Natural Product Sciences
20(2) : 95-101 (2014)

Phytochemical Constituents of *Salsola komarovii* and Their Effects on NGF Induction†

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Abstract – Five lignan glycosides, seven megastigmane glycosides, and seven phenolic compounds were isolated by repeated column chromatography from the MeOH extract of *Salsola komarovii*. Their structures were determined to be lariciresinol-9-*O*-*β*-D-glucopyranoside (**1**), alangilignoside C (**2**), conicaoside (**3**), (+)-lyoniresinol 9'-*O*-*β*-D-glucopyranoside (**4**), (8*S*,8'*R*,7'*R*)-9'-[(*β*-glucopyranosyl)oxy]lyoniresinol (**5**), blumenyl B *β*-D-glucopyranoside (**6**), blumenyl A *β*-D-glucopyranoside (**7**), staphylionoside D (**8**), icariside B_2 (**9**), (6*R*,9*S*)-3-oxo-*α*-ionol *β*-D-glucopyranoside (**10**), 3-oxo-*α*-ionol 9-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (**11**), blumenol B 9-*O*-*β*-D-apiofuranosyl-(1 → 6)-*β*-D-glucopyranoside (**12**), benzyl 6-*O*-*β*-D-apiofuranosyl-*β*-D-glucopyranoside (**13**), canthoside C (**14**), tachioside (**15**), isotachioside (**16**), biophenol 2 (**17**), 2-(3,4-dihydroxy)-phenyl-ethyl-*β*-D-glucopyranoside (**18**), and cuneataside C (**19**) by spectroscopic methods. All the isolated compounds **1 - 19** were reported from this source for the first time. Compounds **2**, **3** and **6** upregulated NGF secretion to 118.8 ± 3.6%, 128.2 ± 9.3% and 111.1 ± 7.1% without significant cell toxicity.
Keywords – *Salsola komarovii*, Chemopodiaceae, NGF regulation, Lignan

Introduction

Exogenous Nerve growth factor (NGF) has therapeutic potential for neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and diabetic polyneuropathy and enhances impaired cholinergic neuron system.[1] As part of our efforts to search bioactive constituents of Korean medicinal plants, we found that the methanol extract of the aerial parts of *Salsola komarovii* induce increases in endogenous NGF levels in C6 glioma cells. *Salsola* species were used for treatments of hypertension, inflammation, cancer, and Alzheimer's disease.[2,3] Previous phytochemical investigations on this genus reported the isolation of flavonoids, alkaloids, coumarins, and sterols.[4] However, only few phytochemical studies on *S. komarovii* have been reported. Thus, we have investigated the bioactive constituents from the aerial parts of *S. komarovii.* Column chromatographic separation of its MeOH extract led to isolation of five lignan glycosides (**1 - 5**), seven megastigmane glycosides (**6 - 12**), and seven phenolic compounds (**13 - 19**). Their structures were identified by physicochemical and spectroscopic methods. All isolates are here reported for the first time from this plant. Also, we evaluated the effects of isolates (**1 - 19**) on NGF induction.

Experimental

General experimental procedures – Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Econosil RP-C_{18} 10 μm column (250 × 10 mm). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) and RP-C_{18} silica gel (YMG GEL ODS-A, 12 nm, S-75 μm) were used for column chromatography. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (v/v).

†Dedicated to Prof. Okpyo Zee of the Sungkyunkwan University for his leading works on Natural Products Research.

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Plant materials – *S. komarovii* (8 kg) were collected at Jejudo, Korea in September 2011, and the plant was identified by one of the authors (K.R. Lee). A voucher specimen (SKKU-NPL 1211) was deposited at the herbarium of School of Pharmacy in Sungkyunkwan University.

