Betulinans A and B, Two Benzoquinone Compounds from *Lenzites betulina*

In-Kyoung Lee,[†] Bong-Sik Yun,[†] Soo-Muk Cho,[†] Won-Gon Kim,[†] Jong-Pyong Kim,[†] In-Ja Ryoo,[†] Hiroyuki Koshino,[‡] and Ick-Dong Yoo*[,†]

Korea Research Institute of Bioscience and Biotechnology, Korea Institute of Science and Technology, P. O. Box 115, Yusong, Taejon 305-600, Korea, and The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama, 351-01, Japan

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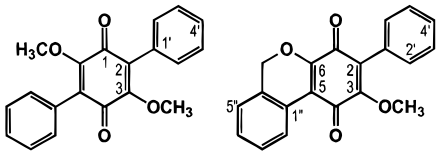
Two lipid peroxidation inhibitors, designated as betulinans A (**1**) and B (**2**), were isolated from the MeOH extract of *Lenzites betulina*. The structures of these compounds have been determined to be 2,5-diphenyl-3,6-dimethoxy-*p*-benzoquinone and 2-phenyl-3-methoxy-[1*H*-2-benzopyran][4,3-*e*][*p*]benzoquinone, respectively, on the basis of various spectral data. Betulinans A and B inhibited lipid peroxidation with IC_{50} values of 0.46 and 2.88 μg/mL, respectively.

Peroxidative disintegration of cells and organellar membranes by free radicals has been implicated in various pathological processes and especially involved in the pathogenesis of diseases such as myocardial and cerebral ischemia, atherosclerosis, diabetes, rheumatoid arthritis, and cancer-initiation and aging processes.[1−3] Thus, free radical scavengers have the potential as protective agents against various diseases. Some free radical scavengers including benthophoenin,[4] naphterpin,[5] benthocyanin,[6] pyrrolostatin,[7] and carazostatin[8] have been isolated from microbial sources. In the course of screening for free radical scavenging substances from basidiomycetes, we isolated two active compounds, betulinans A (**1**) and B (**2**), from the MeOH extract of fruiting bodies of *Lenzites betulina* (L.:Fr.) Fr. (Polyporaceae). We herein describe the isolation, structural determination, and free radical scavenging activities of **1** and **2**.



1 **2**

A MeOH extract of *Lenzites betulina* (1 kg) yielded compounds **1** (6 mg) and **2** (1 mg) by utilizing solvent partition, SiO_2 column chromatography, preparative SiO_2 TLC, and preparative RP-TLC. The molecular formula of **1** was determined to be $C_{20}H_{16}O_4$ by HREIMS (*m*/*z* found 320.1047 M^+, calcd 320.1048) indicating 13 degrees of unsaturation. The IR spectrum of **1** showed absorptions due to a quinone carbonyl group at 1650 (C═O) and 1590 (C═C) cm^{-1} and a mono-substituted benzene ring at 750 and 690 cm^{-1}. The NMR revealed signals attributable to eight protons including methoxy and mono-substituted benzene protons, while only eight carbons were observed in the ^{13}C-NMR spectrum (Table 1). These observations suggested that compound **1** was a symmetrical dimer composed of two quinone carbonyl groups, two mono-substituted benzene rings, two methoxy groups, and four sp^2 quaternary carbons. The structure of **1** was determined to be 2,5-diphenyl-3,6-dimethoxy-*p*-benzoquinone by the combination of the above subunits. The *p*-benzoquinone moiety was suggested by the UV spectrum. The UV absorptions at 247 and 323 nm were similar to that of polyporic acid-related compounds[9] with a *p*-benzoquinone moiety and differed from phlebiarubrone,[10] which has an *o*-benzoquinone moiety. Compound **1** also did not react with *o*-phenylenediamine, a compound that reacts readily with *o*-quinone. The structure of **1** was confirmed by the HMBC data, which had long-range couplings from a methine proton at 7.34 ppm to an sp^2 quaternary carbon at 126.6 ppm and from methoxy protons at 3.82 ppm to an sp^2 quaternary carbon at 154.7 ppm. The ^{1}H- and ^{13}C-NMR data of **1** are summarized in Table 1. Betulinan A is a methylated polyporic acid,[11−13] and this report is the first isolation of this compound from a natural source.

