Non-isoprenoid botryane sesquiterpenoids from basidiomycete 
*Boletus edulis* and their cytotoxic activity

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Abstract: Three non-isoprenoid botryane sesquiterpenoids, named boledulins A–C (1–3), have been isolated from the cultures of basidiomycete *Boletus edulis* Bull. The structures were established by means of spectroscopic methods. Boledulin A (1) exhibited moderate inhibitory activity against five human cancer cell lines.

Keywords: botryane, sesquiterpenoid, boledulin, *Boletus edulis*

Introduction
Botryane sesquiterpenoids possess a non-isoprenoid system skeleton, which have been found limited to several fungi such as *Botrytis cinerea* and *Daldinia concentrica*. The representative botryane sesquiterpenoids are botrydial and its derivatives, which are characterized from phytopathogenic fungus *B. cinerea*. These sesquiterpenoids showed a wide range of biological activities. For instance, they were responsible for the typical infection, and they played an important role in the pathogenicity of the organism in vivo. Botryane sesquiterpenoids attracted great interests of chemists to carry out a large number of investigations including chemical transformations, structure-activity relationships, synthesis, and biosynthesis. Our group has long been focused on the chemical study on higher fungi. Recently, three new botryane sesquiterpenoids, boledulins A–C (1–3), have been isolated from cultures of *Boletus edulis* Bull, an edible basidiomycete collected from southwest of China. The structures were established by extensive spectroscopic data. It is noted that compound 3 is a 15-nor-botryane sesquiterpenoid which was seldom found previously. In addition, compounds 1–3 were evaluated for their cytotoxicity against five human cancer cell lines. This paper reports the isolation, structural elucidation, and cytotoxicity of these compounds.

Results and Discussion

Compound 1 was isolated as optical active white solid ([α]D)30 + 16.8). HRESIMS displayed an [M + Na]+ peak at m/z 337.1990 (calcd 337.1990 for C17H20O3Na) indicating a molecular formula C17H20O3 corresponding to three degrees of unsaturation. IR spectrum revealed the existence of hydroxy and carbonyl groups due to absorption bands at 3439 and 1729 cm⁻¹, respectively. The 13C NMR spectrum gave 17 carbon resonances (Table 1). Besides two methoxy signals at δC 51.6 and 59.4, 15 resonances can be ascribable to four methyls, three sp³ methylenes, four sp³ methines, three sp³ quaternary carbons, and one sp³ quaternary carbon at δC 174.6. These data suggested that compound 1 might be a bicyclic sesquiterpenoid.

In the HMBC spectrum, the correlation of a methoxy signal at δH 3.70 (3H, s) with δC 174.6 (s, C-10) established a methyl ester group. A key correlation of δH 2.45 (1H, d, J = 12 Hz, H-1) with C-10 suggested the linkage of C-10 to the methine. Starting from this methine, a structural fragment was established by the analysis of 1H–1H COSY spectrum as shown in Figure 1. The HMBC correlations of H-1 and δH 1.56 (1H, d, J = 11.0 Hz, H-5) with C-10 suggested the linkage of C-10 to the methine. In addition, a methoxy group placed at...
C-15 was also deduced from the HMBC correlation (Figure 1).

In the ROESY spectrum, the observed cross peaks of H-1/H-5, H-2/H-4, H-1/CH-11, H-4/CH-12 and H-4/CH-14 suggested H-1, H-5, and Me-11 in the same side, while H-2, H-4, Me-12, and Me-14 in the opposite side. Further, these above ROESY cross peaks limited OH-9 to be H-4, Me-12, and Me-14 in the opposite side. Further, these suggested H-1, H-5, and Me-11 in the same side, while H-2, H-4, Me-12, and Me-13, respectively) with δC 150.9 (s, C-5) suggested the double bond placed between C-5 and C-9. The HMBC correlations between H-1, H-4 and C-5, C-9 were also observed. Detailed analysis of other 2D NMR data confirmed that the other parts of 2 were the same as those of 1. Therefore, the structure of 2 (boledulin B) was established.

Compound 3 was isolated as white solid. The molecular formula C18H25O4 was established by HREIMS at m/z 244.1765 [M]+ (calcd for C18H26O4 at m/z 244.1776 [M]+) indicating three degrees of unsaturation. The 1H NMR spectrum displayed similar patterns to those of 1 and 2 including clear signals for four methyl signals (three singlets and one doublet) (Table 2). The 13C NMR spectrum revealed 14 carbon resonances ascribable to four sp3 methyls, three sp3 methylenes, four sp2 methines, one sp3 quaternary carbon, and two sp2 quaternary carbons (Table 1). These data suggested that compound 3 might be a bicyclic nor-sesquiterpenoid.

