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Trichonafurin A: a New γ-Lactone Compound from the Marine-Derived Fungus *Trichoderma atroviride* ZW-7

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Abstract: A new *γ*-lactone compound, trichonafurin A (**1**), was obtained from the culture of *Trichoderma atroviride* ZW-7, an endophyte of the marine brown alga *Sargassum thunbergii*. Its structure was unambiguously assigned by detailed interpretation of 1D/2D NMR and HRESIMS data. Trichonafurin A (**1**) exhibited moderate cytotoxicity against HeLa and HepG2 cell lines with IC_{50} values of 20.2 and 32.1 μM, respectively.

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

1. Fungal Source

Trichoderma atroviride ZW-7 was isolated from the marine brown alga *Sargassum thunbergii* collected from the coastal zone of Qingdao, in July 2022. It was identified by morphological characteristics and by analysis of the ITS regions of its rDNA, whose sequence data have been submitted to GenBank (OQ438000). The fungus was deposited in Qingdao Hospital of Traditional Chinese Medicine, Qingdao, China, and its registration number was ZW-7.

2. Previous Studies

Marine-derived filamentous fungi have made great contributions to natural product research, and marine-derived *Trichoderma* is a vast resource for discovering novel natural products with diverse bioactivities [1,2]. Previous studies have reported that *T. atroviride* can produce structurally diverse natural products, such as cyclopentenone derivative [3], diterpenoids [4,5], alkaloids [6] and so on, and most compounds exhibited definite bioactivities.

3. Present Study

In our ongoing search for structurally new and biologically active secondary metabolites from marine algicolous fungi, one new *γ*-lactone compound, trichonafurin A (**1**), one known epoxy-*δ*-lactone compound, nafuredin (**2**), two known diterpenoids, wickerols A and B (**3**, **4**), and one steroid,

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isoergokonin B (**5**) were isolated from *T. atroviride* ZW-7 via chromatographic techniques and identified by spectroscopic methods. Herein, the details of isolation, structural elucidation, and cytotoxicity of compound **1** are described.

The initial cultures of *T. atroviride* ZW-7 was maintained on PDA medium at 28 °C for 3 days. The mass cultures were performed in 30 × 1 L Erlenmeyer flasks, each containing 300 mL media with 28.5 g fungi medium and 1000 mL natural seawater. After 30 days fermentation at 28 °C, the cultures were filtered to separate the mycelia from the broth, the mycelia were dried, smashed, and extracted with CH_2Cl_2 and MeOH (1:1, v/v). The organic solvents were evaporated under reduced pressure to acquire 20 g of crude residue. Additionally, the filtrate was extracted with EtOAc and concentrated to obtain an extract (15 g). The two parts were combined and subjected to silica gel column chromatography (CC) with step-gradient solvent systems of petroleum ether (PE)/EtOAc (20:1 to 1:1, v/v) and CH_2Cl_2/MeOH (20:1 to 1:1) to afford 10 fractions. Fraction 6 (3.3 g), eluted with CH_2Cl_2/MeOH 20:1, was further purified by CC on RP-18 (MeOH/H_2O, 2:3) and Sephadex LH-20 (MeOH) to yield **1** (2.1 mg).

*Trichonafurin A (**1**):* Colorless oil; $[\alpha]_D^{20}$ = +21.2° (*c* = 0.08, MeOH); ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 355.2251 (calcd for $C_{21}H_{32}O_3Na$, 355.2249).

Cytotoxic Assay: Compound **1** was assayed for cytotoxicity against four human tumour cell lines (HeLa, MCF-7, HepG2, and HCT-8) according to previously reported methods [7-9]. The cells were exposed to the tested compound and the positive control reagent (epirubicin) at gradient concentrations (3.125, 6.25, 12.5, 25, 50, and 100 μM) for 72 h.

