Corrections for background based on reading two values on either side of a maximum as suggested in A.O.C.S. Official Method Cd7-48 (9) will not be valid if there is any overlapping of two cusps that represent different degrees of conjugation. This will be true for absorptions at 315, 328, 346, and 352.5 mμ for mixtures containing hexaenoate.

The elucidation of the nature of the absorption spectra of conjugated fatty acids presented by Figure 4 should prove useful also in studies of the geometric configuration of double bonds in conjugated or alkali-isomerized fatty acids. Conjugated systems which differ in geometric configuration of their double bonds seem to show corresponding systematic differences in the position of their absorption maxima. This may also find application in reaching an understanding of the kinetics and mechanisms involved in the alkali isomerization of unsaturated fatty acids.

Summary

The dependence of the ultraviolet absorption spectra for docosahexaenoate upon conditions used in alkali-isomerization has been studied. The extinction coefficients at all wavelengths varied with sample size when 21% KOH-glycol was used as the isomerization reagent at 180°C.; the effect of sample size was due to the solubility characteristics of 22-carbon fatty acid esters in the isomerization reagent.

Spectrophotometer slit width is an important consideration in the measurement of extinction coefficients at the absorption maximum at 374 mμ.

Certain conditions which must be met for accurate analysis of hexaenoate in fatty acid mixtures are described.

An extension of a theory of Lewis and Calvin has been used to clarify the number, position, and to some extent, intensity of the peaks in the ultraviolet absorption spectra of natural conjugated and alkali-isomerized fatty acids.

REFERENCES

1. Hammond, E. G., and Lundberg, W. O., J. Am. Oil Chem. Soc., *30*, 438-441 (1953).
2. Mitchell, J. H., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem. Anal. Ed., *15*, 1 (1943).
3. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 279 (1952).
4. Herb, S. F., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 456 (1952).
5. Lewis, G. N., and Calvin, M., Chem. Revs., *25*, 273 (1939).
6. Herb, S. F., and Riemenschneider, R. W., Anal. Chem., *25*, 953 (1953).
7. Herb, S. F., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 456 (1952).
8. Klenk, E., and Bongard, W., Z. physiol. Chem., *291*, 104 (1952).
9. Vandenheuvel, F. A., and Richardson, G. H., J. Am. Oil Chem. Soc., *30*, 104 (1953).
10. Herb, S. F., Witnauer, L. P., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *28*, 505 (1951).
11. Front, J. S., and Daubert, B. F., J. Am. Chem. Soc., *67*, 1509 (1945).
12. "Official and Tentative Methods of the American Oil Chemists' Soc.," Cd 7-48, American Oil Chemists' Society, Chicago, Ill., 1948.
13. Mulliken, R. S., J. Chem. Phys., *7*, 364 (1939).
14. Mulliken, R. S., and Rieke, C. A., J. Am. Chem. Soc., *63*, 1770 (1941).
15. Kuhn, W., Helv. Chim. Acta., *31*, 1780 (1948).
16. Henrici, A., Z. physik. Chem., *47B*, 93 (1940).
17. Smakula, A., Angew. Chem., *47*, 657 (1934).
18. Kass, J. P., in "Protective and Decorative Coatings," edited by J. J. Mattiello, Vol. IV, Chapter 12, p. 363, John Wiley and Sons, N. Y., 1941.
19. Nichols, P. L., Herb, S. F., and Riemenschneider, R. W., J. Am. Chem. Soc., *73*, 247 (1951).
20. Kaufmann, H. P., Baltes, J., Volbert, F., and Brockhausen, K., Fette u. Seifen, *52*, 210 (1950).
21. Ferguson, L. N., and Branch, G. E. K., J. Am. Chem. Soc., *66*, 1467 (1944).
22. Carr, E. P., and Walker, M. K., J. Chem. Phys., *4*, 751 (1936).
23. Widmer, C. Jr., and Holman, R. T., Arch. Biochem., *25*, 1 (1950).
24. Wiese, H. F., and Hansen, A. E., Texas Repts. Biol. Med., *9*, 545 (1951).
25. Abu-Nasr, A., and Holman, R. T., J. Am. Oil Chem. Soc. (in press).

