

On the basis of the results shown in Table 5, oxalyl chloride is the only reagent capable of converting the higher unsaturated fatty acids to their acid chlorides in reasonably good yields. The use of phosphorus chloride apparently results in considerable polymerization of the unsaturated acids with corresponding reduction in the yield of acid chloride.

### Summary

Fatty acid chlorides of lauric, myristic, palmitic, stearic, oleic, elaidic, and linoleic acids were prepared using phosphorus tri- and pentachlorides, oxalyl chloride, and thionyl chloride as chlorinating agents. On the basis of yields, ease of handling, and cost of reagent, phosphorus tri- and pentachlorides are superior to other chlorinating agents for the preparation of the higher fatty acid chlorides for use as intermediates in the preparation of pure saturated fatty acid glycerides of known structure. The unsaturated fatty acid chlorides are, however, best prepared from oxalyl chloride.

The use of phosgene, which has been patented for the preparation of acid chlorides, failed to effect efficient conversion of the higher fatty acids to the corresponding chlorides.

The analysis of the fatty acid chlorides by conversion to anilides provides a simple and easy means for ascertaining the extent of conversion of fatty acids to chlorides. By means of this method it is possible to follow readily the course of the reaction in order

to determine when complete conversion of fatty acid to fatty acid chloride has been effected.

### Acknowledgment

The author wishes to acknowledge the assistance of Victor Gastinel, Jr., in the early stages of this work, prior to his induction into military service, and of Miss Maizie Caravella for the preparation of a number of pure acids.

### REFERENCES

1. Adams, Roger and Ulich, L. H., *J. Am. Chem. Soc.* **42**, 599-611 (1920).
2. Averill, H. P., Roche, J. N., and King, C. G., *J. Am. Chem. Soc.* **51**, 866-72 (1929).
3. Black, H. C. and Overley, C. A., *J. Am. Chem. Soc.* **61**, 3051-52 (1939).
4. Bömer, A. and Kappeller, W., *Fette u. Seifen* **44**, 340-43 (1937).
5. Bömer, A. and Stather, J., *Fette u. Seifen* **44**, 29-31 (1937).
6. Daubert, B. F., Fricke, H. H., and Longenecker, H. E., *J. Am. Chem. Soc.* **65**, 2142-44 (1943).
7. Ellis, G. W., *Biochem. J.* **26**, 791-800 (1932).
8. Frankel, J. S., and Brown, J. B., *J. Am. Chem. Soc.* **65**, 415-18 (1943).
9. Hochstetter, A., German Patent 283,896 (April 27, 1915).
- 9a. I. G. Farbenind. Akt.-Ges., British Patent 515,963 (December, 1939).
10. Kraft, F. and Tritschler, F., *Ber.* **33**, 3580-85 (1900).
11. McCutcheon, J. W., *Can. J. Research* **16B**, 158-75 (1938).
12. McMaster, L. and Ahmann, F. F., *J. Am. Chem. Soc.* **50**, 145-49 (1928).
13. Prat, J. and Etienne, A., *Bull. soc. Chim de France* **5T**, 11, 30-34 (1944).
- 13a. Soc. pour l'ind. chim. à Bâle, Swiss Patent 213-047 (April 16, 1941).
14. Taufel, K. and Künkele, F., *Fettechem. Umschau* **42**, 27-29 (1935).
15. Verkade, P. E., *Rec. Trav. Chim. Pas-Bas* **62**, 6, 393-97 (1943).
16. Wheeler, D. H. and Riemenschneider, R. W., *Oil & Soap* **16**, 207-209 (1939).
17. Wood, T. R., Jackson, F. L., Baldwin, A. R., and Longenecker, H. E., *J. Am. Chem. Soc.* **66**, 287-89 (1944).

## Spectral Properties of Linoleic and Linolenic Acids Prepared by Debromination

R. T. O'CONNOR, D. C. HEINZELMAN, MAIZIE CARAVELLA, and S. T. BAUER

Southern Regional Research Laboratory,<sup>1</sup> New Orleans, Louisiana

INCIDENT to studies on the thiocyanogen reagent and method recently described (11) it was found necessary to prepare pure samples of linoleic and linolenic acids. Use of the ultraviolet spectrophotometer to determine the percentage of conjugated isomers present in various preparations of these acids indicated the necessity of introducing certain refinements and precautions into the usual methods of preparation. As a result of these refinements specimens of linoleic and linolenic acids were obtained which were of higher purity spectroscopically than those previously reported (3, 18).

