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Phytochemical Studies on Paeoniae Radix (4) - Cerebrosides and Other Constituents

Yoon Jung Kim[1], Min Hye Yean[1], Eun Ju Lee[1], Ju Sun Kim[1], Je-Hyun Lee[2], and Sam Sik Kang[1,*]

[1]*Natural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[2]*Department of Korean Medicine, Dongguk University, Gyeongju-si, Gyeongbuk 780-714, Korea*

Abstract – A mixture of sixteen cerebrosides, which comprised four cerebroside molecular species (PL-1 ~ PL-4) was separated from the roots of *Paeonia lactiflora*. The structures of cerebrosides were characterized as 1-*O*-β-D-glucopyranosides of phytosphingosines, which comprised a common long-chain base, (2*S*,3*S*,4*R*,8*E/Z*)-2-amino-8-octadecene-1,3,4-triol with eight fatty acids or 2-hydroxy fatty acids of varying chain lengths (C_{16}, C_{18}, $C_{20\text{-}26}$) linked to the amino group. Aralia cerebroside and its 8*Z* isomer (PL-1), 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*, 4*R*,8*E/Z*)-2-[(2'*R*)-2'-hydroxytetracosanoylamino]-8-octadecene-1,3,4-triol (PL-2), 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*, 8*E/Z*)-2-[(2'*R*)-2'-hydroxydocosanoylamino]-8-octadecene-1,3,4-triol (PL-3), and 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*, 8*E/Z*)-2-[(2'*R*)-2'-hydroxytricosanoylamino]-8-octadecene-1,3,4-triol (PL-4) were identified as major components of these cerebroside molecular species. All the major cerebrosides were shown to be a mixture of geometrical isomers (8*E* and 8*Z*) of phytosphingosine-type glucocerebrosides possessing 2*R*-hydroxy fatty acids. In addition, three β-sitosterol derivatives and adenosine were also separated. The structures of these isolates have been determined on the basis of chemical and spectroscopic evidence.
Keywords – *Paeonia lactiflora*, Paeoniaceae, cerebrosides and other constituents, isolation and structure determination

Introduction

In previous papers, we reported the isolation of ten monoterpene glucosides (Kim, *et al*., 2008a; Yean, *et al*., 2008), fourteen phenolic derivatives (Kim, *et al*., 2008b) and nine triterpenoids (Kim, *et al*., 2008c) from Paeoniae Radix, the dry root of *Paeonia lactiflora* Pall. (Paeoniaceae). This paper describes the structural elucidation of four cerebroside molecular species (PL-1 ~ PL-4) together with three β-sitosterol derivatives and adenosine from the same roots of *P. lactiflora* on the basis of various spectroscopic data.

Experimental

General – The optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were obtained on a Jasco FT/IR-300E spectrometer. The FAB-MS was obtained in a 3-nitrobenzyl alcohol matrix in a positive ion mode on a Jeol JMS-700 spectrometer. NMR spectra were measured on a Varian Gemini 2000 (300 MHz) instrument, and chemical shifts were referenced to TMS. GC-MS for fatty acid methyl esters were measured with a Hewlett Packard 5973 N mass selective detector equipped with Hewlett Packard 6890 gas chromatograph. Conditions: DB 5 capillary column (30 m × 0.25 mm × 0.25 mm); column temperature, 160 °C → 350 °C, rate of temperature increase, 5 °C/min; injector and detector (H_2 flame ionization detector) temperature, 280 °C; He flow rate, 1 mL/min. TLC was performed on silica gel 60 F_{254} (Merck) and cellulose plates (art. no. 5716, Merck).

Plant material – The root of *P. lactiflora* was purchased from Asan Oriental Drug store in Seoul which was authenticated by one of authors (JHL). A voucher specimen (LJH2005-12) was deposited in the herbarium of the College of Oriental Medicine, Dongguk University.

Extraction and isolation – The roots of *P. lactiflora* (18 kg) were chopped into small pieces and refluxed with 70% EtOH for 3 hr at 70 - 80 °C (3 L × 5). The 70% EtOH extract was evaporated to dryness under reduced pressure and then partitioned successively between H_2O and CH_2Cl_2, EtOAc (300 g), and then BuOH (680 g). The CH_2Cl_2 fraction was partitioned between 90% aqueous MeOH and hexane to yield hexane (83.5 g) and 90% aqueous MeOH (78.4 g) fractions. The hexane fraction (83.5 g) was fractionated by column chromatography over silica gel with hexane/EtOAc (gradient) to yield

