Natural Product Sciences
7(2) : 27~32 (2001)

Isolation of Soya-cerebroside I from the Roots of *Trichosanthes kirilowii*

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Abstract – In addition to known cucurbitacins, a glucosphingosine type cerebroside and amino acids were isolated from the roots of *Trichosanthes kirilowii*. The structure of cerebroside was determined as soya-cerebroside I by means of spectroscopic methods. Fifteen amino acids were identified as aspartic acid, glutamic acid, serine, glycine, histidine, citrulline, threonine, alanine, proline, tyrosine, valine, isoleucine, leucine, phenylalanine and tryptophan, among which the major components such as citrulline, phenylalanine, leucine/isoleucine and valine were isolated.

Key words – *Trichosanthes kirilowii,* Cucurbitaceae, cerebroside, soya-cerebroside I, amino acids.

Introduction

The roots of *Trichosanthes kirilowii* Maxim. (Cucurbitaceae) have been used for regulation of water balance and for pyretolysis: and the seeds of this plant, on the other hand, have been used as an anti-inflammatory agent, a cough medicine and an expectorant (Namba, 1980; Kim, 1992). The earlier reports on the seeds of this plant deal with the isolation of a variety of substances, including lipids (Huang *et al.*, 2000), sterols, triterpenoids (Akihisa *et al.*, 1988; 1992a, 1992b, 1994a, 1994b; Kimura *et al.*, 1995, 1997; Chao *et al.*, 2000), and its glycosides (Chao *et al.*, 2000). However, a limited number of compounds such as fatty acids, sterols and derivatives (Kanaoka *et al.*, 1982), cucurbitacines (Ryu *et al.*, 1994) and amino acids (Murakami *et al.*, 1965) have been reported from roots. The present study deals with a reinvestigation on the roots of this plant and reports the isolation of a cerebroside and amino acids.

Materials and Methods

General experimental procedures – Melting points were uncorrected. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer in KBr method. EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. The FAB-MS spectra were obtained in a 3-nitrobenzyl alcohol matrix in a positive mode on a VG-VSEQ spectrometer. NMR spectra were measured either on a Varian Gemini 2000 (300 MHz) or a Bruker AMX-500 (500 MHz) instrument, and the chemical shifts were referenced to TMS. TLC was performed on silica gel $60F_{254}$ (Merck).

Plant material – The roots of *Trichosanthes kirilowii* Maxim. were purchased from a crude drug store in Seoul and authenticated by Dr. H.J. Chi. A voucher specimen (SSK98005) was deposited in the herbarium of the Natural Products Research Institute, Seoul National University.

Extraction and isolation – The dry roots (7.5 kg) of *T. kirilowii* were refluxed three times with MeOH in a water bath. The MeOH extract was evaporated to dryness, and the dry residue was partitioned in succession between H_2O and hexane, CH_2Cl_2, EtOAc and then BuOH affording 32.0 g, 9.9 g, 4.9 g, 34.7 g and 43.7 g of the respective extracts. A portion of the CH_2Cl_2 fraction (9 g) was subjected to silica gel column (4 × 70 cm) chromatography. Elution with CH_2Cl_2-MeOH-H_2O (7:1:0.5, 7:2:0.5 and then 7:3:1) gave 18 subfractions (TC-01~18). The subfraction Nos. TC-07 and 10 were further purified by recrystallization from hexane-EtOAc to give **1** and **2**, respectively. Subfraction No. TC-02 (1.2 g) was rechromatographed over silica gel using hexane-EtOAc (gradient, 1% → 25%) as eluant to yield 19 subfractions (TC-02-01~19). Subfraction No. TC-02-07 was recrystallized from hexane-EtOAc to yield **3**. Subfraction No. TC-02-13 (70 mg) was further chromatographed over silica gel using benzene-EtOAc (3:1) to yield 8 subfractions (TC-02-13-01 ~ 13). Subfraction Nos. TC-02-13-02, -04 and -07

