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PHENOLIC DERIVATIVES FROM THE STEMS OF *LAGERSTROEMIA INDICA* AND THEIR BIOLOGICAL ACTIVITY

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Abstract - The purification of a MeOH extract from the stems of *Lagerstroemia indica* (Lythraceae) using repeated column chromatography afforded three new phenolic glycosides (**1-3**), and a new flavone derivative (**4**) along with fifteen known compounds (**5-19**). The structures of new compounds were determined through spectral analysis, including 1D, 2D-NMR and MS data. Compounds **1-19** were evaluated for their inhibitory activities on nitric oxide (NO) production in an activated murine microglial cell line and cytotoxic activities against four human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15).

Lagerstroemia indica (Lythraceae) is a decorative shrub, widely distributed in Korea, Japan, and China.[1] The root of this plant has been used as an astringent, detoxicant and diuretic in traditional Indian medicine.[2] Previous phytochemical investigations of *L. indica* have reported the isolation of alkaloids, triterpenes, and flavonoids.[2-5] Recent studies have reported that the active compound, cytisoside show antimicrobial activity against five pathogenic microorganisms.[6] Antioxidant and aldose reductase inhibitory activity of the its MeOH extract was also reported.[7,8] In the course of continuing to search for biologically active compounds from Korean medicinal plant sources, we performed a phytochemical investigation on the stems of *L. indica*. The $CHCl_3$, EtOAc, and *n*-BuOH fractions were subjected to repeated column chromatography on silica gel, sephadex LH-20, and semi-preparative HPLC separation to yield three new phenolic glycosides (**1**–**3**), named strosides A–C, and a new flavone derivative (**4**), lagerindiol, together with fifteen known compounds (**5**–**19**) (Figure 1). The structures of these new

compounds were determined by spectroscopic methods including 1D, 2D-NMR (COSY, HMQC, and HMBC) and enzyme hydrolysis. The isolated compounds (**1**-**19**) were evaluated for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line and their cytotoxicity against four human cancer cell lines *in vitro* using a sulforhodamine B bioassay.

