

A PHYTOCHEMICAL STUDY OF *IPOMEA PES-CAPRÆ*.^{*1}BY GUSTAV E. CWALINA² AND GLENN L. JENKINS.³

Ipomea Pes-Capræ (L.) Sweet, Family *Convolvulaceæ*, is a perennial plant with a tough woody root as thick as the finger and many feet in length; it abounds in the sand on the seashore; from the enlarged crown of the root grow a number of creeping stems, fleshy and purplish when young but becoming woody as they mature; the shape of the leaf is indicated by the name of the plant.

Detailed botanical descriptions of this plant are given by Small (1), Gray (2), Pardo de Tavera (3), Grisebach (4), Dymock (5) and Schimper (6).

According to Campbell (7), *Ipomea Pes-Capræ* is a denizen of nearly every tropical beach the world over. Guppy (8) states that as a rule this plant monopolizes the thirtieth parallels of north and south longitude. In the United States it is described (1, 2) as growing on sandy beaches from South Carolina to Texas.

Dragendorff (9) states that the leaves are used in New South Wales against rheumatism and dropsy and also for sores and ulcers. The Indian *Materia Medica* (10) gives the following medicinal uses: a decoction (1 to 20) of the roots and leaves is used in rheumatism, gout, gonorrhœa and dropsy in doses of one to two ounces; the plant is supposed to have the power of preventing the effects of age; the juice is given as diuretic in dropsy and the bruised leaves are applied to the parts; the paste of the leaves is applied to boils and carbuncles; the leaves boiled in water are applied locally to painful joints in rheumatism and to the abdomen in colic.

No report of any chemical investigation of this drug has been found.

EXPERIMENTAL.

Ten kilograms of the air-dried aerial portions of *Ipomea Pes-Capræ* were received from T. K. MacDonell in Florida. The stems weighed six Kg.; the leaves and flowers, four Kg. Nine kilograms of this material in the above ratio (6:4) were ground to a fine powder in a Koerner mill. The remaining kilogram was separated into stems and leaves and flowers and ground separately. Representative portions of the stems and of the leaves and flowers were analyzed for the customary drug "constants" using the procedures of the U. S. P. X. The results are recorded below:

For the Stems:

	A.	Per Cent. B.	Average.
Total Volatile at 110° C.	7.70	7.64	7.67
Petroleum Benzin-Soluble Extractive			
Volatile	0.14	0.12	0.13
Non-Volatile	4.50	3.83	4.17
Ether-Soluble Extractive			
Volatile	0.36	0.55	0.46
Non-Volatile	4.50	4.12	4.31
Alcohol-Soluble Extractive	8.67	8.56	8.62

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Diluted Alcohol-Soluble Extractive	18.03	18.43	18.23
Water-Soluble Extractive	13.96	14.23	14.10
Total Ash	7.25	7.41	7.33
Acid-Insoluble Ash	0.05	0.07	0.06

For the Leaves and Flowers:

	A.	Per Cent. B.	Average.
Total Volatile at 110° C.	8.67	8.62	8.65
Petroleum Benzin-Soluble Extractive			
Volatile	0.085	0.096	0.091
Non-Volatile	4.60	4.83	4.72
Ether-Soluble Extractive			
Volatile	0.25	0.39	0.32
Non-Volatile	5.61	6.49	6.05
Alcohol-Soluble Extractive	11.41	11.09	11.25
Diluted Alcohol-Soluble Extractive	22.94	22.31	22.63
Water-Soluble Extractive	17.32	17.88	17.60
Total Ash	14.06	14.07	14.07
Acid-Insoluble Ash	0.11	0.00	0.06

By way of preliminary examination a 49.28-Gm. portion of the mixed drug was extracted successively according to the Dragendorff scheme (11) in a Soxhlet extractor. The extraction was continued for at least ten hours in every case. The solvents were removed on the steam-bath and the residues dried in a desiccator over sulfuric acid to constant weight. The residues were then placed in an oven at 110° C. and heated until their weights again became constant.

	Per Cent.	
	Total.	Non-Volatile at 110° C.
Petroleum Benzin (b. p. 30-60° C.) extracted	3.39	3.36
Ether	1.14	1.00
Chloroform	0.59	0.54
Alcohol	13.51	10.73
Water	16.56	14.90

Tests on the petroleum-benzin extract indicated the presence of sterol, fat and volatile oil. The ether, chloroform and alcohol extracts were of a resinous nature. The alcohol extract, in addition, gave positive tannin reactions. The water extract gave no definite indications.

Tests for alkaloid by maceration with Prollius' fluid as well as with an aqueous acid solution were negative.

Examination by the Lead Method (12) revealed the presence of fat, tannin, red coloring matter and reducing carbohydrate.

Examination of 100 Gm. of the drug by the Stas-Otto Method (13) gave negative tests for alkaloids but did give positive tests for tannin.

