Penostatins F–I, novel cytotoxic metabolites from a *Penicillium* species separated from an *Enteromorpha* marine alga

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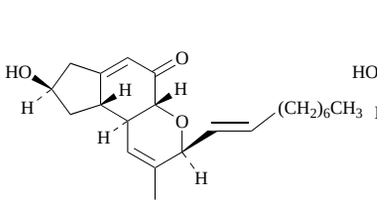
Penostatins F–I have been isolated from a strain of *Penicillium* sp. originally separated from the marine alga *Enteromorpha intestinalis*, and their stereostructures have been established on the basis of spectral analyses. All the compounds exhibit significant cytotoxicity against cultured P388 cells.

Introduction

Marine microorganisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. In our programme devoted to the search for new antitumour metabolites from microorganisms inhabiting the marine environment, we have found a number of antitumour and/or cytotoxic compounds from various fungi and an actinomycete originally isolated from various marine organisms, and elucidated their structures.[1–7] As part of this study, we have previously isolated the cytotoxic communesins,[3] penochalasins[4] and penostatins A (**1**)–D[5] 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 four additional new cytotoxic compounds, penostatins F–I, compounds **2**–**5** respectively.

It is noteworthy that these are a new class of substances with a different ring system from the previously isolated penostatins A (**1**)–D and all the asymmetric centres except one have the opposite absolute configurations between pairs of stereoisomers (**2** and **5**). We report herein the isolation and structure determination of compounds **2**–**5** and their cytotoxic activities.



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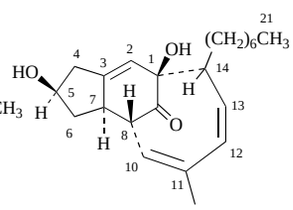

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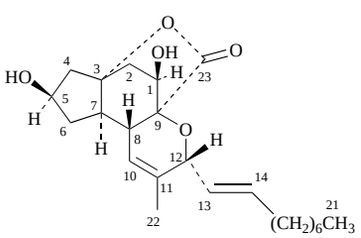

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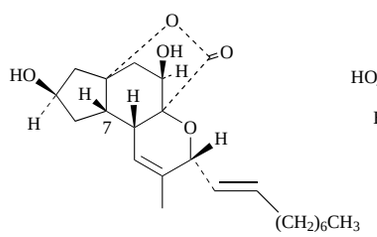

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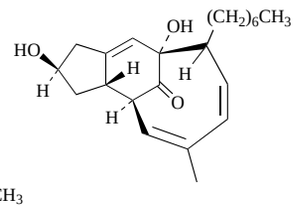

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Results and discussion

In this experiment, the fungal strain was cultivated in a different medium from that used for the previous experiment[3,4] in which communesins and penochalasins were obtained. Namely, it was cultured at 27 °C for 3 weeks in a medium containing 2% glucose, 1% peptone and 2% malt extract in distilled water adjusted to pH 7.5. Although a medium in the previous experiment[3,4] was prepared from seawater, distilled water was used in the preparation of a medium in this case. The MeOH extract of the mycelial cake was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography and HPLC to afford penostatins F–I **2**–**5**.

Penostatin F **2** had its molecular formula, $C_{22}H_{32}O_3$, established by high-resolution electron-impact mass spectrometry (HR-EIMS). Its IR spectrum contained absorption bands at 3451, 1715 and 1610 cm^{-1}, characteristic of a hydroxy group, a ketone and a double bond. A close inspection of the ^1H and ^{13}C NMR spectra of compound **2** (Table 1) by DEPT † and ^1H–^{13}C COSY † experiments revealed the presence of a ketone (C-9), a quaternary sp^3-hybridised carbon (C-1) bearing a hydroxy group, one disubstituted (C-12 and C-13) and two trisubstituted double bonds (C-2 and C-3, and C-10 and C-11), an allylic methyl (C-22), a primary methyl (C-21), eight methylenes (C-4, C-6 and C-15– C-20) and four sp^3 methines (C-5, C-7, C-8 and C-14) including one hydroxymethine (C-5). The EIMS fragment at *m/z* 245 [$M^+ - 99$] implied that the primary methyl (C-21) and the six methylenes (C-15–C-20) constitute an *n*-heptyl group.

The ^1H–^1H COSY analysis (Table 1) for the functional groups thus established led to a partial structure (Fig. 1). The connection of C-3 to both C-4 and C-7 was deduced from cross-peaks attributed to long-range couplings between 2-H and both 4-H and 7-H in the ^1H–^1H COSY, and supported by three-bond 2D heteronuclear multiple-bond connectivity (HMBC) correlations from both 5-H and 6-H to C-3 (Table 1). In addition, the connectivity of C-11 to C-10, C-12 and C-22 was deduced from cross-peaks attributed to long-range couplings between 22-H_3 and both 10-H and 12-H in the ^1H–^1H COSY, and supported by three-bond HMBC correlations from both 8-H and 13-H to

† DEPT = distortionless enhancement by polarization transfer; COSY = chemical-shift correlation spectroscopy.

Table 1 ^{1}H and ^{13}C spectral data of penostatin F **2** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	NOEs (H) [b]	δ_C	HMBC (C)
1					81.95 (q) [c]	
2	5.68q	2.2 (4α, 4β, 7)	4α, 4β, 7	4α, 4β, 15A, 1-OH	125.38 (t)	4, 7
3					144.20 (q)	
4α	2.77br dd	17.0 (4β), 6.5 (5)	2. 4β, 5	2, 5	39.57 (s)	2, 3, 5, 6, 7
β	2.26 ddt	17.0 (4α), 6.5 (5), 2.2 (2, 7)	2, 4α, 5, 7	2, 5, 6β		2, 3, 5, 6, 7
5	4.42 quint	6.5 (4α, 4β, 6α, 6β)	4α, 4β, 6α, 6β	4α, 4β, 6α, 7	72.32 (t)	3, 7
6α	2.57ddt	12.5 (6β), 6.5 (5), 2.2 (2, 7)	5, 6β, 7	5, 7, 8, 10	42.51 (s)	3, 4, 5, 7
β	1.53ddd	12.5 (6α), 10.5 (7), 6.5 (5)	5, 6α	4β, 8		4, 5, 7, 8
7	2.90m		2. 4β, 6α, 8	5, 6α, 10	48.75 (t)	8
8	3.04t	6.0 (7, 10)	7, 10, 22	6α, 6β, 10	50.01 (t)	1, 6, 7, 10, 11
9					211.48 (q)	
10	5.55dq	6.0 (8), 1.5 (22)	8.22	6α, 7, 8, 22	126.62 (t)	7, 8, 9, 11, 12, 13, 22
11					130.66 (q)	
12	5.73d	11.8 (13)	13, 14, 22	13, 22	130.37 (t)	10, 11, 13, 14, 22
13	3.88dd	11.8 (12), 9.0 (14)	12, 14	12, 15B, 1-OH	133.24 (t)	1, 10, 11, 12, 14, 15
14	2.54dt	9.0 (13), 5.0 (15A, 15B)	12, 13, 15A, 15B	15A, 15B	42.93 (t)	1, 12, 13, 15
15A	1.64m		14	2, 14	28.15 (s)	1, 14
B	1.65m		14	13, 14		1, 14
16	1.25br s				27.97 (s)	
17	1.25br s				29.53 (s)	
18	1.25br s				29.16 (s)	
19	1.25br s				31.86 (s)	
20	1.35br s		21		22.66 (s)	
21	0.87t	6.5 (21)	20		14.13 (p)	19, 20
22	1.79d	1.5 (20)	8, 10, 12	10, 12	25.62 (p)	10, 11, 12, 13
1-OH	3.71s			2, 13		
5-OH	1.63br s					

[a] ^{1}H Chemical-shift values (δ ppm from TMS) followed by multiplicity and then the coupling constant (*J*/Hz). Figures in parentheses indicate a proton coupling with that position. [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.

