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The Principles of *Tetragonia tetragonoides* having Anti-ulcerogenic Activity. II. Isolation and Structure of Cerebrosides[1)]

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Compound B_1 (tentative name), isolated from *Tetragonia tetragonoides* as a principle with anti-ulcerogenic activity, was determined to be a mixture of geometrical isomers of 1-*O*-*β*-D-glucopyranosyl-2-*N*-2′-hydroxypalmitoyl-sphinga-4,8-dienine on the basis of chemical and spectral evidence. By repeated chromatography, compound B_1 was separated into compounds $B_{1\text{-a}}$ (major) and $B_{1\text{-b}}$ (minor), which were found to be the 4-*trans*-8-*trans* and 4-*trans*-8-*cis* isomers, respectively. Several cerebrosides and glycolipids from various biological sources were examined for protective activity against the ulcer formation in mice under restraint and water immersion condition.

Keywords——*Tetragonia tetragonoides*; cerebroside; 1-*O*-*β*-D-glucopyranosyl-2-*N*-2′-hydroxypalmitoyl-sphinga-4-*trans*-8-*trans*-dienine; 1-*O*-*β*-D-glucopyranosyl-2-*N*-2′-hydroxypalmitoyl-sphinga-4-*trans*-8-*cis*-dienine; experimental stress ulcer

During a survey of neurotropic components of oriental crude drugs, we have recognized that some of the extracts from these drugs showed a protective effect against the formation of experimental stress ulcers in mice under restraint and water immersion conditions. Some crude drugs, such as Polygalae Radix (Onji), Anemarrhenae Rhizoma (Chimo), *etc.*, have a significant effect.[2)] Interestingly, many of them are generally used as sedative drugs in oriental medicine. In previous reports of this series, we showed that Tetragoniae Herba (Bankyoo; the whole plants of *Tetragonia tetragonoides*) was most effective in protecting against ulcer formation among these drugs,[2)] and compounds A and B_1 (tentative names) were isolated from this drug as the active principles.[3)] Compound A was determined to be a mixture of sterylglucosides.[3)] We have found compound B_1 is a cerebroside mixture consisting of two Δ^8-geometrical isomers, and this mixture has now been separated into its components, compounds $B_{1\text{-a}}$ and $B_{1\text{-b}}$. We report here the structures of compounds $B_{1\text{-a}}$ and $B_{1\text{-b}}$, including the geometry of the olefinic portion. The protective effects against experimental stress ulcer of several cerebrosides and glycolipids obtained from various biological sources were also examined.

Cerebrosides are widely distributed in nature as constituents of brain,[4)] nerves[5)] and other organs,[6)] and are contained in milk,[7)] oyster,[8)] some plants,[9)] *etc.* Cerebrosides contain various sugars, fatty acids and sphingosines as components so that the separation of mixtures of analogues is difficult. The constituents of cerebrosides have been analyzed in order to investigate species differences and organ differences. Cerebrosides gave also been investigated biochemically in connection with lipidoses such as Gaucher's disease[10)] and their immunological properties.[11)] However, very little pharmacological work on cerebrosides has been done.

Compound B_1 (**1**) was obtained as a white solid from MeOH–H_2O[3)] and its molecular formula was determined to be $C_{40}H_{75}NO_9$,[12)] field desorption mass spectrum (FD-MS) *m/z* (%) 736 ($(M+Na)^+$, 100). (Nevertheless, compound B_1 was later recognized to be a mixture of two isomers.) The color reaction of compound B_1 (positive in the anthrone and modified

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Molisch tests) indicated the presence of a sugar moiety in the structure. Hydrolysis of compound B_1 with 2 N HCl afforded glucose. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of compound B_1 exhibited signals of 11—13 protons in the range of 4—5 ppm, including an anomeric signal at 4.82 (1H, d, $J=7$ Hz) ppm. In the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum, signals corresponding to seven tertiary and two secondary carbons in the range of 50—80 ppm and an anomeric carbon at 105.6 ppm were observed. The appearance of the signals at 62.6 (t), 71.5 (d), 75.0 (d), 78.4 (d) and 105.6 (d) ppm[13] indicated that compound B_1 contains an equimolar amount of β-D-glucopyranose. A large singlet-like signal at 1.25 ppm in the ^{1}H-NMR spectrum and the absorptions at 2920, 2850, 1468 and 722 cm^{-1} in the infrared (IR) spectrum are characteristic of the presence of an aliphatic long chain. Two olefinic signals appeared at 5.41 (2H, m) and 5.86 (2H, m) ppm, and the signals were sharpened by irradiation of the methylene signals at *ca.* 2.03 and 2.12 ppm, respectively. One of these olefinic signals (5.86 ppm) was also sharpened by irradiation at near 4.67 ppm. These data revealed the presence of two partial structures including double bonds in compound B_1 as shown in Fig. 1.

Compound B_1 (**1**) was hydrogenated over PtO_2 to yield tetrahydro-compound B_1 (**2**) and was oxidized with OsO_4 to yield tetrahydroxy-compound B_1 (**3**) (Chart 1). These findings also supported the presence of two double bonds in the strucrure. However, the ^{13}C-NMR spectrum of compound B_1 showed five, not four sp^2-carbon signals, which are split into doublets by the off-resonance technique. This finding led us to consider that compound B_1 might be a mixture of two geometrical isomers at a double bond.

