# SCIENTIFIC SECTION



## A PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION OF TRILLIUM ERECTUM.*,**

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Trillium has long been recognized as a medicinal drug and is still retained as an official drug in the sixth revision of the National Formulary. The Fluidextract of Trillium is one of the ingredients of Compound Elixir of Viburnum Opulus, which according to the "Prescription Ingredient Survey" of 1931–1932 (1), was prescribed 2.9 times in every 10,000 prescriptions. Despite the fact that the drug is still used to a fair extent, it is surprising to note that very little is known of its chemical composition and physiological action. If Trillium is to remain as an official drug, certainly something conclusive should be learned of its chemical composition and physiological action. It was with this in mind that the present study was undertaken.

The first chemical examination of Trillium seems to have been made by E. S. Wayne (2). He reports having found gum, resin, extractive, tannic acid, starch, a little volatile oil and a principle analogous to saponin, which has been called "trillin" or "trilline," the two terms being synonymous. Millspaugh (3) claims he has corroborated Wayne's analysis in full.

D. J. Prendergast (4) in an analysis of *Trillium Erectum* found it to contain 6% of moisture, 2.3% ash, 8.2% of fixed oil, 4.8% of resin and 18.9% of alcohol extractive. He also reported that there was present a white, amorphous powder, soluble in chloroform. This powder he found was bitter at first and afterward sweetish to the taste. It turned red with hydrochloric acid and purple with sulfuric acid, was insoluble in ether and produced considerable foam upon agitation with water, in which it was quite soluble. From these scant experiments he concluded that the principle must be convallamarin ($C_{24}H_{44}O_{12}$). He also reported having found gum and small quantities of organic acids, but obtained negative tests for alkaloids and volatile oil.

V. I. Reid (5) from an analysis of the drug concluded that beside the usual plant constituents such as starch, tannin, resin and gum, it contained a small quantity of fixed oil and saponin to the extent of 4.86%. He also claimed to have isolated a crystalline acid material soluble in ether. This principle was colored purplish brown by strong sulfuric acid and light green by sulfuric acid and a crystal of potassium dichromate. He suggested that this principle probably resulted from a decomposition of the saponin.

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EXPERIMENTAL.

Two different lots of the powdered drug were purchased from a reputable dealer and analyzed immediately after the shipment was received for moisture, ash and soluble extractives according to the general directions and procedure given by the U. S. P. X. The results obtained are given in the following table:

| | Per Cent. | | | |
|---|---|---|---|---|
| | Drug Lot 1. | | | Drug Lot 2. |
| | A. | B. | Av. | A. |
| Total ash | 10.88 | 10.74 | 10.81 | 4.15 |
| Acid-insoluble ash | 9.63 | 9.50 | 9.57 | 0.85 |
| Moisture (corr. for vol. const.) | 5.20 | 5.17 | 5.19 | 5.36 |
| Vol. ether-soluble ext. | 0.81 | 0.83 | 0.82 | 0.88 |
| Non-vol. ether-soluble ext. | 7.17 | 7.19 | 7.18 | 8.55 |
| Alcohol-soluble ext. | 21.16 | 19.90 | 20.53 | 26.93 |
| Dilute alcohol-soluble ext. | 15.90 | 15.80 | 15.85 | 34.86 |
| Water-soluble ext. | 12.90 | 13.10 | 13.00 | 26.52 |
| Petroleum benzin ext. | 5.32 | 5.92 | 5.62 | 7.98 |

From the above results it can be seen that drug lot 1 contained a considerable portion of dirt (sand) as shown by the high acid-insoluble matter. Drug lot 2 was apparently a much better lot of drug and gave higher results on extractive matter.

Fifty grams of drug were extracted successively with various solvents. The extracts were dried at 100° C. and weighed. The average results obtained are given below, the solvents being used in the order named in the table.

| Solvent. | Drug Lot 1, Per Cent. | Drug Lot 2, Per Cent. |
|---|---|---|
| Petroleum ether | 5.70 | 6.19 |
| Ether | 0.87 | 3.54 |
| Chloroform | 0.73 | 0.64 |
| Ethyl acetate | 3.98 | 2.68 |
| Alcohol | 19.80 | 20.14 |
| Water | 6.90 | ... |

PETROLEUM-ETHER EXTRACT.

