

THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL

XV. THE C₁₆ AND C₁₈ ACIDS IN ETHER INSOLUBLE BROMIDE AND PETROLEUM ETHER INSOLUBLE BROMIDE OBTAINED FROM THE LOWER FRACTION OF METHYL ESTER OF HIGHLY UNSATURATED ACID CONCENTRATE

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In previous studies on the highly unsaturated acids in sardine oil by one of the authors, Toyama, and Tsuchiya, hiragonic acid (6, 10, 14-hexadecatrienoic acid)¹⁾ and moroctic acid (4, 8, 12, 15-octadecatetraenoic acid)²⁾ were separated as C₁₆ and C₁₈ components, respectively. Also the presence of octadecatrienoic acid³⁾ was indicated. Later, Tsuchiya⁴⁾ separated hexadecatetraenoic and octadecatrienoic acids, and studied the structures of these two acids. The present authors have also undertaken a further study on the highly unsaturated C₁₆ and C₁₈ acids, the results of which are recorded in this paper.

A highly unsaturated acid concentrate was prepared from sardine oil by the sodium salt acetone method, and its methyl ester was fractionated. The lower fraction was brominated, the ether insoluble bromide and the petroleum ether insoluble bromide were separated, and the highly unsaturated acids from both bromides were subjected to a further fractionation by means of several methods. From the highly unsaturated acids from ether insoluble bromide, hiragonic and hexadecatetraenoic acids, as C₁₆ acids, and octadecatrienoic and moroctic acids, as C₁₈ acids, were separated. Hiragonic and octadecatrienoic acids were also found in a relatively high proportion in the highly unsaturated acids from petroleum ether insoluble bromide. This is attributed to the fact that these two acids are contained in a relatively small proportion in the lower methyl ester fraction of highly unsaturated acid concentrate, and the solid bromide formed from these two acids by bromination in ether is not completely precipitated. Thus, a considerable portion of solid bromide as well as liquid bromide from these two acids remains in the ether solution, and when the bromide recovered from the ether solution is treated with petroleum ether, the bromide from these two acids enters largely into the petroleum ether insoluble portion. It should, however, be noted that hiragonic and octadecatrienoic acids obtained from the ether insoluble bromide have not necessarily the same spatial structures as those obtained from the petroleum ether insoluble bromide. Although the highly unsaturated acids of sardine oil, as a whole, seem to undergo more or less geometrical isomerization in the course of bromination-debromination, it is not ascertained in these experiments that to what extent, if at all, such isomerization takes place in the case of hiragonic and octadecatrienoic acids.

Experimental

I. Preparation of Highly Unsaturated Acid Concentrate from Winterized Sardine Oil and Fractionation of its Methyl Ester

Winterized sardine oil obtained by the courtesy of Tohoku Yushi Co. in 1942 was used for these experiments. It had A.V. 1.42, S.V. 189.2, and I.V. (Wijs method) 193.2. On subjecting this oil to the sodium salt acetone method, a highly unsaturated acid concentrate with I.V. 335.0 was obtained from the acetone soluble sodium salts in a yield of 34%. This was converted into methyl ester, and the latter (6.7 kg) was fractionally distilled with the results given in Table 1.

TABLE 1

Fraction	1	2	3	Higher fractions	Residue
Boiling range (°C/below 0.1 mm) } Yield (g)	-170 1,258	170-180 1,235	180-190 1,229	190- 2,556	422
n_D^{20}	1.4718	1.4760	1.4826	1.4874-1.4910	
S.V.	200.1	193.7	180.8	177.8-172.5	
I.V.	251.2	287.7	327.3	365.1-377.3	

Notes: In total, 50 kg of winterized sardine oil was treated by the sodium salt acetone method, giving 17 kg of highly unsaturated acid concentrate. For the present experiments, only a part of this quantity, 6.7 kg, was used. The experiments up to the fractional distillation recorded in Table 1 were completed by the spring of 1943. Further experiments described below were carried out since April, 1951. Till then, Fraction 1 of Table 1 was stored keeping off air in the dark.

