Three Glycosphingolipids Having the Phosphocholine Group from the Crude Drug "Jiryu" (The Earthworm, *Pheretima asiatica*)

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Three glycosphingolipids were isolated in the pure state from the crude drug, "Jiryu" (the earthworm, *Pheretima asiatica*). Their structures were determined as *N*-docosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinato-β-D-galactopyranosyl]-(4*E*)-16-methylheptadecasphingenine (1), *N*-docosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)-phosphinato-β-D-galactopyranosyl]-(4*E*)-sphingenine (2) and *N*-tetracosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)-phosphinato-β-D-galactopyranosyl]-(4*E*)-sphingenine (3). They are zwitterionic glycosphingolipids having a phosphocholine group attached to the sugar moiety, resembling those obtained from two kinds of marine annelid, and one has a branched long-chain base.

Keywords Jiryu; earthworm; *Pheretima asiatica*; zwitterionic glycosphingolipid; galactosylceramide; branched-chain sphingenine

Our recent studies[1,2] on the lipid composition of the crude drugs "Jiryu" and "Suitetsu," the dried body wall of the earthworm, *Pheretima asiatica* and of the leech, *Hirudo nipponica*, respectively, have led to the finding that they contain 1-*O*-alkyl-*sn*-glycero-3-phosphocholines (the precursors of platelet-activating factors, lyso-PAF). Since the earthworm and the leech are land annelids, we attempted a systematic investigation of the lipid composition of the marine annelids *Marphysa sanguinea*[3] and *Neanthes diversicolor*.[4] As expected they were found to contain lyso-PAF.[5] In addition, we isolated two and five novel glycosphingolipids, respectively, that is, galactosylceramides having a phosphocholine group. Recently, Sugita and co-workers[6] have surveyed the polar lipid fraction of another species of the earthworm, *Pheretima hilgendorfi*, and obtained similar compounds as a mixture of homologs.

We have now made a more detailed survey of the lipid composition, particularly of the glycosylceramides, of the crude drugs Jiryu and Suitetsu. Three new glycosphingolipids have been isolated from the former, but none from the latter. This paper deals with the isolation and structure elucidation of the three compounds.

The lipid fraction (fraction 6) described in the previous paper on Jiryu[1] was passed through an Asahipack GS-320 column using MeOH to provide two fractions, fr. 6a and fr. 6b. The former was subjected to conventional high-performance liquid chromatography (HPLC). Its chromatogram showed nine peaks, I—IX, along with several minor peaks (Fig. 1).

Nine fractions (fr. 1—9) corresponding to peaks I—IX were collected, and fr. 5, 6 and 8 were further subjected to HPLC to give **1** from fr. 5, **2** from fr. 6 and **3** from fr. 8. They all exhibited, in their positive ion fast atom bombardment mass spectra (FAB-MS), only one quasimolecular ion peak, and no other $(M+H)^+$ ion peak, such as $(M+H\pm14)^+$ or $(M+H\pm28)^+$, due to any homolog. Each compound gave a positive tailing band with Dragendorff's and Dittmer–Lester's reagents.[7] Accordingly, compounds, **1**, **2** and **3** were all considered to be pure and to contain aminoalkyl and phospho groups.

Compound **1** exhibited the $(M+H)^+$ ion peak at *m/z* 949 in the positive ion FAB-MS, and its negative ion FAB-MS showed characteristic fragment ion peaks at *m/z* 933, 889, 862 and 620 together with the $(M-H)^-$ ion peak at *m/z* 947 (Fig. 2). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum indicated the presence of one phosphocholine, one hexose and one ceramide unit. In addition, it showed signals at δ 0.88 (6H, d, $J=7.0$ Hz) and 1.54 (1H, septet, $J=7.0$ Hz) assignable, respectively, to the secondary methyl and the methine protons. In the ^{13}C-NMR spectrum, all the signals 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, suggesting

