# THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL

## XXI. ULTRAVIOLET ABSORPTION SPECTRA FOR ALKALI-ISOMERIZED POLYETHENOID ACID COMPONENTS OF SARDINE OIL

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Ultraviolet absorption spectra for alkali-isomerized polyethenoid acids in fats and oils have been reported in a number of studies. Among highly unsaturated tetraenoic, pentaenoic and hexaenoic acids, the eicosatetraenoic acid from ox suprarenal lipid has already been studied enough by several authors[1)-4)] to afford currently accepted data on its ultraviolet absorption after alkali-isomerization. Herb, Riemenschneider and co-workers[5)-7)] reported the absorption values after alkali-isomerization of the eicosapentaenoic and docosapentaenoic acids separated from ox suprarenal lipid. Hammond and Lundberg[8)] studied on the alkali-isomerization of the docosahexaenoic acid from hog brain lipid, and presented equations which may be of use for calculating the amounts of polyethenoid acids, including up to hexaethenoid acid, in a mixture containing these acids. However, it requires a further study to verify the applicability of these equations in the case of fish oil fatty acids, since there is some doubt about the identity of the highly unsaturated acids of fish oils with the corresponding members in the lipid of land animals. Abu-Nasr, Potts and Holman[9)10)] reported the absorption values after alkali-isomerization of the eicosapentaenoic, docosapentaenoic and docosahexaenoic acids which were separated as their methyl or ethyl esters from cod liver oil.

In a previous report of this series, docosapentaenoic, eicosapentaenoic, eicosatetraenoic, octadecatetraenoic and hexadecatrienoic acids were separated from sardine oil, and the ultraviolet absorption values of these acids after alkali-isomerization were measured.[11)] Further, several fractions of highly conjugated solid acids were separated from the alkali-isomerization products of a highly unsaturated acid concentrate, a docosapentaenoic acid fraction and a mixture of docosapentaenoic and docosahexaenoic acids, and their spectral characteristics were measured.[12)13)] However, the specimens of highly unsaturated acids used in our previous studies were found to contain some conjugated components even before alkali-isomerization, especially specimens of docosapentaenoic, eicosapentaenoic and eicosatetraenoic acids were contaminated with about 5% of pre-formed conjugated components. Apart from the pre-formed conjugation, the purity of these specimens were not fully examined. Since the specimens of octadecatetraenoic and hexadecatrienoic acids used in our previous studies were prepared by the bromination-debromination procedure, the possibility was not excluded that they had undergone more or less geometrical isomerization. The alkali-isomerization of eicosatetraenoic and octadecatetraenoic acids in our previous studies was not conducted under the condition of 21% KOH-ethylene glycol, 180°C and 15 min. For these reasons, it seems desirable

to separate polyethenoid acid components of a higher purity from sardine oil and determine their spectral characteristics after alkali-isomerization. The present paper records the results of our renewed study in which decosahexaenoic, docosapentaenoic, eicosapentaenoic, eicosatetraenoic, octadecatetraenoic, octadecatrienoic and hexadecatrienoic acids are separated as their methyl esters from sardine oil and their ultraviolet spectral characteristics after alkali-isomerization are measured.

## I. Separation of Polyethenoid Acid Components from Sardine Oil

### 1. Fractional distillation of the methyl ester of polyethenoid acid concentrate and chromatography of the higher fraction

Sardine oil of saponification value 186.8 and iodine value (Wijs) 168.8, produced in Hokkaido, was used in this study. The fatty acids prepared from this oil had neutralization value 196.9 and I.V. 179.3. These were segregated by way of urea adduct in the following way. One kg.-portion of fatty acids was dissolved in 7.5 *l.* of methanol. Three kg. of urea was added to the solution, and the mixture was refluxed until urea was completely dissolved. The solution was cooled at room temperature, and the crystalline urea adducts (1st crop) formed were filtered and washed with a small quantity of methanol. The filtrate and washings were united, 600 g. of urea was added to the united solution, and the mixture was refluxed and then cooled at room temperature. The crystalline urea adducts (2nd crop) formed were filtered and separated from the filtrate (I). The 2nd crop of crystalline urea adducts, a mixed fraction containing urea adducts of highly unsaturated acids besides those of less unsaturated acids, was decomposed with dilute hydrochloric acid to give free fatty acids, and the latter were again subjected to the urea segregation in methanol, giving crystalline urea adducts and the filtrate (II). The filtrates I and II were united, methanol was distilled off, and the residue was decomposed with dilute hydrochloric acid to give a concentrate of highly unsaturated acids. In total, 15 kg. of sardine oil fatty acids were treated, giving 4,517 g. of a highly unsaturated acid concentrate of N.V. 191.7 and I.V. 383.1. In the following, a portion of this concentrate was used.

