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Chemical Constituents of *Impatiens balsamina* Stems and Their Biological Activities

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Abstract – The purification of the MeOH extract from *Impatiens basamina* by repeated column chromatography led to the isolation of one new tetrahydronaphthalene (**1**), together with eleven known compounds (**2 - 12**). The structure of the new compound (**1**) was determined by spectral data analysis (^{1}H and ^{13}C-NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, NOESY, and HR-ESI-MS). Isolated compounds (**1 - 12**) were evaluated for their inhibitory effects on NO production in LPS-activated murine microglial BV-2 cells and their effects on NGF secretion from C6 glioma cells. Compounds **3**, **7,** and **10** reduced NO levels in LPS-activated murine microglial cells with IC_{50} values of 26.89, 25.59, and 44.21 μM, respectively. Compounds **1**, **5,** and **9** upregulated NGF secretion to 153.09 ± 4.66, 156.88 ± 8.86, and 157.34 ± 3.30%, respectively.
Keywords – *Impatiens balsamina,* balsaminaceae, tetrahydronaphthalene, neuroprotective effect

Introduction

Impatiens balsamina (Balsaminaceae), also known as Garden balsam, is widely distributed in Korea, China and India. *I. balsamina* has been used as Chinese medicine to treat anti-cancer and anti-inflammatory herb, also has been used as a Korean traditional medicine for the treatment of scrofulosis, carbuncle, and dysentery.[1-3] Previous phytochemical investigations on *I. balsamina* reported the isolation of triterpenoid saponins, quinones, coumarin, flavonoids, and phenolic compounds, and there were several reports on various biological activities.[4-10] Our earlier phytochemical investigation on white petal of *I. balsamina* resulted in the isolation of anti-neurodegenerative biflavonoid glycosides and anti-inflammatory phenolic compounds.[9,10] In a continuing search for bioactive constituents from Korean medical plant sources, we investigated the MeOH extract of stems of *I. balsamina*. The purification of hexane and EtOAc fractions by repeated column chromatography led to the isolation of one new tetrahydronaphthalene (**1**), together with eleven known compounds (**2 - 12**). The structure of **1** was established on spectral data analysis (^{1}H and ^{13}C-NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY). Isolated compounds (**1 - 12**) were evaluated for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine microglial cells and their effects on NGF secretion from C6 glioma cells.

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Experimental

General experimental procedures – Optical rotations were measured on a Jasco P-2000 polarimeter using methanol solvent. Ultraviolet (UV) spectra were recorded with a Shimadzu UV-1601 UV–vis spectrometer. High Resolution ESI Mass Spectrometer data were obtained with a Waters SYNAPT G2 mass spectrometer. Infrared (IR) spectra were recorded on a Bruker IFS-66/S Fourier-transform IR spectrometer. NMR spectra were recorded on Varian UNITY INOVA 500 NMR spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), and Bruker AVANCE III 700 NMR spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C). Preparative HPLC was performed using a Gilson 321 pump with a Shodex Refractive Index Detector, YMC-Triart C_{18} 5 μm column (250 × 10 mm) and HAISIL 100 silica 5 μm column (250 × 10 mm). Silica gel 60 (Merck, 70 - 230 mesh and 230 – 400 mesh) and RP-C_{18} silica gel (Merck, 230 – 400 mesh) was used for column chromatography. LPLC was performed over a LiChroprep Lobar-A silica 60 column (Merck, 240 mm × 10 mm i.d.) equipped with a FMI QSY-0 pump. Merck precoated

silica gel F_{254} plates and reversed-phase (RP)-18 F_{254s} plates (Merck) were used for thin-layer chromatography (TLC). Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid

