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TETRAHEDRON

Amphidinolide U, Novel 20-Membered Macrolide from Marine Dinoflagellate *Amphidinium* sp.

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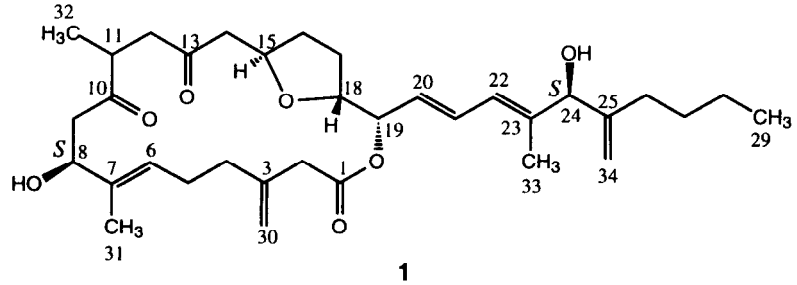
Abstract; A novel 20-membered macrolide, amphidinolide U (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp. and the structure was elucidated on the basis of spectroscopic data. The relative stereochemistry of C-15, C-18, and C-19 was deduced from NOESY correlations, while the absolute configurations at C-8 and C-24 were assigned as both *S* on the basis of modified Mosher's method.

keywords; marine dinoflagellate, macrolide, tetrahydrofuran

Amphidinolides A ~ H and J ~ S are a series of unique macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms *Amphiscolops* spp.[1] Our continuing search for bioactive secondary metabolites from laboratory-cultured marine dinoflagellates[2~5] resulted in the isolation of a novel 20-membered macrolide, amphidinolide U (**1**), from extracts of another strain (Y-56) of the dinoflagellate *Amphidinium* sp. Here we describe the isolation and structure elucidation of **1**.

The acoel flatworm *Amphiscolops* sp. was collected off Zanpa, Okinawa, from which an associated dinoflagellate *Amphidinium* sp. was isolated and mass cultured unialgally at 25 ℃ for 2 weeks in a seawater medium enriched with 1% ES supplement. The harvested algal cells (420 g, wet weight, from 580 L of culture) were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and water. The toluene-soluble materials were subjected to silica gel ($CHCl_3$/MeOH) and C_{18} columns (CH_3CN/H_2O) followed by C_{18} HPLC (CH_3CN/H_2O) to afford



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Stereochemistries of C-15, C-18, and C-19 are relative.

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Table 1. ^{1}H and ^{13}C NMR Data of Amphidinolide U (**1**) in $CDCl_3$.

positn.	δ_C		δ_H (m, Hz)				HMBC (H)
1	170.8	s					2, 19
2	42.8	t	3.07[a]	m			30
3	142.7	s					2, 30
4	35.3	t	2.13[a]	m			2, 5, 30
5	26.4	t	2.20	m	2.17	m	4, 6
6	125.6	d	5.45	m			4, 31
7	135.4	s					9b[c], 31
8	73.1	d	4.33	m			9b[c], 31
8-OH			3.17	br			
9	44.3	t	2.96	dd, 4.6, 12.7	2.72	dd, 6.5, 12.7	11
10	213.4	s					9, 32
11	41.7	d	3.05	m			12a[c], 32
12	46.1	t	2.98	dd, 8.4, 18.1	2.36	dd, 4.3, 18.1	32
13	206.8	s					12, 14
14	48.5	t	2.70	dd, 8.7, 15.8	2.44	dd, 3.6, 15.8	16b[c]
15	75.2	d	4.31	m			14a[c], 16b, 18
16	32.2	t	2.10	m	1.49	m	14a[c]
17	28.2	t	1.96	m	1.64	m	
18	79.77	d	4.09	brq, 7.0			17b[c], 19
19	77.0	d	5.22	brt, 7.3			17b[c], 20, 21
20	127.8	d	5.60	dd, 7.9, 15.1			19, 22
21	130.6	d	6.54	dd, 11.0, 15.1			19, 22
22	125.2	d	6.13	brd, 11.0			20, 24, 33
23	139.9	s					24, 33
24[d]	79.79	d	4.48	s			22, 26, 33, 34
25	149.2	s					24, 26, 27, 34a[c]
26	31.5	t	1.93	m	1.86	m	27, 34
27	30.1	t	1.41[a]	m			26, 29
28	22.5	t	1.31[a]	m			27, 29
29	14.0	q	0.89[b]	t, 7.3			27
30	113.7	t	4.92[a]	s			2
31	13.0	q	1.67[b]	s			
32	16.7	q	1.09[b]	d, 7.1			12a[c]
33	12.6	q	1.66[b]	s			22, 24
34	110.3	t	5.12	s	4.95	s	24, 26

