Three Species of Teleost Fish having Diacyl Glyceryl Ethers in the Muscle as a Major Lipid

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Two species of teleost fish, *Cubiceps gracilis* and *Centrolophus* sp., have been found to possess diacyl glyceryl ethers in the muscle as a major lipid, a condition previously reported only for *Stromateus maculatus*. In these fishes, concentrations of the muscle lipids ranged from 15.2 to 21.0% and those of glyceryl ethers in the muscle lipids from 20.3 to 27.7%. Compositions of glyceryl ethers and total fatty acids were analyzed by gas-liquid chromatography. Chimyl and selachyl alcohols were the major constituents and made up together 74–84% of the glyceryl ethers. The component fatty alcohols detected were of 9–20 carbon atoms and most of them were saturated or monoenoic. Most of the fatty acids were similarly saturated or monoenoic, oleic acid being the main component.

Some of the elasmobranch fish are well known to possess the liver lipids primarily consisting of diacyl glyceryl ethers. These compounds were shown by KARNOVSKY *et al.*[1] to be distributed widely in the different tissues of teleost fish but only in very small amounts. They indicated that in twenty species of teleost fish concentrations of the body lipids were less than 5% and those of glyceryl ethers (as selachyl alcohol) in the body lipids less than 2%. In 1971, however, it was reported by IIDA[2] that the muscle of 'Pampanito', *Stromateus maculatus*, caught off Chili, contained 20.1% lipids. Of these lipids 16.4% was unsaponifiable, and 90.4% of the unsaponifiable fraction was glyceryl ethers. This appears to be the first finding of the peculiar teleost fish in which glyceryl ethers occur as a major lipid.

We have recently confirmed IIDA's observation on *S. maculatus* and found newly two other species with similar lipoidal elaboration, *Cubiceps gracilis* and *Centrolophus* sp. The present paper deals with compositions of glyceryl ethers and total fatty acids of the muscle lipids in these peculiar teleost fishes.

Materials and Methods

Materials One specimen each of *C. gracilis, Centrolophus* sp. and *S. maculatus* was supplied by Dr. T. ABE, Tokai Regional Fisheries Research Laboratory, who also identified them (Fig. 1). The first two were caught by trawlers and the last one by the research vessel, *Oshoromaru*. Specimens of a butterfish, *Peprilus triacanthus*, and dog-

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fish, *Squalus acanthias*, were caught by the trawlers belonging to Nippon Suisan Co. at the North Atlantic. The place of catch, size and body weight are listed in Table 1. They were frozen on boat and kept in our laboratory in a freezer at −20°C until used.

