The Structures of Amphidinolide B Isomers: Strongly Cytotoxic Macrolides Produced by a Free-Swimming Dinoflagellate, *Amphidinium* sp.

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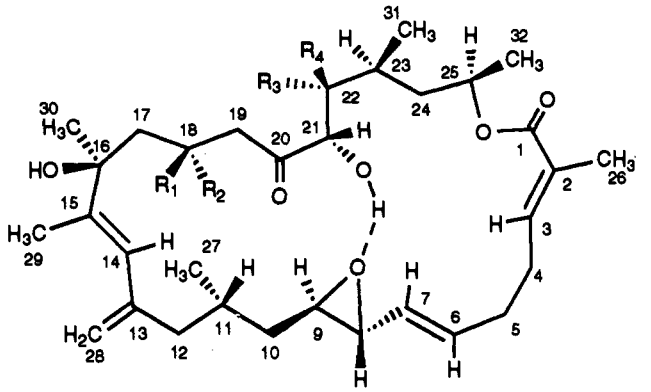
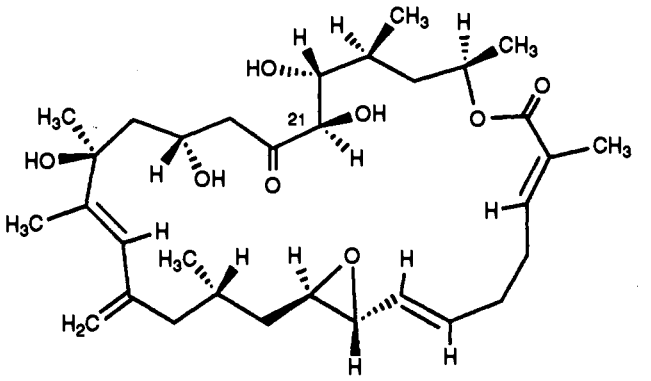
Interesting macrolides have been isolated from dinoflagellates, genus *Amphidinium*, symbiotic with the marine flatworms, *Amphiscolops* spp. Many of them have shown potent toxicity against tumor cell lines and are attracting attention as potential cancer drugs.[1-10] The planar structures of these dinoflagellate macrolides have been elucidated primarily by means of 2D-NMR, but their stereochemistry remains unsolved except for that of the small simple macrolide amphidinolide J.[5] In this communication we report the establishment of the relative stereochemistry of 26-membered C_{32} macrolides in the amphidinolide B group, the most representative and important group of amphidinolides.

The free-swimming dinoflagellate, strain S1-36-5,[11] was collected at Brewers Beach, St. Thomas, U.S. Virgin Islands, cloned, and cultured in seawater enriched with K-supplements.[12] From the freeze-dried algal cells, three isomeric compounds, which we tentatively call amphidinolides B_1, B_2, and B_3 in this paper, were isolated in yields of 0.14%, 0.024%, and 0.0076% of the dried cell weight, respectively.[13]

Chart 1



1. R_1, R_4=H; R_2, R_3=OH
2. R_1, R_3=OH; R_2, R_4=H
3. R_2, R_4=OH; R_1, R_3=H



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Amphidinolide B_1 (**1**, Chart 1) was obtained as needles from *n*-hexane–CH_2Cl_2, mp 82–84 °C; $[\alpha]^{25}_D = -62.5 \pm 0.5°$ (*c* 0.39, $CHCl_3$), $[\alpha]^{25}_D = -71.3 \pm 0.4°$ (*c* 0.39, CH_2Cl_2); HRFABMS *m/z* 563.359 680 ($C_{32}H_{50}O_8$, MH^+, Δ −2.3 ppm); UV λ_{max} = 220 nm (MeOH, ϵ 17 000). The NMR spectral data of **1** were almost identical with those reported for amphidinolide B by Ishibashi *et al.*[2] The structure deduced from the 2D-NMR spectra was also in agreement with the revised planar structure of amphidinolide B by Kobayashi *et al.*[4]

The relative stereochemistry of **1** was obtained from a single crystal X-ray diffraction analysis.[14] Figure 1 shows a perspective view of the final X-ray model. Amphidinolide B_1 has a rectangular shape, which is bridged in the middle by a hydrogen bond (2.02 Å) between 21-OH and the epoxide O. The solution conformation of **1** in $CHCl_3$ and CH_2Cl_2 seems to be close to the

