[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, UPJOHN COMPANY.]

SOME CONSTITUENTS OF VIBURNUM PRUNIFOLIUM.

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The drug "black haw" has been used clinically since about 1870, and at the present time it retains considerable prestige as an anti-spasmodic in menorrhagia and dysmenorrhea. In this sense it is generally spoken of as a uterine sedative.

It does not fall within the scope of this work to discuss the clinical reports upon this drug, but since this subject has received no small amount of attention it appears that a chemical study might be of some assistance on this question. Our present information is based largely upon the work of Van Allen[2] and Sherman[3] who report the presence of (1) a brown resinous body of a bitter taste from which it was impossible to separate the sugar; (2) a greenish-yellow resin, or neutral substance of bitter taste, slightly soluble in water, freely so in alcohol and which was called viburnin; (3) valeric acid; (4) a tannic acid; (5) oxalic acid; (6) citric acid; (7) malic acid; (8) sulfates; (9) chlorides of calcium, magnesium, potassium and iron; (10) some alkaloidal material. Viehoever[4] found that the tannins of the Viburnums gave a green color or precipitate with ferric salts. He also isolated valeric acid and made the copper, zinc, and mercury salts for microscopic identification. A trace of butyric acid was considered to be present.

Black haw is officially described in the latest pharmacopeia as the dried bark of *Viburnum prunifolium*, "without the presence or admixture of more than 5% of wood or other foreign matter." This description has probably completely altered the nature of the pharmacopeial drug because it had previously been defined as the dried bark of the root. We do not know of any experimental reasons for this change and hold that such alterations are unjustifiable unless at least some chemical data can be produced to substantiate the similarity. Our sample of black haw consisted of the dried root bark of the authentic drug and was carefully inspected by our colleague Dr. L. H. Harvey to whom our thanks are due. It was collected in Michigan, and was picked over by hand, so that the examination was conducted upon a selected sample.

The proximate analysis showed that the drug contained 7.1% moisture and 7.3% ash. Ligroin extracted 7.1%; ether, 10.4%; alcohol, 18.7%.

[1] Holder of The Upjohn Coöperative Fellowship at Yale University (1919–1920). This paper is based upon the thesis presented by Charles Barkenbus to the Faculty of the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Van Allen, *Am. J. Pharm.*, **52**, 439 (1880).

[3] Sherman, *Repts. Royal Coll. Phys. Lab. Edinburgh*, **6**, (1897).

[4] Viehoever, *J. Am. Pharm. Assoc.*, **7**, 944 (1918).

The alcohol-insoluble residue had the following composition: crude fiber, 23.3%; pentosans, 16.1%; protein, 2.4%; starch 5.5%; dextrin, 0.5%. The alcoholic extract in which our interest centers contained sucrose, 0.3%; invert sugar, 1.8%, and tannin, 2.0%. When the alcoholic solution was poured inno water a resin was obtained to the amount of 4.6%. This value when taken in conjunction with 7.1% of ligroin soluble (or 10.4% ether-soluble material) indicates the presence of a very considerable amount of material which while being extracted by these volatile solvents in the native state, does not make its appearance later in the water insoluble resin as might be expected.

Other products found in the alcoholic solution and soluble in water besides the sugars and tannin, include formic, acetic and valeric acids; a non-glucosidic, acidic resin soluble in amyl alcohol which readily yields acetic and valeric acids (6.5%) upon saponification; and a second acidic resin which was not extracted by the amyl alcohol procedure, but which yielded 8.1% of acetic acid upon saponification. Traces of salicylic acid and of a hydrocarbon (m. p. 53–54°) resulted upon saponification of this material. While Bourquelot's[1] method appeared to give positive results as to the presence of a glucoside, our exhaustive examination of this solution proved negative in this respect. A trace of amorphous alkaloid is present.

The resin insoluble in water was extracted successively with ligroin, ether, chloroform, ethyl acetate and alcohol. The fat in the ligroin extract yielded a very large amount of unsaponifiable matter, and the chief part of this was a rosin-like solid that could not be distilled. A trace of a mixture of hydrocarbon and a higher alcohol (m. p. 65–72°) was present and also a beautifully crystalline phytosterol, $C_{27}H_{46}O$, that melted at 186–187° and formed an acetate melting at 223–224°. This is a new phytosterol. $[\alpha]_D = +115°$. A small amount of a white crystalline alcohol (m. p. 303°) was found here. Formic, acetic, caproic, caprylic, myristic, palmitic, oleic and linolic acids were identified in the fat. The absence of valeric acid here is interesting.