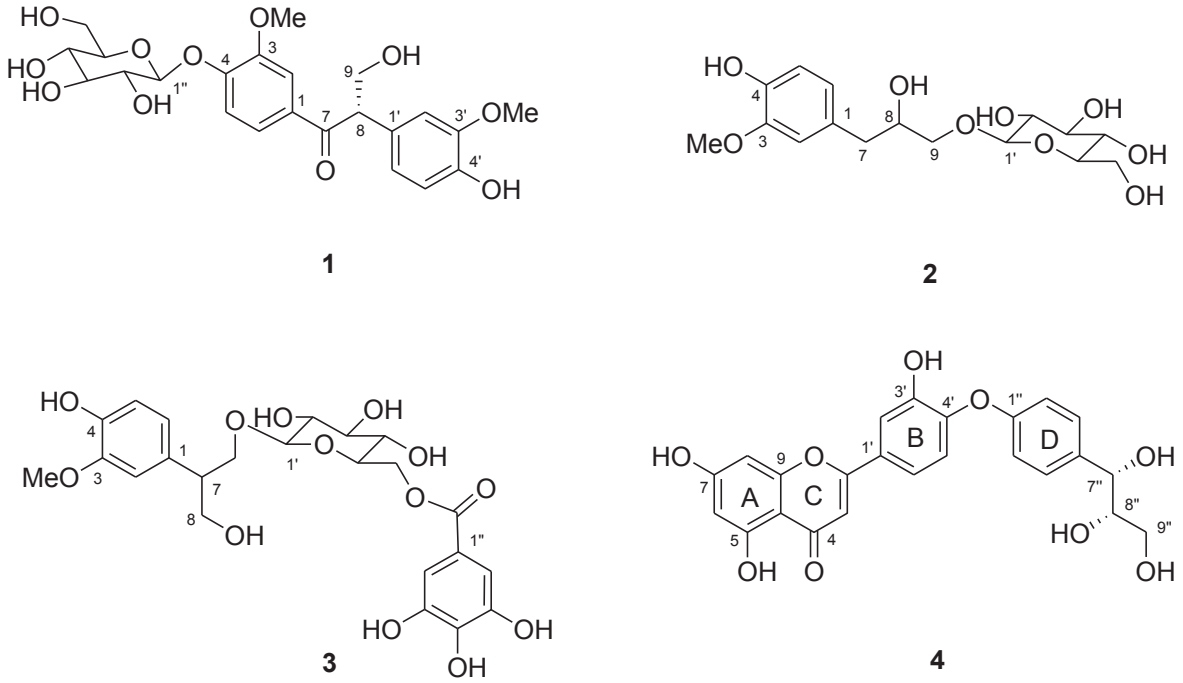

Figure 1. Structures of Compounds **1-4**

Compound **1** was obtained as a brown gum. In its HR-FAB-MS spectrum, the molecular ion $[M + H]^+$ was observed at *m/z* 481.1710 $[M + H]^+$ (calcd. for $C_{23}H_{29}O_{11}$: 481.1710), suggesting the molecular formula was $C_{23}H_{28}O_{11}$ of **1**. The ^{1}H-NMR spectrum showed the presence of two 1,3,4-trisubstitued aromatic rings [δ_H 7.69 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 7.62 (1H, d, *J* = 2.0 Hz, H-2), 7.18 (1H, d, *J* = 8.5 Hz, H-5), 6.90 (1H, d, *J* = 2.0 Hz, H-2'), 6.76 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'), 6.74 (1H, d, *J* = 8.5 Hz, H-5')], one oxymethylene [δ_H 4.27 (1H, dd, *J* = 10.5, 9.0 Hz, H-9a) and 3.73 (1H, dd, *J* = 10.5, 5.0 Hz, H-9b)], and one methine [δ_H 4.78 (1H, dd, *J* = 8.5, 5.0 Hz, H-8)], and two methoxy protons [δ_H 3.89 (3H, s, 3-OCH_3) and 3.84 (3H, s, 3'-OCH_3)]. In the ^{13}C-NMR spectrum, 17 carbon signals, including 12 aromatic carbons, one ketone [δ_C 199.3, (C-7)], one oxymethylene [δ_C 65.5, (C-9)], one methine [δ_C 56.7 (C-8)], and two methoxy groups [δ_C 56.7, (3-OCH_3) and 56.5 (3'-OCH_3)] exhibited, together with a glucose unit [δ_C 101.9 (C-1"), 78.4 (C-3"), 78.0 (C-5"), 74.8 (C-2"), 71.4 (C-4"), and 62.6 (C-6") in the ^{13}C-NMR].[9] The NMR data were very similar to those of evofolin-B (**18**),[10] except for the additional glucose moiety. Enzyme hydrolysis of **1** afforded the aglycone and sugar residue. The aglycone was

confirmed to be evofolin-B (**1a**) by comparison of ^{1}H-NMR and FAB-MS data.[10] The D-glucose was detected by co-TLC comparison and identified by the sign of its specific rotation value.[11] The coupling constant (J = 7.5 Hz) of the anomeric proton at δ_H 5.01 indicated the β-orientation of glucose.[9] The position of glucose was established by an HMBC experiment, in which a long-range correlation was identified between the H-1" (δ_H 5.01) and the C-4 (δ_C 152.3) (Figure 2). The absolute configuration of **1** was assigned as 8*S* by the analysis of circular dichroism (CD) spectrum of **1** showing the negative Cotton effects at 222 and 310 nm and positive Cotton effects 208 and 238 nm.[12] Thus, the structure of compound **1** was determined as shown in Figure 1 and named stroside A.

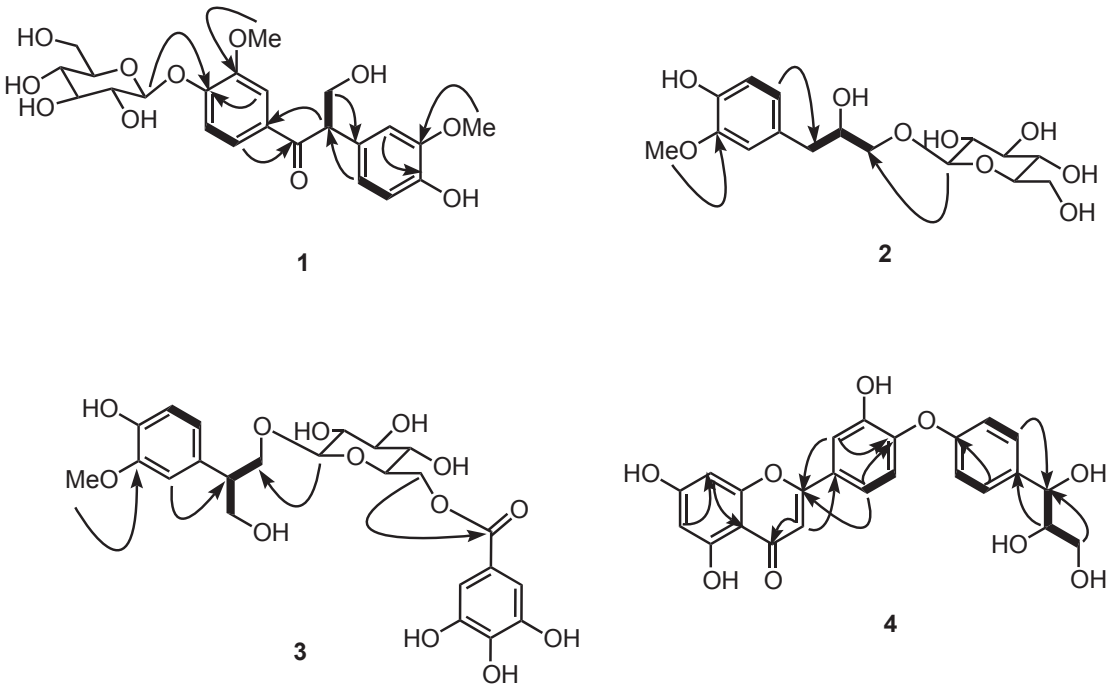

Figure 2. Key HMBC (arrow) and COSY (bold line) correlations of **1-4**

Compound **2** was obtained as a brown gum. The molecular formula $C_{16}H_{24}O_9$ was determined by the HR-FAB-MS *m/z* 361.1499 $[M+H]^+$ (calcd. 361.1498). The ^{1}H- and ^{13}C- NMR spectra were very similar to those of xylocoside A isolated from *Xylosma controversum*.[13] A major difference between them was found to be the location of glucose. The glucose moiety of **2** was confirmed to be located at C-9 by the HMBC correlation between H-1' (δ_H 4.28) and C-9 (δ_C 74.7) (Fig. 2). The J values (J = 7.5 Hz) of the anomeric proton at δ_H 4.46 indicated the presence of a β-glucopyranosyl unit.[9] Enzyme hydrolysis of **2** afforded the aglycone and sugar unit, of which the former was identified by comparison of the ^{1}H-NMR data[13] and the latter by comparison of co-TLC and optical rotation.[11,14] Thus, the structure of compound **2** was determined as shown in Figure 1 and named stroside B.

