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A CHEMICAL STUDY OF THE FIXED OIL OF POKE ROOT.*¹BY SAMUEL W. GOLDSTEIN² AND GLENN L. JENKINS.

The root of *Phytolacca americana* (*decandra*) Linné (poke) has been an official drug since 1820 but no report has been published on the constituents of the fatty oil which this root contains. The present work was undertaken in the course of a complete phytochemical study of poke root.

The root used in this work was supplied by S. B. Penick and Sons and was collected near Asheville, North Carolina.

EXPERIMENTAL.

Twenty-two kilograms of finely powdered poke root were extracted with petroleum benzin (b. p. 30° to 60° C.) in a Lloyd extractor until the drug was exhausted. Most of the benzin was removed by distillation under reduced pressure, and a current of air was passed over the residue to remove the remaining solvent. The residue was dissolved in hot alcohol and the mixture, on standing over night, formed a deposit and a clear supernatant liquid which was removed.

Isolation of a sterol-like Compound, C₂₃H₄₀O.—Small portions of the solid substance gave color reactions with the Liebermann-Burchard (1) and Hager-Salkowski (2) reagents for sterols. In the latter case, however, the red color produced was more pronounced in the sulphuric acid layer than in the chloroform layer. A chloroformic solution of the substance decolorized a bromine solution. The substance was readily soluble in hot ethyl acetate and hot ethyl alcohol, and was deposited from the solutions while still warm, but the coloring matter present behaved in a similar manner. A solution of the substance in hot ethyl acetate was prepared and was boiled with animal charcoal for several minutes, then filtered, while hot, through a Gooch funnel by means of a hydraulic suction pump. On cooling, crystals formed in rosettes. These crystals were removed by filtration and washed with ethyl acetate, then they were dried in an oven for thirty minutes at 95° C. The dried crystals melted at 107–108° C. Tests for nitrogen and sulphur gave negative results.

0.003964 Gm. gave 0.012062 Gm. CO₂ and 0.004326 Gm. H₂O

C = 82.99; H = 12.21 per cent

C₂₃H₄₀O requires C = 83.05; H = 12.13 per cent

0.0280 Gm. dissolved in 14 cc. of chloroform gave $\alpha_D^{26} = +0.14^\circ$ in a 100-mm. tube.

$[\alpha]_D^{26} = +70.0^\circ$.

Two portions of the substance were refluxed for two hours with acetic anhydride and acetyl chloride, respectively. The crystals obtained on cooling in both cases were separated and re-crystallized. The melting points of 107–108° C. indicated that no reaction had occurred and this was confirmed by running mixed melting points of the original substance with the products obtained by the above treatment. The oxygen present in the molecule apparently is not present in the form of a free hydroxyl group.

The alcoholic solution obtained from the petroleum benzin extractive was freed from alcohol by evaporation and the final traces of the solvent were removed by heating the residual oil on a boiling water-bath under reduced pressure. The oil obtained weighed 96.41 Gm., representing 0.44 per cent of the dried root used. The following constants for the oil were determined: Specific gravity_{25°} 0.9209; optical rotation $[\alpha]_D^{26} = +13^\circ$; refractive index $N_D^{26} = 1.4741$; acid number 71.97; saponification number 139.43; ester number 67.46; iodine number 69.14.

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² Abstracted in part from a thesis submitted by Samuel W. Goldstein to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Isolation of Free Fatty Acids.—Fifty-five grams of the oil were dissolved in 1200 cc. of ether and the solution was successively extracted with portions of 5 per cent solutions of ammonium carbonate, sodium carbonate and potassium hydroxide.

The ammonium carbonate shakings were acidified with diluted hydrochloric acid, and the mixture was extracted with ether. The ethereal solution was dried with anhydrous sodium sulphate and then the ether was removed by distillation. The oily residue was dissolved in alcohol and the silver salt was prepared using silver nitrate solution. The silver salt was analyzed.

0.474 Gm. of salt gave on ignition 0.0201 Gm. of Ag

Ag = 42.4 per cent

$C_8H_{16}O_2Ag$ requires Ag = 43.0 per cent.

Although the figures thus obtained are in fairly close agreement with those required for the silver salt of an octanoic acid, it is probable that the oily acid substance was a mixture.

The sodium carbonate shakings were obtained in two portions and these were examined separately.

