Pergamon

Tetrahedron Letters, Vol. 37, No. 9, pp. 1449-1450, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00029-9

Amphidinolide Q, a Novel 12-Membered Macrolide from the Cultured Marine Dinoflagellate *Amphidinium* sp.

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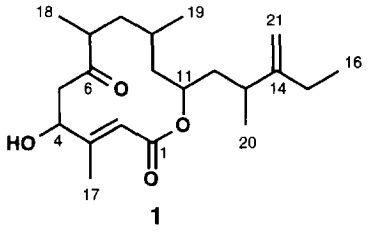
Abstract: Amphidinolide Q (**1**), a novel cytotoxic 12-membered macrolide having an unprecedented carbon-skeleton, was isolated from the cultured marine dinoflagellate *Amphidinium* sp. and the structure elucidated on the basis of spectroscopic data.

We previously isolated a series of cytotoxic macrolides, amphidinolides A ~ H and J ~ P, and a related linear metabolite, amphidinin A, from dinoflagellates of the genus *Amphidinium*, which were symbionts of Okinawan marine flatworms of the genus *Amphiscolops*.[1] We further continued investigation on the constituents of this microalga (strain number, Y-5) and now isolated a novel 12-membered macrolide, amphidinolide Q (**1**), exhibiting moderate cytotoxicity against murine lymphoma L1210 cells in vitro (IC_{50}, 6.4 μg/mL). Here we describe the isolation and structure elucidation of compound **1**, which possesses an unprecedented carbon framework but contains some unique structural or biogenetic features commonly found in other amphidinolides.[2]

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 flash chromatography on ODS (YMC-GEL ODS 60 Å, I-40/60; 85% MeOH) and reversed-phase HPLC (Develosil ODS-5; 75% CH_3CN) yielded amphidinolide Q (**1**) in 0.00005% yield (wet weight).

Amphidinolide Q (**1**), colorless oil; $[\alpha]_D^{20}$ +47° (*c* 0.044, MeOH); IR (film) ν_{max} 3450, 1720, and 1650 cm^{-1}; FABMS (matrix: glycerol) *m/z* 351 $(M+H)^+$, had a molecular formula of $C_{21}H_{34}O_4$ as established by HRFABMS [*m/z* 351.2565, $(M+H)^+$, Δ +3.0 mmu]. The ^1H and ^{13}C NMR spectral data (Table 1) suggested the presence of one ketone, one α,β-unsaturated ester (or lactone), one exomethylene, two oxymethines, three unoxygenated methines, five sp^3 methylenes, and five methyl groups. The ^1H-^1H COSY and spin decoupling experiments of **1** clearly revealed four partial structural units (**a** ~ **d**) shown in Figure 1,



1

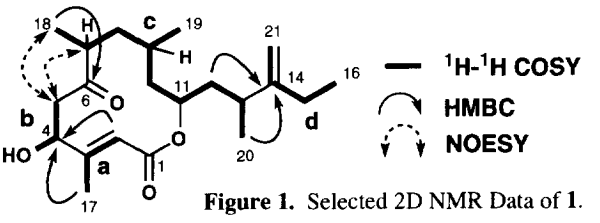


Figure 1. Selected 2D NMR Data of **1**.

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

position	δ_H		δ_C	HMBC correlations	position	δ_H		δ_C	HMBC correlations
1			169.6		11	5.28	m	74.3	H-10a, H_2-12
2	6.25	s	117.4	H_3-17	12 (2H)	1.57	dl	41.8	H_3-20
3			155.4	H_3-17	13	2.35	m	37.2	H_2-12, H_3-20
4	4.07	br s	73.1	H-2, H_3-17	14			155.5	H_2-12, H_2-15,
OH-4	3.68	d							H_3-16, H_3-20
5 (a)	2.38	dl	44.6		15 (a)	2.08	m	27.0	H_3-16, H_3-21
(b)	2.05	dl			(b)	2.02	m		
6			215.1	H_3-18	16 (3H)	1.08	t	12.6	H_2-15
7	1.89	m	50.5	H_3-18	17 (3H)	1.60	s	16.6	H-2
8 (a)	2.29	dl	40.3	H_3-18, H_3-19	18 (3H)	0.75	d	17.9	
(b)	0.90	ddl			19 (3H)	0.74	d	23.0	H-8a
9	0.96	m	33.0	H_3-19	20 (3H)	1.05	d	21.5	H_2-12
10 (a)	1.35	ddl	45.5	H_3-19	21 (a)	4.96	br s	107.3	
(b)	1.12	dt			(b)	4.95	br s		

