June 1994 Chem. Pharm. Bull. 42(6) 1247—1250 (1994) 1247

Phosphocholine-Bonded Galactosylceramides from the Crude Drug "Jiryu" (the Earthworm, *Pheretima asiatica*)

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Received January 17, 1994; accepted February 10, 1994

Eight glycosphingolipids have been isolated in the pure state from the crude drug "Jiryu" (the dried body wall of the earthworm, *Pheretima asiatica*). Their structures have been determined on the basis of chemical and spectroscopic evidence. All the compounds carry a phosphocholine group attached to the sugar moiety, and seven of them consist of ceramide units with branched-chain bases ($C_{18:1}$, $C_{19:1}$ iso type).

Keywords phosphocholine-bonded galactosylceramide; iso-type sphingenine; *Pheretima asiatica*; zwitterionic glycosphingolipid; earthworm; Jiryu

Recent studies on the glycosphingolipids of phylum Annelida, such as *Marphysa sanguinea*,[1] *Neanthes diversicolor*[2] and *Pheretima hilgendorfi*,[3] have revealed that all the galactosylceramides have, in contrast to other glycosylceramides so far reported, a phosphocholine group at the C-6 position of the galactose moiety to form a zwitterionic structure. In our systematic studies on the lipid composition of Annelida, we isolated three phosphocholine-bonded galactosylceramides (**1**—**3**)[4] from the crude drug "Jiryu" (the dried body wall of the earthworm, *P. asiatica*) and characterized their structures. On further examination of the glycosphingolipid fraction, eight additional homologs and/or analogs were obtained. This paper deals with the isolation and structure elucidation of these compounds.

The total lipid fraction was subjected to a combination of silica gel and Cosmosil 75C_{18}-OPN chromatographies with various solvent systems to yield fractions 5 (crude glycerophospholipid fraction) and 6.[4] The latter was further purified with an Asahipack GS-320 column using MeOH to give two crude glycosphingolipid fractions 6a and 6b. Fraction 6a showed nine peaks, I—IX, on the HPLC chromatogram (Fig. 1). Nine fractions (frs. 1—9) corresponding to peaks I—IX were collected and their purity was examined by positive ion FAB-MS. The fractions, which showed several $[M+H]^+$ ion peaks, were further purified by means of HPLC in a recycling mode, and in addition to **1**, **2** and **3**, eight compounds were isolated in the pure state, that is, **4** and **5** from fr. 2, **6** from fr. 3, **7** from fr. 4, **8** from fr. 5, **9** from fr. 6, **10** from fr. 7 and **11** from fr. 9.

The positive ion FAB-MS of each gave a single $[M+H]^+$ ion peak, while the ^1H-NMR spectra showed characteristic signals due to monoglycosylceramide and phosphocholine groups, and except for the methyl signals in the highest field region, the chemical shifts and coupling constants of other signals were almost the same as those of **1**—**3** obtained previously. Furthermore, the ^{13}C-NMR spectra showed two signals ascribable to olefinic carbons, and those due to C-5 and C-6 of the hexosyl unit and to C-1″ and C-2″ of the choline group appeared as doublets owing to coupling with ^{31}P.[1,2] Therefore, these compounds were considered to have the same planar and stereo structures as those of **1**—**3**. With regard to the ceramide unit, the ^1H-NMR spectrum of **8** showed the signals (6H, t, $J=7.0$ Hz) due to two primary methyl groups, while the others exhibited signals (6H, d, $J=7.0$ Hz) and (3H, t, $J=7.0$ Hz) assignable to two secondary and one primary methyl groups, respectively. These findings showed that the ceramide unit of the former one contain only straight carbon chains and the latter seven are composed of straight and branched carbon chains (iso type).

The HF degradation[5] of each compound followed by methanolysis gave a fatty acid methyl ester and a long-chain base together with a methyl galactopyranoside. The liberated fatty acid methyl ester was examined by gas-liquid chromatography (GLC) and electron impact-mass spectra (EI-MS). On GLC, each sample showed a single

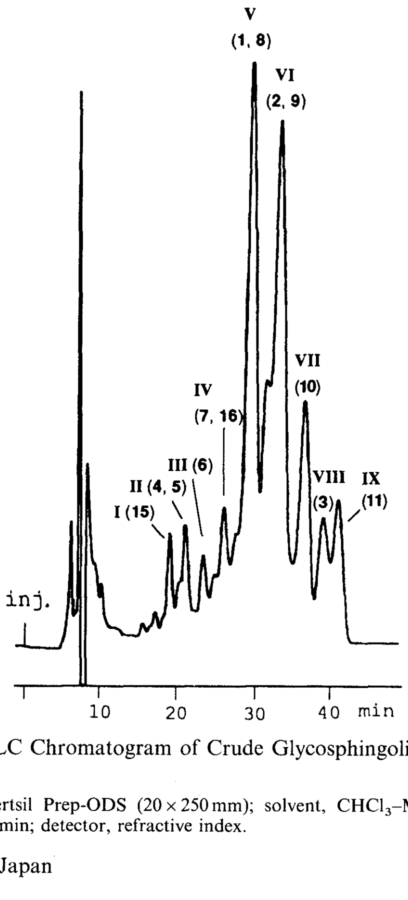

Fig. 1. HPLC Chromatogram of Crude Glycosphingolipids Fraction (Fr. 6a)

Column, Inertsil Prep-ODS (20×250 mm); solvent, $CHCl_3$–MeOH (10:95); flow rate, 3 ml/min; detector, refractive index.

