Natural Product Sciences
12(4) : 193-196 (2006)

Cerebrosides and Triterpenoids from the Roots of *Synurus deltoides*

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Abstract – A mixture of cerebrosides (**1**) and four triterpenoids (**2 - 5**) have been isolated from the hexane- and EtOAc-soluble fractions of the roots of *Synurus deltoides* (Ait.) Nakai (Compositae). Triterpenoid structures were determined as lupeol (**2**), β-amyrin (**3**), α-amyrin (**4**), and ursolic acid (**5**). Synurus cerebrosides (**1**) were characterized as a common long chain base (2*S*,3*S*,4*R*,8*E*)-2-amino-8-octadecene-1,3,4-triol and fatty acyl chains; palmitic acid, (2*R*)-2-hydroxybehenic acid, (2*R*)-2-hydroxytricosanoic acid, (2*R*)-2-hydroxylignoceric acid, (2*R*)-2-hydroxypentacosanoic acid, and (2*R*)-2-hydroxyhexacosanoic acid. The synurus cerebrosides (**1**) were the first isolation from a natural source.

Keywords – *Synurus deltoides*, cerebroside, sturcture determination, triterpenoid

Introduction

Synurus deltoides (Ait.) Nakai (Compositae) is an edible plant widely grown in Korea and China. Three species of the Synurus genus, *S. excelsus*, *S. deltoides*, and *S. palmatopinnatifidus* var. *indivisa*, are distributed widely in Korea (Lee, 1980). This plant is used in Korean and Chinese traditional medicine to treat cystitis, hematemesis, and edema (Nam *et al.*, 2004). Previous chemical and biological studies have been performed concerning Synurus genus. Ursolic acid and scopoletin from *S. deltoides* display an anti-inflammatory activity (Park *et al.*, 2004). Ham *et al.* (1997) showed that the extract of *S. deltoides* exhibited antimutagenicity *in vitro*. Nam *et al.* (2004) reported isolation of terpenoids, coumarins, and flavonoids from *S. excelsus*. As part of our continuing research to find pharmacologically active compounds from Compositae, we isolated four triterpenoids and a mixture of cereborosides from the hexane- and EtOAc-soluble fractions of the roots of *S. deltoides*. This paper deals with the structure elucidation of triterpenoids and new cerebrosides.

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Experimental

General experimental procedures – Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in $CHCl_3$ or MeOH. IR spectra were obtained on a JASCO FT/IR-100 spectrometer. UV spectra were recorded on a Shimadzu UV-2450 spectrometer. ^{1}H- and ^{13}C-NMR spectroscopic data were recorded on Bruker AMX 300 and 600 spectrometers. FAB-MS was measured on a JMS-HX 110/110A spectrometer (JEOL). GC/MS was performed with a GC/MS-QP5050 (Shimadzu); column: DB-5 (30 m × 0.32 mm); column temperature: 120 °C (5 min), increase 7 °C/min, 270 °C (14 min).

Plant material – The roots of *S. deltoides* were collected in August 2000, in Deogyusan, Jeollabuk-do Province, Korea. A voucher specimen (CNU00020) was deposited in the herbarium of the Chungnam National University, Korea.

Extraction and isolation – The dried roots (2.3 kg) of *S. deltoides* were extracted with MeOH (5 L × 3) by refluxing for 3 h to give 100 g of an extract. The MeOH extract was diluted with H_2O (1 L) and partitioned against hexane (1 L × 3) and EtOAc (1 L × 3), successively, to

give the hexane- (23 g) and EtOAc-soluble fractions (45 g), respectively. The hexane-soluble fraction was chromatographed on a silica gel (70 - 230 mesh) with a stepwise gradient of hexane and acetone as eluent to give twelve fractions (H.Fr. 1 - 12). The H.Fr. 5 was further chromatographed with a silica gel (hexane/acetone, 20 : 1) and a preparative HPLC on a RP C-18 (3.9 × 150 mm, 93% MeOH, flow rate 1.0 ml/min) to give **2** (20 mg, R_t 11.0 min), **3** (15 mg, R_t 16.0 min), and **4** (15 mg, R_t 18.5 min). The EtOAc-soluble fraction was chromatographed on a silica gel (70 - 230 mesh) with a stepwise gradient of $CHCl_3$ and MeOH as eluent to give ten fractions (E.Fr. 1 - 10). The E.Fr. 2 was further chromatographed on a silica gel with $CHCl_3$/MeOH (10 : 1) to give **5** (70 mg) and 1 (50 mg).