The molecular formula of **2** was determined to be $C_{20}H_{14}O_4$ on the basis of HREIMS data (*m*/*z* found 318.0885 M^+, calcd 318.0892). The IR spectrum of **2** indicated the presence of a quinone carbonyl group at 1660 (C═O) and 1580 (C═C) cm^{-1} and a mono-substituted benzene ring at 760 and 690 cm^{-1}, suggesting that the structure of **2** was similar to that of **1**. The ^{1}H-NMR spectrum showed peaks attributed to aromatic methine protons (δ 8.33 to 7.13), oxygenated methylene protons (δ 5.34), and methoxy protons (δ 3.82). The ^{13}C-NMR and HMQC spectra contained signals due to quinone carbonyl carbons at δ 192.3 and 185.0; oxygenated sp^2 quaternary carbons at δ 155.3 and 153.0; aromatic methines at δ 130.6, 129.7, 129.0, 128.7, 128.0, 126.5, and 124.4; sp^2 quaternary carbons at δ 129.9, 128.5, 126.1, 125.6, and 116.0; oxygenated methylene at δ 69.9; and methoxy carbon at δ 61.8. The structure of **2** was assigned as shown using HMBC data. The methine proton (C-2′) at δ 7.33 was long-range coupled to sp^2 carbons at δ 126.1 (C-2), 130.6 (C-6′), and 128.7 (C-4′), revealing that the benzene ring was conjugated to a quaternary carbon at δ 126.1. Long-range correlations from the methine proton (C-2″) at δ 8.33 to sp^2 quaternary carbons at δ 128.5 (C-6″) and 116.0 (C-5) and from methylene protons at δ 5.34 to sp^2 quaternary carbons at δ 153.0 (C-6), 128.5 (C-6″), and 125.6 (C-1″) suggested the presence of a 1*H*-2-benzopyran moiety in the structure of **2**. Long-range coupling from methoxy protons at δ 3.82 to a quaternary carbon at δ 155.3 was

* Author to whom correspondence should be addressed. FAX: 82 42 860 4595. E-mail: ybs@geri4680.geri.re.kr.
† Korea Research Institute of Bioscience and Biotechnology.
‡ The Institute of Physical and Chemical Research.
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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Betulinans A (**1**) and B (**2**) in $CDCl_3$

	1		2	
No.	δ_C	δ_H	δ_C	δ_H
1	183.5		192.3[b]	
2	126.6		126.1	
3	154.7		155.3	
3-OCH_3	61.6	3.82 (3H, s)[a]	61.8	3.82 (3H, s)
4	183.5		185.0[b]	
5	126.6		116.0	
6	154.7		153.0	
1′	130.2		129.9	
2′,6′	130.6	7.34 (2H, dd, J = 7.8, 1.5)	130.6	7.33 (2H, dd, J = 7.8, 1.5)
3′,5′	128.0	7.44 (2H, m)	128.0	7.43 (2H, m)
4′	128.6	7.40 (1H, m)	128.7	7.42 (1H, m)
1″	130.2		125.6	
2″	130.6	7.34 (1H, dd, J = 7.8, 1.5)	126.5	8.33 (1H, br d, J = 7.8)
3″	128.0	7.44 (1H, m)	129.0	7.41 (1H, m)
4″	128.6	7.40 (1H, m)	129.7	7.38 (1H, ddd, J = 7.8, 7.8, 1.2)
5″	128.0	7.44 (1H, m)	124.4	7.13 (1H, br d, J = 7.8)
6″	130.6	7.34 (1H, dd, J = 7.8, 1.5)	128.5	
6″-CH_2			69.9	5.34 (2H, s)

[a] Proton resonance multiplicity and coupling constant (J = Hz) are in parenthesis. [b] The assignment is interchangeable.

also observed. The chemical shifts of C-2 and C-3 in **2** were in good agreement with those of the corresponding carbons in **1**, confirming that **2** is a new natural product containing a *p*-quinone moiety.

