

Cerebrosides from Longan Arillus

Jiyoung Ryu, Ju Sun Kim, and Sam Sik Kang

Natural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 110-460, Korea

(Received January 14, 2003)

From the pulp of *Euphoria longana* (Longan Arillus), three cerebroside molecular species have been isolated. Six known cerebroside, soyacerebrosides I and II, 1-O- β -D-glucopyranosyl-(2S,3R,4E,8E)-2-(2'-lignoceroylamino)-4,8-octadecadiene-1,3-diol (longan cerebroside I) and its 8Z isomer (longan cerebroside II), momor-cerebroside I, and phytolacca cerebroside, were identified as major components of these cerebroside molecular species. All the cerebroside were shown to be a mixture of geometrical isomers (8E and 8Z) of sphingosine-type or phytosphingosine-type glucocerebrosides possessing 2-hydroxy fatty acids. The structures of these cerebroside have been determined on the basis of chemical and spectroscopic evidence.

Key words: *Euphoria longana*, Longan Arillus, Sapindaceae, Cerebroside, Isolation, Structure determination

INTRODUCTION

The fruit of *Euphoria longana* Lamarck [= *E. longana* (Lour.) Steud. = *Dimocarpus longan* Lour. = *Nephelium longana* Cambess.] (Sapindaceae) is a sweet food which is available in China and South Asia. Its pulp or flesh (Longan Arillus) is used as a tonic, and for the treatment of amnesia, insomnia, various palpitations due to fright, etc. It is traditionally said to fortify the "heart" and stabilize the "spirit" (Bensky and Gamble, 1993). Several reports have been found on the isolation of the unusual amino acids (Minakata *et al.*, 1985; Sung *et al.*, 1969), dihydroterculic acid (Kleiman *et al.*, 1969), corilagin (Cheng *et al.*, 1995) and acetonylgeraniin (Hsu *et al.*, 1994) from the seeds, β -sitosterol, friedelin, epifriedelanol and 16-hentriacontanol from the leaves (Mahato *et al.*, 1971), and adenosine and the related compounds (Okuyama *et al.*, 1999) from the arillus of this plant. In continuation of our research on the cerebroside (Jung *et al.*, 1996; Kang *et al.*, 1999, 2001; Kim *et al.*, 2001), Longan Arillus was investigated. This paper deals with the isolation and characterization of three cerebroside molecular species together with β -sitosterol and its glucoside, uracil, and adenosine.

MATERIALS AND METHODS

General experimental procedures

Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. The FAB-MS was obtained in a nitrobenzyl alcohol matrix in a positive ion mode on a JEOL DX-300 spectrometer. NMR spectra were measured on either a Varian Gemini 2000 (300 MHz) or a Bruker AMX-500 (500 MHz) instrument, and chemical shifts were referenced to TMS. GC-MS for fatty acids were measured with a Hewlett Packard 5973 mass selective detector equipped with 6890 Series gas chromatograph. Conditions: HP-5MS capillary column (30 m \times 0.25 mm \times 0.25 μ m); column temperature, 80 $^{\circ}$ C-300 $^{\circ}$ C, rate of temperature increase, 10 $^{\circ}$ C/min; injector and detector (H_2 flame ionization detector) temperature, 270 $^{\circ}$ C; He flow rate, 1 mL/min. GC analysis for the determination of absolute configuration of glucose 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 \times 0.32 mm \times 0.25 μ m), column temperature, 200 $^{\circ}$ C; injector and detector temperature, 290 $^{\circ}$ C; He flow rate, 30 mL/min. TLC was performed on silica gel 60F₂₅₄ (Merck) and cellulose plates (Art. No. 5716, Merck).

Plant material

Longan Arillus was purchased from Oriental Drug Markets which was authenticated by Prof. K. S. Lee, College of

Correspondence to: Sam Sik Kang, Natural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 110-460, Korea
E-mail: sskang@snu.ac.kr

Pharmacy, Chungbuk National University.