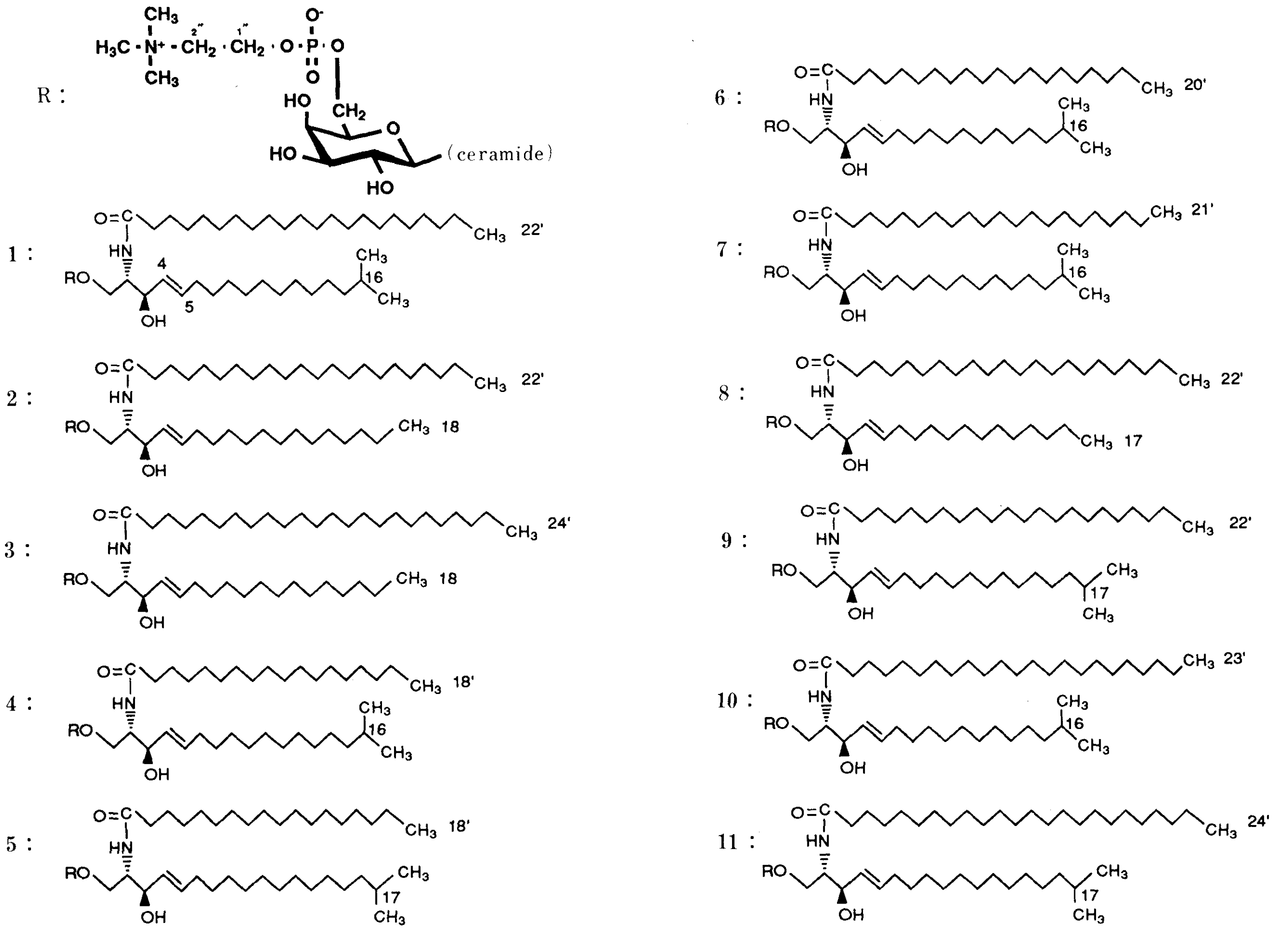

Fig. 2. Structures of Glycosphingolipids (**1**—**11**)

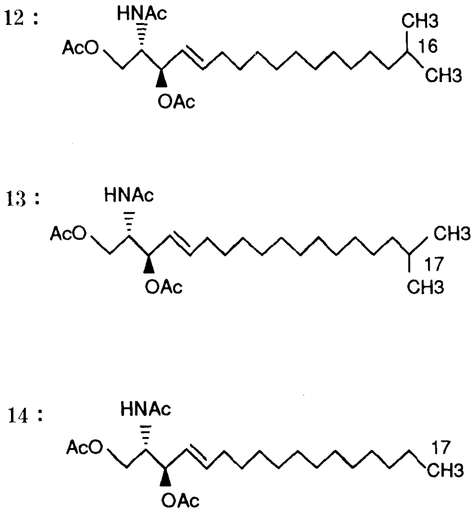

Fig. 3. Structures of **12**, **13** and **14**

peak, which was identified by comparison with authentic samples of fatty acid methyl esters, that is, octadecanoate ($C_{18:0}$) from **4** and **5**, icosanoate ($C_{20:0}$) from **6**, henicosanoate ($C_{21:0}$) from **7**, docosanoate ($C_{22:0}$) from **8** and **9**, tricosanoate ($C_{23:0}$) from **10** and tetracosanoate ($C_{24:0}$) from **11**.