[a]2H. [b]3H. [c]a and b denote low-field and high-field resonances respectively of a geminal pair. [d]24-OH was not detected.

amphidinolide U (**1**, 0.8 mg, 0.0002 %, wet weight) together with two known macrolides, amphidinolides C[6] (**3**, 0.0009 %) and F[7] (0.0006 %).

Amphidinolide U (**1**) had the molecular formula of $C_{34}H_{50}O_7$ as revealed by HRFABMS [*m/z* 593.3462, $(M+Na)^+$, +1.7 mmu]. IR absorptions at 3400 and 1710 cm^{-1} indicated the presence of hydroxyl(s) and carbonyl group(s), respectively. The UV spectrum showed the absorption at 230 nm (ε 20000) due to a conjugated diene chromophore. ^{1}H and ^{13}C NMR data (Table 1) disclosed the existence of two ketones, an ester carbonyl, four sp^2 quaternary carbons, four sp^2 methines, two sp^2 methylenes, six sp^3 methines (five of them bearing an oxygen atom), eleven sp^3 methylenes, and four methyls (two of them attached to olefins). Thus accounting for eight out of ten unsaturations, amphidinolide U (**1**) was inferred to contain two rings. Interpretation of the ^{1}H-^{1}H COSY and TOCSY spectra revealed proton connectivities of the following partial structures: (**a**) from H_2-2 to H_2-30, (**b**) from H_2-4 to H_3-31, (**c**) from 8-OH to H_2-9, (**d**) from H_3-32 to H_2-12, (**e**) from H_2-14 to H_3-

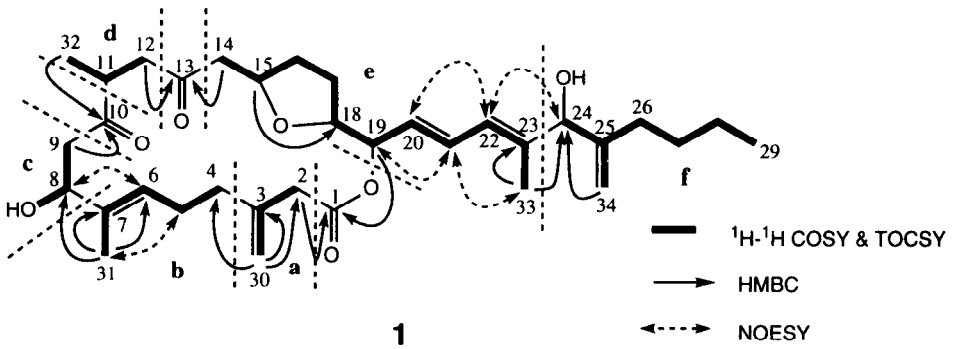


Figure 1. Selected 2D NMR Correlations for Amphidinolide U (**1**)

33, and (**f**) from H-24 to H_3-29 and H_2-34 (Figure 1). Connections among partial structures **a–f** and three quaternary carbons (C-1, C-10, and C-13) were assigned on the basis of ^{1}H-^{13}C long-range correlations observed in the HMBC spectrum. HMBC correlations from H_2-30 to C-2 (δ_C 42.8), C-3 (δ_C 142.7), and C-4 (δ_C 35.3) suggested the connectivity between partial structures **a** and **b** through an *exo*-methylene at C-3. Connections among units **b**, **c**, **d**, **e**, and **f** were deduced from the following HMBC correlations; H_3-31/C-6, H_3-31/C-7, H_3-31/C-8, H_2-9/C-10, H_3-32/C-10, H_2-12/C-13, H_2-14/C-13, H_3-33/C-23, and H_3-33/C-24. The existence of an ester linkage between C-1 and C-19 was implied by HMBC correlations from H_2-2 and H-19 to C-1 (δ_C 170.8). The HMBC cross-peak from H-15 to C-18 suggested that C-15 and C-18 were linked through an oxygen to form a tetrahydrofuran ring. Geometries of three internal olefins at C-6–C-7, C-20–C-21, and C-22–C-23 were assigned as all *E* on the basis of NOESY cross-peaks for H_2-5/H_3-31, H-6/H-8, H-19/H-21, H-20/H-22, H-21/H_3-33, and H-22/H-24. Thus the gross structure of amphidinolide U was elucidated to be **1**.

