Amphidinolide-B, a Novel Macrolide with Potent Antineoplastic Activity from the Marine Dinoflagellate *Amphidinium* sp.

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A novel macrolide, amphidinolide-B with potent antineoplastic activity has been isolated from the marine dinoflagellate *Amphidinium* sp. and its structure elucidated on the basis of spectroscopic studies.

Recently marine micro-organisms have proved to be a new valuable source of bioactive substances, since some symbiotic micro-organisms associated with marine animals have been demonstrated to be responsible for the production of some marine natural products.[1—3] During our studies on bioactive metabolites from marine sources,[4] we have investigated a cultured dinoflagellate *Amphidinium* sp. and reported an antineoplastic macrolide, amphidinolide-A.[5] Our continuing search for more pharmacologically useful substances from this dinoflagellate has now led to the isolation of amphidinolide-B (**1**), a novel macrolide with a very powerful antineoplastic activity. This communication describes the isolation and structure elucidation of (**1**).

The dinoflagellate *Amphidinium* sp., isolated from the Okinawan flatworm *Amphiscolops* sp., was grown unialgally in a sea water medium enriched with Provasoli's ES supplement[6] at 25 °C for two weeks. Harvested cells (375 g from *ca.* 1000 L of culture) were extracted with methanol–toluene (3 : 1) followed by partitioning between toluene and water. The toluene soluble fraction was subjected to repeated silica-gel column chromatography and reversed-phase h.p.l.c. resulting in the isolation of amphidinolide-B (**1**),† $[\alpha]_D^{25}$ −45° (*c* 1, $CHCl_3$), as a white amorphous solid in 0.001% yield (wet weight).

The molecular formula of amphidinolide-B, $C_{32}H_{50}O_8$, was determined by the mass spectra of the parent compound [fast atom bombardment mass spectrometry (f.a.b.m.s.) *m/z* 563 $(M + H)^+$] and its triacetate (**2**)‡ [high resolution electron impact mass spectrometry (h.r.e.i.m.s.) *m/z* 688.3800 (M^+),

† (**1**): f.a.b.m.s. *m/z* 563 (M^+), 545, 527, 511, 509, and 327. I.r. (KBr) 3400, 2920, 1705, 1270, and 1115 cm^{-1}.

‡ (**2**): ^1H n.m.r. ($CDCl_3$) δ 0.90 (3H, d, *J* 6.5 Hz; Me-11), 0.97 (3H, d, *J* 6.8 Hz; Me-23), 1.26 (3H, d, *J* 6.3 Hz; H_3 on C-26), 1.34 (3H, s; Me-16), 1.82 (3H, s; Me-2), 1.85 (3H, s; Me-14), 1.98, 2.08, and 2.14 (each 3H, s; Ac × 3), 2.86 (1H, dd, *J* 17.1 and 6.7 Hz; H-19), 2.91 (1H, td, *J* 5.9 and 2.1 Hz; H-9), 3.07 (1H, dd, *J* 6.9 and 2.1 Hz; H-8), 3.10 (1H, dd, *J* 17.1 and 4.7 Hz; H′-19), 5.01 (1H, m; H-25), 4.85 and 5.03 (each 1H, s; CH_2=13), 5.10, 5.24, and 5.26 (each 1H, m; H-18, 21, and 22), 5.28 (1H, m; H-7), 5.88 (1H, dt, *J* 15.4 and 6.5 Hz; H-6), 5.99 (1H, s; H-15), and 6.70 (1H, td, *J* 6.9 and 1.2 Hz; H-3). E.i.m.s. *m/z* 688 (M^+), 670, 628, 610, and 410.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) n.m.r. spectra of amphidinolide-B (**1**).