Portion I. The reddish brown aqueous solution was treated with animal charcoal, and, after cooling, was acidified with diluted sulphuric acid and extracted with ether. The ethereal solution was dried and the ether was removed by distillation. The orange-colored residue was dissolved in alcohol and the alcoholic solution was kept at 10° C. The crystals which were deposited in the alcoholic solution were separated by filtering through a Gooch funnel with suction. The crystalline solid (1.75 Gm.) was dissolved in alcohol and recrystallized. After drying in a desiccator, the melting point was 72–73° C. Recrystallization from methyl alcohol and then from glacial acetic acid raised the melting point to 76–77° C. The silver salt was prepared and analyzed.

0.0265 Gm. of salt gave on ignition 0.0063 Gm. of Ag

Ag = 23.77 per cent

$C_{20}H_{39}O_2Ag$ requires Ag = 25.67 per cent.

The above-described substance was thus identified as arachidic acid.

The alcoholic mother-liquor from which the arachidic acid had been obtained was concentrated and kept at 5° C. over night. A small amount (0.55 Gm.) of crystalline matter was deposited and this was removed and recrystallized from alcohol. The crystals melted at 61° C. The silver salt was prepared and analyzed.

0.1229 Gm. of salt gave on ignition 0.0361 Gm. of Ag

Ag = 29.37 per cent

$C_{18}H_{35}O_2Ag$ requires Ag = 29.7 per cent.

The crystals were thus identified as palmitic acid.

The alcoholic mother-liquor from which the palmitic acid had been obtained was further concentrated and kept at 10° C. A solid separated in small spherical bundles of crystals. The solid (0.4 Gm.) was removed and recrystallized from alcohol. The crystals melted at 51° C. The silver salt was prepared and analyzed.

0.1371 Gm. of salt gave on ignition 0.0405 Gm. of Ag

Ag = 29.54 per cent

$C_{14}H_{27}O_2Ag$ requires Ag = 30.72 per cent.

The crystals were thus identified as oxymyristic acid.

The alcoholic mother-liquor from which the oxymyristic acid had been obtained was kept at 5° C., when crystals were deposited. The crystals (0.5 Gm.) were removed and, after recrystallization from alcohol, melted at 59–60° C. The silver salt was prepared and analyzed.

0.0632 Gm. of salt gave on ignition 0.0183 Gm. of Ag

Ag = 28.95 per cent

$C_{17}H_{33}O_2Ag$ requires Ag = 28.6 per cent.

The crystals were thus identified as margaric acid.

Two more fractions (0.5 Gm. and 0.8 Gm.) of crystalline matter were obtained from the alcoholic mother-liquor, and, although the melting points of the crystals were 53–56° and 46–48° C., respectively, the analyses of the silver salts corresponded fairly well with the required results of margaric acid. Apparently some other substance had been removed along with the margaric acid to cause a lowering of the melting point.

The residual alcoholic mother-liquor decolorized a chloroformic solution of iodine. The alcohol was removed and the liquid residue was kept for a short time at 10° C., when it solidified. A silver salt was prepared and analyzed.

0.1115 Gm. of salt gave on ignition 0.0279 Gm. of Ag
 Ag = 25.02 per cent
 $C_{18}H_{33}O_2Ag$ requires Ag = 27.72 per cent.

The above-described residue probably consisted of oleic acid and some saturated acid or acids which had not been removed by fractionation.

Portion II. The second portion of the sodium carbonate shakings was treated as under Portion I, and the alcoholic solution obtained was kept at 10° C. The crystals (0.5 Gm.) which were deposited were separated by filtration, and, after recrystallization from glacial acetic acid, they melted at 72–73° C. The silver salt was prepared and analyzed.

0.0155 Gm. of salt gave on ignition 0.0037 Gm. of Ag
 Ag = 23.87 per cent
 $C_{20}H_{39}O_2Ag$ requires Ag = 25.67 per cent.

The crystals were thus identified as arachidic acid.

The alcoholic mother-liquor was concentrated and kept at 10° C. The crystals (1.1 Gm.) which were deposited were removed, and, after recrystallization from glacial acetic acid, melted at 58.5° C. The silver salt was prepared and analyzed.