Plant materials – The dried stems of *I. balsamina* (2 kg) were collected at Asan in Chungcheongnam-Do, Korea, in August 2014. The plants were identified by one of the authors (K.R.L.). A voucher specimen (SKKU-NPL-1425) of the plant was deposited in the herbarium of the School of Pharmacy at Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – The dried stems of *I. balsamina* (2 kg) were extracted with 80% MeOH at room temperature. The filtrate was evaporated *in vacuo* to yield MeOH extract (294 g), which was suspended in distilled H_2O (2.4 L) and then successively partitioned with hexane, $CHCl_3$, EtOAc and *n*-BuOH, yielding 20, 11, 6, and 28 g, respectively. The EtOAc fraction (5 g) was separated over a silica gel column with a solvent system of $CHCl_3$/MeOH/water (7:1:0.1) to yielded five sub-fractions (E1-E5). Sub-fraction E1 (1.8 g) was chromatographed on a RP-C_{18} silica gel column with 30% aqueous MeOH to yield nine sub-fractions (E11-E19). Fraction E12 (430 mg) was purified by RP-C_{18} semi-prep. HPLC (30% MeOH) to yield **2** (9 mg, t_R = 17.1 min). Fraction E2 (1.1 g) was separated by RP-C_{18} silica gel column with 30% aqueous MeOH to give nine sub-fractions (E21-E29). Sub-fraction E23 (161 mg) was purified using RP-C_{18} semi-prep. HPLC (30% MeOH) to yield **1** (20 mg, t_R = 15.1 min), **6** (24 mg, t_R = 21.1 min), and **3** (7 mg, t_R = 22.4 min). E3 (557 mg) was subjected to RP-C_{18} silica gel column with 45% aqueous MeOH to give five sub-fractions (E31-E35). Fraction E31 (148 mg) was purified by RP-C_{18} semi-prep. HPLC (30% CH_3CN) to yield **4** (100 mg, t_R = 11.1 min). Fraction E5 (235 mg) was purified using RP-C_{18} semi-prep. HPLC (20% CH_3CN) to yield **5** (32 mg, t_R = 21.2 min). The hexane soluble fraction was chromatographed on a silica gel column with hexane/EtOAc (20:1 to 1:1) to give eight sub-fractions (H1-H8). Fraction H2 (546 mg) was subjected to using a RP-C_{18} silica gel column with 95% aqueous MeOH to yield **11** (9 mg, t_R = 34.4 min). Fraction H3 (918 mg) was separated to RP-C_{18} silica gel column with 90% aqueous MeOH to yield eight sub-fractions (H31-H38). Subfraction H32 (85 mg) was purified using RP-C_{18} semi-prep. HPLC (90% MeOH) to yield **7** (9 mg, t_R = 36.9 min). Fraction H36 (117 mg) was purified by semi-prep. HPLC ($CHCl_3$/MeOH 120:1) to give **12** (88 mg, t_R = 11.0 min). Fraction H5 (2.4 g) was chromatographed on a RP-C_{18} silica gel column (95% aqueous MeOH) to give twelve sub-fractions (H51-H512). Subfraction H56 (89 mg) was purified using RP-C_{18} semi-prep. HPLC (95% MeOH) to yield **8** (4 mg, t_R = 17.8 min). Fraction H57 (169 mg) was purified by semi-prep. HPLC (hexane/EtOAc 3:1) to yield **9** (3 mg, t_R = 15.3 min). Fraction H512 (112 mg) was purified using semi-prep. HPLC (hexane/EtOAc 3:1) to give **10** (7 mg, t_R = 8.8 min).

1*β*,2*α*,4*β*-Triol-1,2,3,4-tetrahydronaphthalene (1) – Colorless gum. $[\alpha]_D^{25}$: -35.8 (*c* 0.10, MeOH); IR (KBr) ν_{max} cm^{-1}: 3348, 2938, 2836, 1448, 1412, 1055, 1025; UV (MeOH) λ_{max} (log ε) 201 (0.75), 213 (0.45), 217 (0.38), 262 (0.02) nm; ^1H and ^{13}C NMR : see Table 1; HRESIMS *m/z* 181.0865 $[M+H]^+$; (calcd. for $C_{10}H_{13}O_3$, 181.0865).

1*β*,2*β*,4*β*-Triol-1,2,3,4-tetrahydronaphthalene (2) – Colorless gum. $[\alpha]_D^{25}$: -57.8 (*c* 0.11, MeOH); IR (KBr) ν_{max} cm^{-1}: 3340, 2944, 2832, 1460, 1408, 1054, 1021; UV (MeOH) λ_{max} (log ε) 203 (1.28), 211 (1.11), 215 (0.92), 256 (0.04) nm; ^1H and ^{13}C NMR : see Table 1.

