

ON THE OCCURRENCE OF NEW HIGHLY UNSATURATED FATTY ACIDS, $C_{26}H_{40}O_2$ AND $C_{26}H_{42}O_2$, IN TUNNY OIL.

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Tunny oil is obtained from the tunny fish, *Thynnus thynnus*. This fish is known under the Japanese names "Maguro", "Shibi", and "Oyio". The fish is most likely indigene to Japan and considerable number of the fish are caught there. The taste of the fish is very favourable and the fish is used chiefly for edible purpose, the oil, therefore, is scarcely prepared in this country. Concerning the composition of tunny oil, excepting the report on the chemical and physical properties by M. Tsujimoto, it has not been investigated hitherto. Recently, S. Ueno and M. Iwai⁽¹⁾ showed the presence of a highly unsaturated acid $C_{24}H_{38}O_2$ named scoliodonic acid in the liver oil of *scoliodon laticandus*. A little later than this, also Y. Toyama and T. Tsuchiya⁽²⁾ found a highly unsaturated acid $C_{24}H_{36}O_2$ (nisinic acid) from herring oil, cod-liver oil, pilot whale oil, sardine oil, squalus-sucii-liver oil with scoliodonic acid.

After further investigation on the highly unsaturated acids, the present authors have separated a new highly unsaturated acid in a tunny oil having the formula $C_{26}H_{40}O_2$ (\bar{F}_6) and also detected the occurrence of a new acid $C_{26}H_{42}O_2$ (\bar{F}_5), besides scoliodonic acid, nisinic acid, and clupanodonic acid. It has been found that both of these two highly unsaturated fatty acids are new acids and the authors have suggested the names "thynnic" acid for $C_{26}H_{40}O_2$, and "shibic acid" for $C_{26}H_{42}O_2$.

Experimental.

I. **Properties of the Sample Oil.** The tunny oil used in this investigation had the following properties: specific gravity d_4^{20} 0.9264; refractive index n_D^{20} 1.4820; acid value 4.2; iodine value 190.1; saponification value 183.4.

II. **Separation of the Liquid Fatty Acids.** The mixed fatty acids prepared by the usual method were separated from the solid fatty acids by means of the cooling method with cold water and ice water. Yield of the liquid acids 22.7%; sample oil 4 kg; liquid fatty acids 910 g. The liquid fatty acids thus obtained had the following properties: refractive index n_D^{20} 1.4770; iodine value (Wijs) 225.9; neut. value 180.9.

(1) *J. Soc. Chem. Ind., Japan*, **37** (1934), 251 B.

(2) *J. Soc. Chem. Ind., Japan*, **37** (1934), 530 B.

III. Fractional Distillation of Methyl Esters of the Liquid Acids. The liquid fatty acids were esterified with methyl alcohol containing 2% conc. sulphuric acid and then fractionated under a diminished pressure of 4 mm. The results of the fractional distillation are given in Table 1.

Table 1.

Fraction	B.p. (°C./4 mm.)	Yield (g.)
1	-180	49.5
2	180-188	66.0
3	188-200	48.0
4	200-203	56.0
5	203-210	63.0
6	210-215	63.5
7	215-225	55.0
8	225-227	31.0
9	residue and loss	41.0

For convenience of the experimental course, these distillates were classified into two parts and each part was repeatedly fractionated under a diminished pressure of 4 mm. The results of the final distillation and characteristics of each fraction are given in Tables 2, 3, and 4.

Table 2. Redistillation of Fractions -200°C./4 mm. (Sample 184 g.)

Fraction	B.p. (°C./4 mm.)	Yield (g.)	n_D^{20}
1	130-170	4.5	1.4478
2	170-176	15.5	1.4470
3	176-182	27.0	1.4471
4	182-185	24.0	1.4505
5	185-190	25.2	1.4531
6	190-195	30.5	1.4569
7	195-200	20.0	1.4610
8	200-210	19.5	1.4691
9	210-215	10.0	1.4780

Table 3. Redistillation of Fractions 200–225°C./4 mm. (Sample 234 g.)

Fraction	B.p. (°C./4 mm.)	Yield (g.)	n_D^{20}
1	—200	24.5	—
2	200–210	26.5	1.4660
3	210–215	60.2	1.4761
4	210–215	42.5	1.4761
5	215–220	33.4	1.4828
6	220–225	28.5	1.4872
7	225–226	9.0	1.4898

Table 4.