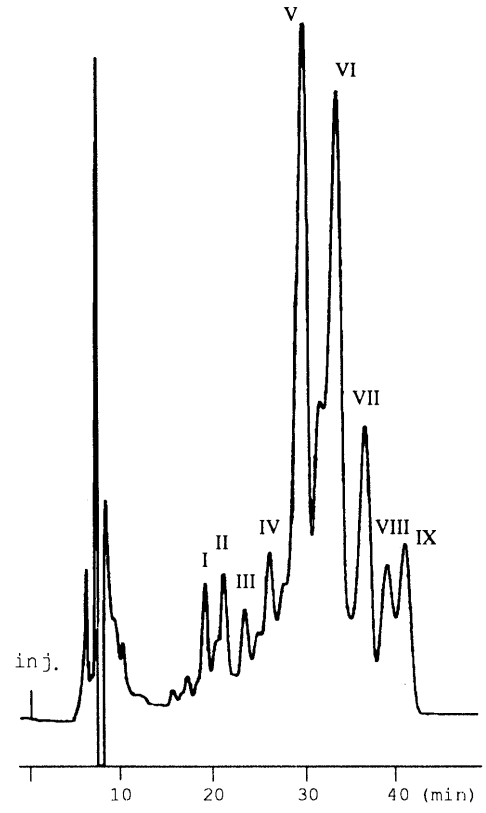

Fig. 1. HPLC Chromatogram of Fr. 6a

Column, inertsil Prep-ODS (20.0 × 250 mm); solvent, $CHCl_3$–MeOH (10:95); flow rate, 3 ml/min; detector, RI.

