Solidification Point Curves of Binary Acid Mixtures

III. Tetracosanoic to Triacontanoic Acids[1]

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The composition of the wax acids and alcohols was a moot question until some thirteen years ago. What are now known to be mixtures of the higher acids from C_{26} to C_{32} had been regarded as individual entities and were designated as such. For example, Chinese insect wax was deemed to consist primarily of a simple ester of ceryl alcohol and cerotic acid. Each of these substances, however, has since been shown to be a mixture consisting of at least three components. Another example of the confused state of knowledge on the subject of wax composition lay in the question of the existence of wax acids and alcohols containing an odd number of carbon atoms.

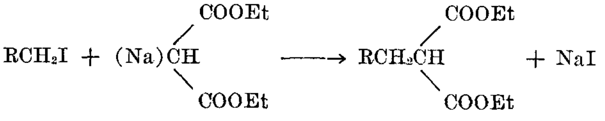
Holde and Bleyberg (5) in 1930 claimed to have identified the "even" acids from C_{24} to C_{32}, inclusive, in various waxes. Shortly thereafter Chibnall *et al* (2) disproved the occurrence of any "odd" acids and alcohols and showed that most of the so-called wax acids previously isolated by others, such as cerotic, montanic and melissic, were mixtures of three or more components in the C_{24}-C_{30} range. This work was essentially qualitative in character and was accomplished by X-ray and melting-point comparisons of synthetic and naturally occurring mixtures. No actual separations were made.

In earlier communications (6) from this Laboratory on the subject of the analytical chemistry of the fatty acids, we have discussed the role of solidification points in their identification and determination and have presented the diagrams for binary mixtures of consecutive pairs of the even acids from decanoic to tetracosanoic. The series having been now extended to triacontanoic acid, pertinent data and diagrams, as before, are herein presented as one phase of an attempt to put the determination of the so-called wax acids on a quantitative basis. Because the isolation of these acids in a pure form from natural sources is impractical, it was necessary to synthesize them. The procedure followed was essentially that used by Bleyburg and Ulrich (1).

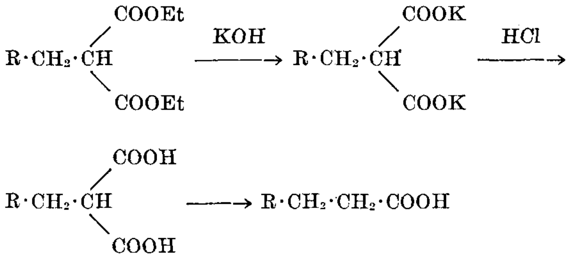
Erucic acid, isolated from rape seed oil, was used as the starting material. Its ethyl ester was converted to ethyl behenate by hydrogenation over Raney nickel catalyst. After that the following sequence of reactions, based on the classic malonic ester synthesis, was carried out:

$$RCOOEt + H_2 \xrightarrow[\text{(copper chromite)}]{\text{250°C., 200 atm.}} RCH_2OH$$

$$RCH_2OH \xrightarrow{PI_3} RCH_2I$$



$$RCH_2I + (Na)CH\begin{matrix}COOEt\\ COOEt\end{matrix} \longrightarrow RCH_2CH\begin{matrix}COOEt\\ COOEt\end{matrix} + NaI$$



$$R\cdot CH_2\cdot CH\begin{matrix}COOEt\\ COOEt\end{matrix} \xrightarrow{KOH} R\cdot CH_2\cdot CH\begin{matrix}COOK\\ COOK\end{matrix} \xrightarrow{HCl}$$

$$R\cdot CH_2\cdot CH\begin{matrix}COOH\\ COOH\end{matrix} \longrightarrow R\cdot CH_2\cdot CH_2\cdot COOH$$

It may be pointed out that the success of such a synthesis, which must be repeated for each of the acids desired, depends on the exercise of great care in every step particularly with respect to purity and yield of the product. The alcohols must be ester-free and the acid finally obtained must be rid of any associated unsaponifiable matter. The use of barium soaps and extraction with petroleum ether fulfilled both these requirements. A representative synthesis is described.

The solidification points (Table 1) of the four "even" acids which were synthesized were found to be in agreement with what are deemed to be the best literature values. The determinations themselves were made as previously described (6). Approximately twenty different mixtures of three pairs of adjacent acids were made (Table 2) and diagrams were constructed (Figure 1) from the pertinent solidification-point composition data, as before.