*Author for correspondence
Fax: +82-2-743-3323; E-mail: sskang@snu.ac.kr

subfractions (Fr. H-01-Fr. H-600). Subfraction 104 was recrystallized from MeOH/CH_2Cl_2 to yield β-sitosterol (132 mg). Subfraction H-368 was further purified on a silica gel column with CH_2Cl_2/MeOH/H_2O (7 : 0.5 : 0.5) to afford β-sitosterol glucoside 6'-*O*-palmitate (353 mg). Fraction H-509 (29 g) was chromatographed on a silica gel column with CH_2Cl_2/MeOH/H_2O (7 : 1 : 0.5 → 520 : 280 : 80) to afford subfraction H-509-101 (445 mg), which was further purified by an RP-18 column with 100% MeOH to afford PL-1 (68 mg), PL-2 (32 mg), PL-3 (6 mg), and then PL-4 (8 mg). The 90% aqueous MeOH (78.4 g) fraction was fractionated by column chromatography over silica gel with CH_2Cl_2/MeOH (gradient) to yield 400 subfractions. Subfraction M-373 was recrystallized from MeOH-CH_2Cl_2 to yield β-sitosterol glucoside (9 mg). The BuOH soluble fraction (437.3 g) was fractionated by silica gel column chromatography with CH_2Cl_2/MeOH/H_2O (7 : 2 : 0.5 → 520 : 280 : 80) to yield 35 fractions (Fr. B-01-Fr. B-35). Fr. B-32 (12.8 g) was purified on a silica gel column with hexane/EtOAc (gradient) to yield subfraction B-32-139, which was further purified by recrystallization with MeOH to yield adenosine (30 mg).

PL-1 – Amorphous white powder. $[\alpha]_D^{20}$ +13.3° (*c* 0.55 in MeOH). IR ν_{max} (KBr) 3393 (OH), 2919, 2851 (CH), 1621, 1538 (amide), 1082, 1036 (glycosidic C-O), 720 [$(CH_2)_n$] cm^{-1}; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.83 (3H, t-like, *J* = 7.2 Hz, CH_3), 0.85 (3H, t-like, *J* = 6.3 Hz, CH_3), 1.25 [s, $(CH_2)_n$], 3.84 (1H, m, H-5"), 3.98 (1H, t, *J* = 8.2 Hz, H-2"), 4.17 (2H, m, H-3", 4), 4.26 - 4.30 (2H, m, H-3, 4"), 4.32 (1H, dd, *J* = 5.1, 11.7 Hz, H-6"a), 4.46 (1H, dd, *J* = 2.7, 11.7 Hz, H-6"b), 4.50 (1H, dd, *J* = 4.5, 10.6 Hz, H-1a), 4.55 (1H, m, H-2'), 4.69 (1H, dd, *J* = 6.6, 10.6 Hz, H-1b), 4.93 (1H, d, *J* = 7.5 Hz, H-1"), 5.26 (1H, m, H-2), 5.40 - 5.54 (2H, m, olefinic H), 8.55 (1H, d, *J* = 9.0 Hz, NH); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 14.2 (Me), 22.9, 25.8, 26.6, 26.7, 27.5 (C-7*Z*), 27.8 (C-10*Z*), 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 32.0 (C-7*E*), 32.1, 32.9 (C-10*E*), 33.2, 33.8, 33.9, 35.5 (C-3'), 51.6 (C-2), 62.5 (C-6"), 70.4 (C-1), 71.4 (C-4"), 72.3 (C-4, 2'), 75.1 (C-2"), 75.8 (C-3), 78.3 (C-3"), 78.5 (C-5"), 105.5 (C-1"), 130.1 (C-9*Z*), 130.3 (C-8*Z*), 130.6 (C-9*E*), 130.8 (C-8*E*), 175.6 (C-1'); FAB-MS *m/z* 754 $[M + Na]^+$, 570 $[(M + H) - 162]^+$, 552 $[M - 179]^+$, 500 [(M + Na) – fatty acid]$^+$, 316 [long-chain base + H]$^+$, 298 $[(M + H) - 179 - 255]^+$.

