ギンザメ Hydrolagus novaezealandiae 肝臓脂質中の中性プラスマロゲンについて

誌名	日本水産學會誌
ISSN	00215392
著者	林, 賢治 高木, 徹
巻/号	46巻8号
掲載ページ	p. 1043-1049
発行年月	1980年8月

Bulletin of the Japanese Society of Scientific Fisheries 46(8), 1043–1049 (1980)

Occurrence of Neutral Plasmalogens in the Liver Lipids of Ratfish, *Hydrolagus novaezealandiae*[*1]

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(Received May 10, 1980)

Neutral plasmalogens isolated from the liver lipids of the deep-sea ratfish *Hydrolagus novaezealandiae* were identified by infrared spectroscopy, nuclear magnetic resonance, and gas liquid chromatography-mass spectrometry. These compounds, which consisted of alk-1-enyl glyceryl ethers and fatty acids, amounted to 12.8% of the liver lipids. The component aldehydes released by acid catalyzed hydrolysis of the alk-1-enyl glyceryl ethers were mostly composed of saturates (53.9%) and monoenes (39.4%) of 14–22 carbon atoms: the major constituents were 18:0 (30.1%), 20:1 (27.2%), 16:0 (14.9%), and 18:1 (7.3%). The fatty acid composition of neutral plasmalogens consisted predominantly of monoenes (56.4%), composed chiefly of 18:1, 16:1 and 20:1 acids.

In a previous study[1] the composition of diacyl glyceryl ethers in the liver lipids of deep-sea ratfish *Hydrolagus novaezealandiae* was determined by thin layer chromatography (TLC), infrared spectroscopy (IR) and gas liquid chromatography (GLC). Besides, the unknown compounds migrating slightly ahead of diacyl glyceryl ethers on silicic acid plates were detected in relatively large amounts in the liver lipids. By TLC, IR, nuclear magnetic resonance (NMR), GLC and GLC-mass spectrometry (GLC–MS), these compounds were determined to be neutral plasmalogens which consisted of alk-1-enyl glyceryl ethers and fatty acids. The present investigation deals with the separation and identification of the neutral plasmalogens, and with the compositions of aldehydes released from the alk-1-enyl glyceryl ethers and of fatty acids from the neutral plasmalogens.

Minute amounts of aldehydogenic neutral lipids (neutral plasmalogens) are distributed in the various mammalian tissues including man[2,3]. On the other hand, in the tissues of marine animals, *e. g.*, shark *Squalus acanthias*,[4] ratfish *Hydrolagus colliei*[5] and *Chimaera monstrosa*,[6] starfish *Asterias forbesi*,[7] and polychaete *Nereis virens*,[8] these compounds have also been found as a relatively large proportion of the total lipids. We found a new natural source of these compounds at a relatively high concentration.

Experimental

Materials

The total lipids extracted from the liver of female ratfish *H. novaezealandiae* described previously[1] were used in this study.

Fractionation of Neutral Plasmalogens

Neutral plasmalogens were separated from total lipids with the column of silicic acid-Celite (2:1), using 6% diethyl ether-hexane after 2% and 4% diethyl ether-hexane as the solvent for elution. It was ascertained that the neutral plasmalogen fraction contained considerable amounts of the contaminating diacyl glyceryl ethers by TLC. Further purification of these compounds was carried out by preparative TLC.

Derivatization

The neutral plasmalogens fractionated were subjected to an alkaline hydrolysis in 1 N ethanolic KOH by boiling under reflux for 1 h. The unsaponifiables (alk-1-enyl glyceryl ethers) were extracted from the saponification mixture with diethyl ether. The fatty acids were recovered after removal of the unsaponifiables by an usual way. The alk-1-enyl glyceryl ethers were treated to release aldehydes with a mixture of concentrated HCl and diethyl ether according to the method reported by ANDERSON *et al.*[9]. Fatty acids were methylated with boron trifluoride-methanol.[10] Prior to GLC, further purification of aldehydes and fatty acid methyl esters was carried out by TLC.

