Unusual Occurrence of Diacyl Glyceryl Ethers in Liver Lipids from Two Species of Gonatid Squids*¹

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The lipid compositions of the livers of two species of gonatid squids, *Berryteuthis magister* and *Gonatopsis makko*, caught in the Japan Sea, were investigated.

The livers yielded 22.4% lipids for *B. magister* and 28.0% for *G. makko*, each of which contained an unusually high percentage of diacyl glyceryl ethers (49.1% and 69.5%, respectively) and small amounts of steryl esters (24.0% and 16.0%), triglycerides (7.1% and 0.9%) and phospholipids (8.6% and 3.5%).

In the two species, the component glyceryl ethers were reasonably similar in composition, consisting mainly of saturates (64.6–70.1%) and monoenes (27.7–33.0%) of 14–22 carbon atoms for the alkyl moiety. The principal components were as follows: 16:0 (58.3–58.5%), 18:1 (23.2–27.7%), 18:0 (4.0–7.6%), and 20:1 (3.4–4.1%). In both species, the fatty acid compositions of diacyl glyceryl ethers consisted predominantly of monoenes with 18:1 acid (28.2–46.6%) as the main component.

For *B. magister*, few differences were found in the percentages of the component fatty acids among the lipid classes of diacyl glyceryl ethers, steryl esters, triglycerides and phospholipids; and for *G. makko* few differences were found among diacyl glyceryl ethers, steryl esters and phospholipids.

Glyceryl ethers of marine origin have proven useful as natural surface-active or softening agents in cosmetics and ointments. Recently, what is more remarkable is that diacyl glyceryl ethers obtained from the ratfish liver lipids act as intermediates in the synthesis of biologically active alkylacetyl glycerophosphocholine (a platelet activating factor).¹⁾

To develop new marine medicinal or cosmetic resources, the characteristics of diacyl glyceryl ethers abundant in the flesh lipids of teleost fish,²⁾ *Serioloba* sp. and *S. punctata*, and in the liver lipids of ratfish,^{3,4)} *Hydrolagus novaezealandiae*, *H. barbouri* and *Rhinochimaera pacifica*, and sharks,^{5,6)} *Dalatias licha* and *Squalus acanthias*, have been described previously. In the present study it was found that the liver lipids of squids belonging to the family Gonatidae, *Berryteuthis magister* and *Gonatopsis makko*, are also good source of diacyl glyceryl ethers.

The present investigation deals with the lipid compositions and the component glyceryl ethers of the livers of the above two species of gonatid squids. In connection with this, the component

glyceryl ethers of diacyl glyceryl ethers present in small amounts in the liver lipids of common squid *Todarodes pacificus* are compared with those of the gonatid species. The component fatty acids of diacyl glyceryl ethers, steryl esters, triglycerides and phospholipids for *B. magister*, *G. makko*, and *T. pacificus* were also determined.

Experimental

Materials

Three species of squids, *B. magister*, *G. makko* and *T. pacificus*, which were 427 g, 105 g and 241 g in body weight, respectively, were caught in the Japan Sea and kept in a frozen state until analysis. The livers from these squids were used for the lipid extraction.

Extraction and Fractionation of Lipids

The total lipids were extracted from the squid livers, following the method of BLIGH and DYER.⁷⁾ Neutral lipids were then separated from phospholipids by means of chromatography on a silicic acid-Celite 545 (2:1 w/w) column. The

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neutral lipids were eluted with chloroform and the phospholipids with methanol.

Thin-Layer Chromatography (TLC)

Qualitative and quantitative analyses of the neutral lipid constituents were carried out by TLC. Fractionation of steryl esters, diacyl glyceryl ethers and triglycerides of the neutral lipids was accomplished on prepared plates. Isopropylidene derivatives of glyceryl ethers were fractionated according to their degree of unsaturation on silicic acid plates impregnated with 15% AgNO₃. Thin layers of silicic acid 0.25 mm thick were used for analytical purposes; those with a thickness of 0.50 mm for preparative purposes. Mixtures of hexane, diethyl ether and acetic acid (90:10:1, 80:20:1, v/v/v) were used as developing solvents. After development, the plates were sprayed with 50% sulfuric acid or alcoholic dichlorofluorescein as visual reagents.

