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Two new sesquiterpenoids from the fungus *Ceriporia alachuana*

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Two new sesquiterpenoids from the fungus *Ceriporia alachuana*

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Two new sesquiterpenoids, tremulenolide D (**1**) and muurolane-10 β ,15-diol (**2**), together with four known sesquiterpenoids, tremulenediol A (**3**), 2 β -hydroxy- α -candinol (**4**), epicubenol (**5**), and 3 β -hydroxy- δ -candinol (**6**), were isolated from cultures of the fungus *Ceriporia alachuana*. The structures of new compounds were determined by extensive spectroscopic analyses. Structurally, compounds **1** and **3** are tremulane-type sesquiterpenoids with an unusual perhydroazulene carbon skeleton.

Keywords: *Ceriporia alachuana*; tremulenolide D; muurolane-10 β ; 15-diol; sesquiterpenoids

1. Introduction

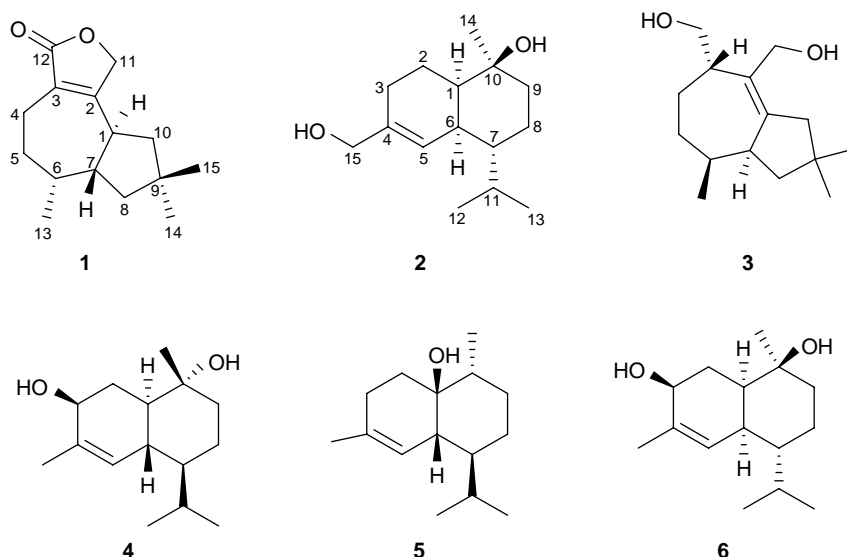
The genus *Ceriporia*, belonging to the family Phanerochaetaceae within Polyporales order, was widely present from boreal to subtropic regions in China [1]. Previous investigations on *Ceriporia* species have reported two new tremulane-type sesquiterpenoids from *Ceriporia lacerate* [2] and the antidiabetic activity of the culture extract of this fungus [3]. However, up to date, little attention was paid to chemical and pharmacological investigation of *Ceriporia alachuana*, a wood-decaying fungus commonly found in China [4–7]. As part of our efforts to search for secondary metabolites from higher fungi [8–12], the study on the EtOAc extract of the cultures of *C. alachuana* led to the two new sesquiterpenoids, tremulenolide D (**1**) and muurolane-10 β ,15-diol (**2**) and the four known sesquiterpenoids, tremulenediol A (**3**) [13], 2 β -hydroxy- α -candinol (**4**) [14], epicubenol (**5**) [15], and 3 β -hydroxy- δ -candinol (**6**) [16] (Figure 1). Compounds **1** and **3** were tremulane-type sesquiter-

penoids with an unusual perhydroazulene carbon skeleton that was initially isolated from the aspen tree rotting fungus *Phellinus tremulae* in 1993 [13], while compounds **2** and **4–6** belonged to candinane type. Herein, we report the isolation and structural elucidation of these compounds.

2. Results and discussion

Compound **1**, obtained as colorless oil, had a molecular formula C₁₅H₂₂O₂ based on HR-ESI-MS at m/z 234.1623, corresponding to five degrees of unsaturation. The IR spectrum revealed the existence of carbonyl groups (1766 cm⁻¹) and double bonds (1632 cm⁻¹). In the ¹³C NMR (DEPT) spectrum, 15 carbons were recognized as three methyls, five methylenes (including an oxygen-bearing one), three methines, and four quaternary carbons (including a carbonyl and a tetrasubstituted double bond). The ¹H and ¹³C NMR spectral data of compound **1** resembled those of tremulenolide A [13], with the main

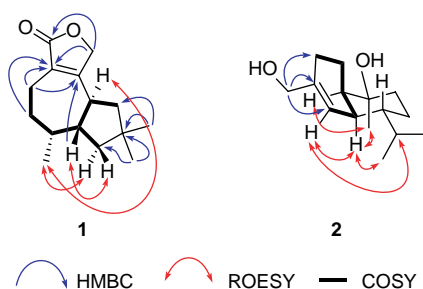
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Figure 1. Structures of compounds **1**–**6**.

