Six Trigalactosylceramides from the Leech (*Hirudo nipponica*)

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Six neutral glycosphingolipids were isolated in the pure state from the leech, *Hirudo nipponica* (Annelida). In contrast to the zwitterionic monogalactosylceramides carrying a choline phosphate group so far obtained, all compounds are non-zwitterionic glycosphingolipids, trigalactosylceramides. Five compounds possess a Galα1–6Galα1–6Galβ1–Cer core, and one is unique in having a Galα1–6Galβ1–6Galβ1–Cer structure. Their full structures have been determined on the bases of chemical and spectral evidence.

Key words glycosphingolipid; trigalactosylceramide; leech; *Hirudo nipponica*; Annelida

Since the finding and isolation of remarkably large amounts of alkylglycerophosphocholines from the earth worm (*Pheretima asiatica*),[1] we have been interested in the constituents, especially the lipid composition, in members of the phylum Annelida. Up to now, about thirty homogenous zwitterionic monogalactosylceramides (ZGSLs)[2] carrying a choline phosphate group, as well as various alkyl ether-type glycerophosphocholines,[3] have been isolated from various annelids. We have continued our preceding study[4] on the lipid composition in members of the phylum, and have isolated six glycosphingolipids (GSLs) in pure form from the leech, *Hirudo nipponica*. Analyses of their spectral data and of the components of the parent GSLs demonstrated that all of them are neutral glycosphingolipids, trigalactosylceramides of Galα1–6Galα1–6Galβ1–Cer or Galα1–6Galβ1–6Galβ1–Cer type. This paper deals with the isolation and structure elucidation of these compounds.

Results and Discussion

The total lipid fraction obtained from the $CHCl_3$–MeOH extract of the whole dried bodies of the materials was subjected to silica gel and Cosmosil 75C_{18}-OPN column chromatographies with various solvents to yield a crude glycolipid fraction (fr. 9).[4] It contains at least 17 components, as revealed by HPLC. We applied repeated-recycling preparative HPLC with a reverse-phase column, and succeeded in isolating six compounds **1**—**6** in a pure state.

The negative ion FAB-MS of **1** exhibited a pseudo-molecular ion and fragment ion peaks arising from cleavage of glycosidic linkages at *m/z* 1186 $[M-H]^-$, 1024 (1186 − hexosyl unit), 862 (1024 − hexosyl unit) and 700 (862 − hexosyl unit). The ^{1}H-NMR spectrum of **1** showed three anomeric proton signals at δ 4.22 (J = 7.3 Hz), 4.85 (J = 4.3 Hz) and 4.99 (J = 4.3 Hz) and six olefinic hydrogens, in addition to signals ascribable to three methylenes next to a double bond (6H, δ 2.03—2.06) and to a methylene located between two double bonds, but it gave no signals due to a phosphocholine unit as in the ZGSLs so far obtained.[2,4] In the ^{13}C-NMR spectrum, the chemical shifts of signals due to a ceramide group (Cer) closely corresponded to those found in the ZGSLs with a tri-unsaturated long-chain base (LCB), suggesting that **1** is a ceramide trisaccharide possessing a 1,4-pentadiene-1,5-diyl (–CH=CH–CH_2–CH=CH–) group.[4]

Methanolysis of **1** with 7.5% HCl–MeOH liberated a fatty acid methyl ester and a sugar unit. The former was analyzed by gas chromatography (GC) and electron impact mass spectrometry (EI-MS), which revealed methyl *n*-tetracosanoate. The latter was converted into a trimethylsilyl ether, which in GC gave peaks identical with those of authentic trimethylsilyl ester of methyl galactoside. By the method of Hara *et al.*,[5] the galactose unit was proved to have D form. From these findings, in conjunction with the molecular weight (M.W. 1187), compound **1** was considered to be a trigalactosylceramide with the same $C_{22:3}$ docosasphingatrienine as that of the ZGSLs reported previously.[4]

According to the Vincenti method,[6] compound **1** was converted into the dimethyl disulfide derivative (**1a**). Its EI-MS exhibited the diagnostically important fragment ion peak at *m/z* 201, which was regarded as being due to a fragment ion produced by cleavage between the C-11 and C-12 sulfided carbons. The two-dimensional shift correlation (^{1}H–^{1}H COSY) spectrum gave a series of correlation peaks from H-3 to H_2-7, and also showed a cross peak between H_2-7 and olefinic hydrogen. These findings showed that the 1,4-pentadiene-1,5-diyl group is located at C-8.