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were recrystallized from hexane-EtOAc to give **4**, **5** and **6**, respectively. The BuOH fraction (7.7 g) was subjected to MCI gel column chromatography. Elution with H_2O, H_2O-MeOH (9:1) and then MeOH gave 10 subfractions (TB-01~10). A small amount of the subfraction No. TB-03 (3 mg) was derivatized as described by Cohen and Strydom (1988) and Heinrikson and Meredith (1984) to give the phenylisothiocynate-derivatized amino acids and then analyzed by using Waters PicoTag System as described by Cohen and Strydom (1988). The amino acid composition thus obtained was shown in Table 1. A portion of the subfraction No. TB-03 (3 g) was rechromatographed over silica gel using CH_2Cl_2-MeOH-H_2O (7:1:0.5, 15:3:1 and then 7:2:0.5) as eluant to yield 17 subfractions (TB-03-01~17). Subfraction No. TB-03-04 was recrystallized from MeOH-H_2O to yield **7** (6 mg). Subfraction Nos. TB-03-06, TB-03-10 and TB-03-06-14 were recrystallized in the same manner as described above to give **8** (1 mg), **9** (8 mg) and **10** (3 mg), respectively.

Compound 1: colorless plate. mp 275~278°C; IR, ν_{max} (KBr) 3424 (OH), 1638 (C=C), 1165, 1074, 1032 (glycosidic C-O), 619 cm^{-1}; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.56 (3H, s, 18-CH_3), 0.71 (3H, s, 19-CH_3), 4.41 (1H, dd, *J* = 5.1, 11.7 Hz, H-6′), 4.59 (1H, dd, *J* = 2.4, 11.7 Hz, H-6′), 5.04 (1H, d, *J* = 7.8 Hz, H-1′), 5.05 (dd, *J* = 8.7, 15.0 Hz, H-23), 5.16 (1H, br s. H-7) 5.20 (dd, *J* = 8.7, 15.0 Hz, H-22); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 37.3 (C-1), 29.5 (C-2), 78.5 (C-3), 34.7 (C-4), 40.2 (C-5), 30.0 (C-6), 117.8 (C-7), 139.6 (C-8), 49.6 (C-9), 34.5 (C-10), 23.4 (C-11), 39.8 (C-12), 43.6 (C-13), 55.2 (C-14), 23.3 (C-15), 28.3 (C-16), 56.3 (C-17), 12.2 (C-18), 13.1 (C-19), 41.1 (C-20), 21.8 (C-21), 138.7 (C-22), 129.6 (C-23), 51.4 (C-24), 32.2 (C-25), 21.3 (C-26), 19.3 (C-27), 25.3 (C-28), 12.1 (C-29), 102.3 (C-1′), 75.4 (C-2′), 77.1 (C-3′), 71.8 (C-4′), 78.7 (C-5′), 62.9 (C-6′); EI-MS, *m/z* 414, 412 (aglycone)$^+$, 399, 397, 273, 271, 255, 229, 213, 203, 173, 147, 133.

Compound 2: amorphous powder, mp 180~183 °C; IR, ν_{max} (KBr) 3368 (OH, NH_2), 2920, 2851 (CH), 1647 (amide), 1541, 1468, 1080, 1047 (glycosidic C-O), 965 (*trans* C=C), 721 [$(CH_2)_n$] cm^{-1}; ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.86 (6H, t-like, *J* = 7.1 Hz, CH_3), 1.26 [br s, $(CH_2)_n$], 1.99~2.03 (1H, m, H-7), 2.13~2.18 (2H, m, H-6, 10), 3.89 (1H, m, H-5″), 4.01 (1H, dd, *J* =7.8, 9.0 Hz, H-2″), 4.17 (1H, m, H-3″), 4.20 (1H, m, H-4″), 4.23 (1H, dd, *J* = 3.9, 10.5 Hz, H-1), 4.33 (1H, dd, *J* = 5.4, 11.8 Hz, H-6″), 4.49 (1H, dd, *J* = 2.5, 11.8 Hz, H-6″), 4.57 (1H, dd, *J* = 3.8, 8.0 Hz, H-2′), 4.69 (1H, dd, *J* = 5.8, 10.5 Hz, H-1), 4.75 (1H, t, *J* = 6.1 Hz, H-3), 4.79 (1H, m, H-2), 4.90 (1H, d, *J* = 7.8 Hz, H-1″), 5.48 (2H, t-like, H-8, 9), 5.92 (1H, dt, *J* = 5.8, 15.4 Hz, H-5), 5.98 (1H, dd, *J* = 5.8, 15.4 Hz, H-4), 8.32 (1H, d, *J* = 8.8 Hz, NH); ^{13}C-NMR (125.8 MHz, pyridine-d_5) δ: 70.2 (C-1), 54.6 (C-2), 72.5 (C-3), 132.0 (C-4), 132.2 (C-5), 33.0 (C-6*), 32.8 (C-7), 130.0 (C-8), 131.2 (C-9), 32.9 (C-10*), 175.7 (C-1′), 72.6 (C-2′), 35.7 (C-3′), 32.2 (C-14′), 105.7 (C-1″), 75.2 (C-2″), 78.5 (C-3″), 71.6 (C-4″), 78.6 (C-5″), 62.7 (C-6″), 14.3 (C-18, 16′), 23.0 (C-15′), 25.9, 29.6, 29.7, 29.9, 30.0, 30.1 (all CH_2) (*may be interchangeable); HRFABMS *m/z* 736.5344 [M + Na]$^+$ (Calcd for $C_{40}H_{75}NO_9$ + Na, 736.5340), *m/z* 482.3095 [long-chain base + glc + Na]$^+$ (Calcd for $C_{24}H_{45}NO_7$ + Na, 482.3094).

