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## Component Glyceryl Ethers in Liver Lipids of Spiny Dogfish and Japanese Cat Shark\*1

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### Abstract

Lipid compositions of liver lipids and the component glyceryl ethers and fatty acids from their lipids, of male and female specimens of spiny dogfish *Squalus acanthias* and Japanese cat shark *Scyliorhinus torazame*, were determined in this study.

The livers of spiny dogfish obtained from the mesopelagic region yielded high lipid contents (67.3%–70.5%): diacyl glyceryl ethers and triglycerides were the prominent lipid classes, accounting for 38.7%–44.6% and 45.3%–47.4%, respectively. Whereas, the livers of Japanese cat shark inhabiting the near-surface, contained 49.8%–58.6% lipids, which were characterized by high percentages of triglycerides (81.8%–91.5%). Consequently, the liver lipids of spiny dogfish contained high levels of unsaponifiables (10.6%–18.1%), consisting of a large amount of glyceryl ethers (67.4%–86.8%) with a small amount of sterols (8.2%–16.2%). On the contrary, the unsaponifiables (1.2%–1.3%) of Japanese cat shark liver lipids were composed of 66.9%–72.4% sterols and 16.2%–17.9% glyceryl ethers.

Of the liver lipids from both shark species, the component glyceryl ethers from diacyl glyceryl ethers for spiny dogfish and from unsaponifiables for Japanese cat shark were found to consist mostly of monoenes (82.8%–85.7%, 63.8%–66.3%) and saturates (12.2%–15.1%, 27.4%–28.0%) of 14–23 carbon atoms for the alkyl moiety. These compounds of spiny dogfish were especially rich in the 18:1 component (58.5%–63.9%) as compared with those of Japanese cat shark (31.7%–36.3%). In comparison between male and female specimens for spiny dogfish, both the component fatty acids of diacyl glyceryl ethers and triglycerides were roughly similar in compositions, consisting predominantly of monoenes with 18:1, 20:1 and 22:1 acids as the major components. In contrast, those of phospholipids contained a considerable amount of polyenes. Of both shark species, the component fatty acids of triglycerides were different in the contents of monoenes and polyenes; the ratios of monoenes to polyenes had 3.0–3.3 for spiny dogfish and 0.8–0.9 for Japanese cat shark, respectively.

### Introduction

The liver lipids of some cartilaginous fish are chemically unusual in that they can contain large amounts of hydrocarbons or diacyl glyceryl ethers, both of which are rare in teleosts or mammals. However, the biological roles of high contents of these lipid classes in marine organisms are not well known. In previous studies<sup>1-4)</sup>, the compositions of the ether-linked lipids such as diacyl glyceryl ethers or neutral

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plasmalogens in liver lipids of deep-sea ratfish, *Hydrolagus novaezealandiae*, *H. barbouri*, and *Rhinochimaera pacifica*, and of deep-sea shark, *Dalatias licha*, were investigated. Subsequently, the liver lipids from male and female specimens of two species of sharks, spiny dogfish *Squalus acanthias* and Japanese cat shark *Scyliorhinus torazame*, were investigated for comparative studies on the ether-linked lipids of ratfish and sharks.

Spiny dogfish are known to possess liver lipids chiefly composed of diacyl glyceryl ethers<sup>5-8</sup>). Little, however, is known with respect to those compounds in liver lipids of Japanese cat shark. Since spiny dogfish and Japanese cat shark inhabit the mesopelagic region and near-surface, respectively, it is of interest to determine the contents and constituents of glyceryl ethers in liver lipids of both shark species, in addition to a comparison with those of male and female specimens.

The present investigation deals with the lipid compositions and the component glyceryl ethers of the male and female liver lipids of spiny dogfish and Japanese cat shark. The component fatty acids of diacyl glyceryl ethers, triglycerides and phospholipids for spiny dogfish, and of triglycerides for Japanese cat shark have also been determined.

