Penochalasins, a novel class of cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga: structure determination and solution conformation

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A novel class of cytochalasans, penochalasins A 1, B and C, have been isolated from a strain of *Penicillium* sp. originally separated from the marine alga *Enteromorpha intestinalis*, and their stereostructures have been established on the basis of NMR spectral analyses and chemical transformations. Different conformations of 1 in $CDCl_3$ and [$^2H_5$]pyridine were determined by analysis of NMR data and molecular modelling. All the compounds exhibited potent cytotoxicity against cultured P388 cells.

Marine microorganisms are potentially a prolific source of highly bioactive secondary metabolites for the development of new pharmaceutical agents. As part of a programme to discover new antitumour metabolites produced by marine microorganisms, we previously reported that antitumour and cytotoxic compounds were produced by microorganisms originally isolated from the marine fish *Halichoeres bleeki*[1] and *Pseudolabrus japonicus*,[2,3] and the marine algae *Sargassum tortile*[4-7] and *Enteromorpha intestinalis*,[8] and their structures were established. Two of them, communesins A and B, were structurally unique cytotoxic metabolites of a strain of *Penicillium* sp. isolated from *E. intestinalis*.[8] Further investigation of metabolites from this fungal strain led to the isolation of three new cytochalasans, designated penochalasins A **1**, B **2** and C **3**, together with known chaetoglobosins A **4**[9-12] and F **5**,[10,12,13] all of which showed potent cytotoxic activity in the P388 lymphocytic leukaemia test system in cell cultures. Chemically the cytochalasans are characterized by a highly substituted perhydroisoindol-1-one fused with a macrocyclic ring, which is a carbocycle, a lactone or a carbonate. A number of cytochalasans have been isolated and classified into several groups according to their structural features.[14] NMR analyses using heteronuclear multiple-bond connectivity (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments and chemical correlations revealed that penochalasins A–C, **1–3**, are a new class of cytochalasans with a macrocyclic ring including a pyrrole moiety. We report herein the stereostructures and cytotoxic activities of compounds **1–3** and the solution conformation of **1**.

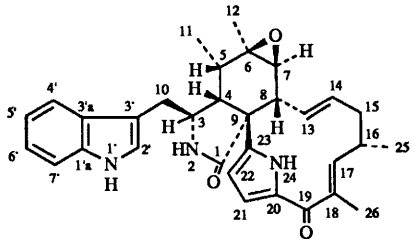
Results and discussion

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. The MeOH extract of the mycelium was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and high-performance liquid chromatography (HPLC) to afford penochalasins A **1**, B **2** and C **3**, and chaetoglobosins A **4** and F **5**. The last two have previously been isolated as metabolites of *Chaetomium* sp.,[9-11,13] but this is the first report of their occurrence as metabolites of *Penicillium* sp.

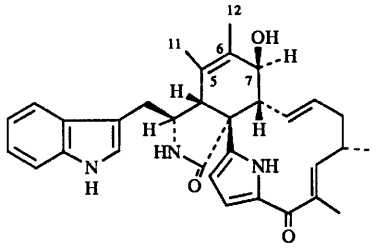
Penochalasin A **1** had the molecular formula $C_{32}H_{35}N_3O_3$ established by high-resolution electron impact mass spectrometry (HREIMS). Its IR spectrum contained absorption bands at 3359, 1690 and 1625 cm^{-1}, characteristic of NH, a carbonyl group and an aromatic ring. A close inspection of the ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$ (Table 1) by distortionless enhancement by polarization transfer (DEPT) and ^1H–^{13}C correlation spectroscopy (COSY) experiments revealed the presence of two secondary methyls (C-11 and C-25), an allylic methyl (C-26), a tertiary methyl (C-12), two methylenes (C-10 and C-15), six sp^3-hybridized methines (C-3, C-4, C-5, C-7, C-8 and C-16), 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 dienone (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 at *m/z* 130, corresponding to an indole ring with a methylene. In addition, the presence of a 2,5-disubstituted pyrrole moiety in **1** was assumed by the NMR signals at δ_H 6.31 (dd, *J* 3.9 and 2.7 Hz), 7.02 (dd, *J* 3.9 and 2.7 Hz) and 10.59 (br s), and at δ_C 109.48, 114.92, 126.79 and 138.90.[15] The appearance of the signals due to an sp^3 methine at δ_H 3.94 and δ_C 53.42 implied that one of the six sp^3 methines is linked to an amido group. This was supported by a cross peak between C-1 and 3-H in an HMBC experiment which was carried out in [$^2H_5$]pyridine because **1** is slightly soluble in $CDCl_3$ (Table 2). The signals due to a methine at δ_H 2.88 and δ_C 61.17 and due to an sp^3 quaternary carbon at δ_C 57.21 in $CDCl_3$ indicated the presence of a trisubstituted epoxide (C-6 and C-7).