difference located at the positions of 1–4 and 11 and 12, which indicated the variation in five-membered lactone part. Further ^1H – ^1H COSY (H-1/H-7/H-6/H-5/H-4) and HMBC (H-5 and H-4 with C-3 and H-4 and H-7 with C-2) experiments revealed that the double bond was located between C-2 and C-3, instead of between C-1 and C-2 in tremulenolide A. The ester carbonyl group was proposed at C-12 from HMBC correlations of H-1/C-3 and C-11, H-4/C-12, and H-11/C-12. Therefore, the planar structure of compound **1** was constructed. In order to determine its relative stereochemistry, ROESY experiment was

carried out, in which the obvious correlations of H-1/Me-13, Me-13/H-8 α , H-6/H-8 β , and H-7/H-8 β were observed, indicating the α orientation of H-1 and Me-13 and β orientation of H-6 and H-7 (Figure 2). With the evidence mentioned above, compound **1** was elucidated as shown in Figure 1, named tremulenolide D.

Compound **2** was isolated as colorless oil. Its molecular formula was determined as $\text{C}_{15}\text{H}_{26}\text{O}_2$ by HR-ESI-MS at m/z 238.1927, with three degrees of unsaturation. The IR spectrum of **2** indicated a hydroxyl group at 3424 cm^{-1} and a $\text{C}=\text{C}$ bond at 1638 cm^{-1} . Its ^1H NMR spectrum exhibited an olefinic proton at δ_{H} 5.81 (d, $J = 4.7\text{ Hz}$, H-5) and two doublet methyls at δ_{H} 0.84 (d, $J = 6.9\text{ Hz}$, Me-12) and 0.88 (d, $J = 6.9\text{ Hz}$, Me-13). Inspection of the ^{13}C NMR (DEPT) spectrum revealed the existence of two sp^2 carbons at δ_{C} 139.4 (C-4, s) and 124.9 (C-5, d), one oxygenated methylene at δ_{C} 66.8 (C-15) and one oxygenated quaternary carbon at δ_{C} 71.4 (C-10), as well as three methyls, four methylenes, and four methines. Comparison of the ^{13}C NMR spectral data of **2** with

Figure 2. Key 2D NMR correlations of compounds **1** and **2**.

those of **6** suggested that they shared the closed structure except for the absence of OH-3 and an additional hydroxymethyl instead of methyl group at C-4. The above assignment was further supported by ^1H – ^1H COSY correlations of H-5/H-6/H-1/H-2/H-3 and HMBC correlations from H-15 to C-3, C-4, and C-5 (Figure 2). The ROESY correlations of H-1/Me-14, H-6/Me-14, H-6/Me-12, H-5/H-6, and H-5/H-11 (Figure 2) indicated that H-1, H-6, and Me-14 were α -orientated and H-7 was β -orientated.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter (Jasco International Co., Ltd, Tokyo, Japan). IR spectra were recorded using a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) with KBr pellets. UV spectroscopic data were obtained by high-pressure liquid chromatography (HPLC). NMR spectra were carried out on Bruker DRX-500 and AV-400 spectrometers (Bruker BioSpin GmbH, Rheinstetten) with tetramethylsilane as an internal standard. ESI-MS and HR-ESI-MS were measured on an API QSTAR Pulsar i mass spectrometer (MDS Sciex, Concord, Ontario, Canada). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and RP-18 gel (40–75 μm , Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan) were used for normal pressure column chromatography (CC). Preparative HPLC (Prep-HPLC) was carried out on an Agilent 1200 liquid chromatography system equipped with a Zorbax SB-C₁₈ column (9.4 \times 150 mm). Pre-coated silica gel GF254 plates (Qingdao Marine Chemical, Inc.) were used for monitoring fractions, and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in ethanol.

3.2 Fungal material and cultivation conditions

The fungus *C. alachuana* was collected at Beijing Botanical Garden and identified by Prof. Yu-Cheng Dai, Beijing Forestry University. The voucher specimen (BJFC005267) and culture have been deposited in the Herbarium of Beijing Forestry University. The liquid culture medium contained saccharine 5%, yeast powder 0.5%, peptone 0.15%, KH₂PO₄ 0.05%, and MgSO₄ 0.05%. Inoculums of *C. alachuana* were prepared in a 15-liter fermentor (Biostar, Shanghai Guoqiang, China) for 6 days under the following conditions: culture temperature 24°C, initial pH 6.0, agitation speed 250 r/min, inoculation volume 10% (by volume), and aeration rate 1.0 vvm.

