# ON THE OIL OF HOKKE (*Pleurogrammus monopterygius*, Pallas), WITH SPECIAL REFERENCE TO THE OCCURRENCE OF NEW HIGHLY UNSATURATED $C_{28}$-FATTY ACIDS.

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"Hokke" (*Pleurogrammus monopterygius*, Pallas) is a fish widely distributed in the sea of the northern part of Honshu and Hokkaido, Japan. According to the growth of the fish, it is classified into kinds of the following names; "Taraba-hokke" (about 420 mm. in length), "Chuhokke" (about 350 mm. in length) and "Rosoku-hokke" (about 300 mm. in length), etc. These fishes contain much quantities of oil, but their flesh is disagreeable to our taste. Hitherto, the Hokke oil has scarcely appeared in the market and the properties of the oil have not been investigated.

A sample of oil (probably of "Taraba-hokke") from Matsumae Hokkaido, had the following properties: acid value, 19.0; $n_D^{20}$, 1.4714; $d_4^{15}$, 0.9153; saponification value, 183.3; iodine value (Wijs), 92.1; oxidised acid, 0.10%; unsaponifiable substances, about 1.5%. The oil was a liquid having a reddish orange colour and separated much solid fat in winter season.

The fatty acids freed from unsaponifiable matters showed the following properties: $n_D^{20}$, 1,4623; neutralisation value, 184.2; iodine value (Wijs), 96.04; ether insoluble polybromide, 9.77%; Br of polybromide, 68.48%:

The solid acids obtained by the lead-salts-alcohol method showed a comparatively high percentage of unsaturated acids: solid acids: yield, 29.2%; iodine value, 20.9; neutralisation value, 190.0; m.p. 41.8–44.7°C. and liquid acids: yield, 70.8%; iodine value, 114.7; neutralisation value, 170.6. As the solid acids are undoubtedly composed mainly of cetoleic acid $C_{22}H_{42}O_2$, the following results can be obtained by calculation: saturated acids: 11.3%; Neutralisation value, 205.0; unsaturated acids: 88.7%.

The results of the fractional distillation of methyl esters of mixed fatty acids freed from unsaponifiable matters are given in Table 1 and shown diagrammatically in Fig. 1.

Table 1.

| Fraction | Distillation temperature (°C. / 5 mm.) | Saponification value | Iodine value | $n_D^{15}$ | Yield (g.) |
|---|---|---|---|---|---|
| 1 | 145—150 | 204.8 | 26.1 | 1.4478 | 47.5 |
| 2 | 150—155 | 216.7 | 27.7 | 1.4451 | 31.4 |
| 3 | 155—160 | 220.2 | 32.8 | 1.4451 | 33.7 |
| 4 | 160—165 | 219.7 | 35.7 | 1.4453 | 36.5 |
| 5 | 165—170 | 216.0 | 39.5 | 1.4461 | 33.3 |
| 6 | 170—175 | 215.2 | 50.4 | 1.4488 | 54.9 |
| 7 | 175—180 | 207.5 | 58.0 | 1.4498 | 79.0 |
| 8 | 180—185 | 208.2 | 67.3 | 1.4518 | 84.0 |
| 9 | 185—190 | 197.3 | 82.7 | 1.4546 | 126.1 |
| 10 | 190—195 | 190.8 | 94.8 | 1.4578 | 73.1 |
| 11 | 195—200 | 186.3 | 105.4 | 1.4598 | 67.5 |
| 12 | 200—205 | 182.4 | 113.1 | 1.4608 | 100.5 |
| 13 | 205—210 | 178.6 | 115.6 | 1.4626 | 104.0 |
| 14 | 210—215 | 175.3 | 114.5 | 1.4638 | 30.6 |
| 15 | 215—220 | 174.1 | 117.7 | 1.4640 | 35.5 |
| 16 | 220—225 | 171.5 | 118.5 | 1.4643 | 51.3 |
| 17 | 225—230 | 168.8 | 122.7 | 1.4651 | 97.9 |
| 18 | 230—235 | 162.8 | 124.3 | 1.4668 | 68.1 |
| 19 | 235—240 | 157.2 | 129.5 | 1.4708 | 7.2 |
| 20 | 240—243 | 152.0 | 135.9 | 1.4788 | 6.2 |
| Residue | — | 145.2 | 117.7 | 1.4833 | 33.8 |

