Constituents of Holothuroidea, 14.[1] Isolation and Structure of New Glucocerebroside Molecular Species from the Sea Cucumber *Stichopus japonicus*

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Five glucocerebroside molecular species, SJC-1-SJC-5, have been isolated from the less polar lipid fraction of a chloroform/methanol extract of the sea cucumber *Stichopus japonicus*. The structures of these glucocerebroside molecular species were determined on the basis of chemical and spectroscopic evidence. SJC-1, SJC-2, and SJC-3 are typical sphingosine- and phytosphingosine-type glucocerebroside molecular species with nonhydroxylated and hydroxylated fatty acyl moieties. SJC-4 and SJC-5 are also sphingosine-type glucocerebroside molecular species with hydroxylated fatty acyl moieties, although they are new glucocerebroside molecular species with unique sphingosine bases.

Key words glycosphingolipid; glucocerebroside; sea cucumber; *Stichopus japonicus*

In our continuing research on biologically active glycosphingolipids (GSLs) from echinoderms, a series of studies on the isolation and structural elucidation of the GSLs from sea cucumber species have been performed in our laboratory.[2—10] In our study of the GSLs of the sea cucumber *Stichopus japonicus* (manamako in Japanese), we reported the isolation and structure of two new ganglioside molecular species.[6,10] Continuing the preceding studies, the isolation and characterization of cerebrosides from *S. japonicus* were performed. In this paper, we report the isolation and characterization of glucocerebrosides from the body walls of *S. japonicus*.

The less polar lipid fraction, which was obtained from the chloroform/methanol extract of the body walls of *S. japonicus*, was subjected to repeated silica gel column chromatography followed by reverse-phase column chromatography to give five cerebroside molecular species, SJC-1-SJC-5, each showing a single spot on silica gel thin-layer chromatography (TLC).

SJC-1, SJC-2, and SJC-3 exhibit strong hydroxy and amide absorptions in their IR spectra, and a series of molecular ion peaks in their positive-ion FAB mass spectra, respectively. In their ^{13}C-NMR spectra (Fig. 1, Table 1), they reveal characteristic signals of a sphingosine-type β-glucocerebroside with an unsubstituted fatty acid (SJC-1), a sphingosine-type β-glucocerebroside with a 2-hydroxy fatty acid (SJC-2), and a phytosphingosine-type β-glucocerebroside possessing a 2-hydroxy fatty acid (SJC-3), respectively. Therefore they are suggested to be the molecular species of three typical types of glucocerebrosides. Their structures shown in Fig. 1 are characterized by comparison of their ^{13}C-NMR spectral data with those of known glucocerebrosides[2—4] hitherto obtained and the results of their chemical degradation, *i.e.*, methanolysis followed by GC-MS analysis of the methanolysis products, fatty acid methyl ester (FAM) and long-chain base (LCB), as shown in Fig. 2 and Experimental. The location and geometry of the double bond in the unsaturated fatty acyl moiety of SJC-1-SJC-3 were determined as follows.

The mass spectra of the dimethyl disulfide (DMDS) derivatives[11,12] of the unsaturated FAMs from SJC-1, (a) and (b) in Fig. 3, show remarkable fragment-ion peaks at *m*/*z*: 173 and 287 for (a) and *m*/*z*: 173 and 301 for (b) due to cleavage of the bonds between the carbons bearing the methylthio groups (Fig. 3). These data indicate that the double bonds in the unsaturated fatty acyl moieties of SJC-1 are located at C-14 and C-15, respectively. On the other hand, the mass spectra of the DMDS derivatives of the unsaturated FAMs from both SJC-2 and SJC-3, shown in (c) and (d) in Fig. 3, reveal notable fragment-ion peaks at *m*/*z*: 173 and 303 for (c) and *m*/*z*: 173 and 317 for (d), which indicates that the position of the double bonds in the unsaturated fatty acyl moieties of both SJC-2 and SJC-3 are C-14 and C-15. Furthermore, the