For the purpose of a more complete examination a larger quantity of the drug was extracted with petroleum ether. In the case of drug lot 1, 9.5 Kg. were extracted in a Lloyd Extractor. Twelve kilograms of drug lot 2 were percolated in a large glass percolator. In the case of drug lot 2, as the first percolate was drawn off and diluted with more petroleum ether, an insoluble resin separated out. This resin was apparently dissolved by the fatty oil in the first percolate which was very thick and upon further dilution with petroleum ether separated out. This resin was separated and only the petroleum-ether soluble material examined as fatty oil. The oil from drug lot 1 having been extracted in the Lloyd Extractor contained this resin in solution. Drug lot 1 yielded 539 Gm. of fatty oil, representing 5.66 per cent of the crude drug; drug lot 2 yielded 697 Gm. of fatty oil, representing 5.81 per cent of the crude drug.

The oils from both lots of drugs were a dark reddish brown color with a greenish fluorescence and an odor similar to that of the drug, indicating the presence of some volatile oil. Both oils on standing at room temperature deposited a solid crystalline material. The oils were easily soluble in absolute, 95 and 90 per cent alcohol. They were also soluble in ether, chloroform, carbon disulfide, carbon tetrachloride and benzene. They were insoluble in water.

The following constants were run on the oils:

| | | | | | |
|---|---|---|---|---|---|
| Specific gravity at 25° C. | 0.9005 | 0.9205 | Unsaponifiable matter | 4.48% | 1.31% |
| Refractive index at 25° C. | 1.4722 | 1.4677 | Acid value | 115.5 | 142.8 |
| Saponification value | 205.3 | 207.9 | Acetyl value | 92.1 | ..... |
| Iodine value | 87.3 | 106.4 | Total volatile acid value | 9.7 | ..... |

The high acid value would indicate that the oil had either undergone hydrolysis in the process of extraction or had already been broken down in the drug and, instead of consisting of triglycerides, now consisted of di- or monoglycerides and free fatty acids. This fact is borne out in the case of the oil from drug lot 1 by the high acetyl value and low volatile acid value. This would also explain why the oils are soluble in alcohol.

A few drops of the oil when spread on a glass slide and left exposed to the air for a period of three weeks, had the same consistency and appearance at the end of this time as before, indicating that it was a non-drying oil. This is in agreement with the iodine value.

The high saponification value and low volatile acid value would indicate the presence of myristic or lauric acids.

A preliminary examination for fatty acids was conducted as follows: after saponification of a portion of the oil and removal of the unsaponifiable matter, the solid and liquid fatty acids were separated by the usual lead-salt-ether method. The solid fatty acids were resolved into two fractions by fractional crystallization from alcohol. One fraction melted at 61.2° C. and the other at 57° C. A 0.2-Gm. portion of the fraction melting at 61.2° C. was neutralized with tenth-normal sodium hydroxide, 7.75 cc. being required. This neutralization value gives a molecular weight of 250.81. The molecular weight of palmitic acid is 256.26. Thus it was concluded that the substance melting at 61.2° C. was palmitic acid. The crystals melting at 57° C. are probably a mixture of palmitic and myristic or lauric acids.

The liquid acids separated by the lead-salt-ether method gave an iodine value of 117.4 indicating the presence of unsaturated fatty acids other than oleic.

The unsaponifiable matter remaining from the saponification of 100 Gm. of the oil from drug lot 2 (amounting to about 1.7 Gm.) was examined as follows: the material was dissolved in hot alcohol and allowed to cool to room temperature. On cooling, a white flocculent precipitate settled out. It was filtered off and recrystallized several times from hot alcohol. Finally only about 10 mg. of a white waxy material remained. This material melted at 75° C. A chloroform solution of it did not decolorize a pale yellow solution of bromine in chloroform, showing the product was a saturated compound. It gave negative reactions for sterol by the Salkowski and Liebermann-Burchard tests. This material was undoubtedly a saturated hydrocarbon. As pentatriacontane ($C_{35}H_{72}$) melts at 75° C., it was concluded that it was this substance.

The residues from the separation of the pentatriacontane were combined and steam distilled until about 400 cc. of distillate were collected. The distillate was shaken repeatedly with ether and upon evaporation of the ether 0.2 Gm. of a pale yellow liquid with a pleasant aromatic odor remained. This residue was a small amount of volatile oil and was too small to examine further.

