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Lignans and Macrolides from the Leaves of *Houttuynia cordata* with Inhibitory Activity against NO Production in Murine Macrophage RAW 264.7 Cells

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Abstract – Eight lignans (**1-8**) and two macrolides (**9-10**) were isolated from the $CHCl_3$-soluble fraction of *Houttuynia cordata* Thumb (Saururaceae). Structures of isolates were elucidated by extensive spectroscopic methods. Fatty acid-derived macrolides (**9-10**) have rarely been reported from nature. All the isolates were tested for their inhibitory activities against NO production in RAW 264.7 cells stimulated by LPS. It was found that compounds **3** and **9** remarkably down-regulated LPS-induced NO production with IC_{50} values, 5.4 and 40.3 μM, respectively, without cytotoxicity.
Keywords – Anti-inflammatory, *Houttuynia cordata*, Lignans, Macrolides

Introduction

Houttuynia cordata Thunb. is the sole species in genus *Houttuynia*, belonging to the family Saururaceae which comprises three genera, *Houttuynia*, *Gymnotheca* and *Saururus*. This plant has been used as traditional medicine to treat the inflammation-related disorders.[1] It has been reported that *H. cordata* contains various compounds including alkaloids, flavonoids and ionones.[2,3] In previous phytochemical studies, the extract of *H. cordata* showed anti-inflammatory activities.[4-6] Inflammation, the major host defense systems, is a key feature of various diseases including metabolic syndrome, cancer, infections, and wound.[7,8] Nitric oxide (NO) in cells involves inflammatory pathway. Moderate levels of NO assist regulation of pro-inflammatory cytokines, such as TNF-α, protecting the cell against stimulus-dependent apoptosis. However, excess NO not only stimulates excessive inflammatory response, such as apoptosis, but also improperly prevents the recruitment and activation of circulating cells, leading to various inflammatory diseases such as sepsis, psoriasis and systemic lupus erythematosus.[9-12] Lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cells have been widely used to explore the anti-inflammatory potency of compounds. The chloroform-soluble fraction of this plant was found to inhibit NO production induced LPS in RAW 264.7 cells, which led us to further investigate the $CHCl_3$-soluble fraction of *H. cordata*. Herein, the isolation of eight lignans (**1-8**) and two macrolides (**9-10**) (Fig. 1), and their inhibitory effects against NO production in RAW 264.7 cells were described.

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Experimental

General experimental procedures – Optical rotation was measured using a Jasco P-2000 digital polarimeter (Jasco, Tokyo, Japan). UV and ECD spectra were obtained with a Chirascan plus Circular Dichroism spectrometer (Chirascan, APL, UK). High-resolution electrospray ionization quadrupole-time-of-flight mass spectroscopy (HR-ESI-qTOF-MS) was performed using a Water Xevo G3 qTOF mass spectrometer (Waters MS Technologies, Manchester, UK). JEOL LA 300 (JEOL, Tokyo, Japan), Bruker GPX-400 and Bruker AVANCE-500 (Bruker, Ettlinger, Germany) were used to obtain NMR spectra. NMR solvents were DMSO-d_6 and methanol-d_4 (Cambridge Isotope Laboratories, Inc. Andover, MA, USA) and the reference peaks were set as δ_H 3.31 and δ_C 49.0 for methanol-d_4 and δ_H 2.50 and δ_C 39.5 for DMSO-d_6. Column chromatography (CC) was performed using Diaion HP-20 (Mitsubishi chemical corporation, Tokyo, Japan) or Kiesegel 60 silica gel (40-63 μm, 230-400 mesh Art. 9385; Merck, Darmstadt, Germany). Medium pressure liquid chromatography (MPLC) was performed on a

