employed. In England and the United States hyoscine was once used to designate the lævo- base. Preference is now given to *l*-scopolamine as the proper name.

Samples of the drug, the alkaloid, the picrate and the aurichloride have been filed for reference.

CONCLUSIONS.

1. The solvent and treatment of final chloroformic extract exert controlling influences upon the results obtained in assays.
2. The high and variable results are not attributable to volatile amines but to an ammonia soap of a resin-like material.
3. The optical rotation, crystal forms of the picrate and aurichloride and their respective melting points prove the alkaloid to be *l*-scopolamine.
4. Lævo-scopolamine was isolated, identified and was the only alkaloid found in *Datura innoxia*, Mill.

A PHYTOCHEMICAL STUDY OF GILLENIA STIPULATA.

BY L. LAVAN MANCHEY.*,[1]

Gillenia stipulata and *Gillenia trifoliata* are North American drugs indigenous to the eastern United States, known to the Indians, and used by the early colonists (1) as a substitute for Ipecac under the name American Ipecac. Of the two species the former has been generally acknowledged as the more efficacious. Although there is no botanical relation between Ipecac and American Ipecac, the roots of both plants, in being annulated, are similar; and this circumstance, no doubt, suggested to the early users of the drug a parallel in therapeutic value.

This drug received recognition in medical practice through the efforts of Barton, Schoepf and others (2, 3) and was official in the United States Pharmacopœia before the sixth revision. It was reputed to be emetic, cathartic, sudorific, expectorant and tonic (4). Ebert (5), however, states that doses of from one to twelve grains of extract prepared from drug obtained through commercial channels were entirely without effect upon himself and upon several young men. Shreeve (6), in 1835, and students (7) of the Philadelphia College of Pharmacy, in 1854, found in the root starch, gum, albumin, volatile oil, wax, fatty resin, lignin, coloring matter, gallotannic acid, lime potash and iron. Stanhope (8), in 1856, isolated a substance from the root cortex which he named "Gillenin," corroborated in 1877 by Wetherill (9). Curry (10), in 1892, reported two glucosides "Gillenin" and "Gillein," neither of which appears to be identical with "Gillenin."

EXPERIMENTAL.

For the present investigation 50-pounds of *G. stipulata*, in the form of a number twenty powder, were procured from commercial sources, and after confirming the

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identity of species a representative sample was analyzed for ash, soluble extractive, etc.

	Per Cent.		
Sample	A.	B.	Average.
Total Ash	22.03	21.95	21.99
Acid-Insoluble Ash	14.99	15.29	15.14
Moisture (corrected for volatile constituents)	7.04	7.04	7.04
Moisture by Xylene Method	6.10		
Volatile Ether-Soluble Extractive	0.54	0.43	0.48
Non-volatile Ether-Soluble Extractive	2.94	2.40	2.67
Alcohol-Soluble Extractive	6.72	6.72	6.72
Diluted Alcohol-Soluble Extractive	13.49	13.81	13.65
Water-Soluble Extractive	5.72	5.70	5.71
Petroleum Benzin Extractive	0.11	0.13	0.12

By way of preliminary examination several tests were undertaken to determine the general character of the constituents:

1. One hundred Gm. of drug, examined by the "Stas-Otto Method," gave negative tests for alkaloid and glucoside.
2. Test for alkaloid by maceration with Prollius' fluid, negative.
3. Since glucoside had been previously reported, a special search for the principle was made by treating an infusion made from 200 Gm. of the drug successively with lead acetate and lead subacetate. The filtrate from the lead precipitates together with the lead precipitates after decomposition with hydrogen sulfide were separately and carefully examined, but all tests for glucoside and saponin were negative.
4. Twenty gr. of the powdered drug when given in capsules to a small dog weighing about 3 Kg. did not produce emesis, nor did the animal evince any unnatural behaviour attributable to drug action. (Subsequently during the examination of the drug, various extractives were administered to dogs in relatively large doses, but in no case was there an apparent response to the drug.)
5. Two hundred and fifty grams of the air-dried drug in the form of a number twenty powder were extracted successively in a Soxhlet apparatus with the following liquids. After removal of the solvent the residual extracts were kept over sulphuric acid until of constant weight.

