Liquid Wax Esters in Liver Oils of the Deep-Sea Teleost Fish *Laemonema longipes*

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The content and the constituent fatty alcohols and acids of wax esters in liver oils of the deep-sea teleost fish *Laemonema longipes* from the northwestern Pacific Ocean were investigated.

An analysis of forty specimens revealed that their liver weights (48.9 ± 14.9 g) accounted for $10.3 \pm 1.9\%$ of their total body weight; the livers consisted of $74.0 \pm 6.3\%$ oils. The liver oils contained high quantities of wax esters ($73.6 \pm 11.6\%$) and low proportions of triglycerides ($25.1 \pm 11.5\%$), and also significant amounts of unsaponifiable materials ($35.4 \pm 5.7\%$), almost entirely consisting of fatty alcohols ($98.8 \pm 0.5\%$). The relationship between the amount of wax esters in the liver (Y, g) and liver weight (X, g) was expressed by the following equation: $Y = 3.241 + 0.477X$, ($r = 0.812$).

The component fatty alcohols of wax esters were found to consist mostly of monoenes ($86.5 \pm 4.2\%$) and saturates ($13.4 \pm 4.1\%$) of 14 to 24 carbon numbers. The principal components found were: 22:1 ($47.4 \pm 4.8\%$), 20:1 ($27.3 \pm 2.6\%$) and 16:0 ($9.5 \pm 2.8\%$) alcohols. On the other hand, the wax ester fatty acids contained 18:1 ($26.9 \pm 3.6\%$), 16:1 ($15.3 \pm 1.1\%$), 20:5 ω 3 ($9.4 \pm 2.7\%$), and 16:0 ($9.3 \pm 0.8\%$) acids, as their major components.

It was concluded that the potential exploitation of *L. longipes* liver oil, unusually rich in liquid wax esters, appears as a possible substitute of sperm whale oil.

Wax esters have been used as the characteristic basis for a wide range of products from cosmetics to extreme-pressure and temperature lubricants, and could also be valuable as a source of fatty alcohols for the chemical industry. As the shortage of sperm whale oil, rich in wax esters, is evident in near future, new sources of commercially desirable wax esters are being sought. Recently more attention has been called for the seed oil of the desert shrub, jojoba *Simmondsia chinensis*,¹⁾ and for the fish oil taken from a certain deep-sea species, orange roughy *Hoplostethus atlanticus*,^{2,3)} both of which contain very high concentrations of wax esters.

On the other hand, Komori and Agawa⁴⁾ first discovered that the oil of a deep-sea fish *Laemonema morosum* contained large amounts of unsaponifiable materials, consisting mainly of 22:1 alcohol. In a previous report, we demonstrated that the liver of a deep-sea teleost fish *Podonema longipes* contained remarkably high amounts of oils, characterized by having a high percentage of wax esters, comprised primarily of 22:1 and 20:1 alcohols.⁵⁾ These two fishes are considered to be the same species belong to the family Moridae *Laemonema longipes*, which is potentially available

in large quantities. Their livers are thus presently an untouched resource as another replacement of sperm whale oil. Recently, Kayama and Iijima⁶⁾ examined *L. longipes* liver oil and tried to separate wax esters by the centrifugation.

The present study attempts to describe in more detail the content of such wax esters and the composition of fatty alcohol moiety in the liver oils of a number of *L. longipes* differing in growth size, caught in the northwestern Pacific Ocean. The component fatty acids of the wax esters were also determined.

Experimental

Materials

Forty *L. longipes* specimens, ranging from 292.0 to 710.0 g (mean $\pm$ standard deviation: 469.2 ± 93.8 g) in body weight, were caught at a depth of ca. 700 m in the northwestern Pacific Ocean in August, 1985. They were stored at -20°C until analysis. The livers of this species were used individually for lipid extraction.