Extraction and isolation

Longan Arillus (2.9 kg) was extracted five times with MeOH in a water bath for 3 h. The MeOH extract was partitioned in succession between H₂O and *n*-hexane (45.34 g), CH₂Cl₂ (9.3 g), EtOAc (6.0 g) and then *n*-BuOH (161.4 g). A portion of the *n*-hexane fraction (45.3 g) was subjected to silica gel column chromatography eluting with *n*-hexane-EtOAc (stepwise, 1, 5, 10, 30, 50, 70, 100%) and gave 28 subfractions. The subfraction Nos. H-13 and H-23 were further purified by recrystallization from *n*-hexane and *n*-hexane-CH₂Cl₂ to give β -sitosterol and β -sitosterol 3-O-glucoside, respectively. The subfraction No. H-01 was rechromatographed over silica gel using CH₂Cl₂-MeOH (gradient, 0.5%, from 1 to 6%) as an elutant to yield 10 subfractions (H-01-01~H-01-10). The subfraction No. H-01-08 was further chromatographed on reversed phase C₁₈ with H₂O-MeOH (90, 5, 3, 1%) to yield three cerebroside molecular species, YA-3-1 (7 mg), YA-3-2 (4 mg) and YA-3-3 (5 mg), each showing a single spot on silica gel thin-layer chromatography. The CH₂Cl₂ and EtOAc fractions were chromatographed on silica gel with CH₂Cl₂-MeOH (from 1 to 10%, from 20 to 50%) to yield β -sitosterol, β -sitosterol 3-O-glucoside, and uracil, respectively. The *n*-BuOH fraction was chromatographed on Sephadex LH-20 with H₂O-MeOH (100, 70, 50%) to yield adenosine. The known compounds were identified by comparison of various data with those reported in the literature.

YA-3-1

Amorphous powder, mp 143-147°C; IR ν_{\max} (KBr) 3398 (OH), 2920, 2851, 1647, 1537 (amide), 1460, 1084 (glycosidic C-O), 730 [(CH₂)_n] cm⁻¹; ¹H-NMR (500 MHz, pyridine-*d*₅) δ : 0.85 (6H, t-like, *J* = 4.2 Hz, 2×CH₃), 1.25 [br s, (CH₂)_n], 1.71-1.99 (2H, m, H-7), 2.00-2.17 (4H, m, H-6, 10), 3.89 (1H, m, H-5''), 4.03 (1H, t, *J* = 7.5 Hz, H-2''), 4.19-4.25 (3H, m, H-1a, 3'', 4''), 4.35 (1H, dd, *J* = 5.4, 11.5 Hz, H-6''a), 4.51 (1H, br d, *J* = 11.5 Hz, H-6''b), 4.57 (1H, m, H-2'), 4.71 (1H, dd, *J* = 6, 10.5 Hz, H-1b), 4.76 (1H, m, H-3), 4.81 (1H, m, H-2), 4.91 (1H, d, *J* = 8 Hz, H-1''), 5.48 (2H, t-like, H-8, 9), 5.92 (1H, dd, *J* = 6, 15.5 Hz, H-4), 5.99 (1H, dd, *J* = 6, 15.5 Hz, H-5), 8.35/8.36 (1H, d, *J* = 9.0/8.8 Hz, N-H); ¹³C-NMR (125.8 MHz, pyridine-*d*₅) δ : 70.2 (C-1), 54.6 (C-2), 72.5 (C-3), 132.0/132.1 (C-4 for *trans/cis*), 132.1/132.2 (C-5 for *trans/cis*), 32.9 (C-6), 32.1/27.6 (C-7 for *trans/cis*), 129.9/129.4 (C-8 for *trans/cis*), 132.1/130.6 (C-9 for *trans/cis*), 32.9/27.4 (C-10 for *trans/cis*), 14.3 (C-18, C-24'), 175.7 (C-1'), 72.6 (C-2'), 35.5 (C-3'), 32.1 (C-4'), 23.0 (C-15'), 105.7 (C-1''), 75.2 (C-2''), 78.5 (C-3''), 72.5 (C-4''), 78.6 (C-5''), 62.7 (C-6''); (+)-FABMS *m/z* 736 [M + Na]⁺, 534 [M - C₆H₁₁O₆]⁺, 482 [long-chain base + glucose + Na]⁺.

