Isolation of Piperine from Black Pepper

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The isolation of natural products from readily available spices has played an important role in exposing students to the laboratory practices of organic chemistry. For example, the isolation of trimyristin (*1*), carvone (*2*), eugenol (*3*), and anethole (*4*) have all been described and introduce the beginning organic chemistry student to a variety of techniques. We wish to add a new and interesting member to this list. Described below is the isolation of the alkaloid piperine (figure) from black pepper.[3] The procedure allows students to be introduced to the use of a reflux apparatus and the techniques of trituration, recrystallization, suction filtration, thin-layer chromatography, and melting point determination using either semimicro- or microscale labware and techniques. The experiment can be completed in a single 3–4 h laboratory period, and as such, is an excellent first experiment for beginning organic chemistry students.

Historical Background

Commercially available black pepper is the dried, full-grown but unripe fruit of the perennial woody vine *Piper nigrum L.*, while white pepper is obtained from the dried ripe fruit by removing the outer portion of the pericarp after soaking in water. Both black and white pepper find their way into homes as flavoring agents and may be bought in many forms such as crude, whole or ground pepper fruits, as an oil,[4] and as an oleoresin.[5] All forms of pepper are generally regarded as safe in FDA regulatory status with the highest average use level reported in nut products (0.42% white pepper) and in baked goods (0.2% oleoresins) (*10*).

Black pepper is known to have a variety of physiological properties including carminative,[6] diaphoretic,[7] diuretic,[8] lipolytic (*11*),[9] and insecticidal (*12–15*) properties, in addition to its widely known activity on the taste buds, which produces a reflex increase in gastric secretion. Folk medicine effects of pepper include its use as a carminative, stimulant and tonic, as well as for treatment of various cancers (*16*). In addition to these uses (*10*), there are Chinese folklore reports of white pepper involved in the treatment of malaria, stomachache, and cholera.[10]

According to Dewein (*17*), the pungency of black pepper was reported by Oerstedt (*18*) in 1821 to be due to the presence of piperine (figure), the structure of which was estab-

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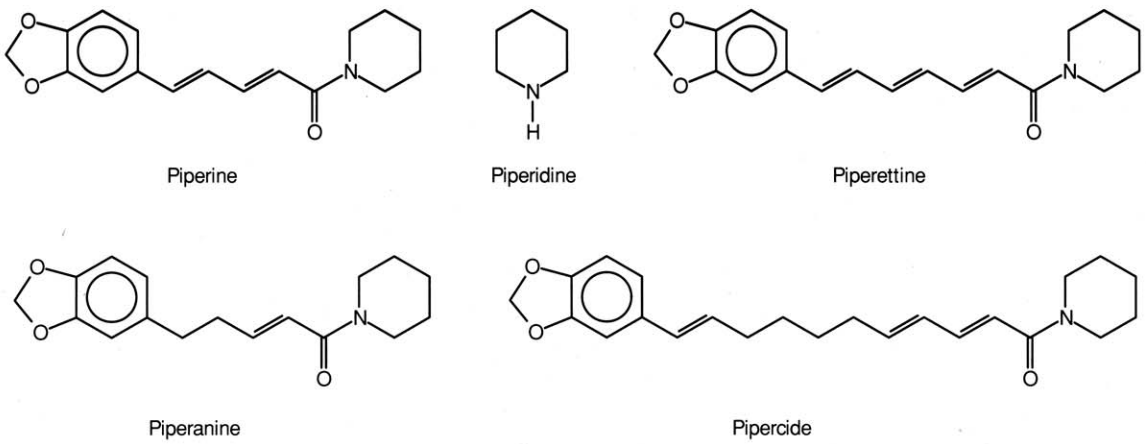
[3]Presented at the 12th Biennial Conference on Chemical Education, Davis, CA, August 1–7, 1992.
[4]Pepper oil is the volatile oil obtained by steam distillation of black pepper and contains a variety of mono- and sesquiterpenes, but none of the pungent agents (*5–9*).
[5]Black pepper oleoresin is obtained by solvent extraction of the crude pepper followed by removal of the solvent and contains the pungent principles as well as the volatile oil in pepper (*10*).
[6]Carminative: Anything used as a remedy for flatulence (an accumulation of gas in the intestinal tract).
[7]Diaphoretic: Any ingested substance that induces perspiration.
[8]Diuretic: An agent that increases the excretion of urine.
[9]Lipolytic: Capable of breaking down and decomposing fat.
[10]Cholera: An acute bacterial disease that principally causes serious intestinal disorders.



Piperine and related alkaloids found in black pepper.

lished by Ladenburg and Scholtz in 1894 (*19*). Since then, it has been determined that black pepper contains approximately 2–4% volatile oils and 5–9% of the alkaloids piperine, piperidine, piperettine, as well as several other minor components, most notably piperanine and pipercide (figure) (*12, 20–25*). Piperine and piperanine are the known pungent agents (*24, 25*), while pipercide is one of three known insecticidal agents present in black pepper (*12, 15*). White pepper has the same pungent principles and alkaloids as black pepper but contains only small amounts of the volatile oils. Because piperine can be isolated only in 2–4% yield from black pepper and is valuable as a pungent agent, several synthetic routes have been devised (*26*).

