SUMMARY AND CONCLUSIONS

The procedures of frontal analysis, elution analysis, and displacement analysis have been applied to several terpenes and terpene mixtures. The results indicate that separations are achieved. In binary mixtures, one component is separated in small quantities before the mixture appears in the eluate.

Frontal analysis appears to be the better method, among the methods and conditions utilized in these experiments, for the separation of terpenes. The method appears to be most effective in the resolution of mixtures of two terpenes when one terpene is in high concentration.

Solvents other than those used in this work are applicable to the separation of terpenes. Petroleum ether was found to be very useful in the separation of menthol and menthone, but qualitative analysis of the eluate was difficult.

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Studies on Species of *Asclepias.* V. Chemistry*,†

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The results of chemical investigations carried out upon the roots of six members of the genus *Asclepias* are reported. The preliminary phase of this study consisted of a proximate analysis of the roots of *A. tuberosa* L., *A. syriaca* L., *A. speciosa* Torr., *A. incarnata* L. var. *alba* Hort., *A. incarnata* L. var. *pulchra* Hort., and *A. curassavica* L. The root of *A. tuberosa* L. was subjected to a phytochemical analysis which resulted in the separation of three fatty acids, a hydrocarbon, a sugar, a phytosterol, an oil, three unidentified crystalline fractions, and the concentration of a potent estrogenic substance into a biologically active fraction.

A REVIEW of the literature on the six species we have studied shows that most chemical investigations have been made on only two of them.

The milky juice of *Asclepias syriaca* L. (*A. cornuti* Decaisne) has been found to contain caoutchouc (1); a resin asclepion (2); esters of butyric (3), acetic, palmitic, cerotic, and erucic acids (4); and α- and β-amyrin (5). From the rhizome have been obtained sucrose (6), a bitter principle or glucoside (7), *l*-nicotine (8), and glucose (9). The stems have been used for fiber and the seed-hairs for textiles (10). Oil from the seeds has been analyzed several times with varying results (11).

The roots of *A. tuberosa* L., besides the usual compounds found in most plants, are presumed to contain a glucoside or saponin (6, 12, 13) and an estrogenic substance (14).

The aerial portions of *A. curassavica* L. contain asclepiadin (15), an amorphous glycoside found in several other plants.

The latex of *A speciosa* Torr. contains a protease (16) which may also be present in other members of the genus.

No investigation has apparently been made of *A. incarnata* L. var. *alba* Hort., or *A. incarnata* L. var. *pulchra* Hort.

EXPERIMENTAL

Proximate Analyses.—Samples of the roots of the six species were gathered from first-year plants in early August prior to any development of the floral parts. They were dried in the air, ground coarsely, and submitted to proximate analysis. The results are given in Table I.

Root of *A. tuberosa*.—The coarsely ground root, amounting to 5 Kg., was extracted according to the method of Harnack for the isolation of asclepiadin from vincetoxicum root (17). This gave 0.001% of a light tan powder which was soluble in water, alcohol, and chloroform, but was insoluble in ether. The aqueous solution possessed a bitter taste. Various attempts to crystallize the material met with failure, as previously found by others investigating asclepiadin. It gave a positive Molisch test for carbohydrate but none with the usual alkaloidal precipitants except tannic acid. It had no reducing

* Received August 31, 1951, from the Massachusetts College of Pharmacy, Boston.
Presented to the Scientific Section, A. PH. A., Buffalo meeting, August, 1951.
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TABLE I.—PROXIMATE ANALYSIS OF SIX SPECIES OF ASCLEPIAS

	A. tuberosa	*A. syriaca*	*A. speciosa*	*A. curassavica*	*A.* var. *alba*	*A.* var. *pulchra*
Moisture	9.0	11.5	10.2	9.5	7.8	8.5
Ash	5.01	8.52	9.97	10.05	7.50	4.79
Acid-insoluble ash	0.63	0.95	1.09	1.13	0.70	0.82
Crude fiber	28.15	30.50	27.52	26.85	21.55	23.19
Successive extractions:						
Benzin	1.25	0.22	1.02	0.48	0.86	0.75
Ether, abs.	1.30	0.31	0.51	0.83	1.01	0.92
Alcohol	3.5	2.9	1.8	7.6	9.5	5.5
Water	15.85	13.28	14.65	16.45	17.40	15.50

properties but, after hydrolysis, gave a reduction of Fehling's solution. The melting range was found to be 90–120°. All of these properties agreed with those described by others for asclepiadin, but they also indicated that the material was a mixture.