The ^1H–^1H COSY analysis (Table 1) for the functional groups thus established led to partial structures A (C-1 to C-5, and C-10 and C-11) and B (C-6 to C-8, C-13 to C-18, and C-25 and C-26) (Fig. 1), which were supported by HMBC correlations (Table 2). The geometry of the double bonds in the partial structure B was deduced from a chemical shift (δ_C 13.52) of the carbon signal of the allylic methyl (C-26),[16] a coupling constant ($J_{13,14}$ 15.3 Hz) and NOEs between 26-H and 16-H, and 26-H and 25-H.

The connection of the partial structures A and B and the remaining functional groups (Fig. 1) was determined on the basis of HMBC correlations (Table 2). The typical HMBC correlations are summarised in Fig. 1. Based on this evidence, the planar structure of **1** was elucidated.



penochalasin A **1**



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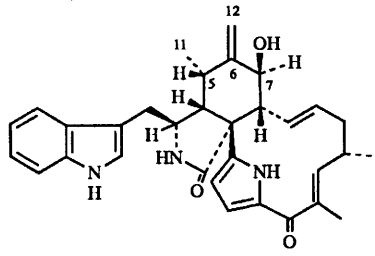
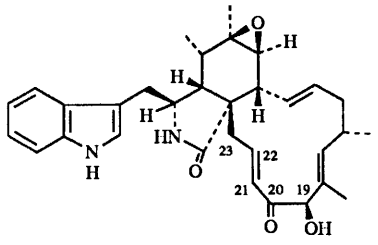
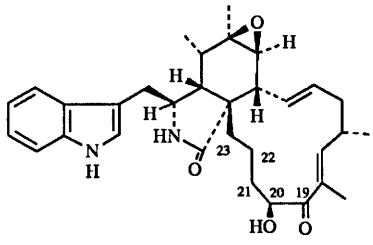


penochalasin C **3**



chaetoglobosin A **4**



chaetoglobosin F **5**

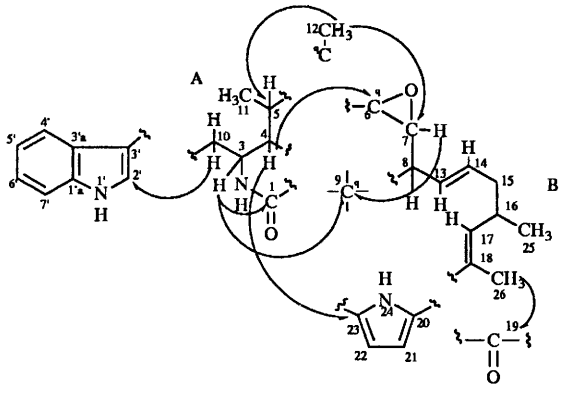


Fig. 1 Partial structures and functional groups of penochalisin A **1** and observed HMBC correlations

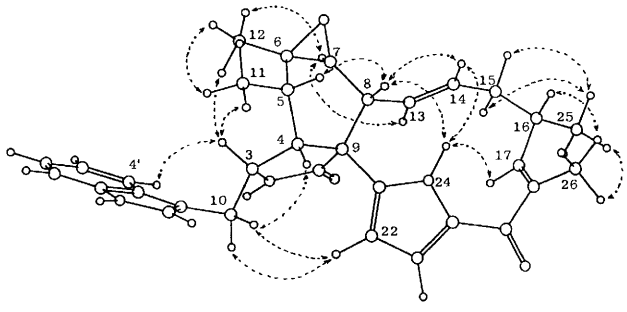


Fig. 2 Conformer **1a** of penochalasin A **1** in [$^2H_5$]pyridine and observed NOEs

