H- and C-nmr Spectral Investigation on Amphidinolide A, an Antileukemic Marine Macrolide

Jun'ichi Kobayashi, Masami Ishibashi, and Hiroshi Hirota

J. Nat. Prod., **1991**, 54 (5), 1435-1439• DOI:
10.1021/np50077a039 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 5, pp. 1435-1439, Sep-Oct 1991

^{1}H- AND ^{13}C-NMR SPECTRAL INVESTIGATION ON AMPHIDINOLIDE A, AN ANTILEUKEMIC MARINE MACROLIDE

JUN'ICHI KOBAYASHI,* MASAMI ISHIBASHI,

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

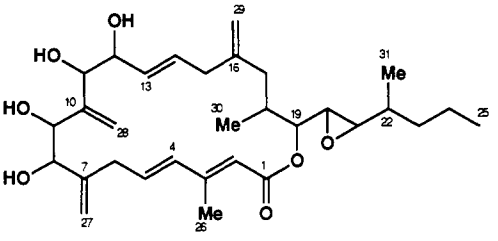
and HIROSHI HIROTA

Department of Chemistry, Faculty of Science, The University of Tokyo, Tokyo 113, Japan

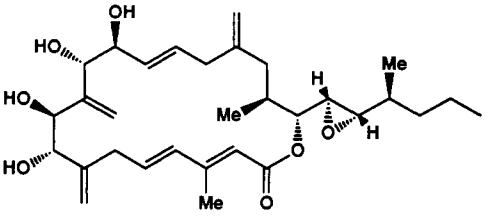
ABSTRACT.—The nmr spectra of amphidinolide A, an antileukemic 20-membered macrolide isolated from a cultured symbiotic marine dinoflagellate of the genus *Amphidinium*, were extensively investigated by utilizing 2D nmr techniques including ^{1}H-^{1}H COSY, NOESY, HMQC, and HMBC experiments to substantiate the previously proposed structure **1**. On the basis of the NOESY data a stereostructure **1a** of amphidinolide A was proposed.

Amphidinolide A [**1**] (1) is the first macrolide of a series of antileukemic compounds isolated from the laboratory-cultured marine dinoflagellate *Amphidinium* sp. which was symbiotically associated with the Okinawan flatworm *Amphiscolops* sp. (2–5). This isolation has demonstrated that symbiotic marine microorganisms are a new promising source of bioactive substances (6). Amphidinolide A [**1**], a 20-membered macrocyclic lactone, shows potent antileukemic activity and structural features of interest, including a contrast of hydrophilic and lipophilic moieties. This macrolide, therefore, has been a subject of total synthesis (7), though the stereochemistry of nine chiral centers in **1** remained undefined. Here we describe the details on the nmr spectral data of amphidinolide A [**1**] including the several types of 2D nmr techniques such as ^{1}H-^{1}H COSY, HMQC (8), HMBC (9), and phase-sensitive NOESY (10) experiments. On the basis of the NOESY results we wish to propose here a stereostructure **1a** of amphidinolide A [**1**].

The ^{1}H-^{1}H COSY spectra recorded in three solvents (C_6D_6, CD_3OD, and $CDCl_3$) clarified the complete proton-proton coupling pattern of amphidinolide A [**1**]. The ^{1}H-nmr chemical shifts and proton-proton coupling constants in the three solvents are presented in Tables



1



1a

1 and 2, respectively. Because the ^1H chemical shifts and the coupling constants did not show particular differences in the three solvents, amphidinolide A [**1**] was considered to adopt the same conformation in these solutions. These COSY spectra clearly revealed the proton connectivities from H-2 to H_3-25 and the positions of three methyl and three exomethylene groups. The chemical shift of H-19 [δ_H ($CDCl_3$) 4.72] indicated that the C-19 is the lactone-terminal position. The ^{13}C-nmr spectrum was obtained in $CDCl_3$ solution and unequivocally assigned on the basis of the HMQC and HMBC spectral data. The complete assignments of ^{13}C signals as well as the long-range ^1H-^{13}C long-range correlations observed through the HMBC spectrum are shown in Table 3. These results were fully consistent with the planar structure **1** for amphidinolide A. The previous assignments of ^{13}C-nmr signals by Kobayashi *et al.* (1), which were based on one-dimensional selective ^1H decoupling experiments, have been corrected as shown in Table 3.

