NOTE

Two 18-membered epothilones from *Sorangium cellulosum* So0157-2

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Epothilones are 16-membered ring macrolides originally isolated from the bacterium *Sorangium cellulosum* Soce90,^{1,2} which were found to kill dividing cells by stabilizing microtubules.³ Previously, epothilone-producing strains of the genus *Sorangium* were isolated and screened in our laboratories. We found that one strain, designated *S. cellulosum* So0157-2, was able to produce epothilones.⁴ The production of epothilones A and B by *S. cellulosum* So0157-2 was optimized to industrial scales.⁵ Recently, five new epothilone glycoside derivatives were isolated from this strain.⁶ As a part of our ongoing search for new epothilones from *S. cellulosum* So0157-2, we focused on the minor and polar components of this strain produced in large-scale fermentations.

The strain *S. cellulosum* So0157-2 was cultivated in 701 of M26 medium.⁷ Epothilones A₉ (**3**) (3.0 mg), A (**4**) (90 mg), B (250 mg) and C (20 mg) were isolated from the 701 fermentation extract by repeated column chromatography (Sephadex LH-20, RP-18 and silica gel, Sephadex, Uppsala, Sweden). The polar epothilone-containing fractions were separated by HPLC (Agilent 1200 instrument, Agilent, Palo Alto, CA, USA; C₁₈ column: Zorbax SB-C18, Agilent, 4.6×250 mm, 5 μm), eluting with 65% methanol at a flow rate of 1.0 ml min⁻¹ to yield compound **1** (t_R 7.8 min, 2.0 mg) and **2** (t_R 10.9 min, 0.8 mg). Their structures were established as epothilone-type 18-membered ring macrolides, named epothilones M (**1**) and N (**2**). In this study, we describe structure elucidation and cytotoxic activity of these two new epothilones.

Compound **1** was isolated as a white powder. The molecular formula was determined to be C₂₆H₃₉NO₇S by HR quadrupole time-of-flight MS (*m/z* 510.2528 [M+H]⁺, 532.2344 [M+Na]⁺; calcd. 509.2447) and NMR data (Table 1). The ¹³C NMR spectrum of **1** displayed 26 carbon signals for five methyl, six methylene, nine methine and six quaternary carbon atoms, including a carbonyl

carbon at δ 171.9 (C-1), together with the ¹H NMR spectrum clearly revealed a typical epothilone structure, including the presence of a thiazole ring, a carbon–carbon double bond, an epoxy function and a carboxyl carbonyl group (Table 1). However, compared with epothilone A (**4**),^{1,2} **1** had one less methyl and one more oxymethylene group as a substituent at the carbon–carbon double bond. Among the various epothilone derivatives, the only known oxymethylene-substituted compounds are represented by epothilones A₉ and C₉.⁸ Indeed, the structure of epothilone A₉ could explain the ¹³C NMR spectrum of **1**. However, the ¹H NMR chemical shifts of the oxymethylene protons (H-17) were observed at δ 5.07 and 5.45 in **1**, but at δ 4.11 and 4.49 in epothilone A₉,⁸ respectively. This difference could be the effect of esterification, indicating that the hydroxymethyl hydroxyl group (17-OH) of epothilone A₉ could be esterified in **1**. Moreover, in our work, epothilone A₉ (**3**) was isolated too. The unambiguous NMR data assignments for **3** provided further support for this esterification (Table 1). The heteronuclear multiple-bond correlations from the oxymethylene protons (H-17) at δ 5.07 and 5.45 to the carbonyl group at δ 171.9 (C-1) indicated the presence of an ester bond between this oxymethylene and the carboxyl group. Therefore, compound **1** was determined to be an 18-membered epothilone-type macrolide, designated as epothilone M.

The relative structure of **1** was assumed to be the same as other epothilones. However, the analysis of NOESY correlations revealed that **1** must have two different conformations (**1a** and **1b**, Figure 1) in CDCl₃ at room temperature, because otherwise the vinyl proton (δ 6.69, H-18) could not simultaneously have NOE correlations with H₂-14 and H-15. Moreover, the observation of an NOE correlation between H-3 and H-6 in **1** was intriguing, which was observed in **3** and **4** as well. This indicates a twist of the epothilone 16-membered macrolide ring (**3**, **4a** and **4b**, Figure 1), which is consistent with that

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Received 28 January 2010; revised 20 April 2010; accepted 21 April 2010; published online 7 July 2010

Table 1 The NMR data for compounds **1**, **2** and **3** (in CDCl₃, δ in p.p.m., *J* in Hz)^a

