A PHYTOCHEMICAL STUDY OF IPOMEA PES-CAPRAE (L.) SWEET

By

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LIBRARY, UNIVERSITY OF MARYLAND
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ACKNOWLEDGEMENT

For suggesting and directing this research

I wish to thank sincerely

Dr. Glenn L. Jenkins.
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A PHYTOCHEMICAL STUDY OF IPOMEA PES-CAPRAE

Ipomea Pes-Caprae (L.) Sweet, Family Convolvulaceae 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 sea-shore; 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-Caprae is a denizen of nearly every tropical beach the world over. Guppy (8) states that as a rule this plant monopolizes coasts between 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.

The medicinal virtues claimed for the plant are many and varied. Dragendorff (9) states that the leaves are used in New South Wales against rheumatism and dropsy and also for sores and ulcers. Dymock (5) describes the drug as being used externally in rheumatism in the form of a decoction and internally in colic. 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, gonorrhea, and dropsy in doses of one to two ounces; it 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 locally applied to painful joints in rheumatism and to the abdomen in colic.

No reports of any chemical investigations of this drug have been found.
EXPERIMENTAL

Ten kilograms of the air-dried aerial portions of Ipomea Pes-Caprae were received from T. K. MacDonall in Florida. The stems weighed six kilograms; the leaves and flowers, four kilograms. 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:

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<th></th>
<th>A</th>
<th>B</th>
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<td>7.64</td>
<td>7.67</td>
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<td></td>
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<tr>
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<td>0.12</td>
<td>0.13</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>7.25</td>
<td>7.41</td>
<td>7.33</td>
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<tr>
<td>Acid-Insoluble</td>
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<td>0.07</td>
<td>0.06</td>
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For the leaves and flowers:

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<tr>
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<tr>
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<td>50% Alcohol Soluble Extractive</td>
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<td>22.31</td>
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<tr>
<td>Water Soluble Extractive</td>
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<td>17.88</td>
<td>17.60</td>
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<tr>
<td>Ash</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>14.06</td>
<td>14.07</td>
<td>14.07</td>
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<tr>
<td>Acid-Insoluble</td>
<td>0.11</td>
<td>0.00</td>
<td>0.06</td>
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By way of preliminary examination a 49.282 Gm. portion of the mixed drug was extracted successively according to the Dragendorff (11) scheme 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.
The petroleum benzin extract was a greenish-brown, greasy wax with an aromatic odor. The extract was redissolved in petroleum benzin and shaken out with 2 per cent sulfuric acid. The acid shakings had a light salmon color but reacted negatively to the usual alkaloidal reagents.

The petroleum benzin extract after being shaken with sulfuric acid solution was evaporated to dryness and treated with 90 per cent ethyl alcohol. The portion insoluble in 90 per cent alcohol was a yellow fat which on solution in chloroform and cooling in the refrigerator deposited a few crystals. These crystals gave a positive reaction when treated with the Liebermann-Burchard reagent for sterols. That portion of the petroleum benzin extract which was dissolved by the 90 per cent alcohol seemed to consist mainly of chlorophyll, fat, wax, and some volatile oil.

The ether extract was of a dark green color and had a pungent aromatic odor. It was liquid when hot but semi-solid at room temperature.

The residue was extracted with water giving a yellow-colored solution which was strongly acid to litmus, gave a green-black precipitate with ferric chloride test solution, green-yellow precipitate with lead acetate and lead subacetate solutions, and white precipitates with strychnine sulphate and gelatin solutions. The addition of 10 per cent ammonia
to the aqueous extract caused an intensification of the yellow color. These reactions indicated the presence of tannin. That portion of the ether extract which was insoluble in water was extracted with various organic solvents. These yielded fat, coloring matter, and resin. All fractions of the ether extract gave negative results when tested for alkaloids.

The chloroform extract was a green-black solid. A yellow-green solution was obtained on extraction with water. This aqueous solution was strongly acid to litmus but gave no reaction with ferric chloride test solution. It yielded precipitates on treatment with solutions of lead, barium, and silver salts. It gave a positive Molisch reaction. The water-insoluble portion of the chloroform extract was dissolved in 95 per cent alcohol. This alcoholic solution on dilution with water threw down a grayish-white resinous precipitate. It gave no other definite reactions. All tests for alkaloids run on the various portions of the chloroform extract were negative.

The 95 per cent alcohol extract was a brittle, glossy solid of a red-brown color. An aqueous extract of the residue gave positive reactions for tannins. A 5 per cent sulfuric acid extract of the residue gave a precipitate with phospho-molybdic acid, phospho-tungstic acid, and gold chloride test solutions but did not produce precipitates on treatment with iodine and mercuric chloride test solutions and Mayer's reagent. A portion of the alcoholic extract when placed in the refrigerator deposited in a few hours a small quantity of a violet-red solid. This substance developed a deep green color in dilute ammonia or potassium hydroxide solution. The green color also developed in the presence of ammonia vapors alone. In 10 per cent sulfuric, hydrochloric, and acetic acid solution
the substance became pink. These color changes suggest an anthocyanin pigment (12). A portion of the original extract redissolved in 95 per cent alcohol and diluted with water produced a gray-white resinous precipitate similar to the one obtained with the chloroform extract.

The distilled water extract was a dark brown solid. It gave positive reactions for tannins and a positive reaction with the Molisch reagent. It produced heavy precipitates with neutral and basic lead acetate solutions and with barium chloride test solution. Alkaloidal reagents gave negative results.

All of the extracts with the exception of the distilled water extract contained chlorophyll.

TESTS FOR ALKALOIDS

Twenty grams of the powdered drug were macerated overnight with a mixture of 120 cubic centimeters of ether, 40 cubic centimeters of chloroform, and 5 cubic centimeters of 10 per cent ammonia. The supernatant liquid was poured off and shaken out with 2 per cent sulfuric acid solution. The acid solution was tested with various alkaloidal reagents but all tests were negative.

Five grams of the powdered drug were extracted with 100 cubic centimeters of a 1 per cent hydrochloric acid solution. The extractive had a pink color which turned green on alkalinization. All alkaloidal tests were negative. This extract seemed to contain the same colored principle which was present in the 95 per cent alcohol extract. The residue from the one per cent hydrochloric acid extract was dissolved in 2 per cent sulfuric acid solution. No colored material could be extracted from this solution by amyl alcohol. According to Wilstatter (12) anthocyanin pig-
ments give the above color changes and are not extracted from a 2 per cent sulfuric acid solution by amyl alcohol.

