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THE COMPOSITION OF CORN WAX[1]

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The chilling of crude corn oil during the refining process causes separation of a small amount of solid material, which forms a thick, yellow, pasty mass after it has been filtered off. This substance was investigated as a possible source of phytosterols during a study of the unsaponifiable matter of corn oil, but a preliminary examination showed that no sterols were present. Although corn oil,[2] as well as its unsaponifiable matter,[3] has been studied by several investigators, no description of this substance is mentioned except the statement that the crude oil is sometimes cooled to effect the separation of stearin[4] and that the latter is disposed of as soap stock.

When a considerable quantity of the crude material became available,[5] it was examined further and found to be a wax. The wax was separated from the oil by mixing the paste with a large amount of ligroin and then centrifuging. After recrystallizing the wax from hot ligroin and amyl alcohol, beautiful shining plates were obtained; m. p., 81–82°. On saponification, the alcohol present was found to be myricyl alcohol which was identical with a sample of this substance prepared from beeswax. The acid obtained had a neutral equivalent corresponding to a C_{23} acid, and the molecular weight of the wax corresponded to that of the myricyl ester of tricosanoic acid.

Since the natural occurrence of such an odd carbon acid would be very unusual, a larger amount of the wax was saponified, the myricyl alcohol was extracted and the acid fraction was converted to the methyl ester. After four fractional distillations of the ester, in a vacuum, two fractions were obtained which corresponded to the methyl esters of a C_{22} and a C_{24} acid, indicating that the original wax was a mixture of the myricyl esters of these acids. All attempts to separate the original wax into its components were unsuccessful. Recrystallization of the wax from a variety of solvents failed to effect any change in the melting point.

The methyl esters obtained by fractionation were saponified and the

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[2] Baughman and Jamieson, THIS JOURNAL, **43**, 2696 (1921). See also Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," MacMillan and Co., New York, **1922**, vol. 2, p. 172.

[3] Anderson and Moore, THIS JOURNAL, **45**, 1944 (1923). Anderson and Shriner, *ibid.*, **48**, 2976 (1926).

[4] Sievers, *U. S. Dept. Agr. Bull.*, No. 904, **1920.**

[5] This material was kindly furnished by the Patent Cereal Co., Geneva, New York.

acids were recrystallized from several solvents including pyridine, according to the method of Brigl and Fuchs,[6] until the melting points were constant. One of the acids melted at 76–77° and had a molecular weight corresponding to a C_{22} acid, and the other melted at 85–86°, its molecular weight indicating a C_{24} acid.

There is considerable confusion in the literature regarding the higher fatty acids, and difficulty was experienced in identifying these substances with other naturally occurring acids previously described. Since palmitic and stearic acids are known to possess straight chains and have been found in corn oil,[2] it might be thought that the higher acids would also have a normal structure. The C_{24} acid was finally identified as *n*-tetracosanoic acid, with which its properties agreed exactly, and a mixed melting point with the synthetic acid prepared by Levene and Taylor[7] showed no depression. We wish to acknowledge our indebtedness to Dr. P. A. Levene for his kindness in comparing these acids for us. This is the first instance that *n*-tetracosanoic acid has been found to occur naturally, all other C_{24} acids reported apparently not having a straight chain. Brigl and Fuchs[6] have reported the separation of lignoceric acid, m. p. 80–81°, from beechwood tar into *n*-tetracosanoic acid, m. p. 85–86°, and an isomeric acid, m p. 74°, but Levene, Taylor and Haller[8] could not confirm this work using lignoceric acid from peanut oil or from cerasin.

A comparison of the properties of the C_{22} acid, m. p. 76–77°, with those of other acids reported in the literature shows that it is not *n*-docosanoic or behenic acid; m. p., 81–82°. It was at first thought that the acid obtained from the wax was impure behenic acid, but after a long series of crystallizations and conversion to the amide and hydrolysis of this derivative, it still melted at the same temperature and hence was undoubtedly pure.

Arachidic acid, m. p. 76–77°, also has been reported to be present in corn oil,[2] but Ehrenstein and Stuewer[9] have obtained evidence that this is really a C_{22} acid and not C_{20}, and state that it is apparently identical with the *iso*behenic acid obtained from lignoceric acid. Holde and Godbole[10] have fractionated "crude arachidic acid" and obtained lignoceric acid and a C_{26} acid corresponding to cerotic acid. Since the status of arachidic acid is so uncertain, no comparison can be drawn between it and the acid isolated in the present research.

Through the courtesy of Dr. C. R. Noller of the University of Illinois, samples of behenic acid, m. p. 81°, and *n*-eicosanoic acid, m. p. 75–76°, were made available, and a series of mixed melting points was made with the C_{22} acid obtained in the present research. Depression of the melting

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[6] Brigl and Fuchs, *Z. physiol. Chem.*, **119**, 280 (1922).

[7] Levene and Taylor, *J. Biol. Chem.*, **59**, 905 (1924).

