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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 4993–4996

Penicillones A and B, two novel polyketides with tricyclo [5.3.1.0^{3,8}] undecane skeleton, from a marine-derived fungus *Penicillium terrestre*

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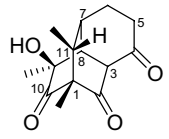
Received 3 March 2005; revised 18 May 2005; accepted 20 May 2005
Available online 13 June 2005

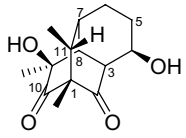
Abstract—Two novel polyketides, penicillones A (**1**) and B (**2**), with tricyclo [5.3.1.0^{3,8}] undecane skeleton, were isolated from *Penicillium terrestre*. Their structures and relative stereochemistries were determined on the basis of spectroscopic methods. The absolute configuration of **2** was established by the modified Mosher's method, while that of **1** was deduced from the similar CD absorptions of **1** and **2**. Compound **1** showed weak cytotoxicities against P338 and A-549 cell lines, while **2** was inactive against P388.

Investigation of structurally novel and biologically active compounds from marine-derived microorganisms has become an important research area in new drug discovery. An increasing number of such compounds have been isolated in recent years.[1] In our search for antitumor compounds from marine-derived microorganisms, a fungus, authenticated as *Penicillium terrestre*, was obtained from the marine sediment in Jiaozhou Bay of Qingdao. Its extract exhibited cytotoxicity against tsFT210 cell line. Studies on the active constituents of this fungus led to the isolation of two novel polyketides, namely, penicillones A (**1**) and B (**2**), together with two known compounds, sorbicillin[2] and trichodimerol.[3] In this letter, we describe the isolation, structure elucidation, and cytotoxicities against P338 and A-549 cell lines of **1** and **2**.

The producing strain was preserved in China Center for Type Culture Collection (patent depositary number: CCTCC M 204077). Fermentation was carried out as follows. A small spoon of spores growing on PDA slant was inoculated into a 250 mL Erlenmeyer flask containing 75 mL sea-water based culture medium (glucose 2%, maltose 2%, monosodium glutamate 10%, beef extract 0.3%, KH_2PO_4 0.05%, and $MgSO_4{\cdot}7H_2O$ 0.03%) and cultured at 28 °C for 2 days on a rotary shaker at 160 rpm. Then, 10 mL of the resultant seed culture was inoculated into a 500 mL Erlenmeyer flask containing 150 mL of the above culture medium and incubated for 7 days at the same conditions.



1



2

Fifty liters of whole broth was filtered through cheesecloth to separate the broth supernatant and mycelia. The former was extracted with ethyl acetate, while the latter was extracted with acetone. The acetone extraction was evaporated under reduced pressure to afford an aqueous solution and then extracted with ethyl acetate. The two ethyl acetate extractions were combined and concentrated in vacuo to give a crude

Keywords: *Penicillium terrestre*; Polyketide; Tricyclo [5.3.1.0^{3,8}] undecane; Penicillone; Microorganism; Fungus.
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0040-4039/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2005.05.087

extract (58.0 g). It was subjected to silica gel column chromatography and eluted with gradient elution of petroleum ether–chloroform and chloroform–methanol, respectively. The fraction eluted with the solvent of chloroform–methanol (150:1) was further purified by HPLC on ODS with 60% methanol to give **1** (36 mg). And the fraction eluted with the solvent of chloroform–methanol (85:1) was separated by HPLC on ODS using 40% methanol as eluting solvent to afford **2** (4 mg).

Compound **1**[4] was obtained as colorless plate crystals. The molecular formula, $C_{14}H_{18}O_4$, was deduced from its ^1H and ^{13}C NMR spectra (Table 1) and was confirmed by HRESIMS. The IR spectrum suggested the presence of hydroxyl (3404 cm^{-1}) and carbonyl (1739, 1714, and 1692 cm^{-1}) groups. Careful analysis of the ^1H,^{13}C NMR, and DEPT data for **1** revealed three carbonyls, two quarternary carbons (one of which was oxygen bearing), four methines, two methylenes, and three methyls. Since three of the six unsaturations were accounted for, it was implied that **1** should contain three rings (Fig. 2).