**EXTRACTION WITH WATER (13)**

Five grams of the powdered drug were warmed with water on the water bath at 50-60°C and the cooled, filtered aqueous extract tested. The extract had a sweet taste, was acid to litmus, gave no definite reaction with ferric chloride test solution, and produced voluminous precipitates with neutral and basic lead acetate solutions. The extract showed heavy reduction of Benedict's and Fehling's solutions and gave a positive Molisch reaction. These tests indicate the presence of a reducing carbohydrate.

**EXAMINATION BY LEAD METHOD (14)**

Since the preliminary tests with lead acetate and basic lead acetate gave positive results it was decided to test a portion of the drug by the Lead Method. Accordingly, 250 grams of ground drug were heated on a steam bath for one hour with two liters of water. The material was then filtered and expressed through cloth, the marc mixed with one liter of hot water and again heated for one hour on the steam bath. The material was once more expressed through cloth, the filtrates combined and filtered through a Buchner funnel. The clear filtrate was heated to boiling and mixed with a slight excess of saturated lead acetate solution. The precipitate (A) so obtained was washed first with lead acetate solution and then with water. The filtrate and washings were concentrated and precipitated with basic lead acetate solution. This treatment yielded a precipitate (B) and a filtrate (B).

The precipitate (A) was extracted three times with hot 95 per cent
ethyl alcohol. The alcoholic solution was freed of lead by treatment with hydrogen sulfide, the hydrogen sulfide removed by passing a stream of carbon dioxide through the solution, and the solution evaporated to dryness. The residue consisted for the most part of a yellow fat. It gave positive tannin reactions. That portion of the precipitate (A) which was not dissolved by hot alcohol was extracted with 10 per cent acetic acid. The resulting red-colored solution was precipitated with hydrogen sulfide. The heavy black precipitate of lead sulfide removed the red-colored principle. The filtrate had only a faint yellow color. On evaporation a sweet-tasting, yellowish, syrupy residue was obtained which gave a positive Molisch reaction. The quantity of the residue was too small to permit of further testing. The lead sulfide precipitate which had carried down the red-colored principle was extracted with ether, chloroform, and ethyl acetate but these solvents did not remove any of the coloring matter. Boiling the precipitate with 10 per cent acetic acid removed the red substance but it could not be induced to crystallize. Alkalization of the acid solution turned the color a light-yellow green.

The precipitate (A) remaining after extraction with 95 per cent alcohol and 10 per cent acetic acid was suspended in water and decomposed with hydrogen sulfide. The filtrate was red colored and deposited a very small amount of glistening white crystals on standing in the refrigerator at \(10^\circ\) C. The quantity of crystals deposited was too small to be filtered. The solution changed to a green-brown color on alkalization with 10 per cent ammonia.

The precipitate (B) was extracted successively with 95 per cent alcohol and chloroform but no significant amount of any material other than sulfur was obtained. The precipitate was suspended in water and decom-
posed with hydrogen sulfide. The filtrate was concentrated and shaken out with ether which removed only traces of brown, amorphous material. The aqueous solution after being shaken out with ether was concentrated on the steam bath. A syrupy brown liquid was obtained. It gave a positive Molisch reaction and reduced Benedict’s solution. No crystalline material could be obtained from the brown syrup.

No definite reactions were given by the residue obtained from the filtrate (B).

The test by the Lead Method gave indications of the presence in the drug of fat, tannin, red coloring matter, and a reducing sugar.

**EXAMINATION BY STAS-OTTO METHOD (15)**

One hundred grams of drug were extracted with 500 cubic centimeters of 95 per cent alcohol and sufficient tartaric acid to make the mixture slightly acid. After evaporation of the alcohol and treatment of the residue with hot water, a water soluble and a water insoluble portion were obtained. Examination of the water insoluble portion revealed the presence therein of chlorophyll, fat, and sterol. The water soluble portion gave no reactions for alkaloids but did give positive tannin reactions.

**DETERMINATION OF TOTAL RESIN**

The method of Power and Rogerson (16) was used in determining the total resin content. Fifty grams of drug were percolated for eleven hours with 95 per cent alcohol. Distilled water was added to the percolate and the alcohol evaporated off on a steam bath. The residue was then washed repeatedly with water. The residual resin weighed 3.6335 grams, representing a total resin content of 7.27 per cent. The water washings gave all the typical tannin reactions.
To determine whether the tannin in the aqueous washings was glycosidal or not the method of Procter (17) was used. The aqueous solution was shaken with ether to remove gallic acid, saturated with sodium chloride, and shaken with ethyl acetate. The ethyl acetate was evaporated off, the last traces being expelled by the repeated addition of small quantities of ether. The residue was dissolved in 2 per cent hydrochloric acid and the solution refluxed for two hours. The solution was cooled, filtered, and the filtrate shaken with ether. The aqueous solution was boiled, neutralized with sodium hydroxide solution, and precipitated with basic lead acetate solution to remove unchanged tannin and coloring matter. The precipitate was filtered off and the excess lead removed by dilute sulfuric acid. The filtrate was neutralized with sodium hydroxide solution and concentrated. This concentrated solution gave no reaction with ferric chloride test solution but did reduce slightly Benedict's and Fehling's solutions.

It is probable therefore that there is a glycosidal tannin present in the drug.

CONSTITUENTS OF THE ASH

Fifty grams of drug were ashed at a temperature not exceeding dull redness. Tests revealed the presence in the ash of the sulphate and chloride radicals and of the following metals: tin, iron, aluminum, magnesium, calcium, sodium, and potassium. The presence of silica was inferred by reason of the fact that all of the ash was not dissolved on treatment with hot nitric acid and from the appearance of the acid-insoluble portion of the ash under the microscope.
PRELIMINARY EXTRACTION WITH ALCOHOL

The main objective of this procedure was to determine whether there was any volatile oil present in the drug. One kilogram of drug was packed in a percolator and exhausted by percolation with 95 per cent alcohol. The alcohol was distilled off on a water bath. Crystals were deposited on the side of the distilling flask. These were found to be soluble in water and were recrystallized from water. The crystals amounted to 0.4 gram and on analysis were found to consist of a mixture of sodium and potassium chlorides. The residue left after the removal of the alcohol was mixed with water and steam distilled. Two liters of distillate were collected. The distillate contained a few oily drops floating on the surface and had an acid reaction. It was extracted repeatedly with ether and the ether removed on the water bath. The light brown, aromatic volatile oil after being dried over sulfuric acid amounted to 0.48 gram. On standing the oil partially solidified. The amount of volatile oil obtained corresponded to a yield of 0.048 per cent.

