

THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL

XVIII. ULTRAVIOLET ABSORPTION MEASUREMENTS OF ALKALI-ISOMERIZED HIGHLY UNSATURATED ACIDS

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Recent development of ultraviolet spectrophotometric analysis of alkali-isomerized product of non-conjugated polyethylenic acids permits a fairly accurate determination of linoleic, linolenic, and arachidonic acids.¹⁾ Concerning the spectrophotometric analysis of alkali-isomerized product of more highly unsaturated acids than linolenic acid, arachidonic acid has hitherto been studied by several authors including Beadle and Kraybill,²⁾ Holman and Burr,³⁾ Potter and Kummerow,⁴⁾ and Herb, Riemenschneider and Donaldson.⁵⁾ Further, Herb and others⁶⁾⁷⁾ studied ultraviolet spectral characteristics of alkali-isomerized eicosapentaenoic and docosapentaenoic acids. According to these authors, spectral characteristics of the alkali-isomerized product of arachidonic and other highly unsaturated acids are much affected by conditions of isomerization. When highly unsaturated acids are isomerized by 1.3 N KOH-glycol (6.5–6.6% KOH-glycol) which is generally used for isomerization of less unsaturated acids such as linoleic acid, the isomerized product show relatively small absorptions in the regions of conjugated tetraene and pentaene, and increasing concentration of the alkali yields a product of larger absorption in these regions, the largest absorption being obtained by an isomerization for 15 minutes at 180° C in 21% KOH-glycol. However, the highly unsaturated acids studied by these authors were prepared from beef suprarenal lipids; the highly unsaturated acids in marine animal oils have not yet been studied in regard to spectral characteristics of their alkali-isomerized product.

The present paper deals with the ultraviolet absorption spectra of the alkali-isomerized product of hiragonic, moroctic, eicosatetraenoic, eicosapentaenoic and clupanodonic acids separated from sardine oil. The data obtained in these experiments are compared with those reported for the highly unsaturated acids from beef suprarenal lipids.

1. Preparation of Highly Unsaturated Acids

Hiragonic acid. The lowest fraction, B.P. –190° C/15 mm, described in Table 4 in the 15th report of this series⁸⁾ was subjected to a further fractionation, and after removing a little forerun and higher fraction, a fraction of S.V. 212.0 and I.V. 283.6 was separated. Saponification of this fraction gave hiragonic acid of d_4^{20} 0.9331, n_D^{20} 1.4795, Mol. Refr. 76.15 (Calc. for $C_{16}H_{26}O_2F_3$, 76.22), N.V. 223.6 (Calc. 224.1) and I.V. 299.6 (Calc. 304.2). Hydrogenation of hiragonic acid yielded palmitic acid.

Moroctic acid. This acid was separated from the fatty acids of Fraction B in the 15th report of this series. It had d_4^{20} 0.9270, n_D^{20} 1.4913, Mol. Refr. 86.40 (Calc. for $C_{18}H_{28}O_2F_4$, 84.99), N.V. 202.6 (Calc. 203.0) and I.V. 366.2 (Calc. 367.3).

Eicosatetraenoic acid. The acid described in the 17th report of this series⁹ was used in these experiments. It had d_4^{20} 0.9266, n_D^{20} 1.4916, Mol. Refr. 95.26 (Calc. for $C_{20}H_{32}O_2F_4$, 94.23), N.V. 183.9 (Calc. 184.3) and I.V. 334.1 (Calc. 333.5).

Eicosapentaenoic acid. While eicosatetraenoic acid was separated from the highly unsaturated methyl ester fraction of B.P. 200°-205° C/1 mm, adjacent fractions (70 g in total, S.V. 175.9, I.V. 363.1) were collected in order to separate eicosapentaenoic acid. The fatty acids from these fractions were fractionally precipitated as sodium salts from acetone, portions of lower iodine value were removed, and portions of higher iodine value were converted into the methyl esters. Fractionation of the methyl esters gave a fraction (21.9 g) of S.V. 177.4 and I.V. 388.0. The fatty acids from this fraction were subjected to further fractional precipitation of sodium salts from acetone, yielding eventually 8.8 g of eicosapentaenoic acid from the final filtrate. It had d_4^{20} 0.9367, n_D^{20} 1.5012, Mol. Refr. 94.96 (Calc. for $C_{20}H_{30}O_2F_5$, 93.88), N.V. 185.7 (Calc. 185.5) and I.V. 412.3 (Calc. 419.7). Hydrogenation of this acid yielded arachidic acid of M.P. 75°-76° C and N.V. 177.6 (Calc. 179.4).