Compound 3: mp 220~223°; IR, ν_{max} (KBr) 3459 (OH), 1721 (OAc), 1696 (C=O), 1372 (CH_3), 1260 (OAc) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 1.18 (3H, s, 29-CH_3), 1.27 (3H, s, 28-CH_3), 1.33 (3H, s, 30-CH_3), 1.40 (3H, s, 21-CH_3), 1.43 (3H, s, 26-CH_3), 1.45 (3H, s, 27-CH_3), 1.95 (3H, s, OAc), 3.12 (1H, d, *J* = 14.7 Hz, H-12α), 3.90 (1H, br s, H-3), 4.30 (1H, t, *J* = 7.5 Hz, H-16), 5.94 (1H, d, *J* = 5.4 Hz, H-6); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 38.8 (C-1), 210.6 (C-2), 80.2 (C-3), 46.7 (C-4), 138.2 (C-5), 121.8 (C-6), 23.8 (C-7), 42.6 (C-8), 48.3 (C-9), 36.2 (C-10), 211.8 (C-11), 48.6 (C-12), 48.2 (C-13), 50.6 (C-14), 45.4 (C-15), 70.9 (C-16), 57.7 (C-17), 20.0 (C-18), 18.6 (C-19), 78.9 (C-20), 24.4 (C-21), 213.9 (C-22), 30.6 (C-23), 34.7 (C-24), 81.2 (C-25), 26.1 (C-26), 25.8 (C-27), 20.9 (C-28), 24.1 (C-29), 19.8 (C-30), 170.3, 22.4 (OAc); EI-MS, *m/z* 482 [M - (HOAc + H_2O)]$^+$, 403, 385, 369, 113 (100%), 95, 69.

Compound 4: colorless rods, mp 230~233°; IR, ν_{max} (KBr) 3567, 3461 (OH), 1721 (OAc), 1696 (C=O), 1628 (C=C), 1458, 1373 (CH_3), 1260 (OAc), 1128, 1059, 1022, 990, 619 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 1.18 (3H, s, 29-CH_3), 1.26 (3H, s, 28-CH_3), 1.33 (3H, s, 30-CH_3), 1.42 (3H, s, 21-CH_3), 1.54, 1.56 (6H, s, 26,27-CH_3), 2.00 (3H, s, OAc), 2.45 (1H, d, *J* = 6.9 Hz, H-17), 2.64 (1H, d, *J* = 14.7 Hz, H-12β), 3.11 (1H, br d, *J* = 14.7 Hz, H-12α), 3.90 (1H, s, H-3), 4.33 (1H, m, H-16), 5.95 (1H, m, H-6), 6.43 (1H, d, *J* = 15.6 Hz, H-23), 7.05 (1H, d, *J* =