The ether extract of the resin yielded a phytosterolin, $C_{33}H_{56}O_6$, that melted at 290°. The acetate melted at 168°. The chloroform extract of the resin consists of an acidic resin that yields valeric acid upon saponification, as does the ethyl acetate soluble resin (18%). The alcohol-soluble resin yielded no valeric, but large amounts of acetic acid upon hydrolysis.

Our chief interest in these results centers in the various different forms in which acetic and valeric acids are present including not only the free acids but the water soluble complex esters, and the resinous esters insoluble

[1] *Arch. Pharm.*, **245**, 172 (1907).

in water. A fluid extract of this drug offers these acids for assimilation in a great variety of combinations.

Some of these valeric ester combinations remind us, in connection with their possible value, of certain synthetic valerates, such as Neobornyval (borneol isovaleryl glycolic acid ester) or Gynoval, (isovaleric ester of isoborneol) with which they probably share the advantage of passing the stomach, and requiring alkaline media for the liberation of the valeric acid. In fact it becomes incomprehensible from a chemical point of view why a large number of simple and synthetic valerates should remain out of the realm of dispute and the discussion should appear to center upon the Viburnums, one of which has already been deleted from the pharmacopeia.

Experimental.

A. Proximate Analysis.—Quantitative extractions were made on the air-dried drug after grinding and sieving.

Solvent.	%.
Ligroin (50–60°)	7.05
Ether	10.46
Ether (110°)	9.82
Alcohol	18.72

The proximate analyses, using the official methods, gave the results tabulated below.

	%.		%.
Moisture	7.09	Protein	2.40
Starch (Diastase)	5.51	Ash	7.32
Pentosans	16.13	Dextrin	0.52
Crude fiber	23.25	Tannin (Hide Powder)	1.97

Concerning this proximate analysis it is to be observed that the sum of the above quantitative determinations of the alcoholic-insoluble plant constituents does not account for this fraction which should amount to 74.2%.

Alcohol-soluble carbohydrates were determined as follows. 250 g. was extracted with ether, and the fat-free drug was then exhausted with boiling neutral 95% alcohol. The combined alcoholic extracts were concentrated under diminished pressure to a small volume and 800 cc. of water was added. Then an excess of basic lead acetate solution was added and the precipitate was filtered off and thoroughly washed with water. The excess of lead was removed from the filtrate with hydrogen sulfide. In the presence of calcium carbonate the filtrate from the lead sulfide was concentrated at 40–45° to 50 cc. This was transferred to a 250 cc. volumetric flask, diluted with 50 cc. of thymol water and 6.25 cc. of 0.5 *N* sodium dihydrogen phosphate solution was added. Enough 0.5 *N* hydrochloric acid was added to make the solution just acid to methyl orange. It was finally made up to exactly 250 cc. and one drop of toluene was added. This

solution showed a rotation of —9.1° V.,[1] at 22° in a 2 dcm. tube. By the Munson and Walker process, 5 cc. gave 0.1953 g. of cuprous oxide, equivalent to 1.79% of invert sugar.

Action of Invertase.—200 cc. of the above solution was treated with 0.25 g. of invertase. After 3 days the reading in a 2-dcm. tube was constant at —11.25° V.,[2] at 22°. 5 cc. gave 0.2180 g. of cuprous oxide. Sucrose, by reduction = 0.19%: by Clerget's formula = 0.34%.[3]

Action of Emulsin.—100 cc. of the above solution was plunged into a boiling, water bath for 10 to 15 minutes to inactivate the invertase. The solution was cooled to room temperature and 0.5 g. of emulsin was added. After 4 days the reading in a 2-dcm. tube was constant at —6.75° V., at 22°. 5 cc. gave 0.258 g. of cuprous oxide. These results indicate inconclusively the presence of a small amount of glucoside, the amount of glucose liberated is calculated to be 0.34%.

Viburnum was examined for the presence of alkaloids and was found in fact to contain traces of such material. An alkaloidal assay was conducted by the process which is official for *Hyoscyamus*,[4] and 15 g. yielded residues weighing 0.0393 g. (0.0398 g.) which were feebly alkaline and required 1.2 cc. of 0.02 *N* acid (1.1 cc.). When the titration was completed it was evident that these residues were conspicuously contaminated with resin, and the acid-soluble part was found to weigh 0.0207 g. (0.0197 g.) This corresponds to about 0.14% of the drug. The acid solutions gave a positive test with Mayer's reagent.

