SCIENTIFIC SECTION

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DIGITALIS FAT—THE PETROLEUM-ETHER EXTRACTIVE OF DIGITALIS PURPUREA LINNÉ.

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(*Continued from page 860, September 1932.*)

Pigmentary Material: Fatty Acids—Fraction I.—The lead salts, insoluble in boiling alcohol, were highly pigmented, having a raven-blackish green color. They give a dark green color to petroleum ether. The material separated in clumps which became brittle upon drying and could be readily pulverized. The weight of this fraction is 148 Gm. and has been set aside for a later study.

The Saturated Fatty Acids: Fraction II.—The fraction of lead salts, soluble in hot alcohol and insoluble in cold alcohol, should contain the saturated or solid fatty acid salts. There were 97 Gm. of a light green powder in this fraction. These salts were dissolved in 2 liters of hot alcohol and while still warm, were decomposed by leading hydrogen sulphide directly into the solution. The lead sulphide was filtered off, the filtrate coming through being of a dull yellowish color; this was subjected to further hydrogen sulphide treatment which precipitated more lead sulphide. The filtrate after the second precipitation came through with a clear, light yellow color. Upon cooling, however, the filtrate became turbid. This alcoholic solution of the fatty acids was partly distilled, the volume being reduced to about one-half. Upon cooling there was a very pronounced clouding of the liquid and partial precipitation. This was filtered off, dissolved in acetic acid and washed with water; it consisted of 8 Gm. of crude fatty acid with a m. p. of 61° C. Upon crystallization from acetic acid, a dark colored ring separated from the fatty acids at the upper surface. This was removed, and as was later discovered, consisted of non-saponifiable material that had not been removed by the ether extraction of the saponified fat. There were one and one-half Gm. of this material, with a m. p. of 65° C.

The filtrate was chilled and kept at 0° C. for five hours which produced a further precipitation. This was filtered off and tested in the same manner as the above. This netted seventeen Gm. of crude fatty acids with a m. p. of 51° to 53° C.

The filtrate was then further concentrated but there was no further precipitation except upon cooling when an additional twenty-eight Gm. of fatty acids separated out. This was taken up in acetic acid, crystallized and then taken up in alcohol, washed with water, yielding a crude fatty acid mixture with a m. p. of 51° to 53° C. This was an attempt to separate the fatty acids by fractional crystallization.

Each of these four fractions of crude fatty acids was then repeatedly crystallized from hot acetic acid, subsequently from alcohol, and washed with water. It was noted that in the acetic acid treatment, a distinct upper and lower layer were formed; this served as a basis for further separation into sub-fractions. As a result of this fractionation the following groupings were made:

	M. P.° C.	Quantity—Gm.	Color.
1. Fatty acids	65	1.0	Creamy white
2. Fatty acids	63	5.5	Creamy, almost white
3. Fatty acids	(The non-saponified portion—1/2 Gm.—yellow)		
4. Fatty acids	71	1.0	Yellow
5. Fatty acids	54	14.5	Yellowish white
6. Fatty acids	61	1.5	White
7. Fatty acids	55	7.0	Creamy white
8. Fatty acids	52	11.0	Light buff

Methyl Esters of the Low M. P. Fatty Acids.—The fatty acids in the above group, having a melting point below 60° C. were combined and the methyl esters were prepared by the Haller method.[1]

The ether solution of the methyl esters was then exposed to the air to remove the ether and permit the crystallization of the methyl esters. Distillation of the methyl esters was deemed inadvisable on account of the small quantity. Upon the removal of all of the ether, there was no separation of methyl esters. The liquid was of a dark red color. It was kept at 29° C. for 5 hours, but there was no congealing or precipitation. This was taken as an indication for the absence of the methyl esters of stearic acid or any other saturated fatty acid higher in the series.

The liquid methyl esters were then cooled to 20° C. after which a solid separated from the liquid. This was filtered off on a force filter. The filtrate was again cooled to 20° C. and more solid matter separated out, which was force filtered and kept as a second fraction. Further cooling of the filtrate at 20° C. produced no more solid matter. The temperature of the solution was then lowered to 8° C. which is 3° above the melting point of the methyl ester of lauric acid. This was force filtered, the filtrate chilled and force filtered, and the process repeated a third time. After the third cooling, no liquid remained.

