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The Lipids of Marine Animals from Various Habitat Depths—VIII

Occurrence of Methoxy Glyceryl Ethers in the Flesh Lipids of Deep-Sea Teleost Fish *Seriollela* sp.

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Methoxy glyceryl ethers isolated from the flesh neutral lipids of the deep-sea teleost fish *Seriollela* sp. were identified by infrared spectroscopy, nuclear magnetic resonance, and gas liquid chromatography-mass spectrometry. These compounds (0.9% in neutral lipids) were composed of ethers with saturated 16 carbon atoms, 1-*O*-(2-methoxyhexadecyl)-glycerol (76.5%), and the homologues saturated 18 carbon atoms (10.4%) in the long alkyl chains, excluding the methoxy group.

In a previous study[1] the composition of the ordinary glyceryl ethers in the flesh neutral lipids of the deep-sea teleost fish *Seriollela* sp. of the family Nomeidae was determined by thin layer chromatography (TLC), infrared spectroscopy (IR) and gas liquid chromatography (GLC). Besides, the unknown compounds more polar than the above glyceryl ethers were found in unsaponifiables of the neutral lipids. By the analyses with TLC, IR, nuclear magnetic resonance (NMR), GLC and GLC-mass spectrometry (GLC-MS), these compounds were determined to be methoxy glyceryl ethers. The present investigation deals with the separation and identification of the methoxy glyceryl ethers.

Methoxy glyceryl ethers were first isolated from Greenland shark liver oil by HALLGREN *et al.*[2] In the lipids of different marine animals, these compounds were distributed in little amount[3] with reference to Greenland shark[2]. Minute amounts of the compounds were also found in the lipids of mammals including man[4]. The methoxy glyceryl ethers had antibiotic activity and inhibited the dissemination and growth of several experimental tumours in mice[5]. We found a new natural source of these compounds in relatively high concentration.

Experimental

Preparation of Unsaponifiables

Neutral lipids obtained from the flesh of *Seriollela* sp. described previously[1] were used in this study. The neutral lipids were subjected to an alkaline hydrolysis in 1 N ethanolic KOH by boiling under reflux for 1 h. The unsaponifiables were extracted from the saponification mixture by diethyl ether.

TLC

Preparative TLC of unsaponifiables was accomplished on 0.50 mm thick plates of silicic acid using a solvent system of hexane: diethyl ether: acetic acid (30: 70: 1). The separated bands were located under long wave UV light after spraying with alcoholic dichlorofluorescein solution. Analytical TLC was conducted on 0.25 mm layers of the same adsorbents and with the same solvent system as above. Spots were visualized by charring with a 50% sulfuric acid spray.

Preparation of Derivatives

Acetates were prepared by the method described in the previous study[1]. The isopropylidene derivatives were prepared by acetonation at room temperature in the presence of $HClO_4$ according to the method reported by MALINS *et al.*[6] Prior to GLC, further purification of these derivatives was carried out by TLC.

Hydrogenation

Each of acetates or isopropylidene derivatives was hydrogenated in a hexane solution with 5% palladium carbon as catalyst. The hydrogenation was performed at atmospheric pressure at 40°C. The samples were shaken for 1 h to achieve complete hydrogenation.

GLC

GLC analyses were carried out with a Yanagimoto G8 instrument equipped with a hydrogen flame ionization detector. Acetates and iso-

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propylidene derivatives, before and after hydrogenation, were analysed on a 1.5 m×3 mm i.d. glass column packed with 5% Silar 10C on Gas Chrom Q. Temperatures of column were 215°C or 190–250°C (programming rate: 2°C/min) for acetates and 210°C for isopropylidene derivatives, respectively. Nitrogen was used as carrier gas. Quantitative analysis was made on the basis of the area percentage of each peak.

IR

IR spectra were determined with a Nippon Bunko model DS-301 spectrometer using CCl_4 as solvent.

NMR

NMR spectra were obtained with a JOEL JNM-PMX model 60 spectrometer on $CDCl_3$ solutions containing tetramethylsilane as an internal standard.