Extraction and isolation – The aerial parts of *S. komarovii* (8 kg) were extracted three times at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (384 g), which was dissolved in water (1.8 L) and then succeccively partitioned with *n*-hexane (20 g), $CHCl_3$ (5 g), EtOAc (7 g), and *n*-BuOH (38 g). The BuOH fraction (38 g) was separated over an RP-C_{18} silica gel column (40% MeOH – 100% MeOH) to yield three fractions (B1-B3). Fraction B1 (18.1 g) was chromatographed on a diaion HP-20 column, eluting with a gradient solvent system consisting of 100% water and 100% MeOH, yielded two subfractions (B11 and B12). Fraction B12 (2.1 g) was subjected to Sephadex LH-20 column chromatography eluted with 50% MeOH to give four fractions (B121-B124). Subfraction B123 (215 mg) was separated over a silica gel column [$CHCl_3$-MeOH-Water, 4 : 1 : 0.1] and further purified with an RP-C_{18} prep. HPLC (20% MeOH) to afford **14** (11 mg, t_R = 10.5 min). Subfraction B124 (654 mg) was separated over a silica gel column [$CHCl_3$-MeOH-Water, 6 : 1 : 0.1] and further purified with an RP-C_{18} prep. HPLC (19% MeOH) to yield **15** (3 mg, t_R = 15.7 min), **16** (6 mg, t_R = 11.0 min), **17** (7 mg, t_R = 14.4 min), **18** (31 mg, t_R = 14.1 min), and **19** (10 mg, t_R = 20.0 min). Fraction B2 (5.6 g) was separated over a silica gel column [$CHCl_3$-MeOH, 10 : 1 → 2 : 1] to yield nine fractions (B21-B29). Subfraction B26 (75 mg) was subjected to column chromatography using Sep-Pak (30% MeOH) and purified with an RP-C_{18} prep. HPLC (33% MeOH) to give **9** (3 mg, t_R = 13.9 min). Subfraction B28 (353 mg) was separated over an RP-C_{18} silica gel column (30% MeOH) and purified with an RP-C_{18} prep. HPLC (10% CH_3CN) to afford **1** (5 mg, t_R = 12.5 min), **2** (5 mg, t_R = 12.3 min), **3** (4 mg, t_R = 12.4 min), **6** (17 mg, t_R = 17.8 min), and **7** (48 mg, t_R = 18.0 min). Subfraction B29 (1.3 g) was subjected to an RP-C_{18} silica gel column (30% MeOH) to give three fractions (B291-293). Compound **8** was obtained from subfraction B292 by the separation of Sep-Pak [$CHCl_3$-MeOH, 8 : 1] and an RP-C_{18} prep. HPLC (14% CH_3CN). Subfraction B293 was purified with an RP-C_{18} prep. HPLC (18% CH_3CN) to yield **4** (12 mg, t_R = 17.1 min), **5** (3 mg, t_R = 17.3 min), and **13** (7 mg, t_R = 15.7 min). Fraction B3 (8.3 g) was separated by Sephadex LH-20 column (70% MeOH) to yield four fractions (B31-B34). Subfraction B32 (537 mg) was subjected to a silica gel column [$CHCl_3$-MeOH-Water, 6:1:0.1] and purified with an RP-C_{18} prep. HPLC (50% MeOH) to afford **10** (22 mg, R_t = 17.6 min), **11** (11 mg, t_R = 22.0 min), and **12** (12 mg, t_R = 25.0 min).

Lariciresinol-9-*O*-*β*-D-glucopyranoside (1) – White powder, mp : 173 ºC; $[\alpha]_D^{25}$: +1.84° (*c* 0.125, MeOH); IR ν_{max} cm^{-1}: 3360, 2946, 2840, 1743, 1375, 1214; FAB-MS *m/z*: 523 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.93 (1H, d, *J* = 1.8 Hz, H-2), 6.80 (1H, d, *J* = 1.8 Hz, H-2'), 6.76 (1H, m, H-5), 6.75 (1H, m, H-6), 6.71 (1H, d, *J* = 8.0 Hz, H-5'), 6.65 (1H, dd, *J* = 8.0, 1.8 Hz, H-6'), 4.74 (1H, d, *J* = 7.5 Hz, H-7), 4.32 (1H, d, *J* = 8.0 Hz, H-Glc), 4.05 (1H, m, H-9'), 4.03 (1H, m, H-9), 3.85 (3H, s, 3-OCH_3), 3.83 (3H, s, 3'-OCH_3), 3.73 (1H, m, H-9), 3.62 (1H, m, H-9'), 2.96 (1H, m, H-7'), 2.77 (1H, m, H-8'), 2.55 (1H, m, H-7'), 2.48 (1H, m, H-8); ^{13}C-NMR (CD_3OD, 125 MHz): δ 147.8 (C-3, 3'), 144.6 (C-4, 4'), 134.4 (C-1), 132.5 (C-1'), 121.1 (C-6'), 118.6 (C-6), 114.9 (C-5'), 114.8 (C-5), 112.3 (C-2'), 109.6 (C-2), 103.6 (Glc C-1), 83.00 (C-7), 76.9 (Glc C-5), 76.8 (Glc C-3), 74.00 (Glc C-2), 72.4 (C-9'), 70.5 (Glc C-4), 67.3 (C-9), 61.5 (Glc C-6), 55.2 (3, 3'-OCH_3), 50.5 (C-8), 42.7 (C-8'), 32.7 (C-7').

Alangilignoside C (2) – Colorless gum, $[\alpha]_D^{25}$: +17.7° (*c* 0.125, MeOH); IR ν_{max} cm^{-1}: 3300, 2905, 1614, 1370, 1210, 1040; FAB-MS *m/z*: 581 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.65 (2H, s, H-2, 6), 6.50 (2H, s, H-2', 6'), 4.85 (1H, d, *J* = 6.5 Hz, H-7), 4.31 (1H, d, *J* = 8.0 Hz, H-1"), 4.08 (1H, m, H-9), 3.99 (1H, m, H-9'), 3.84 (1H, d, *J* = 12.0 Hz, H-6"), 3.83 and 3.82 (12H, s, 3, 3', 5, 5'-OCH_3), 3.75 (2H, m, H-9, 9'), 3.66 (1H, dd, *J* = 12.0, 4.8 Hz, H-6"), 2.96 (1H, dd, *J* = 13.0, 5.0 Hz, H-7'), 2.75 (1H, m, H-8'), 2.52 (2H, m, H-7',8); ^{13}C-NMR (CD_3OD, 125 MHz): δ 149.1 (C-3', 5'), 149.0 (C-3,5), 134.7 (C-4), 133.7 (C-4'), 133.6 (C-1), 131.7 (C-1'), 105.8 (C-2',6'), 103.5 (C-2,6), 103.1 (Glc C-1), 83.2 (C-7), 76.9 (Glc C-5), 76.8 (Glc C-3), 74.0 (Glc C-2), 72.6 (C-9'), 70.5 (Glc C-4), 67.3 (C-9), 61.5 (Glc C-6), 55.6 (3, 3', 5, 5'-OCH_3), 50.6 (C-8), 42.8 (C-8'), 33.1 (C-7').