Table 1. ^1H and ^{13}C NMR data of **1** and **2** in CD_3OD. (δ in ppm, 500 MHz for ^1H and 125 MHz for ^{13}C)[a]

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1	4.41 d (7.3)	73.6	4.64 d (3.5)	70.1
2	4.09 ddd (10.1, 7.3, 3.4)	68.9	3.89 dt (10.9, 3.5)	67.7
3	2.12 ddd (13.5, 10.1, 4.7)	37.1	2.15 ddd (12.2, 10.9, 9.5)	34.9
	2.21 ddd (13.5, 4.7, 3.4)		2.21 dddd (12.2, 6.1, 3.5, 1.0)	
4	4.89 m	66.6	4.73 dd (9.5, 6.1)	67.4
4a		137.5		139.4
5	7.41 dd (7.3, 1.5)	128.1	7.57 d (7.5)	126.7
6	7.34 td (7.3, 1.5)	127.1	7.30 td (7.5, 1.6)	127.9
7	7.29 td (7.3, 1.5)	127.6	7.34 td (7.5, 1.6)	127.3
8	7.56 d (7.3)	127.4	7.41 dd (7.5, 1.6)	129.6
8a		137.6		135.9

[a] *J* values are in parentheses and reported in Hz; the assignments were based on ^1H-^1H COSY, HSQC, and HMBC experiments.

