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Phytochemical Constituents of *Bistorta manshuriensis*

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Abstract – Phytochemical investigation of the MeOH extract of the aerial parts of *Bistorta manshuriensis* resulted in the isolation of two cerebrosides, two lactams, six phenolic compounds and seven flavonoids. Their chemical structures were characterized by spectroscopic methods to be pinelloside (**1**), soyacerebroside I (**2**), pterolactam (**3**), 5-hydroxypyrrolidine-2-one (**4**), vanillic acid (**5**), caffeic acid methyl ester (**6**), protocatechuic acid (**7**), caffeic acid (**8**), 3,5-di-*O*-caffeoyl quinic acid methyl ester (**9**), chlorogenic acid methyl ester (**10**), avicularin (**11**), afzelin (**12**), quercetin (**13**), isoorientin (**14**), quercetin 3-*O*-β-D-glucoside (**15**), quercitrin (**16**), and luteolin (**17**). The isolated compounds (**1 - 4**, **7**, **12**, **14**) were isolated for the first time from this plant source and the compounds **1 - 4**, **9** and **10** were first reported from the genus *Bistorta*. Compound **17** exhibited moderate cytotoxicity and compound **6** exhibited weak cytotoxicity against four human cancer cell lines *in vitro* using an SRB bioassay.
Keywords – *Bistorta manshuriensis*, Polygonaceae, Cerebrosides, Cytotoxicity

Introduction

Bistorta manshuriensis Komarov (Polygonaceae) is a perennial plant, which is widely distributed throughout Korea. The aerial parts of *B. manshuriensis* have been used in traditional Korean medicine for the treatment of bleeding and diarrhea (Lee, 2003). Triterpenes such as 24-methylenecycloartanone and 24(*E*)-ethylidenecycloartanone (Manoharan *et al*., 2005) as well as flavonoids such as catechin and rutin (Liu *et al*., 2006) were reported from this plant. As a part of our continuing search for bioactive constituents from Korean natural resources, we investigated constituents of the aerial parts of *B. manshuriensis*. As a result, we isolated two cerebrosides (**1 - 2**), two lactams (**3 - 4**), six phenolics (**5 -10**) and seven flavonoids (**11 - 17**) from the MeOH extract of the aerial parts of *B. manshuriensis*. All the isolated compounds were tested for their cytotoxic activities against four human cancer cell lines *in vitro* using an SRB bioassay.

Experimental

General – Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer. FAB-MS data were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and Alltech Silica 5 μ column (250 × 10 mm) or Econosil® RP-18 10 μ column (250 × 10 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography. TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) or LiChroprep Lobar®-A RP-18 (240 × 10 mm) columns with a FMI QSY-0 pump (Isco).

Plant materials – The aerial parts of *B. manshuriensis* (2.9 kg) were collected from Mt. Daeduk, Gangwon Province, Korea, in June 2008. A voucher specimen (SKKU-2008-6) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – The aerial parts of *B. manshuriensis* (2.9 kg) were extracted at room temperature with 80% MeOH three times and evaporated under reduced pressure to give a MeOH extract (219 g), which was dissolved in water (800 mL) and then

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successively partitioned with n-hexane, CH_2Cl_2, EtOAc, and n-BuOH yielding 10, 2, 9 and 32 g of residue, respectively. The n-hexane fraction (10 g) was separated over a silica gel column (n-hexane : EtOAc = 7 : 1 → 1 : 1) to yield nine fractions (H1 - H9). Fraction H9 (3 g) was further separated over a silica gel column ($CHCl_3$: MeOH = 10 : 1), separated over a Sephadex LH-20 column (CH_2Cl_2 : MeOH = 1 : 1) and purified with an RP-C_{18} prep. HPLC (Econosil® RP-18 10 μ column, 250 × 10 mm; 55% MeOH) to yield compounds **1** (13 mg, R_t = 19.0 min) and **2** (7 mg, R_t = 20.5 min). The CH_2Cl_2 fraction (2 g) was separated over a silica gel column with a solvent system of CH_2Cl_2 : MeOH (20 : 1 → 1 : 1) as the eluant to yield seven fractions (M1 - M7). Fraction M3 (531 mg) was separated over an RP-C_{18} silica gel column (30% MeOH) and purified with a silica gel prep. HPLC ($CHCl_3$: MeOH = 20 : 1) to give compounds **3** (10 mg, R_t = 25.5 min) and **4** (4 mg, R_t = 27.0 min), respectively. Similarly, Fraction M4 (435 mg) was separated over an RP-C_{18} silica gel column (50% MeOH) and purified by recrystallization using MeOH to give compound **5** (4 mg). Similarly, the EtOAc fraction (9 g) was separated over chromatographic methods (silica gel column, RP-C_{18} silica gel column, Sephadex LH-20 column and RP-C_{18} prep. HPLC) to afford compounds **5 - 16**. The n-BuOH fraction (32 g) was separated over an RP-C_{18} silica gel column (50% MeOH) to yield four fractions (B1 - B4). Fraction B3 (3.1 g) was purified with a Sephadex LH-20 column (90% MeOH) to afford compound **17** (13 mg).

