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9,11-Cycloneren-3,7-diol: a New Cyclonerane Sesquiterpene from the Marine-Sediment-Derived Fungus *Trichoderma harzianum* WH-22

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Abstract: One new cyclonerane sesquiterpene, 9,11-cycloneren-3,7-diol (**1**), along with four known ones, 11-cycloneren-3,7,10-triol (**2**), 9-cycloneren-3,7,11-triol (**3**), 11-methoxy-9-cycloneren-3,7-diol (**4**), and cyclonerodiol (**5**), were obtained from the extract of marine-derived fungus *Trichoderma harzianum* WH-22. The structure of 9,11-cycloneren-3,7-diol (**1**) was elucidated by comprehensive spectroscopic analysis, including 1D/2D NMR and HRESIMS data. 9,11-Cycloneren-3,7-diol (**1**) exhibited weak cytotoxicity against HeLa and HepG2 cancer cell lines with IC_{50} values of 68.2 and 59.7 μM, respectively.

Keywords: cyclonerane sesquiterpene; *Trichoderma harzianum*; structure elucidation; cytotoxic activity.

1. Fungal Source

Trichoderma harzianum WH-22 was isolated from the sediments collected from the coastal zone of Weihai, China in July 2021. It was identified according to morphological characteristics and ITS regions of its rDNA, whose sequence data have been deposited at GenBank (OR534535). The fungus was preserved in Qingdao Hiser Hospital Affiliated of Qingdao University, Qingdao, China, with the number of WH-22.

2. Previous Studies

Secondary metabolites obtained from marine-derived *Trichoderma* spp. exhibited biosynthetic differences with terrestrial-derived ones. A large number of natural products with novel structures and intriguing bioactivities have been discovered from marine-derived fungi, especially for the *Trichoderma* species, which are serving as a repository of new compounds [1,2]. Among them, *T. harzianum* can produce various bioactive secondary metabolites, including terpenoids, macrolides,

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polyketides and so on, which are an important source for searching new drugs [3]. In addition, previous studies had reported that some compounds were isolated from *Trichoderma* spp. derived from the ocean sediments [4], but the secondary metabolites acquired from marine-sediment-derived *T. harzianum* were rarely reported [5].

3. Present Study

During our chemical investigation for functional secondary metabolites from the marine-derived fungi, one new cyclonerane sesquiterpene, 9,11-cycloneren-3,7-diol (**1**), together with four known ones, 11-cycloneren-3,7,10-triol (**2**), 9-cycloneren-3,7,11-triol (**3**), 11-methoxy-9-cycloneren-3,7-diol (**4**), and cyclonerodiol (**5**), were isolated from *T. harzianum* WH-22 by chromatographic separation and identified via spectroscopic methods. Herein, the isolation, structure elucidation, and cytotoxic activity of compound **1** are described in detail.

The mass culture was performed at 26 °C for 30 days in 60 × 1L Erlenmeyer flasks, each containing 40 g of rice and 70 mL of natural seawater. After fermentation, about 100 mL EtOAc was added into each flask to kill the fungi, and the mycelia were collected, dried, and then exhaustively extracted with EtOAc. The crude extract (80.0 g) was obtained after removing the organic solvent by evaporating under reduced pressure. The extract was subjected to silica gel column chromatography (CC) with step-gradient solvent systems involving petroleum ether (PE)/EtOAc and then CH_2Cl_2/MeOH to gain 10 fractions. Fraction 4 (6.3 g), eluted with PE/EtOAc 2:1, was further purified by CC on RP-18 (MeOH/H_2O, 7:3) and Sephadex LH-20 (MeOH) to yeild 9,11-cycloneren-3,7-diol (**1**) (2.1 mg).

*9,11-Cycloneren-3,7-diol (**1**):* Colorless oil; $[\alpha]_D^{20}$ = -25° (*c* = 0.2, MeOH); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HREIMS: *m/z* 239.2018 $[M + H]^+$ (calcd for $C_{15}H_{27}O_2$, 239.2011).