### Materials

Pair specimens of two species of sharks, spiny dogfish *S. acanthias* of the family Squalidae and Japanese cat shark *S. torazame* of the family Scyliorhinidae, were caught by angling from a depth of 200 m and at 50 m respectively off Hakodate in Hokkaido, Japan. The sizes of the examined sharks were as follows: 68.9 cm and 69.2 cm in length, and 2.98 kg and 3.42 kg in weight for each of the male and female specimens of spiny dogfish, and 41 cm–46 cm and 36 cm–43 cm in length, and 0.28 kg–0.39 kg and 0.19 kg–0.40 kg in weight for two males and seven females of Japanese cat shark, respectively. The livers of Japanese cat shark were pooled for each male and female specimen. Male and female livers of these shark species were used for the lipid extraction.

### Experimental Methods

#### *Extraction and Fractionation of Lipids*

Total lipids were extracted from the shark livers, following the method of Bligh and Dyer<sup>9</sup>). From the spiny dogfish liver lipids, neutral lipids were separated from phospholipids by means of chromatography on a silicic acid-Celite 545 (2:1 w/w) column. The neutral lipids were eluted with chloroform; and the phospholipids with methanol.

#### *Thin-Layer Chromatography (TLC)*

The qualitative and quantitative analyses of the total lipid or neutral lipid constituents, and of the component unsaponifiables from total lipids or neutral lipids were carried out by TLC. The fractionations of diacyl glyceryl ethers, triglycerides and steryl esters in the liver neutral lipids of spiny dogfish; of triglycerides in the liver lipids of Japanese cat shark; and of sterols and glyceryl ethers in unsaponifiables of the neutral lipids of the former species and of the total

lipids of the latter ones, were accomplished on preparative plates. Thin layers of silicic acid having a thickness of 0.25 mm were used for analytical purposes; and those of 0.50 mm thickness for preparative purposes. Hexane, diethyl ether, and acetic acid (90:10:1, 70:30:1, 50:50:1 v/v) were used as the developing solvents. After development, plates were sprayed with either 50% sulfuric acid or alcoholic dichlorofluorescein as visual reagents.

#### *Derivatization*

Diacyl glyceryl ethers from the liver neutral lipids of spiny dogfish were subjected to an alkaline hydrolysis in 1 N ethanolic KOH by boiling under reflux for 1 h. The unsaponifiables (glyceryl ethers) were extracted from the saponification mixture with diethyl ether. The glyceryl ethers of Japanese cat shark liver lipids were also obtained by saponification of the total lipids, extraction of the unsaponifiables, and fractionation of the unsaponifiable constituents. The isopropylidene derivatives of glyceryl ethers were prepared by acetonation at room temperature in the presence of  $\text{HClO}_4$  according to the method reported by Malins *et al.*<sup>10)</sup> Fatty acids of diacyl glyceryl ethers, triglycerides and phospholipids obtained from the spiny dogfish liver lipids, and of triglycerides from the liver lipids of Japanese cat shark were methylated with boron trifluoride-methanol<sup>11)</sup>. Prior to gas-liquid chromatography, further purification of these derivatives was carried out by TLC.

#### *Hydrogenation of Derivatives*

Samples were dissolved in hexane containing 5% palladium carbon catalyst and treated with hydrogen at atmospheric pressure and room temperature 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. Fatty acid methyl esters and isopropylidene derivatives of glyceryl ethers were analysed on 1.5 m × 3 mm i.d. glass columns packed with 10 % DEGS on Chromosorb W AW (80/100 mesh) and with 5% Silar 5CP on Gas Chrom Q (100/120 mesh), respectively. The operating conditions were as follows: column temperatures of 185°C for fatty acid methyl esters, and of 230°C or 220°C–260°C (programmed rate, 2°C/min) for isopropylidene derivatives of glyceryl ethers, respectively. Nitrogen was used as a carrier gas. The identification of the components of the aforementioned compounds was accomplished by comparison with known available standards; and by log-plots of retention times against the number of carbon atoms in the chain, before and after hydrogenation. Further identification for the major components of isopropylidene derivatives of glyceryl ethers was carried out by GLC-mass spectrometric analyses. Quantitative analysis was made on the basis of the area percentage of each peak.

