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Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga

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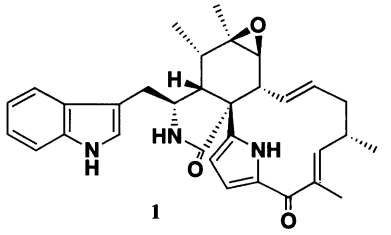
Abstract—New cytochalasans, penochalasins D–H, have been isolated along with chaetoglobosin O from a strain of *Penicillium* sp. originally separated from the marine alga *Enteromorpha intestinalis*, and their stereostructures have been established on the basis of spectral analyses. All the compounds exhibited significant cytotoxicity against cultured P388 cells.

1. Introduction

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have focused our attention on new antitumour materials from microorganisms inhabiting the marine environment.[1–6] As part of this study, we have previously isolated the cytotoxic compounds, communesins,[2] penochalasins A (**1**)–C[3] and penostatins A–I,[4–6] from the mycelia of a strain of *Penicillium* sp. OUPS-79 originally separated from the marine alga *Enteromorpha intestinalis*. Further investigation for metabolites of this fungal strain has now led to the isolation of five additional new cytotoxic metabolites designated penochalasins D–H (**2**–**6**) and known chaetoglobosin O (**7**),[7] which belong to a series of cytochalasans such as cytochalasins and chaotoglobosins.[8] Cytochalasans exhibit some biological activities including marked cytotoxic effects mammalian cells in tissue culture,[8,9] HIV-1 protease inhibitory activity[10] and immunosuppressive activity.[11] These activities have evoked interest in the search for useful leads in the development of new pharmaceutical agents. Although cytochalasans have been isolated from several genera of fungi such as *Chaetomium*, *Phomopsis*, *Diplodia* and *Aspergillus*, their occurrence from *Penicillium* sp. is reported only rarely.[8] Penochalasin D (**2**) isolated from *Penicillium* sp. is chemically one of unique cytochalasans with a macrocyclic ring including a pyrrole moiety in common with penochalasins A (**1**)–C. Furthermore, an investigation on solution conformation of a series of cytochalasans is rarely to be reported. We report herein the stereostructure and cytotoxic activities **2**–**6** and their conformations.



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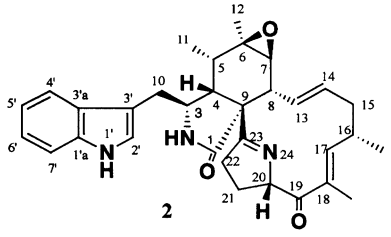

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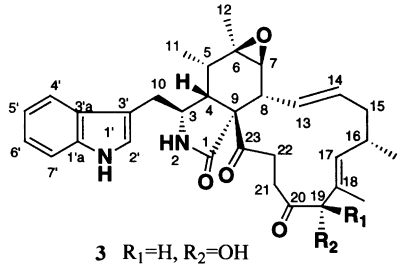
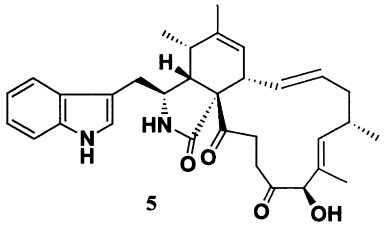


3 R_1=H, R_2=OH
4 R_1=OH, R_2=H



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Keywords: marine fungus; cytochalasan; cytotoxicity.

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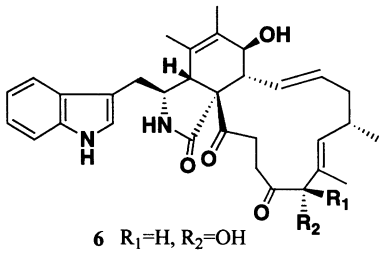

6 R_1=H, R_2=OH
7 R_1=OH, R_2=H

2. Results and discussion

The fungal strain was cultured at 27°C for 3 weeks in a medium containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater as reported previously.[3] The MeOH extract of the mycelium was purified by bioassay-directed fractionation (cytotoxicities against P388 cells) employing a combination of Sephadex LH-20 and silica gel column chromatographies and reversed phase HPLC to afford penochalasins D–H (**2**–**6**), together with known chaetoglobosin O (**7**).[7]

Penochalasin D (**2**) had the molecular formula $C_{32}H_{37}N_3O_3$ established by HREIMS. Its IR spectrum contained absorption bands at 3334, 1695, and 1689 cm^{-1}, characteristic of NH and carbonyl groups. A close inspection of the ^1H and ^{13}C NMR spectra of **2** (Table 1) by DEPT and ^1H–^{13}C COSY experiments revealed the presence of two secondary methyls (C-11 and 16-Me), an allylic methyl (18-Me), a tertiary methyl (C-12), four methylenes (C-10, C-15, C-21 and C-22), seven sp^3-hybridized methines (C-3–C-5, C-7, C-8, C-16 and C-20) including one oxymethine (C-7), two sp^3 quaternary carbons (C-6 and C-9), di- and tri-substituted ethylenes (C-13 and C-14, and C-17 and C-18), a conjugated ketone (C-19), an amide (C-1 and N-2), and a 3-substituted indole, the last feature being supported by the UV spectrum and an EIMS fragment ion (a^+) at *m/z* 130 (Fig. 1), corresponding to an indole ring with a methylene. In addition to the signals for these functional groups, a carbon signal at δ_C 185.77 was observed and ascribed to an sp^2 quaternary carbon (C-23) of an imine by its chemical shift.[12] The appearance of the signals due to an sp^3 methine at δ_H 3.92 and δ_C 55.00 implied that one of the seven sp^3 methines is linked to an amido group. This was supported by an HMBC correlation between C-1 and H-3 (Table 1). The signals due