The total resin content of the drug as determined by the method of Power and Rogerson (14) was 7.27 per cent.

The ash of the drug contained the sulfate, chloride and carbonate radicals and the following metals: tin, iron, aluminum, magnesium, calcium, sodium and potassium. Since the ash was not completely dissolved by treatment with hot dilute nitric acid, silica was probably present also.

One kilogram of drug yielded 0.48 Gm. of volatile oil corresponding to a yield of 0.048 per cent. Most of the oil distilled as a lemon-yellow liquid between 127° C. and 145° C. at 20 mm. The distilled oil had a specific gravity 28° C./28° C. of 0.9626; refractive index at 26° C., 1.4703. An alcoholic solution was neutral to litmus. The oil had an aromatic odor similar to that of the crude drug. It solidified on long standing.

LARGE SCALE EXTRACTION. PETROLEUM-BENZIN EXTRACT.

Nine kilograms of the ground drug, in a number 60 to 80 powder, were extracted by percolation with petroleum benzin (b. p. 30-60° C.). The solvent was recovered from the percolate and

used again for further percolation. Extraction was continued until the percolate came through colorless and left no appreciable residue upon evaporation of a liter of percolate. The petroleum benzin was removed as completely as possible by distillation under reduced pressure over a water-bath. The extract thus obtained weighed 352 Gm., representing 3.91 per cent of the drug. It had a wax-like appearance and consistence, a black-green color and an aromatic odor.

The extract was washed repeatedly with ethyl acetate, being thus separated into an ethyl acetate-soluble and an ethyl acetate-insoluble portion. The solution had a black-green color. The residue was a yellowish wax-like solid and weighed approximately 25 Gm.

Examination of the Ethyl Acetate-Insoluble Material.—This material was washed many times on a Büchner funnel with ethyl acetate. The ethyl acetate was removed completely from the residue and crystallization from various solvents attempted but without success.

The residue was then saponified, first, by the method of Bomer (15) using a 25 per cent solution of potassium hydroxide in 70 per cent alcohol and refluxing for two and a half hours and, secondly, by refluxing for six hours with a 12 per cent solution of potassium hydroxide in 95 per cent alcohol. This treatment yielded, after removal of the ether used, a solid unsaponifiable portion and an alkaline hydro-alcoholic solution of saponifiable material.

The Unsaponifiable Portion.—The unsaponifiable matter was boiled for 2 hours in an acetylation flask with an equal weight of acetic anhydride. At the end of boiling two layers were present in the flask. The upper yellowish oily layer was separated and washed thoroughly with boiling water. It solidified on cooling to room temperature. It would not crystallize from various organic solvents. The lower aqueous layer did not yield anything definite.

Isolation of Pentatriacontane.—The solidified upper layer was now treated by the method of Leys (16) for the separation of hydrocarbons and alcohols. It was dissolved in 100 cc. of iso-amyl alcohol, 100 cc. of concentrated hydrochloric acid were added and the mixture heated to boiling with continuous stirring. The mixture was allowed to cool slowly by immersing the beaker in warm water and allowing it to stand until it had cooled down to room temperature. The solid cake which formed at the surface was lifted off, boiled again with iso-amyl alcohol and concentrated hydrochloric acid, cooled slowly and the cake again lifted off and washed several times with hot water. The lower layers in each of the above procedures consisted of brown, gelatinous masses which on evaporation to dryness on a water-bath became black, wax-like solids. This material could not be purified and was discarded. The solid cake was dissolved in hot iso-amyl alcohol and boiled with charcoal. The solution was filtered through a steam-heated funnel. After removal of the iso-amyl alcohol the residue was dissolved in 300 cc. of ether-chloroform mixture (3:1). The solution on standing over night in a refrigerator threw down a precipitate which was filtered off with suction and washed with cold ether-chloroform mixture. The substance remaining on the filter paper was an almost white powder. It was purified by precipitation from chloroform as follows: the material was dissolved in chloroform at room temperature and placed in the refrigerator at 8° C. for several hours; the precipitate formed was filtered off and washed with cold chloroform. After several repetitions of this procedure, about 0.4 Gm. of a perfectly white powder melting constantly at 74.5° C. was obtained. A carbon tetrachloride solution of a portion of this substance did not decolorize a solution of bromine in carbon tetrachloride either in the hot or in the cold.

3.458 mg. gave 11.115 mg. CO₂ and 4.622 mg. H₂O.

C = 85.44 per cent; H = 14.58 per cent.

C₃₅H₇₂ requires C = 85.27 per cent; H = 14.73 per cent.

The melting point of pentatriacontane is reported in Beilstein (17) as 74.7° C.

The above-described substance from its manner of isolation, its elementary analysis and its melting point was identified as pentatriacontane.