The material non-volatile with steam was further saponified with a small quantity of alcoholic potassium hydroxide in order to remove any traces of unsaponified fatty oil that might still have been present. The unsaponifiable matter from this second saponification was a golden-brown colored oil. It was taken up with hot alcohol and allowed to stand over night. At the end of this time some globules of oil had settled and the supernatant alcoholic solution was decanted off and allowed to stand over night to crystallize. The supernatant alcohol was again poured off and the crystalline material washed with several small portions of cold alcohol. It was then dissolved in warm alcohol and recrystallized several times from this solvent. Finally, a very small amount of a white crystalline substance remained. When allowed to crystallize slowly from alcohol, hexagonal plates were formed. This material was dried at 100° C. and gave a melting point of 130° C. A chloroform solution of the material gave positive Salkowski and Liebermann-Burchard tests for sterol. There was no more material left for further examination; however, the melting point would indicate that it was a phytosterol which melts at 134° C.

All residues remaining from the separation of the sterol were combined and transferred by means of ether to an acetylation flask, the ether evaporated and the residue refluxed with 5 cc. of acetic anhydride for two hours. The warm material was placed in a separatory funnel and allowed to stand over night. Two distinct layers formed, an upper oily layer and a lower layer of acetic anhydride. The oily layer was separated and heated on a steam-bath to remove acetic anhydride. The residue, which was a brown color, was dissolved in ether and transferred to a small distilling flask and distilled. At about 220° C. a few drops distilled over. The distillate was dissolved in ether, placed in a Babcock bottle and the ether evaporated. The residue was

treated with fuming sulfuric acid (6) and after centrifuging, a small globule of oil floated on the surface of the sulfuric acid. As this material was non-saponifiable, could not be acetylated and did not react with fuming sulfuric acid, it must have been a hydrocarbon or mixture of hydrocarbons. It probably consisted of liquid hydrocarbons ranging from undecane to hexadecane.

The acetic anhydride solution remaining after separating the oily hydrocarbon layer was evaporated on the steam-bath to remove the acetic anhydride. The residue was taken up in warm alcohol and cooled in ice water. A trace of precipitate formed; however, it was too small to purify. This precipitate was most likely a sterol acetate.

### ALCOHOL EXTRACT.

The marc left from the extraction with petroleum ether was completely extracted in the Lloyd Extractor with ninety-five per cent alcohol. The alcoholic extract after removal of most of the solvent, amounted in the case of drug lot 1 to about 1900 Gm., in the case of drug lot 2 to about 2800 Gm. These extracts still contained a small amount of alcohol and were of a pilular consistency. The following work, unless otherwise stated, will pertain to the extract of drug lot 2.

*Isolation and Identification of Sucrose.*—During the process of extraction of the drug in the Lloyd Extractor, the percolate was drawn off from time to time in liter beakers. After standing for several days while the remainder of the drug was being extracted, some of the alcohol evaporated and a crystalline material separated out in each of the breakers. The dark brown liquid was decanted and the combined crystals washed repeatedly with small portions of boiling alcohol. The crystals were dissolved in a small quantity of water and filtered. The filtrate was evaporated to a thick syrup and the syrupy liquid poured slowly with constant stirring into absolute alcohol. On standing, a fine crystalline precipitate was obtained. This was filtered and the process repeated until about ten Gm. of a white, sweet tasting, crystalline compound were obtained.

An aqueous solution of the crystals did not reduce Fehling's solution. However, after hydrolysis with dilute hydrochloric acid, the neutralized solution gave a heavy precipitate of cuprous oxide with Fehling's solution.

One-half gram of the crystals left no residue on ignition, showing the absence of inorganic constituents.

The crystals were dried to constant weight at 100° C. and 1.37 Gm. were dissolved in water and made up to 25 cc. in a volumetric flask at 20° C. Direct polarization of this solution in a 200-mm. tube at 20° C. gave a reading of +21.0° Ventzke. The calculated reading for pure sucrose should be +21.08° V. This would indicate that the substance was 99.64 per cent sucrose. Twenty cubic centimeters of the above solution were inverted (7). After standing over night the solution was made to 50 cc. with distilled water. A reading of −2.7° Ventzke was obtained using a 200-mm. tube at 20° C. From the readings before and after inversion the per cent sucrose was calculated using the formula in the A. O. A. C. Book of Methods (7). A value of 99.40 per cent was obtained. The inverted solution gave a positive test for ketoses with Seliwanoff's reagent. From the above results it must be concluded that the crystalline material was sucrose.

