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Structures of New Cytotoxic Antibiotics, Piericidins C_7 and C_8

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Abstract Two new cytotoxic antibiotics, piericidins C_7 (**1**) and C_8 (**2**), were isolated from a marine *Streptomyces* sp. High-resolution FAB-MS established the molecular formulae of **1** and **2** as $C_{28}H_{41}NO_5$ and $C_{29}H_{43}NO_5$, respectively. The planar structures of the piericidins were elucidated by NMR spectral analysis including COSY, HMQC and HMBC. The relative stereochemistry of **2** was determined based on NOESY and coupling constant analyses. **1** and **2** are new members of the piericidin family possessing 11,12-epoxide and 12-butenyl groups.

Keywords piericidin, cytotoxic antibiotic, epoxide

Introduction

In the course of screening for antitumor antibiotics against cells transformed with the adenovirus E1A gene, two new active substances belonging to the piericidin family were isolated from the fermentation broth of a marine *Streptomyces* sp. and were named piericidins C_7 (**1**) and C_8 (**2**). In the preceding paper [1], we have described the fermentation, isolation, physico-chemical properties and biological activities of **1** and **2**, as well as the taxonomy of the producing organism. This paper describes the structure elucidation of **1** and **2**.

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Results and Discussion

Structure Elucidation

High-resolution FAB-MS established the molecular formulae of **1** and **2** as $C_{28}H_{41}NO_5$ (*m/z* 472.3062 $[MH]^+$, $\Delta -0.1$ mmu) and $C_{29}H_{43}NO_5$ (*m/z* 486.3223 $[MH]^+$, $\Delta +0.4$ mmu), respectively. The IR spectra of **1** and **2** revealed the presence of hydroxyl groups (3400 cm^{-1}) and the absence of carbonyl groups. ^{13}C and ^1H NMR data for **1** and **2** are summarized in Table 1. All one-bond ^1H-^{13}C connectivities were confirmed by a heteronuclear multiple-quantum coherence (HMQC) experiment [2].

Three separate proton spin systems in **2** depicted in Fig. 1 by bold lines were identified by a COSY experiment and were expanded to a 3,5,7,9,11,13-hexamethylpentadeca-2,5,7,13-tetraenyl moiety based on ^1H-^{13}C long-range couplings from five singlet methyls (3-Me, 5-Me, 7-Me, 11-Me and 13-Me) to the relevant carbons in the heteronuclear multiple-bond correlation (HMBC) [3] spectrum (Fig. 1). Oxygen substituents were located on C-10, C-11 and C-12 from their chemical shifts (δ 82.7, 66.0 and 66.0, respectively). The remaining five aromatic carbons and a nitrogen atom were required to construct a pyridine moiety. The substitution of the pyridine ring was determined by ^1H-^{13}C long-range correlations from 1-H to C-1′ and C-2′, from a singlet methyl (δ_H 2.03) to C-1′, C-2′ and C-3′, from a methoxyl (δ_H 3.72) to C-4′ and from a methoxyl (δ_H 3.89) to C-5′. A low-field ^{13}C chemical shift (δ 151.5) for C-3′ indicated the presence of a hydroxyl substituent on C-3′. The existence of an epoxide ring between C-11 and C-12 was required by their characteristic ^{13}C chemical shifts (δ 66.0×2) and the molecular formula of **2**. The geometrical configurations were elucidated to be 2*E*, 5*E*, 7*E* and 13*E* from high-field ^{13}C chemical shifts for five allylic methyls and NOESY correlations between 2-H

and 4-H_2, between 4-H_2 and 6-H, and between 6-H and 8-H (Fig. 2).

The relative configurations of **2** were analyzed by NOE correlations and coupling constants. The epoxide stereochemistry was identified as *trans* by NOEs between 10-H and 12-H and between 11-Me and 14-H. A large coupling constant (8.5 Hz) revealed an anti relationship between 9-H and 10-H. NOEs from 9-Me to 10-H and 12-H required these atoms to exist on the same side of a plane representing C-8, C-9, C-10 and C-11 as shown in Fig. 2. An NOE between 9-H and 11-Me arranged them on the opposite side (Fig. 2), thereby establishing the structure of **2** including the relative stereochemistry as shown in Fig. 3.

