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Studies on the Chemistry of the Fatty Acids. VII. The Multiple Nature of the Linoleic and Linolenic Acids Prepared by the Bromination–Debromination Procedure. The Purification of These Acids by Repeated Low-Temperature Crystallization

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The bromination–debromination procedure has been universally used for the isolation of linoleic and linolenic acids because it is the only method which yields products which are pure as evaluated by iodine number. This procedure involves bromination of natural fatty acid mixtures, purification of the solid tetra- or hexabromides, and removal of bromine with zinc. Erdmann and Bedford[1] carried out the debromination reaction in boiling ethyl alcohol. Rollett[2-9] and many others have used strongly acidified alcohol, or have added acid later to decompose zinc soaps and to assure complete esterification. Kaufmann and Mestern[10] used boiling pyridine to avoid ester formation. In several recent reports from this Laboratory we have described low-temperature crystallization procedures for the isolation of these acids in a considerably less pure state than that which results from the debromination method.[11,12,13] By this method, however, the problem of bromide isomerism is not introduced. Brown and Frankel[12] compared the tetrabromide number of debromination linoleic acid with that of 93% crystallization linoleic acid and found them both to be about 91. On the basis of this and other considerations, it was concluded that the linoleic acids prepared by the two methods were identical. In the case of linolenic acid Shinowara and Brown[13] found the hexabromide number of the debromination acid to be 75 while that of the crystallization product was 92 (an average of several preparations). On the basis of these results it was concluded that the linolenic acids prepared by either procedure were dissimilar mixtures of isomeric acids.

The multiple nature of linolenic acid prepared by debromination was suggested in 1909 by Erdmann and Bedford,[1] who considered it to be a mixture of 25% α-linolenic acid which was the true linolenic acid and identical with the natural and 75% of β-linolenic acid, isomeric with the original and yielding no ether-insoluble hexabromide. Rollett disputed this view and believed that debromination linolenic acid was a single acid which yields optically isomeric bromides of widely differing solubility. Rollett's view, as applied to both linoleic and linolenic acids, has been almost universally accepted by modern workers.

The present investigation came about in the course of preparation of very pure linoleic and linolenic acids for use in a general study of the reaction of these acids with thiocyanogen. Crystallization of a specimen of debromination linolenic acid four times from petroleum ether gave a product of higher hexabromide number than the acids recovered in the combined filtrates from the crystallizations.[14] This fact, along with the observation that the crystal and filtrate fractions had different melting points, although the iodine numbers were almost equal, suggested to us that the original debromination acid was a mixture of at least two isomers, one of which was present in predominating amounts, and the other a lower-melting isomer (or mixture of isomers) which gives a lower or zero hexabromide number. Following this, a similar result was found with linoleic acid. A thorough study was then made of specimens of debromination linoleic and linolenic acids in which they were repeatedly crystallized and the yields and constants of the many filtrates and final products were determined. As a result

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(1) Erdmann and Bedford, *Ber.*, **42**, 1324 (1909); *Z. physiol. Chem.*, **69**, 76 (1910).
(2) Rollett, *Z. physiol. Chem.*, **62**, 410, 422 (1909).
(3) Birosel, THIS JOURNAL, **59**, 689 (1937).
(4) Green and Hilditch, *Biochem. J.*, **29**, 1552 (1935).
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(6) McCutcheon, *Can. J. Research*, **16B**, 158 (1938).
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(9) McCutcheon, *Can. J. Research*, **18B**, 231 (1940).
(10) Kaufmann and Mestern, *Ber.*, **69B**, 2684 (1936).
(11) Brown and Stoner, THIS JOURNAL, **59**, 3 (1937).
(12) Brown and Frankel, *ibid.*, **60**, 54 (1938).
(13) Shinowara and Brown, *ibid.*, **60**, 2734 (1938).

(14) Reported at the April (1940) meeting of the American Chemical Society in Cincinnati.