Synurus cerebrosides (1) – White amorphous powder; mp: 105 - 106 °C; $[\alpha]_D$ −58.3 (*c* 0.12, $CHCl_3$); UV λ_{max} nm (logε, MeOH): 204 (3.92); IR (KBr) ν_{max} cm^{-1}: 3350, 1630, 1540; FAB-MS *m/z*: 710 $[M+H]^+$ ($C_{44}H_{88}NO_5$), 696 $[M+H]^+$ ($C_{43}H_{86}NO_5$), 682 $[M+H]^+$ ($C_{42}H_{84}NO_5$), 668 $[M+H]^+$ ($C_{41}H_{82}NO_5$), 654 $[M+H]^+$ ($C_{40}H_{80}NO_5$), and 626 $[M+H]^+$ ($C_{40}H_{68}NO_4$). ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 8.56 (1H, d, J = 9.0 Hz, N-H), 5.51 (2H, m, H-8,9), 5.09 (1H, m, H-2), 4.60 (1H, m, H-2'), 4.49 (1H, dd, J = 10.7, 4.6 Hz, H-1a), 4.40 (1H, dd, J = 10.7, 4.9 Hz, H-1b), 4.32 (1H, m, H-3), 4.26 (1H, m, H-4), 1.25 and 1.33 $(CH_2)_n$, 0.86 (6H, t, J = 6.7 Hz, CH_3 × 2). ^{13}C-NMR (150 MHz, pyridine-d_5) δ: 175.4 (C-1'), 131.0 (C-8), 130.8 (C-9), 77.0 (C-3), 73.1 (C-4), 72.4 (C-2'), 62.2 (C-1), 53.1 (C-2), 35.8 (C-3'), 34.0 (C-5), 33.4 (C-7), 33.1 (C-10), 26.9 (C-6), 26.0 (C-4'), 14.4 (CH_3× 2).

Acid hydrolysis of 1 – Compound **1** (20 mg) was refluxed with 0.9 N HCl in 80% aqueous MeOH (10 ml) for 16 h (Kang *et al.*, 2001). The resulting solution was extracted with hexane, and evaporation of the hexane yielded a fatty acid methyl ester (11 mg). The H_2O layer was neutralized with 28% NH_4OH and extracted with ether. The ether fraction was concentrated to yield a long chain base. The fatty acid methyl ester analyzed by GC/MS. Peak 1 (t_R 14.7 min, palmitic acid methyl ester), EIMS *m/z*: 270 $[M]^+$, 227 $[M - CH_3CO]^+$, 199, 185, 143, 129, 101, 87, 74 $[CH_3OC(OH)=CH_2]^+$ (100), 55. Peak 2 (t_R 24.1 min, 2-hydroxybehenic acid methyl ester), EIMS *m/z*: 370 $[M]^+$, 352, 338, 311 $[M - CH_3COO]^+$, 292, 252, 159, 127, 111, 97, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69, 57 (100). Peak 3 (t_R 25.2 min, 2-hydroxytricosanoic acid methyl ester), EIMS *m/z*: 384 $[M]^+$, 325 $[M - CH_3COO]^+$, 306, 280, 159, 145, 127, 111, 97, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69, 57 (100). Peak 4 (t_R 26.3 min, 2-hydroxylignoceric acid methyl ester), EIMS *m/z*: 398 $[M]^+$, 339 $[M-CH_3COO]^+$, 320, 294, 159, 145, 127, 111, 97, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69, 57 (100). Peak 5 (t_R 27.4 min, 2-hydroxypentacosanoic acid methyl ester), EIMS *m/z*: 412 $[M]^+$, 353 $[M - CH_3COO]^+$, 334, 308, 174, 145, 127, 111, 97, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69, 57 (100). Peak 6 (t_R 28.6 min, 2-hydroxyhexacosanoic acid methyl ester), EIMS *m/z*: 426 $[M]^+$, 394, 367 $[M-CH_3COO]^+$, 348, 311, 285, 241, 195, 145, 127, 111, 97, 90 $[CH_3OC(OH)=CHOH]^+$, 83, 69, 57 (100). The long chain base was analyzed by EIMS (70 eV, rel. int.): 315 $[M]^+$ (0.2), 279 $[M - H_2O]^+$ (19), 261 $[M - 2H_2O]^+$ (1), 167 $[C_{12}H_{23}]^+$ (43), 149 (100), 113 (15), 71 (25), 31 (30). The long chain was identified as 2-amino-1,3,4-trihydroxy-8-octadecene by comparing their literature data with those previously reported (Kang *et al.*, 1999).