The Saponifiable Portion.—Most of the alcohol was removed from the alkaline hydro-alcoholic solution of saponifiable material by distillation over a steam-bath. The concentrated solution was made acid with sulfuric acid (1 to 3) and shaken out with ether. The slightly green-colored ether solution was dried over anhydrous sodium sulfate and the ether removed. The residue, consisting of a few cubic centimeters of a brown liquid, was dissolved in hot 95 per cent alcohol. Upon standing in the refrigerator for a few hours the solution deposited a light brown precipitate. This was filtered off with suction, dissolved in ethyl acetate, and the solution placed in a

refrigerator over night. Several milligrams of light yellow crystals melting at 79–80° C. were obtained. On recrystallization from hot alcohol the crystals although almost white still melted at 79–80° C. The amount of the crystals was too small to permit running further tests. This substance was probably behenic acid which was obtained subsequently in sufficient quantity to be definitely characterized.

The concentrated alkaline liquid after acidification and extraction with ether was evaporated almost to dryness on a steam-bath. The residue was extracted with an ether-alcohol mixture (3 parts of 95 per cent alcohol and 1 part ether). The ether-alcohol solution was boiled twice with charcoal, filtered and evaporated to dryness. The residue was taken up in water and 12 per cent barium chloride solution added until no further precipitation occurred. The filtrate was concentrated to a thick syrup which when heated with potassium bisulfate produced the characteristic pungent choking odor of acrolein. The presence of glycerin was indicated by this test.

Examination of the Ethyl Acetate-Soluble Material.—The portion of the petroleum-benzin extract soluble in ethyl acetate amounted to 325 Gm. Of this, 150 Gm. were refluxed for 6 hours with 1500 cc. of an alcoholic solution of potassium hydroxide (300 Gm. of potassium hydroxide in 1500 cc. of 70 per cent alcohol). Most of the alcohol was distilled off, water added and the mixture extracted repeatedly with 200-cc. portions of ether. This treatment yielded an ether solution of unsaponifiable material and an aqueous alkaline solution of saponifiable material.

The Unsaponifiable Portion.—The ether solutions were combined, washed with water and the ether removed. The yellow-green residue was dissolved in 95 per cent alcohol and placed in the refrigerator over night. A yellow solid separated. This was filtered off and the filtrate concentrated slightly. Another small portion of yellow material was obtained on cooling. The filtrate from this was replaced in the refrigerator. A quantity of gleaming white platelets was slowly deposited over a period of two days. By concentration and further cooling two more fractions of white platelets were obtained. No further crystalline material could be obtained from the mother liquor from which the yellow solid and the white platelets had been obtained.

Isolation of Triacotane.—The two portions of yellow solid material totaling about 2 Gm. were combined and recrystallized several times from 95 per cent alcohol. The top fraction still had a yellow color; therefore it was recrystallized from ethyl acetate. It was obtained as an almost white precipitate melting somewhat indefinitely in the neighborhood of 65° C. It was dissolved in chloroform and the solution boiled with charcoal. On filtration and concentration it was obtained as a perfectly white fine powder melting at 64–65° C. Recrystallization from ether did not change the melting point. The substance did not give positive sterol reactions, it did not react with hot or cold concentrated sulfuric acid and it could not be acetylated. It was insoluble in 10 per cent solutions of hydrochloric acid and sodium hydroxide.

The above-described substance from its manner of isolation and its chemical inactivity was undoubtedly a hydrocarbon. The constancy of its melting point after crystallization from four different solvents indicated that it was probably triacotane, $C_{30}H_{62}$.

Beilstein (17) reports triacotane as melting at 65.2° C.

Isolation of a Sterol.—The three fractions of white platelets which had been obtained from the alcoholic filtrate from the triacotane precipitates were combined and the total (0.6 Gm.) recrystallized from hot 95 per cent alcohol. After drying over night in a vacuum-desiccator over sulfuric acid the crystals melted at 133–134° C. Under the microscope they appeared as transparent, slightly elongated, five-sided rods. They were insoluble in water, in 10 per cent hydrochloric acid and in 10 per cent sodium hydroxide solution. They were soluble in cold concentrated sulfuric acid. The sulfuric acid solution had an orange-red color and showed a heavy green fluorescence by reflected light. The substance gave positive sterol reactions with the Liebermann-Burchard and Salkowski reagents. After four recrystallizations from 95 per cent alcohol the melting point was raised to 135–136° C. Two more crystallizations from absolute alcohol gave a product (about 0.15 Gm.) which melted at 136–137° C.

3.011 mg. gave 9.295 mg. CO_2 and 3.180 mg. H_2O .

C = 84.18 per cent; H = 11.82 per cent.

2.429 mg. gave 7.508 mg. CO_2 and 2.549 mg. H_2O .

C = 84.29 per cent; H = 11.74 per cent.

Average C = 84.24 per cent; H = 11.78 per cent.

$C_{29}H_{49}OH$ requires C = 83.98 per cent; H = 12.16 per cent.