Clupanodonic acid. The higher methyl ester fractions, which were obtained in the fractionations carried out for preparation of eicosatetraenoic and eicosapentaenoic acids, were collected, and subjected to a further fractionation, by which a fraction (6.5 g) of B.P. 215°-218° C/ca 3 mm, S.V. 164.3 and I.V. 370.3 was separated. The fatty acids of this fraction were fractionally precipitated as sodium salts from acetone, and 2.1 g of clupanodonic acid of the following characteristics was obtained: d_4^{20} 0.9360, n_D^{20} 1.5028, Mol. Refr. 104.2 (Calc. for $C_{22}H_{34}O_2F_5$, 103.1), N.V. 170.0 (Calc. 169.8), I.V. 381.5 (Calc. 384.0). Hydrogenation of this acid gave behenic acid of M.P. 79°-80° C and N.V. 166.5 (Calc. 164.7).

Of these five members of highly unsaturated acids, hiragonic and moroctic acids might have undergone geometrical isomerization to some extents since these two acids were subjected twice to bromination-debromination in the course of their preparation. Eicosatetraenoic, eicosapentaenoic and clupanodonic acids were prepared without bromination-debromination procedure, so that these three acids are considered to be free from suffering geometrical isomerization due to bromination-debromination.

2. Alkali-Isomerization

The alkali reagent (KOH-glycol or KOH-glycerol) used in these experiments was prepared following essentially the method prescribed by the American Oil Chemists' Society,¹⁰ and 5.5 g of it was put in a 3 cm × 20 cm test tube fitted with a cork stopper through which a glass tube was inserted. Dry nitrogen was introduced through this glass tube into the test tube in order to avoid the contact of air with the alkali reagent. The test tube was placed in an oil bath maintained at 180° C. After 10 minutes, the test tube was removed from the oil bath, the cork stopper was removed, and 0.0500 g of the sample was added to the reagent. The test tube was again fitted with the cork stopper, and the content of the test

tube was swirled for a half to one minute in order to obtain a clear homogeneous solution. It was then returned to the oil bath. Blank test was made simultaneously by treating a test tube containing the alkali reagent alone without sample. After the desired length of time, the test tube was removed from the oil bath, and cooled rapidly to the room temperature. The content of the test tube was diluted to 500 cc with ethanol. Five cc of this diluted solution was pipetted, and diluted to 50 cc with ethanol. With this final solution, ultraviolet absorption was measured using a Model DU Beckman Spectrophotometer. Experiments with the alkali reagent of 11% KOH-glycerol were carried out without passing nitrogen into the test tube. In the case of alkali-isomerization of moroctic and eicosatetraenoic acids with 11% KOH-glycerol, 0.1000 g of the sample was added to 11 g of the alkali reagent. After isomerization procedure the solution was diluted with water, acidified with hydrochloric acid and extracted with petroleum ether. The free fatty acids obtained from the petroleum ether solution was then dissolved in ethanol and subjected to the ultraviolet absorption measurements.

3. Results and Discussion

Absorption spectra of the original samples and their alkali-isomerized products are shown in Figs. 1-6. Table 1 records specific extinction coefficients of the original samples at wave lengths characteristic to the respective conjugated polyenes. Conjugated components in the original samples were calculated following the method of the American Oil Chemists' Society with the exception that specific extinction coefficients at 310, 316 and 322 $m\mu$ instead of 308, 315 and 322 $m\mu$ were taken for the calculation of conjugated tetraene. Although it is quite doubtful that this method of calculation is strictly applicable for the samples in these experiments, the data in Table 1 indicate the presence of a few percentages of pre-conjugated components in each original sample.