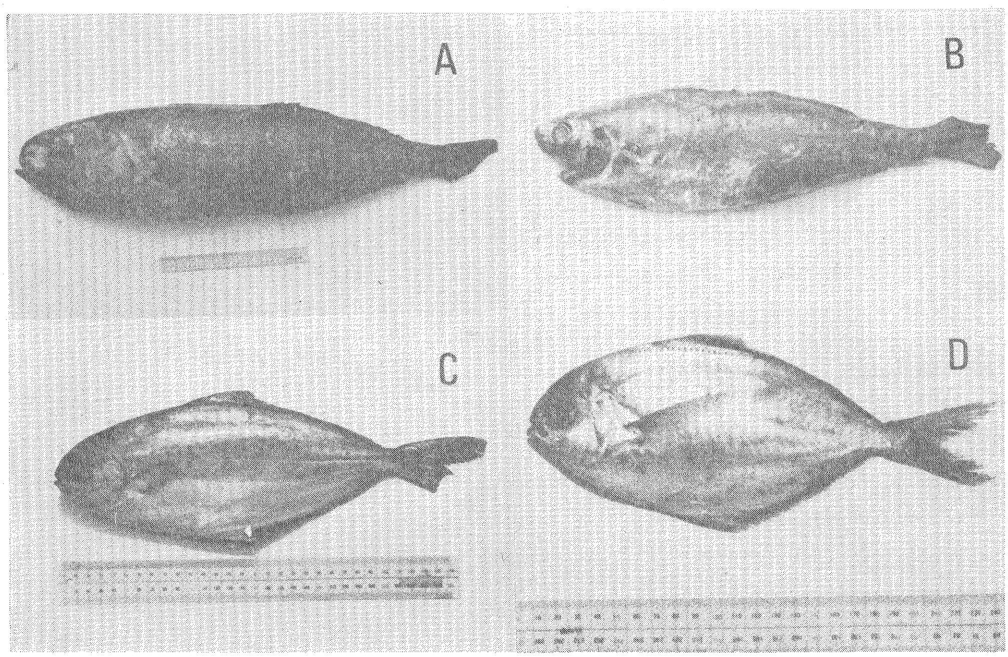

Fig. 1. Four species of teleost fish in the suborder Stromateoidei. A: *Cubiceps gracilis*, B: *Centrolophus* sp., C: *Stromateus maculatus*, D: *Peprilus triacanthus*.

Table 1. Specimens tested.

Japanese name	Scientific name	Place of catch	Body length cm	Body weight kg
	Cubiceps gracilis (LOWE)	Supposed to be off New Zealand	89	14
	Centrolophus sp.	Coast of Tasmania	82	8.1
	Stromateus maculatus CUVIER & VALENCIENNES	North of New Zealand	28	0.41
Shizu	*Peprilus triacanthus*(PECK)	W 70°, N 40°	17*	0.18*
Aburatsunozame	*Squalus acanthias* LINNÉ	W 67°, N 41°	61*	2*

* Average.

Extraction of lipids and estimation of their characteristics Lipids were extracted with acetone from the muscle of teleost fishes and the liver of dogfish and their properties were estimated by the methods as described in our previous paper on the oil fish.[3)]

Thin-layer chromatography Thin-layer chromatography was carried out on Kieselgel or Wako Gel B–10 with solvent systems consisting of n-hexane, diethyl ether, and acetic acid in 90:10:1[4)] by volume for the acetone extracts and in 70:30:1[5)] by volume for the unsaponifiables. Spots were visualized with 50% sulfuric acid.

Florisil column chromatography of unsaponifiables The acetone extracts were saponified by refluxing with 1 N ethanolic potassium hydroxide solution for 1.5 hours. The unsaponifiables were then fractionated by Florisil column chromatography according to the method of SHIMMA and SHIMMA[6]. An approximately 200-mg portion was put onto a column (20 mm × 100 mm) of Florisil (60–100 mesh) and eluted as shown in Fig. 3. Each 10-ml portion was collected.

Gas-liquid chromatography For analysis by gas-liquid chromatography, the glyceryl ethers obtained by Florisil column chromatography were converted to 2,3-0-isopropylidene derivative by acetonation[4,7]; similarly, the total fatty acids formed after saponification from the muscle lipids were converted to methyl esters with methanol-sulfuric acid. Compositions of glyceryl ethers and fatty acids were calculated from the area percentage of each peak.

Analyses by gas-liquid chromatography were carried out with a Hitachi model 063 equipped with a hydrogen flame ionization detector and a 2 m column packed with C-22 fire brick (60–80 mesh, Wilkens Instrument & Research, Inc.) containing 20% of succinate polyester of diethylene glycol. The column temperature was 190°C, injector temperature 250°C and detector temperature 235°C. The flow rate was adjusted to 55 ml/min for nitrogen and 34 ml/min for hydrogen.

Results

Properties of muscle lipids As shown in Table 2, each of three species, *C. gracilis*, *Centrolophus* sp., and *S. maculatus*, contains a large amount of muscle lipids which show a higher value for unsaponifiables and lower iodine value than is usual for fish lipids. In contrast, the butterfish, *P. triacanthus*, shows a high fat content but a very low value for unsaponifiables.

In thin-layer chromatography, the muscle lipids of *C. gracilis, Centrolophus* sp., and *S. maculatus*, reveal two major spots corresponding to diacyl glyceryl ethers and triglycerides, like the liver lipids of the dogfish. The muscle lipids of *P. triacanthus* show

Table 2. Properties of muscle lipids.