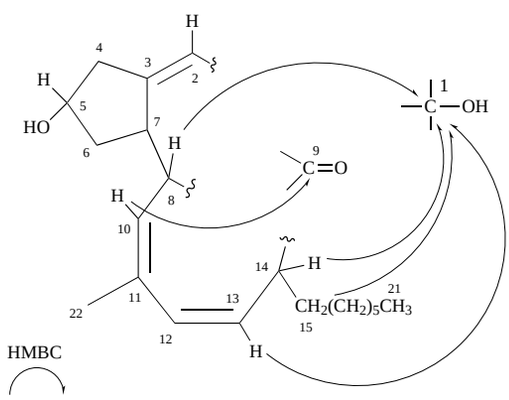


Fig. 1 A partial structure and functional groups of penostatin F **2** and observed HMBC correlations

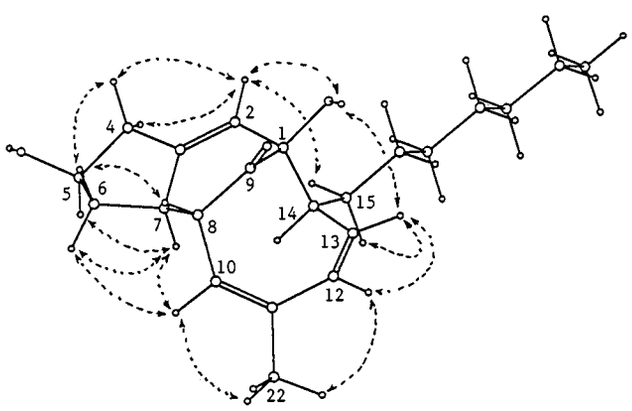


Fig. 2 Energy-minimised conformation of compound **2** and observed NOEs

C-11. HMBC correlations from 10-H to C-9, 8-H to C-1, and both 13-H and 14-H to C-1 implied that C-9 and C-14 are linked to both C-1 and C-8, and to C-1, respectively. Consequently the remaining bonds at C-1 and C-2 should be combined. The geometrical configuration of the conjugated diene (C-10–C-13) was deduced as 10-*z*, 11-*s*-*cis* and 12-*z* from nuclear Overhauser enhancements (NOEs) for 10-H/22-H, 22-H/12-H and 12-H/13-H, the chemical shift of C-22 (δ_C 25.62) [8] and the coupling constant ($J_{12,13}$ 11.8 Hz) (Table 1). The above summarised evidence led to planar structure **2** for penostatin F.

The relative stereochemistry of compound **2** was established by a combination of observed coupling constants, NOE spectroscopy (NOESY) data (Table 1) and selected difference NOE values. The 5-hydroxymethylene proton was observed as a quintet, showing that the coupling constants from 5-H to the four protons of the C-4 and C-6 methylenes are equivalent. Based on the generalized Karplus relationship,[9,10] the observed coupling constant (*J* 6.5 Hz) showed that dihedral angles for 5-H/4-H$^{\alpha}$, 5-H/4-H$^{\beta}$, 5-H/6-H$^{\alpha}$ and 5-H/6-H$^{\beta}$ were approximately 30°, 150°, 30° and 150°, respectively. The dihedral angles were nearly equivalent to those of a minimised-energy structure obtained by the CaChe MM2 method (Fig. 2). Assignments for 4-H$^{\alpha}$, 4-H$^{\beta}$, 6-H$^{\alpha}$ and 6-H$^{\beta}$ were deduced from these dihedral angles, selected difference NOE values between each of these protons and 5-H [5-H/4-H$^{\alpha}$ (2.2%) > 5-H/4-H$^{\beta}$ (0.3%); 5-H/6-H$^{\alpha}$ (1.9%) > 5-H/6-H$^{\beta}$ (≈0%)] and NOEs between 4-H$^{\beta}$ and 6-H$^{\beta}$ (Table 1). The observation of an NOE between 5-H and 7-H in compound **2** (Table 1 and Fig. 2) implied that these protons are in a co-pseudoaxial arrangement, and hence the 5-hydroxy group is oriented pseudoequatorially. Larger NOE values for 8-H/6-H$^{\beta}$ and 7-H/6-H$^{\alpha}$ [8-H/6-H$^{\beta}$ (2.0%) > 8-H/6-H$^{\alpha}$ (0.4%); 7-H/6-H$^{\alpha}$ (4.2%) > 7-H/6-H$^{\beta}$ (≈0%)] indicated that 7-H is arranged *trans* to 8-H and 6-H$^{\beta}$, and consequently, *cis* to the C-8–C-10 bond which should be structurally *cis* to the C-1–C-14 bond. This was supported by dihedral angles for 7-H/6-H$^{\alpha}$, 7-H/6-H$^{\beta}$ and 7-H/8-H (~60°, 160° and 130°, respectively), analyzed by the coupling constants ($J_{6\alpha,7}$ 2.2 Hz, $J_{6\beta,7}$ 10.5 Hz and $J_{7,8}$ 6 Hz). NOEs for 13-H/1-OH, 15-H^{A}/2-H and 15-H^{B}/13-H implied that both the *n*-heptyl and 1-hydroxy groups are on the same side as 13-H. These findings allowed assignment of the relative stereostructure of compound **2**.

Table 2 ^{1}H and ^{13}C spectral data of penostatin I **5** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	NOEs (H) [b]	δ_C
1					81.95 (q) [c]
2	5.67q	2.5 (4α, 4β, 7)	4α, 4β, 7	4α, 4β, 15, 1-OH	125.38 (t)
3					144.20 (q)
4α	2.64br dd	18.0 (4β), 5.0 (5)	2, 4β, 5	2, 5, 6α	39.57 (s)
β	2.41br d	18.0 (4α)	2, 4α, 5, 6β, 7	2, 5	
5	4.53t	5.0 (4α, 6α)	4α, 4β, 6α, 6β	4α, 4β, 6α, 6β	72.32 (t)
6α	1.54td	12.5 (6β, 7), 5.0 (5)	5, 6β, 7	4α, 5, 8	42.51 (s)
β	2.33br ddd	12.5 (6α), 7.0 (7), 2.0 (4β)	4β, 5, 6α, 7	5, 7, 8, 10	
7	3.32m		2, 4β, 6α, 6β, 8	6β, 8, 10, 14	48.75 (t)
8	2.98br t	6.5 (7, 10)	7, 10, 22	6α, 6β, 7, 10	50.01 (t)
9					211.48 (q)
10	5.56dq	6.5 (8), 1.0 (22)	8, 22	6β, 7, 8, 22	126.62 (t)
11					130.66 (q)
12	5.73d	11.8 (13)	13, 14, 22	13, 22	130.37 (t)
13	5.39dd	11.8 (12), 9.0 (14)	12, 14	12, 14, 15, 1-OH	133.24 (t)
14	2.66dt	9.0 (13), 7.0 (15)	12, 13, 15	7, 13, 15	42.93 (t)
15	1.66br q	7.0 (14, 16)	14, 16	2, 13, 14	28.15 (s)
16	1.25br s		15		27.97 (s)
17	1.25br s				29.53 (s)
18	1.25br s				29.16 (s)
19	1.25br s				31.86 (s)
20	1.35m		21		22.66 (s)
21	0.87t	6.5 (20)	20		14.13 (p)
22	1.59br s		8, 10, 12	10, 12	25.62 (p)
1-OH	3.70s			2, 13	
5-OH	1.75br s				

[a,c] As in Table 1. [b] Observed in the selected difference NOE experiment.

