

Amphidinin B, a New Polyketide Metabolite from Marine Dinoflagellate *Amphidinium* sp.

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This paper is dedicated to the memory of Professor Kenneth L. Rinehart at University of Illinois.

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Abstract A new polyketide metabolite, amphidinin B (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp. and the structure and absolute stereochemistry were elucidated on the basis of spectroscopic data and chemical means.

Keywords amphidinin B, dinoflagellate, polyketide

Introduction

Marine dinoflagellates of the genus *Amphidinium* have been recognized as a source of novel secondary metabolites with interesting bioactivities [1–7]. In our continuing search for bioactive metabolites from Okinawan marine organisms, we have investigated extracts of laboratory cultured marine dinoflagellates *Amphidinium* sp., which were symbionts of the Okinawan marine acoel flatworms *Amphiscolops* sp., and isolated a series of cytotoxic macrolides, amphidinolides [1], as well as long chain polyhydroxyl polyketides [3, 6, 7]. Here we describe the isolation and structure elucidation of a new polyketide metabolite, amphidinin B (**1**), which was isolated from extracts of a strain (Y-56) of the dinoflagellate *Amphidinium* sp.

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Materials and Methods

General Procedures

NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd.). The 7.20 and 128.0 ppm resonances of residual C_6D_6 were used as internal references for 1H and ^{13}C NMR spectra, respectively. FABMS spectra were obtained on a JEOL JMS-HX110 spectrometer using glycerol as a matrix in positive mode.

Cultivation and Isolation

The dinoflagellate *Amphidinium* sp. (strain number Y-56) was isolated from inside cells of a marine acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa. The dinoflagellate has been deposited in Graduate School of Pharmaceutical Sciences, Hokkaido University. The dinoflagellate was uniaxially cultured at 25°C for 2 weeks in a seawater medium enriched with 1% ES supplement [8], which consisted of the following elements in 3 liters of distilled water: $NaNO_3$, 10.5 g; $Na_2C_3H_5(OH)_2PO_4 \cdot 5.5H_2O$ (sodium glycerophosphate), 1.5 g; $FeCl_3 \cdot 6H_2O$, 138 mg; $Na_2EDTA \cdot 2H_2O$ (ethylenediaminetetraacetic acid, disodium salt), 900 mg; H_3BO_4 , 150 mg; $MnCl_2$, 30 mg; $ZnCl_2$, 3.8 mg; $CoCl_2$, 0.75 mg; vitamin B_{12} , 0.3 mg; thiamine hydrochloride, 15 mg; D-biotin, 0.15 mg; tris(hydroxymethyl)aminomethane, 15 g. The pH of the supplement was adjusted to 7.8 with 3 M hydrochloric acid, prior to mixing with seawater which was sterilized by autoclaving. The harvested cells of the cultured dinoflagellate (420 g, wet weight, from 580 liters of culture) were extracted with MeOH/toluene (3 : 1). After addition of 1 M NaCl, the mixture was extracted with

toluene. The toluene-soluble fraction was evaporated under reduced pressure to give a residue (3.67 g), which was partially (2.67 g) subjected to a silica gel column (CHCl₃/MeOH, 98:2) and a Sep-Pak C18 cartridge (MeOH/H₂O, 8:2) followed by C18 HPLC [LUNA C18(2), 5 μm, Phenomenex, 10 mm×250 mm; eluent, CH₃CN/H₂O, 85:15; flow rate, 2.5 ml/minute; UV detection at 210 nm] to afford amphidin B (**1**, 0.8 mg, 0.0002%, wet weight) together with known macrolides, amphidinolides T1 (0.005%) [9], T3 (0.0004%) [10], T4 (0.0002%) [10], and T5 (0.0004%) [11].

Reductive Degradation of Amphidin B (**1**)

Amphidin B (**1**, 0.1 mg) was dissolved in MeOH (50 μl) and treated with TMS-CHN₂ at room temperature for 1 hour. The reaction mixture was evaporated *in vacuo* to afford a crude residue. A solution of the residue in THF (20 μl) was treated with LiAlH₄ (1.0 mg) at room temperature for 1 hour. The reaction mixture was partitioned between EtOAc (200 μl×3) and 1 M phosphate buffer (100 μl). The organic phase was evaporated, and the residue was dissolved in 0.1% DMAP solution of CH₂Cl₂ (50 μl). Et₃N (2 μl) and (*R*)-MTPACl (1 μl) were added to the mixture and stirred at room temperature for 20 hours. After addition of *N,N*-dimethyl-1,3-propanediamine (1 μl), the solvent was evaporated *in vacuo*. The residue was purified by C18 HPLC (Wakosil-II 5C18 RS, Wako Pure Chemical Ind., Ltd., 4.6×250 mm; eluent CH₃CN/H₂O, 90:10; flow rate, 1.0 ml/minute; UV detection at 230 nm) to give compounds **2** and **3** [9].