0.0810 Gm. of the salt gave on ignition 0.0239 Gm. of Ag
 Ag = 29.5 per cent
 $C_{16}H_{31}O_2Ag$ requires Ag = 29.7 per cent.

The crystals were thus identified as palmitic acid.

The potassium hydroxide shakings from the ethereal solution of the oil had a red color which changed to yellow on acidification with diluted sulphuric acid. The amount of the substance liberated by the sulphuric acid was too small to investigate.

Saponification of Oil.—The ethereal solution of the oil which had been shaken with the alkalis was washed free of alkali by shaking with water, then dried with anhydrous sodium sulphate, and the ether was removed. The oily residue (33.2 Gm.) was very limpid as compared to the original oil. The oil was saponified according to A. Boemer's method (3). After saponification and addition of 200 cc. of water, the liquid was allowed to cool to room temperature and then was extracted repeatedly with ether. The ethereal extractions were thoroughly washed with water and the ether was removed, leaving a reddish brown solid. The residue was dissolved in hot 98 per cent alcohol, leaving a very small amount of an amber-colored oily substance which did not dissolve.

The substance which did not dissolve in hot alcohol was soluble in hot acetone and cold chloroform. It gave negative Hager-Salkowski and Liebermann-Burchard tests for sterols. The substance may possibly have been a hydrocarbon of low molecular weight.

Isolation of Hentriacontane.—The hot alcohol-soluble portion (300 cc. of solution) of the residue obtained from the ethereal shakings, when allowed to cool and stand over night, deposited a small amount of solid matter. The solid (0.5 Gm.) was separated and, after recrystallization from ethyl acetate, melted at 67.4° C. Using an ethyl acetate solution of the substance, faintly positive tests for sterol were observed. The crystals were not affected by concentrated sulphuric acid, and they did not react with acetic anhydride. After recrystallization from chloroform and then from alcohol, the melting point was found to remain at 67.4° C.

The above-described substance, from its manner of isolation, chemical inactivity and melting point, was identified as hentriacontane.

Isolation of a Sterol (Phytolaccasterol) C₃₀H₅₀O.H₂O.—The alcoholic solution from which the hentriacontane had been obtained was concentrated to one-half its volume (150 cc.) and allowed to stand. Crystals, in the form of platelets, formed in a few hours. The crystals (1.0 Gm.) were removed and recrystallized from a mixture of ethyl acetate and alcohol (1:1), and then from ether. The melting point was 168–169° C. Further recrystallization from ether raised the melting point to 169–170° C. A chloroformic solution of the substance decolorized a bromine solution. Color reactions were observed with the Liebermann-Burchard and Hager-Salkowski reagents for sterols, but in the latter case the sulphuric acid layer exhibited a deeper red than the chloroform layer.

0.004191 Gm. gave 0.012413 Gm. CO₂ and 0.004467 Gm. H₂O

C = 80.78; H = 11.93 per cent

C₃₀H₅₂O₂ requires C = 81.00; H = 11.79 per cent

0.0710 Gm. after heating for 4 hours at 106° C. lost 0.0029 Gm. of H₂O. H₂O = 4.08 per cent

1 molecule of H₂O of hydration = 4.05 per cent

C₃₀H₅₂O₂ = C₃₀H₅₀O.H₂O

0.0303 Gm. dissolved in 15 cc. of chloroform gave $\alpha_D^{26} = +0.07^\circ$ in a 100-mm. tube
 $[\alpha]_D^{26} = +35.0^\circ$.

The compound was boiled with acetic anhydride for 3 hours. On cooling, platelets with a mother of pearl lustre were formed. The crystals were separated from the solution by filtration and after recrystallization from alcohol and then from ether the melting point remained at 183–183.5° C. The acetyl derivative was recrystallized again from petroleum benzine and only perfectly clear platelets were used to determine the melting point which was 183–183.5° C.

0.0518 Gm. reacted with 1.13 cc. of 0.1N KOH

Gram molecular weight = 458.4 Gm.

0.004008 Gm. gave 0.012039 Gm. CO₂ and 0.004326 Gm. H₂O

C = 81.92; H = 11.33 per cent

C₃₂H₅₂O₂ requires C = 81.97; H = 11.19 per cent

Gram molecular weight = 468.4 Gm.

The original compound recovered from the saponification mixture and recrystallized from alcohol melted at 169–170° C.

