Occurrence of Unusually High Level of Wax Esters in Deep-Sea Teleost Fish Muscle, *Hoplostethus atlanticus*

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The lipid composition of the muscle of the deep-sea teleost fish, *Hoplostethus atlanticus*, caught out at sea off New Zealand was determined.

In this species, the muscle contained 4.5% lipids, of which wax esters contained unusually high level of 88.6% with small amounts of triglycerides (1.3%), hydrocarbons (2.7%), sterols and fatty alcohols (2.2%), and phospholipids (5.2%).

The fatty alcohol components of wax esters were composed mostly of saturates (39.8%) and monoenes (53.9%) with 14-24 carbon atoms: the major constituents were 16:0 (28.3%), 20:1 (17.1%), 18:1 (16.2%), 22:1 (14.5%), and 18:0 (7.6%). The fatty acid composition of wax esters consisted predominantly of monoenes with 18:1 (59.4%) as the main component.

Little difference in the fatty acid compositions between wax esters and triglycerides showed that the contents of monoenes in the former and saturates in the latter were relatively large. In contrast, the component fatty acids of phospholipids contained considerable amounts of polyenes.

In most marine teleost fish, triglycerides generally exist as the principal reserves of storage lipids. Hitherto, it has been reported that a considerable amount of wax esters are specifically located in the roe lipids of *Merluccius capensis*¹⁾ and *Mugil cephalus*.¹⁻³⁾ In recent reports, some species of marine teleost fish have been found to possess a large amount of these compounds in the muscle or liver. Those fish are *Lottela phycis*,⁴⁾ *Laemonema morosum*,⁵⁾ *Podonema longipes*,⁶⁾ *Lepidocybium flavobrunneum*,⁷⁾ *Ruvettus pretiosus*,⁷⁻⁹⁾ *Allocyttus verrucosus*,¹⁰⁾ *Latimeria chalumnae*,¹¹⁾ *Hoplostethus islandicus*,¹²⁾ *Hoplostethus gilchristi*,¹³⁾ *Paralepis rissori koyeri*,¹⁴⁾ and several nektonic fish¹⁵⁻¹⁸⁾ of the families Astronesthidae, Cheilodipteridae, Gonostomatidae, Myctophidae, Paralepididae, Scopelosauridae, Sternophychidae and Stomiidae.

The authors have recently found a deep-sea teleost fish of the family Trachichthyidae, *Hoplostethus atlanticus*, which has wax esters predominantly in the muscle lipids with a minor amount of triglycerides. This fish is considered to be the same species, *Hoplostethus gilchristi*, which contained wax esters exclusively in the muscle lipids as demonstrated by MORI *et al.*¹³⁾

The present paper deals with the lipid composition and analyses of fatty alcohol or fatty acid components of wax esters, triglycerides and phospholipids of this peculiar teleost fish muscle. In this connection, fatty alcohol and fatty acid compositions of wax esters are compared with those

of *H. gilchristi*.¹³⁾

Materials

One specimen of male *H. atlanticus*, 39.8 cm in length and 1.13 kg in weight, was caught from ca. 940 m depth off New Zealand by a trawler. The captured fish was brought back to the laboratory in a frozen state. The muscle was used for the lipid extraction.

Experimental Methods

Extraction and Fractionation of Lipids

The total lipids were extracted from the muscle by the method of BLIGH and DYER.¹⁹⁾ Neutral lipids were separated from total lipids by silicic acid-Celite (2:1) column with chloroform, and phospholipids remained in the column were eluted with methanol.

Thin-Layer Chromatography (TLC)

The qualitative and quantitative analyses of the constituents of the muscle lipids were carried out by TLC. The fractionation of wax esters and triglycerides was accomplished on preparative plates. Acetates of fatty alcohols were fractionated according to their degree of unsaturation on silicic acid plates impregnated with 15% AgNO₃. Conditions were: Thin layer of silicic acid was prepared with the thickness of 0.25 mm for analytical purposes and 0.50 mm for preparative pur-

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poses. Hexane, diethyl ether and acetic acid mixture (90:10:1 v/v) or hexane and benzene mixture (30:70 v/v) were used as the developing solvents. Fifty per cent sulfuric acid or alcoholic dichlorofluorescein solution was employed as visual reagents.

