THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL

XX. ULTRAVIOLET ABSORPTION MEASUREMENTS OF HIGHLY CONJUGATED ACIDS OBTAINED BY THE ALKALI-ISOMERIZATION OF CLUPANODONIC ACID AND MIXTURES OF CLUPANODONIC AND DOCOSAHEXAENOIC ACIDS

Yoshiyuki TOYAMA and Tsutomu SHIMO-OKA

Department of Applied Chemistry

In the 19 th report of this series,[1] the highly unsaturated acids prepared from winterized sardine oil were isomerized with alkali, the highly conjugated acids were separated from the isomerized product, and their ultraviolet spectral properties were measured. Among the highly conjugated acids obtained in the previous experiments, the fraction having the highest specific extinction coefficients at the region of conjugated pentaene exhibited the specific extinction coefficients of 300.0 at 330 mμ and 274.7 at 346 mμ along with 222.2 at 316 mμ and 60.7 at 374 mμ. Although this fraction appeared to consist chiefly of C_{22}-acids, it was not a single highly conjugated acid, but was possibly contaminated more or less with oxidized acid, too. In the present experiments, clupanodonic acid (4, 8, 12, 15, 19-docosapentaenoic acid) was prepared from winterized sardine oil and isomerized with alkali. The highly conjugated solid acids were separated from the isomerized product and their ultraviolet absorptions were measured. It should, however, be noted that although the neutralization and iodine values of clupanodonic acid used in these experiments agreed well with the calculated values, the ultraviolet absorption measurements indicated the preformation of a small amount of conjugated triene and tetraene in addition to diene in this clupanodonic acid. On the other hand, the highly conjugated acid fraction obtained by fractionation of the alkali-isomerization product exhibited a small maximum absorption at 374 mμ, indicating the presence of docosahexaenoic acid in the original clupanodonic acid. Hence, taking the iodine value of the original clupanodonic acid into consideration, it should be concluded that the clupanodonic acid used in these experiments is contaminated with less unsaturated acids as well as docosahexaenoic acid. In the previous experiments, the alkali-isomerization product was dissolved ·in hexane, oxidized acids insoluble in hexane at ordinary temperatures were removed, and the solution was cooled to about − 10° C so that the highly conjugated solid acids crystallized out. In the present experiments, however, the highly conjugated solid acids crystallized out from hexane were purified by a further recrystallization from acetone, since the product crystallized out from hexane was afraid of being still contaminated with some oxidized acids. The highly conjugated acid fraction (A_5) thus obtained had M.P. 114–116° C and a hydrogen value which was close to the calculated value for docosapentaenoic acid. On hydrogenation, it yielded behenic acid. Measurements of specific extinction coefficients (Table 5, Fig. 1) at ultraviolet region gave 222.4 at 316 mμ, 253.9 at 330 mμ, 223.5 at 346 mμ and 33.0 at 374 mμ. The fact that a

maximum absorption was distinctly observed at 374 mμ indicates that the fraction (A_5) contains not only conjugated tetraene and pentaene but also hexaene. However, since the specific extinction coefficient of conjugated docosahexaenoic acid is inferred to be at least 260-270 at 374 mμ from the absorption spectra (Table 7, Fig. 2) of the highly conjugated acid fraction (C) obtained from the alkali-isomerization product of a mixture of clupanodonic and docosahexaenoic acids, the content of docosahexaenoic acid in the fraction (A_5) appears to be at most 12-13%, the major components of the fraction (A_5) being conjugated pentaene and tetraene. The specific extinction coefficients (300.0 at 330 mμ and 274.7 at 346 mμ) of the highly conjugated acids described in the previous paper are higher than those (253.9 at 330 mμ and 223.5 at 346 mμ) of the fraction (A_5). However, it can not be concluded that the former contain much cojugated pentaene than the latter since the specific extinction coefficients at these wave lengths are remarkably affected by the content of conjugated hexaene.

As mentioned in the previous paper, the highly conjugated acids are highly susceptible to air oxidation, and the oxidation product exhibits a remarkably smaller absorption at ultraviolet region. It may be also conceivable that the oxidation of conjugated acids is promoted by ultraviolet irradiation during the ultraviolet absorption measurements. In the present experiments, it required less than five minutes to take the ultraviolet spectra of a sample, and the measurements before and after an ultraviolet irradiation for five minutes gave the values only slightly different from each other. However, when a solution of the sample irradiated for five minutes was kept in a well stoppered bottle under nitrogen for five days in a dark place, it showed a considerably smaller absorption, indicating that some chemical changes, such as transition of oxidation product and polymerization, must have occurred during the storage, even if air was strictly excluded.

Experimental

A highly unsaturated acid concentrate (695 g) with I.V. 335.0 was prepared from winterized sardine oil by the sodium salt acetone method, and was converted into the methyl ester which was then subjected to fractional distillation. A fraction of B.P. 210-215° C/ca. 1 mm Hg was separated and saponified, yielding 115 g of a highly unsaturated acid mixture of I.V. 381.8. This acid mixture was fractionally precipitated as sodium salt from acetone, and the following three fractions (Table 1) were finally obtained.