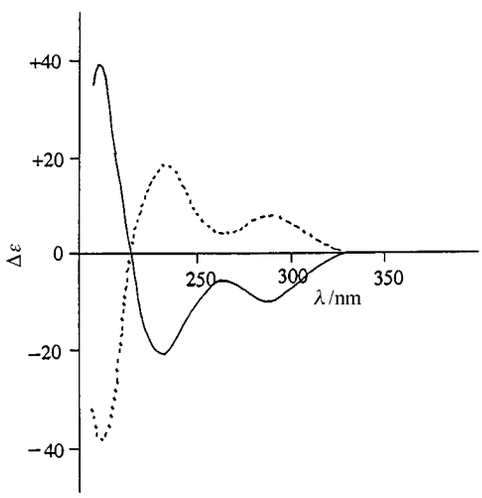
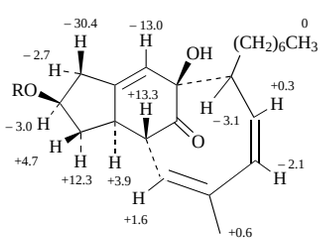


Fig. 3 CD spectra of isomers **2** (——) and **5** (- - -)

In the circular dichroism (CD) spectrum of compound **2** (Fig. 3), there were two negative bands at 285 and 232 nm due to chromophores associated with a dissymmetric β,γ-unsaturated ketone[11] and a twist *cisoid*-conjugated diene,[12] respectively. Application of the negative Cotton effect (285 nm) to the octant rule concerning dissymmetric β,γ-unsaturated ketones[11] allowed assignment of the absolute configuration represented in structure **2**. This assignment was supported by the negative Cotton effect at 232 nm due to the twist *cisoid*-conjugated diene. Based on the modified Mosher method,[13] the ^{1}H chemical-shift differences between the '(*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid' (MTPA) esters **6a** and **6b** (Fig. 4) of compound **2** led to the same absolute stereostructure as established by the CD data for penostatin F.

Penostatin I **5** had the same molecular formula as penostatin F **2**. The general spectral features of compound **5** closely resembled those of its isomer **2** except for the chemical shifts of 4-H, 5-H, 6-H, 7-H and 22-H, and a coupling relationship of 5-H in the NMR spectra (Table 2). This evidence suggested that compound **5** is a stereoisomer of penostatin F **2** at C-5. The appearance of the 5-H signal as a triplet (*J* 5.0 Hz) implied that



6a R = (*R*)-MTPA
6b R = (*S*)-MTPA

Fig. 4 ^{1}H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**6a** and **6b**) of penostatin F (**2**). Δδ-values are expressed in Hz (300 Hz)

the proton is coupling only to one proton each (4-H$^\alpha$ and 6-H$^\alpha$) of the 4- and 6-methylene. Analysis of the observed coupling constant (*J* 5.0 Hz) by the Karplus relationship suggested that dihedral angles for 5-H/4-H$^\alpha$, 5-H/4-H$^\beta$, 5-H/6-H$^\alpha$ and 5-H/6-H$^\beta$ were approximately 40°, 80°, 40° and 80°, respectively, showing that the conformation of the five-membered ring in isomer **5** is different from that in isomer **2** and that consequently the 5-hydroxy group is pseudoaxial (Figs. 2 and 5). Assignments for each proton of the 4- and 6-methylene were supported by a W-type of long-range coupling between 4-H$^\beta$ and 6-H$^\beta$ (Table 2) and NOE values [5-H/4-H$^\alpha$ (3.5%) > 5-H/4-H$^\beta$ (1.4%); 5-H/6-H$^\alpha$ (2.8%) > 5-H/6-H$^\beta$ (1.0%)]. Larger NOE values for 8-H/6-H$^\alpha$ and 7-H/6-H$^\beta$ [8-H/6-H$^\alpha$ (5.3%) > 8-H/6-H$^\beta$ (0.6%); 7-H/6-H$^\beta$ (3.9%) > 7-H/6-H$^\alpha$ (≈0%)] and the coupling constants from 7-H to 8-H, 6-H$^\alpha$ and 6-H$^\beta$ ($J_{6\alpha,7}$ 12.5 Hz, $J_{6\beta,7}$ 7.0 Hz and $J_{7,8}$ 6.5 Hz) (Table 2) implied that 7-H is arranged pseudoaxially and *trans* to 8-H and 6-H$^\alpha$, and consequently *cis* to the 5-hydroxy group with a pseudoaxial arrangement. Observations of NOEs for 7-H/14-H, 13-H/1-OH, 13-H/15-H and 2-H/15-H (Table 2 and Fig. 5) showed the *n*-heptyl and 1-hydroxy groups to be on the same side as 13-H. The above evidence led to relative stereostructure **5**, in which the 5-hydroxy group with a pseudoequatorial arrangement in stereoisomeric compound **2** was now arranged pseudoaxially.

Table 3 ^{1}H and ^{13}C spectral data of penostatin G **3** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	NOEs (H) [b]	δ_C	HMBC (C)
1	4.10dd	9.5 (2α), 4.2 (2β)	2α, 2β, 1-OH	2α, 2β, 1-OH	68.11 (t) [c]	2, 3, 8, 9, 10, 23
2α	2.48br ddd	15.0 (2β), 9.5 (1), 2.0 (7)	1, 2β, 4β, 7	1, 4β	37.99 (s)	1, 3, 4, 7, 9
β	2.16dd	15.0 (2α), 4.2 (1)	1, 2α	1, 4β, 6β, 8		1, 3, 4, 7, 9
3					84.74 (q)	
4α	2.30dd	14.2 (4β), 9.2 (5)	4β, 5	5	43.83 (s)	2, 3, 5, 6, 7
β	1.80br d	14.2 (4α)	2α, 4α, 5	2α, 2β, 5		2, 3, 5, 6, 7
5	4.58br ddd	9,2 (4α), 7.5 (6α), 6.0 (6β)	4α, 4β, 6α, 6β	4α, 4β, 6α, 6β, 7	70.41 (t)	3
6α	2.63dt	13.2 (6β), 7.5 (5, 7)	5, 6β, 7	5, 7	36.18 (s)	3, 4, 5, 7
β	1.56td	13.2 (6α, 7), 6.0 (5)	5, 6α, 7	2β, 5, 8		5, 7, 8
7	1.78m		2α, 6α, 6β, 8	5, 6α, 10	51.02 (t)	2, 3, 6, 8, 10
8	2.93br dq	8.2 (7), 2.2 (22)	7, 22	2β, 6β, 10, 12, 1-OH	30.43 (t)	23
9					78.48 (q)	
10	5.37br s		12, 22	7, 8, 22	120.48 (t)	7, 8, 9, 12, 22
11					133.02 (q)	
12	4.59br d	9.8 (13)	13, 22	8, 14	80.03 (t)	9, 11, 13, 14
13	6.08dd	15.2 (14), 9.8 (12)	12, 14	15, 22	130.27 (t)	11, 12, 14, 15
14	5.71dt	15.2 (13), 6.7 (15)	13, 15	12, 15	135.84 (t)	12, 15, 16
15	2.11m		14, 16	13, 14	32.06 (s)	13, 14, 16
16	1.41m		15		29.12 (s)	
17	1.27br s				29.12 (s)	
18	1.27br s				29,21 (s)	
19	1.27br s				31.83 (s)	
20	1.30br s		21		22.64 (s)	
21	0.87t	6.7 (20)	20		14.10 (p)	19, 20
22	1.57br s		8, 10, 12	10, 13	20.15 (p)	10, 11, 12
23					173.55 (q)	
1-OH	2.41br s		1	1, 8		1, 2
5-OH	1.66br s					

[a–c] As in Table 1.