The absorption spectra have been determined for each of the acids which were obtained by the various modifications applied in their preparation. The data obtained offer a striking example of the utility of the spectrophotometer in following the progress of the preparation of a pure compound. The ultraviolet spectra of the pure fatty acids are of considerable importance in view of the increasing use of the spectrophotometric method of determining the composition of fats and oils and in establishing reference values for use in this type of analysis (1, 2, 7, 14, 15).

### Preparation of the Acids

The acids were separated and purified by modifications of well recognized and generally accepted methods. However, only by observing additional precautions and introducing successive refinements in these methods was it possible to obtain acids of the highest purity.

*Linoleic Acid:* Preparation No. 1: Tetrabromostearic acid (m.p. 114.2°-115.0° C., total immersion thermometer) was prepared from the fatty acids of corn oil according to the method of McCutcheon (12). Debromination was carried out in ethyl ether in accordance with the method of Frankel and Brown (5). Excess ether was removed at room temperature under reduced pressure and the ether-free acid was distilled in a Claisen flask submerged in an oil bath. Distillation in this manner yielded two fractions which were collected over a boiling range of 176°-178° C. at 0.4 mm. pressure.

Preparation No. 2: A survey of the literature showed that melting points of tetrabromostearic acid had been reported which were higher than that used for preparation No. 1. Accordingly, the tetrabromides were subjected to purification by a modification of the method recommended by Kass, Lundberg, and Burr

<sup>1</sup> One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(9), namely, recrystallization from Skellysolve-L with the addition of charcoal to remove color, followed by solution in ether and subsequent precipitation from the ether solution by addition of Skellysolve-F, and finally crystallization from Skellysolve-L. This procedure produced a white crystalline tetrabromostearic acid (m.p. 115.5°-115.8° C., total immersion thermometer) but the yield was low. Debromination was carried out as described for preparation No. 1. However, the recovered linoleic acid was transferred to a molecular pot-still (8) for distillation. Three fractions were collected at temperatures between 115°-113° C. at  $5 \times 10^{-5}$  mm. pressure.

Preparation No. 3: Examination of the first two preparations of linoleic acid indicated the need for introducing special precautions against oxidation. Also improvement in the method of purification was deemed desirable to increase the yield. These improvements were accomplished by recrystallization of the tetrabromostearic acid, once from ethyl ether and twice from Skellysolve-L, using Skellysolve-F to wash the precipitate. Decolorizing carbon (Darco) was added during the first crystallization from Skellysolve-L. The white tetrabromostearic acid (m.p. 115.5°-115.8° C., total immersion thermometer) was debrominated in peroxide-free ether according to the method of Frankel and Brown (5). The ether solution of the regenerated linoleic acid was filtered under an atmosphere of carbon dioxide, dried over sodium sulfate for one hour, and refiltered under the same conditions. The ether was removed under reduced pressure and the pure acid allowed to stand under vacuum overnight. Finally, the acid was fractionated in a molecular pot-still, fractions being collected at 115.0°-113.0° C. at  $4 \times 10^{-5}$  mm. pressure.

Preparation No. 4: The preparation was carried out in every detail as in preparation No. 3 to the point of debrominating the tetrabromostearic acid which was conducted in an atmosphere of oxygen-free nitrogen. The nitrogen was purified by passing through an electrically heated combustion tube packed with copper oxide wire which had been reduced with hydrogen. The entire procedure for freeing and handling the acid was carried out under an atmosphere of nitrogen in order to exclude any possibility of oxidation. The regenerated acid was finally fractionated in a molecular pot-still, during which the fraction reported upon here was collected at a temperature of 113.0° C. at  $1 \times 10^{-4}$  to  $4 \times 10^{-5}$  mm. pressure.

*Linolenic Acid:* Preparation No. 1: Hexabromostearic acid was prepared from linseed oil according to the method of Rollett (16). Crystallization of the hexabromides from Skellysolve-L, followed by recrystallization from xylene, yielded a product melting at 183°-184.5° C., total immersion thermometer. Debromination of the hexabromostearic acid was carried out according to Frankel and Brown (5). The dry, ether-free acid was distilled from a Claissen flask at 180°-182° C. at 0.3 mm. pressure. The product was a pale yellow liquid having somewhat low iodine and thiocyanogen values and an appreciable peroxide value.