#### *Infrared Spectroscopy (IR)*

The IR spectra of diacyl glyceryl ethers and glyceryl ethers were determined with a Nippon Bunko model DS-301 spectrometer using  $\text{CCl}_4$  as a solvent.

*GLC-Mass Spectrometry (GLC-MS)*

The mass spectra of isopropylidene derivatives of glyceryl ethers were recorded with a Hitachi 60M instrument equipped with a glass column (2 m × 3 mm i.d.) of 3% Silar 10C on Gas Chrom Q (100/120 mesh). The mass spectrometer was operated at 20 eV.

**Results and Discussion***Characteristics of Lipids*

The liver lipids of the examined sharks yielded high lipid content; ranging from 67.3% to 70.5% for spiny dogfish, and from 49.8% to 58.6% for Japanese cat shark, respectively (Table 1). Of spiny dogfish liver lipids, neutral lipids were the predominant classes accounting for more than 96% of the total lipid contents and phospholipids less than 4%. Similarly those of Japanese cat shark contained a large amount of neutral lipids and a small of phospholipids.

In TLC, the liver lipids of spiny dogfish revealed relatively large spots corresponding to diacyl glyceryl ethers and triglycerides. Whereas the liver lipids of Japanese cat shark had much larger spots corresponding to triglycerides, and the spots of diacyl glyceryl ethers were not detectable in TLC. In addition, the unsaponifiables obtained from the liver lipids of the examined sharks on silicic acid plates were found to give relatively large spots of glyceryl ethers and small spots of sterols for spiny dogfish. In contrast, Japanese cat shark showed large spots

Table 1. Lipid compositions of the male and female livers of spiny dogfish and Japanese cat shark.

	Spiny dogfish		Japanese cat shark	
	Male	Female	Male	Female
Liver wt g	348	420	32* <sup>1</sup>	30* <sup>1</sup>
Total lipids %* <sup>2</sup>	67.3	70.5	49.8	58.6
Lipid components %* <sup>3</sup>				
Steryl esters	2.0	2.4		
Diacyl glyceryl ethers	44.6	38.7		
Triglycerides	45.3	47.4	91.5	81.8
Phospholipids	3.6	3.5		
Others	4.5* <sup>4</sup>	8.0* <sup>4</sup>	8.5* <sup>5</sup>	18.2* <sup>5</sup>
Unsaponifiables %* <sup>3</sup>	18.1	10.6	1.3	1.2
Unsaponifiable components %* <sup>6</sup>				
Sterols	8.2	16.2	72.4	66.9
Glyceryl ethers	86.8	67.4	16.2	17.9
Others* <sup>7</sup>	5.0	16.4	11.4	15.2

\*1 Averages of two male or seven female specimens. \*2 % to wet wt basis. \*3% to total lipids. \*4 Contain mainly hydrocarbons, fatty acids, sterols and partial glycerides. \*5 Contain mainly hydrocarbons, fatty acids, sterols, partial glycerides and phospholipids. \*6 % to unsaponifiables. \*7 Contain mainly hydrocarbons, methoxy-glyceryl ethers and polar unknown compounds.

of sterols and small spots of glyceryl ethers.

The IR spectra of diacyl glyceryl ethers or glyceryl ethers obtained from liver lipids of the examined sharks gave the following characteristic absorptions. They were:  $1740 \text{ cm}^{-1}$  (C-O) and  $1120 \text{ cm}^{-1}$  (C-O-C) for the former compounds; and  $3440 \text{ cm}^{-1}$  (-OH) and  $1120 \text{ cm}^{-1}$  (C-O-C) for the latter ones, respectively. These spectra were found to be identical with the characteristic absorptions of the respective ether-linked compounds isolated from the flesh lipids of deep-sea teleost fish, *Seriololela* sp., previously reported by the authors<sup>9)</sup>.