			Per Cent.
Petroleum (b. p. 35–40°)	extracted	1.30 Gm.	0.52
Ether	extracted	3.78 Gm.	1.51
Chloroform	extracted	1.43 Gm.	0.57
Alcohol	extracted	17.17 Gm.	6.87
Water	extracted	7.93 Gm.	3.13
	Total	31.61 Gm.	12.60

The petroleum extract was a yellow, fat-like semi-solid having a pleasant odor, and contained much volatile matter which was readily volatilized from paper by gentle heating. A portion of the extract was dissolved in ether and shaken first with water and then with water acidulated with hydrochloric acid. The first shakings were tested for glucoside and the second for alkaloid, but the results in both cases were negative. The ether extract was odoriferous, and both the ether and chloroform extracts were of a resinous nature.

LARGE SCALE EXTRACTION.

For the purpose of complete examination a quantity (14 Kg.) of the ground drug was extracted by continuous percolation with hot alcohol. The quantity of extract thus obtained after drying *in vacuo* amounted to 1745 Gm. Approximately one-half (830 Gm.) of this viscid extract was set aside and reserved for future work. The remainder was mixed with water and steam was passed into the mixture until the volatile products were removed. There then remained in the distillation flask a dark colored aqueous liquid (A) and a quantity of soft, dark, resinous mass (B). The latter was collected, then washed and the washings added to the aqueous liquid.

Examination of Steam Distillate.—The first distillate was slightly opalescent and contained on the surface a small amount of oil which as distillation progressed became partly solid. It was removed with ether and the ethereal liquid dried with anhydrous sodium sulphate. Upon allowing the solvent to evaporate in a desiccator over calcium chloride under slightly reduced pressure, a small quantity of volatile oil was obtained which was insufficient for the determination of its physical constants. The reserve portion of the alcoholic extract was therefore likewise steam-distilled, and the oil thus obtained from the two portions when combined amounted to 3.6 Gm. It was of a light amber color with a slight green fluorescence; possessed an agreeable odor similar to that of the crude drug; and in alcoholic solution was neutral to litmus. Sp. gr. 25°/25°, 0.86. This relatively low value, below 0.9 (11), indicates the presence of terpenes in marked quantity. Refractive index, 1.4804. Specific rotation at 25° C., −6.39. It was soluble in 27 volumes of 75% alcohol, and upon distillation was obtained principally in two fractions, *viz.*, 85–120° and 200–240° C. The higher boiling fractions could not be distilled at ordinary pressures without decomposition.

The aqueous portion of the distillate from which the oil had been removed was faintly acid to litmus, but contained neither formic nor acetic acids.

Examination of the Aqueous Liquid (A).—The aqueous liquid, which as previously mentioned, had been separated from the soft, dark resin amounted to about four liters, and was acid to litmus. It was concentrated at a low temperature to about one-fourth this volume and then shaken many times with ether. The combined ethereal shakings were concentrated to a suitable volume and successively shaken with 3 per cent sodium carbonate and 3 per cent potassium hydroxide solutions. Both alkaline extracts were decomposed with diluted sulphuric acid and the liberated substances removed with ether. Attempts to obtain a crystalline product from the decomposed sodium carbonate and potassium hydroxide extractives by crystallization from various solvents were without success because of the high solubility of the substances. The sodium carbonate extractive, however, when sublimed at 150° C., 20 mm. pressure gave a small quantity of sublimate in the form of white needles which melted at 108–110° C. The product thus obtained was contaminated with a volatile odorous substance, since upon crystallization from petroleum benzin containing a little ether the melting point was raised to 120.5–121°. When esterified with ethyl alcohol and sulphuric acid, it formed ethyl benzoate and was thus identified as benzoic acid.

The residue from the potassium hydroxide was a brown semi-solid possessing a marked pleasant odor, and evidently consisted of a complex mixture.