Lipid Extraction and Analysis

The liver oils were extracted from the tissues by

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the method of Bligh and Dyer.⁷⁾ Wax esters were then fractionated from the liver oils by thin-layer chromatography. The liver oils and wax esters were subjected to hydrolysis in ethanolic potassium hydroxide by boiling under reflux for 1 h. The unsaponifiable material containing fatty alcohols was isolated from the fatty acids by repetitive diethyl ether extraction from the aqueous alkaline solutions. The fatty acids were recovered from the each saponification mixture by a routine method. The quantitative analyses of liver oils and unsaponifiable materials were performed by employing the thin-layer chromatography—flame ionization detector method using an Iatroscan TH-10 instrument with Chromarod S-II rods. Chromatograms were recorded and integrated by a Shimadzu Chromatopack R-1A. Chromarod S-II rods, spotted with a chloroform solution containing liver oils or unsaponifiable materials, were developed in hexane—diethyl ether—formic acid (92: 8: 0.5 v/v/v for the former; 50: 50: 0.5 v/v/v for the latter). The operating conditions were as follows: flow rates of hydrogen and air were 160 ml/min and 2000 ml/min respectively; scan speed was 30 s/scan.

Thin-Layer Chromatography (TLC)

A preliminary qualitative analysis of the liver oil or its unsaponifiable material was performed using TLC. The fractionation of liver oil wax esters was carried out on prepared plates. A portion of the fatty alcohol acetates was fractionated, according to their degree of unsaturation, on silicic acid plates impregnated with 15% silver nitrate. Thin-layers of silicic acid, 0.25 mm thick (20×20 cm), were used for analytical and preparative purposes. Hexane—diethyl ether—acetic acid (90: 10: 1 v/v/v for liver oils and 40: 60: 1 v/v/v for unsaponifiable materials) were used as developing solvents. After development, the plates were sprayed with 50% sulfuric acid or alcoholic rhodamine-6-GO as visual reagents. Lipid constituents were identified by comparison of R_f values with those of corresponding standards.

Derivatization

Fatty alcohols were converted into acetates by dissolving them in a mixture of pyridine and acetic anhydride, and were left to stand in darkness at room temperature overnight. Fatty acids were methylated with 14% boron trifluoride in methanol. Prior to the gas liquid chromatography, further purification of these derivatives

was carried out by TLC.

Hydrogenation

Samples were dissolved in hexane containing 5% palladium catalyst, and treated with hydrogen at room temperature and under atmospheric pressure for 1 h to achieve complete hydrogenation.

Gas Liquid Chromatography (GLC)

Analysis by GLC was carried out using a Shimadzu model GC 8APF gas chromatograph equipped with a dual hydrogen flame ionization detector. The acetates of fatty alcohols and the methyl esters of fatty acids were analyzed on 1.5 m × 3 mm id. glass columns packed with Unisol 3000 on Uniport C (80/100 mesh) and with 10% DEGS on Chromosorb W AW (80/100 mesh), respectively. The operating conditions were as follows: column temperatures were 225°C for the acetates of fatty alcohols, and 180°C for the methyl esters of fatty acids. Nitrogen was used as a carrier gas. The components' identity for fatty alcohols and acids was confirmed by comparison with available known standards as well as by making semilogarithmic plots of the retention times against the carbon numbers with different degrees of double bonds. A quantitative analysis was performed on the basis of the percentage of the total area using a Shimadzu model R-3A Chromatopack.

Results and Discussion

Content of Liver Oil

An examination of the forty *L. longipes* specimens revealed their liver weights ranging from 16.5 to 90.0 g (48.9 ± 14.9 g as mean); the relative weight of the liver accounted for 5.7 to 13.7% ($10.3 \pm 1.9\%$) of the total body weight. The relationship between liver weight (Y, g) and total body weight (X, g) was expressed by the following equation: $Y = -12.408 + 0.131X$, ($r = 0.820$).