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A Methyl Docosahexaenoate: Its Isolation and Characterization[1]

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A study of possible relationships between diet and the highly unsaturated fatty acids (4, 5, and 6 double bonds) of human blood lipids has been started in this laboratory. The presence of fatty acids with five and six double bonds in fish oils (1, 2, 3, 4, 5) and in the lipids of land animals (6, 7) has been known for some years, but relatively few investigations of their implication in lipid metabolism have been made (8, 9, 10).

In order to determine if alkali-conjugation spectrophotometric techniques (11, 12, 13) could be applied in accurate quantitative analysis of the highly unsaturated acids in blood lipids, it was necessary to isolate hexaenoate in pure form and to determine its ultraviolet spectral characteristics following alkali isomerization. Two five-double bond acids have previously been isolated and characterized by Herb *et al.* (14). Knowledge of the ultraviolet spectral characteristics of pure pentaenoate and hexaenoate is also necessary for accurate estimates of the less unsaturated acids in mixtures where penta- and hexaenoates are present. No definite evidence of the existence of fatty acids with more than six double bonds in animal lipids has been reported.

This paper reports the preparation (starting with hog brain), some of the properties, and the probable structure of a methyl docosahexaenoate, together with its behavior under various conditions of alkali isomerization. Some general relationships in the ultraviolet spectral characteristics of various alkali isomerized and conjugated fatty acids were encountered, and a general relationship in the light refractions of unconjugated fatty acid esters was also established, but for convenience these findings will be described in separate communications (15, 16).

Isolation

In preliminary work on fish oils that contain both penta- and hexaenoic acids, spectra were obtained for the C_{20} pentaenoic acids after isomerization which were different from those of the pentaenoic acids isolated from beef adrenals by Herb *et al.* (14). It was therefore decided to turn to a higher animal as a source for hexaenoate. Available evidence indicated

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that brain tissue was one of the better sources of six double bond acids (17, 18). The docosahexaenoate was therefore prepared from hog brain by solvent extraction of dehydrated brain tissue, solvent segregation of a hexaenoate-rich phospholipid fraction, methanolysis, low temperature fractionation of the methyl esters, and chromatography on silica gel columns.

Sixty-eight kilograms of raw hog brain were passed through a meat grinder with ¼ in. openings, and the ground tissue was dehydrated at about 5 mm. pressure and −1 to +4°C. About 12 kg. of dried material was obtained, which was then vacuum-packed in cans and stored at room temperature until used (less than a month).

About 1.5 kg. of the light brown, rubbery, dehydrated brain tissue was cut into small pieces and dropped into 2 liters of petroleum ether (Skellysolve F). The mixture was purged with nitrogen,[2] and the container was then sealed and allowed to stand overnight. Because of difficulty in separating the sediment from the petroleum ether extract 10% of methanol was added, which dissolved additional material and caused the residual undissolved matter to clot into a pasty mass that could be removed by centrifuging. The precipitate was washed with a volume of petroleum ether equal to the supernatant, and the extract and washings were combined. Nitrogen was bubbled through the solutions at all times except during centrifuging.

The petroleum ether extract was concentrated under partial vacuum to about 2 liters, and acetone was added to make the volume 5 liters. The acetone-insoluble material was filtered off and freed from acetone by evaporation with a water pump. It was then suspended in petroleum ether (400 ml./100 grams) and the mixture centrifuged. The precipitate was washed with petroleum ether (100 ml./250 ml. of original suspension), and the extract and washings were combined.

The foregoing operations were repeated on additional batches (1.5 kg.) of dried tissue until the entire 12 kg. of dehydrated brain had been processed. The total lipid extracted amounted to 3.8 kg. Of this 3.13 kg. was recovered as acetone insoluble, and from the latter, 2.07 kg. of petroleum ether-soluble material was obtained. Analysis indicated that the concentration of hexaenoate in the acetone-soluble lipid was less than half that of the insoluble fraction; similarly, in the next step, the concentration of hexaenoate in the petroleum ether-insoluble fraction was only half that of the petroleum ether-soluble fraction.

