

# THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL

## XIX. ULTRAVIOLET ABSORPTION MEASUREMENTS OF HIGHLY CONJUGATED ACIDS OBTAINED BY ALKALI-ISOMERIZATION OF HIGHLY UNSATURATED ACIDS

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Concerning ultraviolet absorption spectra of conjugated acids obtainable by alkali-isomerization of highly unsaturated acids with four or more ethylenic linkages, the conjugated product from arachidonic acid of beef suprarenal lipids has hitherto been studied by many authors including Herb and others<sup>1)</sup> who studied also on the conjugated product from eicosapentaenoic and docosapentaenoic acids of beef suprarenal lipids. In the preceding paper,<sup>2)</sup> the present authors measured the spectral characteristics of alkali-isomerized products of moroctic, eicosatetraenoic, eicosapentaenoic and clupanodonic acids of sardine oil. However, conjugated acids used in previous studies were mixtures of several conjugated components consisting of highly or less conjugated components, though they were obtained by alkali-isomerization of an individual highly unsaturated acid. As for the spectral characteristics of the most highly conjugated component contained in such a conjugated acid mixture, e. i. conjugated polyene corresponding to number of ethylenic linkages of the original highly unsaturated acid, only a little has hitherto been studied. Mowry, Brode and Brown<sup>3)</sup> separated an acid of M.P. 95°-98° C by crystallizing the alkali-isomerized product of methyl arachidonate from petroleum ether and ethanol at -20° C. This acid consisted chiefly of conjugated eicosatetraenoic acid, and its ultraviolet absorption spectra resembled those of 2, 4, 6, 8-decatetraene. It is desirable to know spectral and other properties of each individual component, especially the most highly conjugated component, of conjugated acid mixture obtained by alkali-isomerization in order to apply reasonably the spectrochemical method for determination of respective highly unsaturated acids or to find out a proper utilization of conjugated acids. In the experiments by the present authors described below, the conjugated product obtained by alkali-isomerization of highly unsaturated acids of sardine oil was separated into several fractions, and the ultraviolet absorption characteristics of each fraction were measured.

A sample of highly unsaturated acids of N.V. 176.6 and I.V. 390.0 was isomerized by alkali, and the product was subjected to fractional precipitation of magnesium salt from 95% ethanol, by which a highly conjugated acid fraction of M.P. 98°-103° C and N.V. 173.0 was separated. This fraction appeared to consist chiefly of C<sub>22</sub>-acids from its neutralization value. It was separated further into four fractions *A*, *B*, *C* and *D* by fractional crystallization from hexane. Although each of the four fractions appeared still to consist of a mixture of acids of different degree of

conjugation, the fraction *A* contained the most highly conjugated component and exhibited specific extinction coefficients of 272.1 at 330  $m\mu$  and 249.5 at 346  $m\mu$  in the region of conjugated pentaene and also a considerable absorption in the region of conjugated tetraene and hexaene. Accordingly, the fraction *A* was found to contain a considerable amount of conjugated tetraene and hexaene besides conjugated pentaene. In an attempt to fractionate each fraction further, the samples used for the determination of neutralization value were recovered. But each fraction was exceedingly susceptible to autoxidation, and the recovered samples were contaminated with hexane-insoluble substance. While the changes of ultraviolet absorption spectra of conjugated dienoic and trienoic acids in the course of their oxidation were studied by Holman, Lundberg and Burr,<sup>4)</sup> the hexane-insoluble substance formed by oxidation of the conjugated acids in the present experiments was found to exhibit a considerably small absorption, the specific extinction coefficient at 316  $m\mu$  being only 3.9-37.2. Since the fractions *A-D* are afraid of being contaminated with more or less oxidized product, especially the fraction *A* which was obtained as the first crop of fractional crystallization is suspected to be contaminated with a relatively high proportion of oxidized product, the respective components of these fractions freed from oxidized product would exhibit larger maximum absorption in the region of highly conjugated polyenoic acids. On the other hand, there is a possibility of increasing ultraviolet absorptions in a certain region at a certain stage in the course of oxidation of highly conjugated acid into a hexane-insoluble substance, and it can not be presumably concluded that the contamination of oxidized product decreases always ultraviolet absorption. With the purpose of obtaining a highly conjugated fraction without prolonged procedures which are prone to cause oxidation of the material under examination, the alkali-isomerized product of the same sample of highly unsaturated acids was subjected to a crystallization from hexane at low temperature. The crystalline acid thus obtained showed a remarkably stronger absorption than the fraction *A* in the region of conjugated pentaene, exhibiting the extinction coefficients of 300.0 at 330  $m\mu$  and 274.7 at 346  $m\mu$ .