A portion of the highly unsaturated acid concentrate obtained above was converted to methyl ester, and the latter (1,940 g.) was fractionally distilled, giving fractions shown in Table 1.

TABLE 1. Fractional Distillation of the Methyl Ester of Highly Unsaturated Acid Concentrate

| Fraction | B.p. (°C/ca. 1 mm Hg) | Yield (g.) | Saponif. V. | Iodine V. |
|---|---|---|---|---|
| 1 | -180 | 194 | 205.9 | 253.7 |
| 2 | 180-190 | 239 | 197.1 | 316.5 |
| 3 | 190-200 | 513 | 189.5 | 348.7 |
| 4 | 200-205 | 424 | 178.7 | 382.6 |
| 5 | 205-215 | 390 | 160.8 | 401.5 |
| Residue | — | 180 | — | — |

Notes: The residue had I.V. 270.1. It appeared to contain a considerable amount of the oxidative polymerization product of highly unsaturated methyl ester.

The fraction 5 in Table 1 appeared to be contaminated with a small amount of unsaponifiable matter. A portion of the fraction 5 (about 160 g.) was freed from unsaponifiable matter by separating fatty acids and unsaponifiable matter in the usual way followed by reconversion of the fatty acids to their methyl esters. The methyl esters thus obtained were fractionated by elution chromatography. A 30 g.-portion of methyl esters, as a concentrated solution in hexane, was allowed to pass slowly into an adsorption column, 6 cm. in diameter and 75 cm. in height, packed with silica gel. Hexane containing 2% of ether was used for elution. In total, 145 g. of methyl esters were chromatographed, and seven eluate fractions shown in Table 2 were separated. As is seen from Table 2, the first eluate fraction A-1 has the lowest iodine value followed by the succeeding eluate fractions with increasingly higher iodine values, but the last eluate fraction A-7 has a lower iodine value than the preceding eluate fraction A-6. Calculating from the yields of eluate fractions, it is found that 7.4% of the methyl esters have not been eluted and remain in the adsorption column. The material still adsorbed on silica gel after elution had a dark reddish orange color and was considered to contain some oxidative polymerization products of the methyl esters. While such products may possibly be contained to some extent in the methyl esters before chromatography, they are also possibly formed from the most highly unsaturated methyl ester components in the presence of silica gel during chromatography.

TABLE 2. Chromatography of Highly Unsaturated Methyl Esters (Fraction 5 in Table 1 after Removal of Unsaponifiable Matter)

| Eluate fraction | Yield (g.) | Iodine value |
|---|---|---|
| A-1 | 5.6 | 338.5 |
| A-2 | 27.6 | 345.6 |
| A-3 | 26.2 | 365.6 |
| A-4 | 35.6 | 406.2 |
| A-5 | 10.5 | 413.6 |
| A-6 | 18.6 | 423.3 |
| A-7 | 10.2 | 416.3 |

**2. Separation of methyl decosahexaenoate**

The eluate fractions A-5 to A-7 in Table 2 were united. The united fraction (38 g.) was fractionated by elution chromatography into nine fractions, B-1 to B-9 (Table 3). In this case, iodine values of eluate fractions showed a similar tendency as shown in the case of the fractions A-1 to A-7 in Table 2. The fraction B-6 had the highest iodine value followed by the fractions B-7, B-8 and B-9 of lower iodine values. For the separation of a pure methyl docosahexaenoate fraction, the fractions B-5 and B-6 were united, and 6 g. of the united fraction was subjected to a further chromatography to give the fractions C-1 to C-6 in Table 4. The fraction C-4 in Table 4 had $d_4^{20}$ 0.9401, $n_D^{20}$ 1.4942, S.V. 163.4 and I.V. 442.5 (Calcd. for methyl docosahexaenoate, S.V. 163.8 and I.V. 444.6). The free acid from this fraction had $d_4^{20}$ 0.9484, $n_D^{20}$ 1.5025, N.V. 170.6 and I.V. 460.5 (Calcd. for docosahexaenoic acid, N.V. 170.8 and I.V. 463.6). Hydrogenation of this acid gave a product which after recrystallization from ethanol melted at 79°–79.5°C and was identified with behenic acid by the mixed melting point test.

