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Absolute stereostructures of novel cytotoxic metabolites, penostatins A–E, from a *Penicillium* species separated from an *Enteromorpha* alga

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Abstract : Penostatins A – E have been isolated from a strain of *Penicillium* sp. originally separated from the marine alga *Enteromorpha intestinalis*, and their absolute stereostructures and conformations have been established on the basis of spectral analyses and some chemical transformations. All the compounds except for penostatin D exhibited significant cytotoxicity against cultured P388 cells.

Introduction

In our program devoted to the search for new antitumor metabolites from microorganisms inhabiting the marine environment, we have found a number of antitumor and cytotoxic compounds and elucidated their structures.[1–5] As part of this study, we previously reported that five cytotoxic compounds, communesins A (**1**) and B (**2**)[2] and penochalasins A (**3**)–C,[3] were produced by a strain of *Penicillium* sp. OUPS-79 originally isolated from the marine alga *Enteromorpha intestinalis*, and their structures were established. Further investigation for metabolites of this fungal strain has led to the isolation of nine new compounds, penostatins A (**4**)–E (**8**) and F–I, together with known patulin (**9**)[6] and (+)-epiepoxydon (**10**).[7] Among them, the absolute stereostructures for four compounds, penostatins F–I, were recently reported.[4] We describe herein the details of the absolute stereostructure determination of compounds **4**–**8**, of which the relative stereostructures for **4**–**7** have been briefly reported in a preliminary form.[5] It is noteworthy that all the asymmetric centers except for one position have the opposite absolute configurations between a pair of the stereoisomers (**4** and **5**). All the compounds except for **7** exhibited significant cytotoxic activity in the P388 lymphocytic leukemia test system in cell culture. Among them, patulin (**9**) showed the most potent cytotoxicity.

Results and Discussion

In the present experiment, the fungal strain was cultured at 27°C for 3 weeks in a medium (40 l) containing 2% glucose, 1% peptone and 2% malt extract in distilled water adjusted to pH 7.5, which was different from

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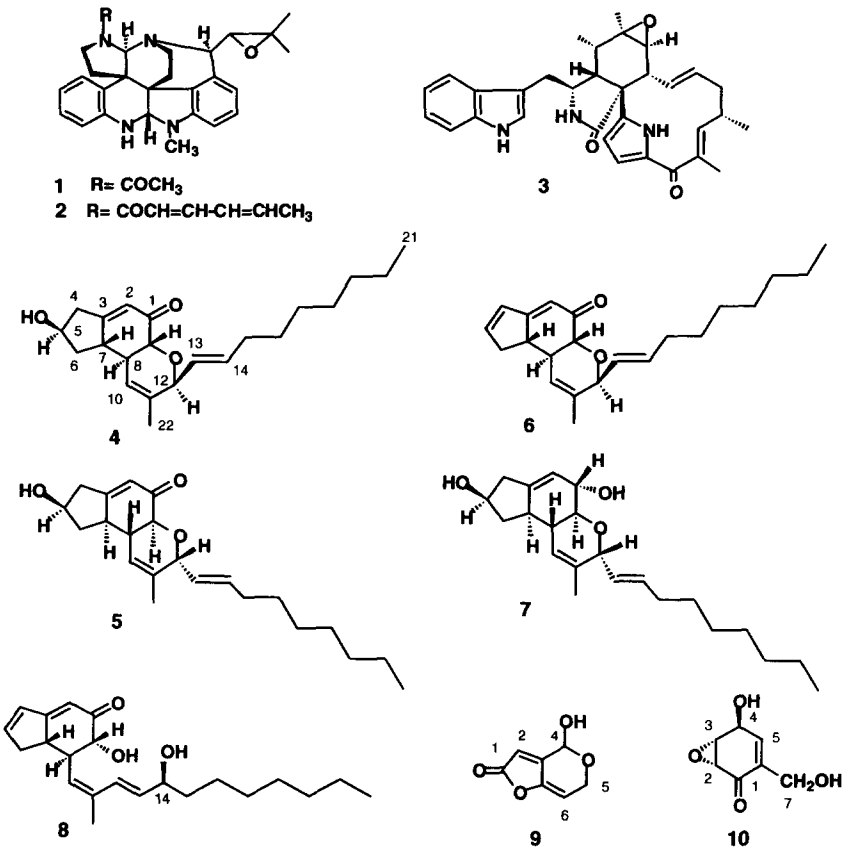

that used for the previous experiment affording communesins and penochalasins (a medium containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater).[2,3] The MeOH extract of the mycelial cake was purified by bioassay-directed fractionation (cytotoxicities against P388 cells) employing a combination of Sephadex LH-20 and silica gel column chromatography and high-performance liquid chromatography (HPLC) to afford penostatins A–E (**4** - **8**) besides communesins A (**1**) and B (**2**). The AcOEt extract of the culture filtrate was purified by the above similar procedures to afford known compounds, patulin (**9**) and (+)-epiepoxydon (**10**), which were identified by analysis of 2D NMR spectra, including 2D heteronuclear multiple-bond connectivity (HMBC) correlations, and comparison of spectral data with published values.[6,7]

Penostatin A (**4**) had the molecular formula $C_{22}H_{32}O_3$ established by high-resolution electron impact mass spectrometry (HREIMS). Its UV and IR spectra exhibited absorption bands at 232 nm, and 3462, 1669 and 1639 cm^{-1}, characteristic of a hydroxy group and an α, β-unsaturated ketone. A close inspection of the ^1H and ^{13}C NMR spectra of **4** (Table 1) by distortionless enhancement by polarization transfer (DEPT) and ^1H-^{13}C correlation spectroscopy (COSY) experiments revealed the presence of a conjugated ketone (C-1), one disubstituted (C-13 and C-14) and two trisubstituted double bonds (C-2 and C-3, and C-10 and C-11), a

Table 1 ^{1}H and ^{13}C NMR data of penostatin A (**4**) and the acetate **11** in $CDCl_3$

	4							**11**			
Position	δ_H[a]			^{1}H-^{1}H COSY	NOEs[b]	δ_C		HMBC (C)	δ_H[a]		
1						196.36	(q)[c]				
2	5.95	q	2.5 (4α, 4β, 7)	4α, 4β, 7	4α, 4β	122.37	(t)	4, 7	5.97	br s	
3						170.39	(q)				
4α	2.84	br dd	19.5 (4β), 5.0 (5)	2, 4β, 5	2	41.67	(s)	2, 3, 6, 7	2.94	br dd	19.0 (4β), 5.0 (5)
β	2.64	br d	19.5 (4α)	2, 4α, 5, 6β	2			2, 3, 5, 6, 7	2.73	br d	19.0 (4α)
5	4.61	br t	5.0 (4α, 6α)	4α, 4β, 6α		70.92	(t)	3, 4, 6, 7	5.37	br t	5.0 (4α, 6α)
6α	1.55	td	12.5 (6β, 7), 5.0 (5)	5, 6β, 7	8, 10	39.15	(s)	3, 5, 7, 8	1.63	m	
β	2.28	ddd	12.5 (6α), 7.0 (7), 2.0 (4β)	4β, 6α, 7	10			3, 4, 5, 7, 8	2.39	m	
7	2.86	m		2, 6α, 6β, 8	9, 10	44.84	(t)	2, 3, 6, 9, 10	2.41	m	
8	2.41	tq	11.0 (7, 9), 1.5 (22)	7, 9, 10, 22	6α	44.75	(t)	6, 7, 9, 11	2.43	m	
9	4.08	d	11.0 (8)	8	7, 13, 14	73.78	(t)	1, 2, 7, 8, 10, 12	4.08	d	11.0 (8)
10	5.55	q	1.0 (22)	8, 22	6α, 6β, 7, 22	121.83	(t)	7, 8, 9, 11, 12, 22	5.54	br s	
11						136.11	(q)				
12	4.60	br d	5.8 (13)	13, 22	14, 22	77.49	(t)	9, 10, 11, 13, 14, 22	4.61	br d	5.8 (13)
13	5.57	dd	14.2 (14), 5.8 (12)	12, 14	9, 15, 22	125.91	(t)	11, 12, 15	5.57	dd	15.5 (14), 5.8 (12)
14	5.68	dt	14.2 (13), 6.5 (15)	13, 15	9, 12, 16, 22	136.49	(t)	12, 15	5.71	dt	15.5 (14), 6.5 (15)
15	2.06	q	6.5 (14, 16)	14, 16	13	32.37	(s)	14, 16	2.09	m	
16	1.37	quint	6.5 (15, 17)	15	14	29.08	(s)	14, 15	1.38	m	
17	1.26	br s				29.08	(s)		1.26	brs	
18	1.26	br s				29.08	(s)		1.26	brs	
19	1.26	br s				31.78	(s)		1.26	brs	
20	1.31	br s		21		22.62	(s)	21	1.26	brs	
21	0.87	t	6.5 (20)	20		14.10	(p)	20	0.88	t	6.5 (20)
22	1.66	br s		8, 10, 12	10, 12, 13, 14	20.06	(p)	10, 11, 12			
5-OH	1.72	br s									
$OCOCH_3$									2.06	s	