The two-dimensional nuclear Overhauser effect (NOESY) spectrum of **1** gave a correlation peak between H-4 and H_2-6, and therefore the geometry of the double bond at C-4 was concluded to be *trans*. On the other hand, because of overlapping of the signals, that of the double bonds at C-8 and C-11 was determined from the chemical shifts of the allylic carbon signals. In the ^{13}C-NMR spectrum, both allylic carbons (C-7 and C-13) were observed at δ 27.8, while the bis-allylic one (the C-10 carbon between the two double bonds, C-8 and C-11) appeared at δ 26.1. On the basis of their chemical shifts,[7,8] the geometry of both two double bonds (C-8 and C-11) was assigned as *cis*.

Hydrogenation over palladium carbon of **1** followed by methanolysis gave a saturated LCB, which was acetylated to give an LCB triacetate (**1b**), which was identified as D-*erythro*-docosasphinganine triacetate by comparison of the optical rotation and spectral data with those of an authentic sample.[4] From the information obtained above, it was clear that the sphingosine unit of **1** was

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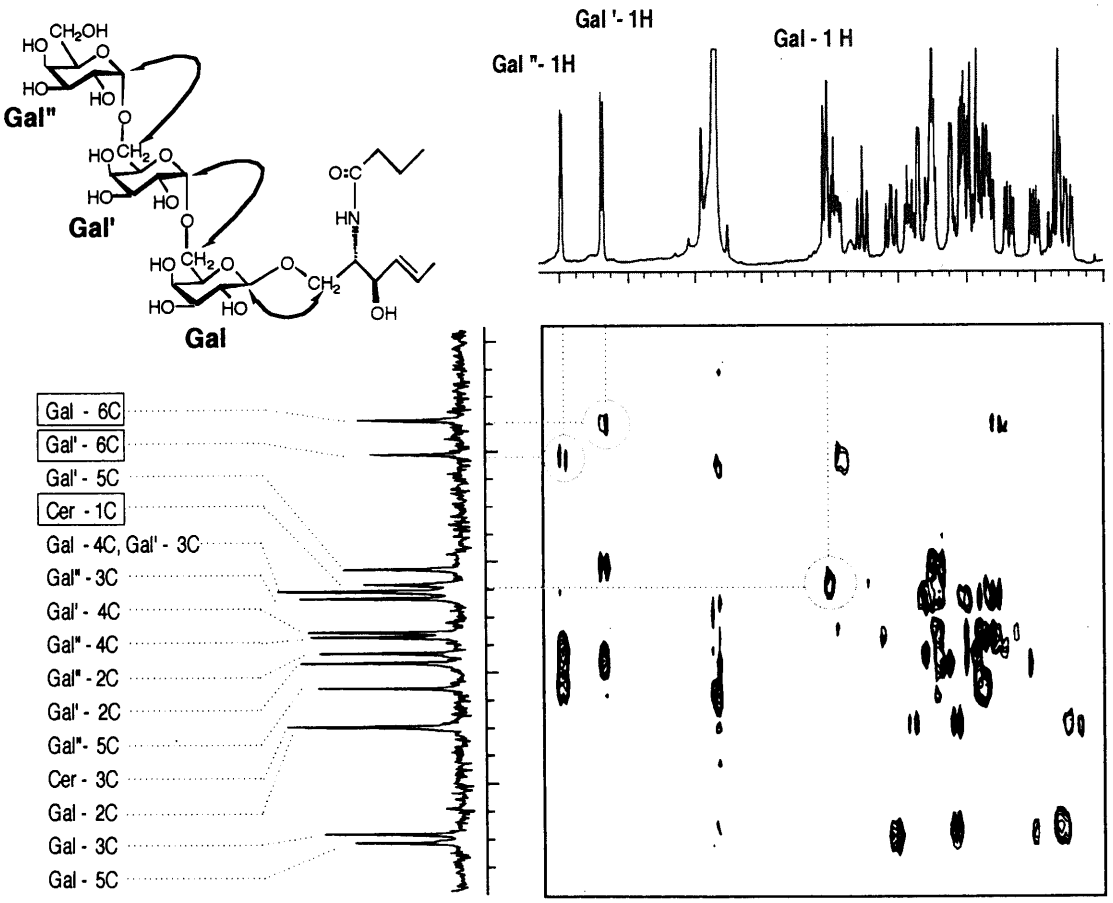

Fig. 1. HMBC Correlations of **1**

D-*erythro*-(4*E*,8*Z*,11*Z*)-docosasphingatrienine.