TABLE 3. Chromatography of the Fractions A-5, A-6 and A-7 in Table 2

| Eluate fraction | Yield (g.) | Saponif. V. | Iodine V. |
|---|---|---|---|
| B-1 | 4.3 | 170.6 | 396.9 |
| B-2 | 6.8 | 168.4 | 410.7 |
| B-3 | 3.5 | 167.9 | 418.1 |
| B-4 | 4.1 | — | 428.6 |
| B-5 | 4.9 | 164.2 | 430.2 |
| B-6 | 2.4 | 163.8 | 436.1 |
| B-7 | 1.9 | — | 430.7 |
| B-8 | 3.0 | — | 428.6 |
| B-9 | 0.4 | — | — |

Notes: All chromatographic fractionations recorded in this paper were conducted using silica gel as adsorbent and hexane containing ether in appropriate proportions as eluant.

TABLE 4. Chromatography of the Fractions B-5 and B-6 in Table 3

| Eluate fraction | Yield (g.) | Iodine value |
|---|---|---|
| C-1 | 1.1 | 420.3 |
| C-2 | 0.9 | 428.2 |
| C-3 | 1.4 | 438.5 |
| C-4 | 1.0 | 442.5 |
| C-5 | 0.6 | 425.6 |
| C-6 | <0.1 | — |

### 3. Separation of methyl docosapentaenoate

The fractions A-2 and A-3 in Table 2 were united. The united fraction (51 g.) was fractionated into nine fractions by elution chromatography. The third eluate fraction (11.6 g.) had an iodine value of 363.2, which is close to the value calculated for methyl docosapentaenoate, but this fraction was judged to contain methyl esters of different unsaturations by the ultraviolet absorption data after alkali-isomerization. This fraction was subjected twice to chromatographic purification, giving eventually a methyl docosahexaenoate fraction (0.7 g.) which had $d_4^{20}$ 0.9307, $n_D^{20}$ 1.4888, S.V. 163.0 (Calcd., 162.8) and I.V. 367.6 (Calcd., 368.4). The free acid from this fraction had N.V. 170.3 and I.V. 383.2 (Calcd. for docosapentaenoic acid, N.V. 169.8 and I.V. 384.0).

### 4. Separation of methyl eicosapentaenoate

Although the fraction 4 among five fractions in Table 1 appeared to be most rich in methyl eicosapentaenoate from its saponification and iodine values, this fraction was used for other purpose. For this study, methyl eicosapentaenoate was separated from the fraction 5 in Table 1 in the following way. The eluate fraction A-4 in Table 2 and the eluate fractions B-1 and B-2 in Table 3 were united, and the united material was fractionally distilled to give a fraction (10.4 g.) of b.p. below 205°C/1 mmHg, S.V. 178.8 and I.V. 395.6. In spite of the proximity of the saponification and iodine values to the corresponding values calculated for methyl eicosapentaenoate, this fraction was found to contain a considerable proportion of hexaenoate by the ultraviolet absorption values after alkali-isomerization. This fraction was again fractionally distilled, and a fraction (5.2 g.) of S.V. 175.8 and I.V. 407.6 was separated and then subjected twice to chromatography. The methyl

eicosapentaenoate fraction (0.4 g.) eventually separated had $n_D^{20}$ 1.4900, S.V. 177.6 (Calcd., 177.3) and I.V. 398.2 (Calcd., 401.0). The fatty acid obtained from a neighbouring eluate fraction yielded a hydrogenation product which, after recrystallization from ethanol, showed m.p. 76°–76.5°C and N.V. 178.5 (Calcd. for arachidic acid, 179.4).

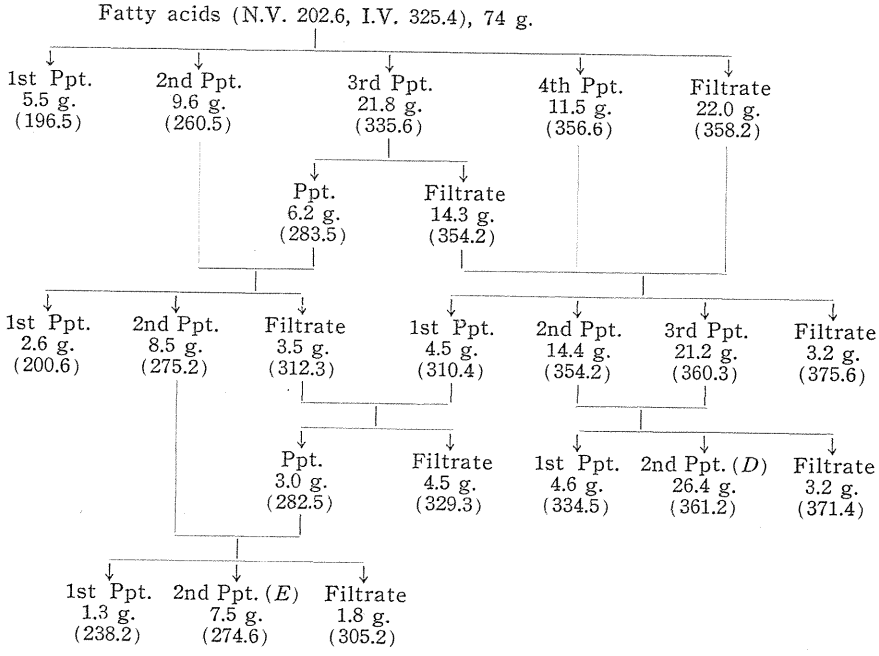
**5. Separation of methyl eicosatetraenoate**