TABLE 1

Fraction	N.V.	I.V.	Yield (g)
a	169.0	384.8	49
b	170.3	402.5	15
c	171.1	424.5	6

The neutralization and iodine values in Table 1 seem to indicate that the fraction (a) is composed of clupanodonic acid (calculated, N.V. 169.8, I.V. 384.0), while the fractions (b) and (c) are composed of mixtures of clupanodonic and docosahexaenoic acids (calculated, N.V. 170.8, I.V. 463.6).

1. Isomerization of clupanodonic acid.   Specific extinction coefficients at the characteristic wave lengths for clupanodonic acid (*a*) in Table 1 are given in Table 2.   The measurements of specific extinction coefficients were made in an ethanol soution of the sample using a Model DU Beckman spectrophotometer.   The contents of conjugated components in clupanodonic acid (*a*) were tentatively calculated from the data in Table 2 in the same way as given in the previous report; 4.8% of diene, 0.12% of triene and 0.09% of tetraene.

TABLE 2

Wave length (mμ)	233	262	268	274	310	316	322
Specific ext. coeff.	5.73	4.17	4.19	4.03	0.55	0.57	0.43

Forty seven g of clupanodonic acid (*a*) was isomerized under the condition of 21% KOH-glycol (1.3 kg), 180° C and 15 minutes with nitrogen.   The isomerized product was cooled, diluted with 1.2 liters of water, and acidified with dilute hydrochloric acid, and the fatty acids liberated were extracted with ether.   Hexane (500 cc) was added to the ether-extract, the portion insoluble in hexane at the room temperature was removed, and 45 g of hexane soluble portion was obtained. This portion consisted chiefly of an oily liquid with a small amount of crystalline solid and exhibited the specific extinction coefficients given in Table 3.

TABLE 3

Wave length (mμ)	233	268	316	330	346
Specific ext. coeff.	74.7	65.8	68.6	73.1	46.3

Hexane soluble portion (44.5 g) obtained above was dissolved in 450 cc of 95% ethanol, and 30 cc of a 10% solution of magnesium acetate in 95% ethanol was added.   After standing overnight, the precipitated magnesium salt was filtered and decomposed with dilute hydrochloric acid, and the free acid mixture liberated was extracted with hexane.   The fatty acid mixture thus obtained was a pale yellow solid of M.P. 96-98° C.   This acid mixture (3.8 g) was dissolved in 30 cc of hexane, cooled to − 10° C, and the crystalline solid (*A*₁) separated was filtered.   Concentration of the filtrate followed by cooling gave further the crystalline solids (*A*₂) and (*A*₃).   From the final filtrate, the crystalline solid (*A*₄) was recovered.   The characteristic properties and specific extinction coefficients of each crystalline fraction are given in Table 4, and the abosorption curves are shown in Fig. 1.

TABLE 4

Fraction	A_1	A_2	A_3	A_4
Yield (g)	0.8	0.6	0.5	1.1
M.P.	116–118	113–115	110–112	—
N.V.	170.3	170.1	169.5	170.3
Specific ext. coeff. at				
316 mμ	198.5	189.2	162.2	98.2
330 〃	236.0	223.5	199.6	116.4
346 〃	208.3	194.2	176.2	107.7
374 〃	30.0	20.2	14.0	16.5

After determining the neutralization values of the fractions A_1–A_3 in Table 4, 1.0 g of conjugated acid mixture was recovered from the soap solutions. It was then dissolved in aqueous acetone (90 parts of acetone and 10 parts of water), and cooled to $-10°$ C, and 0.5 g of pale yellow crystalline solid (A_5) was separated. It had M.P. 114–116° C, N.V. 170.3 and hydrogen value 298.9 * (calculated for docosapentaenoic acid 305.0). It had no characteristic odor unlike the original clupanodonic acid, and exhibited a blue-purple color with antimony trichloride in chloroform. Recry-

FIG. 1

stallization of the hydrogenated product gave behenic acid of M.P. 81-82° C. Specific extinction coefficients at charateristic wave lengths and absorption curve are shown in Table 5 and Fig. 1, respectively.
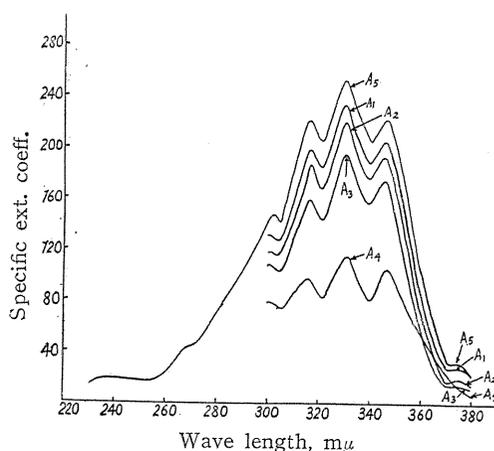
TABLE 5