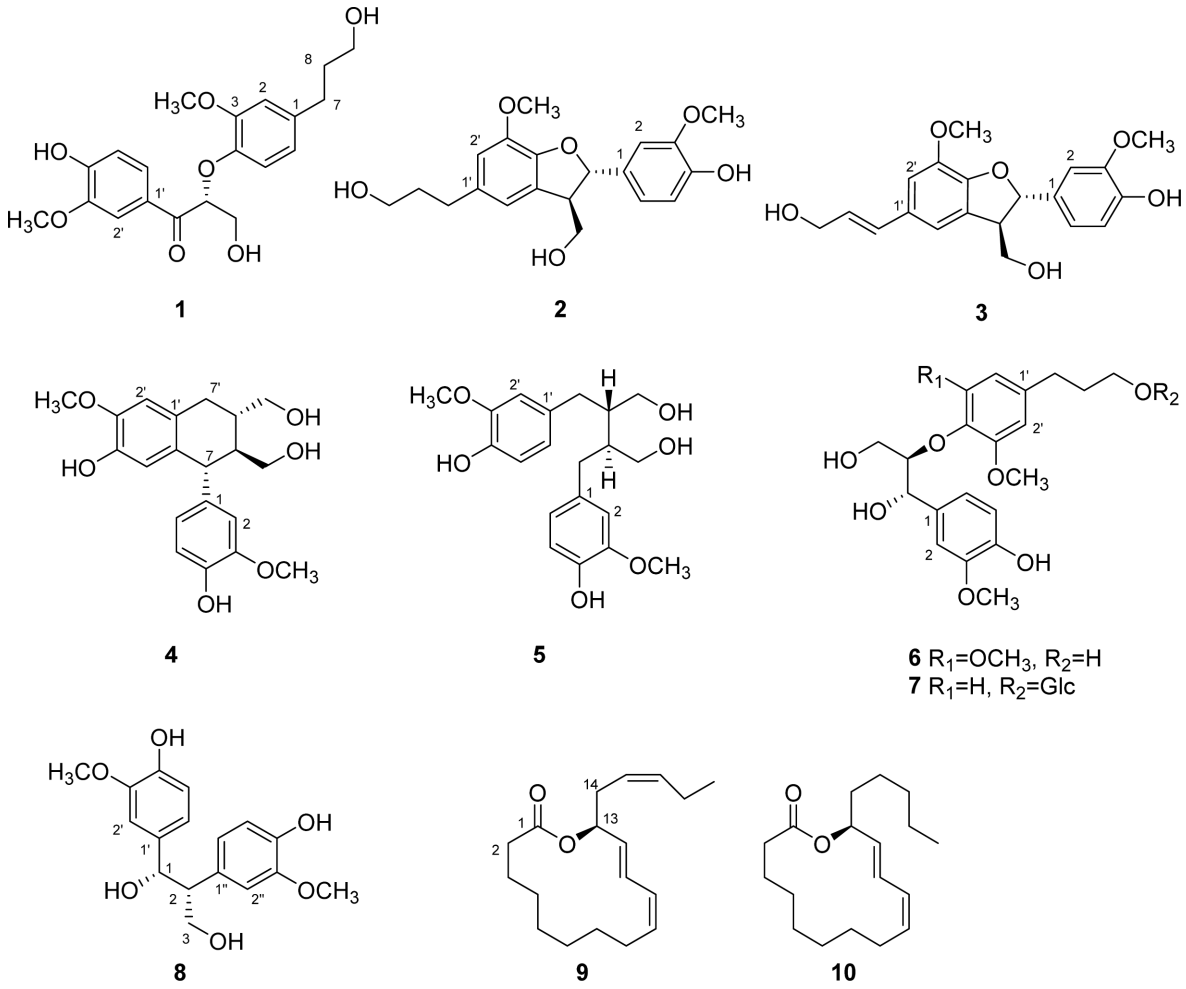

Fig. 1. Structures of compounds **1-10**.

Reveleris C18 reversed phase 40 g column (WR Grace, Columbia, MD, USA) using a Combiflash companion (Isco, Lincoln, NE, USA). High-pressure liquid chromatography (HPLC) was performed on a Gilson HPLC equipped with a Gilson 321 pump and UV/VIS 151 detector (Gilson, Middleton, WI, USA). Semi-preparative ODS columns (Luna 5 μ C18 (2) 100 Å, i.d. 250 × 10 mm, 5 μm Phenomenes Inc., Torrance, CA, USA; Inno C18 column 120 Å, i.d. 250 × 10 mm, 5 μm, Young Jin Biochrom Co., Ltd. Seongnam, Korea) and preparative ODS column (Luna 5 μ C18 (2) 100 Å, i.d. 250 × 21.20 mm, 5 μm Phenomenes Inc., Torrance, CA, USA) were used for separation. HPLC grade solvents were purchased from Fisher Scienific Korea Ltd. (Seoul, Korea). PBS, RPMI, penicillin and streptomycin were purchased from HyClone (Logan, UT, USA). Phosphorylated NF-κB (p-NF-κB), NF-κB (p65), IκBα and β-actin antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Bovine serum albumin, LPS and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material – The dried aerial parts of *Houttuynia cordata* were purchased from the Kyung-Dong oriental herbal market (Seoul, Korea) in August, 2016 and identified by Prof. Jinwoong Kim (Seoul National University, Korea). Specimen of the plant sample (SNUPH2016-02) has been deposited at the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation – The dried aerial parts of *H. cordata* (7.0 kg) were ground to fine powder. Powdered material was extracted three times with 40 L of MeOH and sonicated for 90 min at room temperature. After removal of the solvent under reduced pressure, the chlorophylls were removed using HP-20 with 90% aqueous MeOH. Total extract (900 g) was suspended in H_2O, and then partitioned with $CHCl_3$ and BuOH,

successively. The chloroform-soluble fraction (85.2 g) was subjected to a silica gel column chromatography eluting with gradient mixtures of $CHCl_3$/MeOH (50:1→0:1), and fractionated into 15 fractions (C01-C15). Fraction C09 (2.5 g) was fractionated using an RP-MPLC (40 g) with a stepwise gradient system of aqueous (aq.) MeOH (40%→ 100%), followed by Luna 5 μ preparative HPLC column (70% aq. MeCN) to give compound **9** (163.6 mg, t_R 32.8 min). 25 fractions (C11a-y) and compound **10** (13.6 mg) were obtained by the RP-MPLC (40 g) for C11 (1.1 g) using step-gradient elution with aq. MeOH (30%→ 100%). HPLC separation (Inno column, 20% aq. MeCN) was performed and compounds **7** (5.9 mg, t_R 15.3 min) and **8** (5.8 mg, t_R 20.3 min) were isolated from C11b (21.4 mg). Compound **4** (2.7 mg, t_R 14.2 min) was obtained from C11g (12.5 mg) using an Inno column (20% aq. MeCN). From fraction C11i (8.9 mg), compounds **5** (2.0 mg, t_R 28.0 min) and **6** (2.1 mg, t_R 17.9 min) were isolated using HPLC (Luna 5 μ, 25% aq. MeCN). Fraction C11k (75.2 mg) was further purified on a Luna 5 μ column (25% aq. MeCN) to give compounds **1** (3.0 mg, t_R 29.6 min), **2** (13.6 mg, t_R 40.9 min) and **3** (4.9 mg, t_R 13.5 min).

