SOME CONSTITUENTS OF *VIBURNUM OPULUS*.

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The dried bark of *Viburnum opulus*, known also as cramp bark, was official in U. S. Pharmacopoeia VIII, along with *Viburnum prunifolium* (black haw). Of these the former does not appear in the latest revision but has been relegated along with its fluidextract to the National Formulary.

The bark is used in large doses (2.0–4.0 grams) in considerable quantities in pharmaceutical work, particularly for the preparation of several elixirs. A number of preparations in tablet form are prepared, but these probably lack part of the valerianic acid of the drug. This drug is used medicinally as an antispasmodic or nervine, especially in menorrhagia, and despite some adverse comments upon its use, the drug is a component of some preparations on the market, and physicians continue to prescribe galenicals of *Viburnum opulus*, apparently with desired results.

Sumbul root remains official, although so far as chemical investigation assists our understanding of the therapeutics, it is akin to Viburnum.

Our present information concerning the drug may be summarized as follows: Chevreul[1] found valerianic acid, and this was confirmed by L. von Moro.[2] Krämer[3] reported the presence of tannic acid, a bitter principle (viburnin), brown resin acid, chlorophyll, wax, gum, pectin, potassium malate, calcium malate, calcium sulphate, iron oxide and magnesia.

Our material was obtained from Northern Minnesota through the regular channels, and conformed to the U. S. P. standards. The bark was in pieces 1–5 cm. long, slightly transversely curved, 4–15 mm. wide, and 1 mm. thick. The periderm was young and dark gray in color. Lenticles were few and transverse. The phellogen cells were reddish brown. The inner surface was green, turning to reddish brown in older pieces. The older periderm was flaky with grayish lichen patches common. The fracture was uneven.

In this investigation the dried drug was exhausted with wood alcohol, and the concentrated extract poured into an excess of distilled water. The resin that separated yielded the following substances by fractional extraction. Acetic, valerianic, caproic, caprylic, formic, oleic, linoleic, cerotic and palmitic acids were found in the ligroin extract. From the unsaponifiable material traces of a paraffin (65–70°) and myricyl alcohol that melted at 77°, were obtained.

The ether extract of the resin yielded a quantity of a phytosterolin, $C_{33}H_{56}O_6$, melting at 265–275°, and forming an acetate which melted at 167–168°. A phytosterol agreeing in composition with the formula $C_{27}H_{46}O.H_2O$ and melting at 138° was present. The corresponding acetate melts at 121–123° and has $[\alpha]_D = -37.8°$.

The chloroform and ethyl acetate extracts were uncrystallizable, while the alcoholic extract contained an acidic resin, readily yielding acetic and valerianic acids on saponification.

The drug extractives soluble in the water solution from which the resin had separated, contained acetic and valerianic acids, tannin, sucrose, glucose and an

[1] *Ann. Chim.*, (2) 23, 22, 1817.

[2] *Ann. Chem.*, 55, 330, 1845.

[3] *Arch. Pharm.*, 90, 269, 1844.

ester-like resin yielding acetic and valerianic acids on hydrolysis. The lead subacetate precipitate yielded a crystalline acid, melting at 200–202° ($C_{19}H_{18}O_9$). The biological method of Bourquelot gave indications of the presence of the glucoside in small amount, but it was not isolated.

An alkaloidal assay by the process which is official for Hyoscyamus gave negative results.

EXPERIMENTAL.

A. Proximate Analysis (Mr. Gail Arner).—Quantitative extractions of the air-dried bark with various solvents gave the following results:

Extract.	Percent.
Ligroin (35–55°)	4.0
Ether (110°) (subsequent to above)	1.7
Alcohol absolute	26.87

The proximate analysis resulted as follows:

	Percent.		Percent.
Moisture	8.75	Protein	3.95
Starch (diastase)	7.2, 7.0	Ash	6.95
Pentosans	8.3	Dextrin	0.6
Crude fiber	13.5	Tannin	0.8

Quantitative estimations on the alcohol-soluble carbohydrates were made as follows:

100 Gm. of the finely ground drug were exhausted by repeated extraction with boiling neutral alcohol. The extracts were concentrated to a syrup under diminished pressure and the residue taken up in water. This was precipitated with a slight excess of basic lead acetate solution and the solution made to a volume of 500 cc. An aliquot of this was freed from lead with sodium sulphate and the filtrate was concentrated under reduced pressure to volume of 150 cc (2 cc = 1 Gm. drug).

