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Absolute Stereochemistry of Amphidinolides G and H

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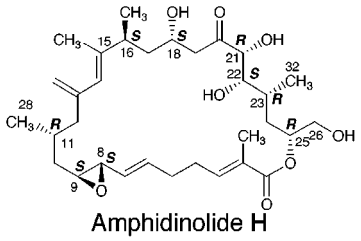
ABSTRACT



Absolute stereochemistry of amphidinolides G (1) and H (2), potent cytotoxic 27- and 26-membered macrolides, respectively, isolated from a marine dinoflagellate *Amphidinium* sp., was determined by X-ray diffraction analysis, synthesis of a degradation product (3) of 2, and interconversion between 1 and 2.

Amphidinolides are a series of unique cytotoxic macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms *Amphiscolops* spp.[1] Amphidinolides G (**1**) and H (**2**), isolated from the marine dinoflagellate *Amphidinium* sp. (Y-25 strain), are potent cytotoxic 27- and 26-membered macrolides, respectively, having unique structural features such as an allyl epoxide or vicinally located one-carbon branches.[2] The gross structures of **1** and **2** have been elucidated primarily by means of 2D NMR data, whereas the stereochemisty remains unsolved. During our search for bioactive and structurally unique secondary metabolites from marine dinoflagellates,[3] a strain (Y-72) of the genus *Amphidinium* producing relatively large amounts of amphidinolides G (**1**) and H (**2**), has been recently separated from the inside cells of the marine acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa.[4] Here we describe the determination of relative and absolute stereochemistry of amphidinolides G (**1**) and H (**2**) on the basis of X-ray diffraction analysis, synthesis of a degradation product (**3**) of **2**, and interconversion between **1** and **2**.

The dinoflagellate *Amphidinium* sp. (Y-72 strain) was cultured unialgally at 25 °C for 2 weeks in a seawater medium enriched with 1% Provasoli's ES supplement. The harvested algal cells (50 g, wet weight, from 120 L of culture) were extracted with MeOH/toluene (4:1), and the extracts were partitioned between toluene and water. The toluene extracts were subjected to a silica gel column followed by C_{18} HPLC to afford amphidinolides G (**1**, 0.046%, wet weight) and H (**2**, 0.082%) (Scheme 1).

Amphidinolide H (**2**) was crystallized from hexane/benzene as colorless needles, mp 131−132 °C. The relative

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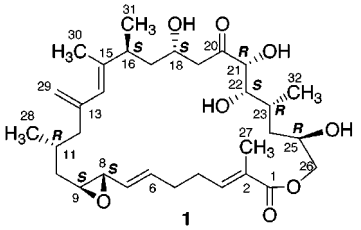
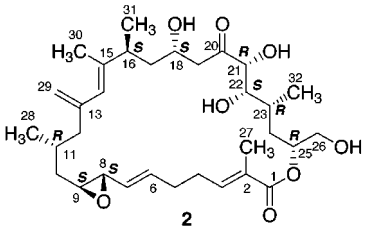
Scheme 1



1



2

stereochemistry of nine chiral centers in **2** was obtained from a single-crystal X-ray diffraction analysis, and the perspective view of the final X-ray model is shown in Figure 1.

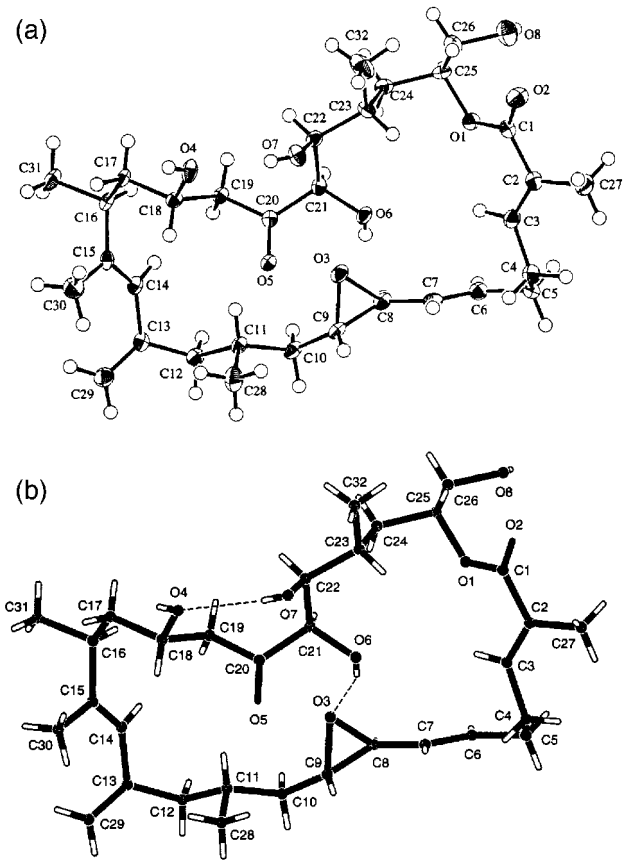
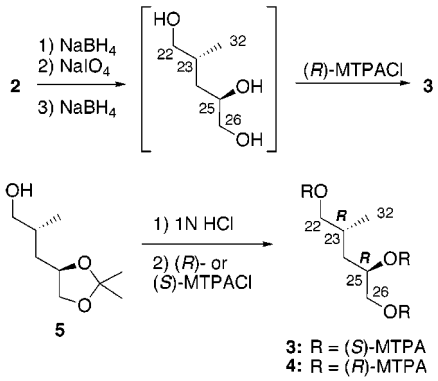


Figure 1. Molecular structures [(a) ORTEP drawing and (b) wire frame model] of amphidinolide H (**2**) obtained by X-ray analysis.

