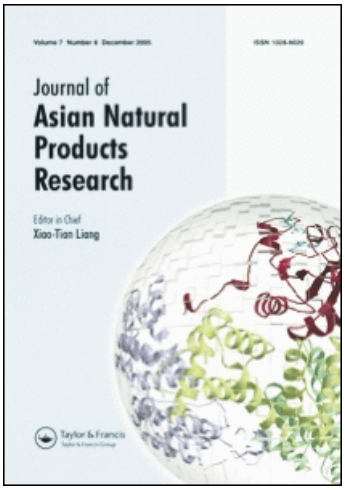


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Microbial transformation of epothilone A by *Aspergillus niger* AS 3.739

Yan-Liang Wang[a]; He Wang[a]; Yu-Xin Lu[a]; Xiao-Chen Cheng[a]; Li-Li Han[a]; Shou-Jun Yuan[a]; De-Xuan Yang[a]; Qing-Lin Zhang[a]; Chu-Tse Wu[a]
[a] Beijing Institute of Radiation Medicine, Beijing, China

To cite this Article Wang, Yan-Liang , Wang, He , Lu, Yu-Xin , Cheng, Xiao-Chen , Han, Li-Li , Yuan, Shou-Jun , Yang, De-Xuan , Zhang, Qing-Lin and Wu, Chu-Tse(2009) 'Microbial transformation of epothilone A by *Aspergillus niger* AS 3.739', Journal of Asian Natural Products Research, 11: 4, 357 — 364
To link to this Article: DOI: 10.1080/10286020902767732
URL: http://dx.doi.org/10.1080/10286020902767732

Journal of Asian Natural Products Research
Vol. 11, No. 4, April 2009, 357–364

Microbial transformation of epothilone A by *Aspergillus niger* AS 3.739

Yan-Liang Wang, He Wang, Yu-Xin Lu, Xiao-Chen Cheng, Li-Li Han, Shou-Jun Yuan, De-Xuan Yang, Qing-Lin Zhang* and Chu-Tse Wu

Beijing Institute of Radiation Medicine, Beijing 100850, China

(*Received 27 October 2008; final version received 26 December 2008*)

The microbiological transformation of epothilone A (**1**) by *Aspergillus niger* AS 3.739 afforded four main metabolites. Their structures were elucidated by ^{1}H, ^{13}C NMR and HSQC, COSY, HMBC, and NOESY spectra as *trans*-12,13-hydroxylated epothilone A (**2**), *cis*-12,13-hydroxylated epothilone A (**3**), *trans*-12,15-epoxidated epothilone A (**4**), and *cis*-12,15-epoxidated epothilone A (**5**). All four compounds were firstly found based on their stereochemistry. These new compounds displayed cytotoxicity against human breast carcinoma cells MCF-7 with IC_{50} 9.88 μg/ml of **2**, 2.52 μg/ml of **3**, 9.88 μg/ml of **4**, and 5.68 μg/ml of **5**.

Keywords: epothilone A; microbial transformation; *Aspergillus niger*; cytotoxicity

1. Introduction

Epothilones are cytotoxic compounds naturally produced by myxobacterium *Sorangium cellulosum* [1] and share a common tubulin-binding site with the cancer drug paclitaxel [2]. In particular, epothilones display dramatic potency against multi-drug-resistant tumor cell lines that are highly resistant to taxol and other anticancer drugs [3,4]. Several epothilones, such as epothilone B, epothilone D, and BMS-247550 [5], are undergoing three clinical trials. Moreover, ixabepilone, one of the epothilone analogs, had been approved by FDA for treatment of colon carcinoma, breast cancer, cancer of kidney, and ovarian cancer in 2007.

Epothilones A and B (Figure 1) were produced as major compounds in *S. cellulosum* fermentation broth. Epothilone A is a main byproduct for the production of epothilone B with A and B yield ratio up to 1:2 or 1:1. By now, a few researches on epothilone A have been published.