As this finding was unexpected we repeated the assay. By exhausting 100 g. of the drug with 95% alcohol, concentrating the combined extracts to 100 cc. and pouring this into a mixture of 10 cc. of *N* sulfuric acid +190 cc. of water, we obtained an acid solution of the alkaloids. The last traces of alcohol were removed by further distillation at low temperatures and the solution was made quantitatively to 250 cc. Of this 200 cc. of the clear acid fluid was exhaustively extracted with ether and then with chloroform. The solution was then rendered ammoniacal but no precipitation could be observed. The solution was exhausted with ether and then with chloroform but the extracts were not alkaloidal and had in fact an acid reaction. The solution was then exhausted with amyl alcohol, first at room temperature, and then at about 90°. The cold extraction yielded a trace of alkaloidal material. It required between 0.6 and 1.1 cc. of 0.05 *N* acid, and gave a positive test with Mayer's reagent. The hot amyl acoholic extraction likewise required 0.44 cc. of 0.1 *N* acid. The

[1] Valenta number.

[2] All polariscopic readings were corrected by proper controls.

[3] As described later the drug contains more sucrose than found here and it is probable that the prolonged alcoholic extraction in the presence of valeric and acetic acids inverted a considerable part.

[4] U. S. Pharmacopeia, IX, p. 225.

ammoniacal liquor was re-acidified, centrifuged to remove resinous precipitation and then precipitated with an excess of Mayer's reagent and it was evident that most of the alkaloid remained in this solution.

The various alkaloidal fractions were united in an acid solution and precipitated with Mayer's reagent. The washed precipitate was suspended in water and decomposed in the usual manner. The filtrate from the mercuric sulfide was concentrated. It yielded a dirty brown solution of a feebly basic nature that contained nothing crystalline. When injected into guinea-pigs it was not fatal but did produce a marked reaction characterized by dyspnea, stupor and some paralysis of the hind quarters. This condition lasted about an hour.

B. Complete Examination of Alcoholic Extract.—For this purpose 29.48 kg. was exhaustively percolated with cold methyl alcohol. The percolate (177.9 liters) was concentrated under diminished pressure to 4.74 liters, giving a dark green viscid syrup. This was shaken into 50 liters of distilled water. On standing for several days a green viscid resin separated but a brown amorphous solid remained suspended in the aqueous layer which was decanted. The resin was washed with water, and the solutions combined. The resin amounted to 1358 g. or 4.6% of the drug.

The Examination of the Water Soluble Constituents.—The aqueous solution was concentrated to a volume of 12 liters under reduced pressure. The acid distillate was rendered alkaline and concentrated to a small volume acidified and steam distilled, and this distillate was extracted with ether, which extracted 53 g. of liquid acid. These were fractionally distilled and collected in 4 fractions (1) 140–155°, (2) 155–165°, (3) 165–170°, (4) 170–175°. The first fractions were small while the chief fraction consisted of pure valeric acid which was further identified by the silver salt.

Calc. for $C_5H_9O_2Ag$: Ag, 51.37. Found: 51.31.

The lower-boiling fractions contained small quantities of acetic acid. Fraction 1 was converted into the silver salt and the most soluble part as well as the less soluble part were analysed.

Found: Ag, 57.0, 51.5.

The steam distillate from which valeric acid had been extracted yielded smaller quantities of acetic acid.

Calc. for $C_2H_3O_2Ag$: Ag, 64.6. Found: 64.0.

The concentrated main solution was freed from the brown amorphous material by filtration, and was then extracted repeatedly with ether. This fraction (39.4 g.) separated upon concentration a small quantity of phytosterolin (0.2 g.). It melted at 280°. Subsequent examination of the ether yielded nothing definite.

Chloroformic extractions of the aqueous layer yielded about 30 g. of a green smear from which nothing was isolated.

