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Content and Composition of Alkyl Glyceryl Ethers in Liver of Gonatid Squid *Berryteuthis magister* from the Northwestern Pacific

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The content and composition of alkyl glyceryl ethers of liver lipids of the gonatid squid *Berryteuthis magister* in the northwestern Pacific were investigated.

Weight of the livers of thirty specimens of this species accounted for 9.6±3.7% of total body weight; 35.2±15.6% of the liver was lipids. The liver lipids contained significant amounts of unsaponifiables (23.8±4.4%), consisting of 76.5±16.2% alkyl glyceryl ethers and 20.2±15.4% sterols. Alkyl glyceryl ethers originated from the constituent diacyl glyceryl ethers (46.5±17.8%) of the liver lipids. The relationship between the amounts (Y, g) of alkyl glyceryl ethers of liver and liver weight (X, g) was expressed by the following equation: $Y=0.002+0.83X$ ($r=0.966$, $P<0.05$).

The component alkyl glyceryl ethers were found to consist mostly of saturates (77.8±3.6%) and monoenes (20.0±3.2%) of 14–20 carbon numbers for the alkyl moiety. The principal components found were: chimyl (16:0, 71.1±4.0%), selachyl (18:1, 17.0±2.5%), and batyl (18:0, 3.5±1.5%) alcohols. It was concluded that the liver of the gonatid squid species was a good source of alkyl glyceryl ethers having chimyl alcohol as its predominant component. In addition, the component fatty acids of the liver lipids were discussed.

Continuing with our research on the ether-linked lipids of marine organisms, we have previously demonstrated that two species of gonatid squids *Berryteuthis magister* and *Gonatopsis makko* in the Sea of Japan contained considerable amounts of alkyl glyceryl ethers in the form of diacyl glyceryl ethers in the livers.[1,2]

Alkyl glyceryl ethers of marine origin have proven useful as natural surface-active or softening agents in cosmetics and ointments. Recent information has shown that diacyl glyceryl ethers isolated from ratfish liver act as intermediates in the synthesis of biologically active alkylacetoyl-glycerophosphocholine (a platelet activating factor).[3]

In the present study we have examined in more detail diacyl glyceryl ether content and content and composition of alkyl glyceryl ethers of the liver lipids from a number of gonatid squids *B. magister* differing in growth size in the northwestern Pacific. The component fatty acids of the liver lipids were also determined.

Experimental

Materials

Thirty specimens of gonatid squid *B. magister*, ranging from 25.0–1170.0 g (mean±standard deviation: 308.8±252.8 g) in body weight, were caught by the trawler from a depth of 500 m at lat. 42°04′N. and long. 143°46′E. of the northwestern Pacific in April 1984 and kept in a frozen state until analysis. The liver of this species was used for lipid extraction.

Lipid Extraction and Analysis

The total lipids were extracted from the tissues by the method of Bligh and Dyer.[4] The lipids were subjected to hydrolysis in ethanolic potassium hydroxide solution by boiling under reflux for 1 h. The unsaponifiables were extracted from the saponification mixture using diethyl ether. Alkyl glyceryl ethers were then fractionated from the unsaponifiables by thin layer chromatography. Fatty acids were recovered from the above saponification mixture by a routine method. The quantitative determination of each of the constituents of total lipids and unsaponifiables was performed by a 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 spotting chloroform solution containing total

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lipids or unsaponifiables were developed with hexane, diethyl ether, and formic acid (92: 8: 0.5 *v/v/v* for the former compounds; 40: 60: 0.5 *v/v/v* for the latter ones). Operating conditions were as follows: flow rates of hydrogen and air were 160 m*l*/min and 2000 m*l*/min respectively; scan speed was 30 s/scan.