	C. gracilis	*Centrolophus* sp.	*S. maculatus*	*P. triacanthus*
Yield %	18.9	21.0	15.2	13.9
Specific gravity d_4^{20}	0.9013	0.9009	0.9191	
Refractive index n_d^{20}	1.4691	1.4684	1.4705	
Acid value	2.6	1.9	15.8	
Saponification value	144.1	134.6	151.9	
Iodine value	87.5	85.2	100.7	
Unsaponifiables %	28.5	30.5	23.7	3.4

the main spot for the triglycerides but only a faint one for diacyl glyceryl ethers. Two distinct spots of wax esters and triglycerides are also observed in the blubber oil of sperm whale.

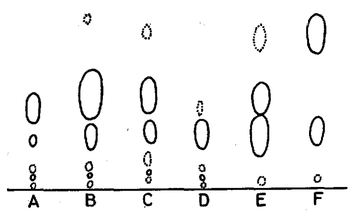

Fig. 2. Thin-layer chromatograms on Wako Gel B–10.
A: *C. gracilis,* B: *Centrolophus* sp., C: *S. maculatus,* D: *P. triacanthus,* E: Liver oil of dogfish, F: Blubber oil of sperm whale.

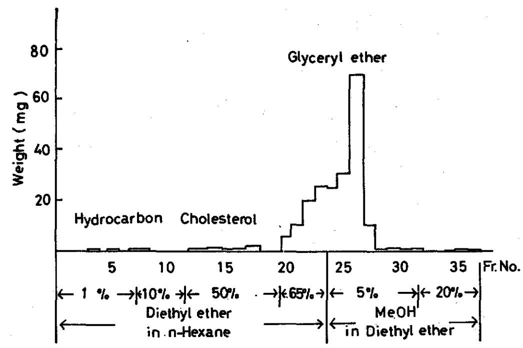

Fig. 3. Elution curve of unsaponifiables from the muscle lipids of *Centrolophus* sp.

Florisil column chromatography of unsaponifiables The elution curve for *Centrolophus* sp. is shown in Fig. 3, as a typical example. Each fraction was identified by thin-layer chromatography. Composition of unsaponifiables is summarized in Table 3. Glyceryl ethers make up approximately 90% of unsaponifiables in each species.

Table 3. Composition of unsaponifiables (%).

	C. gracilis	*Centrolophus* sp.	*S. maculatus*
Hydrocarbons	2.2	3.2	2.0
Cholesterol	6.7	4.8	4.1
Glyceryl ethers	89.1	90.8	85.8
Unknown	2.3	2.1	1.2
Recovery	100.3	100.9	93.1

Composition of glyceryl ethers The yield of isopropylidene glyceryl ethers from the fraction obtained by Florisil column chromatography was 53.6% in *C. gracilis*, 72.0% in *Centrolophus* sp., and 66.1% in *S. maculatus*, respectively. As an example, a gas-liquid chromatogram obtained on the isopropylidene glyceryl ethers from *Centrolophus* sp. is given in Fig. 4. Compositions of glyceryl ethers, calculated from the percentage of peak area, are summarized in Table 4. All of the three species

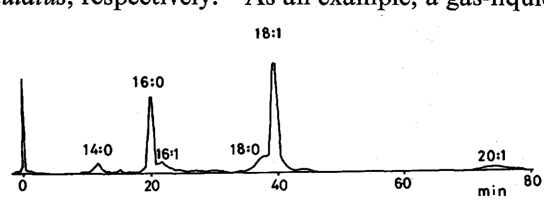

Fig. 4. A representative gas-liquid chromatogram of 2,3-0-isopropylidene derivatives of glyceryl ethers derived from the muscle lipids of *Centrolophus* sp.

contain many components besides chimyl, batyl and selachyl alcohols. The component fatty alcohols detected are of 9–20 carbon atoms and most of them either saturated or monenoic. Odd-numbered, branched, and dienoic chains are present only in small amounts. Chimyl and selachyl alcohols are major constituents and make up together 74–84% of the glyceryl ethers. The ratio of chimyl alcohol to selachyl alcohol is 1.2 in *C. gracilis*, 0.26 in *Centrolophus* sp., and 5.2 in *S. maculatus*.