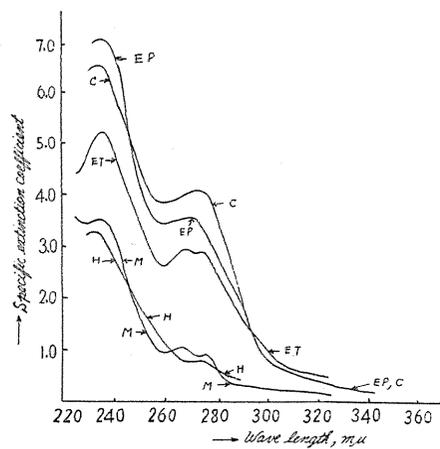


FIG. 1. Original samples.

H: Hiragonic acid; *M*: Moroctic acid; *ET*: Eicosatetraenoic acid; *EP*: Eicosapentaenoic acid; *C*: Clupanodonic acid.

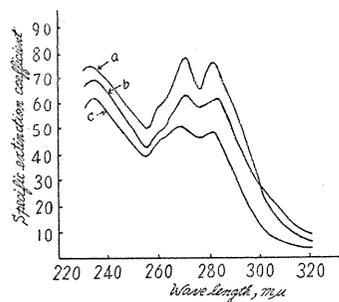


FIG. 2. Hiragonic acid.

a: 21% KOH-glycol, 180° C, 15 minutes; *b*: 11% KOH-glycol, 180° C, 30 minutes; *c*: 6.5% KOH-glycol, 180° C, 45 minutes.

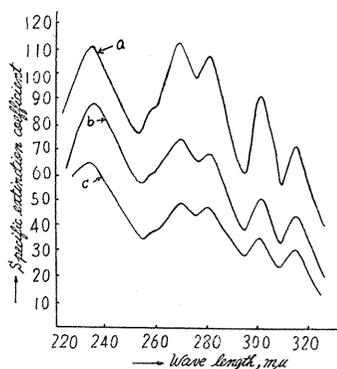


FIG. 3. Moroctic acid.

a: 9.5% KOH-glycol, 180° C, 45 minutes; *b*: 11% KOH-glycerol, 180° C, 45 minutes; *c*: 4.5% KOH-glycol, 180° C, 45 minutes.

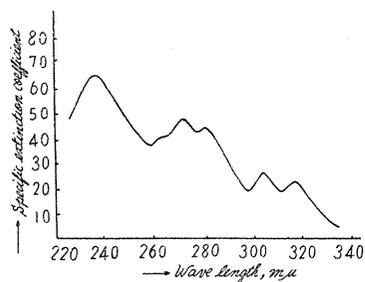


FIG. 4. Eicosatetraenoic acid.

11% KOH-glycerol, 180° C, 45 minutes.

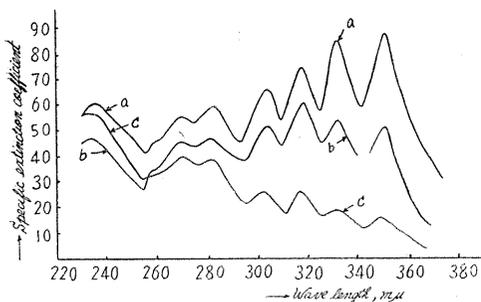


FIG. 5. Eicosapentaenoic acid.

a: 21% KOH-glycol, 180° C, 15 minutes; *b*: 11% KOH-glycol, 180° C, 30 minutes; *c*: 6.5% KOH-glycol, 180° C, 45 minutes.

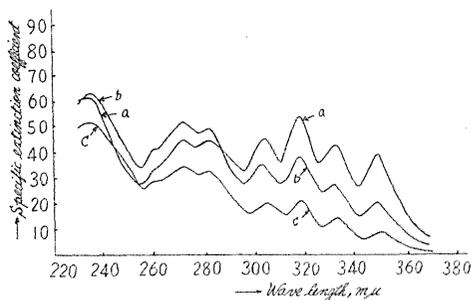


FIG. 6. Clupanodonic acid.

a: 21% KOH-glycol, 180° C, 15 minutes; *b*: 11% KOH-glycol, 180° C, 30 minutes; *c*: 6.5% KOH-glycol, 180° C, 45 minutes.