The water solution was now repeatedly exhausted with warm amyl alcohol of which the first extraction (after washing several times with water) was concentrated to a dark brown syrup, which proved to be miscible with water. It was, therefore, diluted with 1.5 liter amyl alcohol and repeatedly washed in a mechanical shaker to remove soluble sugars, but after many extractions the aqueous layer continued to give a positive test with Fehling's solution. The washed amyl alcoholic solution was concentrated under reduced pressure to a syrup, and the last traces of the solvent removed by blowing steam through. The residue was taken up in 60% alcohol and made up to a volume of 1000 cc. (133.8 g.) When an aliquot of this solution (250 cc.) was hydrolyzed by refluxing for 8 hours with 5% sulfuric acid, there was an increased reducing action by Fehling's test but it was impossible to prepare an osazone from this hydrolysis mixture in the usual manner.

250 cc. (33.5 g.) was heated in the presence of 10% of potassium hydroxide, the alcohol was removed by distillation and then after acidifying and subjecting to steam distillation a quantity of a mixture of valeric and acetic acids were obtained equivalent to 18.1 cc. of *N* sodium hydroxide solution. These acids were identified as silver salts.

Ether soluble acids found: Ag, 51.5. Water soluble acids found: Ag, 62.6.

Considering these to be mostly valeric acid this indicates the presence of 6.5% in ester form.

The acid mixture from which these volatile acids had been steam distilled was examined but nothing isolated.

The total amount of material extracted by amyl alcohol weighed 663 g., equivalent to 2.2% of the drug. These amyl alcohol extracts gave a greenish-black coloration with ferric chloride.

The aqueous liquid which had been extracted with ether, chloroform and with amyl alcohol was freed from the latter by a vigorous steam distillation and then divided into 2 equal aliquots. One-half was precipitated with an excess of basic lead acetate, and the chocolate brown precipitate that separated was filtered off after several days. The precipitate was suspended in water and decomposed with hydrogen sulfide but it contained nothing of interest except for the separation of a small quantity of calcium oxalate and a larger amount of tannin. The solution with ferric chloride gave a dark green coloration that turned to a black precipitate and it gave a heavy precipitate with gelatin solution.

The filtrate from the lead tannate was freed from lead with hydrogen sulfide and concentrated at 40–45° to a volume of 800 cc. This thick cherry-red syrup was allowed to stand for weeks but nothing crystalline separated. It was then taken to dryness and exhausted with alcohol but these solutions failed to crystallize under any conditions.

All this material was united, the alcohol was removed, and then the

residue was taken up in water and made to exactly 1000 cc. with a little toluene.

This solution gave no precipitate with phosphotungstic acid, nor with mercuric acetate solutions.

From 6.5 cc. of the solution we obtained 1.47 g. of *d*-phenyl glucosozone which melted at about 207°.

A trace of pentose sugars was found, since 10 cc. gave 0.0457 g. of phloroglucide (0.03%.)

Inasmuch as the preliminary test for glucoside by Bourquelot's method had indicated the possible presence of such a substance here, we subjected this fraction which had been purified by the removal of 2.2% of the drug by amyl alcohol to a second quantative investigation.

10 cc. of syrup (147 g. drug) was diluted to 100 cc. and 10 cc. (14.7 g.) gave 0.5987 g. of total solids dried at 100°, and 0.0175 g. ash.

5 cc. (7.35 g.) gave 0.2053 g. of Cu_2O = 0.0946 g. of invert sugar.

5 cc. inverted, gave 0.3850 g. of Cu_2O = 0.0783 g. of sucrose.

The solution (1000 cc.) therefore contained approximately 598.7 g. of solids, of which 17.5 g. was ash; 189.2 g. was invert sugar and 156.6 g. was sucrose. The unaccounted material amounted to about 241.6 g. In other words the total solids amounted to 4.07% while the invert sugar equalled 1.29% (1.2) and the sucrose 1.07% (0.94).

In order to find out whether a part of the unaccounted for material might be a glucoside, we repeated the analysis by the biological method. It will be remembered that this solution no longer contains the amyl alcohol soluble fraction which had a high reducing action on Fehling's solution. For this purpose a solution was prepared containing 16.9 cc. of the syrup (250 g. drug) +150 cc. thymol water +6.25 cc. of 0.5 *N* sodium phosphate solution and sufficient 0.5 *N* hydrochloric acid to make the solution just acid to methyl orange. The final volume was exactly 250 cc.

(*a*) *Direct.* Rotation in a 2-dcm. tube at 21° = —1.37° V. 5 cc. gave 0.1383 g. of Cu_2O = 0.0627 g. of invert sugar = 1.25%.

