Novel plasmalogalactosylalkylglycerol from equine brain

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Abstract A novel galactosylalkylglycerol modified with a long-chain cyclic acetal at the sugar moiety, 3-*O*-(4′6′-plasmalogalactosyl) 1-*O*-alkylglycerol, was isolated from equine brain. The presence of cyclic acetal linkage, its linked position, and the length of the acetal chain of the natural plasmalo lipid were determined by proton NMR spectroscopy and fast-atom bombardment–mass spectrometry, as well as gas chromatography–mass spectrometry and gas–liquid chromatography. To identify the isomeric stereostructure of the natural product, the plasmalo derivative was chemically synthesized from 3-*O*-galactosyl 2-*O*-acyl 1-*O*-alkyl glyceride through acetalization after deacylation. As a result, the direction and position of the acetal chain of the natural plasmalo lipid were characterized as an "*endo*"-type 4′,6′-*O*-acetal derivative linked to galactoside by comparison with the NMR data of the synthesized product. The chain lengths of alkyl and acetal groups were C_{14} for the former and C_{16} and C_{18} for the latter, and those for the latter group were mostly similar to those of plasmalogalactosyl ceramide, which was previously isolated from equine brain.—Yachida, Y., M. Kashiwagi, T. Mikami, K. Tsuchihashi, T. Daino, T. Akino, and S. Gasa. **Novel plasmalogalactosylalkylglycerol from equine brain.** ***J. Lipid Res.*** **1999.** 40: **2271–2278.**

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Glycolipids play roles in intercellular recognition and transmembrane signaling (1, 2) as a component of the cell membrane. Of the lipids, some glycosphingolipids are modified with fatty acid and fatty aldehyde to form ester and cyclic acetal linkages, respectively, such as *O*-acylated galactosylceramides (GalCers) in several mammalians (3–5) and fish (6), *O*-fatty acylated glucosylceramide in mammalian epidermis (7), and *O*-fatty acylated sulfatide in equine brain (8). Conjugated lipids with long-chain fatty aldehyde of glycosphingolipid, termed plasmalolipids, were isolated for the first time from normal human brain as novel modified glycolipids (9, 10), in which the modification was reported to occur at 3,4-*O*- and 4,6-*O*- on Gal of GalCer (9) and Gal sphingosine (psychosine) (10) to form cyclic acetal linkage. Their chemical structures, including the presence and chain length of the fatty aldehyde, were characterized mainly with mass spectra using fast-atom bombardment–mass spectrometry (FAB–MS), and by methylation analysis followed by analysis by gas chromatography–mass spectrometry (GC–MS). Furthermore, isomers of plasmalopsychosine have been chemically synthesized from psychosine by acetalization, confirming the isomeric structure of the acetal group to be identical to that of naturally occurring plasmalolipid (11). The isomeric stereostructure of natural plasmaloGalCer from equine brain, which can have two isomers with different directions of the fatty acetal chain, *endo*- and *exo*-types, was characterized as the former type by proton nuclear magnetic resonance spectroscopy (NMR) by us, after chemical synthesis of the plasmalolipid (12). These plasmalo derivatives are modified glycosphingolipids, not glycoglycerolipids. Glycoglycerolipids are known to be a minor component of the glycolipid in comparison to the content of glycosphingolipid in mammalian tissues. The former lipids were reported to be involved in brain and testis as a sulfated derivative termed seminolipid in the latter tissue (13). In the present paper, we describe for the first time isolation from equine brain, synthesis and identification of the stereochemical structure of a plasmalo derivative having a galactosyl glycerol skeleton.

MATERIALS AND METHODS

Chemicals

DEAE-Sephadex, A-25, and LH-20 were purchased from Pharmacia-LKB (Sweden). Iatrobeads (6RS-8060**)** were from Iatron (Tokyo). Precoated thin-layer chromatography (TLC) plates (Silica gel 60) and pyridine-d_5 were obtained from Merck (Germany). Other reagents were of analytical grade.

Isolation of plasmalolipid

The ratio of solvent mixtures is expressed by volume. The isolation of less polar glycolipids from equine brain was performed as described previously (8, 12). Briefly, an acetone powder with a

Abbreviations: CMW, chloroform–methanol–water; FAB–MS, fast atom bombardment spectrometry; GalCer, galactosylceramide; GC–MS, gas chromatography–mass spectrometry; GLC, gas–liquid chromatography; NMR, nuclear magnetic resonance spectroscopy; NOESY, homonuclear Overhauser effect spectroscopy; TLC, thin-layer chromatography.

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dry weight of 104 g was obtained from the whole horse brain (457 g wet weight) after homogenization with acetone (1 g of tissue/9 ml). The neutral glycolipid fraction was extracted with chloroform–methanol–water (CMW), 2:8:1 (1 g of powder/5 ml) three times and isolated from the combined and concentrated extracts with a DEAE-Sephadex, A-25 (acetate form) column (2.5 × 30 cm) by elution with CMW, 40:60:10. The total neutral glycolipids were chromatographed on a silica-gel (Iatrobeads) column (2.5 × 50 cm) by elution with 1,000 ml of each of the solvent mixtures of CM in ratios of 95:5 to 90:10, 85:15, 80:2, 75:25 to 70:30, with a total of 6 l. The fraction that consisted of several less polar glycolipids was eluted with CM, 95:5. The combined eluates were further chromatographed on a silica-gel column with a smaller column size (1.2 × 40 cm) by elution with CM from 98:2 to 96:4 and 94:6, and the chromatography was repeated to obtain homogenous glycolipid. The purified glycolipids were chromatographed on a TLC plate, developed with CMW (90:10:0.5), and visualized by spraying with orcinol–sulfuric acid reagent, for estimation of the purity.

