Sphingolipids and Glycerolipids. I. Chemical Structures and Ionophoretic Activities of Soya-cerebrosides I and II from Soybean

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Two glycosphingolipids named soya-cerebrosides I and II were isolated from soybean, the seeds of *Glycine max* MERRILL (Leguminosae), and their chemical structures have been elucidated on the basis of physicochemical evidence and several chemical degradation reactions. By using a newly constructed liquid membrane-type apparatus (W-08) for measurement of ion-transport and ion-binding activities and by employing a method using human erythrocyte membranes for measurement of ion-permeability, it has been found that soya-cerebroside II exhibits ionophoretic activity for Ca^{2+} ion.

Keywords soya-cerebroside I; soya-cerebroside II; soybean; *Glycine max*; glycosphingolipid; fatty acid analysis; liquid membrane apparatus; ionophoretic activity; ion-transport activity; ion-binding activity

Glycosphingolipids are present at the outer layer of the lipid-bilayer in biological membranes and are thought to participate in antigen–antibody reactions and transmission of biological information.[1] During the course of our studies on biologically active soyasaponins in soybeans, the seeds of *Glycine max* MERRILL,[2] we have noticed the presence of glycosphingolipids in soybeans. Being interested in their biological activities, we have isolated those glycosphingolipids and named them soya-cerebrosides I (**1**) and II (**2**). This paper deals with the structure elucidation of the compounds and an evaluation of their ionophoretic activities by using a newly constructed glass-cell apparatus (designated W-08) equipped with artificial membranes and by employing a method using human erythrocyte membranes.[3]

Chemical Structures of Soya-cerebrosides I and II The defatted methanolic extract of soybean (Tamanishiki strain cultivated in Hokkaido, Japan) was partitioned into a mixture of *n*-butanol and water. Silica gel column chromatography of the *n*-butanol-soluble portion provided a cerebroside fraction together with a fraction containing soyasaponins. Purification of the cerebroside fraction by high-performance liquid chromatography (HPLC) using a reverse-phase solid support afforded soya-cerebrosides I (**1**, 0.0016% from soybean) and II (**2**, 0.0008%).

The infrared (IR) spectra of soya-cerebrosides I (**1**) and II (**2**) showed absorption bands ascribable to hydroxyl (**1**, **2**: 3360 cm^{-1}) and amide (**1**: 1630 cm^{-1}; **2**: 1634 cm^{-1}) groups. The proton nuclear magnetic resonance (^{1}H-NMR) spectra showed signals assignable to four olefinic protons and one anomeric proton (**1**, **2**: δ 4.27, d, J=8.0 Hz) and complex signals due to methylene protons, while the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra showed signals due to four olefinic carbons, one primary and two secondary carbinyl carbons, one amide carbon, one carbon attached to the amide group, and glucosyl carbons. Consequently, it has been found that soya-cerebrosides I (**1**) and II (**2**) are *E*/*Z* isomers in the sphingosine moieties of glycosphingolipids having a β-glucosyl moiety. The chemical shifts of the C_7 and C_{10} carbons in their ^{13}C-NMR spectra [**1**: δ 32.6, 32.8 (C-7, 10); **2**: δ 27.1, 27.4 (C-7, 10)] clearly indicated that **1** is the *E* isomer whereas **2** is the *Z* isomer at the C_8–C_9 double bond.

Methanolysis of both soya-cerebrosides (**1**, **2**) liberated methyl glucoside and a mixture of fatty acid methyl esters.[4] By means of gas chromatography-mass spectrometric (GC-MS) analysis, the composition of the methyl esters liberated from **1** was revealed to be methyl 2-hydroxypalmitate (90%, major), methyl 2-hydroxybehenate (2.0%), methyl 2-hydroxylignocerate (1.5%), methyl palmitate (0.95%) and other minor esters, while that from

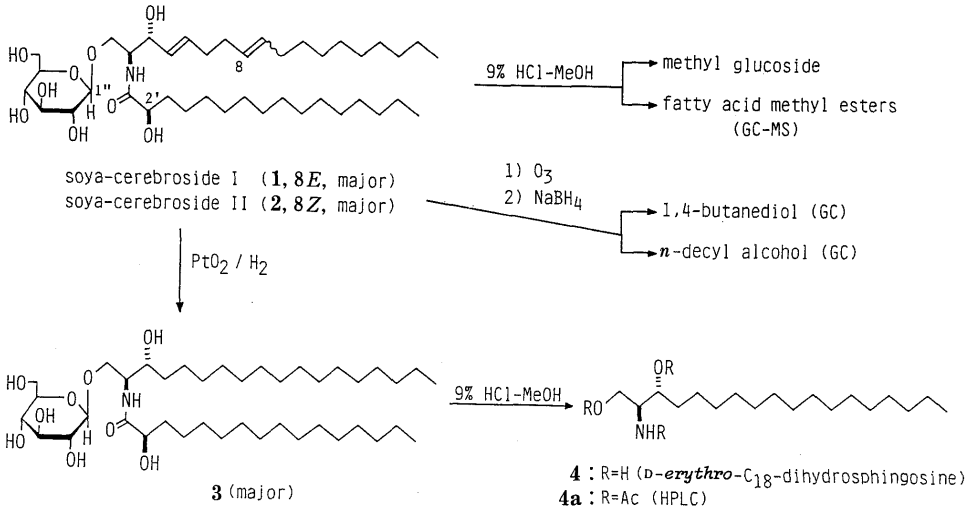


Chart 1

2 was methyl 2-hydroxypalmitate (62%, major), methyl 2-hydroxybehenate (10%), methyl 2-hydroxylignocerate (7.0%), methyl palmitate (2.3%) and other minor esters. Methyl 2-hydroxypalmitate, the major fatty acid methyl ester from both soya-cerebrosides (**1**, **2**) was collected by preparative GC and the absolute configuration at C-2 was determined as *R* from the specific rotation: observed $[\alpha]_D$ $-0.9°$ (ethanol); lit.[5] $[\alpha]_D$ $-1.0°$ (ethanol).