Repeated fractional distillation of the fraction 2 (237 g.) in Table 1 gave a fraction (17 g.) of S.V. 180.6 and I.V. 351.5 as the highest boiling fraction. This was subjected twice to chromatographic fractionation, giving eventually a methyl eicosatetraenoate fraction (0.5 g.) of $n_D^{20}$ 1.4820, S.V. 176.8 (Calcd., 176.2) and I.V. 317.9 (Calcd., 318.8). The fatty acid obtained from a neighbouring eluate fraction yielded a hydrogenation product which was identified with arachidic acid by its m.p. 75°–75.5°C and N.V. 179.2 after recrystallization from ethanol.

**6. Separation of methyl octadecatetraenoate**

After repeated fractional distillation of the fraction 2 in Table 1, a fraction of b.p. 205°–215°C/ca. 10 mmHg, S.V. 193.5 and I.V. 314.1 was separated. The fatty acids (74 g.) from this fraction was fractionally precipitated as their sodium salts from an acetone solution. After repeating the fractional precipitation several times, the fractions given in Table 5 were separated.

TABLE 5. Fractional Precipitation of Fatty Acids as Their Sodium Salts from Acetone




Notes: Yields recorded under the precipitates and filtrates denote those for the fatty acids therefrom. Numerical figures in parentheses denote iodine values.

The fatty acid fraction from the precipitate *D* in Table 5 had an iodine value of 361.2 which is close to the value calculated for octadecatetraenoic acid (367.3), but its neutralization value was found to be 197.6, a little lower than the value calculated for octadecatetraenoic acid (203.0). This fraction was converted to methyl ester, and the latter was fractionally distilled to give a fraction (14.5 g.) of S.V. 191.8 and I.V. 342.8. This methyl ester fraction was fractionated further by chromatography. The eluate fraction (2.2 g.) eventually obtained as octadecatetraenoate had $d_4^{20}$ 0.9220, $n_D^{20}$ 1.4812, S.V. 193.5 (Calcd., 193.2) and I.V. 352.0 (Calcd., 349.6). Hydrogenation of the free acid from this methyl ester fraction gave stearic acid which, after recrystallization from ethanol, had m.p. 69°–69.5°C and N.V. 196.3 (Calcd., 197.2).

**7. Separation of methyl octadecatrienoate**

The fatty acid fraction (N.V. 200.1 and I.V. 274.6) from the precipitate *E* in Table 5 approximately coincides with octadecatrienoic acid (Calcd., N.V. 201.5 and I.V. 273.5) in its neutralization and iodine values. This was converted to methyl ester, and the latter was purified by chromatography. The eluate fraction (0.5 g.) eventually obtained as methyl octadecatrienoate had $n_D^{20}$ 1.4703, S.V. 192.3 (Calcd., 191.8) and I.V. 265.6 (Calcd., 260.4). The fatty acid from a neighbouring eluate fraction yielded stearic acid as its hydrogenation product, which had m.p. 69°–69.5°C and N.V. 196.8 after recrystallization from ethanol.

**8. Separation of methyl hexadecatrienoate**

The fatty acids (181 g.) from the fraction 1 in Table 1 were segregated by way of urea adduct in methanol, and five fraction given in Table 6 were obtained.

TABLE 6. Urea Fractionation of the Fatty Acids from the Fraction 1 in Table 1

| Fraction | Yield (g.) | Neutralization V. | Iodine V. |
|---|---|---|---|
| 1 | 34 | 228.6 | 165.6 |
| 2 | 45 | 225.5 | 220.3 |
| 3 | 21 | 218.3 | 272.4 |
| 4 | 42 | 209.2 | 310.5 |
| 5 | 38 | 206.5 | 330.6 |