(*R*)-1-(3-Methoxy-4-hydroxyphenyl)-2-(3-methoxy-1-hydroxypropylphenoxy)-3-hydroxy-propan-1-one[13] (1) – colorless oil; $C_{20}H_{24}O_7$; $[\alpha]_D^{21}$ +32.2 (c 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 309 (3.79), 280 (3.89), 231 (4.11); CD (MeOH) λ_{max} nm (Δε) 331 (-0.10), 272 (+0.03); ^{1}H NMR (CD_3OD, 500 MHz): δ 6.82 (1H, d, J = 1.8 Hz, H-2), 6.75 (1H, d, J = 8.2 Hz, H-5), 6.63 (1H, dd, J = 8.2, 1.8 Hz, H-6), 2.59 (2H, t, J = 7.6 Hz, H-7), 1.77 (2H, m, H-8), 3.52 (2H, t, J = 6.5 Hz, H-9), 7.61 (1H, d, J = 2.0 Hz, H-2′), 6.86 (1H, d, J = 8.4 Hz, H-5′), 7.68 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 5.50 (1H, dd, J = 5.5, 4.3 Hz, H-8′), 4.01 (2H, m, H-9′), 3.77 (1H, s, 3-OCH_3), 3.87 (1H, s, 3′-OCH_3); ^{13}C NMR (CD_3OD, 125 MHz): 138.1 (C-1), 114.0 (C-2), 151.0 (C-3), 146.6 (C-4), 117.5 (C-5), 121.6 (C-6), 32.6 (C-7), 35.5 (C-8), 62.1 (C-9), 128.6 (C-1′), 112.6 (C-2′), 149.1 (C-3′), 153.9 (C-4′), 115.9 (C-5′), 125.2 (C-6′), 197.4 (C-7′), 84.1 (C-8′), 64.4 (C-9′), 56.3 (3-OCH_3); HRMS (ESI-QTOF) *m/z* 399.1428 $[M+Na]^+$ (calcd. for $C_{20}H_{24}O_7Na$, 399.1414).

(7*S*,8*R*)-Dihydrodehydrodiconiferyl alcohol[14] (2) – colorless oil; $C_{20}H_{24}O_6$; $[\alpha]_D^{21}$ +6.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 281 (3.87), 230 (4.22), 205 (4.84); CD (MeOH) λ_{max} nm (Δε) 294 (-0.64), 261 (+0.00), 242 (-1.65), 226 (+0.70); ^{1}H NMR (CD_3OD, 400 MHz): δ 6.94 (1H, d, J = 1.8 Hz, H-2), 6.75 (1H, d, J = 8.1 Hz, H-5), 6.81 (1H, dd, J = 8.1, 1.8 Hz, H-6), 5.48 (1H, d, J = 6.2 Hz, H-7), 3.45 (1H, m, H-8), 3.81 (1H, overlapped, H-9a), 3.74 (1H, dd, J = 11.0, 7.0 Hz, H-9b), 6.72 (1H, brs, H-2′), 6.72 (1H, brs, H-6′), 2.62 (2H, t, J = 7.6 Hz, H-7′), 1.80 (2H, m, H-8′), 3.56 (2H, t, J = 6.4 Hz, H-9′), 3.80 (3H, s, 3-OCH_3), 3.84 (3H, s, 3′-OCH_3); ^{13}C NMR (CD_3OD, 100 MHz): δ 134.8 (C-1), 110.5 (C-2), 149.1 (C-3), 147.5 (C-4), 116.1 (C-5), 119.7 (C-6), 89.0 (C-7), 55.4 (C-8), 65.0 (C-9), 136.9 (C- 1′), 114.1 (C-2′), 145.2 (C-3′), 147.5 (C-4′), 129.9 (C-5′), 117.9 (C-6′), 32.9 (C-7′), 35.8 (C-8′), 62.2 (C-9′), 56.4 (3-OCH_3), 56.7 (3′-OCH_3); HRMS (ESI-QTOF) *m/z* 383.1470 $[M+Na]^+$ (calcd. for $C_{20}H_{24}O_6Na$, 383.1465).