*Steam Distillation of a Portion of the Alcohol Extract.*—Seven hundred and sixty-five grams of the extract were steam distilled and the distillate after shaking with chloroform and ether, yielded 1.86 Gm. of a golden-brown oil, with an odor of valeric acid. This residue was dissolved in chloroform, shaken with small portions of 8% sodium carbonate solution and then with several portions of 10% sodium hydroxide solution. The chloroform solution remaining was evaporated and yielded 0.37 Gm. of a volatile oil with an aromatic odor similar to that of the drug. The sodium hydroxide shakings gave a negligible residue, showing the absence of phenols.

The sodium carbonate shakings consisted of a mixture of volatile fatty acids. An attempt to fractionally distil the acids was not successful. The mean molecular weight of the acids was 120.8 when determined from the neutralization value. As these acids had a strong odor of valeric acid, they probably consisted of a mixture of the acids from valeric to possibly caprylic acid.

The mother liquor remaining from the steam distillation was separated by the process of centrifuging and decanting into water-soluble and water-insoluble material. The water-insoluble matter was mixed with sand, evaporated to dryness and powdered. It was then percolated with petroleum ether, ether and benzene. These solvents removed only small amounts of fatty and resinous matter. The powdered mixture was then suspended in water and freed from sand by decantation.

*Isolation of a Glucoside.*—The aqueous suspension was treated with about 10 Gm. of barium hydroxide, the mixture evaporated to dryness over a steam-bath and powdered. The powder was extracted with four successive portions of boiling alcohol. The alcohol extracts were combined, cooled, filtered and the alcohol evaporated. The residue, which was insoluble in water, was macerated with water containing a little dilute sulfuric acid, filtered and washed well with water, which removed the yellow color. The residue on the filter was allowed to dry spontaneously and was recrystallized many times from alcohol, until a white, crystalline compound was obtained. The crystals were further purified by treatment with hot ether, benzene and chloroform, these solvents removing only negligible amounts of material, which was discarded. The crystals were again shaken with water and a typical saponin foam was produced. The water was filtered off and the crystals shaken with successive portions of water until a foam was no longer produced on vigorous shaking. The crystals were allowed to dry and recrystallized once more from alcohol. In this manner about 3 Gm. of white crystals were prepared. This compound has been given the name "Trillarin."

*Properties and Composition of "Trillarin."*—This material, when crystallized from ethyl or methyl alcohol, separates in clusters of silky crystals. Under the microscope, they appear as long, flat, rectangular plates; there appear to be no perfect crystals, all apparently being broken at the ends.

The crystals are insoluble in ether, chloroform and benzene. The solubility in ethyl alcohol at 25° C. is 3.49 Gm. per 100 cc.; in acetone 0.99 Gm. per 100 cc. They seem to be slightly more soluble in methyl than in ethyl alcohol. They are slightly soluble in warm ethyl acetate. The crystals are tasteless. When recrystallized from 95% ethyl alcohol, 0.8245 Gm. of the air-dried crystals lost 0.0329 Gm. on drying to constant weight at 100° C., at loss of 3.99%. The anhydrous material is hygroscopic.

The air-dried crystals melted at 197° to 200° C. (uncorr.). The crystals dried at 100° C. soften at about 204° C. and are completely melted at 211° C. (uncorr.). One-tenth gram of the crystals left no residue upon ignition, showing the absence of inorganic impurities. An alcoholic solution of the crystals gave no precipitate with a saturated alcoholic solution of lead acetate or barium hydroxide. A solution of 0.1786 Gm. of the crystals in neutral alcohol gave a permanent pink color to phenolphthalein with one drop of 0.1*N* sodium hydroxide, showing the crystals to be a neutral substance. Both an alcoholic solution and an aqueous suspension of the crystals gave a positive test for carbohydrates by the Molisch reaction. Tests for nitrogen, sulfur, halogens and phosphorus were negative. When a drop of concentrated sulfuric acid is added to the crystals, they turn at first a red-orange color and finally dissolve giving a golden-yellow solution. This sulfuric acid solution of the crystals produces a violet color on standing with a crystal of potassium bromide, a violet color with a solution of bromine in potassium hydroxide and a red color which turns violet on standing with a ferric chloride solution. It is interesting to note that these colors are given by digitalin verum.

