Less polar glycolipids in Alaskan pollack brain: isolation and characterization of acyl galactosyl diacylglycerol, acyl galactosyl ceramide, and acyl glucosyl ceramide

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Abstract We recently reported that glycolipid compositions of gadoid fish nerve tissues are unique in the abundance of *1)* galactosyl diacylglycerol and its sulfate ester; *2)* glucosyl ceramide; and *3)* fatty acid ester of cerebroside (*J. Lipid Res.* 1992. **33:** 1351–1359). The present paper reports the characterization of less polar glycolipids isolated from Alaskan pollack brain. Of twelve glycolipids purified by column chromatography, four were of the galactosyl diacylglycerol type. Chemical analysis, infrared spectrometry, and a permethylation study followed by gas chromatography–mass spectrometry revealed that they were 1,2-di-O-acyl-3-β (6-O-acyl-D-galactopyranosyl)-*sn*-glycerol and 1,2-di-O-acyl-3-β(2'-O-acyl-D-galactopyranosyl)-*sn*-glycerol, and mixtures of these two isomers, with slightly different fatty acid compositions, respectively. The other eight less polar glycolipids were pure forms or mixtures of isomeric forms of cerebroside fatty acid esters in which the substituted position of the acyl group on the hexose moiety varied. The permethylation study revealed that they were 6-O-acyl-β-D-galactopyranosyl, 6-O-acyl-β-D-glucopyranosyl, 2-O-acyl-galactosyl, 2-O-acyl-glucosyl, 3- or 4-O-acyl-galactosyl, and 3-O-acyl-glucosyl ceramides, the 6-O- and 2-O-acyl isomers being predominant. This is the first report of the natural occurrence of *1)* acyl galactosyl diacylglycerol in animal tissues and *2)* acyl glucosyl ceramide in nerve tissues.—**Tamai, Y., K. Nakamura, K. Takayama-Abe, K. Uchida, T. Kasama, and H. Kobatake.** Less polar glycolipids in Alaskan pollack brain: isolation and characterization of acyl galactosyl diacylglycerol, acyl galactosyl ceramide, and acyl glucosyl ceramide. *J. Lipid Res.* 1993. **34:** 601–608.

Supplementary key words glycolipids • fish brain

Glycosphingolipids such as galactosyl ceramide (GalCer) and cerebroside sulfate (sulfatide) are the major components of lipids comprising vertebrate myelin membranes (1, 2). Recently, we reported that nerve membranes of gadoid fishes such as Alaskan pollack and Pacific cod consist of peculiar glycolipid molecular species that are distinctly different from the general composition in the vertebrate nervous system (3). The major characteristics are the abundance of galactosyl diacylglycerol (GalDAG), sulfogalactosyl diacylglycerol, glucosyl ceramide (GlcCer), and cerebroside fatty acid ester (ester cerebroside); it was also noted that hydroxy fatty acids were entirely absent. In the course of detailed investigation of ester cerebroside, we found several species of glycolipids that were eluted from a silicic acid column faster than ester cerebroside. The only glycolipids whose polarities are lower than that of cerebroside so far reported in the vertebrate brain are ester cerebroside and GalDAG (4, 5). The present paper reports the isolation of less polar glycolipids from Alaskan pollack brain and determination of their chemical structures.

MATERIALS AND METHODS

Materials

Alaskan pollack, *Theragra chalcogramma,* were caught in the northern Pacific ocean (cold current), shipped under ice-cold conditions, and supplied through a local fishery. The whole brains were removed and kept frozen until analysis. The following glycolipids, used as references, were prepared in our laboratory: GalCer, sulfatide, and ester cerebroside from bovine brain; GlcCer from Tay-Sachs' brain; and GalDAG and diGalDAG from spinach

Abbreviations: GalDAG, galactosyl diacylglycerol; GalCer, galactosyl ceramide; GlcCer, glucosyl ceramide; sulfatide, cerebroside sulfate; ester cerebroside, fatty acid ester of cerebroside; GLC, gas–liquid chromatography; GC–MS, gas–chromatography–mass spectrometry; IR, infrared; TLC, thin-layer chromatography; TMS, trimethylsilyl.