The petroleum ether-soluble material was subjected to methanolysis. Very mild conditions were necessary to avoid undue destruction of the desired compound. Accordingly the following procedure was adopted. The petroleum ether extract was reduced in volume under partial vacuum until frothing became excessive. For each 2 liters of this concentrate 3 liters of 5% methanolic H_2SO_4 were added. This mixture separated into 2 layers, and a white precipitate formed in the bottom layer. A new layer consisting of esters appeared on standing at room temperature. The solvent was distilled off slowly until the volume was reduced to 2.5 to 3 liters and the precipitate at the bottom of the flask had dissolved. The mixture was then further reduced in volume by vacuum distillation and the methyl esters isolated by the procedure of Shinowara and Brown (19). The unsaturated fatty acid compounds were protected with nitrogen at all times.

In all, about 1,300 g. of methyl esters were obtained, which were estimated to contain 62% of the original hexaenoate and 34% of the total lipid extracted with methanolic petroleum ether. The phospholipid was not completely converted to methyl esters as a small percipitate formed when the methyl esters were dissolved in acetone. The loss however was not large.

The methyl esters were subjected to low temperature fractionation in acetone. By repeated recrystallizations the 1,300 g. of methyl esters yielded 313 g. of brown oil whose iodine value (Wijs) was approximately 225. This oil was later shown to contain about 15% hexaenoate.

The hexaenoate-rich fraction from the low temperature crystallizations was further fractionated on silica gel columns. The technique was essentially the same as that previously used by Riemenschneider *et al.* (20) in the preparation of other unsaturated fatty esters of high purity. Two columns were employed, one 6 cm. in diameter and 104 cm. long and the other 3 cm. in diameter and 74 cm. long. The columns were filled with a mixture containing 80% silica gel [3] and 20% of celite. About 1,300 g. of this mixture was put in the large column and about 200 g. in the small one.

Oxygen was partly removed from the columns by passing nitrogen through them for about 24 hours at room temperature. Petroleum ether was then put into a separatory funnel at the top of each column and flushed with nitrogen for about 10 minutes before being allowed to percolate through.

The methyl esters were chromatographed on the large column in 50-g. batches. Each batch was dissolved in about 250 ml. of petroleum ether that contained 1.75% peroxide-free diethyl ether. The solution was put into the separatory funnel at the top of the column and purged with nitrogen. Then it was allowed to pass slowly into the column, and any residual material in the separatory funnel was washed into the column with the smallest amount of solvent possible. The same solvent was used for elution, and a succession of eluates having volumes of approximately 1.5 liters each were collected until no more esters came through the column. Somewhat better separations could have been obtained by using a lower concentration of diethyl ether in the eluant, but time was saved by using the large column to obtain a rough separation and following this with further fractionation of the enriched fractions on the small column.

The large column was used repeatedly to process five batches of the methyl esters with no apparent decrease in fractionation efficiency even though approximately 15% of the added material remained permanently on the column each time. After one batch had passed through the column, the highly unsaturated acids in subsequent batches were not subject to oxidation by oxygen adsorbed on the silica gel.

Table 1 shows the course of the separation of the first 50-g. lot of esters; these data are typical of all subsequent fractionations except that in later runs the oil began to come through the column in the second elution instead of the sixth, and the iodine values

[2] The nitrogen used throughout this investigation was purified by passage over hot copper to remove residual oxygen.

[3] A special gel supplied by the Davison Chemical Corporation, Baltimore, Md., bearing the number 950-08-08-226.