II. Bromination of Fraction 1 and Fractionation of Bromide

Fraction 1 of Table 1, as seen by its iodine value, contains a considerable amount of less unsaturated methyl esters besides highly unsaturated methyl esters. In order to separate highly unsaturated methyl esters, Fraction 1 was fractionated by way of bromide in the following manner: 100 g of Fraction 1 was dissolved in 1 litre of ether, cooled to -5°C , and brominated. The insoluble bromide formed by bromination was filtered. The filtrate was washed with a solution of sodium bisulfite for the removal of excess bromine. It was then washed with water, and after dehydration with anhydrous sodium sulfate and distillation of ether, the ether soluble bromide was obtained. The ether insoluble bromide was then treated with ethanol. Five hundred cc of ethanol was added for every 100 g of ether insoluble bromide, and the mixture was refluxed. After cooling to room temperature, the insoluble portion was filtered, and again treated with ethanol as before. The ether soluble bromide was treated with petroleum ether. One litre of petroleum ether was added to every 100 g of ether soluble bromide, the mixture was refluxed, and after standing over a night at room temperature, the supernatant solution was separated from the insoluble bromide. By these treatments, 1,200 g of Fraction 1 in total gave 950 g of ether insoluble (ethanol insoluble) bromide and 427 g of petroleum ether insoluble bromide.

III. Debrominated Product from Ether Insoluble Bromide

Ether insoluble (ethanol insoluble) bromide described above was debrominated

with zinc, 5 N-sulfuric acid-ethanol, and ethanol. Since the debrominated product was found to be contaminated with some unsaponifiable substances, it was saponified and the unsaponifiable substances were extracted with ether in the usual way. Fatty acids freed from unsaponifiable substances were converted into methyl esters. Of the ether insoluble bromide (950 g) described above, 100 g was lost by a failure in debromination experiments, and 850 g of the bromide yielded 230 g of methyl esters having S.V. 199.8 and I.V. 338.3. The methyl esters thus obtained were fractionally distilled, and after removing higher fractions, the following two fractions were collected:

Fraction A. 100 g, $-190^{\circ}\text{C}/5$ mm, S.V. 216.7-209.6, I.V. 293.0-326.7.

Fraction B. 89 g, $190-208^{\circ}\text{C}/5$ mm, S.V. 201.2-185.4, I.V. 332.2-375.6.

1. Examination of Fraction A

Ninety-six g of this fraction was again brominated in ether, and insoluble bromide (135 g) formed was filtered. The insoluble bromide was refluxed with 1 litre of benzene for a short time, and filtered while hot. The insoluble portion was treated further twice with benzene as before. The combined benzene solution was cooled to room temperature, and the insoluble bromide formed was filtered. By these treatments, (a) 33 g of bromide insoluble in hot benzene, (b) 52 g of bromide insoluble in cold benzene, and (c) 50 g of bromide soluble in cold benzene were separated.

(a) *Bromide insoluble in hot benzene.* This bromide was debrominated, and the debrominated product was converted into methyl ester (S.V. 201.9 and I.V. 368.5), 7 g of which was fractionated as shown in Table 2.

TABLE 2

Fraction	Boiling range ($^{\circ}\text{C}/5$ mm)	Yield (g)	S.V.	I.V.
1	-185	2.1	210.0	357.1
2	185-190	2.8	205.2	360.5
3	190-200	1.2	190.8	380.0
Residue		0.9	185.2	370.2

The fatty acids (N.V. 222.1 and I.V. 377.2) from Fraction 1 in Table 2 were fractionally precipitated as sodium salts from acetone. Thus, 1.8 g of fatty acids were neutralized with an ethanol solution of sodium hydroxide, and after distilling the bulk of ethanol, 50 cc of acetone was added. The mixture was refluxed, and water was added dropwise until all the sodium salts were dissolved. The solution was cooled with ice, and the precipitate of sodium salts was filtered. The filtrate was added with further 50 cc of acetone, and the second precipitate of sodium salts was filtered. The fatty acid fraction contained in the acetone filtrate was once recovered, and subjected again to a further fractionation by way of sodium salt in acetone. Results recorded in Table 3 were finally obtained.

The fatty acid from the final filtrate in Table 3 had d_4^{20} 0.9364, n_D^{20} 1.4911, and Mol. Refr. 76.81. Calculated for $\text{C}_{16}\text{H}_{24}\text{O}_2\text{F}_4$: N.V. 225.9, I.V. 408.8, and Mol. Refr. 75.76. The ether insoluble bromide obtained from this acid was a white solid with Br content 71.92% (calculated for $\text{C}_{16}\text{H}_{24}\text{O}_2\text{Br}_8$, 72.02%), which melted at about 210°C with darkening. The hydrogenated product of this acid had M.P. 62°C and

TABLE 3

Fatty acid from	Yield (g)	N.V.	I.V.
1st precipitate	0.4	217.1	349.1
2nd precipitate	0.3	219.6	365.3
Precipitate from the filtrate	0.3	220.5	372.4
Final filtrate	0.6	225.9	400.9

N.V. 218.5 after recrystallization from ethanol, and was identified with palmitic acid (M.P. 62°C and N.V. calculated, 218.8).