The presence of the amide chromophore in compound B_1 was suggested by the observation of the amide band at 1640 cm^{-1} in the IR spectrum. The signal of the amide carbonyl group was observed at 176.4 ppm in the ^{13}C-NMR spectrum. Methanolysis of the tetrahydro-compound B_1 (**2**) with 2—4 N HCl in MeOH afforded a fatty acid methyl ester (**5**); $C_{17}H_{34}O_3$, mp 72—92 °C (Chart 2). The fragment pattern in the mass spectrum (MS) of **5** indicated an ester of a C-16 straight chain fatty acid. The ^{1}H-NMR data suggested the presence of –CO–CH(OH)–CH_2– [4.08 (1H, dd, $J=4$, 6 Hz) ppm], while the signal of a methylene adjacent to the carbonyl group was not observed. The ester (**5**) was identified as methyl 2-hydroxypalmitate by comparison of the MS of **5** with the literature values.[14] The

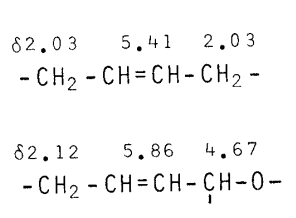

Fig. 1

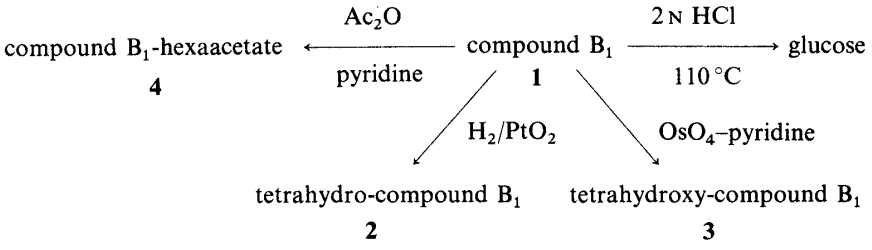

Chart 1

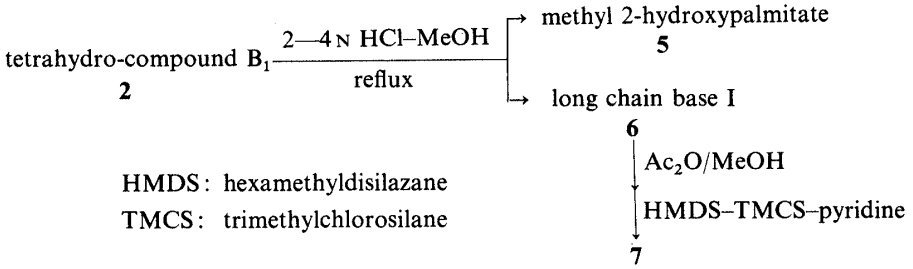

Chart 2. Methanolysis of Tetrahydro-compound B_1 (**2**)

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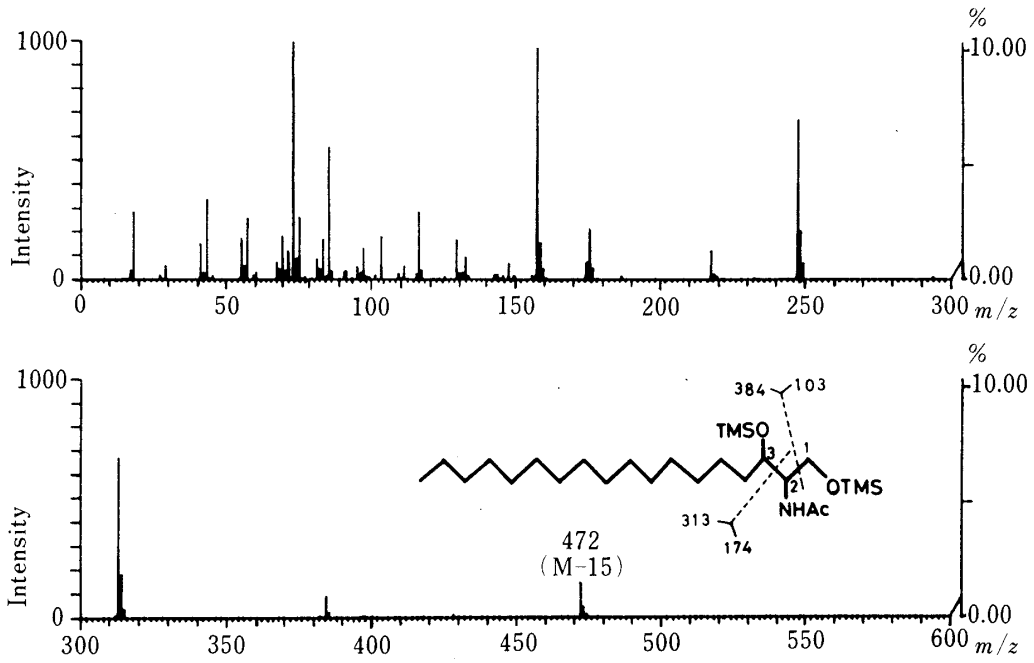

Fig. 2. MS of **7**

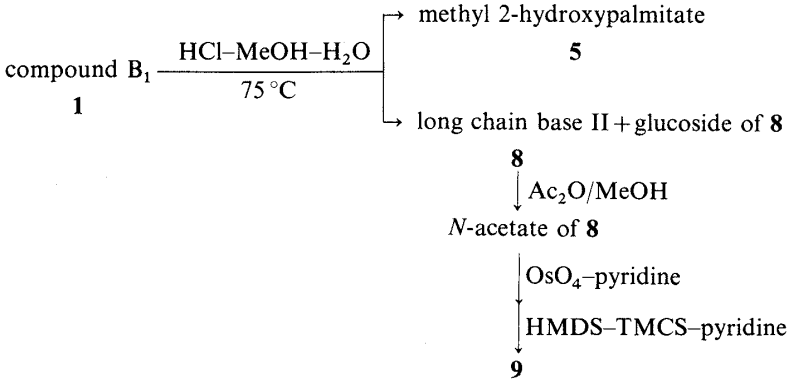

Chart 3. Methanolysis of Compound B_1 (**1**)

long chain base I (**6**) was ninhydrin-positive on thin-layer chromatography (TLC). In the ^{1}H-NMR spectrum of **6**, a triplet-like signal of the methyl group at 0.88 (3H, $J=6$ Hz) ppm and a singlet-like large methylene signal at 1.28 ppm were observed. The above data and the MS (characteristic peaks separated by 14 mass units) indicated that **6** contains a saturated straight chain. The presence of two free hydroxyl groups other than those of the glucose moiety in compound B_1 was suggested by the fact that compound B_1 gave a hexaacetate (**4**) on acetylation (Chart 1). One of these two hydroxyl groups should be present in **6**, since the other is present in the ester **5**, methyl 2-hydroxypalmitate. The long chain base (**6**) also contains an amino group linking to the fatty acid to form the amide and a hydroxyl group bonding to the glucose moiety. The MS of the trimethylsilyl (TMS) derivative of **6** suggested that **6** might be sphinganine. In TLC, **6** was found to be identical with sphinganine which was obtained from commercially available sphingosine by catalytic hydrogenation. The MS of the *N*-acetyl-*O*-TMS-derivative (**7**) of **6** was identical with the reported values for 2-*N*-acetyl-1,3-di-*O*-TMS-sphinganine[15] (Fig. 2). These results suggested that tetrahydro-compound B_1 (**2**) is a cerebroside composed of β-D-glucose, 2-hydroxypalmitic acid and sphinganine.