(*b*) *Action of Invertase.* 200 cc. of the solution + 0.25 g. of invertase after standing 5 days gave a reading of —5.1° V. in a 2-dcm. tube at 21.5°. 5 cc. gave 0.2171 g. of Cu_2O. Sucrose, by reduction = 0.7%; by Clerget's formula = 0.75%.

(*c*) *Action of emulsin.* 100 cc. of the solution was plunged into a boiling water bath for 10 to 15 minutes, cooled to room temperature and 0.5 g. of emulsin added. The reading was difficult to take but averaged —3.5° V. at 22° in a 2-dcm. tube. 5 cc. gave 0.239 g. of Cu_2O.

This change in rotation again indicates the liberation of glucose and there is an increase of 0.0219 g. of Cu_2O (for 5 g. of drug). This is equivalent to 0.182 g. of glucose per 100 g. of viburnum.

It was now attempted to remove the sugars by fermentation[1] and

[1] Fischer and Thierfelder, *Ber.*, **27**, 2031 (1894).

200 cc. of syrup (119.7 g. of solids) containing 36.5 g. of invert sugar and 31.3 g. of sucrose was diluted and titrated to alkalinity with *N* potassium hydroxide solution using phenolphthalein, and then one drop of phosphoric acid was added. To this solution 300 cc. of yeast decoction was added for nutrient material. This solution was filtered through porcelain and the sterile filtrate was inoculated with yeast.[1] It fermented rapidly and was clarified with lead subacetate, filtered and concentrated to 250 cc. after removing the excess of lead.

The material left was an uncrystallizable resin, characterized by an ester-like structure rather than a glucosidic one. When prepared in the dry condition it was not hygroscopic; had a bitter taste and had some reducing action upon Fehling's solution.

17.8 g. was heated upon the steam bath with 25 cc. of 6 *N* sodium hydroxide solution for about 6 hours, acidified and steam distilled. The distillate required 24.0 cc. of 1 *N* potassium hydroxide solution for neutralization, equivalent to 8.1% of acetic acid. (Ag, 63.5%).

A further quantity of the original syrup was fermented as above described and after clarifying with lead subacetate and subsequent removal of the excess of this reagent with hydrogen sulfide, the solution was concentrated, poured upon purified sawdust and dried. This was fractionally extracted with absolute ethyl acetate, and then with absolute alcohol and many fractions separately concentrated, but nothing crystalline was found beyond a few straggling needles in the first ethyl acetate fraction.

All the fractions were united, dissolved in water, and concentrated to remove traces of alcohol and ethyl acetate and then hydrolyzed in the presence of 10% sodium hydroxide solution.

The solution was acidified and steam distilled, to remove quantities of valeric and acetic acids. The acid residue in the flask was agitated with large volumes of ether, which in turn was fractionally extracted with solutions of ammonium carbonate, sodium carbonate, and sodium hydroxide. From the first of these a small quantity of salicylic acid was isolated that melted at 153–154° and gave a violet coloration with ferric chloride. From the ether solution of the neutral substances a quantity (0.2 g.) of a hydrocarbon was isolated. After several crystallizations from alcohol the melting point was elevated from 48–51° to 53–54°.

Calc. for $C_{27}H_{56}$: C, 85.2; H, 14.7. Found: C, 84.5; H, 14.5.

The material appears to be heptacosane.[2]

An acid hydrolysis yielded nothing interesting.

The Examination of the Resin.—The resin (1358 g.) was dissolved in

[1] We are indebted to Dr. Ralph E. Lee of the Fleischmann Company for kindly supplying this material.

[2] The possibility of contamination presents itself because of the difficulty of accounting for this substance here.

wood alcohol and poured upon purified sawdust and dried. The entire material was extracted with the following results:

	G.
Petroleum ether (40–60°)	348
Ether	104
Chloroform	245
Ethyl Acetate	365
Alcohol	168
Total	1230

During the extraction 128 g. had become insoluble, through alteration, possibly of the nature of oxidation.