Synthesis of plasmaloGal alkylglycerol

The chemical acetalization of 3-*O*-galactosyl 2-*O*-acyl 1-*O*-alkyl-*sn*-glyceride was performed according to the method of Sadozai et al. (11, 14) and Yachida et al. (12), with a slight modification. Briefly, the Gal acyl alkylglyceride (15 mg, co-purified from equine brain as described above and the structure characterized by NMR and MS spectra) in dimethylformamide (2 ml) was incubated with *p*-toluene sulfonic acid (2.5 mg) and 1,1-dimethoxyhexadecane (15 mg) at 37°C for 36 h. The reaction mixture was applied on an LH-20 column (1 × 50 cm) with chloroform to remove the reaction solvent and acid. The acetalized products were further purified on a silica-gel column (1 × 40 cm) by elution with 300 ml of each of the CM mixtures with ratios of 100:0 to 99:1, 98:2, 97:3, 96:4, 94:6 and 92:8, for a total of 2,100 ml. An aliquot of the fraction was chromatographed in TLC as above.

The acetalized and purified 4′,6′-*O*-*endo*-hexadecylidene Gal acyl alkylglyceride, the structure of which was characterized by NMR and MS as a deacylated derivative, as described below, was saponified with 0.5% sodium methoxide in methanol (1 ml) for 1 h. The reaction mixture was neutralized with acetic acid, followed by desalting using the LH-20 column described above and running solvent. Through the above two-step reactions, 3-*O*-(4′,6′-*O*-*endo*-hexadecylidene) galactosyl 1-*O*-alkylglycerol (abbreviated S-1, 6 mg) was obtained.

Analysis of lipid and sugar moieties

The fatty aldehyde from acetal linkage and the long chain alkylglycerol both released from synthesized and natural plasmalolipids were separately analyzed from the methanolysates of the purified glycolipids as described previously (12). The constitution ratio of the alkylglycerol species was determined according to a previously reported method (15, 16), using a gas–liquid chromatography (GLC) apparatus (GC-14A, Shimadzu) equipped with a capillary column (0.25 mm × 50 m) coated with 1% DB-5 under programmed temperatures from 150 to 250°C at 5°C per min. The methanolysis of the glycolipid (0.5 mg) was performed in 1 M HCl in methanol at 100°C for 16 h, and the methanolysates were directly applied to a silica-gel column (0.8 × 3 cm) after evaporation of the solvent and acid. The released derivatives of fatty aldehyde (mainly dimethyl acetal, with a minor component of methyl enol ether (12) and fatty acid methyl ester were eluted from the column with 5 ml of $CHCl_3$, and the eluates were evaporated to dryness, dissolved in *n*-hexane and subjected to GLC analysis. The liberated alkylglycerol from plasmaloglycolipid was then eluted with 5 ml of CM, 90:10 from the column, and the eluates were evaporated to dryness and acetylated by adding 0.5 ml of pyridine and 0.1 ml of acetic anhydride followed by standing at 60°C for 20 min. After decomposition of the acetic anhydride with methanol, the solution was evaporated to dryness, and the residue was dissolved in *n*-hexane followed by analysis by GLC. The GLC peaks were characterized using a GC–MS (JEOL JMS-OISG-2) apparatus with electron impact ionization, equipped with a capillary column (0.25 mm × 50 m) coated with 1% OV-1 and the same programmed temperatures as the above GLC, at the NMR–MS Laboratory of the Faculty of Agriculture of Hokkaido University, as described earlier (12).

The sugar species and the substituted sites of the synthesized and natural plasmaloglycolipids were determined by GC–MS as above after derivatization to partially methylated alditol acetate derived from permethylated glycolipid as reported previously (12, 17).

FAB–MS

Positive ion FAB–MS was done on a JEOL JMS-DX300 mass spectrometer equipped with a JMA-DA500 datalizer in the above mentioned NMR–MS laboratory. The sample was bombarded by Xe gas at 6 kV (20 mA) in a matrix of *m*-nitrobenzylalcohol, and the fragments were accelerated at 5 kV, as described previously (18, 19).

NMR spectroscopy

One (1-D)- and two-dimensional (2-D) proton NMR spectra of the glycolipids (0.8 to 6 mg) in 0.3 ml of pyridine-d_5 containing 2% D_2O were obtained at 27°C in a Fourier-transform mode on a Bruker AMX-500 spectrometer at the above laboratory, as described previously (18). Chemical shifts (δ, ppm) were measured using tetramethylsilane as an internal standard.