(7*S*,8*R*)-Dehydrodiconiferyl alcohol[15] (3) – yellowish oil; $C_{20}H_{22}O_6$; $[\alpha]_D^{21}$ -1.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 277 (4.11); CD (MeOH) λ_{max} nm (Δε) 275 (-0.47), 267 (-0.50), 236 (-0.01), 212 (-0.75); ^{1}H NMR (CD_3OD, 300 MHz): δ 6.95 (1H, overlapped, H-2), 6.77 (1H, d, J = 8.1 Hz, H-5), 6.83 (1H, dd, J = 8.1, 1.8 Hz, H-6), 5.52 (1H, d, J = 6.3 Hz, H-7), 3.49 (1H, dd, J = 12.4, 6.3 Hz, H-8), 3.81 (2H, m, H-9), 6.94 (1H, overlapped, H-2′), 6.97 (1H, overlapped, H-6′), 6.54 (1H, d, J = 15.8 Hz, H-7′), 6.23 (1H, ddd, J = 15.8, 5.9, 5.9 Hz, H-8′), 4.21 (1H, d, J = 5.9 Hz, H-9′a), 4.20 (1H, d, J = 5.9 Hz, H-9′b), 3.82 (3H, s, 3-OCH_3), 3.88 (3H, s, 3′-OCH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 134.5 (C-1), 110.5 (C-2), 149.1 (C-3), 147.5 (C-4), 116.1 (C-5), 119.7 (C-6), 89.3 (C-7), 55.2 (C-8), 64.9 (C-9), 132.5 (C- 1′), 112.0 (C-2′), 145.5 (C-3′), 149.2 (C-4′), 129.9 (C-5′), 116.5 (C-6′), 132.0 (C-7′), 127.5 (C-8′), 63.9 (C-9′), 56.3 (3-OCH_3), 56.7 (3′-OCH_3); HRMS (ESI-QTOF) *m/z* 359.1493 $[M+H]^+$ (calcd. for $C_{20}H_{23}O_6$, 359.1489).

(7*R*,8*S*,8′*S*)-Isolariciresinol[16,17] (4) – colorless oil; $C_{20}H_{24}O_6$; $[\alpha]_D^{21}$ +3.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 284 (3.66), 224 (4.04), 202 (4.56); CD (MeOH) λ_{max} nm (Δε) 292 (+0.97), 275 (-0.50), 253 (+0.10), 239 (-0.70), 228 (+0.14), 215 (-1.10); ^{1}H NMR (CD_3OD, 800 MHz): δ 6.65 (1H, d, J = 1.9 Hz, H-2), 6.72 (1H, d, J = 8.0 Hz, H-5), 6.59 (1H, dd, J = 8.0, 1.9 Hz, H-6), 3.78 (1H, d, J = 5.1 Hz, H-7), 1.74 (1H, m, H-8), 3.66 (1H, overlapped, H-9a), 3.37 (1H, dd, J = 11.3, 4.1 Hz, H-9b), 6.63 (1H, s, H-2′), 6.16 (1H, s, H-5′), 2.75 (2H, d, J = 7.7 Hz, H-7′), 1.98 (1H, m, H-8′), 3.71 (1H, dd, J = 11.0, 4.9 Hz, H-9′a), 3.65 (1H, overlapped, H-9′b), 3.75 (3H, s, 3-OCH_3), 3.79 (3H, s, 3′-OCH_3); ^{13}C NMR (CD_3OD, 200 MHz): δ 138.6 (C-1), 113.8 (C-2), 149.0 (C-3), 146.0 (C-4), 116.0 (C-5), 123.2 (C-6), 48.1 (C-7), 48.0 (C-8), 62.2 (C-9), 134.2 (C-1′), 112.4 (C-2′), 147.2 (C-3′), 145.3 (C-4′), 117.4 (C-5′), 129.0 (C-6′), 33.6 (C-7′), 40.0 (C-8′), 65.9 (C-9′), 56.3 (3-OCH_3), 56.4 (3′-OCH_3); HRMS (ESI-QTOF) *m/z* 383.1472 $[M+Na]^+$ (calcd. for $C_{20}H_{24}O_6Na$, 383.1465).

(+)-(8*S*,8′*S*)-Secoisolariciresinol[18] (5) – colorless oil; $C_{20}H_{26}O_6$; $[\alpha]_D^{21}$ +27.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max}

nm (log ε) 280 (3.58), 229 (4.10), 202 (4.69); CD (MeOH) λ_{max} nm (Δε) 280 (-0.12), 256 (+0.05), 242 (-0.52), 229 (+0.06), 213 (-0.20); ^{1}H NMR (CD_3OD, 800 MHz): δ 6.58 (2H, d, *J* = 1.8 Hz, H-2, 2′), 6.65 (2H, d, *J* = 7.9 Hz, H-5, 5′), 6.54 (2H, dd, *J* = 7.9, 1.8 Hz, H-6, 6′), 2.65 (2H, dd, *J* = 13.7, 7.1 Hz, H-7a, 7′a), 2.57 (2H, dd, *J* = 13.7, 7.8 Hz, H-7b, 7′b), 1.89 (2H, m, H-8, 8′), 3.58 (2H, dd, *J* = 11.1, 5.0 Hz, H-9a, 9′a), 3.54 (2H, dd, *J* = 11.1, 4.5 Hz, H-9b, 9′b), 3.73 (6H, s, 3-OCH_3, 3′-OCH_3); ^{13}C NMR (CD_3OD, 200 MHz): δ 133.8 (C-1, 1′), 113.3 (C-2, 2′), 148.8 (C-3, 3′), 145.4 (C-4, 4′), 115.7 (C-5, 5′), 122.7 (C-6, 6′), 36.0 (C-7, 7′), 44.1 (C-8, 8′), 62.1 (C-9, 9′), 56.1 (3-OCH_3, 3′-OCH_3); HRMS (ESI-QTOF) *m/z* 385.1608 $[M+Na]^+$ (calcd. for $C_{20}H_{26}O_6Na$, 385.1622).