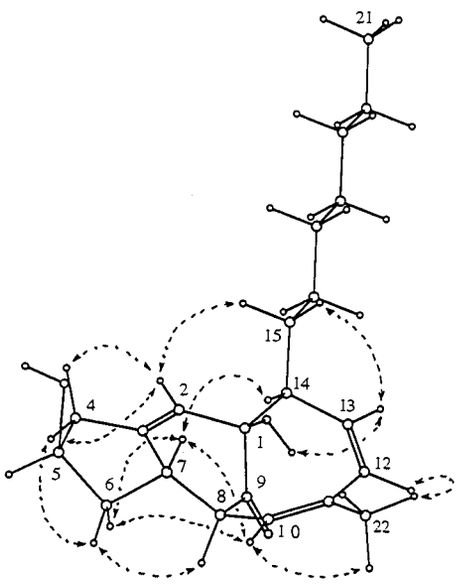


Fig. 5 Energy-minimised conformation of penostatin I **5** and observed NOEs

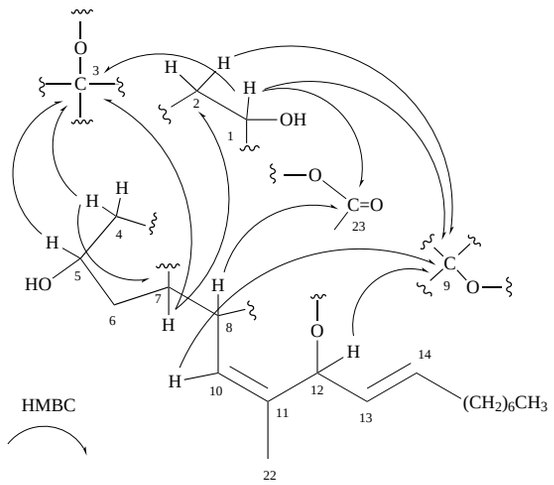


Fig. 6 Partial structures and functional groups of penostatin G **3** and observed HMBC correlations

Compound **5** showed a CD curve (Fig. 3) almost the mirror image of that of its isomer **2**, in which there were two positive Cotton bands at 285 nm and 232 nm due to chromophoric dissymmetric β,γ-unsaturated ketone and twist *cisoid*-conjugated diene moieties, respectively. This finding implied that all the asymmetric centres of compound **5** except for C-5 had the opposite absolute configuration to those of its isomer **2**. The evidence led to absolute stereostructure **5** for penostatin I. The asymmetric centre of C-5 in compound **5** has the same absolute configuration as in isomer **2**, but the conformation of the five-membered ring in compound **5** and consequently the arrangement of the 5-hydroxy group are different from those of isomer **2**. It is of great interest that one fungus produces a pair of the stereoisomers (such as compounds **2** and **5**), in which a number of the asymmetric centres except for one position have opposite absolute configurations. To the best of our knowledge, such examples have never before been reported.

Penostatin G **3** had the molecular formula $C_{23}H_{34}O_5$, established by HR-EIMS. Its IR spectrum contained absorption bands at 3431, 1771 and 1678 cm^{-1}, characteristic of a hydroxy group, a lactone and a double bond. A close inspection of the ^{1}H and ^{13}C NMR spectra of compound **3** (Table 3) by DEPT and ^{1}H–^{13}C COSY experiments revealed the presence of a lactone (C-23), two hydroxylic protons, one disubstituted (C-13 and C-14) and one trisubstituted double bond (C-10 and C-11), an allylic methyl (C-22), a primary methyl (C-21), nine methylenes (C-2, C-4, C-6 and C-15–C-20), five sp^3-methines (C-1, C-5, C-7, C-8 and C-12) including two oxygen-bearing methines, and two quaternary sp^3-carbons bearing oxygen atoms (C-3 and C-9). The EIMS fragment at *m*/*z* 291 [$M^+ - 99$] implied that the primary methyl and six methylenes constitute an *n*-heptyl group.

The ^{1}H–^{1}H COSY analysis for the functional groups thus established led to partial structures shown in Fig. 6. The con-

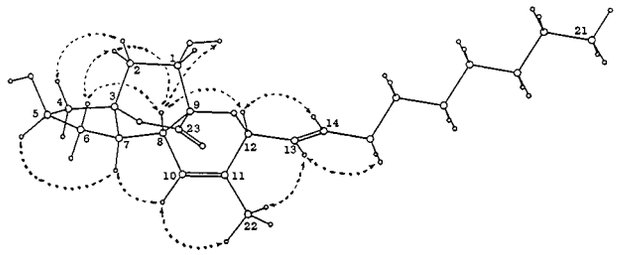
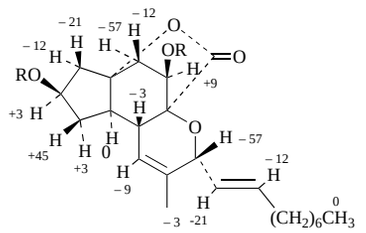


Fig. 7 Energy-minimised conformation of penostatin G **3** and observed NOEs



7a R = (*R*)-MTPA
7b R = (*S*)-MTPA

Fig. 8 ^{1}H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**7a** and **7b**) of penostatin G (**3**). $\Delta\delta$-Values are expressed in Hz (300 Hz)

nectivity of C-11 and C-12 in the partial structure was deduced from a cross-peak attributed to a long-range coupling between 12-H and 22-H in the ^{1}H–^{1}H COSY, and from an HMBC correlation from 22-H to C-12. The geometrical configurations of the 10- and 13-double bonds were respectively deduced as *Z* and *E* from NOEs for 10-H/22-H and 13-H/15-H, the chemical shift of C-22 (δ_C 20.15)[8] and the large coupling constant between 13-H and 14-H (15.2 Hz) (Table 3).

The connection of the partial structures and the remaining functional groups was determined on the basis of HMBC correlations (Table 3 and Fig. 6). An HMBC correlation from 12-H to C-9 suggested that the ether linkage is between C-9 and C-12. The remaining linkages of C-9 to C-1, C-8 and C-23 were deduced from HMBC correlations from 1-H, 2-H and 10-H to C-9, and from 1-H and 8-H to C-23. In addition, the connectivity of C-3 to C-2, C-4 and C-7 is based on HMBC correlations from 1-H, 4-H, 5-H and 7-H to C-3, and 7-H to C-2. The remainder, the carbonyloxy bond of the lactone and the oxygen bond to C-3, suggested the oxygen of the lactone to link to C-3. Based on these considerations, the planar structure **3** was elucidated.

