Biosynthetic Study of Amphidinolide B

Masashi TSUDA, Takaaki KUBOTA, Yusuke SAKUMA, and Jun'ichi KOBAYASHI*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060–0812, Japan.
Received June 7, 2001; accepted July 10, 2001

The biosynthetic origins of amphidinolide B (1) were investigated on the basis of ^{13}C-NMR data of ^{13}C-enriched samples obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in cultures of a dinoflagellate *Amphidinium* sp. These incorporation patterns suggested that 1 was generated from three successive polyketide chains, an isolated C_1 unit from C-2 of acetates, six branched C_1 units from C-2 of acetates, and an "m–m" and an "m–m–m" unit derived only from C-2 of acetates. The labeling patterns of amphidinolide B (1) were different from those of amphidinolide H (2), a 26-membered macrolide closely related to 1.

Key words biosynthesis; marine dinoflagellate; macrolide

Marine dinoflagellates are a rich source of secondary metabolites with unique structures and interesting biological activities. Amphidinolides are a series of macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of the Okinawan marine acoel flatworms *Amphiscolops* spp.[1] Amphidinolides B[2—5] (**1**) and H[6,7] (**2**) (Chart 1) are both 26-membered macrolides first isolated from strains Y-5 and Y-25, respectively, of the dinoflagellate. Their structures including the absolute stereochemistry are similar to each other, and the only different point is the position of a hydroxyl group, which is attached to C-16 in **1**, while it is on C-26 in **2**. Previously we have reported biosynthetic studies of amphidinolide H (**2**) using strain Y-72 of the genus *Amphidinium*, which produces a relatively large amount of **2**.[8] The incorporation patterns obtained by feeding experiments with ^{13}C-labeled acetates suggested that **2** was generated from three unusual C_2 units derived only from C-2 of acetates in addition to three successive polyketide chains. However, biosynthesis of amphidinolide B (**1**) remained to be investigated. Recently a strain Y-71 of the genus *Amphidinium* was revealed to produce a relatively large amount of amphidinolide B (**1**), and acetate incorporation patterns of amphidinolide B (**1**) were investigated using the Y-71 strain to find that the incorporation patterns were different from those of amphidinolide H (**2**).

The dinoflagellate *Amphidinium* sp. (strain Y-71) was cultured in a 50 l nutrient-enriched seawater medium, and feeding experiments were carried out with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates. In the feeding experiments, the dinoflagellate was supplemented with 610 μM of labeled precursors in one portion at 7 d after inoculation, and then the culture was harvested by centrifugation after 14 d. In each case the methanol–toluene extracts of the harvested cells were purified on a silica gel column followed by C_{18} HPLC to afford ^{13}C-labeled amphidinolide B (**1**) in 0.0015% yield on average from the wet weight of the cells.

Assignments of ^{13}C-NMR signals and isotope incorporation results of amphidinolide B (**1**) are presented in Table 1. The ^{13}C-NMR spectrum ($CDCl_3$) of **1** derived from [1-^{13}C] sodium acetate showed significant enrichment of 10 carbons (C-3, C-5, C-7, C-9, C-11, C-13, C-16, C-18, C-23, C-25). On the other hand, enrichment by [2-^{13}C] sodium acetate was observed for 22 carbons (C-1, C-2, C-4, C-6, C-8, C-10, C-12, C-14, C-15, C-17, C-19, C-20, C-21, C-22, C-24, C-26, C-27, C-28, C-29, C-30, C-31, C-32). The ^{13}C–^{13}C correlations observed in the (Incredible Natural Abundance Double Quantum Transfer Experiment) spectra of **1** labeled with [1,2-$^{13}C_2$] sodium acetate showed that 10 acetate units were directly incorporated for C-3/C-4, C-5/C-6, C-7/C-8, C-9/C-10, C-11/C-12, C-13/C-14, C-16/C-17, C-18/C-19, C-23/C-24, and C-25/C-26 (Fig. 1). Two irregular labeling patterns derived only from C-2 of acetates were observed for C-1–C-2 (m–m) and C-20—C-22 (m–m–m), and an isolated C_1 unit from C-2 of acetates was observed for C-15. Six C_1 branches of C-27, C-28, C-29, C-30, C-31, and C-32 were all derived from C-2 of acetates, in which the carbonyl carbons were lost. Three portions of C-3—C-14, C-16—C-19, and C-23—C-26 were likely classical polyketide chains derived from six, two, and two acetate units, respectively.