Of this solution 5 cc (2.5 Gm.) were directly examined by the Walker-Munson process: Cu_2O = 0.0812 Gm. = 0.035 Gm. reducing sugar calculated as glucose. This amounts to 1.4% glucose.

Upon allowing an aliquot to stand over night in the presence of hydrochloric acid a portion equivalent to 1.25 Gm. drug gave 0.1205 Gm. Cu_2O. The percent of sucrose is therefore 2.77.

The polariscopic readings were unsatisfactory, on account of the dark-colored solutions.

Another 50-Gm. sample was first ether-extracted, and then the sugars were extracted with alcohol. The alcohol was removed, and the residue taken up with water and treated with lead subacetate solution in the usual manner; the volume was 250 cc, the direct reading in a 2-dcm. tube was +0.9°. However, upon removing the lead by the addition of a very slight excess of sodium carbonate a solution of the same concentration gave +2.2° at 21° in a 2-dcm. tube.

15 cc (3 Gm.) gave 0.1075 Gm. Cu_2O = 0.0466 Gm. reducing sugar, calculated as glucose = 1.56%.

The lead-free solution was inverted: 100 cc + 5 cc 38% HCl (19 Gm. drug in 100 cc). The reading was +0.2° at 21° in a 2-dcm. tube. At 86° this solution gave an unsatisfactory reading of −0.36°.

10 cc (1.9 Gm. drug) gave 0.1867 Gm. Cu_2O, sucrose = 2.65%.

Calculating the rotations observed to those for a normal solution (26 Gm. in 100 cc) we have, at 21° in a 2-dcm. tube: direct, +2.86°; invert, +0.27°; invert at 86°, −0.5°. By Clerget's formula we calculate, sucrose = 1.95%. The absence of levulose is indicated. Dextrose = 1.2%.

B. Complete Examination of Alcoholic Extract.—For this purpose 34 Kg. were exhausted by percolation with cold wood alcohol. The percolate (410 liters)

was concentrated under diminished pressure to a volume of 8 liters, which was then poured into 72 liters of distilled water. A dark green resin separated. The precipitated resin was removed by filtration and washed with 8 liters of water. The filtrate and washings which were of a dark red color were joined and concentrated under diminished pressure to about 10 liters.

The resin that separated was taken up in alcohol, and the solution concentrated and allowed to stand but there was no evidence of crystallization. The weight of the resin thus precipitated was 1229 Gm., equivalent to 3.6% of the drug. An aliquot (929 Gm.) was poured upon sawdust and dried.

The Examination of the Water-Soluble Constituents.—This extract as stated above was concentrated to a volume of about 10 liters. The distillate was acid. It was rendered alkaline and evaporated on the water-bath to a small volume. It was then again acidified and steam-distilled. The steam distillate was extracted with ether, but most of the acid remained in the aqueous layer. It was neutralized with ammonia and by the addition of successive portions of a 20% silver nitrate solution, several crops of nearly pure silver acetate were separated.

Calculation for $C_2H_3O_2Ag$: Ag = 64.6. Found: 63.8, 64.1, 63.7%.

The ether-soluble volatile acids were joined to the similar fraction obtained when examining the fat.

The concentrated aqueous extract was further reduced in volume and divided into two equal aliquots each of which was exhaustively extracted with ether, which, however, removed only indefinite smeary material. The sodium hydroxide extraction of the ether had a beautiful cherry-red color which turned yellow when acidified. Traces of valerianic acid were present, and sulphur crystals were obtained without difficulty.