Microbial transformation is defined as a reaction catalyzed by microorganisms or enzymes. It has such advantages over chemical synthesis as high stereo- and regio-selectivity as well as mild reaction conditions, simple operation procedure, less cost, and lower pollution. Some reactions that cannot be fulfilled in chemical approach are facile process by microbial transformation [6]. In this work, we used the microorganism *Aspergillus niger* (selected from 11 strains of microorganisms) to biotransform epothilone A and obtained four new compounds with cytotoxicity against human breast carcinoma cells MCF-7. Their structures and configurations were further elucidated by 1D, 2D NMR, and FAB-MS spectra.

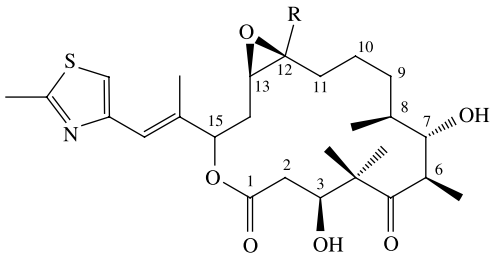
2. Results and discussion

The incubation of epothilone A (**1**) with *A. niger* for 7 days afforded four products **2**–**5**.

*Corresponding author. Email: qinglz@yahoo.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020902767732
http://www.informaworld.com



epothilone A R = H
epothilone B R = CH_3

Figure 1. The structures of epothilone A and epothilone B.

The more polar compound **2** was obtained as white crystalline powder. Its FAB-MS (*m/z*: 512.2 $[M+H]^+$) is in accordance with the formula $C_{26}H_{41}NO_7S$ with 18 amu more than epothilone A (**1**). The H-12 and H-13 signals of epothilone A at δ 2.92 and 3.09 [7] are substituted by the proton signals at δ_H 3.55 and 3.73 in the ^{1}H NMR spectrum of **2**, indicating the epoxy ring between C-12 and C-13 was cleaved and formed two —OH groups. These locations were also confirmed by ^{13}C NMR signals at δ 73.8 and 63.8 assigned with C and H-HSQC (Table 1). So compound **2** was identified to be 12,13-hydroxylated epothilone A. The relative stereochemistry of **2** on C-3, C-6, C-8, C-13, and C-15 were assigned on the basis of NOE correlations between H-3 and H-6, H-3 and H-8, H-3 and H-13, H-13 and H-15. H-12 and H-13 were in opposite stereochemical orientation according to the coupling constants ($J_{12,13} = 7.8$ Hz) and the approximate $J_{12,13}$ value 4.0 Hz supported the presence of a *cis*-substituted configuration [8].

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **2** and **3**.

	2		**3**	
No.	δ_H	δ_C	δ_H	δ_C
1	–	172.2 (C)	–	172.1 (C)
2	2.60 (2H, m)	41.0 (CH_2)	2.50 (1H, m), 2.65 (1H, m)	40.2 (CH_2)
3	4.50 (1H, dd, J = 3.0, 7.8 Hz)	72.3 (CH)	4.29 (1H, dd, J = 3.0, 7.8 Hz)	72.8 (CH)
4	–	54.9 (C)	–	54.4 (C)
5	–	220.5 (C)	–	220.9 (C)
6	3.35 (1H, m)	45.3 (CH)	3.33 (1H, m)	46.0 (CH)
7	3.65 (1H, dd, J = 3.0, 7.8 Hz)	77.7 (CH)	3.70 (1H, dd, J = 3.0, 7.8 Hz)	77.3 (CH)
8	1.34 (1H, m)	37.7 (CH)	1.48 (1H, m)	38.2 (CH)
9	1.21 (1H, m), 1.45 (1H, m)	30.1 (CH_2)	1.25 (1H, m), 1.45 (1H, m)	31.7 (CH_2)
10	1.21 (1H, m), 1.55 (1H, m)	22.4 (CH_2)	1.25 (1H, m), 1.64 (1H, m)	24.9 (CH_2)
11	1.55 (1H, m), 1.78 (1H, m)	34.0 (CH_2)	1.45 (1H, m), 1.64 (1H, m)	34.5 (CH_2)
12	3.55 (1H, dd, J = 7.2, 7.8 Hz)	73.8 (CH)	3.47 (1H, dd, J = 3.6, 4.2 Hz)	74.5 (CH)
13	3.73 (1H, dd, J = 7.2, 7.8 Hz)	68.7 (CH)	3.61 (1H, dd, J = 3.6, 4.2 Hz)	72.4 (CH)
14	1.91 (2H, m)	40.5 (CH_2)	1.91 (1H, m), 2.13 (1H, m)	37.5 (CH_2)
15	5.53 (1H, dd, J = 3.0, 9.0 Hz)	78.2 (CH)	5.53 (1H, dd, J = 3.0, 9.0 Hz)	76.7 (CH)
16	–	140.4 (C)	–	140.0 (C)
17	6.56 (1H, s)	120.0 (CH)	6.56 (1H, s)	119.1 (CH)
18	–	153.4 (C)	–	153.5 (C)
19	7.21 (1H, s)	117.5 (CH)	7.17 (1H, s)	117.3 (CH)
20	–	167.0 (C)	–	166.9 (C)
21	2.68 (3H, s)	18.7 (CH_3)	2.65 (3H, s)	18.7 (CH_3)
22	0.99 (3H, s)	18.1 (CH_3)	1.04 (3H, s)	21.7 (CH_3)
23	1.24 (3H, s)	19.3 (CH_3)	1.31 (3H, s)	22.1 (CH_3)
24	1.16 (3H, d, J = 6.6 Hz)	15.7 (CH_3)	1.16 (3H, d, J = 6.6 Hz)	16.2 (CH_3)
25	0.97 (3H, d, J = 7.2 Hz)	21.9 (CH_3)	0.97 (3H, d, J = 6.6 Hz)	17.6 (CH_3)
27	2.05 (3H, s)	15.5 (CH_3)	2.04 (3H, s)	15.9 (CH_3)

