The Principles of *Tetragonia tetragonooides* having Anti-ulcerogenic Activity. II. Isolation and Structure of Cerebrosides

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Compound B₁ (tentative name), isolated from *Tetragonia tetragonooides* 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-diene on the basis of chemical and spectral evidence. By repeated chromatography, compound B₁ was separated into compounds B₁-a (major) and B₁-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 tetragonooides*; cerebrosides; 1-O-β-D-glucopyranosyl-2-N-2'-hydroxypalmitoyl-sphinga-4-trans-8-trans-diene; 1-O-β-D-glucopyranosyl-2-N-2'-hydroxypalmitoyl-sphinga-4-trans-8-cis-diene; 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. 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 tetragonooides*) was most effective in protecting against ulcer formation among these drugs, and compounds A and B₁ (tentative names) were isolated from this drug as the active principles. Compound A was determined to be a mixture of sterylglucosides. We have found compound B₁ to be a cerebroside mixture consisting of two Δ⁸-g geometrical isomers, and this mixture has now been separated into its components, compounds B₁-a and B₁-b. We report here the structures of compounds B₁-a and B₁-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, nerves and other organs, and are contained in milk, oyster, some plants, 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 and their immunological properties. However, very little pharmacological work on cerebrosides has been done.

Compound B₁ (I) was obtained as a white solid from MeOH–H₂O and its molecular formula was determined to be C₄₆H₇₅NO₉, field desorption mass spectrum (FD-MS) m/z (٪) 736 ([M + Na]⁺, 100). (Nevertheless, compound B₁ was later recognized to be a mixture of two isomers.) The color reaction of compound B₁ (positive in the anthrone and modified
Molisch tests) indicated the presence of a sugar moiety in the structure. Hydrolysis of compound B₁ with 2 N HCl afforded glucose. The ¹H-nuclear magnetic resonance (¹H-NMR) spectrum of compound B₁ exhibited signals of 11—13 protons in the range of 4—5 ppm, including an anomic signal at 4.82 (1H, d, J= 7 Hz) ppm. In the ¹³C-nuclear magnetic resonance (¹³C-NMR) spectrum, signals corresponding to seven tertiary and two secondary carbons in the range of 50—80 ppm and an anomic 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 indicated that compound B₁ contains an equimolar amount of β-D-glucopyranose. A large singlet-like signal at 1.25 ppm in the ¹H-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 B₁ as shown in Fig. 1.

Compound B₁ (1) was hydrogenated over PtO₂ to yield tetrahydro-compound B₁ (2) and was oxidized with OsO₄ to yield tetrahydroxy-compound B₁ (3) (Chart 1). These findings also supported the presence of two double bonds in the structure. However, the ¹³C-NMR spectrum of compound B₁ showed five, not four sp³-carbon signals, which are split into doublets by the off-resonance technique. This finding led us to consider that compound B₁ might be a mixture of two geometrical isomers at a double bond.

The presence of the amide chromophore in compound B₁ 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 ¹³C-NMR spectrum. Methanalysis of the tetrahydro-compound B₁ (2) with 2—4 N HCl in MeOH afforded a fatty acid methyl ester (5); C₁₇H₃₄O₃, 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 ¹H-NMR data suggested the presence of -CO-CH(OH)-CH₂- [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