Conicaoside (3) – Colorless gum, $[\alpha]_D^{25}$: –19.9° (*c* 0.125, pyridine); IR ν_{max} cm^{-1}: 3432, 2945, 1601, 1520, 1466, 1240, 1151, 1040; FAB-MS *m/z*: 575 $[M + Na]^+$; ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 7.25 (1H, d, *J* = 8.0 Hz, H-5'), 7.10 (2H, s, H-2, 6), 7.05 (1H, d, *J* = 1.8 Hz, H-2'), 6.50 (1H, dd, *J* = 8.0, 1.8 Hz, H-6'), 5.70 (1H, d, *J* = 7.0 Hz, H-1"), 5.48 (1H, d, *J* = 5.0 Hz, H-7), 4.38 (1H, m, H-9'), 4.35 (1H, dd, *J* = 10.5, 6.0 Hz, H-9), 4.18 (1H, dd, *J* = 10.5, 7.0 Hz, H-9), 4.10 (1H, t like, *J* = 7.8 Hz, H-9'), 3.72 (9H, s, 3, 3', 5-OCH_3), 3.26 (1H, dd, *J* = 13.0, 4.0 Hz, H-7'), 3.10 (1H, m, H-8'), 2.85 (1H, m, H-7'), 2.76

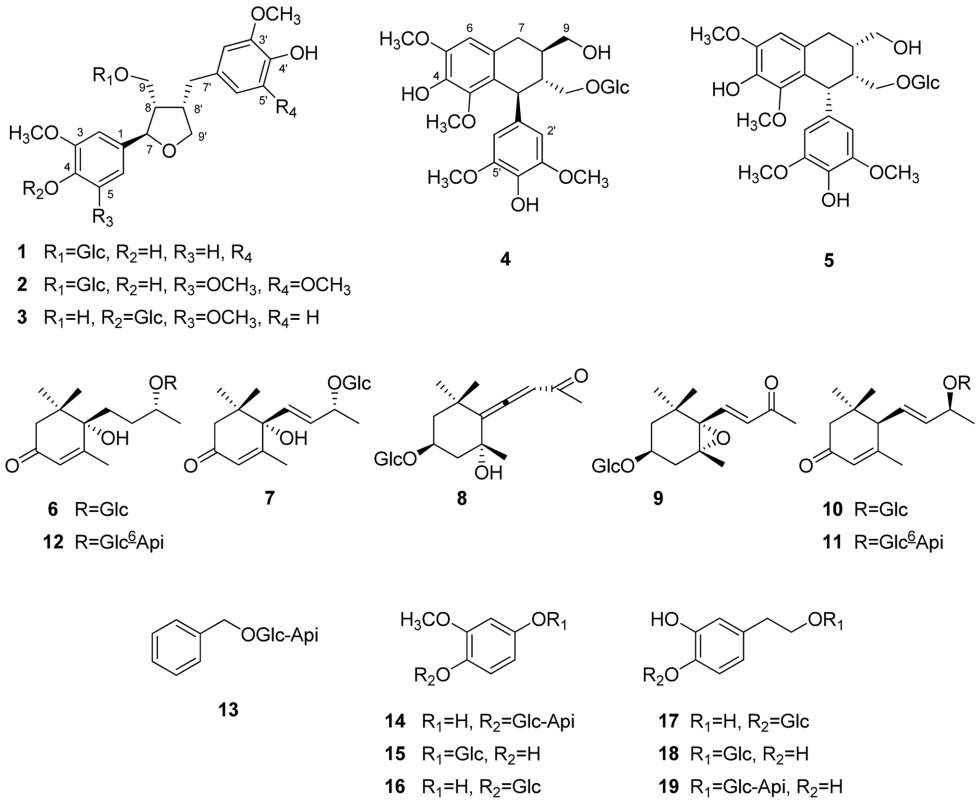

Fig. 1. The structures of **1** - **19** from *S.komarovii.*

(1H, m, H-8'); ^{13}C-NMR (pyridine-d_5, 125 MHz): δ 153.1 (C-3,5), 147.8 (C-3'), 144.7 (C-4'), 140.5 (C-1), 134.3 (C-4), 132.3 (C-1'), 120.9 (C-6'), 115.1 (C-5'), 112.2 (C-2'), 104.3 (Glc C-1), 103.5 (C-2, 6), 82.8 (C-7), 77.2 (Glc C-5), 76.7 (Glc C-3), 74.6 (Glc C-2), 72.6 (C-9'), 70.2 (Glc C-4), 61.4 (Glc C-6), 59.4 (C-9), 55.9 (3, 5-OCH_3), 55.2 (3'-OCH_3), 52.9 (C-8), 42.6 (C-8'), 32.4 (C-7').