TABLE 1
Transition Point Data

Acid	Solidification Point		Resolidification Point[2] (4)
	Observed °C.	Francis and Piper (4) °C.	
Tetracosanoic	83.82 83.45[1]	83.90	83.8
Hexacosanoic	87.38 87.05[1]	87.40	87.2
Octacosanoic	90.48	....	90.4
Triacontanoic	93.10	....	93.2

[1] The two values given here for the C_{24} and C_{26} acids result from different preparations.

[2] The resolidification point is that temperature at which the *partly* molten specimen in the capillary tube commences to resolidify when the temperature is lowered very slowly (4). It is a close approximation of the true solidification point.

Description of a Typical Synthesis

The synthesis of triacontanoic acid from the ethyl ester of octacosanoic acid is described as a typical case.

Conversion of ester to alcohol. The ethyl ester, prepared by the usual method, was reduced to the alcohol by high-pressure hydrogenation (200 at.) over copper chromite at 250°. The reaction mixture—it did not contain more than 5 pct. of unreduced ester in any of the syntheses—was then boiled under reflux with alcoholic potassium hydroxide solution, after which barium chloride solution was added to the hot mixture. The whole was then cooled to 0°C., after which the mixture of crystalline alcohol and precipitated barium soap mixture was filtered off, dried, and extracted with hot petroleum ether in an intermittent

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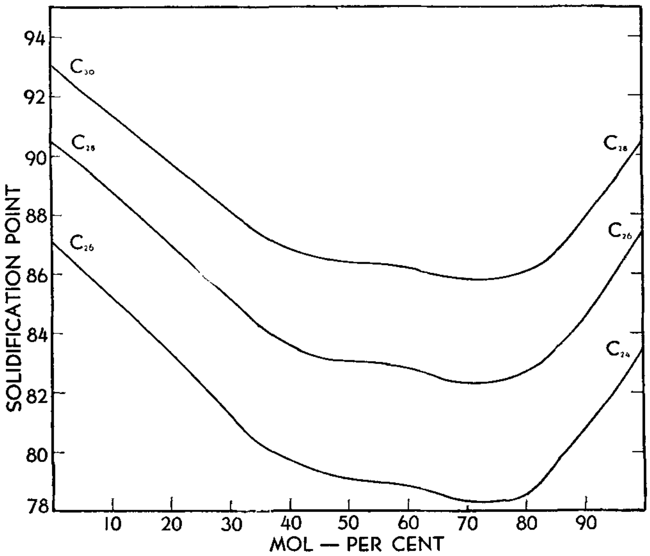

FIG. 1. Solidification Point Diagrams of Binary Mixtures of Tetracosanoic, Hexacosanoic, Octacosanoic, and Triacontanoic Acids.

type of extractor. By this procedure an alcohol free of the corresponding ester was obtained.

Conversion of alcohol to iodide. Octacosanol (25 g.), iodine (10 g.) and red phosphorus (1 g.) were heated for three hours at 180°C. in a flask equipped with stirrer and reflux. The iodide was removed by extraction with petroleum ether; the resulting solution was washed, first with water, then with 5-pct. sodium hydroxide solution, and finally with water. On evaporation, after having dried over calcium chloride, octacosanyl iodide in 96 pct. yield, or 30.5 g. was obtained.

Conversion of iodide to dicarboxylic acid. Sodium (3.4 g.) was dissolved in 30 cc. anhydrous, boiling *n*-butyl alcohol, in a dry three-necked flask equipped with an efficient stirrer, a dropping funnel and a reflux condenser fitted with a calcium chloride tube. To the resulting suspension of sodium butoxide dry diethyl malonate (50 cc.) was added, conversion to sodium malonic ester being insured by refluxing the whole for fifteen minutes. This operation was followed by the addition of the octacosanyl iodide after which the reaction mixture was kept at its boiling point for six hours. To the cooled mixture 500 cc. of ethyl alcohol and a 50-pct. excess of potassium hydroxide were added. The whole was heated for six hours, then chilled and filtered, and the resulting soaps air-dried at room temperature. On boiling a solution of these soaps with an excess of hydrochloric acid, the solid dicarboxylic acid separated in white granular particles. It was filtered off, washed with water until free of salts and hydrochloric acid, then dried *in vacuo* at 110°C. A yield of 28.3 g. equivalent to 97 pct., was obtained.