***erythro*-1-(4-Hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-propane-1,3-diol[19] (6)** – colorless oil; $C_{21}H_{28}O_8$; $[\alpha]_D^{21}$ +0.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 280 (3.78), 229 (4.10); ^{1}H NMR (CD_3OD, 300 MHz): δ 6.98 (1H, d, *J* = 1.7 Hz, H-2), 6.73 (1H, d, *J* = 8.1 Hz, H-5), 6.78 (1H, dd, *J* = 8.1, 1.7 Hz, H-6), 4.91 (1H, d, *J* = 4.9 Hz, H-7), 4.16 (1H, m, H-8), 3.87 (1H, dd, *J* = 12.0, 5.6 Hz, H-9a), 3.52 (1H, dd, *J* = 12.0, 3.6 Hz, H-9b), 6.53 (2H, s, H-2′, 6′), 2.63 (2H, dd, *J* = 8.5, 7.0 Hz, H-7′), 1.81 (2H, m, H-8′), 3.56 (2H, t, *J* = 6.5 Hz, H-9′), 3.83 (3H, s, 3-OCH_3), 3.80 (6H, s, 3′-OCH_3, 5′-OCH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 133.7 (C-1), 111.3 (C-2), 148.7 (C-3), 146.8 (C-4), 115.7 (C-5), 120.5 (C-6), 74.0 (C-7), 87.5 (C-8), 61.5 (C-9), 139.9 (C- 1′), 106.8 (C-2′), 154.3 (C-3′), 134.7 (C-4′), 154.3 (C-5′), 106.8 (C-6′), 33.4 (C-7′), 35.5 (C-8′), 62.1 (C-9′), 56.3 (3-OCH_3), 56.6 (3′-OCH_3), 56.6 (5′-OCH_3); HRMS (ESI-QTOF) *m/z* 431.1683 $[M+Na]^+$ (calcd. for $C_{21}H_{28}O_8Na$, 431.1676).

***erythro*-(7*S*,8*R*)-4,7,9-Trihydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan-9'-*O*-*β*-D-glucopyranoside[20] (7)** – brown oil;$C_{26}H_{36}O_{12}$; $[\alpha]_D^{21}$ +8.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 280 (3.72), 229 (4.07); CD (MeOH) λ_{max} nm (Δε) 252 (+0.12), 239 (-0.30), 227 (+0.34); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.98 (1H, d, *J* = 1.7 Hz, H-2), 6.67 (1H, d, *J* = 8.1 Hz, H-5), 6.76 (1H, dd, *J* = 8.1, 1.7 Hz, H-6), 4.70 (1H, d, *J* = 5.3 Hz, H-7), 4.21 (1H, m, H-8), 3.57 (2H, m, H-9), 6.76 (1H, d, *J* = 1.7 Hz, H-2′), 6.85 (1H, d, *J* = 8.3 Hz, H-5′), 6.65 (1H, dd, *J* = 8.3, 1.7 Hz, H-6′), 2.55 (2H, dd, *J* = 8.1, 8.1 Hz, H-7′), 1.77 (2H, m, H-8′), 3.77 (1H, m, H-9′a), 3.40 (1H, m, H-9′b), 3.72 (3H, s, 3-OCH_3), 3.69 (3H, s, 3′-OCH_3), 4.10 (1H, d, *J* = 7.6 Hz, Glc-1), 2.95 (1H, m, Glc-2), 3.13 (1H, m, Glc-3), 3.04 (1H, m, Glc-4), 3.06 (1H, m, Glc-5), 3.66 (1H, m, Glc-6a), 3.44 (1H, m, Glc-6b); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 133.3 (C-1), 111.4 (C-2), 146.9 (C-3), 145.4 (C-4), 114.6 (C-5), 119.4 (C-6), 71.6 (C-7), 83.9 (C-8), 60.1 (C-9), 134.7 (C- 1′), 113.0 (C-2′), 149.5 (C-3′), 146.0 (C-4′), 116.0 (C-5′), 120.1 (C-6′), 31.1 (C-7′), 31.2 (C-8′), 67.9 (C-9′), 55.4 (3-OCH_3), 55.6 (3′-OCH_3), 103.0 (Glc-1), 73.5 (Glc-2), 76.8 (Glc-3), 70.1 (Glc-4), 76.7 (Glc-5), 61.1 (Glc-6); HRMS (ESI-QTOF) *m/z* 539.2136 $[M-H]^-$ (calcd. for $C_{26}H_{35}O_{12}$, 539.2134).