One-half of the aqueous extract (17 Kg.) was completely precipitated with an excess of basic lead acetate (1500 cc). The light chocolate-colored precipitate was removed, washed, and decomposed with hydrogen sulphide. The filtrate from the lead sulphide was concentrated to a volume of about 500 cc (255 Gm. solids) and allowed to stand in a desiccator, but nothing crystallized.

The solution contained tannin, as it gave a heavy precipitate with gelatin solution, and a dirty green precipitate with ferric chloride solution. A quantity of the solution equal to 28.9 Gm. plant contained 0.2207 Gm. tannin when examined by the hide powder process (0.8% of drug).

Acid hydrolysis of the material gave a mixture of volatile acids (b. p. 110–225°). The material is not glucosidic. Alkaline hydrolysis with 10% alkali for five minutes gave a trace of volatile acids. The hydrolysis mixture was poured into a slight excess of dilute sulphuric acid, and the mixture was cooled and extracted with large volumes of ether. The ether was washed with water and concentrated to a small volume when a brown powder insoluble in absolute ether separated. In two hydrolyses 0.75 and 0.6 Gm., respectively, were obtained from 64 Gm. of the material. It dissolved in ammonium carbonate solution with effervescence and was reprecipitated upon acidification. After repeated recrystallization from boiling water and decolorization with charcoal it was obtained in magnificent dense rhombic plates. It decomposed with effervescence at 199–202° or higher (206°) depending upon the rate of heating. This material is a complex phenolic acid and is at least dibasic.

0.040 Gm. required 3.9 cc $N/20$ alkali for neutralization using methyl red.
0.0689 Gm. required 7.2 cc; 0.0618 required 6.2 cc $N/20$ alkali.
Calculated for 2 NaOH: mol. wt. = 390. Found: 410, 382 (398).

In order to neutralize the phenolphthalein 5.6 cc $N/20$ alkali were required for the first titration. For analysis the samples were dried at 110°.

Calculation for $C_{19}H_{18}O_9$ (390): C = 58.5; H = 4.6. Found: C = 57.9, 58.2; H = 4.4, 4.4.

The solution of this gave a beautiful green coloration with ferric chloride solution. A bluish black precipitate resulted when sodium bicarbonate was added.

The filtrate from the basic lead acetate precipitate was freed from the excess of lead and concentrated to a syrup that measured 1900 cc. A portion (500 cc) was put aside in a desiccator but nothing crystallized after prolonged standing. It was then exhausted with alcohol, but from neither 95% nor absolute alcohol could any crystallization be brought about. The alcoholic solution was dried upon purified sawdust, and several extractions with ethyl acetate were made. A subsequent extraction with absolute alcohol gave crystals of sucrose (2 Gm.), melting at 182–184° and having $[\alpha]_D = 66.5°$. With phosphotungstic acid a practically negative test was obtained. On standing over night some slight precipitation resulted, but the basic fraction is negligible. The total nitrogen in this fraction is 1.61 Gm. The solution gave negative tests with Mayer's reagent and also with mercuric nitrate.

100 cc syrup gave a phosphotungstic acid precipitation containing 0.004 Gm. N.
100 cc of the syrup (895 Gm. drug) gave 0.085 Gm. N.
The chief constituent here is sugar: 25 cc syrup (223.7 Gm. drug) containing 16.67 Gm. solids of which 0.34 Gm. is ash, were diluted to 100 cc. Direct rotation at 19° in 2-dcm. tube = +7.4°. Of this solution 2.5 cc (0.625 cc syrup) gave 0.1732 Gm. Cu_2O = 0.0765 Gm. glucose.
The solution was inverted, 20 cc + 5 cc 40% HCl being diluted to 100 cc. Reading in 2-dcm. tube is +0.5°, and 10 cc gave 0.3556 Gm. Cu_2O = 4.75 Gm. cane sugar.
By calculation we have in 25 cc syrup 3.15 Gm. glucose and 4.75 Gm. cane sugar; this leaves unaccounted for 8.4 Gm.
A 25-cc portion gave 6.0 Gm. *d*-phenylglucozone. This corresponds to approximately 3.0 Gm. glucose. After recrystallization it melted at 204°. Pentose sugar was present in small amount (0.3 Gm. in 25 cc.).
5 cc syrup gave 0.0543 phloroglucid.