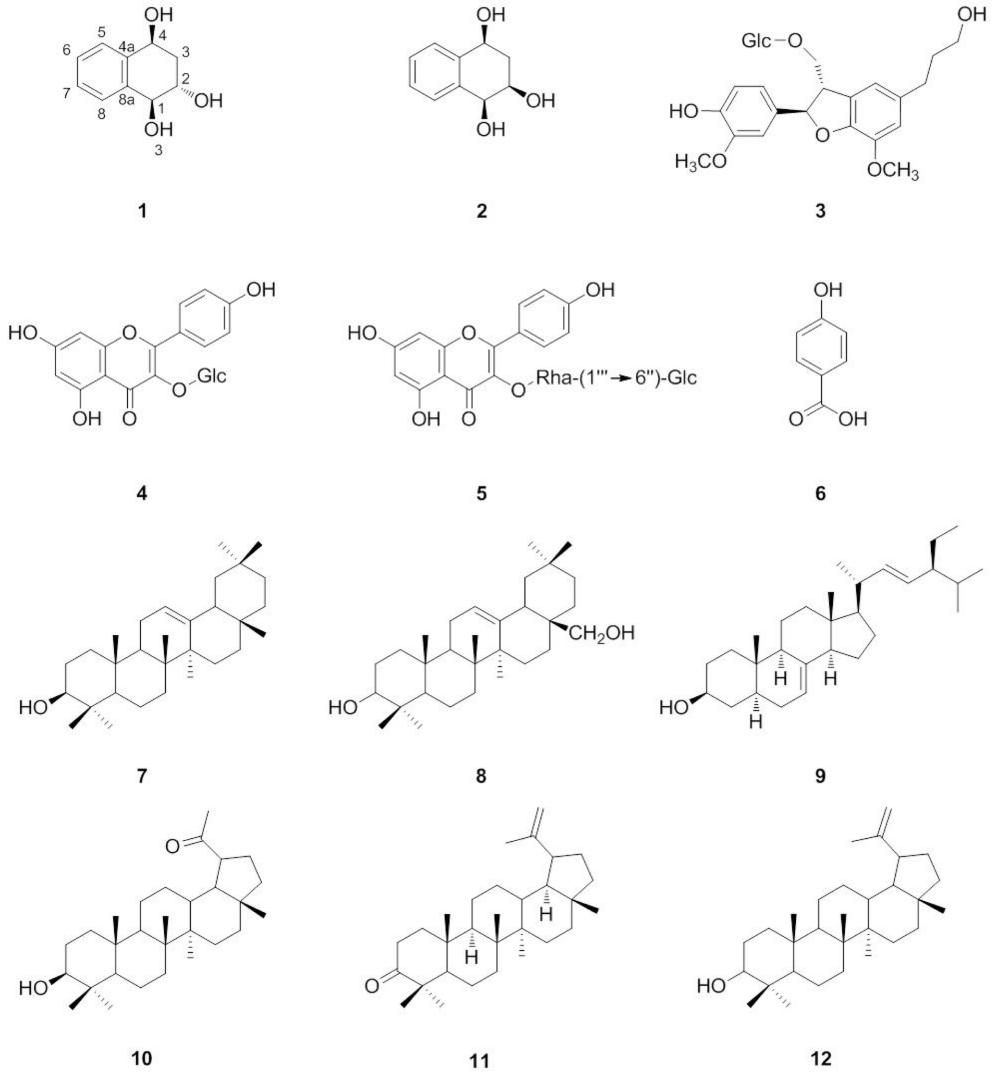

Fig. 1. Structures of compounds **1** - **12** isolated from *I. balsamina.*

(7*R*,8*S*)-Dihydrodehydrodiconiferyl alcohol-9-*β*-*O*-D-glucopyranoside (3) – White gum; ^{1}H NMR (CD_3OD, 500 MHz): δ 6.99 (1H, d, *J* = 1.9 Hz, H-2), 6.85 (1H, dd, *J* = 8.1, 1.9 Hz, H-6), 6.79 (1H, s, H-6'), 6.75 (1H, d, *J* = 8.1 Hz, H-5), 6.72 (1H, s, H-2'), 5.58 (1H, d, *J* = 6.4 Hz, H-7), 4.35 (1H, d, *J* = 7.8 Hz, H-1"), 3.85 (3H, s, 3'-OCH_3), 3.82 (3H, s, 3-OCH_3), 3.56 (2H, t, *J* = 6.5 Hz, H-9'), 2.62 (2H, m, H-7'), 1.81 (2H, tt, *J* = 13.1, 6.5 Hz, H-8'); ^{13}C NMR (CD_3OD, 125 MHz): δ 147.6 (C-3), 146.1 (C-4), 146.0 (C-4'), 143.8 (C-3'), 135.6 (C-1'), 133.2 (C-1), 128.3 (C-5'), 118.4 (C-6), 116.8 (C-6'), 114.6 (C-5), 112.8 (C-2'), 109.4 (C-2), 102.8 (C-1"), 87.8 (C-7), 76.8 (C-5"), 76.7 (C-3"), 73.7 (C-2"), 70.9 (C-9), 70.2 (C-4"), 61.3 (C-6"), 60.8 (C-9'), 55.4 (3'-OCH_3), 55.1 (3-OCH_3), 51.5 (C-8), 34.4 (C-8'), 31.5 (C-7').

Kaempferol-3-*O*-*β*-D-glucopyranoside (4) – Yellow needles; ^{1}H NMR (CD_3OD, 500 MHz): δ 8.08 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.91 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.43 (1H, s, H-8), 6.23 (1H, s, H-6), 5.28 (1H, d, *J* = 7.4 Hz, H-1"); ^{13}C NMR (CD_3OD, 125 MHz): δ 178.1 (C-4), 164.7 (C-7), 161.7 (C-5), 160.1 (C-4'), 157.7 (C-9), 157.1 (C-2), 134.0 (C-3), 130.8 (C-2', 6'), 121.4 (C-1'), 114.7 (C-3', 5'), 104.3 (C-10), 102.7 (C-1"), 98.5 (C-6), 93.6 (C-8), 77.2 (C-5"), 76.7 (C-3"), 74.3 (C-2"), 70.0 (C-4"), 61.2 (C-6").

Nicotiflorin (5) – Yellow needles; ^{1}H NMR (CD_3OD, 500 MHz): δ 8.09 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.91 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.42 (1H, s, H-8), 6.23 (1H, d, *J* = 1.8 Hz, H-6), 5.15 (1H, d, *J* = 7.4 Hz, H-1"), 4.54 (1H, d, *J* = 0.9 Hz, H-1'''), 3.66 (1H, m, H-2'''), 3.55 (1H, dd, *J* = 9.5, 3.3 Hz, H-3'''), 1.14 (3H, d, *J* = 6.2 Hz, H-6'''); ^{13}C