Table 1. ^1H and ^{13}C NMR data of penochalasin D (**2**) in CD_3OD

Position	δ_H[a]		^1H–^1H COSY	NOESY	δ_c	HMBC
1					178.52 (q)[b]	
3	3.92 dd	5.0 (10), 2.5 (4)	4, 10	4, 10, 11, 12, 4′	55.00 (t)	1, 3′
4	2.56 dd	5.7 (5), 2.5 (3)	3, 5	3, 5, 10, 11, 2′, 4′	52.78 (t)	1, 3, 5, 6, 9, 10, 23
5	1.99 qd	7.1 (11), 5.7 (4)	4, 11	4, 8, 11	37.70 (t)	3, 4, 6, 11, 12
6					58.80 (q)	
7	2.77 d	6.2 (8)	8	8, 12, 13	62.86 (t)	6, 8, 12, 13
8	2.54 dd	9.7 (13), 6.2 (7)	7, 13	5, 7, 14	51.84 (t)	1, 7, 9, 13, 14
9					56.65 (q)	
10	2.92 d	5.0 (3)	3	3, 4, 2′, 4′	33.08 (s)	3, 4, 2′, 3′, 3′a
11	1.01 d	7.1 (5)	5	3, 4, 5, 12	13.03 (p)	4, 5, 6
12	1.27 s			3, 7, 11	19.96 (p)	5, 6, 7
13	6.13 ddd	15.1 (14), 9.7 (8), 1.8 (15β)	8, 14	7, 15α	128.12 (t)	15
14	5.17 ddd	15.1 (13), 11.4 (15α), 2.9 (15β)	13, 15α	8, 15β, 16	136.75 (t)	
15α	1.75 dt	13.0 (15β), 11.4 (14, 16)	14, 15β, 16	13, 17, 16-Me	40.96 (s)	13, 14, 16
β	2.40 dddd	13.0 (15α), 5.5 (16), 2.9 (14), 1.8 (13)	15α, 16	14, 16, 16-Me		
16	2.63 m		15α, 15β, 17, 16-Me	14, 15β, 16-Me, 18-Me	33.36 (t)	
17	5.48 br dq	9.7 (16), 1.6 (18-Me)	16, 18-Me	15α, 21B, 22B, 16-Me	146.21 (t)	15, 19, 18-Me
18					134.73 (q)	
19					205.66 (q)	
20	4.19 ddt	9.4 (21A), 6.6 (21B), 3.2 (22A, 22B)	21A, 21B, 22A, 22B	21A	83.20 (t)	
21A	1.76 m		20, 21B, 22B	20	26.33 (s)	19, 20, 23
B	1.94 dddd	15.3 (21A), 10.6 (22B), 6.6 (20), 5.8 (22A)	20, 21A, 22A, 22B	17, 22B		19
22A	1.79 dddd	16.0 (22B), 10.6 (21A), 5.8 (21B), 3.2 (20)	20, 21B, 22B		39.43 (s)	
B	2.57 m	20, 21A, 21B, 22A	17, 21B			23
23					185.77 (q)	
16-Me	0.98 d	6.9 (16-Me)	16	15α, 15β, 16, 17, 18-Me	19.75 (p)	15, 16, 17
18-Me	1.73 d	1.6 (17)	17	16, 16-Me	12.88 (p)	17, 18, 19
1′a					137.94 (q)	
2′	7.03 s			4, 10	125.92 (t)	1′a, 3′, 3′a
3′					110.10 (q)	
3′a					129.32 (q)	
4′	7.50 ddd	8.0 (5′), 1.0 (6′), 0.8 (7′)	5′	3, 4, 10, 5′	119.40 (t)	1′a, 3′, 6′
5′	7.04 dddd	8.0 (4′), 7.0 (6′), 1.0 (7′)	4′, 6′	4′, 6′	120.28 (t)	3′a, 7′
6′	7.12 dddd	8.2 (7′), 7.0 (5′), 1.0 (4′)	5′, 7′	5′, 7′	122.77 (t)	1′a, 4′
7′	7.36 ddd	8.2 (6′), 1.0 (5′), 0.8 (4′)	6′	6′	112.52 (t)	3′a, 5′

[a] ^1H Chemical-shift values (δ ppm from TMS) are followed by the multiplicity of the signals, the coupling constant (*J* Hz) and the coupling proton in parentheses.
[b] Letters p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

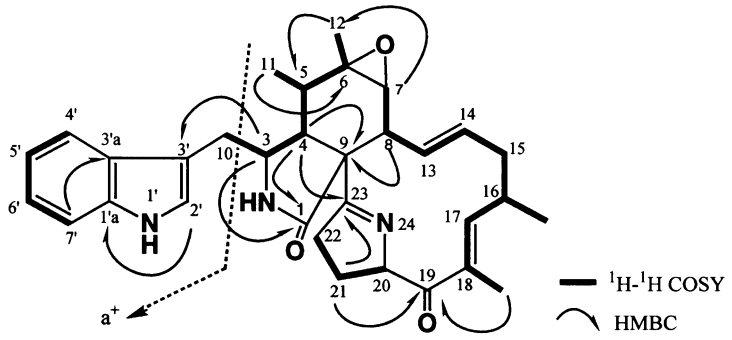

Figure 1. Typical 2D NMR correlations in penochalasin D (**2**) and an observed MS fragment.

to a methine at δ_H 2.77 and δ_C 62.86 and due to a sp^3 quaternary carbon at δ_C 58.80 indicated the presence of a trisubstituted epoxide (C-6 and C-7). The ^{1}H–^{1}H COSY analysis of **2** led to three partial structural units except for a part of the indole moiety as shown by bold-faced lines in Fig. 1, which were supported by HMBC correlations (Table 1). The *E*-geometry of both the Δ^{13}- and Δ^{17}-double bonds was deduced from a coupling constant ($J_{13,14}$=15.1 Hz) of the olefinic protons, a chemical shift (δ_C 12.88) of the allylic methyl (18-Me) carbon signal,[13] and NOEs from H-18-Me to H-16-Me and H-16. The connection of these three units and the remaining functional groups including the indole moiety was determined on the basis of the key HMBC correlations summarized in Fig. 1, and the planar structure of **2** was elucidated.