Preparation of Derivatives

The fatty alcohols were converted into acetates by dissolving them in a mixture of pyridine and acetic anhydride, followed by standing in the dark overnight at room temperature. Fatty acids were methylated with boron trifluoride-methanol.²⁰⁾ Prior to gas liquid chromatography, further purification of these esters was achieved by TLC.

Hydrogenation of Esters

Each ester of fatty alcohols or fatty acids was hydrogenated for qualitative and quantitative examinations. The hydrogenation was performed in a hexane solution with a 5% palladium carbon as a catalyst at atmospheric pressure at 4°C. The samples were shaken for 1 h to achieve complete hydrogenation.

Gas Liquid Chromatography (GLC)

Analyses by GLC were carried out with a Shimadzu model GC 6AM gas chromatograph equipped with a dual hydrogen flame ionization detector. Esters of fatty acids and fatty alcohols were analysed on 1.5 m × 3 mm i.d. glass columns packed with 10% DEGS on Chromosorb WAW (80/100 mesh) and with 5% Silar 10C on Gas Chrom Q (100/120 mesh), respectively. The operating conditions were as follows; temperatures of column, injector and detector: 185°C, 240°C and 240°C for fatty acid methyl esters, and 180°C or 170–230°C (programmed rate, 1°C/min), 250°C and 250°C for fatty alcohol acetates, respectively. Flow rates of N₂, H₂ and air were 40 ml/min, 35 ml/min and 400 ml/min, respectively. The identification of esters was accomplished by comparison with known available standards and by a log-plot of retention times against the number of carbons in the chain, before and after hydrogenation. Quantitative analysis was made on the basis of the area percentage of each peak.

Results and Discussion

Characteristics of Lipids

The muscle of *H. atlanticus* examined in this study contained 4.5% lipids, which contained a

large amount of neutral lipids (94.8%) with some phospholipids (5.2%). As shown in Fig. 1, the muscle lipids had major and minor spots, each corresponding to wax esters and triglycerides, respectively. This indicated that the neutral lipid fraction was chiefly composed of wax esters. The unsaponifiable fraction originated from the major constituent of the muscle lipids gave one spot corresponding to fatty alcohols on TLC plate. The wax ester fraction, therefore, had little contaminant of cholesteryl esters. The argentation TLC of fatty alcohol acetates gave larger spots corresponding to saturates or monoenes with only small of dienes (Fig. 1).

Fig. 1. Thin layer chromatograms of muscle lipids and fatty alcohol acetates of *H. atlanticus*. A: muscle lipids. Adsorbent; silicic acid. Solvent; hexane, diethyl ether, acetic acid (90:10:1 v/v). Hy; hydrocarbons, WE; wax esters, TG; triglycerides, Al; fatty alcohols, St; sterols, PL; phospholipids. B: fatty alcohol acetates. Adsorbent; silicic acid impregnated with 15% silver nitrate. Solvent; hexane, benzene (30:70 v/v). Sat; saturates, Mono; monoenes, Di; dienes.

The lipid composition of the muscle of *H. atlanticus* is given in Table 1. Included in Table 1 for comparison is that of *H. gilchristi* reported by MORI *et al.*¹³⁾ The muscle lipids of *H. atlanticus*

Table 1. The lipid composition of the muscle of *H. atlanticus*

	<i>H. atlanticus</i>	<i>H. gilchristi</i> MORI <i>et al.</i> ¹³⁾
Muscle lipids	4.5%	7.9%
Hydrocarbons	2.7%	} 96.6*%
Wax esters	88.6	
Triglycerides	1.3	trace
Fatty alcohols }	2.2	} 3.4
Sterols }		
Phospholipids	5.2	

* Include only small amounts of hydrocarbons.

were characterized by high level of wax esters (88.6%) and only minute amount of triglycerides (1.3%). Small amounts of hydrocarbons, fatty alcohols, sterols, and phospholipids were also detected in the muscle lipids.