TABLE I

Preliminary Fractionation of 50 g. of Methyl Esters on Silica Gel in the Large Column, Using 1.75% Diethyl Ether in Petroleum Ether (Skellysolve F) as Eluant

Elution number	Eluate	Ester in eluate	Iodine value [a]	Specific extinction coefficient at 374 mu [b]
	ml.	*g.*		
1-5	7,800	trace		
6	1,800	2.56	95	0.07
7	1,500	24.60	214	0.18
8	1,500	6.42	303	6.74
9	1,500	3.50	371	23.0
10	1,500	2.33	393	24.9
11	1,500	1.46	404	25.6
12	1,500	0.67	401	25.8
13	1,500	trace		
Total		41.54		
Original		50.02		5.0

[a] Wijs method, 1 hour.
[b] Isomerized in 21% KOH ethylene glycol under nitrogen for 15 min. at 180°C., using samples of 100 mg.

of the last three elutions usually were about 10 units higher. The eluates were freed of solvent by simultaneously subjecting them to partial vacuum and passing nitrogen through them, after which they were stored under nitrogen in stoppered vessels at —18°C.

The fractions which were rich in hexaenoate, as judged by their iodine values of their spectral characteristics following alkali isomerization, were pooled and further fractionated on the small column. In this case the petroleum ether solvent contained the lowest possible concentration of diethyl ether (0.75%) that was consistent with a reasonable rate of elution. About 7 g. of esters in a small amount of solvent were passed into the column, and, using the same solvent for elution, a series of 250 ml. eluates was collected. In one pass through the small column, fractions were obtained which on the basis of iodine value and spectral absorption after alkali isomerization were judged to be about 95% pure C_{22} hexaenoate, with C_{22} pentaenoate as the probable chief contaminant. A second pass yielded fractions that were C_{22} hexaenoate of high purity as shown in Table 2.

Recoveries of 95% or better could be obtained from the small column if at the end of the run the column was treated with petroleum ether containing 2% diethyl ether to remove strongly adsorbed material of unknown composition. Approximately 90% recoveries were obtained in the eluates collected prior to this treatment. This column was also used repeatedly in order to minimize losses due to irreversible adsorption and oxidation.

Characteristics and Structure of the Ester

The isolated docosahexaenoate was identified, its purity determined, and its structure almost completely established by determination of iodine value, saponification value, melting point of its hydrogenated product alone and mixed with authentic behenate, infra-red and ultraviolet spectral characteristics, refractive index and molar refraction, and ultraviolet spectral characteristics following alkali isomerization.

Within the limits of probable experimental error, the iodine values of the esters from eluates 16 and 18, 444.5 and 442.8 respectively, agree with the theoretical iodine value, 444.7, of a C_{22} six double bond fatty acid methyl ester. Both fractions had saponification values (micro method) of 164, agreeing closely with the theoretical value of 163.8.

A small amount of eluate 16 was hydrogenated, using palladium on charcoal as a catalyst and the hydrogenated ester was separated from the solvent (ethanol) by evaporation of the latter so that no purification by fractional crystallization occurred. The melting point was found to be 50.9-52.8°C., and the mixed melting point with a sample of authentic methyl behenate (m.p. 52.0-52.8°C.) was 51.8-52.8°C. After one recrystallization from acetone the melting point of the hydrogenated product was 51.9-52.3°C. These values were measured in a melting point block, and the beginning and termination of melting were observed through a polarizing microscope. Melting points measured in capillary tubes in a conventional melting point bath gave values which were 1 to 1.5° higher; also, the start and end of the melting were more difficult to judge although approximately the same melting ranges were observed by both methods.

The foregoing data all point to a fatty acid with a straight chain of 22 carbons containing six double bonds.

An infrared spectrum of eluate 16 indicated that all of the double bonds had a *cis* configuration; an observed trace of trans double bonds was probably produced by slight oxidation during the manipulations involved in making the infrared measurements.

Ultraviolet spectra of eluates 16 and 18 showed that very little conjugation was present although 17 showed a considerable amount of conjugation, which was undoubtedly the result of inadvertent exposure to oxygen. Study of the characteristics of the ultraviolet absorption spectra before and after alkali isomerization and infrared absorption spectrum leads to the conclusion that probably no double bond occupies a position nearer the carboxy group than the 4,5 position and that probably none occupies the 21, 22 position. The spectra together with the characteristics described previously suggest that the double bonds are located either in the 4, 7, 10, 13, 16, 19 positions or the 5, 8, 11, 14, 17, 20 positions. The finding of Mead *et al.* (21) that labelled acetate adds to linoleate at the carboxyl end of the chain in the conversion to arachidonate in the rat, together with the finding of Widmer and Holman (8) that feeding of linolenate gives rise to the formation of docosahexaenoate in the rat suggest that the first of these two structures is the more likely. Klenk and Bongard (17) believe that a C_{22} hexaenoate which they detected in brain phosphatides has this structure. An effort will be made shortly to determine definitely the position of the double bonds by the ozonization technique described by Klenk and Bongard (22).