The position and mode of the glycosidic linkages were determined by two-dimensional heteronuclear multiple bond connectivity spectroscopy (HMBC). In the HMBC spectrum, significant correlation peaks, H-1 of the inner galactose (Gal)/C-1 of Cer, H-1 of the middle galactose (Gal′)/C-6 of Gal and H-1 of the outer galactose (Gal″)/C-6 of Gal′ were observed (Fig. 1). The configuration at the C-1 position of each of Gal, Gal′ and Gal″ was concluded to be β, α and α, respectively, judging from the coupling constants and chemical shifts of the corresponding C-1 carbon[9] found in the ^{1}H- and ^{13}C-NMR spectra (Table).

On the basis of all the results described above, the structure of **1** was determined to be *N*-tetracosanoyl-1-*O*-[α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl]-(4*E*,8*Z*,11*Z*)-docosasphingatrienine.

The ^{1}H- and ^{13}C-NMR spectra of compounds **2**—**5** were almost indistinguishable from each other, except for signals due to methyl groups, and signals assignable to the sugar moieties were closely correlated with those of **1**, suggesting that they are analogs of **1**. By analyses of the NMR spectral data and of the degradation products of each compound in the same manner as described for **1**, they were found to have the same oligosaccharide moiety, Galα1–6Galα1–6Galβ1–Cer, as that of **1**. The ceramide part of each of **2**—**5** was identified as *N*-hexadecanoyl-(4*E*)-octadeca- and *N*-hexadecanoyl-(4*E*)-nonadecasphingenines, and *N*-docosadecanoyl-(4*E*)-17-methyloctadecanoyl- and *N*-tetracosanoyl-(4*E*)-17-methyloctadecasphingenines, respectively, by comparison of the physical and spectral data with the corresponding values of authentic samples,[2,4] and their structures were thus characterized as represented in Fig. 2.

The ^{1}H-NMR spectrum of **6**, in contrast to those of **1**—**5**, differed markedly in the chemical shifts of signals arising from the sugar moiety, including those of the three anomeric protons, 4.24 (J=7.3 Hz), 4.25 (J=7.3 Hz) and 4.90 (J=4.3 Hz). Analyses of the components produced by the chemical degradation of **6** revealed that it contains *N*-hexadecanoyl-(4*E*)-octadecasphingenine and that its oligosaccharide consists solely of galactose. These findings demonstrated that **6** is another trigalactosylceramide, differing from **1**—**5** in the oligosaccharide linkage. The HMBC spectrum of **6** gave notable correlation peaks, H-1 of Gal/C-1 of Cer, H-1 of Gal′/C-6 of Gal and H-1 of Gal″/C-6 of Gal′, confirming the positions of the sugar linkage. The configurations of the C-1 positions of Gal, Gal′ and Gal″ were concluded to be β, β and α, respectively, based on the coupling constants of the anomeric proton signals (Table 1). Consequently, **6** was defined as *N*-hexadecanoyl-1-*O*-[α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl]-(4*E*)-octadecasphingenine.

Thus, six trigalactosylceramides were isolated in pure form. Among them, five compounds (**1**—**5**) possess a Galα1–6Galα1–6Galβ1–Cer core,[10] and the last (**6**) is