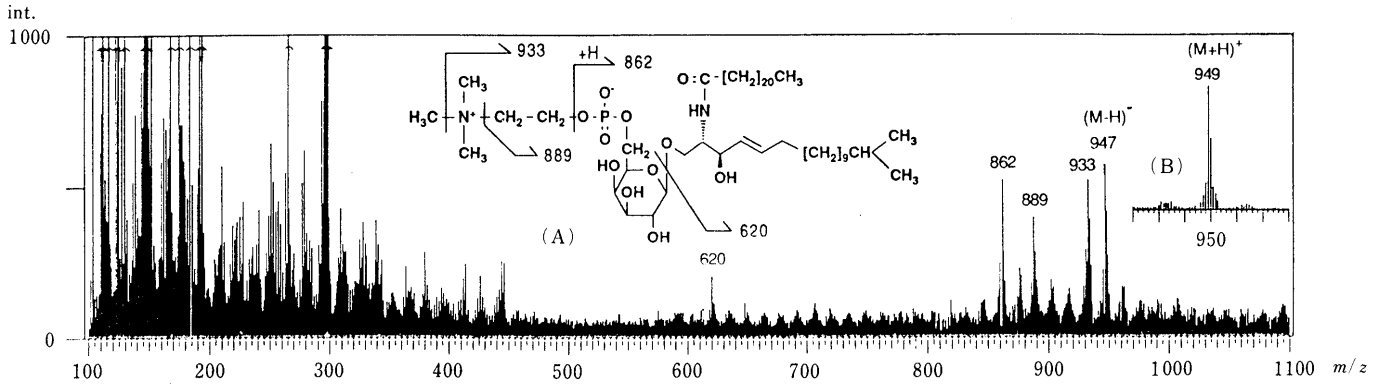

Fig. 2. Negative (A) and Positive (B) Ion FAB-MS of **1**

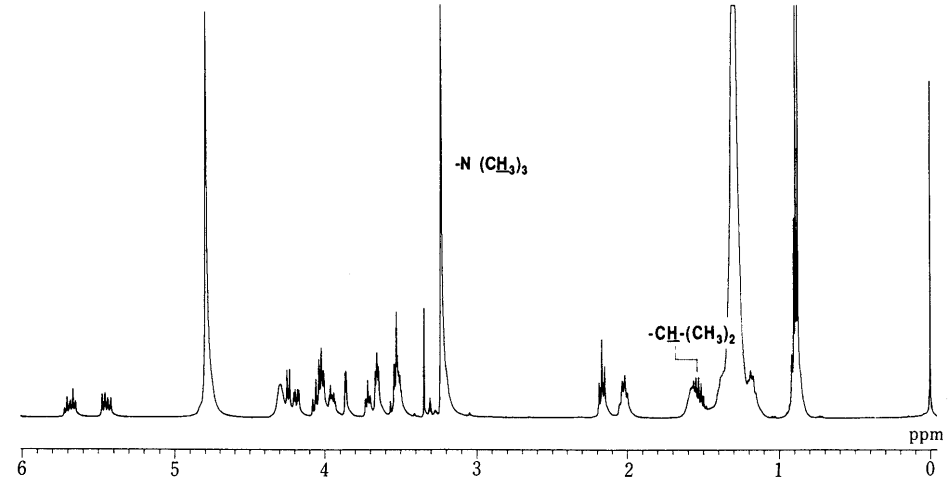

Fig. 3. ^{1}H-NMR Spectrum of **1** (400 MHz in CD_3OD)

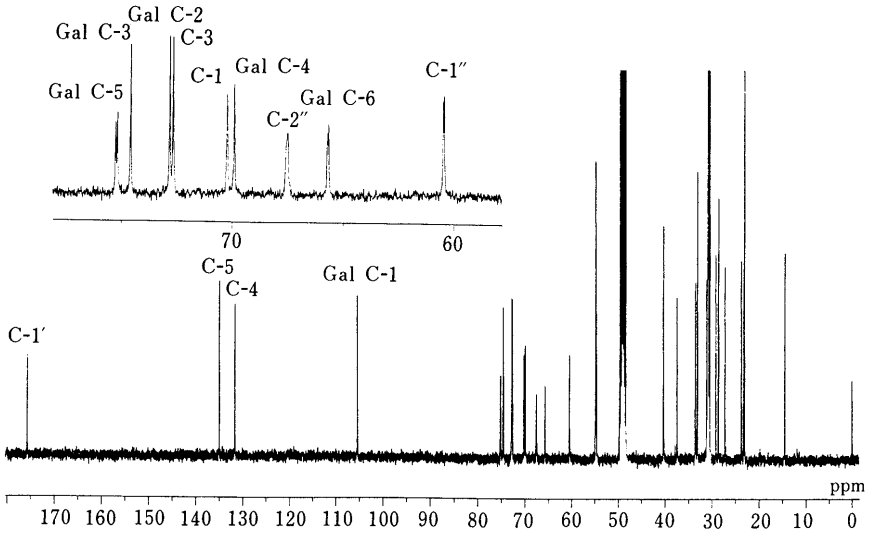

Fig. 4. ^{13}C-NMR Spectrum of **1** (100 MHz in CD_3OD)

that **1** is a monoglycosylceramide carrying the phosphocholine group, that is, an analog of those obtained from *Marphysa sanguinea*,[3] and has a branched carbon chain moiety (Figs. 3 and 4).

Treatment of **1** with HF (47%) (the method of Fischer *et al.*[8]) afforded **1a** (*m/z* 806 $(M+Na)^+$, FAB-MS). The ^{1}H-NMR spectrum of **1a** showed no signal due to the choline group, and resembled those of the monogalactosylceramides reported previously.[3] Compound **1a** was methanolyzed with 7.5% MeOH–HCl to give a methyl hexoside, a fatty acid methyl ester and a long-chain base. The former two were identified as methyl galactoside and methyl *n*-docosanoate by gas chromatography (GC) as well as electron impact mass spectroscopy (EI-MS). The galactose unit was proved to be D form by GC according to the method of Hara *et al.*[9] The long-chain base was

TABLE I. ^{1}H-NMR Spectral Data for **1**, **1c**, **2** and **3** (400 MHz)