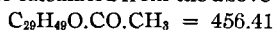
0.1106 Gm. of the sterol dissolved in 10.00 cc. of chloroform gave $\alpha_D^{28} = -0.57^\circ$ in a 100-mm. tube $[\alpha]_D^{28} = -51.54^\circ$.

A chloroform solution of the sterol decolorized a chloroformic solution of bromine.

About 0.15 Gm. of the sterol was acetylated by refluxing with 5 cc. of acetic anhydride for two hours. The acetic anhydride was removed by heating over a water-bath at 10-mm. pressure. The cream-colored crystalline residue was dissolved in chloroform and boiled with charcoal. The filtrate was evaporated to dryness and the white residue recrystallized from absolute alcohol until the crystals melted constantly at 133–134° C.

Since the saponification equivalents of the sterols are said (18) to be more reliable in the determination of the empirical formulas of sterols than combustion analyses, the acetate obtained above was saponified.

0.0793 Gm. of the acetate, dried to constant weight at 105° C., was refluxed for one hour and a half with 5.00 cc. of half-normal alcoholic potassium hydroxide solution. A blank was run simultaneously. The acetate was found to be equivalent to 1.73 cc. of 0.1 normal sulfuric acid. The molecular weight of the acetate calculated from the above was 458.4.



The sterol set free by the saponification was shaken out with chloroform and recrystallized three times from absolute alcohol. The melting point of the crystals rose from 133° to 137° C. The final melting point was not constant but the small amount of the sterol remaining after the third crystallization did not permit of further purification.

From its reactions with bromine, with the Liebermann-Burchard and Salkowski reagents, and with sulfuric acid; from its specific rotation; from its combustion analysis; and from the molecular weight calculated from its saponification value the above-described substance was identified as a sterol with the probable formula: $C_{29}H_{49}OH$.

The Saponifiable Portion.—The aqueous alkaline solution after extraction with ether contained the saponifiable portion of the ethyl acetate-soluble part of the petroleum-benzin extract. It was made acid with sulfuric acid (1 to 3) and extracted with ether. The ether solutions were combined, washed with water, shaken with anhydrous sodium sulfate, filtered and the ether removed. The residue had a rancid odor. It weighed approximately 85 Gm. It was dissolved in 95 per cent alcohol and placed in the refrigerator at 7° C. over night. A quantity of dark green solid material separated out. This was filtered off with suction, washed with cold 95 per cent alcohol, redissolved in hot 95 per cent alcohol and again placed in the refrigerator over night. The material which was deposited now had a light gray color. The filtrates were combined.

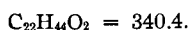
The above treatment produced two fractions: Fraction A, consisting of the saponifiable matter soluble in 95 per cent ethyl alcohol at 7° C. and Fraction B, consisting of the saponifiable matter precipitated from 95 per cent alcohol at 7° C.

By similar treatment of Fraction B with ether there was obtained a Fraction C, readily soluble in ether at 7° C. and a Fraction D, precipitated from ether at 7° C.

Isolation of Behenic Acid.—Fraction C, consisting of the saponifiable matter precipitated from 95 per cent alcohol at 7° but soluble in ether at 7° C., gave a red-brown residue upon removal of the ether. The residue was dissolved in hot alcohol. On cooling down to room temperature, the solution deposited a brown solid from which nothing definite could be isolated. On standing at room temperature, the filtrate from the brown precipitate deposited about 0.3 Gm. of a white solid. This was removed and recrystallized from 95 per cent alcohol until the melting point became constant at 78–79° C.

$C_{22}H_{44}O_2$ has been reported as melting at 79–80° C. (19) and at 81–82° C. (20).

0.0412 Gm. of the acid required 1.20 cc. of 0.1N potassium hydroxide for neutralization. The molecular weight calculated from this data is 343.3.



The silver salt was prepared and analyzed.

0.0135 Gm. of silver salt gave on ignition 0.0032 Gm. of silver.

Ag = 23.70 per cent.

$C_{22}H_{43}O_2Ag$ requires Ag = 24.12 per cent.

The above-described compound was thus identified as behenic acid.

The filtrate from the behenic acid on further concentration deposited another fraction of crystals which melted at 73–74° C. Two recrystallizations from alcohol raised the melting point to 78–79° C. These crystals were evidently a further yield of behenic acid. No other material was obtained from this fraction.

Isolation of Melissic Acid.—Fraction D, consisting of the saponifiable material deposited from alcohol and from ether at 7° C., was dissolved in hot alcohol and cooled in the refrigerator. The white deposit which formed was removed and recrystallized from hot alcohol. The crystals melted at 83–84° C. Further crystallizations from alcohol gave a substance melting constantly at 87° C.

$C_{30}H_{60}O_2$ has been reported as melting at 89–90° C. (21), at 90° C. (22) and 90.6° C. (23).