Derivatization

Fractionated diacyl glyceryl ethers were subjected to alkaline hydrolysis in 1 N ethanolic KOH by boiling under reflux for 1 h. The unsaponifiables (glyceryl ethers) were extracted from the saponification mixture using diethyl ether. Isopropylidene derivatives of glyceryl ethers were prepared by acetonation at room temperature in the presence of HClO₄ according to the method reported by MALINS *et al.*⁸⁾ Fatty acids of diacyl glyceryl ethers, steryl esters, triglycerides, and phospholipids, which were recovered after removal of the unsaponifiables by a routine method, were methylated with boron trifluoride-methanol.⁹⁾ Prior to gas-liquid chromatography (GLC), 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

Analyses by GLC were carried out using a Shimadzu model GC 6AM gas chromatograph, equipped with a dual hydrogen flame ionization detector. Isopropylidene derivatives of glyceryl ethers and fatty acid methyl esters were analysed on 1.5 m × 3 mm i.d. glass columns packed with

5% Silar 5CP on Gas Chrom Q (100/120 mesh) and with 10% DEGS on Chromosorb W AW (80/100 mesh), respectively. The operating conditions were as follows: Column temperatures were 230°C or 220–260°C (programmed rate, 2°C/min) for isopropylidene derivatives of glyceryl ethers, and 185°C for fatty acid methyl esters, respectively. Nitrogen was used as a carrier gas. The identification of the components of the aforementioned compound was accomplished by a comparison of their retention times with those of available standards, as well as by log-plots of retention times against the number of carbon atoms in the chain, before and after hydrogenation. Further identification of the main components of isopropylidene derivatives of glyceryl ethers was carried out by GLC-mass spectrometric analysis. Quantitative analysis was made on the basis of the percentage of the total area under each peak.

Infrared Spectroscopy (IR)

The IR spectra were determined using a Nippon Bunko model DS-301 spectrometer with CCl₄ as a solvent.

GLC-Mass Spectrometry (GLC-MS)

The mass spectra were recorded using 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 livers of the gonatid and common squids examined in this study yielded 22.4% lipids for *B. magister*, 28.0% for *G. makko*, and 37.8% for *T. pacificus*, respectively (Table 1). Of these, neutral lipids formed the predominant class, accounting for more than 91% of the total lipid contents; phospholipids accounted for less than 9%.

The qualitative separation of the lipid constituents is illustrated in Fig. 1. The spot migrating slightly ahead of triglycerides on the silicic acid plates in Fig. 1-A and 1-B was seen as the major constituents of the liver lipids of the gonatid squids. *R_f* of this spot corresponded to that of diacyl glyceryl ethers. The liver lipids of the common squid (Fig. 1-C) contained major and minor constituents, corresponding to triglycerides

Table 1. Lipid composition of the livers of gonatid and common squids

	Gonatid squids		Common squid
	<i>B. magister</i>	<i>G. makko</i>	<i>T. pacificus</i>
Liver lipids	22.4%	28.0%	37.8%
Steryl esters	24.0%	16.0%	4.7%
Diacyl glyceryl ethers	49.1	69.5	1.0
Triglycerides	7.1	0.9	77.7
Fatty acids, Sterols and Partial glycerides	11.2	10.1	9.2
Phospholipids	8.6	3.5	7.4

Fig. 1. Thin-layer chromatograms of the liver lipids of gonatid squids, *B. magister* (A) and *G. makko* (B), and of common squid *T. pacificus* (C). Adsorbent: silicic acid. Solvent: hexane, diethyl ether, acetic acid (90:10:1, v/v/v). SE; steryl esters, DAGE; diacyl glyceryl ethers, TG: triglycerides, FA; fatty acids, ST; sterols, PL; phospholipids.

and diacyl glyceryl ethers, respectively.