The very low-field chemical shifts of a tertiary carbon (δ_C 62.6) and a quaternary carbon (δ_C 67.3) suggested that each of them should be connected with two carbonyls. So there were two β-diketone systems in **1**. Because of only three carbonyls, the two β-diketone systems should comprise partial structure **a**, which was supported by the HMBC correlations of CH_3-1 with C-1, C-2, and C-10, and H-3 with C-2 and C-4. The COSY data extended partial structure **a** to partial structure **b** (Fig. 1). Although there was no cross peak observed between H-7 and H-6 in the COSY spectrum, the connection of C-6 with C-7 could be confirmed by the correlations of H-5 with C-7, H-8 with C-6, and H-11 with C-6 in the HMBC spectrum. And the connection of C-5 with C-4 was supported by the correlations of H-5 with C-3 and C-4. Furthermore, the HMBC correlations of CH_3-9 with C-8, C-9, and C-10, and H-8 with C-9 and C-10 indicated that C-8 was connected to C-10 through C-9 (Table 1). In addition, the ^1H-^{13}C long-range correlations of CH_3-11 with C-1 and CH_3-1 with C-11 unambiguously suggested that C-11 was anchored to C-1. This completed the planar structure of **1**.

In the NOE difference experiment, when CH_3-9 was irradiated, the signals of H-3 and H-8 were enhanced, which revealed the *cis*-relationship between CH_3-9, H-3, and H-8. And the enhancement of H-6 (δ_H 2.06) and H-5 signals upon irradiation of H-11 indicated that the relative configuration of C-11 was as shown in Figure 2, which was further confirmed by the enhancement of the OH-9 signal when CH_3-11 was irradiated. Thus, the relative stereochemistry of **1** was established.

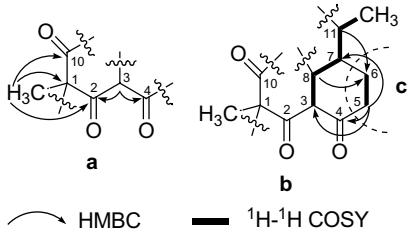

Figure 1. Partial structures **a**, **b**, and **c** in **1** and its selected HMBC and ^1H–^1H COSY data.

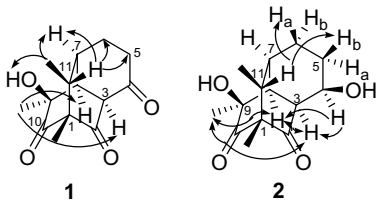

Figure 2. Key NOESY (↔) and NOE (→) correlations for **1** and **2**.

Compound **2**[5] was obtained as colorless needles. The HRESIMS gave a molecular formula of $C_{14}H_{20}O_4$, which differed from that of **1** by two hydrogen atoms. The IR spectrum of **2** showed absorptions of hydroxyl (3321 cm^{-1}) and carbonyl (1732, 1695 cm^{-1}) groups. Interpretation of the ^1H and ^{13}C NMR spectral data revealed the presence of two carbonyls, one oxygenated quaternary carbon, one quaternary carbon, four methines including an oxygen-bearing one, two methylenes, and three methyls. Those data, compared with those of **1**, indicated that **2** had the same structure as **1** except for a carbonyl being hydrogenated. This was in agreement with the COSY and HMBC data (Table 2).

The relative stereochemistry of **2** was established by NOESY and NOE difference experiments (Fig. 2). The cross peaks observed between CH_3-9, H-3, and H-8 in the NOESY spectrum demonstrated that those groups were at the same side of the ring. And the configuration of C-4 was established by the enhancement of H-3 and H-8 signals when H-4 was irradiated. Finally, the enhancement of H_b-5 and H_a-6 signals, upon irradiation on H-11, demonstrated that the configuration of C-11 was as shown in Figure 2. So the relative stereochemistry of **2** was constructed.