The steam distillate after extraction with ether was distinctly acid. It was neutralized with dilute sodium hydroxide solution. A portion of this neutralized liquid was evaporated to dryness and to the residue were added a few drops of concentrated sulfuric acid. The disagreeable rancid odor of butyric acid became perceptible. Another portion of the neutralized liquid was evaporated to dryness and divided into two parts. One part was mixed with ethyl alcohol and sulfuric acid. On slight warming the mixture developed a sharp pine-apple like odor. The other portion of the residue was dissolved in water and a solution of calcium chloride added. The resulting clear solution was concentrated until a precipitate started to form. The mixture was allowed to cool
whereupon the precipitate dissolved. The above reactions and solubilities indicate the presence of butyric acid.

The portion of the alcoholic extract which was not volatile with steam was not investigated at this time.

EXAMINATION OF VOLATILE OIL

The light brown oil was liquefied by gentle warming and distilled under a pressure of 20 millimeters. The bulk of the oil distilled as a lemon-yellow liquid between 127° C. and 145° C. No further distillate was obtained beyond 145° C. but the temperature went up until at 165° C. decomposition began. The residue in the distillation flask had a black-brown color. The distilled oil had a specific gravity 28° C/28° C of 0.9626. Its refractive index at 26° C was 1.4703. It possessed an aromatic odor similar to that of the crude drug. It was neutral to litmus paper. It solidified on standing.
LARGE SCALE EXTRACTION

Petroleum Benzin Extract

Nine kilograms of the ground drug, in a number sixty to eighty powder, were extracted by percolation with petroleum benzin (b.p. 30-60° C). The petroleum benzin was recovered from the percolate by distillation over a water bath and used again for further percolation. Percolation was continued until the percolate came through colorless and left no appreciable residue on evaporating a liter of percolate. The petroleum benzin was removed as completely as possible by distillation under reduced pressure over a water bath. The extract obtained by this procedure weighed 352 grams, representing 3.91 per cent of the drug. It had a wax-like appearance and consistence, a black-green color, and an aromatic odor resembling that of the crude drug.

A portion (5 grams) of the extract was reserved for physiological tests. The remainder was washed repeatedly with ethyl acetate. The petroleum benzin extract was thus separated into an ethyl acetate soluble and an ethyl acetate insoluble portion. The ethyl acetate solution had a black-green color. The material insoluble in ethyl acetate was a yellowish-white wax-like solid and weighed approximately 25 grams.

EXAMINATION OF ETHYL ACETATE INSOLUBLE MATERIAL

The material insoluble in ethyl acetate was washed many times on a Buchner funnel with ethyl acetate. The ethyl acetate was removed completely from the residue and boiling 95 per cent alcohol poured over the material remaining on the Buchner funnel. This treatment left undissolved only a small amount of yellow-brown material. The filtrate on cooling set to a yellowish-white gel. Repeated attempts to obtain crys-
tals from the filtrate by varying the quantity of alcohol and the tem-
perature resulted in failure; therefore, the alcohol was removed from
the filtrate and the residue dissolved successively in petroleum benzin,
benzene, and chloroform. No crystalline material could be obtained by
use of these solvents. Attempted crystallization of the alcohol-insol-
uble portion from various solvents was also unsuccessful.

Since no pure material could be obtained by simple crystallization
methods, all the ethyl acetate insoluble portions were combined and
saponified according to the method of Bomer (18).

Twenty-three grams of ethyl acetate insoluble material were mixed
with 200 cubic centimeters of alcoholic potassium hydroxide solution (60
grams of potassium hydroxide in 200 cubic centimeters of 70 per cent
ethyl alcohol) and refluxed for two and a half hours with frequent vigor-
ous shaking. The mixture was transferred to a separatory funnel, diluted
with 600 cubic centimeters of water, and shaken repeatedly with ether.
A quantity of solid grayish material separated during the shaking. This
was filtered off and washed thoroughly with ether. The above procedure
yielded an ether solution of unsaponified material and a hydro-alcoholic
alkaline solution.

The combined ether solutions were washed with water, shaken thor-
oughly with anhydrous sodium sulfate, and filtered. The ether was re-
moved from the filtrate by distillation over a water bath. Near the end
of the distillation a white solid separated out in the distillation flask
but when all the ether had distilled off the solid melted forming an
orange-colored liquid. As this cooled, fat-like globules appeared
throughout the liquid. Eventually the entire mass became a yellow solid.
The residue was washed repeatedly with hot ethyl alcohol. The filtrate
on cooling deposited a white gelatinous mass (A). The portion insoluble in hot alcohol was a yellow solid (B) at room temperature. The material which had separated out during saponification was similarly separated into a hot alcohol soluble fraction (C) and a hot alcohol insoluble fraction (D). Crystallization of each of the four fractions was attempted from ether, chloroform, benzene, and acetone but without success. Each of the four fractions melted indefinitely between 70 and 80° C. The four fractions were combined and re-saponified by heating under a reflux for six hours with 400 cubic centimeters of a 12 per cent solution of potassium hydroxide in 95 per cent alcohol. The mixture was diluted with water and the alcohol evaporated off on a water bath. The insoluble material which separated during this treatment was filtered off and washed fifteen times with boiling water. The last washing was neutral to litmus paper indicating complete removal of potassium hydroxide. The filtrate resulting from the separation of the insoluble material was shaken out with ether. The ether did not remove anything; therefore, the filtrate was combined with the hydro-alcoholic alkaline solution obtained from the first saponification.

EXAMINATION OF THE UNSAPONIFIABLE PORTION

The unsaponifiable material which had been freed from potassium hydroxide was now 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 obtained from the acetylation was evaporated to
dryness under reduced pressure. The residue was so small that it was discarded.

