SHORT REPORT

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Polyketides and Alkaloids from the Fungus *Penicillium* sp.

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Abstract: The marine-derived fungus SCZ-1 was cultured on rice solid medium. The fermented materials was extracted using ethyl acetate to afford an extract, which was separated by repeated column chromatography to afford three polyketides and five alkaloids, including 2 new polyketides (**1** and **2**). The known compounds were identified to be penipyrol A (**3**), cyclopenin (**4**), cyclopenol (**5**), terretrione D (**6**), viridicatol (**7**), and viridicatin (**8**). The structures of **1** and **2** were determined by extensive analyses of spectroscopic data (1D and 2D NMR, and HRESIMS). The absolute configuration of **2** was assigned by comparison of the experimental ECD spectrum with calculated ECD spectrum. Compound **1**, bearing a two-carbon side chain at C-2, is seldom found among natural isocoumarins. All metabolites were screened for their inhibitory effects toward α-glucosidase, only compound **8** showed weak activity with an IC_{50} value of 548 μM.

Keywords: marine-derived fungus; *Penicillium* sp.; new polyketides.

1. Plant Source

The fungal strain SCZ 4-1 was isolated from sediments collected from the Mirs Bay of Shenzhen. The strain was identified as *Penicillium* sp. by analyses of the ITS region of the rDNA sequence with those of a standard record. The ITS sequence has been sent to the GenBank (http://www.ncbi.nlm.nih.gov) with the accession number KY445779.

2. Previous Studies

In recent years, the metabolites of fungi have been deeply excavated by natural product chemists, leading to the discovery of thousands of molecules with various structures and profound activity [1-6]. The *Penicillium* is an outstanding member which have been proved to be productive. Previous chemical investigation of *Penicillium* strains led to identification of meroterpenoids [7-9], alkaloids [10, 11], polyketides [12, 13], sesquiterpenes and diterpenes [14-17].

In our study, we found that the ^{1}H NMR data of the fermented materials of the marine-derived *Penicillium* sp. SCZ4-1 showed resonances that were probable for alkaloids. Subsequent chromatographic separation of the EtOAc extract led to the isolation of eight compounds including three polyketides (**1**–**3**) and five alkaloids (**4**–**8**). Herein, we reported the isolation and structural identification of these metabolites.

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3. Present Study

The strain was cultured on solid rice medium (80 g rice and 90 mL water) in 20 erlenmeyer flasks (500 mL). The fermentation was carried out for 4 weeks under static conditions at 25 °C. The fermented materials were extracted with EtOAc (1000 mL) for three times and afforded an EtOAc extract (2.1 g), which was separated on a silica gel with petroleum ether/EtOAc (95:5 to 50:50) as eluent to give five fractions (Fr.I–Fr.V). Fra. II was chromatographed over ODS silica gel ($MeOH/H_2O$ = 20:80 to 100:0) to give five fractions (Fr.IIa–Fr.IIe). Fr.IIb was purified by HPLC (a YMC-pack ODS-A column, 2 mL/min) with $MeCN/H_2O$ (50:50) as eluent to give **5** (8.5 mg), **8** (1.8 mg), and **7** (2.3 mg). Fr.IIc was purified on HPLC using 60% acetonitrile/water as eluent to give **2** (2.7 mg), **4** (5.7 mg), and **6** (2.3 mg). Fr.III was applied to the ODS gel ($MeOH/H_2O$ = 20:80 to 100:0) to give five fractions (Fr.IIIa– Fr.IIIe). Fr.IIIb was separated by HPLC ($MeCN/H_2O$ = 45:55) to yield **1** (1.5 mg) and **3** (2.5 mg).

*Peniketide A (**1**)*: Light yellow oil, UV (MeOH) λ_{max} (log ε) 239 (4.36), 244 (4.39) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z: 237.0388 $[M + H]^+$ (calcd. for $C_{11}H_9O_6^+$, 237.0394).

*Methyl ester of penipyrol A (**2**)*: Colorless oil; $[\alpha]^{25}_D$ –34 (c = 0.1, CH_3OH); UV (MeOH) λ_{max} 291 (3.77) nm; ECD (*c* 7.1 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 314 (–0.49), 254 (–1.87), 209 (+1.27) nm;^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 275.1287 $[M - H]^-$ (calcd. for $C_{16}H_{19}O_4^-$, 275.1289)

