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Two Epothilones from *Sorangium cellulosum* Strain So0157-2

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[ABSTRACT] AIM: To study the chemical constituents of *Sorangium cellulosum* So0157-2. **METHODS:** The chemical constituents were isolated and purified by column chromatography, and their structures were identified by spectroscopic analyses including 1D-, 2D-NMR data and MS analyses. **RESULTS:** Two epothilones were purified and identified as *seco*-epothilone A (**1**) and 1-methyl-*seco*-epothilone A (**2**). **CONCLUSION**: **1** and **2** were obtained from this strain for the first time, and **1** was a new natural product.

[KEY WORDS] *Sorangium cellulosum* So0157-2; Epothilones; Spectroscopic analyses

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1 Introduction

Epothilones are exciting new natural products, isolated from the myxobacteria *Sorangium cellulosum* strain So90, with novel molecular structures, important biological properties and an intriguing mechanism of action[1-3]. Epothilones are currently of great interest because of their potential as anticancer agents

As a part of our ongoing search for new epothilones from *Sorangium cellulosum* strain So0157-2, which was isolated from a soil sample collected in Yunnan [4], the chemical constituents of *Sorangium cellulosum* strain So0157-2 have been studied [5], and epothilones A, B and five new epothilone derivatives have been isolated [6]. Further chemical study of this strain led to the isolation of two epothilone analogues (**1** – **2**). In this paper, we describe the fermentation, isolation and structural elucidation of compounds **1** – **2** and their cytotoxicities against human breast carcinoma cell line MDA-MB-435.

2 Apparatus and Material

2.1 *Apparatus and material*

Column chromatography: Qingdao silica gel (200-300 mesh); Merck silica gel 60 Rp-18; Sephadex LH-20: Pharmacia products; TLC: Qingdao precoated plates, silica GF_{254} plates; NMR Spectra: Bruker DRX-600 spectrometer with TMS as internal standard; HR-Q-TOF-MS: API QStar-Pulsar LC-Q-TOF mass spectrometer; in *m/z*.

2.2 *Culture and extraction*

The strain *Sorangium cellulosum* So0157-2 is deposited in the China Center of Typical Culture Collection (CCTCC) with the accession number of CCTCC M 208078. This organism was cultivated in liquid M26 medium at 30 °C for 4 days to prepare the seed cultures [4]. Then the cells were inoculated on CNST medium for the production of epothilones using solid state fermentation. The total volume of the solid fermentation medium was 10 L. After 4-day incubation, XRD-16 resin was added onto the culture for the absorption of epothilone products. The plates were incubated for additional 4-5 days, following which the XRD-16 resin was collected and extracted with methanol exhaustively.

2.3 *Isolation*

The crude extract (1.25 g) was subjected to MPLC (40 g Rp18 silica gel; 50%, 65%, 75% and 100% MeOH, 800 mL for each gradient) to afford four fractions(Frs. 1-4).

Fr1, obtained from elution with MeOH-H_2O (50%), was subjected to column chromatography (CC) over Sephadex LH-20 (in MeOH) twice, and further applied to MPLC CC (Rp18 silica gel; 50%, 60% and 100% MeOH, 400 mL for each gradient) to afford **1** (5 mg).

Fr 3, obtained from elution with MeOH-H_2O (75%), was subjected to CC over Sephadex LH-20 (in MeOH) twice, and

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further applied to silica CC eluted with petroleum ether /acetone (20 : 1, 42 mL; 8 : 1, 45 mL; 5 : 1, 48 mL) to obtain **2** (5 mg).

3 Results

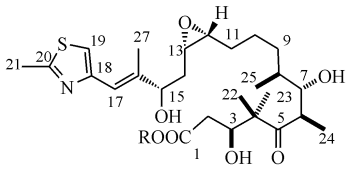
3.1 *Structural elucidation and identification*

Compound **1** was obtained as colorless oil. The molecular formula was determined to be $C_{26}H_{41}NO_7S$ by HR Q-TOF-MS (*m/z* 534.358 9 $[M + Na]^+$ and 512.330 5 $[M + H]^+$) and NMR data (Table 1). The ^{13}C NMR spectrum of **1** displayed 26 carbon signals for six methyls, five methylenes, nine methines, and six quaternary carbon atoms including a carboxyl carbon at δ171.5, respectively. The ^1H and ^{13}C NMR spectra of **1** clearly showed a typical epothilone structure as the presence of a thioazole ring, a carbon-carbon double bond and an epoxy moiety (Table 1). Comparing the ^1H NMR data with those of epothilone A[3], the only difference was that the proton at δ5.44 (H-15) in epothilone A was upshifted to 4.38. The HMBC showed no ^1H-^{13}C long-range correlation from H-15 to C-1, which indicates a structure of *seco*-epothilone A (or epothilone A acid) (Fig. 1).