The IR absorption band at 1771 cm^{-1} was attributable to a γ-lactone, but it was actually the band corresponding to a δ-lactone. In general, a δ-lactone exhibits IR absorption bands at 1735–1750 cm^{-1}, whereas strained lactones, such as tricyclic or spiro-lactones, are known to show absorption bands at 1764–1793 cm^{-1}.[14] The unusual shift of the absorption band of the δ-lactone in compound **3** to high wavenumbers seems to be due to a strain in the lactone.

Analysis of the observed coupling constant of 5-H by the Karplus relationship showed that dihedral angles for 5-H/4-H$^{\alpha}$, 5-H/4-H$^{\beta}$, 5-H/6-H$^{\alpha}$ and 5-H/6-H$^{\beta}$ were approximately 30°, 100°, 20° and 140°, respectively. These dihedral angles and an NOE for 5-H/7-H showed that 5-H and 7-H are arranged co-pseudoaxially and consequently that the 5-hydroxy group is oriented pseudoequatorially (Table 3 and Fig. 7). The coupling constant (8.2 Hz) between 7-H and 8-H, and an NOE for 8-H/6-H$^{\beta}$ implied that 8-H is arranged *trans*-dipseudoaxially to 7-H. An NOE for 8-H/1-OH implied that 8-H is arranged co-pseudoaxially to the 1-hydroxy group, and consequently the C-9–C-23 and C-3–O bonds are both arranged *cis* to 7-H. Furthermore, NOEs for 8-H/12-H, 7-H/10-H and 13-H/22-H

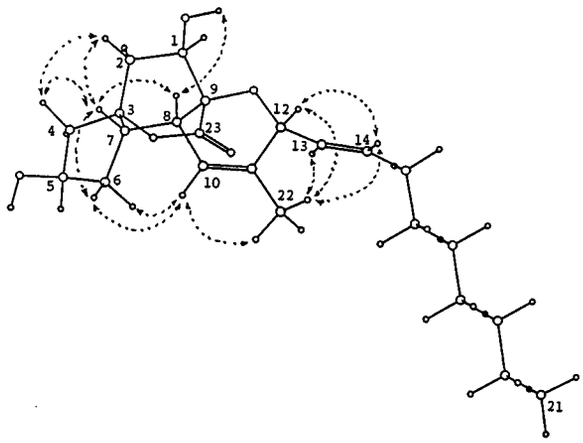


Fig. 9 Energy-minimised conformation of penostatin H **4** and observed NOEs

indicated that the dihydropyran ring of compound **3** exists in a twist-boat conformation with 12-H and the nonenyl group in pseudoaxial and pseudoequatorial arrangements, respectively (Fig. 7). An NOE for 12-H/14-H and the coupling constant (9.8 Hz) between 12-H and 13-H implied that 12-H is oriented *cis* to 14-H and *trans* to 13-H. These findings led to the relative stereostructure of compound **3**.

The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-1,5-bis-MTPA esters (**7a** and **7b**) of compound **3** were found for application of the modified Mosher method as shown in Fig. 8. This result suggested the *R* configuration for the asymmetric centre at C-5 and consequently led to absolute stereostructure **3** for penostatin G. It is known that ^{1}H chemical-shift differences of MTPA esters with axial arrangements are irregularly distributed.[13] In this case, the 1-MPTA ester with an axial arrangement also did not give data in accord with the rule.

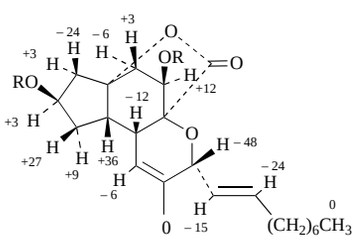
Penostatin H **4** is isomeric with compound **3**. The general spectral features of compound **4** closely resembled those of its isomer **3** except for the chemical shifts of 6-H, 7-H, 8-H, C-1, C-3, C-7, C-10 and C-11 in the NMR spectra (Table 4). This finding suggests compound **4** to be a stereoisomer of penostatin G **3**. In a selected difference NOE experiment, compound **4** showed NOEs from 7-H to 2-H$^{\beta}$, 4-H$^{\beta}$, 6-H$^{\beta}$ and 8-H (Table 4 and Fig. 9), which were not observed in compound **3**. These observed NOEs and the coupling constant (11.5 Hz) between 7-H and 8-H implied that 7-H is arranged co-pseudoaxially to 2-H$^{\beta}$, 4-H$^{\beta}$ and 8-H. In addition, an NOE between 8-H and 1-OH indicated that these have the same co-pseudoaxial arrangement as that of isomer **3**. That the 5-hydroxy group in compound **4** is oriented *cis* to 4-H$^{\beta}$ and 6-H$^{\beta}$ as in isomer **3**, and hence *trans* to 7-H was deduced from selected difference NOE values between 5-H and 4-H or 6-H [5-H/4-H$^{\alpha}$ (1.4%), 5-H/4-H$^{\beta}$ (0.8%), 5-H/6-H$^{\alpha}$ (2.2%) and 5-H/6-H$^{\beta}$ (1.2%)] and from dihedral angles for 5-H/4-H$^{\alpha}$, 5-H/4-H$^{\beta}$, 5-H/6-H$^{\alpha}$ and 5-H/6-H$^{\beta}$ (~20°, 130°, 30° and 100°, respectively), derived from the coupling constants of 5-H to 4-H and 6-H (Table 4). The above summarised evidence suggested that penostatin H **4** is a stereoisomer of penostatin G **3** at C-7.

An NOE from 8-H, but not from 22-H, to 12-H was observed in compound **3**, whereas an NOE from 22-H, but not from 8-H, to 12-H was observed in compound **4**. In addition, an NOE between 8-H and 13-H also was not observed in compound **4**. These observations implied that the dihydropyran ring in compound **4** exists in a twist-chair conformation with the nonenyl group in a pseudoaxial arrangement (Fig. 9). This might be associated with the ^{13}C chemical-shift difference at C-11 between isomers **3** and **4**. An NOE from 12-H to 14-H, but not to 13-H, and the coupling constant (9.6 Hz) between 12-H and 13-H showed 12-H to be oriented *trans* to 13-H. Thus, relative stereostructure **4** was elucidated.