GLC–MS

Mass spectra were recorded with an LKB 9000 instrument equipped with a column of 3% Silar 5CP on Gas Chrom Q.

Results and Discussion

Following TLC of unsaponifiables 5.1% of the neutral lipids, a distinct spot with R_F-value 0.20 which was somewhat lower than that of ordinary glyceryl ethers 0.36 was obtained (Fig. 1), indicating that the former compounds (reffered as compound [I] here after) contained some polar group. This compound [I] amounted to 17.1% of the unsaponifiables, while the ordinary glyceryl ethers amounted to 56.6%, respectively fractionated by the preparative TLC.

The IR spectra of the compound [I] and batyl alcohol are shown in Fig. 2 for comparison. The spectrum of the former compounds gave the following characteristic absorptions; 3400 cm^{-1} (–OH), 2810 cm^{-1} (–OCH_3), 1350 cm^{-1} (–OCH_3) and 1120 cm^{-1} (C–O–C). The absorptions of the compound [I] at 2810 cm^{-1} (slight bend), 1350 cm^{-1} (medium) and 1120 cm^{-1} (very strong) were not found in the spectrum of batyl alcohol and were most probably due to the presence of methoxy group. Besides, the IR spectrum of the compound [I] was identical with the characteristic absorption of methoxy glyceryl ethers isolated from shark liver oil as reported by HALLGREN *et al.*[2]

The NMR spectra of the compound [I] (free) showed a peak about 3.4 ppm which was absent in

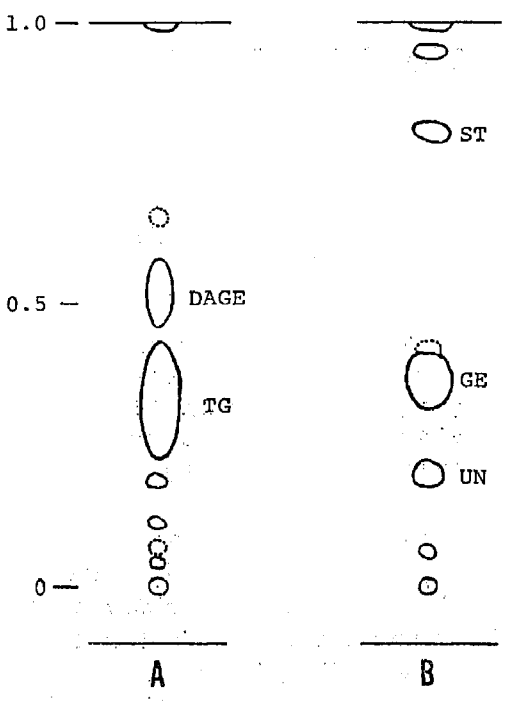

Fig. 1. Thin layer chromatograms of neutral lipids and unsaponifiables. A: neutral lipids. Solvent; hexane-diethylether-aceticacid (90 : 10 : 1). (DAGE); diacyl glyceryl ether, (TG); triglyceride. B: unsaponifiables. Solvent; hexane-diethyl ether-acetic acid (30: 70: 1). (ST); sterol, (GE); glyceryl ether, (UN); the compound [I].

the spectrum of batyl alcohol (Fig. 3). This peak could be due to the presence of a methoxy group[7]. Besides, the NMR spectrum of the compound [I] was very similar to that of the synthesized methoxy glyceryl ethers as reported by HALLGREN *et al.*[2]

The acetates or isopropylidene derivatives of the compound [I], before and after hydrogenation, were subjected to GLC (Fig. 4 and Fig. 5). The retention times of the predominant component of both derivatives were not shifted by hydrogenation, indicating that this component could be saturated. This main component was subjected to mass spectrometry. The mass spectrum of the isopropylidene derivative is shown in Fig. 6. The molecule ion of the component had *m/e* 386. A base peak at *m/e* 241 and other fragment peaks at *m/e* 101, 131 and 255, corresponding to a cleavage of the molecule were obtained. This fragmentation pattern of isopropylidene derivative before hydrogenation was identical with that of the hydrogenated one. The patterns could be explained by a structure with a methoxy group attached to the β-carbon atom of the long alkyl chain. Besides, the fragmentation pattern of isopropylidene derivative of the main component was much simi-