Pinelloside (**1**) – White powder, mp 136 - 138 °C; $[\alpha]_D^{25}$: –13.7° (*c* 0.39, MeOH); FAB-MS *m/z* : 736 $[M + Na]^+$; IR ν_{max} (MeOH) : 3384, 2923, 2850, 1639, 1459, 1027, 723 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 5.76 (1H, dt, *J* = 15.0, 6.5 Hz, H-12), 5.50 (1H, br dt, *J* = 15.0, 6.5 Hz, H-11), 5.38 (1H, dd, *J* = 16.0, 5.5 Hz, H-4), 5.32 (1H, dt, *J* = 16.0, 6.5 Hz, H-5), 4.23 (1H, d, *J* = 7.5 Hz, H-1"), 4.18 (1H, br q, *J* = 5.5 Hz, H-3), 4.11 (1H, dd, *J* = 10.5, 5.5 Hz, H-1a), 4.01 (2H, m, H-2, 2'), 3.87 (1H, dd, *J* = 12.0, 6.0 Hz, H-6"a), 3.77 (1H, dd, *J* = 10.5, 4.0 Hz, H-1b), 3.69 (1H, dt, *J* = 12.0, 6.0 Hz, H-6"b), 3.40 (1H, td, *J* = 9.0, 4.0 Hz, H-3"), 3.34 (1H, td, *J* = 9.0, 4.0 Hz, H-4"), 3.28 (1H, m, H-5"), 3.23 (1H, ddd, *J* = 9.0, 7.5, 4.0 Hz, H-2"), 2.11 (4H, m, H-10, 13), 2.08 (2H, m, H-6), 2.05 (2H, m, H-3'), 1.40-1.35 (6H, m, H-7, 8, 9), 1.35 (28H, m, H-14-16, 4'-14'), 0.97 (6H, t, *J* = 7.0 Hz, H-18, 16'); ^{13}C-NMR (125 MHz, CD_3OD): δ 176.2 (C-1'), 133.3 (C-12), 132.1 (C-4, 11), 130.8 (C-5), 104.7 (C-1"), 77.3 (C-5"), 77.2 (C-3"), 73.8 (C-2"), 72.0 (C-2'), 71.7 (C-3), 70.5 (C-4"), 68.9 (C-1), 62.0 (C-6"), 54.0 (C-2), 34.8 (C-3'), 32.5 (C-6), 31.9 (C-10, 13, 14'), 29.6-26.7 (C-7-9, 14-16, 5'-13'), 25.0 (C-4'), 22.5 (C-17, 15'), 13.3 (C-18), 13.2 (C-16')

Soyacerebroside I (**2**) – White powder, mp 130 - 133 °C; $[\alpha]_D^{25}$: +11.8° (*c* 0.35, MeOH); FAB-MS *m/z* : 736 $[M + Na]^+$; IR ν_{max} (MeOH) : 3381, 2947, 2836, 1454, 1026, 724 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 5.74 (1H, br dt, *J* = 15.0, 6.0 Hz, H-5), 5.50 (1H, dd, *J* = 15.0, 7.0 Hz, H-4), 5.42 (2H, t, *J* = 4.0 Hz, H-8, 9), 4.27 (1H, d, *J* = 7.7 Hz, H-1"), 4.18 (1H, br t, *J* = 7.5 Hz, H-3), 4.11 (1H, dd, *J* = 10.0, 5.5 Hz, H-1b), 4.00 (1H, m, H- 2'), 3.98 (1H, ddd, *J* = 7.5, 5.0, 3.5 Hz, H-2), 3.81 (1H, dd, *J* = 12.0, 4.0 Hz, H-6"b), 3.69 (1H, dd, *J* = 10.0, 3.5 Hz, H-1a), 3.67 (1H, dd, *J* = 12.0, 5.1 Hz, H-6"a), 3.34 (1H, t, *J* = 9.0 Hz, H-3"), 3.28-3.31 (2H, m, H-4", 5"), 3.19 (1H, dd, *J* = 8.8, 7.7 Hz, H-2"), 2.11 (2H, m, H-7), 2.06 (2H, m, H-6), 1.97 (2H, m, H-10), 1.70 (1H, m, H-3'a), 1.58 (1H, ddd, *J* = 14.0, 8.0, 4.0 Hz, H-3'b), 1.42 (2H, m, H-4'), 1.28-1.31 (36H, m, H-11-17, 5'-15'), 0.90 (6H, t, *J* = 6.7 Hz, H-18, 16'); ^{13}C-NMR (125 MHz, CD_3OD): δ 177.2 (C-1'), 134.3 (C-5'), 131.1 (C-8), 130.8 (C-9), 129.8 (C-4), 104.0 (C-1"), 77.3 (C-3", 5"), 73.8 (C-2"), 72.0 (C-2'), 71.9 (C-3), 70.5 (C-4"), 68.9 (C-1), 62.0 (C-6"), 54.0 (C-2), 34.8 (C-3'), 32.5 (C-7), 32.4 (C-10), 32.1 (C-5, 16, 14'), 30.3-29.8 (C-11-15, 5'-13'), 23.2 (C-17, 4', 15'), 14.3 (C-18, 16')