[8] Levene, Taylor and Haller, *ibid.*, **61**, 157 (1924).

[9] Ehrenstein and Stuewer, *J. prakt. Chem.*, [2] **105**, 199 (1923).

[10] Holde and Godbole, *Ber.*, **59**, 36 (1926).

point occurred with both behenic and *n*-eicosanoic. Hence, it was concluded that this substance, melting at 76–77° and possessing the molecular weight of a C_{22} acid, represents an *iso*behenic acid and does not possess a straight chain. It is probably a homolog of the other forked-chain acids such as lignoceric, which on degradation yields an *iso*behenic acid,[11] m. p. 75–76°, and with which it may be identical.

This wax is another example of the numerous plant waxes occurring in nature. It is present in very small amount, 120 g. of pure wax being obtained from 13.5 kg. of thick paste, the latter representing the concentrate from a very large amount of corn oil; hence, the amount actually present in the corn germ must be very small indeed.

It is an interesting fact that one of the constituents of this wax is the myricyl ester of a straight-chain acid, while the other is a myricyl ester of a forked-chain acid. Any theory to account for the synthesis of fatty acids in the plant must be capable of explaining the formation of both straight- and forked-chain acids.

Experimental Part

Purification of Corn Wax.—The wax is present as a very voluminous precipitate in the oil and cannot readily be filtered off. In order to effect its separation, the thick, semi-solid paste obtained during the refining of corn oil was mixed with an equal volume of ligroin and centrifuged. The precipitated paste was then dissolved in hot ligroin, the solution filtered and allowed to cool. Two hundred and twenty g. of solid matter was obtained from 13.5 kg. of the original paste. This material still contained some oil and was recrystallized from amyl alcohol, boiled with acetone, in which the wax is insoluble when cold, filtered and then finally recrystallized from ligroin, from which it separated in feathery crystals; m. p.,[12] 81–82.5°; yield, 120 g.

The sample for analysis was further purified by recrystallization from ligroin and from amyl alcohol, and washed with acetone. The washing with acetone is essential in order to remove the last traces of ligroin and amyl alcohol, which are retained by the wax with great tenacity. The substance still melted at 81–82.5°, and further recrystallizations failed to effect any change in the melting point.

Anal. Subs., 0.1500, 0.1364: CO_2, 0.4488, 0.4094; H_2O, 0.1900, 0.1737. Found: C, 81.60, 81.85; H, 14.17, 14.25.

Mol. wt. Subs., 0.8182, 1.6589; $C_{10}H_8$, 23.600, 23.600: Δt, 0.306°, 0.625°. Found: mol. wt., 781.7, 776.0; av., 778.8.

The calculated molecular weight for a myricyl ester of docosanoic acid is 760.4, of tricosanoic acid, 774.4, and of tetracosanoic acid, 788.4.

The wax is insoluble in cold ether, alcohol, ligroin, amyl alcohol or benzene but is somewhat soluble in carbon tetrachloride and chloroform. A chloroform solution of the wax was optically inactive.

Saponification.—One hundred and twenty g. of the wax, purified as described, was saponified by refluxing with 20 g. of potassium hydroxide in 500 cc. of alcohol for 48 hours. One liter of warm water was added and the hot solution was extracted with eight successive portions of 250 cc. of hot benzene. Upon evaporation of the benzene

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[11] Meyer, Brod and Soyka, *Monatsh.*, **34**, 1113 (1913).

[12] All melting points are corrected.

the myricyl alcohol separated out; m. p., 82–84°. It was again boiled with alcoholic potassium hydroxide, an alcoholic solution of calcium chloride was added to precipitate the last traces of fatty acids, and the hot solution, after it had been filtered, was diluted with water and the myricyl alcohol filtered off. The substance was then recrystallized from alcohol and benzene (1:1) and from ligroin, after which it melted at 87–88°.

Anal. Subs., 0.1204: CO_2, 0.3625; H_2O, 0.1530. Calcd. for $C_{30}H_{61}OH$: C, 82.19; H, 14.15. Found: C, 82.11; H, 14.22.

The acetyl derivative was prepared by boiling with acetic anhydride and crystallizing the resulting acetate from alcohol; m. p., 73°.

Anal. Subs., 0.1185: CO_2, 0.3481; H, 0.1439. Calcd. for $C_{30}H_{61}OCOCH_3$: C, 79.92; H, 13.43. Found: C, 80.11; H, 13.59.

A sample of myricyl alcohol was prepared from beeswax. It melted at 87–88° after purification by the method described above, and a mixed melting point with that obtained from corn wax showed no depression. It should be mentioned that the melting point of myricyl alcohol is often given in the literature[13] as 85°. In order to raise its melting point to 87–88° it is necessary to treat it with alcoholic potassium hydroxide twice and then recrystallize from a mixture of benzene and alcohol (1:1) and finally from ligroin.

