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AMPHIDINOLIDE F, A NEW CYTOTOXIC
MACROLIDE FROM THE MARINE
DINOFLAGELLATE *Amphidinium* sp.

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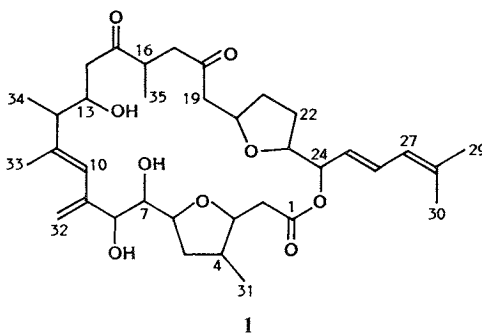
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Marine dinoflagellates have proved to be a subject of considerable attention as a new valuable source of bioactive compounds.¹⁾ During our studies on bioactive substances from Okinawan marine organisms,²⁾ we have isolated five cytotoxic macrolides, named amphidinolides A~E, from the laboratory-cultured dinoflagellate *Amphidinium* sp.^{3,4)} Recently, we further investigated a dinoflagellate of the genus *Amphidinium* which was associated with the Okinawan flatworm *Amphiscolops magniviridis* and a different species from those reported previously.^{1,3)} Here we wish to describe the isolation and structure elucidation of a new cytotoxic 25-membered macrocyclic lactone, named amphidinolide F (**1**).

The host invertebrate *A. magniviridis* was collected at Zanpa, Okinawa, and the associated dinoflagellate *Amphidinium* sp. was isolated and mass cultured unialgally at 25°C for 2 weeks in a sea water medium enriched with 1% ES supplement.⁵⁾ The cultured alga was harvested by centrifugation to yield 740 g of cells (wet weight) from 960 liters of culture. The harvested alga was extracted with MeOH-toluene (3:1) and the extract was partitioned between toluene and 1M aq NaCl. The toluene-soluble fraction was subjected to a silica gel column eluted with 5~30% MeOH in CHCl₃ followed by reversed-phase medium and HPLC's (Develosil LOP-ODS 24S and Develosil ODS-5) with 75% acetonitrile to give a macrolide mixture.

Purification of this mixture by preparative TLC developed twice with acetone-hexane (1:2) afforded amphidinolide F (**1**, 1 × 10⁻⁵%, wet weight) together with previously reported amphidinolides B (8 × 10⁻⁵%)⁶⁾ and C (3 × 10⁻⁵%)⁵⁾.

Amphidinolide F (**1**) was obtained as a colorless amorphous solid, [α]_D²⁰ -57° (c 0.1, CHCl₃). The molecular formula of **1**, C₃₅H₅₂O₉, was established by FAB-MS ((M+H)⁺, m/z 617) and HREI-MS (M⁺, m/z 616.3616, Δ +0.4 mmu). The IR spectrum (film) showed absorptions due to hydroxyl(s) (3400 cm⁻¹) and carbonyl (1700 cm⁻¹) group(s) and the UV absorption maximum (MeOH) at 237 nm (ϵ 22,000) suggested the presence of diene chromophore. The ¹H and ¹³C NMR data (Table 1) of **1** including DEPT experiments revealed the presence of two isolated ketones, an ester carbonyl, four olefins, eleven sp³ methines (eight of them bearing oxygen atoms), seven methylenes, and six methyl groups. These results accounted for seven of the ten unsaturations, suggesting that **1** contains three rings (two ethers and one lactone). The assignment of all protonated carbons was established by the heteronuclear multiple-quantum correlation (HMQC) spectrum.⁷⁾ The detailed analyses of the ¹H-¹H COSY spectrum of **1** revealed the proton connectivities for six segments of C-2~C-4, C-5~C-10, C-12~C-14, C-16~C-17, C-19~C-21, and C-22~C-29. In the COSY spectrum the cross-peaks for 4-H/5-H₂ and 21-H₂/22-H₂ were obscure due to heavily overlapping of the broad methylene signals. The homonuclear Hartmann-Hahn (HOHAHA) spectrum⁸⁾ was, however, quite efficient for clarifying the proton networks of these complex methylene signals, presenting the cross-peaks for these moieties (31-H₃/4-H, 31-H₃/5a-H, and 31-H₃/5b-H; 20-H/21a-H, 20-H/21b-H, 20-H/22a-H,



and 20-H/22b-H) that reveal the connections of C-4/C-5 bond and C-21/C-22 bond. Evidence for the connection between C-10 and C-12 through C-11 was provided by long-range ^1H - ^{13}C correlations obtained from the heteronuclear multiple-bond correlation (HMBC) experiment⁹⁾ (10-H/C-11, 10-H/C-12, 10-H/C-33, 33-H₃/C-10, 33-H₃/C-11, and 33-H₃/C-12). From these observations the six segments based on the COSY data were connected into three partial structures: C-2~C-14, C-16~C-