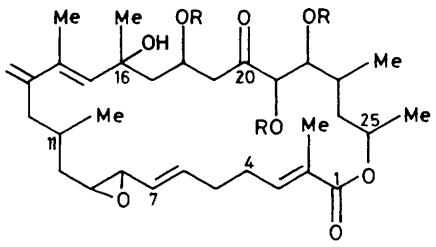
($CDCl_3$, δ in p.p.m.)[a]

Position	H[b]	C[c]	Position	H[b]	C[c]
1		167.65 s	Me-14	1.83 br. s	15.01 q
2		128.33 q	15	5.97 s	135.38 d
Me-2	1.82 br. s	12.37 q	16		75.92 s
3	6.77 td	139.92 d	Me-16	1.42 s	20.94 q
4	2.42 m	30.81 t	OH-16	2.21 s	
4′	2.20 m				
5	2.40 m	26.75 t			
5′	2.15 m				
6	5.92 ddd	124.26 d	17	1.95 m	45.22 t
7	5.16 dd	128.46 d	17′	1.78 dd	
8	3.14 dd	60.04 d	18	4.19 m	66.52 d
9	2.93 dt	59.31 d	OH-18	3.91 d	
10	1.49 ddd	39.39 t	19	2.87 dd	45.85 t
10′	1.27 m		19′	2.79 dd	
11	1.65 m	29.13 d	20		212.39 s
Me-11	0.89 d	18.17 q	21	4.33 dd	77.73 d
12	2.19 m	46.66 t	OH-21	3.87 d	
12′	1.95 m		22	3.71 td	75.53 d
13		144.35 s	OH-22	3.16 d	
CH_2=13	5.03 s(i)	114.79 t	23	1.85 m	33.17 d
	4.83 s(ii)				
14		143.05 s	Me-23	1.01 d	15.59 q
			24	1.95 m	39.29 t
			24′	1.28 m	
			25	5.06 dd	68.34 d
			26	1.28 d(H_3)	28.29 q

[a] The δ 7.27 resonance of residual $CHCl_3$ and δ 76.9 of $CDCl_3$ were used as internal references for ^1H and ^{13}C n.m.r. respectively. [b] Connectivities between the following protons were obtained by the COSY spectrum. H–H [*J* in Hz]: Me(2)–3 [1.2]; Me(2)–4; Me(2)–4′; 3–4 [7.3]; 3–4′ [7.3]; 4–4′; 4–5; 4–5′; 4′–5; 4′–5′; 5–5′; 5–6; 5′–6; 6–7 [15.7]; 7–8 [8.3]; 8–9 [2.2]; 9–10 [3.0]; 9–10′ [8.9]; 10–10′ [13.7]; 10–11 [10.7]; 10′–11; 11–Me(11) [6.5]; 11–12; 11–12′ [10.5]; 12–12′ [14.0]; 12–13(i); 12–13(ii); 13(i)–13(ii); 13(i)–Me(14); 13(ii)–Me(14); 13(i)–15; 13(ii)–15; Me(14)–15; Me(16)–17; 17–17′ [14.6]; 17–18; 17′–18 [5.2]; 18–OH(18); [3.3]; 18–19 [7.4]; 18–19′ [3.2]; 19–19′ [16.0]; 21–OH(21) [5.0]; 21–22 [1.7]; 22–OH(22) [10.3]; 22–23 [10.3]; 23–Me(23) [6.6]; 23–24′; 24–25; 24′–25; 25–26(3H) [6.2]. [c] Multiplicities were determined by DEPT data and assignments were based on selective proton decoupling experiments at 22.5 MHz.

Δ −2.2 (molecular mass units) m.m.u.: $C_{38}H_{56}O_{11}$]. ^1H and ^{13}C n.m.r. spectra revealed that (**1**) possessed four double bonds: an *exo*-methylene, a disubstituted, and two trisubstituted double bonds, both of which bore methyl groups and were (*E*) since the ^{13}C signals for the olefinic methyl groups were at high fields (δ_C 12.37 and 15.01).[7] Comparison with the ^{13}C n.m.r. data of desortomycin[8] along with the u.v. absorption at 222 nm (ε 12000 in MeOH)[9] suggested that one of the trisubstituted olefins constituted an α-methyl-α,β-unsaturated ester moiety (δ_C 167.65). The presence of another carbonyl group was shown by the ^{13}C n.m.r. spectrum of (**1**). The chemical shift (δ_C 212.39) implied that the carbonyl was not conjugated.

Now six of eight unsaturations were thus accounted for. The remaining two were attributed to two rings: a lactone ring (δ_H 5.06; lactone terminal proton) and an epoxide (δ_H 3.14 and 2.93). The ^1H n.m.r. spectrum of (**1**) showed four hydroxy protons: three as doublets and one as a singlet. This suggested the presence of three secondary and one tertiary alcohol groups. The observation of a tertiary methyl signal at δ_H 1.42 (3H, singlet) strongly indicated that the tertiary methyl was



(**1**) R = H

(**2**) R = Ac

attached to the carbon bearing the tertiary hydroxy group [δ_C 75.92, singlet by distortionless enhancements by polarization techniques (DEPT)] since no other quarternary carbon appeared in the sp^3-region in the ^{13}C n.m.r. spectrum of (**1**).