The configuration of the sphingosine base has been clarified in the following manner. Catalytic hydrogenation followed by methanolysis of both soya-cerebrosides (**1**, **2**) gave D-*erythro*-C_{18}-dihydrosphingosine (**4**), which was identical (including the optical properties) with an authentic sample synthesized from (2*Z*)-2-butene-1,4-diol.[6] Furthermore, the triacetate (**4a**) was shown to be identical, by HPLC analysis using a chiral solid support, with the D-*erythro* isomer of the triacetates of D-*erythro*-, L-*erythro*-, D-*threo*-, and L-*threo*-C_{18}-dihydrosphingosines previously synthesized.[6]

Finally, treatment of soya-cerebrosides I (**1**) and II (**2**) with ozone in methanol followed by $NaBH_4$ reduction, yielded 1,4-butanediol and *n*-decyl alcohol which were identified by GC comparison with authentic samples.

On the basis of the above-mentioned evidence, it has been clarified that the basic structures of soya-cerebrosides I and II are 1-*O*-*β*-D-glucopyranosyl-(4*E*,8*E*)-*N*-acyl[7]-4,8-sphingadienine and 1-*O*-*β*-D-glucopyranosyl-(4*E*,8*Z*)-*N*-acyl[7]-4,8-sphingadienine, respectively.[8]

Apparatus W-08 for Measuring Ion-Transport and Ion-Binding Activities Recently, we have constructed a liquid membrane-type glass-cell apparatus designated W-07 for measurement of ion-transport activity for Na^+, K^+, and Ca^{2+} ions[9] and have devised a method for measurement of ion permeability across human erythrocyte membranes.[3] By these means, we have demonstrated the ionophoretic activities of theonellapeptolides,[10] oligopeptide lactones from the Okinawan marine sponge *Theonella swinhoei* (Theonellidae) and merremosides,[11] resin-glycosides from an Indonesian medicinal plant *Merremia mammosa* CHOIS. (Convolvulaceae).[3] Since then, in our continuing study on the improvement of the apparatus W-07 for measuring ion-transport activity on a smaller scale, we have developed a small-sized glass-cell apparatus designated W-08. The new apparatus has the advantage of allowing simultaneous measurement of ion-transport and ion-binding activities for Na^+, K^+, and Ca^{2+} ions.

Apparatus W-08 (Fig. 2) comprises three chambers, two Pyrex glass cells (each of 10 ml volume) for ion-containing water and pure water sandwiching a Teflon cell (effectively a liquid membrane of 4 mm thickness and 0.2 ml volume) holding an organic solution. The left glass cell holds ion-containing water (6 ml, 1 mmol/l) including isotope ions (Na^+ : $^{22}Na^+$ = 12185 : 1, K^+ : $^{42}K^+$ = 4000 : 1, or Ca^{2+} : $^{45}Ca^{2+}$ = 8057 : 1), and the right one contains pure water (6 ml). Two glass cells sandwich the central Teflon cell, which is separated from them by two sheets of artificial membranes (cellulose dialysis membranes of 0.09 mm thickness, Viskase Co.). A sample to be tested is dissolved in chloroform saturated with water (0.01—0.03 mol/l) and injected into the Teflon cell. The whole apparatus is kept at 25 °C and the aqueous phases on both sides are gently stirred.

After 10 h, each phase is sampled. The amount of ions transported from the ion-containing water phase (left cell)

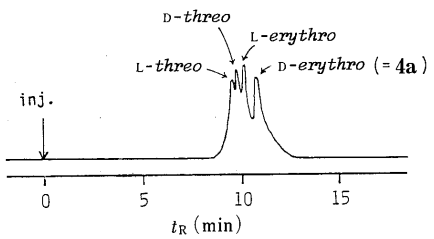


Fig. 1. HPLC (SUMI-PAX OA-4100, *n*-hexane : 2-PrOH = 95 : 5) of Triacetyl-C_{18}-dihydrosphingosines

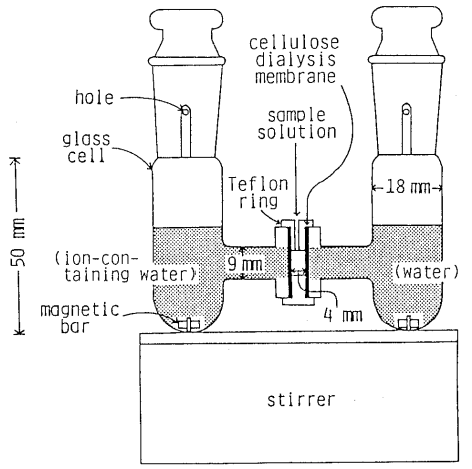


Fig. 2. Apparatus W-08 for Measurements of Ion-Transport and Ion-Binding Activities