TABLE 1

Original sample	Wave length	Specific extinction coefficient	Conjugated component (%)
Hiragonic acid	233	3.32	Diene 2.76
	268	0.90	Triene 0.01
Moroctic acid	233	3.55	Diene 2.96
	268	1.01	Triene 0.14
	316	0.16	Tetraene 0.04
Eicosatetraenoic acid	233	5.28	Diene 4.41
	268	2.95	Triene 0.26
	316	0.53	Tetraene 0.01
Eicosapentaenoic acid	233	7.08	Diene 5.92
	268	3.53	Triene 0.13
	316	0.48	Tetraene 0.04
	346	0.15	Pentaene —
Clupanodonic acid	233	6.53	Diene 5.46
	268	4.07	Triene 0.17
	316	0.48	Tetraene 0.06
	346	0.10	Pentaene —

As shown in Fig. 2, the conjugation of hiragonic acid, particularly the formation of conjugated triene proceeds more markedly with increasing concentration of the alkali reagent. Similarly, the conjugation of moroctic acid (Fig. 3), eicosapentaenoic acid (Fig. 5) and clupanodonic acid (Fig. 6) becomes more remarkable with increasing concentration of the alkali reagent. Especially the formation of conjugated triene and tetraene from moroctic acid and the formation of conjugated tetraene and pentaene from eicosapentaenoic and clupanodonic acids increase notably by using an alkali reagent of higher concentration. Consistent with the results reported by Herb and others, the results of these experiments show that isomerization of the sample for 15 minutes at 180° C in 21% KOH-glycol yields a product having the largest absorption in the region of the most highly conjugated polyene corresponding to the number of ethylenic linkages of the original sample. Isomerization under the conditions of 11% KOH-glycerol, 180° C and 45 minutes was carried out with each of the five samples. While the absorption curves for moroctic and eicosatetraenoic acids after isomerization are shown in Figs. 3 and 4, those for hiragonic, eicosapentaenoic and clupanodonic acids after isomerization are not shown in Figs. 2, 5 and 6 since the respective curves lie closely with the corresponding curves for isomerized products obtained under the isomerization condition of 6.5% KOH-glycol, 180° C and 45 minutes. Results obtained in these experiments for the alkali-isomerized products of eicosatetraenoic, eicosapentaenoic and clupanodonic acids and those obtained by Herb and others are compared in Table 2.

TABLE 2

Isomerized sample	Wave length (m μ)		Specific extinction coefficient	
	The present authors	Herb and others	The present authors	Herb and others
Eicosatetraenoic acid (Arachidonic acid)	235	233	63.1	55.0
	270	268	46.0	46.8
	316	315	22.9	20.3
Eicosapentaenoic acid	235	233	59.0	39.4
	270	268	54.6	41.2
	316	315	75.0	82.4
	348	346	87.8	87.5
Clupanodonic acid (Docosapentaenoic acid)	235	233	60.1	43.5
	270	268	48.7	46.0
	316	315	53.2	56.9
	349	346	39.3	50.4

Notes: (1) Eicosatetraenoic acid was isomerized under the conditions of 11% KOH-glycerol, 180° C and 45 minutes with nitrogen (Herb and others) or without nitrogen (the present authors). Eicosapentaenoic and clupanodonic acids were isomerized under the conditions of 21% KOH-glycol, 180° C and 15 minutes with nitrogen. (2) The wave lengths corresponding to the maximum absorption of the respective conjugated polyenes slightly differ for the samples of Herb and others and the corresponding samples of the present authors.

