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A New Cytotoxic Cadinane Sesquiterpene from *Berberis koreana*

Ki Hyun Kim,* Ho Kyung Kim, Su-Nam Kim,† and Sang Un Choi‡

Natural Product Research Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
**E-mail: khkim83@skku.edu*
†Natural Products Research Center, KIST Gangneung Institute, Gangneung 210-340, Korea
‡Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea
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Berberis koreana Palib. (Berberidaceae), commonly well-known as "Korean barberry", is an endemic species distributed throughout northern Korea. This tree has been used as Korean traditional medicine for the treatment of enteritis, fever, conjunctivitis, and sore throat since ancient times.[1] Numerous alkaloids with medicinal properties have been isolated from the genus *Berberis*,[2] and previous phytochemical investigations on this plant revealed the presence of many alkaloids such as benzylisoquinoline and protoberberine derivatives as well as pyrrole acids.[3-6] In previous biological studies of this plant, an extract of *B. koreana* was reported to be neuroprotective against ischemic damage[7,8] and to exhibit cytotoxic and antioxidant activities.[9] In a preliminary test, we also found that a MeOH extract of the trunk of *B. koreana* exhibited significant cytotoxicity against some human tumor cell lines, which led us to investigate the bioactive extract.[10-14] Our previous phytochemical investigation of *B. koreana* resulted in the isolation and identification of many cytotoxic compounds, biphenyls, lignans, triterpenoids, steroids, and phenolics.[10-14] In our continuing efforts to study the cytotoxic constituents of the MeOH extract, a new cadinane-type sesquiterpene, berkoreanol (**1**) was isolated from the $CHCl_3$-soluble fraction of the MeOH extract using a bioassay guided fractionation technique (Figure 1). The structure of **1** was determined by spectroscopic data interpretation, particularly by extensive 1D and 2D NMR experiments. To the best of our knowledge, several terpenoids from the genus of *Berberis* have been reported today,[11,13,15] but the isolation of a sesquiterpene from this genus is reported in this study for the first time. We report herein the isolation, structural elucidation, and cytotoxicity of the isolated sesquiterpene **1**.

Compound **1** was obtained as a colorless gum. The molecular formula was established as $C_{15}H_{26}O_2$ evidenced from the $[M + H]^+$ peak at *m/z* 239.2015 (calcd. for $C_{15}H_{27}O_2$, 239.2011) in the HR-ESIMS, suggesting three degrees of unsaturation. In compliance with the formula, the presence of hydroxyl group in the molecule could be proposed from the IR absorption band of **1** at 3345 cm^{-1}. The ^1H NMR spectrum displayed two methyl singlets at δ_H 1.28 and 1.66, one methyl doublet at δ_H 0.91, one doublet at δ_H 5.52 attributed to an olefinic methine, and two doublets of doublets at δ_H 3.82 and 3.86 attributed to an oxygenated

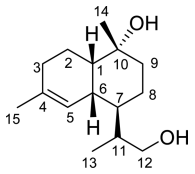

Figure 1. Chemical structure of compound **1**.

methylene group. The ^{13}C NMR spectrum along with the DEPT and HMQC experiments revealed the presence of three methyls, five methylenes, five methines, and two quaternary carbon atoms. A detailed comparison of the above spectral data with those reported for (−)-torreyol revealed the structural resemblance of the two compounds, implying the skeleton of a cadinane sesquiterpenoid of **1**.[16,17] Major differences were the disappearance of a methyl signal and the downfield-shifted signal of C-12 in the NMR spectra of **1** by compared to those of (−)-torreyol, indicating the presence of a hydroxyl group at C-12 in compound **1**. The ^1H-^1H COSY correlations as shown in Figure 2 and the observed HMBC correlations from H-12 to C-7, C-11, and C-13 as well as from H-13 to C-7, C-11, and C-12 proved this conclusion and further confirmed the gross structure of **1** (Figure 2). The relative stereochemistry of **1** was determined by the analysis of NOESY correlations (Figure 2) and coupling constants. The NOESY correlations between H-1, H-6, and H-14 indicated them to be located on the same face of the molecule. The large coupling constant (11.5 Hz) between H-6 and H-7 suggested H-6 and H-7 to be opposite. This orientation was supported by the observed NOESY correlation between H-6 and H-13. The evidence above established the structure of **1** to be 12-hydroxy-(−)-torreyol, named berkoreanol.

In this study, the cytotoxicity of **1** against A549 (a non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon adenocarcinoma) human cancer cell lines was evaluated using the sulforhodamine B (SRB) bioassay *in vitro*.[18] Compound **1** was found to have a moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (IC_{50}: 15.3, 12.7, 14.3 and 15.0 μM, respectively). To our knowledge, several cadinane-type sesquiterpenes with cytotoxic effects have been reported. (−)-(5*R*,6*R*,7*S*,9*R*,10*S*)-Cadinan-3-ene-

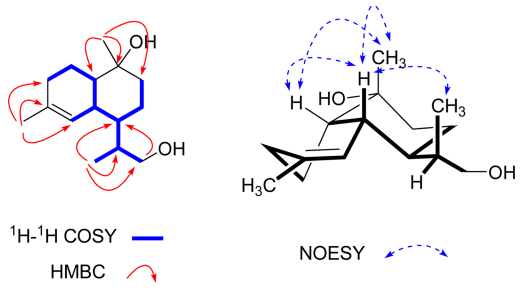

Figure 2. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of **1**.