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leaves. β-Galactosyl glycerol was prepared by deacylation of spinach leaf GalDAG. α-Galactosyl glycerol prepared by Dr. B. Wickberg (University of Lund, Sweden) was a generous gift from Dr. I. Ishizuka (Teikyo University, Tokyo). Various isomers of monomethyl alditols were chemically synthesized (6, 7). Ceramide was a product of Sigma.

Extraction and purification of glycolipids

The brain tissues (about 1400 g wet weight) were extracted with 15 volumes of chloroform–methanol 2:1 (v/v) and then 10 volumes of chloroform–methanol 1:1, as described previously (3). The total lipid extracts (15 g/run) were chromatographed on a column (600 ml) of Florisil (100–200 mesh; Floridin Co.). After cholesterol had been removed with 3 column volumes of chloroform, glycolipids of low polarity were eluted with a discontinuous gradient of chloroform–methanol 98:2, 97:3, and 96:4, 3 column volumes each. The pooled less polar glycolipid fractions were rechromatographed on the same column to separate them from other glycolipids (mainly GalDAG) and nonglycolipid contaminants. The partially purified less polar glycolipids were chromatographed on an Iatrobeads (Iatron Co.) column, with monitoring by TLC with solvent system A (see below). The glycolipids were eluted with chloroform–methanol 99:1, 98:2, 97:3, 96:4, and 95:5 in a stepwise manner. Chromatography was repeated until the less polar glycolipids were each isolated as a single band as judged by TLC.

Thin-layer chromatography

Precoated silica gel 60 HPTLC plates (E. Merck) were used with the following solvent systems:

A, chloroform–methanol–water 90:10:0.5;
B, diethylether–isopropanol–methanol–water 100:4.5:3:1.2;
C, chloroform–methanol–water 65:25:4; and
D, chloroform–methanol–trimethyl borate 50:20:1.

Compositional analysis

The isolated glycolipids were treated with 0.1 M sodium methoxide in methanol. The liberated fatty acid methyl esters were analyzed by GLC. A portion of the deacylated products was separated by TLC with solvent C. The bands corresponding to authentic α-galactosyl glycerol, which were visualized with iodine vapor, were extracted, and then subjected to GLC to determine the anomeric configuration of the glycosidic linkage. The other portion of the alkali-treated glycoside materials was methanolyzed with 3% methanolic hydrochloride, and the resultant fatty acid methyl esters (amide-linked) and hexoses were analyzed by GLC. The long-chain bases were obtained by hydrolysis of the glycosphingolipids with aqueous methanolic hydrochloride (8), and then analyzed as the corresponding aldehydes by GLC (9).

Methylation analysis

To determine the substituted position of the acyl group on hexose in the glycolipid, the acyl group was replaced by a methyl group as described by Yasugi et al. (10), who modified the method of Promé et al. (11). In brief, the glycolipids having fatty acid ester linkages were treated with dihydropyran and *p*-toluene sulfonic acid in ether. The reaction products were extracted with ether, washed with sodium bicarbonate, and then purified on Sep-pak C18, successively. The derivatized glycolipids, in which the hydroxy group of the hexose was masked with tetrahydropyran, were treated with 0.1 M methanolic sodium methoxide. Then the products, the ester bonds having been cleaved, were partitioned with chloroform, methanol, and water. The lower phase lipids were dried and then methylated according to Månsson et al. (12), who modified the method of Ciucanu and Kerek (13). The methyl derivatives of glycolipids finally obtained were methanolyzed with 3% methanolic hydrochloride, reduced with sodium borohydride, and then acetylated with acetic anhydride and pyridine as described previously (14). The partially methylated alditol acetates thus obtained were analyzed by GLC and GC–MS.

