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A New Norsesquiterpene, Nor-bisabolan-1,11-diol, from Marine-Derived Fungus *Trichoderma atroviride* TD-8

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Abstract: A new norsesquiterpene and four known bisabolanes were obtained from the organic extract of *Trichoderma atroviride* TD-8 isolated from ocean sediments. Their structures were assigned by detailed interpretation of 1D/2D NMR and HRESIMS data, and they were determined to be nor-bisabolan-1,11-diol (**1**), (3*R*,6*R*,7*R*)-1,10-bisaboladien-3-ol (**2**), (3*R*,6*R*,7*S*)-1,10-bisaboladien-3,6-diol (**3**), (3*R*,6*S*,7*R*,10*R*)-1-bisabolen-3,10,11-triol (**4**), (3*R*,6*R*,7*S*,10*R*)-1-bisabolen-3,10,11-triol (**5**). Nor-bisabolan-1,11-diol (**1**) exhibited moderate cytotoxicity against HeLa and HCT-8 cell lines with IC_{50} values of 28.6 and 30.3 μM, respectively.

Keywords: *Trichoderma atroviride*; norsesquiterpene; bisabolane; secondary metabolites; cytotoxic activity.

1. Fungal Source

Trichoderma atroviride TD-8 was isolated from ocean sediments collected from the coastal zone of Qingdao, in June 2022. It was identified according to morphological characteristics and analysis of the ITS regions of its rDNA, and its sequence data have been submitted to GenBank (OQ875728). The fungus was deposited in Qingdao Institute of Technology, Qingdao, China, and its registration number was TD-8.

2. Previous Studies

Marine-derived *Trichoderma* have attracted great atterention for natural product research, and a large number of secondary metabolites with novel structures and diverse bioactivities have been discovered [1-3]. In which, *T. atroviride* can produce structurally manifold natural products. Previous

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studies have reported a new γ-lactone [4], three new alkaloids [5], eight new peptaibols [6], and three new cyclopentenoneacrylic acid derivatives [7] with different bioactivities from the *T. atroviride*.

3. Present Study

In our ongoing investigation toward new and bioactive secondary metabolites from marine-derived *Trichoderma* spp., one new norsesquiterpene, nor-bisabolan-1,11-diol (**1**), four known bisabolanes, (3*R*,6*R*,7*R*)-1,10-bisaboladien-3-ol (**2**) [8], (3*R*,6*R*,7*S*)-1,10-bisaboladien-3,6-diol (**3**) [9], (3*R*,6*S*,7*R*,10*R*)-1-bisabolen-3,10,11-triol (**4**) [8], (3*R*,6*R*,7*S*,10*R*)-1-bisabolen-3,10,11-triol (**5**) [8] were isolated from *T. atroviride* TD-8. Herein, the details of isolation and structure elucidation as well as cytotoxicity evaluation of compound **1** are described.

The mass fermentation was performed statically at 28 °C for 30 days in 50 × 1 L Erlenmeyer flasks, each containing 400 mL media by adding 5.0 g peptone, 20.0 g glucose, and 3.5 g yeast extract powder into 1000 mL seawater. At the end of fermentation, the fermentation broth was collected by filtration and was extracted with EtOAc for three times, the organic solvent was evaporated under reduced pressure to obtain 38.5 g of crude residue. The crude extract was subjected to silica gel column chromatography with step-gradient solvent systems of petroleum ether (PE)/EtOAc (50:1 to 0:1, v/v) and CH_2Cl_2/MeOH (20:1 to 1:1) to afford 12 fractions. Fraction 6 (5.6 g) eluted with PE/EtOAc 1:1 was further purified by RP-18 CC (MeOH/H_2O,1:1) and preparative TLC to yield **1** (3.2 mg).

*Nor-bisabolan-1,11-diol (**1**)*: Colorless oil; $[\alpha]_D^{20}$ = +48.0° (*c* = 0.05, MeOH);^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 251.1988 $[M + Na]^+$ (calcd. for $C_{14}H_{28}NaO_2$, 251.1987).

Cytotoxic assay: Compound **1** was tested for cytotoxicity against four human tumour cell lines (HeLa, TPH-1, HCT-8, and HepG2) by previously reported methods [4,10-12]. The cells were exposed to gradient concentrations (100, 50, 25, 12.5, 6.25, and 3.125 μM) of the tested compound and the positive control reagent (epirubicin) for 72 h. The absorbance was recorded at 490 nm to estimate the inhibition activity.

Compound **1** was isolated as colourless oil. HRESIMS analysis gave the molecular formula $C_{14}H_{28}O_2$, suggesting one degree of unsaturation. The ^{1}H NMR spectrum (Table 1) in conjunction with HSQC data displayed notable signals including three methyl doublets, one multiplet and one trible doublet assignable to two oxymethines. The ^{13}C NMR and DEPT spectra exhibited 14 resonances ascribled to three methyls, six methylenes, and five methines. COSY correlations of H_3-12/H-11/H_2-10/H_2-9/H_2-8/H-7/H-6 confirmed the linkage of C-12 to C-6, which extended to C-3 via the COSY correlations of H-6/H-1/H_2-2/H-3 and of H-6/H_2-5/H_2-4/H-3. Me-13 and Me-14 were situated at C-7 and C-3, respectively, affirmed by the COSY correlations of H_3-13 with H-7 and of H_3-14 with H-3 (Figure 2), and HMBC correlations from H_3-13 to C-6, C-7, and C-8 and from H_3-14 to C-2, C-3, and C-4 further confirmed the planar structure of **1**. To satisfy the molecular formula, unsaturation requirement, and the chemical shifts, C-1 and C-11 (δ_C 71.7 and 68.6) were suggested to be hydroxylated. H-1 was deduced to be axial and opposite to H-6 due to the large coupling constants. The NOESY correlation of H-1 with H-3 suggested that they were on the same side of the molecular. The relative configuration around ring A was further confirmed by the identical NMR data with those of bisabolan-1,10,11-triol [13]. In addition, the NOESY correlations of H-7 with H-1 and of H_3-13 with H-1 and H-5b indicated the *S** configuration of C-7 (Figure 2). Unfortunately, the relative configuration of C-11 was unsolved. Thus, compound **1** was named as nor-bisabolan-1,11-diol.