TABLE I

ANALYTICAL DATA ON VARIOUS PREPARATIONS OF LINOLEIC AND LINOLENIC ACIDS

Specimen	Description	Iodine no.	Tetra- or hexabromide no.	Mol. wt.	n^{20}	M. p., °C.
			Linoleic Acid			
...	Calculated	181.2	(214.2)	280.3	....	....
(1)	Debromination acid	180.7		...	....	....
(2)	No. 1, twice cryst.	180.9	94.1	...	1.4689	....
(3)	Debromination acid	181.2	90.6	280.9	1.4699	− 8.8 to − 7.1
(4)	No. 3, 8 times cryst.	181.2	102.0	...	1.4698	....
(5)[c,f]	No. 3, 12 times cryst.	181.0	102.9	280.9	1.4699	− 5.2 to − 5.0
			Linolenic Acids			
...	Calculated	273.7	(272.3)	278.2	....	....
(6)	Debromination acid	272.6	77.4	278.0	1.4800	−12.8[a]
(7)	No. 6, 4 times cryst.	272.3	88.7	...	1.4800	−12.0 to −11.6
(8)	Debromination acid	271.7	81.4	277.3	1.4798	−13.2[a]
(9)	No. 8, 7 times cryst.	272.0	92.1	...	1.4797	....
(10)	No. 8, 10 times cryst.	272.2	93.6	...	1.4797	....
(11)	No. 8, 6 times cryst.	271.5	89.8[b]	...	1.4799	....
(12)[d,f]	Nos. 10 and 11, 4 times cryst.	273.8	96.0	278.1	1.4800	−11.3 to −11.0
(13)[e]	No. 8, 6 times cryst.	272.9	94.0	278.0	1.4800	−11.6 to −11.0

[a] These specimens did not appear entirely solid at −23°. [b] This value is low due to slight mechanical loss. [c] d^{20}_4 0.9022. Rollett reported d^{18}_4 0.9026. [d] d^{20}_4 0.9157. [e] d^{20}_4 0.9164. This specimen is comparable with no. (11), and is included because of its more complete analytical data. [f] Observed molecular refraction of (5) 86.65, calcd. 85.93. Observed molecular refraction of (12) 86.30, calcd. 85.46.

of this work it has been shown that acids prepared by the debromination procedure contain appreciable amounts of isomeric acids, which, we believe, are the result of isomerization during the removal of bromine.

Experimental Part

Preparation of Tetrabromostearic and Hexabromostearic Acids.—Tetrabromostearic acid was prepared as described by Brown and Frankel.[12] It was purified by repeated crystallization from a hot ether solution by cooling to −22°; m. p. 115–115.5°. Hexabromostearic acid was prepared from the fatty acids of perilla oil. It was purified by crystallizing three times from a 10% solution in boiling xylene. The product melted at 183.7–184.5°, when heated with a temperature rise of 1° per minute, starting at 170°. We have found that on longer heating the melting point is lower, which confirms McCutcheon's observation[9] that hexabromides are slowly altered by heat at this temperature. Our observed melting point is considerably higher than that reported by McCutcheon, who purified his bromides by crystallization from dioxane.

The Preparation of Debromination Linoleic Acid and its Separation into Fractions by Crystallization.—Linoleic acid was prepared as described by Brown and Frankel[12] by treatment of pure tetrabromostearic acid in boiling neutral methyl alcohol with an excess of zinc. After vigorous reaction ceased, small amounts of hydrochloric acid were added to assure decomposition of zinc soaps. The product was recovered and distilled; the small amounts of ester formed (4%) were removed by saponification, acidifying and again distilling at 1–2 mm.