GLC and GC–MS analysis

Fatty acid methyl esters were analyzed on a packed column of 4% OV-1 or 15% EGS, or on a capillary column coated with methyl silicone. Hexoses, as the TMS derivatives of methyl glycosides, were identified on a 4% SE-30 column or a methyl silicone-coated capillary column. The anomeric configuration of the glycosidic linkage in galactosyl glycerols, as the TMS derivatives, was determined on a column of 4% OV-101. The partially methylated alditol acetates were analyzed on a 2% OV-225 or Silar 10C column. GC–MS was performed with a Shimadzu LKB-9000 apparatus. The carrier gas was He at a flow rate of 30 ml/min. Mass spectra were recorded at an electron energy of 70 eV, a trap current of 60 μA, and an ion source temperature of 270°C. The long-chain base compositions of glycosphingolipids, as the corresponding aldehydes, were determined on a column of 15% EGS by GLC.

Quantitative analysis

The amount of hexose was determined colorimetrically by the anthrone sulfuric acid method (15), and the ester value by the method of Snyder and Stephens (16).

RESULTS

Isolation of less polar glycolipids

Fig. 1A shows that twelve glycolipids (lanes 5–16) migrating faster than GalDAG (lane 2) were each isolated as a single band on TLC with solvent system A. Although

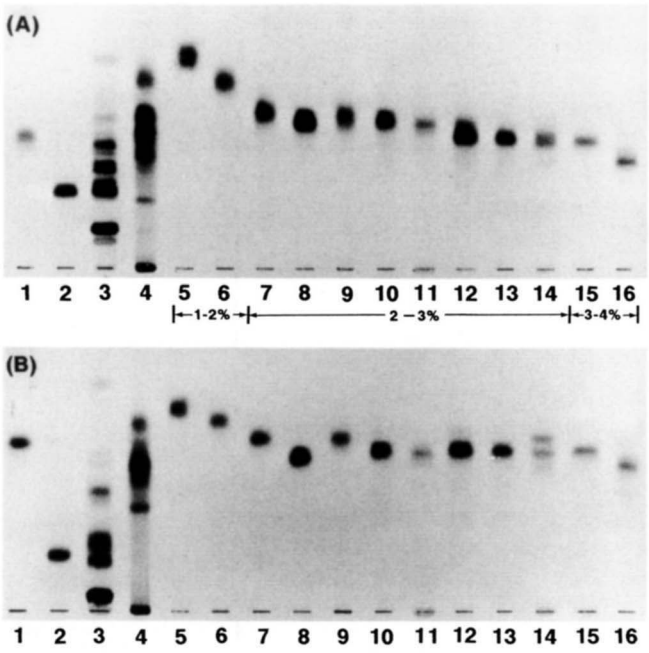

Fig. 1. Thin-layer chromatogram of less polar glycolipids isolated by Iatrobeads column chromatography. Glycolipids were developed on a silica gel HPTLC plate (Merck) with solvent system A (Fig. 1A) or solvent system B (Fig. 1B), and located by spraying the plate with anthrone sulfuric acid reagent, followed by heating. Lane 1, ceramide; lane 2, GalDAG from spinach leaves; lane 3, mixture of total ester cerebrosides and cerebroside from bovine brain; lane 4, less polar glycolipid fraction from Alaskan pollack brain; lanes 5–16, isolated glycolipids. The percentages given under plate A indicate the concentrations of methanol in chloroform as the solvent used for Iatrobeads column chromatography.

some glycolipids migrated almost concomitantly on TLC with this solvent, all glycolipids had been separately eluted from an Iatrobeads column. Most of the less polar glycolipids prepared from Alaskan pollack brain were found to be even less polar than ester cerebroside from bovine brain. When these glycolipids were developed with solvent system B (Fig. 1B), they showed different migration profiles compared to solvent system A. In particular, it was found that GL 5 (lane 9) migrated faster than GLs 4, 6, and 7 (lanes 8, 10, and 11); GLs 6 to 11 (lanes 10 to 5) moved almost concomitantly; and GL 10 exhibited two bands with solvent system B. On the basis of the TLC profiles shown in Fig. 1, the twelve glycolipids were suspected to be different glycolipid species in structure or constituents.

