Isolation of a New Cerebroside from the Root Bark of *Aralia elata*

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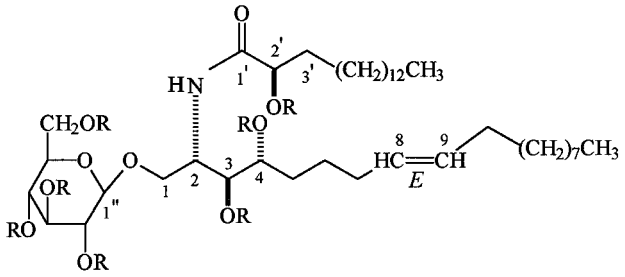
A new cerebroside, named Aralia cerebroside (**1**), was isolated from the root bark of *Aralia elata*. The structure of **1** was determined to be 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*)-2-[(2′*R*)-2′-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol on the basis of physicochemical and spectroscopic studies.

The root bark of *Aralia elata* Seem. (Araliaceae) has long been used in Korean folk medicine to treat cough, cancer, and diabetes.[1] Saponins, oleanolic acid, and *β*-sitosterol 3-*O*-*β*-D-glucoside have been reported from the EtOAc-soluble fraction.[2,3] Among these isolates, oleanolic acid 28-*O*-*β*-D-glucoside was identified as one of the components having hypoglycemic activity.[4] This paper deals with the isolation and characterization of a new cerebroside (**1**) from the EtOAc-soluble fraction of a MeOH extract of this plant.

The dried root bark of *A. elata* was extracted with MeOH. The residue after evaporation of MeOH was dissolved in H_2O and successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc fraction was subjected to Si gel column chromatography to give compound **1**, mp 215−216 °C. The IR spectrum of **1** showed absorption bands typical for hydroxyl, amide, glycosidic C−O, and $(CH_2)_n$ functionalities, and suggestive of a glycosphingolipid. The NMR data of **1** indicated the presence of a sugar (δ_H 4.93, 1H, d, J = 7.9 Hz, anomeric H; δ_C 105.6), an amide linkage (δ_H 8.53, 1H, d, J = 9.1 Hz, N−H; δ_C 175.6), and two long-chain aliphatic moieties. Methanolysis[5] of **1** yielded methyl glucoside, a fatty acid methyl ester, and a long-chain base. The fatty acid methyl ester was identified as methyl 2-hydroxypalmitate by means of GC/MS analysis, and the absolute configuration at C-2 was determined to be *R* from the specific rotation.[6] The presence of a 1,3,4-trihydroxy unsaturated C_{18} long-chain base was deduced from the ^{1}H−^{1}H COSY and MS data. The signal at δ 8.5 gave a cross-peak with the signal at δ 5.26 (H-2) in the ^{1}H−^{1}H COSY spectrum of **1**, which, in turn, showed cross-peaks with methylene protons (H-1) at δ 4.50 and 4.69 and δ 4.27 (H-3). The latter correlated with the signal at δ 4.17 (H-4). The positive FABMS spectrum of **1** showed a molecular ion peak at *m*/*z* 732 $[M + H]^+$ and fragments at *m*/*z* 570 and 553, indicating the loss of glucose. The molecular ion peak of the long-chain base in the EIMS showed at *m*/*z* 315, supporting the above results. The position of the double bond in the long-chain base was determined by EIMS analysis of the corresponding dimethyl disulfide derivative of compound **1** heptaacetate. Characteristic fragment ions at *m*/*z* 932 and 187, from cleavage between the carbons bearing the methylthio group, indicated that the double bond was at C-8.[7,8] The trans (*E*) configuration of the double bond was evidenced by the large vicinal coupling constant (15.6 Hz) as well as by the chemical shifts of the carbons next to the double bond in the heptaacetate (**2**).[9,10] The chemical shift of the H-2 signal and the ^{13}C chemical shifts of C-1−C-4, C-1′, and C-2′ of glucosphingolipids are especially suitable for determination of the absolute stereochemistry of the phytosphingosine moiety.[11,12] The chemical shift of H-2 (δ 5.26) and the carbon chemical shifts at δ 70.5 (C-1), 51.7 (C-2), 75.9 (C-3), 72.4 (C-4), 175.6 (C-1′), and 72.4 (C-2′) in **1** were virtually identical with those of the reported data of other (2*S*,3*S*,4*R*)-phytosphingosine moieties.[11,12] These results clearly indicate that the 1,3,4-trihydroxy phytosphingosine moiety in **1** possesses the 2*S*,3*S*,4*R* configuration. Thus, the structure of **1** was determined to be 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*)-2-[(2′*R*)-2′-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol.