Results

Physico-chemical Properties

Physico-chemical properties of amphidin B (**1**) are summarized in Table 1. Amphidin B (**1**) was soluble in methanol, chloroform, and benzene. The FAB-MS spectrum showed the molecular ion peaks at *m/z* 455 (M+H)⁺, 477 (M+Na)⁺, and 493 (M+K)⁺.

Structure Elucidation of Amphidin B (**1**)

Amphidin B (**1**) had the molecular formula of C₂₅H₄₂O₇ as revealed by HRFABMS [*m/z* 477.2842 (M+Na)⁺, +1.4 mmu]. IR absorptions at 3400~3000, 2700~2500, and 1714 cm⁻¹ indicated the presence of carboxylic acid functionality. ¹H and ¹³C NMR data (Table 2) disclosed the presence of three carbonyl carbons, an *exo*-methylene, six *sp*³ methines, three of which were oxygen-bearing, ten methylenes, and four methyl groups. Since four out of five unsaturations were accounted for, amphidin B (**1**) was

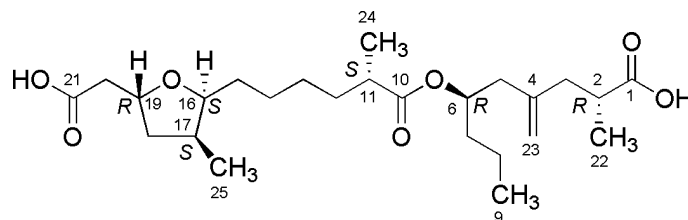
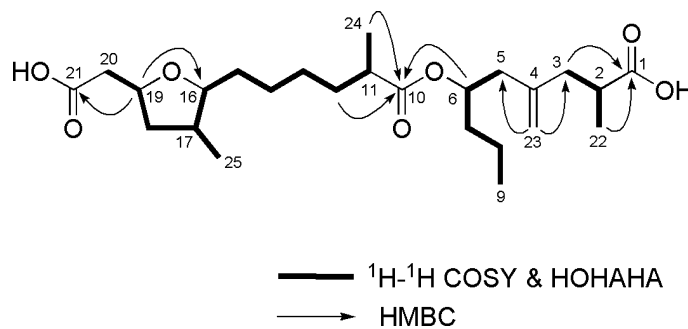
Table 1 Physico-chemical properties of amphidin B (**1**)

Appearance	Colorless oil
[α] _D ²⁴	-6° (c 0.3, CHCl ₃)
FAB-MS (<i>m/z</i>)	477 (M+Na) ⁺
HRFAB-MS (<i>m/z</i>)	
Found	477.2842
Calcd. for (M+Na) ⁺	477.2828
Molecular formula	C ₂₅ H ₄₂ O ₇

inferred to contain one ring. Interpretation of the ¹H-¹H COSY and HOHAHA spectra of **1** revealed proton connectivities of the following units: (**a**) from H-2 to H₂-3 and H₃-22, (**b**) from H₂-5 to H₃-9, and (**c**) from H-11 to H₂-20 and H₃-24, and H-17 to H₃-25 (Fig. 2). Connections among units **a**~**c** and the remaining four carbons (C-1, C-4, C-10, and C-21) were assigned on the basis of ¹H-¹³C long-range correlations observed in the HMBC spectrum as follows. HMBC correlations from H₂-3 (δ_H 2.62 and 2.11) and H₃-22 (δ_H 1.42) to the carbonyl at δ_C 182.4 (C-1), and H-19 (δ_H 4.44) to the carbonyl at δ_C 177.4 (C-21) suggested that C-2 and C-20 were connected to carbonyl carbons. The connection between units **a** and **b** through an *exo*-methylene at C-4 was deduced from HMBC correlations from one proton (δ_H 4.92) of H₂-23 to C-4 (δ 143.3) and C-3 (δ 40.3) and from another proton (δ_H 4.93) of H₂-23 to C-5 (δ 41.0) and C-4. The HMBC correlation from H-19 (δ_H 4.44) to C-16 (δ 81.1) revealed the presence of an ether linkage between C-16 and C-19, thereby constructing a tetrahydrofuran ring. The presence of an ester carbonyl at C-10 was implied by correlations from H₂-12 (δ_H 1.79 and 1.40) and H₃-24 (δ_H 1.21) to C-10 (δ 176.0). The relatively lower-field resonance of H-6 (δ_H 5.27) indicated that C-6 was involved in an ester linkage with C-10. The HMBC correlation from H-6 to C-10 revealed the presence of an ester linkage between C-6 and C-10. Relative stereochemistry of three methine protons (H-16, H-17, and H-19) in the tetrahydrofuran ring was elucidated to be 16,17-*syn* and 16,19-*anti*, since NOESY correlations were observed for H₂-15/H₃-25, H-16/H-20a, and H-19/H₃-25 (Fig. 3). The absolute configurations at six chiral centers in **1** were elucidated as follows. ¹H NMR data of the bis-(*S*)-MTPA esters of C-1~C-9 and C-10~C-21 segments (**2** and **3**, respectively), which were obtained by the successive treatments of **1** with TMS-CHN₂, LiAlH₄, and (*R*)-MTPACl (Scheme 1), were identical with those of bis-(*S*)-MTPA esters of C-13~C-21 and C-1~C-12 segments obtained from amphidinolide T1 [9], respectively. Thus, the absolute configurations at C-2, C-6, C-11, C-16, C-17, and C-19 in **1** were assigned as *R*, *R*, *S*, *S*, *S*, and *R*,