6,7-diol, isolated from the leaves of *Eupatorium adenophorum*, showed cytotoxic activity against the HCT-8, Bel-7402, and A2780 cell lines,[19] and scabralins A obtained from the soft coral *Sinularia scabra* exhibited moderate to weak cytotoxicity against MCF-7, WiDr, Daoy, and HEp 2 cancer cell lines.[20] So far, we have investigated the cytotoxic compounds from *B. koreana* phytochemically, and identified a variety of cytotoxic compounds, including biphenyls, lignans, triterpenoids, steroids, and phenolics.[10-14] The new cadinane-type sesquiterpene, berkoreanol is another type of cytotoxic compounds from *B. koreana.*

Experimental Section

General Experimental Procedures. Optical rotations were measured by a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA). IR spectra were recorded by a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany). Electrospray ionization (ESI) and HR-ESI mass spectra were recorded by a SI-2/LCQ DecaXP Liquid chromatography (LC)-mass spectrometer (Thermo Scientific, West Palm Beach, PL, USA). Nuclear magnetic resonance (NMR) spectra, including ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY experiments, were recorded by a Varian UNITY INOVA 500 NMR spectrometer (Varian, Palo Alto, CA, USA) operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), with chemical shifts given in ppm (δ). Preparative high performance liquid chromatography (HPLC) used a Gilson 306 pump (Gilson, Middleton, WI, USA) with a Shodex refractive index detector (Shodex, New York, NY, USA). Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) and RP-C_{18} silica gel (Merck, 40-63 μm) were used for column chromatography. Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates (Merck, Darmstadt, Germany) were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

Plant Material. The trunk of *B. koreana* was collected on Jeju Island, Korea, in December, 2005. Samples of plant material were identified by Prof. Kang Ro Lee (School of Pharmacy, Sungkyunkwan University, Suwon, Korea). A voucher specimen (SKKU 2005-10) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The trunk of *B. koreana* (2.7 kg) was dried and chopped, and then extracted with 80% aqueous MeOH two times (2 × 4 h) under reflux, and filtered. The filtrate was concentrated under vacuum to obtain a MeOH extract (220 g), which we suspended in distilled water (7.2 L) and then successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, yielding 8, 10, and 50 g of residue, respectively. Each fraction was evaluated for cytotoxicity against human tumor cell lines, the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines, by the SRB bioassay. The $CHCl_3$-soluble fraction showed a significant cytotoxic activity against the tested tumor cell lines. The $CHCl_3$-soluble fraction (10 g) was separated by silica gel (230-400 mesh, 250 g) column chromatography (CC) using a gradient solvent system of *n*-hexane-EtOAc (1:1) and then $CHCl_3$-MeOH (10:1, 5:1) as the eluant to give ten fractions (A-J). Among the fractions, active fractions I and J were consolidated and the mixture (3.0 g) was subjected to RP-C_{18} silica gel (40-63 μm, 200 g) CC using a gradient solvent system of MeOH-H_2O (1:1 → 1:0) to give five subfractions (fr. I1–I5). Fraction I5 (135 mg) was purified by semi-preparative reversed-phase HPLC (Econosil RP-18 10 μm column, 250 × 10.0 mm, 10 μm, flow rate: 2 mL/min) using an isocratic elution with 70% MeOH-H_2O over 30 minutes to afford compound **1** (18 mg).

Berkoreanol (1). Colorless gum; $[\alpha]_D^{25}$: –21.6 (*c* 0.30, MeOH); IR (KBr): ν_{max} = 3345, 2930, 2870, 1662, 1455, 1380, 1289, 1235, 1030 cm^{-1}; UV (MeOH) λ_{max} (log ε) 196 (4.12), 215 (2.85) nm; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; ESIMS: (positive-ion mode) *m/z* = 239 $[M + H]^+$; HR-ESIMS: (positive-ion mode) *m/z* = 239.2015 $[M + H]^+$ (calcd. for $C_{15}H_{27}O_2$, 239.2011).

Table 1. ^{1}H and ^{13}C NMR data of compound **1** in CD_3OD[a]

Position	1	
	δ_H	δ_C
1	1.54 m	45.8 d
2	1.95 m	18.4 t
	1.59 m	
3	2.03 m	31.0 t
	2.00 m	
4		134.6 s
5	5.52 d (5.5)	123.9 d
6	2.08 ddd (11.5, 5.5, 4.5)	36.2 d
7	1.77 m	39.2 d
8	1.45 m	21.5 t
	1.22 m	
9	1.58 m	34.0 t
	1.42 m	
10		71.7 s
11	2.20 m	32.0 d
12	3.86 dd (9.5, 6.5)	71.5 t
	3.82 dd (9.5, 7.0)	
13	0.91 d (7.0)	9.3 q
14	1.28 s	26.8 q
15	1.66 s	22.6 q

[a] ^{1}H and ^{13}C NMR data were recorded at 500 and 125 MHz, respectively. Coupling constants (in Hz) are given in parentheses.

Cytotoxicity Testing. A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each isolated compound against four cultured human tumor cell lines.[18] 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 (purity ≥ 98%, Sigma) 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} 0.16, 0.38, 0.04, and 0.82 μM, respectively.

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Supporting Information. 1D and 2D NMR spectra of **1** are available as Supporting Information.

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