Structural revision of 19, 20-epoxycytochalasin D and its cytotoxic activity

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The structure of 19(αH), 20(αH)-epoxycytochalasin D from the fungus *Xylaria hypoxylon* reported by Espada *et al.*,[1] has been revised to 19(βH), 20(αH)-epoxycytochalasin D (**1**) by spectroscopic methods (^{1}H NMR, ^{13}C NMR, HMQC, HMBC, and NOESY), and single-crystal X-ray diffraction analysis. Cytotoxic activity against tumor cell line was assessed for compounds **1**, and found to show potent cytotoxic activity against tumor cell line P-388.

Keywords: 19, 20-epoxycytochalasin D, cytotoxic, *Engleromyces goetzei*

The cytochalasins are a group of microbial metabolites with pronounced biological activities including effects on mammalian cell morphology and cell division,[2,3] inhibition of HIV-1 protease,[4,5] and antibiotic and antitumor activity.[6,7]

The fungus *Engleromyces goetzei* is mainly distributed in south western China. It has been used in Chinese folk medicine for the treatment of inflammatory diseases, gastric ulcers and cancer.[7] Two cytochalasin-type alkaloids, cytochalasin D[8] and engleromycin[9] have been isolated from this fungus previously. Further studies of the chemical constituents and the related biological activities of this fungus has led to the isolation of 19, 20-epoxycytochalasin D (**1**) and cytochalasin D (**2**). 19, 20-Epoxycytochalasin D was firstly reported by Espada *et al.* from the fungus *Xylaria hypoxylon*. Our extensive spectroscopic studies, including single-crystal X-ray diffraction analysis showed that the stereochemistry 19(αH), 20(αH)-epoxycytochalasin D should be revised to 19(βH), 20(αH)-epoxycytochalasin D (**1**). Compound **1** showed potent cytotoxic activity against tumor cell line P-388 at the IC_{50} levels of 0.16 μM.

Compound **1** was obtained as an optically active ($[\alpha]^{20}_D$ –190.0°) colourless crystal. Its molecular formula was established as $C_{30}H_{37}NO_7$ by HRESIMS at *m/z* 524.2654 $[M + H]^+$ (calcd 524.2648). ^{13}C NMR data ($CDCl_3$, Table 1) revealed the presence of 30 carbon signals, comprising seven quaternary carbons, 16 tertiary carbons, three secondary carbons and four methyls. Comparison of the ^{1}H, ^{13}C NMR and optical rotation data with the data reported in the literature[1] showed that the

Table 1 ^{1}H and ^{13}C NMR data, HMBC and NOESY correlations of **1**[a]