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

vinylic methyl (C-22), a primary methyl (C-21), eight methylenes (C-4, C-6 and C-15–C-20) and five sp^3-hybridized methines (C-5, C-7, C-8, C-9 and C-12) including three oxygen-bearing methines (C-5, C-9 and C-12). Production of the monoacetate derivative (**11**) of **4** by standard acetylation and its ^{1}H NMR signals (Table 1) suggested the presence of one secondary alcohol, linked to the C-5 methine. Consequently, the presence of one ether linkage was clarified as deduced from the molecular formula of **4**. The EIMS fragment at *m/z* 245 [M^+– 99] implied that the primary methyl and the six methylenes constitute a heptyl group.

The ^{1}H-^{1}H COSY analysis (Table 1) led to a partial structure (C-2–C-22). The connection of C-3 to both C-4 and C-7 was deduced from cross-peaks attributed to long-range couplings between H-2 and both H-4 and H-7 in the ^{1}H-^{1}H COSY, and from three-bond HMBC correlations (Table 1) from H-2 to both C-4 and C-7. In addition, the connectivity of C-11 and C-12 was deduced from a cross-peak attributed to a long-range coupling between H-12 and H-22 in the ^{1}H-^{1}H COSY, and from an HMBC correlation from H-22 to C-12. The appearance of a deshielded C-3 carbon signal (δ 170.39) indicated that a ketone is located at the β-position of the C-3 carbon. HMBC correlations from H-9 to C-1, C-2 and C-12, and from H-12 to C-9 implied that C-9 is linked to the ketone (C-1) and that the ether linkage is between C-9 and C-12. The geometrical configuration of the Δ^{13}- olefin was deduced as *E* from a large coupling constant ($J_{13,14}$ 14.2 Hz). The above-summarized evidence led to the planar structure **4** for penostatin A.

The relative stereochemistry for **4** was established by a combination of observed coupling constants, nuclear Overhauser enhancement spectroscopy (NOESY) data (Table 1 and Fig. 1) and selected difference NOE values. Observations of the $J_{7,8}$ and $J_{8,9}$ values (11 Hz each) and an NOE between H-7 and H-9 implied that H-8 is arranged pseudoaxially and *trans* to H-7 and H-9. The 5-hydroxymethine proton was observed as a triplet, implying that the proton couples only to one proton each (H-4α and H-6α) of the 4- and 6-methylenes

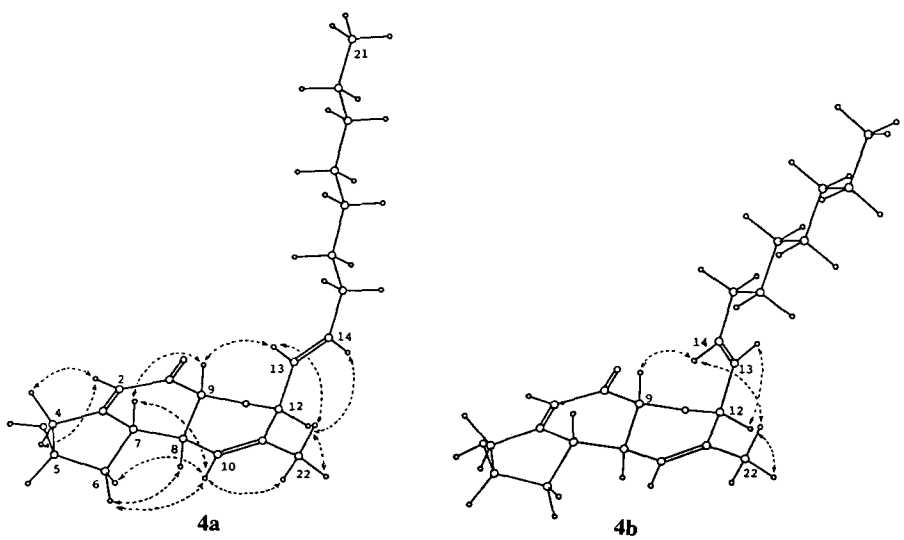

Fig. 1 Energy-minimized conformers **4a** and **4b** of penostatin A (**4**) and observed NOEs.

and their coupling constants are equivalent. Based on the generalized Karplus relationship,[8] the observed coupling constants ($J_{4\alpha,5} = J_{5,6\alpha}$ 5.0 Hz; $J_{4\beta,5} = J_{5,6\beta} \approx 0$ Hz) suggested that the dihedral angles for H-5/H-4α, H-5/H-4β, H-5/H-6α and H-5/H-6β were approximately 38°, 80°, 38° and 80°, respectively, showing that the 5-hydroxy group is oriented pseudoaxially. Assignments for H-4α, H-4β, H-6α and H-6β were deduced from selected difference NOE values between each of these protons and H-5 [H-5/H-4α (2.5 %) > H-5/H-4β (1.5 %); H-5/H-6α (2.6 %) > H-5/H-6β (1.6 %)], and a W-type of long-range coupling between H-4β and H-6β. The observation of an NOE between H-6α and H-8, and coupling constants between H-7 and H-6α or H-6β ($J_{6\alpha,7}$ 12.5 Hz; $J_{6\beta,7}$ 7.0 Hz) suggested that H-6α and H-6β are oriented pseudoaxially and pseudoequatorially, respectively, and that the pseudoaxial 5-hydroxy group is *cis* to H-7. NOEs from H-22 to H-12, H-13 and H-14, and from H-9 to H-13 and H-14 suggested that the dihydropyran ring of **4** exists in a twist-chair conformation with H-12 and the nonenyl group in pseudoequatorial and pseudoaxial arrangements, respectively, indicating the nonenyl group to be *cis* to H-9. The above NOE data also suggested that the C-12–C-13 axis rotates in a $CDCl_3$ solution. In accordance with the relative configuration and the pseudoaxial 5-hydroxy group mentioned above, a stereomodel was created using the CaChe work system, and then its conformational behavior was investigated by computing the potential energy surface as a function of rotational angles for the C-12–C-13 axes using the CaChe MM2 method.[3] The conformational space was sampled by varying rotational angles in steps of 15° for the range from 0° to 360°. At each point a full geometry optimization was carried out, which yielded two local minima. The energies for their minima were 29.36 and 29.58 kcal mol^{-1}, and conformers with their energies corresponded to **4a** (12, 13-*trans*) and **4b** (12, 13-*cis*) (Fig. 1), respectively, between which the energy barrier was a low value (ca. 5.8 – 6.6 kcal mol^{-1}). These data supported the deduction from the NMR data that the C-12–C-13 axis in **4** rotates in a $CDCl_3$ solution. Furthermore, the theoretical dihedral angles for minimized-energy structures (**4a** and **4b**) were nearly equivalent to the dihedral angles for H-5/H-4, H-5/H-6, H-7/H-6α (159°), H-7/H-6β (41°), H-7/H-8 (160°) and H-8/H-9 (160°) analyzed by the coupling constants.[8, 9] The theoretical internuclear distances also supported NOE values (the detailed data are omitted).