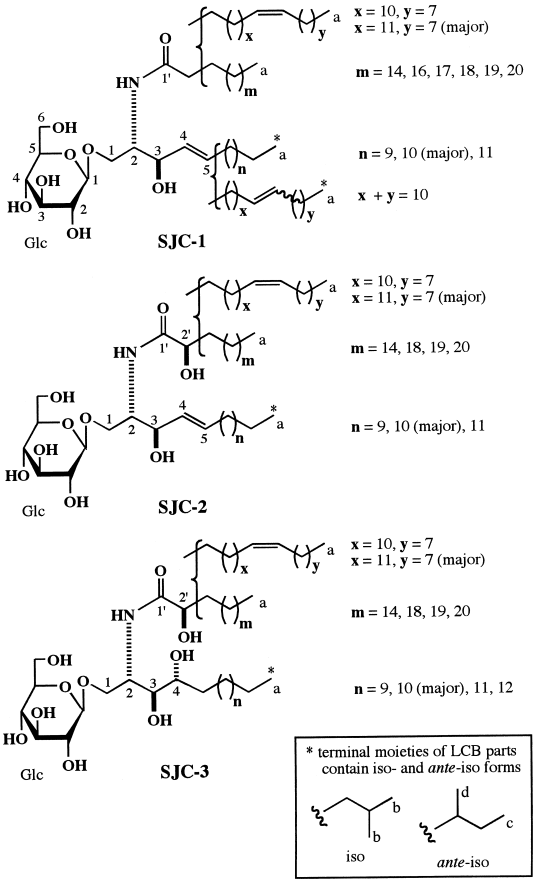

Fig. 1. Structure of SJC-1, SJC-2 and SJC-3

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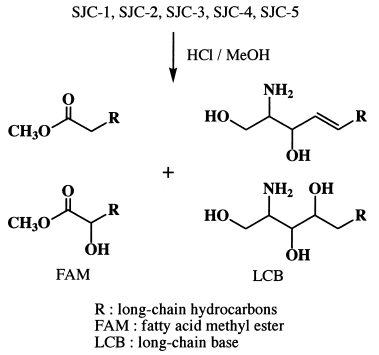

Fig. 2. Methanolysis Products of Cerebrosides

Table 1. ^{13}C-NMR Spectral Data (δ Values) of Glucocerebrosides in C_5D_5N

C		SJC-1	SJC-2	SJC-3	SJC-4	SJC-5
Ceramide						
1	(t)	70.3	70.2	70.4	70.0	70.0
2	(d)	55.1	54.6	51.8	54.6	54.6
3	(d)	72.6	72.3[f)]	75.9	72.4[h)]	72.4[i)]
4	(d)	132.3	131.7	72.6[g)]	131.6	131.6
5	(d)	132.6	132.8		132.7	133.1
8	(d)				76.4	78.0
9	(s)				139.1	75.1
10	(d)				130.6	136.2
11	(d)				67.8	128.2
12	(t)					32.9
1′	(s)	173.5	175.7	175.7	175.7	175.7
2′	(d)		72.5[f)]	72.5[g)]	72.5[h)]	72.5[i)]
=CH	(d)	130.2	130.2	130.2	130.2	130.2
=CHCH_2	(t)	27.5	27.5	27.5	27.5	27.5
CH_3[a)]	(q)	14.3	14.3	14.3	14.3	14.3
CH_3[b)]	(q)	22.8	22.8	22.8		
CH_3[c)]	(q)	11.6	11.6	11.6		
CH_3[d)]	(q)	19.4	19.4	19.4		
CH_3[e)]	(q)				12.5	24.3[j)]
						24.8[j)]
Glc						
1	(d)	105.6	105.6	105.5	105.6	105.5
2	(d)	75.2	75.1	75.1	75.1	75.1
3	(d)	78.4	78.4	78.4	78.4	78.4
4	(d)	71.7	71.5	71.6	71.6	71.5
5	(d)	78.5	78.5	78.5	78.5	78.5
6	(t)	62.8	62.5	62.7	62.7	62.7

a—*d*) Terminal methyl groups in the normal, iso, and *ante*-iso type of side chain (see Fig. 1). *e*) C_9–CH_3 (see Fig. 5). *f*—*i*) Assignments may be interchanged in each vertical column. *j*) Signals derived from C_9 epimers.

geometry (*Z*) of the double bonds of the unsaturated fatty acyl moieties were determined from the δ value (27.5) of the allylic carbon atoms (Table 1) obtained from the ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectra of SJC-1-SJC-3 (Fig. 4a), since allylic carbon signals of the *Z*- and *E*-isomer are observed at δ *ca.* 26—27 and δ *ca.* 31—32, respectively.[13)]

The absolute configuration of their glucose moiety (D-form) was determined using the Hara method[14)] (Experimental).