In GLC-MS analyses, the major components of isopropylidene derivatives of glyceryl ethers obtained from the examined shark liver lipids were recognized from the following characteristic fragment peaks: molecule ion ( $M^+$ ),  $M^+-15$  ( $\text{CH}_3$ )

and  $m/e$  101  $\left( \begin{array}{c} \text{HC-O} \\ | \\ \text{H}_2\text{C-O} \end{array} \right. \text{C} \begin{array}{c} \text{CH}_3 \\ \backslash \\ \text{CH}_3 \end{array} \right)$ , base peak).

The lipid compositions of the male and female livers of both shark species are given in Table 1. The liver lipids and their unsaponifiables in both shark species were roughly similar in composition between male and female specimens. The liver lipids of spiny dogfish obtained from the mesopelagic region were characterized by high percentages of diacyl glyceryl ethers (38.7%–44.6%) and triglycerides (45.3%–47.4%); the ratios of the former compounds to the latter ones were approximately 1.0:1.0–0.8. The contents of sterol esters ranged from 2.0% to 2.4%. Small amounts of hydrocarbons, fatty acids, sterols and partial glycerides were also detected from the liver lipids. Whereas, of the liver lipids of Japanese cat shark obtained from near-surface, triglycerides were the prominent lipid class, accounting for 81.8%–91.5%. Consequently, the liver lipids of spiny dogfish contained high levels of unsaponifiables (10.6%–18.1%), consisting of a large amount of glyceryl ethers (67.4%–86.8%) and a small amount of sterols (8.2%–16.1%). On the contrary, the unsaponifiables (1.2%–1.3%) of Japanese cat shark liver lipids were composed of 66.9%–72.4% sterols and 16.2%–17.9% glyceryl ethers.

The contents of diacyl glyceryl ethers and triglycerides in the liver lipids of spiny dogfish examined were remarkably similar to those of the same species obtained from different waters<sup>5–8)</sup>. Previous studies showed that almost all deep-sea ratfish, which represent a small but interesting group of intermediate fish between sharks and teleost fish, have an abundance of diacyl glyceryl ethers or glyceryl ethers in their livers<sup>1,4,10–17)</sup>. However, it is noteworthy that large amounts of these compounds have been found in livers of a variety of sharks; these particular sharks are limited to a few genera of the Subclass Elasmobranchii<sup>3,5,13,18–20)</sup> and are obtained from the mesopelagic and bathypelagic regions. In addition, the contents and compositions of methoxy-substituted glyceryl ethers of the minor unsaponifiable constituents of both shark species have been investigated elsewhere by the authors<sup>21)</sup>.

#### Compositions of Glyceryl Ethers

The compositions of glyceryl ethers from diacyl glyceryl ethers of spiny dogfish liver lipids, and from unsaponifiables of the liver lipids of Japanese cat shark are given in Table 2. Glyceryl ethers of each shark species were similar in

HAYASHI: Component glyceryl ethers in shark liver lipids

composition between the male and female specimens. The characteristics of these compounds of the examined shark species are as follows. Even numbers of carbon atoms for the alkyl moiety, which ranged from C<sub>14</sub> to C<sub>24</sub>, prevailed as compared with odd ones. The most predominant component for spiny dogfish and Japanese cat shark was 18:1 (58.5%–63.9%, 31.7%–36.3%) followed by 16:1 (14.8%–15.5%, 13.4%–15.2%) and 16:0 (8.0%–8.6%, 14.1%–14.4%), indicating a high monoenoic content (82.8%–85.7%, 63.8%–66.3%). The branched glyceryl ethers (5.7%–8.8%) of Japanese cat shark occurred in a large amount as compared with those (1.0%–1.1%) of spiny dogfish.

The compositions of glyceryl ethers from the liver lipids of the examined spiny dogfish were roughly similar to those of the same species obtained from different waters<sup>5,6,13,20,24</sup>. The component glyceryl ethers of this species obtained from the mesopelagic region were found to be more unsaturated than those found in Japanese cat shark from the near-surface. That is, those of spiny

Table 2. Glyceryl ether compositions of the male and female livers of spiny dogfish and Japanese cat shark.