**ISOLATION OF PENTATRIACONTANE, C_{35}H_{72}**

The material remaining from the acetylation procedure was now treated by the method of Leys (19) for the separation of hydrocarbons and alcohols. It was dissolved in 100 cubic centimeters of iso-amyl alcohol, 100 cubic centimeters 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, gellatinous 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 resulting from the Leys method was dissolved in hot iso-amyl alcohol and boiled with charcoal. The solution was filtered through a steam-heated funnel. On cooling, the filtrate solidified to a cream-colored mass. This was liquefied by heating and the iso-amyl alcohol distilled off. The residue was dissolved in 300 cubic centimeters of ether-chloroform mixture. The solution, on standing overnight 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 then 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 gram 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 cold or in the hot.

3.458 mgm. gave 11.115 mgm. CO₂ and 4.622 mgm. 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 C₃₅H₇₂ is reported in Beilstein (20) as 74.7° C.

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

EXAMINATION OF HYDRO-ALCOHOLIC SOLUTION OF SAPONIFIABLE MATERIAL

Most of the alcohol was removed from the hydro-alcoholic solution by distillation over a steam bath. The concentrated solution was made acid with sulfuric acid (1 to 3) and shaken out with five 100 cubic centimeter portions of ether. The slightly green-colored ether solution was dried over anhydrous sodium sulphate and the ether removed by distillation. 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 the refrigerator overnight. A few milligrams of light yellow crystals melting at 79-80° C. were obtained.
On recrystallization from hot 95 per cent 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, \( \text{CH}_3-(\text{CH}_2)_{20}-\text{C}^=\text{O}-\text{OH} \), 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 95 per cent ethyl 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 was added until no further precipitation occurred. The filtrate was concentrated to a thick syrup which when heated with potassium bisulphate produced the characteristic pungent choking odor of acrolein. The presence of glycerin was indicated by this test.

**EXAMINATION OF ETHYL ACETATE SOLUBLE MATERIAL**

The portion of the petroleum benzin extract soluble in ethyl acetate amounted to 325 grams. Of this, 150 grams were refluxed for 6 hours with 1500 cubic centimeters of an alcoholic solution of potassium hydroxide (300 grams of potassium hydroxide per 1500 cubic centimeters of 70 per cent ethyl alcohol). Most of the alcohol was distilled off, water added, and the mixture extracted repeatedly with 200 cubic centimeter portions of ether. This treatment yielded an ether solution of unsaponifiable matter and an aqueous alkaline solution of saponifiable material.
EXAMINATION OF THE UNSAPONIFIABLE PORTION

The ether solutions were combined, washed with water, and the ether removed by distillation over a water bath. The yellow-green residue was dissolved in 95 per cent ethyl alcohol and placed in the refrigerator overnight. 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 to three days. By slight 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 TRIACONTANE

The two portions of yellow solid material totalling about 2 grams were combined and recrystallized several times from 95 per cent alcohol. The top fraction still had a light yellow color; therefore, it was recrystallized from ethyl acetate. It was obtained as an almost white precipitate melting somewhat indefinitely at about 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 concentrated sulfuric acid either hot or cold, 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, its
chemical inactivity, and its melting point was identified as tri-acontane, C_{30}H_{62}.

According to Beilstein (20) tri-acontane melts at 65.2° C.; nonacosane, C_{29}H_{60} melts at 62-63° C., and hentriacontane, C_{31}H_{64} melts at 68.1 to 68.4° C.

**ISOLATION OF A STEROL**

The three fractions of white platelets which had been obtained from the alcoholic filtrate from the triacontane were combined and the total (0.6 gram) recrystallized from hot 95 per cent alcohol. After drying overnight 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 but were soluble in cold concentrated sulfuric acid. The sulfuric acid solution had a red-orange color and showed a heavy, green fluorescence by reflected light. These solubilities placed the substance in Group V of Kamm's (21) solubility table. This group includes alcohols, aldehydes, ketones, ethers, esters, and anhydrides.

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 gram) which melted at 136-137° C.
3.092 mgm. gave 9.295 mgm. CO₂ and 3.261 mgm. H₂O
C = 82.00 per cent; H = 11.80 per cent

2.494 mgm. gave 7.508 mgm. CO₂ and 2.614 mgm. H₂O
C = 82.09 per cent; H = 11.73 per cent

Average C = 82.05 per cent; H = 11.77 per cent

The crystals which were analyzed for percentage content of carbon and hydrogen had been dried to constant weight in a vacuum desiccator over sulfuric acid prior to analysis; however, it was found that on being heated at 100-105°C to constant weight the desiccator dried crystals lost 2.62 per cent of their weight. When this correction is applied to the above analyses the corrected values become:

C = 84.23 per cent; H = 11.79 per cent

C₂₉H₄₉OH requires C = 83.98 per cent; H = 12.16 per cent

0.1136 gram of the desiccator-dried sterol, corresponding to 0.1106 gram of anhydrous sterol, dissolved in 10.00 cubic centimeters of chloroform gave \( \frac{\infty}{D} = -0.57^\circ \) in a 100 millimeter tube:\( \frac{280}{D} \) = -51.54°.

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

About 0.15 gram of the sterol was acetylated by refluxing with five cubic centimeters of acetic anhydride for two hours. The acetic anhydride was removed by heating over a water bath at ten millimeters pressure. The cream-colored crystalline residue was dissolved in chloroform and boiled with charcoal for a few minutes. The filtrate was evaporated to dryness and the white residue recrystallized from absolute alcohol until the crystals melted constantly at 133 to 134°C.

Since the saponification equivalents of the sterols are said (22)
to be more reliable in the determination of the empirical formulae of sterols than combustion analyses, the acetate obtained above was saponified.

0.0793 gram of the acetate dried to constant weight at 106°C was refluxed for 1.5 hours with 5.00 cubic centimeters of half-normal alcoholic potassium hydroxide solution. A blank was run simultaneously.

The acetate was found to be equivalent to 1.73 cubic centimeters of 0.1 normal sulfuric acid. The molecular weight of the acetate calculated from the above equivalent was 456.4.

\[ C_{29}H_{49} \cdot CO \cdot CH_3 = 456.41 \]

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°C 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, \( C_{29}H_{49} \).

**EXAMINATION OF THE SAPONIFIABLE PORTION**

The aqueous alkaline solution after extraction with ether contained the saponifiable portion of the ethyl acetate soluble part of the petroleum benzol extract. It was made acid with sulfuric acid (1 to 3) and extracted ten times with ether. The ether solutions were combined, washed with water, shaken with anhydrous sodium sulfate, filtered, and
the ether removed by distillation. The residue had a rancid odor. It weighed approximately 85 grams. It was dissolved in 95 per cent alcohol and placed in the refrigerator at 7° C. overnight. 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 placed in the refrigerator overnight. The material 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 were obtained a fraction C, readily soluble in ether at 7° C. and a fraction D, precipitated from ether at 7° C.