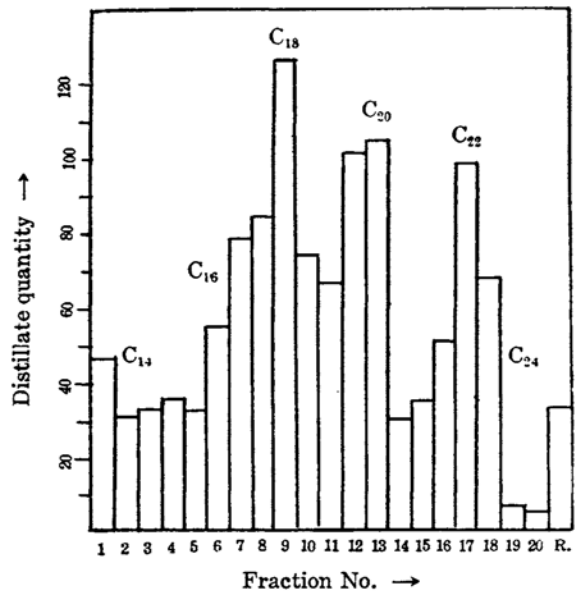


Fig. 1. Diagrammatic representation of the result of fractional distillation of methyl esters.

By comparison with many examples of distillation of methyl esters of such kinds of oils, it will be easily seen that the distillates seem most likely to consist of methyl esters of following fatty acids: (1) Lower fractions 1-8: palmitic acid $C_{16}H_{32}O_2$ (chief component), myristic acid $C_{14}H_{28}O_2$, $C_{16}H_{30}O_2$, etc. (2) Main fractions 9, 10, and 11: oleic acid $C_{18}H_{34}O_2$ (chief component), stearic acid $C_{18}H_{36}O_2$, highly unsaturated fatty acids of $C_{18}$ and $C_{20}$, etc. (3) Fractions 13 and 14: $C_{20}H_{38}O_2$, $C_{20}H_{32}O_2$, $C_{20}H_{30}O_2$, $C_{20}H_{40}O_2$ arachidic acid, etc. (4) Fractions 17 and 18: clupanodonic acid $C_{22}H_{34}O_2$, behenic acid $C_{22}H_{44}O_2$, etc. (5) Higher fractions and residue: fatty acid of $C_{24}$, etc.

**Detection of Highly Unsaturated Fatty Acids of High Molecular Weight.** A sample oil (3500 g.) was methylated by Haller's method and the methyl esters were distilled in vacuo up to 215°C/5 mm. From the distillation residue (450 g.) unsaponifiable substances were removed by means of repeated extraction with ether, and after changing again the acids into methyl esters, they were fractionally distilled. Properties of final distillates are given in Table 2.

Table 2.

| Fraction | Distillation temperature (°C / 3 mm. | Saponification value | Iodine value | $n_D^{15}$ | Yield (g.) |
|---|---|---|---|---|---|
| 1 | 210—215 | 165.3 | 125.0 | 1.4628 | 31.1 |
| 2 | 215—220 | 162.3 | 117.9 | 1.4630 | 12.1 |
| 3 | 220—225 | 159.9 | 118.8 | 1.4640 | 38.3 |
| 4 | 225—230 | 153.2 | 126.9 | 1.4660 | 17.8 |
| Residue | — | 150.0 | 151.5 | — | 28.7 |

Highly unsaturated fatty acids were separated from these fractions as lithium salts which are soluble in 94% acetone. The properties of these fatty acids and their bromides are given in Table 3.