Pterolactam (**3**) – Colorless gum; $[\alpha]_D^{25}$: +38.8° (*c* 0.115, MeOH); FAB-MS *m/z* : 116 $[M + H]^+$; IR ν_{max} (MeOH) : 3381, 2945, 2836, 1681, 1456, 1055, 1028 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 4.85 (1H, dd, *J* = 6.4, 1.2 Hz, H-5), 3.30 (3H, s, OCH_3), 2.45 (1H, m, H-3a), 2.25 (1H, m, H-4a), 2.14 (1H, m, H-3b), 2.00 (1H, m, H-4b); ^{13}C-NMR (125 MHz, CD_3OD): δ 181.6 (C-2), 88.9 (C-5), 54.8 (OCH_3), 30.3 (C-3), 29.5 (C-4)

5-Hydroxypyrrolidine-2-one (**4**) – Colorless gum; $[\alpha]_D^{25}$: +56.0° (*c* 0.075, MeOH); FAB-MS *m/z* : 102 $[M + H]^+$; IR ν_{max} (MeOH) : 3382, 2946, 2837, 1660, 1457 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 5.15 (1H, dd, *J* = 6.3, 1.7 Hz, H-5), 2.38 (1H, m, H-3a), 2.20 (1H, m, H-4a), 2.02 (1H, m, H-3b), 1.90 (1H, m, H-4b); ^{13}C-NMR (125 MHz, CD_3OD): δ 181.5 (C-2), 80.9 (C-5), 29.9 (C-4), 29.2 (C-3)

Vanillic acid (**5**) – Colorless gum; FAB-MS *m/z* : 168 $[M]^+$; IR ν_{max} (MeOH) : 3376, 2945, 2835, 1655, 1457, 1114, 683 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.58 (1H, d, *J* = 2.0 Hz, H-2), 7.54 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 6.82 (1H, d, *J* = 8.5 Hz, H-5), 3.89 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD): δ 167.6 (COOH), 151.4 (C-3), 147.5 (C-4), 124.1 (C-1), 122.9 (C-6), 116.5 (C-2), 114.6 (C-5), 55.2 (OCH_3)

