinergic (Baxter and Chiba, 1999; Gill et al., 2000) frontal and inferior parietal thalamic structures of the right hemisphere (Sturm et al., 1999). Other networks that are involved in the control of arousal and attentional functions are found in posterior parts of the brain, for example, the parietal cortex, superior colliculi, and posterior-lateral thalamus, as well as in anterior regions, for example, the cingular and prefrontal cortices (Posner and Petersen, 1990; Paus, 2001).

10.1.3 Influence of EOs and Fragrances on Brain Potentials Indicative of Arousal

10.1.3.1 Spontaneous Electroencephalogram Activity

Recordings of spontaneous electroencephalogram (EEG) activity during the administration of EOs and fragrances have widely been used to assess stimulant and sedative effects of these substances. Particular attention has been paid to changes within the α and β bands, sometimes also the θ band of the EEG in response to olfactory stimulation since these bands are thought to be most indicative of central arousal processes. Alpha waves are slow brain waves within a frequency range of 8–13 Hz and amplitudes between 5 and 100 μV, which typically occur over posterior areas of the brain in an awake but relaxed state, especially with closed eyes. The α rhythm disappears immediately when subjects open their eyes and when cognitive activity is required, for example, when external stimuli are processed or tasks are solved. This phenomenon is often referred to as α block or desynchronization. Simultaneously with the α block, faster brain waves occur, such as β waves with smaller amplitudes (2–20 μV) and frequencies between 14 and 30 Hz. The β rhythm, which is most evident frontally, is characteristic of alertness, attention, and arousal. In contrast, θ waves are very slow brain waves occurring in fronto-temporal areas with amplitudes between 5 and 100 μV in the frequency range between 4 and 7 Hz. Although the θ rhythm is most commonly associated with drowsiness and light sleep, some researchers found θ activity to correlate with memory processes (Grunwald et al., 1999; Hoedlmoser et al., 2007) and creativity (Razumnikova, 2007). Other authors found correlations between θ activity and ratings of anxiety and tension (Lorig and Schwartz, 1988). With regard to animal olfaction, it has been proposed that the θ rhythm generated by the hippocampus is concomitant to sniffing and allows for encoding and integration of olfactory information with other cognitive and motor processes (Kepecs et al., 2006).

A large number of measures can be derived from recordings of the spontaneous EEG. Time (index) or voltage (power)-based rates of typical frequency bands, as well as ratios between certain frequency bands (e.g., between the α and β band or the θ and β band) within a selected time interval are most commonly used to quantify EEG patterns. Period analysis quantifies the number of waves that occur in the various frequency bands within a distinct time interval of the EEG record and is supposed to be more sensitive to task-related changes than spectral analysis (Lorig, 1989). Other parameters that describe the covariation of a given signal at different electrodes are coherence and neural synchrony. These measures inform on the functional link between brain areas (Oken et al., 2006).

Generally speaking, the pattern of the spontaneous EEG varies with the arousal level of the CNS. Thus, different states of consciousness, such as sleep, wakefulness, or meditation, can be distinguished by their characteristic EEG patterns. For instance, an increase in central activation is typically characterized by a decrease in α and an increase in β activity (Schandry, 1989). More precisely, a decline of α and β power together with a decrease of α index and an increase of β and θ activity have been observed under arousing conditions, that is, in a mental calculation and a psychosocial stress paradigm (Walschburger, 1976). Also when subjects are maximally attentive, frequencies in the α band are attenuated and activity in the β and even higher frequency bands can be observed. Fatigue and performance decrements in situations requiring high levels of attention are often associated with increases in θ and decreases in β activity (Oken et al., 2006). On the other hand, drowsiness and the onset of sleep are characterized by an increase in slow and a decrease in fast EEG waves. However, high activity in the α band, particularly in the range between 7 and 10 Hz, is not

indicative of low arousal states of the brain, such as relaxation, drowsiness, and the onset of sleep, but rather seems to be a component of selective neural inhibition processes that are necessary for a number of cognitive processes, such as perception, attention, and memory (Miller and O'Callaghan, 2006; Palva and Palva, 2007).

Because changes of spontaneous EEG activity accompany a wide range of cognitive as well as emotional brain processes and "EEG measurements […] do not tell investigators what the brain is doing" (Lorig 1989, p. 93), it is somewhat naïve to interpret changes that are induced by the application of an odorant as a result of a single and specific process, particularly when no other correlates of the process of interest are assessed. Nevertheless, this is exactly the approach that has been taken by many researchers to identify stimulant or relaxing effects of odors. The simplest, but in terms of interpretation of the results probably most problematic setup for such experiments is the comparison of spontaneous EEG activity in response to odorants with a no-odor baseline. Using this design, Sugano (1992) observed increased EEG α activity after inhalation of α-pinene (**1**), 1,8-cineole (**2**), lavender, sandalwood, musk, and eucalyptus odors. Considering that traditional aromatherapy discriminates these fragrances by their psychoactive effects, for example, lavender is assigned relaxing properties while eucalyptus is supposedly stimulant (Valnet, 1990); these findings are at least rather curious. Also Ishikawa et al. (2002) recorded the spontaneous EEG in 13 Japanese subjects while drinking either lemon juice with a supplement of lemon odor or lemon juice without it. It was shown that α power was enhanced by supplementation with lemon odor, an indicative of increased relaxation. Again, with regard to aromatherapeutical accounts of the lemon EO this result is somewhat counterintuitive, even more so as in the same study the juice supplemented with lemon odor increased spontaneous locomotion in experimental animals. Haneyama and Kagatani (2007) tested a fragrant spray made from extracts of Chinese spikenard roots [*Nardostachys chinensis* Batalin (Valerianaceae)] in butylene glycol and found increased α activity in subjects under stress. This finding was interpreted by the authors as demonstrating a sedative effect of the extract. However, it is unknown how stress was induced in the subjects and how it was measured. Similarly, Ishiyama (2000) concluded from measurements of the frequency fluctuation patterns of the α band that smelling a blend of terpene compounds, typically found in forests, induced feelings of refreshment and relaxation in human subjects without proper description of how these feelings were assessed.

Inconclusive findings as those described above are not unexpected with such simple experimental designs as there are several problems associated with this kind of experiments. First, a no-odor baseline is often inappropriate as it does not control for cognitive activity of the subject. For instance, subjects might be puzzled by the fact that they do not smell anything eventually focusing attention to the search for an odor. This may lead to quite high arousal levels rather than the intended resting brain state. This was the case with Lorig and Schwarzt (1988), who tested changes of spontaneous EEG in response to spiced apple, eucalyptus, and lavender fragrances diluted in an odorless base: contrary to the authors' expectations it was found that α activity in the no-odor condition was less than that during odor presentation.

Considering the various mechanisms outlined by Jellinek (1997) by which fragrances influence human arousal and behavior, another inherent problem of such simple designs is that little is known about how subjects process stimulus-related information, for example, the pleasantness or intensity of an odorant, and whether or not higher cognitive processes related to the odorant are initiated by the stimulation. For instance, subjects might be able to identify and label some odors but might fail to do so with others; similarly, some odors might trigger the recall of associated memories while others might not. In order to assess psychoactive effects of EOs and fragrances, it seems necessary to control for these factors, for instance by assessing additional variables that inform on the subject's perception of primary and secondary stimulus features and that correlate to the subject's cognitive or emotional arousal state. In the above-mentioned study, Lorig and Schwarzt (1988) collected ratings of intensity and pleasantness of the tested fragrances as well as subjective ratings of a number of affective states in addition to the EEG recordings. Analysis of the

amount of EEG θ activity revealed that the spiced apple odor produced more relaxation than lavender and eucalyptus; the analysis of the secondary variables suggested that this relaxing effect was correlated with subjective estimates of anxiety and tension. As to the EEG patterns, similar results were observed when subjects imagined food odors and practiced relaxation techniques. Thus, the authors conclude that the relaxing effect of spiced apple was probably related to its association with food. These cognitive influences also seem to be a plausible explanation for the increase in α power by lemon odor in the Ishikawa et al. (2002) study. Other studies related to food odors were conducted by Kaneda et al. (2005, 2006). These authors investigated the influence of smelling beer flavors on the frequency fluctuation of α waves in frontal areas of the brain. The results showed relaxing effects of the aroma of hop extracts as well as of linalool (**3**). In addition, in the right hemisphere these fluctuations were correlated with subjective estimates of arousal and with the intensity of the hop aroma. Lee et al. (1994) also found evidence for differential EEG patterns as a function of odor intensity for citrus, lavender, and a floral odor. A 10-min exposure to the weaker intensity of the citrus fragrance in comparison to lavender odor increased the rate of occipital α. Moreover, there was a general trend for citrus to be rated as more comfortable than other fragrances. In contrast, the higher intensity of the floral fragrance increased the rate of occipital β more than the lavender odor.

Several authors have shown influences of odor pleasantness and familiarity on changes of the spontaneous EEG. For instance, Kaetsu et al. (1994) reported that pleasant odors increased the α activity, while unpleasant ones decreased it. In a study on the effects of lavender and jasmine odor on electrical brain activity (Yagyu, 1994), it was shown that changes in the α, β, and θ bands in response to these fragrances were similar when subjects rated them as pleasant, while lavender and jasmine odor led to distinct patterns when they were rated as unpleasant. Increases of α activity in response to pleasant odors might be explained by altered breathing patterns since it has been demonstrated that pleasant odors induce deeper inhalations and exhalations than unpleasant odors, and that this form of breathing by itself increases the activity in the α band (Lorig, 2000). Masago et al. (2000) tested the effects of lavender, chamomile, sandalwood, and eugenol (**4**) fragrances on ongoing EEG activity and self-ratings of comfort and found a significant positive correlation between the degree of comfort and the odorants' potency to decrease the α activity in parietal and posterior temporal regions. In relation to the previously described investigations, this finding is rather difficult to explain, although it differs from the other studies in that it differentiated between electrode sites rather than reporting merely global changes in electrical brain activity. Therefore, this result suggests that topographical differences in electrical brain activity induced by fragrances may be important and need further investigation. In fact, differences in hemispheric localization of spontaneous EEG activity in response to pleasant and unpleasant fragrances seem to be quite consistent. While pleasant odors induced higher activation in left frontal brain regions, unpleasant ones led to bilateral and widespread activation (Kim and Watanuki, 2003) or no differences were observed when an unpleasant odor (valerian) was compared to a no-odor control condition (Kline et al., 2000). Another interesting finding in the study of Kim and Watanuki (2003) was that EEG activity in response to the tested fragrances was observed when subjects were at rest but vanished after performing a mental task. The importance of distinguishing EEG activity arising from different areas of the brain is highlighted by an investigation by Van Toller et al. (1993). These authors recorded α wave activity at 28 sites of the scalp immediately after the exposure to a number of fragrances covering a range of different odor types and hedonic tones at iso-intense concentrations; the odorants had to be rated in terms of pleasantness, familiarity, and intensity. It was shown that in posterior regions of the brain changes in α activity in response to these odors compared to an odorless blank were organized in distinct topographical maps. Moreover, α activity in a set of electrodes at frontal and temporal sites correlated with the psychometric ratings of the fragrances.

As to influences of the familiarity of or experience with fragrances, Kawano (2001) reported that the odors of lemon, lavender, patchouli, marjoram, rosemary, and sandalwood increased α activity over occipital electrode sites in subjects to whom these fragrances were well known. On the

one hand, lag times between frontal and occipital α phase were shorter in subjects less experienced with the fragrances, indicating that these subjects were concentrating more on smelling—and probably identifying—the odorants. These findings were confirmed in an investigation comparing professional perfume researchers, perfume salespersons, and general workers (Min et al., 2003). This study showed that measures of cortico-cortical connectivity, that is, the averaged cross mutual information content, in odor processing were more pronounced in frontal areas with perfume researchers, whereas with perfume salespersons and general workers a larger network of posterior temporal, parietal, and frontal regions was activated. These results could result from a greater involvement of orbitofrontal cortex neurons in perfume researchers, who exhibit high sophistication in discriminating and identifying odors. Moreover, it was shown that the value of the averaged cross mutual information content was inversely related to preference in perfume researchers and perfume salespersons, but not in general workers.

As pointed out above, the administration of EO and fragrances to naïve subjects can lead to cognitive processes that are unknown to the investigator, and sometimes even the subject, but that nevertheless affect spontaneous EEG activity. Some researchers have sought to solve this problem by engaging subjects in a secondary task while the influence of the odorant of interest is assessed. This procedure does not only draw the subject's attention away from the odor stimulus, but also provides the desired information about his/her arousal state. Another benefit of such experimental designs is that the task may control for the subject's arousal state if a certain amount of attention is required to perform it. Measurement of changes of α and θ activity in the presence or absence of 1,8-cineole (**2**), methyl jasmonate (**5**), and *trans*-jasmin lactone (**6**) in subjects who performed a simple visual task showed that the increase in slow wave activity was attenuated by 1,8-cineole (**2**) and methyl jasmonate (**5**), while augmented by *trans*-jasmin lactone (**6**) (Nakagawa et al. 1992). At least in the case of 1,8-cineole (**2**) these findings are supported by results from experimental animals and humans, indicating activating effects of this odorant (Kovar et al., 1987; Nasel et al., 1994; Bensafi et al., 2002). An investigation of the effects of lemon odor EEG α, β, and θ activity during the administration of lemon odor showed that the odor reduced power in the lower α range while it increased power in the higher α, lower β, and lower θ bands (Krizhanovs'kii et al., 2004). These findings of increased arousal were in agreement with better performance in a cognitive task. In addition, it was shown that inhalation of the lemon fragrance was most effective during rest and in the first minutes of the cognitive task, but wore off after less than 10 min. In several experiments, the group of Sugawara demonstrated complex interactions between electrical brain activity induced by the exposure to fragrances, sensory profiling, and various types of tasks (Sugawara et al., 2000; Satoh and Sugawara, 2003). In one study they showed that the odor of peppermint, in contrast to basil, was rated less favorable on a number of descriptors and reduced the magnitude of β waves after as compared to before performance of a cognitive task. In a similar investigation, it was shown that the sensory evaluation as well as changes in spontaneous EEG activity in response to the odors of the linalool enantiomers differed as a function of the molecular structure and the kind of task. For instance, *R*-(−)-linalool (**7**) was rated more favorable and led to larger decreases of β activity after listening to natural sounds than before. In contrast, after as compared to before cognitive effort *R*-(−)-linalool (**7**) was rated as less favorable and tended to increase β power. A similar pattern was found for *RS*-(±)-linalool (**3**), whereas for *S*-(+)-linalool (**8**) the pattern was different, particularly with regard to EEG activity.

In the study of Yagyu (1994), the effects of lavender and jasmine fragrances on the performance in a critical flicker fusion and an auditory reaction time task were assessed in addition to changes of the ongoing EEG. It was demonstrated that in contrast to the EEG findings, lavender decreased performance in both tasks independent of its hedonic evaluation. Jasmine, however, had no effect on task performance. The EEG changes in response to these odorants might well explain their effects on performance: lavender induced decreases of activity in the β band, which is associated with states of low attention regardless of being rated as pleasant or unpleasant; jasmine, on the other hand, increased EEG β activity when it was judged unpleasant but lowered

β activity when judged pleasant, so that overall its effect on performance levelled out. The effects of lavender and rosemary fragrances on electrical brain activity, mood states, and math computations were investigated by Diego, Field, and co-workers (Diego et al., 1998; Field et al., 2005). These investigations showed that the exposure to lavender increased β power, elevated feelings of relaxation, reduced feelings of depression, and improved both speed and accuracy in the cognitive task. In contrast, rosemary odor decreased frontal α and β power, decreased feelings of anxiety, increased feelings of relaxation and alertness, and increased speed in the math computations. The EEG results were interpreted as indicating increased drowsiness in the lavender group and increased alertness in the rosemary group; however, the behavioral data showed performance improvement and similar mood ratings in both groups. These findings suggest that different electrophysiological arousal patterns may still be associated with similar behavioral arousal patterns emphasizing the importance of collecting additional endpoints to evaluate psychoactive effects of EO and fragrances.

10.1.3.2 Contingent Negative Variation

The contingent negative variation (CNV) is a slow, negative event-related brain potential, which is generated when an imperative stimulus is preceded by a warning stimulus and reflects expectancy and preparation (Walter et al., 1964). The amplitude of the CNV is correlated to attention and arousal (Tecce, 1972). Since changes of the magnitude and latency of CNV components have long been associated with the effects of psychoactive drugs (Kopell et al., 1974; Ashton et al., 1977), measurement of the CNV has also been used to evaluate psychostimulant and sedating effects of EOs and fragrances. In a pioneering investigation, Torii et al. (1988) measured CNV magnitude changes evoked by a variety of EOs, such as jasmine, lavender, and rose oil, in male subjects. CNV was recorded at the frontal, central, and parietal sites after the presentation of an odorous or blank stimulus in the context of a cued reaction time paradigm. In addition, physiological markers of arousal, that is, skin potential level and heart rate, were simultaneously measured. Results showed that at frontal sites the amplitude of the early negative shift of the CNV was significantly altered after the presentation of odor stimuli, and that these changes were mostly congruent with stimulating and sedative properties reported for the tested oils in the traditional aromatherapy literature. In contrast to other psychoactive substances, such as caffeine or benzodiazepines, presentation of the EOs neither affected physiological parameters nor reaction times. The authors concluded that the EOs tested influenced brain waves "almost exclusively" while having no effects on other indicators of arousal.

Subsequently, CNV recordings have been used by a number of researchers on a variety of EOs and fragrances to establish effects of odors on the human brain along the activation–relaxation continuum. For instance, Sugano (1992) in the aforementioned study demonstrated that α-pinene (**1**), sandalwood, and lavender odor increased the magnitude of the CNV in healthy young adults, whereas eucalyptus reduced it. It is interesting to note, however, that all of these odors—despite their differential influence on the CNV—increased spontaneous α activity in the same experiment. An increase of CNV magnitude was also observed with the EO from pine needles (Manley, 1993), which was interpreted as having a stimulating effect. Aoki (1996) investigated the influence of odors from several coniferous woods, that is, hinoki [*Chamaecyparis obtusa* (Siebold & Zucc.) Endl. (Cupressaceae)], sugi [*Cryptomeria japonica* D.Don (Cupressaceae)], akamatsu [*Pinus densiflora* Siebold & Zucc. (Pinaceae)], hiba [*Thujopsis dolabrata* var. *hondai* Siebold & Zucc. (Cupressaceae)], Alaska cedar [*Chamaecyparis nootkatensis* (D.Don) Spach (Cupressaceae)], Douglas fir [*Pseudotsuga manziesii* (Mirbel) Franco (Pinaceae)], and Western red cedar [*Thuja plicata* Donn (Cupressaceae)], on the CNV and found conflicting effects: the amplitude of the early CNV component at central sites was decreased by these wood odors, and the α/β-wave ratio of the EEG increased. Moreover, the decrease of CNV magnitude was correlated with the amount of α-pinene (**1**) in the tested wood odors. Also Sawada et al. (2000) measured changes of the early component of the CNV in response to stimulation with terpenes found in the EO of woods and

leaves. These authors noticed a reduction of the CNV magnitude after the administration of α-pinene (**1**), Δ-3-carene (**9**), and bornyl acetate (**10**). However, a more recent investigation (Hiruma et al., 2002) showed that hiba [*Thujopsis dolabrata* Siebold & Zucc. (Cupressaceae)] odor increased the CNV magnitude at frontal and central sites and shortened reaction times to the imperative stimulus in female subjects. These authors thus concluded that the odor of hiba heightened the arousal level of the CNS.

Although the CNV is believed to be largely independent of individual differences, such as age, sex, or race (Manley, 1997), there seem to be cognitive influences that must not be neglected when interpreting the effects of odor stimuli on the CNV. Lorig and Roberts (1990) repeated the study by Torii et al., (1988) and investigated cognitive factors by introducing a manipulation of their subjects as an additional variable into the paradigm. In this experiment subjects were exposed to the original two odors, that is, lavender and jasmine, referred to as odor A and odor B, respectively, as well as to a mixture of the two fragrances. However, in half of the trials in which the mixture was administered subjects were led to believe that they received a low concentration of odor A, whereas in the other half of trials they thought that they would be exposed to a low concentration of odor B. In none of the four conditions were the subjects given the correct odor names. As in the Torii et al. study, lavender reduced the amplitude of the CNV whereas jasmine increased it. When the mixture was administered, however, the CNV magnitude decreased when subjects believed to receive a low concentration of lavender, but increased when they thought they were inhaling a low concentration of jasmine. This means that the alteration of the CNV amplitude was not solely related to the substance that had been administered but also related to the expectation of the subjects. Another point made by Lorig and Roberts (1990) is that in their study self-report data indicated that lavender was actually rated as more arousing than jasmine. Since low CNV amplitudes are not only associated with low arousal but also with high arousal in the context of distraction (Travis and Tecce, 1998), the lavender odor might in fact have led to higher arousal levels than jasmine even though the CNV magnitude was smaller with lavender. Other authors have noted that CNV changes might not only reflect effects of odor stimuli but also the anticipation, expectancy, and the emotional state of the subjects who are exposed to these odorants (Hiruma et al., 2005). The involvement of these and other cognitive factors might well explain why the findings of CNV changes in response to odorants are rather inconsistent.

10.1.4 Effects of EOs and Fragrances on Selected Basic and Higher Cognitive Functions

Psychoactive effects of odorants at the cognitive level have been explored in humans using a large number of methods. A variety of testing procedures ranging from simple alertness or mathematical tasks to tests that assess higher cognitive functions, such as memory or creativity, have been employed to study stimulant or relaxing/sedating effects of EOs and fragrances. Nevertheless, the efficiency of odorants is commonly defined by changes in performance in such tasks as a function of the exposure to fragrances.

10.1.4.1 Alertness and Attention

A number of studies are available on the influence of fragrances on attentional functions. The integrity and the level of the processing efficiency of the attentional systems is a fundamental prerequisite of all higher cognitive functions. Attentional functions can be divided into four categories: alertness, selective and divided attention, and vigilance (Posner and Rafal, 1987; Keller and Groemminger, 1993; Sturm, 1997). Alertness is the most basic form of attention and is intrinsically dependent on the general level of arousal. Selective attention describes the ability to focus on relevant stimulus information while nonrelevant features are neglected; divided attention describes the ability to concomitantly process several stimuli from different sensory modalities. Vigilance refers to the sustenance of attention over longer periods of time. Since the critical stimuli typically occur only rarely

in time, vigilance can be seen as a counterforce against increasing fatigue in boring situations, which is crucial in everyday life, in situations such as long-distance driving (particularly at night), working in assembly lines, or monitoring a radar screen (e.g., in air traffic control).

In a pioneering study, Warm et al. (1991) investigated the influence of peppermint and muguet odors on human visual vigilance. Peppermint, which was rated as stimulant, was expected to increase the task performance, whereas muguet, rated as relaxant, was expected to impair it. After intermittent inhalation, none of these fragrances increased processing speed in the task, but subjects in both odorant conditions detected more targets than a control group receiving unscented air. On the other hand, neither fragrance influenced subjective mood or judgments of workload. Gould and Martin (2001) studied the effects of bergamot and peppermint EOs on human sustained attention, where again peppermint was expected to improve performance, whereas bergamot, characterized as relaxing by an independent sample of subjects, was expected to have a deteriorating effect on vigilance performance. However, only bergamot had a significant influence in the anticipated direction, that is, subjects in this condition detected fewer targets than subjects in the peppermint or a no-odor control condition, which was probably related to subjects' expectation of a relaxing effect.

The influence of the inhalation of a number of EOs, which were expected to have activating effects on performance in an alertness task, was assessed by Ilmberger et al. (2001). Contrary to the authors' hypotheses, the results suggested that these EOs, when compared to an odorless control did not increase the speed of information processing. Even more unexpected, motor learning was impaired in the groups that received EOs; this effect is likely a consequence of distraction induced by the strong odor stimuli, an explanation that was supported by the reaction times that tended to be higher in the EO-treated groups than in the corresponding control groups. Alternatively, these authors argued that a ceiling effect might be responsible for the observed effects. Given that healthy subjects with intact attentional systems already perform at optimal levels of information processing in such basic tasks, it seems likely that activating EOs cannot enhance performance any further. Similarly, performance of healthy subjects may be too robust to be influenced by deactivating fragrances. This hypothesis has been supported by investigations on the EO of peppermint (Ho and Spence, 2005), lavender, and rosemary (Moss et al., 2003). These fragrances rather affected performance in difficult tasks or in tasks testing higher cognitive functions than in simple ones testing basic functions. Another interesting finding of the study of Ilmberger et al. was that changes in performance were correlated with subjective ratings of characteristic odor properties, particularly with pleasantness and efficiency. Similar results were obtained in another study for the EO of peppermint (Sullivan et al., 1998), which showed that in a vigilance task subjects benefited most from the effects of this fragrance when they experienced the task as quite difficult and thought that the EO had a stimulant effect. In addition, the study of Ilmberger and co-workers clearly demonstrated effects of expectation, that is, a placebo effect, as correlations between individual task performance and odor ratings were not only revealed in the EO groups but also in the no-odor control groups. The effects of a pleasant and an unpleasant blend of fragrances on selective attention was studied by Gilbert et al. (1997). No influence of either fragrance blend was found on attentional performance, but the authors observed a sex-specific effect of suggesting the presence of ambient odors. In the presence of a pleasant or no odor in the testing room, male subjects performed better when they were led to believe that no odor was present. Female subjects, however, performed better when they thought that they were exposed to an odorant under the same conditions. No such interaction was found in the unpleasant fragrance condition. These data again emphasize that, in addition to hedonic preferences, expectation of an effect may crucially influence the effects of odorants on human performance and that these factors may affect women and men differently.

Millot et al. (2002) evaluated the influence of pleasant (lavender oil) and unpleasant (pyridine, **11**) ambient odors on performance in a visual or auditory alertness task, and in a divided attention task. The results showed that in the alertness task, irrespective of the tested modality, both fragrances independent of their hedonic valence improved performance by shortening reaction times compared

to an unscented control condition. However, none of the odorants exerted any influence on performance in the selective attention task in which subjects had to attend to auditory stimuli while neglecting visual ones. The authors conclude that pleasant odors enhance task performance by decreasing subjective feelings of stress, that is, by reducing overarousal, while unpleasant fragrances increase activation from suboptimal to optimal levels, thus having the same beneficial effects on cognitive performance. With this explanation the authors, however, presume that subjects in their experimental groups started from dissimilar arousal levels which seems rather unlikely given that subjects were assigned to these groups at random. Moreover, cognitive performance should be affected by alterations of the arousal level more readily with increasing task difficulty. Thus, the interpretation given by the authors does not thoroughly explain why reaction times were influenced by the odorants in the simple alertness task but not in the more sophisticated selective attention task.

Degel and Köster (1999) exposed healthy subjects to either lavender, jasmine, or no fragrance with subjects being unaware of the odorants. Subjects had to perform a mathematical test, a letter counting (i.e., selective attention) task, and a creativity test. The authors expected a negative effect on performance of lavender and a positive effect of jasmine. The results, however, showed that lavender decreased the error rate in the selective attention task, whereas jasmine increased the number of errors in the mathematical test. Ratings of odor valence collected after testing demonstrated that lavender was judged more pleasant than jasmine, independent of which odor had been presented during the testing. Although subjects did not know that a fragrance had been administered, implicit evaluation of odor pleasantness has probably influenced their performance. This relation is supported by the fact that subjects who were not able to correctly identify the odors preferentially associated pictures of the room they had been tested in with the odor that had been present during the testing. Improvement of performance as a result of the inhalation of lavender EO has also been reported in another investigation (Sakamoto et al., 2005). In this study, subjects were exposed to lavender, jasmine or no aroma during phases of rest in-between sessions in which they completed a visual vigilance task involving tracking of a moving target. In the penultimate of five sessions, when fatigue was highest and arousal lowest as estimated from the decrement in performance between sessions of the control group, tracking speed increased and tracking error decreased in the lavender group when compared to the no-aroma group. Jasmine had no effect on task performance. The authors argued that lavender aroma may have decreased arousal during the resting period and hence helped to achieve optimal levels for the following task period. Since no secondary variables indicative of arousal or of subjective evaluation of aroma quality were assessed in this investigation, no inferences can be made on the mechanisms underlying the observed effects. Diego et al. (1998) in the aforementioned investigation studied the influence of lavender and rosemary EOs after a 3-min inhalation period on a mathematical task. In contrast to the authors' expectations, both odorants positively affected performance by increasing calculation speed, although only lavender improved calculation accuracy. In addition, subjects in both fragrance groups reported to be more relaxed. Those in the lavender group had less depressed mood, whereas those in the rosemary group felt more alert and had lower state anxiety scores. These findings were interpreted as indicating overarousal caused by rosemary EO, which led to an increase of calculation speed at the cost of accuracy. In contrast, lavender EO seemed to have reduced the subjects' arousal level and thus led to better performance than rosemary EO. However, since subjects in both fragrance groups felt more relaxed—but obviously only the lavender group benefited from this increase in relaxation—this is a somewhat unsatisfying explanation for the observed results.

Evidence for the influence of physico-chemical odorant properties on visual information processing was supplied by Michael et al. (2005). These authors found that the exposure to both allyl isothiocyanate (AIC, **12**), a mixed olfactory/trigeminal stimulus, and 2-phenyl ethyl alcohol (2-PEA, **13**), a pure olfactory stimulant, impaired performance in a highly demanding visual attention task involving reaction to a target stimulus when a distractor appeared at different intervals after presentation of the target, although different mechanisms were responsible for these effects. In trials without a distractor, only 2-PEA (**13**) significantly increased the reaction times of healthy

subjects; in trials with a distractor, subjects reacted more slowly in both odor conditions as compared to the no-odor control condition. However, AIC impaired performance, independent of the interval between distractor and target, whereas 2-PEA (**13**) only had a negative effect when the interval between target and distractor was short. While 2-PEA (**13**) seemed to have led to performance decrements by decreasing subjects' arousal level, AIC as a strong trigeminal irritant seemed to have affected the shift of attention toward the distractor stimuli, so that they were considered more important than in the other conditions.

Differences in effectiveness of fragrances as a function of the route of administration were explored by Heuberger et al. (2008). In several experiments, these authors investigated the influence of two monoterpenes, that is, 1,8-cineole (**2**) and (±)-linalool (**3**), on performance in a visual sustained attention task after 20 min inhalation and dermal application, respectively. 1,8-cineole (**2**) was expected to induce activation and improve task performance whereas (±)-linalool (**3**) was considered sedating/relaxing thus impairing performance. Since one of the aims of the study was to assess fragrance effects that were not mediated by stimulation of the olfactory system, inhalation of the odorants was prevented in the dermal application conditions. In each condition, subjects rated their mood and well-being. In addition, ratings of odor pleasantness, intensity, and effectiveness were assessed in the inhalation conditions. In regard to performance on the vigilance task, the results showed no difference between the fragrance groups compared to a control group, which had received odorless air. However, 1,8-cineole (**2**) increased feelings of relaxation and calmness whereas (±)-linalool (**3**) led to increased vigor and mood. In addition, individual performance was correlated to the pleasantness of the odor and of expectations of its effect. In contrast, in the dermal application conditions, subjects having received 1,8-cineole (**2**) performed faster than those having received (±)-linalool (**3**). These findings were interpreted as indicating the involvement of different mechanisms after inhalation and nonolfactory administration of fragrances. It seems that psychological effects are predominant when fragrances are applied by means of inhalation, that is, when the sense of smell is stimulated. On the other hand, pharmacological effects of odorants that might be overridden when fragrances are inhaled are evident when processing of odor information is prevented.

10.1.4.2 Learning and Memory

Effects of EOs and fragrances on memory functions and learning have less frequently been explored than influences on more basic cognitive functions. While learning can briefly be defined as "a process through which experience produces a lasting change in behavior or mental processes" (Zimbardo et al., 2003, p. 206), memory is a cognitive system composed of three separate subsystems or stages that cooperate closely to encode, store, and retrieve information. Sensory memory constitutes the first of the three memory stages and is responsible for briefly retaining sensory information. The second stage, working memory, transitorily preserves recent events and experiences. Long-term memory, the third subsystem, has the highest capacity of all stages and stores information based on meaning associated with the information (Zimbardo et al., 2003). A basic form of learning, which has been identified as a potent mediator of fragrance effects in humans (Jellinek, 1997), is conditioning, that is, the (conscious or unconscious) association of a stimulus with a specific response or behavior. For instance, Epple and Herz (1999) demonstrated that children who were exposed to an odorant during the performance of an insolvable task performed worse on other, solvable tasks when the same odorant was presented again. In contrast, no such impairment was observed when no odor or a different odor was presented. These results were interpreted as demonstrating negative olfactory conditioning. Along the same lines, Chu (2008) was able to show positive olfactory conditioning. In his study, children successfully performed a cognitive task, which they believed was insolvable in the presence of an ambient odor. When they were re-exposed to the same odorant, performance on other tasks improved significantly in comparison to another group of children who received a different odor. Since the children in Chu's investigation were described in school reports as underachieving and lacking self-confidence, one might speculate that only children with these specific attributes benefit the influence of fragrances. This seems to be confirmed by the study of

Kerl (1997), who found that, in general, ambient odors of lavender and jasmine did not improve memory functions in school children. However, lavender tended to increase performance in the memory task in children with high anxiety levels, which may have been consequent to the stress-relieving properties of lavender. On the other hand, jasmine impaired performance in lethargic children and this impairment in performance was negatively correlated with the children's rating of the odor. This result might indicate that lethargic children were distracted by the presence of an odorant they liked.

Several studies examined the influence of EOs on a number of memory-related variables in adults (Moss et al., 2003, 2008; Tildesley et al., 2005). These authors reported that lavender reduced the quality of memory and rosemary increased it, while both EOs reduced the speed of memory when compared to a no-odor control condition. At the same time, rosemary increased alertness in comparison to both the control and the lavender group, but exposure to the odorants led to higher contentedness than no scent. Similarly, peppermint enhanced memory quality while ylang-ylang impaired it and reduced processing speed. Ratings of mood showed that peppermint increased alertness while ylang-ylang decreased it and increased subjective calmness. In a third experiment, oral administration of Spanish sage [*Salvia lavandulifolia* Vahl (Lamiaceae)] improved both quality and speed of memory, and increased subjective ratings of alertness, calmness, and contentedness. The beneficial influence of an odorant being present in the learning phase on successive retrieval of information was shown by Morgan (1996). In this study, subjects were exposed or not exposed to a fragrance during the encoding of words unrelated to odor. Recall of the learned material was tested in three unannounced sessions 15 min apart, as well as 5 days after the learning phase. The results showed that performance in those groups that had not been exposed to an odorant in the learning phase declined continuously over time whereas it remained stable in those groups that had learned with ambient odor present. In addition, subjects who had learned under odor exposure performed significantly better when the odor was present during recall than those who had not received an odorant during the learning phase. These findings show that odorants in the encoding phase may serve as cues for later recall of the stored information. Recently, similar results have been reported in subjects who were presented with odorants while asleep (Rasch et al., 2007) demonstrating that fragrances may prompt memory consolidation during sleep. According to a study by Walla et al. (2002), it seems to be crucial whether an odorant in the encoding phase of a mnemonic task is consciously perceived and processed. These authors found differences in brain activation in a word recognition task as a function of conscious versus unconscious olfactory processing in the encoding phase. In other words, when odorants were presented during the learning phase and consciously perceived word recognition was more likely negatively affected than when the odor was not consciously processed. In addition, the same group of researchers demonstrated that word recognition performance was significantly poorer when the odorants were presented simultaneously with the words as opposed to continuously during the encoding phase, and when semantic (deep) as opposed to nonsemantic (shallow) encoding was required. These effects can be explained by a competition of processing resources in brain areas engaged in both language and odor processing (Walla et al., 2003). Similar results were also observed in an experiment involving the encoding of faces with and without odorants present in the learning phase (Walla et al. 2003). Again, recognition accuracy was impaired when an odor was simultaneously presented during encoding.

As discussed above, subjective experience of valence seems to modulate the influence of fragrances on cognition. For instance, Danuser et al. (2003) found no effects of pleasant olfactory stimuli on short-term memory whereas unpleasant odorants reduced the performance of healthy subjects, probably by distracting them. Habel et al. (2007) studied the effect of neutral and unpleasant olfactory stimulation on the performance of a working memory task and found that malodors significantly deteriorated working memory in only about half of the subjects. It was also shown that subjects in the affected group differed significantly in brain activation patterns from those in the unaffected group, that is, the latter showing stronger activation in fronto-parieto-cerebellar networks associated with working memory. In contrast, subjects whose performance was impaired by

the unpleasant odor showed greater activation in areas associated with emotional processing, such as the temporal and medial frontal cortex. The authors concluded that individual differences exist for the influence of fragrances on working memory and that unaffected subjects were better able to counteract the detrimental effect of unpleasant odor stimuli.

Leppanen and Hietanen (2003) tested the recognition speed of happy and disgusted facial expressions when pleasant or unpleasant odorants were presented during the recognition task. This study showed that pleasant olfactory stimuli had no particular influence on the speed of recognition of emotional facial expressions, that is, happy faces were recognized faster than disgusted faces. This result was also observed when no odorant was administered. In the unpleasant condition, however, the advantage for recognizing happy faces disappeared. These findings were interpreted as evidence for the modulation of emotion-related brain structures that form the perceptual representation of facial expressions by unpleasant odorants. Walla et al. (2005) supplied evidence that performance in a face recognition task was only affected when conscious odor processing took place In this study, two olfactory stimuli, that is, 2-PEA (**13**) and dihydrogen sulfide (H_2S), a trigeminal stimulus, that is, carbon dioxide (CO_2), and no odor were presented briefly and simultaneously to the presentation of faces. The results showed that the pure odorants irrespective of their valence improved recognition performance whereas CO_2 decreased it, and only CO_2, which is associated with painful sensations, was processed consciously by the participants of this investigation.

With regard to the content of memory, some researchers have claimed a special relationship between autobiographical, that is, personally meaningful episodic, memories and fragrances. As a result of this special link, it has been observed that memories evoked by olfactory cues are often older, more vivid, more detailed, and more affectively toned than those cued by other sensory stimuli (Chu and Downes, 2002; Goddard et al., 2005; Willander and Larsson, 2007). This phenomenon has been explained by the peculiar neuroanatomical connection of the memory systems with the emotional systems. Evidence for this hypothesis has for instance been supplied by the group of Herz (Herz and Cupchik, 1995; Herz, 2004; Herz et al., 2004), who demonstrated that presentation of odorants resulted in more emotional memories than presentation of the same cue in auditory or visual form. The authors also showed that if the odor cue was hedonically congruent with the item that had to be remembered, memory for associated emotional experience improved. Moreover, personally salient fragrance cues were associated with higher functional activity in emotion-related brain regions, such as the amygdala and the hippocampus.

10.1.4.3 Other Cognitive Tasks

The study of Degel and Köster (1999) described earlier showed that under certain conditions odorants may influence attentional performance even without subjects being aware of their presence. According to an investigation by Holland et al. (2005) the unnoticed presence of odorants may also affect everyday behavior and higher cognitive functions. The authors reported that subliminal concentrations of a citrus-scented cleaning product increased identification of cleaning-related words in a lexical decision task. Moreover, subjects exposed to the subliminal odor listed cleaning-related activities more frequently when asked to describe planned activities during the day and kept their environment tidier during an eating task.

The effects of pleasant suprathreshold fragrances on other higher cognitive functions were, for instance, investigated by Baron (1990). In this study, subjects were exposed to pleasant or neutral ambient odors while solving a clerical coding task and negotiating about monetary issues with a fellow participant. Before performing these tasks subjects indicated self-set goals and self-efficacy. Following the tasks subjects rated the experimental rooms in terms of pleasantness and comfort, as well as their mood. In addition, they were asked which conflict management strategies they would adopt in the future. Although neither fragrance had a direct effect on performance in the clerical task, subjects in the pleasant odor condition set higher goals and adopted a more efficient strategy in the task than those in the neutral odor condition. In addition, male subjects in the pleasant odor condition rated themselves as more efficient than those in the neutral odor condition.

In the negotiation task, subjects in the pleasant odor condition set higher monetary goals and made more concessions than those in the neutral odor condition. Moreover, subjects in the pleasant odor condition were in better mood and reported planning to handle future conflicts less often through confrontation and avoidance. Thus, this study showed that pleasant ambient fragrances offer a potential to create a more comfortable work environment and diminish aggressive behavior in situations involving competition. Gilbert et al. (1997) examined the effect of a pleasant and an unpleasant blend of fragrances on the same clerical coding task but were not able to show any influence of either fragrance on task performance. However, subjects exposed to unpleasant odorants believed that these odorants had negative effects on their performance in simple and difficult mathematical and verbal tasks (Knasko, 1993).

Finally, Ludvigson and Rottman (1989), studying the influence of lavender and clove EOs in ambient air in comparison to a no-odor control condition, found that lavender impaired performance in a mathematical reasoning task, while clove was devoid of effects. However, the lavender effect was only observed in the first of two sessions held one week apart. Also, subjects in the lavender condition rated the experimental conditions more favorably, while clove odor decreased subjects' willingness to return to the second session. Moreover, subjects who were exposed to an odor in one of the two sessions were generally less willing to return and had worse mood than subjects who never received an odor. To further complicate things, these odorant by session interactions were related to personality factors in a highly complex manner.

10.1.5 Conclusions

The presented review of the literature on the effects of EOs and fragrances on human arousal and cognition demonstrates that coherent findings are quite scarce and that we are still far from painting a detailed picture of which effects can be achieved by administering a particular EO and how these effects are precisely exerted. One reason for the inconsistency in results may be that in most studies in humans clear associations between constituents of EOs and observed effects are missing. While exact specifications about the origin, composition, and concentration of the tested oils would be necessary to establish clear pharmacological profiles and dose response curves for specific oils, in many investigations no such details are given. This renders attempts to compare the results from different studies and to generalize findings rather difficult.

Another aspect that clearly contributes to inconclusive findings is the involvement of a variety of mechanisms of action in the effects of EOs on human arousal and cognition. In a very valuable review on the assessment of olfactory processes with electrophysiological techniques, Lorig (2000) points out that EEG changes induced by odorants have to be interpreted with great care, since other direct odor effects may be responsible for changes, particularly when relaxing effects reflected by the induction of slow-wave activity are concerned. Physiological processes, such as altered breathing patterns in response to pleasant versus unpleasant odors, cognitive factors, for example, as a consequence of expectancy or the processing of secondary stimulus features, or an inappropriate baseline condition can lead to changes of the EEG pattern, which are quite unrelated to any psychoactive effect of the tested odorant. Well-designed paradigms are thus necessary to control for cognitive influences that might mask substance-specific effects of fragrances. Also, while EEG and other electrophysiological techniques are highly efficient to elucidate fragrance effects on the CNS in the time domain, we know only little about spatial aspects of such effects. Brain imaging techniques, such as functional magnetic resonance imaging (fMRI), will prove valuable to address this issue. Using fMRI, preliminary results from the author's group (Friedl et al., 2007) have shown that, after prolonged exposure, fragrances alter neuronal activity in distinct regions of the brain in a time-dependent manner and that this influence is sex specific.

When evaluating psychoactive effects of EOs and fragrances on human cognitive functions, the results should be interpreted just as cautiously as those of electrophysiological studies as similar confounding factors, ranging from influences of stimulus-related features, for example, pleasantness,

to expectation of fragrance effects and even personality traits, may be influencing the observed outcome. In regard to higher cognitive functioning, such as language or emotional processing, conscious as opposed to sub- or unconscious processing of odor information seems to differentially affect performance due to differences in the utilization of shared neuronal resources. Again, it seems worthwhile to measure additional parameters that are indicative of (subjective) stimulus information processing and emotional arousal if hypotheses are being built on direct (pharmacological) and cognitively mediated (psychological) odor effects on human behavior. Moreover, comparisons of different forms of application that involve or exclude stimulation of the olfactory system, such as inhalative versus noninhalative, dermal administration, have proven useful in the distinction of pharmacological from psychological mechanisms and will serve to enlarge our understanding of psychoactive effects of EO and fragrances in humans.

10.2 PSYCHOPHARMACOLOGY OF ESSENTIAL OILS

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The use of aromas goes back to ancient times, as implied by the nearly 200 references in the Bible relating the use of aromas for "mental, spiritual, and physical healing" (Perry and Perry, 2006). It is currently accepted that aromas and some of its individual components may in fact possess pharmacological and/or psychological properties, and in many instances the overall effect is likely to result from a combination of both. The following sections review the psychopharmacology of EOs and/or its individual components, as well as its mechanisms of action.

10.2.1 Aromatic Plants Used in Traditional Medical Systems as Sedatives or Stimulants

EOs are generally products of rather complex compositions used contemporaneously in aromatherapy, and for centuries as aromatic medicinal plant species in traditional systems of medicine. Aromatic formulas are used for the treatment of a variety of illnesses, including those that affect the CNS (Almeida et al., 2004). Volatile compounds presenting sedative or stimulatory properties have been and continue to be identified in EOs from aromatic medicinal species spread across different families and genera. The majority of these substances have small structures with less than 12 carbons and present low polarity chemical functions, being therefore quite volatile. Since most natural EOs are formed by complex mixtures, their bioactivity(ies) are obviously dependent on the contribution of their various components. Therefore, studies failing to characterize at least the main components of the EO studied are not discussed in this chapter.

Several *Citrus* EOs contain high proportions of limonene (**14**) as its major component. Orange peels are used as sedative in several countries, and EOs obtained from *Citrus aurantium* L. (Rutaceae) fruit peels can contain as much as 97.8% of limonene (**14**). The anxiolytic and sedative properties of *Citrus* EO suggested by traditional uses have been assessed in mice (Carvalho-Freitas and Costa, 2002; Pultrini et al., 2006) and also shown in a clinical setting (Lehrner et al., 2000). The relaxant effects observed in female patients in a dental office were produced with a *Citrus sinensis* (L.) Osbeck (Rutaceae) EO composed of 88.1% limonene (**14**) and 3.77% myrcene (**15**).

The common and large variability in the composition of natural EOs poses difficulties for the evaluation and the safe and effective use of aromatic medicinal plants. Genetic variations led to the occurrence of chemotypes, as in the case of *Lippia alba* (P. Mill.) N.E. Br. ex Britt. & Wilson (Verbenaceae). Analyses revealed three monoterpenic chemotypes characterized by the prevalence of myrcene (**15**) and citral (**16**) in chemotype I, limonene (**14**) and citral (**16**) in chemotype II, and limonene (**14**) and carvone (**17**) in chemotype III (Matos, 1996). This species is known in Brazil as "*cidreira*"; the aromatic tea from its leaves is traditionally used as a tranquilizer being one of the

most widely known home-made remedies. Pharmacological assays showed anxiolytic (Vale et al., 1999) and anticonvulsant (Viana et al., 2000) effects of EO samples from all three chemotypes. Anticonvulsive and sedative effects in mice were also demonstrated for the three isolated principal constituents of *Lippia alba* oils: limonene (**14**), myrcene (**15**), and citral (**16**) (Vale et al., 2002).

Various *Ocimum* species are used traditionally for sedative and anticonvulsive purposes, and its EOs seem to play an important role for these properties. However, besides the normal variability in the composition of the EOs, the occurrence of chemotypes seems to be generalized in this genus (Grayer et al., 1996; Vieira et al., 2001). The comparison of EO samples from different accessions of *Ocimum basilicum* L. (Lamiaceae) pointed to the occurrence of linalool (**3**) and methylchavicol (**18**) types, and it has been suggested that such data should be taken into account for an intraspecific classification of the taxon (Grayer et al., 1996). An EO of *O. basilicum* L. of the linalool-type containing 44.18% linalool (**3**), 13.65% 1,8-cineole (**2**), and 8.59% eugenol (**4**) as main constituents presented anticonvulsant and hypnotic activities (Ismail, 2006). There are two varieties of *Ocimum gratissimum* L. (Lamiaceae) (*O. gratissimum* L. var. *gratissimum* and *O. gratissimum* var. *macrophyllum* Briq.) that form a polymorphic complex very difficult to differentiate by morphological traits (Vieira et al., 2001). It was clearly demonstrated that the genetic variations of *O. gratissimum* led to three different chemotypes (Vieira et al., 2001): eugenol (**4**), thymol (**19**), and geraniol (**20**). EOs obtained from *O. gratissimum* of the eugenol-type collected during the 4 year seasons contained eugenol (**4**) (44.89–56.10%) and 1,8-cineole (**2**) (16.83–33.67%) as main components, and presented sedative and anticonvulsant activities slightly altered by the composition of each sample (Freire et al., 2006). A dose-dependent sedative effect was observed in mice and rats (Orafidiya et al., 2004) treated with *O. gratissimum* thymol-type EO containing 47.0% thymol (**19**), 16.2% *p*-cymene (**21**), and 6.2% α-terpinene (**22**) as major constituents.

Among Amazonian traditional communities, a widespread recipe that includes *Cissus sicyoides* L. (Vitaceae), *Aeolanthus suaveolens* Mart. ex Spreng. (Lamiaceae), *Ruta graveolens* L. (Rutaceae), and *Sesamum indicum* L. (Pedaliaceae) was identified as the most frequently indicated for the management of epilepsy-like symptoms. The results of pharmacological studies on the traditional preparations and the EO obtained from *Aeolanthus suaveolens* and its principal components led to the suggestion that the volatile lactones could be interesting target compounds in the search for new anticonvulsant agents (de Souza et al., 1997). Linalool (**3**) was identified as one of the principal active components of the *Aeolanthus suaveolens* EO (Elisabetsky et al., 1995b), and proved to play a key role in the central activities of the traditional preparation (Elisabetsky et al., 1995b, 1999; Brum et al., 2001a, b). (*R*)-(–)-Linalool (**7**) is recognized today as the sedative and calming component of numerous traditional and commercial preparations or their isolated natural EOs (Sugawara et al., 1998; Heuberger et al., 2004; Kuroda et al., 2005; Shaw et al., 2007). Epinepetalactone (**23**) is a volatile apolar compound and major component in the EO from *Nepeta sibthorpii* Benth. (Lamiaceae) responsible for the EO anticonvulsant activity (Galati et al., 2004).

Nature continues to reveal its inventiveness in combining different monoterpenes and arylpropenoids to achieve special nuances regarding central activities. An EO obtained from *Artemisia dracunculus* L. (Asteraceae) containing 21.1% *trans*-anethole (**24**), 20.6% α-*trans*-ocimene (**25**), 12.4% limonene (**14**), 5.1% α-pinene (**1**), 4.8% *allo*-ocimene (**26**), and 2.2% methyleugenol (**27**) shows anticonvulsant activity likely to be assigned to a combination of these various monoterpenes (Sayyah et al., 2004). β-Asarone (**28**) and its isomers are recognized as important sedative and anticonvulsive active components as in, for example, drugs based on *Acorus* species in which the total proportion of isomers can reach 90% (Koo et al., 2003; Mukherjee et al., 2007). In the EO from *Acorus tatarinowii* Schott (Acoraceae) rhizome, traditionally used for epilepsy, the combination of monoterpenes and arylpropenoids was found to be ~25% 1,8-cineole (**2**), ~12% linalool (**3**) and ~10% β-asarone (**28**), and isomers (Ye et al., 2006).

Traditional Chinese medicine (TCM) is known to use especially complex preparations, more often than not composed of several plant materials including therefore a number of active principles pertaining to different chemical classes. The TCM prescription SuHeXiang Wan combines different

proportions of as much as 15 crude drugs and is used orally for the treatment of seizure, infantile convulsion, and other conditions affecting the CNS (Koo et al., 2004). The EO obtained by hexane extraction at room temperature from a SuHeXiang Wan composed of nine crude drugs proved to have a relatively simple composition: 21.4% borneol (**29**), 33.3% isoborneol (**30**), 5.9% eugenol (**4**), and other minor components (Koo et al., 2004). The inhalation of this volatile mixture delayed the appearance of pentylenetetrazole (PTZ)-induced convulsions suggesting GABAergic modulation (Koo et al., 2004).

Other monoterpenoid or arylpropenoid derivatives identified as the active components of traditional sedatives include: methyleugenol (**27**) (Norte et al., 2005), isopulegol (**31**) (Silva et al., 2007), and α-terpineol (**32**) (de Sousa et al., 2007). It is worth mentioning that the monoterpene thujone (**33**), the dangerous principle of the ancient Absinthii herba, *Artemisia absinthium* L. (Asteraceae), induces marked central stimulatory effects, especially when used in the form of liqueur. Frequent and excessive use of this drug can cause intoxicated states accompanied by clonic convulsions among other serious consequences (Bielenberg, 2007).

Only a few volatile sesquiterpenes presenting important central activities are currently known. β-Eudesmol (**34**) was found to be one of the volatile active principles of the Chinese medicinal herb *Atractylodes lancea* DC. (Asteraceae) with alleged antagonist properties useful in intoxication by anticholinesterase agents of the organophosphorous type (Chiou et al., 1997). Experimental data show that β-eudesmol (**34**) prevents convulsions and lethality induced by electroshock but not those induced by PTZ or picrotoxin (Chiou et al., 1995). With a very similar chemical structure, α-eudesmol (**35**) protects the development of postischemic brain injury in rats by blocking ϖ-Aga-IVA-sensitive Ca^{2+} channels (Asakura et al., 2000).

The sesquiterpenes caryophyllene oxide (**36**) and β-selinene (=β-eudesmene) (**37**) isolated from the hexane extract from leaves of *Psidium guajava* var. *minor* Mattos (Myrtaceae) potentiated pentobarbital sleep and increased the latency for PTZ-induced convulsions in mice; blockade of extracellular Ca^{2+} was observed in isolated guinea-pig ileum with the hexane extract and its fractions containing both sesquiterpenes (Meckes et al., 1997). The similarity between the chemical structures of the sesquiterpenes (**34**), (**35**), and (**37**) is noteworthy and could indicate relevant characteristic patterns required for central activity.

10.2.2 Effects of EOs in Animal Models

Pure compounds isolated from aromas and complex EOs have been proved to induce a variety of effects on human and other mammalian species. Biological properties such as antispasmodic (Carvalho-Freitas et al., 2002) or other autonomic nervous system-related activities (Haze et al., 2002; Sadraei et al., 2003) are outside the scope of this chapter. Central effects have been extensively documented, and fall more often than not into the sedative (Buchbauer et al., 1991, 1993; Elisabetsky et al., 1995a; Lehrner et al., 2000), anxiolytic (Diego et al., 1998; Cooke and Ernst, 2000; Lehrner et al., 2000; Carvalho-Freitas et al., 2002), antidepressant (Komori et al., 1995a,b), and hypnotic (Diego et al., 1998) categories. As earlier stated, it is likely that the overall effects of EOs in humans results from a combination of physiological (in this case psychopharmacological) and psychological effects. Even when accepting the limitations of the validity of animal models in regard to assessing the effects of drugs on complex human emotional states, in the case of EOs the usefulness of experiments with rodent models is largely limited to clarify the physiological part of the potential effects in humans. Again, the reproducibility of the data compiled in this section would be highly dependent on the composition of EOs, unfortunately not always adequately reported.

Relevant to the data that follows, it has been shown that individual components of EOs administered orally, by means of intraperitoneal or subcutaneous injections, dermally, or by inhalation do reach and adequately cross the blood brain barrier (Kovar et al., 1987; Jirovetz et al., 1990; Buchbauer et al., 1993; Fujiwara et al., 1998; Moreira et al., 2001; Perry et al., 2002). The question of whether psychopharmacological effects in animals are dependent on olfactory functions is surprisingly not

yet entirely clarified. Cedrol (from pine EO) was shown to be sedative in normal rats and rats made anosmic with zinc sulfate (Kagawa et al., 2003). In contrast, a mix of chamomile and lavender oils reduced pentobarbital-induced sleeping time in normal but not anosmic rats and mice (Kagawa et al., 2003).

Rose and lavender oil, administrated intraperitoneally (i.p.) to mice, showed anticonflict effects (Umezu, 1999; Umezu et al., 2002), suggesting anxiolytic properties. Unequivocal anxiolytic properties were also demonstrated for lavender EO (i.p., gerbils) with the elevated plus maze test (Bradley et al., 2007a). Chen et al. (2004) reported that *Angelica sinensis* (Oliv.) Diels (Apiaceae) EO (orally administrated, mice) also has anxiolytic effect in the elevated plus maze, the light/dark model, and the stress-induced hyperthermia paradigms.

The dry rhizomes of *Acorus gramineus* Soland (Acoraceae) are officially listed in the Korean pharmacopoeia for sedative, digestive, analgesic, diuretic, and antifungal effects (Koo et al., 2003). Various CNS effects have been characterized for *Acorus gramineus* EO, including antagonism of PTZ-induced convulsion, potentiation of pentobarbital-induced sleeping time, sedation, and decreased spontaneous locomotion with the water and methanol extracts (mice, i.p.) (Vohora et al., 1990; Liao et al., 1998). Unexpectedly, given that sedative drugs usually impair cognition in animals, the oral administration of *Acorus gramineus* rhizoma EO, with eugenol (**4**) as the principal component, improved cognitive function in aged rats and mice; based on brain amine analyses, the authors suggest that such effects may be related to increased norepinephrine, dopamine, and serotonin relative levels, and to decreased activity of brain acetylcholinesterase (Zhang et al., 2007).

Cymbopogon winterianus Jowitt (Poaceae), popularly known as "citronella" and "java grass," is an important EO yielding aromatic grass, mostly cultivated in India and Brazil. *Cymbopogon winterianus* EO is rich in citronellal (**38**), geraniol (**39**), and citronellol (**40**) (Cassel and Vargas 2006), and has demonstrated anticonvulsant effects (i.p., mice) (Quintans-Júnior et al., 2007). Pharmacological studies with *Cymbopogon citratus* Stapf EO [presenting high percentage of citral (**16**) in its composition] revealed anxiolytic, hypnotic, and anticonvulsant properties when orally administrated to mice (Blanco et al., 2007).

The EO of *Eugenia caryophyllata* Thunb (Myrtaceae), used in Iranian traditional medicine, exhibits anticonvulsant activity against tonic seizures induced by maximal electroshock (MES) (i.p., mice) (Pourgholami et al., 1999a). The leaf EO of *Laurus nobilis* L. (Lauraceae), which has been used as an antiepileptic remedy in Iranian traditional medicine, demonstrated more anticonvulsant activity against experimental seizures induced by PTZ (i.p., in mice) than MES-induced seizures. Components responsible for this effect may include methyleugenol, eugenol, and pinene present in the EO (Sayyah et al., 2002). In the same manner, EO obtained from fruits of *Pimpinella anisum* S.G. Gmel. (Umbelliferae) demonstrated anticonvulsant activity against seizures induced by PTZ or MES in mice (i.p.) (Pourgholami et al., 1999b). As mentioned earlier, since no chemical analysis is reported for the studied EOs one can only speculate on the components that may be responsible for the activities observed with *E. caryophyllata*, *Laurus nobilis*, and *Pimpinella anisum*.

Various *Thymus* species of the Lamiaceae family (including *Thymus fallax* Fisch. & Mey., *Thymus kotschyanus* Boiss. & Hohen., *Thymus pubescens* Boiss. & Kotschy ex Celak, *Thymus vulgaris* M. Bieb.), which are widely distributed as aromatic and medicinal plants in many regions of Iran, have shown CNS effects (Duke et al., 2002). Pharmacological studies in mice (i.p.) with *Thymus fallax, Thymus kotschyanus*, and *Thymus pubescens* containing, respectively, 30.2% of carvacrol (**41**), 18.7% of pulegone (**42**), and 32.1% of carvacrol (**41**), demonstrated that the *Thymus fallax* EO has more antidepressant activity than *Thymus kotschyanus* and *Thymus pubescens* during the forced swimming test (Morteza-Semnani et al., 2007). These results illustrate that minor components of EOs can modify the activity of main components, reaffirming the importance of chemically characterizing EOs in order to understand its overall bioactivity.

An EO fraction obtained from powdered seeds of *Licaria puchury-major* (Mart.) Kosterm. (Lauraceae) containing 51.3% of safrol (**43**), 3.3% of eugenol (**4**), and 2.9% of methyleugenol (**27**) reduced locomotion and anesthetized mice, as well as affording protection against electroshock-induced convulsions (Carlini et al., 1983).

10.2.2.1 Effects of Individual Components

Section 10.2 listed the effects of several EO components found in traditionally used species, including α-pinene (**1**), eugenol (**4**), limonene (**14**), myrcene (**15**), citral (**16**), epi-nepetalactone (**23**), *trans*-anethole (**24**), α-*trans*-ocimene (**25**), *allo*-ocimene (**26**), methyleugenol (**27**), borneol (**29**), isoborneol (**30**), citronellal (**38**), geraniol (**39**), and citronellol (**40**); anticonvulsive, muscle relaxants, anxiolityc and/or hypnotic properties were observed with these compounds when given i.p. to mice (Vale et al., 1999, 2002; Galati et al., 2004; Koo et al., 2004; Sayyah et al., 2004; Cassel and Vargas, 2006; Blanco et al., 2007; Quintans-Júnior et al., 2007).

Linalool (**3**) is a monoterpene commonly found as a major volatile component of EOs in several aromatic plant species, such as *Lavandula angustifolia* Mill (Lamiaceae), *Rosa damascena* Mill. (Rosaceae), *Citrus bergamia* Risso (Rutaceae), *Melissa officinalis* L. (Lamiaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Cymbopogon citratus* DC ex Nees (Poaceae), and *Mentha piperita* L. (Lamiaceae). Interestingly, many linalool-producing species are traditionally used as sedative, analgesic, hypnotic, or anxiolytic remedies in traditional medicine and some as well in aromatherapy (Elisabetsky et al., 1995a).

As mentioned above, *Aeolanthus suaveolens* Mar. ex Spreng. (Lamiaceae) is used as an anticonvulsant through the Brazilian Amazon. The EO obtained from *Aeolanthus suaveolens* and its main component linalool (**3**) proved to be anticonvulsant against several types of experimental convulsions, including those induced by PTZ and transcorneal electroshock (Elisabetsky et al., 1995a), intracerebraly injected quinolinic acid, and i.p. NMDA (Elisabetsky et al., 1999). Moreover, psychopharmacological evaluation of linalool (**3**) showed dose-dependent marked sedative effects, including hypnotic, hypothermic, increased sleeping time, and decreased spontaneous locomotion in mice (i.p.) (Elisabetsky et al., 1995a; Linck et al., 2008). Decreased motor activity was also reported in mice by Buchbauer's group (Buchbauer et al., 1991). Indicating anxiolityc properties linalool (**3**) was reported to have anticonflict effects (mice, i.p.) in the Geller and Vogel tests, and similar findings were reported for lavender oil (Umezu, 2006). Analgesic properties were observed against chemical (rats, p.o.; Barocelli et al., 2004) (mice, s.c.; Peana et al., 2004a, 2004b) and thermal (mice, s.c., Peana et al., 2003) nociceptive stimuli. Since anti-inflammatory activity (rats, s.c.; Peana et al., 2002) has also been reported, it is not clear if linalool-induced analgesia is of central origin. Nevertheless, these experimental data are relevant to clinical studies indicating that aromatherapy with lavender can reduce the demand for opioids during the immediate postoperative period (Kim et al., 2007), and deserve further investigation. Linalool local anesthetic effects were observed *in vivo* by the conjunctival reflex test, and *in vitro* by phrenic nerve-hemidiaphragm preparation (Ghelardini et al., 1999).

10.2.2.2 Effects of Inhaled EOs

Despite the wide and growing use of aromatherapy in the treatment of a diversity of ailments, including those of central origin (Perry and Perry, 2006), and the alleged effects of incenses and other means of ambient aromas, experimental data on psychopharmacological properties of inhaled EOs is surprisingly scarce. Moreover, few of the studies control for inhalation flow and it is difficult to estimate the actual concentration of whatever is being inhaled. However, evidences that EOs and its components are absorbed by inhalation are available. After mice were exposed to a cage loaded with 27 mg of linalool (**3**) for 30, 60, or 90 min, plasma linalool concentrations of, respectively, 1.0, 2.7, and 3.0 ng/mL were found (Buchbauer et al., 1991); these exposure schedules resulted in an exposure-dependent decrease in locomotion. Moreover, detectable plasma concentrations at the nanogram level were reported for as many as 40 different aromatic compounds after 60 min of inhalation (Buchbauer et al., 1993). Therefore, despite the lack of precise measures of inhaled and/or absorbed quantities, it is arguable that animal studies with inhaled aromas are nevertheless informative.

Inhalation of citrus-based aromas [*Citrus sinensis* (L.) Osbeck (Rutaceae)] or fragrances were found to restore stress-induced immunosuppression (Shibata et al., 1990) and antidepressant-like effects in rats (2 mL/min EO in the air flux) (Komori et al., 1995a). A clinical study with depressed

patients revealed that it was possible to reduce the needed antidepressants' doses by inhaling a mixture of citrus oils; moreover, inhalation of the oil by itself was antidepressive and normalized neuroendocrine hormone levels (cortisol and dopamine) in depressive patients (Komori et al., 1995b). Relevant to these findings, inhaled lemon oil [*Citrus limonum* Risso (Rutaceae)] has been shown to increase the turnover of dopamine and serotonin after inhalation in mice (Komiya et al., 2006).

Anxiolytic proprieties were observed in rats with the plus maze model after 7 min rose oil inhalation; this is the only report of inhaled effects in animals after a short period of inhalation, although the procedure of leaving four cotton balls embedded with 2 mL of EO lacks standardization (Almeida et al., 2004). As mentioned above, inhaled cedrol was shown to be sedative in rats (Kagawa et al., 2003), and the inhalation of a volatile mixture from the TCM SuHeXiang Wan composed of 21.4% borneol (**29**), 33.3% isoborneol (**30**), 5.9% eugenol (**4**), and other minor components delayed the appearance of PTZ-induced convulsions suggesting GABAergic modulation (Koo et al., 2004).

Inhaled lavender oil [0–1–2 mL, 25% of linalool (**3**) and 46% of linalyl acetate (**44**)] demonstrated anxiolytic effects in rats, as shown by decreased peripheral movement and defecation in an open field, after at least 30 min of inhalation (Shaw et al., 2007). Similar effects were observed with inhaled lavender containing 38.47% of linalool (**3**) and 43.98% of linalyl acetate (**44**) in gerbils, showing increased exploratory behavior in the elevated plus maze test after 1 or 14 days inhalation (Bradley et al., 2007a). In agreement with studies with inhaled lavender EO, anxiolytic activity was observed in mice after inhaling (±)-linalool (**3**) at 1% or 3% inhaled for 60 min using the light/dark and an immobilization-induced stress paradigms; moreover, after the same inhalation procedure isolated mice exhibited decreased aggression toward an intruder, and increased social interaction was also observed (da Silva et al., 2008).

Finally, antinociceptive (mice) and gastroprotective effects (rats) of orally given or inhaled (60 min in a camera saturated with 2.4 μL/L) *Lavandula hybrida* Rev. (Lamiaceae) EO and its principal constituents linalool (**3**) and linalyl acetate (**44**) have also been reported (Barocelli et al., 2004).

10.2.3 Mechanism of Action Underlying Psychopharmacological Effects of EOs

The mechanisms of action underlying the effects of fragrances as complex mixtures as found *in natura*, or even for isolated components are far from been clarified, but seem to differ among different fragrances (Komiya et al., 2006). If the overall pharmacological effects of an EO depend on the contribution of its various components, the mechanism of action of a complex mixture is far more complex than a simple sum of each component's physiological consequence. Interaction among the various substances present in EOs can modify each other's pharmacodynamic and pharmacokinetic properties; nevertheless, studying the pharmacodynamic basis of isolated components is helpful for a comprehensive understanding of the basis of the physiological and psychopharmacological effects of EOs.

Rats treated (i.p.) with the isolated components of lemon EO, such as *R*-(+)-limonene (**14**), *S*-(−)-limonene (**45**), and citral, did not show the cold stress-induced elevation in norepinephrine and dopamine (Fukumoto et al., 2008); the authors suggested that these effects are related to changes in monoamine release in rat brain slices induced by these compounds (Fukumoto et al., 2003). Complementary to these findings, inhaled lemon oil (cage with a cotton ball with 1 mL, 90 min) has also been reported to increase the turnover of dopamine and serotonin in mice (Komiya et al., 2006).

Inhalation (2 g of fragrance/day, 2 × 3 h/day, for 7, 14, or 30 days at home cages) of *Acorus gramineus* Solander (Acoraceae) EO inhibited the activity of γ-aminobutyric acid (GABA) transaminase (an enzyme critical for metabolizing GABA at synapses), thereby significantly increasing GABA levels; a decrease in glutamate levels was also reported in this study (Koo et al., 2003). Both of these alterations in the inhibitory and excitatory neurotransmitter systems are compatible with and relevant to the sedative and anticonvulsant effects mentioned earlier. Neuroprotective effects on

cultured neurons were also reported for *Acorus gramineus* EO, apparently attained through the blockade of NMDA receptors given that this oil inhibits [^{3}H]-MK801 binding (Cho et al., 2001).

Other examples of EO actions on amino acid neurotransmitters are available. Morrone et al. (2007) using *in vivo* microdialysis showed that bergamot EO (i.p.) increased the levels of aspartate, glycine, taurine, GABA, and glutamate in a Ca^{2+}-dependent manner in rat hippocampus; the authors suggested that these same effects may be relevant for other monoterpenes affecting the CNS. Inhibition of glutamatemediated neurotransmission is in part responsible for the mechanisms of action underlying the above-mentioned anticonvulsant effects of linalool (**3**). Neurochemical assays reveal that (±)-linalool (**3**) acts as a competitive antagonist of L-[^{3}H]-glutamate binding (Elisabetsky et al., 1999), and also shows a dose-dependent noncompetitive inhibition of [^{3}H]-MK801 binding (IC_{50}=2.97 mM) indicating antagonism of NMDA glutamate receptors (Brum et al., 2001a). (±)-Linalool (**3**) also decreases the potassium-stimulated glutamate release and uptake in mice cortical synaptosomes, without affecting basal glutamate release (Brum et al., 2001b). This neurochemical profile explains, for instance, the linalool-induced delay in NMDA-induced convulsions and blockade of quinolinic acid-induced convulsions (Elisabetsky et al., 1999). Eugenol (**4**) was also found to inhibit excitotoxic neuronal effects induced by NMDA, apparently involving modulation of NMDA glutamate receptor, and inhibition of Ca^{2+} uptake (Wie et al., 1997; Won et al., 1998).

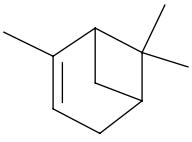
Because (±)-linalool (**3**) is also able to protect against PTZ- and picrotoxin-induced convulsions (Elisabetsky et al., 1999), a GABAergic modulation could also be in place. Nevertheless, (±)-linalool (**3**) did not alter [^{3}H]-muscinol binding (Brum, 2001a) suggesting that, if existing at all, linalool modulation of the GABAergic system is not mediated by $GABA_A$ receptors.

Potentially relevant to the pharmacology profile of linalool (**3**), patch-clamp techniques demonstrated that the monoterpene (no isomer specified) suppressed the Ca^{2+} current in rat sensory neurons and in rat cerebellar Purkinje cells (Narusuye et al., 2005). Based on their study with lavender EO, Aoshima and Hamamoto (1999) suggested that the GABAergic transmission may be of relevance for the mechanism of action of other monoterpenes, such as α-pinene (**1**), eugenol (**4**), citronellal (**38**), citronellol (**40**), and hinokitiol (**46**) (Aoshima and Hamamoto, 1999). Additionally, the antinociceptive activity of *R*-(–)-linalool (**7**) seems to involve several receptors, including opioids, cholinergic M_2, dopamine D_2, adenosine A_1 and A_{2A}, as well as changes in K^+ channels (Peana et al., 2003, 2004a, 2006).

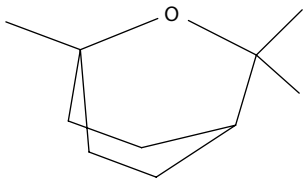
Relevant to studies on cognition, *Salvia lavandulifolia* Vahl (Lamiaceae) EO and isolated monoterpene constituents were shown to inhibit brain acetylcholinesterase and to present antioxidant properties (Perry et al., 2001, 2002).

Overall, its seems reasonable to argue that the modulation of glutamate and GABA neurotransmitter systems are likely to be the critical mechanisms responsible for the sedative, anxiolytic, and anticonvulsant proprieties of linalool and EOs containing linalool (**3**) in significant proportions.

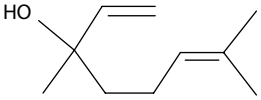
Chemical Structures of Mentioned CNS Active Compounds



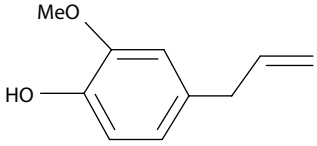
α-Pinene (**1**)



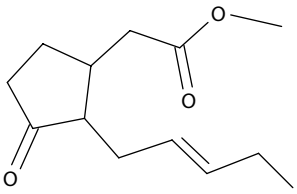
1,8-Cineole (**2**)



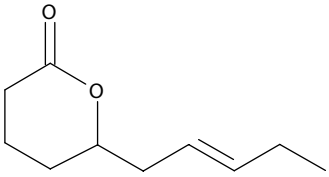
Linalool (**3**)



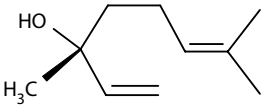
Eugenol (**4**)



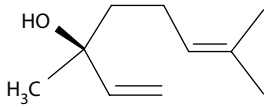
Methyl jasmonate (**5**)



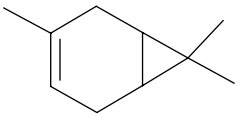
trans-Jasminlactone (**6**)

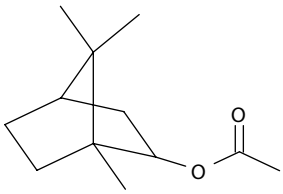


(R)-(–)-linalool (**7**)

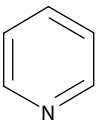


(S)-(+)-linalool (**8**)


Δ-3-Carene (**9**)

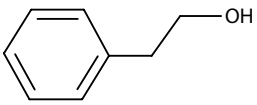


Bornyl acetate (**10**)

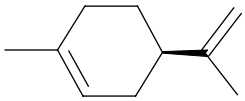


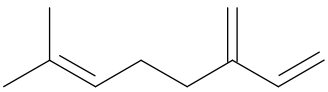
Pyridine (**11**)

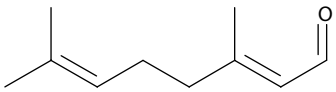
Allyl isothiocyanate (**12**)



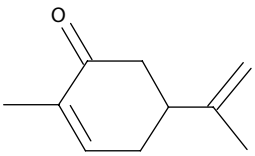
2-Phenyl ethyl alcohol (**13**)


Limonene (**14**)

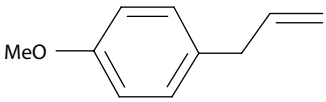

Myrcene (**15**)



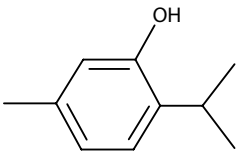
Citral (**16**)



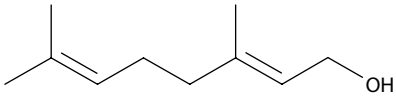
Carvone (**17**)



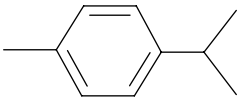
Methylchavicol (**18**)



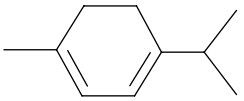
Thymol (**19**)



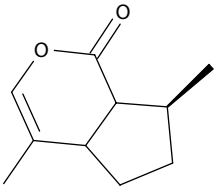
Geraniol (**20**)



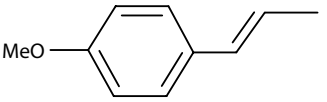
p-Cymene (**21**)



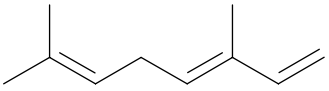
α-Terpinene (**22**)



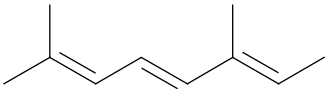
Epi-nepetalactone (**23**)



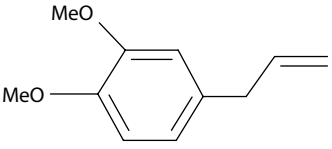
trans-Anethole (**24**)



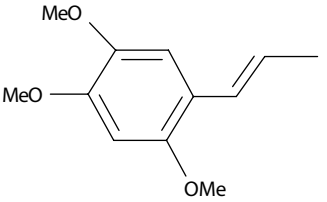
trans-Ocimene (**25**)



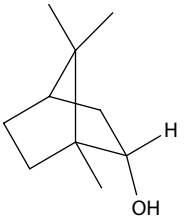
allo-Ocimene (**26**)



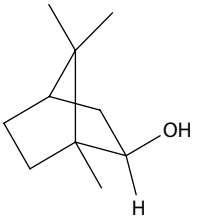
Methyl eugenol (**27**)



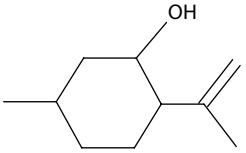
β-Asarone (**28**)



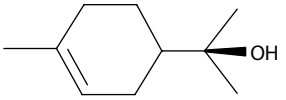
Borneol (**29**)



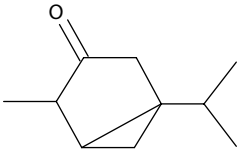
Isoborneol (**30**)



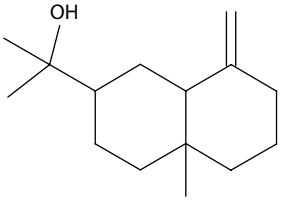
Isopulegol (**31**)



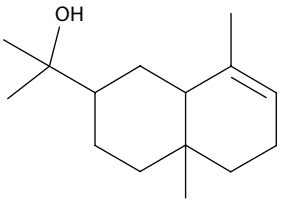
α-Terpineol (**32**)



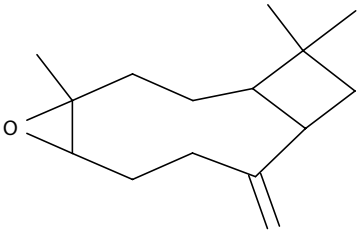
Thujone (**33**)



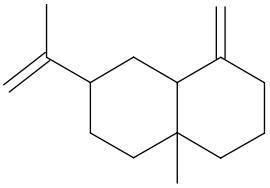
β-Eudesmol (**34**)



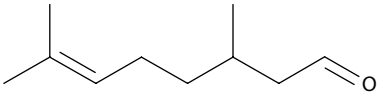
α-Eudesmol (**35**)



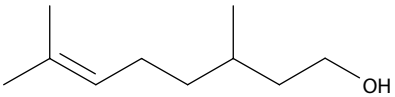
Caryophyllene oxide (**36**)



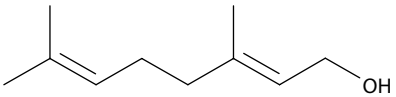
β-Eudesmene (**37**)



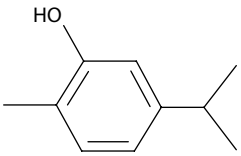
Citronellal (**38**)



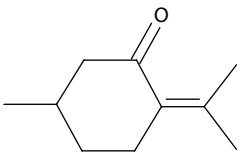
Geraniol (**39**)



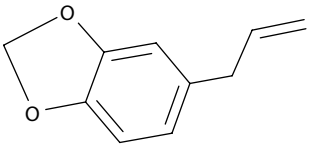
Citronellol (**40**)



Carvacrol (**41**)

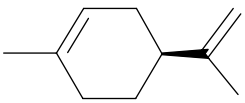


Pulegone (**42**)

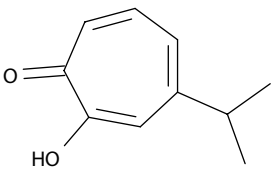


Safrol (**43**)

Linalyl acetate (**44**)



S-(−)-limonene (**45**)



Hinokitiol (**46**)

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11 Phytotherapeutic Uses of Essential Oils

Bob Harris

CONTENTS

11.1 INTRODUCTION

For many, the term "aromatherapy" originally became associated with the concept of the holistic use of essential oils to promote health and well-being. As time has progressed and the psychophysiological effects of essential oils have been explored further, their uses to reduce anxiety and aid sedation have also become associated with the term. This is especially so since the therapy has moved into the field of nursing, where such activities are of obvious benefit to patients in a hospital environment. More importantly, the practice of aromatherapy (in English-speaking countries) is firmly linked to the inhalation of small doses of essential oils and their application to the skin in high dilution as part of an aromatherapy massage.

This chapter is concerned with the medical use of essential oils, given to the patient by all routes of administration to treat specific conditions and in comparably concentrated amounts. Studies that use essential oils in an aromatherapy-like manner, for example, to treat anxiety by essential oil massage, are therefore excluded here.

Of the literature published in peer-reviewed journals over the last 30 years, only a small percentage concerns the administration of essential oils or their components to humans in order to treat disease processes. These reports are listed below in alphabetical order of their activity. The exception is the section on the respiratory tract, where the many activities of the two principal components (menthol and 1,8-cineole) are discussed and related to respiratory pathologies.

All of the references cited are from peer-reviewed publications; a minority is open to debate regarding methodology and/or interpretation of results, but this is not the purpose of this compilation. Reports of individual case studies have been omitted.

11.2 ACARICIDAL ACTIVITY

A number of essential oils have been found to have effective acaricidal activity against infections in the animal world. Recent examples include *Origanum onites* against cattle ticks (Coskun et al.,

2008) and *Cinnamomum zeylanicum* against rabbit mange mites (Fichi et al., 2007). In comparison to veterinary research, there have been few investigations into human acaricidal infections.

The scabies mite, *Sarcoptes scabiei* var. *hominis*, is becoming increasingly resistant to existing acaricidal compounds such as lindane, benzyl benzoate, permethrin, and oral ivermectin. The potential use of a 5% *Melaleuca alternifolia* essential oil solution to treat scabies infections was investigated *in vitro*. It was found to be highly effective at reducing mite survival times and the main active component was terpinen-4-ol. However, the *in vivo* effectiveness was only tested on one individual, in combination with benzyl benzoate and ivermectin (Walton et al., 2004).

A double-blind, randomized, parallel group study was used to compare the effects of 25% w/w benzyl benzoate emulsion with 20% w/w *Lippia multiflora* essential oil emulsion in the treatment of scabies infection in 105 patients. Applied daily, the cure rates for the oil emulsion were 50%, 80%, and 80% for 3, 5, and 7 days, respectively, compared to 30%, 60%, and 70% for the benzyl benzoate emulsion. There were also less adverse reactions to the oil emulsion, leading it to be considered as an additional formulation for the treatment of scabies (Oladimeji et al., 2005).

Although not an infection, the lethal activity of essential oils toward the house dust mite (*Dermatophagoides farina* and *Dermatophagoides pteronyssinus*) is important as these mites are a major cause of respiratory allergies and an etiologic agent in the sensitization and triggering of asthma in children. Numerous studies have been conducted, including the successful inclusion of *Eucalyptus globulus* in blanket washing solutions (Tovey and McDonald, 1997), the high acaricidal activity of clove, rosemary, eucalyptus, and caraway (El-Zemity et al., 2006), and of tea tree and lavender (Williamson et al., 2007).

11.3 ANTICARCINOGENIC

Despite the popularity of *in vitro* experimentation concerning the cellular mechanisms of carcinogenic prevention by essential oil components (mainly by inducing apoptosis), there is no evidence that the direct administration of essential oils can cure cancer. There is evidence to suggest that the mevalonate pathway of cancer cells is sensitive to the inhibitory actions of dietary plant isoprenoids (e.g., Elson and Yu, 1994; Duncan et al., 2005). Animal testing has shown that some components can cause a significant reduction in the incidence of chemically induced cancers when administered before and during induction (e.g., Reddy et al., 1997; Uedo et al., 1999).

Phase II clinical trials have all involved perillyl alcohol. Results demonstrated that despite preclinical evidence, there appeared to be no anticarcinogenic activity in cases of advanced ovarian cancer (Bailey et al., 2002), metastatic colorectal cancer (Meadows et al., 2002), and metastatic breast cancer (Bailey et al., 2008). Only one trial has demonstrated antitumor activity as evidenced by a reduction of tumor size in patients with recurrent malignant gliomas (Orlando da Fonseca et al., 2008).

11.4 ANTIMICROBIAL

Considering that the majority of essential oil research is directed toward antimicrobial activity, there is a surprising lack of corresponding *in vivo* human trials. This is disappointing since the topical and systemic application of essential oils to treat infection is a widespread practice among therapists with (apparently) good results.

11.4.1 Antibacterial

Antibiotics that affect *Propionibacterium acnes* are a standard treatment for acne but antibiotic resistance is becoming prevalent. A preliminary study of 126 patients showed that topical 2% essential oil of *Ocimum gratissimum* (thymol chemotype) in a hydrophilic cream base was more effective than 10% benzyl peroxide lotion at reducing the number of lesions when applied twice daily for 4 weeks (Orafidiya et al., 2002).

In a randomized, single-blind, parallel-group-controlled trial, the same group examined the effects of the addition of aloe vera gel at varying concentrations to the *Ocimum gratissimum* cream and compared its activity with 1% clindamycin phosphate. In the 84 patients with significant acne, it was found that increasing the aloe gel content improved efficacy; the essential oil preparations formulated with undiluted or 50% aloe gels were more effective at reducing lesions than the reference product. The aloe vera gels alone had minimal activity (Orafidiya et al., 2004).

A later report judged the efficacy of a 5% *Melaleuca alternifolia* gel in the amelioration of mild to moderate acne, since a previous study (Raman et al., 1995) had demonstrated the effectiveness of tea tree oil components against *Propionibacterium acnes*. The randomized, double-blind, placebo-controlled trial used 60 patients who were given the tea tree oil gel or the gel alone twice daily for 45 days. The total acne lesion count was significantly reduced by 43.64% and the acne severity index was significantly reduced by 40.49% after the tea tree oil treatment, as compared to the placebo scores of 12.03% and 7.04%, respectively (Enshaieh et al., 2007).

11.4.1.1 Methicillin-Resistant *Staphylococcus aureus*

A number of papers have demonstrated the *in vitro* effects of various essential oils against methicillin-resistant *Staphylococcus aureus* (MRSA); for example, *Lippia origanoides* (Dos Santos et al., 2004), *Backhousia citriodora* (Hayes and Markovic, 2002), *Mentha piperita*, *Mentha arvensis*, and *Mentha spicata* (Imai et al., 2001), and *Melaleuca alternifolia* (Carson et al., 1995). There have been no trials involving the use of essential oils to combat active MRSA infections, although there have been two studies involving the use of tea tree oil as a topical decolonization agent for MRSA carriers.

A pilot study compared the use of 2% mupirocin nasal ointment and triclosan body wash (routine care) with 4% *Melaleuca alternifolia* essential oil nasal ointment and 5% tea tree oil body wash in 30 MRSA patients. The interventions lasted for a minimum of 3 days and screening for MRSA was undertaken at 48 and 96 h post-treatment from sites previously colonized by the bacteria. There was no correlation between length of treatment and outcome in either group. Of the tea tree oil group, 33% were initially cleared of MRSA carriage while 20% remained chronically infected at the end of the treatment; this was in comparison with routine care group of 13% and 53%, respectively. The trial was too small to provide significant results (Caelli et al., 2000).

A randomized, controlled trial compared the use of a standard regime for MRSA decolonization with *Melaleuca alternifolia* essential oil. The 5-day study involved 236 patients. The standard treatment group was given 2% mupirocin nasal ointment thrice daily, 4% chlorhexidine gluconate soap as a body wash once daily, and 1% silver sulfadiazine cream for skin lesions, wounds, and leg ulcers once daily. The tea tree oil group received 10% essential oil cream thrice daily to the nostrils and to specific skin sites and 5% essential oil body wash at least once daily. In the tea tree oil group, 41% were cleared of MRSA as compared to 49% using the standard regime; this was not a significant difference. Tea tree oil cream was significantly less effective at clearing nasal carriage than mupirocin (47% compared to 78%), but was more effective at clearing superficial sites than chlorhexidine or silver sulfadiazine (Dryden et al., 2004).

11.4.2 Antifungal

The essential oil of *Citrus aurantium* var. *amara* was used to treat 60 patients with tinea corporis, cruris, or pedis. One group received a 25% bitter orange (BO) oil emulsion thrice daily, a second group was treated with 20% bitter orange oil in alcohol (BOa) thrice daily, and a third group used undiluted BO oil once daily. The trial lasted for 4 weeks and clinical and mycological examinations were performed every week until cure, which was defined as an elimination of signs and symptoms. In the BO group, 80% of patients were cured in 1–2 weeks and the rest within 2–3 weeks. By using BOa, 50% of patients were cured in 1–2 weeks, 30% in 2–3 weeks, and 20% in 3–4 weeks. With the undiluted essential oil, 25% of patients did not continue treatment, 33.3% were cured in 1 week, 60% in 1–2 weeks, and 6.7% in 2–3 weeks (Ramadan et al., 1996).

A double-blind, randomized, placebo-controlled trial investigated the efficacy of 2% butenafine hydrochloride cream with added 5% *Melaleuca alternifolia* essential oil in 60 patients with toenail onychomycosis. After 16 weeks, 80% of patients in the treatment group were cured, as opposed to none in the control group (Syed et al., 1999). However, butenafine hydrochloride is a potent antimycotic in itself and the results were not compared with this product when used alone.

After an initial *in vitro* study, which showed that the essential oil of *Eucalyptus pauciflora* had a strong fungicidal activity against *Epidermophyton floccosum*, *Microsporum canis*, *Microsporum nanum*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Trichophyton violaceum*, an *in vivo* trial was commenced. Fifty patients with confirmed dermatophytosis were treated with 1% v/v essential oil twice daily for 3 weeks. At the end of the treatment, a cure was demonstrated in 60% of patients with the remaining 40% showing significant improvement (Shahi et al., 2000).

On the surmise that infection with *Pityrosporum ovale* is a major contributing factor to dandruff and that anti-*Pityrosporum* drugs such as nystatin were proven effective treatments, the use of 5% *Melaleuca alternifolia* essential oil was investigated. In this randomized, single-blind, parallel-group study tea tree oil shampoo or placebo shampoo was used daily for 4 weeks by 126 patients with mild to moderate dandruff. In the treatment group, the dandruff severity score showed an improvement of 41%, as compared to 11% in the placebo group. The area involvement and total severity scores also demonstrated a statistically significant improvement, as did itchiness and greasiness. Scaliness was not greatly affected. The condition resolved for one patient in each group and so ongoing application of tea tree oil shampoo was recommended for dandruff control (Satchell et al., 2002a).

For inclusion in a randomized, double-blind, controlled trial, 158 patients with the clinical features of intertriginous tinea pedis and confirmed dermatophyte infection were recruited. They were administered 25% or 50% *Melaleuca alternifolia* essential oil (in an ethanol and polyethylene glycol vehicle) or the vehicle alone, twice daily for 4 weeks. There was an improvement in the clinical severity score, falling by 68% and 66% in the 25% and 50% tea tree oil groups, in comparison with 41% for the placebo. There was an effective cure in the 25% and 50% tea tree oil and placebo groups of 48%, 50%, and 13%, respectively. The essential oil was less effective than standard topical treatments (Satchell et al., 2002b).

The anticandida properties of *Zataria multiflora* essential oil and its active components (thymol, carvacrol, and eugenol) were demonstrated *in vitro* by Mahmoudabadi et al. (2006). A randomized, clinical trial was conducted using 86 patients with acute vaginal candidiasis. They were treated with a cream containing 0.1% *Zataria multiflora* essential oil or 1% clotrimazole once daily for 7 days. Statistically significant decreases in vulvar pruritis (80.9%), vaginal pruritis (65.5%), vaginal burning (73.95), urinary burning (100%), and vaginal secretions (90%) were obtained by the essential oil treatment as compared to the clotrimazole treatment of 73.91%, 56.7%, 82.1%, 100%, and 70%, respectively. In addition, the *Zataria multiflora* cream reduced erythema and satellite vulvar lesions in 100% of patients, vaginal edema in 100%, vaginal edema in 83.3%, and vulvo-vaginal excoriation and fissures in 92%. The corresponding results for clotrimazole were 100%, 100%, 76%, and 88%. In terms of overall efficacy, the rates of improvement were 90% and 74.8% for the *Zataria multiflora* and clotrimazole groups, respectively. Use of the cream alone provided no significant changes (Khosravi et al., 2008).

11.4.3 Antiviral

The *in vitro* studies that have been conducted so far indicate that many essential oils possess antiviral properties, but they affect only enveloped viruses and only when they are in the free state, that is, before the virus is attached to, or has entered the host cell (e.g., Schnitzler et al., 2008). This is in contrast to the majority of synthetic antiviral agents, which either bar the complete penetration of viral particles into the host cell or interfere with viral replication once the virus is inside the cell.

A randomized, investigator-blinded, placebo-controlled trial used 6% *Melaleuca alternifolia* essential oil gel to treat recurrent herpes labialis. It was applied five times daily and continued until re-epithelialization occurred and the polymerase chain reaction (PCR) for Herpes simplex virus was negative for two consecutive days. The median time to re-epithelialization after treatment with tea tree oil was 9 days as compared to 12.5 days with the placebo, which is similar to reductions caused by other topical therapies. The median duration of PCR positivity was the same for both groups (6 days) although the viral titers appeared slightly lower in the oil group on days 3 and 4. None of the differences reached statistical significance, probably due to the small group size (Carson et al., 2001).

Children below 5 years were enrolled in a randomized trial to test a 10% v/v solution of the essential oil of *Backhousia citriodora* against molluscum contagiosum (caused by Molluscipoxvirus). Of the 31 patients, 16 were assigned to the treatment group and the rest to the control of olive oil. The solutions were applied directly to the papules once daily at bedtime for 21 days or until the lesions had resolved. In the essential oil group, five children had a total resolution of lesions and four had reductions of greater than 90% at the end of 21 days. In contrast, none of the control group had any resolution or reduction of lesions by the end of the study period (Burke et al., 2004).

A study was conducted on 60 patients who were chronic carriers of hepatitis B or C. The essential oils of *Cinnamomum camphora* ct 1,8-cineole, *Daucus carota*, *Ledum groelandicum*, *Laurus nobilis*, *Helichrysum italicum*, *Thymus vulgaris* ct thujanol, and *Melaleuca quinquenervia* were used orally in various combinations. They were used as a monotherapy or as a complement to allopathic treatment. The objectives of treatment were normalization of transaminase levels, reduction of viral load, and stabilization or regression of fibrosis. There was an improvement of 100%, when patients with hepatitis C were given bitherapy with essential oils. With essential oil monotherapy, improvements were noted in 64% of patients with hepatitis C and there were two cures of hepatitis B (Giraud-Robert, 2005).

11.4.4 Microbes of the Oral Cavity

The activities of essential oils against disease-producing microbes in the oral cavity have been documented separately because there are numerous reports of relevance. The easy administration of essential oils in mouthrinses, gargles, and toothpastes, and the success of such commercial preparations, has no doubt led to the popularity of this research.

The *in vitro* activities of essential oils against the oral microflora are well documented and these include effects on cariogenic and periodontopathic bacteria. One example is the *in vitro* activity of *Leptospermum scoparium*, *Melaleuca alternifolia*, *Eucalyptus radiata*, *Lavandula officinalis*, and *Rosmarinus officinalis* against *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Streptococcus mutans*. The essential oils inhibited all of the test bacteria, acting bactericidally except for *Lavandula officinalis*. In addition, significant adhesion-inhibiting activity was shown against *Streptococcus mutans* by all essential oils and against *Porphyromonas gingivalis* by tea tree and manuka (Takarada et al., 2004).

There have been at least six *in vivo* studies concerning the activity of individual essential oils against the microflora of the oral cavity. In addition, a review of the literature finds a surprising number of *in vivo* papers that detail the activities of "an essential oil mouthrinse." Closer examination reveals that the essential oil mouthrinse is the commercial product, Listerine. Although Listerine contains 21% or 26% alcohol (depending on the exact product), a 6-month study has shown that it contributes nothing to the efficaciousness of the mouthrinse (Lamster et al., 1983). The active ingredients are 1,8-cineole (0.092%), menthol (0.042%), methyl salicylate (0.06%), and thymol (0.64%). For this reason, a small random selection of such papers is included below.

11.4.4.1 Activity of Listerine against Plaque and/or Gingivitis

An observer-blind, 4-day plaque regrowth, crossover study compared the use of Listerine® with a triclosan mouthrinse and two placebo controls in 32 volunteers. All normal hygiene procedures were suspended except for the rinses. The triclosan product produced a 45% reduction in plaque area and a 12% reduction in plaque index against its placebo, in comparison with 52% and 17%, respectively, for the essential oil rinse. The latter was thus deemed more effective (Moran et al., 1997).

A similar protocol was used to compare the effects of Listerine against an amine fluoride/stannous fluoride-containing mouthrinse (Meridol®) and a 0.1% chlorhexidine mouthrinse (Chlorhexamed®) in inhibiting the development of supragingival plaque. On day 5 of each treatment, the results from 23 volunteers were evaluated. In comparison with their placebos, the median plaque reductions were 12.2%, 23%, and 38.2% for the fluoride, essential oil, and chlorhexidine rinses, respectively. The latter two results were statistically significant (Riep et al., 1999).

After the assessment for the presence of gingivitis and target pathogens (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Veillonella* sp.) and total anaerobes, 37 patients undertook a twice daily mouthrinse with Listerine for 14 days. After a washout period, the study was conducted again using a flavored hydroalcoholic placebo. The results of this randomized, double-blind, crossover study showed that the essential oil rinse significantly lowered the number of all target pathogens by 66.3–79.2%, as compared to the control (Fine et al., 2007).

The effect of adding Listerine mouthrinse to a standard oral hygiene regime in 50 orthodontic patients was examined. The control group brushed and flossed twice daily, whereas the test group also used the mouthrinse twice daily. Measurements of bleeding, gingival, and plaque indices were conducted at 3 and 6 months. All three indices were significantly lowered in the test group as compared to the control at both time intervals (Tufekci et al., 2008).

The same fixed combination of essential oils that is found in Listerine mouthrinse has been incorporated into a dentifrice. Such a dentifrice was used in a 6-month double-blind study to determine its effect on the microbial composition of dental plaque as compared to an identical dentifrice without essential oils. Supragingival plaque and saliva samples were collected at baseline and their microbial content characterized, after which the study was conducted for 6 months. The essential oil dentifrice did not significantly alter the microbial flora and opportunistic pathogens did not emerge, nor was there any sign of developing resistance to the essential oils in tested bacterial species (Charles et al., 2000).

The same dentifrice was examined for antiplaque and antigingivitis properties in a blinded, randomized, controlled trial. Before treatment, 200 patients were assessed using a plaque index, a modified gingival index (GI), and a bleeding index. The dentifrice was used for 6 months, after which another assessment was made. It was found that the essential oil dentifrice had a statistically significant lower whole-mouth and interproximal plaque index (18.3% and 18.1%), mean GI (16.2% and 15.5%), and mean bleeding index (40.5% and 46.9%), as compared to the control. It was therefore proven to be an effective antiplaque and antigingivitis agent (Coelho et al., 2000).

11.4.4.2 Antiviral Listerine

A trial was conducted to examine whether a mouthrinse could decrease the risk of viral crosscontamination from oral fluids during dental procedures. Forty patients with a perioral outbreak of recurrent herpes labialis were given a 30-s mouthrinse with either water or Listerine. Salivary samples were taken at baseline, immediately following the rinse and 30 min after the rinse and evaluated for the viral titer. Infectious virions were reduced immediately to zero postrinse and there was a continued significant reduction 30 min postrinse. The reduction by the control was not significant (Meiller et al., 2005).

11.4.4.3 Activity of Essential Oils

The antibacterial activity of the essential oil of *Lippia multiflora* was first examined *in vitro* for antimicrobial activity against ATCC strains and clinical isolates of the buccal flora. A significant

activity was found, with an MBC of 1/1400 for streptococci and staphylococci, 1/800 for enterobacteria and neisseria, and 1/600 for candida. A mouthwash was prepared with the essential oil at a 1/500 dilution and this was used in two clinical trials.

The buccodental conditions of 26 French children were documented by measuring the percentage of dental surface free of plaque, gum inflammation, and the papillary bleeding index (PBI). After 7 days of rinsing with the mouthwash for 2 min, the test group was found to have a reduction of dental plaque in 69% of cases and a drop in PBI with a clear improvement of gum inflammation in all cases. The second trial was conducted in the Cote d'Ivoire with 60 adult patients with a variety of conditions. After using the mouthwash after every meal for 5 days, it was found that candidiasis had disappeared in most cases, gingivitis was resolved in all patients, and 77% of dental abscesses had resorbed (Pélissier et al., 1994).

Fluconazole-refractory oropharyngeal candidiasis is a common condition in HIV patients. Twelve such patients were treated with 15 mL of a *Melaleuca alternifolia* oral solution (Breath-Away) four times daily for 2 weeks, in a single center, open-label clinical trial. The solution was swished in the mouth for 30–60 s and then expelled, with no rinsing for at least 30 min. Clinical assessment was carried out on days 7 and 14 and also on days 28 and 42 of the follow-up. Two patients were clinically cured and six were improved after the therapy; four remained unchanged and one deteriorated. The overall clinical response rate was thus 67% and was considered as a possible alternative antifungal treatment in such cases (Jandourek et al., 1998).

A clinical pilot study compared the effect of 0.34% *Melaleuca alternifolia* essential oil solution with 0.1% chlorhexidine on supragingival plaque formation and vitality. Eight subjects participated, with a 10-day washout period between each treatment regime of 1 week. The plaque area was calculated using a stain and plaque vitality was estimated using a fluorescence technique. Neither of these parameters was reduced by the tea tree oil treatment (Arweiler et al., 2000).

A gel containing 2.5% *Melaleuca alternifolia* essential oil was used in a double-blind, longitudinal noncrossover trial and compared with a chlorhexidine gel positive control and a placebo gel in the treatment of plaque and chronic gingivitis. The gels were applied as a dentifrice twice daily by 49 subjects for 8 weeks and the treatment was assessed using a gingival index (GI), a PBI, and a plaque staining score. The tea tree group showed a significant reduction in PBI and GI scores, although plaque scores were not reduced. It was apparent that the tea tree gel decreased the level of gingival inflammation more than the positive or negative controls (Soukoulis and Hirsch, 2004).

A mouthcare solution consisting of an essential oil mixture of *Melaleuca alternifolia*, *Mentha piperita*, and *Citrus limon* in a 2:1:2 ratio diluted in water to a 0.125% solution was used to treat oral malodor in 32 intensive care unit patients, 13 of whom were ventilated. The solution was used to clean the teeth, tongue, and oral cavity twice daily. The level of malodor was assessed by a nurse using a visual analogue scale, and volatile sulfur compounds (VSC) were measured via a probe in the mouth, before, 5 and 60 min after treatment. On the second day, the procedure was repeated using benzydamine hydrochloride (BH), which is normally used for oral hygiene, instead of essential oil solution. The perception of oral malodor was significantly lowered after the essential oil treatment but not after the BH treatment. There was a decrease in VSC levels at 60 min for both treatment groups, but not after 5 min for the oil mixture. The results suggested that just one session with the essential oil mixture could improve oral malodor and VSC in intensive care patients (Hur et al., 2007).

The essential oil of *Lippia sidoides* (rich in thymol and carvacrol) was used in a double-blind, randomized, parallel-armed study against gingival inflammation and bacterial plaque. Fifty-five patients used a 1% essential oil solution as a mouthrinse twice daily for 7 days and the results were compared with a positive control, 0.12% chlorhexidine. Clinical assessment demonstrated decreased plaque index and gingival bleeding scores as compared to the baseline, with no significant difference between test and control. The essential oil of *Lippia sidoides* was considered a safe and effective treatment (Botelho et al., 2007).

11.4.5 Controlling Microflora in Atopic Dermatitis

Rarely found on healthy skin, *Staphylococcus aureus* is usually present in dry skin and is one of the factors that can worsen atopic dermatitis. Toxins and enzymes deriving from this bacteria cause skin damage and form a biofilm from fibrin and glycocalyx, which aids adhesion to the skin and resistance to antibiotics. An initial *in vitro* study found that a mixture of xylitol (a sugar alcohol) and farnesol was an effective agent against *Staphylococcus aureus*; xylitol inhibited the formation of glycocalyx whereas farnesol dissolved fibrin and suppressed *Staphylococcus aureus* growth without affecting *Staphylococcus epidermidis* (Masako et al., 2005a).

The same mixture of xylitol and farnesol was used in a double-blind, randomized, placebo-controlled study of 17 patients with mild to moderate atopic dermatitis on their arms. A skin-care cream containing 0.02% farnesol and 5% xylitol or the cream alone was applied to either the left or the right arms for 7 days. The ratio of *Staphylococcus aureus* to other aerobic skin microflora was significantly decreased in the test group compared to placebo, from 74% to 41%, while the numbers of coagulase-negative staphylococci increased. In addition, skin conductance (indicating hydration of skin surface) significantly increased at the test cream sites compared to before application and to the placebo (Masako et al., 2005b).

11.4.6 Odor Management for Fungating Wounds

Fungating wounds may be caused by primary skin carcinomas, underlying tumors or via spread from other tissues. The malodor associated with such necrosis is caused by the presence of aerobic and anaerobic bacteria. The wounds rarely heal and require constant palliative treatment, leading to social isolation of the patients and poor quality of life.

Smell reduction with essential oils was first reported by Warnke et al. (2004) in 25 malodorous patients with inoperable squamous cell carcinoma of the head and neck. A commercial product containing eucalyptus, grapefruit, and tea tree essential oils (Megabac®) was applied topically to the wounds twice daily. Normal medication apart from Betadine disinfection was continued. The smell disappeared completely within 2–3 days and signs of superinfection and pus secretion were reduced in the necrotic areas.

Megabac has also been used in a small pilot study (10 patients) to treat gangrenous areas, being applied via spray thrice daily until granulation tissue formed. The treatment was then continued onto newly formed split skin grafts. All wounds healed within 8 weeks and no concurrent antibiotics were used (Sherry et al., 2003).

Use of essential oils to reduce the smell of fungating wounds in 13 palliative care patients was detailed by another group the following year. *Lavandula angustifolia*, *Melaleuca alternifolia*, and *Pogostemon cablin* essential oils were used alone or in combinations at 2.5–5% concentrations in a cream base. The treatments were effective (Mercier and Knevitt, 2005).

A further study was conducted with 30 patients suffering incurable head and neck cancers with malodorous necrotic ulcers. A custom-made product (Klonemax®) containing eucalyptus, tea tree, lemongrass, lemon, clove, and thyme essential oils was applied topically (5 mL) twice daily. All patients had a complete resolution of the malodor; in addition to the antibacterial activity, an anti-inflammatory effect was also noted (Warnke et al., 2006).

The use of essential oils to treat malodorous wounds in cancer patients is becoming widespread in many palliative care units although no formal clinical trials have been conducted as yet.

11.5 DISSOLUTION OF HEPATIC AND RENAL STONES

11.5.1 Gall and Biliary Tract Stones

Rowachol and Rowatinex are two commercial products that have been marketed for many years and are based on essential oil components. They are sometimes thought of as being the same product but

TABLE 11.1
Composition of Rowachol and Rowatinex Declared by the Manufacturers

Component	Rowachol	Rowatinex
α-pinene	20.0	37.0
β-pinene	5.0	9.0
Camphene	8.0	22.0
1,8-cineole	3.0	4.0
Fenchone	—	6.0
Menthone	9.0	—
Borneol	8.0	15.0
Menthol	48.0	—
anethole	—	6.0

Source: Sybilska, D. and M. Asztemborska, 2002. *J. Biochem. Biophys. Methods*., 54: 187–195.

in fact they are different. The compositions have changed slightly over the years and the most recently disclosed are shown in Table 11.1.

Rowachol has been in use for over 50 years for the dissolution of gallstones and biliary tract stones. There have been many published works on its effects and at least one double-blind trial (Lamy, 1967). It has been stated that although the dissolution rate of Rowachol is not impressive, it is still much greater than Rowatinex and could occur spontaneously (Doran and Bell, 1979). It has been employed alone as a useful therapy for common duct stones (Ellis and Bell, 1981) although improved results were demonstrated when Rowachol was used in conjunction with bile acid therapy (Ellis et al., 1981).

Rowachol has been shown to inhibit hepatic cholesterol synthesis mediated by a decreased hepatic *S*-3-hydroxy-3-methylgutaryl-CoA reductase activity (Middleton and Hui, 1982); the components mostly responsible for this activity were menthol and 1,8-cineole, with pinene and camphene having no significant effect (Clegg et al., 1980). A reduction in cholesterol crystal formation in the bile of gallstone patients has been demonstrated in a small trial using Rowachol (von Bergmann, 1987).

Two early uncontrolled trials reported that Rowachol significantly increased plasma high-density lipoprotein (HDL) cholesterol when administered to patients with low HDL cholesterol; a twofold increase was found in 10 subjects after 6 weeks of treatment (Hordinsky and Hordinsky, 1979), while a progressive increase in HDL of 14 subjects was noted, >100% after 6 months (Bell et al., 1980). This was interesting as low plasma concentrations of HDLs are associated with an elevated risk of coronary heart disease. However, a double-blind, placebo-controlled trial that administered six capsules of Rowachol daily for 24 weeks to 19 men found that there were no significant HDL-elevating effects of the treatment (Cooke et al., 1998). It is currently thought that monoterpenes have no HDL-elevating potential that is useful for disease prevention.

In vitro, a solution of 97% *d*-limonene was found to be 100-fold better at solubilizing cholesterol than sodium cholate. A small trial followed with 15 patients, whereby 20 ml of the *d*-limonene preparation was introduced into the gallbladder via a catheter on alternate days for up to 48 days. The treatment was successful in 13 patients with gallstone dissolution sometimes occurring after three infusions. Side effects included vomiting and diarrhea (Igimi et al., 1976).

A further study was conducted by Igimi et al. (1991) using the same technique with 200 patients. Treatments lasted from 3 weeks to 4 months. Complete or partial dissolution of gallstones was achieved in 141 patients, with complete disappearance of stones in 48% of cases. Epigastric pain was experienced by 168 patients and 121 suffered nausea and vomiting. Further trials have not been conducted.

11.5.2 Renal Stones

While Rowachol is used as a measure against gallstones and biliary tract stones, Rowatinex is used in the treatment of renal stones. The first double-blind, randomized trial was conducted by Mukamel et al. (1987) on 40 patients with acute renal colic. In the Rowatinex group, there was a significantly higher expulsion rate of stones ≥3 mm in diameter in comparison with the placebo (61% and 28%, respectively). There was also a higher overall success rate in terms of spontaneous stone expulsion and/or disappearance of ureteral dilatation in the treatment group compared to placebo (78–52%), but the difference was not statistically significant.

A second double-blind, randomized trial was conducted on 87 patients with ureterolithiasis. Four Rowatinex capsules were prescribed four times a day, the average treatment time being two weeks. The overall stone expulsion rate was significantly higher in the Rowatinex group as compared to placebo; 81% and 51%, respectively. Mild to moderate gastrointestinal disturbances were noted in seven patients. It was concluded that the early treatment of ureteral stones with Rowatinex was preferable before more aggressive measures were considered (Engelstein et al., 1992).

Rowatinex has also been used with success in the removal of residual stone fragments after extracorporeal shock wave lithotriptsy, a situation that occurs in up to 72% of patients when given this therapy. With 50 patients, it was found that Rowatinex decreased the number of calculi debris, reducing the number of late complications and further interventions. By day 28, 82% of patients were free of calculi whereas this situation is normally reached after 3 months without Rowatinex treatment (Siller et al., 1998).

A minor study examined the use of Rowatinex in the management of childhood urolithiasis. Six children aged from 4 months to 5 years were administered varying doses of the preparation from 10 days to 12 weeks. All patients became stone-free with no side effects, although a definite conclusion as to the efficacy of treatment could not be established due to the small patient number involved (Al-Mosawi, 2005).

A comparison of the effects of an α-blocker (tamsulosin) and Rowatinex for the spontaneous expulsion of ureter stones and pain control was undertaken using 192 patients. They were divided into three groups: analgesics only, Rowatinex with analgesics, and tamsulosin with analgesics. For ureter stones less than 4 mm in diameter, their excretion was accelerated by both Rowatinex and tamsulosin. The use of these two treatments also decreased the amount of analgesics required and it was concluded that they should be considered as adjuvant regimes (Bak et al., 2007).

11.6 FUNCTIONAL DYSPEPSIA

Several essential oils have been used in the treatment of functional (nonulcer) dyspepsia. All of the published trials have concerned the commercial preparation known as Enteroplant®, an enteric-coated capsule containing 90 mg of *Mentha* × *piperita*, and 50 mg of *Carum carvi* essential oils.

The combination of peppermint and caraway essential oils has been shown to act locally in the gut as an antispasmodic (Micklefield et al., 2000, 2003) and to have a relaxing effect on the gallbladder (Goerg and Spilker, 2003). The antispasmodic effect of peppermint is well documented and that of caraway essential oil has also been demonstrated (Reiter and Brandt, 1985). The latter alone has also been shown to inhibit gallbladder contractions in healthy volunteers, increasing gallbladder volume by 90% (Goerg and Spilker, 1996).

One of the first studies involved 45 patients in a double-blind, placebo-controlled multicenter trial with the administration of Enteroplant thrice daily for 4 weeks. It was found to be superior to placebo with regard to pain frequency, severity, efficacy, and medical prognosis. Clinical Global Impressions were improved for 94.5% of patients using the essential oil combination (May et al., 1996).

The activity of Enteroplant (twice daily) was compared with that of cisapride (30 mg daily), a serotonin 5-HT_4 agonist that stimulates upper gastrointestinal tract motility, over a 4-week period.

This double-blind, randomized trial found that both products had comparable efficacy in terms of pain severity and frequency, Dyspeptic Discomfort Score, and Clinical Global Impressions (Madisch et al., 1999).

Another double-blind, randomized trial administered either Enteroplant or placebo twice daily for 28 days. Pain intensity and pressure, heaviness, and fullness were reduced in the test group by 40% and 43% as compared to 22% for both in the placebo group, respectively. In addition, Clinical Global Impressions were improved by 67% for the peppermint/caraway combination whereas the placebo scored 21% (May et al., 2000).

Holtmann et al. (2001) were the first to investigate the effect of Enteroplant (twice daily) on disease-specific quality of life as measured by the Nepean Dyspepsia Index. All scores were significantly improved compared to the placebo. In 2002, the same team also demonstrated that patients suffering with severe pain or severe discomfort both responded significantly better in comparison with the placebo.

Approximately 50% of patients suffering from functional dyspepsia are infected with *Helicobacter pylori* (Freidman, 1998). The *Helicobacter* status of 96 patients and the efficacy of Enteroplant were compared by May et al. (2003). They found that patients with *Helicobacter pylori* infection demonstrated a substantially better treatment response than those who were not infected. However, a previous study found no efficacy differences between infected and noninfected functional dyspepsia patients (Madisch et al., 2000) and so the effect of the presence of the bacterium on Enteroplant treatment has yet to be elucidated.

A short review of the literature concluded that treatment with the fixed peppermint/caraway essential oil combination had demonstrated significant efficacy in placebo-controlled trials, had good tolerability and safety, and could thus be considered for the long-term management of functional dyspepsia patients (Holtmann et al.,2003).

11.7 GASTROESOPHAGEAL REFLUX

d-Limonene has been found to be effective in the treatment of gastroesophageal reflux disorder. Nineteen patients took one capsule of 1000 mg *d*-limonene every day and rated their symptoms using a severity/frequency index. After 2 days, 32% of patients had significant relief and by day 14, 89% of patients had complete relief of symptoms (Wilkins, 2002).

A double-blind, placebo-controlled trial was conducted with 13 patients who were administered one 1000 mg capsule of *d*-limonene daily or on alternate days. By day 14, 86% of patients were asymptomatic compared to 29% in the placebo group (Wilkins, 2002).

The mechanism of action of *d*-limonene has not been fully elucidated in this regard but it is thought that it may coat the mucosal lining and offer protection against gastric acid and/or promote healthy peristalsis.

11.8 HYPERLIPOPROTEINEMIA

Girosital is a Bulgarian encapsulated product consisting of rose essential oil (68 mg) and vitamin A in sunflower vegetable oil. Initial animal studies found that rose oil administered at 0.01 and 0.05 mL/kg had a hepatoprotective effect against ethanol. Dystrophy and lipid infiltration were lowered and glycogen tended to complete recovery, suggesting a beneficial effect of rose oil on lipid metabolism (Kirov et al., 1988a).

Girosital was administered to 33 men with long-standing alcohol abuse, twice daily for 3 months. It significantly reduced serum triglycerides and low-density lipoprotein and increased the level of HDL-cholesterin; it was particularly effective for the treatment of hyperlipoproteinemia types IIb and IV. Liver lesions relating to alcohol intoxication improved and subjective complaints such as dyspeptic symptoms and pain were reduced (Konstantinova et al., 1988).

The hypolipidemic effect of Girosital was again studied by giving a capsule once daily for 20 days in 35 patients with hyperlipoproteinemia. In type IIa hyperlipoproteinemia cases, the total lipids were reduced by 23.91% and the total cholesterol by 10.64%. For type IIb patients, the total lipid reduction was 15.93%, triglycerides fell by 25.45%, and cholesterol by 14.06%; in type IV cases the reductions were 33.83%, 25.33%, and 36%, respectively. Girosital was more effective in comparison with the treatment with bezalipe and clofibrate (Stankusheva, 1988).

Thirty-two patients with hyperlipoproteinemia and arterial hypertension were administered one Girosital capsule twice daily for 110 days. A marked reduction in hyperlipoproteinemia was demonstrated in all patients. The hypocholesterolemic effect manifested first in type IIa patients after 20 days, and later in type IIb cases. Reduction of serum triglycerides in type IIb began 50 days after the commencement of treatment (Kirov et al., 1988b).

A further study (Mechkov et al., 1988) examined the effect of Girosital capsules twice daily for 110 days in 30 patients with cholelithiasis, liver steatosis, and hyperlipoproteinemia. Total cholesterol decreased after 20 days of treatment although it tended to rise slightly later in the test period. The triglycerides were most affected in hyperlipoproteinemia types IIb and IV. The β-lipoprotein values were not altered by the treatment.

11.9 IRRITABLE BOWEL SYNDROME

The essential oil of *Mentha* × *piperita* has been used for many years as a natural carminative of the gastrointestinal tract. This effect is principally due to the antispasmodic activity of menthol, which acts as a calcium channel antagonist of the intestinal smooth muscle (Taylor et al., 1984, 1985). Secondary effects include a reduction of gastrointestinal foam by peppermint oil (Harries et al., 1978) and a choleretic activity that is attributed to menthol (Rangelov et al., 1988). The reduction of intestinal hydrogen production caused by bacterial overgrowth has also been demonstrated in patients by enteric-coated peppermint oil (Logan and Beaulne, 2002).

The first clinical trial of peppermint for the treatment of irritable bowel syndrome was conducted by Rees et al. (1979). They prescribed 0.2 mL of peppermint oil in enteric-coated capsules (1–2 capsules depending on symptom severity) thrice daily. Patient assessment considered the oil to be superior to the placebo in relieving abdominal symptoms.

Since then, a further 15 double-blind and two open trials have been conducted; examples of these can be seen in Table 11.2.

Eight studies used the commercial preparation known as Colpermin® and two used Mintoil®, the capsules of which contain 187 and 225 mg of peppermint oil, respectively. The other studies used enteric-coated capsules usually containing 0.2 mL of the essential oil.

The latest trial (Cappello et al., 2007) used a randomized, double-blind, placebo-controlled design to test the efficacy of two capsules of Mintoil twice daily for 4 weeks. The symptoms evaluated before the treatment and at 4 and 8 weeks post-treatment were abdominal bloating, pain or discomfort, diarrhea, constipation, incomplete or urgency of defecation, and the passage of gas or mucus. The frequency and intensity of these symptoms was used to calculate the total irritable bowel syndrome symptoms score. At 4 weeks, 75% of patients in the peppermint oil group demonstrated a >50% reduction of the symptoms score as compared to 38% in the placebo group. At 4 and 8 weeks in the peppermint oil group compared to that before the treatment, there was a statistically significant reduction of the total irritable bowel syndrome symptoms score whereas there was no change with the placebo.

A critical review and meta-analysis of the use of peppermint oil for irritable bowel syndrome was published by Pittler and Ernst (1998). They examined five double-blind, placebo-controlled trials; there was a significant difference between peppermint oil and placebo in three cases and no significant difference in two cases. It was concluded that although a beneficial effect of peppermint oil was demonstrated, its role in treatment was not established.

TABLE 11.2
Examples of Clinical Trials of Peppermint Oil in the Treatment of Irritable Bowel Syndrome

Patients	Treatment	Outcome	Reference
18	0.2–0.4 mL thrice daily for 3 weeks	Superior to placebo in relieving abdominal symptoms	Rees et al. (1979)
29	0.2–0.4 mL thrice daily for 2 weeks	Superior to placebo in relieving abdominal symptoms	Dew et al. (1984)
25	0.2 mL thrice daily for 2 weeks	No significant change in symptoms as compared to placebo	Lawson et al. (1988)
35	One Colpermin thrice daily for 24 weeks	Effective in relieving symptoms	Shaw et al. (1991)
110	One Colpermin 3–4 times daily for 2 weeks	Significant improvement in symptoms as compared to placebo	Liu et al. (1997)
42	1–2 Colpermin thrice daily for 2 weeks	75% of children had reduced pain severity	Kline et al. (2001)
178	Two Mintoil thrice daily for 3 months	Significant improvement in gastroenteric symptoms as compared to placebo (97% versus 33%, respectively)	Capanni et al. (2005)
57	Two Mintoil twice daily for 4 weeks	Significant reduction in overall symptom score	Cappello et al. (2007)

A review of 16 trials was conducted by Grigoleit and Grigoleit (2005). They concluded that there was reasonable evidence that the administration of enteric-coated peppermint oil (180–200 mg) thrice daily was an effective treatment for irritable bowel syndrome when compared to placebo or the antispasmodic drugs investigated (mebeverine, hyoscyamine, and alverine citrate).

A comparison between two commercial delayed release peppermint oil preparations found that there were differences in the pharmacokinetics in relation to bioavailability times and release site. A capsule that is more effective in delivering the peppermint oil to the distal small intestine and ascending colon would be more beneficial in the treatment of irritable bowel syndrome (White et al., 1987). It has also been suggested that the conflicting results in some trials may be due to the inclusion of patients suffering from lactose intolerance, syndrome of small intestinal bacterial overgrowth, and celiac disease, all of which have symptoms similar to irritable bowel disease (Cappello et al., 2007).

11.10 MEDICAL EXAMINATIONS

Although not employed in a treatment context, the antispasmodic activity of peppermint essential oil has been used to facilitate examinations of the upper and lower gastrointestinal tract. A few examples are highlighted below.

Peppermint oil has also been used during double-contrast barium enemas. The study comprised 383 patients in four groups, two being no-treatment and Buscopan groups. The preparation, consisting of 8 mL of essential oil, 0.2 mL of Tween 80 in 100 mL water, was administered in 30 mL quantities via the enema tube or mixed in with the barium meal. Peppermint oil had the same spasmolytic effect as systemic Buscopan in the transverse and descending colon and a stronger effect in the cecum and ascending colon. Both methods of peppermint oil administration were equally effective (Asao et al., 2003).

Orally administered peppermint oil was used in a randomized trial in 430 patients undergoing a double-contrast barium meal examination, without other antispasmodics. A reduction in spasms of the esophagus, lower stomach, and duodenal bulb was found, along with an inhibition of barium flow to the distal duodenum and an improvement of diagnostic quality (Shigeaki et al., 2006).

During endoscopic retrograde cholangiopancreatography, Buscopan or glucagon is used to inhibit duodenal motility but produce adverse effects. Various concentrations of peppermint oil were introduced into the upper gastrointestinal tract of 40 patients undergoing the procedure. Duodenal relaxation was obtained with 20 mL of 1.6% peppermint oil solution and the procedure was performed successfully in 91.4% of patients. The inhibitory effect of peppermint oil appeared to be identical to that of glucagon, but without side effects (Yamamoto et al., 2006).

11.11 NAUSEA

A small study examined a variety of aromatherapy treatments to 25 patients suffering from nausea in a hospice and palliative care facility. Patients were offered the essential oils of *Foeniculum vulgare* var. *dulce*, *Chaemomelum nobile*, and *Mentha* × *piperita*, either singly or in blends, depending on individual preferences. Delivery methods included abdominal compress or massage, personal air spritzer or scentball diffuser. Only 32% of patients reported no response to the treatments and they had all just finished heavy courses of chemotherapy. Using a visual-numeric analogue scale, the remainder of patients experienced an improvement in their nausea symptoms when using the aromatherapy interventions. All patients were also taking antiemetic drugs and so the essential oils were regarded as successful complements to standard medications (Gilligan, 2005).

A 6-month trial investigated the effect of inhaled 5% *Zingiber officinale* essential oil in the prevention of postoperative nausea and vomiting (PONV). All patients were at a high risk for PONV and all used similar combinations of prophylactic intravenous antiemetics. The test group had the essential oil applied to the volar aspects of both wrists via a rollerball immediately prior to surgery. In the recovery room, patients were questioned as to their feelings of nausea. Any patient who felt that they required further medication was considered a "failure." Prevention of PONV by ginger essential oil was effective in 80% of cases, as measured by no complaint of nausea during the recovery period. In those patients who did not receive the essential oil, 50% experienced nausea (Geiger, 2005).

Another experiment used essential oils to prevent PONV, but they were applied after surgery if the patient complained of nausea. An undiluted mixture of *Zingiber officinale*, *Elettaria cardamomum*, and *Artemisia dracunculus* essential oils in equal parts was applied with light friction to the sternocleidomastoid area and carotid-jugular axis of the neck. Of the 73 cases treated, 50 had a positive response, that is, a complete block of nausea and vomiting within 30 min. It was found that the best response (75%) was with patients who had received a single analgesic/anesthetic (de Pradier, 2006).

The use of essential oils to alleviate motion sickness has also been investigated. A blend of *Zingiber officinale*, *Lavandula angustifolia*, *Mentha spicata*, and *Mentha* × *piperita* essential oils in an inhalation dispenser (QueaseEase™) was given to 55 ocean boat passengers with a history of motion sickness. The oil blend was inhaled as needed during the trip and queasiness was assessed using a linear analogue scale. The product was more effective than the placebo in lowering sensations of nausea when the seas were roughest, but was not significant at other times (Post-White and Nichols, 2007).

11.12 PAIN RELIEF

There follows a number of differing conditions that have been treated with essential oils with varying biological activities, such as antispasmodic, anti-inflammatory, and so on. They all share a common effect, that of pain relief.

11.12.1 Dysmenorrhea

The seeds of *Foeniculum vulgare* have been used in traditional remedies for the treatment of dysmenorrhea, an action attributed to the antispasmodic effect of the essential oil. An *in vitro* experiment demonstrated that fennel essential oil inhibited oxytocin- and prostaglandin E_2 (PGE_2)-induced contractions of isolated uterus; the former was considered to have a similar activity to diclofenac, a nonsteroidal anti-inflammatory drug. The overall mechanism of action is still unknown (Ostad et al., 2001).

A randomized, double-blind crossover study examined the effect of oral fennel essential oil at 1% or 2% concentration as compared to placebo for the treatment of 60 women with mild to moderate dysmenorrhea. Up to 1 mL of the solution was taken as required for the pain at intervals of not less than 4 h. In the treatment groups, the severity of the pain was significantly decreased; the efficacy of the 2% fennel oil was 67.4%, which was comparable to the efficacy of nonsteroidal anti-inflammatory drugs (Khorshidi et al., 2003).

Thirty patients with moderate to severe dysmenorrhea took part in a study to compare the activity of mefenamic acid with the essential oil of *Foeniculum vulgare* var. *dulce*. The evaluation was carried out during the first 5 days of three consecutive menstrual cycles. In the first cycle, no intervention was given (control); during the second cycle, 250 mg of mefenamic acid 6 hourly was prescribed; and in the third, 25 drops of a 2% solution of fennel essential oil were given 4 hourly. A self-scoring linear analogue technique was used to determine effect and potency. Both interventions effectively relieved menstrual pain as compared to the control. Mefenamic acid was more potent on the second and third days, but the result was not statistically significant. It was concluded that fennel essential oil was a safe and effective remedy but was probably less effective than mefenamic acid at the dosage used (Jahromi et al., 2003).

A third study used aromatherapy massage for the relief of the symptoms of dysmenorrhea in 67 students. The essential oils of *Lavandula officinalis*, *Salvia sclarea*, and *Rosa centifolia* (2:1:1 ratio) were diluted to 3% in 5 mL of almond oil and applied in a 15-min abdominal massage daily, 1 week before the start of menstruation, and stopping on the first day of menstruation. The control group received no treatment and the placebo group received massage with almond oil only. The results showed a significant improvement of dysmenorrhea as assessed by a verbal multidimensional scoring system for the essential oil group compared to the other two groups (Han et al., 2006).

11.12.2 Headache

The effect of peppermint and eucalyptus essential oils on the neurophysiological, psychological, and experimental algesimetric parameters of headache mechanisms were investigated using a double-blind, placebo-controlled trial with 32 healthy subjects. Measurements included sensitivity to mechanical, thermal, and ischemically induced pain. Four preparations consisting of varying amounts of peppermint and/or eucalyptus oils in ethanol were applied to the forehead and temples. Eucalyptus alone had no effect on the parameters studied. A combination of both oils (10% peppermint and 5% eucalyptus) increased cognitive performance and had a muscle-relaxing and mentally relaxing effect, but did not influence pain sensitivity. Peppermint alone (10%) had a significant analgesic effect with reduction in sensitivity to headache. It was shown to exert significant effects on the pathophysiological mechanisms of clinical headache syndromes (Göbel et al., 1995a).

A second study used the same essential oils when investigating the skin perfusion of the head in healthy subjects and migraine patients. In the former, capillary flow was increased by 225% in comparison with baseline by peppermint oil, while eucalyptus decreased the flow by 16%. In migraine patients, neither essential oil had any effect. It was suggested that the absence of capillary vasodilation (normally caused by menthol) was due to impaired calcium channel function in migraine patients (Göbel et al., 1995b).

11.12.3 Infantile Colic

Since animal studies had demonstrated that the essential oil of *Foeniculum vulgare* reduced intestinal spasm and increased the motility of the small intestine, it was used in a double-blind, randomized, placebo-controlled trial in the treatment of infantile colic. The 125 infants were all 2–12 weeks of age and those in the treatment group received a water emulsion of 0.1% fennel essential oil and 0.4% polysorbate (5–20 mL) up to four times a day. The dose was estimated to provide about 12 mg/kg/day of fennel essential oil. The control group received the polysorbate only. The treatment provided a significant improvement of colic, eliminating symptoms in 65% of infants as compared to 23.7% for the control. No side effects were noted (Alexandrovich et al., 2003).

11.12.4 Joint Physiotherapy

Six sports physiotherapists treated 30 patients suffering from knee or ankle pathologies of traumatic or surgical origin. Two commercial products were used simultaneously, Dermasport® and Solution Cryo®. The former was a gel consisting of the essential oils of *Betula alba*, *Melaleuca leucadendron*, *Cinnamomum camphora*, *Syzygium aromaticum*, *Eucalyptus globulus*, and *Gaultheria procumbens*. Solution Cryo contained the same essential oils minus *Gaultheria procumbens* but with the addition of *Chamaemelum nobile*, *Citrus limon*, and *Cupressus sempervirens*. Both products were at an overall concentration of 6%. Thirty minutes after application a net reduction in movement pain and joint circumference was demonstrated, along with an increase in articular flexion and extension of both joints in all patients (Le Faou et al., 2005).

11.12.5 Nipple Pain

Nipple cracks and pain are a common cause of breastfeeding cessation. In a randomized trial 196 primiparous women were studied during the first 2 weeks postpartum. The test group applied peppermint water (essential oil in water, concentration not given) to the nipple and areola after each breastfeed while the control group applied expressed breast milk. The overall nipple crack rate at the end of the period in the peppermint group was 7% as compared to 23% for the control. Only 2% of peppermint group experienced severe nipple pain in contrast to 23% of the control, with 93% and 71% experiencing no pain, respectively (Melli et al., 2007).

11.12.6 Osteoarthritis

A blend of *Zingiber officinale* (1%) and *Citrus sinensis* (0.5%) essential oils was used in an experimental double-blind study using 59 patients with moderate to severe knee pain caused by osteoarthritis. The treatment group received six massage sessions over a 3-week period; the placebo received the same massage sessions but without the essential oils and the control had no intervention. Assessment of pain intensity, stiffness, and physical functioning was carried out at baseline and at post 1 and 4 weeks. There were improvements in pain and function for the intervention group in comparison with the placebo and control at post 1 week but this was not sustained to week 4. The treatment was suggested for the relief of short-term knee pain (Yip et al., 2008).

11.12.7 Postherpetic Neuralgia

A double-blind crossover study examined the effect of the essential oil of *Pelargonium* spp. on moderate to severe postherpetic pain in 30 subjects. They were assigned to groups receiving 100, 50, or 10% geranium essential oil (in mineral oil), mineral oil placebo, or capsaicin control. Pain relief was measured using a visual analogue scale from 0 to 60 min after treatment. Mean values for the time integral of spontaneous pain reduction was 21.3, 12.7, and 8.0 for the 100%, 50%, and 10% geranium

oils and evoked pain-reduction values were 15.8, 7.7, and 5.9, respectively. Both evoked and spontaneous pains were thus significantly reduced in a dose-dependent manner (Greenway et al., 2003).

The result is interesting because topical capsaicin cream (one of the standard treatments for this condition) relieves pain gradually over 2 weeks, while the essential oil acted within minutes. Geranium essential oil applied cutaneously in animal studies has suppressed cellular inflammation and neutrophil accumulation in inflammatory sites (Maruyama et al., 2006) but postherpetic neuralgia normally occurs after the inflammation has subsided. One of the main components of the essential oil, geraniol, and the minor components of geranial, nerol, and neral, have been shown to interact with the transient receptor potential channel, TRPV1, as does capsaicin (Stotz et al., 2008). This sensory inhibition may explain the efficacy of topical geranium oil.

11.12.8 Postoperative Pain

A randomized, placebo-controlled clinical trial was conducted to determine whether the inhalation of lavender essential oil could reduce opioid requirements after laparoscopic adjustable gastric banding. In the postanesthesia care unit, 54 patients were given either lavender (two drops of a 2% dilution) or nonscented oil in a face mask. It was found that patients in the lavender group required significantly less morphine postoperatively than the placebo group (2.38 and 4.26 mg, respectively). Moreover, significantly more patients in the placebo group required analgesics in comparison with the lavender group; 82% compared to 46% (Kim et al., 2007).

A similar study in the previous year with 50 patients who had undergone breast biopsy surgery had found that lavender essential oil had no significant effect on postoperative pain or analgesic requirements. However, a significantly higher satisfaction with pain control was noted by patients in the lavender group (Kim et al., 2006).

11.12.9 Prostatitis

One study has evaluated the use of Rowatinex for the treatment of chronic prostatitis/chronic pelvic pain syndrome, the rationale being based on the known anti-inflammatory properties of the product. A 6-week, randomized single-blind trial compared the use of Rowatinex 200 mg thrice daily with ibuprofen 600 mg thrice daily in 50 patients. Efficacy was measured by the National Institutes of Health (NIH)-Chronic Prostatitis Symptom Index (NIH-CPSI) that was completed by the patients on four occasions. The decrease in the NIH-CPSI was significant in both groups at the end of treatment and a 25% improvement in the total score was superior in the Rowatinex group (68%) compared to the ibuprofen group (40%). Although the symptomatic response was significant, no patients became asymptomatic (Lee et al., 2006).

11.12.10 Pruritis

Pruritis is one of the most common complications of patients undergoing hemodialysis. Thirteen such patients were given an arm massage with lavender and tea tree essential oils (5% dilution in sweet almond and jojoba oil) thrice a week for 4 weeks. A control group received no intervention. Pruritis score, pruritis-related biochemical markers, skin pH, and skin hydration were measured before and after the study. There was a significant decrease in the pruritis score and blood urea nitrogen level for the test group. The control group showed a decreased skin hydration between pre- and post-test whereas for the essential oil group it was significantly increased (Ro et al., 2002). The lack of a massage only group in the study meant that the effects could not be definitely associated with the essential oils.

11.13 PEDICULICIDAL ACTIVITY

The activity of essential oils against the human head louse, *Pediculus humanus capitis*, has been investigated in a number of reports. Numerous essential oils have been found to exhibit

pediculicidal activity *in vitro*, with common oils such as *Eucalyptus globulus*, *Origanum marjorana*, *Rosmarinus officinalis*, and *Elettaria cardamomum* being comparable to, or more effective than *d*-phenothrin and pyrethrum (Yang et al., 2004). *Melaleuca alternifolia* and *Lavandula angustifolia* have also been found to be highly effective pediculicidal agents (Willimason et al., 2007).

Despite the availability of positive *in vitro* results, only one trial involving application to humans has been documented; a mixture of anise and ylang ylang essential oils in coconut extract (Paranix®) was applied once to five children. Viable lice were not found after 7 days (Scanni and Bonifazi, 2006).

11.14 RECURRENT APHTHOUS STOMATITIS

Recurrent aphthous stomatitis (RAS), also known as canker sores, are the most common oral mucosal lesions and although the process is sometimes self-limiting, the ulcer activity is mostly continuous and some forms may last for 20 years. Predisposing agents include bacteria and fungi, stress, mouth trauma, certain medications, and food allergies. Two essential oils both endemic to Iran have been investigated for treatment of this condition: *Zataria multiflora*, a thyme-like plant containing thymol, carvacrol, and linalool as major components, and *Satureja khuzistanica* containing predominantly carvacrol.

In a double-blind, randomized study, 60 patients with RAS received either 30 mL of an oral mouthwash composed of 60 mg of *Zataria multiflora* essential oil in an aqueous-alcoholic solution or placebo thrice daily for 4 weeks. In the treatment group, 83% of patients responded well while 17% reported a deterioration of their condition. This was compared with 13% and 87% for the placebo group, respectively. A significant clinical improvement with regard to less pain and shorter duration of the condition was found in the essential oil group (Mansoori et al., 2002).

Satureja khuzistanica essential oil 0.2% v/v was prepared in a hydroalcoholic solution and used in double-blind, randomized trial with 60 RAS patients. Its activity was compared with a 25% hydroalcoholic extract of the same plant and a hydroalcoholic placebo. A cotton pad was impregnated with 5 drops of preparation and placed on the ulcers for 1 min (fasting for 30 min afterwards) four times a day. The results of the extract and the essential oil groups were similar, with a significantly lower time for both pain elimination and complete healing of the ulcers in comparison with the placebo (Amanlou et al., 2007). The reported antibacterial, analgesic, antioxidant, and anti-inflammatory activities of this essential oil (Abdollahi et al., 2003; Amanlou et al., 2004, 2005) were thought responsible for the result.

11.15 RESPIRATORY TRACT

Given the volatile nature of essential oils, it should come as no surprise that their ability to directly reach the site of intended activity via inhalation therapy has led to their use in the treatment of a range of respiratory conditions. Moreover, a number of components are effective when taken internally, since they are bioactive at the level of bronchial secretions during their excretion. With the exception of one report, all of the research has used the individual components of either 1,8-cineole or menthol, or has employed them in combination with several other isolated essential oil components within commercial preparations.

11.15.1 Menthol

Menthol-containing essential oils have been used in the therapy of respiratory conditions for many years and the individual component is present in a wide range of over-the-counter medications. Of the eight optical isomers of menthol, *l*-(−)-menthol is the most abundant in nature and imparts a cooling sensation to the skin and mucous membranes.

Menthol is known to react with a temperature-sensitive (8–28°C range) transient receptor potential channel, leading to an increase in intracellular calcium, depolarization and initiation of an action potential (Jordt et al., 2003). This channel, known as TRPM8, is expressed in distinct populations of afferent neurons; primarily thinly myelinated Aδ cool fibers and to a lesser extent, unmyelinated C-fiber nociceptors (Thut et al., 2003). It is the interaction with the TRPM8 thermoreceptor that is responsible for the cooling effect of menthol when it is applied to the skin. This activity is not confined to the dermis, since the presence of TRPM8 has been demonstrated by animal experimentation in the squamous epithelium of the nasal vestibule (Clarke et al., 1992), the larynx (Sant'Ambrogio et al., 1991), and lung tissue (Wright et al., 1998). Thus the activation of cold receptors via inhaled menthol leads to a number of beneficial effects.

11.15.1.1 Antitussive

Despite being used as a component in cough remedies since the introduction of a "vaporub" in 1890, there are few human trials of menthol used alone as being effective. In a citric acid-induced cough model in healthy subjects, Packman and London (1980) found that menthol was effective, although 1,8-cineole was more efficacious. The use of an aromatic unction rather than direct inhalation may have affected the results, since the inhalation of menthol has been shown in animal models to be significantly more effective at cough frequency reduction (28% and 56% at 10 and 30 g/l, respectively) compared to 1,8-cineole (Laude et al., 1994).

A single-blind pseudorandomized crossover trial in 42 healthy children was used to compare the effect of an inhalation of either menthol or placebo on citric acid-induced cough. It was found that cough frequency was reduced in comparison with the baseline but not to that of the placebo (Kenia et al., 2008). However, the placebo chosen was eucalyptus oil, whose main component is 1,8-cineole and known to have similar antitussive properties to menthol.

Along with other ion channel modulators, menthol is recognized as a potential "novel therapy" for the treatment of chronic cough (Morice et al., 2004, p. 489). It is not clear whether the antitussive activity of menthol is due solely to its stimulation of airway cold receptors; it may also involve pulmonary C-fibers (a percentage of which also express TRPM8) or there may be a specific interaction with the neuronal cough reflex.

11.15.1.2 Nasal Decongestant

Menthol is often thought of as a decongestant, but this effect is a sensory illusion. Burrow et al. (1983) and Eccles et al. (1988) showed that there was no change in nasal resistance to airflow during inhalation of menthol, although the sensation of nasal airflow was enhanced. In the former experiment, 1,8-cineole and camphor were also shown to enhance the sensation of airflow, but to a lesser extent than menthol.

In a double-blind, randomized trial subjects suffering from the common cold were given lozenges containing 11 mg of menthol. Posterior rhinomanometry could detect no change in nasal resistance to airflow after 10 min; however, there were significant changes in the nasal sensation of airflow (Eccles et al., 1990).

A single-blind pseudorandomized crossover trial compared the effect of an inhalation of either menthol or placebo. The main outcome measures were nasal expiratory and inspiratory flows and volumes, as measured by a spirometer and the perception of nasal patency, assessed with a visual analogue scale. It was found that there was no effect of menthol on any of the spirometric measurements although there was a significant increase in the perception of nasal patency (Kenia et al., 2008).

Thus it has been demonstrated that menthol is not a nasal decongestant. However, it is useful in therapy since stimulation of the cold receptors causes a subjective sensation of nasal decongestion and so relieves the feeling of a blocked nose. In commercial preparations that include menthol, a true decongestant such as oxymetazoline hydrochloride is often present.

11.15.1.3 Inhibition of Respiratory Drive and Respiratory Comfort

When cold air was circulated through the nose in human breath-hold experiments, subjects were able to hold their breath longer (McBride and Whitelaw, 1981) and inhaling cold air was shown to inhibit normal breathing patterns (Burgess and Whitelaw, 1988). This indicated that cold receptors could be one source of monitoring inspiratory flow rate and volume. Several animal experiments demonstrated that the inhalation of cold air, warm air, plus menthol, or menthol alone (390 ng/mL) significantly enhanced ventilator inhibition (Orani et al., 1991; Sant'Ambrogio et al., 1992).

Sloan et al. (1993) conducted breath-hold experiments with 20 healthy volunteers. The ingestion of a lozenge containing 11 mg of menthol significantly increased the hold time, indicating a depression of the ventilatory drive. It was later postulated by Eccles (2000) that in addition to chemoreceptors detecting oxygen and carbon dioxide in the blood, cold receptors in the respiratory tract may also modulate the drive to breathe.

Eleven healthy subjects breathed through a device that had either an elastic load or a flow-resistive load. Sensations of respiratory discomfort were compared using a visual analogue scale before, during, and after inhalation of menthol. It was found that the discomfort associated with loaded breathing was significantly reduced and was more effective during flow-resistive loading than elastic loading. Inhalation of another fragrance had no effect and so the result was attributed to a direct stimulation of cold receptors by menthol, a reduction in respiratory drive being perhaps responsible (Nishino et al., 1997).

During an investigation of dyspnea, the effect of menthol inhalation on respiratory discomfort during loaded breathing was found to be inconsistent. Further tests found that the effect of menthol was most important during the first few minutes of inhalation and in the presence of high loads (Peiffer et al., 2001). The therapeutic application of menthol in the alleviation of dyspnea has yet to be described.

11.15.1.4 Bronchodilation and Airway Hyperresponsiveness

The spasmolytic activity of menthol on airway smooth muscle has been demonstrated *in vitro* (Taddei et al., 1988). To examine the bronchodilatory effects of menthol, a small trial was conducted on six patients with mild to moderate asthma. A poultice-containing menthol was applied daily for 4 weeks and it was found that bronchoconstriction was decreased and airway hyperresponsiveness improved (Chiyotani et al., 1994b).

A randomized, placebo-controlled trial examined the effects of menthol (10 mg nebulized twice daily for 4 weeks) on airway hyperresponsiveness in 23 patients with mild to moderate asthma. The diurnal variation in the peak expiratory flow rate (a value reflecting airway hyperexcitability) was decreased but the forced expiratory volume was not significantly altered. This indicated an improvement of airway hyperresponsiveness without affecting airflow limitation (Tamaoki et al., 1995). Later *in vivo* research examined the effect of menthol on airway resistance caused by capsaicin- and neurokinin-induced bronchoconstriction; there was a significant decrease in both cases by inhalation of menthol at 7.5 μg/L air concentration. The *in vitro* effect of menthol on bronchial rings was also studied. It was concluded that menthol attenuated bronchoconstriction by a direct action on bronchial smooth muscle (Wright et al., 1997).

In cases of asthma, the beneficial effects of menthol seem to be mainly due to its bronchodilatory activity on smooth muscle; interaction with cold receptors and the respiratory drive may also play an important role.

Recent *in vitro* studies have shown that a subpopulation of airway vagal afferent nerves expresses TRPM8 receptors and that activation of these receptors by cold and menthol excite these airway autonomic nerves. Thus, activation of TRPM8 receptors may provoke an autonomic nerve reflex to increase airway resistance. It was postulated that this autonomic response could provoke menthol- or cold-induced exacerbation of asthma and other pulmonary disorders (Xing et al., 2008). Direct

cold stimulation or inhalation of menthol can cause immediate airway constriction and asthma in some people; perhaps the TRPM8 receptor expression is upregulated in these subjects. The situation is far from clear.

11.15.1.5 Summary

The respiratory effects of menthol that have been demonstrated are as follows:

1. Antitussive at low concentration.
2. Increases the sensation of nasal airflow giving the impression of decongestion.
3. No physical decongestant activity.
4. Depresses ventilation and the respiratory drive at comparatively higher concentration.
5. Reduces respiratory discomfort and sensations of dyspnea.

A number of *in vitro* and animal experiments have demonstrated the bronchomucotropic activity of menthol (Boyd and Sheppard, 1969; Welsh et al., 1980; Chiyotani et al., 1994a), whereas there have been conflicting reports as to whether menthol is a mucociliary stimulant (Das et al., 1970) or is ciliotoxic (Su et al., 1993). Apart from the inclusion of relatively small quantities of menthol in commercial preparations that have known beneficial mucociliary effects, there are no documented human trials to support the presence of these activities.

11.15.2 1,8-Cineole

This oxide has a number of biological activities that make it particularly useful in the treatment of the respiratory tract. 1,8-Cineole has been registered as a licensed medication in Germany for over 20 years and is available as enteric-coated capsules (Soledum®). It is therefore not surprising that the majority of the trials originate from this country and use oral dosing of 1,8-cineole instead of inhalation. Rather than discuss specific pathologies, the individual activities will be examined and their relevance (alone or in combination) in treatment regimes should become apparent.

11.15.2.1 Antimicrobial

The anti-infectious properties of essential oils high in 1,8-cineole content may warrant their inclusion into a treatment regime but other components are more effective in this regard. 1,8-Cineole is often considered to have marginal or no antibacterial activity (Kotan et al., 2007), although it is very effective at causing leakage of bacterial cell membranes (Carson et al., 2002). It may thus allow more active components to enter the bacteria by permeabilizing their membranes.

1,8-Cineole does possess noted antiviral properties compared to the common essential oil components of borneol, citral, geraniol, limonene, linalool, menthol, and thymol; only that of eugenol was greater (Bourne et al., 1999). However, in comparison with the potent thujone, the antiviral potential of 1,8-cineole was considered relatively low (Sivropoulou et al., 1997).

A placebo-controlled, double-blind, randomized parallel-group trial examined the long-term treatment of 246 chronic bronchitics during winter with myrtol standardized Gelomyrtol® forte. This established German preparation consists mainly of 15% α-pinene, 35% limonene, and 47% 1,8-cineole and was administered thrice daily in 300 mg capsules. It was found to reduce the requirement for antibiotics during acute exacerbations; 51.6% compared to 61.2% under placebo. Of those patients needing antibiotics, 62.5% in the test group required them for ≤7 days whereas 76.7% of patients in the placebo group needed antibiotics for more than 7 days. Moreover, 72% of patients remained without acute exacerbations in the test group compared to 53% in the placebo group (Meister et al., 1999).

Although emphasis was given to antibiotic reduction, a significant antimicrobial effect by the preparation is unlikely to have paid an important contribution. Indeed, Meister et al. refer to reduced health impairment due to sputum expectoration and cough, and note other beneficial properties of 1,8-cineole that will be discussed in Sections 11.15.2.2 through 11.15.2.6.

11.15.2.2 Antitussive

The antitussive effects of 1,8-cineole were first proven by Packman and London in 1980, who induced coughing in 32 healthy human subjects via the use of an aerosol spray containing citric acid. This single-blind crossover study examined the effect of a commercially available chest rub containing, among others, eucalyptus essential oil. The rub was applied to the chest in a 7.5 mg dose and massaged for 10–15 s after which the frequency of the induced coughing was noted. It was found that the chest rub produced a significant decrease in the induced cough counts and that eucalyptus oil was the most active component of the rub.

1,8-Cineole interacts with TRPM8, the cool-sensitive thermoreceptor that is primarily affected by menthol. In comparison with menthol, the effect of 1,8-cineole on TRPM8 (as measured by Ca^{2+} influx kinetics) is much slower and declines more rapidly (Behrendt et al., 2004). In a similar manner to menthol, the antitussive activity of 1,8-cineole may be due in part to its stimulation of airway cold receptors.

11.15.2.3 Bronchodilation

In vitro tests using guinea pig trachea determined that the essential oil of *Eucalyptus tereticornis* had a myorelaxant, dose-dependent effect (10–1000 μg/mL) on airway smooth muscle, reducing tracheal basal tone and K^+-induced contractions, as well as attenuating acetylcholine-induced contractions at higher concentrations (Coelho-de-Souza et al., 2005). This activity was found to be mainly due to 1,8-cineole, although the overall effect was thought due to a synergistic relationship between the oxide and α- and β-pinene. Similar results were obtained using the essential oil of *Croton nepetaefolius*, whose major component was also 1,8-cineole (Magalhães et al., 2003).

A double-blind, randomized clinical trial over 7 days compared oral pure 1,8-cineole (3 × 200 mg/day) to Ambroxol (3 × 30 mg/day) in 29 patients with chronic obstructive pulmonary disease (COPD). Vital capacity, airway resistance, and specific airway conductance improved significantly for both drugs, whereas the intrathoracic gas volume was reduced by 1,8-cineole but not by Ambroxol. All parameters of lung function, peak flow, and symptoms of dyspnea were improved by 1,8-cineole therapy, but were not statistically significant in comparison with Ambroxol due to the small number of patients. In addition to other properties, it was noted that the oxide seemed to have bronchodilatory effects (Wittman et al., 1998).

11.15.2.4 Mucolytic and Mucociliary Effects

Mucolytics break down or dissolve mucus and thus facilitate the easier removal of these secretions from the respiratory tract by the ciliated epithelium, a process known as mucociliary clearance. Some mucolytics also have a direct action on the mucociliary apparatus itself.

Administered via steam inhalation to rabbits, 1,8-cineole in concentrations that produced a barely detectable scent (1–9 mg/kg) augmented the volume output of respiratory tract fluid from 9.5% to 45.3% (Boyd and Sheppard, 1971), an effect that they described as "mucotropic." Interestingly, in the same experiment fenchone at 9 mg/kg increased the output by 186.2%, thus confirming the strong effects of some ketones in this regard. Also using rabbits, Zanker (1983) found that oxygenated monoterpenoids reduced mucus deposition and partially recovered the activity of ciliated epithelium.

Because of these early animal experiments, the beneficial effects of 1,8-cineole on mucociliary clearance have been clearly demonstrated in a number of human trials. Dorow et al. (1987) examined the effects of a 7-day course of either Gelomyrtol forte (4 × 300 mg/day) or Ambroxol (3 × 30 mg/day) in 20 patients with chronic obstructive bronchitis. Improved mucociliary clearance was observed in both groups, although improvement in lung function was not detected.

Twelve patients with chronic obstructive bronchitis were given a 4-day treatment with 1,8-cineole (4 × 200 mg/day). By measuring the reduction in percentage radioactivity of an applied radioaerosol, significant improvements in mucociliary clearance were demonstrated at the 60 and 120 min after each administration (Dorow, 1989).

In a small double-blind study, the expectorant effect of Gelomyrtol forte (1 × 300 mg/day, 14 days) was examined in 20 patients with chronic obstructive bronchitis. The ability to expectorate,

frequency of coughing attacks, and shortness of breath were all improved by the therapy, as was sputum volume and color. Both patients and physicians rated the effects of Gelomyrtol forte as better than the placebo, but due to the small group size statistically significant differences could not be demonstrated (Ulmer and Schött, 1991).

A randomized, double-blind, placebo-controlled trial was used to investigate the use of mucolytics to alleviate acute bronchitis (Mattys et al., 2000). They compared Gelomyrtol forte (4 × 300 mg, days 1–14), with Ambroxol (3 × 30 mg, days 1–3; 2 × 30 mg, days 4–14) and Cefuroxime (2 × 250 mg, days 1–6) in 676 patients. By monitoring cough frequency data, regression of the frequency of abnormal auscultation, hoarseness, headache, joint pain, and fatigue, it was shown that Gelomyrtol forte was very efficacious and comparable to the other active treatments. Overall, it scored slightly more than Ambroxol and Cefuroxime and was therefore considered to be a well-evidenced alternative to antibiotics for acute bronchitis.

Several studies have demonstrated a direct effect of 1,8-cineole on the ciliated epithelium itself. Kaspar et al. (1994) conducted a randomized, double-blind three-way crossover 4-day study of the effects of 1,8-cineole (3 × 200 mg/day) or Ambroxol (3 × 30 mg/day) on mucociliary clearance in 30 patients with COPD. Treatment with the oxide resulted in a statistically significant increase in the ciliary beat frequency of nasal cilia, a phenomenon that did not occur with the use of Ambroxol (an increase of 8.2% and 1.1%, respectively). A decrease of "saccharine-time" was clinically relevant and significant after 1,8-cineole therapy (241 s) but not after Ambroxol (48 s). Lung function parameters were significantly improved equally by both drugs.

After the ingestion of Gelomyrtol forte (3 × 1 capsule/day for 4 days) by four healthy persons and one person after sinus surgery, there was a strong increase in mucociliary transport velocity, as detected by movement of a radiolabeled component (Behrbohm et al., 1995).

In sinusitis, the ciliated beat frequency is reduced and 30% of ciliated cells convert to mucus-secreting goblet cells. The impaired mucociliary transport, excessive secretion of mucus, and edema block drainage sites leading to congestion, pain, and pressure.

To demonstrate the importance of drainage and ventilation of sinuses as a therapeutic concept, Federspil et al. (1997) conducted a double-blind, randomized, placebo-controlled trial using 331 patients with acute sinusitis. The secretolytic effects of Gelomyrtol forte (300 mg) over a 6-day period proved to be significantly better than the placebo.

Kehrl et al. (2004) used the known stimulatory effects of 1,8-cineole on ciliated epithelium and its mucolytic effect as a rationale for treating 152 acute rhinosinusitis patients in a randomized, double-blind, placebo-controlled study. The treatment group received 3 × 200 mg 1,8-cineole daily for 7 days. There was a clinically relevant and significant improvement in frontal headache, headache on bending, pressure point sensitivity of the trigeminal nerve, nasal obstruction, and rhinological secretions in the test group, as compared to the control group. It was concluded that 1,8-cineole was a safe and effective treatment for acute nonpurulent rhinosinusitis before antibiotics are indicated.

11.15.2.5 Anti-Inflammatory Activity

The effects of 1,8-cineole on stimulated human monocyte mediator production were studied *in vitro* and compared with that of budesonide, a corticosteroid agent with anti-inflammatory and immunosuppresive effects (Juergens et al., 1998a). At therapeutic levels, both substances demonstrated a similar inhibition of the inflammatory mediators leukotriene B_4 (LTB_4), PGE_2, and interleukin-1β (IL-1β). This was the first evidence of a steroid-like inhibition of arachidonic acid metabolism and IL-1β production by 1,8-cineole.

Later that year, the same team (Juergens et al., 1998b) reported a dose-dependent and highly significant inhibition of tumor necrosis factor-α (TNF-α), IL-1β, thromboxane B_2, and LTB_4 production by 1,8-cineole from stimulated human monocytes *in vitro*.

A third experiment combined *ex vivo* and *in vivo* testing; 10 patients with bronchial asthma were given 3 × 200 mg of 1,8-cineole daily for 3 days. Lung function was measured before the first dose, at the end of the third dose and 4 days after discontinuation of the therapy. At the same time, blood

samples were taken from which monocytes were collected and stimulated *ex vivo* for LTB_4 and PGE_2 production. Twelve healthy volunteers also underwent the treatment and their blood was taken for testing. It was found that by the end of the treatment and 4 days after, the production of LTB_4 and PGE_2 from the monocytes of both asthmatics and healthy individuals was significantly inhibited. Lung function parameters of asthmatic patients were significantly improved (Juergens et al., 1998c).

These three reports suggested a strong anti-inflammatory activity of 1,8-cineole via both the cyclooxygenase and 5-lipooxygenase pathways, and the possibility of a new, well-tolerated treatment of airway inflammation in obstructive airway disease.

Juergens et al. (2003) conducted a double-blind, placebo-controlled clinical trial involving 32 patients with steroid-dependent severe bronchial asthma. The subjects were randomly assigned to receive either a placebo or a 3×200 mg 1,8-cineole daily for 12 weeks. Oral glucocorticosteroids were reduced by 2.5 mg increments every 3 weeks with the aim of establishing the glucocorticosteroid-sparing capacity of 1,8-cineole. The majority of asthma patients receiving oral 1,8-cineole remained clinically stable despite a mean reduction of oral prednisolone dosage of 36%, equivalent to 3.8 mg/day. In the placebo group, where only four patients could tolerate a steroid decrease, the mean reduction was 7%, equivalent to 0.9 mg/day. Compared with the placebo group, 1,8-cineole recipients maintained their lung function four times longer despite receiving lower doses of prednisolone.

Increased mucus secretion often appears as an initial symptom in exacerbated COPD and asthma, where stimulated mediator cells migrate to the lungs to produce cytokines; of particular importance are TNF-α, IL-1β, IL-6, and IL-8 and those known to induce immunoglobulin E (IgE) antibody synthesis and maintain allergic eosinophilic inflammation (IL-4 and IL-5). Therefore, a study was conducted to investigate the role of 1,8-cineole in inhibiting cytokine production in stimulated human monocytes and lymphocytes *in vitro* (Juergens et al., 2004). It was shown that 1,8-cineole is a strong inhibitor of TNF-α and IL-1β in both cell types. At known therapeutic blood levels, it also had an inhibitory effect on the production of the chemotactic cytokines IL-8 and IL-5 and may possess additional antiallergic activity by blocking IL-4 production.

A clinically relevant anti-inflammatory activity of 1,8-cineole has thus been proven for therapeutic use in airway diseases.

11.15.2.6 Pulmonary Function

An inhaler was used to apply 1,8-cineole (Soledum Balm) to 24 patients with asthma or chronic bronchitis in an 8-day-controlled trial. In all but one patient, an objective rise in expiratory peak flow values was demonstrated. The subjective experience of their illness was significantly improved for all subjects (Grimm, 1987).

In an open trial of 100 chronic bronchitics using both inhaled (4×200 mg) and oral (3×200 mg) 1,8-cineole over 7 days, the clinical parameters of forced vital capacity, forced expiratory volume, peak expiratory flow, and residual volume were all significantly improved when compared to initial values before treatment (Mahlo, 1990).

In a randomized, double-blind, placebo-controlled study of 51 patients with COPD, 1,8-cineole (3×200 mg/day) was given for 8 weeks. For the objective lung functions of "airway resistance" and "specific airway resistance," there was a clinically significant reduction of 21% and 26%, respectively. The improvement was attributed to a positive influence on disturbed breathing patterns, mucociliary clearance, and anti-inflammatory effects (Habich and Repges, 1994).

The majority of the *in vivo* trials involving 1,8-cineole report good, if not significant, changes in lung function parameters, whether the investigation concerns the common cold or COPD. This is not a convenient, accidental side effect of treatment but is a direct result of one or more of the factors already discussed that have direct effects on the pathophysiology of the airways. The ability to breathe more effectively and easily is an important consequence of the therapy that is sometimes minimized when dealing with the specific complexities of infection, inflammation, and so on. A compilation of human trials with 1,8-cineole is given in Table 11.3.

Table 11.3
Summary of Human Trials Demonstrating the Beneficial Effects of 1,8-Cineole in Various Respiratory Conditions

Patients	Treatment	Outcome	Reference
		Asthma	
11	Cineole inhalation, 8 days	Objective rise in expiratory peak flow found. Subjective experiences of illness significantly improved	Grimm (1987)
10	Cineole 3 × 200 mg daily, 3 days	LTB_4 and PGE_2 production by monocytes was significantly inhibited. Lung functions were significantly improved	Juergens et al. (1998c)
32	Cineole 3 × 200 mg daily, 12 weeks	Twelve of 16 patients in cineole group remained stable despite a 36% reduction in oral steroid dosage	Juergens et al. (2003)
		Acute bronchitis	
60	Vaporub 3 min	Decreased breathing frequency, suggesting "easier breathing"	Berger et al. (1978a)
676	Gelomyrtol 4 × 300 mg daily, 14 days	Coughing, sputum consistency, well-being, bronchial hyperreactivity, and associated symptoms all improved similarly by Gelomyrtol, Ambroxol, and Cefuroxime	Mattys et al. (2000)
		Chronic bronchitis	
9	Cineole inhalation, 8 days	Objective rise in expiratory peak flow found. Subjective experiences of illness significantly improved	Grimm (1987)
100	Cineole 3 × 200 mg daily, 7 days	All lung function parameters significantly improved	Mahlo (1990)
246	Gelomyrtol 3 × 300 mg daily, 6 months	Reduced acute exacerbations, reduced requirement for antibiotics, reduced treatment times when antibiotics taken. Well-being significantly improved	Meister et al. (1999)
		COPD	
20	Gelomyrtol 4 × 0.3 g daily, 7 days	Improved mucociliary clearance	Dorow et al. (1987)
20	Gelomyrtol 1 × 0.3 g daily, 14 days	All parameters relating to coughing improved. Sputum volume increased	Ulmer and Schött (1991)
12	Cineole 4 × 200 mg, 4 days	Significant improvement of mucociliary clearance	Dorow (1989)
51 including 16 asthmatics	Cineole 3 × 200 mg daily, 8 weeks	Significant improvement in airway resistance (21%), positive effects on sputum output and dyspnea	Habich and Repges (1994)
30	Cineole 1 × 200 mg daily, 4 days	Significant improvements in lung functions of FVC and FEV_1 (Ambroxol and cineole equieffective), significant increase in ciliary beat frequency	Kaspar et al. (1994)
29	Cineole 3 × 200 mg daily, 7 days	All lung function parameters, peak flow and dyspnea improved from day 1 onward	Wittmann et al. (1998)
		Common cold	
24	Eucalyptus oil (9% of a mixture)	Reversed lung function abnormalities in small and large airways	Cohen and Dressler (1982)
		Sinusitis	
331	Gelomyrtol 300 mg, 6 days	Effective treatment instead of antibiotics	Federspil et al. (1997)
152	Cineole 3 × 200 mg daily, 7 days	Effective reduction of symptoms without the need for antibiotics	Kehrl et al. (2004)

11.15.2.7 Summary

Although discussed separately, the multifaceted activities of 1,8-cineole perform together in harmony to provide an effective intervention that can inherently adapt to the needs of the individual patient. As already described, 1,8-cineole is known to possess the following properties:

1. Antimicrobial
2. Antitussive
3. Bronchodilatory
4. Mucolytic
5. Ciliary transport promotion
6. Anti-inflammatory
7. Lung function improvement.

Therefore, it may be seen that a diverse range of respiratory conditions of varying complexities will benefit from the use of pure 1,8-cineole or from essential oils containing this oxide as a major component.

11.15.3 Treatment with Blends Containing both Menthol and 1,8-Cineole

A study measured transthoracic impedance pneumographs of 60 young children (2–40 months) with acute bronchitis before and after a 3-min application of Vaporub® to the back and chest. The data showed an early increase in amplitude up to 33%, which slowly descended during the 70-min post-treatment period to slightly above the control. Breathing frequency progressively decreased during the same period by 19.4%. Clinical observations combined with these results suggested a condition of "easier breathing" (Berger et al., 1978a). Currently, the active ingredients of Vaporub are camphor 4.8%, 1,8-cineole 1.2%, and menthol 2.6, but these components and percentages may have changed over the years.

The same team employed a similar experiment but used the pneumographic data to examine the quiet periods, that is, parts of the pneumogram where changes in the baseline were at least half of the average amplitude in more than five consecutive breathing excursions. It was found that the application of Vaporub increased quiet periods by up to 213.8%, whereas the controls (petroleum jelly application or rubbing only) never exceeded 62.4%. Thus the breathing restlessness of children with bronchitis was diminished and this was confirmed by clinical observations (Berger et al., 1978b).

By the measurement of lung and forced expiratory volumes, nasal, lower, and total airway resistances, closing volume data, the phase III slope of the alveolar plateau, and the maximum expiratory flow volume, peripheral airway dysfunction was confirmed in 24 adults with common colds. In a randomized, controlled trial, an aromatic mixture of menthol, eucalyptus oil, and camphor (56%, 9%, and 35% w/w, respectively) were vaporized in a room where the subjects were seated. Respiratory function measurements were made at baseline, 20 and 60 min after exposure. After the last measurement, phenylephrine was sprayed into the nostrils and the measurements taken again 5–10 min later to determine potential airway responsiveness. The control consisted of tap water. The results showed significant changes in forced vital capacity, forced expiratory volume, closing capacity, and the phase III slope after aromatic therapy as compared to the control. It was concluded that the aromatic inhalation favorably modified the peripheral airway dysfunction (Cohen and Dressler, 1982).

In a randomized, placebo-controlled trial of citric acid-induced cough in 20 healthy subjects, the inhalation of a combination of menthol and eucalyptus oil (75% and 25%, respectively) significantly decreased the cough frequency (Morice et al., 1994).

The effect of an aromatic inunction (Vaporub) was studied by the inhalation of a radioaerosol in a randomized, single-blinded, placebo-controlled crossover trial with 12 chronic bronchitics. It was found that after the application of 7.5 g of the product to the chest, removal of the tracheobronchial

deposit was significantly enhanced at 30 and 60 min postinhalation, although further effects could not be demonstrated during the following 5 h, despite further application of the rub. During the first hour, mucociliary clearance was correlated with the concentration level of the aromatics (Hasani et al., 2003).

Another commercial preparation Pinimenthol®, a mixture of eucalyptus and pine needle oils plus menthol, reduced bronchospasm and demonstrated significant secretolytic effects when insufflated through the respiratory tract and when applied to the epilated skin of animals (Schäfer and Schäfer, 1981). In addition to the known effects of menthol and 1,8-cineole, pine needle oil is considered to be weakly antiseptic and secretolytic (Approved Herbs, 1998).

In a randomized, double-blind 14-day trial, 100 patients with chronic obstructive bronchitis received a combination of theophylline with β-adrenergica 2–3 times daily. The test group also received Pinimenthol. The parameters were investigated were objective (measurement of lung function and sputum) and subjective (cough, respiratory insufficiency, and pulmonary murmur). All differences in the subjective evaluations were statistically significant and of clinical importance; secretolysis was clearly shown. The addition of Pinimenthol showed a clear superiority to the basic combination therapy alone (Linsenmann and Swoboda, 1986).

A postmarketing survey was conducted of 3060 patients prescribed Pinimenthol suffering from cold, acute or chronic bronchitis, bronchial catarrh, or hoarseness. The product was given by inunction (29.6%), inhalation (17.3%), or inunction and inhalation (53.1%). Only 22 patients reported adverse effects and the efficacy of the product was judged as excellent or good by 88.3% of physicians and 88.1% of patients (Kamin and Kieser, 2007).

11.16 ALLERGIC RHINITIS

In a proof of concept study, a nasal spray was made from the essential oil of *Artemisia abrotanum* L. (4 mg/mL) and flavonoid extracts (2.5 μg/mL) from the same plant. The essential oil consisted primarily of 1,8-cineole and davanone at approximately 40% and 50%, respectively. Apart from a spasmolytic activity (Perfumi et al., 1995), little is known about the biological activity of davanone. The flavonoids present were thought to inhibit histamine release and interfere with arachidonic acid metabolism. The nasal spray was self-administered by 12 patients with allergic rhinitis, allergic conjunctivitis, and/or bronchial obstructive disease. They were instructed to use 1–2 puffs in each nostril at the first sign of symptoms, to a maximum of six treatments per day. All patients experienced rapid and significant relief of nasal symptoms and for those with allergic conjunctivitis, a significant relief of subjective eye symptoms was also experienced. Three of six patients with bronchial obstructive disease experienced rapid and clinically significant bronchial relief (Remberg et al., 2004).

11.17 SNORING

A blend of 15 essential oils was developed into a commercial product called "Helps stop snoring" and 140 adult snorers were recruited into a randomized trial using the product as a spray or gargle. Visual analogue scales were completed by the snorers' partners relating to sleep disturbance each night. The treatment lasted for 14 days and results were compared to a pretrial period of the same length. The partners of 82% of the patients using the spray and 71% of patients using the gargle reported a reduction in snoring. This was compared to 44% of placebo users. The mode of action was postulated as being antispasmodic to the soft palate and pharynx (Pritchard, 2004).

11.18 SWALLOWING DYSFUNCTION

A delayed triggering of the swallowing reflex, mainly in elderly people, predisposes to aspiration pneumonia. To improve dysphagia, two different approaches using essential oils have been tried with success.

As black pepper is a strong appetite stimulant, it was postulated that nasal inhalation of the essential oil may stimulate cerebral blood flow in the insular cortex, the dysfunction of which has been reported to play a role in dysphagia. A randomized, controlled study of 105 elderly patients found that the inhalation of black pepper oil for 1 min significantly shortened the delayed swallowing time and increased the number of swallowing movements. Emission computed tomography demonstrated activation of the anterior cingulate cortex by the treatment. The inhalation of lavender essential oil or water had no effects (Ebihara et al., 2006a).

A second study used the established stimulating effects of menthol on cold receptors, since cold stimulation was known to restore sensitivity to trigger the swallowing reflex in dysphagic patients. Menthol was introduced into the pharynx of patients with mild to moderate dysphagia via a nasal catheter. The latent time of swallowing reflex was reduced significantly by menthol in a concentration-dependent manner; 10^{-2} menthol reduced the time to 9.4 s as compared to 13.8 s for distilled water. The use of a menthol lozenge before meals was thought appropriate (Ebihara et al., 2006b).

11.19 CONCLUSION

It is apparent from the diverse range of conditions that have benefitted from the administration of essential oils that their therapeutic potential is vast and yet underdeveloped. Moreover, since they are not composed of a single "magic bullet" with one target, they often have multiple effects that have additive or synergistic properties within a treatment regime.

A great many research papers investigating the bioactivity of essential oils conclude that the results are very encouraging and that clinical trials are the next step. For the majority, this step is never taken. The expense is one limiting factor and it is not surprising that clinical trials are mostly conducted once the essential oils have been formulated into a commercial product that has financial backing. It is evident that many of the claims made for essential oils in therapeutic applications have not been substantiated and an evidence base is clearly lacking. However, there is similarly a lack of research to demonstrate that essential oils are not effective interventions.

With the continuing search for new medicaments from natural sources, especially in the realm of antimicrobial therapy, it is hoped that future research into the efficacy of essential oils will be both stimulated and funded.

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12 *In Vitro* Antimicrobial Activities of Essential Oils Monographed in the European Pharmacopoeia 6th Edition

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CONTENTS

12.1 INTRODUCTION

One of the first systematic *in vitro* examinations of the antimicrobial activity of essential oils dates back to the late nineteenth century when Buchholtz studied the growth inhibitory properties of caraway oil, thyme oil, phenol, and thymol on bacteria having been cultivated in a tabac decoction. In this examination, thymol turned out to be 10-fold stronger than phenol (Buchholtz, 1875), which was in use as surgical antiseptic at that time (Ashhurst, 1927). The German pharmacopoeia "Deutsches Arzneibuch 6" (DAB 6) issued in 1926 and later supplements (1947, 1959) listed together 26 essential oils, and by this it has become obvious that essential oils have a long history in pharmaceutical practice due to their pharmacological activities. The European Pharmacopoeia 6th edition issued in 2007 lists 28 essential oils. Among them are 20 oils already present in DAB 6 (anise, bitter fennel, caraway, cassia, cinnamon bark, citronella, clove, coriander, eucalyptus, juniper, lavender, lemon, matricaria, neroli, peppermint, pine needle, pumilio pine, rosemary, thyme, and turpentine), three oils have been previously listed in the British Pharmacopoeia in the year 1993 (dementholized mint, nutmeg, and sweet orange), one in the French Pharmacopoeia X (star anise), and five oils were added later (cinnamon leaf, clary sage, mandarin, star anise, and tea tree).

Since essential oils are subject for pharmacological studies, tests on their antimicrobial activities have been done frequently. In consequence, a comprehensive data material exists, which, however, has never been compiled together for important essential oils, like such listed actually in the European Pharmacopoeia. Therefore, an attempt was done to collect and examine such information

from scientific literature to obtain an insight into the variability of test parameters, data variation, and the significance of such factors in the interpretation of results.

Among the testing methods used to characterize *in vitro* antimicrobial activity of essential oils, the three main methods turned out to be agar diffusion test, serial broth or agar dilution test, and the vapor phase test. Further tests comprise various kill-time studies, for example, the activity of a compound relative to phenol after 15 min (phenol coefficient) (Rideal et al., 1903), killing time determination after contact to a test compounds using contaminated silk threads (Koch, 1881), recording of growth curves and determination of the amount of a compound being effective to inhibit growth of 50% of test organisms (Friedman et al., 2004), poisoned food techniques in which the delay of microbial growth is determined in presence of growth inhibitors (Kurita et al., 1983; Reiss, 1982), spore germination, and short contact time studies in fungi (Smyth et al., 1932; Mikhlin et al., 1983). Other studies monitor presence or absence of growth by measuring metabolic CO_2 in yeast (Belletti et al., 2004) or visualize growth by indicators, such as sulfur salts from sulfur-supplemented cow milk as growth medium (Geinitz, 1912), 2,3,5-triphenyltetrazolium chloride (Canillac et al., 1996), or *p*-iodonitrophenyltetrazolium violet (Al-Bayati, 2008; Weseler et al., 2005). The bioautographic assay on thin-layer chromatography plates has been developed for identification of active compounds in plant extracts (Rahalison et al., 1994), but was later also taken for the examination of essential oils (Iscan et al., 2002).

12.1.1 Agar Diffusion Test (ADT)

In the agar diffusion test, the essential oil to be tested is placed on top of an agar surface. Two techniques exist: In the first one, the essential oil is placed onto a paper disk; in the second, a hole is made into the agar surface and the essential oil is put into the hole. In the following, the essential oil diffuses from its reservoir through the agar medium, which is seeded with microorganism. Antimicrobially active oils cause an inhibition zone around the reservoir after incubation, respectively, and normally the size of inhibition zone is regarded as measure for the antimicrobial potency of an essential oil. However, lipophilic compounds such as farnesol cause only small inhibition zones against *Bacillus subtilis* in the agar diffusion test (Weis, 1986), although the compound resulted in a strong inhibition in the serial dilution test (MIC = 12.5 µg/ml) (Kubo et al., 1983). Thus, strong inhibitors having low water solubility gave a poor or even negative result in the agar diffusion test. It is therefore wrong to conclude that an essential oil without resulting in an inhibition zone in the agar diffusion test is without any antimicrobially active constituents; or in other words, antimicrobially active compounds are easily overlooked by this method. Another problem is the interpretation of the size of inhibition zones, which depend on both, the diffusion coefficient plus antimicrobial activity of every compound present in an essential oil. Beside generally unknown diffusion coefficients of essential oil constituents, the size of inhibition zones is influenced by several other factors: volatilization of essential oil, disk or hole sizes, amount of compound given to disk or into hole, adsorption by the disk, agar type, agar–agar content, pH, volume of agar, microbial strains tested (Janssen et al., 1987). Taken together, this test method can be used as a pretest, but the results should not be over-rated. In the following data schedule the experimental conditions are briefly given (culture medium, incubation temperature, incubation time, disk or hole size, amount of essential oil on disk or hole). The amount of compound in microgram added to a reservoir (disk or hole) is recalculated from microliter with a density of 1 for all essential oils.

12.1.2 Dilution Test (DIL)

In the dilution test, the essential oil to be tested is incorporated in a semisolid agar medium or liquid broth in several defined amounts. Absence of growth in agar plates or test tubes is determined with

the naked eye after incubation. The minimum inhibitory concentration (MIC) is the concentration of essential oil present in the ungrown agar plate or test tube with the highest amount of test material. When essential oils are tested, the main difficulty is caused by their low water solubility. The addition of solvents (e.g., dimethylsulfoxide and ethanol) or detergents (e.g., Tween 20) to the growth medium are unavoidable, which however influences the MIC (Hili et al., 1997; Hammer et al., 1999; Remmal et al., 1993). Another problem is the volatilization of essential oils during incubation. Working in a chamber with saturated moistened atmosphere (Pauli, 2006) or high water activity levels (Guynot et al., 2005) improved the situation. The MIC is additionally influenced by the selection of growth media, for example, in RPMI 1640, the MIC toward yeast is about 15 times lower than in Sabouraud medium (Jirovetz et al., 2007; McCarthy et al., 1992). Further MIC-influencing test parameters are size of inoculum, pH of growth medium, and incubation time. Nevertheless, the serial dilution test in liquid broth was recommended for natural substances (Boesel, 1991; Hadacek et al., 2000; Pauli, 2007) and is standardized for the testing of antibacterial and antifungal drugs in liquid broth and agar plates (Clinical & Laboratory Standards Institute, 2008). Its use enables a link to data of pharmaceutical drugs and an easier interpretation of test results. In the data section, all concentrations are recalculated in µg/ml. To distinguish between the agar dilution test and the serial dilution test, the growth medium is abbreviated either as agar (A) or broth (B). Test parameters—exceptionally citations—are systematically given and comprise growth medium, incubation time, incubation temperature, and MIC in µg/ml or ppm.

12.1.3 Vapor Phase Test (VP)

In the vapor phase, a standardized method does not exist among tests to study antimicrobial activity of essential oils. In most of the examinations, a reservoir (paper disk, cup, and glass) contains the sample of essential oil, and a seeded agar plate was inverted and covered the reservoir. After inoculation, an inhibition zone is formed, which is the measure of activity. Most of the data listed in the following tables have been worked out by such methods. Because these methods allow only the creation of relative values, a few examiners defined the MIC in atmosphere (MIC_{air}) by using airtight boxes (Inouye et al., 2001; Nakahara et al., 2003), which contained a seeded agar plate and the essential oil on the glass or paper. Otherwise, the results were estimated with +++ = normal growth, ++ = reduced growth, + = visible growth, and NG = no growth. The test parameters given in the following tables are growth medium, incubation time, incubation temperature, and activity evaluation or MIC_{air} in µg/ml or ppm.

To give detailed information, the following abbreviations are used in Tables 12.1 through 12.80: (h), essential oil was given into a hole; BA, Bacto agar; BHA, brain–heart infusion agar; BlA, blood agar; CA, Czapek's agar; CAB, Campylobacter agar base; CDA, Czapek Dox agar; DMSO, dimethylsulfoxide; EtOH, ethanol; EYA, Emerson's Ybss agar; germ., germination; HIB, heart infusion broth; HS, horse serum; inh., inhibition; ISA, Iso-sensitest agar; KBA, King's medium B agar; LA, Laury agar; LSA, Listeria selective agar; MA, malt agar; MAA, medium A agar; MBA, mycobiotic agar; MCA, MacConkey agar; MEB, malt extract broth; MHA, Mueller–Hinton agar; MHA, Mueller–Hinton broth; MIA, minimum inhibitory amount in µg per disk; MIC, minimum inhibitory concentration in µg/ml or ppm; MIC_{air}, minimal inhibitory concentration in µg/ml or ppm in the vapor phase; MYA, malt extract–yeast extract–peptone–glucose agar; MYB, malt extract–yeast extract–glucose–peptone broth; NA, nutrient agar; NB, nutrient broth; OA, oat agar; PB, Pennassay broth; Ref., reference number; SA, Sabouraud agar; SB, Sabouraud broth; sd, saturated disk; SDA, Sabouraud dextrose agar; SGB, Sabouraud glucose broth; SMA, Sabouraud maltose agar; sol., solution; TYA, tryptone–glucose–yeast extract agar; THB, Todd–Hewitt broth; TSA, trypticase soy agar; TSB, trypticase soy broth; TYB, tryptone–glucose–yeast extract broth; VC, various conditions: not given in detail; WA, sucrose–peptone agar; WFA, wheat flour agar; YPB, and yeast extract–peptone–dextrose broth.

TABLE 12.1
Inhibitory Data of Anise Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	4.5	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterococcus faecalis	Bac–	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	7	Janssen et al. (1986)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4.5	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	11	Yousef and Tawil (1980)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Klebsiella aerogenes	Bac–	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	1	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	1.5	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)

Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	6.7	Janssen et al. (1986)
Pseudomonas sp.	Bac–	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4.5	Smith-Palmer et al. (1998)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 100,000	16	Narasimha Rao and Nigam (1970)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	1	Maruzzella and Lichtenstein (1956)
Serratia sp.	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Vibrio cholerae	Bac–	NA, 24 h, 37°C	10 (h), 100,000	17	Narasimha Rao and Nigam (1970)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus sp.	Bac+	Cited	15, 2500	1	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	8	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	12.5	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Lactobacillus delbrueckii	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Lactobacillus plantarum	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactococcus garvieae	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactococcus lactis subsp. *lactis*	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)

continued

TABLE 12.1 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (μg)	Inhibition Zone (mm)	Ref.
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	18	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Staphylococcus aureus	Bac+	Cited	15, 2500	1	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	8.3	Janssen et al. (1986)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	14.5	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	4	Pizsolitto et al. (1975)
Staphylococcus epidermidis	Bac+	NA, 24 h, 37°C	10 (h), 100,000	12	Narasimha Rao and Nigam (1970)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptococcus sp.	Bac+	NA, 24 h, 37°C	10 (h), 100,000	16	Narasimha Rao and Nigam (1970)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	6.35, sd	7	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	35	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	6.35, sd	9	Maruzzella and Liguori (1958)
Aspergillus flavus	Fungi	Cited	—, pure	28	Gangrade et al. (1991)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	6.35, sd	9	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	0	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	6.35, sd	13	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	Cited	—, pure	27	Gangrade et al. (1991)

Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Fusarium oxysporum	Fungi	Cited	—, pure	32	Gangrade et al. (1991)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	15	Pawar and Thaker (2007)
Geotrichum sp.	Fungi	SDA, 2–7 d, 20°C	5 (h), 60,000	17	Kosalec et al. (2005)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	10	Maruzzella and Liguori (1958)
Microsporum canis	Fungi	SDA, 2–7 d, 20°C	5 (h), 60,000	27	Kosalec et al. (2005)
Microsporum gypseum	Fungi	SDA, 2–7 d, 20°C	5 (h), 60,000	21	Kosalec et al. (2005)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	6.35, sd	6	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	12	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	6.35, sd	12	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	6.35, sd	3	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	13	Yousef and Tawil (1980)
Trichophyton mentagrophytes	Fungi	SDA, 2–7 d, 20°C	5 (h), 60,000	23	Kosalec et al. (2005)
Trichophyton rubrum	Fungi	SDA, 2–7 d, 20°C	5 (h), 60,000	25	Kosalec et al. (2005)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	2	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	12	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	15	Yousef and Tawil (1980)
Candida albicans	Yeast	SDA, 2–7 d, 20°C	5 (h), 60,000	29	Kosalec et al. (2005)
Candida glabrata	Yeast	SDA, 2–7 d, 20°C	5 (h), 60,000	21	Kosalec et al. (2005)
Candida krusei	Yeast	SDA, 2–7 d, 20°C	5 (h), 60,000	[a]	Kosalec et al. (2005)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Candida parapsilosis	Yeast	SDA, 2–7 d, 20°C	5 (h), 60,000	30	Kosalec et al. (2005)
Candida pseudotropicalis	Yeast	SDA, 2–7 d, 20°C	5 (h), 60,000	[a]	Kosalec et al. (2005)
Candida tropicalis	Yeast	SDA, 2–7 d, 20°C	5 (h), 60,000	[a]	Kosalec et al. (2005)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	6.35, sd	2	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	9	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)

[a] Fungicidal.

TABLE 12.2
Inhibitory Data of Anise Oil Obtained in the Dilution Test

Microorganism	MO Class	Test Parameters	MIC (µg/mL)	Ref.
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	>10,000	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	TSB, 24 h, 37°C	>10,000	Di Pasqua et al. (2005)
Escherichia coli	Bac–	MHB, DMSO, 24 h, 37°C	>500	Al-Bayati (2008)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Helicobacter pylori	Bac–	Cited, 20 h, 37°C	294.7–589.4	Weseler et al. (2005)
Klebsiella pneumoniae	Bac–	MHB, DMSO, 24 h, 37°C	>500	Al-Bayati (2008)
Proteus mirabilis	Bac–	MHB, DMSO, 24 h, 37°C	125	Al-Bayati (2008)
Proteus vulgaris	Bac–	MHB, DMSO, 24 h, 37°C	62.5	Al-Bayati (2008)
Pseudomonas aeruginosa	Bac–	MHB, DMSO, 24 h, 37°C	>500	Al-Bayati (2008)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Salmonella typhi	Bac–	MHB, DMSO, 24 h, 37°C	500	Al-Bayati (2008)
Salmonella typhimurium	Bac–	TSB, 24 h, 37°C	>10,000	Di Pasqua et al. (2005)
Salmonella typhimurium	Bac–	MHB, DMSO, 24 h, 37°C	250	Al-Bayati (2008)
Bacillus cereus	Bac+	MHB, DMSO, 24 h, 37°C	62.5	Al-Bayati (2008)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Brochotrix thermosphacta	Bac+	M17, 24 h, 20°C	>10,000	Di Pasqua et al. (2005)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	200	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	MHB, DMSO, 24 h, 37°C	125	Al-Bayati (2008)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Alternaria alternata	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Alternaria tenuissima	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)

Aspergillus awamorii	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	PDA, 7–14 d, 28°C	500	Soliman and Badeaa (2002)
Aspergillus flavus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus fumigatus	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus niger	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	83% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus ochraceus	Fungi	PDA, 7–14 d, 28°C	500	Soliman and Badeaa (2002)
Aspergillus ochraceus	Fungi	YES broth, 10 d	82% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	250	Okazaki and Oshima (1953)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus parasiticus	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus parasiticus	Fungi	PDA, 7–14 d, 28°C	500	Soliman and Badeaa (2002)
Aspergillus parasiticus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus sydowi	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus tamari	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Aspergillus terreus	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Botryodiplodia theobromae	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Cephalosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Ceratocystis paradoxa	Fungi	OA, EtOH, 3 d, 20°C	1000	Narasimba Rao et al. (1971)
Cladosporium herbarum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Colletotrichum capsici	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Curvularia lunata	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Curvularia lunata	Fungi	OA, EtOH, 3 d, 20°C	4000	Narasimba Rao et al. (1971)
Curvularia pallescens	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Epicoccum nigrum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)

continued

TABLE 12.2 (continued)

Microorganism	MO Class	Test Parameters	MIC (μg/mL)	Ref.
Fusarium accuminatum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Fusarium culmorum	Fungi	YES broth, 10 d	69% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium equisiti	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Fusarium moniliforme	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Fusarium moniliforme	Fungi	PDA, 7–14 d, 28°C	500	Soliman and Badeaa (2002)
Fusarium moniliforme	Fungi	PDA, 7 d, 23.5°C	52% inh. 10,000	Mueller-Ribeau et al. (1995)
Fusarium moniliforme var. *subglutinans*	Fungi	OA, EtOH, 3 d, 20°C	2000	Narasimba Rao et al. (1971)
Fusarium oxysporum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Fusarium semitectum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Fusarium udum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Geotrichum sp.	Fungi	SGB, 3–7 d, 25°C	15,600	Kosalec et al. (2005)
Helminthosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	4000	Narasimba Rao et al. (1971)
Macrophomina phaesoli	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Microsporum canis	Fungi	SGB, 3–7 d, 25°C	1000	Kosalec et al. (2005)
Microsporum gypseum	Fungi	SGB, 3–7 d, 25°C	2000	Kosalec et al. (2005)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus	Fungi	Cited	250	Okazaki and Oshima (1953)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	400	Yousef and Tawil (1980)
Nigrospora oryzae	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Penicillium chrysogenum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	250	Okazaki and Oshima (1953)
Penicillium citrinum	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Physalospora tucumanensis	Fungi	OA, EtOH, 3 d, 20°C	2000	Narasimba Rao et al. (1971)
Phytophthora capsici	Fungi	PDA, 7 d, 23.5°C	36% inh. 10,000	Mueller-Ribeau et al. (1995)

Rhizoctonia solani	Fungi	PDA, 7 d, 23.5°C	100% inh. 10,000	Mueller-Ribeau et al. (1995)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus nigricans	Fungi	Cited	100% inh. 600	Shukla and Tripathi (1987)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Sclerotina sclerotiorum	Fungi	PDA, 7 d, 23.5°C	100% inh. 10,000	Mueller-Ribeau et al. (1995)
Sclerotium rolfsii	Fungi	OA, EtOH, 6 d, 20°C	2000	Narasimba Rao et al. (1971)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Trichophyton mentagrophytes	Fungi	SGB, 3–7 d, 25°C	7800	Kosalec et al. (2005)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SGB, 3–7 d, 25°C	2000	Kosalec et al. (2005)
Candida albicans	Yeast	SGB, 3–7 d, 25°C	10,000	Kosalec et al. (2005)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Candida glabrata	Yeast	SGB, 3–7 d, 25°C	1000	Kosalec et al. (2005)
Candida krusei	Yeast	SGB, 3–7 d, 25°C	2000	Kosalec et al. (2005)
Candida parapsilosis	Yeast	SGB, 3–7 d, 25°C	1000	Kosalec et al. (2005)
Candida pseudotropicalis	Yeast	SGB, 3–7 d, 25°C	1000	Kosalec et al. (2005)
Candida tropicalis	Yeast	SGB, 3–7 d, 25°C	1000	Kosalec et al. (2005)

TABLE 12.3
Inhibitory Data of Anise Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Test Parameters	Quantity (μg)	Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+	Guynot et al. (2003)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)

TABLE 12.4
Inhibitory Data of Bitter Fennel Obtained in the Agar Diffusion Test

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Agrobacterium tumefaciens	Bac–	WA, 48 h, 25°C	6, 8000	MIA 2880	Cantore et al. (2004)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Burkholderia gladioli pv. *agaricicola*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 7680	Cantore et al. (2004)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	MHA, 48 h, 27°C	6, 15,000	10	Ertürk et al. (2006)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Erwinia carotovora subsp. *atroseptica*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 7680	Cantore et al. (2004)
Erwinia carotovora subsp. *carotovora*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 7680	Cantore et al. (2004)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	14	Schelz et al. (2006)
Escherichia coli	Bac–	MHA, 48 h, 27°C	6, 15,000	25	Ertürk et al. (2006)
Escherichia coli	Bac–	WA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6.5	Deans and Ritchie (1987)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	MHA, 48 h, 27°C	6, 15,000	18	Ertürk et al. (2006)
Pseudomonas agarici	Bac–	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas chichorii	Bac–	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)

continued

TABLE 12.4 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (μg)	Inhibition Zone (mm)	Ref.
Pseudomonas corrugate	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas reactans	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *apata*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *apii*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *atrofaciens*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA 3840	Cantore et al. (2004)
Pseudomonas syringae pv. *Glycinea*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA 960	Cantore et al. (2004)
Pseudomonas syringae pv. *lachrymans*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *maculicola*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *phaseolicola*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *pisi*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *syringae*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas syringae pv. *tomato*	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Pseudomonas tolaasii	Bac−	KBA, 48 h, 25°C	6, 8000	MIA 7680	Cantore et al. (2004)
Pseudomonas viridiflava	Bac−	KBA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Salmonella pullorum	Bac−	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella typhimurium	Bac−	MHA, 48 h, 27°C	6, 15,000	8	Ertürk et al. (2006)
Serratia marcescens	Bac−	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Xanthomonas campestris pv. *campestris*	Bac−	WA, 48 h, 25°C	6, 8000	MIA 5760	Cantore et al. (2004)
Xanthomonas campestris pv. *phaesoli* var. *fuscans*	Bac−	WA, 48 h, 25°C	6, 8000	MIA 720	Cantore et al. (2004)
Xanthomonas campestris pv. *phaesoli* var. *phaesoli*	Bac−	WA, 48 h, 25°C	6, 8000	MIA 480	Cantore et al. (2004)
Xanthomonas campestris pv. *Vesicatoria*	Bac−	WA, 48 h, 25°C	6, 8000	MIA 1440	Cantore et al. (2004)
Yersinia enterocolitica	Bac−	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus megaterium	Bac+	WA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	18	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Clavibacter michiganensis subsp. *Michiganensis*	Bac+	WA, 48 h, 25°C	6, 8000	MIA 7680	Cantore et al. (2004)

Clavibacter michiganensis subsp. *sepedonicus*	Bac+	WA, 48 h, 25°C	6, 8000	MIA 960	Cantore et al. (2004)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Curtobacterium flaccunfaciens pv. *betae*	Bac+	WA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Curtobacterium flaccunfaciens pv. *flaccunfaciens*	Bac+	WA, 48 h, 25°C	6, 8000	MIA >7680	Cantore et al. (2004)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	20	Yousef and Tawil (1980)
Rhodococcus fascians	Bac+	WA, 48 h, 25°C	6, 8000	MIA 1920	Cantore et al. (2004)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	MHA, 48 h, 27°C	6, 15,000	16	Ertürk et al. (2006)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	17	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	MHA, 48 h, 27°C	6, 15,000	12	Ertürk et al. (2006)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), –30,000	15	Schelz et al. (2006)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	6.35, sd	10	Maruzzella and Liguori (1958)
Absidia cormybifera	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	6.35, sd	6	Maruzzella and Liguori (1958)
Alternaria sp.	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Aspergillus candidus	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus flavus	Fungi	PDA, 18 h, 37°C	6, sd	7	Sharma and Singh (1979)
Aspergillus fumigatus	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	6.35, sd	12	Maruzzella and Liguori (1958)
Aspergillus nidulans	Fungi	PDA, 18 h, 37°C	6, sd	9	Sharma and Singh (1979)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Aspergillus niger	Fungi	MHA, 48 h, 27°C	6, 15,000	12	Ertürk et al. (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	31	Yousef and Tawil (1980)
Cladosporium herbarum	Fungi	PDA, 18 h, 37°C	6, sd	12.5	Sharma and Singh (1979)
Cunninghamella echinulata	Fungi	PDA, 18 h, 37°C	6, sd	21	Sharma and Singh (1979)
Fusarium oxysporum	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Helminthosporium sacchari	Fungi	PDA, 18 h, 37°C	6, sd	16	Sharma and Singh (1979)

continued

TABLE 12.4 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	6	Maruzzella and Liguori (1958)
Humicola grisea var. *thermoidea*	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Microsporum gypseum	Fungi	PDA, 18 h, 37°C	6, sd	14.5	Sharma and Singh (1979)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	20	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	6.35, sd	7	Maruzzella and Liguori (1958)
Penicillium aculeatum	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Penicillium chrysogenum	Fungi	CA, 48 h, 27°C	5, sd	15	Nigam and Rao (1979)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	PDA, 18 h, 37°C	6, sd	7	Sharma and Singh (1979)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	7	Maruzzella and Liguori (1958)
Penicillium javanicum	Fungi	CA, 48 h, 27°C	5, sd	25	Nigam and Rao (1979)
Penicillium jensenii	Fungi	CA, 48 h, 27°C	5, sd	10	Nigam and Rao (1979)
Penicillium lividum	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Penicillium notatum	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Penicillium obscurum	Fungi	CA, 48 h, 27°C	5, sd	15	Nigam and Rao (1979)
Penicillium sp. I	Fungi	CA, 48 h, 27°C	5, sd	15	Nigam and Rao (1979)
Penicillium sp. II	Fungi	CA, 48 h, 27°C	5, sd	10	Nigam and Rao (1979)
Penicillium sp. III	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	6.35, sd	3	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	PDA, 18 h, 37°C	6, sd	7	Sharma and Singh (1979)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	20	Yousef and Tawil (1980)
Sporotrichum thermophile	Fungi	EYA, 48 h, 45°C	5, sd	25	Nigam and Rao (1979)
Thermoascus aurantiacis	Fungi	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)

Thermomyces lanuginosa	Fungi	EYA, 48 h, 45°C	5, sd	15	Nigam and Rao (1979)
Thielava minor	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Trichophyton rubrum	Fungi	PDA, 18 h, 37°C	6, sd	8	Sharma and Singh (1979)
Trichothecium roseum	Fungi	PDA, 18 h, 37°C	6, sd	9	Sharma and Singh (1979)
Brettanomyces anomalus	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Candida albicans	Yeast	MHA, 48 h, 27°C	6, 15,000	12	Ertürk et al. (2006)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	24	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	6.35, sd	20	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	10	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	6.35, sd	3	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), −30,000	11	Schelz et al. (2006)
Torula glabrata	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Torula thermophila	Yeast	EYA, 48 h, 45°C	5, sd	20	Nigam and Rao (1979)

TABLE 12.5
Inhibitory Data of Bitter Fennel Oil Obtained in the Dilution Test

Microorganism	MO Class	Test Parameters	MIC (μg/mL)	Ref.
Enterobacter aerogenes	Bac–	MHB, 24 h, 37°C	4880	Ertürk et al. (2006)
Escherichia coli	Bac–	MHB, 24 h, 37°C	1220	Ertürk et al. (2006)
Escherichia coli	Bac–	TYB, 18 h, 37°C	3000	Schelz et al. (2006)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	MHB, 24 h, 37°C	9760	Ertürk et al. (2006)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Salmonella typhimurium	Bac–	MHB, 24 h, 37°C	19,560	Ertürk et al. (2006)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	MHB, 24 h, 37°C	4880	Ertürk et al. (2006)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	TYB, 18 h, 37°C	3000	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	MHB, 24 h, 37°C	9760	Ertürk et al. (2006)
Alternaria alternata	Fungi	MEB, 72 h, 28°C	1500	Mimica-Dukic et al. (2003)
Aspergillus flavus	Fungi	MEB, 72 h, 28°C	1800–2700	Mimica-Dukic et al. (2003)
Aspergillus niger	Fungi	MEB, 72 h, 28°C	1700–2200	Mimica-Dukic et al. (2003)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	200	Yousef and Tawil (1980)
Aspergillus niger	Fungi	MHB, 24 h, 37°C	9760	Ertürk et al. (2006)
Aspergillus ochraceus	Fungi	MEB, 72 h, 28°C	1800–2000	Mimica-Dukic et al. (2003)
Aspergillus oryzae	Fungi	Cited	250	Okazaki and Oshima (1953)
Aspergillus terreus	Fungi	MEB, 72 h, 28°C	1800–2200	Mimica-Dukic et al. (2003)
Aspergillus versicolor	Fungi	MEB, 72 h, 28°C	2000–2200	Mimica-Dukic et al. (2003)
Cladosporium cladosporoides	Fungi	MEB, 72 h, 28°C	1200–1500	Mimica-Dukic et al. (2003)
Epidermophyton floccosum	Fungi	MEB, 72 h, 28°C	1200–1300	Mimica-Dukic et al. (2003)
Fusarium tricinctum	Fungi	MEB, 72 h, 28°C	800–1500	Mimica-Dukic et al. (2003)
Microsporum canis	Fungi	MEB, 72 h, 28°C	1000–1200	Mimica-Dukic et al. (2003)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	400	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)
Penicillium funiculosum	Fungi	MEB, 72 h, 28°C	2800–3000	Mimica-Dukic et al. (2003)
Penicillium ochroyloron	Fungi	MEB, 72 h, 28°C	2500–2800	Mimica-Dukic et al. (2003)
Phomopsis helianthi	Fungi	MEB, 72 h, 28°C	800–1300	Mimica-Dukic et al. (2003)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Trichoderma viride	Fungi	MEB, 72 h, 28°C	2700–3200	Mimica-Dukic et al. (2003)
Trichophyton mentagrophytes	Fungi	MEB, 72 h, 28°C	1000–1200	Mimica-Dukic et al. (2003)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Candida albicans	Yeast	MHB, 24 h, 37°C	4880	Ertürk et al. (2006)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	800	Schelz et al. (2006)

TABLE 12.6
Inhibitory Data of Bitter Fennel Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Test Parameters	Quantity (µg)	Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.7
Inhibitory Data of Caraway Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	30	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterococcus faecalis	Bac–	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TYA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	NA, 24 h, 37°C	4, —	4	El-Gengaihi and Zaki (1982)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	10.3	Janssen et al. (1986)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	18	Yousef and Tawil (1980)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	14.5	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	7.3	Janssen et al. (1986)
Pseudomonas fluorescens	Bac–	NA, 24 h, 37°C	4, —	4	El-Gengaihi and Zaki (1982)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6.5	Deans and Ritchie (1987)
Salmonella sp.	Bac–	NA, 24 h, 37°C	4, —	10	El-Gengaihi and Zaki (1982)

Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Actinomyces sp.	Bac+	NA, 24 h, 37°C	4, —	10	El-Gengaihi and Zaki (1982)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	4, —	4	El-Gengaihi and Zaki (1982)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	9	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	8.3	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	20	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Clostridium butyricum	Bac+	NA, 24 h, 37°C	4, —	0	El-Gengaihi and Zaki (1982)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TYA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus delbrueckii	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactobacillus plantarum	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Lactococcus garvieae	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactococcus lactis subsp. *lactis*	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Micrococcus sp.	Bac+	NA, 48 h, 37°C	4, —	4	El-Gengaihi and Zaki (1982)
Mycobacterium phlei	Bac+	NA, 24 h, 37°C	4, —	10	El-Gengaihi and Zaki (1982)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	28	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Sarcina lutea	Bac+	NA, 48 h, 37°C	4, —	4	El-Gengaihi and Zaki (1982)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TYA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	4, —	4	El-Gengaihi and Zaki (1982)

continued

TABLE 12.7 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	7.7	Janssen et al. (1986)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	17	Yousef and Tawil (1980)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	6.35, sd	7	Maruzzella and Liguori (1958)
Absidia corMYBifera	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	7.4	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Alternaria sp.	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus candidus	Fungi	PDA, 18 h, 37°C	6, sd	13.5	Sharma and Singh (1979)
Aspergillus flavus	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	6.35, sd	10	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Aspergillus nidulans	Fungi	PDA, 18 h, 37°C	6, sd	8	Sharma and Singh (1979)
Aspergillus niger	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	7	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	6.35, sd	13	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	42	Yousef and Tawil (1980)
Cladosporium herbarum	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Cunninghamella echinulata	Fungi	PDA, 18 h, 37°C	6, sd	21	Sharma and Singh (1979)
Fusarium oxysporum	Fungi	PDA, 18 h, 37°C	6, sd	6	Sharma and Singh (1979)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	11	Pawar and Thaker (2007)
Helminthosporium sacchari	Fungi	PDA, 18 h, 37°C	6, sd	6.5	Sharma and Singh (1979)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	12	Maruzzella and Liguori (1958)
Humicola grisea var. *thermoidea*	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Microsporum gypseum	Fungi	PDA, 18 h, 37°C	6, sd	11	Sharma and Singh (1979)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	6.35, sd	9	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	PDA, 18 h, 37°C	6, sd	15	Sharma and Singh (1979)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	28	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)

Penicillium aculeatum	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Penicillium chrysogenum	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	7	Maruzzella and Liguori (1958)
Penicillium digitatum	Fungi	PDA, 18 h, 37°C	6, sd	78	Sharma and Singh (1979)
Penicillium javanicum	Fungi	CA, 48 h, 27°C	5, sd	10	Nigam and Rao (1979)
Penicillium jensenii	Fungi	CA, 48 h, 27°C	5, sd	15	Nigam and Rao (1979)
Penicillium lividum	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Penicillium notatum	Fungi	CA, 48 h, 27°C	5, sd	15	Nigam and Rao (1979)
Penicillium obscurum	Fungi	CA, 48 h, 27°C	5, sd	20	Nigam and Rao (1979)
Penicillium sp. I	Fungi	CA, 48 h, 27°C	5, sd	10	Nigam and Rao (1979)
Penicillium sp. II	Fungi	CA, 48 h, 27°C	5, sd	15	Nigam and Rao (1979)
Penicillium sp. III	Fungi	CA, 48 h, 27°C	5, sd	0	Nigam and Rao (1979)
Rhizopus nigricans	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	6.35, sd	6	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	0	Yousef and Tawil (1980)
Sporotrichum thermophile	Fungi	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)
Thermoascus aurantiacis	Fungi	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)
Thermomyces lanuginosa	Fungi	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)
Thielava minor	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Trichophyton rubrum	Fungi	PDA, 18 h, 37°C	6, sd	7	Sharma and Singh (1979)
Trichothecium roseum	Fungi	PDA, 18 h, 37°C	6, sd	20	Sharma and Singh (1979)
Brettanomyces anomalus	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Candida albicans	Yeast	TYA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	NA, 24 h, 37°C	4, —	0	El-Gengaihi and Zaki (1982)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	9.7	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	30	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Candida utilis	Yeast	NA, 24 h, 37°C	4, —	0	El-Gengaihi and Zaki (1982)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	14	Maruzzella and Liguori (1958)

continued

TABLE 12.7 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	6.35, sd	0	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 37°C	4, —	0	El-Gengaihi and Zaki (1982)
Saccharomyces cerevisiae	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Torula glabrata	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Torula thermophila	Yeast	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)

TABLE 12.8
Inhibitory Data of Caraway Oil Obtained in the Dilution Test

Microorganism	MO Class	Test Parameters	MIC (µg/ml)	Ref.
Bacteria	Bac	5% Glucose, 9 d, 37°C	2000	Buchholtz (1875)
Escherichia coli	Bac−	TYB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac−	TSB, 24 h, 37°C	>10,000	Di Pasqua et al. (2005)
Escherichia coli	Bac−	NA, 1–3 d, 30°C	2000	Farag et al. (1989)
Escherichia coli	Bac−	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Helicobacter pylori	Bac−	Cited, 20 h, 37°C	273.1	Weseler et al. (2005)
Pseudomonas aeruginosa	Bac−	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Pseudomonas fluorescens	Bac−	NA, 1–3 d, 30°C	2000	Farag et al. (1989)
Pseudomonas sp.	Bac−	TSB, 24 h, 37°C	6000	Di Pasqua et al. (2005)
Salmonella typhimurium	Bac−	TSB, 24 h, 37°C	>10,000	Di Pasqua et al. (2005)
Serratia marcescens	Bac−	NA, 1–3 d, 30°C	2500	Farag et al. (1989)
Bacillus subtilis	Bac+	NA, 1–3 d, 30°C	1000	Farag et al. (1989)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Brochotrix thermosphacta	Bac+	M17, 24 h, 20°C	10,000	Di Pasqua et al. (2005)
Corynebacterium sp.	Bac+	TYB, 18–24 h, 37°C	500	Morris et al. (1979)
Micrococcus sp.	Bac+	NA, 1–3 d, 30°C	1000	Farag et al. (1989)
Mycobacterium phlei	Bac+	NA, 1–3 d, 30°C	750	Farag et al. (1989)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	200	Yousef and Tawil (1980)
Sarcina sp.	Bac+	NA, 1–3 d, 30°C	1250	Farag et al. (1989)
Staphylococcus aureus	Bac+	TSB, 24 h, 37°C	10,000	Di Pasqua et al. (2005)
Staphylococcus aureus	Bac+	NA, 1–3 d, 30°C	1250	Farag et al. (1989)
Staphylococcus aureus	Bac+	TYB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Alternaria citri	Fungi	PDA, 8 d, 22°C	>1000	Arras and Usai (2001)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	PDA, 7–14 d, 28°C	2000	Soliman and Badeaa (2002)
Aspergillus flavus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	CA, 7 d, 28°C	84–96% inh. 500	Kumar et al. (2007)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)

continued

TABLE 12.8 (continued)

Microorganism	MO Class	Test Parameters	MIC (μg/ml)	Ref.
Aspergillus ochraceus	Fungi	PDA, 7–14 d, 28°C	3000	Soliman and Badeaa (2002)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus parasiticus	Fungi	PDA, 7–14 d, 28°C	2000	Soliman and Badeaa (2002)
Aspergillus parasiticus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Botrytis cinera	Fungi	PDA, 8 d, 22°C	>1000	Arras and Usai (2001)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Fusarium moniliforme	Fungi	PDA, 7–14 d, 28°C	3000	Soliman and Badeaa (2002)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	800	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	PDA, 8 d, 22°C	>1000	Arras and Usai (2001)
Penicillium italicum	Fungi	PDA, 8 d, 22°C	>1000	Arras and Usai (2001)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Candida albicans	Yeast	TYB, 18–24 h, 37°C	500	Morris et al. (1979)
Saccharomyces cerevisiae	Yeast	NA, 1–3 d, 30°C	1000	Farag et al. (1989)

TABLE 12.9
Inhibitory Data of Caraway Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Test Parameters	Quantity (µg)	Activity	Ref.
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	6.35, sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.10
Inhibitory Data of Cassia Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (μg)	Inhibition Zone (mm)	Ref.
Enterobacter aerogenes	Bac–	MHA, 24 h, 30°C	6, 15,000	18	Rossi et al. (2007)
Escherichia coli	Bac–	BA, 24–48 h, 37°C	12.7, sd	14	Goutham and Purohit (1974)
Escherichia coli	Bac–	MHA, 24 h, 30°C	6, 15,000	21	Rossi et al. (2007)
Proteus vulgaris	Bac–	BA, 24–48 h, 37°C	12.7, sd	17.5	Goutham and Purohit (1974)
Pseudomonas aeruginosa	Bac–	BA, 24–48 h, 37°C	12.7, sd	18	Goutham and Purohit (1974)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 30°C	6, 15,000	19	Rossi et al. (2007)
Salmonella pullorum	Bac–	BA, 24–48 h, 37°C	12.7, sd	13.5	Goutham and Purohit (1974)
Bacillus subtilis	Bac+	BA, 24–48 h, 37°C	12.7, sd	13.5	Goutham and Purohit (1974)
Corynebacterium pyogenes	Bac+	BA, 24–48 h, 37°C	12.7, sd	13.5	Goutham and Purohit (1974)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	20	Lis-Balchin et al. (1998)
Pasteurella multocida	Bac+	BlA, 24–48 h, 37°C	12.7, sd	19	Goutham and Purohit (1974)
Staphylococcus aureus	Bac+	BA, 24–48 h, 37°C	12.7, sd	0	Goutham and Purohit (1974)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 15,000	30	Rossi et al. (2007)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	45	Pawar and Thaker (2007)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	40	Pawar and Thaker (2006)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	37	Pawar and Thaker (2007)

TABLE 12.11
Inhibitory Data of Cassia Oil Obtained in the Dilution Test

Microorganism	MO Class	Test Parameters	MIC (μg/mL)	Ref.
Escherichia coli	Bac−	NA, cited	75–600	Ooi et al. (2006)
Escherichia coli O157:H7	Bac−	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Pseudomonas aeruginosa	Bac−	NA, cited	75–600	Ooi et al. (2006)
Salmonella typhimurium	Bac−	NA, cited	75–600	Ooi et al. (2006)
Salmonella typhimurium	Bac−	BHI, 48 h, 35°C	250	Oussalah et al. (2006)
Vibrio cholerae	Bac−	NA, cited	75–600	Ooi et al. (2006)
Vibrio parahaemolyticus	Bac−	NA, cited	75–600	Ooi et al. (2006)
Yersinia enterocolitica	Bac−	MHA, Tween 20, 24 h, 37°C	300	Rossi et al. (2007)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	300	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	NA, cited	75–600	Ooi et al. (2006)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	250	Oussalah et al. (2006)
Alternaria alternata	Fungi	PDA, 7 d, 28°C	100% inh. 300	Feng and Zheng (2007)
Aspergillus niger	Fungi	YES broth, 10 d	87% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	89% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	500	Okazaki and Oshima (1953)
Aspergillus sp.	Fungi	NA, cited	75–150	Ooi et al. (2006)
Fusarium culmorum	Fungi	YES broth, 10 d	54% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium sp.	Fungi	NA, cited	75–150	Ooi et al. (2006)
Microsporum gypseum.	Fungi	NA, cited	18.8–37.5	Ooi et al. (2006)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)
Trichophyton mentagrophytes	Fungi	NA, cited	18.8–37.5	Ooi et al. (2006)
Trichophyton rubrum	Fungi	NA, cited	18.8–37.5	Ooi et al. (2006)
Candida albicans	Yeast	NA, cited	100–450	Ooi et al. (2006)
Candida glabrata	Yeast	NA, cited	100–450	Ooi et al. (2006)
Candida krusei	Yeast	NA, cited	100–450	Ooi et al. (2006)
Candida tropicalis	Yeast	NA, cited	100–450	Ooi et al. (2006)

TABLE 12.12
Inhibitory Data of Cassia Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Test Parameters	Quantity (μg)	Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Liguori (1958)
Salmonella typhi	Bac–	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Liguori (1958)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	6.35, sd	NG	Maruzzella and Liguori (1958)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	6.35, sd	+	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Liguori (1958)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	6.35, sd	+	Maruzzella and Liguori (1958)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	6.35, sd	+	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.13
Inhibitory Data of Ceylon Cinnamon Bark Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	16.5	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	15	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	18	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	27	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	8.9	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	16	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	5	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	10.1	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	Cited	15, 2500	13	Pizsolitto et al. (1975)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	15.5	Yousef and Tawil (1980)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	14.7	Janssen et al. (1986)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 2000	35	Singh et al. (2007)
Escherichia coli	Bac–	NA, 24 h, 30°C	Drop, 5000	45	Hili et al. (1997)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	22	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	18	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	11	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	17	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	12	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	15.5	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)

continued

TABLE 12.13 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	7.7	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	17.5	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 30°C	Drop, 5000	25	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	10 (h), 2000	60	Singh et al. (2007)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	10.9	Smith-Palmer et al. (1998)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	21.2	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	12	Pizsolitto et al. (1975)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 2000	41	Singh et al. (2007)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	12	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	13	Pizsolitto et al. (1975)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	20	Deans and Ritchie (1987)
Bacillus cereus	Bac+	NA, 24 h, 37°C	10 (h), 2000	27	Singh et al. (2007)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	16	Maruzzella and Lichtenstein (1956)
Bacillus sp.	Bac+	Cited	15, 2500	25	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	16	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	21	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	25	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	23.5	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	10 (h), 2000	56	Singh et al. (2007)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	35	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	19	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	6.8	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	18	Deans and Ritchie (1987)

Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	40	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	10	Janssen et al. (1986)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	7.5	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited	15, 2500	25	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	27.3	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	NA, 24 h, 30°C	Drop, 5000	45	Hili et al. (1997)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 2000	57	Singh et al. (2007)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	20	Pizsolitto et al. (1975)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	8	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	50	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	6.35, sd	12	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SDA, 3 d, 28°C	6, sd	18	Saksena and Saksena (1984)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	6.35, sd	19	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	6.35, sd	16	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	43	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	40	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Keratinomyces afelloi	Fungi	SDA, 3 d, 28°C	6, sd	18	Saksena and Saksena (1984)
Keratinophyton terreum	Fungi	SDA, 3 d, 28°C	6, sd	12	Saksena and Saksena (1984)
Microsporum gypseum	Fungi	SDA, 3 d, 28°C	6, sd	21	Saksena and Saksena (1984)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	6.35, sd	12	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	40	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	6.35, sd	17	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	6.35, sd	7	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	19	Yousef and Tawil (1980)

continued

TABLE 12.13 (continued)

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Trichophyton equinum	Fungi	SDA, 3 d, 28°C	6, sd	28	Saksena and Saksena (1984)
Trichophyton rubrum	Fungi	SDA, 3 d, 28°C	6, sd	28	Saksena and Saksena (1984)
Brettanomyces anomalus	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	18	Conner and Beuchat (1984)
Candida albicans	Yeast	SDA, 3 d, 28°C	6, sd	14	Saksena and Saksena (1984)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	15	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	27	Janssen et al. (1986)
Candida albicans	Yeast	NA, 24 h, 30°C	Drop, 5000	39	Hili et al. (1997)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	48	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	6.35, sd	4	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	21	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	6.35, sd	8	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SDA, 3 d, 28°C	6, sd	21	Saksena and Saksena (1984)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	6.35, sd	18	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	6.35, sd	13	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	29	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	18	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	16	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	15	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	8	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	17	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	28	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	11	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	17	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	6.35, sd	6	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	22	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 30°C	Drop, 5000	53	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	NA, 24 h, 30°C	Drop, 5000	43	Hili et al. (1997)
Torula glabrata	Yeast	MYA, 4 d, 30°C	5, 10% sol. sd	20	Conner and Beuchat (1984)
Torula utilis	Yeast	NA, 24 h, 30°C	Drop, 5000	42	Hili et al. (1997)

TABLE 12.14
Inhibitory Data of Ceylon Cinnamon Bark Oil Obtained in the Dilution Test

Microorganism	MO Class	Test Parameters	MIC (µg/mL)	Ref.
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	500	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NB, 16 h, 37°C	200	Lens-Lisbonne et al. (1987)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Escherichia coli	Bac–	TSB, 24 h, 35°C	500	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	MYB, DMSO, 40 h, 30°C	67% inh. 500	Hili et al. (1997)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	250	Oussalah et al. (2006)
Pseudomonas aeruginosa	Bac–	NB, 16 h, 37°C	300	Lens-Lisbonne et al. (1987)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	MYB, DMSO, 40 h, 30°C	85% inh. 500	Hili et al. (1997)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	500	Smith-Palmer et al. (1998)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	200	Yousef and Tawil (1980)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	300	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	TSB, 10 d, 4°C	300	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	320	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	250	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	NB, 16 h, 37°C	350	Lens-Lisbonne et al. (1987)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	390	Bastide et al. (1987)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	MYB, DMSO, 40 h, 30°C	70% inh. 500	Hili et al. (1997)
Streptococcus faecalis	Bac+	NB, 16 h, 37°C	200	Lens-Lisbonne et al. (1987)
Aspergillus flavus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	PDA, 7–14 d, 28°C	1000	Soliman and Badeaa (2002)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	100	Yousef and Tawil (1980)
Aspergillus ochraceus	Fungi	PDA, 7–14 d, 28°C	1000	Soliman and Badeaa (2002)
Aspergillus parasiticus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)

continued

TABLE 12.14 (continued)

Microorganism	MO Class	Test Parameters	MIC (μg/mL)	Ref.
Aspergillus parasiticus	Fungi	PDA, 7–14 d, 28°C	1000	Soliman and Badeaa (2002)
Botrytis cinera	Fungi	PDA, Tween 20, 7 d, 24°C	25% inh. 1000	Bouchra et al. (2003)
Colletotrichum musae	Fungi	SMKY, EtOH, 7 d, 28°C	300	Ranasinghe et al. (2002)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Fusarium moniliforme	Fungi	PDA, 7–14 d, 28°C	1000	Soliman and Badeaa (2002)
Fusarium proliferatum	Fungi	SMKY, EtOH, 7 d, 28°C	500	Ranasinghe et al. (2002)
Geotrichum citri-aurantii	Fungi	PDA, Tween 20, 7 d, 24°C	30% inh. 1000	Bouchra et al. (2003)
Lasiodiplodia theobromae	Fungi	SMKY, EtOH, 7 d, 28°C	350	Ranasinghe et al. (2002)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	100	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	100	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	PDA, Tween 20, 7 d, 24°C	32% inh. 1000	Bouchra et al. (2003)
Phytophthora citrophthora	Fungi	PDA, Tween 20, 7 d, 24°C	38% inh. 1000	Bouchra et al. (2003)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	400	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	80	Yousef and Tawil (1980)
Candida albicans	Yeast	MYB, DMSO, 40 h, 30°C	72% inh. 500	Hili et al. (1997)
Saccharomyces cerevisiae	Yeast	MYB, DMSO, 40 h, 30°C	59% inh. 500	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	MYB, DMSO, 40 h, 30°C	96% inh. 500	Hili et al. (1997)
Torula utilis	Yeast	MYB, DMSO, 40 h, 30°C	100% inh. 500	Hili et al. (1997)

TABLE 12.15
Inhibitory Data of Ceylon Cinnamon Bark Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Test Parameters	Quantity (µg)	Activity	Ref.
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Escherichia coli	Bac−	BlA, 18 h, 37°C	MIC_{air}	12.5	Inouye et al. (2001)
Haemophilus influenzae	Bac−	MHA, 18 h, 37°C	MIC_{air}	3.13	Inouye et al. (2001)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	6.35, sd	+	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	6.35, sd	++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	6.25	Inouye et al. (2001)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	6.35, sd	+	Maruzzella and Sicurella (1960)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	1.56–3.13	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	6.25	Inouye et al. (2001)
Aspergillus flavus	Fungi	CDA, 6 d	12, 6000	+++	Singh et al. (2007)
Aspergillus niger	Fungi	CDA, 6 d	12, 6000	+	Singh et al. (2007)
Aspergillus ochraceus	Fungi	CDA, 6 d	12, 6000	++	Singh et al. (2007)
Aspergillus terreus	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Fusarium graminearum	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Fusarium moniliforme	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Penicillium citrinum	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Penicillium viridicatum	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.16
Inhibitory Data of Ceylon Cinnamon Leaf Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Test Parameters	Disk Size (mm), Quantity (µg)	Inhibition Zone (mm)	Ref.
Escherichia coli	Bac–	TYA, 18–24 h, 37°C	9.5, 2000	17	Morris et al. (1979)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 2000	25	Singh et al. (2007)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	10 (h), 2000	90	Singh et al. (2007)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 2000	17	Singh et al. (2007)
Bacillus cereus	Bac+	NA, 24 h, 37°C	10 (h), 2000	32	Singh et al. (2007)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	10 (h), 2000	90	Singh et al. (2007)
Corynebacterium sp.	Bac+	TYA, 18–24 h, 37°C	9.5, 2000	12	Morris et al. (1979)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	20	Lis-Balchin et al. (1998)
Staphylococcus aureus	Bac+	TYA, 18–24 h, 37°C	9.5, 2000	18	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 2000	48	Singh et al. (2007)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	50	Pawar and Thaker (2007)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	30	Pawar and Thaker (2006)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	35	Pawar and Thaker (2007)
Candida albicans	Yeast	TYA, 18–24 h, 37°C	9.5, 2000	14	Morris et al. (1979)

TABLE 12.17
Inhibitory Data of Ceylon Cinnamon Leaf Oil Obtained in the Dilution Test

Microorganism	MO Class	Test Parameters	MIC (μg/mL)	Ref.
Escherichia coli	Bac–	TYB, 18–24 h, 37°C	1000	Morris et al. (1979)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Corynebacterium sp.	Bac+	TYB, 18–24 h, 37°C	500	Morris et al. (1979)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	TYB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Aspergillus flavus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Aspergillus niger	Fungi	YES broth, 10 d	95% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	94% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus parasiticus	Fungi	PDA, 6–8 h 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Colletotrichum musae	Fungi	SMKY, EtOH, 7 d, 28°C	500	Ranasinghe et al. (2002)
Fusarium culmorum	Fungi	YES broth, 10 d	73% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium proliferatum	Fungi	SMKY, EtOH, 7 d, 28°C	500	Ranasinghe et al. (2002)
Lasiodiplodia theobromae	Fungi	SMKY, EtOH, 7 d, 28°C	600	Ranasinghe et al. (2002)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Candida albicans	Yeast	TYB, 18–24 h, 37°C	500	Morris et al. (1979)

TABLE 12.18
Inhibitory Data of Ceylon Cinnamon Leaf Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Test Parameters	Quantity (µg)	Activity	Ref.
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Aspergillus flavus	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Aspergillus flavus	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Aspergillus niger	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Aspergillus ochraceus	Fungi	CDA, 6 d	12, 6000	+	Singh et al. (2007)
Aspergillus terreus	Fungi	CDA, 6 d	12, 6000	+	Singh et al. (2007)
Endomyces fibuliger	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium repens	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Fusarium graminearum	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Fusarium moniliforme	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Penicillium citrinum	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Penicillium corylophilum	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Penicillium roqueforti	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Penicillium viridicatum	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.19
Inhibitory Data of Citronella Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0[a]	Möse et al. (1957)
Brucella abortus	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5[a]	Möse et al. (1957)
Campylobacter jejuni	Bac–	Cited	6, 15,000	40[a]	Wannissorn et al. (2005)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0–1[a]	Möse et al. (1957)
Escherichia coli	Bac–	Cited	6, 15,000	10.5[a]	Wannissorn et al. (2005)
Escherichia coli	Bac–	Cited	20,000	0	Lemos et al. (1992)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	Agar, 24 h, 37°C	6, 6000	7	Jirovetz et al. (2006)
Klebsiella pneumonia	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5[a]	Möse et al. (1957)
Klebsiella pneumonia subsp. *oceanae*	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5[a]	Möse et al. (1957)
Klebsiella pneumoniae	Bac–	Agar, 24 h, 37°C	6, 6000	0	Jirovetz et al. (2006)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)
Proteus OX19	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5[a]	Möse et al. (1957)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0[a]	Möse et al. (1957)
Proteus vulgaris	Bac–	Agar, 24 h, 37°C	6, 6000	10	Jirovetz et al. (2006)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0[a]	Möse et al. (1957)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	Agar, 24 h, 37°C	6, 6000	0	Jirovetz et al. (2006)
Pseudomonas fluorescens	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0–1[a]	Möse et al. (1957)
Pseudomonas mangiferae indicae	Bac–	NA, 36–48 h, 37°C	6, sd	11	Garg and Garg (1980)
Salmonella enteritidis	Bac–	Cited	6, 15,000	12.8[a]	Wannissorn et al. (2005)
Salmonella enteritidis	Bac–	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)

continued

TABLE 12.19 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Salmonella paratyphi	Bac−	NA, 36–48 h, 37°C	6, sd	14	Garg and Garg (1980)
Salmonella paratyphi B	Bac−	NA, 24 h, 37°C	5 × 20, 1000	1[a]	Möse et al. (1957)
Salmonella sp.	Bac−	Agar, 24 h, 37°C	6, 6000	0	Jirovetz et al. (2006)
Salmonella typhi	Bac−	NA, 24 h, 37°C	5 × 20, 1000	2–5[a]	Möse et al. (1957)
Salmonella typhi	Bac−	NA, 36–48 h, 37°C	6, sd	18	Garg and Garg (1980)
Salmonella typhimurium	Bac−	Cited	6, 15,000	21[a]	Wannissorn et al. (2005)
Serratia marcescens	Bac−	NA, 24 h, 37°C	5 × 20, 1000	0[a]	Möse et al. (1957)
Serratia marcescens	Bac−	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Vibrio albicans	Bac−	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)
Vibrio cholera	Bac−	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)
Vibrio cholera	Bac−	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Bacillus anthracis	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1[a]	Möse et al. (1957)
Bacillus cereus	Bac+	Cited	20,000	20	Lemos et al. (1992)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	3[a]	Maruzzella and Lichtenstein (1956)
Bacillus mycoides	Bac+	NA, 36–48 h, 37°C	6, sd	12	Garg and Garg (1980)
Bacillus pumilus	Bac+	NA, 36–48 h, 37°C	6, sd	12	Garg and Garg (1980)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1[a]	Möse et al. (1957)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	8[a]	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 36–48 h, 37°C	6, sd	12	Garg and Garg (1980)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	15.5	Yousef and Tawil (1980)
Clostridium perfringens	Bac+	Cited	6, 15,000	39.5[a]	Wannissorn et al. (2005)
Corynebacterium diphtheria	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)
Enterococcus faecalis	Bac+	Agar, 24 h, 37°C	6, 6000	10	Jirovetz et al. (2006)
Listeria monocytogenes	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1[a]	Möse et al. (1957)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	16	Yousef and Tawil (1980)
Sarcina alba	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)
Sarcina beige	Bac+	NA, 24 h, 37°C	5 × 20, 1000	16–23[a]	Möse et al. (1957)
Sarcina citrea	Bac+	NA, 24 h, 37°C	5 × 20, 1000	0[a]	Möse et al. (1957)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)

Sarcina lutea	Bac+	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Sarcina rosa	Bac+	NA, 24 h, 37°C	5 × 20, 1000	11–15[a]	Möse et al. (1957)
Sporococcus sarc.	Bac+	NA, 24 h, 37°C	5 × 20, 1000	26–33[a]	Möse et al. (1957)
Staphylococcus albus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0[a]	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited	20,000	0[a]	Lemos et al. (1992)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5[a]	Möse et al. (1957)
Staphylococcus aureus	Bac+	Agar, 24 h, 37°C	6, 6000	10	Jirovetz et al. (2006)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	11	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 36–48 h, 37°C	6, sd	12	Garg and Garg (1980)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	12.6	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	Cited	20,000	12[a]	Lemos et al. (1992)
Streptococcus haemolyticus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10[a]	Möse et al. (1957)
Streptococcus viridians	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1[a]	Möse et al. (1957)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	15	Yousef and Tawil (1980)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 7–10 d, 28°C	5, 5000	8	Saikia et al. (2001)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Microsporon gypseum	Fungi	SDA, 7–10 d, 28°C	5, 5000	34	Saikia et al. (2001)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	18	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	40	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	1	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	12	Yousef and Tawil (1980)
Sporothrix schenkii	Fungi	SDA, 7–10 d, 28°C	5, 5000	12	Saikia et al. (2001)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	1	Morris et al. (1979)
Candida albicans	Yeast	Cited	20,000	17	Lemos et al. (1992)

continued

TABLE 12.19 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	17	Yousef and Tawil (1980)
Candida albicans	Yeast	Agar, 24 h, 37°C	6, 6000	28	Jirovetz et al. (2006)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Candida albicans	Yeast	SDA, 7–10 d, 28°C	5, 5000	7	Saikia et al. (2001)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	Cited	20,000	0	Lemos et al. (1992)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)

[a] Citronella oil obtained from *Cymbopogon nardus* (Ceylon-type).