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**Fig. 1**

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  \begin{center}
  \begin{tabular}{c c c c c c c c}
    62.03 & 5.41 & 2.03 \\
    CH₂-CH=CH-CH₂-
  \end{tabular}
  \begin{tabular}{c c c c c c c c}
    62.12 & 5.86 & 4.67 \\
    CH₂-CH=CH-CH-O-
  \end{tabular}
  \end{center}
```

**Chart 1**

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\begin{center}
\begin{tikzpicture}
  \node (1) {compound B₁-hexaacetate};
  \node (2) [below of=1] {tetrahydro-compound B₁};
  \node (3) [right of=2] {tetrahydroxy-compound B₁};
  \node (4) [above of=1] {Ac₂O \rightarrow pyridine \rightarrow compound B₁ \rightarrow 2 N HCl \rightarrow glucose \rightarrow 110 °C \rightarrow OsO₄-pyridine};
  \node (5) [below of=2] {methyl 2-hydroxypalmitate};
  \node (6) [below of=5] {HMDS: hexamethyldisilazane; TMCS: trimethylchlorosilane};
  \node (7) [below of=6] {Ac₂O/MeOH \rightarrow HMDS–TMCS–pyridine};
  \node (8) [below of=2] {long chain base I};
  \node (9) [below of=8] {6 \rightarrow 7};
  \node (10) [below of=9] {Chart 2. Methanalysis of Tetrahydro-compound B₁ (2)};

  \draw [->] (1) -- (2);
  \draw [->] (2) -- (3);
  \draw [->] (2) -- (4);
  \draw [->] (4) -- (1);
  \draw [->] (2) -- (5);
  \draw [->] (5) -- (6);
  \draw [->] (6) -- (7);
  \draw [->] (7) -- (8);
  \draw [->] (8) -- (9);
  \draw [->] (9) -- (10);
\end{tikzpicture}
\end{center}
```

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 $\beta$-d-glucose, 2-hydroxypalmitic acid and sphinganine.

Methanolation of compound B$_1$ with 1 N HCl–MeOH–H$_2$O$^{16}$ gave methyl 2-hydroxypal-
...
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 OsO₄. 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 OsO₄ than the other one at C-4. The long chain base II (8) was thus determined to be sphinga-4,8-diene, which has been found in oyster glycolipids⁸⁹ and in certain plant cerebrosides¹⁵ by GC-MS and actually isolated from wheat flour lipids as triacety derivatives.¹⁸ In conclusion, compound B₁ has been identified as a mixture of geometrical isomers of 1-O-β-D-glucopyranosyl-2-N-2′-hydroxypalmitoyl-sphinga-4,8-diene. The MS of the TMS-derivatives of compound B₁ (1) and tetrahydroxy-compound B₁ (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 ¹H-NMR spectrum of compound B₁ (in pyridine-ｄ₅) 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 CDCl₃–CD₃OD 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 B₁ into compounds B₁-a (major) and B₁-b (minor). The 67.80 MHz ¹³C-NMR spectra of compounds B₁-a and B₁-b are illustrated at Fig. 6. Olefinic carbons in compound B₁-b (at 129.20 and 131.24 ppm) are observed at slightly higher magnetic field than those of
Fig. 4. MS of the TMS Derivative of Compound B₁ (1)

Fig. 5. MS of the TMS Derivative of Tetrahydroxy-compound B₁ (3)
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. $\alpha$-Carbons in
TABLE I. Effect of Cerebrosides on Stress-induced Ulcer

<table>
<thead>
<tr>
<th>Source</th>
<th>Protective ratio (%)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bankyoo (compound B₁)</td>
<td>76</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>55</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Soybean</td>
<td>74</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Rice bran</td>
<td>33</td>
<td>N.S.</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>18</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Protective ratio (%)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluco-cerebroside</td>
<td>Gaucher's disease patients</td>
<td>79</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Galacto-cerebroside</td>
<td>Human</td>
<td>89</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Pig cerebroside</td>
<td>Pig spinal cord</td>
<td>43</td>
<td>N.S.</td>
</tr>
<tr>
<td>Kerasine</td>
<td>Whale brain</td>
<td>11</td>
<td>N.S.</td>
</tr>
<tr>
<td>CSE</td>
<td>Whale brain</td>
<td>54</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>GM₂</td>
<td>Tay-Sachs' disease patients</td>
<td>38</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Glyceroglycolipids No. 1</td>
<td>Synthesis</td>
<td>60</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Glyceroglycolipids No. 2</td>
<td>Synthesis</td>
<td>61</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Glyceroglycolipids No. 3</td>
<td>Synthesis</td>
<td>52</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

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

cis olefins are generally shielded relative to those in the corresponding trans isomers. The α-carbons in compound B₁₋₅ (27.34 and 27.90 ppm) are appreciably shielded compared with those in compound B₁₋₄ (32.76 and 33.16 ppm). These data indicated that compound B₁₋₄ is 4-trans-8-trans and compound B₁₋₅ is 4-trans-8-cis.