The results given in Table 6 indicate that the fatty acids from the fraction 1 in Table 1 contain a considerable amount of $C_{14}$- and $C_{18}$-acids besides $C_{16}$-acids. In order to separate $C_{16}$-acids, the fatty acid fractions 2, 3 and 4 in Table 6 were converted to methyl esters, and the latter were fractionally distilled, giving a fraction (10.5 g.) of S.V. 210.5 and I.V. 260.7. This methyl ester fraction was subjected twice to chromatography, and an eluate fraction (0.5 g.) of $n_D^{20}$ 1.4711, S.V. 211.9 (Calcd., 212.4) and I.V. 276.5 (Calcd., 288.3) was eventually separated as methyl hexadecatrienoate, though its iodine value was a little lower than the theoretical value. The fatty acid from a neighbouring eluate fraction yielded palmitic acid as its hydrogenation product which had m.p. 61°–62°C and N.V. 218.0 (Calcd., 218.8) after recrystallization from ethanol.

## II. Ultraviolet Absorption Spectra for Polyethenoid Acid Components

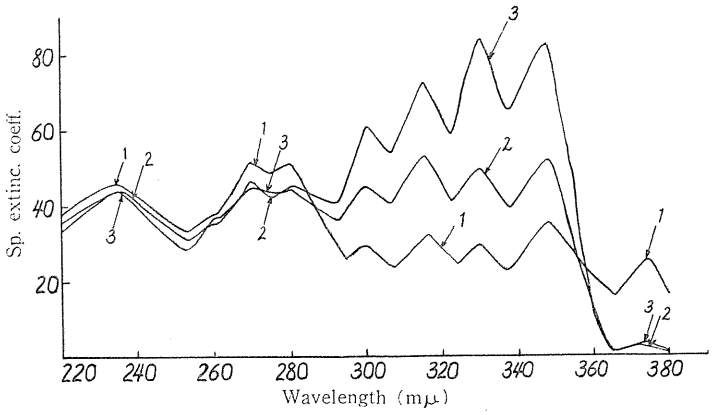
In the first place, conjugated contaminants in the specimens of polyunsaturated methyl esters separated by the foregoing procedures were estimated by measuring ultraviolet absorption spectra in methanol. The specific extinction coefficients of the respective methyl esters at the characteristic wavelengths for diene to hexaene conjugations are shown in Table 7. Gonjugated diene (%) in Table 7 was calculated from the specific extinction coefficient at the characteristic maximum of conjugated diene, 233 mμ or 235 mμ in our experiments, by applying the formula of Brice and Swain.[14] It is, however, questionable that this formula is applicable to the methyl esters of polyethenoid acids in our experiments, and the figures given in Table 7 may be taken merely as approximate levels of conjugated diene content. In the region of characteristic wavelengths for conjugated triene, only a smallest absorption maximum at 268 mμ was observed for the eicosatetraenoate, octadecatrienoate and hexadecatrienoate specimens, suggesting that conjugated triene content for each methyl ester specimen is less than 0.01%. In short, these methyl ester specimens are not completely free from conjugated contaminants, but their amounts in these specimens are considerably small as compared with those in the corresponding specimens in our previous study.[11]

TABLE 7. Ultraviolet Absorption Values of the Methyl Esters of Polyethenoid Acids

| Methyl ester | Specific extinction coefficients | | | | | | | Conjugated diene |
|---|---|---|---|---|---|---|---|---|
| | Wavelength (mμ) | | | | | | | |
| | 233 | 262 | 268 | 274 | 310 | 316 | 322 | (%) |
| Docosahexaenoate | 2.66* | 0.53 | 0.42 | 0.35 | 0.05 | 0.05 | 0.04 | 2.18 |
| Docosapentaenoate | 1.75* | 0.46 | 0.46 | 0.44 | 0.15 | 0.14 | 0.14 | 1.41 |
| Eicosapentaenoate | 1.07* | 0.42 | 0.41 | 0.41 | 0.05 | 0.05 | 0.04 | 0.84 |
| Eicosatetraenoate | 1.35 | 0.42 | 0.46 | 0.40 | 0.10 | 0.09 | 0.08 | 1.08 |
| Octadecatetraenoate | 1.28 | 0.44 | 0.44 | 0.42 | 0.14 | 0.12 | 0.10 | 1.02 |
| Octadecatrienoate | 0.90 | 0.36 | 0.38 | 0.35 | 0.14 | 0.11 | 0.11 | 0.70 |
| Hexadecatrienoate | 0.94 | 0.30 | 0.32 | 0.28 | 0.09 | 0.08 | 0.07 | 0.73 |