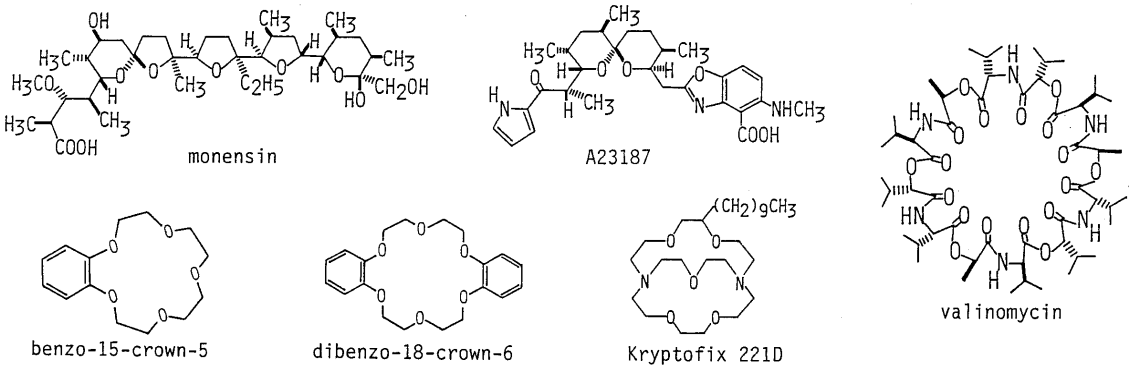


Chart 2

into the pure water phase (right cell) across the organic solution phase (central cell), ions bound by the sample in the organic solution phase and ions remaining in the ion-containing water phase, are respectively determined by counting the radioactivity with a liquid scintillation counter.

By using the apparatus W-08, we first measured ion-transport activity and ion-binding activity of three natural ionophores: monensin[12] (Wako) for Na^+ ion, valinomycin[13] (Sigma) for K^+ ion, and A23187[14] (Fluka) for Ca^{2+} ion, and three synthetic ionophores: benzo-15-crown-5[15] (Merck) for Na^+ ion, dibenzo-18-crown-6[15] (Aldrich) for K^+ ion, and Kryptofix 221D[16] (Merck) for Ca^{2+} ion.

For Na^+ ions, monensin (0.01 mol/l in chloroform) exhibited ion-transport activity (19.91 nmol), as indicated by the amount of ions transported from the ion-containing water phase (left) into the pure water phase (right) across the organic solution phase (central), and the activity was greater than the ion-binding activity (5.53 nmol), which was indicated by the amount of ions in the organic solution phase. On the other hand, benzo-15-crown-5 (0.03 mol/l in chloroform) exhibited ion-binding activity (4.66 nmol) with poor ion-transport activity (0.88 nmol).

In the case of K^+ ions, valinomycin (0.01 mol/l in chloroform) transported K^+ ions efficiently from the ion-containing water phase into the pure water phase (97.54 nmol), while the ions were hardly bound in the organic solution phase (0.26 nmol). Dibenzo-18-crown-6 (0.03 mol/l in chloroform) was found to possess weak ion-transport (5.60 nmol) and ion-binding (11.76 nmol)

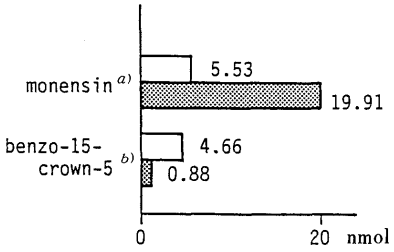


Fig. 3. Na^+-Transport and Na^+-Binding Activities of Monensin and Benzo-15-crown-5

□, amount of ions in the organic solution phase (in central cell); ▨, amount of ions in the pure water phase (in right cell). *a*) 0.01 mol/l in $CHCl_3$, *b*) 0.03 mol/l in $CHCl_3$.

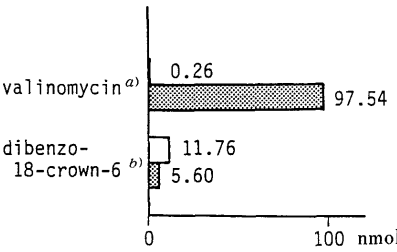


Fig. 4. K^+-Transport and K^+-Binding Activities of Valinomycin and Dibenzo-18-crown-6

□, amount of ions in the organic solution phase (in central cell); ▨, amount of ions in the pure water phase (in right cell). *a*) 0.01 mol/l in $CHCl_3$, *b*) 0.03 mol/l in $CHCl_3$.

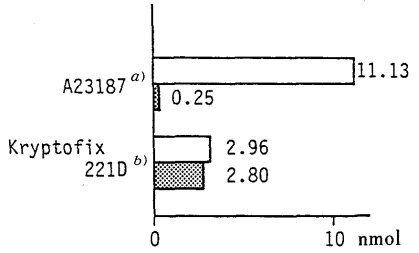


Fig. 5. Ca^{2+}-Transport and Ca^{2+}-Binding Activities of A23187 and Kryptofix 221D

□, amount of ions in the organic solution phase (in central cell); ▨, amount of ions in the pure water phase (in right cell). *a*) 0.01 mol/l in $CHCl_3$, *b*) 0.03 mol/l in $CHCl_3$.

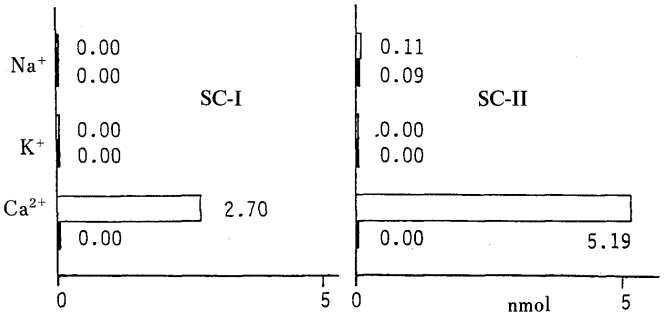


Fig. 6. Ion-Transport and Ion-Binding Activities of Soya-cerebrosides I (**1**, SC-I) and II (**2**, SC-II) with Apparatus W-08

(0.03 M in $CHCl_3$, 10 h): □, in central cell; ■, in right cell.