The relative stereochemistry and conformation for **1** were established by NOESY experiments in [$^2H_5$]pyridine (Table 2). The conformation and the observed key NOEs are summarised in Fig. 2. Analysis of the observed coupling constants (Table 3) in the ^1H NMR spectrum of **1** in [$^2H_5$]pyridine, using a modified Karplus equation,[17] led to the required dihedral angles (Table 3), which except for those between 3-H and 10-H_2 supported the conformation assigned on the basis of NOE experiments. Since the $J_{3,10\alpha}$ and $J_{3,10\beta}$ values are roughly equal, the dihedral angles between 3-H and the 10α- and 10β-Hs should be nearly equal and be both *ca.* 60° or 120°. Selected difference NOE experiments in **1** showed nearly equal NOE values (4.5 and 4.7%, respectively) between 22-H and 10α-H and between 22-H and 10β-H (Table 4), implying that the distance between 22-H and 10α-H is almost the same as that between 22-H and 10β-H and hence both the 3-H/10α-H and 3-H/10β-H dihedral angles are approximately 120°. Considering the generalized Karplus relationship, the $J_{3,10\alpha}$ and $J_{3,10\beta}$ values (8.2 and 6.8 Hz) in **1** are unusually large for coupling constants between protons with a dihedral angle of 120°. It has been reported that values of vicinal coupling constants depend on the electronegativity of a substituent attached to the same carbon atom as one of the vicinally coupled protons.[18] It is therefore most likely that the unusual $J_{3,10\alpha}$ and $J_{3,10\beta}$ values in [$^2H_5$]pyridine are due to the formation of a hydrogen bond between 2-H and [$^2H_5$]pyridine, which is consistent with the fact that the proton signal of 2-H in [$^2H_5$]pyridine was found shifted downfield by 3.71 ppm, relative to that in $CDCl_3$. In addition to NOEs between 22-H and 10-H_2, selected difference NOE experiments around the 10-H_2 of **1** in [$^2H_5$]pyridine exhibited NOEs (2.6 and 2.8%, respectively) between 4'-H and 3-H and between 4-H and 10β-H (Table 4). In accordance with the NMR data mentioned above and listed in Tables 3 and 4, stereomodel **1a** (Figs. 2 and 3) was created as a conformer of **1** in [$^2H_5$]pyridine, using the CaChe work systems. Theoretical dihedral angles and internuclear distances (in part) for **1a** are shown in Tables 3 and 4.

The observed coupling constants in the ^1H NMR spectrum of

Table 1 ^1H and ^{13}C NMR data of penochalasin A **1** in $CDCl_3$

Position	δ_H[a]		^1H–^1H COSY	δ_C
1				175.34 (q)[b]
2	5.84s			
3	3.94dt	9.8 (10β), 4.8 (10α), 4.0 (4)	4, 10α, 10β	53.42 (t)
4	2.68dd	4.0 (3), 3.3 (5)	3, 5	54.29 (t)
5	1.99qd	7.3 (11), 3.3 (4)	4, 11	36.29 (t)
6				57.21 (q)
7	2.88d	5.8 (8)	8	61.17 (t)
8	2.71dd	10.0 (13), 5.8 (7)	7, 13	48.46 (t)
9				50.36 (q)
10α	3.07dd	14.2 (10β), 9.8 (3)	3, 10β	35.35 (s)
β	3.15dd	14.2 (10α), 4.8 (3)	3, 10α	
11	1.19d	7.3 (5)	5	13.37 (p)
12	1.30s			20.05 (p)
13	6.81ddd	15.3 (14), 10.0 (8), 1.8 (15β)	8, 14	132.80 (t)
14	5.78ddd	15.3 (13), 11.5 (15α), 3.6 (15β)	13, 15α	135.53 (t)
15α	2.16dt	13.5 (15β), 11.5 (14, 16)	14, 15β, 16	41.17 (s)
β	2.57dddd	13.5 (15α), 5.4 (16), 3.6 (14), 1.8 (13)	15α, 16	
16	2.88m		15α, 15β, 17, 25	34.11 (t)
17	5.65dq	9.5 (16), 1.4 (26)	16, 26	142.69 (t)
18				134.85 (q)
19				188.47 (q)
20				126.79 (q)
21	7.02dd	3.9 (22), 2.7 (24)	22, 24	114.92 (t)
22	6.31dd	3.9 (21), 2.7 (24)	21, 24	109.48 (t)
23				138.90 (q)
24	10.59br s			
25	1.10d	6.7 (16)	16	19.70 (p)
26	1.94d	1.4 (17)	17	13.52 (p)
1′	8.22br s		2′	
1′a				136.54 (q)
2′	7.10d	2.3 (1′)	1′	122.91 (t)
3′				111.33 (q)
3′a				130.10 (q)
4′	7.56d	8.0 (5′)	5′, 6′	118.19 (t)
5′	7.25t	8.0 (4′, 6′)	4′, 6′, 7′	122.62 (t)
6′	7.18t	8.0 (5′, 7′)	4′, 5′, 7′	120.10 (t)
7′	7.42d	8.0 (6′)	5′, 6′	111.69 (t)

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) 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.

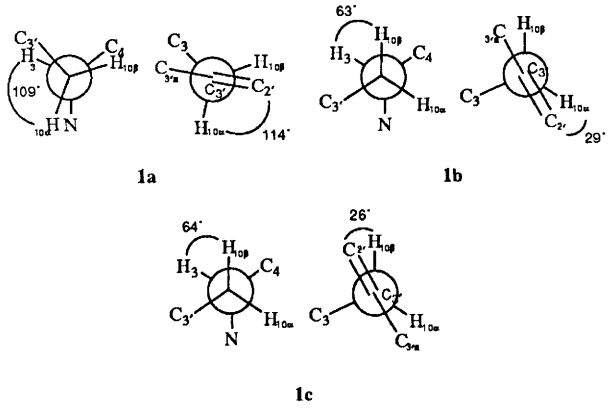


Fig. 3 Newman projection formulae of conformers **1a**, **1b** and **1c** in the C-3–C-10 and C-10–C-3′ bonds