0.0727 Gm. of the acid required 1.61 cc. of 0.1*N* potassium hydroxide for neutralization. The molecular weight calculated from this data is 451.6.

$C_{30}H_{60}O_2 = 452.5.$

The silver salt was prepared and analyzed.

0.0137 Gm. of silver salt gave on ignition 0.0027 Gm. of silver.

Ag = 19.71 per cent.

$C_{30}H_{58}O_2Ag$ requires Ag = 19.29 per cent.

The above-described compound was thus identified as melissic acid, the low melting point being due to the presence of a small amount of impurity.

The filtrate from the melissic acid was evaporated to dryness and the residue dissolved in hot ethyl acetate. On cooling, a few milligrams of yellowish white crystals were obtained. The filtrate was evaporated to dryness giving a light brown residue which melted at 77°. The yellow-white crystals melted at 74–75° C. The quantities of material here were so small that they could not be further worked with. The melting points of the precipitate and residue indicate that some behenic acid may have been present in this fraction as well as in Fraction C.

Fraction A consisted of the saponifiable material soluble in 95 per cent alcohol at 7° C. The alcohol was removed under reduced pressure. The residue weighing about 50 Gm. was mixed with 200 cc. of distilled water and steam distilled. Four 75-cc. fractions were collected. The first three fractions had rancid odors. Fraction 4 had an aromatic odor and contained green oily globules floating on the surface. Each fraction was shaken four times with 25-cc. portions of chloroform. The chloroform shakings from each fraction were combined and the solvent removed by distillation. This treatment gave an aqueous solution of material not removed by shaking with chloroform and a residue of chloroform-soluble material. The residue was mixed with water and titrated with standard sodium hydroxide solution. The neutralized liquid was shaken out with chloroform which removed material (volatile oil) which had not reacted with the sodium hydroxide. The neutralized solution was then evaporated to dryness and the residue, consisting of the sodium salts of the fatty acids, weighed.

The aqueous solution of material not removed by shaking with chloroform was likewise titrated with sodium hydroxide solution, evaporated to dryness and the residue weighed.

Qualitative tests performed on the sodium salt residues indicated the absence of formic and acetic acids.

The quantitative results obtained with the steam-volatile portion of Fraction A are tabulated below.

TABLE I.—ACIDS EXTRACTED FROM STEAM DISTILLATE BY CHLOROFORM.

	Cc. of <i>N</i> /1 Alkali Used.	Gm. of Sodium Salt Obtained.	Gm. of Sodium Salt Equivalent to Alkali Used.			
			Acetate.	Butyrate.	Caproate.	Caprylate.
Fraction I	10.889	1.3565	0.8930	1.1979	1.5039	1.8088
Fraction II	9.567	1.2326	0.7845	1.0524	1.3213	1.5892
Fraction III	5.640	0.7149	0.4625	0.6204	0.7789	0.9368
Fraction IV	3.500	0.4729	0.2870	0.3850	0.4834	0.5814

The results obtained with Fraction I point to a mixture of butyric and caproic acids in almost equimolecular proportions although a mixture of butyric, caproic and caprylic acids in certain proportions would probably correspond to the values given by Fraction I. Fractions II,

III and IV also point to a mixture of butyric and caproic acids with the amount of the higher acid steadily increasing.

Silver salts were prepared from the sodium residues and the per cent of silver in the salts determined. The results are tabulated below.

TABLE II.

	Per Cent Silver in Silver Salt.		Per Cent Silver in Silver Salts of.
Fraction I	50.3	Butyric acid	55.3
Fraction II	48.1	Caproic acid	48.4
Fraction III	46.9	Caprylic acid	43.0
Fraction IV	43.6	Capric acid	38.7

The conclusion as to the composition of Fraction I, Table II, is approximately the same as with Fraction I, Table I; however, since the silver percentage is lower than the percentage required of an equimolecular mixture of butyric and caproic acids the presence of a higher acid, probably caprylic, is indicated. Fractions II, III and IV show a continuously decreasing silver content indicating the presence of a larger ratio of the higher fatty acids.

The quantitative results obtained with the aqueous solution of material not removed by shaking with chloroform are tabulated below.

TABLE III.—ACIDS NOT EXTRACTED FROM STEAM DISTILLATE BY CHLOROFORM.

	Cc. of N/1 Alkali Used.	Gm. of Sodium Salt Obtained.	Gm. of Sodium Acetate.	Salt Equivalent to Butyrate.	Alkali Used. Caproate.
Fraction I	1.284	0.1467	0.1053	0.1412	0.1773
Fraction II	1.260	0.1391	0.1033	0.1386	0.1740
Fraction III	1.007	0.1100	0.0826	0.1108	0.1391
Fraction IV	1.007	0.1154	0.0826	0.1108	0.1391

All four of the above fractions, which contain the volatile fatty acids not extracted from an aqueous solution by chloroform, point consistently to the presence of butyric acid.