According to the modified Mosher's method,[6] two portions (each 1.0 mg) of **2** were treated with (*S*)-(+) and

Table 1. ^1H and ^{13}C NMR, and HMBC data of compound **1** (600, 150 and 600 Hz, $CDCl_3$, TMS, δ ppm)

No.	δ_H (*J* in Hz)	δ_C	HMBC (H→C)
1		67.3 s	
2		201.3 s	
3	3.50 m	62.6 d	2, 4, 7, 8, 9
4		201.7 s	
5	2.45 m	34.8 t	3, 4, 6, 7
6	2.06 m; 2.00 m	28.1 t	4, 5, 11
7	2.58 m	33.5 d	
8	2.52 m	46.7 d	2, 3, 6, 7, 9, 10, 11
9		73.9 s	
10		209.4 s	
11	1.94 m	41.0 d	1, 2, 6, 7, 10, CH_3-11
CH_3-1	1.17 s	9.8 q	1, 2, 10, 11
CH_3-9	1.25 s	22.6 q	8, 9, 10
CH_3-11	1.09 d (7.0)	17.9 q	1, 7, 11
OH	2.86 s		8, 9, 10

Table 2. ^{1}H and ^{13}C NMR, COSY, and HMBC data of compound **2** (600, 150 and 600 Hz, acetone-d_6, TMS, δ ppm)

No.	δ_H (J in Hz)	δ_C	HMBC (H→C)	^{1}H–^{1}H COSY	$\delta_{H(S)}$ (C_5D_5N)[a]	$\delta_{H(R)}$ (C_5D_5N)[a]	$\Delta\delta_{H(S-R)}$[b]
1		68.1 s					
2		213.2 s					
3	2.79 m	51.8 d	2, 4, 5, 7, 8	4, 7, 8	3.4335 br s	3.5025 br s	−0.069
4	3.72 m	73.1 d	2	3, 5	5.3642 m	5.3584 m	+0.0058
5	1.83 m (H_a-5);	29.1 t	4, 6, 7	4, 6	1.9724 m	1.7701 m	+0.2023
	1.28 m (H_b-5)		3, 4, 6		1.7176–1.6205[c]	1.6395–1.5827[c]	—
6	1.73 m (H_a-6);	28.7 t	4, 7, 8	5,7	1.7176–1.6205[c]	1.6395–1.5827[c]	—
	1.58 tt (13.6, 4.0, H_b-6)		11				
7	2.35 m	34.5 d		3, 8, 11, 6	2.6888 br s	2.6820 br s	+0.0068
8	2.05 m	47.3 d	2, 3, 7, 9, 10, 11, CH_3-9	3, 7	2.3683 br s	2.3781 br s	−0.0098
9		74.1 s					
10		210.3 s					
11	1.65 dq (7.3, 4.0)	41.0 d	1, 2, 6, 7, 10, CH_3-11	7, CH_3-11	1.7176–1.6205[c]	1.6395–1.5827[c]	—
CH_3-1	0.98 s	10.4 q	1, 2, 10, 11		1.2445 s	1.2732 s	−0.0287
CH_3-9	1.26 s	22.8 q	8, 9, 10		1.4087 s	1.4374 s	−0.0287
CH_3-11	1.00 d (7.3)	18.2 q	1, 7, 11	11	1.0934 d (7.0)	1.0916 d (7.0)	−0.0018
OH-9	4.73 s		8				

[a] $\delta_{H(S)}$ and $\delta_{H(R)}$ represented the chemical shifts of hydrogens in the (*S*)- and (*R*)-MTPA esters of **2**, respectively.
[b] $\Delta\delta_{H(S-R)} = \delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$.
[c] The signals of H_2-6, H-11, and one of H_2-5 in the (*S*)- and (*R*)-MTPA esters of **2** were overlapped, respectively, but the signals in (*S*)-MTPA esters of **2** were obviously in lower field than those in (*R*)-MTPA esters of **2**.