The fatty acids from Fraction 3 in Table 2 had N.V. 200.3 and I.V. 393.2. The neutralization value is a little lower and the iodine value is considerably higher than the corresponding values for moroctic acid (N.V. 203.0 and I.V. 367.3). They were fractionated by way of sodium salts in acetone, yielding three fractions: (1) with N.V. 202.5 and I.V. 372.3, (2) with N.V. 200.8 and I.V. 398.7, and (3) with N.V. 198.2 and I.V. 409.7. The fraction (1) seems to consist chiefly of moroctic acid. The iodine value of the fraction (3) is very high as compared with that of moroctic acid, but its neutralization value is a little lower than that of moroctic acid. An examination of the hydrogenated product of the fraction (3) revealed that it contained more or less C₂₀ acid besides C₁₅ acid, and hence the high iodine value of the fraction (3) can not be taken as a decisive indication of the presence of octadecapentaenoic acid, although the presence of this acid can not be utterly denied.

(b) *Bromide insoluble in cold benzene.* This bromide was refluxed with 500 cc of benzene-ether (4 : 1), and after cooling the mixture to room temperature, the insoluble bromide was filtered.

The bromide insoluble in benzene-ether (11 g) was debrominated, and the methyl esters (3.0 g, S.V. 201.2, and I.V. 358.3) from the debrominated product were fractionally distilled, by which these were found to consist chiefly of methyl esters of hexadecatetraenoic and moroctic acids. In order to separate hexadecatetraenoic acid, the fatty acids obtained from the fractions excepting a small amount of higher fraction were subjected to the fractional precipitation of sodium salt from acetone, yielding the fatty acid (0.6 g) from the final filtrate having d_4^{20} 0.9360, n_D^{20} 1.4904, N.V. 224.5, and I.V. 400.8. The latter consisted chiefly of hexadecatetraenoic acid. It gave an ether insoluble bromide with Br content 71.83%. Its hydrogenated product yielded palmitic acid of M.P. 61-62°C and N.V. 218.3 after recrystallization from ethanol.

The bromide soluble in benzene-ether (40 g) was debrominated, and the methyl esters (10 g, S.V. 198.0, and I.V. 323.7) were fractionally distilled. After removal of the lower fraction, 4.3 g of a fraction boiling at 180-190°C/5 mm was obtained. The fatty acids from this fraction were fractionated by the fractional precipitation of sodium salt from acetone, and moroctic acid (1.5 g) with N.V. 204.2 and I.V. 370.0 was obtained from the filtrate.

As the results described above indicate that hexadecatetraenoic and moroctic acids are contained in the fatty acids of the bromide soluble in hot benzene (insoluble in cold benzene) as well as the bromide insoluble in hot benzene, the ether insoluble bromides from these two acids are found to be soluble in hot benzene to some extent, though these bromides are octabromides.

(c) *Bromide soluble in cold benzene.* The methyl esters (12 g, S.V. 210.4, and I.V. 278.6) from the debrominated product of this bromide were fractionated as shown in Table 4. Each fraction consists mainly of methyl hiragonate (calculated, S.V. 212.2 and I.V. 288.0).

TABLE 4

Fraction	Boiling range (°C/15 mm)	Yield (g)	S.V.	I.V.
1	-190	3.0	212.7	268.3
2	190-195	5.3	212.5	278.7
3	195-200	2.2	209.2	284.5
Residue		1.5	208.4	279.3

The fatty acid (3.5 g) from Fraction 2 in Table 4 was dissolved in 1 litre of water to which was added a slight excess of potassium hydroxide necessary to neutralize the fatty acid. The solution was cooled with ice, and 1 litre of a 1% solution of potassium permanganate was added in the course of 50 minutes while stirring. The stirring was continued for further 10 minutes, after which a current of sulfur dioxide was passed into the solution until it became discolored. The white precipitate was then filtered, dried, and washed with ether. The product (1.2 g) thus obtained was recrystallized from ethanol-petroleum ether, yielding hexahydroxy palmitic acid with M.P. 121-122°C and N.V. 161.3 (calculated for $C_{16}H_{32}O_8$, N.V. 159.2).