Compound **3** was obtained as a brown gum. The molecular formula was determined to be $C_{23}H_{28}O_{13}$ from

the $[M + H]^+$ peak at *m/z* 513.1602 (calcd. for $C_{23}H_{29}O_{13}$: 513.1608) in the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **3** were very close to those of 1-*β*-D-glucosyloxy-2-(3-methoxy-4-hydroxyphenyl)-propane-1,3-diol, which was isolated from *Juniperus phcenicea*,[15] expect for the additional gallic acid moiety [δ_H 7.11 (2H, s, H-2", 6") in the ^{1}H-NMR: δ_C 168.5 (C-7"), 146.7 (C-3", 5"), 140.0 (C-4"), 121.5 (C-1"), 110.3 (C-2", 6") in the ^{13}C-NMR]. Its position was assigned as C-6' by the HMBC experiment, showing correlation between H-6' [δ_H 4.56 (1H, dd, *J* = 12.0, 2.0 Hz) and 4.42 (1H, dd, *J* = 12.0, 5.0 Hz)] and C-7" (δ_C 168.5) (Figure 2). Thus, the structure of compound **3** was determined as shown in Figure 1 and named stroside C.

Compound **4** was obtained as a yellow gum. An HR-ESI-MS analysis indicated that the molecular formula of **4** was $C_{24}H_{20}O_9$ ($[M+H-H_2O]^+$ *m/z* 435. 1076, calcd 435.1080). The characteristic signals of luteolin[16] were observed at δ_H 7.42 (1H, d, *J* = 1.5 Hz, H-2'), 7.34 (1H, dd, *J* = 8.0, 1.5 Hz, H-6'), 6.88 (1H, d, *J* = 8.0 Hz, H-5'), 6.46 (1H, s, H-3), 6.30 (1H, brs, H-8), 6.08 (1H, brs, H-6) in the ^{1}H-NMR spectrum; at δ_C 182.3 (C-4), 164.9 (C-7), 163.9 (C-2), 161.8 (C-5), 158.0 (C-9), 147.5 (C-4'), 143.9 (C-3'), 123.9 (C-1′), 119.5 (C-6'), 117.4 (C-5'), 114.7 (C-2'), 103.9 (C-10), 103.3 (C-3), 98.9 (C-6), 93.7 (C-8) in the ^{13}C-NMR spectrum, and of *p*-hydroxylphenylglycerol moiety observed in the NMR data [δ_H 7.18 (2H, d, *J* = 8.0 Hz, H-3", 5"), 6.74 (2H, d, *J* = 8.0 Hz, H-2", 6"), 4.84 (1H, d, *J* = 8.5 Hz, H-7"), 3.96 (1H, brs, H-8"), 3.62 (1H, d, *J* = 12.0 Hz, H-9"a), 3.39 (1H, td, *J* = 12.0, 6.0 Hz, H-9"b); δ_C 158.0 (C-1"), 128.7 (C-3", 5"), 126.8 (C-4"), 115.1 (C-2", 6"), 78.6 (C-8"), 76.6 (C-7"), 60.5 (C-9")]. Each partial structures were determined by 2D-NMR spectral data (^{1}H-^{1}H COSY, HMQC, and HMBC data) (Figure 2). The carbon chemical shift at C-4′ in **4** (δ_C 147.5) was upfield shifted comparing to that of luteolin (δ_C 149.8),[16] suggesting that the *p*-hydroxylphenylglycerol moiety was located at the C-4′ position of the luteolin in **4**. The $\Delta\delta_{C8''\text{-}C7''}$ value of **4** in CD_3OD was 2.0 ppm, which indicated that the glycerol moiety of **4** possesses a *threo* relative configuration [*erythro* type : ($\Delta\delta_{C8''\text{-}C7''}$ < 1.0 ppm]. The positive optical rotation ($[\alpha]_D^{25}$ +7.0) of **4** supported that its configuration is 7"*S* and 8"*S*.[17-19] Thus, the structure of compound **4** was determined as shown in Figure 1 and named lagerindiol.

The known compounds were identified as 9,9'-dihydroxy-3,4-methoxylenedioxy-3'-methoxy[7-*O*-4'-8-5']-neolignan (**5**),[20] pterospermin A (**6**),[21] (2*R*,3*S*)-dihydrodehydroconiferyl alcohol (**7**),[22] gochidioboside (**8**),[23] 7*S*,8*R*-dihydrodehydrodiconiferyl alcohol 4-O-*β*-D-glucopyranoside (**9**),[24] hovetrichoside A (**10**),[25] hovetrichoside B (**11**),[25] (1'*S*,2'*R*)-guaiacyl glycerol (**12**),[26] carthamoside B_5 (**13**),[27] (+)-(7*S*,8*S*)-guaiacyl-glycerol 8-O-*β*-D-glucopyranoside (**14**),[19] D-*threo*-guaiacylglycerol 8-*O*-*β*-D-(6'-*O*-galloyl)glycopyrano-side (**15**),[28] alatusol A (**16**),[29] ficusol (**17**),[30] evofolin-B (**18**),[10] and marphenol C (**19**)[31] by comparison of their spectroscopic data with reported data.

