






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

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(Received August 8, 2023; Revised August 31, 2023; Accepted September 04, 2023)

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 IC₅₀ values of 28.6 and 30.3 μM, respectively.

Keywords: *Trichoderma atroviride*; norsesquiterpene; bisabolane; secondary metabolites; cytotoxic activity. © 2023 ACG Publications. All rights reserved.

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 attention 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 CH₂Cl₂/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/H₂O,1:1) and preparative TLC to yield **1** (3.2 mg).

Nor-bisabolan-1,11-diol (1): Colorless oil; $[\alpha]_D^{20} = +48.0^\circ$ ($c = 0.05$, MeOH); ¹H (500 MHz) and ¹³C (125 MHz) NMR data, see Table 1; HRESIMS: m/z 251.1988 [M + Na]⁺ (calcd. for C₁₄H₂₈NaO₂, 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 μ M) 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 C₁₄H₂₈O₂, suggesting one degree of unsaturation. The ¹H NMR spectrum (Table 1) in conjunction with HSQC data displayed notable signals including three methyl doublets, one multiplet and one triple doublet assignable to two oxymethines. The ¹³C NMR and DEPT spectra exhibited 14 resonances ascribed to three methyls, six methylenes, and five methines. COSY correlations of H₃-12/H-11/H₂-10/H₂-9/H₂-8/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/H₂-2/H-3 and of H-6/H₂-5/H₂-4/H-3. Me-13 and Me-14 were situated at C-7 and C-3, respectively, affirmed by the COSY correlations of H₃-13 with H-7 and of H₃-14 with H-3 (Figure 2), and HMBC correlations from H₃-13 to C-6, C-7, and C-8 and from H₃-14 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 H₃-13 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.

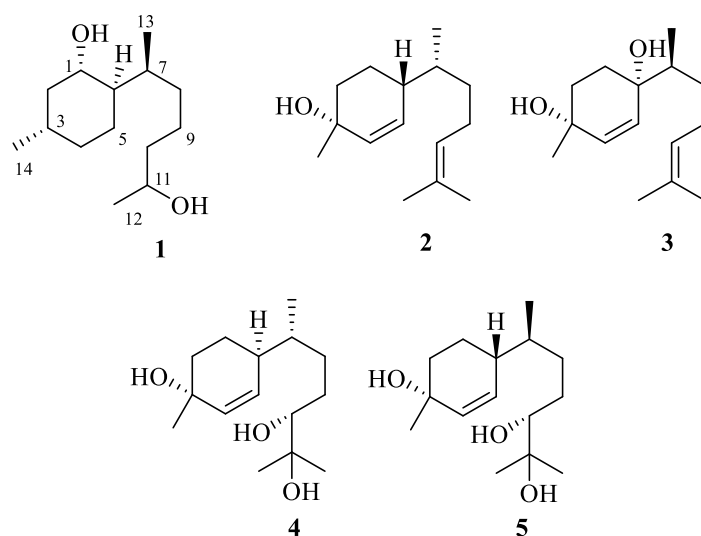


Figure 1. Chemical structures of isolated compounds 1-5

Table 1. ^1H (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

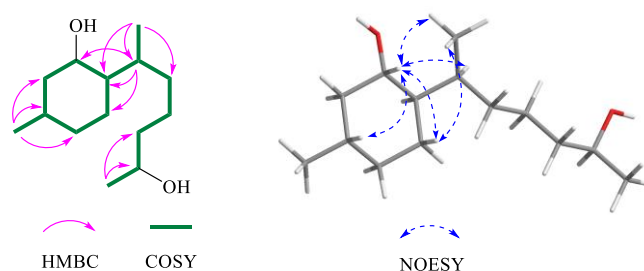


Figure 2. Key ^1H - ^1H -COSY, HMBC, and NOESY correlations of 1

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Nor-bisabolane-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 IC₅₀ values of 28.6 and 30.3 μM, respectively. However, **1** was inactive against TPH-1 and HepG2 cells (IC₅₀ > 100 μM).

Table 2. Cytotoxic activity of nor-bisabolane-1,11-diol (**1**) (IC₅₀, 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 IC₅₀ > 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-bisabolane-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-bisabolane-3,10,11-triol (**4**), (3*R*,6*R*,7*S*,10*R*)-1-bisabolane-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.