Pure silver salts could not be prepared from the above sodium residues.

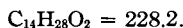
That portion of Fraction A which was not volatile with steam was separated from the water and treated by the lead-ether method for the separation of liquid from solid fatty acids. The method used was that of Tortelli and Ruggeri described by Rosenthaler (24). The ether-soluble and ether-insoluble lead salts were decomposed with 10 per cent hydrochloric acid and shaken out with ether. The ether solutions were washed repeatedly with water and were then dried with anhydrous sodium sulfate. The ether was distilled off from each fraction yielding a residue representing the solid acids and a residue representing the liquid acids.

Isolation of Myristic Acid.—Crystallization from various solvents of the material obtained from the ether-insoluble lead salts, *i. e.*, the solid acids, was attempted but without success. The solvents were removed and the residue amounting to approximately 20 Gm. was distilled under a pressure of 20 mm. Fraction I (up to 105° C.) consisted of a few cubic centimeters of a mixture of the various organic solvents which had been used in attempted crystallizations. Fraction II (up to 150° C.) consisted of half a cubic centimeter of a yellow liquid which could not be characterized. Beyond 150° C. the temperature rose very rapidly to 200° C. Fraction III, therefore, consisted of material distilling between 200° C. and 220° C. and amounted to 5 cc. Fraction IV distilled between 220° C. and 260° C. and amounted to about 3 cc. Decomposition, producing a red-brown distillate, began above 260° C. The residue in the distillation flask was a black-brown, viscous mass from which nothing could be isolated.

Fractions III and IV were both almost colorless liquids which solidified on cooling. Fraction III on crystallization from 95 per cent alcohol melted at 51–52° C. Fraction IV on crystallization from 95 per cent alcohol melted at 52–53° C. The two fractions were combined and recrystallized from alcohol until the melting point became constant at 53.5° C. The pure acid weighed 0.8 Gm.

$C_{14}H_{26}O_2$ has been reported as melting at 53.7° C. (25).

0.7722 Gm. of the acid required 33.11 cc. of 0.1N potassium hydroxide solution for neutralization. The molecular weight calculated from this data is 233.2.



The silver salt was prepared and analyzed.

0.2730 Gm. of silver salt gave 0.0946 Gm. of silver.

Ag = 34.65 per cent.

0.1730 Gm. of silver salt gave 0.0601 Gm. silver.

Ag = 34.74 per cent.

Average: Ag = 34.70 per cent.

$\text{C}_{14}\text{H}_{27}\text{O}_2\text{Ag}$ requires Ag = 33.6 per cent.

The acid was obtained by distillation under a pressure of 20 mm. between 200° C. and 260° C.

$\text{C}_{14}\text{H}_{28}\text{O}_2$ is reported (26) as distilling at 196.5° C. at 15 mm.

The above-described compound was thus identified as myristic acid.

The material obtained from the ether-soluble lead salts, *i. e.*, the liquid acids, was also distilled under a pressure of 20 mm. Only a few drops distilled over up to 105° C. Between 105° C. and 250° C., 2-3 cc. of a slightly red-colored liquid were obtained. This fraction was discarded. Fraction III, distilling between 250° and 258° C., was a yellow liquid amounting to about 8 cc. No further distillate could be obtained without decomposition. The residue in the distillation flask was a black tarry mass from which nothing could be isolated.

Fraction III was dissolved in neutral 95 per cent alcohol and titrated with *N*/1 sodium hydroxide solution of which 14.805 cc. were consumed. The solution was evaporated to dryness and the residue dried to constant weight at 105° C. The residue weighed 3.5935 Gm. The molecular weight of the sodium salt calculated from the above data is 242.7 from which the molecular weight of the acid becomes 220.8.

The sodium salt was converted into the silver salt and the silver salt analyzed.

0.5862 Gm. gave 0.2060 Gm. Ag : Ag = 35.14 per cent.

0.3651 Gm. gave 0.1289 Gm. Ag : Ag = 35.31 per cent.

Average: Ag = 35.22 per cent.

The free acid was regenerated by treating a portion of the silver salt with 10 per cent nitric acid and shaking the mixture out with chloroform. The combined chloroform solutions were washed thoroughly with water and the chloroform evaporated off. The resulting yellow oil was dried over sulfuric acid and the iodine number determined.

0.2432 Gm. of the oil absorbed 0.05673 Gm. of iodine. The iodine number calculated from the above is 23.32. The iodine number of oleic acid, $\text{C}_{18}\text{H}_{34}\text{O}_2$, is 89.96. The low value obtained indicates that the oil was a mixture consisting largely of saturated acids.

The high silver content points to the presence of lower fatty acids, possibly caprylic, $\text{C}_8\text{H}_{16}\text{O}_2$ (per cent Ag in silver salt = 43.0) and capric, $\text{C}_{10}\text{H}_{20}\text{O}_2$ (per cent Ag in silver salt = 38.7). Silver oleate, $\text{C}_{18}\text{H}_{34}\text{O}_2\text{Ag}$ = 27.7 per cent Ag.