The Fatty Acids.—The hot soap solution, freed from myricyl alcohol, was acidified with hydrochloric acid and the solid cake of fatty acids was separated after the mixture had cooled. It was then crystallized from alcohol and acetone, after which the acid melted at 74° and had a neutral equivalent of 357. No unsaturated acids were present, as shown by the fact that the substance did not decolorize a carbon tetrachloride solution of bromine.

The acid was esterified by refluxing with 500 cc. of absolute methyl alcohol and 50 cc. of concd. sulfuric acid for 24 hours. The ester formed an oily layer on top of the methyl alcohol, and after diluting the latter with water and cooling, the ester separated as a solid which was filtered off and dried; yield, 40 g. The ester was fractionally distilled in a vacuum, using a modified Claisen flask.[14] After four fractionations the following fractions were obtained.

	B. p., °C. (1 mm.)	Wt., g.	M. p., °C.
1	175–195	4	45–46
2	200–212	5	52–53
3	209–215	14	52–54
4	215–223	4	55–56
5	223–230	7	57–59

Fractions 1, 3 and 5 were then recrystallized twice from acetone and melted at 47–52°, 53–54° and 58–59°, respectively.

FRACTIONS 1 AND 2.—These consisted chiefly of the methyl ester of the C_{22} acid. On saponification of Fraction 1 and isolation of the acid, it melted at 76–77° after one crystallization from alcohol and had a neutral equivalent of 331.4; calcd. for $C_{22}H_{44}O_2$: 340.4.

FRACTION 3.—This was the largest fraction obtained and since its melting point is the same as that of methyl behenate, 53–54°, it was thought that the acid obtained by saponification of this fraction was impure behenic acid. The acid melted at 76–77°,

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[13] Gascard, *J. Soc. Chem. Ind.*, **12**, 955 (1893); *J. pharm. chim.*, [5] **28**, 49 (1893). Brodie, *Ann.*, **71**, 144 (1849). Schwalb, *Ann.*, **235**, 106 (1886).

[14] Adams, "Organic Syntheses," John Wiley and Sons, New York, **1921**, vol. I, p. 40.

while behenic acid melts at 81–82°. Considerable effort was spent in attempting to purify this acid and it was crystallized from methyl alcohol, ethyl alcohol and acetone; twice from ethyl acetate and pyridine. It was then melted over dil. hydrochloric acid and finally twice crystallized from acetone. The acid still melted at 76–77°.

Anal. Subs., 0.1534: CO_2, 0.4353; H_2O, 0.1779. Calcd. for $C_{22}H_{44}O_2$: C, 77.64; H, 12.94. Found: C, 77.39; H, 12.98.

Mol. wt. Subs., 0.2958: 14.71 cc. of 0.059 *N* alkali. Calcd. for $C_{22}H_{44}O_2$: mol. wt., 340. Found: 340.8.

The amide was prepared by heating 0.5 g. of the acid with 20 g. of thionyl chloride for five minutes and pouring into concd. ammonium hydroxide. Recrystallization from alcohol and ethyl acetate yielded white crystals; m. p., 110–111°.

Anal. Subs., 0.1270: 38.91 cc. of 0.009984 *N* acid. Calcd. for $C_{22}H_{45}ON$: N, 4.12. Found: 4.28.

Hydrolysis of this amide with alcoholic potassium hydroxide yielded an acid having the same melting point as the original, and a mixed melting point with the original showed no depression. It is evident that the sample of acid prepared above was pure and represented a C_{22} acid different from the normal straight-chain behenic acid.

A mixture of this acid, m. p. 76–77°, with behenic acid, m. p. 81–82°, melted at 74–76°, and with *n*-eicosanoic the mixture melted at 70–71°.

FRACTION 4.—This fraction was evidently a mixture of Fractions 3 and 5.

FRACTION 5.—This ester, m. p. 58–59°, corresponds to methyl ester of *n*-tetracosanoic acid. Upon saponification, the acid melted at 79–80°, and on purification by crystallization from pyridine, acetone and methyl alcohol, the melting point rose to 85–86° and was not changed by further crystallization.

Anal. Subs., 0.1221: CO_2, 0.3503; H_2O, 0.1454. Calcd. for $C_{24}H_{48}O_2$: C, 78.17; H, 13.14. Found: C, 78.24; H, 13.32.

Mol. wt. Subs., 0.3736: 17.28 cc. of 0.05900 *N* alkali. Calcd. for $C_{24}H_{48}O_2$: mol. wt., 368. Found: 366.5.

A mixed melting point with *n*-tetracosanoic acid prepared by Levene and Taylor[7] showed no depression, and the properties of the two compounds agreed exactly.

Summary

1. The solid matter separating when crude corn oil is chilled has been found to be a mixture of two waxes.
2. One of these is the myricyl ester of *n*-tetracosanoic acid.
3. The other wax is the myricyl ester of an *iso*behenic acid.

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