The compound C₃₀H₅₀O described above was proved to be a monohydroxy sterol isomeric with the amyryns (m. p. 170° C.) isolated by Tschirch and Cremer (4) from the different sorts of clemis. Vesterberg (5) resolved the amyryns into alpha-amyryn (m. p. 181–181.5° C.) and beta-amyryn (m. p. 193–194° C.). He also prepared the acetyl derivative of the unresolved amyryn and found it to melt at 200° C., and on crystallizing from ligroin solution obtained two crystalline forms, *i. e.*, leaflets and prisms, which he identified as alpha-amyryl acetate (m. p. 220° C.) and beta-amyryl acetate (m. p. 235° C.). Attempts to resolve the acetate of the compound isolated by us into more than one compound were unsuccessful, and the different melting point obtained with clear leaflets from benzine solution indicates that the compound differs from the previously reported isomers.

The mother-liquor from which the sterol had been obtained, on further concentration and standing, yielded another small crop (0.1 Gm.) of the same compound.

Isolation of Combined Fatty Acids.—The alkaline, aqueous solution which had been extracted with ether was acidified with diluted sulphuric acid. The solution was found to contain formic acid by testing with ammoniacal silver nitrate solution. The solution was then extracted repeatedly with ether. The aqueous solution remaining after the ether extractions was neutralized and evaporated to dryness on a water bath. The residue, after extraction with alcohol-ether and removal of the solvent, gave a faintly positive test for the presence of glycerin. The ethereal solution of the liberated fatty acids was dried and the solvent was removed. The residue, on cooling, solidified to a dark reddish brown mass. The solid (1.0 Gm.) that separated was removed, and after recrystallization from alcohol, ethyl acetate and finally from a mixture of ethyl acetate and alcohol, the granular product melted at 75–76° C. The silver salt was prepared and analyzed.

0.0652 Gm. of salt gave on ignition 0.0169 Gm. of Ag
 Ag = 25.6 per cent
 $C_{20}H_{38}O_2Ag$ requires Ag = 25.67 per cent.

The substance was thus identified as arachidic acid.

The mother-liquor was concentrated and kept at 10° C. The solid (0.5 Gm.) which separated was removed by filtration. The solid appeared to have adsorbed some amber-colored oily substance which caused part of the mixture to liquefy when the mixture was heated to about 27° C. The solid was dissolved in alcohol and precipitated as the colorless silver salt, which was analyzed. The colored portion remained in the alcohol.

0.0614 Gm. of salt gave on ignition 0.0173 Gm. of Ag
 Ag = 28.17 per cent
 $C_{17}H_{32}O_2Ag$ requires Ag = 28.6 per cent.

The substance was very probably margaric acid with some impurity which appeared to be some of the original oil that had not been saponified.

The mother-liquor was again concentrated, and, when no solid separated on cooling, the remainder of the solvent was removed. A mixture of solid particles in oil was obtained. An attempt to separate the oil from the acids by forming the sodium salts of the free acids and removing the oil by extracting with ethereal solvents was unsuccessful. The acids were again liberated with diluted sulphuric acid and the mixture was extracted with chloroform. The chloroform was removed, leaving 13.5 Gm. of a reddish brown semi-solid residue. The residue was refluxed on a water bath with 28 cc. of methyl alcohol and 0.7 cc. of concentrated sulphuric acid for 5 hours, and then the excess methyl alcohol was removed. The residue was taken up in ether, the ethereal solution was washed with water to remove the sulphuric acid, then the ethereal solution was dried and the ether removed. The remaining liquid methyl esters were distilled under a pressure of 10 mm. of mercury. A negligible amount of distillate came over between 173° and 189° C.; about 5 cc. of distillate came over at 190° C.; and a small amount of distillate came over between 191° and 215° C. The latter distillate, which was collected separately, was discarded. The distillate which had been collected between 173° and 190° C. was refluxed on a water bath with 10 per cent sodium hydroxide in 70 per cent alcohol for 2 hours. The saponified solution was shaken with ether to remove any unsaponified oil and liberated substances other than the acids. The aqueous solution was then acidified with diluted sulphuric acid and was extracted repeatedly with ether. The ethereal solution was washed with water, dried with anhydrous sodium sulphate, the solvent removed and the solid residue was taken up in warm alcohol. The first crop of crystals, weighing 0.1 Gm., obtained at room temperature melted at 58.5° C. The silver salt was prepared and analyzed.

