ORGANIC LETTERS

2008
Vol. 10, No. 17
3709−3711

Absolute Stereochemistry of Amphidinolide Q

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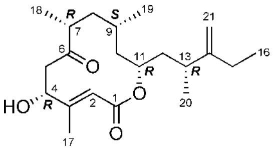
Received June 10, 2008

ABSTRACT



amphidinolide Q (1)

The absolute configurations at five chiral centers in amphidinolide Q (1), a cytotoxic 12-membered macrolide isolated from a marine dinoflagellate *Amphidinium* sp., were elucidated to be 4*R*, 7*R*, 9*S*, 11*R*, and 13*R* on the basis of NMR analyses and a modified Mosher's method.

Amphidinolides are a series of cytotoxic macrolides possessing unique structural features isolated from laboratory cultured marine dinoflagellates *Amphidinium* sp.[1] Amphidinolide Q (**1**) isolated from a dinoflagellate *Amphidinium* sp. (Y-5 strain) is a cytotoxic 12-membered macrolide having C1 branches at vicinal carbons (C-13 and C-14) and an α,*β*-unsaturated ester moiety.[2] The gross structure of **1** has been elucidated by 2D NMR, whereas the stereochemistry remains unsolved due to lack of sample.[2] Quite recently, we could reisolate amphidinolide Q (**1**) from the same strain so that this sample was used to elucidate the stereochemistry of **1**. Here, we describe the elucidation of the absolute configurations at five chiral centers in **1** on the basis of the NMR analysis and a modified Mosher's method.

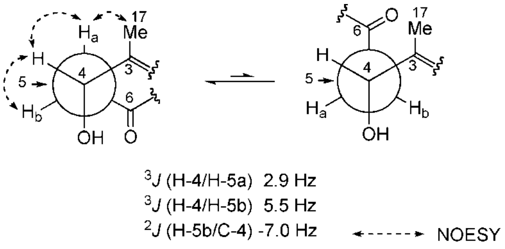


Figure 1. Rotation models for the C-4/C-5 bond of amphidinolide Q (**1**).

The relative stereochemistry for amphidinolide Q (**1**) was elucidated on the basis of the *J*-based configuration analysis (JBCA) method[3] and NOESY correlations. The geminal, vicinal, and long-range *J*(C,H) coupling constants were obtained by the resolution-enhanced ^{1}H spectrum, the HETLOC,[4] and *J*-IMPEACH-MBC[5] spectra in C_6D_6. A small value for ^3J(H-4/H-5a) and a large value for ^2J(H-5b/C-4) indicated that H-4/H-5a and 4-OH/H-5b were both *gauche* (Figure 1). A middle value for ^3J(H-4/H-5b) suggested that the C-4/C-5 bond existed as two conformers, while NOESY correlations for H-4/H-5a, H-4/H-5b, and H-5a/H_3-17 in C_6D_6 indicated that the C-4/C-5 bond existed mainly as a

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(1) (a) Kobayashi, J.; Kubota, T. *J. Nat. Prod.* **2007**, *70*, 451–460. (b) Kobayashi, J. *J. Antibiot.* **2008**, *61*, 271–284. (c) Takahashi, Y.; Kubota, T.; Kobayashi, J. *J. Antibiot.* **2007**, *60*, 376–379. (d) Oguchi, K.; Tsuda, M.; Iwamoto, R.; Okamoto, Y.; Kobayashi, J.; Fukushi, E.; Kawabata, J.; Ozawa, T.; Masuda, A.; Kitaya, Y.; Omasa, K. *J. Org. Chem.* **2008**, *73*, 1567–1570.

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10.1021/ol8013123 CCC: $40.75

conformer with *gauche* relationships for C-3/H-5a and H-4/H-5b. The relative stereochemistry of the C-7 to C-11 moiety in **1** was deduced from analyses of the HETLOC, *J*-IMPEACH-MBC, and NOESY spectra in C_6D_6. The middle values for ^3J(H-7/H-8b) and ^3J(H-9/H-10a) indicated that C-7/C-8 and C-9/C-10 bonds adapted two conformers, while NOESY cross-peaks of H-7/H-9, H-8a/H-10a, H-9/H-11, and H-10b/H_3-19 as well as small *J*-values for H-7/H-8a, H-8b/C-6, H-9/H-10b, and H-10a/C-19 allowed us to assign the major confomer for C-7/C-9 and C-9/C-10 bonds as shown in Figure 2a and 2c, respectively. NOESY correlations for H-7/H-9, H-8a/H-10a, H-9/H-11, and H-10b/H-11 and values for ^3J(H-9/H-8a), ^3J(H-9/H-8b), ^3J(H-8a/C-19), ^3J(H-11/H-10a), ^3J(H-11/H-10b), and ^2J(H-10b/C-11) revealed that C-8/C-9 and C-10/C-11 bonds existed as a single conformer as shown in Figure 2b and 2d, respectively.