The long-chain base liberated by methanolysis was converted into its acetate, which was examined by ^{1}H-NMR and positive ion FAB-MS. The base acetates of **4**, **6**, **7** and **10** were identified as D-*erythro*-(4*E*)-16-methylheptadecasphingenine triacetate (**12**, $C_{18:1}$ iso) by comparison with an authentic sample.[4]

On the other hand, **5**, **9** and **11** each gave another base triacetate (**13**), whose ^{1}H-NMR spectrum was quite similar to that of **12**, but the positive ion FAB-MS showed a $[M+Na]^+$ ion peak at *m*/*z* 462 which was 14 mass units larger than that of **12**. From the above findings and the optical rotation, **13** was proved to be (4*E*)-17-methyloctadecasphingenine triacetate ($C_{19:1}$, iso) having the D-*erythro* configuration.

In the ^{1}H-NMR spectrum of the base triacetate **14** derived from **8**, the chemical shifts of almost all protons were similar to those of **12** and **13**, but two secondary methyl groups in **12** and **13** were replaced by a signal due to a primary methyl group, while in the FAB-MS, the $[M+Na]^+$ ion peak was 14 mass units less than that of D-*erythro*-(4*E*)-octadecasphingenine triacetate.[2,6] From these spectral data and the specific rotation ($[\alpha]_D$ $-16.9°$), **14** was concluded to be D-*erythro*-(4*E*)-heptadecasphingenine triacetate ($C_{17:1}$).

On the basis of the results obtained above, the structures of the eight new compounds were determined to be as shown in Fig. 2.

Substances **15** (*m*/*z*: 879, $[M+H]^+$) and **16** (*m*/*z*: 921, $[M+H]^+$) obtained from fr. 1 and fr. 4, respectively, behaved as homogeneous compounds in their positive ion

FAB-MS, but the former gave, on methanolysis, methyl esters of hexadecanoic, heptadecanoic and octadecanoic acids (ratio, 3 : 1 : 1 on GLC) and the latter gave those of henicosanoic and docosanoic acids (3 : 1). Hence, both **15** and **16** are mixtures of analogs, which have the same molecular mass. Their complete separation into the individual molecular species is in progress.

In our previous studies,[1,2] we found that the phosphocholine-linked galactosylceramides obtained from the marine annelids *M. sanguinea* and *N. diversicolor* consisted of straight-chain bases, mainly *N*-hexadecanoyl-(4*E*)-octadecasphingenine, as the ceramide unit. In contrast, the land annelid, *P. asiatica*, contained a remarkably high proportion (*ca.* 85%) of branched-chain bases.

Experimental

Details of the instruments and chromatographic conditions used throughout this work were the same as described in the previous papers.[2,4] The specific rotations were taken at 24—26 °C.

Isolation of Glycosphingolipids from "Jiryu" The MeOH extractives (75 g) of the dried body walls (1 kg) of the commercial crude drug Jiryu (*P. asiatica*) (purchased from Tochimoto Tenkaido) were treated with CHCl$_3$–MeOH–H$_2$O (1 : 2 : 1, 900 ml), and the lower phase was collected and concentrated to give a brown extract (27 g). This was subjected to silica-gel and Cosmosil 75C$_{18}$-OPN column chromatographies with various solvent systems as described in the preceding paper[4] to give fractions 5 (4 g) and 6 (2 g). Fraction 6 (2.0 g) (positive to Dittmer–Lester's reagent)[7] was applied to an Asahipack GS-320 column (7.6 mm × 50 cm, Asahi Chemical Industry Co., Ltd.) and eluted with MeOH to provide two fractions (crude glycosphingolipid fractions), fr. 6a (1.5 g) and fr. 6b (0.4 g). The former was subjected to conventional HPLC (Inertsil Prep-ODS, GL Sciences; 10 μm, 2 cm × 25 cm; solvent, CHCl$_3$–MeOH, 10 : 95) to give frs. 1—9. Each of frs. 1, 3, 7 and 9 was further purified by HPLC (solvent, CHCl$_3$–MeOH, 10 : 95) to give **6** (13 mg) from fr. 3, **10** (70 mg) from fr. 7, **3** (19 mg) from fr. 8 and **11** (23 mg) from fr. 9. Recycling HPLC was conducted using a JASCO recycling valve (solvent, CHCl$_3$–MeOH, 10 : 95) to give **15** (5 mg) from fr. 1, **4** (13 mg) and **5** (31 mg) from fr. 2 (2 cycles), **7** (27 mg) and **16** (13 mg) from fr. 4 (2 cycles), **1** (247 mg) and **8** (18 mg) from fr. 5 (7 cycles) and **2** (37 mg) and **9** (60 mg) from fr. 6 (6 cycles).