Caffeic acid methyl ester (**6**) – Colorless gum; FAB-

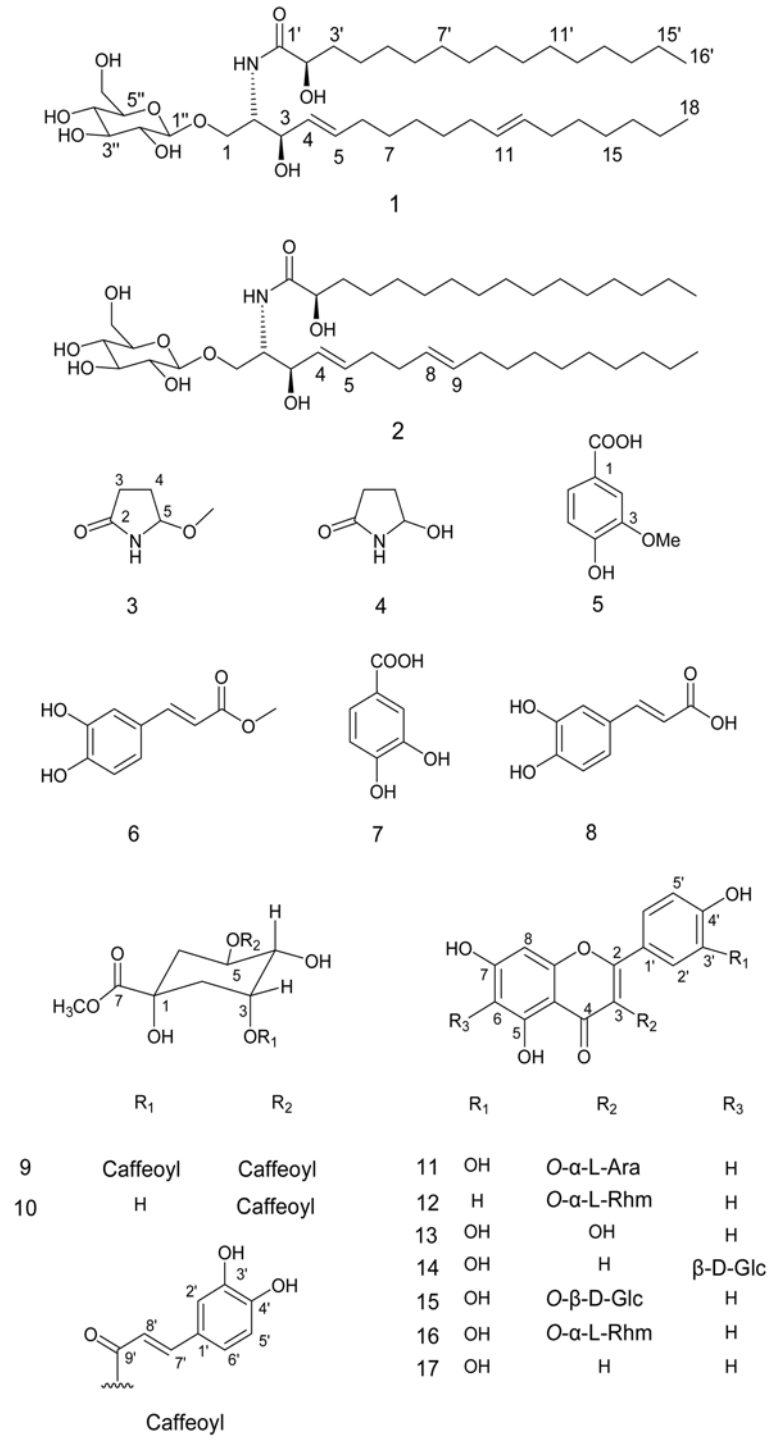

Fig. 1. The structures of **1** - **17** isolated from *B. manshuriensis*.

MS *m/z* : 194 $[M]^+$; IR ν_{max} (MeOH) : 3497, 3320, 2959, 1685, 1635, 1603, 1527, 1280, 1182, 1112, 853, 810 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.53 (1H, d, *J* = 15.5 Hz, H-7), 7.03 (1H, d, *J* = 2.0 Hz, H-2), 6.94 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.78 (1H, d, *J* = 8.0 Hz, H-5), 6.25 (1H, d, *J* = 15.5 Hz, H-8), 3.75 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD): δ 168.6 (C-9), 148.4 (C-4), 145.8 (C-3), 145.7 (C-7), 126.6 (C-1), 121.8 (C-6), 115.3 (C-5), 114.0 (C-2), 113.7 (C-8), 50.8 (OCH_3)

Protocatechuic acid (**7**) – Colorless gum; FAB-MS *m/*

z : 155 $[M + H]^+$; IR ν_{max} (MeOH) : 3381, 2945, 2836, 1681, 1456, 1055, 1028 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.49 (1H, d, *J* = 2.0 Hz, H-2), 7.40 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.80 (1H, d, *J* = 8.0 Hz, H-5); ^{13}C-NMR (125 MHz, CD_3OD): δ 169.0 (C-7), 150.4 (C-4), 144.9 (C-3), 122.8 (C-6), 122.0 (C-1), 116.6 (C-5), 114.6 (C-2)

Caffeic acid (**8**) – Colorless gum; FAB-MS *m/z* : 179 $[M - H]^-$; IR ν_{max} (MeOH) : 3383, 2946, 2836, 1659, 1431, 1280, 1055, 799 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.53 (1H, d, *J* = 16.0 Hz, H-7), 7.05 (1H, d, *J* = 1.5 Hz, H-2), 6.96 (1H, dd, *J* = 8.0, 1.5 Hz, H-6), 6.79 (1H, d, *J* = 8.0 Hz, H-5), 6.22 (1H, d, *J* = 16.0 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD): δ 169.0 (C-9), 148.3 (C-4), 145.8 (C-3), 145.6 (C-7), 126.7 (C-1), 121.7 (C-6), 115.3 (C-5), 114.3 (C-2), 113.9 (C-8)