No.	1	1c	2	3
1	3.54 (1H, dd, 7.0, 10.0)	3.62 (1H, dd, 7.0, 10.0)	3.54 (1H, dd, 7.0, 10.0)	3.52 (1H, dd, 7.0, 10.0)
	4.19 (1H, dd, 4.0, 10.0)	3.82 (1H, dd, 4.0, 10.0)	4.20 (1H, dd, 4.0, 10.0)	4.19 (1H, dd, 4.0, 10.0)
2	3.95 (1H, ddd, 4.0, 7.0, 7.0)	4.30 (1H, ddd, 4.0, 7.0, 7.0)	3.97 (1H, ddd, 4.5, 7.0, 7.0)	3.96 (1H, ddd, 4.0, 7.0, 7.0)
3	4.06 (1H, t, 7.0)	5.26 (1H, t, 7.0)	4.08 (1H, t, 7.0)	4.07 (1H, t, 7.0)
4	5.45 (1H, dd, 7.0, 15.0)	5.42 (1H, dd, 7.0, 15.0)	5.46 (1H, dd, 7.0, 15.0)	5.45 (1H, dd, 7.0, 15.0)
5	5.68 (1H, ddd, 7.0, 7.0, 15.0)	5.79 (1H, ddd, 7.0, 7.0, 15.0)	5.69 (1H, ddd, 7.0, 7.0, 15.0)	5.69 (1H, ddd, 7.0, 7.0, 15.0)
6	2.03 (2H, ddd, 7.0, 7.0, 7.0)	2.03 (2H)	2.03 (2H, ddd, 7.0, 7.0, 7.0)	2.03 (2H, ddd, 7.0, 7.0, 7.0)
16	1.54 (1H, sep, 7.0)	1.53 (1H, sep, 7.0)	*ca.* 1.30	*ca.* 1.29
17	0.88 (3H, d, 7.0)	0.88 (3H, d, 7.0)	*ca.* 1.30	*ca.* 1.29
18	0.88 (3H, d, 7.0)	0.88 (3H, d, 7.0)	0.89 (3H, t, 7.0)	0.89 (3H, t, 7.0)
2′	2.17 (2H, t, 7.0)	2.17 (2H, t, 7.6)	2.17 (2H, t, 7.5)	2.17 (2H, t, 7.5)
3′	1.58 (2H, m)	1.58 (2H, m)	1.59 (2H, m)	1.58 (2H, m)
$-CH_{3'}$	0.90 (3H, t, 7.0)	0.90 (3H, t, 7.0)	0.88 (3H, t, 7.0)	0.89 (3H, t, 7.0)
Gal-1	4.24 (1H, d, 7.0)	4.62 (1H, d, 7.5)	4.24 (1H, d, 7.0)	4.23 (1H, d, 7.0)
2	3.54 (1H, dd, 7.0, 9.5)	5.09 (1H, overlapping)	3.55 (1H, dd, 7.0, 9.5)	3.55 (1H, dd, 7.0, 8.0)
3	3.50 (1H, dd, 3.0, 9.5)	5.09 (1H, overlapping)	3.51 (1H, dd, 2.5, 9.5)	3.51 (1H, dd, 2.5, 8.0)
4	3.86 (1H, dd, 0.7, 3.0)	5.48 (1H, dd, 0.7, 2.0)	3.89 (1H, dd, 0.7, 2.5)	3.87 (1H, dd, 0.7, 2.5)
5	3.72 (1H, td, 0.7, 6.0)	4.05 (1H, m)	3.70 (1H, td, 0.7, 6.0)	3.71 (1H, td, 0.7, 6.0)
6	4.02 (2H, t, 6.0)	4.00 (1H, m)	4.03 (2H, t, 6.0)	4.03 (2H, t, 6.0)
		3.85 (1H, m)		
Cho-1″	4.30 (2H, m)	4.30 (2H, m)	4.30 (2H, m)	4.30 (2H, m)
2″	3.55 (2H, br t, 4.6)	3.64 (2H, br t, 4.6)	3.55 (2H, br t, 4.6)	3.64 (2H, br t, 4.6)
CH_3	3.23 (9H, s)	3.22 (9H, s)	3.23 (9H, s)	3.22 (9H, s)
$COCH_3$		1.94, 1.97, 2.02, 2.15 (each 3H, s)		

Coupling constants (J) in Hz are given in parentheses (solvent: CD_3OD for **1** and **1c**; CD_3OD:$CDCl_3$ (2:1) for **2** and **3**).