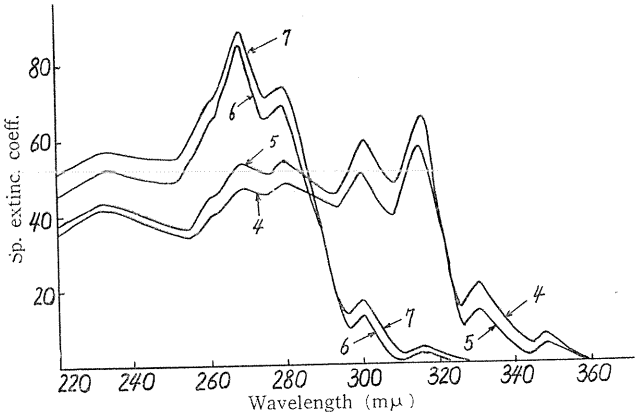
Notes: * Specific extinction coefficient at 235 mμ. The absorption maximum for conjugated diene was observed at 235 mμ for these specimens.

Although it requires a further study to establish the optimal condition for the alkali-isomerization of highly unsaturated acids, the alkali-isomerization in this study was conducted under the condition of 21% KOH-ethylene glycol, 180°C and 15 min. with a current of nitrogen for the sake of the comparison of our results with reported data. The ultraviolet absorption spectra of each methyl ester specimen after alkali-isomerization are shown in Figs. 1 and 2, and the specific extinction coefficients at the characteristic absorption maxima of conjugated polyenes are given in Table 8. The data recorded in Table 8 are expressed on the basis of respective polyethenoid acids instead of their methyl esters. In reference to our data in Table 8, Table 9 contains some data reported in the literature for the polyethenoid acids after alkali-isomerization under the condition of 21% KOH-ethylene glycol, 180°C and 15 min.




1. Methyl docosahexaenoate 2. Methyl docosapentaenoate
3. Methyl eicosapentaenoate

FIG. 1. Ultraviolet absorption spectra of the methyl esters of polyethenoid acids after alkali-isomerization.




4. Methyl eicosatetraenoate 5. Methyl octadecatetraenoate
6. Methyl octadecatrienoate 7. Methyl hexadecatrienoate

FIG. 2. Ultraviolet absorption spectra of the methyl esters of polyethenoid acids after alkali-isomerization.

The methyl docosapentaenoate and methyl eicosapentaenoate in Table 8, after alkali-isomerization, exhibit a small absorption maximum at 374 mμ, indicating the presence of hexaenoate as a contaminant in both specimens. On the other hand, both specimens are considered to be contaminated with some methyl esters of a lower unsaturation than pentaenoate besides hexaenoate, since their iodine values approximate the corresponding theoretical values. The methyl eicosatetraenoate and methyl octadecatetraenoate in Table 8, after alkali-isomerization, exhibit an absorption maximum at 348 mμ, indicating the presence of pentaenoic contaminants in both specimens. Further, the methyl eicosatetraenoate in Table 8 is considered to be contaminated with some methyl esters of lower unsaturation besides penta-

TABLE 8. Ultraviolet Absorption Values for the Polyethenoid Acids after Alkali-isomerization

| Methyl ester | Iodine value of methyl ester | | Specific extinction coefficients (on the basis of free acid) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Wavelength (mμ) | | | | |
| | Observed | Calcd. | 233 | 268 | 316 | 348 | 374 |
| Docosahexaenoate | 442.5 | 444.6 | 45.0* | 54.2* | 32.6 | 35.7 | 26.0 |
| Docosapentaenoate | 367.6 | 368.4 | 47.1* | 47.7* | 55.3 | 54.0 | 2.1 |
| Eicosapentaenoate | 398.2 | 401.0 | 46.2* | 48.6* | 83.2 | 86.0 | 2.7 |
| Eicosatetraenoate | 317.9 | 318.8 | 43.4 | 48.4 | 60.9 | 6.9 | — |
| Octadecatetraenoate | 352.0 | 349.6 | 45.4 | 54.9 | 68.9 | 4.7 | — |
| Octadecatrienoate | 265.6 | 260.4 | 54.9 | 90.7 | 3.1 | — | — |
| Hexadecatrienoate | 276.5 | 288.3 | 60.4 | 93.6 | 4.6 | — | — |

Notes: * Specific extinction coefficients at 235 mμ and 269 mμ in place of 233 mμ and 268 mμ, respectively. The absorption maxima for conjugated diene and triene were observed at 235 mμ and 269 mμ, respectively, for these specimens.