TABLE 12.20
Inhibitory Data of Citronella Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Escherichia coli	Bac–	MHB, 24 h, 36°C	2000–8000	Duarte et al. (2006)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	>8000	Oussalah et al. (2006)
Pseudomonas aeruginosa	Bac–	NA, Tween 80, 24 h, 37°C	>500[a]	Koba et al. (2004)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Pseudomonas cepacia	Bac–	NA, Tween 80, 24 h, 37°C	>500[a]	Koba et al. (2004)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	4000	Oussalah et al. (2006)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	4000	Oussalah et al. (2006)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	1250	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Staphylococcus intermedius	Bac+	NA, Tween 80, 24 h, 37°C	>500[a]	Koba et al. (2004)
Aspergillus candidus	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Aspergillus flavus	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Aspergillus flavus	Fungi	PDA, 28 d, 21°C	4000–10,000	Thanaboripat et al. (2004)
Aspergillus fumigatus	Fungi	NA, Tween 80, 24 h, 37°C	200[a]	Koba et al. (2004)
Aspergillus niger	Fungi	SDB, 7–10 d, 28°C	2500	Saikia et al. (2001)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	6400	Yousef and Tawil (1980)
Aspergillus oryzae	Fungi	Cited	250	Okazaki and Oshima (1953)
Aspergillus versicolor	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Eurotium amstelodami	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Eurotium chevalieri	Fungi	Cited	>250[a]	Nakahara et al. (2003)

continued

TABLE 12.20 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Microsporon canis	Fungi	NA, Tween 80, 24 h, 37°C	200[a]	Koba et al. (2004)
Microsporon gypseum	Fungi	NA, Tween 80, 24 h, 37°C	500[a]	Koba et al. (2004)
Microsporon gypseum	Fungi	SDB, 7–10 d, 28°C	625	Saikia et al. (2001)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	6400	Yousef and Tawil (1980)
Penicillium adametzii	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	250	Okazaki and Oshima (1953)
Penicillium citrinum	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Penicillium griseofulvum	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Penicillium islansicum	Fungi	Cited	>250[a]	Nakahara et al. (2003)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Sporothrix schenkii	Fungi	SDB, 7–10 d, 28°C	1250	Saikia et al. (2001)
Trichophyton mentagrophytes	Fungi	NA, Tween 80, 24 h, 37°C	150[a]	Koba et al. (2004)
Candida albicans	Yeast	NA, Tween 80, 24 h, 37°C	>500[a]	Koba et al. (2004)
Candida albicans	Yeast	SDB, 7–10 d, 28°C	1250	Saikia et al. (2001)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Cryptococcus neoformans	Yeast	NA, Tween 80, 24 h, 37°C	500[a]	Koba et al. (2004)
Malassezia pachydermis	Yeast	NA, Tween 80, 24 h, 37°C	150[a]	Koba et al. (2004)

[a] Citronella oil obtained from *Cymbopogon nardus* (Ceylon-type).

TABLE 12.21
Inhibitory Data of Citronella Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++[a]	Maruzzella and Sicurella (1960)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+[a]	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG[a]	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++[a]	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++[a]	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Aspergillus candidus	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Aspergillus flavus	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Aspergillus flavus	Fungi	PDA, 10 d, 25°C	50	++	Sarbhoy et al. (1978)
Aspergillus fumigatus	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Aspergillus sulphureus	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Aspergillus versicolor	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Eurotium amstelodami	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Eurotium chevalieri	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Mucor fragilis	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Penicillium adametzii	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Penicillium citrinum	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Penicillium griseofulvum	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Penicillium islansicum	Fungi	PDA, 3–5 d, 27°C	250 mg/L air	NG[b]	Nakahara et al. (2003)
Rhizopus stolonifer	Fungi	PDA, 10 d, 25°C	50	++	Sarbhoy et al. (1978)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

[a] Formosan citronella oil.
[b] Citronella oil obtained from *Cymbopogon nardus* (Ceylon-type).

TABLE 12.22
Inhibitory Data of Clary Sage Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Escherichia coli	Bac–	MHA, 24 h, 37°C	6, 10,000	14	Yousefzadi et al. (2007)
Klebsiella pneumoniae	Bac–	MHA, 24 h, 37°C	6, 10,000	10	Yousefzadi et al. (2007)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 37°C	6, 10,000	0	Yousefzadi et al. (2007)
Bacillus pumilus	Bac+	MHA, 24 h, 37°C	6, 10,000	16	Yousefzadi et al. (2007)
Bacillus subtilis	Bac+	MHA, 24 h, 37°C	6, 10,000	17	Yousefzadi et al. (2007)
Enterococcus faecalis	Bac+	MHA, 24 h, 37°C	6, 10,000	9	Yousefzadi et al. (2007)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9–15	Lis-Balchin et al. (1998)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 10,000	15	Yousefzadi et al. (2007)
Staphylococcus epidermidis	Bac+	MHA, 24 h, 37°C	6, 10,000	15	Yousefzadi et al. (2007)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	0	Pawar and Thaker (2007)
Aspergillus niger	Fungi	MHA, 48 h, 30°C	6, 10,000	0	Yousefzadi et al. (2007)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	0	Pawar and Thaker (2006)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	9	Pawar and Thaker (2007)
Candida albicans	Yeast	MHA, 48 h, 30°C	6, 10,000	0	Yousefzadi et al. (2007)
Saccharomyces cerevisiae	Yeast	MHA, 48 h, 30°C	6, 10,000	0	Yousefzadi et al. (2007)

TABLE 12.23
Inhibitory Data of Clary Sage Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Acinetobacter baumannii	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Aeromonas sobria	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Erwinia carotovora	Bac–	NA, 48 h, 22°C	>2000	Maruzzella (1963)
Escherichia coli	Bac–	NA, 48 h, 37°C	>2000	Maruzzella (1963)
Escherichia coli	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Escherichia coli	Bac–	MHB, Tween 80, 24 h, 37°C	15,000	Yousefzadi et al. (2007)
Escherichia coli	Bac–	Cited	1500–2000	Peana et al. (1999)
Klebsiella pneumoniae	Bac–	MHB, Tween 80, 24 h, 37°C	>15,000	Yousefzadi et al. (2007)
Klebsiella pneumoniae	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Proteus vulgaris	Bac–	NA, 48 h, 37°C	>2000	Maruzzella (1963)
Pseudomonas aeruginosa	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Salmonella typhi	Bac–	NA, 48 h, 37°C	>2000	Maruzzella (1963)
Salmonella typhimurium	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Serratia marcescens	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Bacillus cereus	Bac+	NA, 48 h, 37°C	250	Maruzzella (1963)
Bacillus circulans	Bac+	NA, 48 h, 37°C	100	Maruzzella (1963)
Bacillus megaterium	Bac+	NA, 48 h, 37°C	250	Maruzzella (1963)
Bacillus pumilus	Bac+	MHB, Tween 80, 24 h, 37°C	7500	Yousefzadi et al. (2007)
Bacillus subtilis	Bac+	MHB, Tween 80, 24 h, 37°C	7500	Yousefzadi et al. (2007)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 48 h, 37°C	100	Maruzzella (1963)
Enterococcus faecalis	Bac+	MHB, Tween 80, 24 h, 37°C	>15,000	Yousefzadi et al. (2007)
Enterococcus faecalis	Bac+	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Staphylococcus aureus	Bac+	NA, 48 h, 37°C	>2000	Maruzzella (1963)
Staphylococcus aureus	Bac+	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Staphylococcus aureus	Bac+	Cited	1500–2000	Peana et al. (1999)

continued

TABLE 12.23 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	7500	Yousefzadi et al. (2007)
Staphylococcus epidermidis	Bac+	Cited	1500–2000	Peana et al. (1999)
Staphylococcus epidermidis	Bac+	MHB, Tween 80, 24 h, 37°C	7500	Yousefzadi et al. (2007)
Alternaria alternata	Fungi	SDA, 6–8 h, 20°C	500, 62% inh.	Dikshit et al. (1986)
Aspergillus flavus	Fungi	SDA, 6–8 h, 20°C	500, 52% inh.	Dikshit et al. (1986)
Aspergillus niger	Fungi	YES broth, 10 d	– 92% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	– >10,000	Lis-Balchin et al. (1998)
Fusarium culmorum	Fungi	YES broth, 10 d	– 69% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium oxysporum f.sp. *dianthi*	Fungi	PDA, Tween 20, 4 d, 23°C	72% inh. 2000	Pitarokili et al. (2002)
Geotrichum candidum	Fungi	NA, 5 d, 22°C	>2000	Maruzzella (1963)
Gibberella fugikuroi	Fungi	NA, 5 d, 22°C	>2000	Maruzzella (1963)
Helminthosporium truicicum	Fungi	NA, 5 d, 22°C	>2000	Maruzzella (1963)
Microsporum gypseum	Fungi	SDA, 7 d, 30°C	400, 56% inh.	Dikshit and Husain (1984)
Microsporum gypseum	Fungi	SDA, 6–8 h, 20°C	500, 56% inh.	Dikshit et al. (1986)
Penicillium italicum	Fungi	SDA, 6–8 h, 20°C	500, 59% inh.	Dikshit et al. (1986)
Phoma betae	Fungi	NA, 5 d, 22°C	>2000	Maruzzella (1963)
Pityrosporum ovale	Fungi	NA, 5 d, 22°C	>2000	Maruzzella (1963)
Sclerotina cepivorum	Fungi	PDA, Tween 20, 4 d, 23°C	94% inh. 1000	Pitarokili et al. (2002)
Sclerotina sclerotiorum	Fungi	PDA, Tween 20, 4 d, 23°C	1000	Pitarokili et al. (2002)
Trichophyton equinum	Fungi	SDA, 7 d, 30°C	400, 30% inh.	Dikshit and Husain (1984)
Trichophyton mentagrophytes	Fungi	SDA, 6–8 h, 20°C	500, 40% inh.	Dikshit et al. (1986)
Trichophyton rubrum	Fungi	SDA, 7 d, 30°C	400, 43% inh.	Dikshit and Husain (1984)
Trichophyton rubrum	Fungi	SDA, 6–8 h, 20°C	500, 43% inh.	Dikshit et al. (1986)
Candida albicans	Yeast	NA, 48 h, 37°C	>2000	Maruzzella (1963)
Candida albicans	Yeast	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Candida albicans	Yeast	Cited	1500–2000	Peana et al. (1999)

TABLE 12.24
Inhibitory Data of Clary Sage Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 48 h, 37°C	500 µL in cover	+++	Maruzzella (1963)
Proteus vulgaris	Bac–	NA, 48 h, 37°C	500 µL in cover	NG	Maruzzella (1963)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 48 h, 37°C	500 µL in cover	+	Maruzzella (1963)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 48 h, 37°C	500 µL in cover	NG	Maruzzella (1963)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Geotrichum candidum	Fungi	NA, 5 d, 22°C	500 µL in cover	+++	Maruzzella (1963)
Gibberella fugikuroi	Fungi	NA, 5 d, 22°C	500 µL in cover	+++	Maruzzella (1963)
Helminthosporium truicicum	Fungi	NA, 5 d, 22°C	500 µL in cover	+++	Maruzzella (1963)
Phoma betae	Fungi	NA, 5 d, 22°C	500 µL in cover	+++	Maruzzella (1963)
Pityrosporum ovale	Fungi	NA, 5 d, 22°C	500 µL in cover	+++	Maruzzella (1963)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 48 h, 37°C	500 µL in cover	+++	Maruzzella (1963)

Annotation: Clary sage absolute tested (Maruzzella, 1963).

TABLE 12.25
Inhibitory Data of Clove Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	16	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	16.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	1	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	9	Smith-Palmer et al. (1998)
Campylobacter jejuni	Bac–	Cited	6, 15,000	22.5	Wannissorn et al. (2005)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Citrobacter sp.	Bac–	NA, 24 h, 37°C	11, sd	0	Prasad et al. (1986)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Enterobacter sp.	Bac–	NA, 24 h, 37°C	11, sd	16	Prasad et al. (1986)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	14.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	NA, 24 h, 37°C	11, sd	0	Prasad et al. (1986)
Escherichia coli	Bac–	Cited	15, 2500	6	Pizsolitto et al. (1975)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	16	Yousef and Tawil (1980)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	9.7	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	19	Morris et al. (1979)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	13.7	Janssen et al. (1986)
Escherichia coli	Bac–	Cited	6, 15,000	16.5	Wannissorn et al. (2005)
Escherichia coli	Bac–	NA, 24 h, 30°C	Drop, 5000	38	Hili et al. (1997)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	15	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	4	Pizsolitto et al. (1975)

Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	9	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	11	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 30°C	Drop, 5000	23	Hili et al. (1997)
Pseudomonas sp.	Bac–	NA, 24 h, 37°C	11, sd	0	Prasad et al. (1986)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	11.1	Smith-Palmer et al. (1998)
Salmonella enteritidis	Bac–	Cited	6, 15,000	14.3	Wannissorn et al. (2005)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	16	Deans and Ritchie (1987)
Salmonella saintpaul	Bac–	NA, 24 h, 37°C	11, sd	22	Prasad et al. (1986)
Salmonella sp.	Bac–	Cited	15, 2500	7	Pizsolitto et al. (1975)
Salmonella sp. B	Bac–	NA, 24 h, 37°C	11, sd	26	Prasad et al. (1986)
Salmonella typhimurium	Bac–	Cited	6, 15,000	19.5	Wannissorn et al. (2005)
Salmonella weltevreden	Bac–	NA, 24 h, 37°C	11, sd	22	Prasad et al. (1986)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	25	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	6	Pizsolitto et al. (1975)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Bacillus anthracis	Bac+	NA, 24 h, 37°C	11, sd	25	Prasad et al. (1986)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus saccharolyticus	Bac+	NA, 24 h, 37°C	11, sd	20	Prasad et al. (1986)
Bacillus sp.	Bac+	Cited	15, 2500	9	Pizsolitto et al. (1975)
Bacillus stearothermophilus	Bac+	NA, 24 h, 37°C	11, sd	20	Prasad et al. (1986)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	7	Maruzzella and Lichtenstein (1956)

continued

TABLE 12.25 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	16	Janssen et al. (1986)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	12.5	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	11, sd	22	Prasad et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	22.5	Yousef and Tawil (1980)
Bacillus thurengiensis	Bac+	NA, 24 h, 37°C	11, sd	19	Prasad et al. (1986)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	14	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Clostridium perfringens	Bac+	Cited	6, 15,000	20.5	Wannissorn et al. (2005)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	15	Morris et al. (1979)
Lactobacillus casei	Bac+	NA, 24 h, 37°C	11, sd	22	Prasad et al. (1986)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	11	Deans and Ritchie (1987)
Lactobacillus plantarum	Bac+	NA, 24 h, 37°C	11, sd	50	Prasad et al. (1986)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	8.4	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	20	Lis-Balchin et al. (1998)
Micrococcus glutamicus	Bac+	NA, 24 h, 37°C	11, sd	44	Prasad et al. (1986)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	20	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Sarcina lutea	Bac+	NA, 24 h, 37°C	11, sd	32	Prasad et al. (1986)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited	15, 2500	5	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	8	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	11	Janssen et al. (1986)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	14	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	14.3	Yousef and Tawil (1980)

Staphylococcus aureus	Bac+	NA, 24 h, 30°C	Drop, 5000	21	Hili et al. (1997)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	11, sd	30	Prasad et al. (1986)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	10	Pizsolitto et al. (1975)
Staphylococcus sp.	Bac+	NA, 24 h, 37°C	11, sd	26	Prasad et al. (1986)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	1	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	40.5	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	28	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	34	Yousef and Tawil (1980)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	12	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	20	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	47	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	20	Yousef and Tawil (1980)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	18	Conner and Beuchat (1984)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	19	Morris et al. (1979)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	20	Yousef and Tawil (1980)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	28.3	Janssen et al. (1986)
Candida albicans	Yeast	NA, 24 h, 30°C	Drop, 5000	40	Hili et al. (1997)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	27	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)

continued

TABLE 12.25 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	26	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	19	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	19	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	21	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	14	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	15	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	29	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	13	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	18	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	19	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 30°C	Drop, 5000	50	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	NA, 24 h, 30°C	Drop, 5000	34	Hili et al. (1997)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	15	Conner and Beuchat (1984)
Torula utilis	Yeast	NA, 24 h, 30°C	Drop, 5000	39	Hili et al. (1997)

TABLE 12.26
Inhibitory Data of Clove Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Acinetobacter baumannii	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Brucella abortus	Bac–	Cited	250	Okazaki and Oshima (1952)
Brucella melitensis	Bac–	Cited	500	Okazaki and Oshima (1952)
Brucella suis	Bac–	Cited	15	Okazaki and Oshima (1952)
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	500	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	LA, 18 h, 37°C	500–1000	Remmal et al. (1993)
Escherichia coli	Bac–	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	Cited	500	Okazaki and Oshima (1952)
Escherichia coli	Bac–	MPB, DMSO, 40 h, 30°C	74% inh. 500	Hili et al. (1997)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Escherichia coli	Bac–	NA, 1–3 d, 30°C	1250	Farag et al. (1989)
Escherichia coli	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Klebsiella pneumoniae	Bac–	Cited	>500	Okazaki and Oshima (1952)
Klebsiella pneumoniae	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Proteus vulgaris	Bac–	Cited	250	Okazaki and Oshima (1952)
Pseudomonas aeruginosa	Bac–	Cited	>500	Okazaki and Oshima (1952)
Pseudomonas aeruginosa	Bac–	MPB, DMSO, 40 h, 30°C	75% inh. 500	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Pseudomonas fluorescens	Bac–	NA, 1–3 d, 30°C	1500	Farag et al. (1989)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Salmonella haddar	Bac–	LA, 18 h, 37°C	500	Remmal et al. (1993)
Salmonella paratyphi A	Bac–	Cited	500	Okazaki and Oshima (1952)
Salmonella paratyphi B	Bac–	Cited	>500	Okazaki and Oshima (1952)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Serratia marcescens	Bac–	NA, 1–3 d, 30°C	1500	Farag et al. (1989)

continued

TABLE 12.26 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Serratia marcescens	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Shigella dysenteriae I	Bac–	Cited	500	Okazaki and Oshima (1952)
Shigella dysenteriae II	Bac–	Cited	500	Okazaki and Oshima (1952)
Bacillus megaterium	Bac+	LA, 18 h, 37°C	500–1000	Remmal et al. (1993)
Bacillus subtilis	Bac+	NA, 1–3 d, 30°C	500	Farag et al. (1989)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Enterococcus faecalis	Bac+	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Listeria monocytogenes	Bac+	TSB, 10 d, 4°C	200	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	300	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Micrococcus sp.	Bac+	NA, 1–3 d, 30°C	250	Farag et al. (1989)
Mycobacterium phlei	Bac+	NA, 1–3 d, 30°C	500	Farag et al. (1989)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Mycobacterium tuberculosis	Bac+	Cited	125	Okazaki and Oshima (1952)
Sarcina sp.	Bac+	NA, 1–3 d, 30°C	500	Farag et al. (1989)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited	500	Okazaki and Oshima (1952)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	MPB, DMSO, 40 h, 30°C	83% inh. 500	Hili et al. (1997)
Staphylococcus aureus	Bac+	NA, 1–3 d, 30°C	750	Farag et al. (1989)
Staphylococcus aureus	Bac+	LA, 18 h, 37°C	1000	Remmal et al. (1993)
Staphylococcus aureus	Bac+	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Staphylococcus citreus	Bac+	Cited	500	Okazaki and Oshima (1952)
Achorion gypseum	Fungi	Cited, 15 d	125	Okazaki and Oshima (1952)
Alternaria alternata	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	156–312	Tullio et al. (2006)
Alternaria citri	Fungi	PDA, 8 d, 22°C	500	Arras and Usai (2001)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)

Aspergillus flavus	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Aspergillus flavus var. *columnaris*	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250	Tullio et al. (2006)
Aspergillus fumigatus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Aspergillus niger	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	95% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	94% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	250	Okazaki and Oshima (1953)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Botrytis cinera	Fungi	PDA, 8 d, 22°C	500	Arras and Usai (2001)
Cladosporium cladosporoides	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	78–156	Tullio et al. (2006)
Colletotrichum musae	Fungi	SMKY, EtOH, 7 d, 28°C	400	Ranasinghe et al. (2002)
Epidermophyton floccosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	125	Tullio et al. (2006)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Epidermophyton inguinale	Fungi	Cited, 15 d	125	Okazaki and Oshima (1952)
Fusarium culmorum	Fungi	YES broth, 10 d	73% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium oxysporum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	625	Tullio et al. (2006)
Fusarium proliferatum	Fungi	SMKY, EtOH, 7 d, 28°C	500	Ranasinghe et al. (2002)
Lasiodiplodia theobromae	Fungi	SMKY, EtOH, 7 d, 28°C	450	Ranasinghe et al. (2002)
Microsporum canis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	125–500	Tullio et al. (2006)
Microsporum gypseum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	125–250	Tullio et al. (2006)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Mucor racemosus	Fungi	Cited	2	Okazaki and Oshima (1953)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Mucor sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Penicillium chrysogenum	Fungi	Cited	250	Okazaki and Oshima (1953)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	PDA, 8 d, 22°C	500	Arras and Usai (2001)
Penicillium frequentans	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250	Tullio et al. (2006)
Penicillium italicum	Fungi	PDA, 8 d, 22°C	500	Arras and Usai (2001)

continued

TABLE 12.26 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Penicillium lanosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Scopulariopsis brevicaulis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Trichophyton asteroides	Fungi	Cited, 15 d	125	Okazaki and Oshima (1952)
Trichophyton interdigitale	Fungi	Cited, 15 d	125	Okazaki and Oshima (1952)
Trichophyton mentagrophytes	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	250–500	Tullio et al. (2006)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton purpureum	Fungi	Cited, 15 d	125	Okazaki and Oshima (1952)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton schoenleinii	Fungi	Cited, 15 d	125	Okazaki and Oshima (1952)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Candida albicans	Yeast	MPB, DMSO, 40 h, 30°C	61% inh. 500	Hili et al. (1997)
Candida albicans	Yeast	MHA, Tween 20, 48 h, 35°C	1200	Hammer et al. (1999)
Candida albicans	Yeast	MHB, Tween 80, 48 h, 35	1200	Hammer et al. (1998)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Saccharomyces cerevisiae	Yeast	MPB, DMSO, 40 h, 30°C	99% inh. 500	Hili et al. (1997)
Saccharomyces cerevisiae	Yeast	NA, 1–3 d, 30°C	750	Farag et al. (1989)
Schizosaccharomyces pombe	Yeast	MPB, DMSO, 40 h, 30°C	94% inh. 500	Hili et al. (1997)
Torula utilis	Yeast	MPB, DMSO, 40 h, 30°C	95% inh. 500	Hili et al. (1997)

TABLE 12.27
Inhibitory Data of Clove Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	+	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Alternaria alternata	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312–625	Tullio et al. (2006)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Aspergillus flavus	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625–1250	Tullio et al. (2006)
Aspergillus fumigatus	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625–1250	Tullio et al. (2006)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Aspergillus niger	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625–1250	Tullio et al. (2006)
Cladosporium cladosporoides	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78–156	Tullio et al. (2006)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Fusarium oxysporum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312	Tullio et al. (2006)
Microsporum canis	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312–1250	Tullio et al. (2006)
Microsporum gypseum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	156–312	Tullio et al. (2006)
Mucor sp.	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625	Tullio et al. (2006)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Penicillium frequentans	Fungi	RPMI, 7 d, 30°C	MIC_{air}	>10,000	Tullio et al. (2006)
Penicillium lanosum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	>10,000	Tullio et al. (2006)
Rhizopus sp.	Fungi	RPMI, 7 d, 30°C	MIC_{air}	125	Tullio et al. (2006)
Scopulariopsis brevicaulis	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312–1250	Tullio et al. (2006)
Trichophyton mentagrophytes	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78–156	Tullio et al. (2006)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.28
Inhibitory Data of Coriander Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Agrobacterium tumefaciens	Bac–	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Burkholderia gladioli pv. *agaricicola*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 3480	Cantore et al. (2004)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	MHA, 48 h, 27°C	6, 15,000	12	Ertürk et al. (2006)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Erwinia carotovora subsp. *atroseptica*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Erwinia carotovora subsp. *carotovora*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	10	Janssen et al. (1986)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.6	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 30°C	Drop, 5000	13	Hili et al. (1997)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	10.5	Yousef and Tawil (1980)
Escherichia coli	Bac–	MHA, 48 h, 27°C	6, 15,000	20	Ertürk et al. (2006)
Escherichia coli	Bac–	WA, 48 h, 25°C	6, 8000	MIA 870	Cantore et al. (2004)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)

Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 30°C	Drop, 5000	6	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	9	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	MHA, 48 h, 27°C	6, 15,000	12	Ertürk et al. (2006)
Pseudomonas agarici	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 3480	Cantore et al. (2004)
Pseudomonas chichorii	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 6960	Cantore et al. (2004)
Pseudomonas corrugate	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 3480	Cantore et al. (2004)
Pseudomonas reactans	Bac–	KBA, 48 h, 25°C	6, 8000	MIA >6960	Cantore et al. (2004)
Pseudomonas syringae pv. *apata*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 3480	Cantore et al. (2004)
Pseudomonas syringae pv. *atrofaciens*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 6960	Cantore et al. (2004)
Pseudomonas syringae pv. *glycinea*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 870	Cantore et al. (2004)
Pseudomonas syringae pv. *lachrymans*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA >6960	Cantore et al. (2004)
Pseudomonas syringae pv. *maculicola*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 870	Cantore et al. (2004)
Pseudomonas syringae pv. *phaseolicola*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 2610	Cantore et al. (2004)
Pseudomonas syringae pv. *pisi*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 2610	Cantore et al. (2004)
Pseudomonas syringae pv. *syringae*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 3480	Cantore et al. (2004)
Pseudomonas syringae pv. *tomato*	Bac–	KBA, 48 h, 25°C	6, 8000	MIA 3480	Cantore et al. (2004)
Pseudomonas tolaasii	Bac–	KBA, 48 h, 25°C	6, 8000	MIA >6960	Cantore et al. (2004)
Pseudomonas viridiflava	Bac–	KBA, 48 h, 25°C	6, 8000	MIA >6960	Cantore et al. (2004)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	11	Deans and Ritchie (1987)
Salmonella typhimurium	Bac–	MHA, 48 h, 27°C	6, 15,000	7	Ertürk et al. (2006)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Xanthomonas campestris pv. *campestris*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 217	Cantore et al. (2004)
Xanthomonas campestris pv. *phaesoli* var. *fuscans*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 217	Cantore et al. (2004)
Xanthomonas campestris pv. *phaesoli* var. *phaesoli*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 217	Cantore et al. (2004)
Xanthomonas campestris pv. *vesicatoria*	Bac–	WA, 48 h, 25°C	6, 8000	MIA 217	Cantore et al. (2004)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)

continued

TABLE 12.28 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Bacillus megaterium	Bac+	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	7.3	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	11	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	27	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	14	Deans and Ritchie (1987)
Clavibacter michiganensis subsp. *michiganensis*	Bac+	WA, 48 h, 25°C	6, 8000	MIA 374	Cantore et al. (2004)
Clavibacter michiganensis subsp. *sepedonicus*	Bac+	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Curtobacterium flaccunfaciens pv. *betae*	Bac+	WA, 48 h, 25°C	6, 8000	MIA 632	Cantore et al. (2004)
Curtobacterium flaccunfaciens pv. *flaccunfaciens*	Bac+	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	19	Yousef and Tawil (1980)
Rhodococcus fascians	Bac+	WA, 48 h, 25°C	6, 8000	MIA 435	Cantore et al. (2004)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	NA, 24 h, 30°C	Drop, 5000	12	Hili et al. (1997)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	14	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	11.3	Janssen et al. (1986)

Staphylococcus aureus	Bac+	MHA, 48 h, 27°C	6, 15,000	18	Ertürk et al. (2006)
Staphylococcus epidermidis	Bac+	MHA, 48 h, 27°C	6, 15,000	10	Ertürk et al. (2006)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	12	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	18	Maruzzella and Liguori (1958)
Alternaria sp.	Fungi	PDA, 18 h, 37°C	6, sd	9.5	Sharma and Singh (1979)
Aspergillus candidus	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus flavus	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus fumigatus	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	21	Maruzzella and Liguori (1958)
Aspergillus nidulans	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	0	Pawar and Thaker (2006)
Aspergillus niger	Fungi	MHA, 48 h, 27°C	6, 15,000	0	Ertürk et al. (2006)
Aspergillus niger	Fungi	PDA, 18 h, 37°C	6, sd	16	Sharma and Singh (1979)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	21	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Cladosporium herbarum	Fungi	PDA, 18 h, 37°C	6, sd	21.5	Sharma and Singh (1979)
Cunninghamella echinulata	Fungi	PDA, 18 h, 37°C	6, sd	20	Sharma and Singh (1979)
Fusarium oxysporum	Fungi	PDA, 18 h, 37°C	6, sd	0	Sharma and Singh (1979)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	11.5	Pawar and Thaker (2007)
Helminthosporium sacchari	Fungi	PDA, 18 h, 37°C	6, sd	13	Sharma and Singh (1979)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Microsporum gypseum	Fungi	PDA, 18 h, 37°C	6, sd	8	Sharma and Singh (1979)
Mucor mucedo	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	25	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	21	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	28	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°°C	6 (h), pure	60	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	PDA, 18 h, 37°C	6, sd	12	Sharma and Singh (1979)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	14	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	PDA, 18 h, 37°C	6, sd	11	Sharma and Singh (1979)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)

continued

TABLE 12.28 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	19	Yousef and Tawil (1980)
Trichophyton rubrum	Fungi	PDA, 18 h, 37°C	6, sd	10	Sharma and Singh (1979)
Trichothecium roseum	Fungi	PDA, 18 h, 37°C	6, sd	8	Sharma and Singh (1979)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida albicans	Yeast	MHA, 48 h, 27°C	6, 15,000	10	Ertürk et al. (2006)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	11	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	28	Yousef and Tawil (1980)
Candida albicans	Yeast	NA, 24 h, 30°C	Drop, 5000	29	Hili et al. (1997)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	20	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	12	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	8	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 30°C	Drop, 5000	32	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	NA, 24 h, 30°C	Drop, 5000	33	Hili et al. (1997)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Torula utilis	Yeast	NA, 24 h, 30°C	Drop, 5000	37	Hili et al. (1997)

TABLE 12.29
Inhibitory Data of Coriander Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Enterobacter aerogenes	Bac–	MHB, 24 h, 37°C	4315	Ertürk et al. (2006)
Escherichia coli	Bac–	MPB, DMSO, 40 h, 30°C	>500	Hili et al. (1997)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	MHB, 24 h, 37°C	2150	Ertürk et al. (2006)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Pseudomonas aeruginosa	Bac–	MPB, DMSO, 40 h, 30°C	74% inh. 500	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	MHB, 24 h, 37°C	4350	Ertürk et al. (2006)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	MHB, 24 h, 37°C	17,260	Ertürk et al. (2006)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	>8000	Oussalah et al. (2006)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	200	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	MPB, DMSO, 40 h, 30°C	44% inh. 500	Hili et al. (1997)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Staphylococcus aureus	Bac+	MHB, 24 h, 37°C	1070	Ertürk et al. (2006)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	3120	Bastide et al. (1987)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	MHB, 24 h, 37°C	8630	Ertürk et al. (2006)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	CDA, cited	3000	Dubey et al. (1990)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Aspergillus niger	Fungi	MHB, 24 h, 37°C	>20,000	Ertürk et al. (2006)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	800	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	800	Yousef and Tawil (1980)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)

continued

TABLE 12.29 (continued)
Inhibitory Data of Coriander Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	300,625	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Candida albicans	Yeast	MPB, DMSO, 40 h, 30°C	75% inh. 500	Hili et al. (1997)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Candida albicans	Yeast	MHB, Tween 80, 48 h, 35°C	2500	Hammer et al. (1998)
Candida albicans	Yeast	MHB, 24 h, 37°C	4310	Ertürk et al. (2006)
Saccharomyces cerevisiae	Yeast	MPB, DMSO, 40 h, 30°C	68% inh. 500	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	MPB, DMSO, 40 h, 30°C	18% inh. 500	Hili et al. (1997)
Torula utilis	Yeast	MPB, DMSO, 40 h, 30°C	87% inh. 500	Hili et al. (1997)

TABLE 12.30
Inhibitory Data of Coriander Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Escherichia coli	Bac–	BLA, 18 h, 37°C	MIC_{air}	250	Inouye et al. (2001)
Haemophilus influenzae	Bac–	MHA, 18 h, 37°C	MIC_{air}	12.5	Inouye et al. (2001)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.31
Inhibitory Data of Dwarf Pine Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	11	Janssen et al. (1986)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	5	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	8.3	Janssen et al. (1986)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	5	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	16.7	Janssen et al. (1986)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	32.3	Janssen et al. (1986)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)

TABLE 12.32
Inhibitory Data of Dwarf Pine Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)

TABLE 12.33
Inhibitory Data of Dwarf Pine Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)

TABLE 12.34
Inhibitory Data of Eucalyptus Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	8.3	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	MHA, 24 h, 30°C	6, 15,000	7	Rossi et al. (2007)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	8	Janssen et al. (1986)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	10.3	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	Cited	15, 2500	16	Pizsolitto et al. (1975)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	20	Yousef and Tawil (1980)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	22	Schelz et al. (2006)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	10	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	17	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)

continued

TABLE 12.34 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	13	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	9.7	Janssen et al. (1986)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	7.5	Smith-Palmer et al. (1998)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	10	Pizsolitto et al. (1975)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	7	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	13	Pizsolitto et al. (1975)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	10	Maruzzella and Lichtenstein (1956)
Bacillus sp.	Bac+	Cited	15, 2500	4	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	8.3	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	23.5	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	34	Maruzzella and Lichtenstein (1956)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	4.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6–20	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	5.4	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	29	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)

Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited	15, 2500	5	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	8	Janssen et al. (1986)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	8.5	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 15,000	16	Rossi et al. (2007)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	30	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	15	Pizsolitto et al. (1975)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), −30,000	15	Schelz et al. (2006)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	10	Yousef and Tawil (1980)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	10	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	13	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	0	Yousef and Tawil (1980)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	11.7	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	31	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), −30,000	16–21	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)

TABLE 12.35
Inhibitory Data of Eucalyptus Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	>10,000	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISB, Tween 80, 20–24 h, 37°C	10,000	Harkenthal et al. (1999)
Enterobacter aerogenes	Bac–	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	ISB, Tween 80, 20–24 h, 37°C	>40,000	Harkenthal et al. (1999)
Escherichia coli	Bac–	TGB, 18 h, 37°C	2800	Schelz et al. (2006)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Klebsiella pneumoniae	Bac–	ISB, Tween 80, 20–24 h, 37°C	5000	Harkenthal et al. (1999)
Proteus mirabilis	Bac–	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Pseudomonas aeruginosa	Bac–	ISB, Tween 80, 20–24 h, 37°C	>40,000	Harkenthal et al. (1999)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Salmonella choleraesuis	Bac–	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Shigella flexneri	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Bacillus subtilis	Bac+	ISB, Tween 80, 20–24 h, 37°C	10,000	Harkenthal et al. (1999)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Corynebacterium pseudodiphtheriae	Bac+	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Enterococcus durans	Bac+	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Enterococcus faecalis	Bac+	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Enterococcus faecium	Bac+	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Listeria monocytogenes	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	750	Smith-Palmer et al. (1998)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)

Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	1000	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	ISB, Tween 80, 20–24 h, 37°C	20,000	Harkenthal et al. (1999)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	2800	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	ISB, Tween 80, 20–24 h, 37°C	5000	Harkenthal et al. (1999)
Staphylococcus saprophyticus	Bac+	ISB, Tween 80, 20–24 h, 37°C	5000	Harkenthal et al. (1999)
Staphylococcus xylosus	Bac+	ISB, Tween 80, 20–24 h, 37°C	5000	Harkenthal et al. (1999)
Alternaria alternata	Fungi	PDA, 7 d, 28°C	0% inh. 500	Feng and Zheng (2007)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	– 87% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	– 61% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	500	Okazaki and Oshima (1953)
Botrytis cinera	Fungi	PDA, Tween 20, 7 d, 24°C	2% inh. 1000	Bouchra et al. (2003)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	625–1250	Janssen et al. (1988)
Fusarium culmorum	Fungi	YES broth, 10 d	– 78% inh. 10,000	Lis-Balchin et al. (1998)
Geotrichum citri-aurantii	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Mucor racemosus	Fungi	Cited	>500	Okazaki and Oshima (1953)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	25,000	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)
Penicillium digitatum	Fungi	PDA, Tween 20, 7 d, 24°C	2% inh. 1000	Bouchra et al. (2003)
Phytophthora citrophthora	Fungi	PDA, Tween 20, 7 d, 24°C	38% inh. 1000	Bouchra et al. (2003)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	6400	Yousef and Tawil (1980)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	625–1250	Janssen et al. (1988)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	700	Schelz et al. (2006)

TABLE 12.36
Inhibitory Data of Eucalyptus Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.37
Inhibitory Data of Juniper Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Enterobacter aerogenes	Bac–	MHA, 24 h, 30°C	6, 15,000	8	Rossi et al. (2007)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	0	Janssen et al. (1986)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	0	Schelz et al. (2006)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	1	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	MHA, 24 h, 30°C	6, 15,000	11	Rossi et al. (2007)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	10	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	8	Janssen et al. (1986)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	7.7	Janssen et al. (1986)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	5	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	9	Janssen et al. (1986)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 15,000	17	Rossi et al. (2007)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), –30,000	0	Schelz et al. (2006)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	11	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Aspergillus flavus	Fungi	SDA, 72 h, 26°C	8, 25,000	0	Shin (2003)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 72 h, 26°C	8, 25,000	0	Shin (2003)

continued

TABLE 12.37 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	5	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Botrytis cinera	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Cercospora beticola	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Fusarium graminearum	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	7.5	Pawar and Thaker (2007)
Fusarium oxysporum lycopersici	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Helminthosporium oryzae	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Phytophthora capsici	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Pyricularia oryzae	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Pythium ultimum	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Rhizoctonia solani	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Sclerotium rolfsii	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Septoria tritici	Fungi	PDA, few days, 24°C	12.7, sd	0	Angioni et al. (2003)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	15	Janssen et al. (1986)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), −30,000	15–17	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)

TABLE 12.38
Inhibitory Data of Juniper Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Escherichia coli	Bac–	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2003)
Escherichia coli	Bac–	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Escherichia coli	Bac–	TGB, 18 h, 37°C	5400	Schelz et al. (2006)
Escherichia coli O157:H7	Bac–	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Pseudomonas aeruginosa	Bac–	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2003)
Pseudomonas aeruginosa	Bac–	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Salmonella typhimurium	Bac–	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Yersinia enterocolitica	Bac–	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Yersinia enterocolitica	Bac–	MHA, Tween 20, 24 h, 37°C	2500	Rossi et al. (2007)[a]
Bacillus cereus	Bac+	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Enterococcus faecalis	Bac+	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Enterococcus faecium VRE	Bac+	HIB, Tween 80, 18 h, 37°C	>20,000	Nelson (1997)
Listeria monocytogenes	Bac+	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Staphylococcus aureus	Bac+	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2003)
Staphylococcus aureus	Bac+	NB, 24 h, 37°C	>900	Cosentino et al. (2003)
Staphylococcus aureus MRSA	Bac+	HIB, Tween 80, 18 h, 37°C	>20,000	Nelson (1997)
Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	>11,300	Schelz et al. (2006)
Alternaria alternate	Fungi	SDA, 6–8 h, 20°C	500, 13% inh.	Dikshit et al. (1986)
Aspergillus flavus	Fungi	MYB, 72 h, 26°C	>25,000	Shin (2003)
Aspergillus flavus	Fungi	RPMI, 24 h, 37°C	>900	Cosentino et al. (2003)
Aspergillus flavus	Fungi	RPMI, DMSO, 72 h, 37°C	20,000	Cavaleiro et al. (2006)[a]
Aspergillus flavus	Fungi	SDA, 6–8 h, 20°C	500, 11% inh.	Dikshit et al. (1986)
Aspergillus fumigatus	Fungi	RPMI, DMSO, 72 h, 37°C	10,000	Cavaleiro et al. (2006)[a]

continued

TABLE 12.38 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Aspergillus niger	Fungi	MYB, 72 h, 26°C	>25,000	Shin (2003)
Aspergillus niger	Fungi	RPMI, DMSO, 72 h, 37°C	10,000–20,000	Cavaleiro et al. (2006)[a]
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Epidermophyton floccosum	Fungi	RPMI, DMSO, 72 h, 37°C	1250	Cavaleiro et al. (2006)[a]
Microsporum canis	Fungi	RPMI, DMSO, 72 h, 37°C	1250	Cavaleiro et al. (2006)[a]
Microsporum gypseum	Fungi	RPMI, DMSO, 72 h, 37°C	2500	Cavaleiro et al. (2006)[a]
Microsporum gypseum	Fungi	SDA, 6–8 h, 20°C	500, 48% inh.	Dikshit et al. (1986)
Penicillium italicum	Fungi	SDA, 6–8 h, 20°C	500, 20% inh.	Dikshit et al. (1986)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Trichophyton mentagrophytes	Fungi	RPMI, DMSO, 72 h, 37°C	1250	Cavaleiro et al. (2006)[a]
Trichophyton mentagrophytes	Fungi	SDA, 6–8 h, 20°C	500, 48% inh.	Dikshit et al. (1986)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Trichophyton rubrum	Fungi	RPMI, DMSO, 72 h, 37°C	1250	Cavaleiro et al. (2006)[a]
Trichophyton rubrum	Fungi	SDA, 6–8 h, 20°C	500, 53% inh.	Dikshit et al. (1986)
Candida albicans	Yeast	NB, DMSO, 24 h, 30°C	>900	Angioni et al. (2003)
Candida albicans	Yeast	RPMI, DMSO, 24 h, 37°C	1250–10,000	Cavaleiro et al. (2006)[a]
Candida glabrata	Yeast	RPMI, DMSO, 24 h, 37°C	5000	Cavaleiro et al. (2006)[a]
Candida krusei	Yeast	RPMI, DMSO, 24 h, 37°C	5000	Cavaleiro et al. (2006)[a]
Candida parapsilosis	Yeast	RPMI, DMSO, 24 h, 37°C	5000	Cavaleiro et al. (2006)[a]
Candida tropicalis	Yeast	RPMI, DMSO, 24 h, 37°C	20,000	Cavaleiro et al. (2006)[a]
Saccharomyces cerevisiae	Yeast	SB, 24 h, 37°C	>900	Cosentino et al. (2003)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	2700	Schelz et al. (2006)

[a] Juniper communis ssp. *alpine.*

TABLE 12.39
Inhibitory Data of Juniper Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.40
Inhibitory Data of Lavender Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Enterococcus faecalis	Bac–	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	7.7	Janssen et al. (1986)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	0	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas sp.	Bac–	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Salmonella enteritidis	Bac–	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Salmonella typhimurium	Bac–	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)

Serratia marcescens	Bac−	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Shigella flexneri	Bac−	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Yersinia enterocolitica	Bac−	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Yersinia enterocolitica	Bac−	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	17	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	12.5	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	13.7	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	43	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus delbrueckii	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactobacillus plantarum	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Lactobacillus sp.	Bac+	MRS, cited	9, 20,000	20– >90	Pellecuer et al. (1980)
Lactococcus garvieae	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Lactococcus lactis subsp. *lactis*	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0–18	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Listeria monocytogenes	Bac+	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Micrococcus luteus	Bac+	MHA, cited	9, 20,000	0–22	Pellecuer et al. (1980)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Micrococcus ureae	Bac+	MHA, cited	9, 20,000	22	Pellecuer et al. (1980)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	22	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Sarcina ureae	Bac+	MHA, cited	9, 20,000	>90	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	5000 on agar	0	Di Pasqua et al. (2005)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)

continued

TABLE 12.40 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	12.3	Janssen et al. (1986)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	14.3	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	MHA, cited	9, 20,000	14	Pellecuer et al. (1980)
Streptococcus D	Bac+	MHA, cited	9, 20,000	0–29	Pellecuer et al. (1980)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	14	Deans and Ritchie (1987)
Streptococcus micros	Bac+	MHA, cited	9, 20,000	26	Pellecuer et al. (1980)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	15	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	13.3	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Aspergillus flavus	Fungi	SDA, 72 h, 26°C	8, 25,000	4	Shin (2003)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 72 h, 26°C	8, 25,000	2	Shin (2003)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	10	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	32	Yousef and Tawil (1980)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	9.8	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	21	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	16	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	14	Yousef and Tawil (1980)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	11.5	Yousef and Tawil (1980)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	14.3	Janssen et al. (1986)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)

TABLE 12.41
Inhibitory Data of Lavender Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Bordetella bronchiseptica	Bac−	Cited	2000	Pellecuer et al. (1976)
Escherichia coli	Bac−	Cited	500	Pellecuer et al. (1976)
Escherichia coli	Bac−	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac−	TSB, 24 h, 37°C	>10,000	Di Pasqua et al. (2005)
Escherichia coli	Bac−	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Haemophilus influenza	Bac−	Cited	2000	Pellecuer et al. (1976)
Klebsiella pneumoniae	Bac−	Cited	1000	Pellecuer et al. (1976)
Moraxella glucidolytica	Bac−	Cited	2000	Pellecuer et al. (1976)
Neisseria catarrhalis	Bac−	Cited	250	Pellecuer et al. (1976)
Neisseria flava	Bac−	Cited	500	Pellecuer et al. (1976)
Pseudomonas aeruginosa	Bac−	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Salmonella typhimurium	Bac−	TSB, 24 h, 37°C	10,000	Di Pasqua et al. (2005)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	Cited	1000	Pellecuer et al. (1976)
Corynebacterium pseudodiphtheriae	Bac+	Cited	1000	Pellecuer et al. (1976)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Enterococcus faecium VRE	Bac+	HIB, Tween 80, 18 h, 37°C	5000–10,000	Nelson (1997)
Lactobacillus sp.	Bac+	MRS, cited	5	Pellecuer et al. (1980)
Micrococcus flavus	Bac+	Cited	1000	Pellecuer et al. (1976)
Micrococcus luteus	Bac+	MHB, cited	1.25–2.5	Pellecuer et al. (1980)
Micrococcus ureae	Bac+	MHB, cited	2.5	Pellecuer et al. (1980)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	100	Yousef and Tawil (1980)
Sarcina lutea	Bac+	Cited	2000	Pellecuer et al. (1976)
Sarcina ureae	Bac+	MHB, cited	0.31–0.62	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Staphylococcus aureus	Bac+	Cited	2000	Pellecuer et al. (1976)

continued

TABLE 12.41 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Staphylococcus aureus	Bac+	TSB, 3% EtOH, 24 h, 37°C	10,000	Rota et al. (2004)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	12,500	Bastide et al. (1987)
Staphylococcus aureus MRSA	Bac+	HIB, Tween 80, 18 h, 37°C	5000	Nelson (1997)
Staphylococcus epidermidis	Bac+	MHB, cited	5	Pellecuer et al. (1980)
Staphylococcus epidermidis	Bac+	Cited	2000	Pellecuer et al. (1976)
Streptococcus D	Bac+	MHB, cited	0.62–5	Pellecuer et al. (1980)
Streptococcus micros	Bac+	MHB, cited	>5	Pellecuer et al. (1980)
Streptococcus pyogenes	Bac+	Cited	2000	Pellecuer et al. (1976)
Alternaria alternata	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Aspergillus flavus	Fungi	CA, 7 d, 28	5–8% inh. 500	Kumar et al. (2007)
Aspergillus flavus	Fungi	MYB, 72 h, 26°C	3120	Shin (2003)
Aspergillus flavus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Aspergillus flavus var. *columnaris*	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Aspergillus fumigatus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Aspergillus niger	Fungi	Cited	1000	Pellecuer et al. (1976)
Aspergillus niger	Fungi	MYB, 72 h, 26°C	3120	Shin (2003)
Aspergillus niger	Fungi	YES broth, 10 d	– 93% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus niger	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	25,000	Yousef and Tawil (1980)
Aspergillus ochraceus	Fungi	YES broth, 10 d	– 90% inh. 10,000	Lis-Balchin et al. (1998)
Cladosporium cladosporoides	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500–10,000	Tullio et al. (2006)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Epidermophyton floccosum	Fungi	Cited	1000	Pellecuer et al. (1976)
Epidermophyton floccosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Fusarium culmorum	Fungi	YES broth, 10 d	– 89% inh. 10,000	Lis-Balchin et al. (1998)

Fusarium oxysporum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	156	Tullio et al. (2006)
Microsporum canis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500–5000	Tullio et al. (2006)
Microsporum canis	Fungi	MBA, Tween 80, 10 d, 30°C	75– >300	Perrucci et al. (1994)
Microsporum gypseum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000–10,000	Tullio et al. (2006)
Microsporum gypseum	Fungi	MBA, Tween 80, 10 d, 30°C	50– >300	Perrucci et al. (1994)
Microsporum gypseum	Fungi	SDA, 7 d, 30°C	400, 24% inh.	Dikshit and Husain (1984)
Mucor sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Penicillium frequentans	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Penicillium lanosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Rhizopus sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Scopulariopsis brevicaulis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Trichophyton equinum	Fungi	SDA, 7 d, 30°C	400, 10% inh.	Dikshit and Husain (1984)
Trichophyton interdigitale	Fungi	Cited	1000	Pellecuer et al. (1976)
Trichophyton mentagrophytes	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000–10,000	Tullio et al. (2006)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SDA, 7 d, 30°C	400, 21% inh.	Dikshit and Husain (1984)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Candida albicans	Yeast	Cited	2000	Pellecuer et al. (1976)
Candida albicans	Yeast	MHB, Tween 80, 48 h, 35	5000	Hammer et al. (1998)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Candida mycoderma	Yeast	Cited	2000	Pellecuer et al. (1976)
Candida paraspilosis	Yeast	Cited	4000	Pellecuer et al. (1976)
Candida pelliculosa	Yeast	Cited	1000	Pellecuer et al. (1976)
Candida tropicalis	Yeast	Cited	4000	Pellecuer et al. (1976)
Geotrichum asteroides	Yeast	Cited	2000	Pellecuer et al. (1976)
Hansenula sp.	Yeast	Cited	2000	Pellecuer et al. (1976)
Saccharomyces carlsbergensis	Yeast	Cited	2000	Pellecuer et al. (1976)

TABLE 12.42
Inhibitory Data of Lavender Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Escherichia coli	Bac–	BLA, 18 h, 37°C	MIC_{air}	>1600	Inouye et al. (2001)
Haemophilus influenzae	Bac–	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Lactobacillus sp.	Bac+	MRS, cited	Disk, 20,000?	+++	Pellecuer et al. (1980)
Micrococcus luteus	Bac+	MHB, cited	Disk, 20,000?	+++	Pellecuer et al. (1980)
Micrococcus ureae	Bac+	MHB, cited	Disk, 20,000?	++	Pellecuer et al. (1980)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Sarcina ureae	Bac+	MHB, cited	Disk, 20,000?	+	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	100	Inouye et al. (2001)
Staphylococcus epidermidis	Bac+	MHB, cited	Disk, 20,000?	++	Pellecuer et al. (1980)
Streptococcus D	Bac+	MHB, cited	Disk, 20,000?	+++	Pellecuer et al. (1980)

Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus micros	Bac+	MHB, cited	Disk, 20,000?	+++	Pellecuer et al. (1980)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Alternaria alternata	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625–2500	Tullio et al. (2006)
Aspergillus flavus	Fungi	RPMI, 7 d, 30°C	MIC_{air}	2500	Tullio et al. (2006)
Aspergillus fumigatus	Fungi	RPMI, 7 d, 30°C	MIC_{air}	1250–2500	Tullio et al. (2006)
Aspergillus niger	Fungi	RPMI, 7 d, 30°C	MIC_{air}	1250	Tullio et al. (2006)
Cladosporium cladosporoides	Fungi	RPMI, 7 d, 30°C	MIC_{air}	156–312	Tullio et al. (2006)
Fusarium oxysporum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	5000	Tullio et al. (2006)
Microsporum canis	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312–1250	Tullio et al. (2006)
Microsporum gypseum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312	Tullio et al. (2006)
Mucor sp.	Fungi	RPMI, 7 d, 30°C	MIC_{air}	1250	Tullio et al. (2006)
Penicillium frequentans	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625	Tullio et al. (2006)
Penicillium lanosum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	625	Tullio et al. (2006)
Rhizopus sp.	Fungi	RPMI, 7 d, 30°C	MIC_{air}	2500	Tullio et al. (2006)
Scopulariopsis brevicaulis	Fungi	RPMI, 7 d, 30°C	MIC_{air}	125	Tullio et al. (2006)
Trichophyton mentagrophytes	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312–625	Tullio et al. (2006)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.43
Inhibitory Data of Lemon Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	5 × 20, 1000	1–10	Möse et al. (1957)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brucella abortus	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	4.6	Smith-Palmer et al. (1998)
Campylobacter jejuni	Bac–	CAB, 24 h, 42°C	Disk, 10,000	41	Fisher and Phillips (2006)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0–10	Möse et al. (1957)
Escherichia coli	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NA, 24 h, 37°C	Disk, 10,000	21	Fisher and Phillips (2006)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	18.5	Yousef and Tawil (1980)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13.5	Deans and Ritchie (1987)
Klebsiella aerogenes	Bac–	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Klebsiella pneumonia	Bac–	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Klebsiella pneumonia subsp. *oceanae*	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)

Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus OX19	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Proteus sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Pseudomonas fluorescens	Bac–	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Pseudomonas mangiferae indicae	Bac–	NA, 36–48 h, 37°C	6, sd	14	Garg and Garg (1980)
Salmonella enteritidis	Bac–	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Salmonella paratyphi	Bac–	NA, 36–48 h, 37°C	6, sd	16	Garg and Garg (1980)
Salmonella paratyphi B	Bac–	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Salmonella typhi	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 10,0000	0	Narasimha Rao and Nigam (1970)
Salmonella typhi	Bac–	NA, 36–48 h, 37°C	6, sd	28	Garg and Garg (1980)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Serratia marcescens	Bac–	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Serratia sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Vibrio albicans	Bac–	NA, 24 h, 37°C	5 × 20, 1000	11–13	Möse et al. (1957)
Vibrio cholera	Bac–	NA, 36–48 h, 37°C	6, sd	12	Garg and Garg (1980)
Vibrio cholerae	Bac–	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)

continued

TABLE 12.43 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Vibrio cholerae	Bac–	NA, 24 h, 37°C	10 (h), 10,0000	22	Narasimha Rao and Nigam (1970)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus anthracis	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Bacillus cereus	Bac+	BHA, 24 h, 30°C	Disk, 10,000	19	Fisher and Phillips (2006)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus mycoides	Bac+	NA, 36–48 h, 37°C	6, sd	12	Garg and Garg (1980)
Bacillus pumilus	Bac+	NA, 36–48 h, 37°C	6, sd	16	Garg and Garg (1980)
Bacillus sp.	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	5	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 36–48 h, 37°C	6, sd	16	Garg and Garg (1980)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	19.5	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium diphtheria	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Corynebacterium diphtheria	Bac+	NA, 24 h, 37°C	10 (h), 10,0000	0	Narasimha Rao and Nigam (1970)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	3	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	5.3	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	LSA, 24 h, 37°C	Disk, 10,000	41	Fisher and Phillips (2006)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	24	Yousef and Tawil (1980)
Sarcina alba	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Sarcina beige	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)

Sarcina citrea	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Sarcina lutea	Bac+	NA, 36–48 h, 37°C	6, sd	17	Garg and Garg (1980)
Sarcina rosa	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Sporococcus sarc.	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Staphylococcus albus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	11–15	Möse et al. (1957)
Staphylococcus aureus	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	6	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	NA, 36–48 h, 37°C	6, sd	14	Garg and Garg (1980)
Staphylococcus aureus	Bac+	BHA, 24 h, 37°C	Disk, 10,000	14	Fisher and Phillips (2006)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	22	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Staphylococcus epidermidis	Bac+	NA, 24 h, 37°C	10 (h), 100,000	18	Narasimha Rao and Nigam (1970)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Streptococcus haemolyticus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Streptococcus sp.	Bac+	NA, 24 h, 37°C	10 (h), 100,000	0	Narasimha Rao and Nigam (1970)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Streptococcus viridians	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	7.3	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	7	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	18	Yousef and Tawil (1980)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	13.2	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	16	Maruzzella and Liguori (1958)

continued

TABLE 12.43 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	14	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	17	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	0	Yousef and Tawil (1980)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	33.5	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	1	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)

TABLE 12.44
Inhibitory Data of Lemon Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Aerobacter aerogenes	Bac–	NA, pH 7	>2000	Subba et al. (1967)
Campylobacter jejuni	Bac–	CAB, 24 h, 42°C	>40,000	Fisher and Phillips (2006)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	NA, 24 h, 37°C	10,000	Fisher and Phillips (2006)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Escherichia coli	Bac–	NB, 24–72 h, 37°C	98% inh. 10,000	Dabbah et al. (1970)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NB, 24–72 h, 37°C	90% inh. 10,000	Dabbah et al. (1970)
Salmonella schottmuelleri	Bac–	NA, pH 7	>2000	Subba et al. (1967)
Salmonella senftenberg	Bac–	NB, 24–72 h, 37°C	98% inh. 10,000	Dabbah et al. (1970)
Serratia marcescens	Bac–	NA, pH 7	>2000	Subba et al. (1967)
Bacillus cereus	Bac+	BHA, 24 h, 30°C	10,000	Fisher and Phillips (2006)
Bacillus subtilis	Bac+	NA, pH 7	2000	Subba et al. (1967)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Lactobacillus plantarum	Bac+	NA, pH 7	1000	Subba et al. (1967)
Listeria monocytogenes	Bac+	LSA, 24 h, 37°C	2500	Fisher and Phillips (2006)
Micrococcus sp.	Bac+	NA, pH 7	2000	Subba et al. (1967)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	BHA, 24 h, 37°C	>40,000	Fisher and Phillips (2006)
Staphylococcus aureus	Bac+	NB, 24–72 h, 37°C	10,000	Dabbah et al. (1970)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)

continued

TABLE 12.44 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Streptococcus faecalis	Bac+	NA, pH 7	1000	Subba et al. (1967)
Aspergillus awamorii	Fungi	PDA, pH 4.5	>2000	Subba et al. (1967)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	PDA, pH 4.5	>2000	Subba et al. (1967)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus niger	Fungi	PDA, pH 4.5	>2000	Subba et al. (1967)
Aspergillus niger	Fungi	YES broth, 10 d	4% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	800	Yousef and Tawil (1980)
Aspergillus ochraceus	Fungi	YES broth, 10 d	22% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	>500	Okazaki and Oshima (1953)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Botrytis cinera	Fungi	PDA, Tween 20, 7 d, 24°C	4% inh. 1000	Bouchra et al. (2003)
Fusarium culmorum	Fungi	YES broth, 10 d	0% inh. 10,000	Lis-Balchin et al. (1998)
Geotrichum citri-aurantii	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	25,000	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)

Penicillium digitatum	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Penicillium digitatum	Fungi	SDB, 5 d, 20°C, MIC = ED50	500–1000	Caccioni et al. (1998)
Penicillium italicum	Fungi	SDB, 5 d, 20°C, MIC = ED50	1000–2500	Caccioni et al. (1998)
Phytophthora citrophthora	Fungi	PDA, Tween 20, 7 d, 24°C	20% inh. 1000	Bouchra et al. (2003)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Candida albicans	Yeast	MHB, Tween 80, 48 h, 35°C	20,000	Hammer et al. (1998)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Saccharomyces cerevisiae	Yeast	PDA, pH 4.5	500	Subba et al. (1967)
Torula utilis	Yeast	PDA, pH 4.5	1000	Subba et al. (1967)
Zygosaccharomyces mellis	Yeast	PDA, pH 4.5	>2000	Subba et al. (1967)

TABLE 12.45
Inhibitory Data of Lemon Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Campylobacter jejuni	Bac−	CAB, 24 h, 42°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Escherichia coli	Bac−	NA, 24 h, 37°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Escherichia coli	Bac−	BLA, 18 h, 37°C	MIC_{air}	>1600	Inouye et al. (2001)
Haemophilus influenzae	Bac−	MHA, 18 h, 37°C	MIC_{air}	200	Inouye et al. (2001)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus cereus	Bac+	BHA, 24 h, 30°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Listeria monocytogenes	Bac+	LSA, 24 h, 37°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	BHA, 24 h, 37°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	800	Inouye et al. (2001)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	400	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	200	Inouye et al. (2001)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	++	Guynot et al. (2003)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.46
Inhibitory Data of Mandarin Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	1	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Campylobacter jejuni	Bac–	Cited	6, 15,000	11	Wannissorn et al. (2005)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	MHA, 24 h, 30°C	6, 15,000	7	Rossi et al. (2007)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited	6, 15,000	0	Wannissorn et al. (2005)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	MHA, 24 h, 30°C	6, 15,000	8	Rossi et al. (2007)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)

continued

TABLE 12.46 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	9	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Salmonella enteritidis	Bac–	Cited	6, 15,000	0	Wannissorn et al. (2005)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella typhimurium	Bac–	Cited	6, 15,000	9	Wannissorn et al. (2005)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Clostridium perfringens	Bac+	Cited	6, 15,000	35	Wannissorn et al. (2005)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)

Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	4.2	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	15	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	4.1	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 15,000	17	Rossi et al. (2007)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	11	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	12	Maruzzella and Liguori (1958)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	22	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)

TABLE 12.47
Inhibitory Data of Mandarin Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	NB, 24–72 h, 37°C	98% inh. 10,000	Dabbah et al. (1970)
Pseudomonas aeruginosa	Bac–	NB, 24–72 h, 37°C	87% inh. 10,000	Dabbah et al. (1970)
Salmonella senftenberg	Bac–	NB, 24–72 h, 37°C	>10,000	Dabbah et al. (1970)
Yersinia enterocolitica	Bac–	MHA, Tween 20, 24 h, 37°C	2500	Rossi et al. (2007)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Staphylococcus aureus	Bac+	NB, 24–72 h, 37°C	10,000	Dabbah et al. (1970)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)

Annotation: Terpeneless mandarin oil tested Maruzzella and Liguori (1958).

TABLE 12.48
Inhibitory Data of Mandarin Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+	Guynot et al. (2003)

TABLE 12.49
Inhibitory Data of Matricaria Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brucella abortus	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella pneumonia	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Klebsiella pneumonia subsp. *Oceanae*	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Proteus OX19	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas fluorescens	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)

continued

TABLE 12.49 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Salmonella enteritidis	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Salmonella paratyphi B	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella typhi	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Serratia marcescens	Bac–	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Vibrio albicans	Bac–	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Vibrio cholerae	Bac–	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus anthracis	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	13.5	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium diphtheria	Bac+	NA, 24 h, 37°C	5 × 20, 1000	1	Möse et al. (1957)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0–11	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)

Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	15.5	Yousef and Tawil (1980)
Sarcina alba	Bac+	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Sarcina beige	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Sarcina citrea	Bac+	NA, 24 h, 37°C	5 × 20, 1000	0	Möse et al. (1957)
Sarcina rosa	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Sporococcus sarc.	Bac+	NA, 24 h, 37°C	5 × 20, 1000	6–10	Möse et al. (1957)
Staphylococcus albus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	11.5	Yousef and Tawil (1980)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptococcus haemolyticus	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Streptococcus viridians	Bac+	NA, 24 h, 37°C	5 × 20, 1000	2–5	Möse et al. (1957)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	9.7	Pawar and Thaker (2007)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	10	Yousef and Tawil (1980)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	0	Pawar and Thaker (2006)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	9	Pawar and Thaker (2007)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	10	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	11	Yousef and Tawil (1980)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	0	Yousef and Tawil (1980)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	9.5	Yousef and Tawil (1980)

TABLE 12.50
Inhibitory Data of Matricaria Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	NB, Tween 80, 24 h, 37°C	>8000	Aggag and Yousef (1972)
Escherichia coli	Bac–	Agar, cited	10,000	Kedzia et al. (1991)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Helicobacter pylori	Bac–	Cited, 20 h, 37°C	35.7–70.4	Weseler et al. (2005)
Klebsiella pneumoniae	Bac–	Agar, cited	10,000	Kedzia et al. (1991)
Pseudomonas aeruginosa	Bac–	Agar, cited	7500	Kedzia et al. (1991)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	NB, Tween 80, 24 h, 37°C	6000	Aggag and Yousef (1972)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac+	NB, Tween 80, 24 h, 37°C	>8000	Aggag and Yousef (1972)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Staphylococcus aureus	Bac+	Agar, cited	2500	Kedzia et al. (1991)
Staphylococcus aureus	Bac+	NB, Tween 80, 24 h, 37°C	7000	Aggag and Yousef (1972)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Streptococcus faecalis	Bac+	Agar, cited	2500	Kedzia et al. (1991)

Aspergillus flavus	Fungi	PDA, 7–14 d, 28°C	>3000	Soliman and Badeaa (2002)
Aspergillus niger	Fungi	YES broth, 10 d	–63% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Aspergillus ochraceus	Fungi	PDA, 7–14 d, 28°C	>3000	Soliman and Badeaa (2002)
Aspergillus ochraceus	Fungi	YES broth, 10 d	–56% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus parasiticus	Fungi	PDA, 7–14 d, 28°C	>3000	Soliman and Badeaa (2002)
Botrytis cinera	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Fusarium culmorum	Fungi	YES broth, 10 d	–75% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium moniliforme	Fungi	PDA, 7–14 d, 28°C	>3000	Soliman and Badeaa (2002)
Microsporum gypseum	Fungi	Agar, cited	1000	Kedzia et al. (1991)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Phytophthora citrophthora	Fungi	PDA, Tween 20, 7 d, 24°C	2% inh. 1000	Bouchra et al. (2003)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Trichophyton mentagrophytes	Fungi	SDA, 21 d, 20°C	1000	Szalontai et al. (1977)
Trichophyton mentagrophytes	Fungi	SDA, 21 d, 20°C	1000	Szalontai et al. (1977)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Candida albicans	Yeast	SDA, 7 d, 37°C	1000	Szalontai et al. (1977)
Candida albicans	Yeast	Agar, cited	5000	Kedzia et al. (1991)
Candida albicans	Yeast	NB, Tween 80, 24 h, 37°C	7000	Aggag and Yousef (1972)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)

TABLE 12.51
Inhibitory Data of Matricaria Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)

TABLE 12.52
Inhibitory Data of Mint Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	Cited	6, 15,000	90	Wannissorn et al. (2005)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6.5	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Escherichia coli	Bac–	Cited	6, 15,000	14.5	Wannissorn et al. (2005)
Escherichia coli O157	Bac–	Cited	6, 15,000	13.5	Wannissorn et al. (2005)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Proteus sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella agona	Bac–	Cited	6, 15,000	12	Wannissorn et al. (2005)
Salmonella braenderup	Bac–	Cited	6, 15,000	13	Wannissorn et al. (2005)
Salmonella derby	Bac–	Cited	6, 15,000	10	Wannissorn et al. (2005)
Salmonella enteritidis	Bac–	Cited	6, 15,000	13.5	Wannissorn et al. (2005)
Salmonella gallinarum	Bac–	Cited	6, 15,000	52.5	Wannissorn et al. (2005)

continued

TABLE 12.52 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Salmonella hadar	Bac–	Cited	6, 15,000	13.5	Wannissorn et al. (2005)
Salmonella mbandaka	Bac–	Cited	6, 15,000	11.5	Wannissorn et al. (2005)
Salmonella montevideo	Bac–	Cited	6, 15,000	12	Wannissorn et al. (2005)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Salmonella saintpaul	Bac–	Cited	6, 15,000	12	Wannissorn et al. (2005)
Salmonella schwarzergrund	Bac–	Cited	6, 15,000	11.5	Wannissorn et al. (2005)
Salmonella senftenberg	Bac–	Cited	6, 15,000	12	Wannissorn et al. (2005)
Salmonella sp.	Bac–	Cited	15, 2500	4	Pizsolitto et al. (1975)
Salmonella typhimurium	Bac–	Cited	6, 15,000	47.5	Wannissorn et al. (2005)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	4.5	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	6	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6.5	Deans and Ritchie (1987)
Bacillus sp.	Bac+	Cited	15, 2500	10	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	14.5	Deans and Ritchie (1987)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Clostridium perfringens	Bac+	Cited	6, 15,000	90	Wannissorn et al. (2005)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)

Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited	15, 2500	2	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	2	Pizsolitto et al. (1975)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	14	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	1	Pizsolitto et al. (1975)
Absidia corMYBifera	Fungi	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)
Aspergillus flavus	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Aspergillus fumigatus	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Aspergillus sulphureus	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Humicola grisea var. *thermoidea*	Fungi	EYA, 48 h, 45°C	5, sd	15	Nigam and Rao (1979)
Mucor fragilis	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Rhizopus stolonifer	Fungi	PDA, 10 d, 25°C	50	NG	Sarbhoy et al. (1978)
Sporotrichum thermophile	Fungi	EYA, 48 h, 45°C	5, sd	20	Nigam and Rao (1979)
Thermoascus aurantiacis	Fungi	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)
Thermomyces lanuginosa	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Thielava minor	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Torula thermophila	Yeast	EYA, 48 h, 45°C	5, sd	10	Nigam and Rao (1979)

TABLE 12.53
Inhibitory Data of Mint Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Escherichia coli	Bac–	Cited, 24 h	400	Imai et al. (2001)
Helicobacter pylori	Bac–	Cited, 48 h	100	Imai et al. (2001)
Salmonella enteritidis	Bac–	Cited, 24 h	800	Imai et al. (2001)
Enterococcus faecium VRE	Bac+	HIB, Tween 80, 18 h, 37°C	5000–10,000	Nelson (1997)
Staphylococcus aureus MRSA	Bac+	Cited, 24 h	400	Imai et al. (2001)
Staphylococcus aureus MRSA	Bac+	HIB, Tween 80, 18 h, 37°C	5000	Nelson (1997)
Staphylococcus aureus MSSA	Bac+	Cited, 24 h	400	Imai et al. (2001)
Alternaria alternate	Fungi	SDA, 6–8 h, 20°C	63% inh. 500	Dikshit et al. (1986)
Aspergillus flavus	Fungi	CA, 7 d, 28°C	500	Kumar et al. (2007)
Aspergillus flavus	Fungi	Cited	1000	Kumar et al. (2007)
Aspergillus flavus	Fungi	SDA, 6–8 h, 20°C	65% inh. 500	Dikshit et al. (1986)
Aspergillus niger	Fungi	CA, 7 d, 28°C	81% inh. 500	Kumar et al. (2007)
Botryodiplodia theobromae	Fungi	CA, 7 d, 28°C	87% inh. 500	Kumar et al. (2007)
Cladosporium cladosporoides	Fungi	CA, 7 d, 28°C	>500	Kumar et al. (2007)
Fusarium oxysporum	Fungi	CA, 7 d, 28°C	83% inh. 500	Kumar et al. (2007)
Helminthosporium oryzae	Fungi	CA, 7 d, 28°C	500	Kumar et al. (2007)
Helminthosporium oryzae	Fungi	MA, cited[a]	2000	Dikshit et al. (1979)
Helminthosporium oryzae	Fungi	MA, pH 5.0, 6 d, 28°C[a,b]	2000	Dikshit et al. (1982)
Helminthosporium oryzae	Fungi	MA, pH 4.0, 6 d, 28°C[a,b]	500	Dikshit et al. (1982)
Macrophomina phaesoli	Fungi	CA, 7 d, 28°C	94% inh. 500	Kumar et al. (2007)
Microsporum gypseum	Fungi	SDA, 6–8 h, 20°C	64% inh. 500	Dikshit et al. (1986)
Penicillium italicum	Fungi	SDA, 6–8 h, 20°C	65% inh. 500	Dikshit et al. (1986)
Sclerotium rolfsii	Fungi	CA, 7 d, 28°C	500	Kumar et al. (2007)
Trichophyton mentagrophytes	Fungi	SDA, 6–8 h, 20°C	69% inh. 500	Dikshit et al. (1986)
Trichophyton rubrum	Fungi	SDA, 6–8 h, 20°C	36% inh. 500	Dikshit et al. (1986)
Candida albicans	Yeast	Cited, 48 h, 36°C[a]	1100	Duarte et al. (2005)

[a] Mentha arvensis var. *piperascens*.
[b] Dementholized oil.

TABLE 12.54
Inhibitory Data of Neroli Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Imai et al. (2001)
Escherichia coli	Bac–	Cited	15, 2500	0	Imai et al. (2001)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	2	Imai et al. (2001)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	14	Nelson (1997)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 100,000	22	Imai et al. (2001)
Klebsiella aerogenes	Bac–	NA, 24 h, 37°C	10 (h), 100,000	0	Nelson (1997)
Klebsiella sp.	Bac–	Cited	15, 2500	0	Imai et al. (2001)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	2	Dikshit et al. (1986)
Proteus sp.	Bac–	Cited	15, 2500	0	Kumar et al. (2007)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	2	Kumar et al. (2007)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Dikshit et al. (1986)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Kumar et al. (2007)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	12	Kumar et al. (2007)
Salmonella sp.	Bac–	Cited	15, 2500	0	Kumar et al. (2007)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 100,000	19	Kumar et al. (2007)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Kumar et al. (2007)
Serratia sp.	Bac–	Cited	15, 2500	0	Dikshit et al. (1979)
Shigella sp.	Bac–	Cited	15, 2500	1	Dikshit et al. (1982)
Vibrio cholerae	Bac–	NA, 24 h, 37°C	10 (h), 100,000	0	Dikshit et al. (1982)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	7	Kumar et al. (2007)
Bacillus sp.	Bac+	Cited	15, 2500	1	Dikshit et al. (1986)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	19	Dikshit et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	22	Kumar et al. (2007)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	10 (h), 100,000	18	Dikshit et al. (1986)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	11–19	Dikshit et al. (1986)

continued

TABLE 12.54 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	20	Duarte et al. (2005)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Imai et al. (2001)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 100,000	0	Imai et al. (2001)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Imai et al. (2001)
Staphylococcus aureus	Bac+	Cited	15, 2500	2	Nelson (1997)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	13.6	Imai et al. (2001)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	1	Nelson (1997)
Staphylococcus epidermidis	Bac+	NA, 24 h, 37°C	10 (h), 100,000	22	Imai et al. (2001)
Streptococcus sp.	Bac+	NA, 24 h, 37°C	10 (h), 100,000	0	Dikshit et al. (1986)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Kumar et al. (2007)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	9	Kumar et al. (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	9	Dikshit et al. (1986)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	5	Kumar et al. (2007)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	9	Kumar et al. (2007)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	26	Kumar et al. (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	9	Kumar et al. (2007)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	9	Kumar et al. (2007)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	22	Dikshit et al. (1979)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	5	Dikshit et al. (1982)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	56	Dikshit et al. (1982)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	9	Kumar et al. (2007)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	6	Dikshit et al. (1986)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	21	Dikshit et al. (1986)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	8	Kumar et al. (2007)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	30	Dikshit et al. (1986)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	0	Dikshit et al. (1986)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	0	Duarte et al. (2005)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	6	Imai et al. (2001)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	6	Imai et al. (2001)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	4	Imai et al. (2001)

TABLE 12.55
Inhibitory Data of Neroli Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Escherichia coli	Bac−	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac−	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	–86% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	–90% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	250	Okazaki and Oshima (1953)
Cephalosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Ceratocystis paradoxa	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Curvularia lunata	Fungi	OA, EtOH, 3 d, 20°C	4000	Narasimba Rao et al. (1971)
Fusarium culmorum	Fungi	YES broth, 10 d	–71% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium moniliforme var. *subglutinans*	Fungi	OA, EtOH, 3 d, 20°C	2000	Narasimba Rao et al. (1971)
Helminthosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	2000	Narasimba Rao et al. (1971)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	250	Okazaki and Oshima (1953)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	6400	Yousef and Tawil (1980)
Physalospora tucumanensis	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Sclerotium rolfsii	Fungi	OA, EtOH, 6 d, 20°C	20,000	Narasimba Rao et al. (1971)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)

TABLE 12.56
Inhibitory Data of Neroli Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	+++	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.57
Inhibitory Data of Nutmeg Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10.5	Deans and Ritchie (1987)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	11.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	5.5	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10.5	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	8	Janssen et al. (1986)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	6.9	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	18	Yousef and Tawil (1980)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Proteus sp.	Bac–	Cited	15, 2500	5	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	0	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	7.6	Smith-Palmer et al. (1998)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	17	Deans and Ritchie (1987)

continued

TABLE 12.57 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Salmonella sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	18	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10.5	Deans and Ritchie (1987)
Bacillus sp.	Bac+	Cited	15, 2500	5	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	34	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0–12	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	7.7	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	27	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited	15, 2500	3	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	6.5	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	15.5	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	1	Pizsolitto et al. (1975)

Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	8.3	Pawar and Thaker (2007)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	5	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	40	Yousef and Tawil (1980)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	0	Pawar and Thaker (2007)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	28	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	19	Yousef and Tawil (1980)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	13	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	24	Yousef and Tawil (1980)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)

TABLE 12.58
Inhibitory Data of Nutmeg Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	>10,000	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	<100	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	TSB, 10 d, 4°C	500	Smith-Palmer et al. (1998)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Alternaria alternata	Fungi	PDA, 7 d, 28°C	0% inh. 500	Feng and Zheng (2007)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	–88% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	–86% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Fusarium culmorum	Fungi	YES broth, 10 d	–>10,000	Lis-Balchin et al. (1998)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)

continued

TABLE 12.58 (continued)
Inhibitory Data of Nutmeg Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	400	Yousef and Tawil (1980)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	625–1250	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)

TABLE 12.59
Inhibitory Data of Nutmeg Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)

TABLE 12.60
Inhibitory Data of Peppermint Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	14.5	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	16.5	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	7.1	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	11	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	Cited	15, 2500	5	Pizsolitto et al. (1975)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	23	Schelz et al. (2006)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	9.7	Janssen et al. (1986)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	6.8	Smith-Palmer et al. (1998)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	11	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	10	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	0	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	6.3	Smith-Palmer et al. (1998)

Salmonella paratyphi	Bac–	NA, 24 h, 37°C	6, sd	18	Dube and Rao (1984)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	15	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	20	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)
Vibrio cholera	Bac–	NA, 24 h, 37°C	6, sd	16	Dube and Rao (1984)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	13	Deans and Ritchie (1987)
Bacillus anthracis	Bac+	NA, 24 h, 37°C	6, sd	17	Dube and Rao (1984)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus pumilus	Bac+	NA, 24 h, 37°C	6, sd	13	Dube and Rao (1984)
Bacillus sp.	Bac+	Cited	15, 2500	10	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	8	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	21.5	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	14.7	Janssen et al. (1986)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	11	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	13–20	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	5.3	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	16	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	5	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited	15, 2500	8	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	12	Yousef and Tawil (1980)

continued

TABLE 12.60 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	6, sd	13	Dube and Rao (1984)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	19	Janssen et al. (1986)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	6.4	Smith-Palmer et al. (1998)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	10	Pizsolitto et al. (1975)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), –30,000	15	Schelz et al. (2006)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Absidia cormybifera	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	10.6	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Aspergillus flavus	Fungi	PDA, 10 d, 25°C	10	–	Sarbhoy et al. (1978)
Aspergillus fumigatus	Fungi	PDA, 10 d, 25°C	10	–	Sarbhoy et al. (1978)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SDA, 3 d, 28°C	6, sd	10	Saksena and Saksena (1984)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	0	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Aspergillus sulphureus	Fungi	PDA, 10 d, 25°C	10	–	Sarbhoy et al. (1978)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	10	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Humicola grisea var. *thermoidea*	Fungi	EYA, 48 h, 45°C	5, sd	30	Nigam and Rao (1979)
Keratinomyces afelloi	Fungi	SDA, 3 d, 28°C	6, sd	18	Saksena and Saksena (1984)
Keratinophyton terreum	Fungi	SDA, 3 d, 28°C	6, sd	0	Saksena and Saksena (1984)
Microsporum gypseum	Fungi	SDA, 3 d, 28°C	6, sd	10	Saksena and Saksena (1984)
Mucor fragilis	Fungi	PDA, 10 d, 25°C	10	–	Sarbhoy et al. (1978)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	23	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	60	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)

Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	22	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 10 d, 25°C	10	–	Sarbhoy et al. (1978)
Sporotrichum thermophile	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Thermoascus aurantiacis	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Thermomyces lanuginosa	Fungi	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)
Thielava minor	Fungi	EYA, 48 h, 45°C	5, sd	15	Nigam and Rao (1979)
Trichophyton equinum	Fungi	SDA, 3 d, 28°C	6, sd	14	Saksena and Saksena (1984)
Trichophyton rubrum	Fungi	SDA, 3 d, 28°C	6, sd	16	Saksena and Saksena (1984)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	10	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	16	Yousef and Tawil (1980)
Candida albicans	Yeast	SDA, 3 d, 28°C	6, sd	18	Saksena and Saksena (1984)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	1	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SDA, 3 d, 28°C	6, sd	23	Saksena and Saksena (1984)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	9	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), –30,000	12–15	Schelz et al. (2006)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	8	Conner and Beuchat (1984)
Torula thermophila	Yeast	EYA, 48 h, 45°C	5, sd	0	Nigam and Rao (1979)

TABLE 12.61
Inhibitory Data of Peppermint Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Treponema denticola	Bac	HS, 72 h, 37°C	1000	Shapiro et al. (1994)
Treponema vincentii	Bac	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Actinobacillus actinomycetemcomitans	Bac–	HS, 72 h, 37°C	3000	Shapiro et al. (1994)
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	1000	Smith-Palmer et al. (1998)
Capnocytophaga sp.	Bac–	HS, 72 h, 37°C	3000	Shapiro et al. (1994)
Eikenella corrodens	Bac–	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Escherichia coli	Bac–	Cited, 24 h	800	Imai et al. (2001)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGB, 18 h, 37°C	5700	Schelz et al. (2006)
Escherichia coli	Bac–	MHB, 24 h, 36°C	>10,000	Duarte et al. (2006)
Escherichia coli	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Fusobacterium nucleatum	Bac–	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Helicobacter pylori	Bac–	Cited, 48 h	100	Imai et al. (2001)
Helicobacter pylori	Bac–	Cited, 20 h, 37°C	135.6	Weseler et al. (2005)
Porphyromonas gingivalis	Bac–	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Prevotella buccae	Bac–	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Prevotella intermedia	Bac–	HS, 72 h, 37°C	3000	Shapiro et al. (1994)
Prevotella nigrescens	Bac–	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Salmonella enteritidis	Bac–	Cited, 24 h	400	Imai et al. (2001)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Selenomonas artemidis	Bac–	HS, 72 h, 37°C	1000	Shapiro et al. (1994)
Actinomyces viscosus	Bac+	HS, 16–24, 37°C	5000	Shapiro et al. (1994)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	300	Smith-Palmer et al. (1998)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)

Peptostreptococcus anaerobius	Bac+	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	1000	Morris et al. (1979)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	6400	Yousef and Tawil (1980)
Staphylococcus aureus MRSA	Bac+	Cited, 24 h	200	Imai et al. (2001)
Staphylococcus aureus MSSA	Bac+	Cited, 24 h	200	Imai et al. (2001)
Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	5700	Schelz et al. (2006)
Streptococcus sanguinis	Bac+	HS, 16–24, 37°C	6000	Shapiro et al. (1994)
Streptococcus sobrinus	Bac+	HS, 16–24, 37°C	3000	Shapiro et al. (1994)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	800	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	– 98% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	– 93% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	500	Okazaki and Oshima (1953)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Botrytis cinera	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Fusarium culmorum	Fungi	YES broth, 10 d	– >10,000	Lis-Balchin et al. (1998)
Geotrichum citri-aurantii	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Microsporum gypseum	Fungi	SDA, 7 d, 30°C	400, 37% inh.	Dikshit and Husain (1984)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	1600	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	PDA, Tween 20, 7 d, 24°C	0% inh. 1000	Bouchra et al. (2003)
Phytophthora citrophthora	Fungi	PDA, Tween 20, 7 d, 24°C	14% inh. 1000	Bouchra et al. (2003)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)

continued

TABLE 12.61 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	6400	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Trichophyton equinum	Fungi	SDA, 7 d, 30°C	400, 51% inh.	Dikshit and Husain (1984)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	625–1250	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SDA, 7 d, 30°C	400, 61% inh.	Dikshit and Husain (1984)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Candida albicans	Yeast	MHB, Tween 80, 48 h, 35°C	5000	Hammer et al. (1998)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	400	Schelz et al. (2006)

TABLE 12.62
Inhibitory Data of Peppermint Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Escherichia coli	Bac–	BLA, 18 h, 37°C	MIC_{air}	>1600	Inouye et al. (2001)
Haemophilus influenzae	Bac–	MHA, 18 h, 37°C	MIC_{air}	12.5	Inouye et al. (2001)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	++	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	++	Guynot et al. (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+	Guynot et al. (2003)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)

TABLE 12.63
Inhibitory Data of *Pinus sylvestris* Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Bordetella bronchiseptica	Bac–	MHA, 18 h, 37°C	6, 17,500	0	Schales et al. (1993)
Citrobacter freundii	Bac–	MHA, 18 h, 37°C	6, 17,500	0	Schales et al. (1993)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	1	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	11.7	Janssen et al. (1986)
Escherichia coli 1	Bac–	MHA, 18 h, 37°C	6, 17,500	8	Schales et al. (1993)
Escherichia coli 2	Bac–	MHA, 18 h, 37°C	6, 17,500	0	Schales et al. (1993)
Klebsiella pneumoniae	Bac–	MHA, 18 h, 37°C	6, 17,500	0	Schales et al. (1993)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Proteus mirabilis	Bac–	MHA, 18 h, 37°C	6, 17,500	0	Schales et al. (1993)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	MHA, 18 h, 37°C	6, 17,500	0	Schales et al. (1993)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	0	Janssen et al. (1986)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Bacillus sp.	Bac+	MHA, 18 h, 37°C	6, 17,500	11	Schales et al. (1993)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Clostridium perfringens	Bac+	MHA, 18 h, 37°C	6, 17,500	23	Schales et al. (1993)

Enterococcus sp.	Bac+	MHA, 18 h, 37°C	6, 17,500	15	Schales et al. (1993)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	7.7	Janssen et al. (1986)
Staphylococcus sp.	Bac+	MHA, 18 h, 37°C	6, 17,500	6	Schales et al. (1993)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	12	Maruzzella and Liguori (1958)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	12	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	15	Maruzzella and Liguori (1958)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	12	Maruzzella and Liguori (1958)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	16.3	Janssen et al. (1986)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	14	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	18	Maruzzella and Liguori (1958)

TABLE 12.64
Inhibitory Data of *Pinus sylvestris* Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Acinetobacter baumannii	Bac–	MHA, Tween 20, 48 h, 35°C	20,000	Hammer et al. (1999)
Aeromonas sobria	Bac–	MHA, Tween 20, 48 h, 35°C	20,000	Hammer et al. (1999)
Escherichia coli	Bac–	MHB, Tween 80, 24 h, 37°C	>29,000	Chalchat et al. (1989)
Escherichia coli	Bac–	MHA, Tween 20, 48 h, 35°C	20,000	Hammer et al. (1999)
Escherichia coli	Bac–	MHB, Tween 80, 24 h, 37°C	64,300	Bastide et al. (1987)
Klebsiella pneumonia	Bac–	MHB, Tween 80, 24 h, 37°C	3500	Chalchat et al. (1989)
Klebsiella pneumoniae	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Proteus mirabilis	Bac–	MHB, Tween 80, 24 h, 37°C	>29,000	Chalchat et al. (1989)
Pseudomonas aeruginosa	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Pseudomonas aeruginosa	Bac–	MHB, Tween 80, 24 h, 37°C	>29,000	Chalchat et al. (1989)
Salmonella typhimurium	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Serratia marcescens	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Bacillus sp.	Bac+	CA, 7 d, 25°C	5000	Motiejunaite and Peciulyte (2004)
Enterococcus faecalis	Bac+	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Rhodococcus sp.	Bac+	CA, 7 d, 25°C	5000	Motiejunaite and Peciulyte (2004)
Staphylococcus aureus	Bac+	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	3500	Chalchat et al. (1989)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	4000	Bastide et al. (1987)
Alternaria alternata	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Aspergillus flavus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Aspergillus flavus var. *columnaris*	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250	Tullio et al. (2006)
Aspergillus fumigatus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000–10,000	Tullio et al. (2006)
Aspergillus niger	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)
Aspergillus niger	Fungi	CA, 7 d, 25°C	7500–15,000	Motiejunaite and Peciulyte (2004)
Aspergillus versicolor	Fungi	CA, 7 d, 25°C	7500–15,000	Motiejunaite and Peciulyte (2004)

Aureobasidum pullulans	Fungi	CA, 7 d, 25°C	5000–7500	Motiejunaite and Peciulyte (2004)
Chaetomium globosum	Fungi	CA, 7 d, 25°C	5000	Motiejunaite and Peciulyte (2004)
Cladosporium cladosporoides	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250–2500	Tullio et al. (2006)
Cladosporium cladosporoides	Fungi	CA, 7 d, 25°C	5000	Motiejunaite and Peciulyte (2004)
Epidermophyton floccosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250	Tullio et al. (2006)
Fusarium oxysporum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	312	Tullio et al. (2006)
Microsporum canis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250–5000	Tullio et al. (2006)
Microsporum gypseum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500–5000	Tullio et al. (2006)
Mucor sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C		Tullio et al. (2006)
Paecilomyces variotii	Fungi	CA, 7 d, 25°C	>10,000	Thanaboripat et al. (2004) Motiejunaite and Peciulyte (2004)
Penicillium chrysogenum	Fungi	CA, 7 d, 25°C	10,000–25,000	Motiejunaite and Peciulyte (2004)
Penicillium frequentans	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250	Tullio et al. (2006)
Penicillium lanosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Phoma glomerata	Fungi	CA, 7 d, 25°C	10,000–25,000	Motiejunaite and Peciulyte (2004)
Phoma sp.	Fungi	CA, 7 d, 25°C	7500	Motiejunaite and Peciulyte (2004)
Rhizopus sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Rhizopus stolonifer	Fungi	CA, 7 d, 25°C	5000–7500	Motiejunaite and Peciulyte (2004)
Scopulariopsis brevicaulis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Stachybotrys chartarum	Fungi	CA, 7 d, 25°C	10,000–15,000	Motiejunaite and Peciulyte (2004)
Trichoderma viride	Fungi	CA, 7 d, 25°C	5500	Motiejunaite and Peciulyte (2004)
Trichophyton mentagrophytes	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500–5000	Tullio et al. (2006)
Candida albicans	Yeast	MHB, Tween 80, 24 h, 37°C	14,000	Chalchat et al. (1989)
Candida albicans	Yeast	MHA, Tween 20, 48 h, 35°C	20,000	Hammer et al. (1999)
Candida lipolytica	Yeast	CA, 7 d, 25°C	5000	Motiejunaite and Peciulyte (2004)
Geotrichum candida	Yeast	CA, 7 d, 25°C	3500–5000	Motiejunaite and Peciulyte (2004)

Table 12.65
Inhibitory Data of *Pinus sylvestris* Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)

TABLE 12.66
Inhibitory Data of Rosemary Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	15	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Aerobacter tumefaciens	Bac–	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	9.3	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	MHA, 24 h, 30°C	6, 15,000	7	Rossi et al. (2007)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited	(h) 20,000	– to +++	Hethenyi et al. (1989)
Escherichia coli	Bac–	Cited	15, 2500	1	Pizsolitto et al. (1975)
Escherichia coli	Bac–	NA, 24 h, 30°C	Drop, 5000	1	Hili et al. (1997)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Escherichia coli	Bac–	MHA, 24 h, 30°C	6, 15,000	12	Rossi et al. (2007)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	8.7	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), -30,000	15	Schelz et al. (2006)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	13.5	Yousef and Tawil (1980)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 10,0000	18	Narasimha Rao and Nigam (1970)

continued

TABLE 12.66 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Haemophilus influenza	Bac–	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Klebsiella aerogenes	Bac–	NA, 24 h, 37°C	10 (h), 10,0000	20	Narasimha Rao and Nigam (1970)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9.5	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Peptobacterium carotovorum	Bac–	Cited	(h) 20,000	+++	Hethenyi et al. (1989)
Proteus sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	Cited	w 20,000	+++	Hethenyi et al. (1989)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	(h) 20,000	+++	Hethenyi et al. (1989)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 30°C	Drop, 5000	0	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	11	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	11	Janssen et al. (1986)
Pseudomonas pisi	Bac–	Cited	(h) 20,000	++	Hethenyi et al. (1989)
Pseudomonas tabaci	Bac–	Cited	(h) 20,000	+++	Hethenyi et al. (1989)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	9.3	Smith-Palmer et al. (1998)

Salmonella enteritidis	Bac–	Cited, 24 h, 37°C	—	<12	Rota et al. (2004)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 100,000	18	Narasimha Rao and Nigam (1970)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)
Shigella sonnei	Bac–	Cited	(h) 20,000	++	Hethenyi et al. (1989)
Shigella sp.	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)
Vibrio cholerae	Bac–	NA, 24 h, 37°C	10 (h), 100,000	22	Narasimha Rao and Nigam (1970)
Xanthomonas versicolor	Bac–	Cited	(h) 20,000	+++	Hethenyi et al. (1989)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Bacillus sp.	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	Cited	(h) 20,000	–	Hethenyi et al. (1989)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	8	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	8	Janssen et al. (1986)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	20	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	22.5	Yousef and Tawil (1980)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	13.5	Deans and Ritchie (1987)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	10 (h), 100,000	30	Narasimha Rao and Nigam (1970)
Corynebacterium fascians	Bac+	Cited	(h) 20,000	++	Hethenyi et al. (1989)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)

continued

TABLE 12.66 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Lactobacillus sp.	Bac+	MRS, cited	9, 20,000	16.5 – >90	Pellecuer et al. (1980)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8.5	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	7.1	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	16	Lis-Balchin et al. (1998)
Micrococcus luteus	Bac+	MHA, cited	9, 20,000	18–26	Pellecuer et al. (1980)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Micrococcus ureae	Bac+	MHA, cited	9, 20,000	21	Pellecuer et al. (1980)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	23	Yousef and Tawil (1980)
Pneumococcus sp.	Bac+	Cited	(h) 20,000	++	Hethenyi et al. (1989)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Sarcina ureae	Bac+	MHA, cited	9, 20,000	>90	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 10,0000	0	Narasimha Rao and Nigam (1970)
Staphylococcus aureus	Bac+	NA, 24 h, 30°C	Drop, 5000	0	Hili et al. (1997)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	Cited	15, 2500	10	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	12	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	5.9	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 15,000	16	Rossi et al. (2007)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	10.7	Janssen et al. (1986)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	17.9	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), –30,000	0	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	MHA, cited	9, 20,000	12	Pellecuer et al. (1980)
Staphylococcus epidermidis	Bac+	NA, 24 h, 37°C	10 (h), 100,000	22	Narasimha Rao and Nigam (1970)
Streptococcus aegui	Bac+	Cited	(h) 20,000	+	Hethenyi et al. (1989)
Streptococcus D	Bac+	MHA, cited	9, 20,000	0–30	Pellecuer et al. (1980)

Streptococcus faecalis	Bac+	Cited	(h) 20,000	++	Hethenyi et al. (1989)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	15	Deans and Ritchie (1987)
Streptococcus haemolyticus	Bac+	Cited	(h) 20,000	+	Hethenyi et al. (1989)
Streptococcus micros	Bac+	MHA, cited	9, 20,000	18	Pellecuer et al. (1980)
Streptococcus sp.	Bac+	Cited	(h) 20,000	+	Hethenyi et al. (1989)
Streptococcus sp.	Bac+	NA, 24 h, 37°C	10 (h), 100,000	30	Narasimha Rao and Nigam (1970)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	10	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	21	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Aspergillus flavus	Fungi	SDA, 72 h, 26°C	8, 25,000	2	Shin (2003)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 72 h, 26°C	8, 25,000	0	Shin (2003)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	3	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	7	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	10	Yousef and Tawil (1980)
Fusarium moniliforme	Fungi	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	11	Pawar and Thaker (2007)
Fusarium solani	Fungi	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	9	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	18	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Ophiobolus graminis	Fungi	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	20	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	0	Yousef and Tawil (1980)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)

continued

TABLE 12.66 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Candida albicans	Yeast	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Candida albicans	Yeast	NA, 24 h, 30°C	Drop, 5000	7	Hili et al. (1997)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	13	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	24	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	1	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	2	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	8	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	Cited	(h) 20,000	—	Hethenyi et al. (1989)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), −30,000	10	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 30°C	Drop, 5000	12	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	NA, 24 h, 30°C	Drop, 5000	16	Hili et al. (1997)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Torula utilis	Yeast	NA, 24 h, 30°C	Drop, 5000	10	Hili et al. (1997)

TABLE 12.67
Inhibitory Data of Rosemary Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Acinetobacter baumannii	Bac–	MHA, Tween 20, 48 h, 35°C	10,000	Hammer et al. (1999)
Aeromonas sobria	Bac–	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Bordetella bronchiseptica	Bac–	Cited	2500	Pellecuer et al. (1976)
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	500	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	TSB, Tween 80, 48 h, 35°C	40	Panizzi et al. (1993)
Escherichia coli	Bac–	MPB, DMSO, 40 h, 30°C	25% inh. 500	Hili et al. (1997)
Escherichia coli	Bac–	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2004)
Escherichia coli	Bac–	Cited	2500	Pellecuer et al. (1976)
Escherichia coli	Bac–	NA, 1–3 d, 30°C	3500	Farag et al. (1989)
Escherichia coli	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	MHA, Tween 20, 48 h, 35°C	10,000	Hammer et al. (1999)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	25,000	Yousef and Tawil (1980)
Haemophilus influenza	Bac–	Cited	2500	Pellecuer et al. (1976)
Helicobacter pylori	Bac–	Cited, 20 h, 37°C	137	Weseler et al. (2005)
Klebsiella pneumoniae	Bac–	Cited	2500	Pellecuer et al. (1976)
Klebsiella pneumoniae	Bac–	MHA, Tween 20, 48 h, 35°C	20,000	Hammer et al. (1999)
Moraxella glucidolytica	Bac–	Cited	2500	Pellecuer et al. (1976)
Neisseria catarrhalis	Bac–	Cited	1250	Pellecuer et al. (1976)
Neisseria flava	Bac–	Cited	1250	Pellecuer et al. (1976)
Pseudomonas aeruginosa	Bac–	TSB, Tween 80, 48 h, 35°C	>40	Panizzi et al. (1993)
Pseudomonas aeruginosa	Bac–	MPB, DMSO, 40 h, 30°C	54% inh. 500	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2004)
Pseudomonas aeruginosa	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	50,000	Yousef and Tawil (1980)
Pseudomonas fluorescens	Bac–	NA, 1–3 d, 30°C		Thanaboripat et al. (2004) Farag et al. (1989)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	>10,000	Smith-Palmer et al. (1998)

continued

TABLE 12.67 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Salmonella typhimurium	Bac–	TSB, 3% EtOH, 24 h, 37°C	10,000–13,000	Rota et al. (2004)
Salmonella typhimurium	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Serratia marcescens	Bac–	NA, 1–3 d, 30°C	11,000	Farag et al. (1989)
Serratia marcescens	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Shigella flexneri	Bac–	TSB, 3% EtOH, 24 h, 37°C	20,000	Rota et al. (2004)
Yersinia enterocolitica	Bac–	TSB, 3% EtOH, 24 h, 29°C	10,000–15,000	Rota et al. (2004)
Yersinia enterocolitica	Bac–	MHA, Tween 20, 24 h, 37°C	1250	Rossi et al. (2007)
Actinomyces viscosus	Bac+	HS, 16–24, 37°C	>6000	Shapiro et al. (1994)
Bacillus subtilis	Bac+	TSB, Tween 80, 48 h, 35°C	10	Panizzi et al. (1993)
Bacillus subtilis	Bac+	NA, 1–3 d, 30°C	750	Farag et al. (1989)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	Cited	1250	Pellecuer et al. (1976)
Corynebacterium pseudodiphtheriae	Bac+	Cited	1250	Pellecuer et al. (1976)
Enterococcus faecalis	Bac+	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Lactobacillus sp.	Bac+	MRS, cited	5	Pellecuer et al. (1980)
Listeria monocytogenes	Bac+	TSB, 3% EtOH, 24 h, 37°C	7000–10,000	Rota et al. (2004)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	200	Smith-Palmer et al. (1998)
Micrococcus flavus	Bac+	Cited	1250	Pellecuer et al. (1976)
Micrococcus luteus	Bac+	MHB, cited	2.5–5	Pellecuer et al. (1980)
Micrococcus sp.	Bac+	NA, 1–3 d, 30°C	1500	Farag et al. (1989)
Micrococcus ureae	Bac+	MHB, cited	5	Pellecuer et al. (1980)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	400	Yousef and Tawil (1980)
Mycobacterium phlei	Bac+	NA, 1–3 d, 30°C	1250	Farag et al. (1989)
Sarcina lutea	Bac+	Cited	1250	Pellecuer et al. (1976)
Sarcina sp.	Bac+	NA, 1–3 d, 30°C	2000	Farag et al. (1989)
Sarcina ureae	Bac+	MHB, cited	2.5	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	TSB, 3% EtOH, 24 h, 37°C	30,000–50,000	Rota et al. (2004)
Staphylococcus aureus	Bac+	TSB, Tween 80, 48 h, 35°C	20	Panizzi et al. (1993)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	MPB, DMSO, 40 h, 30°C	1% inh. 500	Hili et al. (1997)

Staphylococcus aureus	Bac+	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2004)
Staphylococcus aureus	Bac+	NA, 1–3 d, 30°C	1000	Farag et al. (1989)
Staphylococcus aureus	Bac+	Cited	1250	Pellecuer et al. (1976)
Staphylococcus aureus	Bac+	MHA, Tween 20, 48 h, 35°C	10,000	Hammer et al. (1999)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Staphylococcus epidermidis	Bac+	MHB, cited	5	Pellecuer et al. (1980)
Staphylococcus epidermidis	Bac+	NB, DMSO, 24 h, 37°C	>900	Angioni et al. (2004)
Staphylococcus epidermidis	Bac+	Cited	1250	Pellecuer et al. (1976)
Streptococcus D	Bac+	MHB, cited	2.5–5	Pellecuer et al. (1980)
Streptococcus micros	Bac+	MHB, cited	5	Pellecuer et al. (1980)
Streptococcus pyogenes	Bac+	Cited	625	Pellecuer et al. (1976)
Streptococcus sanguinis	Bac+	HS, 16–24, 37°C	>6000	Shapiro et al. (1994)
Streptococcus sobrinus	Bac+	HS, 16–24, 37°C	>6000	Shapiro et al. (1994)
Absidia glauca	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus chevalieri	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus clavatus	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus flavus	Fungi	MYB, 72 h, 26°C	12,500	Shin (2003)
Aspergillus giganteus	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus niger	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus niger	Fungi	YES broth, 10 d	12% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus niger	Fungi	MYB, 72 h, 26°C	12,500	Shin (2003)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Aspergillus ochraceus	Fungi	YES broth, 10 d	14% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	250	Okazaki and Oshima (1953)
Aspergillus oryzae	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus repens	Fungi	Cited	2000	Pellecuer et al. (1976)
Cephalosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)

continued

TABLE 12.67 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Ceratocystis paradoxa	Fungi	OA, EtOH, 3 d, 20°C	4000	Narasimba Rao et al. (1971)
Cladosporium herbarum	Fungi	Cited	2000	Pellecuer et al. (1976)
Curvularia lunata	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	625–1250	Janssen et al. (1988)
Epidermophyton floccosum	Fungi	Cited	>4000	Pellecuer et al. (1976)
Fusarium culmorum	Fungi	YES broth, 10 d	0% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium moniliforme var. *subglutinans*	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Helminthosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Microsporum canis	Fungi	MBA, Tween 80, 10 d, 30°C	300– >300	Perrucci et al. (1994)
Microsporum gypseum	Fungi	MBA, Tween 80, 10 d, 30°C	>300	Perrucci et al. (1994)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	Cited	1000	Pellecuer et al. (1976)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	6400	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)
Penicillium chrysogenum	Fungi	Cited	2000	Pellecuer et al. (1976)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Penicillium liliacinum	Fungi	Cited	2000	Pellecuer et al. (1976)
Penicillium rubrum	Fungi	Cited	2000	Pellecuer et al. (1976)
Physalospora tucumanensis	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus nigricans	Fungi	Cited	2000	Pellecuer et al. (1976)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)

Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	25,000	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Sclerotium rolfsii	Fungi	OA, EtOH, 6 d, 20°C	4000	Narasimba Rao et al. (1971)
Scopulariopsis brevicaulis	Fungi	Cited	2000	Pellecuer et al. (1976)
Syncephalastrum racemosum	Fungi	Cited	2000	Pellecuer et al. (1976)
Trichophyton interdigitale	Fungi	Cited	>4000	Pellecuer et al. (1976)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Candida albicans	Yeast	SDB, Tween 80, 48 h, 35	10	Panizzi et al. (1993)
Candida albicans	Yeast	MPB, DMSO, 40 h, 30°C	8% inh. 500	Hili et al. (1997)
Candida albicans	Yeast	NB, DMSO, 24 h, 30°C	>900	Angioni et al. (2004)
Candida albicans	Yeast	Cited	1000	Pellecuer et al. (1976)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Candida albicans	Yeast	MHA, Tween 20, 48 h, 35°C	10,000	Hammer et al. (1999)
Candida mycoderma	Yeast	Cited	2000	Pellecuer et al. (1976)
Candida paraspilosis	Yeast	Cited	1000	Pellecuer et al. (1976)
Candida pelliculosa	Yeast	Cited	2000	Pellecuer et al. (1976)
Candida tropicalis	Yeast	Cited	2000	Pellecuer et al. (1976)
Geotrichum asteroides	Yeast	Cited	4000	Pellecuer et al. (1976)
Geotrichum candidum	Yeast	Cited	2000	Pellecuer et al. (1976)
Hansenula sp.	Yeast	Cited	2000	Pellecuer et al. (1976)
Saccharomyces carlsbergensis	Yeast	Cited	4000	Pellecuer et al. (1976)
Saccharomyces cerevisiae	Yeast	SDB, Tween 80, 48 h, 35°C	5	Panizzi et al. (1993)
Saccharomyces cerevisiae	Yeast	MPB, DMSO, 40 h, 30°C	88% inh. 500	Hili et al. (1997)
Saccharomyces cerevisiae	Yeast	NA, 1–3 d, 30°C	2000	Farag et al. (1989)
Schizosaccharomyces pombe	Yeast	MPB, DMSO, 40 h, 30°C	86% inh. 500	Hili et al. (1997)
Torula utilis	Yeast	MPB, DMSO, 40 h, 30°C	2% inh. 500	Hili et al. (1997)
Escherichia coli	Bac–	TGB, 18 h, 37°C	11,300	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	11,300	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	2800	Schelz et al. (2006)

TABLE 12.68
Inhibitory Data of Rosemary Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Aerobacter tumefaciens	Bac−	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	++	Kellner and Kober (1954)
Escherichia coli	Bac−	Cited	Disk, 20,000	NG to +++	Hethenyi et al. (1989)
Haemophilus influenza	Bac−	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Peptobacterium carotovorum	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Proteus vulgaris	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Pseudomonas aeruginosa	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Pseudomonas pisi	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Pseudomonas tabaci	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Shigella sonnei	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Xanthomonas versicolor	Bac−	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Bacillus subtilis	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Corynebacterium fascians	Bac+	Cited	Disk, 20,000	+++	Hethenyi et al. (1989)
Lactobacillus sp.	Bac+	MRS, cited	Disk, 20,000?	++	Pellecuer et al. (1980)
Micrococcus luteus	Bac+	MHB, cited	Disk, 20,000?	++	Pellecuer et al. (1980)
Micrococcus ureae	Bac+	MHB, cited	Disk, 20,000?	+++	Pellecuer et al. (1980)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Pneumococcus sp.	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Sarcina ureae	Bac+	MHB, cited	Disk, 20,000?	++	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Staphylococcus epidermidis	Bac+	MHB, cited	Disk, 20,000?	+++	Pellecuer et al. (1980)

Streptococcus aegui	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Streptococcus D	Bac+	MHB, cited	Disk, 20,000?	++	Pellecuer et al. (1980)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus haemolyticus	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus sp.	Bac+	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Aspergillus flavus	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Endomyces filbuliger	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Fusarium moniliforme	Fungi	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium solani	Fungi	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Ophiobolus graminis	Fungi	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	++	Guynot et al. (2003)
Penicillium corylophilum	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Penicillium roqueforti	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Candida albicans	Yeast	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)
Saccharomyces cerevisiae	Yeast	Cited	Disk, 20,000	NG	Hethenyi et al. (1989)

TABLE 12.69
Inhibitory Data of Star Anise Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5	Deans and Ritchie (1987)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	7	Janssen et al. (1986)
Escherichia coli	Bac–	NA, 24 h, 37°C	10 (h), 2000	18.5	Singh et al. (2006)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	6.7	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	10 (h), 2000	20.3	Singh et al. (2006)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Salmonella typhi	Bac–	NA, 24 h, 37°C	10 (h), 2000	30.1	Singh et al. (2006)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	5.5	Deans and Ritchie (1987)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	9	Deans and Ritchie (1987)
Bacillus cereus	Bac+	NA, 24 h, 37°C	10 (h), 2000	0	Singh et al. (2006)

Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	6.7	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	10 (h), 2000	26.2	Singh et al. (2006)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	10 (h), 2000	0	Singh et al. (2006)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	9	Janssen et al. (1986)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	8	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)

TABLE 12.70
Inhibitory Data of Star Anise Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	300–625	Janssen et al. (1988)

TABLE 12.71
Inhibitory Data of Star Anise Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Aspergillus flavus	Fungi	CDA, 6 d	12, 6000	+	Singh et al. (2006)
Aspergillus niger	Fungi	CDA, 6 d	12, 6000	++	Singh et al. (2006)
Aspergillus ochraceus	Fungi	CDA, 6 d	12, 6000	+++	Singh et al. (2006)
Aspergillus terreus	Fungi	CDA, 6 d	12, 6000	+++	Singh et al. (2006)
Fusarium graminearum	Fungi	CDA, 6 d	12, 6000	+++	Singh et al. (2006)
Fusarium moniliforme	Fungi	CDA, 6 d	12, 6000	NG	Singh et al. (2006)
Penicillium citrinum	Fungi	CDA, 6 d	12, 6000	+	Singh et al. (2006)
Penicillium viridicatum	Fungi	CDA, 6 d	12, 6000	++	Singh et al. (2006)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.72
Inhibitory Data of Sweet Orange Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone in mm	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	CAB, 24 h, 42°C	Disk, 10,000	0	Fisher and Phillips (2006)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	0	Schelz et al. (2006)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	7	Janssen et al. (1986)
Escherichia coli	Bac–	MHA, 24 h, 30°C	6, 15,000	8	Rossi et al. (2007)
Escherichia coli	Bac–	Cited	15, 2500	16	Pizsolitto et al. (1975)
Escherichia coli	Bac–	NA, 24 h, 37°C	Disk, 10,000	18	Fisher and Phillips (2006)
Escherichia coli	Bac–	NA, 18 h, 37°C	6 (h), pure	19.5	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	20	Morris et al. (1979)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	3	Pizsolitto et al. (1975)

continued

TABLE 12.72 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone in mm	Ref.
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	NA, 18 h, 37°C	6 (h), pure	0	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	0	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	MHA, 24 h, 30°C	6, 15,000	6	Rossi et al. (2007)
Pseudomonas mangiferae indicae	Bac–	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Pseudomonas mangiferae indicae	Bac–	NA, 24 h, 28°C	6, sd	9	Kindra and Satyanarayana (1978)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Salmonella paratyphi	Bac–	NA, 36–48 h, 37°C	6, sd	14	Garg and Garg (1980)
Salmonella paratyphi	Bac–	NA, 24 h, 28°C	6, sd	19	Kindra and Satyanarayana (1978)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	15	Pizsolitto et al. (1975)
Salmonella typhi	Bac–	NA, 36–48 h, 37°C	6, sd	24	Garg and Garg (1980)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	2	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	13	Pizsolitto et al. (1975)
Vibrio cholera	Bac–	NA, 36–48 h, 37°C	6, sd	13	Garg and Garg (1980)
Vibrio cholera	Bac–	NA, 24 h, 28°C	6, sd	13	Kindra and Satyanarayana (1978)
Xanthomonas campestris	Bac–	NA, 24 h, 28°C	6, sd	20.5	Kindra and Satyanarayana (1978)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Bacillus anthracis	Bac+	NA, 24 h, 28°C	6, sd	23	Kindra and Satyanarayana (1978)
Bacillus cereus	Bac+	BHA, 24 h, 30°C	Disk, 10,000	36	Fisher and Phillips (2006)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	2	Maruzzella and Lichtenstein (1956)
Bacillus mycoides	Bac+	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Bacillus mycoides	Bac+	NA, 24 h, 28°C	6, sd	17	Kindra and Satyanarayana (1978)

Bacillus pumilus	Bac+	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Bacillus pumilus	Bac+	NA, 24 h, 28°C	6, sd	16	Kindra and Satyanarayana (1978)
Bacillus sp.	Bac+	Cited	15, 2500	7	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	10	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	10.3	Janssen et al. (1986)
Bacillus subtilis	Bac+	NA, 36–48 h, 37°C	6, sd	14	Garg and Garg (1980)
Bacillus subtilis	Bac+	NA, 18 h, 37°C	6 (h), pure	19	Yousef and Tawil (1980)
Bacillus subtilis	Bac+	NA, 24 h, 28°C	6, sd	21.5	Kindra and Satyanarayana (1978)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	8	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	22	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	7.5	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	10	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	LSA, 24 h, 37°C	Disk, 10,000	>90	Fisher and Phillips (2006)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Mycobacterium phlei	Bac+	NA, 18 h, 37°C	6 (h), pure	23.5	Yousef and Tawil (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Sarcina lutea	Bac+	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 36–48 h, 37°C	6, sd	0	Garg and Garg (1980)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	4	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited	15, 2500	6	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	7	Janssen et al. (1986)
Staphylococcus aureus	Bac+	MHA, 24 h, 37°C	6, 15,000	17	Rossi et al. (2007)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	6 (h), pure	20.6	Yousef and Tawil (1980)

continued

TABLE 12.72 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone in mm	Ref.
Staphylococcus aureus	Bac+	BHA, 24 h, 37°C	Disk, 10,000	46	Fisher and Phillips (2006)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), –30,000	0	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	12	Pizsolitto et al. (1975)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Streptococcus viridans	Bac+	Cited	15, 2500	0	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	10	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	8	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	6	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 8 d, 30°C	6 (h), pure	28	Yousef and Tawil (1980)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	10	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Mucor sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	18	Yousef and Tawil (1980)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Penicillium chrysogenum	Fungi	SDA, 8 d, 30°C	6 (h), pure	25	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	7	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	0	Maruzzella and Liguori (1958)
Rhizopus sp.	Fungi	SDA, 8 d, 30°C	6 (h), pure	0	Yousef and Tawil (1980)

Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	0	Morris et al. (1979)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	13	Janssen et al. (1986)
Candida albicans	Yeast	SDA, 18 h, 30°C	6 (h), pure	22.5	Yousef and Tawil (1980)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	5	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	7	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	6	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	4	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), –30,000	7–8	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	0	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	18	Maruzzella and Liguori (1958)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	10	Conner and Beuchat (1984)

TABLE 12.73
Inhibitory Data of Sweet Orange Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Aerobacter aerogenes	Bac–	NA, pH 7	2000	Subba et al. (1967)
Escherichia coli	Bac–	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	>1000	Morris et al. (1979)
Escherichia coli	Bac–	NA, 24 h, 37°C	10,000	Fisher and Phillips (2006)
Escherichia coli	Bac–	NB, 24–72 h, 37°C	93% inh. 10,000	Dabbah et al. (1970)
Escherichia coli	Bac–	TGB, 18 h, 37°C	>11,300	Schelz et al. (2006)
Helicobacter pylori	Bac–	Cited, 20 h, 37°C	65.1	Weseler et al. (2005)
Pseudomonas aeruginosa	Bac–	NB, Tween 20, 18 h, 37°C	>50,000	Yousef and Tawil (1980)
Pseudomonas aeruginosa	Bac–	NB, 24–72 h, 37°C	87% inh. 10,000	Dabbah et al. (1970)
Salmonella heidelberg	Bac–	NB, 1–2 d, 35–37°C	90% inh., 1000	Dabbah et al. (1970)
Salmonella montevideo	Bac–	NB, 1–2 d, 35–37°C	90% inh., 1000	Dabbah et al. (1970)
Salmonella oranienburg	Bac–	NB, 1–2 d, 35–37°C	90% inh., 1000	Dabbah et al. (1970)
Salmonella schottmuelleri	Bac–	NA, pH 7	1000	Subba et al. (1967)
Salmonella senftenberg	Bac–	NB, 24–72 h, 37°C	93% inh. 10,000	Dabbah et al. (1970)
Salmonella typhimurium	Bac–	NB, 1–2 d, 35–37°C	90% inh., 1000	Dabbah et al. (1970)
Serratia marcescens	Bac–	NA, pH 7	>2000	Subba et al. (1967)
Yersinia enterocolitica	Bac–	MHA, Tween 20, 24 h, 37°C	1250	Rossi et al. (2007)
Bacillus cereus	Bac+	BHA, 24 h, 30°C	>40,000	Fisher and Phillips (2006)
Bacillus subtilis	Bac+	NA, pH 7	2000	Subba et al. (1967)
Bacillus subtilis	Bac+	NB, Tween 20, 18 h, 37°C	3200	Yousef and Tawil (1980)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Lactobacillus plantarum	Bac+	NA, pH 7	1000	Subba et al. (1967)
Listeria monocytogenes	Bac+	LSA, 24 h, 37°C	2500	Fisher and Phillips (2006)
Micrococcus sp.	Bac+	NA, pH 7	1000	Subba et al. (1967)
Mycobacterium phlei	Bac+	NB, Tween 20, 18 h, 37°C	800	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	NB, Tween 20, 18 h, 37°C	12,500	Yousef and Tawil (1980)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	NB, 24–72 h, 37°C	10,000	Dabbah et al. (1970)

Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	>11,300	Schelz et al. (2006)
Streptococcus faecalis	Bac+	NA, pH 7	1000	Subba et al. (1967)
Aspergillus awamorii	Fungi	PDA, pH 4.5	2000	Subba et al. (1967)
Aspergillus flavus	Fungi	PDA, pH 4.5	2000	Subba et al. (1967)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Aspergillus niger	Fungi	PDA, pH 4.5	2000	Subba et al. (1967)
Aspergillus niger	Fungi	NB, Tween 20, 8 d, 30°C	50,000	Yousef and Tawil (1980)
Aspergillus niger	Fungi	YES broth, 10 d	0% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	34% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	500	Okazaki and Oshima (1953)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Botrytis cinera	Fungi	PDA, Tween 20, 7 d, 24°C	4% inh. 1000	Bouchra et al. (2003)
Cephalosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	>20,000	Narasimba Rao et al. (1971)
Ceratocystis paradoxa	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Curvularia lunata	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Fusarium culmorum	Fungi	YES broth, 10 d	84% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium moniliforme var. *subglutinans*	Fungi	OA, EtOH, 3 d, 20°C	>20,000	Narasimba Rao et al. (1971)
Geotrichum citri-aurantii	Fungi	PDA, Tween 20, 7 d, 24°C	7% inh. 1000	Bouchra et al. (2003)
Helminthosporium sacchari	Fungi	OA, EtOH, 3 d, 20°C	20,000	Narasimba Rao et al. (1971)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor racemosus	Fungi	Cited	500	Okazaki and Oshima (1953)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Mucor sp.	Fungi	NB, Tween 20, 8 d, 30°C	12,500	Yousef and Tawil (1980)
Penicillium chrysogenum	Fungi	Cited	500	Okazaki and Oshima (1953)
Penicillium chrysogenum	Fungi	NB, Tween 20, 8 d, 30°C	25,000	Yousef and Tawil (1980)
Penicillium digitatum	Fungi	SDB, 5 d, 20°C, MIC = ED50	1000–2400	Caccioni et al. (1998)
Penicillium digitatum	Fungi	PDA, Tween 20, 7 d, 24°C	32% inh. 1000	Bouchra et al. (2003)
Penicillium italicum	Fungi	SDB, 5 d, 20°C, MIC = ED50	3000–5500	Caccioni et al. (1998)

continued

TABLE 12.73 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Physalospora tucumanensis	Fungi	OA, EtOH, 3 d, 20°C	4000	Narasimba Rao et al. (1971)
Phytophthora citrophthora	Fungi	PDA, Tween 20, 7 d, 24°C	13% inh. 1000	Bouchra et al. (2003)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	NB, Tween 20, 8 d, 30°C	3200	Yousef and Tawil (1980)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	>1000	Thompson and Cannon (1986)
Sclerotium rolfsii	Fungi	OA, EtOH, 6 d, 20°C	20,000	Narasimba Rao et al. (1971)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	>1250	Janssen et al. (1988)
Candida albicans	Yeast	NB, Tween 20, 18 h, 37°C	1600	Yousef and Tawil (1980)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Saccharomyces cerevisiae	Yeast	PDA, pH 4.5	1000	Subba et al. (1967)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	2800	Schelz et al. (2006)
Torula utilis	Yeast	PDA, pH 4.5	1000	Subba et al. (1967)
Zygosaccharomyces mellis	Yeast	PDA, pH 4.5	1000	Subba et al. (1967)

TABLE 12.74
Inhibitory Data of Sweet Orange Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Campylobacter jejuni	Bac−	CAB, 24 h, 42°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Escherichia coli	Bac−	NA, 24 h, 37°C	~20,000	+	Kellner and Kober (1954)
Escherichia coli	Bac−	NA, 24 h, 37°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Neisseria sp.	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac−	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus cereus	Bac+	BHA, 24 h, 30°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Listeria monocytogenes	Bac+	LSA, 24 h, 37°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	BHA, 24 h, 37°C	Disk, 10,000	+++	Fisher and Phillips (2006)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Aspergillus flavus	Fungi	Bread, 14 d, 25°C	30,000	NG	Suhr and Nielsen (2003)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Endomyces filbuliger	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium repens	Fungi	Bread, 14 d, 25°C	30,000	+	Suhr and Nielsen (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+++	Guynot et al. (2003)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	+	Guynot et al. (2003)
Penicillium corylophilum	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Penicillium roqueforti	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

TABLE 12.75
Inhibitory Data of Tea Tree Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Escherichia coli	Bac–	NA, 24 h, 30°C	Drop, 5000	14	Hili et al. (1997)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	17	Schelz et al. (2006)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 30°C	Drop, 5000	5	Hili et al. (1997)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	20	Lis-Balchin et al. (1998)
Staphylococcus aureus	Bac+	NA, 24 h, 30°C	Drop, 5000	7	Hili et al. (1997)
Staphylococcus aureus MRSA	Bac+	MHA, 24 h, 37°C	12.7, 30,000	21–33	Carson et al. (1995)
Staphylococcus epidermidis	Bac+	NA, 18 h, 37°C	5 (h), –30,000	14	Schelz et al. (2006)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	15.3	Pawar and Thaker (2007)
Aspergillus flavus	Fungi	SDA, 72 h, 26°C	8, 25,000	9	Shin (2003)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	7	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SDA, 72 h, 26°C	8, 25,000	8	Shin (2003)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	10.5	Pawar and Thaker (2007)
Candida albicans	Yeast	NA, 24 h, 30°C	Drop, 5000	11	Hili et al. (1997)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), –30,000	19–21	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 30°C	Drop, 5000	13	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	NA, 24 h, 30°C	Drop, 5000	20	Hili et al. (1997)
Torula utilis	Yeast	NA, 24 h, 30°C	Drop, 5000	47	Hili et al. (1997)

TABLE 12.76
Inhibitory Data of Tea Tree Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Mycoplasma fermentans	Bac	Cited	100–600	Furneri et al. (2006)
Mycoplasma hominis	Bac	Cited	600–1200	Furneri et al. (2006)
Mycoplasma pneumoniae	Bac	Cited	100	Furneri et al. (2006)
Acinetobacter baumannii	Bac–	HIB, Tween 80, 24 h, 35°C	600–10,000	Hammer et al. (1996)
Actinobacillus actinomycetemcomitans	Bac–	HS, 72 h, 37°C	1100	Shapiro et al. (1994)
Aeromonas sobria	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Bacteroides sp.	Bac–	VC, Tween 80	300–5000	Hammer et al. (1999)
Citrobacter freundii	Bac–	ISB, Tween 80, 20–24 h, 37°C	5000	Harkenthal et al. (1999)
Coliform bacilli	Bac–	MHB, Tween 80, 24 h, 37°C	10,000–20,000	Banes-Marshall et al. (2001)
Coliform bacilli	Bac–	BA, 24 h, 37°C	5000	Banes-Marshall et al. (2001)
Enterobacter aerogenes	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Enterococcus faecalis	Bac–	ISB, Tween 20, 24 h, 37°C	5000–7500	Griffin et al. (2000)
Escherichia coli	Bac–	ISB, Tween 20, 24 h, 37°C	2000	Griffin et al. (2000)
Escherichia coli	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Escherichia coli	Bac–	ISB, Tween 80, 16–20 h, 37°C	2500	Gustafson et al. (1998)
Escherichia coli	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Escherichia coli	Bac–	MPB, DMSO, 40 h, 30°C	28% inh. 500	Hili et al. (1997)
Escherichia coli	Bac–	TGB, 18 h, 37°C	5600	Schelz et al. (2006)
Fusobacterium nucleatum	Bac–	HS, 72 h, 37°C	>6000	Shapiro et al. (1994)
Fusobacterium sp.	Bac–	VC, Tween 80	600–2500	Hammer et al. (1999)
Gardnerella vaginalis	Bac–	VC, Tween 80	600	Hammer et al. (1999)
Klebsiella pneumoniae	Bac–	HIB, Tween 80, 24 h, 35°C	1200–50,000	Hammer et al. (1996)
Klebsiella pneumoniae	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Klebsiella pneumoniae	Bac–	ISB, Tween 20, 24 h, 37°C	3000	Griffin et al. (2000)
Klebsiella pneumoniae	Bac–	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Porphyromonas gingivalis	Bac–	HS, 72 h, 37°C	1100	Shapiro et al. (1994)
Prevotella sp.	Bac–	VC, Tween 80	300–2500	Hammer et al. (1999)

continued

TABLE 12.76 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Proteus mirabilis	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Proteus vulgaris	Bac–	ISB, Tween 20, 24 h, 37°C	3000	Griffin et al. (2000)
Pseudomonas aeruginosa	Bac–	MCA, 24 h, 37	>20,000	Banes-Marshall et al. (2001)
Pseudomonas aeruginosa	Bac–	ISB, Tween 80, 20–24 h, 37°C	>40,000	Harkenthal et al. (1999)
Pseudomonas aeruginosa	Bac–	ISB, Tween 20, 24 h, 37°C	10,000– >20,000	Griffin et al. (2000)
Pseudomonas aeruginosa	Bac–	MHB, Tween 80, 24 h, 37°C	10,000–80,000	Banes-Marshall et al. (2001)
Pseudomonas aeruginosa	Bac–	HIB, Tween 80, 24 h, 35°C	20,000–50,000	Hammer et al. (1996)
Pseudomonas aeruginosa	Bac–	MHB, 18–24 h, 37°C	40,000	Papadopoulos et al. (2006)
Pseudomonas aeruginosa	Bac–	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Pseudomonas aeruginosa	Bac–	MPB, DMSO, 40 h, 30°C	75% inh. 500	Hili et al. (1997)
Pseudomonas fluorescens	Bac–	MHB, 18–24 h, 37°C	40,000	Papadopoulos et al. (2006)
Pseudomonas putida	Bac–	ISB, Tween 20, 24 h, 37°C	>20,000	Griffin et al. (2000)
Pseudomonas putida	Bac–	MHB, 18–24 h, 37°C	10,000	Papadopoulos et al. (2006)
Salmonella choleraesuis	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Salmonella typhimurium	Bac–	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Serratia marcescens	Bac–	ISB, Tween 20, 24 h, 37°C	1000–3000	Griffin et al. (2000)
Serratia marcescens	Bac–	HIB, Tween 80, 24 h, 35°C	2500–50,000	Hammer et al. (1996)
Serratia marcescens	Bac–	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Shigella flexneri	Bac–	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Actinomyces viscosus	Bac+	HS, 16–24, 37°C	6000	Shapiro et al. (1994)
Anaerobic cocci	Bac+	VC, Tween 80	600–2500	Hammer et al. (1999)
Bacillus cereus	Bac+	ISB, Tween 20, 24 h, 37°C	3000	Griffin et al. (2000)
Bacillus subtilis	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Bacillus subtilis	Bac+	ISB, Tween 20, 24 h, 37°C	3000	Griffin et al. (2000)
Corynebacterium pseudodiphtheriae	Bac+	ISB, Tween 80, 20–24 h, 37°C	5000	Harkenthal et al. (1999)
Corynebacterium sp.	Bac+	ISB, Tween 20, 24 h, 37°C	2000–3000	Griffin et al. (2000)
Corynebacterium sp.	Bac+	HIB, Tween 80, 24 h, 35°C	600–20,000	Hammer et al. (1996)
Enterococcus durans	Bac+	ISB, Tween 80, 20–24 h, 37°C	1000	Harkenthal et al. (1999)
Enterococcus faecalis	Bac+	ISB, Tween 80, 20–24 h, 37°C	1000	Harkenthal et al. (1999)

Enterococcus faecalis	Bac+	MHA, Tween 20, 48 h, 35°C	10,000	Hammer et al. (1999)
Enterococcus faecalis	Bac+	MHB, Tween 80, 24 h, 37°C	80,000	Banes-Marshall et al. (2001)
Enterococcus faecium	Bac+	ISB, Tween 80, 20–24 h, 37°C	1000	Harkenthal et al. (1999)
Enterococcus faecium VRE	Bac+	HIB, Tween 80, 18 h, 37°C	5000–10,000	Nelson (1997)
Listeria monocytogenes	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Micrococcus luteus	Bac+	ISB, Tween 20, 24 h, 37°C	2000–3000	Griffin et al. (2000)
Micrococcus luteus	Bac+	HIB, Tween 80, 24 h, 35°C	600–5000	Hammer et al. (1996)
Micrococcus sp.	Bac+	HIB, Tween 80, 24 h, 35°C	600–5000	Hammer et al. (1996)
Micrococcus varians	Bac+	HIB, Tween 80, 24 h, 35°C	5000–10,000	Hammer et al. (1996)
Mobiluncus sp.	Bac+	VC, Tween 80	300–600	Hammer et al. (1999)
Peptostreptococcus anaerobius	Bac+	HS, 72 h, 37°C	2000	Shapiro et al. (1994)
Peptostreptococcus anaerobius	Bac+	VC, Tween 80	600–2500	Hammer et al. (1999)
Propionibacterium acnes	Bac+	Agar, 24 h, 37°C	3100–6300	Raman et al. (1995)
Propionibacterium acnes	Bac+	ISB, Tween 20, 24 h, 37°C	5000	Griffin et al. (2000)
Staphylococcus aureus	Bac+	BA, 24 h, 37°C	10,000	Banes-Marshall et al. (2001)
Staphylococcus aureus	Bac+	HIB, Tween 80, 24 h, 35°C	1200–5000	Hammer et al. (1996)
Staphylococcus aureus	Bac+	ISB, Tween 20, 24 h, 37°C	2000	Griffin et al. (2000)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	20,000	Banes-Marshall et al. (2001)
Staphylococcus aureus	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Staphylococcus aureus	Bac+	MPB, DMSO, 40 h, 30°C	43% inh. 500	Hili et al. (1997)
Staphylococcus aureus	Bac+	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Staphylococcus aureus	Bac+	MHB, Tween 80, 24 h, 37°C	5000	Banes-Marshall et al. (2001)
Staphylococcus aureus	Bac+	Agar, 24 h, 37°C	6300–12,500	Raman et al. (1995)
Staphylococcus aureus MRSA	Bac+	BA, 24 h, 37°C	10,000	Banes-Marshall et al. (2001)
Staphylococcus aureus MRSA	Bac+	MHB, Tween 80, 24 h, 37°C	20,000–40,000	Banes-Marshall et al. (2001)
Staphylococcus aureus MRSA	Bac+	ISB, Tween 20, 24 h, 37°C	2000–3000	Griffin et al. (2000)
Staphylococcus aureus MRSA	Bac+	HIB, Tween 80, 18 h, 37°C	2500	Nelson (1997)
Staphylococcus aureus MRSA	Bac+	Cited	2500	Carson and Messager (2005)
Staphylococcus aureus MRSA	Bac+	HIB, Tween 80, 24 h, 37°c	2500–5000	Carson et al. (1995)
Staphylococcus capitis	Bac+	HIB, Tween 80, 24 h, 35°C	10,000–10,0000	Hammer et al. (1996)
Staphylococcus capitis	Bac+	ISB, Tween 80, 20–24 h, 37°C	1200–2500	Harkenthal et al. (1999)

continued

TABLE 12.76 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Staphylococcus epidermidis	Bac+	HIB, Tween 80, 24 h, 35°C	1200–40,000	Hammer et al. (1996)
Staphylococcus epidermidis	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Staphylococcus epidermidis	Bac+	ISB, Tween 20, 24 h, 37°C	5000	Griffin et al. (2000)
Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	5600	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	Agar, 24 h, 37°C	6300–12,500	Raman et al. (1995)
Staphylococcus haemolyticus	Bac+	HIB, Tween 80, 24 h, 35°C	10,000–40,000	Hammer et al. (1996)
Staphylococcus haemolyticus	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500–5000	Harkenthal et al. (1999)
Staphylococcus hominis	Bac+	HIB, Tween 80, 24 h, 35°C	10,000–40,000	Hammer et al. (1996)
Staphylococcus hominis	Bac+	ISB, Tween 80, 20–24 h, 37°C	1200	Harkenthal et al. (1999)
Staphylococcus saprophyticus	Bac+	HIB, Tween 80, 24 h, 35°C	20,000–30,000	Hammer et al. (1996)
Staphylococcus saprophyticus	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500–5000	Harkenthal et al. (1999)
Staphylococcus warneri	Bac+	HIB, Tween 80, 24 h, 35°C	20,000–80,000	Hammer et al. (1996)
Staphylococcus xylosus	Bac+	HIB, Tween 80, 24 h, 35°C	10,000–30,000	Hammer et al. (1996)
Staphylococcus xylosus	Bac+	ISB, Tween 80, 20–24 h, 37°C	2500	Harkenthal et al. (1999)
Streptococci beta-haemolytic	Bac+	BA, 24 h, 37°C	1200–5000	Banes-Marshall et al. (2001)
Streptococci beta-haemolytic	Bac+	MHB, Tween 80, 24 h, 37°C	80,000	Banes-Marshall et al. (2001)
Streptococci beta-haemolytic Gp.D	Bac+	MHB, Tween 80, 24 h, 37°C	5000–20,000	Banes-Marshall et al. (2001)
Streptococci, faecal	Bac+	MHB, Tween 80, 24 h, 37°C	>80,000	Banes-Marshall et al. (2001)
Streptococci, faecal	Bac+	BA, 24 h, 37°C	10,000	Banes-Marshall et al. (2001)
Streptococcus equi	Bac+	THB, Tween 80, 24 h, 35°C	1200	Carson et al. (1996)
Streptococcus equisimilis	Bac+	THB, Tween 80, 24 h, 35°C	1200	Carson et al. (1996)
Streptococcus pyogenes	Bac+	THB, Tween 80, 24 h, 35°C	1200	Carson et al. (1996)
Streptococcus pyogenes	Bac+	MHB, Tween 80, 24 h, 37°C	20,000	Banes-Marshall et al. (2001)
Streptococcus sobrinus	Bac+	HS, 16–24, 37°C	6000	Shapiro et al. (1994)
Streptococcus sp. group G	Bac+	THB, Tween 80, 24 h, 35°C	1200	Carson et al. (1996)
Streptococcus zooepidemicus	Bac+	THB, Tween 80, 24 h, 35°C	600	Carson et al. (1996)
Alternaria alternata	Fungi	PDA, 7 d, 28°C	62% inh. 500	Feng and Zheng (2007)
Alternaria sp.	Fungi	Cited data	160–1200	Carson et al. (2006)
Alternaria sp.	Fungi	RPMI, Tween 80, 48 h, 30°C	160–1200	Carson and Riley (2002)
Aspergillus flavus	Fungi	Cited data	3100–7000	Carson et al. (2006)

Aspergillus flavus	Fungi	MYB, 72 h, 26°C	3120	Shin (2003)
Aspergillus flavus	Fungi	MA, Tween 20, 24 h, 30°C	4000–5000	Griffin et al. (2000)
Aspergillus flavus	Fungi	MA, Tween 20, 24 h, 30°C	5000–7000	Griffin et al. (2000)
Aspergillus flavus	Fungi	RPMI, Tween 80, 48 h, 35°C	600–1200	Carson and Riley (2002)
Aspergillus fumigatus	Fungi	RPMI, Tween 80, 48 h, 35°C	600–1200	Carson and Riley (2002)
Aspergillus niger	Fungi	Cited data	160–4000	Carson et al. (2006)
Aspergillus niger	Fungi	MA, Tween 20, 24 h, 30°C	3000–4000	Griffin et al. (2000)
Aspergillus niger	Fungi	MYB, 72 h, 26°C	3120	Shin (2003)
Aspergillus niger	Fungi	RPMI, Tween 80, 48 h, 35°C	600–1200	Carson and Riley (2002)
Aspergillus niger	Fungi	YES broth, 10 d	>10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	YES broth, 10 d	91% inh. 10,000	Lis-Balchin et al. (1998)
Blastoschizomyces capitatus	Fungi	Cited data	2500	Carson et al. (2006)
Cladosporium sp.	Fungi	RPMI, Tween 80, 72 h, 30°C	160–1200	Carson and Riley (2002)
Cladosporium sp.	Fungi	Cited data	80–1200	Carson et al. (2006)
Epidermophyton floccosum	Fungi	RPMI, Tween 80, 96 h, 30°C	80–300	Carson and Riley (2002)
Epidermophyton floccosum	Fungi	Cited data	80–7000	Carson et al. (2006)
Fusarium culmorum	Fungi	YES broth, 10 d	76% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium sp.	Fungi	Cited data	80–2500	Carson et al. (2006)
Fusarium sp.	Fungi	RPMI, Tween 80, 48 h, 35°C	80–2500	Carson and Riley (2002)
Malassezia sympodialis	Fungi	Cited data	160–1200	Carson et al. (2006)
Microsporum canis	Fungi	Cited data	300–5000	Carson et al. (2006)
Microsporum canis	Fungi	RPMI, Tween 80, 96 h, 30°C	40–300	Carson and Riley (2002)
Microsporum gypseum	Fungi	RPMI, Tween 80, 96 h, 30°C	160–300	Carson and Riley (2002)
Penicillium sp.	Fungi	Cited data	300–600	Carson et al. (2006)
Penicillium sp.	Fungi	RPMI, Tween 80, 48 h, 35°C	300–600	Carson and Riley (2002)
Pleurotus ferulae	Fungi	SDA, 7 d, 25°C	72–82% inh. 1000	Angelini et al. (2008)
Pleurotus nebrodensis	Fungi	SDA, 7 d, 25°C	64–88% inh. 1000	Angelini et al. (2008)
Pleurotus nebrodensis	Fungi	SDA, 7 d, 25°C	83–88% inh. 1000	Angelini et al. (2008)
Trichophyton interdigitale	Fungi	RPMI, Tween 80, 96 h, 30°C	80–300	Carson and Riley (2002)
Trichophyton mentagrophytes	Fungi	Cited data	1100–4400	Carson et al. (2006)
Trichophyton mentagrophytes	Fungi	MA, Tween 20, 24 h, 30°C	3000–4000	Griffin et al. (2000)
Trichophyton mentagrophytes	Fungi	RPMI, Tween 80, 96 h, 30°C	80–600	Carson and Riley (2002)
Trichophyton rubrum	Fungi	MA, Tween 20, 24 h, 30°C	10,000	Banes-Marshall et al. (2001)

continued

TABLE 12.76 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Trichophyton rubrum	Fungi	Cited data	300–6000	Carson et al. (2006)
Trichophyton rubrum	Fungi	RPMI, Tween 80, 96 h, 30°C	80–300	Carson and Riley (2002)
Trichophyton tonsurans	Fungi	Cited data	40–160	Carson et al. (2006)
Trichophyton tonsurans	Fungi	RPMI, Tween 80, 96 h, 30°C	40–160	Carson and Riley (2002)
Candida albicans	Yeast	RPMI, Tween 80, 48 h, 30°C	1250	Oliva et al. (2003)
Candida albicans	Yeast	MEB, Tween 20, 24 h, 37	2000	Griffin et al. (2000)
Candida albicans	Yeast	RPMI, Tween 80, 48 h, 35°C	2500	Mondello et al. (2006)
Candida albicans	Yeast	MHB, Tween 80, 48 h, 35°C	2500–5000	Hammer et al. (1998)
Candida albicans	Yeast	MPB, DMSO, 40 h, 30°C	37% inh. 500	Hili et al. (1997)
Candida albicans	Yeast	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Candida albicans	Yeast	SDA, 24 h, 37°C	5000–10,000	Banes-Marshall et al. (2001)
Candida capitatus	Yeast	RPMI, Tween 80, 48 h, 30°C	1250–2500	Oliva et al. (2003)
Candida famata	Yeast	SDA, 24 h, 37°C	2500	Banes-Marshall et al. (2001)
Candida glabrata	Yeast	MHB, Tween 80, 48 h, 35°C	1200–5000	Hammer et al. (1998)
Candida glabrata	Yeast	SDA, 24 h, 37°C	2500–5000	Banes-Marshall et al. (2001)
Candida glabrata	Yeast	RPMI, Tween 80, 48 h, 30°C	300–1250	Oliva et al. (2003)
Candida glabrata	Yeast	Cited data	300–8000	Carson et al. (2006)
Candida glabrata	Yeast	RPMI, Tween 80, 48 h, 35°C	600	Mondello et al. (2006)
Candida guilliermondii	Yeast	RPMI, Tween 80, 48 h, 30°C	125ß	Oliva et al. (2003)
Candida incospigua	Yeast	RPMI, Tween 80, 48 h, 30°C	300	Oliva et al. (2003)
Candida krusei	Yeast	RPMI, Tween 80, 48 h, 30°C	1250	Oliva et al. (2003)
Candida krusei	Yeast	RPMI, Tween 80, 48 h, 35°C	2500	Mondello et al. (2006)
Candida krusei	Yeast	SDA, 24 h, 37°C	5000	Banes-Marshall et al. (2001)
Candida lipolytica	Yeast	RPMI, Tween 80, 48 h, 30°C	600–1250	Oliva et al. (2003)

Candida lusitaniae	Yeast	RPMI, Tween 80, 48 h, 30°C	1250	Oliva et al. (2003)
Candida parapsilosis	Yeast	RPMI, Tween 80, 48 h, 35°C	1250	Mondello et al. (2006)
Candida parapsilosis	Yeast	MHB, Tween 80, 48 h, 35°C	2500–5000	Hammer et al. (1998)
Candida parapsilosis	Yeast	Cited data	300–5000	Carson et al. (2006)
Candida parapsilosis	Yeast	RPMI, Tween 80, 48 h, 30°C	600–1250	Oliva et al. (2003)
Candida sp.	Yeast	MHB, Tween 80, 48 h, 35°C	1200–5000	Hammer et al. (1998)
Candida sp.	Yeast	MHB, Tween 80, 24 h, 37°C	5000	Banes-Marshall et al. (2001)
Candida tropicalis	Yeast	Cited data	1200–20,000	Carson et al. (2006)
Candida tropicalis	Yeast	RPMI, Tween 80, 48 h, 35°C	600	Mondello et al. (2006)
Cryptococcus neoformans	Yeast	Cited data	150–600	Carson et al. (2006)
Cryptococcus neoformans	Yeast	RPMI, Tween 80, 48 h, 35°C	300	Mondello et al. (2006)
Malassezia furfur	Yeast	MMA, Tween 20, 7 d	1200–2500	Hammer et al. (2000)
Malassezia furfur	Yeast	Cited data	300–1200	Carson et al. (2006)
Malassezia globosa	Yeast	MMA, Tween 20, 7 d	300–1200	Hammer et al. (2000)
Malassezia obtusa	Yeast	MMA, Tween 20, 7 d	1200	Hammer et al. (2000)
Malassezia slooffiae	Yeast	MMA, Tween 20, 7 d	1200–2500	Hammer et al. (2000)
Malassezia sympodialis	Yeast	MMA, Tween 20, 7 d	160–2500	Hammer et al. (2000)
Rhodotorula rubra	Yeast	Cited data	600	Carson et al. (2006)
Saccharomyces cerevisiae	Yeast	MEB, Tween 20, 24 h, 37°C	2000	Griffin et al. (2000)
Saccharomyces cerevisiae	Yeast	Cited data	2500	Carson et al. (2006)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	2800	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	MPB, DMSO, 40 h, 30°C	69% inh. 500	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	MPB, DMSO, 40 h, 30°C	74% inh. 500	Hili et al. (1997)
Torula utilis	Yeast	MPB, DMSO, 40 h, 30°C	33% inh. 500	Hili et al. (1997)
Trichophyton tonsurans	Yeast	Cited data	1200–2200	Carson et al. (2006)

TABLE 12.77
Inhibitory Data of Tea Tree Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	BLA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Haemophilus influenzae	Bac–	MHA, 18 h, 37°C	MIC_{air}	25	Inouye et al. (2001)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	NG	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	+++	Maruzzella and Sicurella (1960)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	50	Inouye et al. (2001)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)

TABLE 12.78
Inhibitory Data of Thyme Oil Obtained in the Agar Diffusion Test

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Acinetobacter calcoaceticus	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	19	Deans and Ritchie (1987)
Aerobacter aerogenes	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Aeromonas hydrophila	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	22.5	Deans and Ritchie (1987)
Alcaligenes faecalis	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	20	Deans and Ritchie (1987)
Beneckea natriegens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	20	Deans and Ritchie (1987)
Campylobacter jejuni	Bac–	TSA, 24 h, 42°C	4 (h), 25,000	10.4	Smith-Palmer et al. (1998)
Citrobacter freundii	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	21.5	Deans and Ritchie (1987)
Enterobacter aerogenes	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	25.5	Deans and Ritchie (1987)
Erwinia carotovora	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	21.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Escherichia coli	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	8.3	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	NA, 24 h, 37°C	8 (h), pure	16	Fawzi (1991)
Escherichia coli	Bac–	Cited	15, 2500	21	Pizsolitto et al. (1975)
Escherichia coli	Bac–	Cited, 18 h, 37°C	6, 2500	19.3	Janssen et al. (1986)
Escherichia coli	Bac–	NA, 18 h, 37°C	5 (h), –30,000	26	Schelz et al. (2006)
Escherichia coli	Bac–	TGA, 18–24 h, 37°C	9.5, 2000	27	Morris et al. (1979)
Escherichia coli	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	22.5	Deans and Ritchie (1987)
Escherichia coli	Bac–	NA, 24 h, 30°C	Drop, 5000	41	Hili et al. (1997)
Flavobacterium suaveolens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	38	Deans and Ritchie (1987)
Klebsiella pneumoniae	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	19	Deans and Ritchie (1987)
Klebsiella sp.	Bac–	Cited	15, 2500	12	Pizsolitto et al. (1975)
Moraxella sp.	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	24	Deans and Ritchie (1987)
Neisseria perflava	Bac–	NA, 24 h, 37°C	—, sd	3	Maruzzella and Lichtenstein (1956)
Proteus sp.	Bac–	Cited	15, 2500	16	Pizsolitto et al. (1975)
Proteus vulgaris	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Proteus vulgaris	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	20	Deans and Ritchie (1987)
Pseudomonas aeruginosa	Bac–	Cited	15, 2500	0	Pizsolitto et al. (1975)

continued

TABLE 12.78 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 37°C	8 (h), pure	8	Fawzi (1991)
Pseudomonas aeruginosa	Bac–	NA, 24 h, 30°C	Drop, 5000	14	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	Cited, 18 h, 37°C	6, 2500	8.7	Janssen et al. (1986)
Pseudomonas aeruginosa	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	22.5	Deans and Ritchie (1987)
Salmonella enteritidis	Bac–	TSA, 24 h, 35°C	4 (h), 25,000	11.1	Smith-Palmer et al. (1998)
Salmonella pullorum	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	26	Deans and Ritchie (1987)
Salmonella sp.	Bac–	Cited	15, 2500	10	Pizsolitto et al. (1975)
Serratia marcescens	Bac–	NA, 24 h, 37°C	—, sd	4	Maruzzella and Lichtenstein (1956)
Serratia marcescens	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	20.5	Deans and Ritchie (1987)
Serratia sp.	Bac–	Cited	15, 2500	5	Pizsolitto et al. (1975)
Shigella sp.	Bac–	Cited	15, 2500	8	Pizsolitto et al. (1975)
Yersinia enterocolitica	Bac–	ISA, 48 h, 25°C	4 (h), 10,000	23	Deans and Ritchie (1987)
Bacillus cereus	Bac+	NA, 24 h, 37°C	8 (h), pure	21	Fawzi (1991)
Bacillus mesentericus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Bacillus sp.	Bac+	Cited	15, 2500	26	Pizsolitto et al. (1975)
Bacillus subtilis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	12.5	Deans and Ritchie (1987)
Bacillus subtilis	Bac+	NA, 24 h, 37°C	—, sd	25	Maruzzella and Lichtenstein (1956)
Bacillus subtilis	Bac+	Cited, 18 h, 37°C	6, 2500	33.2	Janssen et al. (1986)
Brevibacterium linens	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	24.5	Deans and Ritchie (1987)
Brochotrix thermosphacta	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	16	Deans and Ritchie (1987)
Clostridium sporogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Corynebacterium sp.	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	16	Morris et al. (1979)
Lactobacillus plantarum	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	18	Deans and Ritchie (1987)
Lactobacillus sp.	Bac+	MRS, cited	9, 20,000	25– >90	Pellecuer et al. (1980)
Leuconostoc cremoris	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	0	Deans and Ritchie (1987)
Listeria monocytogenes	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	6–20	Lis-Balchin et al. (1998)
Listeria monocytogenes	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	10	Smith-Palmer et al. (1998)
Micrococcus luteus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	32	Deans and Ritchie (1987)
Micrococcus luteus	Bac+	MHA, cited	9, 20,000	40	Pellecuer et al. (1980)

Micrococcus ureae	Bac+	MHA, cited	9, 20,000	40	Pellecuer et al. (1980)
Sarcina lutea	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Sarcina ureae	Bac+	MHA, cited	9, 20,000	>90	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	—, sd	0	Maruzzella and Lichtenstein (1956)
Staphylococcus aureus	Bac+	TSA, 24 h, 35°C	4 (h), 25,000	8.5	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	Cited	15, 2500	14	Pizsolitto et al. (1975)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	8 (h), pure	19	Fawzi (1991)
Staphylococcus aureus	Bac+	NA, 18 h, 37°C	5 (h), –30,000	24	Schelz et al. (2006)
Staphylococcus aureus	Bac+	TGA, 18–24 h, 37°C	9.5, 2000	25	Morris et al. (1979)
Staphylococcus aureus	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	29	Deans and Ritchie (1987)
Staphylococcus aureus	Bac+	Cited, 18 h, 37°C	6, 2500	33.7	Janssen et al. (1986)
Staphylococcus aureus	Bac+	NA, 24 h, 30°C	Drop, 5000	51	Hili et al. (1997)
Staphylococcus epidermidis	Bac+	Cited	15, 2500	16	Pizsolitto et al. (1975)
Staphylococcus epidermidis	Bac+	MHA, cited	9, 20,000	40	Pellecuer et al. (1980)
Streptococcus D	Bac+	MHA, cited	9, 20,000	17– >90	Pellecuer et al. (1980)
Streptococcus faecalis	Bac+	ISA, 48 h, 25°C	4 (h), 10,000	19.5	Deans and Ritchie (1987)
Streptococcus micros	Bac+	MHA, cited	9, 20,000	>90	Pellecuer et al. (1980)
Streptococcus viridans	Bac+	Cited	15, 2500	3	Pizsolitto et al. (1975)
Streptomyces venezuelae	Bac+	SMA, 2–7 d, 20°C	sd	21	Maruzzella and Liguori (1958)
Alternaria porri	Fungi	PDA, 72 h, 28°C	5, 5000	26.6	Pawar and Thaker (2007)
Alternaria solani	Fungi	SMA, 2–7 d, 20°C	sd	22	Maruzzella and Liguori (1958)
Aspergillus fumigatus	Fungi	SMA, 2–7 d, 20°C	sd	21	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	PDA, 48 h, 28°C	5, 5000	12	Pawar and Thaker (2006)
Aspergillus niger	Fungi	SMA, 2–7 d, 20°C	sd	22	Maruzzella and Liguori (1958)
Aspergillus niger	Fungi	SDA, 3 d, 30	8 (h), pure	35	Fawzi (1991)
Fusarium oxysporum f.sp. *cicer*	Fungi	PDA, 72 h, 28°C	5, 5000	10.5	Pawar and Thaker (2007)
Helminthosporium sativum	Fungi	SMA, 2–7 d, 20°C	sd	20	Maruzzella and Liguori (1958)
Mucor mucedo	Fungi	SMA, 2–7 d, 20°C	sd	22	Maruzzella and Liguori (1958)
Nigrospora panici	Fungi	SMA, 2–7 d, 20°C	sd	16	Maruzzella and Liguori (1958)
Penicillium digitatum	Fungi	SMA, 2–7 d, 20°C	sd	17	Maruzzella and Liguori (1958)
Rhizopus nigricans	Fungi	SMA, 2–7 d, 20°C	sd	14	Maruzzella and Liguori (1958)

continued

TABLE 12.78 (continued)

Microorganism	MO Class	Conditions		Inhibition Zone (mm)	Ref.
Brettanomyces anomalus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	31	Conner and Beuchat (1984)
Candida albicans	Yeast	SMA, 2–7 d, 20°C	sd	14	Maruzzella and Liguori (1958)
Candida albicans	Yeast	TGA, 18–24 h, 37°C	9.5, 2000	14	Morris et al. (1979)
Candida albicans	Yeast	SDA, 24 h, 37°C	8 (h), pure	37	Fawzi (1991)
Candida albicans	Yeast	Cited, 18 h, 37°C	6, 2500	40.7	Janssen et al. (1986)
Candida albicans	Yeast	NA, 24 h, 30°C	Drop, 5000	61	Hili et al. (1997)
Candida krusei	Yeast	SMA, 2–7 d, 20°C	sd	13	Maruzzella and Liguori (1958)
Candida lipolytica	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	18	Conner and Beuchat (1984)
Candida tropicalis	Yeast	SMA, 2–7 d, 20°C	sd	12	Maruzzella and Liguori (1958)
Cryptococcus neoformans	Yeast	SMA, 2–7 d, 20°C	sd	25	Maruzzella and Liguori (1958)
Cryptococcus rhodobenhani	Yeast	SMA, 2–7 d, 20°C	sd	14	Maruzzella and Liguori (1958)
Debaryomyces hansenii	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	15	Conner and Beuchat (1984)
Geotrichum candidum	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	34	Conner and Beuchat (1984)
Hansenula anomala	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	18	Conner and Beuchat (1984)
Kloeckera apiculata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	19	Conner and Beuchat (1984)
Kluyveromyces fragilis	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	17	Conner and Beuchat (1984)
Lodderomyces elongisporus	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	16	Conner and Beuchat (1984)
Metchnikowia pulcherrima	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	38	Conner and Beuchat (1984)
Pichia membranaefaciens	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	34	Conner and Beuchat (1984)
Rhodotorula rubra	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	21	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 20°C	5 (h), −30,000	23–25	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	SMA, 2–7 d, 20°C	sd	16	Maruzzella and Liguori (1958)
Saccharomyces cerevisiae	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	25	Conner and Beuchat (1984)
Saccharomyces cerevisiae	Yeast	NA, 24 h, 30°C	Drop, 5000	80	Hili et al. (1997)
Schizosaccharomyces pombe	Yeast	NA, 24 h, 30°C	Drop, 5000	69	Hili et al. (1997)
Torula glabrata	Yeast	MPA, 4 d, 30°C	5, 10% sol. sd	15	Conner and Beuchat (1984)
Torula utilis	Yeast	NA, 24 h, 30°C	Drop, 5000	67	Hili et al. (1997)

TABLE 12.79
Inhibitory Data of Thyme Oil Obtained in the Dilution Test

Microorganism	MO Class	Conditions	MIC	Ref.
Bacteria	Bac	5% Glucose, 9 d, 37	500–1000	Buchholtz (1875)
Acinetobacter baumannii	Bac–	MHA, Tween 20, 48 h, 35°C	1200	Hammer et al. (1999)
Aeromonas sobria	Bac–	MHA, Tween 20, 48 h, 35°C	1200	Hammer et al. (1999)
Bordetella bronchiseptica	Bac–	Cited	250	Pellecuer et al. (1976)
Campylobacter jejuni	Bac–	TSB, 24 h, 42°C	400	Smith-Palmer et al. (1998)
Enterococcus faecalis	Bac–	M17, 24 h, 20°C	>10,000	Di Pasqua et al. (2005)
Escherichia coli	Bac–	MHA, Tween 20, 48 h, 35°C	1200	Hammer et al. (1999)
Escherichia coli	Bac–	TGB, 18 h, 37°C	1500	Schelz et al. (2006)
Escherichia coli	Bac–	TSB, Tween 80, 48 h, 35°C	2	Panizzi et al. (1993)
Escherichia coli	Bac–	NB, 16 h, 37°C	200	Lens-Lisbonne et al. (1987)
Escherichia coli	Bac–	TSB, 3% EtOH, 24 h, 37°C	3000–8000	Rota et al. (2004)
Escherichia coli	Bac–	LA, 18 h, 37°C	375–500	Remmal et al. (1993)
Escherichia coli	Bac–	NB, 24 h, Tween 20, 37	400	Fawzi (1991)
Escherichia coli	Bac–	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Escherichia coli	Bac–	Cited	500	Pellecuer et al. (1976)
Escherichia coli	Bac–	TSB, 24 h, 35°C	500	Smith-Palmer et al. (1998)
Escherichia coli	Bac–	MHB, DMSO, 24 h, 37°C	62.5	Al-Bayati (2008)
Escherichia coli	Bac–	TSB, 24 h, 37°C	700	Di Pasqua et al. (2005)
Escherichia coli	Bac–	MPB, DMSO, 40 h, 30°C	75% inh. 500	Hili et al. (1997)
Escherichia coli	Bac–	NA, 1–3 d, 30°C	750	Farag et al. (1989)
Escherichia coli	Bac–	MHB, 24 h, 36°C	>10,000	Oussalah et al. (2006)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	>8000	Oussalah et al. (2006)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Escherichia coli O157:H7	Bac–	BHI, 48 h, 35°C	8000	Oussalah et al. (2006)
Haemophilus influenza	Bac–	Cited	1000	Pellecuer et al. (1976)
Helicobacter pylori	Bac–	Cited, 20 h, 37°C	275.2	Weseler et al. (2005)

continued

TABLE 12.79 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Klebsiella pneumoniae	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Klebsiella pneumoniae	Bac–	MHB, DMSO, 24 h, 37°C	500	Al-Bayati (2008)
Klebsiella pneumoniae	Bac–	Cited	5000	Pellecuer et al. (1976)
Moraxella glucidolytica	Bac–	Cited	1000	Pellecuer et al. (1976)
Neisseria catarrhalis	Bac–	Cited	125	Pellecuer et al. (1976)
Neisseria flava	Bac–	Cited	500	Pellecuer et al. (1976)
Proteus mirabilis	Bac–	MHB, DMSO, 24 h, 37°C	62.5	Al-Bayati (2008)
Proteus vulgaris	Bac–	MHB, DMSO, 24 h, 37°C	31.2	Al-Bayati (2008)
Pseudomonas aeruginosa	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Pseudomonas aeruginosa	Bac–	TSB, Tween 80, 48 h, 35°C	>40	Panizzi et al. (1993)
Pseudomonas aeruginosa	Bac–	MHB, DMSO, 24 h, 37°C	>500	Al-Bayati (2008)
Pseudomonas aeruginosa	Bac–	NB, 24 h, Tween 20, 37°C	>50,000	Fawzi (1991)
Pseudomonas aeruginosa	Bac–	MPB, DMSO, 40 h, 30°C	77% inh. 500	Hili et al. (1997)
Pseudomonas aeruginosa	Bac–	NB, 16 h, 37°C	800	Lens-Lisbonne et al. (1987)
Pseudomonas fluorescens	Bac–	NA, 1–3 d, 30°C	1000	Farag et al. (1989)
Pseudomonas sp.	Bac–	TSB, 24 h, 37°C	1500	Di Pasqua et al. (2005)
Salmonella enteritidis	Bac–	TSB, 24 h, 35°C	400	Smith-Palmer et al. (1998)
Salmonella enteritidis	Bac–	TSB, 3% EtOH, 24 h, 37°C	4000–8000	Rota et al. (2004)
Salmonella haddar	Bac–	LA, 18 h, 37°C	500	Remmal et al. (1993)
Salmonella typhi	Bac–	MHB, DMSO, 24 h, 37°C	250	Al-Bayati (2008)
Salmonella typhimurium	Bac–	MHA, Tween 20, 48 h, 35°C	>20,000	Hammer et al. (1999)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	MHB, DMSO, 24 h, 37°C	125	Al-Bayati (2008)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	TSB, 24 h, 37°C	300	Di Pasqua et al. (2005)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	4000	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	BHI, 48 h, 35°C	500	Oussalah et al. (2006)
Salmonella typhimurium	Bac–	TSB, 3% EtOH, 24 h, 37°C	5000	Rota et al. (2004)
Serratia marcescens	Bac–	NA, 1–3 d, 30°C	1250	Farag et al. (1989)

Serratia marcescens	Bac–	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Shigella flexneri	Bac–	TSB, 3% EtOH, 24 h, 37°C	3000–8000	Rota et al. (2004)
Yersinia enterocolitica	Bac–	TSB, 3% EtOH, 24 h, 29°C	3000–5000	Rota et al. (2004)
Bacillus cereus	Bac+	NB, 24 h, Tween 20, 37°C	>50,000	Fawzi (1991)
Bacillus cereus	Bac+	MHB, DMSO, 24 h, 37°C	15.6	Al-Bayati (2008)
Bacillus megaterium	Bac+	LA, 18 h, 37°C	375–500	Remmal et al. (1993)
Bacillus subtilis	Bac+	TSB, Tween 80, 48 h, 35°C	2	Panizzi et al. (1993)
Bacillus subtilis	Bac+	NA, 1–3 d, 30°C	250	Farag et al. (1989)
Bacillus subtilis	Bac+	Cited	500	Pellecuer et al. (1976)
Brochotrix thermosphacta	Bac+	M17, 24 h, 20°C	2500	Di Pasqua et al. (2005)
Corynebacterium pseudodiphtheriae	Bac+	Cited	125	Pellecuer et al. (1976)
Corynebacterium sp.	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Enterococcus faecalis	Bac+	MHA, Tween 20, 48 h, 35°C	5000	Hammer et al. (1999)
Enterococcus faecium VRE	Bac+	HIB, Tween 80, 18 h, 37°C	500–10,000	Nelson (1997)
Lactobacillus delbrueckii	Bac+	MRS, 24 h, 37°C	>10,000	Di Pasqua et al. (2005)
Lactobacillus plantarum	Bac+	MRS, 24 h, 30°C	>10,000	Di Pasqua et al. (2005)
Lactobacillus sp.	Bac+	MRS, cited	310–620	Pellecuer et al. (1980)
Lactococcus garvieae	Bac+	M17, 24 h, 20°C	>10,000	Di Pasqua et al. (2005)
Lactococcus lactis subsp. *lactis*	Bac+	M17, 24 h, 20°C	>10,000	Di Pasqua et al. (2005)
Listeria monocytogenes	Bac+	TSB, 3% EtOH, 24 h, 37°C	<1000–5000	Rota et al. (2004)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	>8000	Oussalah et al. (2006)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	>8000	Oussalah et al. (2006)
Listeria monocytogenes	Bac+	TSB, 24 h, 37°C	1000	Di Pasqua et al. (2005)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Listeria monocytogenes	Bac+	TSB, 24 h, 35°C	200	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	TSB, 10 d, 4°C	200	Smith-Palmer et al. (1998)
Listeria monocytogenes	Bac+	BHI, 48 h, 35°C	2000	Oussalah et al. (2006)
Micrococcus flavus	Bac+	Cited	500	Pellecuer et al. (1976)
Micrococcus luteus	Bac+	MHB, cited	150	Pellecuer et al. (1980)
Micrococcus sp.	Bac+	NA, 1–3 d, 30°C	250	Farag et al. (1989)
Micrococcus ureae	Bac+	MHB, cited	150	Pellecuer et al. (1980)

continued

TABLE 12.79 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Mycobacterium phlei	Bac+	NA, 1–3 d, 30°C	125	Farag et al. (1989)
Sarcina lutea	Bac+	Cited	250	Pellecuer et al. (1976)
Sarcina sp.	Bac+	NA, 1–3 d, 30°C	125	Farag et al. (1989)
Sarcina ureae	Bac+	MHB, cited	78	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	TSB, 3% EtOH, 24 h, 37°C	<1000–5000	Rota et al. (2004)
Staphylococcus aureus	Bac+	Cited	1000	Pellecuer et al. (1976)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	1000	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	NB, 16 h, 37°C	150	Lens-Lisbonne et al. (1987)
Staphylococcus aureus	Bac+	TSB, 24 h, 37°C	1700	Di Pasqua et al. (2005)
Staphylococcus aureus	Bac+	TSB, 24 h, 35°C	200	Smith-Palmer et al. (1998)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	250	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	250	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	MHA, Tween 20, 48 h, 35°C	2500	Hammer et al. (1999)
Staphylococcus aureus	Bac+	MHB, DMSO, 24 h, 37°C	31.2	Al-Bayati (2008)
Staphylococcus aureus	Bac+	NB, 24 h, Tween 20, 37°C	400	Fawzi (1991)
Staphylococcus aureus	Bac+	BHI, 48 h, 35°C	4000	Oussalah et al. (2006)
Staphylococcus aureus	Bac+	TSB, Tween 80, 48 h, 35°C	5	Panizzi et al. (1993)
Staphylococcus aureus	Bac+	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Staphylococcus aureus	Bac+	NA, 1–3 d, 30°C	500	Farag et al. (1989)
Staphylococcus aureus	Bac+	LA, 18 h, 37°C	500	Remmal et al. (1993)
Staphylococcus aureus	Bac+	MPB, DMSO, 40 h, 30°C	95% inh. 500	Hili et al. (1997)
Staphylococcus aureus MRSA	Bac+	HIB, Tween 80, 18 h, 37°C	5000	Nelson (1997)
Staphylococcus epidermidis	Bac+	TGB, 18 h, 37°C	1500	Schelz et al. (2006)
Staphylococcus epidermidis	Bac+	Cited	500	Pellecuer et al. (1976)
Staphylococcus epidermidis	Bac+	MHB, cited	620	Pellecuer et al. (1980)
Streptococcus D	Bac+	MHB, cited	78–620	Pellecuer et al. (1980)
Streptococcus faecalis	Bac+	NB, 16 h, 37°C	150	Lens-Lisbonne et al. (1987)
Streptococcus pyogenes	Bac+	Cited	250	Pellecuer et al. (1976)
Absidia glauca	Fungi	Cited	500	Pellecuer et al. (1976)
Alternaria alternata	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	5000	Tullio et al. (2006)

Alternaria alternata	Fungi	PDA, 7 d, 28°C	62% inh. 500	Feng and Zheng (2007)
Alternaria citri	Fungi	PDA, 8 d, 22°C	500	Arras and Usai (2001)
Aspergillus chevalieri	Fungi	Cited	250	Pellecuer et al. (1976)
Aspergillus clavatus	Fungi	Cited	1000	Pellecuer et al. (1976)
Aspergillus flavus	Fungi	Cited	1000	Pellecuer et al. (1976)
Aspergillus flavus	Fungi	PDA, 7–14 d, 28°C	250	Soliman and Badeaa (2002)
Aspergillus flavus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Aspergillus flavus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Aspergillus flavus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus flavus	Fungi	CA, 7 d, 28°C	74–76% inh. 500	Kumar et al. (2007)
Aspergillus flavus var. *columnaris*	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Aspergillus fumigatus	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Aspergillus giganteus	Fungi	Cited	1000	Pellecuer et al. (1976)
Aspergillus niger	Fungi	Cited	1000	Pellecuer et al. (1976)
Aspergillus niger	Fungi	SB, 72 h, Tween 20, 37°C	200	Fawzi (1991)
Aspergillus niger	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500–5000	Tullio et al. (2006)
Aspergillus niger	Fungi	YES broth, 10 d	–96% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus ochraceus	Fungi	PDA, 7–14 d, 28°C	500	Soliman and Badeaa (2002)
Aspergillus ochraceus	Fungi	YES broth, 10 d	–92% inh. 10,000	Lis-Balchin et al. (1998)
Aspergillus oryzae	Fungi	Cited	2000	Pellecuer et al. (1976)
Aspergillus parasiticus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Aspergillus parasiticus	Fungi	PDA, 7–14 d, 28°C	500	Soliman and Badeaa (2002)
Aspergillus parasiticus	Fungi	PDA, 8 h, 20°C, spore germ. inh.	50–100	Thompson (1986)
Aspergillus repens	Fungi	Cited	250	Pellecuer et al. (1976)
Botrytis cinera	Fungi	PDA, 8 d, 22°C	500	Arras and Usai (2001)
Cladosporium cladosporoides	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250–2500	Tullio et al. (2006)
Cladosporium herbarum	Fungi	Cited	1000	Pellecuer et al. (1976)
Epidermophyton floccosum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Epidermophyton floccosum	Fungi	Cited	1000	Pellecuer et al. (1976)
Epidermophyton floccosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	625	Tullio et al. (2006)
Fusarium culmorum	Fungi	YES broth, 10 d	–86% inh. 10,000	Lis-Balchin et al. (1998)
Fusarium moniliforme	Fungi	PDA, 7–14 d, 28°C	250	Soliman and Badeaa (2002)
Fusarium oxysporum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250	Tullio et al. (2006)

continued

TABLE 12.79 (continued)

Microorganism	MO Class	Conditions	MIC	Ref.
Microsporum canis	Fungi	MBA, Tween 80, 10 d, 30°C	12.5– >300	Perrucci et al. (1994)
Microsporum canis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250–2500	Tullio et al. (2006)
Microsporum gypseum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	1250–2500	Tullio et al. (2006)
Microsporum gypseum	Fungi	MBA, Tween 80, 10 d, 30°C	25– >300	Perrucci et al. (1994)
Mucor hiemalis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Mucor mucedo	Fungi	PDA, 5 d, 27°C	100	Thompson and Cannon (1986)
Mucor mucedo	Fungi	Cited	1000	Pellecuer et al. (1976)
Mucor racemosus f. *racemosus*	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Mucor sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Penicillium chrysogenum	Fungi	Cited	1000	Pellecuer et al. (1976)
Penicillium digitatum	Fungi	PDA, 8 d, 22°C	1000	Arras and Usai (2001)
Penicillium frequentans	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Penicillium italicum	Fungi	PDA, 8 d, 22°C	1000	Arras and Usai (2001)
Penicillium lanosum	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Penicillium liliacinum	Fungi	Cited	500	Pellecuer et al. (1976)
Penicillium rubrum	Fungi	Cited	1000	Pellecuer et al. (1976)
Rhizopus 66-81-2	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus arrhizus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus chinensis	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus circinans	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus japonicus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus kazanensis	Fungi	PDA, 5 d, 27°C	1000	Thompson and Cannon (1986)
Rhizopus nigricans	Fungi	Cited	500	Pellecuer et al. (1976)
Rhizopus oryzae	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus pymacus	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus sp.	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	>10,000	Tullio et al. (2006)
Rhizopus stolonifer	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Rhizopus tritici	Fungi	PDA, 5 d, 27°C	500	Thompson and Cannon (1986)
Scopulariopsis brevicaulis	Fungi	Cited	1000	Pellecuer et al. (1976)
Scopulariopsis brevicaulis	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	10,000	Tullio et al. (2006)
Syncephalastrum racemosum	Fungi	Cited	500	Pellecuer et al. (1976)

Trichophyton interdigitale	Fungi	Cited	1000	Pellecuer et al. (1976)
Trichophyton mentagrophytes	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Trichophyton mentagrophytes	Fungi	RPMI, 1.5% EtOH, 7 d, 30°C	2500	Tullio et al. (2006)
Trichophyton rubrum	Fungi	SA, Tween 80, 21 d, 20°C	<300	Janssen et al. (1988)
Candida albicans	Yeast	SDB, Tween 80, 48 h, 35°C	1	Panizzi et al. (1993)
Candida albicans	Yeast	MPB, DMSO, 40 h, 30°C	500	Hili et al. (1997)
Candida albicans	Yeast	MHA, Tween 20, 48 h, 35°C	1200	Hammer et al. (1999)
Candida albicans	Yeast	NB, 24 h, Tween 20, 37°C	200	Fawzi (1991)
Candida albicans	Yeast	Cited	2000	Pellecuer et al. (1976)
Candida albicans	Yeast	Cited, 48 h, 36°C	2000	Duarte et al. (2005)
Candida albicans	Yeast	TGB, 18–24 h, 37°C	500	Morris et al. (1979)
Candida albicans	Yeast	RPMI, DMSO, 48 h, 35°C	7.5–10.5	12,166
Candida guilliermondii	Yeast	RPMI, DMSO, 48 h, 35°C	7.5	12,166
Candida mycoderma	Yeast	Cited	2000	Pellecuer et al. (1976)
Candida paraspilosis	Yeast	Cited	4000	Pellecuer et al. (1976)
Candida pelliculosa	Yeast	Cited	1000	Pellecuer et al. (1976)
Candida tropicalis	Yeast	Cited	4000	Pellecuer et al. (1976)
Candida tropicalis	Yeast	RPMI, DMSO, 48 h, 35°C	8.5	12,166
Candida utilis	Yeast	RPMI, DMSO, 48 h, 35°C	7.5	12,166
Geotrichum asteroides	Yeast	Cited	2000	Pellecuer et al. (1976)
Geotrichum candidum	Yeast	Cited	2000	Pellecuer et al. (1976)
Hansenula sp.	Yeast	Cited	2000	Pellecuer et al. (1976)
Saccharomyces carlsbergensis	Yeast	Cited	2000	Pellecuer et al. (1976)
Saccharomyces cerevisiae	Yeast	MPB, DMSO, 40 h, 30°C	500	Hili et al. (1997)
Saccharomyces cerevisiae	Yeast	SDB, Tween 80, 48 h, 35°C	2	Panizzi et al. (1993)
Saccharomyces cerevisiae	Yeast	YPB, 24 h, 20°C	400–700	Schelz et al. (2006)
Saccharomyces cerevisiae	Yeast	NA, 1–3 d, 30°C	500	Farag et al. (1989)
Schizosaccharomyces pombe	Yeast	MPB, DMSO, 40 h, 30°C	500	Hili et al. (1997)
Torula utilis	Yeast	MPB, DMSO, 40 h, 30°C	500	Hili et al. (1997)

Annotation: (1) *Thymus vulgaris carvacroliferum*, (2) *Thymus vulgaris linaloliferum*, (3) *Thymus vulgaris thuyanoliferum*, and (4) *Thymus vulgaris thymoliferum.*

TABLE 12.80
Inhibitory Data of Thyme Oil Obtained in the Vapor Phase Test

Microorganism	MO Class	Conditions		Activity	Ref.
Escherichia coli	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Escherichia coli	Bac–	BLA, 18 h, 37°C	MIC_{air}	12.5	Inouye et al. (2001)
Haemophilus influenzae	Bac–	MHA, 18 h, 37°C	MIC_{air}	3.13	Inouye et al. (2001)
Neisseria sp.	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Salmonella typhi	Bac–	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Bacillus megaterium	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Bacillus subtilis var. *aterrimus*	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Corynebacterium diphtheriae	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Lactobacillus sp.	Bac+	MRS, cited	Disk, 20,000 ?	++	Pellecuer et al. (1980)
Micrococcus luteus	Bac+	MHB, cited	Disk, 20,000 ?	++	Pellecuer et al. (1980)
Micrococcus ureae	Bac+	MHB, cited	Disk, 20,000 ?	++	Pellecuer et al. (1980)
Mycobacterium avium	Bac+	NA, 24 h, 37°C	sd	+	Maruzzella and Sicurella (1960)
Sarcina ureae	Bac+	MHB, cited	Disk, 20,000 ?	+	Pellecuer et al. (1980)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Staphylococcus aureus	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Staphylococcus aureus	Bac+	MHA, 18 h, 37°C	MIC_{air}	6.25–12.5	Inouye et al. (2001)
Staphylococcus epidermidis	Bac+	MHB, cited	Disk, 20,000 ?	++	Pellecuer et al. (1980)
Streptococcus D	Bac+	MHB, cited	Disk, 20,000 ?	++	Pellecuer et al. (1980)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus faecalis	Bac+	NA, 24 h, 37°C	sd	++	Maruzzella and Sicurella (1960)
Streptococcus pneumoniae	Bac+	MHA, 18 h, 37°C	MIC_{air}	3.13–6.25	Inouye et al. (2001)
Streptococcus pyogenes	Bac+	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)
Streptococcus pyogenes	Bac+	MHA, 18 h, 37°C	MIC_{air}	3.13–6.25	Inouye et al. (2001)
Alternaria alternata	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78	Tullio et al. (2006)
Aspergillus flavus	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Aspergillus flavus	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Aspergillus flavus	Fungi	RPMI, 7 d, 30°C	MIC_{air}	156–312	Tullio et al. (2006)

Aspergillus fumigatus	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78–312	Tullio et al. (2006)
Aspergillus niger	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Aspergillus niger	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78–156	Tullio et al. (2006)
Botrytis cinera	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Cladosporium cladosporoides	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78	Tullio et al. (2006)
Colletotrichum gleosporoides	Fungi	PDA, 3 d, 25°C	1000	+	Lee et al. (2007)
Endomyces filbuliger	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Eurotium amstelodami	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium herbarum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium repens	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Eurotium repens	Fungi	Bread, 14 d, 25°C	30,000	NG	Suhr and Nielsen (2003)
Eurotium rubrum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Fusarium oxysporum	Fungi	PDA, 3 d, 25°C	1000	+	Lee et al. (2007)
Fusarium oxysporum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	156	Tullio et al. (2006)
Microsporum canis	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78–156	Tullio et al. (2006)
Microsporum gypseum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78	Tullio et al. (2006)
Mucor sp.	Fungi	RPMI, 7 d, 30°C	MIC_{air}	156	Tullio et al. (2006)
Penicillium corylophilum	Fungi	WFA, 42 d, 25°C	Disk, 50,000	NG	Guynot et al. (2003)
Penicillium corylophilum	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Penicillium frequentans	Fungi	RPMI, 7 d, 30°C	MIC_{air}	156	Tullio et al. (2006)
Penicillium lanosum	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312	Tullio et al. (2006)
Penicillium roqueforti	Fungi	Bread, 14 d, 25°C	30,000	+++	Suhr and Nielsen (2003)
Pythium ultimum	Fungi	PDA, 3 d, 25°C	1000	+++	Lee et al. (2007)
Rhizoctonia solani	Fungi	PDA, 3 d, 25°C	1000	+	Lee et al. (2007)
Rhizopus sp.	Fungi	RPMI, 7 d, 30°C	MIC_{air}	312	Tullio et al. (2006)
Scopulariopsis brevicaulis	Fungi	RPMI, 7 d, 30°C	MIC_{air}	78	Tullio et al. (2006)
Trichophyton mentagrophytes	Fungi	RPMI, 7 d, 30°C	MIC_{air}	39–78	Tullio et al. (2006)
Candida albicans	Yeast	NA, 24 h, 37°C	~20,000	NG	Kellner and Kober (1954)

12.2 RESULTS

The results of antimicrobial testing of the 28 essential oils listed in the European Pharmacopoeia 6th edition are shown in Tables 12.1 through 12.80. Although literature is not fully covered, the quantity of available information was in part unexpected.

Anise Oil, *Anisi aetheroleum*

Definition: Essential oil obtained by steam distillation of the dry ripe fruits of *Pimpinella anisum* L.

Bitter-Fennel Fruit Oil, *Foeniculi amari fructus aetheroleum*

Definition: Essential oil obtained by steam distillation of the ripe fruits of *Foeniculum vulgare* Miller, ssp. *vulgare* var. *vulgare*.

Content: fenchone: 12–25%, *trans*-anethole: 55–75%.

Caraway Oil, *Carvi aetheroleum*

Definition: Essential oil obtained by steam distillation of the dry fruits of *Carum carvi* L.

Cassia Oil, *Cinnamomi cassia aetheroleum*

Definition: Cassia oil is obtained by steam distillation of the leaves and young branches of *Cinnamomum cassia* Blume (*Cinnamomum aromaticum* Nees).

Ceylon Cinnamon Bark Oil, *Cinnamomi zeylanicii corticis aetheroleum*

Definition: Ceylon cinnamon bark oil is obtained by steam distillation of the bark of the shoots of *Cinnamomum zeylanicum* Nees (*Cinnamomum verum* J.S. Presl.).

Ceylon Cinnamon Leaf Oil, *Cinnamomi zeylanici folii aetheroleum*

Definition: Essential oil obtained by steam distillation of the leaves of *Cinnamomum verum* J.S. Presl.

Citronella Oil, *Citronellae aetheroleum*

Definition: Essential oil obtained by steam distillation of the fresh or partially dried aerial parts of *Cymbopogon winterianus* Jowitt.

Clary Sage Oil, *Salviae sclaerae aetheroleum*

Definition: Essential oil obtained by steam distillation of the fresh or dried flowering stems of *Salvia sclarea* L.

Clove Oil, *Caryophylli floris aetheroleum*

Definition: Clove oil is obtained by steam distillation of the dried flower buds of *Syzygium aromaticum* (L.) Merill et L. M. Perry (*Eugenia caryophyllus* C. Spreng. Bull. et Harr.).

Coriander Oil, *Coriandri aetheroleum*

Definition: Essential oil obtained by steam distillation of the fruits of *Coriandrum sativum* L.

Dwarf-Pine Oil, *Pini pumilionis aetheroleum*

Definition: Essential oil obtained by steam distillation of the fresh needles, twigs, and branches of *Pinus mugo* ssp. *mugo* ZENARI and/or *Pinus mugo* ssp. *pumilio* (HAENKE) FRANCO.

Pinus mugo Turra [*Pinus mugo* var. *pumilio* (Haenke) Zenari], plant part: leaves and twigs.

Eucalyptus Oil, *Eucalypti aetheroleum*

Definition: Eucalyptus oil is obtained by steam distillation and rectification of the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole. The species mainly used are *Eucalyptus globulus* Labill., *Eucalyptus polybractea* R.T. Baker, and *Eucalyptus smithii* R.T. Baker.

Juniper Oil, *Juniperi aetheroleum*

Definition: Essential oil obtained by steam distillation of the ripe, nonfermented berry cones of *Juniperus communis* L.

Lavender Oil, *Lavandulae aetheroleum*

Definition: Essential oil obtained by steam distillation of the flowering tops of *Lavandula angustifolia* Miller (*Lavandula officinalis* Chaix).

Lemon Oil, *Limonis aetheroleum*

Definition: Essential oil obtained by suitable mechanical means, without the aid of heat, from the fresh peel of *Citrus limon* (L.) Burman fil.

Mandarin Oil, *Citri reticulatae aetheroleum*

Definition: Essential oil obtained without heating, by suitable mechanical treatment of the fresh peel of the fruit of *Citrus reticulata* Blanco var. mandarin.

Matricaria Oil, *Matricariae aetheroleum*

Definition: Blue essential oil obtained by steam distillation of the fresh or dried flower heads or flowering tops of *Matricaria recutita* L. (*Chamomilla recutita* L. Rauschert). There are two types of matricaria oils, which are characterized as rich in bisabolol oxides or rich in (–)-α-bisabolol.

Mint Oil, *Menthae arvensis aetheroleum partim menthol privum*

Definition: Essential oil obtained by steam distillation of the fresh, flowering aerial parts, recently gathered from *Mentha canadensis* L. [syn. *Mentha arvensis* L. var. *glabrata* (Benth) Fern., *Mentha arvensis* var. *piperascens* Malinv. ex Holmes], followed by partial separation of menthol by crystallization.

Neroli Oil, *Neroli aetheroleum*

Definition: Neroli oil is obtained by steam distillation of the fresh flowers of *Citrus aurantium* L. subsp. *aurantium* L. (*Citrus aurantium* L. subsp. *amara* Engl.).

Nutmeg Oil, *Myristicae fragrantis aetheroleum*

Definition: Nutmeg oil is obtained by steam distillation of the dried and crushed kernels of *Myristica fragrans* Houtt.

Peppermint Oil, *Menthae piperitae aetheroleum*

Definition: Essential oil obtained by steam distillation of the fresh aerial parts of the flowering plant of *Mentha piperita* L.

Pinus sylvestris Oil, *Pini sylvestris aetheroleum*

Definition: Essential oil obtained by steam distillation of the fresh needles, twigs, and branches of *Pinus sylvestris* L.

Turpentine Oil, *Pinus pinaster* Type, *Terebinthi aetheroleum ab pinum pinastrum*

Definition: Essential oil obtained by steam distillation, followed by rectification at a temperature below 180°C, from the oleoresin obtained by tapping *Pinus pinaster* Aiton.

Rosemary Oil, *Rosmarinum aetheroleum*

Definition: Essential oil obtained by steam distillation of the flowering aerial parts of *Rosmarinus officinalis* L.

Star Anise Oil, *Anisi stellati aetheroleum*

Definition: Essential oil obtained by steam distillation of the dry ripe fruits of *Illicium verum* Hook. fil.

Sweet Orange Oil, *Aurantii dulcis aetheroleum*

Definition: Essential oil obtained without heating, by suitable mechanical treatment of the fresh peel of the fruit of *Citrus sinensis* (L.) Osbeck (*Citrus aurantium* L. var. *dulcis* L.).

Tea Tree Oil, *Melaleucae aetheroleum*

Definition: Essential oil obtained by steam distillation of the foliage and terminal branchlets of *Melaleuca alternifolia* (Maiden and Betch) Cheel, *Melaleuca linariifolia* Smith, *Melaleuca dissitiflora* F. Mueller, and/or other species of *Melaleuca*.

Thyme Oil, *Thymi aetheroleum*

Definition: Essential oil obtained by steam distillation of the fresh flowering aerial parts of *Thymus vulgaris* L., *T. zygis* Loefl. ex L. or a mixture of both species.

12.3 DISCUSSION

The *in vitro* most antimicrobially active essential oils regularly (or normally) contain substances as main components, which are themselves known to exhibit pronounced antimicrobial properties. These are cinnamic aldehyde (cinnamon bark and cassia oil) and the phenolic compounds eugenol (clove and cinnamon leaf oil) and thymol (thyme oil) (Pauli, 2001). All these essential oils reveal a broadband spectrum of activity in various *in vitro* test systems (agar diffusion, dilution, and vapor phase) due to their considerable water solubility and volatility. The evaluated antimicrobial inhibitory data of the essential oils obtained in agar dilution tests (ADT), serial dilution tests (DIL), and vapor phase (VP) tests are summarized in Table 12.81.

A few essential oils exhibit limited activities and act against a class of microorganism, for example, anise and bitter fennel oil specifically inhibit the growth of filamentous fungi (Table 12.81), or act well against microbial species belonging to an identical genus, for example, caraway oil inhibits *Trichophyton* species. Some essential oils are active only in a specific test system against a defined group of microorganism, for example, dwarf-pine oil and juniper oil preferably inhibit gram-positive bacteria in the vapor phase, but both were of low activity in the agar diffusion or dilution tests. Similarly, citronella oil was not inhibitory toward nine different fungal species in the agar dilution test, but inhibited all of them in the vapor phase test (Nakahara et al., 2003). Yeasts turned out to be susceptible toward essential oil vapors, which is in agreement to observations made with volatile esters and monoterpenes. Possibly, in yeast, the biosynthesis of chitin is inhibited by voilatile compounds emitted from plants (Pauli, 2006).

The inhibitory data itself differ considerably from each other when results of different examiners are compared as it can be seen, for example, by the activities of lemon oil toward *Escherichia coli* (5 tests inactive and 4 tests active) in the agar diffusion test (Table 12.43) or by the MIC values of rosemary oil against *Staphylococcus aureus* (Table 12.67), which cover a range from 20 to 50,000 µg/mL in nine examinations. Even if the unit of the low value of 20 was confused—this might had happened in references Panizzi et al. (1993) and Pellecuer et al. (1980)—the activity range of rosemary oil is 400–50,000 µg/mL. Taken together, exceptionally the above-mentioned relatively strong-acting essential oils, the results are not coordinated and cover frequently the complete activity spectrum from inactive to strong active (evaluation from 0 to 3 in Table 12.81). In part, the results are contradictory and no general rules can be raised from the data shown in Tables 12.1 through 12.80.

Several reasons can be made responsible for this undesirable, but at least normal situation:

- Natural variability in the composition of essential oils.
- Natural variability in the susceptibility of microorganism.
- Different parameters in microbiological testing methods.
- Unknown history of the tested essential oils: production, storage, and age.
- Unsufficient knowledge about exact phytochemical composition.

The composition of essential oils depends on several factors: plant part used, place of growth, climate, natural variation (varieties, subspecies, and chemotypes), harvesting time, production, storage conditions, and analysis parameters in compound identification. Because some of these influencing factors differ from year to year, no constant composition of an essential can be expected, even when it is grown and produced at the same place. Chemotypes possess in part a completely different composition, for example, the MICs of four thyme oil chemotypes (linalool-, thuyanol-, carvacrol, and thymol-type) toward *S. aureus* (Table 12.79) differ from 250 to 4000 µg/mL and low MIC values depended on the presence of thymol (Oussalah et al., 2006). In some literature works, the botanical description of investigated plant material is not defined exactly. The characterization of "fennel oil" is not sufficient to decide between sweet or bitter fennel. The same is true for orange oil, where sweet orange (*Citrus sinensis*) and bitter orange (*Citrus aurantium*) refer to two different botanical

TABLE 12.81
Summary of Results of Antimicrobial Activities of Essential Oils Listed in the European Pharmacopoeia 6th Edition

Essential Oil	Bac–			Bac+			Fungi			Yeast		
Test Method	**ADT**	**DIL**	**VP**	**ADT**	**DIL**	**VP**	**ADT**	**DIL**	**VP**	**ADT**	**DIL**	**VP**
Anise	0–1	0–3	0–2	0–1	0–3	0–2	2–3	0–3	0–2	0–1	1–2	0
Bitter fennel	0–2	1–2	0–3	0–2	1–3	0–2	0–2	2–3	ND	0–2	2	3
Caraway	0–1	0–3	0–3	0–1	1–3	0–3	1–3	0–3	ND	0–1	2	3
Cassia	2	2–3	1–3	2	3	1–3	3	1–3	ND	ND	3	3
Cinnamon bark	1–2	3	1–3	1–3	3	1–3	2–3	2–3	0–3	2–3	2–3	3
Cinnamon leaf	2–3	2	3	2–3	2	3	3	1–3	0–3	2	2	3
Citronella	0–1	1–2	0–3	0–1	2–3	0–3	1–3	1–3	3	1–2	1–3	3
Clary sage	0–1	0–1	0–3	1–2	0–3	0–3	0–1	0–2	0	0	0–2	0
Clove	0–2	0–3	1–3	1–3	0–3	0–3	1–3	0–3	3	1–3	0–2	3
Coriander	0–2	0–2	3	0–2	1–3	0–3	0–2	0–3	ND	0–2	2	3
Dwarf pine	0–1	ND	0–3	0–1	ND	2–3	0	ND	ND	0–1	0	1
Eucalyptus	0–1	0–1	0–3	0–1	1	0–3	0–1	0–2	0	1	2	3
Juniper	0–1	0–2	1–3	0–1	0	2–3	0–1	0–2	0	0–1	2	3
Lavender	0–1	0–3	0–3	0–3	1–3	0–3	0–2	0–3	3	0–1	2	3
Lemon	0–1	0–1	0–3	0–1	0–2	0–3	0–1	0–3	0–1	0–1	2	3
Mandarin	0–1	0–2	ND	0–1	0–1	ND	1	ND	0–2	0–1	2	ND
Matricaria	0–1	0–3	0	0–1	0–3	1–2	0–1	0–2	0	0–1	0–2	0
Mint	0–3	2–3	ND	0–1	2–3	ND	0–3	2	ND	1	2	ND
Neroli	0–1	0	0–3	0–1	1–2	0–3	1–2	1–3	ND	0–1	2	3
Nutmeg	0–1	0–2	0	0–1	0–3	0–1	0–3	0–3	ND	0–1	2	ND
Peppermint	0–1	0–3	0–3	0–1	1–3	2–3	0–3	0–3	1–3	0–1	2–3	2
Pinus sylvestris	0–1	0–1	ND	1	0–2	ND	1	0–2	0	1	1–2	ND
Pinus pinaster	0–1	0–2	1–3	0–1	2	1–3	0–1	2	ND	0–1	0–3	2
Rosemary	0–1	0–3	0–3	0–2	0–3	0–3	0–2	0–2	0–3	0–1	0–2	3
Star anise	0–1	ND	2–3	0–1	ND	2–3	ND	ND	0–3	0–1	2–3	3
Sweet orange	0–2	0–3	0–3	0–2	0–2	0–3	0–2	0–3	0–3	0–1	2	3
Tea tree	1	0–3	0–3	1–2	0–3	0–3	1	1–3	0	1–3	1–3	ND
Thyme	1–2	1–3	2–3	0–3	0–3	1–3	1–3	1–3	0–3	1–3	2–3	3

Evaluation: Agar diffusion test (ADT): 0 = inactive, 1 = weak (most of the inhibition zones up to ~15 mm), 2 = moderate (most of the inhibition zones between 16 and 30 mm), 3 = strong inhibitory (most of the inhibition zones >30 mm); dilution test (DIL): 0 = MIC > 20,000 µg/mL, 1 = >5000–20,000 µg/mL, 2 = 500–5000 µg/mL, 3 = <500 µg/mL; vapor phase test (VP): 0 = no, 1 = weak, 2 = moderate, 3 strong growth reduction.

species. The characterization of essential oils with physical properties alone (e.g., density and solubility) is not sufficient for an unequivocal definition of its constituents. At best distillation, analysis, and pharmacological examination of an essential oil are done in a close-time relationship.

To find out the most appropriate method for antimicrobial testing of essential oils or components thereof, literature inhibitory data of eugenol against *Escherichia coli* were compared with each other and it was concluded that the variation of data as it is obtained in the serial dilution test is tolerable (MIC range from 250 to 600 µg/mL) (Pauli et al., 1996). The same is true for clove oil, which was found to be active in dilution tests against *Escherichia coli* in the range from 400 to 1250 µg/mL in seven of nine examinations (Table 12.26).

Questions concerning disinfectant activity of essential oils, for example, the minimum time needed to kill a given microbial species or the determination of microbial survivors after short time contact, are not answered by agar diffusion or dilution tests. In older literature, the killing concentration relative to phenol was determined after 15 or 30 min exposure of the respective microbials species to the compound to be tested. The so-called carboxylic acid coefficient or phenol coefficient was introduced in 1903 (Rideal et al., 1903) and was also taken for the characterization of the killing activity of essential oils toward microorganism (Martindale, 1910).

Differences in the susceptibility exist between organisms of the identical species as it was shown in experiments with three *Escherichia coli* strains. One of them was inhibited by eugenol methyl ether at a low concentration (MIC = 550 μg/mL), while two other strains tolerated still 8000 μg/mL without any visible growth reduction. Remarkably the MIC of eugenol toward all three strains was almost equal (550–600 μg/mL) (Pauli, 1994). Because these *Escherichia coli* strains never had had the opportunity to develop a specific resistance against eugenol methyl ether before, it is evident that a natural variation of susceptibility toward natural antimicrobials exists.

To improve the data situation of *in vitro* antimicrobial data of essential oils, all aforementioned biological and experimental parameters should be controlled as best as possible. An appropriate microbiological test system should be taken, which allows comparison of inhibitory data with drugs used in the therapy of human infectious diseases. Such worked out and standardized serial dilution tests (Clinical & Laboratory Standards Institute 2008) are already utilized in the examination of natural substances, for example Hostettmann et al. (1999) and Jirovetz et al. (2007). To avoid complications by strains with unknown susceptibilities toward antimicrobials and to make the results from different laboratories comparable to each other, available standard strains from collections (e.g., American Type Culture Collection, ATCC; Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ; and Institute for Fermentation Osaka, IFO) should be taken in the routine analysis of antimicrobial activities of natural compounds and essential oils. Antimicrobials tests should include a greater number of different organisms belonging to the groups: gram-positive, gram-negative bacteria, filamentous fungi, and yeasts.

Two principal reasons for performing the *in vitro* tests are as follows:

1. Identification of antimicrobially active compounds.
2. Control of microbial susceptibilities toward approved antibiotics and antimycotics.

The procedure from identification of antimicrobially active compounds to their use in humans to treat infectious diseases is a multistep pathway, which includes pharmacological (concentration of the active compound at the site of action, half-life time, serum levels, dose–response relationship, etc.) and toxicological (e.g., toxicity, allergic responses, and interactions) aspects.

Essential oils consist frequently of over 100 individual compounds, which themselves plus their metabolic transformation products cannot be followed up in the living body. This fact may explain why pharmacological studies with entire essential oils never have been in the focus of pharmaceutical research.

In animal experiments, it was demonstrated by Imura over 80 years ago that the *in vivo* protection against tuberculosis was not parallel with results *in vitro* (Table 12.82).

The superior protection of tuberculosis-infected guinea pigs by anethole and lemon oil is contradictory to their weak *in vitro* antitubercular activity. By means of these results obtained parallelly *in vivo* and *in vitro* it is obvious that *in vitro* inhibitory data alone cannot be used as an information basis for the treatment of infectious diseases in humans (Table 12.81). Therefore, the recording of so-called aromatograms with patient isolates and a greater number of essential oils—as it is common in "aromatherapy"—is critical (Anonymus, 2008; DHZ-Spektrum, 2007). The selection of the most active essential oil by using *in vitro* testing methods for the therapy of infectious diseases may have success, but at least there is no rational relationship between *in vitro* testing and *in vivo* success (Table 12.81). Besides that, many factors influence the results obtained

TABLE 12.82
Comparison of *In Vitro* Growth Inhibition of Human Type of *Mycobacterium tuberculosis* with the *In Vivo* Protection of Tuberculosis-Infected Guinea Pigs by Essential Oils and Components Thereof

	In Vivo[a]		
Test Materials	**Lymphnodes**	**Viscera**	***In Vitro*[b]**
Anethole	33	50	2500
Lemon oil	49	55	1250
Terpineol	59	57	625
Nutmeg oil, expressed	61	73	312
Geraniol	96	71	625
Eugenol	89	84	1250

Source: Imura, K., 1935. *J. Shanghai Sci. Institute/Section*, 4, 1: 235–270.

[a] Grade of tuberculous change in percent was observed after 10 weeks in lymphnodes and viscera in groups of five infected guinea pigs fed with ~250 mg/kg (recalculated from experimental section) test material per day for 1 week, respectively.

[b] Growth reduction (dry weight tubercle bacilli 5 mg) by a given concentration in µg/mL determined in glycerin-bouillon after 3 weeks incubation at 37°C.

in the agar diffusion test and may lead to wrong interpretation of the results concerning the antimicrobial strength of an essential oil.

It seems to be suitable to discuss a special status of essential oils in concern of their pharmacological activities, since essential oils cannot be followed up by classical pharmacological methods due to their complex nature. Recently, successes of wound treatment with essential oils have been demonstrated under clinical conditions, which cannot be realized with pharmaceuticals (Warnke et al., 2005). In another case, a deep infection of a hip arthoplasty was successfully treated with a pure compound occurring in matricaria oil: (–)-α-bisabolol (Pauli et al., 2007, 2009). Due to its lipophilic nature (–)-α-bisabolol is thought to be taken up by the skin and it enters the blood circulatory system, which should be measureable with pharmacological methods. Interestingly, the toxicity of essential oils toward mammalians decreases significantly with increase of average lipophilicity of their components (Pauli, 2008), while simultaneously the toxicity toward bacteria and fungi increases significantly with increasing lipophilicity (Pauli, 2007), which points to the extraordinary role of essential oils among natural compounds, especially of their highly lipophilic constituents.

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13 Aromatherapy with Essential Oils

Maria Lis-Balchin

CONTENTS

13.1 INTRODUCTION

13.1.1 Aromatherapy Practice in the United Kingdom and the United States

Aromatherapy has become more of an art than a science. This is mostly due to the health and beauty industries, which have taken over the original concept as a money-spinner in the United Kingdom, United States, and almost all other parts of the world. There are virtually thousands of "aromatherapy" products in pharmacies, high street shops, supermarkets, hair salons, and beauty salons. The products are supposedly made with "essential oils" (which are usually perfumes) and include skin creams, hair shampoos, shower gels, moisturizers, bath salts, lotions, candles, as well as essential oils themselves.

Many aromatherapy products, such as perfumes, are also linked with sexual attractiveness. There are numerous "health and beauty" salons or clinics that offer aromatherapy as part of their "treatments" together with waxing, electrolysis, massage (of various types, including "no-hands massage"), facial treatments including botox, manicures and pedicures, eyes and eyebrow shaping, ear-piercing, tanning, and makeup application. Often hundreds of these "therapies" are offered in one small shop, with aromatherapy thrown in. Most people, especially men, consider aromatherapy to be a sensual massage with some perfumes given all over the body by a young lady. This is often the case, although aromatherapy massage is often provided just on the back or even just on the face and hands for busy people. The use of pure essential oils both in such beauty massage and all the aromatherapy products on sale everywhere is very doubtful (because of the cost) but the purchaser believes the advertisements assuring pure oil usage. Beauty consultants/therapists use massage skills and a nice odor simply for relaxation; they sometimes include beautifying treatments using specific essential oils as initiated by Marguerite Maury (1989). Aromatherapy has thus become an art.

However, aromatherapists (who have studied the "science" for 3 h, a week, a year, or even did a 3-year degree) are keen to bring science into this alternative "treatment." The multitude of books written on the subject, aromatherapy journals, and the web sites all consider that there has been enough proof of the scientific merit of aromatherapy. They quote studies that have shown *no* positive or statistically significant effects as proof that aromatherapy works. The actual validity of these claims will be discussed later and several publications criticized this on scientific grounds. Aromatherapy is often combined with "counseling" by a "qualified" therapist, with no counseling qualifications. Massaging is carried out using very diluted plant essential oils (2–5 drops per 10 mL of carrier oil, such as almond oil) on the skin—that is, in almost homeopathic dilutions! But they believe that the essential oils are absorbed and go straight to the target organ where they exert the healing effect. Many aromatherapists combine their practice with cosmology, crystals, colors, music, and so on. These may also be associated with a commercial sideline in selling "own trademark" essential oils and associated items, including diffusers, scented candles, and scented jewelry.

13.2 DEFINITIONS OF AROMATHERAPY

Aromatherapy is defined as "the use of aromatic plant extracts and essential oils in massage and other treatment" (*Concise Oxford Dictionary*, 1995). However, there is no mention of massage or the absorption of essential oils through the skin and their effect on the target organ (which is the mainframe

of aromatherapy in the United Kingdom and the United States) in *Aromatherapie* (Gattefossé, 1937/1993). This was where the term "aromatherapy" was coined after all, by the "father of aromatherapy"—but was actually based on the odor of essential oils and perfumes and their antimicrobial, physiological, and cosmetological properties (Gattefossé, 1928, 1952, 1937/1993). "Pure" essential oils were of no concern to Gattefossé. Recently, definitions have begun to encompass the effects of aromatherapy on the mind as well as on the body (Lawless, 1994; Worwood, 1996, 1998; Hirsch, 1998).

13.3 INTRODUCTION TO AROMATHERAPY CONCEPTS

The original concept of modern aromatherapy was based on the assumption that the volatile, fat-soluble essential oil was equivalent in bioactivity to that of the whole plant when inhaled or massaged onto the skin. Information about the medicinal and other properties of the plants was taken from old English herbals (e.g., Culpeper, 1653), combined with some more esoteric nuances involving the planets and astrology (Tisserand, 1977).

This notion is clearly flawed. As an example, a whole orange differs from just the essential oil (extracted from the rind alone) as the water-soluble vitamins (thiamine, riboflavin, nicotinic acid, and vitamins C and A) are excluded, as are calcium, iron, proteins, carbohydrates, and water. Substantial differences in bioactivity are found in different fractions of plants, for example, the essential oils of *Pelargonium* species produced a consistent relaxation of the smooth muscle of the guinea pig *in vitro*, whereas the water-soluble extracts did not (Lis-Balchin, 2002b). Botanical misinterpretations are also common in many aromatherapy books, for example, "geranium oil" bioactivity is based on Herb Robert, a hardy *Geranium* species found widely in European hedgerows, whereas geranium oil is distilled from species of the South African genus *Pelargonium* (Lis-Balchin, 2002a).

13.3.1 Aromatherapy, Aromatology, and Aromachology

Aromatherapy can now be divided into three "sciences": aromatherapy, aromatology, and aromachology.

Aromachology [coined by the Sense of Smell Institute (SSI), USA, 1982] is based on the interrelationship of psychology and odor, that is, its effect on specific feelings (e.g., relaxation, exhilaration, sensuality, happiness, and achievement) by its direct effect on the brain.

Aromatherapy is defined by the SSI as "the therapeutic effects of aromas on physical conditions (such as menstrual disorders, digestive problems, etc.) as well as psychological conditions (such as chronic depression)." The odor being composed of a mixture of fat-soluble chemicals may thus have an effect on the brain via inhalation, skin absorption, or even directly via the nose.

Aromatology is concerned with the internal use of oils (SSI). This is similar to the use of aromatherapy in most of Europe, excluding the United Kingdom; it includes the effect of the chemicals in the essential oils via oral intake, or via the anus, vagina, or any other possible opening by medically qualified doctors or at least herbalists, using essential oils as internal medicines.

There is a vast difference between aromatherapy in the United Kingdom and that in continental Europe (aromatology): the former is "alternative" while the latter is "conventional." The "alternative" aromatherapy is largely based on "healing," which is largely based on belief (Millenson, 1995; Benson and Stark, 1996; Lis-Balchin, 1997). This is credited with a substantial placebo influence. However, the placebo effect can be responsible for results in both procedures.

13.3.2 Scientifically Accepted Benefits of Essential Oils versus the Lack of Evidence for Aromatherapy

There is virtually no scientific evidence, as yet, regarding the direct action of essential oils, applied through massage on the skin, on specific internal organs—rather than through the odor pathway leading into the mid-brain's "limbic system" and then through the normal sympathetic and

parasympathetic pathways. This is despite some evidence that certain components of essential oils can be absorbed either through the skin or lungs (Buchbauer et al., 1992; Jager et al., 1992; Fuchs et al., 1997).

Many fragrances have been shown to have an effect on mood and, in general, pleasant odors generate happy memories, more positive feelings, and a general sense of well-being (Knasko et al., 1990; Knasko, 1992; Warren and Warrenburg, 1993) just like perfumes. Many essential oil vapors have been shown to depress contingent negative variation (CNV) brain waves in human volunteers and these are considered to be sedative (Torii et al., 1988). Others increase CNV and are considered stimulants (Kubota et al., 1992). An individual with anosmia showed changes in cerebral blood flow on inhaling certain essential oils, just as in people able to smell (Buchbauer et al., 1993c), showing that the oil had a positive brain effect despite the patient's inability to smell it. There is some evidence that certain essential oils (e.g., nutmeg) can lower high blood pressure (Warren and Warrenburg, 1993). Externally applied essential oils (e.g., tea tree) can reduce/eliminate acne (Bassett et al., 1990) and athlete's foot (Tong et al., 1992). This happens, however, using conventional chemical effects of essential oils rather than aromatherapy.

Most clients seeking out aromatherapy are suffering from some stress-related conditions, and improvement is largely achieved through relaxation. An alleviation of suffering and possibly pain, due to gentle massage and the presence of someone who cares and listens to the patient, could be beneficial in such cases as in cases of terminal cancer; the longer the time spent by the therapist with the patient, the stronger the belief imparted by the therapist and the greater the willingness of the patient to believe in the therapy, the greater the effect achieved (Benson and Stark, 1996). There is a need for this kind of healing contact, and aromatherapy with its added power of odor fits this niche, as the main action of essential oils is probably on the primitive, unconscious, limbic system of the brain (Lis-Balchin, 1997), which is not under the control of the cerebrum or higher centers and has a considerable subconscious effect on the person. However, as mood and behavior can be influenced by odors, and memories of past odor associations could also be dominant, aromatherapy should not be used by aromatherapists, unqualified in psychology, and so on in the treatment of Alzheimer's or other diseases of aging (Lis-Balchin, 2006).

Proven uses of essential oils and their components are found in industry, for example, foods, cosmetic products, household products, and so on. They impart the required odor or flavor to food, cosmetics and perfumery, tobacco, and textiles. Essential oils are also used in the paint industry, which capitalizes on the exceptional "cleaning" properties of certain oils. This, together with their embalming properties, suggests that essential oils are very potent and dangerous chemicals—not the sort of natural products to massage into the skin!

Why, therefore, should essential oils be of great medicinal value? They are, after all, just chemicals. However, essential oils have many functions in everyday life ranging from their use in dentistry (e.g., cinnamon and clove oils), as decongestants (e.g., *Eucalyptus globulus*, camphor, peppermint, and cajuput) to their use as mouthwashes (e.g., thyme), also external usage as hyperemics (e.g., rosemary, turpentine, and camphor) and anti-inflammatories (e.g., German chamomile and yarrow). Some essential oils are used internally as stimulants of digestion (e.g., anise, peppermint, and cinnamon) and as diuretics (e.g., buchu and juniper oils) (Lis-Balchin, 2006).

Many plant essential oils are extremely potent antimicrobials *in vitro* (Deans and Ritchie, 1987; Bassett et al., 1990; Lis-Balchin, 1995; Lis-Balchin et al., 1996; Deans, 2002). Many are also strong antioxidant agents and have recently been shown to stop some of the symptoms of aging in animals (Dorman et al., 1995a, 1995b). The use of camphor, turpentine oils, and their components as rubefacients, causing increased blood flow to a site of pain or swelling when applied to the skin, is well known and is the basis of many well-known medicaments such as Vicks VapoRub and Tiger Balm. Some essential oils are already used as orthodox medicines: peppermint oil is used for treating irritable bowel syndrome and some components of essential oils, such as pinene, limonene, camphene, and borneol, given orally have been found to be effective against certain internal ailments,

such as gallstones (Somerville et al., 1985) and ureteric stones (Engelstein et al., 1992). Many essential oils have been shown to be active on many different animal tissues *in vitro* (Lis-Balchin et al., 1997b). There are many examples of the benefits of using essential oils by topical application for acne, Alopecia areata, and Athlete's foot (discussed later in Section 13.21), but this is a treatment using chemicals rather than aromatherapy treatment.

Future scientific studies, such as those on Alzheimer's syndrome (Perry et al., 1998, 1999), may reveal the individual benefits of different essential oils for different ailments, but in practice this may not be of utmost importance as aromatherapy massage for relief from stress. Aromatherapy has had very little scientific evaluation to date. As with so many alternative therapies, the placebo effect may provide the largest percentage benefit to the patient (Benson and Stark, 1996). Many aromatherapists have not been greatly interested in scientific research and some have even been antagonistic to any such research (Vickers, 1996; Lis-Balchin, 1997). Animal experiments, whether maze studies using mice or pharmacology using isolated tissues, are considered unacceptable and only essential oils that are "untested on animals" are acceptable, despite all essential oils having been already tested on animals (denied by assurances of essential oil suppliers) because this is required by law before they can be used in foods.

The actual mode of action of essential oils *in vivo* is still far from clear, and clinical studies to date have been scarce and mostly rather negative (Stevenson, 1994; Dunn et al., 1995; Brooker et al., 1997; Anderson et al., 2000). The advent of scientific input into the clinical studies, rather than aromatherapist-led studies, has recently yielded some more positive and scientifically acceptable data (Smallwood et al., 2001; Ballard et al., 2002; Burns et al., 2000; Holmes et al., 2002; Kennedy et al., 2002). The main difficulty in clinical studies is that it is virtually impossible to do randomized double-blind studies involving different odors as it is almost impossible to provide an adequate control as this would have to be either odorless or else of a different odor, neither of which is satisfactory. In aromatherapy, as practiced, there is a variation in the treatment for each client, based on "holistic" principles, and each person can be treated by an aromatherapist with one to five or more different essential oil mixtures on subsequent visits, involving one to four or more different essential oils in each mixture. This makes scientific evaluation almost useless, as seen by studies during childbirth (Burns and Blaney, 1994; see also Section 13.19). There is also the belief among alternative medicine practitioners that if the procedure "works" in one patient, there is no need to study it using scientific double-blind procedures. There is therefore a great bias when clinical studies in aromatherapy are conducted largely by aromatherapists.