Betulinans A and B inhibited lipid peroxidation with IC_{50} values of 0.46 and 2.88 μg/mL, respectively. Betulinan A was about four times as active as vitamin E (1.68 μg/mL).

Experimental Section

General Experimental Procedures. Melting points of compounds **1** and **2** were determined on a MEL-TEMP II laboratory device and were uncorrected. UV and IR spectra were recorded on a Shimazu UV-260 spectrophotometer and a Laser Precision Analect RFX-65S FT-IR spectrometer, respectively. NMR spectra were obtained using a JEOL JNM-A400 spectrometer in $CDCl_3$ with TMS as an internal standard. The chemical shifts are given in ppm values. EIMS and HREIMS spectra were taken on a Hewlett-Packard Model HP 5989A EIMS system and a JEOL JMS-HX 110A mass spectrometer, respectively, operating at 70 eV. Preparative TLC was performed with Si gel 60 F_{254} and RP-18 F_{254} (Merck).

Fungal Material. *Lenzites betulina* (L.:Fr.) Fr. was collected at Kyeryong Mountain, Chungnam province, Korea, in August 1995, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology, Korea, according to the taxonomic key of Imazeki and Hongo.[14] A voucher specimen is deposited in the Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology. The sample was dried in the dark at room temperature and finely milled for solvent extraction.

Extraction and Isolation. *Lenzites betulina* (1 kg) was extracted twice with 80% aqueous MeOH at room temperature for one day and filtered. The filtrate was concentrated under reduced pressure, and the residue was partitioned between *n*-hexane and H_2O. The *n*-hexane layer was concentrated *in vacuo* and then chromatographed on a SiO_2 column eluted with *n*-hexane/EtOAc (10:1−5:1). The active fractions were concentrated *in vacuo* and subjected to SiO_2 TLC developed with hexane/EtOAc (3:1). Two active bands with R_f values of 0.55 (**1**) and 0.48 (**2**) were removed and extracted with $CHCl_3$/MeOH. The extracts were further purified by reversed-phase TLC with 70% CH_3-CN as a developing solvent to give **1** (6 mg, R_f 0.32) and **2** (1 mg, R_f 0.34).

Betulinan A (1): yellow powder; mp 191−193 °C; UV λ max (EtOH)(ϵ) 247 (8500), 323 (6000) nm; IR (KBr) 1650, 1590, 1440, 1300, 1110, 1040, 750, 690 cm^{-1}; for NMR data, see Table 1; EIMS m/z 320 $[M]^+$, 279, 221, 167, 89; HREIMS m/z $[M]^+$ 320.1047 ($C_{20}H_{16}O_4$ requires 320.1048).

Betulinan B (2): red powder; mp 124−126 °C; UV λ max (EtOH)(ϵ) 238 (6000), 276 (8000), 327 (4000) nm; IR (KBr) 2930, 1740, 1660, 1580, 1450, 1300, 1090, 1040, 760, 690 cm^{-1}; for NMR data, see Table 1; EIMS m/z 318 $[M]^+$, 275, 191, 89; HREIMS m/z $[M]^+$ 318.0885 ($C_{20}H_{14}O_4$ requires 318.0892).

Inhibitory Activity Against Lipid Peroxidation in Rat Liver Microsomes. Rat liver microsomes were prepared according to the method of Ohkawa *et al.*[15] with some modifications and finally suspended in 100 mM Tris−HCl buffer (pH 7.4). Reaction was initiated by the addition of 500 μM $FeSO_4 \cdot 7\ H_2O$. After 30 min at 37 °C under reciprocal agitation, the reaction was stopped by the addition of 3 M trichloroacetic acid in 2.5 N HCl. Lipid peroxidation was evaluated by measuring the thiobarbituric acid reactive products. Inhibitory activity was calculated as follows: [1 − (T−B)/(C − B)] × 100 (%), in which T, C, and B are absorbance values at 530 nm of the drug treatment, the control (peroxidation without a drug), and the 0 time control (no peroxidation), respectively.

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