N-Octadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-16-methylheptadecasphingenine (**4**): Powder, mp 164—165 °C, $[\alpha]_D$ +7.0° (c=0.2, CHCl$_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 893 (M+H)$^+$. Negative ion FAB-MS *m/z* (%): 891 (100) (M−H)$^-$, 877 (80), 833 (50), 806 (70), 564 (30), 265 (100). ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 0.88 (6H, d, *J*=7.0 Hz, H$_3$-17 and 18), 0.89 (3H, t, *J*=7.0 Hz, H$_3$-18′), 1.53 (1H, septet, *J*=7.0 Hz, H-16), 1.58 (2H, m, H$_2$-3′), 2.17 (2H, t, *J*=7.5 Hz, H$_2$-2′), 3.22 (9H, s, N-C$\underline{\text{H}}_3$), 3.51 (1H, dd, *J*=2.5, 8.0 Hz, H-3 of Gal), 3.52 (1H, dd, *J*=7.0, 10.0 Hz, H-1), 3.55 (1H, dd, *J*=7.0, 8.0 Hz, H-2 of Gal), 3.64 (2H, br t, *J*=5.0 Hz, H$_2$-2″), 3.72 (1H, td, *J*=1.0, 6.0 Hz, H-5 of Gal), 3.86 (1H, dd, *J*=1.0, 2.5 Hz, H-4 of Gal), 3.96 (1H, m, H-2), 4.02 (2H, t, *J*=6.0 Hz, H$_2$-6 of Gal), 4.07 (1H, t, *J*=8.0 Hz, H-3), 4.19 (1H, dd, *J*=4.0, 10.0 Hz, H-1), 4.24 (1H, d, *J*=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H$_2$-1″), 5.45 (1H, dd, *J*=7.0, 15.0 Hz, H-4), 5.69 (1H, ddd, *J*=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD$_3$OD, 100 MHz) δ: 14.45 (C-18′), 23.09, 23.76 (C-17 and 18), 27.20 (C-3′), 33.10 (C-6), 37.46 (C-2′), 54.78 (C-2), 54.83 (N-$\underline{\text{C}}$H$_3$), 60.53 (C-1″), 65.79 (C-6 of Gal), 67.50 (C-2″), 69.99 (C-4 of Gal), 70.25 (C-1), 72.77 (C-3), 72.97 (C-2 of Gal), 74.67 (C-3 of Gal), 75.30 (C-5 of Gal), 105.54 (C-1 of Gal), 131.58 (C-4), 135.06 (C-5), 175.91 (C-1′).

N-Octadecanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-17-methyloctadecasphingenine (**5**): Powder, mp 165—167 °C, $[\alpha]_D$ +5.4° (c=0.6, CHCl$_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 907 (M+H)$^+$. Negative ion FAB-MS *m/z* (%): 905 (80) (M−H)$^-$, 891 (90), 847 (50), 820 (70), 578 (30), 297 (100). ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 0.88 (6H, d, *J*=7.0 Hz, H$_3$-18 and 19), 0.89 (3H, t, *J*=7.0 Hz, H$_3$-18′), 1.53 (1H, septet, *J*=7.0 Hz, H-17), 1.58 (2H, m, H$_2$-3′), 2.17 (2H, t, *J*=7.5 Hz, H$_2$-2′), 3.22 (9H, s, N-C$\underline{\text{H}}_3$), 3.51 (1H, dd, *J*=3.0, 10.0 Hz, H-3 of Gal), 3.52 (1H, dd, *J*=7.0, 10.0 Hz, H-1), 3.55 (1H, dd, *J*=7.0, 10.0 Hz, H-2 of Gal), 3.64 (2H, br t, *J*=5.0 Hz, H$_2$-2″), 3.72 (1H, td, *J*=1.0, 3.0 Hz, H-5 of Gal), 3.87 (1H, dd, *J*=1.0, 3.0 Hz, H-4 of Gal), 3.96 (1H, m, H-2), 4.02 (2H, t, *J*=6.0 Hz, H$_2$-6 of Gal), 4.07 (1H, t, *J*=8.0 Hz, H-3), 4.21 (1H, dd, *J*=4.0, 10.0 Hz, H-1), 4.24 (1H, d, *J*=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H$_2$-1″), 5.45 (1H, dd, *J*=7.0, 15.0 Hz, H-4), 5.69 (1H, ddd, *J*=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD$_3$OD, 100 MHz) δ: 14.47 (C-18′), 23.10, 23.75 (C-18 and 19), 27.20 (C-3′), 33.11 (C-6), 37.46 (C-2′), 54.78 (C-2), 54.86 (N-$\underline{\text{C}}$H$_3$), 60.52 (C-1″), 65.76 (C-6 of Gal), 67.64 (C-2″), 69.99 (C-4 of Gal), 70.25 (C-1), 72.77 (C-3), 72.97 (C-2 of Gal), 74.69 (C-3 of Gal), 75.30 (C-5 of Gal), 105.54 (C-1 of Gal), 131.59 (C-4), 135.06 (C-5), 175.91 (C-1′).