In a preliminary experiment 59 g. of debromination acid, no. 1, was twice crystallized from 600 cc. of petroleum ether at −57°. (Acid specimen numbers here and below refer to specimens in Table I.) The crystal fraction, 28 g., no. 2, gave a tetrabromide number of 94.1, a value definitely higher than 90.6 which we have previously reported.[12]

In a carefully controlled recrystallization study, 170 g. of linoleic acid, no. 3, was dissolved in 3400 cc. of petroleum ether (b.p. 30–60°), and cooled to −65° in a methyl alcohol-bath cooled with dry-ice. The solution was fil-

TABLE II

RESULTS OF REPEATED CRYSTALLIZATION OF DEBROMINATION LINOLEIC ACID

	Weight g.	Tetrabromide no.	Isomeric[a] acid, g.	Iodine no.	n^{20}
Original acid	170.0	90.6	20.6	181.2	1.4699
Filtrate I	8.8	44.8	5.0	179.0	1.4700
II	7.4	45.8	5.1	177.5	1.4700
III	8.6	61.9	3.5	179.2	1.4697
IV	5.3	52.2	2.6	179.5	1.4697
V	4.3	53.8	2.0	181.8	1.4698
VI	4.9	57.5	2.2	181.2	1.4699
VII	5.8	53.3	2.8	179.8	1.4699
VIII	3.5	66.7	1.2	179.5	1.4700
IX	5.3	89.7	0.7	180.6	1.4699
X	5.2	93.9	.5	181.1	1.4699
XI	4.1	97.2	.24	180.5	1.4698
XII	5.1	97.9	.25	180.7	1.4700
Final crystal.[b]	92.0	102.9	.0[c]	181.0	1.4699

[a] For the calculation of these weights the tetrabromide number of the "isomeric acid" was assumed to be zero and that of the pure linoleic acid, 102.9. [b] This is 5, Table I. [c] The final crystal product here is assumed to be pure linoleic acid.

tered while still immersed in the bath by inverted suction filtration. Eight such crystallizations were thus carried out consecutively at 3400 cc. volume (crystals are no. 4), and 4 more at −60° from the same volume, making a total of twelve crystallizations. The acids of the individual filtrates and the final crystal fraction, no. 5, were recovered and examined. The data are in Table II.

The multiple nature of debromination linoleic acid is supported by the following facts. Although the crystallizations were carried out at nearly constant volume of solution, the amount of dissolved material decreased from 8.8 g. in the filtrate I to a constant value of 4–5 g. in the final filtrates. From this constant value, the solubility of linoleic acid is about 1.6 g. per liter at −60°. The tetrabromide numbers of the filtrates rose from 44.8 to 97.9; thus, they tend to approach the value 102.9 for the pure acid in the final crystal fraction. These values would be somewhat higher if corrections were made for solubility of the tetrabromides in wash solvent. The methods used in determination of tetra- and hexabromide numbers have been described.[12,13] The variations in tetrabromide numbers and in subsequently described hexabromide numbers are significant because the technique used in the determination of these was maintained constant throughout. Except in filtrate II, the iodine numbers were in all cases above 179, thus showing very little difference between crystal and filtrate fractions. There were unappreciable differences in n^{20} in the several fractions. The melting point of the final product, −5.2° to −5.0°, is significantly higher than any previously reported for linoleic acid; Brown and Frankel, −6.8°; Holde and Gentner,[15] −8 to −7°; McCutcheon,[6] −9 to −8°; Smit,[16] −12 to −11°. We believe this high melting point to be due to removal of a low-melting isomer.

The Preparation and Recrystallization of Debromination Linolenic Acid.—Hexabromostearic acid was reduced by the method employed for linoleic acid, except that the reaction mixture was refluxed for an hour after the last addition of zinc to assure completion of the reaction; yields: 190 g., no. 6, from 600 g. of bromide (86%), and 347 g. of acid, no. 8, from 1000 g. of bromide (94%).

In a preliminary experiment 63.5 g. of no. 6 was twice crystallized from 1200 cc. of petroleum ether at −72° and twice from 600 cc. of this solvent at −62°: yield of final crystals, no. 7, 40 g.; yield of combined filtrates, 22 g.; iodine no. 270.3; hexabromide no., 59.5; n^{20}, 1.4800; liquid at −16.5°. The low hexabromide number and melting point, together with the almost theoretical iodine number of the filtrate acids, seemed to be definite indications of the presence of isomeric acids. Therefore a much more complete series of recrystallizations along with a detailed study of the nature of the resultant filtrates was attempted.