Recent European regulations (the seventh Amendment to the European Cosmetic Directive 76/768/EEC, 2002; see Appendices 27 and 28) have listed 26 sensitizers found in most of the common essential oils used: this could be a problem for aromatherapists as well as clients, both in possibly causing sensitization and also resulting in legal action regarding such an eventuality in the case of the client. Care must be taken regarding the sensitization potential of the essential oils, especially when massaging patients with cancer or otherwise sensitive skin. It should also be borne in mind when considering the use of essential oils during childbirth and in other clinical studies (Burns and Blaney, 1994; Burns et al., 2000) that studies in animals have indicated that some oils cause a decrease in uterine contractions (Lis-Balchin and Hart, 1997).

13.4 HISTORICAL BACKGROUND TO AROMATHERAPY

The advent of "aromatherapy" has been attributed to both the Ancient Egyptians and Chinese over 4500 years ago, as scented plants and their products were used in religious practices, as medicines, perfumes, and embalming agents (Manniche, 1989, 1999), and to bring out greater sexuality (Schumann Antelme and Rossini, 2001). But essential oils as such were unlikely to have been used. In Ancient Egypt, crude plant extracts of frankincense, myrrh, or galbanum, and so on were used in an oily vegetable or animal fat that was massaged onto the bodies of workers building the pyramids or the rich proletariat after their baths (Manniche, 1999). These contained essential oils, water-soluble

extractives, and pigments. Incense smoke from resinous plant material provided a more sacrosanct atmosphere for making sacrifices, both animal and human, to the gods. The incense was often mixed with narcotics like cannabis to anesthetize the sacrificial animals, especially with humans (Devereux, 1997). The frankincense extract in oils (citrusy odor) was entirely different to that burnt (church-like) in chemical composition (Arctander, 1960), and therefore would have entirely different functions.

13.4.1 Scented Plants used as Incense in Ancient Egypt

Frankincense (*Boswellia carterii*; *Boswellia thurifera*) (Burseraceae), Myrrh (*Commiphora myrrha; Balsamodendron myrrha; Balsamodendron opobalsamum*) (Burseraceae), Labdanum (*Cistus ladaniferus*), Galbanum (*Ferula galbaniflua*), Styrax (*Styrax officinalis*), or *Liquidambar orientalis*, Balm of Gilhead (*Commiphora opobalsamum*), Sandalwood (*Santalum album*), and Opoponax (*Opoponax chironium*).

Uses included various concoctions of kyphi, burnt three times a day to the sun god Ra: morning, noon, and sunset, in order for him to come back. The ingredients included raisins, juniper, cinnamon, honey, wine, frankincense, myrrh, burnt resins, cyperus, sweet rust, sweet flag, and aspalanthus in a certain secret proportion (Loret, 1887; Manniche, 1989; Forbes, 1955), as shown on the walls of the laboratory in the temples of Horus at Edfu and Philae. Embalming involved odorous plants such as juniper, cassia, cinnamon, cedarwood, and myrrh, together with natron to preserve the body and ensure safe passage to the afterlife. The bandages in which the mummy was wrapped were drenched in stacte (oil of myrrh) and sprinkled with other spices (for further descriptions and uses, see Lis-Balchin, 2006).

The Chinese also used an incense, *hsiang*, meaning "aromatic," made from a variety of plants, with sandalwood being particularly favored by Buddhists. In India, fragrant flowers including jasmine and the root of spikenard giving a sweet scent were used. The Hindus obtained cassia from China and were the first to organize trading routes to Arabia where frankincense was exclusively found. The Hebrews traditionally used incense for purification ceremonies. The use of incense probably spread to Greece from Egypt around the eighth century BC. The Indians of Mesoamerica used copal, a hard, lustrous resin, obtained from pine trees and various other tropical trees by slicing the bark (*Olibanum americanum*). Copal pellets bound to corn-husk tubes would be burnt in hollows on the summits of holy hills and mountains, and these places, blackened by centuries of such usage, are still resorted to by today's Maya in Guatemala (Janson, 1997) and used medicinally to treat diseases of the respiratory system and the skin.

Anointing also involves incense (Unterman, 1991). Queen Elizabeth II underwent the ritual in 1953 at her coronation, with a composition of oils originated by Charles I: essential oils of roses, orange blossom, jasmine petals, sesame seeds, and cinnamon combined with gum benzoin, musk, civet, and ambergris were used (Ellis, 1960). Similarly, musk, sandalwood, and other fragrances were used by the Hindus to wash the effigies of their gods, and this custom was continued by the early Christians. This probably accounts for the divine odor frequently reported when the tombs of early Christians were opened (Atchley and Cuthbert, 1909). The Christian Church was slow to adopt the use of incense until medieval times, when it was used for funerals (Genders, 1972). The reformation reversed the process as it was considered to be of pagan origin but it still survives in the Roman Catholic Church. Aromatic substances were also widely used in magic (Pinch, 1994).

13.5 PERFUME AND COSMETICS: PRECURSORS OF COSMETOLOGICAL AROMATHERAPY

The word "perfume" is derived from the Latin *per fumare*: "by smoke." The preparation of perfumes in Ancient Egypt was done by the priests, who passed on their knowledge to new priests (Manniche, 1989, 1999). Both high-class people like Nefertiti and Cleopatra used huge amounts of

fragranced materials as unguents, powders, and perfumes and the workers building the great pyramids, who even went on strike when they were denied their allocation of "aromatherapy massage oil" (Manniche, 1999).

13.5.1 Three Methods of Producing Perfumed Oils by the Egyptians

Enfleurage involved steeping the flowers or aromatics in oils or animal fats (usually goat) until the scent from the materials was imparted to the fat. The impregnated fat was often molded into cosmetic cones and used for perfuming hair wigs, worn on festive occasions, which could last for 3 days; the fat would soften and start melting, spreading the scented grease not only over the wig, but also over the clothes and body—more pleasing than the stench of stale wine, food, and excrement (Manniche, 1999).

Maceration was used principally for skin creams and perfumes: flowers, herbs, spices, or resins were chopped up and immersed in hot oils. The oil was strained and poured into alabaster (calcite) containers and sealed with wax. These scented fatty extracts were also massaged onto the skin (Manniche, 1999).

Expression involved putting flowers or herbs into bags or presses, which extracted the aromatic oils. Expression is now only used for citrus fruit oils (Lis-Balchin, 1995). Wine was often included in the process and the resulting potent liquid was stored in jars. These methods are still used today.

Megaleion, an Ancient Greek perfume described by Theophrastus who believed it to be good for wounds, was made of burnt resins and balanos oil, and boiled for 10 days before adding cassia, cinnamon, and myrrh (Groom, 1992). Rose, marjoram, sage, lotus flower, and galbanum perfumes were also made. Apart from these, aromatic oils from basil, celery, chamomile, cumin, dill, fenugreek, fir, henna, iris, juniper, lily, lotus, mandrake, marjoram, myrtle, pine, rose, rue, and sage were sometimes used in perfumes or as medicines taken internally and externally.

Dioscorides, in his *De Materia Medica*, discussed the components of perfumes and their medicinal properties, providing detailed perfume formulae. Alexandrian chemists were divided into three schools, one of which was the school of Maria the Jewess, which produced pieces of apparatus for distillation and sublimation, such as the *bain Marie*, useful for extracting the aromatic oils from plant material. Perfumes became more commonly known in medieval Europe as knights returning from the Crusades brought back musk, floral waters, and a variety of spices.

13.6 MEDICINAL USES: PRECURSORS OF AROMATOLOGY OR "CLINICAL" AROMATHERAPY

The ancient use of plants, not essential oils, can be found in fragments of Egyptian herbals. The names of various plants, their habitats, characteristics, and the purposes for which they were used are included in the following: *Veterinary papyrus* (*ca.* 2000 B.C.), *Gynaecological papyrus* (*ca.* 2000 B.C.), *Papyrus Edwin Smith* (an army surgeon's manual, *ca.* 1600 B.C.), *Papyrus Ebers* (includes remedies for health, beauty, and the home, *ca.* 1600 B.C.), *Papyrus Hearst* (with prescriptions and spells, *ca.* 1400 B.C.), and *Demotic medical papyri* (second century B.C. to first century A.D.).

Magic was often used as part of the treatment and gave the patient the expectation of a cure and thus provided a placebo effect (Pinch, 1994). The term "placing the hand" appears frequently in a large number of medical papyri; this probably alludes to the manual examination in order to reach a diagnosis but could also imply cure by the "laying on of hands," or even both (Nunn, 1997). This could be the basis of modern massage (with or without aromatherapy). It is certainly the basis of many alternative medicine practices at present (Lis-Balchin, 1997).

Plants were used in numerous ways. Onions were made into a paste with wine and inserted into the vagina to stop a woman menstruating. Garlic ointment was used to keep away serpents and

snakes, heal dog-bites, and bruises; raw garlic was given to asthmatics; fresh garlic and coriander in wine was a purgative and an aphrodisiac! Juniper mixed with honey and beer was used orally to encourage defecation; and origanum was boiled with hyssop for a sick ear (Manniche, 1989).

Egyptians also practiced inhalation by using a double-pot arrangement whereby a heated stone was placed in one of the pots and a liquid herbal remedy poured over it. The second pot, with a hole in the bottom through which a straw was inserted, was placed on top of the first pot, allowing the patient to breathe in the steaming remedy (Manniche, 1989), that is, aromatherapy by inhalation.

13.6.1 Middle Ages: Use of Aromatics and Quacks

In the twelfth century, the Benedictine Abbess Hildegard of Bingen (1098–1179) was authorized by the Church to publish her visions on medicine (*Causae et Curae*), dealing with the causes and remedies for illness (Brunn and Epiney-Burgard, 1989). The foul smell of refuse in European towns in the seventeenth century was thought to be the major cause of disease, including the plague (Classen et al., 1994), and aromatics were used for both preventing and in some cases curing diseases; herbs such as rosemary were in great demand and sold for exorbitant prices as a prophylactic against the plague (Wilson, 1925). People forced to live near victims of the plague would carry a pomander, which contained a mixture of aromatic plant extracts. Medical practitioners carried a small cassolette or "perfume box" on the top of their walking sticks, when visiting contagious patients, which was filled with aromatics (Rimmel, 1865). Some physicians wore a device filled with herbs and spices over their nose when they examined plague patients (Wilson, 1925). These became known as "beaks" and it is from this that the term "quack" developed.

Apothecaries were originally wholesale merchants and spice importers, and in 1617 the Worshipful Society of Apothecaries was formed, under the control of the London Royal College of Physicians, which produced an "official" pharmacopoeia specifying the drugs the apothecaries were allowed to dispense. The term "perfumer" occurs in some places instead of "apothecary" (Rimmel, 1865).

John Gerard (1545–1612) and Nicholas Culpeper (1616–1654) were two of the better-known apothecaries of their time. Nicholas Culpeper combined healing herbs with astrology as he believed that each plant, like each part of the body, and each disease, was governed or under the influence of one of the planets: rosemary was believed to be ruled by the Sun, lavender by Mercury, and spearmint by Venus. Culpeper also adhered to the Doctrine of Signatures, introduced by Paracelsus in the sixteenth century, and mythology played a role in many of the descriptive virtues in Culpeper's herbal. This astrological tradition is carried through by many aromatherapists today, together with other innovations such as ying and yang, crystals, and colors.

Culpeper's simple or distilled waters and oils (equivalent to the present hydrosols) were prepared by the distillation of herbs in water in a pewter still, and then fractionating them to separate out the essential or "chymical" oil from the scented plants. The plant waters were the weakest of the herbal preparations and were not regarded as being beneficial. Individual plants such as rose or elderflower were used to make the corresponding waters, or else mixtures of herbs were used to make compound waters (Culpeper, 1826/1981; Tobyn, 1997). Essential oils of single herbs were regarded by Culpeper as too strong to be taken alone, due to their vehement heat and burning, but had to be mixed with other medicinal preparations. Two or three drops were used in this way at a time. Culpeper mentioned the oils of wormwood, hyssop, marjoram, the mints, oregano, pennyroyal, rosemary, rue, sage, thyme, chamomile, lavender, orange, and lemon. Spike lavender, not *Lavandula angustifolia*, is used in aromatherapy nowadays. Herbs such as dried wormwood and rosemary were also steeped in wine and set in the sun for 30–40 days to make a "physical wine." The "herbal extracts" mentioned in the herbals were mostly water soluble and at best, alcoholic extracts, none of which are equivalent to essential oils, which contain many potent chemical components are not found in essential oils.

13.7 MODERN PERFUMERY

In the fourteenth century, alcohol was used for the extraction and preservation of plants, and oleum mirable, an alcoholic extract of rosemary and resins, was later popularized as "Hungary water," without the resins (Müller et al., 1984).

In the sixteenth century, perfumes were made using animal extracts, which were the base notes or fixatives, and made the scent last longer (Piesse, 1855). Among these ingredients were ambergris, musk, and civet.

Perfumes came into general use in England during the reign of Queen Elizabeth (1558–1603). Many perfumes, such as rose water, benzoin, and storax, were used for sweetening the heavy ornate robes of the time, which were impossible to wash. Urinals were treated with orris powder, damask rose powder, and rose water. Bags of herbs, musk, and civet were used to perfume bath water.

Elizabeth I carried a pomander filled with ambergris, benzoin, civet, damask rose, and other perfumes (Rimmel, 1865) and used a multitude of perfumed products in later life. Pomanders, from the French *pomme d'amber* ("ball of ambergris"), were originally hung in silver perforated balls from the ceiling to perfume the room. The ingredients such as benzoin, amber, labdanum, storax, musk, civet, and rose buds could be boiled with gum tragacanth and kneaded into balls; the small ones were made into necklaces.

Various recipes were used for preparing aromatic waters, oils, and perfumes. Some of these were for perfumes and some undoubtedly for alcoholic beverages, as one of the major ingredients for many concoctions was a bottle or two of wine, which when distilled produced a very alcoholic brew.

Ambergris, musk, and civet went out of fashion, as the excremental odors could not be reconciled with modesty (Corbin, 1986). The delicate floral perfumes became part of the ritual of bodily hygiene, gave greater variety, and allowed Louis XV a different perfume every day. Today the sentiment "odours are carried in bottles, for fear of annoying those who do not like them" (Dejeans, 1764) is reemerging as more and more people are becoming sensitive to odors, giving them headaches, asthma, and migraines.

The Victorians liked simple perfumes made of individual plant extracts. Particular favorites were rose, lavender, and violet. These would be steam distilled or extracted with solvents. The simple essential oils produced would often be blended together to produce perfumes like eau de Cologne (1834).

The first commercial scent production was produced in the United Kingdom, in Mitcham, Surrey, in the seventeenth century, using lavender (Festing, 1989). In 1865, cinnamaldehyde, the first synthetic, was made. Adulteration and substitution by the essential oil or component of another plant species became rampant. Aroma chemicals synthesized from coal, petroleum by-products, and terpenes are much cheaper than the equivalent plant products, so perfumes became cheap.

The way was now open for the use of scent in the modern era. It seems therefore a retrograde step to use pure essential oils in "aromatherapy," especially as the "father of aromatherapy," René-Maurice Gattefossé, used scents or deterpenated essential oils.

13.8 AROMATHERAPY PRACTICE

Aromatherapists usually treat their clients (patients) after an initial full consultation, which usually involves taking down a full medical case history. The aromatherapist then decides what treatment to give, which usually involves massage with three essential oils, often one each chosen from those with top, middle, and base perfumery notes, which balances the mixture. Sometimes only "specific" essential oils for the "disease" are used. Most aromatherapists arrange to see the client 3–5 times and the mixture will often be changed on the next visit, if not on each visit, in order to treat all the possible symptoms presented by the client (holistically), or simply as a substitute when no improvement was initially obtained. Treatment may involve other alternative medicine procedures, including chakras.

Many aromatherapists offer to treat any illness, as they are convinced that essential oils have great powers. They embark on the treatment of endometriosis, infertility, asthma, diabetes, and arthritis, even cancer, as they are convinced of the therapeutic nature of essential oils, but are often without the necessary scientific and medical knowledge. "Psychoneuroimmunology" treatment is the current buzzword.

Although aromatherapists consider themselves professionals, there is no Hippocratic oath involved. The aromatherapist, being nonmedically qualified, may not even be acquainted with most of the illnesses or symptoms, so there could be a very serious mistake made as potentially serious illnesses could be adversely affected by being "treated" by a layperson. Some, but not all, aromatherapists ask the patients to tell their doctor of the aromatherapy treatment. Counseling is greatly recommended by aromatherapy schools. Aromatherapists are not necessarily, however, trained in counseling, and with few exceptions could do more damage than good, especially when dealing with psychiatric illness, cases of physical or drug abuse, people with learning difficulties, and so on, where their "treatment" should only be complementary and under a doctor's control (Lis-Balchin, 2006).

13.8.1 Methods of Application of Aromatherapy Treatment

Various methods are used to apply the treatment in aromatherapy. The most usual methods are the following:

- A diffuser, usually powered by electricity, giving out a fine mist of the essential oil.
- A burner, with water added to the fragrance to prevent burning of the essential oil. About 1–4 drops of essential oil are added to about 10 mL water. The burner can be warmed by candles or electricity. The latter would be safer in a hospital or a children's room or even a bedroom.
- Ceramic or metal rings, placed on an electric light bulb with a drop or two of essential oil. This results in a rapid burnout of the oil and lasts for a very short time due to the rapid volatilization of the essential oil in the heat.
- A warm bath with drops of essential oil added. This results in the slow volatilization of the essential oil, and the odor is inhaled via the mouth and nose. Any effect is not likely to be through the absorption of the essential oil through the skin as stated in aromatherapy books, as the essential oil does not mix with water. Droplets either form on the surface of the water, often coalescing, or else the essential oil sticks to the side of the bath. Pouring in an essential oil mixed with milk serves no useful purpose as the essential oil still does not mix with water, and the premixing of the essential oil in a carrier oil, as for massage, just results in a nasty oily scum around the bath.
- A bowl of hot water with drops of essential oil, often used for soaking feet or used as a bidet. Again the essential oil does not mix with the water. This is, however, a useful method for inhaling essential oils in respiratory conditions and colds; the essential oil can be breathed in when the head is placed over the container and a towel placed over the head and container. This is an established method of treatment and has been used successfully with Vicks VapoRub, obas oil, and *Eucalyptus* oils for many years, so it is not surprising that it works with aromatherapy essential oils!
- Compresses using essential oil drops on a wet cloth, either hot or cold, to relieve inflammation, treat wounds, and so on. Again, the essential oil is not able to mix with the water and can be concentrated in one or two areas, making it a possible health hazard.
- Massage of hands, feet, back, or all over the body using 2–4 drops of essential oil (single essential oil or mixture) diluted in 10 mL carrier oil (fixed, oily), for example, almond oil or jojoba oil, grapeseed, wheat-germ oils, and so on. The massage applied is usually by gentle effleurage with some petrissage (kneading), with and without some shiatsu, lymph

drainage in some cases, and is more or less vigorous, according to the aromatherapist's skills and beliefs.

- Oral intake is more like conventional than "alternative" usage of essential oils. Although it is practiced by a number of aromatherapists, this is not to be condoned unless the aromatherapist is medically qualified. Essential oil drops are "mixed" in a tumbler of hot water or presented on a sugar cube or "mixed" with a teaspoonful of honey and taken internally. The inability of the essential oil to mix with aqueous solutions presents a health hazard, as do the other methods, as such strong concentrations of essential oils are involved.

13.9 MASSAGE USING ESSENTIAL OILS

The most popular method of using aromatherapy is through massage. The first written records referring to massage date back to its practice in China more than 4000 years and in Egypt. Hippocrates, the father of modern medicine, wrote, "the physician must be experienced in many things, but most assuredly in rubbing."

Massage has been used for centuries in Ayurvedic medicine in India as well as in China and shiatsu, acupressure, reflexology, and many other contemporary techniques have their roots in these sources. Massage was used for conventional therapeutic purposes in hospitals before World War II and is still used by physiotherapists for various conditions including sports injuries.

René-Maurice Gattefossé, credited as being the founding father of modern aromatherapy, never made a connection between essential oils and massage. It was Marguerite Maury who advocated the external use of essential oils combined with carrier oils (Maury, 1989). She used carefully selected essential oils for cleansing the skin, including that in acne, using a unique blend of oils for each client created specifically for the person's temperament and health situation. Maury's main focus was on rejuvenation; she was convinced that aromas could be used to slow down the aging process if the correct oils were chosen. In recent experiments on animals, it has been shown that the oral intake of some antioxidant essential oils can appear to defer aging, as indicated by the composition of membranes in various tissues (Youdim and Deans, 2000).

Massage *per se* can be a relaxing experience and can help to alleviate the stresses and strains of daily life. In a review of the literature on massage, Vickers (1996) found that in most studies massage had no psychological effect, in a few studies there was arousal, and in an even smaller number of studies there was sedation; some massage has both local and systemic effects on blood flow and possibly on lymph flow and reduction of muscle tension.

It may be that these variable responses are directly related to the variability of massage techniques, of which there are over 200. Massage can be given over the whole body or limited to the face, neck, or just hands, feet, legs—depending on the patient and his or her condition or illness, for example, patients with learning disabilities and many psychiatric patients are often only able to have limited body contact for a short time.

13.9.1 Massage Techniques

Massage is customarily defined as the manual manipulation of the soft tissues of the body for therapeutic purposes, using strokes that include gliding, kneading, pressing, tapping, and/or vibrating (Tisserand, 1977; Price and Price, 1999). Massage therapists may also cause movement within the joints, apply heat or cold, use holding techniques, and/or advise clients on exercises to improve muscle tone and range of motion. Some common massage techniques include Swedish massage, acupressure, craniosacral therapy, deep tissue massage, infant massage, lymph system massage, polarity therapy, reflexology, reiki, rolfing, shiatsu, and therapeutic touch.

Massage usually involves the use of a lubricating oil to help the practitioner's hands glide more evenly over the body. The addition of perfumed essential oils further adds to its potential to relax.

In most English-speaking countries, massage is nowadays seen as an alternative or complementary treatment. However, before World War II, it was regarded as a conventional treatment (Goldstone, 1999, 2000), as it is now in continental Europe. In Austria, for example, most patients with back pain receive (and are usually reimbursed for) massage treatment (Ernst, 2003a).

Not all massage treatments are free of risk. Too much force can cause fractures of osteoporotic bones, and even rupture of the liver and damage to nerves have been associated with massage (Ernst, 2003b). These events are rarities, however, and massage is relatively safe, provided that well-trained therapists observe the contraindications: phlebitis, deep vein thrombosis, burns, skin infections, eczema, open wounds, bone fractures, and advanced osteoporosis (Ernst et al., 2001).

It is not known exactly how massage works, although many theories abound (Vickers, 1996; Ernst et al., 2001). The mechanical action of the hands on cutaneous and subcutaneous structures enhances circulation of blood and lymph, resulting in increased supply of oxygen and removal of waste products or mediators of pain (Goats, 1994). Certain massage techniques have been shown to increase the threshold for pain (Dhondt et al., 1999). Also, most importantly from the standpoint of aromatherapy, a massage can relax the mind and reduce anxiety, which could positively affect the perception of pain (Vickers, 1996; Ernst, 2003a). Many studies have been carried out, most of which are unsatisfactory. It appears that placebo-controlled, double-blind trials may not be possible, yet few randomized clinical trials have been forthcoming.

Different client groups require proper recognition before aromatherapy trials are started or aromatherapy massage is given. For example, for cancer patients, guidelines must be observed (Wilkinson et al., 1999): special care must be taken for certain conditions such as autoimmune disease (where there are tiny bruises present); low blood cell count, which makes the patient lethargic and needing nothing more than very gentle treatment; and lymphoedema, which should not be treated unless the therapist has special knowledge and where enfleurage toward the lymph nodes should not be used.

Recent individual studies to investigate the benefit of massage for certain complaints have given variable results. Many are positive, although the standard of the studies has, in general, been poor (Vickers, 1996). The most successful applications of massage or aromatherapy massage have been in cancer care, and about a third of patients with cancer use complementary/alternative medicine during their illness (Ernst and Cassileth, 1998). Massage is commonly provided within UK cancer services (Kohn, 1999), and although only anecdotal and qualitative evidence is available, it is considered by patients to be beneficial. Only a few small-scale studies among patients with cancer have identified short-term benefits from a course of massage, mainly in terms of reduced anxiety (Corner et al., 1995; Kite et al., 1998; Wilkinson et al., 1999). These studies have been criticized by scientists; however, as they were either nonrandomized, had inadequate control groups or were observational in design (Cooke and Ernst, 2000). Complementary therapy practitioners have criticized medical research for not being sufficiently holistic in approach, focusing on efficacy of treatments in terms of tumor response and survival, rather than quality of life (Wilkinson, 2003).

A general study of the clinical effectiveness of massage by Ernst (1994) used numerous trials, with and without control groups. A variety of control interventions were used in the controlled studies including placebo, analgesics, transcutaneous electrical nerve stimulation (TENS), and so on. There were some positive effects of vibrational or manual massage, assessed as improvements in mobility, Doppler flow, expiratory volume, and reduced lymphoedema in controlled studies. Improvements in musculoskeletal and phantom limb pain, but not cancer pain, were recorded in controlled studies. Uncontrolled studies were invariably positive. Adverse effects included thrombophlebitis and local inflammation or ulceration of the skin.

Different megastudies included massage for delayed-onset muscle soreness—seven trials were included with 132 patients in total (Ernst, 1998); effleurage backrub for relaxation—nine trials were included with a total of 250 patients (Labyak and Metzger, 1997), and massage for low back pain (Ernst, 1999a, 1999b). All gave positive and negative outcomes.

13.10 AROMATHERAPY: BLENDING OF ESSENTIAL OILS

There are numerous suggestions for the use of particular essential oils for treating specific illnesses in books on aromatherapy. However, when collated, each essential oil can treat each illness (Vickers, 1996; compare also individual essential oil monographs in Lis-Balchin, 2006).

A few drops of the essential oil or oils chosen are always mixed with a carrier oil before being applied to the skin for an aromatherapy massage. The exact dilution of the essential oils in the carrier oil is often controversial and can be anything from 0.5% to 20% and more. Either 5, 10, or 20 mL of carrier oil is first poured into a (usually brown) bottle with a stoppered dropper. The essential oil is then added dropwise into the carrier oil, either as a single essential oil or as a mixture of 2–3 different essential oils, and then stoppered.

Volumes of essential oils used for dilutions vary widely in different aromatherapies and the fact that even the size of a "dropper" varies raised the question of possible safety problems (Lis-Balchin, 2006), and a recent article in a nursing journal makes a request for standardization of the measurement of the dropper size (Ollevant et al., 1999).

13.10.1 Fixed Oils

Many fixed oils are used for dilution and all provide a lubricant; many have a high vitamin E and A content. By moistening the skin, they can assist in a variety of mild skin conditions especially where the skin is rough, cracked, or dry (Healey and Aslam, 1996).

Almond (*Prunus amygdalus* var. dulcis)—sweet, cheapest, and most commonly used. Others include apricot kernel (*Prunus armeniaca*), borage seed (*Borago officinalis*), calendula (*Calendula officinalis*), coconut oil (*Cocos nucifera*), evening primrose (*Oenothera biennis)*, grapeseed (*Vitis vinifera*), macadamia nut (*Macadamia integrifolia*), olive (*Olea europaea*), rose hip seed (*Rosa mosqueta*, etc.), soya bean (*Glycine soya*), sunflower (*Helianthus annuus*), wheatgerm (*Triticum vulgare*), and jojoba (*Simmondsia californica*). The latest oil in vogue is emu oil (*Dromiceius novaehol-landiae*), which comes from a thick pad of fat on the bird's back. For centuries, the aborigines of Australia have been applying emu oil to their wounds with excellent results. It is now found in muscle pain relievers, skin care products, and natural soaps.

The exact method of mixing is controversial, but most aromatherapists are taught not to shake the bottle containing the essential oil(s) and the diluent fixed oils, but to gently mix the contents by turning the bottle in the hand. Differences in the actual odor and thereby presumable benefits of the diluted oils made by different aromatherapists can just be due to the different droppers (Lis-Balchin, 2006).

13.11 INTERNAL USAGE OF ESSENTIAL OILS BY AROMATHERAPISTS

Oral intake of essential oils is not true "aromatherapy" as the odor has virtually no effect past the mouth and the effect of the chemical components takes over as odors cannot influence the internal organs (Lis-Balchin, 1998a). Therapy with essential oils is dealt with in another chapter. Most aromatherapists consider that essential oils should only be prescribed by primary care practitioners such as medical doctors or medical herbalists who have intimate knowledge of essential oil toxicology (Tisserand and Balacs, 1995). In the United Kingdom, such "clinical aromatherapy" is rare, unlike on the continent. Maladies treated include arthritis, bronchitis, rheumatism, chilblains, eczema, high blood pressure, and venereal diseases. In clinical aromatherapy, there is a real risk of overdosage due to variable droppers on bottles, which can differ by as much as 200% (Lis-Balchin, 2006); this may be the cause of asphyxiation of a baby, as already shown by peppermint oil (Bunyan, 1998). It is possible that aromatherapists would not be covered by their insurance if there were adverse effects. However, most of us ingest small amounts of essential oils and their components daily in almost all processed foods and drinks, but it does not make us all healthy.

Conventional drugs involving essential oils and their components have been used internally for a long time, for example, decongestants containing menthol, camphor and pine, and various throat drops containing components from essential oils such as lemon, thyme, peppermint, sage, and hyssop.

Essential oils in processed foods are used in very minute amounts of 10 ppm, but can be 1000 ppm in mint confectionery or chewing gum (Fenaroli, 1997). This contrasts greatly with the use of drops of undiluted essential oils on sugar lumps for oral application, or on suppositories in anal or vaginal application. Damage to mucous membranes could result due to the high concentration of the essential oils in certain areas of the applicator.

Essential oils and their components are incorporated into enterically coated capsules to prevent damage and used for treating irritable bowel syndrome (peppermint in Colpermin), a mixture of monoterpenes for treating gallstones (Rowatol) and ureteric stones (Rowatinex); these are under product licenses as medicines (Somerville et al., 1984, 1985; Engelstein et al., 1992).

Some aromatherapists support the use of essential oils in various venereal conditions. However, aromatherapists are either qualified to treat venereal disease conditions, nor can make an accurate diagnosis in the first place, unless they are also medically qualified. Tea tree oil (2–3 drops undiluted) was used on a tampon for candidiasis with apparently very encouraging results (Zarno, 1994). *Candida* treatments also include chamomile, lavender, bergamot, and thyme (Schnaubelt, 1999). Essential oils used in this way, sometimes for months, often produced extremely painful reactions and putrid discharges due to damage to delicate mucosal membranes.

13.12 USE OF PURE OR SYNTHETIC COMPONENTS

Does it really matter whether the essential oil is pure or a synthetic mixture as long as the odor is the same? The perfumers certainly do not see any difference, and even prefer the synthetics as they remain constant. Many of the so-called pure essential oils used today are, however, adulterated (Which Report, 2001; Lis-Balchin et al., 1996, 1998). There is often a difference in the proportion of different enantiomers of individual components that often have different odors and different biological properties (Lis-Balchin, 2002a, 2002b). This was not, however, appreciated by Gattefosse (1937/1993), who worked with perfumes and not with the "pure plant essential oils" (*Formulaires de Parfumerie Gattefossé*, 1906). He studied the antimicrobial and wound-healing properties of essential oils on soldiers during World War I (Arnould-Taylor, 1981). He later worked in hospitals on the use of perfumes and essential oils as antiseptics and other (unstated) applications, and also in dermatology, which led to advances in the development of beauty products and treatments and the publication of *Physiological Aesthetics and Beauty Products* in 1936 (Gattefosse, 1992).

Gattefossé promoted the deterpenization of essential oils because, being a perfumer, he was aware that his products must be stable, have a long shelf-life, and not go cloudy when diluted in alcohol. Terpenes also oxidize rapidly, often giving rise to toxic oxidation products (e.g., limonene of citrus essential oils). But this goes against the use of pure essential oils, as their wholeness or natural synergy is apparently destroyed (Price, 1993). Bergamot and other citrus essential oils obtained by expression are therefore recommended, despite their phototoxicity (Price and Price, 1999). There is no reason why a toxic essential oil should be preferentially used if the nontoxic furanocoumarin-free (FCF) alternative is available. If adverse effects resulted, it is possible that there could be legal implications for the therapist.

13.13 THERAPEUTIC CLAIMS FOR THE APPLICATION OF ESSENTIAL OILS

There are a wide range of properties ascribed to each essential oil in aromatherapy books, without any scientific proof of effectiveness (Vickers, 1996; Lis-Balchin, 2006). The following are a few examples.

Diabetes can be treated by eucalyptus, geranium, and juniper (Tisserand, 1977); clary sage, eucalyptus, geranium, juniper, lemon, pine, red thyme, sweet thyme, vetiver, and ylang ylang (Price,

1993); eucalyptus, geranium, juniper, and onion (Valnet, 1982); and eucalyptus, geranium, cypress, lavender, hyssop, and ginger (Worwood, 1991).

Allergies can be treated by immortelle, chamomile, balm, and rose (Fischer-Rizzi, 1990); lemon balm, chamomile (German and Roman), helichrysum, true lavender, and spikenard (Lawless, 1992); and chamomile, jasmine, neroli, and rose (Price, 1983).

No botanical names are, however, given in the lists, even when there are several possible species. No indication is provided as to why these particular essential oils are used and how they are supposed to affect the condition. Taking the case of diabetes, where there is a lack of the hormone insulin, it is impossible to say how massage with any given essential oil could cure the condition, without giving the hormone itself in juvenile-type diabetes or some blood glucose-decreasing drugs in late-onset diabetes. Unfortunately, constant repetition of a given statement often lends it credence—at least to the layperson, who does not require scientific evidence of its validity.

13.13.1 False Claims Challenged in Court

The false promotion of products for treating not only medical conditions but also well-being generally is now being challenged in the law courts. For example, in 1997, Los Angeles attorney Morsé Mehrban charged that Lafabre and Aroma Vera had violated the California Business and Professions Code by advertising that their products could promote health and well-being, relax the body, relax the mind, enhance mood, purify the air, are antidotes to air pollution, relieve fatigue, tone the body, nourish the skin, promote circulation, alleviate feminine cramps, and do about 50 other things (Barrett, 2000). In September 2000, the case was settled out of court with a $5700 payment to Mehrban and a court-approved stipulation prohibiting the defendants from making 57 of the disputed claims in advertising within California (Horowitz, 2000).

13.14 PHYSIOLOGICAL AND PSYCHOLOGICAL RESPONSES TO ESSENTIAL OILS AND PSYCHOPHYSIOLOGY

Many examples of essential oil effects abound in animal studies, for example, the sedative action of lavender on the overall activity of mice decreased when exposed to lavender vapor (*Lavandula angustifolia* P. Miller); its components linalool and linalyl acetate showed a similar effect (Buchbauer et al., 1992). A possible explanation for the observed sedative effects was shown by Linalool, which produced a dose-dependent inhibition of the binding of glutamate (an excitatory neurotransmitter in the brain) to its receptors on membranes of the rat cerebral cortex (Elisabetsky et al., 1995). More recently, this action was related to an anticonvulsant activity of linalool in rats (Elisabetsky et al., 1999). Other oils with sedative activity were found to be neroli and sandalwood; active components included citronellal, phenylethyl acetate, linalool, linalyl acetate, benzaldehyde, -terpineol, and isoeugenol (in order of decreasing activity).

Stimulant oils included jasmine, patchouli, ylang ylang, basil, and rosemary; active components included fenchone, 1,8-cineole, isoborneol, and orange terpenes (Lis-Balchin, 2006). There was considerable similarity in the sedative and stimulant effects of some essential oils studied physiologically (e.g., their effect on smooth muscle of the guinea pig *in vitro*) and in various psychological assessments, mostly on humans (Lis-Balchin, 2006).

1,8-Cineole when inhaled, showed a decreased blood flow through the brain (measured using computerized tomography) although no changes were found with lavender oil or linalyl acetate (Buchbauer et al., 1993c). Changing electrical activity, picked up by scalp electrodes, in response to lavender odors was considered a measure of brain activity (EEG) (Van Toller et al., 1993). The most consistent responses to odors were in the theta band (Klemm et al., 1992). Many essential oil vapors have been shown to depress CNV brain waves (an upward shift in EEG waves that occurs when people are expecting something to happen) in human volunteers and these are considered to be sedatives; others increase CNV and are considered stimulants: lavender was found to have a sedative

effect on humans (Torii et al., 1988; Kubota et al., 1992; Manley, 1993) and had a "positive" effect on mood, EEG patterns, and maths computations (Diego et al., 1998). It also caused reduced motility in mice (Kovar et al., 1987; Ammon, 1989; Buchbauer et al., 1992, 1993a, 1993b, 1993c; Jaeger et al., 1992). However, Karamat et al. (1992) found that lavender had a stimulant effect on decision times in human experiments.

A large workplace in Japan with odorized air via the whole building showed that citrus smells refreshed the workers first thing in the morning and after the lunch break, and floral smells improved their concentration in between. In the lunch break and during late afternoon, woodland scents were circulated to relax the workers and this increased productivity (Van Toller and Dodd, 1991). It is also possible that the use of a general regime of odorants could have very negative effects on some members of the workforce or on patients in hospital wards, where the use of pleasant odors could mask the usual unpleasant odors providing the smell of fear. Ambient odors have an effect on creativity, mood, and perceived health (Knasco, 1992, 1993) and so does feigned odor (Knasco et al., 1990).

It is very difficult to make simple generalizations concerning the effects of any fragrance on psychological responses, which are based on the immediate perceptual effects, rather than the longer term pharmacological effects because the pharmacological effect is likely to affect people similarly, but the additional psychological mechanisms will create complex effects at the individual level. Odors are perceptible even during sleep, as shown in another experiment; college students were tested with fragrances during the night and the day (Badia, 1991).

Various nonscientific studies have been published in perfumery journals on the treatment of psychiatric patients by psychoaromatherapy in the 1920s (Gatti and Cajola, 1923a, 1923b, 1929; Tisserand, 1997) but there was virtually no information on their exact illnesses. Sedative essential oils or essences were identified as chamomile, melissa, neroli, petitgrain, opoponax, asafoetida, and valerian. Stimulants were angelica, cardamom, lemon, fennel, cinnamon, clove, and ylang ylang. Many aromatherapists have also written books on the effect of essential oils on the mind, giving directives for the use of specific plant oils for treating various conditions, without any scientific proof (Lawless, 1994; Worwood, 1996, 1998; Hirsch, 1998).

13.15 PLACEBO EFFECT OF AROMATHERAPY

The placebo effect is an example of a real manifestation of mind over matter. It does not confine itself to alternative therapies, but there is a greater likelihood of the placebo effect accounting for over 90% of the effect in the latter (Millenson, 1995). Reasons for the potency of the placebo effect are either the patient's belief in the method; the practitioner's belief in the method; or the patient and practitioner's belief in each other, that is, the strength of their relationship (Weil, 1983).

Placebo effects have been shown to relieve postoperative pain, induce sleep or mental awareness, bring about drastic remission in both symptoms and objective signs of chronic diseases, initiate the rejection of warts, and other abnormal growths, and so on (Weil, 1983). Placebo affects headaches, seasickness, and coughs, as well as have beneficial effects on pathological conditions such as rheumatoid and degenerative arthritis, blood cell count, respiratory rates, vasomotor function, peptic ulcers, hay fever, and hypertension (Cousins, 1979). There can also be undesirable side effects, such as nausea, headaches, skin rashes, allergic reactions, and even addiction, that is, a nocebo effect. This is almost akin to voodoo death threats or when patients are mistakenly told that their illness is hopeless—both are said to cause death soon after.

Rats were found to have increased levels of opioids in their brains after inhaling certain essential oils. Opioids are a factor in pain relief (Lis-Balchin, 1998b) and can be increased in the body by autosuggestion, relaxation, belief, and so on.

The use of aromatherapy for pain relief is best achieved through massage, personal concern and touch of the patient, and also listening to their problems. The extra benefit of real "healers" found among aromatherapists is an added advantage.

13.16 SAFETY ISSUE IN AROMATHERAPY

Many aromatherapists and laymen consider natural essential oils to be completely safe. This is based on the misconception that all herbs are safe—because they are "natural," which is a fallacy. The toxicity of essential oils can also be entirely different to that of the herb, not only because of their high concentration, but also because of their ability to pass across membranes very efficiently due to their lipophilicity.

Some aromatherapists erroneously believe that aromatherapy is self-correcting, unlike conventional therapy with medicines, and if errors are made in aromatherapy, they may be resolved through discontinuation of the wrongful application of the oil (e.g., Schnaubelt, 1999).

Many essential oils are inherently toxic at very low concentrations due to very toxic components; these are not normally used in aromatherapy. Many essential oils that are considered to be nontoxic can have a toxic effect on some people; this can be influenced by previous sensitization to a given essential oil, a group of essential oils containing similar components, or some adulterant in the essential oil. It can also be influenced by the age of the person; babies and young children are especially vulnerable and so are very old people. The influence of other medicaments, both conventional and herbal, is still in the preliminary stages of being studied. It is possible that these medicaments, and also probably household products, including perfumes and cosmetics, can influence the adverse reactions to essential oils.

Aromatherapists themselves have also been affected by sensitization (Crawford et al., 2004); in a 12-month period under study, prevalence of hand dermatitis in a sample of massage therapists was 15% by self-reported criteria and 23% by a symptom-based method and included the use of aromatherapy products in massage oils, lotions, or creams. In contrast, the suggestion that aromatherapists have any adverse effects to long-term usage of essential oils was apparently disproved by a nonscientific survey (Price and Price, 1999).

As most essential oils were tested over 30 years ago, the toxicity data may now be meaningless, as different essential oils are now used, some of which contain different quantities of many different synthetic components (Lis-Balchin, 2006).

The major drawbacks of trying to extrapolate toxicity studies in animals to humans concern feelings—from headaches to splitting migraines; feeling sick, vertigo, profound nausea; tinnitus; sadness, melancholia, suicidal thoughts; feelings of hate—which are clearly impossible to measure in animals (Lis-Balchin, 2006). The toxicity of an individual essential oil/component is also tested in isolation in animals and disregards the possibility of modification by other substances, including food components and food additive chemicals, the surrounding atmosphere with gaseous and other components, fragrances used in perfumes, domestic products, in the car, in public transport (including the people), workplace, and so on. These could cause modification of the essential oil/component, its bioavailability, and possibly the enhancement or loss of its function. The detoxification processes in the body are all directed to the production of a more polar product(s), which can be excreted mainly by the kidneys regardless of whether this/these are more toxic or less toxic than the initial substance and differ in different animals.

Most essential oils have GRAS (generally recognized as safe) status granted by the Flavor and Extract Manufacturers Association (FEMA) and approved by the US Food and Drug Administration (FDA) for food use, and many appear in the food chemical codex. This was reviewed in 1996 after evaluation by the expert panel of the FEMA. The assessment was based on data of exposure, and as most flavor ingredients are used at less than 100 ppm, predictions regarding their safety can be assessed from data on their structurally related group(s) (Munro et al., 1996). The no-observed-adverse-effect levels (NOELs) are more than 100,000 times their exposure levels from use as flavor ingredients (Adams et al., 1996). Critical to GRAS assessment are data of metabolic fate and chronic studies rather than acute toxicity. Most essential oils and components have an LD50 of 1–20 g/kg body weight or roughly 1–20 mL/kg, with a few exceptions as follows: Boldo leaf oil 0.1/0.9 (oral/dermal); Calamus 0.8–9/5; Chenopodium 0.2/0.4; Pennyroyal 0.4/4; and Thuja 0.8/4.

Research Institute for Fragrance Materials (RIFM) testing is generally limited to acute oral and dermal toxicity, irritation and dermal sensitization, and phototoxicity of individual materials, and there is little effort to address synergistic and modifying effects of materials in combination (Johansen et al., 1998).

Many materials that were widely used for decades in the past had severe neurotoxic properties and accumulated in body tissues (Spencer et al., 1979; Furuhashi et al., 1994) but most fragrance materials have never been tested for neurological effects, despite the fact that olfactory pathways provide a direct route to the brain (Hastings et al., 1991).

13.17 TOXICITY IN HUMANS

The most recent clinical review of the adverse reactions to fragrances (de Groot and Frosch, 1997) showed many examples of cutaneous reactions to essential oils reported elsewhere (Guin, 1982, 1995). In the United States, about 6 million people have a skin allergy to fragrance and this has a major impact on their quality of life. Symptoms include headaches, dizziness, nausea, fatigue, shortness of breath, and difficulty in concentrating. Fragrance materials are readily absorbed into the body via the respiratory system and once absorbed they cause systemic effects.

Migraine headaches are frequently triggered by fragrances that can act on the same receptors in the brain as alcohol and tobacco, altering mood and function [Institute of Medicine USA, sponsored by the Environmental Protection Agency (EPA)]. Perfumes and fragrances are recognized as triggers for asthma by the American Lung Association. The vast majority of materials used in fragrances are respiratory irritants and there are a few that are known to be respiratory sensitizers. Most have *not* been evaluated for their effects on the lungs and the respiratory system.

Respiratory irritants are known to make the airways more susceptible to injury and allergens, as well as to trigger and exacerbate conditions such as asthma, allergies, sinus problems, and other respiratory disorders. In addition, there is a subset of asthmatics that is specifically triggered by fragrances (Shim and Williams, 1986; Bell et al., 1993; Baldwin et al., 1999), which suggests that fragrances not only trigger asthma, they may also cause it in some cases (Millqvist and Lowhagen, 1996). Placebo-controlled studies using perfumes to challenge people with asthma-like symptoms showed that asthma could be elicited with perfumes without the presence of bronchial obstruction and these were not transmitted by the olfactory nerve as the patients were unaware of the smell (Millqvist and Lowhagen, 1996).

Adverse reactions to fragrances are difficult or even impossible to link to a particular chemical—often due to secrecy rules of the cosmetic/perfumery companies and the enormous range of synthetic components, constituting about 90% of flavor and fragrance ingredients (Larsen, 1998). The same chemicals are used in foods and cosmetics—there is, therefore, a greater impact due to the three different modes of entry: oral, inhalation, and skin.

13.17.1 Increase in Allergic Contact Dermatitis in Recent Years

A study of 1600 adults in 1987 showed that 12% reacted adversely to cosmetics and toiletries, 4.3% of which were used for their odor (i.e., they contained high levels of fragrances). Respiratory problems worsened with prolonged fragrance exposure (e.g., at cosmetic/perfumery counters) and even in churches. In another study, 32% of the women tested had adverse reactions and 80% of these had positive skin tests for fragrances (deGroot and Frosch, 1987). Problems with essential oils have also been increasing. For example, contact dermatitis and allergic contact dermatitis (ACD) caused by tea tree oil has been reported, which was previously considered to be safe (Carson and Riley, 1995). It is unclear whether eucalyptol was responsible for the allergenic response (Southwell, 1997); out of seven patients sensitized to tea tree oil, six reacted to limonene, five to α-terpinene and aromadendrene, two to terpinen-4-ol, and one to *p*-cymene and α-phellandrene (Knight and Hausen, 1994).

Many studies on ACD have been done in different parts of the world (deGroot and Frosch, 1987) and recently more studies have appeared:

- Japan (Sugiura et al., 2000): The patch test with lavender oil was found to be positive in increased numbers and above that of other essential oils in 10 years.
- Denmark (Johansen et al., 2000): There was an 11% increase in the patch test in the last year and of 1537 patients, 29% were allergic to scents.
- Hungary (Katona and Egyud, 2001): Increased sensitivity to balsams and fragrances was noted.
- Switzerland (Kohl et al., 2002): ACD incidence has increased over the years and recently 36% of 819 patch tests were positive to cosmetics.
- Belgium (Kohl et al., 2002): Increased incidence of ACD has been noted.

Occupational increases have also been observed. Two aromatherapists developed ACD: one to citrus, neroli, lavender, frankincense, and rosewood and the other to geraniol, ylang ylang, and angelica (Keane et al., 2000). Allergic airborne contact dermatitis from the essential oils used in aromatherapy was also reported (Schaller and Korting, 1995). ACD occurred in an aromatherapist due to French marigold essential oil, *Tagetes* (Bilsland and Strong, 1990). A physiotherapist developed ACD to eugenol, cloves, and cinnamon (Sanchez-Perez and Garcia Diez, 1999).

There is also the growing problem that patients with eczema are frequently treated by aromatherapists using massage with essential oils. A possible allergic response to a variety of essential oils was found in children with atopic eczema, who were massaged with or without the oils. At first, both massages proved beneficial, though not significantly different; but on reapplying the essential oil massage after a month's break, there was a notable adverse effect on the eczema, which could suggest sensitization (Anderson et al., 2000).

13.17.2 Photosensitizers

Berlocque dermatitis is frequently caused by bergamot or other citrus oil applications on the skin (often due to their inclusion in eau de Cologne) followed by exposure to UV light. This effect is caused by psolarens or furanocoumarins (Klarmann, 1958). Citrus essential oils labeled FCF have no phototoxic effect, but are suspected carcinogens (Young et al., 1990). Other phototoxic essential oils include yarrow and angelica, neroli, petitgrain, cedarwood, rosemary, cassia, calamus, cade, eucalyptus (species not stated), orange, anise, bay, bitter almond, ylang ylang, carrot seed, and linaloe (the latter probably due to linalool, which, like citronellol, has a sensitizing methylene group exposed) (Guin, 1995). Photosensitizer oils include cumin, rue, dill, sandalwood, lemon (oil and expressed), lime (oil and expressed), opoponax, and verbena (the latter being frequently adulterated) (Klarmann, 1958). Even celery soup eaten before UV irradiation has been known to cause severe sunburn (Boffa et al., 1996).

Many of these photosensitizers are now banned or restricted. New International Fragrance Research Association (IFRA) proposals for some phototoxic essential oils include rue oil to be 0.15% maximum in consumer products, marigold oil and absolute to be 0.01%, and petitgrain mandarin oil to be 0.165%.

13.17.3 Commonest Allergenic Essential Oils and Components

The most common fragrance components causing allergy are cinnamic alcohol, hydroxycitronellal, musk ambrette, isoeugenol, and geraniol (Scheinman, 1996). These are included in the eight commonest markers used to check for ACD, usually as a 2% mix. Other components considered allergenic are benzyl salicylate, sandalwood oil, anisyl alcohol, benzyl alcohol, and coumarin.

IFRA and RIFM have forbidden the use of several essential oils and components, including costus root oil, dihydrocoumarin, musk ambrette, and balsam of Peru (Ford, 1991); a concentration

limit is imposed on the use of isoeugenol, cold-pressed lemon oil, bergamot oil, angelica root oil, cassia oil, cinnamic alcohol, hydroxycitronellal, and oakmoss absolute. Cinnamic aldehyde, citral, and carvone oxide can only be used with a quenching agent.

Photosensitivity and phototoxicity occurs with some allergens such as musk ambrette and 6-methyl coumarin that are now removed from skin care products. Children were often found to be sensitive to Peru balsam, probably due to the use of baby-care products containing this (e.g., talcum powder used on nappy rash).

Fragrance materials have been found to interact with food flavorings, for example, a "balsam of Peru-free diet" has been devised in cases where cross reactions are known to occur (Veien et al., 1985). "Newer" sensitizers include ylang ylang (Romaguera and Vilplana, 2000), sandalwood oil (Sharma et al., 1994) but much of this essential oil is adulterated or completely synthetic, lyral (Frosch et al., 1999; Hendriks et al., 1999), and eucalyptol (Vilaplana and Romaguera, 2000).

Some sensitizers have been shown to interact with other molecules. For example, cinnamaldehyde interacts with proteins (Weibel et al., 1989), indicating how the immunogenicity occurs.

There have been very few published reports on neurotoxic aromachemicals such as musk ambrette (Spencer et al., 1984), although many synthetic musks took over as perfume ingredients when public opinion turned against the exploitation of animal products. Musk ambrette was found to have neurotoxic properties in orally fed mice in 1967 and was readily absorbed through the skin. A similar story occurred with acetylethyltetramethyltetralin (AETT), another synthetic musk, also known as versalide, patented in the early 1950s. During routine tests for irritancy in 1975, it was noted that with repeated applications, the skin of the mice turned bluish and they exhibited signs of neurotoxicity. The myelin sheath was damaged irreversibly in a manner similar to that which occurs with multiple sclerosis. Musk xylene, one of the commonest fragrance materials, is found in blood samples from the general population (Kafferlein et al., 1998) and bound to human hemoglobin (Riedel et al., 1999). These musk products have been found to have an effect on the life stages of experimental animals such as the frog, *Xenopus laevis*, the zebra fish, *Danio rerio* (Chou and Dietrich, 1999), and the rat (Christian et al., 1999). The hepatotoxic effect of musks is under constant study (Steinberg et al., 1999).

13.17.4 Toxicity in Young Children: A Special Case

Many aromatherapy books give dangerous advice on the treatment of babies and children, for example, 5–10 drops of "chamomile oil" three times a day in a little warmed milk given to their babies to treat colic with no indication as to which of the three commercially available chamomile oils is to be used and because, depending on the dropper size, the dose could easily approach the oral LD50 for the English and German chamomile oils, this could result in a fatality. Peppermint, often mentioned, could possibly be given by mothers in the form of oil, and has been known to kill a 1-week-old baby (*Evening Standard*, 1998). Dosages given in terms of drops can vary widely according to the size of the dropper in an essential oil.

Many "cosmetics" designed for use by children contain fragrance allergens (Rastogi et al., 1999). In Denmark, samples of children's cosmetics were found to contain geraniol, hydroxycitronellol, isoeugenol, and cinnamic alcohol (Rastogi et al., 1999). Children are more susceptible than adults to any chemical, so the increase in childhood asthma reported in recent years could be caused by fragrance components also found in fast foods. Aromatherapy therefore could be dangerous.

13.17.5 Selected Toxicities of Common Essential Oils and Their Components

Limonene and Linalool are found in a multitude of the commonest aromatherapy oils.

Limonene is a common industrial cleaner and is also the main citrus oil component, which causes ACD, particularly when aged (Chang et al., 1997; Karlberg and Dooms-Goossens, 1997). The major volatile component of lactating mothers' milk in the USA was found to contain *d*-limonene and the component is used as a potential skin penetration promoter for drugs such as indometacin,

especially when mixed with ethanol (Falk-Filipsson et al., 1993). Lastly, cats and dogs are very susceptible to insecticides and baths containing *d*-limonene, giving rise to neurological symptoms including ataxia, stiffness, apparent severe CNS depression, tremors, and coma (von Burg, 1995; see also Beasley, 1999).

Linalool, when oxidized for just 10 weeks, the linalool content fell to 80% and the remaining 20% consisted of a range of breakdown chemicals including linalool hydroperoxide, which was confirmed as a sensitizing agent. The fresh linalool was not a sensitizer; therefore, the EC regulations that are warnings about sensitization potential are looking for potential harm even on storage (Skoeld et al., 2002a, 2002b).

Most cosmetics and perfumes are tested on human "guinea pigs" using similar tests to those described for animals. These are demanded by the RIFM as a final test before marketing a product. Further data are accumulated from notifications from disgruntled consumers who report dermatitis, itching, or skin discoloration after use. These notifications can result in legal claims, although most cases are probably settled out of court and not reported to the general public.

The internet has made it possible for a trusting, although often ill-informed, public to purchase a wide range of dubious plant extracts and essential oils. Even illegal essential oils can now be obtained. Furthermore, unqualified people can offer potentially dangerous advice, such as internal usage or the use of undiluted essential oils on the skin for "mummification," or in order to rid the body of toxic waste. The latter can result in excruciating pain from the burns produced and the subsequent loss of layers of skin.

There is a recipe for suntan oil, including bergamot, carrot seed, and lemon essential oils (all phototoxic) in an aromatherapy book (Fischer-Rizzi, 1990). The author then advises that bergamot oil is added to suntan lotion to get the bonus of the substance called "furocumarin," which lessens the skin's sensitivity to the sun while it helps one to tan quickly. This could cause severe burns. Elsewhere, sassafras (*Ocotea pretiosa*) was said to be only toxic for rats, due to its metabolism and not dangerous to humans (Pénoel, 1991a, 1991b) and a 10% solution in oil was suggested for treating muscular and joint pain and sports injuries. Safrole (and sassafras oil) is, however, controlled under the Controlled Drugs Regulations (1993) and listed as a Category 1 substance, as it is a precursor to the illicit manufacture of hallucinogenic, narcotic, and psychotropic drugs like ecstasy.

French practitioners and other therapists have apparently become "familiar" with untested oils (Guba, 2000). The use of toxicologically untested Nepalese essential oils and the like includes lichen resinoids, sugandha kokila oil, jatamansi oil, and Nepalese lemongrass (*Cymbopogon flexuosa*), also *Tagetes* oil (Basnyet, 1999). *Melaleuca rosalina* (*Melaleuca ericifolia*), 1,8-cineole 18–26%, is apparently especially useful for the respiratory system (Pénoel, 1998), but it is untested and could be a sensitizer.

The Medicines and Healthcare Products Regulatory Agency in the United Kingdom may bring about changes in aromatherapy practice similar to their threat on herbal remedies. Aromatherapists are now using some potentially harmful products in their therapy. This immediately places them at serious risk if there is any untoward reaction to their specific treatment. It virtually means that bottles and containers of essential oils now rank with domestic bleach for labeling purposes and companies are now obliged to self-classify their essential oils on their labels and place them in suitable containers; this applies both to large distributing companies as well as individual aromatherapists reselling essential oils under their own name. Finally, new legislation has gone to the Council of Ministers and may imply that only qualified people will be able to use essential oils, and retail outlets for oils will be pharmacies. Their definition of "qualified" is limited to academic qualifications—doctors or pharmacists.

13.18 CLINICAL STUDIES OF AROMATHERAPY

Very few scientific clinical studies on the effectiveness of aromatherapy have been published to date. Perhaps the main reason is that until recently scientists were not involved and people engaging

in aromatherapy clinical studies had accepted the aromatherapy doctrine in its entirety, precluding any possibility of a nonbiased study. This has been evident in the design and execution of the studies; the main criterion has usually been the use of massage with essential oils and not the effect of the odorant itself. The latter is considered by most aromatherapists as irrelevant to clinical aromatherapy, which implies that it is simply the systemic action of essential oils absorbed through the skin that exerts an effect on specific organs or tissues. Odorant action is considered to be just "aromachology," despite its enormous psychological and physiological impact (Lis-Balchin, 2006). In some studies, attempts are even made to bypass the odorant effect entirely by making the subjects wear oxygen masks throughout (Dunn et al., 1995).

The use of particular essential oils for certain medical conditions is also adhered to, despite the wide assortment of supposed functions for each essential oil claimed by different aromatherapy source materials. In many studies, it is even unclear exactly which essential oil was used; as often the correct nomenclature, chemical composition, and exact purity are not given.

Many aromatherapists feel that they know that aromatherapy works as they have enormous numbers of case studies to prove it. But the production of lists of "positive" results on diverse clients, with diverse ailments, using diverse essential oils in the treatments, and diverse methods of application (which also frequently change from visit to visit for the same client) does not satisfy scientific criteria.

Negative results must surely be among the positive ones, due to the change in essential oils during the course of the treatment, which suggests that they did not work, but these are never stated. There are also no controls in case studies and no attempt to control the bias of the individual aromatherapist and clients.

Double-blind studies are *not* possible in *individual case* studies. Physiological or psychological changes due to the treatment are not properly defined and loose phrases such as "the client felt better" or "happier" are inappropriate for a scientific study.

These faults in the design and interpretation of results of aromatherapy research have been pointed out many times, for example, in Vickers (1996) Kirk-Smith (1996a), Nelson (1997), and Lis-Balchin (2002b). However, the lack of statistically significant results does not prevent many aromatherapists from accepting vaguely positive clinical research results and numerous poor-grade clinical studies are now quoted as factual confirmations that aromatherapy works.

It is almost impossible to do a double-blind study using odorants, as the patient and treatment provider would experience the odor differences and would inevitably react knowingly or unknowingly to that factor alone. The psychological effect(s) could be very diverse, as recall of odors can bring about very acute reactions in different people, depending on the individual's past experiences and on the like (Lis-Balchin, 2006). Lastly, there is potential bias as patients receiving aromatherapy treatment could be grateful for the attention given to them and, not wanting to upset the givers of such attention, would state that they were better and happier than before.

13.19 RECENT CLINICAL STUDIES

13.19.1 Aromatherapy in Dementia

A meticulously conducted double-blind study involved 72 dementia patients with clinically significant agitation treated with *melissa* oil (Ballard et al., 2002). Agitation included anxiety and irritability, motor restlessness, and abnormal vocalization—symptoms that often lead to disturbed behaviors such as pacing, wandering, aggression, shouting, and night-time disturbance, all characterized by appropriate inventories.

Ten percent (by weight) melissa oil (active) or sunflower oil (placebo), combined with a base lotion (*Prunus dulcis* oil, glycerine, stearic acid, cetearyl alcohol, and tocopheryl acetate), was dispensed in metered doses and applied to the face and both arms twice daily for 4 weeks by a care

assistant, the process taking 1–2 min. The patients also received neuroleptic treatment and other conventional treatments when necessary; this was therefore a study of complementary aromatherapy treatment—not an alternative treatment.

The "*melissa* group" showed a higher significant improvement in reducing aggression than the control group by week 4; the total Cohen–Mansfield Agitation Inventory (CMAI) scores had decreased significantly in both groups, from a mean of 68 to 45 (35%; $P < .0001$) in the treatment group and from 61 to 53 (11%; $P < .005$) in the placebo group. Clinically significant reduction in agitation occurred in 60% of the melissa group compared with 14% of placebo responders ($P < .0001$). Neuropsychiatric Inventory (NPI) scores also declined with *melissa* treatment, and quality of life was improved, with less social isolation and more involvement in activities. The latter was in contrast to the usual neuroleptic treatment effects.

The authors concluded that the *melissa* treatment was successful, but pointed out that there was also a significant, but lower, improvement in the control group and suggested that a stronger odor should have been used.

The effect of the *melissa* oil was probably on cholinergic receptors as shown by previous *in vitro* studies (Perry et al., 1999; Wake et al., 2000). The authors also concluded that as most people with severe dementia have lost any meaningful sense of smell, a direct placebo effect due to a pleasant-smelling fragrance, although possible, is an unlikely explanation for the positive effects of *melissa* in this study but others may disagree with this conclusion as it has been shown that subliminal odors can have an effect. The fragrance may have had some impact upon the care staff, and influenced ratings to some degree on the informant schedules.

A further recent study found no support for the use of a purely olfactory form of aromatherapy to decrease agitation in severely demented patients using lavender and thyme oil (Snow et al., 2004).

Other research (Burns et al., 2002) suggested that aromatherapy and light therapy were more effective and gentler alternatives to the use of neuroleptics in patients with dementia. Three studies were analyzed in each category; in the aromatherapy section, it included the study above, plus the use of 2% lavender oil via inhalation in a double-blind study for 10 days (Holmes et al., 2002) and a 2-week single-blind study using either aromatherapy plus massage, aromatherapy plus conversation or massage alone (Smallwood et al., 2001). All of the interventions in the aromatherapy groups proved significantly beneficial. However, so did the light treatment, where patients sat in front of a light box that beamed out 10,000 lux of artificial light, which adjusts the body's melatonin levels, affects the body clock, and is used in the treatment of SAD (seasonal affective disorder).

13.20 PAST CLINICAL STUDIES

In contrast to more recent studies, past clinical trials were often very defective in design and also outcomes. In a recent review, Cooke and Ernst (2000) included only those aromatherapy trials that were randomized and included human patients; they excluded those with no control group or if only local effects (e.g., antiseptic effects of tea tree oil) or preclinical studies on healthy volunteers occurred. The six trials included massage with or without aromatherapy (Buckle, 1993; Stevenson, 1994; Corner et al., 1995; Dunn et al., 1995; Wilkinson, 1995; Wilkinson et al., 1999) and were based on their relaxation outcomes. The authors concluded that the effects of aromatherapy were probably not strong enough for it to be considered for the treatment of anxiety or for any other indication.

A further study included trials with no replicates, and contained six studies. It showed that in five out of six cases the main outcomes were positive; however, these were limited to very specific criteria, such as small airways resistance for common colds (Cohen and Dressler, 1982), prophylaxis of bronchi for bronchitis (Ferley et al., 1989), lessening smoking withdrawal symptoms (Rose and Behm, 1993, 1994), relief of anxiety (Morris et al., 1995), and treatment of alopecia areata (Hay et al., 1998). The alleviation of perineal discomfort (Dale and Cornwell, 1994) was not significant.

Psychological effects, which include inhalation of essential oils and behavioral changes, were already discussed.

13.20.1 Critique of Selected Clinical Trials

The following clinical studies attempted to show that aromatherapy was more efficient than massage alone but they showed mainly negative results; however, in some cases, the authors clearly emphasized some very small positive results and this was then accepted and the report was welcomed in aromatherapy journals as a positive trial that supported aromatherapy.

Massage, aromatherapy massage, or a period of rest in 122 patients in an intensive care unit (ICU) (Dunn et al., 1995) showed no difference between massage with or without lavender oil and no treatment in the physiological parameters and all psychological parameters showed no effects throughout, bar a significantly greater improvement in mood and in anxiety levels between the rest group and essential oil massage group after the first session. The trial had a large number of changeable parameters: it involved patients in the ICU for about 5 days (age range 2–92 years), who received 1–3 therapy sessions in 24 h given by six different nurses. Massage was performed on the back or outside of limbs or scalp for 15–30 min with lavender (*Lavandula vera* at 1% in grapeseed oil, which was the only constant parameter). The patients wore oxygen masks, for some of the time. It seems unlikely that confused patients in ICU could remember the massage or its effects and a child of 2 years could not be expected to answer any pertinent questions.

Massage with and without Roman chamomile in 51 palliative care patients (Wilkinson, 1995) showed that both groups experienced the same decrease in symptoms and severity after three full body massages in 3 weeks. There was, however, a statistically significant difference between the two groups after the first aromatherapy massage and also an improvement in the "quality of life" from pre- to postmassage. German chamomile was likely to have been used, not Roman chamomile as stated, according to the chemical composition and potential bioactivity given.

Aromatherapy with and without massage, and massage alone on disturbed behavior in four patients with severe dementia (Brooker et al., 1997), was an unusual single-case study evaluating the use of "true" aromatherapy (using inhaled lavender oil) for 10 treatments of each, randomly given to each patient over a 3-month period and assessed against 10 no-treatment periods. Two patients became more agitated following their treatment sessions and only one patient seemed to have benefited. According to the staff providing the treatment, however, the use of all the treatments seemed to have been beneficial to the patients, suggesting pronounced bias.

An investigation of the psychophysiological effects of aromatherapy massage following cardiac surgery (Stevenson, 1994) showed experimenter bias due to the statement that "neroli is also especially valuable in the relief of anxiety, it calms palpitations, has an antispasmodic effect and an anti-inflammatory effect … it is useful in the treatment of hysteria, as an antidepressant and a gentle sedative." None of this has been scientifically proven, but as this was not a double-blind study and presumably the author did the massaging, communicating, and collating information alone, bias is probable. Statistical significances were not shown, nor the age ranges of the 100 patients, and no differences between the aromatherapy-only and massage-only groups were shown, except for an immediate increase in respiratory rate when the two control groups (20 min chat or rest) were compared with the aromatherapy massage and massage-only groups.

Atopic eczema in 32 children treated by massage with and without essential oils (Anderson et al., 2000) in a single-case experimental design across subjects showed that this complementary therapy provided no statistically significant differences between the two groups after 8 weeks of treatment. This indicated that massage and thereby regular parental contact and attention showed positive results, which was expected in these children. However, a continuation of the study, following a 3-month period of rest, using only the essential oil massage group showed a possible sensitization effect, as the symptoms worsened.

Massage using two different types of lavender oil on postcardiotomy patients (Buckle, 1993) was proclaimed to be a "double-blind" study but had no controls and the results by the author did not appear to be assessed correctly (Vickers, 1996). The author attempted to show that the "real" lavender showed significant benefits in the state of the patients compared with the other oil. However,

outcome measures were not described and the chemical composition and botanical names of the "real" and "not real" lavender remains a mystery, as three lavenders were stated in the text. Although the results were insignificant, this paper is quoted widely as proof that only "real" essential oils work through aromatherapy massage.

Aromatherapy trails in childbirth have been of dubious design and low scientific merit and, not surprisingly, have yielded confusing results (Burns and Blaney, 1994), mainly due to the numerous parameters incorporated. In the study by Burns and Blaney (1994), many different essential oils were used in various uncontrolled ways during childbirth and assessed using possibly biased criteria as to their possible benefits to the mother and midwife. The first pilot study used 585 women in a delivery suite over a 6-month period using lavender, clary sage, peppermint, eucalyptus, chamomile, frankincense, jasmine, lemon, and mandarin. These oils were either used singly or as part of a mixture where they could be used as the first, second, third, or fourth essential oil. The essential oils were applied in many different ways and at different times during parturition, for example, sprayed in a "solution" in water onto a face flannel, pillow, or bean bag; in a bath; foot bath; an absorbent card for inhalation; or in almond oil for massage. Peppermint oil was applied as an undiluted drop on the forehead and frankincense onto the palm.

Midwives and mothers filled in a form as to the effects of the essential oils including their relaxant value, effect on nausea and vomiting, analgesic action, mood enhancer action, accelerator, or not of labor. The results were inconclusive and there was a bias toward the use of a few oils, for example, lavender was stated to be "oestrogenic and used to calm down uterine tightenings if a woman was exhausted and needed sleep" and clary sage was given to "encourage the establishment of labor." This shows complete bias and a belief in unproven clinical attributes by the authors and presumably those carrying out the study. Which of the lavender, peppermint, eucalyptus, chamomile, or frankincense species were used remains a mystery.

The continuation of this study (Burns et al., 2000) on 8058 mothers during childbirth was intended to show that aromatherapy would "relieve anxiety, pain, nausea and/or vomiting, or strengthen contractions." Data from the unit audit were compared with those of 15,799 mothers not given aromatherapy treatment. The results showed that 50% of the aromatherapy group mothers found the intervention "helpful" and only 14% "unhelpful." The use of pethidine over the year declined from 6% to 0.2% by women in the aromatherapy group. The study also (apparently) showed that aromatherapy may have the potential to augment labor contractions for women in dysfunctional labor, in contrast to scientific data showing that the uterine contractions decrease due to administration of any common essential oils (Lis-Balchin and Hart, 1997).

It is doubtful whether a woman would in her first labor, or in subsequent ones, be able to judge whether the contractions were strengthened or the labor shortened due to aromatherapy. It seems likely that there was some placebo effect (itself a very powerful effector) due to the bias of the experimenters and the "suggestions" made to the aromatherapy group regarding efficacy of essential oils, which were obviously absent in the case of the control group.

Lavender oil (volatilized from a burner during the night in their hospital room) has been successful in replacing medication to induce sleep in three out of four geriatrics (Hardy et al., 1995). There was a general deterioration in the sleep patterns when the medication was withdrawn, but lavender oil seemed to be as good as the original medication. However, the deterioration in the sleep patterns (due to "rebound insomnia"?) may simply have been due to recovery of normal sleep patterns when lavender was given (Vickers, 1996).

The efficacy of peppermint oil was studied on postoperative nausea in 18 women after gynecological operations (Tate, 1997) using peppermint oil or a control, peppermint essence (obviously of similar odor). A statistically significant difference was found between the controls and the test group. The test group required less antiemetics and received less opioid analgesia. However, the use of a peppermint essence as a control seems rather like having two test groups as inhalation was used.

A group of 313 patients undergoing radiotherapy were randomly assigned to receive either carrier oil with fractionated oils, carrier oil only, or pure essential oils of lavender, bergamot, and

cedarwood administered by inhalation concurrently with radiation treatment. There were no significant differences in Hospital Anxiety and Depression Score (HADS) and other scores between the randomly assigned groups. Aromatherapy, as administered in this study, was not found to be beneficial (Graham et al., 2003).

Heliotropin, a sweet, vanilla-like scent, reduced anxiety during magnetic resonance imaging (Redd and Manne, 1991), which causes distress to many patients as they are enclosed in a "coffin"-like apparatus. Patients experienced approximately 63% less overall anxiety than a control group of patients.

A double-blind randomized trial was conducted on 66 women undergoing abortions (Wiebe, 2000). Ten minutes were spent sniffing a numbered container with either a mixture of the essential oils (vetivert, bergamot, and geranium) or a hair conditioner (placebo). Aromatherapy involving essential oils was no more effective than having patients sniff other pleasant odors in reducing preoperative anxiety.

An audit into the effects of aromatherapy in palliative care (Evans, 1995) showed that the most frequently used oils were lavender, marjoram, and chamomile. These were applied over a period of 6 months by a therapist available for 4 h on a weekly basis in the ward. Relaxing music was played throughout, each session to allay fears of the hands-on massage. The results revealed that 81% of the patients stated that they either felt "better" or "very relaxed" after the treatment; most appreciated the music greatly. The researchers themselves confessed that it is uncertain whether the benefits were the result of the patient being given individual attention, talking with the therapist, the effects of touch and massage, the effects of the aromatherapy essential oils, or the effects of the relaxation music.

Aromatherapy massage studied in eight cancer patients did not show any psychological benefit. However, there was a statistically significant reduction in all of the four physical parameters, which suggests that aromatherapy massage affects the autonomic nervous system, inducing relaxation. This finding was supported by the patients themselves, all of whom stated during interview that they felt "relaxed" after aromatherapy massage (Hadfield, 2001).

Forty-two cancer patients were randomly allocated to receive weekly massages with lavender essential oil in carrier oil (aromatherapy group), carrier oil only (massage group), or no intervention for 4 weeks (Soden et al., 2004). Outcome measures included a visual analogue scale (VAS) of pain intensity, the Verran and Snyder–Halpern Sleep Scale (VSH), the Hospital Anxiety and Depression Scale (HADS), and the Rotterdam Symptom Checklist (RSCL). No significant long-term benefits of aromatherapy or massage in terms of improving pain control, anxiety, or quality of life were shown. However, sleep scores improved significantly in both the massage and the combined massage (aromatherapy and massage) groups. There were also statistically significant reductions in depression scores in the massage group. In this study of patients with advanced cancer, the addition of lavender essential oil did not appear to increase the beneficial effects of massage.

A randomized controlled pilot study was carried out to examine the effects of adjunctive aromatherapy massage on mood, quality of life, and physical symptoms in patients with cancer attending a specialist unit (Wilcock et al., 2004). Patients were randomized to conventional day care alone, or day care plus weekly aromatherapy massage using a standardized blend of oils for 4 weeks. At baseline and at weekly intervals, patients rated their mood, quality of life, and the intensity and bother of two symptoms most important to them. However, although 46 patients were recruited to the study, only 11 of 23 (48%) patients in the aromatherapy group and 18 of 23 (78%) in the control group completed all 4 weeks. Mood, physical symptoms, and quality of life improved in both groups but there was *no* statistically significant difference between groups, but all patients were satisfied with the aromatherapy and wished to continue it.

Aromatherapy sessions in deaf and deaf–blind people became an accepted, enjoyable, and therapeutic part of the residents' lifestyle in an uncontrolled series of case studies. It appeared that this gentle, noninvasive therapy could benefit deaf and deaf–blind people, especially as their intact senses can be heightened (Armstrong and Heidingsfeld, 2000).

A scientifically unacceptable study of the effect of aromatherapy on endometriosis, reported only at an aromatherapy conference (Worwood, 1996), involved 22 aromatherapists who treated a total of 17 women in two groups over 24 weeks. One group was initially given massage with essential oils and then not "touched" for the second period, while the second group had the two treatments reversed. Among the many parameters measured were constipation, vaginal discharge, fluid retention, abdominal and pelvic pain, degree of feeling well, renewed vigor, depression, and tiredness. The data were presented as means (or averages, possibly, as this was not stated) but without standard errors of mean (SEM) and lacked any statistical analyses. Unfortunately, the study has been accepted by many aromatherapists as being a conclusive proof of the value in treating endometriosis using aromatherapy.

In all the trials above, there was a more positive outcome for aromatherapy if there were no stringent scientific double-blind and randomized control measures, suggesting that in the latter case, bias is removed.

13.21 USE OF ESSENTIAL OILS MAINLY AS CHEMICAL AGENTS AND NOT FOR THEIR ODOR

The efficacy and safety of capsules containing peppermint oil (90 mg) and caraway oil (50 mg), when studied in a double-blind, placebo-controlled, multicenter trial in patients with nonulcer dyspepsia was shown by May et al. (1996). Intensity of pain was significantly improved for the experimental group compared with the placebo group after 4 weeks.

Six drops of pure lavender oil included in the bath water for 10 days following childbirth was assessed against "synthetic" lavender oil and a placebo (distilled water containing an unknown GRAS additive) for perineal discomfort (Cornwell and Dale, 1995). No significant differences between groups were found for discomfort, but lower scores in discomfort means for days 3 and 5 for the lavender group were seen. This was very unsatisfactory as a scientific study, mainly because essential oils do not mix with water and there was no proof whether the lavender oil itself was pure.

Alopecia areata was treated in a randomized trial using "aromatherapy" carried out over 7 months. The test group massaged a mixture of 2 drops of *Thymus vulgaris*, 3 drops *Lavandula angustifolia*, 3 drops of *Rosmarinus officinalis*, and 2 drops of *Cedrus atlantica* in 3 mL of jojoba and 20 mL grapeseed oil into the scalp for 2 min minimum every night. The control group massaged the carrier oils alone (Hay et al., 1998). There was a significant improvement in the test group (44%) compared with the control group (15%). The smell of the essential oils (psychological/physiological) and/or their chemical nature on the scalp may have achieved these long-term results. On the other hand, the scalp may have healed naturally anyway after 7 months.

Ureterolithiasis was treated with Rowatinex, a mixture of terpenes smelling like Vicks VapoRub in 43 patients against a control group treated with a placebo. The overall expulsion rate of the ureteric stones was greater in the Rowatinex group (Engelstein et al., 1992). Similar mixes have shown both positive and negative results on gallstones over the years.

In a double-blind, placebo-controlled, randomized crossover study involving 332 healthy subjects, four different preparations were used to treat headaches (Gobel et al., 1994). Peppermint oil, eucalyptus oil (species not stated), and ethanol were applied to large areas of the forehead and temples. A combination of the three increased cognitive performance, muscle relaxation, and mental relaxation, but had no influence on pain. Peppermint oil and ethanol decreased the headache. The reason for the success could have been the intense coldness caused by the application of the latter mixture, which was followed by a warming up as the peppermint oil caused counterirritation on the skin; the essential oils were also inhaled.

A clinical trial on 124 patients with acne, randomly distributed to a group treated with 5% tea tree oil gel or a 5% benzoyl peroxide lotion group (Bassett et al., 1990), showed improvement in both groups and fewer side effects in the tea tree oil group. The use of tea tree oil has, however, had detrimental effects in some people (Lis-Balchin, 2006, Chapter 7).

A 10% tea tree oil was used on 104 patients with athlete's foot *Tinea pedis*) in a randomized double-blind study against 1% tolnaflate and placebo creams. The tolnaflate group showed a better effect; tea tree oil was as effective in improving the condition, but was no better than the placebo at curing it (Tong et al., 1992). Surprisingly, tea tree oil is sold as a *cure* for athlete's foot.

13.21.1 Single-Case Studies

In the past few years, the theme of the case studies (reported mainly in aromatherapy journals) has started to change and most of the aromatherapists are no longer announcing that they are "curing" cancer and other serious diseases. Emphasis has swung toward real complementary treatment, often in the area of palliative care. However, the so-called clinical aromatherapists persist in attempting to cure various medical conditions using high doses of oils mainly by mouth, vagina, anus, or on the skin. Many believe that healing wounds using essential oils is also classed as aromatherapy (Guba, 2000) despite the evidence that odor does not kill germs and any effect is due to the chemical activity alone.

Because of the lack of scientific evidence in many studies, we could assume that aromatherapy is mainly based on faith; it works because the aromatherapist believes in the treatment and because the patient believes in the supposed action of essential oils, that is, the placebo effect.

Decreased smoking withdrawal symptoms in 48 cigarette smokers were achieved by black pepper oil puffed out of a special instrument for 3 h after an overnight cigarette deprivation against mint/menthol or nothing (Rose and Behm, 1994).

Chronic respiratory infection was successfully treated when the patient was massaged with tea tree, rosemary, and bergamot oils while on her second course of antibiotics and taking a proprietary cough medicine. She also used lavender and rosemary oils in her bath, a drop of eucalyptus oil and lavender oil on her tissue near the pillow at night, 3 drops of eucalyptus and ginger for inhalations daily, and reduced her dairy products and starches. In a week, her cough was better and by 3 weeks, it had gone (Laffan, 1992). It is unclear which treatment actually helped the patient, and as it took a long time, the infection may well have gone away by then, or sooner, without any medicinal aid.

After just one treatment of aromatherapy massage using rose oil, bergamot, and lavender at 2.5% in almond oil, a 36-year-old woman managed to get pregnant after being told she was possibly infertile following the removal of her right fallopian tube (Rippon, 1993)!

Aromatherapy can apparently help patients with multiple sclerosis, especially for relaxation, in association with many other changes in the diet and also use of conventional medicines (Barker, 1994). French basil, black pepper, and true lavender in evening primrose oil with borage oil was used to counteract stiffness and also to stimulate; this mixture was later changed to include relaxing and sedative oils such as Roman chamomile, ylang ylang, and melissa.

Specific improvements in clients given aromatherapy treatment in dementia include increased alertness, self-hygiene, contentment, initiation of toileting, sleeping at night, and reduced levels of agitation, withdrawal, and wandering. Family carers reported less distress, improved sleeping patterns, and calmness (Kilstoff and Chenoweth, 1998). Other patients with dementia were monitored over a period of 2 months, and then for a further 2 months during which they received aromatherapy treatments in a clinical trial; they showed a significant improvement in motivational behavior during the period of aromatherapy treatment (MacMahon and Kermode, 1998).

13.22 CONCLUSION

Aromatherapy, using essential oils as an odorant by inhalation or massage onto the skin, has not been shown to work better than massage alone or a control. No failures have, however, been reported, although treatment is invariably changed on each visit. Many patients feel better, even if their disease is getting worse, due to their belief in an alternative therapist and this is a good example of "mind over matter," that is, the placebo effect. This effect has been recommended by some members

of the House of Lords Select Committee on Science and Technology, Sixth Report (2000), as a good basis for retaining complementary and alternative medicine, but other members argued that scientific proof of effects is necessary.

It is hoped that aromatherapists do not try to convince their patients of a cure, especially in the case of serious ailments such as cancer, which often recede naturally for a time on their own. Conventional treatment should always be advised in the first instance and retained during aromatherapy treatment with the consent of the patient's primary healthcare physician or consultant. Aromatherapy can provide a useful complementary medical service both in healthcare settings and in private practice, and should not be allowed to become listed as a bogus cure in alternative medicine.

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14 Biotransformation of Monoterpenoids by Microorganisms, Insects, and Mammals

Yoshiaki Noma and Yoshinori Asakawa

CONTENTS

14.1 INTRODUCTION

A large number of monoterpenoids have been detected in or isolated from essential oils and solvent extracts of fungi, algae, liverworts, and higher plants, but the presence of monoterpenoids in fern is negligible. Vegetables, fruits, and spices contain monoterpenoids; however, their fate in human and other animal bodies has not yet been fully investigated systematically. The recent development of analytical instruments makes it easy to analyze the chemical structures of very minor components, and the essential oil chemistry field has dramatically developed.

Since monoterpenoids, in general, show characteristic odor and taste, they have been used as cosmetic materials; food additives; and often for insecticides, insect repellents, and attractant drugs. In order to obtain much more functionalized substances from monoterpenoids, various chemical reactions and microbial transformations of commercially available and cheap synthetic monoterpenoids have been carried out. On the other hand, insect larva and mammals have been used for direct biotransformations of monoterpenoids to study their fate and safety or toxicity in their bodies.

The biotransformation of α-pinene (**4**) by using the black fungus *Aspergillus niger* was reported by Bhattacharyya et al. (1960) half a century ago. During that period, many scientists studied the biotransformation of a number of monoterpenoids by using various kinds of bacteria, fungi, insects, mammals, and cultured cells of higher plants. In this chapter, the microbial transformation of monoterpenoids using bacteria and fungi is discussed. Furthermore, the biotransformation by using insect larva, mammals, microalgae, as well as suspended culture cells of higher plants is also summarized. In addition, several biological activities of biotransformed products are also represented. At the end of this chapter, the metabolite pathways of representative monoterpenoids for further development on biological transformation of monoterpenoids are demonstrated.

14.2 METABOLIC PATHWAYS OF ACYCLIC MONOTERPENOIDS

14.2.1 Acyclic Monoterpene Hydrocarbons

14.2.1.1 Myrcene

The microbial biotransformation of myrcene (**302**) was described with *Diplodia gossypina* ATCC 10936 (Abraham et al., 1985). The main reactions were hydroxylation, as shown in Figure 14.1. On oxidation, myrcene (**302**) gave the diol (**303**) (yield up to 60%) and also a side-product (**304**) that possesses one carbon atom less than the parent compound, in yields of 1–2%.

One of the publications dealing with the bioconversion of myrcene (Busmann and Berger, 1994) described its transformation to a variety of oxygenated metabolites, with *Ganoderma applanatum*, *Pleurotus flabellatus*, and *Pleurotus sajor-caju* possessing the highest transformation activities. One of the main metabolites was myrcenol (**305**) (2-methyl-6-methylene-7-octen-2-ol), which gives a fresh, flowery impression and dominates the sensory impact of the mixture (see Figure 14.1).

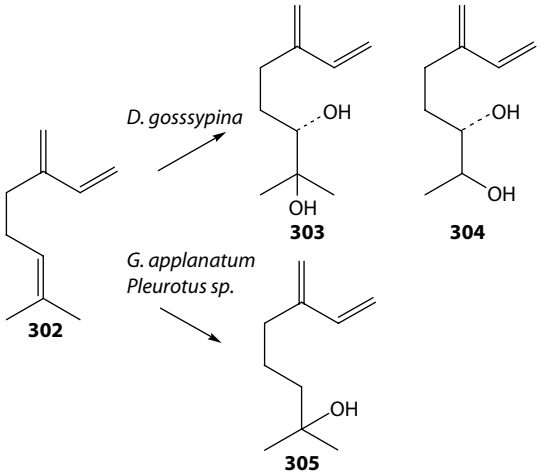


FIGURE 14.1 Biotransformation of myrcene (**302**) by *Diplodia gossypina* (Abraham et al., 1985), *Ganoderma applanatum*, and *Pleurotus* sp. (Modified from Busmann, D. and R.G. Berger, 1994. *J. Biotechol.*, 37: 39–43.)

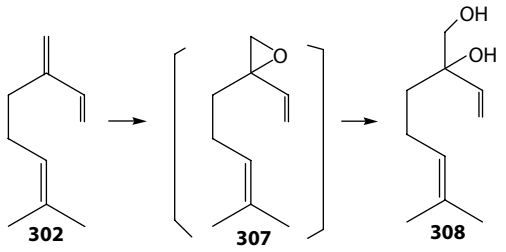


FIGURE 14.2 Biotransformation of myrcene (**302**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1998. *Proc. 42nd TEAC*, pp. 123–125.)

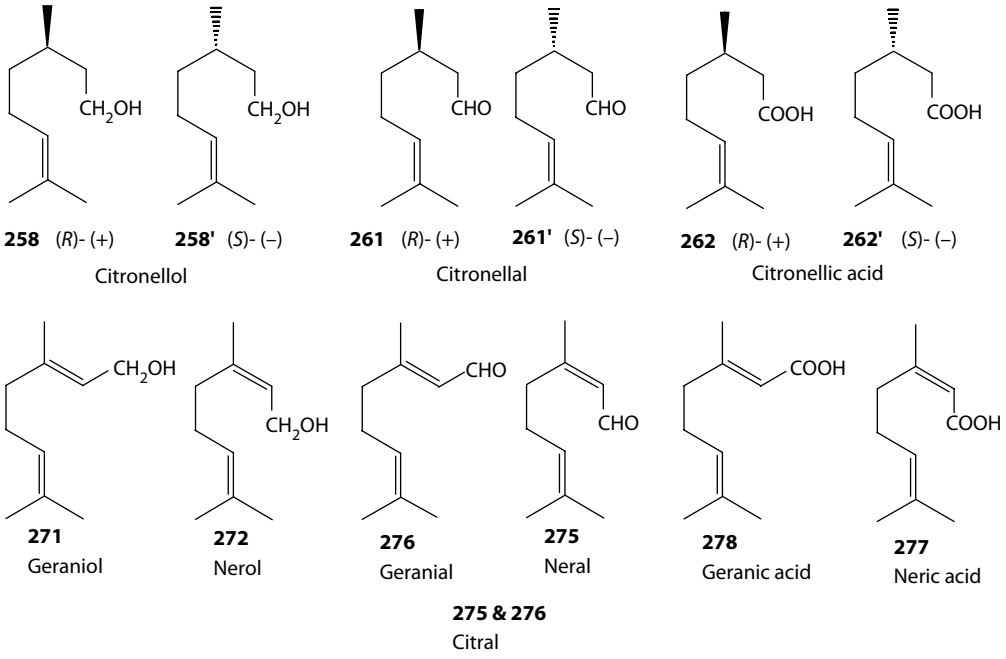
β-Myrcene (**302**) was converted by common cutworm larvae, *Spodoptera litura*, to give myrcene-3,(10)-glycol (**308**) via myrcene-3,(10)-epoxide (**307**) (Figure 14.2) (Miyazawa et al., 1998).

14.2.1.2 Citronellene

(−)-Citronellene (**309**) and (+)-citronellene (**309′**) were biotransformed by the cutworm *Spodoptera litura* to give (3*R*)-3,7-dimethyl-6-octene-1,2-diol (**310**) and (3*S*)-3,7-dimethyl-6-octene-1,2-diol (**310′**), respectively (Takeuchi and Miyazawa, 2005) (Figure 14.3).

14.2.2 Acyclic Monoterpene Alcohols and Aldehydes

14.2.2.1 Geraniol, Nerol, (+)- and (−)-Citronellol, Citral, and (+)- and (−)-Citronellal



The microbial degradation of the acyclic monoterpene alcohols citronellol (**258**), nerol (**272**), geraniol (**271**), citronellal (**261**), and citral (equal mixture of **275** and **276**) was reported in the early part of 1960 (Seubert and Remberger, 1963; Seubert et al., 1963; Seubert and Fass, 1964a, 1964b). *Pseudomonas citronellolis* metabolized citronellol (**258**), citronellal (**261**), geraniol (**271**), and geranic acid (**278**). The metabolism of these acyclic monoterpenes is initiated by the oxidation of the

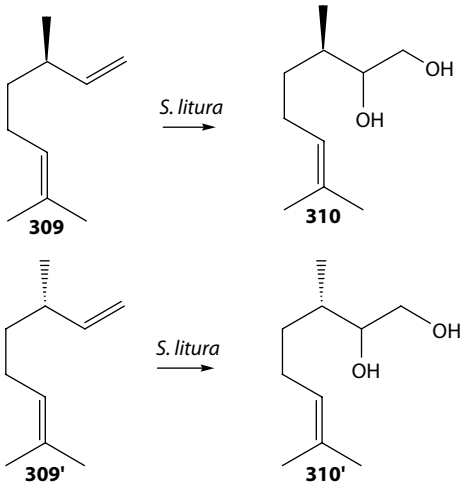


FIGURE 14.3 Biotransformation of (–)-citronellene (**309**) and (+)-citronellene (**309′**) by *Spodoptera litura*. (Modified from Takeuchi, H. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 426–427.)

primary alcohols group to the carboxyl group, followed by the carboxylation of the C-10 methyl group (β-methyl) by a biotin-dependent carboxylase (Seubert and Remberger, 1963). The carboxymethyl group is eliminated at a later stage as acetic acid. Further degradation follows the β-oxidation pattern. The details of the pathway are shown in Figure 14.4 (Seubert and Fass, 1964a).

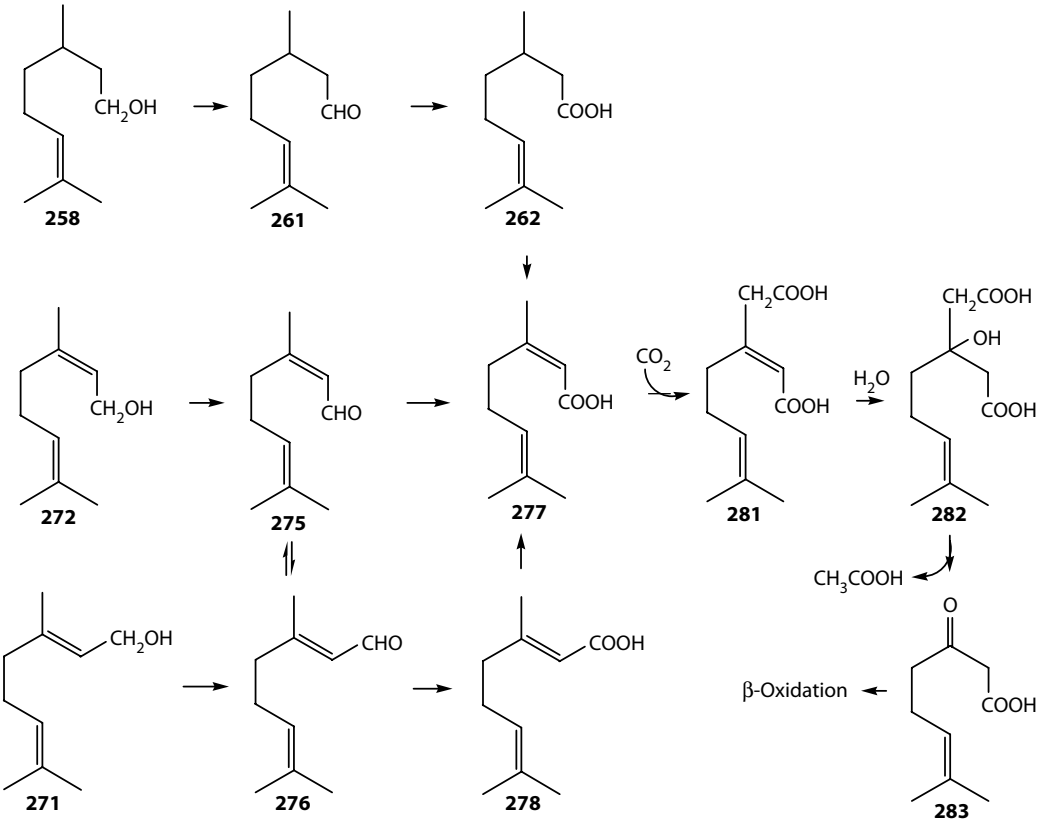


FIGURE 14.4 Biotransformation of citronellol (**258**), nerol (**272**), and geraniol (**271**) by *Pseudomonas citronellolis*. [Modified from Madyastha, K.M. 1984. *Proc. Indian Acad. Sci.* (*Chem. Sci.*), 93: 677–686.]

The microbial transformation of citronellal (**261**) and citral (**275** and **276**) was reported by way of *Pseudomonas aeruginosa* (Joglekar and Dhavlikar, 1969). This bacterium, capable of utilizing citronellal (**261**) or citral (**275** and **276**) as the sole carbon and energy source, has been isolated from soil by the enrichment culture technique. It metabolized citronellal (**261**) to citronellic acid (**262**) (65%), citronellol (**258**) (0.6%), dihydrocitronellol (**259**) (0.6%), 3,7-dimethyl-1,7-octanediol (**260**) (1.7%), and menthol (**137**) (0.75%) (Figure 14.5). The metabolites of citral (**275** and **276**) were geranic acid (**278**) (62%), 1-hydroxy-3,7-dimethyl-6-octen-2-one (**279**) (0.75%), 6-methyl-5-heptenoic acid (**280**) (0.5%), and 3-methyl-2-butenoic acid (**286**) (1%) (Figure 14.5). In a similar way, *Pseudomonas convexa* converted citral (**275** and **276**) to geranic acid (**278**) (Hayashi et al., 1967). The biotransformation of citronellol (**258**) and geraniol (**271**) by *Pseudomonas aeruginosa*, *Pseudomonas citronellolis*, and *Pseudomonas mendocina* was also reported by another group (Cantwell et al., 1978).

A research group in Czechoslovakia patented the cyclization of citronellal (**261**) with subsequent hydrogenation to menthol by *Penicillium digitatum* in 1952. Unfortunately the optical purities of the intermediates pulegol and isopulegol were not determined and presumably the resulting menthol was a mixture of enantiomers. Therefore, it cannot be excluded that this extremely interesting cyclization is the result of a reaction primarily catalyzed by the acidic fermentation conditions and only partially dependent on enzymatic reactions (Babcka et al., 1956) (Figure 14.6).

Based on previous data (Madyastha et al., 1977; Rama and Bhattacharyya, 1977a), two pathways for the degradation of geraniol (**271**) were proposed by Madyastha (1984) (Figure 14.7). Pathway A involves an oxidative attack on the 2,3-double bond, resulting in the formation of an epoxide. Opening of the epoxide yields the 2,3-dihydroxygeraniol (**292**), which upon oxidation forms 2-oxo, 3-hydroxygeraniol (**293**). The ketodiol (**293**) is then decomposed to 6-methyl-5-hepten-2-one (**294**) by an oxidative process. Pathway B is initiated by the oxidation of the primary alcoholic group to geranic acid (**278**) and further metabolism follows the mechanism as proposed earlier for *Pseudomonas citronellolis* (Seubert and Remberger, 1963; Seubert et al., 1963). In the case of nerol (**272**), the *Z*-isomer of geraniol (**271**), degradative pathways analogous to pathways A and B as in geraniol (**271**) are observed. It was also noticed that *Pseudomonas incognita* metabolizes acetates of geraniol (**271**), nerol (**272**), and citronellol (**258**) much faster than their respective alcohols (Madyastha and Renganathan, 1983).

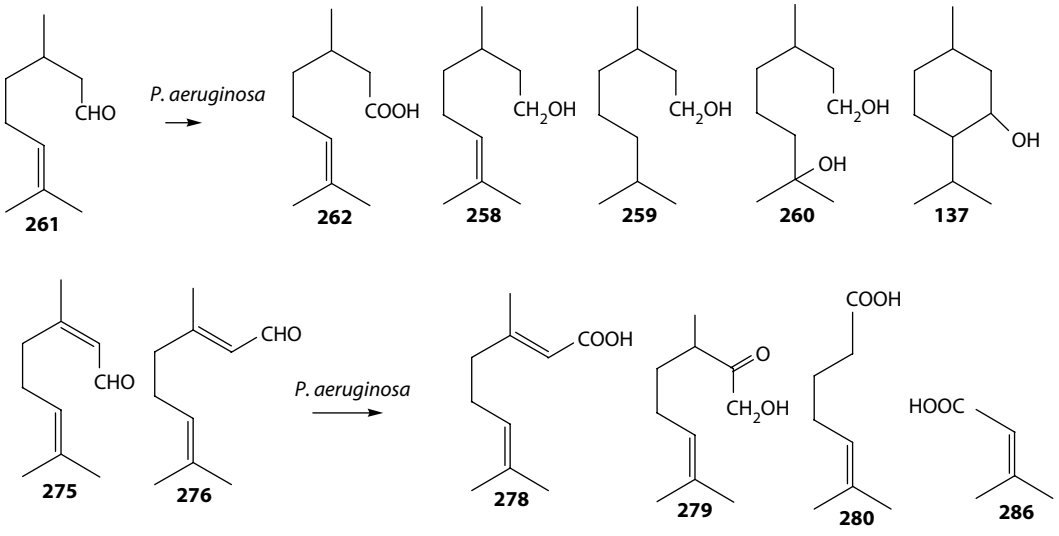


FIGURE 14.5 Biotransformation of citronellal (**261**) and citral (**275** and **276**) by *Pseudomonas aeruginosa*. (Modified from Joglekar, S.S. and R.S. Dhavlikar, 1969. *Appl. Microbiol.*, 18: 1084–1087.)

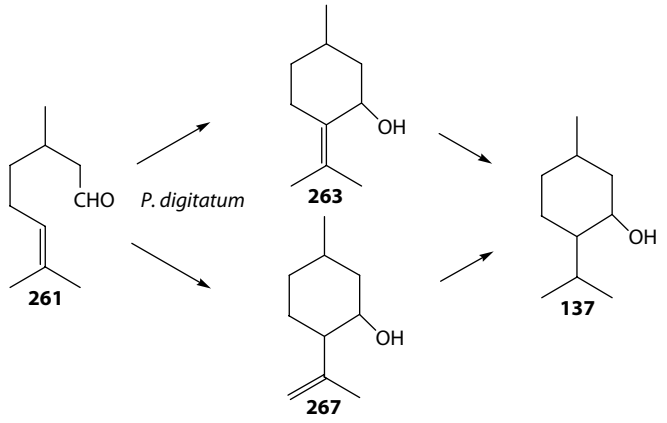


FIGURE 14.6 Biotransformation of citronellal to menthol by *Penicillium digitatum*. (Modified from Babcka, J. et al., 1956. Patent 56-9686b.)

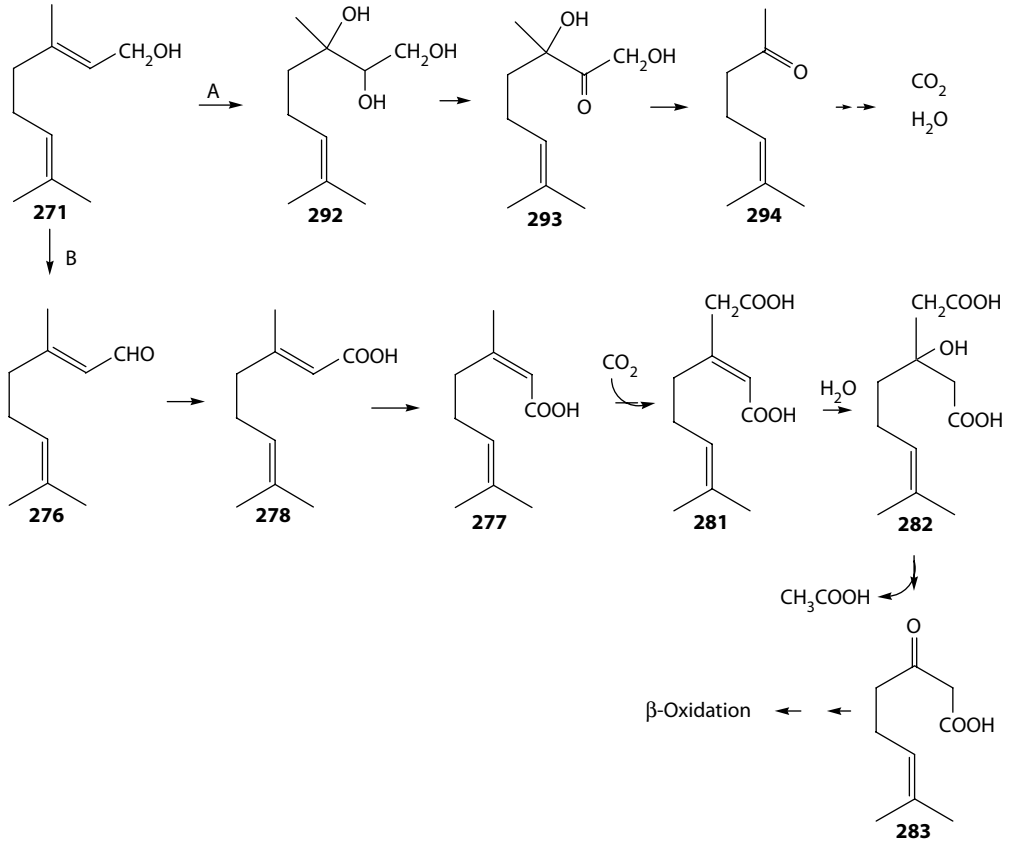


FIGURE 14.7 Metabolism of geraniol (**271**) by *Pseudomonas incognita*. [Modified from Madyastha, K.M. 1984. *Proc. Indian Acad. Sci.* (*Chem. Sci.*), 93: 677–686.]

Euglena gracilis Z converted citral (**275** and **276**, 56:44, peak area in GC) to geraniol (**271**) and nerol (**272**), respectively, of which geraniol (**271**) was further transformed to (+)- and (−)-citronellol (**258** and **258′**). On the other hand, when either geraniol (**271**) or nerol (**272**) was added, both compounds were isomerized to each other and, then, geraniol (**271**) was transformed to citronellol. These results showed that *Euglena* could distinguish between the stereoisomers geraniol (**271**) and nerol (**272**) and hydrogenated geraniol (**271**) selectively. (+)-, (−)-, and (±)-Citronellal (**261**, **261′**, and

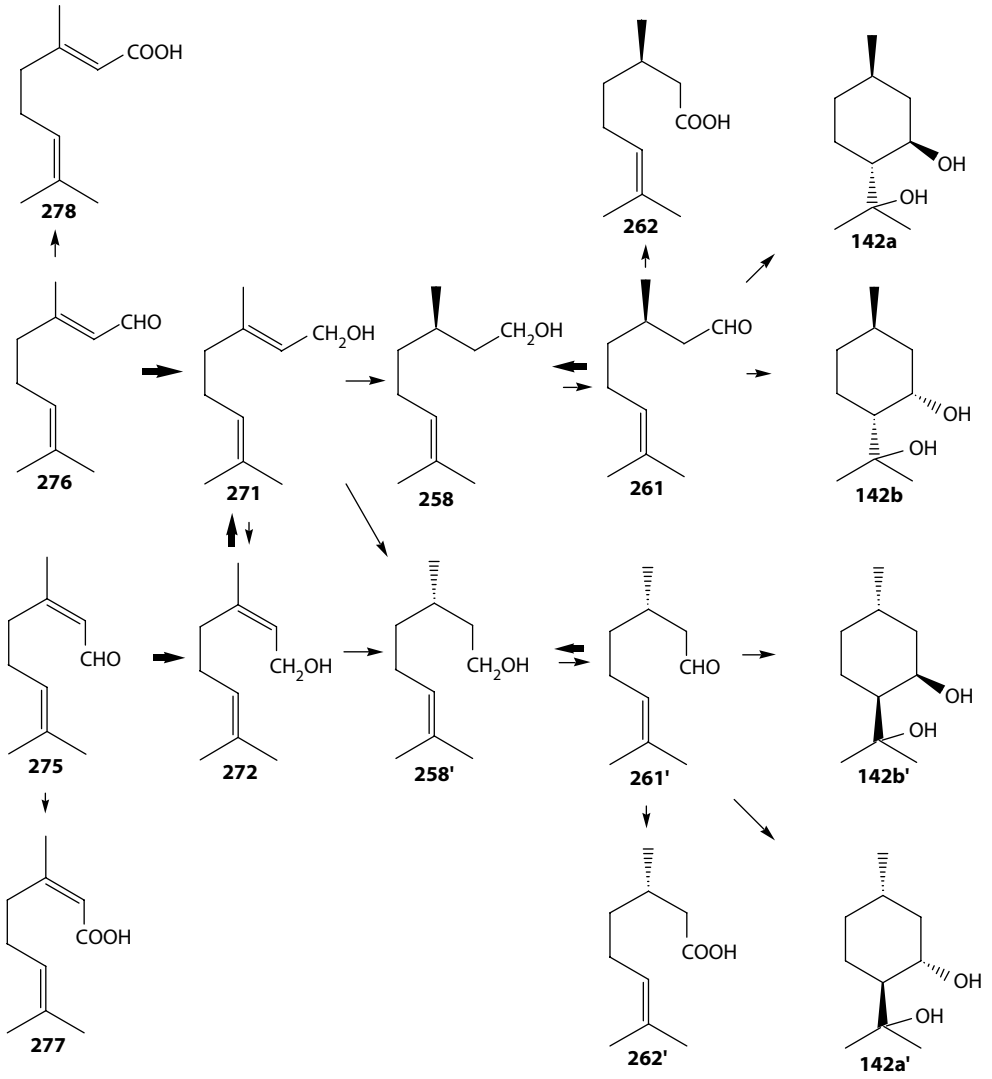


FIGURE 14.8 Metabolic pathways of citral (**275** and **276**) and its metabolites by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151.)

261 and **261′**) were also transformed to the corresponding (+)-, (−)-, and (±)-citronellol (**258**, **258′**, and **258** and **258′**) as the major products and (+)-, (−)-, and (±)-citronellic acids (**262**, **262′**, and **262** and **262′**) as the minor products, respectively (Noma et al., 1991a) (Figure 14.8).

Dunaliella tertiolecta also reduced citral (geranial (**276**) and neral (**275**) = 56:44), (+)-, (−)-, and (±)-citronellal (**261**, **261′**, and **261** and **261′**) to the corresponding alcohols, namely, geraniol (**271**), nerol (**272**), (+)-, (−)-, and (±)-citronellol (**258**, **258′**, and **258** and **258′**) (Noma et al., 1991b, 1992a).

Citral (a mixture of geranial (**276**) and neral (**275**), 56:44 peak area in GC) is easily transformed to geraniol (**271**) and nerol (**272**), respectively, of which geraniol (**32**) is further hydrogenated to (+)-citronellol (**258**) and (−)-citronellol (**258′**). Geranic acid (**278**) and neric acid (**277**) as the minor products are also formed from **276** and **275**, respectively. On the other hand, when either **271** or **272** is used as a substrate, both compounds are isomerized to each other, and then **271** is transformed to citronellol (**258** or **258′**). These results showed the *Euglena* could distinguish between

the stereoisomers, **271** and **272** and hydrogenated selectively **271** to citronellol (**258** or **258′**). (+)-, (−)-, and (±)-Citronellal (**261**, **261′**, and equal mixture of **261** and **261′**) are also transformed to the corresponding citronellol and *p*-menthane-*trans*- and *cis*-3,8-diols (**142a, b, a′** and **b′**) as the major products, which are well known as mosquito repellents and plant growth regulators (Nishimura et al., 1982; Nishimura and Noma, 1996), and (+)-, (−)-, and (±)-citronellic acids (**262**, **262′**, and equal mixture of **262** and **262′**) as the minor products, respectively.

Streptomyces ikutamanensis, Ya-2–1, also reduced citral (geranial (**276**) and neral (**275**) = 56:44), (+)-, (−)-, and (±)-citronellal (**261**, **261′**, and **261** and **261′**) to the corresponding alcohols, namely, geraniol (**271**), nerol (**272**), (+)-, (−)-, and (±)-citronellol (**258**, **258′**, **258** and **258′**). Compounds **271** and **272** were isomerized to each other. Furthermore, terpene alcohols (**258′**, **272**, and **271**) were epoxidized to give 6,7-epoxygeraniol (**274**), 6,7-epoxynerol (**273**), and 2,3-epoxycitronellol (**268**). On the other hand, (+)- and (±)-citronellol (**258** and **258** and **258′**) were not converted at all (Noma et al., 1986) (Figure 14.9).

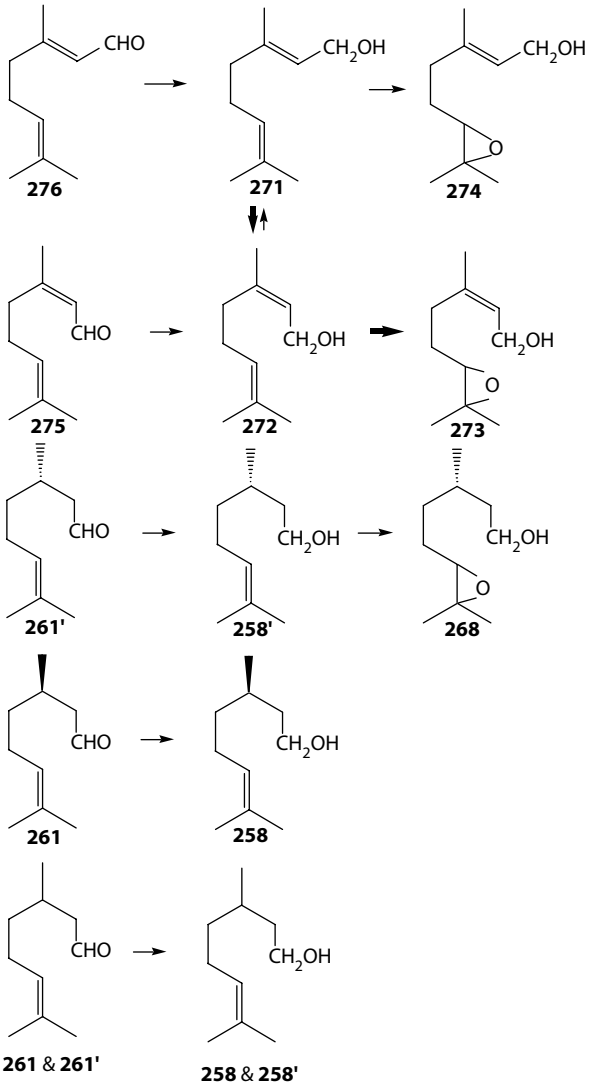


FIGURE 14.9 Reduction of terpene aldehydes and epoxidation of terpene alcohols by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206.)

A strain of *Aspergillus niger*, isolated from garden soil, was able to transform geraniol (**271**), citronellol (**258** and **258′**), and linalool (**206**) to their respective 8-hydroxy derivatives. This reaction was called "ω-hydroxylation" (Madyastha and Krishna Murthy, 1988a, 1988b).

Fermentation of citronellyl acetate with *Aspergillus niger* resulted in the formation of a major metabolite, 8-hydroxycitronellol, accounting for approximately 60% of the total transformation products, accompanied by 38% citronellol. Fermentation of geranyl acetate with *Aspergillus niger* gave geraniol and 8-hydroxygeraniol (50% and 40%, respectively, of the total transformation products).

One of the most important examples of fungal bioconversion of monoterpene alcohols is the biotransformation of citral by *Botrytis cinerea*. *Botrytis cinerea* is a fungus of high interest in wine-making (Rapp and Mandery, 1988). In an unripe state of maturation the infection of grapes by *Botrytis cinerea* is very much feared, as the grapes become mouldy ("gray rot"). With fully ripe grapes, however, the growth of *Botrytis cinerea* is desirable; the fungus is then called "noble rot" and the infected grapes deliver famous sweet wines, such as, for example, Sauternes of France or Tokay Aszu of Hungary (Brunerie et al., 1988).

One of the first reports in this area dealt with the biotransformation of citronellol (**258**) by *Botrytis cinerea* (Brunerie et al., 1987a, 1988). The substrate was mainly metabolized by ω-hydroxylation. The same group also investigated the bioconversion of citral (**275** and **276**) (Brunerie et al., 1987b). A comparison was made between grape must and a synthetic medium. When using grape must, no volatile bioconversion products were found. With a synthetic medium, biotransformation of citral (**275** and **276**) was observed yielding predominantly nerol (**272**) and geraniol (**271**) as reduction products and some ω-hydroxylation products as minor compounds. Finally, the bioconversion of geraniol (**271**) and nerol (**272**) was described by the same group (Bock et al., 1988). When using grape must, a complete bioconversion of geraniol (**271**) was observed mainly yielding ω-hydroxylation products.

The most important metabolites from geraniol (**271**), nerol (**272**), and citronellol (**258**) are summarized in Figure 14.9. In the same year the biotransformation of these monoterpenes by *Botrytis cinerea* in model solutions was described by another group (Rapp and Mandery, 1988). Although the major metabolites found were ω-hydroxylation compounds, it is important to note that some new compounds that were not described by the previous group were detected (Figure 14.9). Geraniol (**271**) was mainly transformed to (2*E*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol (**318**), (*E*)-3,7-dimethyl-2,7-octadiene-1,6-diol (**319**), and (2*E*,6*E*)-2,6-dimethyl-2,6-octadiene-1,8-diol (**300**); nerol (**272**) to (2*Z*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol (**314**), (*Z*)-3,7-dimethyl-2,7-octadiene-1,6-diol (**315**), and (2*E*,6*Z*)-2,6-dimethyl-2,6-octadiene-1,8-diol (**316**). Furthermore, a cyclization product (**318**) that was not previously described was formed. Finally, citronellol (**258**) was converted to *trans*- (**312**) and *cis*-rose oxide (**313**) (a cyclization product not identified by the other group), (*E*)-3,7-dimethyl-5-octene-1,7-diol (**311**), 3,7-dimethyl-7-octene-1,6-diol (**260**), and (*E*)-2,6-dimethyl-2-octene-1,8-diol (**265**) (Miyazawa et al., 1996a) (Figure 14.10).

One of the latest reports in this area described the biotransformation of citronellol by the plant pathogenic fungus *Glomerella cingulata* to 3,7-dimethyl-1,6,7-octanetriol (Miyazawa et al., 1996a).

The ability of fungal spores of *Penicillium digitatum* to biotransform monoterpene alcohols, such as geraniol (**271**) and nerol (**272**) and a mixture of the aldehydes, that is, citral (**276** and **275**), has only been discovered very recently by Demyttenaera and coworkers (Demyttenaera et al., 1996, 2000; Demyttenaera and De Pooter, 1996, 1998). Spores of *Penicillium digitatum* were inoculated on solid media. After a short incubation period, the spores germinated and a mycelial mat was formed. After 2 weeks, the culture had completely sporulated and bioconversion reactions were started. Geraniol (**271**), nerol (**272**), or citral (**276** and **275**) were sprayed onto the sporulated surface culture. After 1 or 2 days, the period during which transformation took place, the cultures were extracted. Geraniol and nerol were transformed into 6-methyl-5-hepten-2-one by sporulated surface cultures of *Pencillium digitatum*. The spores retained their activity for at least 2 months. An overall yield of up to 99% could be achieved.

The bioconversion of geraniol (**271**) and nerol (**272**) was also performed with sporulated surface cultures of *Aspergillus niger*. Geraniol (**271**) was converted to linalool (**206**), α-terpineol (**34**), and

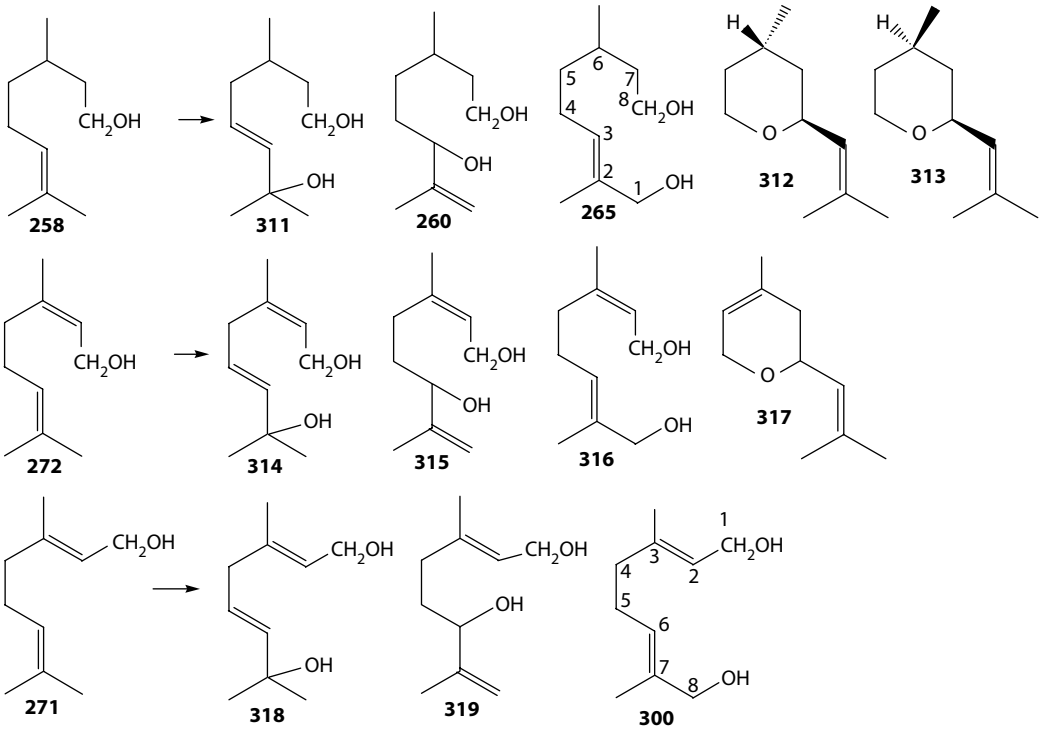


FIGURE 14.10 Biotransformation of geraniol (**271**), nerol (**272**), and citronellol (**258**) by *Botrytis cinerea*. (Modified from Miyazawa, M. et al., 1996a. *Nat. Prod. Lett.*, 8: 303–305.)

limonene (**68**), and nerol (**272**) was converted mainly to linalool (**206**) and α-terpineol (**34**) (Demyttenaera et al., 2000).

The biotransformation of geraniol (**271**) and nerol (**272**) by *Catharanthus roseus* suspension cells was carried out. It was found that the allylic positions of geraniol (**271**) and nerol (**272**) were hydroxylated and reduced to double bond and ketones (Figure 14.11). Geraniol (**271**) and nerol (**272**) were isomerized to each other. Geraniol (**271**) and nerol (**272**) were hydroxylated at C10 to

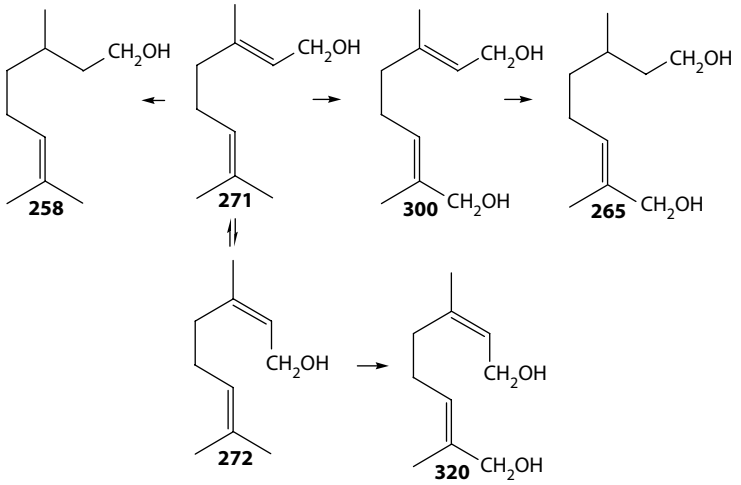


FIGURE 14.11 The biotransformation of geraniol (**271**) and nerol (**272**) by *Catharanthus roseus*. (Modified from Hamada, H. and H. Yasumune, 1995. *Proc. 39th TEAC*, pp. 375–377.)

8-hydroxygeraniol (**300**) and 8-hydroxynerol (**320**), respectively. 8-Hydroxygeraniol (**300**) was hydrogenated to 10-hydroxycitronellol (**265**). Geraniol (**271**) was hydrogenated to citronellol (**258**) (Hamada and Yasumune, 1995).

Cyanobacterium converted geraniol (**271**) to geranic acid (**278**) via geranial (**276**), followed by hydrogenation to give citronellic acid (**262**) via citronellal (**261**). Furthermore, the substrate **271** was isomerized to nerol (**272**), followed by oxidation, reduction, and further oxidation to afford neral (**275**), citronellal (**261**), citronellic acid (**262**), and nerolic acid (**277**) (Kaji et al., 2002; Hamada et al., 2004) (Figure 14.12).

Plant suspension cells of *Catharanthus roseus* converted geraniol (**271**) to 8-hydroxygeraniol (**300**). The same cells converted citronellol (**258**) to 8- (**265**) and 10-hydroxycitronellol (**264**) (Hamada et al., 2004) (Figure 14.13).

Nerol (**272**) was converted by the insect lavae *Spodoptera litura* to give 8-hydroxynerol (**320**), 10-hydroxynerol (**321**), 1-hydroxy-3,7-dimethyl-(2*E*,6*E*)-octadienal (**322**), and 1-hydroxy-3,7-dimethyl-(2*E*,6*E*)-octadienoic acid (**323**) (Takeuchi and Miyazawa, 2004) (Figure 14.14).

14.2.2.2 Linalool and Linalyl Acetate

(+)-Linalool (**206**) [(*S*)-3,7-dimethyl-1,6-octadiene-3-ol] and its enantiomer (**206′**) ((*R*)-3,7-dimethyl-1,6-octadiene-3-ol) occur in many essential oils, where they are often the main component. (*S*)-(+)-Linalool (**206**) makes up 60–70% of coriander oil. (*R*)-(−)-linalool (**206′**), for example, occurs at a concentration of 80–85% in Ho oils from *Cinnamomum camphora*; rosewood oil contains ca. 80% (Bauer et al., 1990).

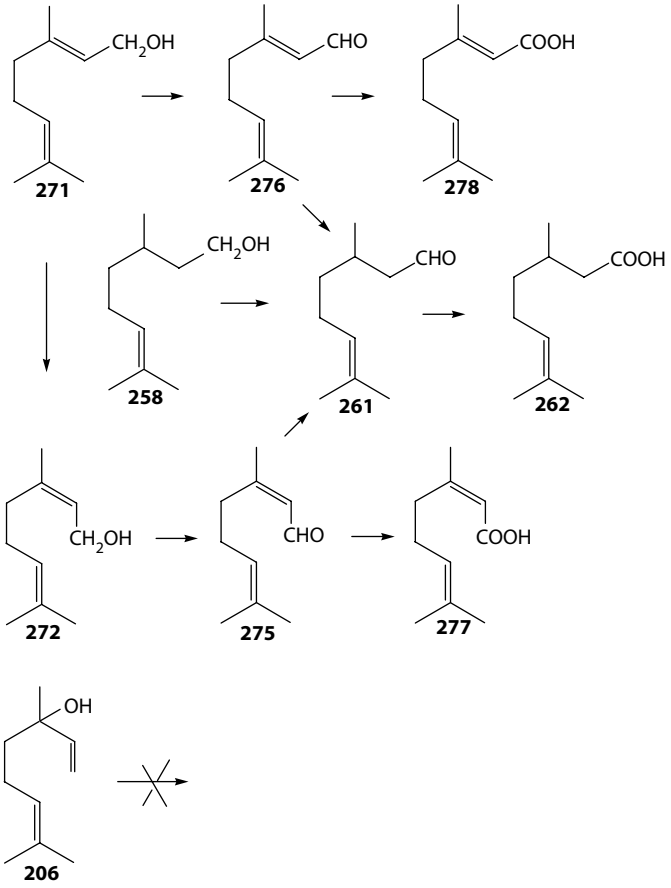


FIGURE 14.12 Biotransformation of geraniol (**271**) and citronellol (**258**) by *Cyanobacterium*.

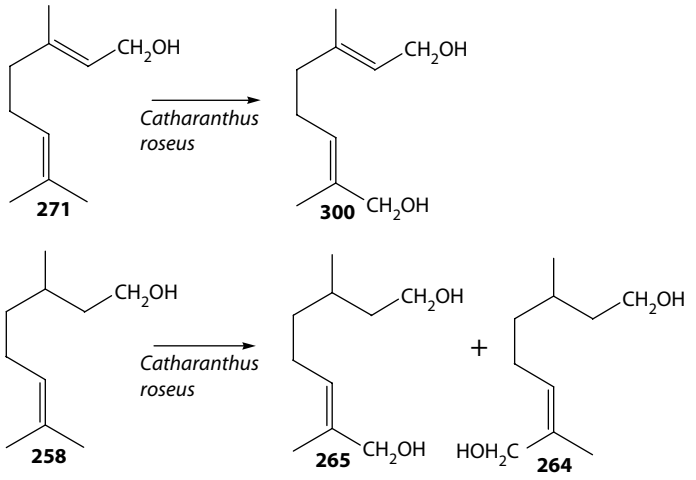


FIGURE 14.13 Biotransformation of geraniol (**271**), citronellol (**258**), and linalool (**206**) by plant suspension cells of *Catharanthus roseus*. (Modified from Hamada, H. et al., 2004. *Proc. 48th TEAC*, pp. 393–395.)

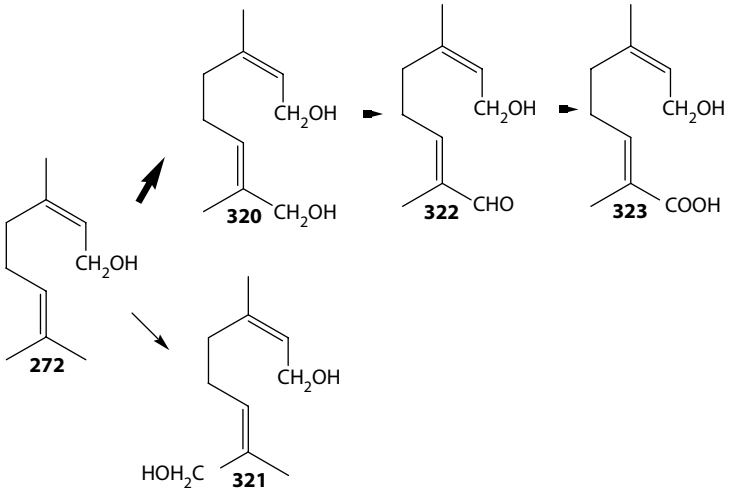


FIGURE 14.14 Biotransformation of nerol (**272**) by *Spodoptera litura*. (Modified from Takeuchi, H. and M. Miyazawa, 2004. *Proc. 48th TEAC*, pp. 399–400.)

Catharanthus roseus converted (+)-linalool (**206**) to 8-hydroxylinalool (**219**) (Hamada et al., 2004) (Figure 14.15).

The biodegradation of (+)-linalool (**206**) by *Pseudomonas pseudomallei* (strain A), which grows on linalool as the sole carbon source, was described in 1973 (Murakami et al., 1973) (Figure 14.16).

Madyastha et al. (1977) isolated a soil Pseudomonad, *Pseudomonas incognita*, by the enrichment culture technique with linalool as the sole carbon source. This microorganism, the "linalool strain" as it was called, was also capable of utilizing limonene (**68**), citronellol (**258**), and geraniol (**271**) but failed to grow on citral (**275** and **276**), citronellal (**261**), and 1,8-cineole (**122**). Fermentation was carried out with shake cultures containing 1% linalool (**206**) as the sole carbon source. It was suggested by the authors that linalool (**206**) was metabolized by at least three different pathways of biodegradation (Figure 14.19). One of the pathways appeared to be initiated by the specific oxygenation of C-8 methyl group of linalool (**206**), leading to 8-hydroxylinalool (**219**), which was further oxidized to linalool-8-carboxylic acid (**220**). The presence of furanoid linalool oxide (**215**) and 2-methyl-2-vinyltetrahydrofuran-5-one (**216**) as the unsaturated lactone in the fermentation medium

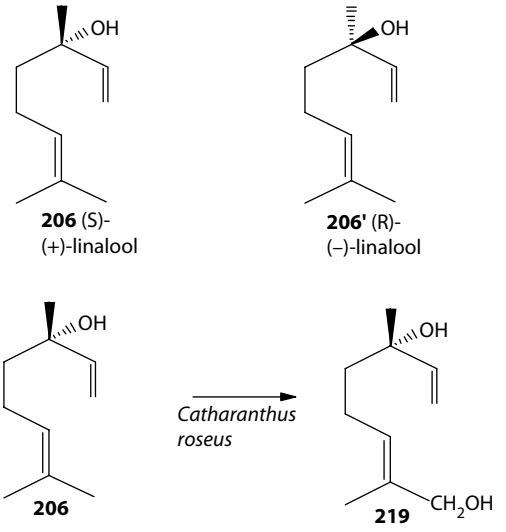


FIGURE 14.15 Biotransformation of linalool (**206**) by plant suspension cells of *Catharanthus roseus*. (Modified from Hamada, H. et al., 2004. *Proc. 48th TEAC*, pp. 393–395.)

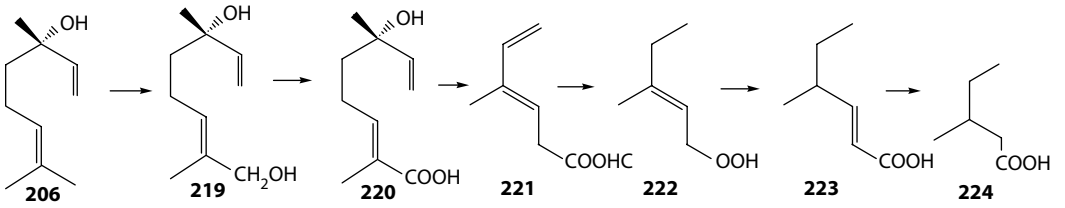


FIGURE 14.16 Degradative metabolic pathway of (+)-linalool (**206**) by *Pseudomonas pseudomallei*. (Modified from Murakami, T. et al., 1973. *Nippon Nogei Kagaku Kaishi*, 47: 699–703.)

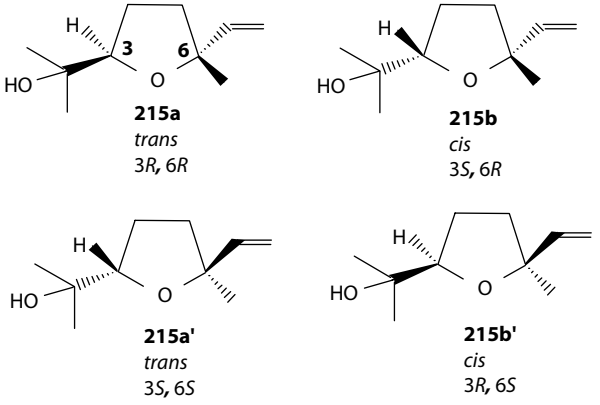


FIGURE 14.17 Four stereoisomers of furanoid linalool oxides. (Modified from Noma, Y. et al., *Proc. 30th TEAC*, pp. 204–206.)

suggested another mode of utilization of linalool (**206**). The formation of these compounds was believed to proceed through the epoxidation of the 6,7-double bond giving rise to 6,7-epoxylinalool (**214**), which upon further oxidation yielded furanoid linalool oxide (**215**) and 2-methyl-2-vinyl-tetrahydrofuran-5-one (**216**) (Figure 14.19).

The presence of oleuropeic acid (**204**) in the fermentation broth suggested a third pathway. Two possibilities were proposed: (3a) water elimination giving rise to a monocyclic cation (**33**), yielding

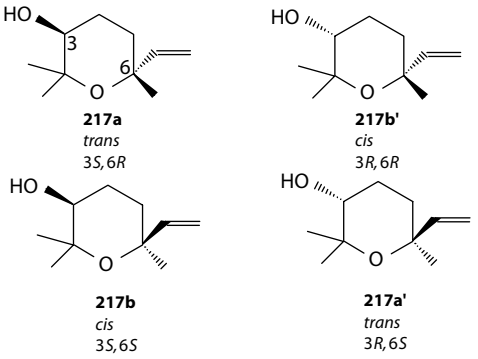


FIGURE 14.18 Four stereoisomers of pyranoid linalool oxides.

α-terpineol (**34**), which upon oxidation gave oleuropeic acid (**204**); (3b) oxidation of the C-10 methyl group of linalool (**206**) before cyclization, giving rise to oleuropeic acid (**204**). This last pathway was also called the "prototropic cyclization" (Madyastha, 1984).

Racemic linalool (**206** and **206′**) is cyclized into *cis*- and *trans*-linalool oxides by various microorganisms such as *Streptomyces albus* NRRL B1865, *Streptomyces hygroscopicus* NRRL B3444, *Streptomyces cinnamonnensis* ATCC 15413, *Streptomyces griseus* ATCC 10137, and *Beauveria sulfurescens* ATACC 7159 (David and Veschambre, 1984) (Figure 14.19).

Aspergillus niger isolated from garden soil biotransformed linalool and its acetates to give linalool (**206**), 2,6-dimethyl-2,7-octadiene-1,6-diol (8-hydroxylinalool (**219a**), α-terpineol (**34**), geraniol (**271**), and some unidentified products in trace amounts (Madyastha and Krishna Murthy, 1988a, 1988b).

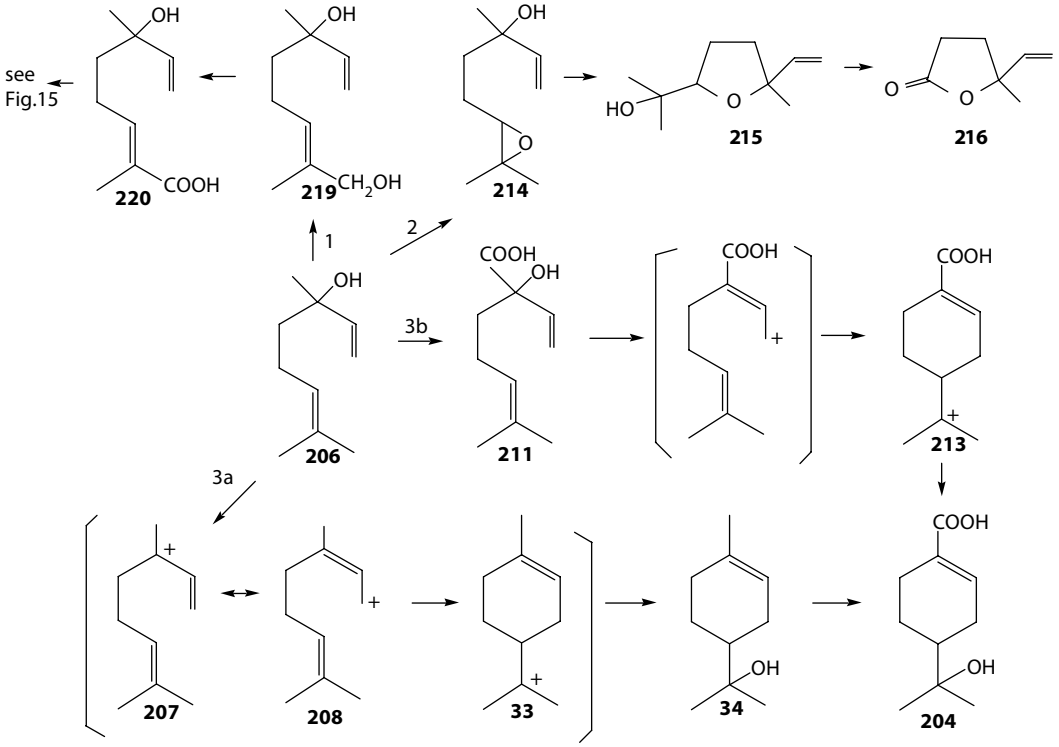


FIGURE 14.19 Biotransformation of linalool (**206**) by *Pseudomonas incognita* (Madyastha et al., 1977) and *Streptomyces albus* NRRL B1865. (Modified from David, L. and H. Veschambre, 1984. *Tetrahadron Lett.*, 25: 543–546.)

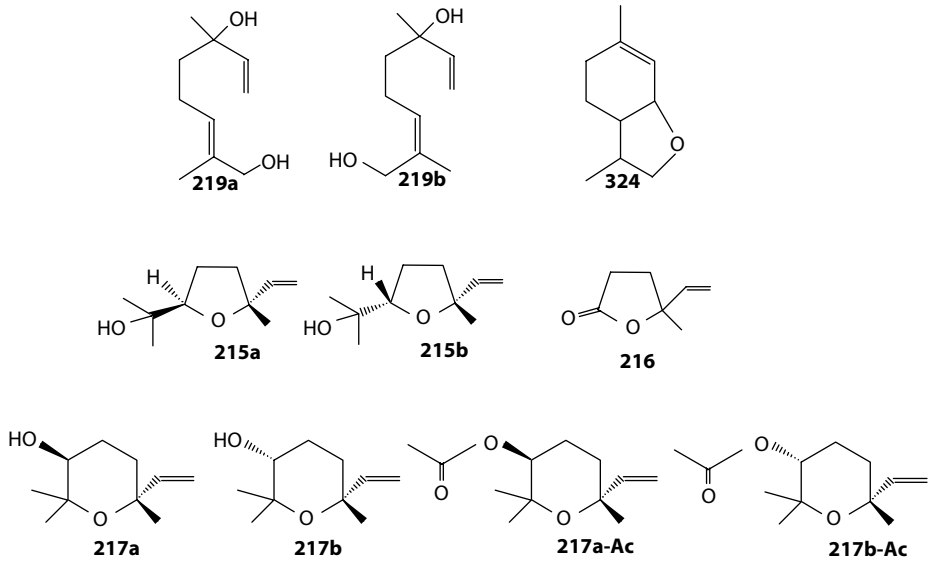


FIGURE 14.20 Biotransformation products of linalool (**206**) by *Botrytis cinerea*. (Modified from Bock, G. et al., 1986. *J. Food Sci.*, 51: 659–662.)

The biotransformation of linalool (**206**) by *Botrytis cinerea* was carried out and identified transformation products such as (*E*)-(**219a**) and (*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol (**219b**), *trans*-(**215a**) and *cis*-furanoid linalool oxide (**215b**), *trans*- (**217a**) and *cis*-pyranoid linalool oxide (**217b**) (Figure 14.18) and their acetates (**217a-Ac**, **217b-Ac**), 3,9-epoxy-*p*-menth-1-ene (**324**) and 2-methyl-2-vinyl-tetrahydrofuran-5-one (**216**) (unsaturated lactone) (Bock et al., 1986) (Figure 14.20). Quantitative analysis, however, showed that linalool (**206**) was predominantly (90%) metabolized to (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol (**219a**) by *Botrytis cinerea*. The other compounds were only found as by-products in minor concentrations.

The bioconversion of (*S*)-(+)-linalool (**206**) and (*R*)-(−)-linalool (**206′**) was investigated with *Diplodia gossypina* ATCC 10936 (Abraham et al., 1990). The biotransformation of (±)-linalool (**206** and **206′**) by *Aspergillus niger* ATCC 9142 with submerged shaking culture yielded a mixture of *cis*- (**215b**) and *trans*-furanoid linalool oxide (**215a**) (yield 15–24%) and *cis*- (**217b**) and *trans*-pyranoid linalool oxide (**217a**) (yield 5–9%) (Demyttenaere and Willemen, 1998). The biotransformation of (*R*)-(−)-linalool (**206a**) with *Aspergillus niger* ATCC 9142 yielded almost pure *trans*-furanoid linalool oxide (**215a**) and *trans*-pyranoid linalool oxide (**217a**) (ee > 95) (Figure 14.21). These conversions were purely biocatalytic, since in acidified water (pH < 3.5) almost 50% linalool (**206**) was recovered unchanged, the rest was evaporated. The biotransformation was also carried out with growing surface cultures.

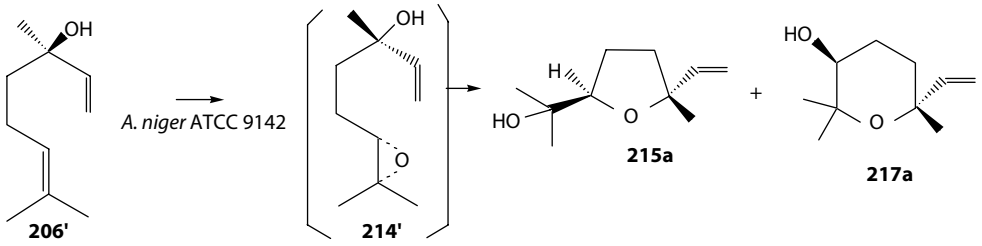


FIGURE 14.21 Biotransformation of (*R*)-(−)-linalool (**206′**) by *Aspergillus niger* ATCC 9142. (Modified from Demyttenaere, J.C.R. and H.M. Willemen, 1998. *Phytochemistry*, 47: 1029–1036.)

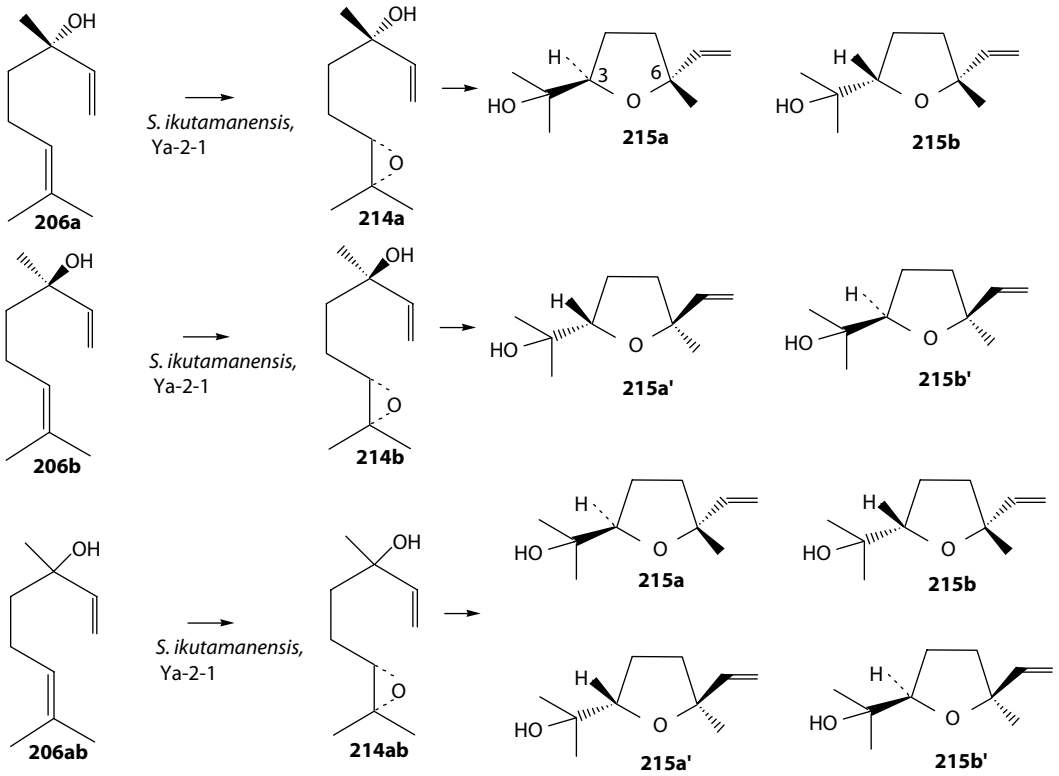


FIGURE 14.22 Metabolic pathway of (+)- (**206**), (–)- (**206′**), and racemic linalool (**206** and **206′**) by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206.)

Streptomyces ikutamanensis, Ya-2-1 also converted (+)- (**206**), (–)- (**206′**), and racemic linalool (**206** and **206′**) via corresponding 2,3-epoxides (**214** and **214′**) to *trans*- and *cis*-furanoid linalool oxides (**215a**, **b**, **a′** and **b′**) (Noma et al., 1986) (Figure 14.22). The absolute configuration at C-3 and C-6 of *trans*- and *cis*-linalool oxides are shown in Figure 14.17.

Biotransformation of racemic *trans*-pyranoid linalool oxide (**217a** and **a′**) and racemic *cis*-linalool-pyranoid (**217b** and **b′**) has been carried out using fungus *Glomerella cingulata* (Miyazawa et al., 1994a). *trans* and *cis*-Pyranoid linalool oxide (**217a** and **217b**) were transformed to *trans*- (**217a′-1**) and *cis*-linalool oxide-3-malonate (**217b′-1**), respectively. In the biotransformation of racemic *cis*-linalool oxide-pyranoid, (+)-(3*R*,6*R*)-*cis*-pyranoid linalool oxide (**217a** and **a′**) was converted to (3*R*,6*R*)-pyranoid-*cis*-linalool oxide-3-malonate (**217a′-1**). (–)-(3*S*, 6*S*)-*cis*-Pyranoid linalool oxide-pyranoid (**217a′**) was not metabolized. On the other hand, in the biotransformation of racemic *trans*-pyranoid linalool oxide (**217b** and **b′**), (–)-(3*R*,6*S*)-*trans*-linalool oxide (**217b′**) was transformed to (3*R*,6*S*)-*trans*-linalool oxide-3-malonate (**217b′-1**) (Figure 14.23). (+)-(3*S*,6*S*)-*trans*-Pyranoid-linalool oxide (**217b**) was not metabolized. These facts showed that *Glomerella cingulata* recognized absolute configuration of the secondary hydroxyl group at C-3. On the basis of this result, it has become apparent the optical resolution of racemic pyranoid linalool oxide proceeded in the biotransformation with *Glomerella cingulata* (Miyazawa et al., 1994a).

Linalool (**206**) and tetrahydrolinalool (**325**) were converted by suspension cells of *Catharanthus roseus* to give 1-hydroxylinalool (**219**) from linalool (**206**) and 3,7-dimethyloctane-3,5-diol (**326**), 3,7-dimethyloctane-3,7-diol (**327**), and 3,7-dimethyloctane-3,8-diol (**328**) from tetrahydrolinalool (**325**) (Hamada and Furuya, 2000; Hamada et al., 2004) (Figure 14.24).

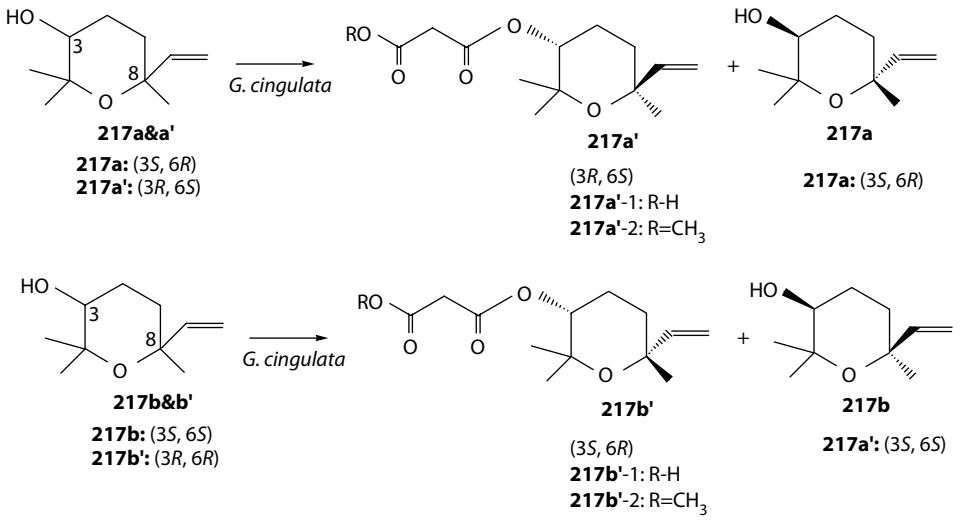


FIGURE 14.23 Biotransformation of racemic *trans*-linalool oxide-pyranoid (**217a** and **a′**) and racemic *cis*-linalool-pyranoid (**217b** and **b′**) by *Glomerella cingulata*. (Modified from Miyazawa, M. et al., 1994a. *Proc. 38th TEAC*, pp. 101–102.)

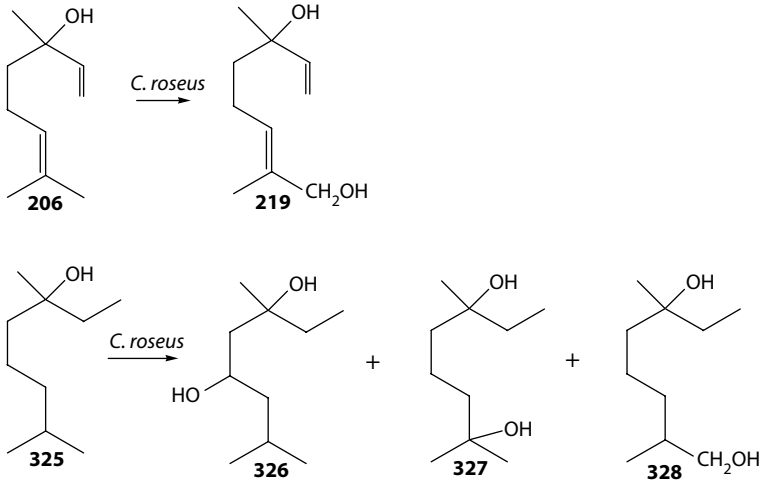


FIGURE 14.24 Biotransformation of linalool (**206**) and tetrahydrolinalool (**325**) by *Catharanthus roseus*. (Modified from Hamada, H. and T. Furuya, 2000. *Proc. 44th TEAC*, pp. 167–168; Hamada, H. et al., 2004. *Proc. 48th TEAC*, pp. 393–395.)

(±)-Linalyl acetate (**206-Ac**) was hydrolyzed to (+)-(*S*)-linalool (**206**) and (±)-linallyl acetate (**206-Ac**) by *Bacillus subtilis*, *Trichoderma S*, *Absidia glauca*, and *Gibberella fujikuroi* as shown in Figure 14.25. But, (±)-dihydrolinallyl acetate (**469-Ac**) was not hydrolyzed by the above microorganisms (Oritani and Yamashita, 1973a).

14.2.2.3 Dihydromycenol

Dihydromyrcenol (**329**) was fed by *Spodptera litura* to give 1,2-epoxydihydro-myrcenol (**330**) as a main products and 3β-hydroxydidyromyrcerol (**331**) as a minor product. Dihydromyrcenyl acetate (**332**) was converted to 1,2-dihydroxydihydromyrcenyl acetate (**333**) (Murata and Miyazawa, 1999) (Figure 14.26).

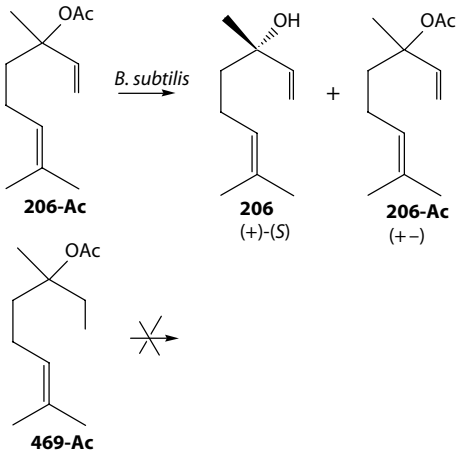


FIGURE 14.25 Hydrolysis of (±)-linalyl acetate (**206-Ac**) by microorganisms. (Modified from Oritani, T. and K. Yamashita, 1973a. *Agric. Biol. Chem*., 37: 1923–1928.)

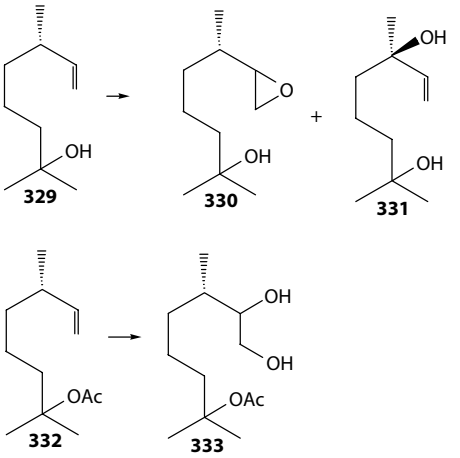
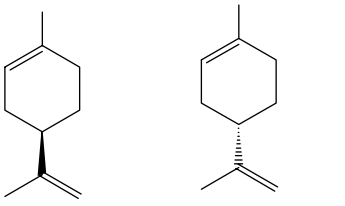


FIGURE 14.26 Biotransformation of dihydromyrcenol (**329**) and dihydromyrcenyl acetate (**332**) by *Spodptera litura*. (Modified from Murata, T. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 393–394.)

14.3 METABOLIC PATHWAYS OF CYCLIC MONOTERPENOIDS

14.3.1 Monocyclic Monoterpene Hydrocarbon

14.3.1.1 Limonene



68
(*R*)-limonene

68'
(*S*)-limonene

Limonene is the most widely distributed terpene in nature after α-pinene (**4**) (Krasnobajew, 1984). (4*R*)-(+)-Limonene (**68**) is present in *Citrus* peel oils at a concentration of over 90%; a low concentration of the (4*S*)-(–)-limonene (**68′**) is found in oils from the *Mentha* species and conifers

(Bauer et al., 1990). The first microbial biotransformation on limonene was carried out by using a soil Pseudomonad. The microorganism was isolated by the enrichment culture technique on limonene as the sole source of carbon (Dhavalikar and Bhattacharyya, 1966). The microorganism was also capable of growing on α-pinene (**4**), β-pinene (**1**), 1-*p*-menthene (**62**), and *p*-cymene (**178**). The optimal level of limonene for growth was 0.3–0.6% (v/v) although no toxicity was observed at 2% levels. Fermentation of limonene (**68**) by this bacterium in a mineral-salts medium resulted in the formation of a large number of neutral and acidic products such as dihydrocarvone (**64**), carvone (**61**), carveol (**60**), 8-*p*-menthene-1,2-*cis*-diol (**65b**), 8-*p*-menthen-1-ol-2-one (**66**), 8-*p*-menthene-1,2-*trans*-diol (**65a**), and 1-*p*-menthene-6,9-diol (**62**). Perillic acid (**69**), β-isopropenyl pimeric acid (**72**), 2-hydroxy-8-*p*-menthen-7-oic acid (**70**), and 4,9-dihydroxy-1-*p*-menthen-7-oic acid (**73**) were isolated and identified as acidic compounds. Based on these data three distinct pathways for the catabolism of limonene (**68**) by the soil Pseudomonad were proposed by Dhavalikar et al. (1966), involving allylic oxygenation (pathway1), oxygenation of the 1,2-double bond (pathway 2), and progressive oxidation of the 7-methyl group to perillic acid (**82**) (pathway 3) (Figure 14.27) (Krasnobajew, 1984). Pathway

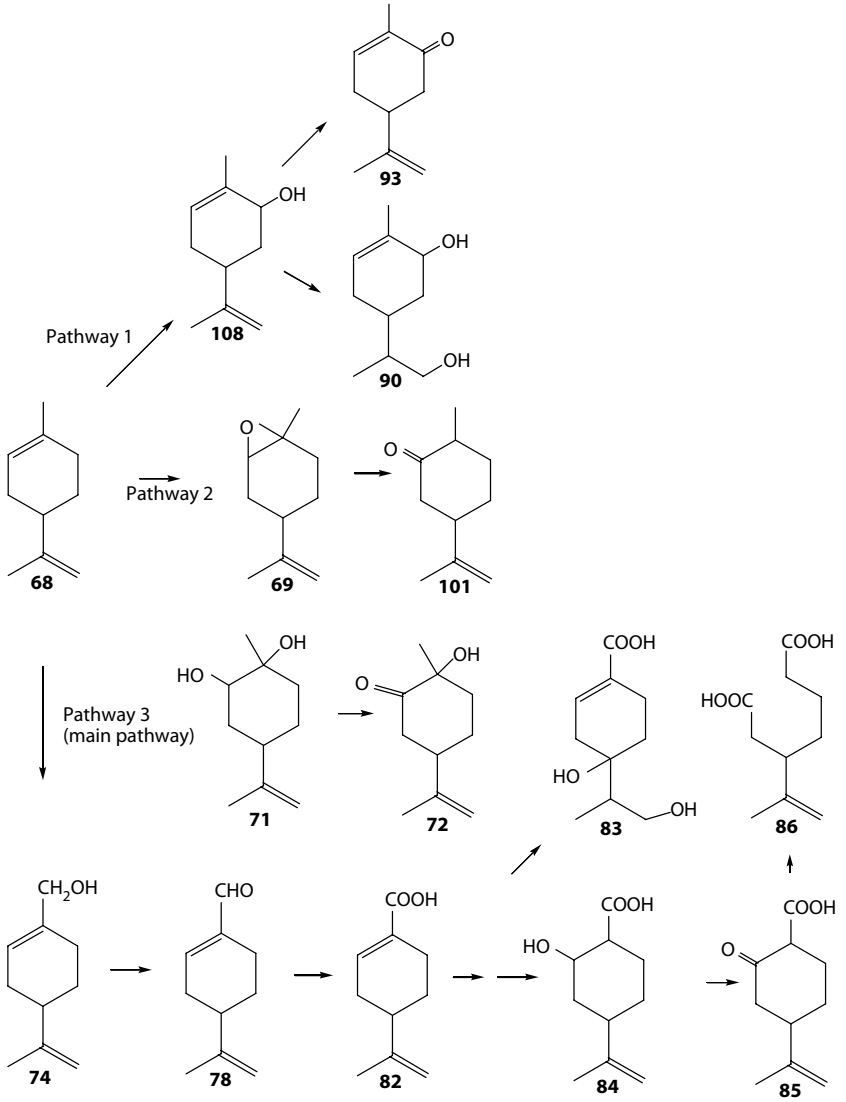


FIGURE 14.27 Pathways for the degradation of limonene (**68**) by a soil Pseudomonad sp. strain (L). (Modified from Krasnobajew, V., 1984. In: *Biotechnology*, K. Kieslich, ed., Vol. 6a, pp. 97–125. Weinheim: Verlag Chemie.)

2 yields (+)-dihydrocarvone (**101**) via intermediate limonene epoxide (**69**) and 8-*p*-menthen-1-ol-2-one (**72**) as oxidation product of limonene-1,2-diol (**71**). The third and main pathway leads to perillyl alcohol (**74**), perillaldehyde (**78**), perillic acid (**82**), constituents of various essential oils and used in the flavour and fragrance industry (Fenaroli, 1975), 2-oxo-8-*p*-menthen-7-oic acid (**85**), β-isopropenyl pimeric acid (**86**), and 4,9-dihydroxy-1-*p*-menthene-7-oic acid (**83**).

(+)-Limonene (**68**) was biotransformed via limonene-1,2-epoxide (**69**) to 8-*p*-menthene 1,2-*trans*-diol (**71b**). On the other hand, (+)-carvone (**93**) was biotransformed via (−)-isodihydrocarvone (**101b**) and 1α-hydroxydihydrocarvone (**72**) to (+)-8-*p*-menthene-1,2-*trans*-diol (**71a**) (Noma et al., 1985a, 1985b) (Figure 14.28). A soil Pseudomonad formed 1-hydroxydihydrocarvone (**72**), 8-*p*-menthene-1,2-*trans*-diol (**71b**) from (+)-limonene (**68**). Dhavalikar and Bhattacharyya (1966) considered that the formation of 1-hydroxy-dihydrocarvone (**66**) is from dihydrocarvone (**64**).

Pseudomonas gladioli was isolated by an enrichment culture technique from pine bark and sap using a mineral salts broth with limonene as the sole carbon source (Cadwallander et al., 1989; Cadwallander and Braddock, 1992). Fermentation was performed during 4–10 days in shake flasks at 25°C using a pH 6.5 mineral salts medium and 1.0% (+)-limonene (**68**). Major products were identified as (+)-α-terpineol (**34**) and (+)-perillic acid (**82**). This was the first report of the microbial conversion of limonene to (+)-α-terpineol (**34**).

The first data on fungal bioconversion of limonene (**68**) date back to the late 1960s (Kraidman et al., 1969; Noma, 2007). Three soil microorganisms were isolated on and grew rapidly in mineral salts media containing appropriate terpene substrates as sole carbon sources. The microorganisms belonged to the class Fungi Imperfecti, and they had been tentatively identified as *Cladosporium*

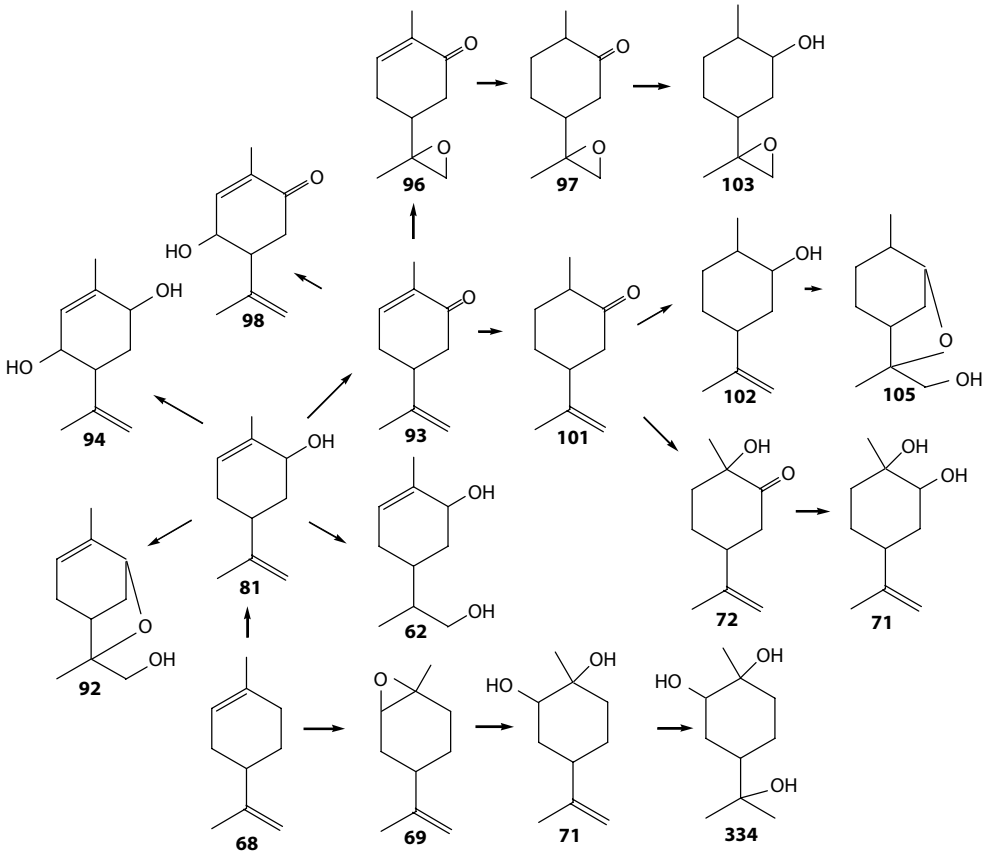


FIGURE 14.28 Formation of (+)-8-*p*-menthene-1,2-*trans*-diol (**71b**) in the biotransformation of (+)-limonene (**68**) and (+)-carvone (**93**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68; Noma, Y. et al., *Proc. 29th TEAC*, pp. 235–237.)

species. One of these strains, designated as *Cladosporium* sp. T_7 was isolated on (+)-limonene (**68a**). The growth medium of this strain contained 1.5 g/L of *trans*-limonene-1,2-diol (**71a**). Minor quantities of the corresponding *cis*-1,2-diol (**71b**) were also isolated. The same group isolated a fourth microorganism from a terpene-soaked soil on mineral salts media containing (+)-limonene as the sole carbon source (Kraidman et al., 1969). The strain, *Cladosporium*, designated T_{12}, was capable of converting (+)-limonene (**68a**) into an optically active isomer of α-terpineol (**34**) in yields of approximately 1.0 g/L.

α-Terpineol (**34**) was obtained from (+)-limonene (**68**) by fungi such as *Penicillium digitatum*, *Pencillium italicum*, and *Cladosporium* and several bacteria (Figure 14.29). (+)-*cis*-Carveol (**81b**), (+)-carvone (**93**) [an important constituent of caraway seed and dill-seed oils (Fenaroli, 1975; Bouwmester et al., 1995), and 1-*p*-menthene-6,9-diol (**90**) were also obtained by *Pencillium digitatum* and *Pencillium italicum*. (+)-(*S*)-Carvone (**93**) is a natural potato sprout inhibiting, fungistatic, and bacteriostatic compound (Oosterhaven et al., 1995a, 1995b). It is important to note that (−)-carvone (**93′**, the "spearmint flavour"] was not yet described in microbial transformation (Krasnobajew, 1984). However, the biotransformation of limonene to (−)-carvone (**93′**) was patented by a Japanese group (Takagi et al., 1972). *Corynebacterium* species grown on limonene was able to produce about 10 mg/L of 99% pure (−)-carvone (**93′**) in 24–48 h.

Mattison et al. (1971) isolated *Penicillium* sp. cultures from rotting orange rind that utilized limonene (**68**) and converted it rapidly to α-terpineol (**34**). Bowen (1975) isolated two common *Citrus* moulds, *Penicillium italicum* and *Penicillium digitatum*, responsible for the postharvest diseases of *Citrus* fruits. Fermentation of *Penicillium italicum* on limonene (**68**) yielded *cis*- (**81b**) and *trans*-carveol (**81a**) (26%) as the main products, together with *cis*- and *trans*-*p*-mentha-2,8-dien-1-ol (**73**) (18%), (+)-carvone (**93′**) (6%), *p*-mentha-1,8-dien-4-ol (**80**) (4%), perillyl alcohol (**74**) (3%), and 8-*p*-menthene-1,2-diol (**71**) (3%). Conversion of **68** by *Penicillium digitatum* yielded the same products in lower yields (Figure 14.29).

The biotransformation of limonene (**68**) by *Aspergillus niger* is a very important example of fungal bioconversion. Screening for fungi capable of metabolizing the bicyclic hydrocarbon terpene α-pinene (**4**) yielded a strain of *Aspergillus niger* NCIM 612 that was also able to transform limonene (**68**) (Rama Devi and Bhattacharyya, 1978). This fungus was able to carry out three types of oxygenative rearrangements α-terpineol (**34**), carveol (**81**), and *p*-mentha-2,8-dien-1-ol (**73**) (Rama Devi and Bhattacharyya, 1978) (Figure 14.30). In 1985, Abraham et al. (1985) investigated the biotransformation of (*R*)-(+)-limonene (**68a**) by the fungus *Penicillium digitatum*. A complete transformation for the substrate to α-terpineol (**34**) by *Penicillium digitatum* DSM 62840 was obtained with 46% yield of pure product.

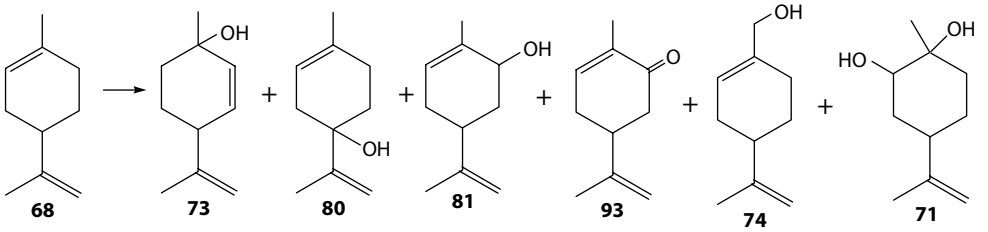


FIGURE 14.29 Biotransformation products of limonene (**68**) by *Penicillium digitatum* and *Penicillium italicum*. (Modified from Bowen, E.R., 1975. *Proc. Fla. State Hortic. Soc.*, 88: 304–308.)

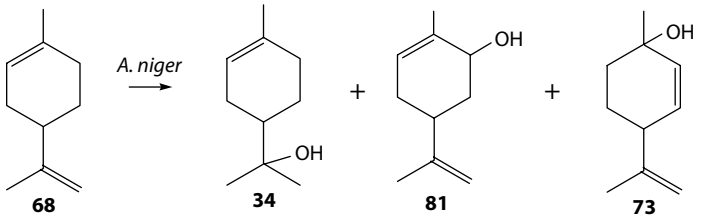


FIGURE 14.30 Biotransformation of limonene (**68**) by *Aspergillus niger* NCIM 612. (Modified from Rama Devi, J. and P.K. Bhattacharyya, 1978. *J. Indian Chem. Soc.*, 55: 1131–1137.)

The production of glycols from limonene (**68**) and other terpenes with a 1-menthene skeleton was reported by *Corynespora cassiicola* DSM 62475 and *Diplodia gossypina* ATCC 10936 (Abraham et al., 1984). Accumulation of glycols during fermentation was observed. An extensive overview on the microbial transformations of terpenoids with a 1-*p*-menthene skeleton was published by Abraham et al. (1986).

The biotransformation of (+)-limonene (**68**) was carried out by using *Aspergillus cellulosae* M-77 (Noma et al., 1992b) (Figure 14.32). It is important to note that (+)-limonene (**68a**) was mainly

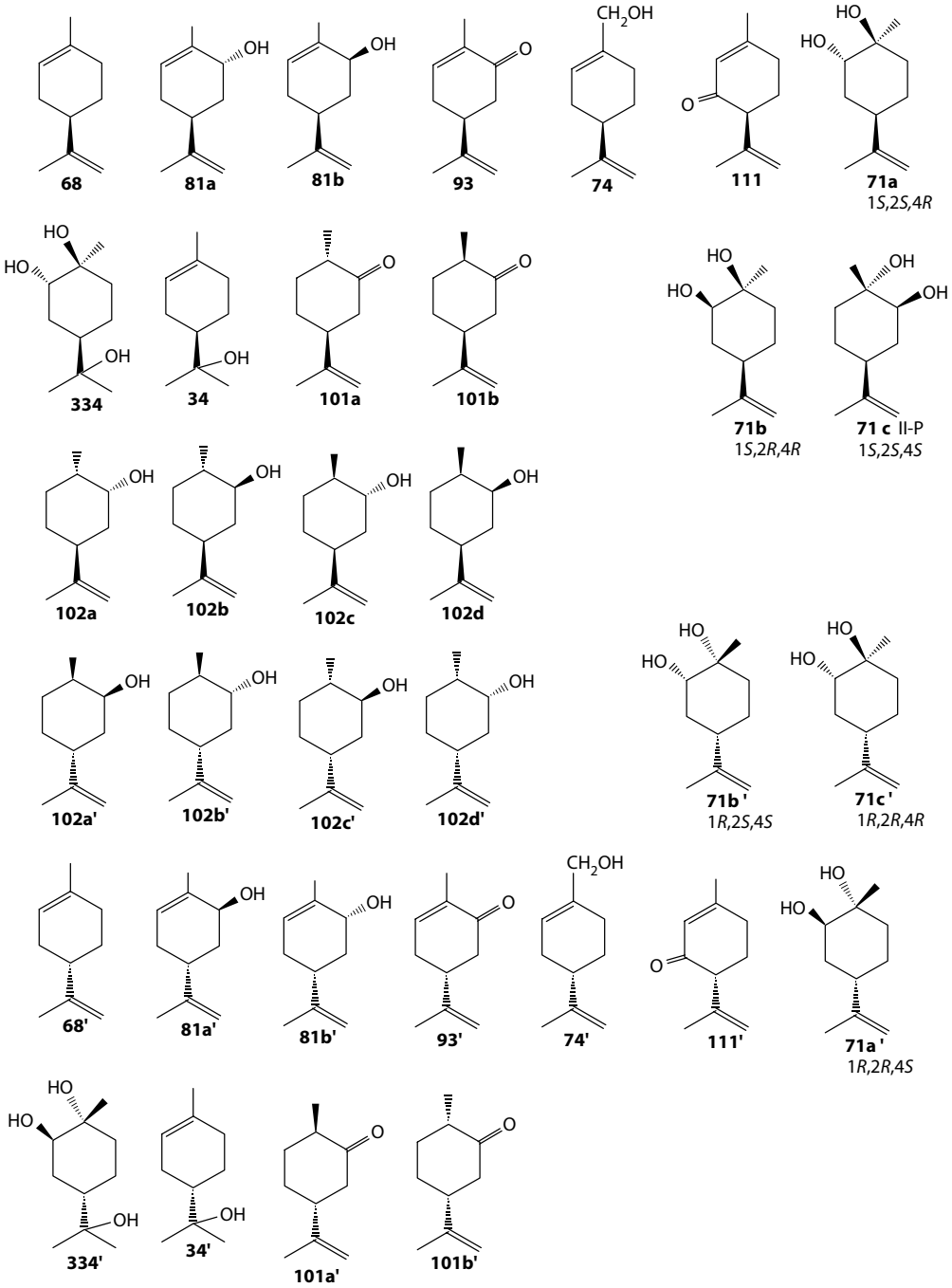


FIGURE 14.31 (+)- and (−)-limonenes (**68** and **68′**) and related compounds.

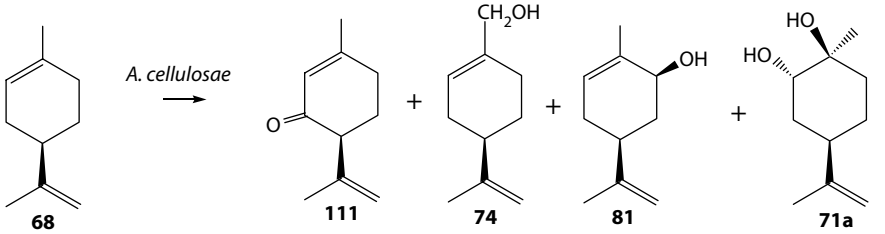


FIGURE 14.32 Biotransformation of (+)-limonene (**68**) by *Aspergillus cellulosae* IFO4040. (Modified from Noma, Y. et al., 1992b. *Phytochemistry*, 31: 2725–2727.)

converted to (+)-isopiperitenone (**111**) (19%) as new metabolite, (1*S*,2*S*,4*R*)-(+)-limonene-1,2-*trans*-diol (**71a**) (21%), (+)-*cis*-carveol (**81b**) (5%), and (+)-perillyl alcohol (**74**) (12%) (Figure 14.32).

(+)-Limonene (**68**) was biotransformed by a kind of *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. to isopiperitenone (**111**, 7% GC ratio), 2α-hydroxy-1,8-cineole (**125b**, 7%), (+)-limonene-1,2-*trans*-diol (**71a**, 6%), and (+)-*p*-menthane-1β,2α,8-triol (**334**, 45%) as main products and (+)-*trans*-sobrerol (**95a**, 2%), (+)-*trans*-carveol (**81a**), (+)-carvone (**93**), (−)-isodihydrocarvone (**101b**), and (+)-*trans*-isopiperitenol (**110a**) as minor products (Noma and Asakawa, 2006a, 2007a) (Figure 14.33). The metabolic pathways of (+)-limonene by *Penicillium digitatum* is shown in Figure 14.34.

On the other hand, (−)-limonene (**68′**) was also biotransformed by a kind of *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. to give isopiperitenone (**111′**), 2α-hydroxy-1,8-cineole (**125b′**), (−)-limonene-1,2-*trans*-diol (**71′**), and *p*-menthane-1,2,8-triol (**334′**) as main products together with (+)-*trans*-sobrerol (**80′**), (+)-*trans*-carveol (**81a′**), (−)-carvone (**93′**), (−)-dihydrocarvone (**101a′**), and (+)-isopiperitenol (**110a′**) as minor products (Noma and Asakawa, 2007b) (Figure 14.35.)

Newly isolated unidentified red yeast, *Rhodotorula* sp., converted (+)-limonene (**68**) mainly to (+)-limonene-1,2-*trans*-diol (**71a**), (+)-*trans*-carveol (**81a**), (+)-*cis*-carveol (**81b**), and (+)-carvone (**93′**) together with (+)-limonene-1,2-*cis*-diol (**71b**) as minor product (Noma and Asakawa, 2007b) (Figure 14.36).

Cladosporium sp. T_7 was cultivated with (+)-limonene (**68**) as the sole carbon source; it converted **68** to *trans*-*p*-menthane-1,2-diol (**71a**) (Figure 14.36) (Mukherjee et al., 1973).

On the other hand, the same red yeast converted (−)-limonene (**68′**) mainly to (−)-limonene-1,2-*trans*-diol (**71a′**), (−)-*trans*-carveol (**81a′**), (−)-*cis*-carveol (**81b′**), and (−)-carvone (**93′**) together with (−)-limonene-1,2-*cis*-diol (**71b′**) as minor product (Noma and Asakawa, 2007b) (Figure 14.37).

The biotransformation of (+)- and (−)-limonene (**68** and **68′**), (+)- and (−)-α-terpineol (**34** and **34′**), (+)- and (−)-limonene-1,2-epoxide (**69** and **69′**), and caraway oil was carried out by *Citrus*

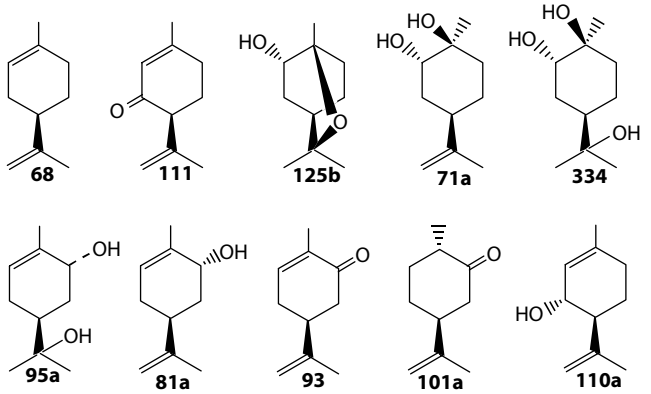


FIGURE 14.33 Metabolites of (+)-limonene (**68**) by a kind of *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. (Modified from Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

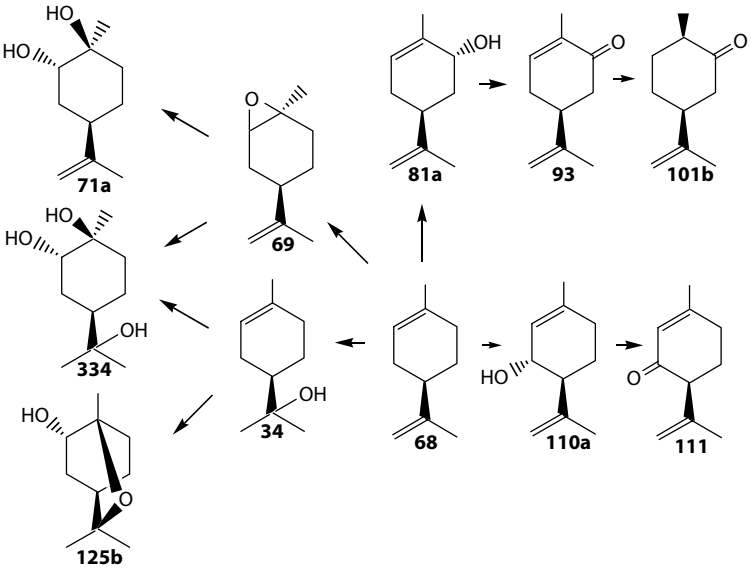


FIGURE 14.34 Biotransformation of (+)-limonene (**68**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. (Modified from Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

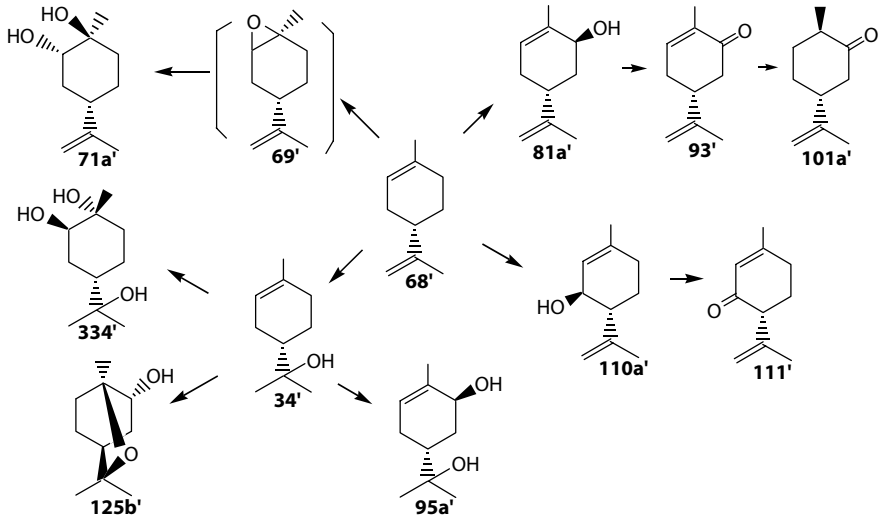


FIGURE 14.35 Biotransformation of (–)-limonene (**68′**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN. (Modified from Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

pathogenic fungi *Penicillium* (Pers.; Fr.) Sacc. KCPYN and newly isolated red yeast, a kind of *Rhodotorula* sp. *Penicillium digitatum* KCPYN converted limonenes (**68** and **68′**) to the corresponding isopiperitone (**111** and **111′**), 1α-hydroxy-1,8-cineole (**125b** and **125b′**), limonene-1,2-*trans*-diol (**71a** and **71a′**), *p*-menthane-1,2,8-triol (**334** and **334′**), and *trans*-sobrerol as main products. (+)- and (–)-α-Terpineol (**34** and **34′**) were the precursors of 2α-hydroxy-1,8-cineole (**125b** and **b′**) and *p*-menthane-1,2,8-triol (**334**). (+)- and (–)-Limonene-1,2-epoxide (**69** and **69′**) were also the precursor of limonene-1,2-*trans*-diol (**71a**). *Rhodotorula* sp. also biotransformed (+)- and (–)-limonene (**68** and **68′**) to the corresponding *trans*- and *cis*-carveols (**81a** and **b**) as main products. This microbe also converted caraway oil, equal mixture of (+)-limonene (**68**) and (+)-carvone (**93**). (+)-Limonene (**68)** disappeared and (+)-carvone (**93)** was produced and accumulated in the cultured broth (Noma and Asakawa, 2007b).

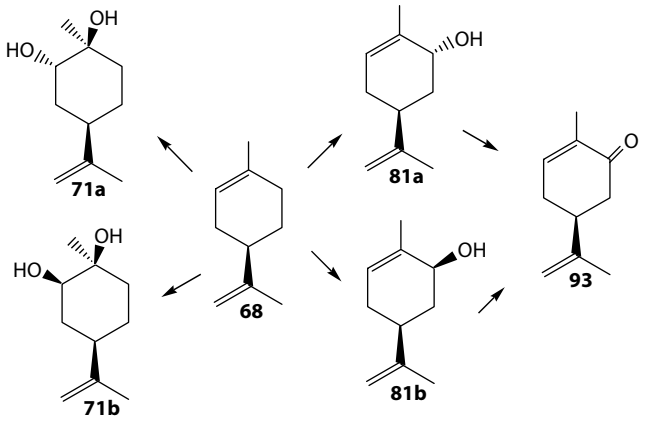


FIGURE 14.36 Biotransformation of (+)-limonene (**68**) by red yeast, *Rhodotorula* sp. and *Cladosporium* sp. T_7. (Modified from Mukherjee, B.B. et al., 1973. *Appl. Microbiol.*, 25: 447–453; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

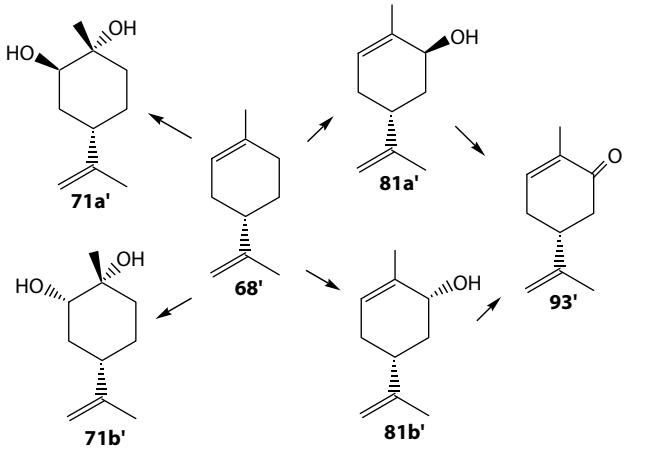


FIGURE 14.37 Biotransformation of (–)-limonene (**68′**) by a kind of *Rhodotorula* sp. (Modified from Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

(4*S*)-(–)- (**68′**) and (4*R*)-(+)-Limonene (**68**) and their epoxides (**69** and **69′**) were incubated by *Cyanobacterium*. It was found that the transformation was enantio- and regioselective. *Cyanobacterium* biotransformed only (4*S*)-limonene (**68′**) to (–)-*cis*- (**81b′**, 11.1%) and (–)-*trans*-carveol (**81a′**, 5%) in low yield. On the other hand, (4*R*)-limonene oxide (**69**) was converted to limonene-1,2-*trans*-diol (**71a′**) and 1-hydroxy-(+)-dihydrocarvone (**72a′**). However, (4*R*)-(+)-limonene (**68**) and (4*S*)-limonene oxide (**69′**) were not converted at all (Figure 14.38) (Hamada et al., 2003).

(+)-Limonene (**68**) was fed by *Spodptera litura* to give (+)-limonene-7-oic acid (**82**), (+)-limonene-9-oic acid (**70**), and (+)-limonene-8,9-diol (**79**); (–)-limonene (**68′**) was converted to (–)-linonene-7-oic acid (**82′**), (–)-limonene-9-oic acid (**70′**), and (–)-limonene-8,9-diol (**79′**) (Figure 14.39) (Miyazawa et al., 1995a).

Kieslich et al. (1985) found a nearly complete microbial resolution of a racemate in the biotransformation of (±)-limonene by *Penicillium digitatum* (DSM 62840). The (*R*)-(+)-limonene (**68**) is converted to the optically active (+)-α-terpineol, $[\alpha]_D = +99°$, while the (*S*)-(–)-limonene (**68′**) is presumably adsorbed onto the mycelium or degraded via unknown pathways (Kieslich et al., 1985) (Figure 14.40).

(4*S*)- and (4*R*)-Limonene epoxides (**69a′** and **a**) were biotransformed by *Cyanobacterium* to give 8-*p*-menthene-1α,2β-ol (**71a**, 68.4%) and 1α-hydroxy-8-*p*-menthen-2-one (**72**, 31.6%) (Hamada et al., 2003) (Figure 14.41).

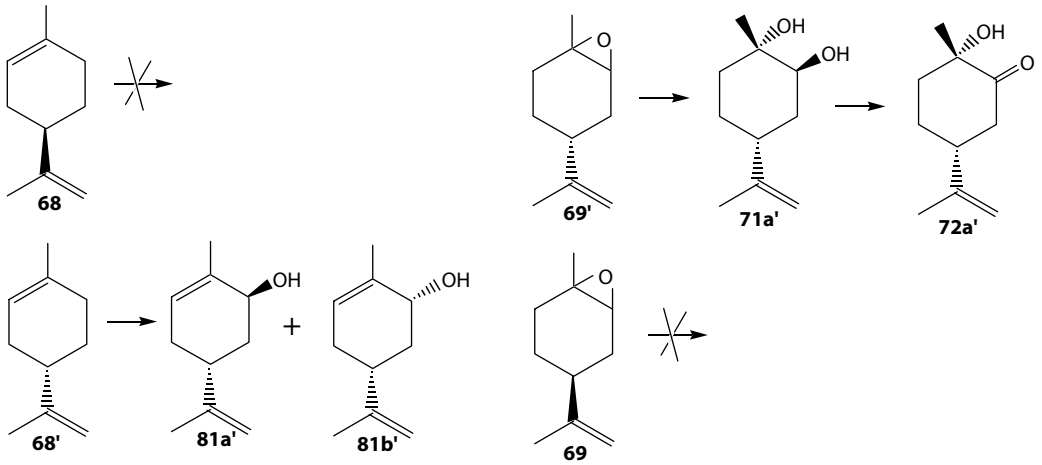


FIGURE 14.38 Biotransformation of (+)- and (−)-limonene (**68** and **68′**) and limonene epoxide (**69** and **69′**) by *Cyanobacterium*. (Modified from Hamada, H. et al., 2003. *Proc. 47th TEAC*, pp. 162–163.)

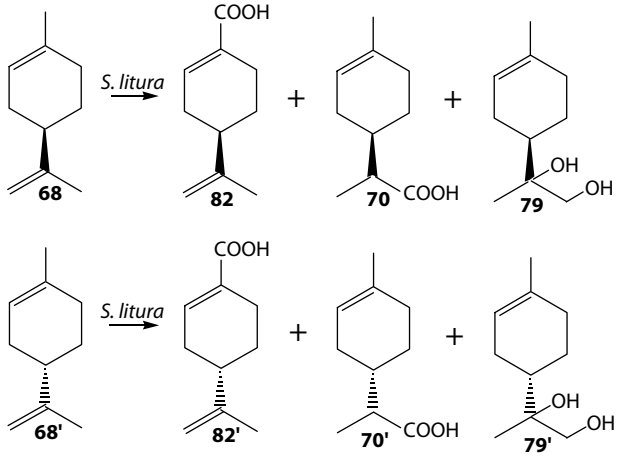


FIGURE 14.39 Biotransformation of (+)-limonene (**68**) and (−)-limonene (**68′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

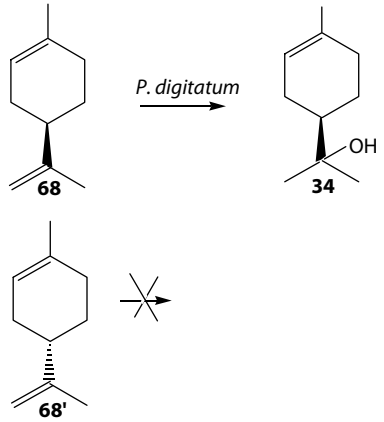


FIGURE 14.40 Microbial resolution of racemic limonene (**68** and **68′**) and the formation of optically active α-terpineol by *Penicillium digitatum*. (Modified from Kieslich, K. et al., 1985. In: *Topics in flavor research*, R.G. Berger, S. Nitz, and P. Schreier, eds, pp. 405–427. Marzling Hangenham: Eichborn.)

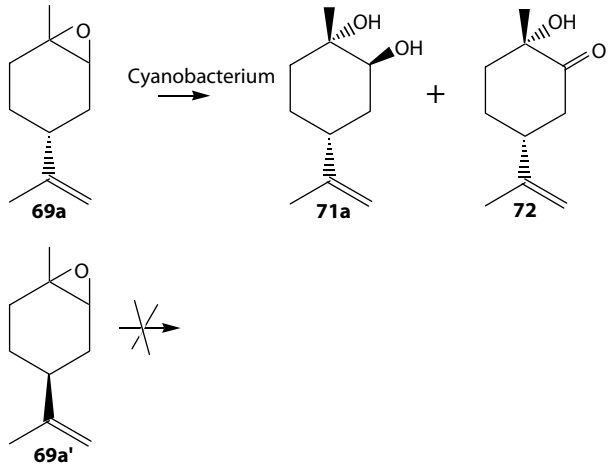
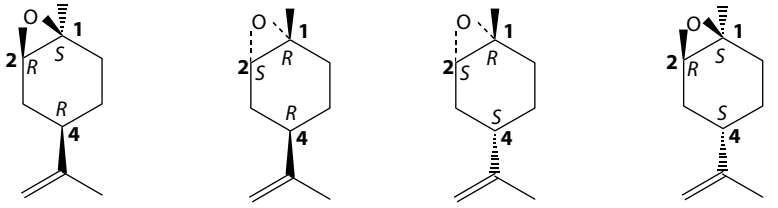


FIGURE 14.41 Enantioselective biotransformation of (4*S*)- (**69a′**) and (4*R*)-limonene epoxides (**69a**) by *Cyanobacterium*. (Modified from Hamada, H. et al., 2003. *Proc. 47th TEAC*, pp. 162–163.)



The mixture of (+)-*trans*- (**69a**) and *cis*- (**69b**), and the mixture of (–)-*trans*- (**69a′**) and *cis*-limonene-1,2-epoxide (**69b′**) were biotransformed by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN to give (1*R*,2*R*,4*R*)- (–)-*trans*-(**71a**) and (1*S*,2*S*,4*S*)-(+)-8-*p*-menthene-1,2-*trans*-diol (**71a′**) and (–)-*p*-menthane-1,2,8-triols (**334a** and **334a′**) (Noma and Asakawa, 2007b) (Figure 14.42).

Biotransformation of 1,8-cineole (**122**) by *Aspergillus niger* gave racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**) (Nishimura et al., 1982). When racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**) was biotransformed by *Glomerella cingulata*, only (–)-2α-hydroxy-1,8-cineole (**125b′**) was selectively esterified with malonic acid to give its malonate (**125b′**-Mal). The malonate was hydrolyzed to give optical pure **125b′** (Miyazawa et al., 1995b). On the other hand, Citrus pathogenic fungi, *Penicillium digitatum*, biotransformed limonene (**68**) to give optical pure **125b** (Noma and Asakawa, 2007b) (Figure 14.43).

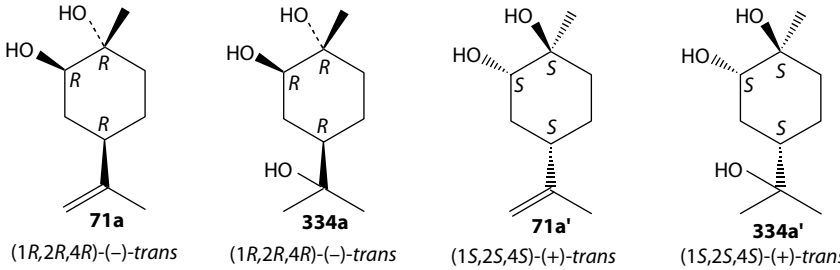


FIGURE 14.42 Biotransformation of (+)-*trans*- (**69a**) and *cis*- (**69b**), and (–)-*trans*- (**69a′**) and *cis*-limonene-1,2-epoxide (**69b′**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN and their metabolites. (Modified from Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

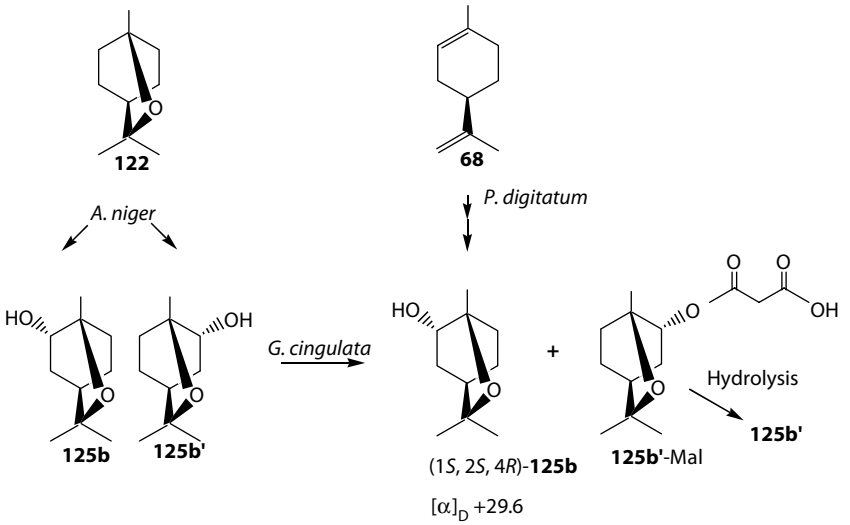


FIGURE 14.43 Formation of optical pure (+)- and (−)-2α-hydroxy-1,8-cineole (**125b** and **b′**) from the biotransformation of 1,8-cineole (**122**) and (+)-limonene (**68**) by *Citrus* pathogenic fungi, *Penicillium digitatum* (Pers.; Fr.) Sacc. KCPYN and *Aspergillus niger* TBUYN-2. (Modified from Nishimura, H. et al., 1982. *Agric. Biol. Chem.*, 46: 2601–2604; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

When monoterpenes, such as limonene (**68**), α-pinene (**4**), and 3-carene (**336**), were administered to the cultured cells of *Nicotiana tabacum*, they were converted to the corresponding epoxides enantio- and stereoselectively. The enzyme (p38) concerning with the epoxidation reaction was purified from the cultured cells by cation exchanged chromatography. The enzyme had not only epoxidation activity but also peroxidase activity. Amino acid sequence of p38 showed 89% homology in their 9 amino acid overlap with horseradish peroxidase (Yawata et al., 1998) (Figure 14.44). It was found that limonene and carene were converted to the corresponding epoxides in the presence of hydrogen

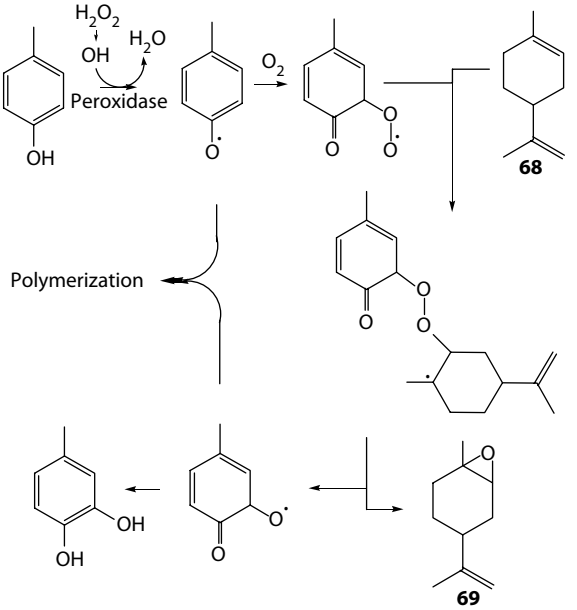


FIGURE 14.44 Proposed mechanism for the epoxidation of (+)-limonene (**68**) with p38 from the cultured cells of *Nicotiana tabacum*. (Modified from Yawata, T. et al., 1998. *Proc. 42nd TEAC*, pp. 142–144.)

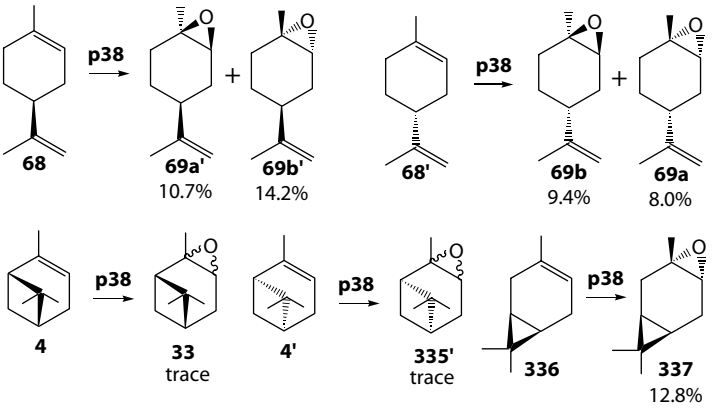


FIGURE 14.45 Epoxidation of limonene (**68**), α-pinene (**4**) and 3-carene (**336**) with p38 from the cultured cells of *Nicotiana tabacum*. (Modified from Yawata, T. et al., 1998. *Proc. 42nd TEAC*, pp. 142–144.)

peroxide and *p*-cresol by a radical mechanism with the peroxidase. (*R*)-limonene (**68**), (*S*)-limonene (**68′**), (1*S*,5*R*)-α-pinene (**4**), (1*R*,5*R*)-α-pinene (**4**), and (1*R*,6*R*)-3-carene (**336**) were oxidized by cultured cells of *Nicotiana tabacum* to give corresponding epoxides enantio- and stereoselectively (Yawata et al., 1998) (Figure 14.45).

14.3.1.2 Isolimonene

Spodptera litura converted (1*R*)-*trans*-isolimonene (**338**) to (1*R*,4*R*)-*p*-menth-2-ene-8,9-diol (**339**) (Miyazawa et al., 1996b) (Figure 14.46).

14.3.1.3 *p*-Menthane

Hydroxylation of *trans*- and *cis*-*p*-menthane (**252a** and **b**) by microorganisms is also very interesting from the viewpoint of the formation of the important perfumes such as (−)-menthol (**137b′**), (−)-carvomenthol (**49b′**), etc., plant growth regulators, and mosquito repellents such as *p*-menthane-*trans*-3,8-diol (**142a**), *p*-menthane-*cis*-3,8-diol (**142b**) (Nishimura and Noma, 1996), and *p*-menthane-2,8-diol (**93**) (Noma, 2007). *Pseudomonas mendocina* strain SF biotransformed **252b** stereoselectively to *p*-*cis*-menthan-1-ol (**253**) (Tsukamoto et al., 1975) (Figure 14.47).

On the other hand, the biotransformation of the mixture of *p*-*trans*- (**252a**) and *cis*-menthane (**252b**) (45:55, peak area in GC) by *Aspergillus niger* gave *p*-*cis*-menthane-1,9-diol (**254**) via *p*-*cis*-menthan-1-ol (**253**). No metabolite was obtained from **252a** at all (Noma et al., 1990) (Figure 14.47).

14.3.1.4 1-*p*-Menthene

Concentrated cell suspension of *Pseudomonas* sp. strain (PL) was inoculated to the medium containing 1-*p*-menthene (**62**) as the sole carbon source. It was degraded to give β-isopropyl pimelic acid (**248**) and methylisopropyl ketone (**251**) (Hungund et al., 1970) (Figure 14.48).

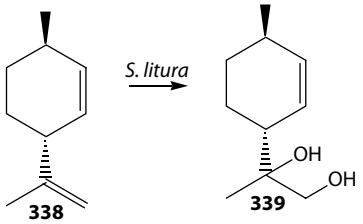


FIGURE 14.46 Biotransformation of (1*R*)-*trans*-isolimonene (**338**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1996b. *Proc. 40th TEAC*, pp. 80–81.)

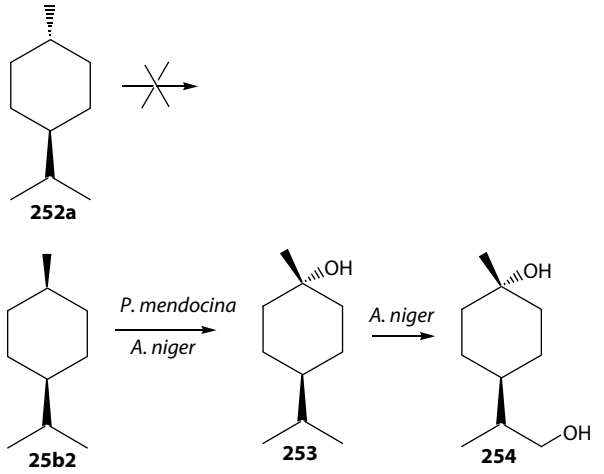


FIGURE 14.47 Biotransformation of the mixture of *trans*- (**252a**) and *cis*-*p*-menthane (**252b**) by *Pseudomonas mendocina* SF and *Aspergillus niger* TBUYN-2. (Modified from Tsukamoto, Y. et al., 1974. *Proc. 18th TEAC*, pp. 24–26; Tsukamoto, Y. et al., 1975. *Agric. Biol. Chem*., 39: 617–620; Noma, Y., 2007. *Aromatic Plants from Asia their Chemistry and Application in Food and Therapy*, L. Jiarovetz, N.X. Dung, and V.K. Varshney, pp. 169–186. Dehradun: Har Krishan Bhalla & Sons.)

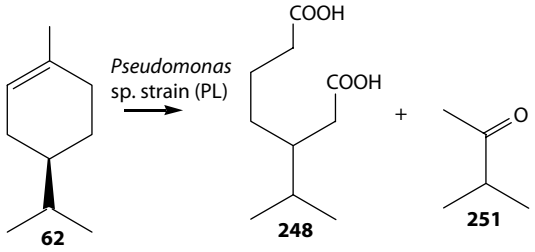


FIGURE 14.48 Biodegradation of (4*R*)-1-*p*-menthene (**62**) by *Pseudomonas* sp. *strain* (PL). (Modified from Hungund, B.L. et al., 1970. *Indian J. Biochem*., 7: 80–81.)

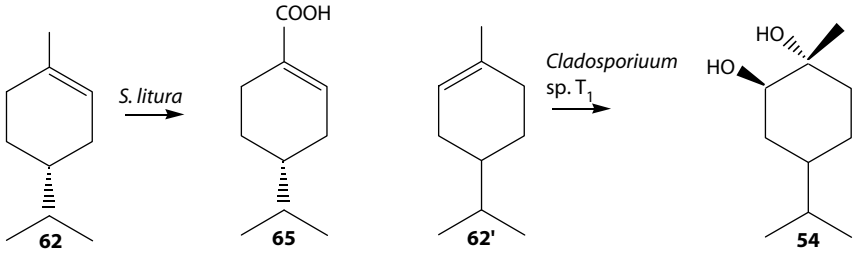


FIGURE 14.49 Biotransformation of (4*R*)-*p*-menth-1-ene (**62**) by *Spodoptera litura* and *Cladosporium* sp. T_1. (Modified from Miyazawa, M. et al., 1996b. *Proc. 40th TEAC*, pp. 80–81; Mukherjee, B.B. et al., 1973. *Appl. Microbiol*., 25: 447–453.)

As shown in Figure 14.49, *Spodoptera litura* converted (4*R*)-*p*-menth-1-ene (**62**) at C-7 position to (4*R*)-phellandric acid (**65**) (Miyazawa et al., 1996b). On the other hand, when *Cladosporium* sp. T_1 was cultivated with (+)-limonene (**68**) as the sole carbon source, it converted **62′** to *trans*-*p*-menthane-1,2-diol (**54**) (Mukherjee et al., 1973).

14.3.1.5 3-*p*-Menthene

When *Cladosporium* sp. T_8 was cultivated with 3-*p*-menthene (**147**) as the sole carbon source, it was converted to *trans*-*p*-menthane-3,4-diol (**141**) as shown in Figure 14.50 (Mukherjee et al., 1973).

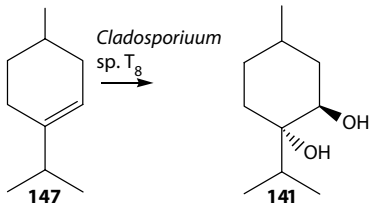


FIGURE 14.50 Biotransformation of *p*-Menth-3-ene (**147**) by *Cladosporium* sp. T_8. (Modified from Mukherjee, B.B. et al., 1973. *Appl. Microbiol.*, 25: 447–453.)

14.3.1.6 α-Terpinene

α-Terpinene (**340**) was converted by *Spodoptera litura* to give α-terpinene-7-oic acid (**341**) and *p*-cymene-7-oic acid (**194**, cuminic acid) (Miyazawa et al., 1995a) (Figure 14.51).

A soil Pseudomonad has been found to grow with *p*-mentha-1,3-dien-7-al (**463**) as the sole carbon source and to produce α-terpinene-7-oic acid (**341**) in a mineral salt medium (Kayahara et al., 1973) (Figure 14.51).

14.3.1.7 γ-Terpinene

γ-Terpinene (**344**) was converted by *Spodoptera litura* to give γ-terpinene-7-oic acid (**345**) and *p*-cymene-7-oic acid (**194**, cuminic acid) (Miyazawa et al., 1995a) (Figure 14.52).

14.3.1.8 Terpinolene

Terpinolene (**346**) was converted by *Aspergillus niger* to give (1*R*)-8-hydroxy-3-*p*-menthen-2-one (**347**), (1*R*)-1,8-dihydroxy-3-*p*-menthen-2-one (**348**), and 5β-hydroxyfenchol (**350b′**). In case of *Corynespora cassiicola* it was converted to terpinolene-1,2-*trans*-diol (**351**) and terpinolene-4,8-diol (**352**). Furthermore, in case of rabbit terpinolene-9-ol (**353**) and terpinolene-10-ol (**354**) were formed from **346** (Asakawa et al., 1983). *Spodoptera litura* also converted **346** to give 1-*p*-menthene-4,8-diol (**352**), cuminic acid (**194**, 29% main product), and terpinolene-7-oic acid (**357**) (Figure 14.53).

14.3.1.9 α-Phellandrene

α-Phellandrene (**355**) was converted by *Spodoptera litura* to give α-phellandrene-7-oic acid (**356**) and *p*-cymene-7-oic acid (**194**, cuminic acid) (Miyazawa et al., 1995a) (Figure 14.54).

14.3.1.10 *p*-Cymene

Pseudomonas sp. strain (PL) was cultivated with *p*-cymene (**178**) as the sole carbon source to give cumyl alcohol (**192**), cumic acid (**194**), 3-hydroxycumic acid (**196**), 2,3-dihydroxycumic acid (**197**),

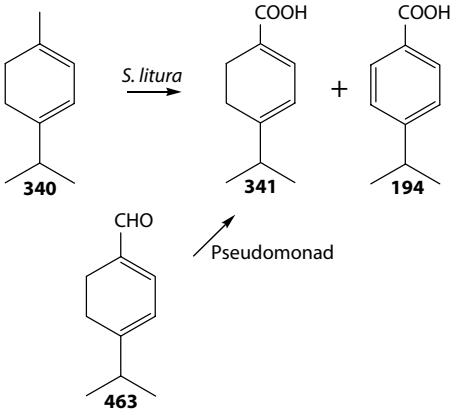


FIGURE 14.51 Biotransformation of α-terpinene (**340**) by *Spodoptera litura* and *p*-mentha-1,3-dien-7-al (**463**) by a soil Pseudomonad. (Modified from Kayahara, H. et al., 1973. *J. Ferment. Technol.*, 51: 254–259; Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

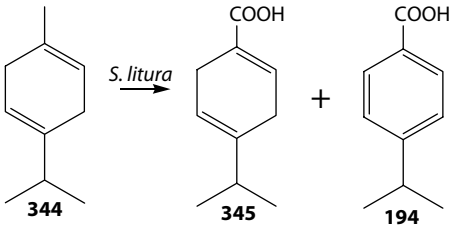


FIGURE 14.52 Biotransformation of γ−terpinene (**344**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

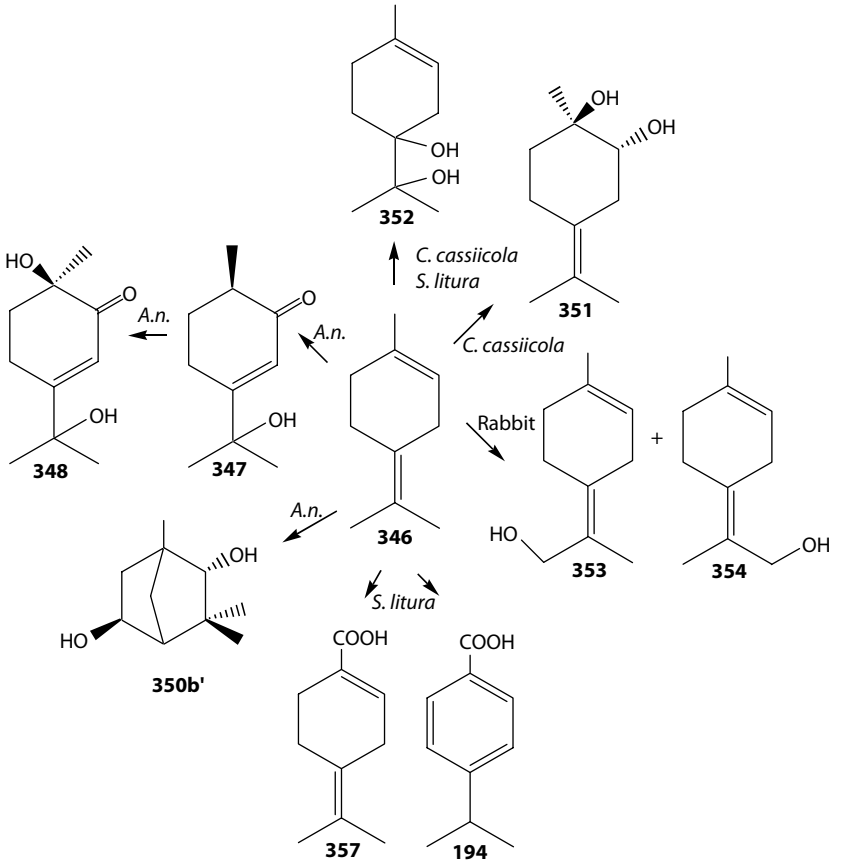


FIGURE 14.53 Biotransformation of terpinolene (**346**) by *Aspergillus niger* (Asakawa et al., 1991), *Corynespora cassiicola* (Abraham et al., 1985), rabbit (Asakawa et al., 1983), and *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

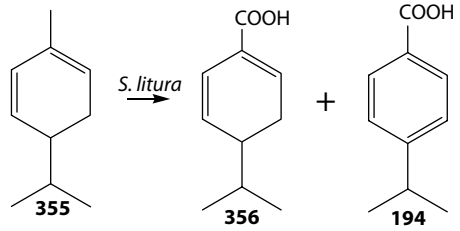


FIGURE 14.54 Biotransformation of α-phellandrene (**355**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1995a. *Proc. 39th TEAC*, pp. 362–363.)

2-oxo-4-methylpentanoic acid (**201**), 9-hydroxy-*p*-cymene (**189**), and *p*-cymene-9-oic acid (**190**) as shown in Figure 14.55 (Madyastha and Bhattacharyya, 1968). On the other hand, *p*-cymene (**178**) was converted regiospecifically to cumic acid (**194**) by *Pseudomonas* sp., *Pseudomonas desmolytica*, and *Nocardia salmonicolor* (Madyastha and Bhattacharyya, 1968) (Figure 14.56).

p-Cymene (**178**) is converted to thymoquinone (**358**) and analogues, **179** and **180**, by various kinds of microorganisms (Demirci et al., 2007) (Figure 14.57).

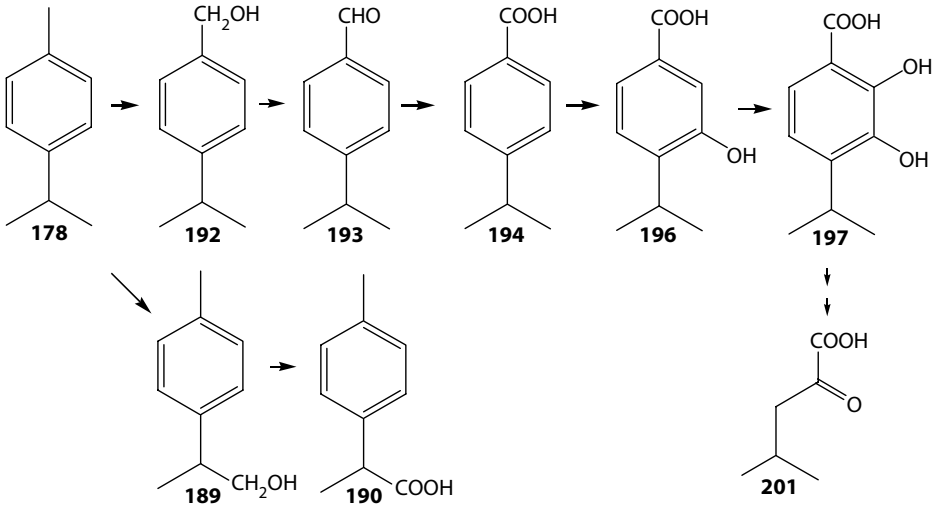


FIGURE 14.55 Biotransformation of *p*-cymene (**178**) by *Pseudomonas* sp. strain (PL). (Modified from Madyastha, K.M. and P.K. Bhattacharyya, 1968. *Indian J. Biochem.*, 5: 161–167.)

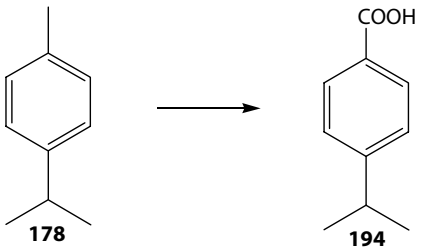


FIGURE 14.56 Biotransformation of *p*-cymene (**178**) to cumic acid (**194**) by *Pseudomonas* sp., *Pseudomonas desmolytica* and *Nocardia salmonicolor.* (Modified from Yamada, K. et al., 1965. *Agric. Biol. Chem.*, 29: 943–948; Madyastha, K.M. and P.K. Bhattacharyya, 1968. *Indian J. Biochem.*, 5: 161–167; Noma, Y., 2000. unpublished data.)

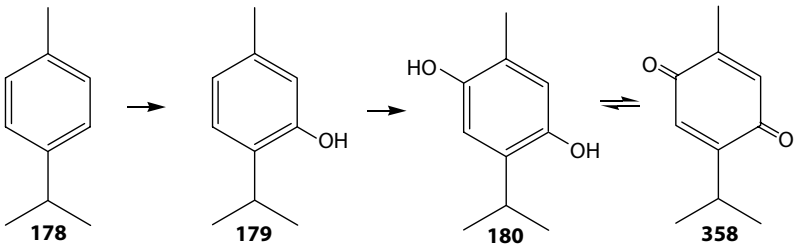
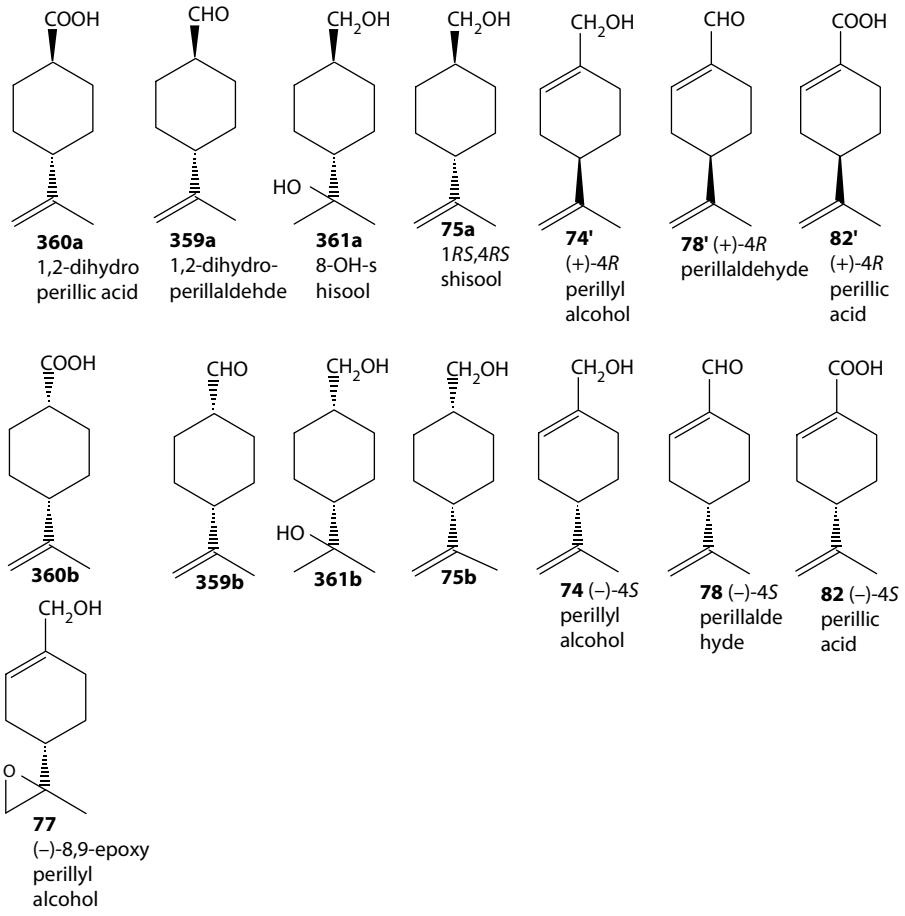


FIGURE 14.57 Biotransformation of *p*-cymene (**178**) to thymoquinone (**358**) and analogues by microorganisms. (Modified from Demirci, F. et al., 2007. *Book of Abstracts of the 38th ISEO*, SL-1, p. 6.)

14.3.2 Monocyclic Monoterpene Aldehyde



14.3.2.1 Perillaldehyde

Biotransformation of (–)-perillaldehyde (**78**), (+)-perillaldehyde (**78′**), (–)-perillyl alcohol (**74**), *trans*-1,2-dihydroperillaldehyde (**359a**) and *cis*-1,2-dihydroperillaldehyde (**359b**), and *trans*-shisoic acid (**360a**) and *cis*-shisoic acid (**360b**) was carried out by *Euglena gracilis* Z. (Noma et al., 1991a), *Dunaliella tertiolecta* (Noma et al., 1991b, 1992a), *Chlorella ellipsoidea* IAMC-27 (Noma et al., 1997), *Streptomyces ikutamanensis* Ya-2-1 (Noma et al., 1984, 1986), and other microorganisms (Kayahara et al., 1973) (Figure 14.58).