The relative stereochemistry and conformation for **2** were deduced from detailed NOE spectral analysis of **2** (Table 1 and Fig. 2). NOEs from H-5 to H-4 and H-8, from H-12 to H-7, H-11and H-3, and from H-11 to H-3 implied that the cyclohexane ring (C-4–C-9) exists in a twist-boat conformation, H-4, H-5 and H-8 on the ring are on the same side, and on the opposite side to H-7, C-11, C-12 and the C-4–C-3 bond. Observation of the NOE between H-12 and H-3 also implied that the C-4–C-3 bond is arranged *cis* to the C-9–C-1 bond, and H-3 *trans* to H-4. NOEs from H-14 to H-8 and H-16 indicated these protons to be on the same side. NOEs from H-15α to H-13 and H-17, and from H-13 to H-7 showed these protons to be on the same side. Furthermore, NOEs for H-17/H-21B, H-17/H-22B and H-20/H-21A implied that H-20 and H-17 are on the opposite side and the dihydropyrrole ring is arranged on a nearly vertical plane to the macrocyclic ring. Based on the evidence summarized above, the relative stereostructure and conformation for penochalasin D (**2**) were elucidated as depicted in Fig. 2.

Penochalasin E (**3**) had the molecular formula $C_{32}H_{38}N_2O_5$ established by HREIMS. Its UV and IR spectra exhibited the absorption bands similar to those of **2**. The general features of its ^{1}H and ^{13}C NMR spectra (Table 2) closely resembled those of **2** except for the absence of the C-23 imine carbon signal, appearance of proton and carbon signals for one hydroxymethine (C-19) and one more carbonyl carbon signal for a ketone, and a chemical-shift difference of some proton and carbon signals. Analysis of ^{1}H–^{1}H COSY and HMBC correlations (H-8/C-9, H-8/C-1, H-4/C-1, H-12/C-5 and H-10/C-3′) (Table 2) suggested the partial structural unit (C-1–C-18-Me and the indole moiety) of **3** to be the same as those of **2**. The connectivity of the partial structural unit (C-1–C-18-Me) with the remaining unit (C-19–C-23) was deduced from the chemical shifts of H-22 (δ_H 1.85 and 2.67) and the typical HMBC correlations (H-4/C-23, H-21A/C-20, H-19/C-20 and H-18-Me/C-19) (Table 2). The presence of the indole ring with a methylene was supported by the UV spectrum and an EIMS fragment at *m/z* 130. The *E*-geometry of the 13,17-diene was deduced from a coupling constant

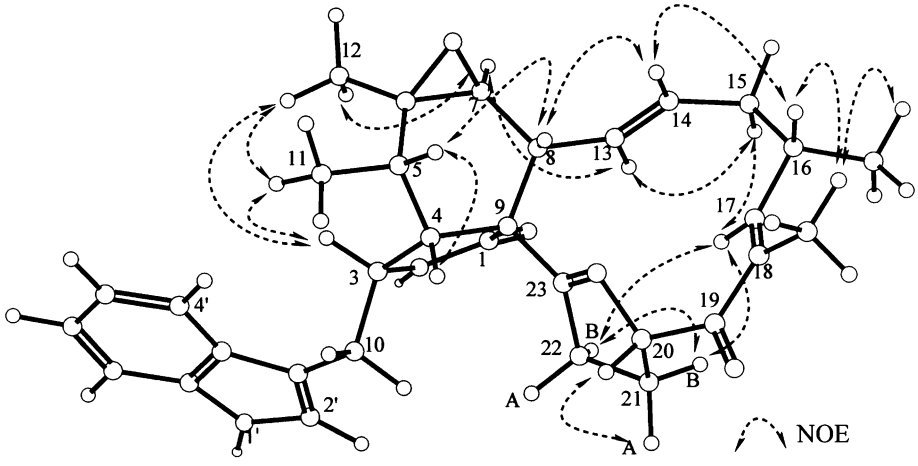

Figure 2. The conformation of penochalasin D (**2**) and observed NOEs.

Table 2. ^{1}H and ^{13}C NMR data penochalasin E (**3**) in CD_3OD

Position	δ_H[a]		^{1}H–^{1}H COSY	NOESY	δ_c	HMBC
1					176.67 (q)[b]	
3	3.85 td	5.0 (10), 2.1 (4)	4, 10	4, 10, 11, 12, 4′	54.37 (t)	5, 3′
4	2.61 dd	5.7 (5), 2.1 (3)	3, 5	3, 5, 10, 11, 2′, 4′	49.67 (t)	1, 5, 6, 10, 23
5	1.72 qd	7.3 (11), 5.7 (4)	4, 11	4, 8, 11	37.84 (t)	3, 4, 6, 11
6					58.94 (q)	
7	2.68 d	5.7 (8)	8	8, 12, 13	63.27 (t)	8, 13
8	2.08 dd	9.8 (13), 5.7 (7)	7, 13	5, 7, 14	50.39 (t)	1, 7, 9, 13, 14
9					65.87 (q)	
10	2.84 d	5.0 (3)	3	3, 4, 2′, 4′	33.46 (s)	3, 4, 2′, 3′, 3′a
11	0.96 d	7.3 (5)	5	3, 4, 5, 12	12.94 (p)	4, 5, 6
12	1.22 s			3, 7, 11	19.71 (p)	5, 6, 7
13	6.08 ddd	15.1 (14), 9.8 (8), 2.1 (15β)	8, 14	7, 15α	128.72 (t)	15
14	5.08 ddd	15.1 (13), 11.0 (15α), 3.0 (15β)	13, 15α, 15β	8, 15β, 16	135.51 (t)	
15α	1.88 dt	14.2 (15β), 11.0 (14, 16)	14, 15β, 16	13, 17, 16-Me	42.30 (s)	
β	2.26 dtd	14.2 (15α), 3.0 (14, 16), 2.1 (13)	14, 15α, 16	14, 16, 16-Me		
16	2.53 m		15α, 15β, 17, 16-Me	14, 15β, 16-Me, 18-Me	33.70 (t)	
17	5.02 br dq	9.6 (16), 1.4 (18-Me)	16, 18-Me	15α, 22B, 16-Me	134.79 (t)	19, 18-Me
18					133.33 (q)	
19	4.27 s			21B, 18-Me	82.68 (t)	17, 18, 20, 18-Me
20					211.36 (q)	
21A	2.14 ddd	15.8 (21B), 9.2 (22B), 7.1 (22A)	21B, 22A, 22B	22A	32.29 (s)	20
B	2.33 ddd	15.8 (21A), 9.4 (22A), 4.3 (22B)	21A, 22A, 22B	19, 22B		
22A	1.85 ddd	19.4 (22B), 9.4 (21B), 7.1 (21A)	21A, 21B, 22B	21A	37.22 (s)	
B	2.67 ddd	19.4 (22A), 9.2 (21A), 4.3 (21B)	21A, 21B, 22A	17, 21B		
23					209.61 (q)	
16-Me	0.93 d	6.9 (16-Me)	16	15α, 15β, 16, 17, 18-Me	21.62 (p)	15, 16, 17
18-Me	1.72 d	1.4 (17)	17	16, 19, 16-Me	16.55 (p)	17, 18, 19
1′a					137.93 (q)	
2′	6.99 s			4, 10	125.83 (t)	1′a, 3′, 3′a
3′					109.88 (q)	
3′a					129.15 (q)	
4′	7.50 dd	7.3 (5′), 1.1 (6′)	5′, 6′	3, 4, 10, 5′	119.46 (t)	1′a, 6′
5′	7.04 td	7.3 (4′, 6′), 1.1 (7′)	4′, 6′, 7′	4′, 6′	120.30 (t)	3′a, 7′
6′	7.08 td	7.3 (5′, 7′), 1.1 (4′)	4′, 5′, 7′	5′, 7′	122.60 (t)	1′a, 4′
7′	7.28 dd	7.3 (6′), 1.1 (5′)	5′, 6′	6′	112.34 (t)	3′a, 5′