(+)-Lyoniresinol-9'-*O*-*β*-D-glucopyranoside (4) – Colorless gum, $[\alpha]_D^{25}$: +39.0° (*c* 0.125, MeOH); IR ν_{max} cm^{-1}: 3430, 2800, 1605, 1520, 1035; FAB-MS *m/z*: 583 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.58 (1H, s, H-6), 6.42 (2H, s, H-2', 6'), 4.20 (1H, d, *J* = 6.0 Hz, H-7'), 4.28 (1H, d, *J* = 8.0 Hz, H-1''), 3.90 (1H, m, H-9'), 3.86 (3H, s, 5-OCH_3), 3.74 (6H, s, 3', 5'-OCH_3), 3.65 (1H, dd, *J* = 11.0, 6.5 Hz, H-9), 3.54 (1H, m, H-9'), 3.34 (3H, s, 3-OCH_3), 2.72 (1H, dd, *J* = 15.0, 4.5 Hz, H-7), 2.62 (1H, dd, *J* = 15.0, 11.5 Hz, H-7), 2.08 (1H, m, H-8'), 1.70 (1H, m, H-8'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 147.8 (C-3', 5'), 147.5 (C-5), 146.4 (C-3), 138.2 (C-4), 137.7 (C-4'), 133.3 (C-1'), 129.0 (C-1), 125.3 (C-2), 106.7 (C-6), 105.8 (C-2', 6'), 103.7 (Glc C-1), 77.1 (Glc C-5), 76.8 (Glc C-3), 74.0 (Glc C-2), 70.5 (C-9'), 70.3 (Glc C-4), 65.1 (C-9), 61.7 (Glc C-6), 58.9 (3-OCH_3), 55.7 (5-OCH_3), 55.4 (3', 5'-OCH_3), 45.5 (C-8'), 41.6 (C-7'), 39.4 (C-8), 32.6 (C-7).

(8*S*,8'*R*,7'*R*)-9'-[(*β*-glucopyranosyl)oxy]lyoniresinol (5) – Colorless gum, $[\alpha]_D^{25}$: −59.0° (*c* 0.125, MeOH); IR ν_{max} cm^{-1}: 3460, 2805, 1615, 1523, 1041; FAB-MS *m/z*: 605 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.57 (1H, s, H-6), 6.42 (2H, s, H-2', 6'), 4.23 (1H, d, *J* = 6.5 Hz, H-7'), 4.13 (1H, d, *J* = 7.5 Hz, H-1''), 3.87 (1H, m, H-9'), 3.87 (3H, s, 5-OCH_3), 3.75 (6H, s, 3', 5'-OCH_3), 3.56 (1H, dd, *J* = 11.0, 6.5 Hz, H-9), 3.44 (1H, m, H-9'), 3.35 (3H, s, 3-OCH_3), 2.66 (2H, m, H-7), 2.11 (1H, m, H-8'), 1.67 (1H, m, H-8'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 147.8 (C-3', 5'), 147.5 (C-5), 146.4 (C-3), 138.3 (C-4), 137.7 (C-4'), 133.5 (C-1'), 129.0 (C-1), 125.1 (C-2), 106.6 (C-6), 106.0 (C-2', 6'), 103.1 (Glc C-1), 77.0 (Glc C-5), 76.8 (Glc C-3), 73.9 (Glc C-2), 70.8 (C-9'), 70.4 (Glc C-4), 65.0 (C-9), 61.5 (Glc C-6), 58.9 (3-OCH_3), 55.7 (5-OCH_3), 55.4 (3', 5'-OCH_3), 45.4 (C-8'), 42.1 (C-7'), 40.1 (C-8), 32.6 (C-7).

Blumenyl B *β*-D-glucopyranoside (6) – Colorless gum, $[\alpha]_D^{25}$: +2.0° (*c* 0.125, MeOH); FAB-MS *m/z*: 411 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.82 (1H, s, H-4), 4.31 (1H, d, *J* = 7.5 Hz, H-1'), 3.82 (1H, m, H-9), 3.79 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'), 3.64 (1H, dd, *J* = 12.0, 5.5 Hz, H-6'), 2.66 (1H, d, *J* = 18.0 Hz, H-2), 2.13 (1H, d, *J* = 18.0 Hz, H-2), 2.04 (3H, d, *J* = 1.0 Hz, H-13), 1.81

(2H, m, H-7), 1.76 (1H, m, H-8), 1.24 (3H, d, J = 6.5 Hz, H-10), 1.09 (3H, s, H-11), 1.01 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 125 MHz): δ 199.8 (C-3), 170.6 (C-5), 125.5 (C-4), 103.2 (C-1'), 78.2 (C-6), 77.2 (C-3'), 77.0 (C-5'), 76.7 (C-9), 74.1 (C-2'), 70.4 (C-4'), 61.6 (C-6'), 49.9 (C-2), 42.0 (C-1), 33.6 (C-7), 31.8 (C-8), 23.2 (C-13), 22.8 (C-12), 21.0 (C-11), 20.9 (C-10).