***erythro*-1,2-Bis-(4-hydroxy-3methoxyphenyl)propane-1,3-diol[21] (8)** – colorless oil; $C_{17}H_{20}O_6$; $[\alpha]_D^{21}$ +15.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 280 (3.79), 226 (4.11); ^{1}H NMR (CD_3OD, 300 MHz): δ 4.92 (1H, d, *J* = 5.7 Hz, H-1), 2.91 (1H, m, H-2), 3.84 (1H, dd, *J* = 10.8, 6.5 Hz, H-3a), 3.68 (1H, m, H-3b), 6.63 (1H, m, H-2′), 6.70 (1H, m, H-5′), 6.67 (1H, m, H-6′), 6.66 (1H, m, H-2″), 6.61 (1H, m, H-5″), 6.62 (1H, m, H-6″), 3.70 (3H, s, 3′-OCH_3), 3.76 (3H, s, 3″-OCH_3); ^{13}C NMR (CD_3OD, 75 MHz): δ 75.5 (C-1), 56.8 (C-2), 64.4 (C-3), 136.4 (C-1′), 111.5 (C-2′), 148.4 (C-3′), 146.5 (C-4′), 115.6 (C-5′), 120.3 (C-6′), 132.2 (C-1″), 114.5 (C-2″), 148.3 (C-3″), 146.1 (C-4″), 115.4 (C-5″), 123.1 (C-6″), 56.3 (3′-OCH_3), 56.2 (3″-OCH_3); HRMS (ESI-QTOF) *m/z* 319.1190 $[M-H]^-$ (calcd. for $C_{17}H_{19}O_6$, 319.1187).

(9*Z*,11*E*,13*S*,15*Z*)-Octadeca-9,11,15-trien-13-olide[22,23] (9) – colorless oil; $C_{18}H_{28}O_2$; $[\alpha]_D^{21}$ +20.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 280 (3.29), 229 (4.76); ^{1}H NMR (CD_3OD, 300 MHz): δ 2.27 (2H, t, *J* = 7.4 Hz, H-2), 1.59 (2H, m, H-3), 1.38~1.30 (8H, m, H-4~7), 2.18 (2H, m, H-8), 5.40 (1H, m, H-9), 5.96 (1H, t, *J* = 11.2 Hz, H-10), 6.50 (1H, dd, *J* = 15.2, 11.2 Hz, H-11), 5.63 (1H, dd, *J* = 15.2, 6.6 Hz, H-12), 4.11 (1H, m, H-13), 2.31 (2H, m, H-14), 5.36 (1H, ddd, *J* = 10.9, 7.1, 1.4 Hz, H-15), 5.47 (1H, ddd, *J* = 10.9, 7.1, 1.3 Hz, H-16), 2.05 (2H, m, H-17), 0.95 (3H, t, *J* = 7.5 Hz, H-18); ^{13}C NMR (CD_3OD, 75 MHz): δ 177.6 (C-1), 35.0 (C-2), 26.1 (C-3), 30.7, 30.3, 30.2, 30.1 (C-4~7), 28.6 (C-8), 133.0 (C-9), 129.3 (C-10), 126.6 (C-11), 136.6 (C-12), 73.2 (C-13), 36.3 (C-14), 125.5 (C-15), 134.5 (C-16), 21.7 (C-17), 14.6 (C-18); HRMS (ESI-QTOF) *m/z* 277.2158 $[M+H]^+$ (calcd. for $C_{18}H_{29}O_2$, 277.2162).

(*S*)-Coriolide[22,23] (10) – colorless oil; $C_{18}H_{30}O_2$; $[\alpha]_D^{21}$ +13.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm (log ε) 280 (3.37), 233 (4.21); ^{1}H NMR (CD_3OD, 300 MHz): δ 2.27 (2H, t, *J* = 7.4 Hz, H-2), 1.59 (2H, m, H-3), 1.42~1.29 (8H, m, H-4~7), 2.19 (2H, m, H-8), 5.41 (1H, dt, *J* = 11.1, 7.3 Hz, H-9), 5.98 (1H, t, *J* = 11.1 Hz, H-10), 6.50 (1H, dd, *J* = 15.1, 11.1 Hz, H-11), 5.62 (1H, dd, *J* = 15.1, 6.3 Hz, H-12), 4.07 (1H, m, H-13), 1.50 (2H, m, H-14), 1.42 (1H, m, H-15a), 1.34 (1H, m, H-15b), 1.30 (2H, m, H-16), 1.32 (2H, m, H-17), 0.91 (3H, dd, *J* = 9.1, 4.7 Hz, H-18); ^{13}C NMR (CD_3OD, 75 MHz): δ 177.9 (C-1), 35.1

(C-2), 26.1 (C-3), 30.6, 30.5, 30.4, 30.2 (C-4~7), 28.6 (C-8), 132.9 (C-9), 129.3 (C-10), 126.5 (C-11), 137.2 (C-12), 73.4 (C-13), 38.4 (C-14), 26.6 (C-15), 32.6 (C-16), 23.6 (C-17), 14.4 (C-18); HRMS (ESI-QTOF) *m/z* 279.2326 $[M+H]^+$ (calcd. for $C_{18}H_{31}O_2$, 279.2319).