Table 2. Inhibitory effects of compounds **1** - **12** on NO production in LPS-activated BV-2 cells

Compound	IC_{50} (μM)[a]	Cell Viability (% of LPS)[b]
1	50.29	89.42 ± 6.36
2	>500	93.08 ± 5.10
3	26.89	109.90 ± 3.47
4	60.27	107.24 ± 2.72
5	>500	92.35 ± 7.51
6	108.55	112.35 ± 4.80
7	25.59	111.09 ± 4.11
8	>500	82.11 ± 2.89
9	>500	91.41 ± 7.19
10	44.21	101.07 ± 2.86
11	94.38	97.45 ± 11.50
12	>500	86.83 ± 4.21
L-NMMA[c]	21.40	100.14 ± 2.54

[a]IC_{50} value of each compound was defined as the concentration (μM) that caused
50% inhibition of NO production in LPS-activated BV-2 cells.
[b]Cell viability after treatment with 20 μM of each compound was determined by
MTT assay and is expressed in percentage (%). The results are averages of three
independent experiments, and the data are expressed as mean ± SD.
[c]L-NMMA as positive control.

NMR (CD_3OD, 125 MHz): δ 178.0 (C-4), 164.8 (C-7), 161.6 (C-5), 160.1 (C-4'), 158.0 (C-9), 157.2 (C-2), 134.1 (C-3), 130.1 (C-2', 6'), 121.4 (C-1'), 114.7 (C-3', 5'), 104.2 (C-10), 103.2 (C-1"), 101.0 (C-1'"), 98.6 (C-6), 93.5 (C-8), 76.8 (C-3"), 75.8 (C-5"), 74.4 (C-2"), 72.5 (C-4'"), 70.9 (C-2'"), 70.7 (C-3'"), 70.0 (C-4"), 68.3 (C-5'"), 67.2 (C-6"), 16.5 (C-6'").

***p*-Hydroxybenzoic acid (6)** – White gum; ^{1}H NMR (CD_3OD, 500 MHz): δ 7.90 (2H, d, J = 8.8 Hz, H-2', 6'), 6.84 (2H, d, J = 8.8 Hz, H-3', 5'); ^{13}C NMR (CD_3OD, 125 MHz): δ 170.1 (C-7), 163.3 (C-4), 133.0 (C-2, 6), 122.8 (C-1), 116.0 (C-3, 5).

***β*-Amyrin (7)** – Colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.19 (1H, t, J = 3.6 Hz, H-12), 3.22 (1H, dd, J = 11.0, 4.6 Hz, H-3), 1.14 (3H, s, H-27), 1.01 (3H, s, H-26), 0.97 (3H, s, H-23), 0.94 (3H, s, H-25), 0.88 (6H, s, H-29, 30), 0.84 (3H, s, H-28), 0.80 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 145.4 (C-13), 121.9 (C-12), 79.3 (C-3), 55.4 (C-5), 47.8 (C-9), 47.4 (C-18), 47.0 (C-19), 41.9 (C-14), 40.0 (C-8), 39.0 (C-4), 38.8 (C-1), 37.3 (C-10), 37.2 (C-22), 34.9 (C-21), 33.5 (C-29), 32.8 (C-7), 32.7 (C-17), 31.3 (C-20), 28.6 (C-28), 28.3 (C-23), 27.3 (C-2), 27.1 (C-16), 26.3 (C-15), 26.2 (C-27), 23.9 (C-30), 23.7 (C-11), 18.5 (C-6), 17.0 (C-26), 15.8 (C-25), 15.7 (C-24).

Table 3. Effects of compounds **1** - **12** on NGF secretion in C6 cells

Compound	NGF secretion (%)[a]	Cell Viability (%)[b]
1	153.09 ± 4.66	98.69 ± 3.69
2	102.90 ± 2.03	98.46 ± 11.25
3	105.83 ± 1.44	97.02 ± 8.02
4	137.97 ± 1.34	96.55 ± 7.38
5	156.88 ± 8.86	96.81 ± 17.29
6	149.21 ± 0.70	86.72 ± 0.02
7	129.76 ± 0.91	91.60 ± 0.70
8	94.75 ± 8.47	91.65 ± 1.20
9	157.34 ± 3.30	95.52 ± 0.38
10	130.55 ± 11.41	98.64 ± 4.12
11	151.05 ± 2.56	103.14 ± 0.62
12	104.93 ± 4.75	83.43 ± 2.48
6-Shogaol[c]	164.74 ± 1.95	100.34 ± 7.91