The absolute stereochemistry for **4** was established by the modified Mosher method[10] and the circular dichroism (CD) spectrum. Treatment of **4** with (*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetyl chloride (MTPACl) in pyridine gave the MTPA esters **12a** and **12b**, respectively, together with compound **6**, identical with penostatin C. The ^{1}H chemical-shift differences between the esters **12a** and **12b** ($\Delta\delta = \delta_S - \delta_R$) are shown in Fig. 2, and the result allowed assignment of the absolute stereostructure **4** with the 5*R* configuration for penostatin A.

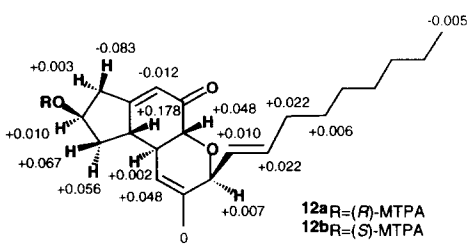

Fig. 2 ^{1}H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**12a** and **12b**) of penostatin A (**4**).

Burgstahler and co-worker have reported that in the CD spectra of a cyclic conjugated enone system

the π-π* Cotton effect in the 200-220 nm region reflects the chirality contribution to the carbonyl group by the pseudoaxial bond on the α'-carbon, while the Cotton effect in the 230–260 nm region is dominated by the allylic axial perturbation of the carbon-carbon double bond.[11] Marumo and co-worker have applied this report to a sesquiterpene.[12] Based on these reports, the positive Cotton effect at 205.5 nm in the CD spectrum (Fig. 3) of **4** implied the 9*R* configuration, identical with the result from the modified Mosher method. On the other hand, the Cotton effect at 241 nm was a negative sign contrary to the expectation of a chilarity contribution of the allylic axial proton (H-7). Thus, the negative Cotton effect at the 230–260 nm region in this case seems to suggest the 7*S* configuration.

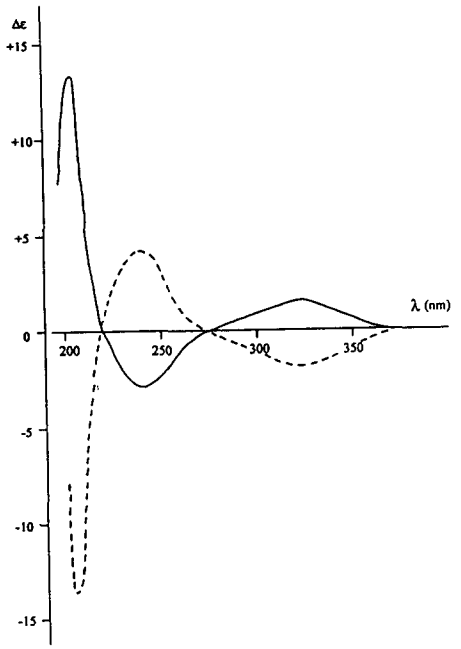

Fig. 3 CD spectra of isomers **4** (—) and **5** (- - -).

Penostatin C (**6**) had a molecular formula ($C_{22}H_{30}O_2$) which contained one molecule of water less than that of **4**. Its UV spectrum exhibited an absorption band shifted to longer wavelength (λ_{max} 283 nm) than that of **4**. The IR spectrum contained absorption bands due to a conjugated ketone (1675 and 1610 cm^{-1}), and not due to a hydroxy group. The general features of the ^1H and ^{13}C NMR spectra of **6** (Table 2) closely resembled those of **4** except that the 4-methylene and 5-hydroxymethine signals in **4** are replaced by those of a double bond (δ_H 6.45 and 6.69; δ_C 132.23 and 147.82) in **6**. These facts led to the planar structure **6** for penostatin C.

NOE data of **6** (Table 2) revealed that the relative chemistry of **6** is the same as that of **4** except for C-5. Formation of **6** from **4** by treatment with MTPACl as mentioned above implied that the absolute configuration of **6** is identical with that of **4** except for C-5. The positive Cotton effect at 203 nm in the CD spectrum (Fig. 4) of **6** indicated the 9*R* configuration, supporting the above-mentioned absolute stereostructure for **6**. In addition, the Cotton effect at 274 nm, which is considered to correspond to that at 241 nm in **4**, was found as a negative sign as in **4**. This result supported the 7*S* configuration of **6**, established by the chemical transformation.

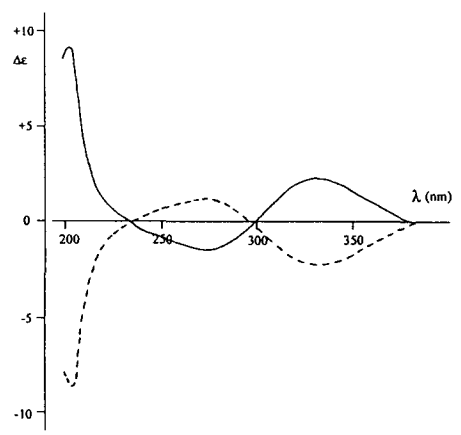

Fig. 4 CD spectra of isomers **6** (—) and **14** (- - -).

Penostatin B (**5**) had the same molecular formula as

Table 2 ^{1}H and ^{13}C NMR data of penostatin C (**6**) in $CDCl_3$

Position	δ_H[a]			^{1}H-^{1}H COSY	NOEs[b]	δ_C		^{1}H-^{13}C long range correlation (H)
1						196.59	(q)[c]	9
2	5.91	d	2.5 (7)	7	4	117.14	(t)	
3						170.96	(q)	4, 5
4	6.45	dt	5.5 (5), 2.0 (6α, 6β)	5, 6β, 6α	2	132.23	(t)	2, 5, 6α, 6β
5	6.69	dt	5.5 (4), 2.5 (6α, 6β)	4, 6β, 6α		147.82	(t)	4, 6α, 6β
6α	2.45	dddd	17.5 (6β), 4.5 (7), 2.5 (5), 2.0 (4)	4, 5, 6β, 7	10	36.44	(s)	4, 5
β	2.86	dddd	17.5 (6α), 7.0 (7), 2.5 (5), 2.0 (4)	4, 5, 6α, 7	10			
7	2.70	dddd	11.2 (8), 7.0 (6β), 4.5 (6α), 2.5 (2)	2, 6α, 6β, 8	9, 10	45.71	(t)	2, 6α, 6β, 9
8	2.53	tq	11.2 (7, 9), 2.0 (22)	7, 9, 10, 22		44.56	(t)	6α, 10
9	4.45	d	11.2 (8)	8, 10	7, 13, 14	75.05	(t)	2, 8, 10, 12
10	5.58	q	1.5 (22)	8, 9, 22	6α, 6β, 7, 22	121.66	(t)	22
11						136.64	(q)	12, 22
12	4.62	br d	6.0 (13)	13, 22	14, 22	77.58	(t)	9, 10, 13, 14, 22
13	5.59	dd	15.5 (14), 6.0 (12)	12, 14	9, 15, 22	126.02	(t)	
14	5.70	dt	15.5 (13), 7.0 (15)	13, 15	9, 12, 22	136.22	(t)	
15	2.06	q	7.0 (14, 16)	14, 16	13	32.37	(s)	13, 14
16	1.38	quint	7.0 (15, 17)	15, 17		29.01	(s)	
17	1.26	br s		16		29.12	(s)	
18	1.26	br s				29.12	(s)	
19	1.26	br s				31.78	(s)	21
20	1.31	br s		21		22.62	(s)	21
21	0.87	t	7.0 (20)	20		14.10	(p)	
22	1.68	br s		8, 10, 12	10, 12, 13, 14	20.09	(p)	10

