Biphenyls from *Berberis koreana*

Ki Hyun Kim,[†] Sang Un Choi,[‡] Sang Keun Ha,[§] Sun Yeou Kim,[§] and Kang Ro Lee*,[†]

Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea, Korea Research Institute of Chemical Technology, Teajeon 305-600, Korea, and Graduate School of East-West Medical Science, Kyung Hee University, Yongin 446-701, Korea

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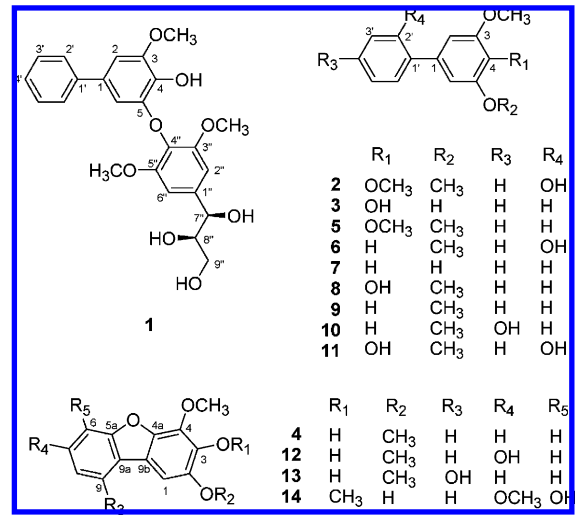
Three new biphenyls, berbekorin A (**1**), 2′-hydroxy-3,4,5-trimethoxybiphenyl (**2**), and 4,5-dihydroxy-3-methoxybiphenyl (**3**), together with 11 known biphenyls (**4**−**14**), were isolated from the *n*-hexane-soluble fraction of the MeOH extract of the trunk of *Berberis koreana*. The structures of **1**−**3** were determined by spectroscopic methods, including 1D and 2D NMR analysis. Compound **1** exhibited cytotoxic activity against the SK-MEL-2 skin melanoma cell line, in particular. Moreover, compounds **2**, **5**, **6**, and **14** inhibited NO production in LPS-activated BV-2 cells, a microglial cell line.

Biphenyl derivatives such as aucuparin and methoxyaucuparin are constitutive components of the heartwood of *Sorbus aucuparia*.[1] These biphenyl derivatives were also isolated and identified from the diseased shoots and leaves of loquat, *Eriobotrya japonica*, as phytoalexins.[2] Moreover, such antifungal biphenyls can be induced when the leaves of *Photinia glabra* and *Raphiolepsis umbellata* are infected with fungi or treated with heavy metal ions.[3,4] This is also true for the sapwood of species of *Cotoneaster*, *Eriobotrya*, *Malus*, and *Sorbus*.[5,6] These plants all belong to the family Rosaceae, with there having been many studies on the formation and identification of biphenyls as phytoalexins of this family.[7−11] However, there have been few reports on biphenyls from plants of other families.

Berberis koreana Palib. (Berberidaceae), commonly known as "Korean barberry", is an endemic species found throughout northern Korea that has been used as a Korean traditional medicine against enteritis, fever, conjunctivitis, and sore throat.[12] Numerous alkaloids with medicinal importance have been isolated from *Berberis* species,[13] and previous phytochemical studies on this plant have led to reports of alkaloids such as benzylisoqunoline and protoberberine derivatives,[14−16] as well as pyrrole acids.[17] An extract of *B. koreana* was reported to be neuroprotective against ischemic damage[18,19] and to exhibit cytotoxic and antioxidant activities.[20]

In a continuing search for bioactive constituents from Korean medicinal plants, we investigated a methanol extract of the trunk of *B. koreana* and have isolated three new biphenyl derivatives (**1**−**3**), together with 11 known biphenyl compounds (**4**−**14**), which are reported herein. The compounds were evaluated for their cytotoxic activities against four human cancer cell lines and for their inhibitory effects on NO production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line.