Preparation No. 2: Another lot of hexabromostearic acid was prepared from linseed oil according to the method of Rollett (16). Decolorization with activated carbon (Darco) and five recrystallizations from toluene gave a white crystalline product (m.p.

185.5°-186.0° C.,<sup>2</sup> total immersion thermometer). Debromination of the hexabromostearic acid was carried out according to the method of Frankel and Brown (5), with peroxide-free ether in an atmosphere of carbon dioxide. Following the removal of the solvent, the acid was fractionated in a molecular pot-still at  $1 \times 10^{-5}$  mm. pressure. The fraction representing the purest acid distilled at 115.5°-116.0° C.

In the distillation of the linoleic and linolenic acid products, various fractions were collected with the object of separating the impurities into the first and residual fractions. Data obtained on these fractions showed that in all cases the middle fraction contained the purest acids. Analytical data obtained on these middle fractions, showing the progressive increase in the purity of the linoleic and linolenic acids prepared by the various modified methods, are given in Table 1.

TABLE 1  
Characteristics of Linoleic and Linolenic Acids

Acid Samples	Iodine Value <sup>a</sup>	Thiocyanogen Value <sup>b</sup>	Peroxide Value <sup>c</sup>
Linoleic No. 1.....	177.0	95.5	....
Linoleic No. 2.....	178.5	94.5	....
Linoleic No. 3.....	180.0	.....	4.0
Linoleic No. 4.....	180.2	97.0	2.7
Linolenic No. 1.....	265.1	165.3	14.2
Linolenic No. 2.....	272.1	167.1	4.0

<sup>a</sup> Iodine values were determined with 150% excess of 0.2 N Wijs reagent and a reaction period of one hour. Theoretical values: linoleic acid, 181.0; linolenic acid, 273.5.

<sup>b</sup> Thiocyanogen values were determined with 0.2 N thiocyanogen reagent containing 25% of CCl<sub>4</sub>. Empirical values: linoleic acid, 96.7; linolenic acid, 167.1 (12).

<sup>c</sup> Milliequivalents of peroxide per kilogram of acid. Peroxide determined by method of Lee (10) modified as follows: Twenty-five ml. of a chloroform-acetic acid solution (2:3) and one gram of powdered potassium iodide were added to the sample in a flask. After standing for one hour in a cool dark cabinet, 50 ml. of water was added and the mixture titrated with 0.1 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>.

In the case of both linoleic and linolenic acids the fractions used for analysis were weighed immediately into micro beakers and transferred to 125-ml. ground-glass stoppered Erlenmeyer flasks. The reagent was pipetted into the flasks at once, thus limiting the possibility of oxidation. The same precaution was observed in all the samples.

#### Spectrophotometric Criteria of Purity

As the product from each method of preparation was obtained, its absorption spectrum in the ultraviolet region from 220  $m\mu$  to 320  $m\mu$  was measured. The spectrophotometric measurements were made with a Beckman quartz spectrophotometer with an effective slit width of from 2.0  $m\mu$  to 0.5  $m\mu$ . Samples of the acids were weighed directly into volumetric flasks, and optically pure cyclohexane (6) was added to give a concentration of approximately 7 grams per liter.

Extinction curves of the four linoleic acids, Figure 1, Curves A to D, and the two linolenic acids, Figure 2, Curves A and B, show successively decreasing absorption owing to the removal of or repression of conjugated impurities by progressively modified and improved methods of purification.

Curve A in Figure 1 shows marked characteristic absorption in the regions of diene conjugation, 232  $m\mu$ , and of triene conjugation, 268  $m\mu$ , and also measurable absorption in the tetraene region, 310  $m\mu$ . Apparently the preparation of linoleic acid by procedure

<sup>2</sup> The sample of brominated acid and thermometer were placed in an oil bath preheated to 170° C. The temperature was increased at the rate of 1°-2° per minute until the melting point of the sample was reached.