IR spectra

The twelve glycolipids could be classified into two groups on the basis of their IR spectra (**Fig. 2**). The spectra of GLs 1, 2, 3, and 5 resembled that of GalDAG from spinach leaves or Alaskan pollack (3), showing the presence of carbonyl ester (1738 cm^{-1}) and sugar (3400 cm^{-1}, 1070 cm^{-1}), but the absence of an acid amide bond (1648 cm^{-1}, 1541 cm^{-1}). On the other hand, the spectra of GLs 4 and 6 to 12 were almost identical with that of ester cerebroside from bovine brain (17), showing the presence of both carbonyl ester and acid amide bonds. It was noted in both groups that the absorption of sugar (3400 cm^{-1}) is considerably small, compared to the doublet absorption (2922 cm^{-1} and 2852 cm^{-1}) due to –CH stretching or the presence of CH_3 and CH_2 chains. From these IR spectra, the former group was considered to be glycoglycerolipids, and the latter group glycosphingolipids having fatty acid ester bonds.

Analysis of glycolipids treated with mild alkali

Fig. 3 shows TLC of the products of glycolipids obtained on mild alkaline treatment. Alkali-treated GLs 1–3 and 5 (lanes 4–8) migrated very slowly and almost concomitantly with authentic galactosyl glycerol and alkali-treated GalDAG (lane 4), while alkali-treated GLs 4 and 6–12 (lanes 9–17) comigrated with authentic cerebroside. These findings coincided with the IR spectra showing that GLs 1–3 and 5 were glycoglycerolipids, and GLs 4 and 6–12 were esterified glycosphingolipids.

The deacylated products of GLs 1–3 and 5 were analyzed by GLC as their TMS derivatives to determine the anomeric configuration of the glycosidic linkage. All deacylated forms were determined to be β-galactosyl glycerol, in comparison with authentic α- and β-galactosyl glycerol (data not shown).

The deesterified products of GLs 4 and 6–12 were separated by TLC with solvent D (**Fig. 4**). All the products were shown to be mixtures of GalCer and GlcCer.

Chemical analysis

Analysis of the hexose compositions of glycolipids by GLC showed that GLs 1–3 and 5 comprised exclusively galactose, whereas GLs 4 and 6–12 comprised galactose and glucose in different ratios in the respective glycolipids (**Table 1**). The results for the latter group coincided with the TLC findings shown in Fig. 4.

The mole ratio of hexose to ester bond in one molecule was determined to be 1:3.2 on average in GLs 1–3 and 5; those in GalDAG and diGalDAG, measured as references, were 1:1.9 (1:2) and 1:1.2 (1:1), respectively (the values in parentheses are the theoretical ones). The hexose to ester ratio in GLs 4 and 6–12 was approximately 1:1. Considering all the data together, GLs 1, 2, 3, and 5 were concluded to be acyl GalDAG, and GLs 4 and 6–12 to be cerebroside having one mole of fatty acid ester.

Substituted position of the acyl group on hexose

The position of the acyl group on galactose or glucose was determined by GLC and GC–MS as the position of the methyl group in partially methylated alditol acetate. Individual peaks of monomethyl alditol acetates were identified from the retention times and the mass spectra by comparison with authentic samples. On the basis of the findings (Table 1), the chemical structures of GLs 2