A portion of the syrup was examined by the biological method of Bourquelot for the presence of glucosides.

Examination of Syrup by Bourquelot's Biochemical Method.[1]—The plant contains only sucrose (1.9%) as the hydrolysis with the invertase showed no interfering sugars. The invert sugar amounts to 1.5% and a substance is present which is very slowly hydrolyzed by emulsin, at least 0.228 Gm. glucose being liberated per 100 Gm. viburnum while the rotation changes from −6.46° to −3.4° V. or +3.06° V. Duplicate gave +2.51° V. This would appear to indicate the presence of about 0.5% of a β-glucoside.

All the following work was accompanied by proper controls.

(*a*) *Direct.*—28 cc syrup (= 250 Gm. viburnum) + 6.25 cc 0.5 N sodium phosphate solution + 12 cc 0.5 N hydrochloric acid + thymol water q. s. to 250 cc (solution is .001 N acid). Rotation in 2-dcm. tube at 22° = +3.39° V. Walker-Munson process: 5 cc = 0.1620 Gm. Cu_2O = 0.0739 Gm. invert sugar = 1.48% of the drug.

[1] *Archiv. d. Pharm.*, 245, 172, 1907; "Handbuch d. Biochem. Arbeit," VII, 766.

(*b*) *Action of Invertase.*—200 cc of above solution + 0.25 Gm. active invertase. After standing several days readings were obtained at 24-hour intervals in 2-dcm. tube at 22.5° equal to −6.46° V. 5 cc gave 0.3821 Gm. Cu_2O. Sucrose: by reduction = 1.93%; by Clerget's formula = 1.93%.

(*c*) *Action of Emulsin.*—100 cc of above solution were plunged into a boiling water-bath for 10–15 minutes to inactivate the invertase. The solution was cooled to room temperature and 0.5 Gm. active emulsin added. After 3 days the rotation was −4.09° V. and 5 cc gave 0.3863 Gm. Cu_2O. Another 0.1 Gm. of emulsin was added and on the fifth day the rotation was −4.08° V. and 5 cc gave 0.3884 Gm. Cu_2O. Emulsin set free 0.055 Gm. glucose for 100 Gm. viburnum.

These solutions were permitted to stand. On the 9th day the rotation was −3.93° V. Reduction: 5 cc = 0.3971 Gm. Cu_2O. On the 16th day the rotation was −3.41° and on the 21st day duplicate reductions gave 5 cc = 0.4069, and 0.4052 Gm. Cu_2O. Emulsin therefore set free 0.20 Gm. per 100 Gm. viburnum.

In another experiment the rotation was more rapidly changed to the right by emulsin. The amount of glucose set free by emulsin was 0.228 Gm. per 100 Gm. viburnum.

Direct Reading = 3.31° V.; *invert reading* = −5.55° V.; *emulsin reading* = −3.04° V. (2 days). The reductions were 0.1675 Gm. Cu_2O; 0.3641 Gm. Cu_2O; and 0.3913 Gm. Cu_2O, respectively.

Acid Inversion.—As the hydrolysis by emulsin did not appear complete even after long standing an acid hydrolysis was made and a very considerably increased yield of reducing substance was obtained, likewise the rotation change was much more positive.

28 cc syrup + 6.25 cc 0.5 *N* sodium phosphate solution + 12 cc 0.5 *N* hydrochloric acid + thymol water 190 cc + 15 cc concentrated hydrochloric acid; stood over night; made up to 250 cc as before. The rotation was constant for days at + 1.3° V. Reduction: 5 cc = 0.4293 Gm. Cu_2O. This value was constant.