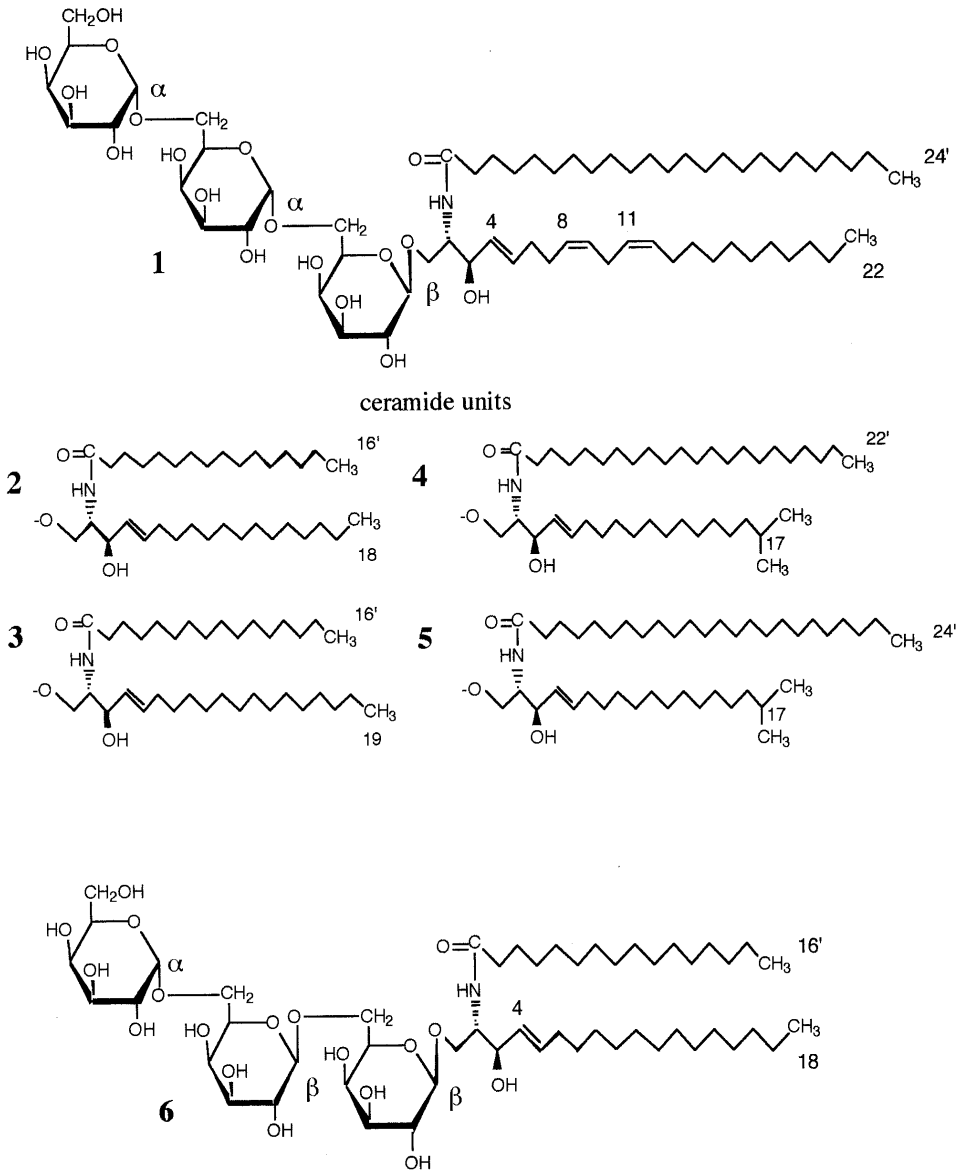

Fig. 2

unique in having a Galα1–6Galβ1–6Galβ1–Cer structure. In view of the similarity of the sphingosine units of compounds **1**—**6** to those of phosphocholine-linked monogalactosylceramides,[4] these neutral GSLs are likely to be precursors of amphoteric galactocerebrosides.

Experimental

The NMR spectra were recorded on a JEOL JMN GSX 400 instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C) at a probe temperature of 35 °C using tetramethylsilane as an internal reference. MS were acquired on a JEOL JMS DX-300 spectrometer (EI-MS: ionization voltage, 30 eV; accelerating voltage, 3—10 kV. Positive ion FAB-MS: accelerating voltage, 3 kV; matrix, glycerol; collision gas, Xe). Optical rotations were measured (24—26 °C) with a JASCO DIP-140 polarimeter. TLC was carried out on silica gel HPTLC with Al sheets (Merck Art. 5556). Spots were visualized with 5% H_2SO_4 in MeOH (by heating). Column chromatography was carried out on Merck Silica gel (230—400 mesh, Art. 9385), and Cosmosil 75C_{18}-OPN (Nacalai Tesque). Preparative HPLC was conducted over an L-column octadecyl silica (10 × 250 mm, Chemicals Inspection & Testing Ins.) on a JASCO PU-980 equipped with a model 504R unit (GL Sciences). Recycling HPLC was carried out on a JASCO PU-980 equipped with a JASCO preparative recycle valve. The analytical GC was carried out with a Hitachi G-3000 equipped with a 30 : 1 splitter and a flame ionization detector.