[a] ^{1}H Chemical-shift values (δ ppm from TMS) are followed by the multiplicity of the signals, the coupling constant (J Hz) and the coupling proton in parentheses.
[b] Letters p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

($J_{13,14}$=15.1 Hz), a chemical shift (δ_C 16.55) of the carbon signal of the allylic methyl (18-Me), and NOEs from H-18-Me to H-16 and H-16-Me. Based on the above evidence, the planar structure of **3** was elucidated. In NOESY experiments (Table 2), the observed NOEs (Fig. 3) in the partial structural unit (C-1–C-18) of **3** implied that the relative configuration and conformation of the unit are the same as those in **2**. In addition, the observation of

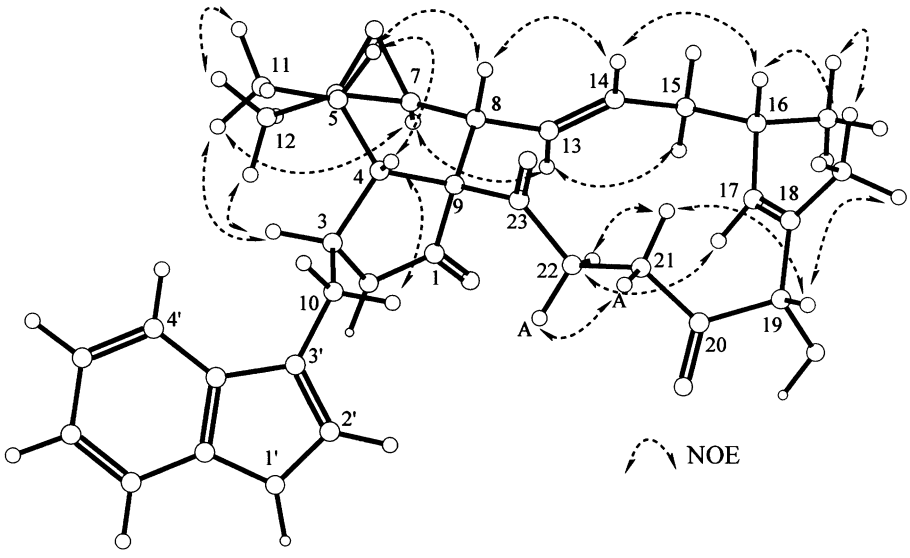

Figure 3. The conformation of penochalasin E (**3**) and observed NOEs.

Table 3. ^{1}H and ^{13}C NMR data penochalasin F (**4**) in CD_3OD

Position	δ_H[a]		^{1}H–^{1}H COSY	NOESY	δ_C	HMBC
1					176.33 (q)[b]	
3	3.91 ddd	4.7 (10B), 3.4 (10A), 2.7 (4)	4, 10A, 10B	4, 10A, 10B, 11, 12	54.42 (t)	
4	2.57 dd	5.5 (5), 2.7 (3)	3, 5	3, 5, 10A, 11, 2′	49.92 (t)	6
5	1.76 qd	7.3 (11), 5.5 (4)	4, 11	4, 8, 11	38.00 (t)	
6					58.79 (q)	
7	2.71 d	5.5 (8)	8	8, 12, 13	63.19 (t)	8, 13
8	2.10 dd	10.1 (13), 5.7 (7)	7, 13	5, 7, 14	50.96 (t)	1, 9, 13, 14
9					65.51 (q)	
10A	2.76 dd	14.6 (10B), 3.4 (3)	3, 10B	3, 4	33.09 (s)	2′, 3′
B	2.98 dd	14.6 (10A). 4.7 (3)	3, 10A	3		2′, 3′, 3′a
11	1.13 d	7.3 (5)	5	3, 4, 5, 12	13.18 (p)	4, 5, 6
12	1.29 s			3, 7, 11	19.83 (p)	5, 6, 7
13	6.00 ddd	15.1 (14), 10.1 (8), 1.8 (15β)	8, 14	7, 15α	129.93 (t)	
14	5.07 ddd	15.1 (13), 11.0 (15α), 3.4 (15β)	13, 15α, 15β	8, 15β, 16	134.35 (t)	
15α	1.91 dt	13.5 (15β), 11.0 (14, 16)	14, 15β, 16	13, 17, 16-Me	42.99 (s)	
β	2.23 dtd	13.5 (15α), 3.4 (14, 16), 1.8 (13)	14, 15α, 16	14, 16, 16-Me		
16	2.42 m		15α, 15β, 17, 16-Me	14, 15β, 16-Me, 18-Me	33.24 (t)	
17	5.40 br dq	8.9 (16), 1.1 (18-Me)	16, 18-Me	15α, 19, 22B, 16-Me	140.63 (t)	18-Me
18					132.92 (q)	
19	4.38 s			17, 22B	83.13 (t)	17, 18, 20
20					211.48 (q)	
21A	1.87 ddd	14.9 (21B), 11.9 (22B), 5.0 (22A)	21B, 22A, 22B		35.43 (s)	
B	2.06 ddd	14.9 (21A), 11.7 (22A), 3.4 (22B)	21A, 22A, 22B	18-Me		
22A	1.03 ddd	19.0 (22B), 11.7 (21B), 5.0 (21A)	21A, 21B, 22B		37.39 (s)	
B	2.80 ddd	19.0 (22A), 11.9 (21A), 3.4 (21B)	21A, 21B, 22A	17, 19		
23					209.52 (q)	
16-Me	0.96 d	6.9 (16-Me)	16	15α, 15β, 16, 17, 18-Me	21.39 (p)	15, 6, 17
18-Me	1.37 d	1.1 (17)	17	16, 21B, 16-Me	10.81 (p)	17, 18, 19
1′a					137.82 (q)	
2′	6.95 s			4	126.36 (t)	1′a, 3′, 3′a
3′					109.27 (q)	
3′a					129.24 (q)	
4′	7.51 dd	7.3 (5′), 1.8 (6′)	5′, 6′	5′	119.70 (t)	6′
5′	7.00 td	7.3 (4′, 6′), 1.8 (7′)	4′, 6′, 7′	4′	120.39 (t)	
6′	7.02 td	7.3 (5′, 7′), 1.8 (4′)	4′, 5′, 7′	7′	122.42 (t)	
7′	7.30 dd	7.3 (6′), 1.8 (5′)	5′, 6′	6′	112.89 (t)	6′