Anal. Found: C 54.21%, H 9.07%.

Calculated for $C_{16}H_{32}O_8$: C 54.53%, H 9.15%.

2. Examination of Fraction B

The fatty acids of this fraction had N.V. 196.2 and I.V. 368.7. Eighty-three g of the fatty acids were fractionally precipitated as sodium salts from acetone by stepwise neutralization with an aqueous solution of sodium hydroxide. The fatty acids from the 1st to 4th precipitates with N.V. 198.7-197.5 and I.V. 309.5-343.0 were combined, and the resulting fatty acid mixture (17 g) with N.V. 197.8 and I.V. 326.0 were subjected to a further fractional precipitation of sodium salt from acetone, as shown in Table 5.

TABLE 5
Fatty acid mixture, 17 g

↓	↓	↓
Fatty acid from 1st ppt. 2.3 g N.V. 199.3 I.V. 301.7	Fatty acid from 2nd ppt. 2.5 g N.V. 198.1 I.V. 308.5	Fatty acid from filtrate 12.0 g N.V. 196.9 I.V. 333.0
↓	↓	
Fatty acid from ppt. 1.5 g N.V. 201.0 I.V. 283.3	Fatty acid from filtrate 2.6 g N.V. 197.2 I.V. 318.7	

The fatty acid from the precipitate of the final fractionation in Table 5 had d_4^{20} 0.9220 and n_D^{20} 1.4794. The neutralization and iodine values coincide with the corresponding values for octadecatrienoic acid (calculated, N.V. 201.5 and I.V. 273.5). The ether insoluble bromide from this acid had Br content 63.60% (calculated for $C_{18}H_{30}O_2Br_6$ 63.27%) and melted at about 215°C with darkening. The hydrogenated product yielded stearic acid of M.P. 67-67.5°C and N.V. 196.5 (calculated, 197.4) after recrystallization from ethanol.

After the separation of octadecatrienoic acid described above, the remainder of the fatty acids of Fraction B was recovered and combined. About 70 g of the resulting mixture was brominated in ether. The ether insoluble bromide, after removing the portion soluble in cold benzene, was debrominated, the methyl ester from the debrominated product was fractionally distilled, and 3.9 g of a fraction boiling at 190-200°C/5 mm was separated as methyl moroctate. The fatty acid from this fraction showed the following constants:

d_4^{20} 0.9270, n_D^{20} 1.4913, Mol. Refr. 86.40, N.V. 202.6, I.V. 366.2 (calculated for $C_{18}H_{28}O_2F_4$: Mol. Refr. 84.99, N.V. 203.0, I.V. 367.3).

Ether insoluble bromide from this acid had Br content 68.92% (calculated for $C_{18}H_{28}O_2Br_8$, 69.82%) and melted at 220°C with darkening. The hydrogenated product yielded stearic acid of M.P. 70°C and N.V. 197.0 after recrystallization from ethanol.

IV. Debrominated Product from Petroleum Ether Insoluble Bromide

Petroleum ether insoluble bromide (427 g) is a viscous liquid with solid deposits. This was brominated with zinc powder and 5 N-sulfuric acid-ethanol in an ethanol solution. The debrominated product was freed from unsaponifiable substances (5 g) and converted into methyl esters (S.V. 202.1 and I.V. 268.0). One hundred and sixty g of methyl esters were fractionally distilled as shown in Table 6.

TABLE 6

Fraction	Boiling range (°C/5-2 mm)	Yield (g)	S.V.	I.V.
1	-160	18.6	214.5	237.5
2	160-170	7.9	212.8	256.6
3	170-175	27.0	210.6	270.1
4	175-180	61.8	207.6	275.3
5	180-185	10.1	190.5	280.1
Residue		34.6	185.6	276.4