(*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (2.5 μL) in deuterated pyridine (0.5 mL) in separate NMR tubes at room temperature. The reactions were monitored by ^{1}H NMR at certain intervals. The reaction of **2** with (*S*)-MTPA chloride was completed after 7 h, while the reaction of **2** with (*R*)-MTPA chloride needed 54 h to finish. The ^{1}H NMR data of the (*S*)- and (*R*)-MTPA esters of **2** were obtained after the end of the slower reaction, and the assignments of the hydrogen signals were established by ^{1}H–^{1}H COSY experiment. The positive $\Delta\delta$ ($\delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$) values of H-5 and H-7, and the negative $\Delta\delta$ values of H-3, H-8, and CH_3-1 indicated that the absolute configuration of C-4 was *R*.[7] Therefore, the absolute stereochemistry of **2** was concluded to be 1*R*,3*S*,4*R*,7*R*,8*S*,9*S*, and 11*R*, as shown in the structure (Fig. 3). The similar CD absorptions[4,5] of **1** and **2** suggested that the absolute configuration of **1** should be 1*R*,3*S*,7*R*,8*S*,9*S*, and 11*R*.

The anticancer activities of two compounds were preliminarily evaluated using P338 and A-549 cell lines by the MTT method.[8] The cell lines were exposed to graded concentrations of **1** and **2**, and cultured in RPMI-1640 medium supplemented with 10% FBS at 37 °C for 72 h. VP16 was used as positive control, and its IC_{50} values to P338 and A-549 cell lines were 0.064 and 1.4 μM, respectively. **1** showed cytotoxicities against P338 and A-549 cell lines with IC_{50} values of 83.0 and 68.4 μM, respectively. At the same conditions, **2** showed IC_{50} values of 97.6 μM to A-549 cells, but inactive to P388 cells. Those data demonstrated that **1** and **2** possessed weak cytotoxicities to the above cell lines.

Compounds with tricyclo [5.3.1.$0^{3,8}$] undecane skeleton are rare in nature. A few have been reported as hydrocarbons and alcohols from several species of plants,[9] while penicillones A (**1**) and B (**2**) we are reporting in this letter are new structures with ketone features. To the best of our knowledge, it is the first time that compounds with tricyclo [5.3.1.$0^{3,8}$] undecane skeleton have been isolated from microorganisms.

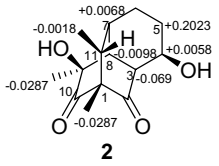

Figure 3. Values of $\Delta\delta$ ($\delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$) of the MTPA esters of **2**.

Acknowledgements

This work was financially supported by the Project of Chinese National Programs for High Technology Research and Development (No.2003AA624020), the Chinese National Natural Science Fund (No. 30472136 and 30470196), and the Shandong Province and Qingdao Natural Science Fund (No. Z2001C01 and 04-2-JZ-81). The antitumor assay was performed at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. We thank Dr. Shichang Miao for valuable discussions and reviewing the manuscript.

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4. Compound **1**: colorless plate crystals (acetone); mp 200–201 °C; $[\alpha]_D^{20}$ +169.7 (*c* 0.2, MeOH); HRESIMS $[M+Na]^+$ *m*/*z* 273.1114, calcd for $C_{14}H_{18}O_4Na$, 273.1103; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 340 (+3.3), 305 (+41.7), 207 (−75.0);

IR (KBr) ν_{max} 3404, 2980, 2941, 1739, 1714, 1692, 1448, 1380, 1145, and 998 cm^{-1}.

5. Compound **2**: colorless needles (acetone); mp 193 °C (dec); $[\alpha]_D^{20}$ −13.7 (*c* 0.2, MeOH); HRESIMS $[(M+Na)]^+$ *m*/*z* 275.1270, calcd for $C_{14}H_{20}O_4Na$, 275.1259; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 339 (+2.5), 300 (+2.7), 203 (−25.0); IR (KBr) ν_{max} 3321, 2925, 1732, 1695, 1576, 1411, 1162, 1054, and 998 cm^{-1}.
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