Thin Layer Chromatography (TLC)

Qualitative analysis of the liver lipid constituents and the unsaponifiable ones was carried out by TLC. Fractionation of alkyl glyceryl ethers of the unsaponifiables was accomplished on prepared plates. Thin layers of silicic acid 0.25 mm thick (20×20 cm) were used for analytical and preparative purposes. Hexane, diethyl ether, and acetic acid (90: 10: 1 *v/v/v* for the liver lipids; 40: 60: 1 *v/v/v* for the unsaponifiables) 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 the Rf values with those of corresponding standards.

Derivatization

Isopropylidene derivatives of alkyl glyceryl ethers were prepared by acetonation at room temperature in the presence of perchloric acid according to the method reported by Malins *et al.*[5] Fatty acid were methylated with boron trifluoride-methanol.[6] 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 catalyst and treated with hydrogen at atmospheric pressure and room temperature 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. Isopropylidene derivatives of alkyl glyceryl ethers and fatty acid methyl esters were analysed on 1.5 m×3 mm i.d. glass columns packed with Unisole 3000 on Uniport C (80/100 mesh) and with 10% DEGS on Chromosorb W AW (80/100 mesh) respectively. Operating conditions were as follows: column temperatures were 230°C for isopropylidene derivatives of alkyl glyceryl ethers, and 185°C for fatty acid methyl esters. Nitrogen was used as a carrier gas. Confirmation of the identity of the components of isopropylidene derivatives of alkyl glyceryl ethers and fatty acid methyl esters was established by comparison with available known standards, as well as by semilogarithmic plots of the retention times against the carbon numbers, before and after hydrogenation. Quantitative determination was made on the basis of the percentages of the total area under each peak using a Shimadzu model R-3A chromatopack.

Results and Discussion

Content of Liver Lipids

Of thirty specimens of the examined gonatid squid *B. magister*, liver weight ranged from 2.0–194.6 g (36.9±44.6 g); the relative weight of the liver accounted for 5.1–17.6% (9.6±3.7%) of the total body weight. The relative weight of the liver to total body weight was identical to that (9.0%) of the same species from the Sea of Japan.[2] The relationship between liver weight (Y, g) and total body weight (X, g) was expressed by the following equation: $Y=-16.411+0.173X$, ($r=0.977$, $P<0.05$).

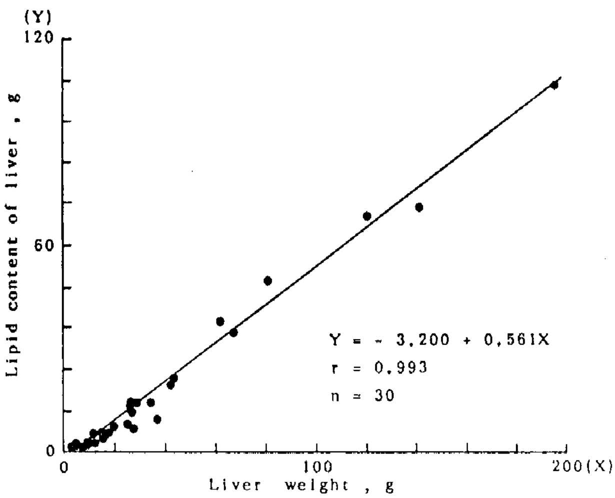

Fig. 1. Relationship between the amount (Y, g) of liver lipids and liver weight (X, g) of the examined gonatid squid.

The livers yielded 0.5–106.0 g (17.6±25.2 g) lipids; the relative weight of the liver lipids accounted for 8.7–60.5% (35.2±15.6%) of the liver weight. As seen in Fig. 1, the relationship between the amount (Y, g) of liver lipids and liver weight (X, g) could be derived as follows: $Y=-3.200+0.561X$, ($r=0.993$, $P<0.05$).