Acknowledgments

This work was funded by the Natural Science Foundation of Dongying City (2023ZR01).

Supporting Information

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

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References

- [1] J. L. Zhang, W. L. Tang, Q. R. Huang, Y. Z. Li, M. L. Wei, L. L. Jiang, C. Liu, X. Yu, H. W. Zhu, G. Z. Chen and X. X. Zhang (2021). *Trichoderma*: a treasure house of structurally diverse secondary metabolites with medicinal importance, *Front. Microbiol.* **12**, 723828.
- [2] M. Jiang, Z. Wu, H. Guo, L. Liu and S. Chen (2020). A review of terpenes from marine-derived Fungi: 2015-2019, *Mar. Drugs* **18**, 321.
- [3] Q. Z. Yin, X. W. Liu, Z. J. Zhang, H. X. Lei and B. Wu (2023). Chemistry and bioactivities of alkaloids isolated from marine fungi (covering 2016-2022), *Fitoterapia* **164**, 105377.
- [4] L. D. Zhai, G. Wei, L. T. Liu, K. Zhang and D. L. Zhang (2023). Trichonafurin A: a new γ-lactone compound from the marine-derived fungus *Trichoderma atroviride* ZW-7, *Rec. Nat. Prod.* **17**, 721-725.
- [5] X. Lu, L. Tian, G. Chen, Y. Xu, H. F. Wang, Z. Q. Li and Y. H. Pei (2012). Three new compounds from the marine-derived fungus *Trichoderma atroviride* G20-12, *J. Asian Nat. Prod. Res.* **14**, 647-651.
- [6] I. Panizel, O. Yarden, M. Ilan and S. Carmeli (2014). Eight new peptaibols from sponge-associated *Trichoderma atroviride*, *Mar. Drugs* **11**, 4937-4960.

- [7] X. X. Tang, S. Z. Liu, Y. Y. Sun, F. M. He, G. X. Xu, M. J. Fang, W. Zhen and Y. K. Qiu (2021). New cyclopentenoneacrylic acid derivatives from a marine-derived fungus *Trichoderma atroviride* H548, *Nat. Prod. Res.* **35**, 3772-3779.
- [8] A. Khrimian, A. Zhang, D. C. Weber, H.-Y. Ho, J. R. Aldrich, K. E. Vermillion, M. A. Sigler, S. Shirali, F. Guzman and T. C. Leskey (2014). Discovery of the aggregation pheromone of the brown marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols, *J. Nat. Prod.* **77**, 1708-1717.
- [9] S. D. Jolad, B. N. Timmermann, J. J. Hoffmann, R. B. Bates, F. A. Camou and J. R. Cole (1988). Sesquiterpenoids from *Coreocarpus arizonicus*, *Phytochemistry* **27**, 3545-3548.
- [10] X. L. Yuan, X. Q. Li, K. Xu, X. D. Hou, Z. F. Zhang, L. Xue, X. M. Liu and P. Zhang (2020). Transcriptome profiling and cytological assessments for identifying regulatory pathways associated with diorcinol N-induced autophagy in A3 cells, *Front. Pharmacol.* **11**, 570450.
- [11] X.Z. Wang, P. L.Hou, Y.B. Qu, R.Z. Huang, Y. Feng, S.F. Zhao, Y.L. Ding and Z.X. Liao (2021). Chemical constituents of Tibetan herbal medicine *Pulicaria insignis* and their in vitro cytotoxic activities, *Rec. Nat. Prod.* **15**, 91-102.
- [12] L.R. Wang, J. Yu, Z.L. Nie and P.P. Shan (2022). Cytotoxic siterpenoids from *Scoparia dulcis*, *Rec. Nat. Prod.* **16**, 453-462.
- [13] Y. P. Song, F. P. Miao, S. T. Fang, X. L. Yin and N. Y. Ji (2018). Halogenated and nonhalogenated metabolites from the marine-alga-endophytic fungus *Trichoderma asperellum* cf44-2, *Mar. Drugs* **16**, 266.

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