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TLC

The fractionation of neutral plasmalogens or alk-1-enyl glyceryl ethers was accomplished on preparative plates (0.50 mm thickness) of silicic acid, using the solvent systems of petroleum ether or hexane, diethyl ether and acetic acid (90: 10: 1, 40: 60: 1, 30: 70: 1 v/v). 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 systems as above. Spots were visualized by charring with a 50% sulfuric spray.

Hydrogenation

Each of fatty acid methyl esters or aldehydes was hydrogenated for qualitative and quantitative examinations. The hydrogenation was performed in hexane solution with 5% palladium carbon as a catalyst at atmospheric pressure at room temperature. The samples were shaken for 1 h to achieve complete hydrogenation.

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 aldehydes 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; temperature of column: 185°C for fatty acid methyl esters, and 210°C or 200–260°C (programmed rate, 2°C/min) for aldehydes, respectively. Nitrogen was used as carrier gas. The identification of the components for above samples was accomplished by comparison with known available standards and by a log-plot of retention times against the number of carbons in the chain, before and after hydrogenation. Further identification for aldehydes was carried out by GLC–MS analyses. Quantitative analysis was made on the basis of the area percentage of each peak.

IR

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

NMR

The NMR spectrum was obtained with a JOEL-PMX model 60 spectrometer on $CDCl_3$ solution containing tetramethylsilane as an internal standard.

GLC–MS

The mass spectra were recorded with an Hitachi 60 M instrument equipped with a column of 3% Silar 10 C on Gas Chrom Q (100/120 mesh). The mass spectrometer was operated at 20 eV.

Results and Discussion

Characteristics of Neutral Plasmalogens and Alk-1-Enyl Glyceryl Ethers

Following TLC of the liver lipids (64.1%) of the ratfish *H. novaezealandiae*, a distinct spot with approximate R_F-value 0.47 which was somewhat higher than that of diacyl glyceryl ethers 0.37 was obtained (Fig. 1-A). The former compounds (neutral plasmalogens) amounted to 12.8% of the liver lipids, whereas the diacyl glyceryl ethers amounted to 65.8%, according to the analyses by

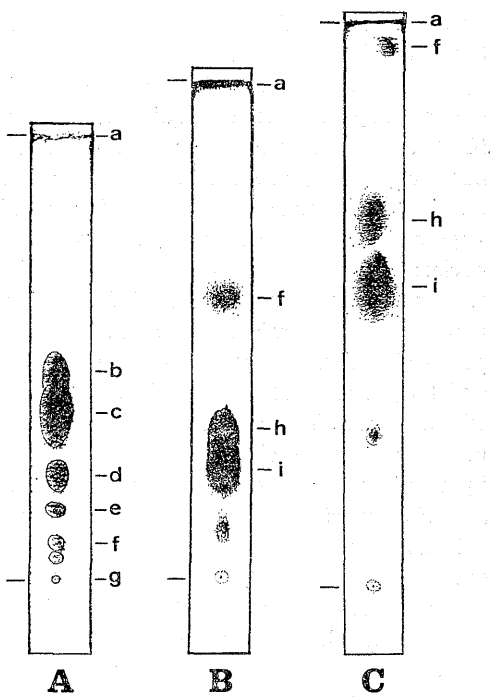


Fig. 1. Thin layer chromatograms of the total lipids and their unsaponifiables from *H. novaezealandiae* liver. A: total lipids. Solvent; petroleum ether-diethyl ether-acetic acid (90: 10: 1 v/v). B: unsaponifiables. Solvent; hexane-diethyl ether-acetic acid (40: 60: 1 v/v). C: unsaponifiables. Solvent; petroleum ether-diethyl ether-acetic acid (30: 70: 1 v/v).

a, hydrocarbons; b, neutral plasmalogens; c, diacyl glyceryl ethers; d, triglycerides; e, fatty acids; f, sterols; g, phospholipids; h, alk-1-enyl glyceryl ethers; i, glyceryl ethers.

a combination of column chromatography and preparative TLC. As shown in Fig. 1-B or 1-C, the spot migrating slightly ahead of glyceryl ethers was detected as the unsaponifiable constituent of the liver lipids. This spot corresponded to the unsaponifiable fraction (alk-1-enyl glyceryl ethers) obtained from the neutral plasmalogens. The behavior of neutral plasmalogens or alk-1-enyl glyceryl ethers isolated from the examined fish on silicic acid plates was consistent with the observation of SCHMID *et al.*[11].