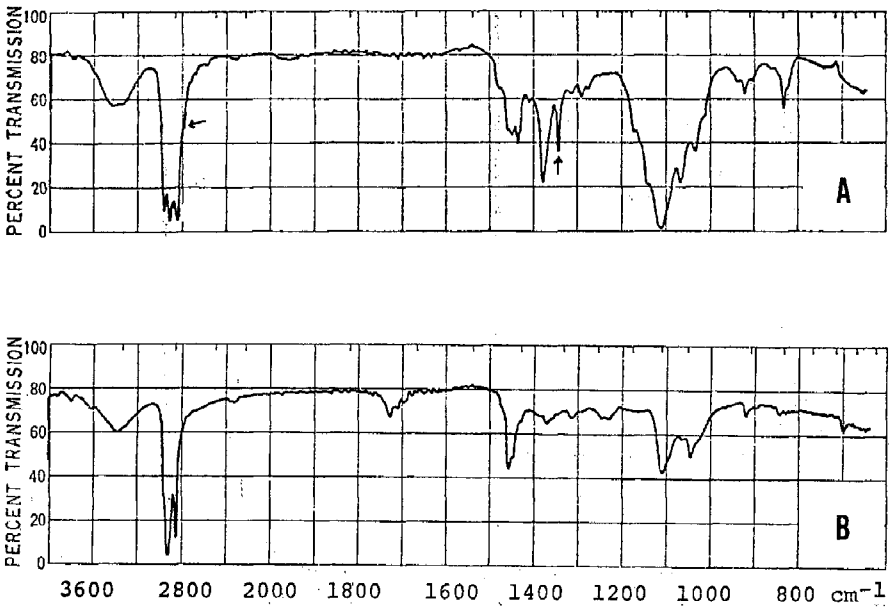

Fig. 2. IR spectra of the compound [I] and batyl alcohol. A: the compound [I], B: batyl alcohol.