The lipid content of the muscle of *H. atlanticus* was somewhat lower compared with that of *H. gilchristi*.¹³⁾ The composition of the muscle lipids, however, agreed reasonably with that of *H. gilchristi*. On the other hand, KAUFMANN *et al.*¹²⁾ have recognized the presence of wax esters in the muscle of *H. islandicus*. It is of interest to note that *H. atlanticus*, *H. gilchristi* and *H. islandicus* belong to the same genus *Hoplostethus*. It is possible, therefore, that wax esters are of a limited usefulness for a chemotaxonomic character, as pointed out previously by MORI *et al.*¹³⁾

Composition of Fatty Alcohols and Fatty Acids

The homologous components of saturated, monoenoic and dienoic fractions of fatty alcohol acetates obtained by argentation TLC were identified by GLC with and without hydrogenation of the unsaturated fractions, respectively. The results obtained are given in Table 2. Included in Table 2 for comparison is that of *H. gilchristi*.¹³⁾ The characteristics of the fatty alcohol components for *H. atlanticus* examined are as follows. Carbon atoms with even number, ranging from C₁₄ to C₂₄, were abundant as compared with odd and branched ones. The predominant components were 16:0 and 18:0 for saturated compounds, and 20:1, 18:1 and 22:1 for monoenoic ones, indicating a high monoenoic content (53.9%). Besides, the dienoic and branched components occurred in a small amount.

Fatty alcohol composition of *H. atlanticus* was similar, on the whole, to that of *H. gilchristi*.¹³⁾ In the terms of the characteristics of fatty alcohol composition, *H. atlanticus* and *H. gilchristi* had large amounts of 20:1 (17.1%, 21.4%) and 22:1

Table 2. The fatty alcohol composition of wax esters from the muscle of *H. atlanticus*

Fatty alcohol*1	<i>H. atlanticus</i>	<i>H. gilchristi</i> MORI <i>et al.</i> ¹³⁾
Peak area %		
14:0	2.1	1.4
15:0	0.7	0.6
16:0	28.3	24.2
17:0	0.2	0.7
18:0	7.6	6.6
19:0	0.2	
20:0	0.5	1.1
22:0	0.2	0.8
23:0		1.2
total	39.8	36.6
14:1	tr*2	0.3
16:1	1.9	1.5
17:1	0.5	0.9
18:1	16.2	9.6
19:1	0.5	0.8
20:1	17.1	21.4
21:1	0.3	0.7
22:1	14.5	21.8
23:1	0.3	0.5
24:1	2.6	3.0
total	53.9	60.5
17:2	0.1	
18:2	0.8	0.8
19:2	0.2	
20:2	1.8	0.7
22:2	1.1	1.0
24:2	0.1	
total	4.1	2.5
15:0 br (I)*3	0.2	
16:0 br (I)	0.4	0.4
17:0 br (I)	0.3	
17:0 br (A)	0.8	
18:0 br (I)	0.5	
total	2.2	0.4

*1 Number of carbon atoms and double bonds.

*2 Trace.

*3 Branched: (I); Iso. (A); anteiso.

(14.5%, 21.8%) compounds. A similar trend has been reported in some deep-sea fish.^{8,10,18)} On the contrary, the fatty alcohol components of *H. islandicus*¹²⁾ consisted of 18:1 (55%), 14:0 (19%) and 16:0 (15%), being extremely different from those of *H. atlanticus* and *H. gilchristi*. This may be due to their different habitats, as the latter inhabits in deep-sea.