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(10) Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753–1770.
(11) Compared to most other *Amphidinium* species, the organism is very large in size (length, 31–43 μm; width, 19–23 μm). Although it best fits the description of *A. carterae* Hulburt, no definite identification has been made. Morphological and ultrastructural studies are under way and will be published elsewhere.
(12) Keller, M. D.; Selvin, R. C.; Claus, W.; Guillard, R. R. L. *J. Phycol.* **1987**, *23*, 633–638.
(13) The toluene–methanol extract of the cells was partitioned between *n*-hexane and 90% MeOH. The methanol layer was chromatographed successively on silica gel (CH_2Cl_2/MeOH, 97:3), C18 silica gel (CH_3CN/H_2O, 8:2), and Hamilton PRP-1 (CH_3CN/H_2O, 8:2) and CN silica gels (Isooctane/2-PrOH, 8:1) to afford pure compounds.

(14) A single crystal of **1** (0.15 × 0.35 × 0.50 mm³) was covered with a thin layer of paratone N oil and mounted in a capillary. Data were collected at −40 °C on a Siemens R3M diffractometer to $2\theta \leq 115°$ (Cu Kα) using variable speed ω scans from 2.50 to 29.30 deg/min. Accurate cell constants, determined by a least-squares fit of 25 well-centered reflections ($25° \leq 2\theta \leq 30°$), were a = 11.417(7), b = 15.220(4), and c = 22.486(6) Å. Systematic extinctions uniquely indicated space group $P2_12_12_1$ with one molecule of $C_{32}H_{50}O_8$ plus a C_6H_{12} solvent constituting the asymmetric unit. Periodically monitored check reflections showed no significant decay. A total of 2991 unique reflections were collected, of which only 1646 (55%) were considered observed ($|F| > 3.0\sigma(F)$). Reflections were corrected for Lorentz and polarization but not absorption. The structure was solved by direct methods using the Siemens SHELXTL PLUS library. Blocked full-matrix least-squares refinements for all positional and thermal parameters for all non-hydrogen atoms and isotropic thermal parameters with partial occupancies for most of the solvent atoms were used. Hydrogens were included using the riding model. Refinement indicates significant thermal disorder for the solvent molecule and serious librational motion within the C3 to C6 region (C4–C5 bond is 1.137 Å, due to this effect). A total of 401 parameters were used in the refinements, to give a final unweighted R = 9.90% and R_w = 13.62%, with $w^{-1} = \sigma^2(F) + 0.0066F^2$. Additional data are available and described in the supplementary material.

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Table 1. Comparison of ^{1}H NMR Chemical Shifts and Coupling Constants of H17–H23 of Amphidinolides B_1 (**1**), B_2 (**2**), and B_3 (**3**) and Coupling Constants Calculated from the Crystal Structure of **1**

	^{1}H NMR: δ (ppm) and J (Hz) obsd			J (Hz) calcd from the crystal structure of **1**[a]	
position	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**1**	structures inverted at C18 (*) and C22 (**)
17b	1.76 dd	1.79 dd	1.75 dd		
	$J_{H17b,18}$ = 5.3	$J_{H17b,18}$ = 7.6	$J_{H17b,18}$ = 5.9	$J_{H17b,18}$ = 2.6	$J_{H17b,18}$ = 4.0*
18	4.16 m	4.19 m	4.16 m		
19a	2.87 dd	3.31 dd	3.03 dd		
	$J_{H19a,18}$ = 7.2	$J_{H19a,18}$ = 2.7	$J_{H19a,18}$ = 8.3	$J_{H19a,18}$ = 8.0	$J_{h19a,18}$ = 2.5*
19b	2.78 dd	2.63 dd	2.87 dd		
	$J_{H19b,18}$ = 3.3	$J_{H19b,18}$ = 8.6	$J_{h19b,18}$ = 3.5	$J_{H19b,18}$ = 1.7	$J_{H19b,18}$ = 8.0*
21	4.33 dd	4.20 dd	4.15 dd		
	$J_{h21,22}$ = 1.9	$J_{H21,22}$ = 2.0	$J_{H21,22}$ = 8.4	$J_{H21,22}$ = 3.4	$J_{H21,22}$ = 7.4**
22	3.71 ddd	3.65 ddd	3.55 ddd		
	$J_{H21,22}$ = 1.9	$J_{H21,22}$ = 2.0	$J_{H21,22}$ = 8.4	$J_{H22,21}$ = 3.4	$J_{H22,21}$ = 7.4**
	$J_{H22,23}$ = 10.1	$J_{H22,23}$ = 10.8	$J_{H22,23}$ = 3.1	$J_{H22,23}$ = 9.9	$J_{H22,23}$ = 2.7**

[a] Calculated according to the modified Karplus equation (Bothner-By, A. B. *Adv. Magn. Reson.* **1965**, *1*, 195) and corrected for the electron negativity of the substituents.