The aqueous liquid (A) after the removal of the ether-soluble constituents had a reddish brown color and a sweet astringent taste becoming bitter when retained in the mouth. It gave a green color with ferric chloride and reduced Fehling's solution readily.

Test for Simple Sugar.—Detection of *d*-glucose: 5 cc. of the aqueous solution was treated with basic lead acetate solution and from the filtrate an osazone was obtained which separated from solution in five minutes, and when crystallized from alcohol melted at 204° C. It was therefore *d*-glucosazone. The remainder of the aqueous liquid was then treated with an excess of lead acetate, and to the filtrate from the lead precipitate a slight excess of basic lead acetate was added. Both lead precipitates were collected on a filter, and washed by the aid of a filter pump with a little water. The filtrate resulting from the lead precipitates, and also the lead acetate and basic lead acetate precipitates after being suspended in water were decomposed by hydrogen sulphide. To exclude the possibility of an active principle having been absorbed by the lead sulphide precipitates they were exhausted by continuous extraction with various solvents, but this treatment afforded only small quantities of inert residues. The final filtrate remaining after treatment with basic lead acetate and also the solutions resulting from the decomposition of the lead precipitates were likewise examined for an active principle by shaking them separately with chloroform. As the result of this treatment, only intractable bitter residues were obtained which were neither glucosidal nor alkaloidal in character.

The three aqueous solutions remaining from the chloroform shakings were allowed to evaporate spontaneously at room temperature until quite concentrated, and the resulting syrupy liquids separately treated with absolute alcohol. The hydroalcoholic solutions thus obtained were decanted from the precipitated matter, and subjected to fractional precipitation with ether. The final ethereal-alcoholic solutions contained practically no material in solution, and the various precipitates obtained by the foregoing procedure consisted only of sugar, tannin, pigments and some amorphous matter.

Examination of the Insoluble Resin (B).—This resin, which was in the form of a nearly black viscous semi-solid, was mixed with a small quantity of alcohol, distributed through Ottawa sand, and when completely dried was subjected to prolonged successive extraction in a large modified Soxhlet apparatus with the following solvents: petroleum bezin (b. p. 35–50°), ether, chloroform, ethyl acetate and alcohol.

Petroleum Benzin Extract of the Resin.—This extract was a viscous green liquid obtained after thirty-five hours of continuous percolation, and amounted to 28 Gm. The entire benzin extract was dissolved in a large volume of ether and shaken successively with ammonium carbonate, sodium carbonate and potassium hydroxide solutions. Each of the alkaline extracts was decomposed with diluted sulphuric acid, and the liberated substances removed with ether.

The decomposed ammonium carbonate extract, from which the ether had been removed upon standing over night, exhibited a readiness to sublime. The method adopted, therefore, in an attempt to isolate this volatile constituent was that of sublimation, the process being conducted at a temperature of 100° C. under 30 mm. pressure. A small quantity of sublimate, melting indefinitely between 50° and 104°, was thus collected. By repeating the process twice more and collecting the sublimate in small fractions the last fraction obtained amounted to about 50 mg. and melted sharply at 121° C. When heated with sulphuric acid and ethyl alcohol it gave the odor of ethyl benzoate, and was therefore benzoic acid which had not been separated during steam distillation.

The quantity of sodium carbonate extractive was negligible and therefore discarded.

The decomposed potassium hydroxide extractive on the other hand amounted to several grams. No crystalline substance could be separated from it by treatment with various solvents. Consequently it was distilled at 20 mm. pressure, redistilled at 25 mm. and the fraction distilling between 100° and 250° C. collected. The presence of small quantities of a phenol were indicated by the formation of an insoluble bromine derivative and by the odor. The principal component of the mixture was a solid at room temperature, and when dissolved in a mixture of methyl alcohol and acetone and allowed to stand it separated in microscopic acicular crystals, melting at 48–49° C. In this crystalline form the compound formed a soap, and no longer possessed phenolic properties. It was precipitated in two fractions from alcoholic solution as silver salt, and analyzed.