No.	1		2		3	
	¹³ C	¹ H ^b	¹³ C	¹ H ^b	¹³ C	¹ H
1	171.9s		172.7s		170.1s	
2	38.1t	2.43 (s), 2.44 (d, 5.2), AA'	38.2t	2.39 (s), 2.41 (d, 4.6), AA'	40.7t	2.13 (dd, 2.9, 14.1), 2.51 (11.6, 14.1)
3	74.0d	4.20 (t, 4.2)	74.9d	4.25 (m)	70.2d	4.30 (dd, 2.8, 11.6)
4	51.7s		51.3s		54.2s	
5	221.4s		221.2s		220.5s	
6	44.0d	3.21 (dq, 3.0, 6.8)	43.9d	3.18 (dq, 3.0, 7.0)	41.2d	3.19 (dq, 3.1, 6.8)
7	75.9d	3.72 (dd, 3.7, 6.2)	75.5d	3.77 (dd, 3.5, 6.8)	73.1d	3.73 (dd, 3.5, 6.8)
8	35.6d	1.67 (m) ^c	34.8d	1.63 (m) ^c	37.5d	1.81 (m)
9	29.4t	1.31 (m) ^c , 1.38 (m) ^c	27.5t	1.30 (m) ^c	31.9t	1.35 (m) ^c , 1.55 (m) ^c
10	23.1t	1.27 (m) ^c , 1.51 (m)	22.2t	1.34 (m) ^c , 1.57 (m) ^c	23.2t	1.37 (m) ^c , 1.54 (m) ^c
11	28.3t	1.42 (m) ^c , 1.71 (m) ^c	33.9t	1.71 (m) ^c , 1.93 (m)	27.9t	1.36 (m) ^c , 1.91 (m) ^c
12	57.6d	2.99 (dt, 4.7, 7.7)	69.8d	3.99 (m) ^c	57.7d	2.95 (dt, 3.4, 9.5) ^c
13	55.2d	3.22 (ddd, 4.6, 6.7)	68.8d	4.01 (m) ^c	55.2d	3.07 (dt, 3.4, 9.8) ^c
14	34.4t	1.80 (br dd, 6.9, 13.9), 1.96 (ddd, 4.9, 10.1, 14.7)	40.4t	1.85 (ddd, 2.7, 6.9, 13.5), 2.12 (dd, 9.1, 13.7)	31.2t	1.86 (m) ^c , 2.06 (br d, 14.8)
15	74.2d	4.53 (d, 10.0)	73.7d	4.63 (br d, 7.0)	76.5d	5.71 (d, 1.7, 11.7)
16	138.1s		138.0s		138.9s	
17	61.1t	5.07 (d, 12.5), 5.45 (d, 12.5)	61.9t	5.09 (d, 12.8), 5.64 (d, 12.8)	57.2t	4.13 (d, 12.9), 4.51 (d, 12.9)
18	124.7d	6.69 (s)	123.6d	6.66 (s)	125.1d	6.62 (s)
19	151.0s		151.6s		150.6s	
20	118.8d	7.07 (s)	118.7d	7.09 (s)	119.4d	7.12 (s)
21	166.0s		166.1s		167.5s	
22	19.3q	2.70 (s, 3H)	19.3q	2.71 (s, 3H)	19.2q	2.74 (s, 3H)
23	18.9q	1.34 (s, 3H)	18.9q	1.33 (s, 3H)	15.8q	1.01 (s, 3H)
24	23.3q	1.07 (s, 3H)	24.2q	1.11 (s, 3H)	23.4q	1.38 (s, 3H)
25	14.8q	1.16 (d, 6.8, 3H)	15.0q	1.17 (d, 6.9, 3H)	13.5q	1.21 (d, 6.7, 3H)
26	16.6q	0.95 (d, 7.0, 3H)	16.3q	0.93 (d, 7.0, 3H)	16.5q	1.04 (d, 7.0, 3H)
3-OH		3.61 (br s)		3.71 (br s)		

^a1D- and 2D-NMR spectra were recorded in CDCl₃ on a Bruker DRX-600 instrument (Bruker, Fällanden, Switzerland).^bProton signal is integrated to 1H except for the otherwise indicated.^cSignals overlapped.

of epothilone A in binding to tubulin.^{9,10} However, in both **3** and **4**, the macrolide ring has only one preferred conformation. But NOESY experiment indicated the rotation of the side chain at C-17 in **4** but not in **3** (Figure 1).¹