[a] ^{1}H Chemical-shift values (δ ppm from TMS) are followed by the multiplicity of the signals, the coupling constant (J Hz) and the coupling proton in parentheses.

[b] Letters p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

NOEs from H-19 to H-21B and H-18-Me, from H-21B to H-18-Me, and from H-17 to H-22B showed H-19 to be oriented on the same side as H-18-Me and on the opposite side to H-17. Thus, the relative configuration and conformation for penochalasin E (**3**) was elucidated as shown in Fig. 3.

Penochalasin F (**4**) had the same molecular formula as **3** as deduced from HREIMS. The general features of its spectral data (Table 3) closely resembled those of **3** except that the signals for H-17, H-21A, H-21B, H-22A, H-18-Me, C-17, C-21 and C-18-Me in the NMR spectra revealed a chemical-shift difference relative to those of **3** (Table 2). In NOESY experiments, **4** showed NOEs from H-19 to H-17 and H-22B, and from H-21B to H-18-Me (Table 3), implying that H-19 is oriented on the same side as H-17. This finding proved penochalasin F (**4**) to be a stereoisomer of **3** at C-19.

Penochalasin H (**6**) was assigned the same molecular formula as **3** as deduced from HREIMS. Its IR, UV and NMR spectra (Table 4) showed close correspondence with those of **3** except that the ^{1}H NMR signal of H-5 in **3** disappeared from **6**, and the signals for H-4, H-7, H-11, H-12, C-3, C-5–C-9, C-11, C-12 and C-14 in **6** revealed a chemical-shift difference relative to those of **3** observed in the same solvent ($CDCl_3$) as that in **6** (see Section 3). The appearance of two allylic methyl proton singlets (H-11 and H-12) at δ_H 1.53 and 1.68 and two quaternary carbon signals (C-5 and C-6) at δ_C 126.23 and 131.52 implied the presence of a tetrasubstituted double bond linked to two methyls in **6**, and the methine proton doublet (H-7) coupled to H-8 was assigned a hydroxymethine on the basis of its chemical shift (δ_H 3.89) and the fact that the signal was sharpened by D_2O exchange. These observations implied that the 5 and 6, and 7 positions in **3** are replaced by a double bond and a hydroxymethine in **6**, respectively. This planar structure was supported by analysis of ^{1}H–^{1}H COSY and HMBC correlations (Table 4).

The observed coupling constants and NOEs in **6** (Table 4) showed that the relative stereochemistry of **6** is the same as that of **3** except for C-5 and the conformation also is similar to that of **3**. The $J_{7,8}$ value (10.3 Hz) in **6** implied that H-7 is