An ethyl alcohol solution of the crystals dried at 100° C., containing 0.3863 Gm. in 25 cc. of solution, gave a rotation of −3.6° angular at 22° C. The specific rotation was therefore −116.3. A molecular weight determination using the elevation of the boiling-point method with absolute ethyl alcohol as the solvent, gave a value of 706. Micro combustion analyses on the crystals dried at 100° C. gave the following results: (1) A 5.371-mg. sample gave 3.851 mg. $H_2O$ and 12.066 mg. $CO_2$. (2) A 4.507-mg. sample gave 3.325 mg. $H_2O$ and 10.094 mg. $CO_2$. These results indicate the formula $C_{37}H_{59}O_{14}$ which has a molecular weight of 727.47.

| | Per Cent Calculated for $C_{37}H_{59}O_{14}$. | Per Cent Found I. | II. |
|---|---|---|---|
| C | 61.03 | 61.26 | 61.07 |
| H | 8.17 | 8.02 | 8.26 |
| O | 30.80 | 30.72 | 30.67 |

*Hydrolysis of "Trillarin."*—The hydrolysis of the glucoside was carried out as follows: 3.0567 Gm. of the crystals dried at 100° C. were dissolved in 150 cc. of ethyl alcohol, 3 cc. of concentrated hydrochloric acid were added and the solution refluxed on a steam-bath for two hours. The alcoholic solution, which had turned slightly yellow during the hydrolysis, was cooled and poured slowly with constant stirring into 500 cc. of water. The mixture was allowed to stand

over night to permit a fine white precipitate of the genin to settle out. The clear aqueous solution was decanted off through a suction filter, the precipitated material washed several times by decantation and finally transferred to the filter and washed thoroughly with water. The precipitate was allowed to dry, dissolved in hot methyl alcohol, transferred to a tared beaker, the alcohol evaporated spontaneously and the residue dried at 100° C. and weighed. The filtrate from the genin was placed in an evaporating dish and heated over a steam-bath until the alcohol was removed and the volume of the solution was about 100 cc. A small amount of genin which had remained in solution had precipitated out and was filtered off and added to the main precipitate. The total weight of the dried genin was 1.6751 Gm. representing 54.8 per cent of the original glucoside.

This genin has been given the name "Trillarigenin."

The filtrate from the genin contained the sugars or sugar split off during hydrolysis.

*Examination of the Sugar Resulting from the Hydrolysis of "Trillarin."*—An excess of silver carbonate was added to the aqueous solution in order to free it from hydrochloric acid. The filtrate was concentrated to a volume of about 20 cc. and shaken several times with chloroform which removed the yellow color and any last traces of the genin. The aqueous solution was concentrated to a thick syrup and dried over calcium chloride for two days. The residue was taken up with warm alcohol and the sugar precipitated with ether. The precipitated sugar was filtered off, dried over sulfuric acid and powdered. A portion of the powder gave a strong reduction with Fehling's solution. An aqueous solution gave a negative test for fructose or other ketoses with Seliwanoff's reagent. A portion of the powder gave a negative test for pentoses when heated with hydrochloric acid and phloroglucinol. An osazone of the material was prepared and melted at 205° C.

The remainder of the material was treated with 30 cc. of nitric acid, specific gravity 1.15, and evaporated on the steam-bath to a volume of about 10 cc. After standing 24 hours, no crystals of mucic acid had separated. The solution was allowed to stand 24 hours longer and still no mucic acid separated; the volume of the solution was now about 5 cc. This test indicates the absence of galactose.

The nitric acid solution remaining from the mucic acid test was concentrated to a volume of about one cc., five cc. of water added and the solution made slightly basic by the cautious addition of solid potassium carbonate. Glacial acetic acid was then added until the solution gave a strong odor of acetic acid. After standing over night a few crystals had formed. These crystals when examined under the microscope were found to be the characteristic rhombic crystals of potassium bisaccharate.

It was concluded from the above results that the sugar split off during the hydrolysis of "Trillarin" was glucose.

*Properties and Composition of "Trillarigenin."*—The dry material was macerated with small portions of water to remove any sugar that might have been present and was then recrystallized from methyl alcohol until white crystals were obtained. The compound crystallized from methyl alcohol in shining wartlike clusters, however, when allowed to dry in the air it gave no definite shape when examined under the microscope.