Aralia cerebroside (**1**) R = H

(**2**) R = Ac

Experimental Section

General Experimental Procedures. Melting points were measured on a Mitamura−Riken apparatus and are uncorrected. Optical rotations were determined on a Rudolph Autopol III automatic polarimeter. IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. EIMS spectra were obtained on either a Hewlett−Packard 5989B mass spectrometer or a VG70-VSEQ mass spectrometer. The FABMS was obtained in a glycerol matrix in a positive ion mode on a JEOL DX-300 spectrometer. NMR spectra were measured on either a Varian FT80A (80 MHz), a Bruker AM-300 (300 MHz), or a Bruker AMX-500 (500 MHz) instrument, and chemical shifts were referenced to TMS. GC analysis was performed with a Hewlett−Packard 5890 Series II gas chromatograph equipped with an H_2 flame ionization detector. Conditions: HP-5 capillary column (30 m × 0.32 mm × 0.25 μm), column temperature, 150 °C for TMS ethers of methyl glycopyranosides; OV-17 on Gas Chrom Q glass column (1.5%, 80−100 mesh, 6 ft), column temperature, 190 °C for fatty acid methyl esters; injector and detector temperature, 280 °C; He flow rate, 30 mL/min. TLC was performed on Si gel 60F_{254} (Merck) and cellulose plates (art no. 5716, Merck).

Plant Material. The root bark of *Aralia elata* was collected in May 1991, in Chungcheon namdo Province, Korea. A voucher specimen (no. SSK-9103) is preserved in the Economic Plant Research Station of our institute.

Extraction and Isolation. Air-dried root bark of *A. elata* (1.8 kg) was chopped into small pieces and extracted with

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MeOH for 3 h four times. The solvent was evaporated to dryness, and the dry residue was partitioned in succession between H_2O and $CHCl_3$, EtOAc, and then *n*-BuOH affording, on evaporation of solvent, 18.5, 9.2, and 230 g of the respective extracts. The EtOAc fraction was subjected to column chromatography on Si gel. Elution with $CHCl_3$−MeOH−H_2O (7:3:0.5) gave 16 subfractions. Subfraction 11 was recrystallized from MeOH to give compound **1** (490 mg).

Aralia cerebroside (1): obtained as an amorphous white powder, mp 215−216 °C; $[\alpha]^{20}_D$ +14.6° (*c* 0.53, MeOH); IR (KBr) ν_{max} 3380 (OH), 1645, 1540 (amide), 1080, 1030 (glycosidic C−O), 720 $[(CH_2)_n]$ cm^{-1}; ^1H NMR (pyridine-d_5, 300 MHz) δ 0.86 (6H, t-like, J = 6.8 Hz, 2 × Me), 1.25 [s, $(CH_2)_n$], 3.85 (1H, m, H-5″), 3.98 (1H, t, J = 7.8 Hz, H-2″), 4.17 (2H, m, H-4, 3″), 4.27 (1H, m, H-3), 4.30 (1H, H-4″), 4.31 (1H, dd, J = 5.1, 12.9 Hz, H-6″), 4.46 (1H, dd, J = 3.1, 12.9 Hz, H-6″), 4.50 (1H, dd, J = 4.7, 10.5 Hz, H-1), 4.55 (1H, m, H-2′), 4.69 (1H, dd, J = 6.6, 10.5 Hz, H-1), 4.93 (1H, d, J = 7.9 Hz, H-1″), 5.26 (1H, m, H-2), 5.45 (1H, dt, J = 5.8, 15.6 Hz, H-8), 5.52 (1H, dt, J = 5.6, 15.6 Hz, H-9), 8.53 (1H, d, J = 9.1 Hz, N−H); ^{13}C NMR (pyridine-d_5, 75.5 MHz) δ 14.3 (Me), 23.0, 25.8, 26.7, 29.5, 29.6, 29.7, 29.8, 30.0, 32.1 (C-7), 33.0 (C-10), 33.3, 33.8, 35.6 (all CH_2), 51.7 (C-2), 62.6 (C-6″), 70.5 (C-1), 71.4 (C-4″), 72.4 (C-4, 2′), 75.1 (C-2″), 75.9 (C-3), 78.4 (C-3″), 78.6 (C-5″), 105.6 (C-1″), 130.6 (C-9), 130.8 (C-8), 175.6 (C-1′); positive FABMS, m/z 732 $[M + H]^+$, 570 $[M + H - 162]^+$, 553 $[M + H - 179]^+$, 477 $[M + H - 255]^+$, 315 $[M + H - 255-162]^+$, 298 $[M + H - 179-255]^+$; EIMS (30 eV) m/z 713 $[M - H_2O]^+$ (1.2), 551 $[M - H_2O - 162]^+$ (4.3), 531 (2.1); HRFABMS m/z 754.5445 $[M + Na]^+$ (calcd for $C_{40}H_{77}NO_{10}$ + Na, 754.5446).