The IR spectra of the neutral plasmalogens and alk-1-enyl glyceryl ethers obtained from the examined fish are shown in Fig. 2. The spectra of these two compounds gave the following characteristic absorptions; 1747 cm^{-1} (-CO-O), 1670 cm^{-1} (C=C) and 1118 cm^{-1} (C-O-C) for the neutral plasmalogens, and 3400-3460 cm^{-1} (-OH), 1670 cm^{-1} (C=C) and 1110 cm^{-1} (C-O-C) for the alk-1-enyl glyceryl ethers. Besides, the IR spectra of these two compounds were identical with the characteristic absorptions of neutral plasmalogens and alk-1-enyl glyceryl ethers, isolated from the ratfish *H. colliei* liver oil reported by SCHMID *et al.*[11]. For the compounds obtained after hydrogenation of the alk-1-enyl glyceryl ethers isolated from the examined fish, the absorption band at 1670 cm^{-1} in the IR spectrum was entirely absent, and was rather similar to the spectrum of batyl alcohol. The absorption band at 1670 cm^{-1} was, therefore, due to a streching vibration of the carbon double bond in the position α to the ether linkage[5]. On the other hand, in the IR spectrum of the compounds released by acid catalyzed hydrolysis of the alk-1-enyl glyceryl ethers from the examined fish the characteristic bands were observed at 1720 cm^{-1} (-CO) and 2720 cm^{-1} (C-H) due to aldehyde group.

The NMR spectrum of the alk-1-enyl glyceryl ethers obtained from the examined fish is shown in Fig. 3. A doublet centered at 5.96 ppm was accounted for the olefinic hydrogen at the carbon in the position α to the ether linkage. This doublet was associated with the hydrogen at the *cis* enol bond, and was consistent with the results that the alk-1-enyl linkage occurred in *cis* configuration[5]. Furthermore the *trans* isomers[12] which would show a doublet near 6.20 ppm and a pair of triplets near 4.78 ppm were not absent. Additional signals were: a peak at 0.89 ppm, accounting for the terminal methyl groups; a single peak at 1.26 ppm, associated with the internal methylene groups of the aliphatic chains; a triplet at 5.39 ppm, representing the isolated olefinic groups. The apparent doublet occurring at 3.82 ppm can be assigned to the CH_2O group of glycerol connected to the ether linkage.

The compounds released by acid catalyzed hydrolysis of the alk-1-enyl glyceryl ethers obtained from the examined fish were subjected to GLC before and after hydrogenation. The typical chromatogram of these compounds before hydrogenation was shown in Fig. 4. The homologous components corresponding to long-chain aliphatic aldehydes were determined. The major components were subjected to mass spectrometry. The typical spectrum and relative intensities of the principal peaks in mass spectra of these components are shown in Fig. 5 and Table 1, respectively. The mass fragmentation patterns of these components were consistent with the observation of fragmentation mechanisms and rearrengements for long-chain aliphatic aldehydes, as demonstrated by CHRISTIANSEN *et al.*[13]. Of all the long-chain

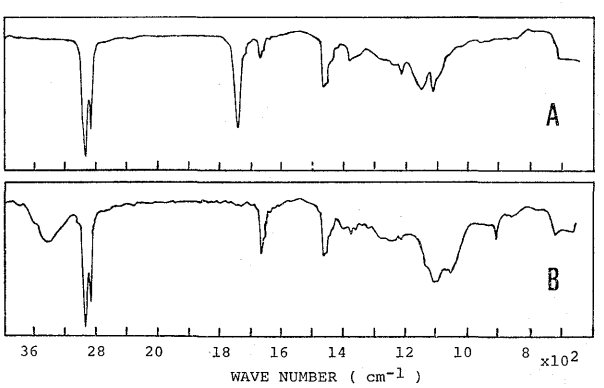


Fig. 2. IR spectra of neutral plasmalogens and alk-1-enyl glyceryl ethers from *H. novaezealandiae* liver.