The dried material gave the same color reactions with sulfuric acid, sulfuric acid and potassium bromide, and sulfuric acid and ferric chloride as were observed for "Trillarin." When dried to constant weight at 100° C., 0.2280 Gm. of the air-dried genin lost 0.0042 Gm. or 1.84 per cent. The dry material appeared to be stable in the air. The 100° C. dried material softened somewhat at 190° C. and melted completely at 197° C. (uncorr.). One-tenth gram of the genin when dissolved in neutral alcohol gave no titration with 0.1*N* sodium hydroxide, showing it was a neutral substance. The specific rotation of the genin dried at 100° C. in ethyl alcohol at 24° C., using a solution of 0.2207 Gm. in 25 cc., was −98.2. Micro-combustion analyses on the genin dried at 100° C. gave the following results: (1) A 3.180-mg. sample gave 2.728 mg. of $H_2O$ and 8.711 mg. of $CO_2$. (2) A 2.991-mg. sample gave 2.590 mg. of $H_2O$ and 8.180 mg. of $CO_2$. These results would indicate the formula $C_{25}H_{39}O_4$ which has a molecular weight of 403.31.

| | Per Cent Calculated for $C_{25}H_{39}O_4$. | Per Cent Found. I. | II. |
|---|---|---|---|
| C | 74.38 | 74.70 | 74.58 |
| H | 9.75 | 9.60 | 9.69 |
| | 15.87 | 15.71 | 15.73 |

The following equation may now be written for the hydrolysis of "Trillarin:"

$$C_{37}H_{59}O_{14} + 2\ H_2O \longrightarrow C_{25}H_{39}O_4 + 2\ C_6H_{12}O_6$$

The theoretical amount of "Trillarigenin" formed should be 55.44 per cent. The actual recovery was 54.8 per cent.

It is of interest to mention that some crystals of "Trillarin" were also obtained from the alcoholic extract of drug lot 1. This principle appears to be a normal constituent of the drug.

*Saponin.*—Lead acetate was added to a portion of the water-soluble material from the alcohol extract but produced only a faint cloudiness. Lead subacetate was then added, the precipitate separated and freed from lead by means of hydrogen sulfide. The lead-free solution was taken to dryness, the residue taken up with alcohol and ether added to the alcoholic solution to complete precipitation. This process of precipitating with alcohol and ether was repeated several times. The dry precipitate was tan colored and produced a typical saponin foam on shaking with water. It readily hemolyzed red blood corpuscles. This material gave some reduction with Fehling's solution, indicating possible contamination with sugars. Further purification of this material will be necessary before analysis.

*Attempt to Isolate Convallamarin.*—As Prendergast (4) had reported isolating a water-soluble, chloroform-soluble, amorphous principle which he said was identical with "convallamarin," a special search for this substance was made both by the procedure used by him and by several other methods. The results were negative and neither the so-called "convallamarin" nor anything like it could be isolated.

*Possible Presence of Alkaloid.*—As preliminary tests had indicated the presence of a substance which gave a precipitate with alkaloidal reagents, the following attempt was made to isolate this substance. One hundred cc. of 10 per cent ammonia and 1500 cc. of ether-chloroform mixture (3 + 1) were added to 500 Gm. of drug lot 2, the mixture was shaken frequently for one hour and allowed to stand over night. The ethereal solution was decanted off through a cotton filter into a large separatory funnel. A fresh portion of ether-chloroform mixture was added to the marc, shaken and macerated over night as before. The solvent from the second extraction was filtered and combined with the first extraction. The combined solutions were then shaken with small successive portions of two per cent sulfuric acid. The combined sulfuric acid shakings were made ammoniacal and again extracted with many small portions of the ether-chloroform mixture. The so-called alkaloid did not seem to be extracted completely by the solvent as ten extractions were made and the tenth extract still gave a positive test with Mayer's and Wagner's reagents. The combined ether-chloroform shakings were then washed several times with water to remove any last traces of ammonia or ammonium salts and then were shaken repeatedly with a one per cent hydrochloric acid solution. The hydrochloric acid apparently completely removed this substance as shown by negative tests with Mayer's reagent on the seventh extraction. The combined hydrochloric acid shakings, which were a deep red-orange color, were allowed to evaporate spontaneously to dryness. The residue amounted to 0.11 Gm. or 0.02 per cent of the drug.

The material was soluble in water, somewhat soluble in alcohol and insoluble in ether and chloroform. When examined under the microscope, the material appeared as red needle-shaped crystals. The crystals did not have a bitter taste. Attempts to recrystallize this material to free it from the red color were unsuccessful. When 0.05 Gm. of the material was run through the usual double extraction procedure for the assay of alkaloids, the residue from the final extraction gave no titration with 0.02*N* sulfuric acid. This shows the non-basicity of this material.