PL-2 – Amorphous white powder. $[\alpha]_D^{20}$ +15.4° (*c* 0.48 in MeOH). IR ν_{max} (KBr) 3397 (OH), 2920, 2851 (CH), 1627, 1538 (amide), 1081, 1035 (glycosidic C-O), 720 [$(CH_2)_n$] cm^{-1}; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.85 (6H, t, *J* = 6.9 Hz, 2 × CH_3), 1.26 [br s, $(CH_2)_n$], 3.84 (1H, m, H-5"), 3.99 (1H, t, *J* = 8.1 Hz, H-2"), 4.16 - 4.19 (3H, m, H-4, 3", 4"), 4.27 (1H, dd, *J* = 6.0, 11.4 Hz, H-3), 4.34 (1H, dd, *J* = 5.4, 11.7 Hz, H-6"a), 4.45 (1H, dd, *J* = 2.1, 11.7 Hz, H-6"b), 4.52 (1H, dd, *J* = 5.7, 10.6 Hz, H-1a), 4.55 (1H, m, H-2'), 4.69 (1H, dd, *J* = 6.9, 10.6 Hz, H-1b), 4.93 (1H, d, *J* = 7.8 Hz, H-1"), 5.27 (1H, m, H-2), 5.43 - 5.54 (2H, m, olefinic H), 8.54 (1H, d, *J* = 9.3 Hz, NH); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 14.1 (Me), 22.8, 25.7, 26.5, 26.7, 27.4 (C-7*Z*), 27.8 (C-10*Z*), 29.4, 29.5, 29.7, 29.7, 29.8, 29.9, 29.9, 32.0 (C-7*E*), 32.8 (C-10*E*), 33.2, 33.7, 33.9, 35.4 (C-3'), 51.6 (C-2), 62.5 (C-6"), 70.3 (C-1), 71.4 (C-4"), 72.3 (C-4, 2'), 75.0 (C-2"), 75.8 (C-3), 78.3 (C-3"), 78.4 (C-5"), 105.4 (C-1"), 130.0 (C-9*Z*), 130.3 (C-8*Z*), 130.5 (C-9*E*), 130.7 (C-8*E*), 175.5 (C-1'); FAB-MS *m/z* 867 $[M + Na + H]^+$, 682 $[(M + H) - 162]^+$, 665 $[(M + H) - 179]^+$, 500 [(M + Na) – fatty acid]$^+$, 316 [long-chain base + H]$^+$.

PL-3 – Amorphous white powder. $[\alpha]_D^{20}$ +10.0° (*c* 0.13 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.85 (6H, t, *J* = 6.6 Hz, 2 × CH_3), 1.26 [br s, $(CH_2)_n$], 3.85 (1H, m, H-5"), 3.99 (1H, t-like, *J* = 8.1 Hz, H-2"), 4.18 - 4.21 (3H, m, H-4, 3", 4"), 4.30 (1H, m, H-3), 4.35 (1H, dd, *J* = 5.1, 11.7 Hz, H-6"a), 4.47 (1H, d, *J* = 2.1, 11.7 Hz, H-6"b), 4.53 (1H, m, H-1a), 4.55 (1H, m, H-2'), 4.70 (1H, dd, *J* = 6.6, 10.5 Hz, H-1b), 4.94 (1H, d, *J* = 8.1 Hz, H-1"), 5.28 (1H, m, H-2), 5.41 - 5.55 (2H, m, olefinic H), 8.56 (1H, d, *J* = 9.0 Hz, NH); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 14.2 (Me), 22.9, 25.8, 26.6, 26.7, 27.6 (C-7*Z*), 27.9 (C-10*Z*), 29.6, 29.8, 29.8, 29.9, 30.0, 32.1 (C-7*E*), 32.9 (C-10*E*), 33.2, 33.7, 33.9, 35.5 (C-3'), 51.7 (C-2), 62.6 (C-6"), 70.4 (C-1), 71.5 (C-4"), 72.4 (C-4, 2'), 75.1 (C-2"), 75.9 (C-3), 78.4 (C-3"), 78.5 (C-5"), 105.6 (C-1"), 130.2 (C-9*Z*), 130.4 (C-8*Z*), 130.6 (C-9*E*), 130.8 (C-8*E*), 175.6 (C-1'); FAB-MS *m/z* 839 $[M + Na + H]^+$, 654 $[(M + H) - 162]^+$, 637 $[(M + H) - 179]^+$, 500 [(M + Na) – fatty acid]$^+$.

PL-4 – Amorphous white powder. $[\alpha]_D^{20}$ +8.0° (*c* 0.2 in MeOH). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.85 (6H, t, *J* = 6.7 Hz, 2 × CH_3), 1.26 [br s, $(CH_2)_n$], 3.85 (1H, m, H-5"), 3.99 (1H, t-like, *J* = 8.2 Hz, H-2"), 4.18 - 4.21 (3H, m, H-4, 3", 4"), 4.30 (1H, m, H-3), 4.35 (1H, dd, *J* = 5.4 11.7 Hz, H-6"a), 4.47 (1H, m, H-6"b), 4.53 (1H, m, H-1a), 4.55 (1H, m, H-2'), 4.70 (1H, dd, *J* = 6.6, 10.6 Hz, H-1b), 4.94 (1H, d, *J* = 7.8 Hz, H-1"), 5.28 (1H, m, H-2), 5.41 - 5.55 (2H, m, olefinic H), 8.56 (1H, d, *J* = 9.0 Hz, NH); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 14.1 (Me), 22.8, 25.7, 26.5, 26.7, 27.4 (C-7*Z*), 27.8 (C-10*Z*), 29.4, 29.5, 29.7, 29.7, 29.8, 29.9, 29.9, 32.0 (C-7*E*), 32.8 (C-10*E*), 33.2, 33.7, 33.9, 35.4 (C-3'), 51.6 (C-2), 62.5 (C-