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

Table 3 ^{1}H and ^{13}C NMR data of penostatin B (**5**) in $CDCl_3$

Position	δ_H[a]			^{1}H-^{1}H COSY	NOEs[b]	δ_C	
1						196.07	(q)[c]
2	5.96	q	2.0 (4α, 4β, 7)	4α, 4β, 7	4α, 4β	122.68	(t)
3						168.82	(q)
4α	2.97	ddd	18.5 (4β), 6.5 (5), 2.0 (2)	2, 4β, 5	2	41.47	(s)
β	2.46	br dd	18.5 (4α), 6.5 (5)	2, 4α, 5, 6α	2		
5	4.52	quint	6.5 (4α, 4β, 6α, 6β)	4α, 4β, 6α, 6β		71.23	(t)
6α	2.54	dt	12.0 (6β), 6.5 (5, 7)	4β, 5, 6β, 7	10	38.61	(s)
β	1.54	ddd	12.0 (6α), 10.5 (7), 6.5 (5)	5, 6α, 7	8		
7	2.51	m		2, 6α, 6β	9	45.12	(t)
8	2.49	br t	10.8 (7, 9)	9, 10, 22	6β	45.27	(t)
9	4.00	d	10.8 (8)	8	7, 13, 14	73.93	(t)
10	5.54	br s		8, 22	6α, 22	121.60	(t)
11						136.42	(q)
12	4.59	br d	6.0 (13)	13, 22	14, 22	77.24	(t)
13	5.55	dd	15.5 (14), 6.0 (12)	12, 14	9, 15, 22	125.95	(t)
14	5.67	dt	15.5 (13), 6.5 (15)	13, 15	9, 12, 22	136.33	(t)
15	2.05	q	6.5 (14, 16)	14, 16	13	32.37	(s)
16	1.39	quint	6.5 (15, 17)	15, 17		29.02	(s)
17	1.25	br s		16		29.12	(s)
18	1.25	br s				29.12	(s)
19	1.25	br s				31.80	(s)
20	1.31	br s		21		22.64	(s)
21	0.87	t	6.5 (20)	20		14.12	(p)
22	1.66	br s		8, 10, 12	10, 12, 13, 14	20.06	(p)
5-OH	1.83	br s					

[a-c] As in Table 2.

penostatin A (**4**). The general spectral features of **5** closely resembled those of **4** except for the chemical shifts of H-4, H-5, H-6, H-7 and C-3, and a coupling relationship of H-5 in the NMR spectra (Table 3). This finding suggested that **5** was a stereoisomer of **4** at C-5. The 5- hydroxymethine proton in **5** was observed as a quintet (J 6.5 Hz), showing that the coupling constants from H-5 to the four protons of the C-4 and C-6 methylenes are equivalent. Analysis of the coupling constant by the modified Karplus relationship[8,9] suggested that the dihedral angles for H-5/H-4α, H-5/H-4β, H-5/H-6α and H-5/H-6β were approximately 30°, 150°, 30° and 150°, respectively, showing that the conformation of the five-membered ring in **5** is different from that in its isomer **4** and that H-5 is oriented pseudoaxially and consequently the 5-hydroxy group is oriented pseudoequatorially. This difference of the conformation of the five-membered ring between **4** and **5** was supported by the following NOE data. NOEs from H-10 to both H-6α and H-6β were observed in **4**, whereas an only NOE between H-10 and H-6α was observed in **5**. The assignment for each proton of the 4- and 6-methylene was supported by NOE values [H-5/H-4α (3.2 %) > H-5/H-4β (1.1 %); H-5/H-6α (4.3 %) > H-5/H-6β (0.8 %)]. The coupling constants from H-7 to H-6α, H-6β and H-8 ($J_{6\alpha,7}$ 6.5 Hz, $J_{6\beta,7}$ 10.5 Hz and $J_{7,8}$ 10.8 Hz) (Table 3) and NOE values from H-8 to H-6α and H-6β [H-8/H-6β (2.2 %) > H-8/H-6α (≈ 0 %)] implied that 7-H is arranged pseudoaxially and *trans* to H-8 and H-6β, and *cis* to H-5. Observations of an NOE between H-7 and H-9, and the coupling constant between H-8 and H-9 ($J_{8,9}$ 10.8 Hz) showed that H-8 and H-9 are arranged pseudoaxially and *trans* to each other. NOEs from H-22 to H-12, H-13 and H-14, and from H-9 to H-13 and H-14 suggested that the conformation of the dihydropyran ring and the arrangement of the nonenyl group in **5** are the same as those of **4** and that the C-12–C-13 axis in **5** also rotates in a $CDCl_3$ solution. The energies for minimized-energy structures (**5a** and **5b**) (Fig. 5) obtained by the same method as in **4** were 29.65 and 29.90 kcal mol^{-1}, respectively, and the energy barrier between them was a low value (ca. 5.3–5.7 kcal mol^{-1}). These data supported the deduction from the NMR data that the C-12–C-13 axis in **5** rotates in a $CDCl_3$ solution. Furthermore, the theoretical dihedral angles and internuclear distances for minimized-energy structures (**5a** and **5b**) supported the dihedral angles analyzed by the coupling constants and selected difference NOE values (the detailed data are omitted).

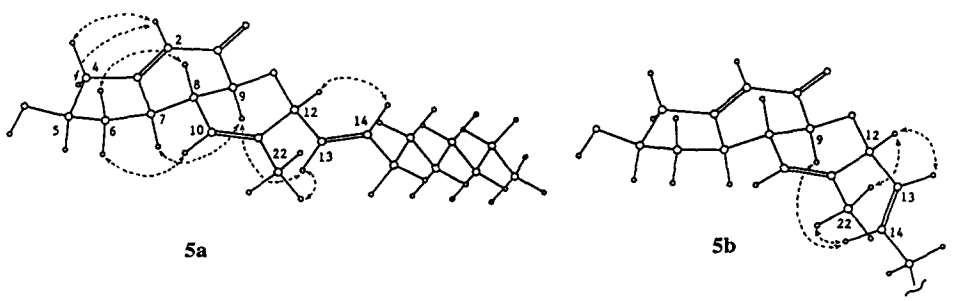

Fig. 5 Energy-minimized conformers **5a** and **5b** of penostatin B (**5**) and observed NOEs.

Treatment of **5** with (*R*)- and (*S*)-MTPACl in pyridine gave the MTPA esters **13a** and **13b**, respectively, together with the enantiomer **14** of **6** (Fig. 4). The ^{1}H chemical-shift differences between the esters **13a** and **13b** (Fig. 6) of **5** and formation of **14** from **5** led to the absolute stereostructure **5** for penostatin B. In the CD spectrum, the curve of **5** (Fig. 3) showed almost a mirror image of that of its isomer **4**, in which the respective negative and positive Cotton effects at 206 nm and 237 nm implied the 9*S* and 7*R* configurations, supporting the absolute stereostructure of **5** established by the modified Mosher method and the chemical transformation. The absolute stereostructure of penostatin B (**5**) thus established implied that all the asymmetric centers of **5** except for C-5 had the opposite absolute configuration to those of its isomer **4**. The asymmetric center of C-5 in **5** had the same absolute configuration as in **4**, but the conformation of the five-membered ring in **5** and consequently the orientation of the 5-hydroxy group were different from those of **4**. It is of great interest that one fungus produces a pair of stereoisomers (such as **4** and **5**), in which all the asymmetric centers except for one position have the opposite absolute configurations. In addition to penostatins A (**4**) and B (**5**), penostatins F and I have been previously reported[4] as examples of this sort of stereoisomers. The absolute stereochemistry of penostatins F and I corresponds to that of penostatins B (**5**) and A (**4**), respectively.