The Ligroin Extract.—This extract, amounting to 348 g., was dissolved in ether and extracted with 10% sodium hydroxide solution. The alkaline solution was acidified and shaken with ether, whereupon a precipitate insoluble in either layer separated and produced an emulsion which was broken by filtration. The green solid that separated (1.0 g.) was hydrolyzed with dil. alcoholic sulfuric acid in the presence of chloroform, whereupon most of the material became converted into a brown amorphous product, but from the chloroform solution a residue was obtained that crystallized gelatinously from ethyl acetate after treating with boneblack and concentrating. With great difficulty this substance was finally obtained as beautiful white silky needles (0.1 g.) from absolute alcohol. It melted at 303° to a clear oil and perhaps belongs to the class represented by oleanol.[1] We attempted to acetylate this product by heating with acetic anhydride in the presence of pyridine but this did not proceed smoothly and we obtained a mixture of substances. From the reaction mixture upon cooling a separation of small plates that melted at 340–350° took place. Upon concentrating gradually a syrup finally resulted which could be crystallized from alcohol. On recrystallization, needles were obtained that melted at 176–178° and effervesced at 190°. We attempted no analysis on account of these small fractions.

The ether solution of acidic substances was extracted with solutions of ammonia carbonate, sodium carbonate, and sodium hydroxide. The sodium carbonate extracted a quantity of free acids and these were studied later. The other solvents yielded nothing crystalline.

While shaking with sodium hydroxide solution, the neutral layer separated 2.0 g. of material from which phytosterolin melting at 280° was readily isolated.

The ether solution of the neutral substances was saponified by boiling for 8 hours with 10% alcoholic potassium hydroxide and the unsaponifiable material separated with ether.

Examination of the Unsaponifiable Matter.—The dried solution

[1] *J. Chem. Soc.*, 93, 891 (1908).

yielded 176 g. of a brownish-red semi-solid mass. It was dissolved in absolute alcohol and a trace (0.05 g.) of a mixture of hydrocarbon and higher alcohol separated that melted at 65–72°. The solution was concentrated sharply and after prolonged standing 13 g. of fine needles separated. This was subjected to a systematic fractional crystallization and the fractions analyzed.

Found: (I, m. p. 182°) C, 83.9; H, 11.6: (IV, m. p. 181°) C, 83.7; H, 11.4.

The results indicate the presence of a predominant amount of phytosterol agreeing in composition for that required by the common formula $C_{27}H_{46}O$.

The material was acetylated and the resulting acetyl derivatives fractionally crystallized from ethyl acetate and alcohol. The top fraction melted at 223–224°. It was saponified with alcoholic potassium hydroxide, and the recovered phytosterol after recrystallization now melted at 186–187° and had a specific rotation in chloroform of +115°.

Calc. for $C_{27}H_{46}O$: C, 83.9; H, 11.9. Found: C, 83.9; H, 11.5.

This alcohol when dissolved in chloroform and acetic anhydride gave a play of colors upon the addition of sulfuric acid resulting in red, purple and finally brown. When moistened with concentrated sulfuric acid it assumed an orange-red color which disappeared upon dilution with water. These color reactions are similar to those described by Power and Tutin[1] for oleasterol.

From the mother liquors of the acetate that melts at 223–224°, a quantity of an acetate was obtained that melted at 200–203°. It was hydrolyzed and an impure phytosterol fraction was obtained. It was repeatedly crystallized but did not melt sharply at 168–172°. It showed a specific rotation of +96.7° and probably consisted largely of the same material as the pure compound first described. (C, 83.3; H, 11.5.)

The alcoholic mother liquors were subjected to a fractional distillation and we obtained a non-distillable solid and brittle residue amounting to about 125 g. The material that distilled was recovered as follows.

Fraction I. (b. p. 100–180° at 28 mm.) This was an orange limpid liquid with aromatic odor and weighed 2 g.

Fraction II. (b. p. 180–210° at 25 mm.) A thick syrupy liquid weighing 5.7 g.

Fraction III. (b. p. 210–250° at 25 mm.) This was a dark red viscid liquid which solidified to a varnish-like mass on cooling.

It should be pointed out that 35.9% of the ligroin extract consisted of the non-distillable unsaponifiable resinous matter as this is unusual.

Examination of the Volatile Fatty Acids.—The alkaline solution from which the above described unsaponifiable matter had been extracted with ether was acidified and steam distilled, and the volatile fatty acids

[1] *Loc. cit.*

were likewise recovered from the acids that occurred free in the fat. The distillate was made alkaline and concentrated, acidified and extracted with ether. These acids were separated by means of their silver salts which were fractionally precipitated.

Fraction I. Calc. for $C_{14}H_{27}O_2Ag$: Ag, 32.24. Found: 31.94.
Fraction II. Calc. for $C_8H_{15}O_2Ag$: Ag, 43.03. Found: 42.5.
Fraction III. Calc. for $C_6H_{11}O_2Ag$: Ag, 48.3. Found: 48.4.