Each of these 5 methyl-ester fractions was then saponified with alcoholic potassium hydroxide, and the free acids liberated by the addition of a 1:10 sulphuric acid solution. The acids were then crystallized from 95% alcohol a number of times, and finally from absolute alcohol. The first fraction yielded four Gm. of an acid with a m. p. of 62.5° C.; this was taken as an indication for palmitic acid. Fractions four and five yielded 2 Gm. of an acid with a m. p. of 53° to 54° C., which was taken as an indication for myristic acid. Fractions two and three of this series were not completely purified; in all probability each is a mixture of palmitic and myristic acids.

Verification of Palmitic Acid ($C_{15}H_{31}COOH$).—That the acid crystallized out from Fraction I is palmitic acid is proven by the following experimental work:

1. The melting point (62.5) coincides with the melting point of palmitic acid—62.8° C.
2. The neutralization value for the acid is 218.98, and the theoretical neutralization value for palmitic acid is recorded as 219.1.
3. The molecular weight as calculated from the acid value is 256.34 and the molecular weight of palmitic acid is 256.26.
4. The acid was obtained by the saponification of a methyl ester having the melting point of methyl palmitate.
5. The acid when mixed with an authentic palmitic acid shows no depression of the melting point.

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[1] E. Haller, *Compt. rend.*, 146 (1908), 250.

Verification of Myristic Acid ($C_{13}H_{27}COOH$).—That the acid crystallized out from Fractions IV and V is myristic acid is proven by the following experimental work:

1. The melting point (53° to 54° C.) coincides with the melting point of myristic acid—53.8° C.
2. The neutralization value for the acid is 245.63 and the neutralization value for myristic acid is recorded as 246.1.
3. The molecular weight of the acid as calculated from the acid value is 228.49, and the molecular weight of myristic acid is 228.22.
4. The acid was obtained by the saponification of a methyl ester having the melting point of methyl myristate.
5. The acid when mixed with authentic myristic acid shows no depression of the melting point.

Fractional Crystallization of the Higher Fatty Acids.—The fractions of acids whose melting points were above 61° C. (page 999) with the exception of Fraction III, were combined (7.5G m.) and crystallized from acetic acid, then alcohol and washed with water a number of times. All of the acids were soluble in the hot alcoholic solution, but upon cooling separation or precipitation would occur that was not a true crystallization. This was filtered off, and the filtrate concentrated from which an acid crystallized. A separation was then attempted on a basis of solubility in cold ether for the purpose of removing any acids in the C_{14} to C_{18} group. The acid which crystallized from the cold alcohol filtrate was completely soluble in the cold ether. The cold alcohol-insoluble acids were also subjected to the hot and cold ether separation with the following results:

1. Cold alcohol, cold ether-soluble fraction—2 Gm., m. p. 69° to 71° C.
2. Cold alcohol insoluble, cold-ether soluble fraction—0.2 Gm., m. p. 71° to 75° C.
3. Cold alcohol and cold ether-insoluble fraction—0.1 Gm., m. p. 82° C.

Each of these fractions was repeatedly crystallized from petroleum ether. The melting point of Fraction I could not be raised above 71° C. The neutralization value for the acid or acids of this fraction was 141.4; the neutralization value for cerotic acid is 141.7 and the melting point is recorded as 77° to 82° C., although F. B. Power reported a cerotic acid having a melting point of 74° to 76° C.[1] In view of the low melting point and the neutralization value of 141.4, it appears as though this fraction is a mixture of possibly cerotic acid and some acids of higher and lower melting points.

Fraction II was finally crystallized from benzene when it had a melting point of 75° C. The neutralization value of it was found to be 138.5. When mixed with an authentic cerotic acid there was no appreciable depression of the melting point. The neutralization value of the authentic cerotic acid was found to be 135.05. The molecular weight of the acid of this fraction was found to be 405.08. The molecular weight of the C_{26} cerotic acid is 396.42. On account of the small amount of material in this fraction, it was impossible to make further determinations. Evidence, however, is sufficient to indicate the possible presence of cerotic acid—$C_{25}H_{51}COOH$.