6"), 70.3 (C-1), 71.4 (C-4"), 72.3 (C-4, 2'), 75.0 (C-2"), 75.8 (C-3), 78.3 (C-3"), 78.4 (C-5"), 105.4 (C-1"), 130.1 (C-9*Z*), 130.3 (C-8*Z*), 130.5 (C-9*E*), 130.7 (C-8*E*), 175.5 (C-1'); FAB-MS *m/z* 853 $[M + Na + H]^+$, 668 $[(M + H) - 162]^+$, 651 $[(M + H) - 179]^+$, 500 $[(M + Na) - \text{fatty acid}]^+$.

Acid hydrolysis PL-1, PL-2, PL-3 and PL-4 – PL-1, PL-2, PL-3 and PL-4 (30, 20, 5 and 6 mg, respectively) were refluxed with 0.9 N HCl in 82% aqueous MeOH (12 mL) for 18 hrs. The resulting solution was extracted with hexane, and 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 conc-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 which was identified by comparison of their spectroscopic data and confirmed by the direct comparison with an authentic sample obtained from *Phytolacca americana* (Kang, *et al*., 2001). The fatty acid methyl esters from PL-1 ~ PL-4 were recrystallized from MeOH to give an amorphous white powder and then analyzed by GC-MS. The methyl glucoside was refluxed with 5% HCl in H_2O for 2 hrs. The reaction solution was evaporated under reduced pressure and then co-chromatographed with authentic sugars. D-Glucose was identified in all hydrolysate.

Fatty acid methyl ester from PL-1 – $[\alpha]_D^{19}$ –2.3° (*c* 0.38 in $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t-like, *J* = 6.9 Hz, CH_3), 1.25 [s, $(CH_2)_n$], 3.79 (3H, s, $COOCH_3$), 4.19 (1H, dd, *J* = 4.2, 7.5 Hz, H-2); peak 1 (t_R 8.196 min, palmitic acid methyl ester, 1.7 %), EIMS *m/z* 270 $[M]^+$, 239, 227 $[M - CH_3CO]^+$, 199, 185, 171, 143, 129, 74 $[CH_3OC(OH) = CH_2]^+$; peak 2 (t_R 10.202 min, 2-hydroxypalmitic acid methyl ester, 98.3 %), EIMS *m/z* 286 $[M]^+$, 254 $[M - CH_3OH]^+$, 227 $[M - CH_3COO]^+$, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$.

Fatty acid methyl ester from PL-2 – $[\alpha]_D^{19}$ –4.6° (*c* 0.2 in $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t-like, *J* = 6.9 Hz, CH_3), 0.92 (3H, t-like, *J* = 7.2 Hz, CH_3), 1.25 [s, $(CH_2)_n$], 3.78 (3H, s, $COOCH_3$), 4.19 (1H, dd, *J* = 4.5, 7.5 Hz, H-2), 4.22 (1H, dd, *J* = 3.6, 5.7 Hz, H-2); peak 1 (t_R 8.166 min, palmitic acid methyl ester, 1.3%), EIMS *m/z* 270 $[M]^+$, 239, 227 $[M - CH_3CO]^+$, 199, 185, 171, 143, 129, 74 $[CH_3OC(OH) = CH_2]^+$; peak 2 (t_R 11.274 min, stearic acid methyl ester, 0.26 %), EIMS *m/z* 298 $[M]^+$, 143, 74 $[CH_3OC(OH) = CH_2]^+$; peak 3 (t_R 21.155 min, 2-hydroxytricosanoic acid methyl ester, 4.4%), EIMS *m/z* 384 $[M]^+$, 325 $[CH_3COO]^+$, 207, 145, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$, 83, 57; peak 4 (t_R 22.425 min, 2-hydroxytetracosanoic acid methyl ester, 89.5 %), EIMS *m/z* 398 $[M]^+$, 366 $[M - CH_3OH]^+$, 339 $[M - CH_3COO]^+$, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$; peak 5 (t_R 24.025 min, 2-hydroxypentacosanoic acid methyl ester, 4.6%), EIMS *m/z* 412 $[M]^+$, 353 $[M - CH_3COO]^+$, 281, 207, 125, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$.