The phase-sensitive NOESY spectra

TABLE 1. ^1H-nmr Chemical Shifts (ppm) of Amphidinolide A [**1**].

Proton	Solvent		
	C_6D_6	CD_3OD	$CDCl_3$
H-2	5.83 s	5.85 s	5.80 s
H-4	5.98 d	6.35 d	6.27 d
H-5	5.71 dd	6.14 ddd	6.09 ddd
H-6a	2.87 m (2H)	3.10 m (2H)	3.20 dd
H-6b			3.13 dd
H-8	4.38 br s	4.21 br s	4.42 br s
H-9	4.44 br s	4.46 br s	4.58 br s
H-11	3.89 br s	3.99 d	4.09 br s
H-12	3.98 br s	4.14 br s	4.22 br s
H-13	5.29 dd	5.46 dd	5.51 dd
H-14	5.65 ddd	5.59 ddd	5.69 dt
H-15a	2.63 dd	2.67 dd	2.76 br s (2H)
H-15b	2.56 dd	2.75 dd	
H-17a	2.36 dd	2.41 dd	2.35 dd
H-17b	1.88 dd	1.92 dd	1.92 dd
H-18	2.19 m	2.14 m	2.17 m
H-19	4.92 dd	4.69 dd	4.72 dd
H-20	2.79 dd	2.91 dd	2.85 dd
H-21	2.85 dd	2.75 dd	2.76 dd
H-22	1.24 m	1.37 m	1.38 m
H-23,-24	1.13–1.49 m (4H)	1.25–1.50 m (4H)	1.25–1.55 m (4H)
H-25 (3H)	0.85 t	0.91 t	0.91 t
H-26 (3H)	2.21 s	2.25 s	2.27 s
H-27a	5.45 s	5.27 t[d]	5.36 s
H-27b	5.01 br s	5.10 br s	5.21 s
H-28a	5.42 t[a]	5.40 t[e]	5.49 s
H-28b	5.15 d[b]	5.35 br s	5.37 s
H-29a	4.86 s	4.85 s	4.88 s
H-29b	4.78 d[c]	4.76 br s	4.79 s
H-30 (3H)	1.09 d	1.06 d	1.06 d
H-31 (3H)	0.83 d	0.92 d	0.93 d

[a] J = 1.3 Hz.
[b] J = 1.3 Hz.
[c] J = 1.5 Hz.
[d] J = 1.2 Hz.
[e] J = 1.3 Hz.

TABLE 2. ^{1}H-^{1}H Coupling Constants (J in Hz) of Amphidinolide A [**1**].

Protons	Solvent		
	C_6D_6	CD_3OD	$CDCl_3$
H-4,-5	15.6	15.4	15.6
H-5,-6a . . .	5.7	5.9	4.8
H-5,-6b . . .	8.9	9.0	8.8
H-6a,-6b . . .	[a]	[a]	14.6
H-8,-9	<1	<1	<1
H-11,-12 . . .	<1	2.8	<1
H-12,-13 . . .	4.4	5.5	4.1
H-13,-14 . . .	15.4	15.6	15.4
H-14,-15a . .	7.4	8.4	7.7
H-14,-15b . .	6.5	5.8	7.7
H-15a,-15b . .	14.4	14.3	[a]
H-17a,-17b . .	14.7	14.3	14.0
H-17a,-18 . .	6.2	5.8	5.5
H-17b,-18 . .	8.9	8.5	9.4
H-18,-19 . . .	3.1	3.2	3.4
H-18,-30 . . .	7.0	7.1	7.0
H-19,-20 . . .	6.8	5.7	5.9
H-20,-21 . . .	2.0	2.2	1.9
H-21,-22 . . .	7.5	7.4	[a]
H-22,-31 . . .	6.8	6.8	6.0
H-24,-25 . . .	7.3	7.2	6.8

[a]Not observable.