Table 3.

| | Yield (g.) | Neutralisation value | Iodine value | $n_D^{15}$ | Yield of ether-insoluble polybromide (%) | Decomposition temp. of polybromide | Bromine content of polybromide (%) |
|---|---|---|---|---|---|---|---|
| Fraction 1 | 5.3 | 157.3 | 318.2 | 1.5003 | 98.0 | about 220° | 72.10 |
| Fraction 3 | 4.0 | 142.1 | 286.5 | 1.5010 | 74.4 | ,, 213 | 70.94 |
| Fraction 4 | 2.6 | 132.2 | 267.7 | 1.5074 | 50.3 | ,, 215 | 65.35 |
| Residue | 3.2 | 133.4 | 239.0 | 1.5120 | 28.4 | ,, 225 | 71.28 |

If only the neutralisation values be considered, the highly unsaturated fatty acid from fraction 1 may be almost identified with $C_{24}$-acids, and the bromine content of the polybromide (72.10%) corresponds to the value between $C_{24}H_{38}O_2$ and $C_{24}H_{36}O_2$ as shown in Table 4.

Table 4.

| | Neutralisation value | Iodine value | Br % of bromide |
|---|---|---|---|
| Found | 157.3 | 318.2 | 72.10 |
| $C_{24}H_{36}O_2$ (Nisinic acid)(1) ($\Gamma_6$) | 157.8 | 427.5 | 72.91 |
| $C_{24}H_{38}O_2$ (Scoliodonic acid)(2) ($\Gamma_5$) | 156.7 | 354.2 | 69.05 |

The unsaturated fatty acid from fraction 3 is similarly identified with $C_{26}$-acids and the bromine content (70.94%) of the bromide corresponds to a mixture of $C_{26}H_{40}O_2Br_{12}$, and $C_{26}H_{42}O_2Br_{10}$ as shown in Table 5.

Table 5.

| | Neutralisation value | Iodine value | Br % of bromide |
|---|---|---|---|
| Found | 142.1 | 286.5 | 70.94 |
| $C_{26}H_{40}O_2$ (Thynnic acid)(3) ($\Gamma_6$) | 146.2 | 396.7 | 71.39 |
| $C_{26}H_{42}O_2$ (Sibic acid)(4) ($\Gamma_5$) | 145.4 | 328.8 | 67.41 |

The saturated acid which was obtained by the hydrogenation of the fatty acid from fraction 2 melted at 70.3–71.5°C and the determination of neutralisation value and ultimate analysis were made. (Found: C, 78.56; H, 13.06; neutralisation value, 138.9. Calculated for $C_{26}H_{52}O_2$: C, 78.8; H, 13.1; neutralisation value, 141.5).

Neutralisation values of fraction 4 and the residue correspond to $C_{23}$-acids. The bromine percentages of the bromides precipitated from the ether solutions of the acids (65.35 and 71.28) nearly agreed respectively with that of $C_{23}H_{46}O_2Br_{10}$ and with that of a mixture $C_{23}\Gamma_7$-, $C_{23}\Gamma_6$-, $C_{23}\Gamma_5$-, $C_{23}\Gamma_4$-acids, etc. as seen from Table 6.

(1) Toyama and Tsuchiya, this Bulletin. **11** (1935), 543.
(2) S. Ueno and M. Iwai, *J. Soc. Chem. Ind.*, Japan, **37** (1934), 251 B.
(3) S. Ueno and C. Yonese, *J. Chem. Soc. Japan*, **57** (1936), 182.
(4) S. Ueno and C. Yonese, *J. Chem. Soc. Japan*, **57** (1936), 182.