Blumenyl A β-D-glucopyranoside (7) – Colorless gum, $[\alpha]_D^{25}$: +34.0° (*c* 0.125, MeOH); FAB-MS *m/z*: 409 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.96 (1H, d, J = 15.0 Hz, H-7), 5.86 (1H, br s, H-4), 5.72 (1H, dd, J = 15.0, 7.0 Hz, H-8), 4.52 (1H, m, H-9), 4.26 (1H, d, J = 8.0 Hz, H-1'), 3.82 (1H, m, H-9), 3.79 (1H, dd, J = 12.0, 1.5 Hz, H-6'), 3.64 (1H, m, H-6'), 2.61 (1H, d, J = 19.0 Hz, H-2), 2.16 (1H, d, J = 18.0 Hz, H-2), 1.94 (3H, s, H-13), 1.28 (3H, d, J = 6.0 Hz, H-10), 1.09 (3H, s, H-11), 1.01 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 125 MHz): δ 200.1 (C-3), 165.9 (C-5), 132.6 (C-7), 132.5 (C-8), 125.9 (C-4), 100.6 (C-1'), 78.8 (C-6), 77.2 (C-3'), 77.0 (C-5'), 73.7 (C-9), 73.4 (C-2'), 70.5 (C-4'), 61.6 (C-6'), 49.6 (C-2), 41.3 (C-1), 23.5 (C-13), 22.4 (C-12), 20.9 (C-11), 18.4 (C-10).

Staphylionoside D (8) – Colorless gum, $[\alpha]_D^{25}$: −58.0° (*c* 0.125, MeOH); FAB-MS *m/z*: 385 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.83 (1H, s, H-8), 4.44 (1H, d, J = 7.5 Hz, H-1'), 3.86 (1H, dd, J = 12.0, 2.0 Hz, H-6'), 3.67 (1H, dd, J = 12.0, 5.0 Hz, H-6'), 2.37 (1H, ddd, J = 13.0, 4.0, 2.0 Hz, H-4), 2.19 (3H, s, H-10), 2.08 (1H, ddd, J = 13.0, 4.0, 2.0 Hz, H-2), 1.46 (2H, m, H-2,4), 1.39 (3H, s, H-13), 1.38 (3H, s, H-11), 1.15 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 125 MHz): δ 210.3 (C-9), 199.6 (C-7), 118.9 (C-6), 101.5 (C-1'), 99.9 (C-8), 76.9 (C-3'), 76.7 (C-5'), 73.9 (C-2'), 71.4 (C-3), 71.2 (C-5), 70.5 (C-4'), 61.6 (C-6'), 48.7 (C-4), 45.5 (C-2), 35.8 (C-1), 31.1 (C-12), 29.6 (C-13), 28.2 (C-11), 25.3 (C-10).

Icariside B_2 (9) – Colorless gum, $[\alpha]_D^{25}$: −96.8° (*c* 0.125, MeOH); FAB-MS *m/z*: 385 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.00 (1H, d, J = 16.0 Hz, H-7), 6.25 (1H, d, J = 15.5 Hz, H-8), 4.39 (1H, d, J = 7.0 Hz, H-1'), 3.89 (1H, m, H-3), 2.43 (1H, m, H-4), 2.28 (3H, s, H-10), 1.73 (1H, m, H-4), 1.66 (1H, m, H-2), 1.38 (1H, m, H-2), 1.20 (3H, s, H-13), 1.18 (3H, s, H-12), 0.98 (3H, s, H-11); ^{13}C-NMR (CD_3OD, 125 MHz): δ 199.1 (C-9), 144.1 (C-7), 132.6 (C-8), 101.8 (C-1'), 76.9 (C-3'), 76.7 (C-5'), 73.9 (C-2'), 71.6 (C-3), 70.5 (C-4'), 69.9 (C-6), 67.2 (C-5), 61.6 (C-6'), 44.0 (C-2), 36.9 (C-4), 34.8 (C-1), 28.3 (C-12), 26.3 (C-11), 24.3 (C-10), 19.0 (C-13).

(6*R*,9*S*)-3-Oxo-α-ionol β-D-glucopyranoside (10) – Colorless gum, FAB-MS *m/z*: 371 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.88 (1H, br s, H-4), 5.75 (1H, dd, J = 15.0, 9.5 Hz, H-7), 5.57 (1H, dd, J = 15.0, 7.5 Hz, H-8), 4.48 (1H, m, H-9), 4.28 (1H, d, J = 7.5 Hz, H-1'), 3.83 (1H, m, H-6'), 3.62 (1H, m, H-6'), 2.69 (1H, d, J = 9.5 Hz, H-6), 2.46 (1H, d, J = 17.0 Hz, H-2), 2.05 (1H, d, J = 16.5 Hz, H-2), 1.98 (3H, d, J = 1.0 Hz, H-13), 1.29 (3H, d, J = 6.5 Hz, H-10), 1.03 (3H, s, H-12), 0.98 (3H, s, H-11); ^{13}C-NMR (CD_3OD, 125 MHz): δ 200.8 (C-3), 164.4 (C-5), 135.9 (C-8), 129.9 (C-7), 125.0 (C-4), 100.6 (C-1'), 77.2 (C-3'), 76.9 (C-5'), 73.8 (C-2'), 73.6 (C-9), 70.6 (C-4'), 61.7 (C-6'), 55.7 (C-6), 48.2 (C-2), 35.9 (C-1), 26.9 (C-12), 26.2 (C-11), 22.7 (C-13), 21.1 (C-10).