The ^{13}C and ^{1}H NMR spectra of **1** showed a loss of one methyl in comparison with **2** (Table 1). A newly appeared olefinic proton (δ 5.55) was coupled with a methylene (4-H_2) and an olefinic methine (6-H), and its *E* geometry was determined by a large vicinal coupling constant (15.5 Hz). The remaining spectral features were essentially identical with those of **2**, indicating that **1** is a 5-demethyl derivative of **2**. The structure of **1** (Fig. 3) was confirmed by COSY, HMQC, HMBC and NOESY experiments (data not shown).

Table 1 NMR data summary for **1** and **2** in CD_3OD

No.	**1**		**2**	
	δ_C	δ_H multiplicity (Hz)	δ_C	δ_H multiplicity (Hz)
1	35.2	3.34 2H d (7.0)	35.2	3.35 2H d (7.0)
2	123.4	5.30 t (7.0)	124.7	5.30 t (7.0)
3	135.9		135.1	
4	43.9	2.75 2H d (7.0)	51.9	2.69 2H s
5	126.7	5.55 dt (15.5, 7.0)	135.0	
6	137.5	6.06 d (15.5)	131.7	5.66 s
7	134.9		134.3	
8	134.7	5.31 d (10.0)	133.3	5.17 d (10.0)
9	37.7	2.68 m	37.7	2.62 m
10	82.6	2.99 d (8.5)	82.7	2.97 d (8.5)
11	65.9		66.0	
12	65.8	3.23 s	66.0	3.25 s
13	130.6		130.5	
14	121.8	5.41 q (7.0)	121.8	5.42 q (7.0)
15	12.8	1.64 3H d (7.0)	12.8	1.64 3H d (7.0)
1′	151.6		151.5	
2′	114.5		114.6	
3′	151.6		151.5	
4′	130.1		130.1	
5′	156.0		156.0	
3-Me	16.7	1.72 3H s	16.0	1.67 3H s
5-Me			17.6	1.65 3H s
7-Me	13.1	1.74 3H s	17.6	1.72 3H s
9-Me	18.2	0.98 3H d (7.0)	18.2	0.99 3H d (7.0)
11-Me	10.6	1.09 3H s	10.6	1.11 3H s
13-Me	13.9	1.65 3H s	13.9	1.65 3H s
2′-Me	10.8	2.03 3H s	10.9	2.03 3H s
4′-OMe	60.8	3.72 3H s	60.9	3.72 3H s
5′-OMe	53.8	3.89 3H s	53.9	3.89 3H s

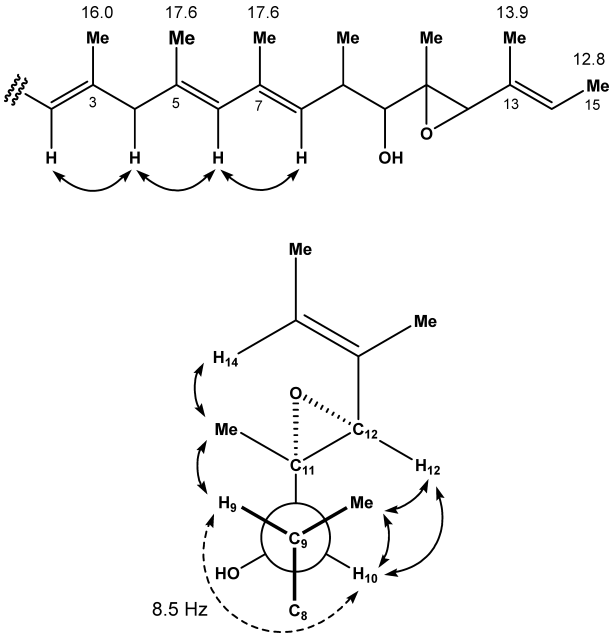

Fig. 2 Relative stereochemistry of **2**.