In the IR and positive-ion FAB mass spectra of SJC-4 and SJC-5, strong hydroxy and amide absorptions and a series of molecular ion peaks are observed, respectively. In their ^{13}C-NMR spectra (Fig. 5, Table 1), they reveal characteristic signals of a sphingosine-type β-glucocerebroside with a 2-hydroxy fatty acid. Therefore they are also suggested to be molecular species of glucocerebrosides like SJC-2. However, SJC-4 shows additional signals due to two secondary hydroxy groups (δ_C: 76.4, 67.8), a trisubstituted olefinic group (δ_C: 139.1, 130.6), and a vinyl methyl group (δ_C: 12.5), and SJC-5 shows a secondary hydroxy group (δ_C: 78.0), a tertiary hydroxy group (δ_C: 75.1), a disubstituted olefinic group (δ_C: 136.2, 128.2), and a methyl group (δ_C: 24.3, 24.8)[15)] by comparison of their ^{13}C-NMR spectra with that of SJC-2 (Table 1).

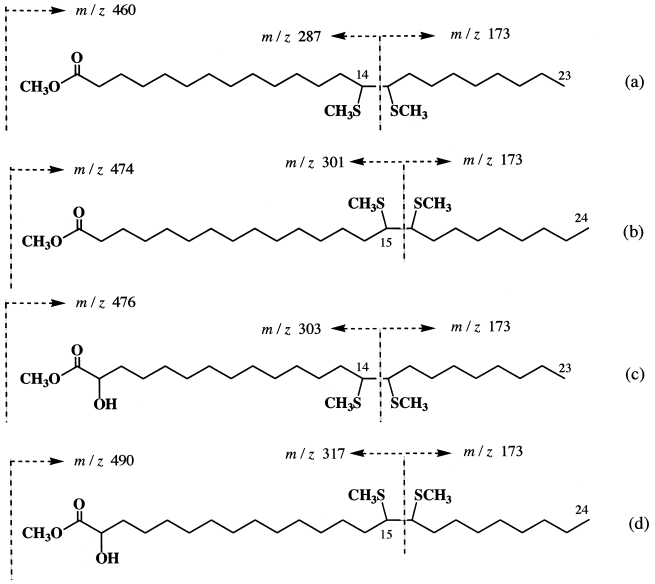

Fig. 3. Mass Fragmentation of DMDS Derivatives of Unsaturated FAMs from SJC-1 (a, b), SJC-2 (c, d), SJC-3 (c, d), SJC-4 (d) and SJC-5 (d)

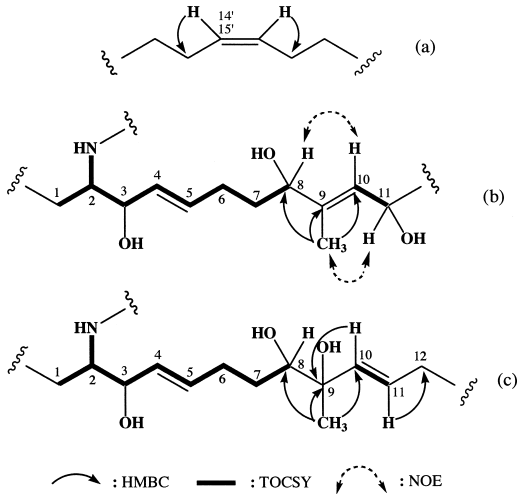

Fig. 4. HMBC, TOCSY and NOE Correlations of Fatty Acyl Moiety of SJC-1—SJC-5 (a) and LCB Moiety of SJC-4 (b) and SJC-5 (c)

When SJC-4 and SJC-5 were methanolyzed and the methanolysis products were analyzed, and the geometry of the double bonds of the unsaturated fatty acyl moieties were determined in HMBC experiments in the same manner as in SJC-2, their acyl moieties were characterized as shown in Fig. 5. Therefore the additional functional groups in SJC-4 and SJC-5 must be located in their LCB moieties. Furthermore, the positive-ion FAB mass spectra of SJC-4 and SJC-5 exhibit remarkable fragment-ion peaks $[504+Na+H]^+$ at