Fraction 1:

0.0378 gave 0.0105 Ag = 27.7 per cent Ag
$C_{18}H_{35}O_2Ag$ requires 27.6 per cent Ag

Fraction 2:

0.0554 gave 0.0155 Ag = 27.9 per cent Ag
$C_{18}H_{35}O_2Ag$ requires 27.6 per cent Ag

The agreement in the analysis of the two fractions, in spite of the low melting point of the free acid, compels the decision that the compound was stearic acid in a slightly impure state.

The filtrate from the stearic acid was allowed to concentrate further until a sufficient amount of crystals separated for analysis.

Silver salt.

0.0400 gave 0.0120 Ag = 30.0 per cent Ag
$C_{16}H_{31}O_2Ag$ requires 29.7 per cent Ag

The per cent of silver found corresponds to the theoretical requirements of palmitic acid.

The mother liquor, containing the more soluble acids, afforded 0.4955 Gm. of silver salt, which upon decomposition yielded 0.0212 Gm. Ag, equivalent to 42.7 per cent Ag. $C_8H_{15}O_2Ag$ requires 42.9 per cent Ag. The more soluble portion, hence, consisted of caprylic acid or a mixture of higher and lower acids the analysis of which subscribes to these requirements.

Saponifiable and Unsaponifiable Constituents of the Petroleum Benzin Extract.—That portion of the petroleum extract which was insoluble in the solutions of the alkalies was freed from ether, and by treatment with hot 95 per cent alcohol was resolved into three fractions, namely: (I) a substance freely soluble in cold alcohol; (II) a flocculent substance soluble in hot, but less soluble in cold alcohol; (III) an insoluble viscous substance.

Fraction (1) was saponified with alcoholic potassium hydroxide, then poured into cold water and the unsaponifiable matter removed with ether. The latter solution, upon removal of the solvent, afforded a quantity of yellow oil becoming partially solid on standing. This was crystallized from a mixture of ethyl acetate and alcohol, from which about 0.4 Gm. separated in the form of long flat plates, melting at 135°.

Upon analysis:
0.0804 gave 0.2457 CO_2 and 0.0897 H_2O; C = 83.3; H = 12.3 per cent. $C_{26}H_{44}O$ requires C = 83.7; H = 11.9 per cent.

It gave Salkowski's reaction for phytosterols, and by the Liebermann-Buchard reaction a transient violet color changing rapidly to blue, turning less rapidly to green and finally to brown.

Its acetate crystallized from acetic anhydride in long lustrous plates which melted at 121°. The compound was thus identified as a phytosterol isomeric with several compounds of the formula $C_{26}H_{44}O$ previously described in the literature (12, 13).

The insoluble flocculent substance (II), like the soluble material, was saponified, the unsaponifiable constituents removed with ether and recrystallized from ethyl acetate. A white substance, amounting to about 0.1 Gm. separated in the form of microscopic needles, melting at 78°.

Upon analysis:
0.0574 gave 0.1715 CO_2 and 0.0766 H_2O; C = 81.4, H = 14.8 per cent. $C_{30}H_{62}O$ requires C = 82.1, H = 14.3 per cent.

The compound, with bromine water, behaved as if saturated, and formed an acetyl derivative which crystallized from acetic anhydride in minute needles melting at 63–63.5°. It was thus identified as a saturated acyclic alcohol isomeric with two similar alcohols previously reported in the literature (14).

An unsaponifiable product was obtained from fraction (III) or the insoluble viscous material, but this gave no crystalline substance upon treatment with ethyl acetate and alcohol. It was distilled under reduced pressure, and found to contain a small quantity of phytosterol.

Fatty Acids.—The alkaline aqueous liquids from which the unsaponifiable constituents had been removed were combined, acidified with diluted sulphuric acid and the liberated fatty acids removed with ether. The ether was evaporated and the residue was distilled under reduced pressure. The distillate was dissolved in glacial acetic acid, decolorized with animal charcoal, and allowed to stand until a quantity of colorless crystals separated which melted at 62.5–63°. The compound was converted into the silver salt and the silver determined with the following result.