Compound **2** was obtained as colorless powder. The HR-ESI-Q-TOF MS revealed the molecular formula of **2** to be $C_{27}H_{43}NO_7S$, (*m/z* 526.823 6 ($[M + H]^+$)). The ^1H and ^{13}C NMR spectra were similar to those of **1**, except for an additional signal [δ 3.73 (s, MeO-)] due to a Me ester, as confirmed by comparison of the ^1H and ^{13}C NMR spectra of **1** and **2**, and corroborated by HMBC between H-1a (δ3.73) and C-1 (δ173.4). Thus, the structure of **2** was determined to be 1-methyl-*seco*-epothilone A (Fig. 1).

Table 1 The NMR data of compounds 1 and 2 in $CDCl_3$ (*J* in Hz)

No.	**1**		**2**	
	^1H	^{13}C	^1H	^{13}C
1	-	171.5 s	-	173.4 s
2	2.42 (m)	36.2 t	2.50 (dd, 1.7, 16.1) 2.43 (m)	36.4 t
3	4.27 (m)	72.5 d	4.28 (dd, 2.4, 10.9)	72.4 d
4		52.2 s		52.0 s
5		222.0 s		222.1 s
6	3.28 (q, 6.2)	41.4 d	3.41 (q,8.9)	41.0 d
7	3.48 (m)	74.7 d	3.49 (d,1.3)	74.6 d
8	1.59 (m)	35.4 d	1.58 (m)	35.5 d
9	1.76(m) 1.27 (m)	32.1 t	1.84 (m) 1.56 (m)	32.7 t
10	1.59 (m)	23.4 t	1.64 (m) 1.40 (m)	23.7 t
11	1.53 (m) 1.38 (m)	28.2 t	1.58 (m)	28.4 t
12	2.99 (q, 4.3)	57.3 d	3.00 (dd, 5.9,10.4)	57.1 d
13	3.15 (m)	54.6 d	3.19 (m)	54.4 d
14	1.89 (m) 1.76 (m)	33.4 t	1.95 (dq, 4.4) 1.76 (dq,4.4)	33.4 t
15	4.38 (m)	75.1 d	4.42 (dd, 4.3, 8.4)	75.4 d
16		142.3 s		141.8 s
117	6.64 (s)	118.6 d	6.62 (s)	118.9 d
18		152.2 s		152.7 s
19	6.97 (s)	115.8 d	6.97 (s)	115.8 d
20		165.3 s		164.7 s
21	2.71 (s)	18.8 q	2.72 (s)	18.9 q
22	1.11 (s)	21.3 q	1.21 (s)	19.1 q
23	1.25 (s)	18.9 q	1.16 (s)	21.3 q
24	1.08 (d, 6.8)	10.6 q	1.08 (d, 6.8)	9.9 q
25	0.89 (d, 6.8)	14.6 q	0.88 (d, 6.8)	15.4 q
27	2.02 (s)	15.6 q	2.09 (s)	14.5 q
-$COOCH_3$			3.73 (s)	52.0 q



1 R =H
2 R = Me

Fig. 1 Structures of compounds 1 and 2

3.2 *Bioactivities of compounds* **1** *and* **2**

The cytotoxicities of **1** and **2** against human breast carcinoma cell line MDA-MB-435 were measured 24 h after treatment by the MTT method[7]. Compounds **1** and **2** displayed weak cytotoxicities with inhibition rates of 7.7% and 27.7% at the dose of 10 μg·mL^{-1}.

4 Discussion

Epothilones A and B were first isolated from *Sorangium cellulosum* Soce90 [1]. The search for new epothilone derivatives, spurred by their potent antitumor activity, resulted in the isolation and identification of dozens of natural epothilone-type 16-membered macrolides [3]. Most recently, five glycosylated derivatives of epothilones were isolated from *S. cellulosum* So0157-2, which represented the first example of epothilone glycosides[6]. However, most epothilones reported previously were 16-membered macrolides except for epothilones I_1-I_6[3]. Those epothilones presumably arise by an extra cycle of chain elongation after the introduction of C-9/C-10. *Seco*-epothilones were obtained only as intermediate in the combinatorial chemical synthesis[8-9]. Our previous isolation and detection work suggested that *seco*-epothilones were produced only in the solid state fermentation extract. *Seco*-epothilone A (**1**) and 1-methy-*seco*-epothilone A (**2**), isolated from a wild type strain *S. cellulosum* So0157-2 in this work, have the same polyketide chain as the 16-membered analogues. In the light of the weak antitumor activities of **1** and **2** *in vitro*, it is worth testing the producing mechanism. Overall, our work points to a new direction for generating novel epothilones for antitumor drug discovery.

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粘细菌 *Sorangium cellulosum* So0157-2 产生的两个埃博霉素类化合物

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【摘 要】 **目的**：对粘细菌 *Sorangium cellulosum* So0157-2 的化学成分进行研究。**方法**: 通过色谱层析对提取物进行分离纯化, 并通过波谱解析(一维、二维的核磁共振谱和质谱)确定了化合物的结构。**结果**：分别鉴定为 *seco*-epothilone A (**1**)和 1-methyl-*seco*-epothilone A (**2**)。**结论**：化合物 **1** 和 **2** 都是首次从该菌株中分离得到。其中 **1** 是首次分离得到的新天然产物。

【关键词】 *Sorangium cellulosum* So0157-2; 埃博霉素; NMR

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