Table 1. Inhibitory Effect on NO Production of Compounds **6, 7** and **16-19** in LPS-Activated BV-2 Cells

Compounds	IC_{50} (μM)[a]	Cell Viability (%)[b]
6	21.4	155.66±6.3
7	14.6	144.59±2.2
16	35.4	137.8±4.1
17	36.0	110.75±5.4
18	22.0	106.4±6.9
19	44.9	111.3±6.8
L-NMMA[c]	18.35	98.2±4.5

[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 is expressed as a percentage (%) of the LPS only treatment group. Results are averages of three independent experiments, and the data are expressed as mean ± SD. Statistical comparisons were performed using a one-way ANOVA test with Student's *t*-test. Only **p*-value < 0.05 was indicated as statistically significant.
[c] NMMA as a positive control.

Then, we evaluated the anti-inflammatory activities of the isolates (**1**–**19**) through the measurement of produced NO levels in murine microglia BV2 cells stimulated by bacterial pathogen, LPS.[32] Among the tested compounds, compound **7** significantly inhibited LPS-stimulated NO production with IC_{50} values of 14.6 μM, which displayed more activity than L-NMMA, a well-known NOS inhibitor. Compound **18** showed the inhibitory activity with an IC_{50} of 22.0 μM in BV-2 cells without cell toxicity (Table 1). The other (**6**, **16**, **17**, and **19**) exhibited week NO production activity in the murine microglia BV-2 cell line. The rest of the compounds did not show any significant inhibitory effects on NO production.

Table 2. Cytotoxicity of compounds **4** and **6** against four cultured human cancer cell lines using the SRB assay *in vitro*

Compounds	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
4	16.59	16.64	17.26	8.83
6	6.51	9.13	11.38	5.87
Doxorubicin[b]	0.026 ± 0.005	0.067 ± 0.003	0.006 ± 0.001	0.013 ± 0.017

[a] IC_{50} value of compounds against cancer cell lines, defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*.
[b] Doxorubicin as positive control.

The isolated compounds (**1-19**) were also performed for cytotoxicity against the A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma) human tumor cell lines *in vitro* using the sulforhodamine B assay.[33] Compounds **4** and **6**

(Table 2), which has flavones skeleton exhibited moderate cytotoxic activity against four human cell lines (IC_{50} (**4**): 16.59, 16.64, 17.26, and 8.83 μM, and IC_{50} (**6**): 6.51, 9.13, 11.38, and 5.87 μM, respectively). The other compounds were inactive (IC_{50} > 30.0 μM).

EXPERIMENTAL

General. Optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer using methanol as a solvent. UV spectra were recorded with a Shimadzu UV-1601 UV-Visible spectrophotometer using methanol as a solvent. HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. HR-ESI mass spectra were recorded on a SI-2/LCQ DecaXP liquid chromatography (LC)-mass spectrometer (Thermo Scientific, West Palm Beach, FL, USA). NMR spectra, including ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY experiments, were recorded on a Varian UNITY INOVA 700 NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C), with chemical shifts given in ppm (δ). Semi-preparative HPLC used a Gilson 306 pump with a Shodex refractive index detector. Silica gel 60 (Merck, 230-400 mesh) and RP-C_{18} silica gel (Merck, 230-400 mesh) were used for column chromatography. Low-pressure LC was performed over a LiChroprep Lobar-A RP-18 (240 × 10 mm i.d.) column with a FMI QSY-O pump (ISCO). The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co. Ltd). Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid. Lipopolysaccharide (LPS) was obtained from Sigma Chemical Company (St. Louis, USA).

Plant material. *L. indica* stems (5 kg) were collected from Goesan in Chungcheongbuk-do, Korea, in May 2012. The plant was authenticated by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL-1203) of the plant was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation. The air dried plant material (5 kg) were extracted with 80% MeOH three times under reflux. The filtrate was evaporated under reduced pressure to yield a MeOH extract (300 g), which was suspended in water (800 mL) and solvent-partitioned to afforded *n*-hexane (17.3 g, yield 5.73%), $CHCl_3$ (9.1 g, yield 3.03%), EtOAc (8.9 g, yield 2.96%), and BuOH (55.0 g, yield 18.33%) fractions. The $CHCl_3$ (9.1 g) fraction was chromatographed on a silica gel (230–400 mesh, 300 g) column eluted with $CHCl_3$-MeOH (30:1, 20:1, 10:1, and 1:1) to yield four fractions (Fr. C1 – Fr. C4). Fr. C1 (2.2 g) was further chromatographed on a silica gel (230–400 mesh, 150 g) column eluted with $CHCl_3$-MeOH (60:1) to yield four fraction (Fr. C11 – Fr. C14). Fr. C12 (1.0 g) was separated over an RP-C_{18} silica gel

