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Amphidinin A, a Novel Amphidinolide-Related Metabolite from the Cultured Marine Dinoflagellate *Amphidinium* sp.

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Abstract: Amphidinin A (1), a novel linear natural product having an unprecedented carbon-skeleton, was isolated from the cultured marine dinoflagellate *Amphidinium* sp. and the structure elucidated on the basis of spectroscopic data. Compound 1 is conceivable to be biogenetically related to amphidinolides.

During our studies on search for new bioactive substances from marine microalgae,[1] we previously isolated a series of cytotoxic macrolides, amphidinolides A ~ N,[1b] from dinoflagellates of the genus *Amphidinium*, which were living inside of Okinawan marine flatworms of the genus *Amphiscolops*. We further continued investigation on the constituents of this microalga (strain number, Y-5) and now succeeded in isolating a novel non-macrolide natural product, amphidinin A (1), exhibiting moderate cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro (IC_{50} values, 3.6 and 3.0 μ g/mL, respectively). Here we describe the isolation and structure elucidation of 1, the structural feature of which was suggestive that compound 1 is biogenetically related to amphidinolides.

The harvested algal cells (878 g, wet weight, from 3420 L of culture) were extracted with MeOH/toluene (3:1) and partitioned between toluene and water. The toluene-soluble fraction was subjected to a silica gel column ($CHCl_3$/MeOH, 95:5) followed by gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1). Further purification by reversed-phase HPLC (ODS; 59% CH_3CN) yielded amphidinin A (1, 0.00006% yield, wet weight), together with amphidinolides A, E, and J.[1]

Amphidinin A (1), colorless oil; $[\alpha]_D^{18}$ -300° (*c* 0.03, MeOH); IR (film) ν_{max} 3400 cm^{-1}; FABMS (matrix: glycerol) *m/z* 367 $(M+H)^+$, had a molecular formula of $C_{22}H_{38}O_4$ as established by HRFABMS [*m/z* 367.2828, $(M+H)^+$, Δ -2.0 mmu]. Although only poor ^{13}C NMR spectrum was obtained for 1 because of the limited sample quantity (no more than 0.5 mg), chemical shifts of carbon signals were able to be assigned from the HMQC[2] and HMBC[3] spectral data (Table 1). The ^1H-^1H COSY spectrum of 1 clearly revealed three proton networks: (i) from OH-1 to H_3-22 (C-1 ~ C-6 unit), (ii) from H_2-21 to H_3-20 (C-7 ~ C-9 unit),[4] and (iii) from H_2-10 to H_3-18 (C-10 ~ C-17 unit), which were firmly substantiated by HOHAHA[5] spectrum. These three units were shown to be connected linearly by the HMBC correlations for H_3-22/C-7, H_2-21/C-6, H_3-20/C-10, and H-10α/C-20. Since the molecule of 1 was inferred to contain one ring from the unsaturation

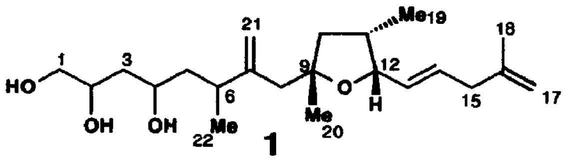

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Table 1. ^1H and ^{13}C NMR Data of Amphidinin A (1) in C_6D_6[a]