(–)-Perillaldehyde (**78**) is easily transformed to give (–)-perillyl alcohol (**74**) and *trans*-shisool (**75a**), which is well known as a fragrance, as the major product, and (–)-perillic acid (**82**) as the minor product. (–)-Perillyl alcohol (**74**) is also transformed to *trans*-shisool (**75a**) as the major product with *cis*-shisool (**75b**) and 8-hydroxy-*cis*-shisool (**361b**). Furthermore, *trans*-shisool (**75a**) and *cis*-shisool (**75b**) are hydroxylated to 8-hydroxy-*trans*-shisool (**361a**) and 8-hydroxy-*cis*-shisool (**361b**), respectively. *trans*-1,2-Dihydroperillaldehyde (**359a**) and *cis*-1,2-dihydroperillaldehyde (**359b**) are also transformed to **75a** and **75b** as the major products and *trans*-shisoic acid (**360a**) and *cis*-shisoic acid (**360b**) as the minor products, respectively. Compound **360a** was also formed from **75a**. In the biotransformation of (±)-perillaldehyde (**74** and **74′**), the same results were obtained as described in the case of **74**. In the case of *Streptomyces ikutamanensis* Ya-2-1, (–)-perillaldehyde (**78**) was converted to (–)-perillic acid (**82**), (–)-perillyl alcohol (**74**), and (–)-perillyl alcohol-8,9-epoxide (**77**) which was the major product.

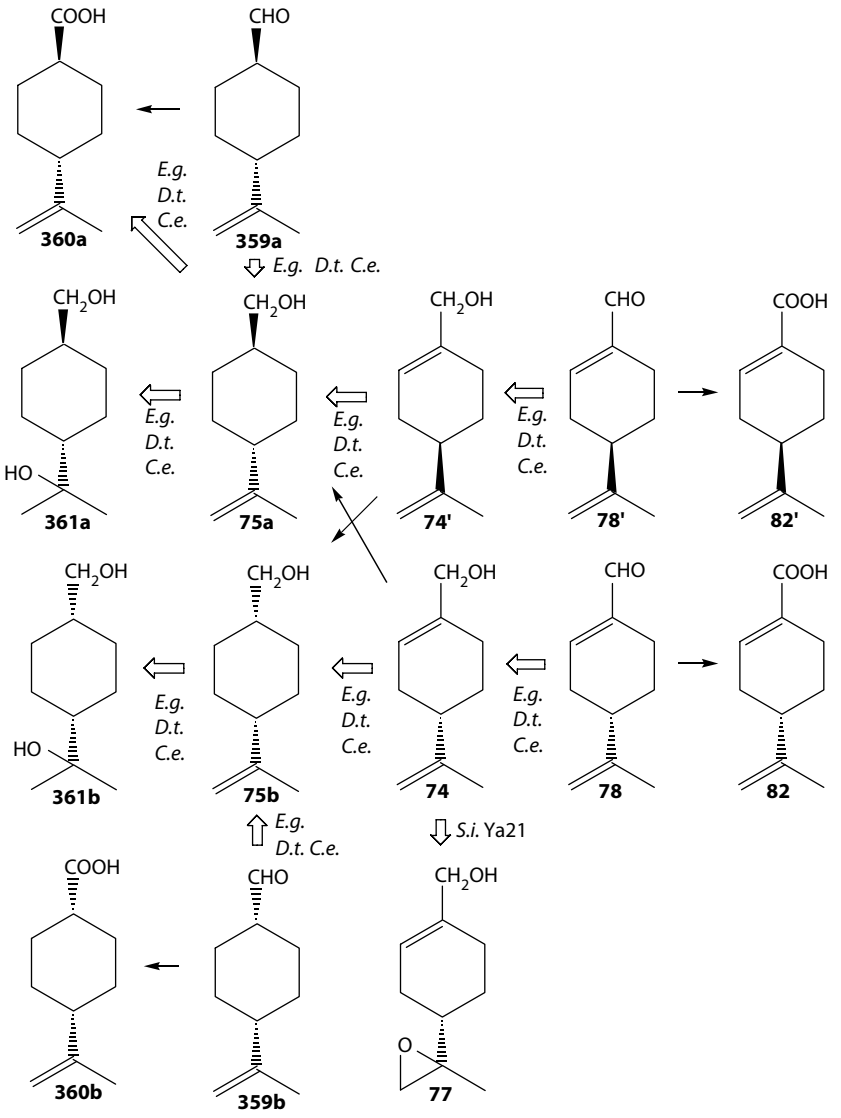


FIGURE 14.58 Metabolic pathways of perillaldehyde (**78** and **78′**) by *Euglena gracilis* Z (Noma et al., 1991a), *Dunaliella tertiolecta* (Noma et al., 1991b; 1992a), *Chlorella ellipsoidea* IAMC-27 (Noma et al., 1997), *Streptomyces ikutamanensis* Ya-2-1 (Noma et al., 1984, 1986), a soil Pseudomonad (Kayahara et al., 1973), and rabbit (Ishida et al., 1981a).

A soil Pseudomonad has been found to grow with (–)-perillaldehyde (**78**) as the sole carbon source and to produce (–)-perillic acid (**82**) in a mineral salt medium (Kayahara et al., 1973).

On the other hand, rabbit metabolized (–)-perillaldehyde (**78**) to (–)-perillic acid (**82**) along with minor shisool (**75a**) (Ishida et al., 1981a).

14.3.2.2 Phellandral and 1,2-Dihydrophellandral

Biotransformation of (–)-phellandral (**64**), *trans*-tetrahydroperillaldehyde (**362a**), and *cis*-tetrahydroperillaldehyde (**362b**) was carried out by microorganisms (Noma et al., 1986, 1991a, 1991b, 1997). (–)-Phellandral (**64**) was metabolized mainly via (–)-phellandrol (**63**) to *trans*-tetrahydroperillyl alcohol (**66a**). *trans*-Tetrahydroperillaldehyde (**362a**) and *cis*-tetrahydroperillaldehyde (**362b**) were also transformed to *trans*-tetrahydroperillyl alcohol (**66a**) and *cis*-tetrahydroperillyl alcohol (**66b**)

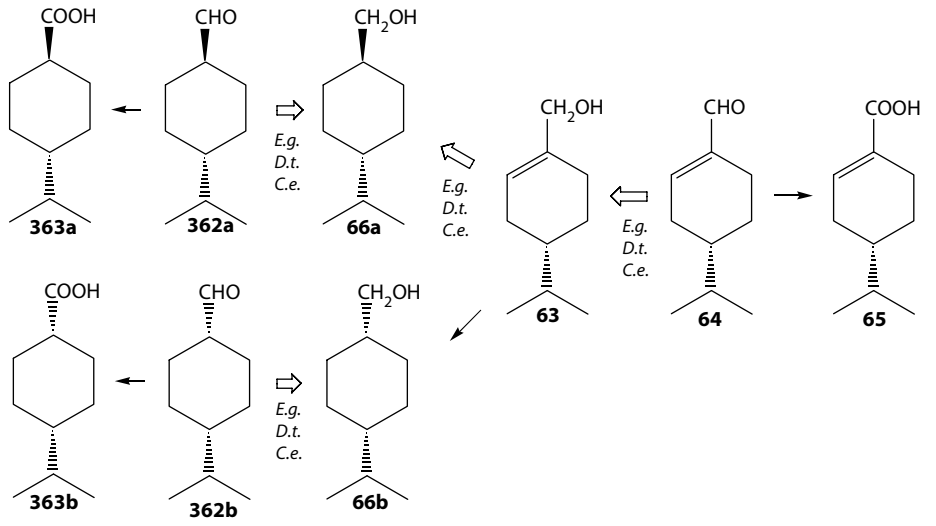


FIGURE 14.59 Metabolic pathways of (–)-phellandral (**64**) by microorganisms. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206; Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151; Noma, Y. et al., 1991b. *Proc. 35th TEAC*, pp. 112–114; Noma, Y. et al., 1997. *Proc. 41st TEAC*, pp. 227–229.)

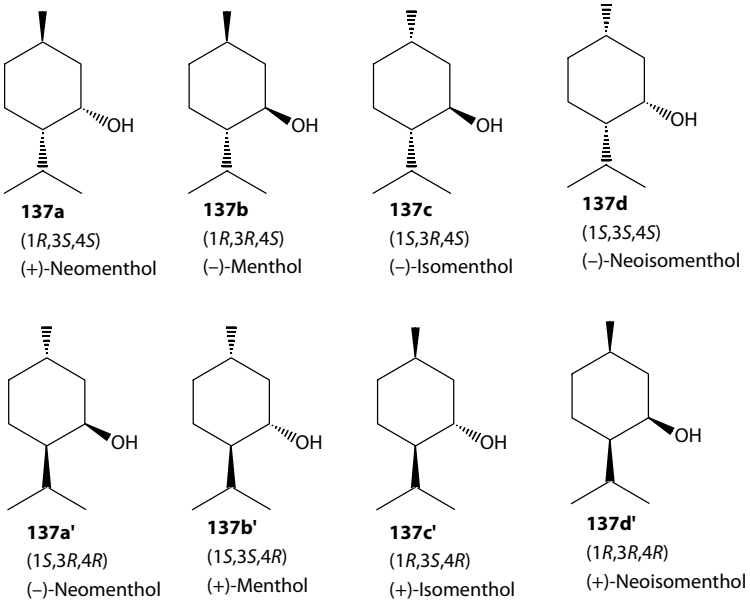
as the major products and *trans*-tetrahydroperillic acid (**363a**) and *cis*-tetrahydroperillic acid (**363b**) as the minor products, respectively (Figure 14.59).

14.3.2.3 Cuminaldehyde

Cumin aldehyde (**193**) is transformed by *Euglena* (Noma et al., 1991a), *Dunaliella* (Noma et al., 1991b), and *Streptomyces ikutamanensis* (Noma et al., 1986) to give cumin alcohol (**192**) as the major product and cuminic acid (**194**) as the minor product (Figure 14.60).

14.3.3 Monocyclic Monoterpene Alcohol

14.3.3.1 Menthol



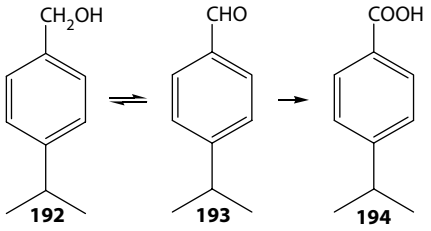


FIGURE 14.60 Metabolic pathway of cumin aldehyde (**193**) by microorganism. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206; Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151; Noma, Y. et al., 1991b. *Proc. 35th TEAC*, pp. 112–114.)

Menthol (**137**) is one of the rare naturally occurring monocyclic monoterpene alcohols that have not only various physiological properties, such as sedative, anesthetic, antiseptic, gastric, and antipruritic, but also characteristic fragrance (Bauer et al., 1990). There are in fact eight isomers with a menthol (*p*-menthan-3-ol) skeleton; (−)-menthol (**137b**) is the most important one, because of its cooling and refreshing effect. It is the main component of peppermint and cornmint oils obtained from the *Mentha piperita* and *Mentha arvensis* species. Many attempts have been made to produce (−)-menthol (**137b**) from inexpensive terpenoid sources, but these sources also unavoidably yielded the (±)-isomers (**137b** and **137b′**): isomenthol (**137c**), neomenthol (**137a**), and neoisomenthol (**137d**) (Krasnobajew, 1984). Japanese researchers have been active in this field, maybe because of the large demand for (−)-menthol (**137b**) in Japan itself, namely 500 t/year (Janssens et al., 1992). Indeed, most literature deals with the enantiomeric hydrolysis of (±)-menthol (**137b** and **137b′**) esters to optically pure *l*-menthol (**137b**). The asymmetric hydrolysis of (±)-menthyl chloroacetate by an esterase of *Arginomonas non-fermentans* FERM-P-1924 has been patented by the Japanese Nippon Terpene Chemical Co. (Watanabe and Inagaki, 1977a, 1977b). Investigators from the Takasago Perfumery Co. Ltd. claim that certain selected species of *Absidia*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Bacillus*, *Pseudomonas*, and others asymmetrically hydrolyze esters of (±)-menthol isomers such as formates, acetates, propanoates, caproates, and esters of higher fatty acids (Moroe et al., 1971; Yamaguchi et al., 1977) (Figure 14.61).

Numerous investigations into the resolution of the enantiomers by selective hydrolysis with microorganisms or enzymes were carried out. Good results were described by Yamaguchi et al. (1977) with the asymmetric hydrolysis of (±)-methyl acetate by a mutant of *Rhodotorula mucilaginosa*, yielding 44 g of (−)-menthol (**137b**) form a 30% (±)-menthyl acetate mixture per liter of cultured medium for 24 h. The latest development is the use of immobilized cells of *Rhodotorula minuta* in aqueous saturated organic solvents (Omata et al., 1981) (Figure 14.62).

Besides the hydrolysis of menthyl esters, the biotransformation of menthol and its enantiomers has also been published (Shukla et al., 1987; Asakawa et al., 1991). The fungal biotransformation of (−)-(**137b**) and (+)-menthols (**137b′**) by *Aspergillus niger* and *Aspergillus cellulosae* was described

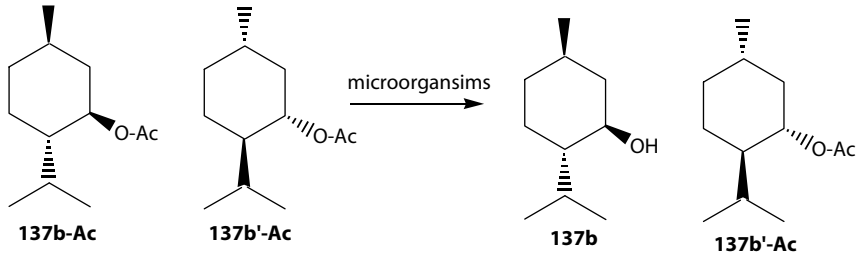


FIGURE 14.61 Asymmetric hydrolysis of racemic menthyl acetate (**137b-Ac** and **137b′-Ac**) to obtain pure (−)-menthol (**137b**). (Modified from Watanabe, Y. and T. Inagaki, 1977a. Japanese Patent 77.12.989. No. 187696x; Watanabe, Y. and T. Inagaki, 1977b. Japanese Patent 77.122.690. No. 87656g; Moroe, T. et al., 1971. Japanese Patent, 2.036. 875. no. 98195t; Oritani, T. and Yamashita, K. 1973b. *Agric. Biol. Chem.*, 37: 1695–1700.)

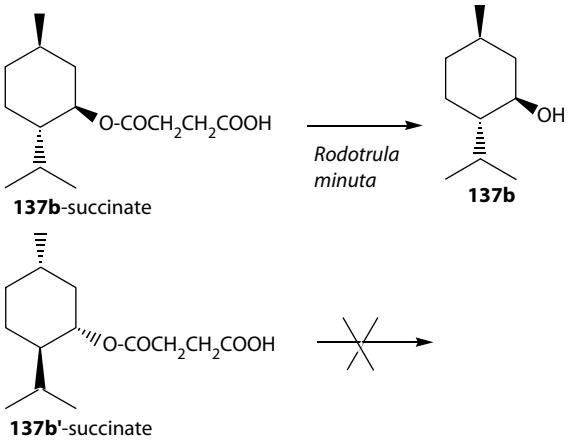


FIGURE 14.62 Asymmetric hydrolysis of racemic menthyl succinate (**137b-** and **137b′-succinates**) to obtain pure (–)-menthol (**137b**). (Modified from Yamaguchi, Y. et al., 1977. *J. Agric. Chem. Soc. Jpn.*, 51: 411–416,)

(Asakawa et al., 1991). *Aspergillus niger* converted (–)-menthol (**137b**) to 1- (**138b**), 2- (**140b**), 6- (**139b**), 7- (**143b**), 9-hydroxymenthols (**144b**), and the mosquito repellent-active 8-hydroxymenthol (**142b**), whereas (+)-menthol (**137b′**) was smoothly biotransformed by the same microorganism to 7-hydroxymenthol (**143b**). The bioconversion of (+)- (**137a′**) and (–)-neomenthol (**137a**) and (+)-isomenthol (**137c′**) by *Aspergillus niger* was studied later by Takahashi et al. (1994), mainly giving hydroxylated products. Noma and Asakawa (1995) reviewed the schematic menthol hydroxylation in detail.

Incubation of (–)-menthol (**137b**) with *Cephalosporium aphidicola* for 12 days yielded 10-acetoxymenthol (**144bb-Ac**), 1α-hydroxymenthol (**138b**), 6α-hydroxy- menthol (**139bb**), 7-hydroxymenthol (**143b**), 9-hydroxymenthol (**144ba**), and 10-hydroxymenthol (**144bb**) (Atta-ur-Rahman et al., 1998) (Figure 14.63).

Aspergillus niger TBUYN-2 converted (–)-menthol (**137b**) to 1α- (**138b**), 2α- (**140b**), 4β- (**141b**), 6α- (**139bb**), 7- (**143b**)-, 9-hydroxymenthols (**144ba**), and the mosquito repellent-active 8-hydroxymenthol (**142b**) (Figure 14.64). *Aspergillus cellulosae* M-77 biotransformed (–)-menthol (**137b**) to 4β-hydroxymenthol (**141b**) predominantly. The formation of **141b** is also observed in *Aspergillus cellulosae* IFO 4040 and *Aspergillus terreus* IFO 6123, but its yield is much less than that obtained from **137b** by *Aspergillus cellulosae* M-77 (Asakawa et al., 1991) (Table 14.1).

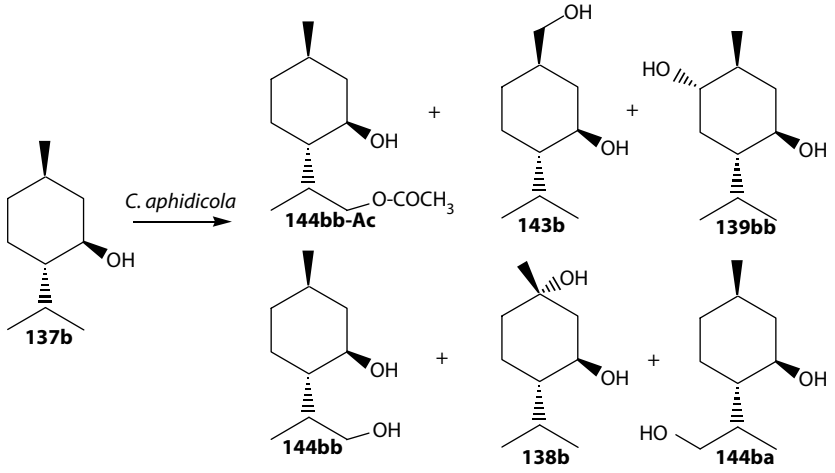


FIGURE 14.63 Biotransformation of (–)-menthol (**137b**) by *Cephalosporium aphidicola*. (Modified from Atta-ur-Rahman, M. et al., 1998. *J. Nat. Prod.*, 61: 1340–1342.)

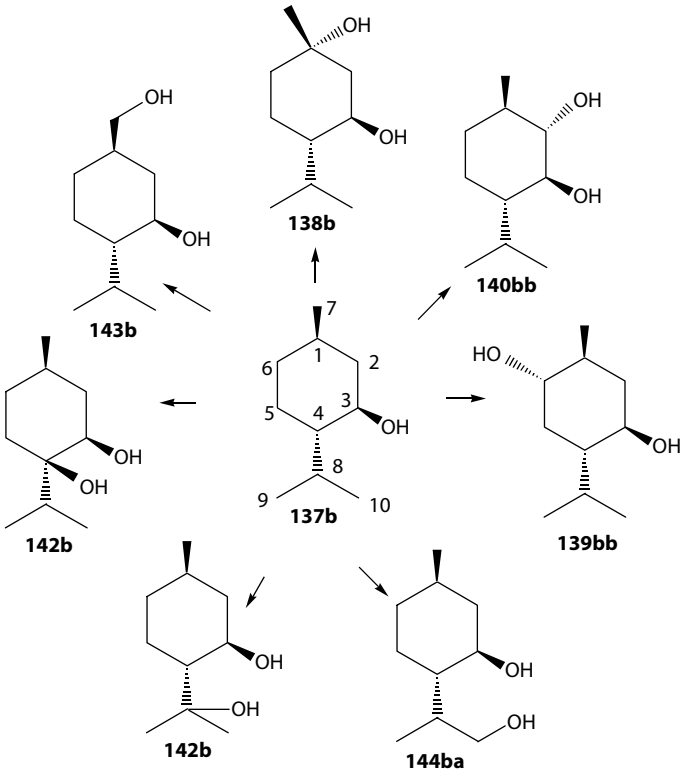


FIGURE 14.64 Metabolic pathways of (–)-menthol (**137b**) by *Aspergillus niger*. (Modified from Asakawa, Y. et al., 1991. *Phytochemistry*, 30: 3981–3987.)

On the other hand, (+)-menthol (**137b′**) was smoothly biotransformed by *Aspergillus niger* to give 1β-hydroxymenthol (**138b′**), 6β-hydroxymenthol (**139ba′**), 2β-hydroxymenthol (**140ba′**), 4α-hydroxymenthol (**141b′**), 7-hydroxymenthol (**143b′**), 8-hydroxymenthol (**142b′**), and 9-hydroxymenthol (**144ba′**) (Figure 14.65) (Table 14.2).

Spodoptera litura converted (–)- and (+)-menthols (**137b** and **137b′**) gave the corresponding 10-hydroxy products (**143b** and **143b′**) (Miyazawa et al., 1997a) (Figure 14.66).

TABLE 14.1
Metabolites of (–)-Menthol (137b) by Various *Aspergillus* spp. (Static Culture)

Microorganisms	138b	142b	139bb	143b	139bb	144ba	141b
A. awamori IFO 4033	+[a]	++	–	+	++	+++	–
A. fumigatus IFO 4400	–	+	–	+	+	+	–
A. sojae IFO 4389	++	+	+	–	–	++++	–
A. usami IFO 4338	–	–	–	+	–	+++	–
A. cellulosae M-77	+	–	–	+	–	++	++++
A. cellulosae IFO 4040	–	+	–	–	–	++	++
A. terreus IFO 6123	+	+	+	–	+	+	–
A. niger IFO 4049	–	+	–	+	–	+++	–
A. niger IFO 4040	–	+	–	+++	–	+++	–
A. niger TBUYN-2	+	++	+	+	++	++	–

[a] Symbols +, ++, +++, etc. are relative concentrations estimated by GC-MS.

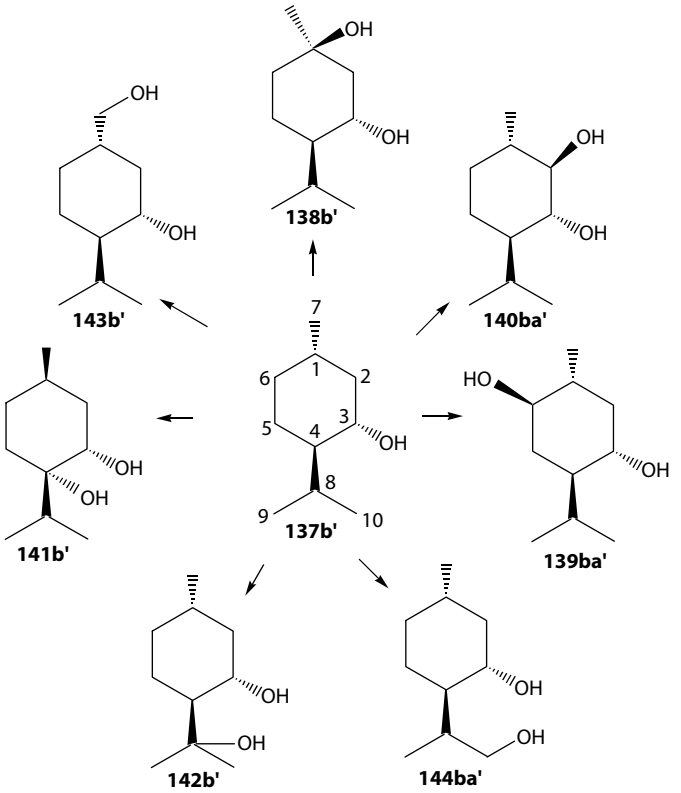


FIGURE 14.65 Metabolic pathways of (+)-menthol (**137b′**) by *Aspergillus niger*. (Modified from Noma, Y. et al., 1989. *Proc. 33rd TEAC*, pp. 124–126; Asakawa, Y. et al., 1991. *Phytochemistry*, 30: 3981–3987.)

(–)-Menthol (**137b**) was glycosylated by *Eucalyptus perriniana* suspension cells to (–)-menthol diglucoside (**364**, 26.6%) and another menthol glycoside. On the other hand, (+)-menthol (**137b′**) was glycosylated by *Eucalyptus perriniana* suspension cells to (+)-menthol di- (**364′**, 44.0%) and triglucosides (**365**, 6.8%) (Hamada et al., 2002) (Figure 14.67).

TABLE 14.2
Metabolites of (+)-Menthol (137b′) by Various *Aspergillus* spp. (Static Culture)

Microorganisms	138b′	142b′	140ba′	143b′	139ba′	144ba′	141b′
A. awamori IFO 4033	+[a]	++	–	+++	–	+++	–
A. fumigatus IFO 4400	+	++	–	+	–	++	–
A. sojae IFO 4389	+	++	–	–	–	+++	–
A. usami IFO 4338	+	–	–	+	–	+++	–
A. cellulosae M-77	–	+	–	–	–	++	++++
A. cellulosae IFO 4040	+	+	–	–	++	+	+
A. terreus IFO 6123	+	+++	+	+	+	++	–
A. niger IFO 4049	+	–	–	–	+	+++	–
A. niger IFO 4040	+	++	–	+	–	++	–
A. niger TBUYN-2	++	+	–	+++++	+	+	–

[a] Symbols +, ++, +++, etc. are relative concentrations estimated by GC-MS.

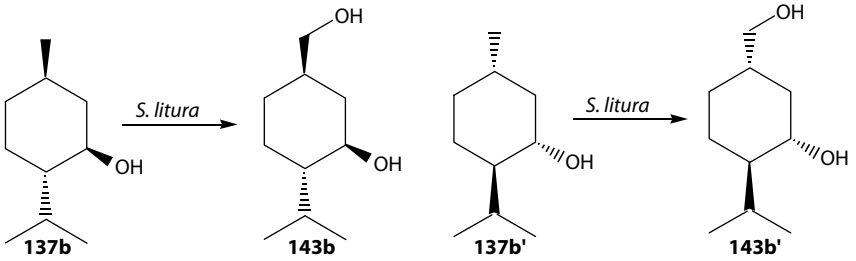


FIGURE 14.66 Biotransformation of (–)- (**137b**) and (+)-menthol (**137b′**) by *Spodoptera litura*. (Modified from Miyazawa, M. et al., 1997a. *Proc. 41st TEAC*, pp. 391–392.)

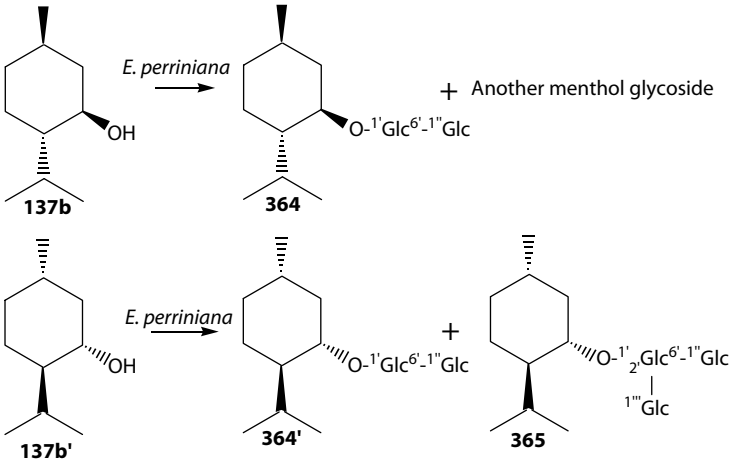


FIGURE 14.67 Biotransformation of (–)- (**137b**) and (+)-menthol (**137b′**) by *Eucalyptus perriniana* suspension cells. (Modified from Hamada, H. et al., 2002. *Proc. 46th TEAC*, pp. 321–322.)

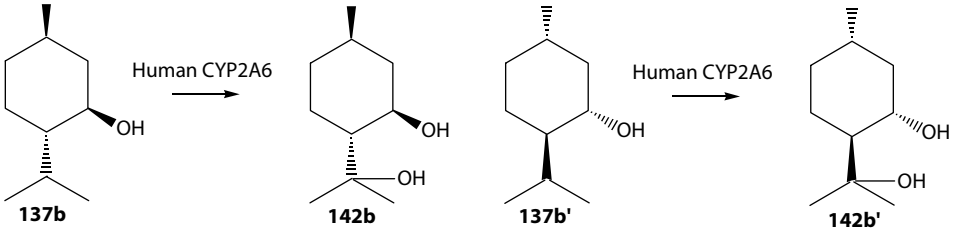


FIGURE 14.68 Biotransformation of (–)-menthol (**137b**) and its enantiomer (**137b′**) by human CYP 2A6. (Modified from Nakanishi, K. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 423–425.)

(–)-Menthol (**137b**) and its enantiomer (**137b′**) were converted to their corresponding 8-hydroxy derivatives (**142b** and **142b′**) by human CYP 2A6 (Nakanishi and Miyazawa, 2005) (Figure 14.68). By various assays, cytochrome P450 molecular species responsible for the metabolism of (–)- (**137b**) and (+)-menthol (**137b′**) was determined to be CYP 2A6 and CYP2B1 in human and rat, respectively. Also, kinetic analysis showed that K_m and V_{max} values for the oxidation of (–)- (**137b**) and (+)-menthol (**137b′**) recombinant CYP2A6 and CYP2B1 were determined to be 28 μM and 10.33 nmol/min/nmol P450 and 27 μM, 5.29 nmol/min/nmol P450, 28 μM and 3.58 nmol/min/nmol P450, and 33 μM and 5.3 nmol/min/nmol P450, respectively (Nakanishi and Miyazawa, 2005) (Figure 14.68).

14.3.3.2 Neomenthol

(+)-Neomenthol (**137a**) is biotransformed by *Aspergillus niger* TBUYN-2 to give five kinds of diols (**138a**, **143a**, **144aa**, **144ab**, and **142a**) and two kinds of triols (**145a** and **146a**) as shown in Figure 14.69 (Takahashi et al., 1994).

(−)-Neomenthol (**137a′**) is biotransformed by *Aspergillus niger* to give six kinds of diols (**140a′**, **139a′**, **143a′**, **144aa′**, **144ab′**, and **142a′**) and a triol (**146a′**) as shown in Figure 14.70 (Takahashi et al., 1994).

14.3.3.3 (+)-Isomenthol

(+)-Isomenthol (**137c**) is biotransformed to give two kinds of diols such as 1β-hydroxy- (**138c**) and 6β-hydroxyisomenthol (**139c**) by *Aspergillus niger* (Takahashi et al., 1994) (Figure 14.71).

(±)-Isomenthyl acetate (**137c-Ac** and **137c′-Ac**) was asymmetrically hydrolyzed to (−)-isomenthol (**137c**) with (+)-isomenthol acetate (**137c′-Ac**) by many microorganisms and esterases (Oritani and Yamashita, 1973b) (Figure 14.72).

14.3.3.4 Isopulegol

(−)-Isopulegol (**366**) was biotransformed by *Spodoptera litura* larvae to give 7-hydroxy-(−)-isopulegol (**367**), 9-hydroxy-(−)-menthol (**144ba**) and 10-hydroxy-(−)-isopulegol (**368**). On the other hand, (+)-isopulegol (**366′**) was biotransformed by the same larvae in the same manner to give 7-hydroxy-(+)-isopulegol (**367′**), 9-hydroxy-(+)-menthol (**144ba′**), and 10-hydroxy-(+)-isopulegol (**368′**) (Ohsawa and Miyazawa, 2001) (Figure 14.73).

Microbial resolution of (±)-isopulegyl acetate (**366-Ac** and **366′-Ac**) was studied by microorganisms. (±)-Isopulegyl acetate (**366-Ac** and **366′-Ac**) was hydrolyzed asymmetrically to give a mixture of (−)-isopulegol (**366**) and (+)-isopulegyl acetate (**366′-Ac**) (Oritani and Yamashita, 1973c) (Figure 14.74).

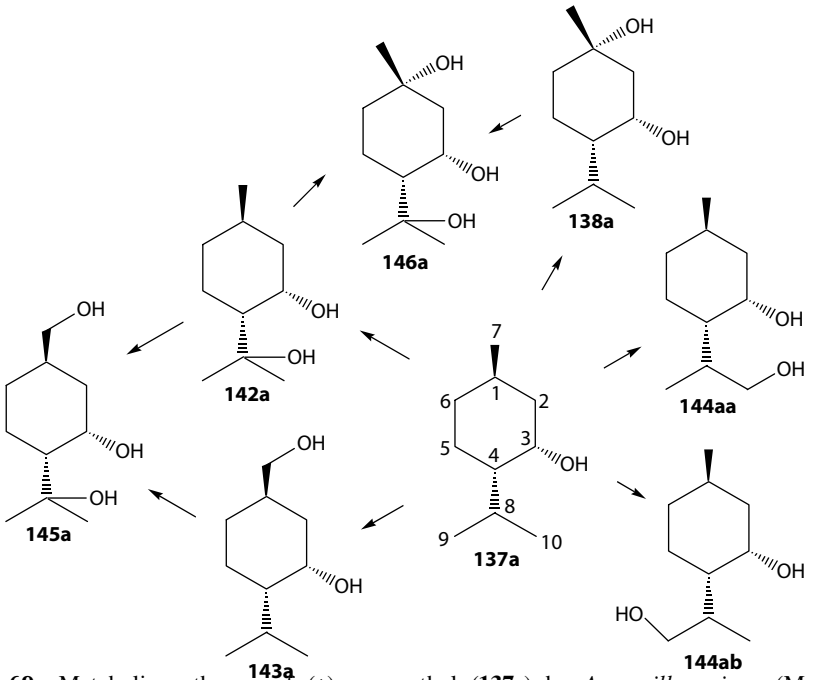


FIGURE 14.69 Metabolic pathways of (+)-neomenthol (**137a**) by *Aspergillus niger*. (Modified from Takahashi, H. et al., 1994. *Phytochemistry*, 35: 1465–1467.)

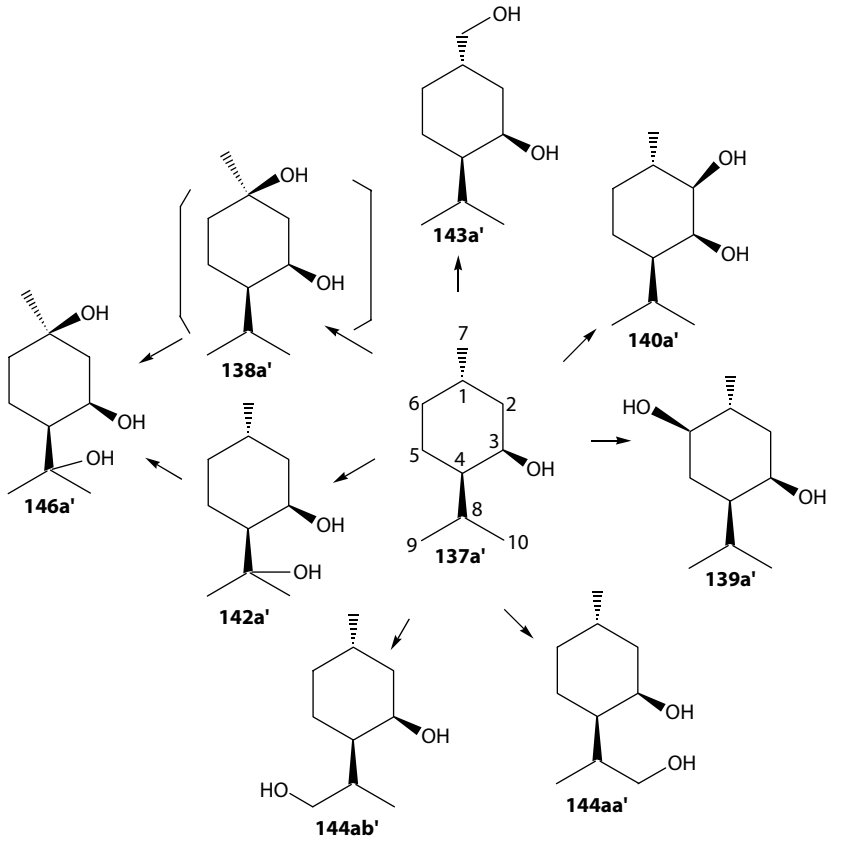


FIGURE 14.70 Metabolic pathways of (–)-neomenthol (**137a′**) by *Aspergillus niger.* (Modified from Takahashi, H. et al., 1994. *Phytochemistry*, 35: 1465–1467.)

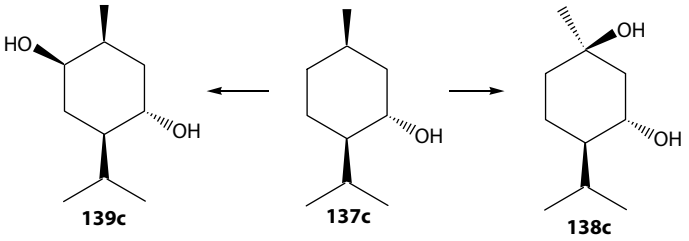


FIGURE 14.71 Metabolic pathways of (+)-isomenthol (**137c**) by *Aspergillus niger.* (Modified from Takahashi, H. et al., 1994. *Phytochemistry*, 35: 1465–1467.)

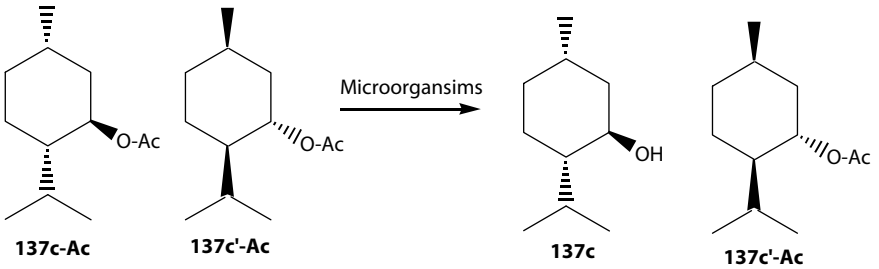


FIGURE 14.72 Microbial resolution of (±)-isomenthyl acetate (**137c-Ac** and **137c′-Ac**) by microbial esterase. (Modified from Oritani, T. and Yamashita, K. 1973b. *Agric. Biol. Chem*., 37: 1695–1700.)

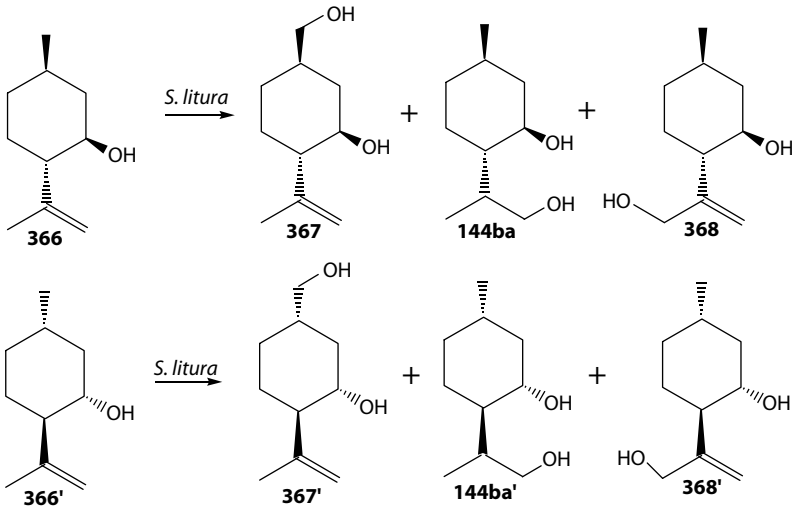


FIGURE 14.73 Biotransformation of (–)- (**366**) and (+)-isopulegol (**366′**) by *Spodoptera litura*. (Modified from Ohsawa, M. and Miyazawa, M. 2001. *Proc. 45th TEAC*, pp. 375–376.)

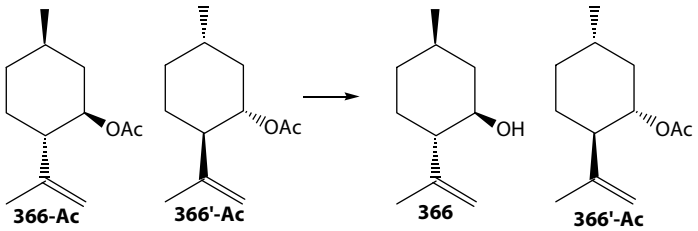


FIGURE 14.74 Microbial resolution of (±)-isopulegyl acetate (**366-Ac** and **366′-Ac**) by microorganisms. (Modified from Oritani, T. and K. Yamashita, 1973c. *Agric. Biol. Chem*., 37: 1687–1689.)

14.3.3.5 α-Terpineol

Pseudomonas pseudomonalli strain T was cultivated with α-terpineol (**34**) as the sole carbon source to give 8,9-epoxy-*p*-menthan-1-ol (**58**) via epoxide (**369**) and diepoxide (**57**) as intermediates (Hayashi et al., 1972) (Figure 14.75).

(+)-α-Terpineol (**34**) was formed from (+)-limonene (**34**) by *Citrus* pathogenic *Pencillium digitatum* (Pers.; Fr.) Sacc. KCPYN, which was further biotransformed to *p*-menthane-1β,2α,8-triol (**334**), 2α-hydroxy-1,8-cineole (**125b**), and (+)-*trans*-sobrerol (**95a**) (Noma and Asakawa 2006a, 2007a) (Figure 14.76). *Penicillium* sp. YuzuYN also biotransformed **34** to **334**. Furthermore, *Aspergillus niger* Tiegh, CBAYN and *Catharanthus roseus* biotransformed **34** to give **95a** and (+)-oleuropeyl alcohol (**204**), respectively (Hamada et al., 2001; Noma and Asakawa 2006a, 2007a) (Figure 14.76).

Gibberella cyanea DSM 62719 biotransformed (–)-α-terpineol (**34′**) to give *p*-menthane-1-β,2α,8-triol (**334′**), 2α-hydroxy-1,8-cineole (**125b′**), 1,2-epoxy-α-terpineol (**369′**), (–)-oleuropeyl alcohol (**204′**), (–)-*trans*-sobrerol (**95a′**), and *cis*-sobrerol (**95b′**) (Abraham et al., 1986) (Figure 14.76). In cases of *Pencillium digitatum* (Pers. Fr.) Sacc. KCPYN, *Penicillium* sp. YuzuYN, *Aspergillus niger* Tiegh, CBAYN **34′** was biotransformed to give **369′**, **95a′**, and **334′**, respectively (Noma and Asakawa 2006a, 2007a) (Figure 14.77). *Catharanthus roseus* biotransformed **34′** to give **95a′** and **204′** (Hamada et al., 2001) (Figure 14.77).

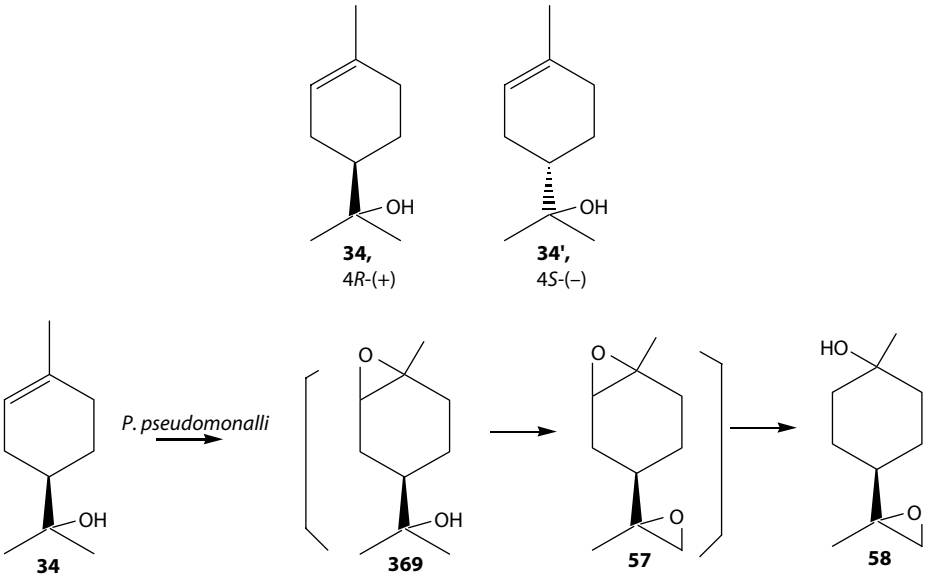


FIGURE 14.75 Biotransformation of (+)-α-terpineol (**34**) to 8,9-epoxy-*p*-menthan-1-ol (**58**) by *Pseudomonas pseudomonalli* strain T. (Modified from Hayashi, T. et al., 1972. *Biol. Chem.*, 36: 690–691.)

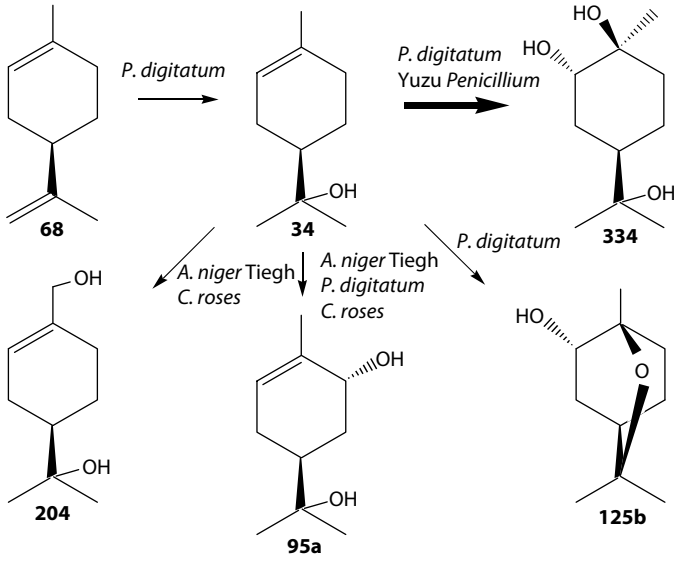


FIGURE 14.76 Biotransformation of (+)-α-terpineol (**34**) by *Citrus* pathogenic fungi, *Pencillium digitatum* (Pers.; Fr.) Sacc. KCPYN, *Penicillium* sp. YuzuYN, *Aspergillus niger* Tiegh, CBAYN. (Modified from Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

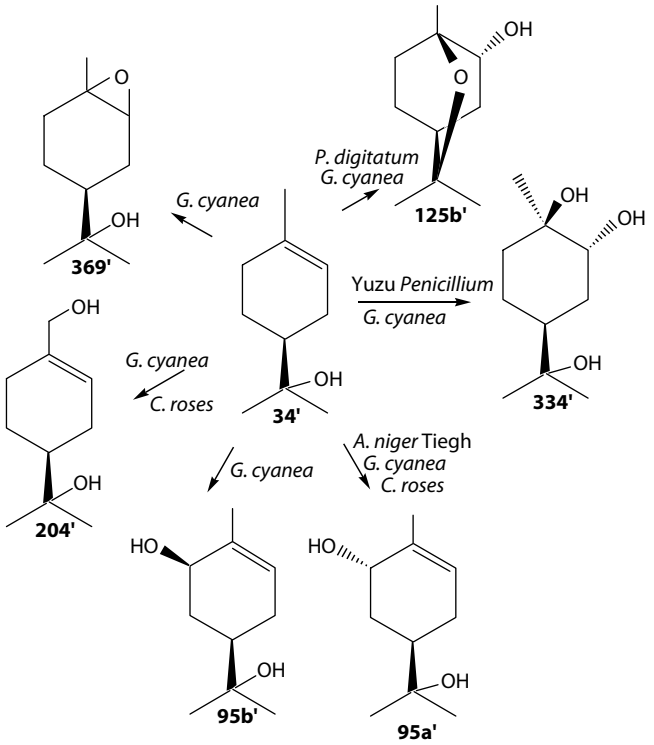
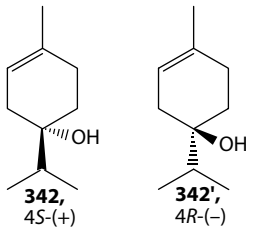


FIGURE 14.77 Biotransformation of (–)-α-terpineol (**34′**) by *Gibberella cyanea* DSM 62719, *Pencillium digitatum* (Pers. Fr.) Sacc. KCPYN, *Penicillium* sp. Yuzu YN, *Aspergillus niger* Tiegh, CBAYN. (Modified from Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol.*, 24: 24–30; Noma, Y. and Y. Asakawa, 2006a. *Proc. 50th TEAC*, pp. 431–433; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

14.3.3.6 (–)-Terpinen-4-ol



Gibberella cyanea DSM 62719 biotransformed (*S*)-(–)-terpinen-4-ol (**342**) (1-*p*-menthen-4-ol) to give 2α-hydroxy-1,4-cineole (**132b**), 1-*p*-menthene-4α,6-diol (**372**), and *p*-menthane-1β,2α,4α-triol (**371**) (Abraham et al., 1986). On the other hand, *Aspergillus niger* TBUYN-2 also biotransformed (–)-terpinen-4-ol (**342**) to give 2α-hydroxy-1,4-cineole (**132b**) and (+)-*p*-menthane-1β,2α,4α-triol (**371**) (Noma and Asakawa 2007b) (Figure 14.78). On the other hand, *Spodoptera litura* biotransformed (*R*)-terpinen-4-ol (**342′**) to (4*R*)-*p*-menth-1-en-4,7-diol (**373′**) (Kumagae and Miyazawa, 1999) (Figure 14.78).

14.3.3.7 Thymol and Thymol Methyl Ether

Thymol (**179**) was converted at the concentration of 14% by *Streptomyces humidus*, Tu-1 to give (1*R*,2*S*)- (**181a**) and (1*R*,2*R*)-2-hydroxy-3-*p*-menthen-5-one (**181b**) as the major products (Noma et al., 1988a) (Figure 14.79). On the other hand, in a *Pseudomonas*, thymol (**179**) was biotransformed to 6-hydroxy- (**180**), 7-hydroxy- (**479**), 9-hydroxy- (**480**), 7,9-dihydroxythymol (**482**), thymol-7-oic acid (**481**), and thymol-9-oic acid (**483**) (Chamberlain and Dagley, 1968) (Figure 14.79).

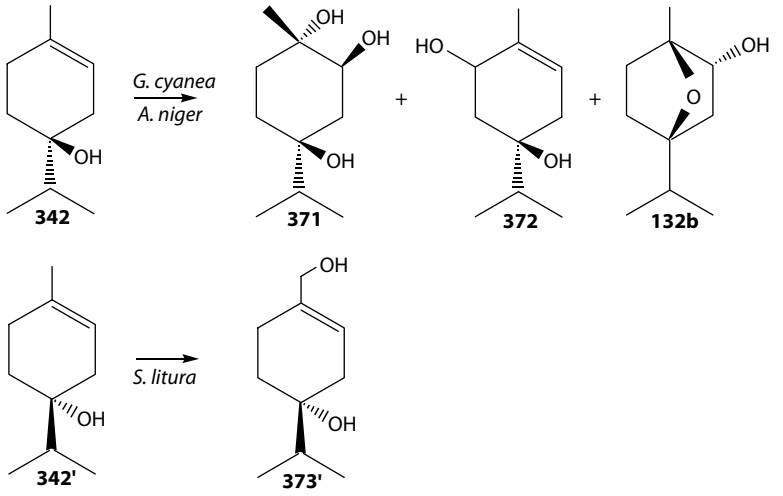


FIGURE 14.78 Biotransformation of (–)-terpinen-4-ol (**342**) by *Gibberella cyanea* DSM 62719, *Aspergillus niger* TBUYN-2, and *Spodoptera litura*. (Modified from Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol*., 24: 24–30; Kumagae, S. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 389–390; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

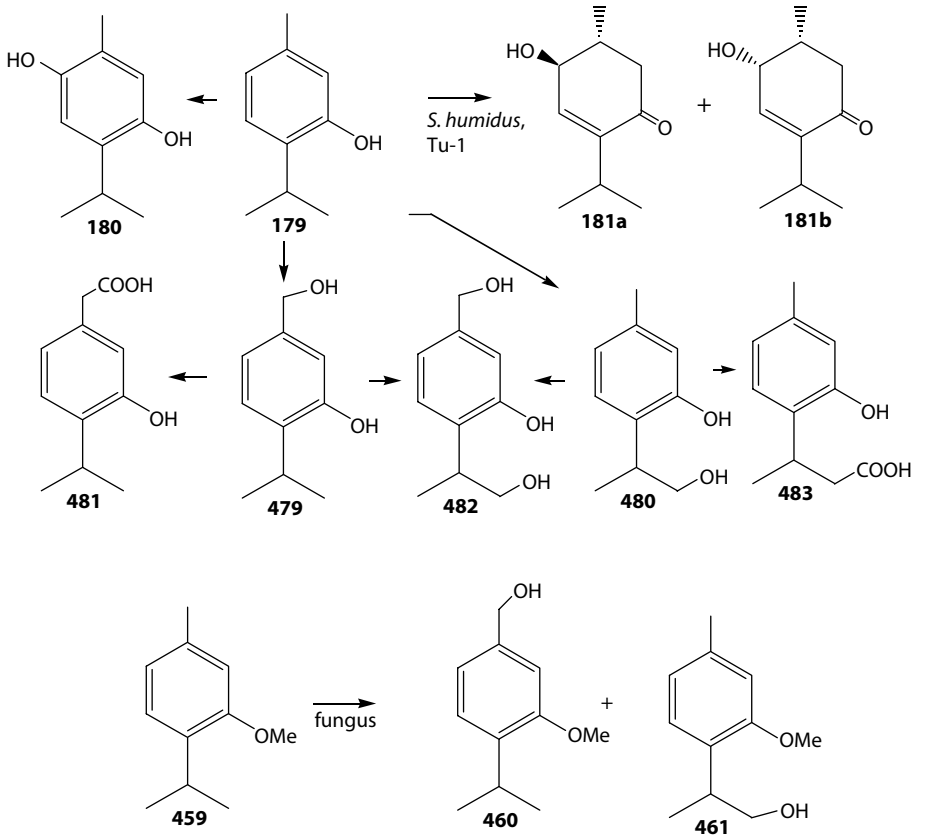


FIGURE 14.79 Biotransformation of thymol (**179**) and thymol methyl ether (**459**) by actinomycetes *Streptomyces humidus*, Tu-1 and fungi *Aspergillus niger*, *Mucor ramannianus*, *Rhizopus arrhizus*, and *Trichothecium roseum*. (Modified from Chamberlain, E.M. and S. Dagley, 1968. *Biochem. J.*, 110: 755–763; Noma, Y. et al., 1988a. *Proc. 28th TEAC*, pp. 177–179; Demirci, F. et al., 2001. *XII Biotechnology Congr.*, Book of abstracts, p. 47.)

Thymol methyl ether (**459**) was converted by fungi, *Aspergillus niger*, *Mucor ramannianus*, *Rhizopus arrhizus*, and *Trichothecium roseum* to give 7-hydroxy- (**460**) and 9-hydroxythymol methyl ether (**461**) (Demirci et al., 2001) (Figure 14.79).

14.3.3.8 Carvacrol and Carvacrol Methyl Ether

When cultivated in a liquid medium with carvacrol (**191**), as a sole carbon source, the bacterial isolated from savory and pine consumed the carvacrol in the range of 19–22% within 5 days of cultivation. The fungal isolates grew much slower and after 13 days of cultivation consumed 7.1–11.4% carvacrol (**191**). Pure strains belonging to the bacterial genera of Bacterium, *Bacillus* and *Pseudomonas* as well as fungal strain from *Aspergillus*, *Botrytis*, and *Geotrichum genera*, were also tested for their ability to grow in medium containing carvacrol (**191**). Among them, only in *Bacterium* sp. and *Pseudomonas* sp. Carvacrol (**191**) uptake was monitored. Both *Pseudomonas* sp. 104 and 107 consumed the substrate in the amount of 19%. These two strains also exhibited the highest cell mass yield and the highest productivity (1.1 and 1.2 g/L per day) (Schwammle et al., 2001).

Carvacrol (**191**) was biotransformed to 3-hydroxy- (**470**), 9-hydroxy (**471**), 7-hydroxy- (**475**), and 8-hydroxycarvacrol (**474**), 8,9-dehydrocarvacrol (**473**), carvacrol-9-oic acid (**472**), carvacrol-7-oic acid (**476**), and 8,9-dihydroxycarvacrol (**477**) by rats (Ausgulen et al., 1987) and microorganisms (Demirci, 2000) including *Trichothecium roseum* and *Cladosporium* sp. (Figure 14.80). Furthermore, carvacrol methyl ether (**191-Me**) was converted by the same fungi to give 7-hydroxy- (**475-Ac**) and

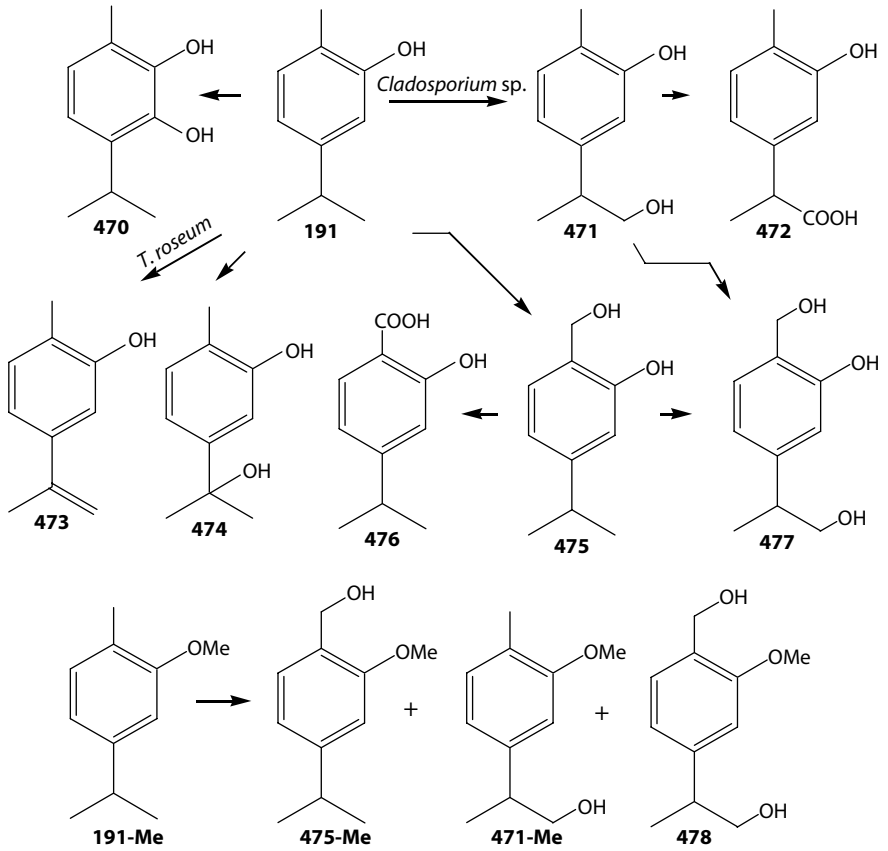
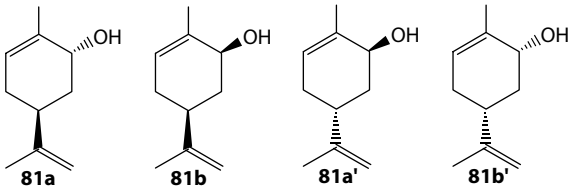


FIGURE 14.80 Biotransformation of carvacrol (**191**) and carvacrol methyl ether (**191-Me**) by rats (Modified from Ausgulen, L.T. et al., 1987. *Pharmacol. Toxicol*., 61: 98–102) and microorganisms (Modified from Demirci, F., 2000. *Microbial transformation of bioactive monoterpenes*. Ph.D. thesis, pp. 1–137. Anadolu University, Eskisehir, Turkey).

9-hydroxycarvacrol methyl ether (**471-Me**) and 7,9-dihydroxycarvacrol methyl ether (**478**) (Demirci, 2000) (Figure 14.80).

14.3.3.9 Carveol



At first, soil Pseudomonad biotransformed (+)-limonene (**68**) to (+)-carvone (**93**) and (+)-1-*p*-menthene-6,9-diol (**90**) via (+)-*cis*-carveol (**81b**) as shown in Figure 14.81 (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966).

Secondary, *Pseudomonas ovalis*, strain 6-1 (Noma, 1977) biotransformed the mixture of (–)-*cis*-carveol (**81b′**) and (–)-*trans*-carveol (**81a′**) (94:6, GC ratio) to (–)-carvone (**93'**) (Noma, 1977), which was further metabolized reductively to give (+)-dihydrocarvone (**101a′**), (+)-isodihydrocarvone (**101b′**), (+)-neodihydrocarveol (**102a),** and (–)-dihydrocarveol (**102b**) (Noma et al., 1984). Hydrogenation at C1, 2-position did not occur, but the dehydrogenation at C6-position occured to give (–)-carvone (**93**) (Figure 14.82).

On the other hand, in *Streptomyces*, A-5-1 and *Nocardia*, 1-3-11, which were isolated from soil, (–)-carvone (**93′)** was reduced to give mainly (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**), respectively. On the other hand, (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**) were dehydrogenated to give **93′** by strain 1-3-11 and other microorganisms (Noma et al., 1986). The reaction between *trans*- and *cis*-carveols (**81a′** and **81b′**) and (–)-carvone (**93′)** is reversible (Noma, 1980) (Figure 14.82).

Thirdly, the investigation for the biotransformation of the mixture of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) (60:40 in GC ratio) was carried out by using 81 strains of soil actinomycetes. All actinomycetes produced (–)-carvone (**93′**) from the mixture of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) (60:40 in GC ratio). However, 41 strains of actinomycetes converted (–)-*cis*-carveol (**81b′**) to give (4*R*,6*R*)-(+)-6,8-oxidomenth-1-en-9-ol (**92a′**), which is named as bottrospicatol after the name of the microorganism, *Streptomyces bottropensis* ⌈Bottro⌋, and (–)-*cis*-carveol (**81b′**) containing *Mentha spicata* ⌈spicat⌋ and alcohol ⌈ol⌋ (Nishimura et al., 1983a) (Figure 14.83).

(+)-Bottrospicatol (**92a′**) was prepared by epoxidation of (–)-carvone (**93′**) with *m*CPBA to (–)-carvone-8,9-epoxide (**96′**), followed by stereoselective reduction with $NaBH_4$ to alcohol, which was immediately cyclized with 0.1 N H_2SO_4 to give diastereo mixture of bottrospicatol (**92a′** and **b′**) (Nishimura et al., 1983a) (Figure 14.84).

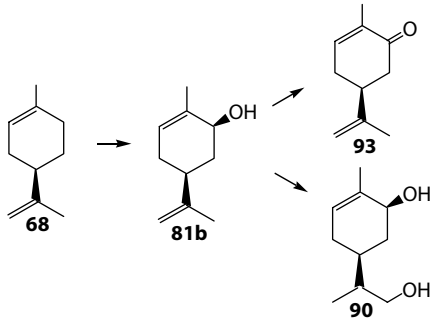


FIGURE 14.81 Proposed metabolic pathway of (+)-limonene (**68**) and (+)-*cis*-carveol (**81b**) by soil Pseudomonad. (Modified from Dhavalikar, R.S. and P.K. Bhattacharyya, 1966. *Indian J. Biochem*., 3: 144–157; Dhavalikar, R.S. et al., 1966. *Indian J. Biochem*., 3: 158–164.)

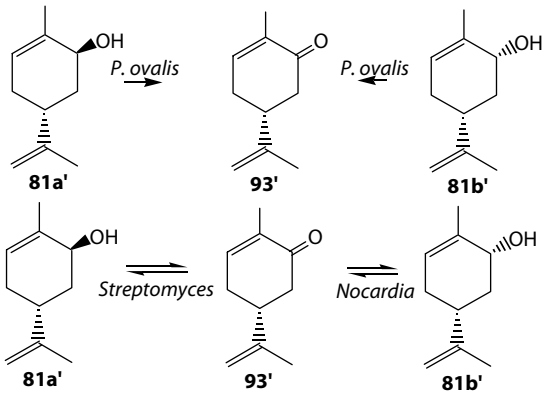


FIGURE 14.82 Biotransformation of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) (6:94, GC ratio) by *Pseudomonas ovalis*, strain 6-1, *Streptomyces* , A-5-1, and *Nocardia*, 1-3-11. (Modified from Noma, Y., 1977. *Nippon Nogeikagaku Kaishi*, 51: 463–470; Noma, Y., 1980. *Agric. Biol. Chem*., 44: 807–812.)

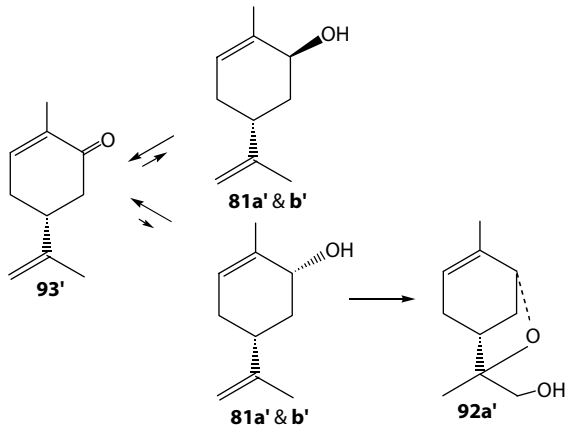


FIGURE 14.83 The Metabolic pathways of *cis*-carveol (**81b′**) by *Pseudomonas ovalis*, strain 6-1 (Modified from Noma, Y., 1977. *Nippon Nogeikagaku Kaishi*, 51: 463–470) and *Streptomyces bottropensis* SY-2-1 and other microorganisms (Modified from Noma, Y. et al., 1982. *Agric. Biol. Chem*., 46: 2871–2872; Nishimura, H. et al., 1983a. *Proc. 27th TEAC*, pp. 107–109).

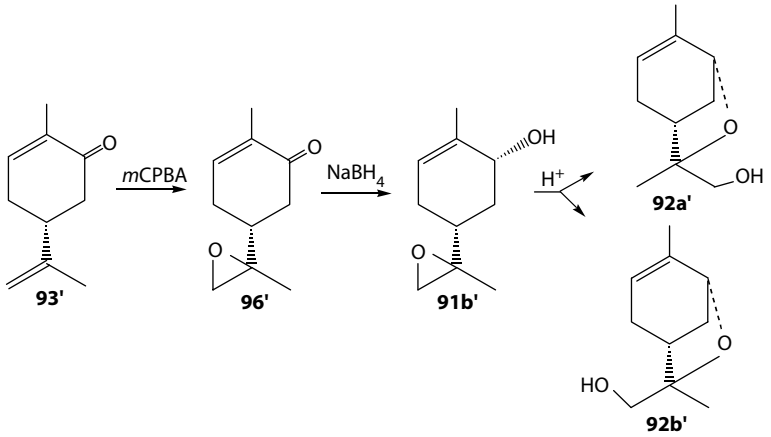


FIGURE 14.84 Preparation of (+)-bottrospicatol (**92a′**) and (+)-isobottrospicatol (**92b′**) from (–)-carvone (**93′**) with *m*CPBA. (Modified from Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp. 173–187. American Chemical Society, Washington, DC.)

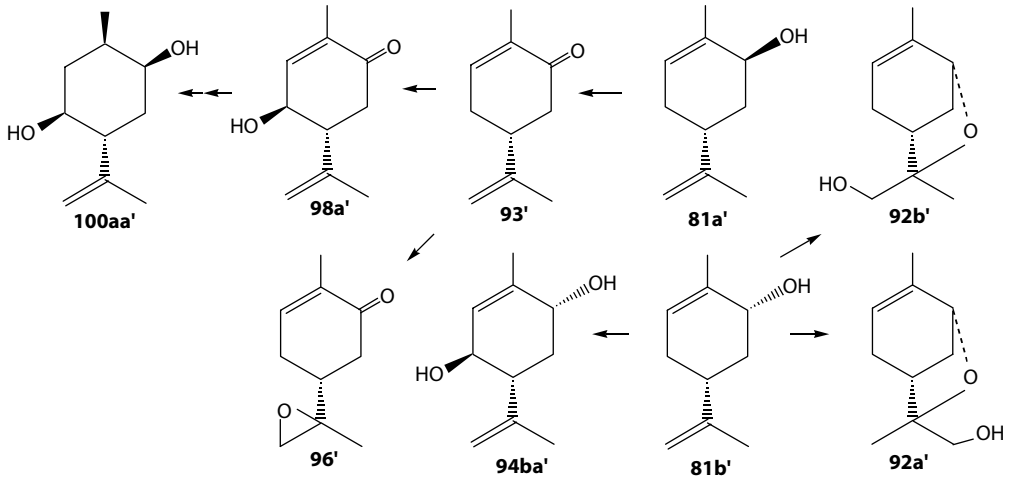


FIGURE 14.85 Biotransformation of (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis* Ya-2-1. (Modified from Noma, Y. et al., 1982. *Agric. Biol. Chem*., 46: 2871–2872; Noma, Y. and H. Nishimura, 1984. *Proc. 28th TEAC*, pp. 171–173; Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849.)

Further investigation showed *Streptomyces bottropensis* SY-2-1 (Noma and Iwami, 1994) has different metabolic pathways for (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**). Namely, *Streptomyces bottropensis* SY-2-1 converted (–)-*trans*-carveol (**81a′**) to (–)-carvone (**93′**), (–)-carvone-8,9-epoxide (**96′**), (–)-5β-hydroxycarvone (**98a′**), and (+)-5β-hydroxyneodihydrocarveol (**100aa′**) (Figure 14.85). On the other hand, *Streptomyces bottropensis* SY-2-1 converted (–)-*cis*-carveol (**81b′**) to give (+)-bottrospicatol (**92a′**) and (–)-5β-hydroxy-*cis*-carveol (**94ba′**) as main products together with (+)-isobottrospicatol (**92b′**) as the minor product as shown in Figure 14.85.

In the metabolism of *cis*-carveol by microorganisms there are four pathways (pathways 1–4) as shown in Figure 14.86. At first, *cis*-carveol (**81**) is metabolized to carvone (**93**) by C2 dehydrogenation (Noma, 1977, 1980) (pathway 1). Secondly, *cis*-carveol (**81b**) is metabolized via epoxide as intermediate to bottrospicatol (**92**) by rearrangement at C2 and C8 (Noma et al., 1982; Nishimura et al., 1983a, 1983b; Noma and Nishimura, 1987) (pathway 2). Thirdly, *cis*-carveol (**81b**) is hydroxylated at C5 position to give 5-hydroxy-*cis*-carveol (**94**) (Noma and Nishimura, 1984) (pathway 3).

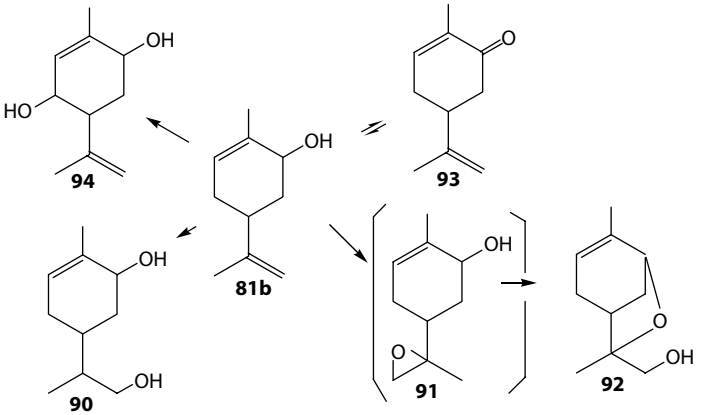


FIGURE 14.86 General metabolic pathways of carveol (**81**) by microorganisms. (Modified from Noma, Y. et al., 1982. *Agric. Biol. Chem*., 46: 2871–2872; Noma, Y. and H. Nishimura, 1984. *Proc. 28th TEAC*, pp. 171–173; Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849; Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.)

TABLE 14.3
Effects of (–)-*cis*- (81b′) and (–)-*trans*-Carveol (81a′) Conversion Products by *Streptomyces bottropensis* SY-2-1 on the Germination of Lettuce Seeds

	Germination Rate (%)	
Compounds	**24 h**	**48 h**
(–)-Carvone (**93′**)	47	89
(+)-Bottrospicatol (**92′**)	3	48
(–)-Carvone-8,9-epoxide (**96′**)	2	77
5β-Hydroxyneodihydrocarveol (**102aa′**)	86	96
5β-Hydroxycarvone (**98a′**)	91	96
Control	95	96

Note: Concentration of each compound was adjusted at 200 ppm.

Finally, *cis*-carveol (**81b**) is metabolized to 1-*p*-menthene-2,9-diol (**90**) by hydroxylation at C9 position (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966) (pathway 4).

Effects of (–)-*cis*- (**81b′**) and (–)-*trans*-carveol (**81a′**) conversion products by *Streptomyces bottropensis* SY-2-1 on the germination of lettuce seeds was examined and the result is shown in Table 14.3. (+)-Bottrospicatol (**92′**) and (–)-carvone-8,9-epoxide (**96′**) showed strong inhibitory activity for the germination of lettuce seeds.

Streptomyces bottropensis SY-2-1 has also different metabolic pathways for (+)-*trans*-carveol (**81a**) and (+)-*cis*-carveol (**81b**) (Noma and Iwami, 1994). Namely, *Streptomyces bottropensis* SY-2-1 converted (+)-*trans*-carveol (**81a**) to (+)-carvone (**93**), (+)-carvone-8,9-epoxide (**96**), and (+)-5α-hydroxycarvone (**98a**) (Noma and Nishimura, 1982, 1984) (Figure 14.87). On the other hand, *Streptomyces bottropensis* SY-2-1 converted (+)-*cis*-carveol (**81b**) to give (–)-isobottrospicatol (**92b**) and (+)-5-hydroxy-*cis*-carveol (**94b**) as the main products and (–)-bottrospicatol (**92a**) as the minor product as shown in Figure 14.88 (Noma et al., 1980, Noma and Nishimura, 1987; Nishimura and Noma, 1996).

Biological activities of (+)-bottrospicatol (**92a′**) and related compounds for plant's seed germination and root elongation were examined towards barnyard grass, wheat, garden cress, radish, green foxtail, and lettuce (Nishimura and Noma, 1996).

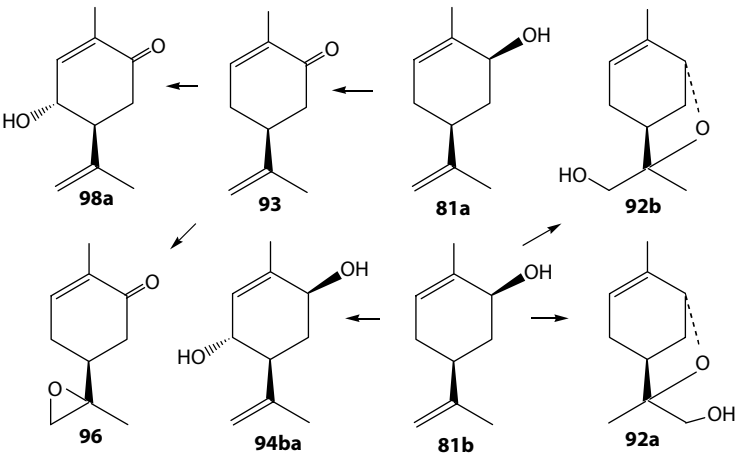


FIGURE 14.87 Metabolic pathways of (+)-*trans*- (**81a**) and (+)-*cis*-carveol (**81b**) by *Streptomyces bottropensis* SY-2-1. (Modified from Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849; Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.)

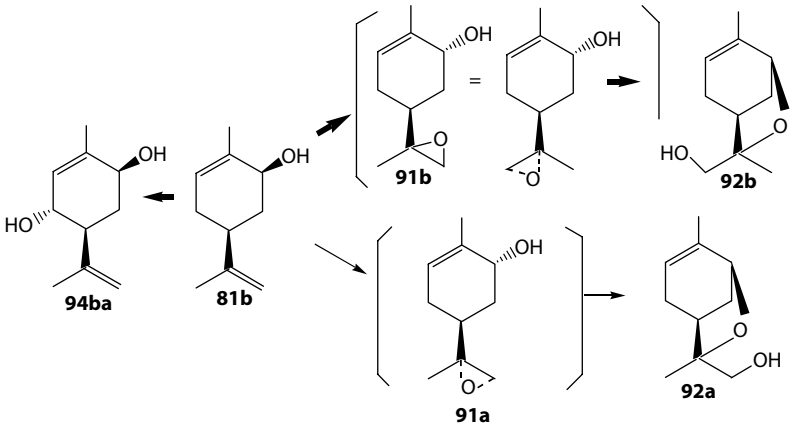


FIGURE 14.88 Metabolic pathways of (+)-*cis*-carveol (**81b**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1987. *Agric. Biol. Chem*., 51: 1845–1849; Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp.173–187. American Chemical Society, Washington, DC.)

Isomers and derivatives of bottrospicatol were prepared by the procedure shown in Figure 14.89. The chemical structure of each compound was confirmed by the interpretation of spectral data. The effects of all isomers and derivatives on the germination of lettuce seeds were compared. The germination inhibitory activity of (+)-bottrospicatol (**92a′**) was the highest of isomers. Interestingly, (−)-isobottrospicatol (**92b**) was not effective even in a concentration of 500 ppm. (+)-Bottrospicatol methyl ether (**92a′**-methyl ether) and esters [**92a′**-methyl (ethyl and *n*-propyl) ester] exhibited weak inhibitory activities. The inhibitory activity of (−)-isodihydrobottrospicatol (**105c′**) was as high as that of (+)-bottrospicatol (**92a′**). Furthermore, an oxidized compound, (+)-bottrospicatal (**374a′**), exhibited higher activity than (+)-bottrospicatol (**92a′**). So, the germination inhibitory activity of (+)-bottrospicatal (**374a′**) against several plant seeds, lettuce, green foxtail, radish, garden cress, wheat, and

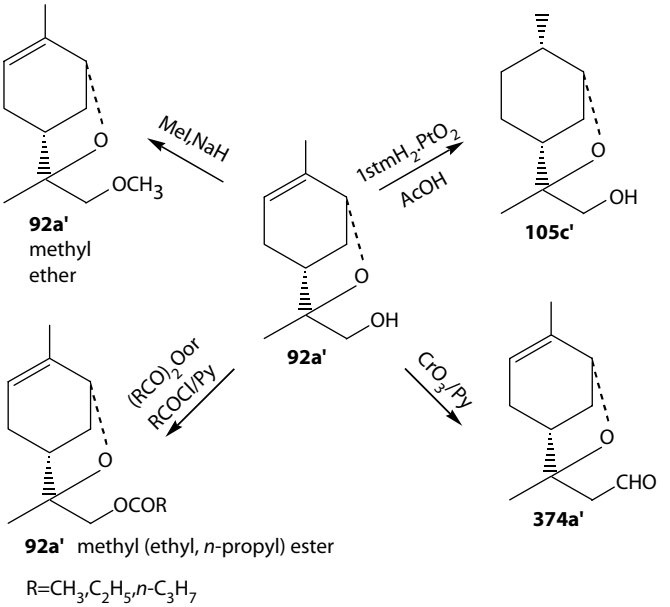


FIGURE 14.89 Preparation of (+)-bottrospicatol (**92a′**) derivatives. (Modified from Nishimura, H. and Y. Noma, 1996. *Biotechnology for Improved Foods and Flavors*, G.R. Takeoka, et al., ACS Symp. Ser. 637, pp. 173–187. American Chemical Society, Washington, DC.)

barnyard grass was examined. The result indicates that (+)-bottrospicatal (**374a′**) is a selective germination inhibitor as follows: lettuce > green foxtail > radish > garden cress > wheat > barnyard grass.

Enantio- and diastereoselective biotransformation of *trans*- (**81a** and **81a′**) and *cis*-carveols by *Euglena gracilis* Z. (Noma and Asakawa 1992) and *Chlorella pyrenoidosa* IAM C-28 was studied (Noma et al., 1997).

In the biotransformation of racemic *trans*-carveol (**81a** and **81a′**), *Chlorella pyrenoidosa* IAM C-28 showed high enantioselectivity for (−)-*trans*-carveol (**81a′**) to give (−)-carvone (**93′**), while (+)-*trans*-carveol (**81a**) was not converted at all. The same *Chlorella pyrenoidosa* IAM C-28 showed high enantioselectivity for (+)-*cis*-carveol (**81b**) to give (+)-carvone (**93**) in the biotransformation of racemic *cis*-carveol (**81b** and **81b′**). (−)-*cis*-Carveol (**81b′**) was not converted at all. The same phenomenon was observed in the biotransformation of mixture of (−)-*trans*- and (−)-*cis*-carveol (**81a′** and **81b′**) and the mixture of (+)-*trans*- and (+)-*cis*-carveol (**81a** and **81b**) as shown in Figure 14.90. The high enantioselectivity and the high diastereoselectivity for the dehydrogenation of (−)-*trans*- and (+)-*cis*-carveols (**81a** and **81b′**) were shown in *Euglena gracilis* Z. (Noma and Asakawa, 1992), *Chlorella pyrenoidosa* IAM C-28 (Noma et al., 1997), *Nicotiana tabacum*, and other *Chlorella* spp.

On the other hand, the high enantioselecivity for **81a′** was observed in the biotransformation of racemic (+)-*trans*-carveol (**81a**) and (−)-*trans*-carveol (**81a′**) by *Chlorella sorokiniana* SAG to give (−)-carvone (**93′**).

It was considered that the formation of (−)-carvone (**93′**) from (−)-*trans*-carveol (**81a′**) by diastereo- and enantioselective dehydrogenation is a very interesting phenomenon in order to produce mosquito repellent (+)-*p*-menthane-2,8-diol (**50a′**) (Noma, 2007).

(4*R*)-*trans*-Carveol (**81a′**) was converted by *Spodptera litura* to give 1-*p*-menthene-6,8,9-triol (**375**) (Miyazawa et al., 1996b) (Figure 14.91).

14.3.3.10 Dihydrocarveol

(+)-Neodihydrocarveol (**102a′**) was converted to *p*-menthane-2,8-diol (**50a′**), 8-*p*-menthene-2, 8-diol (**107a′**), and *p*-menthane-2,8,9-triols (**104a′** and **b′**) by *Aspergillus niger* TBUYN-2 (Noma et al., 1985a, 1985b; Noma and Asakawa, 1995) (Figures 14.92 and 14.93). In case of *Euglena gracilis* Z. mosquito repellent (+)-*p*-menthane-2,8-diol (**50a′**) was formed stereospecifically from (−)-carvone (**93′**) via (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**) (Noma et al., 1993; Noma, 2007). (−)-Neodihydrocarveol (**102a**) was also easily and stereospecifically converted by *Euglena gracilis* Z. to give (−)-*p*-menthane-2,8-diol (**50a**) (Noma et al., 1993).

On the other hand, *Absidia glauca* converted (−)-carvone (**93′**) stereospecifically to give (+)-8-*p*-menthene-2,8-diol (**107a′**) via (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**) (Demirci et al., 2004) (Figure 14.93).

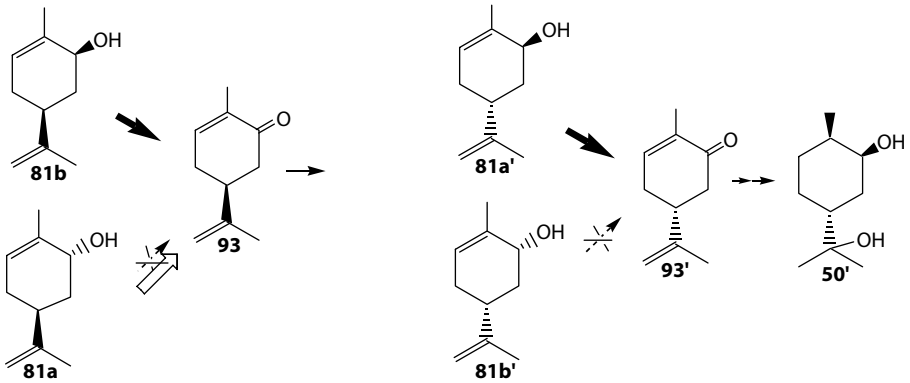


FIGURE 14.90 Enantio- and diastereoselective biotransformation of *trans*- (**81a** and **a′**) and *cis*-carveols (**81b** and **b′**) by *Euglena gracilis* Z and *Chlorella pyrenoidosa* IAM C-28. (Modified from Noma, Y., and Y. Asakawa, 1992. *Phytochem.*, 31: 2009–2011; Noma, Y. et al., 1997. *Proc. 41st TEAC*, pp. 227–229.)

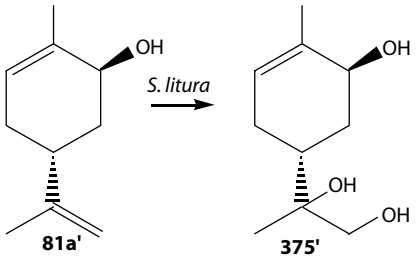


FIGURE 14.91 Biotransformation of (4*R*)-*trans*-carveol (**81a′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1996b. *Proc. 40th TEAC*, pp. 80–81.)

(+)- (**102b**) and (−)-Dihydrocarveol (**102b′**) were converted by 10 kinds of *Aspergillus* spp. to give mainly (+)- (**107b′**) and (−)-10-hydroxydihydrocarveol (**107b**, 8-*p*-menthene-2,10-diol) and (+)- (**50b′**) and (−)-8-hydroxydihydrocarveol (**50b**, *p*-menthane-2,8-diol), respectively (Figure 14.94). The metabolic pattern of dihydrocarveols is shown in Table 14.4.

In case of the biotransformation of *Streptomyces bottropensis*, SY-2-1 (+)-dihydrocarveol (**102b**) was converted to (+)-dihydrobottrospicatol (**105aa**) and (+)-dihydroisobottrospicatol (**105ab**), whereas (−)-dihydrocarveol (**102b′**) was metabolized to (−)-dihydrobottrospicatol (**105aa′**) and (−)-dihydroisobottrospicatol (**105ab′**). (+)-Dihydroisobottrospicatol (**105ab**) and (−)-dihydrobottrospicatol (**105aa′**) are the major products (Noma, 1984) (Figure 14.95).

Euglena gracilis Z. converted (−)-iso- (**102c**) and (+)-isodihydrocarveol (**102c′**) to give the corresponding 8-hydroxyisodihydrocarveols (**50c** and **50c′**), respectively (Noma et al., 1993) (Figure 14.96).

In case of the biotransformation of *Streptomyces bottropensis*, SY-2-1 (−)-neoisodihydrocarveol (**102d**) was converted to (+)-isodihydrobottrospicatol (**105ba**) and (+)-isodihydroisobottrospicatol (**105bb**), whereas (+)-neoisodihydro-carveol (**102d′**) was metabolized to (−)-isodihydrobottrospicatol (**105ba′**) and (−)-isodihydroisobottrospicatol (**105bb′**). (+)-Isodihydroisobottrospicatol (**105bb**) and (−)-isodihydrobottrospicatol (**105ba′**) are the major products (Noma, 1984) (Figure 14.97).

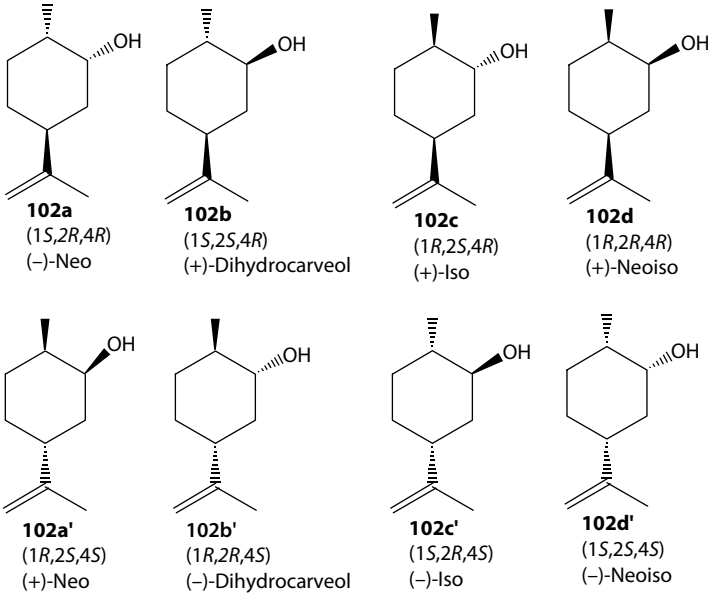


FIGURE 14.92 Chemical structure of eight kinds of dihydrocarveols.

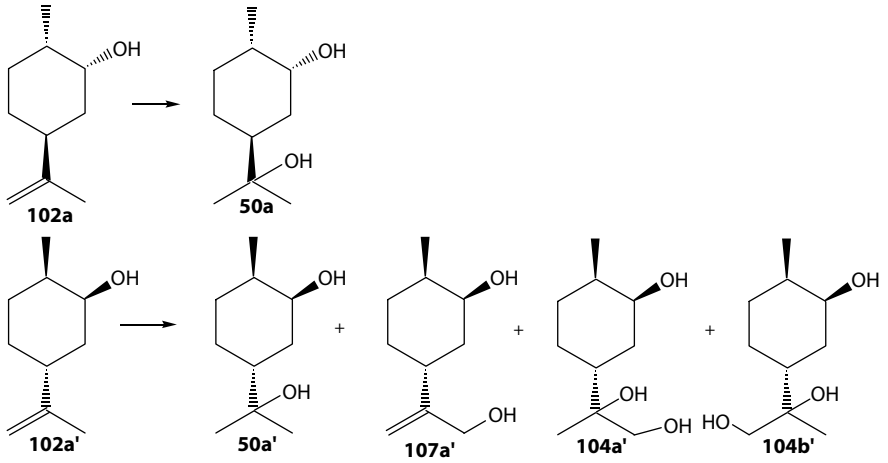


FIGURE 14.93 Biotransformation of (–)- and (+)-neodihydrocarveol (**102a** and **a′**) by *Euglena gracilis* Z, *Aspergillus niger* TBUYN-2, and *Absidia glauca*. (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68; Noma, Y. et al., 1985b. *Proc. 29th TEAC*, pp. 235–237; Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25; Noma, Y., 2007. *Aromatic Plants from Asia their Chemistry and Application in Food and Therapy*, L. Jiarovetz, N.X. Dung, and V.K. Varshney, pp. 169–186. Dehradun: Har Krishan Bhalla & Sons; Noma, Y. and Y. Asakawa, 1995. *Biotechnology in Agriculture and Forestry*, *Vol. 33. Medicinal and Aromatic Plants VIII*, Y.P.S. Bajaj, ed., pp. 62–96. Berlin: Springer; Demirci, F. et al., 2004. *Naturforsch.*, 59c: 389–392.)

Euglena gracilis Z. converted (–)- (**102d**) and (+)-neoisodihydrocarveol (**102d′**) to give the corresponding 8-hydroxyneoisodihydrocarveols (**50d** and **50d′**), respectively (Noma et al., 1993) (Figure 14.98).

Eight kinds of 8-hydroxydihydrocarveols (**50a**–**d** and **50a′**–**d′**; 8-*p*-menthane-2,8-diols) were obtained from carvone (**93** and **93′**), dihydrocarvones (**101a**–**b** and **101a′**–**b′**), and dihydrocarveols (**102a**–**d**, **102a′**–**d′**) by *Euglena gracilis* Z as shown in Figure 14.99 (Noma et al., 1993).

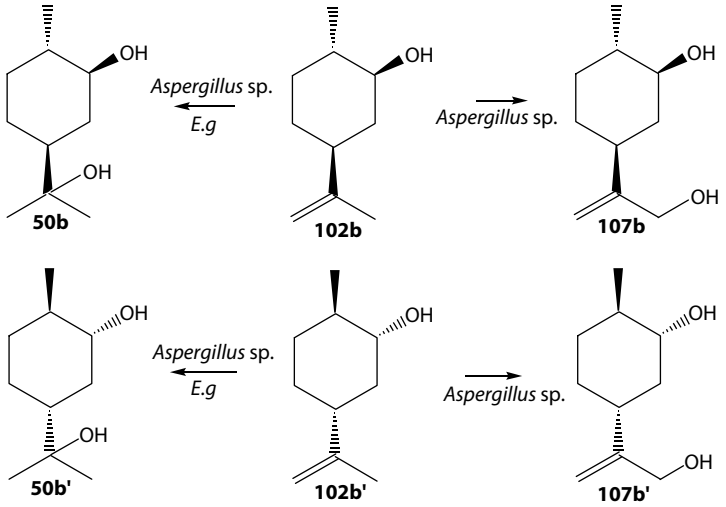


FIGURE 14.94 Biotransformation of (+)- (**102b**) and (–)-dihydrocarveol (**102b′**) by 10 kinds of *Aspergillus* spp. (Modified from Noma, Y., 1988. *The Meeting of Kansai Division of The Agricultural* and *Chemical Society of Japan*, Kagawa, p. 28) and *Euglena gracilis* Z (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25).

TABLE 14.4
Metabolic Pattern of Dihydrocarveols (102b and 102b′) by 10 Kinds of *Aspergillus* spp.

Microorganisms	Compounds					
	107b′	50b′	C.r. (%)	107b	50b	C.r. (%)
A. awamori, IFO 4033	0	98	99	3	81	94
A. fumigatus, IFO 4400	0	14	34	+	6	14
A. sojae, IFO 4389	0	47	59	1	50	85
A. usami, IFO 4338	0	32	52	+	5	7
A. cellulosae, M-77	0	27	52	+	7	14
A. cellulosae, IFO 4040	0	30	55	1	5	8
A. terreus, IFO 6123	0	79	92	+	18	46
A. niger, IFO 4034	0	29	49	+	8	12
A. niger, IFO 4049	4	50	67	9	34	59
A. niger, TBUYN-2	29	68	100	30	53	100

C.r.—conversion ratio.

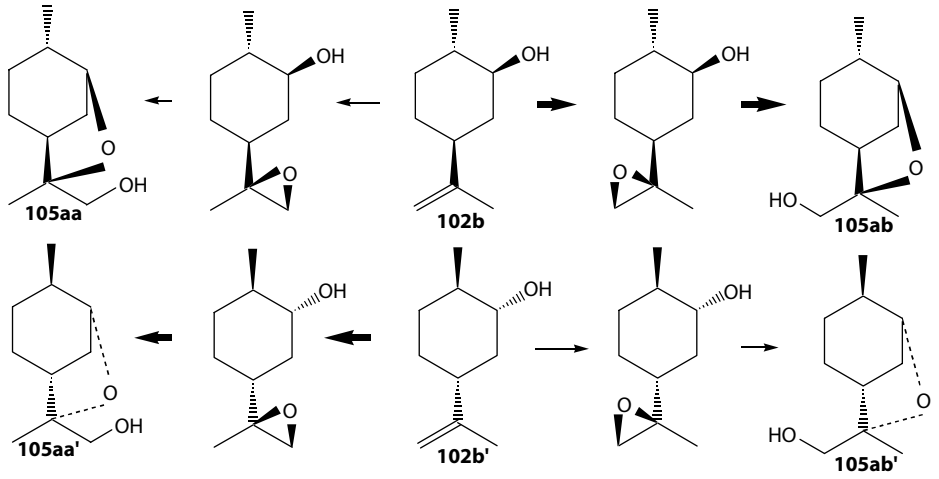


FIGURE 14.95 Biotransformation of (+)- (**102b**) and (–)-dihydrocarveol (**102b′**) by *Streptomyces bottropensis*, SY-2-1. (Modified from Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

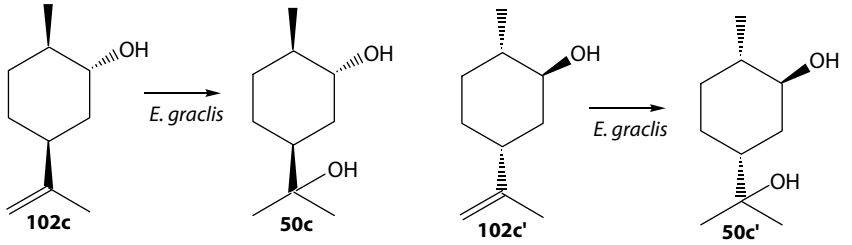


FIGURE 14.96 Biotransformation of (+)-iso- (**102c**) and (–)-dihydrocarveol (**102c′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25.)

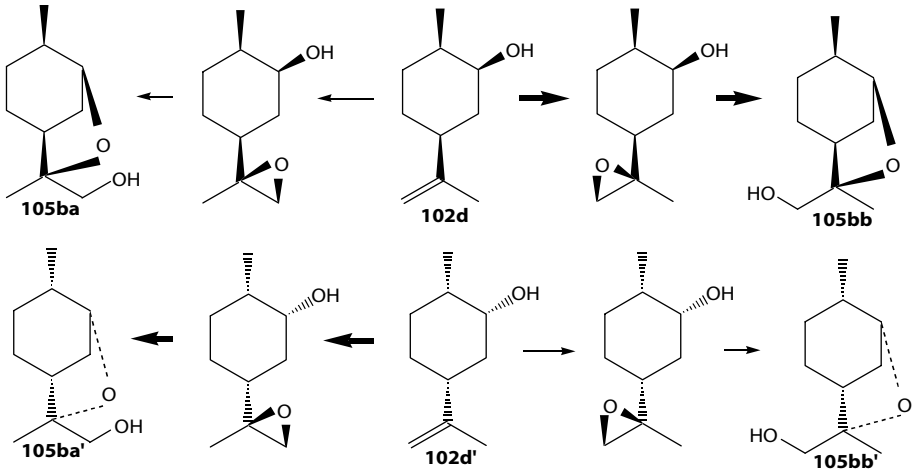


FIGURE 14.97 Formation of dihydroisobottrospicatols (**105**) from neoisodihydrocarveol (**102d** and **d′**) by *Streptomyces bottropensis*, SY-2-1. (Modified from Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

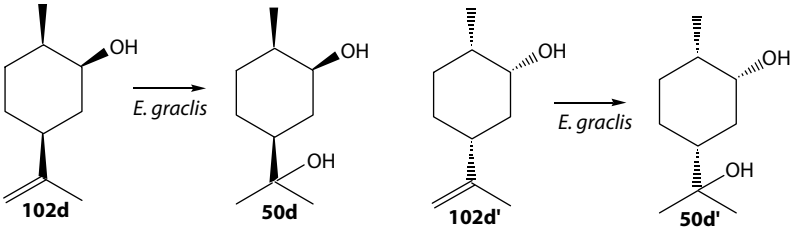
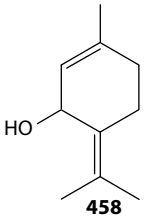


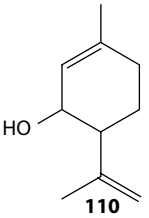
FIGURE 14.98 Biotransformation of (+)- (**102c**) and (−)-neoisodihydrocarveol (**102c′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25.)

14.3.3.11 Piperitenol



Incubation of piperitenol (**458**) with *Aspergillus niger* gave a complex metabolites whose structures have not yet been determined (Noma, 2000).

14.3.3.12 Isopiperitenol



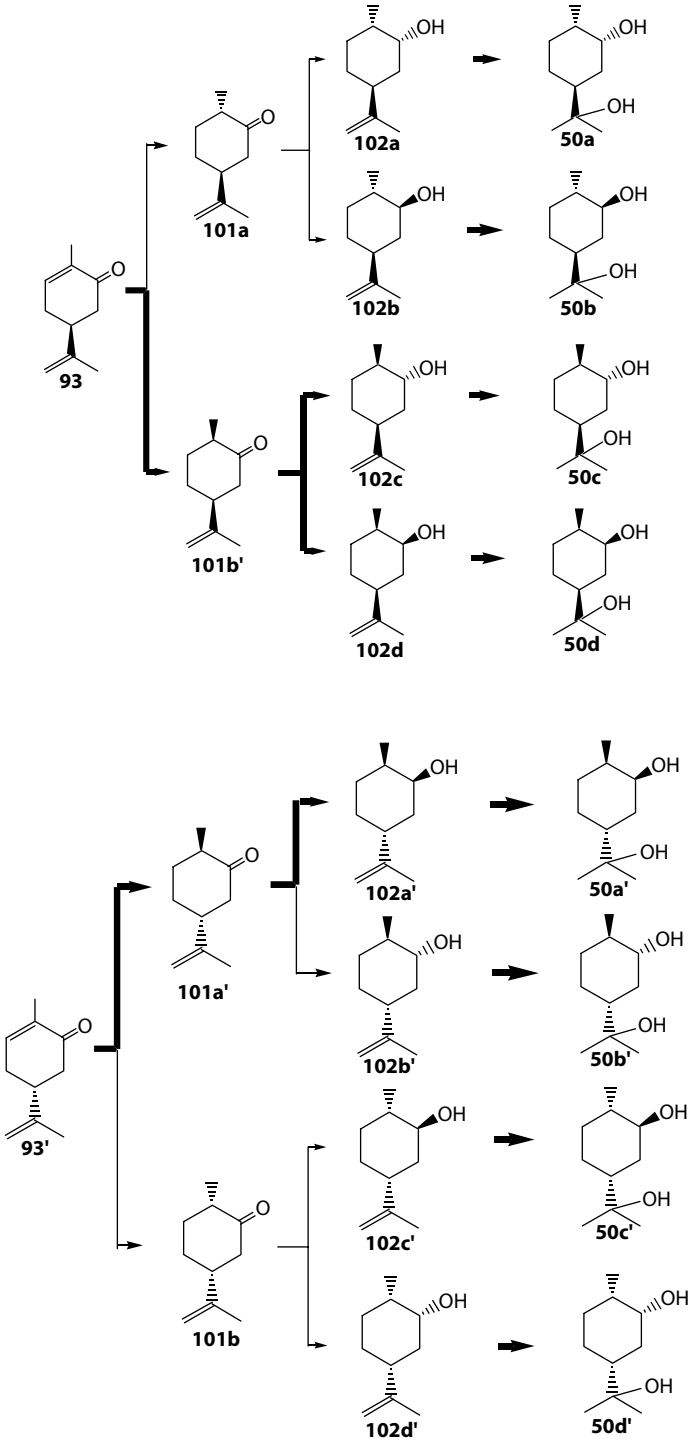
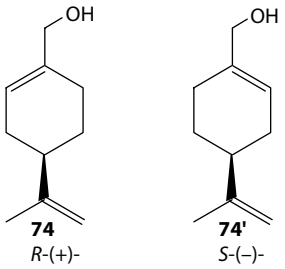


FIGURE 14.99 Formation of eight kinds of 8-hydroxydihydrocarveols (**50a**–**50d**, **50a′**–**50d′**), dihydrocarvones (**101a**–**101b** and **101a′**–**101b′**), and dihydrocarveols (**102a**–**102d** and **102a′**–**102d′**) from (+)- (**93**) and (–)-carvone (**93′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25.)

Piperitenol (**458**) was metabolized by *Aspergillus niger* to give a complex alcohol mixtures whose structures have not yet been determined (Noma, 2000).

14.3.3.13 Perillyl Alcohol



(–)-Perillyl alcohol (**74′**) was epoxidized by *Streptomyces ikutamanensis* Ya-2-1 to give 8,9-epoxy-(–)-perillyl alcohol (**77′**) (Noma et al., 1986) (Figure 14.100).

(–)-Perillyl alcohol (**74′**) was glycosylated by *Eucalyptus perriniana* suspension cells to (–)-perillyl alcohol monoglucoside (**376′**) and diglucoside (**377′**) (Hamada et al., 2002; Yonemoto et al., 2005) (Figure 14.101).

Furthermore, 1-perillly-β-glucopyranoside (**376**) was converted into the corresponding oligosaccharides (**377–381**) using a cyclodextrin glucanotransferase (Yonemoto et al., 2005) (Figure 14.102).

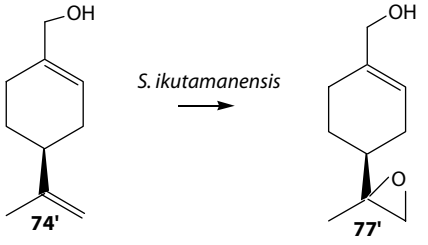


FIGURE 14.100 Biotransformation of (–)-perillyl alcohol (**74′**) by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206.)

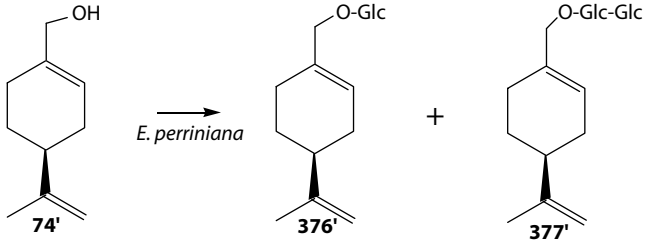


FIGURE 14.101 Biotransformation of (–)-perillyl alcohol (**74′**) by *Eucalyptus perriniana* suspension cell. (Modified from Hamada, H. et al., 2002. *Proc. 46th TEAC*, pp. 321–322; Yonemoto, N. et al., 2005. *Proc. 49th TEAC*, pp. 108–110.)

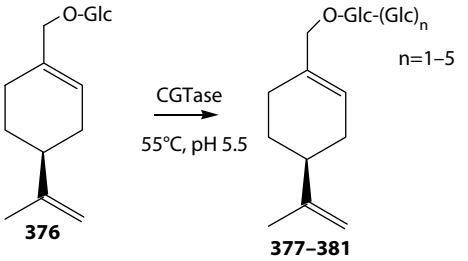
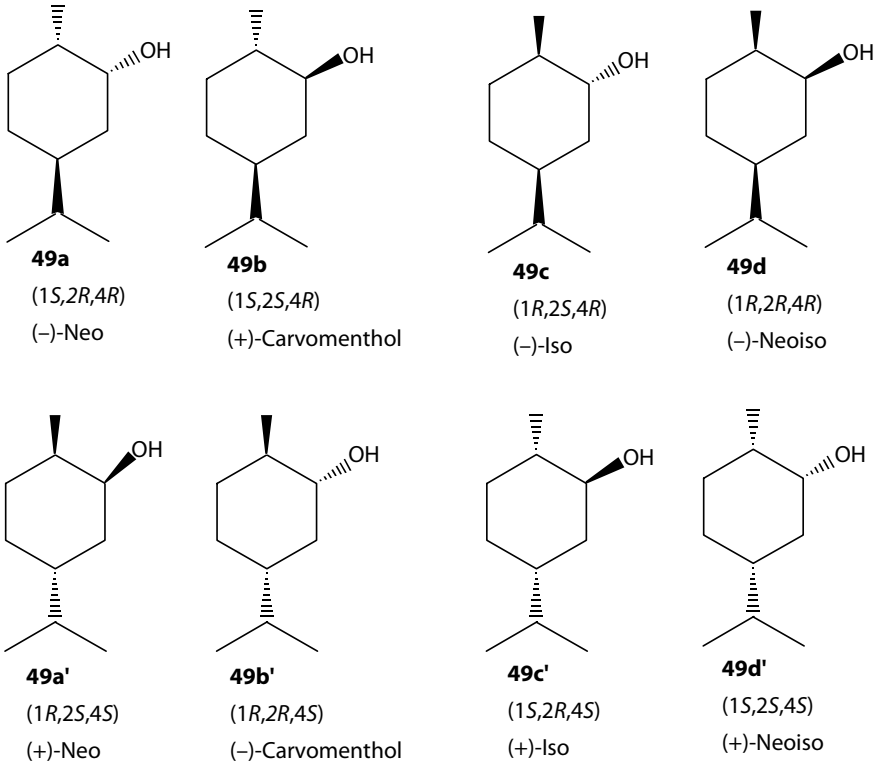


FIGURE 14.102 Biotransformation of (–)-perillyl alcohol monoglucoside (**376**) by CGTase. (Modified from Yonemoto, N. et al., 2005. *Proc. 49th TEAC*, pp. 108–110.)

14.3.3.14 Carvomenthol



(+)-Iso- (**49c**) and (+)-neoisocarvomenthol (**49d**) were formed from (+)-carvotanacetone (**47**) via (–)-isocarvomenthone (**48b**) by *Pseudomonas ovalis*, strain 6-1, whereas (+)-neocarvomenthol (**49a′**) and (–)-carvomenthol (**49b′**) were formed from (–)-carvotanacetone (**47′**) via (+)-carvomenthone (**48a′**) by the same bacteria; of which **48b**, **48a′**, and **49d** were the major products (Noma et al., 1974a) (Figure 14.103).

Microbial resolution of carvomenthols was carried out by selected microorganisms such as *Trichoderma S* and *Bacillus subtilis* var. *niger* (Oritani and Yamashita, 1973d). Racemic carvomenthyl acetate, racemic isocarvomenthyl acetate, and racemic neoisocarvomenthyl acetate were asymmetrically hydrolyzed to (–)-carvomenthol (**49b′**) with (+)-carvomenthyl acetate, (–)-isocarvomenthol (**49c**) with (+)-isocarvomenthyl acetate, and (+)-neoisocarvomenthol (**49d′**) with (–)-neoisocarvomenthyl acetate, respectively; racemic neocarvomenthyl acetate was not hydrolyzed (Oritani and Yamashita, 1973d) (Figure 14.104).

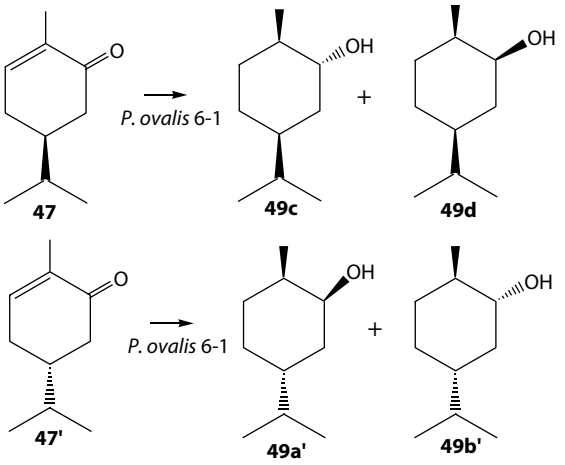


FIGURE 14.103 Formation of (–)-iso- (**49c**), (–)-neoiso- (**49d**), (+)-neo- (**49a′**), and (–)-carvomenthol (**49b′**) from (+)- (**47**) and (–)-carvotanacetone (**47′**) by *Pseudomonas ovalis*, strain 6-1. (Modified from Noma, Y. et al., 1974a. *Agric. Biol. Chem*., 38: 1637–1642.)

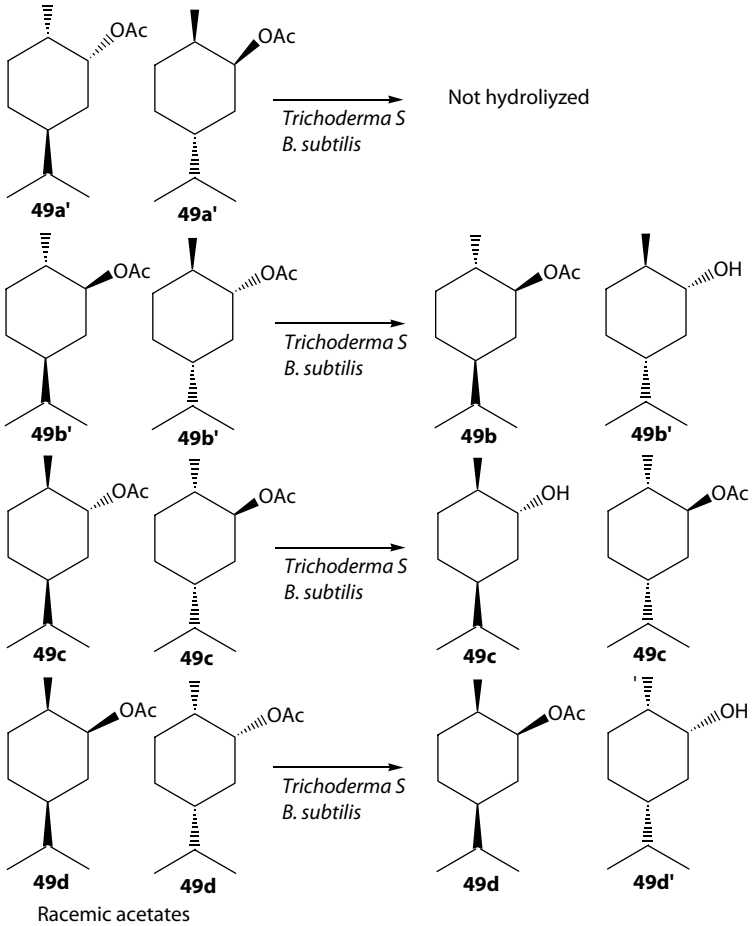
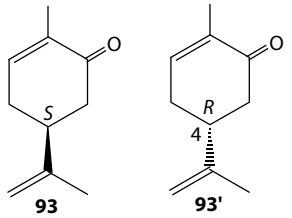


FIGURE 14.104 Microbial resolution of carvomenthols by *Trichoderma S* and *Bacillus. subtilis* var. *niger.* (Modified from Oritani, T. and K. Yamashita, 1973d. *Agric. Biol. Chem*., 37: 1691–1694.)

14.3.4 Monocyclic Monoterpene Ketone

14.3.4.1 α, β-Unsaturated Ketone

14.3.4.1.1 Carvone



93 (4*S*)-(+)-form

93' (4*R*)-(–)-form

Carvone occurs as (+)-carvone (**93**), (–)-carvone (**93′**), or racemic carvone. (*S*)-(+)-Carvone (**93**) is the main component of caraway oil (*ca*. 60%) and dill oil and has a herbaceous odour reminiscent of caraway and dill seeds. (*R*)-(–)-Carvone (**93′**) occurs in spearmint oil at a concentration of 70–80% and has a herbaceous odour similar to spearmint (Bauer et al., 1990).

The distribution of carvone convertible microorganisms is summarized in Table 14.5. When ethanol was used as a carbon source, 40% of bacteria converted (+)- (**93**) and (–)-carvone (**93'**). On the other hand, when glucose was used, 65% of bacteria converted carvone. In case of yeasts, 75% converted (+)- (**93**) and (–)-carvone (**93′**). Of fungi, 90% and 85% of fungi converted **93** and **93′**, respectively. In actinomycetes, 56% and 90% converted **93** and **93′**, respectively.

Many microorganisms except for some strains of actinomycetes were capable of hydrogenating the C=C double bond at C-1, 2 position of (+)- (**93**) and (–)-carvone (**93′**) to give mainly (–)-isodihydrocarvone (**101b**) and (+)-dihydrocarvone (**101a′**), respectively (Noma and Tatsumi, 1973; Noma et al., 1974b; Noma and Nonomura, 1974; Noma, 1976, 1977) (Figure 14.105) (Tables 14.6 and 14.7).

Furthermore, it was found that (–)-carvone (**93′**) was converted via (+)-isodihydrocarvone (**101b′**) to (+)-isodihydrocarveol (**102c′**) and (+)-neoisodihydrocarveol (**102d′**) by some strains of actinomycetes (Noma, 1979a, 1979b). (–)-Isodihydro- carvone (**101b**) was epimerized to (–)-dihydrocarvone (**101a**) after the formation of (–)-isodihydrocarvone (**101b**) from (+)-carvone (**93**) by the growing cells, the resting cells, and the cell-free extracts of *Pseudomonas fragi*, IFO 3458 (Noma et al., 1975).

TABLE 14.5
The Distribution of (+)- (93) and (–)-Carvone (93′) Convertible Microorganisms

Microorganisms	Number of Microorganisms Used	Numbers of Carvone Convertible Microorganisms	Ratio (%)
Bacteria	40	16 (Ethanol, **93**)	40
		16 (Ethanol, **93′**)	40
		26 (Glucose, **93**)	65
		26 (Glucose, **93′**)	65
Yeasts	68	51 (**93**)	75
		51 (**93′**)	75
Fungi	40	34 (**93**)	85
		36 (**93′**)	90
Actinomycetes	48	27 (**93**)	56
		43 (**93′**)	90

Source: Noma, Y. et al., 1993. Part VIII. *Proc. 37th TEAC*, pp. 23–25.

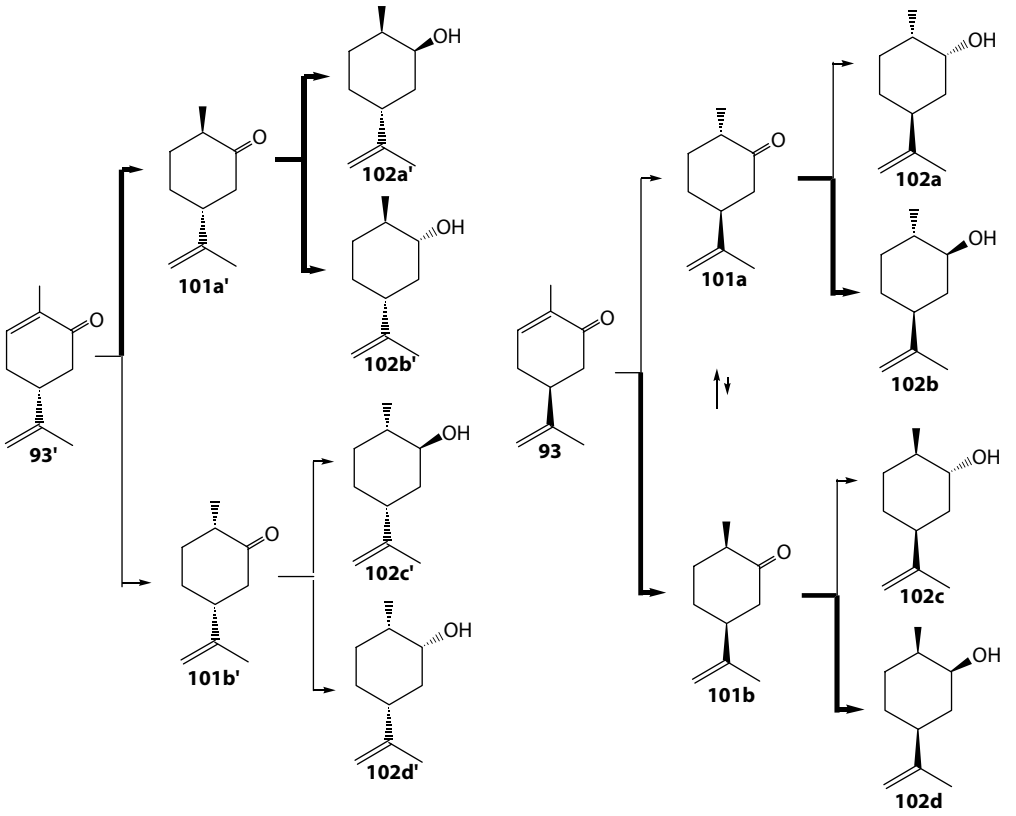


FIGURE 14.105 Biotransformation of (+)- (**93**) and (–)-carvone (**93′**) by various kinds of microorganisms. (Modified from Noma, Y. and C. Tatsumi, 1973. *Nippon Nogeikagaku Kaishi*, 47: 705–711; Noma, Y. et al., 1974b. *Agric. Biol. Chem*., 38: 735–740; Noma, Y. et al., 1974c. *Proc. 18th TEAC*, pp. 20–23; Noma, Y. and S. Nonomura, 1974. *Agric. Biol. Chem*., 38: 741–744; Noma, Y., 1976. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47; Noma, Y., 1977. *Nippon Nogeikagaku Kaishi*, 51: 463–470.)

Consequently, the metabolic pathways of carvone by microorganisms were summarized as the following eight groups (Figure 14.105).

Group 1. (–)-Carvone (**93′**)- (+)-dihydrocarvone (**101a′**)-(+)-neodihydrocarveol (**102a′**)
Group 2. **93′**–**101a′**-(–)-Dihydrocarveol (**102b′**)

TABLE 14.6
Ratio of Microorganisms that Carried Out the Hydrogenation of C=C Double Bond of Carvone by *Si* Plane Attack toward Microorganisms that Converted Carvone

Microorganisms	Ratio (%)
Bacteria	100[a]
	96[b]
Yeasts	74
Fungi	80
Actinomycetes	39

[a] When ethanol was used.
[b] When glucose was used.

Group 3. **93′**–**101a′**-**102a′** and **102b′**
Group 4. **93′**-(+)-Isodihydrocarvone (**101b′**)–**102c′** and **102d**'
Group 5. (+)-Carvone (**93**)-(−)-isodihydrocarvone (**101b**)-(−)-neoisodihydrocarveol (**102d**)
Group 6. **93**–**101b**–**102c**
Group 7. **93**–**101b**–**102c** and **102d**
Group 8. **93**–**101b**–**101a**

The result of the mode action of both the hydrogenation of carvone and the reduction for dihydrocarvone by microorganism is as follows. In bacteria, only two strains were able to convert (−)-carvone (**93′**) via (+)-dihydrocarvone (**101a′**) to (−)-dihydrocarveol (**102b′**) as the major product (Group 3, when ethanol was used as a carbon source, 12.5% of (−)-carvone (**93′**) convertible microorganisms belonged to this group and when glucose was used, 8% belonged to this group) (Noma and Tatsumi 1973; Noma et al., 1975), whereas when (+)-carvone (**93**) was converted, one strain converted it to a mixture of (−)-isodihydrocarveol (**102c**) and (−)-neoisodihydrocarveol (**102d**) (Group 7, 6% and 4% of **93** convertible bacteria belonged to this group, when ethanol and glucose were used, respectively.) and four strains converted it via (−)-isodihydrocarvone (**101b**) to (−)-dihydrocarvone (**101a**) (Group 8, 6% and 15% of (+)-carvone (**93′**) convertible bacteria belonged to this group, when ethanol and glucose were used, respectively.) (Noma et al., 1975). In yeasts, 43% of carvone convertible yeasts belong to group 1, 14% to group 2, and 33% to group 3 (of this group, three strains are close to group 1) and 12% to group 5, 4% to group 6, and 27% to group 7 (of this group, three strains are close to group 5 and one strain is close to group 6). In fungi, 51% of fungi metabolizing (−)-carvone (**93′**) by way of group 1 and 3% via group 3, but there was no strain capable of metabolizing (−)-carvone (**93′**) via group 2, whereas 20% of fungi metabolized (+)-carvone (**93**) via group 5 and 29% via group 7, but there was no strain capable of metabolizeing (+)-carvone (**93**) via group 6. In actinomycetes, (−)-carvone (**93′**) was converted to dihydrocarveols via group 1 (49%), group 2 (0%), group 3 (9%), and group 4 (28%), whereas (+)-carvone (**93**) was converted to dihydrocarveols via group 5 (7%), group 6 (0%), group 7 (19%), and group 8 (0%).

Furthermore, (+)-neodihydrocarveol (**102a′**) stereospecifically formed from (−)-carvone (**93′**) by *Aspergillus niger* TBUYN-2 was further biotransformed to mosquito repellent (1*R*,2*S*,4*R*)-(+)-*p*-menthane-2,8-diol (**50a′**), (1*R*,2*S*,4*R*)-(+)-8-*p*-menthene-2,10-diol (**107a′**), and the mixture of (1*R*,2*S*,4*R*,8*S/R*)-(+)-*p*-menthane-2,8,9-triols (**104aa′** and **104ab′**), while *Absidia glauca* ATCC 22752 gave **107a′** stereoselectively from **102a′** (Demirci et al., 2001) (Figure 14.106).

On the other hand, (−)-carvone (**93′**) was biotransformed stereoselectively to (+)-neodihydrocarveol (**102a′**) via (+)-dihydrocarvone (**101a′**) by a strain of *Aspergillus niger* (Noma and Nonomura 1974), *Euglena gracilis* Z. (Noma et al., 1993), and *Chlorella miniata* (Gondai et al., 1999). Furthermore, in *Euglena gracilis* Z., mosquito repellent (1*R*,2*S*,4*R*)-(+)-*p*-menthane-2,8-diol (**50a′**) was obtained stereospecifically from (−)-carvone (**93′**) via **101a′** and **102a′** (Figure 14.107).

As the microbial method for the formation of mosquito repellentl **50a′** was established, the production of (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**) as the precursor of mosquito repellent **50a′** was investigated by using 40 strains of bacteria belonging to *Escherichia*, *Aerobacter*, *Serratia*, *Proteus*, *Alcaligenes*, *Bacillus*, *Agrobacterium*, *Micrococcus*, *Staphylococcus*, *Corynebacterium*, *Sarcina*, *Arthrobacter*, *Brevibacterium*, *Pseudomonas*, and *Xanthomonas* spp., 68 strains of yeasts belonged to *Schzosaccharomyces*, *Endomycopsis*, *Saccharomyces*, *Schwanniomyces*, *Debaryomyces*, *Pichia*, *Hansenula*, *Lipomyces*, *Torulopsis*, *Saccharomycodes*, *Cryptococcus*, *Kloeckera*, *Trigonopsis*, *Rhodotorula*, *Candida*, and *Trichosporon* spp., 40 strains of fungi belonging to *Mucor*, *Absidia*, *Penicillium*, *Rhizopus*, *Aspergillus*, *Monascus*, *Fusarium*, *Pullularia*, *Keratinomyces*, *Oospora*, *Neurospora*, *Ustilago*, *Sporotrium*, *Trichoderma*, *Gliocladium,* and *Phytophythora* spp., and 48 strains of actinomycetes belonging to *Streptomyces*, *Actinoplanes*, *Nocardia*, *Micromonospora*, *Microbispora*, *Micropolyspora*, *Amorphosporangium*, *Thermopolyspora*, *Planomonospora*, and *Streptosporangium* spp.

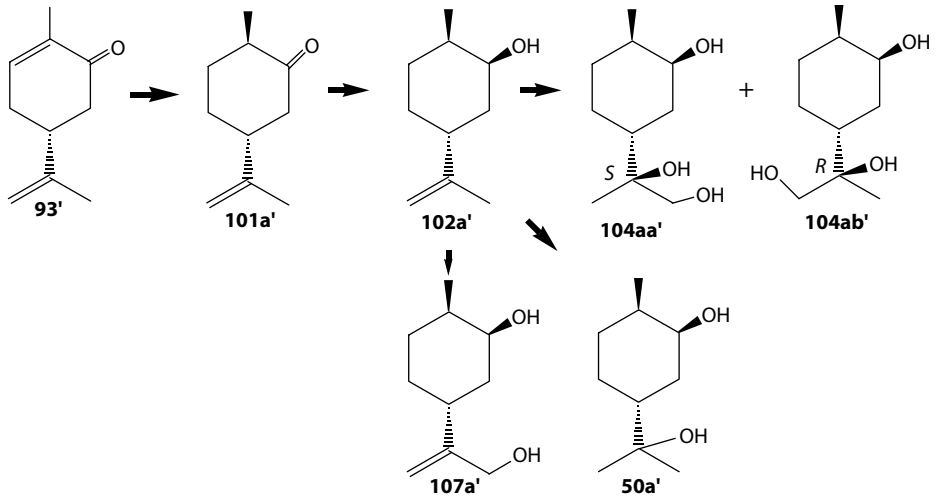


FIGURE 14.106 Metabolic pathways of (–)-carvone (**93′**) by *Aspergillus niger* TBUYN-2 and *Absidia glauca* ATCC 22752. (Modified from Demirci, F. et al., 2001. *XII Biotechnology Congr.*, Book of abstracts, p. 47.)

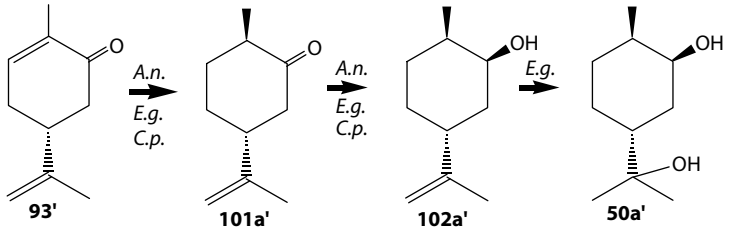


FIGURE 14.107 Metabolic pathway of (–)-carvone (**93′**) by *Aspergillus niger*, *Euglena gracilis* Z, and *Chlorella miniata*. (Modified from Noma, Y. and S. Nonomura, 1974. *Agric. Biol. Chem*., 38: 741–744; Noma, Y. et al., 1993. *Proc. 37th TEAC*, pp. 23–25; Gondai, T. et al., 1999. *Proc. 43rd TEAC*, pp. 217–219.)

As a result, 65% of bacteria, 75% of yeasts, 90% of fungi, and 90% of actinomycetes converted (–)-carvone (**93′**) to (+)-dihydrocarvone (**101a′**) or (+)-neodihydrocarveol (**102a′**) (Figure 14.105). Many microorganisms are capable of converting (–)-carvone (**93′**) to (+)-neodihydrocarveol (**102a′**) stereospecifically. Some of the useful microorganisms are listed in Tables 14.7 and 14.8. There is no good chemical method to obtain (+)-neodihydrocarveol (**102a′**) in large quantity. It was considered that the method utilizing microorganisms is a very useful means and better than the chemical synthesis for the production of mosquito repellent precursor (+)-neodihydrocarveol (**102a′**).

(–)-Carvone (**93**) was biotransformed by *Aspergillus niger* TBUYN-2 to give mainly (+)-8-hydroxyneodihydrocarveol (**50a′**), (+)-8,9-epoxyneodihydrocarveol (**103a′**), and (+)-10-hydroxyneodihydrocarveol (**107a′**) via (+)-dihydrocarvone (**101a′**) and (+)-neodihydrocarveol (**102a′**). *Aspergillus niger* TBUYN-2 dehydrogenated (+)-*cis*-carveol (**81b**) to give (+)-carvone (**93**), which was further converted to (–)-isodihydrocarvone (**101b**). Compound **101b** was further metabolized by four pathways to give 10-hydroxy- (–)-isodihydrocarvone (**106b**), (1*S*,2*S*,4*S*)-*p*-menthane-1,2-diol (**71d**) via 1α-hydroxy- (–)-isodihydrocarvone (**72b**) as intermediate, (–)-isodihydrocarveol (**102c**), and (–)-neoisodihydrocarveol (**102d**). Compound **102d** was further converted to isodihydroisobottrospicatol (**105bb**) via 8,9-epoxy-(–)-neoisodihydrocarveol (**103d**); Compound **105′** was a major product (Noma et al., 1985a) (Figure 14.109).

In case of the plant pathogenic fungus *Absidia glauca* (–)-carvone (**93′**) was metabolized to give the diol, 10-hydroxy-(+)-neodihydrocarveol (**107a′**) (Nishimura et al., 1983b).

(+)-Carvone (**93**) was converted by five bacteria and one fungus (Verstegen-Haaksma et al., 1995) to give (–)-dihydrocarvone (**101a**), (–)-isodihydrocarvone (**101b**), and (–)-neoisodihydrocarveol (**102d**).

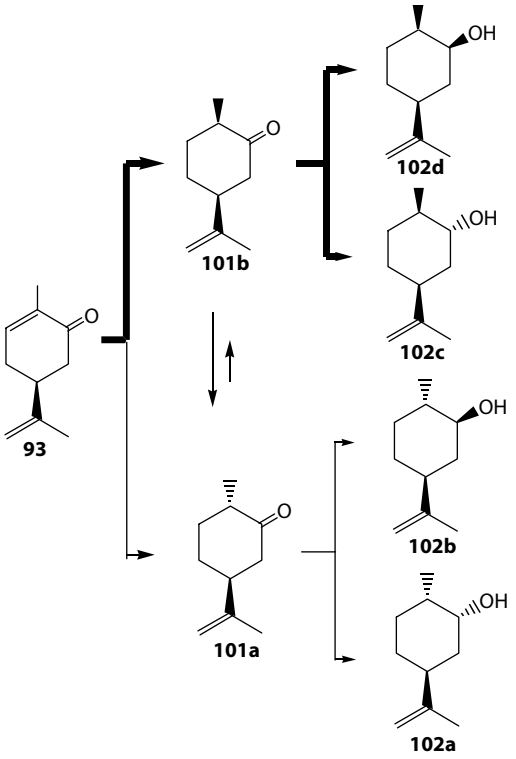


FIGURE 14.108 Metabolic pathways of (+)-carvone (**93**) by *Pseudomonas ovalis*, strain 6-1 and other many microorganisms. (Modified from Noma, Y. et al., 1974b. *Agric. Biol. Chem*., 38: 735–740.)

TABLE 14.7
Summary of Microbial and Chemical Hydrogenation of (–)-Carvone (93′) for the Formation of (+)-Dihdyrocarvone (101a′) and (+)-Isodihydrocarvone (101b′)

	Compounds	
Microorganisms	**101a′**	**101b′**
Amorphosporangium auranticolor	100	0
Microbiospora rosea IFO 3559	86	0
Bacillus subtilis var. *niger*	85	13
Bacillus subtilis IFO 3007	67	11
Pseudomonas polycolor IFO 3918	75	15
Pseudomonas graveolens IFO 3460	74	17
Arthrobacter pascens IFO 121139	73	12
Picha membranaefaciens IFO 0128	70	16
Saccharomyces ludwigii IFO 1043	69	18
Alcalygenes faecalis IAM B-141-1	70	13
Zn-25% KOH–EtOH	73	27
Raney-10% NaOH	71	19

Source: Noma, Y., 1976. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47.

TABLE 14.8
Summary of Microbial and Chemical Reduction of (–)-Carvone (93′) for the Formation of (+)-Neodihydrocarveol (102a′)

	Compounds					
Microorganisms	**101a′**	**101b′**	**102a′**	**102b′**	**102c′**	**102d′**
Torulopsis xylinus IFO 454	0	0	100	0	0	0
Monascus anka var. *rubellus* IFO 5965	0	0	100	0	0	0
Fusarium anguioides Sherbakoff IFO 4467	0	0	100	0	0	0
Phytophthora infestans IFO4872	0	0	100	0	0	0
Kloeckera magna IFO 0868	0	0	98	2	0	0
Kloeckera antillarum IFO 0669	19	4	72	0	0	0
Streptomyces rimosus	+	0	98	0	0	0
Penicillium notatum Westling IFO 464	6	2	92	0	0	0
Candida pseudotropicalis IFO 0882	17	4	79	0	0	0
Candida parapsilosis IFO 0585	16	4	80	0	0	0
$LiAlH_4$	0	0	17	67	2	13
Meerwein–Ponndorf–Verley reduction	0	0	29	55	9	5

Source: Noma, Y., 1976. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 20: 33–47.

Sensitivity of the microorganism to (+)-carvone (**93**) and some of the products prevented yields exceeding 0.35 g/L in batch cultures. The fungus *Trychoderma pseudokoningii* gave the highest yield of (–)-neoisodihydrocarveol (**102d**) (Figure 14.110). (+)-Carvone (**93**) is known to inhibit fungal growth of *Fusarium sulphureum* when it was administered via the gas phase (Oosterhaven et al., 1995a, 1995b). Under the same conditions, the related fungus, *Fusarium solani* var. *coeruleum* was not inhibited. In liquid medium, both fungi were found to convert (+)-carvone (**93**), with the same rate, mainly to (–)-isodihydrocarvone (**101b**), (–)-isodihydrocarveol (**102c**), and (–)-neoisodihydrocarveol (**102d**).

14.3.4.1.1.1 Biotransformation of Carvone to Carveols by Actinomycetes The distribution of actinomycetes capable of reducing carbonyl group of carvone containing α, β-unsaturated ketone to (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) was investigated. Of 93 strains of actinomycetes, 63 strains were capable of converting (–)-carvone (**93′**) to carveols. The percentage of microorganisms that produced carveols from (–)-carvone (**93′**) to total microorganisms was about 71%. Microorganisms that produced carveols were classified into three groups according to the formation of (–)-*trans*-carveol (**81a′**) and (–)-*cis*-carveol (**81b′**): group 1, (–)-carvone-**81b′** only; group 2, (–)-carvone-**81a′** only; and group 3, (–)-carvone-mixture of **81a′** and **81b′**. Three strains belonged to group 1 (4.5%), 34 strains belonged to group 2 (51.1%), and 29 strains belonged to group 3 (44%; of this group two strains were close to group 1 and 14 strains were close to group 2).

Streptomyces, A-5-1 isolated from soil converted (–)-carvone (**93′**) to **101a′**–**102d′** and (–)-*trans*-carveol (**81a′**), whereas *Nocardia*, 1-3-11 converted (–)-carvone (**93′**) to (–)-*cis*-carveol (**81b′**) together with **101a′**–**81a′** (Noma, 1980). In case of *Nocardia*, the reaction between **93′** and **81a′** was reversible and the direction from **81a′** to **93′** is predominantly (Noma, 1979a, 1979b; 1980) (Figure 14.111).

(–)-Carvone (**93′**) was metabolized by actinomycetes to give (–)-*trans*- (**81a′**) and (–)-*cis*-carveol (**81b′**) and (+)-dihydrocarvone (**101a′**) as reduced metabolites. Compound **81b′** was further metabolized to (+)-bottrospicatol (**92a′**). Furthermore, **93′** was hydroxylated at C-5 position and C-8, 9 position to give 5β-hydroxy-(–)-carvone (**98a′**) and (–)-carvone-8,9-epoxide (**96′**), respectively. Compound **98a′** was further metabolized to 5β-hydroxyneodihydrocarveol (**100aa′**) via 5β-hydroxy-dihydrocarvone (**99a′**) (Noma, 1979a, 1979b; 1980) (Figure 14.111).

Metabolic pattern of (+)-carvone (**93**) is similar to that of (–)-carvone (**93′**) in *Streptomyces bottropensis*. (+)-Carvone (**93**) was converted by *Streptomyces bottropensis* to give (+)-carvone-8,9-epoxide

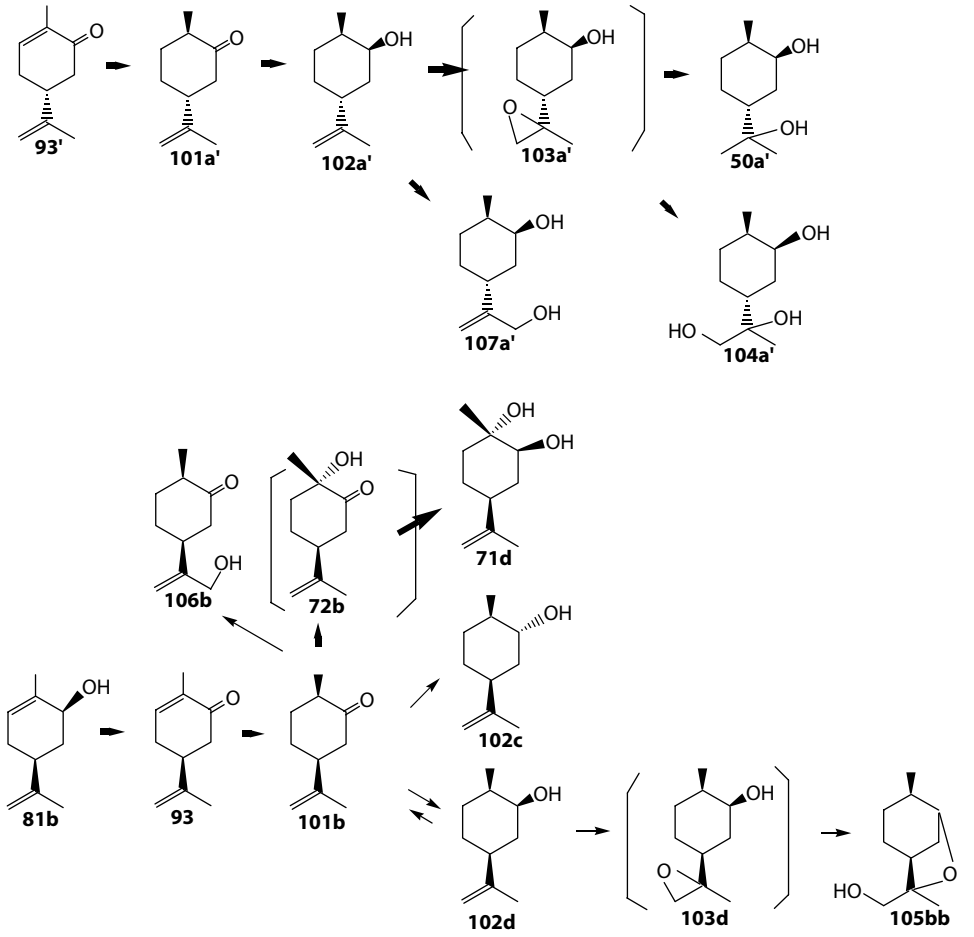


FIGURE 14.109 Possible main metabolic pathways of (–)-carvone (**93′**) and (+)-carvone (**93**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68.)

(**96**) and (+)-5α-hydroxycarvone (**98a**) (Figure 14.112). (+)-Carvone-8,9-epoxide (**96**) has light sweet aroma and has strong inhibitory activity for the germination of lettuce seeds (Noma and Nishimura, 1982).

The investigation of (–)-carvone (**93′**) and (+)-carvone (**93**) conversion pattern was carried out by using rare actinomycetes. The conversion pattern was classified as follows (Figure 14.113):

Group 1. Carvone (**93**)–dihydrocarvones (**101**)–dihydrocarveol (**102**)–dihydrocarveol-8,9-epoxide (**103**)–dihydrobottrospicatols (**105**)–5-hydroxydihydrocarveols (**100**)
Group 2. Carvone (**93**)–carveols (**89**)–bottrospicatols (**92**)–5-hydroxy-*cis*-carveols (**12**)

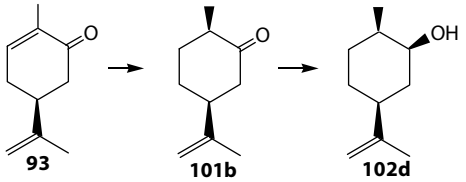


FIGURE 14.110 Biotransformation of (+)-carvone (**93**) by *Trychoderma pseudokoningii*. (Modified from Verstegen-Haaksma, A.A. et al., 1995. *Ind. Crops Prod.*, 4: 15–21.)

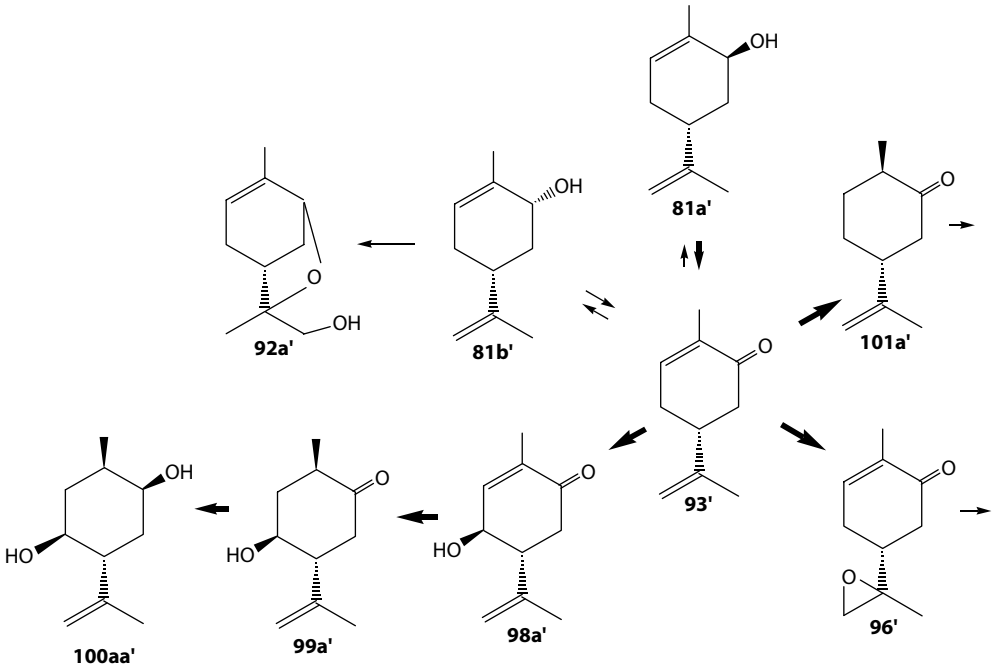


FIGURE 14.111 Metabolic pathways of (–)-carvone (**93′**) by *Streptomyces bottropensis* SY-2-1**,** *Streptomyces ikutamanensis* Ya-2-1, *Streptomyces*, A-5-1, and *Nocardia*, 1-3-11. (Modified from Noma, Y., 1979a. *Nippon Nogeikagaku Kaishi*, 53: 35–39; Noma, Y., 1979b. *Ann. Res. Stud. Osaka Joshigakuen Junior College*, 23: 27–31; Noma, Y., 1980. *Agric. Biol. Chem*., 44: 807–812; Noma, Y. and H. Nishimura, 1983a. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 390; Noma, Y. and H. Nishimura, 1983b. *Proc. 27th TEAC*, pp. 302–305.)

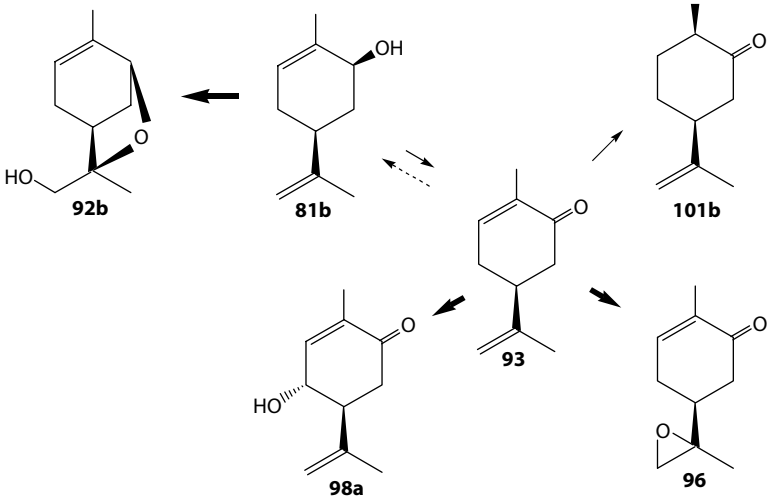


FIGURE 14.112 Metabolic pathways of (+)-carvone (**93′**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis* Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1982. *Proc. 26th TEAC*, pp. 156–159l; Noma, Y. and H. Nishimura, 1983a. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 390; Noma, Y. and H. Nishimura, 1983b. *Proc. 27th TEAC*, pp. 302–305; Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

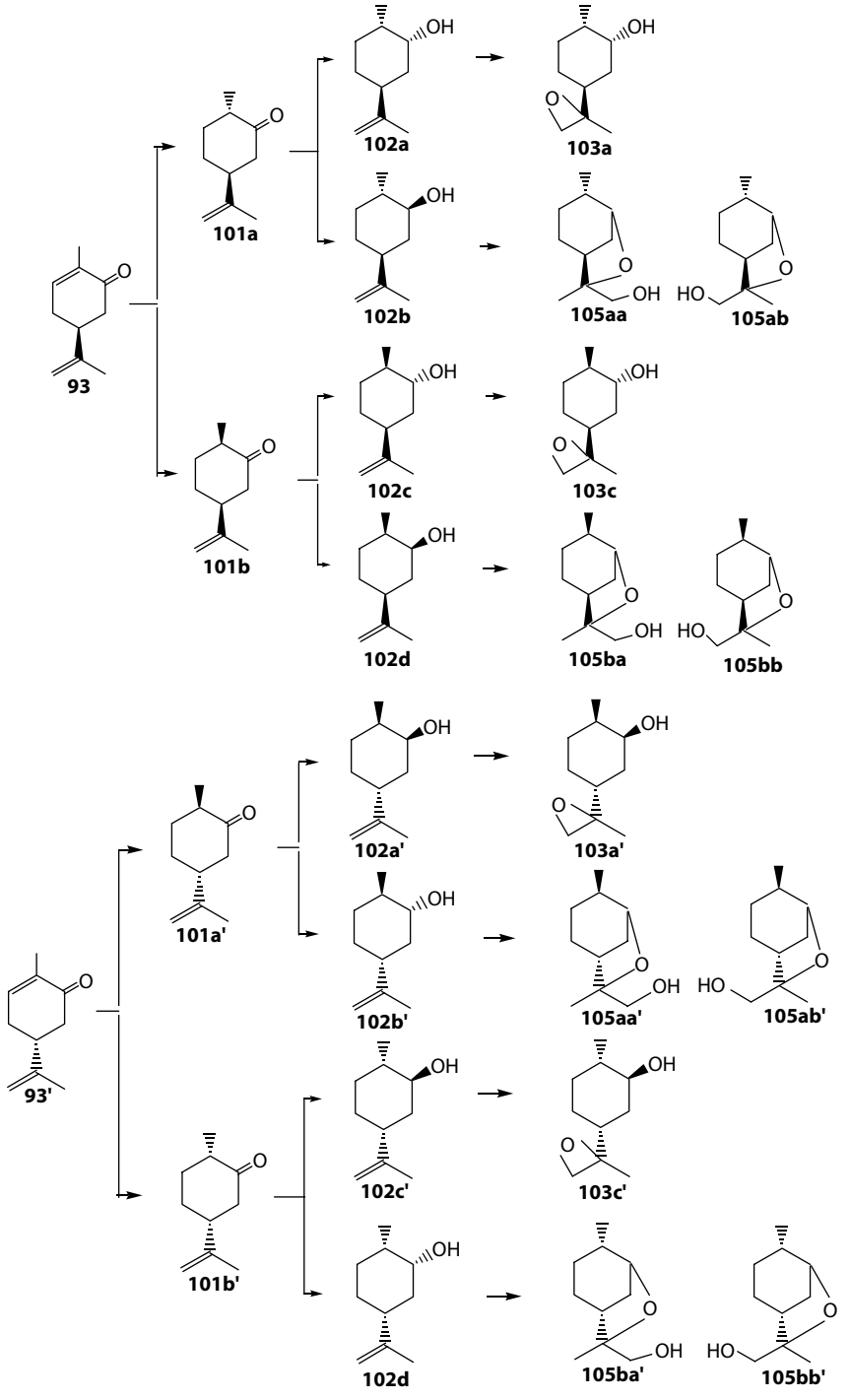


FIGURE 14.113 Metabolic pathways of (+)- (**93**) and (–)-carvone (**93′**) and dihydrocarveols (**102a-d** and **102a′-d′**) by *Streptomyces bottropensis*, SY-2-1 and *Streptomyces ikutamanesi*s, Ya-2-1. (Modified from Noma, Y., 1984. *Kagaku to Seibutsu*, 22: 742–746.)

Group 3. Carvone (**93**)–5-hydroxycarvone (**98**)–5-hydroxyneodihydrocarveols (**15**)
Group 4. Carvone (**93**)–carvone-8,9-epoxides (**96**).

Of 50 rare actinomycets, 22 strains (44%) were capable of converting (–)-carvone (**93′**) to give (–)-carvone-8,9-epoxide (**96′**) via pathway 4 and (+)-5β-hydroxycarvone (**98a′**), (+)-5α-hydroxycarvone (**98b′**), and (+)-5β-hydroxyneodihydrocarveol (**100aa′**) via pathway 3 (Noma and Sakai, 1984).

On the other hand, in case of (+)-carvone (**93**) conversion, 44% of rare actinomycetes were capable of converting (+)-carvone (**93**) to give (+)-carvone-8,9-epoxide (**96**) via pathway 4 and (–)-5α-hydroxycarvone (**98a**), (–)-5β-hydroxycarvone (**98b**), and (–)-5α-hydroxyneodihydrocarveol (**100aa**) via pathway 3 (Noma and Sakai, 1984).

14.3.4.1.1.2 Biotransformation of Carvone by Citrus Pathogenic Fungi, Aspergillus niger Tiegh TBUYN *Citrus* pathogenic *Aspergillus niger* Tiegh (CBAYN) and *Aspergillus niger* TBUYN-2 hydrogenated C=C double bond at C-1, 2 position of (+)-carvone (**93**) to give (–)-isodihydrocarvone (**101b**) as the major product together with a small amount of (–)-dihydrocarvone (**101a**), of which **101b** was further metabolized through two kinds of pathways as follows; namely one is the pathway to give (+)-1α-hydroxyneoisodihydrocarveol (**71**) via (+)-1α-hydroxyisodihydrocarvone (**72**) and the other one is the pathway to give (+)-4α-hydroxy-isodihydrocarvone (**378**) (Noma and Asakawa, 2008) (Figure 14.114).

The biotransformation of enones such as (–)-carvone (**93′**) by the cultured cells of *Chlorella miniata* was examined. It was found that the cells reduced stereoselectively the enones from *si*-face at α-position of the carbonyl group and then the carbonyl group from *re*-face (Figure 14.115).

Stereospecific hydrogenation occurs independent of the configuration and the kinds of the substituent at C-4 position, so that the methyl group at C-1 position is fixed mainly at *R* configuration. [2-^{2}H]- (–)-Carvone ([2-^{2}H]-**93′**) was synthesized in order to clear up the hydrogenation mechanism at C-2 by microorganisms. (Compound [2-^{2}H]-**93** was also easily biotransformed to [2-^{2}H]-8-hydroxy-(+)-neodihydro-carveol (**50a′**) via [2-^{2}H]-(+)-neodihydrocarveol (**102a′**). On the basis of ^{1}H-NMR spectral data of compounds **102a′** and **50a′**, the hydrogen addition of the carbon–carbon double bond at the C_1 and C_2 position by *Aspergillus niger* TBUYN-2, *Euglena gracilis* Z., and *Dunaliella tertiolecta* occurs from the *si* face and *re* face, respectively, namely, *anti* addition (Noma et al., 1995) (Figure 14.115) (Table 14.9).

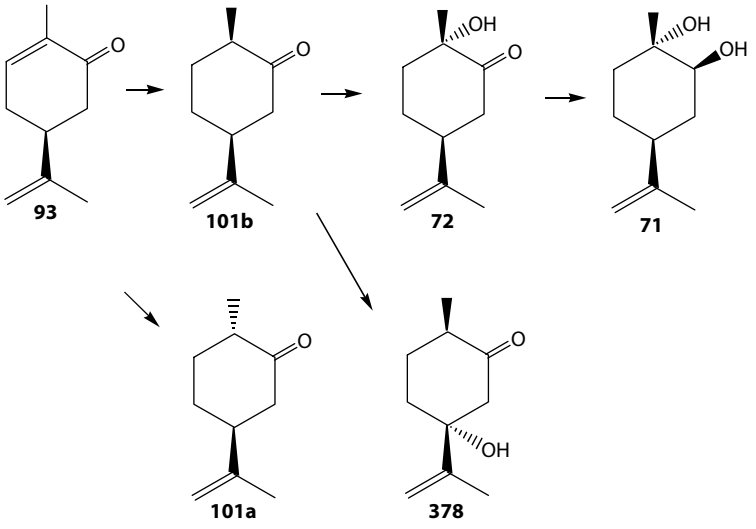


FIGURE 14.114 Metabolic pathways of (+)-carvone (**93**) by *Citrus* pathogenic fungi, *Aspergillus niger* Tiegh CBAYN and *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2008. *Proc. 52nd TEAC*, pp. 206–208.)

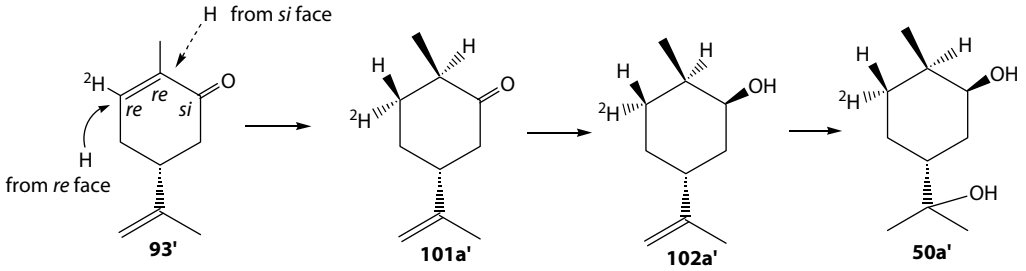


FIGURE 14.115 The stereospecific hydrogenation of the C=C double bond of α, β-unsaturated ketones, the reduction of saturated ketone, and the hydroxylation by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1995. *Proc. 39th TEAC*, pp. 367–368; Noma, Y. and Y. Asakawa, 1998. *Biotechnology in Agriculture and Forestry, Vol. 41. Medicinal and Aromatic Plants X*, Y.P.S. Bajaj, ed., pp. 194–237. Berlin Heidelberg: Springer.)

14.3.4.1.1.3 Hydrogenation Mechanisms of C=C Double Bond and Carbonyl Group In order to understand the mechanism of the hydrogenation of α-, β-unsaturated ketone of (–)-carvone (**93′**) and the reduction of carbonyl group of dihydrocarvone (**101a′**) (–)-carvone (**93′**), (+)-dihydrocarvone (**101a′**) and the analogues of (–)-carvone (**93′**) were chosen and the conversion of the analogues was carried out by using *Pseudomonas ovalis*, strain 6-1. As the analogues of carvone (**93** and **93′**), (–)- (**47′**) and (+)-carvotanacetone (**47**), 2-methyl-2-cyclohexenone (**379**), the mixture of (–)-*cis*- (**81b′**) and (–)-*trans*-carveol (**81a′**), 2-cyclohexenone, racemic menthenone (**148**), (–)-piperitone (**156**), (+)-pulegone (**119**), and 3-methyl-2-cyclohexenone (**381**) were chosen. Of these analogues, (–)- (**47′**) and (+)-carvotanacetone (**47**) were reduced to give (+)-carvomenthone (**48a′**) and (–)-isocarvomenthone (**48b′**), respectively. 2-Methyl-2-cyclohexenone (**379**) was mainly reduced to (–)-2-methylcyclohexanone. But other compounds were not reduced.

The efficient formation of (+)-dihydrocarvone (**101a**), (–)-isodihydrocarvone (**101b′**), (+)-carvomenthone (**48a**), (–)-isocarvomenthone (**48b′**), and (–)-2-methylcyclohexanone from (–)-carvone (**93**), (+)-carvone (**93′**), (–)-carvotanacetone (**47**), (+)-carvotanacetone (**47′**), and 2-methyl-2-cyclohexenone (**379**) suggested at least that C=C double bond conjugated with carbonyl group may be hydrogenated from behind (*si* plane) (Noma, 1977; Noma et al., 1974b) (Figure 14.116).

14.3.4.1.1.4 What is Hydrogen Honor in the Hydrogenation of Carvone to Dihydrocarvone? What is Hydrogen Donor in Carvone Reductase? Carvone reductase prepared from *Euglena gracilis* Z, which catalyzes the NADH-dependent reduction of the C=C bond adjacent to the carbonyl group, was characterized with regard to the stereochemistry of the hydrogen transfer into the substrate. The reductase was isolated from *Euglena gracilis* Z and was found to reduce stereospecifically the C=C double bond of carvone by *anti*-addition of hydrogen from the *si* face at α-position to the carbonyl group and the *re* face at β-position (Table 14.9). The hydrogen atoms participating in the enzymatic reduction at α- and β-position to the carbonyl group originate from the medium and the *pro-4R* hydrogen of NADH, respectively (Shimoda et al., 1998) (Figure 14.117).

TABLE 14.9
The Summary for the Stereospecificity of the Reduction of the C=C Double Bond of [2-^{2}H]-(–)-Carvone ([2-^{2}H]-93) by Various Kinds of Microorganisms

	Stereochemistry at C-2H of Compounds	
Microorganisms	**102a**	**50a**
Aspergillus niger TBUYN-2	β	
Euglena gracilis Z	β	β
Dunaliella tertiolecta	β	
The cultured cells of *Nicotiana tabacum* (Suga et al., 1986)	β	

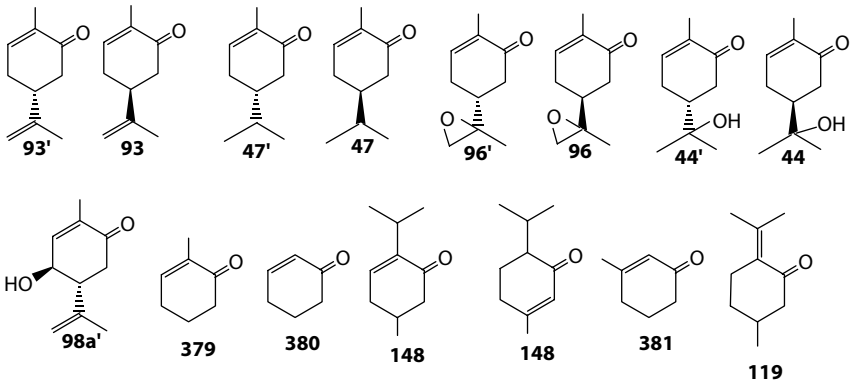


FIGURE 14.116 Substrates used for the hydrogenation of C=C double bond with *Pseudomonas ovalis*, strain 6-1, *Streptomyces bottropensis* SY-2-1, *Streptomyces ikutamanensis* Ya-2-1, and *Euglena gracilis* Z.

TABLE 14.10
Purification of the Reductase from *Euglena gracilis* Z.

	Total Protein (mg)	Total Activity Unit × 10^4	Sp. Act Units per Gram Protein	Fold
Crude extract	125	2.2	1.7	1
DEAE Toyopearl	7	1.5	21	12
AF-Blue Toyopearl	0.1	0.03	30	18

In the case of biotransformation by using *Cyanobacterium* (+)- (**93**) and (–)-carvone (**93′**) were converted with a different type of pattern to give (+)-isodihydrocarvone (**101b′**, 76.6%) and (–)-dihydrocarvone (**101a**, 62.2%), respectively (Kaji et al., 2002) (Figure 14.118). On the other hand, *Catarantus rosea* cultured cell biotransformed (–)-carvone (**93′**) to give 5β-hydroxy- (+)-neodihydrocarveol (**100aa′**, 57.5%), 5α-hydroxy-(+)-neodihydrocarveol (**100ab′**, 18.4%), 5α-hydroxy-(–)-carvone (**98b′**), 4β-hydroxy-(–)-carvone (**384′**, 6.3%), 10-hydroxycarvone (**390′**), 5β-hydroxycarvone (**98′**), 5α-hydroxyneodihydrocarveol (**100ab′**), 5β-hydroxyneodihydrocarveol (**100aa′**), and 5α-hydroxydihydrocarvone (**99b′**) as the metabolites as shown in Figure 14.119, whereas (+)-carvone (**93**) gave 5α-hydroxy-(+)-carvone (**98a**, 65.4%) and 4α-hydroxy-(+)-carvone (**384**, 34.6%) (Hamada and Yasumune, 1995; Hamada et al., 1996; Kaji et al., 2002) (Figure 14.119) (Table 14.11).

(–)-Carvone (**93′**) was incubated with *Cyanobacterium*, enone reductase (43 kDa) isolated from the bacterium and microsomal enzyme to afford (+)-isodihydrocarvone (**101b′**) and (+)-dihydrocarvone

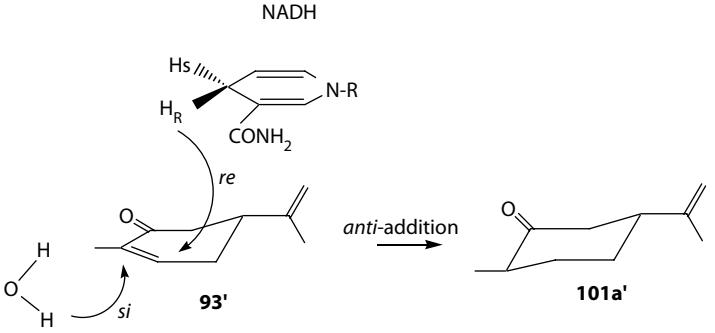


FIGURE 14.117 Stereochemistry in the reduction of (–)-carvone (**93′**) by the reductase from *Euglena gracilis* Z. (Modified from Shimoda, K. et al., 1998. *Phytochem.*, 49: 49–53.)

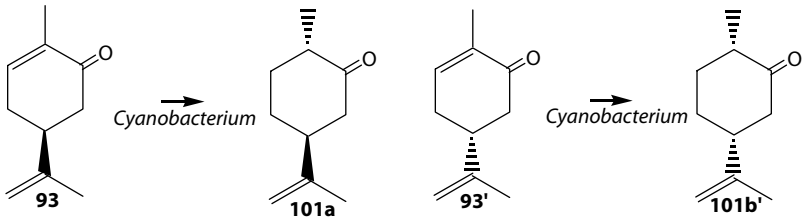


FIGURE 14.118 Biotransformation of (–)- and (+)-carvone (**93** and **93′**) by *Cyanobacterium*. (Modified from Kaji, M. et al., 2002. *Proc. 46th TEAC*, pp. 323–325.)

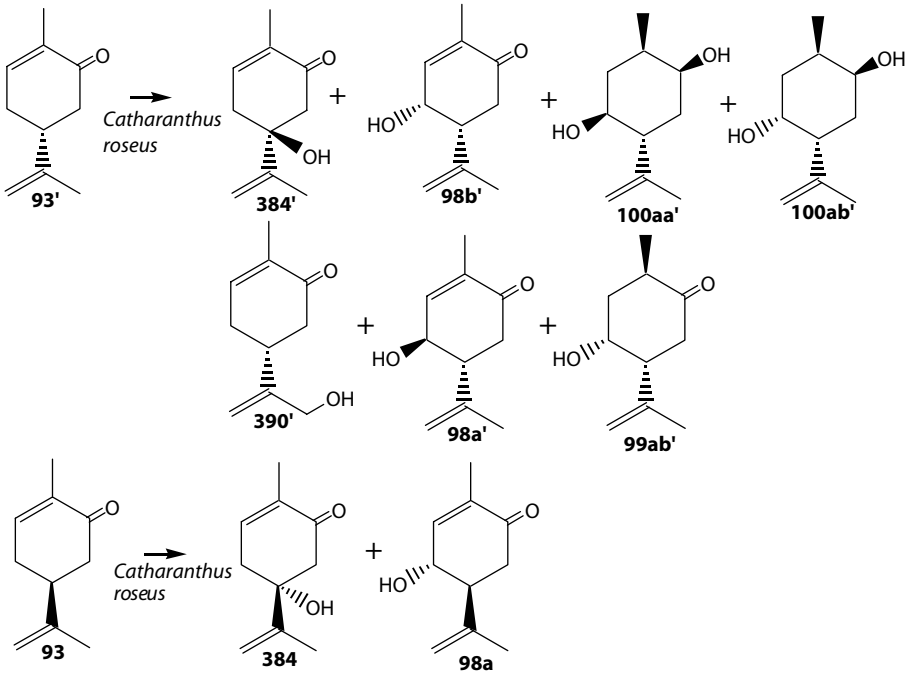


FIGURE 14.119 Biotransformation of (+)- and (–)-carvone (**93** and **93′**) by *Cataranthus roseus*. (Modified from Hamada, H. and H. Yasumune, 1995. *Proc. 39th TEAC*, pp. 375–377; Hamada, H. et al., 1996. *Proc. 40th TEAC*, pp. 111–112; Kaji, M. et al., 2002. *Proc. 46th TEAC*, pp. 323–325.)

(**101a′**). Cyclohexenone derivatives (**379** are **385**) were treated in the same enone reductase with microsomal enzyme to give the dihydro derivative (**382a**, **386a**) with *R*-configuration in excellent *ee* (over 99%) and the metabolites (**382b**, **386b**) with *S*-configuration in relatively high *ee* (85% and 80%) (Shimoda et al., 2003) (Figure 14.120).

TABLE 14.11
Enantioselectivity in the Reduction of Enones (379 and 385) by Enone Reductase

Microsomal Enzyme	Substrate	Product	ee	Configuration[a]
–	**379**	**382a**	>99	R
–	**385**	**386a**	>99	R
+	**379**	**382b**	85	S
+	**385**	**386b**	80	S

[a] Preferred configuration at α-position to the carbonyl group of the products.

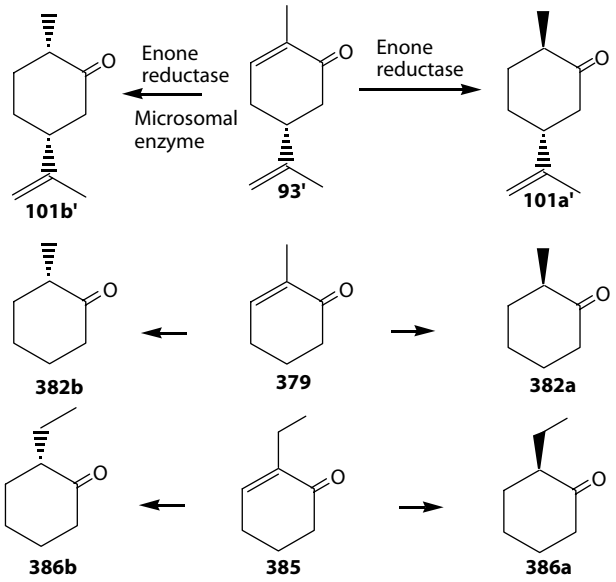
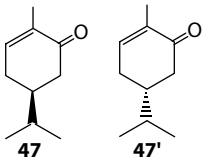


FIGURE 14.120 Biotransformation of 2-methyl-2-cyclohexenone (**379**) and 2-ethyl-2-cyclohexenone (**385**) by enone reductase.

In contrast, almost all the yeasts tested showed reduction of carvone, although the enzyme activity varied. The reduction of (–)-carvone (**93′**) was often much faster than the reduction of (+)-carvone (**93**). Some yeasts only reduced the carbon–carbon double bond to yield the dihydrocarvone isomers (**101a′** and **b′** and **101a** and **b**) with the stereochemistry at C-1 with *R* configuration, while others also reduced the ketone to give the dihydrocarveols with the stereochemistry at C-2 always with *S* for (–)-carvone (**93′**), but sometimes *S* and sometimes *R* for (+)-carvone (**93**). In the case of (–)-carvone (**93′**) yields increased up to 90% within 2 h (van Dyk et al., 1998).

14.3.4.1.2 Carvotanacetone



In the conversion of (+)- (**47**) and (–)-carvotanacetone (**47′**) by *Pseudomonas ovalis*, strain 6-1, (–)-carvotanacetone (**47′**) is converted stereospecifically to (+)-carvomenthone (**48a′**) an the latter compound is further converted to (+)-neocarvomenthol (**49a′**) and (–)-carvomenthol (**49b′**) in small amounts, whereas (+)-carvotanacetone (**47**) is converted mainly to (–)-isocarvomenthone (**48b**) and (–)-neoisocarvomenthol (**49d**), forming (–)-carvomenthone (**48a**) and (–)-isocarvomenthol (**49c**) in small amounts as shown in Figure 14.121 (Noma et al., 1974a).

Biotransformation of (–)-carvotanacetone (**47**) and (+)-carvotanacetone (**47′**) by *Streptomyces bottropensis*, SY-2-1 was carried out (Noma et al., 1985c).

As shown in Figure 14.122, (+)-carvotanacetone (**47**) was converted by *Streptomyces bottropensis,* SY-2-1 to give 5β-hydroxy-(+)-neoisocarvomenthol (**139db**), 5α-hydroxy-(+)-carvotanacetone (**51a**), 5β-hydroxy- (–)-carvomenthone (**52ab**), 8-hydroxy-(+)-carvotanacetone (**44**), and 8-hydroxy-(–)-carvomenthone (**45a**), whereas (–)-carvotanacetone (**47′**) was converted to give 5β-hydroxy-(–)-carvotanacetone (**51a′**) and 8-hydroxy-(–)-carvotanacetone (**44′**).

Aspergillus niger TBUYN-2 converted (–)-carvotanacetone (**47′**) to (+)-carvomenthone (**48a′**), (+)-carvomenthone (**49a′**), diastereoisomeric *p*-menthane-2,9-diols [**55aa′** (8*R*) and **55ab′** (8*S*) in the

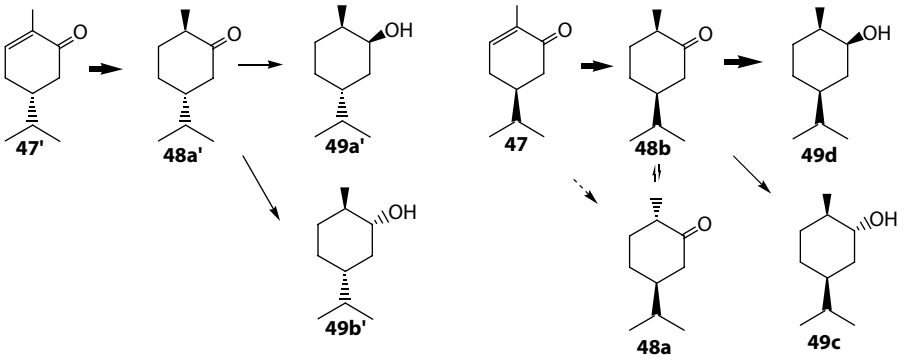


FIGURE 14.121 Metabolic pathways of (–)-carvotanacetone (**47′**) and (+)-carvotanacetone (**47**) by *Pseudomonas ovalis*, strain 6-1. (Modified from Noma, Y. et al., 1974a. *Agric. Biol. Chem*., 38: 1637–1642.)

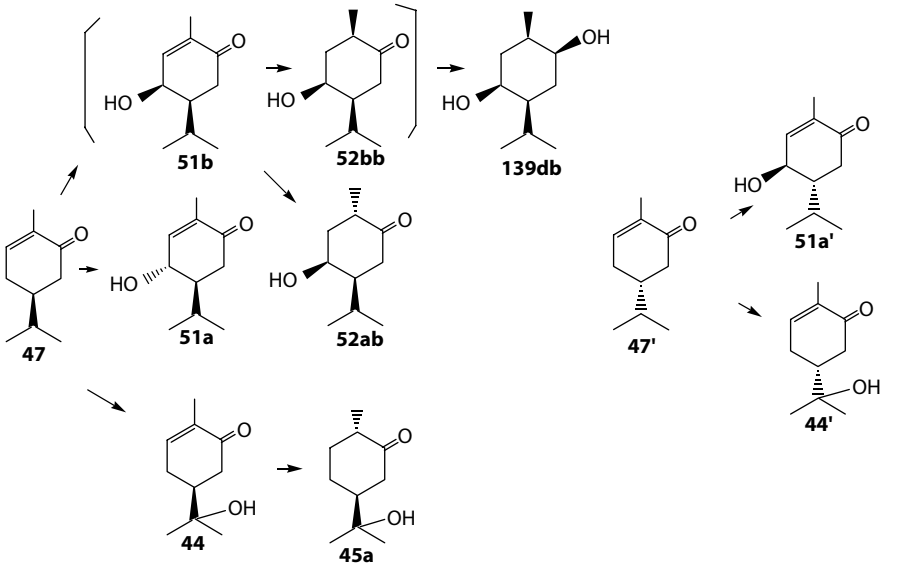
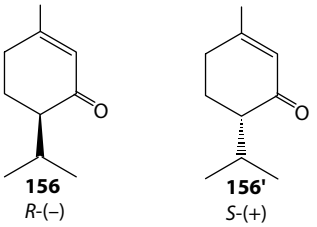


FIGURE 14.122 Proposed the metabolic pathways of (+)-carvotanacetone (**47**) and (–)-carvotanacetone (**47′**) by *Streptomyces bottropensis*, SY-2-1. (Modified from Noma, Y. et al., 1985c. *Proc. 29th TEAC*, pp. 238–240.)

ratio of 3:1], and 8-hydroxy-(+)-neocarvomenthol (**102a′**). On the other hand, the same fungus converted (+)-carvotanacetone (**47**) to (–)-isocarvomenthone (**48b**), 1α-hydroxy-(+)- neoisocarvomenthol (**54**) via 1α-hydroxy-(+)-isocarvomenthone (**53**) and 8-hydroxy-(–)-isocarvomenthone (**45b**) as shown in Figure 14.123 (Noma et al., 1988b).

14.3.4.1.3 Piperitone



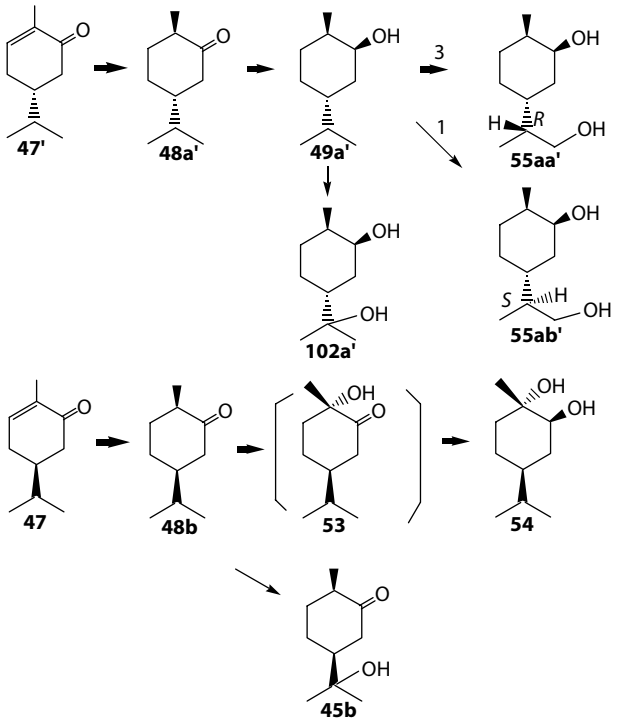


FIGURE 14.123 Proposed metabolic pathways of (–)-carvotanacetone (**47**) and (+)-carvotanacetone (**47′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1988b. *Proc. 32nd TEAC*, pp. 146–148.)

A large number of yeasts were screened for the biotransformation of (–)-piperitone (**156**). A relatively small number of yeasts gave hydroxylation products of (–)-piperitone (**156**). Products obtained from (–)-piperitone (**156**) were 7-hydroxypiperitone (**161**), *cis*-6-hydroxypiperitone (**158b**), *trans*-6-hydroxypiperitone (**158a**), and 2-isopropyl-5-methylhydroquinone (**180**). Yields for the hydroxylation reactions varied between 8% and 60%, corresponding to the product concentrations of 0.04–0.3 g/L. Not one of the yeasts tested reduced (–)-piperitone (**156**) (van Dyk et al., 1998). During the initial screen with (–)-piperitone (**156**) only hydroxylation products were obtained. The hydroxylation products (**161**, **158a**, and **158b**) obtained with nonconventional yeasts from the genera

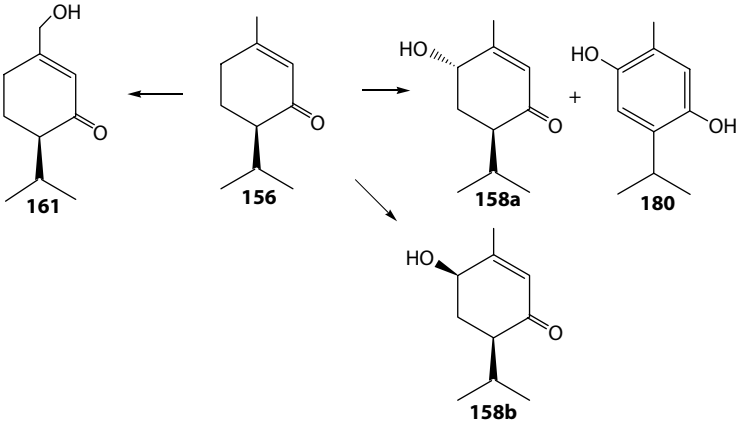


FIGURE 14.124 Hydroxylation products of (*R*)-(–)-piperitone (**156**) by yeast. (Modified from van Dyk, M.S. et al., 1998. *J. Mol. Catal. B: Enzym.*, 5: 149–154.)

Arxula, *Candida*, *Yarrowia*, and *Trichosporon* have recently been described (van Dyk et al., 1998) (Figure 14.124).

14.3.4.1.4 Pulegone

(*R*)-(+)-Pulegone (**119**), with a mint-like odour monoterpene ketone, is the main component (up to 80–90%) of *Mentha pulegium* essential oil (Pennyroyal oil), which is sometimes used in beverages and food additive for human consumption and occasionally in herbal medicine as an abortifacient drug. The biotransformation of (+)-pulegone (**119**) by fungi was investigated (Ismaili-Alaoui et al., 1992). Most fungal strains grown in a usual liquid culture medium were able to metabolize (+)-pulegone (**119**) to some extent in a concentration range of 0.1–0.5 g/L; higher concentrations were generally toxic, except for a strain of *Aspergillus* sp. isolated from mint leaves infusion, which was able to survive to concentrations of up to 1.5 g/L. The predominant product was generally l-hydroxy-(+)-pulegone (**384**) (20–30% yield). Other metabolites were present in lower amounts (5% or less) (see Figure 14.125). The formation of 1-hydroxy-(+)-pulegone (**387**) was explained by hydroxylation at a tertiary position. Its dehydration to piperitenone (**112**), even under the incubation conditions, during isolation or derivative reactions precluded any tentative determination of its optical purity and absolute configuration.

Botrytis allii converted (+)-pulegone (**119**) to (−)-(1*R*)-8-hydroxy-4-*p*-menthen-3-one (**121**) and piperitenone (**112**) (Miyazawa et al., 1991a, 1991b). *Hormonema isolate* (UOFS Y-0067) quantitatively reduced (+)-pulegone (**119**) and (−)-menthone (**149a**) to (+)-neomenthol (**137a**) (van Dyk et al., 1998) (Figure 14.125).

Biotrasformation by the recombinant reductase and the transformed *Escherichia coli* cells were examined with pulegone, carvone, and verbenone as substrates (Figure 14.126). The recombinant reductase catalyzed the hydrogenation of the exocylic C=C double bond of pulegone (**119**) to give menthone derivatives (Watanabe et al., 2007) (Tables 14.12 and 14.13).

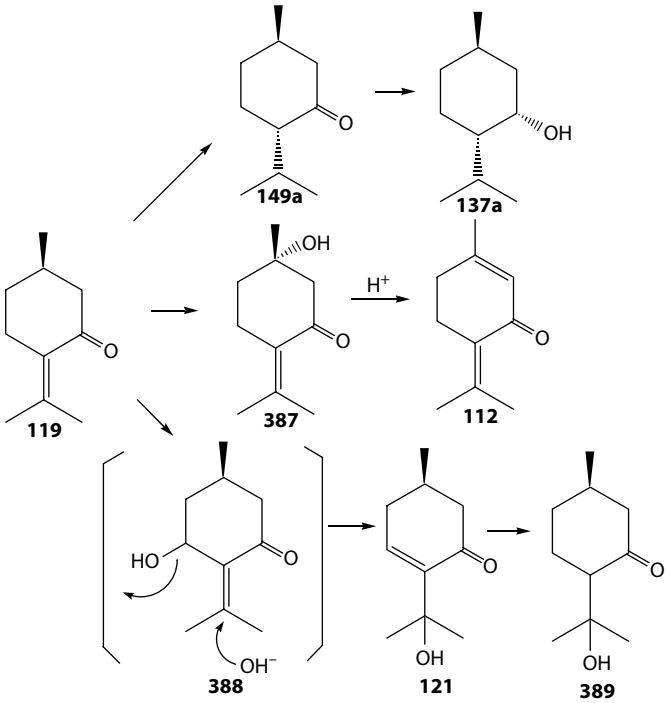


FIGURE 14.125 Biotransformation of (+)-pulegone (**119**) by *Aspergillus* sp., *Botrytis allii*, and *H. isolate* (UOFS Y-0067). (Modified from Miyazawa, M. et al., 1991a. *Chem. Express*, 6: 479–482; Miyazawa, M. et al., 1991b. *Chem. Express*, 6: 873; Ismaili-Alaoui, M. et al., 1992. *Tetrahedron Lett.*, 33: 2349–2352; van Dyk, M.S. et al., 1998. *J. Mol. Catal. B: Enzym.*, 5: 149–154.)

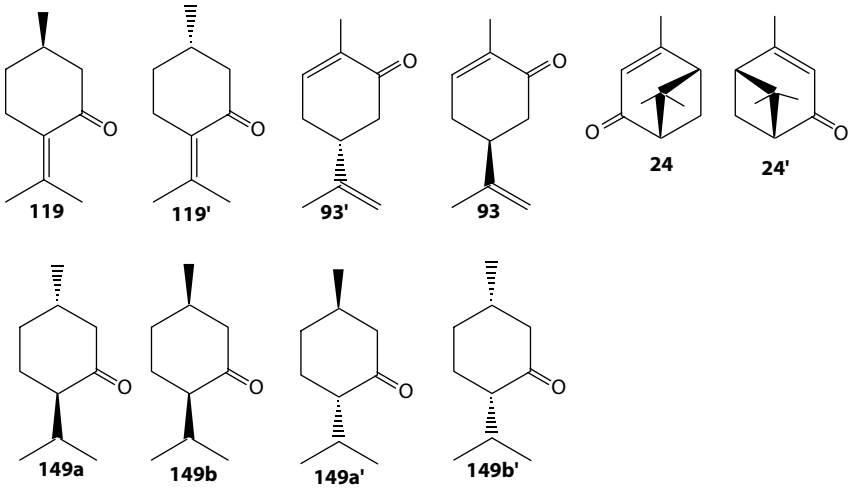


FIGURE 14.126 Chemical structures of substrate reduced by the recombinant pulegone reductase and the transformed *Escherichia coli* cells.

14.3.4.1.5 Piperitenone and Isopiperitenone

Piperitenone (**112**) is metabolized to 5-hydroxypiperitenone (**117**), 7-hydroxypiperitenone (**118**), and 7,8-dihydroxypiperitone (**157**). Isopiperitenol (**110**) is reduced to give isopiperitenone (**111**), which is further metabolized to piperitenone (**112**), 7-hydroxy- (**113**), 10-hydroxy- (**115**), 4-hydroxy- (**114**), and 5-hydroxy-isopiperitenone (**116**). Compounds **111** and **112** are isomerized to each other. Pulegone (**119**) was metabolized to **112**, 8,9-dehydromenthenone (**120**) and 8-hydroxymenthenone (**121**) as shown in the biotransformation of the same substrate using *Botrytis allii* (Miyazawa et al., 1991b) (Figure 14.127).

H. isolate (UOFS Y-0067) reduced (4*S*)-isopiperitenone (**111**) to (3*R*,4*S*)-isopiperitenol (**110**), a precursor of (−)-menthol (**137b**) (van Dyk et al., 1998) (Figure 14.128).

TABLE 14.12
Substrate Specificity in the Reduction of Eenones with the Recombinant Pulegone Reductase

Entry No. (Reaction Time)	Substrates	Products	Conversions (%)
1 (3 h)	(*R*)-(+)-Pulegone (**119**)	(1*R*, 4*R*)-Isomenthone (**149b**)	4.4
2 (12 h)	(*R*)-Pulegone (**119**)	(1*S*, 4*R*)-Menthone (**149a**)	6.8
3 (3 h)	(*S*)-(−)-Pulegone (**119′**)	(1*R*, 4*R*)-Isomenthone (**149b**)	14.3
4 (12 h)	(*S*)-Pulegone (**119′**)	(1*S*, 4*R*)-Menthone (**149a**)	15.7
5 (12 h)	(*R*)-(−)-Carvone (**93′**)	(1*S*, 4*S*)-Isomenthone (**149b′**)	0.3
6 (12 h)	(*S*)-(+)-Carvone (**93**)	(1*R*, 4*S*)-Menthone (**149a′**)	0.5
7 (12 h)	(1*S*, 5*S*)-Verbenone (**24**)	(1*S*, 4*S*)-Isomenthone (**149b′**)	1.6
8 (12 h)	(1*R*, 5*R*)-Verbenone (**24′**)	(1*R*, 4*S*)-Menthone (**149a′**)	2.1
			N.d.
		—	N.d.
		—	N.d.
		—	N.d.
		—	

N.d.—denotes not detected.

TABLE 14.13
Biotransformation of Pulegone (119 and 119′) with the Transformed *Escherichia coli* cells[a]

Substrates	Products	Conversion (%)
(*R*)-(+)-Pulegone (**119**)	(1*R*, 4*R*)-Isomenthone (**149b**)	26.8
(*S*)-(−)-Pulegone (**119′**)	(1*S*, 4*R*)-Menthone (**149a**)	30.0
	(1*S*, 4*S*)-Isomenthone (**149b′**)	32.3
	(1*R*, 4*S*)-Menthone (**149a′**)	7.1

[a] Reaction times of the transformation reaction are 12 h.

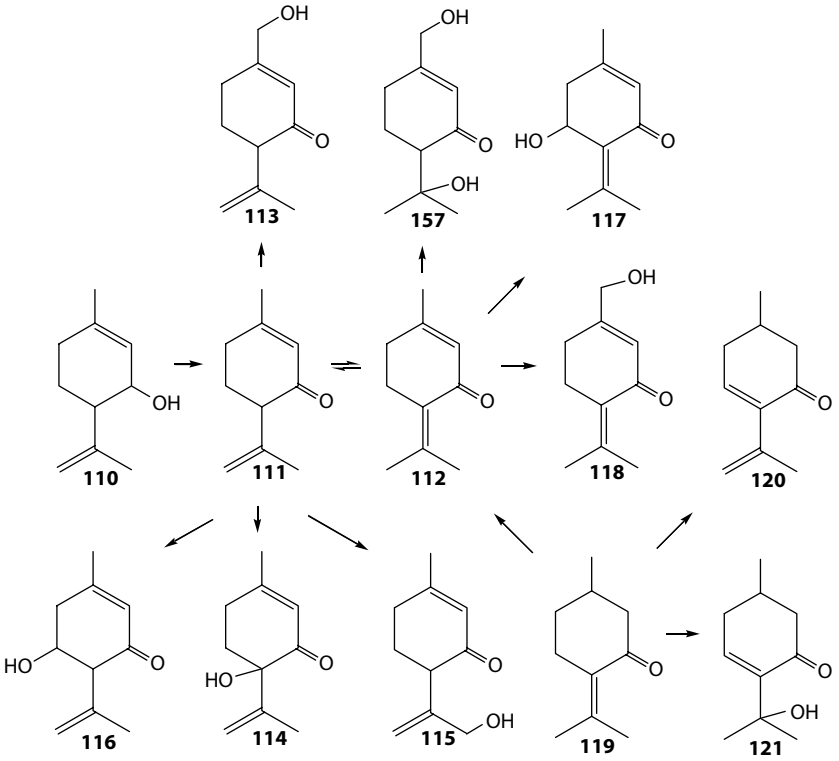


FIGURE 14.127 Biotransformation of isopiperitenone (**111**) and piperitenone (**112**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 1992c. *Proc. 37th TEAC*, pp. 26–28.)

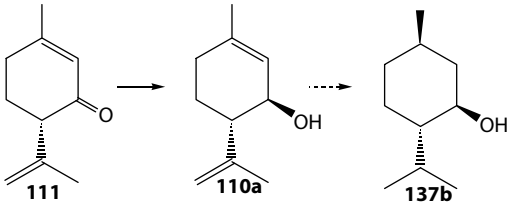
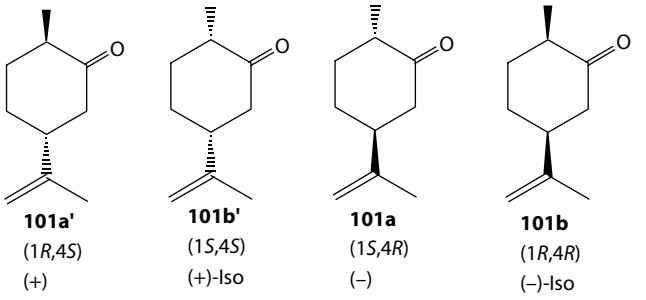


FIGURE 14.128 Biotransformation of isopiperitenone (**111**) by *H. isolate* (UOFS Y-0067). (Modified from van Dyk, M.S. et al., 1998. *J. Mol. Catal. B: Enzym.*, 5: 149–154.)

14.3.4.2 Saturated Ketone

14.3.4.2.1 Dihydrocarvone



In the reduction of saturated carbonyl group of dihydrocarvone by microorganism, (+)-dihydrocarvone (**101a′**) is converted stereospecifically to either (+)-neodihydrocarveol (**102a′**) or (–)-dihydrocarveol (**102b′**) or nonstereospecifically to the mixture of **102a′** and **102b′**, whereas (–)-isodihydrocarvone (**101b**) is converted stereospecifically to either (–)-neoisodihydrocarveol (**102d**) or (–)-isodihydrocarveol (**102c**) or nonstereospecifically to the mixture of **102c** and **102d** by various microorganisms (Noma and Tatsumi, 1973; Noma et al., 1974c; Noma and Nonomura 1974; Noma, 1976, 1977).

(+)-Dihydrocarvone (**101a′**) and (+)-isodihydrocarvone (**101b′**) are easily isomerized chemically to each other. In the microbial transformation of (–)-carvone (**93′**), the formation of (+)-dihydrocarvone (**101a′**) is predominant. (+)-Dihydrocarvone (**101a′**) was reduced to both/either (+)-neodihydrocarveol (**102a′**) and/or (–)-dihydrocarveol (**102b**), whereas in the biotransformation of (+)-carvone (**93**), (+)-isodihydrocarvone (**101b**) was formed predominantly. (+)-Isodihydrocarvone (**101b**) was reduced to both (+)-isodihydrocarveol (**102c**) and (+)-neoisodihydrocarveol (**102d**) (Figure 14.129).

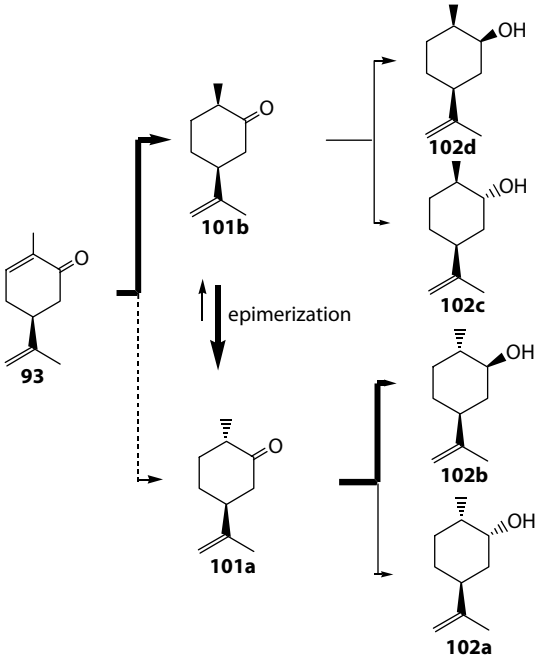


FIGURE 14.129 Proposed metabolic pathways of (+)-carvone (**93**) and (–)-isodihydrocarvone (**101b**) by *Pseudomonas fragi*, IFO 3458. (Modified from Noma, Y. et al., 1975. *Agric. Biol. Chem.*, 39: 437–441.)

However, *Pseudomonas fragi*, IFO 3458, *Pseudomonas fluorescens*, IFO 3081, and *Aerobacter aerogemes*, IFO 3319 and IFO 12059, formed (–)-dihydrocarvone (**101a**) predominantly from (+)-carvone (**93**). In the time course study of the biotransformation of (+)-carvone (**93**), it appeared that predominant formation of (–)-dihydrocarvone is due to the epimerization of (–)-isodihydrocarvone (**101b′**) by epimerase of *Pseudomonas fragi* IFO 3458 (Noma et al., 1975).

14.3.4.2.2 Isodihydrocarvone Epimerase

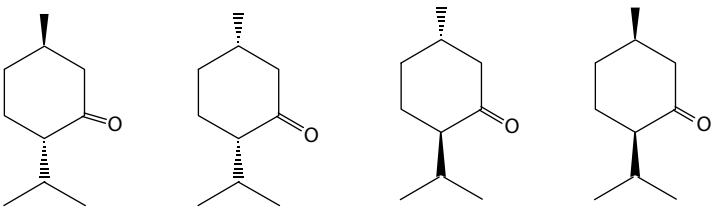
14.3.4.2.2.1 Preparation of Isodihydrocarvone Epimerase The cells of *Pseudomonas fragi* IFO 3458 were harvested by centrifugation and washed five times with 1/100 M KH_2PO_4–Na_2HPO_4 buffer (pH 7.2). Bacterial extracts were prepared from the washed cells (20 g from 3-L medium) by sonic lysis (Kaijo Denki Co., Ltd., 20Kc., 15 min, at 5–7°C) in 100 mL of the same buffer. Sonic extracts were centrifuged at 25, 500 *g* for 30 min at –2°C. The opalescent yellow supernatant fluid had the ability to convert (–)-isodihydrocarvone (**101b**) to (–)-dihydrocarvone (**101a**). On the other hand, the broken cell preparation was incapable of converting (–)-isodihydrocarvone (**101b**) to (–)-dihydrocarvone (**101a**). The enzyme was partially purified from this supernatant fluid about 56-fold with heat treatment (95–97°C for 10 min), ammonium sulfate precipitation (0.4–0.7 saturation), and DEAE-Sephadex A-50 column chromatography.

The reaction mixture consisted of a mixture of (–)-isodihydrocarvone (**101b**) and (–)-dihydrocarvone (**101a**) (60:40 or 90:10), 1/30 M KH_2PO_4–Na_2HPO_4 buffer (pH 7.2), and the crude or partially purified enzyme solution. The reaction was started by the addition of the enzyme solution and stopped by the addition of ether. The ether extract was applied to analytical GLC (Shimadzu Gas Chromatograph GC-4A 10% PEG-20M, 3 m × 3 mm, temperature 140–170°C at the rate of 1°C a min, N_2 35 mL/min), and epimerization was assayed by measuring the peak areas of (–)-isodihydrocarvone (**101b**) and (–)-dihydrocarvone (**101a**) in gas liquid chromatography (GLC) before and after the reaction.

The crude extract and the partially purified preparation were found to be very stable to heat treatment; 66% and 36% of the epimerase activity remained after treatment at 97°C for 60 and 120 min, respectively (Noma et al., 1975).

A strain of *Aspergillus niger* TBUYN-2 hydroxylated at C-1 position of (–)-isodihydrocarvone (**101b**) to give 1α-hydroxyisodihydrocarvone (**72b**), which was easily and smoothly reduced to (1*S*, 2*S*, 4*S*)-(–)-8-*p*-menthene-1,2-*trans*-diol (**71d**), which was also obtained from the biotransformation of (–)-*cis*-limonene-1,2-epoxide (**69**) by microorganisms and decomposition by 20% HCl (Figure 14.127) (Noma et al., 1985a, 1985b). Furthermore, *Aspergillus niger* TBUYN-2 and *Aspergillus niger* Tiegh (CBAYN) biotransformed (–)-isodihydrocarvone (**101b**) to give (–)-4α-hydroxyisodihydrocarvone (**378b**) and (–)-8-*p*-menthene-1,2-*trans*-diol (**71d**) as the major products together with a small amount of 1α-hydroxyisodihydrocarvone (**72b**) (Noma and Asakawa, 2008) (Figure 14.130).

14.3.4.2.3 Menthone and Isomenthone



149a (1*R*,4*S*) (–)-Menthone

149b (1*S*,4*S*) (–)-Isomenthone

149a' (1*S*,4*R*) (+)-Menthone

149b' (1*R*,4*R*) (+)-Isomenthone

The growing cells of *Pseudomonas fragi* IFO 3458 epimerized 17% of racemic isomenthone (**149b** and **b′**) to menthone (**149a** and **a′**) (Noma et al., 1975). (–)-Menthone (**149a**) was converted

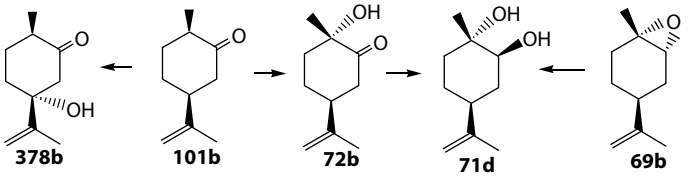
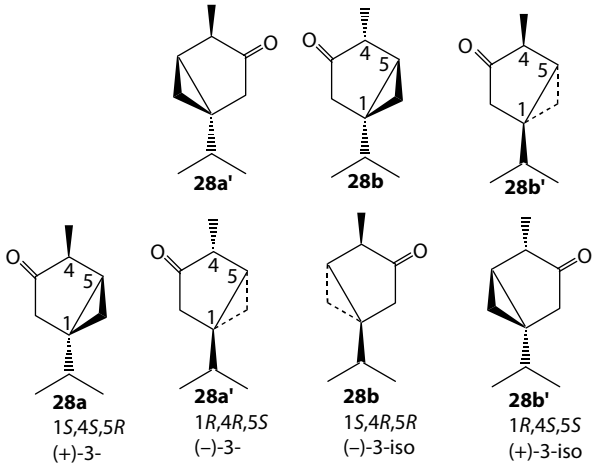


FIGURE 14.130 Biotransformation of (+)-carvone (**93**), (–)-isodihydrocarvone (**101b**), and (–)-*cis*-limonene-1,2-epoxide (**69b**) by *Aspergillus niger* TBUYN-2 and *Aspergillus niger* Tiegh (CBAYN). (Modified from Noma, Y. et al., 1985a. *Annual Meeting of Agricultural and Biological Chemistry*, Sapporo, p. 68; Noma, Y. and Y. Asakawa, 2008. *Proc. 52nd TEAC*, pp. 206–208.)

by *Pseudomonas fluorescens* M-2 to (–)-3-oxo-4-isopropyl-1-cyclohexanecarboxylic acid (**164a**), (+)-3-oxo-4-isopropyl-1-cyclohexanecarboxylic acid (**164b**), and (+)-3-hydroxy-4-isopropyl-1-cyclohexanecarboxylic acid (**165ab**). On the other hand, (+)-menthone (**149a′**) was converted to give (+)-3-oxo-4-isopropyl-1-cyclohexane carboxylic acid (**164a′**) and (–)-3-oxo-4-isopropyl-1-cyclohexane carboxylic acid (**164b′**). Racemic isomenthone (**149b** and **b′**) was converted to give racemic 1-hydroxy-1-methyl-4-isopropylcyclohexane-3-one (**150**), racemic piperitone (**156**), racemic 3-oxo-4-isopropyl-1-cyclohexene-1-carboxylic acid (**162**), 3-oxo-4-isopropyl-1-cyclohexane carboxylic acid (**164b**), 3-oxo-4-isopropyl-1-cyclohexane carbxylic acid (**164a**), and (+)-3-hydroxy-4-isopropyl-1-cyclohexane carboxylic acid (**165ab**) (Figure 14.131).

Soil plant pathogenic fungi, *Rhizoctonia solani* 189 converted (–)-menthone (**149a**) to 4β-hydroxy-(–)-menthone (**392**, 29%) and 1 α, 4 β-dihydroxy-(–)-menthone (**393**, 71%) (Nonoyama et al., 1999) (Figure 14.131). (–)-Menthone (**149a**) was transformed by *Spodoptera litura* to give 7-hydroxymenthone (**151a**), 7-hydroxyneomenthol (**165c**), and 7-hydroxy-9-carboxymenthone (**394a**) (Hagiwara et al., 2006) (Figure 14.132). (–)-Menthone (**149a**) gave 7-hydroxymenthone (**151a**) and (+)-neomenthol (**137c**) by human liver microsome (CYP2B6). Of 11 recombinant human P450 enzymes (express in *Trichoplusia ni* cells) tested, CYP2B6 catalyzed oxidation of (–)-menthone (**149a**) to 7-hydroxymenthone (**151a**) (Nakanishi and Miyazawa, 2004) (Figure 14.132).

14.3.4.2.4 Thujone



β-Pinene (**1**) is metabolized to 3-thujone (**28**) via α-pinene (**4**) (Gibbon and Pirt, 1971). α-Pinene (**4**) is metabolized to give thujone (**28**). Thujone (**28**) was biotransformed to thujoyl alcohol (**29**) by *Aspergillus niger* TBUYN-2 (Noma, 2000). Furthermore, (–)-3-isothujone (**28b**) prepared from *Armois* oil was biotransformed by plant pathogenic fungus, *Botrytis allii* IFO 9430 to give 4-hydroxythujone (**30**) and 4,6-dihydroxythujone (**31**) (Miyazawa et al., 1992a) (Figure 14.133).

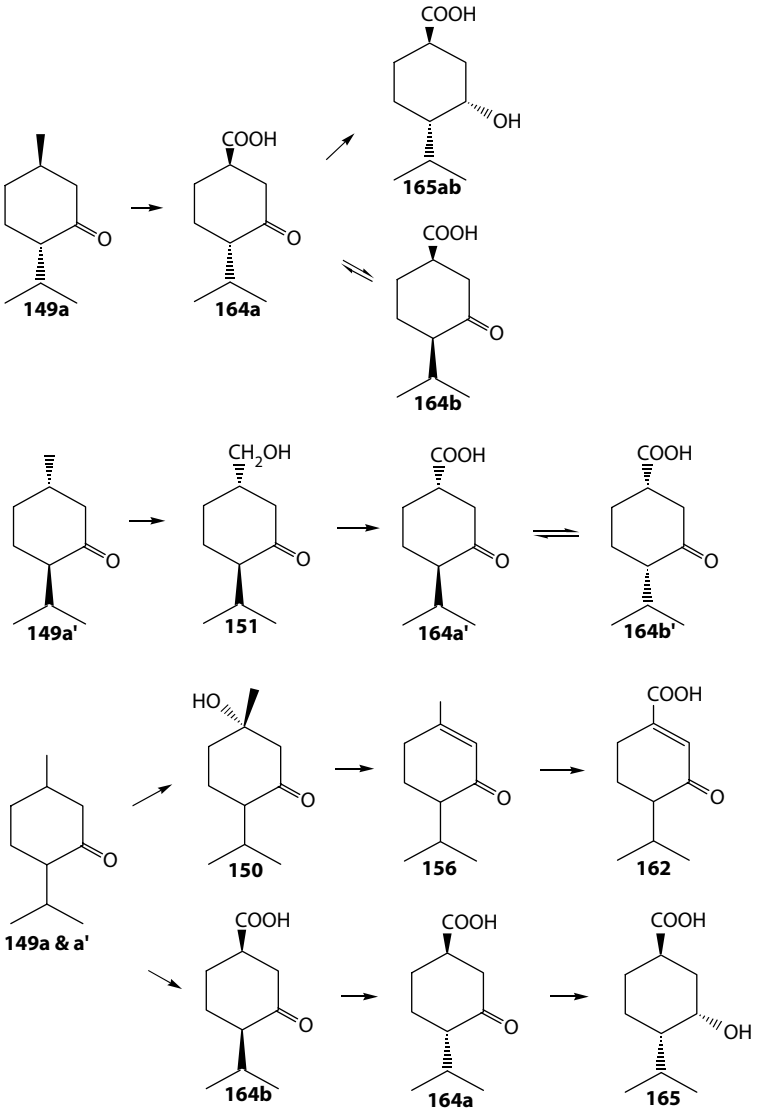


FIGURE 14.131 Biotransformation of (–)- (**149a**) and (+)-menthone (**149a′**) and racemic isomenthone (**149b** and **149b′**) by *Pseudomonas fluorescens* M-2. (Modified from Sawamura, Y. et al., 1974. *Proc. 18th TEAC*, pp. 27–29.)

14.3.4.3 Cyclic Monoterpene Epoxide

14.3.4.3.1 1,8-Cineole

1,8-Cineole (**122**) is a main component of the essential oil of *Eucalyptus adiata* var. *australiana* leaves, comprising *ca.* 75% in the oil, which corresponds to 31 mg/g fr.wt. leaves (Nishimura et al., 1980).

The most effective utilization of **122** is very important in terms of renewable biomass production. It would be of interest, for example, to produce more valuable substances, such as plant growth regulators, by the microbial transformation of **122**. The first reported utilization of **122** was presented by MacRae et al. (1979), who showed that it was a carbon source for *Pseudomonas flava* growing on *Eucalyptus* leaves. Growth of the bacterium in a mineral salt medium containing **122** resulted in the oxidation at the C-2 position of **122** to give the metabolites (1*S*,4*R*,6*S*)-(+)-2α-hydroxy-1,8-cineole (**225a**), (1*S*,4*R*,6*R*)-(–)-2β-hydroxy-1,8-cineole (**125a**), (1*S*,4*R*)-(+)-2-oxo-1,8-cineole (**126**), and (–)-(*R*)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2(3H)-one (**128**) (Figure 14.134).

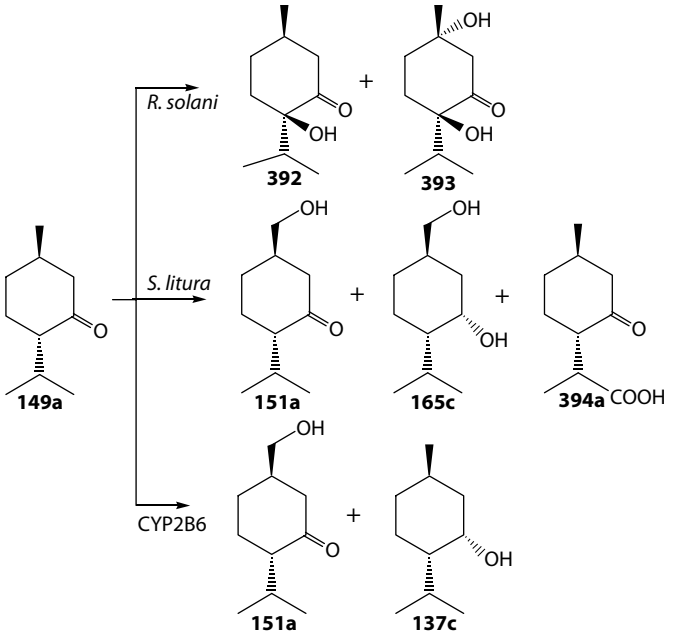


FIGURE 14.132 Metabolic pathway of (–)-menthone (**149a**) by *Rhizoctonia solani* 189, *Spodptera litura* and human liver microsome (CYP2B6). (Modified from Nonoyama, H. et al., 1999. *Proc. 43rd TEAC*, pp. 387–388; Nakanishi, K. and M. Miyazawa, 2004. *Proc. 48th TEAC*, pp. 401–402; Hagiwara, Y. et al., 2006. *Proc. 50th TEAC*, pp. 279–280.)

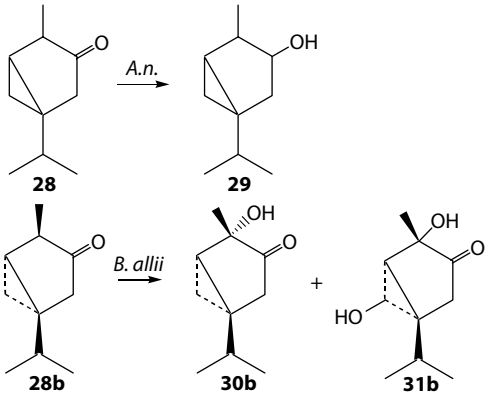


FIGURE 14.133 Biotransformation of (–)-3-isothujone (**28b**) by *Aspergillus niger* TBUYN-2 and plant pathogenic fungus, *Botrytis allii* IFO 9430. (Modified from Gibbon, G.H. and S.J. Pirt, 1971. *FEBS Lett.*, 18: 103–105; Miyazawa, M. et al., 1992a. *Proc. 36th TEAC*, pp. 197–198.)

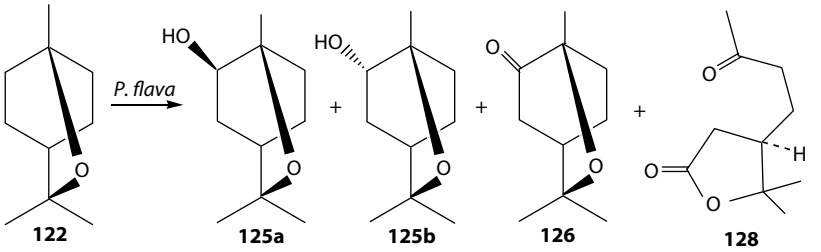


FIGURE 14.134 Biotransformation of 1,8-cineole (**84**) by *Pseudomonas flava*. (Modified from MacRae, I.C. et al., 1979. *Aust. J. Chem*., 32: 917–922.)

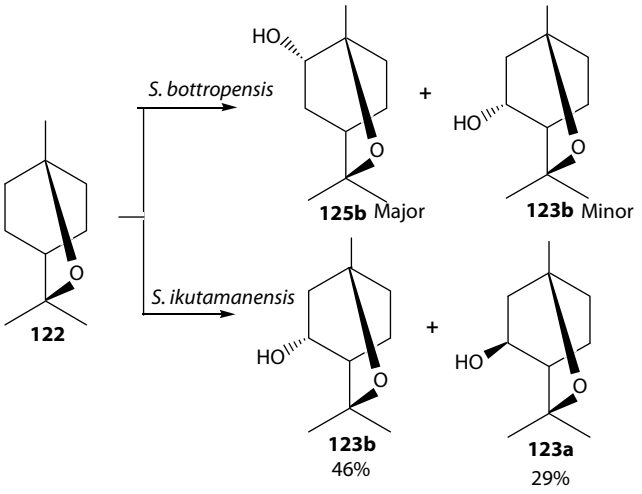


FIGURE 14.135 Biotransformation of 1,8-cineole (**122**) by *Streptomyces bottropensis* SY-2-1 and *Streptomyces ikutamanensis* Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1980. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 28; Noma, Y. and H. Nishimura, 1981. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 196.)

Streptomyces bottropensis, SY-2-1 biotransformed 1,8-cineole (**122**) stereochemically to (+)-2α-hydroxy-1,8-cineole (**125b**) as the major product and (+)-3α-hydroxy-1,8-cineole (**123b**) as the minor product. Recovery ratio of 1,8-cineole metabolites as ether extract was *ca.* 30% in *Streptomyces bottropensis*, SY-2-1 (Noma and Nishimura, 1980, 1981) (Figure 14.135).

In case of *Streptomyces ikutamanensis*, Ya-2-1 1,8-cineole (**122**) was biotransformed regioselectively to give (+)-3α-hydroxy-1,8-cineole (**123b**, 46%) and (+)-3β-hydroxy-1,8-cineole (**123b**, 29%) as the major product. Recovery ratio as ether extract was *ca.* 8.5% in *Streptomyces ikutamanensis*, Ya-2-1 (Noma and Nishimura, 1980, 1981) (Figure 14.135).

When (+)-3α-hydroxy-1,8-cineole (**123b**) was used as substrate in the cultured medium of *Streptomyces ikutamanensis*, Ya-2-1, (+)-3β-hydroxy-1,8-cineole (**123a**, 32%) was formed as the major product together with a small amount of (+)-3-oxo-1,8-cineole (**126a**, 1.6%). When (+)-3β-hydroxy-1,8-cineole (**123a**) was used, (+)-3-oxo-1,8-cineole (**126a**, 9.6%) and (+)-3α-hydroxy-1,8-cineole (**123b**, 2%) were formed. When (+)-3-oxo-1,8-cineole (**126a**) was used, (+)-3α-hydroxy- (**123b**, 19%) and (+)-3β-hydroxy-1,8-cineole (**123a**, 16%) were formed.

Based on the above results, it is obvious that (+)-3β-hydroxy-1,8-cineole (**123b**) is formed mainly in the biotransformation of 1,8-cineole (**122**), (+)-3α-hydroxy-1,8-cineole (**123b**), and (+)-3-oxo-1,8-cineole (**126a**) by *Streptomyces ikutamanensis*, Ya-2-1. The production of (+)-3β-hydroxy-1,8-cineole (**123b**) is interesting, because it is a precursor of mosquito repellent, *p*-menthane-3,8-diol (**142aa′**) (Noma and Nishimura, 1981) (Figure 14.136).

When *Aspergillus niger* TBUYN-2 was cultured in the presence of 1,8-cineole (**122**) for 7 days, it was transformed to three alcohols [racemic 2α-hydroxy-1,8-cineoles (**125b** and **b′**), racemic 3α-hydroxy- (**123b** and **b′**), and racemic 3β-hydroxy-1,8-cineoles (**123a** and **123a′**)] and two ketones [racemic 2-oxo- (**126** and **126′**) and racemic 3-oxo-1,8-cineoles (**124** and **124′**)] (Figure 14.135). The formation of 3α-hydroxy- (**123b** and **b′**) and 3β-hydroxy-1,8-cineoles (**123a** and **123a′**) is of great interest not only due to the possibility of the formation of *p*-menthane-3,8-diol (**142** and **142′**), the mosquito repellents and plant growth regulators that are synthesized chemically from 3α-hydroxy- (**123b** and **b′**) and 3β-hydroxy-1,8-cineoles (**123a** and **123a′**), respectively, but also from the viewpoint of the utilization of *Eucalyptus adiata* var. *australiana* leaves oil as biomass. An Et_2O extract of the culture broth (products and **122** as substrate) was recovered in 57% of substrate (w/w) (Nishimura et al., 1982; Noma et al., 1996) (Figure 14.137).

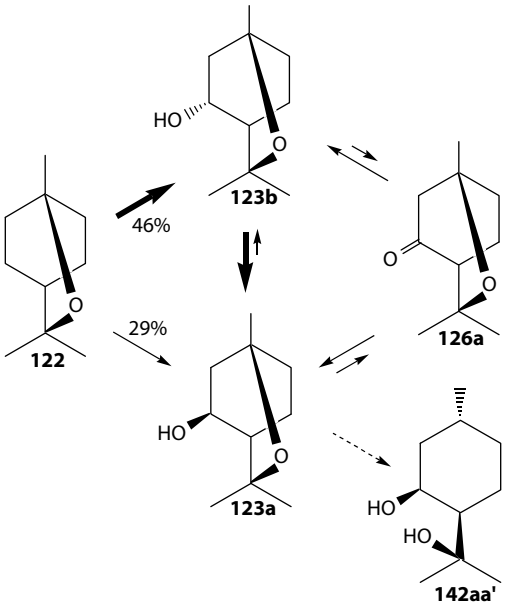


FIGURE 14.136 Biotransformation of 1,8-cineole (**122**), (+)-3α-hydroxy-1,8-cineole (**123b**), (+)-3β-hydroxy-1,8-cineole(**123a**), and (+)-3-oxo-1,8-cineole (**126a**) by *Streptomyces ikutamanensis*, Ya-2-1. (Modified from Noma, Y. and H. Nishimura, 1981. *Annual Meeting of Agricultural and Biological Chemical Society*, Book of abstracts, p. 196.)

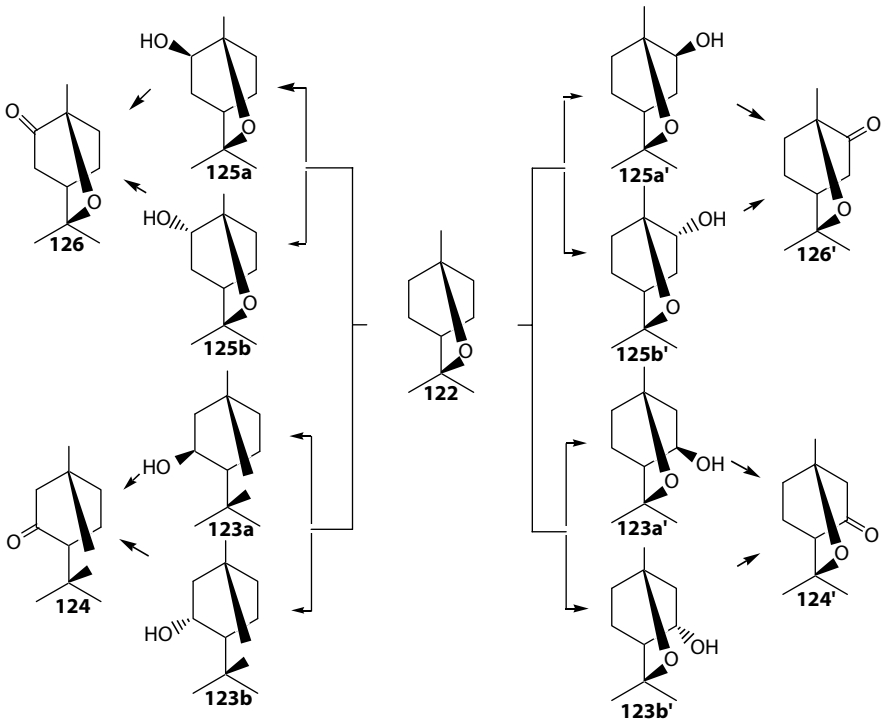


FIGURE 14.137 Biotransformation of 1,8-cineole (**122**) by *Aspergillus niger* TBUYN-2. (Modified from Nishimura, H. et al., 1982. *Agric. Biol. Chem.*, 46: 2601–2604; Noma, Y. et al., 1996. *Proc. 40th TEAC*, pp. 89–91.)

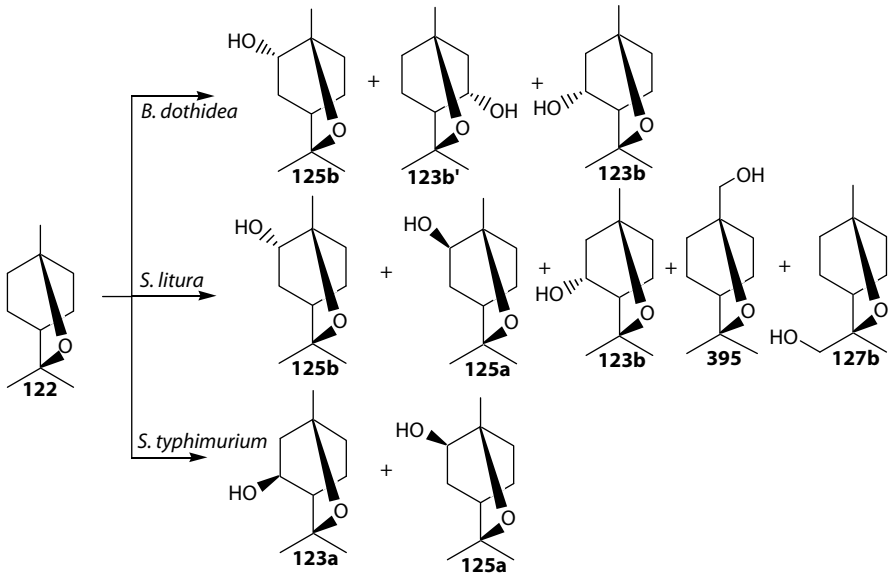


FIGURE 14.138 Biotransformation of 1,8-cineole (**122**) by *Botryosphaeria dothidea*, *Spodptera litura*, and *Salmonella typhimurium*. (Modified from Noma, Y. et al., 1996. *Proc. 40th TEAC*, pp. 89–91; Saito, H. and M. Miyazawa, 2006. *Proc. 50th TEAC*, pp. 275–276; Hagiwara, Y. and M. Miyazawa, 2007. *Proc. 51st TEAC*, pp. 304–305.)