YA-3-2

Amorphous powder, mp 185-197°C; IR ν_{\max} (KBr) 3410 (OH), 2922, 2852, 1653, 1541 (amide), 1458, 1039 (glycosidic C-O), 730 [(CH₂)_n] cm⁻¹; ¹H-NMR (500 MHz, pyridine-*d*₅) δ : 0.86 (6H, m, 2×CH₃), 1.26 [br s, (CH₂)_n], 1.61-1.80 (2H, m, H-7), 2.02-2.17 (4H, m, H-6, 10), 3.91 (1H, m, H-5''), 4.04 (1H, t, *J* = 7.5 Hz, H-2''), 4.22-4.25 (3H, m, H-1a, 3'', 4''), 4.36 (1H, dd, *J* = 5.4, 11.5 Hz, H-6''a), 4.52 (1H, br d, *J* = 11.5 Hz, H-6''b), 4.58 (1H, m, H-2'), 4.71 (1H, dd, *J* = 6, 10.5 Hz, H-1b), 4.76 (1H, m, H-3), 4.82 (1H, m, H-2), 4.91 (1H, d, *J* = 8 Hz, H-1''), 5.49 (2H, t-like, H-8, 9), 5.92 (1H, dd, *J* = 5.7, 15.3 Hz, H-4), 6.00 (1H, dd, *J* = 6, 15.4 Hz, H-5), 8.34/8.37 (1H, d, *J* = 9.0/8.8 Hz, N-H); ¹³C-NMR (125.8 MHz, pyridine-*d*₅) δ : 70.2 (C-1), 54.6 (C-2), 72.3 (C-3), 131.5/131.8 (C-4 for *trans/cis*), 132.1/132.2 (C-5 for *trans/cis*), 32.9 (C-6), 32.1/27.6 (C-7 for *trans/cis*), 129.8/129.6 (C-8 for *trans/cis*), 131.0/130.3 (C-9 for *trans/cis*), 32.9/27.4 (C-10 for *trans/cis*), 14.3 (C-18, C-24'), 175.7 (C-1'), 72.6 (C-2'), 35.7 (C-3'), 105.7 (C-1''), 75.2 (C-2''), 78.5 (C-3''), 71.6 (C-4''), 78.6 (C-5''), 62.7 (C-6''); (+)-FABMS *m/z* 848 [M + Na]⁺, 646 [M - C₆H₁₁O₆]⁺, 482 [long-chain base + glucose + Na]⁺.

YA-3-3

Amorphous powder, mp 127°C; IR ν_{\max} (KBr) 3408 (OH), 2920, 2851, 1655, 1543 (amide), 1458, 1078 (glycosidic C-O), 723 [(CH₂)_n] cm⁻¹; ¹H-NMR (500 MHz, pyridine-*d*₅) δ : 0.86 (6H, t-like, *J* = 6.8 Hz, 2×CH₃), 1.27 [br s, (CH₂)_n], 2.09 (2H, m, H-10), 2.29 (2H, m, H-7), 3.86 (1H, m, H-5''), 4.01 (1H, t, *J* = 7.8 Hz, H-2''), 4.17-4.20 (3H, m, H-4, 3'', 4''), 4.29 (1H, dd, *J* = 5, 10.5 Hz, H-3), 4.36 (1H, dd, *J* = 6.6, 12.3 Hz, H-6''a), 4.49 (1H, br d, *J* = 12.3 Hz, H-6''b), 4.53 (1H, dd, *J* = 4.6, 10.6 Hz, H-1a), 4.72 (1H, dd, *J* = 6.8, 10.6 Hz, H-1b), 4.58 (1H, m, H-2'), 4.90 (1H, d, *J* = 7.8 Hz, H-1''), 5.30 (1H, m, H-2), 5.45-5.53 (2H, olefinic H), 8.57 (1H, d, *J* = 9.1 Hz, N-H); ¹³C-NMR (125.8 MHz, pyridine-*d*₅) δ : 70.4 (C-1), 51.7 (C-2), 75.8 (C-3), 72.4 (C-4), 33.2 (C-6), 33.8/27.9 (C-7 for *trans/cis*), 130.3/130.1 (C-8 for *trans/cis*), 130.6/130.4 (C-9 for *trans/cis*), 33.9/27.5 (C-10 for *trans/cis*), 14.2 (C-18, C-24'), 175.6 (C-1'), 72.4 (C-2'), 37.1 (C-3'), 105.6 (C-1''), 75.1 (C-2''), 78.5 (C-3''), 71.4 (C-4''), 78.6 (C-5''), 62.6 (C-6''); (+)-FABMS *m/z* 866 [M + Na]⁺, 844 [M + H]⁺, 682 [(M + H)-C₆H₁₀O₅]⁺, 664 [M - C₆H₁₁O₆]⁺, 500 [long-chain base + glucose + Na]⁺.