^{1}H NMR: 600 MHz in CD_3OD (δ, J value in Hz); ^{13}C NMR: 150 MHz in CD_3OD (δ); s, singlet; d, doublet; t, triplet; m, multiplet.

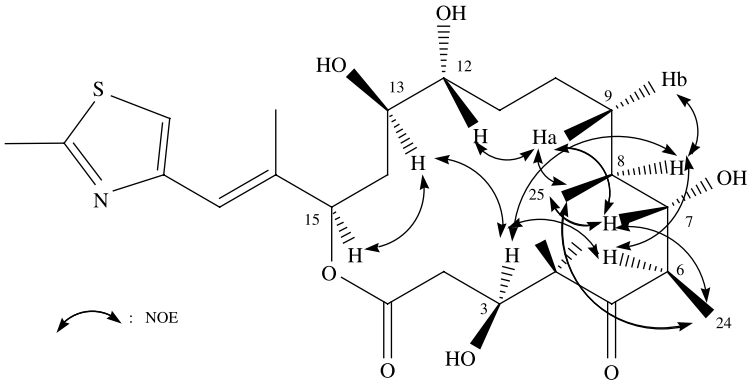


Figure 2. Key NOE correlations of **2**.

Furthermore, NOEs between H-25 (δ 0.97) and H-9a (δ 1.45), H-25 and H-24, H-7 (δ 3.65) and H-9a (δ 1.45), H-9a (δ 1.45) and H-12 (δ 3.55) indicated that H-7, H-12, H-24, and H-25 were in the outward direction of 16-ring plane as shown in Figure 2. Thus, compound **2** was identified to be *trans*-12,13-hydroxylated epothilone A.

Compound **3** was obtained as colorless solid powder. Its MS (*m/z*: 512.2 $[M + H]^+$) was the same as compound **2**. The ^1H and ^{13}C NMR spectral data were almost identical to those of compound **2**, except for the high-field shifts of H-12 (δ 3.48) and H-13 (δ 3.61), compared with H-12 at δ 3.55 and H-13 at δ 3.73 of compound **2** in the ^1H NMR spectrum, and the downfield shift of C-13 at δ 72.4 in the ^{13}C NMR spectrum, compared with C-13 at δ 68.7 of compound **2**. These shifts indicated that compound **3** was a stereoisomer of compound **2**. The configuration of compound **3** was also determined by NOESY spectrum. The NOE correlations between H-3 and H-6, H-6 and H-8, H-3 and H-12, H-12 and H-15, H-12 and H-13, and H-13 and H-15 indicated that H-3, H-6, H-8, H-12, H-13, and H-15 were in the inside of the molecular plane (Figure 3). H-12 and H-13 were in the same stereochemical orientation based on the coupling constant ($J_{12,13} =$ 4.2 Hz). So compound **3** was determined to be *cis*-12,13-hydroxylated epothilone A.

