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

Received July 27, 2009

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 *B. koreana* trunk and were identified to be the structures of 1–3. 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*. These biphenyl derivatives were also isolated and identified from the diseased shoots and leaves of loquat, *Eriobotrya japonica*, and previous phytochemical studies on this plant have also true for the sapwood of species of *Cotoneaster*, *Eriobotrya*, *Malus*, and *Sorbus*. This is also true for the sapwood of species of *Cotoneaster*, *Eriobotrya*, *Malus*, and *Sorbus*. These plants all belong to the family Rosaceae, with there being many studies on the formation and identification of biphenyls as phytoalexins of this family. However, there have been few reports on biphenyls from plants of other families.

*Berberis koreana* Barlb. (*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. Numerous alkaloids are infected with fungi or treated with heavy metal ions. This is "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. Numerous alkaloids...12

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 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 was...
Compound 2 was obtained as a viscous oil. The molecular formula was determined to be C₁₅H₁₆O₄ from the molecular ion peak [M⁺] at m/z 260.1045 (calcd for C₁₅H₁₆O₄, 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 1,3,4,5-arylglycerol moieties without substituent(s) at C-7 and/or C-8 [ethyroyl (ΔδCye-c-τ < 1.0 ppm) and threo (ΔδCye-c-τ ≥ 2.0 ppm) arylglycerols]. The ΔδCye-c-τ value of 1 in CDOD was 2.5 ppm, which indicated that the glycerol moiety of 1 possesses a threo relative configuration. The negative optical rotation ([α]D⁻27.2°) of 1 confirmed that its configuration is 7R and 8R. Thus, the structure of 1 was determined as (7R,8R)-3,4,5-trihydroxy-4′-O-5-biphenylenic ester, and it has been named berberin A.

Compound 3 was also obtained as a viscous oil. The molecular formula was determined to be C₁₅H₁₆O₄ from the molecular ion peak [M⁺] at m/z 261.0794 (calcd for C₁₅H₁₆O₄, 261.0786) 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 13C 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 (δC 144.7) and C-4 (δC 132.7) signals in compound 1 were shifted downfield to δC 147.1 and 138.8, respectively, in compound 3. The OCH₃-3 (δOCH₃ 3.95) signal showed a cross-peak with C-5 (δC 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 IC₅₀ values of 8.2, 4.4, and 7.0 µM, respectively. All other compounds were inactive (IC₅₀ > 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. 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 IC₅₀ 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 (13C), 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 x 22 mm) or Econosil RP-18 10 µm column (250 x 22 mm). Silica gel 60 (Merck, Darmstadt, 70–230 and 230–400 mesh) and RP-C₃ silica gel (Merck, 230–400 mesh) were used for column chromatography. TLC was performed using Merck precoated silica gel F₂₅₄ plates and RP-18 F₂₅₄ plates. The packing material for molecular sieves column chromatography was Sephadex LH-20 (Pharmacia Co., Sweden). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar-A Si gel 60 (240 x 10 mm) and LiChroprep Lobar-A RP-18 (240 x 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 twice (2 x 4 h) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a MeOH extract (220 g), which was suspended in distilled H₂O (7.2 L) and then successively partitioned with n-hexane, CHCl₃, 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 further separated on a LiChroprep Lobar-A Si gel 60 (240 x 10 mm) column and eluted with n-hexane–CH₂Cl₂–EtOAc (15:5:1) 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 x 10 mm) column and eluted with n-hexane–CH₂Cl₂–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–CH₂Cl₂–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 x 10 mm) column and eluted with n-hexane–CH₃OH–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–CH₂Cl₂–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–CH₂Cl₂–EtOAc (5:1:1), to yield compounds 3 (3 mg) and 7 (6 mg). Fraction F (350 mg) was separated on an RP-C₁₈ silica gel (230–400 mesh, 100 g) 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–CH₂Cl₂–EtOAc, 5:1:1) column and purified by preparative reversed-phase HPLC, using a solvent system of aqueous MeOH, to yield compounds 6 (1 mg) and 11 (8 mg).

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-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 NO₂⁻ concentration. Cell viability was measured using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay.¹² N⁰-N-Monomethyl-L-arginine (L-NMMA, Sigma), a well-known NOS inhibitor, was tested as a positive control. The IC₅₀ value for the positive control, L-NMMA, was 16.8 µM.

References and Notes