Acid hydrolysis YA-3-1, YA-3-2, and YA-3-3

YA-3-1, YA-3-2, and YA-3-3 (3, 2 and 3 mg, respectively) were refluxed with 0.9 N HCl in 82% aqueous MeOH (12 ml) for 18 h. The resulting solution was extracted with *n*-hexane, and combined organic phase was dried over Na₂SO₄. Evaporation of the hexane yielded a fatty acid methyl ester. The H₂O layer was neutralized with conc-NH₄OH and extracted with ether. The ether layer was dried

over Na_2SO_4 , filtered and then concentrated to yield a long chain base. The fatty acid methyl ester of YA-3-1 was recrystallized from MeOH to give an amorphous white powder and then analyzed by GC-MS. Peak 1 (t_{R} 17.27 min, palmitic acid methyl ester), EI-MS m/z : 270 $[\text{M}]^+$, 239, 227 $[\text{M} - \text{CH}_3\text{CO}]^+$, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$, 55. Peak 2 (t_{R} 18.58 min, 2-hydroxypalmitic acid methyl ester), EI-MS m/z : 286 $[\text{M}]^+$, 254 $[\text{M} - \text{CH}_3\text{OH}]^+$, 227 $[\text{M} - \text{CH}_3\text{COO}]^+$, 208, 145, 127 $[\text{C}_9\text{H}_{19}]^+$, 111 $[\text{C}_8\text{H}_{15}]^+$, 97 $[\text{C}_7\text{H}_{13}]^+$, 90 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$. Peak 3 (t_{R} 18.96 min, oleic acid methyl ester), EI-MS m/z : 296 $[\text{M}]^+$. Peak 4 (t_{R} 19.17 min, stearic acid methyl ester), EI-MS m/z : 298 $[\text{M}]^+$, 267, 255 $[\text{M} - \text{CH}_3\text{CO}]^+$, 241, 227, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$, 55. The fatty acid methyl ester of YA-3-2 was recrystallized from MeOH to give an amorphous white powder and then analyzed by GC-MS. Peak 1 (t_{R} 17.27 min, palmitic acid methyl ester), EI-MS m/z : 270 $[\text{M}]^+$, 239, 227 $[\text{M} - \text{CH}_3\text{CO}]^+$, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$, 55. Peak 2 (t_{R} 18.96 min, oleic acid methyl ester), EI-MS m/z : 296 $[\text{M}]^+$. Peak 3 (t_{R} 19.17 min, stearic acid methyl ester), EI-MS m/z : 298 $[\text{M}]^+$, 267, 255 $[\text{M} - \text{CH}_3\text{CO}]^+$, 241, 227, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$, 55. Peak 4 (t_{R} 24.06 min, lignoceric acid methyl ester), EI-MS m/z : 382 $[\text{M}]^+$, 351, 339 $[\text{M} - \text{CH}_3\text{CO}]^+$, 283, 269, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$, 55. Peak 5 (t_{R} 25.17 min, 2-hydroxylignoceric acid methyl ester), EI-MS m/z : 398 $[\text{M}]^+$, 366 $[\text{M} - \text{CH}_2\text{OH}]^+$, 339 $[\text{M} - \text{CH}_3\text{COO}]^+$, 320, 125, 111, 97, 90 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CHOH}]^+$, 83, 57. The fatty acid methyl ester of YA-3-3 was recrystallized from MeOH to give an amorphous white powder and then analyzed by GC-MS. Peak 1 (t_{R} 17.27 min, palmitic acid methyl ester), EI-MS m/z : 270 $[\text{M}]^+$, 239, 227 $[\text{M} - \text{CH}_3\text{CO}]^+$, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$. Peak 2 (t_{R} 18.96 min, oleic acid methyl ester), EI-MS m/z : 296 $[\text{M}]^+$. Peak 3 (t_{R} 19.17 min, stearic acid methyl ester), EI-MS m/z : 298 $[\text{M}]^+$, 267, 255 $[\text{M} - \text{CH}_3\text{CO}]^+$, 241, 227, 213, 199, 185, 171, 157, 143, 129, 87, 74 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CH}_2]^+$, 55. Peak 4 (t_{R} 25.17 min, 2-hydroxylignoceric acid methyl ester), EI-MS m/z : 398 $[\text{M}]^+$, 366 $[\text{M} - \text{CH}_2\text{OH}]^+$, 339 $[\text{M} - \text{CH}_3\text{COO}]^+$, 320, 125, 111, 97, 90 $[\text{CH}_3\text{OC}(\text{OH}) = \text{CHOH}]^+$, 83, 57. The methyl glucoside was refluxed with 5% HCl in H_2O for 2 hrs. The reaction solution was evaporated under reduced pressure and the absolute configuration of the glucose from the hydrolysate was determined according to the method reported by Hara *et al.* (1987) using GC. The t_{R} was 22.5 min for D-glucose.