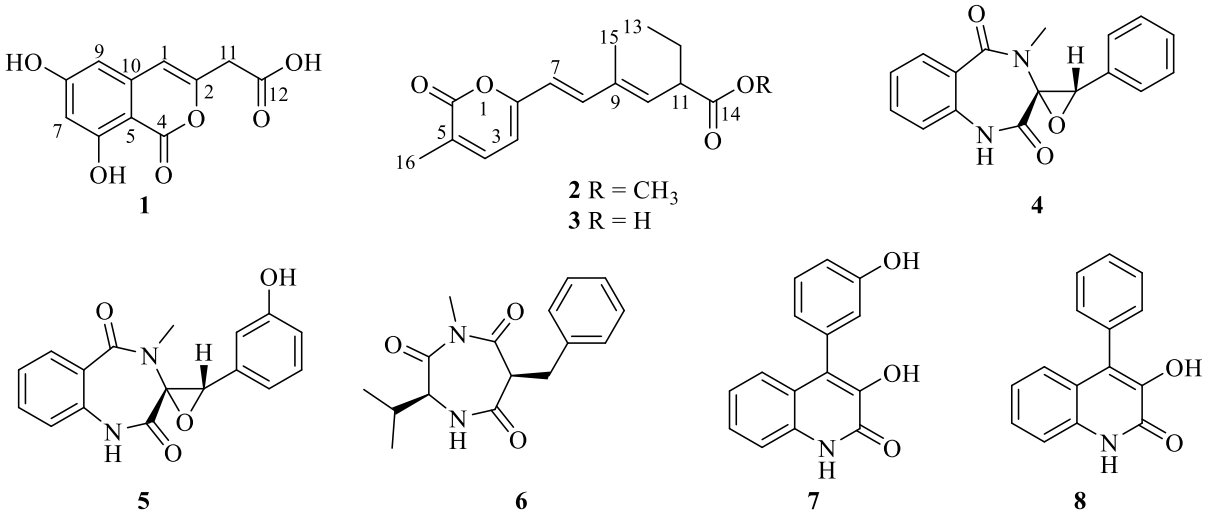

Figure 1. Structures of compounds **1**–**8** from the strain *Penicillium* sp.

Compound **1** had the molecular formula of $C_{11}H_8O_6$ as assigned by the HRESIMS (237.0388 $[M + H]^+$, calcd. for $C_{11}H_9O_6^+$, 237.0394), indicating eight sites of unsaturation. The ^{1}H NMR spectrum displayed the signals for three aromatic protons [δ_H 6.47 (1H, br s), 6.34 (2H, br s)] and two protons for a methylene [δ_H 3.52 (2H, br s)]. The ^{13}C NMR exhibited 11 carbon resonances in total, which were attributed by HSQC spectrum to three sp^2 methine (δ_C 107.8, 104.0, 102.9), seven sp^2 non-protonated carbons (δ_C 173.0, 167.5, 167.3, 164.6, 152.6, 141.0, 99.8), and a sp^3 methylene carbon (δ_C 40.3). The above-mentioned data indicated that the structure of **1** contained a benzene ring, a carboxyl carbon, an ester carbon, and two carbons for a double bond. As the unsaturated groups accounted for seven of the eight sites of unsaturation, the remaining one required the presence of an additional ring in the structure. These data were very similar to those of a reported natural analog (**1a**) except for the presence of a methylene (δ_H 3.52; δ_C 40.3) and an additional carbonyl carbon (δ_C 173.0) instead of the allylic methyl (δ_H 2.22; δ_C 19.0) in **1a** [18], suggesting that the methyl in **1a** was replaced by an acetic acid moiety in **1**. The deduced structure was further confirmed by 2D NMR analyses (Figure 2) and MS data, especially the HMBC correlations from methylene protons H_2-11 (δ_H

3.52) to C-2 (δ_C 152.6), and the carboxylic carbon C-12 (δ_C 173.0) and from the olefinic proton H-1 (δ_H 6.47) to C-2, C-11 (δ_C 40.3), and C-12. Thus, the structure of **1** was determined as 7,9-dihydroxy-3-acetic acidisocoumarin, interestingly, isocoumarin bearing a two-carbon side chain at C-2 was rarely found in nature. Compound **1** was named peniketide A.

Table 1. ^{1}H (400 Hz) and ^{13}C NMR (100 Hz) Data of **1**–**3** and **1a** in methanol-d_4 (δ in ppm)

No.	**1**		**1a**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	6.47, br s	107.8	6.47	102.1				
2		152.6		155.7		165.2		165.1
3						124.6		124.4
4		167.3		166.9	7.32, d (6.9)	142.4	7.30, d (6.9)	142.3
5		99.8		99.8	6.26, d (6.9)	106.7	6.25, d (6.9)	106.6
6		164.6		165.0		159.0		159.0
7	6.34, br s	102.9	6.30	103.8	6.25, d (15.8)	119.6	6.23, d (15.8)	119.4
8		167.5		167.0	7.04, d (15.8)	138.7	7.04, d (15.8)	138.8
9	6.34, br s	104.0	6.33	105.9		137.0		136.6
10		141.0		141.8	5.75, d (9.9)	136.0	5.78, d (9.8)	136.6
11	3.52, br s	40.3	2.22	19.0	3.42, ddd (9.9, 7.2, 7.2)	48.0	3.34, ddd (9.8, 7.2, 7.2)	47.1
12		173.0			1.83, m 1.61, m	27.1	1.83, m 1.61, m	27.2
13					0.93, t (7.4)	11.9	0.94, t (7.4)	12.0
14						175.7		177.2
15					1.88, s	12.7	1.89, s	12.7
16					2.06, s	16.6	2.06, s	16.7
OCH_3					3.70, s	52.4		