Fraction III was likewise crystallized from benzene but it was impossible to obtain a pure white acid and further crystallizations had to be abandoned on

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[1] *Jour. Chem. Soc.*, 93 (1908), 1658.

account of the limited amount of material. The m. p. of this fraction was 84° C. Melissic acid has a melting point of 88.5° C. The neutralization value for this fraction was 83.68 while that of melissic acid is 124.1. The possibility that this fraction might consist of impure melissic acid suggested itself for the following reasons:

1. The m. p. approaches that of montanic acid (86° C.) but because of the yellowish appearance of the acid it was thought the impurity would cause a greater depression of the melting point. For melissic acid there would be a difference of 4.5°.
2. The neutralization value indicates the presence of considerable impurity which factor would again rule out the possibility of montanic acid.
3. Since the saturated alcohol and the saturated hydrocarbon of C_{30} composition have been isolated, it would seem logical that the corresponding saturated acid might also be present.

Verification of Cerotic Acid ($C_{25}H_{51}COOH$).—That the acid crystallized from the cold alcohol-insoluble cold, ether-soluble fraction is cerotic acid is proven by the following experimental work:

1. The melting point (75° C.) coincides with a melting point of cerotic acid reported by F. B. Power—74° to 76° C.
2. The neutralization value for the acid is 138.5 while the neutralization value for authentic cerotic acid is 135.05.
3. The molecular weight of the acid as calculated from the acid value is 405.08, and the molecular weight of cerotic acid is 396.42.
4. The acid when mixed with authentic cerotic acid shows no appreciable depression of the melting point.

The Unsaturated Fatty Acids: Fractions III and IV.—The fraction consisting of the solution of the lead salts soluble in cold alcohol (Fraction IV) was evaporated to one-half its volume (5 liters) and cooled for a day at 15° C. No precipitate appeared. The lead salts in solution were decomposed by means of hydrogen sulphide led directly into the solution. The solution was warmed in order to coagulate the lead sulphide and then filtered. A greenish colored filtrate was obtained. This filtrate was again treated with hydrogen sulphide with a further precipitation of lead sulphide. When warmed and filtered again, the filtrate was a clear, orange to golden-orange liquid. The lead-sulphide precipitate was thoroughly washed with hot alcohol; it evidently retained the greater amount of pigmentary material.

The alcohol was recovered from the filtrate and washings under reduced pressure and the last traces of acetic acid were removed by heating on a steam-bath under reduced pressure. Carbon dioxide was passed into the vacuum distillation apparatus during the distillation; 157 Gm. of a red oil were obtained.

Fraction III was treated in exactly the same manner from which 29 Gm. of a dark red oily liquid were obtained.

Bromination of the Unsaturated or Liquid Fatty Acids: Fraction IV.—The bromination and separation of the unsaturated fatty acids was carried out according to the process described by L. Rosenthaler.[1] The hexabromides were thrown out of solution when the brominated solution had been kept at 5° C. for twenty-four hours. They were filtered off by suction, washed with a cold mixture of equal parts of glacial acetic acid and ether and then with 200 cc. of absolute alco-

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[1] L. Rosenthaler, "Chemical Investigation of Plants," 1st Edition (1930), page 83.

hol. The hexabromides were then crystallized in a white mass from benzene. Twelve grams of the hexabromide were obtained; the melting point (181–182° C.) coincides with the recorded melting point of linolenic hexabromide.

Characterization of Linolenic Acid.—The hexabromide was analyzed for bromine content by the Stepenow method.[1] The liberated halogen was then titrated according to the Volhard method.[2]

	Sample 1.	Sample 2.	Average.
Per cent bromine present...	63.122 per cent	62.987 per cent	63.054 per cent

The theoretical percentage of bromine in linolenic hexabromide is 63.32%.

Verification of Linolenic Acid ($C_{17}H_{29}COOH$).—That linolenic acid is present is proven by the preparation of the hexabromide derivative.

1. The prepared hexabromide has a m. p. (181° to 182° C.) which coincides with the recorded m. p. for linolenic hexabromide (180° to 181° C.).
2. The bromine content of the prepared hexabromide (average 63.05%) compares favorably with the theoretical bromine content of linolenic hexabromide (63.32%).