Fraction	B.p. (°C./4mm.)	Yield (g.)	n_D^{20}	Iodine value (Wijs)	Sap. value	Colour
1	130–170	4.5	1.4478	75.1	—	pale yellow
2	170–176	15.5	1.4470	37.8	—	„
3	176–182	27.0	1.4471	84.0	—	„
4	182–185	24.0	1.4505	95.8	—	„
5	185–190	25.2	1.4531	109.3	—	„
6	190–195	30.5	1.4569	131.9	—	„
7	195–200	20.0	1.4610	150.0	—	„
8	200–210	46.0	1.4660	163.0	—	„
9	210–215	112.7	1.4761	259.2	176.5	„
10	215–220	33.4	1.4828	271.3	180.6	„
11	220–225	28.5	1.4871	331.6	169.9	yellow
12	225–227	9.0	1.4898	319.5	166.3	„
13	225–227	31.0				

IV. Separation of Highly Unsaturated Fatty Acids. (1) *Separation from Fractions 12 and 13.* The mixed acids (30 g.) obtained from methyl esters by the usual method, were freed from unsaponifiable matter and then treated by sodium-salt-acetone-method:

	Yield (g.)	Colour
Fatty acid from soluble part	15	orange-red
Fatty acid from insoluble part	6	yellow

The highly unsaturated fatty acid prepared from these fractions had the following properties: d_4^{20} 0.9443; n_D^{20} 1.5042, n_D^{15} 1.5062; iodine value (Wijs) 372.1; neut. value 148.1. Bromine content of the ether insoluble polybromide: 71.27, 71.22% (calculated for $C_{26}H_{40}O_2Br_{12}$: 71.41; $C_{26}H_{42}O_2Br_{10}$: 67.42%). The calculation gives the following numbers:

	Neut. value	Iodine value
$C_{24}H_{38}O_2$ (F_5)	156.7	354.2
$C_{24}H_{40}O_2$ (F_6)	157.8	427.5
$C_{26}H_{42}O_2$ (F_5)	145.4	328.8
$C_{26}H_{40}O_2$ (F_6)	146.2	396.7

The isolated highly unsaturated acid was exhaustively hydrogenated at room temperature in the presence of Adams platinum catalyst. The hydrogenation product, after being decolourised with active charcoal and recrystallised from ether, formed shining plates, melting point 76–77°C., which were identified as pure cerotic acid ($C_{26}H_{52}O_2$) (pure cerotic acid melts at 77.8°C.). (Found: C, 78.20; H, 13.40. Calculated for $C_{26}H_{52}O_2$: C, 78.8; H, 13.1%.)

As stated above, the neutralisation value of the highly unsaturated acid obtained from these fractions corresponds to that of the C_{26} -series, the iodine value lies between the calculated values for $C_{26}H_{40}O_2$ and $C_{26}H_{42}O_2$, and bromine percentage of the bromide closely agrees with the calculated value for $C_{26}H_{40}O_2Br_{12}$. Accordingly, it may be considered to consist chiefly of $C_{26}H_{40}O_2$ (F_6) and include a new acid $C_{26}H_{42}O_2$ (F_5).

As these acids are not found in the literatures, the present authors propose the names "thynnac acid" for the acid $C_{26}H_{40}O_2$ (F_6) and "shibic acid" for the acid $C_{26}H_{42}O_2$ (F_5), after the fish names already described.

(2) *Separation from Fraction 11.* The mixed acids (24 g.) obtained from methyl esters (26.5 g.) were separated into soluble and insoluble parts by sodium-salt-acetone-method:

	Yield (g.)	Colour
Fatty acids from soluble part	17	orange-red
Fatty acids from insoluble part	4	yellow

The characteristics of the fatty acids from the soluble part: n_D^{20} 1.5007; iodine value (Wijs) 367.0; neut. value 155.0. The liquid fatty acids prepared above were further treated by means of lithium-salt-acetone-method and the acids from the soluble part had the following properties: n_D^{15} 1.5046, n_D^{20} 1.5018; d_4^{20} 0.9419; iodine value (Wijs) 369.1 (calculated for $C_{24}H_{38}O_2$ (F_5) and $C_{24}H_{36}O_2$ (F_6): neut. value, 156.7 and 157.8; iodine value, 354.2 and 427.5, respectively). Bromine content of ether insoluble polybromide: 72.13% (calculated for $C_{24}H_{36}O_2Br_{12}$ and $C_{24}H_{38}O_2Br_{10}$: 72.91 and 69.05% respectively).

The experimental results described above show that the highly unsaturated acid obtained from this fraction corresponds to the C_{24} -series and it is a mixture of scoliodonic acid $C_{24}H_{38}O_2$ (F_5) and nisinic acid $C_{24}H_{36}O_2$ (F_6).