N-Icosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-16-methylheptadecasphingenine (**6**): Powder, mp 160—163 °C, $[\alpha]_D$ +7.0° (c=0.6, CHCl$_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 921 (M+H)$^+$. Negative ion FAB-MS *m/z* (%): 919 (70) (M−H)$^-$, 905 (52), 861 (37), 834 (47), 592 (20), 297 (100). ^{1}H-NMR (CD$_3$OD–CDCl$_3$, 2 : 1, 400 MHz) δ: 0.89 (6H, d, *J*=7.0 Hz, H$_3$-17 and 18), 0.90 (3H, t, *J*=7.0 Hz, H$_3$-20′), 1.53 (1H, septet, *J*=7.0 Hz, H-16), 1.58 (2H, m, H-3′), 2.17 (2H, t, *J*=7.5 Hz, H$_2$-2′), 3.22 (9H, s, N-C$\underline{\text{H}}_3$), 3.52 (1H, dd, *J*=7.0, 10.0 Hz, H-1), 3.51 (1H, dd, *J*=3.0, 9.5 Hz, H-3 of Gal), 3.55 (1H, dd, *J*=7.0, 9.5 Hz, H-2 of Gal), 3.64 (2H, br t, *J*=5.0 Hz, H$_2$-2″), 3.72 (1H, td, *J*=1.0, 6.0 Hz, H-5 of Gal), 3.87 (1H, dd, *J*=1.0, 3.0 Hz, H-4 of Gal), 3.97 (1H, m, H$_2$-2), 4.02 (2H, t, *J*=6.0 Hz, H$_2$-6 of Gal), 4.08 (1H, t, *J*=7.0 Hz, H-3), 4.20 (1H, dd, *J*=4.0, 10.0 Hz, H-1), 4.24 (1H, d, *J*=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H$_2$-1″), 5.45 (1H, dd, *J*=7.0, 15.0 Hz, H-4), 5.69 (1H, ddd, *J*=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD$_3$OD–CDCl$_3$, 2 : 1, 100 MHz) δ: 14.42 (C-20′), 23.07, 23.66 (C-17 and 18), 27.11 (C-3′), 33.42 (C-6), 37.38 (C-2′), 54.63 (C-2), 54.83 (N-$\underline{\text{C}}$H$_3$), 60.40 (C-1″), 65.65 (C-6 of Gal), 67.58 (C-2″), 69.84 (C-4 of Gal), 70.14 (C-1), 72.66 (C-3), 72.86 (C-2 of Gal), 74.58 (C-3 of Gal), 75.26 (C-5 of Gal), 105.42 (C-1 of Gal), 131.42 (C-4), 135.02 (C-5), 175.80 (C-1′).

N-Henicosanoyl-1-*O*-[6-*O*-(2-methylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-16-methylheptadecasphingenine (**7**): Powder, mp 165—168 °C, $[\alpha]_D$ +7.8° (c=1.0, CHCl$_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 935 (M+H)$^+$. Negative ion FAB-MS *m/z* (%): 933 (65) (M−H)$^-$, 919 (78), 875 (50), 848 (70), 606 (23), 297 (100). ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 0.88 (6H, d, *J*=7.0 Hz, H$_3$-17 and 18), 0.90 (3H, t, *J*=7.0 Hz, H$_3$-21′), 1.53 (1H, septet, *J*=7.0 Hz), 1.60 (2H, m, H$_2$-3′), 2.18 (2H, t, *J*=7.0 Hz, H$_2$-2′), 3.22 (9H, s, N-C$\underline{\text{H}}_3$), 3.52 (1H, dd, *J*=7.0, 10.0 Hz, H-1), 3.52 (1H, dd, *J*=3.0, 10.0 Hz, H-3 of Gal), 3.54 (1H, dd, *J*=7.0, 10.0 Hz, H-2 of Gal), 3.64 (2H, br t, *J*=5.0 Hz, H$_2$-2″), 3.72 (1H, td, H-5 of Gal), 3.87 (1H, dd, *J*=1.0, 3.0 Hz, H-4 of Gal), 3.72 (1H, td, *J*=1.0, 6.0 Hz, H-5 of Gal), 3.98 (1H, m, H-2), 4.02 (2H, t, *J*=6.0 Hz, H$_2$-6 of Gal), 4.11 (1H, t, *J*=7.0 Hz, H-3), 4.18 (1H, dd, *J*=4.0, 10.0 Hz, H-1), 4.23 (1H, d, *J*=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H$_2$-1″), 5.48 (1H, dd, *J*=7.0, 15.0 Hz, H-4), 5.70 (1H, ddd, *J*=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD$_3$OD, 100 MHz) δ: 14.38 (C-21′), 23.09, 23.63 (C-17 and 18), 27.17 (C-3′), 33.04 (C-6), 37.38 (C-2′), 54.73 (C-2), 54.78 (N-$\underline{\text{C}}$H$_3$), 60.48 (C-1″), 65.65 (C-6 of Gal), 67.48 (C-2″), 69.88 (C-4 of Gal), 70.21 (C-1), 72.66 (C-3), 72.81 (C-2 of Gal), 74.57 (C-3 of Gal), 75.24 (C-5 of Gal), 105.44 (C-1 of Gal), 131.60 (C-4), 134.88 (C-5), 175.69 (C-1′).