The most polar compound **4** was obtained as colorless powder. Its MS (*m/z*: 494 $[M+ H]^+$) was in accordance with the formula $C_{26}H_{39}NO_6S$, which was the same as epothilone A. However, the downfield shift at δ 4.23 and 5.27 indicated that there should

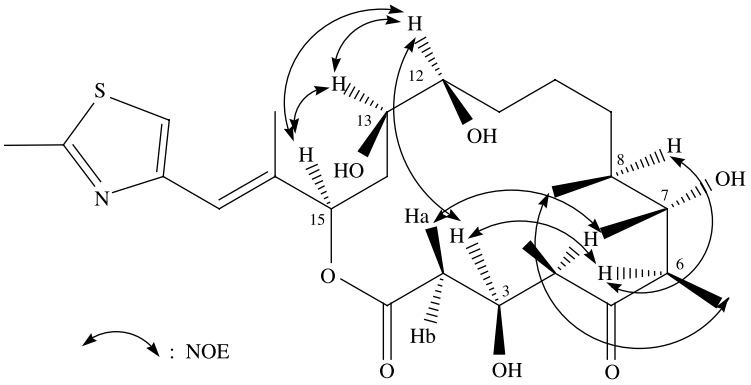


Figure 3. Key NOE correlations of **3**.

Table 2. ^{1}H and ^{13}C NMR spectral data of compounds **4** and **5**.

	4		**5**	
No.	δ_H	δ_C	δ_H	δ_C
1	–	172.1 (C)	–	174.0 (C)
2	2.43 (2H, m)	40.8 (CH_2)	2.37 (2H, m)	43.4 (CH_2)
3	4.39 (1H, dd, $J = 3.6$, 7.2 Hz)	70.2 (CH)	4.37 (1H, dd, $J = 3.6$, 7.2 Hz)	72.9 (CH)
4	–	55.2 (C)	–	57.7 (C)
5	–	220.1 (C)	–	222.6 (C)
6	3.35 (1H, m)	45.2 (CH)	3.35 (1H, m)	47.6 (CH)
7	3.64 (1H, dd, $J = 3.6$, 6.6 Hz)	75.7 (CH)	3.64 (1H, dd, $J = 3.6$, 6.6 Hz)	78.2 (CH)
8	1.36 (1H, m)	39.4 (CH)	1.34 (1H, m)	41.7 (CH)
9	1.36 (1H, m), 1.53 (1H, m)	32.3 (CH_2)	1.34 (1H, m), 1.55 (1H, m)	34.9 (CH_2)
10	1.53 (1H, m)	24.1 (CH_2)	1.05 (1H, m), 1.55 (1H, m)	26.6 (CH_2)
11	1.88 (2H, m)	33.2 (CH_2)	1.86 (2H, m)	35.2 (CH_2)
12	4.23 (1H, dd, $J = 3.6$, 4.2 Hz)	82.1 (CH)	3.90 (1H, dd, $J = 3.6$, 4.2 Hz)	84.0 (CH)
13	5.27 (1H, dd, $J = 3.6$, 4.2 Hz)	77.3 (CH)	5.22 (1H, dd, $J = 3.6$, 4.2 Hz)	79.5 (CH)
14	2.89 (1H, m), 2.21 (1H, m)	39.5 (CH_2)	2.68 (1H, m), 1.83 (1H, m)	42.4 (CH_2)
15	4.60 (1H, dd, $J = 6.6$, 9.6 Hz)	83.1 (CH)	4.28 (1H, dd, $J = 6.6$, 10.2 Hz)	85.5 (CH)
16	–	141.0 (C)	–	143.4 (C)
17	6.55 (1H, s)	119.3 (CH)	6.61 (1H, s)	121.9 (CH)
18	–	153.5 (C)	–	156.1 (C)
19	7.18 (1H, s)	117.1 (CH)	7.20 (1H, s)	119.0 (CH)
20	–	166.9 (C)	–	169.6 (C)
21	2.67 (3H, s)	18.7 (CH_3)	2.63 (3H, s)	22.0 (CH_3)
22	0.97 (3H, s)	18.0 (CH_3)	0.90 (3H, s)	20.6 (CH_3)
23	1.34 (3H, s)	22.6 (CH_3)	1.24 (3H, s)	25.2 (CH_3)
24	1.12 (3H, d, $J = 7.2$ Hz)	15.4 (CH_3)	1.07 (3H, d, $J = 7.2$ Hz)	17.8 (CH_3)
25	0.99 (3H, d, $J = 7.2$ Hz)	18.6 (CH_3)	0.93 (3H, d, $J = 7.2$ Hz)	21.2 (CH_3)
27	1.95 (3H, s)	17.4 (CH_3)	1.92 (3H, s)	17.4 (CH_3)

