

Amphidinolactone B, a New 26-Membered Macrolide from Dinoflagellate *Amphidinium* sp.

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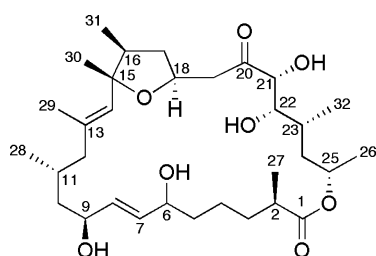
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Abstract A new 26-membered macrolide, amphidinolactone B, has been isolated from a marine dinoflagellate *Amphidinium* sp., and the structure and relative stereochemistry were elucidated on the basis of spectroscopic data. Amphidinolactone B (**1**) showed modest cytotoxicity.

Keywords dinoflagellate, *Amphidinium*, 26-membered macrolide, amphidinolactone B

Introduction

Marine dinoflagellates of the genus *Amphidinium* have been recognized as a source of novel secondary metabolites with interesting structures and bioactivities [1~4]. In our continuing search for bioactive metabolites from Okinawan marine organisms, we have investigated extracts of laboratory cultured dinoflagellates *Amphidinium* sp., which were symbionts of the Okinawan marine acoele flatworms

Amphidinolactone B (**1**)

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Amphiscolops sp., and isolated a series of cytotoxic macrolides, amphidinolides, as well as long chain polyhydroxy polyketides [1]. Here we describe the isolation and structure elucidation of a new 26-membered macrolide, amphidinolactone B (**1**), from a strain (Y-25) of the dinoflagellate *Amphidinium* sp.

Experimental

General

IR and UV spectra were recorded on a Shimadzu UV-1600PC and a JASCO FT/IR-5300 spectrophotometers, respectively. ¹H-, ¹³C- and 2D NMR spectra were measured on a Bruker AMX-600 spectrometer using 2.5 mm micro cells for C₆D₆ (Shigemi Co., Ltd.). Positive-mode ESI-MS were obtained on a JEOL JMS 700-TZ spectrometer using a sample dissolved in MeOH.

Cultivation and Isolation

The dinoflagellate was uniaxially cultured at 25°C for 2 weeks in a seawater medium enriched with 1.0% Provasoli's Erd-Schreiber (ES) [5] supplement. The harvested cells of the cultured dinoflagellate (713 g, wet weight, from 3000 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 (1.13 g), which was subjected to a silica gel column (CHCl₃/MeOH, 1 : 0→0 : 1) and a Sep-Pak C₁₈ cartridge (CH₃CN/H₂O, 7 : 3) followed by C₁₈ HPLC [YMC Pack Pro C₁₈, 5 μm, YMC Co., Ltd., 10 mm×250 mm; eluent, MeOH/H₂O, 80 : 20; flow rate, 2.0 ml/minute; UV detection at 210 nm] to afford **1**, (80 μg, 0.000011%, wet weight).

Amphidinolactone B (1)

Colorless amorphous solid; IR ν_{\max} (neat) cm^{-1} 3360 and 1720; ESI-MS m/z 589 ($M+\text{Na}$)⁺; HRESI-MS (m/z

589.3712 [$(M+\text{Na})^+$; calcd for $\text{C}_{32}\text{H}_{54}\text{O}_8\text{Na}$, 589.3716]). ¹H- and ¹³C-NMR data see Table 1.

Table 1 ¹H- and ¹³C-NMR data of amphidinolactone B (**1**) in C_6D_6

No	δ_{H}	δ_{C}
1		175.6 s
2	2.48 (1H, m)	41.7 d
3a	1.85 (1H, m)	35.4 t
3b	1.35 (1H, m)	
4a	1.60 (1H, m)	24.5 t
4b	1.40 (1H, m)	
5	1.55 (2H, m)	38.2 t
6	4.03 (1H, dd, 12.8, 5.3)	72.5 d
7	5.74 (1H, dd, 15.4, 5.3)	134.1 d
8	5.71 (1H, dd, 15.4, 5.1)	134.1 d
9	4.16 (1H, m)	70.1 d
10a	1.60 (1H, m)	43.4 t
10b	1.18 (1H, m)	
11	1.97 (1H, m)	28.7 d
12a	1.97 (1H, m)	49.5 t
12b	1.75 (1H, m)	
13		137.5 s
14	5.18 (1H, s)	131.9 d
15		85.2 s
16	1.97 (1H, m)	43.8 d
17a	1.75 (1H, m)	40.6 t
17b	1.05 (1H, ddd, 12.1, 9.8, 9.8)	
18	4.31 (1H, m)	74.3 d
19a	2.54 (1H, dd, 13.6, 9.0)	45.6 t
19b	2.23 (1H, dd, 13.6, 3.6)	
20		208 ^a s
21	4.15 (1H, d, 1.3) ^b	79.2 d
22	3.75 (1H, dd, 8.8, 1.3) ^b	75.2 d
23	2.11 (1H, m)	33.3 d
24a	1.97 (1H, m)	40.6 t
24b	1.12 (1H, m)	
25	5.29 (1H, m)	68.3 d
26	1.21 (3H, d, 6.1)	21.3 q
27	1.17 (3H, d, 7.2)	17.5 q
28	1.00 (3H, d, 6.1)	21.3 q
29	1.75 (3H, s)	18.5 q
30	1.12 (3H, s)	23.7 q
31	0.77 (3H, d, 7.0)	15.5 q
32	1.18 (3H, d, 7.0)	17.5 q