A, neutral plasmalogens; B, alk-1-enyl glyceryl ethers.

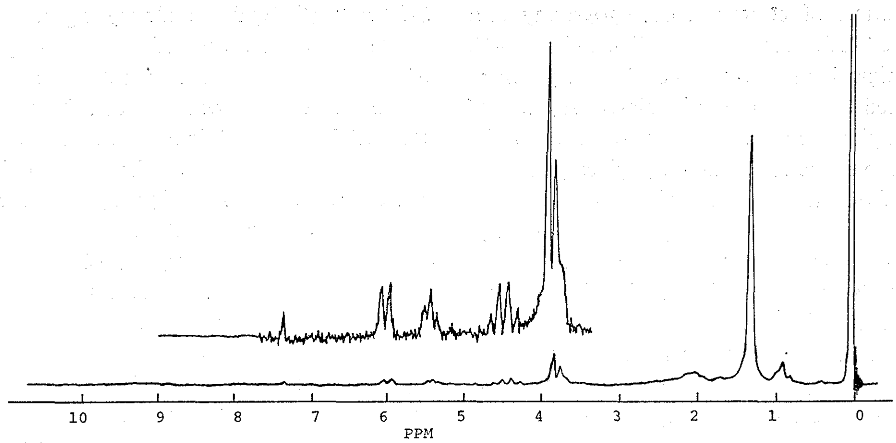


Fig. 3. NMR spectrum of the alk-1-enyl glyceryl ethers from *H. novaezealandiae* liver.

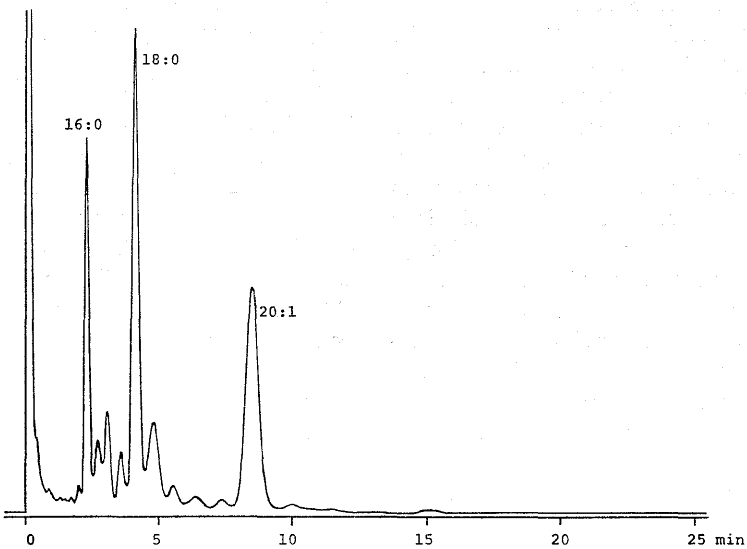
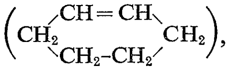


Fig. 4. Gas liquid chromatogram of the long-chain aldehydes released from the alk-1-enyl glyceryl ethers of *H. novaezealandiae* liver.

aldehydes, the base peak in the spectra was observed at *m/e* 82 at low ionization voltage. This prominent peak, having a ring structure  , might arise from a primary decomposition of the molecular ion.[13] The saturated aldehydes gave small but recognizable molecular ion peaks as compared with those of monoenoic ones. The fragments at *m/e* M^+–18, M^+–44 and M^+–46 were also present in the mass spectra of long-chain aldehydes but with relatively low intensities. A peak at *m/e* M^+–18 was presumably due to loss of water from the molecular ion. The formation of the ion at *m/e* M^+–44 $(\genfrac{}{}{0pt}{}{HO}{H}{>}C{=}CH_2)$ was postulated to involve a β-cleavage and a hydrogen transfer from γ-position to the oxygen,[13] and the ion at *m/e* M^+–46 might to loss of $18+28(H_2O+CH_2{=}CH_2)$ from the molecular ion. Besides the normal series of hydrocarbon fragments, a series of peaks with even mass number, was apparent ($C_5H_8^+=68$; $68+14n$, where $n=0, 1, 2\ldots$).