Two hundred grams of linolenic acid, no. 8, in 4 liters of petroleum ether, was cooled to −71° and the solution filtered. The crystal fraction from seven such crystallizations at temperatures of −68 to −71° is no. 9.

A similar eighth crystallization followed; filtrates I–VIII are described in Table III. There was accidental contamination of the solution with methyl alcohol from the bath during the ninth crystallization, so that the filtrates of the ninth and tenth crystallizations were not examined. The crystal fraction of the tenth crystallization is no. 10. To it was added 20 g. of six times recrystallized debromination acid, and the mixture, 91 g., was carried through four more crystallizations at −70° in 3700 cc. portions of solvent. Filtrates are A–D, Table III. The final crystal fraction, 75 g., no. 12, represents acid, most of which had been crystallized 14 times. Its melting point, −11.3 to −11.0°, is the highest so far reported for linolenic acid, as is its hexabromide number, 96.0. We believe this linolenic acid to be the purest specimen of the acid so far isolated, and that it is identical with the linolenic acid present in linseed and perilla oils.

TABLE III

RESULTS OF REPEATED CRYSTALLIZATION OF DEBROMINATION LINOLENIC ACID

	Weight g.	Hexabromide no.	Isomeric acid g.[a]	Iodine no.	Mol. wt.	n^{20}
Orig. acid		81.4	15.2 (%)	271.7	277.3	....
Filtrate I	13.9[c]	40.5[b]	8.0	264.9	284.0	1.4802
II	12.7[c]	42.8[b]	7.5	269.0	...	1.4801
III	10.5	54.5	5.0	265.5	...	1.4795
IV	7.8	61.8	2.8	267.3	...	1.4795
V	5.4	65.8	1.7	269.0	...	1.4799
VI	4.1	68.0	1.2	265.0	...	1.4798
VII	7.0	82.7	1.0	268.9	279.2	1.4798
VIII	4.7	67.8	0.9	267.0	...	1.4795
A	4.6	83.2	.6	262.8	...	1.4816
B	3.3	88.8	.4	266.4	...	1.4807
C	4.6	90.2	.28	269.5	279.2	1.4801
D	3.4	92.2	.14	269.9	...	1.4801

[a] The content of isomeric acid is calculated from the hexabromide no., assuming the isomeric acid to have a hexabromide no. of 0, and pure linolenic acid 96.0. [b] The bromides from filtrates I and II were mixed. The m. p. was 177–178°, the same value as obtained from the bromides of the final crystal acid. [c] These two filtrates remained liquid at −22°.

Our reasons for believing debromination linolenic acid to be a mixture of isomers are analogous to those presented for linoleic acid; they may be summarized as follows: (1) An even more decided decrease occurred in the weights of filtrates. From the weights of the final filtrates it is evident that the solubility of linolenic acid is about 1.0 g. per liter at −70°. (2) The hexabromide numbers of the filtrates rose from 40.5 to 92.2, the last value approximating the value 96.0 of the final crystal fraction. Concurrently, the hexabromide numbers of the crystal fractions rose. (3) The iodine numbers, except in Filtrates I and A, were above 265, showing the filtrates were essentially as unsaturated as the crystal fractions,[17] and it is apparent that the isomers react quantitatively with the Wijs reagent. n^{20} variations, likewise, except for A and B, did not indicate any appreciable impurity.[4] With reference to melting point, filtrates I and II remained

(15) Holde and Gentner, *Ber.*, **58**, 1067 (1925).

(16) Smit, *Rec. trav. chim.*, **49**, 539 (1930).