(3) *Separation from Fraction 10.* The mixed acids (28 g.) obtained from methyl esters (32 g.) were separated into soluble and insoluble parts by means of sodium salt-acetone-method.

	Yield (g.)	Colour
Fatty acids from soluble part	20	orange-red
Fatty acids from insoluble part	4	yellow

The properties of the liquid acids from soluble part: n_D^{20} 1.4958, n_D^{15} 1.4978; d_4^{20} 0.9365; iodine value (Wijs) 333.4; neut. value 166.3 (calculated for $C_{22}H_{36}O_2$ (\bar{F}_4) and $C_{22}H_{34}O_2$ (\bar{F}_5): neut. value, 168.9 and 170.0; iodine value 305.6 and 384.3, respectively). The clupanodonic acid of M. Tsujimoto had the following properties: d_4^{15} 0.9385; d_4^{20} 0.9356; n_D^{20} 1.5020; b.p. 236°C./5 mm.; methyl ester b.p. 222°C./5 mm. By the experimental results it was shown that the highly unsaturated fatty acids prepared from this fraction correspond to the C_{22} -series, consisting of clupanodonic acid $C_{22}H_{34}O_2$ and an acid $C_{22}H_{36}O_2$.

(4) *Separation from Fraction 9.* The mixed acids (86 g.) obtained from methyl esters by the usual method were separated into the acetone soluble and insoluble parts:

	Yield (g.)	Colour
Fatty acids from soluble part	62.0	orange-red
Fatty acids from insoluble part	11.0	pale-yellow

The liquid acids from soluble part had the following properties: n_D^{20} 1.4855; iodine value (Wijs) 278.5. The highly unsaturated acids which were further separated by means of lithium-salt-acetone-method from the liquid acids prepared above had the following properties: n_D^{15} 1.4952, n_D^{20} 1.4936; d_4^{20} 0.9363; neut. value 166.2; iodine value (Wijs) 332.9. Bromine content of ether insoluble polybromide: 70.01 (calculated for $C_{22}H_{34}O_2Br_{10}$ and $C_{22}H_{36}O_2Br_8$: 70.01 and 65.80%, respectively). The characteristics and the analytical results showed that the properties of the highly unsaturated acid from this fraction agreed with that of the liquid acids from fraction 10 and clupanodonic acid is the main constituent of the highly unsaturated acid obtained from fractions 10 and 9. Accordingly, it was confirmed that clupanodonic acid exists in a large amount in tunny oil as in the cases of other fish oils.

(5) *Separation from Fractions 7 and 8.* The mixed acids (45 g.) obtained from methyl esters (64 g.) were separated by means of sodium-salt-acetone-method:

	Yield (g.)	Colour
Fatty acids from soluble part	33	orange-red
Fatty acids from insoluble part	4	yellow

The liquid fatty acids prepared in the same way had the following properties: n_D^{20} 1.4789, n_D^{15} 1.4791; neut. value 170.6; iodine value (Wijs) 220.9. From the properties described above, it was assumed that the liquid acids correspond to the C_{20} -series consisting of the acids of the $C_nH_{2n-4}O_2$ and the $C_nH_{2n-6}O_2$ series.

(6) *Separation from Fraction 6.* The mixed acids (27 g.) obtained from methyl esters (28 g.) were separated:

	Yield (g.)	Colour
Fatty acids from soluble part	7	yellow
Fatty acids from insoluble part	12	white

The properties of the fatty acids from soluble part: n_D^{20} 1.4712; neut. value 194.9; iodine value (Wijs) 185.0. From the properties, the authors assumed that the liquid acids from this fraction correspond to the lower unsaturated acids of the C_{18} -series.

(7) *Separation from Fractions 1, 2, 3, 4, and 5.* The liquid part from these fractions has not yielded highly unsaturated fatty acids.

Summary.

The mixed liquid acids obtained from the sample oil by cooling were converted into methyl esters which were fractionally distilled under a diminished pressure of 4 mm. From the highest boiling fraction, the authors separated a new highly unsaturated acid $C_{26}H_{40}O_2$ (I_6) and also detected the occurrence of another new acid $C_{26}H_{42}O_2$ (I_5) besides scoliodonic acid ($C_{24}H_{38}O$), nisinic acid ($C_{24}H_{36}O_2$) and clupanodonic acid ($C_{22}H_{34}O_2$). The present authors propose the names "thynnic acid" for the acid $C_{26}H_{40}O_2$ and "shibic acid" for the acid $C_{26}H_{42}O_2$.

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