Part of the syrup (500 cc) was fermented with yeast. The solution was clarified with lead subacetate and the excess of lead removed with hydrogen sulphide. The sugar-free resinous residue was not crystalline.

A quantity (86 Gm.) was slightly acidified, and steam-distilled. The distillate required 38 cc *N* alkali. The acids were valerianic and acetic. (Ag = 49.0, 50.0, 56.6, 64.0, 63.6.) The solution was then concentrated to 100 cc and hydrolyzed in the presence of 10% sodium hydroxide. It was acidified and again distilled. The distillate required 23.9 cc *N* alkali. (Ag = 49.25, 50.0, 63.0, 64.1.) The volatile acids amount to about 4 or 5% of the ester-like resin which is very similar to the resin in *Viburnum prunifolium.*

Examination of the Resin.—The resin (929 Gm.) after thorough drying upon purified sawdust was transferred to a continuous extractor and extracted with the following results:

Petroleum ether (40–60°)	365 Gm.
Ether	160 Gm.
Chloroform	23 Gm.
Ethyl acetate	95 Gm.
Alcohol	128 Gm.
Total	771 Gm.

It is observed that 158 Gm. (16% of the resin) was not recovered, alteration having rendered some of it insoluble in alcohol.

The Ligroin Extract.—This was concentrated to a syrup and redissolved in ether. The solution was extracted with 10% sodium hydroxide solution. The alkaline solution was acidified and again extracted with ether and then this was fractionally extracted with solutions of ammonium carbonate, potassium carbonate, and potassium hydroxide. The potassium carbonate extract was acidified and steam-distilled. The volatile acids in the distillate were joined with those obtained

upon hydrolysis of the fat. The non-volatile fatty acids were shaken out with ether, dried over sodium sulphate and distilled. They boiled chiefly at 253–260° at 35–45 mm. The weight of distilled acids was 30 Gm. (Iodine No. = 88.2). Neither potassium hydroxide extract nor the ammonium carbonate extract previously mentioned removed anything of interest.

The original ether extract of the neutral solution of fat was evaporated to dryness and saponified with alcoholic potash for five hours. The alcohol was removed and water added to completely precipitate the unsaponifiable matter which was shaken out with ether.

Examination of Unsaponifiable Matter.—The dried solution contained about 99 Gm. of a deep orange-colored syrup which when dissolved in alcohol and gradually concentrated yielded only traces (0.03 Gm.) of a paraffin-like solid that melted indefinitely at 65–70°. The alcohol was removed and the material was distilled under diminished pressure.

Fraction I (b. p. 80–200° at 25 mm.). Reddish oil, 4 Gm.
Fraction II (b. p. 200–225° at 15 mm.). Light brown oil, 10 Gm.
Fraction III (b. p. 225–270° at 15 mm.). Thick viscous liquid, 13 Gm.
Fraction IV (b. p. 270–305° at 15 mm.). 10 Gm.
Fraction V (b. p. 305–320° at 15 mm.). 10 Gm.

The residue weighed 43 Gm. There separated from III some material melting at 58–61°. Several crystallizations raised this to 75°. The same material was obtained from IV. It crystallized from ethyl acetate in needles. The melting point was 77° but lack of material prevented further purification.

Calculation for $C_{30}H_{62}O$: C, 82.2; H 14.1. Found: C, 83.1; H, 13.7.

The material is therefore myricyl alcohol.

Examination of the Volatile Fatty Acids.—The alkaline solution from which the unsaponifiable matter had been extracted with ether was acidified and steam-distilled. The distillate was extracted with ether. By titration the aqueous layer showed the presence of 4.4 Gm. acetic acid which was isolated as the silver salt, and some reduction indicated the probable presence of formic acid.

Calculation for $C_2H_3O_2Ag$: Ag = 64.6. Found: 62.9, 65.0%.