**ISOLATION OF REMENIC ACID**

Fraction C, consisting of the saponifiable matter precipitated from 95 per cent alcohol at 7° C. but soluble in ether gave a red-brown residue upon removal of the ether by distillation. The residue was dissolved in hot 95 per cent alcohol. On cooling down to room temperature, the solution deposited a green-brown solid from which nothing definite could be isolated. The filtrate from the brown material on standing at room temperature deposited about 0.3 gram of a white solid. The solid was removed and recrystallized from 95 per cent ethyl 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. (24) and at 81–82° C. (25).
0.0412 gram of the acid required 1.20 cubic centimeters of 0.1 N potassium hydroxide for neutralization. The molecular weight calculated from this data is 343.3.

\[ C_{22}H_{44}O_2 = 340.4 \]

The silver salt was prepared and analyzed.

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

\[ Ag = 23.70 \text{ per cent} \]

\[ C_{22}H_{44}O_2Ag \text{ requires } Ag = 24.12 \text{ per cent} \]

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 76-79° 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 recrystallizations from hot alcohol gave a substance melting constantly at 87° C.

\[ C_{30}H_{60}O_2 \text{ has been reported as melting at 89-90° C. (26), at 90° C. (27), and at 90.6° C. (28).} \]

0.0727 gram of the acid required 1.61 cubic centimeters of 0.1 N potassium hydroxide for neutralization. The molecular weight calculated from this data is 451.6.
The silver salt was prepared and analyzed.

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

\[ \text{Ag} = 19.71 \text{ per cent} \]

\[ C_{30}H_{59}O_2Ag \text{ requires } \text{Ag} = 19.29 \text{ per cent.} \]

The above described compound was thus identified as melissic acid, probably containing 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 crystalline matter were obtained. The melting point was 74-75° C. The filtrate was evaporated to dryness giving a light brown residue which melted at 77° C. The quantities of material here were so small that they could not be worked with any further. 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 was a greenish-black, viscous mass with a rancid odor. It weighed approximately 50 grams. It was mixed with 200 cubic centimeters of distilled water and steam distilled. Four 75 cubic centimeter 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 cubic centimeter portions of chloroform. The chloroform shakings were combined and the solvent removed by distillation. This treatment gave an aqueous solution of material not removed by shaking with chloroform and a res-
idue of chloroform soluble material. The residue was mixed with dis-
tilled 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 neu-
tralized solution was then evaporated to dryness and the residue, con-
sisting of the sodium salts of the fatty acids, weighed.

The aqueous solution of material not removed by shaking with chlo-
roform 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>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACIDS EXTRACTED FROM STEAM DISTILLATE BY CHLOROFORM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cubic Centimeters of Sodium Salt</th>
<th>Grams of Sodium Salt Equivalent to Alkali Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/1 alkali obtained</td>
<td>Acetate</td>
</tr>
</tbody>
</table>

| Fraction I | 10.869 | 1.3565 | 0.8930 | 1.1979 | 1.5039 | 1.8088 |
| " II | 9.567 | 1.2325 | 0.7845 | 1.0524 | 1.3213 | 1.5892 |
| " III | 5.640 | 0.7149 | 0.4625 | 0.6204 | 0.7789 | 0.9358 |
| " 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 portions although it is probable that a mixture of butyric, caproic, and caprylic acids in certain proportions would correspond to the values given in fraction I. Fractions II, III, and IV also point to a mixture of butyric and caproic acids or of butyric, caproic, and caprylic acids with the amount of the higher acids 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

<table>
<thead>
<tr>
<th></th>
<th>Per cent Silver found in Silver Salt</th>
<th>Per cent Silver in Silver Salts of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction I</td>
<td>50.3</td>
<td>Butyric Acid 55.3</td>
</tr>
<tr>
<td>&quot; II</td>
<td>48.1</td>
<td>Caproic Acid 48.4</td>
</tr>
<tr>
<td>&quot; III</td>
<td>46.9</td>
<td>Caprylic Acid 43.0</td>
</tr>
<tr>
<td>&quot; IV</td>
<td>43.6</td>
<td>Capric Acid 38.7</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>ACIDS NOT EXTRACTED FROM STEAM DISTILLATE BY CHLOROFORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cubic Centimeters of N/1 alkali used</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fraction I</td>
</tr>
<tr>
<td>&quot; II</td>
</tr>
<tr>
<td>&quot; III</td>
</tr>
<tr>
<td>&quot; IV</td>
</tr>
</tbody>
</table>

All four of the above fractions, which contain the volatile fatty acids not extractable 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 (29). 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 till they were free of hydrochloric acid and were
then dried with anhydrous sodium sulphate. The ether was distilled off over a water bath yielding a residue representing the solid acids and a residue representing the liquid acids.

**ISOLATION OF MYRICSTIC 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 by distillation and the residue amounting to approximately 20 grams was distilled under a pressure of about 20 millimeters. 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 the procedures described above. Fraction II (up to 150° C.) consisted of about half a cubic centimeter of a yellow colored 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 about 5 cubic centimeters. Fraction IV distilled between 220° C. and 260° C. and amounted to about 3 cubic centimeters. Decomposition, producing a red-brown distillate, began above 260° C. The residue in the distillation flask was a black-brown, thick viscous mass from which nothing could be isolated.

Fractions III and IV were both practically colorless liquids which solidified on cooling. Fraction III on crystallization from 95% ethyl alcohol melted at 51-52° C. Fraction IV on crystallization from 95% ethyl 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 amount of the pure acid was approximately 0.8 gram.
$C_{14}H_{28}O_2$ has been reported as melting at $53.7^\circ C$. (30).

0.7722 gram of the acid required 35.11 cubic centimeters of 0.1 N potassium hydroxide for neutralization. The molecular weight calculated from this data is 233.2.

$$C_{14}H_{28}O_2 = 228.2$$

The silver salt was prepared and analyzed.