3,5-Di-*O*-caffeoyl quinic acid methyl ester (**9**) – Brown gum; $[\alpha]_D^{25}$: –8.41° (*c* 0.01, MeOH); FAB-MS *m/z* : 531 $[M + H]^+$; IR ν_{max} (MeOH) : 3389, 2971, 1687, 1604, 1521, 1449, 1369, 1268, 1176, 814 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.61 (1H, d, *J* = 16.0 Hz, H-7"), 7.59 (1H, d, *J* = 16.0 Hz, H-7'), 7.06 (1H, d, *J* = 2.0 Hz, H-2"), 7.05 (1H, *J* = 2.0 Hz, H-2'), 6.96 (2H, d, *J* = 8.0 Hz, H-5', 5"), 6.78 (2H, dd, *J* = 8.0, 2.0 Hz, H-6', 6"), 6.33 (1H, d, *J* = 16.0, H-8"), 6.25 (1H, d, *J* = 16.0 Hz, H-8'), 5.39 (1H, m, H-5), 5.30 (1H, m, H-3), 3.97 (1H, dd, *J* = 6.5, 3.5 Hz, H-4), 3.70 (3H, s, OCH_3), 2.33 (1H, dd, *J* = 13.2, 3.2 Hz, H-2b), 2.31 (1H, dd, *J* = 13.8, 6.6 Hz, H-6a), 2.18 (1H, dd, *J* = 13.8, 3.9 Hz, H-6b), 2.14 (1H, dd, *J* = 13.2, 8.0 Hz, H-2a); ^{13}C-NMR (125 MHz, CD_3OD): δ 174.5 (COO), 168.7 (C-9"), 168.0 (C-9'), 148.6 (C-4"), 148.4 (C-4'), 146.3 (C-7"), 146.0 (C-7'), 145.7 (C-3"), 145.6 (C-3'), 126.7 (C-1"), 126.5 (C-1'), 121.9 (C-6"), 121.8 (C-6'), 116.5 (C-5"), 116.4 (C-5'), 115.2 (C-2"), 115.1 (C-2'), 114.3 (C-8"), 114.1 (C-8'), 73.5, (C-1), 71.1 (C-5), 71.0 (C-3), 70.8 (C-4), 51.9 (OCH_3), 35.5 (C-6), 34.5 (C-2)

Chlorogenic acid methyl ester (**10**) – Colorless gum; $[\alpha]_D^{25}$: –42.3° (*c* 0.175, MeOH); FAB-MS *m/z* : 391 $[M + Na]^+$; IR ν_{max} (MeOH) : 3389, 2972, 1732, 1521, 1370, 1275, 1056, 1012 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.53 (1H, d, *J* = 16.0 Hz, H-7'), 7.05 (1H, d, *J* = 1.5 Hz, H-2'), 6.96 (1H, dd, *J* = 8.0, 1.5 Hz, H-6'), 6.79 (1H, d, *J* = 8.0 Hz, H-5'), 6.22 (1H, d, *J* = 16.0 Hz, H-8'), 5.27 (1H, dd, *J* = 10.0, 4.5 Hz, H-3), 4.13 (1H, br d, *J* = 3.0 Hz, H-5), 3.73 (1H, m, H-4), 3.70 (3H, s, $COOCH_3$), 2.21 (1H, dd, *J* = 14.0, 3.0 Hz, H-2a), 2.08 (2H, m, H-2b, 6a), 1.99 (1H, br d, *J* = 14.0 Hz, H-6b); ^{13}C-NMR (125 MHz, CD_3OD): δ 174.3 (C-7), 167.1 (C-9'), 148.5 (C-4'), 146.0 (C-7'), 145.7 (C-3'), 126.5 (C-1'), 121.8 (C-6'), 115.4 (C-5'), 114.0 (C-8'), 113.9 (C-2'), 74.7 (C-1), 71.4 (C-3), 71.0 (C-4), 69.2 (C-5), 51.8 (OCH_3), 36.9 (C-6), 36.6 (C-2)

Avicularin (**11**) – Yellow powder, mp 180 - 183 °C; FAB-MS *m/z* : 435 $[M + H]^+$; IR ν_{max} (MeOH) : 3258, 1656, 1605, 1504, 1448, 1364, 1305, 1026, 824 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.62 (1H, s, 5-OH), 7.57 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.48 (1H, d, *J* = 2.0 Hz, H-2'), 6.85 (1H, d, *J* = 8.0 Hz, H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.59 (1H, d, *J* = 1.0 Hz, H-1"); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 178.4 (C-4), 164.1 (C-7), 161.9 (C-5), 157.6 (C-9), 157.1 (C-2), 149.2 (C-4'), 145.8 (C-3'), 134.1 (C-3), 122.4 (C-1'), 121.7 (C-6'), 116.3 (C-5'), 116.2 (C-2'), 108.6 (C-1"), 104.7 (C-10), 99.4 (C-6), 94.3 (C-8), 86.6 (C-4"), 82.8 (C-2"), 77.8 (C-3"), 61.4 (C-5")