MTPA esterification of 2-hydroxy fatty acid methyl ester

To a solution of fatty acid methyl ester in pyridine (40 μL) was added (+)-MTPA chloride (4.6 μL), and the solution

was allowed to stand at 25°C overnight. 3-[(Dimethylamino)propyl]amine (2.8 μL) was added, and after 10 min of standing, the solvent was evaporated. The residue was dissolved in CCl_4 and used for $^1\text{H-NMR}$ measurement. The signals in the aliphatic region appeared at δ 0.893 (3H, t, $J = 6.6$ Hz, CH_3), 1.259 (s, $(\text{CH}_2)_n$), 3.648 (3H, s, OCH_3), 3.795 (3H, s, COOCH_3), 5.084 (2H, t, $J = 5.4$ Hz, CH_2O).

RESULTS AND DISCUSSION

The hexane fraction from the MeOH extract of Longan Arillus was subjected to repeated silica gel and reversed phase C_{18} column chromatography to give three cerebroside molecular species, YA-3-1, YA-3-2, and YA-3-3, each showing a single spot on silica gel thin-layer chromatography. YA-3-1, YA-3-2, and YA-3-3 exhibited strong hydroxyl and amide absorptions in their IR spectra, and a molecular ion peak in the positive FAB mass spectra, respectively. In their NMR spectra, they reveal characteristic signals of a sphingosine-type β -glucocerebroside possessing a 2-hydroxy fatty acid for YA-3-1 and YA-3-2, and a phytosphingosine-type β -glucocerebroside possessing a 2-hydroxy fatty acid for YA-3-3, respectively. Therefore, they are suggested to be molecular species of two typical types of glucocerebrosides. Their structures were characterized by comparison of their $^{13}\text{C-NMR}$ spectral data with those of the known glucocerebrosides (Kang *et al.*, 2001; Kim *et al.*, 2001; Kang *et al.*, 1999; Jung *et al.*, 1996), and by means of the results of their chemical degradations, namely methanolysis followed by the GC-MS analysis of the methanolysis products, fatty acid methyl ester and long-chain base. The absolute configurations of glucose as well as of 2-hydroxy fatty acid methyl esters as D- and R-forms, respectively were determined by the Hara method (Hara *et al.*, 1987; Kang *et al.*, 1999) and by $^1\text{H-NMR}$ chemical shift of OCH_3 group of MTPA esters of the mixture of 2-hydroxy fatty acid methyl esters (Yasuhara and Yamaguchi, 1980; Kang *et al.*, 2001), respectively.

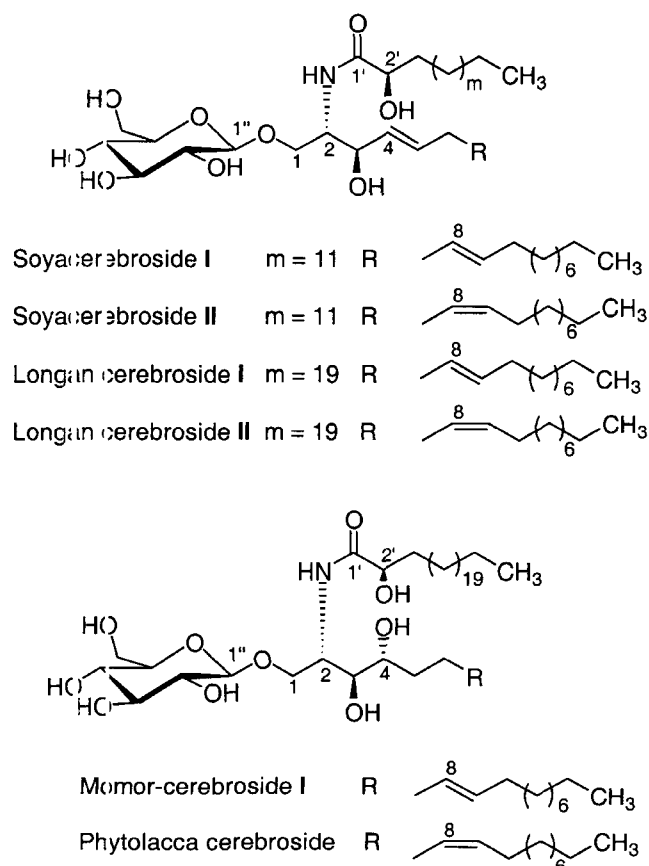
The positive FAB mass spectrum of YA-3-1 showed of a $[\text{M} + \text{Na}]^+$ ion peak at m/z 736 together with ions at m/z 534 $[\text{M} - \text{C}_6\text{H}_{11}\text{O}_6]^+$, 482 [long-chain base + glucose + Na] $^+$. The NMR data of YA-3-1 were in good agreement with those of known β -glucocerebrosides (Kim *et al.*, 2001; Jung *et al.*, 1996), which is composed of (2S,3R,4E)-sphingosine, 2-hydroxy fatty acid, and β -glucopyranose. Methanolysis of YA-3-1 yielded methyl glucoside, a mixture of fatty acid methyl esters and a long-chain base. The fatty acid methyl esters were identified as methyl palmitate, methyl 2-hydroxypalmitate, methyl oleate and methyl stearate by GC-MS analysis. The major fatty acid methyl ester was methyl 2-hydroxypalmitate. The long-chain base was identified as a mixture of stereoisomers (8E and 8Z) of (2S,3R,4E)-2-amino-1,3-dihydroxyoctadeca-4,8-diene by direct