The alcoholic extract of the root was exhausted with ether to remove fatty material, and was decolorized with activated charcoal. The solution was allowed to evaporate spontaneously, and after about two-thirds of the solvent had vaporized, large tan-colored crystals were deposited. After purification these crystals were found to be sucrose, the total yield amounting to about 0.11% of the dried root.

Several samples of the air-dried root (about 700 Gm. each) were extracted for about ten hours with petroleum ether in a Soxhlet apparatus. The solutions were collected and the solvent was removed by distillation at reduced pressure. The dark, olive-green, oily residue, after being partly decolorized by treatment with purified siliceous earth, amounted to about 1.5% of the dried root. The decolorized oil had a specific gravity of 0.9376 at 20°, a refractive index of 1.4860 at 15°, a saponification number of 185.5, an acid value of 106.8, and an iodine number of 132.5.

The oil, after standing at room temperature, deposited a solid fat-like material, and the amount of this deposit was increased by refrigeration to about 36% of the oil. The solid, after repeated recrystallization from methyl alcohol, had a melting point of 28°, was light yellowish-white in color, and possessed no odor but had a characteristic fatty taste. The material was placed in hot 0.2 *N* alcoholic potassium hydroxide and the mixture was boiled for a few minutes. Most of it went into solution, but a small amount of unsaponifiable matter was collected by filtration. Upon acidification of the solution, there was obtained a white, fluffy precipitate which was soluble in petroleum ether, ether, acetone, alcohol, amyl alcohol, benzene, carbon tetrachloride, and hot methyl alcohol. This precipitate had a refractive index of 1.3490 at 42°, a neutralization equivalent of 193.25, a molecular weight (by the Rast method) of 195.86, an acid value of 40.88. It melted at about 40°, and gave no test for unsaturation. Since these properties pointed to lauric acid, a *p*-bromophenacyl ester was prepared in the usual way. The melting point of this ester was 74°, confirming that the acid was lauric acid and indicating that the original deposit in the oil was trilaurin. The small amount of unsaponifiable matter was separated into two portions by fractional precipitation of an alcoholic solution by water. One was a light yellow, brittle solid that melted at 58–60°, had a molecular weight (by the Rast method) of 132, and gave positive Liebermann-Burchard and Salkowski reactions for sterols. The other was a dark greenish-brown mass that weighed about 0.4 Gm., melted at about 28°, and had a molecular weight of approximately 247. These properties indicated that it was a saturated hydrocarbon.

By refrigerating the remaining petroleum ether extract for two weeks, there was obtained a further deposit of a light yellow-green solid, which was removed by filtration. This crude solid, which had a melting range of 58–61°, was purified by dissolving it in hot acetone with activated charcoal and refluxing the solution for two hours. The mixture was then filtered, concentrated, and rapidly cooled. From the cold solution there crystallized a light yellow solid with a melting point of 62°, a neutralization equivalent of 249, and a molecular weight of 246.8. The conclusion that this was palmitic acid was confirmed by preparing the *p*-bromophenacyl ester, which melted at 84°.