3-Oxo-α-ionol 9-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (11) – Colorless gum, FAB-MS *m/z*: 501 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.93 (1H, br s, H-4), 5.78 (1H, dd, J = 15.0, 9.5 Hz, H-7), 5.61 (1H, dd, J = 15.0, 7.5 Hz, H-8), 5.04 (1H, d, J = 2.0 Hz, H-1"), 4.46 (1H, m, H-9), 4.31 (1H, d, J = 7.5 Hz, H-1'), 2.73 (1H, d, J = 9.5 Hz, H-6), 2.50 (1H, d, J = 17.0 Hz, H-2), 2.07 (1H, d, J = 16.5 Hz, H-2), 2.01 (3H, d, J = 1.0 Hz, H-13), 1.33 (3H, d, J = 6.5 Hz, H-10), 1.06 (3H, s, H-12), 1.02 (3H, s, H-11); ^{13}C-NMR (CD_3OD, 125 MHz): δ 200.3 (C-3), 163.4 (C-5), 135.3 (C-8), 130.2 (C-7), 125.1 (C-4), 111.2 (C-1"), 100.6 (C-1'), 81.2 (C-3"), 77.2 (C-3'), 77.0 (C-2"), 76.9 (C-5'), 73.9 (C-4"), 73.8 (C-2'), 73.6 (C-9), 70.6 (C-4'), 67.7 (C-6'), 55.7 (C-6), 48.4 (C-2), 36.4 (C-1), 26.9 (C-12), 26.2 (C-11), 22.1 (C-13), 20.6 (C-10).

Blumenol B 9-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (12) – Colorless gum, FAB-MS *m/z*: 543 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.83 (1H, s, H-4), 5.02 (1H, d, J = 2.0 Hz, H-1"), 4.32 (1H, d, J = 7.5 Hz, H-1'), 3.82 (1H, m, H-9), 2.52 (1H, d, J = 18.0 Hz, H-2), 2.13 (1H, d, J = 18.0 Hz, H-2), 2.04 (3H, d, J = 1.0 Hz, H-13), 1.81 (2H, m, H-7), 1.76 (1H, m, H-8), 1.23 (3H, d, J = 6.5 Hz, H-10), 1.15 (3H, s, H-11), 1.02 (3H, s, H-12); ^{13}C-NMR (CD_3OD, 125 MHz): δ 199.8 (C-3), 170.6 (C-5), 125.5 (C-4), 110.9 (C-1"), 103.2 (C-1'), 80.8 (C-3"), 78.2 (C-6), 77.2 (C-3'), 77.1 (C-2"), 77.0 (C-5'), 74.0 (C-4"), 76.7 (C-9), 74.0 (C-2'), 70.4 (C-4'), 66.6 (C-6'), 49.9 (C-2), 42.0 (C-1), 33.6 (C-7), 31.8 (C-8), 23.2 (C-13), 22.8 (C-12), 21.0 (C-11), 20.9 (C-10).

Benzyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside (13) – Colorless gum, FAB-MS *m/z*: 401 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.43-7.25 (5H, m, H-2, 3, 4, 5, 6), 5.05 (1H, d, J = 2.5 Hz, H-1"), 4.90 (1H, d, J = 11.5 Hz, H-7), 4.66 (1H, d, J = 12.0 Hz, H-7), 4.32 (1H, d, J = 8.0 Hz, H-1'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 137.8 (C-1), 128.1 (C-3, 5), 128.1 (C-2), 127.5 (C-6), 109.7 (C-1"), 102.6 (C-1'), 79.3 (C-3"), 76.9 (C-2"), 76.8 (C-3'), 75.7 (C-5'), 73.8 (C-4"), 73.7 (C-2'), 70.5 (C-4'), 69.4 (C-7), 67.6 (C-5"), 64.4 (C-6).

Canthoside C (14) – Colorless gum, FAB-MS *m/z*: 435 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.76 (1H, d, *J* = 2.5 Hz, H-2), 6.71 (1H, d, *J* = 8.0 Hz, H-5), 6.60 (1H, dd, *J* = 8.0, 2.5 Hz, H-6), 4.98 (1H, d, *J* = 2.0 Hz, H-1"), 4.70 (1H, d, *J* = 7.5 Hz, H-1'), 3.76 (3H, s, 3-OCH_3) ; ^{13}C-NMR (CD_3OD, 125 MHz): δ 151.6 (C-1), 148.1 (C-3), 141.9 (C-4), 114.9 (C-5), 109.8 (C-1"), 108.9 (C-6), 102.9 (C-2), 102.6 (C-1'), 79.3 (C-3"), 76.9 (C-2"), 76.8 (C-3'), 75.7 (C-5'), 73.8 (C-4"), 73.7 (C-2'), 70.5 (C-4'), 67.6 (C-5"), 64.4 (C-6'), 55.4 (5-OCH_3).

Tachioside (15) – Colorless gum, FAB-MS *m/z*: 302 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.80 (1H, d, *J* = 3.0 Hz, H-2), 6.73 (1H, d, *J* = 8.5 Hz, H-5), 6.57 (1H, dd, *J* = 8.5, 3.0 Hz, H-6), 4.80 (1H, d, *J* = 7.5 Hz, H-1'), 3.68 (3H, s, 3-OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 152.1 (C-4), 149.7 (C-2), 141.2 (C-1), 119.4 (C-6), 106.5 (C-5), 103.2 (C-1'), 100.7 (C-3), 76.9 (C-3'), 76.6 (C-5'), 73.9 (C-2'), 70.3 (C-4'), 61.4 (C-6'), 55.6 (3-OCH_3).