Table 4. ^{1}H and ^{13}C NMR data penochalasin H (**6**) in $CDCl_3$

Position	δ_H[a]		^{1}H–^{1}H COSY	NOESY	δ_C	HMBC
1					174.14 (q)[b]	
2	5.69 br s		3	3, 10A		9
3	3.55 br ddd	8.3 (10A), 6.2 (10B), 1.6 (4)	2, 4, 10A, 10B	2, 4, 10A, 10B, 11, 4′	57.47 (t)	1, 5
4	3.31 br s		3, 7, 11, 12	3, 8, 10A, 10B, 11, 2′	49.37 (t)	1
5					126.23 (q)	
6					131.52 (q)	
7	3.89 br d	10.3 (8),	4, 8, 11, 12	8, 12, 13	68.54 (t)	
8	2.05 t	10.3 (7, 13)	7, 13	4, 7, 14	52.84 (t)	1, 7, 9, 13, 14, 23
9					61.74 (q)	
10A	2.63 dd	14.2 (10B), 8.3 (3)	3, 10B	2, 3, 4, 2′, 4′	33.17 (s)	3, 4, 2′, 3′. 3′a
B	2.79 dd	14.2 (10A), 6.2 (3)	3, 10A	3, 4, 2′, 4′		3, 4, 2′, 3′, 3′a
11	1.53 s		4, 7, 12	3, 4, 12, 4′	17.34 (p)	4, 5, 6
12	1.68 s		4, 7, 11	7, 11	14.12 (p)	5, 6, 7
13	6.31 ddd	15.3 (14), 10.3 (8), 2.0 (15β)	8, 14	7, 15α, 22B	127.87 (t)	
14	5.29 ddd	15.3 (13), 11.0 (15α), 3.4 (15β)	13, 15α, 15β	8, 15β, 16	136.78 (t)	
15α	2.05 dt	14.3 (15β), 11.0 (14, 16)	14, 15β, 16	13, 17, 16-Me	41.28 (s)	
β	2.30 dtd	14.3 (15α), 3.0 (14, 16), 2.0 (13)	14, 15α, 16	14, 16, 16-Me		
16	2.52 m		15α, 15β, 17, 16-Me	14, 15β, 17, 16-Me, 18-Me	32.72 (t)	
17	4.98 dq	8.9 (16), 1.4 (18-Me)	16, 18-Me	15α, 16, 22B, 16-Me	135.00 (t)	19
18					133.37 (q)	
19	4.43 br s		19-OH	21B, 18-Me, 19-OH	81.64 (t)	
20					209.64 (q)	
21A	2.37 ddd	15.3 (21B), 10.6 (22B), 7.1 (22A)	21B, 22A, 22B	22A	31.38 (s)	20
B	2.97 dd	15.3 (21A), 10.5 (22A)	21A, 22A, 22B	19, 22B, 18-Me		20
22A	2.91 ddd	17.6 (22B), 10.5 (21B), 7.1 (21A)	21A, 21B, 22B	21A	37.08 (s)	20, 23
B	3.25 dd	17.6 (22A), 10.6 (21A)	21A, 21B, 22A	13, 17, 21B		20, 23
23					207.50 (q)	
16-Me	0.98 d	6.9 (16)	16	15α, 15β, 16, 17, 18-Me	21.25 (p)	15, 16
18-Me	1.9 d	1.4 (17)	17	16, 19, 21B, 16-Me	17.65 (p)	17, 18, 19
7-OH	1.75 br s					
19-OH	3.47 br s		19			
1′	8.22 br s		2′	2′, 7′		
1′a					136.34 (q)	
2′	7.02 d	2.3(1′)	1′	2, 3, 4, 10A, 10B, 1′	122.94 (t)	1′a, 3′, 3′a
3′					111.07 (q)	
3′a					126.81 (q)	
4′	7.51 br d	8.0 (5′)	5′	3, 10A, 10B, 5′	118.31 (t)	1′a, 6′
5′	7.15 ddd	8.0 (4′), 7.0 (6′), 0.9 (7′)	4′, 6′	4′, 6′	119.84 (t)	3′a, 7′
6′	7.22 ddd	8.0 (7′), 7.0 (5′), 1.1 (4′)	5′, 7′	5′, 7′	122.42 (t)	1′a, 4′, 7′
7′	7.38 dd	8.0 (6′), 0.9 (5′)	6′	1′, 6′	111.42 (t)	3′a, 5′

[a] ^{1}H Chemical-shift values (δ ppm from TMS) are followed by the multiplicity of the signals, the coupling constant (J Hz) and the coupling proton in parentheses.

[b] Letters p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

arranged pseudoaxially and *trans* to H-8. This evidence led to the relative stereostructure **6** for penochalasin H. The observed NOEs for H-19/H-18-Me, H-19/H-21B, H-17/H-22B, and H-21B/H-18-Me in **6** revealed that the conformation of the macrocyclic ring in **6** is the same as that of **3**. This compound is a C-19 epimer of chaetoglobosin O which was already isolated by Ichihara and co-workers from a fungul strain of *Cylindrocladium floridanum*, a causal fungus of alfalfa black rot disease.[7] Compound **7** obtained in this experiment was identified as chaetoglobosin O by comparison of spectral data with published values.[7] The observed NOEs for H-17/H-19, H-17/H-22B, H-19/H-22B and H-21A/H-22A in **7** revealed that the conformation of the macrocyclic ring in **7** is the same as that of **4**.

Penochalasin G (**5**) was assigned a molecular formula which contained one oxygen atom less than that of **4**. Comparison of its ^{1}H and ^{13}C NMR signals (Table 5) with those of **4** (Table 3) revealed that the epoxide (C-6 and C-7) in **4** is replaced by a trisubstituted ethylene (δ_H 5.32; δ_C 141.27 and 126.60) in **5**. The observed coupling constants and NOEs (Table 5) in **5** showed its configuration and conformation to be the same as that of **4** except for C-6 and C-7. This finding led to the relative stereostructure **5** for penochalasin G.

The absolute configuration for compounds **2**–**6** has not been established independently, but is assumed as depicted herein by the co-occurrence of known structurally related metabolites, chaetoglobosins A and F, which were reported previously.[3]

The cytotoxic activities of penochalasins D-H (**2**–**6**) and chaetoglobosin O (**7**) were examined in the P388 lymphocytic leukaemia test system in cell culture, according to the method reported previously.[14] All the compounds (**2**–**7**) tested exhibited significant cytotoxic activity (ED_{50} 3.2, 2.1, 1.8, 1.9, 2.8 and 2.4 $\mu g\ ml^{-1}$, respectively).