N-Docosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-heptadecasphingenine (**8**): Powder, mp 187—189 °C, $[\alpha]_D$ +7.4° (c=0.8, CHCl$_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 935 (M+H)$^+$. Negative ion FAB-MS *m/z* (%): 933 (80) (M−H)$^-$, 919 (83), 875 (55), 848 (75), 606 (24), 297 (100). ^{1}H-NMR (CD$_3$OD–CDCl$_3$, 2 : 1, 400 MHz) δ: 0.88 (6H, t, *J*=7.0 Hz, H$_3$-17 and 22′), 1.60 (2H, m, H$_2$-3′), 2.18 (2H, t, *J*= 7.0 Hz, H$_2$-2′), 3.22 (9H, s, N-C$\underline{\text{H}}_3$), 3.52 (1H, dd, *J*=7.0, 10.0 Hz, H-1), 3.52 (1H, dd, *J*=3.0, 10.0 Hz, H-3 of Gal), 3.54 (1H, dd, *J*=7.0, 10.0 Hz, H-2 of Gal), 3.64 (2H, br t, *J*=5.0 Hz, H$_2$-2″), 3.72 (1H, td, *J*=1.0, 6.0 Hz, H-5 of Gal), 3.87 (1H, dd, *J*=1.0, 3.0 Hz, H-4 of Gal), 3.98 (1H, m, H-2), 4.02 (2H, t, *J*=6.0 Hz, H$_2$-6 of Gal), 4.11 (1H, t, *J*=7.0 Hz, H-3), 4.18 (1H, dd, *J*=4.0, 10.0 Hz, H-1), 4.23 (1H, d, *J*=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H$_2$-1″), 5.48 (1H, dd, *J*=7.0, 15.0 Hz, H-4), 5.70 (1H, ddd, *J*=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD$_3$OD–CDCl$_3$, 2 : 1, 100 MHz) δ: 14.42 (C-17 and 22′), 27.05 (C-3′), 33.36 (C-6), 37.36 (C-2′), 54.65 (C-2), 54.80 (N-$\underline{\text{C}}$H$_3$), 60.34 (C-1″), 65.59 (C-6 of Gal), 67.55 (C-2″), 69.76 (C-4 of Gal), 70.08 (C-1), 72.60 (C-3), 72.80 (C-2 of Gal), 74.50 (C-3 of Gal), 75.12 (C-5 of Gal), 105.33 (C-1 of Gal), 131.33 (C-4), 134.97 (C-5), 175.73 (C-1′).

N-Docosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-*β*-D-galactopyranosyl-(4*E*)-17-methyloctadecasphingenine (**9**): Powder, mp 163—166 °C, $[\alpha]_D$ +9.0° (c=1.5, CHCl$_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 963 (M+H)$^+$. Negative ion FAB-MS *m/z* (%): 961 (75) (M−H)$^-$, 947 (90), 903 (70), 876 (93), 634 (37), 297 (100). ^{1}H-NMR

(CD_3OD–$CDCl_3$, 2 : 1, 400 MHz) δ: 0.88 (6H, d, J=7.0 Hz, H_3-18 and 19), 0.90 (3H, t, J=7.0 Hz, H_3-22′), 1.54 (1H, septet, J=7.0 Hz), 1.59 (2H, m, H_2-3′), 2.17 (2H, t, J=7.5 Hz, H_2-2′), 3.22 (9H, s, N-C$\underline{H}_3$), 3.50 (1H, dd, J=3.0, 10.0 Hz, H-3 of Gal), 3.54 (1H, dd, J=7.5, 10.0 Hz, H-1), 3.55 (1H, dd, J=7.0, 10.0 Hz, H-2 of Gal), 3.64 (2H, br t, J=5.0 Hz, H_2-2″), 3.72 (1H, td, J=1.0, 6.0 Hz, H-5 of Gal), 3.87 (1H, dd, J=1.0, 3.0 Hz, H-4 of Gal), 3.97 (1H, m, H-2), 4.02 (2H, t, J=6.0, H_2-6 of Gal), 4.08 (1H, t, J=7.0 Hz, H-3), 4.20 (1H, dd, J=4.0, 10.0 Hz, H-1), 4.23 (1H, d, J=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H_2-1″), 5.45 (1H, dd, J=7.0, 15.0 Hz, H-4), 5.69 (1H, ddd, J=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD_3OD–$CDCl_3$, 2 : 1, 100 MHz) δ: 14.28 (C-22), 22.9, 23.40 (C-18 and 19), 27.75 (C-3′), 33.01 (C-6), 37.06 (C-2′), 54.22 (C-2), 54.56 (N-$\underline{C}H_3$), 59.84 (C-1″), 64.97 (C-6 of Gal), 67.02 (C-2″), 69.10 (C-4 of Gal), 69.66 (C-1), 72.20 (C-3), 72.41 (C-2 of Gal), 74.62 (C-3 of Gal), 74.70 (C-5 of Gal), 104.74 (C-1 of Gal), 130.62 (C-4), 134.76 (C-5), 175.24 (C-1′).