The livers yielded 11.1 to 65.5 g (36.4 ± 12.1 g) of oils; the relative weight of the liver oils amounted to 50.1 to 82.9% ($74.0 \pm 6.3\%$) of the liver weight and 3.8 to 10.6% ($7.7 \pm 1.6\%$) of the total body weight, respectively. As seen in Fig. 1, the relationship between the amount of liver oils (Y, g) and liver weight (X, g) could be expressed as follows: $Y = -1.350 + 0.772X$, ($r = 0.956$).

Component of Liver Oil and Unsaponifiable Material

TLC of the *L. longipes* liver oil revealed relatively

Fig. 1. Relationship between the amount of liver oils (Y, g) and liver weight (X, g) of the examined *L. longipes*.

Table 1. Lipid class composition of liver of the examined *L. longipes*

Component	Mean $\pm$ SD %*
Wax esters	73.6 $\pm$ 11.6
Triglycerides	25.1 $\pm$ 11.5
Fatty acids	0.3 $\pm$ 0.1
Sterols	0.2 $\pm$ 0.1
Partial glycerides	0.2 $\pm$ 0.1
Phospholipids	0.7 $\pm$ 1.1

* N=40.

large spots corresponding to wax esters and triglycerides. The composition of the liver oils is presented in Table 1. The liver oils were characterized by a high level of 73.6 $\pm$ 11.6% wax esters, followed by 25.1 $\pm$ 11.5% triglycerides. It is noteworthy that even the small-sized *L. longipes* examined had an abundant amount of wax esters in their livers. The wax ester content in the liver oils of the present *L. longipes* was comparatively higher than that reported previously (47.7%).⁵⁾

The wax ester content of the livers examined in this study ranged from 7.6 to 47.9 g (26.5 $\pm$ 8.8 g). The relationship between the amount of wax esters in the liver (Y, g) and liver weight (X, g) was expressed by the following equation: $Y = 3.241 + 0.477X$, ($r = 0.812$) (Fig. 2). Thus, substituting the weight of *L. longipes* liver for this expression, it is possible to estimate the absolute amount of wax ester of the individual liver.

The liver oils were also characterized by a high level of unsaponifiable materials ranging from 23.2 to 48.4% (35.4 $\pm$ 5.7%). The content of unsaponifiable material in the livers accounted

Fig. 2. Relationship between the amount of wax esters in the liver (Y, g) and liver weight (X, g) of the examined *L. longipes*.

Table 2. Composition of unsaponifiable materials of liver oils of the examined *L. longipes*

Component	Mean $\pm$ SD %*
Fatty alcohols	98.8 $\pm$ 0.5
Sterols	0.7 $\pm$ 0.3
Glycerol ethers	0.2 $\pm$ 0.3
Methoxy glycerol ethers	0.1 $\pm$ 0.1
Polar compounds	0.2 $\pm$ 0.1

* N=40.

for 3.6 to 22.9 g (12.8 $\pm$ 4.2 g). The constituents of unsaponifiable materials are as shown in Table 2. The unsaponifiable materials were almost entirely fatty alcohols (98.8 $\pm$ 0.5%). Small quantities of sterols and glycerol ethers were also detected. The content of the fatty alcohols in the livers ranged from 3.6 to 22.6 g (12.6 $\pm$ 4.2 g). The relationship between the amount of fatty alcohols in the liver (Y, g) and liver weight (X, g) could be derived as follows: $Y = 1.549 + 0.227X$, ($r = 0.808$). TLC revealed that the small spot migrating slower than the glycerol ether spot on the silicic acid plate corresponded to that of methoxy glycerol ethers isolated from ratfish liver lipids.⁸⁾

Composition of Fatty Alcohol and Acid of Wax Ester

The component fatty alcohols of the liver oil wax esters are as shown in Table 3. Even numbers of carbon atoms, ranging from C₁₄ to C₂₄, were abundant as compared with odd ones. The most predominant component found was 22:1 alcohol (47.4 $\pm$ 4.8%), followed by 20:1 (27.3 $\pm$ 2.6%) and

