Biosynthetic Study of Amphidinolide W

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The biosynthetic origins of amphidinolide W (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 acetate in cultures of a strain Y-42 of the dinoflagellate *Amphidinium* sp. These incorporation patterns suggested that 1 was generated from a hexaketide chain, two acetate units, four isolated C_1 units from C-2 of acetates, and four branched C_1 units from C-2 of acetates. The acetate-incorporation patterns for C-1–C-2–(C-21) and C-8–C-18–(C-23, C-24) of 1 corresponded well to those for C-1–C-2–(C-27) and C-5–C-15–(C-28, C-29) of amphidinolide H (2) isolated from this strain.

Key words biosynthesis; marine dinoflagellate; macrolide

Marine dinoflagellates have been recognized as a rich source of polyketides possessing interesting biological activity.[1—3] Most of dinoflagellate-derived polyketides possess unique and complex structures. Amphidinolides are a group of structurally-unique macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms *Amphiscolops* spp.[4,5] Amphidinolide W[6] (**1**), which was recently isolated from a strain Y-42 of the genus *Amphidinium* dinoflagellate, is the first macrolide without an exomethylene unit among the all amphidinolides obtained so far. During our continuing studies of biosynthesis of polyketides from dinoflagellates of the genus *Amphidinium*,[7,8] biosynthetic origins of amphidinolide W (**1**) were investigated by ^{13}C-NMR data of the ^{13}C-enriched samples obtained by feeding experiments with ^{13}C-lableled acetates in culture of the Y-42 strain of *Amphidinium* sp. Here we describe unusual labeling patterns of **1** with acetates.

The dinoflagellate *Amphidinium* sp. (strain Y-42) was cultured in a 100 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 acetate. In feeding experiments, the dinoflagellate was supplemented with 610 μM of labeled precursors in one portion at 4 d after inoculation, and then the culture was harvested by centrifugation after 14 d. In each case, the extracts of the harvested cells were chromatographed by a silica gel column to give a macrolide-containing fraction, which was treated with trimethylsilyldiazomethane and then subjected to silica gel column chromatography to separate a large amount of fatty acid-related compounds. The fraction including macrolides was purified by C_{18} HPLC to afford ^{13}C-labeled amphidinolide W (**1**) in 0.003% yield as an average from wet weight of the cells.

Assignments of isotope incorporation results of **1** derived from ^{13}C-labeled sodium acetate were shown in Table 1. The ^{13}C-NMR spectrum ($CDCl_3$) of **1** derived from [1-^{13}C] sodium acetate showed significant enrichment of 8 carbons (C-3, C-6, C-8, C-10, C-12, C-14, C-16, C-19). On the other hand, enrichment by [2-^{13}C] sodium acetate was observed for 16 carbons (C-1, C-2, C-4, C-5, C-7, C-9, C-11, C-13, C-15, C-17, C-18, C-20, C-21, C-22, C-23, C-24). Thus, the all 24 carbon signals contained in amphidinolide W (**1**) were shown to be labeled by acetates (Fig. 1). The ^{13}C–^{13}C correlations observed in the Incredible Natural Abundance Double Quantum Transfer Experiment (INADEQUATE) spectra of **1** labeled with [1,2-$^{13}C_2$] sodium acetate showed that 8 acetate units were directly incorporated for C-3/C-4, C-6/C-7, C-8/C-9, C-10/C-11, C-12/C-13, C-14/C-15, C-16/C-17, and C-19/C-20. The four C_1 branches at C-21, C-22, C-23, and C-24 were all derived from C-2 of acetates, in which the carbonyl carbons were lost. The C-6–C-17 portion was likely classical polyketide chains derived from six acetate units. Therefore, the incorporation patterns suggested that amphidinolide W (**1**) was a unique non-successive mixed polyketide

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

Positn.	δ_C	Intensity ratio (labeled/unlabeled)[b]		
		[1-^{13}C]-Acetate	[2-^{13}C]-Acetate	Assignment c or m[c]
1	175.31 s	0.81	2.03	m
2	39.36 d	1.13	2.11	m
3	25.86 t	5.68	1.53	c
4	35.97 t	1	2.09	m
5	212.79 s	0.92	3.40	m
6	45.80 d	4.55	1	c
7	32.32 t	0.89	3.00	m
8	32.39 t	4.55	1.64	c
9	138.24 d	0.78	3.12	m
10	127.30 d	5.34	1.65	c
11	79.03 d	0.85	2.42	m
12	70.63 d	4.38	1.26	c
13	41.01 d	0.86	3.21	m
14	28.86 d	3.86	0.98	c
15	135.54 d	0.88	2.68	m
16	133.54 s	3.86	1.65	c
17	133.80 d	0.93	2.06	m
18	129.81 d	0.93	2.25	m
19	26.50 t	3.70	1.44	c
20	13.86 q	0.99	3.21	m
21	16.47 q	1.48	2.71	m
22	18.53 q	1.02	3.39	m
23	21.76 q	1.01	3.60	m
24	12.70 q	1.06	2.81	m

a) The ^{13}C-NMR spectra were recorded in $CDCl_3$ solution at 150 MHz with sweep width of 35700 Hz using 'zgpg'. Numbers of scans for unlabeled and labeled **1** were 10000 and 2000, respectively. *b*) Intensity of each peak in the labeled **1** divided by that of the corresponding signal in the unlabeled **1**, normalized to give a ratio of 1 for unenriched peak (C-4 for [1-^{13}C]-acetate labeling and C-6 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.

