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Liver Lipids of Gonatid Squid *Berryteuthis magister*: a Rich Source of Alkyl Glyceryl Ethers

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

Weight of the livers of thirty-four specimens of this species accounted for 9.0±1.8% of total body weight; 23.8±9.1% of the liver was lipids. The liver lipids contained high quantities of unsaponifiables (26.7±4.0%), consisting of 55.2±11.4% alkyl glyceryl ethers and 41.9±11.7% sterols. Alkyl glyceryl ethers and sterols originated from diacyl glyceryl ethers (27.5±9.0%) and steryl esters (12.0±5.6%), respectively. The relationship between the amount (Y, g) of alkyl glyceryl ethers of liver and liver weight (X, g) was expressed by the following equation: $Y = -0.556 + 0.077X$, ($r = 0.961$, $P < 0.05$).

The component alkyl glyceryl ethers were found to consist mostly of saturates (69.8±4.9%) and monoenes (27.4±4.7%) of 14–20 carbon numbers for the alkyl moiety. The principal components found were: chimyl (16:0, 58.7±6.4%), selachyl (18:1, 24.6±4.6%), and batyl (18:0, 6.6±1.9%) alcohols. This indicated that the liver of the gonatid squid species was a good source of alkyl glyceryl ethers having chimyl alcohol as its predominant component.

Regarding the development of new potential marine, medicinal or cosmetic resources, the characteristics of diacyl glyceryl ethers have been reported previously. These compounds were found in abundance in the flesh lipids of teleost fish[1] *Seriollela* sp. and *S. punctata* and in the liver lipids of ratfish[2,3] *Hydrolagus novaezealandiae, H. barbouri* and *Rhinochimaera pacifica* and of sharks[4,5] *Dalatias licha* and *Squalus acanthias*. In previous studies designed to find a new source of alkyl glyceryl ethers, we have demonstrated two other species of gonatid squids *Berryteuthis magister* and *Gonatopsis makko*, both of which contained considerable amounts of the compounds in the form of diacyl glyceryl ethers in the liver lipids.[6]

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 glycceryl ethers isolated from ratfish liver lipids act as intermediates in the synthesis of biologically active alkylacetoylglycerophosphocholine (a platelet activating factor).[7]

In this paper, we described in detail diacyl glyceryl ether content and content and composition of alkyl glyceryl ethers of the liver lipids from a number of gonatid squid *B. magister*. In addition, the component fatty acids of the liver lipids were discussed.

Experimental

Materials

Thirty-four specimens of gonatid squid *B. magister*, ranging from 91.1–503.0 g (mean ± standard deviation: 169.3±79.3 g) in body weight, were caught in the Sea of Japan 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.[8] The lipids were subjected to hydrolysis in 1 N ethanolic KOH 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 deter-

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mination 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 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 sec/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 dichlorofluorescein as visual reagents.

Derivatization

Isopropylidene derivatives of alkyl glyceryl ethers were prepared by acetonation at room temperature in the presence of $HClO_4$ according to the method reported by MALINS *et al.*[9] Fatty acids were methylated with boron trifluoride-methanol.[10] 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 6AM gas chromatograph equipped with a dual hydrogen flame ionization detector. Isopropylidene derivatives of alkyl glyceryl ethers and fatty acid methyl esters were analyzed 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.

Results and Discussion

Content of Liver Lipids

Of thirty-four specimens of the examined gonatid squid *B. magister*, liver weight ranged from 7.9–58.8 g (15.6±9.9 g); the relative weight of the liver accounted for 5.3–13.1% (9.0±1.8%) of the total body weight. The relationship between liver weight (Y, g) and total body weight (X, g) was expressed by the following equation: $Y=-4.585+0.119X$, ($r=0.957$, $P<0.05$).

The livers yielded 0.9–26.0 g (4.1±4.5 g) lipids; the relative weight of the liver lipids accounted for 9.9–44.2% (23.8±9.1%) 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 drived as follows: $Y=-2.697+0.441X$, ($r=0.961$, $P<0.05$).