Figure 7 shows the 270 MHz ¹H-NMR spectra of these isomers. The geminal coupling constants of the anomic portions, ¹H-NMR 159 (compound B₁₋₄) and 162 (compound B₁₋₅) Hz
support the view that compounds B_{1-a} and B_{1-b} are β-anomers. The configuration around C-2
and C-3 in the sphingine parts of compounds B_{1-a} and B_{1-b} is expected to be 1,2-erythro, since
sphingosines from natural sources are known to be predominantly erythro.\textsuperscript{22}

The anti-ulcerogenic activities of some plant cerebroside are indicated in Table I. The
cerebroside from Bankyoo, alfalfa and soybean have such activity. The activities of animal
cerebroside and some glyceroxyglycolipids 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\textsubscript{2} from Tay–Sachs’ disease patients and synthetic glyceroxyglycolipids also show such
activity, although it is not very potent. Keratin from whale brain and cerebroside from pig
spinal cord have no effect.\textsuperscript{25} These results can be summarized as follows. Cerebroside
containing different sugars, gluco- and galacto-cerebroside, exhibit activity. Compound B\textsubscript{1},
which contains a hydroxy group at the C (2)-position in its fatty acid portion, is active. However,
cerebroside from pig and rice, which also contain hydroxy fatty acids as the major
fatty acid part,\textsuperscript{23} are inactive. Compound B\textsubscript{1} contains sphingadienine, as does cerebroside
from rice bran,\textsuperscript{26} but the latter cerebroside does not show a protective effect. Thus, the
structure-activity relationship for anti-ulcerogenic activity remains unclear.

Compound B\textsubscript{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. \textsuperscript{1}H-NMR and \textsuperscript{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
rotary 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\textsubscript{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\textsubscript{1}—Colorless solid (MeOH–H\textsubscript{2}O), mp 185 °C. FD-MS m/z (%): 736 ([M + Na]\textsuperscript{+}),
101, 713 ([M + Na]\textsuperscript{+}), 715, 722. \textsuperscript{1}H-NMR in pyridine-d\textsubscript{6}: 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\textsubscript{2}O), 8.25 (1H, br d,
J = 7 Hz). \textsuperscript{13}C-NMR (in CD\textsubscript{3}OD–CDCl\textsubscript{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). \textsuperscript{13}C-NMR
in pyridine-d\textsubscript{6}: 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). \textsuperscript{13}C-
NMR (in CD\textsubscript{2}OD–CDCl\textsubscript{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\textsubscript{1} (1)—Compound B\textsubscript{1} (1, 1 mg) in 2% 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 p-glucose by TLC using microcrystalline cellulose (AcOEt–pyridine–H\textsubscript{2}O–EtOH: 12:5:4:2; AcOEt–
pyridine–H\textsubscript{2}O–AcOH: 5:5:3:1) and silica gel impregnated with 0.02% NaOAc (acetic–H\textsubscript{2}O: 9:1).

