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AMPHIDINOLACTONE A, A NEW 13-MEMBERED MACROLIDE FROM DINOFLAGELLATE *AMPHIDINIUM* SP.

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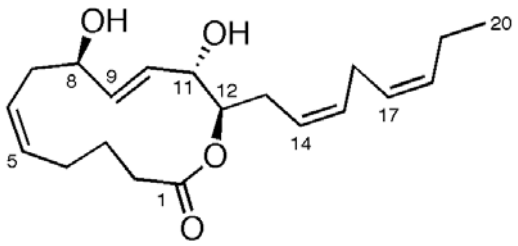
Abstract - A new 13-membered macrolide, amphidinolactone A (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp., and the structure and relative stereochemistry were elucidated by spectroscopic data. Amphidinolactone A (**1**) is the first macrolide without a branched methyl or an exomethylene among all the macrolides isolated from the dinoflagellates so far.

INTRODUCTION

Marine dinoflagellates of the genus *Amphidinium* have been recognized as a source of novel secondary metabolites with interesting structures and bioactivities.[1-7] 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,[1] as well as long chain polyhydroxyl polyketides.[2,6,7] Here we describe the isolation and structure elucidation of a new 13-membered macrolide, amphidinolactone A (**1**), which was isolated from extracts of a strain (Y-25) of the dinoflagellate *Amphidinium* sp.



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RESULTS AND DISCUSSION

The dinoflagellate *Amphidinium* sp. (strain number Y-25) was isolated from inside cells of a marine acoel flatworm *Amphiscolops breviviridis* collected off Sunabe, Okinawa. The harvested cells of the cultured dinoflagellate 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, which was subjected to a silica gel column and a Sep-Pak C_{18} cartridge followed by C_{18} HPLC to afford amphidinolactone A (**1**, 0.1 mg, 0.000014%, wet weight).

Table 1. ^{1}H and ^{13}C NMR Data of Amphidinolactone A (**1**) in C_6D_6.

Position	δ_H		δ_C	
1	-		[α]	s
2a	2.19	(1H, m)	32.3	t
2b	2.10	(1H, m)		
3a	1.86	(1H, m)	22.2	t
3b	1.26	(1H, m)		
4a	2.32	(1H, m)	25.5	t
4b	1.86	(1H, m)		
5	5.30	(1H, ddd, 11.0, 9.6, 4.6 Hz)	130.2	d
6	5.66	(1H, m)	124.4	d
7a	2.32	(1H, m)	35.6	t
7b	2.19	(1H, m)		
8	4.00	(1H, ddd,7.6, 6.7, 2.9 Hz)	71.8	d
9	5.53	(1H, dd, 15.8, 7.6 Hz)	135.9	d
10	5.23	(1H, dd, 15.8, 7.6 Hz)	130.1	d
11	3.83	(1H, dd, 8.1, 7.6 Hz)	73.5	d
12	5.02	(1H, ddd, 8.8, 7.3, 3.7 Hz)	73.5	d
13a	2.68	(1H, ddd, 14.5, 3.7, 3.7 Hz)	29.0	t
13b	2.50	(1H, ddd, 14.5, 7.3, 7.3 Hz)		
14	5.58	(1H, m)	131.5	d
15	5.60	(1H, m)	124.8	d
16	2.88	(2H, m)	25.6	t
17	5.46	(1H, m)	126.7	d
18	5.46	(1H, m)	132.4	d
19	2.06	(2H, m)	20.0	t
20	0.96	(3H, t, 7.5 Hz)	14.3	q

[α] not observed.

Amphidinolactone A (**1**) had the molecular formula of $C_{20}H_{30}O_4$ as revealed by HRESIMS [*m/z* 357.2057 $(M+Na)^+$, +1.6 mmu]. IR absorptions at 3370 and 1720 cm^{-1} indicated the presence of hydroxy group and ester carbonyl group. ^1H and ^{13}C NMR data (Table 1) (obtained from the HMQC spectrum) of **1** disclosed the presence of eight sp^2 methines, three oxymethines, seven sp^3 methylenes, and one methyl group. Considering the molecular formula, the existence of a carbonyl unit (C=O) was indicated. Since five out of six unsaturations were accounted for, amphidinolactone A (**1**) was inferred to contain one ring. Detailed analyses of the ^1H-^1H COSY spectrum of **1** established connectivities of a long carbon chain from C-2 to C-20. ^1H and ^{13}C chemical shifts of CH_2-2 (δ_H 2.19, 2.10 ; δ_C 32.3) and CH-12 (δ_H 5.02 ; δ_C 73.0) suggested that C-12 was involved in an ester linkage with C-1. ^1H-^1H couplings ($J_{5,6}$ = 11.0 Hz, $J_{9,10}$ = 15.8 Hz) of two disubstituted double bonds at C-5 and C-9 indicated the *Z* and *E* geometry, respectively. Geometries of two disubstituted double bonds at C-14 and C-17 were assigned as both *Z* from NOESY correlations of H_2-13/H_2-16 and H_2-16/H_2-19 as well as the carbon chemical shifts of C-16 (δ_C 25.6), which was typical value for a methylene carbon between two *Z* double bonds.[8] Thus, the gross structure of amphidinolactone A (**1**) was elucidated as shown in Fig. 1.