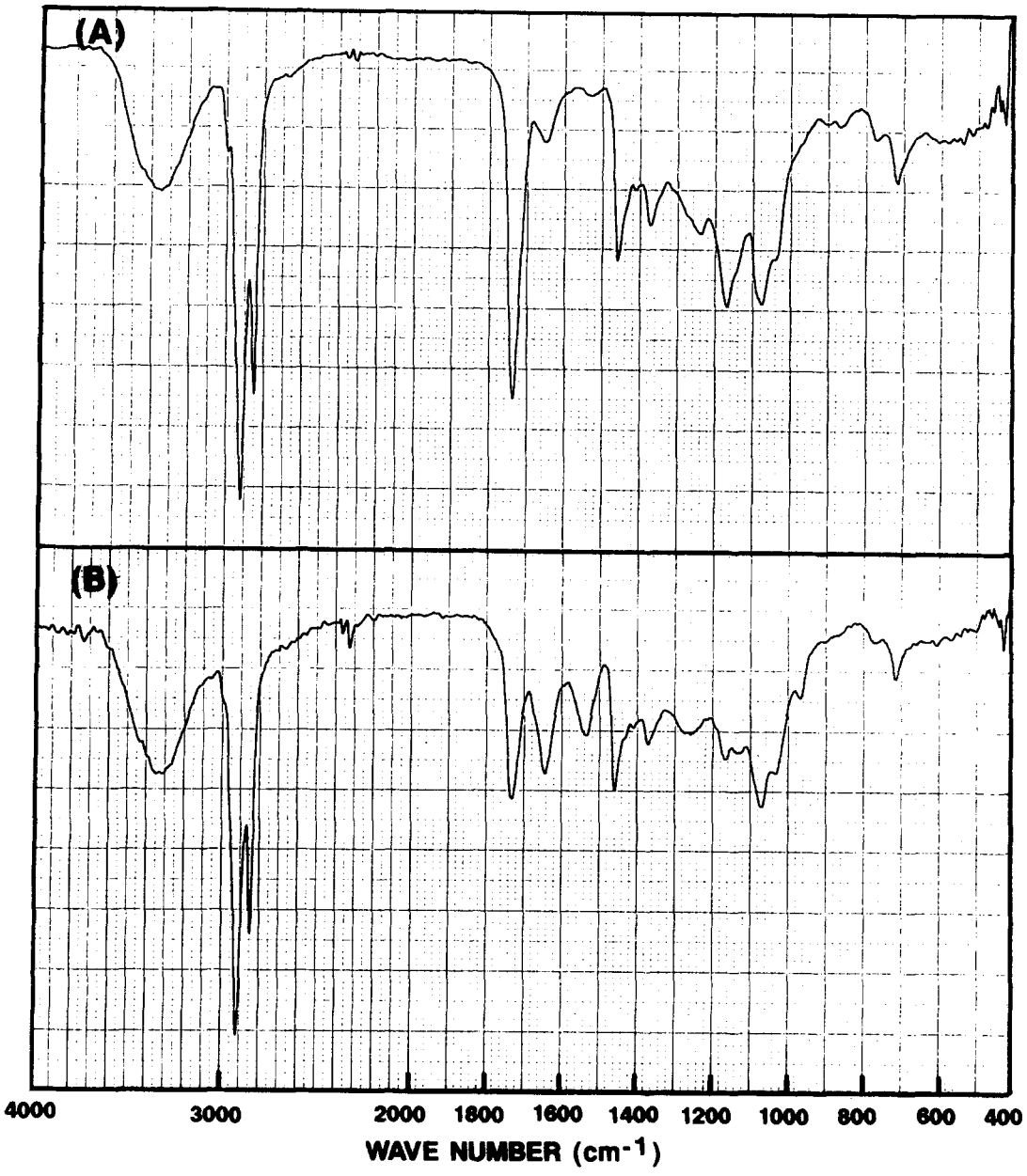

Fig. 2. IR spectra of less polar glycolipids. IR absorption spectrometry of glycolipids was performed with a JASCO DR-81 Infrared Spectrometer equipped with a diffuse reflectance attachment (Japan Spectroscopic Co.). (A) IR spectrum of GL 2. The spectra of GLs 1, 3, and 5 were almost identical to that of GL 2. (B) IR spectrum of GL 7. The spectra of GLs 4 and 6–8 were almost identical to that of GL 7.

and 5 were determined to be 1,2-di-O-acyl-3-β(6-O-acyl-D-galactopyranosyl)-*sn*-glycerol and 1,2-di-O-acyl-3-β(2'-O-acyl-D-galactopyranosyl)-*sn*-glycerol, respectively; and GLs 1 and 3 were mixtures of these two isomers.

The alkylacyl form of acyl galactosyl glyceride was not determined in this study. In our previous study (3), we detected small amounts (3% of the diacyl form) of 1-O-alkyl-2-O-acyl-3-O-galactosyl-*sn*-glycerol, a possible precursor of the acyl galactosyl derivative. A band that migrated slightly slower than cerebroside (possibly alkyl galactosyl glycerol) was faintly observed on TLC of alkali-treated materials (see Fig. 3, lanes 5–8). Thus, it is possible that the alkylacyl type of this glycolipid is present in Alaskan pollack brain.