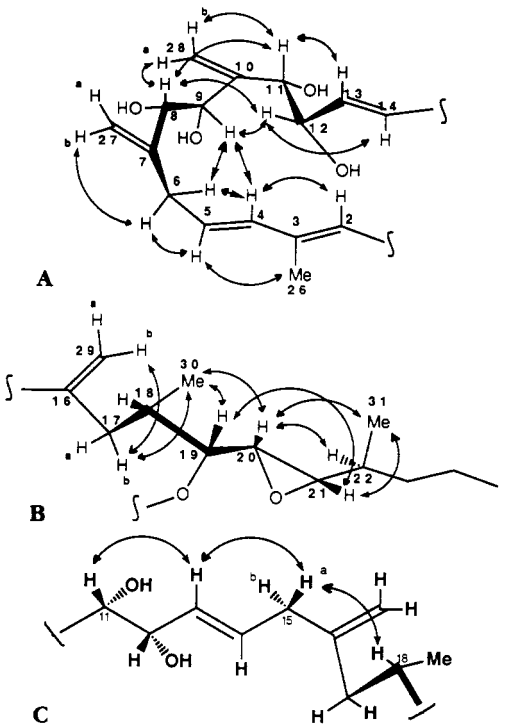


FIGURE 1. Segments **A**, **B**, and **C** with nOe correlations in arrows.

TABLE 3. ^{13}C-nmr Chemical Shifts and the HMBC Correlations of Amphidinolide A [**1**] in $CDCl_3$.[a,b]

Position	δ_C	Multiplicity	H coupled with C (HMBC correlations)
1	165.8	s	
2	118.6	d	H-4, H_3-26
3	152.7	s	H_3-26
4	136.3	d	H-2, H-6b, H_3-26
5	134.7	d	H-4, H-6b
6	39.0	t	H-4, H_2-27
7	144.8	s	H_2-6
8	72.5	d	H_2-27, H-6a
9	70.6	d	H_2-28
10	147.2	s	
11	75.8	d	H_2-28
12	73.5	d	H-14
13	130.5	d	H_2-15
14	130.9	d	H_2-15
15	39.7	t	H-14, H-17b, H_2-29
16	145.0	s	H_2-15, H_2-17
17	36.2	t	H_2-15, H_2-29, H_3-30
18	33.3	d	H_2-17, H_3-30
19	74.7	d	H-17b, H-20, H_3-30
20	54.2	d	H-19
21	61.8	d	H-22, H_3-31
22	35.3	d	H-21, H_3-31
23	36.7	t	H_3-25, H_3-31
24	19.9	t	H_3-25
25	14.2	q	
26	13.9	q	H-2, H-4
27	114.6	t	H_2-6
28	116.0	t	
29	112.8	t	H_2-15, H_2-17
30	14.9	q	H_2-17
31	15.8	q	

[a]Previously (1), the ^{13}C signals for C-9 and C-19, C-10 and C-16, C-11 and C-12, C-13 and C-14, and C-30 and C-31 were inversely assigned, respectively.

[b]"s", "d", "t", and "q" denote singlet, doublet, triplet, and quartet, respectively. Multiplicities were determined by DEPT experiments.

of amphidinolide A [**1**] were recorded in the three solvents (C_6D_6, CD_3OD, and $CDCl_3$) and the observed NOESY cross-peaks are listed in Table 4. Although interpretation of the relative stereochemistry of chiral centers of macrocyclic compounds by spectral means is still not easy, some suggested information for the relative configurations may be provided by the nOe data (11,12). We propose here a three-dimensional drawing **1a** of amphidinolide A that contains the relative configurations of the nine chiral centers of **1**, that most sufficiently satisfy the NOESY data described in Table 4.

TABLE 4. NOe Correlations Observed through Phase-Sensitive NOESY Spectra.