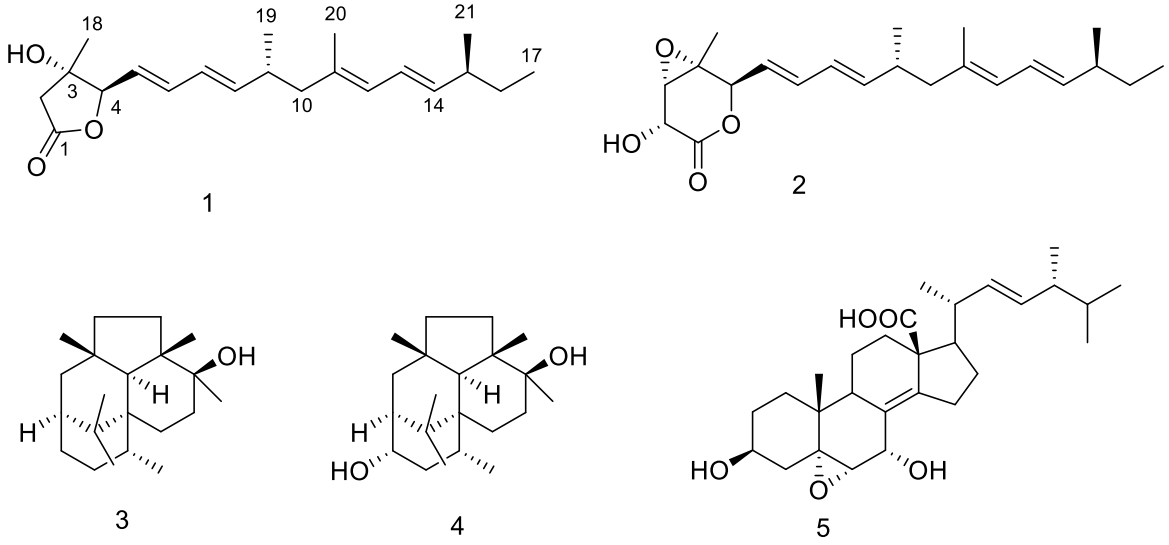

Figure 1. Chemical structures of isolated compounds **1-5**

Compound **1** was obtained as colourless oil. HRESIMS (*m/z* 355.2251 $[M + Na]^+$) analysis gave the molecular formula $C_{21}H_{32}O_3$. The ^1H NMR spectrum (Table 1) displayed notable proton signals involving seven olefinic protons at δ = 6.31 (dd, *J* = 15.2, 10.4, H-6), δ = 6.17 (dd, *J* = 15.2, 10.2, H-13), δ = 6.02 (dd, *J* = 15.2, 10.4, H-7), δ = 5.76 (d, *J* = 10.2, H-12), δ = 5.80 (dd, *J* = 15.2, 6.7, H-8), δ = 5.46 (dd, *J* = 15.0, 7.5, H-14), and δ = 5.44 (dd, *J* = 15.2, 7.3, H-5), one oxygenated methine proton at δ = 4.75 (d, *J* = 7.3, H-4), five methyl groups, sorted into two methyl singlets at δ = 1.71 (s, H_3-20) and 1.36 (s, H_3-18), two methyl doublets at δ = 0.99 (d, *J* = 6.7 Hz, H_3-21) and 0.97 (d, *J* = 6.7 Hz, H_3-19), and one methyl triplet at δ = 0.86 (t, *J* = 7.5 Hz, H_3-17). The ^{13}C NMR spectrum (Table 1) exhibited 21 resonances, including five methyls, three methylenes, ten methines [including seven olefinic at δ = 143.8 (C-8), δ = 139.0 (C-14), δ = 135.5 (C-6), δ = 127.1 (C-12), δ = 126.6 (C-7), δ = 124.8 (C-13), and δ = 122.8 (C-5), one oxygenated sp^3 at δ = 89.5 (C-4)], and three quaternary carbons

[including one olefinic at δ = 134.0 (C-11) and one ester carbonyl at δ = 174.4 (C-1)]. The above NMR data displayed high similarities with those of nafuredin (**2**) [10]. The main difference was the γ-lactone in **1** replacing the expoxy-δ-lactone in nafuredin (**2**), validated by the HMBC correlations from H_3-18 to C-2, C-3, and C-4, from H_2-2 to C-1, from H-4 to C-1 and C-3. The structure of methylated olefinic side chain was confirmed by HMBC correlations from H_3-20 to C-10, C-11, and C-12, and COSY correlations from H-4 to H-10/H_3-19 and from H-12 to H-17/H_3-21. Other HMBC correlations further corroborated the planar structure of **1**. The relative configuration of **1** was determined by NOESY correlations. The NOE correlations of H-5 with H-18 indicated them to be on the same face of the molecule (Figure 2). The NOE correlations of H-7 with H-5 and H-9, of H-12 with H_2-10 and H-14 allowed *E* configurations of the two dienes (Figure 2). The relative configurations of C-9 and C-15 were same as those of nafuredin (**2**) by their identical NMR data. Compound **1** was named trichonafurin A.