The 13C NMR signal at δC 60.5 (t, C-10) allowed the existence of an oxygen-containing methylene, and starting from which, two structural fragments of CH2=CH- and CH2CHCH2CH2CHCH2- were established by the 1H–1H COSY spectrum as shown in Figure 1. In the HMBC spectrum, the key correlations of δH 1.83 (1H, m, H-1) with δC 131.4 (s, C-9) and δH 2.04 (1H, d, J = 6.4 Hz, H-5) with δC 131.4 (s, C-9) suggested the link of C-9 to C-1 and C-5, respectively. Hence, the structure of ring A was established. The HMBC correlation

Figure 1. Selected 2D NMR correlations of 1 and 3.

Table 1. 13C NMR data for boledulins A–C (1–3).

| position | 1 | 2 | 3 |
|----------|---|---|---|
| 1        | 61.3, CH | 44.2, CH | 48.4, CH |
| 2        | 29.9, CH | 33.1, CH | 31.7, CH |
| 3        | 43.6, CH3 | 37.9, CH3 | 44.8, CH3 |
| 4        | 70.2, CH | 67.3, CH | 70.4, CH |
| 5        | 68.4, CH | 150.9, C | 65.8, CH |
| 6        | 36.7, qC | 44.9, qC | 37.7, qC |
| 7        | 53.0, CH3 | 51.5, CH3 | 56.6, CH3 |
| 8        | 49.4, qC | 50.3, qC | 129.4, qC |
| 9        | 87.8, qC | 136.4, qC | 131.4, qC |
| 10       | 174.6, qC | 175.3, qC | 60.5, CH3 |
| 11       | 21.1, CH3 | 19.4, CH3 | 20.0, CH3 |
| 12       | 28.3, CH3 | 30.1, CH3 | 24.8, CH3 |
| 13       | 36.3, CH3 | 30.6, CH3 | 31.2, CH3 |
| 14       | 21.8, CH3 | 23.6, CH3 | 15.0, CH3 |
| 15       | 78.0, CH3 | 68.6, CH3 |  |
| COOCH3   | 51.6, CH3 | 52.3, CH3 |  |
| OCH3     | 59.4, CH3 |  |  |

*Measured in CDCl3 at 100 MHz; †Measured in CDCl3 at 150 MHz.

Accordingly, the absolute configuration of 1 could be determined as 1S, 2R, 4S, 5R, 8S, 9S. Therefore, the structure of 1 (boledulin A) was established.

Compound 2 was isolated as a colorless oil, that gave an [M + Na]2 peak at m/z 305.1720 (calcd for C19H25O4Na, 305.1728) in the positive ion HRESIMS, consistent with the molecular formula C19H24NaO4 indicating four degrees of unsaturation. The 1D NMR spectroscopic data (Tables 1 and 2) suggested that the backbone of 2 was the same as that of 1. Differences between them were identified to be a new double bond (δC 136.4 and 150.9) and the loss of a methoxy group at C-15 in 2. The HMBC correlations of δH 0.93 (3H, s, Me-14) with δC 136.9 (s, C-9) and δH 1.16 and 1.34 (each 3H, s, Me-12 and Me-13, respectively) with δC 150.9 (s, C-5) suggested the double bond placed between C-5 and C-9. The HMBC correlations between H-1, H-4 and C-5, C-9 were also observed. Detailed analysis of other 2D NMR data confirmed that the other parts of 2 were the same as those of 1. Therefore, the structure of 2 (boledulin B) was established.

Table 2. 1H NMR Data for Boledulins A–C (1–3).

| position | 1 | 2 | 3 |
|----------|---|---|---|
| 2        | 1.83, m | 1.89, m | 1.60, m |
| 3a       | 1.08, d (12.0) | 1.58, m | 1.20, m |
| 3b       | 1.92, m | 1.92, m |  |
| 4        | 3.92, dd | 4.32, dd | 3.56, dd |
| 5        | 1.56, d (11.0) | 2.04, d (6.4) |  |
| 7        | 1.15, d (12.8) | 1.49, d (13.2) | 2.05, d (16.8) |
| 10       | 2.42, d (12.8) | 1.98, d (13.2) | 2.19, d (16.8) |
| 11       | 0.87, d (6.6) | 0.95, d (6.6) | 1.00, d (6.4) |
| 12       | 1.28, s | 1.16, s | 1.08, s |
| 13       | 1.26, s | 1.34, s | 1.17, s |
| 14       | 0.97, s | 0.93, s | 1.78, s |
| 15a      | 3.01, d (10.2) | 3.07, d (10.4) |  |
| 15b      | 3.31, d (10.2) | 3.09, d (10.4) |  |
| COOCH3   | 3.70, s | 3.69, s |  |
| OCH3     | 3.27, s |  |  |

*Recorded in CDCl3 at 400 MHz; †Recorded in CDCl3 at 600 MHz.
Optical rotations were measured with a Jasco P-1020 polarimeter. IR spectra were obtained on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectra were run on an AV-400 MHz or a Bruker avance III-600 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) are expressed in ppm with reference to solvent signals. HREIMS were recorded on a Waters Auto Premier P776 spectrometer. HRESIMS were recorded on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao), and spots were visualized by 10% H2SO4 in ethanol.

### Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a Lecos P-1020 polarimeter. IR spectra were obtained on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. 1D and 2D NMR spectra were run on an AV-400 MHz or a Bruker avance III-600 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) are expressed in ppm with reference to solvent signals. HREIMS were recorded on a Waters Auto Premier P776 spectrometer. HRESIMS were recorded on an API QSTAR Pulsar i spectrometer. Column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People’s Republic of China). An Agilent 1100 series instrument equipped with an Agilent ZORBAX SB-C18 column (5 μm, 4.6 mm × 150 mm) was used for high-performance liquid chromatography (HPLC) analysis, and a semipreparative Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 150 mm) was used for the sample preparation. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao), and spots were visualized by 10% H2SO4 in ethanol.

**Fungal Material and Cultivation Conditions.** The fungi *Boletus edulis* Bull. were collected from Ailao Mountain, Yunnan province, China. A voucher specimen was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. The mycelial cultures were derived from tissue plugs. Culture PDA medium: potato (peeled), 200 g, glucose, 20 g, KH2PO4, 3 g, MgSO4, 1.5 g, citric acid, 0.1 g, and thiamin hydrochloride, 10 mg, in 1 L of deionized H2O. The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out on a shaker at 25 °C and 150 rpm for 20 days.

### Extraction and Isolation.

The culture broth (20 L) was extracted three times with EtOAc. The EtOAc was evaporated in vacuo to yield an extract (8.6 g). The latter was subjected to a silica gel column eluted with petroleum ether-acetone (1:0 to 1:1) to afford fractions 1–5. Fraction 2 (1.8 g) was separated by silica gel CC (petroleum ether-Me2CO, 10:1→3:1) to afford two subfractions a and b. Fraction a (100 mg) was separated repeatedly by silica gel CC (petroleum ether-EtOAc, 7:1) to afford 3 (10.5 mg). Fraction b (30 mg) was separated further by HPLC (acetonitrile-H2O, 40:60 to 60:40) to yield 1 (2.2 mg) and 2 (1.1 mg).

**Boledulin A (1):** white solid; [α]20 D + 16.8 (c 0.19, CHCl3); IR (KBr) ν max 3439, 2924, 2855, 1729, 1629, 1177, 1088 cm−1; 13C (150 MHz) and 1H NMR (600 MHz) data (CDCl3), see Tables 1 and 2, respectively; positive ion HRESIMS m/z 337.1990 (calcd for C14H10O2Na [M + Na]+, 337.1990)

**Boledulin B (2):** colorless oil; [α]20 D + 10.3 (c 0.17, CHCl3); 13C (150 MHz) and 1H NMR (600 MHz) data (CDCl3), see Tables 1 and 2, respectively; positive ion HRESIMS m/z 305.1720 (calcd for C14H10O2Na [M + Na]+, 305.1728)

**Boledulin C (3):** white solid; [α]20 D − 1.0 (c 0.18, CHCl3); IR (KBr) ν max 3251, 2977, 2828, 1699, 1432, 1037 cm−1; 13C (100 MHz) and 1H NMR (400 MHz) data (CDCl3), see Tables 1 and 2, respectively; HRESIMS m/z 224.1765 (calcd for C14H10O2 [M]+, 224.1776)

**Cytotoxicity Assay.** Five human cancer cell lines, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA). An Agilent 1100 series instrument equipped with Agilent ZORBAX SB-C18 column (5 μm, 4.6 mm × 150 mm) was used for high-performance liquid chromatography (HPLC) analysis, and a semipreparative Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 150 mm) was used for the sample preparation. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao), and spots were visualized by 10% H2SO4 in ethanol.

### Table 3. Cytotoxicity for Boledulins A-C (1-3) (IC50, μM).

| Compd. | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW480 |
|--------|-------|-----------|-------|-------|-------|
| 1      | 2.6   | 8.4       | 8.3   | 3.4   | 3.5   |
| 2      | >40   | >40       | >40   | >40   | >40   |
| 3      | >40   | >40       | >40   | >40   | >40   |
| cisplatin | 1.1   | 14.5      | 12.8  | 13.0  | 12.6  |
| taxol  | <0.008| <0.008    | <0.008| <0.008| <0.008|

All compounds were evaluated for their cytotoxicties against five human cancer cell lines using the MTT method as reported previously.7 The result displayed that compound 1 showed moderate cytotoxicity against five human cancer cell lines using cisplatin as the positive control, while compounds 2–3 were inactive against all the tested cell lines with IC50 values of more than 40 μM (Table 3).
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