TABLE II. ^{13}C-NMR Chemical Shifts (δ) of **1**, **2** and **3** (100 MHz)

No.	1	2	3
1	70.23	69.72	69.95
2	54.75	54.16	54.39
3	72.68	72.28	72.46
4	131.62	130.71	131.12
5	134.90	134.78	134.91
6	33.09	33.06	33.26
16	28.60		
17	23.15	23.29	23.50
18	23.15	14.28	14.36
1′	175.71	175.33	175.56
2′	37.40	37.13	37.26
3′	27.19	26.69	26.93
$CH_{3'}$	14.51	14.28	14.36
Gal-1	105.46	104.83	105.13
2	72.83	72.53	72.68
3	74.59	74.10	74.38
4	69.90	69.20	69.57
5	75.26[a]	74.75[a]	74.95[a]
6	65.67[a]	65.03[a]	65.45[a]
Cho-1″	60.50[a]	59.92[a]	60.19[a]
2″	67.50[a]	67.27[a]	67.44[a]
CH_3	54.80	54.65	54.71

Solvent: CD_3OD for **1**, CD_3OD:$CDCl_3$ (2:1) for **2** and **3**. *a*) The signals appear as doublets (J=5–8 Hz) because of the coupling with ^{31}P.

acetylated to give a triacetate (**1b**, $[\alpha]_D$ −12.2°, m/z 448 $(M+Na)^+$, FAB-MS). Its ^{1}H-NMR spectrum was almost identical with that of D-*erythro*-(4*E*)-sphingenine triacetate ($[\alpha]_D$ −11.4°) reported by Shibuya and co-workers,[10] but the signal due to the primary methyl protons in the latter was replaced by that at δ 0.86 ascribable to the secondary methyl protons in **1b**. Thus, **1b** was regarded as a branched-chain D-*erythro*-(4*E*)-sphingenine. Usual acetylation of **1** gave a peracetate (**1c**), and its ^{1}H-NMR spectrum showed, when compared with that of **1**, considerable downfield shifts of the H-2, H-3 and H-4 protons (1.55, 1.59, 1.62 ppm, respectively) of the galactose unit and of H-3 (1.20 ppm) of the ceramide moiety, suggesting that the phosphocholine group is located at C-6 of the galactose residue. Compound **1** was treated with 7.5% methanolic HCl for 20 h. The products showing positive spots with Dittmer–Lester's reagent were isolated by HPLC and acetylated to give compounds **1d** and **1e**, which were identified as the methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranoside 6-phosphocholine and its β-anomer, respectively, by comparison of their ^{1}H-NMR spectra with those of authentic samples,[3] confirming the location of the phosphocholine group. The coupling constants and chemical shifts of the signals due to the galactose unit in **1** indicated that the configuration of the glycosidic linkage is β in $^4C_1$ conformation (Tables I and II).

On the basis of all the above chemical and spectral data, **1** was assigned the structure *N*-docosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinato-β-D-galactopyranosyl]-(4*E*)-16-methylheptadecasphingenine (**1**). Compound **2** exhibited in its positive and negative ion FAB-MS the same pseudo-molecular ion peaks at m/z 949 $(M+H)^+$ and 947 $(M-H)^-$, respectively, as those of **1**, and gave the same ^{1}H- and ^{13}C-NMR spectra as those of **1** except for the primary methyl signals at δ 0.89 (3H, t, J=7.0 Hz) instead of the secondary methyl signals (6H, d) in **1**.

The HF treatment of **2** followed by methanolysis in the same manner as for **1** afforded methyl α- and β-galactosides, a fatty acid methyl ester (m/z 354 $(M)^+$, EI-MS) and a long-chain base. Acetylation of the base gave an acetate (**2a**, $[\alpha]_D$ −12.0°), of which the ^{1}H-NMR and FAB-MS as well as specific rotation value were all identical with those of D-*erythro*-(4*E*)-sphingenine triacetate. The fatty acid methyl ester was identified as methyl *n*-docosanoate by GC

