THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL. XII. A FURTHER EXPERIMENT ON THE SEPARATION OF OCTADECATRIENOIC ACID $C_{18}H_{30}O_2$.

By **Yoshiyuki TOYAMA** and **Tomotaro TSUCHIYA.**

Received August 20th, 1936. Published November 28th, 1936.

In the 1st report(1) of this series the highly unsaturated C_{16}- and C_{18}-acids of sardine oil were examined with the results that hiragonic acid $C_{16}H_{26}O_2$ and moroctic acid $C_{18}H_{28}O_2$ were isolated from C_{16}- and C_{18}-acids, respectively, and the presence of an octadecatrienoic acid $C_{18}H_{30}O_2$ was also indicated. The constitutions of hiragonic and moroctic acids were established in the 2nd and 3rd reports(2) of this series.

The presence of an octadecatrienoic acid in sardine oil was first pointed out by Fahrion.(3) He analysed a fraction of the bromides which was separated from the brominated products of the sardine oil fatty acids as the portion insoluble in petroleum ether, and he believed it to be a hexabromide derived from an octadecatrienoic acid which he named jecoric acid, but there is little doubt that the fraction of the bromides analysed by him is not really a single compound. Although the presence of an octadecatrienoic acid in various kinds of marine animal oils besides sardine oil has been also frequently reported, the evidence for the presence of this acid seems, in most cases, to rest on inadequate experimental results. However, Tsujimoto(4) brought out a strong

---

(1) This Bulletin, **4** (1929), 83.
(2) This Bulletin, **10** (1935), 192, 232.
(3) *Chem. Ztg.*, **17** (1893), 521.
(4) *Rept Tokyo Imp. Ind. Research Lab.*, **6** (1911), 17; **12** (1917), 169.

evidence for the presence of an octadecatrienoic acid in carp oil and snapping-turtle oil by the finding that the bromine content of ether-insoluble bromides obtained from the fatty acids of these oils lay close to the calculated value for $C_{18}H_{30}O_2Br_6$. In a recent investigation on the oil from *Chanos chanos*, Kafuku and Hata(5) reported the separation of bromides obtained from the liquid fatty acids and found that the fraction which was insoluble in ether but soluble in benzene consisted of a hexabromide which was identical with that derived from linolenic acid. These authors seem to be the first, so far as we know, who indicated the presence of linolenic acid in fish oils with certainty. In the following we describe the results of our further experiments on the separation of octadecatrienoic acid starting with 60 kg. of the ethyl esters of sardine oil fatty acids. In these experiments sufficient indications of the presence of octadecatrienoic acid were obtained, but the separation and the identification of octadecatrienoic acid were not attained. A fraction of octadecatrienoic acid was separated from the debrominated product obtained from the bromides which were insoluble in ether but soluble in benzene, but it could not be freed from the contamination of moroctic and hiragonic acids together with the products of intramolecular polymerisation of these acids. Judging from the results of these experiments, octadecatrienoic acid forms an extremely minor constituent of sardine oil fatty acids.

Experimental.

Sixty kg. of the mixed ethyl esters prepared by the ethanolysis of sardine oil were fractionated, and 28.5 kg. of a fraction boiling below 215°/10 mm. was collected. This fraction was saponified, the fatty acids liberated were dissolved in 85% methanol, and the solution was cooled. The crystalline deposit consisting chiefly of saturated acids was removed by filtration, and the filtrate was neutralised with aqueous solution of lithium hydroxide. The insoluble lithium soaps consisting mainly of the lithium soaps of saturated and mono-ethylenic acids were removed by filtration, and 7.5 kg. of fatty acids were recovered from the filtrate in the usual way. The fatty acids thus obtained were converted into the methyl esters, which were then fractionated yielding 5.9 kg. of a fraction boiling below 215°/15 mm. This fraction was brominated in ethereal solution, and the ether-insoluble bromides were separated and then treated with benzene yielding 1.5 kg. of the bromides soluble in benzene, from which 463 g. of the methyl esters were regenerated by debromination. The methyl esters were fractionated with the following results: (1) below 190°/15 mm. 98 g., (2) 190–205°/15 mm. 175 g., (3) 205–215°/15 mm. 120 g., (4) 215–221°/15 mm. 40 g., (5) residue 30 g. For the details of the above experiments the 2nd report(6) of this series should be consulted, in which the fraction (1) was subjected to a further fractionation for the separation of methyl hiragonate. In the present experi-

---

(5) *J. Soc. Chem. Ind., Japan,* **38** (1935), 1414.