The trunk of *B. koreana* was collected in the Jeju Island area and was dried, chopped, and extracted with 80% aqueous MeOH under reflux. The *n*-hexane-soluble fraction of the MeOH extract showed cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT15 cells in a sulforhodamine B (SRB) assay. Purification of the *n*-hexane extract led to the isolation of 14 biphenyl derivatives (**1**−**14**). Among these, the known compounds were identified as the previously reported substances eriobofuran (**4**),[11] methoxyaucuparin (**5**),[1] 3,5-dimethoxybiphenyl-2′-ol (**6**),[21] 3-hydroxy-5-methoxybiphenyl (**7**),[22] aucuparin (**8**),[23] 3,5-dimethoxybiphenyl (**9**),[24] 3,5-dimethoxybiphenyl-4′-ol (**10**),[25] 2′-hydroxyaucuparin (**11**),[23] δ-cotonefuran (**12**),[11] 9-hydroxyeriobofuran (**13**),[11] and



ε-cotonefuran (**14**),[10] by comparison of their spectroscopic and physical data with previously reported values.

Compound **1** was obtained as a viscous oil. The molecular formula was determined to be $C_{24}H_{26}O_8$ from the molecular ion peak $[M + H]^+$ at *m*/*z* 443.1727 (calcd for $C_{24}H_{27}O_8$, 443.1706) in the positive-ion HRFABMS. The IR spectrum indicated that **1** possesses hydroxy (3382 cm^{-1}), phenyl (2946, 1454 cm^{-1}), and ether (1245 cm^{-1}) functional groups. The ^1H NMR spectrum revealed the signals for a monosubstituted benzene ring [δ_H 7.56 (2H, dd, J = 8.0, 1.5 Hz, H-2′, 6′), 7.42 (2H, td, J = 8.0, 1.5 Hz, H-3′, 5′), 7.30 (1H, tt, J = 8.0, 1.5 Hz, H-4′)], two 1,3,4,5-tetrasubstituted benzene rings [δ_H 6.92 (1H, d, J = 2.0 Hz, H-6), 6.80 (1H, d, J = 2.0 Hz, H-2); 6.72 (2H, s, H-2″, 6″), respectively], four oxygenated protons [δ_H 5.02 (1H, d, J = 8.0 Hz, H-7″), 4.05 (1H, m, H-8″), 3.93 (1H, dd, J = 11.5, 4.0 Hz, H-9″a), 3.61 (1H, dd, J = 11.5, 6.5 Hz, H-9″b)], and three methoxy groups [δ_H 3.98 (3H, s, OCH_3-3), 3.94 (6H, s, OCH_3-3″, 5″)]. The ^{13}C NMR spectroscopic data (including HMQC and DEPT) indicated 24 carbon signals of **1** to be composed of 18 aromatic carbons (including nine quaternary carbons), two oxygenated methine carbons, an oxygenated methylene carbon, and three methoxy carbons. The signals for a monosubstituted benzene ring [δ_C 141.0 (C-1′), 128.9 (C-3′, 5′), 127.3 (C-4′), 127.0 (C-2′, 6′)], a 1,3,4,5-tetrasubstituted benzene ring [δ_C 149.2 (C-3), 144.7 (C-5), 134.3 (C-1), 132.7 (C-4), 108.9 (C-6), 103.7 (C-2)], and a methoxy group [δ_C 56.4 (OCH_3-3)] implied that compound **1** is a biphenyl derivative on the basis of its NMR data.[21−25] This was reconfirmed by the HMBC spectrum (Figure 1). Additionally, the signals for a 1,3,4,5-tetrasubstituted benzene ring [δ_C 147.5 (C-3″, 5″), 135.5 (C-1″), 128.2 (C-4″), 104.3 (C-2″, 6″)/δ_H 6.72 (H-2″, 6″)], a

* Corresponding author. Tel: +82-31-290-7710. Fax: +82-31-290-7730. E-mail: krlee@skku.ac.kr.
[†] Sungkyunkwan University.
[‡] Korea Research Institute of Chemical Technology.
[§] Kyung Hee University.

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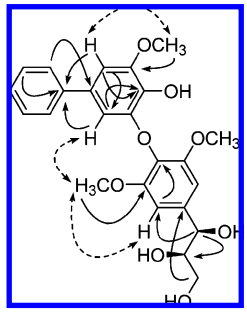

Figure 1. Key HMBC (→) and NOESY (↔) correlations of **1**.

