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Amphidinolide V, novel 14-membered macrolide from marine dinoflagellate *Amphidinium* sp.

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Abstract

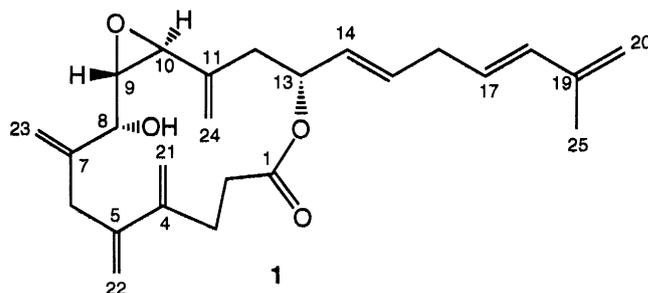
A novel 14-membered polyene macrolide, amphidinolide V (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp., and the structure was elucidated by spectroscopic data. The relative stereochemistry of C-8, C-9, C-10, and C-13 was elucidated by NOESY correlations. Amphidinolide V (**1**) possessing five *exo*-methylenes and one epoxide exhibited cytotoxicity against tumor cell lines. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: marine dinoflagellate; macrolide; polyene; cytotoxic.

Marine microorganisms have proven to produce a variety of chemically interesting and biologically significant secondary metabolites.^{1–3} During our search for bioactive substances from marine dinoflagellates of the genus *Amphidinium*,⁴ we have examined extracts of the laboratory-cultured marine dinoflagellate *Amphidinium* sp. (strain number Y-5) and isolated thirteen macrolides with various backbone skeletons and biogenetically unusual structural features.⁵ Here we describe the isolation and structure elucidation of a novel 14-membered polyene macrolide possessing five *exo*-methylenes, amphidinolide V (**1**), from the same dinoflagellate (Y-5).

The harvested algal cells (357 g, wet weight from 1500 L of culture) were extracted with toluene:methanol (1:3), and the extracts were partitioned between toluene and 1 M NaCl. Toluene-soluble materials were subjected to silica gel (CHCl₃/MeOH) and ODS columns (MeOH/H₂O) followed by C₁₈ HPLC (CH₃CN/H₂O) to afford amphidinolide V (**1**, 0.2 mg, 0.00005%, wet weight) together with known macrolides, amphidinolides A,⁶ C,⁷ E,⁸ and J.⁹

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Amphidinolide V¹⁰ (**1**) showed the molecular ion peak at m/z 419 [(M+Na)⁺] in the ESIMS spectrum,¹¹ and the molecular formula, C₂₅H₃₂O₄, was established by HRESIMS [m/z 419.2188, (M+Na)⁺, Δ -1.1 mmu]. The IR and UV spectra indicated the presence of hydroxy (ν_{\max} 3450 cm⁻¹), ester (ν_{\max} 1730 cm⁻¹), and diene [λ_{\max} 230 nm (ϵ 11000)] functionalities. ¹H and ¹³C NMR data (Table 1) of **1** revealed the presence of an ester, five *sp*² quaternary carbons, four *sp*² methines, five *sp*² methylenes, four oxymethines (two of them bearing an epoxide ring), five methylenes, and a methyl. Detailed analyses of the ¹H-¹H COSY, TOCSY, and HMQC spectra disclosed one isolated methylene (H₂-6) and three proton networks from H₂-2 to H₂-3, from 8-OH to H-10, and from H₂-12 to H-18. Five *exo*-methylene carbons (C-20–C-24) were assigned by one-bond C–H couplings observed for the HMQC spectrum. HMBC correlations from H₂-21 to C-3 and C-5 and from H₂-22 to C-4 and C-6 implied the connectivity from C-3 to C-6 through two *exo*-methylene units (C-4–C-21 and C-5–C-22). Connectivities among C-6, C-8, and C-23 through C-7 were elucidated by HMBC correlations from H₂-6 to C-7 and from H₂-23 to C-6 and C-8. The presence of an *exo*-methylene unit at C-11 was elucidated by HMBC correlations from