(230–400 mesh) column (150 g, 1.5 × 30 cm) using a solvent system of 100% MeOH to give seven subfractions (Fr. C121 – Fr. C127). Fr. C121 (200 mg) was purified by semi-preparative reversed-phase HPLC using a 250 mm × 10 mm i.d., 10 μm, Econosil RP-18 column (Alltech) with a solvent system of 35% MeCN (1 L, flow rate; 2 mL/min) to give compounds **16** (3 mg), **17** (9 mg), and **19** (3 mg). Fr. C13 (200 mg) was separated over an RP-C_{18} silica gel (230–400 mesh) column (100 g, 1.5 × 30 cm) using a solvent system of 60% MeOH to give seven subfractions (Fr. C131 – Fr. C137). Fr. C131 (50 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 40% MeCN to yield compound **18** (9 mg). Fr. C14 (900 mg) was separated over an RP-C_{18} silica gel (230–400 mesh) column (100 g, 1.5 × 30 cm) using a solvent system of 90~100% MeOH to give four subfractions (Fr. C141 – Fr. 144). Fr. C142 (340 mg) was loaded on a Sephadex LH-20 column (450 g, 2 × 50 cm) and eluted with 80% MeOH and purified by semi-preparative reversed-phase HPLC with a solvent system of 55% MeCN to yield compound **7** (9 mg). The EtOAc (8.8 g) was separated over an RP-C_{18} silica gel (230–400 mesh) column (300 g, 3 × 30 cm) using a solvent system of 30~100% MeOH to give ten fractions (Fr. E1 – Fr. E10). Fr. E6 (1.0 g) was loaded on a Sephadex LH-20 column (450 g, 2 × 50 cm) and eluted with 80% MeOH to yield five subfractions (Fr. E61 – Fr. E65). Fr. E65 (220 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 50% MeOH to yield compound **5** (1.5 mg). Fr. E7 (470 mg) was loaded on a Sephadex LH-20 column (450 g, 2 × 50 cm) and eluted with 80% MeOH and purified by semi-preparative reversed-phase HPLC, as described above, with a solvent system of 55% MeCN to yield compounds **4** (2 mg) and **6** (5 mg). The BuOH (17.0 g) was chromatographed on a silica gel (230–400 mesh, 300 g) column eluted with $CHCl_3$-MeOH (5:1 and 1:1) to yield four fraction (Fr. B1 – Fr. B4). Fr. B2 (2.7 g) was separated over an RP-C_{18} silica gel (230–400 mesh) column (200 g, 1.5 × 30 cm) using a solvent system of 37% MeOH to give nine fractions (Fr. B21 – Fr. B29). Fr. B21 (520 mg) was loaded on a Sephadex LH-20 column (450 g, 2 × 50 cm) and eluted with 80% MeOH and purified by semi-preparative reversed-phase HPLC with a solvent system of 10% MeCN to yield compounds **12** (2 mg) and **14** (16 mg). Fr. B22 (500 mg) was loaded on a Sephadex LH-20 column (450 g, 2 × 50 cm) and eluted with 80% MeOH to yield six subfractions (Fr. B221 – Fr. B226). Fr. B222 (200 mg) was separated using a Lobar-A Si gel 60 (240 × 10 mm) column ($CHCl_3$/MeOH = 5:1) and purified by semi-preparative reversed-phase HPLC with a solvent system of 15% MeCN to yield compounds **2** (3 mg), **10** (5 mg), **11** (9 mg) and **13** (4 mg). Fr. B223 (60 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 15% MeCN to yield compound **1** (5 mg). Fr. B225 (40 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 20% MeCN to yield compounds **3** (3 mg) and **15** (3 mg). Fr. B25 (160 mg) was loaded on a Sephadex LH-20 column (450 g, 2 × 50 cm) and eluted with 80% MeOH and purified by semi-preparative reversed-phase HPLC with a solvent system of 20% MeCN to yield compound **9** (4 mg). Fr. B26 (170 mg) was loaded on a Sephadex LH-20 column

(450 g, 2 × 50 cm) and eluted with 80% MeOH and purified by semi-preparative reversed-phase HPLC with a solvent system of 20% MeCN to yield compound **8** (6 mg).

Stroside A (**1**): Brown gum, $[\alpha]_D^{25}$ +3.0 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 272 (0.7), 227 (1.3), 207 (1.8) nm; IR (KBr) ν_{max} 3375, 2950, 1653, 1513, 1454, 1419, 1270, 1076, 1018, 713 cm^{-1}; CD (MeOH) λmax ($\Delta\varepsilon$) 208 (+11.1), 222 (–9.3), 238 (+16.1), and 310 (–5.96) nm; ^{1}H-NMR (CD_3OD, 700 MHz) δ_H 7.69 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 7.62 (1H, d, *J* = 2.0 Hz, H-2), 7.18 (1H, d, *J* = 8.5 Hz, H-5), 6.90 (1H, d, *J* = 2.0 Hz, H-2'), 6.76 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'), 6.74 (1H, d, *J* = 8.5 Hz, H-5'), 5.01 (1H, d, *J* = 7.5 Hz, H-1"), 4.78 (1H, dd, *J* = 8.5, 5.0 Hz, H-8), 4.27 (1H, dd, *J* = 10.5, 9.0 Hz, H-9a), 3.90 (1H, m, H-6"a), 3.89 (3H, s, 3-OCH_3), 3.84 (3H, s, 3'-OCH_3), 3.73 (1H, dd, *J* = 10.5, 5.0 Hz, H-9b), 3.69 (1H, dd, *J* = 12.5, 6.0 Hz, H-6"b), 3.52 (1H, m, H-2"), 3.48 (1H, m, H-5"), 3.46 (1H, m, H-3"), 3.40 (1H, m, H-4"); ^{13}C-NMR (CD_3OD, 175 MHz) δ_C 199.3 (C-7), 152.3 (C-4), 150.7 (C-3), 149.5 (C-3'), 147.2 (C-4'), 132.9 (C-1), 129.7 (C-1'), 124.6 (C-6), 122.3 (C-6'), 116.7 (C-5'), 116.3 (C-5), 113.2 (C-2), 112.9 (C-2'), 101.9 (C-1"), 78.4 (C-3"), 78.0 (C-5"), 74.8 (C-2"), 71.4 (C-4"), 65.5 (C-9), 62.6 (C-6"), 56.7 (C-8, 3-OCH_3), 56.5 (3'-OCH_3); HR-FAB-MS *m/z* 481.1710 $[M+H]^+$ (calcd for 481.1710).