Wave length (mμ)	233	268	316	330	346	374
Specific ext. coeff.	17.5	40.5	222.4	253.9	223.5	33.0

On subjecting an ethanol solution of fraction (A_5) to an ultraviolet irradiation for five minutes in the spectrophotometer, its specific extinction coefficient at 330 mμ became 252.8, which is smaller than the value 253.9 in Table 5 only by 1.1 units. However, when the same solution after irradiation was kept in a well stoppered bottle under nitrogen for five days in a dark place, the specific extinction coefficient at 330 mμ was decreased to 230.6, which is smaller than the value in Table 5 by 23.3 units.

2. *Isomerization of mixture of clupanodonic and docosahexaenoic acids* (*I.V.* *402.5*). Fourteen g of the fraction (*b*) in Table 1 was isomerized under the same

TABLE 6

Fraction	B_1	B_2	B_3
Yield (g)	0.3	0.2	0.3
M.P. (°C)	120–122	116–120	—
N.V.	171.0	170.7	170.8
Specific ext. coeff. at			
233 mμ	14.1	26.4	36.6
268 //	16.5	24.0	36.5
316 //	258.2	228.6	134.7
330 //	285.1	247.8	150.0
346 //	275.2	240.0	138.4
374 //	145.0	101.0	70.9

* This value seems to be a little lower.

condition as described above for clupanodonic acid, and 1.1 g of highly conjugated solid acid was obtained from the isomerized product. This was subjected to further fractional crystallizations from hexane, and the fractions B_1, B_2 and B_3 (recovered from the final filtrate) were separated. Characteristic properties and absorption curves of each fraction are given in Table 6 and Fig. 2, respectively.

   3. Isomerization of a mixture of clupanodonic and docosahexaenoic acids (I.V. 424.5).   The fraction (c) in Table 1 was isomerized under the same condition as described above, and 0.8 g of highly conjugated acid was obtained from 5.2 g of the fraction (c). This was dissolved in 200 cc of aqueous acetone and cooled to − 10° C, and 0.4 g of a pale yellow crystalline solid was obtained. It had M.P. 124–126° C and hydrogen value 347.2. Calculating from the hydrogen value, it consists of 33% of C_{22}-pentaenoic acid (hydrogen value, calculated, 305.0) and 67% of C_{22}-hexaenoic acid (hydrogen value, calculated, 368.2). Specific extinction coefficients at characteristic wave lengths and absorption curve are given in Table 7 and Fig. 2, respectively.
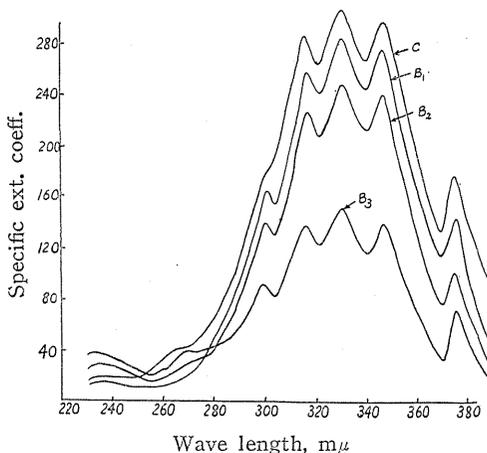

FIG. 2

TABLE 7

Wave length (mμ)	233	268	316	330	346	374
Specific ext. coeff.	18.1	39.8	284.5	307.0	295.8	177.0

Summary

   Clupanodonic acid, which is contaminated more or less with docosahexaenoic and other acids, and mixtures of clupanodonic and docosahexaenoic acids were separated from winterized sardine oil, and these were isomerized under the condition of 21% KOH-glycol, 180° C and 15 minutes with nitrogen. Highly conjugated solid acids were separated from the isomerized products and their ultraviolet absorptions were measured. A highly conjugated acid fraction of pale yellow color obtained from clupanodonic acid had M.P. 114–116° C and specific extinction coefficients of 222.4, 253.9, 223.5 and 33.0 at 316, 330, 346 and 374 mμ, respectively. Although it exhibited distinctly a maximum absorption at 374 mμ besides 316, 330 and 346 mμ, it appeared to consist predominantly of conjugated pentaene and tetraene. A highly conjugated acid fraction of M.P. 124–126° C obtained from a mixture of clupanodonic and docosahexaenoic acids contained pentaenoic and hexaenoic acids in a proportion of 33 : 67 when calculated from its hydrogen value. It had maximum absorptions at 316, 330, 346 and 374 mμ, the specific extinction coef-

ficients being 284.5, 307.0, 295.8 and 177.0, respectively.

References

1) Y. Toyama and T. Shimo-oka : *Memoirs Faculty of Engineering, Nagoya Univ.* 5, 330 (1953).