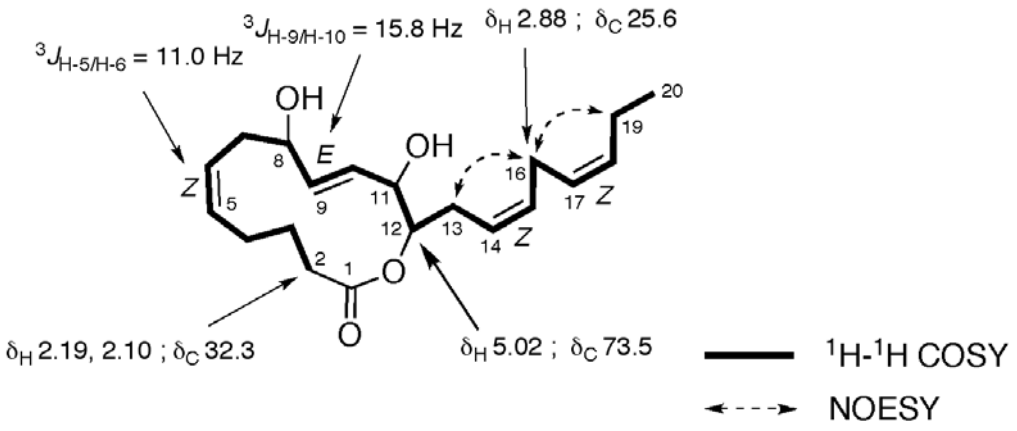

Figure 1. Key chemical shifts and 2D NMR correlations for amphidinolactone A (**1**).

The relative stereochemistry of C-8, C-11, and C-13 in **1** was deduced from NOESY correlations as shown in Figure 2. NOESY correlations for H-6/H-8, H-8/H-10, and H-10/H-12 implied that H-8 and H-12 were both α-oriented. On the other hand, NOESY correlations were observed for H-7b/H-9, H-9/H-11, and H-11/H-13, suggesting that H-11 was β-oriented. Therefore, the relative stereochemistry of C-8, C-11, and C-12 of amphidinolactone A was elucidated to be **1**.

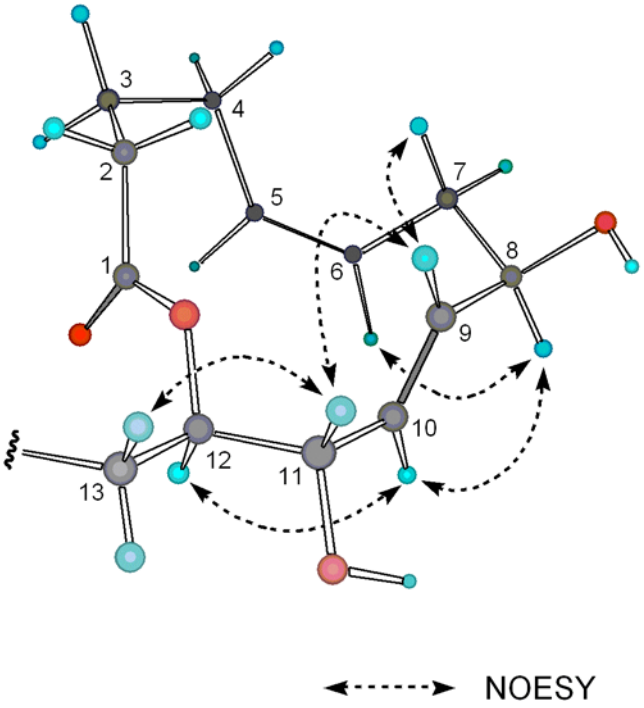

Figure 2. Selected NOESY correlations and relative stereochemistry for amphidinolactone A (**1**).

Amphidinolactone A (**1**) is a new 13-membered macrolide consisting a C_{20} carbon-chain possessing four disubstituted double bonds and two hydroxy groups. Amphidinolactone A (**1**) is the first macrolide without a branched methyl or an exomethylene among all the macrolides isolated from the dinoflagellates *Amphidinium* sp. so far. Amphidinolactone A (**1**) showed cytotoxicity against L1210 murine leukemia cells (IC_{50}, 8 μg/mL), while **1** did not show such activity against human epidermoid carcinoma KB cells (IC_{50} > 10 μg/mL) in vitro.

EXPERIMENTAL

General Experimental Procedures.

IR spectrum was recorded on a JASCO FT/IR-230. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm symmetrical microtubes (DMS-0025, Shigemi Co., Ltd). The 7.20 and 128.0 ppm resonances of residual C_6D_6 were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS- 700TZ spectrometer.

Material.

The dinoflagellate *Amphidinium* sp. (strain number Y-25) was isolated from inside cells of a marine acoel flatworm *Amphiscolops breviviridis* collected off Sunabe, Okinawa. The dinoflagellate has been deposited in Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation.

The dinoflagellate was unialgally cultured at 25 ^{o}C for 2 weeks in a seawater medium enriched with 1% ES supplement.[9] The harvested cells of the cultured dinoflagellate (713 g, wet weight, from 3000 L 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, 10 mm x 250 mm; eluent, MeOH/H_2O, 80:20; flow rate, 2.0 mL/min; UV detection at 210 nm] to afford amphidinolactone A (**1**, 0.1 mg, 0.000014 %, wet weight).

Amphidinolactone A (1): colorless amorphous solid; $[\alpha]_D^{19}$ -62 (*c* 0.065, C_6H_6); IR (film)ν_{max} 3370 and 1720 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 357 $(M+Na)^+$; HRESIMS *m*/*z* 357.2057 (M+Na; calcd for $C_{20}H_{30}O_4Na$, 357.2041).

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