16:0 ($9.5 \pm 2.8\%$) alcohols, indicating a very high concentration of monoenoic ($86.5 \pm 4.2\%$). Branched fatty alcohols also occurred but only in

Table 3. Fatty alcohol composition of wax esters in liver oils of the examined *L. longipes*

Component ^{*1}	Mean $\pm$ SD % ^{*2}
14:0	2.3 ± 1.1
15:0	0.2 ± 0.1
16:0	9.5 ± 2.8
17:0	0.2 ± 0.1
18:0	0.7 ± 0.1
19:0	0.2 ± 0.1
20:0	0.3 ± 0.1
16:1	4.6 ± 1.3
17:1	0.1 ± 0.1
18:1	2.6 ± 0.6
19:1	0.1 ± 0.1
20:1	27.3 ± 2.6
21:1	0.1 ± 0.1
22:1	47.4 ± 4.8
23:1	0.2 ± 0.1
24:1	4.2 ± 0.7
15:0 br ^{*3}	0.1 ± 0.1
16:0 br	0.1 ± 0.1
Saturates	13.4 ± 4.1
Monoenes	86.5 ± 4.2
Branched	0.1 ± 0.2

^{*1} No. of carbon atoms: no. of double bonds.

^{*2} N=40.

^{*3} Branched compounds.

Table 4. Fatty acid composition of wax esters in liver oils of the examined *L. longipes*

Component ^{*1}	Mean $\pm$ SD % ^{*2}
14:0	6.8 ± 1.3
16:0	9.3 ± 0.8
18:0	1.3 ± 0.2
14:1	0.5 ± 0.2
16:1	15.3 ± 1.1
17:1	1.7 ± 0.2
18:1	26.9 ± 3.6
20:1	9.6 ± 2.6
22:1	7.6 ± 2.9
18:2 ω 6	1.8 ± 0.3
18:4 ω 3	2.4 ± 0.6
20:4 ω 6	0.2 ± 0.1
20:4 ω 3	0.9 ± 0.3
20:5 ω 3	9.4 ± 2.7
22:6 ω 3	1.5 ± 0.4
Saturates	18.3 ± 2.0
Monoenes	62.4 ± 4.1
Polyenes	18.7 ± 3.7
Unknowns	0.5 ± 0.5

^{*1} No. of carbon atoms: no. of double bonds.

^{*2} N=40.

small amounts. However, no polyunsaturated fatty alcohols were detected.

The component fatty alcohols were in good agreement in composition to those of the same species reported previously.⁵⁾ It should be noted that the aforementioned fatty alcohols obtained from the liver oil wax esters of *L. longipes* were extremely different in composition to those of the jojoba seed oil,¹⁾ orange roughy oil,^{2,3)} and sperm whale oil.³⁾

The major fatty acid composition of the *L. longipes* wax esters studied is given in Table 4. As shown in Table 4, 18:1, 16:1, 20:1, 20:5 ω 3, and 16:0 acids were characteristic, and their contents were $26.9 \pm 3.6\%$, $15.3 \pm 1.1\%$, $9.6 \pm 2.6\%$, $9.4 \pm 2.7\%$, and $9.3 \pm 0.8\%$ of the total fatty acids, respectively. The high proportion of 20:5 ω 3 acid in the *L. longipes* wax esters were extremely different from the wax ester fatty acids found in jojoba seed oil,¹⁾ orange roughy oil,^{2,3)} and sperm whale oil,³⁾ which were lacking of this polyunsaturated fatty acid.

Although the extent of the deep-sea teleost fish *L. longipes* resource is at present unknown, it is large (past catches: 14,000 t in 1985), and it is possible that a fishery could be developed to process these fish. It can be concluded therefore that the potential exploitation of *L. longipes* liver oil, unusually rich in liquid wax esters, appears as a possible substitute of sperm whale oil.

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