(6) This Bulletin, **10** (1935), 192.

ment the fraction (3) was fractionated repeatedly, and the following two fractions were collected separately.

Fraction	B.p./15 mm.	Saponif. v.	Iodine v. (Wijs)	Yield (g.)
(a)	205–210°	190.1	291.0	23
(b)	210–215°	190.9	292.4	28

Since the characteristics of the fractions (a) and (b) show no appreciable difference, they were united and subjected to the fractional precipitation of sodium soaps in acetone in the following manner: 45 g. of the fatty acids obtained from the united fractions were dissolved in 360 c.c. of acetone, neutralised with an approximately 4 N alcoholic solution of sodium hydroxide, and alcohol was added until the total volume of the alcoholic solution of sodium hydroxide and the alcohol subsequently added measured 54 c.c. Then 36 c.c. of water and 450 c.c. of acetone were added to the solution, and after heating for a short time the insoluble sodium soaps were precipitated by cooling and filtered. The precipitate and the filtrate were worked up to separate the fatty acids in the usual way.

	Neutr. v.	Iodine v.	Yield (g.)
Fatty acids from the precipitate	201.7	293.8	30.5
Fatty acids from the filtrate	198.6	331.9	14.5

The fatty acids from the precipitate were again fractionated by the sodium-soap-acetone method in the following way: 30 g. of the fatty acids from the precipitate were dissolved in 240 c.c. of acetone, neutralised with an approximately 4 N alcoholic solution of sodium hydroxide, and alcohol was added until the total volume of the 4 N alcoholic sodium hydroxide solution required for neutralisation and the alcohol added measured 30 c.c. Then 30 c.c. of water and 300 c.c. of acetone were added, and after heating for a short time the solution was cooled down to separate the precipitate of insoluble sodium soaps which was filtered and decomposed with hydrochloric acid yielding 23 g. of fatty acids with neutr. value 202.2 and iodine value 284.2. These were fractionated as their methyl esters, (22 g.), and the following fractions were separated.

Fraction	B.p./15 mm.	d_4^{15}	n_D^{15}	Saponif. v.	Iodine v.	Yield (g.)
(1)	199–205°	—	—	—	—	0.4
(2)	205–208°	0.9143	1.4810	197.5	277.4	3.3
(3)	208–212°	0.9144	1.4815	195.4	274.7	4.5
(4)	212–215°	0.9159	1.4825	193.0	267.4	9.9
(5)	215–217°	—	—	—	—	0.6
Residue	—	—	—	—	—	3.3 (diff.)

While the specific gravity and the refractive index, as is seen from the above table, become higher as the boiling point becomes higher, the iodine value falls slightly as the boiling point becomes higher. This is possibly due to a contamination of polymerised methyl esters in the high boiling fractions. The fraction (4) has the characteristics which are close to the calculated values for methyl octadecatrienoate $C_{18}H_{29}O_2CH_3$ (saponif. value 192.0, iodine value 260.6), but it was found from the results of bromination to contain more highly unsaturated methyl esters together with some polymerised methyl esters besides methyl octadecatrienoate. On brominating 7.0 g. of the fraction (4), there were obtained 4.2 g. (60%) of ether-insoluble bromides. These were heated with 400 c.c. of benzene on the water bath under a reflux condenser and then cooled down to about 35°. The insoluble portion was filtered, and on distilling off benzene from the filtrate there remained benzene-soluble bromides with Br-content 65.28% which were higher in Br-content than $C_{18}H_{29}O_2CH_3Br_6$ (62.13% Br). The benzene-soluble bromides were again refluxed with 300 c.c. of benzene, and after cooling the solution to about 30°, a small amount of insoluble bromides was filtered. From the filtrate, 2.5 g. of benzene-soluble bromides were obtained, which had Br-content 64.78% and melted at 195° with slight darkening. The Br-content seems to indicate that the bromides thus obtained contain more highly unsaturated methyl esters besides $C_{18}H_{29}O_2CH_3Br_6$.

Summary.

After having found in a previous study that sardine oil contains an octadecatrienoic acid as a minor constituent of highly unsaturated C_{18}-acids, we have attempted in the present experiment to separate the octadecatrienoic acid starting with a large amount of sardine oil. The results of the present experiment seem to indicate that the octadecatrienoic acid giving an ether-insoluble bromide is contained only in far lesser proportion than presumed before.

Tokyo Imperial Industrial Research Laboratory,
Hatagaya, Shibuya-ku, Tokyo.