RESULTS

Isolation of plasmalolipid

The individual, less polar, glycolipid from equine brain migrating faster than GalCer on TLC analysis was isolated and purified by repeated silica-gel column chromatography. The whole brain yielded 2 mg of 4′,6′-*O*-*endo*-plasmaloGalCer (abbreviated as Pc-1 in **Fig. 1**, which was previously characterized (12)), 5 mg of 3-*O*-(4′,6′-*O*-*endo*-plasmaloGal) 1-*O*-alkylglycerol (Pg-1 in Fig. 1, the structure described below), 12 mg of 6-*O*′-acyl GalCer, 8 mg of 2-*O*′-acyl GalCer, 16 mg of 3-*O*-Gal 2-*O*-acyl 1-*O*-alkylglyceride (GalAAG in the figure), 15 mg of 3-*O*-Gal 1,2-*di*-*O*-acyl glyceride, 655 mg of GalCer and 4 mg of 3-*O*-Gal 1-*O*-alkylglycerol (lysoGalAAG in the figure) in reverse order of the polarity on TLC, as shown in Fig. 1. The structures of 6-*O*′-acyl GalCer, 2-*O*′-acyl GalCer and 3-*O*-Gal 2-*O*-acyl 1-*O*-alkylglyceride were identified by proton NMR, GLC and MS (data not shown). The migration of a major glycolipid involved in Pg-1 was not observed on TLC analysis after saponification with 0.2 m sodium methoxide in methanol (data not shown), by which an alikali-labile group such as the *O*-acyl group was released, indicating no modification with an alkali-labile group in the major glycolipid. However, a minor sugar-positive spot was detected at the origin in a TLC plate after the saponification, suggested that Pg-1 had a minor, alkali-labile contaminant. The Pg-1 was, therefore, further chromatographed by TLC and on a silica-gel column using a solvent mixture other than CMW, but sep-

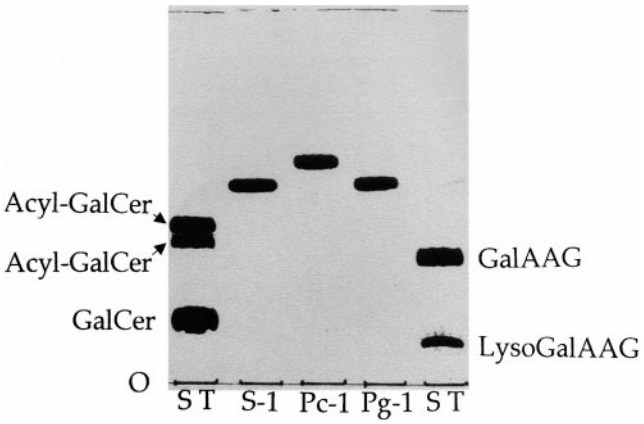


Fig. 1. TLC of plasmaloglycolipids. The TLC plate was developed with CMW (90:10:0.5) and visualized by staining with orcinol-sulfuric acid reagent under heating. Lane ST indicates authentic glycolipid mixtures from equine brain. Lanes S-1 and Pg-1 show synthesized and natural 1-*O*-alkyl 3-*O*-(4′,6′-*O*-*endo*-cyclic fatty acetal Gal1β) glycerol, respectively. Pc-1 is 4′,6′-*O*-*endo*-cyclic acetal GalCer (plasmaloGalCer) isolated previously from equine brain (12). GalAAG and LysoGalAAG are abbreviations of 1-*O*-alkyl 2-*O*-acyl 3-*O*-(Gal1β)-*sn*-glycerol and 1-*O*-alkyl 3-*O*-(Gal1β)-*sn*-glycerol, respectively.

aration was unsuccessful. The cyclic acetal linkage isomers due to 3′, 4′-*O*-*endo*- and -*exo*-type were not detected in TLC analysis using synthesized 3-*O*-(3′,4′-*O*-*endo*- and (-*exo*-plasmaloGal) 2-*O*-acyl 1-*O*-alkylglyceride (see below) as a reference.

Synthesis of 3-*O*-plasmalolGal alkyl glycerol

The chemical synthetic pathway of plasmaloGal alkylglycerol is illustrated in **Fig. 2**, demonstrating, first, acetalization of 3-*O*-Gal 2-*O*-acyl 1-*O*-alkylglyceride with dimethoxyhexadecane and, second, saponification of the acetalized product. The acetalization reaction afforded 3-*O*-(4′,6′-*O*-*endo* hexadecylidene Gal) 2-*O*-acyl 1-*O*-alkylglyceride (the structure was determined as the saponified product, S-1, see below) with a yield of 12.4 mg, 3′,4′-*O*-*endo* hexadecylidene derivative with one of 1.6 mg, 3′,4′-*O*-*exo* hexadecylidene derivative, 0.8 mg and 1.2 mg of an unknown product, and starting 3-*O*-Gal 2-*O*-acyl 1-*O*-alkylglyceride with a yield of 0.5 mg from 18 mg of the starting material. The structures of 3′,4′-*O*-*endo* and -*exo* hexadecylidene derivatives were separately confirmed by 1-D and 2-D proton NMR (data not shown) as reported previously (12). The acetalized 3-*O*-Gal 2-*O*-acyl 1-*O*-alkylglyceride due to a 4′,6′-*O*-*endo* hexadecylidene derivative was next saponified to remove the *O*-acyl group, to give S-1 with a quantitative yield.