Measurement of Cytotoxicity: Cytotoxic assay toward the human tumor cell lines, including HeLa, HepG2, and MCF-7, was carried out with reported methods [6,7]. The cells were exposed to the tested compound with various concentrations (3.125, 6.25, 12.5, 25, 50, and 100 μM) in 96-well plates for 72 h, and the cell viability was determined by the CCK-8 Cell Proliferation and Cytotoxicity Assay Kit, and the absorbance was recorded by a microplate spectrophotometer at 490 nm. Epirubicin was provided as the positive control.

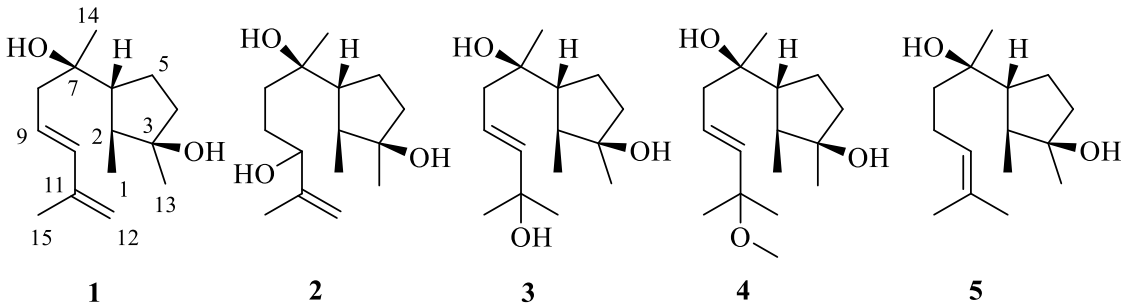

Figure 1. Chemical structures of compounds **1-5** isolated from *T. harzianum* WH-22

Compound **1** was acquired as colorless oil. HRESIMS analysis gave the molecular formula $C_{15}H_{26}O_2$, suggesting three degrees of unsaturation. The ^{1}H NMR spectrum exhibited notable proton signals involving four olefinic protons at δ = 6.21 (br d, *J* = 15.6 Hz, H-10), 5.69 (dt, *J* = 15.6 and 7.6 Hz, H-9), 4.92 (br s, H-12a), and 4.91 (br s, H-12b), and four methyls including three methyl singlets at δ = 1.86 (s, H-15), δ = 1.27 (s, H-13), and δ = 1.16 (s, H-14) and one methyl doublet at δ = 1.06 (d, *J* = 6.8 Hz, H-1). The ^{13}C NMR spectrum displayed 15 resonances, assigned to four methyls, four methylenes, four methines, and three quaternary carbons by DEPT experiments. Its NMR data was

high similar to those of 11-cycloneren-3,7,10-triol (**2**) [8], except for the presence of signals for a *trans* double bond (C-9 = C-10) and lack of signals for a methylene (C-9) and an oxygenated methine (C-10), which was validated by the COSY correlations of H-9 with H-8 and H-10 and the HMBC correlations from H-12 to C-2, C-11, and C-15. Other COSY and HMBC correlations (Figure 2), including HMBC correlations from H-13 to C-2, C-3, and C-4 and from H-14 to C-6, C-7, and C-8 and COSY correlations of H-1/H-2/H-6/H-5/H-4 and of H-8/H-9/H-10, further confirmed the planar structure of **1**. The geometry of double bond at C-9 was proposed to be *trans* by the large coupling constant (J = 15.6 Hz) between H-9 and H-10. H-6 was *syn* to C-1 by the NOE correlation of H-6 with H-1, while C-13 was opposite to C-1 by the NOE correlation of H-2 with H-13 (Figure 2). The relative configuration of C-14 and the absolute configuration of **1** were deduced to be the same as those of cyclonerodiol (**5**) [9,10] and 9-cycloneren-3,7,11-triol (**3**) [8], confirmed by their identical NMR data and specific optical rotation values [$[\alpha]_D^{20}$ -25 (c = 0.2, MeOH) for **1**; $[\alpha]_D^{20}$ -21 (c = 0.1, MeOH or $CDCl_3$) for cyclonerodiol; $[\alpha]_D^{20}$ -22 (c = 0.04, MeOH) for 9-cycloneren-3,7,11-triol].