Isolation of GSLs 1—6 The $CHCl_3$–MeOH extractives (72.2 g) of the crushed powder (1 kg) of the leech, *Hirudo nipponica* (sold as a crude drug "Suitetsu," purchased from Tochimoto Tenkaido, 1993), were treated with $CHCl_3$–MeOH–H_2O (1 : 1 : 1, 600 ml), and the lower layer was collected and concentrated to give a total lipid fraction (55.6 g). It was placed on a silica gel column and eluted successively with $CHCl_3$–MeOH (8 : 2→7 : 3)→$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5→6 : 4 : 1→5 : 5 : 1) to give six fractions, fr. 1 (38.3 g), fr. 2 (3.3 g), fr. 3 (1.9 g), fr. 4 (4.6 g), fr. 5 (1.8 g) and fr. 6 (4.5 g). Fraction 4 was further separated by silica gel column chromatography with $CHCl_3$–MeOH (7 : 3) to give fr. 5 (2.7 g), fr. 6 (1.1 g) and fr. 7 (0.7 g). Fraction 6 was subjected to chromatography on a 75C_{18}-OPN column using MeOH→$CHCl_3$–MeOH (1 : 1) as the eluent to yield three fractions, fr. 8 (270 mg), fr. 9 (464 mg) and fr. 10 (62 mg). Fraction 9 was separated by HPLC (mobile phase: $CHCl_3$–MeOH, 1.5 : 10) to give seventeen GSL fractions, 9-1 (14.6 mg), 9-2 (19.8 mg), 9-3 (15.4 mg), 9-4 (19.1 mg), 9-5 (11.4 mg), 9-6 (7.7 mg), 9-7 (42.0 mg), 9-8 (10.0 mg), 9-9 (25.0 mg), 9-10 (8.0 mg), 9-11 (15.0 mg), 9-12 (20.0 mg), 9-13 (35.0 mg), 9-14 (8.0 mg), 9-15 (10.0 mg), 9-16 (13.0 mg) and 9-17 (8.0 mg). Among them, selected fractions (9-2, 9-3, 9-9, 9-12 and 9-13) were purified by HPLC in a recycling mode by use of $CHCl_3$–MeOH–H_2O (5 : 20 : 1). The solvent of each fraction was evaporated under a nitrogen stream to yield compounds **1** and **5** (11.0 and 10.0 mg, 8 cycles from fr. 9-13), **2** and **6** (3.8 and 4.4 mg, 17 cycles from fr. 9-2), **3** (5.0 mg, 30 cycles) and **4** (11.3 mg, 19 cycles from fr. 9-9). **1**: mp 135—146 °C, $[\alpha]_D$ +46.7° (c = 1.1, $CHCl_3$–MeOH, 1 : 1). Negative ion FAB-MS m/z (rel. int.): 1186 $[M-H]^-$ (100), 1024 (32),

Table 1. ^{13}C- and ^{1}H-NMR Spectral Data for Glycosphingolipids ($CDCl_3$–CD_3OD)