The ether-soluble volatile acids (10 Gm.) with which a small quantity of sulphur was admixed were dried and fractionally distilled: (1) up to 129°; (2) 175–186°; (3) 186–205°; (4) 205–240°; (5) 240–270°. The first fraction was converted into silver salts and the presence of formic and acetic acids was indicated. The remaining fractions were redistilled and a small amount (0.2 Gm.) of the second fraction distilled below 175°. To this residue the third fraction was added and a fraction was collected (2.5 Gm.) at 175–190° with reference to the boiling point of valerianic acid which is 186°.

Calculation for $C_5H_{10}O_2$: C = 58.8; H = 9.8. Found: C = 58.4; H = 9.8.

To the residue not distilling at 190° the higher boiling fraction was added and fractions were prepared as follows: (*a*) −196° (0.2 Gm.); (*b*) 196–212° (0.7 Gm.); and (*c*) 212–225° (0.4 Gm.).

The fraction boiling at 196–212° was collected with reference to the boiling point of caproic acid (204–205°) but the fraction contains also caprylic acid.

Calculation for $C_6H_{12}O_2$: C = 62.1; H = 10.3; for $C_8H_{16}O_2$: C = 66.7, H = 11.1. Found: C = 64.5; H = 10.4.

The highest boiling fraction (212–225°) is chiefly caprylic acid, containing a small quantity of unsaturated acid, which reduced alkaline permanganate solution. The silver salt was fractionally crystallized and analyzed.

Calculation for $C_8H_{15}O_2Ag$: Ag = 43.0. Found: Ag = 42.2, 42.0.

The volatile acids therefore consist essentially of acetic and valerianic acids. A trace of formic acid is present and the presence of caproic and caprylic acids is proved.

Examination of the Non-Volatile Fatty Acids.—The acid mixture which had been steam-distilled was cooled and extracted with ether. From the dried solution the ether was removed and the residue of non-volatile fatty acids distilled under diminished pressure. They were collected in two fractions: (1) 235–250° at 25–35 mm., (2) 260–280° at 25 mm. The former which amounted to 80% of the material had an iodine number of 82.5, the latter 101.3.

The solidified non-volatile acids derived from the potassium carbonate extract were separated through the lead salts into the solid and liquid acids, whereupon 21.3 Gm. yielded 7.3 Gm. solid acids and 13.2 Gm. liquid acids (62%). The solid acids still had an iodine number of 24. The liquid acids boiled chiefly but not completely at 226–270° at 17 mm. and this fraction had an iodine number of 119.3. The entire liquid acids were now esterified and a fractional distillation of the methyl esters showed that 90% of these distilled between 200–215° at 15 mm. This is therefore a mixture of the methyl esters of linoleic acid (208° at 11 mm.) and oleic acid (213° at 15 mm.). The iodine number was 122.35.

Calculation for $C_{19}H_{36}O_2$: I. V. = 85.8; $C_{19}H_{34}O_2$: I. V. = 172.7. Found: I. V. = 122.35.

A part of the non-volatile acids obtained upon hydrolysis of the glycerides were likewise separated by means of the lead salts in order to prepare a larger quantity of the acids. The liquid acids boiled smoothly at 215–245° at 15 mm., mostly at 232–236°.

Calculation for $C_{18}H_{34}O_2$: C = 76.6; H = 12.1; I. V. = 90.1; for $C_{18}H_{32}O_2$: C = 77.1; H = 11.4; I. V. = 181.4. Found: C = 76.95; H = 11.6; I. V. = 128.

This agrees with the values required for a mixture of oleic acid (58%) and linoleic acid (42%).

The solid saturated acids were crystallized from absolute alcohol. There was isolated in the first fraction, cerotic acid melting at 76°.

Calculation for $C_{26}H_{52}O_2$: C = 78.8; H = 13.1; N. V. = 141.7. Found: C = 78.3; H = 13.35; N. V. = 144.9.