The fatty acid compositions of wax esters, triglycerides and phospholipids for *H. atlanticus* are presented in Table 3. Included in Table 3 for comparison is that of wax esters of *H. gilchristi*.¹³⁾ For wax esters and triglycerides of *H. atlanticus*,

Table 3. The fatty acid composition of each lipid class from the muscle of *H. atlanticus*

Fatty acid* ¹	<i>H. atlanticus</i>		<i>H. gilchristi</i> MORI <i>et al.</i> ¹³⁾	
	PL	TG	WE	WE
	Peak area %			
12:0			0.1	
14:0	1.9	3.9	1.4	0.9
15:0	0.7	0.8	0.1	0.1
16:0	21.7	16.4	2.1	1.3
17:0	1.3	1.5	0.4	1.5
18:0	3.3	1.4	0.7	0.5
19:0				0.6
21:0				0.3
total	28.9	24.0	4.8	5.2
14:1	0.1	0.3	0.4	0.3
15:1	0.5		0.1	
16:1	19.4	5.8	13.4	7.7
17:1	1.3	0.2	0.5	0.9
18:1	22.2	31.1	59.4	52.8
19:1	0.5	0.5	0.6	0.8
20:1	1.2	14.1	12.4	19.1
21:1	0.6	0.6	0.2	
22:1	3.3	13.6	5.4	10.6
23:1	0.1			
24:1	0.2	4.6	0.2	0.5
total	49.4	70.8	92.6	92.7
18:2	2.8	1.1	1.3	1.1
18:3	2.7	0.6	0.4	0.5
18:4	0.9	0.4		
20:2	0.6	0.2	0.1	0.4
20:3				tr* ²
20:4	4.6	0.5	0.1	tr
20:5	3.9	0.2	tr	tr
22:6	4.5	tr	tr	
total	20.0	3.0	1.9	2.0
14 br (I)			0.1	
15 br (I)	0.1			
15 br (A)	0.1	0.4	0.1	
16 br (I)	0.6			
16 br (A)		0.7		0.1
17 br (I)			0.3	
17 br (A)	0.2	0.3		
18 br (I)	0.2	0.6		
20 br (I)		0.2		
20 br (A)	0.5		0.2	
total	1.7	2.2	0.7	0.1
mono/poly	2.5	23.6	48.7	

*¹ Number of carbon atoms and double bonds. *² Trace.
 PL: phospholipids, TG: triglycerides, WE: wax esters,
 mono: monoenes, poly: polyenes, br: branched, (I): *iso*,
 (A): *anteiso*.

the contents of monoenoic acids, composed chiefly of 18:1, 20:1, 16:1 and 22:1 acids, were predominant as compared with those of saturated and

polyenoic acids. In wax esters, in particular, 18:1 acid was made up 59.4% of the whole acids. Little difference in the fatty acid compositions between wax esters and triglycerides showed that the contents of monoenes in the former and saturates in the latter were relatively large. In contrast, the component fatty acids of phospholipids contained considerable amounts of polyenes.

The fatty acid components of wax esters of *H. atlanticus* were somewhat similar in composition to those of *H. gilchristi*.¹³⁾ As given in Table 3, the ratios of monoenoic to polyenoic acids were 48.7 for wax esters and 23.6 for triglycerides, respectively. This corresponded to the characteristics in the fatty acid composition of the neutral lipids from a number of deep-sea fish, as previously reported by the authors.²¹⁻²³⁾

It has been suggested that possible functions for large amounts of wax esters in marine organisms were buoyancy, thermal insulation and reserve energy storage.^{11,24,25)} On the other hand, the biochemical pathways leading to the formation of wax esters are not well understood. NEVENZEL²⁶⁾ has postulated that fatty acids from either exogenous food sources or from *de novo* synthesis are the precursors for wax esters, and suggested that fatty acids are reduced to alcohols which are then esterified to other fatty acids thus forming the wax esters. Recently, BENSON *et al.*²⁶⁾ have demonstrated that marine planktonic copepods first utilized the triglyceride components of their stored lipids and then more slowly metabolized their wax ester reserves. It is of interest to note from viewpoint of physiological or ecological role that the deep-sea organisms are abundant in wax esters.

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