Methanolysis of compound B_1 with 1 N HCl–MeOH–H_2O[16] gave methyl 2-hydroxypal-

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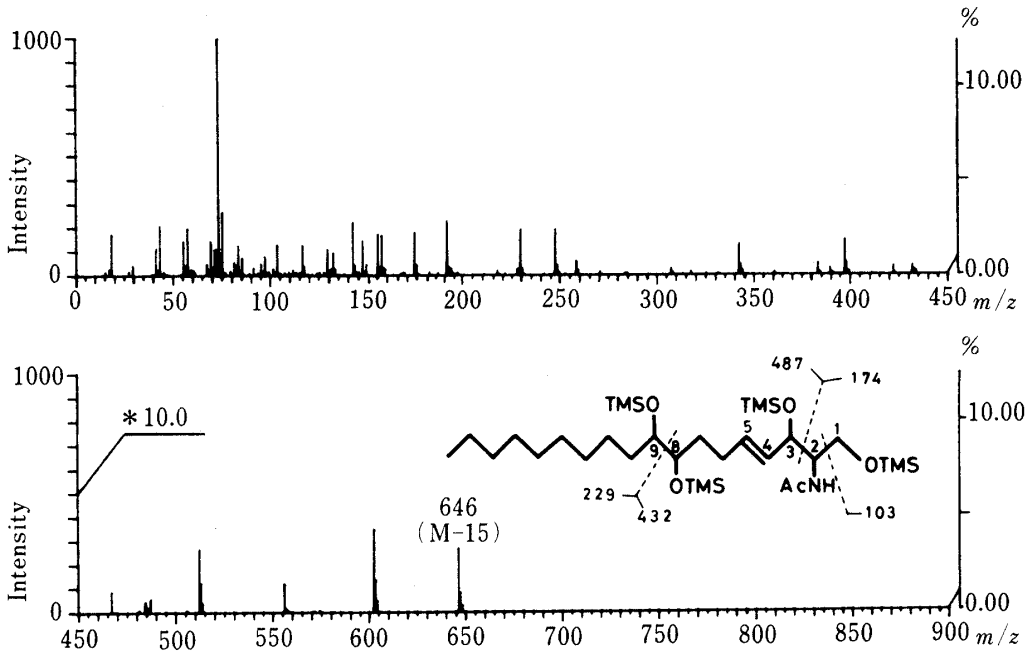

Fig. 3. MS of **9**

mitate (**5**) and long chain base II (**8**) in a low yield (Chart 3). The base (**8**) contains two double bonds. One of the double bonds should be present at C-4, since it was already found to be linked to a carbon bearing oxygen as shown in Fig. 1. The position of the other double bond was determined as follows. The base (**8**) was converted into the *N*-acetyldihydroxylate through *N*-acetylation followed by oxidation with an equimolar amount of OsO_4. The MS of the TMS derivative (**9**) showed characteristic fragments (*m/z* (%) 229 (23) and 432 (9)) possibly formed by cleavage between C-8 and C-9 (Fig. 3). The double bond at C-8 was more easily oxidized with OsO_4 than the other one at C-4. The long chain base II (**8**) was thus determined to be sphinga-4,8-dienine, which has been found in oyster glycolipids[8] and in certain plant cerebrosides[17] by GC-MS and actually isolated from wheat flour lipids as triacetyl derivatives.[18] In conclusion, compound B_1 has been identified as a mixture of geometrical isomers of 1-*O*-*β*-D-glucopyranosyl-2-*N*-2′-hydroxypalmitoyl-sphinga-4,8-dienine. The MS of the TMS-derivatives of compound B_1 (**1**) and tetrahydroxy-compound B_1 (**3**) support the proposed structure (Figs. 4 and 5).

The stereochemistry of double bonds in sphingosines has often been assessed by comparison of the intensities of the bands of *cis* and/or *trans* double bonds in their IR spectra. However, the geometry is not exactly determined by this method. We found that the olefinic signal at 5.86 (2H, m) ppm in the ^{1}H-NMR spectrum of compound B_1 (in pyridine-d_5) assigned to H–C (4) and H–C (5) was observed as two signals at 5.38 (1H, m) and 5.70 (1H, m) ppm when measured in $CDCl_3$–CD_3OD solvent system. On irradiation at 2.06 ppm, the multiplet signals of H–C (4) and H–C (5) were changed to a doublet of doublets ($J = 16$, 6 Hz) and a doublet ($J = 16$ Hz), respectively. The coupling constant between them indicated that the geometry in this double bond is *trans*. On the other hand, the coupling between H–C (8) and H–C (9) is not observed because the signals overlap. We therefore tried to separate the two stereoisomers.

Chromatography using a silica gel pre-packed column resulted in the separation of compound B_1 into compounds $B_{1\text{-a}}$ (major) and $B_{1\text{-b}}$ (minor). The 67.80 MHz ^{13}C-NMR spectra of compounds $B_{1\text{-a}}$ and $B_{1\text{-b}}$ are illustrated at Fig. 6. Olefinic carbons in compound $B_{1\text{-b}}$ (at 129.20 and 131.24 ppm) are observed at slightly higher magnetic field than those of