Isotachioside (16) – Colorless gum, FAB-MS *m/z*: 302 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.02 (1H, d, *J* = 9.0 Hz, H-5), 6.80 (1H, d, *J* = 3.0 Hz, H-2), 6.28 (1H, dd, *J* = 9.0, 3.0 Hz, H-6), 4.80 (1H, d, *J* = 7.5 Hz, H-1'), 3.7.0 (3H, s, 3-OCH_3) ; ^{13}C-NMR (CD_3OD, 125 MHz): δ 153.8 (C-4), 150.9 (C-2), 139.9 (C-1), 119.4 (C-6), 106.5 (C-5), 103.2 (C-1'), 100.7 (C-3), 76.9 (C-3'), 76.6 (C-5'), 73.9 (C-2'), 70.3 (C-4'), 61.4 (C-6'), 55.4 (3-OCH_3).

Biophenol 2 (17) – Colorless gum, FAB-MS *m/z*: 339 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.10 (1H, d, *J* = 8.5 Hz, H-5), 6.73 (1H, d, *J* = 2.0 Hz, H-2), 6.65 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 4.69 (1H, d, *J* = 8.0 Hz, H-1'), 3.69 (2H, t, *J* = 7.0 Hz, H-8), 2.71 (2H, t, *J* = 7.0 Hz, H-7); ^{13}C-NMR (CD_3OD, 125 MHz): δ 147.2 (C-4), 144.1 (C-3), 135.1 (C-1), 120.2 (C-2), 118.1 (C-5), 116.5 (C-6), 103.6 (C-1'), 77.1 (C-5'), 76.9 (C-3'), 73.9 (C-2'), 70.2 (C-4'), 63.1 (C-8), 61.3 (C-6'), 38.5 (C-7).

2-(3,4-Dihydroxy)-phenyl-ethyl-O-β-D-glucopyranoside (18) – Colorless gum, FAB-MS *m/z*: 339 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.73 (1H, d, *J* = 2.0 Hz, H-2), 6.67 (1H, d, *J* = 8.0 Hz, H-5), 6.54 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 4.28 (1H, d, *J* = 8.0 Hz, H-1'), 4.02 (1H, m, H-8), 3.69 (1H, m, H-8), 2.76 (2H, m, H-7); ^{13}C-NMR (CD_3OD, 125 MHz): δ 144.7 (C-3), 143.3 (C-4), 130.1 (C-1), 119.9 (C-6), 115.7 (C-5), 114.9 (C-2), 102.9 (C-1'), 76.7 (C-3'), 76.5 (C-5'), 73.7 (C-2'), 70.7 (C-4'), 70.2 (C-8), 61.3 (C-6'), 35.2 (C-7).

Cuneataside C (19) – Colorless gum, FAB-MS *m/z*: 471 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.68 (1H, d, *J* = 2.0 Hz, H-2), 6.67 (1H, d, *J* = 8.0 Hz, H-5), 6.55 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 5.00 (1H, d, *J* = 3.0 Hz, H-1"), 4.27 (1H, d, *J* = 7.5 Hz, H-1'), 4.02 (1H, m, H-8), 3.69 (1H, m, H-8), 2.76 (2H, m, H-7); ^{13}C-NMR (CD_3OD, 125 MHz): δ 144.7 (C-3), 143.3 (C-4), 130.0 (C-1), 119.9 (C-6), 115.7 (C-5), 114.9 (C-2), 109.6 (C-1"), 103.0 (C-1'), 79.2 (C-3"), 76.6 (C-2"), 75.5 (C-3'), 73.7 (C-5'), 73.6 (C-4"), 70.8 (C-2'), 70.3 (C-8), 67.3 (C-4'), 64.1 (C-5"), 62.9 (C-6'), 35.2 (C-7).

NGF and cell viability assays – C6 glioma cells were used to measure NGF release into the medium.11 C6 cells were purchased from the Korean Cell Line Bank and maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator with 5% CO_2. To measure NGF content in medium and cell viability, C6 cells were seeded into 24-well plates (1×10^5 cells/well). After 24 h, the cells were treated with DMEM containing 2% FBS and 1% penicillin-streptomycin with 20 μM of each isolated compound for one day. Media supernatant was used for the NGF assay using an ELISA development kit (R&D Systems). Cell viability was assessed by the MTT assay.