Table 5. ^{1}H and ^{13}C NMR data penochalasin G (**5**) in CD_3OD

Position	δ_H[a]		^{1}H–^{1}H COSY	NOESY	δ_C	HMBC
1					176.54 (q)[b]	
3	3.48 q	3.3 (4, 10A, 10B)	4, 10A, 10B	4, 10A, 10B, 11, 12	55.50 (t)	1, 4, 3′
4	2.63 dd	5.3 (5), 3.3 (3)	3, 5	3, 5, 10A, 11, 2′, 4′	51.51 (t)	1, 3, 5, 6, 9, 2′, 3′, 3′a
5	2.42 m		4, 7, 11	4, 8, 11	36.53 (t)	
6					141.27 (q)	
7	5.32 qt	3.7 (12), 1.6 (5, 8)	5, 8, 12	8, 12, 13	126.60 (t)	
8	2.69 dquint	10.1 (13), 1.6 (7, 12)	7, 12, 13	5, 7, 14	49.21 (t)	
9					68.56 (q)	
10A	2.73 dd	14.6 (10B), 3.3 (3)	3, 10B	3, 4, 11, 2′, 4′	32.61 (s)	3, 2′, 3′a
B	3.01 dd	14.6 (10A). 3.3 (3)	3, 10A	3, 4′		3, 4, 2′, 3′, 3′a
11	1.29 d	7.3 (5)	5	3, 4, 5, 10A, 12	13.94 (p)	4, 5, 6
12	1.79 dd	3.7 (7), 8 (1.6)	7, 8	3, 7, 11	20.16 (p)	5, 6, 7
13	5.88 ddd	15.1 (14), 10.1 (8), 1.8 (15β)	8, 14	7, 15α	132.00 (t)	
14	4.96 ddd	15.1 (13), 11.0 (15α), 3.4 (15β)	13, 15α, 15β	8, 15β, 16	132.29 (t)	15
15α	1.86 dt	13.5 (15β), 11.0 (14, 16)	14, 15β, 16	13, 17, 16-Me	42.91 (s)	
β	2.19 dtd	13.5 (15α), 3.4 (14, 16), 1.8 (13)	14, 15α, 16	14, 16, 16-Me		
16	2.38 m		15α, 15β, 17, 16-Me	14, 15β, 16-Me, 18-Me	33.32 (t)	
17	5.40 br dq	8.9 (16), 1.1 (18-Me)	16, 18-Me	15α, 19, 22B, 16-Me	140.92 (t)	15, 16, 19, 18-Me
18					132.68 (q)	
19	4.36 s			17. 22B	82.94 (t)	17, 18, 20, 18-Me
20					211.63 (q)	
21A	1.75 ddd	15.1 (21B), 11.7 (22B), 5.5 (22A)	21B, 22A, 22B	22A	35.62 (s)	19, 20, 22
B	2.01 ddd	15.1 (21A), 11.2 (22A), 3.2 (22B)	21A, 22A, 22B	22B, 18-Me		23
22A	0.88 ddd	19.0 (22B), 11.2 (21B), 5.5 (21A)	21A, 21B, 22B	21A, 4′	37.77 (s)	23
B	2.85 ddd	19.0 (22A), 11.7 (21A), 3.2 (21B)	21A, 21B, 22A	17, 19, 21B		
23					210.46 (q)	
16-Me	0.94 d	6.9 (16-Me)	16	15α, 15β, 16, 17, 18-Me	21.38 (p)	15, 16, 17
18-Me	1.35 d	1.1 (17)	17	16, 21B, 16-Me	10.76 (p)	17, 18, 19
1′a					137.83 (q)	
2′	6.96 s			4, 10A	126.35 (t)	1′a, 3′, 3′a
3′					109.76 (q)	
3′a					129.31 (q)	
4′	7.51 dd	7.1 (5′), 1.5 (6′)	5′, 6′	4, 10A, 10B, 22A, 5′	119.97 (t)	1′a, 6′
5′	6.99 td	7.1 (4′, 6′), 1.5 (7′)	4′, 6′, 7′	4′, 6′	120.29 (t)	
6′	7.01 td	7.1 (5′, 7′), 1.5 (4′)	4′, 5′, 7′	5′, 7′	122.35 (t)	
7′	7.29 dd	7.1 (6′), 1.5 (5′)	5′, 6′	6′	112.69 (t)	5′

[a] ^{1}H Chemical-shift values (δ ppm from TMS) are followed by the multiplicity of the signals, the coupling constant (J Hz) and the coupling proton in parentheses.

[b] Letters p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

3. Experimental

3.1. General procedures

UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin Elmer FT-IR spectrometer 1720X. Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter. NMR spectra were recorded at 27°C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7 MHz for ^{1}H and ^{13}C, respectively, with tetramethylsilane (TMS) as an internal reference. EIMS was determined using a Hitachi M-4000H mass spectrometer. Liquid chromatography over silica gel (mesh 230–400) was performed in a medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm×20 mm i. d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent system CH_2Cl_2–MeOH (19:1), and compounds were viewed under UV lamp and sprayed with 10% H_2SO_4 followed by heating.

3.1.1. Culturing and isolation of metabolites. As reported previously,[3,4] a strain of *Penicillium* sp. OUPS-79 isolated from the marine alga *Enteromorpha intestinalis* (Linne) Link (Ulvaceae) was grown in a liquid medium (20 l) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5 for 3 weeks at 27°C. The culture was filtered under suction and the mycelium collected was extracted thrice with MeOH. The combined extracts were evaporated under reduced pressure. The resulting extract (63 g) was passed through Sephadex LH-20, using MeOH–CH_2Cl_2 (1:2) as the eluent. The third fraction (28.2 g) was chromatographed on a silica gel column with a CH_2Cl_2–MeOH gradient as the eluent. The MeOH–CH_2Cl_2 (1:199) and (1:99) eluates were collected as Fr. 1 (659 mg) and Fr. 2 (310 mg), respectively. Fr. 1 was purified by HPLC (ODS) using MeOH–water (3:1) as the eluent to afford **2** (3.3 mg). Fr. 2 afforded **3** (3.1 mg), **4** (1.5 mg), **5** (1.9 mg), **6** (2.2 mg) and **7** (3.0 mg), respectively, after purification by HPLC (ODS) using MeOH–water (4:1) as the eluent.

3.1.2. Penochalasin D (2). Obtained as colourless oil

$[\alpha]_D$=+10.8° (*c* 0.19, $CHCl_3$); λ_{max} (EtOH)/nm: 221 (log ϵ 4.33), 242 sh (3.72), 280 (3.58) and 292 (3.48); ν_{max} (liquid)/cm^{-1}: 3334 (NH), 1698, 1692 and 1684 (C=O, C=N); *m/z* (EI): 511 (M^+, 72%), 496 (37), 469 (79), 456 (16), 381 ($M^+-C_9H_8N$, 11), 185 (20) and 130 (a^+, C_9H_8N, 100); *m/z* (HREI): 511.2834 [M^+] (Calcd for $C_{32}H_{37}N_3O_3$: 511.2833). ^1H and ^{13}C NMR data are listed in Table 1.