Proton	Solvent		
	C_6D_6	CD_3OD	$CDCl_3$
H-2	4	4	4, 15, 17a, 19
H-4	2, 6, 9	2, 6, 9	2, 6b, 9
H-5	6, 26	6, 26	6a, 8, 26
H-6 (2H)	4, 5, 9, 27b	4, 5, 9, 27b	(a) 5, 27b (b) 4, 9, 27b
H-8	11, 12, 28a	11, 12, 28a	5, 9, 11, 12, 27a, 28a
H-9	4, 6, 12, 28a	4, 6, 11, 12, 28a	4, 6b, 8, 11, 12, 27a, 28a
H-11	8, 13, 28b	8, 9, 13, 28b	8, 9, 12, 13, 28a, 28b
H-12	8, 9, 14	8, 9, 14	8, 9, 11, 13, 14, 28a, 28b
H-13	11	11	11, 12, 14, 15
H-14	12	12	12, 13, 15, 17a
H-15a	18, 29a	18	(2H) 2, 13, 14, 29a
H-15b	29a		
H-17a			2, 14, 17b, 19
H-17b	20, 29b, 30	29b, 30	17a, 20, 29b, 30
H-18	15a, 29b	15a, 29b	19, 21, 29b, 30
H-19	21, 30	21, 30	2, 17a, 18, 20, 21, 30
H-20	17b, 22, 30, 31	22, 30, 31	17b, 19, 22, 30, 31
H-21	19, 31	19, 31	18, 19, 31
H-22	20	20	20
H-23,-24 (4H)			
H-25 (3H)			
H-26	5	5	5
H-27a			8, 9
H-27b	6	6	6a, 6b
H-28a	8, 9	8,9	8, 9, 11, 12
H-28b	11	11	11, 12
H-29a	15a, 15b		15
H-29b	17b, 18	17b, 18	17b, 18, 30
H-30 (3H)	17b, 19, 20	17b, 19, 20	17b, 18, 19, 20, 29b
H-31 (3H)	20, 21	20, 21	20, 21

In Figure 1, three partial segments **A**, **B**, and **C** with NOESY correlations in arrows are shown to support the stereostructure **1a**.

These NOESY results of amphidinolide A [**1**] might be helpful as basic data for making a working hypothesis of total synthesis of this macrolide and also for consideration of the structure-activity relationships.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded on a Bruker AM-500 spectrometer. The sample was prepared in 5 mm tubes. Amphidinolide A [**1**] (1) (0.9 mg) was used, and spectra were internally referenced to the solvent resonances.

The phase-sensitive NOESY spectra were obtained by using standard pulse sequences (10) at 300K. The original 512 × 1024 data matrix were used for a 4400 Hz spectral width, and the mixing time was 500 msec.

LITERATURE CITED

1. J. Kobayashi, M. Ishibashi, H. Nakamura, Y. Ohizumi, T. Yamasu, T. Sasaki, and Y. Hirata, *Tetrahedron Lett.*, **27**, 5755 (1986).
2. M. Ishibashi, Y. Ohizumi, M. Hamashima, H. Nakamura, Y. Hirata, T. Sasaki, and J. Kobayashi, *J. Chem. Soc., Chem. Commun.*, 1127 (1987).
3. J. Kobayashi, M. Ishibashi, M.R. Wälchli, H. Nakamura, Y. Hirata, T. Sasaki, and Y. Ohizumi, *J. Am. Chem. Soc.*, **110**, 490 (1988).
4. J. Kobayashi, M. Ishibashi, H. Nakamura, Y. Ohizumi, T. Yamasu, Y. Hirata, T. Sasaki, T. Ohta, and S. Nozoe, *J. Nat. Prod.*, **52**, 1036 (1989).
5. J. Kobayashi, M. Ishibashi, T. Murayama,

M. Takamatsu, M. Iwamura, Y. Ohizumi, and T. Sasaki, *J. Org. Chem.*, **55**, 3421 (1990).
6. J. Kobayashi, *J. Nat. Prod.*, **52**, 225 (1989).
7. S.J. O'Connor and P.G. Willard, *Tetrahedron Lett.*, 30, 4637 (1989).
8. A. Bax and S. Subramanian, *J. Mag. Reson.*, **67**, 565 (1986).
9. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
10. G. Bodenhausen, H. Kogler, and R.R. Ernst, *J. Mag. Reson.*, **58**, 370 (1984).
11. J.-M. Lancelin and J.-M. Beau, *J. Am. Chem. Soc.*, **112**, 4060 (1990).
12. P. Sowinski, P. Gariboldi, J.K. Pawlak, and E. Borowski, *J. Antibiot.*, **42**, 1639 (1989).

Received 5 March 1991