J. Antibiot. 60(6): 376–379, 2007

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

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

Yohei Takahashi, Takaaki Kubota, Jun'ichi Kobayashi

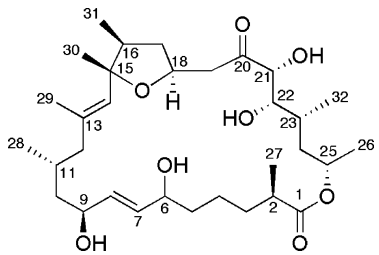
Received: April 6, 2007 / Accepted: June 5, 2007

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 acoel flatworms *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.



Amphidinolactone B (**1**)

J. Kobayashi (Corresponding author)**, Y. Takahashi, T. Kubota:** Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan,
E-mail: jkobay@pharm.hokudai.ac.jp

Experimental

General

IR and UV spectra were recorded on a Shimadzu UV-1600PC and a JASCO FT/IR-5300 spectrophotometers, respectively. ^{1}H-, ^{13}C- and 2D NMR spectra were measured on a Bruker AMX-600 spectrometer using 2.5 mm micro cells for C_6D_6 (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 unialgally 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 ($CHCl_3$/MeOH, 1 : 0→0 : 1) and a Sep-Pak C_{18} cartridge (CH_3CN/H_2O, 7 : 3) followed by C_{18} HPLC [YMC Pack Pro C_{18}, 5 μm, YMC Co., Ltd., 10 mm×250 mm; eluent, MeOH/H_2O, 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 $(M+Na)^+$; HRESI-MS (*m/z* 589.3712 $[(M+Na)^+$; calcd for $C_{32}H_{54}O_8Na$, 589.3716]). ^1H- and ^{13}C-NMR data see Table 1.

Table 1 ^1H- and ^{13}C-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 ^{13}C 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] $^3J_{H/H}$ 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 C_{18} cartridge followed by C_{18} HPLC to afford **1**, 80 μg, 0.000011%, wet weight).

1 had the molecular formula of $C_{32}H_{54}O_8$ as revealed by HRESI-MS [*m/z* 589.3712 $(M+Na)^+$, −0.4 mmu]. IR absorptions at 3370 and 1720 cm^{-1} indicated the presence of hydroxy and carbonyl functionalities. ^1H- and ^{13}C-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 H_3-27 (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

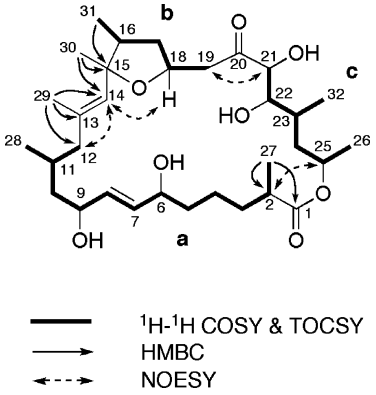


Fig. 1 Selected 2D NMR correlations for amphidinolactone B (**1**).

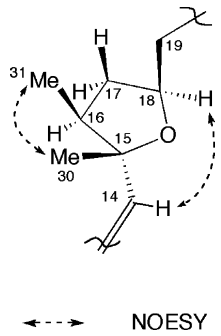


Fig. 2 Selected NOESY correlations and relative stereochemistry for tetrahydrofuran ring in amphidinolactone B (**1**).

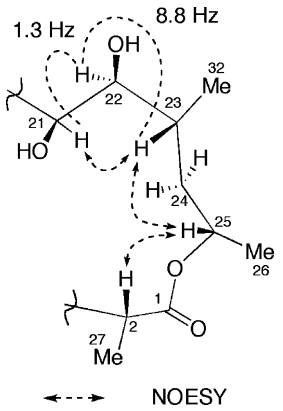


Fig. 3 Selected NOESY correlations and ^{1}H-^{1}H couplings and relative stereochemistries for amphidinolactone B (**1**) (C-21～C-25 and C-1～C-2 moieties).