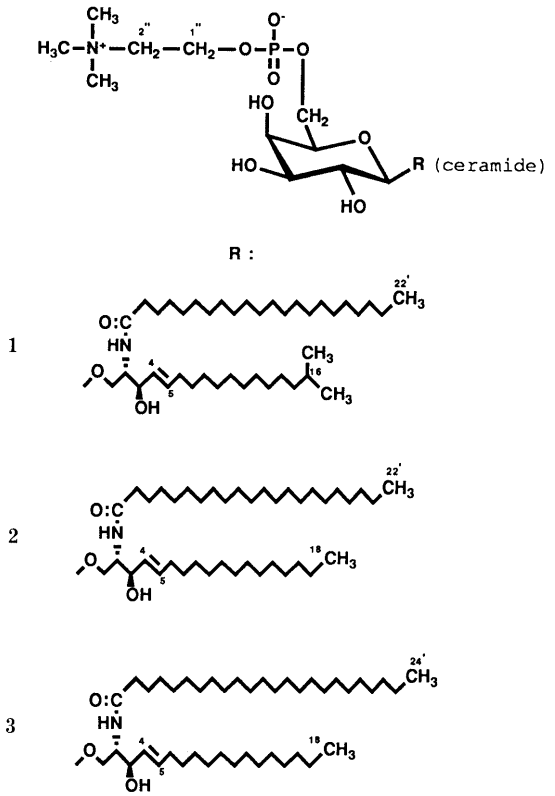

Fig. 5. Structures of **1**, **2** and **3**

analysis. Accordingly, compound **2** differs from **1** only in the sphingenine moiety and is assigned the structure *N*-docosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinato-*β*-D-galactopyranosyl]-(4*E*)-sphingenine (**2**).

Compound **3** showed in the positve ion FAB-MS the $(M+H)^+$ ion peak at *m/z* 977, which is 28 mass units more than that of **2**, and in the ^{1}H- and ^{13}C-NMR spectra gave the same signals as those of **2** except for those ascribable to the fatty acid moiety.

The HF degradation of **3** followed by methanolysis in the same manner as for **1** gave methyl α-, *β*-galactosides and a methyl *n*-tetracosanoate, together with a long-chain base. The base was derived into a peracetate, which was identical with the D-*erythro*-(4*E*)-sphingenine (**2a**) liberated from **2**. Hence, **3** has the same sphingenine and sugar moieties as **2** and differs only in the fatty acid unit. The docosanoyl group in **2** is replaced by a tetracosanoyl group in **3**.

Therefore the structure of **3** is defined as *N*-tetracosanoyl-1-*O*-[6-*O*-(2-trimethylammonioethoxy)phosphinato-*β*-D-galactopyranosyl]-(4*E*)-sphingenine (**3**). Figure 5 shows the structures.

It is of interest that galactosylceramides analogous to those obtained from two kinds of marine annelid were also found in a land annelid, the earthworm (*Pheretima asiatica*), but not in the leech (*Hirudo nipponica*). Compound **1** is unique in that the ceramide moiety has a branched-chain base.

Experimental

Details of the instruments and chromatographic conditions used throughout this work were the same as described in the previous papers[1,2] except in the following respect: specific rotations were measured at 24—26 °C.

Isolation of Compounds 1—3 from Jiryu The fractionation of the MeOH extractives of the crushed powder (1 kg) of the commercial crude drug Jiryu (*P. asiatica*) was described in the preceding paper.[1] Fraction 6 (2.0 g) was passed through an Asahipack GS-320 column (7.6 mm × 50 cm, Asahi Chemical Industry Co., Ltd.) by using MeOH as the eluent to provide two 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 fr. 1—9. Fractions 5, 6 and 8 were further purified by HPLC to give compounds **1** (247 mg, from fr. 5), **2** (37 mg from fr. 6) and **3** (19 mg from fr. 8). **1**: powder, mp 178—181 °C, $[\alpha]_D$ +5.5° (*c*=1.2, MeOH). Positive and negative ion FAB-MS *m/z*: Fig. 2. ^{1}H- and ^{13}C-NMR *δ*: Tables I and II. **2**: powder, mp 163—166 °C, $[\alpha]_D$ +5.5° (*c*=0.8, MeOH). Positive ion FAB-MS *m/z*: 949 $(M+H)^+$. Negative ion FAB-MS *m/z* (%): 947 $(M-H)^-$ (67), 933 (53), 889 (40), 862 (53), 620 (21), 255 (100). ^{1}H- and ^{13}C-NMR *δ*: Tables I and II. **3**: powder, mp 183—187 °C, $[\alpha]_D$ +7.6° (*c*=0.4, MeOH). Positive ion FAB-MS *m/z*: 977 $(M+H)^+$. Negative ion FAB-MS *m/z* (%): 975 $(M-H)^-$ (74), 961 (51), 917 (40), 890 (54), 648 (23), 255 (100). ^{1}H- and ^{13}C-NMR *δ*: Tables I and II.