No.		1		2		3	
		^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Cer	1	69.4	3.50 (dd, 7.0, 10.0) 4.18 (dd, 4.0, 10.0)	69.6	3.51 (dd, 7.0, 10.0) 4.19 (dd, 4.0, 10.0)	69.6	3.49 (dd, 7.0, 10.0) 4.18 (dd, 4.0, 10.0)
Cer	2	53.6	3.97 (m)	53.9	3.96 (m)	53.8	3.96 (m)
Cer	3	72.0	4.12 (t, 7.0)	71.6	4.11 (t, 7.0)	71.5	4.10 (t, 7.0)
Cer	4	130.4	5.44 (dd, 7.0, 15.0)	130.6	5.45 (dd, 7.0, 15.0)	130.5	5.44 (dd, 7.0, 15.0)
Cer	5	134.7	5.70 (ddd, 7.0, 8.0, 15.0)	134.8	5.70 (ddd, 7.0, 8.0, 15.0)	134.8	5.69 (ddd, 7.0, 8.0, 15.0)
Cer	6	33.0	2.03	32.6	2.02	32.5	2.04
Cer	7	27.8	2.06	—	*ca.* 1.28	—	*ca.* 1.27
Cer	8	130.6	5.30—5.40	—	*ca.* 1.28	—	*ca.* 1.27
Cer	9	128.5	5.30—5.40	—	*ca.* 1.28	—	*ca.* 1.27
Cer	10	26.1	2.78 (2H, t, 7.0)	—	*ca.* 1.28	—	*ca.* 1.27
Cer	11	128.4	5.30—5.40	—	*ca.* 1.28	—	*ca.* 1.27
Cer	12	130.5	5.30—5.40	—	*ca.* 1.28	—	*ca.* 1.27
Cer	13	27.8	2.06	—	*ca.* 1.28	—	*ca.* 1.27
–CH–$(CH_3)_2$		—	—	—	—	—	—
CH_3		14.2	0.88 (3H, t, 7.0)	14.3	0.89 (3H, t, 7.0)	14.3	0.88 (3H, t, 7.0)
FA	2″	175.1	2.17 (2H, t, 7.0)	175.3	2.17 (2H, t, 7.0)	175.2	2.14 (2H, t, 7.0)
CH_3		14.2	0.88 (3H, t, 7.0)	14.3	0.89 (3H, t, 7.0)	14.3	0.88 (3H, t, 7.0)
Gal	1	104.5	4.22 (d, 7.3)	104.7	4.20 (d, 7.3)	104.7	4.18 (d, 7.3)
Gal	2	72.3	3.53	72.2	3.53	72.2	3.51
Gal	3	73.9	3.52	74.2	3.52	74.1	3.50
Gal	4	69.5	3.85 (dd, 1.0, 3.0)	69.7[a]	3.85 (dd, 1.0, 3.0)	69.7	3.84 (dd, 1.0, 3.0)
Gal	5	74.1	3.73	74.3	3.73	74.2	3.74
Gal	6	66.4	3.60 (dd, 4.5, 10.5) 4.02 (dd, 7.5, 10.5)	66.7	3.60 (dd, 4.5, 10.5) 4.02 (dd, 7.5, 10.5)	66.6	3.58 (dd, 4.5, 10.5) 4.01 (dd, 7.5, 10.5)
Gal′	1	98.4	4.85 (d, 4.3)	99.0	4.87 (d, 4.3)	98.9	4.86 (d, 4.3)
Gal′	2	70.9	3.80	71.0	3.80	70.9	3.78
Gal′	3	69.5	3.80	69.8[a]	3.80	69.7	3.78
Gal′	4	70.3	3.90	70.6	3.91	69.8	3.90
Gal′	5	69.2	4.18 (m)	69.4	4.18 (m)	68.3	4.16 (m)
Gal′	6	67.0	3.68 (dd, 4.5, 10.5) 3.90	67.3	3.67 (dd, 4.5, 10.5) 3.91	67.2	3.66 (dd, 4.5, 10.5) 3.90
Gal″	1	98.4	4.99 (d, 4.3)	98.8	4.98 (d, 4.3)	98.7	4.98 (d, 4.3)
Gal″	2	70.6	3.80	70.9	3.80	70.8	3.80
Gal″	3	69.6	3.78	69.9	3.78	69.7	3.78
Gal″	4	70.4	3.94 (dd, 1.0, 3.0)	70.9	3.94 (dd, 1.0, 3.0)	70.6	3.94 (dd, 1.0, 3.0)
Gal″	5	71.3	3.90	71.0	3.91	71.0	3.90
Gal″	6	62.3	3.73	62.4	3.73	62.4	3.74

862 (32), 700 (36). Positive ion FAB-MS *m/z*: 1211 $[M+Na+H]^+$. **2**: mp 130—138 °C, $[\alpha]_D$ +51.6° (*c*=0.4, $CHCl_3$–MeOH, 1:1). Positive ion FAB-MS *m/z*: 1047 $[M+Na+H]^+$. **3**: mp 130—140 °C, $[\alpha]_D$ +41.7° (*c*=0.5, $CHCl_3$–MeOH, 1:1). Positive ion FAB-MS *m/z*: 1061 $[M+Na+H]^+$. **4**: mp 133—146 °C, $[\alpha]_D$ +55.4° (*c*=1.1, $CHCl_3$–MeOH, 1:1). Positive ion FAB-MS *m/z*: 1145 $[M+Na+H]^+$. **5**: mp 137—145 °C, $[\alpha]_D$ +52.8° (*c*=1.0, $CHCl_3$–MeOH, 1:1). Positive ion FAB-MS *m/z*: 1173 $[M+Na+H]^+$. **6**: mp 132—138 °C, $[\alpha]_D$ +8.3° (*c*=0.8, $CHCl_3$–MeOH, 1:1). Positive ion FAB-MS *m/z*: 1047 $[M+Na+H]^+$. ^{1}H- and ^{13}C-NMR spectral data of **1**—**6** ($CDCl_3$–CD_3OD, 1:1) δ: see Table.