Table 4 ^{1}H and ^{13}C spectral data of penostatin H **4** in $CDCl_3$

Position	δ_H [a]	*J*/Hz	^{1}H–^{1}H COSY	NOEs (H) [b]	δ_C
1	4.18br dd	9.8 (2α), 2.0 (2β)	2α, 2β	2α, 2β, 1-OH	77.50 (t) [c]
2α	2.62dd	14.5 (2β), 9.8 (1)	1, 2β	1, 4β	38.53 (s)
β	1.83br dd	14.5 (2α), 2.0 (1)	1, 2α	1, 7	
3					90.29 (q)
4α	2.51dd	15.0 (4β), 7.0 (5)	4β, 5	5	44.66 (s)
β	1.68br dd	15.0 (4α), 3.0 (5)	4α, 5	2α, 5, 7	
5	4.59br ddd	7.0 (4α), 6.0 (6α), 3.0 (4β)	4α, 4β, 6α	4α, 4β, 6α, 6β	71.43 (t)
6α	2.02td	13.0 (6β, 7), 6.0 (5)	5, 6β, 7	5, 10	36.56 (s)
β	1.86dd	13.0 (6α), 7.5 (7)	6α, 7	5, 7, 10	
7	2.86ddd	13.0 (6α), 11.5 (8), 7.5 (6β)	6α, 6β	2β, 4β, 6β, 8	43.22 (t)
8	3.34br dq	11.5 (7), 2.5 (22)	22	7, 10, 1-OH	29.95 (t)
9					78.03 (q)
10	5.46br s		12, 22	6α, 6β, 8, 22	118.95 (t)
11					135.16 (q)
12	4.59br d	9.6 (13)	10, 13	14, 22	80.09 (t)
13	6.12dd	15.2 (14), 9.6 (12)	12, 14	15, 22	130.07(t)
14	5.70dt	15.2 (13), 6.7 (15)	13, 15	12, 15, 22	135.97 (t)
15	2.12m		14, 16	13, 14	32.14 (s)
16	1.41m		15, 17		29.21 (s)
17	1.27br s		16		29.21 (s)
18	1.27br s				29.21 (s)
19	1.27br s				31.89 (s)
20	1.31br s		21		22.71 (s)
21	0.88t	6.7 (20)	20		14.16 (p)
22	1.59br s		8, 10	10, 12, 13, 14	20.40 (p)
23					173.13 (q)
1-OH	2.38br s			1, 8	
5-OH	1.60br s				

[a–c] As in Table 2.



8a R = (*R*)-MTPA
8b R = (*S*)-MTPA

Fig. 10 ^{1}H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**8a** and **8b**) of penostatin H (**4**). $\Delta\delta$-Values are expressed in Hz (300 Hz)

The ^{1}H chemical-shift differences between the (*R*)- and (*S*)-1,5-bis-MTPA esters (**8a** and **8b**) of penostatin H **4** are shown in Fig. 10. The result suggested that the asymmetric centre at C-5 has the *R* configuration. The ^{1}H chemical-shift differences due to the 1-MTPA ester with an axial arrangement did not allow us to suggest any absolute configuration at C-1, as in penostatin G **3**. Based on the above evidence, the absolute stereostructure of penostatin H was established as **4**.

As partly described above, all the dihedral angles of vicinal protons in penostatins F–I **2**–**5** obtained from coupling constants were nearly equivalent to those of each minimised-energy structure (Figs. 2, 5, 7 and 9) obtained by the CaChe MM2 method, and their experimental NOE-values corresponded nearly to the theoretical distances.

The cytotoxic activities of penostatins F–I **2**–**5** were examined in the P388 lymphocytic leukaemia test system in cell culture, according to the method reported previously.[15] Compounds **2**–**5** exhibited potent cytotoxic activity (ED_{50} 1.4, 0.5, 0.8 and 1.2 μg/cm^3, respectively).

Experimental

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 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 ^{1}H and ^{13}C, respectively, with tetramethylsilane (TMS) as internal reference. *J*-Values are given in Hz. The ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra were recorded on a Varian XL-300 spectrometer, and the HMBC and NOESY spectra on a Bruker DMX-500 spectrometer and a Varian UNITY INOVA-500 spectrometer, with the usual parameters. EIMS was determined using a Hitachi M-4000H mass spectrometer. Liquid chromatography over silica gel (mesh 230–400) was performed at medium pressure. High-performance liquid chromatography (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 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. OUPS-79 isolated from the marine alga *Enteromorpha intestinalis* (Linne) Link (Ulvaceae)[3,4] was grown in a liquid medium (40 dm^3) containing 2% glucose, 1% peptone and 2% malt extract in distilled water adjusted to pH 7.5 for 3 weeks at 27 °C. The culture was filtered under suction and the mycelium was collected, and extracted thrice with MeOH. The combined extracts were evaporated under reduced pressure and the resulting concentrate (45 g) was passed through Sephadex LH-20, using MeOH–CH_2Cl_2 (1 : 1) as the eluent. The second fraction (23.2 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), (1 : 99) and (1 : 49) eluates were collected as 2 fractions [Fr. 1 (366 mg), and Fr. 2 (207 mg)], 4 fractions [Fr. 3 (130 mg), Fr. 4 (50 mg), Fr. 5 (51 mg) and Fr. 6 (54 mg)] and 1 fraction [Fr. 7 (239 mg)], respectively. Fr. 2 was purified by HPLC (ODS) using MeOH–water (9 : 1) as the

eluent to afford penostatin I **5** (12 mg). Fr. 3, Fr. 5 and Fr. 6 afforded penostatin F **2** (9 mg), penostatin G **3** (5 mg) and penostatin H **4** (5 mg), respectively, after purification by HPLC (ODS) using MeOH–water (4:1) as the eluent.

Penostatin F 2. Obtained as an *oil*, $[\alpha]_D$ −12.5 (*c* 0.24, $CHCl_3$); λ_{max}(EtOH)/nm 232sh (log ε/dm^3 mol^{-1} cm^{-1} 3.93) and 281 (2.81); ν_{max}(liquid)/cm^{-1} 3451 (OH), 1715 (CO) and 1610 (C=C); *m*/*z* (EI) 344 (M^+, 74%), 326 ($M^+ - H_2O$, 9), 316 (M^+ − CO, 16), 248 (38), 245 [$M^+ - (CH_2)_6CH_3$, 9], 227 [$M^+ - (CH_2)_6CH_3 - H_2O$, 11] and 217 [$M^+ - (CH_2)_6CH_3$ − CO, 100] [*m*/*z* (HREI) Found: M^+, 344.2347. $C_{22}H_{32}O_3$ requires *M*, 344.2351]; CD λ (*c* 4.74×10^{-5} mol dm^{-3} in EtOH)/nm 202 ($\Delta\varepsilon$ +44.13), 218 (0), 232 (−23.34), 263 (−6.72), 285 (−9.59) and 324 (0). ^{1}H and ^{13}C NMR data are listed in Table 1.

Penostatin G 3. Obtained as an *oil*, $[\alpha]_D$ −35.1 (*c* 0.29, $CHCl_3$); ν_{max}(liquid)/cm^{-1} 3431 (OH), 1771 (lactone) and 1678 (C=C); *m*/*z* (EI) 390 (M^+, 36%), 345 ($M^+ - CO_2H$, 80), 291 [$M^+ - (CH_2)_6CH_3$, 75] and 121 (100) [*m*/*z* (HREI) Found: M^+, 390.2406. $C_{23}H_{34}O_5$ requires *M*, 390.2406]. ^{1}H and ^{13}C NMR data are listed in Table 3.

Penostatin H 4. Obtained as an *oil*, $[\alpha]_D$ −11.4 (*c* 0.18, $CHCl_3$); ν_{max}(liquid)/cm^{-1} 3405 (OH), 1776 (lactone) and 1678 (C=C); *m*/*z* (EI) 390 (M^+, 23%), 345 ($M^+ - CO_2H$, 100), 291 [$M^+ - (CH_2)_6CH_3$, 18] and 121 (24) [*m*/*z* (HREI) Found: M^+, 390.2407]. ^{1}H and ^{13}C NMR data are listed in Table 4.