Afzelin (**12**) – Yellow powder, mp 180 - 185 °C; FAB-MS *m/z* : 433 $[M + H]^+$; IR ν_{max} (MeOH) : 3354, 1656, 1605, 1504, 1450, 1365, 1305, 1025, 819 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.62 (1H, s, 5-OH), 7.76 (2H, d, *J* = 7.3 Hz, H-2', 6'), 6.92 (2H, d, *J* = 7.3 Hz, H-3', 5'), 6.50 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.29 (1H, br s, H-1"), 0.80 (3H, d, *J* = 6.0 Hz, H-6"); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 178.4 (C-4), 164.1 (C-7), 161.9 (C-5), 160.6 (C-4'), 158.0 (C-2), 157.2 (C-9), 134.9 (C-3), 131.3 (C-2, 6'), 121.7 (C-1'), 116.3 (C-3', 5'), 104.7 (C-10), 102.5 (C-1"), 99.4 (C-6), 94.3 (C-8), 71.8 (C-4"), 71.3 (C-5"), 71.0 (C-3"), 70.8 (C-2"), 18.2 (C-6")

Quercetin (**13**) – Yellow powder, mp 183 - 190 °C; FAB-MS *m/z* : 303 $[M + H]^+$; IR ν_{max} (MeOH) : 3395, 2946, 2835, 1658, 1609, 1522, 1452, 1025, 823 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.48 (1H, s, 5-OH), 7.68 (1H, d, *J* = 1.5 Hz, H-2'), 7.53 (1H, dd, *J* = 1.5, 8.5 Hz, H-6'), 6.88 (1H, d, *J* = 8.5 Hz, H-5'), 6.40 (1H, d, *J* = 1.5 Hz, H-8), 6.20 (1H, d, *J* = 1.5 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 176.5 (C-4), 164.6 (C-7), 161.4 (C-5), 156.8 (C-9), 148.4 (C-4'), 147.5 (C-2), 145.8 (C-3'), 136.4 (C-3), 122.7 (C-1'), 120.7 (C-6'), 116.3 (C-5'), 115.8 (C-2'), 103.7 (C-10), 98.9 (C-6), 94.1 (C-8)

Isoorientin (**14**) – Yellow powder, mp 180 - 190 °C; FAB-MS *m/z* : 449 $[M + H]^+$; IR ν_{max} (MeOH) : 3387, 2974, 1653, 1456, 1357, 1302, 1054, 1025, 823 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 13.56 (1H, s, 5-OH), 7.43 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 7.42 (1H, d, *J* = 2.0 Hz, H-2'), 6.89 (1H, d, *J* = 8.0 Hz, H-5'), 6.67 (1H, d, *J* = 2.0 Hz, H-3), 6.47 (1H, d, *J* = 2.0 Hz, H-8), 4.59 (1H, d, *J* = 10.0 Hz, H-1"), 4.10 (1H, t, *J* = 8.0 Hz, H-2"); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 182.6 (C-4), 164.6 (C-2), 164.0 (C-7), 161.4 (C-5), 156.8 (C-9), 150.4 (C-4'), 146.4 (C-3'), 122.7 (C-1'), 120.7 (C-6'), 116.3 (C-5'), 114.0 (C-2'), 110.0 (C-6), 104.1 (C-10), 103.5 (C-3), 94.2 (C-8), 82.3

(C-5"), 80.0 (C-3"), 73.7 (C-1"), 71.3 (C-2"), 70.9 (C-4"), 62.2 (C-6")

Quercetin 3-*O*-β-D-glucoside (**15**) – Yellow powder, mp 183 - 185 °C; FAB-MS *m/z* : 465 $[M+H]^+$; IR ν_{max} (MeOH) : 3387, 1656, 1448, 1362, 1305, 1054, 1025, 825 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.60 (1H, s, 5-OH), 7.67 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 7.66 (1H, d, *J* = 2.0 Hz, H-2'), 6.86 (1H, d, *J* = 8.0 Hz, H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.41 (1H, d, *J* = 8.0 Hz, H-1"); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 178.2 (C-4), 164.8 (C-7), 162.0 (C-5), 157.0 (C-2), 156.9 (C-9), 149.2 (C-4'), 145.5 (C-3'), 135.0 (C-3), 122.3 (C-6'), 121.9 (C-1'), 116.9 (C-5'), 115.9 (C-2'), 104.6 (C-10), 101.6 (C-1"), 99.4 (C-6), 94.2 (C-8), 78.3 (C-5"), 77.2 (C-3"), 74.8 (C-2"), 70.7 (C-4"), 61.7 (C-6")