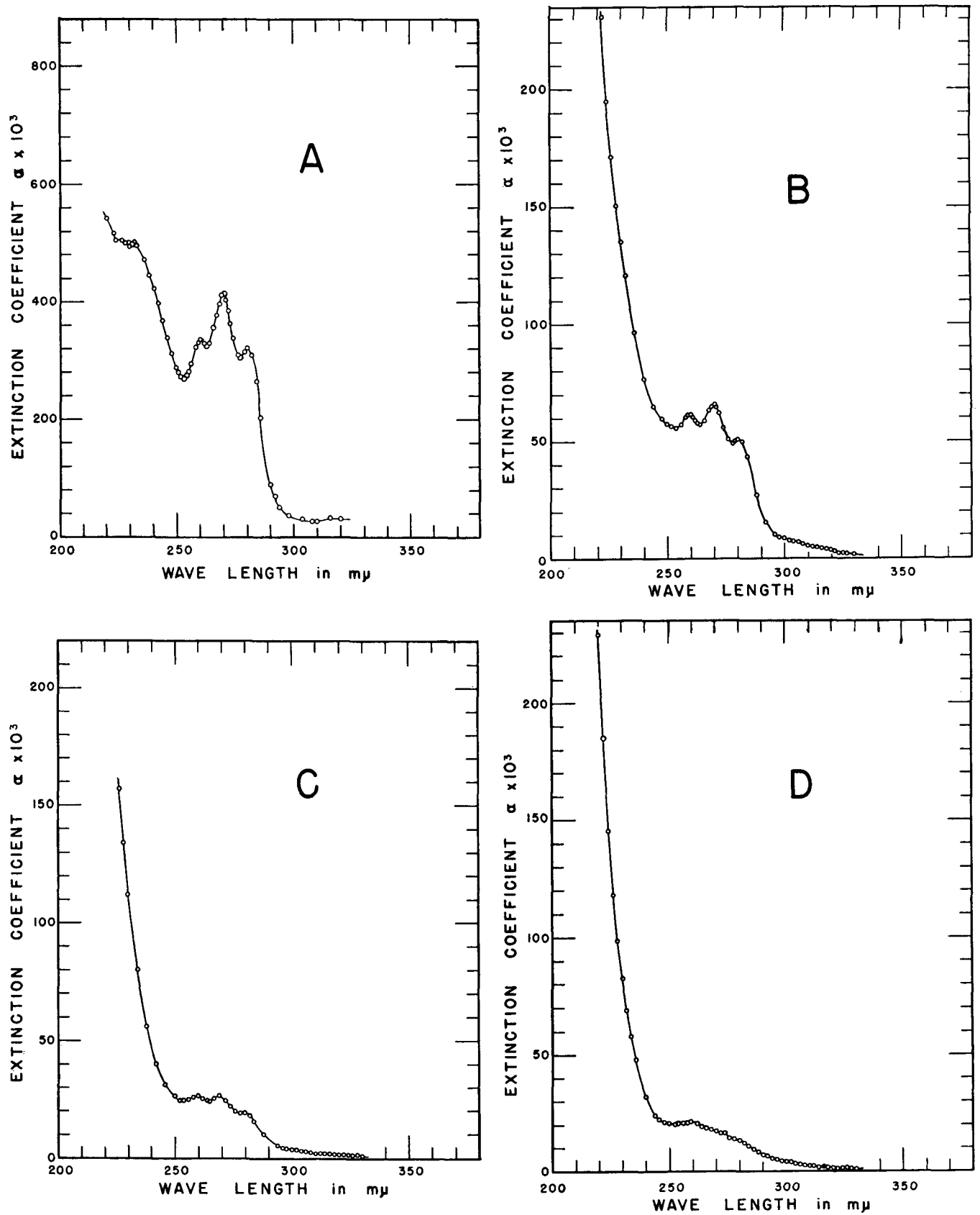


FIG. 1. Ultraviolet Absorption of Linoleic Acids.

- A—Preparation No. 1
- B—Preparation No. 2
- C—Preparation No. 3
- D—Preparation No. 4

