SHORT REPORT

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A New Antibacterial Diterpene with a Fused 6-5-6-6 Ring System, Trichodermanin I, Isolated from the Soil-Derived Fungus *Trichoderma atroviride* YD-13

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Abstract: One new diterpene with a fused 6-5-6-6 ring system, trichodermanin I (**1**), along with three known ones, wickerols A and B and trichodermanin F (**2**-**4**), were acquired from the extract of *Trichoderma atroviride* YD-13 isolated from soil. Their chemical structures were determined by interpretation of 1D/2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HRESIMS) data. Compound **1** was evaluated for inhibiting the growth of four human pathogenic bacteria (*Clostridium botulinum*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*) and exhibited potential antibacterial activity against *C. botulinum*, *E. coli*, and *S. aureus* with MIC values of 8.0 μg/mL, 32 μg/mL, and 16 μg/mL, respectively.

Keywords: *Trichoderma atroviride*; diterpene; secondary metabolites; antibacterial activity.

1. Fungal Source

Trichoderma atroviride YD-13 was isolated from soil collected from Wenzhou, in July 2023. This fungus was identified as *Trichoderma atroviride* through analysis of its internal transcribed spacer (ITS) regions of rDNA, and the sequence data were submitted to GenBank (PQ044562). The fungus was deposited in the Third Affiliated Hospital of Wenzhou Medical University, China, with the registration number YD-13.

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2. Previous Studies

Trichoderma sp. can produce a large number of secondary metabolites with high diversity in chemical structures and biological activities, among which, terpenoids play an important role in these metabolites [1-4]. To date, ten diterpenes with a fused 6-5-6-6 ring system have been found, namely, wickerols A and B and trichodermanins A-H [5-8]. All of these diterpenes were isolated from *Trichoderma* fungi, including *T. atroviride* and *T. harzianum*. These diterpenes displayed various kinds of biological activities, such as anti-influenza virus and inhibition of human tumour cell lines [5-8].

3. Present Study

In the continuing investigation toward new secondary metabolites with biological activities from *Trichoderma* spp., we isolated four diterpenes with a fused 6-5-6-6 ring system, including one new compound, trichodermanin I (**1**), and three known ones, wickerols A and B and trichodermanin F (**2**-**4**) [5,8], from the organic extract of *Trichoderma atroviride* YD-13. Herein, the details of isolation, structure elucidation, and antibacterial activity of compound **1** are described.

Mass fermentation was incubated statically at 25 °C in a liquid medium (18 L) containing 2% glucose, 0.5% yeast extract powder, and 0.5% peptone in sterilized water. After fermentation for 30 days, EtOAc was added into flasks to kill the mycelia, and the culture broth was extracted with EtOAc three times, the combined organic phase was concentrated under reduced pressure to afford 31.4 g of crude extract. Then, the extract was subjected to silica gel column chromatography (CC) with step-gradient solvent systems of petroleum ether (PE)–EtOAc and CH_2Cl_2–MeOH (from 20:1 to 1:1), yielding 10 fractions. Fraction 5 (2.7 g), eluted with PE–EtOAc (1:1), was further separated by RP-18 CC (MeOH–H_2O, 3:1) and silica gel (PE–EtOAc, from 5:1 to 1:1), followed by preparative thin layer chromatography (TLC), to yield **1** (4.1 mg).

*Trichodermanin I (**1**):* Colorless oil; $[\alpha]_D^{20}$ = +3.6 (*c* = 0.3, MeOH); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 307.2634 $[M + H]^+$ (calcd for $C_{20}H_{35}O_2$, 307.2637).

Antibacterial activity assay: The antibacterial activity against four human pathogenic bacteria (*Clostridium botulinum*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*) of compound **1** was assayed by 96-well microtiter plates method [9]. The tested bacteria were cultivated in the Mueller-Hinton broth medium at 37 °C, and their concentration was adjusted to 1.5×10^8 CFU/mL. Compound **1** and positive control (chloramphenicol) were dissolved in dimethyl sulfoxide (DMSO), then 5 μL of the sample solution and 95 μL of prepared bacteria suspension were added into the 96-well plates (the final sample concentrations were 64, 32, 16, 8, 4, 2, 1, 0.5 μg/mL) and incubated at 37 °C for 24 h. The DMSO was the negative control. The optical density was measured at 600 nm using a multi-detection microplate reader.

Compound **1** was acquired as colorless oil. Its molecular formula was assigned to be $C_{20}H_{34}O_2$ by analysis of HRESIMS (*m/z* 307.2634 $[M + H]^+$, calcd for $C_{20}H_{35}O_2$, 307.2637, mass error = -1.0 ppm), implying four degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) exhibited distinct proton signals corresponding to one oxygenated methine proton at δ = 3.57 (d, *J* = 5.9 Hz, H-9), four methyl singlets at δ = 1.20 (s, H_3-16), 1.06 (s, H_3-18), 0.99 (s, H_3-19), and 0.97 (s, H_3-20), and one methyl doublet at δ = 1.04 (d, *J* = 7.0 Hz, H_3-17). The ^{13}C NMR and DEPT spectra displayed 20 resonances classified into five methyls, six sp^3-methylenes, five sp^3-methines [including one oxygenated at δ = 79.6 (C-9)], and four nonprotonated sp^3-carbons [including one oxygenated at δ = 73.8 (C-15)]. Its ^{1}H and ^{13}C NMR data were highly similar to those of wickerol A (**2**) [5], with the main difference being the presence of an oxymethine (δ_H 3.57, δ_C 79.6) in **1** instead of a methylene group. Therefore, **1** was deduced to be a hydroxylated derivative of wickerol A at C-9, which was confirmed by the HMBC correlations from H_3-20 to C-7, C-8, C-9, and C-12, from H-9 to C-12 and by the ^{1}H-^{1}H COSY correlations from H-9 to H-12 (Figure 2). ^{1}H-^{1}H COSY experiment established the other two partial