Components of Liver Lipids and Unsaponifiables

In TLC, the liver lipids of *B. magister* revealed relatively large spots each of which corresponded to diacyl glyceryl ethers, triglycerides, fatty acids and phospholipids. The composition of the liver lipids is presented in Table 1. The liver lipids were characterized by a high level of 46.5±17.8% diacyl glyceryl ethers, followed by 16.5±12.3% triglycerides. The diacyl glyceryl ether content found in the liver lipids of the examined squid, being relatively higher than that (27.5%) in the same species from the Sea of Japan,[2] was similar to those of the ratfish liver lipids (54–66%)[7,8] and shark liver lipids (28–45%).[9,10]

Table 1. Lipid composition of liver of the examined gonatid squid

Component	Mean±SD %*
Steryl esters	3.9± 2.9
Diacyl glyceryl ethers	46.5±17.8
Triglycerides	16.5±12.3
Fatty acids	13.2± 7.0
Diglycerides	3.5± 3.1
Sterols	3.0± 1.2
Alkyl glyceryl ethers Monoglycerides	3.4± 0.5
Phospholipids	10.0± 8.7

* N=30.

Small amounts (3.9%) of steryl esters found in the liver lipids of the examined squid were extremely different from those (12.0%) of the same species from the Sea of Japan,[2] being considered to be related to their dietary lipids.

The content of diacyl glyceryl ethers of the livers examined in this study ranged from 0.01–39.3 g (8.0±9.6 g). The relationship between the amount (Y, g) of diacyl glyceryl ethers of liver and liver weight (X, g) was expressed by the following equation: $Y=0.417+0.206X$, ($r=0.939$, $P<0.05$).

Appreciable quantities (13.3±7.0%) of fatty acids in the free state were found in the liver lipids. Nonesterified fatty acids do not occur in nature in high concentration in normal tissues. Therefore, the high levels of the above compounds suggested that they may be produced by hydrolysis of triglycerides of the liver during frozen storage and thawing.

Table 2. Unsaponifiable composition of liver lipids of the examined gonatid squid

Component	Mean±SD %*
Hydrocarbons	1.3± 0.4
Sterols	20.2±15.4
Alkyl glyceryl ethers	76.5±16.2
Methoxy glyceryl ethers	0.7± 0.5
Polar compounds	1.3± 0.2

* N=30.

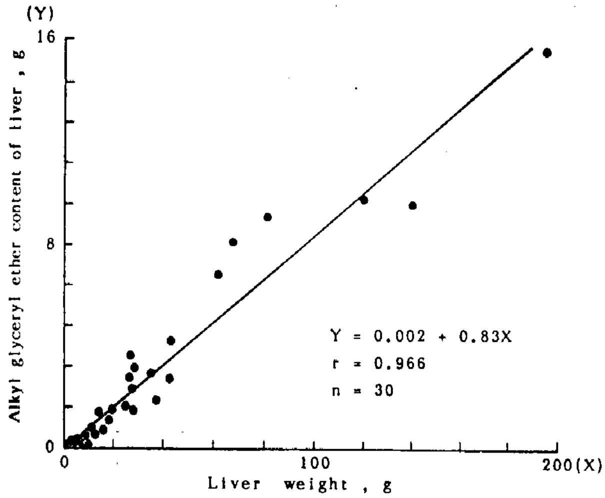

Fig. 2. Relationship between the amount (Y, g) of alkyl glyceryl ethers of liver and liver weight (X, g) of the examined gonatid squid.

The liver lipids were also characterized by a high level of unsaponifiables ranging from 15.9–32.9% (23.8±4.4%). The content of the unsaponifiables of the livers accounted for 0.1–18.6 g (3.6±4.4 g). The constituents of the unsaponifiables were tabulated in Table 2. The unsaponifiables contained significant amounts of 76.5±16.2% alkyl glyceryl ethers and 20.2±15.4% sterols. The amounts of alkyl glyceryle thers found in the examined species was relatively high when compared with those (55.2%) of the same species from the Sea of Japan.[2] The content of alkyl glyceryl ethers of the livers ranged from 0.02–15.6 g (3.1±3.8 g). The relationship between the amount (Y, g) of alkyl glyceryl ethers of liver and liver weight (X, g) could be derived as follows: $Y=0.002+0.83X$, ($r=0.966$, $P<0.05$) (Fig. 2).