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

No	δ_H (*J* in Hz)	δ_C, type
1		174.4, C
2	2.65, d (17.2); 2.58, d (17.2)	43.1, CH_2
3		77.1, C
4	4.75, d (7.3)	89.5, CH
5	5.44, dd (15.2, 7.3)	122.8, CH
6	6.31, dd (15.2, 10.4)	135.5, CH
7	6.02, dd (15.2, 10.4)	126.6, CH
8	5.80, dd (15.2, 6.7)	143.8, CH
9	2.41, m	34.9, CH
10	2.09, dd (13.1, 6.7); 1.96, dd (13.5, 7.8)	47.5, CH_2
11		134.0, C
12	5.76, d (10.2)	127.1, CH
13	6.17, dd (15.0, 10.2)	124.8, CH
14	5.46, dd (15.0, 7.5)	139.0, CH
15	2.07, m	38.8, CH
16	1.32, m	30.0, CH_2
17	0.86, t (7.5)	12.0, CH_3
18	1.36, s	23.4, CH_3
19	0.97, d (6.7)	19.7, CH_3
20	1.71, s	16.7, CH_3
21	0.99, d (6.7)	20.3, CH_3

*δ in ppm in $CDCl_3$

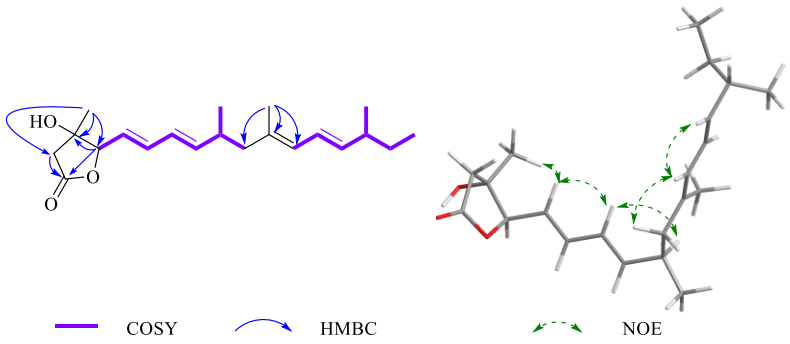

Figure 2. Key ^{1}H-^{1}H-COSY, HMBC, and NOE correlations of **1**

Trichonafurin A (**1**) was evaluated for cytotoxic effect against HeLa, MCF-7, HepG2, and HCT-8 cell lines. The result showed that **1** displayed moderate cytotoxicity against HeLa and HepG2 cells with IC_{50} values of 20.2 and 32.1 μM, respectively. However, **1** exhibited no activities against MCF-7 and HCT-8 cells (IC_{50} > 100 μM).

Table 2. Cytotoxic activity of trichonafurin A (**1**) (IC_{50}, μM)

Compounds	HeLa	MCF-7	HepG2	HCT-8
1	20.2 ± 0.7[a]	–[b]	32.1± 0.3	–
Epirubicin[c]	4.5 ± 0.2	3.5 ± 0.1	4.6 ± 0.3	5.8 ± 0.2

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

4. Discussion

Chemical investigation towards the marine algicolous *T. atroviride* ZW-7 resulted in the isolation of one new *γ*-lactone compound, trichonafurin A (**1**), one known epoxy-*δ*-lactone compound, nafuredin (**2**), two known diterpenoids, wickerols A and B (**3**, **4**), and one steroid, isoergokonin B (**5**). The above compounds increased the molecular diversity of *T. atroviride*. Compound **1** was evaluated for cytotoxicity against four human tumour cell lines and exhibited moderate cytotoxicity against HeLa and HepG2 cells, and its cytotoxicity may be improved after optimizing structure.

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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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