Fatty acid methyl ester from PL-3 – $[\alpha]_D^{26}$ –1.0° (*c* 0.2 in $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t-like, CH_3), 1.25 [s, $(CH_2)_n$], 3.79 (3H, s, $COOCH_3$), 4.18 (1H, dd, *J* = 4.2, 7.5 Hz, H-2); peak 1 (t_R 11.000 min, palmitic acid methyl ester, 11.1 %), EIMS *m/z* 270 $[M]^+$, 239, 227 $[M - CH_3CO]^+$, 199, 185, 171, 143, 129, 74 $[CH_3OC(OH) = CH_2]^+$; peak 2 (t_R 23.279 min, 2-hydroxydocosanoic acid methyl ester, 59 %), EIMS *m/z* 370 $[M]^+$, 338 $[M - CH_3OH]^+$, 311 $[M - CH_3COO]^+$, 292, 266, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$; peak 3 (t_R 24.657 min, 2-hydroxytricosanoic acid methyl ester, 7.4 %), EIMS *m/z* 384 $[M]^+$, 325 $[M - CH_3COO]^+$, 207, 145, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$, 83, 57; peak 4 (t_R 26.099 min, 2-hydroxytetracosanoic acid methyl ester, 12.6 %), EIMS *m/z* 398 $[M]^+$, 366 $[M - CH_3OH]^+$, 339 $[M - CH_3COO]^+$, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$; peak 5 (t_R 27.631 min, 2-hydroxypentacosanoic acid methyl ester, 5.4%), EIMS *m/z* 412 $[M]^+$, 353 $[M - CH_3COO]^+$, 281, 207, 125, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$; peak 6 (t_R 29.470 min, 2-hydroxyhexacosanoic acid methyl ester, 4.5%), EIMS *m/z* 426 $[M]^+$, 367 $[M - CH_3COO]^+$, 281, 207, 125, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$.

Fatty acid methyl ester from PL-4 – $[\alpha]_D^{19}$ –2.1° (*c* 0.18 in $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t-like, CH_3), 0.92 (3H, t-like, CH_3), 1.25 [s, $(CH_2)_n$], 3.78 (3H, s, $COOCH_3$), 4.19 (1H, dd, *J* = 4.5, 6.4 Hz, H-2), 4.22 (1H, dd, *J* = 2.4, 4.9 Hz, H-2); peak 1 (t_R 10.981 min, palmitic acid methyl ester, 5.7%), EIMS *m/z* 270 $[M]^+$, 239, 227 $[M - CH_3CO]^+$, 199, 185, 171, 143, 129, 74 $[CH_3OC(OH) = CH_2]^+$; peak 2 (t_R 23.190 min, 2-hydroxydocosanoic acid methyl ester, 10.6%), EIMS *m/z* 370 $[M]^+$, 338 $[M - CH_3OH]^+$, 311 $[M - CH_3COO]^+$, 292, 266, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$; peak 3 (t_R 24.792 min, 2-hydroxytricosanoic acid methyl ester, 69.6%), EIMS *m/z* 384 $[M]^+$, 325 $[CH_3COO]^+$, 207, 145, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$, 83, 57; peak 4 (t_R 26.106 min, 2-hydroxytetracosanoic acid methyl ester, 14.1%), EIMS *m/z* 398 $[M]^+$, 366 $[M - CH_3OH]^+$, 339 $[M - CH_3COO]^+$, 111, 97, 90 $[CH_3OC(OH) = CHOH]^+$.

β-Sitosterol (stigmast-5-en-3β-ol) – Colorless needles. $[\alpha]_D^{26}$ –56.0° (*c* 0.2 in $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.68 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 3.53 (1H, m, H-3), 5.35 (1H, d, *J* = 5.1 Hz, H-6); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-

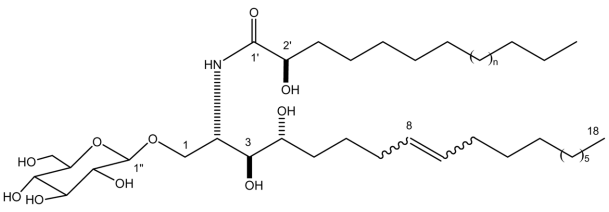
12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.9 (C-18), 19.4 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.6 (C-26), 19.8 (C-27), 23.1 (C-28), 12.0 (C-29); EIMS (rel. int., %) *m/z* 414 $[M]^+$ (31), 396 $[M - H_2O]^+$ (11), 381 $[M - (H_2O + CH_3)]^+$ (7), 329 (18), 303 (12), 273 (12), 255 (13), 231 (12), 213 (17), 107 (53), 105 (66), 57 (100).