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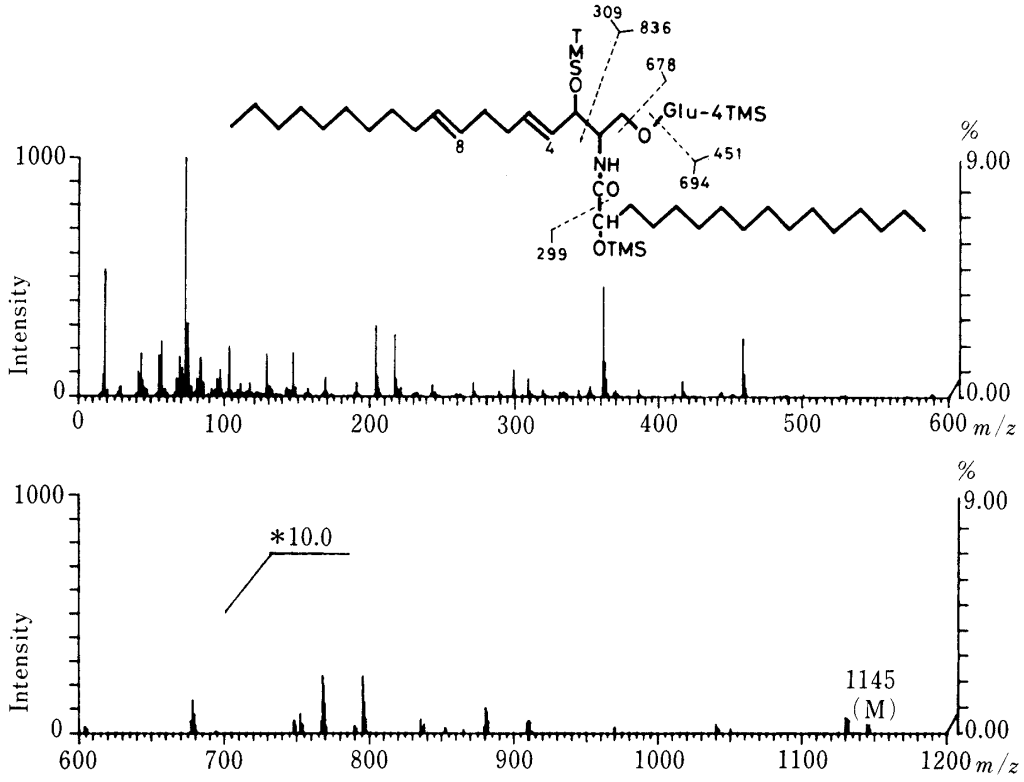

Fig. 4. MS of the TMS Derivative of Compound B_1 (**1**)

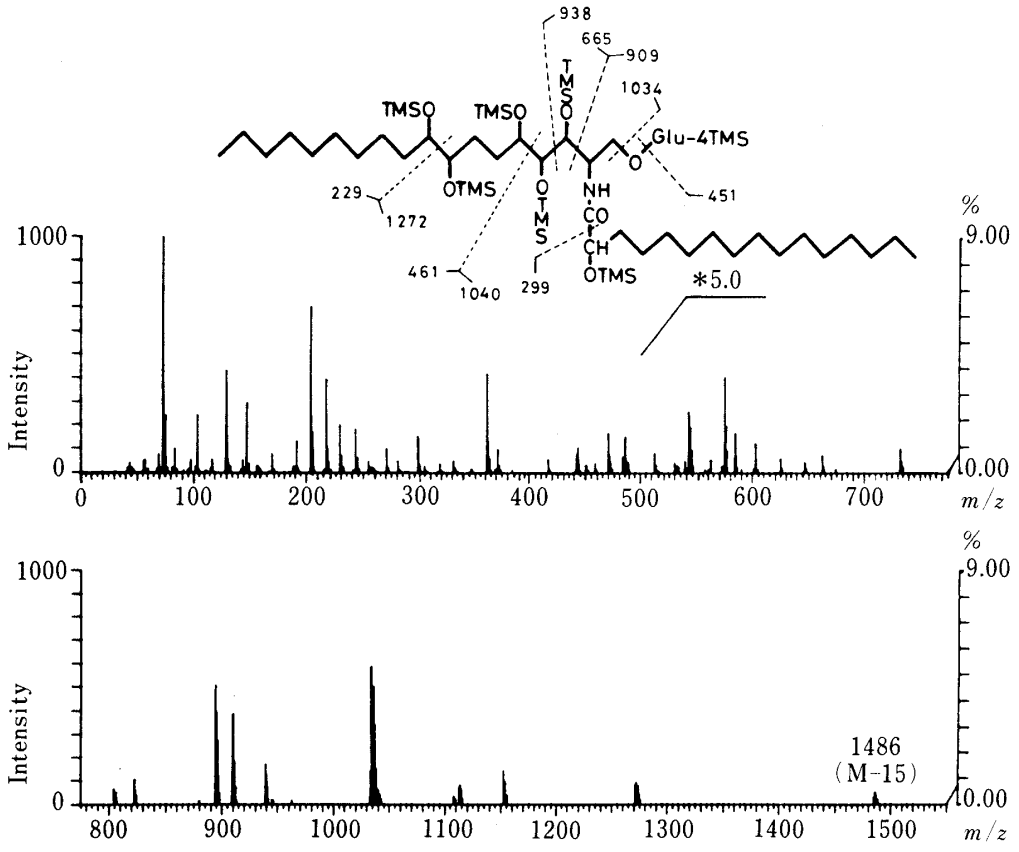

Fig. 5. MS of the TMS Derivative of Tetrahydroxy-compound B_1 (**3**)

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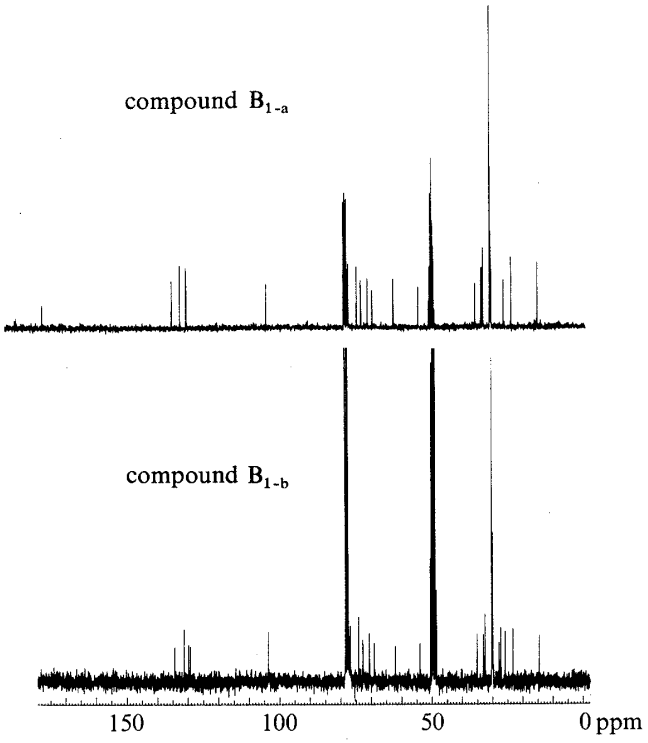