Table 1
¹H and ¹³C NMR data of amphidinolide V (**1**)

positn.	δ_C^a	δ_H^a		δ_H^b	
1	171.3 ^b	s			
2	33.5	t	2.45 ^c m	2.17 m	2.05 ddd, 14.4, 4.7, 3.8
3	30.2	t	2.73 m	2.57 ddd, 14.1, 12.1, 3.8	2.38 dt, 14.1, 4.7
4	144.5	s			
5	141.4	s			
6	39.0	t	3.25 d, 16.4	3.11 d, 16.4	3.05 d, 16.3
7	144.7	s			2.88 d, 16.3
8	71.1	d	4.50 m	3.97 m	
8-OH			2.15 brs	1.88 brs	
9	63.2	d	2.80 m	2.82 dd, 6.5 2.2	
10	57.7	d	3.46 brs	3.55 d, 2.2	
11					
12	38.9	t	2.41 ^c m	2.29 dd, 14.5, 3.3	2.18 m
13	74.3	d	5.43 m	5.62 dt, 7.1, 3.3	
14	127.6	d	5.42 m	5.47 dd, 15.5, 7.1	
15	131.2	d	5.72 m	5.74 dt, 15.5, 6.6	
16	34.9	t	2.83 ^c m	2.70 ^c t, 6.6	
17	127.1	d	5.60 dt, 15.6, 6.7	5.57 dt, 15.6, 6.6	
18	133.3	d	6.12 d, 15.6	6.21 d, 15.6	
19	141.5	s			
20	114.8	t	4.89 ^c s	5.01 s	4.94 s
21	114.4	t	5.13 s	5.08 s	4.97 s
22	113.6	t	5.26 s	5.10 s	4.92 s
23	114.4	t	5.46 s	5.14 s	5.68 s
24	114.8	t	5.19 s	4.93 s	5.10 s
25	18.3	q	1.83 ^d s	1.78 ^d s	4.76 s

^ain CDCl₃. ^bin benzene-*d*₆. ^c2H. ^d3H. ^enot observed.

H₂-24 to C-10 and C-12. HMBC correlations from H₂-20 to C-18 and C-25 and from H₃-25 to C-18 and C-19 suggested that the side chain possessed an isopropenyl terminus. The ester carbonyl (C-1) was shown to be attached to C-2 by the HMBC correlation from H₂-2 to C-1. On the other hand, the HMBC correlation from low-field resonance of H-13 to C-1 was observed, indicating that an ester linkage was formed between C-1 and C-13. Geometries of two disubstituted double bonds at C-14–C-15 and C-17–C-18 were both assigned as *E* by NOESY correlations for H-13/H-15, H-14/H-16, and H-16/H-18 as well as ¹H–¹H coupling constants ($J_{14,15}=15.5$ Hz and $J_{17,18}=15.6$ Hz). Thus the gross structure of amphidinolide V was concluded to be **1** (Fig. 1).

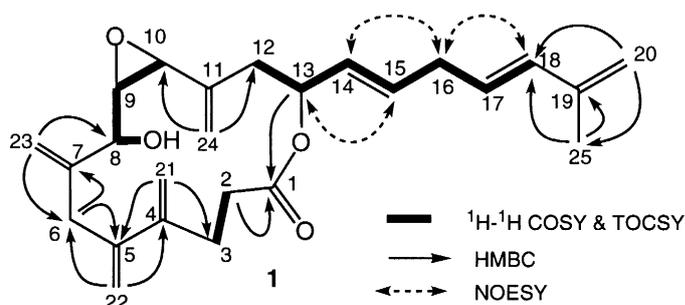


Fig. 1. Selected 2D NMR correlations of amphidinolide V (**1**)

The relative stereochemistry of C-8, C-9, C-10, and C-13 in **1** was deduced from ¹H–¹H coupling constants and NOESY correlations in benzene-*d*₆ (Fig. 2). The epoxide ring was assigned as *trans* on the basis of the ¹H–¹H coupling constant ($J_{9,10}=2.2$ Hz).¹² The existence of an *S-trans* diene moiety at C-4(C-21)–C-5(C-22) was elucidated on the basis of NOESY correlations for H-3a/H-21a and H-3b/H-22b.¹³ The NOESY correlation for H-2a/H-8 indicated that H-8 was directed to the inside of the macrolactone ring. NOESY correlations for H-6a/H-9, H-6a/H-21b, H-9/H-24a and H-13/H-24b implied that H-6a, H-9, and H-13 were all β -orientated. On the other hand, NOESY correlations were observed for H-8/H-10, H-8/H-22a, and H-10/H-12b, suggesting that H-10 was α -orientated. Therefore the relative stereochemistry of C-8, C-9, C-10, and C-13 of amphidinolide V was elucidated to be **1**.^{14,15}