Analysis of lipid and sugar moieties

The lipid compositions of Pg-1 and S-1 are summarized in **Table 1**. The individual composition of fatty aldehyde was calculated with the sum of dimethyl acetal and methyl enol ether derivatives, since both derivatives were converted from the acetal residue through anhydrous methanolysis, but fatty aldehyde itself was hardly detected (12). For the analysis of the aldehyde composition of Pg-1, S-1

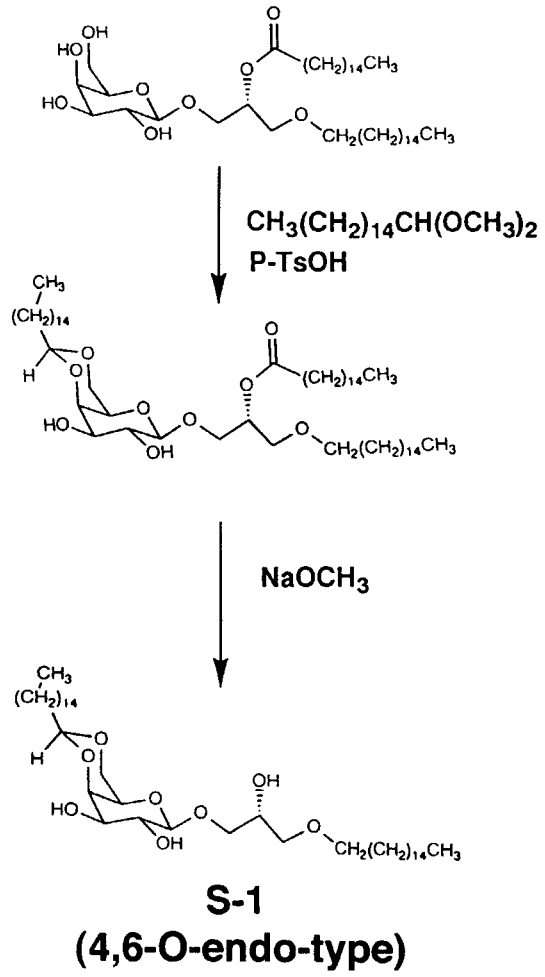


Fig. 2. Synthetic pathway of 1-*O*-alkyl 3-*O*-(4′,6′-*O*-*endo*-cyclic fatty acetal Gal1β)-glycerol (S-1). P-TsOH, *p*-toluene sulfonic acid.

was used as an authentic plasmaloglycolipid because of its exclusive hexadecilydene structure of the acetal linkage (Table 1). The major aldehydes of Pg-1 were hexadecanal and octadecanal, each with a saturated derivative and minor ones were their unsaturated derivatives. In addition, these aldehyde derivatives were liberated from Pg-1 and S-1 in equimolar amounts. In the GLC analysis of the fatty aldehyde derivative fraction, fatty acid methyl ester contamination was 18%, demonstrating that the mono *O*-acyl derivative of Gal-glycerol was involved in Pg-1 with a ratio

TABLE 1. Lipid composition of plasmaloGal alkylglycerol from equine brain

	Aldehyde				Glyceryl Ether			Fatty Acid from Glyceryl Ester		
	16:0	16:1	18:0	18:1	14:0	16:0	18:0	14:0	16:0	18:0
	%				%			%		
Pg-1	54	5	37	4	9	72	tr	2	16	tr
S-1	100	0	0	0	12	88	tr	0	0	0

(Carbon number): (saturated (0) or monounsaturated (1); tr, trace (>1%).

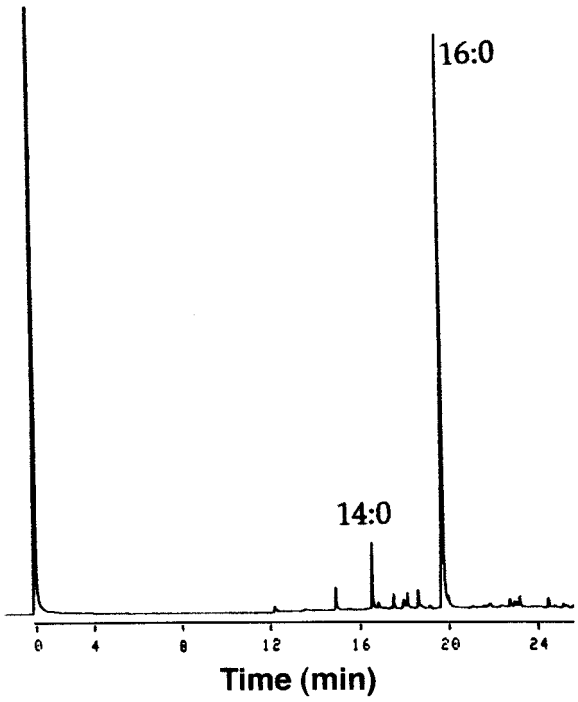


Fig. 3. Gas chromatogram of alkylglycerol liberated from Pg-1. The alkylglycerol was analyzed by GLC after purification with silica-gel chromatography from methanolysates of Pg-1; 14:0 and 16:0 show 1-*O*-tetradecylglycerol and 1-*O*-hexadecylglycerol, respectively.

of 18%. This contamination was in agreement with the proton NMR spectrum of Pg-1 (see below). On the other hand, mono alkylglycerol released from plasmaloglycolipid after methanolysis was analyzed as a peracetylated derivative by GLC, as shown in **Fig. 3**. The GLC peaks were analyzed by GC–MS (data not shown), indicating the ethers to be 1-*O*-alkyl glycerol, not 2-*O*-alkyl glycerol. Tetradecylglyceryl ether (α-tetradecylglycerol, 14:0 in Table 1 and Fig. 3) as a minor ether and hexadecylglyceryl ether (α-hexadecylglycerol of chimyl alcohol, 16:0) as a major one were observed both in Pg-1 and S-1. The sum of these alkyl glycerols in Pg-1 was, however, approximately 80% as compared to those in S-1, suggesting contamination by another glycolipid in Pg-1, which is labile to methanolysis like *O*-acyl glycerol derivatives.