Stroside B (**2**): Brown gum, $[\alpha]_D^{25}$ +8.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 281 (0.5), 230 (1.2), 208 (1.9) nm; IR (KBr) ν_{max} 3377, 2945, 1645, 1518, 1456, 1432, 1375, 1275 1158, 1078, 1026, 742 cm^{-1}; ^{1}H-NMR (CD_3OD, 700 MHz) δ_H 6.86 (1H, d, *J* = 2.0 Hz, H-2), 6.73 (1H, d, *J* = 7.5 Hz, H-5), 6.69 (1H, dd, *J* = 7.5, 2.0 Hz, H-6), 4.28 (1H, d, *J* = 7.5 Hz, H-1'), 3.98 (1H, m, H-8), 3.93 (1H, dd, *J* = 10.5, 3.5 Hz, H-9a), 3.87 (3H, s, 3-OCH_3), 3.86 (1H, m, H-6a'), 3.68 (1H, dd, *J* = 12.0, 5.5 Hz, H-6'b), 3.42 (1H, dd, *J* = 10.0, 7.0 Hz, H-9b), 3.37 (1H, m, H-5'), 3.30 (1H, m, H-4'), 3.27 (1H, m, H-3'), 3.24 (1H, m, H-2'), 2.76 (1H, dd, *J* = 13.0, 6.0 Hz, H-7a), 2.70 (1H, dd, *J* = 14.5, 7.0 Hz, H-7b); ^{13}C-NMR (CD_3OD, 175 MHz) δ_C 148.9 (C-3), 146.1 (C-4), 131.2 (C-1), 123.0 (C-6), 116.2 (C-5), 114.3 (C-2), 105.0 (C-1'), 78.1 (C-3'), 78.0 (C-5'), 75.3 (C-2'), 74.7 (C-9), 73.2 (C-8), 71.7 (C-4'), 62.7 (C-6'), 56.5 (3-OCH_3), 40.5 (C-7); HR-FAB-MS *m/z* 361.1499 $[M+H]^+$ (calcd for361.1498).

Stroside C (**3**): Brown gum, $[\alpha]_D^{25}$ +9.3 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 275 (0.5), 228 (0.8), 206 (1.5); IR (KBr) ν_{max} 3374, 2929, 2856, 1641, 1519, 1440, 1378, 1261, 1077, 1020, 638cm^{-1}; ^{1}H-NMR (CD_3OD, 700 MHz) δ_H 7.11 (2H, s, H-2", 6"), 6.85 (1H, d, *J* = 1.5 Hz, H-2), 6.71 (1H, d, *J* = 8.5 Hz, H-5), 6.66 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 4.56 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'a), 4.42 (1H, dd, *J* = 12.0, 5.0 Hz, H-6'b), 4.36 (1H, d, *J* = 7.5 Hz, H-1'), 4.06 (1H, dd, *J* = 10.0, 6.0 Hz, H-9a), 3.86 (1H, dd, *J* = 10.0, 7.5 Hz, H-9b), 3.83 (1H, dd, *J* = 11.0, 6.0 Hz, H-8a), 3.77 (1H, m, H-8b), 3.58 (1H, m, H-5'), 3.42 (1H, m,

H-4'), 3.40 (1H, m, H-3'), 3.23 (1H, m, H-2'), 3.03 (1H, m, H-7); ^{13}C-NMR (CD_3OD, 175 MHz) δ_C 168.5 (C-7''), 148.9 (C-3), 146.7 (C-3'', 5''), 146.3 (C-4), 140.0 (C-4''), 133.4 (C-1), 121.9 (C-6), 121.5 (C-1''), 116.2 (C-5), 113.0 (C-2), 110.3 (C-2'', 6''), 105.0 (C-1'), 78.1 (C-3'), 75.7 (C-5'), 75.1 (C-2'), 72.5 (C-9), 71.8 (C-4'), 65.2 (C-8), 64.9 (C-6'), 56.5 (3-OCH_3), 49.6 (C-7); HR-FAB-MS *m/z* 513.1602 $[M+H]^+$ (calcd for 513.1608).