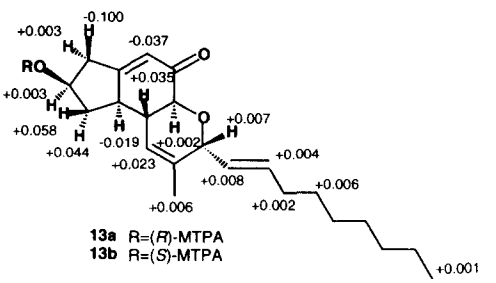

Fig. 6 ^{1}H chemical-shift differences ($\Delta\delta=\delta_S-\delta_R$) between the (*R*)- and (*S*)-MTPA esters (**13a** and **13b**) of penostatin B (**5**).

Penostatin D (**7**) had the molecular formula ($C_{22}H_{34}O_3$) which contained two proton atoms more than that of **4** and **5**. Comparison of the ^{1}H and ^{13}C NMR data of **7** (Table 4) with those of **5** suggested that the ketone (C-1) in **5** is replaced by a hydroxymethine in **7**. The coupling constants from H-5 to H-4 and H-6 ($J_{4\alpha,5}$ 7.5 Hz, $J_{4\beta,5}$ 5.0 Hz and $J_{5,6\alpha}$ 6.5 Hz) and an NOE between H-5 and H-7 implied that H-5 is arranged pseudoaxially and *cis* to H-7 as in **5**. The relative configuration of C-8, C-9 and C-12 and conformation of the dihydropyran ring in **7** were shown to be the same as those of **5** by the coupling constant between H-8 and H-9 ($J_{8,9}$ 9.5 Hz), and NOEs from H-7 to H-9, and from H-9 and H-22 to H-13 and H-14. These NOEs also showed that the C-12–C-13 axis in **7** rotates in a $CDCl_3$ solution. Observations of NOEs for H-1/H-8 and H-9/1-OH, and the coupling constant between H-1 and H-9 ($J_{1,9}$ 8.0 Hz) implied that H-1 and H-9 are oriented pseudoaxially and *trans* to each other. Compound **7** was treated with Ac_2O-CrO_3[13] to give the oxidation product **5**, identical with the natural product in all respects, including the CD spectrum. The above-summarized evidence allowed assignment of the absolute stereostructure **7** to penostatin D.

Penostatin E (**8**) was assigned the molecular formula $C_{22}H_{32}O_3$ as deduced from a molecular ion peak in HREIMS, indicating that it had one molecule of H_2O more than **6**. Its UV spectrum exhibited absorption bands at 235 and 281 nm. The IR spectrum contained absorption bands due to a hydroxy group (3464 cm^{-1}) and a conjugated ketone (1660 and 1620 cm^{-1}). The general features of the ^{1}H and ^{13}C NMR spectra of **8** (Table 5)

Table 4 ^{1}H and ^{13}C NMR data of penostatin D (**7**) in $CDCl_3$

Position	δ_H[a]			^{1}H-^{1}H COSY	NOEs[b]	δ_C	
1	4.34	m		2, 9	8	71.73	(t)[c]
2	5.47	quintet	2.0 (4α, 4β, 7, 1)	1, 4α, 4β, 7	4β	120.13	(t)
3						145.18	(q)
4α	2.71	br dd	16.5 (4β), 7.5 (5)	2, 4β, 5		40.30	(s)
β	2.19	br dd	16.5 (4α), 5.0 (5)	2, 4α, 5	2		
5	4.37	m		4α, 4β, 6α, 6β	7	71.87	(t)
6α	2.40	dt	13.0 (6β), 6.5 (5, 7)	5, 6β, 7	10	39.53	(s)
β	1.33	m		5, 6α, 7	10		
7	2.07	m		2, 6α, 6β	5, 9	43.43	(t)
8	2.03	m		9, 10, 22	1	41.50	(t)
9	3.45	dd	9.5 (8), 8.0 (1)	1, 8	7, 13, 14, 1-OH	75.50	(t)
10	5.50	q	1.5 (22)	8, 22	6α, 6β, 22	122.00	(t)
11						135.05	(q)
12	4.41	d	6.5 (13)	13, 22	14, 22	77.27	(t)
13	5.57	dd	15.5 (14), 6.5 (12)	12, 14	9, 15, 22	126.84	(t)
14	5.69	dt	15.5 (13), 6.5 (15)	13, 15	9, 12, 22	135.56	(t)
15	2.06	q	6.5 (14, 16)	14, 16	13	32.39	(s)
16	1.37	m		15, 17		29.13	(s)
17	1.27	br s		16		29.13	(s)
18	1.27	br s				29.13	(s)
19	1.27	br s				31.81	(s)
20	1.30	m		21		22.65	(s)
21	0.88	t	6.5 (20)	20		14.10	(p)
22	1.63	br s		8, 10, 12	10, 12, 13, 14	20.06	(p)
1-OH	2.34	br s			9		
5-OH	1.60	br s					

[a–c] As in Table 2.

Table 5 ^{1}H and ^{13}C NMR data of penostatin E (**8**) in $CDCl_3$

Position	δ_H[a]			^{1}H-^{1}H COSY	NOEs[b]	δ_C	
1						199.21	(q)[c]
2	6.02	d	2.5 (7)	7	4	114.86	(t)
3						173.49	(q)
4	6.49	dt	6.0 (5), 2.5 (6α, 6β)	5, 6α, 6β	2	132.06	(t)
5	6.73	dt	6.0 (4), 3.0 (6α, 6β)	4, 6α, 6β		149.86	(t)
6 α	2.29	dddd	18.0 (6β), 4.5 (7), 3.0 (5), 2.5 (4)	4, 5, 6β, 7		37.65	(s)
β	2.70	dddd	18.0 (6α), 7.0 (7), 3.0 (5), 2.5 (4)	4, 5, 6α, 7			
7	2.87	dddd	10.0 (8), 7.0 (6β), 4.5 (6α), 2.5 (2)	2, 6α, 6β	9, 10	45.86	(t)
8	3.02	q	10.0 (7, 9, 10)	9, 10	12, 9-OH	48.21	(t)
9	3.97	dd	10.0 (8), 1.5 (9-OH)	8	7, 10	77.23	(t)
10	5.41	br d	10.0 (8)	8, 12, 22	7, 9, 22	129.22	(t)
11						135.21	(q)
12	6.61	d	15.5 (13)	10, 13	8, 14	127.03	(t)
13	5.78	dd	15.5 (12), 6.5 (14)	12, 14	22	134.20	(t)
14	4.18	q	6.5 (13, 15)	13, 15	12	73.17	(t)
15	1.53	q	6.5 (14, 16)	14, 16		37.50	(s)
16	1.27	br s		15		29.52	(s)[c]
17	1.27	br s				29.25	(s)[c]
18	1.27	br s				25.45	(s)[c]
19	1.27	br s				31.80	(s)
20	1.27	br s		21		22.65	(s)
21	0.88	t	6.5 (20)	20		14.11	(p)
22	1.94	d	1.5 (10)	10	10, 13	21.06	(p)
9-OH	3.89	d	1.5 (9)		8		
14-OH	1.55	br s					

[a–c] As in Table 2.

closely resembled those of **6** except that the signal of two hydroxy groups appeared in **8** and the coupling relationships of the protons of one disubstituted double bond in **8** are different from those of **6**. This evidence suggested that **8** is a dihydroxy compound which is derived from fission of the dihydropyran ring in **6** and has a conjugated diene. Analysis of ^{1}H-^{1}H COSY (H-8/H-9, H-8/H-10, H-10/H-12 and H-13/H-14 etc.) and the EIMS fragment at *m/z* 227 [$M^+ - H_2O - C_7H_{15}$] led to the planar structure **8** with $\Delta^{10,12}$-diene and 9, 14-dihydroxy groups for penostatin E. The geometrical configuration of $\Delta^{10,12}$-diene was deduced as 10-*cis*, 11-s-*trans* and 12-*trans* from the NOEs for H-10/H-22 and H-22/H-13, and the chemical shift of C-22 (δ_C 21.06)[14] and the large coupling constant ($J_{12,13}$ 15.5 Hz) between H-12 and H-13.