0.0413 Gm. of salt gave on ignition 0.0124 Gm. of Ag
 Ag = 30.0 per cent
 $C_{18}H_{34}O_2Ag$ requires Ag = 29.7 per cent.

The compound was very likely palmitic acid with a small amount of impurity.

The mother-liquor was concentrated and kept at 10° C. The solid (2.0 Gm.) which separated was removed, and after recrystallization from alcohol melted at 62–62.5° C. The silver salt was prepared and analyzed.

0.1010 Gm. of salt gave on ignition 0.0308 Gm. of Ag
 Ag = 30.0 per cent

$C_{16}H_{32}O_2Ag$ requires Ag = 29.7 per cent

The acid melts at 62–62.5° C.

$C_{16}H_{32}O_2$ melts at 62.6° C.

The methyl ester was collected at 190° C. (10 mm.)

$C_{16}H_{31}O_2CH_3$ boils at 196° C. (15 mm.)

0.0596 Gm. of the acid required 2.34 cc. of 0.1*N* KOH; corresponding to 220.3 mg. of KOH per Gm. of acid

$C_{16}H_{32}O_2$ has a neutralization value of 219.1 mg. of KOH per Gm. of acid

Molecular weight determined from the acid value is 253.9

$C_{16}H_{32}O_2 = 256.34$.

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

The mother-liquor was concentrated and kept at 10° C. A crop of crystals (1.0 Gm.) was obtained, which, after recrystallization from ethyl acetate, melted at 61–62° C. This, undoubtedly, was a further yield of palmitic acid.

The mother-liquor was found to react readily with bromine. The alcohol was removed, leaving a residue which was liquid at room temperature and which solidified when kept at 10° C. The liquefied substance gave a positive elaidin reaction when treated with concentrated nitric acid and copper wire. The silver salt was prepared and analyzed.

0.1756 Gm. of salt gave on ignition 0.0503 Gm. of Ag

Ag = 28.6 per cent

$C_{18}H_{33}O_2Ag$ requires Ag = 27.7 per cent.

Methyl oleate distils at about the same temperature as methyl palmitate.

The above-described residue probably consisted mainly of oleic acid together with some acid or acids which had not been removed by fractionation.

SUMMARY AND CONCLUSIONS.

1. The fatty oil obtained by extracting dried poke root with petroleum benzin was found to have the following constants: Specific gravity_{25°} = 0.9209; optical rotation, $[\alpha]_D^{26°} = +13°$; refractive index, $N_D^{26°} = 1.4741$; acid number, 71.97; saponification number, 139.43; ester number, 67.46; iodine number, 69.14.

2. Chemical investigation of the fatty oil proved it to be a complex mixture containing a large proportion of free fatty acids, some esters of fatty acids with glycerol, and wax-like esters of fatty acids with a sterol.

(a) A sterol-like compound, $C_{23}H_{40}O$, melting at 107–108° C.; and having an optical activity of $[\alpha]_D^{26°} = +70.0°$, was isolated. The oxygen present in the molecule does not behave as a part of a free hydroxyl group, since boiling with acetic anhydride and with acetyl chloride had no effect on the compound.

(b) A sterol (phytolaccasterol), $C_{30}H_{50}O \cdot H_2O$, melting at 169–170° C., and having an optical activity of $[\alpha]_D^{26°} = +35.0°$, was isolated. It is isomeric with the amyriins isolated by Tschirch and Cremer (4), but the differences in the behavior of the acetates prove them to be dissimilar. Phytolaccasterol was proved to be a monohydroxy compound by the saponification of its acetate, from which procedure a molecular weight of 458.4 was calculated as compared with 468.4 as determined by analysis.

(c) A hydrocarbon, hentriacontane ($C_{31}H_{64}$), was isolated and identified by its manner of separation and by its inactivity with chemical reagents, and its melt ing point of 67.4° C.

(d) The following fatty acids were isolated: Arachidic, 5.91 per cent; palmitic, 8.63 per cent; margaric, 4.19 per cent; oxymyristic, 0.72 per cent. The presence of oleic acid, acids of low molecular weight and glycerol was proved.

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