^{1}H NMR: 600 MHz in CD_3OD (δ, J value in Hz); ^{13}C NMR: 150 MHz in CD_3OD (δ); s, singlet; d, doublet; t, triplet; m, multiplet.

be epoxy ring cleavage between C-12 and C-13 compared with the ^{1}H NMR of epothilone A (**1**). This ring cleavage was also confirmed by the ^{13}C NMR spectral data (δ 82.1 and 77.3 assigned with C and H-HSQC, Table 2). C-13 was identified to be linked with an acyl group by HMBC spectrum, where there was correlation between H-13 and C-1 (Figure 4). There were also correlations between H-12 (δ 4.23) and C-15 (δ 83.1), H-15 (δ 4.60) and

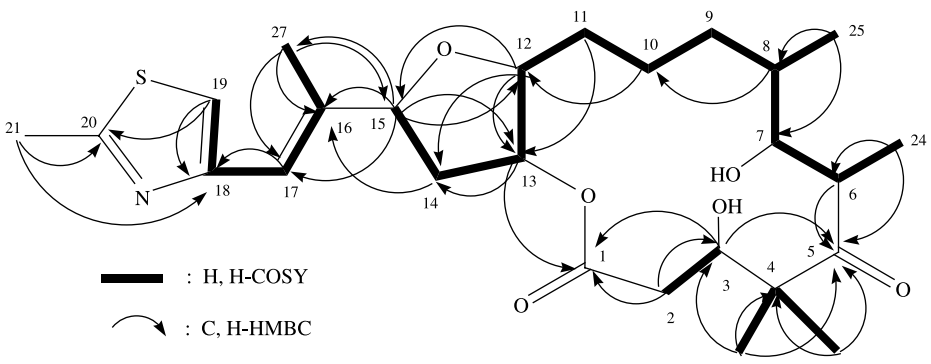


Figure 4. Key ^{1}H, ^{1}H-COSY, and ^{13}C, ^{1}H-HMBC correlations of **4**.

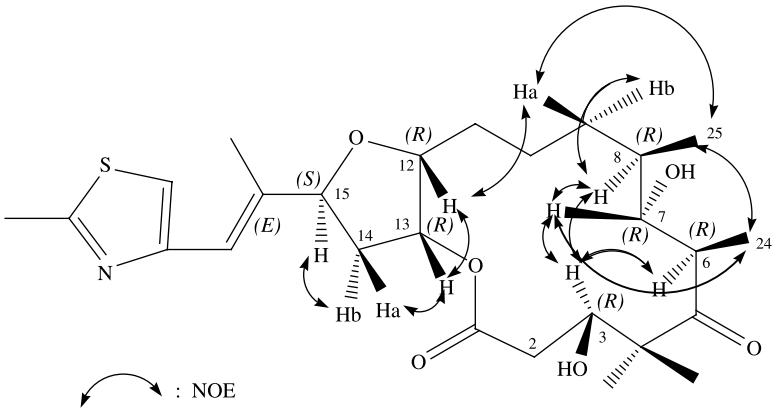


Figure 5. Key NOE correlations of **4**.