The low molecular weight, 220.8, likewise indicates the presence in the oil of lower fatty acids.

Oleic acid, $\text{C}_{18}\text{H}_{34}\text{O}_2 = 282.3.$

ALCOHOL EXTRACT.

The marc from the petroleum-benzin extraction was freed of solvent and exhausted in a Lloyd extractor with hot 95 per cent alcohol. Most of the alcohol was recovered in the Lloyd extractor. The concentrated extract was dried *in vacuo* over calcium chloride. The weight of the alcoholic extract thus obtained was 669 Gm. representing 7.43 per cent of the drug. The extract was a very thick green mass. It was incorporated intimately with the aid of alcohol with purified sand and sawdust. The sand and sawdust used had been previously extracted successively with petroleum benzin, ether, chloroform and alcohol. The mixed material was spread out in a thin layer and the alcohol allowed to evaporate at room temperature. The dry residue was packed in a percolator and extracted successively with benzin, ether, chloroform, ethyl acetate and 95 per cent alcohol. In each case percolation was continued until one liter of percolate gave no appreciable residue on removal of the solvent.

Petroleum Benzin-Soluble Portion of Alcohol Extract.—This extract was a dark green, soft mass weighing 64 Gm., representing 9.6 per cent of the alcohol extract. Two cubic centimeters of volatile oil were separated by steam distillation but no other definite substance could be obtained.

A portion of the extract was hydrolyzed by refluxing it for 2 hours with a 5 per cent solution of sulfuric acid in alcohol. Hydrolysis did not liberate a reducing sugar; therefore, material of a glycosidal nature was not present in the extract.

Ether-Soluble Portion of Alcohol Extract.—The residue after removal of the solvent was a dark green viscous liquid and weighed 42 Gm., representing 6.3 per cent of the alcohol extract. Thirty grams of the extract were dissolved in 95 per cent alcohol and allowed to stand. A green precipitate was thrown down. This was filtered off, dissolved in ethyl acetate and the solution again allowed to stand. About 2.5 Gm. of green material was deposited from the ethyl acetate solution. The green material was probably chlorophyll although it did not give the "brown phase" reaction (27). This reaction is given only by intact chlorophyll, that is, chlorophyll which has not undergone changes in its lactam groups.

The alcohol solution from which the chlorophyll had been deposited yielded on further treatment volatile oil and a soft resin. Here also there was no material of a glycosidal nature.

Chloroform-Soluble Portion of Alcohol Extract.—This extract was a green-black liquid weighing 31 Gm., representing 4.6 per cent of the alcohol extract. Only intractable resinous material was obtained from the extract.

Ethyl Acetate-Soluble Portion of Alcohol Extract.—This extract was a dark brown solid weighing 78 Gm., representing 11.7 per cent of the alcohol extract. The extract was washed with 95 per cent alcohol. There remained undissolved several grams of yellow-white crystals. These were found to be soluble in water and were recrystallized from water. The last crystallization yielded symmetrical cubes. The crystals were inorganic. Qualitative tests revealed the presence of sodium, potassium and chloride ions. Gravimetric and volumetric tests with silver nitrate indicated that the crystals were a mixture of sodium and potassium chlorides in the ratio of 80 parts of sodium chloride to 20 parts of potassium chloride.

The portion of the ethyl-acetate extract which dissolved in 95 per cent alcohol did not yield any crystalline material. The alcohol was removed, the residue mixed with water, and the mixture steam distilled. The distillate contained volatile oil and butyric acid.

The non-volatile material in the distilling flask consisted of a milky yellow supernatant liquid and a soft black resin.

Nothing could be isolated from the resin.

The supernatant liquid deposited a red-black solid on standing. This solid was soluble in 10 per cent sodium hydroxide solution and was reprecipitated from the alkaline solution on acidification. It may have been a phlobaphene.

After the deposition of the above substance the supernatant solution had a clear amber color. The solution gave a heavy black-green precipitate with ferric chloride solution, a cream-colored precipitate with strychnine sulfate, yellow precipitates with lead acetate and lead subacetate solutions, a white precipitate and a red ring at the interface with sulfuric acid, a brown precipitate with bromine water, a gray precipitate with gelatin solution and a white precipitate turning dark brown with potassium dichromate solution. The solution was strongly acid to litmus, had an astringent taste and assumed a dark brown color on alkalization with ammonia. It reduced Fehling's solution. With ferric chloride solution, a very dilute solution of the substance produced a green precipitate which turned violet on addition of sodium acetate solution. The above reactions indicated the presence of a catechol tannin.