Cell culture of RAW 264.7cells – Murine macrophages, RAW 264.7 purchased from the Korean Research Institute of Bioscience and Biotechnology (Daejeon, Korea) were grown in RPMI medium with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin sulfate in a humidified 5% CO_2 atmosphere at 37ºC.

Measurement of NO production – The nitrite concentration in the culture medium was measured using NO indicator according to the Griess reaction.[24] RAW 264.7 cells (2×10^5 cells/well) were cultured with RPMI in 96-well plates with no phenol red, then pretreated with samples for 1 hour. Cellular NO production was induced by the addition of LPS and incubation for 24 hours. After incubation, 100 μL of media aliquot was added to the same volume of Griess reagent and incubated for 15 minutes. The absorbance at 540 nm was measured on ELISA microplate reader (Benchmark, Bio-Rad Laboratories, CA, USA). The concentration of nitrite was determined using nitrite standard curve.

MTT assay for cell viability – Cells (5×10^4 cells/well) were grown in 96-well plates and incubated with growth media in the presence of indicated concentration of samples. After incubation for 25 h, ten microliter of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (5 mg/mL in saline) was added. After further incubation for 4 h, supernatant was aspirated, and the formazan crystals were then solubilized in dimethyl sulfoxide (DMSO). Absorbance was measured at 540 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Benchmark, Bio-Rad Laboratories, CA, USA). Relative cell viability was determined compared with untreated control culture. All experiments were performed in triplicate.

Immunoblot analysis – Protein expression was assessed by Western blotting according to general procedure. Briefly, RAW 264.7 cells (2×10^6 cells/mL) were cultured in 60 mm culture dishes, with pretreatment of samples. After cells were washed twice in ice cold PBS (pH 7.4), it was resuspended in lysis buffer on ice for 15 min, and then centrifuged. Protein concentration was measured using protein assay reagent (Bio-Rad) according to the manufacturer's instructions. Protein (20 – 30 μg) was mixed with same volume of 5x sample buffer (20% glycerol, 4% SDS, 10% 2-ME, 0.05% bromophenol blue, and 1.25 M Tris [pH 6.8]), then run at 150 v for 90 min onto 8% SDS-polyacrylamide gel electrophoresis gels. The proteins-transferred membranes were incubated overnight with the primary antibody (diluted 1:500–1:1000) in Tris-buffered saline containing 0.1% Tween 20 and 5% skim milk. After that, the blots were washed with Tris-buffered saline with 0.1% Tween 20 three times and incubated for 1 h with a horseradish peroxidase conjugated secondary anti-IgG antibody (diluted 1:2000 – 1:20,000). It was washed three times with Tris-buffered saline (0.1% Tween 20), and immunoreactive bands were obtained using the chemiluminescent substrate ECL Plus (Amersham Biosciences, Piscataway, NJ, USA). A ChemiDoc Imaging system (ChemiDoc™ XRS system with Image Lab™ software 3.0; Bio-Rad, Hercules, CA, USA) was used to obtain the images.

Results and Discussion

Eight lignans and two macrolides were isolated from *H. cordata* leaves. Their structures were determined to be (*R*)-1-(3-methoxy-4-hydroxyphenyl)-2-(3-methoxy-1-hydroxypropylphenoxy)-3-hydroxy-propan-1-one (**1**), (7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol (**2**), (7*S*,8*R*)-dehydrodiconiferyl alcohol (**3**), (7*R*,8*S*,8′*S*)-isolariciresinol (**4**), (+)-(8*S*,8′*S*)-secoisolariciresinol (**5**), *erythro*-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxy phenoxy]-propane-1,3-diol (**6**), *erythro*-(7*S*,8*R*)-4,7,9-trihydroxy-3,3'-dimethoxy-8-*O*-4'-neolignan-9'-*O*-*β*-D-glucopyranoside (**7**), 1,2-bis-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol (**8**), (9*Z*, 11*E*, 13*S*, 15*Z*)-octadeca-9,11,15-trien-13-olide (**9**) and (*S*)-coriolide (**10**) by comparing their measured spectroscopic data with the literature. All compounds (**1**-**10**) were isolated for the first time from the family Saururaceae. Compounds **9** and **10** were fatty acid-derived macrolides, which have been rarely reported from nature. Compound **9** has been isolated only from the scent glands of the tropical butterflies *Heliconius cydno* and *H. pachinus*,[23] while (*S*)-coriolide (**10**) has been found previously in *Monnina emarginata* seed oil, the fungus *Stagonospora* and the scent glands of the tropical butterflies *Heliconius cydno* and *H. pachinus*.[23,25,26] The biological effects of these macrolides are not yet known, except for the cytotoxic activity of **10** against P388 mouse leukemia cells.