As this substance which gave precipitates with Mayer's and Wagner's reagents was not basic, could not be completely extracted with solvents from an ammoniacal solution, had no bitter taste and was physiologically inactive, it was concluded that it was not an alkaloid. It might have been an impure amino acid or some complex coloring principle.

## PHARMACOLOGY.

*Historical.*—Rafinesque (8) claims to have introduced the drug into Materia Medica. It was described as a medicinal in "Henry's Herbal," published in 1812. The earliest therapeutic use of the drug goes back to the time of the Indians, the Indian women using it after parturition (8). The early colonists probably learned of the virtues of the drug through the Indians and increased its uses. Williams (9) cites several cases in which he obtained great and decided benefits with the

drug in menorrhagia and hæmophtysis. He also cites cases in which it was beneficial in scrofulous eruptions. Hubbard (10) claims to have used the drug successfully in hemorrhages. He states: "From the experience I have had I should think the Trillium had a more decided effect in uterine hemorrhage of the passive kind than all other remedies." Du Kate (11) states that in large doses Trillium is emetic, expectorant, diuretic and laxative; in smaller doses it is astringent and possesses great power in controlling hemorrhages and morbid mucous secretions. He gives two cases in which the drug was used successfully in hemoptysis and hematuria.

Some of the many properties that have been proclaimed for the drug are: Tonic, alterative, emmenagogue, antiseptic, etc. It has also been used internally in cases of hectic fever, asthma, catarrhal coughs, diarrhoea and dysentery. Externally it has been used as a cure for carbuncles, ulcers, tumors, etc. It has also been used by the eclectics in conjunction with bugle weed (Lycopus Virginicus) for diabetes.

In spite of the many uses that have been attributed to Trillium there seems to be no satisfactory evidence in the literature that would justify its being used.

*Experimental.*—For the purpose of a preliminary pharmacological examination, three different lots of the whole drug, each weighing two pounds, were obtained from J. L. Hopkins and Co., New York. About 500 Gm. of a representative sample of the three lots were ground to a number twenty powder. A tincture was prepared from the powdered drug using a menstruum of three parts of alcohol and one part of water. Eight cubic centimeters of this tincture represented one Gm. of the drug.

An assay of this tincture was made on frogs using the mortality curve frog method of Chapman and Morrell (12). It was assayed in terms of the U. S. P. XI reference digitalis powder. By this method the reference digitalis powder was shown to be 16.6 times more toxic than the drug.

The alcohol from 200 cc. of the tincture was evaporated by blowing a current of air over it until the volume was 50 cc. This material was filtered and administered to an anesthetized cat through the femoral vein. One and one-half cc. of the solution, representing 0.75 Gm. of the drug, killed the cat, which weighed 1.86 Kg., within 5 minutes. Another cat, weighing 2.95 Kg., also received 1.5 cc. of this solution and died within 15 minutes.

Another 200-cc. portion of the tincture was evaporated, by means of a current air, to 50 cc. and then diluted with water to 200 cc. An anesthetized cat was given 0.1 cc. of this solution intravenously and a marked lowering of the carotid blood pressure resulted. When the blood pressure had returned to normal 20 cc. of the solution was given orally and 20 cc. rectally and no activity was observed after two hours. However, when additional doses of the solution were again injected intravenously into the same anesthetized cat, a marked lowering of the blood pressure again resulted and when 6.1 cc. had been administered, the cat died. The above experiments were repeated on another anesthetized cat with the same results and 5.7 cc. produced death.

Sixty cubic centimeters of the above solution equivalent to 7.5 Gm. of drug were given orally to a pregnant cat and no action whatever was evident.

The toxicity observed by the frog method and intravenous cat method as contrasted with the negative results obtained by oral administration of massive doses, indicated that the toxicity was produced by an anaphylactoid circulatory shock due to a saponin-like or protein-like substance.

A solution of the glucoside "Trillarin" was assayed by the frog method of Chapman and Morrell (12) and carried to the point where it was less than one one-thousandth as active as ouabain and still showed no action. It was thus concluded that this glucoside was physiologically inactive.

A one to one-thousand solution of the so-called alkaloid gave no action on frogs when injected in doses as high as 0.035 cc. per Gm. body weight of frog. It can be concluded from this result and from the fact that oral administration of massive doses of an aqueous extract of the drug produced no action, that this material was not alkaloidal in character and that the drug contains no active alkaloids.