The sugar moieties of Pg-1 and S-1 were analyzed by GC–MS after derivatization to partially methylated alditol acetates, revealing that both glycolipids afforded only 1,4,5,6-*tetra*-*O*-acetyl 2,3-*di*-*O*-methyl galactitol (data not shown). This sugar derivative was similarly detected from permethylated plasmaloGalCer as reported earlier (12), indicating substitution at C-4, 6-*O* of the Gal in Pg-1 and S-1.

FAB–MS

The major and minor ions at *m*/*z* 723 and 695 of S-1 were assigned to a pseudo molecular ion of the plasmalohexosyl alkyl glycerol structure having hexadecylidene (16:0) Gal and hexadecyl glycerol (16:0) for the former ion and hexadecylidene Gal and tetradecyl glycerol (14:0) for the latter ion, respectively, as demonstrated in **Fig. 4A**. Furthermore, the presence of the ion at *m*/*z* 385 confirmed the structure due to hexadecylidene–hexose, and the ion at 317 was suitable for the structure with [H^+ + hexadecyl glycerol] in S-1. On the other hand, the spectrum of Pg-1 had additional ions in comparison to that of S-1, such as peaks at *m*/*z* 751 and 413 as shown in Fig. 4B. These additional ions were responsible for the heterogeneity of chain length of the acetal group of Pg-1, since the length of the alkyl chain of the alkyl glycerol was similar to that of S-1 with 16:0 and 14:0 (Table 1). Regarding the lipid components of Pg-1, the ions at *m*/*z* 723, 751 and 695 were, therefore, assigned to the plasmalohexosyl alkyl glycerol structures having hexadecylidene plus hexadecyl glycerol and/or octadecylidene (18:0) plus tetradecyl glycerol for *m*/*z* 723, octadecylidene plus hexadecyl glycerol for *m*/*z* 751 and hexadecylidene plus tetradecyl glycerol for *m*/*z* 695, respectively. In addition to the ion at *m*/*z* 385 due to the hexadecylidene–hexose structure, the ion at *m*/*z* 413, suitable for the octadecylidene–hexose structure was observed in Pg-1, agreeing with the above lipid data. Further minor peaks at *m*/*z* 709 ("a" in Fig. 4B), 737 ("b") and 765 ("c") were detected in the spectrum of Pg-1. These minor peaks disappeared in the spectrum of saponified Pg-1 (data not shown), demonstrating the peaks originated from an alkali-labile moiety like the acylglycerol derivative contaminating Pg-1. These minor peaks could consequently be identified to originate from plasmalohexosyl glycerol structures of hexadecylidene with tetradecanoyl glycerol for *m*/*z* 709, hexadecylidene with hexadecanoyl glycerol and/or octadecylidene with tetradecanoyl glycerol for *m*/*z* 737, and octadecyldene with hexadecanoyl glycerol for *m*/*z* 765.

NMR spectroscopy

As a measuring solvent for the NMR spectrum of plasmaloGal alkylglycerol, pyridine-d_5 containing D_2O was used instead of dimethylsulfoxide-d_6 containing D_2O, which was previously used for the spectrum of plasmaloGalCer (12), as the plasmaloGal glycerol derivative was hardly soluble in the latter solvent mixture even at 90°C, and further, the derivative was labile at a high temperature.

The 1-D proton NMR spectra of Pg-1 (A) and S-1 (B) were mostly similar without contamination, with minor signals in the former spectrum as shown in **Fig. 5**, indicating that they were similar glycolipids. These contaminating signals (asterisked peaks in Fig. 5A) disappeared in the spectrum of Pg-1 after saponification, and the resultant spectrum was completely coincident to that of S-1 (data not shown). The protons of these glycolipids were assigned by 2-D chemical shift-correlated spectroscopy as summarized in **Table 2**, and the assignments were demonstrated in the spectrum of Pg-1 (Fig. 5A). The Gal moiety was assigned as the β-anomer from the coupling constant of the anomeric proton. The acetal proton at the C-1 position (H-1′′, numbering for the protons is demonstrated in **Fig. 6**) resonated at δ4.797 at 27°C (Table 2) and that at δ4.725 at 90°C (spectrum, not shown) both in Pg-1 and S-1. The latter chemical shift was comparable to that at δ4.682 at 90°C of 4′,6′-*O*-*endo*-plasmaloGalCer, suggesting