0.0868 gave 0.0256 Ag = 29.5 per cent Ag
$C_{16}H_{31}O_2Ag$ requires Ag = 29.7 per cent Ag

This result thus indicated that the substance was palmitic acid.

Ether Extract of the Resin.—This extract, as originally obtained, was a dark green ethereal solution containing a sparingly soluble amorphous substance in suspension and a more soluble viscous substance which collected on the bottom of the extraction flask as the solution cooled. The amorphous substance and the viscous matter amounting, respectively, to 6 and 7 Gm. were separately collected, leaving the soluble material which after removal of the solvent weighed 53 Gm.

The amorphous substance was not sufficiently soluble in the common organic solvents to be purified by crystallization, but by prolonged continuous extraction with hot absolute ethyl alcohol it was obtained as a perfectly white powder. It was not decomposed by diluted acids or alkalies, even on boiling. It separated from a hot 50 per cent aqueous pyridine solution, upon cooling, in an imperfectly crystalline condition. It did not contain nitrogen, and after being dried in an oven at 110° it decomposed at approximately 265°.

Upon analysis:
0.0612 gave 0.1573 CO_2 and 0.0588 H_2O. $C = 70.1$; $H = 10.6$ per cent.
0.0395 gave 0.1018 CO_2 and 0.0378 H_2O. $C = 70.3$; $H = 10.6$ per cent.

A small amount of the substance was converted into an acetyl derivative by boiling, for one hour, with acetic anhydride. The ester separated as the solution cooled, and when removed and dried, melted at 154–159°. It crystallized from ethyl alcohol in small rectangular plates, and the melting point was raised to 162°. By the Liebermann-Buchard reaction, it gave a transient pink color changing rapidly to blue and then to green.

Upon analysis.
0.0818 gave 0.2056 CO_2 and 0.0713 H_2O. $C = 68.4$; $H = 9.7$ per cent. $C_{34}H_{56}O_8$ requires $C = 68.8$; $H = 9.5$ per cent.

A portion of the acetyl derivative was hydrolyzed with alcoholic potassium hydroxide, and the liberated compound collected and analyzed. It decomposed at about 265°.

0.1123 gave 0.2957 CO_2 and 0.1087 H_2O. $C = 71.8$; $H = 10.8$ per cent. $C_{28}H_{50}O_5$ requires $C = 72.0$; $H = 10.8$ per cent.

These results were interpreted as indicating that the compound was a trihydric alcohol, $C_{28}H_{47}O_2(OH)_3$, and that the variation in percentage composition before and after acetylization might be attributable to more complete purification through hydrolysis of the acetic acid ester.

The acid constituents of the material readily soluble in ether were removed from an ether solution with diluted alkalies. Ammonium carbonate extracted small quantities of unsaturated fatty acids; sodium carbonate and potassium hydroxide only intractable resinous matter. By saponification of the residual acid-free extract an additional quantity of the phytosterol previously described was isolated, and also an acid which when crystallized from glacial acetic acid melted at 53.5°. Its silver salt was prepared and analyzed.

0.1124 gave 0.0363 $Ag = 32.3$ per cent Ag
$C_{14}H_{27}O_2Ag$ requires 32.2 per cent Ag

It was therefore myristic acid.

The sparingly soluble viscous substance was subjected to the same treatment as described in connection with the petroleum extract, but without success in removing any compound other than those already isolated.

Chloroform and Ethyl Acetate Extracts of the Resin.—These extracts amounted to 7 and 68 Gm., respectively. Both were brown resinous products from which no pure substance could be obtained.

Alcohol Extract of the Resin.—The alcoholic solution of this extract was poured into a large volume of water, the precipitated matter collected, dried and extracted with benzene. The benzene was allowed to evaporate spontaneously, leaving a small amount of a crystalline compound lodged in a soft resinous mass. Upon treatment with warm alcohol it remained undissolved in the form of white needles which melted with decomposition between 237° and 241°. The quantity of this substance was too small to permit further investigation.