Characterization of Linolic Acid.—The filtrate from the linolenic hexabromide was poured into a large volume of water (6 liters), the precipitate filtered off, washed with saturated sodium thiosulphate solution to remove the excess bromine, and then repeatedly washed with water. The precipitate was then dissolved in ether, the ether removed by distillation and the residue extracted with hot petroleum ether. This petroleum-ether extract was chilled at 0° C. and maintained at that temperature for two hours, when the tetrabromide derivative of an unsaturated acid came out of solution. The tetrabromide was filtered off and the filtrate concentrated to half its volume and again chilled at 0° C. After two hours, a second precipitate appeared which was filtered off and combined with the first precipitate. The filtrate was again lowered to 0° C. but no further precipitation occurred. The tetrabromide was dissolved in boiling petroleum ether from which it crystallized, then crystallized from ether, and finally from petroleum ether again. The crystals were white, had a m. p. of 111° to 112° C. which m. p. could not be raised by further recrystallizations. The yield was 2.5 Gm. Lewkowitsch records the m. p. for linolic tetrabromide as 113° to 114° C.

A bromine determination according to the Stepenow method showed the following results:

	Sample 1.	Sample 2.	Average.
Per cent bromine present...	52.73 per cent	53.001 per cent	52.865 per cent

The theoretical amount of bromine in linolic tetrabromide is calculated as 53.33%.

Verification of Linolic Acid ($C_{17}H_{31}COOH$).—That linolic acid is present is proven by the preparation of the tetrabromide derivative.

1. The prepared tetrabromide has a m. p. (111° to 112° C.) which compares favorably with the recorded m. p. for linolic tetrabromide (113° to 114° C.)
2. The bromine content of the prepared tetrabromide (average 52.865%) also compares favorably with the recorded theoretical bromine content of linolic tetrabromide (53.33%).

Characterization of Oleic Acid.—After the removal of the tetrabromide by filtration, the filtrate contained the dibromides. This filtrate was exposed to the

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[1] *Ber. d. d. chem. Gesels.*, 39 (1906), 4056.

[2] H. Bassett, "Theory of Quant. Anal.," 1st Edition (1925), page 25.

air and the petroleum ether evaporated, which left a yellowish red thick liquid weighing about twenty Gm. Bromine determinations were made of this dibromide material with the following results:

	Sample 1.	Sample 2.	Average.
Per cent bromine present...	38.93 per cent	41.03 per cent	39.98 per cent

The calculated theoretical amount of bromine in oleic dibromide is only 36.8%; apparently all of the tetrabromide had not been thrown out of solution during the chilling process.

Oleic acid was liberated from this dibromide according to the method of Heiduschka and Lueft,[1] in which the dibromide is decomposed by zinc filings in the presence of ethyl alcohol, forming the ethyl ester of the acid. The ester is saponified with potassium hydroxide and the potassium soap formed is decomposed with 1:10 sulphuric acid. This free fatty acid was shaken out with ether, dried over sodium sulphate and the ether removed by distillation leaving the acid. This was of a very dark red color, so it was treated with charcoal to clarify it after which it had a light yellowish color. The yield was about seven Gm.

Verification of Oleic Acid ($C_{17}H_{33}COOH$).—That oleic acid is present is proven by the preparation of the dibromide from which the free acid is again liberated.

1. The prepared dibromide has a bromine content of 39.98% which is slightly higher than the theoretical amount of bromine in oleic dibromide (36.8%) but which is probably due to the presence of a small amount of linolic tetrabromide.
2. Oleic acid was liberated from the dibromide and responded to the following tests for oleic acid:[2]
   - *a.* Solidifying point—3° to 4° C.; theoretical—4° C.
   - *b.* The acid in sulphuric acid, layered with a 1% vanillin solution shows a violet ring at the line of contact.
   - *c.* The addition of the acid drop by drop into sulphuric acid containing a carbohydrate and followed by adding some water produces an intense red color, which when still further diluted with water becomes violet, and finally disappears leaving a black carbonized mass.

Fraction III yielded the same unsaturated fatty acids as Fraction IV. Their respective bromides were prepared with the following results:

	Melting Point.		Bromine %.	
	Exper.	Theor.	Exper.	Theor.
1. Linolenic hexabromide	181° C.	180°–181° C.	63.04	63.32
2. Linolic tetrabromide	111°–112°	113°–114°	53.32	53.33
3. Oleic dibromide	......	......	40.10	36.80

The Non-Saponifiable Fraction.—The non-saponifiable material (300 Gm.) was heated to the point of liquefaction with five hundred cc. of 95% alcohol. There was a separation into two distinct layers:

I. An upper layer of orange color—the hot alcohol-soluble fraction.
II. A lower layer of deep red color—the hot alcohol-insoluble fraction.