Compound **2** was obtained as a white powder. The molecular formula C₂₆H₄₀ClNO₇S determined by the HR quadrupole time-of-flight MS data (*m/z* 546.2295 [M+H]⁺, 568.2065 [M+Na]⁺; calcd. 545.2214), together with the NMR data (Table 1), indicated that **2** had nearly identical structural features as those found in **1**, except that the C-12/C-13 epoxy function in **1** was opened in **2**. The HR MS data showed isotopic quasi-molecular peak triplets at *m/z* 546/547/548 and 568/569/570, with the ratio of [M]/[M+2] being equal to 3:1 (Supplementary Information), indicating that opening of the epoxy ring in **2** is due to chlorine-substitution. This conclusion was supported by the ¹³C NMR downfield shifts from δ 55.2 (C-12) and 57.7 (C-13) in **1** to δ 69.8 (C-12) and 68.8 (C-13) in **2** (Table 1), and NMR data comparison with *trans*-12,13-hydroxylated epothilone A and *cis*-12,13-hydroxylated epothilone A.¹¹ Unambiguous NMR assignments were made on the basis of heteronuclear multiple-bond correlation, heteronuclear single-quantum coherence and ¹H-¹H COSY experiments. Thus, the structure of **2** was established to be 12,13-depoxy-12-chloro-13-hydroxyl epothilone M, designated as epothilone N. The relative configurations of C-12 and C-13 in **2** were determined by NOESY experiment. The NOEs between H-15 and

H-14 α (δ 1.85), and H-14 β (δ 2.12) and H-13 (δ 4.01) indicated the α -orientation of 13-OH (Figure 1). The relative configuration of 12-Cl was assumed to be β , because the opening of the C-12/C-13 epoxy ring by chlorination would presumably proceed by an S_N2 mechanism.

The cytotoxic activity of epothilones M (**1**), N (**2**) and A₉ (**3**) against the MDA-MB-435 cell line was assayed by the MTT method.¹² Compounds **1** and **3** showed similar activities with IC₅₀ of 0.12 and 0.26 μ M, respectively, which is consistent with the values reported for epothilone A₉ against the mouse fibroblast cell line L929 (IC₅₀ 0.20 μ M).⁸ In contrast, compound **2**, an epoxide-opened derivative, showed only modest activity with an IC₅₀ of 2.47 μ M, which is similar to the C-12/C-13 diol derivatives of epothilones A and B.^{11,13}

Epothilones A and B were first isolated from *S. cellulorum* Soce90 in 1993.^{1,2} 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.^{8,14,15} The structural variations of natural epothilone-type macrolides are mainly due to the presence of a C-12/C-13 epoxide ring or a corresponding carbon-carbon double bond, and the different methyl substitutions and dehydrogenations in the macrolide ring. Variations of the thiazole side chain at C-15 observed were hydroxylation at the vinyl methyl group in epothilones A₉ and C₉, and at the thiazole methyl group in epothilones E and F.⁸ One ethyl thiazole derivative,

Figure 1 Structures of compounds **1**, **2**, **3** and **4**, and the preferred conformations determined by the NOEs as indicated (H ↔ H).

epothilone B₁₀, was isolated as well.⁸ 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 I₁–I₆, which are produced by the mutant strain *S. cellulosum* So ce90/B2.⁸ Those epothilones presumably arise from an extra cycle of chain elongation after the introduction of C-9/C-10.

Epothilones M and N, isolated from a wild-type strain *S. cellulosum* So0157-2 in this work, are 18-membered epothilones having the same polyketide chain as the 16-membered analogs. In light of the significant cytotoxic activities of epothilones M and N *in vitro*, it is worth attempting to produce more 18-membered ring derivatives by bioengineering of *S. cellulosum* So0157-2. Although both of the two novel 18-membered epothilones are 10–100 times less potent than epothilones A and B, it would still be worth testing their *in vivo* antitumor activity, because some antitumor natural products have been reported to have low activity in *in vitro* test systems, but a dramatic *in vivo* antitumor activity.¹⁶ Overall, our work points to a new direction for generating novel epothilones for antitumor drug discovery.

Epothilone M (1). white powder; $[\alpha]_{\text{D}}^{20} +104.27$ (c 0.12, methanol). IR (KBr): ν_{max} 3384, 2963, 2931, 1726, 1691 cm^{-1} ; HR quadrupole time-of-flight MS m/z : 510.2528 $[\text{M}+\text{H}]^+$, 532.2344 $[\text{M}+\text{Na}]^+$; ^1H and ^{13}C NMR data, see Table 1.

Epothilone N (2). white powder; $[\alpha]_{\text{D}}^{20} +31.0$ (c 0.1, methanol); IR (KBr): ν_{max} 3440, 3471, 2958, 2924, 1725, 1722 cm^{-1} ; HR quadrupole time-of-flight MS m/z : 546.2295 $[\text{M}+\text{H}]^+$, 568.2065 $[\text{M}+\text{Na}]^+$; ^1H and ^{13}C NMR data, see Table 1.

ACKNOWLEDGEMENTS

This study was partially supported by the National Natural Science Fund for Distinguished Young Scholars (30325044 to YS, 30825001 to YL), Key Program of International Cooperation, Ministry of Science and Technology of China (2007DFA30970) and the 863 Program of China (20060102Z1083).

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (<http://www.nature.com/ja>)