TABLE II

Fractionation of 3.58 g. of Hexaenoate Concentrate on Silica Gel (Second Pass on Small Column), Using 0.75% Diethyl Ether in Petroleum Ether as Eluant

Elution number	Eluate	Ester in eluate	Iodine value [a]
	ml.	*g.*	
1-14	3,500	trace	
15	250	0.32	
16	250	0.65	444.5 [b]
17	250	0.50	433.8 [c]
18	250	0.49	442.8
19	250	0.39	
20	500	0.47	440.00
21	750	0.41	433.5
Total		3.23	
Original		3.58	

[a] Wijs method, 1 hour.
[b] Theoretical iodine value for pure methyl docosahexaenoate, 444.7.
[c] This value is low, probably because of accidental oxidation that occurred after elution, as indicated by ultraviolet absorption in the conjugated diene region of spectrum.

There was a very low absorption maximum in the conjugated tetraene region of the ultraviolet spectra of all the eluates examined, but the origin of this is unknown. It does not appear to have been due to oxidation since oxidation of a small amount of hexaenoate produced diene and triene absorption, but no tetraene. Also, even the purest ester fractions had a trace of yellow coloration which could not be removed by the chromatographic methods employed.

Refractive Index and Molar Refraction

The observed values for the refractive index, n_D^{20}, and the molar refraction, R_m, were 1.49736 and 108.49, respectively. The latter was based on the refractive index at 25°C. (obtained by subtracting 0.00038 per degree from the value measured at 20° (23) and on the measured value of the density at 25° 0.9214 g. per cc.

Farmer and Vandenheuvel have reported a considerably lower value, 1.49300, for the n_D^{20} of a methyl docosahexaenoate that they prepared from cod liver oil (4). From this they calculated the molar refraction to be 107.25. According to them, higher values may be attributed to conjugation that develops when such materials are fractionally distilled through ordinary types of fractionation columns. However the docosahexaenoate prepared in the present investigation was not distilled. Moreover its ultraviolet absorption spectrum indicated an absence of any significant conjugation.

Farmer and Vandenheuvel have used their value for the n_D^{20} of methyl docosahexaenoate together with values for methyl esters of other polyene acids to establish a straightline relationship between refractive index and iodine value (24). The straight line characteristic was apparently regarded as validation of their observed value for docosahexaenoate. In establishing this relationship, any possible dependence of refractive index on carbon chain length except insofar as the latter affects iodine value, was evidently not considered.

Values for molar refraction calculated from commonly employed atomic constants are also lower than the value observed in the present investigation. The well known constants of Eisenlohr (25) yield a value of 106.17, and the constants of Swientoslowski (26) together with Eisenlohr's constant for each double bond, 1.733, yield the value 107.77. However the constant for the double bond has been criticized by Bauer and Fajans (27) who suggest that a more realistic interpretation of Eisenlohr's data requires the assignment of a somewhat indefinite value, 1.7 ± 0.2. With six double bonds this alone would lead to an uncertainty of ± 1.2 units in the calculated molar refraction.

Although the observed molar refraction in the present investigation is higher than the value found by Farmer and Vandenheuvel and the calculated values, it is believed to be more accurate. This assertion is justified primarily by its agreement with a simple empirical relationship between molar refraction, carbon chain length, and unsaturation. This relation, to be fully described elsewhere (16), is quite general in character and covers the reported refraction values for a considerable number and variety of fatty acid methyl esters. The good agreement in the case of the docosahexaenoate sample was regarded as an indication of its relatively high purity.