C-12 (δ 82.1), which indicated epoxidation between C_{12}—OH and C_{15}—OH. Compound **4** was identified to be 12,15-epoxidated epothilone A. The relative stereochemistry on C-3, C-6, and C-8 was assigned on the basis of NOE correlations between H-3 and H-6, H-3 and H-8. Furthermore, NOEs between H-25 (δ 0.99) and H-24 (δ 1.12), H-25 (δ 0.99) and H-9a (δ 1.53), H-9a (δ 1.53) and H-12 (δ 4.23), H-7 (δ 3.64) and H-24 (δ 1.12), and H-9b (δ 1.45) and H-12 (δ 3.55) indicated that H-7, H-12, H-24, and H-25 were in the outside of the 16-ring plane as shown in Figure 5. The configuration of compound **4** was determined to be *trans*-12,15-epoxidated epothilone A.

The less polar compound **5** was obtained as colorless powder. Its MS (*m*/*z*: 494 $[M + H]^+$) indicated the same molecular formula of $C_{26}H_{39}NO_6S$ as compound **4**. We concluded that compound **5** was a stereoisomer of **4** because all signals in the ^{1}H NMR spectrum were consistent with those of **4** except for H-14a, 14b, and H-15 (δ 2.68, 1.83, and 4.28). NOESY spectrum showed the NOE correlations between H-12 and H-15, H-13 and H-15, H-13 and H-10b, H-10b and H-7, H-7 and H-24, and H-24 and H-25, which indicated that H-7, H-12, H-13, H-15, H-24, and H-25 were in the outside of the 16-ring plane, and H-3, H-6, and H-8 were in the inward direction (Figure 6). So the

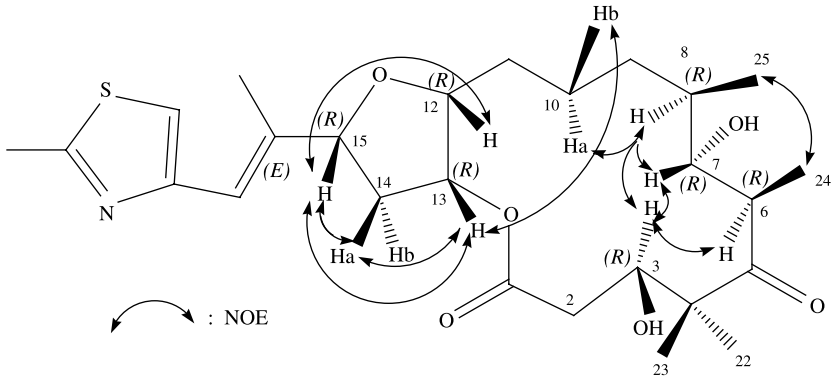


Figure 6. Key NOE correlations of **5**.

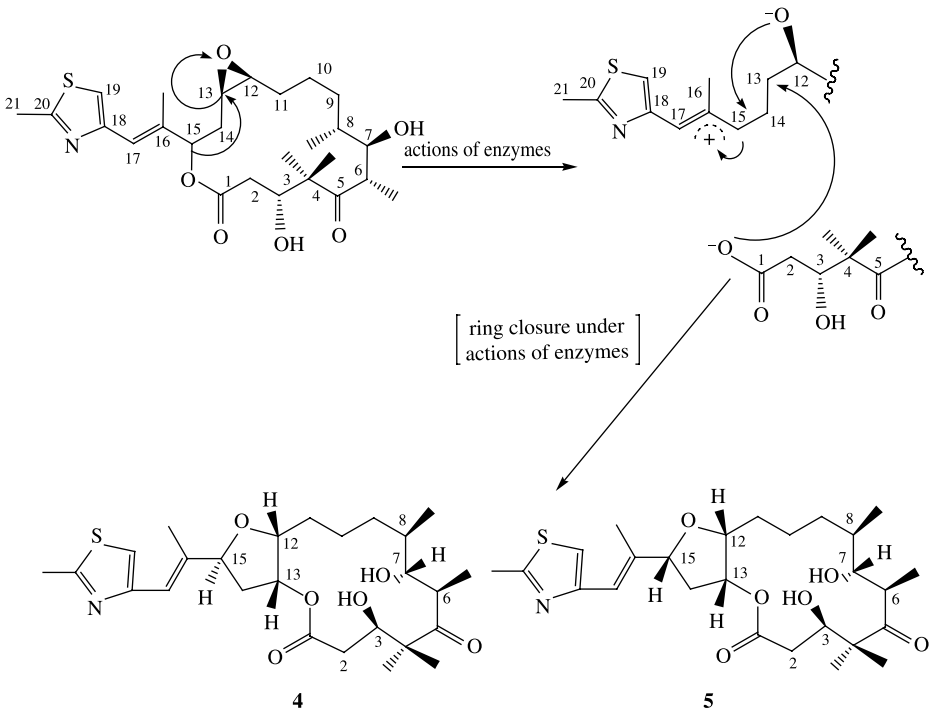


Figure 7. Main ring rearrangements of compounds **4** and **5**.

configuration of compound **5** was identified to be *cis*-12,15-epoxidated epothilone A.

