THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL X. THE SEPARATION OF HIGHLY UNSATURATED C_{24}-ACIDS.

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After having examined the highly unsaturated C_{16}-, C_{18}-, C_{20} and C_{22}- acids in sardine oil, we extended our investigations to the highly unsaturated C_{24}-acids. Concerning the highly unsaturated C_{24}-acids in sardine oil, nothing has hitherto been known. However, the highly unsaturated C_{24}-acids in marine animal oils other than sardine oil have been studied by a few authors. Bull[1] stated to have found an acid $C_{24}H_{40}O_2$ in herring oil. Ueno and Iwai[2] have recently shown the presence of a highly unsaturated acid $C_{24}H_{38}O_2$, which they named scoliodonic acid, in

(1) *Chem. Ztg.*, **23** (1899), 996.
(2) *J. Soc. Chem. Ind., Japan*, **37** (1934), 562.

the liver oil of *Scoliodon laticaudus*. We[3] separated a highly unsaturated acid $C_{24}H_{36}O_2$, named nisinic acid, from herring oil, and found the same acid also in cod-liver oil, pilot-whale oil and in the liver oil from *Squalus sucklii*.

For the separation of highly unsaturated C_{24}-acids in the present experiment, the residue from the distillation of the highly unsaturated methyl esters described in the 7th report[4] of this series was used as the starting material. The fatty acids liberated from it consisted mainly of highly unsaturated C_{24}-acids and some polymerised acids. These were separated into the fractions of different degrees of unsaturation by the fractional precipitation of sodium soaps in acetone, and there was obtained nisinic acid $C_{24}H_{36}O_2$[5] from the fraction of the highest degree of unsaturation. Examination of each fraction seemed to indicate the presence of some other highly unsaturated C_{24}-acids, such as scoliodonic acid $C_{24}H_{38}O_2$ and also an acid $C_{24}H_{40}O_2$, besides nisinic acid, but no individual acid other than nisinic acid was separated.

Experimental.

1. **Separation of Highly Unsaturated C_{24}-Acids.** In the previously reported experiment[6] of the separation of highly unsaturated C_{22}-acids, 10 kg. of sardine oil was treated by means of sodium-soap-acetone method, and the concentrated fraction of highly unsaturated acids was obtained from the sodium soaps soluble in acetone. This was converted into the methyl esters and the latter were subjected to the fractional distillation which was continued up to 214°/2 mm. For the present experiment the residue from the distillation was used as the starting material. It was saponified, the unsaponifiable matter was removed by extraction with ether, and the soap solution on acidification yielded free fatty acids. These were reconverted into the methyl esters and the latter were distilled up to 215°/2 mm., yielding about 69 g. of a residue which had saponif. value 150.5 and iodine value 308.3.[7] The residue was saponified, and the fatty acids obtained on acidification were treated with charcoal in petroleum ether. The fatty acids (62. g.) thus obtained showed neutr. value 156.8 and iodine value 324.5. The product of hydrogenation of these fatty acids showed neutr. value 151.5 (calc. for $C_{24}H_{48}O_2$: 152.3) and m.p. 81.5–82° after recrystallisation from alcohol. The melting point was not lowered when the substance was admixed in various proportions with a specimen of *n*-tetracosanic acid, m.p. 84–84.5°, prepared from behenic acid

(3) *J. Soc. Chem. Ind., Japan*, **37** (1934), 1176.

(4) This Bulletin, **10** (1935), 433.

(5) Strictly speaking, nisinic acid or an acid having the same composition as nisinic acid.

(6) This Bulletin, **10** (1935), 433.

(7) Unless otherwise stated, the iodine values recorded in this paper were determined by the Wijs method.

by malonic ester synthesis. It follows from these results that the fatty acids having m.p. 81.5–82° consist mainly of *n*-tetracosanic acid, and consequently the highly unsaturated acids prior to hydrogenation consist chiefly of C_{24}-acids.

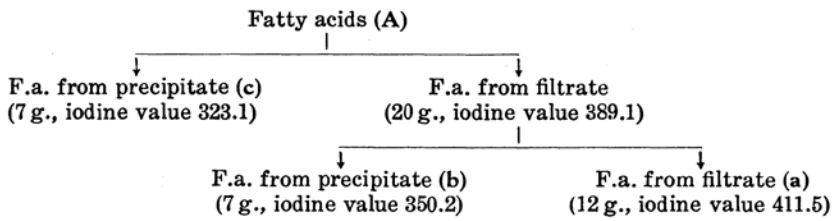
2. **Fractional Precipitation of Sodium Soaps in Acetone.** The highly unsaturated C_{24}-acids (neutr. value 156.8 and iodine value 324.5) obtained above were neutralised with a portion of sodium hydroxide solution prepared by dissolving 10 g. of sodium hydroxide in 20 c.c. of water and 40 c.c. of 95% alcohol, and then 60 c.c. of acetone was added by which a precipitate of insoluble sodium soaps was formed. The precipitate was filtered, and the fatty acids were separated from the precipitate and the filtrate, respectively, in the usual way. Fifty-nine grams of the highly unsaturated C_{24}-acids gave the following results (Table 1).

Table 1.