On the other hand, the incorporation patterns of amphidinolide H (**2**) (Fig. 1) have been reported to be generated from three successive polyketide chains and three unusual "m–m" units derived only from C-2 of acetates.[8] The C-16—C-20 portion of amphidinolide H (**2**) is labeled as "m(m)–c–m(O)–c–m(O)", while the labeling pattern of the C-16—C-20 moiety in amphidinolide B (**1**) was revealed to be "c(m)(O)–m–c(O)–m–m(O)" containing a diketide in the C-16—C-19 portion. Furthermore, C-16 and C-18 in **1** were labeled by both C-1 of acetate, whereas those in **2** were labeled by both C-2 of acetate. The vicinally located one-carbon branches [C-15(C-30)–C-16(C-31)] of **1** were labeled as "m(m)–c(m)", which corresponds to the labeling patterns of the C-3(C-21)–C-4(C-22) portion in amphidinolide J[9] and C-8(=CH_2)–C-9(–CH_3) portion of goniodomin A.[10] However, the vicinally located one-carbon branches of amphidinolides C[11] and H[8] (**2**) were labeled as "m(m)–m(m)". Though the unusual labeling patterns such as "m–m" in **1** and **2** may be explained by the Favorski-type or Tiffeneau–Demjanov reaction proposed by Wright *et al.*[12] or Rawlings,[13] respectively, experimental evidences seem to be essential for its application.

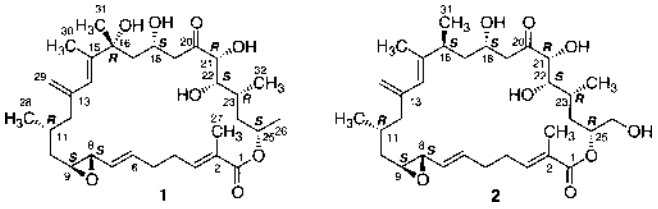

Chart 1. Structures of Amphidinolides B (**1**) and H (**2**)

* To whom correspondence should be addressed. e-mail: jkobay@pharm.hokudai.ac.jp

Experimental

General Methods The NMR samples of ^{13}C-labeled **1** were prepared in 2.5 mm micro cells for $CDCl_3$ (Shigemi Co., Ltd., Japan) by dissolving 2.5 mg each in 99.98% $CDCl_3$ 100 μl, while for the sample of unlabeled **1**, 8 mg in 99.98% $CDCl_3$ 100 μl was used. All ^{13}C-NMR spectra were recorded using the pulse sequence 'zgpg' on a Bruker AMX-600 spectrometer, sweep widths were 35700 Hz, and numbers of scans were 8000. INADEQUATE spectra were obtained by a Bruker 'inadsy' pulse sequence. The repetition delay and the delay for creating antiphase C–C magnetization ($1/2J_{CC}$) were 2.0 s and 11.4 ms, respectively. The F_1 and F_2 spectral widths were both 25000 Hz. For each 256 t_1 increments, 32 transients (with four dummy scans) were accumulated in 2K data points. Zero-filling to 512 points for F_1 and multiplication with unshifted sine-bell windows were performed in both dimensions prior to two-dimensional Fourier transformation. The resulting data matrix was 2K×512. The total measuring time was *ca*. 10 h.

Table 1. Isotope Incorporation Results Based on ^{13}C-NMR Data of Amphidinolide B (**1**)[a]

Positn.	δ_C	Intensity ratio (labeled/unlabeled)[b]		
		[1-^{13}C]-acetate	[2-^{13}C]-acetate	Assignment c or m[c]
1	167.77 s	1.07	3.41	m
2	128.44 s	1.07	2.76	m
3	140.04 d	3.05	1.10	c
4	26.87 t	1.20	2.94	m
5	30.92 t	3.13	1.01	c
6	135.45 d	1.12	3.51	m
7	128.60 d	3.05	1.00	c
8	60.11 d	1	3.67	m
9	59.43 d	3.51	1	c
10	39.51 t	1.09	3.60	m
11	29.26 d	3.05	1.08	c
12	46.96 t	1.17	3.23	m
13	144.47 s	2.54	1.01	c
14	124.37 d	1.21	2.69	m
15	143.16 s	1.26	3.40	m
16	76.01 s	2.49	1.13	c
17	45.36 t	1.05	3.44	m
18	66.58 d	2.58	1.07	c
19	45.97 t	0.87	2.80	m
20	212.49 s	1.07	3.11	m
21	77.84 d	1.03	2.65	m
22	75.62 d	0.89	2.82	m
23	33.27 d	3.23	1.03	c
24	39.45 t	1.24	3.52	m
25	68.46 d	3.42	1.12	c
26	21.04 q	1.11	3.65	m
27	12.48 q	1.26	3.86	m
28	18.33 q	1.21	3.91	m
29	114.89 t	1.10	2.86	m
30	15.71 q	1.26	3.61	m
31	28.40 q	0.93	3.17	m
32	15.10 q	1.10	3.78	m

a) The ^{13}C-NMR spectra were recorded in $CDCl_3$ solution. *b*) Intensity of each peak in the labeled **1** devided by that of the corresponding signal in the unlabeled **1**, respectively, normalized to give a ratio of **1** for unenriched peak (C-8 for [1-^{13}C]-acetate labeling and C-9 for [2-^{13}C]-acetate labeling). *c*) c denotes the carbon derived from C-1 of acetate, while m indicates the carbon derived from C-2 of acetate.