## SUMMARY AND CONCLUSIONS.

A literature review of the chemical and physiological reports on Trillium has been presented.

Results obtained on moisture, ash and soluble extractives on two different lots of drug are tabulated, as well as the results obtained by the successive extraction of the drug with different solvents.

A fatty oil was obtained from each of two lots of drug. A description of these oils is given and their constants compared. The fatty oils consist chiefly of free fatty acids with some mono or diglycerides. The solid fatty acids consist chiefly of palmitic acid, with probably some myristic or lauric acids. The liquid fatty acids have not been examined but the iodine number would indicate other unsaturated acids than oleic acid. A mixture of volatile fatty acids was isolated, which seemed to range between valeric and caprylic acids.

Examination of the unsaponifiable matter disclosed the presence of a solid hydrocarbon, pentatriacontane $C_{35}H_{72}$, liquid hydrocarbons possibly ranging from undecane to hexadecane, volatile oil and a sterol probably phytosterol.

Sucrose was isolated and identified.

A glucoside, which has been given the name "Trillarin," was isolated and its properties recorded. From combustion analyses and a molecular weight determination, the formula $C_{37}H_{59}O_{14}$ was given to this compound. A survey of the literature has disclosed no compound of this formula.

The glucoside upon hydrolysis yields glucose and a genin which has been given the name "Trillarigenin."

The properties of "Trillarigenin" have been recorded and combustion analyses gave the formula $C_{25}H_{39}O_4$. A search of the literature has failed to show a compound of this formula.

The hydrolysis of "Trillarin" may be represented by the following equation:

$$C_{37}H_{59}O_{14} + 2\ H_2O \longrightarrow C_{25}H_{39}O_4 + 2\ C_6H_{12}O_6$$

This glucoside has proven to be physiologically inactive.

A small amount of material was present which gave precipitates with alkaloidal reagents. It was concluded that this material was not an alkaloid and that the positive tests with alkaloidal reagents were probably due to an amino acid or a coloring principle.

A saponin is present but was not isolated in a pure enough state to determine its composition.

A preliminary pharmacological examination has shown that oral administration of massive doses of a de-alcoholized tincture to a pregnant cat had no effect whatever. Both oral and rectal administration to anesthetized cats also had no effect.

Very small doses of the de-alcoholized tincture produced death when injected into frogs or intravenously to cats. It is believed, however, that death is produced in these cases by an anaphylactoid circulatory shock due to a saponin-like or a protein-like substance.

The usual plant constituents such as resin, starch, dextrin mucilage and oxalate are present. The presence of tannin was not established.

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# TOXICITY OF PROPYLENE GLYCOL.*

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## INTRODUCTION.

During the past several years suggestions have been made that propylene glycol is a suitable solvent for a variety of purposes, pharmaceutical and otherwise. As a result of this interest several papers (1, 2) have appeared in which attention was directed particularly to toxicological studies on this substance. In connection with another investigation involving the use of propylene glycol it was considered advisable to extend some of these studies for the purpose of completing our own data on this subject. Some of the results to be described confirm those of other authors; and some are believed to be new observations not previously described in the literature. The propylene glycol used in these studies was the alpha form (1, 2 propane diol) obtained from the Carbide and Carbon Chemicals Corporation.

Seidenfeld and Hanzlik (1) reported acute fatal doses for propylene glycol when given intramuscularly and intravenously to white rats and rabbits. They also investigated the effects in growing rats of the continued drinking of water containing various amounts of propylene glycol. The intramuscular fatal doses were found to be about 14 Gm. per Kg. body weight for rats, and about 7 Gm. per Kg. for rabbits. The intravenous fatal doses were found to be about 16 Gm. per Kg. for rats, and about 5 Gm. per Kg. for rabbits. Practically no effects were observed in rats from drinking water containing less than 10% propylene glycol; but with higher concentrations the fluid intake was greatly restricted, and the animals lived only a few days. No definite microscopic changes were observed in organs of the animals sacrificed at the end of the experimental period of 140 days. It is interesting to note that these authors found the intravenous fatal dose for rats to be greater than the intramuscular fatal dose, a relationship rarely observed. This is particularly significant since the intravenous fatal dose for another rodent (rabbit) was found to be considerably less than the intramuscular fatal dose, which is the usual relationship.

Braun and Cartland (2) investigated the acute toxicity of propylene glycol for rats by subcutaneous, intramuscular and intravenous injections, and obtained results which were in satisfactory agreement with those reported by Seidenfeld and

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