I. *The Hot Alcohol-Soluble Fraction.*—This fraction upon cooling precipitated an orange-colored solid, which was the basis for a further subdivision of this fraction into:

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[1] *Arch. d. Pharm.*, 257 (1919), 51.

[2] J. Lewkowitsch, "Chem. Tech. Fats, Oils and Waxes," 6th Edition, 1 (1921), 183. L. Rosenthaler, "Chem. Invest. of Plants," 1st Edition (1930), page 84. A. Benedikt and R. Ulzer, "Anal. d. Fette u. Wachse," 4th Edition (1903), page 25.

a. The cold alcohol-soluble fraction—deep red color.
b. The cold alcohol-insoluble fraction—orange color.

The solid Fraction *b.* was washed with repeated five hundred-cc. portions of hot 95% alcohol, effecting a solution which upon cooling would precipitate the cold alcohol-insoluble material. But with each washing and subsequent precipitation the solid became lighter in color, changing from the orange to a deep yellow, through the various shades of yellow to cream and finally a white crystalline substance. The yield of this was 4.5 Gm. It was recrystallized from ethyl acetate several times. The melting point was 81° to 83° C. The crystals were very light and fluffy, tasteless and completely soluble in acetic anhydride. Since this was non-saponifiable material, the solubility in the anhydride classified it as an alcohol, ruling out the possibility of a hydrocarbon.

The crystalline mass was dissolved in chloroform and crystallized from it, which raised the m. p. to 84–85° C. Melissyl alcohol is recorded as having a m. p. of 85° C. Accordingly the acetate of this alcohol was prepared according to the method described by J. Lewkowitsch.[1] The acetate was dissolved in ethyl acetate from which it crystallized in very short acicular crystals. Upon filtering, however, these crystals matted together in a parchment-like film which could be readily peeled from the filter paper. The m. p. of the acetate was 72° to 73° C. which is in agreement with the recorded m. p. of melissyl acetate; Lewkowitsch records it as melting at 73° to 75° C. The acetate was recrystallized from absolute alcohol in an attempt to get away from the film-like acetate, but upon filtering it again massed together. The m. p., however, was raised to 73° to 74° C.

Verification of Melissyl Alcohol ($C_{30}H_{61}OH$).—That the substance isolated from this fraction is melissyl alcohol is proven by the following:

1. It is non-saponifiable material entirely soluble in acetic anhydride which characterizes it as an alcohol.
2. The m. p. is 84° to 85° C. which is in accord with the recorded m. p. of 85° C. for melissyl alcohol.
3. The alcohol fails to give a positive Liebermann-Burchard and Salkowski test, indicating that it was not an unsaturated alcohol or sterol.
4. The crystals in ethyl acetate medium are very short and acicular.
5. The acetate prepared from it has a m. p. of 73° to 74° C.; the m. p. recorded in the literature for melissyl acetate is 73° to 75° C.

The cold alcohol-soluble Fraction *a.* (above) together with the alcohol washings of the fraction that yielded the melissyl alcohol, was subjected to distillation for recovery of the alcohol and uncovering the substances in solution. Distillation was carried on under reduced pressure. After the bulk of the liquid had been reduced to one-half its volume and cooled, a mass of rusty-colored crystals appeared. After three successive crystallizations from 95% alcohol, a mass of small white, lustrous, flaky crystals was obtained having a m. p. of 124° C. The yield was seven Gm. About three Gm. of these crystals were dissolved in ether from which they crystallized in needles; the m. p. was raised to 135° to 136° C.

These crystals gave a positive Liebermann-Burchard test[2] as well as positive

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[1] J. Lewkowitsch, "Chem. Tech. Fats, Oils and Waxes," 6th Edition (1921), page 613.

[2] A. Mathews, "Physiol. Chem.," 3rd Edition (1920), page 82.

tests for the Salkowski[1] and Hesse[2] reactions which characterize the crystals as a sterol or unsaturated alcohol. To confirm this characterization, the acetate was prepared according to Boemer's method.[3] The acetate was crystallized from absolute alcohol, washed with 95% alcohol and then recrystallized four times from absolute alcohol. The acetate had a m. p. of 128° to 129° C. which conforms with the m. p. of the phytosterol acetate recorded in the literature (125° to 137° C.). The acetate crystallized from absolute alcohol in very lustrous, flaky, mother-of-pearl plates bearing somewhat of a resemblance to fragments of mica.