General Feeding Experiments of ^{13}C-Labeled Precursors The dinoflagellate cultured in a 50 l nutrient-enriched seawater medium was supplemented with [1-^{13}C], [2-^{13}C], or [1,2-$^{13}C_2$] sodium acetate (610 μM) in one portion at 7 d after inoculation, and then the culture was harvested by centrifugation after 14 d to obtain cells of the dinoflagellate (80 g as an average, wet weight). Extraction and isolation of amphidinolide B (**1**) from the harvested cells were carried out by the same procedure as described previously.[2] The ^{13}C-labeled amphidinolide B (**1**) was obtained in 0.0015% yield as an average from wet weight of the cells.

Acknowledgments We thank Professor T. Yamasu, University of the Ryukyus, and Professor M. Ishibashi, Chiba University, for help with dinoflagellate collection. This work was partly supported by a Grant-in-Aid from the Japan Securities Scholarship Foundation, a Grant-in-Aid from the Uehara Memorial Foundation, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References and Notes

1) Ishibashi M., Kobayashi J., *Heterocycles*, **44**, 543—572 (1997).
2) Ishibashi M., Ohizumi Y., Hamashima M., Nakamura H., Hirata Y., Sasaki T., Kobayashi J., *J. Chem. Soc*., *Chem. Commun*., **1987**, 1127—1129.
3) Kobayashi J., Ishibashi M., Nakamura H., Ohizumi Y., Yamasu T., Hirata, Y., Sasaki T., Ohta T., Nozoe S., *J. Nat. Prod*., **52**, 1036—1041 (1989).
4) Bauer I., Maranda L., Shimizu Y., Peterson R. W., Cornell, L., Steiner J. R., Clardy J., *J. Am. Chem. Soc*., **116**, 2657—2658 (1994).
5) Ishibashi M., Ishiyama H., Kobayashi J., *Tetrahedron Lett.*, **35**, 8241—8242 (1994).
6) Kobayashi J., Shigemori H., Ishibashi M., Yamasu T., Hirota H., Sasaki T., *J. Org. Chem*. **56**, 5221—5224 (1991).
7) Kobayashi J., Shimbo K., Sato M., Shiro M., Tsuda M., *Org. Lett*., **2**, 2805—2807 (2000).
8) Sato M., Shimbo K., Tsuda M., Kobayashi J., *Tetrahedron Lett*., **41**, 503—506 (2000).
9) Kobayashi J., Takahashi M., Ishibashi M., *J. Chem. Soc*., *Chem. Commun*., **1995**, 1639—1640.
10) Murakami M., Okita Y., Matsuda H., Okino T., Yamaguchi K., *Phytochemistry*, **48**, 85—88 (1998).
11) Kubota T., Tsuda M., Kobayashi J., *Tetrahedron*, **57**, 5955—5957 (2001).
12) Wright J. L. C., Hu T., Mclachlan J. L., Needaham J., Walter J. A., *J. Am. Chem. Soc*., **118**, 8757—8758 (1996).
13) Rawlings B. J., *Nat. Prod. Rep*., **16**, 425—584 (1999).

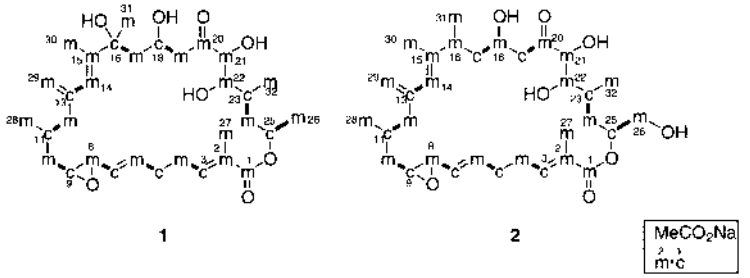

Fig. 1. Labeling Patterns of Amphidinolides B (**1**) and H (**2**) Resulting from Feeding Experiments with ^{13}C-Labeled Acetates