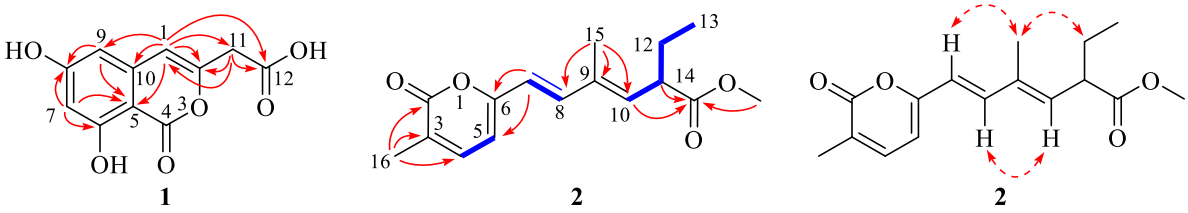

Figure 2. Key HMBC (⟶), COSY (—), NOESY (⟷) correlations of **1** and **2**

The molecular formula of compound **2** was assigned as $C_{16}H_{20}O_4$ on the basis of the HRESIMS (275.1287 [M – H]$^-$, calcd. for $C_{16}H_{19}O_4^-$, 275.1289) and NMR data (Table 1), requiring seven degrees of unsaturation. The ^{1}H NMR spectrum showed the resonances for two olefinic methyls (δ_H 2.06 and 1.89), a triplet of methyl (δ_H 0.94), a singlet of methoxy group (δ_H 3.70), five olefinic protons (δ_H 7.30, 7.04, 6.25, 6.23, 5.78) including two for a *trans* double bond [δ_H 7.04 (15.8 Hz), 6.23 (15.8 Hz)], and three aliphatic protons (δ_H 3.34, 1.83, 1.61), while the ^{13}C NMR and HSQC spectra presented 15 carbon resonances totally, involving eight olefinic carbons (δ_C 165.2, 142.4, 138.7, 137.0, 136.0, 124.6, 106.7, 119.6), two carbonyl carbons (δ_C 175.7, 159.0), four methyls (δ_C 16.6, 12.7, 11.9), a methoxy (δ_C 52.4), one methylene (δ_C 27.1), one methine (δ_C 48.0). The aforementioned data were similar to the co-isolated know compound penipyrol A (**3**) with only distinction owing to the presence of a methoxy (δ_H 3.70, δ_C 52.4), suggesting **2** was the methyl derivative of **3** [19]. The speculated structure of **2** was confirmed by 2D NMR analyses. Specifically, the HMBC correlation from the methoxy protons to the carbonyl carbon at δ_C 175.7 established the methyl ester moiety. The geometries of Δ^7 and Δ^{10} in **2** were both assigned as *E*-configuration by NOESY experiments (Figure

2) and by comparison of its NMR data with those of **3**. The absolute configuration of **2** was determined by comparison of experimental ECD spectrum with the calculated ECD spectrum, which led to the assignment of the 11*R* configuration for **2** (Figure 3).

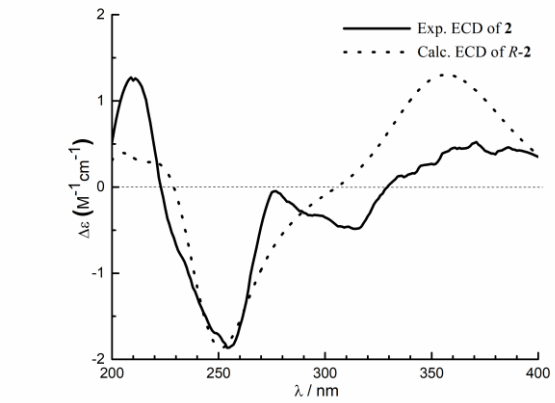

Figure 3. Experimental ECD spectrum of **2** in MeOH and the calculated ECD spectra of *R*-2 at the b3lyp/6-31+g(d,p) level

Besides, the other known compounds were elucidated to be penipyrol A (**3**) [19], cyclopenin (**4**) [20], cyclopenol (**5**) [20], terretrione D (**6**) [21], viridicatol (**7**) [22], viridicatin (**8**) [20], by comparing their ^{1}H and ^{13}C NMR data with reported data in the literature.

All the isolated metabolites were tested for the activity toward the α-glucosidase according to the literature [23], only compound **8** showed weak inhibitory effect with an IC_{50} value of 548 μM (the positive control acarbose: 217 μM).

Supporting Information

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

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