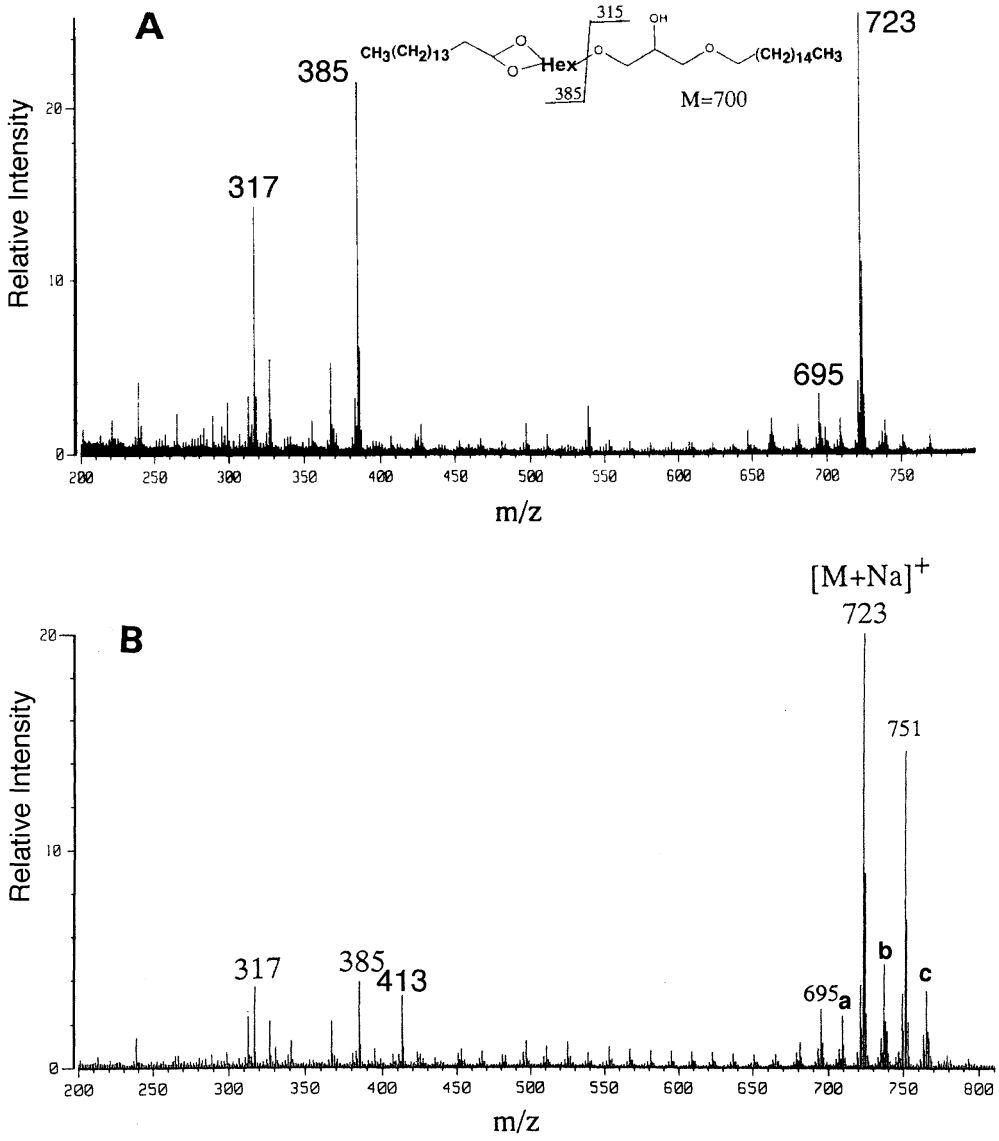


Fig. 4. Positive ion FAB-MS spectra of synthesized and natural plasmaloglycolipids. Panels A and B demonstrate spectra of synthesized (S-1) and natural (Pg-1) plasmaloglycolipids, respectively. Ions "a", "b" and "c" originated from contaminating glycolipids in Pg-1.

that the Pg-1 had the *endo*-type stereostructure of acetal linkage because of a chemical shift higher than δ4.500 of the 4′,6′-*O*-*exo* isomer as mentioned previously (12). Furthermore, significant down-field shifts of H-4′ and H-6′a, but a weak shift of H-6′b, on the galactose moieties (underlined chemical shifts in Table 2) of Pg-1 and S-1 in comparison to those of 3-*O*-Gal 1-*O*-alkyl glycerol (Table 2) enabled us to identify the substitution site of the acetal linkage at C-4 and 6-*O* positions on the galactose. This substituted position is supported by the data from methylation analysis of the sugar moiety of the plasmaloglycolipids as described above.

The direction of the hydrocarbon chain of the acetal group of Pg-1 and S-1 was identified by 2-D homonuclear Overhauser effect spectroscopy (NOESY). The NOE signals of acetal H-1′′ to Gal H-4′ as contour 4′-1′′ and to Gal H-6′a as 6′a-1′′ were observed in the spectrum of S-1 as shown in Fig. 5C. These contours indicated the presence of acetal H-1′′ near Gal H-4′ and Gal H-6′a, and consequently S-1 and Pg-1 were estimated to be *endo*-stererisomers as demonstated in Fig. 6, not *exo*-isomers. If these glycolipids were *exo*-isomers, acetal H-1′′ would have an NOE signal with Gal H-2′ as reported previously (12). It is unclear whether or not NOE peak 4′-1′′ overlapped that of 6′b-1′′ as the chemical shifts of Gal H-4′ and Gal H-6′b were very narrow.

Thus, the results from GLC, GC–MS, FAB–MS, and NMR data indicated the chemical structure of S-1 and the major glycolipid of Pg-1 to be 3-*O*-(4′,6′-*O*-*endo*-cyclic acetal Galβ1)-*sn*-1-*O*-alkyl glycerol, as shown in Fig. 6. The structure of the minor glycolipid involved in Pg-1 was speculated to be 3-*O*-(4′,6′-*O*-*endo*-cyclic acetal Galβ1)-*sn*-1-*O*-acyl glycerol from the NOESY spectrum of the intact Pg-1 (data not shown). The chemical structure of the contaminant glycolipid requires further investigation.