^a calculated value. The ¹³C chemical shift of C-20 in **1** obtained from ChemNMR ver 10.0 (CambridgeSoft) was 208 ppm. Actually, those of the corresponding carbons in amphidinolide B-type macrolides are observed in the range of 210–215 ppm [1]. ^b $^3J_{\text{H}/\text{H}}$ values observed in CDCl_3 .

Results and Discussion

The dinoflagellate *Amphidinium* sp. (strain number Y-25) was isolated from inside cells of the marine acoel flatworm *Amphiscolops breviviridis* collected off Sunabe, Okinawa. The harvested cells of the cultured dinoflagellate were extracted with MeOH/toluene (3 : 1), and 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, which was subjected to a silica gel column and a Sep-Pak C₁₈ cartridge followed by C₁₈ HPLC to afford **1**, 80 μg , 0.000011%, wet weight).

1 had the molecular formula of $\text{C}_{32}\text{H}_{54}\text{O}_8$ as revealed by HRESI-MS [m/z 589.3712 ($M+\text{Na}$)⁺, -0.4 mmu]. IR absorptions at 3370 and 1720 cm^{-1} indicated the presence of hydroxy and carbonyl functionalities. ¹H- and ¹³C-NMR data (Table 1) of **1** disclosed the presence of one ester carbonyl, one sp^2 quaternary carbon, one sp^3 oxygenated quaternary carbon, three sp^2 methines, ten sp^3 methines (six of which were bearing an oxygen atom), eight sp^3 methylenes, and seven methyl groups. Considering the molecular formula, the existence of a keto carbonyl was indicated [6]. Since four out of six unsaturations were accounted for, **1** was inferred to contain two rings. Detailed analyses of the ¹H-¹H COSY and TOCSY spectra of **1** revealed connectivities of three partial structures, **a** (C-2 to C-12, C-2 to C-27, and C-11 to C-28), **b** (C-16 to C-19 and C-16 to C-31), and **c** (C-21 to C-26 and C-23 to C-32) as shown in Fig. 1. HMBC correlations of H₃-27 (δ_{H} 1.17) to C-1 (δ_{C} 175.6) and C-2 (δ_{C} 41.7) indicated connectivities of C-1 to C-2 and C-2 to C-27. Connections between C-12

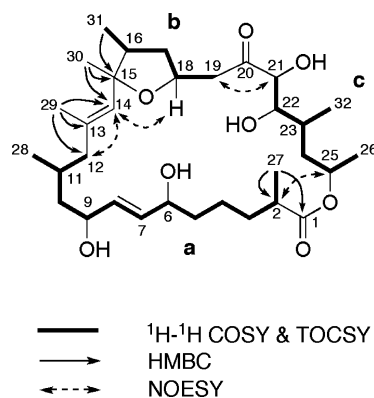


Fig. 1 Selected 2D NMR correlations for amphidinolactone B (**1**).

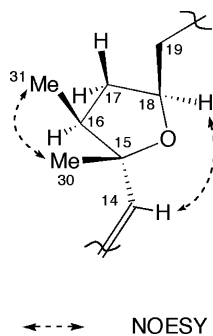


Fig. 2 Selected NOESY correlations and relative stereochemistry for tetrahydrofuran ring in amphidinolactone B (**1**).

