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A New Cytotoxic Sesquiterpenoid from *Penicillium oxalicum* 2021CDF-3

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Abstract: During the chemical studies on the target fungus *Penicillium oxalicum* 2021CDF-3, four compounds (**1**−**4**) were isolated and characterized from the culture of the solid rice medium. Their chemical structures were definitely determined by using HRESIMS and NMR experiments. Among them, compound **1** was reported as a new vetivane-type sesquiterpenoid bearing a tricyclic 7/5/5 framework. Compounds **1**−**4** were measured for their cytotoxicities against A549, MCF7, MKN-45, and HCT 116 cells. Compounds **2** and **4** displayed considerable inhibitory activity on the gastric cancer MKN-45 cell with IC_{50} values of 8.0 ± 0.5 and 11.9 ± 0.6 μM, respectively.

Keywords: *Penicillium oxalicum;* endophytic fungus; secondary metabolites; sesquiterpenoid; cytotoxicity.

1. Fungal Source

The endophytic fungus characterized as *Penicillium oxalicum* 2021CDF-3 was acquired from the inner tissue of *Rhodomela confervoides* (Rhodomelaceae), a marine red alga collected from Lianyungang, China. The fungal identification was performed by analysis of its ITS rDNA gene sequence with those previously reported sequences. The sequence of the strain 2021CDF-3, which has been submitted to GenBank with an accession number of OP349593, showed a high identity (99.8%) with that of *P. oxalicum* (accession No. KY400080). This fungus has been deposited at the School of Food and Pharmacy, Zhejiang Ocean University.

2. Previous Studies

Endophytic fungi have a unique secondary metabolism and can produce an abundance of important specialized metabolites [1,2]. Nowadays, many metabolites with biological significance have been isolated from endophytic fungi [3−5]. Many metabolites and their derivatives have become targets of agrochemicals and drugs [6,7]. During our continuous search for innovative specialized metabolites from endophytic fungi [8−11], an algal-derived endophytic species *P. oxalicum* 2021CDF-3 was chemically investigated. Phytochemical studies on this fungal strain have yielded many interesting metabolites. For example, this fungus, when cultured in PDB medium, produced cytotoxic

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indole derivatives including two new prenylated indole alkaloids asperinamide B and peniochroloid B [9] as well as a new cytotoxic ergostane steroid peniciloxatone A [10]. In addition, this fungus cultured in rice medium was found to produce two new cytotoxic polyketides oxalichroman A and oxalihexane A [11]. This study was a continuation of the previous studies to report on the new compound isolated from this fungal strain.

3. Present Study

The fungus *P. oxalicum* 2021CDF-3 was cultured in liquid PDB medium for 5 days shaking at 28 °C with 200 rpm to prepare the seed cultures. Then, 2 mL seed was transferred into 500 mL Erlenmeyer flask preloaded with 100 g of rice and 100 mL of distilled water. A total of 50 flasks (5 kg of rice) was cultured in stationary state at room temperature for 40 days. Subsequently, the rice medium was extracted for three times with equivalent EtOAc. The EtOAc extracts were combined and dried under reduced pressure to afford 22.6 g of brown paste. The obtained paste was sectionalized by silica gel chromatography with CH_2Cl_2-MeOH elution mixtures from 100:1 to 10:1 (v/v). The elution products of different polarity were combined by TLC analysis to give six fractions. Fraction 3 eluting with CH_2Cl_2-MeOH = 60:1 was subjected to C_{18} reversed-phase silica gel column chromatography (MeOH-H_2O mixtures from 10% to 100%, v/v) to yield five subfractions. Compounds **1** (2.9 mg, t_R = 10.6 min) and **2** (4.8 mg, t_R = 7.1 min) were separated from subfractions 3.4 (eluted with 50% MeOH-H_2O) by semipreparative RP-HPLC (55% MeOH-H_2O). It should be pointed out that compound **2** was isolated from fraction 5, which was eluted with CH_2Cl_2-MeOH 20:1, in our previous studies [12]. In this study, compound **2** was also isolated from fraction 3 eluting with CH_2Cl_2-MeOH = 60:1. Fraction 4 eluting with CH_2Cl_2-MeOH = 40:1 was purified by silica gel column chromatography (CH_2Cl_2-MeOH = 20:1, v/v) to afford compounds **3** (7.5 mg) and **4** (5.6 mg).