Ester cerebrosides were found to be mixtures of various isomers: GLs 4 and 6 were mainly of the 6-O-acyl galactose type; and the most abundant components of GLs 7, 8, 9, 10, and 12 were 6-O-acyl galactose (42%), 2-O-acyl glucose (47%), 6-O-acyl glucose (48%), 3-O-acyl glucose (66%), and 2-O-acyl glucose (47%), respectively. As a whole, the most abundant molecular species was 6-O-acyl galactose, followed by 2-O-acyl galactose or glucose, 3-O-

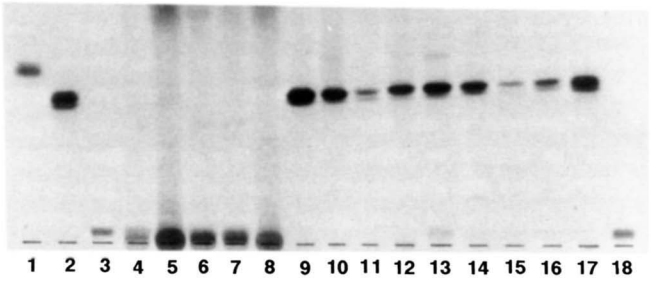

Fig. 3. Thin-layer chromatogram of alkali-treated glycolipids. The materials were developed with solvent system C; detection was as described in the legend to Fig. 1. Lane 1, GalDAG from Alaskan pollack brain; lane 2, cerebroside; lanes 3 and 18, synthetic α-galactosyl glycerol; lane 4, alkali-treated lane 1 material; lanes 5–8, products of alkali-treated GLs 1, 2, 3, and 5, respectively; lanes 9–17, products of alkali-treated GLs 4 and 6–12, respectively.

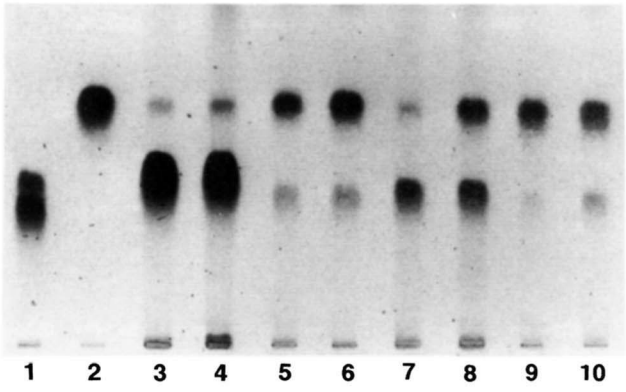

Fig. 4. Thin-layer chromatogram of alkali-treated glycosphingolipids. Glycolipids were developed with solvent system D; detection was as described in the legend to Fig. 1. Lane 1, GalCer; lane 2, GlcCer from Tay-Sachs' brain; lanes 3–10, products of alkali-treated GLs 4 and 6–12. Note that GalCer was predominant in lanes 3, 4, and 7; GlcCer was predominant in lanes 5, 6, 9, and 10; and the material in lane 8 comprised both glycolipids in comparable amounts.

acyl glucose, 6-O-acyl glucose, and 3 (or 4)-O-acyl galactose. All-acetyl galactitol or glucitol was not detected, indicating that the acyl ester linkage is on the galactose or glucose and not on the hydroxy group of sphingosine.

Fatty acid and long-chain base composition

The major fatty acid components of acyl GalDAG were 18:1 and 16:0, the latter increasing as the migration rate on TLC decreased (**Table 2**); conversely, the content of longer chain fatty acids (C < 20) decreased with decreasing mobility. It was suspected, therefore, that the four fractions of acyl GalDAG were separated mainly due to the difference in the fatty acid composition.

