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NOTE

Epothilone O, a new member of this family from *Sorangium cellulosum* strain So0157-2

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Epothilones are a new class of 16-membered macrolides with taxol-like effects produced by myxobacterium *Sorangium cellulosum*.[1,2] In contrast to taxol, they are active against taxol-resistant tumors and are water soluble. Following the isolation and characterization of the major metabolites, epothilones A and B from the strain *S. cellulosum* So ce90, some natural and synthetic epothilone variants have been reported,[3–7] and a few of them have undergone preclinical or clinical evaluations.[8] As a result, ixabepilone, a lactam analog of epothilone B, has been approved for clinical use for treatment of certain forms of breast cancer.[9] In our previous investigation, five new epothilone glycosides and two new epothilone congeners have been isolated from the epothilones A and B producing strain *S. cellulosum* strain So0157-2.[10,11] As part of our ongoing research on the metabolites produced by the *S. cellulosum* strain So0157-2, a new 16-membered macrolide, designed as epothilone O (**1**), was obtained from the 120 l fermentation broth of *S. cellulosum* strain So0157-2. Herein, we describe the isolation, structure elucidation and biological activity of the new compound.

The producing organism *S. cellulosum* strain So0157-2 was provided by Professor Yuezhong Li at the Shandong University, China, and was deposited in the China Center of Typical Culture Collection (CCTCC) with accession No.CCTCC M 208078. The culture and fermentation of *S. cellulosum* strain So0157-2 were conducted according to the procedure as described in the literature.[10] The adsorber resin XAD-1600 (Rohm and Haas, Philadelphia, PA, USA; 2.4 l) was separated from the 120 l fermentation broth with a process filter. After washing the resin with water, the resin was eluted with four bed volumes of 95% ethanol. The ethanol eluent was diluted to about 30% ethanol and charged to a XAD-1600 resin column eluting with 30, 40, 50, 60 and 70% ethanol (two bed volumes each). The fractions eluting with 60 and 70% ethanol were pooled and evaporated at 50 °C to give a mixture, which was dissolved in $CHCl_3$ and chromatographed on silica gel (100–200 mesh) eluting with petroleum ether/acetone solvent gradient from 70:30 to 40:60. The polar fraction eluting with petroleum ether/acetone (60:40) was further chromatographed on a Sephadex LH-20 (GE Healthcare, Glies, UK) column and eluted with ethanol and detected by TLC developed with petroleum ether/acetone (3:2) and visualized under UV light at 254 nm (Rf = 0.46) to give a subfraction. The subfraction was further isolated by semipreparative HPLC (Agilent 1100 instrument, Agilent, Palo Alto, CA, USA; C18 column: Zorbax SB-C18, Agilent, 9.4 × 250 mm, 5 μm) using CH_3CN/H_2O (40:60) with a flow rate of 1.5 ml min^{-1} at a room temperature to give compound **1** (t_R 37.80 min, 3.5 mg).

Compound **1** was isolated as colorless oil. Its molecular formula was determined to be $C_{27}H_{41}NO_6S$ by the HRESIMS *m/z* 506.2572 [M-H]$^-$, (calcd for $C_{27}H_{40}NO_6S$, 506.2582), indicating 8 degrees of unsaturation. Compound **1** is thus an isomer of epothilone B. The UV spectrum of **1** showed absorption maxima at 248 nm. The IR spectrum indicated the presence of hydroxyl (3436 cm^{-1}) and carbonyl (1636 cm^{-1}) groups. In the ^{1}H NMR spectrum (Table 1), signals for two aliphatic doublet methyls (δ_H 0.97 (d, J = 6.7 Hz), 1.14 (d, J = 7.1 Hz)), two aliphatic singlet methyls (δ_H 1.13 (s), 1.26 (s)), three olefinic methyls (δ_H 1.65 (s), 2.10 (d, J = 1.0 Hz), 2.70 (s)), four oxygenated methine protons (δ_H 3.97 (m), 4.08 (m), 4.10 (m), 5.16 (dd, J = 8.4, 4.0 Hz)) and three olefinic protons (δ_H 5.55 (t, J = 6.5 Hz), 6.53 (s), 6.97 (s)) were easily identified. The ^{13}C NMR and DEPT spectra (Table 1) displayed 27 carbon resonances, which corresponded to seven methyls (δ_C 12.3, 13.0, 15.1, 16.0, 19.1, 21.0, 21.3), four methylenes (δ_C 23.3, 29.1, 39.0, 39.2), nine methines (four oxygenated and three olefinic) and seven quaternary carbons (one ketone, one ester carbonyl, four olefinic). The above ^{1}H and ^{13}C NMR data indicated that compound **1** possessed a structural skeleton similar to epothilone D.[3] Detailed comparisons of the NMR data of **1** with those of epothilone D revealed that the C-11-C-13 moieties of these two compounds were different. In the ^{1}H–^{1}H COSY spectrum, the correlations of H-14 (δ_H 2.03, 2.06) with H-15 (δ_H 5.16) and H-13 (δ_H 4.10), and the correlations of H-10 (δ_H 2.10, 2.24) with H-11 (δ_H 5.55) and H-9 (δ_H 1.47, 1.78) established the subunits of C-13-C-15 and C-9-C-11, respectively. The observed HMBC correlations from δ_H 1.65 (H-26) to C-11 (δ_C 128.0), C-12 (δ_C 136.5) and C-13 (δ_C 76.0) established the connectivity of the two

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Table 1 ^{1}H and ^{13}C NMR data for compound 1 (in $CDCl_3$)