to C-14 *via* C-13 and C-13 to C-29 were implied by HMBC cross-peaks for H₃-29 (δ_{H} 1.75) to C-12 (δ_{C} 49.5), C-13 (δ_{C} 137.5), and C-14 (δ_{C} 131.9). Connectivities of C-14 to C-16 *via* C-15 and C-30 to C-15 were derived from HMBC cross-peaks for H₃-30 (δ_{H} 1.12) to C-14 and C-15 (δ_{C} 85.2), and H₃-31 (δ_{H} 0.77) to C-15. ¹H and ¹³C chemical shifts of C-25 (δ_{H} 5.29; δ_{C} 68.3) indicated that C-25 was involved in an ester linkage with C-1. The NOESY correlation for H-2/H-25 also supported the connectivity of C-25 to C-2. The connectivity of C-19 to C-21 through a remaining keto carbonyl at C-20 was deduced from the molecular formula of **1** and the NOESY correlation for H₂-19/H-21. The ¹H and ¹³C chemical shifts of CH₂-19 and CH-21 (Table 1) in **1** corresponded well to those of CH₂-19 (δ_{H} 2.87, 2.78; δ_{C} 45.9) and CH-21 (δ_{H} 4.33; δ_{C} 77.7) in amphidinolide B [7], supporting that **1** possessed the same partial structure for C-19~C-21 including a ketone at C-20 as amphidinolide B. The presence of a tetrahydrofuran ring was deduced from deuterium-induced shift [8] of oxymethine carbons in the HSQC spectra of **1** measured in C₆D₆/CD₃OD (95:5) and C₆D₆/CD₃OH (95:5), respectively, as follows. Four oxymethine signals for C-6 (δ_{C} 72.5), C-9 (δ_{C} 70.1), C-21 (δ_{C} 79.2), and C-22 (δ_{C} 75.2) showed significant deuterium-induced shifts, whereas two oxymethine signals for C-18 (δ_{C} 74.3) and C-25 did not show such deuterium-induced shift, implying that C-18 was connected to C-15 through an ether linkage, and that C-25 was involved in an ester linkage with C-1. The ¹H-¹H coupling ($J_{7,8}$ = 15.4 Hz) of the disubstituted double bond at C-7 and C-8 indicated the *E* geometry. The *E* geometry of the double bond at C-13 and C-14 was deduced from the NOESY correlation observed for H-12/H-14 as well as the ¹³C chemical shift of C-29 (δ_{C} 18.5). Thus, the gross structure of amphidinolactone B was elucidated to be **1**.

The relative stereochemistry of C-15, C-16, and C-18 in the tetrahydrofuran ring was deduced from NOESY

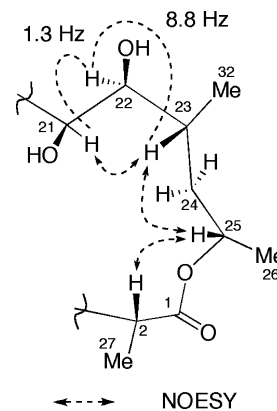


Fig. 3 Selected NOESY correlations and ¹H-¹H couplings and relative stereochemistries for amphidinolactone B (**1**) (C-21~C-25 and C-1~C-2 moieties).

correlations as shown in Fig. 2. NOESY correlations for H-14/H-18 implied that C-14 and H-18 were both α -oriented, while NOESY correlations observed for H₃-30/H₃-31 suggested that C-30 and C-31 were both β -oriented (Fig. 2).

The relative stereochemistry of C-2, C-22, C-23, and C-25 was elucidated from ¹H-¹H couplings and NOESY correlations (Fig. 3). The values for ³ $J_{\text{H-21/H-22}}$ (1.3 Hz) and ³ $J_{\text{H-22/H-23}}$ (8.8 Hz) indicated a *syn* relationship for H-21 and H-22 and an *anti* relationship for H-22 and H-23, respectively. NOESY correlations of H-23/H-25 and H-25/H-2 suggested that H-2, H-23, and H-25 were oriented toward the same direction. Furthermore, considering conformation of the macrocyclic ring, the relative stereochemistries of the C-21~C-25 and C-1~C-2 moieties were elucidated as shown in Fig. 3.

Since the carbon skeleton of **1** is the same as those of amphidinolide B-type macrolides [11], the stereochemistry of C-9 and C-11 in **1** may be the same as those of amphidinolide B-type macrolides. The stereochemistry of C-6 remains to be defined, due to a very limited amount of the sample (80 μg).

1 is a new 26-membered macrolide possessing a tetrahydrofuran ring, a keto carbonyl, four hydroxyl groups, and six branched methyls. **1** showed cytotoxicity against L1210 murine leukemia cells and human epidermoid carcinoma KB cells (IC₅₀, 3.3 and 5.3 $\mu\text{g}/\text{ml}$, respectively) *in vitro*.

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