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Biosynthetic study of amphidinolide C

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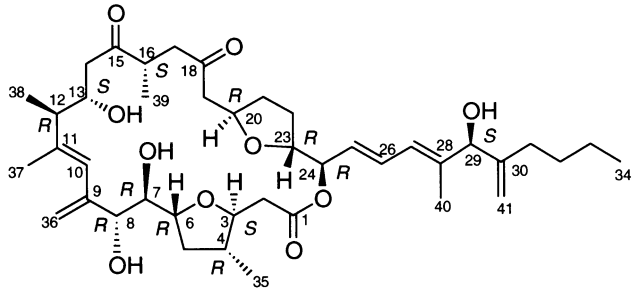
Abstract—The biosynthetic origins of amphidinolide C (**1**) were investigated on the basis of ^{13}C NMR data of ^{13}C enriched samples obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in cultures of a dinoflagellate *Amphidinium* sp. These incorporation patterns suggested that amphidinolide C (**1**) was generated from four diketide chains, four acetate units, five isolated C_1 unit from C-2 of acetates, seven branched C_1 units from C-2 of acetates, and a '**m**–**m**' and a '**m**–**m**–**m**' units derived only from C-2 of acetates.

1. Introduction

Marine dinoflagellates have been recognized as a rich source of secondary metabolites possessing unique structures and interesting bioactivity.[1] Amphidinolides C[2,3] (**1**) and F,[4] first isolated from dinoflagellates *Amphidinium* sp. (Y-5 and Y-26 strains, respectively), are unique 25-membered macrolides having two tetrahydrofuran rings and vicinally located one-carbon branches. Particularly, amphidinolide C (**1**) exhibited potent cytotoxicity against tumor cells. During our search for bioactive and structurally unique secondary metabolites from marine dinoflagellates,[5] a strain (Y-71) of the genus *Amphidinium* producing relatively large amount of amphidinolides C (**1**), B,[6–8] and T1,[9] has been recently separated from the inside cells of a marine acoel flatworm *Amphiscolops* sp. collected off Sunabe, Okinawa. The biosynthetic origins of amphidinolide C (**1**) were investigated by ^{13}C NMR data of the ^{13}C-enriched samples obtained by feeding experiments with ^{13}C-labeled acetates in culture of the Y-71 strain of *Amphidinium* sp. Here we describe unusual labeling patterns of **1** with acetates.

2. Results and discussion

The dinoflagellate *Amphidinium* sp. (strain Y-71) 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 7 days after inoculation, and then the culture was harvested by centrifugation after 14 days. In each case the extracts of the harvested cells were purified by a silica gel column followed by C_{18} HPLC to afford ^{13}C-labeled amphidinolide C (**1**) (Chart 1) in 0.0008% yield from as an average from wet weight of the cells.



1

Chart 1. Structure of amphidinolide C (**1**).

Assignments of ^{13}C NMR signals and isotope incorporation results of **1** derived from ^{13}C-labeled sodium acetate were shown in Table 1 and Fig. 1. The ^{13}C NMR spectra ($CDCl_3$ and C_6D_6) of **1** derived from [1-^{13}C] sodium acetate showed significant enrichment of 12 carbons (C-1, C-4, C-7, C-9, C-13, C-16, C-18, C-21, C-24, C-26, C-31, and C-33). On the other hand, enrichment by [2-^{13}C] sodium acetate was observed for 29 carbons (C-2, C-3, C-5, C-6, C-8, C-10, C-11, C-12, C-14, C-15, C-17, C-19, C-20, C-22, C-23, C-25, C-27, C-28, C-29, C-30, C-32, C-34, C-35, C-36, C-37, C-38, C-39, C-40, and C-41). Thus all the 41 carbon signals contained in **1** were shown to be labeled by acetates. The ^{13}C NMR spectrum of **1** labeled with [1,2-$^{13}C_2$] sodium acetate showed enriched carbon signals flanked by two strong satellite signals. One-bond J_{CC} coupling constants measured in benzene-d_6 indicated the definite incorporation of 12 acetate units for C-1/C-2 (58.3 Hz), C-4/C-5 (32.7 Hz), C-7/C-8 (39.2 Hz), C-9/C-10 (54.5 Hz), C-13/C-14 (38.7 Hz),

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Table 1. Isotope incorporation results based on the ^{13}C NMR data of amphidinolide C (**1**)