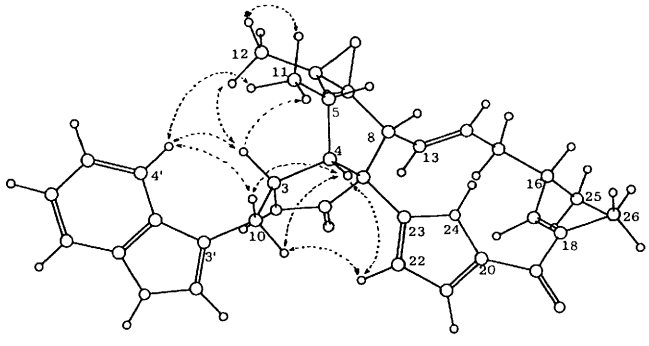


Fig. 4 Energy minimized conformer **1b** of penochalasin A **1** in $CDCl_3$ and observed NOEs

1 in $CDCl_3$ (Tables 1 and 3) showed close similarities with those in [$^2H_5$]pyridine (Table 2) except that the coupling constants between 3-H and 10β-H and between 3-H and 10α-H are 4.8 and 9.8 Hz, respectively. Analysis of the observed coupling constants in $CDCl_3$ by the modified Karplus relationship showed dihedral angles approximate to 60 and 180° between 3-H and 10β-H and between 3-H and 10α-H, respectively, in this case (Table 3). In selected difference NOE experiments of **1** in $CDCl_3$, NOEs for 22-H/10α-H and 3-H/10β-H were observed together with those for 4′-H/3-H and 4′-H/11-H, whereas no NOE for 22-H/10β-H and 3-H/10α-H was found (Table 4). Based on this evidence, **1b** (Figs. 3 and 4) was elucidated as a conformer of **1** existing in $CDCl_3$, which is formed from **1a** by rotation of the C-3–C-10 and C-10–C-3′ axes.

The conformational behaviour of **1** was investigated by computing the potential energy surface as a function of rotational angles (x and y) for the C-3–C-10 and C-10–C-3′ axes using the CaChe MM2 method. The conformational space was sampled by varying x and y in steps of 15° for the range from 0° to 360°. At each point a full geometry optimization was carried out, which yielded ten local minima. The energy for one of the minima was 124.51 kcal mol^{-1}† and a conformer with this energy corresponded to conformer **1b** in $CDCl_3$. The energy of the lowest minimum in the energy surface was 122.98 kcal mol^{-1} and a conformer **1c** with this energy showed the 3-H/10β-H and 10β-H/C-3′–C-2′ dihedral angles of −63.9 and 26.0°, respectively (Figs. 3 and 5). Though the energy (129.39 kcal mol^{-1}) of conformer **1a** in [$^2H_5$]pyridine was found to be higher

† 1 cal = 4.184 J.

Table 2 ^1H and ^{13}C NMR data of penochalasin A **1** in [$^2H_5$]pyridine

Position	δ_H[a]		NOEs[b]	δ_C	HMBC (C)
1				176.75 (q)[c]	
2	9.55br s		12		1, 4, 9
3	4.26ddd	8.2 (10α), 6.8 (10β), 2.2 (4)	11, 12, 4′	54.32 (t)	1, 4, 5, 9, 3′
4	2.80dd	5.2 (5), 2.2 (3)	10β, 11, 22	54.32 (t)	1, 3, 5, 6, 9, 10, 23
5	1.94qd	7.2 (11), 5.2 (4)	8	36.95 (t)	3, 4, 6
6				57.27 (q)	
7	3.30d	6.0 (8)	12, 13	62.10 (t)	6, 8, 9, 12, 13
8	3.30dd	9.5 (13), 6.0 (7)	5, 14, 24	48.11 (t)	1, 6, 7, 9, 14, 23
9				51.64 (q)	
10α	3.51dd	14.0 (10β), 8.2 (3)	22	35.65 (s)	3, 2′, 3′, 3′a
β	3.38dd	14.0 (10α), 6.8 (3)	4, 22		
11	0.92d	7.2 (5)	3, 4, 12	12.84 (p)	4, 5, 6
12	1.26s		2, 3, 7, 11	19.84 (p)	5, 6, 7
13	7.18ddd	15.5 (14), 9.5 (8), 1.8 (15β)	7, 17	132.97 (t)	7, 8, 9, 14, 15
14	5.89ddd	15.5 (13), 11.5 (15α), 3.6 (15β)	8, 16, 24	135.16 (t)	8, 15
15α	1.86dt	13.0 (15β), 11.5 (14, 16)	17, 25	41.23 (s)	13, 14, 16, 17, 25
β	2.37dddd	13.0 (15α), 5.4 (16), 3.6 (14), 1.8 (13)	25		13, 14, 16, 17
16	2.65m		14, 26	33.59 (t)	17, 18, 25
17	5.55dq	11.2 (16), 1.5 (26)	13, 15α, 24	146.46 (t)	15, 16, 25, 26
18				135.80 (q)	
19				189.65 (q)	
20				131.65 (q)	
21	7.36dd	4.0 (22), 2.5 (24)		114.37 (t)	22, 23
22	6.77dd	4.0 (21), 2.5 (24)	4, 10α, 10β	109.53 (t)	20, 21, 23
23				139.88 (q)	
24	11.06br s		8, 14, 17		20, 21, 22, 23
25	0.84d	6.7 (16)	15α, 15β, 26	19.42 (p)	15, 16, 17
26	1.90d	1.5 (17)	16, 25	12.84 (p)	17, 18, 19
1′	12.08br d	2.5 (2′)	7′		1′a, 2′, 3′, 3′a
1′a				137.76 (q)	
2′	7.49d	2.5 (1′)		124.74 (t)	1′a, 3′a, 3′, 10
3′				111.67 (q)	
3′a				128.44 (q)	
4′	7.85d	7.4 (5′)	3	118.83 (t)	1′a, 3′, 6′
5′	7.30td	7.4 (4′, 6′), 1.5 (7′)		119.60 (t)	3′a, 7′
6′	7.35t	7.4 (5′, 7′)		121.98 (t)	1′a, 4′
7′	7.65dd	7.4 (6′), 1.5 (5′)	1′	112.32 (t)	5′, 3′a