Fusion with Potassium Hydroxide.—Fifty grams of potassium hydroxide were dissolved in 50 cc. of water in a porcelain dish, 10 Gm. of resin were added and the mixture heated until the temperature of the fused mass was 230°. It was dissolved in about 250 cc. of water, acidified with sulphuric acid and steam passed into the mixture to remove the volatile products of decomposition. Formic acid was identified in the first distillate. The liquid remaining after steam-distillation was filtered, decolorized with animal charcoal and allowed to evaporate spontaneously until a white crystalline substance separated. This compound decomposed at 197°, formed an insoluble lead salt and with ferric chloride gave a green color which turned red upon the addition of sodium carbonate, thus indicating its identity as protocatechuic acid.

Treatment with Dilute Alcoholic Sulphuric Acid.—As a final test for glucoside, a portion of the resin was hydrolyzed by boiling it for eight hours with a 5 per cent solution of sulphuric acid in alcohol. Hydrolysis did not liberate a reducing sugar and there is, therefore, no evidence of the presence of such a constituent in the drug.

SUMMARY AND CONCLUSIONS.

1. The most important constituents of the root of American Ipecac (*Gillenia stipulata*), as obtained in commerce include:

a. A volatile oil consisting largely of terpenes.

b. Water-soluble constituents.—*d*-Glucose, tannin, protocatechuic acid, benzoic acid and red coloring matter.

c. Benzin-soluble constituents.—Wax, associated with a phytosterol, $C_{26}H_{44}O$, a saturated monohydric alcohol, $C_{30}H_{62}O$, stearic and palmitic acids, small quantities of saturated fatty acids of low molecular weight and some unidentified unsaturated fatty acids.

d. Constituents removed by ether.—A trihydric alcohol, $C_{28}H_{47}O_2(OH)_3$ and myristic acid.

e. An unidentified white, crystalline compound present in the resin removed by ethyl alcohol.

2. The commercial drug contains neither alkaloid nor glucoside.

3. Although three glucosides have been reported previously by Stanhope and Curry such constituents cannot, in view of the evidence gathered from the present investigation, be regarded as components of the drug of commerce.

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DIFFERENCES IN SPECIES OF TAXUS.*

BY IVOR JONES AND E. V. LYNN.

During the course of an extended investigation into the conifers of Washington, attention was ultimately called to the western yew, *Taxus brevifolia*. This tree is little known except to the woodsmen who call it simply "yew." It inhabits the forests on the coast from central British Columbia to central California, being usually found on the shaded banks of mountain streams, deep gorges or damp ravines. Most of the thriving and finest trees occur directly under larger conifers. Whenever they are accidentally exposed to direct sunlight, the leaves acquire a yellowish brown, apparently unhealthy color. The largest specimens are encountered in Oregon and Washington, particularly on the Olympic Peninsula.

The tree attains a height of 20 to 75 feet and a diameter of 6 to 30 inches, but the growth is very slow. One specimen cut down in Washington was found to be over 100 years old, although only 4.4 inches in diameter. The trunk is straight and conical with long, slightly dropping branches which give the tree a weeping appearance. The bark is conspicuously thin, rarely over one-fourth inch thick, and covered with a layer of thin, papery, purple, easily detached scales, beneath which the newer bark is a clear rose color. There are no pitchy pockets as is characteristic of most western conifers.

The leaves, which usually remain on the branches for three or four years, are dark yellowish green on top and much paler on the under surface and are very soft to the touch. They are usually about one-half inch long and one-sixteenth inch wide. The midribs are proportionately large and prominent and the petioles are slender and yellow.

The fruit is a bright red berry which differs from that of other conifers in consisting of red pulp enclosing a single seed which is almost as large, about the size of a small pea. It becomes ripe in September and begins to fall in October.

The wood, like that of other yews, is heavy, hard, strong, brittle, closely grained and slightly resinous. It is of a light red color with a thin white sap-wood and obscure medullary rays. By the natives and pioneers it was very highly prized for bows, paddles, spears, fence posts and many other articles.

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* Scientific Section, A. Ph. A., Toronto meeting, 1932.