In our study, we have first reported that biotransformation of epothilone A with microorganism, *A. niger* could afford the four compounds **2**, **3**, **4**, and **5** and ^{1}H, ^{13}C NMR spectral data of them were supplied.

We found that there were rearrangements at C-12, C-13, C-1, and C-15 of compounds **4** and **5**. It was considered that the bonds between the oxygen and C-12 and C-13 were weakened, respectively, under actions of some enzymes possibly, such as hydrolase and oxidase. The C13—O bond broke and a C-13 lactone was formed under inversion of the stereocenter as assisted by the carboxyl group. Meanwhile, a cation was generated and intramolecular ring was brought out to lead to compounds **4** and **5** as epimers at C-15 (Figure 7).

Cytotoxicity assays were carried out on human breast carcinoma cells MCF-7 to test the biotransformed compounds. Table 3 summarizes the *in vitro* activity (IC_{50}) of compounds. All biotransformed compounds

Table 3. Cytotoxicity of compounds **1**–**5** against human breast cancer MCF-7 cells.

Compound	Cytotoxicity (IC_{50}, μg/ml)
Epothilone A (**1**)	0.005
trans-12,13-Hydroxylated epothilone A (**2**)	9.88
cis-12,13-Hydroxylated epothilone A (**3**)	2.52
trans-12,15-Epoxidated epothilone A (**4**)	9.88
cis-12,15-Epoxidated epothilone A (**5**)	5.68

IC_{50} values in μg/ml represent the average of two or three independent 72 h growth inhibition assays. IC_{50} is defined as the drug concentration that reduces cell protein by 50%.

are less active than epothilone A; however, compounds **3** and **5** are more active than compounds **2** and **4**.

These results indicated that the epoxide ring cleavage between C-12 and C-13 reduced the biological activity. The *cis*-configuration compounds are more active than *trans*-configuration compounds.

3. Experimental

3.1 General experimental procedures

The 1D and 2D NMR spectra were recorded in CD_3OD on a Varian Inova 600 instrument at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR, respectively. FAB-MS spectra were obtained with a Micromass ZabSpec mass spectrometer. TLC analysis was carried out using silica gel GF254 (Qingdao Haiyang Chemical Group Co., Qingdao, China). HPLC was carried out on a Waters 600 model system and a 996-diode array detector.

3.2 Materials

Epothilone A (**1**) was isolated from the fermentation broth of UV mutant of myxobacterium *S. cellulosum* and its structure was determined by comparing its ^1H and ^{13}C NMR spectral data with those reported previously in the literature [7].

3.3 Microorganism

All microorganisms were purchased from China General Microbiological Culture Collection Center, Beijing.

3.4 Culture and biotransformation procedures

Biotransformation screening was carried out in several 50 ml flasks containing 20 ml liquid medium (glucose 20 g, potato starch 20 g, H_2O 1 l). The flask was agitated at 160 rpm at 27°C. After 2 days, the substrate (epothilone A, 0.1 ml of 20 mg/ml in alcohol) was added and incubated under the same condition for an additional 7 days. We screened 11 microorganism strains for biotransformation. The strains were *Aspergillus oryzae*, *flavus link*, *niger*, the mutant strain of *niger*, *Rhizopus nigricans*, *Curvularia lunata*, *Cunninghamella elegans*, *blakesleana*, *Mucor spinosus*, *Streptomyces albulus*, and *Absidia coerulea*. Of these strains, *A. oryzae*, *A. niger*, and *A. flavus link* were able to transform epothilone A. However, *A. niger* was selected for further study because the TLC analysis indicated that it was able to biotransform epothilone A completely.