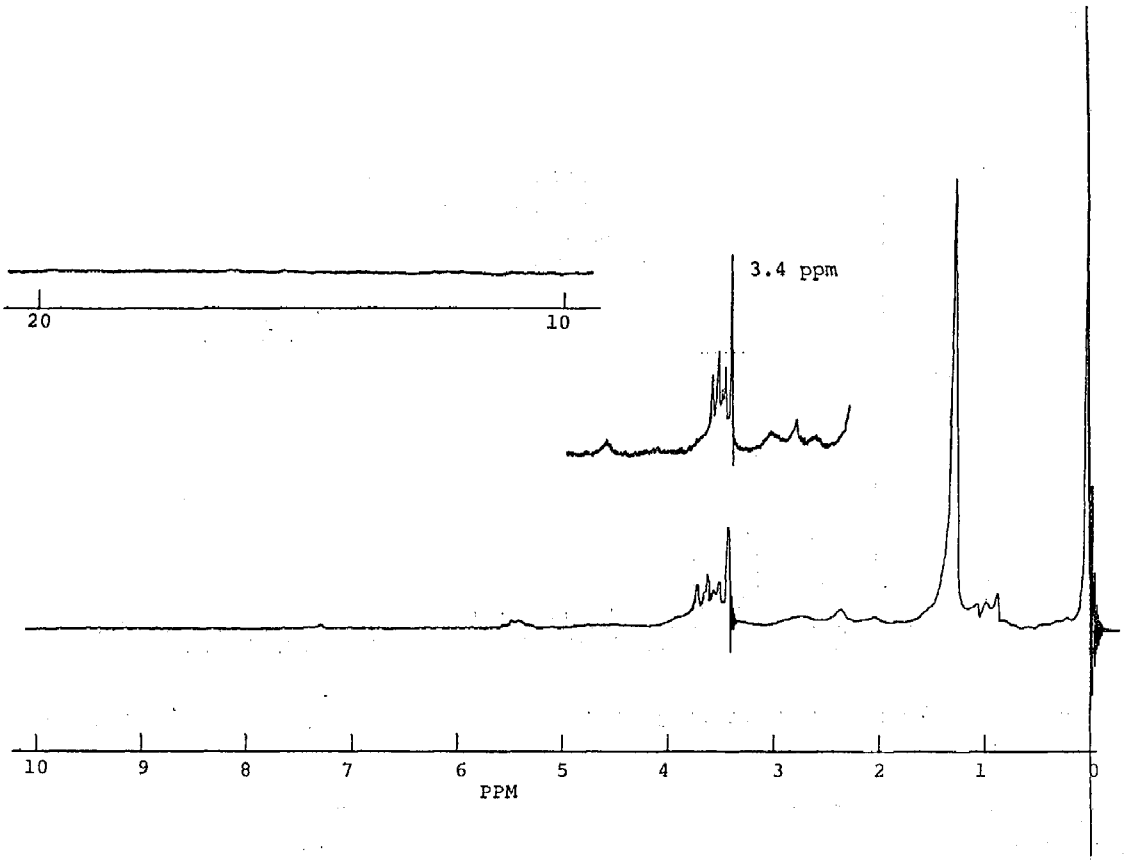

Fig. 3. NMR spectrum of the compound [I].

lar to that of the synthesized 2,3-*O*-isopropylidene-1-*O*-(2-methoxyhexadecyl)-glycerol as reported by HALLGREN *et al.*[2]

The data from GLC–MS, NMR and IR showed that the structure of main component of the methoxy glyceryl ethers was 1-*O*-(2-methoxyhexadecyl)-glycerol. From the results shown in Fig. 4 and Fig. 5, the homologues compounds in both acetates and isopropylidene derivatives were determined by comparing with each peak area and

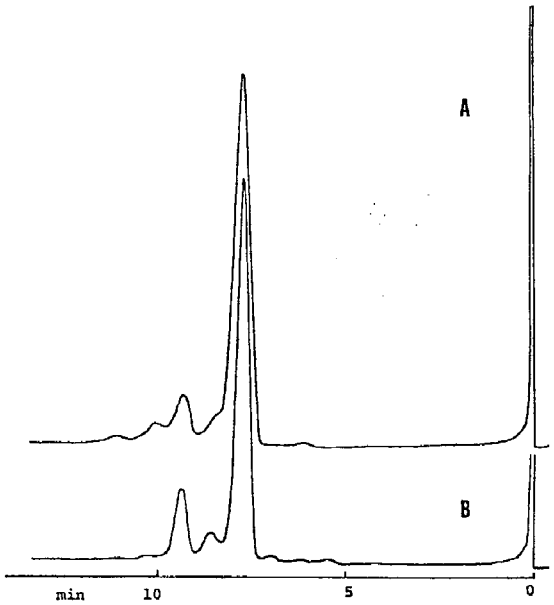

Fig. 4. Gas liquid chromatogram of acetates of the compound [I]. A: before hydrogenation, B: after hydrogenation. Temperature of column; 190–250°C (programming rate, 2°C/min).

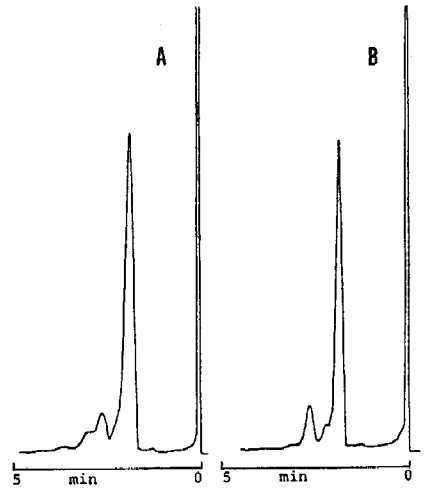

Fig. 5. Gas liquid chromatogram of isopropylidene derivatives of the compound [I]. A: before hydrogenation, B: after hydrogenation. Temperature of column; 210°C.