β-Sitosterol glucoside 6'-*O*-palmitate (daucosterol 6'-*O*-palmitate) – Amorphous white powder. $[\alpha]_D^{26}$ –24.1° (*c* 0.25 in $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.68 (3H, s, CH_3-18), 0.81 (3H, d, *J* = 6.3 Hz, CH_3-26), 0.83 (3H, d, *J* = 6.6 Hz, CH_3-27), 0.84 (3H, t, *J* = 7.2 Hz, CH_3-29), 0.88 [3H, t, *J* = 6.9 Hz, $CH_3(CH_2)_n$], 0.92 (3H, d, *J* = 6.3 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 1.25 [br s, $(CH_2)_n$], 2.33 (2H, br t, *J* = 7.5 Hz, -CH_2CH_2CO-), 3.31 - 3.59 (5H, m, H-3, 2' - 5'), 4.34 (1H, d, *J* = 7.8 Hz, H-1'), 4.40 (2H, m, H-6'), 5.36 (1H, m, H-6); FAB-MS *m/z* 837 $[M + Na]^+$ (palmitic acid), 823 $[M + Na]^+$ (pentadecanoic acid).

β-Sitosterol glucoside (daucosterol) – Amorphous white powder. $[\alpha]_D^{26}$ –41.7° (*c* 0.2 in pyridine). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.65 (3H, s, CH_3-18), 0.85 (3H, d, *J* = 6.6 Hz, CH_3-26), 0.87 (3H, d, *J* = 6.6 Hz, CH_3-27), 0.88 (3H, t, *J* = 7.5 Hz, CH_3-29), 0.92 (3H, s, CH_3-19), 0.98 (3H, d, *J* = 6.3 Hz, CH_3-21), 2.47 (1H, br t, *J* = 13.5 Hz, H-4a), 2.73 (1H, ddd, *J* = 2.0, 5.0, 13.5 Hz, H-4b), 3.93 (1H, m, H-5'), 3.99 (1H, m, H-3), 4.06 (1H, t, *J* = 8.7 Hz, H-2'), 4.28 (1H, t, *J* = 9.0 Hz, H-3'), 4.31 (1H, t, *J* = 8.7 Hz, H-4'), 4.42 (1H, dd, *J* = 5.1, 12.0 Hz, H-6'a), 4.57 (1H, dd, *J* = 2.1, 12.0 Hz, H-6'b), 5.06 (1H, d, *J* = 7.5 Hz, H-1'), 5.34 (1H, d, *J* = 4.8 Hz, H-6).

Adenosine – Amorphous white powder. $[\alpha]_D^{26}$ –60.3° (*c* 0.55 in H_2O). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.54 (1H, ddd, *J* = 3.6, 7.2, 12.0 Hz, H-5'a), 3.66 (1H, td, *J* = 3.9, 12.0 Hz, H-5'b), 3.95 (1H, dd, *J* = 3.3, 6.9 Hz, H-4'), 4.13 (1H, dd, *J* = 4.5, 7.8 Hz, H-3'), 4.60 (1H, dd, *J* = 6.6, 11.4 Hz, H-2'), 5.86 (1H, d, *J* = 6.3 Hz, H-1'), 7.32 (2H, br s, NH_2), 8.12 (1H, s, H-2), 8.33 (1H, s, H-8); ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 61.9 (C-5'), 70.9 (C-3'), 73.6 (C-2'), 86.1 (C-4'), 88.1 (C-1'), 119.6 (C-5), 140.1 (C-8), 149.3 (C-4), 52.6 (C-2), 56.4 (C-6); FABMS *m/z* 268 $[M + H]^+$.

Alkaline hydrolysis of β-sitosterol glucoside 6'-*O*-palmitate – β-Sitosterol glucoside 6'-*O*-palmitate (100 mg) was treated with 3% NaOH in MeOH (50 mL) for 4 hrs. After concentration the MeOH solution, the residue was suspended in H_2O and filtered. The filtrate was acidified with *d*-HCl and extracted with ether, and combined organic phase was dried over Na_2SO_4. Evaporation of the ether yielded a residue, which was methylated with CH_2N_2 to yield a fatty acid methyl ester and then analyzed by GC-MS. Peak 1 (t_R 5.088 min, pentadecanoic acid methyl ester, 3 %), EIMS *m/z* 256 $[M]^+$, 213 $[M - CH_3COO]^+$, 129, 74 $[CH_3OC(OH) = CH_2]^+$. Peak 2 (t_R 8.196 min, palmitic acid methyl ester, 97%), EIMS *m/z* 270 $[M]^+$, 239, 227 $[M - CH_3CO]^+$, 199, 185, 171, 143, 129, 74 $[CH_3OC(OH) = CH_2]^+$. The precipitate was recrystallized from MeOH-CH_2Cl_2 to give β-sitosterol glucoside which was identified by direct comparison with an authentic sample.