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) are followed by the multiplicity of the signals, the coupling constant (J/Hz) and the coupling proton in parentheses. [b] Observed in the NOESY experiment. [c] Letters p, s, t and q in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

Table 3 Experimental and theoretical coupling constants and dihedral angles in compound **1**

	[$^2H_5$]pyridine		**1a**	$CDCl_3$		**1b**
Atom numbers	J (Hz)	Dihedral angles (°)[a]	Dihedral angles (°)[b]	J (Hz)	Dihedral angles (°)[a]	Dihedral angles (°)[b]
3-H/4-H	2.2	114.9	125.8	4.0	124.6	127.0
3-H/10α-H	8.2	35.6 or 144.4	109.2	9.8	152.8	177.1
3-H/10β-H	6.8	42.2 or 135.5	−133.0	4.8	51.5	−63.2
4-H/5-H	5.2	49.6	50.2	3.3	58.9	51.8
7-H/8-H	6.0	134.1	152.7	5.8	133.2	153.6
8-H/13-H	9.5	151.1	166.6	10.0	153.9	165.6
13-H/14-H	15.5	180.0[c]	−179.7	15.3	180.0[c]	−179.8
14-H/15α-H	11.5	164.4	178.2	11.5	164.4	178.8
14-H/15β-H	3.6	57.4	−64.8	3.6	57.4	−64.1
15α-H/16-H	11.5	164.4	172.8	11.5	164.4	172.7
15β-H/16-H	5.4	48.7	55.2	5.4	48.7	55.0
16-H/17-H	11.2	161.9	152.1	9.5	151.1	150.8
10α-H/C-3′–C-2′			113.7			−29.0
10β-H/C-3′–C-2′			−0.5			−146.8
C-8–C-9/C-23–N-24			−5.7			−4.0
C-18–C-19/C-20–N-24			−3.8			−4.8

[a] Calculated by a modified Karplus equation ($J = 12.4 \cos^2 \varphi$; $0° \leqslant \varphi \leqslant 180°$).[17] [b] Theoretical. [c] Deduced from a *trans* coupling constant in an olefin.

than that of the minimum energy conformers in the CaChe MM2 calculations, it seems most likely that the energy is actually in lower levels due to other factors such as the solvent effect in [$^2H_5$]pyridine.

The absolute configuration assigned to **1** has not been established independently, but is assumed to be the same as for its co-metabolites, chaetoglobosins A **4** and F **5**.

Penochalasin C **3** had the same molecular formula as **1** as deduced from HREIMS. The general features of its UV, IR and NMR spectra (Table 5) closely resembled those of **1** (Table 1)

Table 4 Experimental NOE values in compound **1** and theoretical distances in conformers **1a** and **1b**