*Penicivetivane B (**1**)*: colorless oil; $[\alpha]^{20}_D$ +76.9 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.03) nm; ^{1}H and ^{13}C NMR data, shown in Table 1; HRESIMS *m/z* 237.1846 $[M + H]^+$ (calcd for $C_{15}H_{25}O_2^+$, 237.1849).

Cytotoxic Activity Test: The isolated compounds **1**−**4** were evaluated for their cytotoxicities against A549, MCF7, MKN-45, and HCT 116 cell lines by using Cell Counting Kit (CCK-8) method. The details were reported by Yuan et al. [13]. Single concentration (20 μM) was set for each compound in preliminary screening, while seven concentration gradients (0, 3.125, 6.25, 12.5, 25, 50, and 100 μM) were used for the IC_{50} determination. Doxorubicin was selected as the positive control.

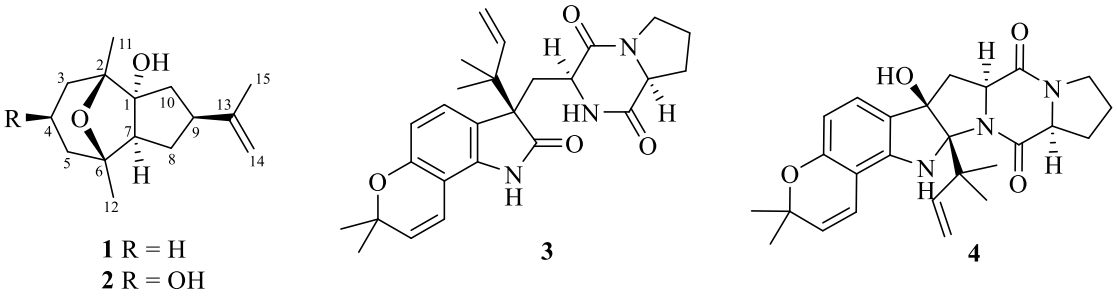

Figure 1. Chemical structures of the isolated compounds **1**−**4**

Compound **1** was obtained as colorless oil through the above-described separation process. Compound **1** was assigned the molecular formula of $C_{15}H_{24}O_2$ based on the detection of the ion peak at *m/z* 237.1846 ($[M + H]^+$, calcd for $C_{15}H_{25}O_2^+$, 237.1849) in HRESIMS spectrum. In the ^{1}H NMR spectrum of **1**, two terminal olefinic protons resonating at δ_H 4.72 (1H, br s, H_2-14a) and 4.69 (1H, br s, H_2-14b), two methine protons at δ_H 2.64 (1H, tq, *J* = 8.8, 4.9 Hz, H-9) and 2.12 (1H, t, *J* = 8.9 Hz, H-7), ten protons supposed for five methylene groups at δ_H 1.16−2.09, three methyl groups at δ_H 1.72 (3H, s, H_3-15), 1.00 (3H, s, H_3-12), and 0.99 (3H, s, H_3-11), as well as one exchangeable proton at δ_H

4.64 (1H, s, 1-OH) were observed (Table 1). The ^{13}C NMR spectrum (Table 1), together with the HSQC spectrum of **1**, clearly showed 15 carbons, which were differentiated as three methyls, six methylenes with one olefinic at δ_C 109.1 (C-14), two methines, and four quaternary carbons including with one olefinic group at δ_C 147.9 (C-13) and three oxygenated carbons at δ_C 91.2 (C-1), 80.8 (C-2), and 78.5 (C-6). Inspection of the 1D NMR data of **1** indicated that **1** possessed the same skeleton type as penicivetivane A (compound **2**), a vetivane-type sesquiterpenoid with a tricyclic 7/5/5 scaffold [12]. The main difference between **1** and **2** was that the oxygenated methine at C-4 in **2** was replaced by a methylene group in **1**. The seven-membered ring in **1** was established by the ^{1}H–^{1}H COSY correlations of H_2-3/H_2-4/H_2-5, as well as by the key HMBC correlations from H_2-3 to C-1 and C-5, from H-7 to C-5, from H_3-11 to C-1 and C-3, from H_3-12 to C-5 and C-7, and from 1-OH to C-1, C-2, and C-7 (Figure 2). Moreover, ^{1}H–^{1}H COSY correlations of H-7/H_2-8/H-9/H_2-10 indicated the presence of the $CHCH_2CHCH_2$ spin system made up of the five-membered ring. The seven-membered ring was fused with the five-membered ring at C-1 and C-7 supported by the correlations from H-7 to C-8, from H_2-8 to C-6 and C-10, and from 1-OH to C-10 (Figure 2). An epoxy ring between the oxygenated quaternary carbons C-2 and C-6 was deduced by the molecular formula $C_{15}H_{24}O_2$ and relevant degrees of unsaturation. Aided by the other HMBC correlations shown in Figure 2, the planar structure of **1** was established. Compound **1** possessed a rare vetivane-type skeleton with a tricyclic 7/5/5 scaffold [14]. The vetivane-type sesquiterpenoid was previously reported in 1960. Compounds **1** and **2** were elucidated as the second occurrence of this kind of skeleton [14].