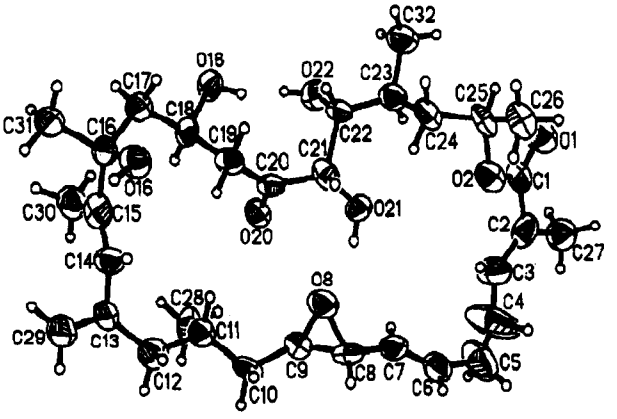

Figure 1. Perspective view of X-ray crystallographically-determined structure of amphidinolide B1 (**1**).

crystal conformation, because the observed spin–spin coupling constants match the values calculated from dihedral angles obtained from the crystal structure.

Both amphidinolide B_2 (**2**), an amorphous solid, $[\alpha]^{25}_D = -43.9 \pm 0.2°$ (c 0.42, $CHCl_3$); HRFABMS m/z 563.359 680 ($C_{32}H_{50}O_8$, MH^+, Δ −2.3 ppm); UV: λ_{max} = 220 nm (ϵ 16 000, MeOH), and amphidinolide B_3 (**3**), $[\alpha]^{25}_D = -69.4 \pm 1.1°$ (c 0.16, $CHCl_3$); HRFABMS m/z 563.359 680 ($C_{32}H_{50}O_8$, MH^+, Δ −2.3 ppm); λ_{max} = 220 nm (ϵ = 16 000, MeOH), showed NMR spectra very similar to those of **1**. In the spectra of **2**, the only significant differences are in the chemical shifts and coupling constants of H17 and H19 (Table 1). If **2** has the same conformation as **1**, as is likely in view of their chemical shifts and coupling constants of the rest of the molecule, these differences can be best explained if **2** is the C18 stereoisomer of **1**. Similarly, differences in the NMR spectra of **3** are confined around C22. There are differences in the coupling constants and chemical shifts of H22, and $J_{H22,H23}$ and $J_{H22,H21}$ are close to the calculated values for the C22 epimer (Table 1). Formulating **3** as the 22 epimer also explains the downfield shifts observed for the C19 methylene hydrogens, because, in the 22 epimeric form, the hydroxyl group comes closer (~3 Å) to C19 methylene hydrogens.

Comparison of the ^{1}H and ^{13}C NMR data strongly indicates that amphidinolide B_1 (**1**) and amphidinolide B_2 (**2**) are identical with amphidinolide B[2] and amphidinolide D[4], respectively. However, Kobayashi *et al.* assigned the structure of 21-*epi*-amphidinolide B (**4**) to amphidinolide D on the basis that amphidinolide D showed prominent NOEs between H21 and H19, as well as between H21 and 23-methyl protons. In amphidinolide B, such NOEs were absent.[4] If the compounds are identical with **1** and **2**, this result cannot be predicted on the basis of the crystal conformation. In the X-ray structure of **1**, H21 is located very close to both H19a,b, and a strong NOE is expected. The stereochemistry of the 23-methyl group is such that no NOE seems possible with H21 in either 21 epimer. Additional experiments as well as caution will be needed to secure the unequivocal identities of these isomeric compounds.

Amphidinolides B_1, B_2, and B_3 exhibited potent cytotoxicity against human colon tumor cell line HCT 116 (IC_{50} 0.122, 7.5, and 0.206 μg/mL, respectively). However, the level of the activity was significantly lower than that reported for amphidinolide B and amphidinolide D against a different cell line, murine leukemia L1210 (IC_{50} 1.4×10^{-4} and 1.9×10^{-2} μg/mL, respectively).[2,4]

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra and tables containing assignments and coupling constants of the compounds **1**, **2** and **3**, and X-ray data for **1** (37 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.