PL-1 [1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-(2'-hydroxypalmitoyl amino)-8-octadecene-1,3,4-triol] n= 6

PL-2 [1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-(2'-hydroxylignoceroyl amino)-8-octadecene-1,3,4-triol] n= 14

PL-3 [1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-(2'-hydroxydocosanoyl amino)-8-octadecene-1,3,4-triol] n= 12

PL-4 [1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-(2'-hydroxytricosanoyl amino)-8-octadecene-1,3,4-triol] n= 13

Fig. 1. Structures of the major cerebrosides from Paeoniae Radix.

Results and Discussion

The *n*-hexane fraction from the 70% EtOH extract of Paeoniae Radix was subjected to repeated silica gel and RP-18 column chromatography to give four cerebroside molecular species, PL-1, PL-2, PL-3 and PL-4 together with β-sitosterol and β-sitosterol glucoside 6'-*O*-palmitate, each showing a single spot on silica gel thin-layer chromatography. The structures of β-sitosterol and β-sitosterol glucoside 6'-*O*-palmitate were determined by direct comparisons with authentic samples (Jung, *et al.*, 2008). PL-1 exhibited strong hydroxyl, amide and glycosidic C-O and $(CH_2)_n$ absorptions in IR spectrum, and a pseudomolecular ion peak at *m/z* 754 $[M + Na]^+$ in the positive FAB mass spectrum, respectively. The NMR data of PL-1 indicated the presence of a sugar (δ_H 4.93, 1H, d, *J* = 7.5 Hz, anomeric H; δ_C 105.5), an amide linkage (δ_H 8.55, 1H, d, *J* = 9.0 Hz, N-H; δ_C 175.6) and two long chain aliphatic moieties which was essentially identical to those of aralia cerebroside (Kang, *et al.*, 1999), suggesting a glycosphingolipid nature. The structure was characterized by comparison of the ^{13}C-NMR spectral data with those of the known glucocerebrosides

(Jung *et al*, 1996; Kang *et al*., 1999; Kang *et al*., 2001; Ryu, *et al*., 2003), and by means of the results of the chemical degradation (Gaver and Sweeley, 1965). The PL-1 showed characteristic ^{13}C-NMR signals due to C-1 ~ C-4, C-1', C-2' and C-1" 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 5.26 and the carbon chemical shifts at δ 70.4 (C-1), 51.6 (C-2), 75.8 (C-3), 72.3 (C-4), 175.6 (C-1') and 72.3 (C-2') in PL-1 were virtually identical with those of the reported data of other (2*S*,3*S*,4*R*)-phytosphingosine moieties (Kang, *et al*., 1999; 2001). The positive FAB mass spectrum of PL-1 showed of an $[M + Na]^+$ ion peak at *m/z* 754 together with characteristic ions at *m/z* 570 $[(M + H) - 162]^+$, 552 $[M - 179]^+$, 500 [(M + Na) – fatty acid]$^+$, 316 [long-chain base + H]$^+$, and 298 $[(M + H) - 179 - 255]^+$ as shown in Fig. 2 (Falsone, *et al*., 1993; 1994). Methanolysis of PL-1 afforded a long-chain base together with methyl 2-hydroxypalmitate (98.3%) and very small amount of methyl palmitate (1.7%) by GC-MS analysis. The presence of a 2*S*,3*S*,4*R*,8*E*/*Z*-2-amino-1,3,4-trihydroxyoctadeca-8-ene long-chain base was confirmed by the direct comparison with an authentic sample obtained from *Phytolacca americana* (Kang, *et al*., 2001). Accordingly, the structure of PL-1 was identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-[2'-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol which was isolated from *Panax notoginseng* (Cho, *et al*., 2006). The *Z* (*cis*) isomer, 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*)-2-[2'-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol has been isolated from the *Phytolacca americana* as one of the major cerebrosides (Kang, *et al*., 2001) and *Sida spinosa* (Darwish and Reinecke, 2003), but the *E* (*trans*) isomer, 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*)-2-[2'-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol, has been found to be identical to aralia cerebroside isolated from the roots of *Aralia elata* (Kang, *et al*., 1999) and *Serratula chinensis* (Ling, *et al*., 2006).