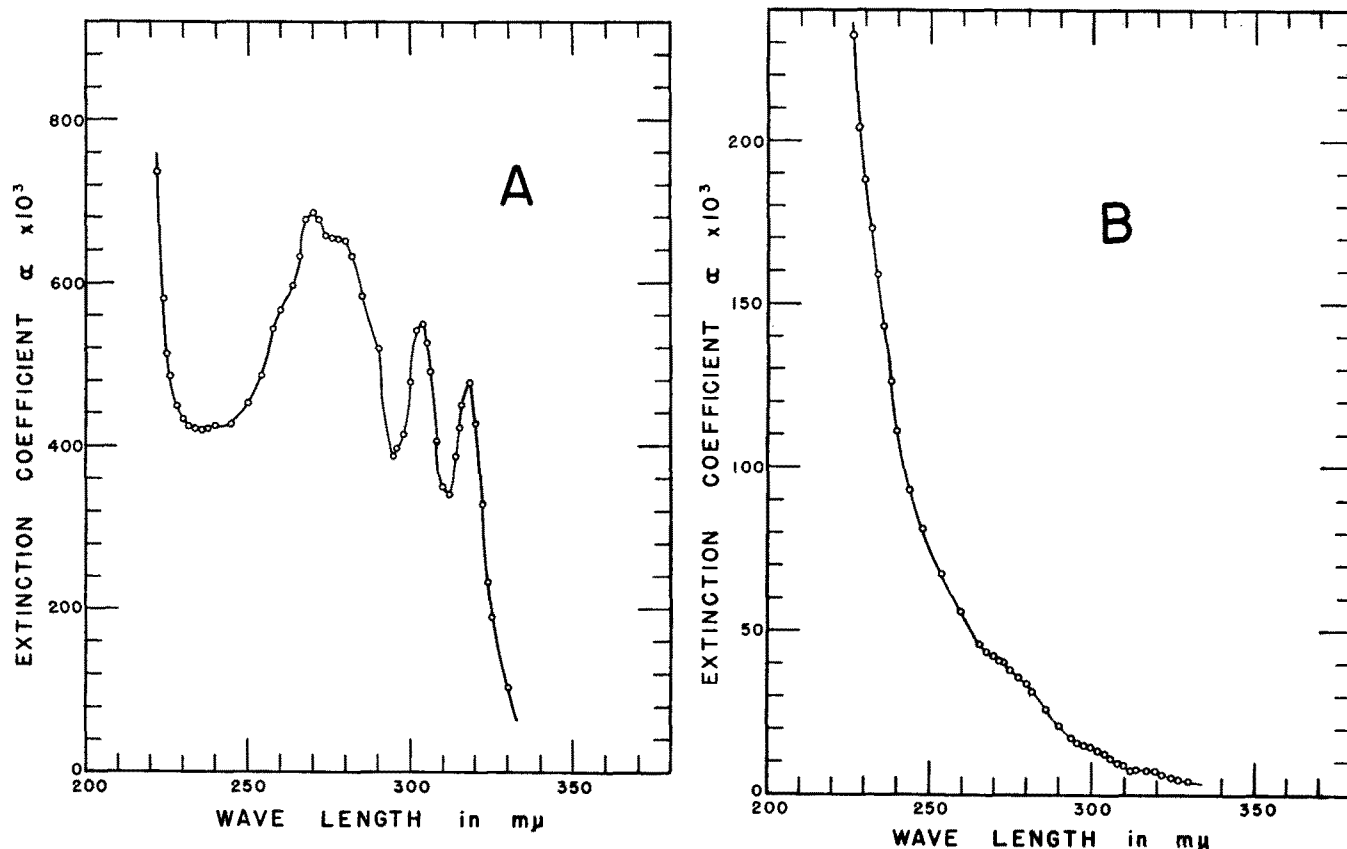


FIG. 2. Ultraviolet Absorption of Linolenic Acids.

A—Preparation No. 1

B—Preparation No. 2

No. 1 is accompanied by the formation of relatively large quantities of conjugated constituents.

The second curve of Figure 1, Curve B, shows the effect of molecular distillation used in purifying preparation No. 2. Diene conjugated impurities were almost entirely eliminated and triene and tetraene conjugated constituents were greatly reduced. However, there was still a measurable quantity of triene conjugated acid in the sample.

Curves C and D of Figure 1 illustrate the continual reduction in conjugated components, particularly triene conjugation, with the introduction of additional precautions to preclude oxidation from the method of preparation. As indicated by Curve C, the use of carbon dioxide in preparation No. 3 further decreased the quantity of triene conjugated material. However, only when purified nitrogen was used in preparation No. 4 was it possible to completely eliminate the triene and tetraene conjugated acids, as indicated by Curve D. The acid from preparation No. 4 showed no measurable amounts of diene, triene, or tetraene conjugated acids, as is evident from absorption curve D of Figure 1.

Figure 2 illustrates in a similar manner the increase in purity of the linolenic acid attained in preparation No. 2 compared to that from preparation No. 1. Curve A of this figure (preparation No. 1) indicates the presence in the sample of relatively large quantities of triene and tetraene conjugated impurities. Molecular distillation and the use of an atmosphere of carbon dioxide in preparation No. 2 reduced the amount of conjugated material to negligible quantities.