1-substituted 1,2,3-propantriol [δ_C 78.7 (C-8″), 76.5 (C-7″), 61.7 (C-9″)/δ_H 5.02 (H-7″), 4.05 (H-8″), 3.93 (H-9″a), 3.61 (H-9″b)], and two methoxy groups [δ_C 56.6 (OCH_3-3″, 5″)/δ_H 3.94 (OCH_3-3″, 5″)] were observed. On the basis of these data, it was deduced that **1** contains a syringylglycerol moiety.[26,27] Determination of the final structure of **1**, including the location of the syringylglycerol group, was accomplished by HMBC and NOESY experiments (Figure 1). Thus, HMBC correlations between H-7″ (δ_H 5.02) and C-2″, 6″ (δ_C 104.3) and C-8″ (δ_C 78.7) were detected. The cross-peaks observed in the NOESY spectrum from H-6 (δ_H 6.92) to OCH_3-3″, 5″ (δ_H 3.94) confirmed that the syringylglycerol moiety is located at C-5. The chemical shift differences of C-7″ and C-8″ ($\Delta\delta_{C8''-C7''}$) were directly applicable in distinguishing *threo*- and *erythro*-arylglycerol moieties without substituent(s) at C-7″ and/or C-8″ [*erythro* ($\Delta\delta_{C8''-C7''}$ <1.0 ppm) and *threo* ($\Delta\delta_{C8''-C7''} \geq 2.0$ ppm) arylglycerols].[26,27] The $\Delta\delta_{C8''-C7''}$ value of **1** in CD_3OD was 2.5 ppm, which indicated that the glycerol moiety of **1** possesses a *threo* relative configuration. The negative optical rotation ($[\alpha]^{25}_D$ −37.2) of **1** confirmed that its configuration is 7″*R* and 8″*R*.[26,27] Thus, the structure of **1** was determined as (7″*R*,8″*R*)-syringylglycerol-4″-*O*-5-biphenyl ether, and it has been named berbekorin A.

Compound **2** was obtained as a viscous oil. The molecular formula was determined to be $C_{15}H_{16}O_4$ from the molecular ion peak $[M]^+$ at *m*/*z* 260.1045 (calcd for $C_{15}H_{16}O_4$, 260.1049) in the positive-ion HRFABMS. The IR spectrum of **2** showed hydroxy (3382 cm^{-1}), phenyl (2946, 1454 cm^{-1}), and ether (1281 cm^{-1}) absorption bands. The ^1H NMR spectrum displayed the signals for a 1,2-disubstituted benzene ring [δ_H 7.29 (1H, td, J = 7.5, 1.2 Hz, H-6′), 7.25 (1H, td, J = 7.5, 1.2 Hz, H-4′), 7.00 (1H, dd, J = 7.5, 1.2 Hz, H-3′), 6.98 (1H, dd, J = 7.5, 1.2 Hz, H-5′)], a 1,3,4,5-tetrasubstituted benzene ring [δ_H 6.67 (2H, s, H-2, 6)], and three methoxy groups [δ_H 3.92 (3H, s, OCH_3-4), 3.90 (6H, s, OCH_3-3, 5)]. The ^{13}C NMR spectrum indicated the presence of six quaternary carbons at δ_C 154.1 (C-3, 5), 152.6 (C-2′), 137.9 (C-4), 128.8 (C-1′), and 128.3 (C-1), six methine carbons at δ_C 130.1 (C-6′), 129.4 (C-4′), 120.7 (C-5′), 115.9 (C-3′), and 106.2 (C-2, 6), and three methoxy signals at δ_C 61.1 (OCH_3-4) and 56.4 (OCH_3-3, 5). These data were found to be very similar to those of **11**,[23] with the exception of an additional methoxy signal (δ_H 3.92/δ_C 61.1) in the NMR spectra, which was confirmed to be at C-4 from the HMBC correlation between OCH_3-4 (δ_H 3.92) and C-4 (δ_C 137.9). Thus, it was concluded that **2** is 2′-hydroxy-3,4,5-trimethoxybiphenyl.