[a]C6 cells were treated with 20 μM of the compounds. After 24 h, the content of
NGF secreted into C6-conditioned media was measured by ELISA. The level of
secreted NGF is expressed as a percentage of the untreated control (set at 100%).
Data are the mean ± SD of three independent experiments performed in triplicate.
[b]Cell viability after treatment with 20 μM of each compound was determined by
the MTT assay and is expressed as a percentage (%). Results are the mean of three
independent experiments, and the data are expressed as the mean ± SD.
[c]6-shogaol was used as positive control.

Erythrodiol (8) – Colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.20 (1H, t, J = 3.5 Hz, H-12), 3.55 (1H, d, J = 10.9 Hz, H-28a), 3.22 (1H, d, J = 10.9 Hz, H-28b), 3.22 (1H, d, J = 10.9 Hz, H-3), 1.17 (3H, s, H-27), 1.00 (3H, s, H-23), 0.95 (3H, s, H-26), 0.94 (3H, s, H-25), 0.89 (3H, s, H-29), 0.88 (3H, s, H-30), 0.79 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 144.2 (C-13), 122.4 (C-12), 79.0 (C-3), 69.7 (C-28), 55.2 (C-5), 47.6 (C-9), 46.5 (C-19), 42.4 (C-18), 41.7 (C-14), 39.8 (C-8), 38.9 (C-4), 38.6 (C-1), 37.0 (C-10, 17), 34.1 (C-21), 33.2 (C-29), 32.6 (C-7), 31.0 (C-20), 30.9 (C-22), 28.0 (C-23), 27.2 (C-2), 25.9 (C-27), 25.6 (C-15), 23.6 (C-11), 23.5 (C-30), 22.0 (C-16), 18.3 (C-6), 16.7 (C-26), 15.6 (C-24), 15.5 (C-25).

***α*-Spinasterol (9)** – White needles; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.16 (1H, dd, J = 15.7, 8.7 Hz, H-22), 5.03 (1H, dd, J = 15.2, 8.6 Hz, H-23), 3.60 (1H, tt, J = 10.9, 4.5 Hz, H-3), 1.03 (3H, d, J = 6.6 Hz, H-3), 0.85 (3H, d, J = 6.5 Hz, H-26), 0.56 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 139.8 (C-8), 139.4 (C-22), 129.7 (C-23), 117.7 (C-7), 71.3 (C-3), 56.1 (C-17), 55.4 (C-14), 51.5 (C-24), 49.7 (C-9), 43.5 (C-13), 41.0 (C-20), 40.5 (C-5), 39.7 (C-12), 38.2 (C-4), 37.4 (C-1), 34.4 (C-10), 32.1 (C-

25), 31.7 (C-2), 29.9 (C-6), 28.7 (C-16), 25.6 (C-28), 23.2 (C-15), 21.8 (C-11), 21.6 (C-26), 21.3 (C-21), 19.2 (C-27), 13.2 (C-19), 12.5 (C-29), 12.3 (C-18).

29-Nor-20-oxolupeol (10) – White gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.19 (1H, dd, *J* = 11.4, 4.8 Hz, H-3), 2.58 (1H, td, *J* = 11.3, 6.0 Hz, H-19), 2.15 (3H, s, H-30), 1.02 (3H, s, H-26), 0.97 (6H, s, H-23, 27), 0.83 (3H, s, H-25), 0.78 (3H, s, H-28), 0.76 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 213.1 (C-20), 79.2 (C-3), 55.5 (C-5), 52.9 (C-19), 50.5 (C-9), 49.9 (C-18), 43.3 (C-14), 42.9 (C-17), 41.0 (C-8), 40.1 (C-22), 39.1 (C-4), 38.9 (C-1), 37.4 (C-10), 37.3 (C-13), 35.2 (C-16), 34.4 (C-7), 29.4 (C-30), 28.2 (C-23), 27.9 (C-21), 27.6 (C-15), 27.5 (C-2), 27.4 (C-12), 21.1 (C-11), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.1 (C-26), 15.6 (C-24), 14.7 (C-27).