Extinction coefficients ( $\alpha = E_{1\text{ cm.}}^{s/l}$ ) for the four samples of linoleic acid and the two samples of lino-

lenic, throughout the regions of diene, triene, and tetraene absorption, are tabulated in Table 2. The percentages of conjugated acids which were present in the various preparations and represented by the absorption bands of Figures 1 and 2 were calculated by the methods proposed by Brode, Patterson, Brown, and Frankel (3), and by Brice and Swain (2), and are also included in Table 2. These data further illustrate the effect of the different procedures for the purification of linoleic and linolenic acids, and the importance of the use of spectrophotometric measurements as an additional criterion of purity. The rearrangement of double bonds from nonconjugated to conjugated systems during heat bodying of a drying oil was first proposed by Scheiber (17) and has since been considered to be one of the important factors in the polymerization mechanism. Recently, Farmer and co-workers (4) have shown by spectrographic measurements that esters such as ethyl linolenate develop conjugation during the course of auto-oxidation by atmospheric oxygen in the illumination of the laboratory at a temperature of about 20° C. The present investigation further emphasizes the necessity of eliminating contact of the unsaturated fatty acids with oxygen during their preparation. It would appear that oxygen may have had more effect on the formation of conjugated isomers than the zinc bromide, which is present during the debromination as postulated by Brode *et al.* (3).

#### Summary

Modified procedures are described for the preparation of linoleic and linolenic acids which remove and/or prevent the formation of conjugated acids.

TABLE 2  
Spectrophotometric Measurements of Linoleic and Linolenic Acids

Sample No.	Extinction Coefficient $a = E \frac{g./l.}{1 \text{ cm.}}$									Conjugated Acids					
	Wave Length $m\mu$									Diene <sup>1</sup>	Triene <sup>1</sup>	Tetraene <sup>1</sup>	Diene <sup>2</sup>	Triene <sup>2</sup>	Tetraene <sup>2</sup>
	232	233	262	268	274	280	310	316	322						
Linoleic No. 1	0.499	0.494	0.328	0.396	0.339	0.332	0.0270	0.0321	0.0261	0.39	0.24	0.013	0.39	0.082	0.0063
Linoleic No. 2	0.121	0.115	0.059	0.063	0.055	0.051	0.0056	0.0046	0.0032	0.092	0.038	0.0016	0.077	0.0078	0.00023
Linoleic No. 3	0.095	0.088	0.025	0.026	0.022	0.020	0.0023	0.0018	0.0016	0.072	0.015	0.0008	0.055	0.0033	0.00
Linoleic No. 4	0.062	0.056	0.015	0.013	0.012	0.010	0.0031	0.0026	0.0024	0.046	0.0069	0.0012	0.027	0.00	0.00
Linolenic No. 1	0.424	0.422	0.581	0.677	0.658	0.650	0.349	0.450	0.328	0.31	0.40	0.16	0.33	0.075	0.13
Linolenic No. 2	0.083	0.078	0.022	0.020	0.018	0.016	0.0058	0.0061	0.0049	0.064	0.011	0.0024	0.045	0.00	0.00085

<sup>1</sup> Calculated according to method of Brode *et al.* (3).

<sup>2</sup> Calculated according to method of Brice and Swain (2).

The importance of spectrophotometric measurements as a criterion of purity is emphasized.

### Acknowledgments

The authors are indebted to Madeline G. Lambou for the iodine, thiocyanogen, and peroxide values, and to Earl B. Schuman for the preparation of the drawings.

### REFERENCES

1. Beadle, B. W., and Kraybill, H. R., *J. Am. Chem. Soc.* **66**, 1232 (1944).
2. Brice, B. A., and Swain, M. L., *J. Opt. Soc. Amer.* **35**, 532 (1945).
3. Brode, W. R., Patterson, J. W., Brown, J. B., and Frankel, J., *Ind. Eng. Chem., Anal. Ed.*, **16**, 77 (1944).
4. Farmer, E. Harold, Koch, H. P., and Sutton, Donald A., *J. Chem. Soc.*, **1943**, 541-547.