0.2730 gram of silver salt gave 0.0946 gram silver.

$$Ag = 34.65 \text{ per cent}$$

0.1730 gram of silver salt gave 0.0601 gram silver.

$$Ag = 34.74 \text{ per cent}$$

Average: $Ag = 34.7$ per cent

$C_{14}H_{27}O_2Ag$ requires $Ag = 33.6$ per cent

The acid was obtained by distillation under a pressure of about 20 millimeters between $200^\circ C.$ and $250^\circ C.$

$C_{14}H_{28}O_2$ is reported (30) as distilling at $196.5^\circ C.$ under a pressure of 15 millimeters.

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 about 20 millimeters. This residue amounted to approximately 25 grams. Only a few drops distilled over up to $105^\circ C.$ Between $105^\circ C.$ and $250^\circ C.$, two to three cubic centimeters of a slightly red colored liquid were obtained. This fraction was discarded. Fraction III distilling between $250^\circ C.$ and $258^\circ C.$ was a yellow liquid and amounted to about 8 cubic centimeters. 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 kept overnight in a vacuum desiccator containing sulfuric acid. It was then dissolved in neutral 95 per cent ethyl alcohol and titrated with normal sodium hydroxide solution of which 14.805 cubic centimeters were consumed. The solution was evaporated to dryness and the residue dried to constant weight at 105 °C. The residue weighed 3.5935 grams. 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 gram gave 0.2060 gram Ag; Ag = 35.14 per cent
0.3651 gram gave 0.1289 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 several times with chloroform. The combined chloroform solutions were washed thoroughly with water. The chloroform was evaporated off giving a yellow oil. This was dried over sulfuric acid and the iodine number determined.

0.2432 gram of the oil absorbed 0.05673 gram of iodine. The iodine number calculated from the above is 23.32. The iodine number of oleic acid, $C_{18}H_{34}O_2$, 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 capryllic, $C_8H_{16}O_2$ (per cent Ag in silver salt = 43.0) and capric, $C_{10}H_{20}O_2$ (per cent Ag in silver salt = 38.7). Silver oleate, $C_{18}H_{34}O_2Ag = 27.7$ 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

Another portion of free acid was regenerated from the silver salt as before and boiled with concentrated nitric acid for 30 minutes. The mixture was diluted with water and shaken out with chloroform, the chloroform solutions thoroughly washed with water, and the chloroform evaporated off. The residue consisted of a mixture of solid and oil. The oil was filtered off with strong suction and the residue washed with ether. The residue was dissolved in hot water. Upon cooling, the solution deposited a quantity of white crystals. These were filtered off and dried over sulfuric acid. They melted at 130° C. Another crystallization from hot water did not change the melting point. The crystals could not be identified. They were titratable but the molecular weight calculated from the titration (312 for a di-basic acid) did not conform with any substance having a melting point in the neighborhood of 130° C.
A fluid extract was prepared from 100 grams of drug using 95 per cent alcohol as the menstruum. The first 85 cubic centimeters of percolate were set aside. Percolation was continued until the percolate came through colorless and left no appreciable residue on evaporation of 50 cubic centimeters of percolate. This second percolate was concentrated at a low temperature under reduced pressure and the residue dissolved in the 85 cubic centimeters of reserve percolate. The volume was then made up to 100 cubic centimeters.

One cubic centimeter of the above fluid extract per kilogram was given orally by capsule to two pregnant cats. No action was observed within twenty-four hours.

The marc remaining from the preparation of the fluid extract was exhausted by percolation with 50 per cent alcohol. The latter portions of the percolate were concentrated as above.

All of the percolates including the 95 per cent alcohol fluid extract were combined and concentrated as far as possible by means of a current of air at room temperature. The viscous resinous residue amounted to 16 grams. Of this residue, 1.8 grams were given orally by capsule to a female cat weighing 2.55 kilograms. This represented a dose of 4.4 grams of drug per kilogram. No effects were observed within 72 hours.

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

The chloroform soluble portion of the alcohol soluble extractive contained a substance which gave positive alkaloidal reactions; therefore, the chloroform soluble extract was tested physiologically. Eight and four-tenths grams of the drug, represented by 0.62 grams of the extract, was given by capsule to a female cat weighing 2.7 kilograms. The dose of drug per kilogram was 3.1 grams. No effects were observed within 72 hours.
ALCOHOL EXTRACT

The marc from the petroleum benzin extraction was spread out in a thin layer and the solvent allowed to evaporate. The marc, freed from solvent, was 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 grams 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. In each case percolation had been continued until the percolate came through colorless and until one liter of percolate gave no appreciable residue on evaporation. The mixed material was spread out in a thin layer at room temperature and the alcohol allowed to evaporate. The dry residue was packed in a percolator and extracted successively with petroleum benzin (b.p. 30–60° C), ether, chloroform, ethyl acetate, and 95 per cent ethyl alcohol. In each case percolation was continued until one liter of percolate gave no appreciable residue on removal of the solvent. The extracts obtained were extremely difficult to analyze because each extract was a complex mixture of resins, fats, green pigments, and volatile oil.

PETROLEUM BENZIN SOLUBLE PORTION OF ALCOHOL EXTRACT

This extract was a dark green, soft mass weighing 64 grams representing 9.6 per cent of the alcohol extract. Forty-five grams of the extract were dissolved in alcohol and crystallization attempted but without success.
The alcoholic solution was refluxed for five hours with an alcoholic solution of potassium hydroxide (30 grams in 300 cubic centimeters of 95 per cent alcohol). Water was added and most of the alcohol evaporated off on the water bath. The resulting mixture was transferred to a large separatory funnel and shaken out several times with 100 cubic centimeter portions of chloroform. The combined chloroform shakings were washed with water and the chloroform distilled off on a water bath. The residue (A) was a dark brown liquid with an aromatic odor and amounted to 12 grams. The alkaline liquid which had been shaken out with chloroform was now made acid with sulfuric acid (1 to 3) and again shaken out with chloroform. The chloroform solutions were washed with water and the chloroform removed giving a green-black residue (B) having a rancid odor and weighing 26 grams.

The unsaponifiable residue (A) was dissolved in hot alcohol and allowed to stand. About one gram of amorphous green-gray material was thrown down. It could not be characterized. The alcohol was removed from the unsaponifiable material, the residue mixed with distilled water, and the mixture steam distilled. Two liters of steam distillate were collected. About two cubic centimeters of a light yellow volatile oil were recovered from the distillate. The portion of the unsaponifiable material which was not volatile with steam was recovered by extraction with chloroform and crystallization from alcohol again attempted. Only small amounts of intractable matter were obtained.