Highly conjugated solid acid fractions obtained in these experiments are light yellow in color. Among the polyene compounds which have been synthesized by previous authors, pentaene and hexaene compounds are generally colored, methyl ester of 2,4,6,8,10-dodecapentaenoic acid,<sup>5)</sup> for instance, being colored yellow. Although the color of such highly conjugated compounds seems to be inherent, the possibility is not excluded that the color of the highly conjugated solid fraction obtained in these experiments should be attributed to a contamination of colored oxidized product.

### Experimental

A highly unsaturated acid concentrate separated from winterized sardine oil by means of the sodium salt acetone method was converted into methyl esters, and the latter were fractionally distilled. A fraction of B.P. 218°-220° C/ca. 1 mm was separated. Saponification of this fraction yielded a highly unsaturated acid mixture of N.V. 176.6 and I.V. 390.0. Its spectral characteristics were measured in ethanol with the results shown in Table 1. Calculating the amounts of conjugated components in the same manner as described in the preceding paper, the sample of Table 1 contains 4.4% of diene, 0.05% of triene and 0.01% of tetraene.

Although these results can not be considered strictly correct, it is seen that the sample contains at least a few percentages of conjugated diene.

TABLE 1

Wave length, $m\mu$	233	262	268	274	310	316	322
Specific extinction coefficient	5.27	3.22	3.25	3.21	0.688	0.695	0.684

Twenty g of this highly unsaturated acid mixture was isomerized by heating with 550 g of 21% KOH-glycol for 15 minutes at 180° C with stirring under the atmosphere of nitrogen. After isomerization the solution was cooled, diluted with 500 cc of water, acidified with hydrochloric acid, and extracted with ether. The ether-extract was then treated with hexane, hexane-insoluble portion was removed, and 18.5 g of hexane-soluble portion was obtained. This was an oily substance which deposited solid at ordinary temperature. Specific extinction coefficients at characteristic wave lengths are given in Table 2.

TABLE 2

Wave length, $m\mu$	233	268	316	346	374
Specific extinction coefficient	65.3	50.6	56.4	40.2	10.5

The hexane-soluble portion (18 g) was dissolved in 180 cc of 95% ethanol, and 10 cc of a 10% solution of magnesium acetate in 95% ethanol was added. After standing over a night, the precipitate of magnesium soaps was separated and decomposed with hydrochloric acid. The resulting fatty acid mixture from magnesium soaps was taken up with hexane; yield 1.4 g. It was a crystalline solid of light yellow color and had M.P. 98°-103° C and N.V. 173.0. It was subjected to further fractional crystallizations in the following way. On cooling a solution of 1.2 g of this fatty acid mixture in 20 cc of hexane to -10° C, the first fraction *A* crystallized out from the solution. The 2nd and 3rd fractions, *B* and *C*, were obtained from the filtrate by concentration and cooling. The fraction *D* was recovered from the final filtrate. Properties and spectral characteristics of each fraction are given in Table 3. The absorption curves of each fraction are shown in Fig. 1. Each fraction has a light yellow color and is exceedingly susceptible to

TABLE 3

Fraction	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Yield, g	0.2	0.3	0.2	0.3
M.P., °C	110-116	107-112	100-103	—
N.V.	172.0	172.4	173.6	174.4
Sp. ext. coef. at, $m\mu$				
233	22.0	13.9	11.2	45.8
268	45.6	13.2	33.6	55.6
316	230.1	203.0	180.1	109.7
330	272.1	240.0	210.2	108.6
346	249.5	219.8	200.8	92.1
374	114.9	38.0	19.0	37.6

oxidation when exposed to air. The material recovered by adding hydrochloric acid to the soap solution of the sample used for the determination of neutralization value was found to be contaminated with a considerable amount of hexane-insoluble substance which exhibited a very weak absorption in the ultraviolet region as shown by the curves *a* and *b* in Fig. 2.