In TLC, the small spot migrating slower than the spots of alkyl glyceryl ethers on the silicic acid plate corresponded to that of methoxy glyceryl ethers isolated from the ratfish liver lipids.[11]

Compositions of Alkyl Glyceryl Ethers and Fatty Acids

The component alkyl glyceryl ethers of the liver lipids are given in Table 3. Even numbers of carbon atoms, ranging from C_{14} to C_{20}, prevailed for the alkyl moiety. The most predominant component found was chimyl alcohol (16:0, 71.1±4.0%), followed the selachyl (18:1, 17.0±2.5%) and batyl (18:0, 3.5±1.5%) alcohols, in-

Table 3. Alkyl glyceryl ether composition of liver lipids of the examined gonatid squid

Component*1	Mean±SD %*2
14:0	2.0±1.0
15:0	0.3±0.1
16:0	71.1±4.0
17:0	0.5±0.3
18:0	3.5±1.5
19:0	0.4±0.2
14:1	0.1±0.1
16:1	0.5±0.4
18:1	17.0±2.5
19:1	0.3±0.1
20:1	2.0±0.9
22:1	0.2±0.2
15 Br*3	0.1±0.1
16 Br	0.2±0.1
17 Br	0.9±0.8
18 Br	0.9±0.5
Saturates	77.8±3.6
Monoenes	20.1±3.2
Branched	2.1±1.2

*1 Indicated by chain length and double bond of alkyl moiety.
*2 N=30.
*3 Branched compounds.

Table 4. Fatty acid composition of liver lipids of the examined gonatid squid

Component*1	Mean±SD %*2
14:0	2.5±0.8
16:0	11.1±2.6
18:0	1.8±0.4
16:1	4.2±1.4
18:1	28.5±3.4
20:1	9.8±2.9
22:1	6.1±1.6
20:4ω6	0.9±0.2
20:5ω3	12.7±1.8
22:6ω3	8.8±1.5
Saturates	18.7±3.2
Monoenes	51.7±5.0
Polyenes	29.5±3.2

*1 No. of carbon atoms: no. of double bonds.
*2 N=30.

dicating a high content of saturated (77.8±3.6%). The branched alkyl glyceryl ethers also occurred but only in small amounts (2.1±1.2%).

The component alkyl glyceryl ethers were in good agreement in composition to those of the gonatid squid reported previously.[1,2] It should be noted that the aforementioned alkyl glyceryl ethers, being characteristically rich in chimyl alcohol, of the liver lipids of *B. magister* were extremely different from those of the liver lipids of ratfish and sharks, *i.e.*, the component alkyl glyceryl ethers for ratfish and sharks are rich in monoenes with selachyl alcohol (54–64%).[7-10]

The content of chimyl alcohol of the livers of the examined squids ranged from 0.01–9.68 g (2.1±2.5 g). The relationship between the amount (Y, g) of chimyl alcohol of liver and liver weight (X, g) was estimated by the following equation: $Y=0.100+0.055X$, ($r=0.962$, $P<0.05$).

The major fatty acid composition of the liver lipids of the gonatid squid studied is given in Table 4. As for the liver of *B. magister*, 18:1, 20:5ω3, 16:0, 20:1 and 22:6ω3 acids were characteristic, their contents being 28.5±3.4%, 12.7±1.8%, 11.1±2.6%, 9.8±2.9%, and 8.8±1.5% of the total fatty acids respectively.

In conclusion, the liver lipids of the gonatid squid *B. magister* contained alkyl glyceryl ethers rich in chimyl alcohol in unusually high concentrations. Taking into consideration the resource potential of *B. magister*,[12] it is recommended that the liver lipids of this gonatid squid species are a good source of alkyl glyceryl ethers.

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