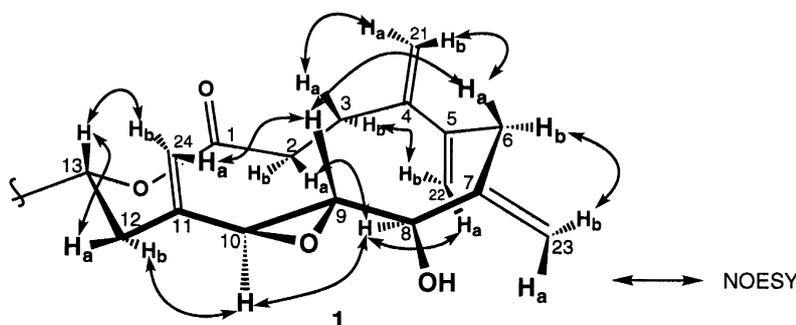


Fig. 2. Relative stereochemistry of amphidinolide V (**1**) based on ¹H–¹H coupling constants^a and NOESY correlations (benzene-*d*₆). ^aThe coupling constants (H/H in Hz) for this moiety are as follows: 2a/3a=12.1, 2a/3b=4.7, 2b/3a=3.8, 2b/3b=4.7, 8/9=6.5, 9/10=2.2, 12a/13=3.3, and 12b/13=3.3

Amphidinolide V (**1**) is a unique 14-membered polyene macrolide possessing five *exo*-methylenes and one epoxide. A vicinally located one-carbon branch, which is one of structural features characteristic of known amphidinolides,^{1b} exists as both *exo*-methylenes at C-4/C-5 position in **1**. Amphidinolide V (**1**) exhibited cytotoxicity against murine lymphoma L1210 (IC₅₀, 3.2 μ g/mL) and epidermoid carcinoma KB cells (IC₅₀, 7 μ g/mL) *in vitro*.

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10. Amphidinolide V (**1**): colorless oil; IR ν_{\max} 3450 and 1730 cm^{-1} ; UV λ_{\max} 230 nm (ϵ 11000); ^1H and ^{13}C NMR (Table 1); ESIMS m/z 419 ($\text{M}+\text{Na}^+$); HRESIMS m/z 419.2188 [calcd for $\text{C}_{25}\text{H}_{32}\text{O}_5\text{Na}$ ($\text{M}+\text{Na}^+$), 419.2199].
11. Positive mode ESIMS spectra were measured on a Jeol JMS-700TZ spectrometer at -80 V as a focus voltage using a sample dissolved in MeOH with flow rate of 200 $\mu\text{L}/\text{min}$.
12. J values for *cis*- (4 Hz) and *trans*-epoxides (1.5 Hz): In *Handbook of Proton NMR Spectra and Data*; Asahi Research Center, Ed.; Academic Press: Tokyo, 1985; Vol. 1, p. 86.
13. a and b denote low-field and high-field resonances, respectively, of a geminal pair.
14. Relative configurations at C-8, C-9, C-10, and C-13 were R^* , S^* , S^* , and R^* , respectively.
15. Molecular mechanics calculations for the four possible diastereomers with respect to C-8, C-9/C-10, and C-13 of **1** were carried out using MacroModel program ver. 6.0 (MM2* force-field). The most stable conformation (total energy; 35.70 kcal/mol) having 8*R*, 9*S*, 10*S*, and 13*R*-configurations fitted well the conformation shown in Fig. 2, while those of three other possible diastereomers could not be explained from the NOE data of **1**. For example, in the most stable conformation of the diastereomer having 8*R*, 9*R*, 10*R*, and 13*R*-configurations shown below, observation of NOE's for H-6a/H-9 and H-13/H-24b seems to be difficult.