15.6 Hz, H-24); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 38.8 (C-1), 210.6 (C-2), 80.2 (C-3), 46.7 (C-4), 138.1 (C-5), 121.9 (C-6), 23.9 (C-7), 42.7 (C-8), 48.4 (C-9), 36.3 (C-10), 211.8 (C-11), 48.6 (C-12), 47.9 (C-13), 50.6 (C-14), 45.3 (C-15), 71.3 (C-16), 58.1 (C-17), 19.9 (C-18), 18.8 (C-19), 78.1 (C-20), 23.8 (C-21), 202.4 (C-22), 120.2 (C-23), 152.0 (C-24), 79.3 (C-25), 26.4 (C-26), 25.9 (C-27), 21.0 (C-28), 24.1 (C-29), 20.1 (C-30), 170.2, 21.9 (OAc); EI-MS, *m/z* 498 [M - HOAc]$^+$, 480, 455, 403, 385, 369, 111, 105, 96 (100%), 69.

Compound 5: mp 178~179°; IR, ν_{max} (KBr) 3447 (OH), 1721 (OAc), 1696 (C=O), 1628 (C=C), 1458, 1372 (CH_3), 1262 (OAc), 1127, 1094, 1057, 1022, 990, 617 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3),1.08 (3H, s, 29-CH_3), 1.31 (3H, s, 30-CH_3), 1.38 (3H, s, 28-CH_3), 1.41 (3H, s, 21-CH_3), 1.53, 1.55 (6H, s, 26,27-CH_3), 2.01 (3H, s, OAc), 2.65 (1H, d, J = 14.4 Hz, H-12β), 3.12 (1H, br d, J = 14.4 Hz, H-12α), 4.11 (1H, br s, H-3), 4.34 (1H, m, H-16), 5.93 (1H, m, H-6), 6.45 (1H, d, J = 15.6 Hz, H-23), 7.05 (1H, d, J = 15.6 Hz, H-24); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 32.2 (C-1), 211.0 (C-2), 79.4 (C-3), 40.8 (C-4), 139.9 (C-5), 122.0 (C-6), 23.9 (C-7), 42.4 (C-8), 48.2 (C-9), 36.4 (C-10), 212.4 (C-11), 48.7 (C-12), 47.9 (C-13), 50.5 (C-14), 45.5 (C-15), 71.3 (C-16), 58.1 (C-17), 19.0 (C-18), 18.5 (C-19), 78.1 (C-20), 23.7 (C-21), 202.4 (C-22), 120.3 (C-23), 151.9 (C-24), 79.3 (C-25), 26.4 (C-26), 25.9 (C-27), 24.4 (C-28), 27.6 (C-29), 19.9 (C-30), 170.3, 21.9 (OAc); EI-MS, *m/z* 498 [M - HOAc]$^+$, 455, 403, 385, 369, 113, 96 (100%), 69.

Compound 6: colorless needles. mp 173~174°; IR, ν_{max} (KBr) 3447 (OH), 1721 (OAc), 1696 (C=O), 1626 (C=C), 1460, 1373 (CH_3), 1256 (OAc), 1128, 1059, 1022, 986, 617 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.98 (3H, s, 18-CH_3), 1.08 (3H, s, 30-CH_3), 1.28 (3H, s, 29-CH_3), 1.34 (3H, s, 28-CH_3), 1.36 (3H, s, 21-CH_3), 1.44 (3H, s, 19-CH_3), 1.56 (6H, s, 26,27-CH_3), 2.01 (3H, s, OAc), 2.69 (1H, d, J = 14.1 Hz, H-12β), 3.24 (1H, br d, J = 14.1 Hz, H-12α), 4.32~4.44 (2H, m, H-2,16), 5.79 (1H, m, H-6), 6.47 (1H, d, J = 15.6 Hz, H-23), 7.06 (1H, d, J = 15.6 Hz, H-24); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 36.0 (C-1), 71.6 (C-2), 212.1 (C-3), 50.2 (C-4), 140.4 (C-5), 120.3 (C-6), 23.9 (C-7), 42.3 (C-8), 48.4 (C-9), 33.7 (C-10), 213.1 (C-11), 48.6 (C-12), 48.1 (C-13), 45.3 (C-15), 50.7 (C-14), 71.3 (C-16), 58.2 (C-17), 17.8 (C-18), 18.9 (C-19), 78.2 (C-20), 23.8 (C-21), 202.4 (C-22), 120.4 (C-23), 152.0 (C-24), 79.3 (C-25), 26.4 (C-26), 25.9 (C-27), 21.2 (C-28), 29.3 (C-29), 20.0 (C-30), 170.3, 21.9 (OAc); EI-MS, *m/z* 498 [M - HOAc]$^+$, 480, 455, 403, 385, 369, 113, 96 (100%), 69.