Acetylation of 1.[13] Aralia cerebroside (**1**, 50 mg) in pyridine (1 mL) was treated with Ac_2O (1 mL) at room temperature overnight. Evaporation of the resulting soultion under a N_2 stream gave chromatographically homogeneous heptaacetate (**2**) as an amorphous white powder: mp 57−59 °C; IR (KBr) ν_{max} 3430, 1746, 1231 (OAc), 1665, 1528 (amide), 1373, 1047 (glycosidic C−O), 720 $[(CH_2)_n]$ cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86 (6H, t-like, J = 6.7 Hz, 2 × Me), 1.24 [s, $(CH_2)_n$], 1.59 (2H, m, H-3′), 1.81−1.84 (1H, m, H-5), 1.92−1.96 (2H, m, H-7, 10), 3.65−3.69 (1H, m, H-5″), 3.66 (1H, dd, J = 3.5, 10.9 Hz, H-1), 3.84 (1H, dd, J = 3.2, 10.9 Hz, H-1), 4.11 (1H, dd, J = 2.3, 12.4 Hz, H-6″), 4.22−4.26 (1H, m, H-2), 4.23 (1H, dd, J = 4.5, 12.4 Hz, H-6″), 4.46 (1H, d, J = 8.0 Hz, H-1″), 4.85−4.88 (1H, m, H-2′), 4.88 (1H, dd, J = 8.3, 9.6 Hz, H-2″), 5.04 (1H, t, J = 9.8 Hz, H-4″), 5.09−5.12 (2H, m, H-3, 4), 5.16 (1H, t, J = 9.5 Hz, H-3″), 5.32 (1H, dt, J = 5.8, 15.6 Hz, H-8), 5.38 (1H, dt, J = 5.4, 15.6 Hz, H-9), 6.70 (1H, d, J = 8.8 Hz, N−H), 1.97 (MeCO), 1.99 (MeCO), 2.02 (2 × MeCO), 2.04 (MeCO), 2.06 (MeCO), 2.21 (MeCO); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.0 (Me), 22.6, 24.9, 25.6, 27.9, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.8 (all CH_2), 66.6 (C-1), 48.2 (C-2), 74.0 (C-3), 72.7 (C-4), 31.7 (C-5), 32.5 (C-7), 131.1 (C-8), 129.2 (C-9), 32.2 (C-10), 73.1 (C-2′), 100.4 (C-1″), 71.3 (C-2″), 71.9 (C-3″), 68.1(C-4″), 71.7 (C-5″), 61.7(C-6″), 20.5 (3 × MeCO), 20.6 (2 × MeCO), 20.9 (2 × MeCO), 169.2, 169.3, 169.7, 169.9, 170.1, 170.2, 170.5, 171.0 (all Me*C*O, NH*C*O); EIMS m/z 1025 $[M]^+$ (43.6), 966 $[M - OAc]^+$ (29.2), 965 $[M - HOAc]^+$ (39.2), 906 $[M - OAc - HOAc]^+$ (7.2), 775 (21.5), 774 (34.6), 724 (8.4), 695 (33.3), 679 (43.4), 678 (55.9), 677 (37.6), 664 (34.0), 635 (19.7), 634 (21.3), 620 (12.4), 618 (13.5), 604 (11.1), 562 (15.9), 424 (11.8), 390 (29.4), 332 (55.9), 331 $[glc(OAc)_4]^+$ (32.4), 271 (48.6), 229 (38.0), 211 (42.4), 170 (52.8), 169 (25.5), 145 (48.5), 139 (61.4), 127 (71.0), 109 (97.5), 81 (100); HRFABMS m/z 1048.6216 $[M + Na]^+$ (calcd for $C_{54}H_{91}NO_{17}$ + Na, 1048.6185).

Acid Hydrolysis of 1.[5] Aralia cerebroside **1** (50 mg) was refluxed with 0.9 N HCl in 82% aqueous MeOH (12 mL) for 18 h. The resulting solution was extracted with *n*-hexane, and the combined organic phase was dried over Na_2SO_4. Evaporation of the hexane yielded a fatty acid methyl ester. The H_2O layer was neutralized with NH_4OH and extracted with ether. The ether layer was dried over Na_2SO_4, filtered, and then concentrated to yield a long-chain base. The H_2O layer was evaporated under a N_2 stream. The residue was dissolved in pyridine (0.05 mL), then the solution was trimethylsilylated with TMS−HT (0.1 mL) at 60 °C for 30 min. After addition of *n*-hexane and H_2O, the *n*-hexane layer was removed and analyzed by GC. The retention times (t_R) of the peaks were 26.7 and 29.4 min for methyl glucopyranoside.