NOESY correlations were observed for H-14a/H-16a, H-14a/H-17a, H-15/H-17b, H-16a/H-18, H-17a/H-19, and H-17b/H-20, indicating that relative stereochemistries between H-15 and H-18 and between H-18 and H-19 were *anti*- and *syn*-oriented, respectively (Figure 2). The absolute

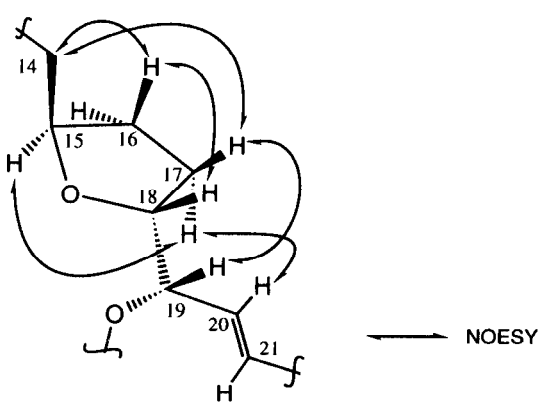
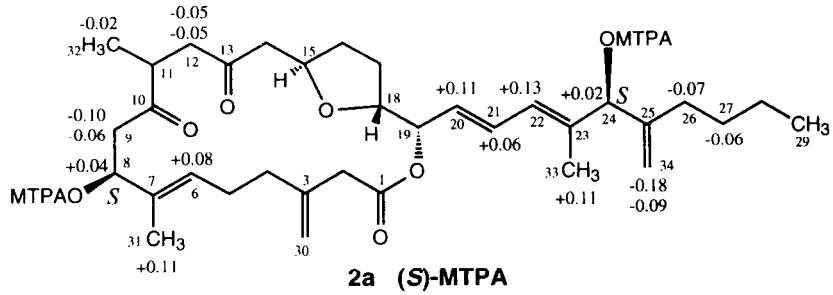


Figure 2. Relative Stereochemistry of Tetrahydrofuran Ring in Amphidinolide U (**1**)



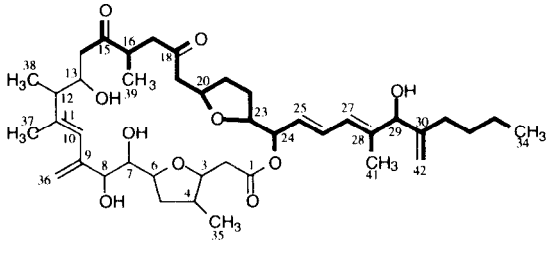
2a (*S*)-MTPA

2b (*R*)-MTPA

Figure 3. Δδ values [Δδ (in ppm) = δ_S -δ_R] obtained for (*S*)- and (*R*)-MTPA ester (**2a** and **2b**, respectively) of amphidinolide U (**1**).
Stereochemistries of C-15, C-18, and C-19 are relative.

configurations at C-8 and C-24 were assigned by modified Mosher's method[8] as follows (Figure 3). Amphidinolide U (**1**) was treated with (*R*)-(-)- or (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) to afford (*S*)-(-)- and (*R*)-(+)-MTPA ester (**2a** and **2b**, respectively) of **1**. Δδ values of H-6 (+0.06) and H_3-31 (+0.11) showed positive signs, while those of H_2-9 (-0.10 and -0.06) H_2-12 (-0.05 and -0.05), and H_3-32 (-0.02) were negative values, thus indicating 8*S*-configuration. On the other hand, 24*S*-configuration was determined on the basis of Δδ values of H-20 (+0.11), H-21 (+0.06), H-22 (+0.13), H_2-26 (-0.07),H_2-27 (-0.06), H_3-33 (+0.11), and H_2-34 (-0.18 and -0.09). Thus the absolute configurations at C-8 and C-24 and the relative stereochemistry of C-15, C-18, and C-19 of amphidinolide U were elucidated to be **1**.