structures: –CH_2(C-7)–CH(C-6)–CH_2(C-1)–CH_2(C-2)–CH(C-3)–CH_3(C-17)– and –CH_2(C-13)–CH_2(C-14)– (Figure 2). HMBC correlations from H_3-18 and H_3-19 to C-4, C-5, and C-6 suggested the existence of a geminal dimethyl system, and H_3-16 was located at C-15 based on the cross-peaks of H_3-16 to C-11, C-14, and C-15. Further HMBC correlations confirmed the planar structure of **1** (Figure 2). The relative configuration of **1** was determined by analyzing coupling constants and the NOESY spectrum. The large coupling constant (J = 13.6) between H-11 and H-12 indicated that H-11 was *anti* to H-12. The NOESY correlations of H_3-16 with H-12 and H_3-19 and of H-2b with H_3-17 and H_3-18 indicated that these protons were located on the same side of the molecule. Additionally, the NOESY correlations of H_3-20 with H-3, H-9 and H-11 and of H-3 with H-11, suggested that C-20 was *syn* to H-9, H-11, and H-3 (Figure 2).

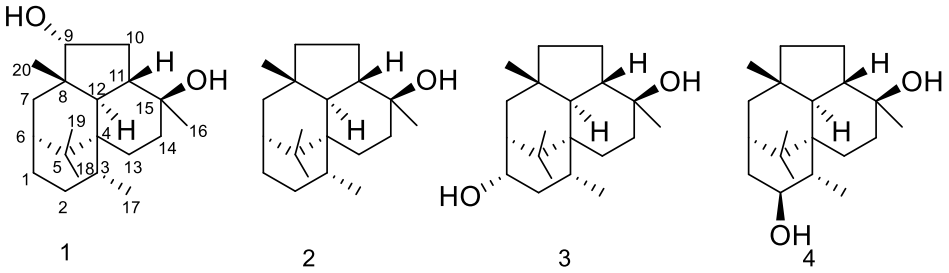

Figure 1. Chemical structures of compounds **1-4** isolated from *T. atroviride* YD-13

Table 1. ^{1}H (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**
1a	2.14, m	26.2, CH_2
1b	1.63, m	
2a	2.04, m	28.7, CH_2
2b	1.47, m	
3	2.17, m	27.2, CH
4		38.5, C
5		38.7, C
6	1.59, m	40.8, CH
7a	2.05, m	35.0, CH_2
7b	1.15, dd (13.1, 2.7)	
8		43.9, C
9	3.57, d (5.9)	79.6, CH
10a	2.25, ddd (15.1, 9.6, 5.9)	33.2, CH_2
10b	1.39, dd (15.1, 6.1)	
11	1.86, ddd (13.6, 9.7, 6.1)	43.9, CH
12	1.74, d (13.6)	46.2, CH
13a	1.71, dt (14.1, 3.4)	26.7, CH_2
13b	1.26, ddd (14.1, 14.1, 3.4)	
14a	1.59, m	41.1, CH_2
14b	1.46, m	
15		73.8, C
16	1.20, s	21.2, CH_3
17	1.04, d (7.0)	23.2, CH_3
18	1.06, s	24.8, CH_3
19	0.99, s	25.5, CH_3
20	0.97, s	19.6, CH_3

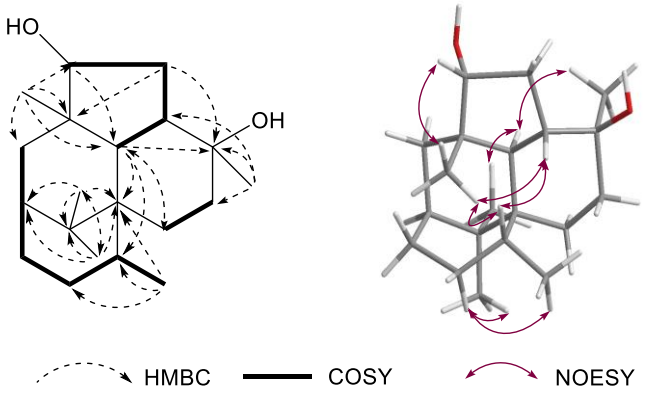

Figure 2. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of **1**

Trichodermanin I (**1**) was assayed for antibacterial activity against four human pathogenic bacteria: *Clostridium botulinum*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*. The results (Table 2) indicated that **1** displayed potential activity against *C. botulinum*, *E. coli*, and *S. aureus* with MIC values of 8.0 μg/mL, 32 μg/mL, and 16 μg/mL, respectively. However, **1** did not inhibit the growth of *Salmonella* (MIC > 64 μg/mL).

Table 2. Antibacterial activity of trichodermanin I (**1**) (MIC, μg/mL)

Compounds	*C. botulinum*	*E. coli*	*Salmonella*	*S. aureus*
1	8.0	32	– [a]	16
chloramphenicol	1.0	2.0	2.0	1.0

[a] MIC > 64 μg/mL

In conclusion, four diterpenes with a fused 6-5-6-6 ring system were isolated from soil-derived fungus *T. atroviride* YD-13, including one new compound, trichodermanin I (**1**), and three known ones, wickerols A and B and trichodermanin F (**2**-**4**). Based on the previous reports and this study, diterpenes with a fused 6-5-6-6 ring system were consistently acquired from *Trichoderma* spp., suggesting that wickerol-type diterpenes may be the typical secondary metabolites of this genus. In the antibacterial assay, compound **1** exhibited potential activity against *C. botulinum*, *E. coli*, and *S. aureus*, which may be developed as an antibacterial agent in the future.

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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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