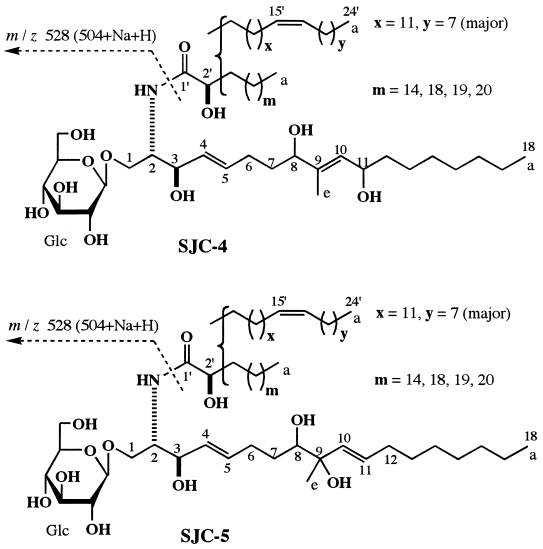

Fig. 5. Structures of SJC-4 and SJC-5

m/*z*: 528 due to cleavage of the amide bond, indicating that their LCB moieties are octadecane (C_{18}) derivatives as shown in Fig. 5. The structures of the side chains of the sphingosine base moieties of SJC-4 and SJC-5 were determined with the aid of the 2D-NMR experiments as shown in Fig. 4.

Totally correlated spectroscopy (TOCSY) of SJC-4 indicates the existence of a secondary hydroxy group at C-8. The HMBC correlation from the vinyl methyl group (δ_H: 1.90) to C-8 (δ_C: 76.4) and olefinic carbons (δ_C: 139.1, 130.6) shows the trisubstituted olefinic group located at C-9 and C-10. Another secondary hydroxy group must be located at C-11, as shown by the TOCSY correlation. The geometry of the double bond is assumed to be *E* on the basis of the ^{13}C-NMR chemical shift of C_9–CH_3 (δ_C: 12.5)[16] and the nuclear Overhauser effect (NOE) correlation between C_9–CH_3 (δ_H: 1.90) and 11-H (δ_H: 4.78), and between 8-H (δ_H: 4.37) and 10-H (δ_H: 5.97). Therefore the structure of the side chain of the LCB moiety of SJC-4 is represented as shown in Fig. 4(b).

In the same manner as in SJC-4, the side-chain moiety of SJC-5 is characterized as shown in Fig. 4(c). The TOCSY correlation suggests the existence of a secondary hydroxy group at C-8. HMBC correlations are observed from the tertiary methyl group [C_9–CH_3, δ_H: 1.60 (s) and 1.64 (s)][15] to C-8 (δ_C: 78.0), hydroxy-bearing tertiary carbon (C-9, δ_C: 75.1), and olefinic carbon (C-10, δ_C: 136.2), and from the olefinic proton (10-H, δ_H: 6.02) to C-9. The geometry (*E*) of the olefinic group is determined from the allylic carbon signals (C-12, δ_C: 32.9) assigned based on the HMBC correlation as described above.

The absolute configuration (D-form) of the glucose moiety of both SJC-4 and SJC-5 was determined using the same method as for SJC-2. Additionally, the terminal moieties of the LCB of both cerebrosides are the normal form based on the terminal methyl signal (δ_C: 14.3) in their ^{13}C-NMR spectra (Table 1).

Accordingly, the structures of SJC-4 and SJC-5 are represented as shown in Fig. 5. Although the stereochemistry of the side-chain moieties of LCB could not be determined except for the geometry of the double bonds in this study, to the best of our knowledge, cerebrosides with such unique LCB moieties have not previously been obtained. Pure cerebroside components from each molecular species should be isolated in the future. However, the isolation and structure of SJC-4 and SJC-5 are reported here since the results of this study are interesting and noteworthy.

Experimental

Optical rotations were measured with a Jasco Dip-370 digital polarimeter at 25 °C. IR spectra were obtained on a Jasco FT/IR-410 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol GX-270 spectrometer (270, 67.8 MHz) or a Varian Unity-500 spectrometer (500, 125 MHz). Positive-ion FAB-MS spectra were acquired with a Jeol JMS-SX-102 mass spectrometer (xenon atom beam; matrix, *m*-nitrobenzyl alcohol). GC-MS were recorded with a Shimadzu QP-5050A [EI mode; ionizing potential, 120 eV; column, TC-1701 (0.25 mm×30 m, GL Science Inc.); carrier gas, He]. GC was performed on a Shimadzu GC-14B [FID mode; column, fused silica capillary column DB-17 (0.32 mm×30 m, J & W Scientific); carrier, N_2].