Acetylation of Compound B\textsubscript{1} (1)—Compound B\textsubscript{1} (1, 32 mg) in Ac\textsubscript{2}O (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\textsubscript{30}H\textsubscript{47}N\textsubscript{15}O\textsubscript{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)\textsuperscript{100}, 966 (M + 1)\textsuperscript{70}, 926 (M + 1)\textsuperscript{22}, 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\textsubscript{max} cm\textsuperscript{-1}: 3370, 2960, 2920, 2850, 1747, 1678, 1532, 1372, 1230, 1036, 976. \textsuperscript{1}H-
NMR (in CDCl\textsubscript{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)—Compound B (1, 34 mg) in MeOH (9 ml) was stirred with PtO₂ (10 mg) under H₂ gas for 135 min. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by crystallization from CHCl₃—MeOH to give tetrahydro-compound B₂ (2) as colorless gelatinous crystals (25 mg), mp 201—203°C. FD-MS m/z (%): 718 ([M + 1]⁺, 100). IR vKBr cm⁻¹: 3330, 2910, 2845, 1648, 1538, 1466, 1074, 720. ¹H-NMR (in pyridine-d₅): 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 D₂O), 7.06 (br, disappeared with D₂O), 8.31 (1H, br d, J = 8 Hz, disappeared with D₂O).

**OsO₄-oxidation of Compound B** (1)—Compound B (1, 37 mg) in OsO₄—pyridine (50 mg/10 ml) was stirred at room temperature for 2h. A solution of NaHSO₃—H₂O—pyridine (20 ml) [prepared from NaHSO₃ (1.8 g), pyridine (20 ml) and H₂O (30 ml)] was added to the reaction mixture with continued stirring for 15 min. The whole was extracted with CHCl₃—MeOH after the addition of H₂O (5 ml). The extract was evaporated in vacuo and the residue was dissolved in CHCl₃—MeOH. The soluble part was evaporated and the residue was crystallized from MeOH to obtain tetrahydrox-compound B (3) as white solid (16 mg), mp 210°C. FD-MS m/z (%): 804 ([M + Na]⁺, 100). IR vKBr cm⁻¹: 3350, 2920, 2845, 1632, 1524, 1467, 1080, 720. ¹H-NMR (in pyridine-d₅): δ: 0.83 (6H, t-like, J = 5 Hz), 1.23 (s-like), 1.70 (m), 216 (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 D₂O).

**Methodan of Tetrahydro-compound B** (2)—Tetrahydro-compound B (2, 28 mg) in 2—4N HCl—MeOH (17.5 ml) was refluxed for 9h. 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 C₁₉H₃₄O₄): 286.2507, 100. El—MS m/z (%): 286 (M⁺, 100), 268 (M⁺—16, 100), 250 (M⁺—32, 100), 222 (M⁺—48, 100), 186 (M⁺—80, 100), 168 (M⁺—96, 100), 150 (M⁺—112, 100), 132 (M⁺—128, 100), 114 (M⁺—144, 100), 96 (M⁺—160, 100), 78 (M⁺—176, 100), 60 (M⁺—192, 100), 42 (M⁺—208, 100), 24 (M⁺—224, 100), 12 (M⁺—236, 100).

**N-Acetylation followed by Tetramethylsililation of Long Chain Base I** (6)—A mixture of long chain base I (6, 1 mg), MeOH (0.4 ml) and Ac₂O (0.4 ml) was allowed to stand for 17.5 h at room temperature. The reaction solution was poured into water and extracted with CHCl₃ and ether. The product was purified by preparative TLC (Kieselgel G, CHCl₃—MeOH—2N NH₄OH 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, El—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).

**Manipulation of Compound B** (1)—Compound B (1, 111 mg) was stirred with 1N HCl—MeOH containing H₂O (40 ml) at 75°C for 15 h.¹⁰ The reaction solution was chromatographed with resin (Amberlite CG-400) to give methyl 2-hydroxypalmitate (25, 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, CHCl₃—MeOH—2N NH₄OH 40:10:1). Glucoside of 8, FD-MS m/z (%): 460 ([M + H]⁺, 100), ¹H-NMR (in pyridine-d₅): δ: 0.84 (3H, t-like, J = 6 Hz), 2.14 (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), ¹H-NMR (in CDC₁₃—CD₃OD): 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 Ac₂O (1 ml) was allowed to stand overnight at room temperature. The reaction mixture was poured into water and extracted with ether and CHCl₃. The extracts were separated by preparative TLC (Kieselgel H, CHCl₃—MeOH—2N NH₄OH 40:10:1) to give the N-acetate (4 mg) and the O,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-Diaceitate of 8, El—MS m/z (%): 381 ([M + 1]⁺, 0.5), 365 (0.1), 321 (3), 304 (0.3), 290 (0.2), 280 (0.2), 262 (4), 252 (1), 237 (0.7), 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).