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** (δ in ppm) in $CDCl_3$

No	**δ_H (J in Hz)**	**δ_C, type**
1	1.06, d (J = 6.8)	14.6, CH_3
2	1.64, m	44.5, CH
3		81.5, C
4a	1.69, m	40.5, CH_2
4b	1.59, m	
5a	1.87, m	24.5, CH_2
5b	1.61, m	
6	1.86, m	54.4, CH
7		74.9, C
8a	2.32, dd (J = 13.9, 7.6)	44.1, CH_2
8b	2.27, dd (J = 13.9, 7.9)	
9	5.69, dt (15.6, 7.6)	125.5, CH
10	6.21, br d (15.6)	137.0, CH
11		142.0, C
12a	4.92, br s	115.6, CH_2
12b	4.91, br s	
13	1.27, s	26.2, CH_3
14	1.16, s	25.6, CH_3
15	1.86, s	18.9, CH_3

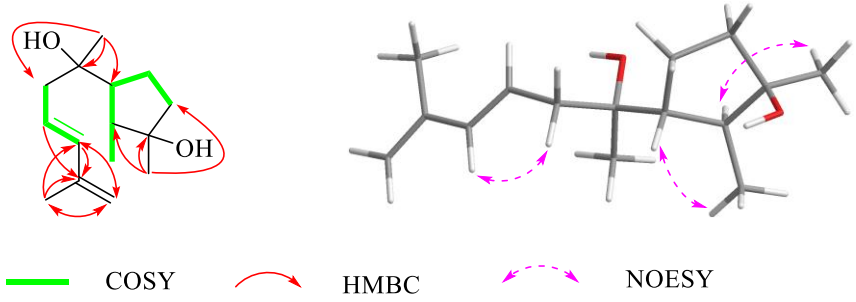

Figure 2. Key ^1H-^1H-COSY, HMBC, and NOE correlations for **1**

9,11-Cycloneren-3,7-diol (**1**) was evaluated for cytotoxic effect against HeLa, HepG2, and MCF-7 cell lines. The result showed that **1** exhibited weak cytotoxicity against HeLa and HepG2 cells with IC_{50} values of 68.2 and 59.7 μM, respectively. However, **1** displayed no activity toward MCF-7 cells (IC_{50} > 100 μM).

Table 2. Cytotoxic activity of 9,11-cycloneren-3,7-diol (IC_{50}, μM)

Compounds	HeLa	HepG2	MCF-7
1	68.2 ± 3.1	59.7 ± 4.6	–[a]
Epirubicin	5.9 ± 0.2	6.2 ± 0.7	5.8 ± 0.4

[a] No cytotxit effect at 100 μM.

Secondary metabolites from marine-sediment-derived *Trichoderma* have been rarely studied. In our chemical investigation towards the marine-sediment-derived fungus *T. harzianum* WH-22, one new cyclonerane sesquiterpene, 9,11-cycloneren-3,7-diol (**1**), and four known ones, 11-cycloneren-3,7,10-triol (**2**), 9-cycloneren-3,7,11-triol (**3**), 11-methoxy-9-cycloneren-3,7-diol (**4**), and cyclonerodiol (**5**), were isolated and elucidated. Cyclonerane sesquiterpenes were always found in some *Trichoderma* spp., such as *T. asperellum* and *T. harzianum*, and they can act as metabolite-mark of *Trichoderma*. In addition, 9,11-cycloneren-3,7-diol (**1**) was evaluated for inhibitory activity against three human tumour cell lines and exhibited cytotoxicity against HeLa and HepG2 cells. This study enriches research contents of marine natural products and makes a contribution to utilization of marine bio-resource.

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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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