17, and C-19~C-29. These three fragments were shown to be linked through three carbonyls (δ 213.58, 207.47, and 171.16) on the basis of HMBC correlations (2-H₂/C-1, 24-H/C-1, 14-H₂/C-15, 35-H₃/C-15, 17-H₂/C-18, and 19-H₂/C-18). In particular the observation of the 24-H/C-1 cross-peak confirmed the fact that the ester bond is present between C-1 and C-24 to construct a 25-membered macrocyclic lactone ring. This finding was firmly ascertained by the comparison of the ^1H and ^{13}C

Table 1. ^1H and ^{13}C NMR data of amphidinolide F (I).^a

Position	^1H	J (Hz)	^{13}C	^1H coupled with ^{13}C (HMBC correlations)
1			171.16 s	2-H ₂ , 24-H
2	2.49 (2H) m		38.65 t	
3	3.81 dt	2.4, 6.8	81.26 d	2-H ₂ , 5a-H, 31-H ₃
4	1.81 m		39.67 d	2-H ₂ , 5a-H, 31-H ₃
5a	2.11 m		36.81 t	31-H ₃
5b	1.39 m			
6	3.78 dt	2.1, 7.3	79.08 d	7-H, 8-H
7	3.53 m		76.71 d	8-H
8	4.05 d	4.0	76.71 d	7-H, 32a-H, 32b-H
9			144.37 s	7-H, 8-H, 32a-H
10	5.98 br s		124.62 d	8-H, 32a-H, 32b-H
11			140.00 s	10-H, 12-H, 33-H ₃ , 34-H ₃
12	2.25 m		49.46 d	10-H, 14a-H, 33-H ₃ , 34-H ₃
13	3.93 dt	2.0, 9.8	70.50 d	12-H, 14a-H, 34-H ₃
14a	2.74 dd	9.3, 15.1	45.65 t	
14b	2.51 m			
15			213.58 s	14a-H, 14b-H, 17a-H, 17b-H, 35-H ₃
16	3.15 m		42.93 d	17a-H, 17b-H, 35-H ₃
17a	3.04 dd	9.3, 17.1	45.81 t	35-H ₃
17b	2.29 m			
18			207.47 s	17a-H, 17b-H, 19a-H, 19b-H
19a	2.73 dd	8.8, 16.6	48.45 t	
19b	2.50 m			
20	4.36 m		74.82 d	19a-H, 19b-H, 21b-H
21a	2.08 m		31.84 t	19a-H, 19b-H
21b	1.47 m			
22a	1.90 m		28.46 t	21a-H
22b	1.54 m			
23	4.08 dd	7.8, 14.8	79.87 d	24-H, 25-H
24	5.17 t	7.8	77.93 d	22a-H, 22b-H, 23-H, 26-H
25	5.31 dd	7.8, 14.7	123.97 d	24-H, 26-H
26	6.50 dd	11.2, 14.7	132.09 d	24-H, 25-H
27	5.76 br d	11.2	124.06 d	25-H, 29-H ₃ , 30-H ₃
28			138.25 s	26-H, 29-H ₃ , 30-H ₃
29	1.75 (3H) s		26.00 q	30-H ₃
30	1.73 (3H) s		18.43 q	29-H ₃
31	1.00 (3H) d	6.3	15.39 q	
32a	5.14 br s		116.16 t	8-H, 10-H
32b	4.93 br s			
33	1.67 (3H) s		13.94 q	12-H
34	1.03 (3H) d	7.3	15.29 q	12-H
35	1.10 (3H) d	6.8	16.20 q	17a-H, 17b-H

^a Recorded on a Bruker AM-500 spectrometer in CDCl₃.

NMR data of amphidinolide C, a 25-membered macrolide previously isolated from the different species of the dinoflagellate belonging to the genus *Amphidinium*.⁵⁾ The side chain of amphidinolide F (**1**) was shown to consist of a diene bearing two methyl groups, C₆-unit shorter than the side chain of amphidinolide C. The phase-sensitive NOESY spectrum of **1** showed cross-peaks for 24-H/26-H, 25-H/27-H, 26-H/30-H₃, and 27-H/29-H₃, which clearly accounted for the diene structure (25*E*-configuration, $J_{25,26}=14.7$ Hz). Thus the gross structure of amphidinolide F (**1**) was concluded to be **1**. Since the ¹H and ¹³C NMR chemical shifts as well as the ¹H-¹H coupling constants of amphidinolide F (**1**) correspond well to those of amphidinolide C, it was suggested that in compound **1** the THF rings are also present at C-3/C-6 and C-20/C-23 positions and the stereochemistries of 11 chiral centers present in **1** correspond to those of amphidinolide C.

Amphidinolide F (**1**) is considered to be biogenetically closely related to amphidinolide C, the former possibly being a precursor of the latter. Amphidinolide F (**1**) exhibited cytotoxic activity against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells *in vitro* with IC₅₀ values of 1.5 and 3.2 μg/ml, respectively.

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