**OsO₄ Oxidation of the N-Acetate of 8**—The N-acetate of 8 (4 mg) in OsO₄—pyridine (3.5 mg/0.7 ml) was stirred for 2 h at room temperature. A mixture of NaHSO₃—pyridine—H₂O (1.5 ml), which had been prepared from NaHSO₃ (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 CHCl₃—MeOH to obtain a pale yellow amorphous solid (2 mg), which was purified by preparative TLC (Kieselgel H, CHCl₃—MeOH—2N NH₄OH 40:10:1). After trimethylsilylation to 9, the MS was measured (see Fig. 3).

**Catalytic Hydrogenation of D-Sphingosine** —Commercial sphingosine (Sigma) (3 mg) in MeOH (2 ml) was
hydrogenated with PtO₂ (3 mg) under H₂ gas for 140 min. The reaction solution was filtered and evaporated to give a colorless amorphous solid (3 mg).

Separation of Compound B₁₇,₈ and B₁₃,₄—Compound B₁ (54 mg) was chromatographed on a Kusano C.I.G. GPS-153 column eluted with CHCl₃-MeOH—benzene (25:1:1) at a pressure of 40 kg/cm². Compound B₁₇,₈ main fraction (15 mg), compound B₁₃,₄ (22 mg) and their mixture (24 mg, wet) were obtained. Compound B₁₇,₈ main fraction (15 mg) was rechromatographed to give compounds B₁₃,₈ (6 mg), B₁₃,₄ (4 mg) and their mixture (7 mg). Compound B₁₇,₈ white granules (MeOH), mp 184—186 °C. ORD (c = 6.48 mg/ml, MeOH—CHCl₃ 1.5:1) [α]D²⁵ (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 νcm⁻¹: 3350, 2955, 2920, 2850, 1643, 1466, 1080, 963, 720. Compound B₁₃,₈, 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 GM₁ 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 cerebroside 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. 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 ulcer was calculated according to the following formula.

\[
\text{Protective ratio} (\%) = 100 - \left( \frac{\text{ulcer index (test compound)}}{\text{ulcer index (control)}} \right) \times 100
\]

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

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References and Notes

1) A part of this work was presented at the 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, April 1981.
12) These data were obtained from a consideration of the hexa-acetate, C₅₂H₇₁NO₁₅, because the hydrosopic nature of compound B made elemental analysis difficult.
18) R. Lain and O. Renkonen, Biochemistry, 12, 1106 (1973).
19) In the $^{13}$C-NMR spectrum of compound $B_{1,4}$, the amide carbonyl carbon could not be observed, probably due to the small amount of the sample.
23) Pig cerebroside: hydroxy fatty acids, 92.13%; normal fatty acids, 7.97%.$^{24}$ Rice bran cerebroside: 2-hydroxy fatty acids, 89%; normal fatty acids, 11%.$^{25}$
26) Rice bran cerebroside: sphinga-4,8-diene, 70%.$^{17}$
28) Recent reexamination of the cerebroside from pig spinal cord for the protective activity against the experimental stress ulcer showed the protective ratio of 65% ($p < 0.01$) at the intraperitoneal dose of 100 mg/kg. The test solution was prepared carefully by homogenizing with a small amount of lecithin (which was confirmed no protective effect) as vehicle.