Glyceryl ether*1	Spiny dogfish		Japanese cat shark	
	Male	Female	Male	Female
	Peak area %			
14:0	1.8	3.0	1.7	2.3
15:0	0.2	0.3	3.2	3.8
16:0	8.0	8.6	14.1	14.4
17:0	0.1	0.3	1.3	0.9
18:0	1.9	2.6	6.4	6.1
20:0	0.1	0.1	0.7	0.5
16:1	15.5	14.8	13.4	15.2
17:1	1.0	1.5	1.2	1.3
18:1	63.9	58.5	31.7	36.3
19:1	0.2	0.4	1.7	1.9
20:1	3.2	5.3	6.1	6.9
21:1	0.6	0.8	1.4	1.1
22:1	0.5	0.7	6.1	3.0
23:1	—	—	0.8	0.2
18:2	1.0	0.9	—	—
14 br*2	—	—	1.3	0.6
15 br	0.4	0.3	1.2	0.2
16 br	0.1	0.2	2.4	3.4
18 br	0.2	0.3	0.7	0.7
21 br	—	—	3.2	0.8
Σ Minor components *3	1.2	1.3	—	—
Σ Saturates	12.2	15.1	27.4	28.0
Σ Monoenes	85.7	82.8	63.8	66.3
Σ Dienes	1.1	1.0	—	—
Σ Branched	1.0	1.1	8.8	5.7

\*1 Indicated by chain length and double bonds of alkyl moiety.

\*2 Branched components were detected and identified in GLC chromatograms after hydrogenation.

\*3 Components of less than 0.5%.

dogfish were characterized by a much larger amount of the 18:1 component. A similar trend has been reported that glyceryl ethers in the liver lipids of deep-sea ratfish and sharks are rich in monoenes which consist abundantly of the 18:1 component (52.7%–73.8%).<sup>1,3,14,16,17,20,22,23)</sup>

### Compositions of Fatty Acids

Of the male and female liver lipids, the fatty acid compositions of triglycerides for Japanese cat shark, and of diacyl glyceryl ethers, triglycerides and phospholipids for spiny dogfish are given in Table 3. Between male and female specimens of Japanese cat shark, the component fatty acids of triglycerides were similar in composition. Also in comparison between male and female specimens of spiny

Table 3. Fatty acid compositions of the different lipid classes of the male and female livers of spiny dogfish and Japanese cat shark.

Fatty acid*1	Spiny dogfish						Japanese cat shark	
	Male			Female			Male	Female
	DAGE	TG	PL	DAGE	TG	PL	TG	TG
Peak area %								
14:0	2.5	2.4	3.4	2.5	3.6	7.4	5.4	5.4
15:0	0.4	0.4	0.5	0.4	0.4	0.6	0.7	0.6
16:0	27.4	17.1	17.9	19.6	16.3	20.0	20.1	19.6
17:0	1.3	1.8	1.2	0.9	0.8	0.6	1.3	1.2
18:0	2.7	3.5	5.3	1.8	2.8	4.4	2.9	3.1
16:1	5.6	6.3	7.4	5.3	7.1	11.2	7.5	7.3
17:1	0.8	0.9	0.7	0.5	0.6	0.7	0.8	0.8
18:1	21.0	30.4	20.8	16.5	23.1	13.7	15.2	14.1
20:1	9.5	9.5	5.8	12.7	12.2	3.4	4.6	4.3
22:1	12.2	7.4	3.8	18.2	11.2	2.5	2.3	2.2
24:1	2.7	2.4	0.9	2.5	1.6	0.6	1.7	1.8
18:2	1.0	1.4	1.7	0.8	1.6	1.6	1.4	1.4
18:3	0.8	0.8	1.4	1.2	1.5	1.9	0.9	0.9
18:4	0.7	1.0	2.4	0.4	1.6	5.7	1.5	1.7
20:2	0.2	0.2	0.2	0.2	0.3	0.1	0.6	0.8
20:4	1.1	1.9	5.3	1.7	2.1	4.2	3.5	3.6
20:5	2.5	3.8	8.1	2.2	3.7	10.8	8.2	8.5
21:5	1.4	0.8	0.8	0.5	0.5	0.8	2.1	2.0
22:5	1.7	1.0	1.8	4.5	1.6	2.5	3.8	3.4
22:6	3.6	6.3	8.5	5.7	5.5	5.5	13.9	15.8
Σ Minor components*2	0.9	0.7	2.1	1.9	1.9	1.8	1.6	1.5
Σ Saturates	34.3	25.2	28.6	25.4	24.0	33.5	30.7	30.2
Σ Monoenes	52.4	57.6	39.9	56.0	56.4	32.5	33.4	31.7
Σ Polyenes	13.2	17.2	30.2	17.2	18.7	33.1	35.9	38.1
Σ Branched	0.1	tr*3	1.3	1.4	0.9	0.9	tr	tr
Mono/Poly	4.0	3.3	1.3	3.3	3.0	1.0	0.9	0.8