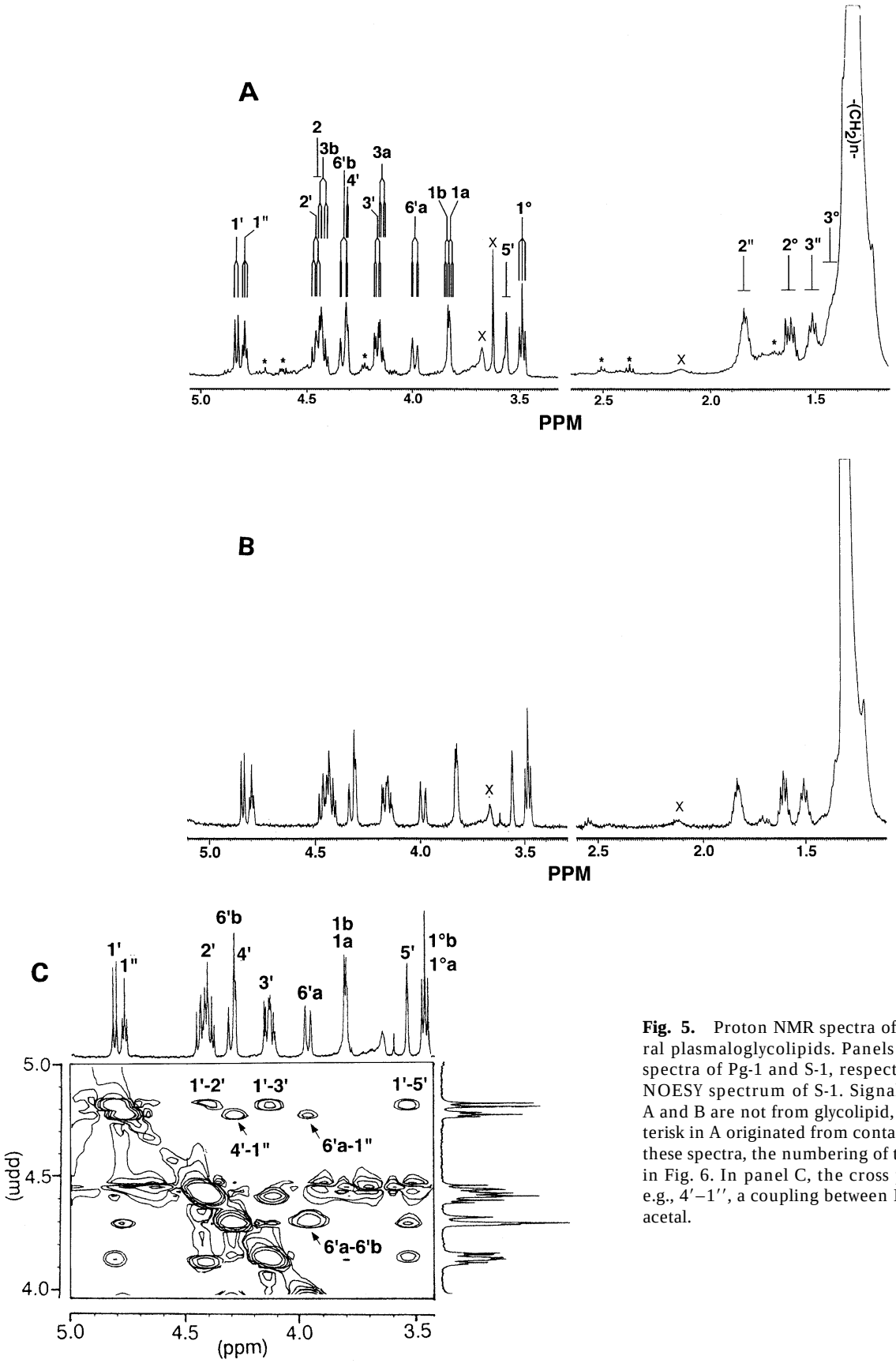


Fig. 5. Proton NMR spectra of synthesized and natural plasmaloglycolipids. Panels A and B indicate 1-D spectra of Pg-1 and S-1, respectively. Panel C is a 2-D NOESY spectrum of S-1. Signals marked with "x" in A and B are not from glycolipid, and signals with an asterisk in A originated from contaminating glycolipid. In these spectra, the numbering of the protons is as shown in Fig. 6. In panel C, the cross peaks are assigned as, e.g., 4′–1′′, a coupling between H-4 on Gal and H-1 on acetal.

TABLE 2. Chemical shifts and coupling constants in the proton NMR spectra of Pg-1, S-1 and 3-*O*-Gal 1-*O*-alkyl glycerol (LysoGalAAG)