N-Tricosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-β-D-galactopyranosyl-(4*E*)-16-methylheptadecasphingenine (**10**): Powder, mp 173—175 °C, $[\alpha]_D$ +7.5° (c=1.5, $CHCl_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 977 $(M+H)^+$. Negative ion FAB-MS *m/z* (%): 975 (100) $(M-H)^-$, 961 (75), 917 (50), 890 (67), 648 (30). ^{1}H-NMR (CD_3OD–$CDCl_3$, 2 : 1, 400 MHz) δ: 0.88 (6H, d, J=7.0 Hz, H_3-17 and 18), 0.90 (3H, t, J=7.0 Hz, H_3-23′), 1.54 (1H, septet, J=7.0 Hz), 1.58 (2H, m, H_2-3′), 2.17 (2H, t, J=7.0 Hz, H_2-2′), 3.22 (9H, s, N-C$\underline{H}_3$), 3.52 (1H, dd, J=7.0, 10.0 Hz, H-1), 3.52 (1H, dd, J=3.0, 10.0 Hz, H-3 of Gal), 3.54 (1H, dd, J=7.0, 10.0 Hz, H-2 of Gal), 3.64 (2H, br t, J=5.0 Hz, H_2-2″), 3.72 (1H, td, J=1.0, 6.0 Hz, H-5 of Gal), 3.88 (1H, dd, J=1.0, 3.0 Hz, H-4 of Gal), 3.96 (1H, m, H-2), 4.02 (2H, t, J=6.0 Hz, H_2-6 of Gal), 4.07 (1H, t, J=7.0 Hz, H-3), 4.21 (1H, dd, J=4.0, 10.0 Hz, H-1), 4.23 (1H, d, J=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H_2-1″), 5.45 (1H, dd, J=7.0, 15.0 Hz, H-4), 5.69 (1H, ddd, J=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD_3OD–$CDCl_3$, 2 : 1, 100 MHz) δ: 14.47 (C-23), 23.11 (C-17), 23.11 (C-18), 26.90 (C-3′), 33.26 (C-6), 37.55 (C-2′), 54.30 (C-2), 54.79 (N-$\underline{C}H_3$), 60.16 (C-1″), 65.26 (C-6 of Gal), 67.37 (C-2″ of Gal), 69.43 (C-4 of Gal), 69.92 (C-1), 72.28 (C-3), 72.65 (C-2 of Gal), 74.83 (C-3 of Gal), 74.96 (C-5 of Gal), 105.05 (C-1 of Gal), 130.96 (C-4), 134.98 (C-5), 175.50 (C-1′).

N-Tetracosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinate-β-D-galactopyranosyl-(4*E*)-16-methylheptadecasphingenine (**11**): Powder, mp 184—187 °C, $[\alpha]_D$ +7.5° (c=0.5, $CHCl_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 991 $(M+H)^+$. Negative ion FAB-MS *m/z* (%): 989 (88) $(M-H)^-$, 975 (87), 931 (55), 904 (80), 662 (25), 297 (100). ^{1}H-NMR (CD_3OD–$CDCl_3$, 2 : 1, 400 MHz) δ: 0.89 (6H, d, J=7.0 Hz, H_3-18 and 19), 0.89 (3H, t, J=7.0 Hz, H_3-24′), 1.53 (1H, septet, J=7.0 Hz), 1.58 (2H, m, H_2-3′), 2.17 (2H, t, J=7.5 Hz, H_2-2′), 3.22 (9H, s, N-C$\underline{H}_3$), 3.51 (1H, dd, J=3.0, 10.0 Hz, H-3 of Gal), 3.52 (1H, dd, J=7.0, 10.0 Hz, H-1), 3.55 (1H, dd, J=7.0, 10.0 Hz, H-2 of Gal), 3.64 (2H, br t, J=5.0, H_2-2″), 3.72 (1H, td, J=1.0, 6.0 Hz, H-5 of Gal), 3.87 (1H, dd, J=1.0, 3.0 Hz, H-4 of Gal), 3.96 (1H, m, H-2), 4.02 (2H, t, J=6.0 Hz, H_2-6 of Gal), 4.07 (1H, t, J=7.0 Hz, H-3), 4.21 (1H, dd, J=4.0, 10.0 Hz, H-1), 4.23 (1H, d, J=7.0 Hz, H-1 of Gal), 4.30 (2H, m, H_2-1″), 5.45 (1H, dd, J=7.0, 15.0 Hz, H-4), 5.69 (1H, ddd, J=7.0, 8.0, 15.0 Hz, H-5). ^{13}C-NMR (CD_3OD–$CDCl_3$, 2 : 1, 100 MHz) δ: 14.38 (C-24), 23.51 (C-18), 23.51 (C-19), 26.94 (C-3′), 33.29 (C-6), 37.28 (C-2′), 54.75 (C-2), 54.72 (N-$\underline{C}H_3$), 60.22 (C-1″), 65.43 (C-6 of Gal), 67.40 (C-2″), 69.61 (C-4 of Gal), 69.98 (C-1), 72.69 (C-2 of Gal), 72.38 (C-3), 72.49 (C-3 of Gal), 75.00 (C-5 of Gal), 105.18 (C-1 of Gal), 131.16 (C-4), 134.91 (C-5), 175.59 (C-1′).