comparison with an authentic sample (Kim *et al.*, 2001), which was obtained from soya-cerebroside I. Therefore, YA-3-1 was designated as a sphingosine-type ceramide glucoside molecular species, composed of the aforementioned fatty acids and long-chain bases, as depicted in Scheme 1. The major components are soyacerebrosides I and II, which have been previously isolated from *Phaseolus angularis* (Ohnishi and Fujino, 1981; Kojima *et al.*, 1993), soybean (Ohnishi *et al.*, 1982; Shibuya *et al.*, 1990), *Tetragonia tetragonoides* (Okuyama and Yamazaki, 1983), *Pisum sativum* (Ito *et al.*, 1985), *Acer negundo* (Inoue *et al.*, 1992), *Prunus jamasakura* (Yoshioka *et al.*, 1990), and *Allium sativum* (Inagaki *et al.*, 1998). On the other hand, soyacerebroside I from *Momordica charantia* (Xiao *et al.*, 2000) and soyacerebroside II from *Datura metel* (Sahai *et al.*, 1999) and *Psychotria correae* (Achenbach *et al.*, 1995) were isolated and identified. Very recently, soyacerebroside I was also isolated from the same genus *Dimocarpus fumatus* stem bark (Voutquenne *et al.*, 1999).

The ^1H - and ^{13}C -NMR spectra of YA-3-2 were essentially identical with those of YA-3-1, suggesting only subtle differences in the chain length of the fatty acid moiety and/or long-chain base. In the positive FAB-MS spectrum, a pseudomolecular ion at m/z 848 $[\text{M} + \text{Na}]^+$ together with

ions at m/z 646 $[\text{M} - \text{C}_6\text{H}_{11}\text{O}_6]^+$ and 482 $[\text{long-chain base} + \text{glucose} + \text{Na}]^+$ were observed. These results are different by 112 ($8 \times \text{CH}_2$) mass units higher than YA-3-1. Therefore, YA-3-2 is suggested to be a molecular species of a sphingosine-type ceramide glucoside possessing a 2-hydroxy C_{24} fatty acid moiety. In fact, YA-3-2 gives methyl 2-hydroxylignocerate along with minor fatty acid methyl esters such as methyl palmitate, methyl oleate, methyl stearate, and methyl lignocerate and a mixture of stereoisomers (8E and 8Z) of (2S,3R,4E)-2-amino-1,3-dihydroxy octadeca-4,8-diene upon methanolysis. Accordingly, the structures of the major components of YA-3-2 were identified as 1-O- β -D-glucopyranosyl-(2S,3R,4E,8E)-2-(2'-hydroxylignoceroylamino)-4,8-octadecadiene-1,3-diol (longan cerebroside I) and its 8Z isomer (longan cerebroside II). Therefore, YA-3-2 was designated as a sphingosine-type ceramide glucoside molecular species, composed of the aforementioned fatty acids and long-chain bases, as depicted in Scheme 1. This molecular species has been identified from the seed of *Vigna angularis* by means of GC-MS analysis (Kojima *et al.*, 1998). To the best of our knowledge, this is the first time that the cerebroside has been isolated and characterized from natural sources.