These properties of the isolated phytosterol fairly well meet the description of sitosterol whose m. p. is 137° to 138° C. and whose acetate has a m. p. of 125.6° to 137° C. Sitosterol crystallizes in plates from alcohol and in anhydrous needles from ether.[4]

A 1% alcoholic solution of digitonin formed a precipitate when mixed with a solution of the sterol. This property of the sterol to form an addition compound with digitonin is the basis of Windaus' sterol determination.[5] The digitonide comes out as a colorless mass of fine crystals.

Verification of the Phytosterol—Sitosterol ($C_{27}H_{45}OH$).—That the above-mentioned isolated substance is a phytosterol, and more specifically sitosterol, is proven by the following experimental results:

1. It gives positive sterol tests—Liebermann-Burchard, Salkowski and Hesse.
2. It crystallizes in plates from alcohol and in needles from ether.
3. Its melting point is 135° to 136° C.; the recorded m. p. for sitosterol is 135° to 137.5° C.[6]
4. It is precipitated as the colorless crystals of the digitonide when mixed with a 1% alcoholic digitonin solution.
5. The acetate melts at 128° to 129° C.; the sitosterol acetate has a m. p. varying from 125.6° to 137° C. depending upon the source of the sitosterol.[7]

The mother liquor yielding the phytosterol was concentrated to half of its volume when about one Gm. more of the sterol was crystallized. Further concentrations failed to yield more phytosterol, and the last traces of alcohol were removed from it on the steam-bath. The residue is a thick viscous liquid, retaining the deep blood-red color, and from which no precipitation has occurred over a period of several months. It is soluble in ether and in petroleum ether, but no crystallizations were obtained from these solutions.

II. *The Hot Alcohol-Insoluble Fraction.*—In hot alcohol, this fraction separated out as a chocolate-red liquid at the bottom of the beaker. Upon cooling it hardened into a body of a waxy consistency having a waxy feel and showing a waxy luster when cut with a knife and a dull rugged fracture when broken. This fraction was readily separated from the hot alcohol-soluble fraction since the latter was of a mushy consistency when cooled, whereas the waxy fraction became hard and solid. This fraction was repeatedly heated with alcohol to the point of lique-

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[1] L. Rosenthaler, "Chem. Invest. of Plants," 1st Edition (1930), page 78.

[2] *Ztschr. f. untersuch. d. Nahr. u. Genussm.*, 4 (1901), 865; 5 (1902), 1013.

[3] J. Lewkowitsch, "Chem. Tech. Fats, Oils and Waxes," 6th Edition, Vol. 1 (1921), page 280.

[4] *Ber. d. d. chem. Gesels.*, 42 (1909), 238.

[5] *Ibid.*, 39 (1906), 4379.

[6] J. Lewkowitsch, "Chem. Tech. Fats, Oils and Waxes," 6th Edition, Vol. 1 (1921).

[7] J. Lewkowitsch, *Ibid.*, 6th Edition, Vol. 1 (1921), page 600.

faction, and a similar separation made as described above. Of the hot alcohol-soluble extraction, the portion insoluble in the cold alcohol was filtered off and added to the melissyl alcohol yielding fraction (page 1000). The filtrate of this was added to the phytosterol yielding fraction (page 1001). This procedure was continued until no further extractive was yielded to hot alcohol.

The final alcohol-insoluble fraction was soluble in acetone from which it crystallized as a very light and fluffy mass of somewhat gummy consistency, but it was difficult to free it of all of the yellow color. All of the red color, however, was removed and was contained in the filtrate obtained by force filtering the crystalline mass.

The acetone filtrate with the red coloring matter was set aside, the acetone evaporated, leaving a residue of deep red color and of more or less greasy feel, non-waxy and of a soft consistency. This was washed with alcohol several times some of it going into solution from which a white crystalline substance was precipitated which gave positive sterol tests. Nothing further was done with the dark red residue. The crystals upon recrystallization from ether had a m. p. of 130° C. Apparently this is the same sterol identified as sitosterol but lack of material prevented further work on this sterol.