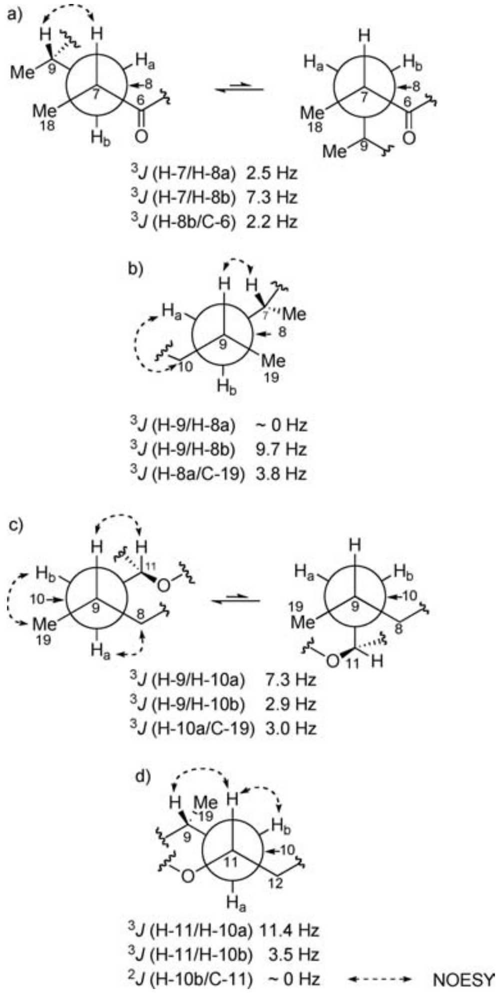

Figure 2. Rotation models for (a) C-7/C-8, (b) C-8/C-9, (c) C-9/C-10, and (d) C-10/C-11 bonds of amphidinolide Q (**1**).

Relative configurations at C-4 and C-7 were deduced from NOESY correlations for H-2/H-8a, H_3-17/H-5a, H-4/H_2-5, and H-5a/H-7 for **1** as shown in Figure 3.

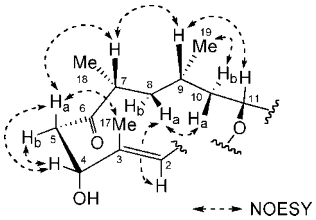

Figure 3. Selected NOESY correlations and relative stereochemistry for the C-2~C-11 portion of amphidinolide Q (**1**).

The relationship of C-11 and C-13 could not be deduced from the JBCA method due to the overlapping of signals for H-12a and H-12b in C_6D_6, whereas these signals were slightly separated in $CDCl_3$. A small value for ^3J(H-11/H-12a) and a large value for ^3J(H-11/H-12b) suggested a *gauche* relationship of H-11/H-12a and an *anti* relationship for H-11/H-12b, respectively. In addition, an *anti* relationship of H-12a/11-O deduced from a small *J*-value of H-12a/C-11 and NOESY correlations for H-11/H-12a and H-11/H-13 revealed that the C-11/C-12 bond existed as a single conformer as shown in Figure 4a. On the other hand, NOESY cross-peaks of H-11/H-13, H-12a/H-21a, and H-12b/H_3-20 as well as the large value for ^3J(H-13/H-12a) and middle values for ^3J(H-13/H-12b) and ^3J(H-12a/C-20) indicated that the C-12/C-13 bond existed as a pair of two conformers as shown in Figure 4b.

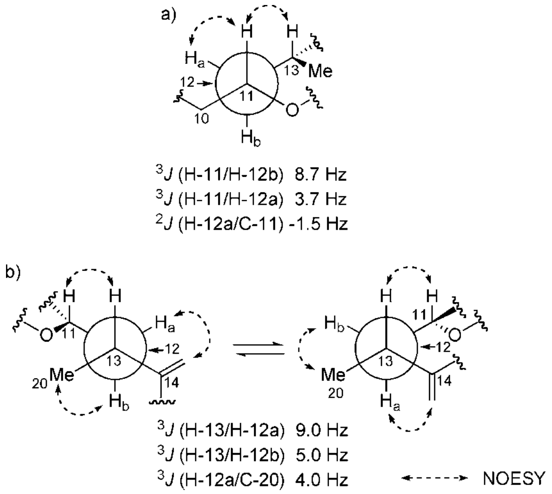

Figure 4. Rotation models for (a) C-11/C-12 and (b) C-12/C-13 bonds of amphidinolide Q (**1**).