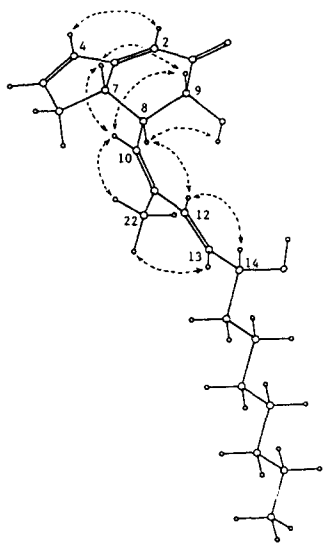

Fig. 7 Energy-minimized conformation of penostatin E (**8**) and observed NOEs.

The relative stereochemistry for **8** was established by a combination of the observed coupling constants and NOE data (Table 5 and Fig. 7). The observation of an NOE between H-7 and H-9, and coupling constants from H-8 to H-7 and H-9 ($J_{7,8}$ 10 Hz; $J_{8,9}$ 10 Hz) suggested that H-7, H-8 and H-9 are oriented pseudoaxially and H-8 is *trans* to H-7 and H-9. In addition, NOEs for H-8/H-12, H-7/H-10 and H-9/H-10 suggested that the side chain of **8** exists in a conformation in which H-10 is *trans* to H-8, and H-12 is on the same side as H-8. The dihedral angles analyzed by the coupling constants as mentioned above with compounds **4** and **5** are nearly equivalent to those of a minimized-energy structure (Fig. 7) obtained by the CaChe MM2 method on the basis of the NMR data. The absolute configuration of C-14 was established by the modified Mosher method. Treatment of **8** with (*R*)- and (*S*)-MTPACl in pyridine gave the 14-MTPA monoesters **15a** and **15b**, respectively, between which the ^{1}H chemical-shift differences (Fig. 8) suggested that the asymmetric center at C-14 has the *S* configuration. In the CD spectrum of compound **8**, there was a negative band at 290 nm as observed in **6**, implying the 7*S* configuration. Thus, the absolute stereostructure of **8** was elucidated.

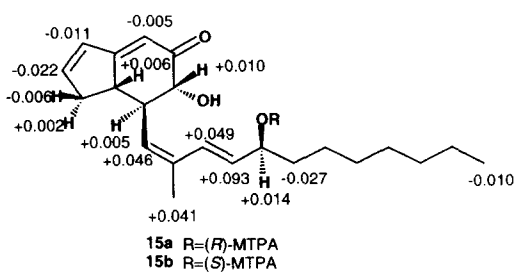

Fig. 8 ^{1}H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**15a** and **15b**) of penostatin E (**8**).

The cytotoxic activities of the compounds obtained herein were examined in the P388 lymphocytic leukemia test system in cell culture. As shown in Table 6, all the compounds except for **7** exhibited significant cytotoxic activity and patulin (**9**) showed the most potent cytotoxicity. The

result shown in Table 6 suggested that the cyclic conjugated enone system plays an important role for the enhancement of the cytotoxic activities in penostatins. Penostatin C (**6**) and patulin (**9**) also were tested against a panel of human tumor cell lines (39 kinds of the human tumor cells) and displayed significant cytotoxicity against seven kinds of the tumor cells shown in Table 6 and against almost all the human tumor cells including those shown in Table 6, respectively.

Table 6. Cytotoxicity of compounds **4-10** against tumor cells[a]

	4	5	6	7	8	9	10
P388	0.8	1.2	1.0	11.0	0.9	0.06	0.2
BSY-1[b]			2.0			0.34	
MCF-7[b]			1.6			0.65	
HCC2998[c]			2.0			1.54	
NCI-H522[d]			2.5			0.30	
DMS114[d]			1.9			0.57	
OVCAR-3[e]			2.4			0.37	
MKN1[f]			1.7			0.39	

[a] Data are expressed as ED_{50} values (μg/ml). [b] Human brain tumor. [c] Human colon cancer. [d] Human lung cancer. [e] Human ovarian cancer. [f] Human stomach cancer.

Experimental Section

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. 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 Varian UNITY INOVA-500 spectrometer, with the usual parameters. EIMS was 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 x 20 mm i. d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent CH_2Cl_2-MeOH (19:1), and compounds were viewed under 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. As reported previously,[4] a strain of *Penicillium* sp. isolated from the marine alga *Enteromorpha intestinalis* (Linne) Link (Ulvaceae) was grown in a liquid medium (40 l) 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 collected was extracted thrice with MeOH. The combined extracts were evaporated under reduced pressure to give an MeOH concentrate (45 g). On the other hand, the culture filtrate was extracted thrice with AcOEt and the combined extracts were evaporated under reduced pressure to give an AcOEt concentrate (32 g).

The MeOH concentrate 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 3 fractions [Fr. 7 (239 mg), Fr. 8 (106.3 mg) and Fr. 9 (201.1 mg)], respectively. Fr. 1 was purified by HPLC (ODS) using MeOH–water (9 : 1) as the eluent to afford **6** (37 mg). Fr. 3, Fr. 6 and Fr. 7 afforded **2** (33 mg), **4** (45 mg) and **8** (10 mg), **5** (9 mg), and **1** (28 mg) and **7** (6 mg), respectively, after purification by HPLC (ODS) using MeOH-water (4 : 1) as the eluent. Communesins A (**1**) and B (**2**) were identified by direct comparison with an authentic sample.

The AcOEt concentrate was passed through Sephadex LH-20, using MeOH–CH_2Cl_2 (1: 1) as the eluent. The second fraction (11.8 g) was chromatographed on a silica gel column with a CH_2Cl_2–MeOH gradient as the eluent. The CH_2Cl_2 and CH_2Cl_2–MeOH (1: 49) eluates gave patulin (**9**) (3 g) and (+)-epiepoxydon (**10**) (330 mg), respectively.

Penostatin A (4). Obtained as colorless needles, mp 73–75 °C (from MeOH), $[\alpha]_D$ +133.3° (*c* 0.18 in $CHCl_3$); λ_{max} (EtOH)/nm 232 (logε 4.22); ν_{max} (KBr)/ cm^{-1} 3462 (OH), 1669 (C=C-CO) and 1639 (C=C); *m/z* (EI) 344 (32%, M^+), 245 (100, $M^+-(CH_2)_6CH_3$), 227 (18, $M^+-(CH_2)_6CH_3-H_2O$), 220 (16), 124 (68) and 106 (77); (Found: M^+, 344.2356. $C_{22}H_{32}O_3$ requires M^+, 344.2351); CD λ (*c* 1.08 x 10^{-4} M in EtOH)/nm 205.5 ($\Delta\varepsilon$ +13.38), 221 (0), 241 (–2.96), 274 (0), 323 (+1.55) and 373 (0). ^1H and ^{13}C NMR data are listed in Table 1.

Penostatin B (5). Obtained as a colorless powder, mp 63–66°C, $[\alpha]_D$ –103.1° (*c* 0.49 in $CHCl_3$); λ_{max} (EtOH)/nm 230 (logε 4.10); ν_{max} (KBr)/cm^{-1} 3465 (OH), 1673 (C=C-CO) and 1637 (C=C); *m/z* (EI) 344 (50%, M^+), 245 (100, $M^+-(CH_2)_6CH_3$), 227 (9, $M^+-(CH_2)_6CH_3-H_2O$), 220 (16), 124 (34) and 106 (12); (Found: M^+, 344.2353. $C_{22}H_{32}O_3$ requires M^+, 344.2351); CD λ (*c* 1.09 x 10^{-4} M in EtOH)/nm 206 ($\Delta\varepsilon$ –14.87), 220 (0), 237 (+4.17), 270 (0), 320 (–1.81) and 375 (0). ^1H and ^{13}C NMR data are listed in Table 3.