Table 4. Composition of glyceryl ethers (%).

Glyceryl ether*	*C. gracilis*	*Centrolophus* sp.	*S. maculatus*
9:0		tr	0.1
10:0	0.3	tr	0.1
11:0	0.5	0.1	0.3
12:0	0.5	0.1	0.1
12:1	0.2		
13:0br	0.1	tr	
13:0	0.2	0.3	
14:0	4.3	2.2	9.1
15:0br	0.2	0.1	
15:0	1.9	1.3	1.4
16:0br	0.2	0.1	0.1
16:0	40.1	16.0	68.6
16:1	5.0	4.2	2.8
17:0br	1.8	0.8	1.2
17:0	1.0	0.9	0.4
17:1	0.7	0.9	
18:0br	1.1	0.3	
18:0	6.6	6.7	2.7
18:1	33.7	62.5	13.1
18:2	0.2	0.3	
20:1	1.4	3.2	tr

* Indicated by chain length and double bond of alkyl moiety.

Composition of total fatty acids As shown in Table 5, the muscle lipids of the three species are characterized by high proportion of monoenoic acids and small proportion of polyenoic acids. Oleic acid is the main component in all species. The major constituents are eicosenoic acid in *C. gracilis*, palmitic acid in *Centrolophus* sp., and palmitic and docosenoic acids in *S. maculatus*.

Discussion

Our results on *S. maculatus* were almost identical with those reported by IIDA[2], though the origin of specimens was different.

Table 5. Composition of fatty acids (%).

Fatty acid	*C. gracilis*	*Centrolophus* sp.	*S. maculatus*
12:0		0.1	0.5
12:1			tr
13:0		tr	tr
14:0br		tr	tr
14:0	2.3	4.2	5.9
14:1	0.3	0.6	0.7
15:0	0.5	0.8	0.4
16:0br	0.1	0.2	0.1
16:0	11.1	22.7	13.6
16:1	4.9	7.2	6.6
17:0	1.5	2.1	1.6
17:1	1.1	1.0	1.0
18:0	6.3	6.1	4.3
18:1	44.4	34.7	32.1
18:2	1.3	1.0	1.2
18:3	0.6	0.5	1.0
18:4		0.3	2.2
19:1	1.1	0.4	0.4
20:0	0.3	0.7	0.5
20:1	19.1	8.0	4.7
20:2	0.1		tr
20:3	0.6	1.0	0.6
20:5	tr	0.5	2.7
22:1 (20:4)	2.4	4.8	12.0
22:3	0.3		0.2
22:5	0.3	tr	0.5
22:6	0.6	0.6	4.3
24:1 (22:4)	0.8	2.5	2.9

In *C. gracilis*, *Centrolophus* sp., and *S. maculatus*, levels of the muscle lipids were as high as 18.9, 21.0, and 15.2% and those of glyceryl ethers in the muscle lipids 25.4, 27.7, and 20.3%, respectively. Their behavior in thin-layer chromatography clearly indicated that the glyceryl ethers are present as diacyl derivatives in the muscle. The method for separation of diacyl glyceryl ethers from these fishes is now under examination.

It is interesting that these peculiar teleost fishes all belong to the suborder Stromateoidei according to HAEDRICH[8], who assigned *Centrolophus* to the family Centrolophidae, *Cubiceps* to the family Nomeidae and both *Stromateus* and *Peprilus* to the family Stromateidae. Differing from a member of the same family, *S. maculatus*, the butterfish, *P. triacanthus* was found to have the muscle lipids containing only a very small amount of glyceryl ethers. These compounds, therefore, may be of a limited usefulness as a chemotaxonomic character.

Glyceryl ethers in the liver[4,9–17], flesh[4] and roe[18] lipids from many species of elasmobranch fishes have been analyzed in detail in recent years. In these elasmobranch fishes, both chimyl and selachyl alcohols are the main components, with selachyl alcohol always being the more abundant. Preponderance of both alcohols in three species of teleost fish reported here was also recognized, but their relative ratio greatly differed from species to species.

