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Boletunones A and B, Highly Functionalized Novel Sesquiterpenes from *Boletus calopus*

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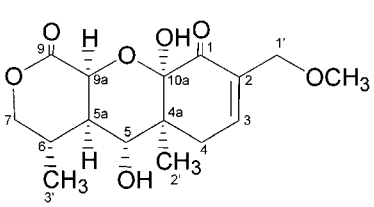
ABSTRACT



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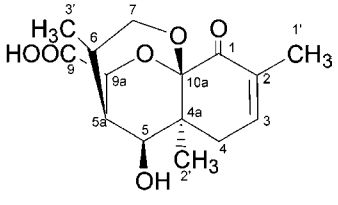

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Highly functionalized novel sesquiterpenes, named boletunones A and B, were isolated from the methanolic extract of the fruiting body of the mushroom *Boletus calopus*. Their structures and relative stereochemistry were established by various spectral analyses.

Mushrooms have proved to be a rich source of secondary metabolites with unusual structures as well as interesting biological activities. Despite their potential for drug development, few bioactive metabolites have been reported from mushrooms as compared with higher plants and microbes. We have screened biologically active and chemically novel compounds from the fruiting bodies of Korean basidiomycetes.[1,2] In the course of our continuing research, highly functionalized novel sesquiterpenes, named boletunones A (**1**) and B (**2**), have been isolated from the mushroom *Boletus calopus* Pers.: fr. (Boletaceae). *Boletus* spp. are known to produce a variety of amino acid analogues such as 2-amino-4-hydroxypentanoic acid[3] and 2-amino-4-methyl-5-hexenoic acid,[4] prenylated phenolics such as asiaticusin,[5] peptaibols such as boletusin,[6] benzoquinones such as boviquinone,[7] thelephoric acid precursors such as cyclovariegatin,[8] polyene dicarboxylic acids such as boletocrocin[9] and dodecapentaenedioic acid, macrolide phenolics such as ornatipolide,[10] and hydroxylated pulvinic acids such as xerocomic acid, variegatic acid, variegatorubin, and pulviquinone.[11] In this paper, we present the isolation and structure determination of **1** and **2**.

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Table 1. ^{13}C (125 MHz) and ^{1}H (600 MHz) NMR spectral data of **1** and **2** in DMSO-d_6

	1		2	
C	δ C	δ H (J, Hz)	δ C	δ H (J, Hz)
1	198.7 s		198.6 s	
2	133.1 s		131.7 s	
3	146.5 d	6.94 (m)	145.0 d	6.77 (br, s)
4	28.1 t	H_α 2.50 (dd, 20.8, 2.3)	30.1 t	H_α 2.46 (d, 19.7)
		H_β 2.61 (dd, 20.8, 5.6)		H_β 2.56 (d, 19.7)
4_a	56.0 s		54.5 s	
5	76.2 d	4.35 (s)	71.9 d	3.77 (s)
5_a	45.9 d	2.14 (m)	46.0 d	2.57 (m)
6	29.8 d	2.01 (m)	27.6 d	2.08 (m)
7	71.5 t	H_α 4.04 (dd, 10.5, 3.6)	66.6 t	H_α 4.10 (t, 11.5)
		H_β 3.88 (t, 10.5)		H_β 3.35 (dd, 11.5, 6.8)
9	171.3 s		173.0 s	
9_a	73.2 d	4.49 (d, 9.6)	77.4 d	4.34 (d, 3.6)
10_a	106.3 s		107.0 s	
10_a-OH		5.92 (s)		
1′	68.6 t	H_α 4.01 (d, 13.5)	15.2 t	1.63 (s)
		H_β 3.94 (d, 13.5)		
1′-OCH_3	58.4 q	3.36 (s)		
2′	21.7 q	1.57 (s)	21.0 q	0.94 (s)
3′	17.0 q	0.79 (d, 6.2)	15.0 q	0.83 (d, 7.1)

Specimens of *Boletus calopus* were collected at Odae mountain, Kangwon province, Korea, in July to August, 2002, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology, Korea, according to the taxonomic key of Imazeki and Hongo. A voucher specimen is deposited in the Laboratory of Antioxidants, Korea Research Institute of Bioscience and Biotechnology. Fresh fruiting bodies of *Boletus calopus* (1.8 kg) were extracted twice with MeOH at room temperature for 3 days and filtered. The methanolic extract was concentrated under reduced pressure, and the aqueous residue was partitioned between hexane, $CHCl_3$, EtOAc, and BuOH. The EtOAc extract was concentrated in vacuo, and the crude residue was subjected to silica gel column chromatography followed by stepwise elution with $CHCl_3$−MeOH (50:1, 25:1,15:1, 5:1). The fractions eluted with $CHCl_3$−MeOH (5:1) were pooled and concentrated in vacuo. The residue was applied again to a Sephadex LH-20 and then eluted with MeOH to give two active fractions. One fraction dissolved in MeOH was further purified by ODS HPLC column chromatography eluted with MeOH−H_2O (45:55) to yield compound **1**[12] as a white powder. The other fraction was purified by preparative ODS TLC developed with MeOH−H_2O (45:55) to afford compound **2**[13] as a white powder.