Analysis of the Fatty Acids Each (*ca.* 2 mg) of **1**—**6** was treated with 5% methanolic HCl at 90 °C for 1 h. The fatty acid methyl ester produced was extracted with *n*-hexane, and analyzed by GC (fused silica capillary column Bonded MPS-50, Quadrex, 0.25 mm × 50 m; column temperature, 230 °C (hold, 12 min)→240 °C at 1 °C/min; carrier gas, He at 33.4 ml/min): t_R (min): 6.54 (methyl *n*-hexadecanoate from **2**, **3** and **6**), 21.02 (methyl *n*-docosanoate from **4**), 31.75 (methyl *n*-tetracosanoate from **1** and **5**). Each fatty acid methyl ester was identified by EI-MS comparison with an authentic sample of the corresponding methyl ester.

Analysis of the Sugar Unit The MeOH layer obtained above was evaporated to dryness to give a methyl glycoside fraction. A part of the fraction was treated with *N*-trimethylsilylimidazole and the product was examined by GC (fused silica capillary column Bonded MPS-50, Quadrex, 0.25 mm × 50 m; column temperature, 180 °C; carrier gas, He at 33.4 ml/min). t_R (min): 11.0, 12.2, 12.6, 13.7. The peaks were identical with those of authentic methyl galactose derivatives.

The remaining methyl glycoside fraction was heated with 1 ml of 2 N HCl at 90 °C for 1 h. The reaction mixture was neutralized with Ag_2CO_3. The precipitate was removed by centrifugation and the supernatant was shaken with $CHCl_3$ (1 ml). The $CHCl_3$ layer was separated and evaporated to dryness under a nitrogen stream. The residue was applied to a Sephadex LH-20 column and eluted with MeOH to give a sugar fraction. This was analyzed by GC according to the method described in the preceding paper,[4] and identified as the D-galactose derivative (t_R, 19.45 and 19.52 min).

Preparation of the Dimethyl Disulfide Derivative Carbon disulfide (0.2 ml) and iodine (1 mg) were added to **1** (2 mg) in dimethyl disulfide (0.2 ml), and the mixture was kept at 60 °C for 40 h. The reaction was quenched with 5% aqueous solution of sodium thiosulfate, then the mixture was shaken with $CHCl_3$–MeOH (1:1, 3 ml). The lower layer was separated and concentrated under a nitrogen stream. The product (**1a**) was subjected to analysis by EI-MS. **1a**: EI-MS *m/z*: 201 $[C_{12}H_{25}S]$.

Hydrogenation of the LCB Part Compound **1** (10 mg) was hydrogenated over 10% palladium carbon (100 mg) in $CHCl_3$–MeOH (1:4, 50 ml). The catalyst was filtered off and the filtrate was evaporated to dryness to give a product. This was methanolyzed with 7.5% methanolic HCl at 90 °C for 2 h. The fatty acid formed was extracted with *n*-hexane and the methanolic layer was neutralized by adding a small excess of Ag_2CO_3. After centrifugation, the supernatant was evaporated to dryness to give a residue. The residue was chromatographed on a Sephadex LH-20 column with MeOH, yielding a product. This was acetylated with acetic anhydride–pyridine (1:1, 1 ml) at room temperature for 1 d to give an LCB triacetate (**1b**, 3 mg). **1b**: $[\alpha]_D$ +8.0° (*c*=0.1, $CHCl_3$). Positive ion FAB-MS *m/z*: 484 $[M+H]^+$. The ^{1}H-NMR was in accord with that of D-*erythro*-docosasphinganine. Each of

Table 1. (continued)