The YA-3-3 showed characteristic ^{13}C -NMR signals due to C-1-C-4, C-1', C-2', C-1'' and C-2'' of a 1-O- β -glucopyranoside of a phytosphingosine-type ceramide possessing a 2-hydroxy fatty acid. The chemical shift of the H-2 at δ 5.30 and the carbon chemical shifts at δ 70.4 (C-1), 51.7 (C-2), 75.8 (C-3), 72.4 (C-4), 175.6 (C-1') and 72.4 (C-2') in YA-3-3 were virtually identical with those of the reported data of other (2S,3S,4R)-phytosphingosine moieties (Kang *et al.*, 2001). The positive FAB-MS spectrum of YA-3-3 showed pseudomolecular ions at m/z 866 $[\text{M} + \text{Na}]^+$ and 844 $[\text{M} + \text{H}]^+$, and fragments at m/z 682 $[(\text{M} + \text{H}) - \text{C}_6\text{H}_{10}\text{O}_5]^+$, 664 $[\text{M} - \text{C}_6\text{H}_{11}\text{O}_6]^+$ and 500 $[\text{long-chain base} + \text{glucose} + \text{Na}]^+$. Methanolysis of YA-3-3 afforded methyl palmitate, methyl oleate, methyl stearate, and methyl 2-hydroxylignocerate by GC-MS analysis. The major fatty acid methyl ester was methyl 2-hydroxylignocerate. Accordingly, the structure of the major cerebroside of YA-3-3 was identified as 1-O- β -D-glucopyranosyl-(2S,3S,4R,8E/Z)-2-[2'-hydroxylignoceroylamino]-8-octadecene-1,3,4-triol. The Z (*cis*) isomer, 1-O- β -D-glucopyranosyl-(2S,3S,4R,8Z)-2-[2'-hydroxylignoceroylamino]-8-octadecene-1,3,4-triol has been isolated from the *Phytolacca* species as one of the major cerebrosides (Kang *et al.*, 2001), but the E (*trans*) isomer, 1-O- β -D-glucopyranosyl-(2S,3S,4R,8E)-2-[2'-hydroxylignoceroylamino]-8-octadecene-1,3,4-triol, has been found to be identical to momor-cerebroside I isolated from *Momordica charantia* (Xiao *et al.*, 2000). Therefore, YA-3-3 was designated as a phytosphingosine-type ceramide glucoside molecular species, composed of the aforementioned fatty acids and long-chain bases, as depicted in Scheme 1. The biological



Scheme 1. Structures of the major cerebrosides from Longan Arillus

activities of these components will be examined in future studies.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-002). FAB-MS and NMR (500 MHz) data were recorded at KBSI (Seoul). The assistance of the staffs there is gratefully acknowledged.