Components of Liver Lipids and Unsaponifiables

In TLC, the liver lipids of *B. magister* revealed

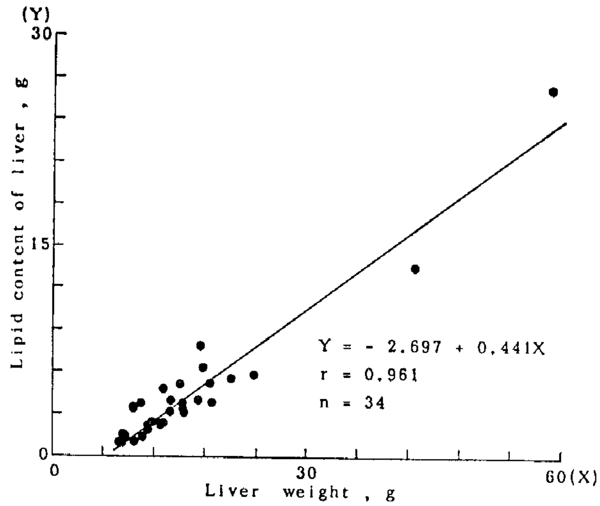

Fig. 1. Relationship between the amount (Y, g) of liver lipids and liver weight (X, g) of gonatid squid.

Table 1. Lipid composition of the gonatid squid liver

Component	Range %	Mean ±SD %*
Steryl esters	3.3–32.1	12.0±5.6
Diacyl glyceryl ethers	5.2–48.5	27.5±9.2
Triglycerides	0.3–19.8	6.0±4.8
Fatty acids	15.7–45.2	24.3±6.3
Diglycerides	4.4–19.2	9.0±3.2
Sterols	2.7– 9.4	5.2±1.4
Alkyl glyceryl ethers } Monoglycerides }	0.2– 4.3	2.5±0.8
Phospholipids	5.6–27.3	13.6±5.2

* N=34.

Table 2. Unsaponifiable composition of liver lipids of gonatid squid

Component	Range %	Mean ±SD %*
Hydrocarbons	0.2– 3.1	0.8± 0.6
Sterols	25.3–79.9	41.9±11.7
Alkyl glyceryl ethers	17.5–70.3	55.2±11.4
Methoxy glyceryl ethers	0.4– 1.5	0.7± 0.3
Polar compounds	0.5– 3.1	1.4± 0.8

* N=34.

relatively large spots each of which corresponded to diacyl glyceryl ethers, steryl esters and triglycerides. This result was very similar to that demonstrated previously for liver lipids of the same species.[6] The composition of the liver lipids is presented in Table 1. The liver lipids were characterized by a high level of 27.5±9.2% diacyl glyceryl ethers followed by 12.0±5.6% steryl esters and 6.0±4.8% triglycerides. The diacyl glyceryl ether content was somewhat lower compared with those of the ratfish liver lipids (54–66%)[2,3] and shark liver lipids (28–45%).[4,5] The content of diacyl glyceryl ethers of the livers examined in this study ranged from 0.1–8.0 g (1.2±1.5 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: $Y=-1.049+0.146X$, ($r=0.955$, $P<0.05$).

Appreciable quantities of fatty acids and diglycerides 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 result from lipolysis of triglycerides of the liver during frozen storage and thawing.

The liver lipids were also characterized by a high level of unsaponifiables ranging from 14.8–35.4% (26.7±4.0%). The content of the unsaponifiables of the livers accounted for 0.2–7.1 g (1.1±1.2 g). The constituents of the unsaponifiables were tabulated in Table 2. The unsaponifiables contained significant amounts of 55.2±11.4% alkyl glyceryl ethers and 41.9±11.7% sterols. The content of alkyl glyceryl ethers of the livers ranged from 0.1–4.5 g (0.6±0.8 g). The relationship between the amount (Y, g) of alkyl glyceryl ethers of liver and liver weight (X, g) could be derived as follows: $Y=-0.556+0.077X$, ($r=0.961$, $P<0.05$) (Fig. 2).