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The molecular mechanics calculations of **1** were carried out by using Sybyl 6.5 (MMFF94 force-field).[6] One of the most stable confomers explained well the NOESY correations observed for **1** (Figure 5). Thus, the relative stereochemistry of amphidinolide Q (**1**) was elucidated as shown.

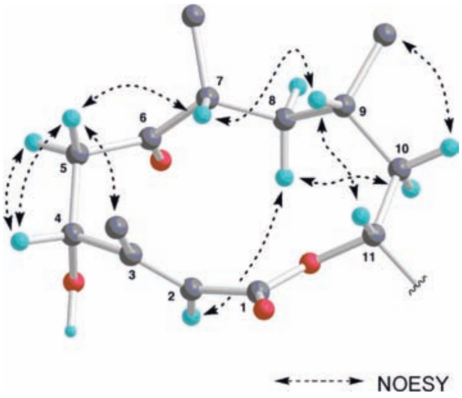
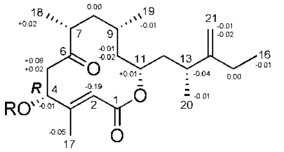


Figure 5. Selected NOESY correlations and a stable conformer for macrolactone ring of amphidinolide Q (**1**) obtained from the molecular mechanics calculations (hydrogen atoms of methyl groups were omitted).

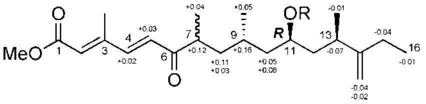


2a: R = (*S*)-MTPA
2b: R = (*R*)-MTPA

Figure 6. Δδ values [Δδ (in ppm) $= \delta_S - \delta_R$] obtained for the (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of amphidinolide Q (**1**).

To elucidate the absolute configurations of amphidinolide Q (**1**), a modified Mosher's method[7] was applied as follows. Treatment of **1** with (*R*)-(−)- and (*S*)-(+)-2-methoxy-2-trifluoro-2-phenylacetyl chloride (MTPACl) gave the (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of **1**. Δδ values (Δ$\delta = \delta_S - \delta_R$) obtained from ^{1}H NMR data of **2a** and **2b** are shown in Figure 6. The Δδ values for H_2-5 and H_3-18 are positive, while negative Δδ values are observed for H-2, H_2-10, H-13, H_3-16, H_3-17, H_3-19, H_3-20, and H_2-21.[8] These results indicated that the absolute configuration at C-4 was *R*.

The relative stereochemistry of **1** was elucidated as described above, while the absolute configuration at C-11 was elucidated directly by application of a modified Mosher method to the linear methyl ester derivatives of amphidinolide Q (**1**). Treatment of **1** with K_2CO_3 in MeOH yielded a mixture of two linear methyl esters generated by dehydration of C-4 and C-5 and epimerization of C-7. The mixture was treated with (*R*)- and (*S*)- MTPACl, and the resulting bis-(*S*)- and (*R*)-MTPA esters were obtained by HPLC purification. Δδ values for **3a** and **3b** suggested the absolute configuration at C-11 to be *R* (Figure 7). Thus, the absolute configurations at the five chiral centers in **1** were concluded to be 4*R*, 7*R*, 9*S*, 11*R*, and 13*R*, respectively.



3a: R = (*S*)-MTPA
3b: R = (*R*)-MTPA

Figure 7. Δδ values [Δδ (in ppm) $= \delta_S - \delta_R$] obtained for the (*S*)- and (*R*)-MTPA esters (**3a** and **3b**, respectively) of the linear methyl ester derivatives of amphidinolide Q (**1**).

Acknowledgment. The authors thank Ms. S. Oka, Center for Instrumental Analysis, Hokkaido University, for measurements of ESIMS. This work was partly supported by grants from a Takeda Science Foundation, a Mochida Memorial Foundation for Medical and Pharmaceutical Research, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Supporting Information Available: Experimental procedures and one- and two-dimensional NMR spectra for **1**, **2a**, **2b**, **3a**, and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8013123

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(8) A positive Δδ value for H-11 seems to be due to the anisotropic effect of the ester carbonyl group at C-1.