Compound **3** was also obtained as a viscous oil. The molecular formula was determined to be $C_{13}H_{12}O_3$ from the molecular ion peak $[M]^+$ at *m*/*z* 216.0794 (calcd for $C_{13}H_{12}O_3$, 216.0786) in the positive-ion HRFABMS. The IR spectrum exhibited absorptions of hydroxy (3382 cm^{-1}), phenyl (2946, 1454 cm^{-1}), and ether (1280 cm^{-1}) groups. The ^1H and ^{13}C NMR and DEPT spectroscopic data of **3** were similar to those of **1**, except for the absence of signals assignable to the syringylglycerol moiety of **1**. This suggested that **3** has the same biphenyl structure but without the syringylglycerol moiety. The C-5 (δ_C 144.7) and C-4 (δ_C 132.7) signals in compound **1** were shifted downfield to δ_C 147.1 and 138.8, respectively, in compound **3**. The OCH_3-3 (δ_H 3.95) signal showed a cross-peak with C-3 (δ_C 150.4) in the HMBC spectrum. The biphenyl structure of **3** was confirmed by the cross-peaks in the $^1H-^1H$ COSY and HMBC spectra. Thus, **3** was determined as 4,5-dihydroxy-3-methoxybiphenyl.

The isolated compounds **1**−**14** were evaluated for their cytotoxic activities against the A549, SK-OV-3, SK-MEL-2, and HCT15 human tumor cell lines using the SRB assay. Compound **1** exhibited cytotoxicity against three of the cell lines, namely, A549, SK-MEL-2, and HCT-15, with IC_{50} values of 8.2, 4.4, and 7.0 μM, respectively. All other compounds were inactive (IC_{50} > 10 μM) for all cell lines.

It has been reported that an extract of *B. koreana* is neuroprotective by inhibiting postischemic inflammation and *N*-methyl-D-aspartate receptor activation in animal models of ischemic brain injury.[18,19] Thus, the inhibitory effects of isolated compounds **1**−**14** from *B. koreana* were evaluated for NO production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line. Compounds **2**, **5**, **6**, and **14** inhibited LPS-induced NO production, with IC_{50} values of 50.6, 21.9, 32.1, and 33.0 μM, respectively. All other compounds were less active in this assay. Compound **1** was cytotoxic at 20 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded using a Shimadzu UV-1601 UV−visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ). FAB and HRFAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and an Apollo Silica 5 μm column (250 × 22 mm) or Econosil RP-18 10 μm column (250 × 22 mm). Silica gel 60 (Merck, Darmstadt, 70−230 and 230−400 mesh) and RP-C_{18} silica gel (Merck, 230−400 mesh) were used for column chromatography. TLC was performed using Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co., Sweden). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar-A Si gel 60 (240 × 10 mm) and LiChroprep Lobar-A RP-18 (240 × 10 mm) columns with a FMI QSY-0 pump (ISCO).

Plant Material. The trunk of *B. koreana* was collected on Jeju Island, Korea, in December 2005, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2005-10) has been deposited in the herbarium of the College of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The trunk of *B. koreana* (2.7 kg) was dried, chopped, and extracted with 80% aqueous MeOH two times (2 × 4 h) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a MeOH extract (220 g), which was suspended in distilled H_2O (7.2 L) and then successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, yielding 8, 10, and 50 g of residue, respectively. The *n*-hexane-soluble fraction (8 g) was separated on a silica gel (230−400 mesh, 250 g) column and eluted with *n*-hexane−EtOAc (3:1), to yield seven fractions (A−G). Fraction B (200 mg) was separated further on a LiChroprep Lobar-A Si gel 60 (240 × 10 mm) column and eluted with *n*-hexane−CH_2Cl_2−EtOAc (15:0.5:1) to give four subfractions (B1−B4). Compound **9** (40 mg) was obtained from subfraction B1 (100 mg) by separation with preparative reversed-phase HPLC, using a solvent system of 85% aqueous MeOH. Fraction C (150 mg) was separated on a LiChroprep Lobar-A Si gel 60 (240 × 10 mm) column and eluted with *n*-hexane−$CHCl_3$−EtOAc (8:1:1) to give three subfractions (C1−C3). Subfraction C2 (80 mg) was separated by preparative normal-phase HPLC, using a solvent system of *n*−hexane−CH_2Cl_2−EtOAc (7:1:1), to yield compound **10** (12 mg). Fraction D (210 mg) was purified on a LiChroprep Lobar-A Si gel 60 (240 × 10 mm) column and eluted with *n*−hexane−$CHCl_3$−EtOAc (8:1:1) to give three subfractions (D1−D3). Compounds **5** (8 mg) and **6** (35 mg) were obtained from subfraction D1 (25 mg) and D2 (100 mg), respectively, by separation with preparative HPLC, using a solvent