It was reported that a very small quantity of diacyl glyceryl ethers may be growth-stimulating for some of the mammalian cells[19,20] and a bacterium, *Lactobacillus lactis*[21]. On the other hand, SMITH[22] described 'the flesh of most is excellent' on the family Stromateidae (not identical but nearly equivalent to the suborder Stromateoidei of HAEDRICH[8]) and 'flesh delicate and tasty, but does not keep well' on *Stromateus fiatola*. However, IIDA[2] reported that some of the volunteers who had tried to eat *S. maculatus* suffered from diarrhea. By using feeding experiments, KANEDA *et al.*[23] demonstrated that the diacyl glyceryl ethers obtained by molecular distillation from the liver lipids of 'Kokonohoshi-gin-zame', *Chimaera barbouri*, are far inferior to soybean oil in supporting the growth of rats but less hazardous to the same test animals than wax esters. From the dietary point of view, it may be worth studying whether fish possessing diacyl glyceryl ethers in quantity are edible or not.

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References

1) M. L. KARNOVSKY, W. S. RAPSON and M. BLACK: *J. Soc. Chem. Ind.*, **65**, 425–428 (1946).
2) H. IIDA: This Bull., **37**, 338 (1971).
3) M. MORI, T. SAITO, Y. NAKANISHI, K. MIYAZAWA and Y. HASHIMOTO: *ibid.*, **32**, 137–145 (1966).
4) D. C. MALINS, J. C. WEKELL and C. R. HOULE: *J. Lipid Res.*, **6**, 100–105 (1965).
5) H. K. MANGOLD and D. C. MALINS: *J. Amer. Oil Chem. Soc.*, **37**, 383–385 (1960).
6) Y. SHIMMA and H. SHIMMA: *Bull. Tokai Reg. Fish. Res. Lab.*, **44**, 49–54 (1965).
7) D. J. HANAHAN, J. EKHOLM and C. M. JACKSON: *Biochemistry*, **2**, 630–641 (1963).
8) R. L. HAEDRICH: *Bull. Museum, Comp. Zool.*, **135**, No. 2, 31–139 (1967).
9) Y. SHIMMA, H. SHIMMA and R. KIKUCHI: *Bull. Tokai Reg. Fish. Res. Lab.*, **53**, 103–113 (1968).
10) D. C. MALINS: *Chem. Ind., London*, 1359–1360 (1960).
11) B. HALLGREN and S. LARSSON: *J. Lipid Res.*, **3**, 31–38 (1962).

12) K. E. GUYER, W. A. HOFFMAN, L. A. HARROCKS and D. G. CORNWELL: *ibid.,* **4**, 385–391 (1963).
13) M. KAYAMA: This Bull., **34**, 646–649 (1968).
14) H. SHIMMA and Y. SHIMMA: *Bull. Tokai Reg. Fish. Res. Lab.,* **59**, 101–110 (1969).
15) H. SHIMMA and Y. SHIMMA: This Bull. **36**, 1159–1162 (1970).
16) M. KAYAMA, Y. TSUCHIYA and J. C. NEVENZEL: *ibid.,* **37**, 111–118 (1971).
17) F. SPENER and H. K. MANGOLD: *J. Lipid Res.,* **12**, 12–16 (1971).
18) H. SHIMMA and Y. SHIMMA: This Bull., **34**, 1015–1021 (1968).
19) A. BROHULT: *Nature,* **181**, 1484–1485 (1958).
20) B. HALLGREN and S. LARSSON: *J. Lipid Res.,* **3**, 39–43 (1962).
21) A. BROHULT: *Nature,* **188**, 591–592 (1960).
22) J. L. B. SMITH: The Sea Fishes of Southern Africa, 302–303, Central News Agency, Ltd., South Africa (1950).
23) T. KANEDA, H. SAKAI, S. ISHII and K. ARAI: *Bull. Tokai Reg. Fish. Res. Lab.,* **12**, 1–73 (1955).