3.3 Extraction and isolation

The entire culture broth of *C. alachuana* (20 liters) was initially filtered, and the filtrate was extracted with EtOAc for three times. The organic layer was concentrated under reduced pressure to give a crude residue (15 g), which was subjected to CC over silica gel using a petroleum ether–Me₂CO gradient (1:0 \rightarrow 0:1) to afford fractions A–G. Fraction B (910.0 mg) was subjected to CC over silica gel eluted with a petroleum ether–Me₂CO system (20:1) to get **5** (4.0 mg). Fraction D (801.5 mg) was separated by CC over silica gel (petroleum ether–Me₂CO) and Sephadex LH-20 (MeOH), and then purified by preparative HPLC (MeCN/H₂O; 0 \rightarrow 30 min, 30:70 \rightarrow 50:50; 10 ml/min; detected at 205 nm; t_R = 9.5 min) to give **1** (5.0 mg). Fraction F (2.50 g) was subjected to CC over silica gel to provide five subfractions (F₁–F₅). Each subfraction was further purified by repeated CC and preparative HPLC (MeCN/H₂O; 10 ml/min; detected at 205 nm). Subsequently, compound **2** (7.0 mg; 0 \rightarrow 20 min, 20:80 \rightarrow 40:60; t_R = 12.0 min) was obtained from subfraction F₁, compound

Table 1. Spectroscopic data for compounds **1** and **2**.

Pos.	1		2	
	δ_C	δ_H	δ_C	δ_H
1	38.5, d	2.82 (m)	47.2, d	1.54–1.58 (m)
2	164.3, s		18.8, t	2.07 (m)/1.49 (m)
3	127.7, s		27.5, t	2.07–2.14 (m)
4	19.8, t	2.34 (m, 4 α) 2.47 (m, 4 β)	139.4, s	
5	33.4, t	1.67–1.69 (m)	124.9, d	5.81 (d, 4.7)
6	32.2, d	2.09–2.11 (m)	37.1, d	2.07 (m)
7	49.1, d	2.11–2.17 (m)	45.0, d	1.33 (tt, 11.7, 3.8)
8	44.6, t	1.50 (dd, 12.7, 8.0, 8 α) 1.42 (dd, 12.7, 9.8, 8 β)	22.1, t	1.13 (m, 8 α) 1.48 (m, 8 β)
9	36.5, s		35.8, t	1.58 (m, 9 α) 1.46 (m, 9 β)
10	44.0, t	1.36 (dd, 12.2, 9.0, 10 α) 1.72 (dd, 12.2, 7.4, 10 β)	71.4, s	
11	70.3, t	4.62 (d, 16.6, 11 α) 4.54 (d, 16.6, 11 β)	27.1, d	1.90–1.99 (m)
12	175.5, s		15.5, q	0.84 (d, 6.9)
13	11.7, q	0.95 (d, 7.0)	22.0, q	0.88 (d, 6.9)
14	31.0, q	1.04 (s)	28.5, q	1.25 (s)
15	31.3, q	1.06 (s)	66.8, t	3.91 (s)

6 (3.0 mg; 0 \rightarrow 30 min, 20:80 \rightarrow 40:60; t_R = 12.5 min) was isolated from subfraction F₂, compound **3** (4.4 mg; 0 \rightarrow 20 min, 15:85 \rightarrow 30:70; t_R = 9.0 min) was yielded from subfraction F₃, and compound **4** (6.5 mg; 0 \rightarrow 30 min, 20:80 \rightarrow 40:60; t_R = 13.0 min) was generated from subfraction F₄.

3.3.1 Tremulenolide D (**1**)

Colorless oil; $[\alpha]_D^{19}$ –13.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.10) nm; IR (KBr) ν_{max} 2957, 2938, 1766, 1715, 1632, 1455, 1041 cm^{–1}; ¹H and ¹³C NMR spectroscopic data, see Table 1; ESI-MS: m/z 234 [M]⁺, 219, 137, 95; HR-ESI-MS: m/z 234.1623 (calcd for C₁₅H₂₂O₂, 234.1620).

3.3.2 Muurolane-10 β ,15-diol (**2**)

Colorless oil; $[\alpha]_D^{19}$ +22.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (2.08), 202 (2.67) nm; IR (KBr) ν_{max} 3440, 3424, 2958, 2936, 2891, 2871, 1638, 1462, 1384, 1128 cm^{–1}; ¹H and ¹³C NMR spectroscopic data, see Table 1; ESI-MS:

m/z 238 [M]⁺, 220, 177, 159, 135, 83; HR-ESI-MS: m/z 238.1927 (calcd for C₁₅H₂₆O₂, 238.1933).

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