position	δ_H		δ_C	HMBC (^1H)	position	δ_H		δ_C	HMBC (^1H)
1 (a)	3.65	m	68.5		10 (β)	1.61	dd		
(b)	3.57	m			11	2.09	m	36.7	H-10α, H_3-19
1-OH	2.30	br s			12	4.15	dd	83.7	H_3-19
2	4.02	m	73.5		13	5.36	dd	129.4	H_2-15
2-OH	4.81	br s			14	5.57	dt	132.7	H_2-15
3 (a)	1.73	dt	39.8		15 (2H)	2.62	d	41.7	H-17a, H_3-18
(b)	1.39	br d			16			144.2	H_2-15, H_3-18
4	4.10	m	69.5	H-3a, H_2-5	17 (a)	4.86	s	111.2	H_2-15, H_3-18
4-OH	4.86	br s			(b)	4.85	s		
5 (2H)	1.49	dd	45.0	H_3-22	18 (3H)	1.69	s	22.6	H_2-15, H-17b
6	2.77	m	35.7	H_2-5, H_2-8, H_2-21, H_3-22	19 (3H)	0.77	d	15.6	H-10α
7			151.3	H_2-5, H_2-8	20 (3H)	1.08	s	26.0	H-8a, H-10α
8 (a)	2.18	d	51.0	H_3-20, H_2-21	21 (a)	4.88	s	112.0	H_2-8
(b)	2.12	d			(b)	4.83	s		
9			83.0	H-8a, H_3-20	22 (3H)	1.06	d	24.5	
10 (α)	1.20	dd	47.7	H_3-19, H_3-20					

[a] J (H/H) in Hz: 1a/1b = 10.8; 1a/2 = 4.0; 1b/2 = 5.6; 2/3a = 7.1; 2/3b = 1.3; 3a/3b = 13.1; 3a/4 = 7.6; 3b/4 = 1.6; 4/5(2H) = 6.1; 5(2H)/6 = 8.4; 6/22 = 6.9; 8a/8b = 13.2; 10α/10β = 12.3; 10α/11 = 9.2; 10β/11 = 7.3; 11/12 = 7.8; 11/19 = 7.0; 12/13 = 8.4; 13/14 = 15.2; 14/15(2H) = 6.9.

degrees, the oxygenated quaternary carbon at C-9 and the oxymethine at C-12 were suggested to be linked through an ether oxygen to form a tetrahydrofuran (THF) ring. This inference was supported by the following characteristic NOESY cross-peaks: H-8b/H_3-19, H-10β/H_3-20, H-10α/H-13, H-10α/H_3-19, H-11/H-12, H-12/H_3-20, and H-13/H_3-19,[6] from which the relative configurations of H-12, Me-19, and Me-20 on the THF moiety were deduced as β, α, and β, respectively. The geometry of $\Delta^{13,14}$-double bond was *E* on the basis of the coupling constant ($J_{13,14}$ = 15.2 Hz). Thus, the structure of amphidinin A was concluded as **1**.

A variety of macrolides with new carbon skeletons have been isolated from dinoflagellates of the genus *Amphidinium*.[1] Amphidinin A (**1**), a non-macrolide compound,[7] also possesses an unprecedented carbon framework, having some structural relationships to previously isolated amphidinolides as follows: (i) vicinally located methyl and exomethylene groups (C-6 ~ C-7 moiety) are also contained in amphidinolides J and K,[1] and (ii) 2-methyl-1,4-pentadiene unit (C-13 ~ C-17 moiety) corresponds to C-22 ~ C-26 positions of amphidinolide E.[1] Studies on defining the relative and absolute stereochemistries of chiral centers of **1** based on synthesis are currently under investigation.

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4. The COSY spectrum showed cross-peaks due to allyl couplings between H_2-21 and H_2-8, while one of the methylene protons (δ_H 2.18, H-8a) exhibited a *W*-type coupling with the singlet methyl protons (H_3-20). These observations were further confirmed by the HMBC correlations (*e.g.*, H_3-20/C-8 and H_2-21/C-8).
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6. Other NOESY correlations clearly observed (H/H; mixing time, 800 msec): 1a/2, 1b/2, 1a/3b, 1b/3a, 1b/3b, 2/3b, 2/4, 3a/5, 3b/4, 4/5, 4/21a, 5/6, 5/21a, 5/22, 6/8a, 6/22, 8a/10α, 8a/22, 8b/10α, 8b/10β, 8b/20, 8b/21b, 12/14, 12/13, 13/15, 14/15, 15/17b, 17a/18, 21a/22, and 21b/22.
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