3.1.3. Penochalasin E (3). Obtained as colourless oil $[\alpha]_D$=−73.0° (*c* 0.14, $CHCl_3$); λ_{max} (EtOH)/nm: 221 (log ϵ 4.43), 241sh (3.97), 280 (3.84) and 291 (3.77); ν_{max} (liquid)/cm^{-1}: 3361 (NH, OH), 1696 and 1684 (C=O); *m/z* (EI): 530 (M^+, 16%), 445 (20), 385 (19), 185 (20) and 130 (C_9H_8N, 100); *m/z* (HREI): 530.2779 [M^+] (Calcd for $C_{32}H_{38}N_2O_5$: 530.2779); ^1H NMR ($CDCl_3$): δ 0.95 (3H, d, *J*=6.6 Hz, H-16-Me), 1.17 (3H, d, *J*=7.1 Hz, H-11), 1.22 (3H, s, H-12), 1.84 (1H, qd, *J*=7.1., 5.7 Hz, H-5), 1.85 (3H, d, *J*=1.4 Hz, H-18-Me), 2.00 (1H, dt, *J*=14.4, 11.0 Hz, H-15α), 2.06 (1H, dd, *J*=10.3, 5.7 Hz, H-8), 2.10 (1H, ddd, *J*=15.8, 10.3, 6.6 Hz, H-21A), 2.26 (1H, dtd, *J*=14.4, 3.0, 2.0 Hz, H-15β), 2.42 (1H, ddd, *J*=19.9, 10.5, 6.6 Hz, H-22A), 2.48 (1H, m, H-16), 2.63 (1H, dd, *J*=14.4, 7.6 Hz, H-10A), 2.76 (1H, ddd, *J*=15.8, 10.5, 2.2 Hz, H-21B), 2.77 (1H, d, *J*=5.7 Hz, H-7), 2.83 (1H, dd, *J*=5.7, 2.1 Hz, H-4), 2.88 (1H, dd, *J*=14.4, 4.6 Hz, H-10B), 3.09 (1H, ddd, *J*=19.9, 10.3 Hz, H-22B), 3.44 (1H, br d, *J*=4.0 Hz, 19-OH), 3.78 (1H, m, H-3), 4.35 (1H, br d, *J*=4.0 Hz, H-19), 4.94 (1H, br dq, *J*=8.9, 1.4 Hz, H-17), 5.14 (1H, ddd, *J*=15.3, 11.0, 3.0 Hz, H-14), 5.93 (1H, br s, H-2), 6.20 (1H, ddd, *J*=15.3, 10.3, 2.0 Hz, H-13), 7.01 (1H, d, *J*=2.0 Hz, H-2′), 7.18 (1H, td, *J*=8.1, 7.1, 1.0 Hz, H-5′), 7.23 (1H, td, *J*=8.1, 7.1, 1.1 Hz, H-6′), 7.36 (1H, br d, *J*=8.1 Hz, H-7′), 7.50 (1H, dd, *J*=8.1, 1.1 Hz, H-4′) and 8.23 (1H, br s, H-1′); ^{13}C NMR ($CDCl_3$):δ 12.87 (C-11), 17.60 (C-18-Me), 19.54 (C-12), 21.17 (C-16-Me), 31.26 (C-21), 32.73 (C-16), 34.46 (C-10), 36.36 (C-5), 37.21 (C-22), 41.03 (C-15), 48.74 (C-4), 49.58 (C-8), 52.21 (C-3), 57.47 (C-6), 61.92 (C-7), 64.20 (C-9), 81.63 (C-19), 110.33 (C-3′), 111.37 (C-7′), 118.38 (C-4′), 120.13 (C-5′), 122.58 (C-6′), 123.43 (C-2′), 127.00 (C-3′a), 127.69 (C-13), 133.02 (C-18), 134.07 (C-14), 135.10 (C-17), 136.25 (C-1′a), 174.25 (C-1), 207.05 (C-23) and 209.56 (C-20). ^1H and ^{13}C NMR data in CD_3OD are listed in Table 2.

3.1.4. Penochalasin F (4). Obtained as colourless oil $[\alpha]_D$=−80.0° (*c* 0.13, $CHCl_3$); λ_{max} (EtOH)/nm: (log ϵ): 220 (4.68), 240sh (4.28), 280 (4.17) and 291 (4.10); ν_{max} (liquid)/cm^{-1}: 3358(NH, OH), 1697 and 1685 (C=O); *m/z* (EI): 530 (M^+, 5%), 456 (48), 385 (71), 185 (60) and 130 (C_9H_8N, 100); *m/z* (HREI): 530. 2767 [M^+] (Calcd for $C_{32}H_{38}N_2O_5$: 530.2779). ^1H and ^{13}C NMR data are listed in Table 3.

3.1.5. Penochalasin G (5). Obtained as colourless powder, mp 124–126°C $[\alpha]_D$=−143.6° (*c* 0.19, $CHCl_3$); λ_{max} (EtOH)/nm: 220 (log ϵ 4.42), 240sh (3.82) and 280 (3.80); ν_{max} (KBr)/cm^{-1}: 3412 (NH, OH), 1702, 1688 and 1678 (C=O); *m/z* (EI): 514 (M^+, 7%), 417 (7), 389 (8), 302 (12), 212 (48), 183 (13), 130 (C_9H_8N, 63) and 121 (100); *m/z* (HREI): 514.2810 [M^+] (Calcd for $C_{32}H_{38}N_2O_4$: 514.2829). ^1H and ^{13}C NMR data are listed in Table 5.

3.1.6. Penochalasin H (6). Obtained as colourless powder mp 180–182°C, $[\alpha]_D$=−72.7° (*c* 0.18, $CHCl_3$); λ_{max} (EtOH)/nm: 220 (log ϵ 4.16), 243sh (3.97), 281 (3.52) and 290 (3.50); ν_{max} (KBr)/cm^{-1}: 3414(NH, OH), 1701 and 1692 (C=O); *m/z* (EI): 530 (M^+, 2%), 200 (9) and 130 (C_9H_8N, 100); *m/z* (HREI): 530.2763 [M^+] (Calcd for $C_{32}H_{38}N_2O_5$: 530.2779). ^1H and ^{13}C NMR data are listed in Table 4.

3.1.7. Chaetoglobosin O (7). Obtained as colourless oil $[\alpha]_D$=−139.1° (*c* 0.23, $CHCl_3$); λ_{max} (EtOH)/nm: 222 (log ϵ 4.47), 243sh (3.95), 281 (3.78) and 291 (3.74); ν_{max} (liquid)/cm^{-1}: 3398 (NH, OH), 1698 and 1689 (C=O); *m/z* (EI): 530 (M^+, 3%), 200 (8), 130 (C_9H_8N, 100); *m/z* (HREI): 530.2764 [M^+] (Calcd for $C_{32}H_{38}N_2O_5$: 530.2779). ^1H and ^{13}C NMR data were in accord with the published values.[7]

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