Table 6.

| Calculated for | Neutralisation value | Iodine value | Br % of bromide |
|---|---|---|---|
| $C_{28}H_{42}O_2$ ($F_7$) | 136.7 | 433.1 | 73.16 |
| $C_{28}H_{44}O_2$ ($F_6$) | 136.1 | 369.4 | 69.94 |
| $C_{28}H_{46}O_2$ ($F_5$) | 135.4 | 306.3 | 65.86 |
| $C_{28}H_{48}O_2$ ($F_4$) | 134.7 | 243.4 | 60.56 |

The saturated acid (m.p. 72.0–73.5°C.) obtained from the mixture of fraction 4 and the residue corresponds to $C_{28}H_{56}O_2$ in the results of ultimate analysis and neutralisation value. (Found: C, 79.12; H, 13.69; neutralisation value, 134.7. Calculated for $C_{28}H_{56}O_2$: C, 79.20; H, 13.10; neutralisation value, 132.2).

**Detection of Cetoleic Acid $C_{22}H_{42}O_2$.** The saponification values of fractions 1,2,3, etc. (Table 2) are respectively higher than those of the corresponding methyl esters of highly unsaturated acids in the literatures. This is explained if lower acids of lower unsaturation are present. The authors have isolated an acid $C_{22}H_{42}O_2$ (m.p. 33.0–33.7°) from the lithium salt of the fractions 2 and 3 (Table 2) insoluble in 94% acetone (Found: C, 77.76; H, 12.51; neutralisation value, 166.0; iodine value, 75.1. Calculated for $C_{22}H_{42}O_2$: C, 78.03; H, 12.51; neutralisation value, 165.8; iodine value, 75.0). By means of the mixed melting point the acid has been identified with cetoleic acid (m.p. 33–34°): mixed m.p. with errucic acid (m.p. 33–34°) from rape seed oil, 26.7–27.3°; mixed m.p. with cetoleic acid (m.p. 32.0–32.5°) from Menuke-fish oil[5], 32.0–32.9°.

**Investigation of the Unsaponifiable Constituents.** The unsaponifiable matter is a reddish brown mass having many crystals of cholesterol (35.4%). The sample (20.2 g.) was subjected to fractional crystallisation from methanol as follows: Fraction 1: m.p. 142.3–144.0°, yield 6.7 g. Fraction 2: m.p. 139.5–140.1°, yield 1.5 g. Residue: liquid, yield 10.1 g.

The crystalline fractions were recrystallised several times from 95% alcohol and a substance with a melting point 146–147°C was obtained, which was identified with cholesterol $C_{27}H_{46}O$. The liquid portion, which still contained a small quantity of cholesterol, was acetylated, and the product was distilled in vacuo as given in Table 7.

(5) S. Ueno and M. Iwai, *J. Soc. Chem. Ind., Japan*, **37** (1934), 52 B.

Table 7.

| Fraction | Distillation temperature (°C/8 mm.) | Saponification value | Iodine value | Yield (g.) | Color |
|---|---|---|---|---|---|
| 1 | 135–160 | 51.8 | 29.6 | 0.9 | Pale yellow liquid |
| 2 | 160–180 | 142.7 | 57.2 | 0.8 | " " " (with some crystals) |
| 3 | 180–200 | 183.3 | 92.6 | 1.4 | Orange |
| 4 | 200–230 | 159.9 | 104.7 | 1.8 | " |
| 5 | 230–245 | 136.8 | 103.6 | 2.4 | " |
| Residue | | | | 1.5 | Black |

| Acetyl derivative of | Formula | Saponification value | Iodine value |
|---|---|---|---|
| Cholesterol | $C_{27}H_{45}O\cdot COCH_3$ | 131.0 | 59.2 |
| Oleyl alcohol | $C_{18}H_{33}O\cdot COCH_3$ | 182.0 | 82.3 |

From these results, the liquid unsaponifiable matter seems to consist chiefly of oleyl alcohol $C_{18}H_{33}OH$.