The extensive analyses of the COSY spectrum[10] of (**1**) allowed assignment of all protons and established the proton connectivities of the following three fragments: C-1 ~ C-15, C-17 ~ C-19, and C-21 ~ C-26. The signal for Me-2 showed clear off-diagonal peaks due to allylic couplings to H-3 and methylene protons on C-4. The *exo*-methylene protons on C-13 showed obvious cross peaks with one of C-12 methylene protons, Me-14, and H-15. Other vicinal couplings were relatively easily obtained (Table 1). The configurations of (6*E*) and 8,9-*trans* were suggested by the coupling constants ($J_{6,7}$ 15.7 Hz and $J_{8,9}$ 2.2 Hz)[5] and confirmed by the 2D NOESY spectrum (cross peaks: H-6 and H-8; H-7 and H-9). The NOESY spectrum also revealed the (*S*)-*trans* conformation of Δ^{13} and $\Delta^{14(15)}$-double bonds [cross peaks: H-12 (δ_H 2.19) and H-15; CH_2=13 (δ_H 5.03) and Me-14]. The ester oxygen on C-1 was shown to be connected to C-25 by the chemical shift of H-25 (δ_H 5.06). Other oxymethine protons (H-18, 21, and 22) were shifted about 1—1.5 p.p.m. downfield in the ^1H n.m.r. spectrum of the triacetate (**2**). To construct a whole molecule those three fragments were considered to be connected by two quarternary carbons, the isolated ketone and the carbon bearing the tertiary hydroxy and the tertiary methyl groups. The chemical shift of the C-19 methylene protons (δ_H 2.87 and 2.79) indicated that this methylene carbon was located at the α-position to the carbonyl group. Thus the carbonyl was assigned on C-20. The quarternary carbon bearing the tertiary methyl group was deduced to be at C-16 position by the difference nuclear Overhauser enhancement (n.O.e.) experiment. On irradiation of the tertiary methyl protons (δ_H 1.42) the signal for H-15 and Me-14 showed enhancement in area by 9% and 3%, respectively. Accordingly, the connections from C-16 to C-17 and from C-20 to C-21 now remained to be elucidated. The fragment C-21 ~ C-26, the one end of the molecule, had to be connected to a quaternary carbon, because the signal for H-21 showed cross peaks only with H-22 and OH on C-21. The chemical shift of the C-17 methylene protons (δ_H 1.78 and 1.95) implied that this carbon was not the α-position of the carbonyl group. These observations suggested that C-21 was the α-position to C-20 carbonyl group and C-17 was connected to C-16 by a process of elimination. This was supported by the fact that in the COSY spectrum a slight but consistent cross peak appeared between Me-16 and one of H-17 (δ_H 1.95) probably due to the W-type coupling. Hence on the basis of the discussions developed above we concluded that amphidinolide-B has structure (**1**).

Amphidinolide-B (**1**) is a new twenty six-membered macrolide.§ Its molecular constitution and substitution pattern are

§ The stereochemistry at all chiral centres in (**1**) remains undefined.

quite different from those of amphidinolide-A previously obtained from the same dinoflagellate.[5] Their biogenetical relationships and biological roles appear to raise intriguing questions, since amphidinolide-B (**1**) possessed a powerful antitumor activity against L1210 murine leukemia cells *in vitro* with the $I.C._{50}$ (50% inhibitory concentration) value of 0.14 ng/ml. Considering the $I.C._{50}$ values, amphidinolide-B (**1**) was about 10 000 times as potent as amphidinolide-A.

We thank Professor T. Yamasu (University of the Ryukyus) for providing the dinoflagellate, Mr. T. Hayase (Mitsubishi Chemical Industry Ltd.) for help with n.m.r. measurements, Professor T. Miyazawa and Dr. T. Higashijima (University of Tokyo) for the NOESY experiment, and the Science and Technology Agency of the Japanese Government for financial support.

Received, 9th March 1987; Com. 290

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