This material, therefore, consists of a mixture of caproic, caprylic and myristic acids.

The aqueous liquid from which the above described acids had been ether-extracted consisted chiefly of formic acid with a trace of acetic acid.

Examination of the Non-Volatile Fatty Acids.—The acid mixtures which had been steam distilled were cooled and extracted with ether and the fatty acid fractions were united. 50 g. was distilled, and the distillate collected in 2 fractions; (*a*) b. p. 195–230° at 23 mm. weighing 21.5 g., showed an iodine number of 23.8, (*b*) b. p. 230–265° at 23–60 mm. weighed 11.5 g. and had an iodine number of 82.0. The residue in the distillation flask was considerable.

The distillates were united and the solid and liquid acids were separated by the lead salt method. From 23 g. there was obtained 7 g. of liquid acids (30%) while 14.8 g. of solid acids (64%) was recovered.

The solid acids were prepared in larger quantity and material having an iodine number of 15.5 to 19.3 was obtained and this was subjected to a systematic fractional crystallization. The top fractions were palmitic acid (m. p. 60–61°).

Calc. for $C_{16}H_{32}O_2$: C, 75.0; H, 12.5; N. V.,[1] 219.1. Found: C, 75.0; H, 12.35; N. V., 219.2.

The lower fractions consisted essentially of palmitic acid with a small amount of myristic acid. The fourth fraction melted at 49°.

Calc. for $C_{14}H_{28}O_2$: C, 73.7; H, 12.3; N. V., 246.1. Found: C, 74.1; H, 12.2; N. V., 221.4.

The liquid acids were prepared in larger quantities and converted into their methyl esters by the method of Phelps and Phelps[2] and of these 102 g. were distilled. These boiled at 185–280° at 15 mm., the lower fractions contained some palmitic ester. The unsaturated esters boiled chiefly at 205–280° at 15 mm.

Calc. for $C_{17}H_{33}COOCH_3$: C, 77.0; H, 12.2; iodine No., 85.1. Calc. for $C_{17}H_{31}COOCH_3$: C, 77.55; H, 11.6; iodine No., 172.8. Found: C, 77.7; H, 11.5; iodine No., 95–106.1.

The liquid acids, therefore, consist of linolic and oleic acids.

[1] Neutralization value.

[2] *Am. J. Sci.*, **24**, 194 (1907).

The Ether Extract of the Resin, upon standing deposited 25.5 g. of a green solid. From this precipitate phytosterolin was readily obtained by crystallization from dilute pyridine. It melted at 290°.

Calc. for $C_{33}H_{56}O_6$: C, 72.3; H, 10.2. Found: C, 72.6; H, 10.4.

The material gave the characteristic color tests and was converted into its acetyl derivative that melted at 168°.

A systematic examination of the ethereal filtrate yielded nothing crystalline.

The Chloroform Extract of the Resin was divided into several parts, one of which was systematically examined by extraction with the usual alkaline solutions, but nothing was isolated. When subjected to acid hydrolysis with dil. alcoholic 5% sulfuric acid it was shown to be non-glucosidic, but no definite products resulted.

This extract is characterized by its ester-like structure and readily yielded valeric acid upon alkaline hydrolysis. It amounted to 7.2% of the resin and was identified as silver valerate. (Ag, 51.5%) No acetic acid was found.

The hydrolysis solution from which valeric acid had been removed by distillation was examined, but with the exception of a minute quantity (0.05 g.) of a crystalline product that melted at 99–102° nothing was found. This material occurred in the ammonium carbonate extract of the ether soluble fraction.

The Ethyl Acetate Extract of the Resin could not be directly crystallized. It constantly underwent spontaneous alteration leading to the precipitation of increasing amounts of insoluble amorphous material. The insoluble material proved to be soluble in alcohol but was not crystalline. Acid hydrolysis gave no crystalline products but an hydrolysis with 10% alcoholic potash yielded large quantities of valeric acid. Calculated on the basis of titration values found in the steam distillate it amounts to 18.5% of the resin that remained soluble in ethyl acetate. It was readily identified as the silver salt. (Ag, 51.6%.) This resinous material is an acidic resin.

The Alcohol Extract of the Resin was systematically examined but yielded nothing of interest except when subjected to alkaline hydrolysis it yielded large quantities of acetic acid (Ag, 64.4%) and practically no valeric acid.

KALAMAZOO, MICH.