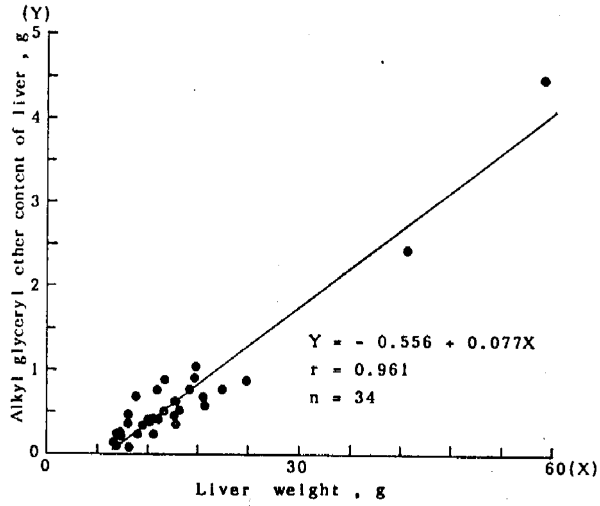

Fig. 2. Relationship between the amount (Y, g) of alkyl glyceryl ethers of liver and liver weight (X, g) of gonatid squid.

In TLC, the small spot migrating slower than the spot 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, 58.7±6.4%) followed by selachyl (18:1, 24.6±4.6%) and batyl (18:0, 6.6±1.9%) alcohols, indicating a high content of saturated (69.8±4.9%). The branched alkyl glyceryl ethers also occurred but only in small amounts (2.8±0.7%). The content of chimyl alcohol of the livers ranged from 0.1–2.2 g (0.4±0.4 g). The relationship between the amount (Y, g) of chimyl alcohol of liver and liver weight (X, g) was estimated by the following equation: $Y=-0.248+0.040X$, (r=

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

Component*1	Range %	Mean±SD %*2
14:0	0.6– 1.9	1.1±0.4
15:0	0.5– 0.9	0.7±0.1
16:0	49.1–69.9	58.7±6.4
17:0	1.1– 2.6	1.7±0.4
18:0	3.6–11.4	6.6±1.9
19:0	0.3– 1.8	0.9±0.3
16:1	0.1– 1.4	0.5±0.3
18:1	16.2–35.4	24.6±4.6
19:1	0.1– 2.4	0.8±0.6
20:1	0.8– 2.5	1.5±0.3
16:0 Br*3	0.1– 0.4	0.2±0.1
17:0 Br	1.2– 3.1	1.6±0.4
18:0 Br	0.5– 2.4	1.0±0.4
Saturates	52.9–77.0	69.8±4.9
Monoenes	19.4–38.6	27.4±4.7
Branched	1.9– 5.6	2.8±0.7

*1 Indicated by chain length and double bond of alkyl moiety.
*2 N=34.
*3 Branched compounds.

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

Component*1	Range %	Mean±SD %*2
14:0	1.3– 5.5	3.1±1.0
16:0	5.6–15.3	8.1±2.2
18:0	1.3– 3.3	1.9±0.4
16:1	3.3–11.1	5.9±1.4
18:1	21.9–33.9	27.2±3.5
20:1	3.4– 8.7	5.7±1.1
22:1	0.8– 7.8	3.8±1.7
20:4ω6	1.0– 4.9	2.7±1.0
20:5ω3	8.4–22.2	14.1±3.6
22:6ω3	9.2–20.8	14.6±3.2
Saturates	10.7–24.1	15.1±3.2
Monoenes	38.8–54.7	46.2±3.2
Polyenes	28.8–47.3	38.6±3.8

*1 No. of carbon atoms: no. of double bonds.
*2 N=34.

0.957, $P<0.05$).

The component alkyl glyceryl ethers were in good agreement in composition to those of the gonatid squid.[6] It should be noted that the aforementioned alkyl glyceryl ethers of the liver lipids of *B. magister* examined in this study 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 (18:1, 53.7–63.9%).[2–5]

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 and 22:6ω3 acids were characteristic, their content being 27.2±3.5%, 14.1±3.6% and 14.6±3.2% 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 (16:0 component) in unusually high concentrations. Taking into consideration the resource potential of *B. magister*,[12,13] it is recommended that the liver lipids of this gonatid squid species are a good source of alkyl glyceryl ethers.

Acknowledgments

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