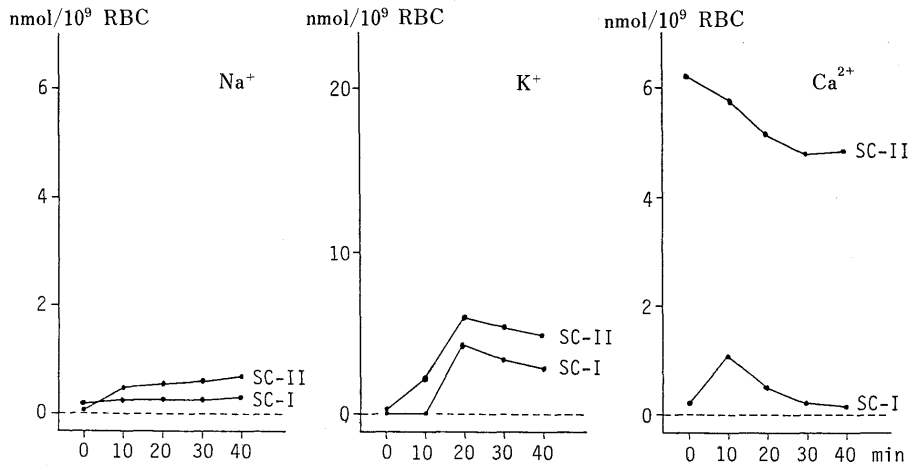
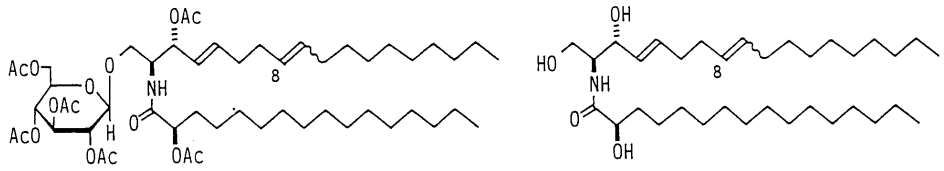


Fig. 7. Ion-Permeability of Soya-cerebrosides I (**1**, SC-I) and II (**2**, SC-II) in Human Erythrocyte Membranes (0.10 μmol/10^9 RBC)



5 (8*E*, major)
6 (8*Z*, major)

7 (8*E*, major)
8 (8*Z*, major)

Chart 3

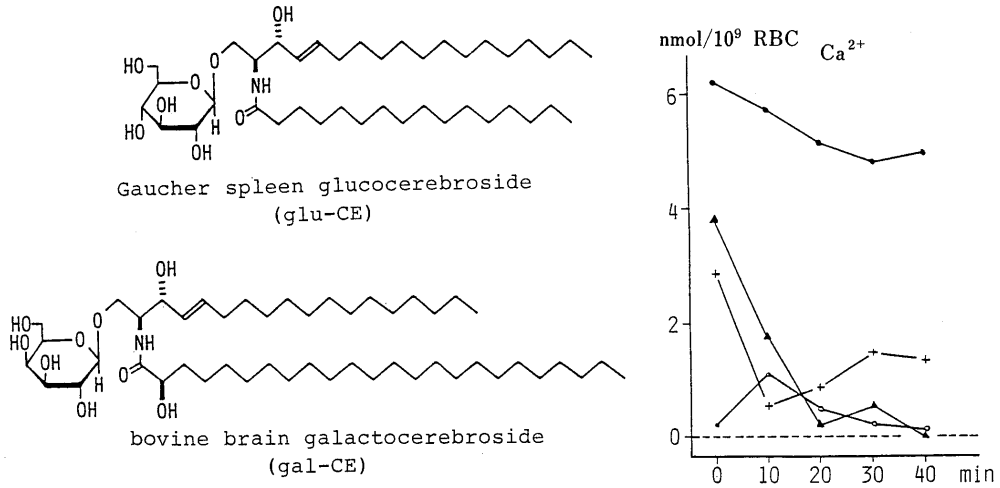


Fig. 8. Ion-Permeability of Gaucher Spleen Glucocerebroside and Bovine Brain Galactocerebroside in Human Erythrocyte Membranes (0.01 μmol/10^9 RBC)

—●—, II (**2**, SC-II); —▲—, gal·CE; —+—, glu-CE; —○—, I (**1**, SC-I).

activities.

For Ca^{2+} ions, A23187 (0.01 mol/l in chloroform) exhibited much stronger ion-binding activity (11.13 nmol) than ion-transport activity (0.25 nmol), while Kryptofix 221D (0.03 mol/l in chloroform) exhibited almost equal ion-transport (2.80 nmol) and ion-binding (2.96 nmol) activities.

Ionophoretic Activities of Soya-cerebrosides I and II We next examined the ionophoretic activities of soya-cerebrosides I (**1**) and II (**2**) by using the apparatus W-08 and by employing the human crythrocyte membrane method.