Compositions of Long-Chain Aldehydes and Fatty Acids

By GLC analyses, the homologous components of long-chain aldehydes originated from the alk-1-enyl glyceryl ethers and the fatty acid components

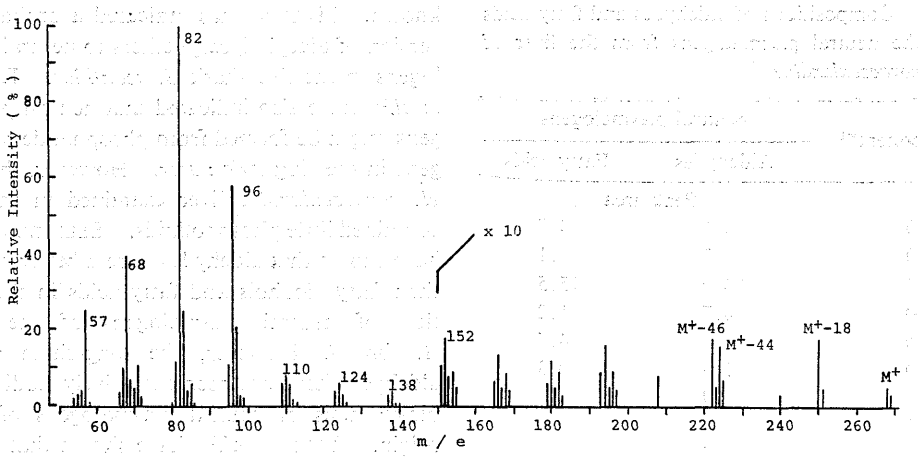


Fig. 5. GLC–MS spectrum of the major component (octadecanal) of the long-chain aldehydes released from the alk-1-enyl glyceryl ethers of *H. novaezealandiae* liver.

Table 1. Relative intensities* of the principal peaks in mass spectra of long-chain aldehydes released from alk-1-enyl glyceryl ethers

Carbon no.: no. of double bond	16:0	17:0	18:0	20:0	18:1	19:1	20:1
Molecular weight	240	254	268	296	266	280	294
M^+	0.4	0.4	0.5	0.4	0.8	2.7	9.4
M^+-18	1.2	0.9	2.2	2.3	1.4	3.8	10.9
M^+-44	2.1	1.4	1.6	1.2	1.6	2.5	4.2
M^+-46	2.3	1.2	1.9	1.8	0.5	—	—
43	3.5	2.2	1.8	1.2	4.4	4.0	8.7
57	39.8	25.4	19.5	11.0	19.4	27.9	26.8
68	41.8	27.0	36.5	27.2	17.9	23.4	24.6
82	100.0	100.0	100.0	100.0	100.0	100.0	100.0
96	53.0	55.2	70.2	73.0	50.9	50.8	96.1
110	5.6	7.5	8.4	9.0	9.8	17.4	22.2
124	5.3	5.7	6.2	7.0	7.3	12.1	13.8
138	3.3	3.1	3.7	4.4	4.3	7.7	10.2
152	1.7	1.8	1.8	2.1	2.2	3.8	6.5

* Expressed as per cent of base peak.
M^+: Molecular ion.

from the neutral plasmalogens were determined by comparing with each peak area and retention time before and after hydrogenation, respectively. The quantitative distribution of the components for aldehydes or fatty acids is given in Table 2.