\*1 No. of carbon atoms: no. of double bonds. \*2 Components of less than 0.5%. \*3 Trace. DAGE: diacyl glyceryl ethers, TG: triglycerides, PL: phospholipids, Mono:monoenes, Poly: polyenes.



dogfish, the component fatty acids of diacyl glyceryl ethers and triglycerides were roughly similar in composition, consisting predominantly of monoenes with 18:1, 20:1 and 22:1 acids as the major component. In contrast, those of phospholipids contained a considerable amount of polyenes. The fatty acids of diacyl glyceryl ethers and triglycerides of spiny dogfish examined were roughly similar to those of the same species obtained from different water<sup>5)</sup>. In both examined shark species, the component fatty acids of triglycerides were different in the contents of monoenes and polyenes; the ratios of monoenes to polyenes was 3.0–3.3 for spiny dogfish and 0.8–0.9 for Japanese cat shark, respectively. These results for spiny dogfish obtained from the mesopelagic region were close to the characteristics of the fatty acids of neutral lipids, which consisted mainly of triglycerides, from a number of the teleost fish obtained from the bathypelagic regions as previously reported by the authors<sup>25–27)</sup>.

Friedberg *et al.*<sup>28)</sup> have found in their study about the biosynthesis of diacyl glyceryl ethers using isolated stomach of spiny dogfish to have incorporated long-chain alcohols into diacyl glyceryl ethers. Malins<sup>29)</sup> has observed that in the liver of spiny dogfish 1-<sup>14</sup>C-palmitic acid is reductively incorporated *in vivo* into the alkyl moiety of diacyl glyceryl ethers but not extensively. These studies clearly indicate that fatty alcohols are important precursors in the biosynthesis of alkyl moiety of diacyl glyceryl ethers. Nevertheless, no component of wax esters or fatty alcohols was detected at all in liver lipids or in the unsaponifiable fraction of spiny dogfish examined in this study. But, it is noteworthy that considerable amounts of wax esters, as well as triglycerides, diacyl glyceryl ethers and steryl esters, are present in the serum of spiny dogfish<sup>7)</sup>.

On the other hand, the biological roles of high contents of diacyl glyceryl ethers in marine organisms are not well known. Malins *et al.*<sup>30)</sup> have postulated that ether-linked lipids are deposited at the expense of triglycerides to offset the increase in body weight. Also a regulatory mechanism might have been in the spiny dogfish body system involving the selective metabolism of diacyl glyceryl ethers and triglycerides for the delicate attenuation of near neutral buoyancy. From the viewpoint of physiological or ecological roles in the marine environment, it is interesting to note that deep-sea shark and ratfish species abundantly contain diacyl glyceryl ethers in their livers.

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HAYASHI: Component glyceryl ethers in shark liver lipids

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