Penostatin I 5. Obtained as an *oil*, $[\alpha]_D$ +13.3 (*c* 0.30, $CHCl_3$); λ_{max}(EtOH)/nm 230sh (log ε/dm^3 mol^{-1} cm^{-1} 4.01) and 278 (3.68); ν_{max}(liquid)/cm^{-1} 3435 (OH), 1713 (CO) and 1610 (C=C); *m*/*z* (EI) 344 (M^+, 37%), 326 ($M^+ - H_2O$, 10), 316 (M^+ − CO, 22), 248 (83), 217 [$M^+ - (CH_2)_6CH_3$ − CO, 92] and 121 (100) [*m*/*z* (HREI) Found: M^+, 344.2346. $C_{22}H_{32}O_3$ requires *M*, 344.2350], CD λ (*c* 1.74×10^{-5} mol dm^{-3} in EtOH)/nm 202 ($\Delta\varepsilon$ −38.22), 219 (0), 232 (+16.51), 263 (+3.47), 285 (+6.08) and 325 (0). ^{1}H and ^{13}C NMR data are listed in Table 2.

Formation of the (*R*)- and (*S*)-MTPA esters 6a and 6b from penostatin F 2

(*R*)-MTPA (5 mg), dicyclohexylcarbodiimide (DCC) (5 mg) and 4-(dimethylamino)pyridine (DMAP) (3 mg) were added to a CH_2Cl_2 solution (1 cm^3) of penostatin F **2** (1.2 mg), and the reaction mixture was left for 6 h. The solvent was evaporated off under reduced pressure, and the residue was purified by HPLC (ODS) using MeOH to afford ester **6a** (1.0 mg). The same reaction with compound **2** (1.4 mg) using (*S*)-MTPA (5 mg) gave ester **6b** (1.2 mg).

Ester 6a. Obtained as an oil; *m*/*z* (EI) 560 (M^+); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.5, 21-H_3), 1.25 (10 H, br s, 16–20-H_2), 1.62 (2 H, m, 15-H_2), 1.68 (1 H, ddd, *J* 12.5, 9.5 and 6.5, 6-H$^\beta$), 1.78 (3 H, d, *J* 1.0, 22-H_3), 2.49 (1 H, br dd, *J* 17.0 and 6.5, 4-H$^\beta$), 2.55 (1 H, dt, *J* 9.0 and 5.0, 14-H), 2.69 (1-H, ddd, *J* 12.5, 8.0 and 6.5, 6-H$^\alpha$), 2.89 (1 H, br dd, *J* 17.0 and 6.5, 4-H$^\alpha$), 2.93 (1 H, br t, *J* 6.0, 8-H), 2.96 (1 H, m, 7-H), 3.49 (3 H, s, OMe), 3.68 (1 H, s, 1-OH), 5.38 (1 H, dd, *J* 11.5 and 9.0, 13-H), 5.42 (1 H, quint, *J* 6.5, 5-H), 5.51 (1 H, dq, *J* 6.0 and 1.0, 10-H), 5.70 (1 H, q, *J* 2.0, 2-H), 5.73 (1 H, d, *J* 11.5, 12-H), 7.40 (3 H, m, ArH) and 7.45 (2 H, m, ArH).

Ester 6b. Obtained as an oil; *m*/*z* (EI) 560 (M^+); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.5, 21-H_3), 1.25 (10 H, br s, 16–20-H_2), 1.63 (2 H, m, 15-H_2), 1.69 (1 H, ddd, *J* 12.5, 9.5 and 6.5, 6-H$^\beta$), 1.78 (3 H, *J* 1.0, 22-H_3), 2.39 (1 H, br dd, *J* 17.0 and 6.5, 4-H$^\beta$), 2.54 (1 H, dt, *J* 9.0 and 5.0, 14-H), 2.73 (1-H, ddd, *J* 12.5, 8.0 and 6.5, 6-H$^\alpha$), 2.88 (1 H, br dd, *J* 17.0 and 6.5, 4-H$^\alpha$), 2.97 (1 H, m, 7-H), 2.97 (1 H, br d, *J* 6.0, 8-H), 3.50 (3 H, s, OMe), 3.64 (1 H, s, 1-OH), 5.38 (1 H, dd, *J* 11.5 and 9.0, 13-H), 5.41 (1 H, quint, *J* 6.5, 5-H), 5.52 (1 H, br d, *J* 6.0, 10-H), 5.66 (1 H, q, *J* 2.0, 2-H), 5.72 (1 H, d, *J* 11.5, 12-H), 7.41 (3 H, m, ArH) and 7.46 (2 H, m, ArH).

Formation of the (*R*)- and (*S*)-MTPA esters 7a and 7b from penostatin G 3

Using the same procedure as above with compound **2**, penostatin G **3** (1.0 and 1.1 mg) was treated with (*R*)-MTPA (10 mg) and (*S*)-MTPA (10 mg) to afford esters **7a** (0.9 mg) and **7b** (1.0 mg), respectively.

Ester 7a. Obtained as an oil; *m*/*z* (EI) 822 (M^+); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.5, 21-H_3), 1.26 (8 H, br s, 17–20-H_2), 1.41 (2 H, br s, 16-H_2), 1.43 (1 H, td, *J* 14.0 and 5.5, 6-H$^\beta$), 1.57 (3 H, s, 22-H_3), 1.91 (1 H, m, 7-H), 1.93 (1 H, dd, *J* 15.5 and 3.5, 2-H$^\beta$), 1.95 (1 H, br d, *J* 15.0, 4-H$^\beta$), 2.14 (2 H, m, 15-H), 2.41 (1 H, dd, *J* 15.0 and 9.0, 4-H$^\alpha$), 2.59 (1 H, ddd, *J* 15.5, 9.5 and 2.0, 2-H$^\alpha$), 2.78 (1 H, br d, *J* 8.2, 8-H), 2.81 (1 H, dt, *J* 14.0 and 7.5, 6-H$^\alpha$), 3.47 (3 H, s, OMe), 3.51 (3 H, s, OMe), 4.50 (1 H, br d, *J* 9.5, 12-H), 5.30 (1 H, br s, 10-H), 5.32 (1 H, dd, *J* 9.5 and 3.5, 1-H), 5.52 (1 H, ddd, *J* 9.0, 7.5 and 5.5, 5-H), 5.64 (1 H, dt, *J* 15.5 and 6.5, 14-H), 6.01 (1 H, dd, *J* 15.5 and 9.5, 13-H), 7.37 (6 H, m, ArH), 7.45 (2 H, m, ArH) and 7.58 (2 H, m, ArH).

Ester 7b. Obtained as an oil; *m*/*z* (EI) 822 (M^+); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.5, 21-H_3), 1.26 (8 H, br s, 17–20-H_2), 1.41 (2 H, br s, 16-H_2), 1.57 (3 H, s, 22-H_3), 1.58 (1 H, td, *J* 14.0 and 5.5, 6-H$^\beta$), 1.88 (1 H, br d, *J* 15.0, 4-H$^\beta$), 1.89 (1 H, dd, *J* 15.5 and 3.5, 2-H$^\beta$), 1.91 (1 H, m, 7-H), 2.10 (2 H, m, 15-H_2), 2.37 (1 H, dd, *J* 15.0 and 9.0, 4-H$^\alpha$), 2.40 (1 H, ddd, *J* 15.5, 9.5 and 2.0, 2-H$^\alpha$), 2.77 (1 H, br d, *J* 8.2, 8-H), 2.82 (1 H, dt, *J* 14.0 and 7.5, 6-H$^\alpha$), 3.47 (3 H, s, OMe), 3.51 (3 H, s, OMe), 4.31 (1 H, br d, *J* 9.5, 12-H), 5.27 (1 H, br s, 10-H), 5.35 (1 H, dd, *J* 9.5 and 3.5, 1-H), 5.53 (1 H, ddd, *J* 9.0, 7.5 and 5.5, 5-H), 5.60 (1 H, dt, *J* 15.5 and 6.5, 14-H), 5.94 (1 H, dd, *J* 15.5 and 9.5, 13-H), 7.40 (6 H, m, ArH), 7.46 (2 H, m, ArH) and 7.51 (2 H, m, ArH).