Amphidinolide H (**2**) was revealed to have a rectangular shape, which was bridged in the middle by an intramolecular hydrogen bond (1.99 Å) between O(O6)H and epoxide O(O3). The bond length of one C−O bond [O3−C8; 1.470-(3) Å] at the epoxide ring was longer than that of the other C−O bond [O3−C9; 1.448(3) Å], probably because of the effect of the intramolecular hydrogen bond. By another intramolecular hydrogen bond (1.92 Å) between O(O7)H-22 and O(O4)-18, an envelope-boat-shaped eight-membered ring was constructed at the C18−C19−C20 −C21−C22−O7−O(O7)H−O4 portion. In this crystalline structure the *S-cis* diene portion at C15−C14−C13−C29 was revealed to be twisted [torsion angle, −35.6(5)°].

To determine the absolute stereochemistry of amphidinolide H (**2**), **2** was treated with $NaBH_4$ followed by $NaIO_4$ oxidation, $NaBH_4$ reduction, esterification with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlolide (MTPACl), and separation using C_{18} HPLC to afford the tris-(*S*)-MTPA ester (**3**) of the C22−C26 segment (Scheme 2).

Scheme 2



On the other hand, both tris-(*S*)- and (*R*)-MTPA esters (**3** and **4**) of the C22−C26 segment were prepared from the (2*R*,4*R*)- acetonide (**5**), which was synthesized from methyl

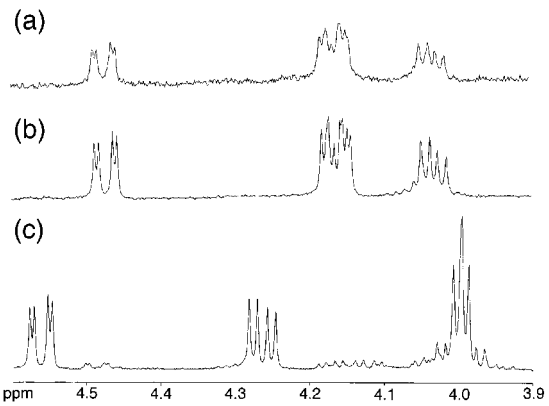


Figure 2. ^{1}H NMR spectra (partial) of (a) tris-(*S*)-MTPA ester (**3**) derived from amphidinolide H (**2**), (b) tris-(*S*)-MTPA ester (**3**) of the synthetic C22−C26 segment, and (c) tris-(*R*)-MTPA ester (**4**) of the synthetic C22−C26 segment.

(2*S*)-3-hydroxy-2-methylpropionate reported previously.[5] In the ^{1}H NMR spectra (Figure 2 and Table 1) of tris-(*S*)- and (*R*)-MTPA esters (**3** and **4**) of the synthetic segment, significant differences were observed for signals due to methylene protons at C22 (**3**, δ_H 4.16 and 4.03; **4**, δ_H 4.00, 2H). ^{1}H NMR data of the tris-(*S*)-MTPA ester (**3**) derived from the natural product (**2**) were identical with those of the synthetic tris-(*S*)-MTPA ester (**3**), indicating 23*R*- and 25*R*-configurations. Therefore, the absolute configurations of amphidinolide H (**2**) were concluded to be 8*S*, 9*S*, 11*R*, 16*S*, 18*S*, 21*R*, 22*S*, 23*R*, and 25*R*.

Table 1. Selected ^{1}H NMR Data of Tris-(*S*)- and (*R*)-MTPA Esters (**3** and **4**, respectively) of the Synthetic C22−C26 Segment

position	**3**	**4**
H_2-22	4.16, 4.03	4.00[a]
H-23	1.36	1.28
H_2-24	1.77[a]	1.70, 1.60
H-25	5.37	5.35
H_2-26	4.47, 4.17	4.56, 4.26
H_3-32	0.92[b]	0.86[b]

[a] 2H. [b] 3H.

The absolute stereochemistry of amphidinolide G (**1**) was determined by interconversion between **1** and amphidinolide H (**2**). Treatment of **2** with K_2CO_3 in EtOH at 4 °C for 18 h yielded a 1:1 mixture of **1** and **2**. All spectral data of amphidinolide G (**1**) isolated from this mixture were identical with those of natural product (**1**). On the other hand, treatment of amphidinolide G (**1**) with K_2CO_3 also gave a 1:1 mixture of **1** and **2**. Thus the absolute configurations of amphidinolide G (**1**) were the same as those of amphidinolide H (**2**).

The absolute stereochemistry and the X-ray structure of amphidinolide H (**2**) correspond well to those of amphidinolide B,[6] which has been isolated from the Y-5 strain of *Amphidinium* sp. by our group[7] and also from a free-swimming *Amphidinium* sp. by Shimizu *et al.*[8] Both amphidinolides B and H (**1**) showed potent cytotoxicity (IC_{50} 0.0045−0.00014 μg/mL) against cultured tumor cells,[2,7a] which may result from not only the characteristic functionalities such as the allyl epoxide and the *S*-*cis*-diene but also the unique 3D structures

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Supporting Information Available: X-ray data of **2**, procedures of degradation, synthesis, and derivatization, and spectral data of **3** and **4**. This material is available via the Internet at http://pubs.acs.org.

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