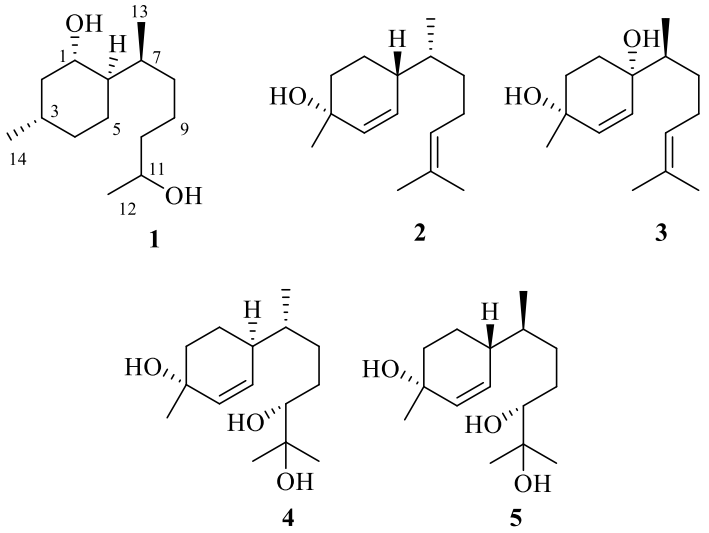

Figure 1. Chemical structures of isolated compounds **1-5**

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** (δ in ppm) in MeOD

No	δ_H (*J* in Hz)	δ_C, type
1	3.37, td (10.6, 4.2)	71.7, CH
2a	1.94, dddd (12.2, 5.7, 4.0, 2.4)	46.0, CH_2
2b	0.95, ddd (12.1, 12.1, 10.7)	
3	1.40, m	33.0, CH
4a	1.67, m	35.8, CH_2
4b	0.84, m	
5a	1.57, dq (13.1, 3.3)	24.2, CH_2
5b	1.02, qd (13.0, 3.4)	
6	1.19, m	49.2, CH
7	2.05, m	31.6, CH
8a	1.29, m	36.6, CH_2
8b	1.25, m	
9a	1.46, m	25.0, CH_2
9b	1.31, m	
10	1.42, m	40.4, CH_2
11	3.71, m	68.6, CH
12	1.14, d (6.2)	23.5, CH_3
13	0.79, d (6.9)	14.2, CH_3
14	0.92, d (6.6)	22.7, CH_3

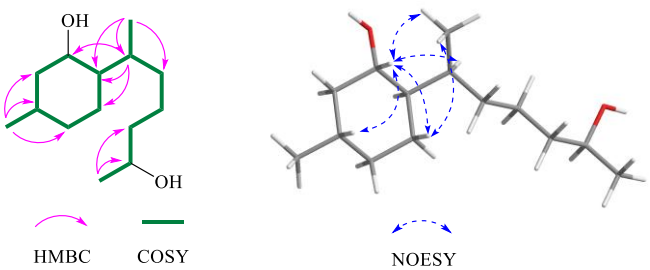

Figure 2. Key ^{1}H-^{1}H-COSY, HMBC, and NOESY correlations of **1**

A New nor-bisabolan type sesquiterpenoid

Nor-bisabolan-1,11-diol (**1**) was assayed for cytotoxic effect against HeLa, TPH-1, HCT-8, and HepG2 cell lines. The results (Table 2) showed that **1** exhibited moderate cytotoxicity against HeLa and HCT-8 cells with IC_{50} values of 28.6 and 30.3 μM, respectively. However, **1** was inactive against TPH-1 and HepG2 cells (IC_{50} > 100 μM).

Table 2. Cytotoxic activity of nor-bisabolan-1,11-diol (**1**) (IC_{50}, μM)

Compounds	HeLa	TPH-1	HCT-8	HepG2
1	28.6 ± 0.5[a]	–[b]	30.3± 0.2	–
Epirubicin[c]	3.9 ± 0.1	4.1 ± 0.1	4.8 ± 0.4	6.2 ± 0.3

[a] mean ± SD, n = 3; [b] IC_{50} > 100 μM; [c] positive control.

In conclusion, chemical investigation towards the marine-derived fungus *T. atroviride* TD-8 resulted in the isolation of one new norsesquiterpene, nor-bisabolan-1,11-diol (**1**), four known bisabolanes, (3*R*,6*R*,7*R*)-1,10-bisaboladien-3-ol (**2**), (3*R*,6*R*,7*S*)-1,10-bisaboladien-3,6-diol (**3**), (3*R*,6*S*,7*R*,10*R*)-1-bisabolen-3,10,11-triol (**4**), (3*R*,6*R*,7*S*,10*R*)-1-bisabolen-3,10,11-triol (**5**). Bisabolane sesquiterpenes and their derivatives were rarely discovered in the fungus *T. atroviride*, the above compounds greatly increase the molecular diversity of *T. atroviride*. In addition, compound **1** was evaluated for cytotoxicity against four human tumour cell lines and displayed moderate cytotoxicity against HeLa and HCT-8 cells.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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