Quercitrin (**16**) – Yellow powder, mp 180 - 187 °C; FAB-MS *m/z* : 449 $[M+H]^+$; IR ν_{max} (MeOH) : 3388, 2974, 1656, 1608, 1507, 1450, 1363, 1306, 1055, 826 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.65 (1H, s, 5-OH), 7.31 (1H, d, *J* = 1.5 Hz, H-2'), 7.30 (1H, dd, *J* = 1.5, 8.5 Hz, H-6'), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 6.40 (1H, d, *J* = 1.5 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.21 (1H, br s, H-1"), 0.80 (3H, d, *J* = 6.0 Hz, H-6"); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 178.2 (C-4), 165.0 (C-7), 162.0 (C-5), 158.0 (C-2), 157.2 (C-9), 149.2 (C-4'), 145.9 (C-3'), 134.9 (C-3), 121.8 (C-6'), 121.5 (C-1'), 116.4 (C-5'), 116.2 (C-2'), 104.8 (C-10), 102.6 (C-1"), 99.4 (C-6), 94.2 (C-8), 71.9 (C-4"), 71.3 (C-2"), 71.1 (C-3"), 70.8 (C-5"), 18.2 (C-6")

Luteolin (**17**) – Yellow powder, mp 180 - 186 °C; FAB-MS *m/z* : 287 $[M+H]^+$; IR ν_{max} (MeOH) : 3381, 2965, 1654, 1608, 1507, 1450, 1363, 1306, 1055, 683 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.97 (1H, s, 5-OH), 7.39 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 7.37 (1H, d, *J* = 2.0 Hz, H-2'), 6.86 (1H, d, *J* = 8.0 Hz, H-5') 6.60 (1H, s, H-3), 6.44 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 182.7 (C-4), 165.2 (C-2), 164.8 (C-7), 162.0 (C-5), 158.2 (C-9), 149.8 (C-4'), 145.9 (C-3'), 122.5 (C-1'), 119.1 (C-2'), 115.6 (C-5'), 113.0 (C-6'), 104.1 (C-10), 102.7 (C-3), 98.9 (C-6), 93.8 (C-8)

Test for cytotoxicity *in vitro* – Sulforhodamin B bioassay (SRB) was used as for cytotoxicity screening (Skehan *et al.*, 1990). The *in vitro* cytotoxicity of each compound against four cultured human tumor cells was assessed at the Korean Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT cell lines were IC_{50} 0.003, 0.034, 0.0013, and 0.221 μM, respectively.

Results and Discussion

Compounds **5 - 17** were identified by comparing the ^{1}H-, ^{13}C-NMR, and MS spectral data with the literature values to be vanillic acid (**5**) (Harrison *et al.*, 1995), caffeic acid methyl ester (**6**) (Lee *et al.*, 2007), protocatechuic acid (**7**) (Kwak *et al.*, 2009), caffeic acid (**8**) (Sun *et al.*, 2006), 3,5-di-*O*-caffeoyl quinic acid methyl ester (**9**) (Choi *et al.*, 2004), chlorogenic acid methyl ester (**10**) (Meena *et al.*, 1998), avicularin (**11**) (Kim *et al.*, 2006), afzelin (**12**) (Kim *et al.*, 2008), quercetin (**13**) (Lee *et al.*, 2004), isoorientin (**14**) (Ju *et al.*, 1998), quercetin 3-*O*-β-D-glucoside (**15**) (Jung *et al.*, 2002), quercitrin (**16**) (Lee *et al.*, 2002) and luteolin (**17**) (Markham *et al.*, 1978).

The following describes the structural elucidation of compounds **1 - 4**, which were for the first time isolated from the genus *Bistorta.*

Compound **1** was obtained as a white powder with a negative optical rotation ($[\alpha]_D^{25}$: –13.7° in MeOH). From the FAB-MS (*m/z* 736 $[M+Na]^+$), ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **1** was deduced to be $C_{40}H_{75}NO_9$. The ^{1}H- and ^{13}C-NMR NMR spectra showed signal patterns of typical cerebrosides (Laurence *et al.*, 1999; Hirotaka *et al.*, 1993). The ^{1}H-NMR spectrum of **1** showed two double bonds at δ 5.76 (1H, dt, *J* = 15.0, 6.5 Hz, H-12), 5.50 (1H, br dt, *J* = 15.0, 6.5 Hz, H-11), 5.38 (1H, dd, *J* = 16.0, 5.5 Hz, H-4), 5.32 (1H, dt, *J* = 16.0, 6.5 Hz, H-5) and two terminal methyl groups at δ 0.97 (6H, t, *J* = 7.0 Hz, H-18, H-16'). The anomeric proton peak at δ 4.23 (H-1") of D-glucose was observed to have β form based on the coupling constant (*J* = 7.5 Hz) (Stephen *et al.*, 1977). In The ^{13}C-NMR spectrum, one ketone group at δ 176.2 (C-1'), two double bonds at δ 133.3 (C-12), 132.1 (C-4, 11), 130.8 (C-5), a glucosyl moiety at δ 104.7 (C-1"), 77.3 (C-5"), 77.2 (C-3"), 73.8 (C-2"), 70.5 (C-4"), 62.0 (C-6"), a methine carbon attached to nitrogen at δ 54.0 (C-2) and three vicinal methylene carbons at δ 32.5 (C-6), 31.9 (C-10, 13) were observed. Based on the above data and the comparison of the data with those in a previous literature (Laurence *et al.*, 1999), the structure of **1** was identified as pinelloside.