The unsaponifiable matter was now mixed with twice its weight of acetic anhydride and boiled for two hours. The hot solution was homogeneous but on cooling it deposited some solid material. This was fil-
tered off but it could not be purified. It was saponified by refluxing with alcoholic potassium hydroxide for two hours. The alkaline mixture was shaken out with chloroform, the chloroform removed, and crystallization from various solvents attempted but without success. The material was transferred to a distilling flask and distillation under reduced pressure attempted. The material did not distil without decomposition. Further work on this fraction was abandoned.

The saponifiable residue (B) was dissolved in a mixture of three parts of ether and one part of chloroform and shaken out first with 10 per cent ammonium carbonate solution and then with 10 per cent sodium hydroxide solution. After extraction with sodium hydroxide solution there remained in the ether-chloroform mixture only a very small amount of reddish-brown material which was discarded.

The material soluble in ammonium carbonate solution was made acid and shaken out with ether. The ether was removed. Attempts at crystallization of the resinous residue from various solvents met with failure. Distillation of the residue under reduced pressure produced only a few cubic centimeters of distillate which had an aromatic odor and was acid to litmus. A pure silver salt could not be prepared. The residue in the distillation flask was a green-black resin from which nothing crystalline could be obtained.

The sodium hydroxide soluble material yielded results similar to those obtained with the ammonium hydroxide soluble material.

Nineteen grams of the original petroleum benzine soluble portion of the alcohol extract were dissolved in alcohol. Sulfuric acid and water were added in such proportions as to make the content of H₂SO₄ five per cent. The mixture was refluxed for two hours. It then had the pro-
nounced fruity odor of ethyl butyrate. The alcohol was removed and water added. The mixture was filtered, sulfuric acid removed from the filtrate with barium hydroxide, and the filtrate from this concentrated to a thick syrup. The syrup did not reduce Fehling's or Benedict's solution. Since acid hydrolysis did not liberate a reducing sugar, the absence of material of a glycosidal nature in the petroleum benzin soluble extract may be assumed.

**ETHER SOLUBLE PORTION OF ALCOHOL EXTRACT**

This extract was a dark green viscous liquid weighing 42 grams 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 grams of green material were deposited from the ethyl acetate solution. The green material was probably chlorophyll although it did not give the "brown-phase" reaction (32). 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 was concentrated to a small volume, mixed with water, and steam distilled. The steam distillate was shaken with ether. The combined ether shakings on removal of the ether gave a very small amount of a light green volatile oil having an aromatic odor but unpleasant taste. An alcoholic solution of the oil was acid to litmus. The distillate after the removal of the ether soluble constituents was still acid in reaction. A portion (150 cubic centimeters) was neutralized with sodium hydroxide
solution and evaporated to dryness. The residue, mixed with alcohol and concentrated sulfuric acid, gave off the characteristic fragrant odor of ethyl butyrate.

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

The supernatant liquid was strongly acid to litmus and gave a dark green precipitate with ferric chloride solution. Extraction with ether yielded a very small amount of green coloring matter. After extraction with ether, the supernatant liquid was evaporated to dryness. The residue consisted of more chlorophyll mixed with brown fatty material.

The black viscous resin yielded nothing definite.

Hydrolysis of ten grams of the original ether extract with 5 per cent sulfuric acid in aqueous alcohol yielded no reducing sugar. Absence of glycosidic material in the ether soluble portion of the alcohol extract was thus indicated.

CHLOROFORM SOLUBLE PORTION OF ALCOHOL EXTRACT

This extract was a green-black liquid weighing 31 grams representing 4.6 per cent of the alcohol extract. As nothing could be directly separated from a solution of this extract in various solvents, 25 grams of it were mixed with water, and steam distilled. The steam distillate was found to contain volatile oil and butyric acid as in the case of the ether soluble extract.

The non-volatile material in the distilling flask consisted of a clear, amber colored supernatant liquid and a black solid resin.

The dried resin burned with a smoky flame and left no perceptible residue. Nothing definite could be isolated from it.
The amber colored supernatant liquid had a bitter astringent taste and was acid to litmus. It yielded precipitates with solutions of phospho-molybdic acid and phospho-tungstic acid, Mayer's reagent, iodine test solution, and gold chloride solution.

The supernatant liquid was made alkaline with 10 per cent ammonia and shaken out with chloroform. The combined chloroform solutions were shaken out with 2 per cent sulfuric acid. The combined acid solutions were made alkaline whereupon a cloudy white precipitate appeared. This was completely shaken out with chloroform. The chloroform solutions were evaporated to dryness. A very small amount of a brown, varnish-like residue was obtained. A portion of the residue dissolved in slightly acidulated distilled water gave precipitates with the reagents named above. The remainder of the residue was dissolved in neutral alcohol and titration attempted. An excess of standard acid was added but the amount of standard alkali required for back titration was exactly equivalent to the acid added. The substance was recovered from the titration mixture and dissolved in a small volume of alcohol. The amount of the substance was so small that solutions of it had to be evaporated to dryness to obtain any of it. Slow evaporation of solutions of the substance in ether, chloroform, and alcohol produced fan-shaped clusters of very fine needles contaminated with brown coloring matter. This coloring matter could not be removed by charcoal. Under the microscope the impure crystals appeared as uniformly grained, long flat crystals with irregular ends. The micromelting point of the impure crystals was 160-163° C.

Hydrolysis of five grams of the original chloroform extract with 5 per cent sulfuric acid in aqueous alcohol yielded no reducing sugar.
Absence of glycosidic material in the chloroform soluble portion of the alcohol extract was thus indicated.

**ETHYL ACETATE SOLUBLE PORTION OF ALCOHOL EXTRACT**

This extract was a dark brown solid weighing 78 grams representing 11.7 per cent of the alcohol extract.

**Isolation of Sodium and Potassium Chloride**

The extract was mixed with 95 per cent alcohol. There remained undissolved several grams of yellowish-white crystalline material. It was found to be soluble in water and was recrystallized from water. The last crystallization yielded perfectly symmetrical cubes. These crystals were inorganic and had a salty taste. Qualitative tests revealed the presence of sodium, potassium, and chloride ions. Lithium and ammonium ions were absent.

The following quantitative experiments were performed with the crystals:

- 0.1928 gram produced 0.4517 gram of silver chloride
- 0.3188 " " 0.7484 " "

Assuming the crystals to be sodium chloride the per cent of NaCl in the crystals is respectively 95.54 and 95.73.