Lagerindiol (**4**): Yellow gum, $[\alpha]_D^{25}$ +7.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 335 (0.5), 269 (0.8), 206 (1.3); IR (KBr) ν_{max} 3359, 2940, 2831, 1652, 1607, 1498, 1440, 1350, 1252, 1164, 1032, 833, 638 cm^{-1}; ^{1}H-NMR (CD_3OD, 700 MHz) δ_H 7.42 (1H, d, *J* = 1.5 Hz, H-2'), 7.34 (1H, dd, *J* = 8.0, 1.5 Hz, H-6'), 7.18 (2H, d, *J* = 8.0 Hz, H-3'', 5''), 6.88 (1H, d, *J* = 8.0 Hz, H-5'), 6.74 (2H, d, *J* = 8.0 Hz, H-2'', 6''), 6.46 (1H, s, H-3), 6.30 (1H, brs, H-8), 6.08 (1H, brs, H-6), 4.84 (1H, d, *J* = 8.5 Hz, H-7''), 3.96 (1H, brs, H-8''), 3.62 (1H, d, *J* = 12.0 Hz, H-9''a), 3.39 (1H, td, *J* = 12.0, 6.0 Hz, H-9''b); ^{13}C-NMR (CD_3OD, 175 MHz) δ_C 182.3 (C-4), 164.9 (C-7), 163.9 (C-2), 161.8 (C-5), 158.0 (C-9, 1''), 147.5 (C-4'), 143.9 (C-3'), 128.7 (C-3'', 5''), 126.8 (C-4''), 123.9 (C-1′), 119.5 (C-6'), 117.4 (C-5'), 115.1 (C-2'', 6''), 114.7 (C-2'), 103.9 (C-10), 103.3 (C-3), 98.9 (C-6), 93.7 (C-8), 78.6 (C-8''), 76.6 (C-7''), 60.5 (C-9''); HR-ESI-MS *m/z* 435.1076 $[M+H-H_2O]^+$ (calcd for 435.1080).

Enzyme hydrolysis and sugar identification Compounds **1** (1.0 mg) and **2** (1.5 mg) in H_2O (2 mL) were hydrolyzed with *β*-glucosidase (30 mg, Emulsin) at 37 °C for 2 days. The $CHCl_3$ layers of **1** and **2** were followed by purification on a silica gel Waters Sep-Pak Vac 6cc ($CHCl_3$-MeOH, 10:1) to afford **1a** (0.2 mg) and **2a** (0.4 mg). The sugar in the water layer was identified as D-glucose by co-TLC ($CHCl_3$:MeOH:H_2O = 2:1:0.2, R_f value: 0.2 for glucose) and optical rotation {$[\alpha]_D^{25}$ +46.6 (*c* 0.02, H_2O) from **1** and $[\alpha]_D^{25}$ +43.1 (*c* 0.10, H_2O) from **2**}.

Evofolin-B (1a): brown gum; FAB-MS: *m/z* = 319 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 700 MHz) δ_H 7.62 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 7.58 (1H, d, *J* = 2.0 Hz, H-2), 6.91 (1H, d, *J* = 2.0 Hz, H-2'), 6.81 (1H, d, *J* = 8.0 Hz, H-5), 6.77 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.74 (1H, d, *J* = 8.0 Hz, H-5'), 4.77 (1H, dd, *J* = 8.5, 2.0 Hz, H-8), 4.26 (1H, dd, *J* = 11.0, 8.0 Hz, H-9a), 3.88 (3H, s, 3-OCH_3), 3.84 (3H, s, 3'-OCH_3),3.62 (1H, dd, *J* = 10.0, 5.0 Hz, H-9b).

3-(4-Hydroxy-3-methoxy)-phenyl-1,2-propandiol (2a): brown gum; FAB-MS: *m/z* = 199 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 700 MHz) δ_H 6.72 (1H, d, *J* = 2.0 Hz, H-2), 6.60 (1H, d, *J* = 2.0 Hz, H-5), 6.55 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 3.73 (3H, s, 3-OCH_3), 3.68 (1H, m, H-8), 3.39 (1H, dd, *J* = 11.0, 4.5 Hz, H-7a),

3.31 (1H, dd, *J* = 11.5, 4.0 Hz, H-7b), 2.62 (1H, dd, *J* = 14.0, 5.5 Hz, H-9a), 2.49 (1H, dd, *J* = 13.0, 6.5 Hz, H-9b).

Measurement of NO Production and Cell Viability The BV-2 mouse microglial cell line has been extensively used in published studies as an *in vitro* culture system for the investigation of primary microglial function. In this study, BV-2 cells were maintained in DMEM supplemented with 5% FBS and 1% PS. To measure nitric oxide (NO) production, BV-2 cells were plated into a 96 well plate (3×10^4 cells/well) and treated with 100 ng/mL lipopolysaccharide (LPS) in the presence or absence of isolates (**1-19**) for 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide and 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 assessed by the MTT assay. In this study, N^G-mono-methyl-L-arginine (L-NMMA, Sigma-Aldrich), a well-known nitric oxide synthase (NOS) inhibitor, was tested as a positive control.[32]

Cytotoxicity Assay A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each compound isolated against four cultured human tumor cell lines.[33] The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines were IC_{50} 0.026 ± 0.005, 0.067 ± 0.003, 0.006 ± 0.001, and 0.013 ± 0.017 μM, respectively.

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REFERENCES

1. Y. N. Lee, 'Flora of Korea', Kyohaksa, Seoul, 1996, p 520.
2. I. S. Lee, U. J. Youn, H. J. Kim, B. S. Min, J. S. Kim, and K. H. Bae, *Planta Med.*, 2011, **77**, 2037.