Amphidinolide U (**1**) is a novel 20-membered macrolide possessing a tetrahydrofuran ring, two *exo*-methylenes, three branched methyls, two ketones, two hydroxyl groups, and a C_{10} linear side-chain. The gross structure of C-9–C-29 unit of amphidinolide U (**1**) corresponds to that of C-14–C-34 of amphidinolide C (**3**), while the carbon skeleton of C-1–C-8 unit of **1** is very close to that of C-1 ~ C-8 of amphidinolide A[9] (**4**). This observation suggests that amphidinolide U (**1**) may be biogenetically related to amphidinolides C (**3**) and A (**4**). Amphidinolide U (**1**) exhibited weak cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro* with IC_{50} values of 12 and 20 μg/mL, respectively.



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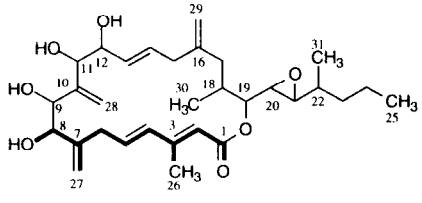

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Experimental Section

General Methods. The IR and UV spectra were taken on a JASCO FT/IR-5300 and a JASCO Ubest-35 spectrophotometers, respectively. ^{1}H and 2D NMR spectra were recorded on a Bruker AMX-600 spectrometer, and ^{13}C NMR spectra were measured on a Bruker ARX-500 spectrometer. Positive-mode FAB mass spectra were obtained on a JEOL JMS HX-110 using *p*-nitrobenzyl alcohol as a matrix.

Cultivation and Isolation. The dinoflagellate *Amphidinium* sp. (strain number Y-56) was separated from the inside cells of the marine acoel flatworm *Amphiscolops* sp., which was collected off Zanpa, Okinawa. The dinoflagellate was unialgally cultured at 25 °C for two weeks in seawater medium enriched with 1% ES supplement. The harvested cells (420 g, wet weight, from 580 L of culture) were extracted with MeOH/toluene (3:1, 3 L x 3). After addition of 1 M NaCl aq. (1 L), the mixture was extracted with toluene (4 L x 3). The toluene-soluble fractions were evaporated under reduced pressure to give a residue (3.67 g), which was subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) and a Sep-Pak cartridge C_{18} (MeOH/H_2O, 8:2) followed by C_{18} HPLC [LUNA C18(2), 5 μm, Phenomenex®, 10 x 250 mm; eluent, CH_3CN/H_2O (75:25); flow rate, 2.5 mL/min; UV detection at 254 nm] to afford amphidinolides U (**1**, 0.8 mg, 0.0002 %, wet weight, t_R 13.2 min), C (**3**, 0.0009 %, t_R 16.5 min), and F (0.0006 %, t_R 15.0 min).

Amphidinolide U (1): UV (MeOH) λ_{max} 230 nm (ε 20000); IR (KBr) ν_{max} 3400, 2935, and 1710 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); FABMS *m/z* 570 $(M+H)^+$ and 593 $(M+Na)^+$; HRFABMS *m/z* 593.3662 [calcd for $C_{34}H_{50}O_7Na$ $(M+Na)^+$, 593.3645].