Separation of SJC-1-SJC-5 The body walls of the sea cucumber *S. japonicus* (46.9 kg) were chopped and extracted with $CHCl_3$/MeOH [1 : 4, 18 l and 1 : 2, 18 l (two times)]. The combined extracts were concentrated *in vacuo* to give an aqueous solution (15 l), which was extracted three times with *n*-hexane (6 l). The *n*-hexane phase was concentrated *in vacuo* to give a residue (418.5 g), which was washed with cold acetone to give an acetone-insoluble fraction (less polar lipid fraction, 326.2 g). The less polar lipid fraction was chromatographed on silica gel (solvent $CHCl_3$/MeOH/H_2O, 85 : 15 : 0 to 4 : 6 : 1) to give five fractions. Successive column chromatography of fractions 2, 3 and 4 (silica gel, solvent $CHCl_3$/MeOH, 87 : 13 to 1 : 1) afforded SJC-1 (3.59 g) (*Rf*=0.58), SJC-2 (7.76 g) (*Rf*=0.50) and SJC-3 (1.56 g) (*Rf*=0.41), respectively. Fraction 5 was further chromatographed on reverse-phase (C18) (solvent 94% MeOH to 100% MeOH) to afford SJC-4 (74 mg) (*Rf*=0.29) and SJC-5 (38 mg) (*Rf*=0.32) [silica gel TLC, solvent $CHCl_3$/MeOH/H_2O (83 : 17 : 2)].

SJC-1: Amorphous powder, $[\alpha]_D$ −1.6° (*c*=1.00, 1-PrOH). IR (KBr) cm^{-1}: 3369 (OH), 1647, 1545 (amide). Positive-ion FAB-MS *m*/*z*: 770—840 $[M+Na]^+$ series. ^{1}H-NMR (C_5D_5N) δ: 4.96 (1H, d, *J*=7.6 Hz, glucose 1-H), 5.51 (*ca.* 2H, m, olefinic H of fatty acyl moiety). ^{13}C-NMR: See Table 1.

SJC-2: Amorphous powder, $[\alpha]_D$ +6.6° (*c*=1.00, 1-PrOH). IR (KBr) cm^{-1}: 3367 (OH), 1645, 1545 (amide). Positive-ion FAB-MS *m*/*z*: 800—860 $[M+Na]^+$ series. ^{1}H-NMR (C_5D_5N) δ: 4.91 (1H, d, *J*=7.6 Hz, glucose 1-H), 5.51 (*ca.* 2H, m, olefinic H of fatty acyl moiety). ^{13}C-NMR: See Table 1.

SJC-3: Amorphous powder, $[\alpha]_D$ +8.1° (*c*=1.00, 1-PrOH). IR (KBr) cm^{-1}: 3370 (OH), 1645, 1548 (amide). Positive-ion FAB-MS *m*/*z*: 820—880 $[M+Na]^+$ series. ^{1}H-NMR (C_5D_5N) δ: 4.97 (1H, d, *J*=7.6 Hz, glucose 1-H), 5.51 (*ca.* 1H, m, olefinic H of fatty acyl moiety). ^{13}C-NMR: See Table 1.

SJC-4: Amorphous powder, $[\alpha]_D$ +28.4° (*c*=1.00, 1-PrOH). IR (KBr) cm^{-1}: 3386 (OH), 1649, 1534 (amide). Positive-ion FAB-MS *m*/*z*: 830—910 $[M+Na]^+$ series, 528 $[504+Na+H]^+$. ^{1}H-NMR (C_5D_5N) δ: 8.35 (1H, d, *J*=8.0 Hz, NH), 4.24, 4.67 (each 1H , m, 1-H_2), 4.78 (1H, m, 2-H), 4.78 (1H, m, 3-H), 6.04 (1H, m, 4-H), 6.00 (1H, m, 5-H), 2.35, 2.44 (each 1H, m, 6-H_2), 1.82, 1.91 (each 1H, m, 7-H_2), 4.37 (1H, m, 8-H), 5.97 (1H, m, 10-H), 4.78 (1H, m, 11-H), 1.90 (3H, s, C_9–CH_3), 0.88 (6H, m, terminal methyl groups), 4.91 (1H, d, *J*=7.6 Hz, glucose 1-H), 5.50 (*ca.* 2H, m, olefinic H of fatty acyl moiety). ^{13}C-NMR: See Table 1.