The fatty acid methyl ester was recrystallized from MeOH to give an amorphous white powder (15 mg) and then analyzed by GC. The retention time (t_R) of the peak was 5.77 min; mp 40 °C; $[\alpha]^{20}_D$ −1.2° (*c* 0.6, $CHCl_3$); IR (KBr) ν_{max} 3400 (OH), 1738 (C=O), 1466, 1283, 721 $[(CH_2)_n]$; ^1H NMR ($CDCl_3$, 80 MHz) δ 0.88 (3H, t-like, Me), 1.25 [s, $(CH_2)_n$], 3.78 (3H, s, COOMe), 4.18 (1H, t, J = 6 Hz, H-2); EIMS (30 eV) m/z 286 $[M]^+$ (4.8), 254 $[M - MeOH]^+$ (2.7), 227 $[M - COOMe]^+$ (63.5), 208 (3.4), 159, 145, 127 $[C_9H_{19}^+]$ (19.1), 111 $[C_8H_{15}^+]$ (41.7), 97 $[C_7H_{13}^+]$ (88.0), 90 [MeO−C−O$^+$H=CHOH] (86.2), 83 $[C_6H_{11}^+]$ (100), 69 $[C_5H_9^+]$ (96.5), 57 (94.1). The fatty acid methyl ester was identified as (2*R*)-2-hydroxy palmitic acid methyl ester by comparison with literature data.[5,14]

The long-chain base was analyzed by EIMS (30 eV) m/z 315 $[M]^+$ (0.1), 297 $[M - H_2O]^+$ (0.1), 279 $[M - 2H_2O]^+$ (9.4), 261 $[M - 3H_2O]^+$ (0.4), 167 $[C_{12}H_{23}]^+$ (38.8), 149 (100), 113 (10). A portion of the long-chain base was heated with TMCS (1.6 mL) and HMDS (2.6 mL) in pyridine (2 mL) at 70 °C for 30 min, and the reaction mixture was analyzed by EIMS (30 eV) m/z 516 $[M - Me]^+$ (0.4), 456 $[M - (Me)_2Si{=}O^+H]$ (0.1), 441 [456 − Me]$^+$ (0.2), 428 $[M - CH_2OSi(Me)_3]^+$ (0.5), 368 {M − [Me + $(Me)_3Si^+$ + $(Me)_2Si{=}O^+H$]} (0.6), 338 (6.9), 297 [Me−$(CH_2)_8$-CH=CH−$(CH_2)_3$−CH=O$^+$Si$(Me)_3$] (0.7), 279 (14.1), 167 $[C_{12}H_{23}]^+$ (38.8), 149 (100), 132 $[(Me)_3SiO{-}CH_2{-}CH{-}NH_2]^+$ (31.1). A portion of the long-chain base was acetylated with Ac_2O and pyridine (1:1, 0.3 mL each) at 70 °C for 1.5 h. Concentration to dryness under a N_2 stream yielded a hexaacetate. EIMS (30 eV) m/z 483 $[M]^+$ (0.1), 423 $[M - HOAc]^+$ (0.1).

Dimethyl Disulfide Derivative[8] of Aralia Cerebroside Heptaacetate. Heptaacetate **2** (11 mg) was dissolved in carbon disulfide (1 mL) and dimethyl disulfide (1 mL). Iodine (10 mg) was added, and the reaction mixture was kept at 70 °C for 48 h in a small sealed vial. The reaction was quenched with aqueous $Na_2S_2O_3$ (5%) and the reaction mixture extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4, filtered, and then concentrated to give a dimethyl disulfide derivative. EIMS m/z 1119 $[M]^+$ (8.7), 1072 $[M - SMe]^+$ (18.4), 1024 $[M - (SMe + CH_4S)]^+$ (8.0), 1012 $[M - SMe - HOAc]^+$ (8.7), 932 $[M - C_{11}H_{23}S]^+$ (22.6), 872 $[M - C_{11}H_{23}S - HOAc]^+$ (20.3), 772 (25.3), 740 (34.1), 680 (38.9), 331 $[glc(Ac)_4]^+$ (64.2), 187 $[C_{11}H_{23}S]^+$ (66.7), 109 (100).

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