system of *n*-hexane−CH_2Cl_2−EtOAc (6:1:1) and 75% aqueous MeOH, respectively. Fraction E (500 mg) was filtered and separated on a LiChroprep Lobar-A RP-18 (240 × 10 mm) column and eluted with 80% aqueous MeOH to afford three subfractions (E1−E3). Subfraction E1 (40 mg) was separated by preparative normal-phase HPLC, using a solvent system of *n*-hexane−CH_2Cl_2−EtOAc (5:1:1), to yield compounds **3** (3 mg) and **7** (6 mg). Fraction F (350 mg) was separated on an RP-C_{18} silica gel (230−400 mesh, 100 g) column and eluted with 90% aqueous MeOH to give four subfractions (F1−F4). Subfraction F1 (180 mg) was subjected to passage over a LiChroprep Lobar-A Si gel 60 (*n*-hexane−CH_2Cl_2−EtOAc, 5:1:1) column and purified by preparative reversed-phase HPLC, using a solvent system of 75% aqueous MeOH, to yield compounds **2** (5 mg), **4** (15 mg), and **8** (45 mg). Fraction G (2.0 g) was separated on an RP-C_{18} silica gel (230−400 mesh, 150 g) column, eluted with 80% aqueous MeOH, to give six subfractions (G1−G6). Subfraction G1 (80 mg) was purified by preparative normal-phase HPLC (*n*-hexane−EtOAc, 2:1) to yield compound **12** (10 mg). Subfraction G3 (300 mg) was separated on a Sephadex LH-20 column (100% MeOH) and purified by preparative reversed-phase HPLC, using a solvent system of 85% aqueous MeOH, to obtain **11** (7 mg), **13** (4 mg), and **14** (7 mg). Subfraction G5 (150 mg) was purified by preparative normal-phase HPLC, using a solvent system of $CHCl_3$−MeOH (80:1), to obtain **1** (5 mg).

Berbekorin A (1): viscous oil; $[\alpha]^{25}_D$ −37.2 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3382, 2946, 2835, 1725, 1665, 1454, 1245, 1031, 699 cm^{-1}; UV (MeOH) λ_{max} (log ε) 280 (4.2), 268 (4.4), 230 (4.6) nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.56 (2H, dd, J = 8.0, 1.5 Hz, H-2′, 6′), 7.42 (2H, td, J = 8.0, 1.5 Hz, H-3′, 5′), 7.30 (1H, tt, J = 8.0, 1.5 Hz, H-4′), 6.92 (1H, d, J = 2.0 Hz, H-6), 6.80 (1H, d, J = 2.0 Hz, H-2), 6.72 (2H, s, H-2″, 6″), 5.02 (1H, d, J = 8.0 Hz, H-7″), 4.05 (1H, m, H-8″), 3.98 (3H, s, OCH_3-3), 3.94 (6H, s, OCH_3-3″, 5″), 3.93 (1H, dd, J = 11.5, 4.0 Hz, H-9″a), 3.61 (1H, dd, J = 11.5, 6.5 Hz, H-9″b); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.2 (C-3), 147.5 (C-3″, 5″), 144.7 (C-5), 141.0 (C-1′), 135.5 (C-1″), 134.3 (C-1), 132.7 (C-4), 128.9 (C-3′, 5′), 128.2 (C-4″), 127.3 (C-4′), 127.0 (C-2′, 6′), 108.9 (C-6), 104.3 (C-2″, 6″), 103.7 (C-2), 78.7 (C-8″), 76.5 (C-7″), 61.7 (C-9″), 56.6 (OCH_3-3″, 5″), 56.4 (OCH_3-3); positive FABMS *m*/*z* 443 $[M + H]^+$; positive HRFABMS *m*/*z* 443.1727 $[M + H]^+$ (calcd for $C_{24}H_{27}O_8$, 443.1706).