Solid arrows show NOE correlations and dashed arrows indicate ^{1}H-^{1}H couplings.

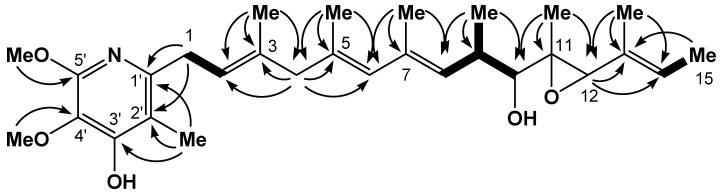

Fig. 1 ^{1}H-^{1}H COSY and HMBC analyses for piericidin C_8 (**2**).

Bold lines show ^{1}H spin networks and arrows indicate ^{1}H-^{13}C long-range correlations.

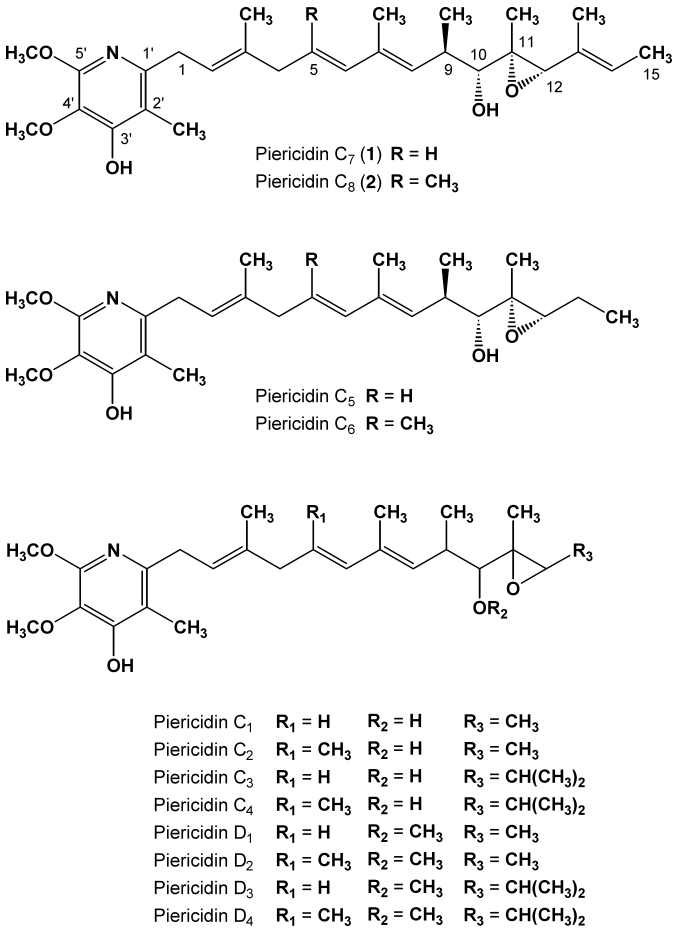

Fig. 3 Structures of piericidins C_7 (**1**) and C_8 (**2**) and related compounds.

A series of piericidins possessing an epoxide moiety have been isolated from *Streptomyces* sp. and *Nocardioides* sp., *e.g.*, piericidins C_1~C_6 and piericidins D_1~D_4 (Fig. 3) [4~7]. **1** and **2** represent two new members of this family and are the first examples produced by a marine actinomycete.

Experimental

Spectroscopic Measurements

Mass spectra were obtained on a JEOL SX-102A spectrometer in the FAB mode using *m*-nitrobenzyl alcohol as matrix and polyethylene glycol as internal standard. IR spectra were measured on a JASCO FT/IR-410 spectrometer. NMR spectra were obtained on a JEOL JNM-LA400 spectrometer with ^{1}H NMR at 400 MHz and with ^{13}C NMR at 100 MHz. Chemical shifts are given in ppm relative to CHD_2OD at 3.30 ppm for ^{1}H NMR and CD_3OD at 49.0 ppm for ^{13}C NMR.

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