Results and Discussion

Compounds **1 - 19** were identified as lariciresinol-9-*O*-β-D-glucopyranoside (**1**),[6] alangilignoside C (**2**),[7] conicaoside C (**3**),[8] (+)-lyoniresinol 9'-*O*-β-D-glucopyranoside (**4**),[9, 10] (8*S*,8'*R*,7'*R*)-9'-[(β-glucopyranosyl)oxy]lyoniresinol (**5**),[11] blumenyl B β-D-glucopyranoside (**6**),[12] blumenyl A β-D-glucopyranoside (**7**),[12] staphylionoside D (**8**),[13] Icariside B_2 (**9**),[14] (6*R*,9*S*)-3-oxo-α-ionol β-D-glucopyranoside (**10**),[15] 3-oxo-α-ionol 9-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**11**),[16] blumenol B 9-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**12**),[16] benzyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside (**13**),[17] canthoside C (**14**),[18] tachioside (**15**),[19] isotachioside (**16**),[20] biophenol 2 (**17**),[20] 2-(3,4-dihydroxy)-phenyl-ethyl-O-β-D-glucopyranoside (**18**),[21] and cuneataside C (**19**)[22] by comparing the ^{1}H-, ^{13}C-NMR, and MS spectral data with the literature values. All the isolated compounds **1 - 19** were reported from this source for the first time. The following describes the structural elucidation of compound **3**, which upregulated NGF secretion without significant cell toxicity.

Compound **3** was obtained as a colorless gum. From the FAB-MS (*m/z*: 575 $[M + Na]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **3** was deduced to be $C_{27}H_{36}O_{12}$. The ^{1}H-NMR spectrum showed five aromatic protons at δ_H 7.25 (1H, d, *J* = 8.0 Hz), 7.10 (2H, s), 7.05 (1H, d, *J* = 1.8 Hz), and 6.50 (1H, dd, *J* = 8.0, 1.8 Hz), three methoxy groups at δ_H 3.72 (9H, s). The coupling patterns of five aromatic protons suggested the

Table. 1. Effects of Compounds **1** - **19** on NGF secretion and cell viability in C6 cells[a]

Compounds	NGF secretion (%)	Cell viability (%)
1	100.5 ± 0.4	88.7 ± 2.0
2	118.8 ± 3.6	84.5 ± 4.5
3	128.2 ± 9.3	85.4 ± 18.0
4	88.7 ± 6.0	104.6 ± 6.8
5	127.3 ± 10.3	62.9 ± 4.7
6	111.1 ± 7.1	104.9 ± 2.0
7	101.0 ± 6.7	96.6 ± 4.4
8	64.8 ± 0.6	102.0 ± 0.7
9	32.2 ± 14.4	41.3 ± 3.0
10	51.5 ± 1.4	101.9 ± 2.6
11	72.2 ± 0.2	94.5 ± 3.0
12	89.8 ± 5.5	93.2 ± 1.1
13	51.8 ± 5.1	111.9 ± 0.8
14	53.2 ± 13.6	111.5 ± 0.2
15	52.0 ± 0.7	114.1 ± 2.9
16	71.1 ± 6.0	104.3 ± 4.3
17	71.7 ± 30.5	103.3 ± 0.9
18	75.3 ± 6.0	104.5 ± 1.9
19	57.6 ± 13.2	106.4 ± 3.1
6-Shogaol[b]	126.5 ± 8.5	103.8 ± 4.1

[a] C6 cells were treated with 20 μM of compounds **1** - **19**. After 24 h, the content of NGF secretion in C6-conditioned media was measured by ELISA, and the cell viability was determined by MTT assay. The level of secreted NGF and viable cells are expressed as percentage of the untreated control. The data shown represent the means ± SD of three independent experiments performed in triplicate. [b] 6- Shogaol as positive control.

existence of typical 1,3,4-trisubstituted and 1,3,4,5-tetrasubstituted benzene rings. In addition, signals attributable to sugar moiety were observed at δ_H 5.70 (1H, d, J = 7.0 Hz) and 4.50-4.00 (5H, m) in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum demonstrated the presence of 27 carbon signals, consisting of 12 aromatic carbon signals including 5 oxygenated aromatic carbon signals [δ_C 153.1 (×2), 147.8, 144.7, and 134.3] and three methoxy carbon signals [δ_C 55.9 (×2), 55.2]. The presence of anomeric carbon signal at δ_C 104.3 and five oxygenated carbon signals (δ 77.2, 76.7, 74.6, 70.2, and 61.4) suggested the presence of D-glucose (Stephen *et al*., 1977). The coupling constant (J = 7.0 Hz) of the anomeric proton of D-glucose indicated that it was the β-form.[23] Based on the above evidences, the structure of **3** was determined to be conicaoside.[8]

The isolated compounds **1** - **19** were evaluated for their effects on NGF induction using C6 glial cells. As shown in Table 1, compound **5** was stimulants of NGF secretion in C6 cells (127.3 ± 10.3%). However, compound **5** induced significant cytotoxicity (62.9 ± 4.7%) at a concentration of 20 μM. Also, Compounds **2**, **3** and **6** increased NGF secretion to 118.8 ± 3.6%, 128.2 ± 9.3% and 111.1 ± 7.1% of untreated control and nomal cell viability (84.5 ± 4.5%, 85.4 ± 18.0 and 104.9 ± 2.0, respectively).

The most potent stimulant of NGF release, conicaoside (**3**), may have a potential for neuroprotection via inducing NGF secretion and may deserve further investigation as a candidate for regulation of neurodegenerative diseases and diabetic polyneuropathy. The apparent activity of multiple components from this plant suggests the possibility of additive or synergistic effects which merits further investigation.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20120009850).

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Received May 2, 2014
Revised June 5, 2014
Accepted June 6, 2014