SJC-5: Amorphous powder, $[\alpha]_D$ +21.3° (*c*=1.00, 1-PrOH). IR (KBr) cm^{-1}: 3367 (OH), 1645, 1545 (amide). Positive-ion FAB-MS *m*/*z*: 850—910 $[M+Na]^+$ series, 528 $[504+Na+H]^+$. ^{1}H-NMR (C_5D_5N) δ: 8.35 (1H, d, *J*=8.2 Hz, NH), 4.23, 4.66 (each 1H , m, 1-H_2), 4.77 (1H, m, 2-H), 4.77 (1H, m, 3-H), 6.05 (1H, m, 4-H), 6.05 (1H, m, 5-H), 2.43, 2.69 (each 1H, m, 6-H_2), 1.80, 2.00 (each 1H, m, 7-H_2), 3.86 (1H, m, 8-H), 6.02 (1H, m, 10-H), 6.02 (1H, m, 11-H), 1.60, 1.64 (each 1.5H, s, C_9-CH_3), 0.88 (6H, m, terminal methyl groups), 4.90 (1H, d, *J*=7.6 Hz, glucose 1-H), 5.51 (*ca*. 2H, m, olefinic H of fatty acyl moiety). ^{13}C-NMR: See Table 1.

Methanolysis of SJC-1 SJC-1 (5.0 mg) was heated with 10% HCl in MeOH (1 ml) at 70 °C for 18 h. The reaction mixture was then extracted with *n*-hexane, and the extract was concentrated *in vacuo* to yield a mixture of FAM. The MeOH layer was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated *in vacuo* to give a mixture of LCB and methyl glycoside.

FAM: ^{13}C-NMR (C_5D_5N) δ: 14.3 (terminal methyl group), 51.5 (OCH_3), 130.2 (olefinic C), 173.9 (CO).

GC-MS Analysis of FAM from SJC-1 A FAM mixture from SJC-1 was subjected to GC-MS [column temp. 150—250 °C (rate of temperature increase 5 °C/min)]. The results were as follows: methyl octadecanoate, t_R [min] (ratio of peak areas)=16.5 (1.3), *m/z*: 298 (M^+), 255 $(M-43)^+$; methyl icosanoate, t_R=20.0 (0.3), *m/z*: 326 (M^+), 283 $(M-43)^+$; methyl heneicosanoate, t_R=21.6 (0.7), *m/z*: 340 (M^+), 297 $(M-43)^+$; methyl docosanoate, t_R=23.4 (17.9), *m/z*: 354 (M^+), 311 $(M-43)^+$; methyl tricosenoate, t_R=25.2 (7.2), *m/z*: 366 (M^+), 323 $(M-43)^+$; methyl tricosanoate, t_R=25.5 (3.9), *m/z*: 368 (M^+), 325 $(M-43)^+$; methyl tetracosenoate, t_R=27.7 (67.8), *m/z*: 380 (M^+), 337 $(M-43)^+$; methyl tetracosanoate, t_R=28.0 (1.0), *m/z*: 382 (M^+), 339 $(M-43)^+$.

GC-MS Analysis of TMS Ethers of LCB from SJC-1 A mixture of LCB and methyl glycoside from SJC-1 was heated with 1-(trimethylsilyl) imidazole–pyridine (1 : 1) for 15 min at 70 °C, and the reaction mixture (TMS ethers) was analyzed using GC-MS [column temperature 180—250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: 2-amino-1,3-dihydroxy-4-hexadecene, t_R [min] (ratio of peak areas)=12.6 (7.3), *m/z*: 312 $(M-103)^+$, 283 $(M-132)^+$, 132; 2-amino-1,3-dihydroxy-4-heptadecene, t_R=13.6 (57.4), *m/z*: 326 $(M-103)^+$, 297 $(M-132)^+$, 132; 2-amino-1,3-dihydroxy-octadecadiene, t_R=15.1 (6.3), *m/z*: 338 $(M-103)^+$, 309 $(M-132)^+$, 132; 2-amino-1,3-dihydroxy-4-octadecene, t_R=15.5 (29.0), *m/z*: 340 $(M-103)^+$, 311 $(M-132)^+$, 132.