All the isolates were tested for their inhibitory effects on LPS-induced NO production in RAW 264.7 cells (Fig. 2). Compounds **3**, **9** and **10** showed moderate inhibitory activity against LPS-stimulated NO production with IC_{50} values of 5.4, 40.3 and 7.5 μM, respectively. Of them, compound **10** showed cytotoxicity to RAW 264.7 cells

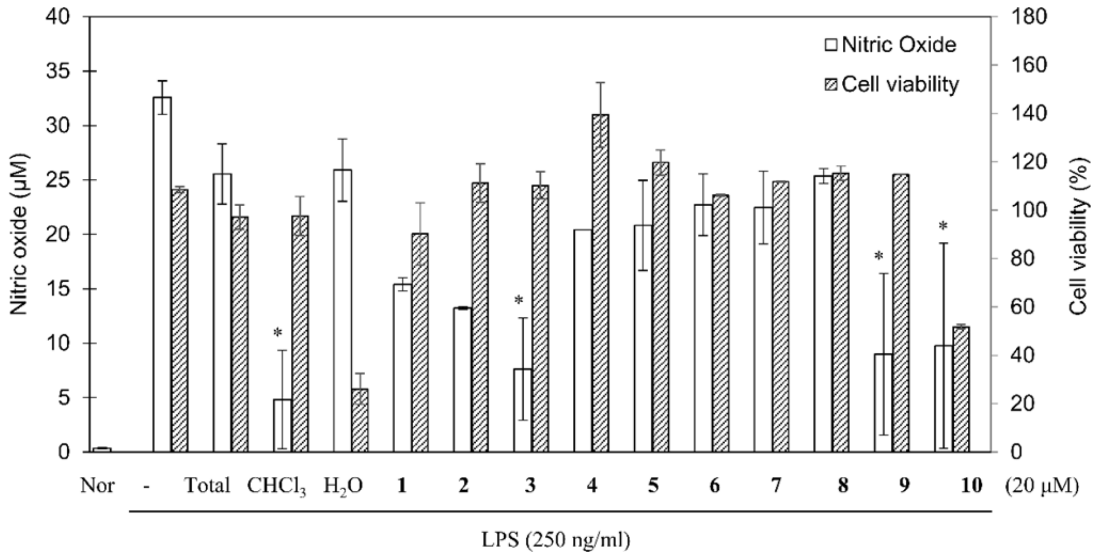

Fig. 2. Effects of extract, solvent-soluble fractions, and compounds (20 μM) on the NO production and viability of RAW 264.7 cells. The values shown are the means ± SEM of data from two experiments. *$P < 0.01$ indicates significant differences from the control group.

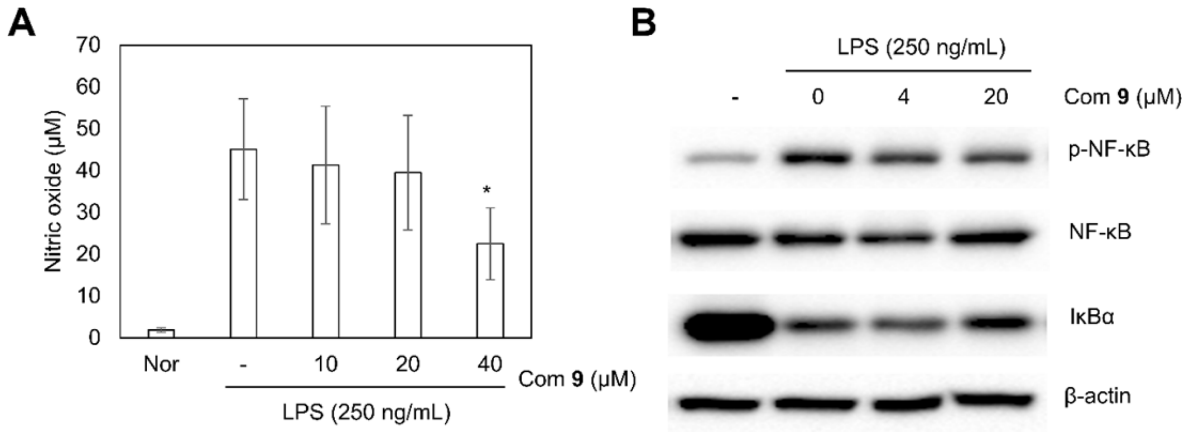

Fig. 3. Effects of compound **9** on activating the NF-κB pathway in the LPS-stimulated RAW 264.7 cells. (A) The effect of **9** on NO production in LPS-stimulated RAW 264.7 cells. The values shown are the mean ± SEM of data from three independent experiments. *$P < 0.01$ indicates significant differences from the control group. (B) The pNF-κB protein levels decreased with treatment of **9**.

(< 20 μM). We further evaluated the effects of **9** on activating the NF-κB pathway in the RAW 264.7 cell by Western blot. The pNF-κB protein levels were decreased by treatment of compound **9** (Fig. 3).

(7*S*,8*R*)-Dehydrodiconiferyl alcohol (**3**) was previously reported to have anti-inflammatory activities, anti-oxidative and anti-adipogenic activities in osteoblast, macrophage, lymphocyte and macroglia cell types.[24,27-31] It has previously been reported that compound **3** showed the effects on the regulation of various pro-inflammatory mediators, such as TNF-α, IL-6, IL-1β, NO, PGE_2 and ROS in LPS-stimulated RAW 264.7 cells.[27]

Herein, the diverse constituents including macrolides from the family Saururaceae were discovered. Macrolides have rarely been reported from plants. It is also noteworthy that **9** has anti-inflammatory potential in term of the undetermined bioactivities of fatty acid-derived macrolides.

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