Atom numbers	**1** ([$^2H_5$]pyridine) NOEs	**1a** Distances (Å)	**1** ($CDCl_3$) NOEs	**1b** Distances (Å)
3-H/10α-H	≈0	2.86	≈0	3.10
3-H/10β-H	≈0	2.98	2.2 (3-H), 5.6 (10β-H)	2.57
3-H/11-H	2.8 (3-H), 2.5 (11-H)[a]	2.21	3.6 (3-H), 8.6 (11-H)	2.21
3-H/12-H	3.0 (3-H)	2.21	4.9 (3-H)	2.17
4-H/10α-H	≈0	3.42	2.5 (4-H), 2.8 (10α-H)	2.54
4-H/10β-H	1.9 (4-H), 2.8 (10β-H)	2.16	3.1 (4-H), 4.4 (10β-H)	2.65
11-H/12-H	2.4 (11-H)	2.46	—[b]	2.55
22-H/4-H	2.6 (22-H), 2.3 (4-H)	2.73	3.3 (22-H), 4.0 (4-H)	2.78
22-H/10α-H	2.9 (22-H), 4.5 (10α-H)	2.92	2.2 (22-H), 5.9 (10α-H)	2.25
22-H/10β-H	4.0 (22-H), 4.7 (10β-H)	2.56	≈0	3.84
4'-H/3-H	1.2 (4'-H), 2.6 (3-H)	2.50	3.1 (4'-H), 4.9 (3-H)	2.86
4'-H/10β-H	≈0	4.20	3.5 (4'-H), 5.4 (10β-H)	2.51
4'-H/11-H	≈0	4.12	0.4 (4'-H), 5.3 (11-H)	2.64
4'-H/12-H	≈0	3.56	≈0	4.06

[a] Protons irradiated in parentheses. [b] The NOE could not be evaluated because the signals for 11-H and 12-H are close together.

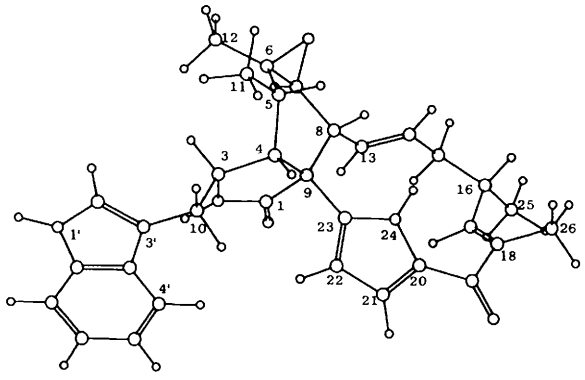


Fig. 5 Lowest minimum energy conformer **1c**

except that the signals for 5-H, 7-H, 12-H, C-1, C-5, C-6, C-7, C-12 and C-14 revealed chemical shifts different from those of **1**. The typical ^{13}C NMR signals for an epoxide at δ_C 57.21 (C-6) and 61.17 (C-7) in **1** were replaced by those due to a sp^2 quaternary carbon and a hydroxymethine at δ_C 147.92 and 68.62 in **3**, respectively. The methine proton doublet (7-H, δ_H 4.02) coupled to 8-H was sharpened by D_2O exchange, implying that it is due to a hydroxymethine. In addition, the C-12 methyl proton singlet (δ_H 1.30) in **1** was missing from **3** and replaced by the typical signals due to a terminal methylene at δ_H 5.25 and 5.48 in **3**. This was supported by the ^{13}C NMR signals (δ_C 142.92 and 114.62) for C-6 and C-12. The above summarised evidence led to planar structure **3** for penochalasin C.

Penochalasin B **2** was assigned the same molecular formula as **1** and **3** as deduced from HREIMS. Its IR, UV and NMR spectra (Table 5) showed close correspondence with those of **1** (Table 1) except that the ^1H NMR signal of 5-H in **1** disappeared from **2** and the signals for 7-H, 11-H, 12-H, C-4–C-7, C-9, C-12, C-14 and C-17 in **2** were found shifted downfield, relative to those of **1**. The appearance of two allylic methyl proton singlets (11-H and 12-H) at δ_H 1.77 and 1.66 and two quaternary carbon signals (C-5 and C-6) at δ_C 127.23 and 131.48 implied the presence of a tetrasubstituted double bond linked to two methyls in **2**, and the methine proton doublet (7-H) coupled to 8-H was assigned as a hydroxymethine on the basis of its chemical shift (δ_H 3.98) and the fact that the signal was sharpened by D_2O exchange. These observations allowed assignment of planar structure **2** to penochalasin B.

Treatment of **1** with 2% HCl in MeOH–CH_2Cl_2 (2:1) afforded **2** and **3** in a ratio of 2:1. Based on this finding, the absolute stereostructures of penochalasins B and C are represented as **2** and **3**, respectively. These were supported by the observed coupling constants in **2** and **3**.

The cytotoxic activities of penochalasins A **1**, B **2** and C **3** were examined in the P388 lymphocytic leukaemia test system in cell cultures, according to the method reported previously.[19] Compounds **1**, **2**, **3**, **4** and **5** all exhibited potent cytotoxic activity (ED_{50} 0.4, 0.3, 0.5, 0.6 and 0.9 μg cm^{-3}, respectively).