Table 2 ^1H and ^{13}C NMR data for amphidinin B (**1**) in C_6D_6

Position	δ_{C}	δ_{H} (m, Hz)	Position	δ_{C}	δ_{H} (m, Hz)
1	182.4		13	26.7	1.49 (m)
2	38.2	2.72 (m)			1.34 (m)
3	40.3	2.62 (dd, 14.9, 7.8) 2.11 (m)	14	27.3	1.44 (m) 1.35 (m)
4	143.3		15	30.2	1.49 (m)
5	41.0	2.32 (dd, 14.5, 8.2) 2.14 (m)	16	81.1	3.76 (m)
6	71.6	5.27 (m)	17	36.1	1.90 (m)
7	36.8	1.54 (m) 1.44 (m)	18	39.7	1.47 ^a (m)
8	18.9	1.42 (m) 1.34 (m)	19	73.0	4.44 (m)
9	14.1	0.92 ^b (t, 7.3)	20	41.4	2.45 (dd, 14.9, 8.2) 2.24 (m)
10	176.0		21	177.4	
11	40.0	2.50 (m)	22	17.0	1.42 ^b (d, 7.1)
12	34.0	1.79 (m) 1.40 (m)	23	114.2	4.93 (s) 4.92 (s)
			24	17.3	1.21 ^b (d, 6.7)
			25	14.0	0.74 ^b (d, 7.1)

^a2H, ^b3H.**Fig. 1** Structure of amphidinin B (**1**).**Fig. 2** Selected 2D NMR correlations for amphidinin B (**1**).

respectively. Thus, the structure of amphidinin B (**1**) was elucidated as shown in Fig. 1.

Discussion

Amphidinin B (**1**) is a new polyketide metabolite consisting

two linear carbon-chain units of C-1~C-9 (C-22 and C-23) and C-10~C-21 (C-24 and C-25) through an ester linkage (C-6 and C-10), possessing a tetrahydrofuran ring, one *exo*-methylene, three branched methyls, and two carboxylic acid groups.

This is the third isolation of polyketide metabolites with a low molecular weight and no macrocyclic lactone ring from marine dinoflagellates *Amphidinium* sp., although amphidin A [12] and amphidinoketides [13] have been isolated from dinoflagellates of the same genus. The backbone of **1** was the same as the carbon frameworks of 19-membered macrolides, amphidinolides T1, T3~T5. Biogenetically, amphidin B (**1**) may be related to amphidinolides T1, T3~T5.

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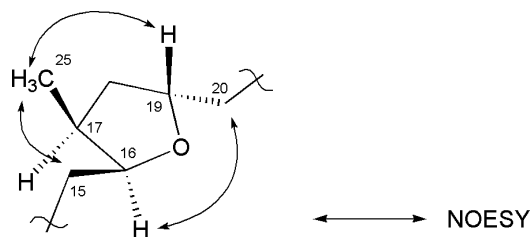
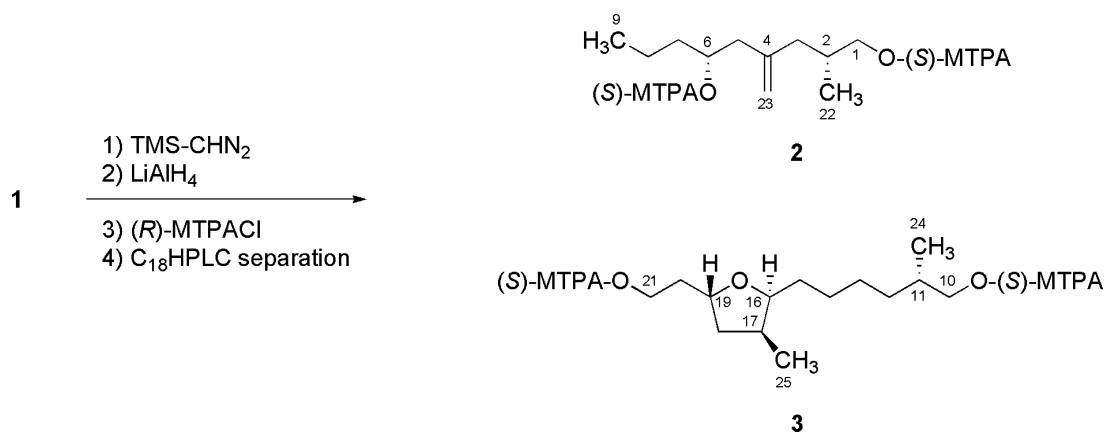


Fig. 3 Selected NOESY correlations and relative stereochemistry for tetrahydrofuran ring of amphidin B (**1**).

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Scheme 1 Reductive degradation of amphidin B (**1**) and preparation of the MTPA esters (**2**) and (**3**) of degradation products.

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