Survey for Galactosylceramides in Suitetsu The commercial crude drug Suitetsu (*H. nipponica*) (500 g) was crushed and extracted in the same manner as described for that fr. 6 from Jiryu, but no galactosylaceramide could be obtained.

HF Treatment of 1 Degradation of **1** was carried out by the method of Fischer *et al.*,[8] HF (47%, 5 ml) was added to a mixture of the sample (35 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 a Sephadex LH-20 column using MeOH to give a product. It was purified by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (7:3:0.1) to give **1a** (20 mg). mp 155—160 °C, $[\alpha]_D$ +1.0° (*c*=0.4, $CHCl_3$–MeOH, 1:1). Negative ion FAB-MS *m/z*: 783 $(M)^-$, positive ion FAB-MS *m/z*: 806 $(M+Na)^+$. ^{1}H-NMR ($CD_3OD+CDCl_3$, 2:1, 400 MHz) *δ*: 0.88 (6H, d, *J*=7.0 Hz), 0.90 (3H, t, *J*=7.0 Hz), 1.53 (1H, sep, *J*=7.0 Hz), 1.60 (2H, m, H-3′), 2.18 (2H, t, *J*=7.0 Hz, H-2′), 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), 3.61 (1H, dd, *J*=4.0, 11.0 Hz, H-6 of Gal), 3.77 (2H, H-5, H-6 of Gal), 3.87 (1H, dd, *J*=1.0, 3.0 Hz, H-4 of Gal), 3.98 (1H, m, H-2), 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), 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).

Methanolysis of 1a Compound **1a** (10 mg) was heated with 7.5% methanolic HCl 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 (2% OV-17; 3.2 mm × 3 m glass column, column temperature; 170—230 °C at 3 °C/min, carrier gas: N_2 1.6 kg/cm^2). t_R (min): 26.7 (methyl *n*-docosanoate). EI-MS *m/z* 354 $(M)^+$.

The remaining MeOH layer was neutralized by adding a small excess of Ag_2CO_3. After centrifugation, the supernatant was evaporated to dryness under nitrogen. The residue was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (7:3:0.5) to give a long-chain base and a methyl glycoside. The long-chain base (4 mg) was acetylated with acetic anhydride–pyridine (1:1) at room temperature for 1 d to give **1b** (2.2 mg). $[\alpha]_D$ −12.2° (*c*=0.2, MeOH). Positive ion FAB-MS *m/z*: 448 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) *δ*: 0.86 (6H, d, *J*=7.0 Hz), 2.01, 2.06, 2.06 ($COCH_3$, 3H, s), 4.06 (H-1, 1H, dd, *J*=4.0, 12.0 Hz), 4.30 (H-1, 1H, dd, *J*=6.0, 12.0 Hz), 4.42 (H-2, 1H, m), 5.28 (H-3, t, *J*=6.5 Hz), 5.40 (H-4, 1H, m), 5.62 (NH, 1H, m), 5.79 (H-5, 1H, ddd, *J*=7.0, 8.0, 15.0 Hz).