Fig. 6. ^{13}C-NMR Spectra of Compounds B_{1-a} and B_{1-b}[19]

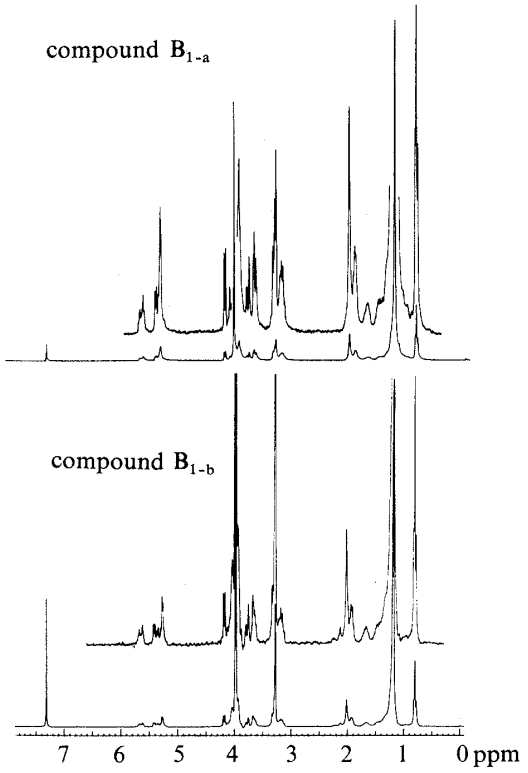

Fig. 7. ^{1}H-NMR Spectra of Compounds B_{1-a} and B_{1-b}

compound B_{1-a} (at 129.58 and 131.69 ppm). The methylene signals in compound B_{1-a} showed a similar pattern (same shifts) to those of compound B_{1-b}, except for two signals at 32.76 and 33.16 ppm. In the spectrum of compound B_{1-b}, two methylene signals corresponding to the above two signals (32.76 and 33.16 ppm) are observed at 27.34 and 27.90 ppm. α-Carbons in

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TABLE I. Effect of Cerebrosides on Stress-induced Ulcer

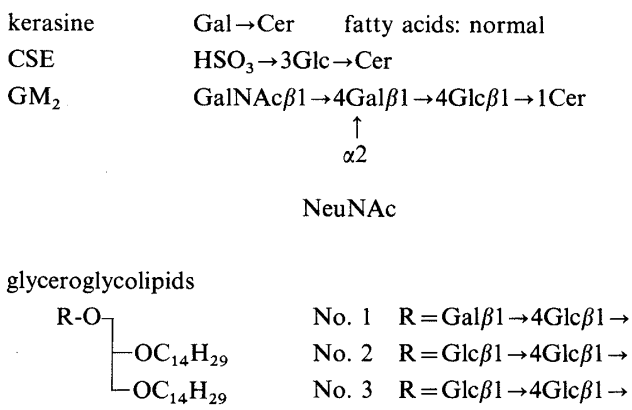
Source	Protective ratio (%)	Statistical significance
Bankyoo (compound B_1)	76	$p<0.01$
Alfalfa	55	$p<0.05$
Soybean	74	$p<0.001$
Rice bran	33	N.S.
Wheat bran	18	N.S.

Test compounds were administered intraperitoneally at the dose of 100 mg/kg. N.S.: no significance.

TABLE II. Effect of Animal Cerebrosides and Synthetic Glycolipids on Stress-induced Ulcer in Mice

Compound	Source	Protective ratio (%)	Statistical significance
Gluco-cerebroside	Gaucher's disease patients	79	$p<0.01$
Galacto-cerebroside	Human	89	$p<0.001$
Pig cerebroside	Pig spinal cord	43	N.S.
Kerasine	Whale brain	11	N.S.
CSE	Whale brain	54	$p<0.05$
GM_2	Tay-Sachs' disease patients	38	$p<0.05$
Glyceroglycolipids No. 1	Synthesis	60	$p<0.05$
Glyceroglycolipids No. 2	Synthesis	61	$p<0.05$
Glyceroglycolipids No. 3	Synthesis	52	N.S.

Test compounds were administered intraperitoneally at the dose of 100 mg/kg. N.S.: no significance.



Cer, ceramide; GalNAc, *N*-acetylgalactosamine; NeuNAc, *N*-acetylneuraminic acid

Fig. 8

cis olefins are generally shielded relative to those in the corresponding *trans* isomers.[20] The α-carbons in compound $B_{1\text{-}b}$ (27.34 and 27.90 ppm) are appreciably shielded compared with those in compound $B_{1\text{-}a}$ (32.76 and 33.16 ppm). These data indicated that compound $B_{1\text{-}a}$ is 4-*trans*-8-*trans* and compound $B_{1\text{-}b}$ is 4-*trans*-8-*cis*.

Figure 7 shows the 270 MHz ^{1}H-NMR spectra of these isomers. The geminal coupling constants of the anomeric portions,[21] 159 (compound $B_{1\text{-}a}$) and 162 (compound $B_{1\text{-}b}$) Hz

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support the view that compounds $B_{1\text{-a}}$ and $B_{1\text{-b}}$ are β-anomers. The configuration around C-2 and C-3 in the sphingenine parts of compounds $B_{1\text{-a}}$ and $B_{1\text{-b}}$ is expected to be D-*erythro*, since sphingosines from natural sources are known to be predominantly *erythro*.[22]

The anti-ulcerogenic activities of some plant cerebrosides are indicated in Table I. The cerebrosides from Bankyoo, alfalfa and soybean have such activity. The activities of animal cerebrosides and some glyceroglycolipids have also been examined, and the results are shown in Table II (Fig. 8). Gluco-cerebroside from human brain exhibits activity. CSE from whale brain, GM_2 from Tay–Sachs' disease patients and synthetic glyceroglycolipids also show such activity, although it is not very potent. Kerasine from whale brain and cerebroside from pig spinal cord have no effect.[28] These results can be summarized as follows. Cerebrosides containing different sugars, gluco- and galacto-cerebrosides, exhibit activity. Compound B_1, which contains a hydroxyl group at the C (2)-position in its fatty acid portion, is active. However, cerebrosides from pig and rice, which also contain hydroxy fatty acids as the major fatty acid part,[23] are inactive. Compound B_1 contains sphingadienine, as does cerebroside from rice bran,[26] but the latter cerebroside does not show a protective effect. Thus, the structure-activity relationship for anti-ulcerogenic activity remains unclear.