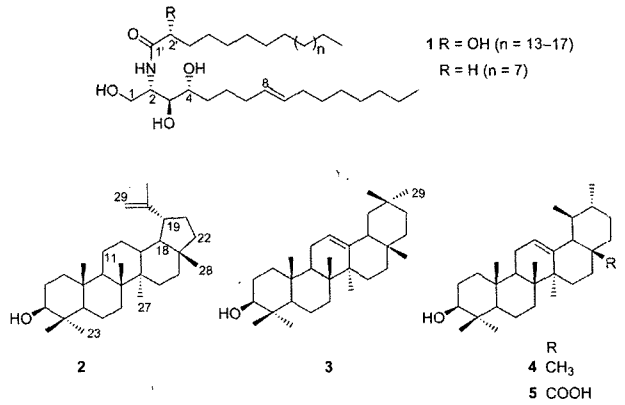

Fig. 1. The structures of the compounds from *S. deltoides*.

Lupeol (2) – White amorphous powder; mp: 215 °C; $[\alpha]_D$ +25.4 (*c* 0.2, MeOH); UV λ_{max} nm (logε, MeOH): 204 (3.88); IR (KBr) ν_{max} cm^{-1}: 3300, 2950, 1640, 1450, 1385; FAB-MS: 425.30 $[M - H]^-$; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.82, 0.88, 0.97, 1.03, 1.04, 1.23, 1.73 (each 3H, s, CH_3), 3.44 (1H, m, H-3), 4.72 (1H, s, H-29a), 4.87 (1H, s, H-29b); ^{13}C-NMR data: see Table 1.

β-Amyrin (3) – White amorphous powder; mp: 197 - 198 °C; $[\alpha]_D$ +88.3 (*c* 0.23, MeOH); UV λ_{max} nm (logε, MeOH): 204 (3.72); IR (KBr) ν_{max} cm^{-1}: 3300, 2950, 1460, 1380; FAB-MS: 425.29 $[M - H]^-$; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.90, 0.91, 0.92, 0.97, 1.01, 1.06, 1.21, 1.25 (each 3H, s, CH_3), 3.44 (1H, m, H-3), 5.25 (1H, t, J = 3.5 Hz, H-12); ^{13}C-NMR data: see Table 1.

α-Amyrin (4) – White amorphous powder; mp: 186 - 188 °C; $[\alpha]_D$ +82.4 (*c* 0.33, MeOH); UV λ_{max} nm (logε, MeOH): 204 (3.72), 244 (3.29); IR (KBr) ν_{max} cm^{-1}: 3300, 2920, 1470, 1380; FAB-MS: 425.30 $[M - H]^-$; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.81, 0.89, 0.91, 0.98, 1.05, 1.06, 1.16, 1.25 (each 3H, s, CH_3), 3.45 (1H, m, H-3), 5.21 (1H, t, J = 3.5 Hz, H-12); ^{13}C-NMR data: see

Table 1. ^{13}C-NMR spectroscopic data of compounds **2** - **5** (75 MHz, Pridine-d_5)

carbon	2	3	4	5
1	39.5	39.3	39.5	39.1
2	28.0	28.3	27.1	27.1
3	78.3	78.3	78.3	79.1
4	39.7	39.6	39.6	37.3
5	56.0	55.9	56.0	55.7
6	19.0	19.0	19.0	18.7
7	34.9	33.2	33.5	33.4
8	41.3	40.3	40.5	39.0
9	51.0	48.2	48.3	48.0
10	37.7	37.4	37.4	39.9
11	21.3	24.1	23.9	23.6
12	25.8	121.6	125.1	125.9
13	38.5	145.4	140.1	138.6
14	43.2	42.1	42.5	42.4
15	28.5	26.7	28.5	28.4
16	36.0	27.3	27.1	24.6
17	43.4	32.9	34.2	48.1
18	48.8	47.7	59.5	53.3
19	48.2	47.3	40.0	39.5
20	151.2	31.4	40.1	39.3
21	30.0	35.1	31.6	31.0
22	41.3	37.6	42.0	37.2
23	28.8	28.8	29.1	28.3
24	16.4	15.9	16.1	15.6
25	16.6	16.7	16.8	15.8
26	16.5	17.2	17.3	17.2
27	14.9	26.3	23.7	23.8
28	18.3	28.9	29.0	180.9
29	110.1	33.6	17.9	17.2
30	19.6	24.0	21.7	21.3

Table 1.

Ursolic acid (5) – White amorphous powder; mp: 287 - 288 °C; $[\alpha]_D$ +66.4 (*c* 0.33, MeOH); UV λ_{max} nm (logε, MeOH): 204 (3.91); IR (KBr) ν_{max} cm^{-1}: 3300, 2920, 1470, 1380; FAB-MS: 425.30 [M - H]$^-$; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.77 - 1.09 (each 3H × 7, tertiary and secondary methyl), 3.18 (1H, t, J = 7.5 Hz, H-3), 5.23 (1H, t, J = 3.5 Hz, H-12); ^{13}C-NMR data: see Table 1.