Position	δ_C	Intensity ratio (labeled/unlabeled)[a]		δ_C	Intensity ratio (labeled/unlabeled)[b]		Assignment c or m[c]
		[1-^{13}C]-Acetate	[2-^{13}C]-Acetate		[1-^{13}C]-Acetate	[2-^{13}C]-Acetate	
1	171.09 s	2.69	0.92	171.09 s	2.89	1.18	c
2	38.61 t	1.47	4.28	39.22 t	0.80	2.62	m
3	81.17 d	1.44	3.82	81.77 d	0.78	3.12	m
4	30.68 d	3.56	1.04	40.22 d	3.01	1.33	c
5	36.64 t	1.56	4.05	37.04 t	0.90	2.86	m
6	78.60 d	1.61	3.96	79.17 d	0.83	3.05	m
7	76.18[d] d			76.54 d	2.89	1.09	c
8	76.73[d] d			77.73 d	0.82	3.38	m
9	144.28 s	3.50	1	145.98 s	2.01	0.75	c
10	124.35 d	1.60	4.07	125.16 d	0.94	3.05	m
11	139.88[e] s			140.66 s	0.82	2.49	m
12	48.93 d	1.18	2.96	49.25 d	0.75	2.52	m
13	70.53 d	4.02	1.15	71.07 d	2.84	0.92	c
14	45.24 t	1.36	4.09	45.85 t	0.70	3.02	m
15	213.74 s	1	2.52	213.27 s	1	3.42	m
16	42.43 d	4.43	1.12	42.44 t	2.74	1	c
17	45.88 t	1.26	3.74	46.39 t	1.01	3.16	m
18	207.80 s	3.08	1.01	207.45 s	2.42	1.29	c
19	48.23 t	1.51	4.09	48.73 t	0.90	2.68	m
20	74.88 d	1.36	3.88	75.71 d	1.04	3.27	m
21	31.75 t	3.87	1.19	32.28 t	2.94	0.96	c
22	28.05 t	1.39	3.44	28.31 t	0.94	2.90	m
23	79.43[f] d			80.04 d	0.78	2.75	m
24	77.14[d] d			77.25 d	2.82	1.07	c
25	127.06 d	1.36	4.01	128.53[d] d			m
26	130.67 d	4.41	1.22	131.13 d	2.29	0.85	c
27	124.84 d	1.44	3.57	125.50 d	0.92	2.98	m
28	140.08[e] s			140.92 s	0.82	2.98	m
29	79.59[f] d			79.90 d	0.96	2.45	m
30	148.95 s	0.53	2.00	149.43 s	0.90	2.23	m
31	31.25 t	3.13	0.85	31.74 t	2.92	0.98	c
32	29.91 t	0.74	2.09	30.42 t	1.18	3.11	m
33	22.28 t	2.77	0.73	22.80 t	3.87	1.04	c
34	13.78 q	0.77	2.32	14.17 q	0.88	2.68	m
35	15.32 q	1.06	2.77	15.39[g] q			m
36	115.70 t	1.87	5.42	115.17 t	0.90	3.38	m
37	14.41 q	1.53	3.52	15.36[g] q			m
38	15.32 q	0.87	2.84	15.78 q	0.94	2.36	m
39	16.00 q	0.79	2.88	16.24 q	0.78	2.99	m
40	12.36 q	1.05	3.17	12.60 q	1.21	3.08	m
41	109.99 q	1.37	3.61	110.35 t	1.10	2.55	m

The ^{13}C NMR spectra were recorded at 125 MHz with sweep width of 35,700 Hz using 'zgpg'. Number of scans were 8000.

[a] In $CDCl_3$, intensity of each peak in the labeled **1** devided by that of the corresponding signal in the unlabeled **1**, normalized to give a ratio of **1** for unenriched peak (C-15 for [1-^{13}C]-acetate labeling and C-9 for [2-^{13}C]-acetate labeling).

[b] In C_6D_6, intensity normalized to give a ratio of **1** for unenriched peak (C-15 for [1-^{13}C]-acetate labeling and C-16 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.

[d] These signals were overlapped with the signals of the solvent.

[e] These signals were overlapped with each other.

[f] These signals were overlapped with each other.

[g] These signals were overlapped with each other.