TABLE 9. Reported Data on Ultraviolet Absorption Values for the Polyethenoid Acids after Alkali-isomerization

| Polyethenoid acid | Source | Specific extinction coefficients | | | | | Reference |
|---|---|---|---|---|---|---|---|
| | | Wavelength (mμ) | | | | | |
| | | 233 | 268 | 315 | 346 | 374 | |
| Docosahexaenoic | Hog brain lipid | 40.4 | 50.4 | 28.4 | 26.6 | 28.1 | 8) |
| Docosahexaenoic | Cod liver oil | 48.0 | 46.4 | 36.9 | 30.4 | 16.8 | 10) |
| Docosapentaenoic | Ox suprarenal lipid | 43.5 | 46.0 | 56.9 | 50.4 | — | 7) |
| Docosapentaenoic | Cod liver oil | 42.7 | 42.8 | 54.7 | 40.8 | 8.7 | 10) |
| Docosapentaenoic | Sardine oil | 60.1 | 48.7 | 53.2 | 39.3 | — | 11) |
| Eicosapentaenoic | Ox suprarenal lipid | 39.4 | 41.2 | 82.4 | 87.5 | — | 7) |
| Eicosapentaenoic* | Cod liver oil | 43.0 | 45.7 | 47.4 | 48.0 | 10.3 | 10) |
| Eicosapentaenoic | Sardine oil | 59.0 | 54.6 | 75.0 | 87.8 | — | 11) |
| Eicosatetraenoic | Ox suprarenal lipid | 39.7 | 48.2 | 60.6 | — | — | 7) |
| Eicosatetraenoic | Hog liver lipid | 42.0 | 46.1 | 60.9 | 12.8 | — | 15) |
| Octadecatrienoic** | Vegetable oils | 47.5 | 90.5 | — | — | — | 7) |
| Hexadecatrienoic | Sardine oil | 75.6 | 78.9 | — | — | — | 11) |

Notes: * A methyl eicosapentaenoate fraction of I.V. 405. ** Linolenic acid. Specific extinction coefficients for the docosapentaenoic and eicosapentaenoic acids from sardine oil and eicosatetraenoic acid from hog liver lipid are the values at 235, 270, 316 and 348-349 mμ in place of 233, 268, 315 and 346 mμ, respectively. The specific extinction coefficient, 16.8, for the docosahexaenoic acid from cod liver oil is the value at 375 mμ in place of 374 mμ.

enoate since its iodine value is close to the theoretical value. The methyl octadecatrienoate in Table 8 has a little higher iodine value than the theoretical value and exhibits an absorption maximum at 316 mμ after alkali-isomerization, indicating the presence of tetraenoic contaminants in this specimen. The methyl hexadecatrienoate in Table 8 has an iodine value somewhat lower than the theoretical value, yet it exhibits an absorption maximum at 316 mμ after alkali-isomerization, indicating the presence of both tetraenoic and lower unsaturated contaminants in this specimen. Thus, the methyl ester specimens in Table 8, except methyl docosahexaenoate, are not pure specimens. Accordingly the specific extinction coefficients

of these specimens after alkali-isomerization, given in Table 8, should be corrected for the purity of these specimens and the influence of polyethenoid contaminants before they can be taken as data for the corresponding pure specimens.

Comparing our data in Table 8 and reported data in Table 9, the following may be mentioned. Among the values reported for the specific extinction coefficient of isomerized docosahexaenoic acid at 374–375 mμ, the value reported by Hammond and Lundberg[8] for the acid from hog brain lipid is the highest. The value obtained in this study for the acid from sardine oil is slightly lower than the value reported by Hammond and Lundberg, but it is considerably high as compared with the value reported by Abu-Nasr, Holman and co-workers[10] for the acid from cod liver oil. As for the specific extinction coefficients of isomerized docosapentaenoic and eicosapentaenoic acids at 346-348 mμ, the values obtained in this study for the acids from sardine oil are comparable with the corresponding values reported by Herb, Riemenschneider and co-workers[7] for the acids from ox suprarenal lipid, whereas the values reported for the acids from cod liver oil are lower than the corresponding values for the acids from sardine oil and ox suprarenal lipid. Especially the value for the eicosapentaenoic acid from cod liver oil is exceedingly low. Abu-Nasr, Holman and co-workers considered a relatively large absorption at 375 mμ for their specimens of docosapentaenoic and eicosapentaenoic acids, after alkali-isomerization, to be an end absorption of conjugated pentaene, but our specimens of docasapentaenoate and eicosapentaenoate after alkali-isomerization do not exhibit such a large end absorption at 374 mμ. They exhibit sharply a small absorption maximum at 374 mμ indicating the presence of hexaenoic contaminants in these specimens.