Conversion of dicarboxylic acid to n-acid. The dicarboxylic acid thus obtained was placed in a vacuum distillation apparatus and heated until the *n*-aliphatic acid refluxed on the sides of the flask (250°C.). Most of the decarboxylation took place at 140-160°C. when the pressure rose to several millimeters of mercury; after this, however, the pressure dropped to 0.1 mm. Approximately 25 g. of crude triacontanoic acid with a solidification point of 92.5°C. (about .7° low) was obtained. The yield, dicarboxylic acid to triacontanoic acid, was 100 per cent.

Purification of the acid. The crude acid was dissolved in 500 cc. of ethyl alcohol. A 50 pct. excess of potassium hydroxide was then added. The resultant suspension of potassium soaps in hot alcohol was put in solution by adding hot water. A solution of barium chloride sufficient to precipitate all the acid was added and the suspension refluxed for fifteen minutes. The barium soaps were filtered off, dried, and then extracted with hot petroleum ether in an intermittent extractor to remove unsaponifiable matter. The barium soaps were acidified with hydrochloric acid, taken up in petroleum ether, washed, dried, filtered, and crystallized to yield 23.2 g. of pure triacontanoic acid (solidification point 93.10°C.) which did not change in solidification point on recrystallization. The yield, crude acid to pure acid, was 92 pct. The overall yield from octacosanol to triacontanoic acid was 88 pct.

TABLE 2

Solidification Points of Binary Acid Mixtures

Tetracosanoic-Hexacosanoic Acids		Hexacosanoic-Octacosanoic Acids		Octacosanoic-Triacontanoic Acids	
Composition C_{24}-acid mol-pct.	Solidification Point °C. (corr.)	Composition C_{26}-acid mol-pct.	Solidification Point °C. (corr.)	Composition C_{28}-acid mol-pct.	Solidification Point °C. (corr.)
0.00	87.05	0.00	90.48	0.00	93.10
4.96	86.21	8.16	89.17	7.94	91.79
9.97	85.33	15.96	87.79	15.80	90.54
14.84	84.34	23.63	86.38	23.67	89.25
19.81	83.35	31.40	85.00	31.40	87.96
23.95	82.52	36.63	83.91	36.45	87.13
29.95	81.29	41.58	83.48	41.53	86.74
34.85	80.27	44.22	83.30	44.00	86.55
39.35	79.76	46.80	83.11	46.54	86.42
44.54	79.37	49.10	83.04	49.14	86.35
49.79	79.04	51.81	82.98	51.67	86.34
54.44	78.94	56.61	82.89	56.61	86.31
59.90	78.84	61.79	82.80	61.62	86.10
64.23	78.60	66.48	82.41	66.41	85.83
69.15	78.35	71.40	82.28	71.38	85.75
74.63	78.38	73.81	82.31	73.84	85.78
79.51	78.43	76.33	82.37	76.10	85.85
85.95	79.82	80.95	82.67	80.93	86.08
91.12	80.96	85.92	83.52	85.78	86.74
95.76	82.21	92.92	85.31	92.92	88.59
100.00	83.45	100.00	87.38	100.00	90.48

TABLE 3

Distillation Data on Ethyl Esters of Acids of Chinese Wax

Fraction	Boiling Point at 0.25 mm. °C.	Weight g.	Melting Point of Esters °C.	Solidification Point of Acids °C.
0		115.0	60.0	77.85
1	204-217	28.0	55.0	76.30
2	217-222	29.2	59.0	79.75
3	222-226	21.0	60.8	80.70
4	226-228	7.7	61.7	81.30
5	228-229	7.7	62.5	81.70
6	229-229	6.4	64	84.00
Residue	....	14.1	......	84.00

Application

These solidification-point diagrams can be applied to the wax alcohols by converting the alcohols to acids either by direct oxidation or by a malonic ester synthesis. Of immediate interest, however, was an attempt at separation of the acids of a typical wax, such as Chinese insect wax, into binary mixtures. Repeated attempts at separation by distillation of the ethyl esters through a Widmer column proved unsuccessful for reasons which will become apparent by reference to the distillation. Data in Table 3.