5. Frankel, J. S., and Brown, J. B., *J. Amer. Chem. Soc.*, **65**, 415 (1943).
6. Graff, M. M., O'Connor, R. T., and Skau, E. L., *Ind. Eng. Chem., Anal. Ed.*, **16**, 556 (1944).
7. Hilditch, T. P., Morton, R. A., and Riley, J. P., *The Analyst*, **70**, 68 (1945).
8. Hickman, K. C. D., *Chem. Rev.*, **34**, 51 (1944).
9. Kass, J. P., Lundberg, W. O., and Burr, G. O., *Oil & Soap*, **17**, 50 (1940).
10. Lee, C. H., "Rancidity in Edible Fats," 1st Edition, page 107, Chemical Publishing Company, Inc., New York, N. Y.
11. Lambou, M. G., and Dollear, F. G., *Oil & Soap*, **32**, 226 (1945).
12. McCutcheon, J. W., *Can. J. Research*, **16B**, 158 (1938).
13. Mehlenbacher, V. C., *Chem. and Eng. News*, **22**, 606-608 (1944).
14. Mitchell, J. H., Jr., Kraybill, H. R., and Zscheile, F. P., *Ind. Eng. Chem., Anal. Ed.*, **15**, 1 (1943).
15. O'Connor, R. T., Heinzelman, D. C., and Dollear, F. G., *Oil & Soap*, **10**, 257 (1945).
16. Rollett, A., *Z. Physiol. Chem.*, **62**, 422 (1909).
17. Scheiber, J., *Farbe und Lack*, 585-587 (1929).
18. Wood, Thomas R., Jackson, F. L., Baldwin, A. R., and Longenecker, H. E., *J. Am. Chem. Soc.*, **66**, 287-289 (1944).

## The Fatty Acids of Menhaden Oil.

### III. Distillation of the Hydrogenated Methyl Esters and Evaluation of the Carbon Series\*

FRANK A. SMITH\*\* and J. B. BROWN

Laboratory of Physiological Chemistry, Ohio State University, Columbus, Ohio

IN THE first paper (1, 2) of this series the methyl esters of menhaden oil were distilled through an efficient column and the  $C_{12}$ ,  $C_{14}$ ,  $C_{16}$ , and  $C_{18}$  fractions were studied mainly by low temperature crystallization. It was pointed out that not all of the  $C_{18}$  esters of the specimen were included in the  $C_{18}$  main fraction resulting from this distillation because we did not try to distill the high boiling fractions containing the highly unsaturated esters. In view of the work of Frammer and van den Heuvel (3) and of our experience in this laboratory the polyethylenic esters of this oil, especially those of the  $C_{20}$  and  $C_{22}$  series, are seriously altered by long exposure to the conditions of distillation with the resultant formation of polymerised and cyclized material. In view of these facts the usual ester distillation method for the evaluation of the carbon series cannot be applied to the methyl esters of menhaden oil directly. Accordingly we have attempted to evaluate the composition by analysis of the fractions resulting from distillation of the hydrogenated methyl esters. The results thus obtained are slightly distorted, especially in the higher series, because of their high original unsaturation and of the fact that addition of hydrogen to the  $C_{20}$  and  $C_{22}$  series results in an appreciable increase

in the amounts of these fractions actually reported below.

#### Experimental

Two gallons of the specimen of menhaden oil used in our previous work was hydrogenated for us in the laboratory of the Procter and Gamble Company through the courtesy of Dr. A. S. Richardson. Fourteen hundred and fifty g. of this fat was esterified in the usual manner. The crude esters were distilled: B.P. 180-238° at 2 mm. The residue from this distillation was transferred to a smaller flask and an additional distillate (14.6 g.) obtained; m.m.w. 456 and I.N. 33.9. The yield of esters was practically quantitative. The main product, not including the 14.6 g., analyzed as follows: m.m.w. 300.9; I.N. 6.9. Six hundred and four g. of these esters was distilled through our packed column. The results of the distillation, analyses of the fractions, and calculations of component carbon series are shown in Table I; 6.8 g. of the original 15 g. residue was apportioned to the residue fraction of this distillation and included as "above  $C_{26}$ " material in the table.

The carbon series of menhaden oil as shown in Table I include acids from  $C_{14}$  through and above  $C_{26}$ . The 0.1% of  $C_{12}$  esters found in our former work (1) is not apparent from the distillation of this comparatively small amount of esters but must be considered as present. Since the saturated and monoethylenic

\* Presented in partial fulfillment of the requirements for the degree of doctor of philosophy in the Graduate school.

\*\* Present address: School of Medicine and Dentistry, University of Rochester, Rochester, New York.