Results and Discussion

Repeat column chromatography of the hexane- and EtOAc-soluble fractions of the MeOH extract of the roots of *S. deltoides* led to the isolation of four triterpenoids and a mixture of cerebrosides. Four triterpenoids were identified as lupeol (**2**) (Lee and Lee, 1999), β-amyrin (**3**) (Park *et al.*, 2004), α-amyrin (**4**) (Park *et al.*, 2004), and ursolic acid (**5**) (Min *et al.*, 2000).

Compound **1** was obtained as amorphous powder and a series of quasimolecular ion peaks were observed at *m/z* 710, 696, 682, 668, 654, and 626. The NMR data of **1** showed the characteristic signals for 2-amino-1,3,4-triol in hydrocarbon chain at δ_H 4.26 (1H, m), 4.32 (1H, m), 4.40 (1H, dd, J = 10.7, 4.6 Hz), 4.49 (1H, dd, J = 10.7, 4.6 Hz) and 5.09 (1H, m), and at δ_C 53.1, 62.2, 73.1 and 77.0. In addition, the ^{1}H-NMR spectrum showed an amide linkage at δ 8.56 (1H, d, J = 9.0 Hz), two olefinic signals at δ 5.51 (2H, m), and two long chain aliphatic moieties. This observation was further supported by the ^{13}C-NMR spectral assignments; a carbonyl carbon at δ 175.4, two olefinic carbons at δ 131.0 and 130.8, and two terminal methyl groups in aliphatic hydrocarbon chains at δ 14.4, which were assignable to a sphingolipid, compared with those of cerebrosides isolated from Phytolaccae Radix and *Aster scaber* (Kang *et al.*, 2001; Kwon *et al.*, 2003). The acid hydrolysis of **1** yielded a mixture of fatty acid methyl esters and a long chain base. The fatty acid methyl esters were identified as methyl palmitate (6.4%), 2-hydroxybehenic acid (6.4%), 2-hydroxytricosanoic acid (12.3%), 2-hydroxylignoceric acid (45.0%), 2-hydroxypentacosanoic acid (20.8%), and 2-hydroxyhexacosanoic acid (9.0%) by GC/MS analysis. The absolute configuration at C-2 of 2-hydroxy fatty acid was determined to be *R* from the specific rotation (−58.3°) (Shibuya *et al.*, 1990). The presence of an unsaturated C18 long chain 2-amino-1,3,4-triolglyceride was deduced from the ^{1}H-^{1}H COSY-NMR spectrum and MS data. The signal at δ 8.56 (N-H) was coupled to a methine proton at δ 5.09 (H-2) in the ^{1}H-^{1}H COSY spectrum, which in turn, were coupled to a methylene protons at δ 4.49 and 4.40 (H_2-1), and 4.32 (H-3). The latter signal was further coupled to another methine proton at δ 4.26 (H-4). The H-2 chemical shift and the ^{13}C chemical shifts of C-1 (δ 62.2), C-2 (δ 53.1), C-3 (δ 77.0), C-4 (δ 73.1), C-1' (δ 175.4), and C-2' (δ 72.4) were very similar to (2*S*,3*S*,4*R*)-*N*-2-sphingosine skeleton, compared with those of poke-weed cerebrosides isolated from Phytolaccae Radix (Kang *et al.*, 2001). This result supported that the 1,3,4-trihydroxy phytosphingosine moiety in **1** possessed the 2*S*,3*S*,4*R* configuration. The *trans* (*E*) geometry of a double bond of long chain base was confirmed by the ^{13}C-NMR chemical shifts of the carbons next to the double bond at δ 33.4 (C-7) and 33.1 (C-10) in **1** (Inagaki *et al.*, 1998). On the basis of the above findings, synurus cerebroside (**1**) was determined to be (2*S*,3*S*,4*R*,8*E*)-2-(palmitoylamino)-8-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*E*)-2-[(2*R*)-2-hydroxybehenoylamino]-8-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*E*)-2-[(2*R*)-2-hydroxytricosanoylamino]-8-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*E*)-2-[(2*R*)-2-hydroxylignoceroylamino]-8-octadecene-1,3,4-triol, (2*S*,3*S*,4*R*,8*E*)-2-[(2*R*)-2-hydroxypentacosanoylamino]-8-octadecene-1,3,4-triol, and (2*S*,3*S*,4*R*,8*E*)-2-[(2*R*)-2-hydroxyhexacosanoylamino]-8-octadecene-1,3,4-triol.

Acknowledgements

This research was supported by a grant (PF0320302-00) from the Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science. We are grateful to KBSI for ^{1}H and ^{13}C NMR spectral measurements. The authors would like to thank Dr. Kim, J. C. Biofunction Research Team, Korea Research Institute of Chemical Technology, Korea, for his instrumental assistance in fatty acid methyl ester determination.

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(Accepted October 4, 2006)