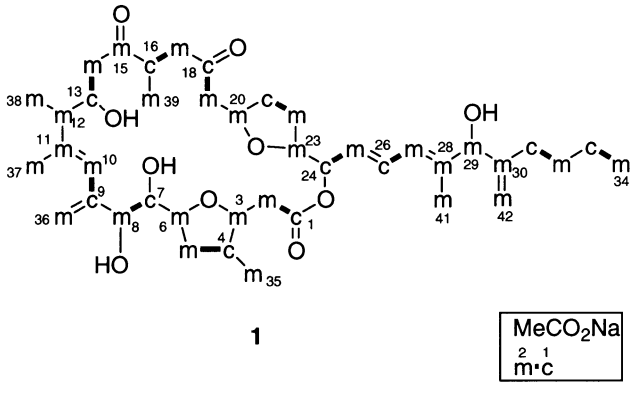

Figure 1. Labeling patterns of amphidinolide C (**1**) resulting from feeding experiments with ^{13}C-labeled acetates.

C-16/C-17 (37.1 Hz), C-18/C-19 (39.8 Hz), C-21/C-22 (32.2 Hz), C-24/C-25 (50.1 Hz), C-26/C-27 (56.7 Hz), C-31/C-32 (34.9 Hz), and C-33/C-34 (34.9 Hz). This was also supported by one-bond $^{13}C-^{13}C$ correlations observed in the INADEQUATE spectrum of **1** labeled with [1,2-$^{13}C_2$] sodium acetate. These results suggested that four parts from C-7 to C-10, C-16 to C-19, C-24 to C-27, and from C-31 to C-34 were likely to be diketide chains. Two irregular labeling patterns derived only from C-2 of acetates were observed for C-11-C-12 (**m**–**m**) and C-28-C-30 (**m**–**m**–**m**), while five isolated C_1 units from C-2 of acetates were observed for C-3, C-6, C-15, C-20, and C-23. The C_1 branches at C-35, C-36, C-37, C-38, C-39, C-40, and C-41 were all derived from C-2 of acetates, in which the carbonyl carbons were lost.

The incorporation patterns suggest that amphidinolide C (**1**) is a unique non-successive mixed polyketide consisting of four diketide chains, four acetate units, five isolated C_1 unit from C-2 of acetates, and a '**m**–**m**' and a '**m**–**m**–**m**' units derived only from C-2 of acetates. The previous biosynthetic studies of 26- and 15-, and 19-membered macrolides, amphidinolides H,[10] J,[11,12] and T1,[9] respectively, have revealed that these are also generated through non-successive mixed polyketides. The C-28–C-30 portion in **1** was labeled as unique '**m**(**m**)– **m**(O)–**m**(**m**)', and such labeling patterns have been reported for those of C-39–C-41 part in yessotoxin from a dinoflagellate *Protoceratium reticulatum*.[13] The C-9–C-12 portion including the vicinally located one-carbon branches in amphidinolide C (**1**) disclosed the same labeling pattern, '**c**(**m**)–**m**–**m**(**m**)–**m**(**m**)', as those of amphidinolides G and H.[10] Two tetrahydrofuran portions in **1** showed the '**m**–**c**–**m**–**m**' labeling pattern, while that of amphidinolide T1 has been revealed to be '**m**–**c**–**m**–**c**'.[6] On the other hand, okadaic acid[14] and goniodomin A[15] also possess a tetrahydrofuran ring, which has been labeled as '**m**–**c**–**m**–**c**' and '**c**–**m**–**c**–**m**', respectively.

3. Experimental

3.1. General methods

^{13}C NMR spectra of **1** and **2** were recorded on a Bruker ARX-500 spectrometer. INADEQUATE spectra were obtained by a Bruker indasy pulse sequence. The repetition delay and the deray 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 25,000 Hz, respectively. 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 2D Fourier transformation. The resulting data matrix was 2K×512. The total measuring time was ca. 10 h.

3.2. 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 7 days after inoculation, and then the culture was harvested by centrifugation after 14 days. Sodium ^{13}C-bicarbonate (1.2 mM) was added to 100 L culture of the dinoflagellate at 10 days, and then the culture was harvested by centrifugation after 14 days. Extraction and isolation of amphidinolide C (**1**) from the harvested cells were carried out by the same procedure as described previously.[2] The ^{13}C-labeled amphidinolide C (**1**) was obtained in 0.0010 yield as an average from wet weight of the cells.

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