Compound 7: IR, ν_{max} (KBr) 3434, 3034, 2630 (NH_3^+), 1609 (NH_3^+), 1589 (COO^-), 1522 (NH_3^+), 1402, 1321, 739 (oop), 696 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) δ: 3.11 (1H, dd, J = 8.0, 14.4 Hz, CH_2), 3.28 (1H, dd, J = 5.1, 14.4 Hz, CH_2), 3.98 (1H, dd, J = 5.1, 8.0 Hz, CH), 7.30~7.45 (5H, m, aromatic H).

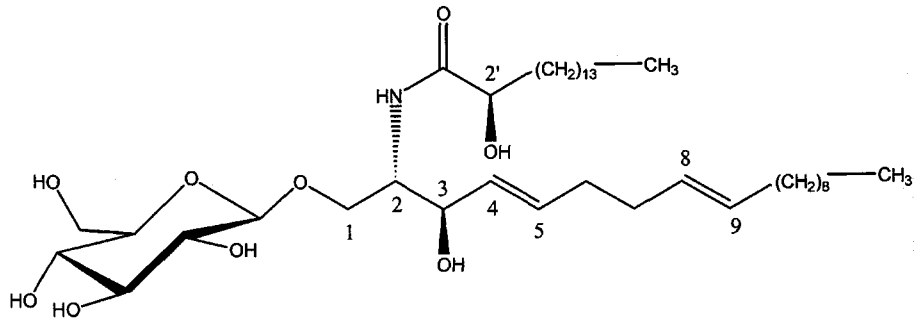
Compound 8: IR, ν_{max} (KBr) 3437, 2959 (NH_3^+), 1618 (NH_3^+), 1584 (COO^-), 1520 (NH_3^+), 1408 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) δ: 0.89~1.0 (CH_3), 1.62~1.75 (m, CH_2), 3.64 (d, J = 3.9 Hz, isoleucine C<u>H</u>NH_2). 3.70 (t, J = 5 Hz, leucine C<u>H</u>NH_2).

Compound 9: IR, ν_{max} (KBr) 3436, 2969, 2629 (NH_3^+), 1618 (NH_3^+), 1588 (COO^-), 1510 (NH_3^+), 1397 (CH_3), 1329 (CH), 775 (COO^-), 718 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) δ: 0.94 (3H, d, J = 6.9 Hz, CH_3), 0.99 (3H, d, J = 7.2 Hz, CH_3), 2.22 [1H, m, C<u>H</u>$(CH_3)_2$], 3.55 (1H, d, J = 4.2 Hz, CH).

Compound 10: IR, ν_{max} (KBr) 3436, 3358, 3113, 2956 (NH_3^+), 1649 (NH_3^+, amide), 1588 (COO^-, amide), 1414, 1350 (amide) cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) δ: 1.53 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.83 (2H, m, $C\underline{H}_2CH$), 3.10 (2H, t, J = 6.8 Hz, $NHC\underline{H}_2$), 3.71 (1H, t, J = 6 Hz, C<u>H</u>NH_2).

Results and Discussion

The CH_2Cl_2 fraction of MeOH extract from *T. kirilowii* was separated by silica gel column chromatography to afford a sterol glucoside (**1**), and a cerebroside (**2**), in addition to four known cucurbitacins (**3~6**). The structures of a mixture of α-spinasterol and Δ^7-stigmastenol glucosides (Woo and Kang, 1973; Gomes and Alegrio, 1998; Kanaoka *et al.*, 1982), 23,24-dihydroisocucurbitacin B (**3**), isocucurbitacin B (**4**), 3-epiisocucurbitacin B (**5**), and cucurbitacin B (**6**) (Ryu *et al.*, 1994; Kitajima *et al.*, 1989; Arisawa *et al.*, 1984; Monte *et al.*, 2000) were determined by a combination of spectroscopic analysis and comparison with reported data. Compound **2** was obtained as an amorphous powder. The molecular formula was established as $C_{40}H_{75}NO_9$ based on the molecular ion at *m/z* 736.5344 [M + Na]$^+$ in the high resolution FAB-MS. In the IR spectrum of **2**, strong absorption bands typical for hydroxyl, amide, glycosidic C-O, and $(CH_2)_n$ functionalities were observed. The NMR data of **2** indicated the presence