No.	δ_C	δC[b]	δH, multi, *J*/Hz	HMBC	
				H→C	NOESY H→H
1	173.52	173.43	–	–	–
2	–	–	5.58 (1H, brs)	3, 4, 9	3
3	53.94	53.90	3.25 (1H. m)	–	2, 27, 31
4	50.70	50.71	2.27 (1H, t, 4.9)	6, 10, 21	5, 8, 10a, 10b
5	32.58	32.56	2.60 (1H, m)	4, 6, 11	4
6	147.48	147.35	–	–	–
7	69.99	69.98	3.83 (1H, d, 10.3)	6, 12, 13	12b, 13
8	46.55	46.54	2.64 (1H, m)	1, 7, 9, 13, 14	4
9	52.53	52.45	–	–	–
10	45.16	45.18	*a*: 2.87 (1H, dd, 13.4, 5.1)	3, 4, 1', 2', 6'	4, 27, 31
			b: 2.75 (1H, dd, 13.4, 9.0)	3, 4, 1', 2', 6'	4, 27, 31
11	13.50	13.49	0.90 (3H, d, 6.7)	4, 5, 6	2, 3, 5, 12a
12	114.35	114.44	*a*: 5.29 (1H, s);	5, 7	11
			b: 5.07 (1H, s)	5, 7	7
13	131. 20	131.17	5.90 (1H, dd, 15.5, 9.8)	14, 15	7, 14, 15a
14	133.42	133.47	5.71 (1H, ddd, 15.5, 10.0, 5.8)	8, 13	13, 15a, 15b,
16					
15	37.44	37.40	*a*: 2.68 (1H, m)	16, 20	13, 14, 15b
			b: 2.11 (1H, dd, 12.0, 5.8)	13, 14, 16, 22	14, 15a, 22
16	41.93	41.89	3.22 (1H, m)	14, 15, 17, 22	14, 22
17	215.29	215.28	–	–	–
18	76.36	76.32	–	–	–
19	59.65	59.63	3.16 (1H, d, 1.8)	18, 20	23, 25
20	52.76	52.72	3.54 (1H, brs)	19, 21	3, 21
21	74.12	74.06	5.52 (1H, s)	4, 8, 19, 20, 25	20, 25
22	19.16	19.17	1.21 (3H, d, 6.7)	15, 16, 17	15b, 16
23	21.86	21.86	1.54 (3H, s)	18, 19	19
24	169.81	169.82	–	–	–
25	20.66	20.68	2.16 (3H, s)	24	19, 21
26	137.13	137.11	–	–	–
27, 31	129.18	129.14	7.17 (2H, d, 7.2)	10, 2', 3', 4', 5, 6'	3, 10, 28, 29,
28, 30	128.96	128.97	7.32 (2H, m)	1', 2', 6'	27, 29
29	127.12	127.13	7.25 (1H, m)	2', 3', 5, 6'	27, 28

[a]Measured in $CDCl_3$ at 500 MHz; [b]literature data.

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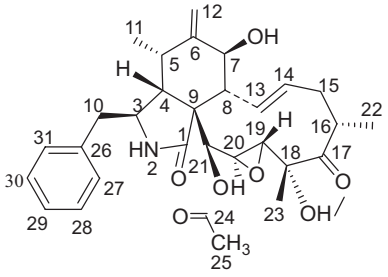

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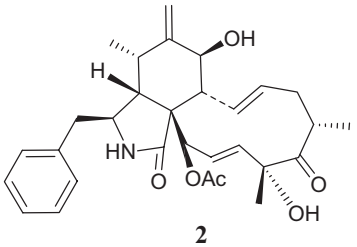

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Fig. 1 The structures of **1** and **2**.

compound of **1** was identical with 19, 20-epoxycytochalasin D, which was confirmed by the intensive analysis of 2D NMR data. The relative stereochemistry of **1** was deduced from the NOESY (Table 1). In the NOESY spectrum, the cross-peaks observed between the proton pairs of H-3/H-21, H-21/H-20 indicated that H-3, H-20, and H-21 were α-oriented. The H-19 was assigned to be β-configuration judged from the NOESY correlations of H-19 with H_3-23 and H_3-25. The recrystallisation of **1** from methanol afforded colourless cubic crystals of $C_{30}H_{37}NO_7.H_2O$. The structure and relative stereochemistry of **1** were finally determined by a single-crystal X-ray diffraction[11] as showed in Fig. 2, which was in good agreement with the structure established by two-dimensional NMR techniques. Herein, **1** was identified as 19(βH), 20(αH)-epoxycytochalasin D. Though a number of cytochalasin-type alkaloids with epoxy group have been reported,[1,12] to the best of our knowledge, this is the first report of cytochalasin-type alkaloids with a *trans*-epoxy group. Compound **1** was evaluated its cytotoxic activity according to standard protocols,[13] and the natural anticancer agent pseudolaric acid B[14] was used as positive control. Compounds **1** showed potent cytotoxic activity against P-388 tumor cell line ($IC_{50} = 1.6 \times 10^{-7}$ mol).