Compound B_1 administered intraperitoneally elongated pentobarbital-induced sleeping time and delayed the starting time of tremorine-induced tremor, although the results are not described here.

Our pharmacological studies on cerebroside are continuing. Additional information will be presented and discussed elsewhere.

Experimental

All melting points are uncorrected. IR and ultraviolet (UV) spectra were obtained with Hitachi 323 and Hitachi EPI-G3 spectrometers, respectively. MS were taken with a JEOL JMS O1SG-2 spectrometer equipped with a JMA 2000 mass data analysis system. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL JNM PS-100 and JEOL JNM PFT-100 spectrometers with tetramethylsilane as an internal standard, and chemical shifts are expressed in δ-values (ppm). The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Optical rotatory dispersion (ORD) spectra were measured on a JASCO ORD/CD J-20 unit. TLC was carried out on 0.25 mm precoated Kieselgel 60 F_{254} plates (Merck); the plates used for preparative TLC were coated with Kieselgel G or H (Merck, 0.5 mm thickness).

Properties of Compound B_1——Colorless solid (MeOH–H_2O), mp 185 °C. FD-MS *m/z* (%): 736 ($(M+Na)^+$, 100), 713 ($(M+1)^+$, 8). UV λ_{max}^{MeOH} nm: end absorption. IR ν_{max}^{KBr} cm^{-1}: 3360, 2920, 2850, 1640, 1535, 1468, 1081, 967, 722. ^{1}H-NMR (in pyridine-d_5) δ: 0.87 (6H, t-like, $J=5$ Hz), 1.25 (s-like), 1.73 (2H, m), 2.03 (4H, m), 2.12 (2H, m), 3.68—4.76 (m), 4.82 (1H, d, $J=7$ Hz), 5.41 (2H, m), 5.86 (2H, m), 6.36 (br, disappeared with D_2O), 8.25 (1H, br d, $J=7$ Hz). ^{1}H-NMR (in CD_3OD–$CDCl_3$ 1 : 2) δ: 0.88 (6H, t-like, $J=6$ Hz), 1.30 (s-like), 2.00 (4H, m), 2.06 (2H, m), 3.14—4.16 (m), 4.22 (1H, d, $J=7$ Hz), 5.33 (2H, m), 5.38 (1H, m), 5.70 (1H, m), 7.48 (1H, br d, $J=6$). ^{13}C-NMR (in pyridine-d_5): 14.3 (q), 22.9 (t), 25.8 (t), 27.3 (t), 27.5 (t), 29.6 (t), 30.0 (t), 32.2 (t), 32.9 (t), 35.6 (t), 54.6 (d), 62.6 (t), 70.2 (t), 71.5 (d), 72.4 (d), 75.0 (d), 78.4 (d), 105.6 (d), 129.4 (d), 129.8 (d), 130.6 (d), 131.0 (d), 132.0 (d), 175.6 (s). ^{13}C-NMR (in CD_3OD–$CDCl_3$ 9 : 11) δ: 14.2 (q), 23.1 (t), 25.6 (t), 27.2 (t), 27.7 (t), 30.1 (t), 32.3 (t), 32.9 (t), 35.1 (t), 53.8 (d), 61.8 (t), 68.8 (t), 70.5 (d), 72.3 (d), 73.9 (d), 76.8 (d), 103.6 (d), 129.0 (d), 129.7 (d), 130.9 (d), 131.4 (d), 134.1 (d), 176.4 (s).

Acid Hydrolysis of Compound B_1 (1)——Compound B_1 (**1**, 1 mg) in 2 N HClaq. (1 ml) was stirred at 110 °C for 2—3 h. After the evaporation of the solvent, the sugar in the reaction mixture was shown to be identical with authentic D-glucose by TLC using microcrystalline cellulose (AcOEt–pyridine–H_2O–EtOH 12 : 5 : 4 : 2, AcOEt–pyridine–H_2O–AcOH 5 : 5 : 3 : 1) and silica gel impregnated with 0.02 N NaOAc (acetone–H_2O 9 : 1).

Acetylation of Compound B_1 (1)——Compound B_1 (**1**, 32 mg) in Ac_2O (0.25 ml) and pyridine (0.25 ml) was allowed to stand overnight at room temperature. The reaction solution was poured into water and extracted with AcOEt. Acetate (**4**) was purified by preparative TLC (Kieselgel H, benzene–acetone 6 : 1) and recrystallization from MeOH to give colorless needles (16 mg), mp 70—73 °C. *Anal.* Calcd for $C_{52}H_{87}NO_{15}$: C, 64.64; H, 9.07; N, 1.45. Found: C, 64.72; H, 9.26; N, 1.46. FD-MS *m/z* (%): 966 ($(M+1)^+$, 100). EI-MS *m/z* (%): 965 (M^+, 1), 905 (5) 846 (1), 798 (2), 739 (1), 709 (1), 686 (7), 634 (2), 618 (4), 604 (1), 558 (3), 390 (10), 331 (98), 271 (16), 262 (6), 229 (3), 211 (6), 169 (95), 127 (9), 109 (30), 43 (100). IR ν_{max}^{KBr} cm^{-1}: 3370, 2960, 2920, 2850, 1747, 1678, 1532, 1372, 1230, 1036, 976. ^{1}H-NMR (in $CDCl_3$) δ: 0.88 (6H, t-like, $J=5$ Hz), 1.29 (s-like), 1.60—2.30 (m), 2.01 (3H, s), 2.04 (9H, s), 2.09 (3H, s),

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2.17 (3H, s), 3.48—4.40 (1H, d, $J=7$ Hz), 4.80—5.88 (9H, m), 6.29 (1H, d, $J=8$).