The crystalline mass which was obtained from the acetone and was insoluble in hot 95% alcohol, was melted and hot 95% alcohol added. This again threw the mass to the bottom of the beaker and upon cooling formed a hard, solid waxy mass suggestive of beeswax. There were 86 Gm. of this material. This was all dissolved in ethyl acetate and crystallized from it. After six successive crystallizations, a nearly white product was obtained, but the gummy or sticky feel was still in evidence but less pronounced. This crystalline material was then boiled with 95% alcohol for two minutes. The major portion of the fraction went into solution but rapidly settled out upon cooling below the boiling point as a pure white, amorphous, very light and caky mass.

Crystallizing from various solvents was attempted, the best results being obtained with chloroform; the gummy property was still a part of the crystalline mass.

These crystals were next treated for hydrocarbon separation according to the method of A. Leys,[1] in which the amyl alcoholic solution of the crystals was boiled with fuming hydrochloric acid. Upon cooling a solid layer separated out on the upper surface which was lifted off and purified by a repetition of the process. The amyl alcohol-hydrochloric acid mother liquor was mixed with a large quantity of water so as to wash out the acid. The amyl alcohol was then concentrated and a small amount of melissyl alcohol crystallized from it.

The solid layer, hydrocarbon in nature, crystallized from chloroform in silky, lustrous white scales or laminæ, having a m. p. of 69° to 69.5° C. Repeated attempts to make an acetate by heating with acetic anhydride under reflux for several hours failed to produce any results. The hydrocarbon would invariably crystallize out from chloroform or ethyl acetate with an unchanged melting point. It was not affected by concentrated sulphuric acid; it reacted negatively to the sterol tests; and it failed to decolorize a 1% permanganate solution. All of these

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[1] *Jour. de pharm. et de chim.*, Ser. 7, 5 (1912), 577. L. Rosenthaler, "Nachweis. d. Org. Verbind." (1923), 13.

tests are indications that the substance is hydrocarbon, and according to its melting point it may be characterized as triacontane which is reported in the literature as having a m. p. of 69° to 70° C. The yield was thirty-five Gm.

Verification of Triacontane ($C_{30}H_{62}$).—That the substance isolated is a hydrocarbon and that it might be triacontane is indicated by the following experimental work:

1. The substance is highly inactive, failing to form an acetate.
2. It is non-saponifiable and gives a negative test for sterol.
3. It is not affected by concentrated sulphuric acid.
4. It does not decolorize a 1% permanganate solution.
5. It was obtained in a manner that Leys prescribes for the isolation of hydrocarbon material.
6. While these 5 points classify it as a hydrocarbon, the m. p. of 69° to 69.5° C. classifies it as triacontane.

SUMMARY.

I. The percentage of total fat (petroleum-ether extractive) in digitalis leaves varies from 1.023% to 1.750%, a value much lower than any previously reported.

II. Organic compounds isolated from the fat and not previously reported in the literature are:

a. Fatty Acids—Saturated
  1. Myristic Acid
  2. Palmitic Acid
  3. Cerotic Acid
  4. Melissic Acid (?)

b. Fatty Acids—Unsaturated
  1. Oleic Acid
  2. Linolic Acid
  3. Linolenic Acid

c. Alcohols—Saturated
  1. Glycerol
  2. Melissyl Alcohol

d. Alcohols—Unsaturated (Sterols)
  1. Sitosterol

e. Hydrocarbons—Saturated
  1. Triacontane.

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IX. THE STANDARDIZATION AND STABILIZATION OF ERGOT PREPARATIONS.*

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The stability of the fluidextract of ergot has been a serious difficulty in its pharmaceutical and therapeutic use. Previously, Haskell and Eckler (1), Garcia (2), Prybill and Maurer (3), Wokes (4), Thompson (5), Swanson (6), Smith and Stohlman (7), Rowe and Scoville (8) and Swanson, Powell, Stevens and Stuart (9) have reported rapid deterioration of ergot preparations. The purpose of this investigation is a continuation of previous stability tests (6 and 9) and an attempt to ascertain definitely (*a*) whether or not the hydrogen-ion concentration factor, (*b*) buffer salts or (*c*) reducing agents prevent the deterioration of ergot and its alkaloids.

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* Scientific Section, Toronto meeting, 1932.