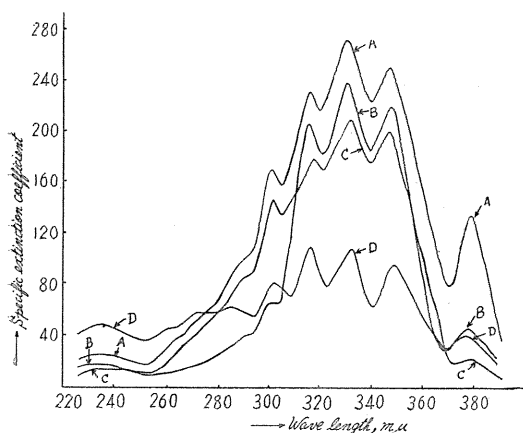


Fig. 1

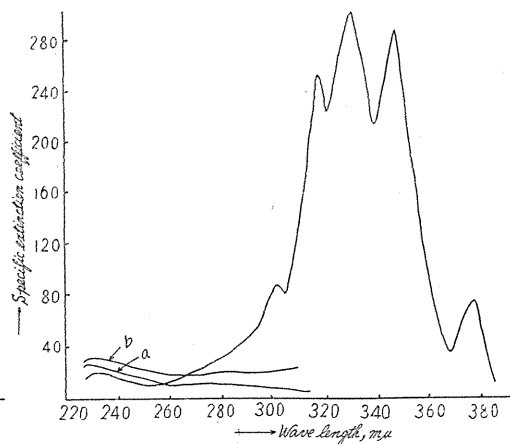


Fig. 2

In another experiment, the highly unsaturated acid mixture was isomerized under the same condition as described above. The isomerized product was dissolved in hexane, and the insoluble substance was removed at room temperature. The clear solution was then cooled to  $-10^{\circ}\text{C}$ , and there was obtained 0.5 g of crystalline solid which had N.V. 174.4 and melted substantially at  $110^{\circ}\text{--}120^{\circ}\text{C}$ . The specific extinction coefficients (Fig. 2) at 316, 330, 346 and 374  $m\mu$  were 222.2, 300.0, 274.7 and 60.7, respectively.

It is to be noted that highly conjugated solid acids obtained in these experiments are not perfectly odorless, but they are free from the characteristic odor of original highly unsaturated acids. When several color reactions were tested with a chloroform solution of highly conjugated solid acids, sulfuric acid developed a blue-violet to red-violet color. A saturated solution of antimony trichloride in chloroform developed a blue-violet color, while the Wijs solution was colored red without forming precipitations unlike the case with the original highly unsaturated acids.

### Summary

A highly unsaturated acid fraction of N.V. 176.6 and I.V. 390.0 was prepared from winterized sardine oil, and isomerized by heating for 15 minutes at  $180^{\circ}\text{C}$  in 21% KOH-glycol under the atmosphere of nitrogen. From the isomerized product, a highly conjugated solid fraction which consisted chiefly of  $\text{C}_{22}$ -acids and had a light yellow color was separated. This fraction was subjected to further fractional crystallizations, and ultraviolet absorption spectra of each fraction were measured. The fraction showing the most strong absorption in the region of conjugated pentaene melted substantially at  $110^{\circ}\text{--}120^{\circ}\text{C}$  and exhibited the specific extinction coef-

ficients of 300.0 at 330  $m\mu$  and 274.7 at 346  $m\mu$  with the specific extinction coefficients of 222.2 at 316  $m\mu$  and 60.7 at 374  $m\mu$ . Highly conjugated fractions obtained in these experiments are exceedingly susceptible to oxidation on standing in the air. The oxidation decreases the absorption in the region corresponding to highly conjugated polyene.

#### References

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