The absorption characteristics for both corresponding samples differ somewhat from each other. In the case of eicosapentaenoic acid, the absorption in the region of conjugated diene and triene is larger for the sample of the present authors,

while the absorption in the region of conjugated tetraene is smaller for the sample of the present authors. The absorption in the region of conjugated pentaene is nearly the same for both samples. In the case of clupanodonic acid, the sample of the present authors shows a larger absorption in the region of conjugated diene and a smaller absorption in the region of conjugated pentaene. Although the cause of these discrepancies is not clear, they might be attributed to the dissimilarity of purity or structure (position of ethylenic linkage) of the original samples or to a slight dissimilarity of isomerization condition.

Comparing eicosapentaenoic and clupanodonic acids under the same isomerization condition (21% KOH-glycol), the isomerized product of eicosapentaenoic acid shows a larger absorption in the region of conjugated tetraene and pentaene. Also moroctic acid, though this acid was subjected to bromination-debromination in the course of its preparation, yields an isomerized product of higher degree of conjugation than does eicosatetraenoic acid under the same isomerization condition (11% KOH-glycerol). It appears from these results that among the polyethylenic acids having the same number of ethylenic linkages those of lesser carbon atoms undergo a greater degree of conjugation than those of larger carbon atoms. But this relation does not necessarily hold for all cases, since hiragonic acid, as shown in Table 3, yields a smaller amount of conjugated triene than linolenic acid¹¹⁾ when isomerized by using 21% KOH-glycol, while the formation of conjugated diene under the same isomerization condition is larger for hiragonic acid. Thus the progress of conjugation depends more or less upon the relative position of each ethylenic linkage of the original samples.

TABLE 3

	Wave length (m μ)	Specific extinction coefficient	
		Hiragonic acid	Linolenic acid
11% KOH-glycerol	233	61.8	58.6
	268	51.3	48.6
21% KOH-glycol	233	75.6	47.5
	268	78.9	90.5

Notes: In the case of 11% KOH-glycerol, hiragonic acid was isomerized without using nitrogen.

Summary

Five members of highly unsaturated acids separated from sardine oil, hiragonic, moroctic, eicosatetraenoic, eicosapentaenoic and clupanodonic acids were subjected to alkali-isomerization, and the ultraviolet absorption of isomerized acids was measured. Conjugation proceeded to a greater extent with increasing concentration of the alkali used; especially the formation of the most highly conjugated polyene corresponding to the number of ethylenic linkages in the original acids became more marked with increasing concentration of the alkali used. The condition for isomerization (21% KOH-glycol, 180° C, and 15 minutes) given by Herb and Riemenschneider¹²⁾ was found to be suitable also for the highly unsaturated acids from sardine oil. Among acids having the same number of ethylenic linkages, acids with lesser carbon atoms appeared, in most cases, to undergo isomerization

to a greater extent. But this relation did not necessarily hold for all cases, since the tendency to conjugation appeared to depend upon the relative position of each ethylenic linkage in the original acids.

References

- 1) The American Oil Chemists' Society, "Official and Tentative Methods," Cd 7-48.
- 2) B. W. Beadle and H. R. Kraybill: *J. Amer. Chem. Soc.* **66**, 1232 (1944).
- 3) R. T. Holman and G. O. Burr: *Arch. Biochem.* **19**, 474 (1948).
- 4) G. C. Potter and F. A. Kummerow: *J. Amer. Oil Chem. Soc.* **27**, 190 (1950).
- 5) S. F. Herb, R. W. Riemenschneider and J. Donaldson: *J. Amer. Oil Chem. Soc.* **28**, 55 (1951).
- 6) S. F. Herb, L. P. Witnauer and R. W. Riemenschneider: *J. Amer. Oil Chem. Soc.* **28**, 505 (1951).
- 7) S. F. Herb and R. W. Riemenschneider: *J. Amer. Oil Chem. Soc.* **29**, 456 (1952).
- 8) Y. Toyama and T. Yamamoto: *Memoirs Faculty of Engineering, Nagoya Univ.* **5**, 114 (1953).
- 9) Y. Toyama and T. Yamamoto: *Memoirs Faculty of Engineering, Nagoya Univ.* **5**, 319 (1953).
- 10) *l.c.*, 1).
- 11) *l.c.*, 7).
- 12) *l.c.*, 7).