No.	δ_H *(mult, J in Hz)*	δ_C	*No.*	δ_H *(mult, J in Hz)*	δ_C
1		171.0 s	14	2.06 (m)	39.2 t
2	2.52 (dd, 14.2, 8.9)	39.0 t		2.03 (m)	
	2.42 (dd, 14.2, 2.8)		15	5.16 (dd, 8.4, 4.0)	78.2 d
3	4.08 (m)	73.8 d	16		137.9 s
4		52.4 s	17	6.53 (s)	120.0 d
5		222.0 s	18		152.3 s
6	3.18 (m)	43.2 d	19	6.97 (s)	116.2 d
7	3.79 (m)	74.6 d	20		164.8 s
8	1.76 (m)	34.3 d	21	2.70 (s)	19.1 q
9	1.78 (m)	29.1 t	22	1.26 (s)	21.0 q
	1.47 (m)		23	1.13 (s)	21.3 q
10	2.24 (m)	23.3 t	24	1.14 (d, 7.1)	13.0 q
	2.10 (m)		25	0.97 (d, 6.7)	16.0 q
11	5.55 (t, 6.5)	128.0 d	26	1.65 (s)	12.3 q
12		136.5 s	27	2.10 (d, 1.0)	15.1 q
13	4.10 (m)	76.0 d			

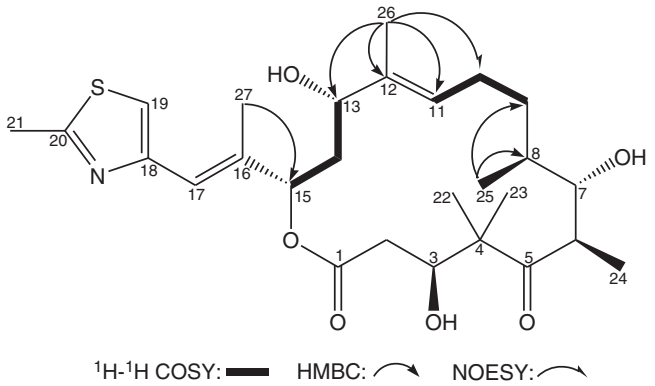

Figure 1 The structure and key ^{1}H–^{1}H COSY, HMBC and NOESY correlations of compound **1**.

subunits. Consequently, the planar structure of **1** was assigned as shown in Figure 1. The NOESY correlation between H-26 and H-10 indicated the geometry of Δ^{11} olefin was *E*. Except the configuration of C-13, the other chiral centers in **1** were assigned in analogy with epothilones A and B. The orientation of the 13-OH was assigned as α based on the correlation of H-13 and H-15 in the NOESY experiment.

Epothilone O (**1**) was evaluated for cytotoxic activity against human lung carcinoma A549 using the CCK-8 colorimetric method as described previously.[10] Bioassay result showed that compound **1** was cytotoxic with an IC_{50} of 7.9 $\mu g\,ml^{-1}$.

Epothilone O (**1**): Colorless oil; $[\alpha]_D^{25}$-6.25 (*c* 0.016, EtOH); UV (EtOH) λ_{max} 248 (log ε 3.74) nm; IR (KBr): ν_{max} 3436, 2925, 1636, 1460, 1374, 1267, 1050 cm^{-1}; ^{1}H and ^{13}C NMR spectral data see Table 1; ESIMS: *m/z* 506 $[M\text{-}H]^-$; HRESIMS: *m/z* 506.2572 $[M\text{-}H]^-$ (calcd for $C_{27}H_{40}NO_6S$, 506.2582).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Gerth, K., Bedorf, N., Höfle, G., Irschik, H. & Reichenbach, H. Epothilons A and B: antifungal and cytotoxic compounds from *Sorangium cellulosum* (myxobacteria). Production, physico-chemical and biological properties. *J. Antibiot.* **49,** 560–563 (1996).
2 Bollag, D. M. *et al.* Epothilones, a new class of microtubule stabilizing agents with a taxol-like mechanism of action. *Cancer Res.* **55,** 2325–2333 (1995).
3 Hardt, I. H. *et al.* New natural epothilones from *Sorangium cellulosum*, strains So ce90/B2 and So ce90/D13: isolation, structure elucidation, and SAR studies. *J. Nat. Prod.* **64,** 847–856 (2001).
4 Arslanian, R. L. *et al.* A new cytotoxic epothilone from modified polyketide synthases heterologously expressed in *Myxococcus xanthus*. *J. Nat. Prod.* **65,** 1061–1064 (2002).
5 Starks, C. M., Zhou, Y., Liu, F. & Licari, P. J. Isolation and characterization of new epothilone analogues from recombinant *Myxococcus xanthus* fermentations. *J. Nat. Prod.* **66,** 1313–1317 (2003).
6 Nicolaou, K. C., Roschangar, F. & Vourloumis, D. Chemical biology of epothilones. *Angew. Chem. Int. Ed. Engl.* **37,** 2014–2045 (1998).
7 Dong, S. D. *et al.* Rapid access to epothilone analogs via semisynthetic degradation and reconstruction of epothilone D. *Tetrahedron Lett.* **45,** 1945–1947 (2004).
8 Lee, J. J. & Swain, S. M. The epothilones: translating from the laboratory to the clinic. *Clin. Cancer Res.* **14,** 1618–1624 (2008).
9 Lechleider, R. J. *et al.* Ixabepilone in combination with capecitabine and as monotherapy for treatment of advanced breast cancer refractory to previous chemotherapies. *Clin. Cancer Res.* **14,** 4378–4384 (2008).
10 Wang, J. D. *et al.* Five new epothilone metabolites from *Sorangium cellulosum* strain So0157-2. *J. Antibiot.* **62,** 483–487 (2009).
11 Wang, J. D. *et al.* New epothilone congeners from *Sorangium cellulosum* strain So0157-2. *Nat. Prod. Res.* **25,** 1707–1712 (2011).