1. *Separation of hiragonic acid.* Fraction 4 in Table 6 was redistilled, and the lower fraction boiling below 175°C/5 mm was separated. This lower fraction was combined with Fractions 1, 2, and 3. The combined fraction (61 g, N.V. 212.7, and S.V. 262.5) was brominated, and the bromide insoluble in ether and soluble in cold benzene was separated. This bromide was debrominated, the methyl ester from the debrominated product was fractionally distilled, and a fraction boiling at 185-190°C/15 mm was separated. The fatty acid from this fraction appears to consist chiefly of hiragonic acid from its N.V. 223.0 and I.V. 295.6. Four g of this fatty acid was further fractionated by the fractional precipitation of sodium salt from acetone, the fatty acid portion (N.V. 222.5 and I.V. 280.5) from the precipitate was removed, and the fatty acid portion (2.7 g) from the filtrate was obtained as hir-

agonic acid. It had d_4^{20} 0.9293, n_D^{20} 1.4848, Mol. Refr. 77.16, N.V. 223.9, and I.V. 302.3 (calculated for $C_{16}H_{26}O_2F_3$: Mol. Refr. 76.22, N.V. 224.1, and I.V. 304.2). On hydrogenation, it gave palmitic acid of M.P. 60.5-61.5°C and N.V. 217.9 (calculated, 218.8) after recrystallization from ethanol.

Notes: The refractive index of the hiragonic acid obtained above appears somewhat too high, and hence its purity is not considered so high, though its neutralization and iodine values agree well with the calculated values.

2. *Separation of octadecatrienoic acid.* Fractions 4 and 5 in Table 6 were repeatedly fractionated, and a fraction (8.5 g, S.V. 192.7, and I.V. 271.1) boiling at 185-195°C/5 mm was finally separated. The fatty acid (8 g) from this fraction was subjected to the fractional precipitation of sodium salt from acetone, with the results given in Table 7.

TABLE 7

Fatty acid from	Yield (g)	N.V.	I.V.
1st precipitate	1.0	197.9	263.1
2nd precipitate	2.1	202.3	279.5
3rd precipitate	2.5	202.6	282.3
Filtrate	2.3	205.7	295.6

The neutralization and iodine values of the fatty acids from the 2nd and 3rd precipitates agree with the calculated values for octadecatrienoic acid (calculated, N.V. 201.5 and I.V. 273.5). The fatty acid from the 2nd precipitate had d_4^{20} 0.9226, n_D^{20} 1.4796, and Mol. Refr. 85.73 (calculated for $C_{18}H_{30}O_2F_3$ 85.46). The ether insoluble bromide from this fatty acid had Br content 63.58% (calculated for $C_{18}H_{30}O_2Br_6$ 63.27%). On hydrogenation, it gave stearic acid of M.P. 68.5-69°C and N.V. 197.7 after recrystallization from ethanol.

The fatty acid (2.0 g) from the 3rd precipitate was added with 500 cc of water containing a slight excess of potassium hydroxide necessary to neutralize the fatty acid. A 1.5% solution of potassium permanganate was gradually added while stirring. After 200 cc of permanganate solution had been added, stirring was continued further for a while. A current of sulfur dioxide was passed into the solution in order to reduce the oxides of manganese and the excess of permanganate, and the solution was concentrated by evaporation. Acidification with sulfuric acid formed a white precipitate (0.6 g). This was filtered, washed with ether, and then recrystallized from ethanol-petroleum ether, yielding hexahydroxy stearic acid of M.P. 178-179°C and N.V. 146.3 (calculated, 147.5).

Summary

1. Winterized sardine oil was treated by the sodium salt acetone method, and a highly unsaturated acid concentrate was separated. The methyl ester prepared from the latter was fractionally distilled, and a lower fraction was separated. This fraction was brominated, and the ether insoluble bromide and the petroleum ether insoluble bromide were separated.

2. The highly unsaturated acid components of the ether insoluble bromide were separated by applying the fractional distillation of methyl ester, the solvent-frac-

tionation of bromide, and the fractional precipitation of sodium salt from acetone. Hiragonic acid and hexadecatetraenoic acid as C_{16} acids, and octadecatrienoic acid and moroctic acid as C_{18} acids were separated. The presence of octadecapentaenoic acid was not decisively indicated, but the presence of this acid could not be utterly denied. From the highly unsaturated acid components of the petroleum ether insoluble bromide, hiragonic acid and octadecatrienoic acid were separated.

3. Hydroxylation by Hazura's method yielded a hexahydroxy palmitic acid of M.P. 121-122°C from hiragonic acid and a hexahydroxy stearic acid of M.P. 178-179°C from octadecatrienoic acid.

4. Ether insoluble octabromides of hexadecatetraenoic and moroctic acids are soluble in hot benzene to some extent.

References

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