The IR spectra of diacyl glyceryl ethers and glyceryl ethers obtained from the liver lipids gave the following characteristic absorptions: 1740 cm^{-1} (C=O) and 1120 cm^{-1} (C-O-C) for the former compounds and 3440 cm^{-1} (-OH) and 1120 cm^{-1} (C-O-C) for the latter ones. These absorptions were identical to those of the respective ether-linked compounds isolated from the flesh lipids of the deep-sea teleost fish, *Seriellela* sp., as previously reported by the authors.²⁾

In GLC-MS analysis, the main components of isopropylidene derivatives of glyceryl ethers obtained from the liver lipids were found to give the

Table 2. Relative intensities*¹ of the principal peaks in mass spectra of the main components of isopropylidene derivatives of glyceryl ethers

Glyceryl ether* ²	M ⁺		M ⁺ -15	
	m/e	%	m/e	%
14:0	328	0.1	313	33.7
16:0	356	—	341	37.9
17:0	370	—	355	32.8
18:0	384	—	369	43.1
16:1	354	4.2	339	29.5
18:1	382	4.3	367	37.9
20:1	410	4.7	430	32.1

*¹ Expressed as percent of base peak (m/e 101).

*² Indicated by chain length and double bond of alkyl moiety. M⁺: Molecular ion.

following characteristic peaks: molecular ion (M⁺), M⁺-15 (CH₃) and m/e 101

The lipid compositions of the livers of the two species of gonatid squids are given in Table 1. For comparison, data concerning the common squid are included in the same table. The liver lipids of the gonatid squids were characterized by high levels of diacyl glyceryl ethers (*B. magister*, 49.1%; *G. makko*, 69.5%) with only minute amounts of triglycerides (7.1% and 0.9%, respectively). Small amounts of steryl esters, fatty acids, sterols, and phospholipids were also detected in the liver lipids. On the contrary, the liver lipids of the common squid had large amounts of triglycerides (77.7%) and minute amounts of diacyl glyceryl ethers (1.0%), similar to various other marine organisms. No study has, hitherto, been reported on cephalopod species having an unusual abundance of diacyl glyceryl ethers in their livers. The diacyl glyceryl ether content (49–70%) of the liver lipids of the gonatid squid examined was comparable to those of the ratfish liver lipids (54–66%)^{3,4)} and shark liver

lipids (28–45%),^{5,9)} In addition, it is of interest to note that *B. magister* and *G. makko* belong to the same family, Gonatidae. Diacyl glyceryl ethers might, therefore, prove somewhat useful in regard to chemotaxonomic character.

Glyceryl Ether Composition

The main components of glyceryl ethers from diacyl glyceryl ethers of the liver lipids of the gonatid and common squids are given in Table 3. Glyceryl ethers of the gonatid species, *B. magister* and *G. makko*, were reasonably similar in composition. Even, as opposed to odd, numbers of carbon atoms, ranging from C₁₄ to C₂₂, prevailed for the alkyl moiety. The most predominant component for both gonatid squid species was 16:0 (58.3–58.5%) followed by 18:1 (23.2–27.7%) and 18:0 (4.0–7.6%), indicating a high content of saturates (64.6–70.1%). The branched glyceryl ethers occurred only in small amounts (less than 2.2%). As for the common squid *T. pacificus*, the component glyceryl ethers from diacyl glyceryl ethers of its liver lipids were similar in composition to those of the gonatid squids, except for the percentage of 14:0. Similar findings have been reported for the squid *Loligo* sp. in which phosphorylated glyceryl ethers (7.9% of the phospholipid content) contained 16:0 component at high percentage (59%).¹⁰⁾

It should be noted that the aforementioned

Table 3. Glyceryl ether composition of the liver lipids of gonatid and common squids

Glyceryl ether ^{*1}	Gonatid squids		Common squid
	<i>B. magister</i>	<i>G. makko</i>	<i>T. pacificus</i>
	Peak area %		
14:0	0.7	1.0	13.0
15:0	0.3	0.5	2.4
16:0	58.3	58.5	50.3
17:0	1.9	0.6	1.4
18:0	7.6	4.0	7.7
19:0	1.1	tr ^{*2}	tr
14:1	0.1	tr	1.2
16:1	0.5	0.5	2.2
18:1	23.2	27.7	12.4
20:1	3.4	4.1	2.8
17:0br ^{*3}	—	1.2	—
18:0br	1.7	0.5	1.3
Saturates	70.1	64.6	75.4
Monoenes	27.7	33.0	20.9
Branched	2.2	2.1	1.7
Unknown	—	0.3	2.0

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

*2 Trace.