	Chemical Shift (δ, ppm)													
	Glycerol					Gal							Acetal		
	H-1a	H-1b	H-2	H-3a	H-3b	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′a	H-6′b	H-1′′	H-2′′	H-3′′
Pg-1	3.820	3.832	4.449	4.143	4.421	4.840	4.461	4.166	4.313	3.560	3.985	4.326	4.797	1.835	1.507
S-1	3.820	3.832	4.446	4.142	4.420	4.840	4.462	4.163	4.310	3.561	3.985	4.326	4.797	1.836	1.506
LysoGalAAG	3.789	3.789	4.407	4.129	4.357	4.850	4.468	4.122	4.529	4.043	4.400	4.400	—	—	—
	Coupling Constant (J, Hz)													
	$J_{1a,1b}$	$J_{1a,2}$	$J_{1b.2}$	$J_{2.3a}$	$J_{2.3b}$	$J_{3a,3b}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6'a}$	$J_{5',6'b}$	$J_{6'a,6'b}$	$J_{1'',2''}$	
Pg-1	10.8	3.7	6.4	3.7	5.7	11.1	7.7	9.4	3.7	<1.5	<1.5	<1.5	12.3	5.1	
S-1	10.8	3.7	6.5	3.5	5.7	11.1	7.7	9.4	3.7	<1.5	<1.5	<1.5	12.3	5.1	
LysoGalAAG	eq[a]	5.4	5.4	3.5	5.7	10.6	7.7	9.4	3.7	<1.5	5.9	5.6	eq[a]	—	

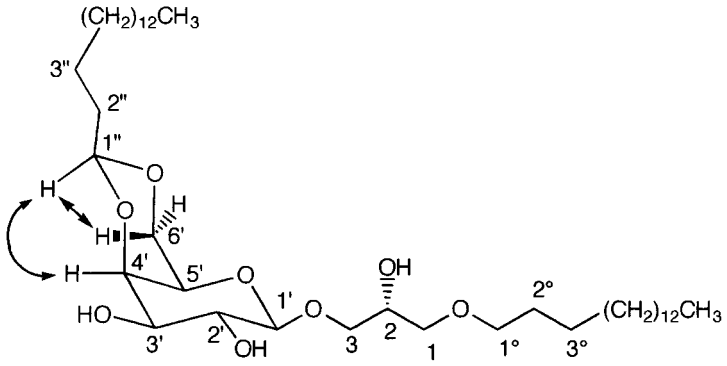
[a] Equivalent.

DISCUSSION

As plasmaloglycolipids, 3′,4′-*O*- and 4′,6′-*O*-*endo*-cyclic acetal derivatives on Gal of GalCer (9) and of Gal sphingosine (10) have been found in human brain, and in equine brain for the former glycolipid (12). These plasmalolipids were classified as sphingolipids, not glycerolipids. A plasmaloglycolipid having a glyceride skeleton was isolated from equine brain and characterized for the first time in this report. As a modification of glycoglycerolipid, *O*-acyl Gal diacylglycerol was earlier isolated from pollack brain, though the structure was only partly characterized (6). The *endo*-stereoisomeric direction of the long chain aldehyde in plasmaloGal alkyl glycerol was exclusively determined by observation of NOE peaks in the proton NMR spectrum, as in the case of plasmaloGalCer (12). The length and binding site of the long chain alcohol to glycerol were analyzed by GLC and GC–MS and found to be an acetate derivative of the alkyl glyceryl ether, revealing 1-hexadecyl- and 1-tetradecyl-glycerol, not 2-alkyl glycerol. However, the plasmaloglycerolipid was approximately 20% contaminated by an acyl-type glycerolipid, probably 3-*O*-plasmaloGal-1-*O*-acyl glycerol, judging from the mobility on TLC of the lipid after saponificatin, GLC analysis and the NMR spectrum. The contaminant was hardly removed by chromatography using a silica-gel column with several solvent mixtures other than the mixture mentioned as above or HPLC using a reversed-phase column (data not shown). The plasmalo sugar moiety with 4′,6′-*O*-Gal was, however, homogeneous between alkyl ether-type glycerol and acyl-type glycerol judging from the NMR spectrum.

As a result, both the plasmalosphingoglycolipids and plasmaloglyceroglycolipids were found to have similar common linkage at the 4′,6′-*O* position of the Gal moiety with an acetal group and similar *endo*-type stereoisomer (9, 12) as a major plasmalolipid. In addition, the acetal residues of these plasmalolipids were estimated to have similar chain lengths with hexadecanal and octadecanal as the major aldehydes. These similarities led us to presume the existence of similar biosynthetic mechanisms, at least, acetalization of Gal moiety, in sphingo- and glycero-plasmalolipids, though the mechanism is not established yet. However, the presence of a free amino group in Gal sphingosine should not be disregarded in the biosynthetic pathway of both plasmaloglycolipids if acetalization directly occurs in Gal-sphingosine and -alkyl glycerol.

The biological information available about plasmaloglycolipid is very limited at present. Both the plasmaloGal alkyl glycerol and plasmaloGal sphingosine were found



1-*O*-hexadecyl-3-*O*-(4,6-*endo*-hexadecylidene-Galβ1)-*sn*-glycerol

Fig. 6. Chemical structure of Pg-1 from equine brain. The interaction between protons observed in 2-D NOESY is demonstrated by arrows.

only in mammalian brains as described above and in this report, whereas its probable mother glycolipid, at least for the former lipid, localizes in testis and spermatozoa in addition to the central nervous system (13). The subcellular distribution of these plasmaloglycolipids is not clear either, though their unmodified glycolipids are exclusively distributed in the plasma membrane in many mammalian cells. The biological function of plasmaloGal sphingosine in prevention of apoptosis of neural cells was recently reported (20). The homology of the external appearance of plasmaloGal alkyl glycerol with that of plasmaloGal sphingosine might indicate biological activity similar to that of the latter lipid; however, a free amino residue would be critically important in the particular structure of the latter lipid. Further investigation of biological function of the plasmaloglycolipid is required.

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