(17) We cannot agree with the observation of McCutcheon (*Ind. Eng. Chem., Anal. Ed.*, **12**, 465 (1940)) that the Wijs method gives results 98.8% of the theory with the several common unsaturated acids. In this Laboratory we have repeatedly obtained values which are almost exactly equal to the theoretical with oleic, linoleic and linolenic acids and their esters.

liquid at −22°. The melting points of the unrecrystallized specimens of the original debromination acid have ranged in this Laboratory from −12.8 to −14.5°. McCutcheon has recently reported a value of −16.25 to −17°. We are convinced that these variations in melting points are due to the presence of varying amounts of the contaminating isomer.

Discussion

In the preceding work we have shown that debromination linoleic and linolenic acids, prepared by reduction of the corresponding bromo-acids in neutral methyl alcohol, contain about 12 and 15%, respectively, of isomeric acids which give nearly theoretical iodine numbers for C_{18} two and three bond acids but lower melting points and much lower tetrabromide and hexabromide numbers than the original debromination acids. It does not seem likely that the isomeric acids have conjugated bonds. Attempts to further isolate the contaminating isomer in the case of linolenic acid have been so far unsuccessful, because the differences in solubility are too small. It seems logical to conclude that the contaminating isomers are of the *cis–trans* type, which yield no tetrabromides or hexabromides insoluble in petroleum ether or ether, respectively. The origin of these isomeric acids is not certain. The high melting points of our bromides make it improbable that they contain appreciable amounts of isomeric bromides, which might give rise to other acids. We believe that the contaminating isomeric acids arise from isomerization during the debromination procedure. It has been our experience in this Laboratory that linoleic and linolenic acids, prepared in a neutral alcoholic reduction medium, such as we have employed above, do not differ from those prepared in a strongly acid medium in such properties as melting point, iodine number, and yield of insoluble bromides. Therefore, it seems likely though not certain that preparations of debromination acids, previously reported in the literature, have been more or less contaminated with these isomeric acids and that the final crystal fractions described above are the purest acids so far prepared. The crystallization procedure is the only method at present available for removing these isomeric acids.

Summary

Linoleic and linolenic acids, prepared by the debromination procedure, have been shown by repeated low temperature crystallizations to contain 12 to 15%, respectively, of isomeric acids of low melting points and low or zero tetra- and hexabromide numbers. The constants of the highly purified acids are described. The origin of the isomeric acids is discussed.

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An Amino Analog of Vitamin B_1

BY DONALD PRICE AND FRANK D. PICKEL

Bergel and Todd[1] synthesized a number of analogs of vitamin B_1, none of which showed any measurable vitamin activity in animal tests. They, moreover, stated that any significant alteration in the structure of the vitamin would cause almost complete loss of activity, and their statement has been largely borne out by later work.

Finkelstein and Elderfield[2] reported two pyridine analogs of the vitamin which were inactive toward polyneuritic rats at levels of 100 γ per rat. Schmelkes[3] announced the preparation of 2-methyl-3-β-hydroxyethyl-N-((2-methyl-6-amino-pyrimidyl-(5))-methyl)-pyridinium bromide hydrobromide, the exact pyridine analog of vitamin B_1 and stated that it showed activity. He later published[4] his synthesis of this substance but gave no further data concerning its activity. The same substance prepared by a different synthesis was also reported by Baumgarten and Dornow,[5] who stated that a 26-fold quantity was required for activity equal to that of vitamin B_1. In a later paper the same authors[6] showed that their previously published structure as well as that of Schmelkes was in error due to the Clemmensen reduction having taken an unforeseen course, in each case the hydroxyl group in the pyridine side-

(1) F. Bergel and A. R. Todd, *J. Chem. Soc.*, 1504 (1937).
(2) J. Finkelstein and R. C. Elderfield, *J. Org. Chem.*, **4**, 365 (1939).
(3) F. C. Schmelkes, *Science*, **90**, 113 (1939).
(4) F. C. Schmelkes and R. R. Joiner, THIS JOURNAL, **61**, 2562 (1939).
(5) P. Baumgarten and A. Dornow, *Ber.*, **73**, 44 (1940).
(6) *Ibid.*, **73**, 353 (1940).