It is to be noted that while the melting points of the esters indicate a possible separation, the solidification points of the acids are not high enough to correspond to a binary mixture of the expected acids. This can be readily seen by reference to the diagrams which indicate the minimum solidification point a binary mixture of acids may have. As the work of Chibnall *et al* (2) indicates the presence of the C_{26}, C_{28} and C_{30} acids in Chinese insect wax, fraction 2, if separation had been achieved might be expected to be a mixture of C_{26} and C_{28} acids. The minimum solidification point for such a binary mixture would be 82.3° while that actually observed for fraction 2 was 79.75°. This is a clear indication that no worth-while fractionation had been secured.

Despite these data, there is an excellent possibility that by the use of a more efficient fractionating column separation at least into binary mixtures could be accomplished with these higher fatty acids. The fact that use of the ordinary Widmer column led to a certain amount of separation as evidenced by the spread in the melting point of the esters and the solidification points of the acids encourages the view that these acids can be analyzed by ester fractionation combined with the use of solidification-point diagrams. Work by others also tends to indicate that fractionation is possible. Collins (3), in an attempt at preparing pure hexacosanoic acid from Chinese insect wax also tried to fractionate the mixed esters. The use of a fractionating column gave results analogous to those obtained here and molecular distillation gave further separation but did not give pure components. Holde and Bleyburg (5) isolated several of the higher fatty acids from C_{24} to C_{30} by fractional distillation and recrystallization but details of their apparatus are not given.

Summary

The solidification point diagrams of binary mixtures of tetracosanoic to triacontanoic acids have been presented along with a representative synthesis of one of the acids. The possibility of using these diagrams for the analysis of the wax acid mixtures has been discussed, but an attempt to separate the acids of Chinese insect wax by ester fractionation into binary mixtures proved unsuccessful.

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The Viability, Chemical Composition and Internal Microflora of Frost Damaged Soybeans[1]

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Unseasonable early frosts in September, 1942, followed by warm dry weather in the more northerly soybean producing areas of Iowa, Illinois and Minnesota, resulted in much of the crop reaching the market at normal moisture levels but containing varying percentages of immature and frost-damaged beans. As this widespread condition was a new experience since soybeans became a major crop in these areas, questions arose as to the storage behavior, viability, feeding and processing values of such soybeans. In connection with a study of the respiratory behavior of soybeans of varying grade, a limited number of 1942-crop samples were collected. Incidental to the respiration studies, certain analytical data were obtained which appear of interest as an index of the value of immature and frosted soybeans for seed, feeding and industrial purposes. The data include commercial grade, viability, proximate feedingstuffs analyses, acidity and iodine value of the oil, phosphate acidity, amino-acid acidity, nonprotein nitrogen, reducing- and nonreducing sugars. In addition, a qualitative and semiquantitative study was made of the internal aerobic microflora.

Materials and Methods

The experimental material comprised two series of 1942-crop samples, one from Minnesota and the other from Illinois. The Minnesota series consisted of 7 composites, showing increasing increments of immature and frost-damaged kernels, which were prepared from 84 samples obtained from the various growing areas of the state. The Illinois series comprised 7 samples taken at market points from farmers' deliveries.

Size of seed was determined in triplicate by counting and weighing 100 seeds; the weights were calculated to a dry-matter basis. Official grades, including test weight per bushel and damaged kernel content, were supplied by the Minneapolis office of the Grain Products Branch, Food Distribution Administration, U. S. Department of Agriculture. Germination tests were made by the Minnesota Seed Testing Laboratory. Proximate feedingstuffs analyses were carried out by the official methods of the Association of Official Agricultural Chemists (1), with the exception of oil content. This departure involved extracting the ground samples with petroleum ether (Skellysolve F) for 12 hours, regrinding with sand, and re-extracting for an additional 14 hours. Iodine values were calculated from the refractive indices of the oils, determined with a Zeiss refractometer equipped with a water jacketed prism head (n^{25}_{D} 1.46680 to 1.50210), using the following equation of Majors and Milner (2):

$$\text{Iodine Value} = 8626.877\, n^{25}_{D} - 12575.226.$$

Oil acidity, phosphate acidity, and *amino-acid acidity* were determined by the methods outlined by Zeleny and Coleman (3) for cereals. Nonprotein nitrogen

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