REFERENCES

- Achenbach, H., Lottes, M., Waibel, R., Karikas, G. A., Correa, M. D., and Gupta, M. P., Alkaloids and other compounds from *Psychotria correae*. *Phytochemistry*, 38, 1537-1545 (1995).
- Bensky, D. and Gamble, A., *Materia Medica*, Revised Ed., Eastland Press, Seattle, p. 335, (1993).
- Cheng, J.-T., Lin, T.-C., and Hsu, F.-L., Antihypertensive effect of corilagin in the rat. *Can. J. Physiol. Pharmacol.*, 73, 1425-1429 (1995).
- Hara, S., Okabe, H., and Mihashi, K., Gas-liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(R)-carboxylates. *Chem. Pharm. Bull.*, 35, 501-506 (1987).
- Hsu, F.-L., Lu, F.-H., and Cheng, J.-T., Influence of acetylgeraniin, a hydrolyzable tannin from *Euphoria longana*, on orthostatic hypotension in a rat model. *Planta Med.*, 60, 297-300 (1994).
- Inagaki, M., Harada, Y., Yamada, K., Isobe, R., Higuchi, R., Matsuura, H. and Itakura, Y., Isolation and structure determination of cerebrosides from garlic, the bulbs of *Allium sativum* L. *Chem. Pharm. Bull.*, 46, 1153-1156 (1998).
- Inoue, T., Sakurai, N., Nagai, S., and Nagai, M., Studies on the constituents of Aceraceae Plants (X). Isolation of flavonoid glycosides and a cerebroside from the leaves of *Acer negundo*. *Shoyakugaku Zasshi*, 46, 261-264 (1992).
- Ito, S., Ohnishi, M., and Fujino, Y., Investigation of sphingolipids in pea seeds. *Agric. Biol. Chem.*, 49, 539-540 (1985).
- Jung, H. J., Kim, C.-O., Kim, Y. C., and Kang, S. S., New bioactive cerebrosides from *Arisaema amurense*. *J. Nat. Prod.*, 59, 319-322 (1996).
- Kang, S. S., Kim, J. S., Xu, Y. N., and Kim, Y. H., Isolation of new cerebroside from the root bark of *Aralia elata*. *J. Nat. Prod.*, 62, 1059-1060 (1999).
- Kang, S. S., Kim, J. S., Son, K. H., Kim, H. P., and Chang, H. W., Cyclooxygenase-2 inhibitory cerebrosides from *Phytolacca radix*. *Chem. Pharm. Bull.*, 49, 321-323 (2001).
- Kim, J. S., Byun, J. H., and Kang, S. S., Isolation of soya-cerebroside from the roots of *Trichosanthes kirilowii*. *Nat. Prod. Sci.*, 7, 27-32 (2001).
- Kleiman, R., Earle, F. R., and Wolff, I. A., Dihydrosterculic acid, a major fatty acid component of *Euphoria longana* seed oil. *Lipids*, 4, 317-320 (1969).
- Kojima, M., Suzuki, H., Ohnishi, M., and Ito, S., Effects of growth temperature on lipids of adzuki bean cells. *Phytochemistry*, 47, 1483-1487 (1998).
- Mahato, S. B., Sahu, N. P., and Chakravarti, R. N., Chemical investigation on the leaves of *Euphoria longana*. *Phytochemistry*, 10, 2847-2848 (1971).
- Minakata, H., Komura, H., Tamura, S. Y., Ohfuné, Y., Nakanishi, K., and Kata, T., Antimutagenic unusual amino acids from plants. *Experientia*, 41, 1622-1623 (1985).
- Ohnishi, M. and Fujino, Y., Chemical composition of ceramide and cerebroside in azuki bean seeds. *Agric. Biol. Chem.*, 45, 1283-1284 (1981).
- Ohnishi, M. and Fujino, Y., Sphingolipids in immature and mature soybeans. *Lipids*, 17, 803-810 (1982).
- Okuyama, E. and Yamazaki, M., The principles of *Tetragonia tetragonoides* having anti-ulcerogenic activity. II. Isolation and structure of cerebrosides. *Chem. Pharm. Bull.*, 31, 2209-2219 (1983).
- Okuyama, E., Ebihara, H., Takeuchi, H., and Yamazaki, M., Adenosine, the anxiolytic-like principle of the arillus of *Euphoria longana*. *Planta Med.*, 65, 115-119 (1999).
- Sahai, M., Manickam, M., Gupta, M., Srivastava, A., and Ray, A. B., Characterization of a cerebroside isolated from the leaves of *Datura metel*. *J. Indian Chem. Soc.*, 76, 95-97 (1999).
- Shibuya, H., Kawashima, K., Sakagami, M., Kawanishi, H., Shimomura, M., Ohashi, K., and Kitagawa, I., Sphingolipids and Glycerolipids. I. Chemical structures and ionophoretic activities of soya-cerebrosides I and II from soybean. *Chem. Pharm. Bull.*, 38, 2933-2938 (1990).
- Sung, M.-L., Fowden, L., Millington, D. S., and Sheppard, R. C., Acetylenic amino acids from *Euphoria longana*. *Phytochemistry*, 8, 1227-1233 (1969).
- Voutquenne, L., Lavaud, C., Massiot, G., Sevenet, T., and Hadi, H. A., Cytotoxic polyisoprenes and glycosides of long-chain fatty alcohols from *Dimocarpus fumatus*. *Phytochemistry*, 50, 63-69 (1999).
- Xiao, Z.-Y., Chen, D.-H., and Si, J.-Y., Studies on the chemical constituents from *Momordica charantia*. *Chin. Trad. Herbal Drugs (Zhongcaoyao)*, 31, 571-573 (2000).
- Yasuhara, Y. and Yamaguchi, S., Determination of absolute configuration and enantiomeric purity of 2- and 3-hydroxy-carboxylic acid ester. *Tetrahedron Lett.*, 21, 2827-2830 (1980).
- Yoshioka, A., Etoh, H., Yagi, A., Sakata, K., and Ina, K., Isolation of Flavonoids and cerebrosides from bark of *Prunus jamasakura* as repellents against the blue mussel, *Mytilus edulis*. *Agric. Biol. Chem.*, 54, 3355-3356 (1990).