Classically, piperine is isolated by ethanol extraction of ground pepper followed by an overnight precipitation of piperine from 10% alcoholic potassium hydroxide (*27*). Because of the relatively short time available during most laboratory periods, an alternate method involving the extraction of piperine into dichloromethane and its precipitation via trituration of the crude oils with diethyl ether was developed.

Experimental[11]

Extraction

Add 5.0 g of pure ground pepper[12] and 10 mL of dichloromethane to a 50-mL round-bottomed flask. Use the round-bottomed flask as the basis for a reflux apparatus having a water-cooled condenser and heating mantle. Heat the sample to reflux, and then maintain a gentle reflux for 20 min. After the required reflux period, lower the heating mantle and allow the reflux apparatus to cool for 5 min. Suction filter the slurry with the aid of a 4.5-cm Büchner funnel, washing the pepper grounds once with 5 mL of dichloromethane. Remove two or three drops of the extract and place it in a capped vial for use in the thin-layer chromatographic analysis.

Trituration/Isolation

Transfer the extract obtained above to a clean 25-mL round-bottomed flask and concentrate *in vacuo*.[13] The resulting olive-brown, viscous oil should be cooled in an ice-bath and then 3 mL of cold ether added to the oil while gently stirring for 3–4 min. Some piperine may precipitate at this point, but remove the solvent *in vacuo* anyway. Once again cool the resulting oil in an ice-bath and then add 3 mL of cold ether to the oil while gently stirring to promote the precipitation of piperine.[14] Allow the flask to cool for an additional 10 min with occasional stirring.[15] Isolate the straw-yellow crystals of crude piperine by suction filtration with the aid of a 1.5-cm Hirsch funnel. Wash the crystals twice with 2-mL portions of cold ether. Place a small portion of the filtrate in a capped vial for use in the thin-layer chromatographic analysis.

Recrystallization

Place the crude piperine isolated above into a 13 × 100 mm test tube and dissolve it in a minimum amount of hot 3:2 acetone:hexane solution. Once all the solid has dissolved, allow the test tube to sit undisturbed for 15 min at room temperature. Rod-like, yellow crystals of piperine should be present. Cool the solution for an additional 30 min in an ice-bath before isolating the purified piperine by suction filtration with the help of a 1.5-cm Hirsch funnel. Wash the crystals once with a 2-mL portion of cold ether, allow them to air dry for several minutes, and then obtain the weight of the crystals.[16] The melting point[17] of the purified piperine now can be determined and the identity of the product confirmed by mixed melting point, thin-layer chromatographic analysis, or spectral analyses.[18]

Thin-Layer Chromatographic Analysis

Transfer a small portion of the purified piperine crystals to a small vial and dissolve them in a drop or two of acetone. Prepare a silica-gel thin-layer chromatography plate[19] for the spotting of four samples. With the aid of a capillary, place a sample of the crude oil remaining from the extraction procedure, a sample of the filtrate from the trituration/isolation procedure, the sample prepared from the purified piperine, and a sample of a piperine standard on separate points of the thin-layer plate. Develop the plate using 3:2 acetone:hexane. Visualize under UV illumination and stain in an iodine chamber.

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[11]The experimental procedure can be adapted for use with microscale glassware after the extraction has been completed.

[12]We have found that the use of inexpensive grades, and certain national brands, of ground black pepper do not yield isolable amounts of piperine when used. However, we have found that the Schilling brand of *pure ground* black pepper marketed by McCormick & Co., Inc. gives consistent and reliable results.

[13] The use of a rotary evaporator is ideal. However, any method may be utilized as long as all the solvent is removed. Any remaining traces of CH_2Cl_2 will prevent the precipitation of piperine upon trituration with Et_2O.

[14]This procedure assists in removing the last traces of CH_2Cl_2 that may be present and that prevent complete precipitation of the extracted piperine.

[15]If a copious amount of precipitate is not present, concentrate the sample *in vacuo* again and repeat the trituration. Generally, a student who failed to obtain a precipitate the first time should now obtain an appropriate amount of material. If not, concentrate and triturate as needed to precipitate the product.

[16]Typically, yields of approximately 2% or 100 mg are obtained.

[17]Literature melting point 130–132.5 °C: Weast, R. C., Ed. *CRC Handbook of Chemistry and Physics*, 58th ed.; CRC Press: West Palm Beach, FL, 1978.

[18]Aldrich Chemical Company of Milwaukee, WI, sells piperine in 5 g and 25 g lots. The material is of sufficient purity (97%) for preparing TLC standards and as a reference standard for mixed melting points determinations (mp 131–134 °C).

[19]Kieselgel 60 F_{254} plates having a 0.2 mm coating thickness are recommended.