The relative configurations were determined by the analysis of NOESY spectrum of **1**. As demonstrated in Figure 2, 1-OH showed mutual NOE correlations with H-7 and H-9, revealing these protons were placed in the same side. Moreover, key NOE correlations between H-7 and H_3-12 as well as between H-10a and 1-OH/H_3-11 implied that the methyl groups of H_3-11 and H_3-12 were also situated at the same orientation as that of 1-OH and H-7 (Figure 2). These signals were in accordance with those from penicivetivane A (compound **2**) [12]. Considering the same biosynthetic pathways between **1** and **2**, the stereochemistry of **1** was temporarily assigned to be the same as **2**.

Compounds **3** and **4** were characterized as notoamides C and D, respectively, by comparing their NMR data with the reported literature [15].

Table 1. ^{1}H and ^{13}C NMR data (500 and 125 MHz, respectively) of compound **1** (measured in DMSO-d_6)

No	**1**	
	δ_H (J in Hz)	δ_C, type
1		91.2, C
2		80.8, C
3	1.73, m; 1.16, td (12.8, 5.5)	33.0, CH_2
4	2.09, m; 1.46, m	18.0, CH_2
5	1.38, m	37.4, CH_2
6		78.5, C
7	2.12, t (8.9)	56.6, CH
8	1.82, m; 1.27, m	33.8, CH_2
9	2.64, tq (8.8, 4.9)	45.5, CH
10	1.56, ddd (11.9, 4.9, 2.5); 1.38, m	43.3, CH_2
11	0.99, s	22.0, CH_3
12	1.00, s	24.0, CH_3
13		147.9, C
14	4.72, br s; 4.69, br s	109.1, CH_2
15	1.72, s	21.9, CH_3
1-OH	4.64, s	

A new cytotoxic sesquiterpenoid

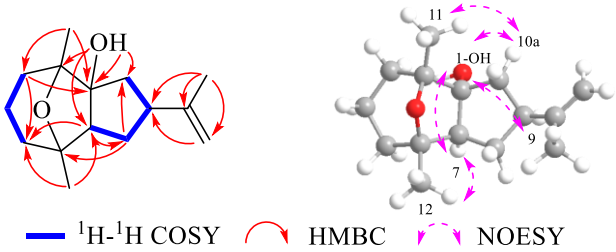

Figure 2. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of **1**

The isolated compounds **1**−**4** were measured for their cytotoxicities against A549, MCF7, MKN-45, and HCT 116 cells. Consequently, compounds **2** and **4** displayed the equal inhibitory activity on the MKN-45 cell as the positive control doxorubicin (IC_{50} = 5.0 ± 0.2 μM), with IC_{50} values of 8.0 ± 0.5 μM and 11.9 ± 0.6 μM, respectively (Table 2). Compared with compound **1** (IC_{50} = 20.5 ± 1.0 μM) toward the MKN-45 cell, compound **2** showed higher activity, suggesting that the OH group may play an important role in cytotoxicity. In addition, our previous studies indicated that compound **2** possessed strong antimicrobial properties against human pathogens [12]. Considering the structural similarity, it may be interesting to investigate antimicrobial results of compound **1**.

Table 2. Cytotoxic activities of compounds **1**−**4** (IC_{50}, μM)

Compound	A549	MCF7	MKN-45	HCT 116
1	> 100 μM	48.5 ± 1.2	20.5 ± 1.0	> 100 μM
2	> 100 μM	> 100 μM	8.0 ± 0.5	60.6 ± 1.8
3	> 100 μM	> 100 μM	> 100 μM	23.4 ± 1.0
4	> 100 μM	> 100 μM	11.9 ± 0.6	> 100 μM
doxorubicin	2.3 ± 0.2	4.9 ± 0.6	5.0 ± 0.2	1.8 ± 0.3

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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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