Catalytic Hydrogenation of Compound B_1 (1)——Compound B_1 (**1**, 34 mg) in MeOH (9 ml) was stirred with PtO_2 (10 mg) under H_2 gas for 135 min. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by crystallization from $CHCl_3$–MeOH to give tetrahydro-compound B_1 (**2**) as colorless gelatinous crystals (25 mg), mp 201—203 °C. FD-MS *m/z* (%): 718 ($(M+1)^+$, 100). IR ν_{max}^{KBr} cm^{-1}: 3330, 2910, 2845, 1648, 1538, 1466, 1074, 720. ^{1}H-NMR (in pyridine-d_5) δ: 0.86 (6H, t-like, $J=6$ Hz), 1.31 (s-like), 1.80 (m), 2.06 (m), 3.74—4.74 (m), 4.80 (1H, d, $J=8$ Hz), 6.30 (br, disappeared with D_2O), 7.06 (br, disappeared with D_2O), 8.31 (1H, br d, $J=8$ Hz, disappeared with D_2O).

OsO_4-oxidation of Compound B_1 (1)——Compound B_1 (**1**, 37 mg) in OsO_4–pyridine (50 mg/10 ml) was stirred at room temperature for 2 h. A solution of $NaHSO_3$–H_2O–pyridine (20 ml) [prepared from $NaHSO_3$ (1.8 g), pyridine (20 ml) and H_2O (30 ml)] was added to the reaction mixture with continued stirring for 15 min. The whole was extracted with $CHCl_3$–MeOH after the addition of H_2O (5 ml). The extract was evaporated *in vacuo* and the residue was dissolved in $CHCl_3$–MeOH. The soluble part was evaporated and the residue was crystallized from MeOH to obtain tetrahydroxy-compound B_1 (**3**) as white solid (16 mg), mp 210 °C. FD-MS *m/z* (%): 804 ($(M+Na)^+$, 100). IR ν_{max}^{KBr} cm^{-1}: 3350, 2920, 2845, 1632, 1524, 1467, 1080, 720. ^{1}H-NMR (in pyridine-d_5) δ: 0.83 (6H, t-like, $J=5$ Hz), 1.23 (s-like), 1.70 (m), 2.16 (m), 3.68—4.80 (m), 4.87 (1H, d, $J=7$ Hz), 5.30 (1H, br), 8.54 (1H, br d, $J=10$ Hz, disappeared with D_2O).

Methanolysis of Tetrahydro-compound B_1 (2)——Tetrahydro-compound B_1 (**2**, 28 mg) in 2—4 N HCl–MeOH (17.5 ml) was refluxed for 9 h. The reaction solution was extracted with petroleum ether. The extract was purified by preparative TLC (Kieselgel H, benzene–acetone 10 : 1) to give a white solid (**5**, 12 mg). The solution was made alkaline with 10% NaOH aq. and extracted with ether. The pale yellow amorphous solid thus obtained (14 mg) was crystallized from AcOEt to yield a white powder (**6**, 3 mg). Methyl 2-hydroxypalmitate (**5**), mp 47—48 °C. High resolution MS 286.2489 (Calcd for $C_{17}H_{34}O_3$: 286.2507). FD-MS *m/z* (%): 286 (M^+, 100). EI-MS *m/z* (%): 286 (M^+, 85), 254 (9), 227 (100), 208 (19), 182 (5), 159 (6), 156 (6), 145 (14), 141 (4), 139 (10), 127 (13), 125 (19), 113 (9), 111 (34), 103 (14), 97 (55), 90 (43), 85 (14), 83 (60), 71 (25), 69 (54), 57, (46), 55 (45), 43 (44), 41 (31). IR ν_{max}^{KBr} cm^{-1}: 3375, 2915, 2850, 1752, 1740, 1466, 1280, 725. ^{1}H-NMR (in $CDCl_3$) δ: 0.85 (3H, t-like, $J=6$ Hz), 1.22 (s-like), 1.64 (br), 3.08 (br, disappeared with D_2O), 3.71 (3H, s), 4.08 (1H, q, $J=7$, 5 Hz). Long chain base I (**6**), mp 72—92 °C. FD-MS *m/z* (%): 302 ($(M+1)^+$, 100) IR ν_{max}^{KBr} cm^{-1}: 3335, 2915, 2845, 1600, 1509, 1462, 1060, 731, 720. TMS-derivative of **6**, EI-MS *m/z* (%): 430 ($(M-15)^+$, 1), 342 (29), 313 (24), 149 (3), 147 (7), 132 (100), 129 (6), 125 (2), 116 (10), 115 (4), 111 (4), 103 (7), 101 (1), 99 (2), 97 (8), 85 (6), 83 (9), 73 (49), 71 (9), 69 (10), 60 (26), 57 (15), 55 (10), 43 (20), 41 (8).

***N*-Acetylation followed by Tetramethylsilylation of Long Chain Base I (6)**——A mixture of long chain base I (**6**, 1 mg), MeOH (0.4 ml) and Ac_2O (0.4 ml) was allowed to stand for 17.5 h at room temperature. The reaction solution was poured into water and extracted with $CHCl_3$ and ether. The product was purified by preparative TLC (Kieselgel G, $CHCl_3$–MeOH–2 N NH_4OH 40 : 10 : 1), then trimethylsilylated with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) in pyridine (0.2 ml) for 1 h at room temperature to give **7**, EI-MS *m/z* (%): 472 ($M-15)^+$, 14), 384 (10), 313 (68), 247 (68), 217 (12), 175 (22), 157 (97), 129 (17), 116 (28), 103 (18), 97 (13), 85 (55), 73 (100).