0.1497 gram of the crystals consumed 24.445 cubic centimeters of N/10 silver nitrate solution.

Calculated on the basis of sodium chloride the per cent of NaCl = 95.45.

Since the qualitative tests showed the crystals to consist of sodium chloride and potassium chloride the per cent of each in the crystals was calculated from the results of the first two determinations as follows:
0.5116 gram of crystals produced 1.2001 grams AgCl.

If \( X \) = grams of NaCl in 0.5116 gram of crystals, then

\[
(0.5116 - X) = \text{grams of KCl in 0.5116 gram of crystals.}
\]

\[
\left[ \frac{\text{AgCl}}{\text{NaCl}} \times X \right] + \left[ \frac{\text{AgCl}}{\text{KCl}} \times (0.5116 - X) \right] = 1.2001
\]

\[
X = 0.4087 \text{ grams of NaCl}
\]

\[
0.5116 - 0.4087 = 0.1029 \text{ grams of KCl}
\]

\[
\frac{0.4087}{0.5116} \times 100 = 79.9 \text{ per cent NaCl in crystals}
\]

\[
\frac{0.1029}{0.5116} \times 100 = 20.1 \text{ per cent KCl in crystals.}
\]

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 whole steam distilled. This steam distillate as the previous ones 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 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 solution, 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 strongly. With ferric chloride, a very dilute solution produced a green precipitate which turned to violet on addition of sodium acetate solution. The above reactions indicated the presence of a catechol tannin.

To determine whether the tannin was a glycoside or not the method of Procter (17) was used. One third of the supernatant solution (one hundred cubic centimeters) was used for the determination. A portion of the clear, colorless neutral filtrate obtained at the end was boiled with Fehling's solution. A small amount of cuprous oxide was produced. Another portion of the filtrate gave a positive Molisch reaction. An osazone could not be prepared. These tests indicated the presence of a reducing carbohydrate. This in turn leads to the conclusion that the tannin was a glycosidal tannin.

A quantitative determination of the tannin present in the supernatant liquid was made by the method described by Rosenthaler (33). Twenty cubic centimeters of tannin solution were evaporated to dryness on the water bath, then dried to constant weight at 100° C. The weight of the residue = amount of total soluble matter \( G \) = 2.1506 grams. The residue was converted to ash and the amount of ash ascertained \( A \) = 0.0220 gram. \( G - (A) \) = amount of soluble organic matter \( O \) in 20 cubic centimeters of tannin solution = 2.1506 - 0.0220 = 2.1286 grams. Twenty cubic centimeters of tannin solution were mixed
with 130 cubic centimeters of water and digested for one hour with 10 grams of hide powder with frequent stirring, the mass pressed out, and the filtrate again treated for 24 hours with 5 grams of hide powder. The filtrate from this gave no reaction with ferric chloride solution. Fifty cubic centimeters of the filtrate were evaporated to dryness and the residue dried to constant weight. It was then converted into ash and the weight of the ash subtracted. The difference in the weights multiplied by 3 gives the quantity of non-tannin \( (N) \) present in 150 cubic centimeters of the diluted tannin solution: 

\[
1.4686 - 0.0259 = 1.4429 \times 3 = 4.3287 \text{ grams.}
\]

From this is deducted the weight of the organic matter dissolved out of the hide powder, as determined by an experiment under the same conditions. This weight = 4.3086 grams. Then, 

\[
4.3287 - 4.3086 = 0.0201 \text{ gram, weight of non-tannin in 20 cubic centimeters of tannin solution.}
\]

The quantity of tannin present in the original twenty cubic centimeters of the tannin solution is finally obtained from \( (0) - (N) \) : 

\[
2.1286 - 0.0201 = 2.1085 \text{ grams.}
\]

The total amount of tannin present in the supernatant liquid calculated from the above = 

\[
2.1085 \times 15 = 31.6 \text{ grams.}
\]

Concentration of the remaining tannin solutions 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.

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 extracted with 95 per cent alcohol. Most of the alcohol was removed by
distillation. The thick brown-black residue was mixed with water and the volatile oil removed by steam distillation.

The brown-colored 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 grams of greasy green material were deposited. This material 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 grams. 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. Its acid value was 116.5; 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.

**SUMMARY AND CONCLUSIONS**

1. A short botanical description of *Ipomea Pes-Caprae* has been given, followed by reports of medicinal uses.

2. The United States Pharmacopoeia X "constants" for crude drugs have been determined for *Ipomea Pes-Caprae*.

3. A preliminary extraction by the Dragendorff scheme indicated that petroleum benzin and 95 per cent alcohol were the best extractants for the drug. The various extracts obtained by the Dragendorff method were described.
4. Tests on small amounts of drug for the presence of alkaloids were negative.

5. The test by the Lead Method gave indications of the presence in the drug of fat, tannin, red coloring matter, and a reducing sugar.

6. The total resin content of the drug was found to be 7.27 per cent. The aqueous washings from this determination responded to reactions for a glycosidal tannin.

7. The ash from a portion of the drug was found to contain tin, iron, aluminum, magnesium, calcium, sodium, potassium, and silica. The sulfate, chloride, and carbonate radicals were also present.

8. The drug was found to contain 0.048 per cent volatile oil. The volatile oil had a specific gravity of 0.9626 at $\frac{25^\circ C}{25^\circ C}$ and a refractive index at 25° C. of 1.4703.

9. Extraction of nine kilograms of the drug with petroleum benzin yielded 3.91 per cent of extractive. The extract yielded the following substances: pentatriacontane, $C_{36}H_{72}$; triacontane, $C_{30}H_{62}$; a sterol, $C_{29}H_{49}OH$; behenic acid, $C_{21}H_{43}COOH$; melissic acid, $C_{29}H_{59}COOH$; butyric acid, $C_{3}H_{7}COOH$; and myristic acid, $C_{13}H_{27}COOH$. The presence of glycerol, of saturated fatty acids of low molecular weight, and of an unsaturated fatty acid was demonstrated.

10. The marc from the petroleum benzin extraction was extracted with 95 per cent alcohol and yielded 7.43 per cent of extractive. The extract yielded volatile oil; butyric acid; chlorophyll; resin; a substance melting at 160-163° C. and responding to alkaloidal reactions; inorganic crystals consisting of sodium and potassium chlorides; and a catechol tannin which yielded a reducing carbohydrate on hydrolysis.
II. 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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