With the apparatus W-08 (0.03 mol/l in chloroform), it has been found that soya-cerebrosides I (**1**) and II (**2**) exhibit ion-binding activity for Ca^{2+} ion (**1**: 2.70 nmol; **2**: 5.19 nmol, after 10 h) (Fig. 6). By employing the method with human erythrocyte membranes (0.10 μmol/10^9 RBC), soya-cerebroside I (**1**) did not exhibit any ion-permeability for the three ions, while soya-cerebroside II (**2**) exhibited ion-permeability specifically for Ca^{2+} ion (Fig. 7).

On the other hand, five derivatives prepared from soya-cerebrosides I (**1**) and II (**2**), *i.e.* the tetrahydro derivative (**3**), the hexaacetates (**5**, **6**), and their ceramides (**7**, **8**), did not show any ion-permeability across the erythrocyte membranes for Ca^{2+} ion. We have also examined the ion-permeability of Gaucher spleen glucocerebroside[17] (Sigma) and bovine brain galactocerebroside[18] (Sigma), and have found that both cerebrosides show approximately half the activity at the beginning of sample application as compared with soya-cerebroside II (**2**), but with less durability (Fig. 8). These results indicate that i) the double bonds in the sphingosine base and the glucosyl moiety are essential for soya-cerebroside II (**2**) to exhibit Ca^{2+}-ionophoretic activity and ii) the chemical structure of the ceramide moiety is concerned in the durability of the action.

Soya-cerebroside II (**2**) seems to be the first example of a glycosphingolipid having ionophoretic activity for Ca^{2+} ion.

Experimental

Melting points were determined on a Yanagimoto micro-meltingpoint apparatus and are recorded as observed. Optical rotations were measured in a 0.5 dm tube with a JASCO DIP-370 polarimeter. Electron impact (EI)-MS and GC-MS were taken on a JEOL JMS-D300 spectrometer. Fast atom bombardment (FAB)-MS were taken on a JEOL JMS-SX102 spectrometer. IR spectra were taken on a Hitachi 260-30 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL FX-90Q (90 MHz) and GX-500 (500 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. Coupling constants (J values) are given in Hz. GC was carried out on Shimadzu GC-9A and Hitachi 164T gas chromatographs. Radioactivity was counted with an Aloka LSC-950 liquid scintillation counter. HPLC was carried out on Shimadzu LC-5A, LC-6A and Waters C-201 chromatographs. Column chromatography was performed on Kieselgel 60 (Merck, 70—230 mesh). Thin-layer chromatography (TLC) was carried out with pre-coated Kieselgel 60F_{254} plates (Merck).

Isolation of Soya-cerebrosides I (1) and II (2) from Soybean Powdered soybeans (Tamanishiki strain cultivated in Hokkaido, Japan, 5 kg) were extracted with MeOH three times (18 l each, with heating under reflux for 5 h). Removal of the solvent from the combined MeOH solution under reduced pressure gave the MeOH extract (700 g). The MeOH extract was defatted by washing with *n*-hexane to afford the defatted MeOH extract (350 g), which was partitioned into a mixture of *n*-BuOH and H_2O (1 : 1). Removal of the solvent from the *n*-BuOH-soluble portion under reduced

pressure provided the *n*-BuOH extract (180 g), which was subjected to column chromatography (SiO_2 2 kg, $CHCl_3$: MeOH = 15 : 2) to provide a cerebroside fraction (160 mg) together with a soyasaponin-containing fraction (45 g). The cerebroside fraction was then purified by reverse-phase HPLC [YMC AM-312 (10 × 250 mm) + YMC AL-312 (10 × 250 mm), $CHCl_3$: MeOH : H_2O = 2 : 20 : 1] to furnish soya-cerebrosides I (**1**, 80 mg, 0.0016% from soybean) and II (**2**, 40 mg, 0.0008%).

Soya-cerebroside I (**1**): A white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3360, 2915, 2850, 1630. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.90 (6H, t-like, $CH_3 \times 2$), 4.27 (1H, d, J = 8.0 Hz, 1″-H), 5.43 (2H, m, 8-H, 9-H), 5.47 (1H, dd, J = 15.0, 7.5 Hz, 4-H), 5.73 (1H, d-like, 5-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ: for sphingosine moiety: 32.6, 32.8 (C-7, 10), 32.8 (C-6), 54.4 (C-2), 69.9 (C-1), 72.4 (C-3), 129.8, 131.0, 131.8, 132.0 (C-4, 5, 8, 9); for fatty acid moiety: 72.2 (C-2′), 175.6 (C-1′); for sugar moiety: 62.5 (C-6″), 71.4 (C-4″), 74.9 (C-2″), 78.3 (C-3″, 5″), 105.4 (C-1″). FAB-MS *m/z*: 736 $(M+Na)^+$ for 2-hydroxypalmitoyl-type (major). High-resolution FAB-MS *m/z*: Calcd for $C_{40}H_{75}NO_9$ + H: 714.5516. Found: 714.5531 $(M+H)^+$ for 2-hydroxypalmitoyl-type (major).

Soya-cerebroside II (**2**): A white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3360, 2915, 2850, 1634. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.90 (6H, t-like, $CH_3 \times 2$), 4.27 (1H, d, J = 8.0 Hz, 1″-H), 5.37 (2H, m, 8-H, 9-H), 5.49 (1H, dd, J = 15.0, 7.5 Hz, 4-H), 5.74 (1H, dt, J = 15.0, 6.5 Hz, 5-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ: for sphingosine moiety: 27.1, 27.4 (C-7, 10), 32.7 (C-6), 54.3 (C-2), 69.8 (C-1), 72.3 (C-3), 129.2, 131.4, 131.8, 131.9 (C-4, 5, 8, 9); for fatty acid moiety: 72.0 (C-2′), 175.6 (C-1′); for sugar moiety 62.4 (C-6″), 71.3 (C-4″), 74.8 (C-2″), 78.1 (C-5″), 78.2 (C-3″), 105.2 (C-1″). FAB-MS *m/z*: 736 $(M+Na)^+$ for 2-hydroxypalmitoyl type (major). High-resolution FAB-MS *m/z*: Calcd for $C_{40}H_{75}NO_9$ + H: 714.5516. Found: 714.5524 $(M+H)^+$ for 2-hydroxypalmitoyl type (major).