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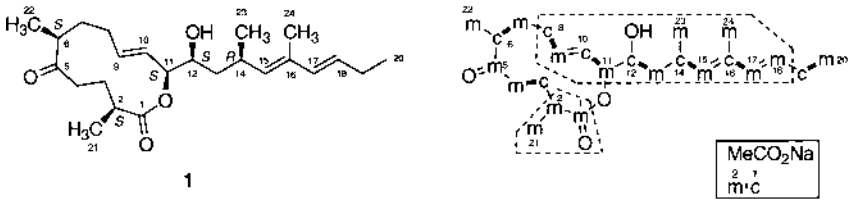

Fig. 1. Structure and Labeling Patterns of Amphidinolide W (**1**) Resulting from Feeding Experiments with ^{13}C-Labeled Acetates
Labeling patterns within dotted line corresponding to those of amphidinolide H (**2**) as shown in Fig. 2.

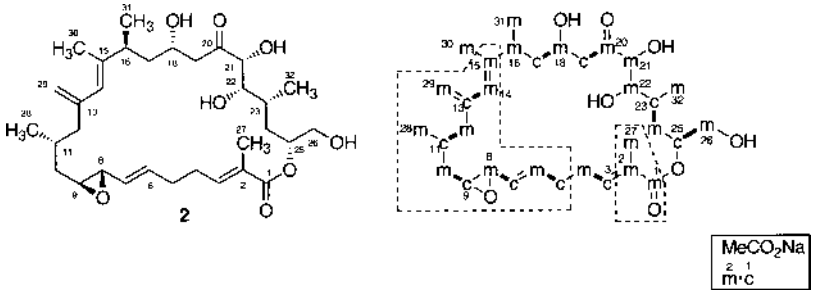

Fig. 2. Structure and Labeling Patterns of Amphidinolide H (**2**) Resulting from Feeding Experiments with ^{13}C-Labeled Acetates[11]
Labeling patterns within dotted line corresponding to those of amphidinolide W (**1**) as shown in Fig. 1.

consisting of a hexaketide chain, two acetate units, four isolated C_1 units from C-2 of acetates, and four branched C_1 units from C-2 of acetates.

From the dinoflagellate *Amphidinium* (strain Y-42), amphidinolide W (**1**) in addition to amphidinolide G and H[9,10] (**2**) and their related macrolides were isolated previously.[5] The acetate-incorporation patterns for C-1–C-2–(C-21) and C-8–C-18–(C-23, C-24) of **1** corresponded well to those for C-1–C-2–(C-27) and C-5–C-15–(C-28, C-29) of **2**. This observation suggests that amphidinolide W (**1**) may be biogenetically related to amphidinolides G and H (**2**).[11]

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% $CDCl_3$ 40 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 2000. INADEQUATE spectra were obtained by a Bruker 'inadsy' pulse sequence.

General Feeding Experiments of ^{13}C-Labeled Precursors The dinoflagellate cultured in a 100 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 4 d after inoculation, and then the culture was harvested by centrifugation after 14 d to obtain cells of the dinoflagellate (70 g as an average, wet weight). The harvested cells were extracted with MeOH/toluene (3 : 1, 400 ml×3). After addition of 1 M NaCl aq. (200 ml), the mixture was extracted with toluene (200 ml×3). The toluene-soluble fractions were evaporated under reduced pressure to give a residue, which was subjected to a silica gel column ($CHCl_3$/MeOH, 98 : 2) and then a Sep-Pak cartridge C_{18} (MeOH/H_2O, 8 : 2→MeOH). The fraction eluted with MeOH/H_2O (8 : 2) was treated with 2 M trimethylsilyldiazomethane in hexane (2 ml) at room temperature for 5 h, and the mixture was passed through a silica gel column (hexane/EtOAc, 2 : 1). The fraction containing amphidinolide W (**1**) was purified by C_{18} HPLC [Mightysil RP-18, 5 μm, Kanto Chemical Co., Inc., 10×250 mm; eluent, CH_3CN/H_2O (85 : 15); flow rate, 3 ml/min; UV detection at 220 nm] to afford amphidinolide W (**1**, t_R 15.4 min). The ^{13}C-labeled amphidinolide W (**1**) was obtained in 0.003% yield as an average from wet weight of the cells.

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