3. H. J. Kim, I. S. Lee, U. J. Youn, Q. C. Chen, T. M. Ngoc, D. T. Ha, H. Liu, B. S. Min, J. Y. Lee, R. S. Seong, and K. H. Bae, *J. Nat. Prod*., 2009, **72**, 749.
4. S. Jeelani and M. A. Khuroo, *Chem. Nat. Compd*., 2014, **50**, 681.
5. K. N. Vinod, Puttaswamy, K. N. Ninge Gowda, and R. Sudhakar, *Chin. J. Chem*., 2010, **28**, 1091.
6. Y. Diab, K. Atalla, and K. Elbanna, *Drug Discov. Ther*., 2012, **6**, 212.
7. L. Fu, B. T. Xu, X. R. Xu, X. S. Qin, R. Y. Gan, and H. B. Li, *Molecules*, 2010, **15**, 8602.
8. S. H. Jung, S. S. Lim, S. Lee, Y. S. Lee, K. H. Shin, and Y. S. Kim, *Nat. Prod. Sci*., 2003, **9**, 34.
9. K. W. Woo, E. Moon, S. Y. Park, S. Y. Kim, and K. R. Lee, *Bioorg. Med. Chem. Lett*., 2012, **22**, 7465.
10. T. S. Wu, J. H. Yeh, and P. L. Wu, *Phytochemistry*, 1995, **40**, 121.
11. K. H. Kim, S. U. Choi, and K. R. Lee, *J. Nat. Prod*., 2009, **72**, 1121.
12. J. Q. Zhao, Y. M. Wang, J. J. Lv, H. T. Zhu, D. Wang, C. R. Yang, M. Xu, and Y. J. Zhang, *J. Braz. Chem. Soc*., 2014, **25**, 1446.
13. Z. R. Xu, X. Y. Chai, C. C. Bai, H. Y. Ren, Y. N. Lu, H. M. Shi, and P. F. Tu, *Helv. Chim. Acta*, 2008, **91**, 1346.
14. M. D. Greca, M. Ferrara, A. Fiorentino, P. Monaco, and L. Previtera, *Phytochemistry*, 1998, **49**, 1299.
15. G. Comte, D. P. Allais, A. J. Chulia, J. Vercauteren, and N. Pinaud, *Phytochemistry*, 1997, **44**, 1169.
16. B. Feryal, B. Ahcene, A. Souad, M. Eric, B. Fadila, and B. Samir, *Der Pharmacia Lettre*, 2014, **6**, 50.
17. K. H. Kim, S. U. Choi, S. K. Ha, S. Y. Kim, and K. R. Lee, *J. Nat. Prod*., 2009, **72**, 206.
18. S. Lin, S. Wang, M. Liu, M. Gan, S. Li, Y. Yang, Y. Wang, W. He, and J. Shi, *J. Nat. Prod*., 2007, **70**, 817.
19. M. Gan, Y. Zhang, S. Lin, M. Liu, W. Song, J. Zi, Y. Yang, X. Fan, J. Shi, J. Hu, J. Sun, and N. Chen, *J. Nat. Prod*., 2008, **71**, 647.
20. W. H. Lin, J. M. Fang, and Y. S. Cheng, *Phytochemistry*, 1999, **50**, 653.
21. P. Dixit, M. P. Khan, G. Swarnkar, N. Chattopadhyay, and R. Maurya, *Bioorg. Med. Chem. Lett*., 2011, **21**, 4617.
22. J. S. Jiang, Z. M. Feng, Y. H. Wang, and P. C. Zhang, *Chem. Pharm. Bull*., 2005, **53**, 110.
23. Y. Takeda, C. Mima, T. Masuda, E. Hirata, A. Takashi, and H. Otsuka, *Phytochemistry*, 1998, **49**, 2137.
24. N. Matsuda, H. Sato, Y. Yaoita, and M. Kikuchi, *Chem. Pharm. Bull*., 1996, **44**, 1122.
25. K. Yoshikawa, N. Mimura, and S. Arihara, *J. Nat. Prod*., 1998, **61**, 1137.
26. T. Ishikawa, E. Fujimatu, and J. Kitajima, *Chem. Pharm. Bull*., 2002, **50**, 1460.

27. Y. Z. Zhou, H. Chen, L. Qiao, X. Lu, H. M. Hua, and Y. H. Pei, *Helv. Chim. Acta*, 2008, **91**, 1277.
28. K. Ishimaru, G. I. Nonaka, and I. Nishioka, *Phytochemistry*, 1987, **26**, 1147.
29. K. H. Kim, S. K. Ha, S. U. Choi, S. Y. Kim, and K. R. Lee, *Planta Med.*, 2013, **79**, 361.
30. Y. C. Li and Y. H. Kuo, *Phytochemistry*, 1998, **49**, 2417.
31. G. Y. Yang, R. R. Wang, H. X. Mu, Y. K. Li, X. N. Li, L. M. Yang, Y. T. Zheng, W. L. Xiao, and H. D. Sun, *J. Nat. Prod*., 2013, **76**, 250.
32. D. W. Reif and S. A. McCreedy, *Arch. Biochem. Biophys*., 1995, **1**, 170.
33. P. Skehan, R. Stroreng, D. Scudiero, A. Monks, J. Mcmahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, and M. R. Boyd, *J. Nat. Cancer Inst*., 1990, **82**, 1107.