to C-14 *via* C-13 and C-13 to C-29 were implied by HMBC cross-peaks for H_3-29 (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 H_3-30 (δ_H 1.12) to C-14 and C-15 (δ_C 85.2), and H_3-31 (δ_H 0.77) to C-15. ^{1}H and ^{13}C 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 H_2-19/H-21. The ^{1}H and ^{13}C chemical shifts of CH_2-19 and CH-21 (Table 1) in **1** corresponded well to those of CH_2-19 (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 C_6D_6/CD_3OD (95 : 5) and C_6D_6/CD_3OH (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 ^{1}H-^{1}H 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 ^{13}C 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 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 H_3-30/H_3-31 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 ^{1}H-^{1}H couplings and NOESY correlations (Fig. 3). The values for $^{3}J_{H\text{-}21/H\text{-}22}$ (1.3 Hz) and $^{3}J_{H\text{-}22/H\text{-}23}$ (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 (IC_{50}, 3.3 and 5.3 μg/ml, respectively) *in vitro*.

Acknowledgment The authors thank Ms. S. Oka, Center for Instrumental Analysis, Hokkaido University, for measurements of ESI-MS. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. (a) Kobayashi J, Kubota T. Bioactive macrolides and polyketides from marine dinoflagellates of the genus *Amphidinium*. J Nat Prod 70: 451–460 (2007)
   (b) Takahashi Y, Kubota T, Kobayashi J. Amphidinolactone A, a new 13-membered macrolide from dinoflagellate *Amphidinium* sp. Heterocycles 72: 567–572 (2007)
2. (a) Satake M, Murata M, Yasumoto T, Fujita T, Naoki H. Amphidinol, a polyhydroxypolyene antifungal agent with an unprecedented structure, from a marine dinoflagellate, *Amphidinium klebsii*. J Am Chem Soc 113: 9859–9861 (1991)
   (b) Paul G. K, Matsumori N, Murata M, Tachibana K. Isolation and chemical structure of amphidinol 2, a potent hemolytic compound from marine dinoflagellate *Amphidinium klebsii*. Tetrahedron Lett 36: 6279–6282 (1995)
3. Huang X, Zhao D, Guo Y, Wu H, Trivellone E, Cimino G. Lingshuiols A and B, two new polyhydroxy compounds from the Chinese marine dinoflagellate *Amphidinium* sp. Tetrahedron Lett 45: 5501–5504 (2004)
4. Washida K, Koyama T, Yamada K, Kita M, Uemura D. Karatungiols A and B, two novel antimicrobial compounds, from the symbiotic marine *Amphidinium* sp. Tetrahedron Lett 47: 2521–2525 (2006)
5. Provasoli L. *In*: Culture and Collection of Algae. Watanabe A, Hattori A. (eds.) Japanese Society of Plant Physiology, Tokyo, pp. 63–75 (1968)
6. For detection of the keto carbonyl, treatment of **1** with 2,4-dinitrophenylhydrazine gave the 2,4-dinitrophenylhydrazone; ESI-MS *m*/*z* 745 $(M-H)^-$; HRESI-MS (*m*/*z* 745.4028 $[(M-H)^-$; calcd for $C_{38}H_{57}O_{11}N_4$, 745.4024]).
7. Ishibashi M, Ohizumi Y, Hamashima M, Nakamura H, Hirata Y, Sasaki T, Kobayashi J. Amphidinolide-B, a novel macrolide with potent antineoplastic activity from the marine dinoflagellate *Amphidinium* sp. J Cem Soc, Chem Commun: 1127–1129 (1987)
8. (a) Pfeffer PE, Valentine KM, Parrish W. Deuterium-induced differential isotope shift carbon-13 NMR. 1. Resonance reassignments of mono- and disaccharides. J Am Chem Soc 101: 1265–1274 (1979)
   (b) Reuben J. Isotopic multiplets in the carbon-13 NMR spectra of polyols with partially deuterated hydroxyls. 4. Molecular structure as reflected in the carbon-13 NMR spectra of oligosaccharides with partially deuterated hydroxyls. J Am Chem Soc 107: 1747–1755 (1985)