A part of the methyl glycoside was treated with *N*-trimethylsilylimidazole and the derivative was examined by GC on a Hitachi G-3000 equipped with a 30:1 splitter and a flame ionization detector (fused silica capillary column Bonded MPS-50, Quadrex, 0.25 mm × 50 m, column temperature; 180 °C, carrier gas; He: 33.4 ml/min). t_R (min): 11.0, 12.2, 12.6, 13.7. These peaks were identical with those of authentic methyl galactose derivatives.

Acetylation of 1 Compound **1** (4 mg) was dissolved in acetic anhydride–pyridine (3 ml, 1:1) and the solution was left standing for 1 d at room temperature to give **1c** (4 mg). ^{1}H-NMR (CD_3OD) *δ*: Table I.

Determination of the Configuration of the Galactose Unit Compound **1a** (1 mg) was heated with 2 ml of 2 N HCl at 90 °C for 2 h. The reaction mixture was neutralized with Ag_2CO_3. The precipitate was removed by centrifugation and the supernatant was shaken with 2 ml of $CHCl_3$. The solvent was evaporated off and the residue was passed through a Sephadex LH-20 column using MeOH, yielding a sugar fraction. According to the

method of Hara *et al.*,[9] the conversion of a sugar with L-cysteine methyl ester hydrochloride to a methyl thiazolidine 4(*R*)-carboxylate derivative was followed by trimethylsilylation of hydroxy groups in the sugar with *N*-trimethylsilylimidazole. The product was examined by GC under the same analytical condition as for the trimethylsilyl derivative of the methyl glycoside except for the column temperature (230 °C). t_R (min): 19.45, 19.52.

Isolation of Galactopyranose 6-Phosphocholine Compound **1** (40 mg) was methanolyzed with 3 ml of 7.5% methanolic HCl at 100 °C for 20 h. The reaction mixture was extracted with *n*-hexane and the remaining methanolic layer was neutralized with Ag_2CO_3. The precipitate was removed by centrifugation, and the supernatant was evaporated to dryness. The residue was chromatographed on a Sephadex LH-20 column using MeOH to give products (12 mg), which showed a positive spot with the Dittmer–Lester's reagent. The products were subjected to preparative HPLC on a gel permeation column (size, 7.6 mm × 50 cm, Asahipak GS-320, Asahi Chemical Industry Co., Ltd.) using MeOH. The eluates showing positive spot at *Rf* 0.2 with Dittmer–Lester's reagent on TLC ($CHCl_3$–MeOH–H_2O, 1 : 9 : 2) were combined and evaporated to dryness. The residue was dissolved in acetic anhydride/pyridine (1 : 1) and the mixture was allowed to stand at room temperature for 4 d. The solution was evaporated to dryness under nitrogen and the residue was purified by preparative HPLC using 55% MeOH to give two products, **1d** (5 mg) and **1e** (4 mg). **1e**: $[\alpha]_D$ +50.0° (c=0.4, MeOH). Negative ion FAB-MS *m/z* (%): 484 $(M-H)^-$ (11), 470 (100), 425 (75), 399 (89). ^{1}H-NMR (CD_3OD) δ: 1.93, 2.04, 2.15 ($OCOCH_3$, 3H, s), 3.22 (N-Me, 9H, s), 3.48 (OCH_3, 3H, s), 3.62 (H_2-2″, 2H, m), 3.87, 4.05 (H-6 of Gal, each, 1H, m), 4.07 (H-5 of Gal, 1H, m), 4.25 (H_2-1″, 2H, m), 4.55 (H-1 of Gal, 1H, d, J=8.0 Hz), 5.07 (H-2, 3 of Gal), 5.49 (H-4 of Gal, 1H, dd, J=1.5, 3.0 Hz). Compound **1d** was identical with an authentic sample of α-D-galactopyranoside 6-phosphocoline.[3]

Determination of the Components of 2 and 3 Each of **2** (3 mg) and **3** (3 mg) was treated with 5 ml of HF (47%), to give **2a** (2 mg), which was identified as D-*erythro*-(4*E*)-sphingenine.[3] Methanolysis of this was followed by treatment and analysis in the same manner as for **1**. t_R (min): 26.8 (methyl *n*-docosanoate), 38.4 (methyl *n*-tetracosanoate).

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