15: Powder, mp 163—165 °C, $[\alpha]_D$ +6.4° (c=0.2, $CHCl_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 879 $(M+H)^+$. Negative ion FAB-MS *m/z* (%): 877 (44) $(M-H)^-$, 863 (45), 819 (30), 792 (40), 550 (10), 265 (100).

16: Powder, mp 165—168 °C, $[\alpha]_D$ +6.5° (c=0.4, $CHCl_3$–MeOH, 1 : 1). Positive ion FAB-MS *m/z*: 921 $(M+H)^+$. Negative ion FAB-MS *m/z* (%): 919 (50) $(M-H)^-$, 905 (52), 861 (40), 834 (50), 592 (25), 297 (100).

Analysis of the Components Degradation was carried out by the method of Fisher *et al.*[5] Five ml of HF (47%) was added to each sample (3—9 mg) and 1 ml of $CHCl_3$–MeOH (1 : 10) at 0 °C in a polyethylene tube fitted with a cap, and the reaction mixture was allowed to stand at 0 °C for 36 h. The pH of the solution was then brought to 6.5 with a saturated LiOH solution. The precipitate was filtered off and the filtrate was passed through Sephadex LH-20 column using MeOH to give a product (2—8 mg). The product was heated with 7.5% methanolic HCl (0.5 ml) at 90 °C for 1 h. The fatty acid methyl ester formed was extracted with *n*-hexane. The *n*-hexane layer was analyzed by GC (fused silica capillary column bonded MPS-50 (Quadrex), 0.25 mm × 50 m; column temperature 230 (hold 12 min)-240 °C at 1 °C/min; carrier gas, N_2 2.0 kg/cm^2; t_R (min): 9.23 (methyl *n*-octadecanoate) from **4** and **5**, 13.80 (methyl *n*-icosanoate) from **6**, 21.40 (methyl *n*-henicosanoate) from **7**, 21.44 (methyl *n*-docosanoate) from **8** and **9**, 26.02 (methyl *n*-tricosanoate) from **10**, 32.39 (methyl *n*-tetracosanoate) from **11**. Substances **15** and **16** were treated and analyzed in the same manner as described for **4**—**11**. The former gave three peaks, 6.34 (methyl *n*-hexadecanoate), 7.73 (methyl *n*-heptadecanoate) and 9.22 (methyl *n*-octadecanoate), and the latter gave two peaks, 21.40 (methyl *n*-henicosanoate) and 21.44 (methyl *n*-docosanoate). Each peak substance was identified as the corresponding methyl ester by EI-MS comparison with an authentic sample.

The remaining MeOH layer obtained above was neutralized by adding a small excess of Ag_2CO_3. After centrifugation, the supernatant was evaporated to dryness under a nitrogen stream at room temperature. The residue was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5) to give a methyl glycoside and a long-chain base. The former was treated with *N*-trimethylsilylimidazole and the derivative was analyzed by GC according to the method described in the preceding paper.[4] Those peaks (t_R: 11.0, 12.2, 12.6, 13.7 min) were identical with those of trimethylsilyl derivatives derived from methyl galactoside.

The latter (1—3 mg) was acetylated with acetic anhydride–pyridine (1 : 1) at room temperature for 1 d to give a product (**12** from **4**, **6**, **7** and **10**; **13** from **5**, **9** and **11**; **14** from **8**). Compound **12** was identified as D-*erythro*-(4*E*)-16-methylheptadecasphingenine triacetate by ^{1}H-NMR and FAB-MS comparison.

13: $[\alpha]_D$ −16.1° (c=0.3, $CHCl_3$). Positive ion FAB-MS *m/z*: 462 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, d, J=7.0 Hz, H_3-18 and 19), 1.98, 2.06, 2.06 (3H, s, COC$\underline{H}_3$), 4.07 (1H, dd, J=4.0, 12.0 Hz, H-1), 4.30 (1H, dd, J=6.0, 12.0 Hz, H-1), 4.42 (1H, m, H-2), 5.29 (1H, t, J=6.5 Hz, H-3), 5.39 (1H, m, H-4), 5.62 (1H, m, N$\underline{H}$), 5.78 (1H, ddd, J=7.0, 8.0, 15.0 Hz, H-5).

14: $[\alpha]_D$ −16.9° (c=0.1, $CHCl_3$). Positive ion FAB-MS *m/z*: 434 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.0 Hz, H_3-17), 1.98, 2.06, 2.06 (3H, s, COC$\underline{H}_3$), 4.06 (1H, dd, J=4.0, 12.0 Hz, H-1), 4.30 (1H, dd, J=6.0, 12.0 Hz, H-1), 4.43 (1H, m, H-2), 5.28 (1H, t, J=6.5 Hz, H-3), 5.39 (1H, m, H-4), 5.62 (1H, m, N$\underline{H}$), 5.79 (1H, ddd, J=7.0, 8.0, 15.0 Hz, H-5).

Acknowledgment The authors thank Professor Toshio Kawasaki of this university for his valuable advice. Thanks are also due to Dr. Du Xiaoming of the Institute of Medical & Pharmaceutical Sciences Dalian, China for identification of the crude drug, the earthworm.

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