Lupenone (11) – Colorless gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.69 (1H, d, *J* = 2.4 Hz, H-29a), 4.57 (1H, dd, *J* = 2.4, 1.4 Hz, H-29b), 1.69 (3H, brs, H-30), 1.07 (6H, s, H-24, 26), 1.03 (3H, s, H-23), 0.96 (3H, s, H-27), 0.93 (3H, s, H-25), 0.80 (3H, s, H-28); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 218.2 (C-3), 150.9 (C-20), 109.4 (C-29), 54.9 (C-5), 49.8 (C-9), 48.2 (C-18), 47.9 (C-19), 47.3 (C-4), 43.0 (C-14), 42.9 (C-17), 40.8 (C-8), 40.0 (C-22), 39.6 (C-2), 38.2 (C-13), 36.9 (C-10), 35.5 (C-16), 34.1 (C-1), 33.6 (C-7), 29.8 (C-21), 27.4 (C-15), 26.6 (C-23), 25.1 (C-12), 21.5 (C-11), 21.0 (C-24), 19.7 (C-6), 19.3 (C-30), 18.0 (C-28), 16.0 (C-25), 15.8 (C-26), 14.5 (C-27).

Lupeol (12) – White gum; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.69 (1H, d, *J* = 2.3 Hz, H-29a), 4.57 (1H, dd, *J* = 2.4, 1.4 Hz, H-29b), 3.19 (1H, dd, *J* = 11.4, 4.9 Hz, H-3), 2.37 (1H, td, *J* = 11.1, 5.8 Hz, H-19), 1.68 (3H, brs, H-30), 1.03 (3H, s, H-26), 0.97 (3H, s, H-27), 0.95 (3H, s, H-23), 0.83 (3H, s, H-25), 0.79 (3H, s, H-24), 0.76 (3H, s, H-28); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 151.2 (C-20), 109.4 (C-29), 79.1 (C-3), 55.6 (C-5), 50.5 (C-9), 48.7 (C-19), 48.3 (C-18), 43.2 (C-17), 43.1 (C-14), 41.1 (C-8), 40.3 (C-22), 39.0 (C-1, 4), 38.3 (C-13), 37.2 (C-10), 35.6 (C-16), 34.4 (C-7), 30.1 (C-21), 28.4 (C-23), 27.9 (C-15), 27.7 (C-2), 25.4 (C-12), 21.3 (C-11), 19.7(C-30), 18.6 (C-6), 18.3(C-28), 16.5 (C-25), 16.3 (C-26), 15.7 (C-24), 14.7 (C-27).

Measurement of NO Production and Cell Viability – Murine microglial BV-2 cells were plated into a 96-well plate (3×10^4 cells/well). After 24 h, cells were pretreated with samples for 30 min and then stimulated with 100 ng/mL of LPS for another 24 h. Nitrite, a soluble oxidation product of NO, was measured in culture media using the Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 540 nm was measured using a microplate reader. Sodium nitrite was used as a standard to calculate the NO_2^- concentration. Cell viability was tested by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. N^G-Mono-methyl-L-arginine was evaluated as a positive control.

NGF and cell viability assays – C6 glioma cells were used to measure NGF release into the medium. 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. 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 sample for one day. The cell viability of the C6 cells was tested via an MTT assay. 6-shogaol was evaluated as a positive control.

Result and Discussion

The structures of compounds **2** - **12** were determined by comparison of their spectroscopic data with those in the literatures to be 1*β*,2*β*,4*β*-triol-1,2,3,4-tetrahydronaphthalene (**2**),[11] (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol-9-*O*-*β*-D-glucopyranoside (**3**),[12] kaempferol-3-*O*-*β*-D-glucopyranoside (**4**),[13] nicotiflorin (**5**),[14] *p*-hydroxybenzoic acid (**6**),[15] *β*-amyrin (**7**),[16] erythrodiol (**8**),[17] *α*-spinasterol (**9**),[18] 29-nor-20-oxolupeol (**10**),[19] lupenone (**11**),[20] and lupeol (**12**).[21]