J (H/H) in Hz: 4/OH-4=7.8; 4/5a=2.9; 4/5b=5.6; 5a/5b=12.7; 7/18=7.2; 7/8a=3.1; 7/8b=6.8; 8a/8b=13.5; 8a/9=*ca.* 0; 8b/9=3.7; 9/10a=7.2; 9/10b=2.6; 9/19=6.7; 10a/10b=14.1; 10a/11=5.0; 10b/11=3.1; 11/12=5.1; 12/13=7.2; 13/20=7.0; 15/21=1.5; 15a/15b=15.3; 15/16=7.4.

and connections of these four units and remaining two carbonyl carbons (C-1 and C-6) were suggested by the HMBC correlations [H-2/C-4 and H_3-17/C-4 (**a/b**); H_3-18/C-6 (C-6/**c**); H_2-12/C-14 and H_3-20/C-14 (**c/d**)] as well as the following observations. The ^1H chemical shifts of H_2-5 (Table 1) implied that the C-5 was adjacent to an sp^2 carbon, and the NOESY correlations observed for H-5a/H-7 and H-5a/H_3-18 were indicative of the connection of **b** and **c** units through the C-6 ketone. Selected key HMBC and NOESY correlations were also shown in Figure 1. The ^{13}C chemical shift of the C-17 methyl (δ_C 16.6) argued that the Δ^2-olefin was *E*, and this double bond was suggested to be conjugated with the C-1 ester carbonyl from the ^{13}C chemical shifts (C-2: δ_C 117.4; C-3: δ_C 155.4), which was also consistent with the UV absorption data of **1** (MeOH, λ_{max} 222 nm, ε 10300). Since the molecule of **1** was inferred to contain one ring from the unsaturation degrees, the C-1 carbonyl had to be linked to the C-11 oxymethine to form a 12-membered lactone ring, which was coincident with the low-field resonance of H-11 (δ_H 5.28). The planar structure of amphidinolide Q was thus elucidated as **1**. Among the NOESY correlations considerably observed for **1**,[3] cross-peaks for H-2/H-8a, H-7/H-9, H-8a/H-10a, and H-9/H-11 were noteworthy, which may suggest that the H-7, H-9, and H-11 are oriented to the same side of the macrocycle plane whereas the H-2, H-8a, and H-10a are directed otherwise. Further convincing evidences, however, have not been provided thus far for stereochemical assignment of the molecule of **1**.

A variety of macrolides with unprecedented carbon skeletons have been isolated from dinoflagellates of the genus *Amphidinium*.[1] Amphidinolide Q (**1**) also possesses a backbone skeleton hitherto unknown, while the vicinal location of the C1 branches (methyl and exomethylene groups; C-13 ~ C-14 moiety of **1**) is one of the unusual structural features of the amphidinolides and other microalgal metabolites.[2]

Acknowledgment: We thank Prof. T. Sasaki, Kanazawa University, for cytotoxicity tests. 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 and Notes

1. Ishibashi, M.; Takahashi, M.; Kobayashi, J. *J. Org. Chem.* **1995**, *60*, 6062-6066 and references cited therein.
2. Kobayashi, J.; Takahashi, M.; Ishibashi, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1639-1640.
3. NOESY cross-peaks distinctly observed for **1** in C_6D_6 solution (H/H; mixing time, 800 msec): 2/4-OH, 2/8a, 4/5(2H), 4/17, 5a/5b, 5a/7, 5a/17, 5a/18, 7/9, 7/18, 8a/8b, 8a/10a, 8b/18, 9/11, 9/19, 10a/10b, 10b/11, 10(2H)/12, 10b/19, 11/12, 11/13, 11/21a, 12/13, 12/20, 12/21a, 13/20, 13/21a, 15a/15b, 15(2H)/16, 15(2H)/21b, 16/21b, and 20/21a.

(*Received in Japan* 4 *December* 1995; *revised* 26 *December* 1995; *accepted* 27 *December* 1995)