Methanolysis of Soya-cerebroside I (1) a) For the Sugar Moiety Analysis: A solution of soya-cerebroside I (**1**, 5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 3 h. The reaction mixture was neutralized with Ag_2CO_2 powder and the inorganic precipitate was removed by filtration. The solvent was removed from the filtrate under reduced pressure to yield the product, which was dissolved in pyridine (0.1 ml) and treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml) for 10 min. The reaction product was then analyzed by GC to identify the TMS derivative of methyl glucoside. GC 1) 1.5% silicone SE-30 on Chromosorb WAW DMCS (80—100 mesh); 3 mm × 1 m glass column; column temperature, 150 °C; N_2 flow rate, 35 ml/min; t_R, 14 min 54 s, 18 min 5 s. 2) 1.5% OV-1 on Chromosorb WAW DMCS (80—100 mesh); 3 mm × 1 m glass column; column temperature, 140 °C; N_2 flow rate, 35 ml/min; t_R, 9 min 26 s, 10 min 38 s.

b) For the Fatty Acid Moiety Analysis: A solution of soya-cerebroside I (**1**, 5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 3 h. After addition of H_2O (3 ml), the whole was extracted with petroleum ether (bp 30—80 °C) three times. Removal of the solvent from the combined petroleum ether solution furnished the product. The product was then analyzed by GC-MS. GC-MS: 1.5% silicone SE-52 on Uniport HP (60—80 mesh); 3 mm × 2 m glass column; column temperature, 150—220 °C (3 °C/min); He_2 flow rate, 35 ml/min; ionization, 22 eV; t_R, methyl palmitate 7 min 24 s (0.95%), methyl 2-hydroxypalmitate 9 min 24 s (90%), methyl 2-hydroxybehenate 19 min 26 s (2.0%), and 2-hydroxylignocerate 22 min 24 s (1.5%).

Methanolysis of Soya-cerebroside II (2) a) For the Sugar Moiety Analysis: A solution of soya-cerebroside II (**2**, 5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 3 h. The sugar-containing portion was worked up and analyzed by GC (as the TMS derivative), as described above for the methanolysis product of **1**, to identify methyl glucoside.

b) For the Fatty Acid Moiety Analysis: A solution of soya-cerebroside II (**2**, 5 mg) was heated under reflux for 3 h. The fatty acid portion was worked up and analyzed by GC-MS, as described above for the methanolysis product of **1**, to detect methyl palmitate (2.3%), methyl 2-hydroxypalmitate (62%), methyl 2-hydroxybehenate (10%), and methyl 2-hydroxylignocerate (7.0%).

Purification of Methyl 2-Hydroxypalmitate (3) The above-described fatty acid portion (28 mg) obtained from soya-cerebroside I (**1**, 50 mg) was purified by preparative GC [2% silicone SE-52 on Uniport HP (0.3 mm × 2 m); He_2 flow rate, 25 ml/min; column temperature, 180 °C] to afford methyl (2*R*)-2-hydroxypalmitate [6 mg, mp 45 °C (*n*-hexane), $[\alpha]_D$ −0.9° (c = 0.3, EtOH); lit.[5] mp 45—46 °C, $[\alpha]_D$ −1.0° (EtOH)].

Catalytic Hydrogenation of Soya-cerebroside I (1) A solution of soya-cerebroside I (**1**, 50 mg) in MeOH (10 ml) was treated with PtO_2 (20 mg) and the mixture was stirred vigorously under an H_2 atmosphere (1 atm) at room temperature for 2 h. After removal of the catalyst by filtration, the solvent was evaporated off under reduced pressure to yield the product (55 mg). Purification of the product by column chromatography (SiO_2 3 g, $CHCl_3$: MeOH = 9 : 1) furnished the tetrahydro derivative (**3**, 47 mg). **3**: a white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3330, 2910, 1648. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.87 (6H, t, J = 6.5 Hz, $CH_3 \times 2$), 3.4—4.6 (11H, m), 4.72 (1H, d, J = 6.5 Hz, 1″-H), 8.41 (1H, d, J = 8.0 Hz, amide proton). FAB-MS *m/z*: 718 $(M+H)^+$ for 2-hydroxypalmitoyl type (major). High-resolution FAB-MS *m/z*: Calcd for $C_{40}H_{79}NO_9$ + H: 718.5528. Found: 718.5511 $(M+H)^+$ for 2-hydroxypalmitoyl type (major).

Methanolysis of 3 A solution of **3** (45 mg) in 9% HCl–dry MeOH (30 ml) was heated under reflux for 5 h. The reaction mixture was adjusted to pH 8 with 10% aqueous NaOH and the whole was extracted with ether. Removal of the solvent from the combined ethereal solution gave the product (28 mg). Purification of the product by column chromatography (SiO_2 5 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10) furnished D-*erythro*-C_{18}-dihydrosphingosine (**4**, 6 mg), which was found to be identical with an authentic sample[6] by comparisons of the physical data [IR (KBr) and ^{1}H-NMR (pyridine-d_5)] and specific rotation.