Formation of the (*R*)- and (*S*)-MTPA esters 8a and 8b from penostatin H 4

Using the same procedure as above with compound **2**, penostatin H **4** (0.9 and 0.8 mg) was treated with (*R*)-MTPA (10 mg) and (*S*)-MTPA (10 mg) to afford esters **8a** (0.9 mg) and **8b** (0.9 mg), respectively.

Ester 8a. Obtained as an oil; *m*/*z* (EI) 822 (M^+); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.5, 21-H_3), 1.27 (8 H, br s, 17–20-H_2), 1.43 (2 H, m, 16-H_2), 1.57 (3 H, br s, 22-H_3), 1.70 (1 H, dd, *J* 15.5 and 1.0, 2-H$^\beta$), 1.84 (1 H, dd, *J* 16.0 and 3.0, 4-H$^\beta$), 1.99 (1 H, dd, *J* 13.0 and 7.2, 6-H$^\beta$), 2.14 (1 H, td, *J* 13.0 and 6.0, 6-H$^\alpha$), 2.15 (2 H, m, 15-H_2), 2.50 (1 H, ddd, *J* 13.0, 11.0 and 7.2, 7-H), 2.67 (1 H, dd, *J* 16.0 and 7.0, 4-H$^\alpha$), 2.82 (1 H, dd, *J* 15.5 and 9.0, 2-H$^\alpha$), 3.16 (1 H, br dq, *J* 11.0 and 2.5, 8-H), 3.50 (3 H, s, OMe), 3.52 (3 H, s, OMe), 4.51 (1 H, br d, *J* 9.5, 12-H), 5.40 (1 H, br s, 10-H), 5.41 (1 H, dd, *J* 9.0 and 1.0, 1-H), 5.54 (1 H, ddd, *J* 7.0, 6.0 and 3.0, 5-H), 5.70 (1 H, dt, *J* 15.5 and 6.5, 14-H), 6.04 (1 H, dd, *J* 15.5 and 9.5, 13-H), 7.42 (6 H, m, ArH), 7.48 (2 H, m, ArH) and 7.59 (2 H, m, ArH).

Ester 8b. Obtained as an oil; *m*/*z* (EI) 822 (M^+); δ_H(300 MHz; $CDCl_3$) 0.88 (3 H, t, *J* 6.5, 21-H_3), 1.27 (8 H, br s, 17–20-H_2), 1.40 (2 H, m, 16-H_2), 1.55 (3 H, br s, 22-H_3), 1.71 (1 H, dd, *J* 15.5 and 1.0, 2-H$^\beta$), 1.76 (1 H, dd, *J* 16.0 and 3.0, 4-H$^\beta$), 2.08 (1 H, dd, *J* 13.0 and 7.2, 6-H$^\beta$), 2.10 (2 H, m, 15-H_2), 2.17 (1 H, td, *J* 13.0 and 6.0, 6-H$^\alpha$), 2.62 (1 H, ddd, *J* 13.0, 11.0 and 7.2, 7-H), 2.68 (1 H, dd, *J* 16.0 and 7.0. 4-H$^\alpha$), 2.80 (1 H, dd, *J* 15.5 and 9.0, 2-H$^\alpha$), 3.12 (1 H, br dq, *J* 11.0 and 2.5, 8-H), 3.50 (3 H, s, OMe), 3.52 (3 H, s, OMe), 4.35 (1 H, br d, *J* 9.5, 12-H), 5.38 (1 H, br s, 10-H), 5.45 (1 H, dd, *J* 9.0 and 1.0, 1-H), 5.55 (1 H, ddd, *J* 7.0, 6.0 and 3.0, 5-H), 5.62 (1 H, dt, *J* 15.5 and 6.5, 14-H), 5.99 (1 H, dd, *J* 15.5 and 9.5, 13-H), 7.42 (6 H, m, ArH), 7.49 (2 H, m, ArH) and 7.53 (2 H, m, ArH).

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References

1 C. Takahashi, A. Numata, Y. Ito, E. Matsumura, H. Araki, H. Iwaki and K. Kushida, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 1859.
2 C. Takahashi, K. Minoura, T. Yamada, A. Numata, K. Kushida, T. Shingu, S. Hagishita, H. Nakai, T. Sato and H. Harada, *Tetrahedron*, 1995, **51**, 3483.
3 A. Numata, C. Takahashi, Y. Ito, T. Takada, K. Kawai, Y. Usami, E. Matsumura, M. Imachi, T. Ito and T. Hasegawa, *Tetrahedron Lett.*, 1993, **34**, 2355.
4 A. Numata, C. Takahashi, Y. Ito, K. Minoura, T. Yamada, C. Matsuda and K. Nomoto, *J. Chem. Soc.*, *Perkin Trans. 1*, 1996, 239.
5 C. Takahashi, A. Numata, T. Yamada, K. Minoura, S. Enomoto, K. Konishi, M. Nakai, C. Matsuda and K. Nomoto, *Tetrahedron Lett.*, 1996, **37**, 655.
6 C. Takahashi, T. Matsushita, M. Doi, K. Minoura, T. Shingu, Y. Kumeda and A. Numata, *J. Chem. Soc.*, *Perkin Trans. 1*, 1995, 2345.
7 C. Takahashi, T. Takada, T. Yamada, K. Minoura, K. Uchida, E. Matsumura and A. Numata, *Tetrahedron Lett.*, 1994, **35**, 5013.
8 G. Englert, *Helv. Chim. Acta*, 1975, **58**, 2367.
9 M. Karplus, *J. Chem. Phys.*, 1959, **30**, 11.
10 R. J. Abraham and J. S. E. Holker, *J. Chem. Soc.*, 1963, 807.
11 A. Moscowitz, K. Mislow, M. A. W. Glass and C. Djerassi, *J. Am. Chem. Soc.*, 1962, **84**, 1945; E. Bunnenberg, C. Djerassi, K. Missiow and A. Moscowitz, *J. Am. Chem. Soc.*, 1962, **84**, 2823.
12 U. Weiss, H. Ziffer and E. Charney, *Tetrahedron*, 1965, **21**, 3105.
13 T. Kusumi, I. Ohtani, Y. Inouye and H. Kakisawa, *Tetrahedron Lett*, 1988, **29**, 4731; I. Ohtani, T. Kusumi, O. M. Ishitsuka and H. Kakisawa, *Tetrahedron Lett.*, 1989, **30**, 3147.
14 L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*, Methuen, London, 2nd edn., 1958, p. 186.
15 A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae and E. Fujita, *Chem. Pharm. Bull.*, 1989, **37**, 648.

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