Experimental

General procedures

Mps were obtained on a Yanagimoto micromelting point apparatus and are uncorrected. 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 and are given in units of 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded on a JASCO J-500A spectrometer. NMR spectra were recorded at 27 °C on a Varian XL-300 spectrometer, operating at 300 and 75.4 MHz for ^1H and ^{13}C, respectively, with tetramethylsilane (TMS) as an internal reference. The ^1H–^1H and ^1H–^{13}C COSY spectra were recorded on a Varian XL-300 spectrometer, and the HMBC and NOESY spectra on a Bruker DMX spectrometer with the usual parameters. EIMS were determined using a Hitachi M-80 spectrometer. Liquid chromatography over silica gel (mesh 230–400) was performed under 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 id). 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 a UV lamp and sprayed with 10% H_2SO_4 followed by heating. MM2 calculations were carried out using the CaChe work system on a Macintosh platform.

Culturing and isolation of metabolites

A strain of *Penicillium* sp. was initially separated from the marine alga *Enteromorpha intestinalis* (Linne) Link (Ulvaceae) collected from Tanabe Bay, Japan. The marine alga was homogenized with sterile artificial seawater and applied to the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting colonies provided a pure strain of *Penicillium* sp. The fungal strain was grown in a liquid medium (20 dm^3) 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 which was filtered off was extracted (3×) with MeOH. The combined extracts were evaporated under reduced pressure and the resulting concentrate (63 g) was passed through Sephadex

Table 5 ^1H and ^{13}C NMR data of penochalasins B **2** and C **3** in $CDCl_3$

	2			**3**		
Position	δ_H[a]		δ_C	δ_H		δ_C
1			175.25 (q)[b]			169.87 (q)
2	5.75br s			5.80br s		
3	3.61ddd	9.0 (10α), 5.8 (10β), 2.5 (4)	53.91 (t)	3.54dt	10.2 (10α), 4.0 (4, 10β)	53.16 (t)
4	3.00br d	2.5 (3)	60.23 (t)	2.75t	4.0 (3, 5)	53.00 (t)
5			127.23 (q)	2.98qd	6.5 (11), 4.0 (4)	32.32 (t)
6			131.48 (q)			147.92 (q)
7	3.98br d	10.0 (8)	67.47 (t)	4.02br d	10.0 (8)	68.62 (t)
8	2.64t	10.0 (7, 13)	47.71 (t)	3.05t	10.0 (7, 13)	49.82 (t)
9			53.68 (q)			49.82 (q)
10α	3.22dd	14.0 (10β), 9.0 (3)	34.35 (s)	2.98dd	14.0 (10β), 10.2 (3)	34.85 (s)
β	3.24dd	14.0 (10α), 5.8 (3)		3.16dd	14.0 (10α), 4.0 (3)	
11	1.77s		13.05 (p)	1.24d	6.5 (11)	15.10 (p)
12	1.66s		17.87 (p)	5.25s		114.62 (s)
				5.48s		
13	6.69dd	15.5 (14), 10.0 (8), 1.6 (15β)	132.06 (t)	6.67ddd	15.5 (14), 10.0 (8), 1.6 (15β)	132.63 (t)
14	5.74ddd	15.5 (13), 11.5 (15α), 3.2 (15β)	137.94 (t)	5.82ddd	15.5 (13), 11.5 (15α), 3.2 (15β)	138.08 (t)
15α	2.17dt	13.5 (15β), 11.5 (14, 16)	41.35 (s)	2.19dt	13.5 (15β), 11.5 (14, 16)	41.27 (s)
β	2.60dddd	13.5 (15α), 4.8 (16), 3.2 (14), 1.6 (13)		2.61dddd	13.5 (15α), 4.8 (16), 3.2 (14), 1.6 (13)	
16	2.85m		33.93 (t)	2.91m		34.09 (t)
17	5.61dq	9.0 (16), 2.0 (26)	145.10 (t)	5.68 dq	9.4 (16), 1.8 (26)	142.07 (t)
18			135.13 (q)			135.08 (q)
19			189.49 (q)			188.04 (q)
20			126.81 (q)			126.90 (q)
21	6.97dd	3.9 (22), 2.7 (24)	114.46 (t)	7.02dd	3.9 (22), 2.7 (24)	115.07 (t)
22	6.41dd	3.9 (21), 2.7 (24)	109.47 (t)	6.18dd	3.9 (21), 2.7 (24)	109.17 (t)
23			138.50 (q)			139.81 (q)
24	9.89br s			10.78br s		
25	1.12d	7.0 (16)	19.52 (p)	1.10d	7.0 (16)	19.78 (p)
26	1.95d	2.0 (17)	13.77 (p)	1.95d	2.0 (17)	13.68 (p)
7-OH	1.90br s			2.00br s		
1′	8.13br s			8.21br s		
1′a			136.45 (q)			136.51 (q)
2′	7.09d	2.0 (1′)	122.57 (t)	7.09d	2.3 (1′)	122.86 (t)
3′			111.53 (q)			111.47 (q)
3′a			130.88 (q)			129.77 (q)
4′	7.50dd	8.0 (5′), 1.0 (6′)	118.41 (t)	7.55dd	8.0 (5′), 1.0 (6′)	118.44 (t)
5′	7.21td	8.0 (4′, 6′). 1.0 (7′)	122.57 (t)	7.25td	8.0 (4′, 6′), 1.0 (7′)	122.60 (t)
6′	7.15td	8.0 (5′, 7′), 1.0 (4′)	119.91 (t)	7.15td	8.0 (5′, 7′), 1.0 (4′)	119.99 (t)
7′	7.39dd	8.0 (6′), 1.0 (5′)	111.69 (t)	7.40dd	8.0 (6′), 1.0 (5′)	111.62 (t)