Acetylation of 4 A solution of **4** (3 mg) in Ac_2O–pyridine (1 : 2, 0.6 ml) was allowed to stand at room temperature for 1 h. The reaction mixture was poured into ice-water, and the whole was extracted with EtOAc. Work-up of the EtOAc extract in the usual manner gave the product, which was purified by column chromatography (SiO_2 1 g, *n*-hexane : EtOAc = 1 : 2) to furnish triacetyl-D-*erythro*-C_{18}-dihydrosphingosine (**4a**, 2.8 mg). **4a** was identical with an authentic sample[6] by judged from HPLC on a chiral solid support (SUMI-PAX OA-4100; *n*-hexane : 2-PrOH = 95 : 5; flow rate, 1.3 ml/min; (RI detector × 8).

Catalytic Hydrogenation of Soya-cerebroside II (2) A solution of soya-cerebroside II (**2**, 25 mg) in MeOH (5 ml) was treated with PtO_2 (10 mg) and the mixture was stirred vigorously under an H_2 atmosphere (1 atm) at room temperature for 2 h. After removal of the catalyst by filtration, the solvent was evaporated off under reduced pressure to yield the product (27 mg). Purification of the product by column chromatography (SiO_2 2 g, $CHCl_3$: MeOH = 9 : 1) furnished the hydrogenated product (20 mg), which was shown to be identical with **3** by IR (KBr) and ^{1}H-NMR (pyridine-d_5) comparisons.

Ozone Oxidation Followed by $NaBH_4$ Reduction of Soya-cerebroside I (1) A solution of soya-cerebroside I (**1**, 3 mg) in MeOH (5 ml) was bubbled through with ozonized oxygen at −78 °C for 30 min. The cooled solution was then bubbled through with nitrogen to remove excess ozone. After warming gradually to room temperature, the reaction mixture was treated with $NaBH_4$ (10 mg) at room temperature for 10 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was separated by filtration. The filtrate was evaporated under reduced pressure to give the product (2.5 mg), which was analyzed by GC to identify 1,4-butanediol and *n*-decyl alcohol. GC: 15% PEGS on Chromosorb WAW 80—100 mesh); 3 mm × 2 m glass column; column temperature, 150 °C; N_2 flow rate, 35 ml/min; t_R, 1,4-butanediol 4 min 46 s, *n*-decyl alcohol 6 min 30 s.

Ozone Oxidation Followed by $NaBH_4$ Reduction of Soya-cerebroside II (2) A solution of soya-cerebroside II (**2**, 2 mg) in MeOH (5 ml) was bubbled through with ozonized oxygen at −78 °C for 30 min. The cooled solution was then bubbled through with nitrogen to remove excess ozone. After warming gradually to room temperature, the reaction mixture was treated with $NaBH_4$ (10 mg) at room temperature for 10 min. Work-up of the reaction mixture as described above for the ozone oxidation product of **1**, furnished the product (2 mg), which was analyzed by GC to identify 1,4-butanediol and *n*-decyl alcohol.

Procedure for Ion-Transport and Ion-Binding Activity Tests Tests using the apparatus W-08 were carried out according to the procedure described in the text. The molar ions in the pure water phase and those in the organic solution phase were calculated by the following equations.

$$M_R = \frac{X_R}{X_L} \cdot \frac{v_L}{v_R} \cdot C_L \cdot V_R \qquad M_C = \frac{X_C}{X_L} \cdot \frac{v_L}{v_C} \cdot C_L \cdot V_C$$

where, M_R = molar ions in the pure water phase (μmol); M_C = molar ions in the organic solution phase (μmol); X_R = observed amount of the isotope ions in v_R ml of the pure water (cpm); X_C = observed amount of the isotope ions in v_C ml of organic solution (cpm); X_L = observed amount of the isotope ions in v_L ml of ion-containing water (cpm); C_L = initial concentration of ion-containing water (mmol/l); V_R = volume of pure water (ml); and V_C = volume of organic solution (ml).

Acetylation of Soya-cerebroside I (1) A solution of soya-cerebroside I (**1**, 10 mg) in pyridine (1 ml) was treated with Ac_2O (0.5 ml) and the mixture was left standing at room temperature for 4 h, then poured into ice-water

and extracted with EtOAc. work-up of the EtOAc extract in the usual manner gave **5** (10 mg). **5**: a white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1747, 1676. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.89 (6H, t, J=7.0 Hz, $CH_3\times2$), 2.00, 2.04, 2.10, 2.18 (3H each, all s, $OCOCH_3\times4$), 2.03 (6H, s, $OCOCH_3\times2$), 3.62 (1H, dd, J=4.5, 10.5 Hz, 1-H_a), 3.70 (1H, ddd, J=2.5, 4.5, 10.0 Hz, 5″-H), 3.94 (1H, dd, J=4.0, 10.5 Hz, 1-H_b), 4.15 (1H, dd, J=4.5, 12.5 Hz, 6″-H_a), 4.24 (1H, dd, J=4.5, 12.5 Hz, 6″-H_b), 4.30 (1H, m, 2-H), 4.48 (1H, d, J=8.0 Hz, 1″-H), 4.96 (1H, dd, J=8.0, 9.5 Hz, 2″-H), 5.08 (1H, t-like, 3″-H or 4″-H), 5.15 (1H, dd, J=5.0, 7.0 Hz, 2′-H), 5.19 (1H, t-like, 3″-H or 4″-H), 5.32 (1H, t-like, 3-H), 5.3—5.5 (3H, m, 4-H, 8-H, 9-H), 5.82 (1H, dt, J=15.5, 6.0 Hz, 5-H), 6.33 (1H, d, J=9.0 Hz, –NHCO–). FAB-MS m/z: 988 $(M+Na)^+$, 966 $(M+H)^+$ for 2-hydroxypalmitoyl type (major). High-resolution FAB-MS m/z: Calcd for $C_{52}H_{88}NO_{15}+H$: 966.6157. Found: 966.6173 $(M+H)^+$ for 2-hydroxypalmitoyl type (major).