Plant pathogenic fungus *Botryosphaeria dothidea* converted 1,8-cineole (**122**) to optical pure (+)-2α-hydroxy-1,8-cineole (**125b**) and racemic 3α-hydroxy-1,8-cineole (**123b** and **b′**), which were oxidized to optically active 2-oxo- (**126**) (100% ee) and racemic 3-oxo-1,8-cineole (**124** and **124′**), respectively (Table 14.14). Cytochrome P-450 inhibitor, 1-aminobenzotriazole, inhibited the hydroxylation of the substrate (Noma et al., 1996) (Figure 14.138). *Spodptera litura* also converted 1,8-cineole (**122**) to give three secondary alcohols (**123b, 125a,** and **b**) and two primary alcohols (**395** and **127**) (Hagihara and Miyazawa, 2007). *Salmonella typhimurium* OY1001/3A4 and NADPH-P450 reductase hydroxylated 1,8-cineole (**122**) to 2β-hydroxy-1,8-cineole (**125a**, $[\alpha]_D + 9.3$, 65.3% ee) and 3β-hydroxy-1, 8-cineole (**123a**, $[\alpha]_D$–27.8, 24.7% ee) (Saito and Miyazawa, 2006).

Extraction of the urinary metabolites from brushtail possums (*Trichosurus vulpecula*) maintained on a diet of fruit impregnated with 1,8-cineole (**122**) yielded *p*-cresol (**129**) and the novel C-9 oxidated products 9-hydroxy-1,8-cineole (**127a**) and 1,8-cineole-9-oic acid (**462a**) (Flynn and Southwell, 1979; Southwell and Flynn, 1980) (Figure 14.139).

1,8-Cineole (**122**) gave 2β-hydroxy-1,8-cineole (**125a**) by CYP-450 human and rat liver microsome. Cytochrome P450 molecular species responsible for metabolism of 1,8-cineole (**122**) was determined to be CYP3A4 and CYP3A1/2 in human and rat, respectively. Kinetic analysis showed that K_m and V_{max} values for the oxidation of 1,8-cineole (**122**) by human and rat treated with

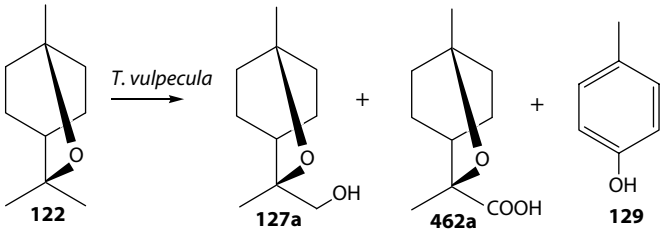


FIGURE 14.139 Metabolism of 1,8-cineole in *Trichosurus vulpecula*. (Modified from Southwell, I.A. and T.M. Flynn, 1980. *Xenobiotica*, 10: 17–23.)

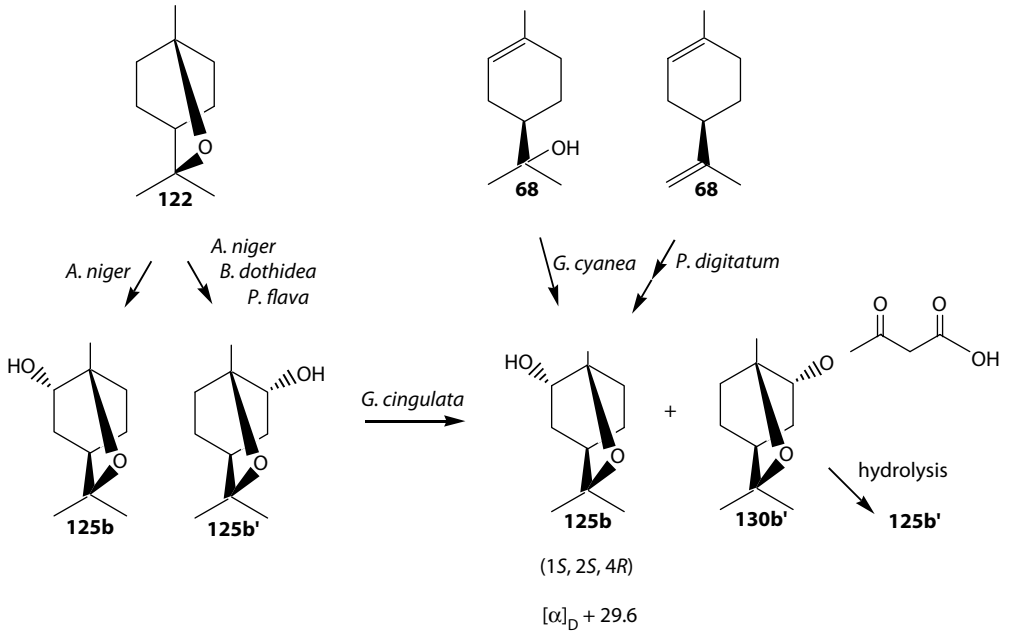


FIGURE 14.140 Formation of 2α-hydroxy-1,8-cineoles (**125b** and **b′**) from 1,8-cineole (**122**) and optical resolution by *Glomerella cingulata* and *Aspergillus niger* TBUYN-2 and **125b′** from (+)-limonene (**68**) by *Pencillium digitatum*. (Modified from Nishimura, H. et al., 1982. *Agric. Biol. Chem*., 46: 2601–2604; Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol*., 24: 24–30; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353; Noma, Y. et al., 1986. *Proc. 30th TEAC*, pp. 204–206; Noma, Y. and Y. Asakawa, 2007a. *Book of Abstracts of the 38th ISEO*, p. 7.)

pregnenolone-16α-carbonitrile (PCN), recombinant CYP3A4 were determined to be 50 μM and 90.9 nmol/min/nmol P450, 20 μM and 11.5 nmol/min/nmol P450, and 90 μM and 47.6 nmol/min/nmol P450, respectively (Shindo et al., 2000).

Microbial resolution of racemic 2α-hydroxy-1,8-cineoles (**125b** and **b′**) was carried out by using *Glomerella cingulata*. The mixture of **125b** and **b′** was added to a culture of *Glomerella cingulata* and esterified to give after 24 h (1*R*,2*R*,4*S*)-2α-hydroxy-1,8-cineole-2-yl-malonate (**130b′**) in 45% yield (ee 100%). The recovered alcohol showed 100% ee of the (1*S*,2*S*,4*R*)-enantiomer (**125b**) (Miyazawa et al., 1995b). On the other hand, optically active (+)-2α-hydroxy-1,8-cineole **(125b)** was also formed from (+)-limonene (**68**) by a strain of *Citrus* pathogenic fungus *Penicillium digitatum* (Saito and Miyazawa 2006, Noma and Asakawa 2007a) (Figure 14.140).

Esters of racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**) were prepared by a convenient method (Figure 14.141). Their odours were characteristic. Then products were tested against antimicrobial activity and their microbial resolution was studied (Hashimoto and Miyazawa, 2001) (Table 14.15).

1,8-Cineole (**122**) was glucosylated by *Eucalyptus perriniana* suspension cells to 2α-hydroxy-1,8-cineole monoglucoside (**404**, 16.0% and **404′**, 16.0%) and diglucosides (**405**, 1.4%) (Hamada et al., 2002) (Figure 14.142).

14.3.4.3.2 1,4-Cineole

Regarding the biotransformation of 1,4-cineole (**131**), *Streptomyces griseus* transformed it to 8-hydroxy-1,4-cineole (**134**), whereas *Bacillus cereus* transformed 1,4-cineole (**131**) to 2α-hydroxy-1,4-cineole (**132b**, 3.8%) and 2β-hydroxy-1,4-cineoles (**132a**, 21.3%) (Liu et al., 1988) (Figure 14.144). On the other hand, a strain of *Aspergillus niger* biotransformed 1,4-cineole (**131**) regiospecifically to 2α-hydroxy-1,4-cineole (**132b**) (Miyazawa et al., 1991c) and (+)-3α-hydroxy-1,4-cineole (**133b**) (Miyazawa et al., 1992b) along with the formation of 8-hydroxy-1,4-cineole (**134**) and 9-hydroxy-1,4-cineole (**135**) (Miyazawa et al., 1992c) (Figure 14.144).

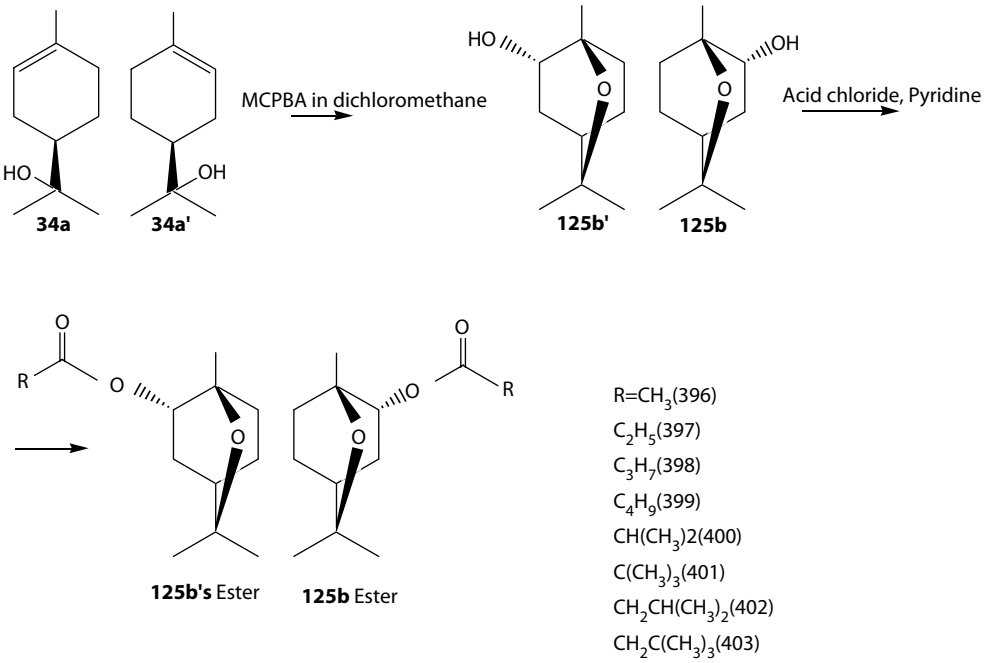


FIGURE 14.141 Chemical synthesis of esters of racemic 2α-hydroxy-1,8-cineole (**125b** and **b′**). (Modified from Hashimoto Y. and M. Miyazawa, 2001. *Proc. 45th TEAC*, pp. 363–365.)

Microbial optical resolution of racemic 2α-hydroxy-1,4-cineoles (**132b** and **b′**) was carried out by using *Glomerella cingulata* (Liu et al., 1988). The mixture of 2α-hydroxy-1,4-cineoles (**132b** and **b′**) was added to a culture of *Glomerella cingulata* and esterified to give after 24 h (1*R*,2*R*,4*S*)-2α-hydroxy-1,4-cineole-2-yl malonate (**136′**) in 45% yield (ee 100%). The recovered alcohol showed an ee of 100% of the (1*S*,2*S*,4*R*)-enantiomer (**132b**). On the other hand, optically active (+)-2α-hydroxy-1,4-cineole (**132b**) was also formed from (–)-terpinen-4-ol (**342**) by *Gibberella cyanea* DSM (Abraham et al., 1986) and *Aspergillus niger* TBUYN-2 (Noma and Asakawa, 2007b) (Figure 14.145).

TABLE 14.14
Stereoselectivity in the Biotransformation of 1,8-Cineole (122) by *Aspergillus niger, Botryosphaeria dothidea*, and *Pseudomonas flava*

	Products
Microorganisms	**125a and a′, 125b and b′, 123b and b′, 123a and a′**
Aspergillus niger TBUYN-2	2:43:49:6
	50:50 41:59
Botryosphaeria dothidea	4:59:34:3
	100:0 53:47
Pseudomonas flava	29:71:0:0
	100:0

Source: Noma, Y. et al., 1996. *Proc. 40th TEAC*, pp. 89–91.

TABLE 14.15
Yield and Enantiomer Excess of Esters of Racemic 2α-Hydroxy-1,8-Cineole (125b and b′) on the Microbial Resolution by *Glomerella cingulata*

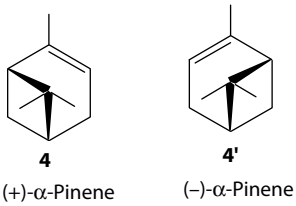
	0 h	24 h		48 h	
Compounds	**%ee**	**%ee**	**Yield (%)**	**%ee**	**Yield (%)**
396	(−)36.3	(+)85.0	24.0	(+)100	14.1
397	(−)36.9	(+)73.8	18.6	(+)100	8.6
398	(−)35.6	(+)33.2	13.7	(+)75.4	3.5
399	(−)36.8	(+)45.4	14.4	(+)100	2.3
400	(−)35.4	(−)21.4	25.2	(+)20.6	8.0
401	(−)36.7	(−)37.8	31.5	(−)40.6	15.2
402	(−)36.1	(−)29.8	46.8	(−)15.0	24.0
403	(−)36.3	(−)37.6	72.2	(−)39.0	36.9

Source: Hashimoto Y. and M. Miyazawa, 2001. *Proc. 45th TEAC*, pp. 363–365.

14.4 METABOLIC PATHWAYS OF BICYCLIC MONOTERPENOIDS

14.4.1 Bicyclic Monoterpene

14.4.1.1 α-Pinene



α-Pinene (**4** and **4′**) is the most abundant terpene in nature and obtained industrially by fractional distillation of turpentine (Krasnobajew, 1984). (+)-α-Pinene (**4**) occurs in oil of *Pinus palustris* Mill. at concentrations of up to 65%, and in oil of *Pinus caribaea* at concentrations of 70% (Bauer et al., 1990). On the other hand, *Pinus caribaea* contains (–)-α-pinene (**4′**) at the concentration of 70–80% (Bauer et al., 1990).

The biotransformation of (+)-α-pinene (**4**) was investigated by *Aspergillus niger* NCIM 612 (Bhattacharyya et al., 1960, Prema and Bhattacharyya, 1962). A 24 h shake culture of this strain metabolized 0.5% (+)-α-pinene (**4**) in 4–8 h. After the fermentation of the culture broth contained (+)-verbenone (**24**) (2–3%), (+)-*cis*-verbenol (**23b**) (20–25%), (+)-*trans*-sobrerol (**43a**) (2–3%), and

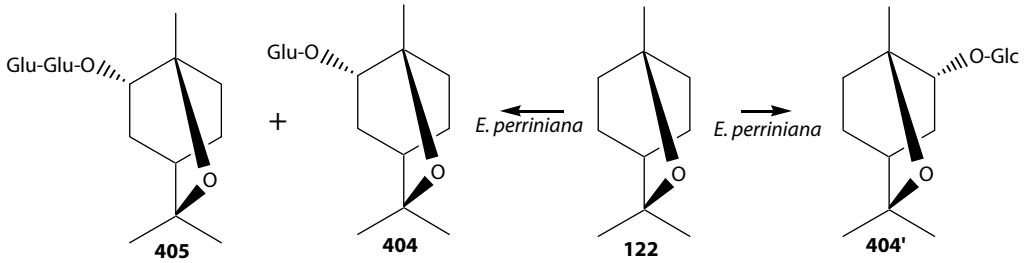


FIGURE 14.142 Biotransformation of 1,8-cineole (**122**) by *Eucalyptus perriniana* suspension cell. (Modified from Hamada, H. et al., 2002. *Proc. 46th TEAC*, pp. 321–322.)

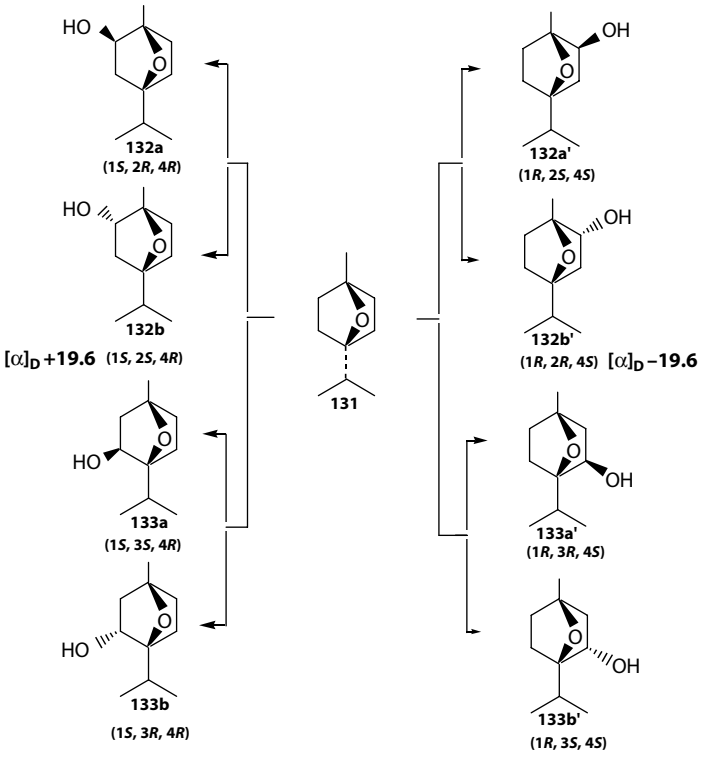


FIGURE 14.143 Metabolic pathways of 1,4-cineole (**131**) by microorganisms

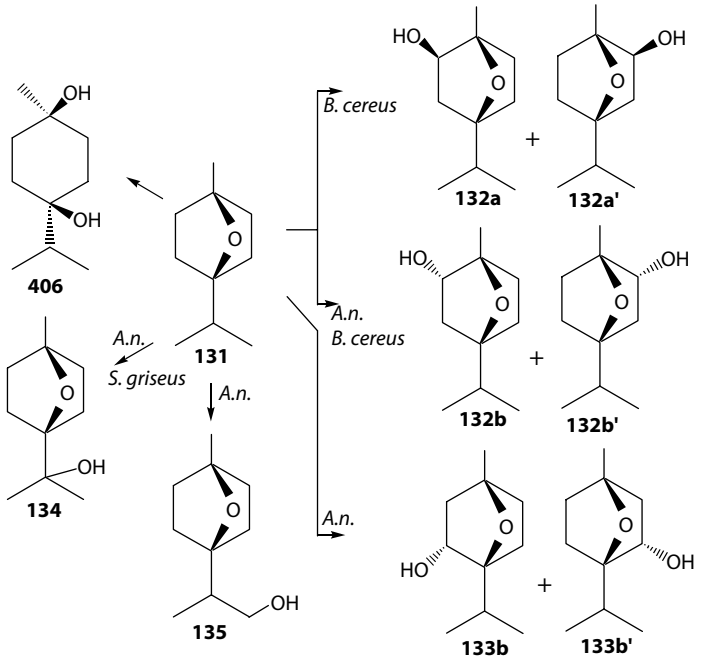


FIGURE 14.144 Metabolic pathways of 1,4-cineole (**131**) by *Aspergillus niger* TBUYN-2, *Bacillus cereus*, and *Streptomyces griseus*. (Modified from Liu, W. et al., 1988. *J. Org. Chem*., 53: 5700–5704; Miyazawa, M. et al., 1991c. *Chem. Express*, 6: 771–774; Miyazawa, M. et al., 1992b. *Chem. Express*, 7: 305–308; Miyazawa, M. et al., 1992c. *Chem. Express*, 7: 125–128; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353.)

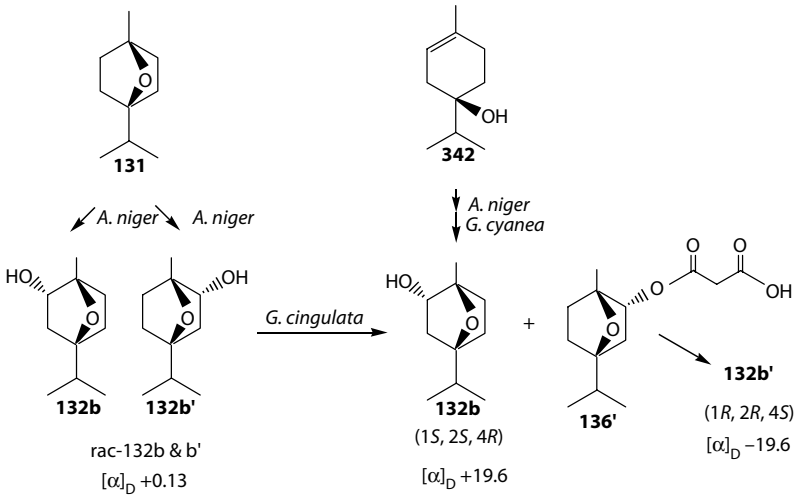


FIGURE 14.145 Formation of optically active 2α-hydroxycineole from 1,4-cineole (**131**) and terpinene-4-ol (**342**) by *Aspergillus niger* TBUYN-2, *Gibberella cyanea*, and *Glomerella cingulata*. (Modified from Abraham, W.-R. et al., 1986. *Appl. Microbiol. Biotechnol.*, 24: 24–30; Miyazawa, M. et al., 1991c. *Chem. Express*, 6: 771–774; Miyazawa, M. et al., 1995b. *Proc. 39th TEAC*, pp. 352–353; Noma, Y. and Y. Asakawa, 2007b. *Proc. 51st TEAC*, pp. 299–301.)

(+)-8-hydroxycarvotanacetone (**44**) (Bhattacharyya et al., 1960; Prema and Bhattacharyya 1962 (Figure 14.146).

The degradation of (+)-α-pinene (**4**) by a soil *Pseudomonas* sp. (PL strain) was investigated by Hungund et al. (1970). A terminal oxidation pattern was proposed, leading to the formation of organic acids through ring cleavage. (+)-α-Pinene (**4**) was fermented in shake cultures by a soil *Pseudomonas* sp. (PL strain) that is able to grow on (+)-α-pinene (**4**) as the sole carbon source, and borneol (**36**), myrtenol (**5**), myrtenic acid (**84**), and α-phellandric acid (**65**) (Shukla and Bhattacharyya, 1968) (Figure 14.147) were obtained.

The degradation of (+)-α-pinene (**4**) by *Pseudomonas fluorescens* NCIMB11671 was studied and a pathway for the microbial breakdown of (+)-α-pinene (**4**) was proposed as shown in Figure 14.148 (Best et al., 1987; Best and Davis, 1988). The attack of oxygen is initiated by enzymatic oxygenation of the 1,2-double bond to form α-pinene epoxide (**38**), which then undergoes rapid rearrangement to produce a unsaturated aldehyde, occurring as two isomeric forms. The primary product of the reaction (*Z*)-2-methyl-5-isopropylhexa-2,5-dien-1-al (**39**, isonovalal) can undergo chemical isomerization to the *E*-form (novalal, **40**). Isonovalal (**39**), the native form of the aldehyde, possesses *citrus*, woody, spicy notes, whereas novallal (**40**) has woody, aldehydic, and cyclone notes. The same biotransformation was also carried out by *Nocardia* sp. strain P18.3 (Griffiths et al., 1987a, b).

Pseudomonas PL strain and PIN 18 degradated α-pinene (**4**) by the pathway proposed in Figure 14.149 to give two hydrocarbon, limonene (**68**) and terpinolene (**346**), and neutral metabolite,

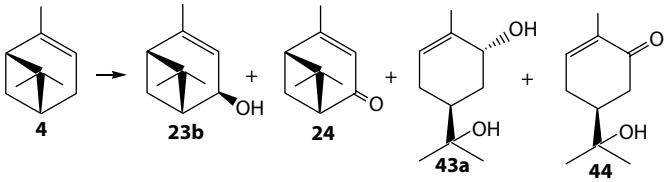


FIGURE 14.146 Biotransformation of (+)-α-pinene (**4**) by *Aspergillus niger* NCIM 612. (Modified from Bhattacharyya, P.K. et al., 1960. *Nature*, 187: 689–690; Prema, B.R. and P.K. Bhattachayya, 1962. *Appl. Microbiol.*, 10: 524–528.)

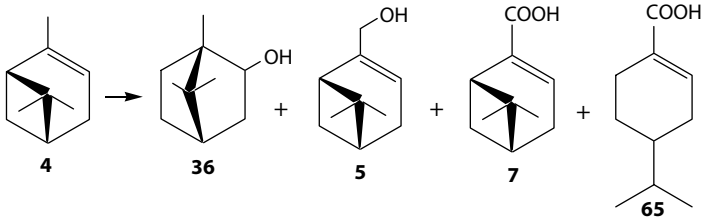


FIGURE 14.147 Biotransformation of (+)-α-pinene (**4**) by *Pseudomonas* sp. (PL strain). (Modified from Shukla, O.P., and P.K. Bhattacharyya, 1968. *Indian J. Biochem*., 5: 92–101.)

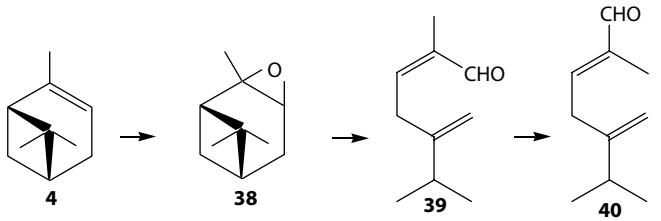


FIGURE 14.148 Biotransformation of (+)-α-pinene (**4**) by *Pseudomonas fluorescens* NCIMB 11671. (Modified from Best, D.J. et al., 1987. *Biotransform*., 1: 147–159.)

borneol (**36**). A probable pathway has been proposed for the terminal oxidation of β-isopropylpimelic acid (**248**) in the PL strain and PIN 18 (Shukala and Bhattacharyya, 1968).

Pseudomonas PX 1 biotransformed (+)-α-pinene (**4**) to give (+)-*cis*-thujone (**29**) and (+)-*trans*-carveol (**81a**) as major compounds. Compounds **81a**, **171**, **173**, and **178** have been identified as fermentation products (Gibbon and Pirt, 1971; Gibbon et al., 1972) (Figure 14.150).

Aspergillus niger TBUYN-2 biotransformed (–)-α-pinene (**4′**) to give (–)-α-terpineol (**34′**) and (–)-*trans*-sobrerol (**43a′**) (Noma et al., 2001). The mosquitocidal (+)-(1*R*,2*S*,4*R*)-1-*p*-menthane-2,8-diol (**50a′**) was also obtained as a crystal in the biotransformation of (–)-α-pinene (**4′**) by *Aspergillus niger* TBUYN-2 (Noma et al., 2001; Noma, 2007) (Figure 14.151).

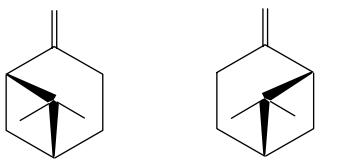
(1*R*)-(+)-α-Pinene (**4**) and its enantiomer (**4′**) were fed to *Spodptera litura* to give the corresponding (+)- and (–)-verbenones (**24** and **24′**) and (+)- and (–)-myrtenols (**5** and **5′**) (Miyazawa et al., 1996c) (Figure 14.152).

(–)-α-Pinene (**4′**) was treated in human liver microsomes CYP 2B6 to afford (–)-*trans*-verbenol (**23′**) and (–)-myrtenol (**5′**) (Sugie and Miyazawa, 2003) (Figure 14.153).

In rabbit, (+)-α-pinene (**4**) was metabolized to (–)-*trans*-verbenols (**23**) as the main metabolites together with myrtenol (**5**) and myrtenic acid (**7**). The purities of (–)-verbenol (**23**) from (–)- (**4′**), (+)- (**4**), and (+/–)-α-pinene (**4** and **4′**) was 99%, 67%, and 68%, respectively. This means that the biotransformation of (–)-**4′** in rabbit is remarkably efficient in the preparation of (–)-*trans*-verbenol (**23a**) (Ishida et al., 1981b) (Figure 14.154).

(–)-α-Pinene (**4′**) was biotransformed by the plant pathogenic fungus *Botrytis cinerea* to afford 3α-hydroxy-(–)-β-pinene (**2a′**, 10%), 8-hydroxy-(–)-α-pinene (**434′**, 12%), 4β-hydroxy-(–)-pinene-6-one (**468′**, 16%), and (–)-verbenone (**24′**) (Farooq et al., 2002) (Figure 14.155).

14.4.1.2 β-Pinene



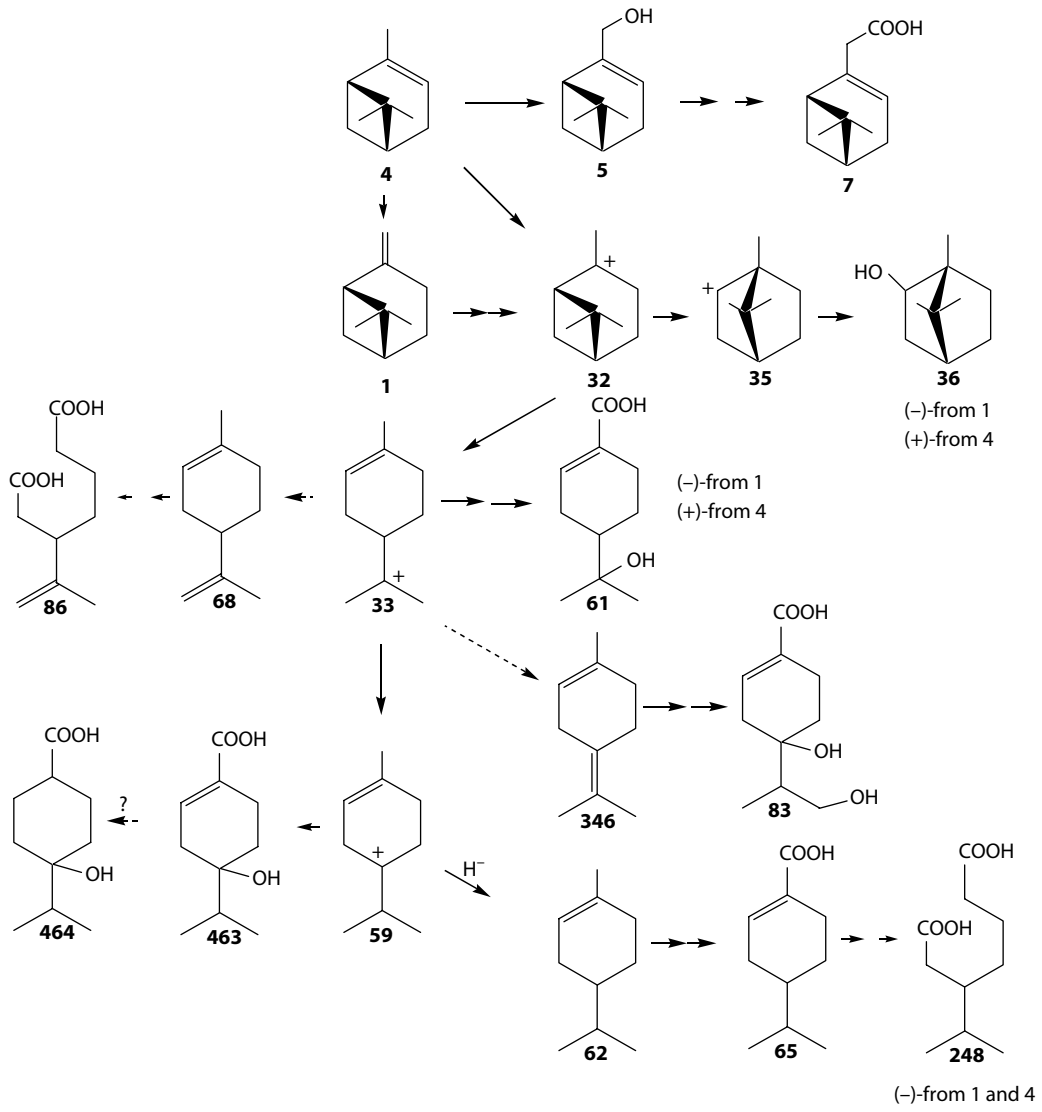


FIGURE 14.149 Metabolic pathways of degradation of α- and β-pinene by a soil Pseudomonad (PL strain) and *Pseudomonas* PIN 18. (Modified from Shukla, O.P., and P.K. Bhattacharyya, 1968. *Indian J. Biochem*., 5: 92–101.)

(+)-β-Pinene (**1**) is found in many essential oils. Optically active and racemic β-pinene are present in turpentine oils, although in smaller quantities than (+)-α-pinene (**4**) (Bauer et al., 1990).

Shukla et al. (1968) obtained a similarly complex mixture of transformation products from (–)-β-pinene (**1′**) through degradation by a *Pseudomonas* sp/(PL strain). On the other hand, Bhattacharyya and Ganapathy (1965) indicated that *Aspergillus niger* NCIM 612 acts differently and more specifically on the pinenes by preferably oxidizing (–)-β-pinene (**1′**) in the allylic position to form the interesting products pinocarveol (**2′**) and pinocarvone (**3′**), besides myrtenol (**5′**) (see Figure 14.156). Furthermore, the conversion of (–)-β-pinene (**1′**) by *Pseudomonas putida-arvilla* (PL strain) gave borneol (**36′**) (Rama Devi and Bhattacharyya, 1978) (Figure 14.156).

Pseudomonas pseudomallai isolated from local sewage sludge by the enrichment culture technique utilized caryophyllene as the sole carbon source (Dhavlikar et al., 1974). Fermentation of (–)-β-pinene (**1′**) by *Pseudomonas pseudomallai* in a mineral salt medium (Seubert's medium) at

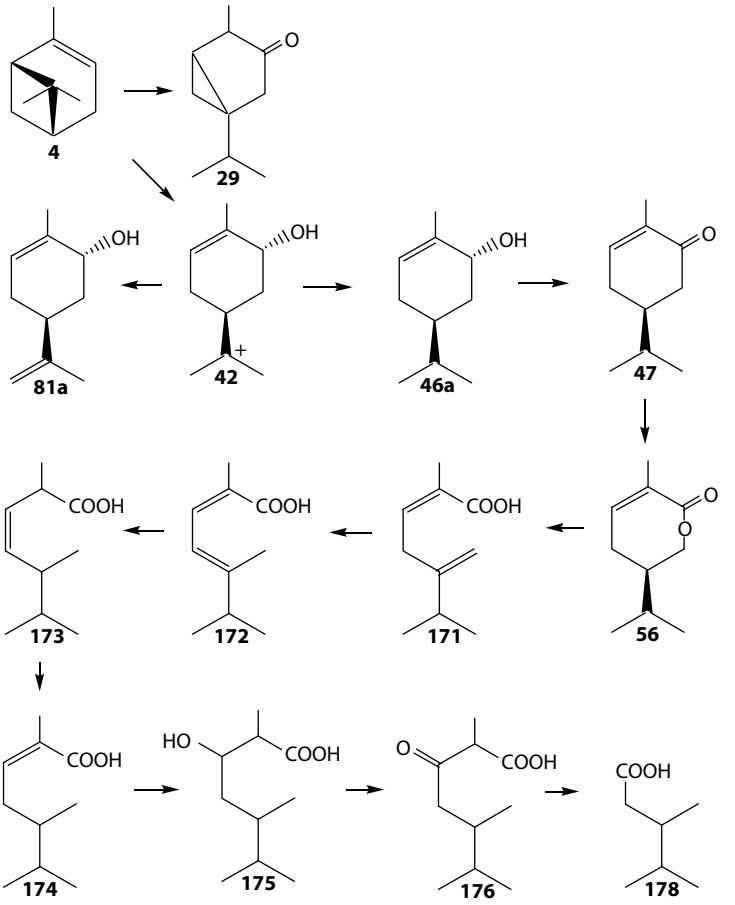


FIGURE 14.150 Proposed metabolic pathways for (+)-α-pinene (**4**) degradation by *Pseudomonas* PX 1. (Modified from Gibbon, G.H. and S.J. Pirt, 1971. *FEBS Lett.*, 18: 103–105; Gibbon, G.H. et al., 1972. *Proc. IV IFS, Ferment. Technol. Today*, pp. 609–612.)

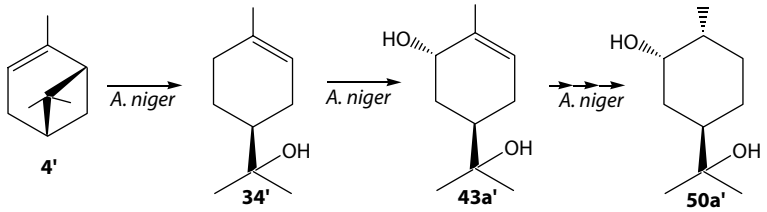


FIGURE 14.151 Biotransformation of (–)-α-pinene (**4**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2001. *Proc. 45th TEAC*, pp. 88–90.)

30°C with agitation and aeration for 4 days yielded camphor (**37′**), borneol (**36a′**), isoborneol (**36b′**), α-terpineol (**34′**), and β-isopropyl pimelic acid (**248′**) (see Figure 14.154). Using modified Czapek-Dox medium and keeping the other conditions the same, the pattern of the metabolic products was dramatically changed. The metabolites were *trans*-pinocarveol (**2′**), myrtenol (**5′**), α-fenchol (**11′**), α-terpineol (**34′**), myrtenic acid (**7′**), and two unidentified products (see Figure 14.157).

(–)-β-Pinene (**1′**) was converted by plant pathogenic fungi, *Botrytis cinerea*, to give four new compounds such as (–)-pinane-2α,3α-diol (**408′**), (–)-6β-hydroxypinene (**409′**), (–)-4α,5-dihydroxypinene (**410′**), and (–)-4α-hydroxypinen-6-one (**411′**) (Figure 14.158).

This study progressed further biotransformation of (–)-pinane-2α,3α-diol (**408′**) and related compounds by microorganisms as shown in Figure 14.158.

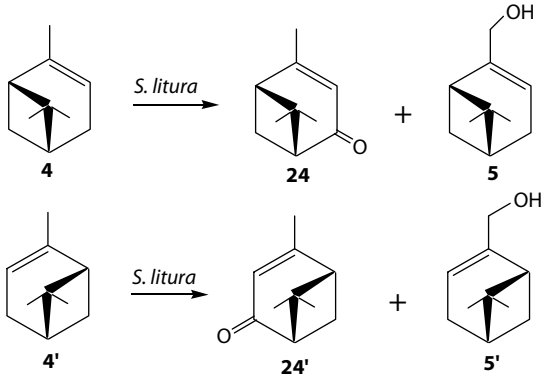


FIGURE 14.152 Biotransformation of (+)- (**4**) and (–)-α-pinene (**4′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1996c. *Proc. 40th TEAC*, pp. 84–85.)

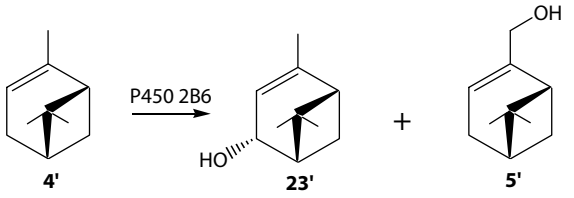


FIGURE 14.153 Biotransformation of (–)-α-pinene (**4′**) by human liver microsomes CYP 2B6. (Modified from Sugie, A. and M. Miyazawa, 2003. *Proc. 47th TEAC*, pp. 159–161.)

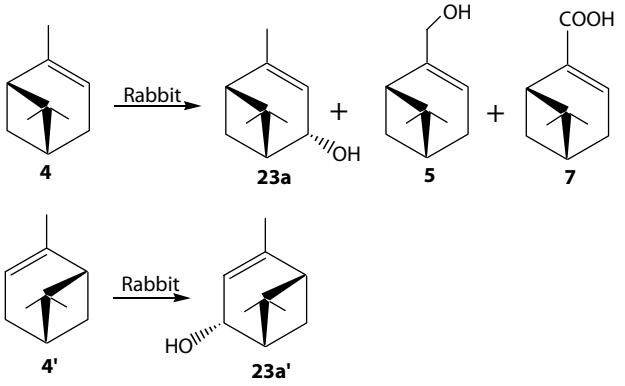


FIGURE 14.154 Biotransformation of α-pinene by rabbit. (Modified from Ishida, T. et al., 1981b. *J. Pharm. Sci.*, 70: 406–415.)

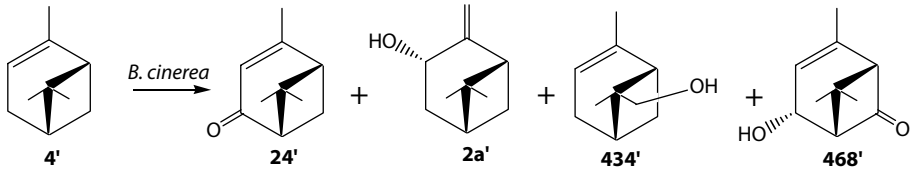


FIGURE 14.155 Microbial transformation of (–)-α-pinene (**4′**) by *Botrytis cinerea*. (Modified from Farooq, A. et al., 2002. *Z. Naturforsch.*, 57c: 686–690.)

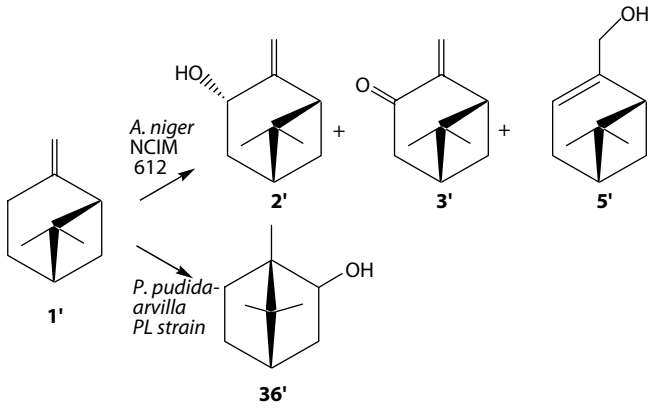


FIGURE 14.156 Biotransformation of (–)-β-pinene (**1′**) by *Aspergillus niger* NCIM 612 and *Pseudomonas putida-arvilla* (PL strain). (Modified from Bhattacharyya, P.K. and K. Ganapathy, 1965. *Indian J. Biochem.*, 2: 137–145; Rama Devi, J. and P.K. Bhattacharyya, 1978. *J. Indian Chem. Soc*., 55: 1131–1137.)

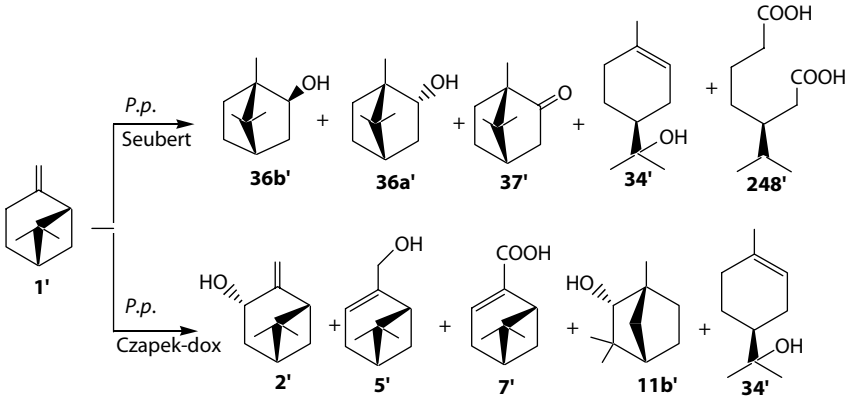


FIGURE 14.157 Biotransformation of (–)-β-pinene (**1′**) by *Pseudomonas pseudomallai*. (Modified from Dhavalikar, R.S. et al., 1974. *Dragoco Rep.*, 3: 47–49.)

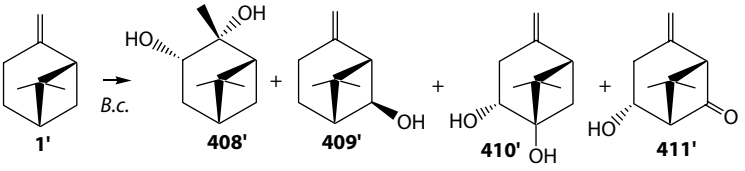


FIGURE 14.158 Biotransformation of (–)-β-pinene (**1′**) by *Botrytis cinerea*. (Modified from Farooq, A. et al., 2002. *Z. Naturforsch.*, 57c: 686–690.)

As shown in Figure 14.159, (+)- (**1**) and (–)-β-pinenes (**1′**) were biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-α-terpineol (**34**) and (+)-oleuropeyl alcohol (**204**) and their antipodes (**34′** and **204′**), respectively. The hydroxylation process of α-terpineol (**34**) to oleuropeyl alcohol (**204**) was completely inhibited by 1-aminotriazole as cyt.P-450 inhibitor.

(–)-β-Pinene (**1′**) was at first biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-*trans*-pinocarveol (**2a′**) (**274**). (+)-*trans*-Pinocarveol (**2a′**) was further transformed by three pathways: firstly, (+)-*trans*-pinocarveol (**2a′**) was metabolized to (+)-pinocarvone (**3′**), (–)-3-isopinanone (**413′**), (+)-2α-hydroxy-3-pinanone (**414′**), and (+)-2α,5-dihydroxy-3-pinanone (**415′**). Secondly, (+)-*trans*-pinocarveol (**2a′**) was metabolized to (+)-6β-hydroxyfenchol (**349ba′**) and thirdly (+)-*trans*-pinocarveol (**2a′**) was metabolized to (–)-6β,7-dihydroxyfenchol (**412ba′**) via epoxide and diol as intermediates (Noma and Asakawa, 2005a) (Figure 14.160).

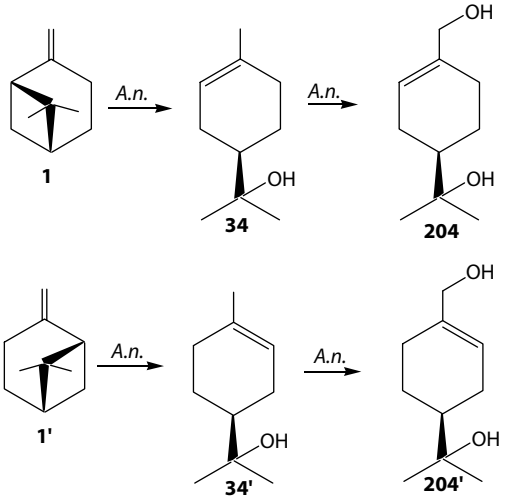


FIGURE 14.159 Biotransformation of (+)- (**1**) and (–)-β-pinene (**1′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2001. *Proc. 45th TEAC*, pp. 88–90.)

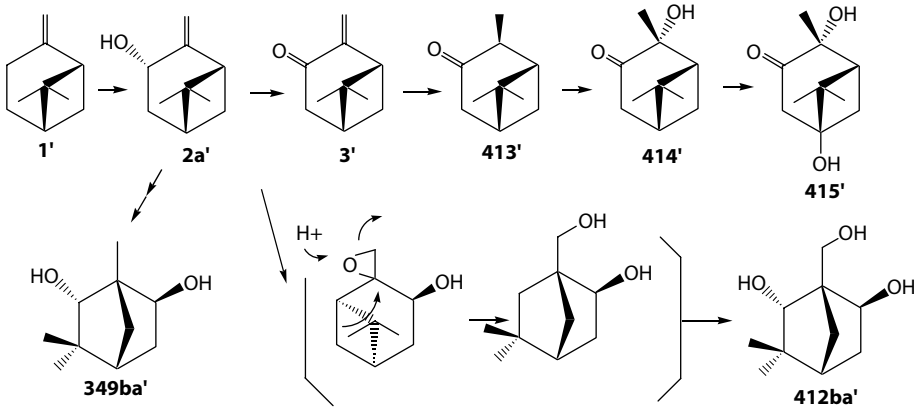


FIGURE 14.160 The metabolism of (–)-β-pinene (**1′**) and (+)-*trans*-pinocarveol (**2a′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

(–)-β-Pinene (**1′**) was metabolized by *Aspergillus niger* TBUYN-2 with three pathways as shown in Figure 14.154 to give (–)-α-pinene (**4′**), (–)-α-terpineol (**34**'), and (+)-*trans*-pinocarveol (**2a′**). (–)-α-Pinene (**4′**) is further metabolized by three pathways. At first (–)-α-pinene (**4′**) was metabolized via (–)-α-pinene epoxide (**38′**), *trans*-sobrerol (**43a′**), (–)-8-hydroxycarvotanacetone (**44′**), (+)-8-hydroxycarvomenthone (**45a**) to (+)-*p*-menthane-2,8-diol (**50a′**), which was also metabolized in (–)-carvone (**93′**) metabolism. Secondly, (–)-α-pinene (**4′**) is metabolized to myretenol (**83′**), which is metabolized by rearrangement reaction to give (–)-oleuropeyl alcohol (**204′**). (–)-α-Terpineol (**34′**), which is formed from β-pinene (**1′**), was also metabolized to (–)-oleuropeyl alcohol (**204′**) and (+)-*trans*-pinocarveol (**2a′**), formed from (–)-β-pinene (**1′**), was metabolized to pinocarvone (**3′**), 3-pinanone (**413′**), 2α-hydroxy-3-pinanone (**414′**), 2α,5-dihydroxy-3-pinanone (**415′**), and 2α,9-dihydroxy-3-pinanone (**416′**). Furthermore, (+)-*trans*-pinocarveol (**2a′**) was metabolized by rearrangemet reaction to give 6β-hydroxyfenchol (**349ba′**) and 6β,7-dihydroxyfenchol (**412ba′**) (Noma and Asakawa, 2005a) (Figure 14.161).

(–)-β-Pinene (**1′**) was metabolized by *Aspergillus niger* TBUYN-2 to give (+)-*trans*-pinocarveol (**2a′**), which was further metabolized to 6β-hydroxyfenchol (**349ba′**) and 6β, 7-dihydroxyfenchol

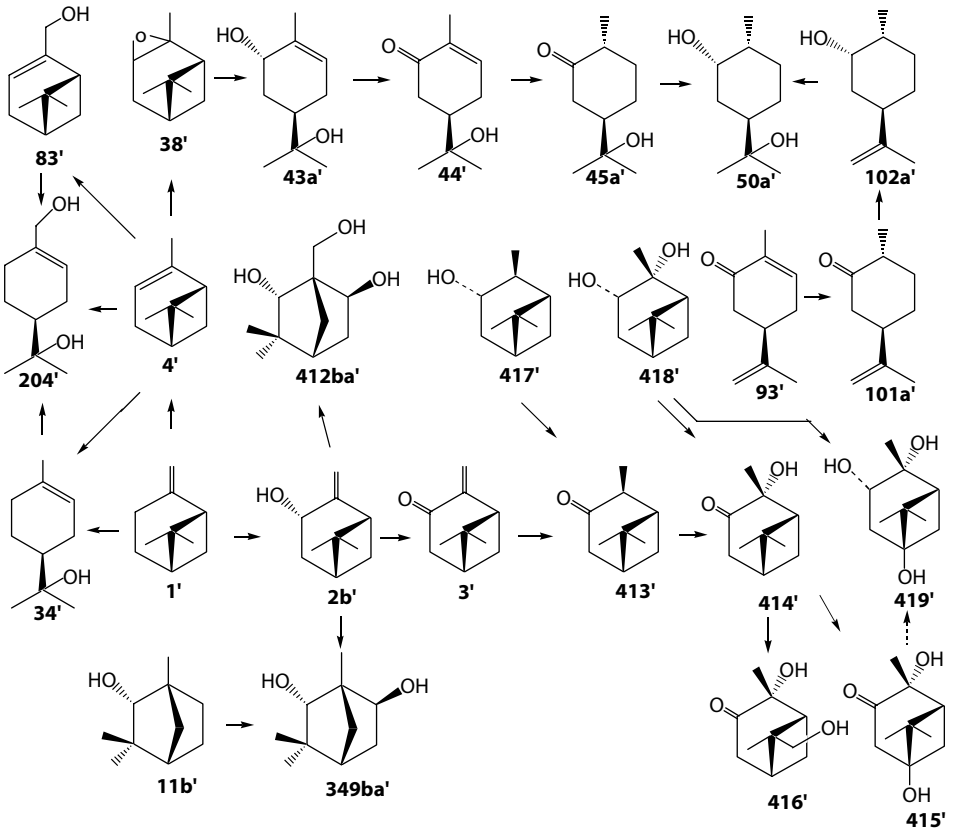


FIGURE 14.161 Biotransformation of (–)-β-pinene (**1′**), (–)-α-pinene (**4′**), and related compounds by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

(**412ba′**) by rearrangement reaction (Noma and Asakawa, 2005a) (Figure 14.162). 6β-Hydroxy-fenchol (**349ba′**) was also obtained from (–)-fenchol (**11b′**). (–)-Fenchone was hydroxylated by the same fungus to give 6β- (**13a′**) and 6α-hydroxy-(–)-fenchone (**13b′**). There is a close relationship between the metabolism of (–)-β-pinene (**1′**) and those of (–)-fenchol (**11′**) and (–)-fenchone (**12′**).

(–)-β-Pinene (**1′**) and (–)-α-pinene (**4′**) were isomerized to each other. Both are metabovlized via (–)-α-terpineol (**34**') to (–)-oleuropeyl alcohol (**204′**) and (–)-oleuropeic acid (**61′**). (–)-Myrtenol (**5′**) formed from (–)-α-pinene (**1′**) was further metabolized via cation to (–)-oleuropeyl alcohol (**204′**) and (–)-oleuropeic acid (**61′**). (–)-α-Pinene (**4′**) is further metabolized by *Aspergillus niger* TBUYN-2 via (–)-α-pinene epoxide (**38′**) to *trans*-sobrerol (**43a′**), (–)-8-hydroxycarvotanacetone (**44′**), (+)-8-hydroxycarvomenthone (**45a**), and mosquitocidal (+)-*p*-menthane-2,8-diol (**50a′**) (Battacharyya et al., 1960; Noma et al., 2001, 2002, 2003) (Figure 14.163).

The major metabolites of (–)-β-pinene (**1′**) were *trans*-10-pinanol (myrtanol) (**8ba′**) (39%) and (–)-1-*p*-menthene-7,8-diol (oleuropeyl alcohol) (**204′**) (30%). In addition, (+)-*trans*-pinocarveol (**2a′**) (11%) and (–)-α-terpineol (**34′**) (5%), verbenol (**23a** and **23b**) and pinocarveol (**2a′**) were oxidation products of α- (**4**) and β-pinene (**1**), respectively, in bark beetle, *Dendroctonus frontalis*. (–)-*Cis*- (**23b′**) and (+)-*trans*-verbenols (**23a′**) have pheromonal activity in *Ips paraconfussus* and *Dendroctonus brevicomis*, respectively (Ishida et al., 1981b) (Figure 14.164).

14.4.1.3 (±)-Camphene

Racemate camphene (**437** and **437′**) is a bicyclic monoterpene hydrocarbon found in *Liquidamar* species, *Chrysanthemum*, *Zingiber officinale*, *Rosmarinus officinalis*, and among other plants. It

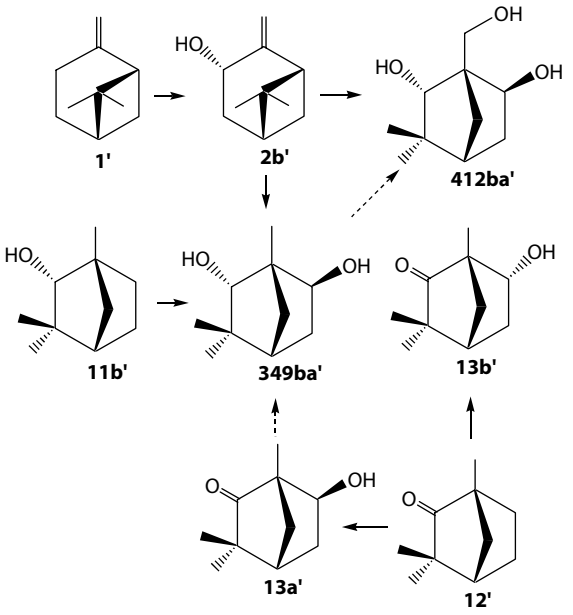


FIGURE 14.162 Relationship of the metabolism of (–)-β-pinene (**1′**), (+)-fenchol (**11′**) and (–)-fenchone (**12′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

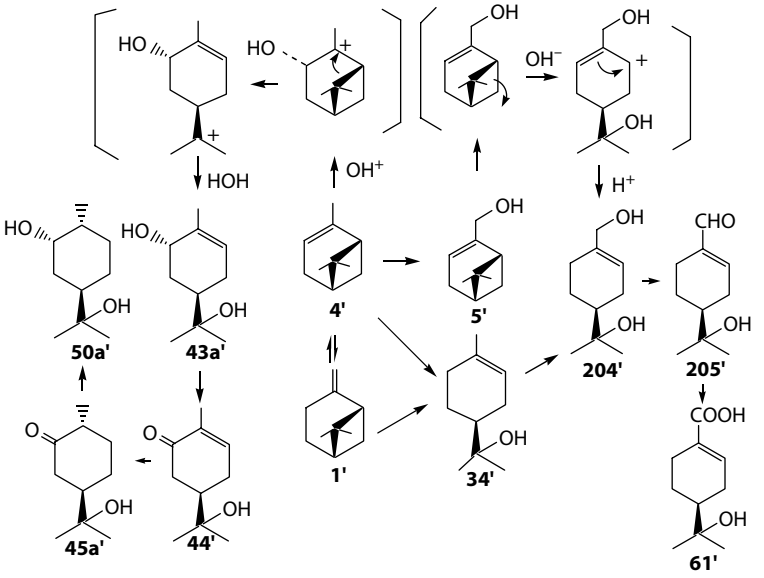


FIGURE 14.163 Metabolic pathways of (–)-β-pinene (**1′**) and related compounds by *Aspergillus niger* TBUYN-2 . (Modified from Bhattacharyya, P.K. et al., 1960. *Nature*, 187: 689–690; Noma, Y. et al., 2001. *Proc. 45th TEAC*, pp. 88–90; Noma, Y. et al., 2002. *Book of Abstracts of the 33rd ISEO*, p. 142; Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93.)

was administered into rabbits. Six metabolites, camphene-2,10-glycols (**438a, 438b**), which were the major metabolites, together with 10-hydroxytricyclene (**438c**), 7-hydroxycamphene (**438d**), 6-exo-hydroxycamphene **(438e**), and 3-hydroxytricyclene (**438f**) were obtained (Ishida et al., 1979). On the basis of the production of the glycols (**438a** and **438b**) in good yield, these alcohols might be formed through their epoxides as shown in Figure 14.165. The homoallyl camphene oxidation products (**438c**–**f**) apparently were formed through the non-classical cation as the intermediate.

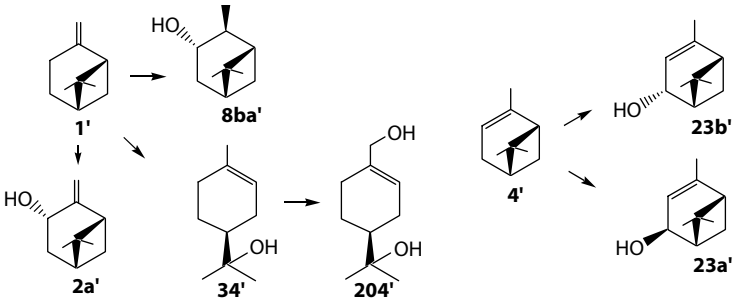


FIGURE 14.164 Metabolism of β-pinene (**1**) by bark beetle, *Dendroctonus frontalis*. (Modified from Ishida, T. et al., 1981b. *J. Pharm. Sci.*, 70: 406–415.)

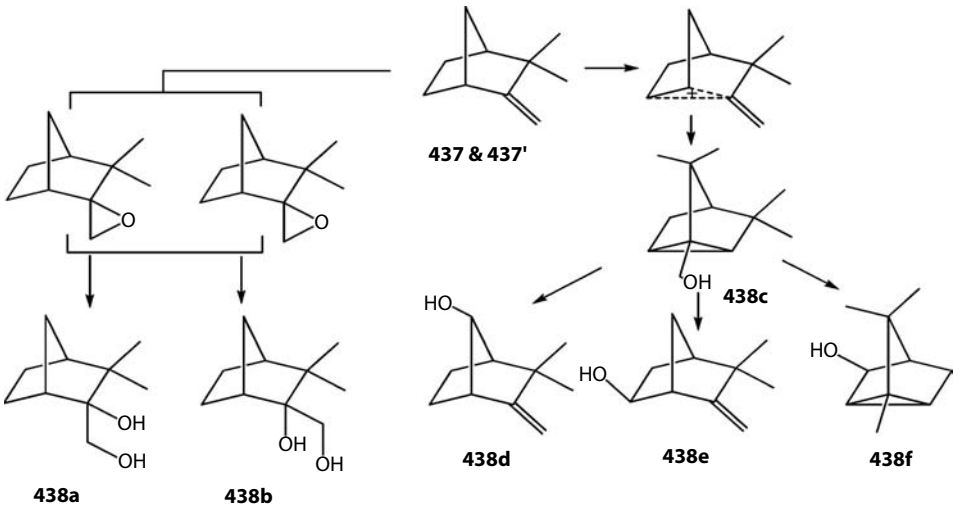
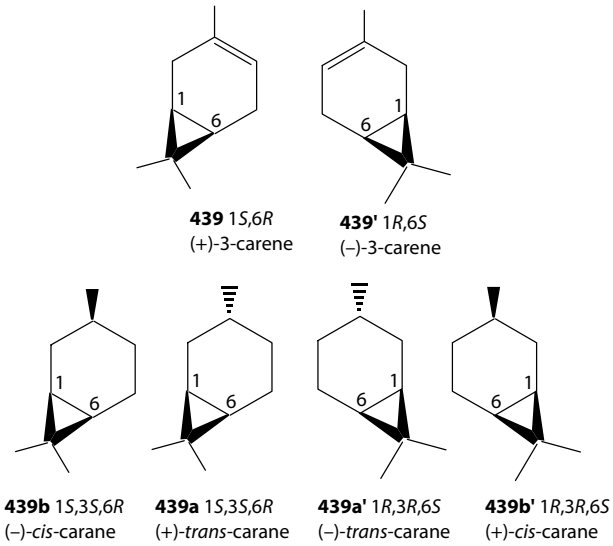


FIGURE 14.165 Biotransformation of (±)-camphene (**437** and **437′**) by rabbits. (Modified from Ishida, T. et al., 1979. *J. Pharm. Sci.*, 68: 928–930.)

14.4.1.4 3-Carene and Carane



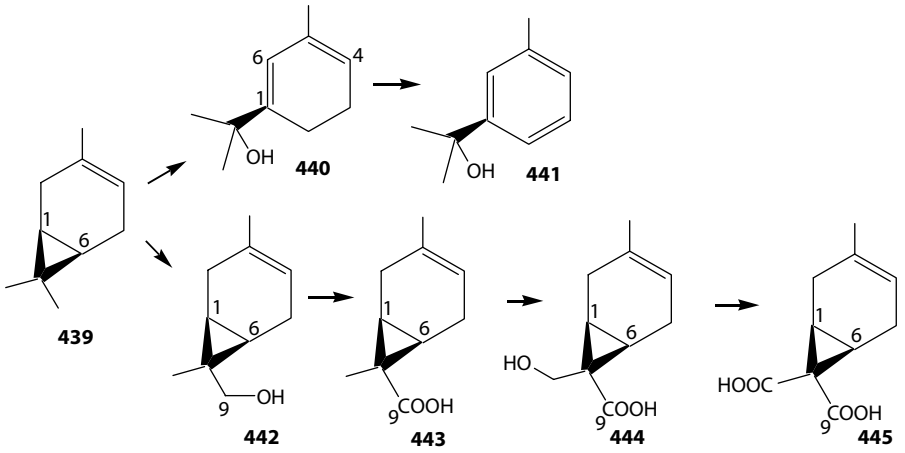


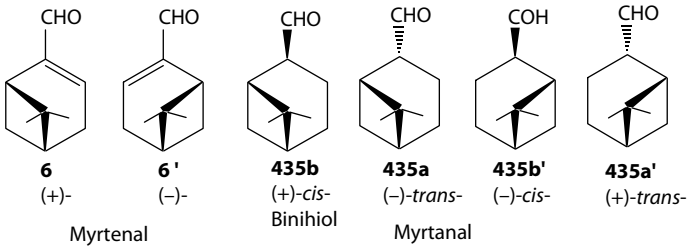
FIGURE 14.166 Metabolic pathways of (+)-3-carene (**439**) by rabbit (Modified from Ishida, T. et al., 1981b. *J. Pharm. Sci*., 70: 406–415). 3-(+)-Carene (**439**) was converted by *Aspergillus niger* NC 1M612 to give either hydroxylated compounds of 3-carene-2-one or 3-carene-5-one, which was not fully identified. (Modified from Noma, Y. et al., 2002. *Book of Abstracts of the 33rd ISEO*, p. 142) (Figure 14.167).

(+)-3-Carene (**439**) was biotransformed by rabbits to give *m*-mentha-4,6-dien-8-ol (**440**) (71.6%) as the main metabolite together with its aromatized *m*-cymen-8-ol (**441**). The position of C-5 in the substrate is thought to be more easily hydroxylated than C-2 by enzymatic systems in the rabbit liver. In addition to ring opening compound, 3-carene-9-ol (**442**), 3-carene-9-carboxylic acid (**443**), 3-carene-9,10-dicarboxilic acid (**445**), chamic acid, and 3-caren-10-ol-9-carboxylic acid (**444**) were formed. The formation of such compounds is explained by stereoselective hydroxylation and carboxylation of *gem*-dimethyl group (Ishida et al., 1981b) (Figure 14.166). In case of (–)-*cis*-carane (**446**), two C-9 and C-10 methyl groups were oxidized to give dicarboxylic acid (**447**) (Ishida et al., 1981b) (Figure 14.166).

3-(+)-Carene (**439**) was converted by *Aspergillus niger* NC 1M612 to give either hydroxylated compounds of 3-carene-2-one or 3-carene-5-one, which was not fully identified (Noma et al., 2002) (Figure 14.167).

14.4.2 Bicyclic Monoterpene Aldehyde

14.4.2.1 Myrtenal and Myrtanal



Euglena gracilis Z biotransformed (–)-myrtenal (**6′**) to give (–)-myrtenol (**5′**) as the major product and (–)-myetenoic acid (**7′**) as the minor product. However, further hydrogenation of (–)-myrtenol (**5′**) to *trans*- and *cis*-myrtanol (**8a** and **8b**) did not occur even at a concentration less than ca. 50 mg/L. (*S*)-*Trans* and (*R*)-*cis*-myrtanal (**435a′** and **435b′**) were also transformed to *trans*- and *cis*-myrtanol (**8a′** and **8b′**) as the major products and (*S*)-*trans*- and (*R*)-*cis*-myrtanoic acid (**436a′** and **436b′**) as the minor products, respectively (Noma et al., 1991a) (Figure 14.168).

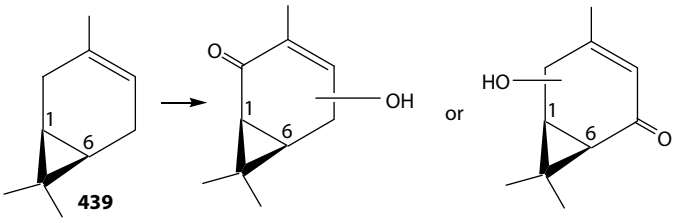


FIGURE 14.167 Metabolic pathways of (+)-3-Carene (**439**) by *Aspergillus niger* NC 1M612. (Modified from Noma, Y. et al., 2002. *Book of Abstracts of the 33rd ISEO*, p. 142.)

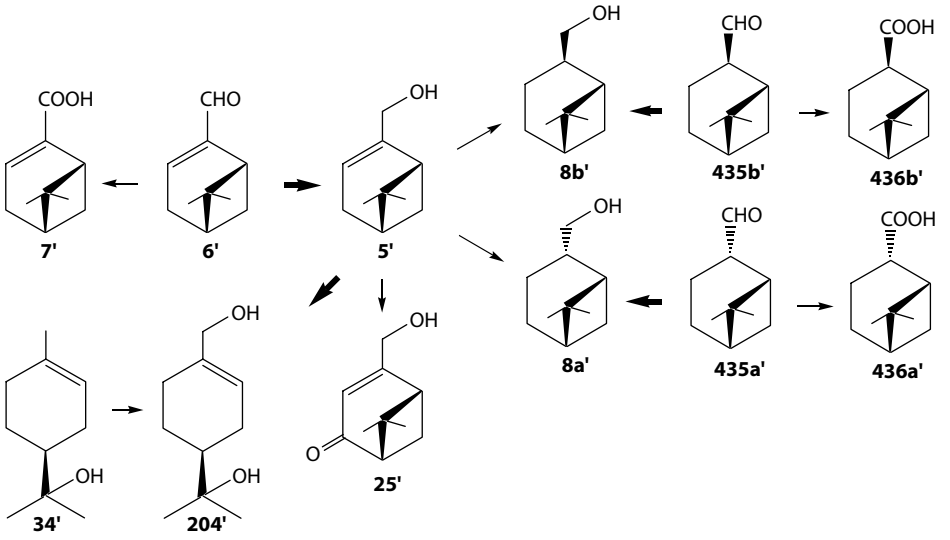


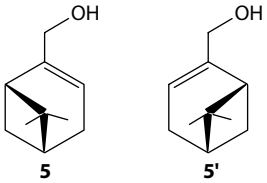
FIGURE 14.168 Biotransformation of (–)-myrtenal (**6′**) and (+)-*trans*- (**435a′**) and (–)-*cis*-myrtanal (**435b′**) by microorganisms. (Modified from Noma, Y. et al., 1991a. *Phytochem.*, 30: 1147–1151; Noma, Y. and Y. Asakawa, 2005b. *Proc. 49th TEAC*, pp. 78–80; Noma, Y. and Y. Asakawa, 2006b. *Book of Abstracts of the 37th ISEO*, p. 144.)

In case of *Aspergillus niger* TBUYN-2, *Aspergillus sojae*, and *Aspergillus usami*, (–)-myrtenol (**5′**) was further metabolized to 7-hydroxyverbenone (**25′**) as a minor product together with (–)-oleuropeyl alcohol (**204′**) as a major product (**279, 280**). (–)-Oleuropeyl alcohol (**204′**) is also formed from (–)-α-terpineol (**34**) by *Aspergillus niger* TBUYN-2 (Noma et al., 2001) (Figure 14.168).

Rabbits metabolized myrtenal (**6′**) to myrtenic acid (**7′**) as the major metabolite and myrtanol (**8a′** or **8b′**) as the minor metabolite (Ishida et al., 1981b) (Figure 14.168).

14.4.3 Bicyclic Monoterpene Alcohol

14.4.3.1 Myrtenol



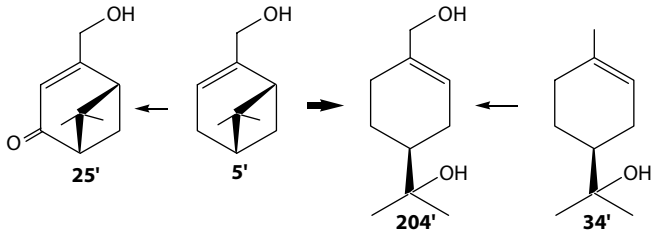


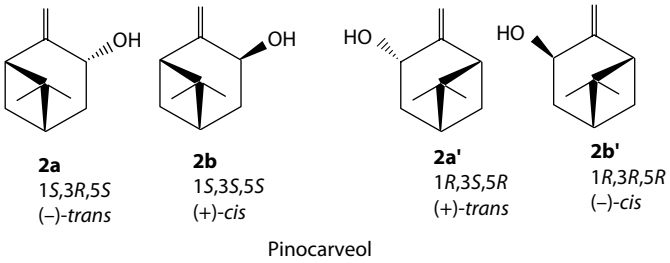
FIGURE 14.169 Biotransformation of (–)-myrtenol (**5′**) and (–)-α-terpineol (**34′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005b. *Proc. 49th TEAC*, pp. 78–80.)

(–)-Myrtenol (**5′**) was biotransformed mainly to (–)-oleuropeyl alcohol (**204′**), which was formed from (–)-α-terpineol (**34′**) as a major product by *Aspergillus niger*, TBUYN-2. In case of *Aspergillus sojae* IFO 4389 and *Aspergillus usami* IFO 4338, (–)-myrtenol (**5′**) was metabolized to 7-hydroxyverbenone (**25′**) as a minor product together with (–)-oleuropeyl alcohol (**204′**) as a major product (Noma and Asakawa, 2005b) (Figure 14.169).

14.4.3.2 Myrtanol

Spodoptera litura converted (–)-*trans*-myrtanol (**8a**) and its enantiomer (**8a′**) to give the corresponding myrtanic acid (**436** and **436′**) (Miyazawa et al., 1997b) (Figure 14.170).

14.4.3.3 Pinocarveol



(+)-*trans*-Pinocarveol (**2a′**) was biotransformed by *Aspergillus niger* TBUYN-2 to the following two pathways. Namely, (+)-*trans*-pinocarveol (**2a′**) was metabolized via (+)-pinocarvone (**3′**),

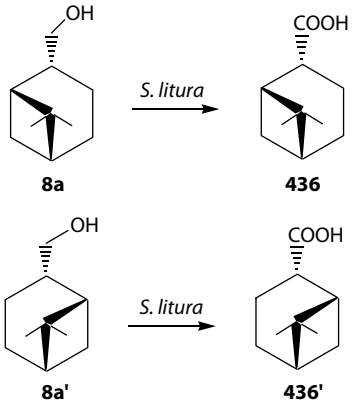
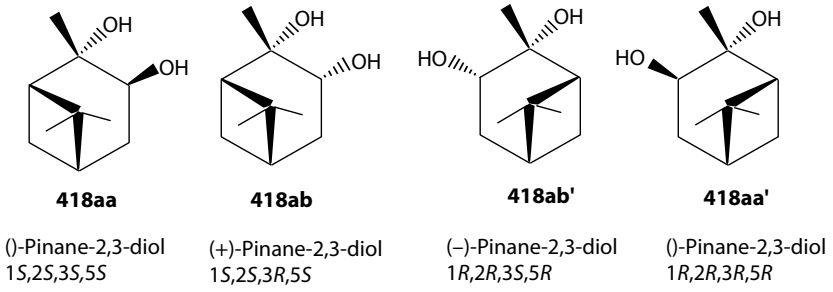


FIGURE 14.170 Biotransformation of (–)-*trans*-myrtanol (**8a**) and its enantiomer (**8a′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1997b. *Proc. 41st TEAC*, pp. 389–390.)

(–)-3-isopinanone (**413′**), and (+)-2α-hydroxy-3-pinanone (**414′**) to (+)-2α,5-dihydroxy-3-pinanone (**415′**) (pathway 1). Furthermore, (+)-*trans*-pinocarveol (**2a′**) was metabolized to epoxide followed by rearrangement reaction to give 6β-hydroxyfenchol (**349ba′**) and 6β,7-dihydroxyfenchol (**412ba′**) (Noma and Asakawa, 2005a) (Figure 14.171). *Spodoptera litura* converted (+)-*trans*-pinocarveol (**2a′**) to (+)-pinocarvone (**3′**) as a major product (Miyazawa et al., 1995c) (Figure 14.171).

14.4.3.4 Pinane-2,3-diol



This results led us to study the biotransformation of (–)-pinane-2,3-diol (**418ab′**) and (+)-pinane-2,3-diol (**418ab**) by *Aspergillus niger* TBUYN-2. (–)-Pinane-2,3-diol (**418ab′**) was easily biotransformed to give (–)-pinane-2,3,5-triol (**419ab′**) and (+)-2,5-dihydroxy-3-pinanone (**415a′**) as the major products and (+)-2-hydroxy-3-pinanone (**414a′**) as the minor product.

On the other hand, (+)-pinane-2,3-diol (**418ab**) was also biotransformed easily to give (+)-pinane-2,3,5-triol (**419ab**) and (–)-2,5-dihydroxy-3-pinanone (**415a**) as the major products and (–)-2-hydroxy-3-pinanone (**414a**) as the minor product (Noma et al., 2003) (Figure 14.172). *Glomerella cingulata* transformed (–)-pinane-2,3-diol (**418ab′**) to a small amount of (+)-2α-hydroxy-3-pinanone (**414ab′**, 5%) (Kamino and Miyazawa, 2005), whereas (+)-pinane-2,3-diol (**418ab**) was transformed to a small amount of (–)-2α-hydroxy-3-pinanone (**414ab**, 10%) and (–)-3-acetoxy-2α-pinanol (**433ab-Ac**, 30%) (Kamino et al., 2004) (Figure 14.172).

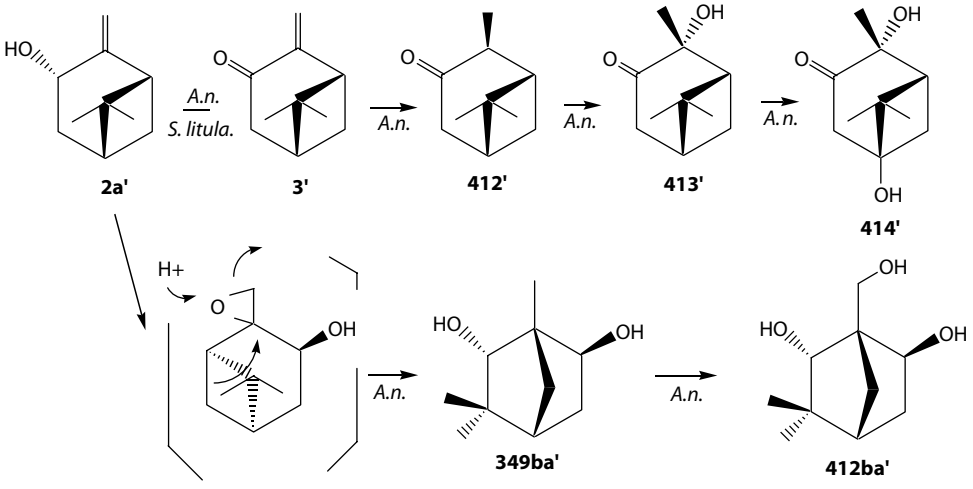


FIGURE 14.171 Biotransformation of (+)-*trans*-pinocarveol (**2a′**) by *Aspergillus niger* TBUYN-2 and *Spodptera litura*. (Modified from Miyazawa, M. et al., 1995c. *Proc. 39th TEAC*, pp. 360–361; Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

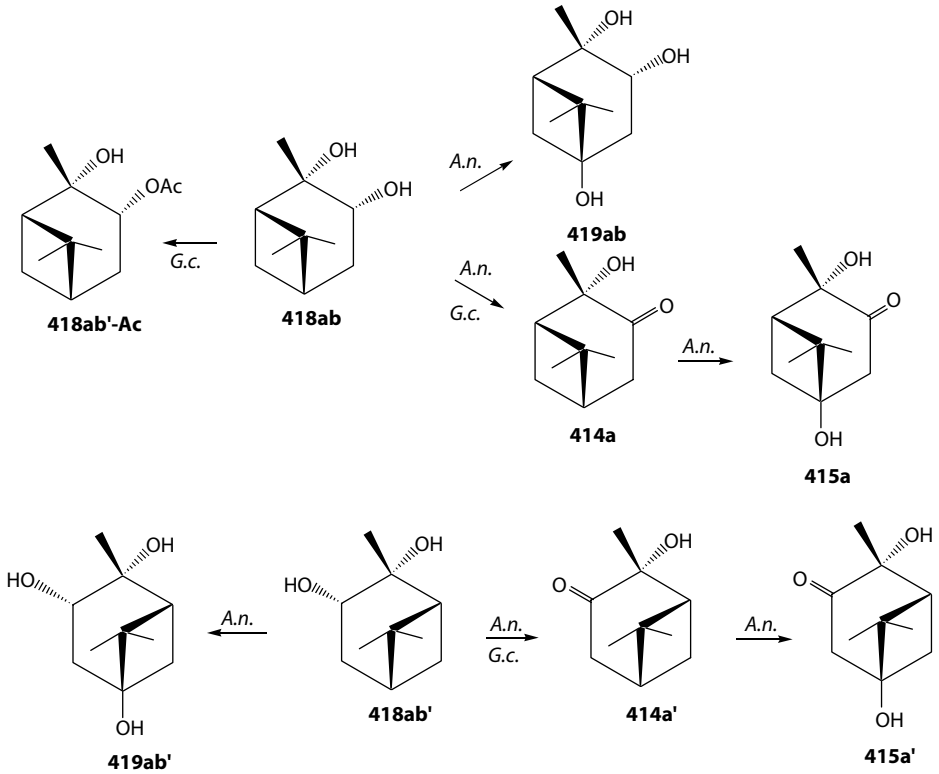
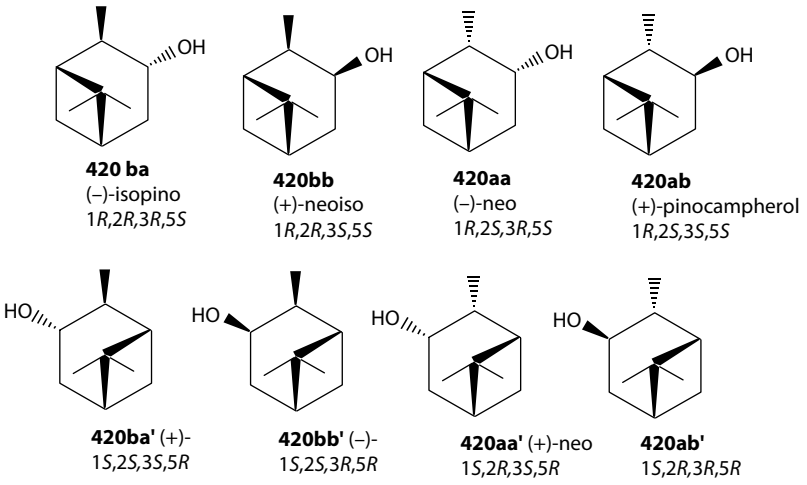


FIGURE 14.172 Biotransformation of (+)-pinane-2,3-diol (**418ab′**) and (–)-pinane-2,3-diol (**418ab′**) by *Aspergillus niger* TBUYN-2(**276**)] and *Glomerella cingulata*. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Kamino, F. et al., 2004. *Proc. 48th TEAC*, pp. 383–384; Kamino, F. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 395–396.)

14.4.3.5 Isopinocampheol (3-Pinanol)



*14.4.3.5.1 Chemical Structure of (–)-Isopinocampheol (**420ba**) and (+)-Isopinocampheol (**420ba′**)*

Biotransformation of isopinocampheol (3-pinanol) with 100 bacterial and fungal strains yielded 1-, 2-, 4-, 5-, 7-, 8-, and 9-hydroxyisopinocampheol besides three rearranged monoterpenes, one

of them bearing the novel isocarene skeleton. A pronounced enantioselectivity between (–)-(**420ba**) and (+)-isopinocampheol (**420ba′**) was observed. The phylogenetic position of the individual strains could be seen in their ability to form the products from (+)-isopinocampheol (**420ba′**). The formation of 1,3-dihydroxypinane (**421ba′**) is a domain of bacteria, while 3,5-(**415ba′**) or 3,6-dihydroxypinane (**428baa′**) was mainly formed by fungi, especially those of the phylum *Zygomycotina*. The activity of *Basidiomycotina* towards oxidation of isopinocampheol was rather low. Such informations can be used in a more effective selection of strains for screening (Wolf-Rainer, 1994) (Figure 14.173).

(+)-Isopinocampheol (**420ba′**) was metabolized to 4β-hydroxy-(+)-isopino-campheol (**424′**), 2β-hydroxy-(+)-isopinocampherol acetate (**425ba′-Ac**), and 2α-methyl,3-(2-methyl-2-hydroxypropyl)-cyclopenta-1β-ol (**432′**) (Wolf-Rainer, 1994) (Figure 14.174).

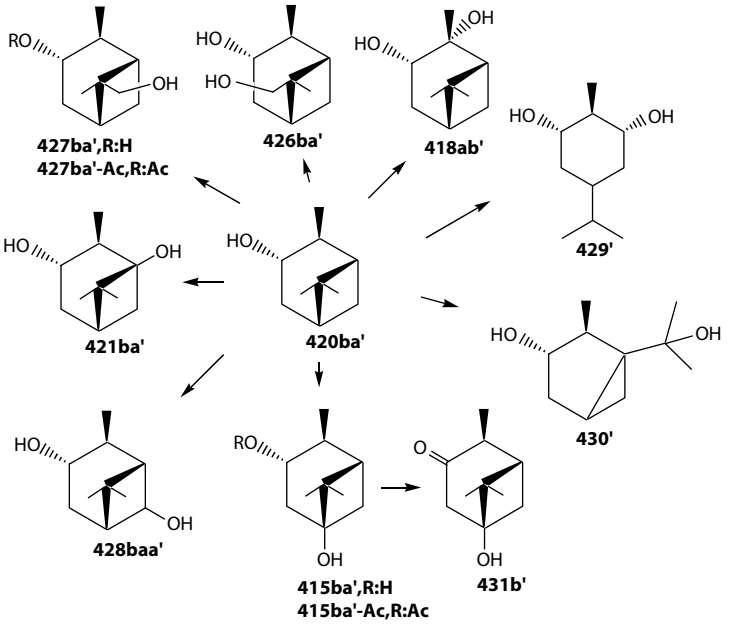


FIGURE 14.173 Metabolic pathways of (+)-isopinocampheol (**420ba′**) by microorganisms. (Modified from Wolf-Rainer, A., 1994. *Naturforsch*., 49c: 553–560.)

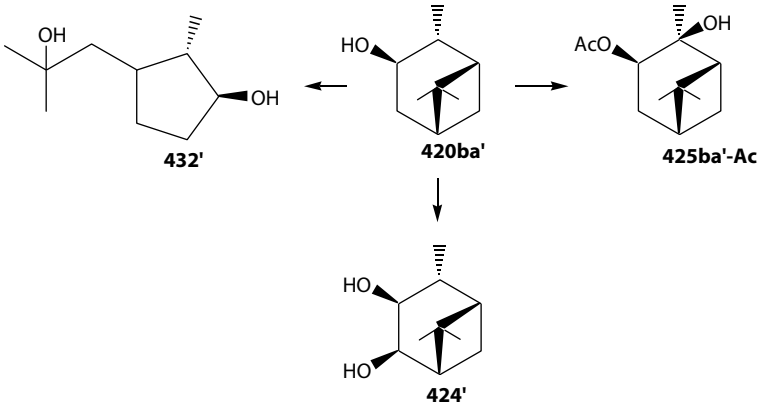


FIGURE 14.174 Metabolic pathways of (+)-isopinocampheol (**420ba′**) by microorganisms. (Modified from Wolf-Rainer, A., 1994. *Naturforsch*., 49c: 553–560.)

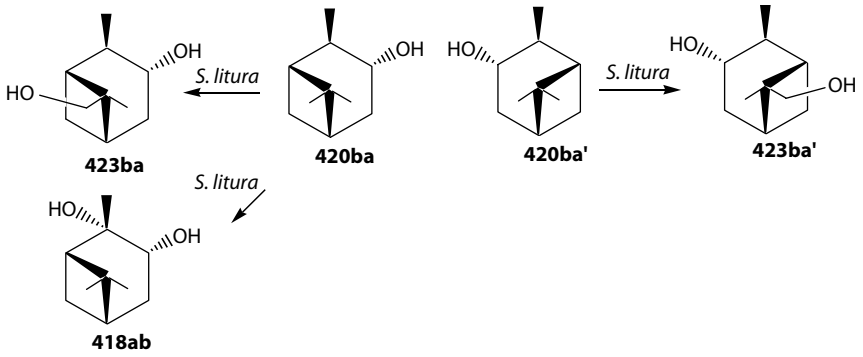


FIGURE 14.175 Biotransformation of (−)- (**420ba**) and (+)-isopinocampheol (**420ba′**) by *Spodptera litura*. (Modified from Miyazawa, M. et al., 1997c. *Phytochemistry*, 45: 945–950.)

(−)-Isopinocampheol (**420ba**) was converted by *Spodoptera litura* to give (1*R*,2*S*,3*R*,5*S*)-pinane-2,3-diol (**418ba**) and (−)-pinane-3,9-diol (**423ba**), whereas (+)-isopinocampheol (**420ba′**) was converted to (+)-pinane-3,9-diol (**423ba′**) (Miyazawa et al., 1997c) (Figure 14.175).

(−)-Isopinocampheol (**420ba**) was biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-(1*S*,2*S*,3*S*,5*R*)-pinane-3,5-diol (**422ba**, 6.6%), (−)-(1*R*,2*R*,3*R*,5*S*)-pinane-1,3-diol (**421ba**, 11.8%), and pinane-2,3-diol (**418ba**, 6.6%), whereas (+)-isopinocampheol (**420ba′**) was biotransformed by *Aspergillus niger* TBUYN-2 to give (+)-(1*S*,2*S*,3*S*,5*R*)-pinane-3,5-diol (**422ba′**, 6.3%) and (−)-(1*R*,2*R*,3*R*,5*S*)-pinane-1,3,-diol (**421ba′**, 8.6%) (Noma et al., 2009) (Figure 14.176). On the other hand, *Glomerella cingulata* converted (−)- (**420ba**) and (+)-isopinocampheol (**420ba′**) mainly to (1*R*,2*R*,3*S*,4*S*,5*R*)-3,4-pinanediol (**484ba**) and (1*S*,2*S*,3*S*,5*R*,6*R*)-3,6-pinanediol (**485ba′**), respectively, together with (**418ba**), (**422ba**), (**422ba′**), and (**486ba′**) as minor products (Miyazawa et al., 1997c) (Figure 14.176). Some similarities exist between the main metabolites with *Glomerella cingulata* and *Rhizoctonia solani* (Miyazawa et al., 1997c) (Figure 14.176).

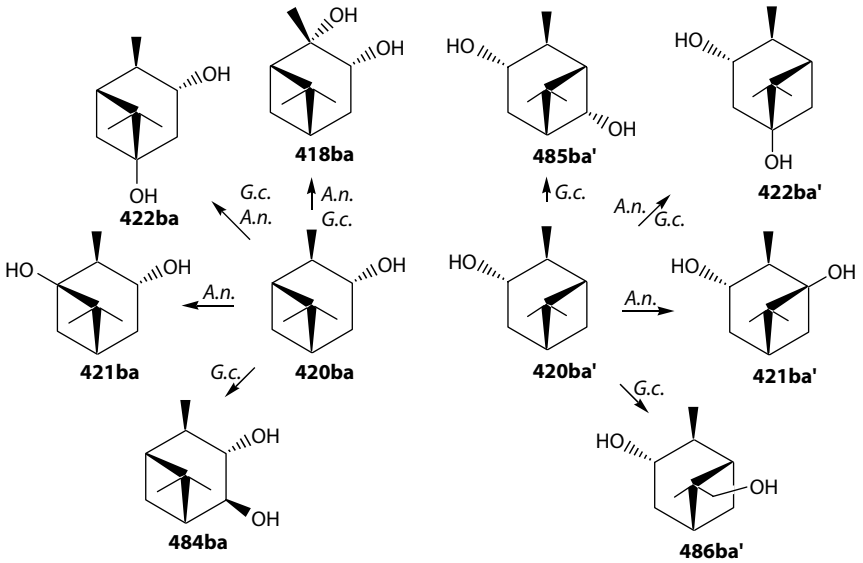
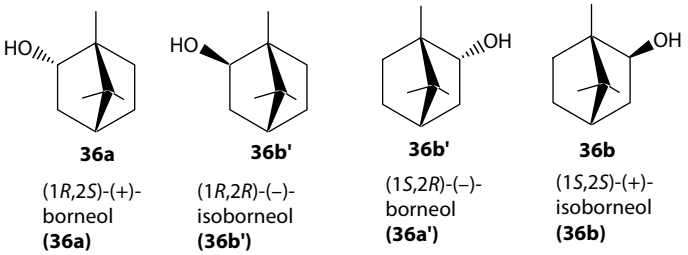


FIGURE 14.176 Biotransformation of (−)- (**420ba**) and (+)-isopinocampheol (**420ba′**) by *Aspergillus niger* TBUYN-2 and *Glomerella cingulata*. (Modified from Miyazawa, M. et al., 1997c. *Phytochemistry*, 45: 945–950; Noma, Y. et al., 2009. unpublished data.)

14.4.3.6 Borneol and Isoborneol



(–)-Borneol (**36a′**) was biotransformed by *Pseudomonas pseudomonallei* strain H to give (–)-camphor (**37′**), 6-hydroxycamphor (**228′**), and 2,6-diketocamphor (**229′**) (Hayashi et al., 1969) (Figure 14.177).

Euglena gracilis Z. showed enantio- and diastereoselectivity in the biotransformation of (+)- (**36a**), (–)- (**36a′**), and (±)-racemic borneols (equal mixture of **36a** and **36a′**) and (+)- (**36b**), (–)- (**36b′**), and (±)-isoborneols (equal mixture of **36b** and **36b′**). The enantio- and diastereoselective dehydrogenation for (–)-borneol (**36a′**) was carried out to give (–)-camphor (**37′**) at *ca.* 50% yield (Noma et al., 1992d; Noma and Asakawa, 1998). The conversion ratio was always *ca.* 50% even at different kinds of concentration of (–)-borneol (**36a′**). When (–)-camphor (**37′**) was used as a substrate, it was also converted to (–)-borneol (**36a′**) in 22% yield for 14 days. Furthermore, (+)-camphor (**37**) was also reduced to (+)-borneol (**36a**) in 4% and 18% yield for 7 and 14 days, respectively (Noma et al., 1992d, Noma and Asakawa, 1998) (Figure 14.178).

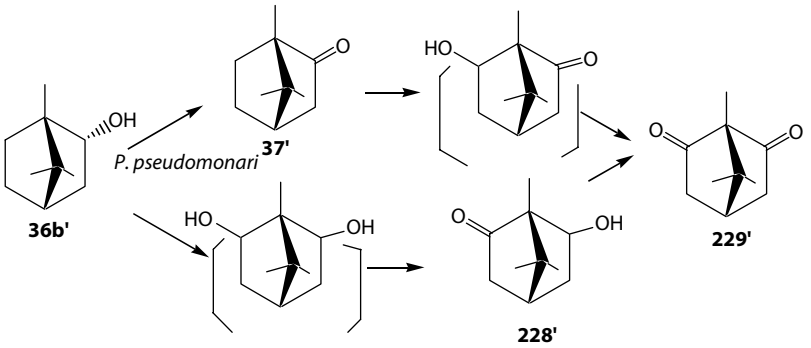


FIGURE 14.177 Biotransformation of (–)-borneol (**36a′**) by *Pseudomonas pseudomonallei* strain. (Modified from Hayashi, T. et al., 1969. *J. Agric. Chem. Soc. Jpn*., 43: 583–587.)

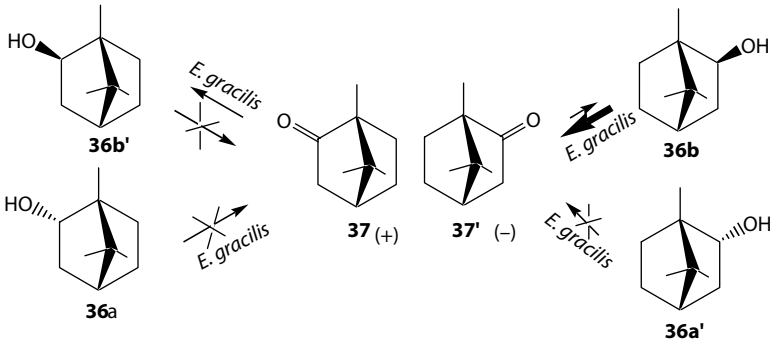


FIGURE 14.178 Enantio- and diastereoselectivity in the biotransformation of (+)- (**36a**) and (–)-borneols (**36a′**) by *Euglena gracilis* Z. (Modified from Noma, Y. et al., 1992d. *Proc. 36th TEAC*, pp. 199–201; Noma, Y. and Y. Asakawa, 1998. *Biotechnology in Agriculture and Forestry, Vol. 41. Medicinal and Aromatic Plants X*, Y.P.S. Bajaj, ed., pp. 194–237. Berlin Heidelberg: Springer.)

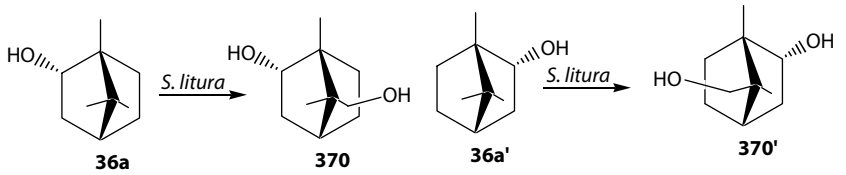
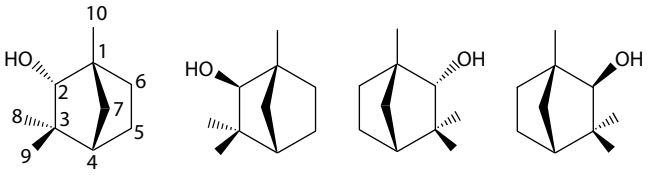


FIGURE 14.179 Biotransformation of (+)- (**36a**) and (–)-borneols (**36a′**) by *Spodptera litura*. (Modified from Miyamoto, Y. and M. Miyazawa, 2001. *Proc. 45th TEAC*, pp. 377–378.)

(+)- (**36a**) and (–)-Borneols (**36a′**) were biotransformed by *Spodoptera litura* to (+)- (**370a**) and (–)-bornane-2,8-diols (**370a′**), respectively (Miyamoto and Miyazawa, 2001) (Figure 14.179).

14.4.3.7 Fenchol and Fenchyl Acetate



(1*R*,2*R*,4*S*)-(+)-Fenchol (**11a**) was converted by *Aspergillus niger* TBUYN-2 and *Aspergillus cellulosae* IFO 4040 to give (–)-fenchone (**12**), (+)-6β-hydroxyfenchol (**349ab**), (+)-5β-hydroxyfenchol (**350ab**) and 5α-hydroxyfenchol (**350aa**) (Noma and Asakawa, 2005a) (Figure 14.180).

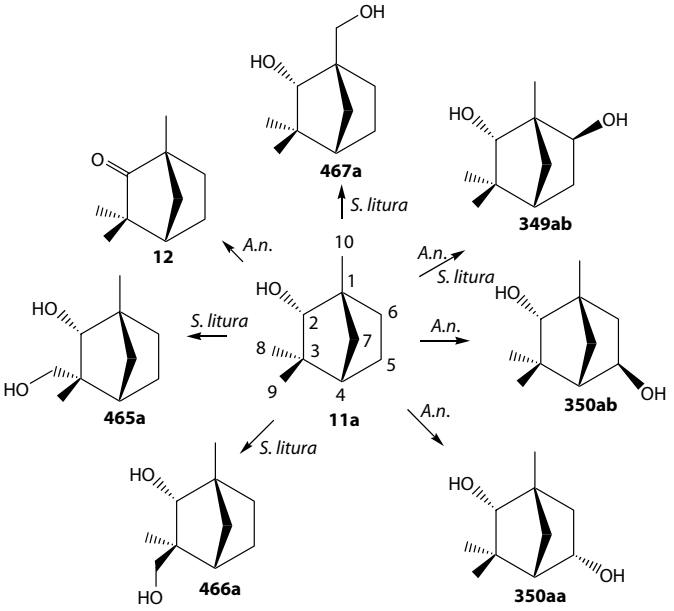


FIGURE 14.180 Biotransformation of (+)-fenchol (**11a**) by *Aspergillus niger* TBUYN-2, *Aspergillus cellulosae* IFO 4040, and the larvae of common cutworm, *Spodptera litura*. (Modified from Miyazawa, M. and Y. Miyamoto, 2004. *Tetrahadron*, 60: 3091–3096; Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

The larvae of common cutworm, *Spodoptera litura*, converted (+)-fenchol (**11a**) to (+)-10-hydroxyfenchol (**467a**), (+)-8-hydroxyfenchol (**465a**), (+)-6β-hydroxyfenchol (**349ab**), and (−)-9-hydroxyfenchol (**466a**) (Miyazawa and Miyamoto, 2004) (Figure 14.180).

(+)-*trans*-Pinocarveol (**2**), which was formed from (−)-β-pinene (**1**), was metabolized by *Aspergillus niger* TBUYN-2 to 6β-hydroxy- (+)-fenchol (**349ab**) and 6β,7-dihydroxy-(+)-fenchol (**412ba′**). (−)-Fenchone (**12**) was also metabolized to 6α-hydroxy- (**13b**) and 6β-hydroxy- (−)-fenchone (**13a**). (+)-Fenchol (**11**) was metabolized to 6β-hydroxy-(+)-fenchol (**349ab**) by *Aspergillus niger* TBUYN-2. Relationship of the metabolisms of (+)-*trans*-pinocarveol (**2**), (−)-fenchone (**12**), and (+)-fenchol (**11**) by *Aspergillus niger* TBUYN-2 is shown in Figure 14.181 (Noma and Asakawa 2005a).

(+)-α-Fencyl acetate (**11a-Ac**) was metabolized by *Glomerella cingulata* to give (+)-5-β-hydroxy-α-fencyl acetate (**350a-Ac**, 50%) as the major metabolite and (+)-fenchol (**11a**, 20%) as the minor metabolite (Miyazato and Miyazawa 1999). On the other hand, (−)-α-fencyl acetate (**11a′-Ac**) was metabolized to (−)-5-β-hydroxy-α-fencyl acetate (**350a′-Ac**, 70%) and (−)-fenchol (**11a′**, 10%) as the minor metabolite by *Glomerella cingulata* (Miyazato and Miyazawa, 1999) (Figure 14.182).

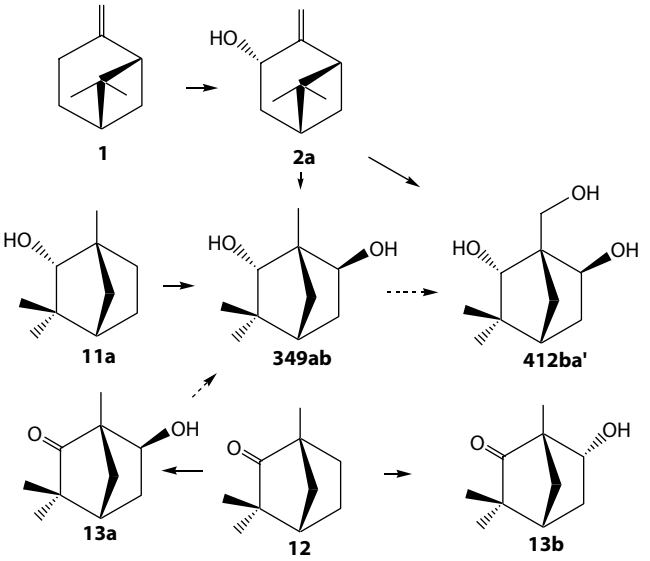


FIGURE 14.181 Metabolism of (+)-*trans*-pinocarveol (**2**), (−)-fenchone (**12**), and (+)-fenchol (**11**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

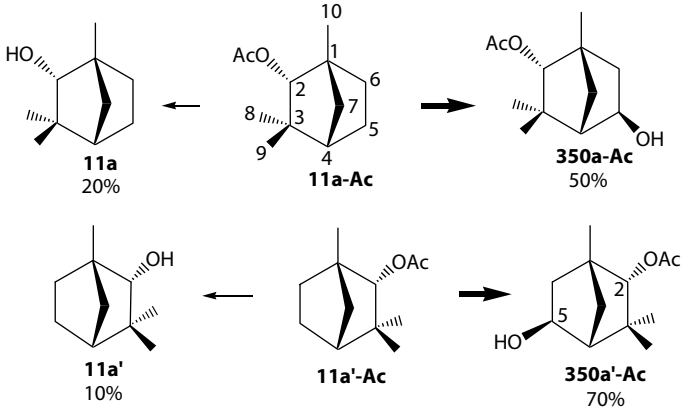
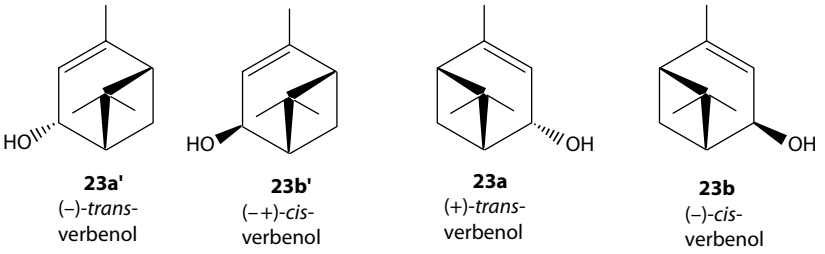


FIGURE 14.182 Biotransformation of (+)- (**11a-Ac**) and (−)-α-fencyl acetate (**11a′-Ac**) by *Glomerella cingulata*. (Modified from Miyazato, Y. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 213–214.)

14.4.3.8 Verbenol



(–)-*trans*-Verbenol (**23a′**) was biotransformed by *Spodoptera litura* to give 10-hydroxyverbenol (**451a′**). Furthermore, (–)-verbenone (**24′**) was also biotransformed in the same manner to give 10-hydroxyverbenone (**25′**) (Yamanaka and Miyazawa, 1999) (Figure 14.183).

14.4.3.9 Nopol and Nopol Benzyl Ether

Biotransformation of (–)-nopol (**452′**) was carried out at 30°C for 7 days at the concentration of 100 mg/200 mL medium by *Aspergillusniger* TBUYN-2, *Aspergillus sojae* IFO 4389, and *Aspergillus usami* IFO 4338. (–)-Nopol (**452′**) was incubated with *Aspergillus niger* TBUYN-2 to give 7-hydroxymethyl-1-*p*-menthen-8-ol (**453′**). In cases of *Aspergillus sojae* IFO 4389 and *Aspergillus usami* IFO 4338, (–)-nopol (**452′**) was metabolized to 3-oxonopol (**454′**) as a minor product together with 7-hydroxymethyl-1-*p*-menthen-8-ol (**453′**) as a major product (Noma and Asakawa, 2005b; 2006c) (Figure 14.184).

Biotransformation of (–)-nopol benzyl ether (**455′**) was carried out at 30°C for 8–13 days at the concentration of 277 mg/200 mL medium by *Aspergillus niger* TBUYN-2, *Aspergillus sojae* IFO 4389, and *Aspergillus usami* IFO 4338. (–)-Nopol benzyl ether (**455′**) was biotransformed by *Aspergillus niger* TBUYN-2 to give 4-oxonopl-2′, 4′-dihydroxy benzyl ether (**456′**), and (–)-oxonopol (**454′**). 7-Hydroxymethyl-1-*p*-menthen-8-ol benzyl ether (**457′**) was not formed at all (Figure 14.185).

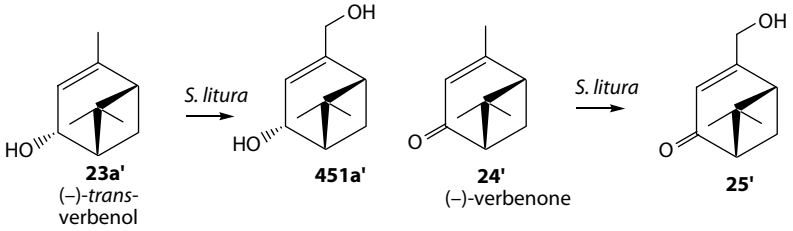


FIGURE 14.183 Metabolism of (–)-*trans*-verbenol (**23a′**) and (–)-verbenone (**24′**) by *Spodptera litura*. (Modified from Yamanaka, T. and M. Miyazawa, 1999. *Proc. 43rd TEAC*, pp. 391–392.)

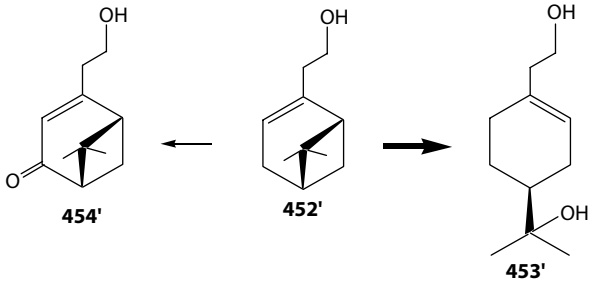


FIGURE 14.184 Biotransformation of (–)-nopol (**452′**) by *Aspergillus niger,* TBUYN-2, *Aspergillus sojae* IFO 4389 and *Aspergillus usami* IFO 4338. (Modified from Noma, Y. and Y. Asakawa, 2005b. *Proc. 49th TEAC*, pp. 78–80; Noma, Y. and Y. Asakawa, 2006c. *Proc. 50th TEAC*, pp. 434–436.)

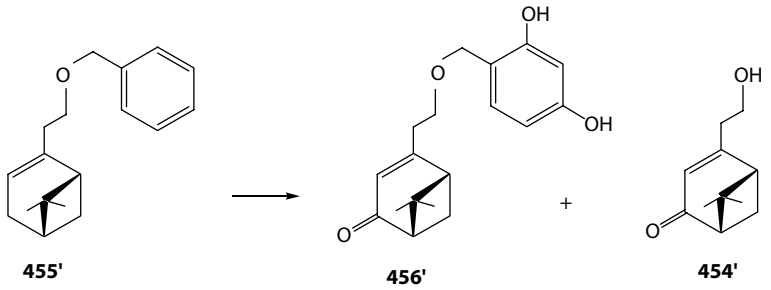


FIGURE 14.185 Biotransformation of (–)-Nopol benzyl ether (**455′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2006b. *Book of Abstracts of the 37th ISEO*, p. 144; Noma, Y. and Y. Asakawa, 2006c. *Proc. 50th TEAC*, pp. 434–436.)

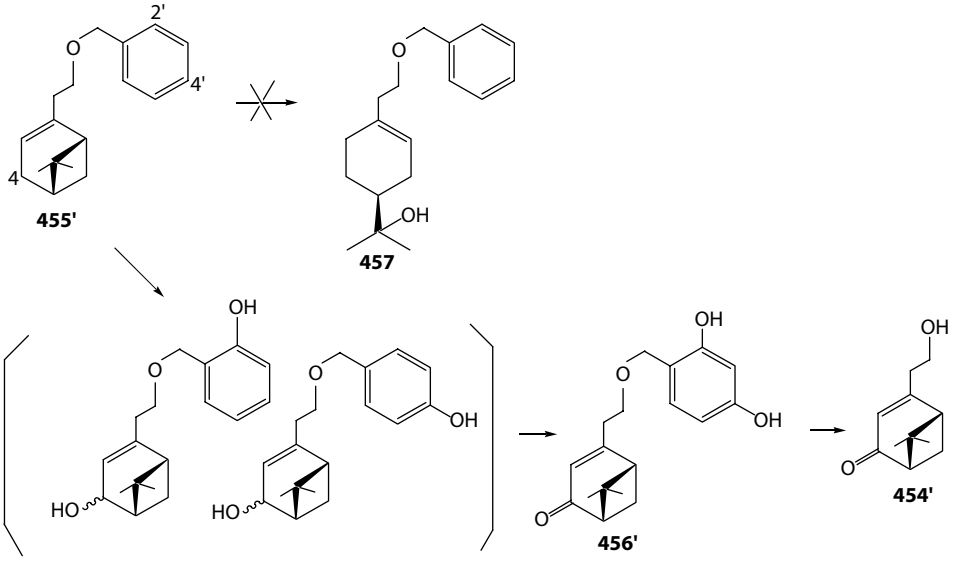


FIGURE 14.186 Proposed metabolic pathways of (–)-nopol benzyl ether (**455′**) by microorganisms. (Modified from Noma, Y. and Y. Asakawa, 2006b. *Book of Abstracts of the 37th ISEO*, p. 144; Noma, Y. and Y. Asakawa, 2006c. *Proc. 50th TEAC*, pp. 434–436.)

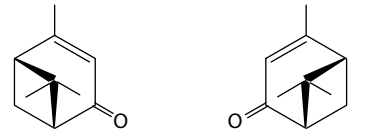
4-Oxonopol-2',4'-dihydroxybenzyl ether (**456′**) shows strong antioxidative activity (IC_{50} 30.23 μM). Antioxidative activity of 4-oxonopol-2',4'-dihydroxybenzyl ether (**456′**) is the same as that of butyl hydroxyl anisol (BHA) (Noma and Asakawa, 2006b,c).

Citrus pathogenic fungi, *Aspergillus niger* Tiegh (CBAYN) also transformed (–)-nopol (**452′**) to (–)-oxonopol (**454′**) and 4-oxonopol-2',4'-dihydroxybenzyl ether (**456′**) (Noma and Asakawa, 2006b,c) (Figure 14.186).

14.4.4 Bicyclic Monoterpene Ketones

14.4.4.1 α-, β-Unsaturated Ketone

14.4.4.1.1 Verbenone



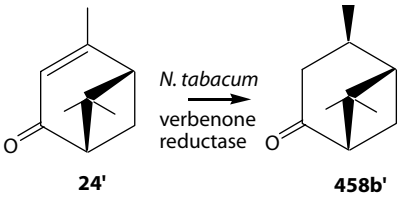


FIGURE 14.187 Hydrogenation of (–)-verbenone (**24′**) to (–)-isoverbanone (**458b′**) by verbenone reductase of *Nicotiana tabacum*. (Modified from Suga, T. and T. Hirata, 1990. *Phytochemistry*, 29: 2393–2406; Shimoda, K. et al., 1996. *J. Chem. Soc., Perkin Trans.* 1, 355–358; Shimoda, K. et al., 1998. *Phytochem.*, 49: 49–53; Shimoda, K. et al., 2002. *Bull. Chem. Soc. Jpn*., 75: 813–816; Hirata, T. et al., 2000. *Chem. Lett*., 29: 850–851.)

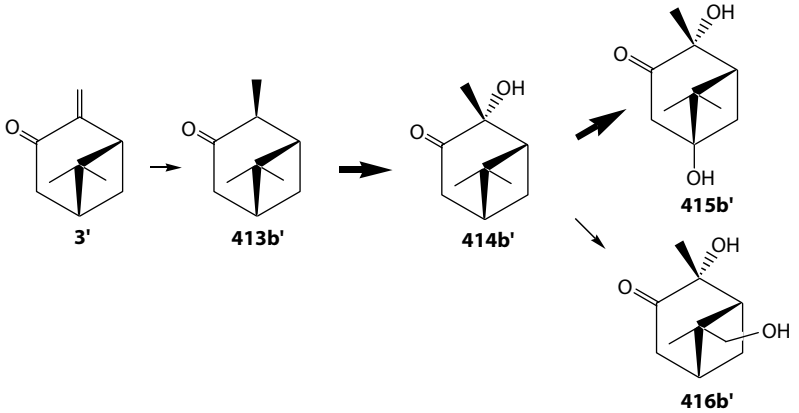
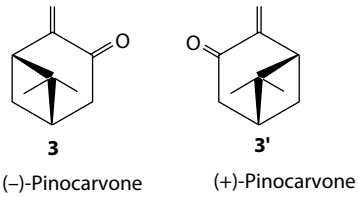


FIGURE 14.188 Biotransformation of (+)-pinocarvone (**3′**) by *Aspergillusniger* TBUYN-2. (Modified from Noma, Y. and Y. Asakawa, 2005a. *Book of Abstracts of the 36th ISEO*, p. 32.)

(–)-Verbenone (**24′**) was hydrogenated by reductase of *Nicotiana tabacum* to give (–)-isoverbanone (**458b′**) (Suga and Hirata, 1990; Shimoda et al., 1996, 1998, 2002; Hirata et al., 2000) (Figure 14.187).

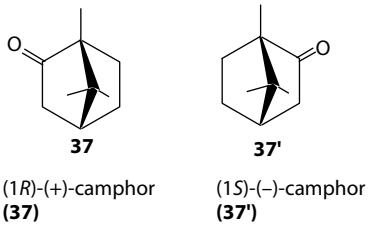
14.4.4.1.2 Pinocarvone



Aspergillus niger TBUYN-2 transformed (+)-pinocarvone (**3′**) to give (–)-isopinocamphone (**413b′**), 2α-hydroxy-3-pinanone (**414b′**), 2α, 5-dihydroxy-3-pinanone (**415b′**) together with small amounts of 2α, 10-dihydroxy-3-pinanone (**416b′**) (Noma and Asakawa, 2005a) (Figure 14.188).

14.4.4.2 Saturated Ketone

14.4.4.2.1 Camphor



(+)- (**37**) and (–)-Camphor (**37′**) are found widely in nature, of which (+)-camphor (**37**) is more abundant. It is the main component of oils obtained from the camphor tree *Cinnamomum camphora* (Bauer et al., 1990). The hydroxylation of (+)-camphor (**37**) by *Pseudomonas putida* C_1 was described (Abraham et al., 1988). The substrate was hydroxylated exclusively in its 5-exo- (**235b**) and 6-exo- (**228b**) positions.

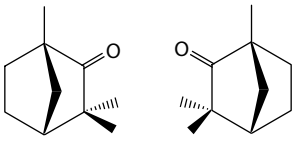
Although only limited success was achieved in understanding the catabolic pathways of (+)-camphor (**37**), key roles for methylene group hydroxylation and biological Baeyer–Villiger monooxygenases in ring cleavage strategies were established (Trudgill, 1990). A degradation pathway of (+)-camphor (**37**) by *Pseudomonas putida* ATCC 17453 and *Mycobacaterium rhodochorus* T_1 was proposed (Trudgill, 1990).

The metabolic pathway of (+)-camphor (**37**) by microorganisms is shown in Figure 14.189. (+)-Camphor (**37**) is metabolized to 3-hydroxy- (**243**), 5-hydroxy- (**235**), 6-hydroxy- (**228**), and 9-hydroxycamphor (**225**) and 1,2- campholide (**237**). 6-Hydroxycamphor (**228**) is degradatively metabolized to 6-oxocamphor (**229**) and 4-carboxymethyl-2,3,3-trimethylcyclopentanone (**230**), 4-carboxymethyl-3,5,5-trimethyltetrahydro-2-pyrone (**231**), isohydroxy-camphoric acid (**232**), isoketocamphoric acid (**233**), and 3,4,4-trimethyl-5-oxo-*trans*-2-hexenoic acid (**234**), whereas 1,2-campholide (**237**) is also degradatively metabolized to 6-hydroxy-1,2-campholide (**238**), 6-oxo-1, 2-campholide (**239**), and 5-carboxymethyl-3,4,4-trimethyl-2-cyclopentenone (**240**), 6-carboxymethyl-4,5,5-trimethyl-5,6-dihydro-2-pyrone (**241**) and 5-carboxymethyl-3,4,4-trimethyl-2-heptene-1,7-dioic acid (**242**). 5-Hydroxycamphor (**235**) is metabolized to 6-hydroxy-1,2-campholide (**238**), 5-oxo-camphor (**236**), and 6-oxo-1,2-campholide (**239**). 3-Hydroxycamphor (**243**) is also metabolized to camphorquinone (**244**) and 2-hydroxyepicamphor (**245**) (Bradshaw et al., 1959; Conrad et al., 1961, 1965a, 1965b; Gunsalus et al., 1965; Chapman et al., 1966; Hartline and Gunsalus, 1971; Oritani and Yamashita, 1974) (Figure 14.189).

Human CYP 2A6 converted (+)-camphor (**37**) and (–)-camphor (**37′**) to 6-*endo*-hydroxycamphor (**228a**) and 5-*exo*-hydroxycamphor (**235b**), while rat CYP 2B1 did 5-*endo*- (**235a**), 5-*exo*- (**235b**) and 6-*endo*-hydroxycamphor (**228a**) and 8-hydroxycamphor (**225**) (Gyoubu and Miyazawa 2006) (Figure 14.190).

(+)-Camphor (**37**) was glycosylated by *Eucalyptus perriniana* suspension cells to (+)-camphor monoglycoside (3 new, 11.7%) (Hamada et al., 2002) (Figure 14.191).

14.4.4.2.2 Fenchone



12 (+)- **12'** (–)-

(+)-Fenchone (**12**) was incubated with *Corynebacterium* sp. (Chapman et al., 1965) and *Absidia orchidis* (Pfrunder and Tamm, 1969a) give 6β-hydroxy- (**13a**) and 5β-hydroxyfenchones (**14a**) (Figure 14.191). On the other hand, *Aspergillus niger* biotransformed (+)-fenchone (**12**) to (+)-6α-(**13b**) and (+)-5α-hydroxyfenchones (**14b**) (Miyazawa et al., 1990a, 1990b) and 5-oxofenchone (**15**), 9-formylfenchone (**17b**), and 9-carboxyfenchone (**18b**) (Miyazawa et al., 1990a, 1990b) (Figure 14.192).

Furthermore, *Aspergillus niger* biotransformed (–)-fenchone (**12′**) to 5α-hydroxy- (**14b′**) and 6α-hydroxyfenchones (**13b′**) (Yamamoto et al., 1984) (Figure 14.193).

(+)- and (–)-Fenchone (**12** and **12′**) were converted to 6β-hydroxy- (**13a**, **13a′**), 6α-hydroxyfenchone (**13b**, **13b′**), and 10 hydroxyfenchone (**4**, **4′**) by P-450. Of the 11 recombinant human P450 enzymes tested, CYP2A6, CYP2B6 catalyzed oxidation of (+)- (**12**) and (–)-fenchone (**12′**) (Gyoubu and Miyazawa, 2005) (Figure 14.194).

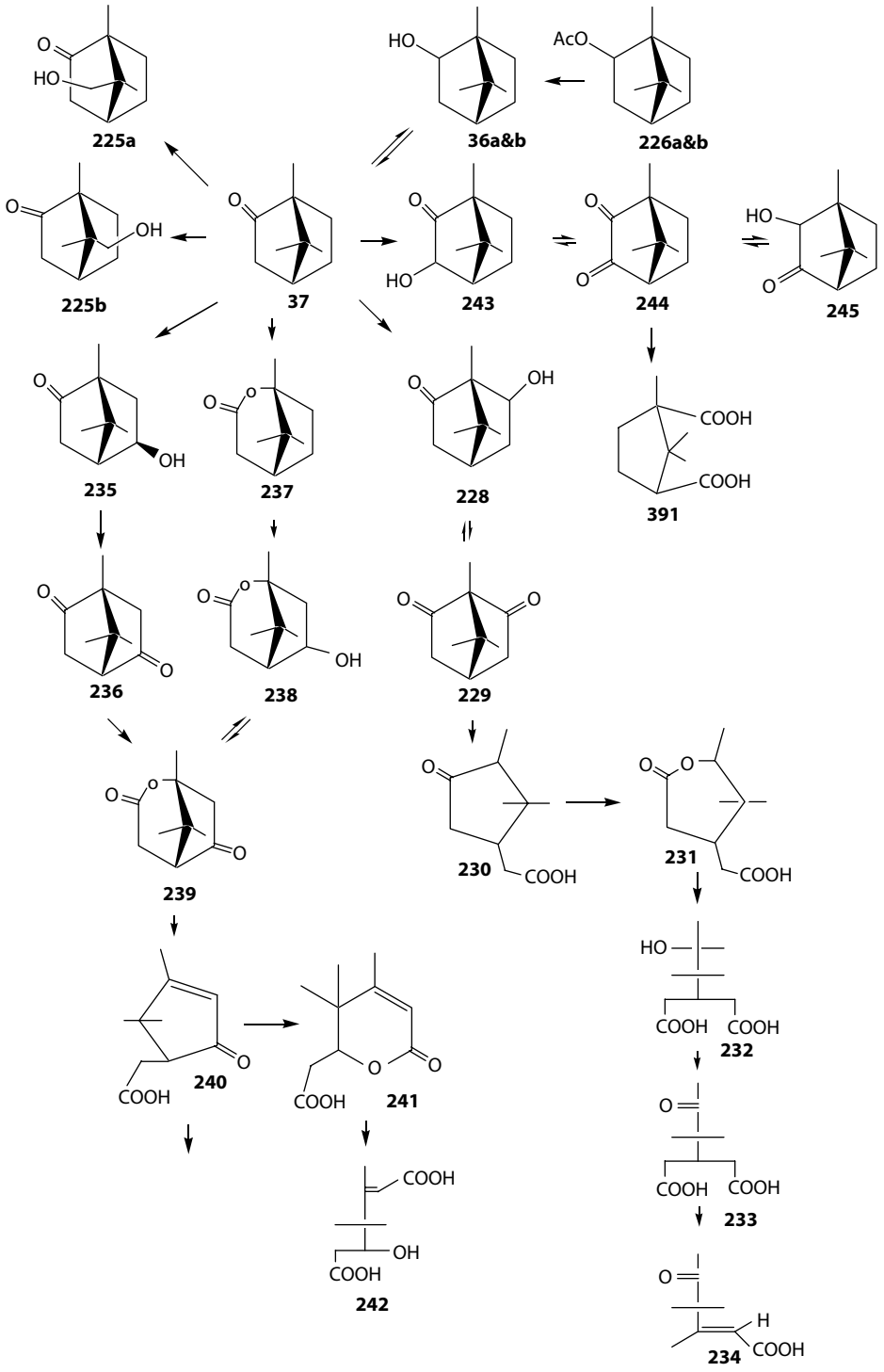


FIGURE 14.189 Metabolic pathways of (+)-camphor (**37**) by *Pseudomonas putida* and *Corynebacterium diphtheroides*. (Modified from Bradshaw, W.H. et al., 1959. *J. Am. Chem. Soc*., 81: 5507; Conrad, H.E. et al., 1961. *Biochem. Biophys. Res. Commun*., 6: 293–297; Conrad, H.E. et al., 1965a. *J. Biol. Chem*., 240: 495–503; Conrad, H.E. et al., 1965b. *J. Biol. Chem*., 240: 4029–4037; Gunsalus, I.C. et al., 1965. *Biochem. Biophys. Res. Commun*., 18: 924–931; Chapman, P.J. et al., 1966. *J. Am. Chem. Soc*., 88: 618–619; Hartline, R.A. and I.C. Gunsalus, 1971. *J. Bacteriol*., 106: 468–478; Oritani, T. and K. Yamashita, 1974. *Agric. Biol. Chem*., 38: 1961–1964.)

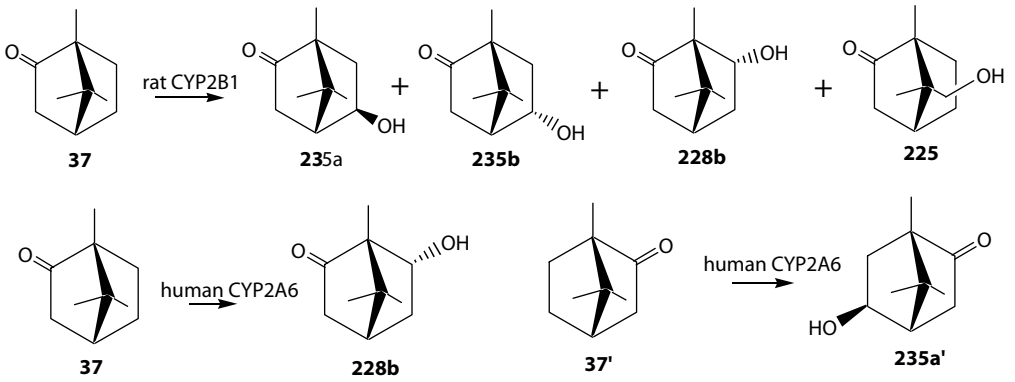


FIGURE 14.190 Biotransformation of (+)-camphor (**37**) by rat P450 enzyme (above) and (+)- (**37**) and (−)-camphor (**37′**) by human P450 enzymes.

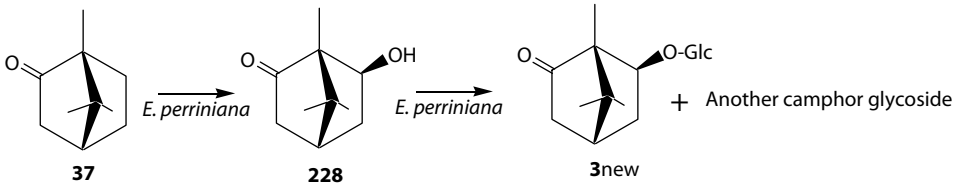


FIGURE 14.191 Biotransformation of (+)-camphor (**37**) by *Eucalyptus perriniana* suspension cell.

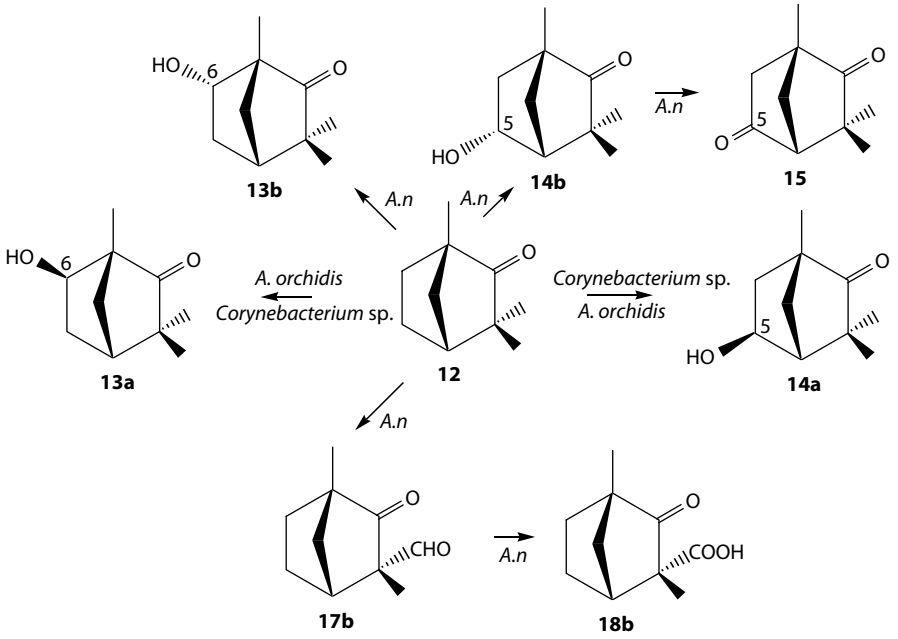


FIGURE 14.192 Metabolic pathways of (+)-fenchone (**12**) by *Corynebacterium* sp., *A. orchidis* and *Aspergillus niger* TBUYN-2. (Modified from Chapman, P.J. et al., 1965. *Biochem. Biophys. Res. Commun*., 20: 104–108; Pfrunder, B. and Ch. Tamm, 1969a. *Helv. Chim. Acta*., 52: 1643–1654; Miyazawa, M. et al., 1990a. *Chem. Express*, 5: 237–240; Miyazawa, M. et al., 1990b. *Chem. Express*, 5: 407–410.)

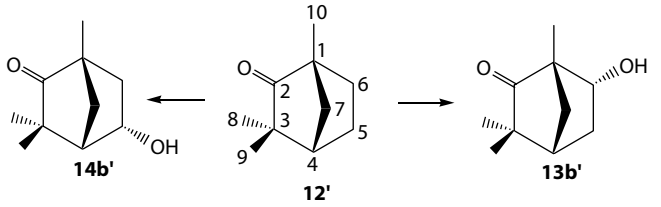


FIGURE 14.193 Metabolic pathways of (–)-fenchone (**12′**) by *Aspergillus niger* TBUYN-2. (Modified from Yamamoto, K. et al., 1984. *Proc. 28th TEAC*, pp. 168–170.)

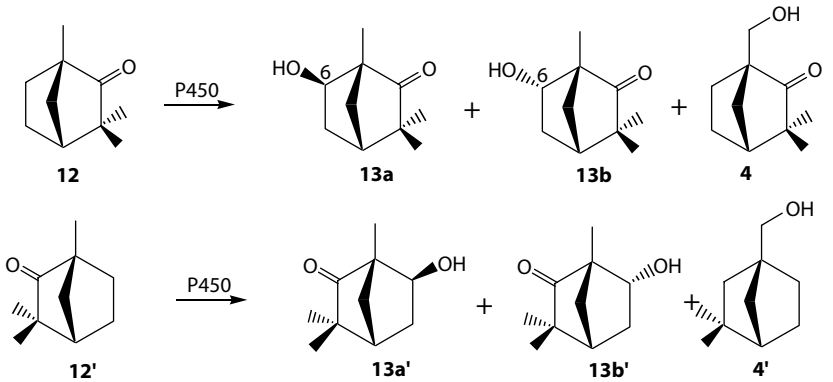
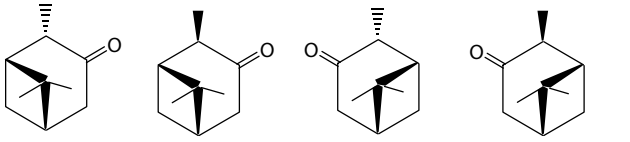


FIGURE 14.194 Biotransformation of (+)-fenchone (**12**) and (–)-fenchone (**12′**) by P-450 enzymes. (Modified from Gyoubu, K. and M. Miyazawa, 2005. *Proc. 49th TEAC*, pp. 420–422.)

14.4.4.2.3 3-Pinanone (Pinocamphone and Isopinocamphone)



413a 1*R*2*S*5*S* (+)-pino-camphone

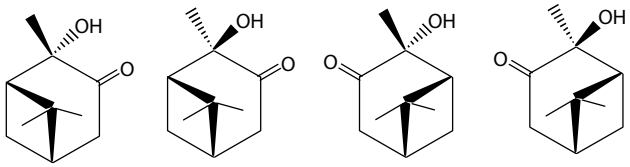
413b 1*R*,2*R*,5*S* (+)-isopino-camphone

413a' 1*S*2*R*5*R* (–)-pino-camphone

413b' 1*S*2*S*5*R* (–)-isopino-camphone

(+)- (**413**) and (–)-Isopinocamphone (**413′**) were biotransformed by *Aspergillus niger* to give (–)-(**414**) and (+)-2-hydroxy-3-pinanone (**414′**) as the main products, respectively, which inhibit strongly germination of lettuce seeds, and (–)- (**415**) and (+)-2,5-dihydroxy-3-pinanone (**415′**) as the minor components, respectively (Noma et al., 2003, 2004) (Figure 14.195).

14.4.4.2.4 2-Hydroxy-3-Pinanone



(1*S*,2*R*,5*S*) (–)-2-OH-3-pinanone **414a**

(1*S*,2*R*,5*S*) (+)-2-OH-3-pinanone **414b**

(1*R*,2*R*,5*R*) (+)-2-OH-3-pinanone **414a'**

(1*R*,2*S*,5*R*) (+)-2-OH-3-pinanone **414b'**

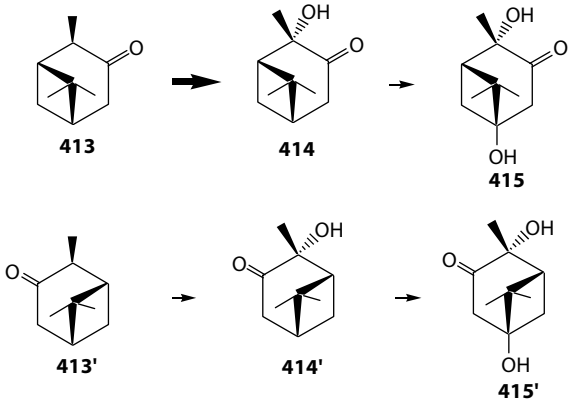


FIGURE 14.195 Biotransformation of (+)-isopinocamphone (**413b**) and (−)-isopinocamphone (**413b′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

(−)-2α-Hydroxy-3-pinanone (**414**) was incubated with *Aspergillus niger* TBUYN-2 to give (−)-2α, 5-dihydroxy-3-pinanone (**415**) predominantly, whereas the fungus converted (+)-2α-hydroxy-3-pinanone (**414′**) mainly to 2α, 5-dihydroxy-3-pinanone (**415′**), 2α,9-dihydroxy-3-pinanone (**416′**), and (−)-pinane-2α,3α,5-triol (**419ba′**) (Noma et al., 2003, 2004) (Figure 14.196).

Aspergillus niger TBUYN-2 metabolized β-pinene (**1**), isopinocamphone (**414b**), 2α-hydroxy-3-pinanone (**414a**), and pinane-2,3-diol (**419ab**) as shown in Figure 14.197. On the other hand, *Aspergillus niger* TBUYN-2 and *Botrytis cinerea* metabolized β-pinene (**1′**), isopinocamphone (**414b′**), 2α-hydroxy-3-pinanone (**414a′**), and pinane-2,3-diol (**419ab′**) as shown in Figure 14.198. Relationship of the metabolism of β-pinene (**1**, **1′**), isopinocamphone (**414b, 414b′**), 2α-hydroxy-3-pinanone (**414a**, **414a′**), and pinane-2,3-diol (**419ab**, **419ab′**) in *Aspergillus niger* TBUYN-2 and *Botrytis cinerea* is shown in Figures 14.197 and 14.198.

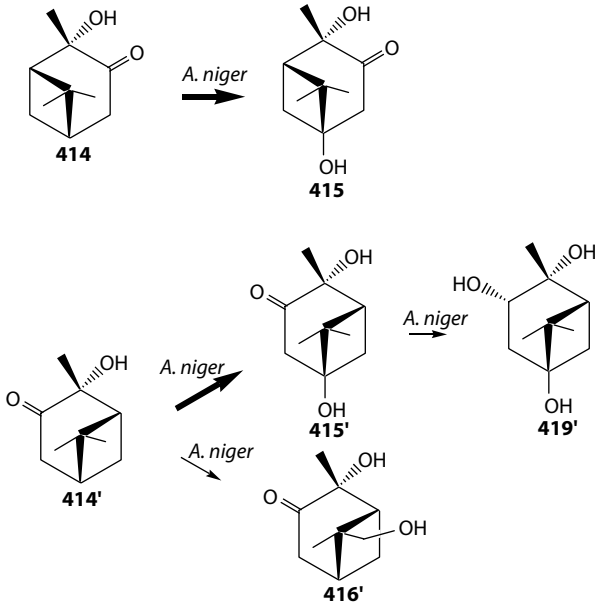


FIGURE 14.196 Biotransformation of (−)- (**414**) and (+)-2-hydroxy-3-pinanone (**414′**) by *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

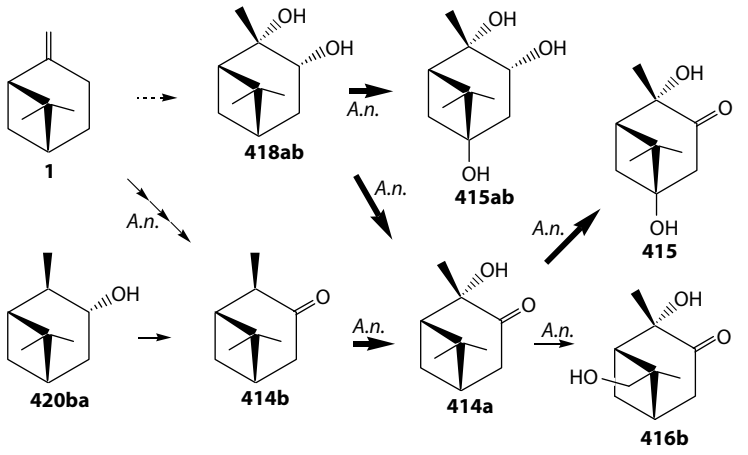


FIGURE 14.197 Relationship of the metabolism of β-pinene (**1**), isopinocamphone (**414b**), 2α-hydroxy-3-pinanone (**414a**), and pinane-2,3-diol (**419ab**) in *Aspergillus niger* TBUYN-2. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

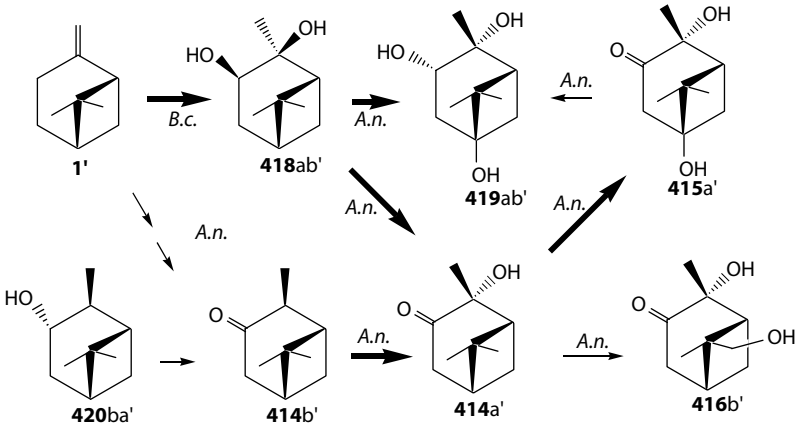


FIGURE 14.198 Relationship of the metabolism of β-pinene (**1′**), isopinocamphone (**414b′**), 2α-hydroxy-3-pinanone (**414a′**), and pinane-2,3-diol (**419ab′**) in *Aspergillus niger* TBUYN-2 and *Botrytis cinerea*. (Modified from Noma, Y. et al., 2003. *Proc. 47th TEAC*, pp. 91–93; Noma, Y. et al., 2004. *Proc. 48th TEAC*, pp. 390–392.)

14.4.4.2.4.1 Mosquitocidal and Knock-Down Activity Knock-down and mortality activity toward mosquito, *Culex quinequefasciatus*, was carried out for the metabolites of (+)- (**418ab**) and (−)-pinane-2,3-diols (**418ab′**) and (+)- and (−)-2-hydroxy-3-pinanones (**414** and **414′**) by Dr. Radhika Samarasekera, Industrial Technology Institute, Sri Lanka. (−)-2-Hydroxy-3-pinanone (**414′**) showed the mosquito knock-down activity and the mosqutocidal activity at the concentration of 1% and 2% (Table 14.16).

14.4.4.2.4.2 Antimicrobial Activity The microorganisms were refreshed in Mueller Hilton Broth (Merck) at 35–37°C, and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, UK) media for preparation of inoculum. *Escherichia coli* (NRRL B-3008), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (NRRL 3567), *Salmonella typhimurium* (NRRL B-4420), *Staphylococcus epidermidis (*ATCC 12228), Methicillin-resistant *Staphylococcus aureus* (MRSA, Clinical isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey), and *Candida albicans* (Clinical Isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey)

TABLE 14.16
Knock-down and Mortality Activity Toward Mosquito[a]

Compounds	Knock-Down (%)	Mortality (%)
(+)-2,5-Dihydroxy-3-pinanone (**415**, 2%)	27	20
(−)-2,5-Dihydroxy-3-pinanone (**415′**, 2%)	NT	7
(+)-2-Hydroxy-3-pinanone (**414**, 2%)	40	33
(−)-2-Hydroxy-3-pinanone (**414′**, 2%)	100	40
(−)-2-Hydroxy-3-pinanone (**414′**, 1%)	53	7
(+)-Pinane-2,3,5-triol (**419**, 2%)	NT	NT
(−)-Pinane-2,3,5-triol (**419**, 2%)	13	NT
(+)-Pinane-2,3-diol (**418**, 2%)	NT	NT
(−)-Pinane-2,3-diol (**418′**, 2%)	NT	NT

[a] The results are against Culex quinequefasciatus.

were used as pathogen test microorganisms. Microdilution broth susceptibility assay (*R1*, *R2*) was used for the antimicrobial evaluation of the samples. Stock solutions were prepared in DMSO (Carlo-Erba). Dilution series were prepared from 2 mg/mL in sterile distilled water in micro-test tubes from where they were transferred to 96-well micro-titer plates. Overnight grown bacterial and candial suspensions in double strength Mueller–Hilton broth (Merck) was standardized to approximately 10^8 CFU/mL using McFarland No:0.5 (10^6 CFU/mL for *Candida albicans*). A volume of 100 µL of each bacterial suspension was then added to each well. The last row containing only the serial dilutions of samples without microorganism was used as negative control. Sterile distilled water, medium, and microorganisms served as a positive growth control. After incubation at 37°C for a 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC), chloramphenicol (Sigma), ampicillin (sigma), and ketoconazole (Sigma) were used as standard antimicrobial agents (Koneman et al., 1997; Amsterdam, 1997) (Table 14.17).

TABLE 14.17
Biological Activity of Pinane-2,3,5-Triol (419 and 419′), 2,5-Dihydroxy-3-Pinanone (415 and 415′), and 7-Hydroxymethyl-1-*p*-menthene-8-ol (453′) Toward MRSA

	MIC (mg/mL)							
	Compounds					Control		
Microorganisms	419	415′	415	419′	453′	ST1	ST2	ST3
Escherichia coli	0.5	0.5	0.25	0.5	0.25	0.007	0.0039	Nt
Pseudomonas aeruginosa	0.5	0.125	0.125	0.25	0.25	0.002	0.0078	Nt
Enterobacter aerogenes	0.5	0.5	0.25	0.5	1.00	0.007	0.0019	Nt
Salmonella typhimurium	0.25	0.125	0.125	0.25	0.25	0.01	0.0019	Nt
Candida albicans	0.5	0.125	0.125	0.25	1.00	Nt	Nt	0.0625
Staphylococcus epidermidis	0.5	0.5	0.25	0.5	1.00	0.002	0.0009	Nt
MRSA	0.25	0.125	0.125	0.25	0.125	0.5	0.031	Nt

Source: Iscan (2005, unpublished data).
MRSA, methicillin-resistant Staphylococcus aureus; Nt, not tested; ST1, ampicillin-Na (Sigma); ST2, chloramphenicol (Sigma); ST3, ketoconazole (sigma).

14.5 SUMMARY

14.5.1 Metabolic Pathways of Monoterpenoids by Microorganisms

About 50 years are over since the hydroxylation of α-pinene (**4**) was reported by *Aspergillus niger* in 1960 (Bhattacharyya et al., 1960). During these years many investigators have studied the biotransformation of a number of monoterpenoids by using various kinds of microorganisms. Now we summarize the microbiological transformation of monoterpenoids according to the literatures listed in the references including the metabolic pathways (Figures 14.199 through 14.206) for the further development of the investigation on microbiological transformation of terpenoids.

Metabolic pathways of β-pinene (**1**), α-pinene (**4**), fenchol (**11**), fenchone (**12**), thujone (**28**), carvotanacetone (**47**), and sobrerol (**43**) are summarized in Figure 14.199. In general, β-pinene (**1**) is metabolized by six pathways. At first, β-pinene (**1**) is metabolized via α-pinene (**4**) to many metabolites such as myrtenol (**5**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968), verbenol (**23**) (Bhattacharyya et al., 1960; Prema and Bhattacharyya, 1962), and thujone (**28**) (Gibbon and Pirt, 1971). Myrtenol (**5**) is further metabolized to myrtenal (**6**) and myrtenic acid (**7**). Verbenol (**23**) is further metabolized to verbenone (**24**), 7-hydroxyverbenone (**25**), 7-hydroxyverbanone (**26**), and 7-formyl verbanone (**27**). Thujone (**28**) is further metabolized to thujoyl alcohol (**29**), 1-hydroxythujone (**30**), and 1,3-dihydroxythujone (**31**). Secondly, β-pinene (**1**) is metabolized to pinocarveol (**2**) and pinocarvone (**3**) (Ganapathy and Bhattacharyya, unpublished data). Pinocarvone (**3**) is further metabolized to isopinocamphone (**413**), which is further hydroxylated to give 2-hydroxy-3-pinanone (**414**). Compound **414** is further metabolized to give pinane-2,3-diol (**419**), 2,5- dihydroxy- (**415**), and 2,9-dihydroxy-3-pinanone (**416**). Thirdly, β-pinene (**1**) is metabolized to α-fenchol (**11**) and fenchone (**12**) (Dhavlikar et al., 1974), which are further metabolized to 6-hydroxy- (**13**) and 5-hydroxyfenchone (**14**), 5-oxofenchone (**15**), fenchone-9-al (**17**), fenchone-9-oic acid (**18**) via 9-hydroxyfenchone (**16**), 2,3-fencholide (**21**), and 1,2-fencholide (**22**) (Pfrunder and Tamm, 1969a, 1969b; Yamamoto, et al., 1984; Christensen and Tuthill, 1985; Miyazawa et al., 1990a, 1969b). Fenchol (**12**) is also metabolized to 9-hydroxyfenchol (**466**) and 7-hydroxyfenchol (**467**), 6-hydroxyfenchol (**349**), and 6,7-dihydroxyfenchol (**412**). Fourthly, β-pinene (**1**) is metabolized via fenchoquinone (**19**) to 2-hydroxyfenchone (**20**) (Pfrunder and Tamm 1969b; Gibbon et al., 1972). Fifthly, β-pinene (**1**) is metabolized to α-terpineol (**34**) via pinyl cation (**32**) and 1-*p*-menthene-8-cation (**33**) (Hosler, 1969; Hayashi et al., 1972; Saeki and Hashimoto, 1968, 1971). α-Terpineol (**34**) is metabolized to 8,9-epoxy-1-*p*-menthanol (**58**) via diepoxide (**57**), terpine hydrate (**60**), and oleuropeic acid (**204**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hosler 1969; Hungund et al., 1970; Hayashi et al., 1972; Saeki and Hashimoto, 1968, 1971). As shown in Figure 14.202, oleuropeic acid (**204**) is formed from linalool (**206**) and α-terpineol (**34**) via **204**, **205**, and **213** as intermediates (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970) and degradatively metabolized to perillic acid (**82**), 2-hydroxy-8-*p*-menthen-7-oic acid (**84**), 2-oxo-8-*p*-menthen-7-oic acid (**84**), 2-oxo-8-*p*-menthen-1-oic acid (**85**), and β-isopropyl pimelic acid (**86**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970). Oleuropeic acid (**204**) is also formed from β-pinene (**1**) via α-terpineol (**34**) as the intermediate (Noma et al., 2001). Oleuropeic acid (**204**) is also formed from myrtenol (**5**) by rearrangement reaction by *Aspergillus niger* TBUYN-2 (Noma and Asakawa 2005b). Finally, β-pinene (**1**) is metabolized to borneol (**36**) and camphor (**37**) via two cations (**32** and **35**) and to 1-*p*-menthene (**62**) via two cations (**33** and **59**) (Shukla and Bhattacharyya, 1968). 1-*p*-Menthene (**62**) is metabolized to phellandric acid (**65**) via phellandrol (**63**) and phellandral (**64**), which is further degradatively metabolized through **246**–**251** and **89** to water and carbon dioxide as shown in Figure 14.204 (Shukla et al., 1968). Phellandral (**64**) is easily reduced to give phellandrol (**63**) by *Euglena* sp. and *Dunaliella* sp. (Noma et al., 1984, 1986, 1991a, 1991b; 1992d). Furthermore, 1-*p*-menthene (**62**) is metabolized to 1-*p*-menthen-2-ol (**46**) and *p*-menthane-1,2-diol (**54**) as shown in Figure 14.204. Perillic acid (**82**) is easily formed from perillandehyde (**78**) and perillyl alcohol (**74**) (Figure 14.19) (Swamy et al., 1965; Dhavalikar and

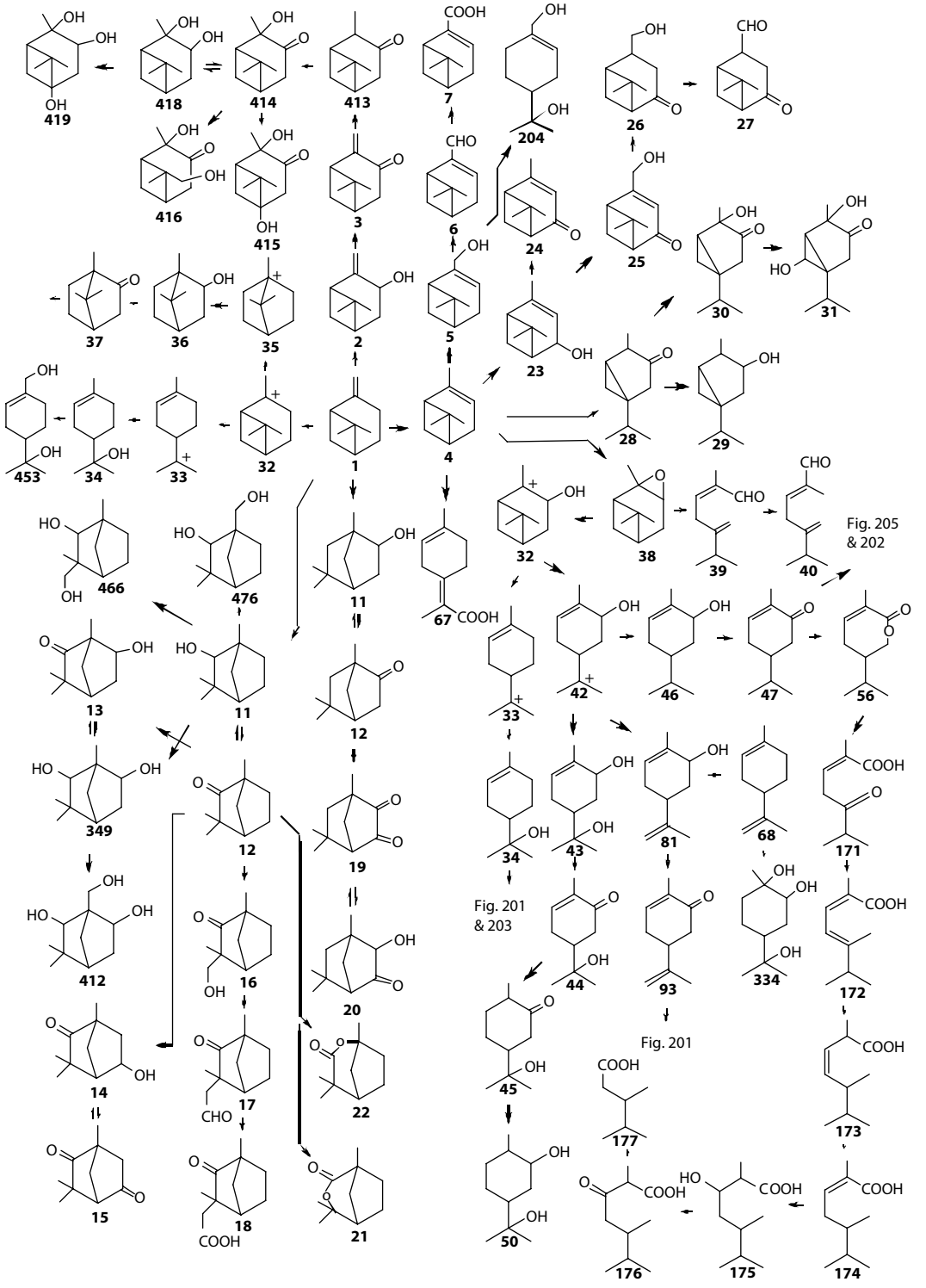


FIGURE 14.199 Metabolic pathways of β-pinene (**1**), α-pinene (**4**), fenchone (**9**), thujone (**28**), and carvotanacetone (**44**) by microorganisms.

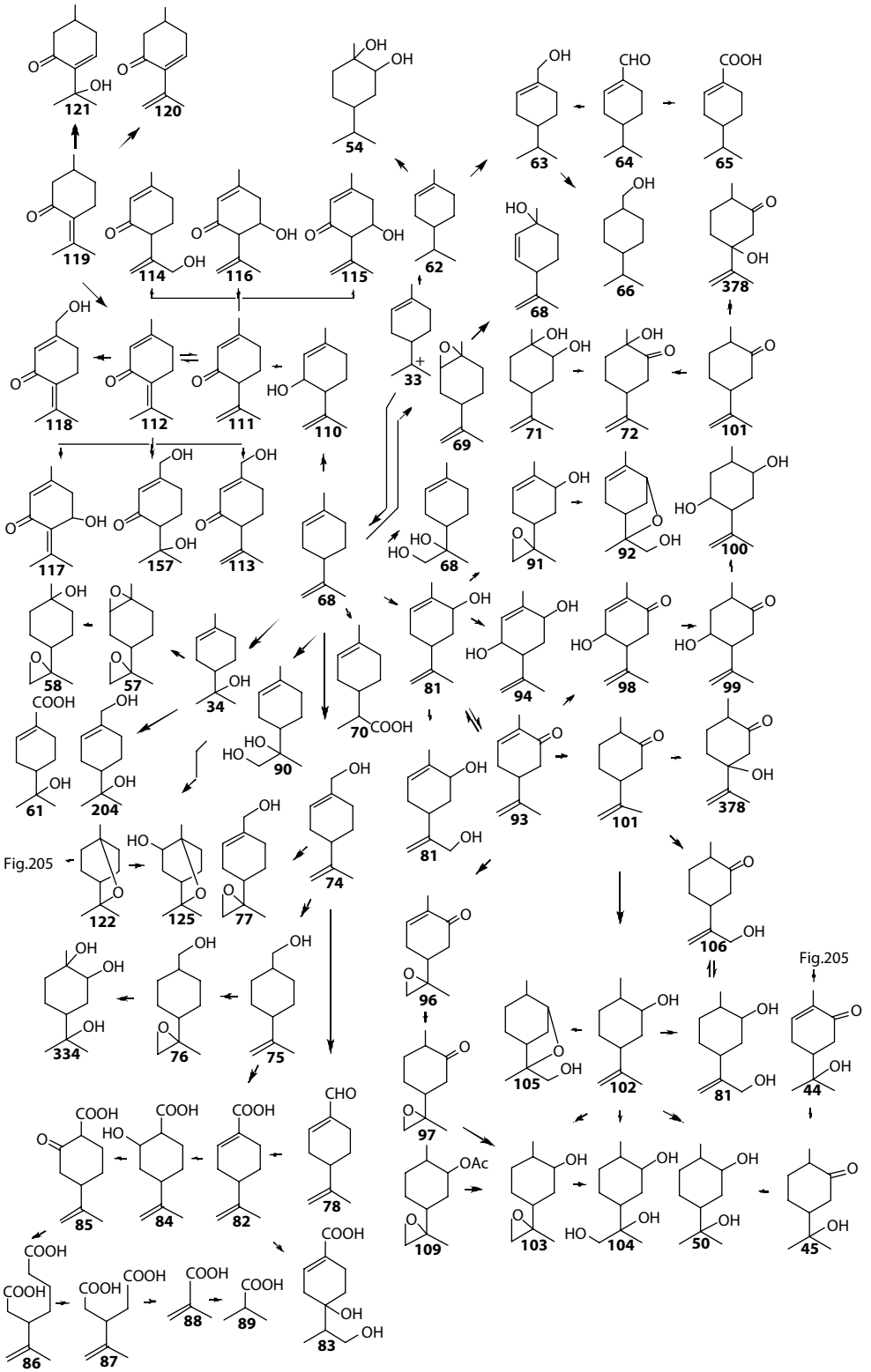


FIGURE 14.200 Metabolic pathways of limonene (**68**), perillyl alcohol (**74**), carvone (**93**), isopiperitenone (**111**), and piperitenone (**112**) by microorganisms.

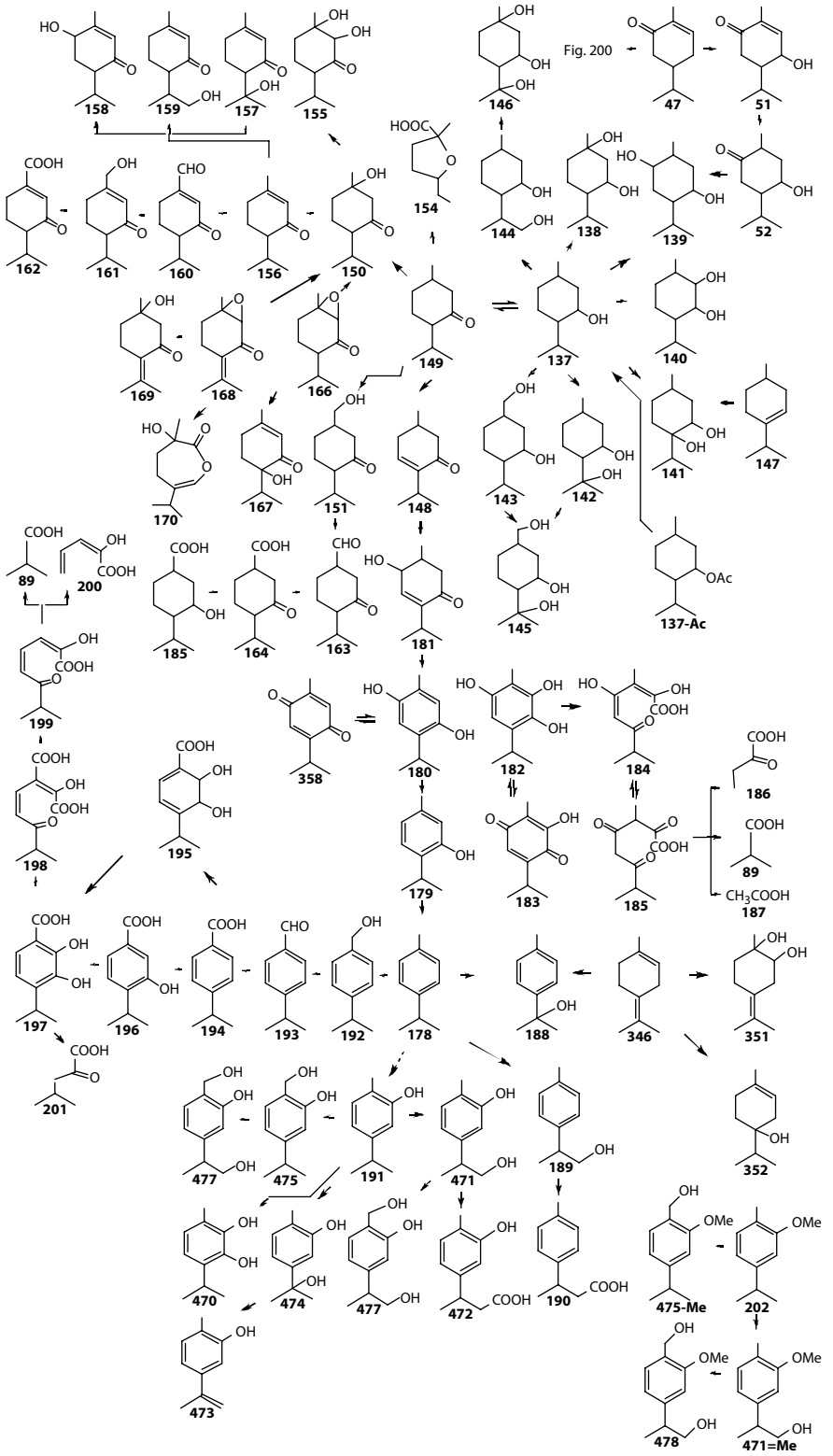


FIGURE 14.201 Metabolic pathways of menthol (**137**), menthone (**149**), *p*-cymene (**178**), thymol (**179**), carvacrol methyl ether (**201**), and carvotanacetone (**47**) by microorganisms and rabbit.

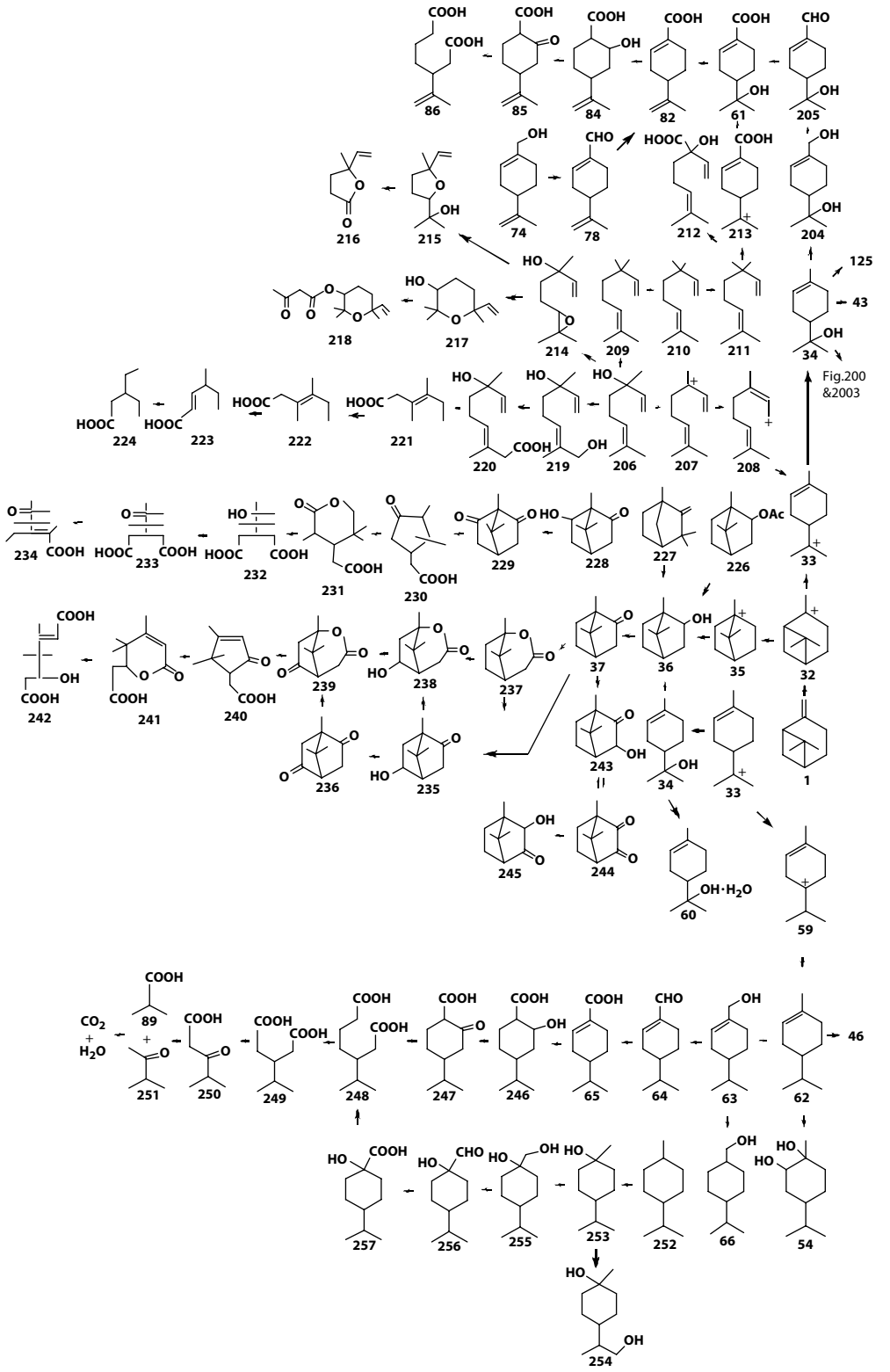


FIGURE 14.202 Metabolic pathway of borneol (**36**), camphor (**37**), phellandral (**64**), linalool (**206**), and *p*-menthane (**252**) by microorganisms.

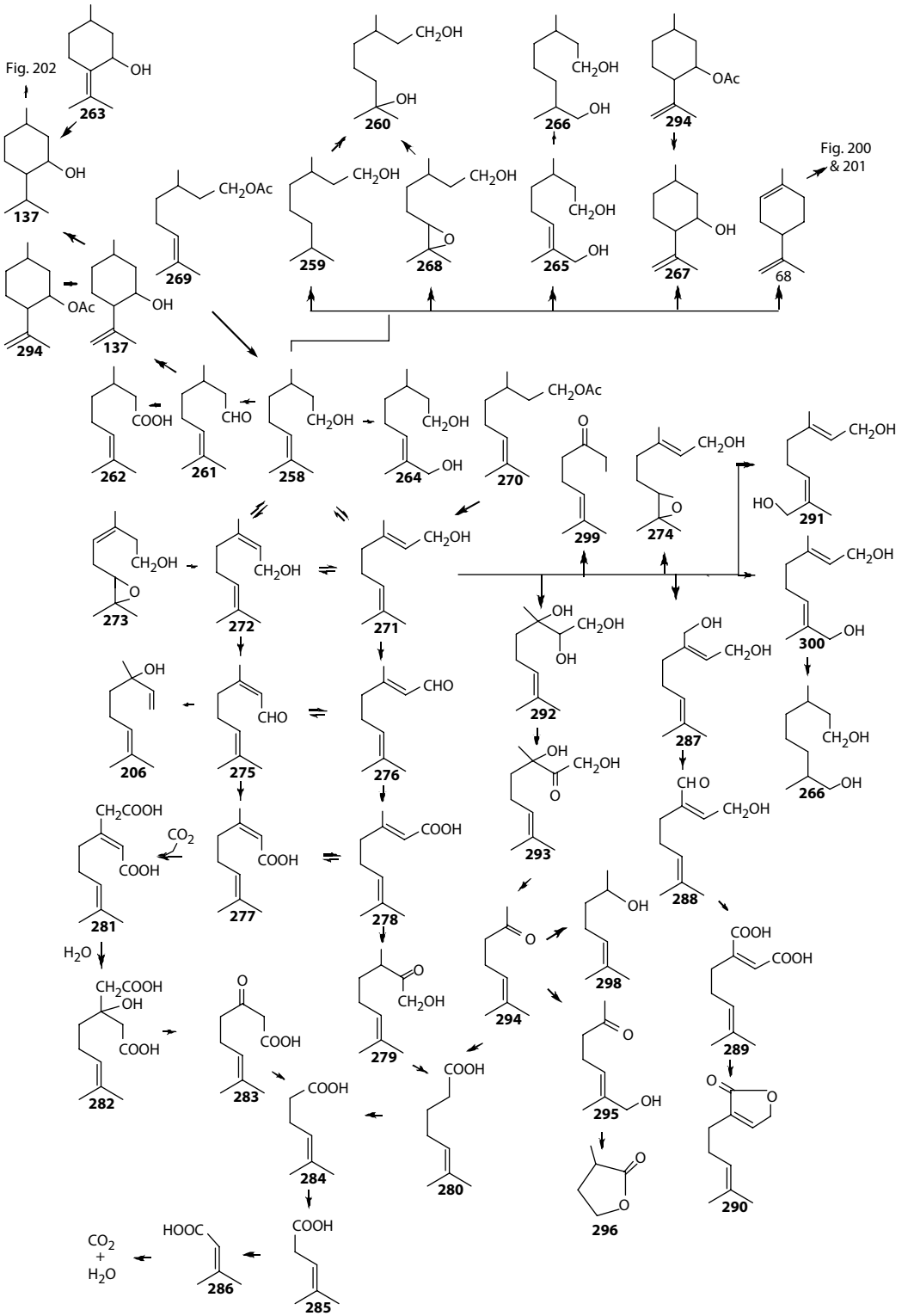


FIGURE 14.203 Metabolic pathways of citronellal (**258**), geraniol (**271**), nerol (**272**), and citral (**275** and **276**) by microorganisms.

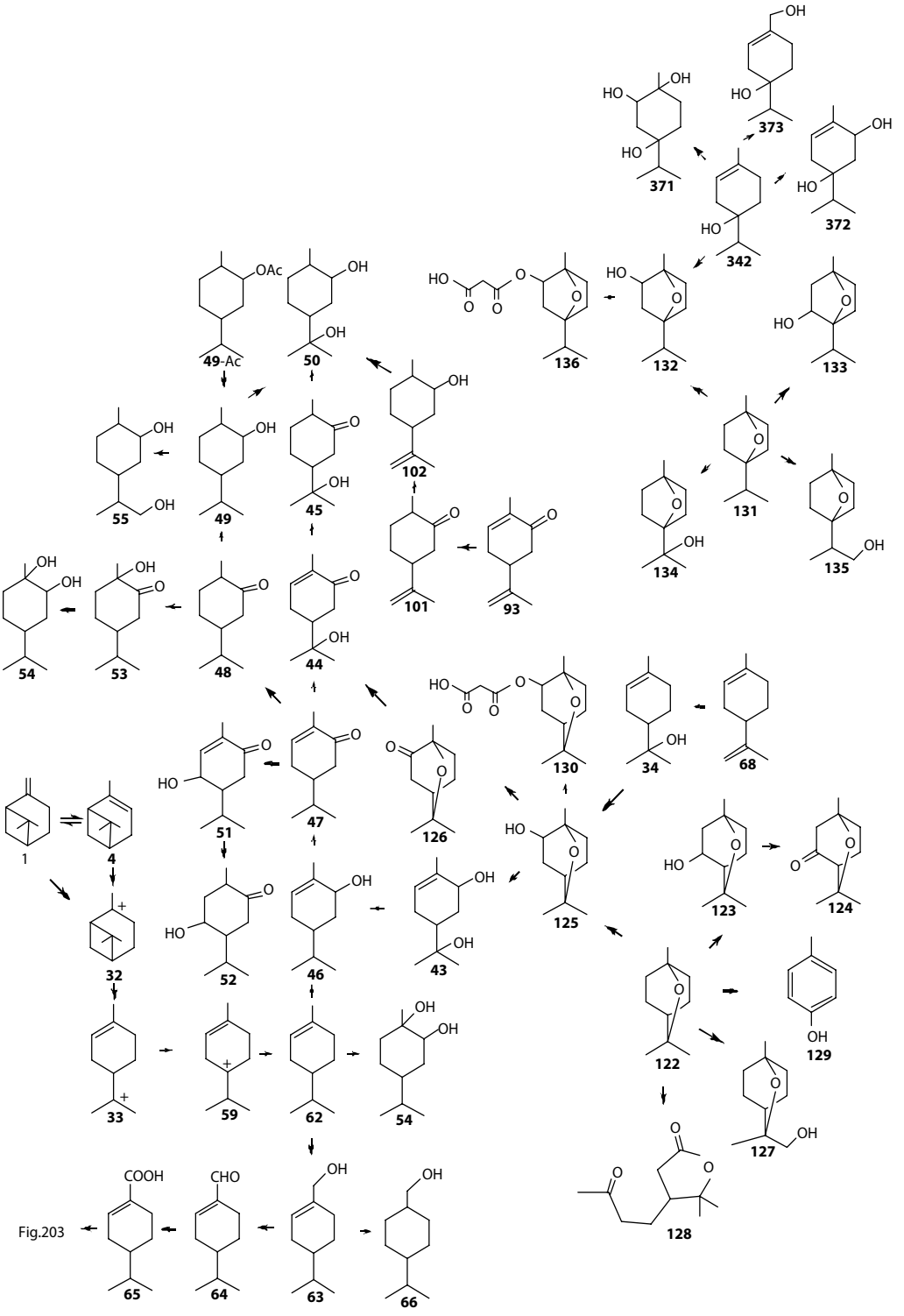


FIGURE 14.204 Metabolic pathways of 1,8-cineole (**122**), 1,4-cineole (**131**), phellandrene (**62**), and carvotanacetone (**47**) by microorganisms.

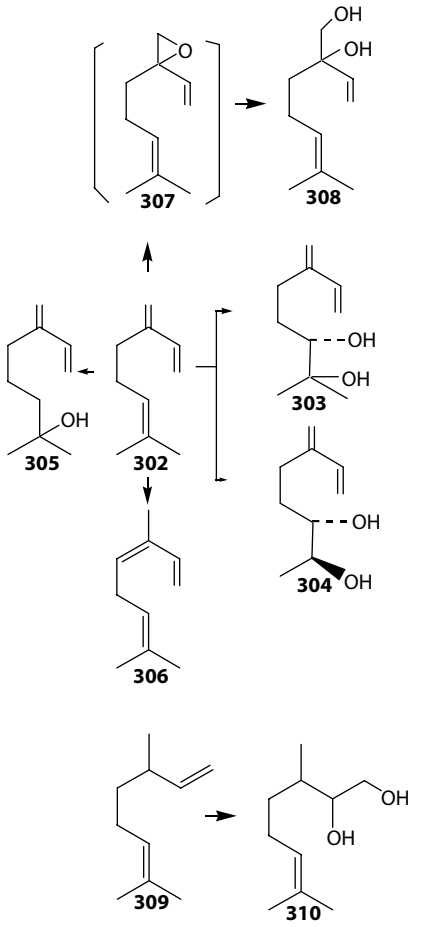


FIGURE 14.205 Metabolic pathways of myrcene (**302**) and citronellene (**309**) by rat and microorganisms.

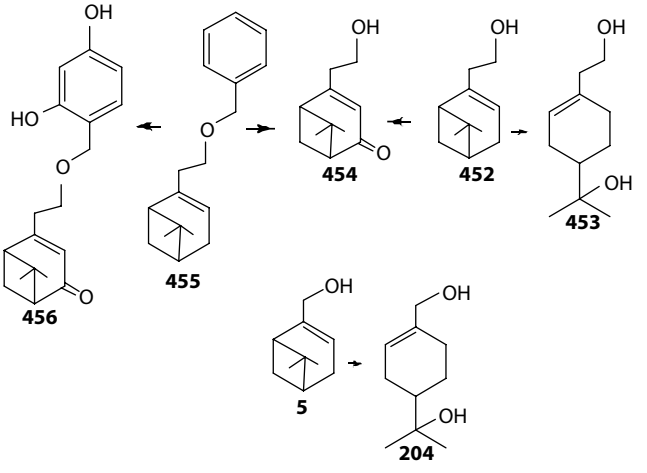


FIGURE 14.206 Metabolic pathways of nopol (**452**) and nopol benzyl ether (**455**) by microorganisms.

Bhattacharyya, 1966; Dhavalikar et al., 1966; Ballal et al., 1967; Kayahara et al., 1973; Shima et al., 1972). α-Terpineol (**34**) is also formed from linalool (**206**). α-Pinene (**4**) is metabolized by five pathways as follows: firstly, α-pinene (**4**) is metabolized to myrtenol (**5**), myrtenal (**6**), and myratenoic acid (**7**) (Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970; Ganapathy and Bhattacharyya, unpublished results). Myrtenal (**6**) is easily reduced to myrtenol (**5**) by *Euglena* and *Dunaliella* spp., (Noma et al., 1991a, 1991b; 1992d). Myrtanol (**8**) is metabolized to 3-hydroxy- (**9**) and 4-hydroxymyrtanol (**10**) (Miyazawa et al., 1994b). Secondly, α-pinene (**4**) is metabolized to verbenol (**23**), verbenone (**24**), 7-hydroxyverbenone (**25**), and verbanone-7-al (**27**) (Bhattacharyya et al., 1960; Prema and Bhattacharyya, 1962; Miyazawa et al., 1991d). Thirdly, α-pinene (**4**) is metabolized to thujone (**28**), thujoyl alcohol (**29**), 1-hydroxy- (**30**), and 1,3-dihydroxythujone (**31**) (Gibbon and Pirt, 1971; Miyazawa et al., 1992a; Noma, 2000). Fourthly, α-pinene (**4**) is metabolized to sobrerol (**43**) and carvotanacetol (**46**, 1-*p*-menthen-2-ol) via α-pinene epoxide (**38**) and two cations (**41** and **42**). Sobrerol (**43**) is further metabolized to 8-hydroxycarvotanacetone (**44**, carvonhydrate), 8-hydrocarvomenthone (**45**), and *p*-menthane-2,8-diol (50) (Prema and Bhattacharyya, 1962; Noma, 2007). In the metabolism of sobrerol (**43**), 8-hydroxycarvotanacetone (**44**), and 8-hydroxycarvomenthone (**45**) by *Aspergillus niger* TBUYN-2, the formation of *p*-menthane-2,8-diol (**50**) is very high enantio- and diastereoselective in the reduction of 8-hydroxycarvomenthone (Noma, 2007). 8-Hydroxycarvotanacetone (**44**) is a common metabolite from sobrerol (**43**) and carvotanacetone (**47**). Namely, carvotanacetone (**47**) is metabolized to carvomenthone (**48**), carvomenthol (**49**), 8-hydroxycarvomenthol (**50**), 5-hydroxycarvotanacetone (**51**), 8-hydroxycarvotanacetone (**44**), 5-hydroxycarvomenthone (**52**), and 2,3-lactone (**56**) (Gibbon and Pirt, 1971; Gibbon et al., 1972, Noma et al., 1974a; 1985c; 1988b). Carvomenthone (**48**) is metabolized to **45**, 8-hydroxycarvomenthol (**50**), 1-hydroxycarvomenthone (**53**), and *p*-menthane-1,2-diol (**54**) (Noma et al., 1985b, 1988b). Compound **52** is metabolized to 6-hydroxymenthol (**139**), which is the common metabolite of menthol (**137**) (see Figure 14.201). Carvomenthol (**49**) is metabolized to 8-hydroxycarvomenthol (**50**) and *p*-menthane-2, 9-diol (**55**). Finally, α-pinene (**4**) to borneol (**36**) and camphor (**37**) via **32** and **35** and to phellandrene (**62**) via **32** and two cations (**33** and **59**) as mentioned in the metabolism of β-pinene (**1**). Carvotanacetone (**47**) is also metabolized degradatively to 3,4-dimethylvaleric acid (**177**) via **56** and **158**-**163** as shown in Figure 201 (Gibbon and Pirt, 1971; Gibbon et al., 1972). α-Pinene (**4**) is also metabolized to 2-(4-methyl-3-cyclohexenylidene)-propionic acid (**67**) (Figure 14.199).

Metabolic pathways of limonene (**68**), perillyl alcohol (**74**), carvone (**93**), isopiperitenone (**111**), and piperitenone (**112**) are summarized in Figure 14.199. Limonene (**68**) is metabolized by eight pathways. Namely, limonene (**68**) is converted into α-terpineol (**34**) (Savithiry et al., 1997), limonene-1,2-epoxide (**69**), 1-*p*-menthene-9-oic acid (**70**), perillyl alcohol (**74**), 1-p-menthene-8,9-diol (**79**), isopiperitenol (**110**), *p*-mentha-1,8-diene-4-ol (**80**, 4-terpineol), and carveol (**81**) (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966; Bowen, 1975; Miyazawa et al., 1983; Van der Werf et al., 1997; Savithrry et al., 1997; Van der Werf and de Bont, 1998a, 1998b; Noma et al., 1982, 1992d). Dihdyrocarvone (**101**), limonene-1,2-diol (**71**), 1-hydroxy-8-*p*-menthene-2-one (**72**), and *p*-mentha-2,8-diene-1-ol (**73**) are formed from limonene (**68**) via limonene epoxide (**69**) as intermediate. Limonene (**68**) is also metabolized via carveol (**78**), limonene-1,2-diol (**71**), carvone (**93**), 1-*p*-menthene-6,9-diol (**95**), 8,9-dihydroxy-1-*p*-menthene (**90**), α-terpineol (**34**), 2α-hydroxy-1,8-cineole (**125**), and *p*-menthane-1,2,8-triol (**334**). Bottrospicatol (**92**) and 5-hydroxycarveol (**94**) are formed from *cis*-carveol by *Streptomyces bottropensis* SY-2-1 (Noma et al., 1982; Nishimura et al., 1983a; Noma and Nishimura, 1992; Noma and Asakawa, 1992). Carveyl acetate and carveyl propionate (both are shown as **106**) are hydrolyzed enantio- and diastereoselectively to carveol (**78**) (Oritani and Yamashita, 1980; Noma, 2000). Carvone (**93**) is metabolized through four pathways as follows: firstly, carvone (**93**) is reduced to carveol (**81**) (Noma, 1980). Secondly, it is epoxidized to carvone-8,9-epoxide (**96**), which is further metabolized to dihydrocarvone-8,9-epoxide (97), dihydrocarveol-8,9-epoxide (**103**), and menthane-2,8,9-triol (**104**) (Noma, 2000; Noma et al., 1980; Noma and Nishimura, 1982). Thirdly, **93** is hydroxylated to 5-hydroxycarvone (**98**), 5-hydroxydihydrocarvone

(**99**), and 5-hydroxydihydrocarveol (**100**) (Noma and Nishimura, 1982). Dihydrocarvone (**101**) is metabolized to 8-*p*-menthene-1,2-diol (**71**) via l-hydroxydihydrocarvone (**72**), 10-hydroxydihydrocarvone (**106**), and dihydrocarveol (**102**), which is metabolized to 10-hydroxydihydrocarveol (**107**), *p*-menthane-2,8-diol (**50**), dihydrocarveol-8,9-epoxide (**100**), *p*-menthane-2,8,9-triol (**104**), and dihydrobottrospicatol (**105**) (Noma et al., 1985a, 1985b). In the biotransformation of (+)-carvone by plant pathogenic fungi, *Aspergillus niger* Tiegh, isodihydrocarvone (**101**) was metabolized to 4-hydroxyisodihydrocarvone (**378**) and 1-hydroxyisodihydrocarvone (**72**) (Noma and Asakawa, 2008). 8,9-Epoxydihydrocarveyl acetate (**109**) is hydrolyzed to 8,9-epoxydihydrocarveol (**103**). Perillyl alcohol (**74**) is metabolized through three pathways to shisool (**75**), shisool-8,9-epoxide (**76**), perillyl alcohol-8,9-epoxide (**77**), perilladehyde (**78**), perillic acid (**82**), and 4,9-dihydroxy-1-*p*-menthen-7-oic acid (**83**). Perillic acid (**82**) is metabolized degradatively to **84**–**89** as shown in Figure 14.200 (Swamy et al., 1965; Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966; Ballal et al., 1967; Shukla et al., 1968; Shukala and Bhattacharyya, 1968; Hungund et al., 1977; Kayahara et al., 1973; Shima et al., 1972). Isopiperitenol (**110**) is reduced to isopiperitenone (**111**), which is metabolized to 3-hydroxy- (**115**), 4-hydroxy- (**116**), 7-hydroxy- (**113**) and 10-hydroxy-isopiperitenone (**114**), and piperitenone (**112**). Compounds isopiperitenone (**111**) and piperitenone (**112**) are isomerized to each other (Noma et al., 1992c). Furthermore, piperitenone (**112**) is metabolized to 8-hydroxypiperitone (**157**), 5-hydroxy- (**117**) and 7-hydroxypiperitenone (**118**). Pulegone (**119**) is metabolized to **112**, 8-hydroxymenthenone (**121**), and 8,9-dehydromenthenone (**120**).

Metabolic pathways of menthol (**137**), menthone (**149**), thymol (**179**), and carvacryol methyl ether (**202**) are summarized in Figure 14.201. Menthol (**137**) is generally hydroxylated to give 1-hydroxy- (**138**), 2-hydroxy- (**140**), 4-hydroxy- (**141**), 6-hydroxy- (**139**), 7-hydroxy- (**143**), 8-hydroxy- (**142**), and 9-hydroxymenthol (**144**) and 1,8-dihydroxy- (**146**) and 7,8-dihydroxymenthol (**148**) (Asakawa et al., 1991; Takahashi et al., 1994; Van der Werf et al., 1997). Racemic menthyl acetate and menthylchloroacetate are hydrolyzed asymmetrically by an esterase of microorganisms (Brit Patent, 1970; Moroe et al., 1971; Watanabe and Inagaki, 1977a, 1977b). Menthone (**149**) is reductively metabolized to **137** and oxidatively metabolized to 3,7-dimethyl-6-hydroxyoctanoic acid (**152**), 3,7-dimethyl-6-oxooctanoic acid (**153**), 2-methyl-2,5-oxidoheptenoic acid (**154**), 1-hydroxymenthone (**150**), piperitone (**156**), 7-hydroxymenthone (**151**), menthone-7-al (**163**), menthone-7-oic acid (**164**), and 7-carboxylmenthol (**165**) (Sawamura et al., 1974). Compound **156** is metabolized to menthone-1,2-diol (**155**) (Miyazawa et al., 1991e, 1992d,e). Compound **148** is metabolized to 6-hydroxy- (**158**), 8-hydroxy- (**157**), and 9-hydroxypiperitone (**159**), piperitone-7-al (**160**), 7-hydroxypiperitone (**161**), and piperitone-7-oic acid (**162**) (Lassak et al., 1973). Compound **149** is also formed from menthenone (**148**) by hydrogenation (Mukherjee et al., 1973), which is metabolized to 6-hydroxymenthenone (**181**, 6-hydroxy-4-*p*-menthen-3-one). 6-hydroxymenthenone (**181**) is also formed from thymol (**179**) via 6-hydroxythymol (**180**). 6-Hydroxythymol (**180**) is degradatively metabolized through **182**–**185** to **186**, **187**, and **89** (Mukherjee et al., 1974). Piperitone oxide (**166**) is metabolized to 1-hydroxymenthone (**150**) and 4-hydroxypiperitone (**167**) (Lassak et al., 1973; Miyazawa et al., 1991e). Piperitenone oxide (**168**) is metabolized to 1-hydroxymenthone (**150**), 1-hydroxypulegone (**169**), and 2,3-seco-p-menthalacetone-3-en-1-ol (**170**) (Lassak et al., 1973; Miyazawa et al., 1991e). *p*-Cymene (**178**) is metabolized to 8-hydroxy- (**188**) and 9-hydroxy-p-cymene (**189**), 2- (4-methylphenyl)-propanic acid (**190**), thymol (**179**), and cumin alcohol (**192**), which is further converted degradatively to *p*-cumin aldehyde (**193**), cumic acid (**194**), *cis*-2,3-dihydroxy-2,3-dihydro-*p*-cumic acid (**195**), 2,3-dihydroxy-*p*-cumic acid (**197**), **198**–**200,** and **89** as shown in Figure 14.3 (Chamberlain and Dagley, 1968; DeFrank and Ribbons, 1977a, 1977b; Hudlicky et al., 1999; Noma, 2000). Compound **197** is also metabolized to 4-methyl-2-oxopentanoic acid (**201**) (DeFrank and Ribbons, 1977a). Compound **193** is easily metabolized to 192 and **194** (Noma et al., 1991a, 1992). Carvacrol methyl ether (**202**) is easily metabolized to 7-hydroxycarvacrol methyl ether (**203**) (Noma, 2000).

Metabolic pathways of borneol (**36**), camphor (**37**), phellandral (**64**), linalool (**206**), and *p*-menthane (**252**) are summarized in Figure 14.202. Borneol (**36**) is formed from β-pinene (**1**), α-pinene

(**4**), **34**, bornyl acetate (**226**), and camphene (**229**) and it is metabolized to **36**, 3-hydroxy- (**243**), 5-hydroxy- (**235**), 6-hydroxy- (**228**), and 9-hydroxycamphor (**225**), and 1,2-campholide (**23**). Compound **228** is degradatively metabolized to 6-oxocamphor (**229**) and **230**–**234**, whereas **237** is also degradatively metabolized to 6-hydroxy-1,2-campholide (**238**), 6-oxo-1,2-campholide (**239**), and **240**–**242**. 5-Hydroxycamphor (**235**) is metabolized to **238**, 5-oxocamphor (**236**), and 6-oxo-1,2-campholide (**239**). Compound **243** is also metabolized to camphorquinone (**244**) and 2-hydroxyepicamphor (**245**) (Bradshaw et al., 1959; Conrad et al., 1961, 1965a, 1965b; Gunsalus et al., 1965; Chapman et al., 1966; Hartline and Gunsalus, 1971; Oritani and Yamashita, 1974). 1-p-Menthene (**62**) is formed **1** and **4** via three cations (**32**, **33**, and **59**) and metabolized to phellandrol (**63**) (Noma et al., 1991a) and *p*-menthane-1,2-diol (**54**). Compound **63** is metabolized to phellandral (**64**) and 7-hydroxy-*p*-menthane (**66**). Compound **64** is furthermore metabolized degradatively to CO_2 and water via phellandric acid (**65**), **246**–**251**, and **89** (Dhavalikar and Bhattacharyya, 1966; Dhavalikar et al., 1966; Bahhal et al., 1967; Shukla et al., 1968; Shukla and Bhattacharyya, 1968; Hungund et al., 1970). Compound **64** is also easily reduced to phellandrol (**63**) (Noma et al., 1991a, 1992a). *p*-Menthane (**252**) is metabolized via 1-hydroxy-p-menthane (**253**) to *p*-menthane-1,9-diol (**254**) and *p*-menthane-1,7-diol (**255**) (Tukamoto et al., 1974, 1975; Noma et al., 1990). Compound **255** is degradatively metabolized via **256**–**248** to CO_2 and water through the degradation pathway of phellandric acid (**65**, **246**–**251**, and **89**) as mentioned above. Linalool (**206**) is metabolized to α-terpineol (**34**), camphor (**37**), oleuropeic acid (**61**), 2-methyl-6-hydroxy-6-carboxy-2,7-octadiene (**211**), 2-methyl-6-hydroxy-6-carboxy-7-octene (**199**), 5-methyl-5-vinyltetrahydro-2-furanol (**215**), 5-methyl-5-vinyltetrahydro-2-furanone (**216**), and malonyl ester (**218**). 1-Hydroxylinalool (**219**) is metabolized degradatively to 2,6-dimethyl-6-hydroxy-*trans*-2,7-octadienoic acid (**220**), 4-methyl-*trans*-3,5-hexadienoic acid (**221**), 4-methyl-*trans*-3,5-hexadienoic acid (**222**), 4-methyl-*trans*-2-hexenoic acid (**223**), and isobutyric acid (**224**). Compound **206** is furthermore metabolized via **213** to **61**, **82**, and **84**–**86** as shown in Figure 14.2 (Mizutani et al., 1971; Murakami et al., 1973; Rama Devi and Bhattacharyya, 1977a, 1977b; Rama Devi et al., 1977; Madyastha et al., 1977; David and Veschambre, 1985; Miyazawa et al., 1994a, 1994b).

Metabolic pathways of citronellol (**258**), citronellal (**261**), geraniol (**271**), nerol (**272**), citral [neral (**275**) and geranial (**276**)], and myrcene (**302**) are summarized in Figure 14.203 (Seubert and Fass, 1964; Hayashi et al., 1968; Rama Devi and Bhattacharyya, 1977a, 1977b). Geraniol (**271**) is formed from citronellol (**258**), nerol (**272**), linalool (**206**), and geranyl acetate (**270**) and metabolized through 10 pathways. Namely, compound **271** is hydrogenated to give citronellol (**258**), which is metabolized to 2,8-dihydroxy-2,6-dimethyl octane (**260**) via 6,7-epoxycitronellol (**268**), isopulegol (**267**), limonene (**68**), 3,7-dimethyloctane-1,8-diol (**266**) via 3,7-dimethyl-6-octene-1,8-diol (**265**), **267**, citronellal (**261**), dihydrocitronellol (**259**), and nerol (**272**). Citronellyl acetate (**269**) and isopulegyl acetate (**301**) are hydrolyzed to citronellol (**258**) and isopulegol (**267**), respectively. Compound **261** is metabolized via pulegol (**263**) and isopulegol (**267**) to menthol (**137**). Compound **271** and **272** are isomerized to each other. Compound **272** is metabolized to **271**, **258**, citronellic acid (**262**), nerol-6-,7-epoxide (**273**), and neral (**275**). Compound **272** is metabolized to neric acid (**277**). Compounds **275** and **276** are isomerized to each other. Compound **276** is completely decomposed to CO_2 and water via geranic acid (**278**), 2,6-dimethyl-8-hydroxy-7-oxo-2-octene (**279**), 6-methyl-5-heptenoic acid (**280**), 7-methyl-3-oxo-6-octenoic acid (**283**), 6-methyl-5-heptenoic acid (**284**), 4-methyl-3-heptenoic acid (**284**), 4-methyl-3-pentenoic acid (**285**), and 3-methyl-2-butenoic acid (**286**). Furthermore, compound **271** is metabolized via 3-hydroxymethyl-2,6-octadiene-1-ol (**287**), 3-formyl-2,6-octadiene-1-ol (**288**), and 3-carboxy-2,6-octadiene-1-ol (**289**) to 3- (4-methyl-3-pentenyl)-3-butenolide (**290**). Geraniol (**271**) is also metabolized to 3,7-dimethyl-2,3-dihydroxy-6-octen-1-ol (**292**), 3,7-dimethyl-2-oxo-3-hydroxy-6-octen-1-ol (**293**), 2-methyl-6-oxo-2-heptene (**294**), 6-methyl-5-hepten-2-ol (**298**), 2-methyl-2-heptene-6-one-1-ol (**295**), and 2-methyl-γ-butyrolactone (**296**). Furthermore, **271** is metabolized to 7-methyl-3-oxo-6-octanoic acid (**299**), 7-hydroxymethyl-3-methyl-2,6-octadien-1-ol (**291**), 6,7-epoxygeraniol (**274**), 3,7-dimethyl-2,6-octadiene-1,8-diol (**300**), and 3,7-dimethyloctane-1,8-diol (**266**).

Metabolic pathways of 1,8-cineole (**122**), 1,4-cineole (**131**), phellandrene (**62**), carvotanacetone (**47**), and carvone (**93**) by micororganisms are summarized in Figure 14.204.

1,8-Cineole (**112**) is biotransformed to 2-hydroxy- (**125**), 3-hydroxy- (**123**), and 9-hydroxy-1,8-cineole (**127**), 2-oxo- (**126**) and 3-oxo-1,8-cineole (**124**), lactone [**128**, (*R*)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2-(3H)-one] and *p*-hydroxytoluene (**129**) (MacRae et al., 1979, Nishimura et al., 1982; Noma and Sakai, 1984). 2-Hydroxy-1,8-cineole (**125**) is further converted into 2-oxo-1,8-cineole (**126**), 1,8-cineole-2-malonyl ester (**130**), sobrerol (**43**), and 8-hydroxycarvotanacetone (**44**) (Miyazawa et al., 1995b). 2-Hydroxy-1,8-cineole (**125**) and 2-oxo-1,8-cineole (**126**) are also biodegradated to sobrerol (**43**) and 8-hydroxycarvotanacetone (**44**), respectively. 2-Hydroxy-1,8-cineole (**125**) was esterified to give malonyl ester (**130**). 2-Hydroxy-1,8-cineole (**125**) was formed from limonene (**68**) by Citrus pathogenic fungi, *Penicillium digitatum* (Noma and Asakawa 2007b). 1,4-Cineole (**131**) is metabolized to 2-hydroxy- (**132**), 3-hydroxy- (**133**), 8-hydroxy- (**134**), and 9-hydroxy-1,4-cineole (**135**). Compound **132** is also eaterified to malonyl ester (**136**) as well as **125** (Miyazawa et al., 1995b). Terpinen-4-ol (342) is metabolized to 2-hydroxy-1,4-cineole (**132**), 2-hydroxy- (**372**) and 7-hydroxyterpinene-4-ol (**342**), and p-mentane-1,2,4-triol (**371**) (Abraham et al., 1986; Noma and Asakawa, 2007a; Kumagae and Miyazawa, 1999). Phellandrene (**62**) is metabolized to carvotanacetol (**46**) and phellandrol (**63**). Carvotanacetol (**46**) is further metabolized through the metabolism of carvotanacetone (**47**). Phelandrol (**63**) is also metabolized to give phellandral (**64**), phellandric acid (**65**), and 7-hydroxy-p-menthane (**66**). Phellandric acid (**65**) is completely degradated to carbon dioxide and water as shown in Figure 14.202.

Metabolic pathways of myrcene (**302**) and citronellene (**309**) by microorganisms and insects are summarized in Figure 14.205. β-Myrcene (**302**) was metabolized with *Diplodia gossypina* ATCC 10936 (Abragam et al., 1985) to the diol (**303**) and a side-product (**304**). β-Myrcene (**302**) was metabolized with *Ganoderma applanatum*, *Pleurotus flabellatus*, and *Pleurotus sajor-caju* to myrcenol (**305**) (2-methyl-6-methylene-7-octen-2-ol) and **306** (Busmann and Berger, 1994).

β-Myrcene (**302**) was converted by common cutworm larvae, *Spodoptera litura*, to give myrcene-3, (10)-glycol (**308**) via myrcene-3,(10)-epoxide (**307**) (Miyazawa et al., 1998). Citronellene (**309**) was metabolized by cutworm *Spodoptera litura* to give 3,7-dimethyl-6-octene-1,2-diol (**310**) (Takechi and Miyazawa, 2005). Myrcene (**302**) is metabolized to two kinds of diols (**303** and **304**), myrcenol (**305**), and ocimene (**306**) (Seubert and Fass, 1964; Abraham et al., 1985). Citronellene (**309**) was metabolized to (**310**) by *Spodoptera litura* (Takeuchi and Miyazawa, 2005).

Metabolic pathways of nopol (**452**) and nopol benzyl ether (**455**) by microorganisms are summarized in Figure 14.206. Nopol (**452**) is metabolized mainly to 7-hydroxyethyl-α-terpineol (453) by rearrangement reaction and 3-oxoverbenone (454) as minor metabolite by *Aspergillus* spp. including *Aspergillus niger* TBUYN-2 (Noma and Asakawa, 2006b,c). Myrtenol (**5**) is also metabolized to oleoropeic alcohol (**204**) by rearrangement reaction. However, nopol benzyl ether (**455**) was easily metabolized to 3-oxoverbenone (**454**) and 3-oxonopol-2′,4′-dihydroxybenzylether (**456**) as main metabolites without rearrangement reaction (Noma and Asakawa 2006c).

14.5.2 Microbial Transformation of Terpenoids as Unit Reaction

Microbiological oxidation and reduction patterns of terpenoids and related compounds by fungi belonging to *Aspergillus* spp. containing *Aspergillus niger* TBUYN-2 and *Euglena gracilis Z* are summarized in Tables 14.18 and 14.19, respectively. Dehydrogenation of secondary alcohols to ketones, hydroxylation of both nonallylic and allylic carbons, oxidation of olefins to form diols and triols via epoxides, reduction of both saturated and α,β-unsaturated ketones and hydrogenation of olefin conjugated with the carbonyl group were the characteristic features in the biotransformation of terpenoids and related compounds by *Aspergillus* spp.

Compound names: **1**, β-pinene; **2**, pinocarveol; **3**, pinocarvone; **4**, α-pinene; **5**, myrtenol; **6**, myrtenal; **7**, myrtenoic acid; **8**, myrtanol; **9**, 3-hydroxymyrtenol; **10**, 4-hydroxymyrtanol; **11**, α-fenchol; **12**, fenchone; **13**, 6-hydroxyfenchone; **14**, 5-hydroxyfenchone; **15**, 5-oxofenchone; **16**,

TABLE 14.18
Microbiological Oxidation and Reduction Patterns of Monoterpenoids by *Aspergillus niger* TBUYN-2

	Microbiological Oxidation	
Oxidation of alcohols	Oxidation of primary alcohols to aldehydes and acids	
	Oxidation of secondary alcohols to ketones	(−)-*trans*-Carveol (**81a′**), (+)-*trans*-carveol (**81a**), (−)-*cis*-carveol (**81b′**), (+)-*cis*-carveol (**81b**), 2α-hydroxy-1,8-cineole (**125b**), 3α-hydroxy-1,8-cineole (**123b**), 3β-hydroxy-1,8-cineole (**123**a)
Oxidation of aldehydes to acids		
Hydroxylation	Hydroxylation of nonallylic carbon	(−)-Isodihydrocarvone (**101c′**), (−)-carvotanacetone (**47′**), (+)-carvotanacetone (**47**), *cis*-*p*-menthane (**252**), 1α-hydroxy-*p*-menthane (**253**), 1,8-cineole (**122**), 1,4-cineole (**131**), (+)-fenchone (**12**), (−)-fenchone (**12′**), (−)-menthol (**137b′**), (+)-Menthol (**137b**), (−)-neomenthol (**137a**), (+)-neomenthol (**137a**), (+)-isomenthol (**137c**)
	Hydroxylation of allylic carbon	(−)-Isodihydrocarvone (**101b**), (+)-neodihydrocarveol (**102a′**), (−)-dihydrocarveol (**102b′**), (+)-dihydrocarveol (**102b**), (+)-limonene (**68**), (−)-limonene (**68′**)
Oxidation of olefins	Formation of epoxides and oxides	
	Formation of diols	(+)-Neodihydrocarveol (**102a′**), (+)-dihydrocarveol (**102b**), (−)-dihydrocarveol (**102b′**), (+)-limonene (**68**), (−)-limonene (**68′**)
	Formation of triols	(+)-Neodihydrocarveol (**102a′**)
Lactonization		
	Microbiological Reduction	
Reduction of aldehydes to alcohols		
Reduction of ketones to alcohols	Reduction of saturated ketones	(+)-Dihydrocarvone (**101a′**), (−)-isodihydrocarvone (**101b**), (+)-carvomenthone (**48a′**), (−)-isocarvomenthone (**48b**)
	Reduction of α,β-unsaturated ketones	
Hydrogenation of olefins	Hydrogenation of olefin conjugated with carbonyl group	(−)-Carvone (**93′**), (+)-carvone (**93**), (−)-carvotanacetone (**47′**), (+)-carvotanacetone (**47**)
	Hydrogenation of olefin not conjugated with a carbonyl group	

TABLE 14.19
Microbiological Oxidation, Reduction, and Another Reaction Patterns of Monoterpenoids by *Euglena gracilis Z*

	Microbiological Oxidation	
Oxidation of alcohols	Oxidation of primary alcohols to aldehydes and acids	
	Oxidation of secondary alcohols to ketones	(−)-*trans*-Carveol (**81a′**), (+)-*cis*-carveol (**81b**), (+)-isoborneol (**36b**) *Diastereo- and enantioselective dehydrogenation is observed in carveol, borneol, and isoborneol
Oxidation of aldehydes to acids		Myrtenal (**6**), myrtanal, (−)-perillaldehyde (**78**), *trans*- and *cis*-1,2-dihydroperillaldehydes (**261a** and **261b**), (−)-phellandral (**64**), *trans*- and *cis*-tetrahydroperillaldehydes, cuminaldehyde (**193**), (+)- and (−)-citronellal (**261** and **261′**) *Acids were obtained as minor products
Hydroxylation	Hydroxylation of nonallylic carbon	
	Hydroxylation of allylic carbon	(+)-Limonene (**68**), (−)-limonene (**68′**)
Oxidation of olefins	Formation of epoxides and oxides	
	Formation of diols	
	Formation of triols	(+)-and (−)-Neodihydrocarveol (**102a′** and **a**), (−)- and (+)-dihydrocarveol (**102b′** and **b**), (+)- and (−)-isodihydrocarveol (**102c′** and **c**), (+)- and (−)-neoisodihydrocarveol (**102d′** and **d**)
Lactonization		
	Microbiological Reduction	
Reduction of aldehydes to alcohols	Reduction of terpene aldehydes to terpene alcohols	Myrtenal (**6**), myrtanal, (−)-perillaldehyde (**78**), *trans*- and *cis*-1,2-dihydroperillaldehydes (**261a** and **261b**), phellandral (**64**), *trans*- and *cis*-1,2-dihydroperillaldehydes (**261a** and **261b**), *trans*- and *cis*-tetrahydroperillaldehydes, cuminaldehyde (**193**), citral (**275** and **276**), (+)- (**261**) and (−)-citronellal (**261′**)
	Reduction of aromatic and related aldehydes to alcohols	
	Reduction of aliphatic aldehydes to alcohols	
Reduction of ketones to alcohols	Reduction of saturated ketones	(+)-Dihydrocarvone (**101a′**), (−)-isodihydrocarvone (**101b**), (+)-carvomenthone (**48a′**), (−)-isocarvomenthone (**48b**), (+)-dihydrocarvone-8,9-epoxides (**97a′**), (+)-isodihydrocarvone-8,9-epoxides (**97b′**), (−)-dihydrocarvone-8,9-epoxides (**97a**)
	Reduction of α,β-unsaturated ketones	
Hydrogenation of olefins	Hydrogenation of olefin conjugated with carbonyl group	(−)-Carvone (**93′**), (+)-carvone (**93**), (−)-carvotanacetone (**47′**), (+)-carvotanacetone (**47**), (−)-carvone-8,9-epoxides (**96′**), (+)-carvone-8,9-epoxides (**96**)
	Hydrogenation of olefin not conjugated with a carbonyl group	

continued

TABLE 14.19 (continued)
Microbiological Oxidation, Reduction, and Another Reaction Patterns of Monoterpenoids by *Euglena gracilis Z*

	Hydrolysis	
Hydrolysis	Hydrolysis of ester	(+)-*trans*- and *cis*-Carveyl acetates (**108a** and **b**), (−)-*cis*-carveyl acetate (**108b′**), (−)-*cis*-carveyl propionate, geranyl acetate (**270**)
	Hydration	
Hydration	Hydration of C=C bond in isopropenyl group to tertiary alcohol	(+)-Neodihydrocarveol (**102a′**), (−)-dihydrocarveol (**102b′**), (+)-isodihydrocarveol (**102c′**), (+)-neoisodihydrocarveol (**102d′**) (−)-neodihydrocarveol (**102a**), (+)-dihydrocarveol (**102b**), (−)-isodihydrocarveol (**102c**), (−)-neoisodihydrocarveol (**102d**), *trans*- and *cis*-shisools (**75a** and **75b**)
	Isomerization	
Isomerization		Geraniol (**271**), nerol (**272**)

9-hydroxyfenchone; **17**, fenchone-9-al; **18**, fenchone-9-oic acid; **19**, fenchoquinone; **20**, 2-hydroxyfenchone; **21**, 2,3-fencholide; **22**, 1,2-fencholide; **23**, verbenol; **24**, verbenone; **25**, 7-hydroxyverbenone; **26**, 7-hydroxyverbenone; **27**, verbanone-4-al; **28**, thujone; **29**, thujoyl alcohol; **30**, 1-hydroxythujone; **31**, 1,3-dihydroxythujone; **32**, pinyl cation; **33**, 1-*p*-menthene-8-cation; **34**, α-terpineol; **35**, bornyl cation; **36**, borneol; **37**, camphor; **38**, α-pinene epoxide; **39**, isonovalal; **40**, novalal; **41**, 2-hydroxypinyl cation; **42**, 6-hydroxy-1-*p*-menthene-8-cation; **43**, *trans*-sobrerol; **44**, 8-hydroxycarvotanacetone; (carvonehydrate); **45**, 8-hydraocarvomenthone; **46**, 1-*p*-menthen-2-ol; **47**, carvotanacetone; **48**, carvomenthone; **49**, carvomenthol; **50**, 8-hydroxycarvomenthol; **51**, 5-hydroxycarvotanacetone; **52**, 5-hydroxycarvomenthone; **53**, 1-hydroxycarvomenthone; **54**, *p*-menthane-1,2-diol; **55**, *p*-menthane-2,9-diol; **56**, 2,3-lactone; **57**, diepoxide; **58**, 8,9-epoxy-1-*p*-menthanol; **59**, 1-*p*-menthene-4-cation; **60**, terpine hydrate; **61**, oleuropeic acid (8-hydroxyperillic acid); **62**, 1-*p*-menthene; **63**, phellandrol; **64**, phellandral; **65**, phellandric acid; **66**, 7-hydroxy-*p*-menthane; **67**, 2-(4-methyl-3-cyclohexenylidene)-propionic acid; **68**, limonene; **69**, limonene-1,2-epoxide; **70**, 1-*p*-menthene-9-oic acid; **71**, limonene-1,2-diol; **72**, 1-hydroxy-8-*p*-menthene-2-one; **73**, 1-hydroxy-*p*-menth-2,8-diene; **74**, perillyl alcohol; **75**, shisool; **76**, shisool-8,9-epoxide; **77**, perillyl alcohol-8,9-epoxide; **78**, perillandehyde; **79**, 1-p-menthene-8,9-diol; **80**, 4-hydroxy-*p*-menth-1,8-diene (4-terpineol); **81**, carveol; **82**, perillic acid; **83**, 4,9-dihydroxy-1-*p*-menthene-7-oic acid; **84**, 2-hydroxy-8-*p*-menthen-7-oic acid; **85**, 2-oxo-8-*p*-menthen-7-oic acid; **86**, β-isopropyl pimelic acid; **87**, isopropenylglutaric acid; **88**, isobutenoic acid; **89**, isobutyric acid; **90**, 1-*p*-menthene-8,9-diol; **91**, carveol-8,9-epoxide; **92**, bottrospicatol; **93**, carvone; **94**, 5-hydroxycarveol; **95**, 1-*p*-menthene-6,9-diol; **96**, carvone-8,9-epoxide; **97**, dihydrocarvone-8,9-epoxide; **98**, 5-hydroxycarvone; **99**, 5-hydroxydihydrocarvone; **100**, 5-hydroxydihydrocarveol; **101**, dihydrocarvone; **102**, dihydrocarveol; **103**, dihydrocarveol-8,9-epoxide; **104**, *p*-menthane-2,8,9-triol; **105**, dihydrobottrospicatol; **106**, 10-hydroxydihydrocarvone; **107**, 10-hydroxydihydrocarveol; **108**, carveyl acetate and carveyl propionate; **109**, 8,9-epoxydihydrocarveyl acetate; **110**, isopiperitenol; **111**, isopiperitenone; **112**, piperitenone; **113**, 7-hydroxyisopiperitenone; **114**, 10-hydroxyisopiperitenone; **115**, 4-hydroxyisopiperitenone; **116**, 5-hydroxyisopiperitenone; **117**, 5-hydroxypiperitenone; **118**, 7-hydroxypiperitenone; **119**, pulegone; **120**, 8,9-dehydromenthenone; **121**, 8-hydroxymenthenone; **122**, 1,8-cineole; **123**, 3-hydroxy1,8-cineole; **124**, 3-oxo-1,8-cineole; **125**, 2-hydroxy-1,8-cineole; **126**, 2-oxo-1,8-cineole;

127, 9-hydroxy-1,8-cineole; **128**, lactone (*R*)-5,5-dimethyl-4-(3′-oxobutyl)-4,5-dihydrofuran-2-(3H)-one; **129**, *p*-hydroxytoluene; **130**, 1,8-cineole-2-malonyl ester; **131**, 1,4-cineole; **132**, 2-hydroxy-1,4-cineole; **133**, 3-hydroxy-1,4-cineole; **134**, 8-hydroxy-1,4-cineole; **135**, 9-hydroxy-1, 4-cineole; **136**, 1,4-cineole-2-malonyl ester; **137**, menthol; **138**, 1-hydroxymenthol; **139**, 6-hydroxymenthol; **140**, 2-hydroxymenthol; **141**, 4-hydroxymenthol; **142**, 8-hydroxymenthol; **143**, 7-hydroxymenthol; **144**, 9-hydroxymenthol; **145**, 7,8-dihydroxymenthol; **146**, 1,8-dihydroxymenthol; **147**, 3-*p*-menthene; **148**, menthenone; **149**, menthone; **150**, 1-hydroxymenthone; **151**, 7-hydroxymenthone; **152**, 3,7-dimethyl-6-hydroxyoctanoic acid; **153**, 3,7-dimethyl-6-oxoactanoic acid; **154**, 2-methyl-2,5-oxidoheptenoic acid; **155**, menthone-1,2-diol; **156**, piperitone; **157**, 8-hydroxypiperitone; **158**, 6-hydroxypiperitone; **159**, 9-hydroxypiperitone; **160**, piperitone-7-al; **161**, 7-hydroxypiperitone; **162**, piperitone-7-oic acid; **163**, menthone-7-al; **164**, menthone-7-oic acid; **165**, 7-carboxylmenthol; **166**, piperitone oxide; **167**, 4-hydroxypiperitone; **168**, piperitenone oxide; **169**, 1-hydroxypulegone; **170**, 2,3-seco-*p*-menthylacetone-3-en-1-ol; **171**, 2-methyl-5-isopropyl-2,5-hexadienoic acid; **172**, 2,5,6-trimethyl-2,4-heptadienoic acid; **173**, 2,5,6-trimethyl-3-heptenoic acid; **174**, 2,5,6-trimethyl-2-heptenoic acid; **175**, 3-hydroxy-2,5,6-trimethyl-3-heptanoic acid; **176**, 3-oxo-2,5,6-trimethyl-3-heptanoic acid; **177**, 3,4-dimethylvaleric acid; **178**, *p*-cymene; **179**, thymol; **180**, 6-hydroxythymol **181**, 6-hydroxymenthenone, 6-hydroxy-4-*p*-menthen-3-one; **182**, 3-hydroxythymo1,4-quinol; **183**, 2-hydroxythymoquionone; **184**, 2,4-dimethyl-6-oxo-3,7-dimethyl-2,4-octadienoic acid; **185**, 2,4,6-trioxo-3,7-dimethyl octanoic acid; **186**, 2-oxobutanoic acid; **187**, acetic acid; **188**, 8-hydroxy-*p*-cymene; **189**, 9-hydroxy-*p*-cymene; **190**, 2-(4-methylphenyl)-propanoic acid; **191**, carvacrol; **192**, cumin alcohol; **193**, *p*-cumin aldehyde; **194**, cumic acid; **195**, *cis*-2,3-dihydroxy-2, 3-dihydro-*p*-cumic acid; **196**, 3-hydroxycumic acid; **197**, 2,3-dihydroxy-*p*-cumic acid; **198**, 2-hydroxy-6-oxo-7-methyl-2,4-octadien-1,3-dioic acid; **199**, 2-methyl-6-hydroxy-6-carboxy-7-octene; **201**, 4-methyl-2-oxopentanoic acid; **202**, carvacrol methyl ether; **203**, 7-hydroxycarvacrol methyl ether; **204**, 8-hydroxyperillyl alcohol; **205**, 8-hydroxyperillaldehyde; **206**, linalool; **207**, linalyl-6-cation; **208**, linalyl-8-cation; **209**, 6-hydroxymethyl linalool; **210**, linalool-6-al; **211**, 2-methyl-6-hydroxy-6-carboxy-2,7-octadiene; **212**, 2-methyl-6-hydroxy-7-octen-6-oic acid; **213**, phellandric acid-8-cation; **214**, 2,3-epoxylinalool; **215**, 5-methyl-5-vinyltetrahydro-2-furanol; **216**, 5-methyl-5-vinyltetrahydro-2-furanone; **217**, 2,2,6-trimethyl-3-hydroxy-6-vinyltetrahydropyrane; **218**, malonyl ester; **219**, 1-hydroxylinalool (3,7-dimethyl-1,6-octadiene-8-ol); **220**, 2,6-dimethyl-6-hydroxy-*trans*-2,7-octadienoic acid; **221**, 4-methyl-*trans*-3,5-hexadienoic acid; **222**, 4-methyl-trans-3,5-hexadienoic acid; **223**, 4-methyl-*trans*-2-hexenoic acid; **224**, isobutyric acid; **225**, 9-hydroxycamphor; **226**, bornyl acetate; **228**, 6-hydroxycamphor; **229**, 6-oxocamphor; **230**, 4-carboxymethyl-2,3,3-trimethylcyclopentanone; **231**, 4-carboxymethyl-3,5,5-trimethyltetrahydro-2-pyrone; **232**, isohydroxycamphoric acid; **233**, isoketocamphoric acid; **234**, 3,4,4-trimethyl-5-oxo-trans-2-hexenoic acid; **235**, 5-hydroxycamphor; **236**, 5-oxocamphor; **237**, **238**, 6-hydroxy-1, 2-campholide; **239**, 6-oxo-1,2-campholide; **240**, 5-carboxymethyl-3,4,4-trimethyl-2-cyclopentenone; **241**, 6-carboxymethyl-4,5,5-trimethyl-5,6-dihydro-2-pyrone; **242**, 5-hydroxy-3,4,4-trimethyl-2-heptene-1,7-dioic acid; **243**, 3-hydroxycamphor; **244**, camphorquinone; **245**, 2-hydroxyepicamphor; **246**, 2-hydroxy-*p*-menthan-7-oic acid; **247**, 2-oxo-*p*-menthan-7-oic acid; **248**, 3-isopropylheptane-1,7-dioic acid; **249**, 3-isopropylpentane-1,5-dioic acid; **250**, 4-methyl-3-oxopentanoic acid; **251**, methyl-isopropyl ketone; **252**, *p*-menthane; **253**, 1-hydroxy-*p*-menthane; **254**, *p*-menthane-1,9-diol; **255**, *p*-menthane-1,7-diol; **256**, 1-hydroxy-*p*-menthene-7-al; **257**, 1-hydroxy-*p*-menthene-7-oic acid; **258**, citronellol; **259**, dihydrocitronellol; **260**, 2,8-dihydroxy-2,6-dimethyl octane; **261**, citronellal; **262**, citronellic acid; **263**, pulegol; **264**, 7-hydroyxmethyl-6-octene-3-ol; **265**, 3,7-dimethyl-6-octane-1,8-diol; **266**, 3,7-dimethyloctane-1,8-diol; **267**, isopulegol; **268**, 6,7-epoxycitronellol; **269**, citronellyl acetate; **270**, geranyl acetate; **271**, geraniol; **272**, nerol **273**, nerol-6,7-epoxide; **274**, 6,7-epoxygeraniol; **275**, neral; **276**, geranial; **277**, neric acid; **278**, geranic acid; **279**, 2,6-dimethyl-8-hydroxy-7-oxo-2-octene; **280**, 6-methyl-5-heptenoic acid; **281**, 7-methyl-3-carboxymethyl-2,6-octadiene-1-oic acid; **282**, 7-methyl-3-hydroxy-3-carboxymethyl-6-octen-1-oic acid; **283**, 7-methyl-3-oxo-6-octenoic acid; **284**, 6-methyl-5-heptenoic acid; **284**, 4-methyl-3-heptenoic acid; **285**, 4-methyl-3-pentenoic acid; **286**, 3-methyl-2-butenoic acid; **287**, 3-hydroxymethyl-2,6-octadiene-1-ol; **288**, 3-formyl-2,6-

octadiene-1-ol; **289**, 3-carboxy-2,6-octadiene-1-ol; **290**, 3-(4-methyl-3-pentenyl)-3-butenolide; **291**, 7-hydroxymethyl-3-methyl-2,6-octadien-1-ol; **292**, 3,7-dimethyl-2,3-dihydroxy-6-octen-1-ol; **293**, 3,7-dimethyl-2-oxo-6-octene-1,3-diol; **294**, 6-methyl-5-hepten-2-one; **295**, 6-methyl-7-hydroxy-5-heptene-2-one; **296**, 2-methyl-γ-butyrolactone; **297**, 6-methyl-5-heptenoic acid; **298**, 6-methyl-5-hepten-2-ol; **299**, 7-methyl-3-oxo-6-octanoic acid; **300**, 3,7-dimethyl-2,6-octadiene-1,8-diol; **301**, isopulegyl acetate; **302**, myrcene; **303**, 2-methyl-6-methylene-7-octene-2,3-diol; **304**, 6-methylene-7-octene-2,3-diol; **305**, myrcenol; **306**, ocimene; **307**, myrcene-3,(10)-epoxide; **308**, myrcene-3,(10)-glycol; **309**, (−)-citronellene; **309′**, (+)-citronellene; **310**, (3*R*)-3,7-dimethyl-6-octene-1,2-diol; **310′**, (3*S*)-3,7-dimethyl-6-octene-1,2-diol; **311**, (*E*)-3,7-dimethyl-5-octene-1,7-diol; **312**, *trans*-rose oxide; **313**, *cis*-rose oxide; **314**, (2*Z*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol; **315**, (Z)-3,7-dimethyl-2,7-octadiene-1,6-diol; **316**, (2*E*,6*Z*)-2,6-dimethyl-2,6-octadiene-1,8-diol; **317**, a cyclization product; **318**, (2*E*,5*E*)-3,7-dimethyl-2,5-octadiene-1,7-diol; **319**, (*E*)-3,7-dimethyl-2,7-octadiene-1,6-diol; **320**, 8-hydroxynerol; **321**, 10-hydroxynerol; **322**, 1-hydroxy-3,7-dimethyl-2*E*,6*E*-octadienal; **323**, 1-hydroxy-3,7-dimethyl-2*E*,6*E*-octadienoic acid; **324**, 3,9-epoxy-*p*-menth-1-ene; **325**, tetrahydrolinalool; **326**, 3,7-dimethyloctane-3,5-diol; **327**, 3,7-dimethyloctane-3,7-diol; **328**, 3,7-dimethyl-octane-3,8-diol; **329**, dihydromyrcenol; **330**, 1,2-epoxydihydromyrcenol; **331**, 3β-hydroxydihydromyrcerol; **332**, dihydromyrcenyl acetate; **333**, 1,2-dihydroxydihydromyrcenyl acetate; **334**, (+)-*p*-menthane-1-β,2α,8-triol; **335**, α-pinene-1,2-epoxide; **336**, 3-carene; **337**, 3-carene-1,2-epoxide; **338**, (1*R*)-*trans*-isolimonene; **338**, (1*R*,4*R*)-*p*-menth-2-ene-8,9-diol; **339**, (1*R*,4*R*)-*p*-menth-2-ene-8,9-diol; **340**, α-Terpinene; **341**, α-terpinene-7-oic acid; **342**, (−)-terpinen-4-ol; **343**, *p*-menthane-1,2,4-triol; **344**, γ-terpinene; **345**, γ-terpinene-7-oic acid; **346**, terpinolene; **347**, (1*R*)-8-hydroxy-3-*p*-menthen-2-one; **348**, (1*R*)-1,8-dihydroxy-3-*p*-menthen-2-one; **349**, 6β-hydroxyfenchol; **350**, 5β-hydroxyfenchol (a,5β,b,5α); **351**, terpinolene-1,2-*trans*-diol; **352**, terpinolene-4,8-diol; **353**, terpinolene-9-ol; **354**, terpinolene-10-ol; **355**, α-phellandrene; **356**, α-phellandrene-7-oic acid; **357**, terpinolene-7-oic acid; **358**, thymoquinone; **359**, 1,2-dihydroperillaldehyde; **360**, 1,2-dihydroperillic acid; **361**, 8-hydroxy-1,2-dihydroperillyl alcohol; **362**, tetrahydroperillaldehyde (a *trans*, b *cis*); **363**, tetrahydroperillic acid (a *trans*, b *cis*); **364**, (−)-menthol monoglucoside; **365**, (+)-menthol diglucoside; **366**, (+)-isopulegol; **367**, 7-hydroxy-(+)-isopulegol; **368**, 10-hydroxy-(+)-isopulegol; **369**, 1,2-epoxy-α-terpineol; **370**, bornane-2,8-diol; **371**, *p*-menthane-1α,2β,4β-triol; **372**, 1-*p*-menthene-4β,6-diol; **373**, 1-*p*-menthene-4a,7-diol; **374**, (+)-bottrospicatal; **375**, 1-*p*-menthene-2β,8,9-triol; **376**, (−)-perillyl alcohol monoglucoside; **377**, (−)-perillyl alcohol diglucoside; **378**, 4α-hydroxy-(−)-isodihydrocarvone; **379**, 2-methyl-2-cyclohexenone; **380**, 2-cyclohexenone; **381**, 3-methyl-2-cyclohexenone; **382**, 2-methylcyclohexanone; **383**, 2-methylcyclohexanol (a, *trans* b, *cis*); **384**, 4-hydroxycarvone; **385**, 2-ethyl-2-cyclohexenone; **386**, 2-ethylcyclohexenone (a1*R*) (b1*S*); **387**, 1-hydroxypulegone; **388**, 5-hydroxypulegone; **389**, 8-hydroxymenthone; **390**, 10-hydroxy-(−)-carvone; **391**, 1,5,5-trimethylcyclopentane-1,4-dicarboxylic acid; **392**, 4β-hydroxy-(−)-menthone; **393**, 1α,4β-dihydroxy-(−)-menthone; **394**, 7-hydroxy-9-carboxymenthone; **395**, 7-hydroxy-1,8-cineole; **396**, methyl ester of 2α-hydroxy-1,8-cineole; **397**, ethyl ester of 2α-hydroxy-1,8-cineole; **398**, *n*-propyl ester of 2α-hydroxy-1,8-cineole; **399**, *n*-butyl ester of 2α-hydroxy-1,8-cineole; **400**, isopropyl ester of 2α-hydroxy-1,8-cineole; **401**, tertiary butyl ester of 2α-hydroxy-1,8-cineole; **402**, methylisopropyl ester of 2α-hydroxy-1,8-cineole; **403**, methyl tertiary butyl ester of 2α-hydroxy-1,8-cineole; **404**, 2α-hydroxy-1,8-cineole monoglucoside (404 and 404'); **405**, 2α-hydroxy-1,8-cineole diglucoside; **406**, *p*-menthane-1,4-diol; **407**, 1-*p*-menthene-4β,6-diol; **408**, (−)-pinane-2α,3α-diol; **409**, (−)-6β-hydroxypinene; **410,** (−)-4α,5-dihydroxypinene; **411**, (−)-4α-hydroxypinen-6-one; **412**, 6β,7-dihydroxyfenchol; **413**, 3-oxo-pinane; **414**, 2α-hydroxy-3-pinanone; **415**, 2α, 5-dihydroxy-3-pinanone; **416**, 2α,10-dihydroxy-3-pinanone; **417**, *trans*-3-pinanol; **418**, pinane-2α,3α-diol; **419**, pinane-2α, 3α, 5-triol; **420**, isopinocampheol (3-pinanol); **421**, pinane-1,3α-diol; **422**, pinane-3α,5-diol; **423**, pinane-3β,9-diol; **424**, pinane-3β,4β,-diol; **425**, **426**, pinane-3α,4β-diol; **427**, pinane-3α,9-diol; **428**, pinane-3α,6-diol; **429**, *p*-menthane-2α,9-diol; **430**, 2-methyl-3α-hydroxy-1-hydroxyisopropyl cyclohexane propane; **431**, 5-hydroxy-3-pinanone; **432**, 2α-methyl,3-(2-methyl-2-hydroxypropyl)-cyclopenta-1β-ol; **433**, 3-acetoxy-2α-pinanol; **434**, 8-hydroxy-α-pinene;**435**, **436**, myrtanoic acid; **437**, camphene; **438**, camphene gylcol; **439**, (+)-3-carene; **440**, *m*-mentha-4,6-dien-8-ol; **441**,

m-cymen-8-ol; **442**, 3-carene-9-ol; **443**, 3-carene-9-carboxylic acid; **444**, 3-caren-10-ol-9-carboxylic acid; **445**, 3-carene-9,10-dicarboxylic acid; **446**, (–)-*cis*-carane; **447**, dicarboxylic acid of (–)-*cis*-carane; **448**, (–)-6β-hydroxypinene; **449**, (–)-4α,5-dihydroxypinene; **450**, (–)-4α-hydroxypinen-6-one; **451**, 10-hydroxyverbenol; **452**, (–)-nopol; **453**, 7-hydroxymethyl-1-*p*-menthen-8-ol; **454**, 3-oxonopol; **455**, nopol benzyl ether; **456**, 4-oxonopl-2′,4′-dihydroxybenzyl ether; **457**, 7-hydroxymethyl-1-*p*-menthen-8-ol benzyl ether; **458**, piperitenol; **459**, thymol methyl ether; **460**, 7-hydroxythymol methyl ether; **461**, 9-hydroxythymol methyl ether; **462**, 1,8-cineol-9-oic acid; **463**, 4-hydroxyphellandric acid; **464**, 4-hydroxydihydrophellandric acid; **465**, (+)-8-hydroxyfenchol; **466**, (–)-9-hydroxyfenchol; **467**, (+)-10-hydroxyfenchol, **468**, 4α-hydroxy-6-oxo-α-pinene; **469**, dihydrolinalyl acetate; **470**, 3-hydroxycarvacrol; **471**, 9-hydroxycarvacrol; **472**, carvacrol-9-oic acid; **473**, 8,9-dehydrocarvacrol; **474**, 8-hydroxycarvacrol; **475**, 7-hydroxycarvacrol; **476**, carvacrol-7-oic acid; **477**, 8,9-dihydroxycarvacrol; **478**, 7,9-dihydroxycarvacrol methyl ether; **479**, 7-hydroxythymol; **480**, 9-hydroxythymol; **481**, thymol-7-oic acid; **482**, 7,9-dihydroxythymol; **483**, thymol-9-oic acid; **484,** (1*R*,2*R*,3*S*,4*S*,5*R*)-3,4-pinanediol.