The characteristics of long-chain aldehydes are as follows. Carbon atoms with even number, ranging from C_{14} to C_{22}, were abundant as compared with odd and branched ones. The major constituents were 18:0 (30.1%), 20:1 (27.2%), 16:0 (14.9%), and 18:1 (7.3%), indicating a high saturated content (53.9%) followed after monoenoic (39.4%). Besides, the branched components occurred in a small amount (6.7%). The aldehydes released from the alk-1-enyl glyceryl ethers of the ratfish *H. novaezealandiae* liver were somewhat similar in composition to those found in the ratfish *C. monstrosa*[6] and *H. colliei*.[11]

On the other hand, the fatty acid composition of the neutral plasmalogens consisted predominantly of monoenes (56.4%), composed chiefly of 18:1, 16:1 and 20:1 acids, as compared with those of saturates or polyenes. The fatty acid composition of the neutral plasmalogens from the examined fish was somewhat similar, on the whole, to that of diacyl glyceryl ethers from the same ratfish *H. novaezealandiae* liver.[1] However, the neutral plasmalogens from the examined fish were shown to have relatively high concentrations of polyenes such as 18:2, 18:3, 20:4 and 20:5 acids as

Table 2. Compositions of aldehydes and fatty acids of the neutral plasmalogens from the liver of *H. novaezealandiae*

Component*1	Neutral plasmalogens	
	Aldehydes	Fatty acids
	Peak area %	
14:0	0.1	4.7
15:0	0.3	1.1
16:0	14.9	15.5
17:0	4.7	1.2
18:0	30.1	4.7
19:0	1.8	0.3
20:0	0.9	
21:0	0.9	
22:0	0.2	
total	53.9	27.5
14:1	tr*2	0.7
15:1	tr	0.5
16:1	2.2	11.0
17:1	0.4	1.3
18:1	7.3	29.6
19:1	1.4	0.5
20:1	27.2	10.8
21:1	0.3	0.8
22:1	0.6	0.4
24:1		0.8
total	39.4	56.4
18:2		1.3
18:3		2.5
20:2		0.3
20:4		4.1
20:5		3.4
21:5		0.4
22:5		tr
22:6		0.3
total		12.3
15 br (*A*)*3	0.1	0.2
16 br (*A*)	0.7	
16 br (*I*)		1.1
17 br (*A*)	2.0	0.4
17 br (*I*)		0.3
18 br (*A*)		1.2
18 br (*I*)	2.8	0.3
19 br (*I*)	1.0	
20 br (*A*)		0.3
20 br (*I*)	0.1	
total	6.7	3.8

*1 No. of carbon atoms: no. of double bonds.
*2 Trace.
*3 Branched compounds were detected and identified in GLC chromatograms after hydrogenation. (*A*), *anteiso*; (*I*), *iso*.

compared with those of the ratfish *C. monstrosa*[8] and *H. colliei*.[11]

The biosynthesis and metabolism of the neutral plasmalogens in marine organisms are not well known. MALINS[4] has indicated a probable conversion of diacyl glyceryl ethers to neutral plasmalogens in the live shark *S. acanthias*. THOMPSON *et al.*[14] have also indicated that neutral plasmalogens might be formed from phosphatide plasmalogens in the slug *Arion ater*. However, the ratfish *H. novaezealandiae* liver examined in this study contained little phospholipids. ELLINGBOE *et al.*[15] have shown that aldehydes were a better precursor than fatty alcohols and fatty acids in the formation of neutral plasmalogens of the starfish *A. forbesi*. However, the long-chain aliphatic aldehydes have not been extensively studied in the biosynthesis of neutral plasmalogens of marine origin. ROOTS *et al.*[16] have shown that environmental temperature as stress had a striking effect on the levels of phosphatide plasmalogens in goldfish *Carassius auratus*. It is interest to note from viewpoint of their physiological or ecological role in the marine environment that some ratfish *H. colliei*,[5] *C. monstrosa*,[11] and *H. novaezealandiae* examined in this study have relatively large amounts of neutral plasmalogens in their livers.

Acknowledgements

The authors thank to Mr. K. KURATA, Hakodate Tech. Coll., for his kind measurement of NMR spectrum. This work was supported in part by a grant from the Ministry of Education.

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