2 Soya-cerebroside I

of β-D-glucose (δ_H 4.90, 1H, d, J = 7.8 Hz, anomeric H; δ_C 105.7), an amide linkage (δ_H 8.32, 1H, d, J = 8.8 Hz, N-H; δ_C 175.7) and two long chain aliphatic moieties which was essentially identical to those of cerebrosides from *Arisaema amurense* (Jung *et al.*, 1996), suggesting a sphingosine-type cerebroside nature (Jung *et al.*, 1996; Inagaki *et al.*, 1998). The positive FAB-MS spectrum of **2** showed an ion [long-chain base + glucose + Na]$^+$ peak at *m/z* 482.3095 typical for amide bond cleavage in cerebrosides (Kang *et al.*, 2001; Inagaki *et al.*, 1998). Therefore, **2** was expected to be a sphingosine-type cerebroside having 2-hydroxypalmitic acid β-D-glucopyranose residue. The amide signal at δ 8.32 gave a cross peak with the H-2 multiplet signal at δ 4.79 in the ^{1}H-^{1}H COSY spectrum of **2**, which in turn showed cross peaks with methylene protons (H-1) at δ 4.23 and 4.69 and δ 4.75 (H-3). The latter correlated with two olefinic proton signals at δ 5.92 (H-5) and 5.98 (H-4). The double bond at C-4, 5 was found to be *trans* (*E*), as evidenced by the large coupling constant (J = 15.4 Hz). These results were in good agreement with those of known (2*S*,3*R*,4*E*)-sphingosine-type cerebrosides (Jung *et al.*, 1996; Inagaki *et al.*, 1998), which were further supported by the ^{13}C NMR data. The chemical shifts of three methylene carbons (C-6, 7 and 10) adjacent to the olefinic carbons were observed at δ 32.0~δ 33.0, supporting the *trans* (*E*) double bond at C-8 and 9 (Kang *et al.*, 2001; Inagaki *et al.*, 1998). The relative configurations of C-2, 3 and C-2′ of **2** were established on the basis of ^{13}C NMR data [δ 54.6 (C-2), 72.5 (C-3) and δ 72.5 (C-2′)], which were in good agreement with those published for 2*S*,3*R*,2′*R* configuration (Jung *et al.*, 1996; Inagaki *et al.*, 1998). In light of the above evidences, the structure of **2** was deduced to be 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*, 4*E*,8*E*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-4,8-octadecadiene-1,3-diol. This compound was found to be identical with the known soya-cerebroside I, which has been previously isolated from *Phaseolus angularis* (Ohnishi and Fujino, 1981), soybean (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), *Allium sativum* (Inagaki *et al.*, 1998), *Dimocarpus fumatus* (Voutquenne *et al.*, 1999). and *Momordica charantia* (Xiao *et al.*, 2000). This seems to be the first instance of the isolation of soya-cerebroside I from this plant. Murakami *et al.* (1965) reported the isolation of amino acids such as citrulline, arginine, glutamic acid and aspartic acid from this plant. Reinvestigation on the amino acid composition by reversed-phase HPLC analysis has resulted in the

Table 1. Amino acid composition of *Trichosanthes kirilowii*

Amino acid	Mole %	Amino acid	Mole %
Aspartic acid	0.52	Glutamic acid	1.54
Serine	1.29	Glycine	1.51
Histidine	0.94	Citrulline	43.29
Threonine	0.33	Alanine	12.14
Proline	3.84	Tyrosine	1.98
Valine	8.52	Isoleucine	11.41
Leucine	9.67	Phenylalanine	1.71
Tryptophan	1.33		

identification of 15 amino acids as shown in Table 1, among which phenylalanine (**7**), leucine/isoleucine (**8**), valine (**9**) and citrulline (**10**) were isolated and identified by spectroscopic means.

Acknowledgements

This work was partially supported by the Brain Korea 21 project. We are grateful to KBSI for NMR, HRFAB measurements and amino acid analysis.

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(Accepted April 13, 2001)