The molecular formula of **1** was determined to be $C_{16}H_{22}O_7$ on the basis of high-resolution ESI-MS [$(M + Na)^+$, 349.12546 m/z (−0.31 mmu error)] in combination with ^{1}H and ^{13}C NMR data. The IR data suggested the presence of a δ-lactone (1735 cm^{-1}), a carbonyl (1668 cm^{-1}), and a hydroxyl (3422 cm^{-1}) moiety. The ^{1}H and ^{13}C NMR data (Table 1) with DEPT, $^{1}H-^{1}H$ COSY, and HMQC data suggested the presence of $-C(CH_2-O-)=CH-CH_2-$, $-C(-O-)H-CH-CH(CH_3)-CH_2-$, a hydroxylated methine, an isolated methyl, a methoxy, a carboxylic carbon, a carbonyl, two sp^3 quaternary carbons, and two exchangeable protons. In the HMBC spectrum (Figure 1), long-range couplings were observed from the olefinic proton at δ 6.94 (3−H) of the $-^{2}C(^{1'}CH_2-O-)=^{3}CH-^{4}CH_2-$ group to the

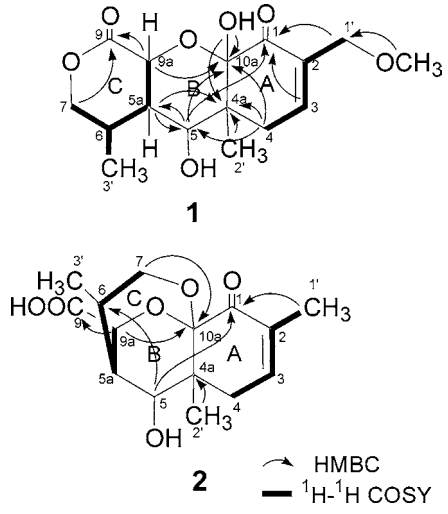

Figure 1. Key HMBC and $^{1}H-^{1}H$ COSY correlations of Boletunones A (**1**) and B (**2**).

(12) Data for boletunone A (**1**): white powder; mp 255 °C; $[\alpha]_D$ +50° (c 0.6 mg/mL, MeOH); UV (MeOH) $\lambda_{max}(\epsilon)$ 236 (14855); IR (KBr) 3422, 2923, 2853, 1735, 1668, 1598, 1459 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESI-MS m/z 349.1254 $(M + Na)^+$, $C_{16}H_{22}O_7Na$ requires 349.1257.

(13) Data for boletunone B (**2**): white powder; mp 282 °C; $[\alpha]_D$ +32° (c 1.2 mg/mL, MeOH); UV (MeOH) $\lambda_{max}(\epsilon)$ 242 (21009); IR (KBr) 3429, 2927, 1722, 1667, 1595, 1411 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESI-MS m/z 319.1132 $(M + Na)^+$, $C_{15}H_{20}O_6Na$ requires 319.1158.

carbonyl carbon (C-1, δ 198.7), C-4 (δ 28.1), and one sp^3 quaternary carbon (C-4a, δ 56.0). The methylene proton (4−H_β) at δ 2.61 of the $-^2C(^{1'}CH_2-O-)=^3CH-^4CH_2-$ group was long-range coupled to C-4a, the other sp^3 quaternary carbon at δ 106.3 (C-10a), and the hydroxylated methine at δ 76.2 (C-5). The hydroxylated methine at δ 4.12 (5-H), in turn, was long-range coupled to C-4a and C-10a, suggesting together with the ^{13}C chemical shift of C-10a that C-10a might be connected to C-1. The long-range correlation of 5-H with C-1 was observed in the HMBC using CD_3OD, indicating that C-10a should be connected to C-1. These spectral data indicated the presence of the six-membered ring A incorporating α,β-unsaturated carbonyl group in its structure. Long-range couplings were observed from the protons at δ 2.14 (5a−H), δ 4.49 (9a−H) and δ 4.04 (7-H_α) of the $-^{9a}C(-O-)H-^{5a}CH-^6CH(CH_3)-^7CH_2-$ group to the carboxylic carbon at δ 171.3 (C-9). This indicated the presence of the δ-lactone ring C. Long-range couplings were observed from 5-H to the methine carbon at δ 45.9 (C-5a) of the ring C as well as C-4a and C-10a of the ring A and from 5a−H to C-4a and C-5. In addition, the oxygenated proton at 4.49 (9a−H) of the ring C was long-range coupled to C-10a of the ring A. These data clearly indicated that the ring B is present between rings A and C. Thus, the planar structure of boletunone A was determined as **1** having an unique skeleton as shown in Figure 1.