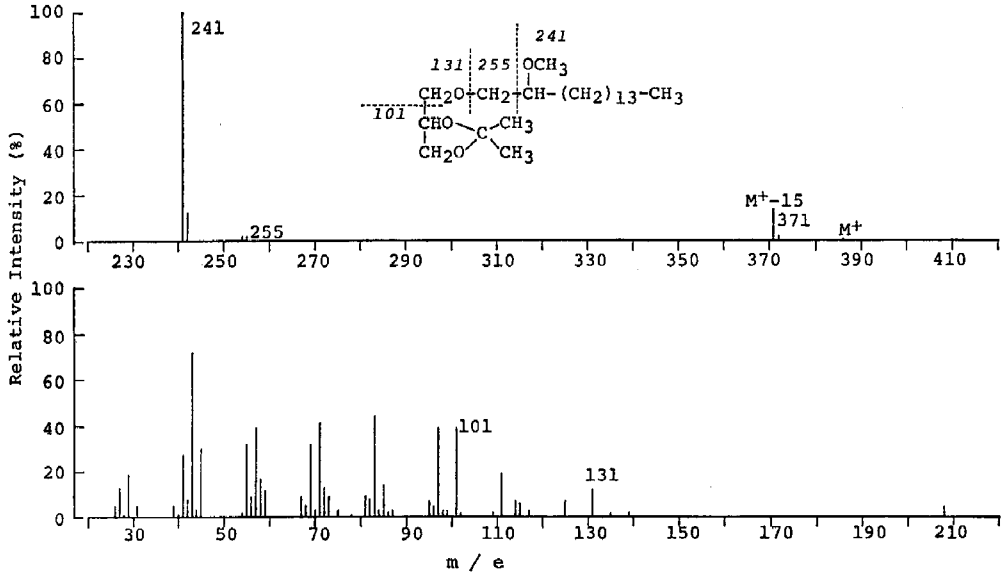

Fig. 6. GLC–MS spectrum of the major component of the compound [I] (isopropylidene derivative).

retention time, before and after hydrogenation. The quantitative distribution within the mixture of the methoxy glyceryl ethers are given in Table 1. The methoxy glyceryl ethers had a high content of ethers with saturated 16 carbon atoms, 1-*O*-(2-methoxyhexadecyl)-glycerol (76.5%), and the homologues saturated 18 carbon atoms (10.4%) in the long alkyl chains excluding the methoxy group. The composition of the methoxy glyceryl ethers were somewhat different from that of the ordinary glyceryl ethers isolated from *Seriollela* sp.[1], which were composed chiefly of monounsaturated C_{20}, C_{18}, C_{16}, C_{22} and saturated C_{16}, C_{18}, and C_{14} in the long alkyl chains.

The neutral lipids separated from the flesh lipids of the deep-sea teleost fish *Seriollela* sp. yielded 0.9% of methoxy glyceryl ethers. In Greenland shark liver oil[2], about 4% of methoxy glyceryl

Table 1. Percentage composition of the methoxy glyceryl ethers isolated from the flesh lipids of *Seriollela* sp.

Long chain component*	Peak area %
14:0	0.8
16:0	76.5
16:1	1.4
17:0	5.2
18:0	10.4
18:1	2.1
19:0	2.2
20:0	1.4

* Excluding the methoxy group.

ethers was found. This was of higher level as compared to the results of the present study. On the other hand, the neutral lipids from other marine animals[3] yielded 0.01–0.17% of methoxy glyceryl ethers, which were of lower level than that of the present study. Of the methoxy glyceryl ethers in the flesh neutral lipids of *Seriollela* sp., the major components were saturated 16 and 18 carbon atoms at 76.5% and 10.4%, respectively. This was somewhat different from the components of 16:1 (60%), 16:0 (15%) and 18:1 (20%) in the shark liver oil[2], and in contrast, was similar to those in the sea mussel or Fresh-water crayfish.[3]

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