GC Analysis of TMS Ethers of Methyl Glycoside from SJC-1 The mixture of TMS ethers of the LCB and methyl glycoside was analyzed using GC [column temperature: 100—250 °C (rate of temperature increase 5 °C/min)]: t_R [min]=17.9 and 18.1 (methyl α- and β-glucopyranoside).

Determination of Absolute Configuration of Glucose Moiety of SJC-1 (Hara Method) SJC-1 (1 mg) was heated with 4 N H_2SO_4 (0.5 ml) at 100 °C for 24 h in a sealed vial. The reaction mixture was then extracted with *n*-hexane, and the acidic aqueous phase was neutralized with $Ba(OH)_2$, centrifuged, and the clear supernatant solution was concentrated. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (1.5 mg) and pyridine (0.1 ml) at 60 °C for 1 h. Then 0.1 ml of 1-(trimethylsilyl) imidazole was added, and the mixture was heated at 60 °C for a further 0.5 h to yield trimethylsilyl ether of the methyl (4*R*)-thiazolidine-4-carboxylate derivative. The derivative was analyzed using GC [column temperature: 100—250 °C (rate of temperature increase 5 °C/min)]; t_R=12.2 min (derivative of D-glucose, 12.2 min; L-glucose, 12.8 min). In the same way, the absolute configuration of the glucose moiety (D-form) of SJC-2—SJC-5 was determined.

Methanolysis of SJC-2 In the same manner as described for SJC-1, SJC-2 was methanolyzed and the reaction mixture was worked up to give a mixture of FAM and a residue composed of the LCB and methyl glycoside.

FAM: ^{13}C-NMR (C_5D_5N) δ: 14.3 (terminal methyl group), 51.5 (OCH_3), 71.2 (CHOH), 130.2 (olefinic C), 175.9 (CO).

GC-MS Analysis of FAM from SJC-2 A FAM mixture from SJC-2 was subjected to GC-MS [column temperature 180—250 °C (rate of temperature increase 5 °C/min)]. The results were as follows: methyl 2-hydroxyoctadecanoate, t_R [min] (ratio of peak areas)=13.8 (2.4), *m/z*: 314 (M^+), 255 $(M-59)^+$; methyl 2-hydroxydocosanoate, t_R=21.6 (12.4), *m/z*: 370 (M^+), 311 $(M-59)^+$; methyl 2-hydroxytricosenoate, t_R=24.2 (4.6), *m/z*: 382 (M^+), 323 $(M-59)^+$; methyl 2-hydroxytricosanoate, t_R=24.6 (6.7), *m/z*: 384 (M^+), 325 $(M-59)^+$; methyl 2-hydroxytetracosenoate, t_R=28.1 (73.7), *m/z*: 396 (M^+), 337 $(M-59)^+$; methyl 2-hydroxytetracosanoate, t_R=28.8 (0.2), *m/z*: 398 (M^+), 339 $(M-59)^+$.

GC-MS and GC Analyses of TMS Ethers of LCB and Methyl Glycoside from SJC-2 The residue (mixture of the LCB and methyl glycoside) from SJC-2 was trimethylsilylated, and the reaction mixture was analyzed using GC-MS and GC in the same manner as described for SJC-1. LCB (GC-MS): 2-amino-1,3-dihydroxy-4-hexadecene (ratio of peak areas, 11.9), 2-amino-1,3-dihydroxy-4-heptadecene (77.8), and 2-amino-1,3-dihydroxy-4-octadecene (10.3) were detected. Methyl glycoside (GC): methyl α- and β-glucopyranosides were detected.

Methanolysis of SJC-3 SJC-3 was methanolyzed and the reaction mixture was worked up in the same way as SJC-1. A mixture of FAM and a residue composed of the LCB and methyl glycoside were obtained.

FAM: ^{13}C-NMR (C_5D_5N) δ: 14.3 (terminal methyl group), 51.5 (OCH_3), 71.2 (CHOH), 130.2 (olefinic C), 175.9 (CO).