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15 Biotransformation of Sesquiterpenoids, Ionones, Damascones, Adamantanes, and Aromatic Compounds by Green Algae, Fungi, and Mammals

Yoshinori Asakawa and Yoshiaki Noma

CONTENTS

15.1 INTRODUCTION

Recently, environment-friendly green or clean chemistry is emphasized in the field of organic and natural product chemistry. Noyori's high-efficient production of (−)-menthol using (*S*)-BINAP-Rh

catalyst is one of the most important green chemistries (Tani et al., 1982; Otsuka and Tani, 1991) and 1000 ton of (−)-menthol has been produced by this method in 1 year. On the other hand, enzymes of microorganisms and mammals are able to transform a huge variety of organic compounds, such as mono- sesqui-, and diterpenoids, alkaloids, steroids, antibiotics, and amino acids from crude drugs and spore-forming green plants to produce pharmacologically and medicinally valuable substances.

Since Meyer and Neuberg (1915) studied the microbial transformation of citronellal, there are a great number of reports concerning biotrasformation of essential oils, terpenoids, steroids, alkaloids, and acetogenins using bacteria, fungi, and mammals. In 1988 Mikami (Mikami, 1988) reported the review article of biotransformation of terpenoids entitled "Microbial Conversion of Terpenoids." Lamare and Furstoss (1990) reviewed biotransformation of more than 25 sesquiterpenoids by microorganisms. In this chapter, the recent advances in the biotransformation of natural and synthetic compounds; sesquiterpenoids, ionones, α-damascone, and adamantanes, and aromatic compounds, using microorganisms including algae and mammals are described.

15.2 BIOTRANSFORMATION OF SESQUITERPENOIDS BY MICROORGANISMS

15.2.1 Highly Efficient Production of Nootkatone (2) from Valencene (1)

The most important and expensive grapefruit aroma, nootkatone (**2**), decreases the somatic fat ratio (Haze et al., 2002), and therefore its highly efficient production has been requested by the cosmetic and fiber industrial sectors. Previously, valencene (**1**) from the essential oil of Valencia orange was converted into nootkatone (**2**) by biotransformation using *Enterobacter* species only in 12% yield (Dhavlikar and Albroscheit, 1973), *Rodococcus* KSM-5706 in 0.5% yield with a complex mixture (Okuda et al., 1994), and using Cytochrome P450 (CYP450) in 20% yield with other complex products (Sowden et al., 2005). Nootkatone (**2**) was chemically synthesized from valencene (**1**) with $AcOOCMe_3$ in three steps and chromic acid in low yield (Wilson and Saw, 1978) and using surface-functionalized silica supported by metal catalysts such as Co^{2+}, Mn^{2+}, and so on with *tert*-butyl hydroperoxide in 75% yield (Salvador and Clark, 2002). However, these synthetic methods are not safe because they involve toxic heavy metals. An environment-friendly method for the synthesis of nootkatone that does not use any heavy metals such as chromium and manganese must be designed. The commercially available and cheap sesquiterpene hydrocarbon (+)-valencene (**1**) ($[\alpha]_D$ + 84.6°, c = 1.0) obtained from Valencia orange oil was very efficiently converted into nootkatone (**2**) by biotransformations using *Chlorella* (Hashimoto et al., 2003a), *Mucor* species (Hashimoto et al., 2003), *Botryosphaeria dothidea*, and *Botryodiplodia theobromae* (Furusawa et al., 2005, 2005a; Noma et al., 2001a).

Chlorella fusca var. *vacuolata* IAMC-28 (Figure 15.1) was inoculated and cultivated while stationary under illumination in Noro medium $MgCl_2{\cdot}6H_2O$ (1.5 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), KCl (0.2 g), $CaCl_2{\cdot}2H_2O$ (0.2 g), KNO_3 (1.0 g), $NaHCO_3$ (0.43 g), TRIS (2.45 g), K_2HPO_4 (0.045 g), Fe-EDTA (3.64 mg), EDTA-2Na (1.89 mg), $ZnSO_4{\cdot}7H_2O$ (1.5 g), H_3BO_2 (0.61 mg), $CoCl_2{\cdot}6H_2O$ (0.015 mg), $CuSO_4{\cdot}5H_2O$ (0.06 mg), $MnCl_2{\cdot}4H_2O$ (0.23 mg), and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.38 mg), in distilled H_2O 1 L (pH 8.0). Czapek-peptone medium [1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.1% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.05% KCl, and 0.001% $FeSO_4{\cdot}7H_2O$, in distilled water (pH 7.0)] was used for the biotransformation of substrate by microorganism. *Aspergillus niger* was isolated in our laboratories from soil in Osaka prefecture, and was identified according to its physiological and morphological characters.

(+)-Valencene (**1**) (20 mg/50 mL) isolated from the essential oil of Valencia orange was added to the medium and biotransformed by *Chlorella fusca* for a further 18 days to afford nootkatone (**2**) [gas chromatography-mass spectrometry (GC-MS) peak area: 89%; isolated yield: 63%] (Figure 15.2) (Furusawa et al., 2005, 2005a; Noma et al., 2001a). The reduction of **2** with $NaBH_4$ and $CeCl_3$ gave

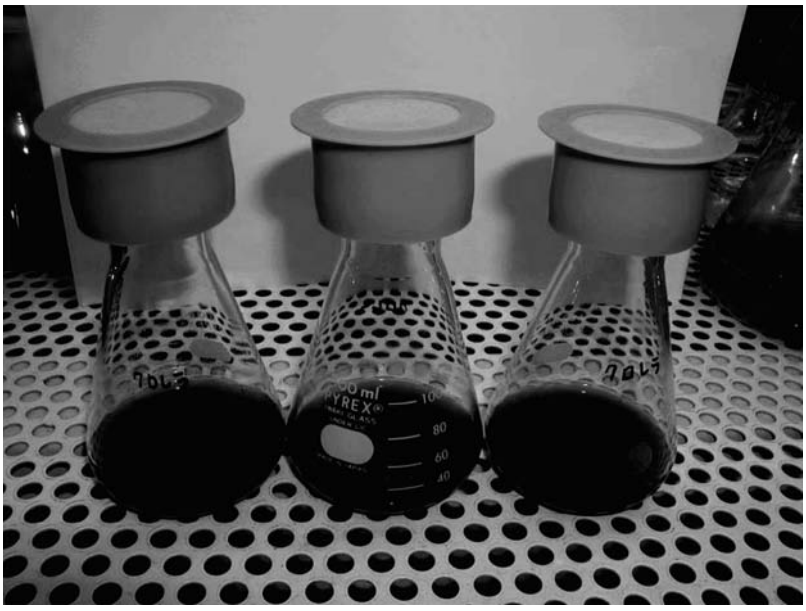


FIGURE 15.1 *Chlorella fusca* var. *vacuolata*.

2α-hydroxyvalencene (**3**) in 87% yield, followed by Mitsunobu reaction with *p*-nitrobenzoic acid, triphenylphosphine, and diethyl azodicarboxylate to give nootkatol (2β-hydroxyvalencene) (**4**), possessing calcium-antagonistic activity isolated from *Alpinia oxyphylla* (Shoji et al., 1984) in 42% yield. Compounds **3** and **4** thus obtained were easily biotransformed by *Chlorella fusca* and *Chlorella pyrenoidosa* for only 1 day to give nootkatone (**2**) in good yield (80–90%), respectively.

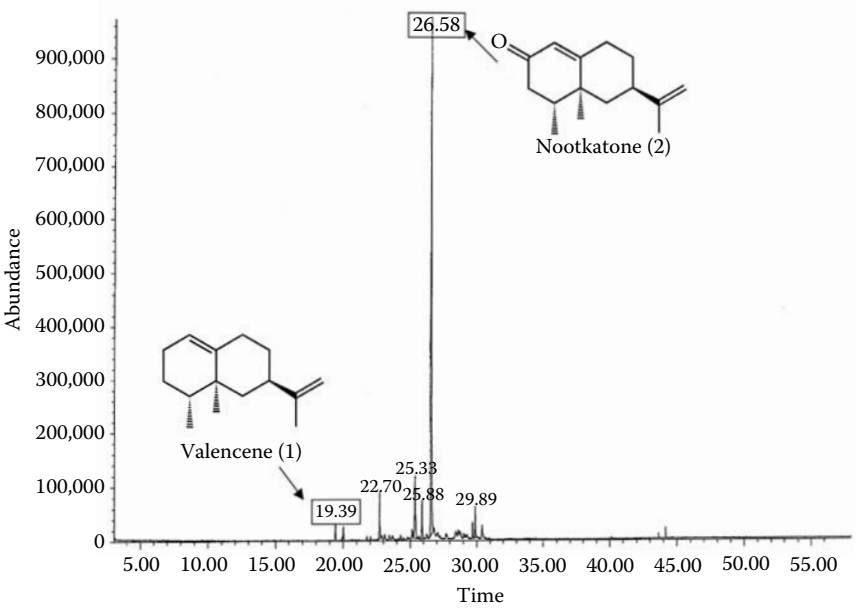


FIGURE 15.2 Total ion chromatogram of metabolites of valencene (**1**) by *Chlorella fusca* var. *vacuolata*.

TABLE 15.1
Conversion of Valencene (1) to Nootkatone (2) by *Chlorella* sp. for 14 Days

	Metabolites (% of the Total in GC-MS)				
***Chlorella* sp.**	**Valencene (1)**	**2α-Nootkatol (3)**	**2β-Nootkatol (4)**	**Nootkatone (2)**	**Conversion Ratio (%)**
C. fusca	11	0	0	89	89
C. pyrenoidosa	7	0	0	93	93
C. vulgaris	0	0	0	100	100

The biotransformation of compound **1** was further carried out by *Chlorella pyrenoidosa* and *Chlorella vulgaris* (Furusawa et al., 2005, 2005a) and soil bacteria (Noma et al., 2001) to give nootkatone in good yield (Table 15.1).

In the time course of the biotransformation of **1** by *Chlorella pyrenoidosa*, the yield of nootkatone (**2**) and nootkatol (**4**) without 2α-hydroxyvalencene (**3**) increased with the decrease in that of **1**, and subsequently the yield of **2** increased with decrease in that of **3**. In the metabolic pathway of valencene (**1**), **1** was slowly converted into nootkatol (**4**), and subsequently **4** was rapidly converted into **2**, as shown in Figure 15.3.

A fungus strain from the soil adhering to the thalloid liverwort *Pallavicinia subciliata*, *Mucor* species, which was inoculated and cultivated statically in Czapek-peptone medium (pH 7.0) at 30°C for 7 days. Compound **1** (20 mg/50 mL) was added to the medium and incubated for a further 7 days.

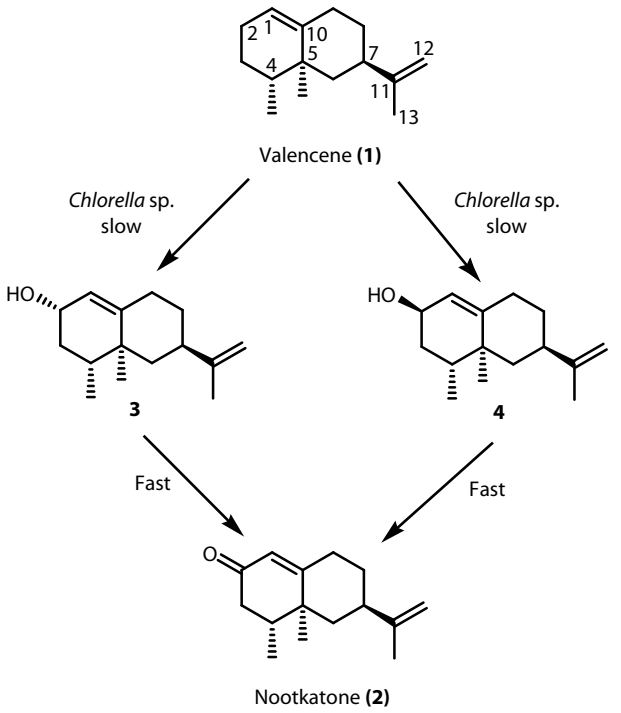


FIGURE 15.3 Biotransformation of valencene (**1**) by *Chlorella* species.

Nootkatone (**2**) was then obtained in very high yield (82%) (Furusawa et al., 2005; Noma et al., 2001a).

The biotransformation from **1** to **2** was also examined using the plant pathogenic fungi *Botryosphaeria dothidea* and *Botryodiplodia theobromae* (a total of 31 strains) separated from fungi infecting various types of fruit, and so on. *Botryosphaeria dothidea* and *Botryodiplodia theobromae* were both inoculated and cultivated while stationary in Czapek-peptone medium (pH 7.0) at 30°C for 7 days. The same size of the substrate **1** was added to each medium and incubated for a further 7 days to obtain nootkatone (42–84%) (Furusawa et al., 2005).

The expensive grapefruit aromatic, nootkatone (**2**) used by cosmetic and fiber manufacturers was obtained in high yield by biotransformation of (+)-valencene (**1**), which can be cheaply obtained from Valencia orange, by *Chlorella* species, fungi such as *Mucor* species, *Botryosphaeria dothidea*, and *Botryodiplodia theobromae*. This is a very inexpensive and clean oxidation reaction, which does not use any heavy metals, and thus this method is expected to find applications in the industrial production of nootkatone.

15.2.2 Biotransformation of Valencene (1) by *Aspergillus niger* and *Aspergillus wentii*

Valencene (**1**) from Valencia orange oil was cultivated by *Aspergillus niger* in Czapek-peptone medium, for 5 days to afford six metabolites **5** (1.0%), **6** and **7** (13.5%), **8** (1.1%), **9** (1.5%), **10** (2.0%), and **11** (0.7%), respectively. Ratio of compounds **6** (11*S*) and **7** (11*R*) was determined as 1:3 by HPLC analysis of their thiocarbonates (**12** and **13**) (Noma et al., 2001a) (Figure 15.4).

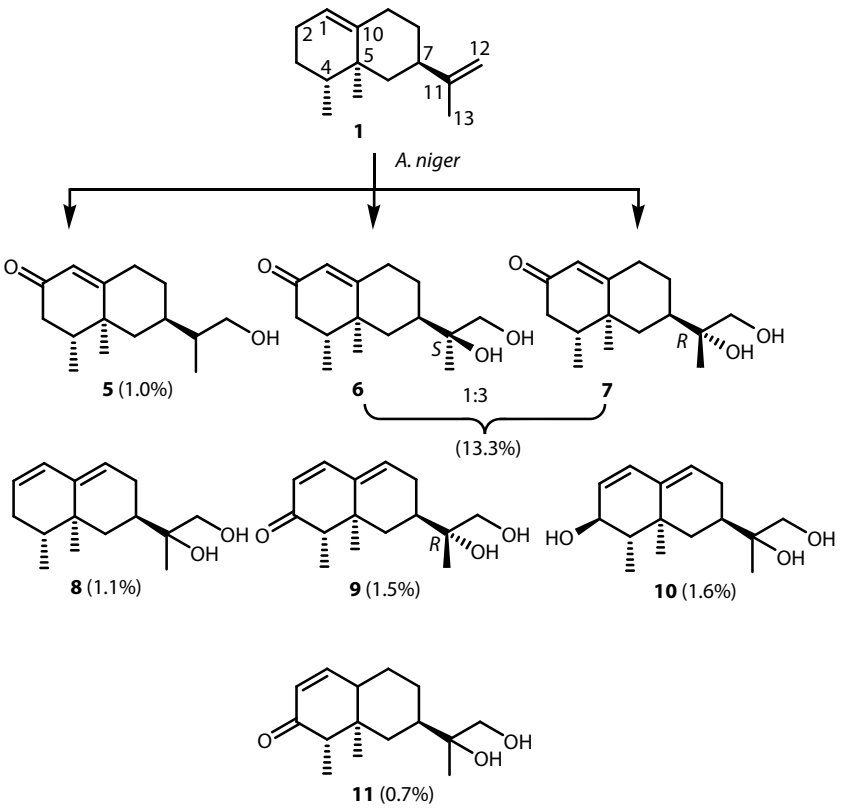


FIGURE 15.4 Biotransformation of valencene (**1**) by *Aspergillus niger*.

Compounds **8**–**11** could be biosynthesized by elimination of a hydroxy group of 2-hydroxyvalencenes (**3**, **4**). Compound **3** was biotransformed for 5 days by *Aspergillus niger* to give three metabolites **6** and **7** (6.4%), **8** (34.6%), and **9** (5.5%), respectively. Compound **4** was biotransformed for 5 days by *Aspergillus niger* to give three metabolites: **6** and **7** (21.8%), **9** (5.5%), and **10** (10.4%), respectively (Figure 15.5).

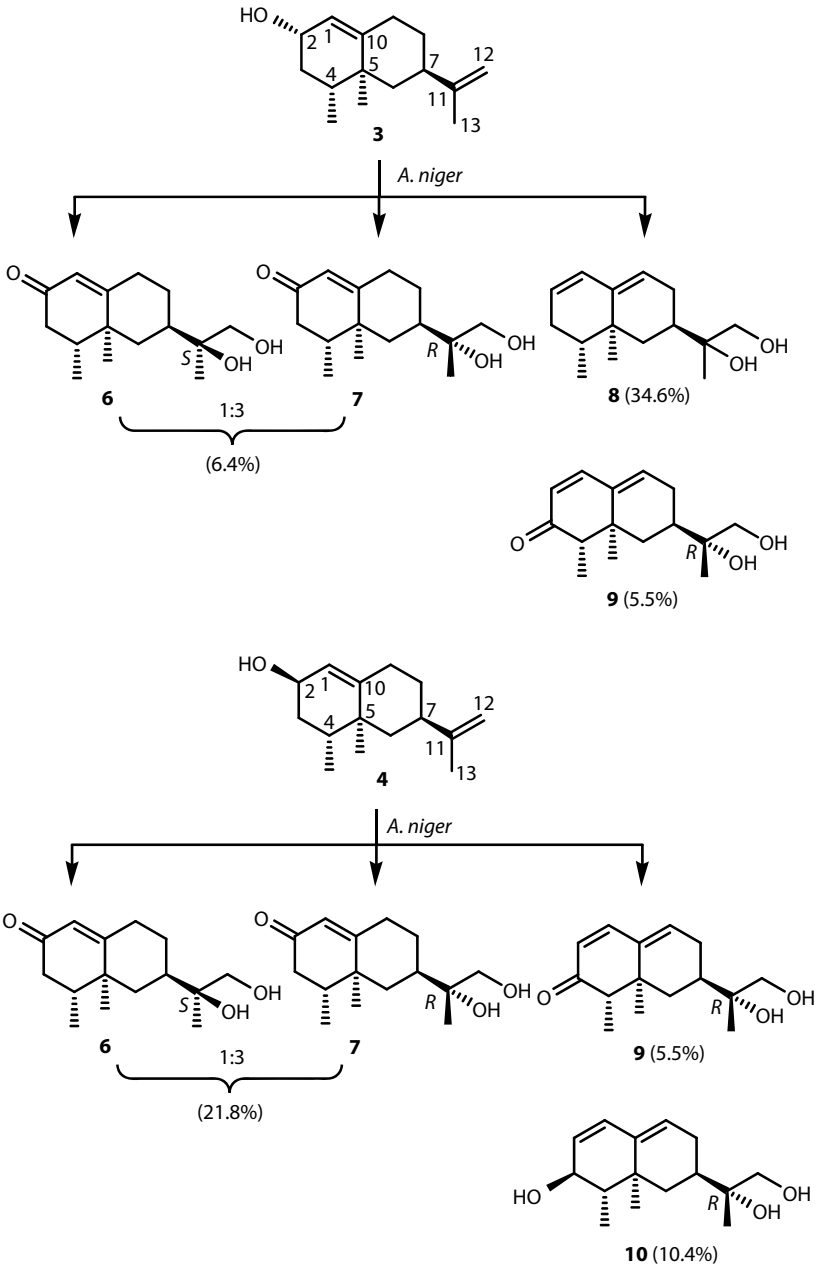


FIGURE 15.5 Biotransformation of 2α-hydroxyvalencene (**3**) and 2β-hydroxyvalencene (**4**) by *Aspergillus niger*.

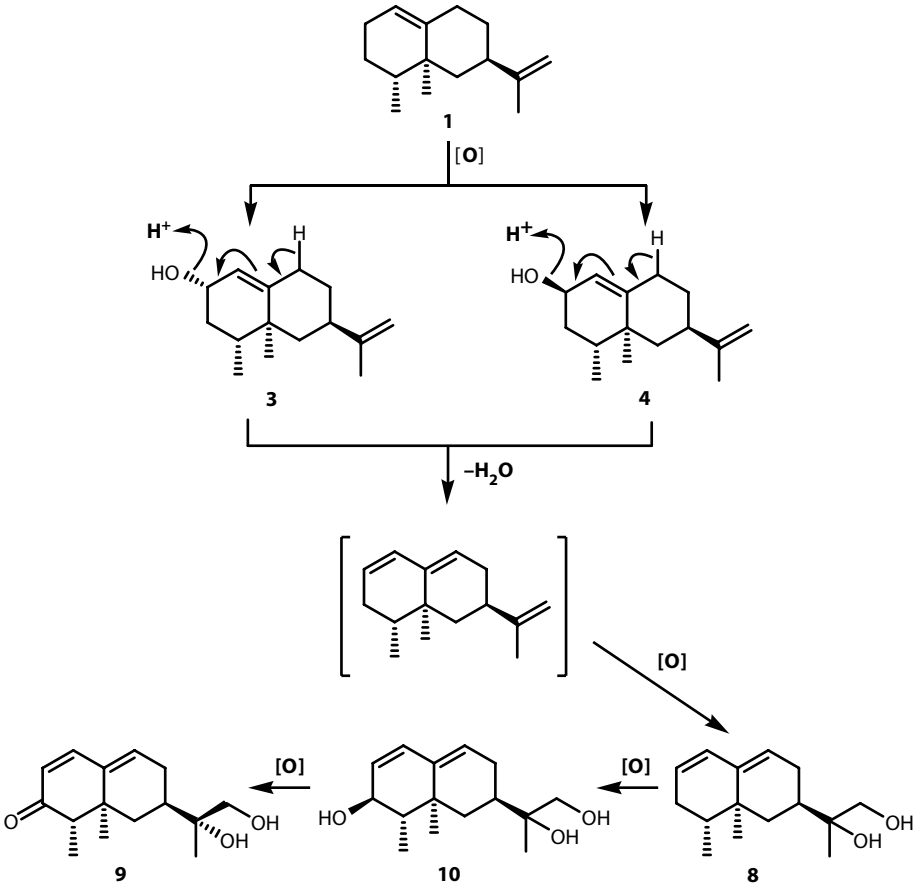


FIGURE 15.6 Possible pathway of biotransformation of valencene (**1**) by *Aspergillus niger.*

Both ratios of **6** (11*S*) and **7** (11*R*) obtained from **3** and **4** were 1:3, respectively. From the above results, plausible metabolic pathways of valencene (**1**) and 2-hydroxyvalencene (**3**, **4**) by *Aspergillus niger* are shown in Figure 15.6 (Noma et al., 2001a).

Aspergillus wentii and *Eurotrium purpurasens* converted valencene (**1**) to 11,12-epoxide (**14a**) and the same diol (**6**, **7**) (Takahashi and Miyazawa, 2005) as well as nootkatone (**2**) and 2α-hydroxyvalencene (**3**) (Takahashi and Miyazawa, 2006).

Kaspera et al. (2005) reported that valencene (**1**) was incubated in submerged cultures of the ascomycete *Chaetomium globosum*, to give nootkatone (**2**), 2α-hydroxyvalencene (**3**), and valencene 11,12-epoxide (**14a**), together with a valencene ketodiol, valencenediols, a valencene ketodiol, a valencene triol, or valencene epoxydiol that were detected by liquid chromatography-mass spectrocopy (LC-MS) spectra and GC-MS of trimethyl silyl derivatives. These metabolites are accumulated preferably inside the fungal cells (Figure 15.7).

The metabolites of valencene, nootkatone (**2**), (**3**), and (**14a**), indicated grapefruit with sour and citrus with bitter odor, respectively. Nootkatone 11,12-epoxide (**14**) showed no volatile fragrant properties.

15.2.3 Biotransformation of Nootkatone (2) by *Aspergillus niger*

Aspergillus niger was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium at 30°C for 7 days. (+)-Nootkatone (**2**), ($[\alpha]_D$ + 193.5°, *c* = 1.0), (80 mg/200 mL), which was isolated

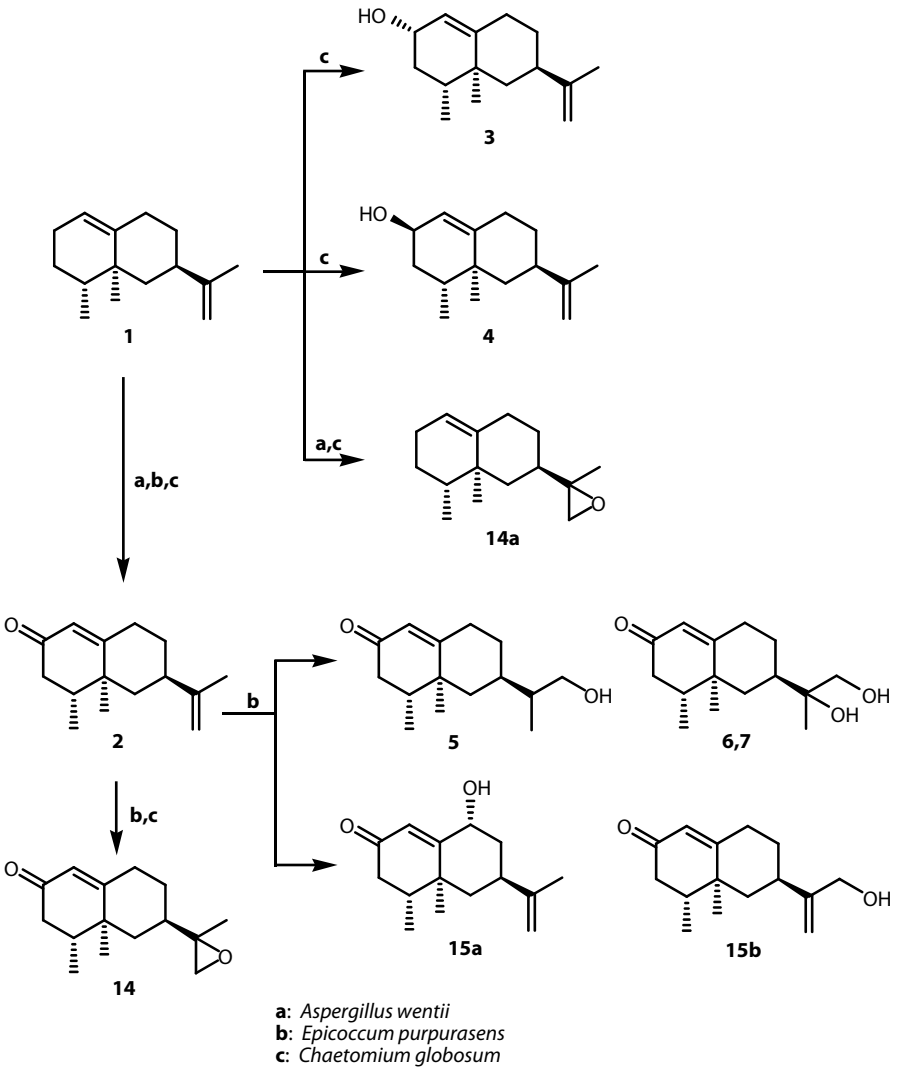


FIGURE 15.7 Biotransformation of valencene (**1**) and nootkatone (**2**) by *Aspergillus wentii, Epicoccum purpurascens,* and *Chaetomium globosum*.

from the essential oil of grapefruit, was added to the medium and further cultivated for 7 days to obtain two metabolites, 12-hydroxy-11,12-dihydronootkatone (**5**) (10.6%) and C11 stereo-mixtures (51.5%) of nootkatone-11*S*,12-diol (**6**) and its 11*R* isomer (**7**) (11*R*:11*S* = 1:1) (Hashimoto et al., 2000a; Noma et al., 2001a; Furusawa et al., 2003) (Figure 15.8).

11,12-epoxide (**14**) obtained by epoxidation of nootkatone (**2**) with *m*CPBA was biotransformed by *Aspergillus niger* for 1 day to afford **6** and **7** (11*R*:11*S* = 1:1) in good yield (81.4%). 1-aminobenzotriazole, an inhibitor of CYP450, inhibited the oxidation process of **1** into compounds **5**–**7** (Noma et al., 2001a). From the above results, possible metabolic pathways of nootkatone (**2**) by *Aspergillus niger* might be considered as shown in Figure 15.9.

The same substrate was incubated with *Aspergillus wentii* to produce diol (**6**, **7**) and 11,12--epoxide (**14**) (Takahashi and Miyazawa, 2005).

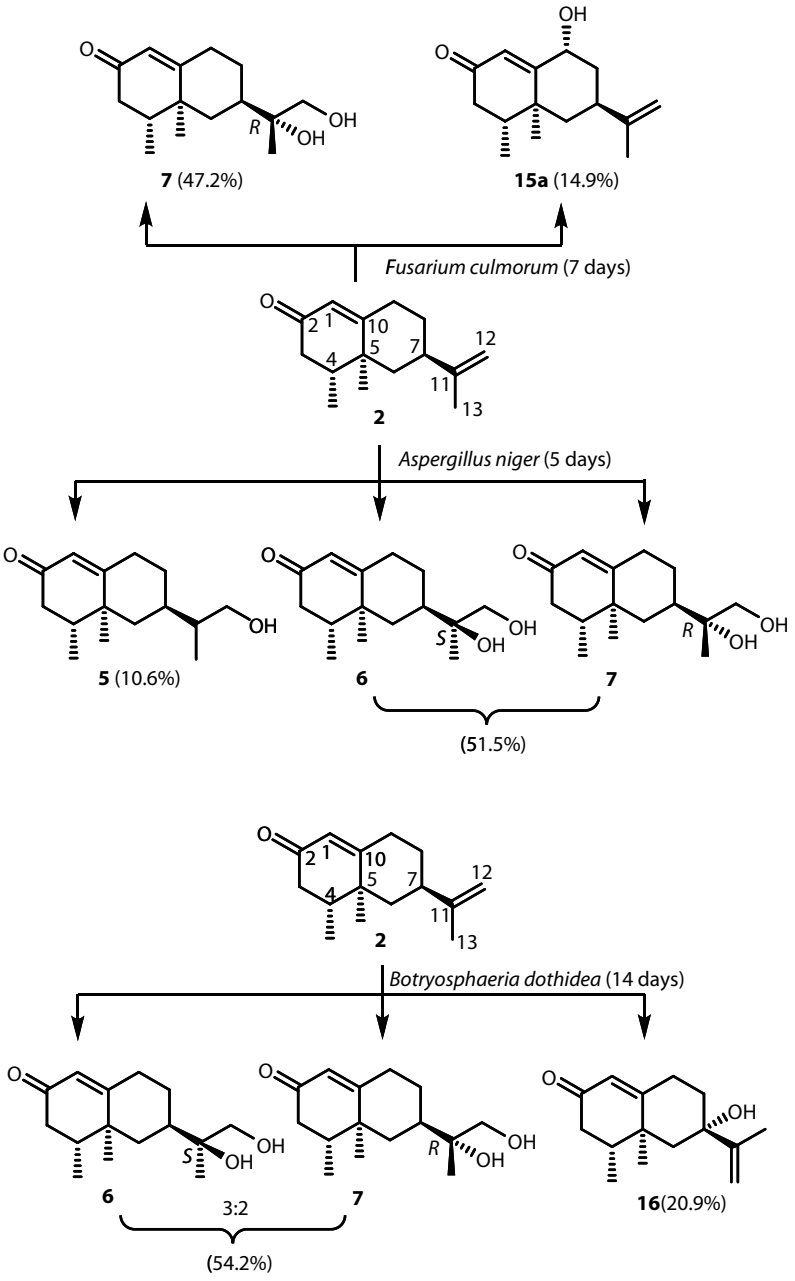


FIGURE 15.8 Biotransformation of nootkatone (**2**) by *Fusarium culmorum*, *Aspergillus niger*, and *Botryosphaeria dothidea*.

15.2.4 Biotransformation of Nootkatone (2) by *Fusarium culmorum* and *Botryosphaeria dothidea*

(+)-Nootkatone (**2**) was added to the same medium as mentioned above including *Fusarium culmorum* to afford nootkatone-11*R*,12-diol (**7**) (47.2%) and 9β-hydroxynootkatone (**15**) (14.9%) (Noma et al., 2001a).

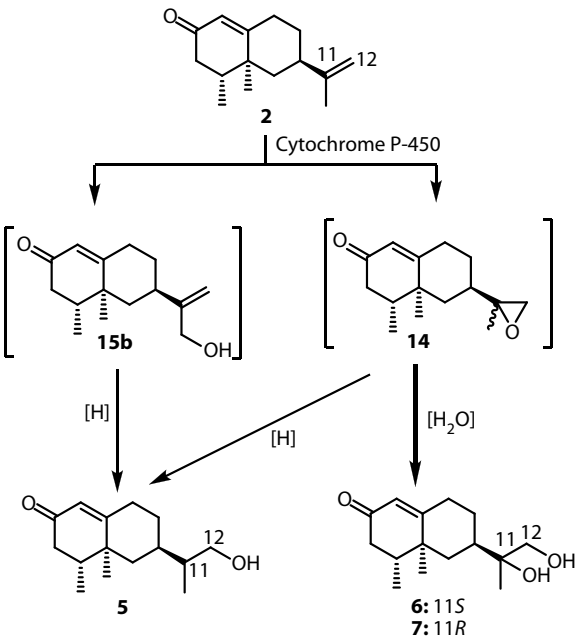


FIGURE 15.9 Possible pathway of biotransformation of valencene (**1**) by Cytochrome P-450.

Compound **7** was stereospecifically obtained at C11 by biotransformation of **1**. Purity of compound **7** was determined as ca. 95% by high performance liquid chromatography (HPLC) analysis of the thiocarbonate (**13**).

The biotransformation of nootkatone (**2**) was examined by the plant pathogenic fungus, *Botryosphaeria dothidea* separated from the fungus that infected the peach. (+)-Nootkatone (**2**) was cultivated with *Botryosphaeria dothidea* (Peach PP8402) for 14 days to afford nootkatone diols (**6** and **7**) (54.2%) and 7α-hydroxynootkatone (**16**) (20.9%). Ratio of compounds **6** and **7** was determined as 3:2 by HPLC analysis of the thiocarbonates (**12**, **13**) (Noma et al., 2001a). Nootkatone (**2**) was administered into rabbits to give the same diols (**6**, **7**) (Asakawa et al., 1986; Ishida, 2005).

Epicoccum purpurascens also biotransformed nootkatone (**2**) to **5**–**7**, **14**, and **15a** (Takahashi and Miyazawa, 2006).

The biotransformation of **2** by *Aspergillus niger* and *Botryosphaeria dothidea* resembled to that of the oral administration to rabbit since the ratio of the major metabolites 11*S*- (**6**) and 11*R*-nootkatone-11,12-diol (**7**) was similar. It is noteworthy that the biotransformation of **2** by *Fusarium culmorum* affords stereospecifically nootkatone-11*R*, 12-diol (**7**) (Noma et al., 2001a) (Figure 15.10).

Metabolites **3**–**5**, **12**, and **13** from (+)-nootkatone (**2**) and **14**–**17** from (+)-valencene (**1**) did not show an effective odor.

Dihydronootkatone (**17**), which shows that citrus odor possesses antitermite activity, was also treated in *Aspergillus niger* to obtain 11*S*-mono- (**18**) and 11*R*-dihydroxylated products (**19**) (the ratio 11*S* and 11*R* = 3:2). On the other hand, *Aspergillus cellulosae* reduced ketone group at C2 of **17** to give 2α- (**20**) (75.7%) and 2β-hydroxynootkatone (**21**) (0.7%) (Furusawa et al., 2003) (Figure 15.11).

Tetrahydronootkatone (**22**) also shows antitermite and mosquito-repellant activity. It was incubated with *Aspergillus niger* to give two similar hydroxylated compounds (**23**, 13.6% and **24**, 9.9%) to those obtained from **17** (Furusawa, 2006) (Figure 15.12).

8,9-Dehydronootkatone (**25**) was incubated with *Aspergillus niger* to give four metabolites, a unique acetonide (**26**, 15.6%), monohydroxylated (**27**, 0.2%), dihydroxylated (**28**, 69%), and a carboxyl derivative (**29**, 0.8%) (Figure 15.13).

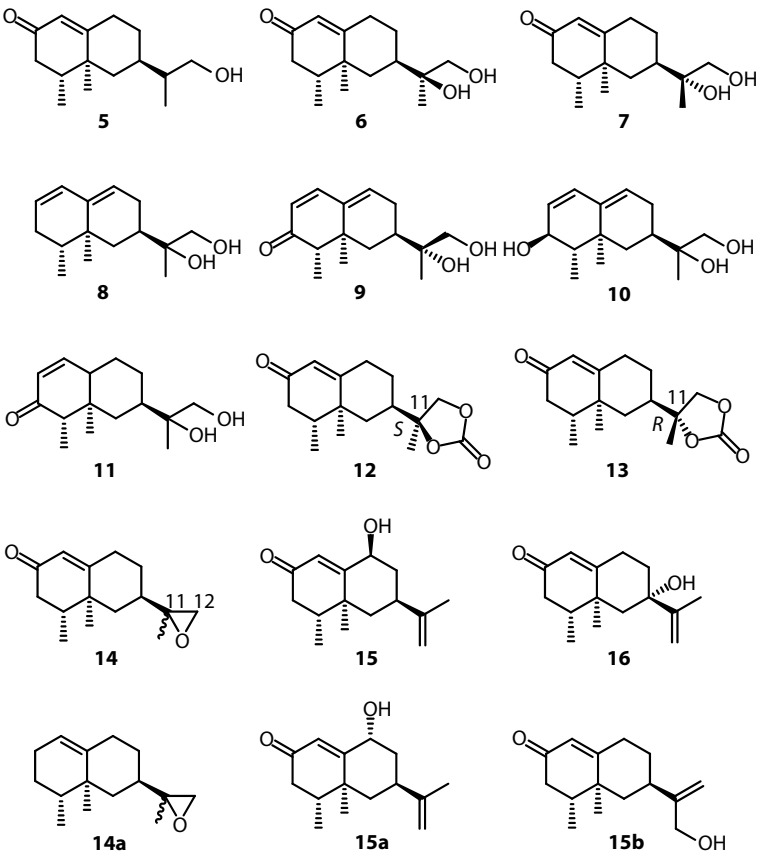


FIGURE 15.10 Metabolites (**5**–**11**, **14**–**15b**) from valencene (**1**) and nootkatone (**2**) by various microorganisms.

When the same substrate was treated in *Aspergillus sojae* IFO 4389, compound **25** was converted to the different monohydroxylated product (**30**, 15.8%) from that mentioned above. *Aspergillus cellulosae* is an interesting fungus since it did not give any same products as mentioned above; in place, it produced trinorsesquitepene ketone (**31**, 6%) and nitrogen-containing aromatic compound (**32**) (Furusawa et al., 2003) (Figure 15.14).

Mucor species also oxidized compound **25** to give three metabolites, 13-hydroxy-8,9-dehydronootkatone (**33**, 13.2%), an epoxide (**34**, 5.1%), and a diol (**35**, 19.9%) (Furusawa et al., 2003). The same substrate was investigated with cultured suspension cells of the liverwort, *Marchantia polymorpha* to afford **33** (Hegazy et al., 2005) (Figure 15.15).

Although *Mucor* species could give nootkatone (**21**) from valencene (**1**), this fungus biotransformed the same substrate (**25**) to the same alcohol (**30**, 13.2%) obtained from the same starting compound (**25**) in *Aspergillus sojae*, a new epoxide (**34**, 5.1%) and a diol (**35**, 9.9%).

The metabolites (**3**, **4**, **20**, **21**) inhibited the growth of lettuce stem, and **3** and **4** inhibited germination of the same plant (Hashimoto and Asakawa, 2007).

Valerianol (**35a**), from *Valeriana officinalis* whose dried rhizome is traditionally used for its carminative and sedative properties, was biotransformed by *Mucor plumbeus*, to produce three metabolites, a bridged ether (**35b**), and a triol (**35c**), which might be formed via C1–C10 epoxide, and **35d** arises from double dehydration (Arantes et al., 1999). In this case, allylic oxidative compounds have not been found (Figure 15.16).

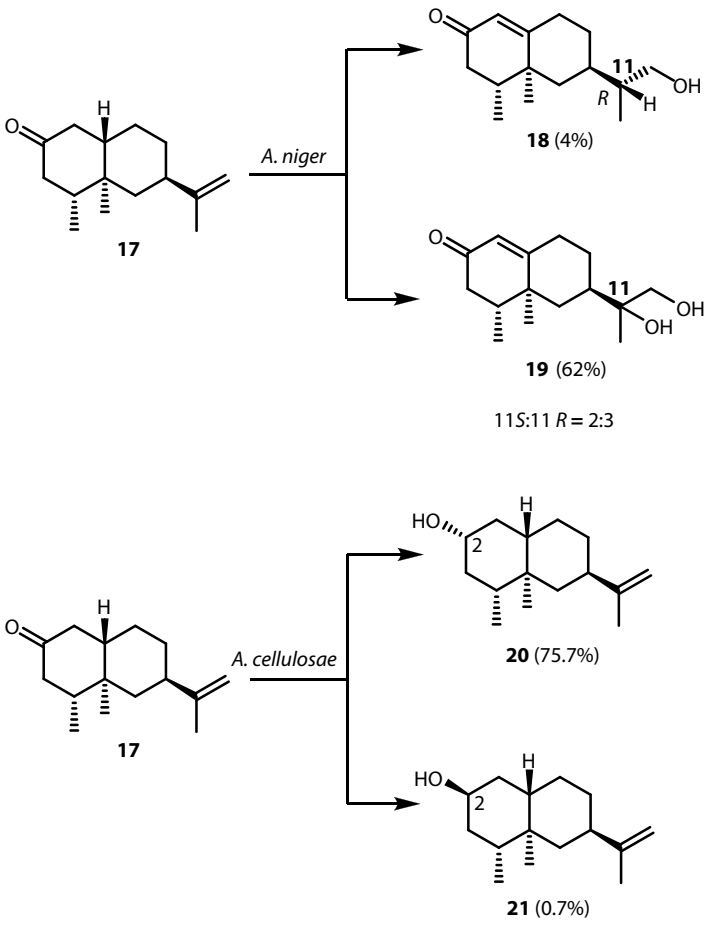


FIGURE 15.11 Biotransformation of dihydronootkatone (**17**) by *Aspergillus niger* and *Aspergillus cellulosae*.

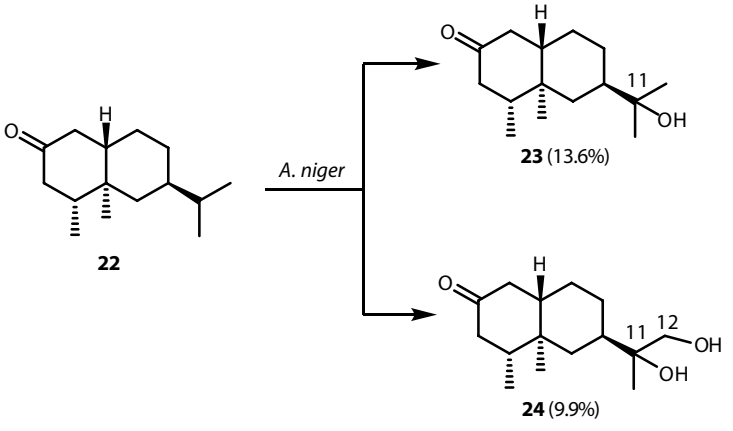


FIGURE 15.12 Biotransformation of tetrahydronootkatone (**22**) by *Aspergillus niger*.

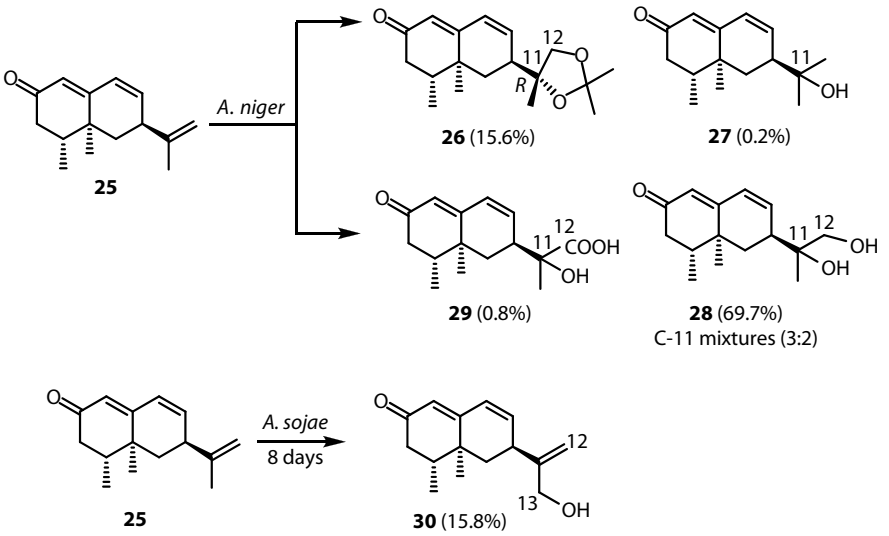


FIGURE 15.13 Biotransformation of 8,9-dehydronootkatone (**25**) by *Aspergillus sojae*.

15.2.5 Biotransformation of (+)-1(10)-Aristolene (36) from the Crude Drug *Nardostachys chinensis* by *Chlorella fusca*, *Mucor* species, and *Aspergillus niger*

The structure of sesquiterpenoid, (+)-1(10)-aristolene (= calarene) (**36**) from the crude drug *Nardostachys chinensis* was similar to that of nootkatone. 2-Oxo-1(10)-aristolene (**38**) shows antimelanin inducing activity and excellent citrus fragrance. On the other hand, the enantiomer (**37**) of **36** and (+)-aristolone (**41**) were also found in the liverworts as the natural products. In order

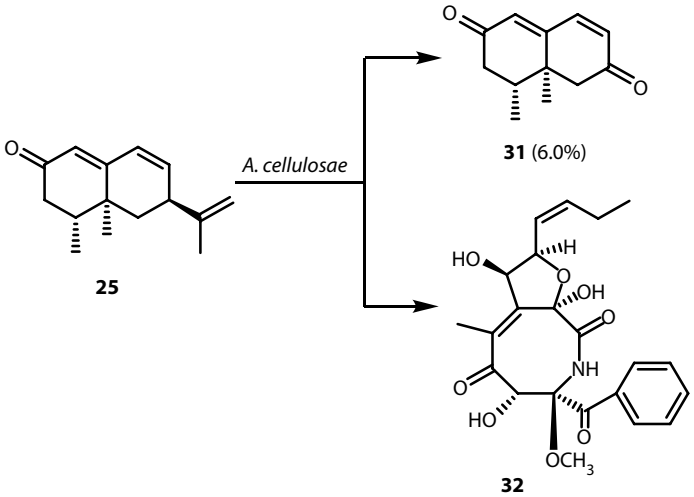


FIGURE 15.14 Biotransformation of 8,9-dehydronootkatone (**25**) by *Aspergillus cellulosae*.

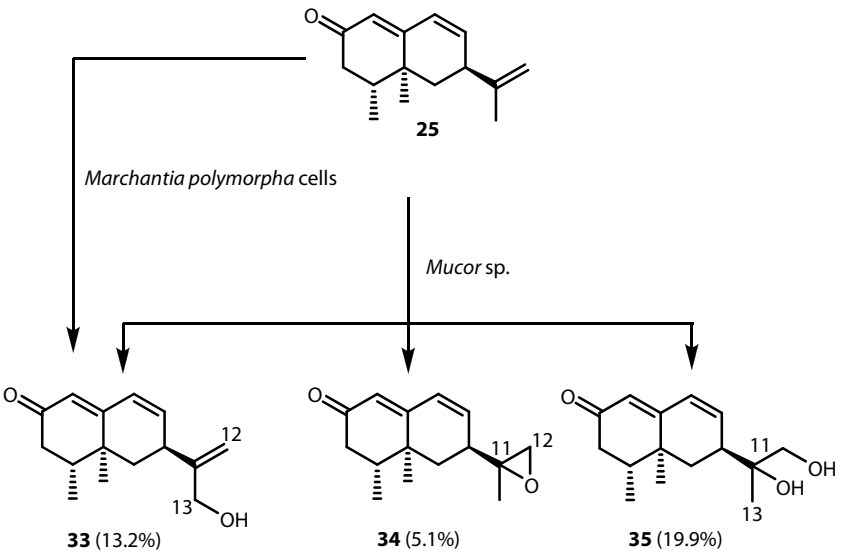


FIGURE 15.15 Biotransformation of 8,9-dehydronootkatone (**25**) by *Marchantia polymorpha* and *Mucor* species.

to obtain compound **38** and its analogues, compound **36** was incubated with *Chlorella fusca* var. *vacuolata* IAMC-28, *Mucor* species, and *Aspergillus niger* (Furusawa et al., 2006a) (Figure 15.17).

Chlorella fusca was inoculated and cultivated stationary in Noro medium (pH 8.0) at 25°C for 7 days and (+)-1(10)-aristolene (**36**) (20 mg/50 mL) was added to the medium and further incubated for 10–14 days and cultivated stationary under illumination (pH 8.0) at 25°C for 7 days to afford 1(10)-aristolen-2-one (**38**, 18.7%), (−)-aristolone (**39**, 7.1%), and 9-hydroxy-1(10)-aristolen-2-one (**40**). Compounds **38** and **40** were found in *Aristolochia* species (Figure 15.18).

Mucor species was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0) at 30°C for 7 days. (+)-1(10)-Aristolene (**36**) (100 mg/200 mL) was added to the medium and further for 7 days. The crude metabolites contained **38** (0.9%) and **39** (0.7%) as very minor products (Figure 15.19).

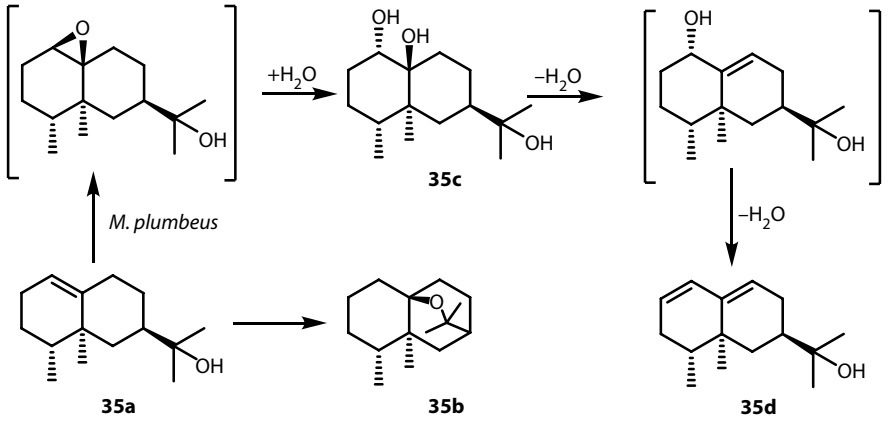


FIGURE 15.16 Biotransformation of valerianol (**35a**) by *Mucor plumbeus.*

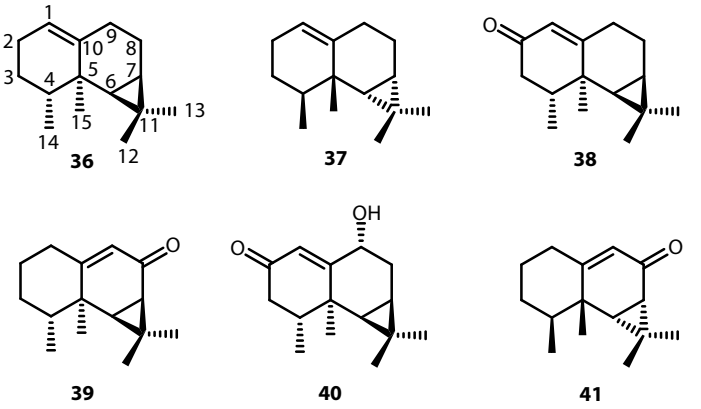


FIGURE 15.17 Naturally occurring aristolane sesquiterpenoids.

Although *Mucor* species produced a large amount of nootkatone (**2**) from valencene (**1**), however, only poor yield of similar products as those from valencene (**1**) was seen in the biotransformation of tricyclic substrate (**36**). Possible biogenetic pathway of (+)-1(10)-aristolene (**36**) is shown in Figure 15.20.

Aspergillus niger was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0) at 30°C for 3 days. (+)-1(10)-Aristolene (**36**) (100 mg/200 mL) was added to the medium and further for 7 days. From the crude metabolites four new metabolic products (**42**, 1.3%), (**43**, 3.2%), (**44**, 0.98%), and (**45**, 2.8%) were obtained in very poor yields (Figure 15.21). Possible metabolic pathways of **36** by *Aspergillus niger* are shown in Figure 15.22.

Commercially available (+)-1(10)-aristolene (**36**) was treated with *Diplodia gossypina* and *Bacillus megaterium*. Both microorganisms converted **36** to four (**46**–**49**; 0.8%, 1.1, 0.16%, 0.38%) and six metabolites, (**40**, **50–55**; 0.75%, 1.0%, 1.0%, 2.0%, 1.1%, 0.5%, 0.87%), together with **40** (0.75%) respectively (Abraham et al., 1992) (Figure 15.23).

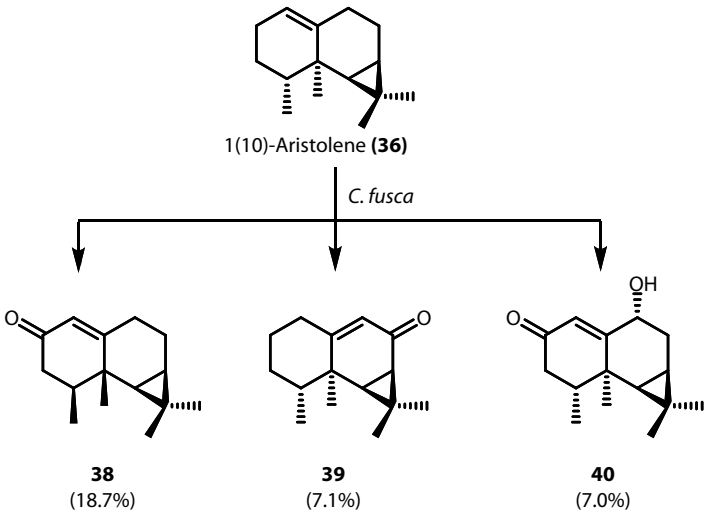


FIGURE 15.18 Biotransformation of 1(10)-aristolene (**36**) by *Chlorella fusca*.

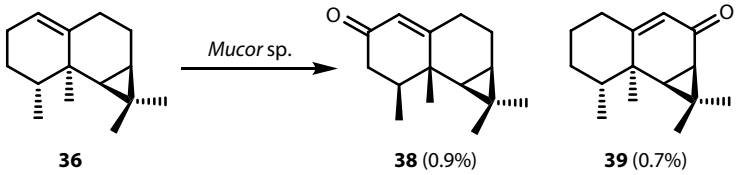


FIGURE 15.19 Biotransformation of 1(10)-aristolene (**36**) by *Mucor* species.

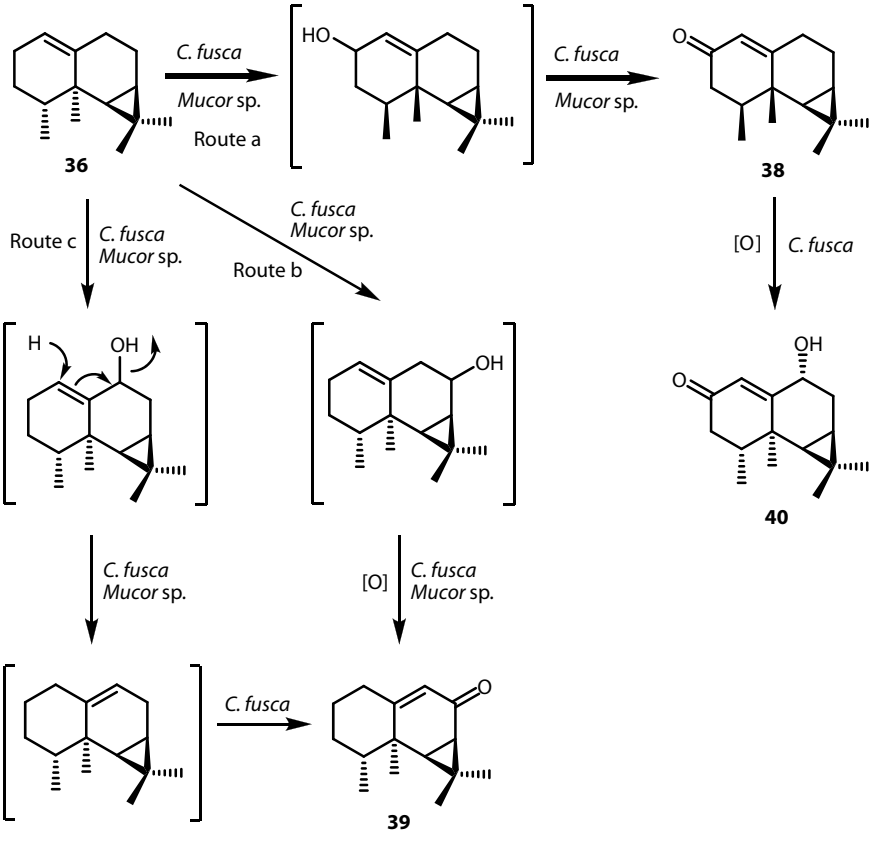


FIGURE 15.20 Possible pathway of biotransformation of 1(10)-aristolene (**36**) by *Chlorella fusca* and *Mucor* species.

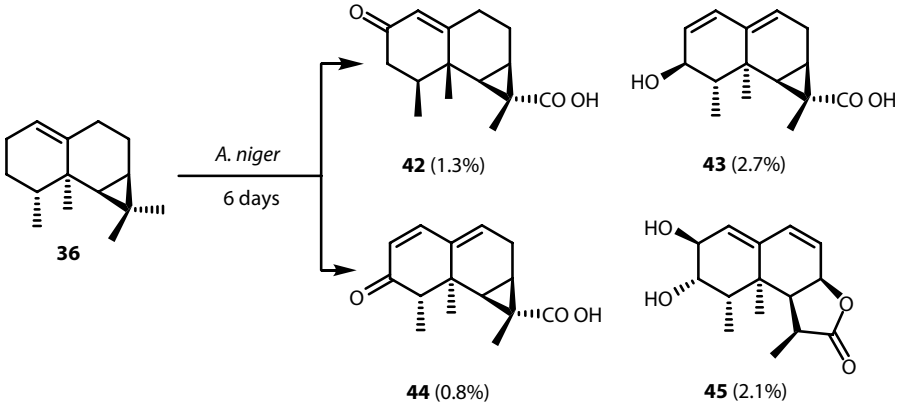


FIGURE 15.21 Biotransformation of 1(10)-aristolene (**36**) by *Aspergillus niger.*

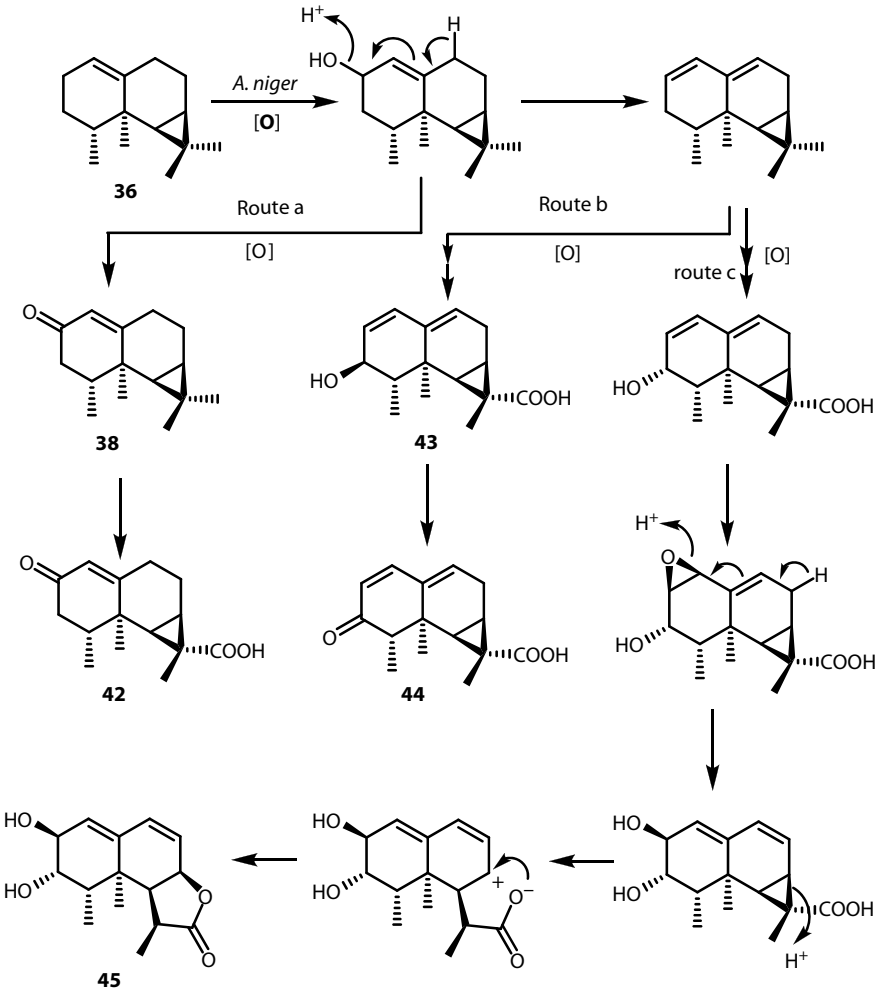


FIGURE 15.22 Possible pathway of biotransformation of 1(10)-aristolene (**36**) by *Aspergillus niger.*

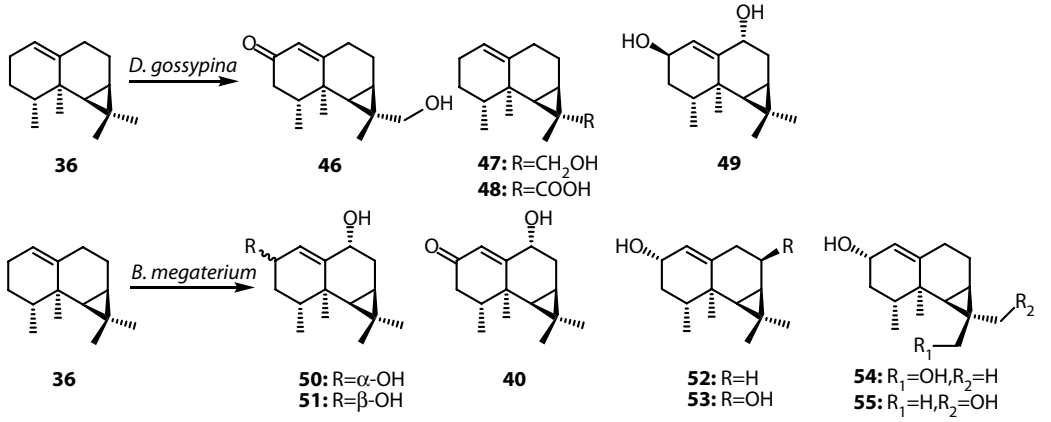


FIGURE 15.23 Biotransformation of 1(10)-aristolene (**36**) by *Diplodia gossypina* and *Bacillus megaterium.*

It is noteworthy that *Chlorella* and *Mucor* species introduce hydroxyl group at C2 of the substrate (**36**) as seen in the biotransformation of valencene (**1**) while *Diplodia gossypina* and *Bacillus megaterium* oxidizes C2, C8, C9, and/or 1,1-dimethyl group on a cyclopropane ring. *Aspergillus niger* oxidizes not only C2 but also stereoselectively oxidized one of the gem-dimethyl groups on cyclopropane ring. Stereoselective oxidation of one of gem-dimethyl of cyclopropane and cyclobutane derivatives is observed in biotransformation using mammals (see later).

15.2.6 Biotransformation of Various Sesquiterpenoids by Microorganisms

Aromadendrane-type sesquiterpenoids have been found not only in higher plants but also in liverworts and marine sources. Three aromadendrenes (**56**, **57**, **58**) were biotransformed by *Diplodia gossypina*, *Bacillus megaterium*, and *Mycobacterium smegmatis* (Abraham et al., 1992). Aromadendrene (**56**) (800 mg) was converted by *Bacillus megaterium* to afford a diol (**59**) and a triol (**60**) of which **59** (7 mg) was the major product. The triol (**60**) was also obtained from the metabolite of (+)-(1*R*)-aromadendrene (**56**) by the plant pathogen *Glomerella cingulata* (Miyazawa et al., 1995a). *allo*-Aromadendrene (**57**) (1.2 g) was also treated in *Mycobacterium smegmatis* to afford **61** (10 mg) (Abraham et al., 1992) (Figure 15.24).

The same substrate was also incubated with *Glomerella cingulata* to afford C10 epimeric triol (**62**) (Miyazawa et al., 1995a). Globulol (**58**) (400 mg) was treated in *Mycobacterium smegmatis* to give only a carboxylic acid (**63**) (210 mg). The same substrate (**58**) (1 g) was treated in *Diplodia gossypina* and *Bacillus megaterium* to give two diols, **64** (182 mg), **65** and a triol (**66**) from the former and **67**–**69** from the latter organism among which **64** (60 mg) was predominant (Abraham et al., 1992). *Glomerella cingulata* and *Botrytis cinerea* also bioconverted globulol (**58**) to diol (**64**) regio- and stereoselectively (Miyazawa et al., 1994) (Figures 15.25 and 15.26).

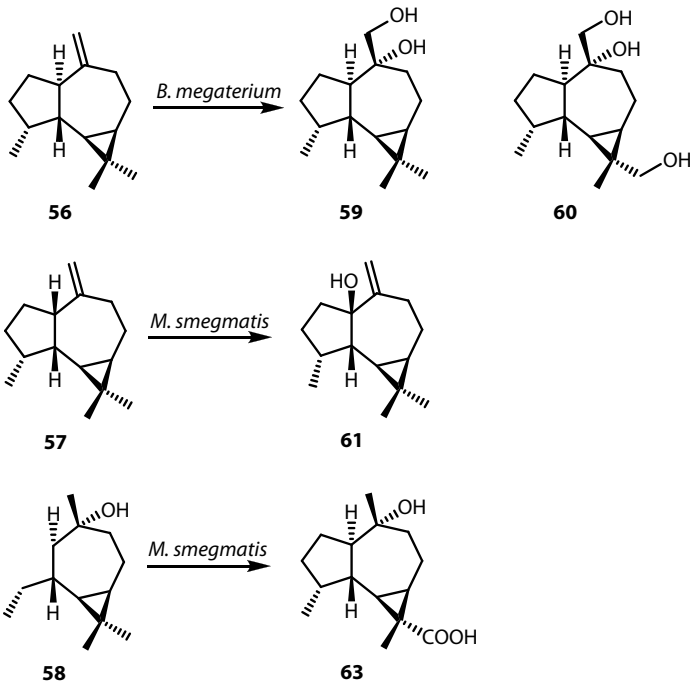


FIGURE 15.24 Biotransformation of aromadendrene (**56**), alloaromadendrene (**57**), and globulol (**58**) by *Bacillus megaterium* and *Mycobacterium smegmatis*.

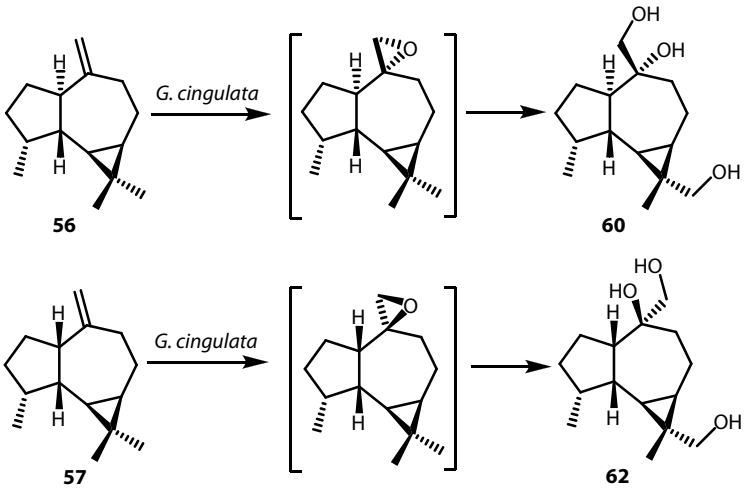


FIGURE 15.25 Biotransformation of aromadendrene (**56**) and alloaromadendrene (**57**) by *Glomerella cingulata*.

Globulol (**58**) (1.5 g) and 10-epiglobulol (**70**) (1.2 mL) were separately incubated with *Cephalosporium aphidicola* in shake culture for 6 days to give the same diol **64** (780 mg) as obtained from the same substrate by *Bacillus megaterium* mentioned above and **71** (720 mg), (Hanson et al., 1994). *Aspergillus niger* also converted globulol (**58**) and epiglobulol (**70**) to a diol (**64**) and

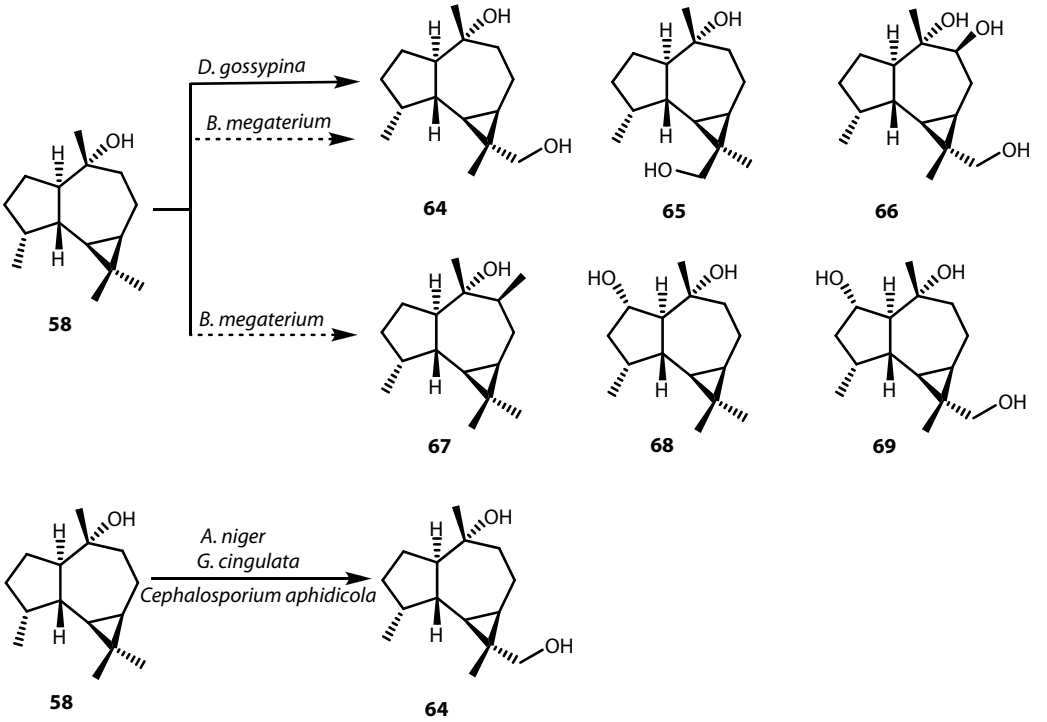


FIGURE 15.26 Biotransformation of globulol (**58**) by various microorganisms.

three 13-hydroxylated globulol (**71**, **72**, **74**) and 4α-hydroxylated product (**73**). The epimerization at C4 is very rare example (Hayashi et al., 1998).

Ledol (**75**), an epimer at C1 of globulol was incubated with *Glomerella cingulata* to afford C13 carboxylic acid (**76**) (Miyazawa et al., 1994) (Figure 15.27).

Squamulosone (**77**), aromadendr-1(10)-en-9-one isolated from *Hyptis verticillata* (Labiatae), was reduced chemically to give **78**–**82**, which were incubated with the fungus *Curvularia lunata* in two different growth media (Figure 15.28).

From **78**, two metabolites **80** and **83** were obtained. Compound **79** and **80** were metabolized to give ketone **81** as the sole product and **78** and **83**, respectively. From compound **81**, two metabolites, **79** and **84** were obtained (Figure 15.29). From the metabolite of the substrate (**82**), five products (**84**–**88**) were isolated (Collins, Reynold, and Reese, 2002) (Figure 15.30).

Squamulosone (**77**) was treated in the fungus *Mucor plumbeus* ATCC 4740 to give not only cyclopentanol derivatives (**89**, **90**) but also C12 hydroxylated products (**91**–**93**) (Collins, Ruddock, et al., 2002) (Figure 15.31).

Spathulenol (**94**), which is found in many essential oils, was fed by *Aspergillus niger* to give a diol (**95**) (Higuchi et al., 2001). *Ent*-10β-hydroxycyclocolorenone (**96**) and myli-4(15)-en-9-one (**96a**) isolated from the liverwort *Mylia taylorii* were incubated with *Aspergillus niger* IFO 4407 to give C10 epimeric product (**97**) (Hayashi et al., 1999) and 12-hydroxylated product (**96b**), respectively (Nozaki et al., 1996) (Figures 15.32 and 15.33).

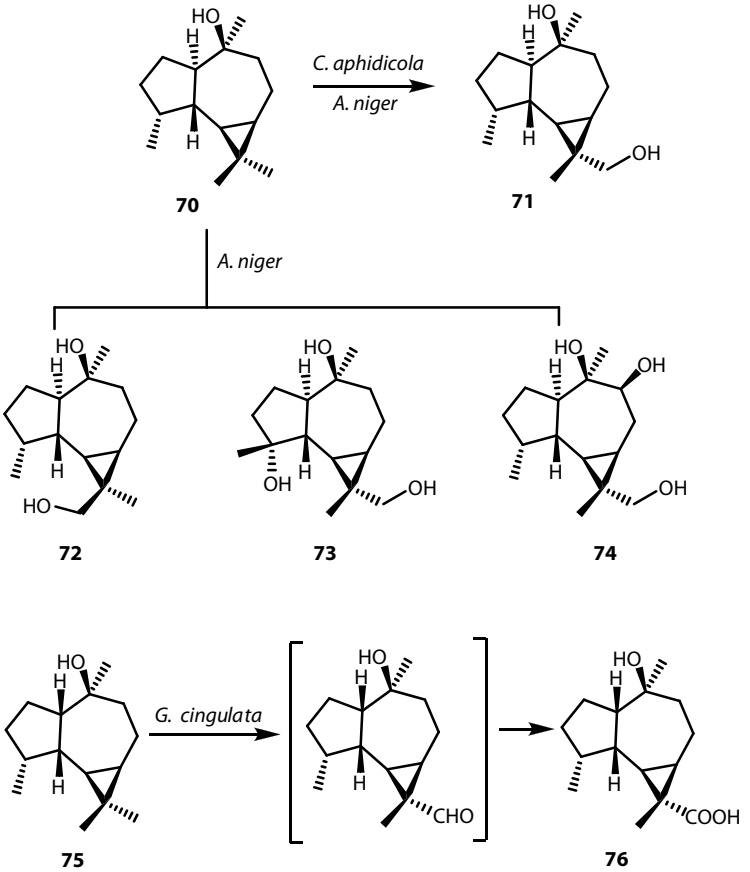


FIGURE 15.27 Biotransformation of 10-epi-glubulol (**70**) and ledol (**75**) by *Cephalosporium aphidicola*, *Aspergillus niger*, and *Glomerella cingulata*.

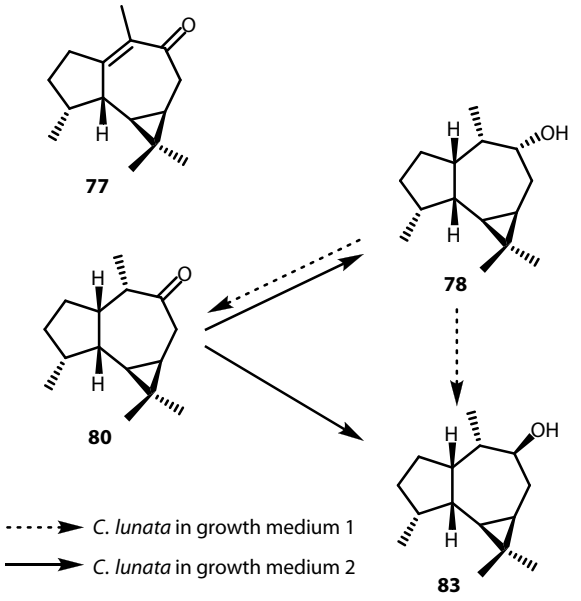


FIGURE 15.28 Biotransformation of aromadendra-9-one (**80**) by *Curvularia lunata*.

(+)-*ent*-Cyclocolorenone (**98**) $[\alpha]_D - 405°$ (c = 8.8, EtOH), one of the major compounds isolated from the liverwort *Plagiochila sciophila* (Asakawa, 1982, 1995), was treated by *Aspergillus niger* to afford three metabolites, 9-hydroxycyclocolorenone (**99**, 15.9%) 12-hydroxy-(+)-cyclocolorenone (**100**, 8.9%) and a unique cyclopropane-cleaved metabolite, 6β-hydroxy-4,11-guaiadien-3-one (**101**, 35.9%), and 6β,7β-dihydroxy-4,11-guaiadien-3-one (**102**, trace), of which **101** was the major component. The enantiomer (**103**) $[\alpha]_D + 402°$ (c = 8.8, EtOH) of **98** isolated from *Solidago altissima* was biotransformed by the same organism to give 13-hydroxycycolorenone (**103a**, 65.5%), the enantiomer of **100**, 1β,13-dihydroxycyclocolorenone (**103b**, 5.0%), and its C11-epimer (**103c**)

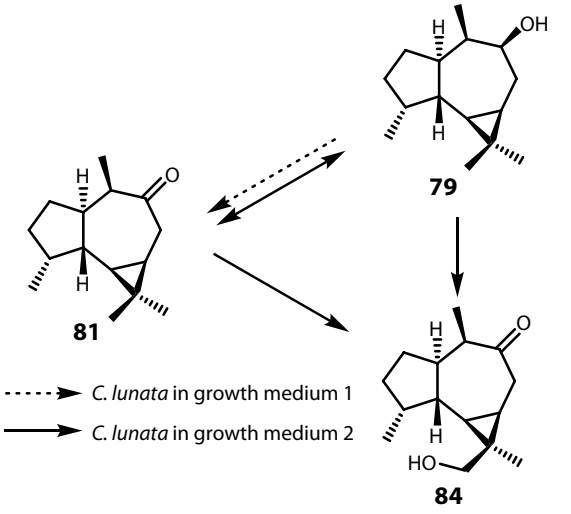


FIGURE 15.29 Biotransformation of 10-epi-aromadendra-9-one (**81**) by *Curvularia lunata*.

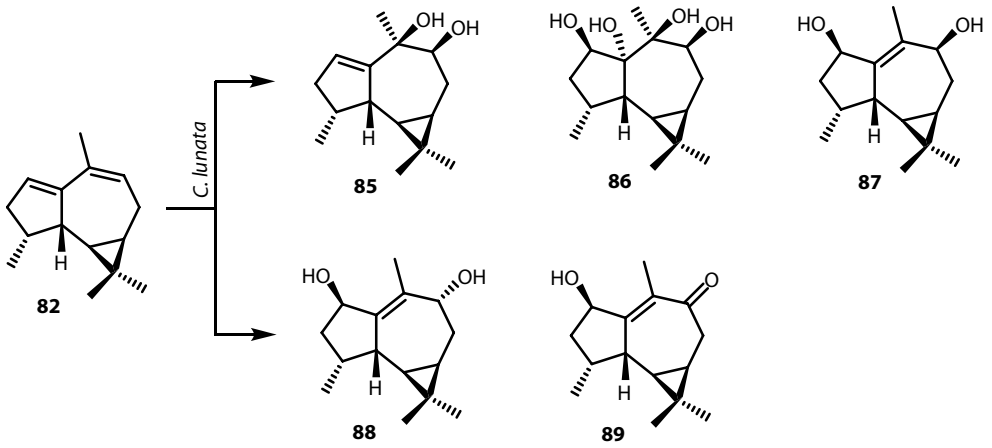


FIGURE 15.30 Biotransformation of aromadendr-1(10),9-diene (**82**) by *Curvularia lunata*.

(Furusawa et al., 2005b, 2006a). It is noteworthy that no cyclopropane-cleaved compounds from **103** have been detected in the crude metabolites even in GC-MS analysis (Figure 15.34).

Plagiochiline A (**104)** that shows potent insect antifeedant, cytotoxicity, and piscidal activity are very pungent 2,3-secoaromadendrane sesquiterpenoids having 1,1-dimethyl cyclopropane ring, isolated from the liverwort *Plagiochila fruticosa*. Plagiochilide (**105**) is the major component of this liverwort. In order to get more pungent component, the lactone (**105**, 101 mg) was incubated with

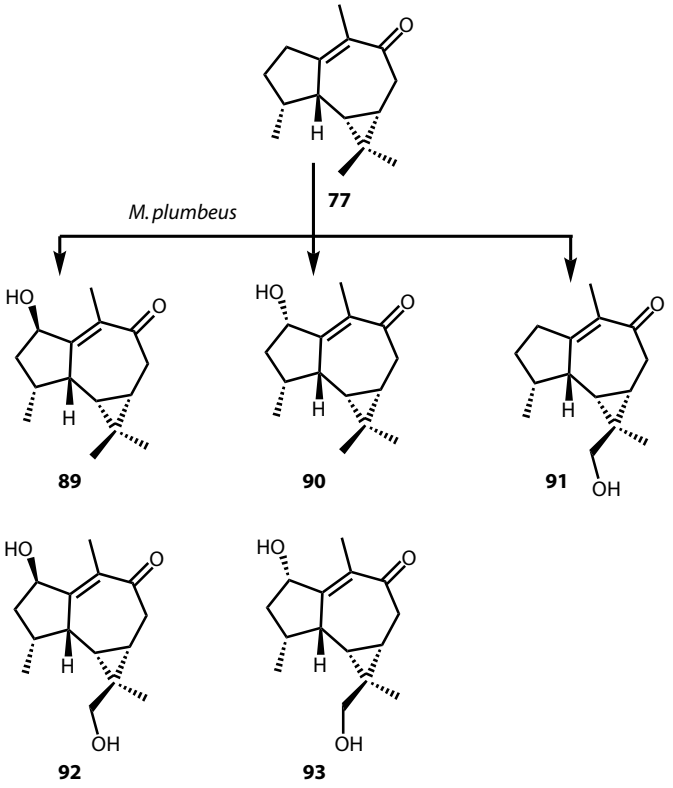
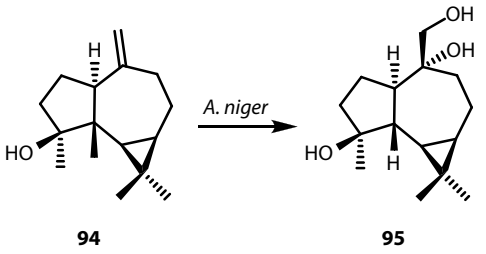


FIGURE 15.31 Biotransformation of squamulosone (**77**) by *Mucor plumbeus*.



94 **95**

FIGURE 15.32 Biotransformation of spathulenol (**94**) by *Aspergillus niger.*

Aspergillus niger to give two metabolites **106** (32.5%) and **107** (9.7%). Compound **105** was incubated in *Aspergillus niger* including 1-aminobenzotriazole, the inhibitor of CYP450, to produce only **106**, since this enzyme plays an important role in the formation of carboxylic acid (**107**) from primary alcohol (**106**). Unfortunately, two metabolites show nothing hot taste (Hashimoto et al., 2003c; Furusawa et al., 2006) (Figure 15.35).

Partheniol, 8α-hydroxybicyclogermacrene (**108**) isolated from *Parthenium argentatum* × *Parthenium Tometosa*, was cultured in the media of *Mucor circinelloides* ATCC 15242 to afford six metabolites, a humulane (**109**), three maaliane- (**110**, **112**, **113**), an aromadendrane- (**111**), and a tricylohumulane triol (**114**), the isomer of compound (**111**). Compounds **110**, **111**, and **114** were isolated as their acetates (Figure 15.36).

Compounds **110** might originate from the substrate by acidic transannular cyclization since the broth was pH 6.4 just before extraction (Maatooq, 2002).

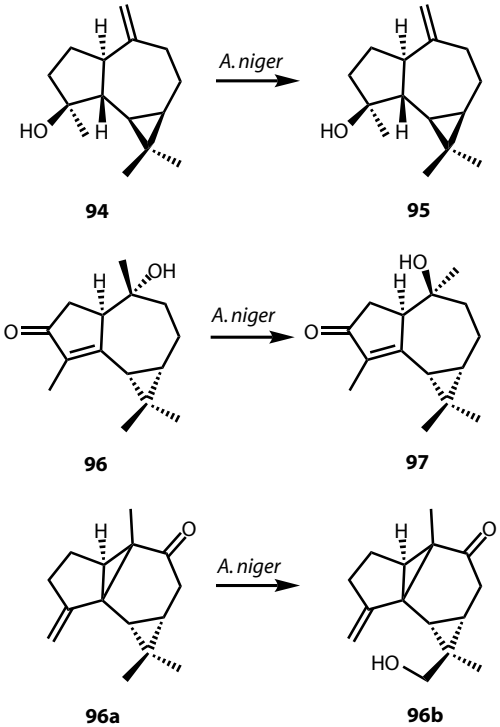


FIGURE 15.33 Biotransformation of spathulenol (**94**), *ent*-10β-hydroxycyclocolorenone (**96**) and myli-4-(15)-en-9-one (**96a**) by *Aspergillus niger.*

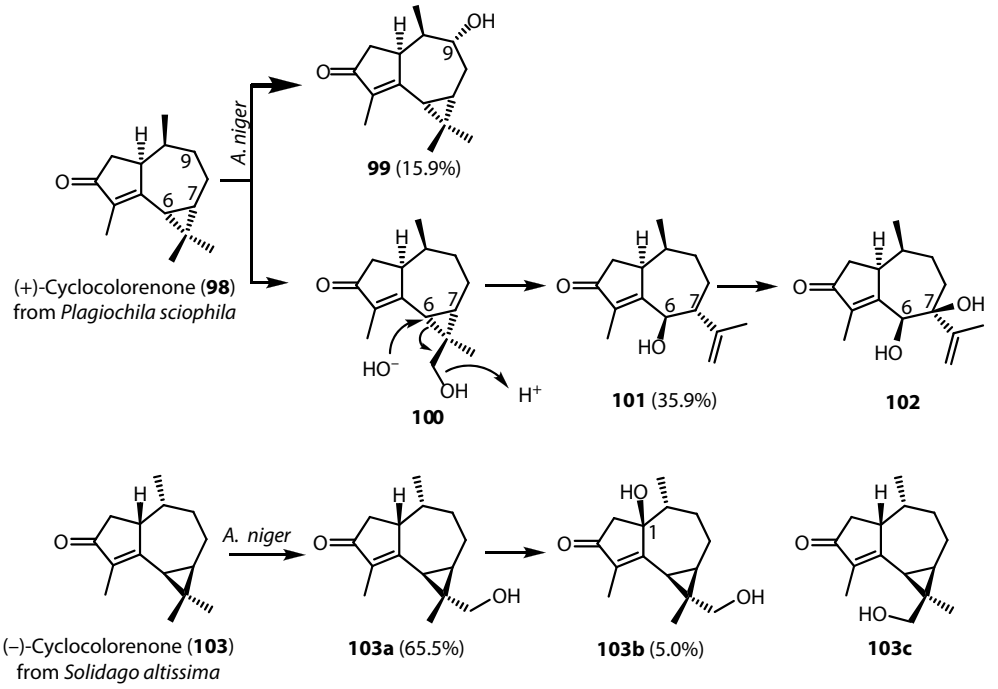


FIGURE 15.34 Biotransformation of (+)-cyclocolorenone (**98**) and (–)-cyclocolorenone (**103**) by *Aspergillus niger*.

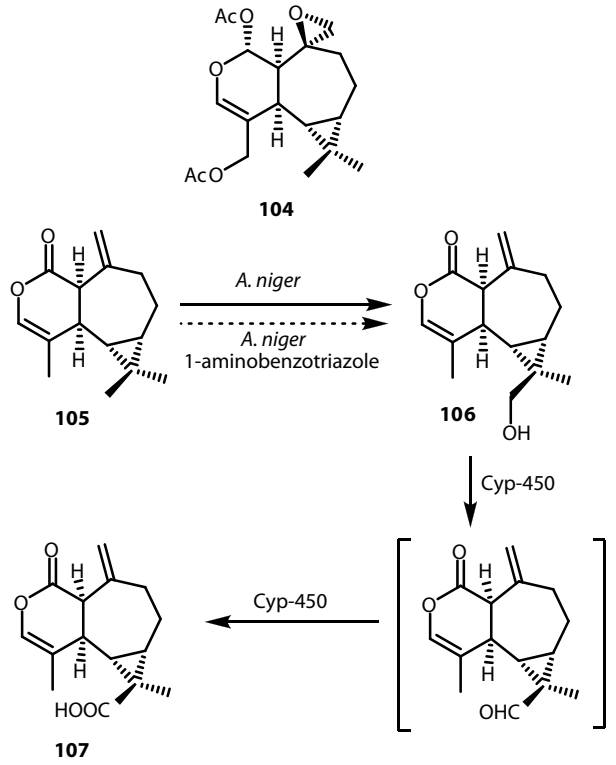


FIGURE 15.35 Biotransformation of plagiochiline C (**104**) by *Aspergillus niger*.

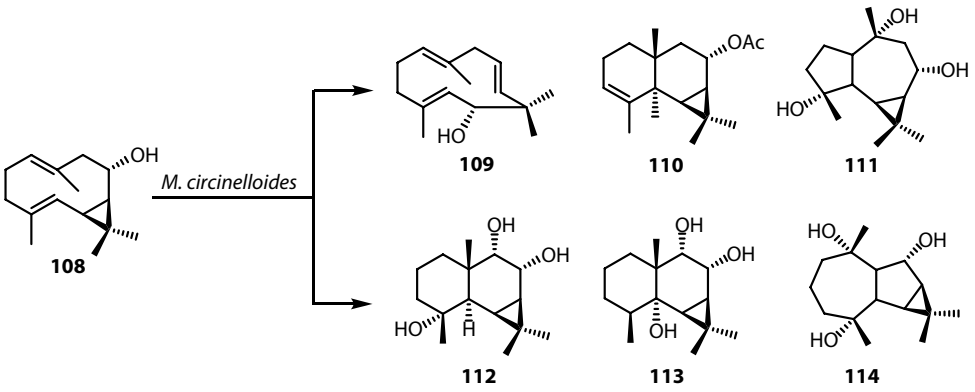


FIGURE 15.36 Biotransformation of 8α-hydroxybicyclogermacrene (**108**) by *Mucor circinelloides*.

The same substrate (**108**) was incubated with the fungus *Calonectria decora* to afford six new metabolites (**108a**–**108f**). In these reactions, hydroxylation, epoxidation, and *trans*-annular cyclization were evidenced (Maatooq, 2002b) (Figure 15.37).

ent-Maaliane-type sesquiterpene alcohol, 1α-hydroxymaaliene (**115**), isolated from the liverwort *Mylia taylorii*, was treated in *Aspergillus niger* to afford two primary alcohols (**116**, **117**) (Morikawa et al., 2000). Such an oxidation pattern of 1,1-dimethyl group on the cyclopropane ring has been found in aromadendrane series as described above, and mammalian biotransformation of a monoterpene hydrocarbon, Δ^3-carene (Ishida et al., 1981) (Figure 15.38).

9(15)-Africanene (**117a**), a tricyclic sesquiterpene hydrocarbon isolated from marine soft corals of *Simularia* species, was biotransformed by *Aspergillus niger* and *Rhizopus oryzae* for 8 days to give 10α-hydroxy- (**117b**) and 9α,15-epoxy derivative (**117c**) (Venkateswarlu et al., 1999) (Figure 15.39).

Germacrone (**118**), (+)-germacrone-4,5-epoxide (**119**), and curdione (**120**) isolated from *Curcuma aromatica*, which has been used as crude drug, was incubated with *Aspergillus niger.* From compound **119** (700 mg), two naturally occurring metabolites, zedoarondiol (**121**) and isozedoarondiol (**122**), were obtained (Takahashi, 1994). Compound (**119**) was cultured in callus of *Curcuma zedoaria* and *Curcuma aromatica* to give the same secondary metabolites **121**, **122**, and **124** (Sakui et al., 1988) (Figures 15.40 and 15.41).

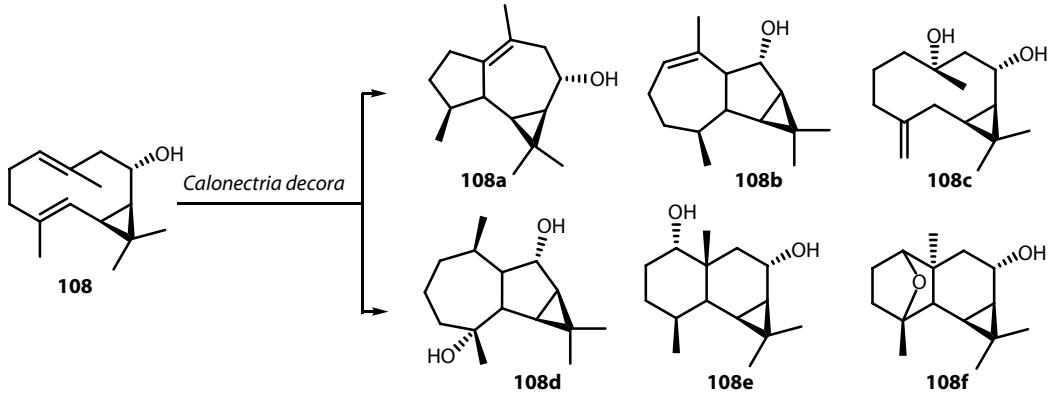


FIGURE 15.37 Biotransformation of 8α-hydroxybicyclogermacrene (**108**) by *Calonectria decora*.

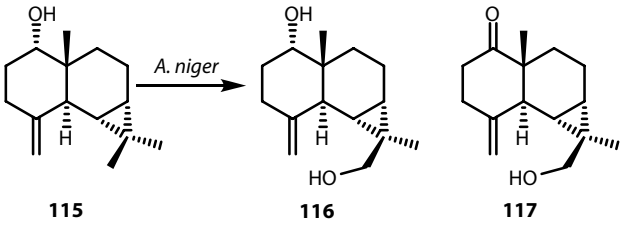


FIGURE 15.38 Biotransformation of 1α-hydroxymaaliene (**115**) *Aspergillus niger.*

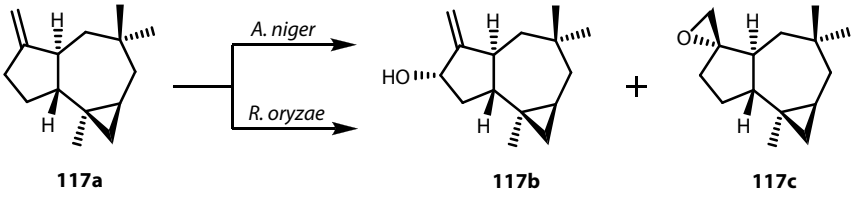


FIGURE 15.39 Biotransformation of 9(15)-africanene (**117a**) by *Aspergillus niger* and *Rhizopus oryzae.*

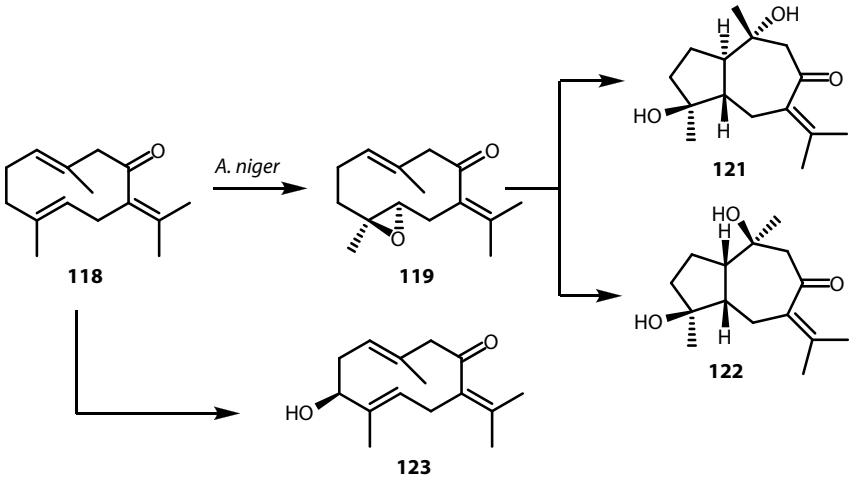


FIGURE 15.40 Biotransformation of germacrone (**118**) by *Aspergillus niger.*

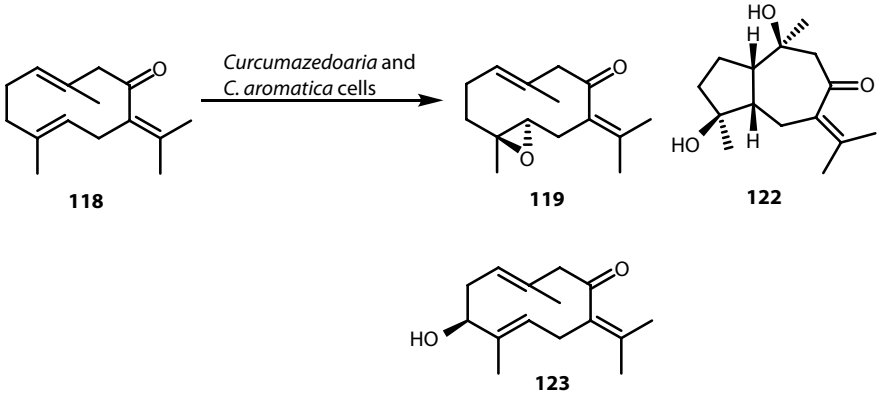


FIGURE 15.41 Biotransformation of germacrone (**118**) by *Curcuma zedoaria* and *Curcuma aromatica* cells.

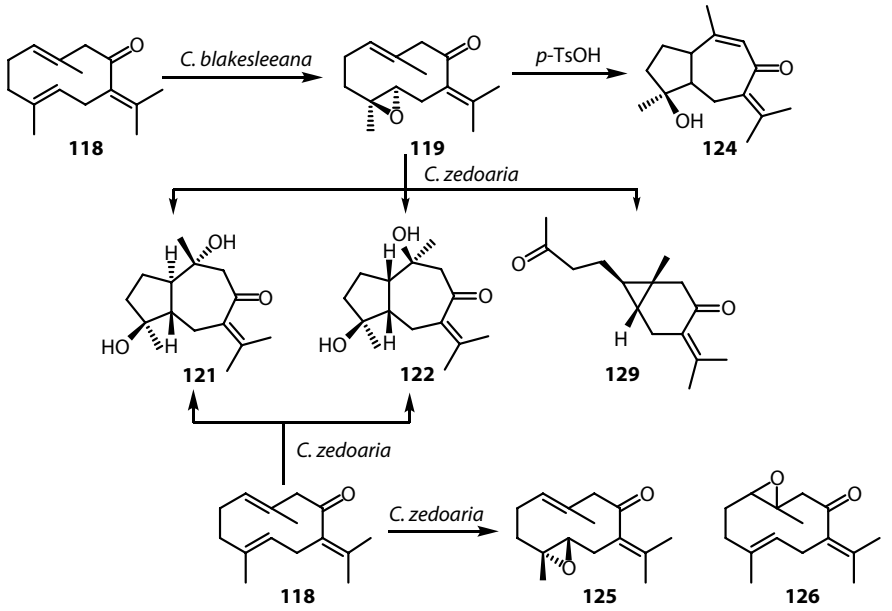


FIGURE 15.42 Biotransformation of germacrone (**118**) by *Cunninghamella blakeseeana* and *Curcuma zedoaria* cells.

Aspergillus niger biotransformed germacrone (**118**, 3g) to very unstable 3β-hydroxygermacrone (**123**), and 4,5-epoxygermacrone (**119**) which was further converted to two guaiane sesquiterpenoids (**121**) and (**122**) through *trans*-annular-type reaction (Takahashi, 1994). The same substrate was incubated in the microorganism, *Cunninghamella blakeseeana* to afford germacrone-4,5-epoxide (**119**) (Hikino et al., 1971) while the treatment of **118** in the callus of *Curcuma zedoaria* gave four metabolites **121**, **122**, **125**, and **126** (Sakamoto et al., 1994) (Figure 15.42).

The same substrate (**118**) was treated in plant cell cultures of *Solidago altissima* (Asteraceae) for 10 days to give various hydroxylated products (**121**, **127**, **125**, **128**–**132**) (Sakamoto et al., 1994). Guaiane (**121**) underwent further rearrangement C4–C5, cleavage and C5–C10 *trans*-annular cyclization to the bicyclic hydroxyketone (**128**) and diketone (**129**) (Sakamoto et al., 1994) (Figure 15.43).

Curdione (**120**) was also treated in *Aspergillus niger* to afford two allylic alcohols (**133**, **134**) and a spirolactone (**135**). *Curcuma aromatica* and *Curcuma wenyujin* produced spirolactone (**135**) which might be formed from curdione via *trans*-annular reaction *in vivo* was biotransformed to spirolactone diol (**135**) (Asakawa et al., 1991; Sakui et al., 1992) (Figure 15.44).

Aspergillus niger also converted shiromodiol diacetate (**136**) isolated from *Neolitsea sericea* to 2β-hydroxy derivative (**137**) (Nozaki et al., 1996) (Figure 15.45).

Twenty strains of filamentous fungi and four species of bacteria were screened initially by thin layer chromatography (TLC) for their biotransformation capacity of curdione (**120**). *Mucor spinosus*, *Mucor polymorphosporus*, *Cunninghamella elegans*, and *Penicillium janthinellum* were found to be able to biotransform curdione (**120**) to more polar metabolites. Incubation of curdione with *Mucor spinosus*, which was most potent strain to produce metabolites, for 4 days using potato medium gave five metabolites (**134**, **134a**–**134d**) among which compounds **134c** and **134d** are new products (Ma et al., 2006) (Figure 15.46).

Many eudesmane-type sesquiterpenoids have been biotransformed by several fungi and various oxygenated metabolites obtained.

β-Selinene (**138**) is ubiquitous sesquiterpene hydrocarbon of seed oil from many species of Apiaceae family; for example, *Cryptotenia canadensis* var. *japonica*, which is widely used as vegetable for Japanese soup. β-Selinene was biotransformed by plant pathogenic fungus *Glomerella*

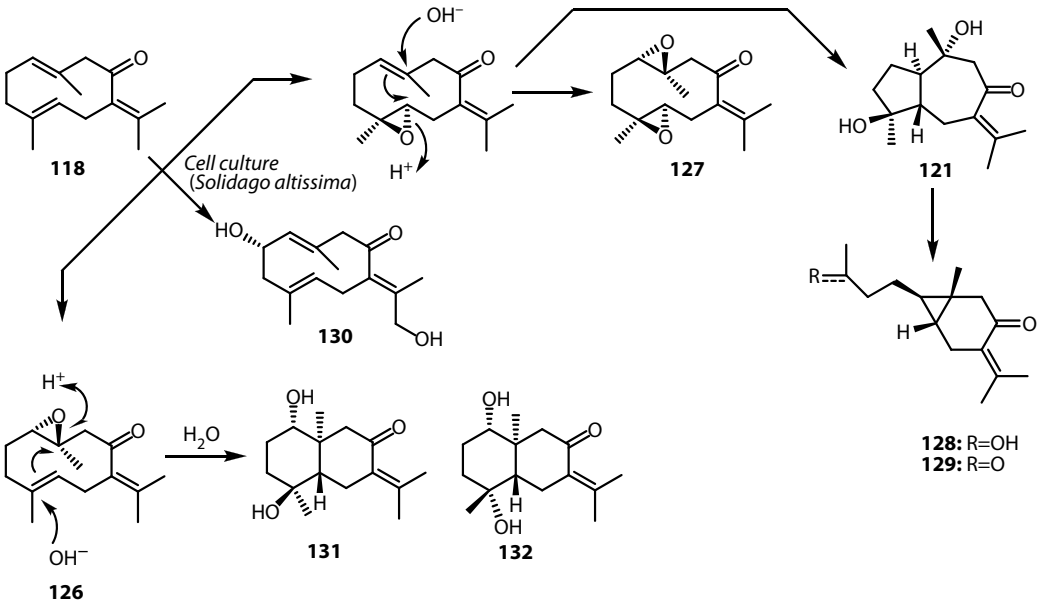


FIGURE 15.43 Biotransformation of germacrone (**118**) by *Solidago altissima* cells.

cingulata to give an epimeric mixtures (1:1) of 1β,11,12-trihydroxy product (**139**) (Miyazawa et al., 1997a). The same substrate was treated in *Aspergillus wentii* to give 2α,11,12-trihydroxy derivative (**140**) (Takahashi et al., 2007).

Eudesm-11(13)-en-4,12-diol (**141**) was biotransformed by *Aspergillus niger* to give 3β-hydroxy derivative (**142**) (Hayashi et al., 1999).

α-Cyperone (**143**) was fed by *Collectotrichum phomoides* (Lamare and Furstoss, 1990) to afford 11,12-diol (**144**) and 12-manool (**145**) (Higuchi et al., 2001) (Figure 15.47).

The filamentous fungi *Gliocladium roseum* and *Exserohilum halodes* were used as the bioreactors for 4β-hydroxyeudesmane-1,6-dione (**146**) isolated from *Sideritis varoi* subsp. *cuatrecasasii*. The former fungus transformed **146** to 7α-hydroxyl- (**147**), 11-hydroxy- (**148**), 7α,11-dihydroxy- (**149**), 1α,11-dihydroxy- (**150**), and 1α,8α-dihydroxy derivatives (**151**) while *Exserohilum halodes* gave only 1α-hydoxy product (**152**) (Garcia-Granados et al., 2001) (Figure 15.48).

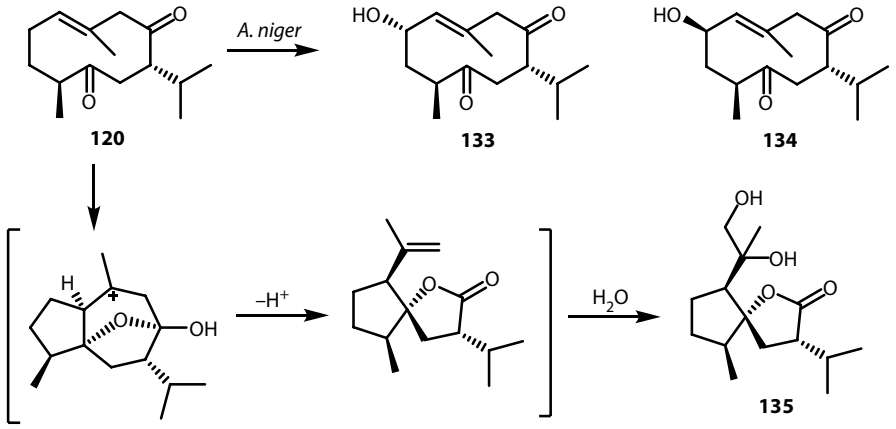
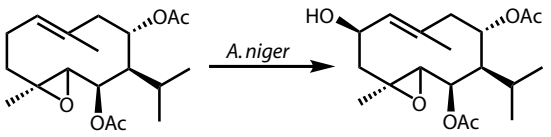


FIGURE 15.44 Biotransformation of curdione (**120**) by *Aspergillus niger*.



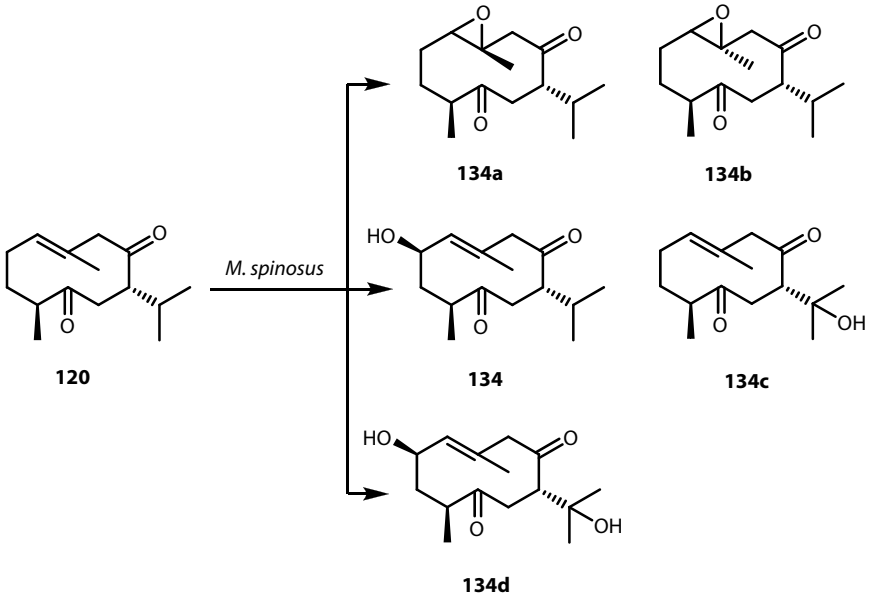
136 137

FIGURE 15.45 Biotransformation of shiromodiol diacetate (**136**) by *Aspergillus niger.*

Orabi (2000) reported that *Beauvaria bassiana* is the most efficient microorganism to metabolize plectanthone (**152a**) among 20 microorganisms, such as *Absidia glauca, Aspergillus flavipes, Beauvaria bassiana, Cladosporium resinae, Penicillium frequentans*, and so on. The substrate (**152a**) was incubated with *Beauvaria bassiana* to give metabolites **152b** (2.1%), **152c** (21.2%), **152d** (2.5%), **152e** (no data), and **152f** (1%) (Figure 15.49).

(–)-α-Eudesmol (**153**) isolated from the liverwort *Porella stephaniana* was treated by *Aspergillus cellulosae* and *Aspergillus niger* to give 2-hydroxy (**154**) and 2-oxo derivatives (**155**), among which the latter product was predominantly obtained. This bioconversion was completely blocked by 1-aminobenzotriazole, CYP450 inhibitor. Compound **155** has been known as natural product, isolated from *Pterocarpus santalinus* (Noma et al., 1996). Biotransformation of α-eudesmol (**153**) isolated from the dried *Atractylodes lancea* was reinvestigated by *Aspergillus niger* to give 2-oxo-11,12-dihydro-α-eudesmol (**156**) together with 2-hydroxy- (**154**), and 2-oxo-α-eudesmol (**155**). β-Eudesmol (**157**) was treated in *Aspergillus niger*, with the same culture medium to afford 2α- (**158**) and 2β-hydroxy-α-eudesmol (**159**) and 2α,11,12-trihydroxy-β-eudesmol (**160**) and 2-oxo derivative (**161**), which was further isomerized to compound **162** (Noma et al., 1996, 1997) (Figure 15.50).

Three new hydroxylated metabolites (**157b**–**157d**) along with a known **158** and (**157e**–**157g**) were isolated from the biotransformation reaction of a mixture of β- (**157**) and γ-eudesmols (**157a**) by *Gibberella suabinetii*. The metabolites proved a super activity of the hydroxylase, dehydrogenase, and isomerase enzymes. The hydroxylation is a common feature; on the contrary, cyclopropyl ring formation like compound (**158d**) is very rare (Maatooq, 2002a) (Figure 15.51).



120 134a 134b 134 134c 134d

FIGURE 15.46 Biotransformation of curdione (**120**) by *Mucor spinosus.*

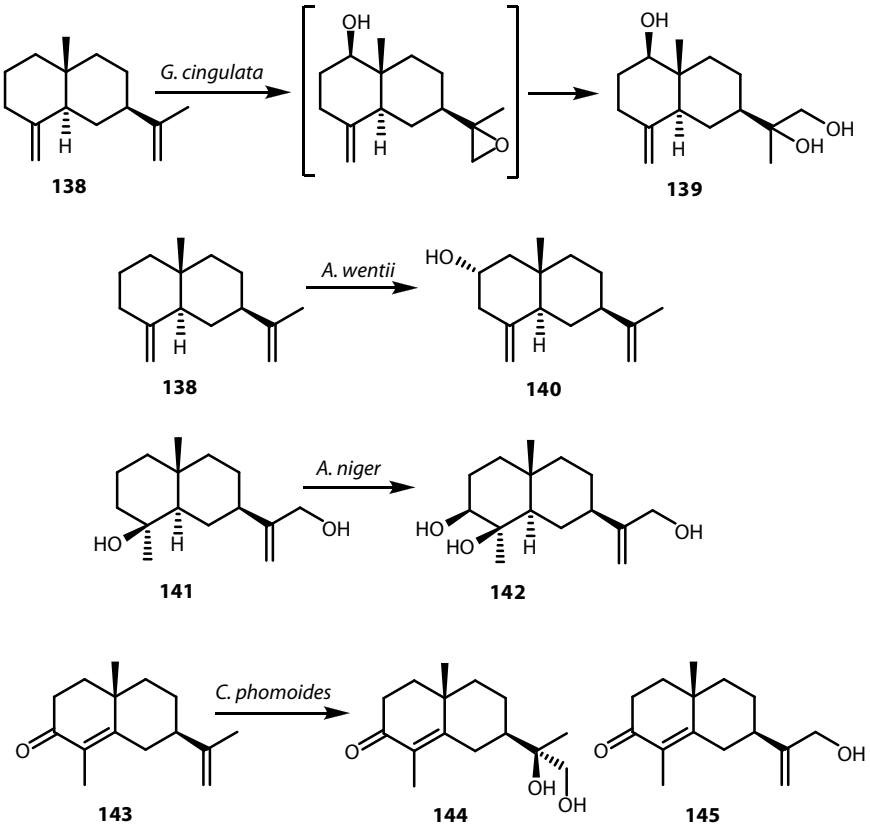


FIGURE 15.47 Biotransformation of eudesmenes (**138**, **141**, **143**) by *Aspergillus wentii*, *Glomerella cingulata*, and *Collectotrium phomoides*.

A furanosesquiterpene, atractylon (**163**) obtained from *Atractylodis* rhizoma was treated with the same fungus to yield atractylenolide III (**164**) possessing inhibition of increased vascular permeability in mice induced by acetic acid (Hashimoto et al., 2001).

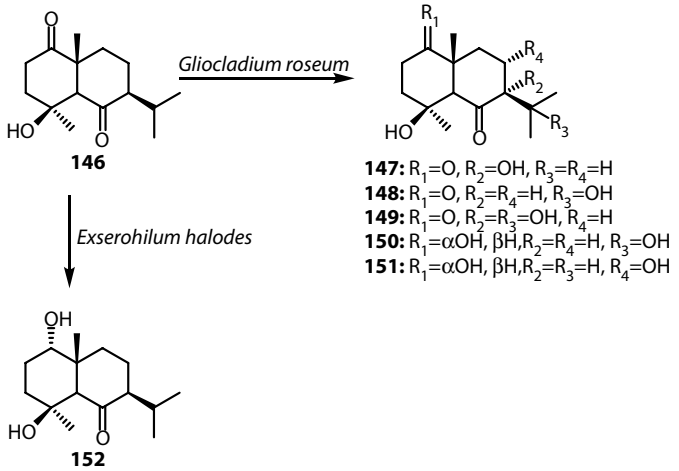


FIGURE 15.48 Biotransformation of 4β-hydroxy-eudesmane-1,6-dione (**146**) by *Gliocladium roseum* and *Exserohilum halodes*.

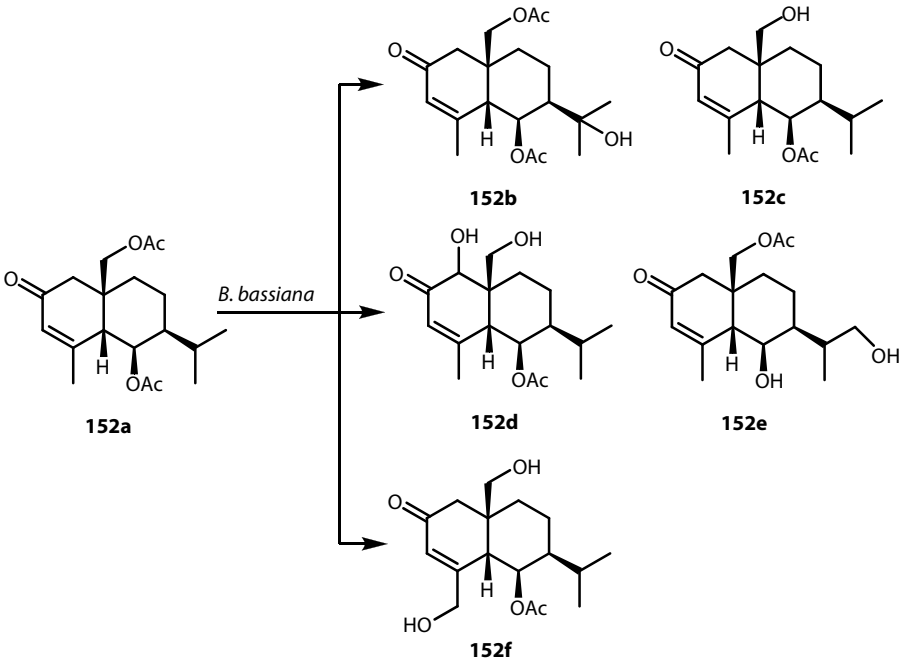


FIGURE 15.49 Biotransformation of eudesmenone (**152a**) by *Beauvaria bassiana*.

The biotransformation of sesquiterpene lactones have been carried out by using different microorganisms.

Costunolide (**165**), a very unstable sesquiterpene γ-lactone, from *Saussurea radix*, was treated in *Aspergillus niger* to produce three dihydrocostunolides (**166**–**168**) (Clark and Hufford, 1979). Costunolide is easily converted into eudesmanolides (**169**–**172**) in diluted acid, thus **166**–**168** might be biotransformed after being cyclized in the medium including the microorganisms. If the crude

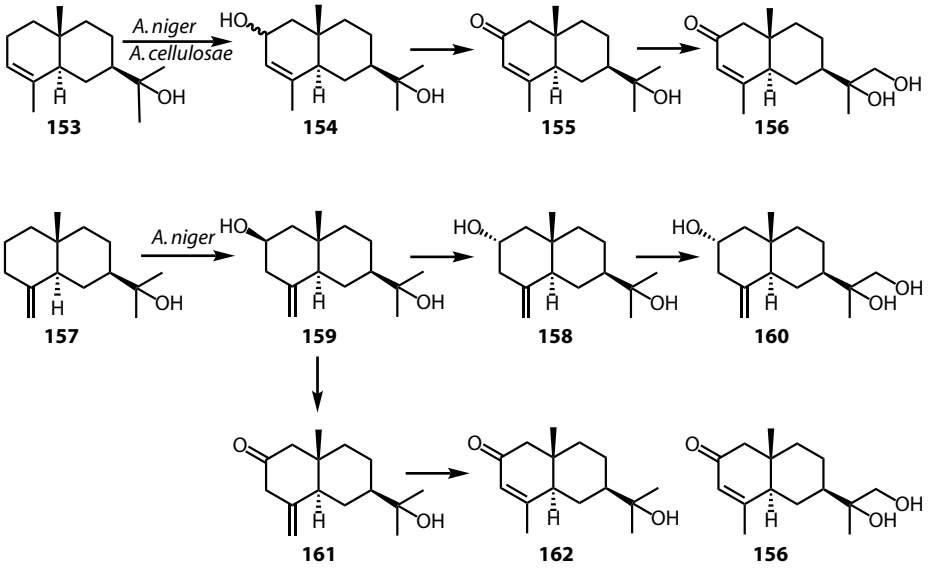


FIGURE 15.50 Biotransformation of α-eudesmol (**153**) and β-eudesmol (**157**) by *Aspergillus niger* and *Aspergillus cellulosae*.

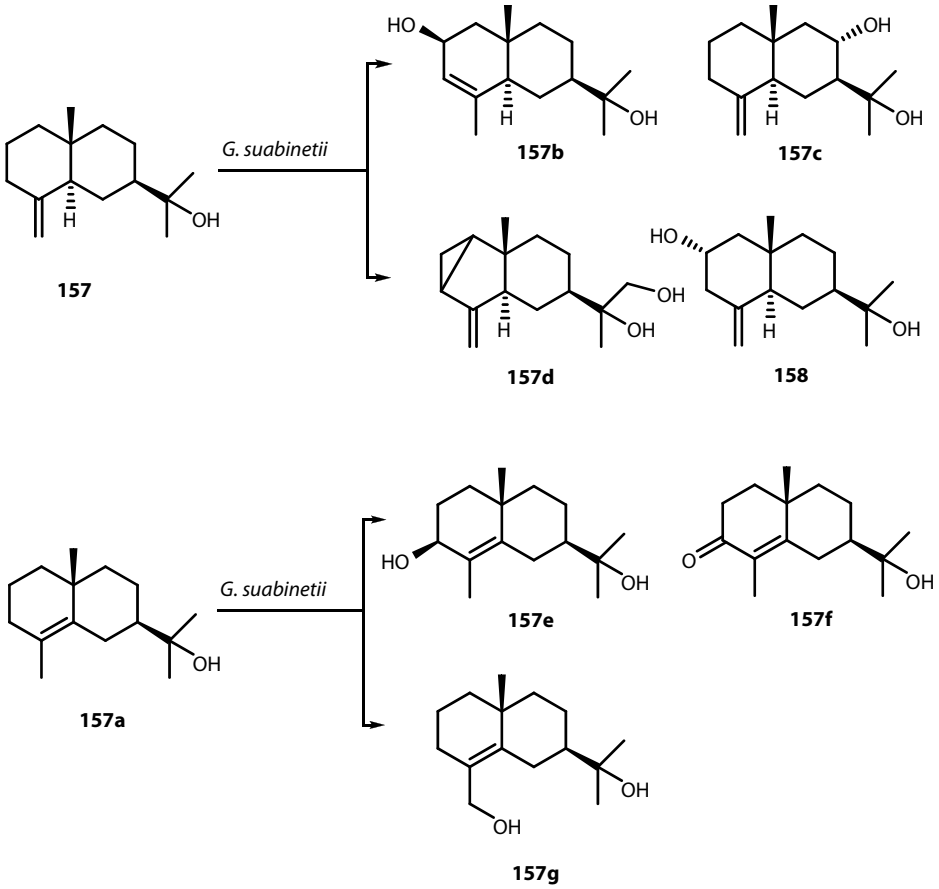


FIGURE 15.51 Biotransformation of β-eudesmol (**157**) and γ-eudesmol (**157a**) by *Gibberella suabinetii*.

drug including costunolide (**165**) is orally administered, **165** will be easily converted into **169**–**172** by stomach juice (Figure 15.52).

(+)-Costunolide (**165**), (+)-cnicin (**172a**), and (+)-salonitgenolide (**172b**) were incubated with *Cunninghamella echinulata* and *Rhizopus oryzae*.

The former fungus converted compound **165**, to four metabolites, (+)-11β,13-dihydrocostunolide (**165a**), 1β-hydroxyeudesmanolide, (+)-santamarine (**166a**), (+)-reynosin (**166b**), and (+)-1β-hydroxyarbusculin A (**168a**), which might be formed from 1β,10α-epoxide (**166c**). Treatment of **172a** with *Cunninghamella echinulata* gave (+)-salonitenolide (**172b**) (Barrero et al., 1999) (Figure 15.53).

α-Cyclocostunolide (**169**), β-cyclocostunolide (**170**), and γ-cyclocostunolide (**171**) prepared from costunolide were cultivated in *Aspergillus niger*, respectively. From the metabolite of **169**, four dihydro lactones (**173**–**176**) were obtained, among which sulfur-containing compound (**176**) was predominant (Figure 15.54).

The same substrate (**169**) was cultivated for 3 days by *Aspergillus cellulosae* to afford a sole metabolite, 11β,13-dihydro-α-cyclocostunolide (**177**). Possible metabolic pathways of **169** by both microorganisms were shown in Figure 15.55.

A double bond at C11–C13 of **169** was firstly reduced stereoselectively to afford **177**, followed by oxidation at C2 to give **173**, and then further oxidation occurred to furnish two hydroxyl derivatives (**174**, **175**) in *Aspergillus niger*. The sulfide compound (**176**) might be formed from **175** or by Michel condensation of ethyl 2-hydroxy-3-mercaptopropanate, which might originate from Czapek-peptone medium into exomethylene group of α-cyclocostunolide (Hashimoto et al., 1999a, 2001).

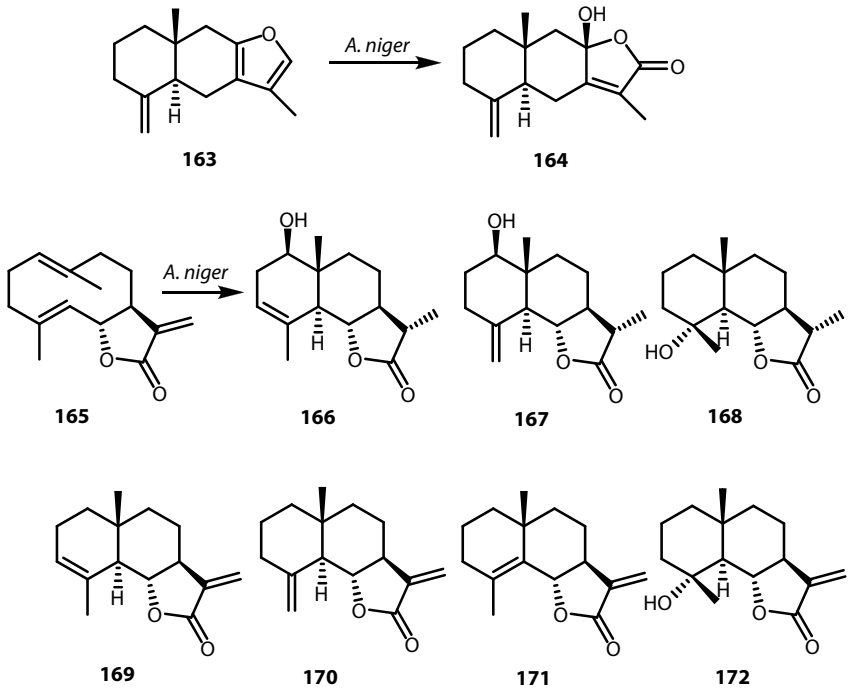


FIGURE 15.52 Biotransformation of atractylon (**163**) and costunolide (**165**) by *Aspergillus niger.*

Aspergillus niger converted β-cyclocostunolide (**170**) to 2-oxygenated metabolites (**173**, **174**, **178**–**181**) of which **173** was predominant. It is suggested that compound **173** and **174** might be formed during biotransformation period since metabolite media after 7 days was acidic (pH 2.7). Surprisingly, *Aspergillus cellulose* gave a sole 11β,13-dihydro-β-cyclocostunolide (**182**), which was abnormally folded in the mycelium of *Aspergillus cellulosae* as a crystal form after biotransformation of **170**. On the other hand, the metabolites were normally liberated in medium outside of the mycelium of *Aspergillus niger* and *Botryosphaeria dothidea* (Hashimoto et al., 1999a, 2001) (Figure 15.56).

Botryosphaeria dothidea has no stereoselectivity to reduce C11–C13 double bond of β-cyclocostunolide (**170**) since this organism gave two dihydro derivatives **182** (16.7%) and **183** (37.8%), respectively, as shown in Figure 15.57.

It is noteworthy that both α- and β-cyclocostunolides were biotransformed by *Aspergillus niger* to give the sulfur-containing metabolites (**176**, **181**). Possible biogenetic pathway of **170** is shown in Figure 15.58.

When γ-cyclocostunolide (**171**) was cultivated in *Aspergillus niger* to give dihydro-α-santonin (**187**, 25%) and its related C11,C13 dihydro derivatives (**184**–**186**, **188**, **189**) were obtained as a small amount. Compound **186** was recultivated for 2 days by the same organism as mentioned above to afford **187** (25%) and 5β-hydroxy-α-cyclocostunolide (**189**, 54%). Recultivation of **185** for 2 days by *Aspergillus niger* afforded compound **187** as a sole metabolite. During the biotransformation of **171**, no sulfur-containing product was obtained. Both *Aspergillus cellulosae* and *Botryosphaeria dothidea* produced only dihydro-γ-cyclocostunolide (**184**) from the substrate (**171**) (Hashimoto et al., 1999a, 2001) (Figure 15.59).

Santonin (**190**) has been used as vermicide against round warm. *Cunninghamella blakesleeana* and *Aspergillus niger* converted **190**–**187** (Atta-ur Rahman et al., 1998). When **187** was fed by *Aspergillus niger* for one week to give 2β-hydroxy-1,2-dihydro-α-santonin (**188**, 39%) as well as 1β-hydroxy-1,2-dihydro-α-santonin (**195**, 6.5%), 9β-hydroxy-1,2-dihydro-α-santonin (**196**, 6.9%), and α-santonin (**190**, 5.4%), which might be obtained from dehyroxylation of **188**, as a minor

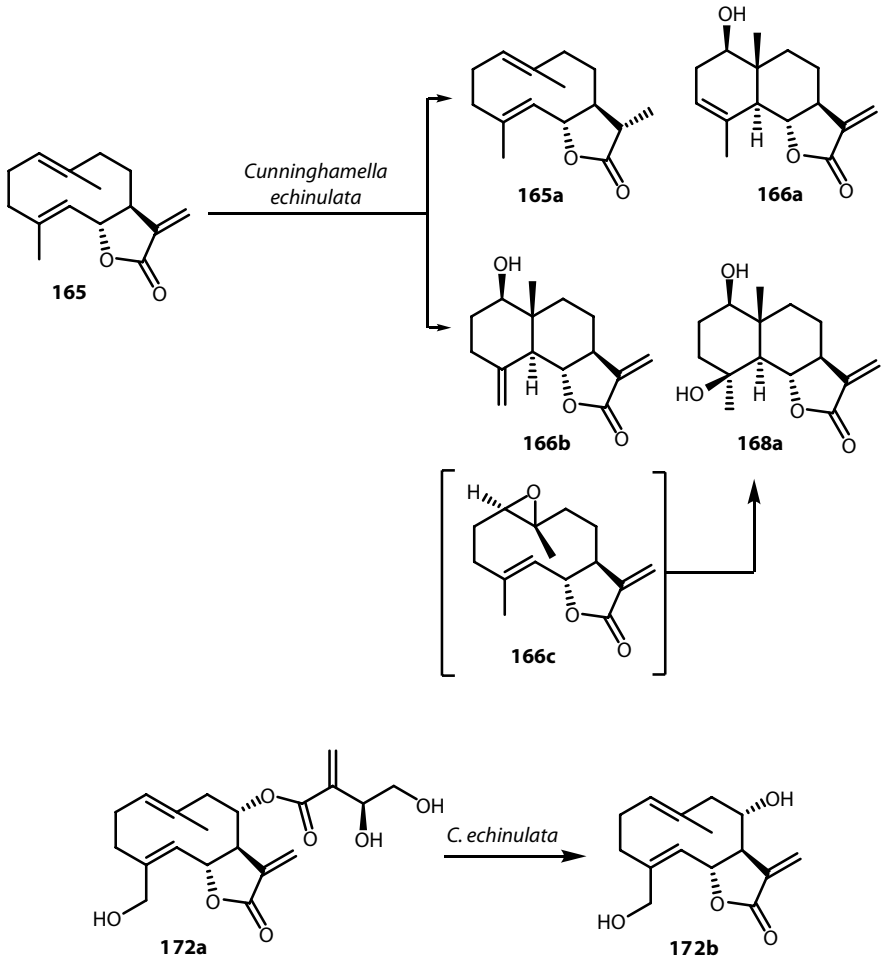


FIGURE 15.53 Biotransformation of costunolide (**165**) and its derivative (**172a**) by *Cunninghamella vechinulata* and *Rhizopus oryzae*.

component (Hashimoto et al., 2001). Compound **188** was isolated from the crude metabolite of γ-cyclocostunolide (**171**) by *Aspergillus niger* as mentioned above (Figure 15.60).

It was treated with *Aspergillus niger* for 7 days to give **191** (18.3%), **192** (2.3%), **193** (19.3%), and **194** (3.5%) of which **193** was the major metabolite. Compound **191** was isolated from dog's urine after the oral administration of **190**. The structure of compound **194** was established as lumisantonin obtained by the photoreaction of **190**. α-Santonin **190** was not converted into 1,2-dihydro derivative by *Aspergillus niger*, whereas the other strain of *Aspergillus niger* gave a single product, 1,2-dihydro-α-santonin (**187**) (Hashimoto et al., 2001) (Figure 15.61).

Ata and Nachtigall (2004) reported that α-santonin (**190**) was incubated with *Rhizopus stolonifer* to give (**187a**), and (**183b**), while with *Cunninghamella bainieri*, *Cunninghamella echinulata*, and *Mucor plumbeus* to afford the known 1,2-dihydro-α-santonin (**187**) (Figure 15.62).

α-Santonin (**190**) and 6-*epi*-α-santonin (**198**) were cultivated in *Absidia coerulea* for 2 days to give 11β-hydroxy- (**191**, 71.4%) and 8α-hydroxysantonin (**197**, 2.0%), while 6-*epi*-santonin (**198**) afforded four major products (**199**–**201**, **206**) and four minor analogues (**202**, **203**–**205**). *Asparagus officinalis* also biotransformed α-santonin (**190**) into three eudesmanolides (**187**, **207**, **208**) and a guaianolide (**209**) in a small amount. 6-*Epi*-santonin (**198**) was also treated in the same bioreactor

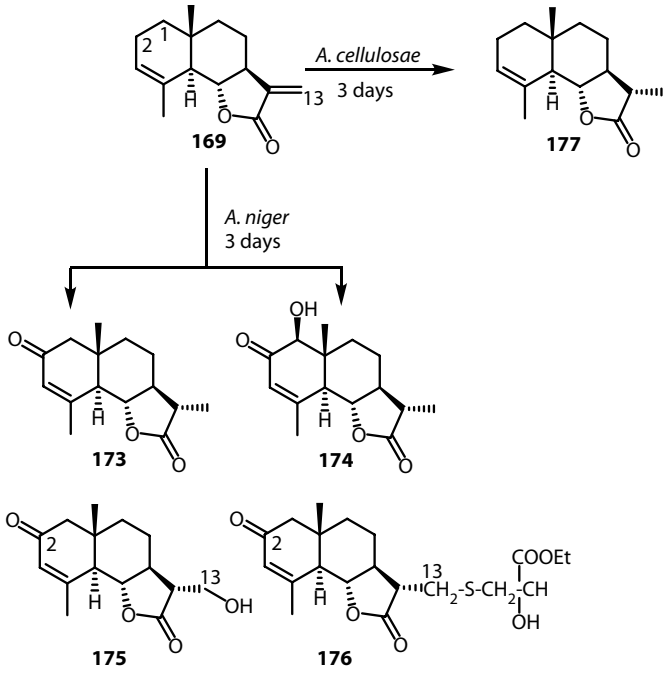


FIGURE 15.54 Biotransformation of α-cyclocostunolide (**169**) by *Aspergillus niger* and *Aspergillus cellulosae*.

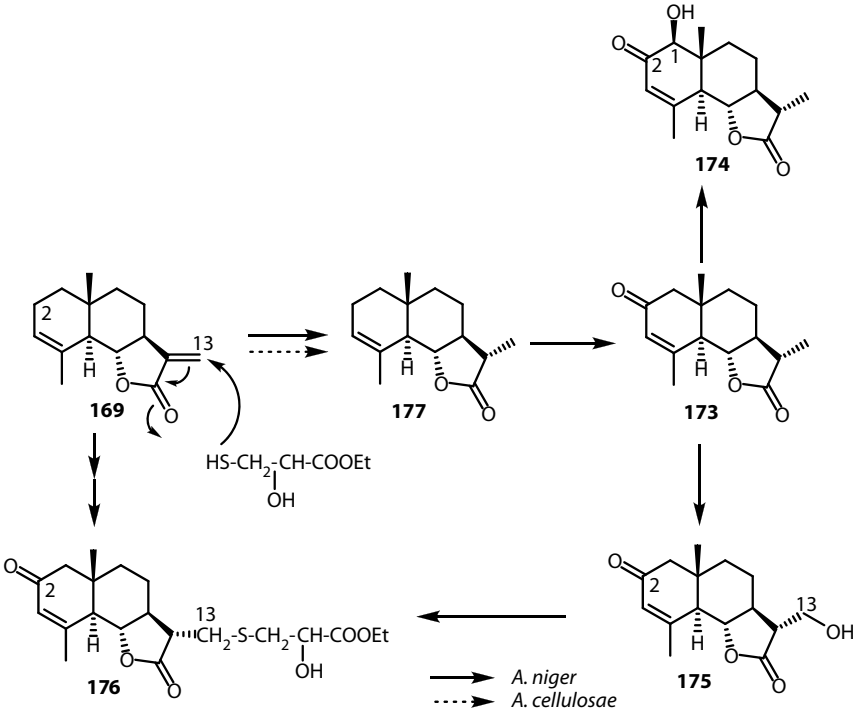


FIGURE 15.55 Possible pathway of biotransformation of α-cyclocostunolide (**169**) by *Aspergillus niger* and *Aspergillus cellulosae*.

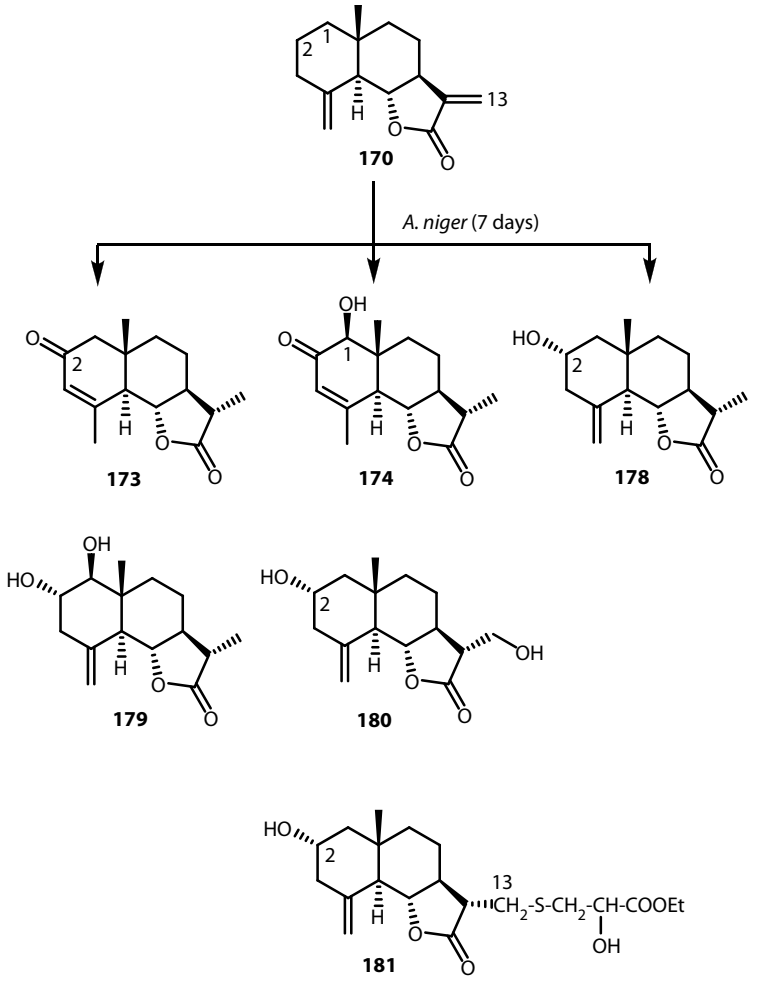


FIGURE 15.56 Biotransformation of β-cyclocostunolide (**170**) by *Aspergillus niger*.

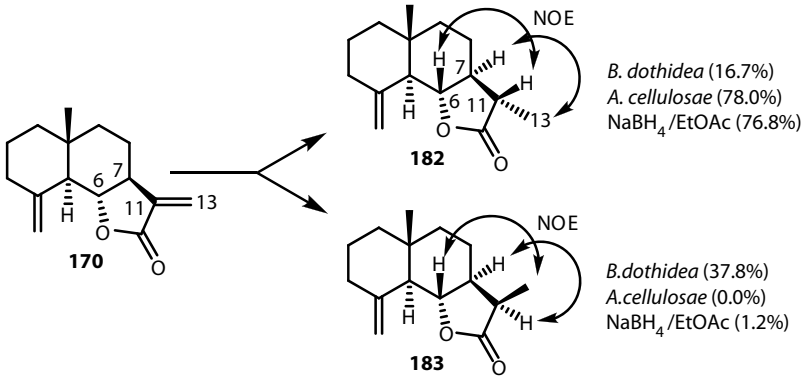


FIGURE 15.57 Biotransformation of β-cyclocostunolide (**170**) by *Aspergillus cellulosae* and *Botryosphaeria dothidea*.

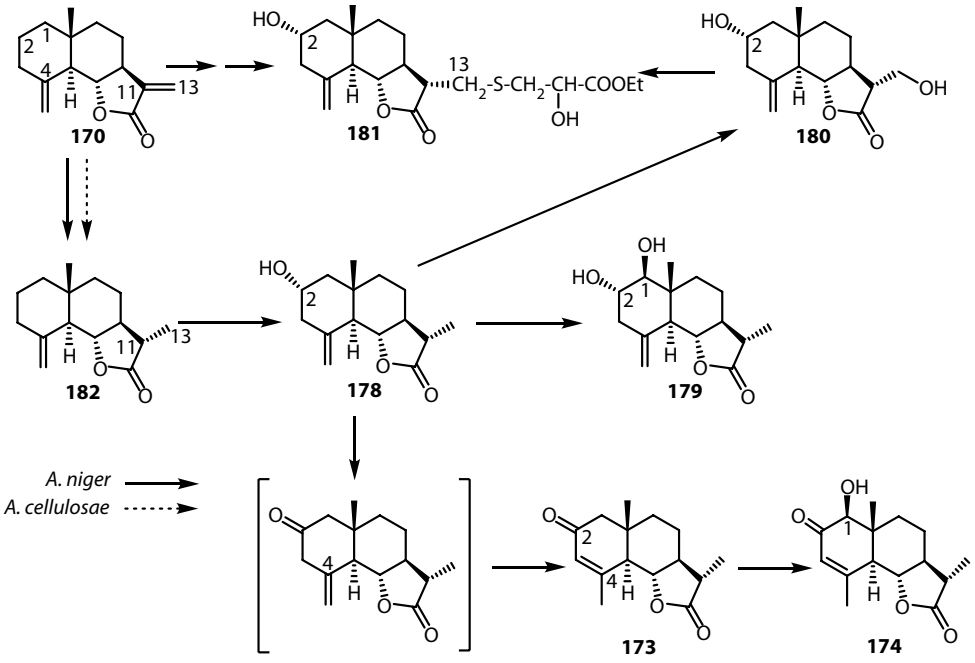


FIGURE 15.58 Possible pathway of biotransformation of β-cyclocostunolide (**170**) by *Aspergillus niger* and *Aspergillus cellulosae*.

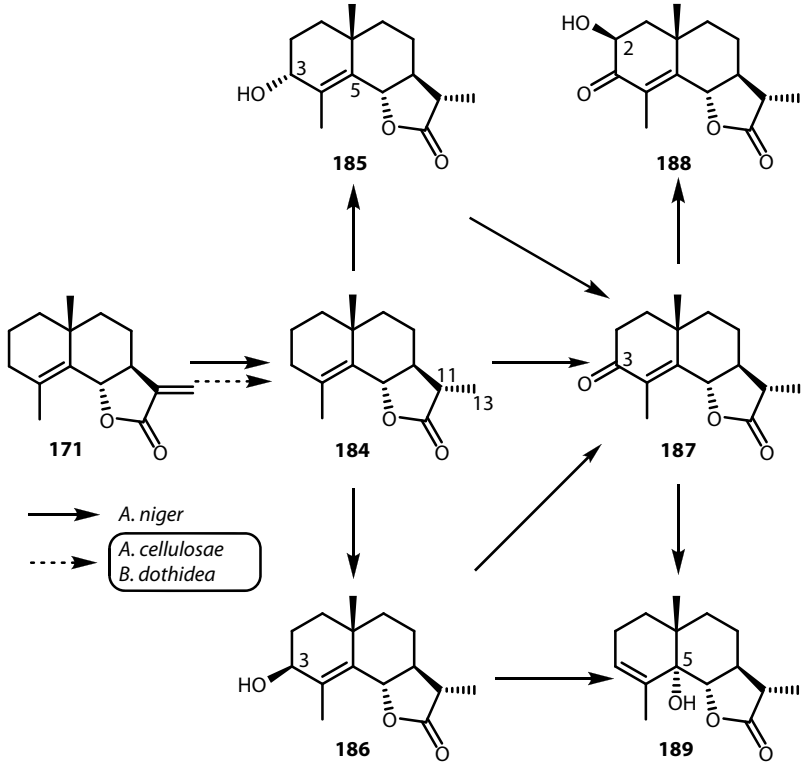


FIGURE 15.59 Biotransformation of γ-cyclocostunolide (**171**) by *Aspergillus niger*, *Aspergillus cellulosae*, and *Botryosphaeria dothidea*.

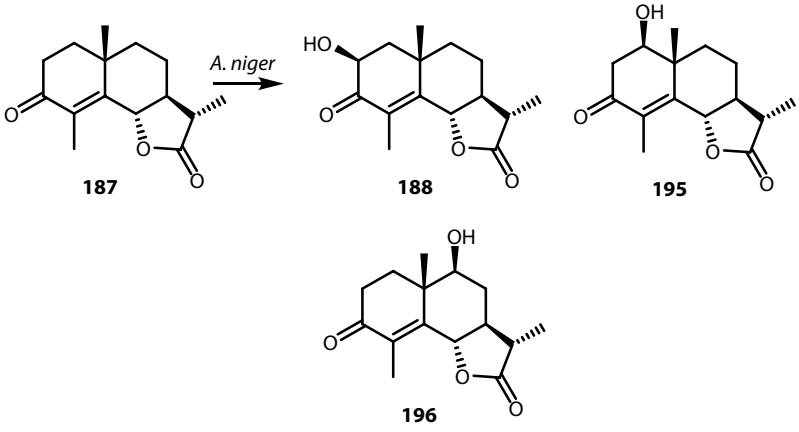


FIGURE 15.60 Biotransformation of dihydro-α-santonin (**187**) by *Aspergillus niger.*

as mentioned above to give **199** and **206**, the latter of which was obtained as a major metabolite (44.7%) (Yang et al., 2003) (Figure 15.63).

α-Santonin (**190**) was incubated in the cultured cells of *Nicotiana tabacum* and the liverwort *Marchantia polymorpha*. *Nicotiana tabacum* cells gave 1,2-dihydro-α-santonin (**187**) (50%) for 6 days. The latter cells also converted α-santonin to 1,2-dihydro-α-santonin, but conversion ratio was only 28% (Matsushima et al., 2004) (Figure 15.64).

6-*Epi*-α-santonin (**198**) and its tetrahydro analogue (**210**) were also incubated with fungus *Rhizopus nigricans* to give 2α-hydroxydihydro-α-santonin (**211**) (Amate et al., 1991), the epimer of **188** obtained from the biotransformation of dihydro-α-santonin (**187**) by *Aspergillus niger* (Hashimoto et al., 2001). The product **211** might be formed via 1,2-epoxide of **198**. Compound **210** was converted through carbonyl reduction to furnish **212** and **213** under epimerization at C4 (Amate et al., 1991) (Figure 15.65).

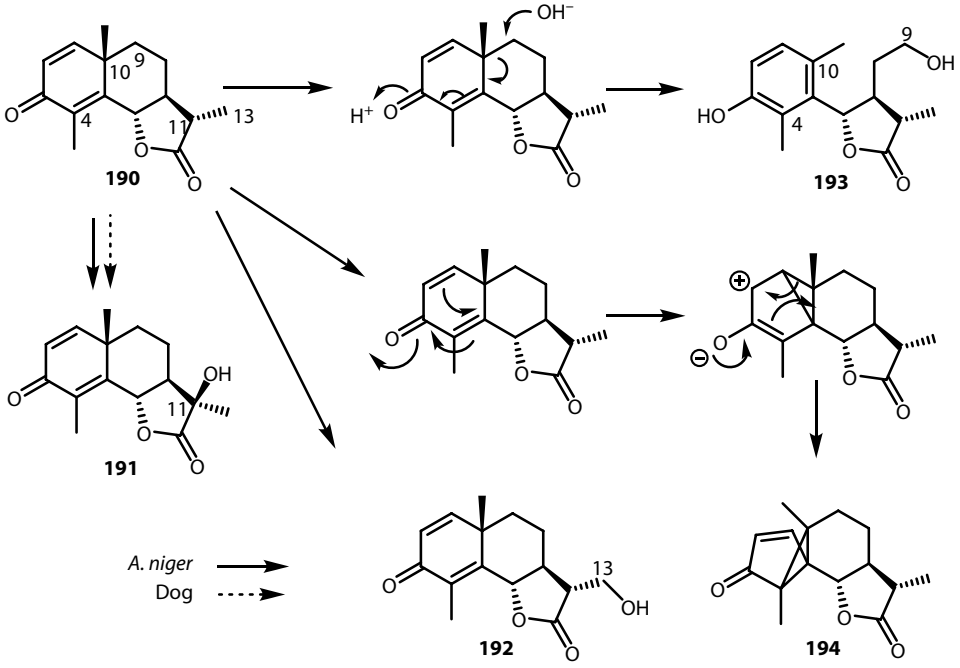


FIGURE 15.61 Biotransformation of α-santonin (**190**) by *Aspergillus niger* and dogs.

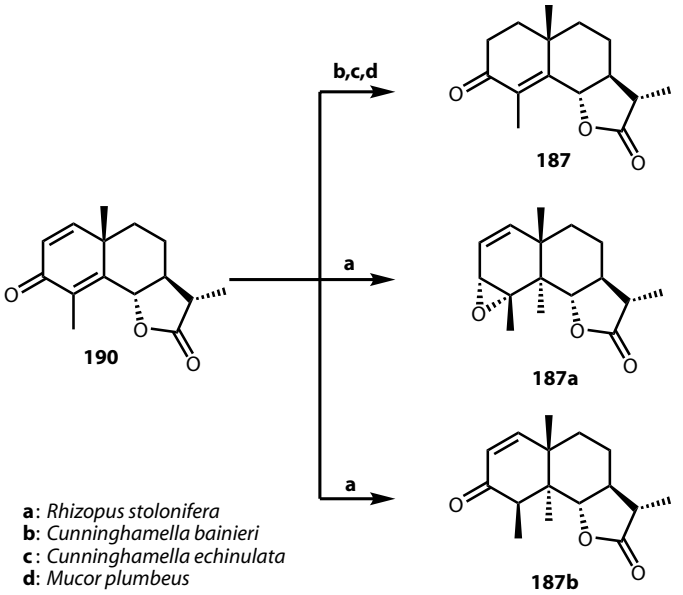


FIGURE 15.62 Biotransformation of α-santonin (**190**) by *Rhizopus stolonifera, Cunninghamella bainieri, Cunninghamella echinulata*, and *Mucor plumbeus*.

1,2,4β,5α-Tetrahydro-α-santonin (**214**) prepared from α-santonin (**190**) was treated with *Aspergillus niger* to afford six metabolites (**215**–**220**) of which **219** was the major product (21%). When the substrate (**214**) was treated with CYP450 inhibitor, 1-aminobenzotriazole, only **215** was obtained without its homologues, **216**–**220**, while the C4 epimer (**221**) of **214** was converted by the same microorganism to afford a single metabolite (**222**) (73%). Further oxidation of **222** did not occur. This reason might be considered by the steric hindrance of β (axial) methyl group at C4 (Hashimoto et al., 2001) (Figure 15.66).

7α-Hydroxyfrullanolide (**223**) possessing cytotoxicity and antitumor activity, isolated from *Sphaeranthus indicus* (Asteraceae), was bioconverted by *Aspergillus niger* to afford 13*R*-dihydro

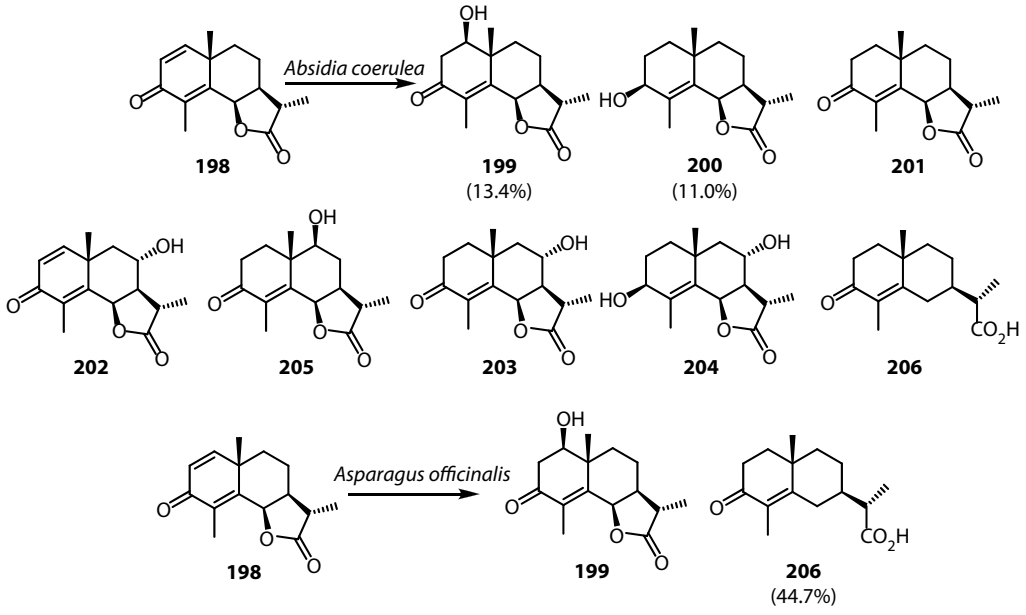


FIGURE 15.63 Biotransformation of α-epi-santonin (**198**) by *Absidia coerulea* and *Asparagus officinalis*.

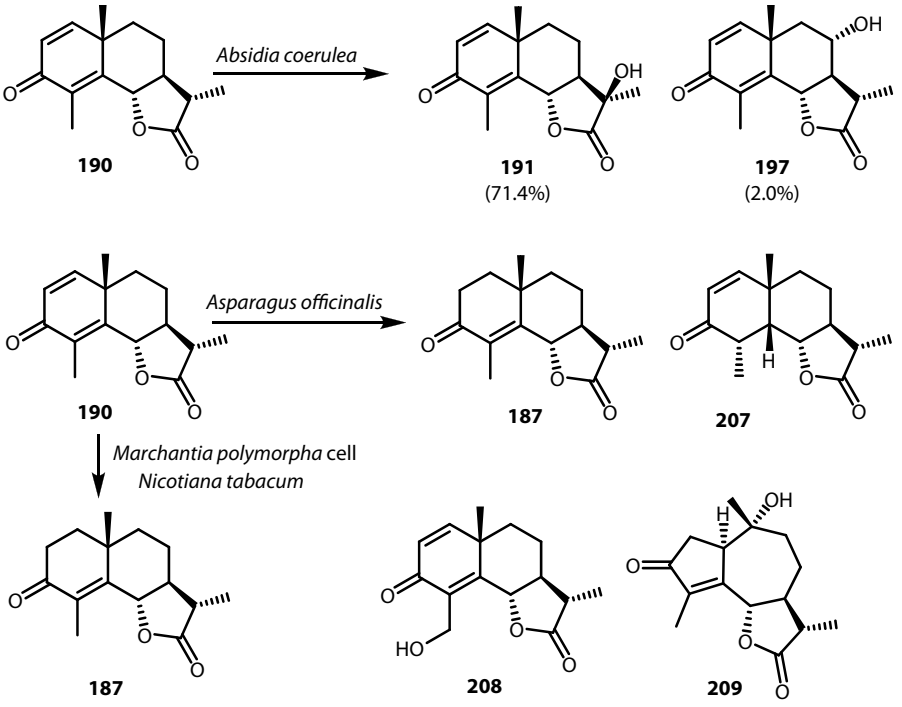


FIGURE 15.64 Biotransformation of 6-epi-α-santonin (**190**) by *Absidia coerulea, Asparagus officinalis, Marchantia polymorpha*, and *Nicotiana tabacum*.

derivative (**224**). The same substrate was also treated in *Aspergillus quardilatus* (wild type) to give 13-acetyl product (**225**) (Atta-ur Rahman et al., 1994) (Figure 15.67).

Incubation of (−)-frullanolide (**226**), obtained from the European liverwort, *Frullania tamarisci* subsp. *tamarisci* causes a potent allergenic contact dermatitis, was incubated by *Aspergillus niger* to give dihydrofrullanolide (**227**), nonallergenic compound in 31.8% yield. In this case, C11–C13 dihydro derivative was not obtained (Hashimoto et al., 2005).

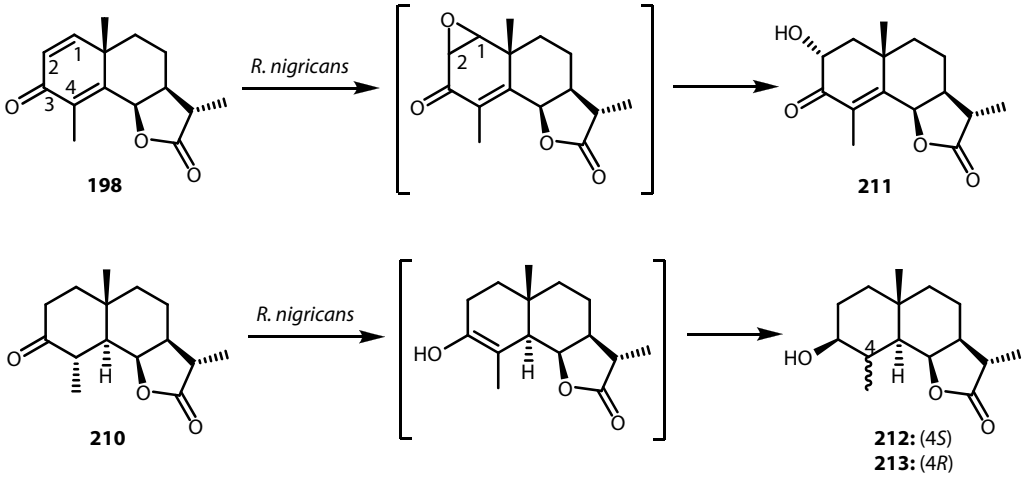


FIGURE 15.65 Biotransformation of α-episantonin (**198**) and tetrahydrosantonin (**210**) by *Rhizopus nigricans*.

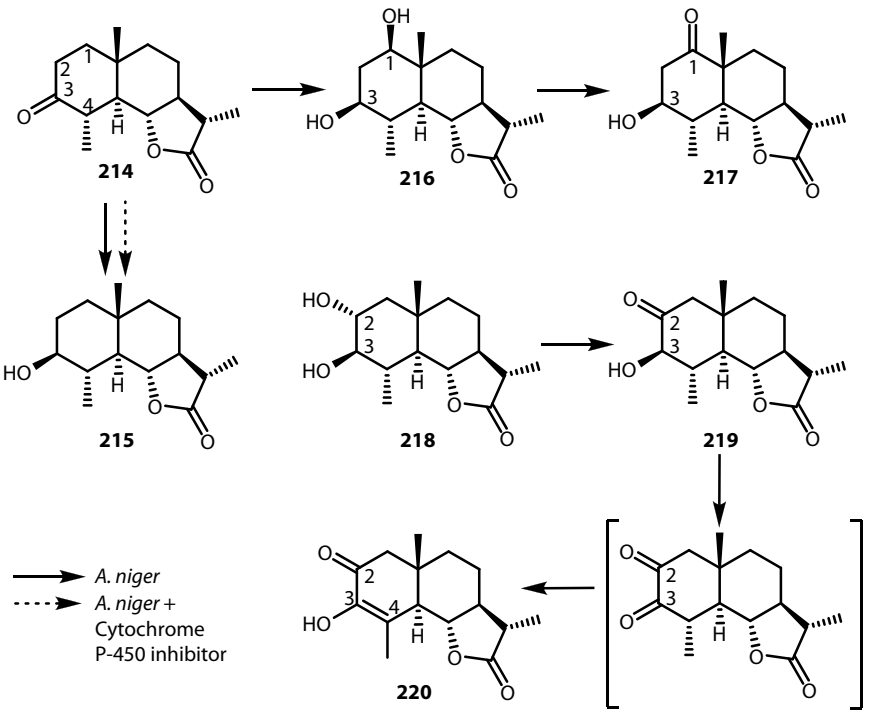


FIGURE 15.66 Biotransformation of 1,2,4β,5α-tetrahydro-α-santonin (**214**) by *Aspergillus niger.*

Guaiane-type sesquiterpene hydrocarbon, (+)-γ-gurjunene, (**228**) was treated in plant pathogenic fungus *Glomerella cingulata* to give two diols, (1*S*,4*S*,7*R*,10*R*)-5-guaien-11,13-diol (**229**), and (1*S*,4*S*,7*R*,10*S*)-5-guaien-10,11,13-triol (**230**) (Miyazawa et al., 1997, 1998) (Figure 15.68).

Glomerella cingulata converted guaiol (**231**) and bulnesol (**232**) to 5,10-dihydroxy (**233**) and 15-hydroxy derivative (**234**), respectively (Miyazawa et al., 1996) (Figure 15.69).

When *Eurotium rubrum* was used as the bioreactor of guaiene (**235**), rotunodone (**236**) was obtained (Sugawara and Miyazawa, 2004). Guaiol (**231**) was also transformed by *Aspergillus niger* to give a cylopentane derivative, pancherione (**237**) and two dihydroxy guaiols (**238**, **239**) (Morikawa et al., 2000), of which **237** was obtained from the same substrate using *Eurotium rubrum* for 10 days (Sugawara and Miyazawa, 2004; Miyazawa and Sugawara, 2006) (Figure 15.70).

Parthenolide (**240**), a germacrane-type lactone, isolated from the European feverfew (*Tanacetum parthenium*) as a major constituent shows cytotoxic, antimicrobial, and antifungal, anti-inflammatory, antirheumatic activity, apoptosis inducing, and NF-κB and DNA binding inhibitory activity. This substrate was incubated with *Aspergillus niger* in Czapek-peptone medium for 2 days to give six metabolites (**241**, 12.3%, **242**, 11.3%, **243**, 13.7%, **244**, 5.0%, **245**, 9.6%, **246**, 5.1%) (Hashimoto et al., 2005) (Figure 15.71). Compound **244** was a naturally occurring lactone from *Michelia champaca* (Jacobsson et al., 1995). The stereostructure of compound **243** was established by x-ray crystallographic analysis.

When parthenolide (**240**) was treated in *Aspergillus cellulosae* for 5 days, two new metabolites, 11β,13-dihydro-(**247**, 43.5%) and 11α,13-dihydroparthenolides (**248**, 1.6%) were obtained together with the same metabolites (**241**, 5.3%, **243**, 11.2%, **245**, 10.4%) as described above (Figure 15.72). Possible metabolic root of **240** has been shown in Figure 15.73 (Hashimoto et al., 2005).

Galal et al. (1999) reported that *Streptomyces fulvissimus* or *Rhizopus nigricans* converted parthenolide (**240**) into 11α-methylparthenolide (**247**) in 20–30% yield while metabolite 11β-hydroxyparthenolide (**248**) was obtained by incubation of **240** with *Rhizopus nigricans* and

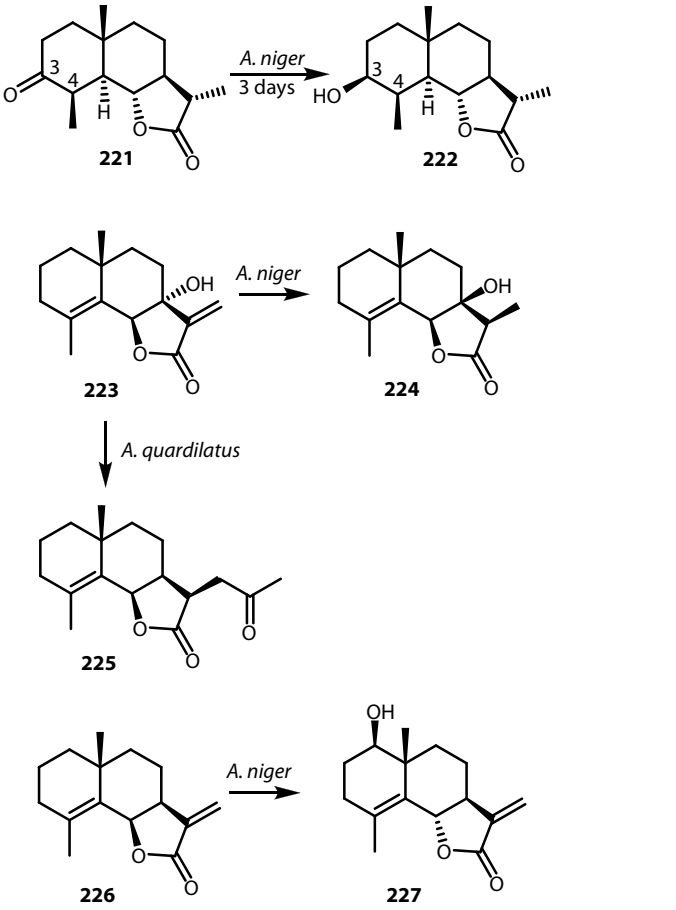


FIGURE 15.67 Biotransformation of C4-epimer (**221**) of **214**, 7α-hydroxyfrullanolide (**223**), and frullanolide (**226**) by *Aspergillus niger* and *Aspergillus quardilatus.*

Rhodotorula rubra. In addition to the metabolite **247**, *Streptomyces fulvissimus* gave minor polar metabolite, 9β-hydroxy derivative (**248a**) in low yield (3%). The same metabolite (**248a**) was obtained from **247** by fermentation of *Streptomyces fulvissimus* as a minor constituent. Furthermore, 14-hydroxyparthenolide (**248b**) was obtained from **240** and **247** as a minor component (4%) by *Rhizopus nigricans* (Figure 15.74).

Pyrethrosin (**248c**), a germacranolide, was treated in the fungus *Rhizopus nigricans* to afford five metabolites (**248d**–**248h**). Pyrethrosin itself and metabolite **248e** displayed cytotoxic activity against human malignant melanoma with IC_{50} 4.20 and 7.5 μg/mL, respectively. Metabolite **248h** showed significant *in vitro* cytotoxic activity against human epidermoid carcinoma (KB cells) and against human ovary carcinoma with $IC_{50} < 1.1$ and 8.0 μg/mL, respectively. Compounds **248f** and

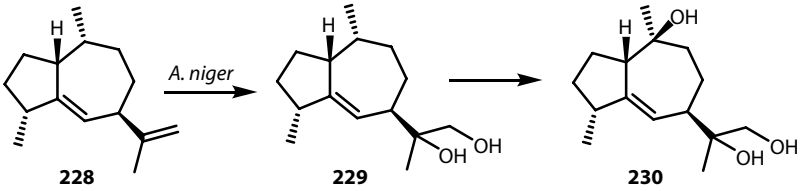


FIGURE 15.68 Biotransformation of (+)-γ-gurjunene (**228**) by *Glomerella cingulata.*

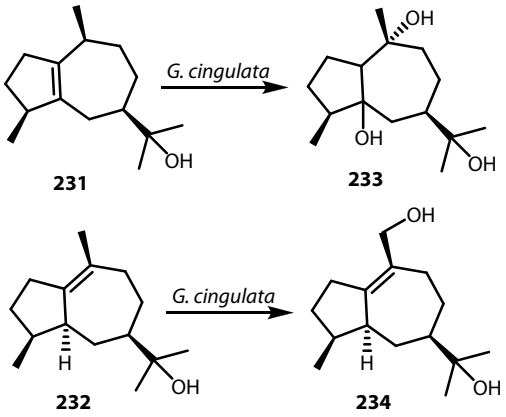


FIGURE 15.69 Biotransformation of guaiol (**221**) and bulnesol (**232**) by *Glomerella cingulata*.

248i were active against *Cryptococcus neoformans* with IC_{50} 35.0 and 25 μg/mL, respectively while **248a** and **248g** showed antifungal activity against *Candida albicans* with IC_{50} 30 and 10 μg/mL. Metabolites **248g** and its acetate (**248i**), derived from **248g** showed antiprotozoal activity against *Plasmodium falciparum* with IC_{50} 0.88 and 0.32 μg/mL, respectively without significant toxicity. Compound **248i** also exhibited pronounced activity against the chloroquine-registant strain of *Plasmodium falciparum* with IC_{50} 0.38 μg/mL (Galal, 2001) (Figure 15.75).

(–)-Dehydrocostuslactone (**249**), inhibitors of nitric oxide synthases and TNF-α, isolated from *Saussurea* radix, was incubated with *Cunninghamella echinulata* to afford (+)-11α,13-dihydrodehydrocostuslactone (**250a**). The epoxide (**251**) and a C11 reduced compound (**250**) were obtained by the above microorganisms (Galal, 2001).

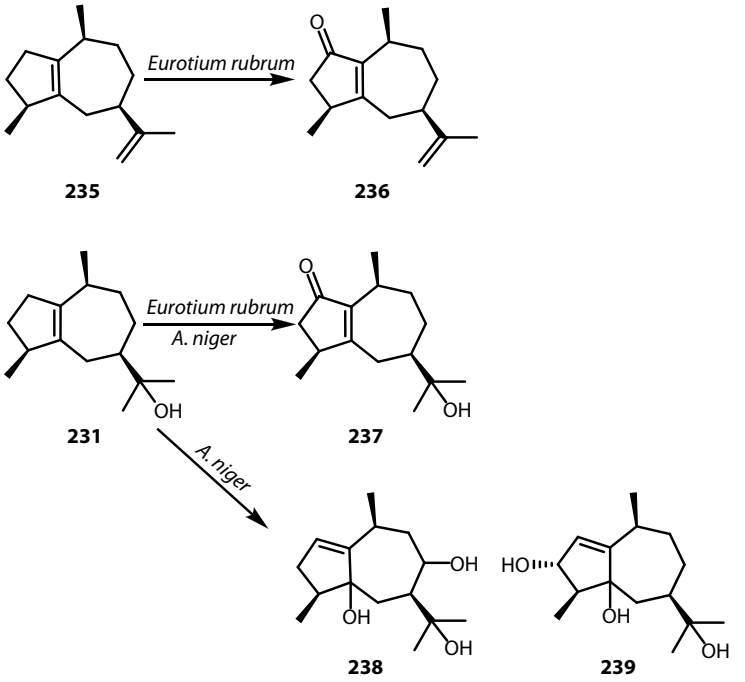


FIGURE 15.70 Biotransformation of guaiene (**235**) by *Eurotium rubrum* and guaiol (**231**) by *Aspergillus niger* and *Eurotium rubrum*.

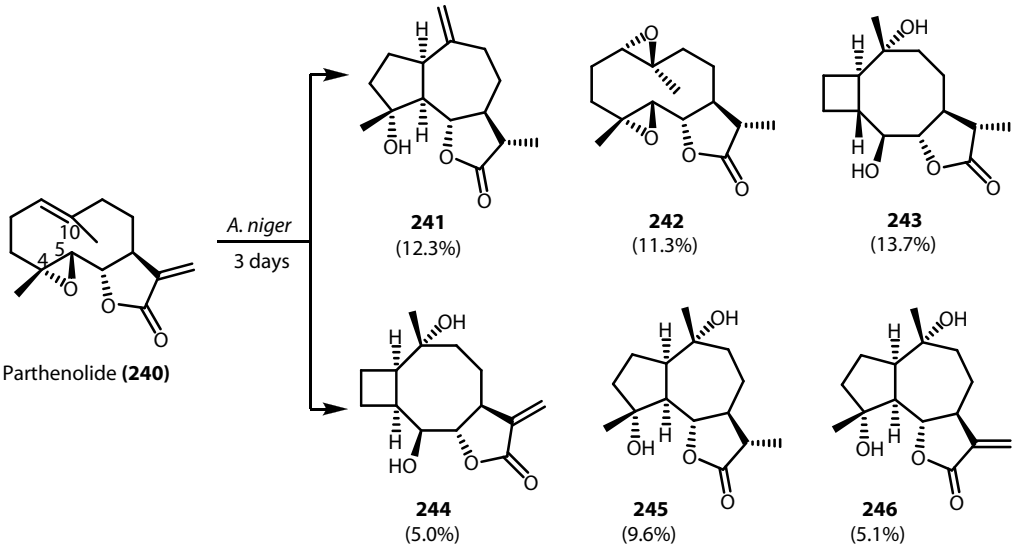


FIGURE 15.71 Biotransformation of parthenolide (**240**) by *Aspergillus niger.*

Cunninghamella echinulata and *Rhizopus oryzae* bioconverted **249** into C11/C13 dihydrogenated (**250**) and C10/C14 epoxidated product (**251**). Treatment of **252a** in *Cunninghamella echinulata* and *Rhizopus oryzae* gave (–)-16-(1-methyl-1-propenyl) eremantholide (**252b**) (Galal, 2001) (Figure 15.76).

The same substrate (**249**) was fed by *Aspergillus niger* for 7 days to afford four metabolites costuslactone (**250**), and their derivatives (**251**–**253**), of which **251** was the major product (28%) while the same substrate was cultivated with *Aspergillus niger* for 10 days, two minor metabolites (**254**, **255**) were newly obtained in addition to **252** and **253** of which the latter lactone was predominant (20.7%) (Hashimoto et al., 2001) (Figure 15.77).

When compound (**249**) was treated with *Aspergillus niger* in the presence of 1-aminobenzotriazole, **249** was completely converted into 11β,13-dihydro derivative (**250**) for 3 days; however, further biodegradation did not occur for 10 days (Hashimoto et al., 1999, 2001). The same substrate (**249**) was cultivated with *Aspergillus cellulosae* IFO to furnish 11,13-dihydro- (**250**) (82%) for only one day and then the product (**250**) slowly oxidized into 11,13-dihydro-8β-hydroxycostuslactone (**256**) (1.6%) from 8 days (Hashimoto et al., 1999, 2001) (Figure 15.78).

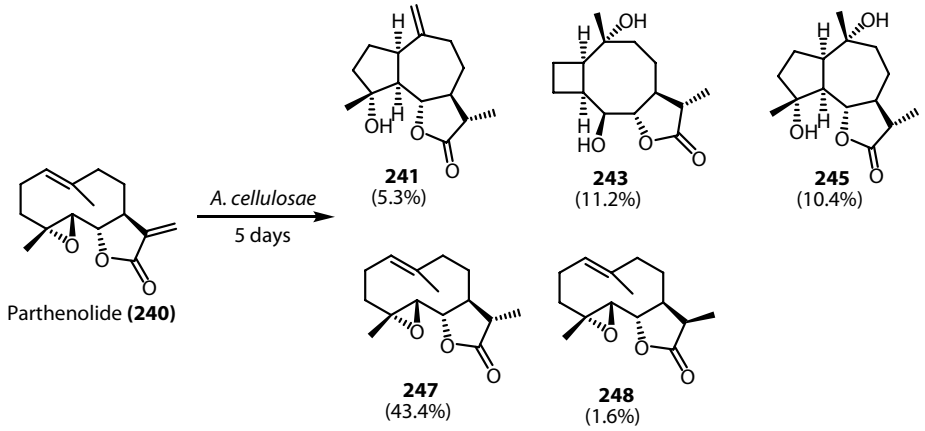


FIGURE 15.72 Biotransformation of parthenolide (**240**) by *Aspergillus cellulosae.*

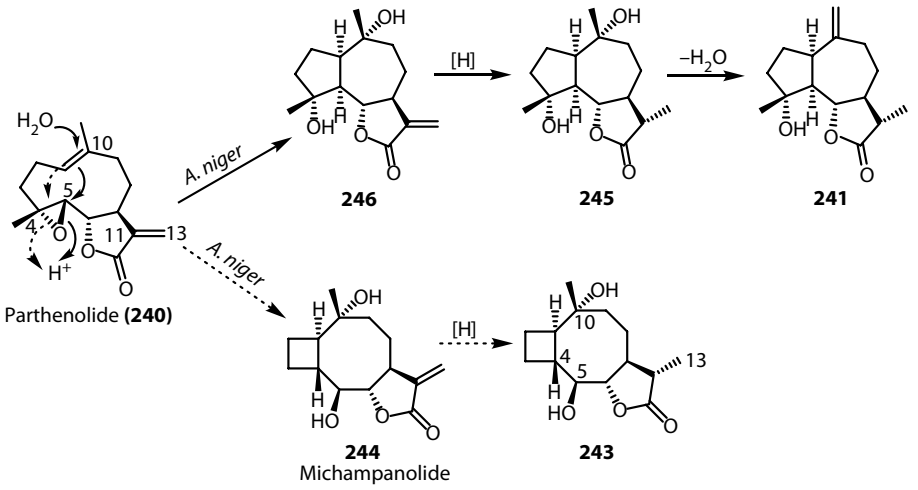


FIGURE 15.73 Possible pathway of biotransformation of parthenolide (**240**).

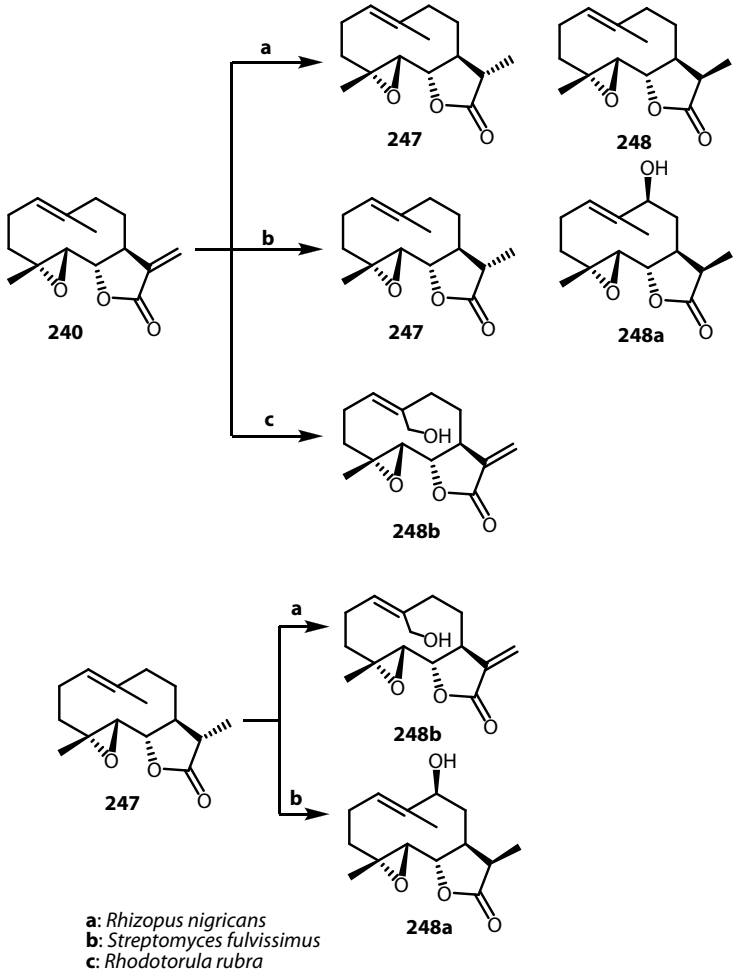


FIGURE 15.74 Biotransformation of parthenolide (**240**) and its dihydro derivative (**247**) by *Rhizopus nigricans*, *Streptomyces fulvissimus*, and *Rhodotorula rubra*.

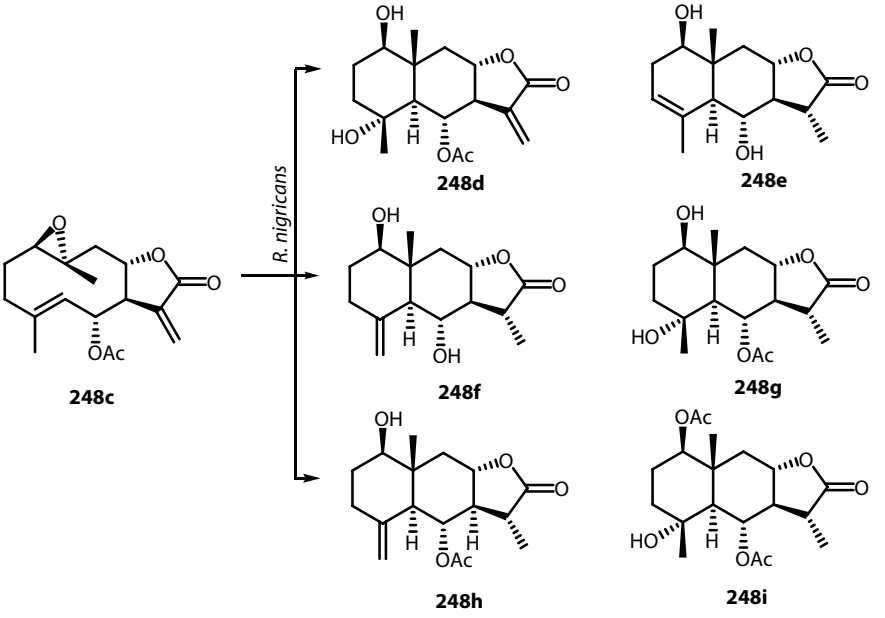


FIGURE 15.75 Biotransformation of pyrethrosin (**248c**) by *Rhizopus nigricans.*

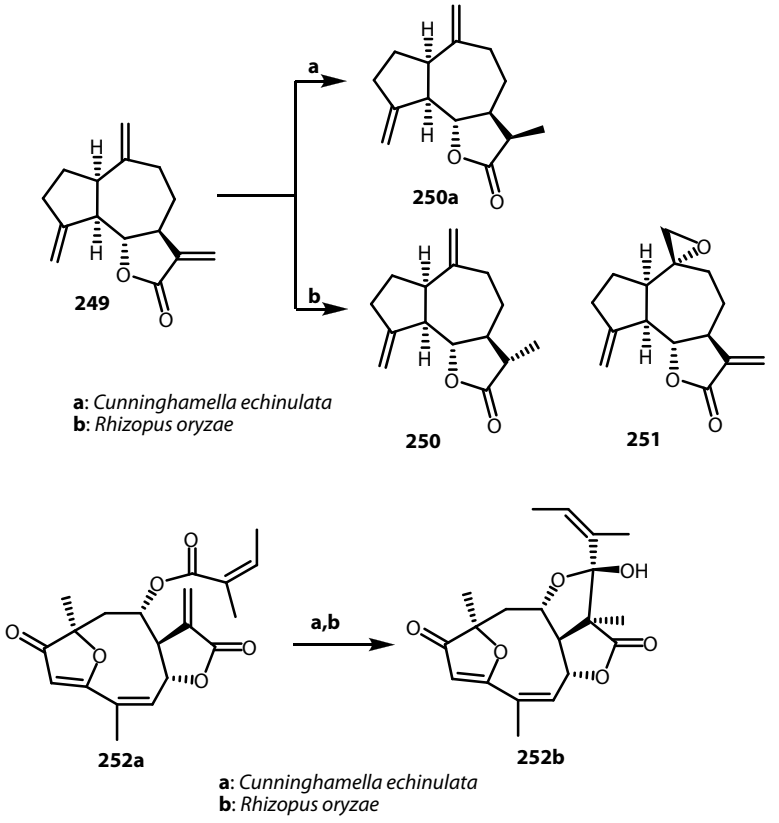


FIGURE 15.76 Biotransformation of (−)-dehydrocostuslactone (**249**) and rearranged guaianolide (**252a**) by *Cunninghamella echinulata* and *Rhizopus oryzae*.

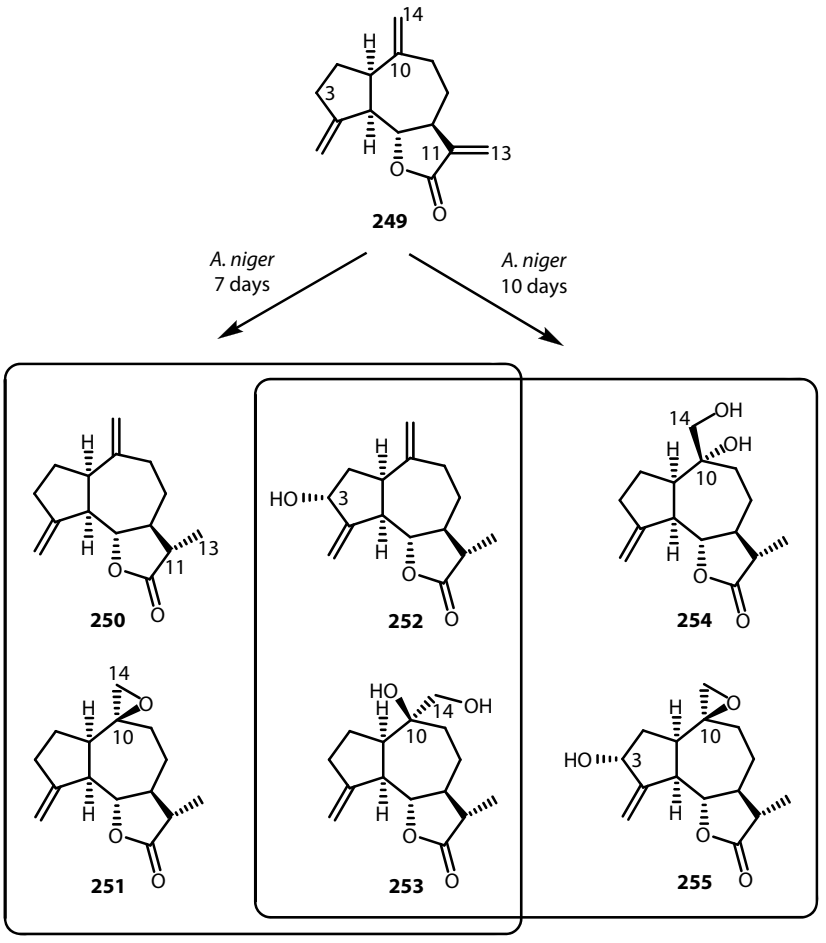


FIGURE 15.77 Biotransformation of (−)-dehydrocostuslactone (**249**) by *Aspergillus niger.*

The lactone (**249**) was biodegraded by the plant pathogen *Botryosphaeria dothidea* for 4 days to give the metabolites (**250**) (37.8%) and **257** (8.6%) while *Aspergillus niger* IFO-04049 (4 days) and *Aspergillus cellulosae* for 1 day gave only **250**. Thus *Botryosphaeria dothidea* demonstrated low stereoselectivity to reduce C11–C13 double bond (Hashimoto et al., 2001). Furthermore three *Aspergillus* species, *Aspergillus niger* IFO 4034, *Aspergillus awamori* IFO 4033, and *Aspergillus terreus* IFO6123 were used as bioreactors for compounds **249**. *Aspergillus niger* IFO 4034 gave three products (**250**–**252**), of which **252** was predominant (56% in GC-MS). *Aspergillus awamori* IFO 4033 and *Aspergillus terreus* IFO 6123 converted **249** to give **250** (56% from *Aspergillus awamori*, 43% from *Aspergillus terreus*) and **252** (43% from *Aspergillus awamori*, 57% from *Aspergillus terreus*), respectively (Hashimoto et al., 2001) (Figure 15.79).

Vernonia arborea (Asteraceae) contains zaluzanin D (**258**) in high content. Ten microorganisms were used for the biotransformation of compound **258**. *Botrytis cinerea* converted **258** into **259** and **260** (85:15%) and *Fusarium equiseti* gave **259** and **260** (33:66%). *Curvularia lunata*, *Colletotrichum lindemuthianum*, *Alternaria alternate*, and *Phyllosticta capsici* produced **259** as the sole metabolite in good yield while *Sclerotinia sclerotiorumn* and *Rhizpctonia solani* gave deactyl product (**261**) as a sole product, and **260**, **262**–**264** among which **263** and **264** are the major products, respectively. Reduction of C11–C13 exocylic double bond is the common transformation of α-methylene γ-butyrolactone (Kumari et al., 2003) (Figure 15.80).

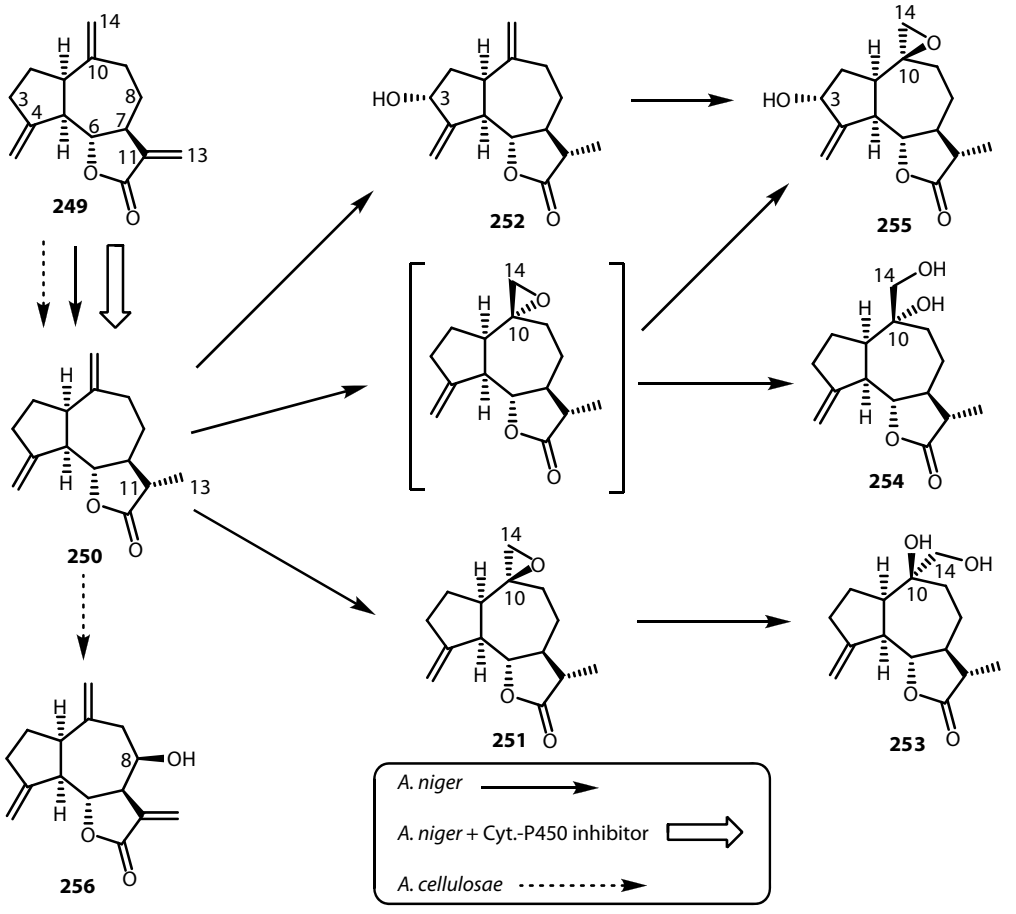


FIGURE 15.78 Possible pathway of biotransformation of (–)-dehydrocostuslactone (**249**) by *Aspergillus niger* and *Aspergillus cellulosae*.

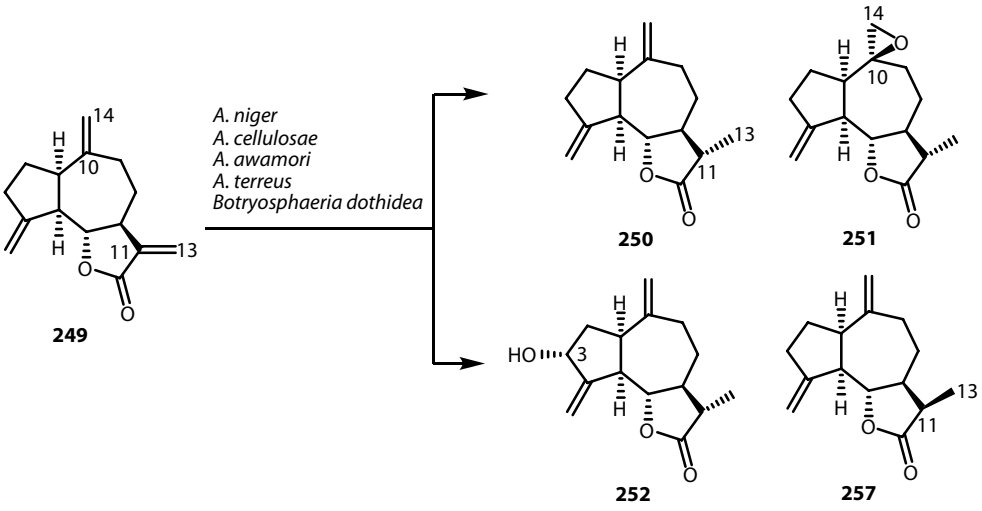


FIGURE 15.79 Biotransformation of (–)-dehydrocostuslactone (**249**) by *Aspergillus* species and *Botryosphaeria dothidea*.

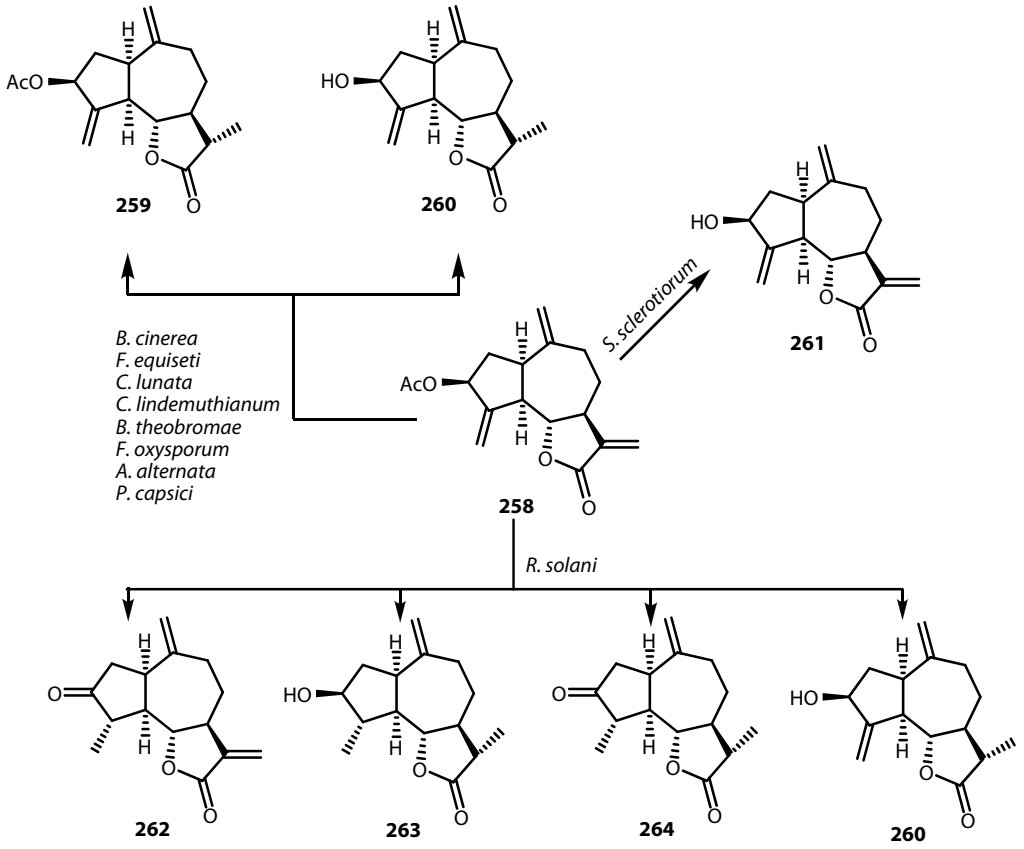


FIGURE 15.80 Biotransformation of zaluzanin D (**258**) by various fungi.

Incubation of parthenin (**264a**) with the fungus *Beauveria bassiana* in modified Richard's medium gave C11–C13 reduced product (**264b**) in 37% yield, while C11 α-hydroxylated product (**264c**) was obtained in 32% yield from the broth of the fungus *Sporotrichum pulverulentum* using the same medium (Bhutani and Thakur, 1991) (Figure 15.81).

Cadina-4,10(15)-dien-3-one (**265**) possessing insecticidal and ascaricidal activity, from Jamaican medicinal plant *Hyptis verticillata* was metabolized by *Curvularia lunat*a ATCC 12017 in potato dextrose to give its 12-hydoxy- (**266**), 3α-hydroxycadina-4,10(15)-dien (**267**), and 3α-hydroxy-4,5-dihydrocadinenes (**268**) while **265** was incubated by the same fungus in peptone, yeast, and beef extracts and glucose medium, only **267** and **268** were obtained. Compound **267** derived synthetically was treated in the same fungus *Curvularia lunata* to afford three metabolites (**269**–**271**) (Collins and Reese, 2002) (Figure 15.82).

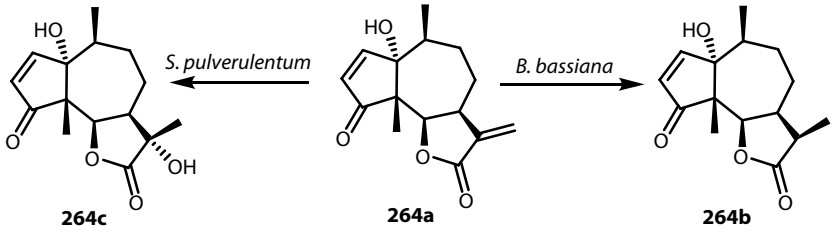


FIGURE 15.81 Biotransformation of parthenin (**264a**) by *Sporotrichum pulverulentum* and *Beauveria bassiana*.

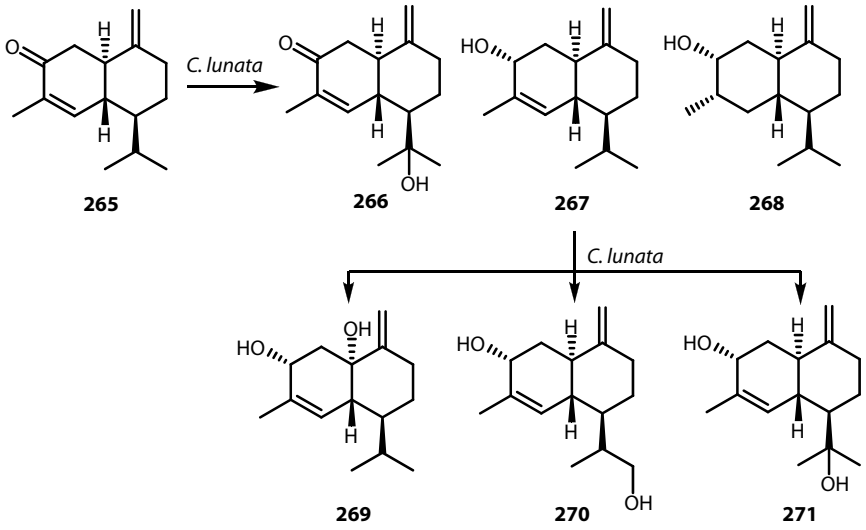


FIGURE 15.82 Biotransformation of cadina-4,10(15)-dien-3-one (**265**) by *Curvularia lunata*.

The incubation of the same substrate (**265**) in *Mucor plumbeus* ATCC 4740 in high iron-rich medium gave **270**, which was obtained from *Curvularia lunata* mentioned above, **268**, **272**, **273**, **277**, **278**, and **279**. In low iron medium, this fungus converted the same substrate **265** into three epoxides (**274**–**276**), a tetraol (**280**) with common metabolites (**268**, **273**, **277**, **278**), and **271**, which was the same metabolite used by *Curvularia lunata* (Collins, Reynold, and Reese, 2002). It is interesting to note that only epoxides were obtained from the substrate (**265**) by *Mucor* fungus in low iron medium (Figure 15.83).

The same substrate (**265**) was incubated with the deuteromycete fungus, *Beauveria bassiana,* which is responsible for the muscaridine disease in insects, in order to obtain new functionalized analogues with improved biological activity. From compound **265**, nine metabolites were obtained.

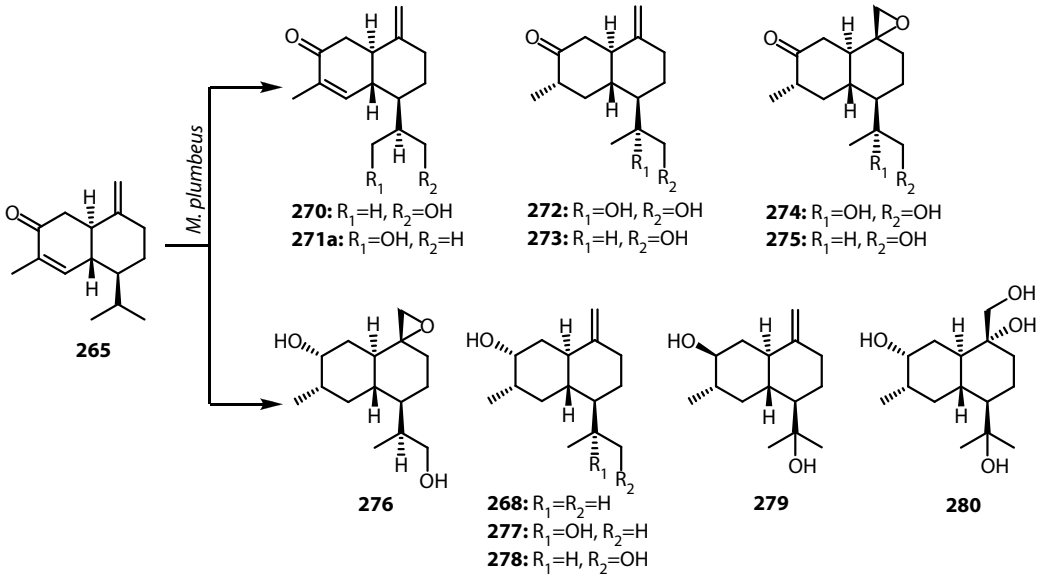


FIGURE 15.83 Biotransformation of cadina-4,10(15)-dien-3-one (**265**) by *Mucor plumbeus*.

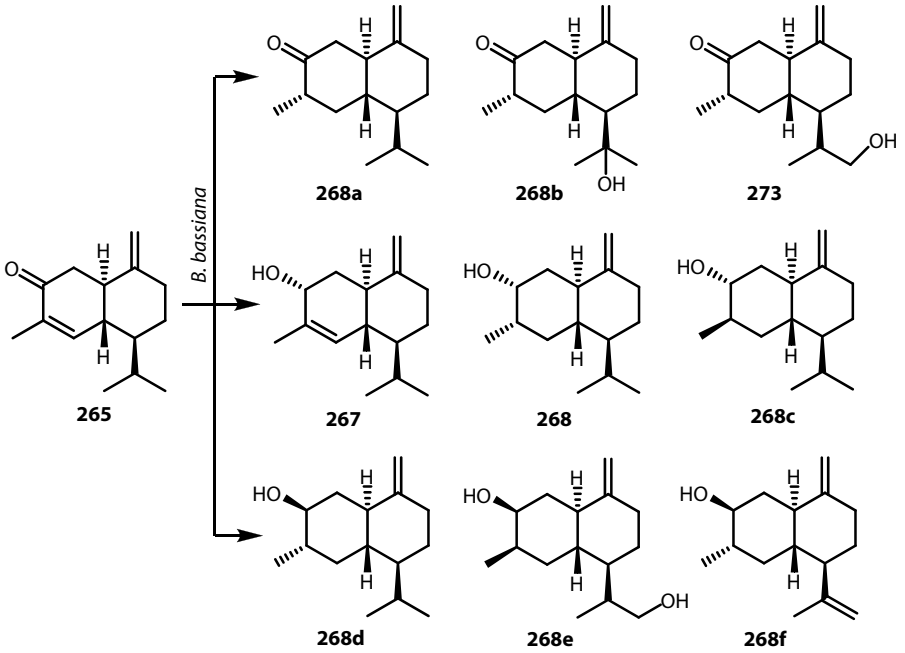


FIGURE 15.84 Biotransformation of cadina-4,10(15)-dien-3-one (**265**) by *Beauveria bassiana*.

The insecticidal potential of the metabolites (**267**, **268**, **268a**–**268f**) were evaluated against *Cylas formicarius*. The metabolites (**273**, **268**, **268d**) showed enhanced activity compared with the substrate (**265**). The plant growth regulatory activity of the metabolites against radish seeds was tested. All the compounds showed inhibitory activity; however, their activity was less than colchicine (Buchanan et al., 2000) (Figure 15.84).

Cadinane-type sesquiterpene alcohol (**281**) isolated from the liverwort *Mylia taylorii* gave a primary alcohol (**282**) by *Aspergillus niger* treatment (Morikawa et al., 2000) (Figure 15.85).

Fermentation of (−)-α-bisabolol (**282a**) possessing anti-inflammatory activity with plant pathogenic fungus *Glomerella cingulata* for 7 days yielded oxygenated products (**282b**–**282e**) of which compound **282e** was predominant. 3,4-Dihydroxy products (**282b**, **282d**, **282e**) could be formed by hydrolysis of the 3,4-epoxide from **282a** and **282c** (Miyazawa et al., 1995b) (Figure 15.86).

El Sayed et al. (2002) reported microbial and chemical transformation of (*S*)-(+)-curcuphenol (**282g**) and curcudiol (**282n**), isolated from the marine sponges, *Didiscus axeata*. Incubation of compound **282g** with *Kluyveromyces marxianus* var. *lactis* resulted in the isolation of six metabolites (3–8, **282h**–**282j**). The same substrate was incubated with *Aspergillus alliaceus* to give the metabolites (**282p**, **282q**, **282s**) (Figure 15.87).

Compounds **282g** and **282n** were treated in *Rhizopus arrhizus* and *Rhodotorula glutinus* for 6 and 8 days to afford glucosylated metabolites, 1α-D-glucosides (**282o**) and **282r**, respectively. The

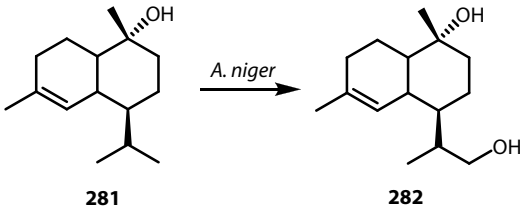


FIGURE 15.85 Biotransformation of cadinol (**281**) by *Aspergillus niger*.

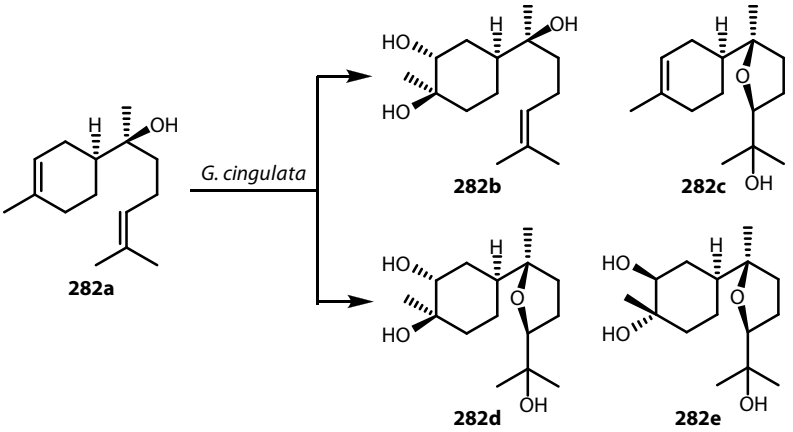


FIGURE 15.86 Biotransformation of β-bisabolol (**282a**) by *Glomerella cingulata*.

substrate itself showed antimicrobial activity against *Candia albicans, Cryptococcus neoformans*, and MRSA-resistant *Staphylococcus aureus* and *Staphylococcus aureus* with MIC and MFC/MBC ranges of 7.5–25 and 12.5–50 μg/mL, respectively. Compounds **282g** and **282h** also exhibited *in vitro* antimalarial activity against *Plasmodium falciparium* (D6 clone) and *Plasmodium falciparium* (W2 clone) of 3600 and 3800 ng/mL (selective index (S.I.) > 1.3), and 1800 (S.I. > 2.6), and 2900 (S.I. > 1.6), respectively (El Sayed et al., 2002) (Figure 15.87).

Artemisia annua is one of the most important Asteraceae species as antimalarial plant. There are many reports of microbial biotransformation of artemisinin (**283**), which is active antimalarial rearranged cadinane sesquiterpene endoperoxide, and its derivatives to give novel antimalarials with increased activities or differing pharmacological characteristics.

Lee et al. (1989) reported that deoxoartemisinin (**284**) and its 3α-hydroxy derivative (**285**) were obtained from the metabolites of artemisinin (**283**) incubated with *Nocardia corallina* and *Penicillium chrysogenum* (Figure 15.88).

Zhan et al. (2002) reported that incubation of artemisinin (**283**) with *Cunninghamella echinulata* and *Aspergillus niger* for 4 days at 28°C resulted in the isolation of two metabolites, 10β-hydroxyartemisinin (**287a**) and 3α-hydroxydeoxyartemisinin (**285**), respectively.

Compound **283** was also biotransformed by *Aspergillus niger* to give four metabolites, deoxyartemisinin (**284**, 38%), 3α-hydroxydeoxyartemisinin (**285**, 15%), and two minor products (**286**, 8% and **287**, 5%) (Hashimoto et al., 2003b).

Artemisinin (**283**) was also bioconverted by *Cunninghamella elegans*. During this process, 9β-hydroxyartemisinin (**287b**, 78.6%), 9β-hydroxy-8α-artemisinin (**287c**, 6.0%), 3α- hydroxydeoxoartemisinin (**285**, 5.4%), and 10β-hydroxyartemisinin (**287d**, 6.5%) have been formed. On the basis of quantitative structure-activity relationship (QSAR) and molecular modeling investigations, 9β-hydoxy derivatization of artemisinin skeleton may yield improvement in antimalarial activity and may potentially serve as an efficient means of increasing water solubility (Parshikov et al., 2004) (Figure 15.89).

Albicanal (**288**) and (–)-drimenol (**289**) are simple drimane sesquiterpenoids isolated from the liverwort, *Diplophyllum serrulatum*, and many other liverworts and higher plants. The latter compound was incubated with *Mucor plumbeus* and *Rhizopus arrhizus*. The former microorganism converted **289** to 6,7α-epoxy- (**290**), 3β-hydroxy- (**291**), and 6α-drimenol (**292**) in the yields of 2%, 7%, and 50%, respectively. On the other hand, the latter species produced only 3β-hydroxy derivative (**291**) in 60% yield (Aranda et al., 1992) (Figure 15.90).