The ^{1}H- and ^{13}C-NMR spectra of PL-2 were essentially identical with those of PL-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* 867 $[M + Na + H]^+$ together with ions at *m/z* 682 $[(M + H) - 162]^+$, 665 $[(M + H) - 179]^+$, 500 [(M + Na) – fatty acid]$^+$ and 316 [long-chain base + H]$^+$ were observed. These results are different by 112 (8 × CH_2) mass unit higher than PL-1. Therefore, PL-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, PL-2 gives methyl

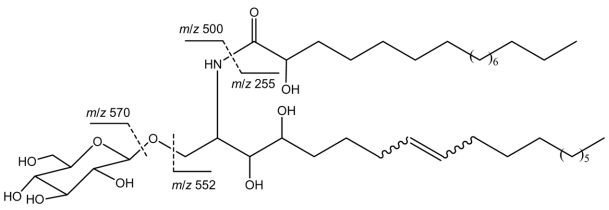


Fig. 2. Key FAB-MS fragmentation of PL-1.

2-hydroxytetracosanoate (85.9%) along with minor fatty acid methyl esters such as methyl palmitate (1.3%), methyl stearate (0.26%), methyl 2-hydroxytricosanoate (4.4%) and methyl 2-hydroxypentacosanoate (4.6%) and a mixture of stereoisomers (8*E* and 8*Z*) of (2*S*,3*S*,4*R*)-2-amino-1,3,4-trihydroxyoctadeca-8-ene upon methanolysis. Accordingly, the structures of the major components of PL-2 were identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*R*,8*E*/*Z*)-2-(2'-hydroxytetracosanoylamino)-8-octadecene-1,3,4-triol (longan cerebroside I) and its 8*Z* isomer (longan cerebroside II). This molecular species has recently been isolated from Longan Arillus (Ryu, *et al*., 2003) and *Vigna angularis* (Kojima, *et al*., 1998). The *Z* isomer of PL-2 has been isolated from the *Phytolacca americana* as one of the major cerebrosides (Kang, *et al*., 2001), *Euphorbia nicaeensis* (Cateni, *et al*., 2003a), *Euphorbia peplis* (Cateni, *et al*., 2003b) and *Desmodium gangeticum* (Mishra, *et al*., 2005), but the *E* isomer has been found to be identical to the cerebroside isolated from *Momordica charantia* (Xiao, *et al*., 2000) and *Cordia platythyrsa* (Tapondjou, *et al*., 2005).

The NMR and FAB-MS data of PL-3 and 4 were virtually identical with those of PL-1 and 2, suggesting only subtle differences in the chain length of the fatty acid moieties. The positive FAB-MS spectra of PL-3 and 4 showed pseudomolecular ions $[M + Na + H]^+$ at *m/z* 839 and 853, respectively, together with the common fragment ions such as $[(M + H) - 162]^+$, $[(M + H) - 179]^+$ and [(M + Na) – fatty acids]$^+$. Methanolysis of PL-3 afforded methyl palmitate (11.1%), methyl 2-hydroxydocosanoate (59%), methyl 2-hydroxytricosanoate (7.4%), methyl 2-hydroxytetracosanoate (12.6%), methyl 2-hydroxypentacosanoate (5.4%) and 2-hydroxyhexacosanoate (4.5%) by GC-MS analysis. The major fatty acid methyl ester was methyl 2-hydroxydocosanoate. Accordingly, the structure of the major cerebroside of PL-3 was identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-[2'-hydroxydocosanoylamino]-8-octadecene-1,3,4-triol. The *Z* (*cis*) isomer, 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*)-2-[2'-hydroxydocosanoylamino]-8-octadecene-1,3,4-triol has been isolated from *Phytolacca americana* as one of the major

cerebrosides (Kang, *et al*., 2001) and *Euphorbia nicaeensis* (Cateni, *et al*., 2003a). The *E* isomer has been isolated from *Serratula chinensis* (Ling, *et al*., 2006). Methanolysis of PL-4 afforded methyl palmitate (5.7%), methyl 2-hydroxydocosanoate (10.6%), methyl 2-hydroxytricosanoate (69.6%) and methyl 2-hydroxytetracosanoate (14.1%) by GC-MS analysis. The major component of a mixture of fatty acid methyl esters was methyl 2-hydroxytricosanoate. Accordingly, the structure of the major cerebroside of PL-4 was identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*/*Z*)-2-[2'-hydroxytricosanoylamino]-8-octadecene-1,3,4-triol. The *Z* (*cis*) isomer, 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*)-2-[2'-hydroxytricosanoylamino]-8-octadecene-1,3,4-triol has been isolated from the *Phytolacca americana* (Kang, *et al*., 2001). Occurrence of cerebrosides in *Paeonia* is reported here for the first time.

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