Methanolysis of Compound B_1 (1)——Compound B_1 (**1**, 111 mg) was stirred with 1 N HCl–MeOH containing H_2O (40 ml) at 75 °C for 15 h.[16] The reaction solution was chromatographed with resin (Amberlite CG-400) to give methyl 2-hydroxypalmitate (**5**, 21 mg) and base-containing fraction (68 mg). The base fraction (52 mg) was separated into long chain base II (**8**, 7 mg) and its glucoside (30 mg) by preparative TLC (Kieselgel H, $CHCl_3$–MeOH–2 N NH_4OH 40 : 10 : 1). Glucoside of **8**, FD-MS *m/z* (%): 460 ($(M+1)^+$, 100). ^{1}H-NMR (in pyridine-d_5) δ: 0.84 (3H, t-like, $J=5$ Hz), 1.24 (s-like), 2.01 (4H, m), 2.05 (2H, m), 3.64—4.69 (m), 4.76 (1H, q, $J=6$, 5 Hz), 4.85 (1H, d, $J=8$ Hz), 5.04 (1H, d, $J=4$ Hz), 5.37 (2H, m), 5.83 (2H, m). ^{1}H-NMR (in $CDCl_3$–CD_3OD) δ: 0.85 (3H, t-like, $J=5$ Hz), 1.14 (s-like), 1.96 (4H, m), 2.05 (2H, m), 3.08—4.20 (m), 4.25 (1H, d, $J=7$ Hz), 4.64 (1H, d, $J=3$ Hz), 5.29 (2H, m), 5.34 (1H, m), 5.73 (1H, m).

***N*-Acetylation of Long Chain Base II (8)**——A mixture of long chain base II (**8**, *ca.* 8 mg), MeOH (0.5 ml) and Ac_2O (1 ml) was allowed to stand overnight at room temperature. The reaction mixture was poured into water and extracted with ether and $CHCl_3$. The extracts were separated by preparative TLC (Kieselgel H, $CHCl_3$–MeOH–2 N NH_4OH 40 : 10 : 1) to give the *N*-acetate (4 mg) and the *N,O*-diacetate (2 mg). *N*-Acetate of **8**, FD-MS *m/z* (%): 340 ($(M+1)^+$, 100). EI-MS *m/z* (%): 339 (M^+, 0.2), 321 (0.4), 308 (0.3), 102 (35), 85 (100), 82 (11), 70 (18), 67 (12), 60 (71), 57 (15), 55 (26), 43 (53), 41 (32). *N,O*-Diacetate of **8**, EI-MS *m/z* (%): 381 (M^+, 0.5), 363 (0.1), 321 (3), 304 (0.3), 290 (0.2), 280 (0.2), 262 (4), 252 (1), 237 (0.3), 219 (0.7), 214 (1), 156 (2), 144 (10), 102 (67), 85 (100), 70 (40), 67 (18), 60 (80), 57 (16), 55 (29), 43 (89), 41 (49).

OsO_4 Oxidation of the *N*-Acetate of 8——The *N*-acetate of **8** (4 mg) in OsO_4–pyridine (3.5 mg/0.7 ml) was stirred for 2 h at room temperature. A mixture of $NaHSO_3$–pyridine–H_2O (1.5 ml), which had been prepared from $NaHSO_3$ (1.8 g), pyridine (20 ml) and water (30 ml), was added to the reaction solution. The whole was stirred for 50 min at room temperature and extracted with $CHCl_3$–MeOH to obtain a pale yellow amorphous solid (2 mg), which was purified by preparative TLC (Kiesegel H, $CHCl_3$–MeOH–2 N NH_4OH 40 : 10 : 1). After trimethylsilylation to **9**, the MS was measured (see Fig. 3).

Catalytic Hydrogenation of D-Sphingosine——Ccommercial sphingosine (Sigma) (3 mg) in MeOH (2 ml) was

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hydrogenated with PtO_2 (3 mg) under H_2 gas for 140 min. The reaction solution was filtered and evaporated to give a colorless amorphous solid (3 mg).

Separation of Compound $B_{1\text{-}a}$ and $B_{1\text{-}b}$——Compound B_1 (54 mg) was chromatographed on a Kusano C.I.G. GPS-153 column eluted with $CHCl_3$–MeOH–benzene (25 : 1 : 1) at a pressure of 40 kg/cm^2. Compound $B_{1\text{-}b}$ main fraction (15 mg), compound $B_{1\text{-}a}$ (22 mg) and their mixture (24 mg, wet) were obtained. Compound $B_{1\text{-}b}$ main fraction (15 mg) was rechromatographed to give compounds $B_{1\text{-}b}$ (6 mg), $B_{1\text{-}a}$ (4 mg) and their mixture (7 mg). Compound $B_{1\text{-}a}$, white granules (MeOH), mp 184—186 °C. ORD (c=6.48 mg/ml, MeOH–$CHCl_3$ 1.5 : 1) $[\alpha]^{15.5}$ (nm): +5.4 (589), +8.1 (500), +12.0 (400), +24.7 (300). FD-MS m/z (%): 736 ($(M+Na)^+$, 100), 714 ($(M+1)^+$, 12). IR ν_{max}^{KBr} cm^{-1}: 3350, 2955, 2920, 2850, 1643, 1466, 1080, 963, 720. Compound $B_{1\text{-}b}$, FD-MS m/z (%): 736 ($(M+Na)^+$, 100), 714 ($(M+1)^+$, 31).

Bioassay

Animals and Materials——Female STD-ddY mice weighing 20 to 26 g were used. Test compounds were suspended in normal saline containing 0.5—1% carboxymethylcellulose (CMC). Gluco-cerebroside from Gaucher's disease patients and galacto-cerebroside from human brain were kindly given by Prof. S. Nojima, University of Tokyo; kerasine, CSE and GM_2 were provided indirectly by Prof. T. Yamakawa through Dr. H. Otsuka, University of Tokyo; synthetic glyceroglycolipids by Dr. T. Ogawa, the Institute of Physical and Chemical Research; and plant cerebrosides and pig cerebroside by Ryukakusan Co., Ltd.

Experimental Stress-induced Ulcer——Groups of 3 to 10 female mice were tested. Test compounds and vehicle were intraperitoneally administered at the dose of 100 mg/kg 30 min before the experiment. The mice were immobilized in a restraint cage and immersed to the depth of the xiphoid level in a water bath maintained at 25 °C for 18 h according to the method described by Yano and Harada.[27] The stomachs from sacrificed mice were fixed with 3% formalin solution. The ulcer index was evaluated as the sum of the length of erosions in the glandular stomach. Protective effectiveness of test compounds against experimental stress uncler was calculated according to the following formula.

$$\text{Protective ratio (\%)} = 100 - \frac{\text{ulcer index (test compound)}}{\text{uncler index (control)}} \times 100$$

Statistical Analysis——The significance of results obtained was evaluated by using Student's t test.

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