From the residual petroleum ether extract no crystalline bromide could be separated, indicating the absence of linoleic and linolenic glycerides. This residue was saponified with alcoholic potassium hydroxide and acidified to liberate the fatty acids in the form of a dark brown oil. Two drops of the oil was dissolved in sulfuric acid, and a dilute solution of vanillin was superimposed. A violet-colored ring appeared at the junction of the two layers, indicating that oleic acid was present. The remainder of the acid mixture was neutralized with potassium hydroxide, and to the dilute solution, 10% potassium permanganate was added dropwise with constant stirring, until the pink color was permanent. The solution was acidified to dissolve the manganese dioxide and to liberate the fatty acids. There precipitated a small quantity of a white substance, which was collected on a filter and dried. It melted at 133–135°, sufficiently close to the recorded melting point of dihydroxystearic acid (136.5°) to indicate that oleic acid was present in the acid mixture. It would appear that glycerides of oleic acid were contained in the original extract.

Estrogenic Substances.—Costello and Lynn reported that the root of *Asclepias tuberosa* has estrogenic activity (14), and experiments were designed to confirm this statement and to test other species. For this purpose the material was extracted to the total phenol fraction by the modified Pincus method. The biological assay was that of Allan and Doisy (18) and the results were also checked with a Beckman quartz spectrophotometer by comparing the ultraviolet absorption curves with the typical curve for estriol.

No estrogenic activity could be demonstrated in the stems, leaves, pods, or seeds of *A. tuberosa*, but the roots possessed a significant amount. The total phenol fraction produced a positive state of estrus in 50% of the test animals after administration in doses of 15–25 mg. per rat, and gave the typical absorption curve. Based upon a yield of 0.6% of the root for the total phenol fraction, this represents a substantial estrogenic activity.

No activity could be proved in the leaves, flowers, seed hairs, or pods of *A. syriaca*. The stems and roots did produce a typical absorption curve, but the total phenol fraction did not produce estrus in doses as large as 150 mg.

No significant amount of estrogenic activity could be found in various parts of the other four species, although a weak biological response was shown by the stems in two cases. The total phenol fraction of *A. speciosa* appeared to be somewhat toxic to the rats.

SUMMARY

1. The results of proximate analyses on the roots of six members of the genus are presented.

2. From the root of *Asclepias tuberosa* were separated a substance similar to asclepiadin, sucrose, trilaurin, palmitic acid, an ester of oleic acid, and probably a sterol and a saturated hydrocarbon. Estrogenic activity of the root was confirmed, but tests of various parts of this and other species showed little or no activity.

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A Colorimetric Determination of Streptomycin and Dihydrostreptomycin*

By F. MONASTERO†

A colorimetric method of assay is described involving the streptidine moiety of the streptomycin and dihydrostreptomycin molecule. The method is simple, reproducible, and rapid. Comparative results between the chemical assay and the microbiological assay are presented.

SINCE the discovery of streptomycin and dihydrostreptomycin, several microbiological and chemical methods of assay have been proposed. The microbiological assay methods utilizing strains of *Bacillus subtilis* (1) and *Escherichia coli* (2) have been found advantageous in determining the potencies of these antibiotics in body fluids and in various dosage forms. Similarly, the chemical assay methods for streptomycin of Boxer, *et al.* (3), and Scudi, *et al.* (4), have been equally applicable to the determination of the concentration of these antibiotics in biological systems and in various dosage forms. The periodate oxidation method proposed by Garlock and Grove (5) for assaying dihydrostreptomycin has been found satisfactory. However, the lengthy procedure involved has made this method inapplicable for the routine testing of large numbers of samples.

The chemical assays for streptomycin have been reactions dealing with either (*a*) the alkaline hydrolysis of the streptose moiety to form maltol (2-methyl-3-hydroxy-γ-pyrone) which produces a stable purple-red color when reacted with ferric ions in acid solution (3), or (*b*) with *n*-methyl l-glucosamine, the amino sugar moiety of the streptomycin molecule which produces a pink color with the Elson-Morgan reagent (4).

A colorimetric method of assay for streptomycin and dihydrostreptomycin involving the

* Received October 25, 1951, from the Analytical Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.
† The author wishes to thank Mr. F. H. Hedger, Mr. T. C. Grenfell, and Dr. J. A. Means for their helpful comments and constructive criticisms of this paper.