Although the absorption values for pure specimens of respective polyethenoid acids after alkali-isomerization can not be conclusively established from the data in Table 8, the approximate values can be tentatively derived from the data in Table 8 if several assumptions on the fatty acid composition of the specimens used in this study and some other respects be permitted. Table 10 records the absorption values after alkali-isomerization for pure polyethenoid acids calculated from the data in Table 8 on the following assumptions. Taking into consideration that the saponification and iodine values of each methyl ester specimen used in this study are close to the theoretical values, the acid components of each methyl ester specimen are assumed, in most cases, to contain a next more unsaturated acid of two more carbon numbers and a next less unsaturated acid of two lesser carbon numbers besides the main acid component. The fatty acid composition of each methyl ester specimen is then calculated from the observed iodine value of methyl ester specimen and the content of more unsaturated companion estimated by the absorption value after alkali-isomerization. In the case of the octadecatrienoate and hexadecatrienoate specimens, the less unsaturated companion in the fatty acid components is taken as hexadecenoic and tetradecenoic acids instead of hexadecadienoic and tetradecadienoic acids, respectively, since the latter two acids occur in sardine oil only in the smallest amount if they occur at all. For the calculation of the absorption values after alkali-isomerization of pure polyethenoid acids, the docosahexaenoate in Table 8 is regarded as a pure specimen. Further, although there are no reported data on the absorption values after alkali-isomerization of the tetracosahexaenoic acid which is assumed as a companion in the fatty acids of the docosapentaenoate specimen, they are tentatively assumed to be the same as the corresponding values for docosahexaenoic acid.

TABLE 10. Ultraviolet Absorption Values after Alkali-isomerization for Pure Polyethenoid Acids Calculated from the Data in Table 8 on Some Assumptions

| Polyethenoid acid | Specific extinction coefficients for pure acid | | | | | Assumed composition of the polyethenoid acid components of the methyl ester specimen |
|---|---|---|---|---|---|---|
| | Wavelength (mμ) | | | | | |
| | 235 | 269 | 316 | 348 | 374 | |
| Docosahexaenoic | 45.0 | 54.2 | 32.6 | 35.7 | 26.0 | Docosahexaenoic 100% |
| Docosapentaenoic | 48.0 | 47.6 | 56.0 | 61.3 | — | Docosapentaenoic 83.4%, tetracosahexaenoic 8.1%, eicosatetraenoic 8.5% |
| Eicosapentaenoic | 46.4 | 46.6 | 93.1 | 109.2 | — | Eicosapentaenoic 75.4%, docosahexaenoic 10.4%, octadecatetraenoic 14.2% |
| Eicosatetraenoic | 41.1 | 42.2 | 70.3 | — | — | Eicosatetraenoic 77.6%, docosapentaenoic 11.3%, octadecatrienoic 11.1% |
| Octadecatetraenoic | 45.7 | 55.5 | 67.5 | — | — | Octadecatetraenoic 95.7%, eicosapentaenoic 4.3%, hexadecatrienoic 0%* |
| Octadecatrienoic | 55.5 | 92.9 | — | — | — | Octadecatrienoic 95.6%, eicosatetraenoic 4.4%, hexadecenoic 0%* |
| Hexadecatrienoic | 67.9 | 106.4 | — | — | — | Hexadecatrienoic 84.4%, octadecatetraenoic 6.8%, tetradecenoic 8.8% |

Notes: Absorption maxima of alkali-isomerized polyethenoid acids were observed in most cases of our experiments at 235, 269, 316, 348 and 374 mμ instead of 233, 268, 315, 346 and 374 mμ usually reported in the literature.

* The percentages for these hexadecatrienoic and hexadecenoic acids obtained by calculation showed small negative values, −0.3% and −1.6%, respectively, but these were taken as zero.

The specific extinction coefficient of alkali-isomerized eicosatetraenoic acid at 316 mμ is expected to be smaller than the corresponding values for alkali-isomerized octadecatetraenoic acid, but the reverse is the case in Table 10. This arises from the foregoing assumption that the more unsaturated acid companion is taken as docosapentaenoic acid in the case of eicosatetraenoate specimen while as eicosapentaenoic acid in the case of octadecatetraenoate specimen.

## III. Summary

Docosahexaenoic, docosapentaenoic, eicosapentaenoic, eicosatetraenoic, octadecatetraenoic, octadecatrienoic and hexadecatrienoic acids were separated as their methyl esters from sardine oil. The methyl esters were isomerized under the condition of 21% KOH-ethylene glycol, 180°C and 15 min., and the ultraviolet absorption values of isomerized polyethenoid acids were estimated with a view of supplementing our previous studies on the same subject. Further, the approximate values of the ultraviolet absorption after alkali-isomerization for the respective pure polyethenoid acids were tentatively calculated from the observed data for the specimens used in this study by making several assumptions on the fatty acid composition of the respective specimens and some other respects.

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