No.	4		5		6	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Cer 1	69.6	3.50 (dd, 7.0, 10.0) 4.19 (dd, 4.0, 10.0)	70.1	3.50 (dd, 7.0, 10.0) 4.19 (dd, 4.0, 10.0)	69.4	3.62 (dd, 7.0, 10.0) 4.19 (dd, 4.0, 10.0)
Cer 2	54.0	3.98 (m)	54.3	3.98 (m)	54.1	3.97 (m)
Cer 3	71.6	4.13 (t, 7.0)	72.6	4.13 (t, 7.0)	72.5	4.12 (t, 7.0)
Cer 4	130.7	5.45 (dd, 7.0, 15.0)	130.9	5.44 (dd, 7.0, 15.0)	130.2	5.45 (dd, 7.0, 15.0)
Cer 5	134.8	5.71 (ddd, 7.0, 8.0, 15.0)	135.4	5.70 (ddd, 7.0, 8.0, 15.0)	134.7	5.70 (ddd, 7.0, 8.0, 15.0)
Cer 6	32.6	2.02	33.1	2.03	32.5	2.03
Cer 7	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
Cer 8	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
Cer 9	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
Cer 10	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
Cer 11	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
Cer 12	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
Cer 13	—	*ca.* 1.28	—	*ca.* 1.27	—	*ca.* 1.28
$-\underline{CH}-(CH_3)_2$	27.8	1.55[b]	28.5	1.54[b]	—	—
CH_3	22.9	0.89 (6H, d, 7.0)	23.8	0.88 (6H, d, 7.0)	14.2	0.89 (3H, t, 7.0)
FA 2″	175.3	2.16 (2H, t, 7.0)	175.7	2.17 (2H, t, 7.0)	175.2	2.17 (2H, t, 7.0)
CH_3	14.3	0.89 (3H, t, 7.0)	14.9	0.88 (3H, t, 7.0)	14.3	0.89 (3H, t, 7.0)
Gal 1	104.8	4.22 (d, 7.3)	105.1	4.21 (d, 7.3)	104.6	4.24 (d, 7.3)
Gal 2	72.3	3.53	72.7	3.53	72.0	3.53
Gal 3	74.2	3.52	74.6	3.52	74.2	3.53
Gal 4	69.8	3.85 (dd, 1.0, 3.0)	70.1[a]	3.85 (dd, 1.0, 3.0)	69.3	3.88 (dd, 1.0, 3.0)
Gal 5	74.3	3.73	74.7	3.73	74.1[a]	3.75
Gal 6	66.8	3.60 (dd, 4.5, 10.5) 4.00 (dd, 7.5, 10.5)	67.1	3.60 (dd, 4.5, 10.5) 4.00 (dd, 7.5, 10.5)	68.5	3.88 3.99 (dd, 7.5, 10.5)
Gal′ 1	99.1	4.88 (d, 4.3)	99.3	4.88 (d, 4.3)	104.2	4.25 (d, 7.3)
Gal′ 2	70.9	3.80	71.5	3.80	72.0	3.54
Gal′ 3	69.8	3.80	70.2[a]	3.80	74.2	3.54
Gal′ 4	70.6	3.92	70.9	3.90	69.3	3.90
Gal′ 5	69.5	4.18 (m)	69.8	4.18 (m)	74.6[a]	3.75
Gal′ 6	67.4	3.69 (dd, 4.5, 10.5) 3.92	67.8	3.68 (dd, 4.5, 10.5) 3.90	67.5	3.65 (dd, 4.5, 10.5) 3.93
Gal″ 1	98.9	4.99 (d, 4.3)	99.1	4.99 (d, 4.3)	99.8	4.90 (d, 4.3)
Gal″ 2	70.9	3.80	71.3	3.80	69.7	3.80
Gal″ 3	69.8	3.78	70.2	3.78	70.9	3.78
Gal″ 4	70.6	3.94 (dd, 1.0, 3.0)	71.1	3.95 (dd, 1.0, 3.0)	70.4	3.94 (dd, 1.0, 3.0)
Gal″ 5	71.1	3.92	71.9	3.90	71.6	3.91
Gal″ 6	62.4	3.73	63.0	3.73 3.80	62.2	3.74 3.80

Coupling constants (J) in Hz are given in parentheses. Cer, ceramide unit; FA, fatty acid unit; Gal, galactose unit. *a*) The assignments may be interchanged. *b*) Signals appeared as a septet ($J=7.0$ Hz) because of overlapping.

2—**6** was treated in the same manner as described for **1**, and the long-chain bases formed were identified as D-*erythro*-octadeca- (from **2** and **6**), D-*erythro*-nonadeca- (from **3**), and D-*erythro*-17-methyloctadeca-sphinganines (from **4** and **5**).

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References and Notes

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