In the ester cerebrosides, 24:1 was the most abundant acid, amounting to almost half of the total fatty acids (**Table 3**). Ester-linked fatty acids comprised mainly 16:0, 18:0, and 18:1, the relative contents being slightly different in glycolipid species; 24:1 was the predominant component of amide-linked fatty acids in all glycolipids. Hydroxy fatty acids were not detected. As a whole, the fatty acid compositions of ester cerebrosides were very similar to each other.

TABLE 1. Percentage composition of hexoses and partially methylated alditol acetates derived from acyl GalDAG and ester cerebrosides

		Total Hexoses[a]		Partially Methylated Alditol Acetates[b]					
GL	Type	Galactose	Glucose	6-O-Methyl Galactitol Acetate	6-O-Methyl Glucitol Acetate	2-O-Methyl Galactitol Acetate	2-O-Methyl Glucitol Acetate	3-O-Methyl Galactitol Acetate[c]	3-O-Methyl Glucitol Acetate
1	GG	100	-	73	-	27	-	-	-
2	GG	100	-	100	-	-	-	-	-
3	GG	97	3	54	-	45	-	-	-
4	CE	96	4	98	-	2	-	-	-
5	GG	94	6	-	-	100	-	-	-
6	CE	96	4	78	-	14	-	8	-
7	CE	87	13	42	-	13	-	14	31
8	CE	33	67	24	20	-	47	5	4
9	CE	23	77	21	48	5	9	-	17
10	CE	18	82	8	3	1	4	19	66
11	CE	63	37						
12	CE	27	73	25	-	-	47	-	28

Values are averages of two determinations and percentages as to the total hexoses (*a*) determined as the TMS derivatives of the methanolyzates or as to the total alditol acetates (*b*). Partially methylated galactitol or glucitol acetates were analyzed by GLC on a column of 2% OV-225 at a constant temperature of 230°C. The 2-O-methylgalactitol and glucitol acetates, which were incompletely separated on the OV-225 column, were resolved on a Silar 10C column, and the values obtained with the former column being calculated from the ratios obtained with the latter column. A methylation study of GL 11 was not performed because of insufficient amount. GG, galactosyl diacylglycerol type; CE, cerebroside type. Dash (-) indicates negligible amount or not detected.

[c] 3-O-Methyl and 4-O-methyl galactitol acetates were not separated in this study.

TABLE 2. Fatty acid composition of acyl GalDAG

Fatty Acid	GL1	GL2	GL3	GL5
		% of total fatty acids		
16:0	19	27	29	36
16:1	11	12	11	11
18:0	10	8	8	9
18:1	40	39	40	36
20:1	12	10	8	7
22:1	7	4	3	2

Values are averages for two or more determinations by GLC. Minute amounts of 14:0, 20:0, and 22:0 were also present.

The long-chain bases of ester cerebrosides consisted of almost only sphingenine.

Contents of acyl GalDAG

The total content of acyl GalDAG was 22.4 nmol/g brain, GL 2 amounting to nearly half of the total (**Table 4**). This value corresponded to 0.4% of the total glycoglycerolipids and 0.2% of the total glycolipids of Alaskan pollack brain (3); ester cerebrosides amounted to 90 nmol/g brain, corresponding to 1.6% of the total cerebrosides and 0.8% of the total glycolipids (3).

DISCUSSION

Acyl GalDAG was first isolated from spinach leaves (18, 19) and soft wheat flour (20); and the additional fatty acid was determined to be linked to the 6-position of galactose. This compound, however, has been proved to be an artifactual product enzymatically formed on the homogenization of plant leaves in phosphate buffer or during preparation of the chloroplast envelope fraction, but not detected in extracts from intact leaves (21–23). On the other hand, acyl derivatives of GalDAG have not been found to date in animal tissues. In this study, lipids were extracted directly with a mixture of chloroform and methanol. The enzymatic synthesis of glycolipids during the extraction is not likely to occur under these conditions, as shown in plants. Thus, this is the first report showing that this glycolipid is present in nature as a structural component of nerve membranes, and not an artifactual product.