	Yield (g.)	Iodine value
Fatty acids from filtrate (A)	29	371.7
Fatty acids from precipitate (B)	29	277.6
Loss	1	—

Fatty acids from filtrate (A). These were dissolved in a little acetone, partially neutralised by using a portion of sodium hydroxide solution prepared by dissolving 10 g. of sodium hydroxide in 10 c.c. of water and 20 c.c. of 95% alcohol, and a sufficient quantity of acetone was added, by which a precipitate of insoluble sodium soaps was formed. The precipitate was once brought into solution by warming the solution on the water-bath, reprecipitated on cooling, and then filtered. The fatty acids were separated from the precipitate and the filtrate, respectively, in the usual way, and those obtained from the filtrate were again treated as before to effect a further separation. The results of these treatments are given below (Table 2).

Table 2.



For the sake of comparison, the neutralisation value and the iodine value for the highly unsaturated C_{24}-acids and also the Br-content of the corresponding bromo-derivatives are shown in Table 3.

The iodine value of the fatty acids (a) is close to the calculated value for $C_{24}H_{36}O_2$. The fatty acids (b) showed an iodine value which was close to the calculated value for $C_{24}H_{38}O_2$, but the ether insoluble bromide obtained from them showed

Table 3.

Acid	Neutr. v.	Iodine v.	Bromo-derivative	Br-content (%)
$C_{24}H_{36}O_2$	157.5	427.5	$C_{24}H_{36}O_2Br_{12}$	72.91
$C_{24}H_{38}O_2$	156.6	354.2	$C_{24}H_{38}O_2Br_{10}$	69.05
$C_{24}H_{40}O_2$	155.7	281.8	$C_{24}H_{40}O_2Br_8$	63.96
$C_{24}H_{42}O_2$	154.9	210.2	$C_{24}H_{42}O_2Br_6$	56.96

Br-content 70.84%, which lies between the calculated values for $C_{24}H_{36}O_2Br_{12}$ and $C_{24}H_{38}O_2Br_{10}$. Accordingly the fatty acids (**b**) are believed to be a mixture of acids of different degrees of unsaturation. The fatty acids (**c**) seem to contain some less unsaturated acids than $C_{24}H_{38}O_2$ according to their iodine value.

The fatty acids (**a**) were partially neutralised with sodium hydroxide solution in acetone as before, and the precipitate of insoluble sodium soaps was removed. The fatty acids obtained from the filtrate were again subjected to the same treatment, and these separative operations were repeated several times, until the fatty acids obtained from the precipitate and the filtrate showed iodine values which did not differ much from one another. The fatty acids from the final filtrate had the following constants and were considered to consist chiefly of nisinic acid $C_{24}H_{36}O_2$: d_4^{15} 0.9486, d_4^{20} 0.9451, n_D^{15} 1.5140, n_D^{20} 1.5120, mol. refraction 113.1 (calc. for $C_{24}H_{36}O_2$: 111.8), neutr. value 157.0, iodine values by the Wijs and the Rosenmund-Kuhnhenn methods 422.5 and 407.2 respectively. Bromination in ethereal solution yielded 138% of an ether insoluble bromide which showed Br-content 72.76% and turned black at about 240° without melting.

Fatty acids from precipitate (**B**). Twenty-eight grams of these fatty acids were neutralised by using a portion of sodium hydroxide solution prepared by dissolving 10 g. of sodium hydroxide in 30 c.c. of water and 60 c.c. of 95% alcohol, and then 300 c.c. of acetone was added by which a precipitate of insoluble sodium soaps was formed. The fatty acids (18.5 g.) liberated from the insoluble sodium soaps had iodine value 260.1 and n_D^{15} 1.5208. The iodine value lies between the calculated values for $C_{24}H_{40}O_2$ and $C_{24}H_{42}O_2$. Bromination in ethereal solution yielded, however, an ether insoluble bromide having Br-content 68.09% which was higher than the calculated value for $C_{24}H_{40}O_2Br_8$, indicating that the fatty acids from the insoluble sodium soaps contained some more highly unsaturated acids than $C_{24}H_{40}O_2$. After distilling off the solvent from the filtrate from ether insoluble bromide, the residue was treated with petroleum ether, and there was separated a petroleum ether insoluble bromide which melted at about 70° and had Br-content 57.55% which was close to the calculated value for $C_{24}H_{42}O_2Br_6$. It should, however, be noted that the fatty acids obtained from the insoluble sodium soaps showed an abnormally high refractive index in comparison with their iodine value, giving an indication of contamination of some polymerised fatty acids, and consequently it is not excluded that the petroleum ether insoluble bromide obtained above may have been derived from polymerised fatty acids, but not from $C_{24}H_{42}O_2$.

Summary.

A concentrated fraction of highly unsaturated acids was separated from sardine oil by means of sodium-soap-acetone method, and was distilled as its methyl esters up to 215°/2 mm. The residue from the distillation consisted mainly of the methyl esters of highly unsaturated C_{24}-acids and some polymerised products. The free fatty acids liberated from the residue were separated into the fractions of different degrees of unsaturation by means of fractional precipitation of sodium soaps in acetone. The fraction of the highest degree of unsaturation consisted of nisinic acid $C_{24}H_{36}O_2$. Examination of other fractions seemed to indicate the presence of some less unsaturated acids, such as $C_{24}H_{38}O_2$ and $C_{24}H_{40}O_2$, but no individual acids other than nisinic acid were separated.

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