Penostatin C (6). Obtained as a colorless powder, mp 63–65°C, $[\alpha]_D$ +120.0° (*c* 1.00 in $CHCl_3$); λ_{max} (EtOH)/nm 283 (logε 4.30); ν_{max} (KBr)/ cm^{-1} 1675 (C=C-CO) and 1610 (C=C-C=C); m/z (EI) 326 (45%, M^+), 227 (61, $M^+-(CH_2)_6CH_3$), 220 (29) and 106 (100); (Found: M^+, 326.2245. $C_{22}H_{30}O_2$ requires M^+, 326.2244); CD λ (*c* 5.67 x 10^{-5} M in EtOH)/nm 203 ($\Delta\varepsilon$ +9.08), 238 (0), 274 (–1.60), 300 (0), 327 (+2.14), 385 (0). ^1H and ^{13}C NMR data are listed in Table 2.

Penostatin D (7). Obtained as a colorless powder, mp 106–110°C, $[\alpha]_D$ -26.7° (*c* 0.14 in $CHCl_3$); ν_{max} (KBr)/ cm^{-1} 3343 (OH) and 1634 (C=C); *m/z* (EI) 346 (38%, M^+), 247 (100, $M^+-(CH_2)_6CH_3$), 220 (14), 151 (20) and 108 (21); (Found: M^+, 346.2504. $C_{22}H_{34}O_3$ requires M^+, 346.2508). ^1H and ^{13}C NMR data are listed in Table 4.

Penostatin E (8). Obtained as a colorless oil, $[\alpha]_D$ +48.5° (*c* 0.16 in $CHCl_3$); λ_{max} (EtOH)/nm 235 (logε 4.40) and 281 (4.23); ν_{max} (liquid film)/ cm^{-1} 3464 (OH), 1660 (C=C-CO) and 1620 (C=C-C=C); *m/z* (EI) 344 (0.6 %, M^+), 326 (3, M^+-H_2O), 227 (19, $M^+-(CH_2)_6CH_3-H_2O$) and 106 (100); (Found: M^+, 344.2352. $C_{22}H_{32}O_3$ requires M^+, 344.2351); CD λ (*c* 5.81 x 10^{-5} M in EtOH)/nm 211 ($\Delta\varepsilon$ –1.30), 229 (–2.61), 244 (0), 255 (+2.35), 271 (0), 290 (–3.39), 308 (0), 328 (+2.61) and 380 (0). ^1H and ^{13}C NMR data are listed in Table 5.

Patulin (9). Obtained as a colorless oil. *m/z* (EI) 154 (M^+). δ_H ($CDCl_3$) 4.40 (1H, ddd, *J* 17.8, 3.8 and 0.8 Hz, H-5A), 4.50 (1H, br s, OH), 4.70 (1H, ddd, *J* 17.8, 4.5 and 1.0 Hz, H-5B), 5.95 (1H, m, H-6), 6.00 (1H, m, H-4), 6.03 (1H, br s, H-2). Its spectal data were identical with published values.[6]

(+)-Epiepoxydon (10). Obtained as a colorless oil, $[\alpha]_D$ +114° (*c* 0.92, CH_3OH). *m/z* (EI) 156 (M^+). δ_H ($CDCl_3$) 2.16 (1H, t, *J* 6.3 Hz, 7-OH), 2.22 (1H, d, *J* 8.8 Hz, 4-OH), 3.51 (1H, dd, *J* 3.7 and 1.1 Hz, H-2), 3.82 (1H, m, H-3), 4.24 (1H, dd, *J* 14.5 and 6.3 Hz, H-7), 4.40 (1H, dd, *J* 14.5 and 6.3 Hz, H-7), 4.75 (1H, m, H-4), 6.69 (1H, m, H-5). Its spectal data were identical with published values.[7]

Acetylation of Penostatin A (4). Ac_2O (0.5 ml) was added to a pyridine solution (0.2 ml) of penostatin A (**4**) (2.0 mg), and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure, and the residue was purified by HPLC using MeOH as the eluent to afford the acetate **11** (1.5 mg) as an oil. *m/z* (EI) 386 (M^+). ^1H NMR data are listed in Table 1.

Formation of the (*R*)- and (*S*)-MTPA esters 12a and 12b from penostatin A (4). (*R*)-MTPACl (0.1 ml) was added to a pyridine solution (0.5 ml) of penostatin A (**4**) (5.8 mg), and the reaction mixture was left at room temperature for 18 h. The solvent was evaporated off under reduced pressure, and the residue was purified by chromatography on a silica gel column with a hexane-CH_2Cl_2 gradient as the eluent. The hexane-CH_2Cl_2 (3:1) eluate was purified by HPLC (ODS) using MeOH to afford the ester **12a** (1.7 mg) and penostatin C (**6**) (2.5 mg). The same reaction with **4** (4.0 mg) using (*S*)-MTPACl (0.1 ml) gave the ester **12b** (1.0 mg) and penostatin C (**6**) (1.5 mg). Penostatin C (**6**) was identified by direct comparison with an authentic sample.

12a : Obtained as a colorless oil; *m/z* (EI) 560 (M^+); δ_H (300 MHz; $CDCl_3$) 0.87 (3H, t, *J* 6.5 Hz, H-21),1.26 (8H, br s, H-17-20), 1.37 (2H, quintet, *J* 6.5 Hz, H-16), 1.67 (1H, m, H-6α), 1.67 (3H, s, H-22), 2.04 (2H, q, *J* 6.5 Hz, H-15), 2.38 (1H, m, H-6β), 2.41 (1H, m, H-7), 2.41 (1H, m, H-8), 2.81 (1H, br d, *J* 19.5 Hz, H-4β), 3.01 (1H, br dd, *J* 19.5 and 5.0 Hz, H-4α), 3.56 (3H, s, OMe), 3.96 (1H, d, *J* 11.0 Hz, H-9), 4.58 (1H, br d, *J* 6.0 Hz, H-12), 5.44 (1H, br s, H-10), 5.54 (1H, dd, *J* 15.0 and 6.0 Hz, H-13), 5.61 (1H, br t, *J* 5.0 Hz, H-5), 5.66 (1H, dt, *J* 15.0 and 6.5 Hz, H-14), 5.96 (1H, q, *J* 2.5 Hz, H-2), 7.42 (3H, m, Ar-H) and 7.51 (2H, m, Ar-H).

12b : Obtained as a colorless oil; *m/z* (EI) 560 (M^+); δ_H (300 MHz; $CDCl_3$) 0.87 (3H, t, *J* 6.5 Hz, H-21), 1.26 (8H, br s, H-17-20), 1.37 (2H, quintet, *J* 6.5 Hz, H-16), 1.67 (3H, s, H-22), 1.72 (1H, td, *J* 12.5 and 5.0 Hz, H-6α), 2.06 (2H, q, *J* 6.5 Hz, H-15), 2.42 (1H, br tq, *J* 11.0 and 1.5 Hz, H-8), 2.45 (1H, ddd, *J* 12.5, 7.0 and 2.0 Hz, H-6β), 2.59 (1H, m, H-7), 2.73 (1H, br d, *J* 19.5 Hz, H-4β), 3.01 (1H, br dd, *J* 19.5 and 5.0 Hz, H-4α), 3.54 (3H, s, OMe), 4.01 (1H, d, *J* 11.0 Hz, H-9), 4.59 (1H, br d, *J* 6.0 Hz, H-12), 5.48 (1H, br s, H-10), 5.55 (1H, dd, *J* 15.0 and 6.0 Hz, H-13), 5.62 (1H, br t, *J* 5.0 Hz, H-5), 5.68 (1H, dt, *J* 15.0 and 6.5 Hz, H-14), 5.95 (1H, q, J 2.5 Hz, H-2), 7.42 (3H, m, Ar-H) and 7.51 (2H, m, Ar-H).