(–)-Polygodial (**293**) possessing piscicidal, antimicrobial, and mosquito-repellant activity is the major pungent sesquitepene dial isolated from *Polygonum hydropiper* and the liverwort, *Porella vernicosa* complex. Polygodial was incubated with *Aspergillus niger*, however, because of its

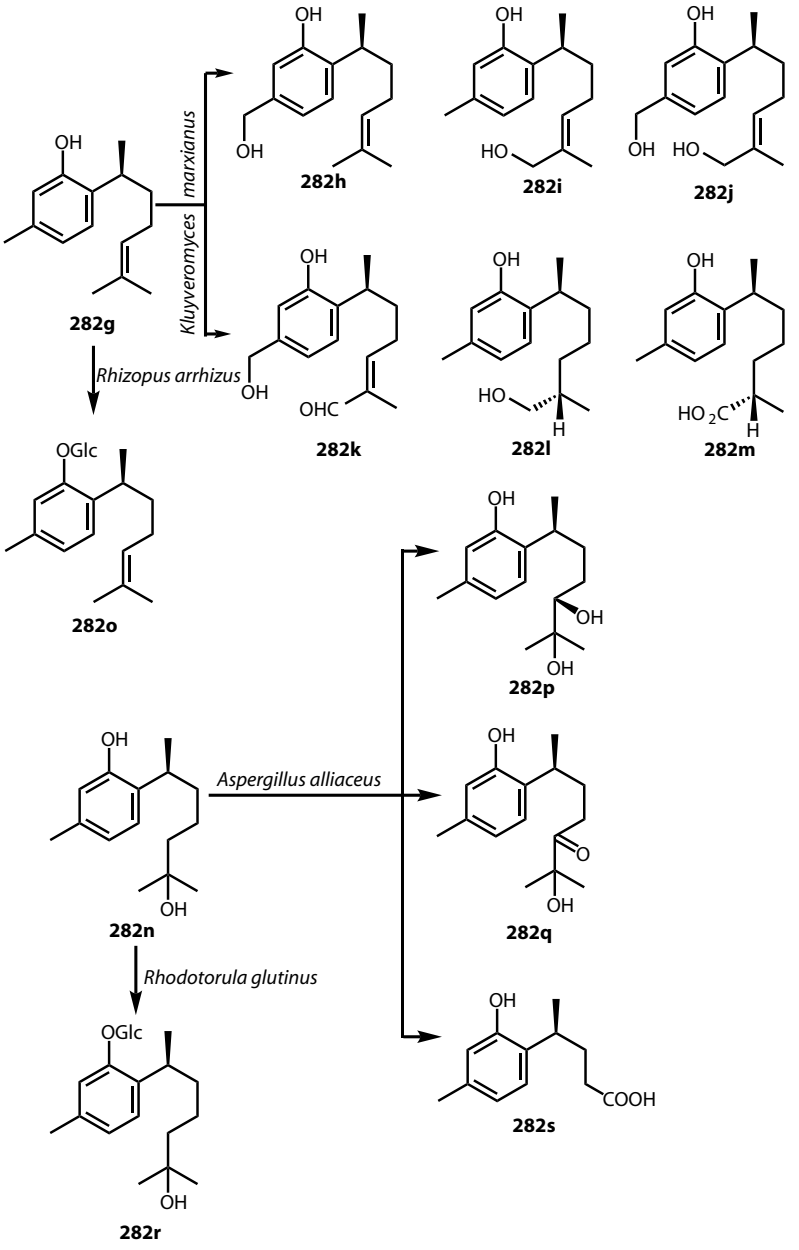


FIGURE 15.87 Biotransformation of (*S*)-(+)-curcuphenol (**282g**) by *Kluyveromyces marxianus* and *Rhizopus arrhizus* and curcudiol (**282n**) by *Aspergillus alliaceus* and *Rhodotorula glutinus*.

antimicrobial activity, nothing metabolite was obtained (Sekita et al., 2005). Polygodiol (**295**) prepared from polygodial (**293**) was also treated in the same manner as described above to afford 3β-hyrdoxy- (**297**), which was isolated from *Marasmius oreades* as antimicrobial activity (Ayer and Craw, 1989) and 6α-hydroxypolygodiol (**298**) in 66–70% and 5–10% yields, respectively (Aranda et al., 1992). The same metabolite (**297**) was also obtained from polygodiol (**295**) as a sole metabolite from the culture broth of *Aspergillus niger* in Czapek-peptone medium for 3 days in 70.5% yield (Sekita et al., 2005), while the C9 epimeric product (**296**) from isopolygodial (**294**) was incubated with *Mucor plumbeus* to afford 3β-hydroxy- (**299**) and 6α-hydroxy derivative (**300**) in low yields,

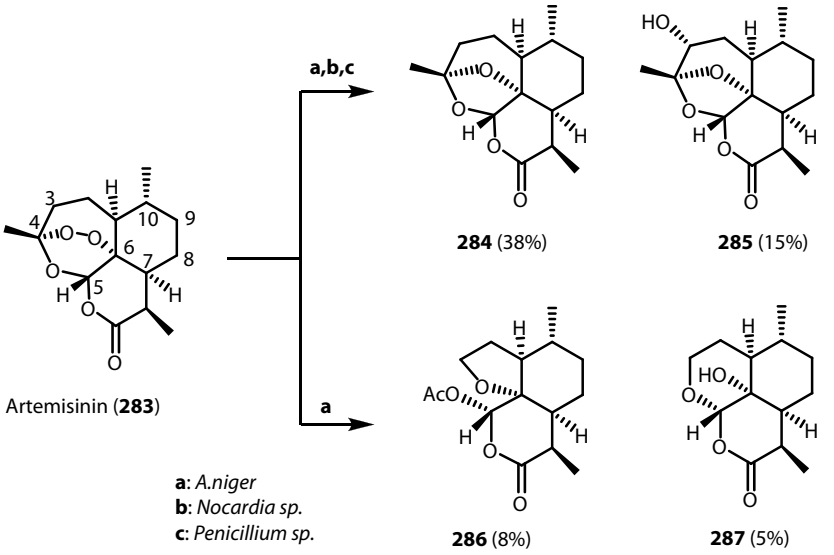


FIGURE 15.88 Biotransformation of artemisinin (**283**) by *Aspergillus niger*, *Nocardia corallina*, and *Penicillium chrysogenum*.

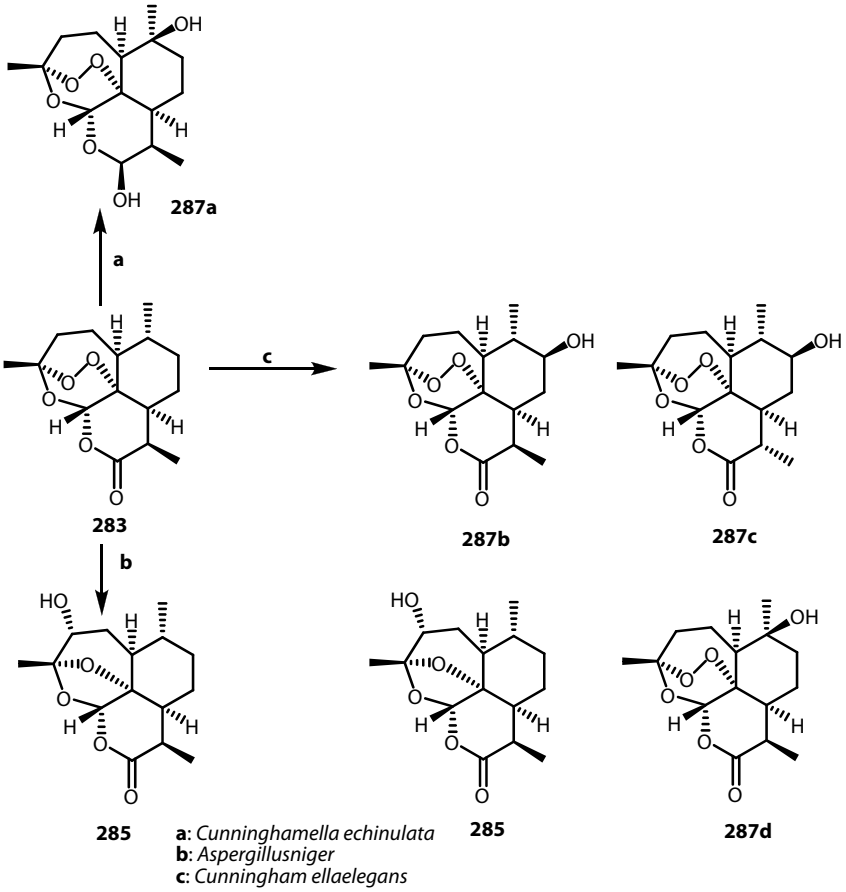


FIGURE 15.89 Biotransformation of artemisinin (**283**) by *Cunninghamella echinulata*, *Cunninghamella elegans*, and *Aspergillus niger*.

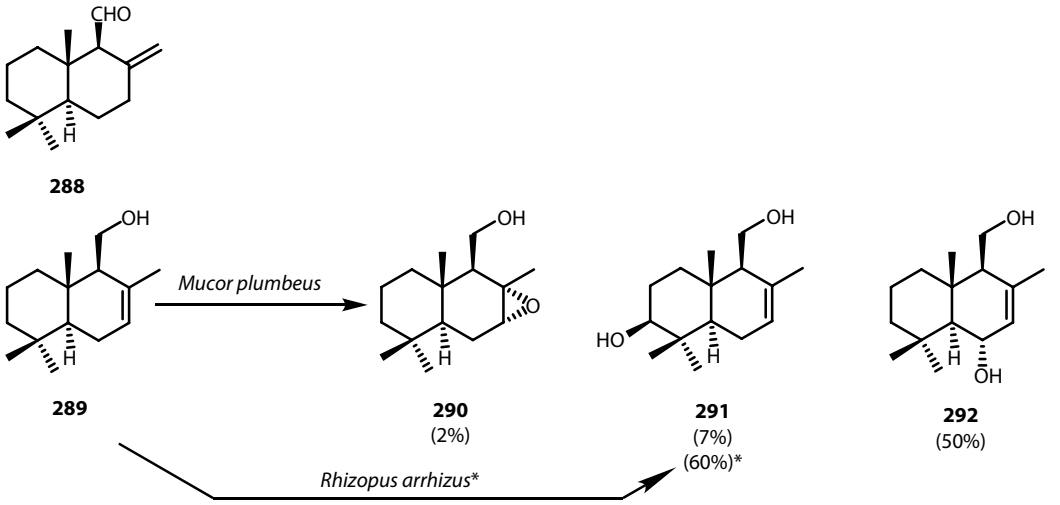


FIGURE 15.90 Biotransformation of drimenol (**289**) by *Mucor plumbeus* and *Rhizopus arrhizus*.

7% and 13% (Aranda et al., 1992). Drim-9α-hydroxy-11β,12-diacetoxy-7-ene (**301**) derived from polygodiol (**295**) was treated in the same manner as described above to yield its 3β-hydroxy derivative (**302**, 42%) (Sekita et al., 2005) (Figures 15.91 and 15.92).

Cinnamodial (**303**) from the Malagasy medicinal plant, *Cinnamosma fragrans*, was also treated in the same medium including *Aspergillus niger* to furnish three metabolites, respectively in very law yields (**304**, 2.2%; **305**, 0.05%; and **306**, 0.62%). Compound **305** and **306** are naturally occurring cinnamosmolide, possessing cytotoxicity and antimicrobial activity, and fragrolide. In this case, the introduction of 3β-hydroxy group was not observed (Sekita et al., 2006) (Figure 15.93).

Naturally occurring rare drimane sesquiterpenoids (**307–314**) were biosynthesized by the fungus *Cryptoporus volvatus* with isocitric acids. Among these compounds, in particular, cryptoporic acid E (**312**) possesses antitumor promoter, anticolon cancer, and very strong super oxide anion radical scavenging activities (Asakawa et al., 1992). When the fresh fungus allowed standing in moisture condition, olive fungus *Paecilomyces varioti* grows on the surface of the fruit body of this

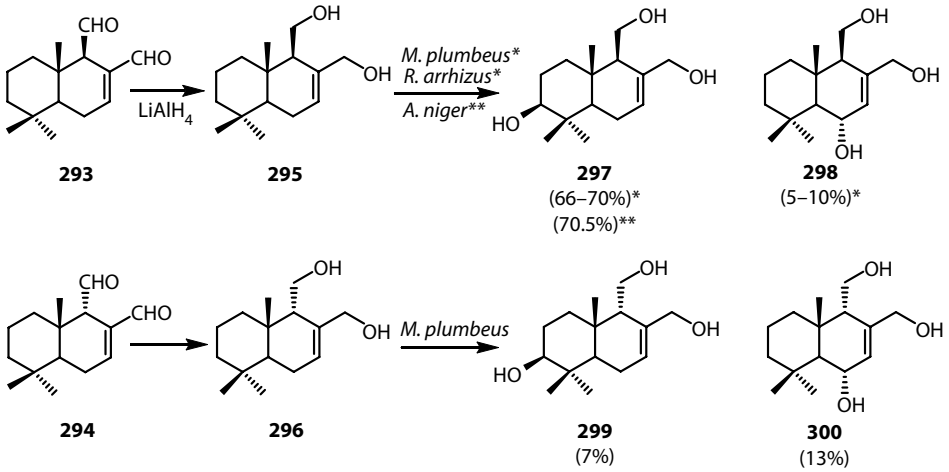
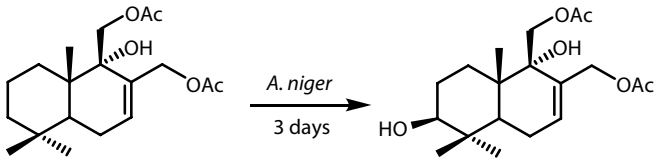


FIGURE 15.91 Biotransformation of polygodiol (**295**) by *Mucor plumbeus*, *Rhizopus arrhizus*, and *Aspergillus niger*.



301 **302** (47.2%)

FIGURE 15.92 Biotransformation of drim-9α-hydroxy-11,12-diacetoxy-7-ene (**301**) by *Aspergillus niger.*

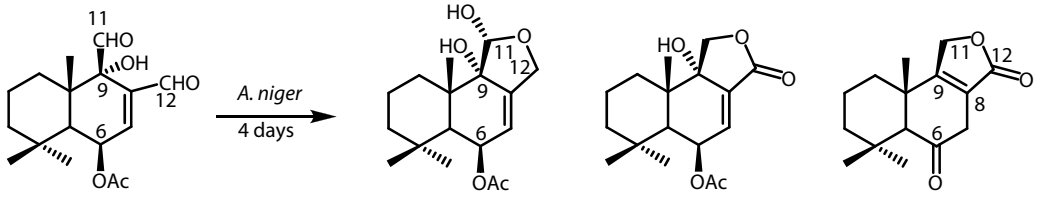
fungus. 2 kg of the fresh fungus was infected by *Cryptoporus volvatus* for 1 month, followed by the extraction of methanol to give the crude extract, then purification using silica gel and Sephadex LH-20 to give five metabolites (**316**, **318**–**321**), which were not found in the fresh fungus (Takahashi et al., 1993a). Compound **318** was also isolated from the liverworts, *Bazzania* and *Diplophyllum* species (Asakawa, 1982, 1995) (Figure 15.94).

Liverworts produce a large number of enantiomeric mono-, sesqui-, and diterpenoids to those found in higher plants and lipophilic aromatic compounds. It is also noteworthy that some liverworts produce both normal and its enantiomers. The more interesting phenomenon in the chemistry of liverworts is that the different species in the same genus, for example, *Frullania tamarisci* subsp. *tamarisci* and *Frullania dilatata* produce totally enantiomeric terpenoids. Various sesqui- and diterpenoids, bibenzyls, and bisbibenzyls isolated from several liverworts show characteristic fragrant odor, intensely hot and bitter taste, muscle relaxing, antimicrobial, antifungal, allergenic contact dermatitis, antitumor, insect antifeedant, superoxide anion release inhibitory, piscicidal, and neurotrophic activity (Asakawa, 1982, 1990, 1995, 1999, 2007, 2008; Asakawa and Ludwiczuk, 2008). In order to obtain the different kind of biologically active products and to compare the metabolites of both normal and enantiomers of terpenoids, several secondary metabolites of specific liverworts were biotransformed by *Penicillium sclerotiorum*, *Aspergillus niger*, and *Aspergillus cellulosae.*

(−)-Cuparene (**322**) and (−)-2-hydroxycuparene (**323**) have been isolated from the liverworts, *Bazzania pompeana* and *Marchantia polymorpha*, while its enantiomer (+)-cuparene (**324**) and (+)-2-hydroxycuparene (**325**) from the higher plant, *Biota orientalis* and the liverwort *Jungermannia rosulans.* (*R*)-(−)-α-Cuparenone (**326**) and grimaldone (**327**) demonstrate intense flagrance. In order to obtain such compounds from both cuparene and its hydroxy compounds, both enatiomers mentioned above were cultivated with *Aspergillus niger* (Hashimoto et al., 2001a) (Figure 15.95).

From (−)-cuparene (**322**), five metabolites (**328**–**332**) all of which contained cyclopentanediols or hydroxycyclopentanones were obtained. An aryl methyl group was also oxidized to give primary alcohol, which was further oxidized to afford carboxylic acids (**329**–**331**) (Hashimoto et al., 2001a) (Figure 15.96).

From (+)-cuparene, six metabolites (**333**–**338**) were obtained. These are structurally very similar to those found in the metabolites of (−)-cuparene, except for the presence of an acetonide (**336**), but they are not identical. All metabolites possess benzoic acid moiety.



Cinnamodial (**303**) **304** (2.2%) **305** (0.05%) Cinnamosmolide **306** (0.20%) Fragrolide

FIGURE 15.93 Biotransformation of cinnamodial (**303**) by *Aspergillus niger.*

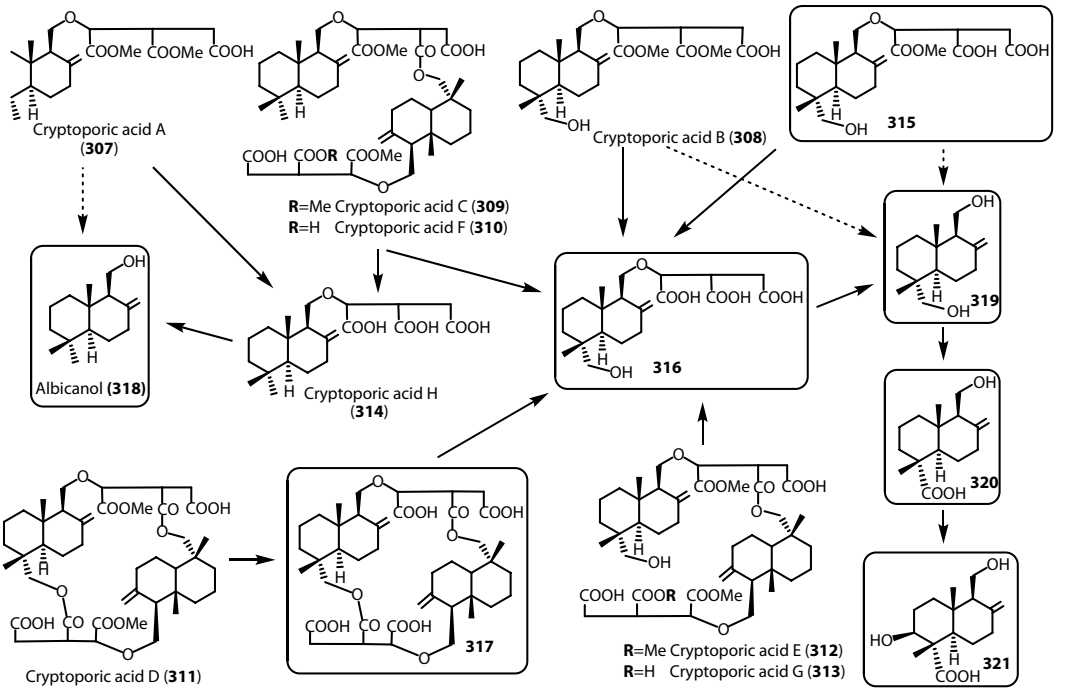


FIGURE 15.94 Biotransformation of cryptoporic acids (**307**–**317**, **316**) by *Paecilomyces varioti.*

The possible biogenetic pathways of (+)-cuparene (**324**) has been proposed in Figure 15.97. Unfortunately, none of the metabolites show strong mossy odor (Hashimoto et al., 2006). The presence of an acetonide in the metabolites has also been seen in those of dehydronootkatone (**25**) (Furusawa et al., 2003) (Figure 15.98).

The liverwort *Herbertus adancus*, *Herbertus sakuraii*, and *Mastigophora diclados* produce (−)-herbertene, the C3 methyl isomer of cuparene, with its hydroxy derivatives, for example, herbertanediol (**339**), which shows NO production inhibitory activity (Harinantenaina et al., 2007) and herbertenol (**342**). Treatment of compound (**339**) in *Penicillium sclerotiorum* in Czapek-polypeptone medium gave two dimeric products, mastigophorene A (**340**) and mastigophorene B (**341**), which showed neurotrophic activity (Harinantenaina et al., 2005).

When (−)-herbertenol (**342**) was biotransformed for 1 week by the same fungus, no metabolic product was obtained; however, five oxygenated metabolites (**344**–**348**) were obtained from its methyl ether (**343**). The possible metabolic pathway is shown in Figure 15.99. Except for the presence of the ether (**348**), the metabolites from **342** resemble those found in (−)- and (+)-cuparene (Hashimoto et al., 2006) (Figures 15.100 and 15.101).

Maalioxide (**349**), mp 65–66°, $[\alpha]_D^{21}$–34.4°, obtained from the liverwort, *Plagiochila sciophila* was inoculated and cultivated rotatory (100 rpm) in Czapek-peptone medium (pH 7.0) at 30°C for 2 days. (−)-Maalioxide (**349**) (100 mg/200 mL) was added to the medium and further cultivated for 2 days to afford three metabolites, 1β-hydroxy-(**350**), 1β,9β-dihydroxy- (**351**), and 1β,12-dihydroxymaalioxides (**352**), of which **351** was predominant (53.6%). When the same substrate was cultured with *Aspergillus cellulosae* in the same medium for 9 days, 7β-hydroxymaalioxide (**353**) was obtained as a sole product in 30% yield. The same substrate (**349**) was also incubated with the fungus *Mucor plumbeus* to obtain a new metabolite, 9β-hydroxymaalioxide (**354**), together with two known hydroxylated products (**350**, **353**) (Wang et al., 2006).

Maalioxide (**349**) was oxidized by *m*-chloroperbenzoic acid to give a very small amount of **353** (1.2%), together with 2α-hydroxy- (**355**, 2%) and 8α-hydroxymaalioxide (**356**, 1.5%), which

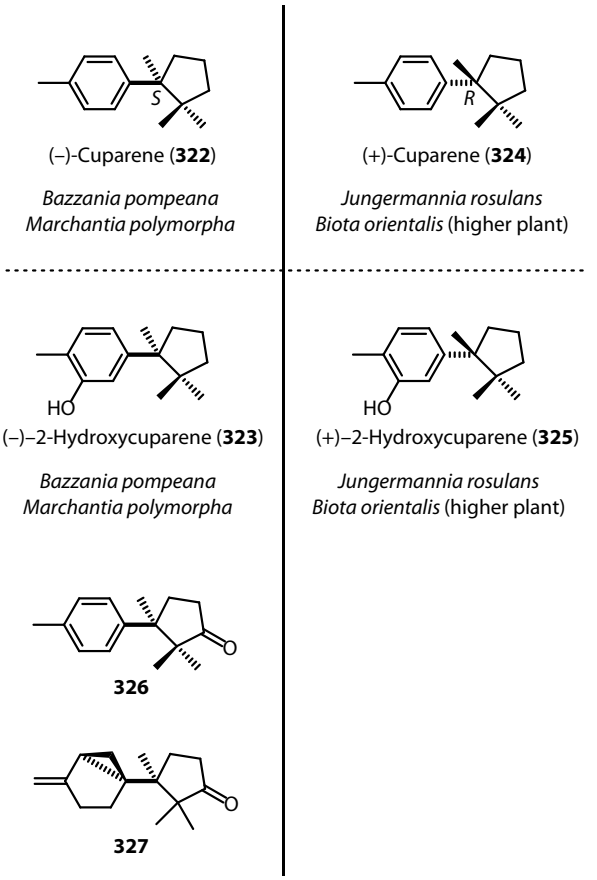


FIGURE 15.95 Naturally occurring cuparene sesquiterpenoids (**322**–**327**).

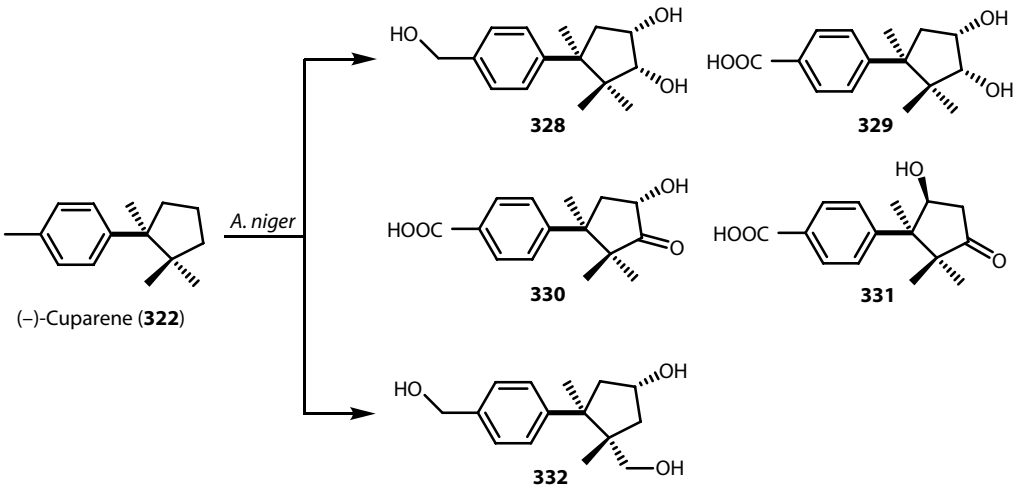


FIGURE 15.96 Biotransformation of (–)-cuparene (**322**) by *Aspergillus niger.*

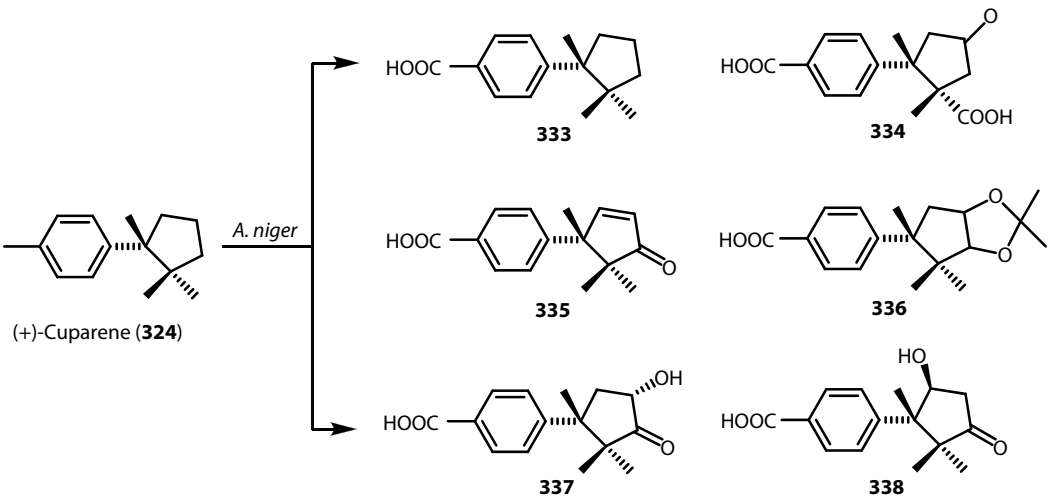


FIGURE 15.97 Biotransformation of (+)-cuparene (**324**) by *Aspergillus niger.*

have not been obtained in the metabolite of **349** in *Aspergillus niger* and *Aspergillus cellulosae* (Tori et al., 1990) (Figure 15.102).

Plagiochila sciophila is one of the most important liverworts, since it produces bicyclohumulenone (**357**), which possesses strong mossy note and is expected to manufacture compounding perfume. In order to obtain much more strong scent, **357** was treated in *Aspergillus niger* for 4 days to give 4α,10β-dihydroxybicyclohumulenone (**358**, 27.4%) and bicyclohumurenone-12-oic acid (**359**). An epoxide (**360**) prepared by *m*-chloroperbenzoic acid was further treated in the same fungus as described above to give 10β-hydoxy derivative (**361**, 23.4%). Unfortunately, these metabolites possess only faint mossy odor (Hashimoto et al., 2003c) (Figure 15.103).

The liverwort *Reboulia hemisphaerica* biosynthesizes cyclomyltaylanoids like **362** and also *ent*-1α-hydroxy-β-chamigrene (**367**). Biotransformation of cyclomyltaylan-5-ol (**362**) in the same

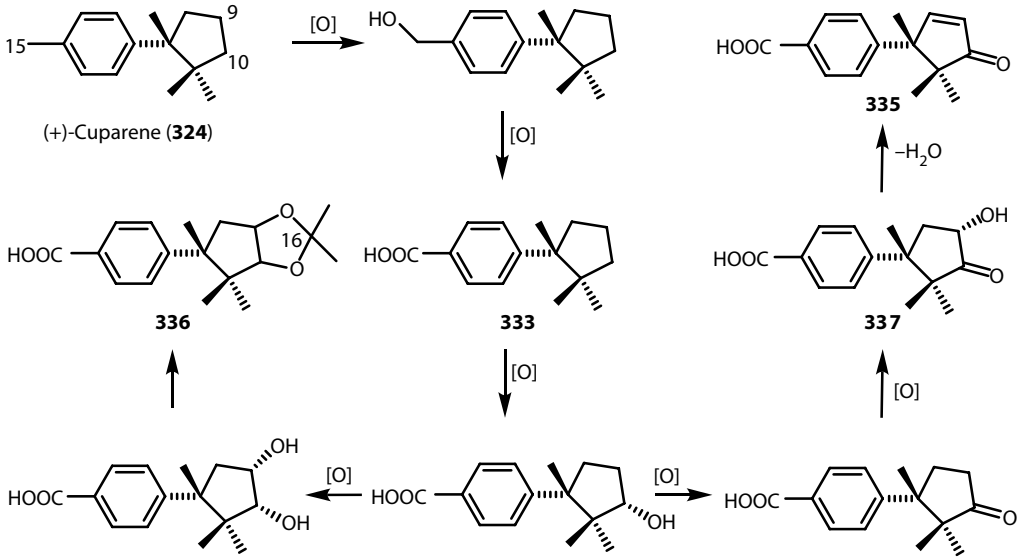


FIGURE 15.98 Possible pathway of biotransformation of (+)-cuparene (**324**) by *Aspergillus niger.*

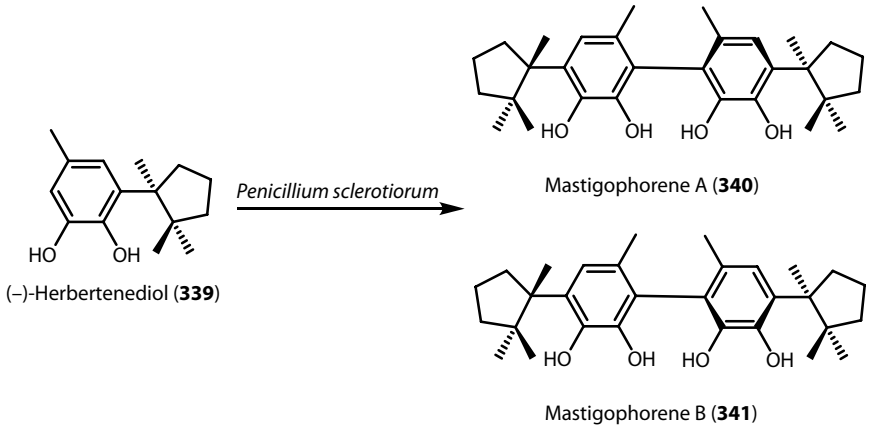


FIGURE 15.99 Biotransformation of (–)-herbertenediol (**339**) by *Penicillium sclerotiorum*.

medium including *Aspergillus niger* gave four metabolites, 9β-hydroxy- (**363**, 27%), 9β,15-dihydroxy- (**364**, 1.7%), 10β-hydroxy- (**365**, 10.3%), and 9β,15-dihydroxy derivative (**366**, 12.6%). In this case, the stereospecificity of alcohol was observed, but the regiospecificity of alcohol moiety was not seen in this substrate (Furusawa et al., 2005b, 2006b) (Figure 15.104).

The biotransformation of spirostructural terpenoids was not carried out. *Ent*-1α-hydroxy-β-chamigrene (**367**) was inoculated in the same manner as described above to give three new metabolites (**368**–**370**), of which **370** was the major product (46.2% in isolated yield). The hydroxylation of vinyl methyl group has been known to be very common in the case of microbial and mammalian biotransformation (Furusawa et al., 2005, 2006) (Figure 15.105).

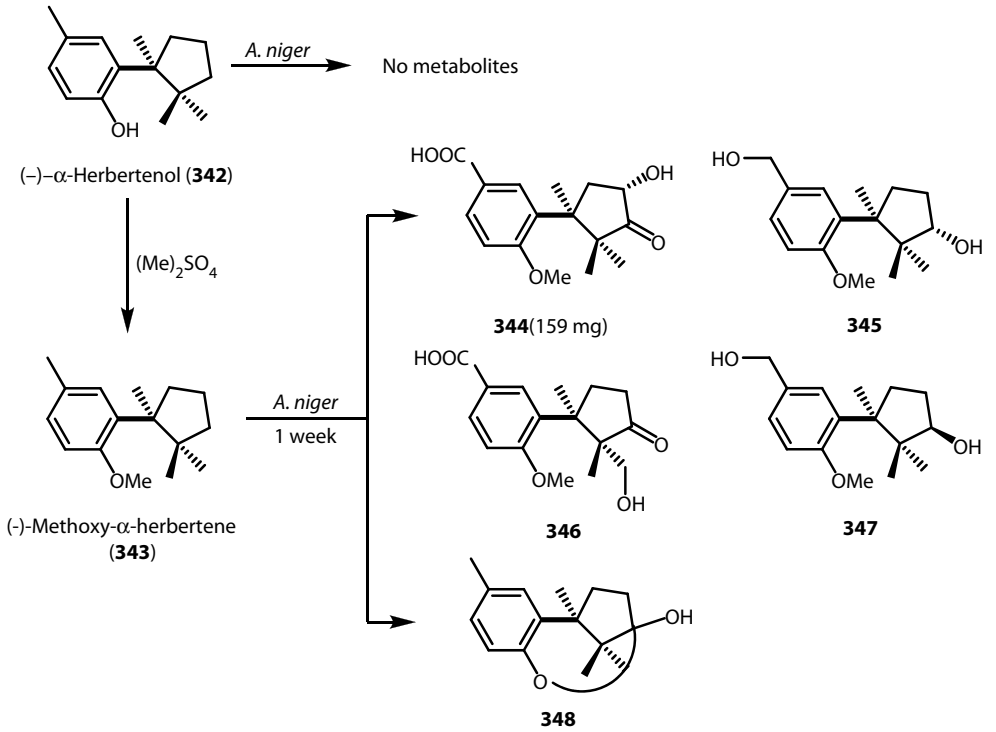


FIGURE 15.100 Biotransformation of (–)-methoxy-α-herbertene (**343**) by *Aspergillus niger*.

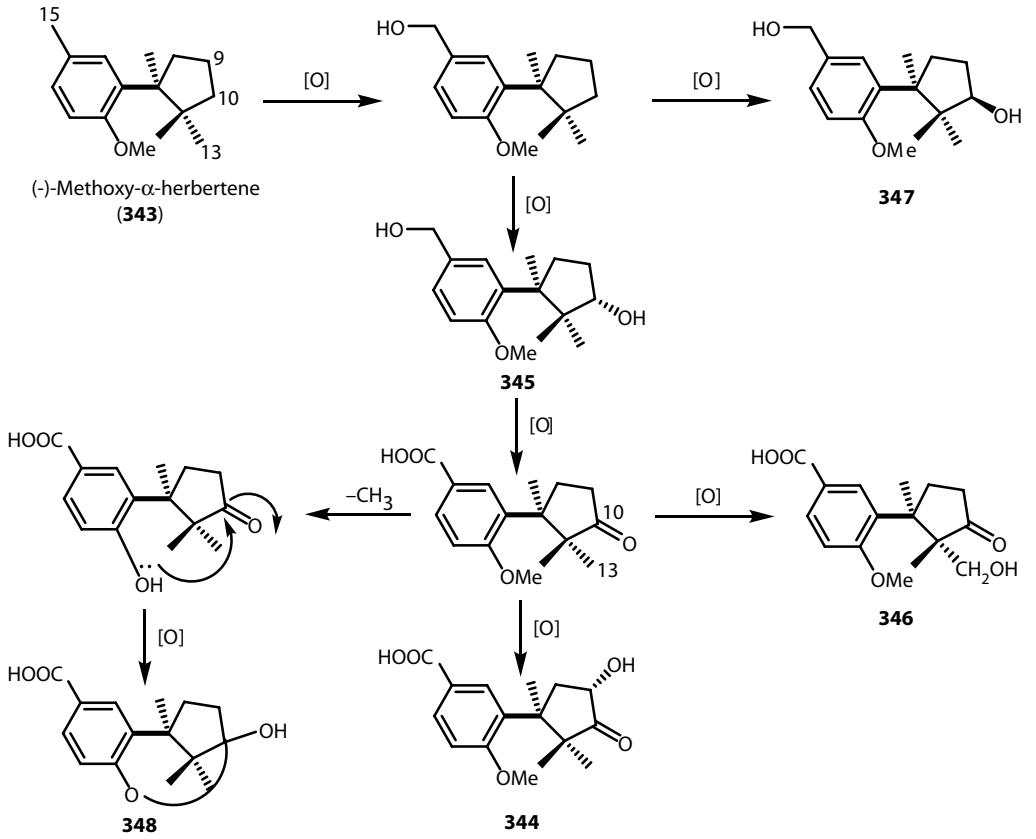


FIGURE 15.101 Possible pathway of biotransformation of (–)-methoxy-α-herbertene (**343**) by *Aspergillus niger.*

β-Barbatene (= gymnomitrene) (**4**), a ubiquitous sesquiterpene hydrocarbon, from liverwort like *Plagiochila sciophila* and many others. Jungermanniales liverworts was treated in the same manner using *Aspergillus niger* for 1 day gave a triol, 4β,9β,10β-trihydoxy-β-barbatene (**27**, 8%) (Hashimoto et al., 2003c).

Pinguisane sesquiterpenoids have been isolated from the Jungermanniales, Metzgeriales, and Marchantiales. In particular, the Lejeuneaceae and Porellaceae are rich sources of this unique type of sesquiterpenoids. One of the major furanosesquitepene (**373**) was biodegradated by *Aspergillus niger* to afford primary alcohol (**375**), which might be formed from **374** as shown in Figure 15.106 (Lahlou et al., 2000) (Figure 15.107).

In order to obtain more pharmacologically active compounds, the secondary metabolites from crude drugs and animals, for example, nardosinone (**376**) isolated from the crude drug, *Nardostachys chinensis*, which has been used for headache, stomachache, and diuresis possesses antimalarial activity. Hinesol (**384**), possessing spasmolytic activity, obtained from *Atractylodis lanceae* rhizoma, animal perfume (–)-ambrox (**391**) from ambergris were biotransformed by *Aspergillus niger, Aspergillus cellulosae*, *Botryosphaeria dothidea*, and so on.

Nardosinone (**376**) was incubated in the same medium including *Aspergillus niger* as described above for 1 day to give six metabolites (**377**, 45%; **378**, 3%; **379**, 2%; **380**, 5%; **381**, 6%; and **382**, 3%). Compounds **380**–**382** are unique trinorsesquiterpenoids although their yields are very poor. Compound **380** might be formed by the similar manner to that of phenol from cumene (**383**) (Figure 15.108) (Hashimoto et al., 2003b) (Figure 15.109).

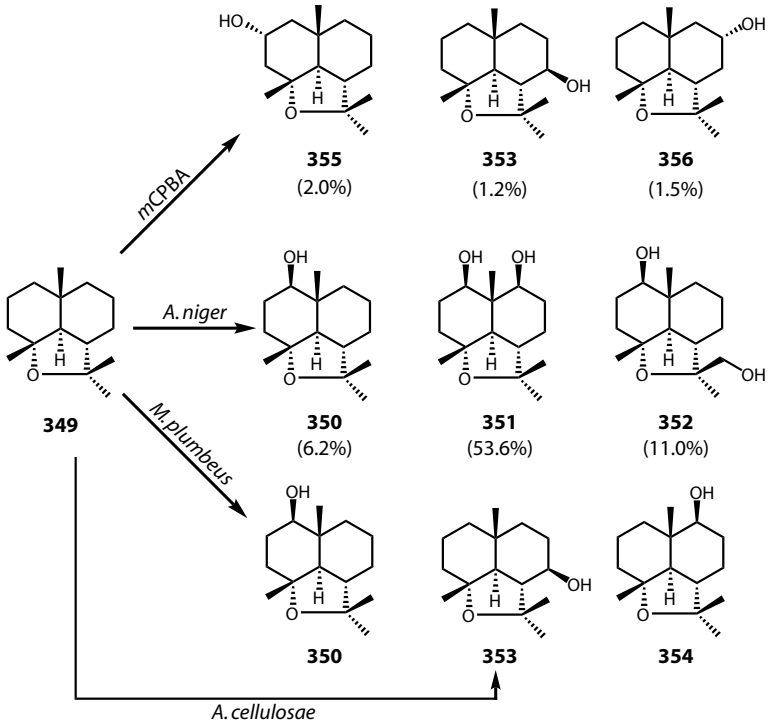


FIGURE 15.102 Biotransformation of maalioxide (**349**) by *Aspergillus niger*, *Aspergillus cellulosae*, and *Mucor plumbeus*.

From hinesol (**384**), two allylic alcohols (**386**, **387)** and their oxygenated derivative (**385**), and three unique metabolites (**388**–**390**) having oxirane ring were obtained. The metabolic pathway is very similar to that of oral administration of hinesol since the same metabolites (**395**–**387**) were obtained from the urine of rabbits (Hashimoto et al., 1998, 1999b, 2001) (Figure 15.110).

To obtain a large amount of ambrox (**391**), a deterrence, labda-12,14-dien-7α,8-diol obtained from the liverwort, *Porella pettottetiana* as a major component, was chemically converted into

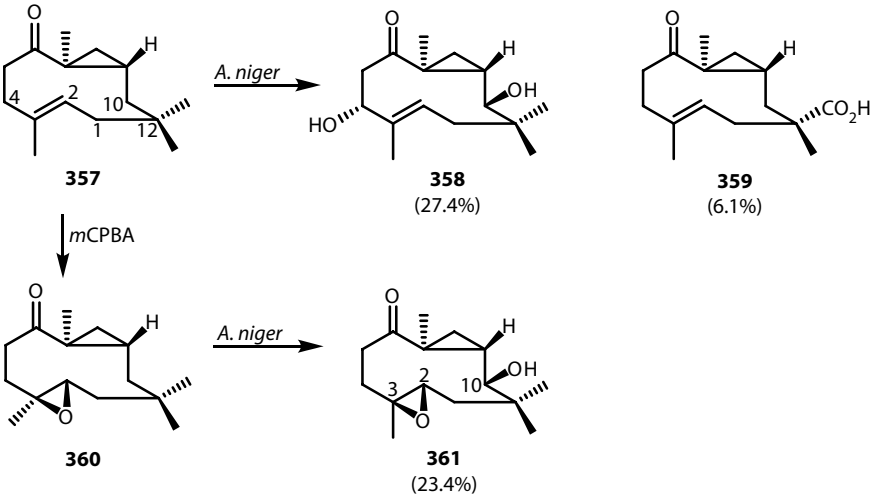


FIGURE 15.103 Biotransformation of bicyclohumulenone (**357**) by *Aspergillus niger*.

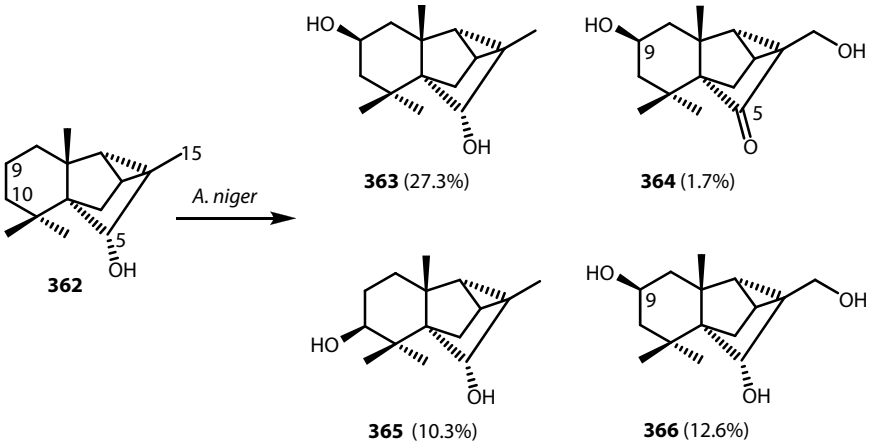


FIGURE 15.104 Biotransformation of cyclomyltaylan-5-ol (**362**) by *Aspergillus niger*.

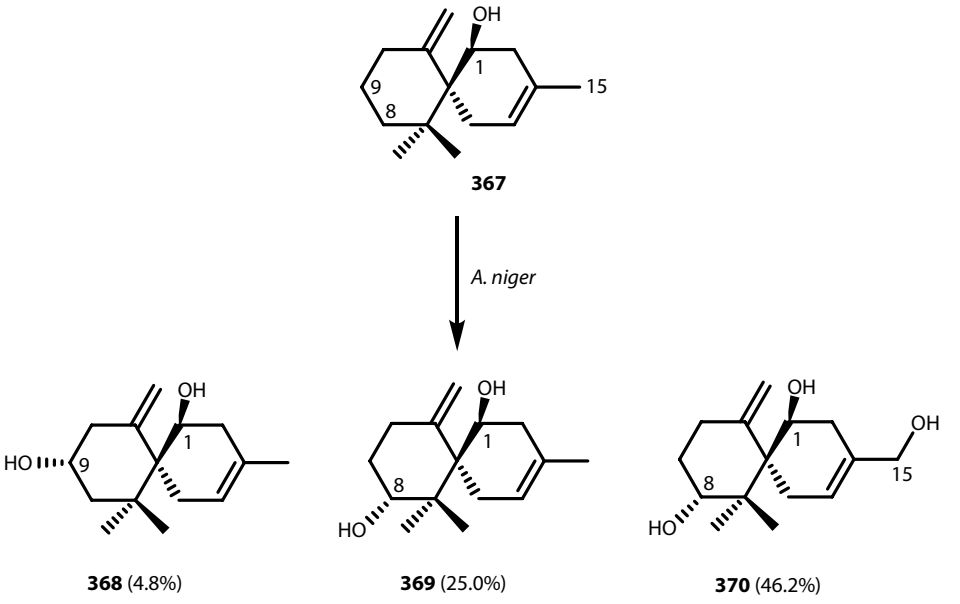


FIGURE 15.105 Biotransformation of *ent*-1α-hydroxy-β-chamigrene (**367**) by *Aspergillus niger*.

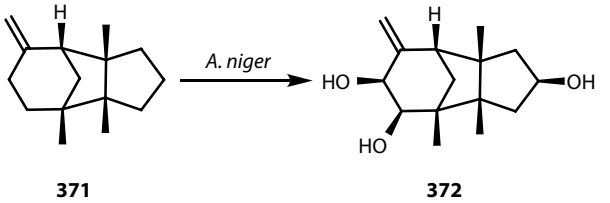


FIGURE 15.106 Biotransformation of β-barbatene (**371**) by *Aspergillus niger*.

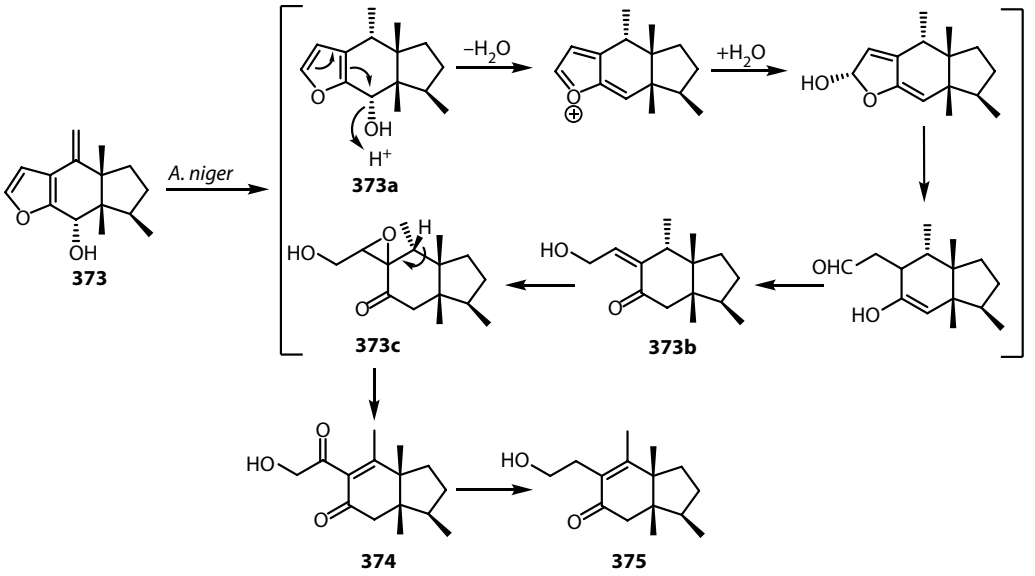


FIGURE 15.107 Biotransformation of pinguisanol (**373**) by *Aspergillus niger.*

(–)-ambrox via six steps in relatively high yield (Hashimoto et al., 1998a). Ambrox was added to Czapek-peptone medium including *Aspergillus niger*, for 4 days, followed by chromatography of the crude extract to afford four oxygenated products (**392**–**395**), among which the carboxylic acid (**393**, 52.4%) is the major product (Hashimoto et al., 2001) (Figure 15.111).

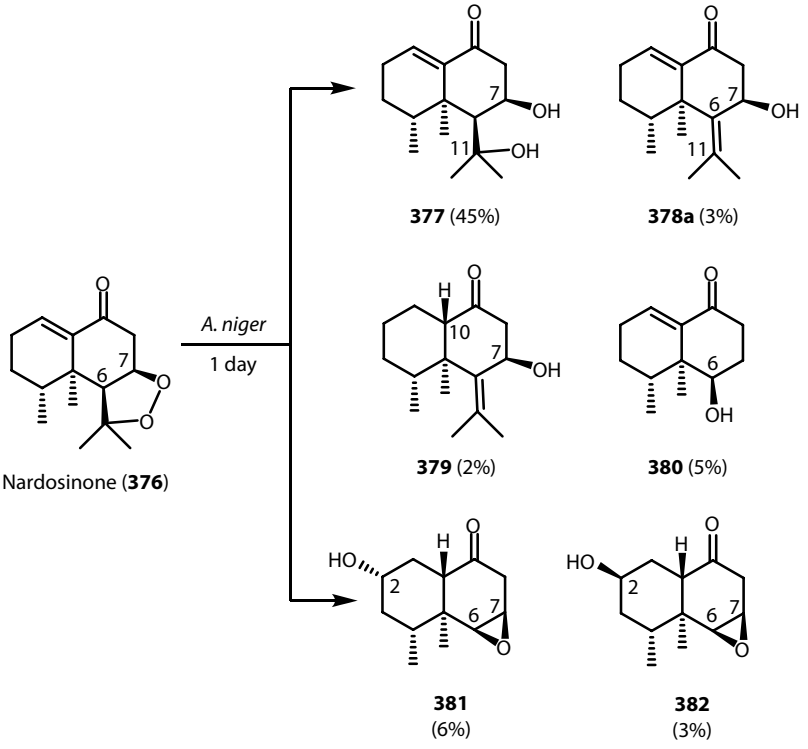


FIGURE 15.108 Biotransformation of nardosinone (**376**) by *Aspergillus niger.*

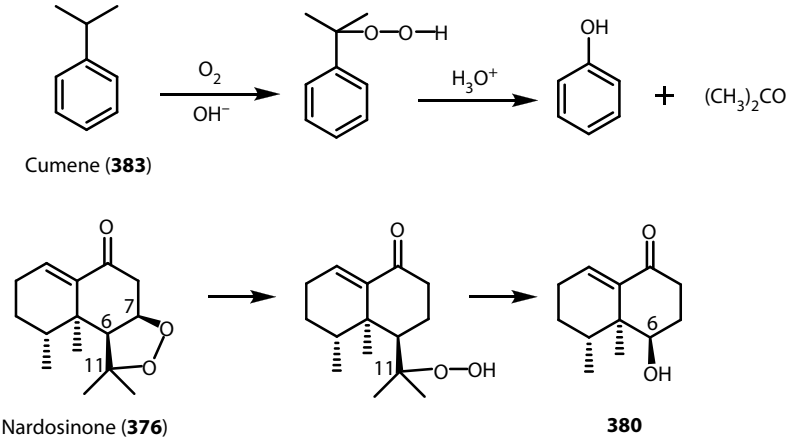


FIGURE 15.109 Possible pathway of biotransformation of nardosinone (**376**) to trinornardosinone (**380**) by *Aspergillus niger.*

When ambrox (**391**) was biotransformed by *Aspergillus niger* for 9 days in the presence of 1-aminobenzotriazole, an inhibitor of CYP450, compounds **396** and **397** were obtained instead of the metabolites (**392**–**395**), which were obtained by incubation of ambrox in the absence of the inhibitor. Ambrox was cultivated by *Aspergillus cellulosae* for 4 days in the same medium to afford

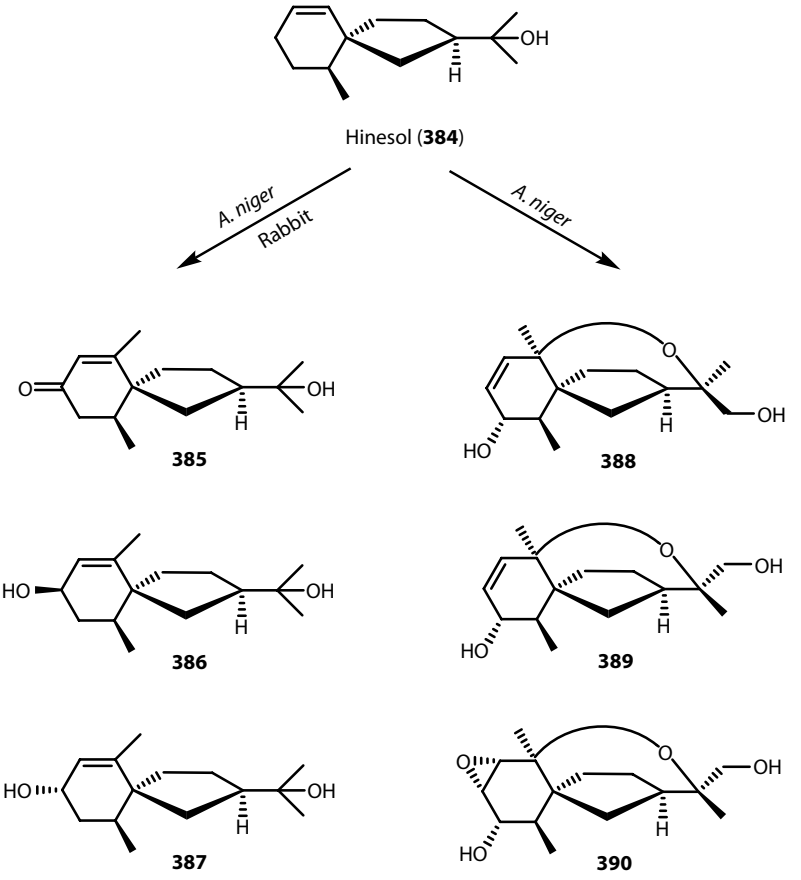


FIGURE 15.110 Biotransformation of hinesol (**384**) by *Aspergillus niger.*

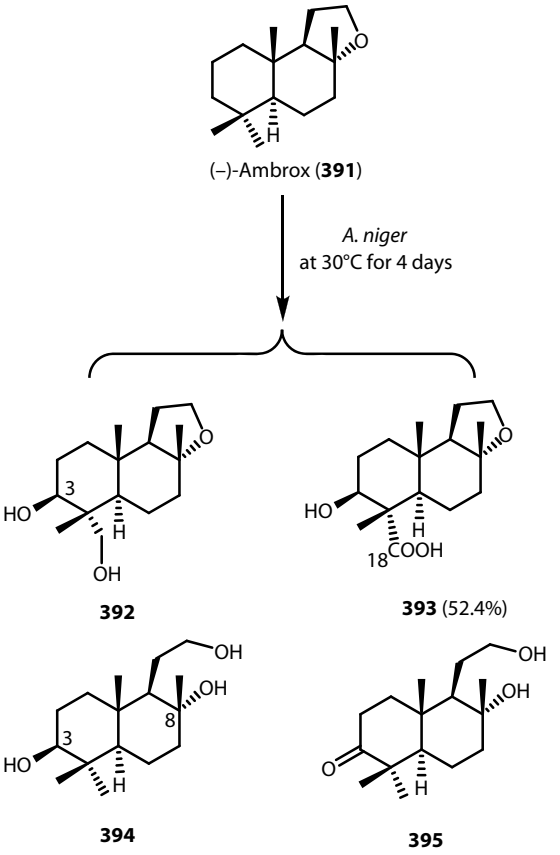


FIGURE 15.111 Biotransformation of (–)-ambrox (**391**) by *Aspergillus niger.*

C1 oxygenated products (**398** and **399**), the former of which was the major product (41.3%) (Hashimoto et al., 2001) (Figure 15.112).

The metabolite pathways of ambrox are quite different between *Aspergillus niger* and *Aspergillus cellulosae*. Oxidation at C1 occurred in *Aspergillus cellulosae* to afford **398** and **399**, which was also afforded by John's oxidation of **398**, while oxidation at C3 and C18 and ether cleavage between C8 and C12 occurred in *Aspergillus niger* to give **392**–**395**. Ether cleavage seen in *Aspergillus niger* is very rare.

Fragrance of the metabolites (**392**–**395**) and 7α-hydroxy-(–)-ambrox (**400**) and 7-oxo-(–)-ambrox (**401**) obtained from labdane diterpene diol were estimated. Only **399** demonstrated a similar odor to ambrox (**391**) (Hashimoto et al., 2001) (Figure 15.113).

(–)-Ambrox (**391**) was also microbiotransformed with *Fusarium* lini to give mono-, di-, and trihydroxylated metabolites (**401a**–**401d**), while incubation of the same substrate with *Rhizopus stolonifera* afforded two metabolites (**394**, **396**), which were obtained from **391** by *Aspergillus niger* as mentioned above, together with **397** and **401e** (Choudhary et al., 2004) (Figure 15.114).

The sclareolide (**402**) which is C12 oxo derivative of ambrox was incubated with *Mucor plumbeus* to afford three metabolites 3β-hydroxy- (**403**, 7.9%), 1β-hydroxy- (**404**, 2.5%), and 3-ketosclareolide (**405**, 7.9%) (Aranda et al., 1991) (Figure 15.115).

Aspergillus niger in the same medium as mentioned above converted sclareolide (**402**) into two new metabolites (**406**, **407**), together with known compounds (**403**, **405**), of which 3β-hydroxysclareolide (**403**) is preferentially obtained (Hashimoto et al., 2007) (Figure 15.116).

From the metabolites of sclareolide (**402**) incubated with *Curvularia lunata* and *Aspergillus niger*, five oxidized compounds, (**403**, **404**, **405**, **405a**, **405b**) were obtained. Fermentation of **402** with *Gibberella fujikuroii* afforded (**403**, **404**, **405**, **405a**). The metabolites, **403** and **405a** were

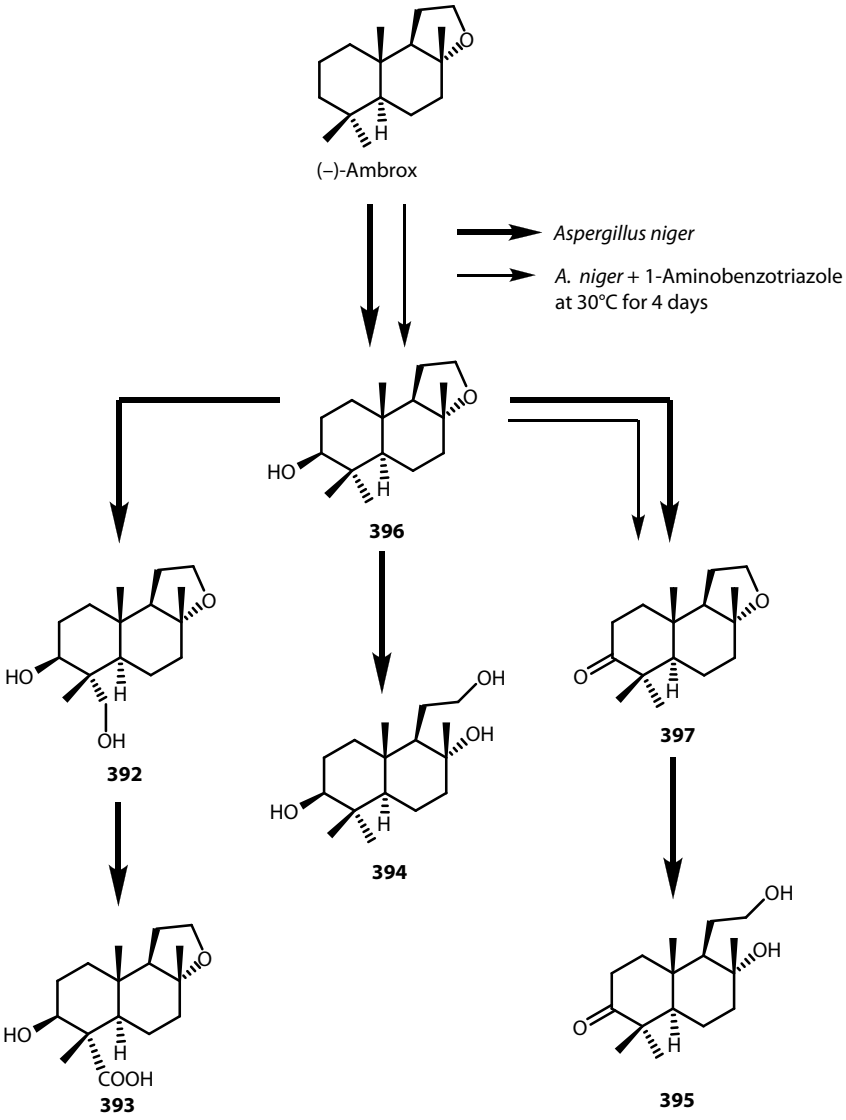


FIGURE 15.112 Possible pathway of biotransformation of (–)-ambrox (**391**) by *Aspergillus niger.*

formed from the same substrate by the incubation of *Fusarium lini*. No microbial transformation of **402** was observed with *Pleurotus ostreatus* (Atta-ur Rahman et al., 1997) (Figure 15.117).

Compound **391** treated in *Curvularia lunata* gave metabolites **401e** and **396**, while *Cunninghamella elegans* yielded compounds **401e** and **396** and (+)-sclareolide (**402**) (Figure 15.113). The metabolites (**401a**–**401e**, **396**) from **391** do not release any effective aroma when compared to **391.** Compound **394** showed a strong sweet odor quite different from the amber-like odor (Choudhary et al., 2004).

Sclareolide (**402**) exhibited phytotoxic and cytotoxic activity against several human cancer cell lines. *Cunninghamella elegans* gave new oxidized metabolites (**403**, **404**, **405a**, **405c**, **405d**, **405e**), resulting from the enantioselective hydroxylation. Metabolites **403**, **404**, and **405a** have been known as earlier as biotransformed products of **402** by many different fungi and have shown cytotoxicity against various human cancer cell lines. The metabolites (**403**, **404**, and **405a**) indicated significant phytotoxicity at higher dose against *Lemna minior* L. (Choudhary et al., 2004) (Figure 15.117).

Ambrox (**391**) and sclareolide (**402**) were incubated with the fungus *Cephalosporium aphidicola* for 10 days in shake culture to give 3β-hydroxy- (**396**), 3β,6β-dihydroxy- (**401g**), 3β,12-dihydroxy- (**401h**),

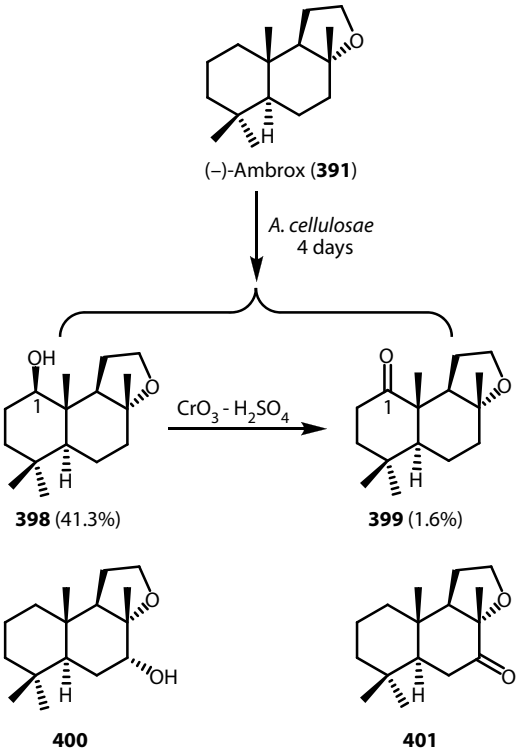


FIGURE 15.113 Biotransformation of (–)-ambrox (**391**) by *Aspergillus cellulosae*.

and sclareolide 3β,6β-diol (**401f**), and 3β-hydroxy- (**403**), 3-keto- (**405**), and sclareolide 3β,6β-diol (**401f**), respectively (Hanson and Truneh, 1996) (Figure 15.118).

Zerumbone (**408**), which is easily isolated from the wild ginger, *Zingiber zerumbet* and its epoxide (**409**) were incubated with *Fusarium culmorum* and *Aspergillus niger* in Czapek-peptone medium, respectively. The former fungus gave (1R,2R)-(+)-2,3-dihydrozerumbol (**410**) stereospecifically via either 2,3-dihydrozerumbone (**408a**) or zerumbol (**408b**) or both and accumulated **410** in the mycelium. The facile production of optically active **410** will lead a useful material of woody fragrance, namely 2,3-dihydrozerumbone. *Aspergillus niger* biotransformed **408** via epoxide (**409**) to several metabolites containing zerumbone-6,7-diol as a main product. The same fungus converted the epoxide (**409**) into three major metabolites containing (2R,6S,7S,10R,11S)-1-oxo7,9-dihydroxyisodaucane (**413**) via dihydro derivatives (**411**, **412**). However, *Aspergillus niger* biotransformed **409** only into **412** in the presence of CYP450 inhibitor, 1-aminobenzotriazole (Noma et al., 2002).

The same substrate was incubated in the *Aspergillus niger*, *Aspergillus orysae*, *Candia rugosa*, *Candia tropicalis, Mucor mucedo, Bacillus subtilis*, and *Schizosaccharomyces pombe*; however, any metabolites have been obtained. All microbes except for the last organism, zerumbone epoxide (**409**), prepared by *m*CPBA, bioconverted into two diastereoisomers, 2R,6S,7S-dihydro- (**411**) and 2R,6R,7R-derivative (**412**), whose ratio was determined by gas chromatography (GC) and their enantio-excess was over 99% (Nishida and Kawai, 2007) (Figure 15.119).

Several microorganisms and a few mammals (see later) for the biotransformation of (+)-cedrol (**414**) which is widely distributed in the cedar essential oils were used. Plant pathogenic fungus *Glomerella cingulata* converted cedrol (**414**) into three diols (**415**–**417**) and 2α-hydroxycedrene (**418**) (Miyazawa et al., 1995). The same substrate (**414**) was incubated with *Aspergillus niger* to give **416** and **417** together with a cyclopentanone derivative (**419**) (Higuchi et al., 2001). Human skin microbial flora, *Staphylococcus epidermidis* also converted (+)-cedrol into 2α-hydroxycedrol (**415**) (Itsuzaki et al., 2002) (Figure 15.120).

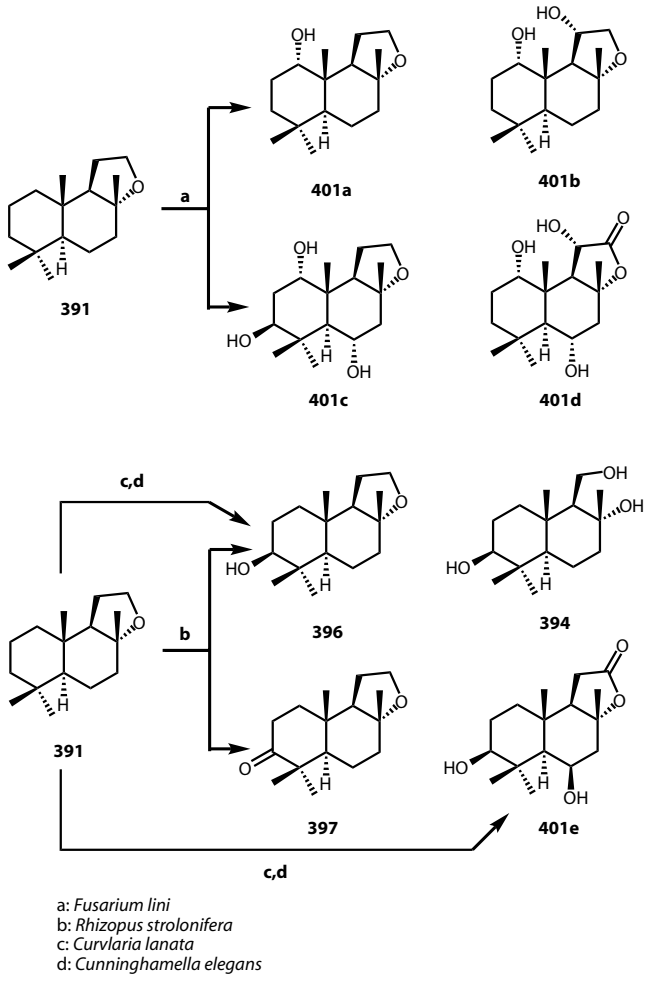


FIGURE 15.114 Biotransformation of (–)-ambrox (**391**) by *Fusarium lini* and *Rhizopus stronifera*.

Cephalosporium aphidicola bioconverted cedrol (**414**) into **417** (Hanson and Nasir, 1993). On the other hand, *Corynesphora cassiicola* produced **419** in addition to **417** (Abraham et al., 1987). It is noteworthy that *Botrytis cinerea* that damages many flowers, fruits and vegetables biotransformed cedrol into different metabolites (**420**–**422**) from those mentioned above (Aleu et al., 1999).

4α-Hydroxycedrol (**424**) was obtained from the metabolite of cedrol acetate (**423**) by using *Glomerella cingulata* (Matsui et al., 1999) (Figure 15.121).

Patchouli alcohol (**425**) was treated in *Botrytis cinerea* to give three metabolites two tertiary alcohols (**426**, **427**), four secondary alcohols (**428**, **430**, **430a**), and two primary alcohols (**430b**,

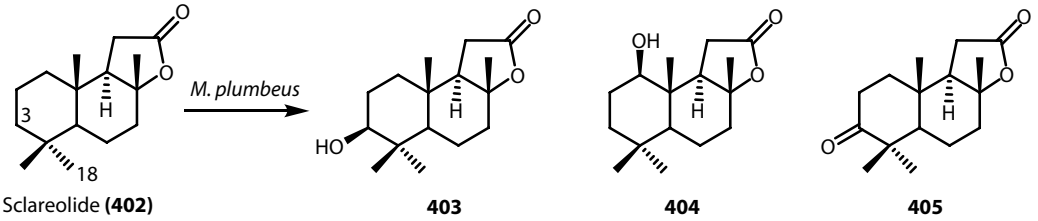


FIGURE 15.115 Biotransformation of (+)-sclareolide (**402**) by *Mucor plumbeus*.

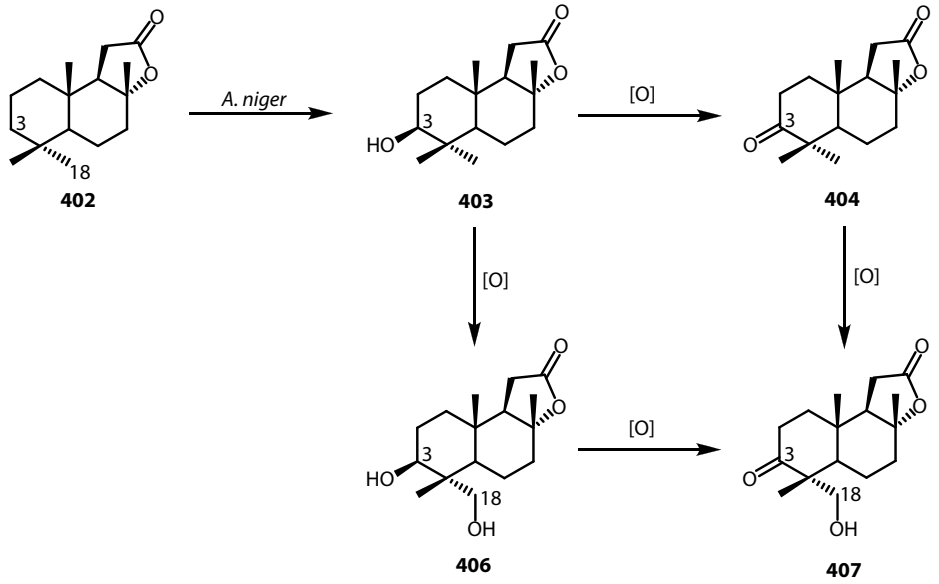


FIGURE 15.116 Biotransformation of (+)-sclareolide (**402**) by *Aspergillus niger.*

430c) of which compounds **425**, **427**, and **428** are the major metabolites (Aleu et al., 1999) while plant pathogenic fungus *Glomerella cingulata* converted the same substrate to 5-hydroxy- (**426**) and 5,8-dihydroxy derivative (**429**) (Figure 15.122).

In order to confirm the formation of **429** from **426**, the latter product was reincubated in the same medium including *Glomerella cingulata* to afford **429** (Miyazawa et al., 1997b) (Figure 15.123).

Patchouli acetate (**431**) was also treated in the same medium to give **426** and **429** (Matsui and Miyazawa, 2000). 5-Hydroxy-α-patchoulene (**432**) was incubated with *Glomerella cingulata* to afford 1α-hydroxy derivative (**426**) (Miyazawa et al., 1998a).

(−)-α-Longipinene (**433**) was treated with *Aspergillus niger* to afford 12-hydroxylated product (**434**) (Sakata et al., 2007).

Ginsenol (**435**), which was obtained from the essential oil of *Panax ginseng,* was incubated with *Botrytis cinerea* to afford four secondary alcohols (**436**–**439**) and two cyclohexanone derivatives (**440**) from **437** and **441** from **438** or **439**. Some of the oxygenated products were considered as potential antifungal agents to control *Botrytis cinerea* (Aleu et al., 1999a) (Figures 15.124 and 15.125).

(+)-Isolongifolene-9-one (**442**), which was isolated from some cedar trees was treated in *Glomerella cingulata* for 15 days to afford two primary alcohols (**443**, **444**) and a secondary alcohol (**445**) (Sakata and Miyazawa, 2006) (Figure 15.126).

Choudhary et al. (2005) reported that fermentation of (−)-isolongifolol (**445a**) with *Fusarium lini* resulted in the isolation of three metabolites, 10-oxo- (**445b**), 10α-hydroxy- (**445c**), and 9α-hydroxyisolngifolol (**445d**). Then the same substrate was incubated with *Aspergillus niger* to yield the products **445c** and **445d**. Both **445c** and **445d** showed inhibitory activity against butylcholinesterease enzyme in a concentration-dependent manner with IC_{50} 13.6 and 299.5 μM, respectively (Figure 15.127).

(+)-Cycloisolongifol-5β-ol (**445e**) was fermented with *Cunninghamella elegans* to afford three oxygenated metabolites: 11-oxo- (**445f**), 3β-hydroxy- (**445g**), and 3β,11α-dihydroxy derivative (**445h**) (Choudhary et al., 2006a). (Figure 15.128).

A daucane-type sesquiterpene derivative, lancerroldiol *p*-hydroxybenzoate (**446**) was hydrogenated with cultured suspension cells of the liverwort, *Marchantia polymorpha* to give 3,4-dihydrolancerodiol (**447**) (Hegazy et al., 2005) (Figure 15.129).

Widdrane sesquiterpene alcohol (**448**) was incubated with *Aspergillus niger* to give an oxo and an oxy derivatives (**449**, **450**) (Hayashi et al., 1999) (Figure 15.130).

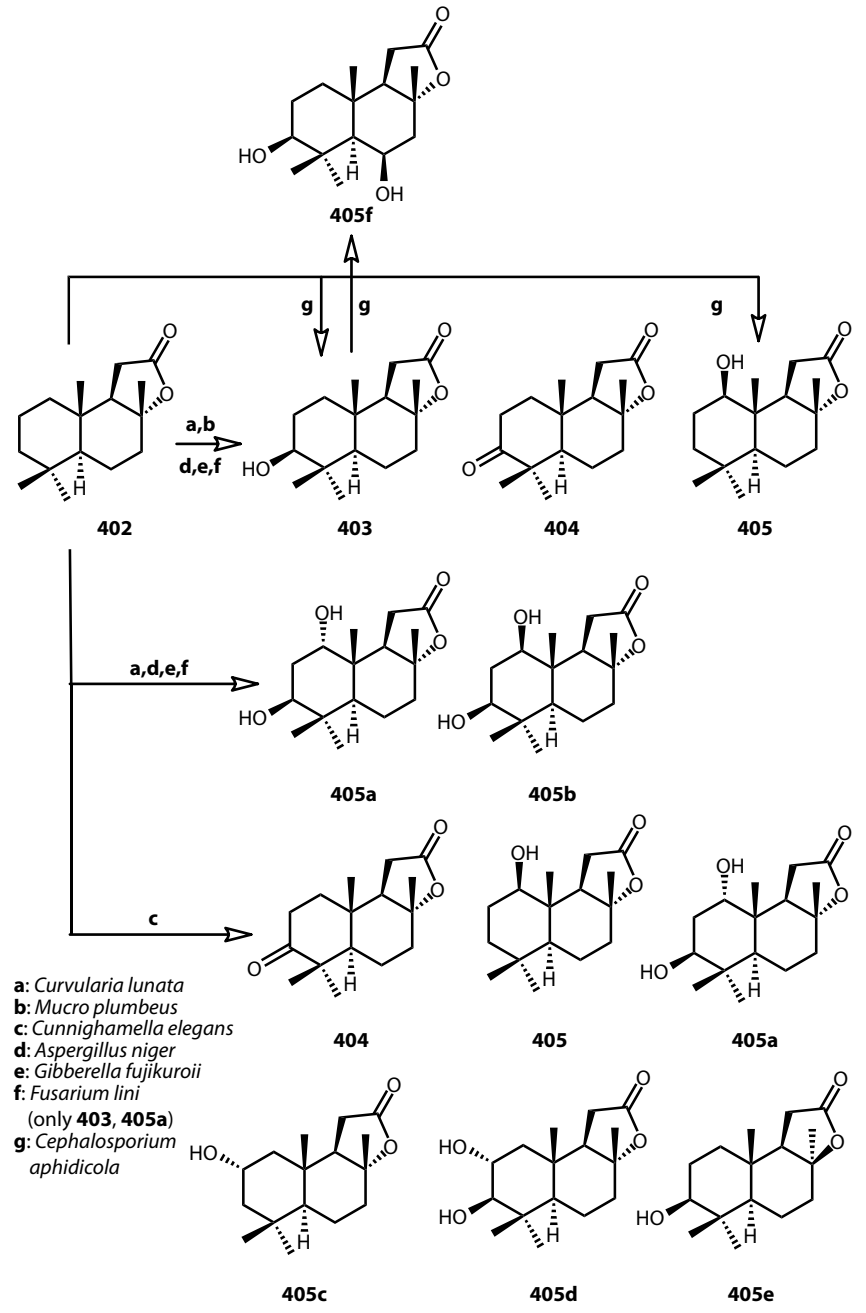


FIGURE 15.117 Biotransformation of (+)-sclareolide (**402**) by various fungi.

(–)-β-Caryophyllene (**451**), one of the ubiquitous sesquiterpene hydrocarbons found not only in higher plants but also in liverworts, was biotransformed by *Pseudomonas cruciviae*, *Diplodia gossypina*, and *Chaetomium cochlioides* (Lamare and Furstoss, 1990). *Pseudomonas cruciviae* gave a ketoalcohol (**452**) (Devi, 1979), while the latter two species produced the 14-hydroxy-5,6-epoxide (**454**), its carboxylic (**455**), and 3α-hydroxy- (**456**) and norcaryophyllene alcohol (**457**), all of which might be formed from caryophyllene C5,C6-epoxide (**453**). Oxidation pattern of (–)-β-caryophyllene by the fungi is very similar to that by mammals (see later) (Figure 15.131).

Fermentation of (–)-β-caryophyllene (**451**) with *Diplodia grossypina* afforded **14** different metabolites (**453**–**457j**), among which 14-hydroxy-5,6-epoxide (**454**) and the corresponding acid

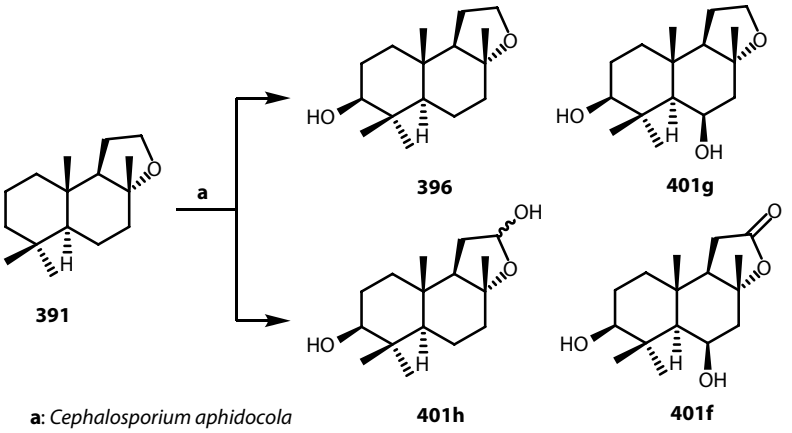


FIGURE 15.118 Biotransformation of (–)-ambrox (**391**) by *Cephalosporium aphidicola*.

(**455**) were the major metabolites. Compound **457j** is structurally very rare and found in *Poronia punctata*. The main reaction path is epoxidation at C5, C6 as mentioned above and selective hydroxylation at C4 (Abraham et al., 1990) (Figure 15.132).

(–)-β-Caryophyllene epoxide (**453**) was incubated with *Cephalosporium aphidicola* for 6 days to afford two metabolites (**457l**, **457m**) while *Macrophomina phaseolina* biotransformed the same substrate to 14- (**454**) and 15-hydroxy derivatives (**457k**). The same substrate was treated in *Aspergillus niger*, *Gibberella fujikuroii*, and *Rhizopus stolonifera*, for 8 days and *Fusarium lini* for 10 days to afford the metabolites **457n**, **457o**, **457p** and **457q**, and **457r**, respectively. All metabolites were estimated for butyrylcholine esterase inhibitory activity and compound **457k** was found to show potency similar activity to galanthamine HBr (IC_{50} 10.9 versus 8.5 μM) (Choudhary et al., 2006) (Figure 15.133).

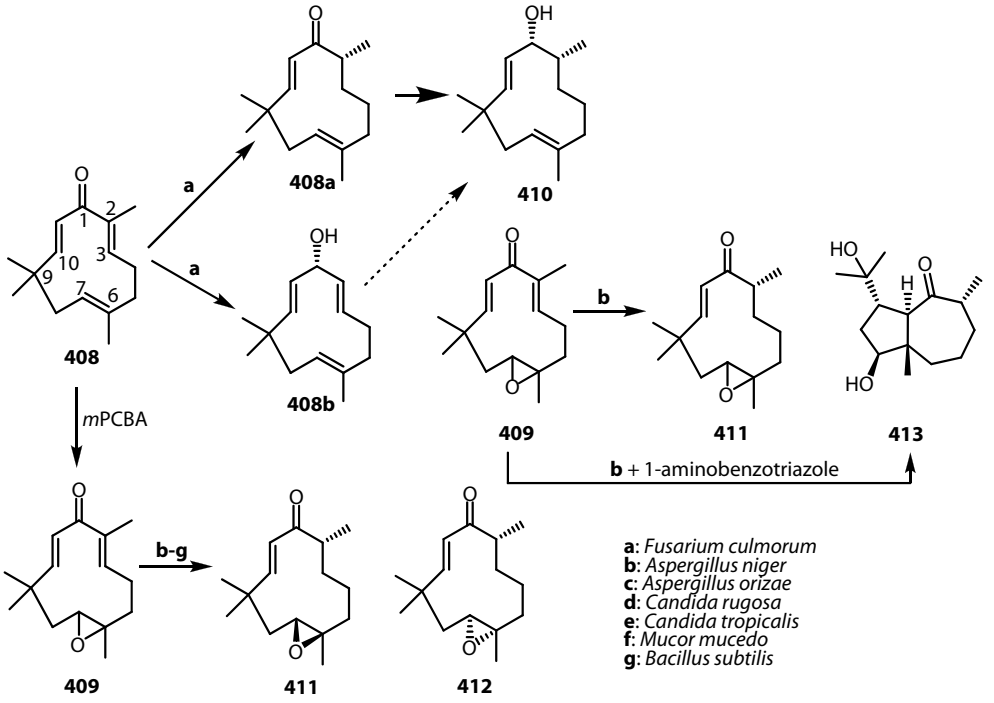


FIGURE 15.119 Biotransformation of zerumbone (**408**) by various fungi.

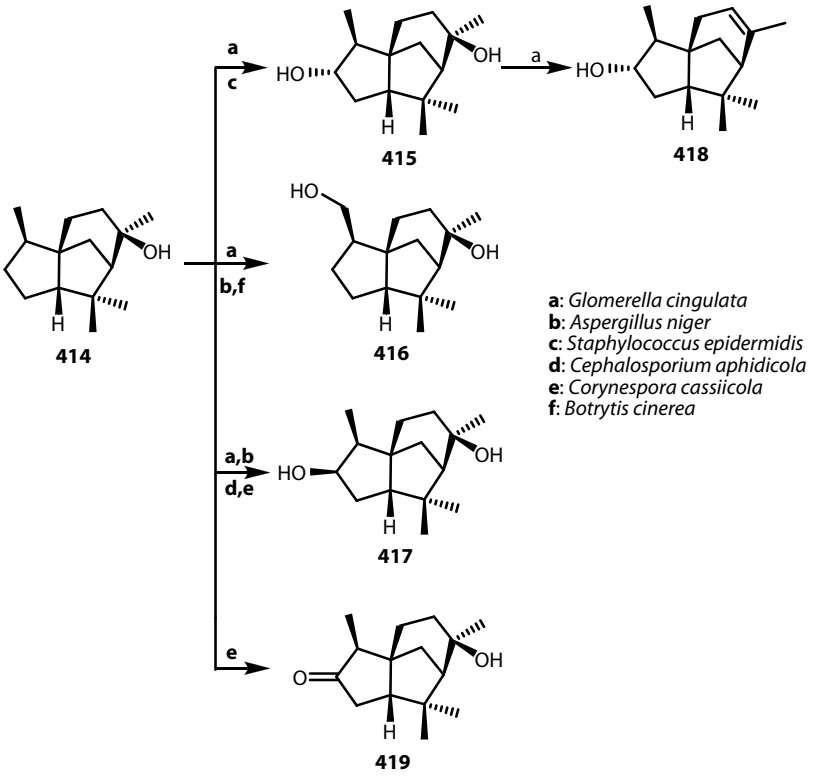


FIGURE 15.120 Biotransformation of cedrol (**414**) by various fungi.

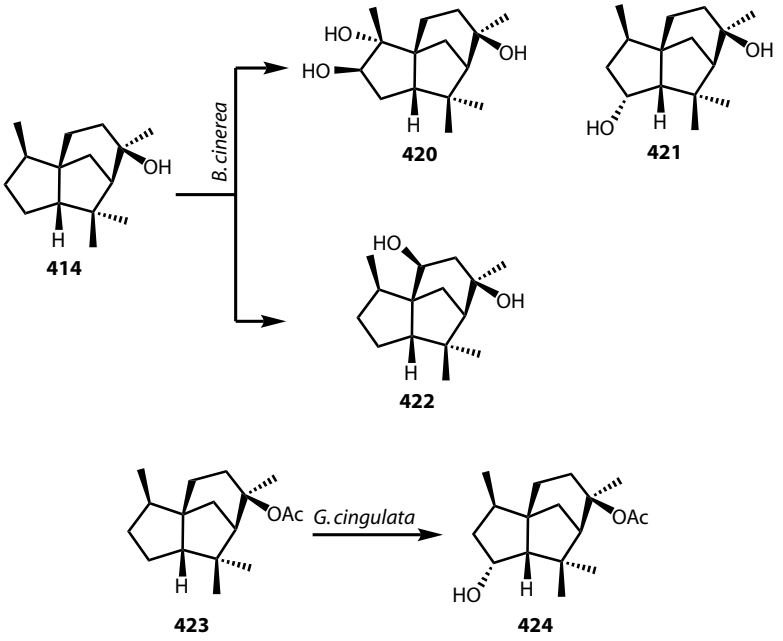


FIGURE 15.121 Biotransformation of cedrol (**414**) by *Botrytis cinerea and Glomerella cingulata*.

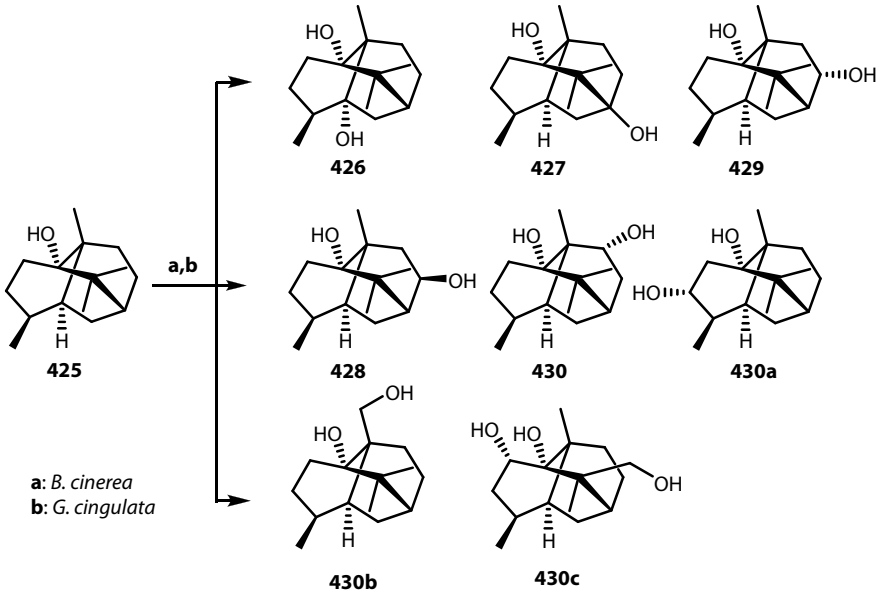


FIGURE 15.122 Biotransformation of patchoulol (**425**) by *Botrytis cinerea*.

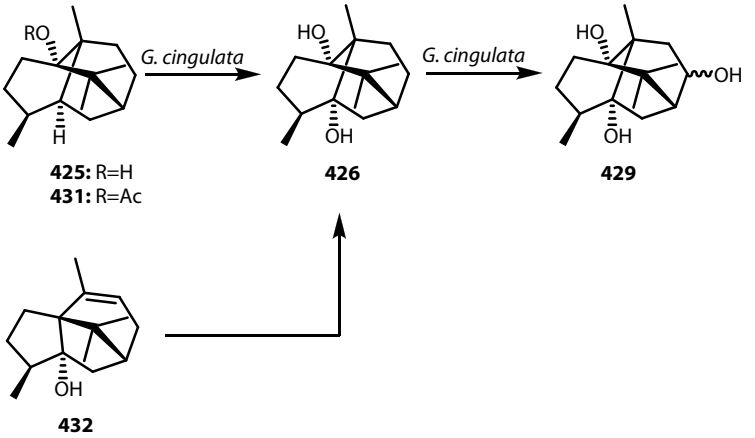


FIGURE 15.123 Biotransformation of patchoulol (**425**) by *Glomerella cingulata*.

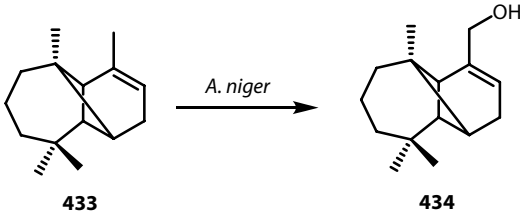


FIGURE 15.124 Biotransformation of α-longipinene (**433**) by *Aspergillus niger*.

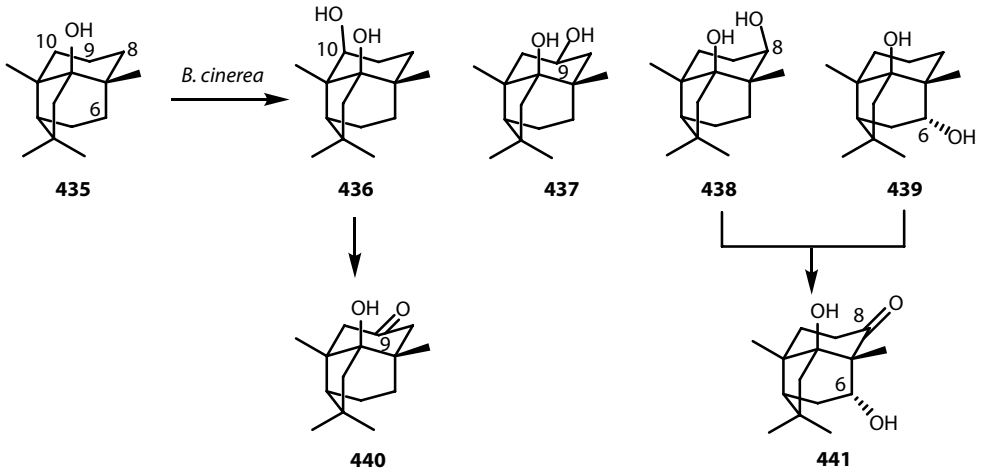


FIGURE 15.125 Biotransformation of ginsenol (**435**) by *Botrytis cinerea*.

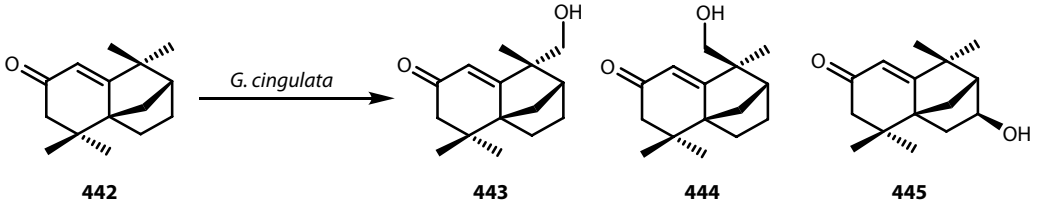


FIGURE 15.126 Biotransformation of (+)-isolongifolene-9-one (**442**) by *Glomerella cingulata*.

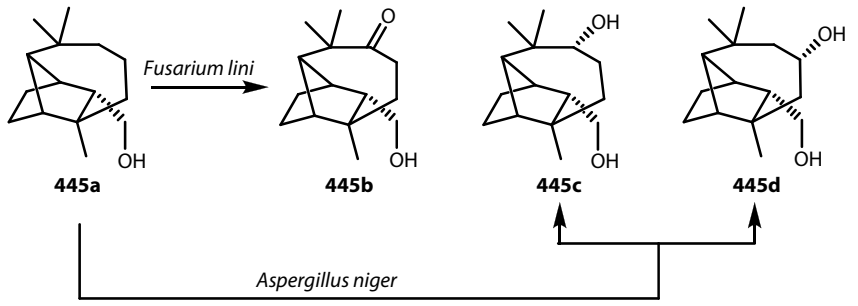


FIGURE 15.127 Biotransformation of (−)-isolongifolol (**445a**) by *Aspergillus niger* and *Fusarium lini*.

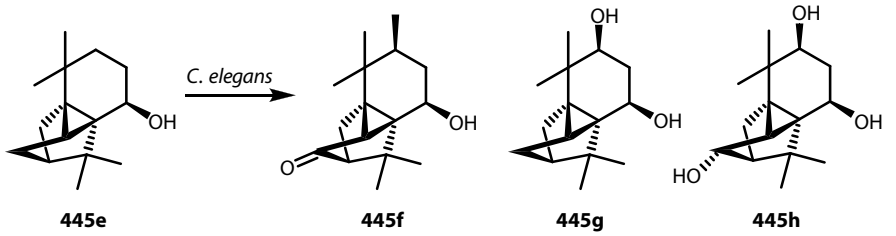


FIGURE 15.128 Biotransformation of (+)-cycloisolongifol-5β-ol (**445e**) by *Cunninghamella elegans*.

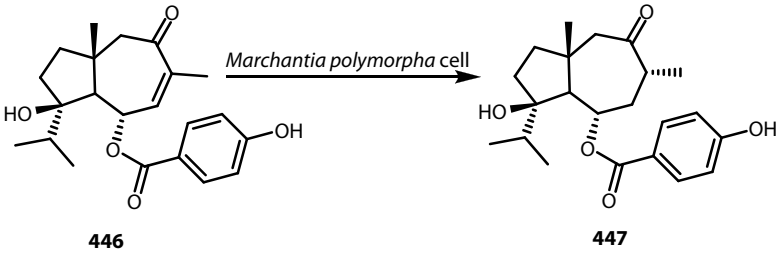


FIGURE 15.129 Biotransformation of lancerodiol *p*-hydroxybenzoate (**446**) by *Marchantia polymorpha* cells.

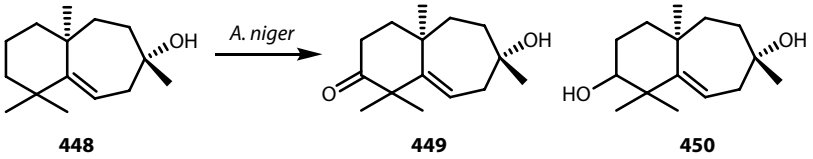


FIGURE 15.130 Biotransformation of widdrol (**448**) by *Aspergillus niger.*

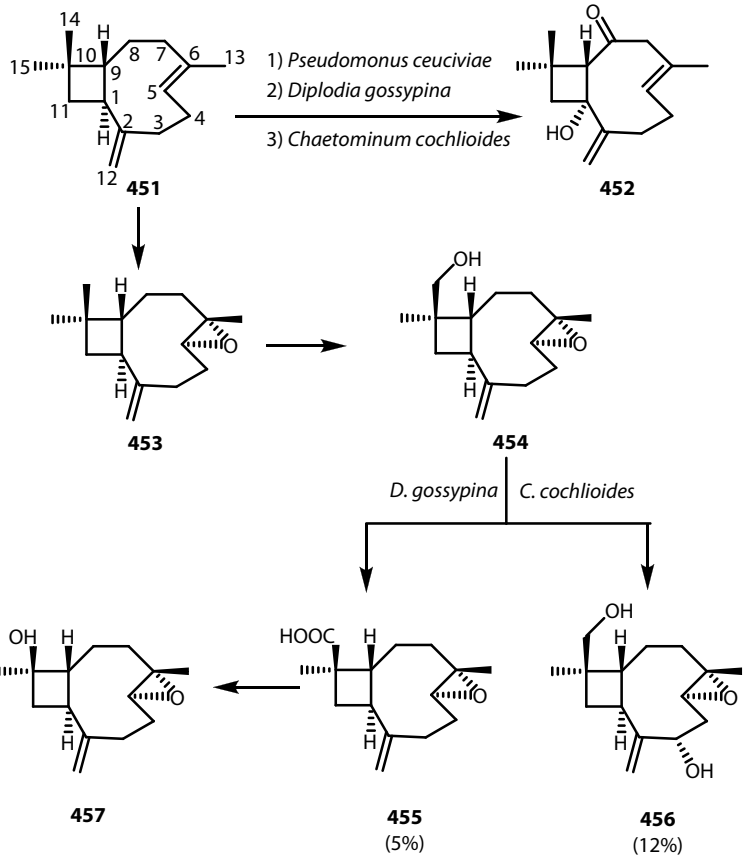


FIGURE 15.131 Biotransformation of (–)-β-caryophyllene (**451**) by *Pseudomonas ceuciviae*, *Diplodia gossypina*, and *Chaetomium cochlioides.*

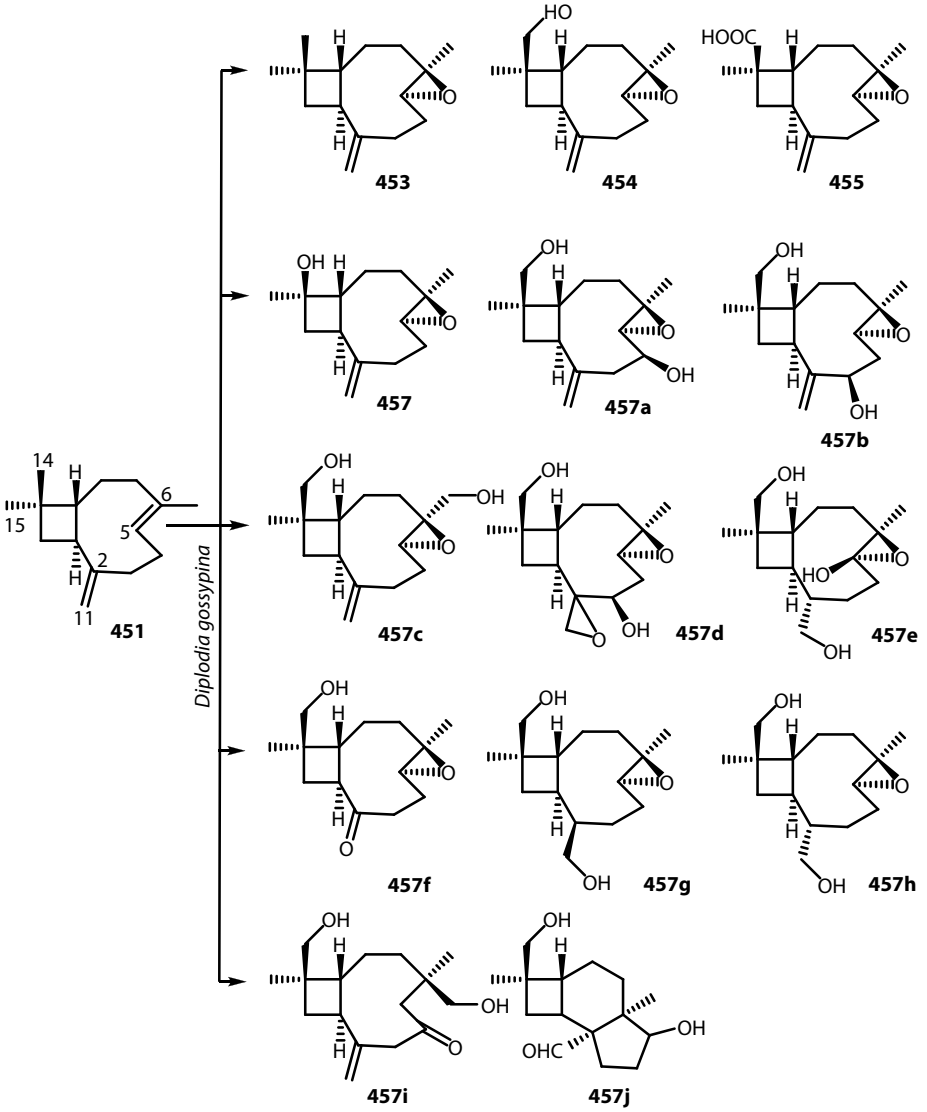


FIGURE 15.132 Biotransformation of (–)-β-caryophyllene (**451**) by *Diplodia gossypina*.

The fermentation of (–)-β-caryophyllene oxide (**453**) using *Botrytis cinerea* and the isolation of the metabolites were carried out by Duran et al. (1999) Kobuson (**457w**) was obtained with fourteen products (**457s**–**457u**, **457x**). Diepoxides **457t** and **457u** could be the precursors of epimeric alcohols **457q** and **457y** obtained through reductive opening of the C2,C11-epoxide. The major reaction paths are stereoselective epoxidation and introduction of hydroxyl group at C3. Compound **457ae** has a caryolane skeleton (Figure 15.134).

When isoprobotryan-9α-ol (**458**) produced from isocaryophyllene was incubated with *Botrytis cinerea*, it was hydroxylated at tertiary methyl groups to give three primary alcohols (**459**–**461**) (Aleu et al., 2002) (Figure 15.135).

Acyclic sesquiterpenoids, racemic *cis*-nerolidol (**462**), and nerylacetone (**463**) were treated by the plant pathogenic fungus, *Glomerella cingulata* (Miyazawa et al., 1995a). From the former substrate, a triol (**464**) was obtained as the major product. The latter was bioconverted to give the two methyl ketones (**465**, **467**) and a triol (**468**), among which **465** was the predominant. The C10,C11 diols (**464**, **465**) might be formed from both epoxides of the substrates, followed by the hydration although no C10,C11-epoxides were detected (Figure 15.136).

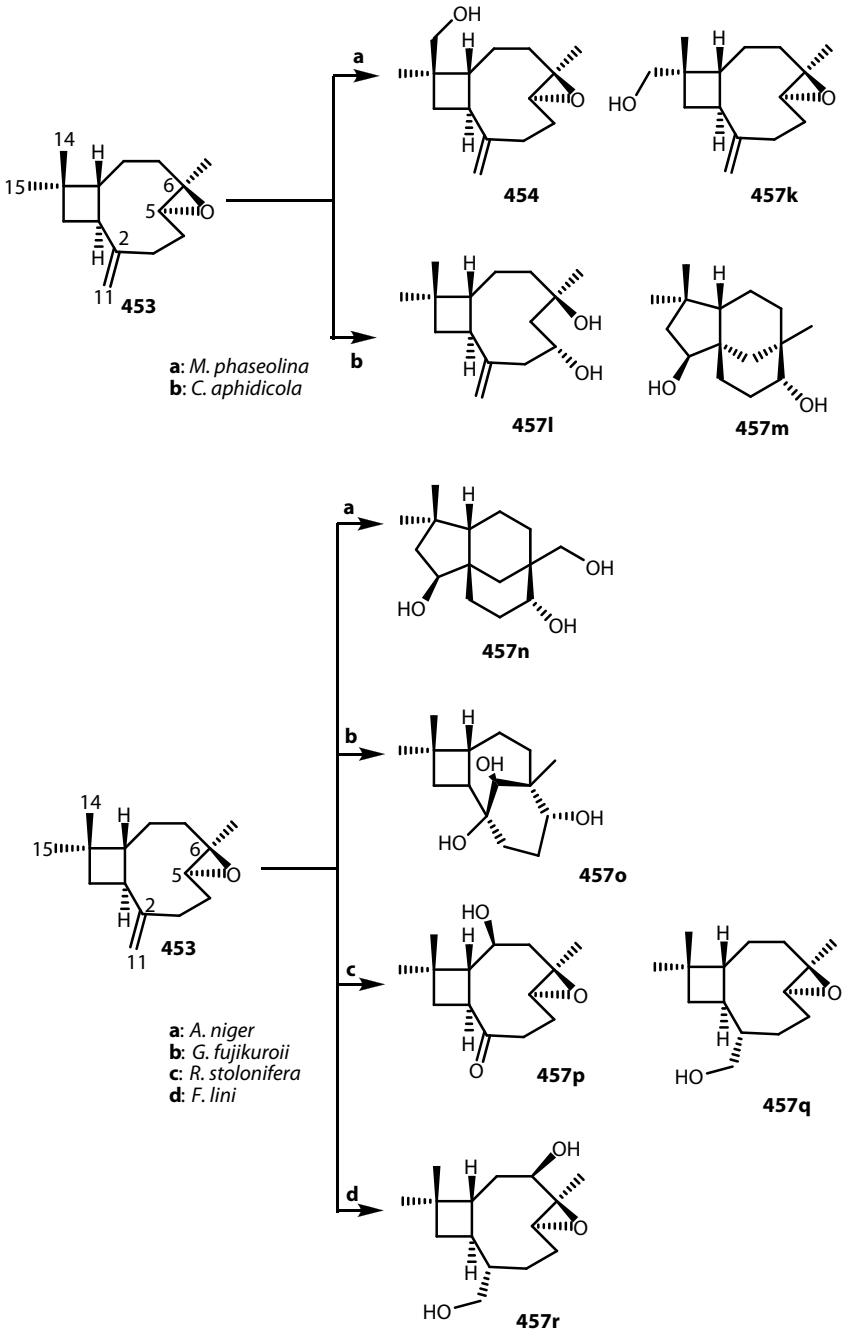


FIGURE 15.133 Biotransformation of (–)-β-caryophyllene epoxide (**453**) by various fungi.

Racemic *trans*-nerolidol (**469**) was also treated in the same fungus to afford ω–2 hydroxylated product (**471**) and C10,C11 hydroxylated compounds (**472**) as seen in racemic *cis*-nerolidol (**462**) (Miyazawa et al., 1996a) (Figure 15.137).

12-Hydroxy-*trans*-nerolidol (**472a**) is an important precursor in the synthesis of interesting flavor of α-sinensal. Hrdlicka et al. (2004) reported the biotransformation of *trans*-(**469**) and *cis*-nerolidol

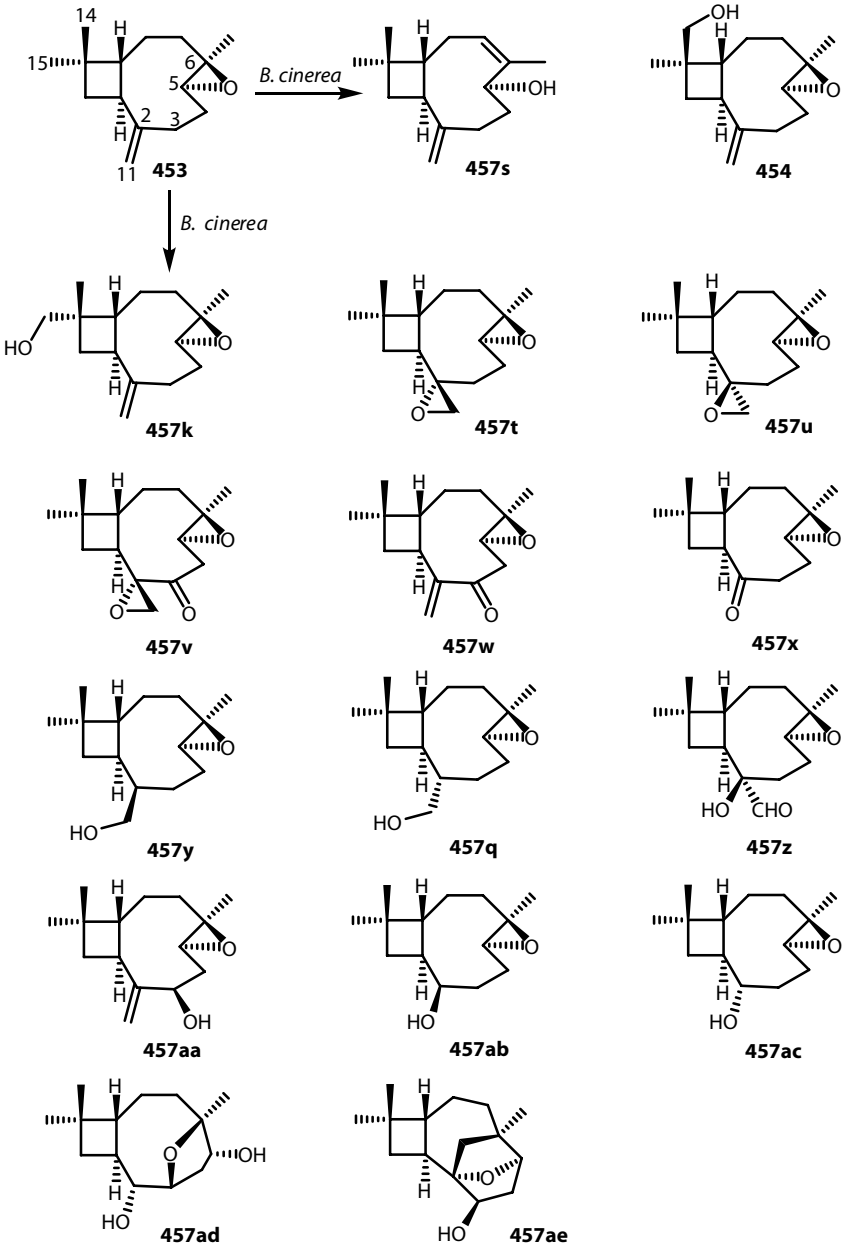


FIGURE 15.134 Biotransformation of (–)-β-caryophyllene epoxide (**453**) by *Botrytis cinerea*.

(**462**) and *cis-/trans*-mixture of nerolidol using repeated batch culture of *Aspergillus niger* grown in computer-controlled bioreactors. *Trans*-nerolidol (**469**) gave **472a** and **472** and *cis*-isomer (**462**) afforded **464a** and **464**. From a mixture of *cis*- and *trans*-nerolidol, 12-hydroxy-*trans*-neroridol **472a** (8%) was obtained in postexponential phase at high dissolved oxygen. At low dissolved oxygen condition, the mixture gave **472a** (7%) and **464a** (6%) (Figure 15.138).

From geranyl acetone (**470**) incubated with *Glomerella cingulata*, four products (**473**–**477**) were formed. It is noteworthy that the major compounds from both substrates (**469**, **470**) were ω–2

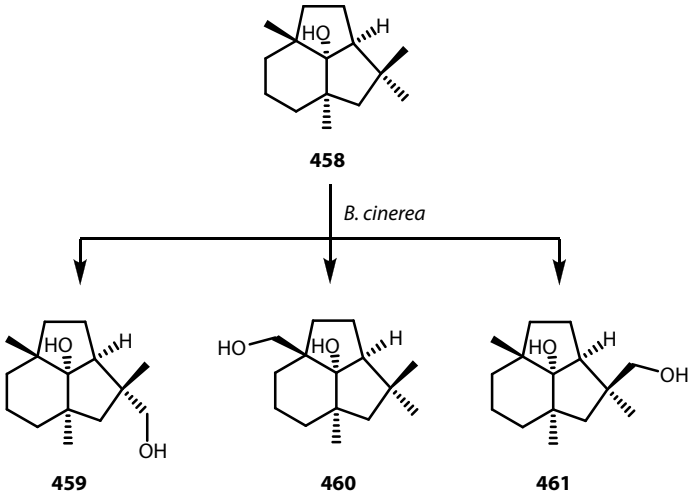


FIGURE 15.135 Biotransformation of isoprobotryan-9α-ol (**458**) by *Botrytis cinerea*.

hydroxylated products, but not C10,C11 dihydroxylated products as seen in *cis*-nerolidol (**462**) and nerylacetone (**463**) (Miyazawa et al., 1995c) (Figure 15.136).

The same fungus bioconverted (2*E*,6*E*)-farnesol (**478**) to four products, ω−2 hydroxylated product (**479**), which was further oxidized to give C10,C11 dihydroxylated compound (**480**) and 5-hydroxy derivative (**481**), followed by isomerization at C2,C3 double bond to afford a triol (**482**) (Miyazawa et al., 1996b) (Figure 15.140).

The same substrate was bioconverted by *Aspergillus niger* to afford two metabolites, 10,11-dihydroxy- (**480**) and 5,13-hydroxy derivative (**480a**) (Madyastha and Gururaja, 1993).

The same fungus also converted (2*Z*,6*Z*)-farnesol (**483**) to three hydroxylated products: 10,11-dihydroxy-(2*Z*,6*Z*)- (**484**), 10,11-dihydroxy (2*E*,6*Z*)-farnesol (**485**), and (5*Z*)-9,10-dihydroxy-6,10-dimethyl-5-undecen-2-one (**486**) (Nankai et al., 1996) (Figure 15.140).

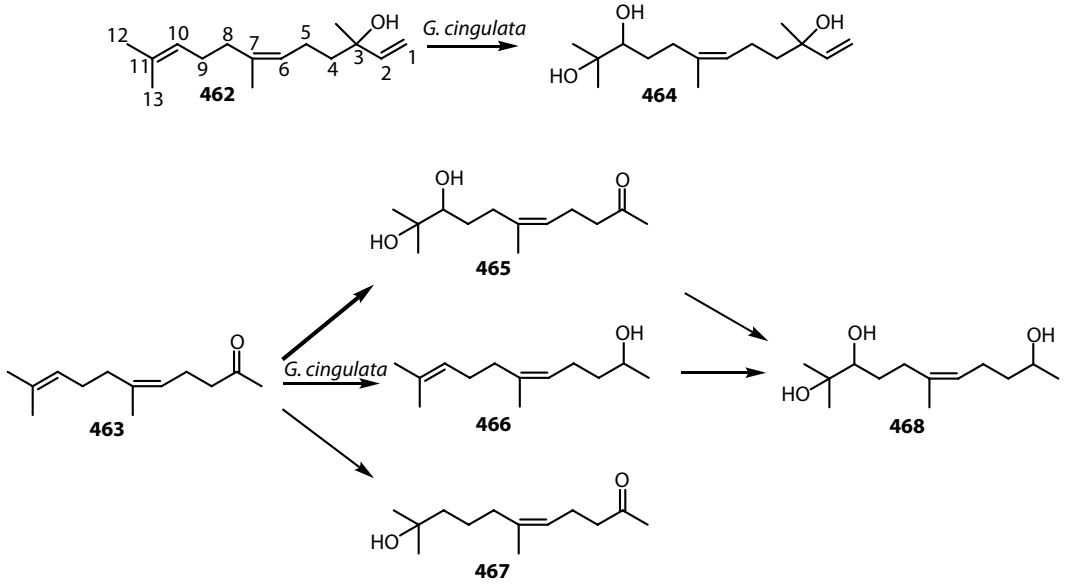


FIGURE 15.136 Biotransformation of *cis*-nerolidol (**462**) and *cis*-geranyl acetone (**463**) by *Glomerella cingulata*.

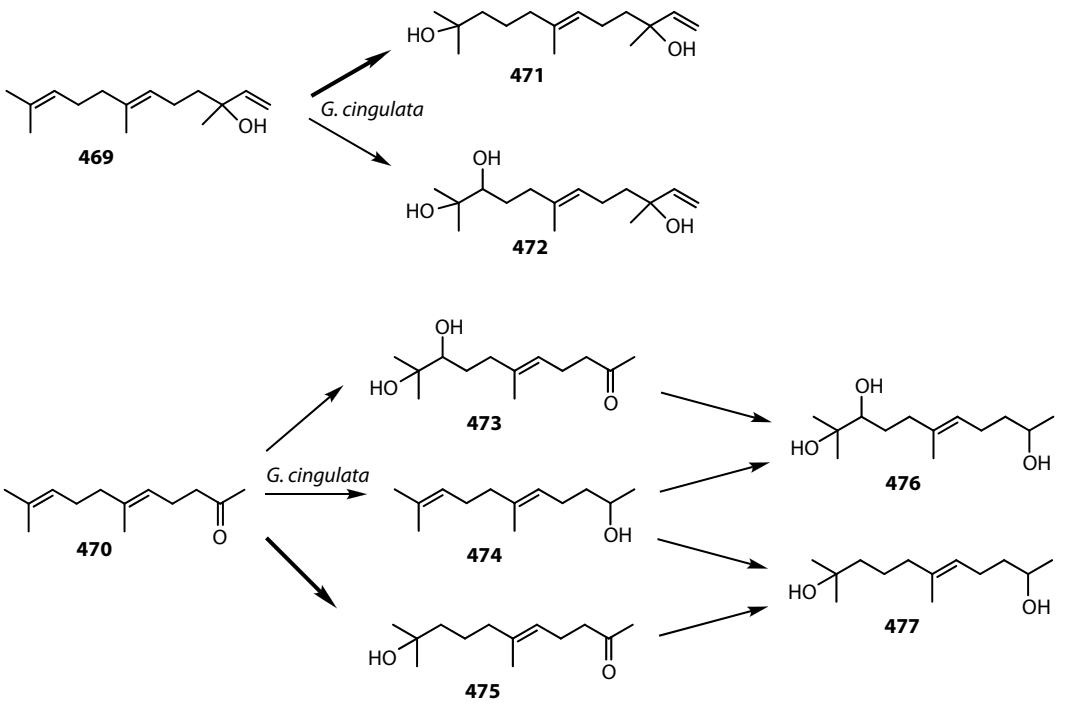


FIGURE 15.137 Biotransformation of *trans*-nerolidol (**469**) and *trans*-geranyl acetone (**470**) by *Glomerella cingulata*.

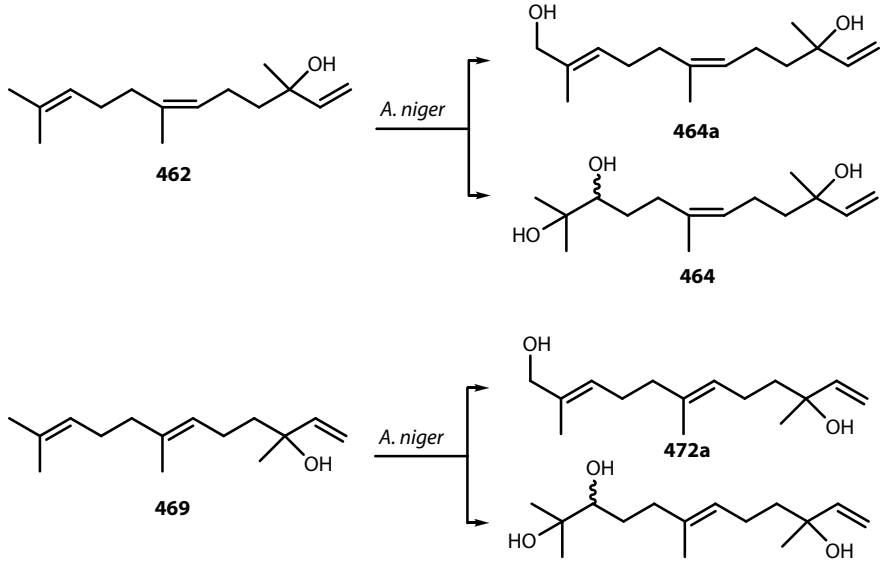


FIGURE 15.138 Biotransformation of *cis*- (**462**) and *trans*-nerolidol (**469**) by *Aspergillus niger*.

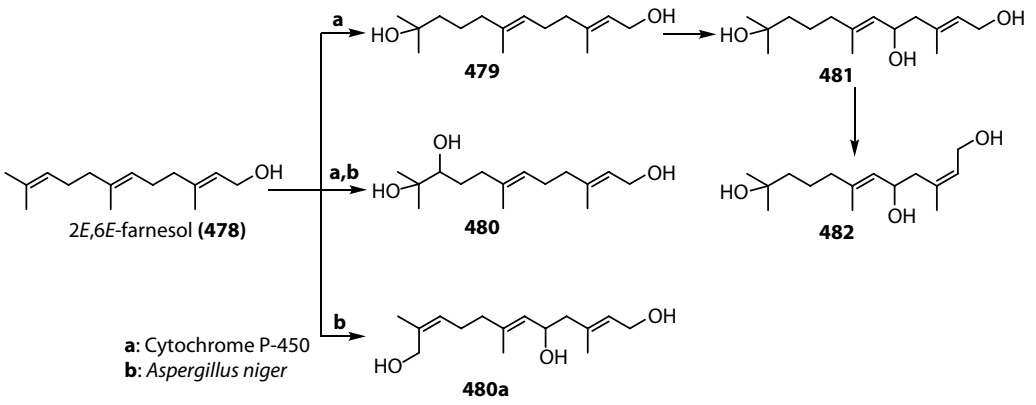


FIGURE 15.139 Biotransformation of *2E,6E*-farnesol (**478**) by Cytochrome P-450 and *Aspergillus niger*.

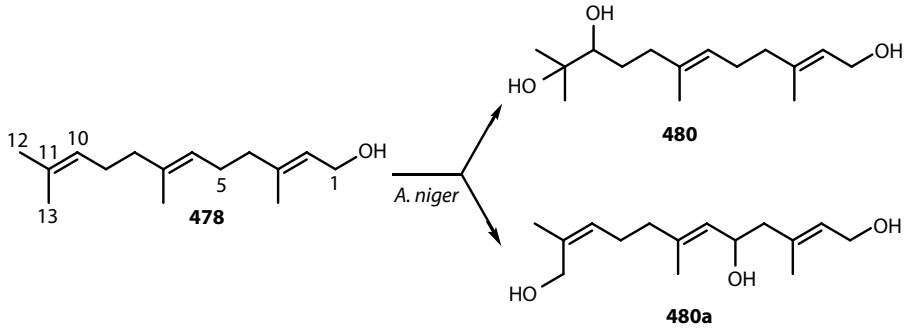


FIGURE 15.140 Biotransformation of *2E,6E*-farnesol (**478**) by *Aspergillus niger*.

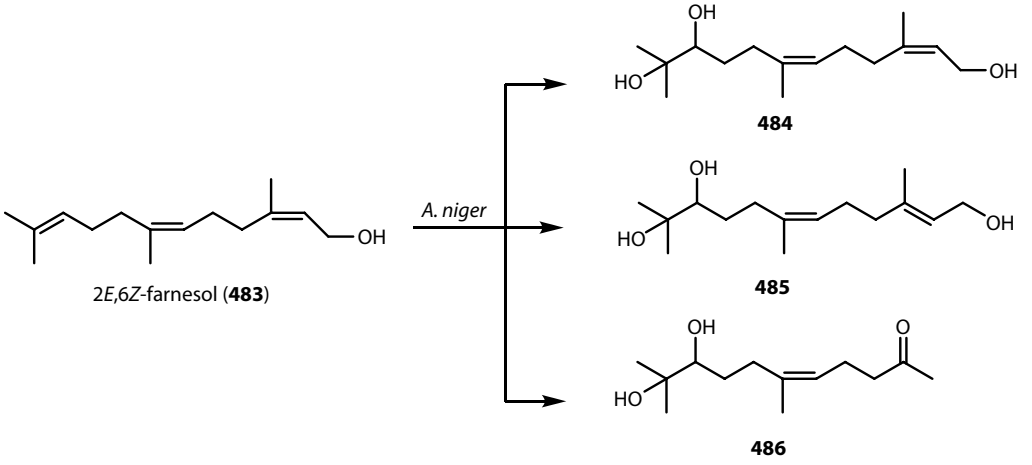


FIGURE 15.141 Biotransformation of *2Z,6Z*-farnesol (**478**) by *Aspergillus niger*.

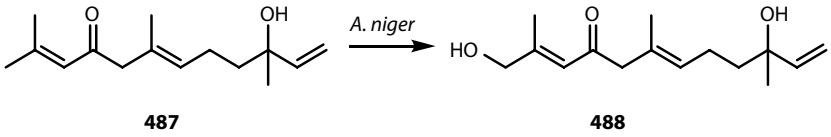


FIGURE 15.142 Biotransformation of 9-oxo-*trans*-nerolidol (**487**) by *Aspergillus niger*.

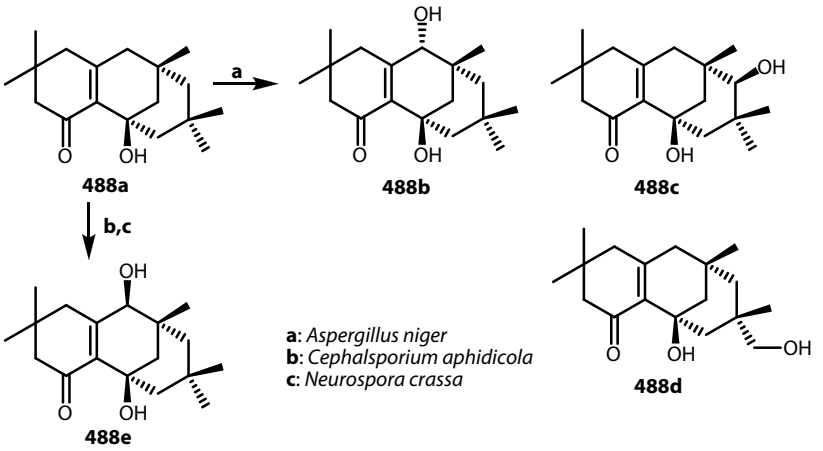


FIGURE 15.143 Biotransformation of diisophorone (**488a**) by *Aspergillus niger, Cephalosporium aphidicola,* and *Neurospora crassa.*

A linear sesquiterpene 9-oxonerolidol (**487**) was treated in *Aspergillus niger* to give ω−1 hydroxylated product (**488**) (Higuchi et al., 2001) (Figure 15.142).

Racemic diisophorone (**488a**) dissolved in ethanol was incubated with the Czapek–Dox medium of *Aspergillus niger* to afford 8α- (**488b**), 10β- (**488c**), and 17-hydroxydiisophorone (**488d**) (Kiran et al., 2004).

On the other hand, the same substrate was fed with *Nicotiana crassa* and *Cephalosporium aphidicola* to afford only 8β-hydroxydiisophorone (**488e**) in 20% and 10% yield, respectively (Kiran et al., 2005) (Figure 15.143).

From the metabolites of 5β,6β-dihydroxypresilphiperfolane 2β-angelate (**488f**) using the fungus *Mucor ramannianus*, 2,3-epoxyangeloyloxy derivative (**488g**) was obtained (Orabi, 2001) (Figure 15.144).

15.3 BIOTRANSFORMATION OF SESQUITERPENOIDS BY MAMMALS, INSECTS, AND CYTOCHROME P-450

15.3.1 Animals (Rabbits) and Dosing

Six male albino rabbits (2–3 kg) were starved for 2 days before experiment. Monoterpene were suspended in water (100 mL) containing polysorbate 80 (0.1 g) and were homogenized well. This solution (20 mL) was administered to each rabbit through a stomach tube followed by water (20 mL). This dose of sesquiterpenoids corresponds to 400–700 mg/kg. Rabbits were housed in stainless steal metabolism cages and were allowed rabbit food and water *ad libitum*. The urine was collected daily for 3 days after drug administration and stored at 0–5°C until the time of analysis. The urine was centrifuged to remove feces and hairs at 0°C and the supernatant was used for the experiments.

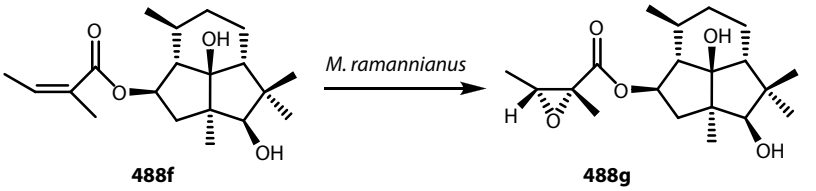


FIGURE 15.144 Biotransformation of 5β,6β-dihydroxypresilphiperfolane 2β-angelate (**488f**) by *Mucor ramannianus.*

The urine was adjusted to pH 4.6 with acetate buffer and incubated with β-glucuronidase-arylsufatase (3 mL/100 mL of fresh urine) at 37°C for 48 h, followed by continuous ether extraction for 48 h. The ether extracts were washed with 5% $NaHCO_3$ and 5% NaOH to remove the acidic and phenolic components, respectively. The ether extract was dried over $MgSO_4$, followed by evaporation of the solvent to give the neutral crude metabolites (Ishida et al., 1981).

15.3.2 Sesquiterpenoids

Wild rabbits (hair) and deer damage the young leaves of *Chamaecyparis obtusa*, one of the most important furniture and house-constructing tree in Japan. The essential oil of the leaves contains a large amount of (–)-longifolene (**489**). Longifolene (36 g) was administered to 18 of rabbits to obtain the metabolites (3.7 g) from which an aldehyde (**490**) (35.5%) was isolated as pure state. In the metabolism of terpenoids having an exomethylene group, glycol formation was often found, but in the case of longifolene such as a diol was not formed. Introduction of an aldehydes group in biotrans formation is very remarkable. Stereoselective hydroxylation of the gem dimethyl group on a seven-membered ring is first time (Ishida et al., 1982) (Figure 15.145).

(–)-β-Caryophyllene (**451**) is one of the ubiquitous sesquiterpene hydrocarbons in plant kingdom and the main component of beer hops and clove oil, and is being used as a culinary ingredient and as a cosmetic in soaps and fragrances. (–)-β-Caryophyllene also has cytotoxic against breast carcinoma cells and its epoxide is toxic to *Planaria* worms. It contains unique 1,1-dimethylcyclobutane skeleton. (–)-β-Caryophyllene (3 g) was treated in the same manner as described above to afford the crude metabolite (2.27 g) from which (10*S*)-14-hydroxycaryophyllene-5,6-oxide (**491**) (80%) and a diol (**492**) were obtained (Asakawa et al., 1981). Later, compound (**491**) was isolated from the Polish mushroom, *Lactarius camphorates* (Basidiomycetes) as a natural product (Daniewski et al., 1981). 14-Hyroxy-β-callyophyllene and 1-hydroxy-8-keto-β-caryophyllene have been found in Asteraceae and *Pseudomonas* species, respectively. In order to confirm that caryophyllene epoxide (**453**) is the intermediate of both metabolites, it was treated in the same manner as described above to give the same metabolites (**491**) and (**492**), of which **491** was predominant (Asakawa et al., 1981, 1986) (Figure 15.146).

The grapefruit aroma, (+)-nootkatone (**2**) was administered into rabbits to give 11,12-diol (**6**, **7**). The same metabolism has been found in that of biotransformation of nootkatone by microorganisms as mentioned in the previous paragraph. Compounds (**6**, **7**) were isolated from the urine of hypertensive subjects and named urodiolenone. The endogenous production of **6**, **7** seem to occur interdentally from the administrative manner of nootkatone or grapefruit. Synthetic racemic nootkatone epoxide (**14**) was incubated with rabbit-liver microsomes to give 11,12-diol (**6**, **7**) (Ishida, 2005). Thus, the role of the epoxide was clearly confirmed as an intermediate of nootkatone (**2**).

(+)-*ent*-Cyclocolorenone (**98**) and its enantiomer (**103**) were biotransformed by *Aspergillus* species to give cyclopropane-cleaved metabolites as described in the previous paragraph.

In order to compare the metabolites between mammals and microorganisms, the essential oil (2 g/rabbit) containing (–)-cyclocolorenone (**103**) obtained from *Solidago altissima* was administered in rabbits to obtain two metabolites; 9β-hydroxycyclocolorenone (**493**) and 10-hydroxycyclocolorenone (**494**) (Asakawa et al., 1986). 10-Hydroxyaromadendrane-type compounds are well

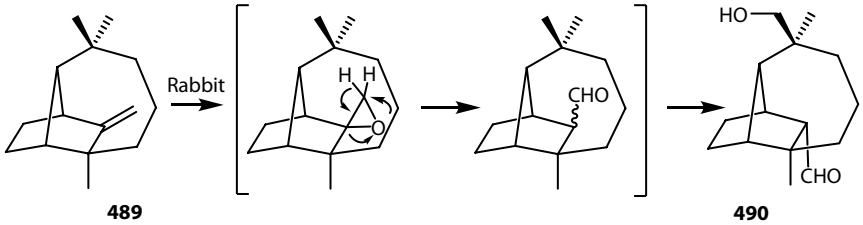


FIGURE 15.145 Biotransformation of longifolene (**489**) by rabbit.

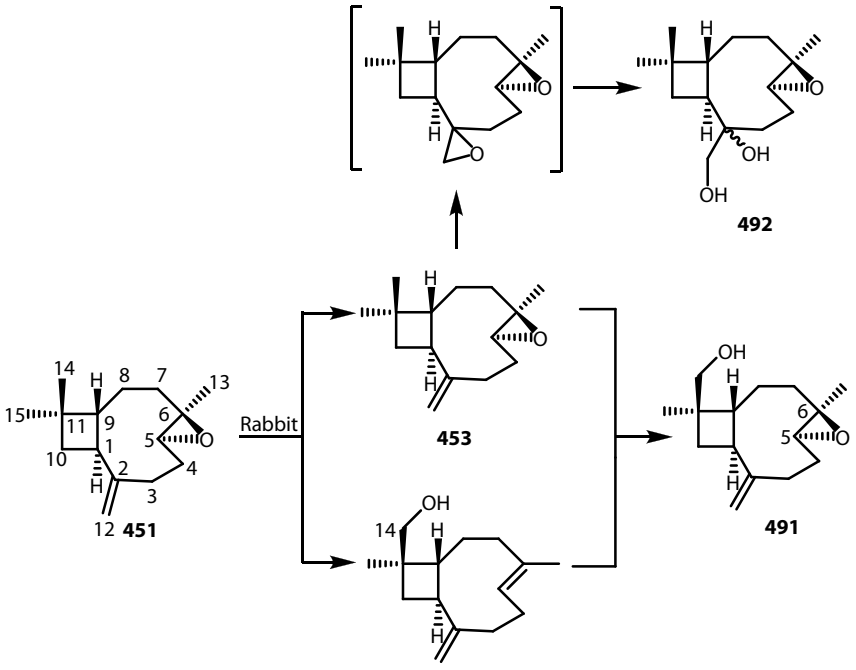


FIGURE 15.146 Biotransformation of (–)-β-caryophyllene (**451**) by rabbit.

known as the natural products. No oxygenated compound of cyclopropane ring was found in the metabolites of cyclocolorenone in rabbit (Figure 15.147).

From the metabolites of elemol (**495**) possessing the same partial structures of monoterpene hydrocarbon, myrcene, and nootkatone, one primary alcohol (**496**) was obtained from rabbit urine after the administration of **495** (Asakawa et al., 1986) (Figure 15.148).

Components of cedar wood such as cedrol (**414**) and cedrene shorten the sleeping time of mice. In order to search for a relationship between scent, olfaction, and detoxifying enzyme induction, (+)-cedrol (**414**) was administered to rabbits and dogs. From the metabolites from rabbits, two C3 hydroxylated products (**418** and **497**) and a diol (**415** or **416**), which might be formed after the hydrogenation of double bond. Dogs converted cedrol (**414**) into the different metabolite products,

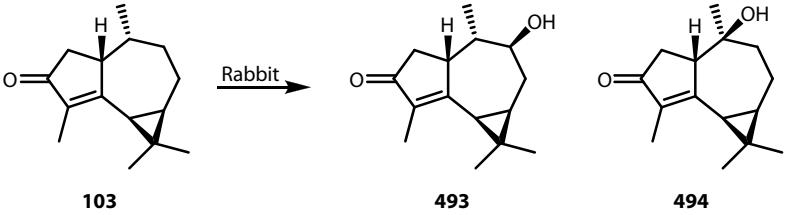


FIGURE 15.147 Biotransformation of (+)-*ent*-cyclocolorenone (**101**) by rabbit.

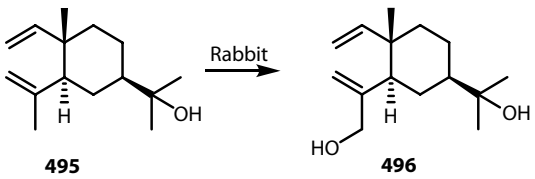


FIGURE 15.148 Biotransformation of elemol (**495**) by rabbit.

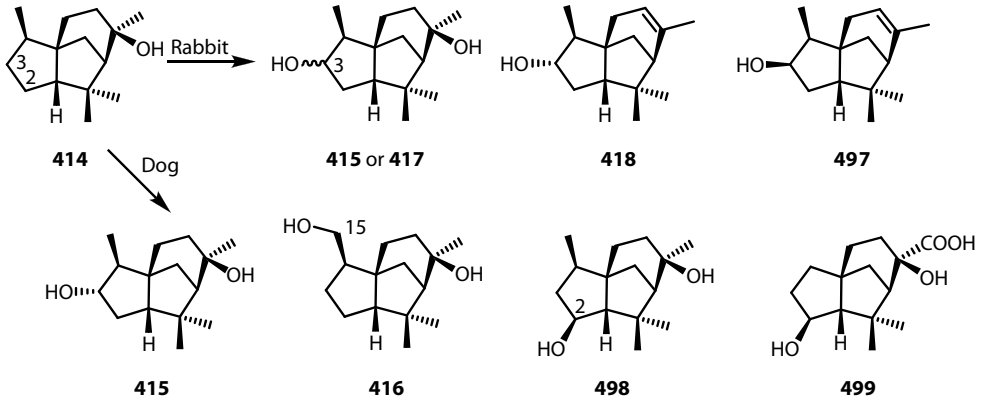


FIGURE 15.149 Biotransformation of cedrol (**414**) by rabbits or dogs.

C2 (**498**) and C2/C14 hydroxylated products (**499**), together with the same C3 (**415**) and C15 hydroxylated products (**416**) as those found in the metabolites of microorganisms and rabbits. The above species-specific metabolism is very remarkable (Bang and Ourisson, 1975).

The microorganisms, *Cephalosporium aphidicoda*, *Corynespora cassicoda*, *Botrytis cinerea*, and *Glomerella cingulata* also biotransformed cedrol to various C2, C3, C4, C6, and C15 hydroxylated products as shown in the previous paragraph. The microbial metabolism of cedrol resembles that of mammals (Figure 15.149).

Patchouli alcohol (**425**) with fungi static properties is one of the important essential oils in perfumery industry. Rabbits and dogs gave two oxidative products (**500**, **501**) and one norpatchoulen-1-one (**502**) possessing a characteristic odor. Plant pathogen, *Botrytis cinerea* causes many diseases for vegetables and flowers. This pathogen gave totally different five metabolites (**426**–**430**) from those found in the urine metabolites of mammals as described above (Bang et al., 1975) (Figure 15.150).

Sandalwood oil contains mainly α-santalol (**503**) and β-santalol. Rabbits converted α-santalol to three diastereomeric primary alcohols (**504**–**506**) and dogs did carboxylic acid (**507**) (Zundel, 1976) (Figure 15.151).

(2*E*,6*E*)-Farnesol (**478**) was treated in cockroach Cytochrome P-450 (CYP4C7) to form region- and diastereospecifically ω-hydroxylated at the C12 methyl group to the corresponding diol (**508**) with 10*E*-configuratation (Sutherland et al., 1998) (Figure 15.152).

Juvenile hormone III (**509**) was also treated in cockroach CYP4C7 to the corresponding 12-hydroxylated product (**510**) (Sutherland et al., 1998).

The African locust converted the same substrate (**509**) into a 7-hydroxy product (**511**) and a 13-hydroxylated product (**512**). It is noteworthy that the African locust and cockroach showed clear species specificity for introduction of oxygen function (Darrouzet et al., 1997).

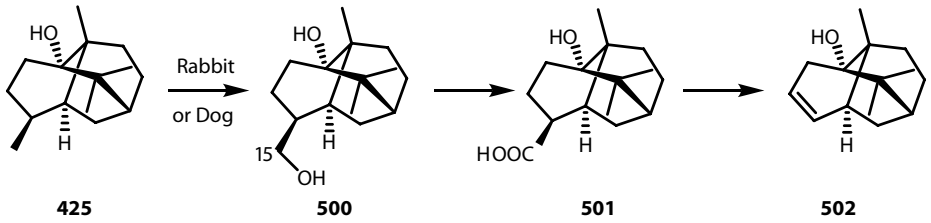


FIGURE 15.150 Biotransformation of patchouli alcohol (**425**) by rabbits or dogs.

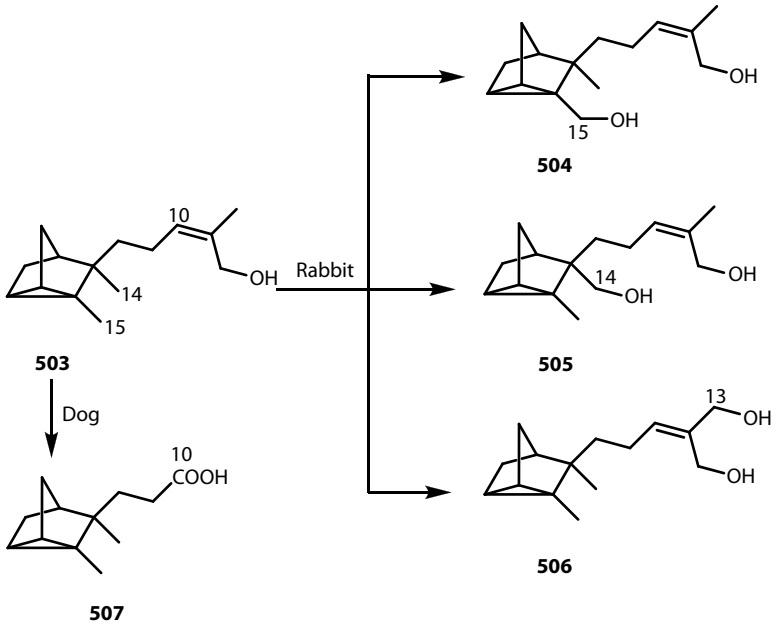


FIGURE 15.151 Biotransformation of santalol (**503**) by rabbits or dogs.

15.4 BIOTRANSFORMATION OF IONONES, DAMASCONES, AND ADAMANTANES

Racemic α-ionone (**513**) was converted to 4-hydroxy-α-ionone (**514**), which was further dehydrogenated to 4-oxo-α-ionone (**515**) by *Chlorella ellipsoidea* IAMC-27 and *Chlorella vulgaris* IAMC-209. α-Ionone (**513**) was reduced preferentially to α-ionol (**516**) by *Chlorella sorokiniana* and *Chlorella salina* (Noma et al. 1997a).

α-Ionol (**516**) was oxidized by *Chlorella pyrenoidosa* to afford 4-hydroxy-α-ionol (**524**). The same substrate was fed by the same microorganism and *Aspergillus niger* to furnish α-ionone (**513**) (Noma and Asakawa, 1998) (Figure 15.153).

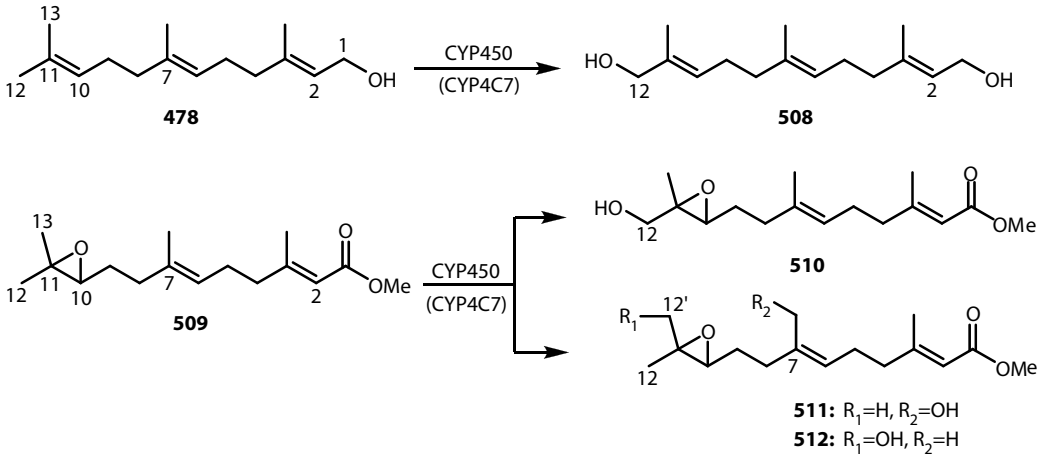


FIGURE 15.152 Biotransformation of *2E,6E*-farnesol (**478**) by cockroach Cytochrome P-450 and 10,11-epoxyfarnesic acid methyl ester (**509**) by African locust Cytochrome P-450.

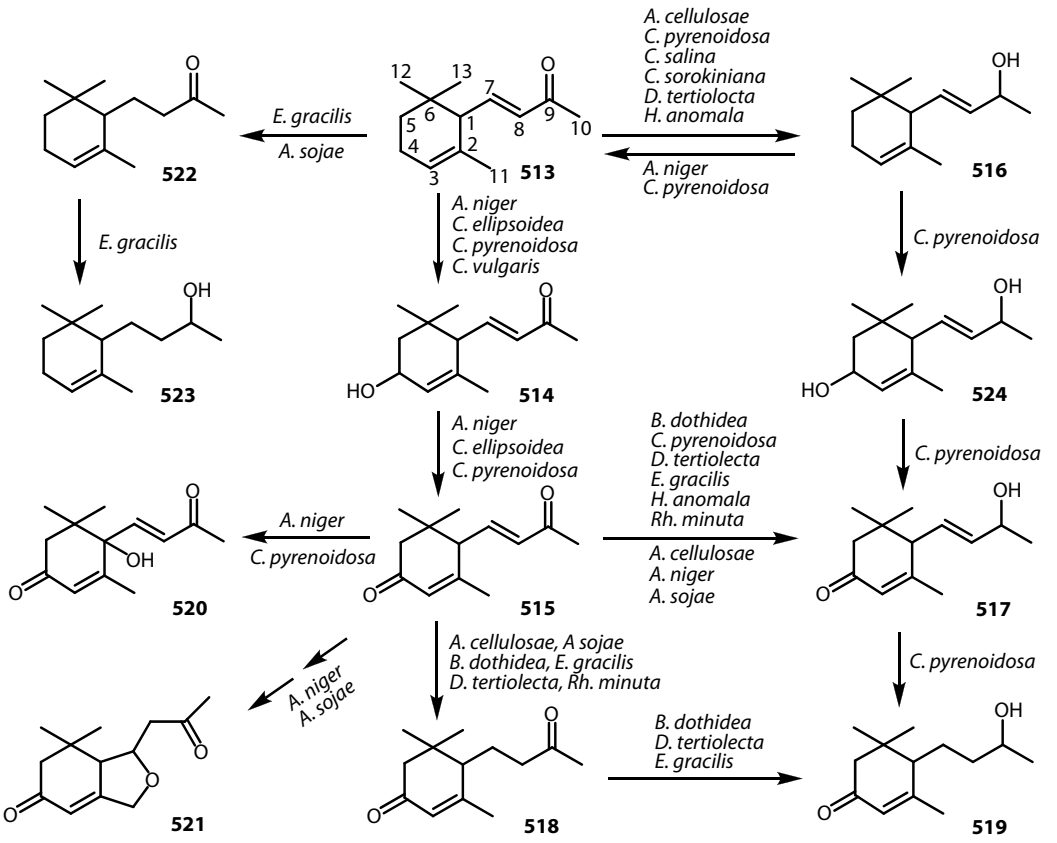


FIGURE 15.153 Biotransformation of α-ionone (**513**) by various microorganisms.

4-Oxo-α-ionone (**515**), which is one of the major product of α-ionone (**513**) by *Aspergillus Niger,* was transformed reductively by *Hansenula anomala, Rhodotorula minuta, Dunaliella tertiolecta, Euglena gracilis, Chlorella pyrenoidosa* C28 and other eight kinds of *Chlorella* species, *Botryosphaeria dothidea*, *Aspergillus cellulosae* IFO 4040 and *Aspergillus sojae* IFO 4389 to give 4-oxo-α-ionol (**517**), 4-oxo-7,8-dihydro-α-ionone (**518**), and 4-oxo-7,8-dihydro-α-ionol (**519**). Compound **515** was also oxidized by *Aspergillus niger* and *Aspergillus sojae* to give 1-hydroxy-4-oxo-α-ionone (**520**) and 7,11-oxido-4-oxo-7,8-dihydro-α-ionone (**521**). C7–C8 Double bond of α-ionone (**513**), 4-oxo α-ionone (**515**), and 4-oxo-α-ionol (**517**) were easily reduced to their corresponding dihydro products (**522**, **518**, **519**), respectively, by *Euglena, Aspergillus, Botrysphaeria*, and *Chlorella* species. The metabolite (**522**) was further reduced to **523** by *Euglena gracilis* (Noma et al., 1998).

Biotransformation of (+)-1*R*-α-ionone (**513a**), $[\alpha]_D$+386.5°; 99% ee and (–)-1*S*-α-ionone (**513a′**), $[\alpha]_D$–361.6°, 98% ee, which were obtained by optical resolution of racemic α-ionone (**513**), was fed by *Aspergillus niger* for 4 days in Czapek-peptone medium. From (**513a**), 4α-hydroxy-α-ionone (**514a**), 4β-hydroxy-α-ionone (**514b**), and 4-oxo-α-ionone (**515a**) were obtained, while from compound **513a′**, the enantiomers (**514a′**, **514b′**, **515a′**) of the metabolites from **513a** were obtained; however, the difference of their yields were observed. In case of **513a**, 4α-hydroxy-α-ionone (**514a**) was obtained as the major product, while **515a′** was predominantly obtained from **513a′**. This oxidation was inhibited by 1-aminobenzotriazole, thus CYP-450 is contributed to this oxidation process (Hashimoto et al., 2000) (Figure 15.154).

α-Damascone (**525**) was incubated with *Aspergillus niger* and *Aspergillus terreus*, in Czapek-peptone medium to give *cis*- (**525**) and *trans*-3-hydroxy-α-damascones (**527**) and 3-oxo-α-damscone

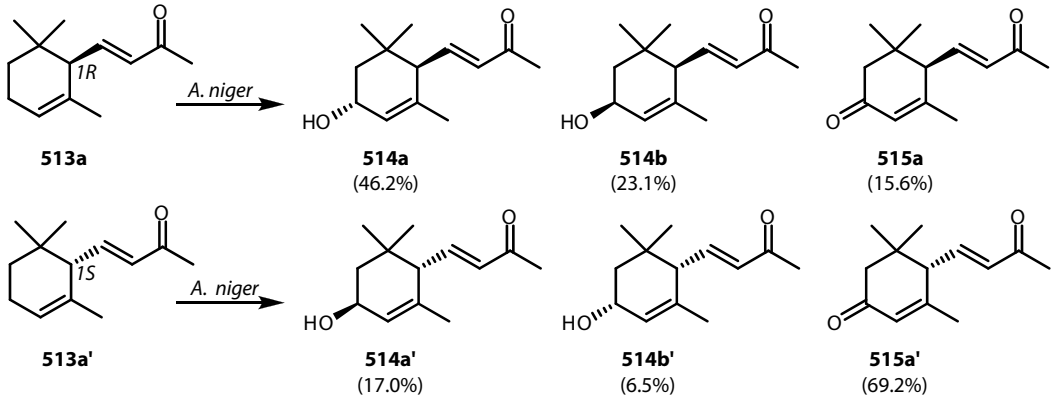


FIGURE 15.154 Biotransformation of (*1R*)-α-ionone (**513a**) and (*1S*)-α-ionone (**513a′**) by *Aspergillus niger.*

(**528**), while the latter *Aspergillus* species afforded 3-oxo-8,9-dihydro-α-damascone (**529**). The hydroxylation process of **525**–**527** was inhibited by CYP-450 inhibitor. *Hansenula anomala* reduced α-damascone (**525**) to α-damascol (**530**). *Cis*- (**526**) and *trans*-4-hydroxy-α-damascone (**527**) were fed by *Chlorella pyrenoidosa* in Noro medium to give 4-oxodamascone (**528**) (Noma et al., 2001a) (Figure 15.155).

β-Damascone (**531**) was also treated in *Aspergillus niger* to afford 5-hydroxy-β-damascone (**532**), 3-hydroxy-β-damascone (**533**), 5-oxo- (**534**), 3-oxo-β-damscone (**535**), and 3-oxo-1, 9-dihydroxy-1,2-dihydro-β-damascone (**536**) as the minor components. In case of *Aspergillus terreus*, 3-hydroxy-8,9-dihydro-β-damascone (**537**) was also obtained (Figure 15.156).

Adamantane derivatives have been used as many medicinal drugs. In order to obtain the drugs, adamantanes were incubated by many microorganisms, such as *Aspergillus niger*, *Aspergillus awamori, Aspergillus cellulosae, Aspergillus fumigatus, Aspergillus sojae, Aspergillus terreus,*

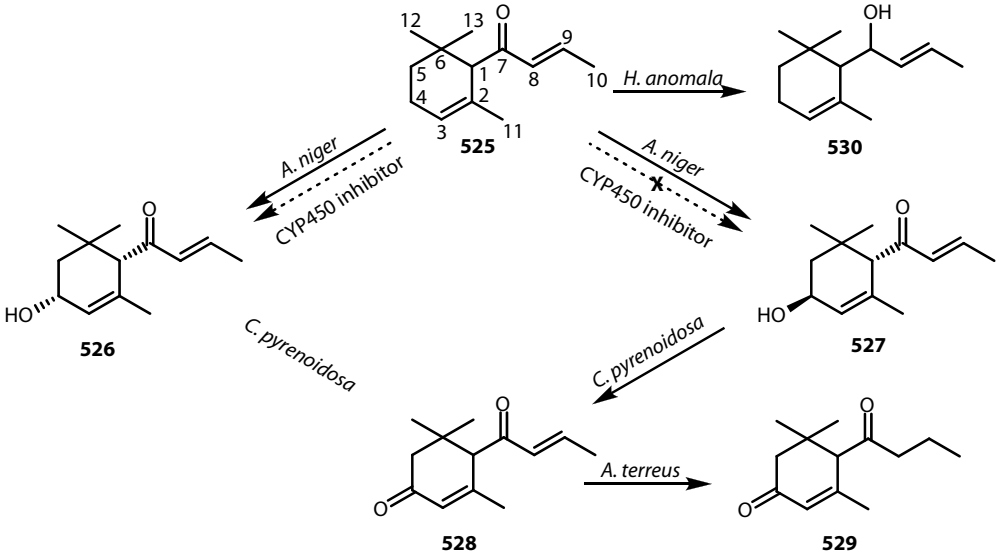


FIGURE 15.155 Biotransformation of α-damascone (**525**) by various microorganisms.

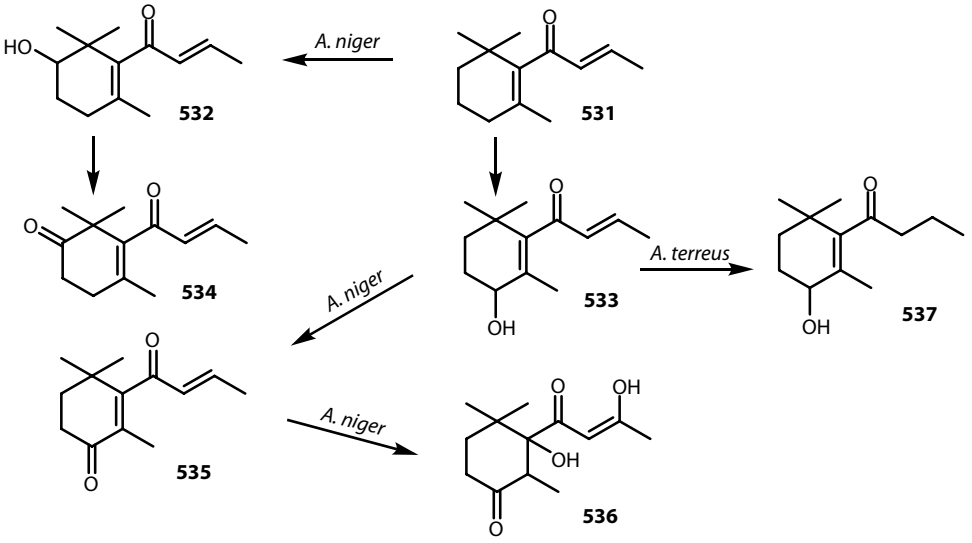


FIGURE 15.156 Biotransformation of β-damascone (**531**) by *Aspergillus niger* and *Aspergillus terreus.*

Botryospherica dothidea, Chlorella pyrenoidosa IMCC-28*, Chlorella sorokiriana, Fusarium culmurorum, Euglena glacilis*, and *Hansenula anomala* (Figure 15.157).

Adamantane (**538**) was incubated with *Aspergillus niger, Aspergillus Cellulosea,* and *Botryosphaeria dothidea* in Czapek-peptone medium. The same substrate was also treated in *Chlorella pyrenoidosa* in Noro medium. Compound **538** was converted into both 1-hydroxy- (**539**) and 9α-hydroxyadamantane (**540**) by all four microorganisms, followed by oxidation oxidized to give 1,9α-dihydroxyadamantanol (**541**) by *Aspergillus Niger,* which was further oxidized to 1-hydroxyadamantane-9-one (**542**), which was reduced to afford 1,9β-hydroxyadamantane (**544**). *Aspergillus niger* gave the metabolite (**541**) as the major product in 80% yield. *Aspergillus cellulosae* converted **538** to **539** and **540** in the ratio of 81:19. *Chlorella pyrenoidosa* gave **539**, **540** and adamantane-9-one (**543**) in the ratio 74:16:10. 4α-Adamantanol (**540**) was directly converted by *Chlorella pyrenoidosa*, *Aspergillus niger*, and *Aspergillus cellulosae* to afford **543**, which was also reduced to 9α-adamantanol (**540**) by *Aspergillus niger.* The biotransformation of adamantane, however, did not occur by the microorganisms: *Hansenula anomala, Chlorella sorokiriana, Dunaliella tertiolecta*, and *Euglena glacilis* (Noma et al., 1999).

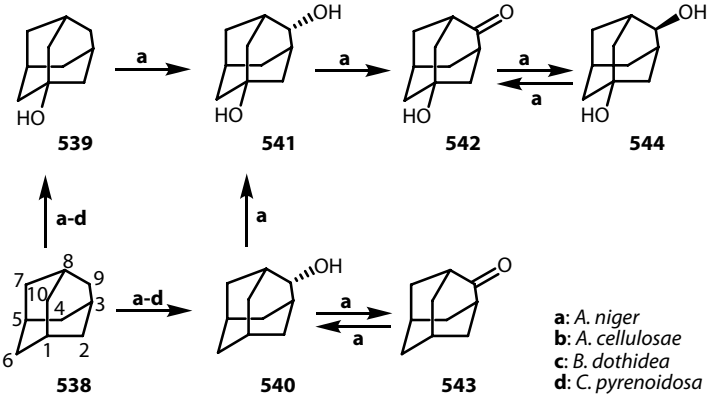


FIGURE 15.157 Biotransformation of adamantane (**538**) by various microorganisms.

Adamantanes (**538**–**543**, **542**) were also incubated with various fungi including with *Fusarium culmurorum*. 1-Hydroxyadamantane-9-one (**542**) was reduced stereoselectively to **541** by *Aspergillus niger, Aspergillus cellulosae*, *Botryosphaeria dothidea*, and *Fusarium culmurorum*. On the contrary, *Fusarium culmurorum* reduced **541**–**542**. *Aspergillus cellulosae* and *Botryosphaeria dothidea* bioconverted **542** to 1,9β-hydroxyadamantane (**544**) stereoselectively. Adamantane-9-one (**543**) was treated by *Aspergillus niger* to give nonsteroselectively **545**–**547** that were further converted into diketone (**548**, **549**) and a diol (**550**). It is noteworthy that oxidation and reduction reactions were observed between ketoalcohol (**547**) and diols (**551**, **552**). The same phenomenon was also seen between **546** and **553**. The latter diol was also oxidized by *Aspergillus niger* to furnish diketone (**549**) (Noma et al., 2001b, 2003). Direct hydroxylation at C3 of 1-hydroxyadamantane- 9-one (**542**) was seen in the incubation of **539** with *Aspergillus niger.*

4-Adamantanone (**543**) showed promotion effect of cell division of the fungus, while 1-adamantanol (**539**) and adamantane-9-one (**543**) inhibited germination of lettuce seed. 1-Hydroxyadamantane-9-one (**542**) inhibited the elongation of root of lettuce while and adamantane-1,4-diol (**544**) and adamantane itself (**538**) promoted root elongation (Noma et al., 1999, 2001b) (Figure 15.158).

Stereoselective reduction of racemic bicycle[3.3.1]nonane-2,6-dione (**555a**, **555a′**) was carried out by *Aspergillus awamori, Aspergillus fumigatus, Aspergillus cellulosae, Aspergillus sojae, Aspergillus terreus, Aspergillus niger, Botryosphaeria dothidea*, and *Fusarium culmorum* in Czapek-peptone, *Hansenula anomala* in yeast, *Euglena glacilis* in Hunter, and *Dunaliella tertiolecta* in Noro medium, respectively. All microorganisms reduced **555** and **555a′** to give

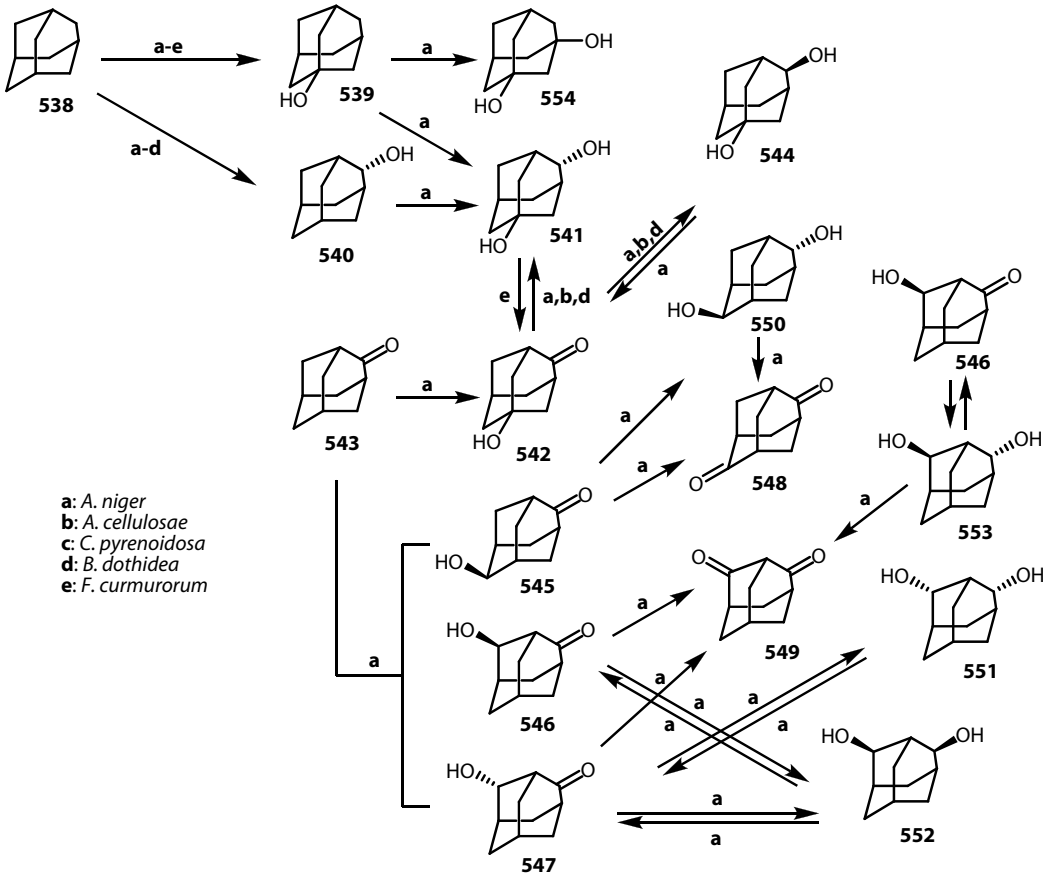


FIGURE 15.158 Biotransformation of adamantane (**538**) and adamantane-9-one by various microorganisms.

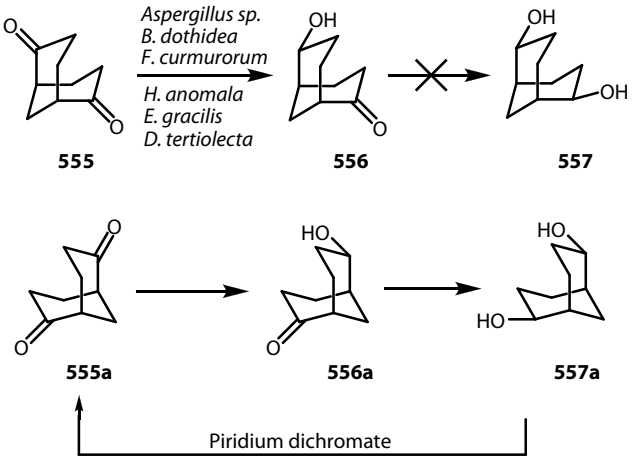


FIGURE 15.159 Biotransformation of bicyclo[3.3.1]nonane-2,6-dione (**555a**, **555a′**) by various microorganisms.

corresponding monoalcohol (**556**, **556a**) and optical-active (–)-diol (**557a**) ($[\alpha]_D$ –71.8° in the case of *Aspergillus terreus*), which was formed by enantioselective reduction of racemic monool, namely **556** and **556a** (Noma et al., 2003) (Figure 15.159).

15.5 BIOTRANSFORMATION OF AROMATIC COMPOUNDS

Essential oils contain aromatic compounds, such as *p*-cymene, carvacrol, thymol, vanillin, cinnamaldehyde, eugenol, chavicol, safrole, and asarone (**558**), among others.

Takahashi (1994) reported that simple aromatic compounds, propylbenzene, hexylbenene, decylbenzene, *o*- and *p*-hydroxypropiophenones, *p*-methoxypropiophenone, 4-hexylresorcinol, and methyl 4-hexylresorcionol were incubated with *Aspergillus niger.* From hexyl- and decylbenzenes, ω1-hydroxylated products were obtained, whereas from propylbenzene, ω2 hydroxylated metabolites were obtained (Takahashi, 1994).

Asarone (**558**) and dihydroeugenol (**562**) were not biotransformed by *Aspergillus niger.* However, dihydroasarone (**559**) and methyldihyroeugenol (**563**) were biotransformed by the same fungus to produce a small amount of 2-hydroxy (**560**, **561**) and 2-oxo derivatives (**564**, **565**), respectively. The chirality at C2 was determined to be *R* and *S* mixtures (1:2) by the modified Mosher method (Takahashi, 1994) (Figure 15.160).

Chlorella species are excellent microalgae as oxidation bioreactors as mentioned earlier. Treatment of monoterpene aldehydes and related aldehydes were reduced to the corresponding primary alcohols, indicating that these green algae possess reductase.

A microalgae, *Euglena gracillis* Z. also contains reductase. The following aromatic aldehydes were treated in this organism. Benzaldehyde, 2-cyanobenzaldehyde, *o*-, *m*-, and *p*-anisaldehyde, salicylaldehyde, *o*-, *m*-, and *p*-tolualdehyde, *o*-chlorobenzaldehyde, *p*-hydroxybenzaldehyde, *o*-nitro-, *m*-, and *p*-nitrobenaldeyde, 3-cyanobenzaldehyde, vanillin, isovanillin, *o*-vanillin, nicotine aldehyde, 3-phenylpropionaldehyde, ethyl vanillin. Veratraldehyde, 3-nitrosalicylaldehde, penylacetaldehyde, and 2-phenylproanaldehyde gave their corresponding primary alcohols. 2-Cyanobenzaldehyde gave its corresponding alcohol with phthalate. *m*- and *p*-Chlorobenzaldehyde gave its corresponding alcohols and *m*- and *p*-chlorobenzoic acids. *o*-Phthaldehyde and *p*-phthalate and iso- and terephthaldehydes gave their corresponding monoalohols and dialcohols. When cinnamaldehyde and α-methyl cinnamaldehyde were incubated in *Euglena gracilis*, cinnamyl alcohol and 3-phenylpropanol, and 2-methylcinnamyl alcohol, and 2-methyo-3-phenylpropanol were obtained

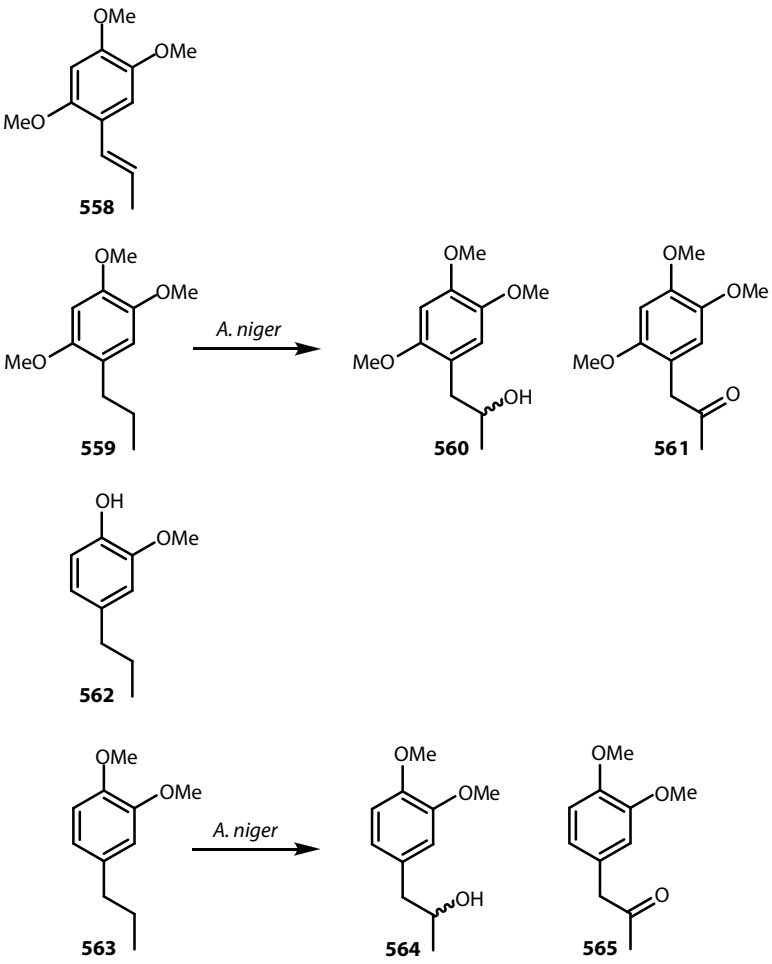


FIGURE 15.160 Biotransformation of dihydroasarone (**559**) and methyldihydro eugenol (**563**) by *Aspergillus niger*.

in good yield. *Euglena gracilis* could convert acetophenone to 2-phenylethanol; however, its enantio-excess is very poor (10%) (Takahashi, 1994).

Raspberry ketone (**566**) and zingerone (**574**) are the major components of raspberry (*Rubus idaeus*) and ginger (*Zingeber officinale*) and these are used as food additive and spice. Two substrates were incubated with the *Phytolacca americana* cultured cells for 3 days to produce two secondary alcohols (**567**, **568**) as well as five glucosides (**569**–**572**) from **566**, and a secondary alcohol (**576**) and four glycoside products (**575**, **577**–**579**) from **574**. In the case of raspberry ketone, phenolic hydroxyl group was preferably glycosylated after the reduction of carbonyl group of the substrate occurred. It is interesting to note that one more hydroxyl group was introduced into the benzene ring to give **568**, which were further glycosylated one of the phenolic hydroxyl groups and no glycocide of the secondary alcohol at C2 were obtained (Figure 15.161).

On the other hand, zingerone (**574**) was converted into **576**, followed by glycosylation to give both glucosides (**577**, **578**) of phenolic and secondary hydroxyl groups and a diglucoside (**579**) of both phenolic and secondary hydroxyl group in the molecule. It is the first report on the introduction of individual glucose residues onto both phenolic and secondary hydroxyl groups by cultured plant cells (Shimoda et al., 2007) (Figure 15.162).

Thymol (**580**), carvacrol (**583**), and eugenol (**586**) were glucosylated by glycosyl transferase of cell-cultured *Eucalyptus perriniana* to each glucoside (**581**, 3%; **584**, 5%; **587**, 7%) and gentiobioside

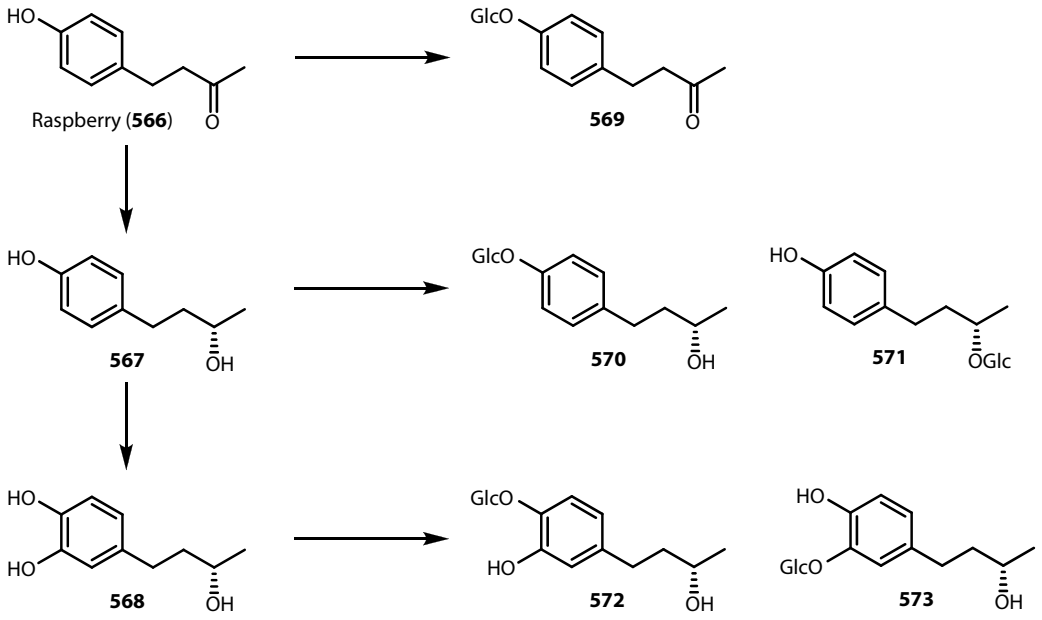


FIGURE 15.161 Biotransformation of raspberry ketone (**566**) by *Phytolacca americana* cells.

(**582**, 87%; **585**, 56%; **588**, 58%). The yield of thymol glycosides was 1.5 times higher than that of carvacrol and 4 times higher than that of eugenol. Such glycosylation is useful to obtain higher water-soluble products from natural and commercially available secondary metabolites for food additives and cosmetic fields (Shimoda et al., 2006) (Figure 15.163).

Hinokitiol (**589**), which is easily obtained from cell suspension cultures of *Thujopsis dolabrata* and possesses potent antimicrobial activity, was incubated with cultured cells of *Eucalyptus perriniana* for 7 days to give its monoglucosides (**590**, **591**, 32%) and gentiobiosides (**592**, **593**) (Furuya et al., 1997, Hamada et al., 1998) (Figure 15.164).

(–)-Nopol benzyl ether (**594**) was smoothly biotransformed by *Aspergillus niger, Aspergillus cellulosae, Aspergillus sojae, Aspergillus Usami*, and *Penicillium* species in Czapek-peptone medium to give (–)-4-oxonopol-2′,4′-dihydroxybenzyl ether (**595**, 23% in the case of *Aspergillus niger*), which demonstrated antioxidant activity (ID_{50} 30.23 μ/M), together with a small amount of nopol (6.3% in *Aspergillus niger*). This is very rare direct introduction of oxygen function on the phenyl ring (Noma and Asakawa, 2006) (Figure 15.165).

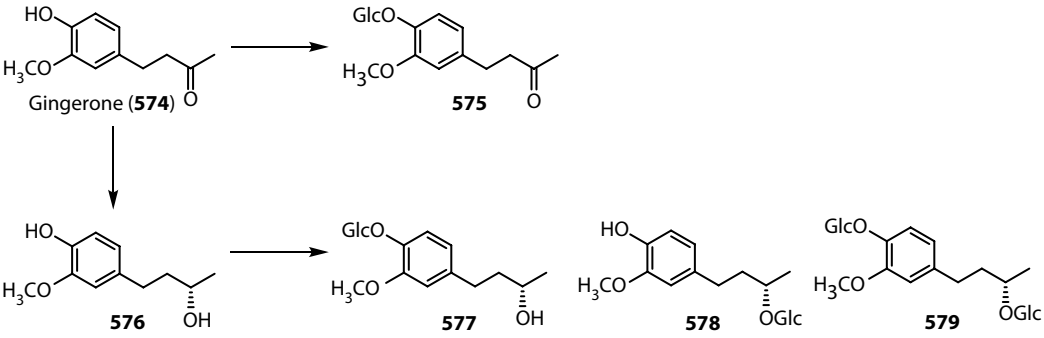


FIGURE 15.162 Biotransformation of zingerone (**574**) by *Phytolacca americana* cells.

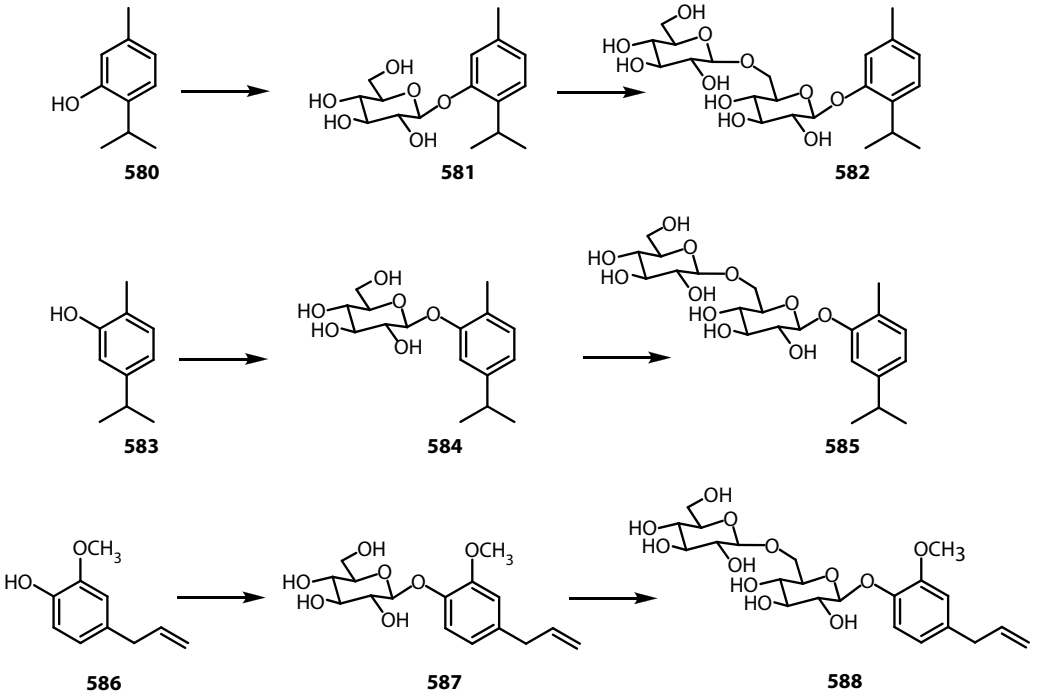


FIGURE 15.163 Biotransformation of thymol (**580**), carvacrol (**583**), and eugenol (**586**) by *Eucalyptus perriniana* cells.

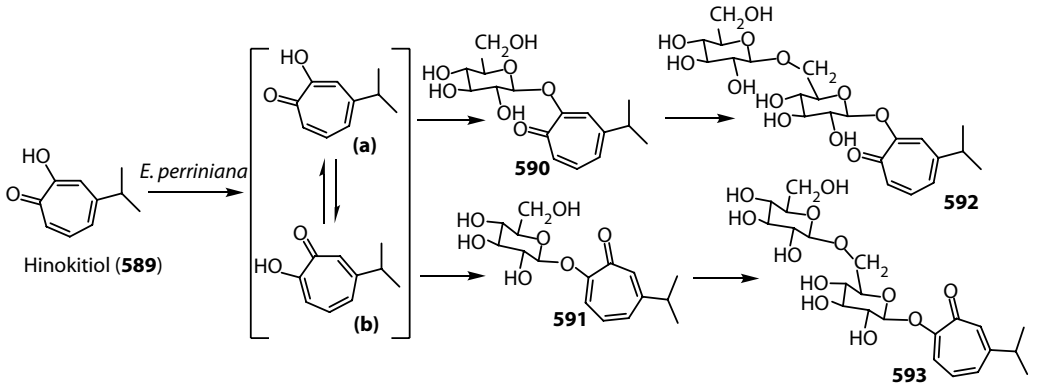


FIGURE 15.164 Biotransformation of hinokitiol (**589**) by *Eucalyptus perriniana* cells.

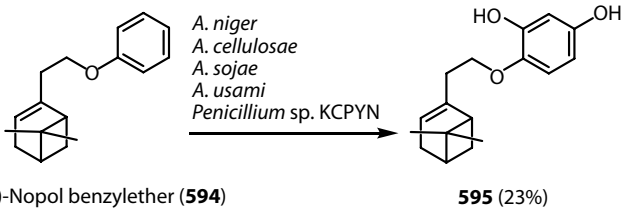


FIGURE 15.165 Biotransformation of nopol benzylether (**531**) by *Aspergillus* and *Penicillium* species.

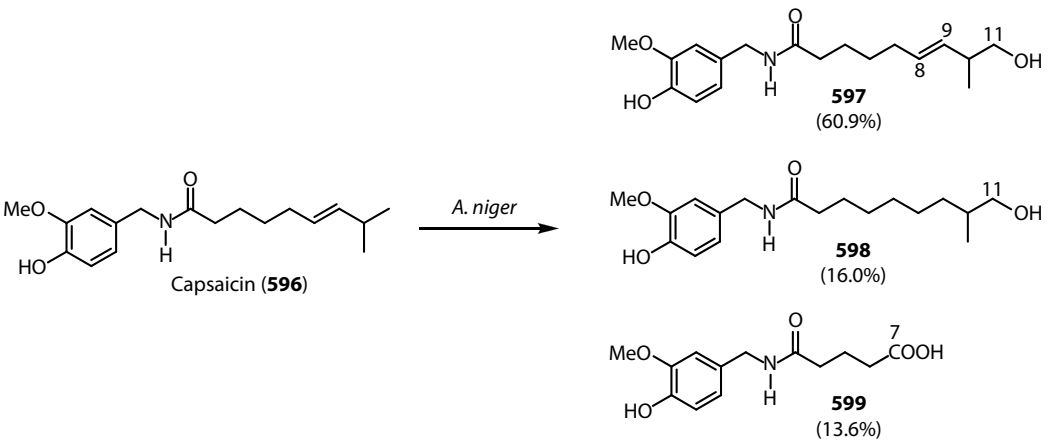


FIGURE 15.166 Biotransformation of capsaicin (**596**) by *Aspergillus niger.*

Capsicum annuum contains capsaicin (**596**) and its homologues having an alkylvanillylamides possess various interesting biological activities such as anti-inflammation, antioxidant, saliva, and stomach juice inducing activity, analgesic, antigenotoxic, antimutagenic, anticarcinogenic, antirheunatoid arthritis, diabetic neuropathy, and used as food additives. On the other hand, because of potent pungency and irritation on skin and mucous membrane, it has not yet been permitted as medicinal drug. In order to reduce this typical pungency and application of nonpungent capsaicin metabolites to the crude drug, capsaicin (**596**) (600 mg) including 30% of dihydrocapsaicin (**600**) was incubated in Czapek-peptone medium including *Aspergillus niger* for 7 days to give three metabolites, ω1-hydroxylated capsaicin (**597**, 60.9%), 8,9-dihydro-ω1-hydroxycapsaicin (**598**, 16%), and a carboxylic acid (**599**, 13.6%). All of the metabolites do not show pungency (Figure 15.166).

Dihydrocapsaicin (**600**) was also treated in the same manner as described above to afford ω1-hydroxydihyrocpsaicin (**598**, 80.9%) in high yield and the carboxylic acid (**599**, 5.0%). Capsaicin itself showed carbachol-induced contraction of 60% in the bronchus at a concentration of 1 μmol/L. 11-Hydroxycapsicin (**85**) retained this activity of 60% at a concentration of 30 μmol/L. Dihydrocapsicin (**600**) showed the same activity of contraction in the bronchus, at the same concentration as that used in capsaicin. However, the activity of contraction in the bronchus of 11-hydroxy derivative (**598**) showed weaker (50% at 30 μmol/L) than that of the substrate. Since both metabolites (**597** and **598**) are tasteless, these products might be valuable for the crude drug although the contraction in the bronchus is weak. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity test of capsaicin and dihydrocapsaicin derivatives was carried out. 11-Hydroxycapsaicin (**597**), 11-dihydrocapsaicin (**598**), and capsaicin (**596**) showed higher activity than (±)-α-tocopherol and 11-dihydroxycapsaicin (**598**) displayed the strong scavenging activity (IC_{50} 50 μmol/L) (Hashimoto, Asakawa, unpublished results) (Figure 15.167).

Shimoda et al. (2007a) reported the bioconversion of capsaicin (**596**) and 8-nordihydrocapsaicin (**601**) by the cultured cell of *Catharathus roseus* to give more water-soluble capsaicin derivatives. From capsaicin, three glycosides, capsaicin 4-*O*-β-D-glucopyranoside (**602**), which was one of the capsaicinoids in the fruit of *Capsicum* and showed 1/100 weaker pungency than capsaicin, 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucoside (**603**) and 4-*O*-(6-*O*-α-L-arbinosyl)-β-D-glucopyranoside (**604**) were obtained. 8-Nor-dihydrocapsaicin (**601**) was also incubated with the same cultured cell to afford the similar products (**605**–**607**) all of which reduced their pungency and enhanced water solubility. Since many synthetic capsaicin glycosides possess remarkable pharmacological activity, such as decrease of liver and serum lipids, the present products will be used for valuable prodrugs (Figure 15.168).

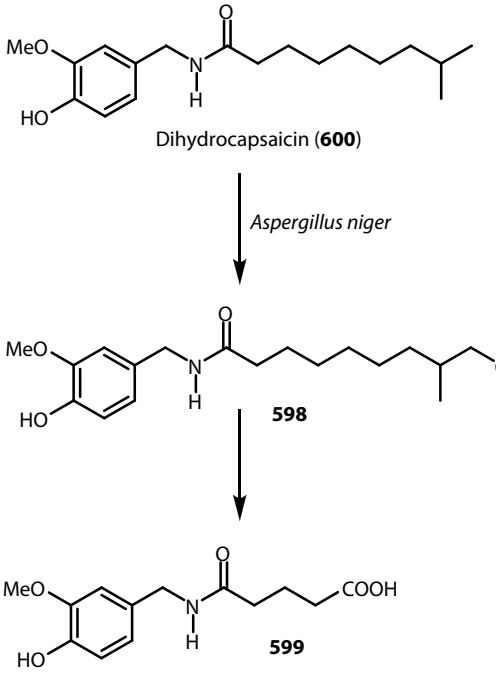


FIGURE 15.167 Biotransformation of dihydrocapsaicin (**600**) by *Aspergillus niger.*

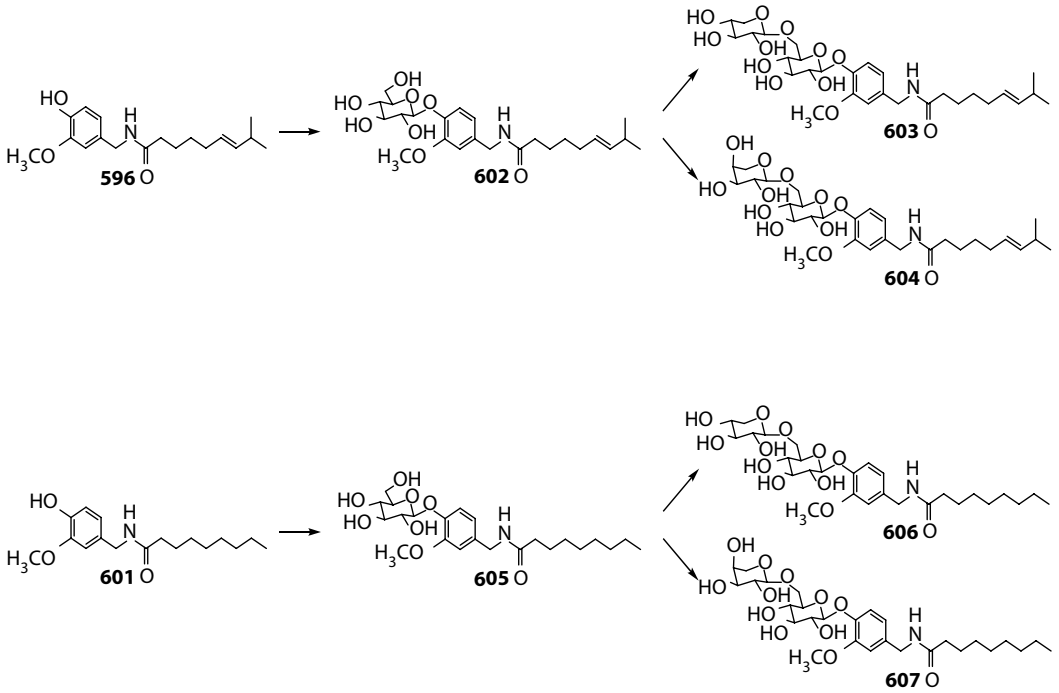


FIGURE 15.168 Biotransformation of capsaicin (**596**) and 8-nor-dihydrocapsaicin (**601**) by *Catharanthus roseus* cells.

Zingiber officinale contains various sesquiterpenoids and pungent aromatic compounds such as 6-shogaol (**608**) and 6-gingerol (**613**) and their pungent compounds that possess cardio tonic and sedative activity. 6-Shogaol (**608**) was incubated with *Aspergillus niger* in Czapek-peptone medium for 2 days to afford ω1-hydroxy-6-shagaol (**609**, 9.9%), which was further converted to 8-hydroxy derivative (**610**, 16.1%), a γ-lactone (**611**, 22.4%), and 3-methoxy-4-hydroxyphenylacetic acid (**612**, 48.5%) (Figure 15.169).

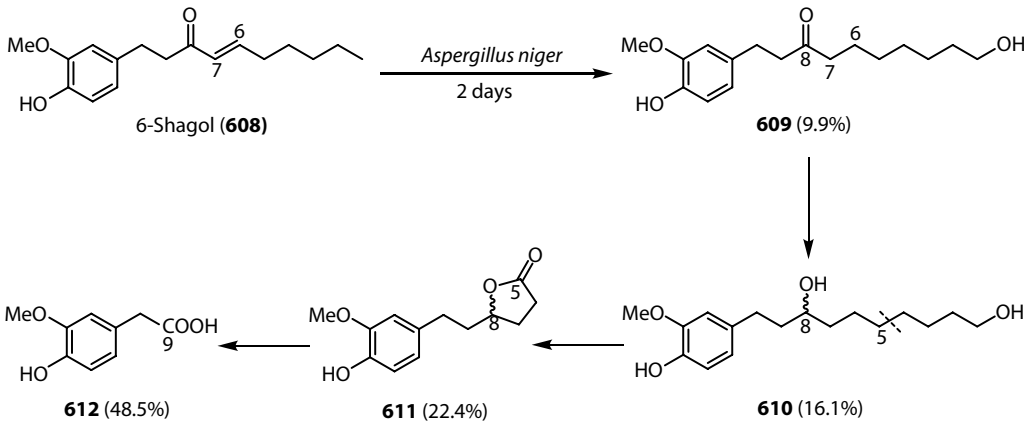


FIGURE 15.169 Biotransformation of 6-shogaol (**608**) by *Aspergillus niger*.

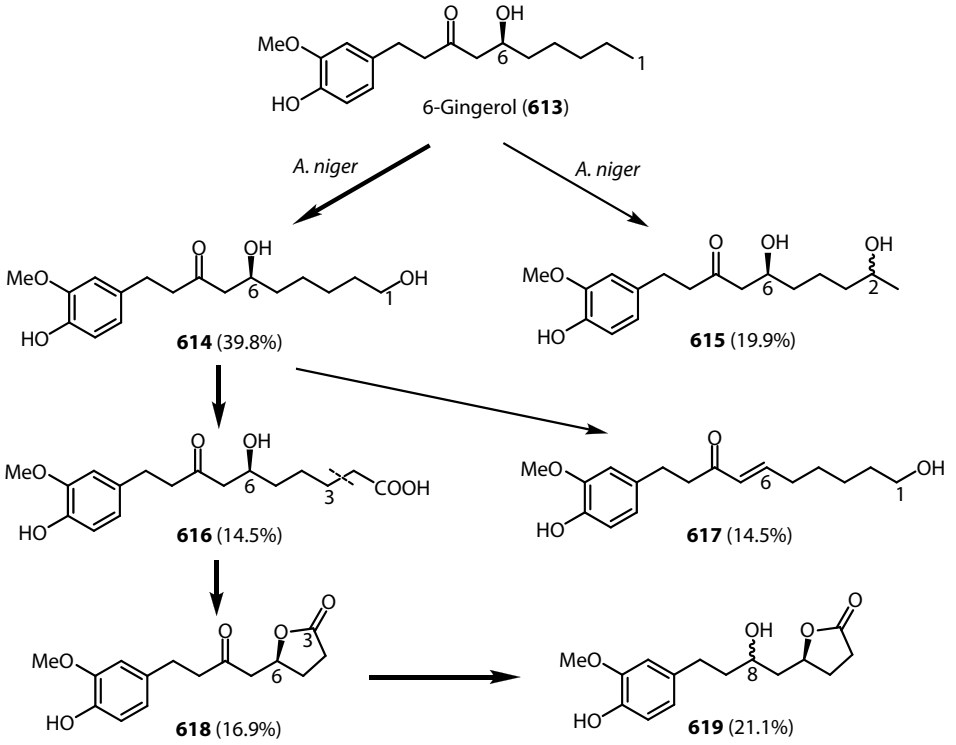


FIGURE 15.170 Biotransformation of 6-gingerol (**613**) by *Aspergillus niger*.

6-Gingerol (**613**) (1 g) was treated in the same condition as mentioned above to yield six metabolites, ω1-hydroxy-6-gingerol (**614**, 39.8%), its carboxylic derivative (**616**, 14.5%), a γ-lactone (**618**) (16.9%) that might be formed from (**616**), its 8-hydoxy-γ-lactone (**619**, 12.1%), ω2-hydroxy-6-gingerol (**615**, 19.9%), and 6-deoxy-gingerol (**617**, 14.5%) (Takahashi et al., 1993).

The metabolic pathway of 6-gingerol (**613**) resembles that of 6-shagaol (**608**). That of 6-shogaol and dihydrocapsaicin (**600**) is also similar since both substrates gave carboxylic acids as the final metabolites (Takahashi et al., 1993) (Figure 15.170).

In conclusion, a number of sesquiterpenoids were biotransformed by various fungi and mammals to afford many metabolites, several of which showed antimicrobial and antifungal, antiobesity, cytotoxic, neurotrophic, and enzyme inhibitory activity. Microorganisms introduce oxygen atom at allylic position to give secondary hydroxyl and keto groups. Double bond is also oxidized to give epoxide, followed by hydrolysis to afford a diol. These reactions precede stereo- and regiospecifically. Even at nonactivated carbon atom, oxidation reaction occurs to give primary alcohol. Some fungi like *Aspergillus niger* cleave the cyclopropane ring with a 1,1-dimethyl group. It is noteworthy that *Aspergillus niger* and *Aspergillus cellulosae* produce the totally different metabolites from the same substrates. Some fungi occurs reduction of carbonyl group, oxidation of aryl methyl group, phenyl coupling, and cyclization of a 10-membered ring sesquiterpenoids to give C6/C6- and C5/C7-cyclic or spiro compounds. Cytochrome P-450 is responsible for the introduction of oxygen function into the substrates.

The present methods are very useful for the production of medicinal and agricultural drugs as well as fragrant components from commercially available cheap, natural, and unnatural terpenoids or a large amount of terpenoids from higher medicinal plants and spore-forming plants like liverworts and fungi.

The methodology discussed in this chapter is a very simple one-step reaction in water, nonhazard, and very cheap, and it gives many valuable metabolites possessing different properties from those of the substrates.

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16 Industrial Uses of Essential Oils

W. S. Brud

CONTENTS

16.1 INTRODUCTION

The period when essential oils were used first on an industrial scale is difficult to identify. The nineteenth century is generally regarded as the commencement of the modern phase of industrial application of essential oils. However, the large-scale usage of essential oils dates back to ancient Egypt. In 1480 BC, Queen Hatshepsut of Egypt sent an expedition to the country of Punt (now Somalia) to collect fragrant plants, oils, and resins as ingredients for perfumes, medicaments, and flavors and for the mummification of bodies. Precious fragrances have been found in many Egyptian archeological excavations, as a symbol of wealth and social position.

If significant international trade of essential oil-based products is the criterion for industrial use, "Queen of Hungary Water" was the first alcoholic perfume in history. This fragrance, based on rosemary essential oil distillate, was created in the mid-fourteenth century for the Polish-born Queen Elisabeth of Hungary. Following a special presentation to King Charles V, The Wise of France in 1350, it became popular in all medieval European courts. The beginning of the eighteenth century saw the introduction of "Eau de Cologne," based on bergamot and other citrus oils, which remains widely used to this day. This fresh citrus fragrance was the creation of Jean Maria Farina, a descendant of Italian perfumers who came to France with Catherine de Medici and settled in Grasse in the sixteenth century. According to the city of Cologne archives, Jean Maria Farina and Karl Hieronymus Farina, in 1749, established factory (Fabriek) of this water, which sounds very "industrial." The "Kölnisch Wasser" became the first unisex fragrance rather than one simply for men, known and used all over Europe, and it has been repeated subsequently in innumerable countertypes as a fragrance for men.

16.2 THE HISTORY

The history of production of essential oils dates back to ca. 3500 BC when the oldest known water distillation equipment for essential oils was employed, and may be seen today in the Texila museum in Pakistan. Ancient India, China, and Egypt were the locations where essential oils were produced and widely used as medicaments, flavors, and fragrances. Perfumes came to Europe most probably from the East at the time of the crusades, and perfumery was accorded a professional status by the approval of a French guild of perfumers in Grasse by King Philippe August in 1190. For centuries, Grasse remained the center of world perfumery and was also the home of the first ever officially registered essential oils-producing company—Antoine Chiris—in 1768. (It is worth noting that not much later, in 1798, the first American essential oil company—Dodge and Olcott Inc.—was established in New York.)

About 150 years earlier, in 1620, an Englishman, named Yardley, obtained a concession from King Charles I to manufacture soap for the London area. Details of this event are sparse, other than the high fee paid by Yardley for this privilege. Importantly, however, Yardley's soap was perfumed with English lavender, which remains the Yardley trademark today, and it was probably the first case of use of an essential oil as a fragrance in large-scale soap production.

The use of essential oils as food ingredients has a history dating back to ancient times. There are many examples of the use of citrus and other squeezed (manually or mechanically expressed) oils for sweets and desserts in ancient Egypt, Greece, and the Roman Empire. Numerous references exist to flavored ice creams in the courts of the Roman Emperor Nero and of China. The reintroduction of recipes in Europe is attributed to Marco Polo on his return from traveling to China. In other stories, Catherine de Medici introduced ice creams in France, whereas Charles I of England served the first dessert in the form of frozen cream. Ice was used for freezing drinks and food in many civilizations and the Eastern practice of using spices and spice essential oils both as flavoring ingredients and as food conservation agents was adopted centuries ago in Europe.

Whatever may be regarded as the date of their industrial production, essential oils, together with a range of related products—pomades, tinctures, resins, absolutes, extracts, distillates, concretes, and so on—were the only ingredients of flavor and fragrance products until the late nineteenth century. At this stage, the growth in consumption of essential oils as odoriferous and flavoring materials stimulated the emergence of a great number of manufacturers in France, the United Kingdom, Germany, Switzerland, and the United States (Table 16.1).

The rapid development of the fragrance and flavor industry in the nineteenth century was generally based on essential oils and related natural products. In 1876, however, Haarman and Reimer started the first production of synthetic aroma chemicals—vanillin, then coumarin, anisaldehyde, heliotropin, and terpineol. Although aroma chemicals made a revolution in fragrances with top discoveries in the twentieth century, for many decades both flavors and fragrances were manufactured with constituents of natural origin, the majority of which were essential oils.

16.3 FRAGRANCES

The main reason for the expansion of the essential oils industry and the growing demand for products was the development of the food, soap, and cosmetics industries. Today's multinational companies, the main users of fragrances and flavors, have evolved directly from the developments during the mid-nineteenth century.

In 1806, William Colgate opened his first store for soaps, candles, and laundry starch on Dutch Street in New York. In 1864, B.J. Johnson in Milwaukee started the production of soap, which came to be known as Palmolive from 1898. In 1866, Colgate launched its first perfumed soaps and perfumes. In 1873, Colgate launched toothpaste in a glass jug on the market and in the tube first in 1896. In 1926, two soap manufacturers—Palmolive and Peet—merged to create Palmolive–Peet, which 2 years later merged with Colgate to establish the Colgate–Palmolive–Peet company (renamed as the Colgate–Palmolive Company in 1953).

TABLE 16.1
The First Industrial Manufacturers of Essential Oils, Flavors, and Fragrances

Company Name	Country	Established
Antoine Chiris	France (Grasse)	1768
Cavallier Freres	France (Grasse)	1784
Dodge & Olcott Inc.	USA (New York)	1798
Roure Bertrand Fils and Justin Dupont	France (Grasse)	1820
Schimmel & Co.	Germany (Leipzig)	1829
J. Mero-Boyveau	France (Grasse)	1832
Stafford Allen and Sons	United Kingdom (London)	1833
Robertet et Cie	**France (Grasse)**	**1850**
W.J. Bush	United Kingdom (London)	1851
Payan-Bertrand et Cie	**France (Grasse)**	**1854**
A. Boake Roberts	United Kingdom (London)	1865
Fritsche-Schimmel Co	USA (New York)	1871
V. Mane et Fils	**France (Grasse)**	**1871**
Haarman&Reimer	Germany (Holzminden)	1874
R.C. Treatt Co.	**United Kingdom (Bury)**	**1886**
N.V. Polak und Schwartz	Holland (Zaandam)	1889
Ogawa and Co.	**Japan (Osaka)**	**1893**
Firmenich and Cie	**Switzerland (Geneve)**	**1895**
Givaudan S.A.	**Switzerland (Geneve)**	**1895**
Maschmeijer Aromatics	Holland (Amsterdam)	1900

Note: Companies continuing to operate under their original name are printed in bold.

In October 1837, William Procter and James Gamble signed a formal partnership agreement to develop their production and marketing of soaps (Gamble) and candles (Procter). "Palm oil," "rosin," "toilet," and "shaving" soaps were listed in their advertisements. An "oleine" soap was described as having a violet odor. Only 22 years later, Procter & Gamble (P&G) sales reached 1 million dollars. In 1879, a fine but inexpensive "ivory" white toilet soap was offered to the market with all purpose applications as a toilet and laundry product. In 1890, P&G was selling more than 30 different soaps.

The story of a third player started in 1890 when William Hesket Lever created his concept of the Sunlight Soap, which revolutionized the idea of cleanliness and hygiene in Victorian Britain.

The very beginning of twentieth century marked the next big event when the young French chemist Eugene Schueller prepared his first hair color in 1907 and established what is now L'Oreal. These were the flagships in hundreds of emerging (and disappearing by fusions, takeovers, or bankruptcy) manufacturers of perfumes, cosmetics, toiletries, detergents, household chemicals, and related products, the majority of which were and are perfumed with essential oils.

16.4 FLAVORS

Over the same time period, another group of users of essential oils entered the markets. In 1790, the term "soda water" for carbon dioxide saturated water as a new drink appeared for the first time in the United States and in 1810, the first U.S. patent was issued for the manufacture of imitations of natural gaseous mineral waters. Only 9 years later the "soda fountain" was patented by Samuel Fahnestock. In 1833, carbonated lemonade flavored with lemon juice and citric acid was on sale in England. In 1835, the first bottled soda water appeared in the United States. It is, however, interesting that the first flavored sparkling drink—Ginger Ale—was created in Ireland in 1851. The milestones in flavored soft drinks

appeared 30 years later: 1881—the first cola-flavored drink in the United States; 1885—Dr Pepper was invented by Charles Aderton in Waco, Texas; 1886—Coca-Cola by Dr John S. Pemberton in Atlanta, Georgia; and in 1898—Pepsi-Cola, created by Caleb Bradham, known from 1893 as "Brad's Drink."

Dr Pepper was advertised as the king of beverages, free from caffeine (which was added to it later on), was flavored with black cherry artificial flavor, and was first sold in the Old Corner Drug Store owned by Wade Morrison. Its market success and position as one of the most popular U.S. soft drinks started by a presentation during the St Louis World's Fair, where some other important flavor-consuming products—ice cream cones, hot dog rolls, and hamburger buns—were also shown. All of them remain major users of natural flavors based on essential oils. Hundred years later after the merger with another famous lemon–lime drink 7UP in 1986, it finally became a part of Cadbury.

Dr. John Pemberton was a pharmacist and he mixed up a combination of lime, cinnamon, coca leaves, and cola to make the flavor for his famous drink, first as a remedy against headache (Pemberton French Wine Coca) and then reformulated according to the prohibition law and used it to add taste to soda water from his "soda fountain." The unique name and logo was created by his bookkeeper Frank Robinson and Coca-Cola was advertised as a delicious, exhilarating, refreshing and invigorating temperance drink. Interestingly, the first year of sales resulted in $20 loss, as the cost of the flavor syrup used for the drink was higher than the total sales of $50. In 1887, another pharmacist, Asa Candler, bought the idea and with aggressive marketing in 10 years introduced his drink all over the United States and Canada by selling syrup to other companies licensed to manufacture and retail the drink. Until 1905, Coca-Cola was known as a tonic drink and contained the extract of cocaine and cola nuts and with the flavoring of lime and sugar.

Like Pemberton, Caleb Bradham was a pharmacist and in his drugstore, he offered soda water from his "soda fountain." To promote sales, he flavored the soda with sugar, vanilla, pepsin, cola, and "rare oils"—obviously the essential oils of lemon and lime—and started selling it as a cure for dyspepsia, "Brad's Drink" than Pepsi-Cola.

The development of the soft drinks industry is of great importance because it is a major consumer of essential oils, especially those of citrus origin. It is enough to say that nowadays, according to their web pages, only Coca-Cola-produced beverages are consumed worldwide in a quantity exceeding 1 billion drinks per day. If we consider that the average content of the appropriate essential oil in the final drink is about 0.001–0.002%, and the standard drink is ca. 0.31 (300 g), we approach a daily consumption of essential oils by this company alone at the level of 3–6 tons per day, which gives an annual usage well over 2000 tons. Although all other brands of the food industry use substantial quantities of essential oils in ice creams, confectionary, bakery, and a variety of fast foods (where spice oils are used), these together use less oils than the beverage manufacturers.

There is one special range of products that can be situated between the food and cosmetic–toiletries industry sectors and it is a big consumer of essential oils, especially of all kinds of mint, eucalyptus, and some other herbal and fruity oils. These are oral care products, chewing gums, and all kinds of mouth refreshing confectioneries. As mentioned above, toothpastes appeared on the market in the late nineteenth century in the the United States. Chewing gums or the custom of chewing certain plant secretions were known to the ancient Greeks (e.g., mastic tree resin) and to ancient Mayans (e.g., sapodilla tree gum). Chewing gum, as we know it now, started in America around 1850 when John B. Curtis introduced flavored chewing gum, which was first patented in 1859 by William Semple. In 1892, William Wrigley used chewing gum as a free gift with sales of baking powder in his business in Chicago and very soon he realized that chewing gum has real potential. In 1893, Juicy Fruit gum came into market and was followed in the same year by Wrigley's Spearmint; today, both products are known and consumed worldwide and their names are global trademarks.

16.5 PRODUCTION AND CONSUMPTION

This brief and certainly incomplete look into the history of industrial usage of essential oils as flavor and fragrance ingredients shows that the real industrial scale of flavor and fragrance industry

developed in the second half of the nineteenth century together with transformation of "manufacture" into "industry."

There are no reliable data on the scale of consumption of essential oils in specific products. On the basis of different sources, it can be estimated that the world market for the flavors and fragrances has a value of 10–12 billion euro, being equally shared by each group of products. It is very difficult to estimate the usage of essential oils in each of the groups. More oils are used in flavors than in fragrances which today are mainly based on aroma chemicals, especially in large volume compounds used in detergents and household products. Table 16.2 presents estimated data on world consumption of major essential oils (each used over 500 tons per annum).

TABLE 16.2
Estimated World Consumption of the Major Essential Oils

Oil Name	Consumption (tons)	Approximate Value (€ million)[a]	Major Applications[b]
Orange	50,000	275	Soft drinks, sweets, fragrances
Cornmint (*Mentha arvensis*)[c]	25,000	265	Oral care, chewing gum, confectionery, fragrances, menthol crystals
Peppermint	4500	120	Oral care, chewing gum, confectionery, liquors, tobacco, fragrances
Eucalyptus (*Eucalyptus globulus*)	4000	22	Oral care, chewing gum, confectionery, pharmaceuticals, fragrances
Lemon	3500	21	Soft drinks, sweets, diary, fragrances, household chemicals
Citronella	3000	33	Perfumery, toiletries, household chemicals
Eucalyptus (*Eucalyptus citriodora*)	2100	10	Confectionery, oral care, chewing gum, pharmaceuticals, fragrances
Clove leaf	2000	24	Condiments, sweets, pharmaceuticals, tobacco, toiletries, household chemicals
Spearmint (*Mentha spicata*)	2000	46	Oral care, chewing gum, confectionery
Cedarwood (*Virginia*)	1500	22	Perfumery, toiletries, household chemicals
Lime	1500	66	Soft drinks, sweets, diary, fragrances
Lavandin	1000	15	Perfumery, cosmetics, toiletries
Litsea cubeba	1000	20	Citral for soft drinks, fragrances
Cedarwood (China)	800	11	Perfumery, toiletries, household chemicals
Camphor	700	3	Pharmaceuticals
Coriander	700	40	Condiments, pickles, processed food, fragrances
Grapefruit	700	9	Soft drinks, fragrances
Star anise	700	7	Liquors, sweets, bakery, household chemicals
Patchouli	600	69	Perfumery, cosmetics, toiletries
Basil	500	12	Condiments, processed food, perfumery, toiletries
Mandarine	500	30	Soft drinks, sweets, liquors, perfumery, toiletries

[a] Based on average prices offered in 2007.
[b] Almost all of the major oils are used in alternative medicine.
[c] Main source of natural menthol.

The following oils are used in quantities between 100 and 500 tons per annum: bergamot, cassia, cinnamon leaf, clary sage, dill, geranium, lemon petitgrain, lemongrass, petitgrain, pine, rosemary, tea tree, and vetivert. It must be emphasized that most of the figures given above on the production volume are probably underestimates because no reliable data are available on the domestic consumption of essential oils in major producing countries, such as China, India, and Indonesia. Therefore quantities presented in various sources are sometimes very different. For example, consumption of *Mentha arvensis* is given as 5000 and 25,000 tons per annum. The lower one probably relates to the direct usage of the oil, the higher includes the oil used for the production of menthol crystals. In Table 16.2, the highest available figures are presented. Considering the above and general figures for flavors and fragrances, it can be estimated that the total value of essential oils used worldwide is somewhere between 2 and 3 billion euro. Price fluctuations (e.g., the patchouli oil price jump in mid-2007) and many other unpredictable changes cause any estimation of essential oils consumption value to be very risky and disputable. The figures given in the table are based on average trade offers. Table 16.2 does not include turpentine, which is sometimes added into essential oils data. Being used mainly as a chemical solvent or a raw material in the aroma chemicals industry, it has no practical application as an essential oil, except in some household chemicals.

As noted earlier, the largest world consumer of essential oils is the flavor industry, especially for soft drinks. However, this is limited to a few essential oils, mainly citrus (orange, lemon, grapefruit, mandarin, lime), ginger, cinnamon, clove, and peppermint. Similar oils are used in confectionery, bakery, desserts, and dairy products, although the range of oils may be wider and include some fruity products and spices. The spicy oils are widely used in numerous salted chips, which are commonly consumed along with beverages and long drinks. Also, the alcoholic beverage industry is a substantial user of essential oils; for example, anis in numerous specialties of the Mediterranean region; herbal oils in liqueurs; ginger in ginger beer; peppermint in mint liquor; and in many other flavored alcohols.

Next in importance to beverages in the food sector are the sweet, dairy, confectionery, dessert (fresh and powdered), sweet bakery, and cream manufacturing sector, for which the main oils used are citrus, cinnamon, clove, ginger, and anis. Many other oils are used in an enormous range of very different products in this category.

The fast food and processed food industries are also substantial users of essential oils, although the main demand is for spicy and herbal flavors. Important oils here are coriander (especially popular in the United States), pepper, pimento, laurel, cardamom, ginger, basil, oregano, dill, and fennel, which are added to the spices with the aim of strengthening and standardizing the flavor.

The major users of essential oils are the big compounders—companies that emerged from the historical manufacturers of essential oils and fragrances and flavors and new ones established by various deals between old players in the market or, like International Flavors and Fragrances (IFF), were created by talented managers who left their parent companies and started on their own. Today's big 10 are listed in Table 16.3.

Out of the 20 companies listed in Table 16.1, seven were located in France but by 2007, out of 10 largest, only two are from France. Also, only four of today's big 10 are over a century old with two leaders—Givaudan and Firmenich—from Switzerland and Mane and Robertet from France.

The flavor and fragrance industry is the one where the majority of oils are introduced into appropriate flavor and fragrance compositions. Created by flavorists and perfumers, an elite of professionals in the industry, the compositions, complicated mixtures of natural and nature identical ingredients for flavoring, and natural and synthetic components for fragrances, are offered to end users. The latter are the manufacturers of millions of very different products from luxurious "haute couture" perfumes, and top-class-flavored liquors and chocolate pralines through cosmetics, household chemicals, sauces, condiments, cleaning products, air fresheners, and aroma marketing.

It is important to emphasize that a very wide range of essential oils are used in alternative or "natural" medicine with aromatherapy—treatment of many ailments with the use of essential oils as bioactive ingredients—being the leading outlet for the oils and products in which they are applied as major active components. The ideas of aromatherapy from a niche area dominated by lovers of

TABLE 16.3
Leading Producers of Flavors and Fragrances

Position	Company Name (Headquarters)	Sales in Million (€)[a]
1	Givaudan S.A. (Vernier, Switzerland)	2550
2	Firmenich S.A. (Geneve, Switzerland)	1620
3	International Flavors and Fragrances (New York, USA)	1500
4	Symrise AG (Holzminden, Germany)	1160
5	Takasago International Corporation (Tokyo, Japan)	680
6	Sensient Technologies Flavors&Fragrances (Milwaukee, USA)	400
7	T. Hasegawa Co. Ltd (Tokyo, Japan)	280
8	Mane S.A. (Le Bar-sur-Loup, France)	260
9	Frutarom Industries Ltd (Haifa, Israel)	220
10	Robertet S.A. (Grasse, France)	210

[a] Estimated data based on web pages of the companies, various reports, and journals.

nature and some kind of magic, although based on very old and clinically proved experience, came into mass production appearing as an advertising "hit" in many products including global ranges. Examples include Colgate–Palmolive liquid soaps, a variety of shampoos, body lotions, creams, and so on by many other producers, and fabric softeners emphasizing the benefits to users' mood and condition from the odors of essential oils (and other fragrant ingredients) remaining on fabrics. Aromatherapy and "natural" products, where essential oils are emphasized as "the natural" ingredients, are a very fast developing segment of the industry and this is a return to what was a common practice in ancient and medieval times.

16.6 CHANGING TRENDS

Until the second half of the nineteenth century, formulas of perfumes and flavors (although much less data are available on flavoring products in history) were based on essential oils and some other naturals (musk, civet, amber, resins, pomades, tinctures, extracts, etc.). Now, some 150 years later, old formulations are being taken out of historical books and are advertised as the "back to nature" trend. Perfumery handbooks published until the early twentieth century listed essential oils, and none or only one or two aroma chemicals (or isolates from essential oils). A very good illustration of the changes that affected the formulation of perfumes in the twentieth century is a comparison of rose fragrance as recorded in perfumery handbooks. Dr Heinrich Hirzel in his *Die Toiletten Chemie* (1892, p. 384) gave the following formula for high-quality white rose perfume:

400 g of rose extract
200 g of violet extract
150 g of acacia extract
100 g of jasmine extract
120 g of iris infusion
25 g of musk tincture
5 g of rose oil
10 drops of patchouli oil.

Felix Cola's milestone work *Le Livre de Parfumeur* (1931, p. 192) recorded a white rose formula containing only 1% of rose oil, 2% of rose absolute, 7.5% other oils, and aroma chemicals.

Rose Blanche	
Rose oil	10 g
Rose absolute	20 g
Patchouli oil	25 g
Bergamot oil	50 g
Linalool	60 g
Benzyl acetate	7 g
Phenylethyl acetate	75 g
Citronellol	185 g
Geraniol	200 g
Phenylethyl alcohol	300 g

In the mid-twentieth century, perfumers were educated to consider chemicals as the most convenient, stable, and useful ingredients for fragrance compositions. Several rose fragrance formulas with less than 2% rose oil or absolute can be found in F.V. Wells and M. Billot's *Perfumery Technology*, (1975), and rose fragrance without any natural rose product is nothing curious in a contemporary perfumers' notebook. However, looking through descriptions of new fragrances launched in the last few years, one can observe a very strong tendency to emphasize the presence of natural ingredients—oils, resinoids, and absolutes—in the fragrant mixture. The "back to nature" trend creates another area for essential oils usage in many products.

A very fast growing group of cosmetics and related products today are the so-called organic products. These are based on plant ingredients obtained from wild harvesting or from "organic cultivation" and which are free of pesticides, herbicides, synthetic fertilizers, and other chemicals widely used in agriculture. According to different sources, sales of "organic" products in 2007 will reach 4–5 billion U.S. dollars. The same "organic raw materials" are becoming more and more popular in the food industry, which in consequence will increase the consumption of "organic flavors" based on "organic essential oils." "Organic" certificates, available in many countries (in principle for agricultural products, although they are institutions that also certify cosmetics and related products), are product passports to a higher price level and selective shops or departments in supermarkets. The importance of that segment of essential oils consumption can be illustrated by comparison of the average prices for standard essential oils as listed in Table 16.4 and the same oils claimed as "organic."

The consumption of essential oils in perfumed products varies according to the product (Table 16.5): from a very high level in perfumes (due to the high concentration of fragrance compounds in perfumes and the high content of natural ingredients in perfume fragrances) and in a wide range of "natural" cosmetics and toiletries to relatively low levels in detergents and household chemicals, in which fragrances are based on readily available low-priced aroma chemicals. However, it must be emphasized that although the concentration of essential oils in detergents and related products is low, the large volume sales of these consumer products result in substantial consumption of the oils.

Average values given for fragrance dosage in products and for the content of oils in fragrances are based on literature data and private communications from the manufacturers. It should be noted that in many cases the actual figures for individual products can be significantly different. "Eau Savage" from Dior is a very good example: analytical data indicate a content of essential oils (mainly bergamot) of over 70%. Toothpastes are exceptional in that the content of essential oils in the flavor is in some cases nearly 100% (mainly peppermint, spearmint cooled with natural menthol).

While the average dosage of fragrances in the final product can be very high, flavors in food products are used in very low dosages, well below 1%. The high consumption of essential oils by this sector results from the large volume of sales of flavored foods. Average dosages of flavors and the content of essential oils in the flavors are given in Table 16.6.

As in the case of fragrances, the average figures given in Table 16.6 vary in practice in individual cases, both in the flavor content in the product and much more in the essential oils

TABLE 16.4
Prices of Selected Standard and "Organic" Essential Oils

Oil Name	Standard Quality (€/kg)[a]	Organic Quality (€/kg)[a]
Orange	5.50	35
Cornmint (*M. arvensis*)	10.50	50
Peppermint	27.00	100
Eucalyptus (*E. globulus*)	5.50	26
Lemon	6.00	30
Citronella	11.00	23
Eucalyptus (*E. citriodora*)	5.00	34
Clove leaf	12.00	60
Spearmint (*M. spicata*)	23.00	40
Cedarwood (*Virginia*)	15.00	58
Lime	44.00	92
Lavandin	15.00	36
Litsea cubeba	20.00	44
Cedarwood (China)	14.00	53
Camphor	4.50	24
Coriander	57.00	143
Grapefruit	13.00	170
Patchouli	115.00	250

[a] Average prices based on commercial offers in 2007.

TABLE 16.5
Average Dosage of Fragrances in Consumer Products and Content of Essential Oils in Fragrance Compounds

Position	Product	Average Dosage of Fragrance Compound in Product (%)	Average Content of Essential Oils in Fragrance (%)
1	Perfumes	10.0–25.0	5–30[a]
2	Toilet waters	3.0–8.0	5–50[a]
3	Skin care cosmetics	0.1–0.6	0–10
4	Deodorants (inclusive deoparfum)	0.5–5.0	0–10
5	Shampoos	0.3–2.0	0–5
6	Body cleansing products (liquid soaps)	0.5–3.0	0–5
7	Bath preparations	0.5–6.0	0–10
8	Soaps	0.5–3.0	0–5
9	Toothpastes	0.5–2.5	10–50[b]
10	Air fresheners	0.5–30.0	0–20
11	Washing powders and liquids	0.1–0.5	0–5
12	Fabric softeners	0.1–0.5	0–10
13	Home care chemicals	0.5–5.0	0–5
14	Technical products	0.1–0.5	0–5
15	Aromatherapy and organic products	0.1–0.5	100

[a] Traditional perfumery products contained more natural oils than modern ones.
[b] Mainly mint oils.