2′-Hydroxy-3,4,5-trimethoxybiphenyl (2): viscous oil; IR (KBr) ν_{max} 3382, 2946, 2835, 1725, 1665, 1454, 1281, 1031, 699 cm^{-1}; UV (MeOH) λ_{max} (log ε) 292 (4.3), 266 (4.2) nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.29 (1H, td, J = 7.5, 1.2 Hz, H-6′), 7.25 (1H, td, J = 7.5, 1.2 Hz, H-4′), 7.00 (1H, dd, J = 7.5, 1.2 Hz, H-3′), 6.98 (1H, dd, J = 7.5, 1.2 Hz, H-5′), 6.67 (2H, s, H-2, 6), 5.35 (1H, br s, OH-2′), 3.92 (3H, s, OCH_3-4), 3.90 (6H, s, OCH_3-3, 5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.1 (C-3, 5), 152.6 (C-2′), 137.9 (C-4), 130.1 (C-6′), 129.4 (C-4′), 128.8 (C-1′), 128.3 (C-1), 120.7 (C-5′), 115.9 (C-3′), 106.2 (C-2, 6), 61.1 (OCH_3-4), 56.4 (OCH_3-3, 5); positive FABMS *m*/*z* 260 $[M]^+$; positive HRFABMS *m*/*z* 260.1045 $[M]^+$ (calcd for $C_{15}H_{16}O_4$, 260.1049).

4,5-Dihydroxy-3-methoxybiphenyl (3): viscous oil; IR (KBr) ν_{max} 3382, 2946, 2835, 1725, 1665, 1454, 1280, 1031, 699 cm^{-1}; UV (MeOH) λ_{max} (log ε) 275 (4.2), 230 (4.6) nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.58 (2H, dd, J = 8.0, 1.5 Hz, H-2′, 6′), 7.45 (2H, td, J = 8.0, 1.5 Hz, H-3′, 5′), 7.36 (1H, tt, J = 8.0, 1.5 Hz, H-4′), 6.84 (1H, d, J = 2.0 Hz, H-2), 6.80 (1H, d, J = 2.0 Hz, H-6), 3.95 (3H, s, OCH_3-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 150.4 (C-3), 147.1 (C-5), 140.7 (C-1′), 138.8 (C-4), 131.6 (C-1), 129.0 (C-3′, 5′), 128.3 (C-4′), 127.3 (C-2′, 6′), 110.2 (C-6), 104.7 (C-2), 56.5 (OCH_3-3); positive FABMS *m*/*z* 217 $[M + H]^+$; positive HRFABMS *m*/*z* 216.0794 $[M]^+$ (calcd for $C_{13}H_{12}O_3$, 216.0786).

Eriobofuran (4): viscous oil; IR (KBr) ν_{max} 3382, 2946, 2835, 1725, 1665, 1454, 1281, 1031, 699 cm^{-1}; UV (MeOH) λ_{max} (log ε) 316 (sh, 3.8), 302 (4.1), 293 (4.0), 260 (4.1) nm; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.82 (1H, dd, J = 8.0, 1.0 Hz, H-9), 7.54 (1H, dd, J = 8.0, 1.0 Hz, H-6), 7.38 (1H, td, J = 8.0, 1.0 Hz, H-7), 7.28 (1H, td, J = 8.0, 1.0 Hz, H-8), 7.10 (1H, s, H-1), 5.82 (1H, s, OH-3), 4.26 (3H, s, OCH_3-4), 3.99 (3H, s, OCH_3-2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.4 (C-5a), 145.0 (C-2), 142.9 (C-4a), 137.8 (C-3), 132.7 (C-4), 125.9 (C-7), 124.9 (C-9a), 122.8 (C-8), 119.8 (C-9), 116.3 (C-9b), 111.7 (C-6), 96.5 (C-1), 61.2 (OCH_3-4), 57.0 (OCH_3-2); positive FABMS *m*/*z* 245 $[M + H]^+$.

Cytotoxicity Testing. A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each compound isolated against four cultured human cancer cell lines.[28] 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 HCT15 (colon adenocarcinoma). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines were IC_{50} 0.16, 0.38, 0.04, and 0.82 μM, respectively.

Measurement of NO Production and Cell Viability. The BV-2 murine microglia cell line was stimulated with 100 ng/mL of lipopolysaccharide (LPS) in the presence or absence of each test compound for 24 h. Nitrite in the culture medium, a soluble oxidation product of NO, was determined 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-napthylethylenediamine 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 measured using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay.[29] N^G-Monomethyl-L-arginine (L-NMMA, Sigma), a well-known NOS inhibitor, was tested as a positive control. The IC_{50} value for the positive control, L-NMMA, was 16.8 μM.

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Supporting Information Available: 1D and 2D NMR data of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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