(*S*)-MTPA Ester (2a) of Amphidinolide U (1). To a CH_2Cl_2 solution (10 μL) of amphidinolide U (**1**, 0.2 mg) were added 4-dimethylaminopyridine (0.01 mg), triethylamine (1 μL), and (*R*)-(-)-MTPACl (0.5 μL) at room temperature, and stirring was continued for 2 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (0.5 μL) and evaporation of the solvent, the residue was passed through a silica gel column (hexane/EtOAc, 1:1) to afford the (*S*)-MTPA ester (**2a**, 0.1 mg) of **1**. **2a:** ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, *J* = 7.3 Hz, H_3-29), 1.09 (3H, d, *J* = 7.1 Hz, H_3-32), 1.28 (2H, m, H_2-28), 1.36 (2H, m, H_3-27), 1.53 (1H, m, H-16b), 1.65 (3H, s, H_3-31), 1.66 (1H, m, H-17b), 1.67 (3H, s, H_3-33), 1.83 (2H, m, H_2-26), 1.99 (1H, m, H-17a), 2.08 ~ 2.20 (5H, m, H_2-4, H_2-5, and H-16a), 2.31 (1H, m, H-12b), 2.41 (1H, m, H-14b), 2.69 (1H, m, H-14a), 2.71 (1H, m, H-9b), 2.90 (1H, m, H-12a), 2.95 (1H, m, H-9a), 3.02 (1H, d, *J* = 16.3 Hz, H-2b), 3.08 (1H, d, *J* = 16.3 Hz, H-2a), 3.16 (1H, m, H-11), 3.53 (6H, s), 4.07 (m, H-18), 4.32 (1H, m, H-15), 4.91 (2H, s, H-30b and H-34b), 4.93 (2H, s, H-30a and H-34a), 5.21 (1H, m, H-19), 5.61 (1H, dd, *J* = 7.9 and 15.1 Hz, H-20), 5.69 (1H, m, H-6), 5.78 (1H, s, H-24), 5.88 (1H, m, H-8), 6.11 (1H, d, *J* = 11.0 Hz, H-22), 6.50 (1H, dd, *J* = 11.0 and 15.1 Hz, H-21), 7.35 ~ 7.43 (6H, m), and 7.48 ~ 7.54 (4H, m); FABMS *m/z* 1025 $(M+Na)^+$; HRFABMS *m/z* 1025.4220 [calcd for $C_{54}H_{64}O_{11}F_7Na$ $(M+Na)^+$, 1025.4250].

(*R*)-MTPA Ester (2b) of Amphidinolide U (1). Amphidinolide U (**1**, 0.2 mg) was treated with (*S*)-(+)-MTPACl (1 μL) by the same procedure as described above to afford the (*R*)-MTPA ester (**2b**, 0.1 mg) of **1**. **2b**: ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, *J* = 7.3 Hz, H_3-29), 1.11 (3H, d, *J* = 7.1 Hz, H_3-32), 1.28 (2H, m, H_2-28), 1.42 (2H, m, H_3-27), 1.49 (1H, m, H-16b), 1.56 (6H,

s, H_3-31 and H_3-33), 1.65 (1H, m, H-17b), 1.90 (2H, m, H_2-26), 1.97 (1H, m, H-17a), 2.07 ~ 2.18 (5H, m, H_2-4, H_2-5, and H-16a), 2.36 (1H, m, H-12b), 2.42 (1H, m, H-14b), 2.67 (1H, m, H-14a), 2.81 (1H, m, H-9b), 2.95 (1H, m, H-12a), 3.01 (1H, m, H-9a), 3.03 (1H, d, J = 16.3 Hz, H-2b), 3.08 (1H, d, J = 16.3 Hz, H-2a), 3.13 (1H, m, H-11), 3.54 (6H, s), 4.05 (m, H-18), 4.32 (1H, m, H-15), 4.89 (1H, s, H-30b) 4.92 (1H, s, H-30a), 5.00 (1H, s, H-34b), 5.11 (1H, s, H-34a), 5.20 (1H, m, H-19), 5.50 (1H, dd, J = 7.9 and 15.1 Hz, H-20), 5.61 (1H, m, H-6), 5.76 (1H, s, H-24), 5.84 (1H, m, H-8), 5.98 (1H, d, J = 11.0 Hz, H-22), 6.44 (1H, dd, J = 11.0 and 15.1 Hz, H-21), 7.35 ~ 7.43 (6H, m), and 7.48 ~ 7.54 (4H, m); FABMS *m/z* 1025 $(M+Na)^+$; HRFABMS *m/z* 1025.4217 [calcd for $C_{54}H_{64}O_{11}F_7Na$ $(M+Na)^+$, 1025.4250].

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