## Summary.

The chemical and physical characteristics of a sample of the oil of "Hokke" (*Pleurogrammus monopterygius*, Pallas) have been determined. In the investigation of higher fractions and distillation residue, the authors have recognised the probable existence of highly unsaturated fatty acids of higher molecules such as nisinic acid $C_{24}H_{36}O_2$ ($\Gamma_6$), scoliodonic acid $C_{24}H_{38}O_2$ ($\Gamma_5$), thynnic acid $C_{26}H_{40}O_2$ ($\Gamma_6$), sibic acid $C_{26}H_{42}O_2$ ($\Gamma_5$), and highly unsaturated fatty $C_{28}$-acid such as $C_{28}H_{46}O_2$ ($\Gamma_5$), etc. In addition to these compounds, it appears also to contain $C_{28}H_{42}O_2$ ($\Gamma_7$), $C_{28}H_{44}O_2$ ($\Gamma_6$), etc. Highly unsaturated $C_{28}$-fatty acids have not yet been reported as occurring in any other oils.

Since the authors found shortly before scoliodonic acid $C_{24}H_{38}O_2$ ($\Gamma_5$) in a shark liver oil (*Scoliodon laticaudus* Müller and Henle), the research of these kinds of highly unsaturated fatty acids of higher molecules became to arouse interest in our country. Thus, Y. Toyama and T. Tsuchiya[6] found nisinic acid $C_{24}H_{36}O_2$ and scoliodonic acid $C_{21}H_{38}O_2$ in herring oil, sardine oil and other

(6) Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind., Japan*, **37** (1934), 530 B.

fish oils, S. Ueno and C. Yonese[7] detected not only these new acids, but also thynnic acid $C_{23}H_{40}O_2$, sibic acid $C_{26}H_{42}O_2$, etc.

In consideration of the distribution of constituent acids in several kinds of fish oils, the general distribution curves of fatty acid shown in Fig. 2 can

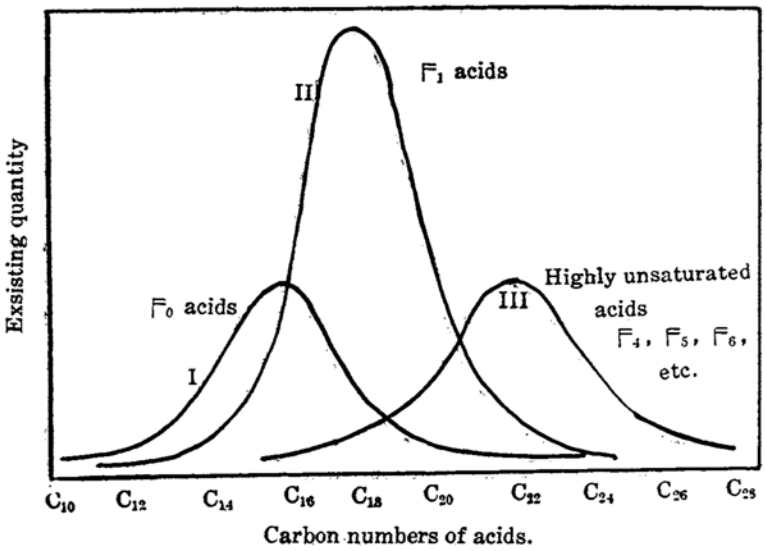


Fig. 2. General distribution curves of fatty acids in fish oil.

be assumed. From curve III, it is evident that the highly unsaturated $C_{28}$-acids have a possible existence. Nevertheless, to our vexation in the present experiment, these compounds have not been isolated in a pure state, but their occurrence could only be suggested by the bromine contents of bromides.

Unsaponifiable substances contain 35.4% of cholesterol $C_{27}H_{46}O$, and the remainder, which is in liquid state, seems to be composed chiefly of oleyl alcohol $C_{18}H_{33}OH$.

It is a characteristic of this oil that it contains comparatively large quantities of higher molecular $C_{20}$- and $C_{22}$-acids, among which the authors have isolated an acid $C_{22}H_{42}O_2$, undoubtedly cetoleic acid.

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(7) S. Ueno and C. Yonese, *J. Chem. Soc. Japan*, **57** (1936), 322.