The relative stereochemistry of **1** was determined by NOESY experiments as shown in Figure 2. NOEs were observed among 4-H_α, 4a-Me, and 10a-OH. NOE effects between 5-H and 4-H_β were also observed. These data indicated that the relative stereochemistries of C-4a, C-5, and C-10a should be *S**, *R**, and *R**, respectively. Also, NOEs were observed among 9a−H, 5a−H, and 7-H_β. 6-H has a NOE correlation to 7-H_α, while 6-Me has NOE correlations to both 7-H_α and 7-H_β, suggesting that H-6 and 7-H_β are in anti configurations. Very interestingly, NOEs from 4-H_β to 6-H were observed. These data, with the aid of computer-generated three-dimensional drawings, indicated that **1** has a peculiar stereostructure of a U shape. From these spectral data, the relative stereochemistries of C-5a, C-6, and C-9a were determined to be *R**, *R**, and *S**, respectively.

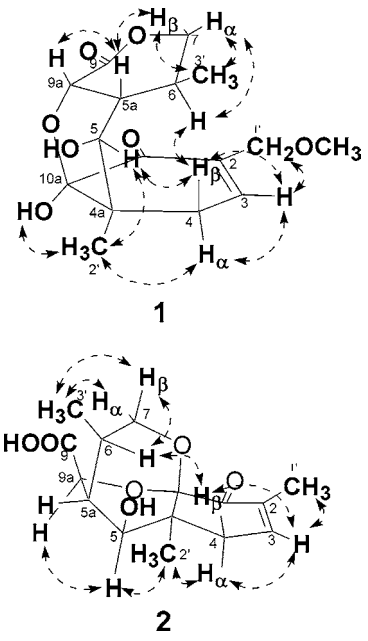

Figure 2. Key NOE correlations of Boletunones A (**1**) and B (**2**).

The molecular formula of **2** was determined to be $C_{15}H_{20}O_6$ on the basis of high-resolution ESI-MS [$(M + Na)^+$, 319.1132 *m*/*z* (−2.6 mmu error)] in combination with ^1H and ^{13}C NMR data. The IR data suggested the presence of a carboxylic (1723 cm^{-1}), a carbonyl (1667 cm^{-1}), and a hydroxyl (3429 cm^{-1}) moiety. The ^1H and ^{13}C NMR data (Table 1) of **2** with DEPT, $^1H-^1H$ COSY, and HMQC data were similar to those of **1**. One difference in the ^1H and ^{13}C NMR data was that one more methyl was observed in **2**, while the methoxy and one methylene of **1** disappeared, suggesting that the methoxymethylene residue of **1** was replaced by a methyl in **2**. This was confirmed by the long-range correlations from the methyl (1′-H) at δ 1.63 to a carbonyl carbon (δ 198.6, C-1), an sp^2 quaternary carbon (δ 131.7, C-2), and an olefinic carbon (δ 145.0, C-3). The remaining methylene (δ_H 4.10 and δ_H 3.57, δ_C 66.6, C−7) was determined to be present as $-CH-CH-CH(CH_3)-CH_2-$ like in **1** by $^1H-^1H$ COSY and HMBC data (Figure 1). The other difference in the ^1H and ^{13}C NMR data was that the methylene carbon of C-7 was downfield-shifted from δ 71.5 to 66.6 and the exchangeable proton of 10a-OH disappeared in **2**. In addition, the ^{13}C chemical shift of the methine at C-9a was also changed from δ 73.2 to 77.4. Considering the higher polarity of **2** than **1**, these spectral data suggested that the methylene of C-7 might be connected as an ether linkage with C-10a, not as an ester bond with the carboxylic acid, resulting in a free carboxylic acid. This was confirmed by HMBC experiments. The proton of 7-H_β at δ 3.57 was long-range coupled to two methine carbons at δ 47.5 (C-5a) and δ 29.9 (C-6) and one sp^3 quaternary carbon at δ 107.0 (C-10a), not the carboxylic acid at δ 173.0. These spectral data clearly indicated that the methylene of C-7 should be connected as the ether bond with C-10a. The remaining structure was also confirmed by HMBC spectral data. Therefore, the planar structure of **2** was determined as shown in Figure 1.

The stereochemistry of **2** was determined by NOESY experiments (Figure 2). NOEs were observed among 4-H_α, 4a-Me, 5-H, and H-9a. 6-H correlates to 7-H_β, while 6-Me correlates to both 7-H_α and 7-H_β, suggesting that H-6 and 7-H_α are in anti configurations. Like in **1**, NOEs between 6-H and 4-H_β were also observed. These observations indicated that 4-Ha, 4a-Me, 5-H, and 9a−H were all β-oriented, while 5a-H has α-orientation. This configuration was supported by the small coupling constant between 5a-H and 9a-H (3.6 Hz) and the high-field-shifted chemical shift (δ_H 0.94) of 4a-Me, which could be induced by an

anisotrophic effect. Therefore, the relative stereochemistries of C-4a, C-5, C-5a, C-6, C-9a, and C-10a should be *S**, *S**, *S**, *R**, *S**, and *S**, respectively.

Boletunone A and B are novel sesquiterpene compounds incorporating highly oxygenated skeleton in their molecules. Tricyclic sesquiterpenes of unique skeleton and stereostructure were found in boletunones A and B which have not been previously described as far as we know. Boletunone A and B exhibited free-radical scavenging activity in a dose-dependent fashion with IC_{50} (μM) values of 4.97 and 5.39 in a DPPH assay, respectively.

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