Cytochalasin D (**2**) was identified by comparison of its spectral data with literature data (^{1}H, ^{13}C NMR).[10]

Experimental

Optical rotations were determined on a Perkin-Elmer 341 polarimeter (λ 589 nm). IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disc. NMR spectra were measured on a Bruker AM-500 spectrometer with TMS as internal standard. ESIMS was recorded on a Finnigan LCQ^{DECA} Mass spectrometer. In X-ray crystallography, cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections measure on a Rigaku AFC7R four circle diffractometer employing graphite monochromated $Mo_{K\alpha}$ radiation (λ = 0.71073Å) and operating in the φ–ω scan mode. Data reduction and empirical absorption corrections (ψ-scans) were performed with the SHELXS-97 package. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200-300 mesh) was used for column chromatography, and pre-coated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, People's Republic of China) were used for TLC.

Plant material

The fungus *Engleromyces goetzei* was collected from the Hangzhou area of Zhejiang Province of China and authenticated by Prof. Yong-

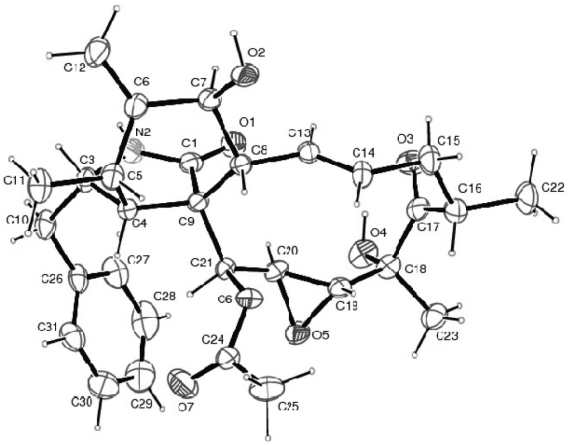

Fig. 2 Single-crystal X-ray structure of 19, 20-epoxycytochalasin D (**1**).

Hong Zhang of the Fujian Medical University. The voucher specimen (ZUTE200505) has been deposited in the College of Pharmaceutical Science, Zhejiang University of Technology.

Extraction and purification

The fresh bodies of the fungus of *E. goetzei* (10 kg) were extracted three times with 95% EtOH at room temperature. The extract was evaporated to dryness under reduced pressure and the residue (203 g), which was then dissolved in water (3 l) to form a suspension, and was extracted with ethyl acetate to afford an ethyl acetate soluble fraction E (88 g). The fraction E was subjected to column chromatography eluted with petrol containing increasing amount of acetone to afford fractions 1–6. The fraction 4 containing mainly alkaloids was subjected to a column chromatography on silica gel eluted with petroleum ether–EtOAc–HCOOH (1 : 1 : 0.1) to afford **1** (6.3 mg) and **2** (705 mg).

19(βH), 20(αH)-epoxycytochalasin D (**1**): A colourless cubic crystal (CH_3OH); $[\alpha]^{20}_D$ –190° (*c* 0.08, $CHCl_3$); IR (KBr) ν_{max} 3433 (OH), 2929, 1747, 1691, 1373, 1225, 1014 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); Positive ESIMS *m/z* 524 $[M + H]^+$; Positive HRESIMS *m/z* 524.2654 $[M + H]^+$ (cacld for $C_{30}H_{38}NO_7$ 524.2648).

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11 Crystal data: $C_{30}H_{37}NO_7.H_2O$, M = 541.64, monoclinic system, space group P 1 21 1; (a) 10.5850 (3); (b) 12.1369 (4); (c) 10.9613 (4) Å, V) 1408.18 (8) Å^3, Z) 2: (d) 1.277 g/cm^3. A crystal of dimensions 0.45 × 0.30 × 0.30 mm was used for measurements on a Rigaku/MSC four circle diffractometer. Crystallagraphic data for the structure of 1 has been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 286810). Copies of these data can be obtained, free of charge, on application to the CCDC via www.ccdc.com.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK; fax: + 44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).
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