Ester cerebrosides have been widely found in vertebrate nerve tissues (4), although in only small amounts, since the first report by Norton and Brotz (24). The only hexose component of this glycolipid is galactose, except for Alaskan pollack brain (3), but the position of the acyl ester linkage on galactose varies with the animal species: e.g., the 6-position in pig brain (25); the 6- and 3-positions in bovine brain (17, 26); and the 6-, 2-, 4-, and 3-positions in whale, bovine, and human brain (10, 27, 28). On the other hand, acyl GlcCer, the glucose type of ester cerebroside, has been reported so far only in Gaucher's spleen (29). In this study, the chemical structures of isomeric forms of acyl GlcCer from nerve tissues were determined for the first time.

It was previously shown that the possibility of acyl group migration during permethylation in the procedure used in this experiment is negligible (10). Thus, the isomers with different acyl group substitution obtained in this study can be considered not to be artifactual products. Gadoid fishes have been found to accumulate a large amount of GlcCer in the brain, unlike other vertebrate species (3). Consequently, acyl GlcCer is presumed to occur in significant amounts, as shown in this study, because ester cerebrosides are presumably synthesized enzymatically through the acylation of cerebroside.

Previously it was reported that glycoglycerolipid-containing liposomes show a lower transition temperature than those containing glycosphingolipids (30). Such differences of physicochemical properties between these

TABLE 3. Fatty acid composition of ester cerebrosides

	Total								Ester-Linked								Amide-Linded							
Fatty Acid	GLs 4	6	7	8	9	10	11	12	4	6	7	8	9	10	11	12	4	6	7	8	9	10	11	12
16:0	14	10	16	10	10	18	11	18	29	19	30	18	15	30	17	29	1	2	3	2	1	2	2	8
16:1	3	3	3	3	3	2	2	2	6	6	2	5	2	2	2	2	-	-	-	-	-	-	-	-
18:0	8	7	12	8	9	13	10	13	12	8	15	10	15	15	17	13	6	6	16	8	8	12	8	15
18:1	15	17	13	17	16	13	16	11	29	37	28	36	34	20	32	19	-	1	1	2	2	1	1	4
20:1	3	3	2	3	3	2	3	2	5	7	5	8	8	8	9	8	-	-	-	-	-	-	-	-
22:1	6	5	6	5	5	5	6	5	2	3	2	3	3	3	3	-	12	10	12	9	9	9	13	7
24:1	48	48	45	49	52	44	49	45	16	20	14	20	20	22	21	23	80	82	69	81	81	79	77	66
Others	3	7	3	5	2	3	3	4	1	4	4	-	3	-	-	6	-	-	-	-	-	-	-	-

Values are averages of two or more determinations by GLC of percentages of total ester-linked or amide-linked fatty acids. The total fatty acids were obtained by methanolysis of materials with methanolic hydrochloride, ester-linked fatty acids by treatment of materials with sodium methoxide in methanol, and amide-linked fatty acids by methanolysis of de-esterified glycolipids. Others include 14:0, 22:0, 24:0, and 26:1. Dash (-), negligible amount or not detected.

TABLE 4. Content of acyl GalDAG in Alaskan pollack brain

Glycolipid	nmol/g brain
Total acyl GalDAG	22.4
GL 1	0.67
GL 2	12.9
GL 3	6.3
GL 5	2.5
Total ester cerebroside	90.0

Values are averages of three determinations. The contents of individual ester cerebrosides were not determined.

two glycolipid groups may be speculated to be due to the presence or absence of acyl ester linkages in the molecules. Acyl GalDAG and ester cerebrosides are considered to be components constituting myelin membranes as well as their respective precursor, GalDAG and cerebrosides. Together with the absence of hydroxy fatty acids, the nerve membranes of Alaskan pollack used in this study are reasonably considered to be extremely fluid. Alaskan pollack belongs to 'Gadidae', and all fishes in this order are deep-sea fishes. The present findings can be interpreted that in the process of evolution, gadoid fishes, adapting to the living conditions of low temperature and high water-pressure, have acquired the ability to synthesize glycolipids rich in the ester linkage by which nerve membranes function properly. A study to elucidate the relationship between the structures of glycolipids and the functions and properties of myelin membranes may answer the hypothesis raised above.

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