TABLE 16.6
Average Content of Flavors in Food Products and of Essential Oils in Flavors

Position	Food Products	Flavor Dosage in Food Product (%)	Essential Oils Content in Flavor (%)
1	Alcoholic beverages	0.05–0.15	3–100
2	Soft drinks	0.10–0.15	2–5
3	Sweets (confectionery, chocolate, etc.)	0.15–0.25	1–100
4	Bakery (cakes, biscuits, etc.)	0.10–0.25	1–50
5	Ice creams	0.10–0.30	2–100
6	Diary products, desserts	0.05–0.25	1–50
7	Meat and fish products (also canned)	0.10–0.25	10–20
8	Sauces, ketchup, condiments	0.10–0.50	2–10
9	Food concentrates	0.10–0.50	1–25
10	Snacks	0.10–0.15	2–20

percentage in the flavor. Again "natural" or "organic" products contain only essential oils, since it is unacceptable to include any synthetic aroma chemicals or so-called nature identical food flavors. It should be noted that a substantial number of flavorings are oleoresins: products that are a combination of essential oils and other plant-derived ingredients, which are especially common in hot spices (pepper, chili, pimento, etc.) containing organoleptically important pungent components that do not distill in steam. This group of oleoresin products must be included in the total consumption of essential oils.

For many years after World War II, aroma chemicals were considered the future for fragrance chemistry and there was strong, if unsuccessful, pressure by the manufacturers to get approval for the wide introduction of synthetics (especially those regarded as "nature identical") in food flavors. The very fast development of production and usage of aroma chemicals caused increasing concern over safety issues for the human health and for the environment. One by one certain products were found harmful either for human health (e.g., nitro musks) or for nature. This resulted in wide research on the safety of the chemicals and the development of new safe synthetics. Concurrently, the attention of perfumers and producers turned in the direction of essential oils, which as derived from natural sources and known and used for centuries were generally considered safe. According to recent research, however, this belief is not entirely true and some, fortunately very few, oils and other fragrance products obtained from plants have been found dangerous, and their use has been banned or restricted. However, these are exceptional cases and the majority of essential oils are found safe both for use on the human body as cosmetics and related products as well as for consumption as food ingredients.

It is important to appreciate that the market for "natural," "organic," and "ecological" products both in body care and food industries has changed from a niche area to a boom in recent years with the growth exceeding 30% per annum. The estimated value of sales for "organic" cosmetics and toiletries is 600–800 million euro in Europe, the United States, and Japan and will grow steadily together with organic foods. This creates a very sound future for the essential oils industry, which as such or as isolates derived from the oils will be widely used for fragrance compounds in cosmetic and related products as well as for flavors.

Furthermore, the modernization of agricultural techniques and the growth of plantation areas result in better economical factors for the production of essential oil-bearing plants, creating workplaces in developing countries of Southeast Asia, Africa, and South America as well as further development of modern farms in the United States and Europe (Mediterranean area, Balkans). Despite some regulatory restrictions (EU, REACH, FDA, etc.), essential oils are and will have an

important and growing share in the fragrance and flavor industry. The same will be true for the usage of essential oils and other products of medicinal plants in pharmaceutical products. It is well known that the big pharmaceutical companies invest substantial resources in studies of folk and traditional medicine as well as in research on biologically active constituents of plant origin. Both of these areas cover applications of essential oils. The same is observed in cosmetic and toiletries using essential oils as active healing ingredients.

16.7 CONCLUSIONS

It can be concluded that the industrial use of essential oils is a very promising area and that regular growth shall be observed in future. Much research work will be undertaken both on the safety of existing products and on development of new oil-bearing plants that are used locally in different regions of the world both as healing agents and as food flavorings. Both directions are equally important. Global exchange of tastes and customs shall not lead to unification by Coca-Cola or McDonalds. With all the positive aspects of these products, there are many local specialties that can become world property, like basil-oregano-flavored pizza, curry dishes, spicy kebab, or the universal and always fashionable Eau de Cologne. With the growth of the usage of the commonly known essential oils, new ones coming from exotic flowers of the Amazon jungle or from Indian Ayurveda books can add new benefits to the flavor and fragrance industry.

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17 Encapsulation and Other Programmed Release Techniques for Essential Oils and Volatile Terpenes

Jan Karlsen

CONTENTS

17.1 INTRODUCTION

In order to widen the applications of volatiles (essential oils), it is necessary to lower the volatility of the compounds to obtain a longer shelf life of products made with these compounds. By lowering the volatility, one can also imagine a possibility to better test the biological effects of these compounds. The encapsulation processes are means by which a liquid essential oil is enclosed in a carrier matrix to provide a dry, free-flowing powder. However, for the prolonged effect of volatile compounds many other techniques are used, where methods are copied from other fields of research when one wants to control the release of active ingredients.

To lower the volatility, one needs to encapsulate the volatiles into a polymer matrix, utilize complex formation, use the covalent bonding to a matrix—to mention a few techniques. We therefore need to formulate the volatiles and take many of these techniques from areas where controlled release formulations have already been in use for many years. The area with the maximum number of applications of controlled release formulations is the field of drug delivery. In this area of research, there exists a large number of publications as well as a large number of patents where one can find inspiration for the formulation of programmed release of volatiles (Deasy, 1984).

So far in the area of volatile terpenes/essential oils, we have seen a large number of investigations that focused on plant selection, volatiles isolation techniques, separation of the volatiles isolated, identification of isolated compounds, and the biochemical formation of terpenes. The formulation

of volatiles into products has been seen as an area of industrial research. This has naturally led to a large number of patents but very few scientific publications on the formulation of essential oils and lower terpenes.

The idea of this chapter is to give an introduction to the area of making a controlled release product of volatiles and, in particular, of essential oils and their constituents.

17.2 CONTROLLED RELEASE OF VOLATILES

The main interest of volatiles encapsulation is the possibility to extend the biological effect of the compounds. For essential oils, we want to prolong the activity by lowering the evaporation of the volatile compounds. During the last 10 years, there are not many publications on this topic in the scientific literature but there are quite a number of patents that describe the various ways of prolonging the effect of volatiles (Porzio, 2008; Sair and Sair, 1980a; Sair, 1980b; Zasypkin and Porzio, 2004; Fulger and Popplewell, 1997, 1998; McIver, 2005). One reason for this fact is that the prolonging of the effect of volatile compounds is regarded as so close to practical applications and therefore the area of industrial research where the results will be bonded in patent applications. However, there are signs that this idea is changing. In order to lower the volatility, thus prolonging the effect of essential oils and terpenes, we have to look into another area of scientific research. In the field of drug delivery, many techniques have been studied for the controlled delivery of active molecules.

The reasons for controlled release (encapsulation of volatiles) may be the following:

Changing the impact of fragrance and flavors
Adding fragrance to textiles
Stabilizing specific compounds
Tailoring the fragrance to the intended use of a product
Lowering the volatility and thereby prolonging the shelf life of a fragrance product.

The slow or controlled release of volatiles is achieved by:

Encapsulation
Solution/dispersion in a polymer matrix
Complex formation
Covalent bonding to another molecule or matrix.

For essential oils and lower terpenes, the following techniques can be utilized depending on the volatiles and the intended use of the final product:

Microcapsule production
Microparticle production
Melt extrusion
Melt injection
Complex formation
Liposomes
Micelles
Covalent bonding to a matrix
Combination of nanocapsules into larger microcapsules.

Since the making of one of the above-mentioned type of products and techniques will influence the activity toward the human biological membranes in one way or the other. Therefore, the relevant sizes of biological units are listed in Table 17.1 and the average sizes of units produced in consumer products, where volatile compounds are involved are listed in Table 17.2.

TABLE 17.1
Size Diameters of Biological Entities

Human blood cells	7000–8000 nm
Bacteria	800–2000 nm
Human cell nucleus	1000 nm
Nanoparticles that can cross biomembranes	60 nm
Virus	17–300 nm
Hemoglobin molecule	3.3 nm
Nanoparticle that can cross blood–brain barrier	4 nm
DNA helix	3 nm
Water molecule	0.3 nm

TABLE 17.2
Average Size of Formulation Units in nm (Sizes below 150 nm may be Invisible to the Naked Eye)

Solutions	0.1
Micellar solutions	0.5
Macromolecular solutions	0.5
Microemulsions	5–20
Liposomes SUV	20–150
Nanospheres	100–500
Nanocapsules	100–500
Liposomes LUV	200–500
Liposomes MLV	200–1000
Microcapsules	5000–30,000
Simple emulsions	500–5000
Multiple emulsions	10,000–100,000

Abbreviations: SUV is small unilamellar vesicles, LUV is large unilamellar vesicles, and MUV is multilamellar vesicles.

Since the introduction of the encapsulation of volatiles (essential oils/lower terpenes), the number of applications has multiplied. Encapsulation of volatiles gives us a more predictable and long-lasting effect of the products. The areas of applications are large and the industry of essential oils and terpenes foresee many prospects for microencapsulated products.

Application markets of encapsulated essential oils and terpenes are:

Medicine
Food, household items, and personal care
Biotechnology
Pharmaceuticals
Electronics
Photography
Chemical industry
Textile industry
Cosmetics.

It is therefore easy to understand that the encapsulation procedures will open up a much larger market for essential oil/terpene products. Experience from all the areas mentioned above can be applied to the study of volatile compounds in products.

In the area of essential oils and lower terpenes, simple encapsulation procedures from the area of drug delivery are applied. The essential oils or single active constituents are mixed with a hydrophilic polymer and spray-dried using a commercial spray-drier. Depending on whether we have an emulsion or a solution of the volatile fraction in the polymer, we obtain monolithic particles or a normal microcapsule.

The most usual polymers used for encapsulation are:

Oligosaccharides from α-amylase
Modified starches from maize, cassava, rice, and potato
Acacia gum
Gum arabic
Alginate
Chitosan.

Many different emulsifiers are used to solubilize the essential oils totally or partly, prior to the encapsulation procedure. This can result in a monolithic particle or a usual capsule, where the essential oil is surrounded by a hydrophilic coating. When the mixture of an essential oil and a hydrophilic polymer is achieved, the application of a spray-drying procedure of the resulting mixture will result in the formation of microcapsules. The techniques for achieving an encapsulated product in high efficiency will depend on many technical parameters and can be found in the patent literature. Normally a mixture of essential oil:hydrophilic polymer (4:1) can be used, but this will also depend on the type of equipment used. The reader is advised to refer to the parameters given for the polymer used in the experiment. To achieve the encapsulated product, a mixture of low pressure and temperature is used in the spray-drying equipment and a loss of essential oil/volatiles is inevitable. However, a recovery of more than 70% can be achieved by carefully monitoring the production conditions.

17.3 USE OF HYDROPHILIC POLYMERS

In product development, one tends to use cheap derivatives of starches or other low-grade quality polymers. Early studies with protein-based polymers such as gelatine, gelatine derivatives, soy proteins, and milk-derived proteins gave reasonable technical quality of the products. However, even if these materials show stable emulsification properties with essential oils, they have some unwanted side effects in products. We have seen that a more careful control of the polymer used can result in real high-tech products, where the predictability of the release of the volatiles can be assured like a programmed release of drug molecules in drug delivery devices. The polymer quality to be used will, of course, depend on the intention of the final product. In the cosmetic industry, where one is looking for an essential oil product, free-flowing and dry, to mix with a semisolid or a solid matrix, the use of simple starch derivatives will be very good. For other applications, where the release of the volatiles needs to be controlled or predicted more accurately, it is recommended that a more thorough selection of a well-characterized polymer is done. One example of a very good and controllable polymer is alginate. This polymer is available in many qualities and can be tailored to any controlled release product. The chemistry of alginate is briefly discussed below as this discussion will allow the reader to decide whether to opt for an alginate of technical quality or, if a high-tech product is the aim, to choose a better characterized hydrocolloid.

17.4 ALGINATE

Alginates are naturally occurring polycarbohydrates consisting of the monomers α-L-glucuronic acid (G) and β-D-mannuronic acid (M). The relative amounts of these two building blocks will influence the total chemistry of the polymer. The linear polymer is water soluble due to its polarity. Today the alginate can be produced by the bacteria that allow us to control the composition of the monomers (G/M ratio). The chemical composition of the alginate is dependent on the origin of the raw material. The marine species display seasonal differences in the composition and different parts of the plant produce different alginates. Alginates may undergo epimerization to obtain the preferred chemical composition. This composition (G/M ratio) will determine the diffusion rate through the swollen alginate gel, which surrounds the encapsulated essential oils (Elias, 1997; Amsden, 1998a, 1998b; Ogston, 1973). An important structure parameter is also the distribution of the carboxyl groups along the polymer chain. The molecular weight of the polymer is equally important, and molecular weights between 12,000 and 250,000 are readily available in the market. The alginate polymer can form a swollen gel by hydrophobic interaction or by cross-linking with divalent ions like calcium. The G/M ratio determines the swelling rate and therefore also the release of encapsulated compounds. The diffusion of different substances has been studied and references can be made to essential oil encapsulation. The size of alginate capsules can also vary from 100 μm or more down to the nanometer range depending on the production procedure chosen (Draget et al., 1994, 1997; Donati et al., 2005; Tønnesen and Karlsen, 2002; Shilpa, 2003).

17.5 STABILIZATION OF ESSENTIAL OIL CONSTITUENTS

The encapsulation of essential oils in a hydrophilic polymer may stabilize the constituents of the oil but a better technique for this purpose will often be to use cyclodextrins in the encapsulation process. The use of cyclodextrins will lead to a complexation of the single compounds, which will again stabilize the complexed molecule. Complex formation with cyclodextrins is often used in drug delivery to promote solubility of lipophilic compounds; however, in the case of volatiles containing compounds that may oxidize, the complex formation will definitely prolong the shelf life of the finished product. A good review of the flavor encapsulation advantages is given by Risch and Reineccius (1988). The most important aspect of essential oil encapsulation in a hydrophilic polymer is that the volatility is lowered. Lowering the volatility will result in longer shelf life of products and a better stability of the finished product in this respect.

17.6 CONTROLLED RELEASE OF VOLATILES FROM NONVOLATILE PRECURSORS

The limited effect of volatiles for olfactive perception has led to the development of encapsulated volatiles and also to the development of covalent-bonded fragrance molecules to matrices. In this way, molecules release their fragrance components by the cleavage of the covalent bond. Mild reaction conditions met in practical life initiated by light, pH, hydrolysis, temperature, oxygen, and enzymes may release the flavors. The production of "profragrances" is a very active field for the industry and has led to numerous patents. The plants producing essential oils have invented means by which the volatiles are produced, stored, and released into the atmosphere at predestined times related to the environmental factors. The making of a profragrance involves mimicking these natural procedures into flavor products. However, we are simplifying the process by using only one parameter in this release process, that is, the splitting of a covalent bond. In theory, the making of a long-lasting biological impact and the breakdown of a constituent are contradictory reactions. However, in practice the use of a covalent bond and thereafter the control of the splitting of this bond by parameters such as light, humidity, temperature, and so on can be built into suitable flavor

and fragrance products. Naturally, this technique using covalent bonding is only applicable to single essential oil constituents but constitutes a follow-up of essential oil encapsulation (Herrmann, 2004, 2007; Powell, 2006).

17.7 CYCLODEXTRIN COMPLEXATION OF VOLATILES

Cyclodextrin molecules are modified carbohydrates that have been used for many years to modify the solubility properties of drug molecules by complexation. The cyclodextrin can also be applied to volatiles to protect them against the environmental hazards and thus prolong the shelf life of these compounds. Cyclodextrin complexation will also modify the volatility of the essential oils and prolong the bioactivity. The cyclodextrins will give a molecular encapsulation by the complexation reaction with volatile molecules. The complexation of the volatiles with cyclodextrin may improve the heat stability, improve the stability toward oxygen, and improve the stability against light (Szente, 1988). A significant lowering of the volatility has been observed for the complexation with essential oils (Risch and Reineccius, 1988). The complexation of essential oils by the use of cyclodextrins will also result in increased heat stability. This is in contrast with the stability of volatiles that have been adsorbed on a polymer matrix. The use of cyclodextrins can protect the volatiles against

Loss of volatiles upon storage of a finished product
Light-induced instability
Heat decomposition
Production of free-flowing "dry" powders
Oxidation.

17.8 CONCLUDING REMARKS

The encapsulation/complexation of essential oils, volatiles, or single oil constituents will result in a remarkable lowering of the volatility, stabilize the constituents, improve the shelf life of finished products, and prolong the biological activity. The control of these parameters will depend on the nature of the volatiles to be encapsulated. Most of the literature on the encapsulation of volatiles is found in the patent literature. Techniques described in the literature allow the user of essential oils to choose the polymer matrix in which to encapsulate an essential oil according to the use of the finished product. The effect of controlled delivery of flavor and fragrance molecules opens up large areas of applications, which previously were limited due to the volatility of the essential oils and their constituents. The encapsulation or lowering the volatility of compounds like essential oil ingredients will allow for more relevant studies of the biological effects of volatile compounds.

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18 Aroma-Vital Cuisine
Healthy and Delightful Consumption by the Use of Essential Oils

Maria M. Kettenring and Lara-M. Vucemilovic-Geeganage

CONTENTS

> Your nourishment ought to be your remedies and your medicaments shall be your food.
>
> **—Hippocrates**

Certainly, the value of our nutrition, in terms of nutritional physiology, is not only conditioned by its nutrient and calorie contents. Moreover, also health-conscious and constitutional eating habits require an adequate preparation of meals as well as an appropriate form of presentation. Early sophisticated civilizations and their health doctrines, like that of the Traditional Chinese Medicine (TCM), Ayurveda in Southeast Asia, and for instance the medical schools during the ancient Greek period examined individuals and their reaction on life circumstances, habits, nutrition, and substances, to contribute to a long-lasting health. To support a person's balance the aim was to develop a conscious way of using the senses and a balanced sensory perception.

Thus fragrances are a kind of soul food, as the information of scents can be perceived in every section of our self, physical, energetic as well as intellectual, from a holistic point of view. Adding spice with essential oils according to the Aroma-Vital cuisine combines sensuality with sanative potential.

People across continents and cultures have experimented with the healing virtues of "nature's bouquet" or just simply tried to enhance the flavor and vitality of their meals. The ancient Egyptian civilization reverted to an elaborated dinner ceremony by using the efficacy of essential oils to get

the participants in the mood for the meal. Before the food was served, heated chalices with scented fats, enriched with a variety of herbs and spices were provided, not only to spread pleasant smells, rather as a kind of odorous aperitif to activate ones saliva to prepare for digestion. Meals that have been enriched with essential oils or expressed oils, rebound to a conscious awareness of consuming food, are well-nigh comparable, like going on a culinary expedition. This fare is perceived as a composition of tastes, which is not only tastefully ingenious, but also might be able to raise the food's virtue.

In this regard the entropy rather than the potency of the condiment is significant. The abundance of nuances, the art of adding flavor on the cusp of being noticeable, becomes more important than giving aroma officiously. The scents hovering above the meals, almost like a slight breeze, compound the food's own natural flavor in a subtle manner. "Less is more" is the economic approach which in this context is indicative.

The sensation of satiety is taking place early on. Due to this desire to savor to the fullest, the taste is excited and leads to longer chewing. This in turn activates α-amylase (amylolytic enzyme, already working in the oral cavity). Conditionally on the high bioavailability, especially of the monoterpenes, which are significant and available in the paring of citrus fruits and some kind of herbs, in a sense the Aroma-Vital cuisine shows aspects of the salutary genesis (Salutogenese). The savoriness of the food, pleasant smell, and appetizing appearance plays a prominent role here, at last the appetite regulates between physiological needs and pleasure and thus variety and vitally enhanced meals are in demand.

18.1 BASIC PRINCIPLES OF THE AROMA-VITAL CUISINE

18.1.1 The Heart of Culinary Arts is Based on Exquisite Ingredients and an Accomplished Rounding

Natural aromas, from blossoms, herbs, seeds, and spices, extracted in artificial pure essential oils, delicately accompany the elaborate cuisine. They are not supposed to supersede fresh herbs, rather complementing them. If, however, herbs are not available, natural essences are delightfully suited to add nuances. They are giving impetus to and are flexible assistants for preparing last-minute menus. One should use this rich source to compile a first-aid assortment of condiments or even a mobile spice rack.

18.1.2 Quality Criteria and Specifics that have to be Adhered to, while Handling Essential Oils for Food Preparation

The regional legal regulations of the food chemical codex or the local food legislation might differ and if one is going to use essential oils professionally, one has to be firm with them, but still there are certain basics that deserve attention and lead to a safe and healthy way of practicing this subtle culinary art.

For cooking, solely 100% pure essential oils from controlled organic cultivation should be used. Oils that are not available of controlled organic origin, particularly those that are cold-pressed, a residue check should be guaranteed by the manufacturer to ensure that the product does not contain harmful amounts of pesticides. The label should not only contain name, contents, and quantity but also

- Latin definition
- Country of origin
- Description of used plant parts
- Used method of extraction

- Date of expiry
- If the oil has been thinned, the exact ratio of mixture
- If solvents have been used, they should be mentioned.

For the Aroma-Vital cuisine, the only acceptable solvent would be alcohol. As the oil is used in very small and thinned concentrations it would not be harmful to children. Less qualitative oils from industrial origin sometimes might even contain other substances. It should be indicated that natural flavorings used in food production should be pure and free of animal by-products such as gelatin or glycerin, which has been obtained by saponification of animal fat.

18.1.3 Storage

Essential oils are very sensible to the disposure of light, air and temperature; therefore they should be stored adequately. In this way, long-lasting essential oils keep their aroma as well as their ingredients and might even develop their bouquet. Foods or processed foods with essential oils may not be stored in tin boxes. Very important: essential oils should be kept away from children.

18.1.4 Quantity

The internal use of essential oils has to be practiced carefully. This subtle art is an amazing tool, but swallowed in too huge amounts, they are bad for one's health. One should never add the pure concentrate of essential oils to foods; it should not be forgotten that 1 drop is often comparable to a huge amount of plant material. Therefore, they ought to be always thinned and the dilution should be used teaspoon by teaspoon.

18.1.5 Emulsifiers and Forms of Administering

Essential oils are not water soluble; therefore, emulsifiers are necessary to spread their aroma, they are for example

1. Basic oils, special oils, or macerated oils
2. Butter, milk, curd, egg yolk, and mayonnaise
3. Alcohol and vinegar
4. Syrups, molasses, honeys, treacles, and sugars
5. Salt
6. Tofu, soy sauce and tamarind sauce
7. Avocado, lemon juice, and coconut
8. Sesame seeds, sunflower seeds, almonds, and walnuts.

On the basis of these emulsifiers and a mixture of essential oils, a variety of "culinary assistants" can be conjured up: spiced oils, spiced butter or mayonnaises, spiced alcohols, spiced syrups, spiced sauces, or even spiced salts. These blends can be prepared in advance and stored to use them for everyday meals. Another nice variation is the use of hydrolates (a partial extract of plant material extracted by distillation) such as rose water, for food preparation.

18.1.6 To Add Spice with Natural Aromas in a Balanced Way

To know how food and essential oils interact is a great help to create a harmonic assembly of foods, which is nourishing us from a holistic point of view. In this manner, the sun-pervaded seed oils of anise, bay, dill, fennel, or caraway might be able to aerate the earthy corm- and root-vegetable. Salads can be enhanced and prepared to be more digestive by adding pure natural essential oils such as thyme, rosemary, and clementine to the marinade, or another rather Asian variation would be to add ginger, pepper, and lemon grass.

18.1.7 Essential Oils are able to Lift Our Spirits as well

A condiment ensemble of orange, vanilla extract, cacao extract, and rose for example, is able to support soul foods such as milk rice, milk shakes, and desserts in their attitude to supply security and confidence.

18.2 A SMALL CULINARY TRIP: AROMA-VITAL CUISINE RECIPES AND INTRODUCTION

TABLE 18.1
Basic Spice Rack of Essential Oils: How to Prepare Essential Oil Mixtures and Essential Oil Seasonings

Basic Essential Oils	Mixtures	Emulsifier Seasonings		Recipes Example
		EURO ASIA		
Lime (*Citrus aurantiifolia*)	5 drops	1. Oil	50 mL sesame oil	Asian style
		2. Dairy prod.	50 mL mayonnaise	Eggs
Coriander seed (*coriandrum sativum*)	1 drop	3. Vinegar	50 mL rice vinegar	Sushi
		4. Sweetener	50 mL agave syrup	Chutney
Ginger (*Zingiber officinalis*)	2 drops	5. Salt	50 mg sea-salt	Spice
		6. Tofu and co	50 mL soy sauce	Marinated fried tofu
Lemongras (*Cymbopogon citratus*)	1 drop	7. Vegetables and fruits	50 mL coconut milk	Rice and curry
		8. Nuts and seeds	50 mg sesame seeds	Spice
Green pepper (*Piper nigrum*)	1 drop			
		O SOLE MIO		
		1.	50 mL olive oil	Pasta
Thyme linalool (*Thymus vulgaris*)	1 drop	2.	50 mL egg yolk	Omelette
		3.	50 mL balmy vinegar	Salad
Rosemary cineole (*Rosmarinus officinalis*)	1/2 drop	4.	50 mL honey	Cuisine Provencal
		5.	50 mg sea-salt	Spice
Clementine (*Citrus deliciosa*)	5 drops	6.	50 mg tofu	Grilled tofu
		7.	50 mg avocado	Guacamole
		8.		Pesto
		CAPRI		
Orange (*Citrus sinensis*)	5 drops	1.	50 mL hazelnut oil	Desserts
		2.	50 mL buttermilk	Drink
Lemon (*Citrus limon*)	3 drops	3.	50 mL cider vinegar	Salad
		4.	100 mL maple syrup	Desserts
		5.	50 mg sea-salt	Spice
		6.	50 mL apple vinegar	Fruit salad
		7.	50 mg avocado	Sauce
		8.	50 mg walnuts	Cakes
		BERGAMOT-GRAND MANIER		
Grapefruit (*Citrus paradisi*)	5 drops	1.	50 mL walnut oil	Salad
		2.	50 mg butter	Cake

continued

TABLE 18.1 (continued)
Basic Spice Rack of Essential Oils: How to Prepare Essential Oil Mixtures and Essential Oil Seasonings

Basic Essential Oils	Mixtures	Emulsifier Seasonings		Recipes Example
Orange (*Citrus sinensis*)	5 drops	3.	1 L white vine	Beverage
		4.	50 mg raw sugar	Sweets
Limon (*Citrus limon*)	2 drops	5.	50 mg sea-salt	Spice
		6.	50 mL tamarind sauce	Thai cuisine
Bergamot (*Citrus bergamia*)	2 drops	7.	50 mL lemon juice	Drink
		8.	50 mg pumpkin seeds	Soup
		MAGIC ORANGE		
Orange (*Citrus sinensis*)	5 drops	1.	50 mL almond oil	Sweets
		2.	50 mg goat cheese	Oriental
Vanillaextract (*Vanilla planifolia*)	3 drops	3.	50 mL raspberry vinegar or balsamic vinegar	Fruit salad
		4.	100 mL honey/treacle	Sweets
Kakaoextract (*Theobroma cacao*)	3 drops	5.	—	
		6.	50 mL seitan tofu	Oriental
Rose (*Rosa damascena*)	1/2 drop	7.	50 mg bananas	Desserts
		8.	50 mg almonds	Spice
		CLARY SAGE AND BERGAMOT		
Clary sage (*Salvia sclarea*)	2 drops			Spice
Bergamot (*Citrus bergamia*)	5 drops	5.	50 g sea-salt	
		PEPPERMINT		
Peppermint (*Mentha piperita*)	Rather less—2 drops per 100 mL/mg	4.	100 mL maple syrup	Drink
		LAVENDER		
Lavender (*Lavandula officinalis*)	Rather less—2 drops per 100 mL/mg	4.	100 mL honey	Cuisine Provencal

MENU

Basics

Crispy Coconut Flakes (Flexible Asian Spice Variation)
Gomasio (Sesame Sea-Salt Spice)
Honey Provencal

Beverages

Aroma Shake with Herbs
Earl Grey at His Best
Lara's Jamu
Rose-Cider
Syrup Mint-Orange

Entrees

a. Soups:
   Peppermint Heaven
   Perky Pumpkin Soup
b. Salads:
   Melon-Plum Purple Radish Salad
   Salad with Goat Cheese and Ricotta

Appetizer and Finger Food

Crudities—Flavored Crispy Raw Vegetables
Maria's Dip
Tapenade
Tofu Aromanaise
Vegetable Skewer

Main Course

Celery—Lemon Grass Patties
Chèvre Chaude-Goat Cheese "Provence" with Pineapple
Crispy Wild Rice-Chapatis
Mango–Dates–Orange Chutney
Prawns Bergamot

Dessert, Cakes, and Baked Goods

Apple Cake Rose
Chocolate Fruits and Leaves
Homemade Fresh Berry Jelly
Rose Semifreddo
Sweet Florentines
(Chocolate should not be heated up more than 40°. Essential oils are best at 40° as well.)

AROMA-VITAL CUISINE RECIPES

Basics

Crispy Coconut Flakes (Flexible Asian Spice Variation)

Nice with Asian flavored dishes or sweet baked goods.
Ingredients:

- 50 g dried coconut flakes
- 10 drops EURO ASIA intermixture (spicy variation) or 10 drops MAGIC ORANGE intermixture (sweet variation)
- 1 preserving jar.

Preparation: Roast the coconut flakes in a frying pan. Lightly scatter the chosen essential oils into the empty jar. Spread the oil well, then fill in the roasted coconut rasps and shake it well.

Gomasio (A Sesame Sea-Salt Spice)

Gomasio is a secret of the Middle Eastern cuisine, which completes your spice rack and gives a subtle salty flavor to the dish. Nice to combine with soy sauce, fresh thyme leaves, or cumin.

Ingredients:

- 50 g sesame seeds
- 1 teaspoon EURO ASIA seasoning salt no. 5
- 1 preserving jar.

Preparation: Roast the sesame seeds in a frying pan, then mix the seeds with the salt in a mortar. Crush them lightly with a pestle to release the flavor. Fill into a preserving jar and shake it well. If necessary add a few more drops of EURO ASIA intermixture.

Honey Provencal

A great basic for the cuisine Provencal.
Ingredients:

- 100 ml acacia honey
- 5 drops O SOLE MIO intermixture
- 2 drops LAVENDER pure essential oil
- 1 drop EURO ASIA intermixture
- 1 drop CLARY SAGE AND BERGAMOT intermixture.

Preparation: Emulsify the ingredients well. Use the honey to brush grilled vegetables, tofu, goat and sheep cheese, or to season gratins, to add a fabulous distinctly French flavor to a simple dish.

Beverages

Aroma Shake with Herbs

This green fruity flavored cleansing juice certainly is a great rejuvenator.
Ingredients:

- 500 mL organic buttermilk
- 100 mL organic soy milk
- 5 tablespoons sprouts (alfalfa, adzuki bean sprouts, and cress)
- 100 mL carrot juice
- 3 drops CAPRI intermixture
- 2 drops EURO ASIA intermixture
- 1 tablespoon maple syrup
- 1 tablespoon parsley finely chopped.

Preparation: Pour the buttermilk and soy milk in a blender and process for a few minutes until combined. Add the carrot juice, then emulsify the essential oils with the maple syrup and stir it into the mixture. Fill into iced tall glasses and serve chilled. A decorative idea is to dive the top of the glasses into lemon juice and then into the finely chopped parsley, before filling in the shake.

Earl Grey at His Best

Ingredients:

- 1 preserving jar (100 g capacity)
- 100 g Darjeeling tea “first flush”
- 10 drops BERGAMOT basic essential oil.

Preparation: Lightly scatter the "BERGAMOT" basic essential oil into the empty jar. Add the tea, close the jar and shake it well. Repeat the procedure to shake the jug for the next 5–10 days; then this incredible sort of flavored tea will be ready to serve.

Lara's Jamu

Jamu is a kind of herbal tonic from Southeast Asia. Every country and family has their own recipes. This one is a tasty booster for the immune system.
Ingredients:

- The rind of two limes in thin shreds
- Juice of two limes
- 2 tablespoons freshly grated ginger
- 1 handful fresh or dried nettle
- 50 ml maple treacle
- 2 teaspoons curcuma powder
- 500 ml water
- 750 ml of sparkling water (optional)
- 5 drops EURO ASIA intermixture
- 2 drops PEPPERMINT basic essential oil
- 3 drops CAPRI intermixture.

Courtesy of Subash J. Geeganage.

Preparation: Boil the mixture of lime, ginger, and nettle with 500 ml water for 10 min; then let it cool down a bit to be able to sieve it later into decorative chalices. Mix the curcuma powder with fresh lime juice and the EURO ASIA basic essential oil and stir it into the herbal mixture. Now the maple treacle mixed with PEPPERMINT basic essential oil will be stirred in as a sweetener. Serve hot or chilled with sparkling water, fresh mint sprigs, and sliced lime.

Rose-Cider

Refreshing and inspiring.
Ingredients:

- 1 L cider
- 1/2 drop rose basic essential oil or 1 tablespoon organic rose water.

Preparation: Stir in the rose oil or rose water. Serve cold.

Syrup Mint-Orange

A refreshing hot summer drink.
Ingredients:

- 50 mL PEPPERMINT seasoning syrup no. 4
- 5 drops CAPRI intermixture.

Preparation: Simply mix the ingredients and you have a refreshing basic syrup, which can be used for drinks, baked goods, to pour it into soda water, tea juices, or even into ice cubes. To serve, garnish the drinks with some fresh peppermint leaves.

Entrees

Soups

Peppermint Heaven
Ingredients:

- 500 mL vegetable stock
- Fresh peppermint leaves for decoration
- 2–3 drops PEPPERMINT basic essential oil
- 1 drop BERGAMOT basic essential oil
- 150 mL cream
- O SOLE MIO salt no. 5 or regular salt to season to taste.

Preparation: Whip the cream; then add the basic essential oils to it. Meanwhile boil the vegetable stock; then stir in the cream. Ladle into soup bowls to serve and garnish each with a little bit whipped cream and fresh mint leaves.

Perky Pumpkin Soup
Warm and spicy—the perfect autumn dinner.
Ingredients:

- 2 drops CAPRI intermixture
- 1 large onion, finely chopped
- 2 carrots, sliced finely
- 1 tablespoon pumpkin seed-oil or butter
- 500 g peeled pumpkin, finely chopped into cubes
- 200 mL vegetable stock
- 50 mL cream
- 1 teaspoon curry powder
- 1 tablespoon EURO ASIA seasoning oil no. 1
- Fresh coriander to garnish
- 1 tablespoon CAPRI seasoning salt no. 5
- A little bit sherry.

Preparation: Heat the pumpkin seed oil in a saucepan. Add the onion and carrots and cook over moderate heat until it softens. Stir in the pumpkin pieces and cook until the pumpkin is soft. Process the mixture in a blender and pour it to the pan. Stir in the vegetable stock and cream and season with the essential oils, salt, and sherry. Ladle into warm soup bowls and garnish each with some fresh coriander leaves.

Salads

Melon-Plum Purple Radish Salad

A refreshing hot summer party dish.
Ingredients:

- 1 mid-size watermelon or 2 Galia melons
- 1 handful radishes rinsed and chopped
- 1 bell pepper rinsed and sliced
- 3 pears rinsed and chopped
- Juice of 1 lemon
- 1 tablespoon CAPRI or O SOLE MIO seasoning oil no. 1
- 250 g sour cream
- 150 g curd
- Salt
- Freshly ground black pepper
- Some fresh summer herbs like thyme, cress or lemon balm.

Courtesy of Ulla Mayer-Raichle.

Preparation: Half the melon in a zigzag manner, separate the halves, remove the seeds from the melon halves, and use a melon baller to scoop out even-sized balls. Place the half of the melon balls, radishes, bell pepper, and pears in a large salad bowl and marinade the salad with lemon juice. Then store the melon halves and the salad in the fridge for at least half an hour. Meanwhile mix the seasoning oil of your choice with sour cream and curd and season with salt and pepper. Stir the mixture into the salad carefully and fill the salad into the melon halves. Garnish them with herbs and some of the extra melon balls.

Salad with Goat Cheese and Ricotta

A refreshing companion for spicy foods.
Ingredients:

- 1 red bell pepper rinsed, sliced
- 1 green bell pepper rinsed, sliced
- 1 scallion, chopped
- 1 head salad greens (Aragula, Sorrel, Dandelion, etc.), rinsed, dried, and chopped.

For the salad dressing:

- 1 drop O SOLE MIO intermixture
- 3 drops CAPRI intermixture
- 4 tablespoons dark olive oil
- Juice of 1 lemon
- Sea-salt
- 100 g goat cheese or ricotta, chopped
- 1/2 handful fresh eatable spring blossoms (daisies, primroses, etc.), rinsed
- 2 handfuls fresh herbs of your choice (coriander, parsley, basil, etc.), rinsed
- Roasted sesame.

Preparation: Emulsify the essential oil intermixtures with the olive oil; add the lemon juice and season with salt. Place the dressing in a large bowl, marinade the cheese, and add the salad leaves, bell peppers, and scallion. Mix well and garnish with the herbs and blossoms and the roasted sesame.

Appetizer and Finger Food

Crudities—Flavored Crispy Raw Vegetables

Simple and delicious.
Ingredients:

- 750 g vegetables well rinsed and cut into crudities (radishes, scallions, chicory, carrots, etc.)
- Juice of 1 lemon
- 5 drops CAPRI intermixture.

Preparation: Emulsify the CAPRI intermixture into the lemon juice, fill it into a spray flacon, and spread it on top of the sliced vegetables. Serve with dip and breadsticks or baguette.

Maria's Dip

Ingredients:

- 3 drops CAPRI intermixture
- 1 tablespoon creme fraiche
- 1/2 teaspoon salt
- 250 g sour cream.

Preparation: Emulsify the CAPRI essential oil intermixture into the creme fraiche. Stir in the salt and sour cream until combined. Ready to serve with bread, toast, and for example, the flavored crudities.

Tapenade

An Italian secret simple to make and perfect for dipping or seasoning.
Ingredients:
For the olives:

- 200 g pitted green or black olives, rinsed and halved
- 100 mL dark olive oil
- 1 handful fresh rosemary
- 10 drops O SOLE MIO intermixture.

For the tapenade:

- 60 g capers
- 1 crushed garlic clove
- Freshly ground black pepper.

Preparation: Marinate the olives in a mixture of olive oil, rosemary, and O SOLE MIO intermixture for at least 1 h. Place the olives, capers, and garlic in a food processor or blender and process until combined. Gradually add the flavored marinade and blend to a coarse paste; season with pepper. Keep stored in the fridge for up to 1 week.

Tofu Aromanaise

Served with the veggie skewers—a truly impressive dinner party dish.
Ingredients:

- 200 g organic pure tofu or smoked tofu
- 3 tablespoons sunflower oil
- 2 tablespoons EURO ASIA seasoning oil no. 1
- EURO ASIA seasoning salt no. 5
- A few chives.

Preparation: Put the tofu in a blender and process it until the tofu is smooth. Transfer the creamy tofu to a bowl and stir in the sunflower oil very slowly, then add the EURO ASIA seasoning oil, and season with EURO ASIA salt. Garnish the top with chopped chives. Serve cold.

Veggie Skewers

A tasty idea for your next barbecue.
Ingredients:

- 20 skewers
- 1000 g fresh young vegetables

(tomatoes, fennel, eggplants, carrots, bell peppers, scallions, etc.).
For the marinade:

- 5 tablespoons dark olive oil
- 3 tablespoons either O SOLE MIO or EURO ASIA seasoning oil no. 1
- Freshly grounded pepper
- 1 handful fresh chopped herbs (basil, thyme, parsley, etc.) or dried herbs.

Courtesy of Ulla Mayer-Raichle.

Preparation: Prepare the vegetables and cut them into cubes. Mix all the marinade ingredients in a shallow dish and add the vegetable cubes. Spoon the marinade over the vegetables and leave to marinate in the fridge for at least 1 h. Then thread the cubes onto skewers. Brush with the marinade and broil or grill until golden, turning occasionally. Serve with baguette, tofu aromannaise tapenade, or any other dip.

Main Course

Celery Lemon Grass Patties

Delicious, little, and flexible to combine.
Ingredients:

- 1–2 large celery
- 250 mL liquid vegetable stock
- 1 organic free range egg
- 4 lemon slices
- 1 pinch of BERGAMOT CLARY SAGE no. 5.

Asian variation:

- 3 tablespoons coconut flakes
- 2 tablespoons EURO ASIA seasoning no. 1
- Coconut oil or roasted sesame oil to fry.

Mediterranean variation:

- 2 tablespoons O SOLE MIO seasoning no. 1
- 3 tablespoons sesame seeds
- Soy oil to fry.

Preparation: Blanche the washed and sliced celery roots in the vegetable stock. Choose your favorite cookie cutter, like heart or star, and cut them out of the blanched celery. Whisk the egg and stir in the essential oil variation of your choice. Marinate the celery stars and hearts, then coat them with coconut flakes or sesame seeds and fry them until they have a delicious golden brown color. To serve, top them with a small amount of the essential oil seasoning. They are great to accompany salads, baked potatoes with sour cream, and other vegetarian dishes or if you prefer, beef creations.

Chévre Chaude-Goat Cheese "Provence" with Pineapple

Ingredients:

- 4 slices of fresh pineapple
- 1 tablespoon sunflower oil or butter or ghee
- 1 teaspoon "O SOLE MIO honey" no. 4
- 1 tablespoon CAPRI honey no. 4
- 2 tablespoons honey PROVENCAL (basics)
- 2–3 small goat or sheep cheese
- A little bit fresh or dried thyme to garnish
- Sour cream
- Salad or Parma ham (optional).

Courtesy of Ulla Mayer-Raichle.

Preparation: Halve the pineapple slices and fry them on both sides. Lower the heat and top them with CAPRI honey. Preheat the oven to 180°C. Halve the cheese and place them on top of each of the two pineapple slices. Drop a little bit of honey PROVENCAL on each portion and bake it shortly until the cheese starts to caramelize. Serve immediately with the rest of the aromatized honeys dispersed on the surface, fresh herbs above, the sour cream on top, and with Parma ham or fresh salad aside.

Crispy Wild Rice-Chapatis

Ingredients:

- 200 g wild rice
- 400–500 mL warm water
- 1 laurel leaf
- 1 small onion or 3 scallions, finely chopped
- 1 teaspoon EURO ASIA seasoning oil no. 1
- 1 tablespoon EURO ASIA seasoning soy sauce no. 6
- 2 organic or free range eggs
- Curry powder
- Lemon juice as you like
- Around 2 tablespoons oil or ghee to fry.

Preparation: Steam the wild rice briefly, then fill it up with the rest of the warm water, and add the laurel leave. Cook it for another 15–20 min, then turn the heat down and stir in the EURO ASIA seasoning oil no. 1. Cover it, leave it and let it chill until firm. Then stir all ingredients into the wild rice. Divide the mixture into walnut-sized balls; then flatten them slightly. Heat the oil or ghee in a pan and fry the chapatis until golden brown on each side. Drain on paper towels and serve at once. These crispy wild rice-chapatis taste delicious with steamed vegetables and dips or even salads. They are ideal as a snack or a nice idea for the next picnic.

Mango–Dates–Orange Chutney

A spice dip-trip to Asia.
Ingredients:
For 1000 g you need

- 250 g organic well-scrubbed oranges (e.g., sweet and juicy sorts like Valencia)
- 250 g onions

- 250 g sliced mangoes
- 350 mL acacia honey
- If this is not available choose any other treacle or honeys, which is neutral in taste and of organic origin
- 50 mL maple syrup
- 2 teaspoons CAPRI essential oil seasoning salt no. 5
- A little bit of chile powder or 1 fresh chile pepper
- 350 mL cider vinegar
- 250 mg chopped dates
- 50 mL of either EURO ASIA
- or MAGIC ORANGE essential oil seasoning vinegar no. 3
- 2 tablespoons CAPRI essential oil seasoning syrup no. 4
- 5 drops pure EURO ASIA condiment intermixture.

Preparation: Remove long, thin shreds of orange rind, using a grater (zester). Scrape it firmly along the surface of the fruit. Remove the white layer of the oranges; then slice the oranges and remove the pits. Finely chop the onions. Peel the mangoes and cut them into small chunks. Mix honey, syrup, chile powder, and vinegar with 1 teaspoon of the CAPRI salt no. 5 and boil it in a huge saucepan until the honey melts, stir it well. Add mangoes, onions, dates, oranges, and the half of the shredded orange rind. Then lower the heat and let it simmer for 1 h, until the mixture has formed a thick mass. Stir in the rest of the shredded orange rind and the chosen essential oil vinegar no. 3. Then emulsify the pure EURO ASIA condiment intermixture into the CAPRI syrup no. 4 and stir it in the chutney. Use the rest of the CAPRI salt no. 5 to add spice. Fill the mixture into sterilized warm preserving jars, store them cold and dark. Nice to serve with the Chévre chaude or the crispy wild rice-chapatis and veggie skewers.

Prawns Bergamot

Ingredients:

- 500 g large prawns

Marinade:

- 5 drops pure CAPRI essential oil intermixture
- 1 small onion
- 1/2 crushed garlic clove
- 1 handful flat leaf parsley
- 3 scallions
- Juice of a lemon
- 2 drops pure BERGAMOT essential oil
- 1/2 teaspoon fennel seed
- 6 tablespoons olive oil
- Salt and fresh pepper
- 3 tablespoons BERGAMOT–GRAND MANIER vine no. 3.

Preparation: Prepare and wash the prawns as usual. Slice the onions and garlic, chop the parsley finely and cut the scallions into quarters. Take a teaspoon of lemon juice and emulsify the essential oils in it and mix in the rest of the ingredients. Let the prawns soak in the marinade and keep it in the fridge for 1 h. Then separate the prawns from the marinade; filter the marinade and keep the parts separately. Fry the prawns inside of the liquid parts of the marinade, then add the rest.

Stir it well for another minute, season with salt, pepper, and BERGAMOT vine and let it simmer slowly. Nice to serve with baguette or the crispy wild rice chapatis and vegetables like green asparagus tips.

Dessert, Cakes, and Baked Goods

Apple Cake Rose

This classic combination is an apples favorite destiny. Suited even for diabetics.
Ingredients:

- 250 g spelt flour
- 120 g finely sliced cold butter
- 1 organic or free range egg
- 1 tablespoon CAPRI essential oil seasoning no. 1
- Salt
- 50–100 mL warm water
- 1000 g sweet ripe apples
- Juice of a half lemon
- 1 tablespoon organic rose water.

For the topping:

- 250 ml cream
- 1 egg yolk of an organic or free range egg
- 5–7 drops MAGIC ORANGE pure seasoning intermixture
- 1 tablespoon organic rose water
- 50 g sliced almonds to garnish the top of the cake.

Preparation: Sift the flour, butter, egg, warm water, and the CAPRI seasoning no. 1 into a large mixing bowl. Mix everything together until combined; then store the cake mixture in the fridge for a half hour. In the meanwhile, peel and core the apples, slice them into wedges, and slice the wedges thinly. Combine lemon juice with rose water and splash it over the apples. For the topping, beat the egg yolk with the cream and the pure essential oil intermixture MAGIC ORANGE. Then pour the cake mixture into the prepared pan, smooth the surface, then make a shallow hollow in a ring around the edge of the mixture. Arrange the apple slices on top of the cake mixture. Pour the topping carefully above the apple slices and garnish the sliced almonds above. Cover the cake with aluminum foil. Bake for 30–40 min, until firm and the mixture comes away from the side of the pan. Lower the heat, remove the foil, and bake it for another 5 min. Serve warm.

Chocolate Fruits and Leaves

A delicious way to consume your favorite fruits, dried fruits, nuts, or even leaves like rose leaves.
Ingredients:

- 250 g organic chocolate couverture (bitter chocolate)
- 5 drops MAGIC ORANGE or BERGAMOT–GRAND MANIER or CAPRI intermixture – or 2–3 drops PEPPERMINT, LAVENDER, or GINGER pure basic essential oil, depending on your taste—spicy, mint, or fruity.

Courtesy of Ulla Mayer-Raichle.

Preparation: Warm up the chocolate couverture until you have a creamy consistency. Stir in your choice of basic essential oils or intermixture. Dive in the fruits, and let them dry. Serve chilled.

Homemade Fresh Berry Jelly

Ingredients:

- 500 gm mixed berries
- (blue berries; rasp berries; red, white, and black currant; black berries; strawberries, cranberries, cherries)
- 100 mL water
- 1 tablespoon agar or 2 tablespoons kuzu or sago (binding agent)
- 1–2 tablespoons cold water
- 12 drops MAGIC ORANGE intermixture
- 3 tablespoons maple syrup.

Preparation: Take the clean fruits and boil them in the water. Stir the binding agent into the small amount of cold water, then add it to the warm fruits and let them boil for another 3–5 min before you lower the heat, then leave the mixture to cool. Emulsify the essential oils intermixture with the maple honey; then stir it into the jelly. Serve cool with fresh berries or a spoonful of whipped cream with mint leaves.

Rose Semifreddo

Romantic and delicate aromatic dessert.
Ingredients:

- 150 g creme fraiche
- 75 g low fat quark
- 100 mL acacia honey
- 1 tablespoon rose water
- Rose leaves from 2 roses (organic farming)
- 2 tablespoons cognac

- Nonalcoholic alternative—1 drop pure MAGIC ORANGE intermixture in 2 tablespoons maple syrup
- 150 mL whipped cream
- 1 drop of pure MAGIC ORANGE intermixture.

Preparation: Place the creme fraiche and the quark in a bowl and cream together. Keep some rose leaves for decoration aside, process the rest of the leaves in a food processor until smooth, then transfer them into the bowl; add the acacia honey and stir to mix. Whisk in the rose water and either the cognac or the MAGIC ORANGE maple syrup. Fold in the whipped cream and the pure MAGIC ORANGE intermixture gently, being careful not to over mix. Pour the mixture into some small plastic containers, cover and freeze until the ice is firm. Transfer the ice to the refrigerator about 20 min before serving to allow it to soften a little. Serve in scoops decorated with rose leaves and berries.

Sweet Florentine

Sweet almond munchies.
Ingredients:

- 500 g butter
- 200 g sugar
- 2 packages organic bourbon vanilla sugar
- 250 mL cream
- 300 g sliced almonds
- 30 g spelt or wheat grain
- 15–20 drops MAGIC ORANGE or CAPRI intermixture emulsified in 1 tablespoon maple treacle
- 100 g chocolate couverture with 5–8 drops CAPRI or MAGIC ORANGE intermixture.

Preparation: Caramelize the sugar, then stir in the bourbon vanilla, butter until the sugar has been melted, then stir in almonds and flour. Preheat the oven to 180°C, then spoon the mixture on a baking tray and bake for 10 min. Do not worry, it is in their nature to melt. To serve, just cut them into diamonds after cooling down and remove them from the pan. Dive them halfway into the chocolate couverture only (the lower smooth side) then let them dry. Serve chilled or iced.

RÉSUMÉ

Aroma-vital cuisine is an aspect of aroma culture and therefore an art and cultivation of using the senses especially taste and smell.

19 Essential Oils Used in Veterinary Medicine

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19.1 INTRODUCTION

Essential oils are volatile constituents of aromatic plants. These liquid oils are generally complex mixtures of terpenoid and/or nonterpenoid compounds. Mono-, sesqui-, and sometimes diterpenoids, phenylpropanoids, fatty acids and their fragments, benzenoids, and so on may occur in various essential oils (Baser and Demirci, 2007).

Except for citrus oils obtained by cold pressing, all other essential oils are obtained by distillation. Products obtained by solvent extraction or supercritical fluid extraction are not technically considered as essential oils (Baser, 1995).

Essential oils are used in perfumery, food flavoring, pharmaceuticals, and sources of aromachemicals.

Essential oils exhibit a wide range of biological activities and 31 essential oils have monographs in the latest edition of the European Pharmacopoeia (Table 19.1).

TABLE 19.1
Essential Oil Monographs in the European Pharmacopoeia (6.5 Edition, 2009)

English Name	Latin Name	Plant Name
Anise oil	*Anisi aetheroleum*	*Pimpinella anisum* L. fruits
Bitter-fennel fruit oil	*Foeniculi amari fructus aetheroleum*	*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*
Bitter-fennel herb oil	*Foeniculi amari herba aetheroleum*	*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*
Caraway oil	*Carvi aetheroleum*	*Carum carvi* L.
Cassia oil	*Cinnamomi cassiae aetheroleum*	*Cinnamomum cassia* Blume (*Cinnamomum aromaticum* Nees)
Cinnamon bark oil, Ceylon	*Cinnamomi zeylanici corticis aetheroleum*	*Cinnamomum zeylanicum* Nees
Cinnamon leaf oil, Ceylon	*Cinnamomi zeylanici folium aetheroleum*	*Cinnamomum verum* J.S. Presl.
Citronella oil	*Citronellae aetheroleum*	*Cymbopogon winterianus* Jowitt
Clarysage oil	*Salviae sclareae aetheroleum*	*Salvia sclarea* L.
Clove oil	*Caryophylli aetheroleum*	*Syzigium aromaticum* (L.) Merill et L.M. Perry (*Eugenia caryophyllus* C.S. Spreng. Bull. et Harr
Coriander oil	*Coriandri aetheroleum*	*Coriandrum sativum* L.
Dwarf pine oil	*Pini pumilionis aetheroleum*	*Pinus mugo* Turra.
Eucalyptus oil	*Eucalypti aetheroleum*	*Eucalyptus globulus* Labill.
Juniper oil	*Juniperi aetheroleum*	*Juniperus communis* L. meyvesi
Lavender oil	*Lavandulae aetheroleum*	*Lavandula angustifolia* P. Mill. (*Lavandula officinalis* Chaix.)
Lemon oil	*Limonis aetheroleum*	*Citrus limon* (L.) Burman fil.
Mandarin oil	*Citri reticulatae aetheroleum*	*Citrus reticulata* Blanco
Matricaria oil	*Matricariae aetheroleum*	*Matricaria recutita* L. (*Chamomilla recutita* (L.) Ranschert)
Mint oil, partly dementholized	*Menthae arvensis aetheroleum partim mentholi privum*	*Mentha canadensis* L. (*Mentha arvensis* L. var. *glabrata* (Benth.) Fern, *Mentha arvensis* L. var. *piperascens* Malinv. ex Holmes) **Japanese mint**
Neroli oil (formerly bitter-orange flower oil)	*Neroli aetheroleum* (formerly *Aurantii amari floris aetheroleum*)	*Citrus aurantium* L. subsp. *aurantium* (*Citrus aurantium* L. subsp. *amara* Engl.)
Nutmeg oil	*Myristicae fragrantis aetheroleum*	*Myristica fragrans* Houtt.
Peppermint oil	*Menthae piperitae aetheroleum*	*Mentha* × *piperita* L.
Pine silvestris oil	*Pini silvestris aetheroleum*	*Pinus silvestris* L.
Rosemary oil	*Rosmarini aetheroleum*	*Rosmarinus officinalis* L.
Spanish sage oil	*Salviae lavandulifoliae aetheroleum*	*Salvia lavandulifolia* Vahl.
Spike lavender oil	*Spicae aetheroleum*	*Lavandula latifolia* Medik.
Star anise oil	*Anisi stellati aetheroleum*	*Illicium verum* Hooker fil.
Sweet orange oil	*Aurantii dulcis aetheroleum*	*Citrus sinensis* (L.) Osbeck (*Citrus aurantium* L. var. *dulcis* L.)
Tea tree oil	*Melaleucae aetheroleum*	*Melaleuca alternifolia* (Maiden et Betch) Cheel, *Melaleuca linariifolia* Smith, *Melaleuca dissitiflora* F. Mueller and other species
Thyme oil	*Thymi aetheroleum*	*Thymus vulgaris* L., *T. zygis* L.
Turpentine oil, *Pinus pinaster* type	*Terebinthini aetheroleum ab pinum pinastrum*	*Pinus pinaster* Aiton. (**Maritime pine**)

Antimicrobial activities of many essential oils are well documented (Bakkali et al., 2008). Such oils may be used singly or in combination with one or more oils. For the sake of synergism this may be necessary.

Although many are generally regarded as safe (GRAS), essential oils are generally not recommended for internal use. However, their much diluted forms (e.g., hydrosols) obtained during oil distillation as a by-product may be taken orally.

Topical applications of some essential oils (e.g., oregano and lavender) in wounds and burns bring about fast recovery without leaving any sign of cicatrix. By inhalation, several essential oils act as a mood changer and have effect especially on respiratory conditions.

Several essential oils (e.g., citronella oil) have been used as pest repellents or as insecticides and such uses are frequently encountered in veterinary applications.

In recent years, especially after the ban on the use of antibiotics in animal feed in the European Union since January 2006, essential oils have emerged as a potential alternative to antibiotics in animal feed.

Essential oils used in veterinary medicine may be classified as follows:

1. Oils attracting animals
2. Oils repelling animals
3. Insecticidal, pest repellent, and antiparasitic oils
4. Oils used in animal feed
5. Oils used in treating diseases in animals.

19.2 OILS ATTRACTING ANIMALS

Valeriana oils (and valerianic and isovalerianic acids) and nepeta oils (and nepetalactones) are well-known feline-attractant oils. Their odor attracts male cats.

Douglas fir oil and its monoterpenes have been claimed to attract deer and wild boar (Buchbauer et al., 1994).

Dogs are normally drawn to floral oils and usually choose to take these by inhalation only. Monoterpene-rich oils are usually too strong for dogs, with the exception of bergamot, *Citrus bergamia*.

Cats also usually select only floral oils for inhalation. Cats do not have metabolic mechanism to break down essential oils due to the lack of enzyme glucuronidase. Therefore, they should not be taken by mouth and should not be generally applied topically (http://www.ingraham.co.uk).

19.3 OILS REPELLING ANIMALS

Peppermint oil (*Mentha piperita*) repels mice. It can be applied under the sink in the kitchen or applied in staples to prevent mice annoying horses and livestock. A few drops of peppermint oil in a bucket of water used to scrub out a stall and sprinkling a few drops around the perimeter and directly on straw or bedding is said to eliminate or severely curtail the habitation of mice (Anonymous, 2001).

A patent (United States Patent 4961929) claims that a mixture of methyl salicylate, birch oil, wintergreen oil, eucalyptus oil, pine oil, and pine-needle oil repels dogs.

Another patent (United States Patent 4735803) claims the same using lemon oil and α-terpinyl methyl ether.

Another similar formulation (United States Patent 4847292) claims that a mixture of citronellyl nitrile, citronellol, α-terpinyl methyl ether, and lemon oil repels dogs.

A mixture of black pepper and capsicum oils and the oleoresin of rosemary is claimed to repel animals (United States Patent 6159474).

Citronella oil repels cats and dogs (Moschetti, 2003).

Repellents alleged to repel cats include allyl isothiocyanate (oil of mustard), amyl acetate, anethole, capsaicin, cinnamaldehyde, citral, citronella, citrus oil, eucalyptus oil, geranium oil, lavender

oil, lemongrass oil, menthol, methyl nonyl ketone, methyl salicylate, naphthalene, nicotine, paradichlorobenzene, and thymol. Oil of mustard, cinnamaldehyde, and methyl nonyl ketone are said to be the most potent.

Essential oils comprised of 10 g/L solutions of cedarwood, cinnamon, sage, juniper berry, lavender, and rosemary, each were potent snake irritants. Brown tree snakes exposed to a 2-s burst of aerosol of these oils exhibited prolonged, violent undirected locomotory behavior. In contrast, exposure to a 10 g L^{-1} concentration of ginger oil aerosol caused snakes to locomote, but in a deliberate, directed manner. The 10 g/L solutions delivered as aerosols of *m*-anisaldehyde, *trans*-anethole, 1,8-cineole, cinnamaldehyde, citral, ethyl phenylacetate, eugenol, geranyl acetate, or methyl salicylate acted as potent irritants for brown tree snakes (*Boiga irregularis*) (Clark and Shivik, 2002).

19.4 OILS AGAINST PESTS

19.4.1 Insecticidal, Pest Repellent, and Antiparasitic Oils

The essential oil of bergamot (*Citrus bergamia*), anise (*Pimpinella anisum*), sage (*Salvia officinalis*), tea tree (*Melaleuca alternifolia*), geranium (*Pelargonium* sp.), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), hyssop (*Hyssopus officinalis*), rosemary (*Rosmarinus officinalis*), and white clover (*Trifolium repens*) can be used to control certain pests on plants. They have been shown to reduce the number of eggs laid and the amount of feeding damage by certain insects, particularly lepidopteran caterpillars. Sprays made from Tansy (*Tanacetum vulgare*) have demonstrated a repellent effect on imported cabbageworm on cabbage, reducing the number of eggs laid on the plants. Teas made from wormwood (*Artemisia absinthium*) or nasturtiums (*Nasturtium* spp.) are reputed to repel aphids from fruit trees, and sprays made from ground or blended catnip (*Nepeta cataria*), chives (*Allium schoenoprasum)*, feverfew (*Tanacetum parthenium*), marigolds (*Calendula*, *Tagetes*, and *Chrysanthemum* spp.), or rue (*Ruta graveolens*) have also been used by gardeners against pests that feed on leaves (Moschetti, 2003).

19.4.2 Fleas and Ticks

Dogs, cats, and horses are plagued by fleas and ticks. One to two drops of citronella or lemongrass oils added to the shampoo will repel these pests. Alternatively, 4–5 drops of cedarwood oil and pine oil is added to a bowl of warm water and a bristle hair brush is soaked with this solution to brush the pet down with it. Eggs and parasites gathered in the brush are rinsed out. This is repeated several times. This solution can be used similarly for livestock after adding citronella and lemon grass oils to this mixture.

Flea collar can be prepared by a mixture of cedarwood (*Juniperus virginiana*), lavender (*Lavandula angustifolia*), citronella (*Cymbopogon winterianus* (Java)), thyme oils, and 4–5 garlic (*Allium sativum*) capsules. This mixture is thinned with a teaspoonful of ethanol and soaked with a collar or a cotton scarf. This is good for 30 days (Anonymous, 2001).

Ticks can be removed by applying 1 drop of cinnamon or peppermint oil on Q-tip by swabbing on it.

Carvacrol-rich oil (64%) of *Origanum onites* and carvacrol was found to be effective against the tick *Rhipicephalus turanicus*. Pure carvacrol killed all the ticks following 6 h of exposure, while 25% and higher concentrations of the oil were effective in killing the ticks by the 24-h posttreatment (Coskun et al., 2008).

19.4.3 Mosquitoes

Catnip oil (*Nepeta cataria*) containing nepetalactones can be used effectively as a mosquito repellent. It is said to be 10 times more effective than DEET (Moschetti, 2003). *Juniperus communis* berry oil

is a very good mosquito repellent. Ocimum volatile oils including camphor, 1,8-cineole, methyl eugenol, limonene, myrcene, and thymol strongly repelled mosquitoes (Regnault-Roger, 1997).

Citronella oil repels mosquitoes, biting insects, and fleas.

Essential oils of *Zingiber officinale* and *Rosmarinus officinalis* were found to be ovicidal and repellent, respectively, toward three mosquito species (Prajapati et al., 2005). Root oil of *Angelica sinensis* and ligustilide was found to be mosquito repellent (Wedge et al., 2009).

19.4.4 Moths

Cedarwood oil is used in mothproofing. A large number of patents have been assigned to the preservation of cloths from moths and beetles: Application of a solution containing clove (*Syzigum aromaticum*) essential oil on woolen cloth; filter paper containing *Juniperus rigida* oil, and tablets of *p*-dichlorobenzene mixed with essential oils to be placed in wardrobe.

19.4.5 Aphids, Caterpillars, and Whiteflies

19.4.5.1 Garlic Oil

Essential oils effective in insect pest control (Regnault-Roger, 1997).

19.4.6 Ear Mites

Peppermint oil is applied to a Q-tip and swabbed inside of the ear.

19.4.7 Antiparasitic

A patent (United States Patent 6800294) on an antiparasitic formulation comprising eucalyptus oil (*Eucalyptus globulus*), cajeput oil (*Melaleuca cajeputi*), lemongrass oil, clove bud oil (*Syzigium aromaticum*), peppermint oil (*Mentha piperita*), piperonyl, and piperonyl butoxide. The formulation can be used for treating an animal body, in the manufacture of a medicament for treating ectoparasitic infestation of an animal, or for repelling parasites.

Two essential oils derived from *Lavandula angustifolia* and *Lavandula* × *intermedia* were investigated for any antiparasitic activity against the human protozoal pathogens *Giardia duodenalis* and *Trichomonas vaginalis* and the fish pathogen *Hexamita inflata*, all of which have significant infection and economic impacts. The study has demonstrated that low (≤1%) concentrations of *Lavandula angustifolia* and *Lavandula* × *intermedia* oil can completely eliminate *Trichomonas vaginalis*, *Giardia duodenalis*, and *Hexamita inflata in vitro*. At 0.1% concentration, *Lavandula angustifolia* oil was found to be slightly more effective than *Lavandula* × *intermedia* oil against *Giardia duodenalis* and *Hexamita inflata* (Moon et al., 2006).

The antiparasitic properties of essential oils from *Artemisia absinthium*, *Artemisia annua*, and *Artemisia scoparia* were tested on intestinal parasites, *Hymenolepis nana*, *Lambli intestinalis*, *Syphacia obvelata*, and *Trichocephalus muris* [*Trichuris muris*]. Infested white mice were injected with 0.01 ml/g of the essential oils (6%) twice a day for 3 days. The effectiveness of the essential oils was observed in 70–90% of the tested animals (Chobanov et al., 2004).

Parasites, such as head lice and scabies, as well as internal parasites, are repelled by oregano oil (86% carvacrol). The oil can be added to soaps, shampoos, and diluted in olive oil for topical applications. By taking a few drops daily under the tongue, one can gain protection from waterborne parasites, such as *Cryptosporidium* and *Giardia*. Internal dosages also are effective in killing parasites in the body (http://curingherbs.com/wild_oregano_oil.htm) (Foster, 2002).

Essential oils from *Pinus halepensis*, *Pinus brutia*, *Pinus pinaster*, *Pinus pinea*, and *Cedrus atlantica* were tested for molluscicidal activity against *Bulinus truncatus*. The oil from *Cedrus*

atlantica was found the most active (LC 50 = 0.47 ppm). Among their main constituents, α-pinene, β-pinene, and myrcene exhibited potent molluscicidal activity (LC 50 = 0.49; 0.54, and 0.56 ppm, respectively). These findings have important application of natural products in combating schistosomiasis (Lahlou, 2003).

Origanum essential oils have exhibited differential degrees of protection against myxosporean infections in gilthead and sharpsnout sea bream tested in land-based experimental facilities (Athanassopoulou et al., 2004a, 2004b).

19.5 ESSENTIAL OILS USED IN ANIMAL FEED

Essential oils can be used in feed as appetite stimulant, stimulant of saliva production, gastric and pancreatic juices production enhancer, and antimicrobial and antioxidant to improve broiler performance. Antimicrobial effects of essential oils are well documented. Essential oils due to their potent nature should be used as low as possible levels in animal nutrition. Otherwise, they can lead to feed intake reduction, gastrointestinal (GIT) microflora disturbance, or accumulation in animal tissues and products. Odor and taste of essential oils may contribute to feed refusal; however, encapsulation of essential oils could solve this problem (Gauthier, 2005).

Generally, Gram-positive bacteria are considered more sensitive to essential oils than Gram-negative bacteria because of their less complex membrane structure (Lis-Balchin, 2003).

Carvacrol, the main constituent of oregano oils, is a powerful antimicrobial agent (Baser, 2008). It asserts its effect through the biological membranes of bacteria. It acts through inducing a sharp reduction of the intercellular ATP pool through the reduction of ATP synthesis and increased hydrolysis. Reduction of the membrane potential (transmembrane electrical potential), which is the driving force of ATP synthesis, makes the membrane more permeable to protons. A high level of carvacrol (1 mM) decreases the internal pH of bacteria from 7.1 to 5.8 related to ion gradients across the cell membrane. 1 mM of carvacrol reduces the internal potassium (K) level of bacteria from 12 mmol/mg of cell protein to 0.99 mmol/mg in 5 min. K plays a role in the activation of cytoplasmic enzymes and in maintaining osmotic pressure and in the regulation of cytoplasmic pH. K efflux is a solid indication of membrane damage (Ultee et al., 1999).

It has been shown that the mode of action of oregano oils is related to an impairment of a variety of enzyme systems, mainly involved in the production of energy and the synthesis of structural components. Leakage of ions, ATP, and amino acids also explain the mode of action. Potassium and phosphate ion concentrations are affected at levels below the MIC concentration (Lambert et al., 2001).

19.5.1 Ruminants

A recent review compiled information on botanicals including essential oils used in ruminant health and productivity (Rochfort et al., 2008). Unfortunately, there are few reports on the effects of essential oils and natural aromachemicals on ruminants. It was demonstrated that the consumption of terpene volatiles such as camphor and α-pinene in "tarbush" (*Flourencia cernua*) effected feed intake in sheep (Estell et al., 1998). *In vitro* and *in vivo* antimicrobial activities of essential oils have been demonstrated in ruminants (Cardozo, 2005; Elgayyar et al., 2001; Moreira et al., 2005; Wallace et al., 2002). Synergistic antinematodal effects of essential oils and lipids were demonstrated (Ghisalberti, 2002). Other nematocidal volatiles reported are as follows: benzyl isothiocyanate (goat), ascaridole (goat and sheep) (Githiori et al., 2006; Ghisalberti, 2002), geraniol, eugenol (Githiori et al., 2006; Chitwood, 2002), and menthol, 1,8-cineole (Chitwood, 2002).

Methylsalicylate, the main component of the essential oil of *Gaultheria procumbens* (Wintergreen), is topically used as emulsion in cattle, horses, sheep, goats, and poultry in the treatment of muscular and articular pain. The recommended dose is 600 μg/kg bw twice a day. The duration of treatment is usually less than 1 week (EMEA, 1999). It is included in Annex II of

Council Regulation (EEC) N. 2377/90 as a substance that does not need an MRL level. *Gaultheria procumbens* should not to be used as flavoring in pet food since salicylates are toxic to dogs and cats. As cats metabolize salicylates much more slowly than other species, they are more likely to be overdosed. Use of methylsalicylate in combination with anticoagulants such as warfarin can result in adverse interactions and bleedings (Chow et al., 1989; Ramanathan, 1995; Tam et al., 1995; Yip et al., 1990).

The essential oil of *Lavandula angustifolia* (*Lavandulae aetheroleum*) is used in veterinary medicinal products for topical use together with other plant extracts or essential oils for antiseptic and healing purposes. The product is used in horses, cattle, sheep, goats, rabbits, and poultry. It is included in Annex II of Council Regulation (EEC) N. 2377/90 as a substance that does not need an MRL level (EMEA, 1999; Franz et al., 2005).

The outcomes of *in vitro* studies investigating the potential of *Pimpinella anisum* essential oil as a feed additive to improve nutrient use in ruminants are inconclusive, and more and larger preferably *in vivo* studies are necessary for evaluation of efficacy (Franz et al., 2005).

Carvacrol, carvone, cinnamaldehyde, cinnamon oil, clove bud oil, eugenol, and oregano oil have resulted in a 30–50% reduction in ammonia N concentration in diluted ruminal fluid with a 50:50 forage concentrate diet during the 24-h incubation (Busquet et al., 2006).

Carvacrol has been suggested as a potential modulator of ruminal fermentation (Garcia et al., 2007).

19.5.2 Poultry

19.5.2.1 Studies with CRINA Poultry

Dietary addition of essential oils in a commercial blend (CRINA® Poultry) showed a decreased *Escherichia coli* population in ileo-cecal digesta of broiler chickens. Furthermore, in high doses, a significant increase in certain digestive enzyme activities of the pancreas and intestine was observed in broiler chickens (Jang et al., 2007).

In another study, CRINA Poultry was shown to control the colonization of the intestine of broilers with *Clostridium perfringens* and the stimulation of animal growth was put down to this development (Losa, 2001).

Commercial essential oil blends CRINA Poultry and CRINA Alternate were tested in broilers infected with viable oocysts of mixed *Eimeria* spp. It was concluded that these essential oil blends may serve as an alternative to antibiotics and/or ionophores in mixed *Eimeria* infections in non-cocci-vaccinated broilers, but no benefit of essential oil supplementation was observed for vaccinated broilers against coccidia (Oviedo-Rondon et al., 2006).

19.5.2.1.1 Other Studies

Supplementation of 200 ppm essential oil mixture (EOM) that included oregano, clove, and anise oils (no species name or composition given!) in broiler diets was said to significantly improve the daily live weight gain and feed conversion ratio (FCR) during a growing period of 5 weeks (Ertas et al., 2006). Similar results were obtained with 400 mg/kg anise oil (composition not known!) (Ciftci et al., 2005).

A total of 50 and 100 mg/kg of feed of oregano oil* were tested on broilers. No growth-promoting effect was observed. At 100 mg/kg of feed, antioxidant effect was detected on chicken tissues (Botsoglou et al., 2002a).

Positive results were also reported for oregano oil added in poultry feed (Bassett, 2000).

* Oregano essential oil was in the form of a powder called Orego-Stim. This product contains 5% oregano essential oil (Ecopharm Hellas, SA, Kilkis, Greece) and 95% natural feed grade inert carrier. The oil of *Origanum vulgare* subsp. *hirtum* used in this product contains 85% carvacrol + thymol.

Antioxidant activities of rosemary and sage oils on lipid oxidation of broiler meat have been shown. Following dietary administration of rosemary and sage oils to the live birds, a significant inhibition of lipid peroxidation was reported in chicken meat stored for 9 days (Lopez-Bote et al., 1998). A dietary supplementation of oregano essential oil (300 mg/kg) showed a positive effect on the performance of broiler chickens experimentally infected with *Eimeria tenella*. Throughout the experimental period of 42 days, oregano essential oil exerted an anticoccidial effect against *Eimeria tenella*, which was, however, lower than that exhibited by lasalocid. Supplementation with dietary oregano oil to *Eimeria tenella*-infected chickens resulted in body weight gains and feed conversion ratios not differing from the noninfected group, but higher than those of the infected control group and lower than those of chickens treated with the anticoccidial lasalocid (Giannenas et al., 2003).

Inclusion of oregano oil at 0.005% and 0.01% in chicken diets for 38 days resulted in a significant antioxidant effect in raw and cooked breast and thigh muscle stored up to 9 days in refrigerator (Botsoglou et al., 2002b).

Oregano oil (55% carvacrol) exhibited a strong bactericidal effect against lactobacilli and following the oral administration of the oil MIC values of amicain, apramycin, and streptomycinand neomyc against *Escherichia coli* strains increased (Horosova et al., 2006).

An *in vitro* assay measuring the antimicrobial activity of essential oils of *Coridothymus capitatus*, *Satureja montana*, *Thymus mastichina, Thymus zygis*, and *Origanum vulgare* was carried out against poultry origin strains of *Escherichia coli, Salmonella enteritidis*, and *Salmonella essen*, and pig origin strains of enterotoxigenic *Escherichia coli* (ETEC), *Salmonella choleraesuis*, and *Salmonella typhimurium*. *Origanum vulgare* (MIC ≤ 1% v/v) oil showed the highest antimicrobial activity against the four strains of *Salmonella*. It was followed by *Thymus zygis* oil (MIC ≤ 2% v/v). *Thymus mastichina* oil inhibited all the microorganisms at the highest concentration, 4% (v/v). Monoterpenic phenols carvacrol and thymol showed higher inhibitory capacity than the monoterpenic alcohol linalool. The results confirmed potential application of such oils in the treatment and prevention of poultry and pig diseases caused by salmonella (Penalver et al., 2005).

In another study, groups of male, 1-day-old Lohmann broilers were given maize–soya bean meal diets, with oils extracted from thyme, mace, and caraway or coriander, garlic, and onion (0, 20, 40, and 80 mg/kg) for 6 weeks. The average daily gain and FCR were not different between the broilers fed with the different oils; meat was not tainted with flavor or smell of the oils (Vogt and Rauch, 1991).

19.5.2.2 Studies with Herbromix

Essential oils from oregano herb (*Origanum onites*), laurel leaf (*Laurus nobilis*), sage leaf (*Salvia fruticosa*), fennel fruit (*Foeniculum vulgare*), myrtle leaf (*Myrtus communis*), and citrus peel (rich in limonene) were mixed and formulated as feed additive after encapsulation. It is marketed in Turkey as poultry feed under the name Herbromix®.

The following three *in vivo* experiments with this product were recently accomplished.

19.5.2.2.1 In Vivo Experiment 1

In this study, 1250 sexed 1-day-old broiler chicks obtained from a commercial hatchery were randomly divided into five treatment groups of 250 birds each (negative control, antibiotic, and essential oil combination (EOC) at three levels). Each treatment group was further subdivided into five replicates of 50 birds (25 males and 25 females) per replicate. Commercial EOC at three different levels (24, 48, and 72 mg) and antibiotic (10 mg avilamycin) per kg were added to the basal diet. There were significant effects of dietary treatments on body weight, feed intake (except at day 42), FCR, and carcass yield at 21 and 42 days. Body weights were significantly different between the treatments. Birds fed on diet containing 48 mg essential oil/kg being the highest and this treatment was followed by chicks fed on the diet containing 72 mg essential oil/kg, antibiotic, negative control, and 24 mg essential oil/kg at day 42.

Supplementation with 48 mg EOC/kg to the broiler diet significantly improved the body weight gain, FCR, and carcass yield compared to other dietary treatments on 42 days of age. EOC may be considered as a potential growth promoter in the future of the new era, which agrees with producer needs for increased performance and today's consumer demands for environment-friendly broiler production. The EOC can be used cost effectively when its cost is compared with antibiotics and other commercially available products in the market.

19.5.2.2.2 In Vivo Experiment 2

In this study, 1250 sexed 1-day-old broiler chicks were randomly divided into five treatment groups of 250 birds each (negative control, organic acid, probiotic, and EOC at two levels). Each treatment group was further subdivided into five replicates of 50 birds (25 males and 25 females) per replicate. The oils in the EOC were extracted from different herbs growing in Turkey. The organic acid at 2.5 g/kg diet, the probiotic at 1 g/kg diet, and the EOC at 36 and 48 mg/kg diet were added to the basal diet.

The results obtained from this study indicated that the inclusion of 48 mg EOC/kg broiler diet significantly improved the body weight gain, FCR, and carcass yield of broilers compared to organic acid and probiotic treatments after a growing period of 42 days. The EOC may be considered as a potential growth promoter like organic acids and probiotics for environment-friendly broiler production.

19.5.2.2.3 In Vivo Experiment 3

The aim of the present study was to examine the effect of essential oils and breeder age on growth performance and some internal organs weight of broilers. A total of 1008 unsexed 1-day-old broiler chicks (Ross-308) originating from young (30 weeks) and older (80 weeks) breeder flocks were randomly divided into three treatment groups of 336 birds each, consisting of control and two EOMs at a level of 24 and 48 mg/kg diet. There were no significant effects of dietary treatments on body weight gain of broilers at days 21 and 42.

On the other hand, there were significant differences on the feed intake at days 21 and 42. The addition of 24 or 48 mg/kg EOM to the diet reduced significantly the feed intake compared to the control. The groups fed with the added EOM had significantly better FCR than the control at days 21 and 42. Although, there was no significant effect of broiler breeder age on body weight gain at day 21, significant differences were observed on body weight gain at 42 days of age. Broilers originating from young breeder flock had significantly higher body weight gain than those originating from old breeder flock at 42 days of age. No difference was noticed for carcass yield, liver, pancreas, proventriculus, gizzard, and small intestine weight. Supplementation with EOM to the diet in both levels significantly decreased mortality at days 21 and 42.

The results indicated that the Herbromix may be considered as a potential growth promoter. However, more trials are needed to determine the effect of essential oil supplementation to diet on the performance of broilers with regard to variable management conditions including different stress factors, essential oils and their optimal dietary inclusion levels, active substances of oils, dietary ingredients, and nutrient density (Cabuk et al., 2006a, 2006b; Alcicek et al., 2003, 2004; Bozkurt and Baser, 2002a, 2002b).

19.5.3 Pigs

CRINA® Pigs was tested on pigs. The results for the first 21-day period showed that males grew faster, ate less, and exhibited superior FCR compared to females. Although female carcass weight was higher, males had a significantly lower carcass fat than females (Losa, 2001).

The addition of fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*) oils was not found beneficial for weaned piglets. In feed choice conditions, fennel oil caused feed aversion (Schoene et al., 2006).

Oregano oil was found to be beneficial for piglets (Molnar and Bilkei, 2005).

In a preliminary investigation, the effects of low-level dietary inclusion of rosemary, garlic, and oregano oils on pig performance and pork quality were carried out. Unfortunately, no information on the species from which the oils were obtained and their composition existed in the paper. The pigs appeared to prefer the garlic-treated diet, and the feed intake and the average daily gain were significantly increased although no difference in the feed efficiency was observed. Carcass and meat quality attributes were unchanged, although a slight reduction of lipid oxidation was noted in oregano-fed pork. Since the composition of the oils is not clear, it is not possible to evaluate the results (Janz et al., 2007).

A study revealed that the inclusion of essential oil of oregano in pigs' diet significantly improved the average daily weight gain and FCR of the pigs. Pigs fed with the essential oils had higher carcass weight, dressing percentage, and carcass length than those fed with the basal and antibiotic-supplemented diet. The pigs that received the essential oil supplementation had a significantly lower fat thickness. Also lean meat and ham portions from these pigs were significantly higher. Therefore, the use of *Origanum* essential oil as feed additive improves the growth of pigs and has greater positive effects on carcass composition than antibiotics (Onibala et al., 2001).

Ropadiar®, an essential oil of the oregano plant, was supplemented in the diet of weaning pigs as alternative for antimicrobial growth promoters (AMGPs), observing its efficacy on the performance of the piglets. Ropadiar liquid contains 10% oregano oil and has been designed to be added to water. Compared to the negative control (without AMGP), Ropadiar® improved performance only during the first 14 days after weaning. Based on the results of this trial, it cannot be argued about the usefulness of Ropadiar® as an alternative for AMGP in diets of weanling pigs. However, its addition in prestarter diets could improve performance of these animals (Krimpen and Binnendijk, 2001).

The objective of another trial was to ascertain the effect on nutrient digestibilities and N-balance, as well as on parameters of microbial activity in the gastrointestinal tract of weaned pigs after adding oregano oil to the feed. The apparent digestibility of crude nutrients (except fiber) and the N-balance of the weaned piglets in this study were not influenced by feeding piglets restrictively with this feed additive. By direct microbiological methods, no influence of the additive on the gut flora could be found (Moller, 2001).

The inclusion of essential oil of spices in the pigs' diet significantly improved the average daily weight gain and FCR of the pigs in Groups 3, 4, and 5, as compared to Groups 1 and 2 ($P < 0.01$). Furthermore, pigs fed with the essential oils had higher carcass weight ($P < 0.01$), dressing percentage ($P < 0.01$), and carcass length ($P < 0.01$) than those fed with the basal and antibiotic-supplemented diet. In Groups 3, 4, and 5, backfat thickness was significantly lower than those in Groups 1 and 2. Moreover, lean meat and ham portions from pigs in Groups 3, 4, and 5 were significantly higher than those from pigs in Groups 1 and 2. In conclusion, the use of essential oils as feed additives improves the growth of pigs and has greater positive effects on carcass composition than antibiotics (Onibala et al., 2001).

19.6 ESSENTIAL OILS USED IN TREATING DISEASES IN ANIMALS

There is scarce scientific information on the use of essential oils in treating diseases in animals. Generally, the oils used in treating diseases in humans are also recommended for animals.

Internet literature is abound with valid and/or suspicious information in this issue. We have tried to compile relevant information using the reachable resources. The information may not be concise or comprehensive but should be seen as an effort to combine the available information in a short period of time.

The oil of *Ocimum basilicum* has been reported as an expectorant in animals. The combined oils of *Ocimum micranthum* and *Chenopodium ambrosioides* is claimed to treat stomach ache and colic in animals (http://www.ansci.cornell.edu/plants/medicinal/basil.html).

Bad breath as a result of gum disease and bacterial buildup on the teeth of pets can be treated by brushing their teeth with a mixture of a couple of tablespoons of baking soda, 1 drop of clove oil and 1 drop of aniseed oil. Lavender, myrrh, and clove oils can also be directly applied to their gums.

For wounds, abscesses, and burns, lavender and tea tree oils are used by topical application. Skin rashes can be treated with tea tree, lavender, and chamomile oils.

Earache of pets can be healed by dripping a mixture of lavender, chamomile, and tea tree oils (1 drop each) dissolved in a teaspoonful of grapeseed or olive oil in the infected ears.

Hoof rot in livestock can be treated with a hot compress made up of 10 drops of chamomile, 15 drops of thyme, and 5 drops of melissa oils diluted in about 100 ml of vegetable oil (e.g., grapeseed oil).

Intestinal worms of horses can be expelled by applying 3–4 drops of thyme oil and tansy leaves to each feed. Melissa oil can be added to feed to increase milk production of both cows and goats (http://scentsnsensibility.com/newsletter/Apr0601.htm).

Aromatic plants such as *Pimpinella isaurica*, *Pimpinella aurea*, and *Pimpinella corymbosa* are used as animal feed to increase milk secretion in Turkey (Tabanca et al., 2003).

To calm horses, chamomile oil is added to their feed. Pneumonia in young elephants caused by *Klebsiella* is claimed to be healed by *Lippia javanica* oil. Rose and yarrow oils bring about emotional release in donkeys by licking them. Wounds in horses are treated with *Achillea millefolium* oil; sweet itch is treated with peppermint oil. *Matricaria recutita* and *Achillea millefolium* oils are used to heal the skin with inflammatory conditions (Anonymous, 2008).

A study evaluated the effect of dietary oregano etheric oils as nonspecific immunostimulating agents in growth-retarded, low-weight growing-finishing pigs. A group of pigs were fed with commercial fattening diet supplemented with 3000 ppm oregano additive (Oregpig®, Pecs, Hungary), composed of dried leaf and flower of *Origanum vulgare*, enriched with 500 g/kg cold-pressed essential oils of the leaf and flower of *Origanum vulgare*, and containing 60 g carvacrol and 55 g thymol/kg. Dietary oregano improved growth in growth-retarded growing-finishing pigs and had nonspecific immunostimulatory effects on porcine immune cells (Walter and Bilkei, 2004).

Menthol is often used as a repellent against insects and in lotions to cool legs (especially for horses) (Franz et al., 2005).

Milk cows become restless and aggressive each time a group of cows are separated and regrouped. This can last a few days putting cows in more stress resulting in a drop in milk production. Two Auburn University scientists could solve this problem by spraying anise oil (*Pimpinella anisum*) on the cows. Treated animals could not distinguish any differences among the cows in new or old groupings. They were mellower and kept their milk production up. Among many other oils tested but only anise seemed to work (Anonymous, 1990).

Essential oils have been found effective in honeybee diseases (Ozkirim, 2006; Ozkirim et al., 2007).

In this review, we tried to give you an insight into the use of essential oils in animal health and nutrition. Due to the paucity of research in this important area there is not much to report. Most information on usage exists in the form of not-so-well-qualified reports. We hope that this rather preliminary report can be of use as a starting point for more comprehensive reports.

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20 Trade of Essential Oils

Hugo Bovill

The essential oil industry is highly complex and fragmented. There are at least 100 different producing countries, as can be seen from the map Essential Oils of the World (Figure 20.1). Many of these producing countries have been active in these materials for many decades. They are often involved in essential oils due to historical colonization, for example, clove oil from Madagascar has traditionally been purchased via France, nutmeg from Indonesia through Holland, and West Indian and Chinese products through Hong Kong and the United Kingdom. The main markets for essential oils are the United States (New Jersey), Germany, the United Kingdom, Japan, and France (Paris and Grasse). Within each producing country, there is often a long supply chain starting with the small peasant artisanal producer, producing just a few kilos, who then sells it to a collector who visits different producers and purchases the different lots that are then bulked together to form an export lot, which is then often exported by a firm based in the main capital or main seaport of that country. This exporter is equipped with the knowledge of international shipping regulations, in particular for hazardous goods, which applies to many essential oils. They also are able to quote in US$ or Euros, which is often not possible for small local producers (Figure 20.2).

Producers of essential oils can vary from the very large, such as an orange juice factory where orange oil is a by-product, down to a small geranium distiller (Figures 20.3 and 20.4).

The business is commenced by sending type samples that are examples of the production from the supplier and should be typical of the production that can be made going forward. Lot samples are normally provided to the purchaser in the foreign country to enable them to chemically analyze the quality organoleptically both on odor and flavor. It is essential that the qualities remain constant as differing qualities are not acceptable and there is normally no such thing as a "better" quality; it is either the same or it is not good. This is the key to building close relationships between suppliers in the country of origin and the purchaser.

Many suppliers try to improve their processes by adapting their equipment and modernizing. In Paraguay, petitgrain distillers replaced wooden stills with stainless steel stills on the advice of overseas aid noncommercial organizations (NCOs). This led to a change in quality and the declining usage of petitgrain oil. The quality issues made customers unhappy, and in fact the Paraguayan distillers reverted back to their traditional wooden stills (Figure 20.5).

Market information, as provided by the processor, is essential to developing long-term relationships. To enable the producer to understand market pricing, he should appreciate that when receiving more enquiries for an oil, it is likely that the price is moving upward and it is by these signs of demand that he can establish that there are potential shortages in the market (Figure 20.6).

Producers and dealers exporting oil should be prepared to commit to carry inventory to ensure carryover and adequate delivery reliability. It is important to note that with climate change, weather and market conditions are becoming increasingly important, and prior to planting, advice should be sought from the buyer as to their intentions, for short, medium, and long term. Long- and medium-term contracts are unusual and it is becoming increasingly common for flavor and fragrance companies not to commit over 1 year but to buy hand to mouth and purely give estimated volume needs going forward. This strengthens the role of the essential oil dealers, of whom there are very few remaining in the main trading centers of the world, such as the United States, France, the United Kingdom, Germany, and Japan.

FIGURE 20.1 **(See color fold-out insert at the back of the book)** World map showing production centers of essential oils. Courtesy of Treatt PLC.

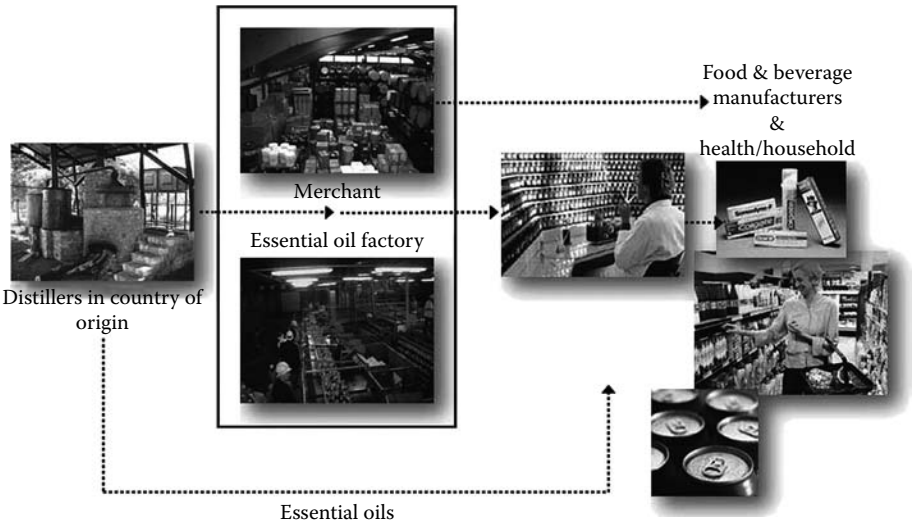

FIGURE 20.2 Flowchart showing the supply chain from distiller to finished product.

FIGURE 20.3 South American orange juice factory. (Photograph by kind permission of Sucocitrico Cutrale Ltd.)

FIGURE 20.4 Copper Still in East Africa.

FIGURE 20.5 Petitgrain still.

TREATT
PLC

Treatt Market Report
July 2007

Orange Oil Sweet

Strong demand currently for orange oil of all origins as we reach a period of the year where Florida plants are off season and Brazil is just beginning processing but oil of acceptable aldehyde is not yet available in volume for shipment from Brazil. The Brazilian crop this year is expected to be a very similar size to last season which is the first time the bi-annual cycle has been broken for 8 years. Better crop management including increased irrigation of groves and favourable weather conditions are two reasons cited for the better than expected crop in Brazil. Prices are moderately firm due to strong demand but this may subside as volume begins to come through in Brazil.

The 2006/07 crop in Florida was very low indeed at just 129 million boxes which contrasts markedly with the record crop of 1997/98 at 244 million boxes for example. As regularly reported in this column a significant hurricane event in Florida could result in a very firm market.

Lemon Oil

As volume availability improves, thanks to South American new crop the market price is showing signs of stabilising.

High quality oil continues to be in strong demand and discerning buyers are advised to carefully monitor the quality of their oils.

Lime Oil Distilled

Better fruit availability at the peak of the crop in Mexico has moved prices to lower levels as the market comes off the top of the cycle. However, strong fresh fruit demand is expected to keep the market firm compared with what we have seen in the last decade.

FIGURE 20.6 Market information.

To quote from *Marketing Essential Oils* (n.d.) by W.A. Ennever of R.C. Treatt & Co. Ltd, London in the 1960s, "The dealer serves as a buffer between these two interests (producer and essential oil merchant house) by purchasing and carrying stocks of oils for his own account and risk when the producer and/or merchant house is unable to wait for the user's demand and hold stocks until the latter is ready to purchase. The risk of market fluctuations to the essential oil dealer or merchant in this practice, is quite considerable, but naturally, is reduced by his knowledge and experience of the trade. He is equipped to handle large or small quantities and a range of qualities, as a buyer or seller. Thus through the dealer's participation, the producer has a larger number of outlets for his production and the user can be reasonably certain of finding supplies of the oils required when he considers it necessary to purchase." The dealer is aware of world markets and potential shortages that other producers may not be aware of, as these are happening in different continents. They can also have the knowledge of increasing demand and movements in consumer tastes.

Some essential oils are produced for their chemical constituents, whereas most are produced for their aromatic parts, and it is important that suppliers understand what is expected of them by their customer, whether it is chemical constituents naturally occurring or whether it is the aroma and flavor. Examples of this are turpentine oil, litsea cubeba oil, sassafras oil, clove leaf oil, and coriander oil.

There is greater demand for ethical supplies, but it should be borne in mind that these surprisingly often do not receive a premium and when entering the essential oil industry it is important to note that it is not always the highest priced oils that give the best return as these are often those that are the most popular for new entrants to produce. Before entering into production of an essential oil, it is important to fully verify the market. It may be that there is good supply locally of the herb, for example, but maybe this is for a traditional purpose such as local medicinal use, producing local foodstuffs, or liqueurs.

Origins are constantly changing and moving, as can be seen from the following: peppermint oil Mitcham production went from England to the United States; mint came from China, then went to Brazil and Paraguay, back to China and now to India.

Within the essential oil market, there are generally four different types of buyers: aromatherapy, the flavor and fragrance industries, and dealers. Many of these can be contacted through agents who would not pay for the goods themselves but would take a nominal commission of, say, 5%. The end users range from aromatherapists selling very small volumes of high, fine quality, natural essential oils to flavor and fragrance companies, and in a few cases, consumer product companies. The main markets are the essential oils dealers, of which there are probably 10 or 20 major companies remaining in the world, some of which are also involved in the manufacture of flavors or fragrances. To avoid conflicts of interest, it is perhaps better to work with those who concentrate solely on raw materials. Several of these companies have been established for many years and have a good trading history. Some information about them can be gained from their websites, but without meeting them in person, it is not easy to establish their credentials.

Conditions of trade are normally done on a FOB or a CIF basis, and the price should be given before samples are sent. With each sample, a Material Safety Data Sheet (MSDS), a Child Labor Certificate, and a Certificate of Analysis should be sent. It should be noted that the drums should be sealed and that the sample should be fully topped with nitrogen or be full to ensure that there is no oxygen present, in order to make sure that oxidation is avoided. The sample bottles should be made from glass and not from plastic to avoid contamination by phthalates. The lots should be bulked before sampling and a flashpoint test should be obtained to guarantee that it is within the law to send the sample by mail or by air freight with the correct labeling.

Many customers are able to give advice on production, but dealers in particular are best placed to advise. To enable contact with such dealers, it is worthwhile attending international meetings such as the International Federation of Essential Oils and Aroma Trades (IFEAT) annual conference or reading the *Perfumer and Flavorist* magazine, which gives full details of brokers, dealers, and essential oil suppliers. There is no reference site that is 100% reliable in pricing for essential

oils; this information should be gained by working with a variety of buyers, and from this a knowledge of the market can be acquired.

The essential oil industry is very traditional and even though there have been changes in analytical methods and demands, the knowledge required in 1950 by buyers such as Mr Ennever of Treatt (as can be seen from his quotation earlier in this chapter) are not too different from today. There is greater demand for organically certified, Kosher, Halal, and other standards. The market can change far quicker now than in the past, thanks to the worldwide web. Producers are often their own worst enemies and can destroy their own successful markets by communicating with their neighboring farmers, thereby encouraging them to enter the market. This can depress prices as a result of increased supply, but on the other hand, it can sometimes be in the interest of a sole producer to have other producers participating in the supply, to ensure guarantees of supply and to lower the costs of production, which in turn encourages buyers to use the oil. Oils such as patchouli and grapefruit have had significant changes in price, as can be seen in the price graphs in Figures 20.7 through 20.9.

These price movements have reduced demand as major buyers of these products have had to look for alternatives to replace them as they are unable to cope with the massively increased prices from US$10 to US$100 for grapefruit and from US$12.5 to US$70 per kilo for peppermint oil. It can be seen, therefore, that stable pricing can lead to increased demand. Unstable pricing can lead to the death of essential oils. This is an important reason for holding inventory so that producers can enter into long-term associations with essential oil buyers to ensure good relationships.

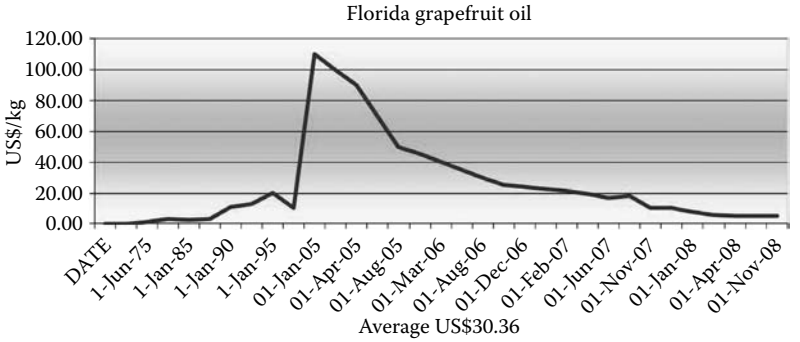

FIGURE 20.7 Price graph of grapefruit oil.

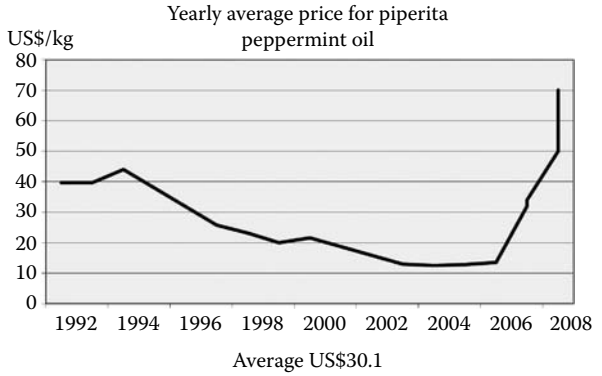

FIGURE 20.8 Price graph of peppermint oil (piperita).

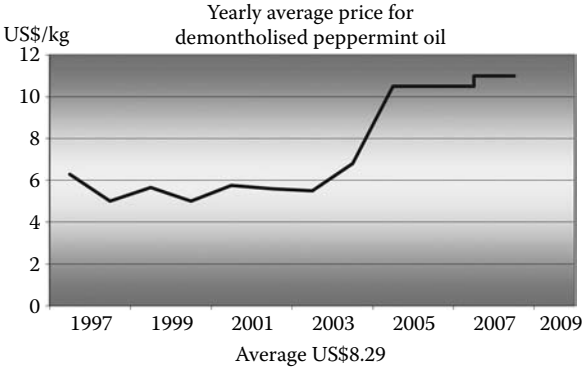

FIGURE 20.9 Price graph of peppermint oil (arvensis).

In the 1970s, there was considerable fraud of millions of dollars, caused by the shipment of essential oils from Indonesia to the major buyers. The oils were in fact water, despite analysis certificates from Indonesian Government laboratories showing them to be the named essential oil. Payment had been made by letters of credit and this fraudulent practice has discouraged buyers from opening letters of credit to suppliers today. Terms of trade should normally be cash against shipping documents or payment after receipt and quality control of goods.

The United States produces import statistics for essential oils and these can often be useful sources of information, and the European Union (EU) also has such statistics. The EU statistics cover a wide range of essential oils in each tariff; therefore the information is very vague and should not be used to make decisions. These statistics give no clues as to the quality of the product and it is that which can determine the price. The production of essential oils, as can be seen in the quotation by V.A. Beckley OBE, MC, Senior Agricultural Chemist, Kenya, who said during a meeting in 1931 in Nairobi, is perhaps more chancy than most farming propositions; it most certainly requires more attention and supervision than most, and, with certain rare exceptions, does not pay much more highly is still valid to this day, despite this being said in 1935.

The essential oil industry is a very small, tightly knit circle of traders, dealers, producers and consumers, and apart from some notable exceptions there is a very strong trade ethos. As it is a relatively small industry in terms of global commodities, statistics are not produced and it is by relationships with customers that information becomes available. Much that is on the Internet is misleading as it is for small quantities or is often written by consultants, and this information can be rapidly out-of-date as prices can move extremely quickly in either direction.

21 Storage and Transport of Essential Oils

Klaus-Dieter Protzen

CONTENTS

21.1 MARKETING OF ESSENTIAL OILS: THE FRAGRANT GOLD OF NATURE POSTULATES PASSION, EXPERIENCE, AND KNOWLEDGE

The trade of essential oils is affected more and more by Legal regulations related to Safety Aspects. The knowledge and the compliance with these superseding regulations have today become a *Conditio Sine Qua Non* (precondition) to ensure trouble-free global business relation as far as regulatory requirements are concerned as these requirements often may adversely affect usual commercial aspects.

When placing essential oils on the market in the EU for use as flavors and fragrances in foods, animal feed, cosmetic pharmaceuticals, aromatherapy, and so on, among others, the following regulations have to be observed (Dueshop, 2008):

- Council Directive 79/831/EEC—Dangerous Substances (see Section 21.6)
- Dangerous Preparations 99/45/EEC
- EU Flavouring Directive No. 88/388/EEC and a new EU flavor regulation in the stage of announcement
- Novel Food Directive No. 258/97/EEC
- Labelling Directive 2000/13/EC—food allergens
- EU Food Regulation No. 178/2002/EU
- New Pesticide Provisions—Regulation No. 396/2005/EU
- New EU Cosmetic Regulations Amending Directive No. 76/768/EEC—restrictions and bans (see Table 21.2 at the end of this chapter)
- Detergent Use Regulation No. 648/2004/EC
- EU Pharmaceutical Legislation—GMP aspects
- Biocide Use Directive No. 98/8/EEC
- Dangerous Substance Directive DSD 67/584/EEC

Essential oils are agro-based products that are generally manufactured by or collected from small individual producers. A large-scale production would require capital investment, which is rarely attracted as investors evidently realize the problems that no quick return of money is ensured because of too many factors influencing the market negatively like the dependency on weather conditions affecting the size of a crop over the whole vegetation period, competing crops challenging the acreage, a keen global competition striving for market shares, and narrow margins that do not compensate the involved risks. These aggravating factors also have an impact on the trade of essential oils.

A major part of the essential oil industry and the trade of these articles are dominated by small-scale and medium-sized family enterprises as only entrepreneurs with passion, a personal engagement and a persistent dedication as well as a long-standing experience nerve themselves to stay successfully in this business of the liquid gold of nature.

Success in the field of essential oils depends on enthusiasm and hard work, on a broad knowledge of the market situation, in spending a lot of time and cost to investigate new ideas of state-of-the-art conditions of processing raw materials that affect yield and quality and the return of investment, the adherence to comply with ever changing administrative regulations.

Essential oils are natural substances mainly obtained from vegetable raw materials either by distillation with water or steam or by mechanical process (expression) from the epicarp of citrus fruits. They are concentrated fragrance and flavor materials of complex composition, in general volatile alcohols, aldehydes, ethers, esters, ketones, hydrocarbons, and phenols of the group of mono- and sesquiterpenes or phenylpropanes as well as nonvolatile lactones.

A definition of the term essential oils and related fragrance/aromatic substances is given in the ISO-Norm 9235 Aromatic Natural Raw Materials (International Standard Organization, Geneva, 1997).

Because of their composition essential oils are classified by regulatory authorities in the EU as "Natural" but also as "Chemical Substances" (Dueshop, 2007).

The classification of chemical Substances is laid down in the Council Directive 67/548 and subsequent amendments but in particular in Council Directive 79/831/EEC of 18-09-1979. This 6th amendment is the basis of all existing regulations for dangerous/hazardous chemicals as it earmarked the beginning of a new era.

The topic REACH will not be covered in this chapter because of its complexity and too many open questions and answers respectively at the time of this writing. I hope, however, that in exchange a brief introduction to the historic development of the existing regulatory framework can be of help to understand the Safety Aspects, which are the background of the actual regulations as well as the forthcoming impediments in connection with REACH.

REACH is the abbreviation for *R*egistration, *E*valuation, *A*uthorization of *C*hemicals. It is another impeding Regulation in Europe—the consistent continuation of the existing rules to satisfy the EU administration of a perfect system to safeguard absolute security to protect humans and the environment regarding the use of chemicals within the EU.

For the trade, that is, the industry as well as importers and dealers of essential oils, REACH is a heavy burden demanding, already in the forefront, an unbelievable amount of time to clarify questions regarding the required product information for an appropriate registration of the so-called natural complex substances (NCS).

Before the publication of Directive 79/831/EEC only a few people were aware of the aftermath of a centralized European administration. Regulations regarding transport of dangerous goods were adhered—the trade of essential oils was well aware of the risk of flammability of many of the oils but most people, however, were caught more or less unprepared with regard to the new classification that natural essential oils have to be considered as "chemicals." The new Directive with its detailed regulations came as a surprise. It terminated the familiar view that essential oils because of their natural origin (and the fact they were used for centuries in medicines, flavors, and fragrances) could continue to exist as a special group of natural products like a sleeping beauty in the reality of a hostile world of administrative regulations. Now, all of a sudden it caused essential

oils to be considered as chemical substances of which a major part had to be classified as hazardous "chemical" substances.

21.2 THE IMPACT AND CONSEQUENCES ON THE CLASSIFICATION OF ESSENTIAL OILS AS NATURAL BUT CHEMICAL SUBSTANCES

The bell for the new era sounded when chemical substances in use within the EC during a reference period of 10 years had to be notified for European Inventory of Existing Commercial Chemical Substances (EINECS).

At that time EINECS enabled the EC administration not only to dispose of, for the first time, a survey of all chemical substances that had been in use in the EC between January 1, 1971 and September 18, 1981, but also to distinguish between "known substances" and "new substances."

"KNOWN" substances are all chemicals notified for EINECS, whereas all chemical substances that were not notified (and subsequently registered as "known substances" in EINECS) are considered by the EU administration as "new chemicals."

EINECS is a "closed list"—"New" chemical substances to be placed on the market in the EU after the deadline of September 18, 1981, therefore have to be notified for the European List of Notified Chemical Substances (ELINCS), the list complementing EINECS.

NEW chemical substances can be placed on—and used in—the market of the EU only after clearance according to uniform EC standards by competent (national) authorities. Thus, from the beginning, all potential risks of a (new) chemical substance are ascertained for a proper labeling for handling to avoid risks for humans as well as to protect the environment.

"Known" chemical substances (notified for EINECS) enjoyed, in a transitional phase, temporary exemption from the obligation to furnish the same safety data required for new chemical substances. Based on the experience gathered during their use, for quite a while it was assumed (Dueshop, 2007) that the temporary continuation of their use could be tolerated according to the hitherto used older standards of safety—and in view of the fact that a short-term clearance of approximately 100,000 chemical substances registered in EINECS could not be effected overnight.

Because these products have been notified for EINECS and therefore known to the regulative agencies in the EC, they are screened step by step either depending on their potential risk or according to the volumes produced or imported respectively to make sure that the known substances also comply with the new safety standards according to the following volume bands:

<100 kg
100–1000 kg
1–10 tons
10–100 tons
100–1000 tons
1000 tons plus.

Once new chemical substances have been cleared by the competent EC authorities, an ELINCS notification number—and later on an ELINCS registration number—is allocated. The names of the substances are published in regular intervals as newly registered chemical substances in ELINCS.

Responsible for the registration of substances in EINECS—and later on for ELINCS—was (is) the ECB/JRC (the European Chemical Bureau/Joint Research Centre of the European Commission at ISPRA). This agency was commissioned by the EC administration to allocate an EINECS registration number after having collected, evaluated, and arranged in proper order all notified substances.

To perform this task, the EU administration made use of the principles of the CAS system and arranged for the majority of essential oils and other UVCBs notified for EINECS the allocation of (new) CAS numbers.

But ATTENTION—the CAS number is an identification number for a chemical substance allotted by a private enterprise in the United States and must not be confused with the EINECS registration number.

EINECS and ELINCS numbers are registration numbers allocated by the EU administration, that is, ECB/JRC at ISPRA.

CAS numbers are assigned by the (private) CAS organization in the United States with the purpose of identification of (defined) chemical substances. A CAS number is allocated to a new (defined) chemical substance only after thorough examination of the product as per IUPAC Rules by the CAS organization to make sure that irrespective of different chemical descriptions and/or coined names that have been given to a product, a substance can be clearly related by the allocated CAS number according to the (CAS) principle "one substance—one number."

Using the CAS number system to register also chemical substances in EINECS that are not defined chemicals, the problem had to be sorted out how to register, for example, essential oils as they are products of complex composition. It was therefore necessary to extend the CAS system for this reason to allot a CAS number also to the so-called UVCBs, that is, substances that have been summed up under this abbreviation as substances of "*u*nknown or *v*ariable composition, *c*omplex reaction products, and *b*iological materials."

Essential oils are eventually registered as NCS by their botanical origin as for example:

Lavender oil: Lavender—*Lavandula angustifolia* ext.
EINECS registration no. 289-995-2—CAS no. (Einecs) 90063-37-9 extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, terpenes, terpene-free fractions, distillates, and residues from *Lavandula angustifolia*—Labiatae (Lamiaceae)

Lavender oil: Lavender—*Lavandula angustifolia* ext.
EINECS registration no. 283-994-0—CAS no. (Einecs) 84776-65-8 extractives … from *Lavandula angustifolia angustifolia*— Labiatae (Lamiaceae)

Lavender concrete/absolute: Lavender—*Lavandula angustifolia* ext.
EINECS registration no. 289-995-2—CAS no. (Einecs) 90063-37-9 extractives and their physically modified derivatives such as tinctures, *concretes, absolutes*, essential oils, terpenes, terpene-free fractions, distillates, and residues from *Lavandula angustifolia*—Labiatae (Lamiaceae)

Lavandin oil: *Lavandula hybrida* ext.
EINECS registration no. 294-470-6—CAS no. (Einecs) 91722-69-9 extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, terpenes, terpene-free fractions, distillates, and residues from *Lavandula hybrida*—Labiatae (Lamiaceae)

Lavandin oil abrialis: *Lavandula hybrida abrial* ext.
EINECS registration no. 297-384-7—CAS no. (Einecs) 93455-96-0 extractives and … from *Lavandula hybrida abrial*—Labiatae (Lamiaceae)

Lavandin oil grosso: *Lavandula hybrida grosso* ext.
EINECS registration no. 297-385-2—CAS no. (Einecs) 93455-97-1 extractives and … from *Lavandula hybrida grosso*—Labiatae (Lamiaceae).

Since essential oils are registered as extractives under their botanical origin, concretes/absolutes and other natural extractives of the same botanical origin have the same EINECS and CAS numbers as the essential oil.

When checking an EINECS number it is important to investigate in the official original documentation as in the secondary literature there exist too many inaccuracies.

TABLE 21.1
Examples of Different CAS-Numbers used in USA and EINECS in EU

	CAS No. USA	CAS No. EINECS	EC Registration No.
Eucalyptus oil	8000-48-4	84625-32-1	283-406-2
Eucalyptus globulus Lab.—Myrtaceae			
Lavender oil	8000-28-0	90063-37-9	289-995-2
Lavandula angustifolia—Labiatae			
Lavandula angustifolia angustifolia—Labiatae		84776-65-8	283-994-0
Lemon oil	8008-56-8	8028-48-6	284-515-8
		84929-31-7	284-515-8
Citrus limon L.—Rutaceae			
Orange oil	8008-52-9	8028-48-6	232-433-8
Citrus sinensis—Rutaceae			
Peppermint oil	8006-90-4	98306-02-6	308-770-2
Mentha piperita L.—Lamiaceae			
Manuka oil tairawhiti	—	223749-44-8	425-630-7
Leptospermum scoparium—Myrtaceae			(ELINCS)

Due to the lack of rules for an uniform classification of UVCBs (as an example the correct identification of the botanical origin of an essential oil), it happened that against the principles of the CAS organization in some cases several CAS numbers had been allocated to essential oils of the same denomination and in addition:

- An older CAS number allocated for an (earlier) registration of the product in the USA.
- A new CAS number allocated for registration in the EC for EINECS/ELINCS, respectively.

Once again, a CAS number does not mean that the product is registered in the European EINECS—the CAS number is just an identification number of a chemical substance allotted upon request by the (private) CAS organization.

Table 21.1 is exemplifying the allocation of several CAS numbers for the same essential oils but in connection with EINECS only the CAS number (EINECS) is of relevance.

Manuka Oil from New Zealand is the first (and only) essential oil that had to be notified for ELINCS as a new chemical substance after the Council Directive 79/831/EEC became effective on September 18, 1981 (Dueshop, 2007). It is quoted here only for the sake of completeness and curiosity.

This brief reflection on the background of EINECS and ELINCS is made as an introduction of the actual situation with regard to safety requirements and to alert new players in the field of essential oils to make sure that before intending to place a fragrance or flavor raw material on the European market they check whether or not this product is listed in EINECS or ELINCS respectively or is marketed in compliance with the Regulations of REACH for new substances. Placing of chemical substances in the states of the EU that are not meeting these requirements is a breach of law that can even be prosecuted as an offense with a penalty or a fine up to euros 100,000.

21.3 DANGEROUS SUBSTANCES AND DANGEROUS GOODS

There is a significant difference between the similar sounding words and regulations regarding DANGEROUS SUBSTANCES and DANGEROUS (HAZARDOUS) GOODS.

Both regulations are targeted to protect humans and the environment, but the term “Dangerous Substance” refers to the risks connected with the properties of the substance, that is, the potential risk of a direct contact with the product during production, packaging, and use.

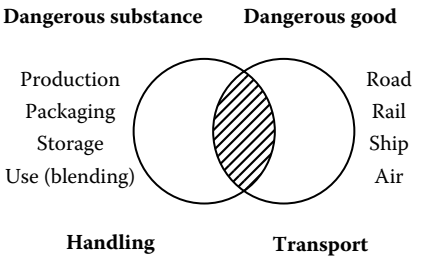

FIGURE 21.1 Interrelationship between dangerous substances and dangerous goods. (Friendly permission, Paul Kaders, Hamburg.)

Dangerous Substances are chemicals that fall into the categories quoted in article 2 of the already mentioned Council Directive 79/831/EEC—the 6th amendment of Directive 67/548. They are categorized as

Explosive
Oxidizing
Flammable (extremely flammable—highly flammable—flammable)
Toxicity (very toxic—toxic—harmful)
Corrosive (corrosive—irritant)
Dangerous for the environment (ecotoxicity)
Carcinogenic—teratogenic—mutagenic (CMR).

To protect humans and the environment—but principally the workers using them—articles that fall in these categories have to be classified as "Dangerous Substances" and labeled as per the subsequent Dangerous Substances Directive.

The term **Dangerous Goods** refer to dangerous substances properly packed and labeled for storage and transport by road, rail, sea, or air (Figure 21.1).

As per the rules and recommendations developed by a UN Committee of Experts regarding the transport of dangerous goods or substances they are defined as articles or substances that are capable of posing a risk to health, safety, property, or the environment.

Dangerous goods are classified into the following groups (classes of relevance for essential oils have been marked in bold font):

Class 1: Explosives
Class 2: Gases
Class 3: Flammable liquids
Class 4: Flammable solids
Class 5: Oxidizing substances and organic peroxides
Class 6: Toxic and infectious substances—eventually "***poison***"
Class 7: Radioactive material
Class 8: Corrosives
Class 9: Miscellaneous dangerous goods.

21.4 PACKAGING OF DANGEROUS GOODS

Dangerous goods must be transported in UN-approved packaging, which has been tested for sufficient stability and graded in the packing groups (PGs) I, II, and III.

PG III (low risk)—Suitable for dangerous goods having a low-risk classification only.
PG III corresponds to the UN packing code "Z"

PG II (medium risk)—This type of packing matches the requirements for most of the essential oils.
PG II corresponds to UN packing code "Y"—PG II includes PG III.
PG I (high risk) corresponds to UN packing code "X".
PG I includes PG II and III—this type of packing has the highest stability.

All dangerous goods have to be packed in the so-called UN-approved packing.

Essential oils that are classified as dangerous goods and shipped in bulk, that is, drum lots for example, will only be accepted for transport if they are packed in UN-approved iron drums. These drums with a bunghole for example bear the following UN code:

UN 1A1/Y/1.4/150/(06)/(NL)/(VL824)

This specification reveals the following details:

1A1	Steel drum—nonremovable head
Y	PG II
1.4	Maximum relative density at which the packing has been tested
150	Test pressure
(0.6)	Year of manufacture
(NL)	State (country)
(VL 123)	Code number of manufacturer

The potential risks of dangerous substances or goods respectively have to be declared in the relevant transport documentation. In addition to this information, also warning labels have to be used on the packages to alert workers regarding the nature of the goods they are handling.

The aim of dangerous goods regulations is not only to protect persons occupied with the conveyance of dangerous substances but to also serve, for example, fire brigades, who in case of an accident or fire are called and have to be aware of the risks.

In this connection, a few words are due on the so-called UN/ID numbers for dangerous goods. These UN numbers are assigned to dangerous goods according to their hazard classification and composition. These UN (hazard identification) numbers should not be confused with the number of UN packaging.

UN numbers are listed in all regulations for transport of dangerous goods and are identical for all types of transport.

Approximately 170 essential oils have to be classified as dangerous substances/goods. According to their composition, the following UN numbers have been assigned to these oils:

65	UN no. 1169—extracts, aromatic, and liquid
52	UN no. 3082—environmentally hazardous substance, liquid, n.o.s.
14	UN no. 1272—pine oil(s)
6	UN no. 1992—flammable liquid, toxic, n.o.s.
6	UN no. 2810—toxic liquid organic n.o.s.
5	UN no. 2319—terpene hydrocarbons

and others are distributed among the UN nos. 2811 (3), 2924 (3), 1545 (2), 1130 (1), 1197 (1), 1201 (1), 1299 (1), 1990 (1), and 3077 (1).

Details can be found in EFFA's Code of Practice (CoP, 2008, et seq.), which is described later on.

Consignments of dangerous substances (and dangerous goods respectively) must be accompanied by a so-called Material Safety Data Sheet. For this purpose, the International Standard

Organization (ISO) has developed a standard form that—divided into 16 headings—provides basically information on

Name of the supplier
Name and identification of the substance/preparation
Composition/components of the article
Hazard identification
First aid measures
Fire fighting measures
Accidental release measures
Ecological information
Transport information
Regulatory information and so on

to inform users and forwarders about the risks in connection with the chemical substance.

Not only producers but also suppliers have the responsibility that the MSDS Form (material safety datasheet) is properly completed.

21.5 LABELING

IFRA and IOFI have regularly informed their members as well as stakeholders in the industry and trade for more than five decades about potential health risks that have been assessed for natural and synthetic raw materials used in flavors and fragrances in research and tests.

Since a couple of years ago, the European Association of the Flavour and Fragrance Industry in Europe (EFFA) has been publishing a Code of Practice (CoP, 2008) with recommendations regarding a proper classification and labeling of aromatic chemicals and essential oils.

This "CoP" is complementing the information of IFRA and IOFI. It is continuously updated by experts of the industry and the trade by the Hazard Communication Working Group (HCWG) and furnishes for the disposal of people all over the world occupied in handling essential oils and aromatic chemicals; an up-to-date recommendation for a proper classification and labeling of hazardous fragrance and flavor raw materials (Protzen, 1989).

The actual version of this CoP 2009 is available on the internet free of charge from the homepage of EFFA: **http://www.effa.be/**.

Because of the compiled state-of-the-art expertise, EFFA's CoP has almost obtained in practice the quality of an official documentation. Therefore not only the trade but also the port and transport authorities who are in charge of controlling the compliance of safety regulations for transport of dangerous goods are today referring to this guideline (Protzen, 1998).

For approximately. 1200 aromatic chemicals used in the flavor and fragrance industry and 220 commercially used essential oils as well as information on 60 natural extracts like absolutes and resinoids, the CoP contains a guideline detailing information on

EC registration number
CAS number relevant in the EC/EU
CAS number relevant in the USA
Commercial name
Content of hydrocarbons (%)
Warning labels
UN Transport Regulations (dangerous goods class, required class of packing group class, appropriate UN number)

R (Risk) phrases
S (Safety) phrases.

Before the Council Directive 79/831/EEC was issued, flammability of essential oils was considered the main danger emanating from these articles. Today's knowledge of potential risks of essential oils is extended. As a precaution very rigid safety regulations that consider extreme conditions often exceeding empirical and practical experience require that from the 220 essential oils listed in the CoP 2008, approximately 70%, that is, 170 essential oils, are classified as dangerous Substances and therefore must be labeled accordingly for storage, use, and transport, as for example:

Harmful

Dangerous for the environment

Irritant

The following warning labels cover the majority of risks:

190	Xn	Harmful—a St. Andrew's Cross (Xn)
174	N	Dangerous for the environment
60	Xi	Irritant—a St. Andrew's Cross (Xi)
12	T	Toxic—a skull and cross-bones (T)
3	C	Corrosive—the symbol showing the damaging effect of an acid

In addition to this information also R-phases and R labels must be used on the packaging. A list that explains the meaning of R + S phrases required for labeling essential oils as per the CoP is enclosed for further perusal.

A statistical evaluation of the R (Risk) labels to be used is shown in the following differentiation to have a better and detailed idea of the potential risks:

205	R-43	May cause sensation by skin contact
158	R-65	Harmful—may cause lung damage if swallowed
103	R-51/53	Toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
95	R-38	Harmful if swallowed
88	R-50/53	Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
80	R-10	Flammable
40	R-52/53	Harmful to aquatic organisms—may cause long-term adverse effects on the aquatic environment
38	R-22	Harmful if swallowed.

Flammability as a major risk of essential oils is today outnumbered by the potential risks emanating from these concentrated fragrances and flavors causing harm to the skin, to the health risk if swallowed, and their ecotoxicity.

The cumulative frequency of occurrence reveals that the majority of essential oils have to be handled with care and workers should use a protection particularly when a contact of these concentrated volatile natural fragrance and flavor materials with the skin is possible.

Special care and attention should be given when handling essential oils labeled with

R-50/53	Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-34	Causes burns (oils containing thymol)
R-45	May cause cancer (oils containing safrol)
R-68	Possible risk of irreversible effects

Safety starts at the point of production but in the chain of supply each party involved is directly responsible for proper handling, that is, declaration and labeling of goods. In Europe, a special transport police is in the ports and on the roads intensifying the controls for correct declaration, packaging and labeling of dangerous goods and heavy fines are imposed:

Risk phrases applicable for storage and transport of essential oil—data as per EFFA CoP 2008:

R-10	Flammable
R-20	Harmful by inhalation
R-21	Harmful in contact with the skin
R-22	Harmful if swallowed
R-23	Toxic by inhalation
R-24	Toxic in contact with the skin
R-25	Toxic if swallowed
R-26	Very toxic by inhalation
R-27	Very toxic in contact with the skin
R-34	Causes burns
R-36	Irritating to eyes
R-37	Irritating to the respiratory system
R-38	Irritating to the skin
R-41	Risk of serious damage to eyes
R-43	May cause sensation by skin contact
R-45	May cause cancer
R-65	Harmful—may cause lung damage if swallowed
R-66	Repeated exposure may cause skin dryness or cracking
R-68	Possible risk of irreversible effects
R-21/22	Harmful in contact with skin and if swallowed
R-36/38	Irritating to eyes and skin
R-50/53	Very toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-51/53	Toxic to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-52/53	Harmful to aquatic organisms—may cause long-term adverse effects on the aquatic environment
R-68/22	Harmful—possible risk of irreversible effects if swallowed

21.6 LIST OF REGULATIONS FOR THE CONSIDERATION OF DOING BUSINESS IN THE EU

- Council Directive 79/831/EEC of 18 September 1979 amending for the sixth time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances
  OJ L 259, 15.10.1979, p. 10–28 (DA, DE, EN, FR, IT, NL)
- Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations
  OJ L 200, 30.7.1999, p. 1–68 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Council Directive 88/388/EEC of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production
  OJ L 184, 15.7.1988, p. 61–66 (ES, DA, DE, EL, EN, FR, IT, NL, PT)
- Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients
  OJ L 43, 14.2.1997, p. 1–6 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs
  OJ L 109, 6.5.2000, p. 29–42 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety
  OJ L 31, 1.2.2002, p. 1–24 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EECText with EEA relevance.
  OJ L 70, 16.3.2005, p. 1–16 (ES, CS, DA, DE, ET, EL, EN, FR, IT, LV, LT, HU, MT, NL, PL, PT, SK, SL, FI, SV)
- Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents (Text with EEA relevance)
  OJ L 104, 8.4.2004, p. 1–35 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market
  OJ L 123, 24.4.1998, p. 1–63 (ES, DA, DE, EL, EN, FR, IT, NL, PT, FI, SV)
- Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances
  OJ 196, 16.8.1967, p. 1–98 (DE, FR, IT, NL) English special edition: Series I Chapter 1967 P. 0234
- Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products
  (OJ L 262, 27.9.1976, p. 169)

For amendments see Table 21.2.

TABLE 21.2

COUNCIL DIRECTIVE
of 27 July 1976
on the approximation of the laws of the Member States relating to cosmetic products
(76/768/EEC)
(OJ L 262, 27.9.1976, p. 169)

Amended by		Official Journal No	Page	Date
▶ **M1**	Council Directive 79/661/EEC of 24 July 1979	L 192	35	31.7.1979
▶ **M2**	Commission Directive 82/147/EEC of 11 February 1982	L 63	26	6.3.1982
▶ **M3**	Council Directive 82/368/EEC of 17 May 1982	L 167	1	15.6.1982
▶ **M4**	Commission Directive 83/191/EEC of 30 March 1983	L 109	25	26.4.1983
▶ **M5**	Commission Directive 83/341/EEC of 29 June 1983	L 188	15	13.7.1983
▶ **M6**	Commission Directive 83/496/EEC of 22 September 1983	L 275	20	8.10.1983
▶ **M7**	Council Directive 83/574/EEC of 26 October 1983	L 332	38	28.11.1983
▶ **M8**	Commission Directive 84/415/EEC of 18 July 1984	L 228	31	25.8.1984
▶ **M9**	Commission Directive 85/391/EEC of 16 July 1985	L 224	40	22.8.1985
▶ **M10**	Commission Directive 86/179/EEC of 28 February 1986	L 138	40	24.5.1986
▶ **M11**	Commission Directive 86/199/EEC of 26 March 1986	L 149	38	3.6.1986
▶ **M12**	Commission Directive 87/137/EEC of 2 February 1987	L 56	20	26.2.1987
▶ **M13**	Commission Directive 88/233/EEC of 2 March 1988	L 105	11	26.4.1988
▶ **M14**	Council Directive 88/667/EEC of 21 December 1988	L 382	46	31.12.1988
▶ **M15**	Commission Directive 89/174/EEC of 21 February 1989	L 64	10	8.3.1989
▶ **M16**	Council Directive 89/679/EEC of 21 December 1989	L 398	25	30.12.1989
▶ **M17**	Commission Directive 90/121/EEC of 20 February 1990	L 71	40	17.3.1990
▶ **M18**	Commission Directive 91/184/EEC of 12 March 1991	L 91	59	12.4.1991
▶ **M19**	Commission Directive 92/8/EEC of 18 February 1992	L 70	23	17.3.1992
▶ **M20**	Commission Directive 92/86/EEC of 21 October 1992	L 325	18	11.11.1992
▶ **M21**	Council Directive 93/35/EEC of 14 June 1993	L 151	32	23.6.1993
▶ **M22**	Commission Directive 93/47/EEC of 22 June 1993	L 203	24	13.8.1993
▶ **M23**	Commission Directive 94/32/EC of 29 June 1994	L 181	31	15.7.1994
▶ **M24**	Commission Directive 95/34/EC of 10 July 1995	L 167	19	18.7.1995
▶ **M25**	Commission Directive 96/41/EC of 25 June 1996	L 198	36	8.8.1996
▶ **M26**	Commission Directive 97/1/EC of 10 January 1997	L 16	85	18.1.1997
▶ **M27**	Commission Directive 97/18/EC of 17 April l997	L 114	43	1.5.1997
▶ **M28**	Commission Directive 97/45/EC of 14 July 1997	L 196	77	24.7.1997
▶ **M29**	Commission Directive 98/16/EC of 5 March 1998	L 77	44	14.3.1998
▶ **M30**	Commission Directive 98/62/EC of 3 September 1998	L 253	20	15.9.1998
▶ **M31**	Commission Directive 2000/6/EC of 29 February 2000	L 56	42	1.3.2000
▶ **M32**	Commission Directive 2000/11/EC of 10 March 2000	L 65	22	14.3.2000
▶ **M33**	Commission Directive 2000/41/EC of 19 June 2000	L 145	25	20.6.2000
▶ **M34**	Commission Directive 2002/34/EC of 15 April 2002	L 102	19	18.4.2002
▶ **M35**	Commission Directive 2003/1/EC of 6 January 2003	L 5	14	10.1.2003
▶ **M36**	Commission Directive 2003/16/EC of 19 February 2003	L 46	24	20.2.2003
▶ **M37**	Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003	L 66	26	11.3.2003
▶ **M38**	Commission Directive 2003/80/EC of 5 September 2003	L 224	27	6.9.2003

continued

TABLE 21.2 (continued)

Amended by		Official Journal No	Page	Date
▶ **M39**	Commission Directive 2003/83/EC of 24 September 2003	L 238	23	25.9.2003
▶ **M40**	Commission Directive 2004/87/EC of 7 September 2004	L 287	4	8.9.2004
▶ **M41**	Commission Directive 2004/88/EC of 7 September 2004	L 287	5	8.9.2004
▶ **M42**	Commission Directive 2004/94/EC of 15 September 2004	L 294	28	17.9.2004
▶ **M43**	Commission Directive 2004/93/EC of 21 September 2004	L 300	13	25.9.2004
▶ **M44**	Commission Directive 2005/9/EC of 28 January 2005	L 27	46	29.1.2005
▶ **M45**	Commission Directive 2005/42/EC of 20 June 2005	L 158	17	21.6.2005
▶ **M46**	Commission Directive 2005/52/EC of 9 September 2005	L 234	9	10.9.2005
▶ **M47**	Commission Directive 2005/80/EC of 21 November 2005	L 303	32	22.11.2005
▶ **M48**	Commission Directive 2006/65/EC of 19 July 2006	L 198	11	20.7.2006
▶ **M49**	Commission Directive 2006/78/EC of 29 September 2006	L 271	56	30.9.2006
▶ **M50**	Commission Directive 2007/1/EC of 29 January 2007	L 25	9	1.2.2007
▶ **M51**	Commission Directive 2007/17/EC of 22 March 2007	L 82	27	23.3.2007
▶ **M52**	Commission Directive 2007/22/EC of 17 April 2007	L 101	11	18.4.2007
▶ **M53**	Commission Directive 2007/53/EC of 29 August 2007	L 226	19	30.8.2007
▶ **M54**	Commission Directive 2007/54/EC of 29 August 2007	L 226	21	30.8.2007
▶ **M55**	Commission Directive 2007/67/EC of 22 November 2007	L 305	22	23.11.2007
▶ **M56**	Commission Directive 2008/14/EC of 15 February 2008	L 42	43	16.2.2008
▶ **M57**	Commission Directive 2008/42/EC of 3 April 2008	L 93	13	4.4.2008
▶ **M58**	Commission Directive 2008/88/EC of 23 September 2008	L 256	12	24.9.2008
▶ **M59**	Commission Directive 2008/123/EC of 18 December 2008	L 340	71	19.12.2008
▶ **M60**	Directive 2008/112/EC of the European Parliament and of the Council of 16 December 2008	L 345	68	23.12.2008
▶ **M61**	Commission Directive 2009/6/EC of 4 February 2009	L 36	15	5.2.2009
Amended by				
▶ **A1**	Act of Accession of Greece	L 291	17	19.11.1979
▶ **A2**	Act of Accession of Spain and Portugal	L 302	23	15.11.1985
Corrected by				
▶ **C1**	Corrigendum, OJ L 255, 25.9.1984, p. 28 (84/415/EEC)			
▶ **C2**	Corrigendum, OJ L 157, 24.6.1988, p. 38 (88/233/EEC)			
▶ **C3**	Corrigendum, OJ L 199, 13.7.1989, p. 23 (89/174/EEC)			
▶ **C4**	Corrigendum, OJ L 273, 25.10.1994, p. 38 (94/32/EC)			
▶ **C5**	Corrigendum, OJ L 341, 17.12.2002, p. 71 (2002/34/EC)			
▶ **C6**	Corrigendum, OJ L 151, 19.6.2003, p. 44 (2002/34/EC)			
▶ **C7**	Corrigendum, OJ L 58, 26.2.2004, p. 28 (2003/83/EC)			
▶ **C8**	Corrigendum, OJ L 97, 15.4.2005, p. 63 (2004/93/EC)			
▶ **C9**	Corrigendum, OJ L 258, 4.10.2007, p. 44 (2007/54/EC)			
▶ **C10**	Corrigendum, OJ L 136, 24.5.2008, p. 52 (2008/42/EC)			

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22 Recent EU Legislation on Flavors and Fragrances and Its Impact on Essential Oils

Jan C.R. Demyttenaere

CONTENTS

22.1 INTRODUCTION

In the last years, several new European Regulations and Directives have been adopted or announced in relation to flavors and fragrances. As essential oils and extracts are very important ingredients for flavoring and fragrance applications, these new regulations will have a major impact on the trade and use in commerce of these essential oils and extracts.

This chapter will focus on some pieces of legislation that are of major importance for the Flavour and Fragrance (F&F) Industry, such as the Cosmetic Directive 76/768/EC and especially its Seventh Amendment (2003/15/EC) and the first amendment of the Detergent Regulation (June 2006), which make the labeling of 26 specific fragrance ingredients (the so-called 26 "alleged" allergens) mandatory: the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of "complex ingredients" such as extracts and essential oils).

Some attention will be paid to the new Flavouring Regulation (part of the so-called Food Improvement Agents Package) that will replace the current Flavouring Directive 88/388/EEC and that is currently under discussion at the EU Commission, EU Parliament and Council levels.

Also the issue of hazard classification and labeling of dangerous substances and preparations, and essential oils containing hazardous components will be addressed and some examples will be given. This relates to the recent publication of the Commission Directive 2006/8/EC amending the Dangerous Preparations Directive 1999/45/EC.

22.2 COSMETIC AND DETERGENT LEGISLATION AND ALLERGEN LABELING

22.2.1 History and Background

In recent years (the late 1980s and 1990s), there has been a scientific debate on the safety of fragrance (perfumery) ingredients. Dermatologists have highlighted the risk of contact allergy from fragrance ingredients (Santussi et al., 1987; Becker et al., 1994), and actions to prevent the disease have been requested (Frosch et al., 1995; Larsen et al., 1996; SCCNFP 0017/98).

As a result of this and in response to a question from a Member State (MS) and members of the European parliament, the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) has been asked by DG Enterprise (EU Commission) to respond to the following mandate in relation to the safety of fragrance ingredients and to answer (among others) the following questions:

- It is proposed that all known fragrance allergens are labeled on cosmetics if used in the products. Does the SCCNFP agree to this proposal? If so,
  - Which chemicals fall under this classification?
  - Is there a maximum concentration of each chemical permissible without the requirement for labeling?
- Restrictions are proposed for the three most common fragrance allergens (cinnamic aldehyde, isoeugenol, and hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so
  - Which fragrance materials should be subject to restrictions?
  - What are the conditions for restrictions (maximum concentration, fields of applications, etc.)?

Other questions were related to industry-restricted and industry-prohibited substances.

In its Interim position on Fragrance allergy SCCNFP/0202/99 adopted at the SCCNFP session of June 23, 1999, the SCCNFP already stated: "Contact allergy to fragrance substances is an important clinical problem. Up to 10% of individuals with eczema are allergic to fragrance substances and possibly 1–2% of the general population."

In the same Interim position, SCCNFP considered that the mandate from the European Commission could be usefully divided into the following two sections:

1. Identification of those fragrance ingredients that are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy as a means to avoid contact with an allergen. An opinion as to whether such an identification can be related to concentrations present in a product when elicitation levels are known.
2. An opinion on the adoption of industry-prohibited substances into Annex 2 and adoption of industry-restricted substances into Annex 3. Consideration as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such an inclusion in Annex 22.3. Whether there are additional substances that should be subject to inclusion in an annex.

Taking into account the importance and enormity of the mandate, it was concluded that the first section should be considered initially.

As a result, the SCCNFP published, as a follow-up to the Interim position, its opinion SCCNFP/0017/98 (adopted by the SCCNFP during the plenary session of December 8, 1999) entitled "Fragrance allergy in consumers—A review of the problem: Analysis of the need for appropriate consumer information and Identification of consumer allergens."

This opinion relates to the first section mentioned above and consists of

- A critical review of the problem of fragrance allergy in consumers.
- Identification of those fragrance ingredients that are well recognized as consumer allergens.
- An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

Allergy to natural ingredients (such as oakmoss) was not addressed in this opinion but was analyzed separately (see SCCNFP opinion of October 24, 2000).

It was the opinion of the SCCNFP that

- Fragrance ingredients have to be considered as an important cause of contact allergy.
- Based on criteria restricted to dermatological data reflecting the clinical experience, it was possible to identify 24 fragrance ingredients, which correspond to the most frequently recognized allergens. Thirteen of these have been reported more frequently; these are well-recognized contact allergens in consumers and are thus of most concern; 11 others are less well documented.

In the opinion (SCCNFP/0017/98), two lists were given: a List A with 13 fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognized consumer allergens, and a List B with 11 fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens.

Tables 22.1 and 22.2 review the substances of Lists A and B.

In addition, the SCCNFP stated in its opinion that information should be provided to consumers about the known presence in cosmetic products of fragrance ingredients with a well-recognized potential to cause contact allergy: "Information regarding these fragrance chemicals should be given to consumers if deliberately added to a fragrance formulation either in the form of a chemical or as an identified constituent of an ingredient."

TABLE 22.1
List A (SCCNFP/0017/98)—13 Most Frequently Reported Allergens (CAS No.)

Amyl cinnamal (122-40-7)	Amylcinnamyl alcohol (101-85-9)
Benzyl alcohol (100-51-6)	**Benzyl salicylate (118-58-1)**
Cinnamyl alcohol (104-54-1)	**Cinnamal (104-55-2)**
Citral (5392-40-5)	**Coumarin (91-64-5)**
Eugenol (97-53-0)	**Geraniol (106-24-1)**
Hydroxycitronellal (107-75-5)	Hydroxymethylpentylcyclohexene-carboxaldehyde (HMPCC) (31906-04-4)
Isoeugenol (97-54-1)	

Note: Substances highlighted in bold are naturally occurring fragrance materials and the other substances are synthetic fragrance ingredients that are not known to occur in nature.

TABLE 22.2
List B (SCCNFP/0017/98)—11 Less Frequently Reported Allergens (CAS No.)

Anisyl alcohol (105-13-5)	**Benzyl benzoate (120-51-4)**
Benzyl cinnamate (103-41-3)	**Citronellol (106-22-9)**
Farnesol (4602-84-0)	Hexyl cinnamaldehyde (101-86-0)
Lilial (80-54-6)	***d*-Limonene (5989-27-5)**
Linalool (78-70-6)	Methyl heptine carbonate (111-12-6)

3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one (=alpha-iso-methylionone) (127-51-5)

Note: Substances highlighted in bold are naturally occurring fragrance materials and the other substances are synthetic fragrance ingredients that are not known to occur in nature.

Additionally, as mentioned above, also two natural ingredients, oakmoss and tree moss extracts, were addressed in a separate SCCNFP opinion (adopted during the 14th plenary meeting of October 24, 2000).

These two natural mosses are identified as follows: oakmoss extracts derived from the lichen, *Evernia prunastri* (L.) Arch. (Usneaceae), growing primarily on oak trees, and tree moss extracts derived from a mixture of lichens, mainly *Evernia furfuracea* (L.) Arch. (Usneaceae) growing on *Pinus* species.

Oakmoss extract has CAS no. 90028-68-5 and EINECS no. 289-861-3.

Tree moss extract has CAS no. 90028-67-4 and EINECS no. 289-860-8.

The term "labeling" comes from the EU Commission (DG Enterprise) and whether a fragrance ingredient should be labeled or not is a Risk Manager's decision. In its *memorandum* of 2001 (SCCNFP/0450/01), the SCCNFP (being the Risk Assessor) clearly states that "because of the lack of dose/elicitation data for these substances, the SCCNFP has been unable to provide recommendations on levels above which the information to the consumer would be necessary." Nevertheless, SCCNFP mentions in its memorandum that it is "aware that for practical risk management reasons there is a need for threshold levels for the provision of information." There is a proposal that for leave-on products, this threshold level should be 10 ppm in the finished cosmetic product, whereas for rinse-off products, the SCCNFP would consider a working level 10 times higher than that recommended for leave-on products to be reasonable, being 100 ppm.

22.2.2 Cosmetic Directive and its Seventh Amendment

The EU Commission has implemented the above-mentioned SCCNFP opinions in the 7th Amendment of the Cosmetic Directive 76/768/EC (2003/15/EC) by adding the following restrictions [limitations and requirements (for labeling)] to 26 fragrance substances in Annex III, Part 1: "The presence of the substance must be indicated in the list of ingredients referred to in Article 6(1)(g) when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products."

However, no further restrictions (such as maximum authorized concentrations in the finished cosmetic products), except the labeling requirements were introduced at that time.

This means that the presence of any of the 26 alleged allergens (sensitizers) must be indicated (labeled) in the list of ingredients on the packaging of the finished cosmetic products when its concentration exceeds 10 ppm (leave-on products) or 100 ppm (rinse-off products), according to Art. 6.1(g) of the Cosmetic Directive, 7th Amendment.

However, it is important to note here that the Fragrance Industry is self-regulating by issuing the International Fragrance Association Code of Practice (IFRA CoP), which is published by the IFRA. This CoP consists of Standards (the so-called IFRA Standards) for the fragrance ingredients with certain restrictions/limitations and in some cases bans to which the International Fragrance Industry should comply. The last amendment of the IFRA CoP is the 44th Amendment. The IFRA CoP and

its 44th Amendment and the IFRA Standards can be found on the homepage of the International Fragrance Association: www.ifraorg.org.

22.2.3 Impact on Extracts and Essential Oils and Aromatic Natural Raw Materials

The mandatory labeling requirement for the 26 alleged allergens is irrespective of the source of the allergen or the way by which it has been introduced in the final cosmetic product. In other words, the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of "complex ingredients" such as extracts and essential oils). This means that the use of essential oils containing them in formulations may lead to the presence of such allergens and the labeling requirement will apply.

Sixteen of the 24 alleged allergenic substances are naturally occurring (see substances indicated in bold in Tables 22.1 and 22.2), the other eight substances are synthetic fragrance ingredients that do not occur in nature as far as known.

The structures of the 16 naturally occurring allergenic substances are depicted in Figures 22.1 and 22.2.

The remaining two alleged allergens are aromatic natural raw materials by themselves: oakmoss (*E. prunastri*) and tree moss (*E. furfuracea*).

According to the current knowledge of the F&F Industry, these 16 allergens occur in about 180 natural raw materials (extracts and essential oils) (EFFA CoP, 2007).

A list of aromatic natural raw materials containing any of the 16 naturally occurring sensitizers and their presence (if >0.1%) or concentration can be found in Annex 22.1 to this chapter—this is based on earlier internal communication (2004) of the F&F industries related to a former version of the EFFA CoP.

One of the key challenges for the Fragrance Industry is the analysis and identification of the 16 naturally occurring allergens in the natural raw materials (extracts and essential oils) and fragrance compounds (mixtures and preparations). To address this work, the Fragrance Industry has established an Analytical Working Group of IFRA where methods of analysis are developed. A recommended method of analysis for gas chromatography-mass spectrometry (GC-MS) quantification of suspected allergens in fragrance compounds has been published by this group in 2003 (Chaintreau et al., 2003). Some further work on the investigation of the GC-MS determination of allergens (GC-MS quantification of allergens in fragrances and data treatment strategies and method performances) was published more recently by the same group (Chaintreau et al., 2007).

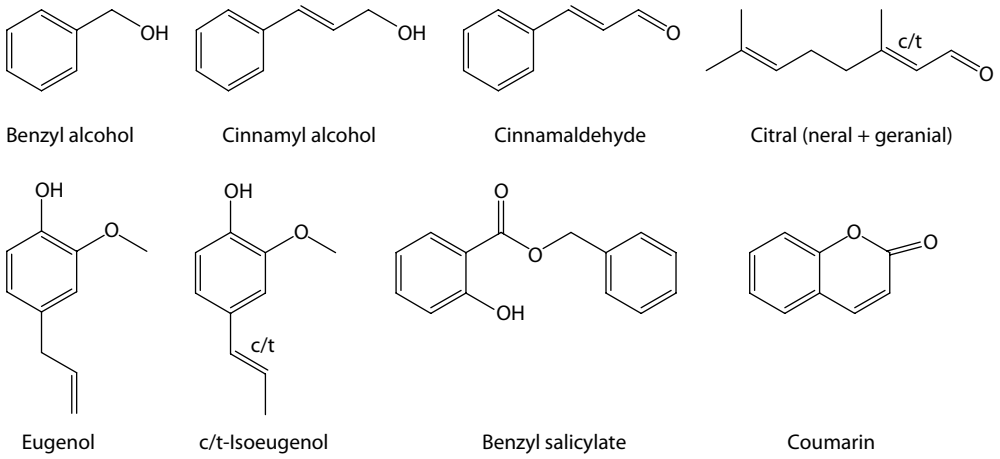

FIGURE 22.1 Structures of the 16 naturally occurring alleged allergenic substances (part 1).

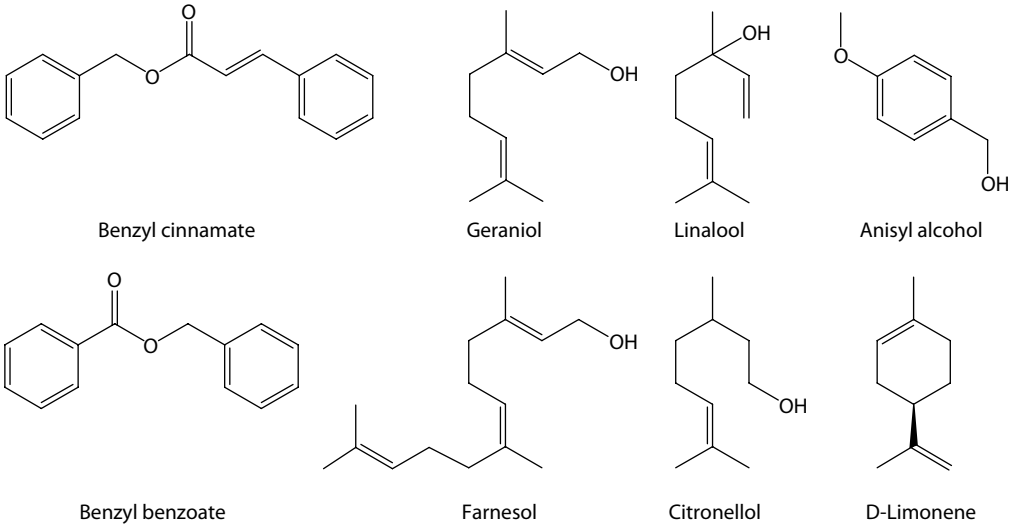

FIGURE 22.2 Structures of the 16 naturally occurring alleged allergenic substances (part 2).

22.2.4 Recent Data on Sensitization to Fragrances

Recently a new study on the sensitization to the 26 fragrance ingredients (24 single substances and two natural extracts) that have to be labeled according to the European Regulation was published by the group of Schnuch et al. (2007). This study was part of the multicenter project Information Network of Departments of Dermatology (IVDK) (Schnuch et al., 1997, 2004). The aim was to study the frequency of sensitization to these 26 alleged allergenic fragrances, in particular the actual frequencies of contact allergy to these 26 fragrances. To test this, the fragrance ingredients were patch tested in consecutive, unselected patients (in total 21,325 patients) by the IVDK network during a 2-year period, consisting of four periods of 6 months. The number of patients tested with each of the fragrance substances ranged from 1658 to 4238.

The frequency of sensitization was expressed by the proportion of patients reacting allergic (% pos.), that is, the number of allergic patients compared to the number of patients tested (*n* pos./*n* tested, %) and the frequency of allergic reactions was then standardized for age and sex.

The "allergenic" fragrances were divided into three groups, depending on the frequency of sensitization, based on the 95% confidence interval (CI).

The first group of ingredients with the upper CI > 1.0% could be regarded as important allergens and was called Group I. This group includes the two natural extracts, oakmoss and tree moss, and the substances HMPCC, hydroxycitronellal, isoeugenol, cinnamic acid, and farnesol.

Another group of ingredients with an upper CI between 0.5% and 1.0% was found to be clearly allergenic but less important in terms of sensitization frequency (Group II). This group comprises cinnamic alcohol, citral, citronellol, geraniol, eugenol, coumarin, lilial, amyl-cinnamic alcohol, and benzyl cinnamate.

On the other hand, the third group (Group III) comprises substances that have turned out to be (extremely) rare sensitizers in this study, or which in other instances may even be considered as nonsensitizers, according to the authors. This group with an upper CI of less than 0.5% contains 10 materials: benzyl alcohol, linalool, methylheptin carbonate, α-amyl-cinnamic aldehyde, α-hexyl-cinnamic aldehyde, limonene, benzyl salicylate, γ-methylionone, benzyl benzoate, and anisyl alcohol. It was further concluded that sensitization to allergens of the first group is significantly more frequent than sensitization to allergens of the third group.

Regarding Group III it is also worth noting that some molecules are not allergens as such, but only turn into allergens after substantial oxidation, for example, limonene and linalool (Karlberg and Dooms-Goossens, 1992; Karlberg et al., 1992; Hagvall and Karlberg, 2006).

It is interesting to note that there is a difference in the classification of the allergens (reported frequency) according to the opinion of the SCCP (SCCP/0017/98) and the classification in groups by Schnuch et al. For example one substance that is an important allergen according to the study of Schnuch (Group I), farnesol, is according to SCCP "less frequently reported." The same applies to two substances of Group II that are according to SCCP "less frequently reported," namely citronellol and benzyl cinnamate. On the other hand, two materials that are according to the study of Schnuch (extremely) rare sensitizers (Group III) are according to SCCP "frequently reported," namely benzyl alcohol and benzyl salicylate. A comparison of the classifications is given in Table 22.3. Differences in classification according to the two sources are highlighted in bold.

It is important to focus in some more depth on the allergenic potential of the natural ingredients, oakmoss and tree moss. In contrast to oakmoss, which is known to be a potent sensitizer since a long time ago, tree moss had not been systematically tested in cosmetic patch test series in the past, and the study by Schnuch et al. (2007) is claimed to be the first study in which tree moss was tested in a larger population. In this study, tree moss was found to be the most frequent allergen. Earlier study reports had already identified atranol and chloroatranol (degradation products of atranorin and chloroatranorin) as the most potent allergens (Johansen et al., 2003, 2006). The chemical structures of atranol and chloroatranol are depicted in Figure 22.3.

TABLE 22.3
Classification of Alleged Allergens According to SC Opinion (Frequently or Less Frequently Reported)

Name	Frequency (SCCNFP/0017/98) (Except for the Mosses (SCCNFP opinion of October 24, 2000; SCCP (SCCP/00847/04))	Group (Schnuch et al., 2007)	Frequency of Sensitization[a]
Tree moss extract 1	SCCNFP/0202/99 and SCCP/00847/04: potent	Group I	2.4
Oakmoss extract 1	SCCNFP/0202/99 and SCCP/00847/04: potent	Group I	2.0
Isoeugenol	Frequently reported	Group I	1.1
Cinnamal	Frequently reported	Group I	1.0
Farnesol	**Less frequently reported**	Group I	0.9
Cinnamyl alcohol	Frequently reported	Group II	0.6
Citral	Frequently reported	Group II	0.6
Citronellol	**Less frequently reported**	Group II	0.5
Eugenol	Frequently reported	Group II	0.4
Coumarin	Frequently reported	Group II	0.4
Geraniol	Frequently reported	Group II	0.4
Benzyl cinnamate	**Less frequently reported**	Group II	0.3
Benzyl alcohol	**Frequently reported**	Group III	0.3
Linalool	Less frequently reported	Group III	0.2
Benzyl salicylate	**Frequently reported**	Group III	0.1
d-Limonene	Less frequently reported	Group III	0.1
Anisyl alcohol	Less frequently reported	Group III	0.0
Benzyl benzoate	Less frequently reported	Group III	0.0

Source: SCCNFP opinions versus the publication by Schnuch et al., 2007.

[a] Frequency of sensitization (%) = *n* test/*n* pos. (standardized for age and sex).

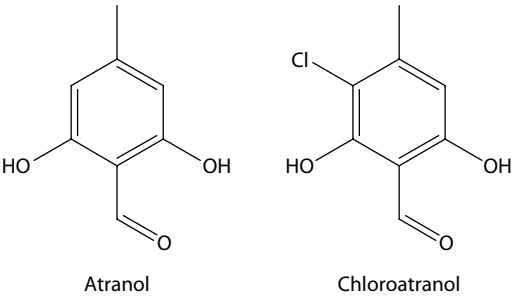

FIGURE 22.3 Structures of atranol and chloroatranol, most potent allergens in oakmoss and tree moss.

This also seems to be in line with the opinion of the SCCP (SCCP/00847/04) on atranol and chloroatranol present in natural extracts (e.g., oakmoss and tree moss extract) where both constituents were regarded as very potent allergens. Because chloroatranol was shown to cause elicitation of reactions by repeated open exposure at the ppm level (0.0005%) and at the ppb level on patch testing (50% elicit at 0.000015%), the SCCP concluded that "chloro-atranol and atranol should not be present in cosmetic products."

As a result, today the Fragrance Industry is producing oakmoss and tree moss with reduced levels of atranol and chloroatranol (i.e., oakmoss and tree moss absolutes treated for the selective removal of atranol and chloroatranol).

The authors concluded in their paper that the study again emphasizes the need for a "different look" on fragrances as contact allergens, which is a confirmation of previous findings (Schnuch et al., 2002). The authors propose a differentiated evaluation of ingredients of each group for overall evaluation, considering not only frequency of sensitization, but also the amount of exposure or use, as well as allergenic potency—also exposure to (highly) oxidized materials could be taken into account.

In particular, for Group I substances the authors agree that a regulation in terms of restrictions (or even ban) and labeling is needed, whereas for Group II substances labeling alone may be adequate enough for the purpose of prevention. But according to the authors, for some of the ingredients of Group III, neither restrictions nor labeling seems justified.

Thus, the authors express their opinion, justified by the findings of this study, that the Commission Decision on the labeling of all 26 "alleged allergens" should be revised.

The Fragrance Industry in turn has taken note of this study and comes to the same conclusion. Based on this, the Industry would now like to propose a pragmatic and different approach for the three Groups of "alleged fragrance ingredients": for example for Group III materials, Industry would advocate no labeling requirements and only restrictions where needed based on scientifically justified concern and for Group II and Group I materials, the Industry would propose appropriate and adequate measures based on scientific data. The Industry would like to avoid overregulation and overlabeling for alleged sensitizers.

Also the Commission took note of the publication of this study and as a consequence DG Enterprise sent a mandate to the SCCP with a request for an updated scientific opinion on the fragrance substances hydroxycitronellal (CAS 107-75-5), isoeugenol (CAS 97-54-1), and *d*-limonene (CAS 5989-27-5).

Currently, the presence of hydroxycitronellal and isoeugenol needs to be labeled in the final cosmetic product according to Annex III, Part 1 of the Cosmetic Directive (Entries 72 and 73, respectively). However, in the future, restrictions to these fragrance ingredients may be proposed, because the Commission is considering a maximum concentration of 1.0% of hydroxycitronellal and of 0.02% of *cis*- and *trans*-isoeugenol (or their sum) in finished cosmetic products (except oral care products). DG Enterprise has asked SCCP its opinion whether they consider these concentrations to be safe for

consumers when used in cosmetic products taking into account the scientific data provided. In fact such restrictions would be more in line with the self-regulating policy and principles of the Fragrance Industry, as applied through the IFRA CoP and its Standards, as explained above.

Regarding limonene, DG Enterprise has asked SCCP to re-evaluate the level of peroxides for the limonenes in cosmetic products. In parallel, the Fragrance Industry through the Research Institute for Fragrance Materials (RIFM) is conducting some local lymph node assay (LLNA) work on limonene and some other key materials for a better scientific substantiation of the maximum peroxide level. RIFM is planning to test limonene with different (low) levels of peroxide to determine the EC3 value (equivalent to the human NOEL). This is a project with the University of Göteburg, Sweden (Professor. A.-T. Karlberg). Some of the goals of this research are to investigate the fundamental scientific basis of the auto-oxidation of four important structurally related fragrance ingredients (e.g., limonene) and one essential oil; to look more closely at the sensitization potential of limonene (and hence to challenge the current sensitization hazard classification of R43 of limonene, which itself is not a sensitizer, and essential oils rich in limonene such as orange oil); and to challenge the sensitization hazard classification of other essential oils, containing another important fragrance ingredient, labeled as allergen, namely linalool. This is possible if it can be demonstrated that linalool oxidizes differently in an essential oil as compared to the pure compound.

The impact of hazard classification (e.g., R43 Risk Phrase) of fragrance ingredients on the classification of essential oils containing them will be discussed in more detail in the section on Hazard Classification and Labeling.

The impact of allergen labeling requirements on essential oils is very important and a different approach of the regulators toward labeling based on the new scientific data available could be very high. If for example no further labeling requirements would apply to Group III materials (which include the following six naturally occurring substances: benzyl alcohol, linalool, benzyl salicylate, *d*-limonene, anisyl alcohol, and benzyl benzoate), the number of affected natural raw materials (extract and essential oils) would be reduced from about 180 to only 80 extracts/essential oils that would need to be taken into account for labeling purposes (see Annex 22.2 to this chapter), according to the EFFA CoP. This would also have a favorable impact on the analytical burden: much less essential oils would have to be analyzed for the presence and concentration of the allergens; also the number of target analytes (allergens) would be reduced from 16 to 10 naturally occurring ones.

The issue on allergen labeling can also have detrimental business impact as some customers (clients) of the fragrance industry (being the cosmetic and detergent industry) are requesting fragrances (i.e., perfume mixtures and preparations) that are "allergen-free." This would mean that the suppliers of essential oils would need to produce "allergen-free" essential oils and extracts, that is, natural materials that do not contain any of the 16 naturally occurring "alleged allergens." This is of course practically impossible. Moreover, generally producing extracts and essential oils without or even with reduced levels of the 16 naturally occurring "allergens" (which are very important fragrance constituents by themselves), for example, by selectively removing them, would have a very high and negative impact on the organoleptic and sensory properties of the essential oils and hence on the fine fragrances and perfumes containing them. As mentioned above, only in particular cases (e.g., for oakmoss and tree moss extracts, which are of high importance to the perfumer but also very potent allergens) the Industry is successfully producing new qualities with reduced levels of allergens (*in casu* atranol and chlorotranol) to reduce the sensitization potential of the mosses. It is worthwhile to mention here that a considerable number of IFRA Standards (IFRA prohibited materials) are part of the Cosmetics Directive (banned materials under Annex II of the Cosmetics Directive).

22.2.5 First Amendment of the Detergent Regulation and Allergen Labeling

In line with the 7th Amendment of the Cosmetic Directive 76/768/EEC as just discussed above in the previous paragraph, the first amendment of the Detergent Regulation (from June 2006) makes

the labeling of the 26 alleged "allergenic" materials mandatory: the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of "complex ingredients" such as essential oils).

This first amendment is the Commission Regulation (EC) No. 907/2006 (20/06/06) amending Regulation (EC) No. 648/2004 on detergents. The recital (whereas) (4) of this regulation states the following:

> *(4) There is a requirement to declare allergenic fragrances if they are added in the form of pure substances. However there is no requirement to declare them if they are added as constituents of complex ingredients such as essential oils or perfumes. To ensure better transparency to the consumer, allergenic fragrances in detergents should be declared irrespective of the way they are added to the detergent.*

The threshold for labeling is defined as 0.01% by weight (100 ppm), according to the adaptation of Annex VII (for labeling and ingredient data sheet) as follows:

> *If added at concentrations exceeding 0.01% by weight, the allergenic fragrances that appear on the list of substances in Annex III, Part 1 to Directive 76/768/EEC, as a result of its amendment by Directive 2003/15/EC of the European Parliament and of the Council to include the allergenic perfume ingredients from the list first established by the Scientific Committee on Cosmetics and Non-food Products (SCCNFP) in its opinion SCCNFP/0017/98, shall be listed using the nomenclature of that Directive, as shall any other allergenic fragrances that are subsequently added to Annex III, Part 1 to Directive 76/768/EEC by adaptation of that Annex to technical progress.*

This text for Annex VII is written in such a way to ensure that the presence of the 26 alleged fragrance materials above the given threshold has to be declared irrespective of the way they are added (i.e., as such or as being part of "complex ingredients" such as essential oils).

In that way according to the first amendment of the Detergent Regulation, the same rules apply for detergents as for cosmetic end products for the requirements of allergen labeling.

22.3 CURRENT FLAVOURING DIRECTIVE AND FUTURE FLAVOURING REGULATION: IMPACT ON ESSENTIAL OILS

In the European Union for flavorings, the current Flavouring Directive 88/388/EC still applies. This is the Council Directive of June 22, 1988, on the approximation of the laws of the MS relating to flavorings for use in foodstuffs and to source materials for their production, as published in the Official Journal on 15/07/88 (OJ L 184, p. 61). It has been amended once by the Commission Directive 91/71/EEC of 16/01/91 (OJ L 42, p. 25, 15/02/91). As this is a Directive, it is up to the EU MS to take the necessary measures to ensure that flavorings may not be marketed or used if they do not comply with the rules laid down in this Directive, as stated in Art. 3 of this Directive.

However since recent years (around 2002) a Proposal for a new Regulation of the European Parliament and of the Council on flavorings and certain food ingredients with flavoring properties for use in and on foods is under discussion. The last version of the Commission Proposal that was the basis for further discussions and Amendments from EU Parliament was issued in July 2006. As many essential oils and extracts either contain flavoring substances or are regarded as "food ingredients with flavoring properties," this new Flavouring Regulation will have an impact on essential oils and their use as flavoring ingredients for food products. Extracts and essential oils contain certain constituents (substances) that according to this regulation "should not be added as such to food" or to which maximum levels apply. They are often referred to as "biologically active substances" or "active principles." Especially the application of maximum levels of these substances will have an impact on how and when extracts, essential oils but also herbs and spices may or can be applied to food. Also the definitions for "natural" have drastically changed. The difference

between the current Directive 88/388/EC and the future Flavour Regulation will be outlined in the next paragraphs.

22.3.1 Current Flavouring Directive 88/388/EC

22.3.1.1 Maximum Levels of "Biologically Active Substances"

In the current Flavouring Directive 88/388/EC, Annex II sets maximum levels (limits) for certain substances obtained from flavorings and other food ingredients with flavoring properties in foodstuffs as consumed in which flavorings have been used. Art. 4 (c) stipulates that

> *(c) the use of flavourings and of other food ingredients with flavouring properties does not result in the presence of substances listed in Annex II in quantities greater than those specified therein.*

The limits apply to Foodstuffs and Beverages (mg/kg) — exceptions apply: for example, alcoholic beverages and confectionaries. In Table 22.4, the maximum levels (without the exceptions) for these substances for foodstuffs in general and beverages are given. A more detailed table (with all the exceptions) is given in Annex 22.3 to this chapter.

This means that for essential oils, extracts, complex mixtures containing these "biologically active substances" (e.g., nutmeg, cinnamon, peppermint, and sage oils) and when added to food and flavorings, maximum levels apply. The same applies to herbs and spices containing these "biologically active substances" as herbs and spices are also "food ingredients with flavoring properties."

22.3.1.2 Definition of "Natural"

Also important is how the current Flavouring Directive addresses "naturalness" of flavors and how "natural" is defined for the purpose of labeling. This is stipulated by Art. 9a.2 (amending the original Art. 9.2 of 88/388/EC by 91/71/EEC):

> *2. the word 'natural', or any other word having substantially the same meaning, may be used only for flavourings in which the flavouring component contains exclusively flavouring substances as defined in Article 1 (2) (b) (i) and/or flavouring preparations as defined in Article 1 (2) (c). If the sales description of the flavourings contains a reference to a foodstuff or a flavouring source, the word 'natural' or any other word having substantially the same meaning, may not be used unless the flavouring component has been isolated by appropriate physical processes, enzymatic or microbiological processes or traditional food-preparation processes solely or almost solely from the foodstuff or the flavouring source concerned.*

TABLE 22.4
Annex II of 88/388/EC—Maximum Levels (mg/kg) for Certain Substances in Foodstuffs and Beverages

Substance	Foodstuffs and Beverage	Substance	Foodstuffs	Beverages
Agaric acid	20	Aloin	0.1	0.1
β-Asarone	0.1	Berberine	0.1	0.1
Coumarin	2	Hydrocyanic acid	1	1
Hypericine	0.1	Pulegone	25	100
Quassine	5	Safrole and isosafrole	1	1
Santonin	0.1	Thujone (α and β)	0.5	0.5

How a "natural flavoring substance" can be obtained is thus defined in Art. 1.2 (b) (i):

(b) 'flavouring substance' means a defined chemical substance with flavouring properties which is obtained:
(i) by appropriate physical processes (including distillation and solvent extraction) or enzymatic or microbiological processes from material of vegetable or animal origin either in the raw state or after processing for human consumption by traditional food-preparation processes (including drying, torrefaction and fermentation),

How a "flavoring preparation" can be obtained is defined in Art. 1.2 (c):

(c) 'flavouring preparation' means a product, other than the substances defined in (b) (i), whether concentrated or not, with flavouring properties, which is obtained by appropriate physical processes (including distillation and solvent extraction) or by enzymatic or microbiological processes from material of vegetable or animal origin, either in the raw state or after processing for human consumption by traditional food-preparation processes (including drying, torrefaction and fermentation);

The above means that a "flavoring preparation" is by default always "natural" and that extracts and essential oils (obtained by appropriate physical processes such as distillation and solvent extraction) from material of vegetable origin (e.g., plant material) can be considered as "flavoring preparation" and thus "natural."

22.3.2 Future Flavouring Regulation

As mentioned above, a Proposal for a new Flavouring Regulation is under discussion since the last years at three levels: the EU Commission, the EU Parliament, and the Council (MS-level). The full title of this proposal is *Proposal for a Regulation of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in foods and amending Council Regulation (EEC) No. 1576/89, Council Regulation (EEC) No. 1601/91, Regulation (EC) No. 2232/96 and Directive 2000/13/EC*. The last (amended) proposal from the Commission dates from 24/10/2007 (Commission Directive 91/71/EEC).

With this new Regulation, the former Council Directive 88/388/EEC of June 22, 1988, as well as its amendment Directive 91/71/EEC and the Commission Decision 88/389/EEC will be repealed.

This Flavouring Regulation is part of a larger package, called the "Food Improvement Agents Package" (FIAP), comprising the Flavouring, Additives and Enzymes Regulation and the Common Authorisation Procedure. The drafting of the entire package started at Commission level, has undergone a tremendous amount of amendments, as issued and adopted by the European Parliament, and was at the time of the preparation of the manuscript for this chapter under discussion with three parties: the EU Commission, the EU Parliament, and the Council under Portuguese Presidency. The last chance for the parties to come to a political agreement and to reach a common position under the first Reading was in December 2007. However, a common position could not be reached by the end of 2007 and there was a second Reading (Plenary session) in July 2008: under Slovenian Presidency.

For a long time (at the time of the preparation of the manuscript of this chapter) there was not one final document but two major draft versions: the last amended Commission Proposal of 24/10/2007 (Commission Directive 91/71/EEC) and the last Council Proposal for Political Agreement of 10/12/2007 (Commission Directive 93/21/EEC), which were the basis for discussion for the Council meeting (Agriculture and Fisheries) on December 17–18, 2007. The major differences between the two versions (Commission Proposal and Council Proposal) available at the end of 2007 (at the time of the preparation of this manuscript), and the impact on essential oils will be outlined below. As a final Proposal of the European Parliament and the Council had just come available shortly before submission of this manuscript for publication (Council Proposal, July 15, 2008), also this final

Council Proposal will be discussed briefly, in order to be as much as possible up-to-date. Meanwhile at the time of the publication of this book, the final version of the new Flavouring Regulation has been published in the Official Journal on 31 December 2008 (OJ L 354, 31.12.2008, p. 34): Regulation (EC) No 1334/2008. In essence this Regulation as published is the same as the final Council Proposal which was published on July 15, 2008. It has entered into force on January 20, 2009 and will apply as from January 20, 2011. As of this application date, the current Flavouring Directive 88/388/EEC will be repealed.

22.3.2.1 Maximum Levels of "Biologically Active Substances"

Apart from the fact that the current Directive 88/388/EC will turn into a Regulation, there are many changes that will have an impact on how essential oils and extracts will be used as source of flavors.

The most important issue is how the so-called biologically active substances are addressed.

This is addressed by Art. 5 of the Draft Council Proposal (Art. 6 of Commission Proposal): "Presence of certain substances," which refers to Annex III with the same title. Both Council and Commission proposals clearly state in the first paragraph that "Substances listed in Part A of Annex III shall *not* be added *as such* to food."

However, when it comes to the levels of these substances coming from the use of flavorings and food ingredients with flavoring properties (such as extracts, essential oils, herbs, and spices), the Commission and Council proposals differ slightly.

Art. 6.2 in the Commission Proposal reads as follows:

> *2. Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in the compound foods listed in Part B of Annex III shall not be exceeded as a result of the use of flavourings and food ingredients with flavouring properties in and on those foods.*
>
> *The maximum levels shall apply to the compound foods as offered ready for consumption or as prepared according to the instructions of the manufacturer.*

Art. 5.2 in the Council Proposal (December 10, 2007) reads as follows:

> *2. Without prejudice to Council Regulation No. 1576/89 maximum levels of certain substances, naturally present in flavourings and/or food ingredients with flavouring properties, in the compound foods listed in Part B of Annex III shall not be exceeded as a result of the use of flavourings and/or food ingredients with flavouring properties in and on those foods.*
>
> *The maximum levels of the substances set out in Annex III apply to foods as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels apply to the food as reconstituted according to the instructions on the label, taking into account the minimum dilution factor.*

The wording in the latest Council Proposal of July 15 (Art. 6) is essentially the same as the wording of the Council Proposal of December 10 (Art. 5).

This means that maximum levels of these substances also apply when the substances come from any type of food ingredients with flavoring properties; the only difference between the Commission Proposal and the Council proposals is that in the Council proposals an exception is given to dried and/or concentrated foods that can have higher levels before they are diluted/reconstituted. Upon dilution/reconstitution, the normal maximum levels apply again.

The main difference between the current Flavouring Directive 88/388 and the future Flavouring Regulation is that in the Directive 88/388 there is only one list (Annex II) of substances to which the maximum levels apply—all those substances may not be added *as such* to food. In contrast, in the future Flavouring Regulation, the Annex III is split into two parts: Part A with "Substances which may *not* be added *as such* to food" and Part B establishing "Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in certain compound food as consumed to which flavourings and/or food ingredients with flavouring properties have been added."

TABLE 22.5
Annex III, Part A: Substances that May *Not* be Added *As Such* to Food

Agaric Acid	Aloin	**Capsaicin**
1,2-Benzopyrone, coumarin	**Hypericine**	**β-Asarone**
1-Allyl-4-methoxybenzene, estragole	**Hydrocyanic acid**	**Menthofuran**
4-Allyl-1,2-dimethoxybenzene, methyleugenol	**Pulegone**	**Quassin**
1-Allyl-3,4-methylene dioxy benzene, safrole	**Teucrin A**	**Thujone (α and β)**

Note: Substances in bold are those that are in Part A, Annex III of both the Council and Commission proposals—aloin and coumarin are *not* included in Annex III, Part A of the Commission proposal.

Part A contains 15 substances (according to the Council proposals) or 13 substances (according to the Commission Proposal—aloin and coumarin are not in), whereas Part B contains 11 substances (according to the Council proposals) or 10 substances (according to the Commission Proposal—coumarin is not in).

Table 22.5 lists the Substances of Part A of Annex III "which may not be added as such to food" and Table 22.6 lists the 11 substances of Part B with their respectively maximum levels in the various compound foods according to the Council proposals.

There are some major differences between the Part B of Annex III in the Council proposals and the list in the Commission Proposal:

- As mentioned above, maximum levels for coumarin are only set in the Council proposals and not in the Commission Proposal.
- For Teucrin A different levels are set in the Council proposals for different compound foods, whereas in the Commission Proposal only for one category, namely alcoholic beverages, a maximum level of 2 mg/kg applies.
- Regarding the chemical names, in the Commission Proposal no trivial name is given for 1-allyl-4-methoxybenzene (estragol) and 4-allyl-1,2-dimethoxy-benzene (methyleugenol) in contrast to the Council Proposal (synonyms given).
- But the most important and major difference is the statement in the Council Proposal of December 10 on top of the table, which is *not* in the Commission Proposal, which reads as follows:

"These maximum levels shall not apply to compound foods which are prepared and consumed on the same site, contain no added flavourings and contain only herbs and spices as food ingredients with flavouring properties."

This statement is to allow the unrestricted use of herbs and spices to foods that are prepared and consumed on the same site, for example, restaurants and catering services.

However, in the latest Council Proposal of July 15, this statement has disappeared.

Instead another footnote has been introduced applying to three of the substances that are marked with an asterisk (*): estragol, safrole, and methyl eugenol. This footnote reads as follows (Council Proposal, July 15):

**The maximum levels shall not apply where a compound food contains no added flavourings and the only food ingredients with flavouring properties which have been added are fresh, dried or frozen herbs and spices. After consultation with the Member States and the Authority, based on data made available by the Member States and on the newest scientific information, and taking into account the*

TABLE 22.6
Maximum Levels of Certain Substances, Naturally Present in Flavorings and Food Ingredients with Flavoring Properties, in Certain Compound Foods Consumed to which Flavorings and/or Food Ingredients with Flavoring Properties have been Added

Name of the Substance	Compound Food in which the Presence of the Substance is Restricted	Maximum Level (mg/kg)
β-Asarone	Alcoholic beverages	1.0
1-Allyl-4-methoxybenzene, estragol	Dairy products	50
	Processed fruits, vegetables (including mushrooms, fungi, roots, tubers, pulses, and legumes), nuts, and seeds	50
	Fish products	50
	Nonalcoholic beverages	10
Hydrocyanic acid	Nougat, marzipan, or its substitutes or similar products	50
	Canned stone fruits	5
	Alcoholic beverages	35
Menthofuran	Mint/peppermint containing confectionery, except micro breath freshening confectionery	500
	Micro breath freshening confectionery	3000
	Chewing gum	1000
	Mint/peppermint containing alcoholic beverages	200
4-Allyl-1,2-dimethoxy-benzene, methyleugenol	Dairy products	20
	Meat preparations and meat products, including poultry and game	15
	Fish preparations and fish products	10
	Soups and sauces	60
	Ready-to-eat savouries	20
	Nonalcoholic beverages	1
Pulegone	Mint/peppermint containing confectionery, except micro breath freshening confectionery	250
	Micro breath freshening confectionery	2000
	Chewing gum	350
	Mint/peppermint containing nonalcoholic beverages	20
	Mint/peppermint containing alcoholic beverages	100
Quassin	Nonalcoholic beverages	0.5
	Bakery wares	1
	Alcoholic beverages	1.5
1-Allyl-3,4-methylene dioxy benzene, safrole	Meat preparations and meat products, including poultry and game	15
	Fish preparations and fish products	15
	Soups and sauces	25
	Nonalcoholic beverages	1
Teucrin A	**Bitter-tasting spirit drinks or bitter[a]**	**5**
	Liqueurs[b] with a bitter taste	**5**
	Other alcoholic beverages	**2**
Thujone (α and β)	Alcoholic beverages, except those produced from *Artemisia* species	10
	Alcoholic beverages produced from *Artemisia* species	35
	Nonalcoholic beverages produced from *Artemisia* species	0.5

continued

TABLE 22.6 (continued)
Maximum Levels of Certain Substances, Naturally Present in Flavorings and Food Ingredients with Flavoring Properties, in Certain Compound Foods Consumed to which Flavorings and/or Food Ingredients with Flavoring Properties have been Added

Name of the Substance	Compound Food in which the Presence of the Substance is Restricted	Maximum Level (mg/kg)
COUMARIN	Traditional and/or seasonal bakery ware containing cinnamon in the labeling	50
	"Breakfast cereals" including muesli	20
	Fine bakery ware with exception of traditional and/or seasonal bakery ware containing cinnamon in the labeling	15
	Desserts	5

[a] As defined by Article 1.4 (p) of EC Regulation 1576/89.
[b] As defined by Article 1.4 (r) of EC Regulation 1576/89.

use of herbs and spices and natural flavouring preparations, the Commission, if appropriate, proposes amendments to this derogation.

This means that the maximum levels do not apply to estragol, safrole, and methyl eugenol when only fresh, dried, or frozen herbs and spices are added! However when "food ingredients with flavoring properties" such as essential oils are added, or when essential oils and/or other flavorings are added in combination with herbs and spices, the levels do apply.

It is anticipated that nothing will change anymore in Art. 6 of the Council Proposal of July 15 until the adoption of the final text and that also the Annex III will remain as it is now. However, as stipulated in the footnote under the Annex III (applying to the three substances with an asterisk) amendments to the current derogations for herbs and spices can be expected.

It is also important to note that according to Art. 30 of the Flavouring Regulation (*Entry into Force*), which will only apply 24 months after its Entry into Force, Art. 22 shall apply from the date of its Entry into Force. Art. 22 concerns the Amendments to Annexes II through V. This means that if the Flavouring Regulation would be adopted by the end of 2008 and hence would apply end of 2010, the Annexes can be amended immediately, if necessary.

Whereas the Art. 6 of the Council Proposal of July 15 discussed above (i.e., Art. 6 of the Commission Proposal) relates to "certain substances," Art. 7 of the Council Proposal of July 15 (i.e., Art. 7 of the Commission Proposal) relates to "Use of certain source materials," which is even more important in relation to herbs, spices, extracts, and essential oils. This article refers to Annex IV of the Regulation, which is a new annex that was not in the current Flavouring Directive 88/388/EC entitled "List of source materials to which restrictions apply for their use in the production of flavourings and food ingredients with flavouring properties." This Annex IV consists of two parts:

- Part A: Source materials that shall not be used for the production of flavorings and food ingredients with flavoring properties.
- Part B: Conditions of use for flavorings and food ingredients with flavoring properties produced from certain source materials.

The complete Annex IV according to the current Draft proposals (there is no difference between the Draft Council and Commission proposals regarding the content of Annex IV) is given in Annex 22.4 to this chapter.

Art. 7 of the Council Proposal of July 15 (Art. 7 of the Commission Proposal) stipulates the following:

1. *Source materials listed in Part A of Annex IV shall not be used for the production of flavourings and/or food ingredients with flavouring properties.*
2. *Flavourings and/or food ingredients with flavouring properties produced from source materials listed in Part B of Annex IV may only be used under the conditions indicated in that Annex.*

With the exception of the fact that "and/or" in "flavourings *and/or* food ingredients" in both paragraphs in the Council Proposal is replaced by "and" ("flavourings *and* food ingredients") in the Commission Proposal, the remainder of the article is exactly the same. It is anticipated that nothing will change anymore on this article and the related Annex IV for the final version of the Flavouring Regulation.

22.3.2.2 Definition of "Natural"

Regarding "naturalness" of flavors and how "natural" is defined for the purpose of labeling, the situation has drastically changed since the current Flavouring Directive 88/388/EC.

For example today according to 88/388/EC there are three categories of flavoring substances: natural, nature-identical (NI), and artificial. However, with the new Flavouring Regulation there will be only two categories: natural and not natural, meaning that the difference between the former categories NI and artificial will disappear and these two will merge in one category of "synthetic flavorings."

Also the position of the Council is clearly different from the position of the Commission. In both proposals, "natural flavoring substance" is defined by Art. 3.2 (c).

This definition according to the *Commission* proposals (December 10 and July 15) reads as follows:

> *(c) 'natural flavouring substance' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II.*

This definition according to the Council Proposal reads as follows:

> *(c)'natural flavouring substance' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II; Natural flavouring substances correspond to substances that are naturally present and have been identified in nature;*

Important is the additional line, according to the Council proposals, stating that a substance has to be identified in nature before it can be regarded as "natural," so it is not only sufficient to produce it "in a natural way": it has to be identical to something that is present in nature. This is to avoid the problem that arises when an enzymatic or microbial process is developed by which a flavoring substance can be produced "by enzymatic or microbial processes from material of vegetable origin" (i.e., natural source materials) that up to then has never been identified in nature (and is not naturally occurring), such as ethylvanillin, then such a material would be labeled as a "natural flavoring substance."

More important than the definition of "natural" as such (Art. 3.2 (c)), however, is how "appropriate physical process" is defined.

Annex II to the Flavouring Regulation gives a list of "traditional food preparation processes" by which (natural) flavoring substances and *natural* flavoring preparations are obtained (in the title of

Annex II in the Commission Proposal the wording "*natural* flavouring preparations" is used, which is confusing since flavoring preparations are, as per definition, natural, see below). The full list of *traditional food preparation processes* is given in Annex 22.5 to this chapter.

The definition of "appropriate physical process" is different between the two proposals and is described in Art. 3.2 (k).

This definition according to the *Commission* Proposal reads as follows:

(k) 'appropriate physical process' shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, without prejudice to the listing of traditional food preparation processes in Annex II, and does not involve the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

This definition according to the *Council* Proposal (December 10, 2007) reads as follows:

(k) 'appropriate physical process' shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring and does not involve among others the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

It can be noted that the definition according to the Council Proposal does no longer refer to the processes listed in Annex II. In contrast to the Commission Proposal where all *traditional food preparation processes* as listed in Annex II also fall under the definition of "appropriate physical processes" (*cf.* the wording "without prejudice to the listing of … "), according to the Council Proposal only processes that *do not intentionally modify the chemical nature of the components* of the flavoring are considered to be "appropriate physical processes" in order to obtain a "natural flavouring substance."

This means that distillation and certain extraction techniques that *do* modify the chemical nature of the components are not regarded as "appropriate physical processes" for obtaining natural flavoring substances.

Fortunately thanks to very strong and effective, successful lobbying by the European Flavour Industry, this has been rectified and an amendment to this definition (Art. 3.2 (k)) has been accepted by Council, Commission, and European Parliament.

According to the latest Council Proposal (July 15, 2008), this definition reads as follows:

(k) "appropriate physical process" shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, ***without prejudice to the listing of traditional food preparation processes in Annex II****, and does not involve, inter alia, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.*

This definition again refers to the Annex II, which means that all processes listed in Annex II also fall again under the definition of "*appropriate physical processes*."

When looking at the situation for "flavouring preparations" (such as essential oils and extracts), the wording of the Commission Proposal is slightly different than that of the Council Proposal.

According to Art. 16.2 of the Council Proposal (July 15) and Art. 15.2 of the Commission Proposal, the term "natural" may be used for the description of a flavoring if the flavoring component comprises only flavoring preparations, which means that a "flavoring preparation" can be regarded as "natural" by definition. In other words, there is no such thing as a "synthetic flavoring preparation."

The definition for "flavouring preparation" according to the *Commission* Proposal reads as follows (Art. 3.2 (d)):

(d) 'flavouring preparation' shall mean a product, other than a flavouring substance, obtained from:

(i) food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II and/or appropriate physical processes;

and/or

> *(ii) material of vegetable, animal or microbiological origin, other than food, obtained by one or more of the traditional food preparation processes listed in Annex II and/or appropriate physical, enzymatic or microbiological processes;*

The definition for "flavouring preparation" according to the *Council* proposals (versions December 10 and July 15 being identical) reads as follows (Art. 3.2(d)):

> *(d) 'flavouring preparation' shall mean a product, other than a flavouring substance, obtained from:*
>
> *(i) food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II;*

and/or

> *(ii) material of vegetable, animal or microbiological origin, other than food, by appropriate physical, enzymatic or microbiological processes, the material being taken as such or prepared by one or more of the traditional food preparation processes listed in Annex II;*

Although the wording of Commission and Council differ on this definition, it could be concluded that according to the latter definition (according to Council Proposal) essential oils and extracts obtained from plant material (*material of vegetable origin*) prepared by distillation (which is a *traditional food preparation process* listed in Annex II) followed by an *appropriate physical process* can be considered as a "flavoring preparation" and thus natural as long as the chemical nature of the components is not intentionally modified during the physical process.

However, it can also be argued that if the physical process after the distillation (e.g., extraction, drying, evaporation, condensation, dilution, etc.) intentionally modifies the chemistry of the components (which is often the case), then the end product can no longer be regarded as *flavoring preparation* and thus natural, according to the Council Proposal.

In that respect, the wording of the Commission Proposal (part (ii)) is much broader and less ambiguous and will not lead to these restrictions. According to the Commission Proposal, an extract, essential oil, absolute or concrete, … obtained from material of vegetable origin (flower, root, fruit, etc.) by one or more of the processes listed in Annex II complies with the definition of "flavoring preparation" and can thus be regarded as a "natural."

However, it is anticipated that the latest version, being the Council Proposal of July 15, 2008, is the blueprint of the final Flavouring Regulation, and will be the text as it will most probably be adopted by the end of the year and published. Entry into force of the new EU Flavouring Regulation will be on the twentieth day following that of its publication in the Official Journal of the European Union. It is anticipated that this will be at the earliest by the end of 2008 or the beginning of 2009. As stated above, it shall apply 24 months after the entry into force of this Regulation.

22.4 HAZARD CLASSIFICATION AND LABELING OF FLAVORS AND FRAGRANCES

This section describes the rules for hazard classification and labeling of F&F substances and preparations, including natural raw materials, such as extracts and essential oils, containing hazardous constituents, according to the EU regulations.

For trade of F&F (including pure substances and mixtures or preparations thereof and natural raw materials) within the European Union, certain rules apply within the European Industry which are established by the European Flavour and Fragrance Association Code of Practice (EFFA CoP). The following general considerations are taken over from the Introductory note to the EFFA CoP, which is published yearly on the EFFA website: www.effa.be. It should be noted that the most recent

version of the EFFA CoP (version of 2009) for the first time also takes into account the Globally Harmonized System of Classification and Labeling of Chemicals (UN-GHS). This GHS has now also been implemented in the EU with the Publication of the EU-GHS Regulation (so-called "CLP Regulation": Classification, Labelling and Packaging of substances and mixtures) [Regulation (EC) No. 1336/2008, OJ L 354, 31.12.2008, p. 60]. It has entered into force on 20 January 2009 and the current Directive 67/548/EEC (DSD) and Directive 1999/45/EC (DPD) shall be repealed with effect from 1 June 2015. However, Annex I of the DSD has already been repealed and transferred into Annex VI of the EU-CLP Regulation, with exception of the last two technical adaptations (ATP 30 and ATP 31) to the DSD.

However, the following section on classification on labeling is still based on the currently applicable DSD and DPD.

Within the European Union, substances and preparations have to be classified and, if dangerous according to criteria laid down in the regulations, have to be labeled according to certain rules. The classification and labeling of substances are either prescribed in Annex I to the Dangerous Substances Directive 67/548/EEC (DSD) or have to be done by the supplier using the criteria of Annex VI of this Directive. For preparations, like F&F compounds, it is done according to the Dangerous Preparations Directive 1999/45/EC (DPD).

Several substances of interest to the fragrance and flavor industry are mentioned in Annex I of the DSD. They are included in the respective attachments to the EFFA CoP with their Annex I number next to their CAS and EU numbers. The label mentioned in the attachment has to be used in the MS of the European Union.

Special emphasis is put on Classification of aspiration hazard (Xn; R65) of both substances that can easily reach the lungs upon ingestion and cause lung damage (substances with low viscosity and low surface tension) and mixtures/preparations with a high hydrocarbon (HC) content and low kinematic viscosity that will pose the same hazard.

Based on measurement results for a number of natural raw materials (e.g., extracts and essential oils) with HC contents between 10% and 90+% and on similar measurements of some F&F compounds, a dedicated Working Group of the F&F Industry has come to the conclusion that in practice, substances and preparations containing more than 10% of HC(s) fall within the criteria for viscosity and surface tension.

Therefore, the European F&F Industry through its EFFA CoP recommends

- To determine the HC content of substances (supplier information, analysis) and preparations (including extracts and essential oils) (calculation) and to classify as Xn; R65 if more than 10% HC is present.
- That nonclassification should only be possible if viscosity and/or surface tension measurement results are available for a specific substance or preparation (including extracts and essential oils).

In addition, classification and labeling of skin sensitizers is addressed in the CoP: the issue on skin sensitization (and labeling of the alleged allergens for the purpose of the Cosmetic Directive, 7th Amendment) has been in depth discussed in the first section. However, it should be noted here that classification of substances and essential oils or extracts as sensitizers (R43) has nothing to do with the requirement to label the 26 alleged allergens on the final cosmetic products according to the Cosmetic Directive (7th Amendment).

Following the EFFA CoP, skin sensitizers are labeled Xi, R43. According to the CoP, it is recommended to use the administrative limit concentration of 1% when classifying preparations (including extracts and essential oils) containing them in all cases, unless a different threshold is laid down in Annex I to the DSD.

In the EFFA CoP, special attention is paid to the hazard classification and labeling of natural raw materials, referred to as "natural complex substances" (NCSs) in the CoP. The terminology *Natural*

Complex Substance is used because in some cases the natural raw material (the complex) is regarded as a single substance, rather than a complex mixture.

NCSs (e.g., essential oils, and extracts from botanical and animal sources) require special procedures due to the fact that they might have quite different chemical compositions (and therefore hazard classifications) under the same designation. This may occur even when this differentiates between species, cultivars and chemotypes and different production procedures (e.g., absolutes, resinoids, and distilled oils).

There are two ways of classifying and labeling NCSs such as extracts and essential oils: either based on the data known and available on the natural raw material as such (NCS is regarded as a single "substance") or based on the hazardous constituents they are composed of (NCS is regarded as a complex mixture).

In the first case, an NCS may be classified on the basis of the data obtained by testing the NCS. The test results of an NCS, even if containing classified constituents, are evaluated in accordance with the DSD. The health and environmental hazard classifications derived following this approach are quality dependent, which is also indicated in the EFFA CoP.

In the second case, for grades of NCSs and for endpoints for which reliable test data are lacking, the EU's Labelling Guide (Annex VI to the DSD) incorporates a requirement introduced by Commission Directive 93/21/EEC, whereby the hazard classification of complex substances shall be evaluated on the basis of levels of their known chemical constituents. Where knowledge about constituents exists, for example, on substances limited as per Annex II of Directive 88/388/EC (the so-called biologically active substances—see above) or on substances with sensitizing, toxic, harmful, corrosive, and environmentally hazardous properties, the classification and labeling of these NCSs according to the requirements of the European Union should follow the rules for preparations (= mixtures) as prescribed by the DPD.

One dedicated section of the EFFA CoP also provides a list with the composition of the NCSs (extracts, essential oils, concretes, absolutes, etc.) in terms of the presence (content in %) of hazardous constituents and HCs in the NCSs that have to be taken into account for the classification and labeling of the NCSs or a preparation containing these NCSs, based on the DPD.

F&F compounds that are preparations (i.e., compounded mixtures, formulations, or compositions) should be classified and labeled according to the EU's DPD 1999/45/EC and its articles 6 and 7.

In practice, test data on the flavor or fragrance compounds (preparations) are not available or collected. Therefore the classification of these preparations should be based on the chemical composition and should include the contributions of hazardous substances present as constituents in the NCSs present in the formulation. This is another reason why the composition of the NCSs in terms of presence of the hazardous constituents is also part of the EFFA CoP.

Examples of important constituents to take into account for classification:

- Sensitizers (R43) → NCSs (essential oils and extracts) to be classified as R43 if the content (%) of the sensitizer (if one) or the content of their sum (if more than one) is greater than or equal to 1%.
- CMRs (carcinogenic, mutagenic, and reprotoxic materials: R45; R46; R68) → NCSs to be classified as CMR if the content of the CMR substance(s) is greater than or equal to 0.1%.

The final classification and labeling of an essential oil can be totally different depending on the approach used for the classification, either based on data on the essential oil as such (the first case described above) or based on the hazardous constituents in the essential oil (the second case described above). This is illustrated below with two examples: orange oil, containing mainly limonene [which is classified as both a sensitizer (R43) and very toxic for the environment (R50/53)], and nutmeg oil, containing safrole which is a CMR (T; R45-22-68).

TABLE 22.7
Composition of Orange Oil with Main Hazardous Constituents

Constituent	Concentration (%)	Classification
d-Limonene	96.2	R10-38-43-50/53
Linalool	0.5	Not classified
Citral	0.2	R38-43

Orange oil: Table 22.7 below depicts the composition of orange oil with the classification of the main constituents.

Resulting classification of orange oil:

- Classification and labeling "as a single substance" (based on data on the oil as such):

Xn	Harmful
R10	Flammable
R65	Harmful: may cause lung damage if swallowed

- Classification based on its constituents:

R10	Flammable
R38	**Irritating to skin**
R43	**May cause sensitization by skin contact**
R50/53	**Very toxic to aquatic organisms (environment)**
R65	Harmful: may cause lung damage if swallowed

- Labeling (pictograms) based on its constituents:

Xn: Harmful 

N: dangerous for the environment 

Nutmeg oil: Table 22.8 below depicts the composition of nutmeg oil with the classification of the main constituents.

Resulting classification of nutmeg oil:

- Classification and labeling "as a single substance" (based on data on the oil as such):

Xn	Harmful
R10	Flammable
R65	Harmful: may cause lung damage if swallowed

TABLE 22.8
Composition of Nutmeg Oil with Main Hazardous Constituents

Constituent	Concentration (%)	Labeling and Classification
Pinenes	40	Xi; R43, N; R50/53
Limonene	7	Xi; R38-43, N; R50/53
Safrole	2	T; R45, Xn; R22-68
Isoeugenol	1	Xn; R21/22, Xi; R36/38-43

- Classification based on its constituents:

R10	Flammable
R43	**May cause sensitization by skin contact**
R45	**May cause cancer**
R50/53	**Very toxic to aquatic organisms (environment)**
R65	Harmful: may cause lung damage if swallowed
R68	**Possible risk of irreversible effects**

- Labeling (pictograms) based on its constituents:

T: Toxic (CMR) 

N: Dangerous for the environment 

So with these examples, it can be demonstrated that the final Classification and Labeling (C&L) of essential oils will change significantly depending on the approach used: based on existing data (for the various endpoints) on the essential oil as such or based on the hazardous constituents. It should however be underlined that according to the rules of the EFFA CoP, C&L of natural raw materials or NCSs can only be done for the endpoints for which data (on the NCS as such) are available (e.g., skin irritation, sensitization, environmental toxicity, etc.)—if no data are available, then the constituents must be taken into account for the classification for these endpoints.

22.5 CONCLUSION

As described and outlined above, several new European Regulations and Directives have been adopted during the last years, and other regulations are currently under discussion and will soon enter into force in relation to flavors and fragrances and cosmetics. NCSs or raw materials (such as essential oils and extracts) are very important ingredients for flavoring and fragrance applications. As a result, these new regulations will have a major impact on the trade and use in commerce of these essential oils and extracts, in particular on labeling issues, as has been demonstrated with the 7th Amendment of the Cosmetic Directive (labeling of alleged allergens) and the coming new Flavouring Regulation (labeling of "natural"). The labeling issue is especially important because of its impact on consumer behavior: consumers do not want to buy cosmetic end products that are labeled with potentially allergenic ingredients. Overlabeling should always be avoided. Therefore it is essential to lobby for a pragmatic approach toward allergen labeling and to advocate no labeling requirements for fragrance ingredients that are proven to be (extremely) rare sensitizers or no sensitizers at all, especially if these are naturally occurring constituents of a wide variety of essential oils and extracts. With respect to food, consumers prefer natural flavorings to synthetic ones. Good and pragmatic definitions in the Flavouring Regulation that will soon replace the current Flavouring Directive are essential to ensure that all natural raw materials such as essential oils and extracts can be labeled as natural.

ANNEX 22.1
Aromatic Natural Raw Materials Containing Any of the 16 Naturally Occurring Alleged Aensitizers

Aromatic Natural Raw Materials	Type	Benzyl Alcohol	Benzyl Salicylate	Cinnamyl Alcohol	Cinnamal	Citral	Coumarin	Eugenol	Geraniol	Isoeugenol	Anisyl Alcohol	Benzyl Benzoate	Benzyl Cinnamate	Citronellol	Farnesol	Limonene	Linalool	Total %
Ambrette		*	*	*	*	*	*	*	*	*	*	*	*	*	5	*	1	6
Angelica root		*	*	*	*	*	*	*	*	*	*	*	*	*	*	18	0.3	18.3
Angelica seed		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Star Anise		*	*	*	*	*	*	*	*	*	*	*	*	*	*	3	1.5	4.5
Anise		*	*	*	*	*	*	*	*	*	*	*	*	*	*	2	0.1	2.1
Armoise		*	*	*	*	*	*	*	*	*	*	*	*	0.2	*	2	*	2.2
Basil	Linalol	*	*	*	*	*	*	15	0.2	*	*	*	*	0.3	*	1	62	78.5
Basil	Me-chavicol	*	*	*	*	*	*	0.5	*	*	*	*	*	*	*	1	1.1	2.6
Bay		*	*	*	*	*	*	56	*	*	*	*	*	*	*	4	3	63
Benzoin	note 1	*	*	*	*	*	*	*	*	*	*	0.2	0.8	*	*	*	*	1
Bergamot (s) cold press		*	*	*	*	0.7	*	*	*	*	*	*	*	*	*	45	15	60.7
Bergamot bergapten-free		*	*	*	*	0.7	*	*	*	*	*	*	*	*	*	45	15	60.7
Bergamot distilled			*	*	*	0.4	*	*	*	*	*	*	*	*	*	40	40	80.4
Bitter orange		*	*	*	*	0.1	*	*	*	*	*	*	*	*	*	95	0.2	95.3
Buchu (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	30	0.5	30.5
Cabreuva		*	*	*	*	*	*	*	*	*	*	*	*	*	3	*	*	3
Cajuput		*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	10	3.6	14
Camphor		*	*	*	*	*	*	*	*	*	*	*	*	*	*	25	0.5	25.5
Cananga		*	3	*	*	*	*	0.7	1.5	*	*	5	*	*	2	*	3	15.2
Caraway		*	*	*	*	*	*	*	*	*	*	*	*	*	*	45	*	45
Cardamom		*	*	*	*	0.6	*	*	1.2	*	*	*	*	*	*	4	4	9.8
Carrot		*	*	*	*	*	*	*	2	*	*	*	*	*	*	3	2	7
Cascarilla		*	*	*	*	*	*	0.3	*	*	*	*	*	*	*	5	5	10.3
Cassia		*	*	1	90	*	4	0.5	*	*	*	1	*	*	*	0.1	*	96.6
Cedarwood (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Celery		*	*	*	*	*	*	*	*	*	*	*	*	*	*	79	0.1	79.1
Chamomile	Roman	*	*	*	*	*	*	*	0.7	*	*	*	*	0.7	*	5	0.8	7.2

Chamomile	Blue	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0.4	1.4
Cinnamon bark		*	*	0.2	75	*	0.3	6	*	*	*	1.5	*	*	*	1	6	90
Cinnamon leaf		*	*	1	3	*	0.3	85	*	*	*	4	*	*	*	*	4	97.3
Cistus (Rockrose)		*	*	*	*	*	*	*	1.2	*	*	*	*	*	*	4	0.5	5.7
Sri Lanka Citronella		*	*	*	*	1.1	*	*	23	*	*	*	*	8.5	*	12	1	45.6
Java Citronella		*	*	*	*	1.3	*	1	25	*	*	*	*	14	1	5	1.5	48.8
Clary Sage (s)		*	*	*	*	*	*	*	2.2	*	*	*	*	*	*	1	24	27.2
Clove (s)		*	*	*	*	*	*	92	*	0.5	*	*	*	*	*	*	*	92.5
Copaiba		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Coriander	fruit	*	*	*	*	*	*	*	3	*	*	*	*	*	*	5	78	86
Coriander	leaf	*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	1	25	26.4
Corn mint	Arvensis	*	*	*	*	*	*	*	*	*	*	*	*	*	*	7	*	7
Cubeb		*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	1.2	2.2
Cypress (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	14	0.8	14.8
Davana		*	*	*	*	*	*	0.1	*	*	*	*	*	*	*	0.1	1	1.2
Dill (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	45	4	49
Elemi		*	*	*	*	*	*	*	*	*	*	*	*	*	*	72	*	72
Estragon	Tarragon	*	*	*	*	*	*	0.2	*	*	*	*	*	*	*	6	*	6.2
Eucalyptus globulus		*	*	*	*	*	*	*	*	*	*	*	*	*	*	8	*	8
Eucalyptus dives		*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	1	2
Eucalyptus citriodora		*	*	*	*	*	*	*	*	*	*	*	*	0.7	*	1	0.3	2
Fennel	Bitter	*	*	*	*	*	*	*	*	*	*	*	*	*	*	35	*	35
Fennel	Sweet	*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Galbanum		*	*	*	*	*	*	*	*	*	*	*	*	*	*	3.4	*	3.4
Geranium	N. Africa	*	*	*	*	1.5	*	*	18	*	*	*	*	36	*	1	8.5	65
Geranium	Bourbon	*	*	*	*	1.5	*	*	20	*	*	*	*	26	*	1	11	59.5
Geranium	China	*	*	*	*	1.2	*	*	12	*	*	*	*	43	*	1	4.5	61.7
Ginger (s)		*	*	*	*	0.7	*	*	*	*	*	*	*	*	*	2	0.6	3.3
Grapefruit (s)		*	*	*	*	0.15	*	*	*	*	*	*	*	*	*	95	0.1	95.25
Guaiac wood		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Gurjun		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Hay	note 1	*	*	*	*	*	8	*	*	*	*	*	*	*	*	*	*	8
Ho (s)		*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	0.2	90	90.6
Hop		*	*	*	*	*	*	*	0.2	*	*	*	*	*	*	1	0.6	1.8

continued

ANNEX 22.1 (continued)

Aromatic Natural Raw Materials	Type	Benzyl Alcohol	Benzyl Salicylate	Cinnamyl Alcohol	Cinnamal	Citral	Coumarin	Eugenol	Geraniol	Isoeugenol	Anisyl Alcohol	Benzyl Benzoate	Benzyl Cinnamate	Citronellol	Farnesol	Limonene	Linalool	Total %
Hyssop		*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Immortelle		*	*	*	*	*	*	*	*	*	*	*	0.2	*	*	3.8	6	10
Juniper berry		*	*	*	*	*	*	*	*	*	*	*	*	*	*	20	0.2	20.2
Labdanum		*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Laurel (s)		*	*	*	*	*	*	2	0.3	*	*	*	*	*	*	5	11	18.3
Lavandin (s)		*	*	*	*	*	0.1	*	0.6	*	*	*	*	*	*	2	38	40.7
Lavandin absolute		*	*	*	*	*	5	*	1.5	*	*	*	*	*	*	1	42	49.5
Lavender absolute		*	*	*	*	*	8	*	1.5	*	*	*	*	*	*	0.3	42	51.8
Lavender (s)		*	*	*	*	*	*	*	1.1	*	*	*	*	*	*	1	45	47.1
Spike lavender		*	*	*	*	*	0.2	0.1	0.2	*	*	*	*	0.3	*	3	50	53.8
Lemon (s)		*	*	*	*	3	*	*	0.2	*	*	*	*	*	*	73	0.3	76.5
Lemongrass (s)		*	*	*	*	90	*	0.3	7	*	*	*	*	0.8	*	4	2	100
Lime (s) distilled		*	*	*	*	3	*	*	0.4	*	*	*	*	*	*	50	0.2	53.6
Lime (s) cold press		*	*	*	*	6.5	*	*	0.4	*	*	*	*	*	*	55	0.2	62.1
Litsea cubeba		*	*	*	*	78	*	*	1.5	*	*	*	*	1.5	*	15	**3**	99
Mace		*	*	*	*	*	*	1.1	*	0.2	*	*	*	*	*	4	0.4	5.7
Mandarin (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	75	0.3	75.3
Marjoram sweet		*	*	*	*	*	*	*	0.3	*	*	*	*	*	*	5	25	30.3
Marjoram wild	Spanish	*	*	*	*	*	*	*	*	*	*	*	*	*	*	6	48	54
Mentha citrata (s)		*	*	*	*	*	*	*	2	*	*	*	*	*	*	1	50	53
Myrrh		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Myrtle		*	*	*	*	*	0.2	0.7	0.8	*	*	*	*	0.3	*	12	2	16
Neroli		*	*	*	*	0.3	*	*	3.5	*	*	*	*	*	4	18	44	69.8
Niaouli		*	*	*	*	*	*	*	*	*	*	*	*	*	*	10	0.2	10.2
Nutmeg		*	*	*	*	*	*	0.6	0.4	1	*	*	*	0.2	*	7	0.4	9.6
Oakmoss	note 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Olibanum	note 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	18.5	*	18.5
Opoponax	note 1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0

Sweet orange (s)		*	*	*	*	0.1	*	*	*	*	*	*	*	*	*	95	0.4	95.5
Origanum	Spanish	*	*	*	*	0.5	*	0.3	*	0.3	*	*	*	*	*	1	15	17.1
Palmarosa (s)		*	*	*	*	1	*	*	85	*	*	*	*	*	1.2	1	4	92.2
Parsley (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	5	*	5
Patchouli (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Pepper (s)	Black	*	*	*	*	*	*	*	*	*	*	*	*	*	*	23	1	24
Peppermint	Piperita	*	*	*	*	*	*	*	*	*	*	*	*	*	*	3	0.4	3.4
Peru oil	note 1	4.5	*	*	*	*	*	*	*	*	*	78	9	*	*	*	*	91.5
Bitter orange petitgrain		*	*	*	*	0.3	*	*	4	*	*	*	*	*	*	6	30	40.3
Lemon petitgrain		*	*	*	*	26	*	*	3.5	*	*	*	*	*	*	42	2.5	74
Mandarin petitgrain		*	*	*	*	0.1	*	*	*	*	*	*	*	*	*	11	0.7	11.8
Bergamot petitgrain		*	*	*	*	1	*	*	4	*	*	*	*	*	*	1.5	30	36.5
Petitgrain	Paraguay	*	*	*	*	1	*	*	4.5	*	*	*	*	*	*	2	30	37.5
Pimento (s)		*	*	*	*	*	*	85	*	1	*	*	*	*	*	2	0.5	88.5
Pine (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	6	0.2	6.2
Rose oil	Bulgaria	*	*	*	*	1	*	1.5	22	*	*	*	*	34	1	*	1.4	60.9
Rose oil	Maroc	*	*	*	*	1	*	1.5	47	*	*	*	*	20	1	*	1.4	71.9
Rose oil	China	*	*	*	*	1.5	*	1.5	18.3	*	*	*	*	31	1	*	1.3	54.6
Rose oil (s)	Turkey	*	*	*	*	2	*	1.5	20	*	*	*	*	49	1	*	1.3	74.8
Rosemary (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	6	0.8	6.8
Rosewood		*	*	*	*	*	*	*	2.5	*	*	1.6	*	*	*	1	90	95.1
Sage (s)		*	*	*	*	*	*	*	0.4	*	*	*	*	*	*	5	9	14.4
Sandalwood		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Schinus molle (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	15	0.5	15.5
Spearmint (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	24	0.2	24.2
Spikenard		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Styrax resin	note 1	0.1	*	3.5	*	*	*	*	*	*	*	*	1.5	*	*	*	*	5.1
Styrax oil		*	*	54	1.1	*	*	*	*	*	*	*	*	*	*	*	*	55.1
Tagete (s)		*	*	*	*	*	*	0.2	*	*	*	*	*	3	*	9	0.5	12.7
Tangerine (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	95	0.5	95.5
Tarragon	Estragon	*	*	*	*	*	*	0.2	*	*	*	*	*	*	*	6	*	6.2
Tea tree		*	*	*	*	*	*	*	*	*	*	*	*	*	*	4	*	4
Red thyme	Spanish	*	*	*	*	0.5	*	*	0.5	*	*	*	*	*	*	1	6.5	8.5
Tolu	note 1	0.5	*	0.6	1.7	*	*	0.1	*	*	*	37.5	4.6	*	*	*	*	45

continued

ANNEX 22.1 (continued)

Aromatic Natural Raw Materials	Type	Benzyl Alcohol	Benzyl Salicylate	Cinnamyl Alcohol	Cinnamal	Citral	Coumarin	Eugenol	Geraniol	Isoeugenol	Anisyl Alcohol	Benzyl Benzoate	Benzyl Cinnamate	Citronellol	Farnesol	Limonene	Linalool	Total %
Tonka		*	*	*	*	*	65	*	*	*	*	*	*	*	*	*	*	65
Treemoss	note1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Turpentine (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	7	*	7
Valerian		*	*	*	*	*	*	*	*	*	*	*	*	*	*	2	*	2
Vetiver (s)		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
Ylang extra super		0.5	3.5	*	*	*	*	0.5	0.7	0.5	*	6	*	*	2	*	13	26.7
Ylang extra (s)		0.5	4	*	*	*	*	0.5	3	0.5	*	8	*	*	3	*	24	43.5
Ylang I (s)		0.5	4	*	*	*	*	0.5	2.6	0.5	*	9.2	*	*	3	*	19	39.3
Ylang II (s)		0.5	4	*	*	*	*	0.5	2.4	0.5	*	10	*	*	4	*	9.5	31.4
Ylang III (s)		0.5	5	*	*	*	*	0.5	0.8	0.5	*	8.5	*	*	4	*	40	59.8
Legend		* = <0.1%			% = Actual value					ISO standardized essential oils, INCI chemical names used in this list								

Source: Internal communication (2004) regarding former version of EFFA CoP.

ANNEX 22.2
Natural Raw Materials (Natural Complex Substances) Containing the 10 Naturally Occurring Alleged Allergens of Groups I and II According to Schnuch et al. (2007)

Ambrette	Clove stem	Linaloe wood	Rose absolute
Armoise (*A. alba*)	Coriander leaf/herb	*Litsea cubebe*	Rose, Bulg
Armoise (*A. vulgar.*)	Coriander seed	Mace	Rose, China
Artemisia	Deertongue leaf abs	Melissa (lem. balm)	Rose, Maroc
Basil, linal. type	Euc. Citriodora	Mentha citrata	Rose, Turkey
Bay	Flouve	Myrtle	Rosewood
Cabreuva	Geranium, Bourbon	Neroli	Sandalwood Aus. (*S. spicatum*)
Cananga	Geranium, Chin.	Nutmeg	Snakeroot
Cardamon	Geranium, N. Afr.	Orange flower abs	Styrax
Carnation abs	Hay abs	Osmanthus abs	Thyme, wild (*T. serpyllum*)
Carrot seed	Hyacinth absolute	Palmarosa	Tolu abs
Cassia	Labdanum	Peru balsam oil	Tonka abs
Cassie abs (Acacia)	Laurel (Sweet bay)	Peru balsam resinoid	Tuberose abs
Cinnamon bark	Lavandin abs	Petit grain, bergamot	Turmeric
Cinnamon leaf	Lavender	Petit grain, lemon	Verbena abs
Citronella Ceylon	Lavender abs	Petit grain, orange	Verbena oil
Citronella Java	Lemon	Petit grain, Paraguay	Ylang extra sup.
Clary sage	Lemongrass	Pimento berry	Ylang extra, Com. (Mad.)
Clove bud	Lime, dist	Pimento leaf	Ylang, Com., (Mad.)
Clove leaf	Lime, expr.	Rhodinol	

Source: EFFA-CoP.

ANNEX 22.3
Maximum Limits for Certain Substances Obtained from Flavorings and other Food Ingredients with Flavoring Properties Present in Foodstuffs as Consumed in which Flavorings have been Used (Annex II of 88/388/EC)

Substances	Foodstuffs (mg/kg)	Beverages (mg/kg)	Exceptions and/or Special Restrictions
Agaric acid (1)	20	20	100 mg/kg in alcoholic beverages and foodstuffs containing mushrooms
Aloin (1)	0.1	0.1	50 mg/kg in alcoholic beverages
β-Asarone (1)	0.1	0.1	1 mg/kg in alcoholic beverages and seasonings used in snack foods
Berberine (1)	0.1	0.1	10 mg/kg in alcoholic beverages
Coumarin (1)	2	2	10 mg/kg in certain types of caramel confectionery 50 mg/kg in chewing gum 10 mg/kg in alcoholic beverages
Hydrocyanic acid (1)	1	1	50 mg/kg in nougat, marzipan or its substitutes or similar products 1 mg/% volume of alcohol in alcoholic beverages 5 mg/kg in canned stone fruit
Hypericine (1)	0.1	0.1	10 mg/kg in alcoholic beverages 1 mg/kg in confectionery

continued

ANNEX 22.3 (continued)
Maximum Limits for Certain Substances Obtained from Flavorings and other Food Ingredients with Flavoring Properties Present in Foodstuffs as Consumed in which Flavorings have been Used (Annex II of 88/388/EC)

Substances	Foodstuffs (mg/kg)	Beverages (mg/kg)	Exceptions and/or Special Restrictions
Pulegone (1)	25	100	250 mg/kg in mint or peppermint-flavored beverages 350 mg/kg in mint confectionery
Quassine (1)	5	5	10 mg/kg in confectionery in pastille form 50 mg/kg in alcoholic beverages
Safrole and isosafrole (1)	1	1	2 mg/kg in alcoholic beverages with not more than 25% volume of alcohol 5 mg/kg in alcoholic beverages with more than 25% volume of alcohol 15 mg/kg in foodstuffs containing mace and nutmeg
Santonin (1)	0.1	0.1	1 mg/kg in alcoholic beverages with more than 25% volume of alcohol
Thuyone (α and β) (1)	0.5	0.5	5 mg/kg in alcoholic beverages with not more than 25% volume of alcohol 10 mg/kg in alcoholic beverages with more than 25% volume of alcohol 25 mg/kg in foodstuffs containing preparations based on sage 35 mg/kg in bitters

(1) May not be added as such to foodstuffs or to flavorings. May be present in a foodstuff either naturally or following the addition of flavorings prepared from natural raw materials

ANNEX 22.4
List of Source Materials to which Restrictions Apply for Their Use in the Production of Flavorings and Food Ingredients with Flavoring Properties (Annex IV of Draft Flavouring Regulation, According to Council Proposal, December 10, 2007)

Part A: Source materials which shall not be used for the production of flavorings and food ingredients with flavoring properties

Source Material	
Latin Name	Common Name
Tetraploid form of *Acorus calamus*	Tetraploid form of Calamus

Part B: Conditions of use for flavorings and food ingredients with flavoring properties produced from certain source materials

Source Material		
Latin Name	**Common Name**	**Conditions of Use**
Quassia amara L. and *Picrasma excelsa* (Sw)	Quassia	Flavorings and food ingredients with flavoring properties produced from the source material may only be used for the production of beverages and bakery wares
Laricifomes officinales (Vill.: Fr) Kotl. et Pouz or *Fomes officinalis*	White agaric mushroom	Flavorings and food ingredients with flavoring properties produced from the source material may only be used for the production of alcoholic beverages
Hypericum perforatum	St Johns wort	
Teucrium chamaedrys	Wall germander	

ANNEX 22.5
List of Traditional Food Preparation Processes (Annex II of Draft Flavouring Regulation, According to Council Proposal)

Chopping	Coating
Heating, cooking, baking, frying (up to 240°C at atmospheric pressure) and pressure cooking (up to 120°C)	Cooling
Cutting	Distillation/rectification
Drying	Emulsification
Evaporation	Extraction, including solvent extraction in accordance with Directive 88/344/EEC
Fermentation	Filtration
Grinding	
Infusion	Maceration
Microbiological processes	Mixing
Peeling	Percolation
Pressing	Refrigeration/freezing
Roasting/grilling	Squeezing
Steeping	

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Note: (Alphanumeric/numeric character) indicates figure subset serial number.

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FIGURE 4.3 Lavender drying on the field.

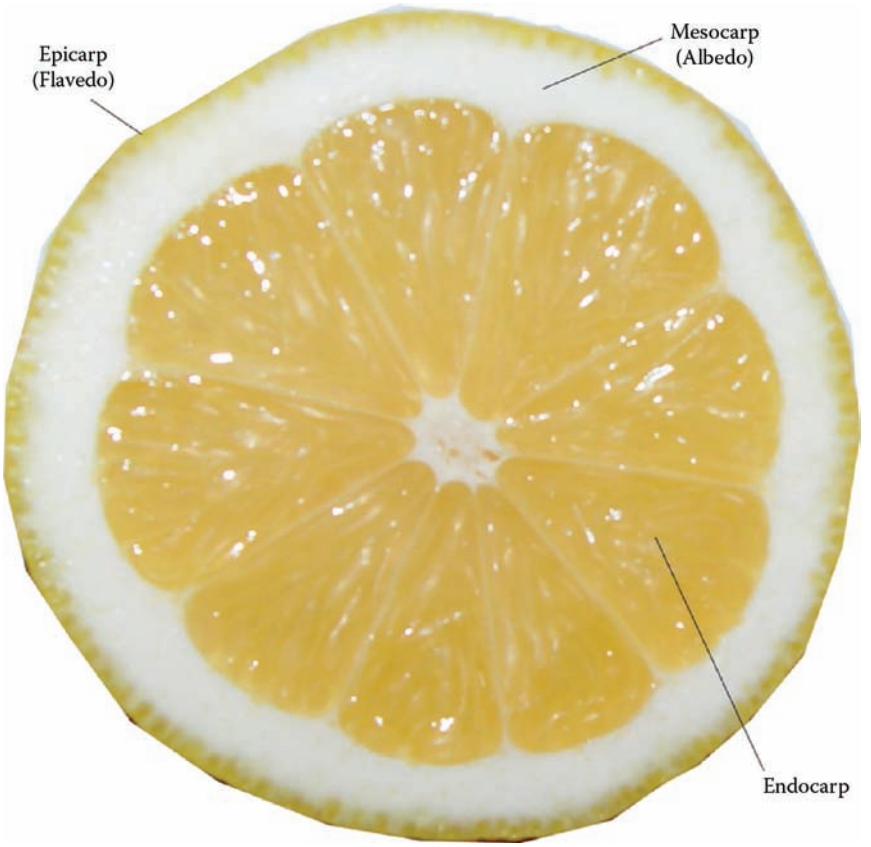

FIGURE 4.4 Parts of a citrus fruit.

FIGURE 4.5 "Pellatrici method." The spiked Archimedes screw with lemons, washed with water.

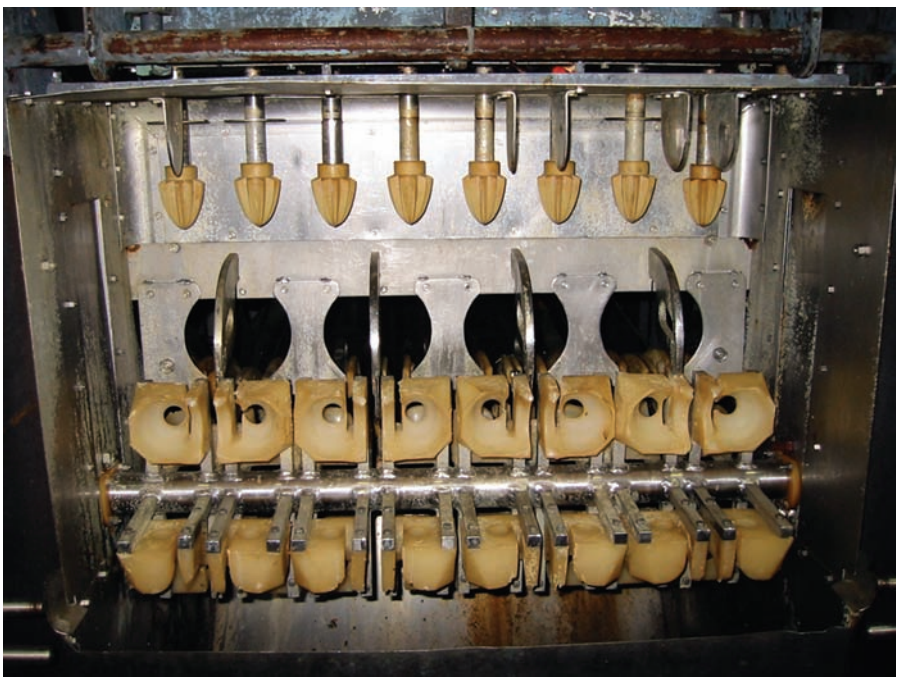

FIGURE 4.6 "Brown" process. A battery of eight juice squeezers waiting for fruits.

FIGURE 4.7 FMC extractor.

FIGURE 4.18 Oil and muddy water in the Florentine flask.

GENERAL CHEMISTRY

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Science, Technology, and Applications

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