Preparative scale fermentation was carried out in five 1000 ml flasks, each containing 400 ml of medium under the same circumstances and conditions as the screening fermentation. A total of 200 mg of epothilone A was used (40 mg/flask).

3.5 Extraction and isolation of the metabolites

The culture broth was vigorously mixed with two volumes of ethyl acetate and separated. The ethyl acetate extract was concentrated to dryness under vacuum. The crude extract (1.9 g) was separated and purified by preparative TLC eluted with $CHCl_3$–MeOH (30:1), and obtained compound **5** (9.8 mg). The remains (1.05 g) collected from preparative TLC silica gel plate were subjected to silica gel column chromatography (2 × 100 cm) and eluted with $CHCl_3$–MeOH on the stepwise gradient elution from 90:1 to 1:1. The fractions were further purified by preparative reverse phase HPLC (250 × 10 mm, Kromasil C_{18}, 5 μm, MeOH–H_2O = 3:2, UV 210 nm) to obtain compounds **2** (15.3 mg), **3** (6.5 mg), and **4** (40 mg).

3.5.1 Compound **2**

White powder; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectral data are shown in Table 1; FAB-MS: *m/z* 512.2 $[M+H]^+$.

3.5.2 Compound **3**

White powder; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectral

data are shown in Table 1; FAB-MS: *m/z* 512.2 $[M+H]^+$.

3.5.3 Compound **4**

White powder; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectral data are shown in Table 2; FAB-MS: *m/z* 494.0 $[M+H]^+$.

3.5.4 Compound **5**

White powder; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectral data are shown in Table 2; FAB-MS: *m/z* 494.0 $[M+H]^+$.

3.6 Cytotoxicity assay

Cells were maintained in a 5% CO_2-humidified atmosphere at 37°C in RPMI 1640 medium supplemented with 10% fetal bovine serum.

Ninety-six-well microtiter plates were implanted tumor cells with 3×10^4 cells/ml. After 18 h, 100 μl of compounds diluted in the growth medium ranging from 0.001 to 20 μg/ml was added into each well. After 72-h incubation, the cells were fixed with 100 μl of 0.5 mg/ml methyl thiazolyl tetrazolium. Then the precipitate was dissolved with DMSO and agitated for 15 min. The IC_{50} values were determined by OD at 570 nm.

Acknowledgement

We wish to thank the NMR Laboratory, National Center of Biomedical Analysis for suggestions and preparation of NMR spectrum.

References

[1] K. Gerth, N. Bedorf, G. Höfle, H. Irschik, H. Reichenbach, H. Irschik, and H. Reichenbach, *J. Antibiot.* **49**, 560 (1996).
[2] P. Giannakakou, R. Gussio, E. Nogales, K.H. Downing, D. Zaharevitz, B. Bollbuck, G. Poy, D. Sackett, K.C. Nicolaou, and T. Fojo, *Proc. Natl Acad. Sci. USA* **97**, 2904 (2000).
[3] T.C. Chou, X.C. Zhang, A. Balog, D.S. Su, D.F. Meng, K. Savin, J. Bertino, and S. Danishyefsky, *Proc. Natl Acad. Sci. USA* **95**, 9642 (1998).
[4] T.C. Chou, X.C. Zhang, C. Harris, S. Kuduk, A. Balog, K. Savin, J. Bertino, and S. Danishyefsky, *Proc. Natl Acad. Sci. USA* **95**, 15798 (1998).
[5] F. Lee, R. Borzilleri, R. Fairchild, S.H. Kim, B.H. Long, R.S. Carmen, G.D. Vite, W.C. Rose, and R.A. Kramer, *Clin. Cancer Res.* **7**, 1429 (2001).
[6] J.X. Zhan, H.Z. Guo, J.G. Dai, Y.X. Zhang, and D.A. Guo, *J. Chin. Pharm. Sci.* **10**, 187 (2001).
[7] H. Hardt, H. Steinmetz, K. Gerth, F. Sasse, H. Reichenbach, and G. Höfle, *J. Nat. Prod.* **64**, 847 (2001).
[8] S.F. Victory, D.G. Vandervelde, R. Jalluri, G.L. Grunewald, and G.I. Georg, *Bioorg. Med. Chem. Lett.* **6**, 893 (1996).