Compound **2** was obtained as a white powder with a positive optical rotation ($[\alpha]_D^{25}$: +11.8° in MeOH). From the FAB-MS (*m/z* 736 $[M+Na]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **2** was deduced to

be $C_{40}H_{75}NO_9$. The ^{1}H- and ^{13}C-NMR spectral data of **2** were very similar to those of **1**, except for chemical shift values in the double bonds region. In the ^{1}H-NMR spectrum, peaks at δ 5.76 (1H, dt, J = 15.0, 6.5 Hz, H-12), 5.50 (1H, br dt, J = 15.0, 6.5 Hz, H-11), 5.38 (1H, dd, J = 16.0, 5.5 Hz, H-4), 5.32 (1H, dt, J = 16.0, 6.5 Hz, H-5) of **1** were replaced to δ 5.74 (1H, br dt, J = 15.0, 6.0 Hz, H-5), 5.50 (1H, dd, J = 15.0, 7.0 Hz, H-4), 5.42 (2H, t, J = 4.0 Hz, H-8, 9) of **2**. Also in the ^{13}C-NMR spectrum, peaks at δ 133.3 (C-12), 132.1 (C-4, 11), 130.8 (C-5) of **1** were changed to δ 134.3 (C-5), 131.1 (C-8), 130.8 (C-9), 129.8 (C-4) of **2**. The difference of chemical shift values between two compounds indicated that positions of double bonds are different. Based on the comparison of the ^{1}H- and ^{13}C-NMR and MS spectral data in a previous paper (Voutquenne *et al.,* 1999), the structure of **2** was determined to be soyacerebroside I.

Compound **3** was obtained as a colorless gum with a positive optical rotation ($[\alpha]_D^{25}$: +38.8° in MeOH). From the FAB-MS (*m/z* 116 $[M+H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **3** was deduced to be $C_5H_9NO_2$. The ^{1}H-NMR spectrum showed one oxygenated proton at δ 4.85 (1H, dd, J = 6.4, 1.2 Hz, H-5), one methoxy protons at δ 3.30 (OCH_3), and four methylene protons at δ 2.45 (1H, m, H-3a), 2.25 (1H, m, H-4a), 2.14 (1H, m, H-3b), 2.00 (1H, m, H-4b). In the ^{13}C-NMR spectrum, one ketone group at δ 181.6 (C-2), one oxygenated carbon at δ 88.9 (C-5), one methoxy carbon at δ 54.8 (OCH_3) and two methylene carbons at δ 30.3 (C-3), 29.5 (C-4) were observed. The peaks at δ 4.85 (1H, dd, J = 6.4, 1.2 Hz, H-5) and δ 2.45 (1H, m, H-3a), 2.25 (1H, m, H-4a), 2.14 (1H, m, H-3b), 2.00 (1H, m, H-4b) in ^{1}H-NMR as well as peaks at δ 181.6 (C-2) and δ 88.9 (C-5) in ^{13}C-NMR indicated it to possess lactam ring structure (Kuhnt *et al.,* 1995). Based on the comparison of the ^{1}H- and ^{13}C-NMR and MS spectral data in a previous paper (Song *et al.,* 2008), the structure of **3** was determined to be pterolactam.

Compound **4** was obtained as a colorless gum with a positive optical rotation ($[\alpha]_D^{25}$: +56.0° in MeOH). From the FAB-MS (*m/z* 102 $[M+H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **4** was deduced to be $C_4H_7NO_2$. The NMR spectra of **4** were very similar to those of **3**, but there was no signal at δ 3.30 (OCH_3) in ^{1}H-NMR and δ 54.8 (OCH_3) in ^{13}C-NMR spectra of **4**. This indicated it to have no methoxy group in **4**. Based on the comparison of the ^{1}H- and ^{13}C-NMR and MS spectral data in a previous paper (Staubmann *et al.,* 1999), the structure of **4** was determined to be 5-hydroxypyrrolidine-2-one.

The isolated compounds (**1 - 17**) were tested *in vitro* for cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cells using the SRB assay. Compound **17** exhibited moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2 and HCT15 cell lines (IC_{50} : 9.5, 12.2, 13.0 and 10.8 μM, respectively). And compound **6** showed weak cytotoxicity against A549, SK-OV-3, SK-MEL-2 and HCT15 cell lines (IC_{50} : 29.1, 70.6, 49.1 and 33.3 μM, respectively). The other compounds showed little cytotoxic activity against cancer cell lines tested (IC_{50} > 100 μM).

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