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) 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.

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 system as the eluent. The MeOH–CH_2Cl_2 (1:199) eluate (659 mg) was purified by HPLC [MeOH–water (4:1)] to afford compounds **1** (14.4 mg), **2** (9.6 mg), **3** (4.9 mg), **4** (10.2 mg) and **5** (9.1 mg), of which the last two were identified by comparison of their UV, IR and ^1H and ^{13}C NMR data with those reported previously.[10–13]

Penochalasin A 1. Obtained as colourless needles, mp 222–224 °C (from acetone), $[\alpha]_D$ −10 (*c* 0.20 in $CHCl_3$); λ_{max}(EtOH)/nm 206 (log ε 4.58), 222 (4.66), 285 (4.40), 292 (4.09) and 313 (4.13); ν_{max}(KBr)/cm^{-1} 3359 (NH), 1690 (CON, C=C–C=O), 1623 (Ar–C–C); *m/z* (EI) 509 (33%, M^+), 442 (5), 380 (13), 243 (19), 202 (15), 171 (22), 131 (38) and 130 (100, C_9H_8N); [*m/z* (HREI) Found: M^+, 509.2677. $C_{32}H_{35}N_3O_3$ requires *M*, 509.2677]; CD λ(*c* 9.00 × 10^{-5} mol dm^{-3} in EtOH)/nm 225 (Δε +1.7), 233 (0), 240 (−1.1), 247 (0), 263 (+2.0), 295 (+3.4), 317 (0), 338 (−3.0) and 397 (0). ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

Penochalasin B 2. Obtained as a colourless powder, mp 177–179 °C, $[\alpha]_D$ −6.2 (*c* 0.2 in $CHCl_3$); λ_{max}(EtOH)/nm 206 (log ε 4.10), 222 (4.10), 284 (4.08), 293 (4.50) and 312 (4.45); ν_{max}(KBr)/cm^{-1} 3410 (NH, OH), 1693 (CON, C=C–C=O) and 1628 (Ar–C–C); *m/z* (EI) 509 (33%, M^+), 491 (4), 380 (6), 379 (8), 362 (4), 361 (4), 306 (5), 305 (4), 131 (67) and 130 (100, C_9H_8N); [*m/z* (HREI) Found: M^+, 509.2686. $C_{32}H_{35}N_3O_3$ requires *M*, 509.2677]; ^1H and ^{13}C NMR data are listed in Table 2.

Penochalasin C 3. Obtained as a colourless powder, mp 173–178 °C, $[\alpha]_D$ −6.2 (*c* 0.1 in $CHCl_3$); λ_{max}(EtOH)/nm 208 (log ε 4.50), 222 (4.59), 284 (3.92), 293 (3.96) and 315 (3.87); ν_{max}(KBr)/cm^{-1} 3371 (NH, OH), 1693 (CON, C=C–C=O), 1628 (Ar–C–C); *m/z* (EI) 509 (33%, M^+), 380 (1), 243 (3), 131 (36) and 130 (100, C_9H_8N); [*m/z* (HREI) Found: M^+, 509.2668. $C_{32}H_{35}N_3O_3$ requires *M*, 509.2677]; ^1H and ^{13}C NMR data are listed in Table 2.

Formation of Penochalasins B 2 and C 3 from Penochalasin A 1

A solution of 10% aqueous HCl (0.1 cm^3) was added to a solution of **1** (4.0 mg) in CH_2Cl_2 and MeOH (1:2; 0.4 cm^3). The reaction mixture was stirred for 30 min at room temp. after which it was diluted with water, neutralised with aqueous NH_3 and extracted with CH_2Cl_2. Evaporation of the extract under reduced pressure followed by HPLC (ODS) using MeOH–water (4:1) afforded **2** (2.1 mg) and **3** (1.2 mg), which were identified by comparison with authentic samples.

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