GC-MS Analysis of FAM from SJC-3 A FAM mixture from SJC-3 was subjected to GC-MS under the same conditions as described for the FAM mixture obtained from SJC-2. The results were as follows: methyl 2-hydroxyoctadecanoate (ratio of peak areas, 6.1), methyl 2-hydroxydocosanoate (30.7), methyl 2-hydroxytricosenoate (3.0), methyl 2-hydroxytricosanoate (18.5), methyl 2-hydroxytetracosenoate (40.5), methyl 2-hydroxytetracosanoate (7.3).

GC-MS and GC Analyses of TMS Ethers of LCB and Methyl Glycoside from SJC-3 In the same way as SJC-1, a mixture of the LCB and methyl glycoside from SJC-3 was trimethylsilylated, and the reaction mixture was analyzed using GC-MS and GC. LCB (GC-MS): 2-amino-1,3,4-trihydroxy-hexadecane, t_R [min] (ratio of peak areas)=14.9 (16.5), *m/z*: 312 $(M-193)^+$, 271 $(M-234)^+$, 132; 2-amino-1,3,4-trihydroxy-heptadecane, t_R=15.9 (70.8), *m/z*: 326 $(M-193)^+$, 285 $(M-234)^+$, 132; 2-amino-1,3,4-trihydroxy-octadecane, t_R=17.4 (10.5), *m/z*: 340 $(M-193)^+$, 299 $(M-234)^+$, 132; 2-amino-1,3,4-trihydroxy-nonadecane, t_R=19.6 (2.2), *m/z*: 354 $(M-193)^+$, 313 $(M-234)^+$, 132. Methyl glycoside (GC): methyl α- and β-glucopyranoside were detected.

Methanolysis of SJC-4 and SJC-5 Conducted in the same manner as in SJC-1, SJC-4 and SJC-5 were methanolyzed and the reaction mixture was worked up to give a mixture of FAM and a residue composed of the LCB and methyl glycoside, respectively.

GC-MS and GC Analyses of FAM and TMS Ethers of Methyl Glycoside from SJC-4 and SJC-5 Each FAM mixture from SJC-4 and SJC-5 was subjected to GC-MS under the same conditions as described for the FAM mixture obtained from SJC-2. The results were as follows (ratio of peak areas of FAM from SJC-4 and SJC-5): methyl 2-hydroxyoctadecanoate (6.1, 4.1), methyl 2-hydroxydocosanoate (16.7, 20.4), methyl 2-hydroxytricosanoate (8.7, 7.5), methyl 2-hydroxytetracosenoate (66.2, 63.2), and methyl 2-hydroxytetracosanoate (2.3, 4.3). Each mixture of LCB and methyl glycoside from SJC-4 and SJC-5 was trimethylsilylated and each reaction mixture was analyzed by GC-MS and GC in the same manner as for SJC-1, and methyl α- and β-glucopyranoside were detected from both reaction mixtures. However, the LCB could not be detected.

DMDS Derivatives of FAM from SJC-1-SJC-5 Each FAM mixture (0.7 mg) from SJC-1—SJC-5 was dissolved in carbon disulfide (0.2 ml), and DMDS (0.2 ml) and iodine (1 mg) were added to the solution. The resulting mixture was kept at 70 °C for 40 h in a small-volume sealed vial. The reaction was subsequently quenched with aqueous $Na_2S_2O_3$ (5%), and the mixture was extracted with *n*-hexane (0.4 ml). The extract was concentrated and the residue (DMDS derivative) was analyzed using GC-MS [Shimadzu QP-1000: EI mode; ionizing potential, 70 eV; column, TC-1701 (0.53 mm× 15 m, GL Science Inc.); carrier gas, He; column temperature 250 °C]: DMDS derivative of SJC-1 FAM, t_R [min]=10.2, *m/z*: 460 (M^+), 287, 173 (a), t_R=13.4, *m/z*: 474 (M^+), 301, 173 (b); SJC-2 FAM, t_R=16.8, *m/z*: 476 (M^+), 303, 173 (c), t_R=22.7, *m/z*: 490 (M^+), 317, 173 (d); SJC-3 FAM, (c) and (d); SJC-4 and SJC-5 FAM, (d) (see Fig. 3).

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