Compound **1** was obtained as a colorless gum. The molecular formula was determined to be $C_{10}H_{12}O_3$, based on molecular ion peak $[M+H]^+$ at *m/z* 181.0865 (calcd for $C_{10}H_{13}O_3$, 118.0865) in the positive-ion HR-ESI-MS. The IR spectrum of **1** indicated the presence of hydroxy group (3348 cm^{-1}). The ^{1}H NMR spectrum of **1** showed the presence of a 1,2-disubstituted aromatic ring protons at δ_H 7.56 (1H, d, *J* = 7.3 Hz), 7.41 (1H, dd, *J* = 7.3, 1.5 Hz), 7.34 (1H, td, *J* = 7.3, 1.5 Hz), and 7.29 (1H, td, *J* = 7.3, 1.5 Hz), three oxygenated proton signals at δ_H 4.89 (1H, m), 4.41 (1H, d, *J* = 7.3 Hz), and 4.09 (1H, ddd, *J* = 10.1, 7.3, 3.4 Hz) and one methylene proton signal at δ_H 2.21 (1H, ddd, *J* = 13.5, 4.7, 3.4 Hz), and 2.12 (1H, ddd, *J* = 13.5, 10.1, 4.7 Hz). The ^{13}C NMR spectrum displayed 10 carbon signals, including aromatic carbon signals at δ_C 137.6, 137.5, 128.1, 127.6, 127.4, and 127.1, three oxygenated carbon signals at δ_C 73.6, 68.9, and 66.6, one methylene carbon signal at δ_C 37.1. These NMR data were similar to 1*α*,2*α*,4*β*-triol-1,2,3,4-tetrahydronaphthalene, except for chemical shifts and coupling constants of methine protons.[22] The location of hydroxyl groups was confirmed by ^{1}H-^{1}H COSY correlations and HMBC data

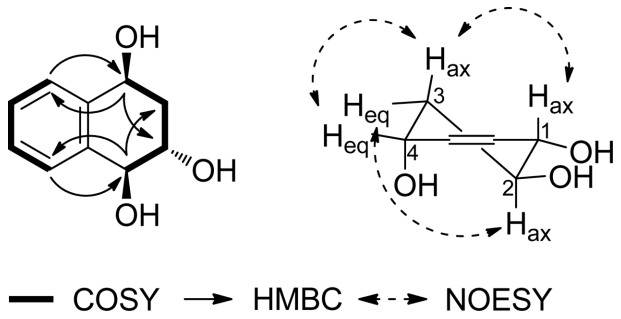

Fig. 2. Key ^{1}H-^{1}H COSY (▬), HMBC (⟶) and NOESY (⇠⋯⇢) correlations of **1.**

(Fig. 2). The relative configuration of **1** established based on coupling constants ($^3J_{1,2}$ = 7.3 Hz, $^3J_{2,3}$ = 10.1, 3.4 Hz, and $^3J_{3,4}$ = 4.7 Hz) of ^{1}H-NMR spectra, leading oxygenated protons at C-1, C-2, and C-4 to be as axial, axial, and equatorial positions, respectively (Fig. 2).[22,23] In the NOESY spectrum, correlations of H-3_{ax} with H-1 and H-4 indicated that OH groups at C-1 and C-4 were to be in the same orientation, and cross peak H-2/H-3_{eq} showed that OH groups at C-1 and C-2 were to be in the opposite orientation (Fig. 2). Thus, the structure of **1** was established as 1*β*,2*α*,4*β*-triol-1,2,3,4-tetrahydronaphthalene.

The isolated compounds (**1 - 12**) were evaluated for their inhibitory effects on NO production in LPS-activated murine microglial cells and their effects on NGF secretion from C6 glioma cells. Among them, compounds **3**, **7**, and **10** reduced NO levels in LPS-activated murine microglial BV-2 cells with IC_{50} values of 26.89, 25.59, and 44.21 μM, respectively. Compounds **1**, **5**, and **9** upregulated NGF secretion to 153.09 ± 4.66, 156.88 ± 8.86, and 157.34 ± 3.30%, respectively.

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