The filtrate yielded a mixture of acids, consisting chiefly of palmitic melting at about 51–53°.

Calculation for $C_{14}H_{28}O_2$: C = 73.7; H = 12.3; for $C_{16}H_{32}O_2$: C = 75.0; H = 12.5; for $C_{18}H_{36}O_2$: C = 76.1; H = 12.7. Found: C = 74.65; H = 12.2.

The Ether Extract of the Resin separated a quantity (38 Gm.) of insoluble material. Recrystallized from dilute pyridine a white microcrystalline product consisting of a phytosterolin was obtained. It melted at 265–275° and was identified as the acetate which melted at 167–8° sharply, and had the characteristic crystalline appearance of this compound.

Calculation for $C_{33}H_{52}O_6(COCH_3)_4$: C = 68.7; H = 8.9. Found: C = 68.6; H = 8.8.

The ethereal filtrate when extracted with the usual alkaline solvents yielded nothing of interest, most of the fraction separating as a green chlorophylaceous mass insoluble in the ether and in the solvent. Repeated filtration removed this and the ethereal solution after being extracted was concentrated and a quantity of a neutral substance separated from the ether. This was crystallized from alcohol four times, finally melting at 138° sharply. It crystallized in nacreous plates, and gave the color reactions characteristic for the phytosterols although the appearance of the final permanent green was long delayed by the intermediate blue coloration.

The material was converted into the corresponding acetyl derivative, and after crystallization from acetic anhydride it was fractionally crystallized from ethyl acetate. The acetate melts at 121–123°. The phytosterol was regenerated from this and melted at 137.5–138° after recrystallization from alcohol.

Calculation for $C_{27}H_{46}O.H_2O$: H_2O, 4.8. Found: 4.8, 4.8.
Calculation for $C_{27}H_{46}O$: C, 83.9; H, 11.9. Found: C, 83.95, 83.35; H, 11.8, 12.2.

0.3351 Gm. acetate made up to 20 cc with chloroform showed a rotation of $-1.266°$ in a 2-dcm. tube, whence $[\alpha]\,^{23}_{D} = -37.8°$.

The ethereal filtrate diluted with 95% alcohol gave green-colored mixtures consisting in part of this phytosterol. This was not further studied.

The Chloroform Extract.—This was a dark green resin (23 Gm.) and yielded nothing of interest. It was not glucosidic.

The Ethyl Acetate Extract (95 Gm.) was concentrated to dryness and redissolved in alcohol, but no crystallization could be effected after prolonged standing. It yielded nothing crystalline. When boiled with dilute, hydrochloric acid, it turned raspberry-red in color but no evidence of a glucoside was obtained.

The Alcoholic Extract of the Resin (128 Gm.) yielded nothing crystalline. It was not glucosidic.

5 cc of the alcohol solution (0.4 Gm.) were precipitated with 45 cc water and the alcohol removed on the steam-bath. The precipitation resin was centrifuged off and repeatedly washed with water. The resin was dissolved in 10 cc 10% alcoholic hydrochloric and boiled for 1 hour. The alcohol was distilled off, and the residues taken up with water. The solutions and rinsings were made up to 100 cc. Fifty cc gave 0.0117 Gm. $Cu_2O = 0.0051$ Gm. glucose $= 2.6\%$ of the resin.

The entire extract was treated with 200 cc alcoholic potash (10%) and proved to be almost completely precipitated. The addition of water rendered it more soluble. After the addition of enough alkali to make 10% of the volume, the mixture was boiled for about five hours on reflux. The alcohol was distilled off, and the liquid acidified and steam-distilled. The distillate (= 85 cc *N* alkali) contained a quantity of volatile acids, which were converted into the silver salts and analyzed (acetic and valerianic acid).

Found: Ag = 48.3, 49.2, 63.8, 64.0%.

The material from which these acids had been steam-distilled was extracted with ether, but nothing was isolated.

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