Acetylation of Soya-cerebroside II (2) A solution of soya-cerebroside II (**2**, 10 mg) in pyridine (1 ml) was treated with Ac_2O (0.5 ml) and the mixture was left standing at room temperature for 4 h, then poured into ice-water and extracted with EtOAc. Work-up of the EtOAc extract in the usual manner gave **6** (10 mg). **6**: a white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1747, 1691. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.89 (6H, t, J=7.0 Hz, $CH_3\times2$), 2.00, 2.04, 2.10, 2.18 (3H each, all s, $OCOCH_3\times4$), 2.03 (6H, s, $OCOCH_3\times2$), 3.62 (1H, dd, J=4.5, 10.5 Hz, 1-H_a), 3.70 (1H, ddd, J=2.5, 4.5, 10.0 Hz, 5″-H), 3.94 (1H, dd, J=4.0, 10.5 Hz, 1-H_b), 4.15 (1H, dd, J=2.5, 12.5 Hz, 6″-H_a), 4.24 (1H, dd, J=4.5, 12.5 Hz, 6″-H_b), 4.31 (1H, m, 2-H), 4.48 (1H, d, J=8.0 Hz, 1″-H), 4.96 (1H, dd, J=8.0, 9.5 Hz, 2″-H), 5.08 (1H, t-like, 3″-H or 4″-H), 5.15 (1H, dd, J=4.5, 7.0 Hz, 2′-H), 5.19 (1H, t-like, 3″-H or 4″-H), 5.33 (1H, t-like, 3-H), 5.3—5.5 (3H, m, 4-H, 8-H, 9-H), 5.83 (1H, dt, J=15.5, 6.0 Hz, 5-H), 6.33 (1H, d, J=9.0 Hz, –NHCO–). FAB-MS m/z: 988 $(M+Na)^+$, 966 $(M+H)^+$ for 2-hydroxypalmitoyl type (major). High-resolution FAB-MS m/z: Calcd for $C_{52}H_{88}NO_{15}+H$: 966.6157. Found: 966.6182 $(M+H)^+$ for 2-hydroxypalmitoyl type (major).

Acid Hydrolysis of Soya-cerebroside I (1) to Give 7 A suspension of soya-cerebroside I (**1**, 18 mg) in benzene (2 ml) was treated with 10% aqueous H_2SO_4 (1 ml) and the whole was heated under reflux with stirring for 9 h. After cooling, the reaction mixture was poured into aqueous saturated $NaHCO_3$ and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the product (18 mg), which was purified by column chromatography (SiO_2 1 g, $CHCl_3$: MeOH=10:1) to furnish **7** (5.6 mg). **7**: a white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3350, 1653. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.81 (6H, t, J=7.0 Hz, $CH_3\times2$), 3.7—4.2 (4H, m, 1-H_2, 2-H, 3-H), 4.33 (1H, t-like, 2′-H), 5.40 (2H, m, 8-H, 9-H), 5.55 (1H, dd, J=6.5, 15.5 Hz, 4-H), 5.79 (1H, dt, J=15.5, 6.5 Hz, 5-H), 7.15 (1H, d, J=7.5 Hz, –NHCO–). FAB-MS m/z: 574 $(M+Na)^+$, 552 $(M+H)^+$ for 2-hydroxypalmitoyl type (major). High-resolution FAB-MS m/z: Calcd for $C_{34}H_{65}NO_4+H$: 552.4994. Found: 552.4989 $(M+H)^+$ for 2-hydroxypalmitoyl type (major).

Acid Hydrolysis of Soya-cerebroside II (2) to Give 8 A suspension of soya-cerebroside II (**2**, 15 mg) in benzene (4 ml) was treated with 10% aqueous H_2SO_4 (2 ml) and the whole was heated under reflux with stirring for 5 h. After cooling, the reaction mixture was poured into aqueous saturated $NaHCO_3$ and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated NaCl, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the product (17 mg), which was purified by column chromatography (SiO_2 1 g, $CHCl_3$: MeOH=10:1) to furnish **8** (4.6 mg). **8**: a white amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 3340, 1652. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.81 (6H, t, J=7.0 Hz, $CH_3\times2$), 3.7—4.2 (4H, m, 1-H_2, 2-H, 3-H), 4.33 (1H, t-like, 2′-H), 5.3—5.5 (2H, m, 8-H, 9-H), 5.58 (1H, dd, J=6.5, 15.5 Hz, 4-H), 5.81 (1H, m, 5-H), 7.11 (1H, d, J=8.5 Hz, –NHCO–). FAB-MS m/z: 574 $(M+Na)^+$, 552 $(M+H)^+$ for 2-hydroxypalmitoyl type (major). High-resolution FAB-MS m/z: Calcd for $C_{34}H_{65}NO_4+H$: 552.4994. Found: 552.4948 $(M+H)^+$ for 2-hydroxypalmitoyl type (major).

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