Ultraviolet Spectral Absorption following Alkali Isomerization

Samples of the methyl docosahexaenoate isomerized at 180°C. in 21% KOH-glycol showed absorption peaks at 233, 268, 279, 301, 315, 333, 352.5, and 374 mμ. In addition to such factors as time of heating and alkali concentration, which are known to affect the intensities of the absorption bands developed in other unsaturated fatty acids during alkali isomerization, it was found that the size of sample isomerized and the spectrophotometer slit width used for readings at 374 mμ markedly affected the absorption spectrum of isomerized docosahexaenoate. A study of factors involved in the analysis of hexaenoate by the alkali conjugation-spectrophotometric technique and a further treatment of the ultraviolet absorption spectra of conjugated fatty acids based on the work of Lewis and Calvin (28) will be published separately (15).

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Summary

A methyl docosahexaenoate has been prepared from hog brain phosphatides and its purity established. From its physical and chemical characteristics as well as other considerations, the double bonds, all of which are in the *cis* configuration, have been tentatively assigned to positions 4, 7, 10, 13, 16, and 19 in the carbon chain.

REFERENCES

1. Toyama, Y., and Tsuchiya, T., Bull. Chem. Soc., Japan, *10*, 241, 296, 301, 433, 441, 539, 543. 547 (1935).
2. Tsujimoto, M., J. Soc. Chem. Ind., Japan, *23*, 1007 (1920).
3. Ueno, S., and Yonase, C., J. Chem. Soc., Japan, *57*, 180 (1936).
4. Farmer, E. H., and Vandenheuvel, F. A., J. Chem. Soc., *1938*, 427.
5. Brown, J. B., and Beall, G. D., J. Am. Chem. Soc., *45*, 1289 (1923).
6. Nunn, L. C. A., and Smedley-McLean, I., Biochem. J., *32*, 2178 (1938).
7. Burr, G. O., unpublished work.
8. Widmer, C. Jr., and Holman, R. T., Arch. Biochem., *25*, 1 (1950).
9. Reiser, R., J. Nutrition, *42*, 325 (1950); *44*, 159 (1951).
10. Wiese, H. F., and Hansen, A. E., Texas Repts. Biol. Med., *9*, 545 (1951).
11. Mitchell, J. H., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem., Anal ed., *15*, 1 (1943).
12. Brice, B. A., Swain, M. L., Herb, S. F., Nichols, P. L., Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 279 (1952).
13. Herb, S. F., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *29*, 456 (1952).
14. Herb, S. F., Witnauer, L. P., and Riemenschneider, R. W., J. Am. Oil Chem. Soc., *28*, 505 (1951).
15. Hammond, E. G., and Lundberg, W. O., J. Am. Oil Chem. Soc., *30*, 000 (1953).
16. Hammond, E. G., and Lundberg, W. O., (paper in preparation).
17. Klenk, E., and Bongard, W., Z. physiol. Chem., *291*, 104 (1952).
18. Holman, R. T., and Greenberg, S., unpublished data.
19. Shinowara, G. Y., and Brown, J. B., Oil and Soap, *15*, 151 (1938).
20. Riemenschneider, R. W., Herb, S. F., and Nichols, P. L., J. Am. Oil Chem. Soc., *26*, 371 (1949).
21. Mead, J. F., Steinberg, G., Howton, D. R., Federation Proc., *12*, 244 (1953).
22. Klenk, E., and Bongard, W., Z. physiol. Chem., *290*, 181 (1952).
23. Mattil, K. F., and Longenecker, A. E., Oil and Soap, *21*, 16 (1944).
24. Vandenheuvel, F. A., and Farmer, E. H., J. Am. Oil Chem. Soc., *28*, 512 (1951).
25. Eisenlohr, F., Z. physik. Chem., *75*, 585 (1910); *79*, 129 (1912).
26. Swientoslowski, W., J. Am. Chem. Soc., *42*, 1945 (1920).
27. Bauer, N., and Fajans, K., in "Physical Methods of Organic Chemistry," edited by A. Weissberger, Vol. I, part 2, p. 1168, Interscience, New York, 1949.
28. Lewis, G. N., Calvin, M., Chem. Revs., *25*, 273 (1939).

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