*3 Branched compounds.

diacyl glyceryl ethers of the liver lipids from the gonatid and common squids were extremely different in their component glyceryl ethers from those of the liver lipids of ratfish and sharks. That is, the component glyceryl ethers for ratfish

Table 4. Fatty acid composition of each lipid class of the liver lipids of gonatid and common squids

Com- ponent*1	Gonatid squids							Common squid			
	<i>B. magister</i>				<i>G. makko</i>			<i>T. pacificus</i>			
	DAGE	SE	TG	PL	DAGE	SE	PL	DAGE	SE	TG	PL
	Peak area %										
14:0	0.9	0.7	2.2	1.1	4.4	21.6	9.0	3.4	4.0	5.0	3.9
16:0	4.9	2.9	12.8	18.2	5.4	8.3	15.8	9.0	8.3	20.6	43.8
18:0	1.6	0.9	5.2	5.5	1.3	1.5	2.1	2.1	1.9	4.0	10.7
16:1	1.9	3.4	3.1	2.1	4.9	7.6	3.3	5.2	8.0	4.9	3.6
18:1	28.2	24.3	30.7	12.2	46.6	23.0	12.2	16.8	17.6	19.5	11.4
20:1	12.5	9.2	13.1	9.6	13.8	7.9	9.0	7.9	5.8	6.5	8.4
22:1	5.1	3.7	5.5	1.1	0.1	0.6	0.7	2.4	1.8	2.4	1.1
20:4	3.4	6.2	3.6	9.4	10.0	9.9	5.1	2.8	2.2	1.9	1.4
20:5	9.5	14.1	6.8	17.8	tr*2	1.9	17.2	9.5	14.1	8.2	4.5
22:6	21.1	27.6	6.8	16.8	0.4	18.2	tr	32.8	27.4	18.5	4.4
Saturates	9.2	6.1	22.3	27.2	17.0	36.6	27.7	16.5	16.6	31.4	61.7
Monoenes	49.6	42.9	54.5	25.9	67.8	43.2	26.8	34.2	35.4	35.2	26.5
Polyenes	41.2	51.0	23.2	46.9	14.9	19.8	45.1	49.3	48.0	33.4	11.8

*1 No. of carbon atoms: no. of double bonds.

*2 Trace.

DAGE: Diacyl glyceryl ethers, SE: Steryl esters, TG: Triglycerides, PL: Phospholipids.

and sharks are rich in monoenes with 18:1 component (53.7–63.9%).^{3–6)}

Fatty Acid Composition

Fatty acid compositions of diacyl glyceryl ethers of the gonatid and common squids are given in Table 4. For comparison, fatty acid compositions of steryl esters, triglycerides and phospholipids of the liver lipids for the three species of squids are included in the same table. For the gonatid squids, *B. magister* and *G. makko*, the fatty acid compositions of diacyl glyceryl ethers consisted predominantly of monoenes with 18:1 acid (28.2–46.6%) as the main component. Also, these lipid classes were roughly similar in the fatty acid compositions. However, diacyl glyceryl ethers of *G. makko* were extremely different in the component fatty acid percentages from those of *B. magister* and *T. pacificus*. While *G. makko* showed 20:5 and 22:6 acids at a level of 0.4%, the other two species contained 30.6–42.3% of these acids.

Few differences in the percentages of the component fatty acids were found among the lipid classes of diacyl glyceryl ethers, steryl esters, triglycerides and phospholipids for the squid species, *B. magister*, *G. makko* and *T. pacificus*.

In conclusion, the liver lipids of the gonatid squids, *B. magister* and *G. makko*, contain glyceryl ethers rich in the 16:0 component at an unusually

high concentration. Taking into consideration at the potential resources of *B. magister*,^{11,12)} it is recommended that the liver lipids of this gonatid squid be made available for use as a new source for the isolation of glyceryl ethers.

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