Formation of the (R)- and (S)-MTPA esters 13a and 13b from penostatin B (5). Using the same procedure as above with compound 4, penostatin B (5) (3.8 and 3.5 mg) was treated with (R)-MTPACl (0.1 ml) and (S)-MTPACl (0.1 ml) to afford the ester 13a (1.0 mg) and compound 14 (1.5 mg), and the ester 13b (0.8 mg) and compound 14 (1.2 mg), respectively. Compound 14 showed a CD curve (Fig. 4) almost a mirror image of that

of its isomer 6.

13a: Obtained as a colorless oil; m/z (EI) 560 (M+); _ H (300 MHz; CDCl3) 0.87 (3H, t, J 6.5 Hz, H-21), 1.25 (8H, br s, H-17-20), 1.36 (2H, m, H-16), 1.65 (3H, s, H-22), 1.66 (1H, ddd, J 12.0, 10.5 and 6.5 Hz, H-6 _), 2.05 (2H, q, J 6.5 Hz, H-15), 2.39 (1H, dq, J 10.5 and 2.0 Hz, H-8), 2.49 (1H, br tdd, *J* 10.5, 6.5 and 2.0 Hz, H-7), 2.66 (1H, br dd, *J* 18.5 and 6.5 Hz, H-4β), 2.67 (1H, dt, *J* 12.0 and 6.5 Hz, H-6α), 3.07 (1H, ddd, *J* 18.5, 6.5 and 2.0 Hz, H-4α), 3.52 (3H, s, OMe), 3.99 (1H, d, *J* 10.5 Hz, H-9), 4.58 (1H, br d, *J* 6.0 Hz, H-12), 5.48 (1H, br s, H-10), 5.48 (1H, quint, *J* 6.5 Hz, H-5), 5.54 (1H, dd, *J* 15.0 and 6.0 Hz, H-13), 5.67 (1H, dt, *J* 15.0 and 6.5 Hz, H-14), 5.95 (1H, q, *J* 2.0 Hz, H-2), 7.42 (3H, m, Ar-H) and 7.50 (2H, m, Ar-H).

13b: Obtained as a colorless oil; *m/z* (EI) 560 (M^+); δ_H (300 MHz; $CDCl_3$) 0.87 (3H, t, *J* 6.5 Hz, H-21), 1.26 (8H, br s, H-17-20), 1.37 (2H, m, H-16), 1.66 (3H, s, H-22), 1.72 (1H, ddd, *J* 12.0, 10.5 and 6.5 Hz, H-6β), 2.05 (2H, q, *J* 6.5 Hz, H-15), 2.42 (1H, dq, *J* 10.5 and 2.0 Hz, H-8), 2.47 (1H, br tdd, *J* 10.5, 6.5 and 2.0 Hz, H-7), 2.56 (1H, br dd, *J* 18.5 and 6.5 Hz, H-4β), 2.71 (1H, dt, *J* 12.0 and 6.5 Hz, H-6α), 3.08 (1H, ddd, *J* 18.5, 6.5 and 2.0 Hz, H-4α), 3.52 (3H, s, OMe), 4.00 (1H, d, *J* 10.5 Hz, H-9), 4.59 (1H, br d, *J* 6.0 Hz, H-12), 5.49 (1H, quint, *J* 6.5 Hz, H-5), 5.50 (1H, br s, H-10), 5.55 (1H, dd, *J* 15.0 and 6.0 Hz, H-13), 5.67 (1H, dt, *J* 15.0 and 6.5 Hz, H-14), 5.92 (1H, q, *J* 2.0 Hz, H-2), 7.42 (3H, m, Ar-H) and 7.50 (2H, m, Ar-H).

Formation of penostatin B (5) from penostatin D (7). Chromium trioxide (3 mg) was added to a mixture of acetic anhydride (0.5 ml), glacial acetic acid (1 ml) and benzene (0.5 ml). One drop of the above reagent was added to a benzene solution (0.4 ml) of penostatin D (**7**) (1.2 mg) with stirring, and immediately after that, the mixture was neutralized with 10% aqueous $NaHCO_3$ and extracted with benzene. The extract was evaporated off under reduceed pressure, and the residue was purified by HPLC (ODS) using MeOH to afford penostatin B (**5**) (1.0 mg), which was identified by direct comparison with an authentic sample.

Formation of the (*R*)- and (*S*)-MTPA esters (15a and 15b) from penostatin E (8). (*R*)-MTPA (5 mg), dicyclohexylcarbodiimide (DCC) (5 mg) and 4-(dimethylamino)pyridine (DMAP) (2 mg) were added to a CH_2Cl_2 solution (1 ml) of penostatin E (**8**) (1.0 mg), and the reaction mixture was left at room temperature for 6 h. The solvent was evaporated off under reduced pressure, and the residue was purified by a silica gel column chromatography with a CH_2Cl_2-MeOH gradient and then HPLC (ODS) using MeOH to afford **15a** (0.8 mg) The same reaction with **8** (1.2 mg) using (*S*)-MTPA (5 mg) gave **15b** (0.6 mg).

15a : Obtained as a colourless oil; *m/z* (EI) 560 (M^+); δ_H (300 MHz; $CDCl_3$) 0.88 (3H, t, *J* 6.5 Hz, H-21), 1.26 (10H, br s, H-16-20), 1.75 (2H, q, *J* 6.5 Hz, H-15), 1.88 (3H, s, H-22), 2.20 (1H, br d, *J* 18.0 Hz, H-6α), 2.66 (1H, br d, *J* 18.0 Hz, H-6β), 2.87 (1H, m, H-7), 2.89 (1H, q, *J* 10.0 Hz, H-8), 3.55 (3H, s, OMe), 3.95 (1H, d, *J* 10.0 Hz, H-9), 5.43 (1H, br d, *J* 10.0 Hz, H-10), 5.53 (1H, dd, *J* 15.0 and 6.5 Hz, H-14), 5.64 (1H, dd, *J* 15.0 and 6.0 Hz, H-13), 6.03 (1H, br s, H-2), 6.50 (1H, br s, H-4), 6.63 (1H, br d, *J* 15.0 Hz, H-12), 6.70 (1H, m, H-5), 7.42 (3H, m, Ar-H), 7.50 (2H, m, Ar-H).

15b : Obtained as a colorless oil; *m/z* (EI) 560 (M^+); δ_H (300 MHz; $CDCl_3$) 0.87 (3H, t, *J* 6.5 Hz, H-21), 1.25 (10H, br s, H-16-20), 1.73 (2H, q, *J* 6.5 Hz, H-15), 1.92 (3H, s, H-22), 2.20 (1H, br d, *J* 18.0 Hz, H-6α), 2.65 (1H, br d, *J* 18.0 Hz, H-6β), 2.88 (1H, m, H-7), 2.89 (1H, q, *J* 10.0 Hz, H-8), 3.54 (3H, s, OMe), 3.96 (1H, d,

J 10.0 Hz, H-9), 5.48 (1H, br d, *J* 10.0 Hz, H-10), 5.55 (1H, dd, *J* 15.0 and 6.5 Hz, H-14), 5.74 (1H, dd, *J* 15.0 and 6.0 Hz,H-13), 6.03 (1H, br s, H-2), 6.49 (1H, br s, H-4), 6.67 (1H, br d, *J* 15.0 Hz, H-12), 6.68 (1H, m, H-5), 7.42 (3H, m, Ar-H), 7.50 (2H, m, Ar-H).

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