Concentration of the remaining tannin solution under reduced pressure resulted in the production of a brown uncrystallizable syrup. On being kept over sulfuric acid in a desiccator the syrup was converted into an amorphous brown powder. This was set aside for further investigation.

Portion of Alcohol Extract Not Dissolved by Petroleum Benzin, Ether, Chloroform and Ethyl Acetate.—The alcoholic extractive which remained in the sand and sawdust was removed with 95 per cent alcohol. Most of the alcohol was recovered from the percolate. The thick brown-black residue was mixed with water and the volatile oil removed by steam distillation.

The brown supernatant liquid in the distilling flask gave no definite reactions. The insoluble brown mass in the distilling flask was dissolved in alcohol and allowed to stand. About

4 Gm. of greasy, green material was deposited. This was filtered off and the alcoholic filtrate concentrated to the consistency of a thin syrup and poured slowly into a large volume of hot water. The precipitated resin was allowed to settle and the supernatant liquid decanted. The resin was redissolved in alcohol and reprecipitated. It was collected and dried. The dry resin amounted to 84 Gm. It was a black brittle solid with a smooth, shiny fracture. It had a slightly astringent taste and burned with a smoky flame. It was freely soluble in 10 per cent ammonia and in 6 per cent potassium hydroxide solution. Its acid value was 116.3; its saponification value, 227.4. It lost 6.6 per cent of its weight on being dried to constant weight at 105° C. It had an ash content of 0.32 per cent.

PHYSIOLOGICAL TESTS.

One cc. per Kg. of a fluidextract was given orally by capsule to two pregnant cats. No action was observed within 24 hours.

The marc remaining from the preparation of the fluidextract was exhausted by percolation with 50 per cent alcohol. This percolate was combined with the remainder of the fluidextract (the fluidextract had been prepared with 95 per cent alcohol as the menstruum). The combined extractives were concentrated as far as possible by means of a current of air at room temperature. The viscous resinous residue amounted to 16 Gm. Of this residue 1.8 Gm. was given orally by capsule to a female cat weighing 2.55 Kg. This represented a dose of 4.4 Gm. of drug per Kg. No effects were observed within 72 hours.

The remaining 14.2 Gm. of residue were mixed with alcohol and water to give a mixture which could be injected intraperitoneally. The total volume of the mixture was 40 cc. Five cubic centimeters were injected intraperitoneally into a female cat weighing 2.6 Kg. The dose of drug per Kg. calculated from the above was 4.3 Gm. No action was observed within 24 hours.

SUMMARY AND CONCLUSIONS.

1. A short botanical description of *Ipomea Pes-Capræ* has been given, followed by reports of medicinal uses.
2. The U. S. P. X "constants" for crude drugs have been determined for *Ipomea Pes-Capræ*.
3. Tests on small amounts of the drug for the presence of alkaloids were negative.
4. The total resin content of the drug was found to be 7.27 per cent.
5. The ash of the drug contained the sulfate, chloride and carbonate radicals and the following metals: tin, iron, aluminum, magnesium, calcium, sodium and potassium. Silica was also present.
6. The drug yielded 0.048 per cent volatile oil. The oil distilled between 127° and 145° C. at 20 mm.; specific gravity 28° C./28° C. = 0.9626; refractive index at 26° C. = 1.4703.
7. Extraction of 9 Kg. of the drug with petroleum benzin yielded 3.91 per cent of extractive. The following substances were isolated from the extract: pentatriacontane; triacontane; a sterol: C₂₉H₄₉OH; behenic acid, melissic acid, butyric acid and myristic acid. The presence of glycerol, of saturated fatty acids of low molecular weight and of an unsaturated fatty acid was demonstrated.
8. The marc from the petroleum-benzin extraction was percolated with 95 per cent alcohol and yielded 7.43 per cent of extractive. The extract yielded volatile oil; butyric acid; chlorophyll; resin; inorganic crystals consisting of sodium and potassium chlorides; and a catechol tannin.
9. During the examination of the drug various extractives were administered to cats but in no case was there an apparent response to the drug.

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PREPARATION OF RESIN OF PODOPHYLLUM.*

BY A. H. UHL.¹

According to Lloyd (1), King was the first to have prepared the so-called resin of Podophyllum. The discovery was accidental. Its precipitation resulted when the hydroalcoholic extract was mixed with an infusion of the same drug (2). The earlier work on the "resin" threw no light on its chemical composition except in so far as the observation of its solubility in hot water caused Maisch to express the opinion that it was not a true resin (3). As the therapeutically important constituent of the drug, its preparation and its properties received special attention, more particularly when, in 1860, the resin of Podophyllum was made official in the United States Pharmacopœia.

EXPERIMENTAL.

The powdered dried roots and rhizomes of *Podophyllum peltatum* were extracted to exhaustion with 95 per cent alcohol in a Lloyd extractor. A portion of the alcohol was recovered leaving a syrupy, nearly black extract. On standing over night a greenish oily-looking material

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