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Biosynthetic studies of amphidinolides G and H: unusual labeling patterns in feeding experiments with ^{13}C -labeled acetates

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Abstract

The biosynthetic origins of amphidinolides G (**1**) and H (**2**) were investigated on the basis of 2D NMR data of ^{13}C -enriched samples obtained by feeding experiments with $[1-^{13}\text{C}]$, $[2-^{13}\text{C}]$, and $[1,2-^{13}\text{C}_2]$ sodium acetates in cultures of a marine dinoflagellate *Amphidinium* sp. These incorporation patterns suggested that **1** and **2** were generated from three unusual C_2 units derived only from C-2 of acetates in addition to three successive polyketide chains. Furthermore, it is noted that six oxygenated carbons of C-1, C-18, C-20, C-21, C-22, and C-26 in **1** and **2** were not derived from the C-1 carbonyl but from the C-2 methyl of acetates. © 2000 Elsevier Science Ltd. All rights reserved.

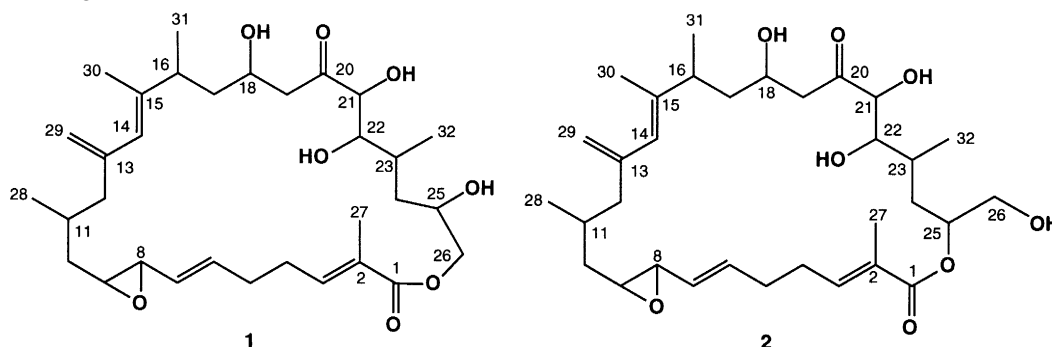
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Amphidinolides are a series of unique cytotoxic macrolides obtained from marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms *Amphiscolops* spp.¹ Among them the structurally-related 26-membered, amphidinolides B,² D,³ and H,⁴ or 27-membered macrolides, amphidinolides G⁴ and L,⁵ with potent cytotoxicity have unique structural units such as an allyl epoxide or vicinally located one-carbon branches. During our search for bioactive and structurally-unique secondary metabolites from marine dinoflagellates,⁶ a strain (Y-72) of the genus *Amphidinium* producing relatively large amounts of amphidinolides G (**1**) and H (**2**), which were first isolated from another strain (Y-25),⁴ has been recently separated from the inside cells of the marine acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa. Here we describe the unusual labeling patterns of amphidinolides G (**1**) and H (**2**) on the basis of stable isotope incorporation experiments using this Y-72 strain.

The dinoflagellate *Amphidinium* sp. (strain Y-72) was cultured in a 20 L nutrient-enriched seawater medium, and feeding experiments were carried out with $[1-^{13}\text{C}]$, $[2-^{13}\text{C}]$, and $[1,2-^{13}\text{C}_2]$ sodium acetate, $[1-^{13}\text{C}]$ sodium propionate, $[methyl-^{13}\text{C}]$ -L-methionine, and $[2-^{13}\text{C}]$ sodium pyruvate. In feeding experiments for monolabeled precursors, the dinoflagellate was supplemented with sodium acetate (610 μM),

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sodium propionate (520 μM), or L-methionine (340 μM) in one portion at 12 days after inoculation, and then the culture was harvested by centrifugation after 14 days. On the other hand, $[1,2-^{13}\text{C}_2]$ sodium acetate (610 μM) was fed to the algal culture at 11 days, then 24 h later the culture was harvested. The feeding experiment for $[2-^{13}\text{C}]$ sodium pyruvate (450 μM) was carried out under the following schedule: the precursor was fed at 7 days followed by harvest of the culture after 14 days. The feeding experiment with $[2-^{13}\text{C}]$ sodium acetate was also performed under the same schedule as that of pyruvate feeding. 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 amphidinolides **1** and **2** in 0.03 and 0.06% yields, respectively, as an average from wet weight of the cells.



Assignments of ^{13}C NMR signals and isotope incorporation results of amphidinolide **H** (**2**) are presented in Table 1. The ^{13}C NMR spectrum (CDCl_3) of **2** derived from $[1-^{13}\text{C}]$ sodium acetate showed the significant enrichment of 10 carbons (C-3, C-5, C-7, C-9, C-11, C-13, C-17, C-19, C-23, and C-25), while in the ^{13}C NMR spectrum of **2** derived from $[2-^{13}\text{C}]$ sodium acetate, enrichment was observed

Table 1
Isotope incorporation results based on the ^{13}C NMR data of amphidinolide **H** (**2**)^a

positrn	δ_{C}	intensity ratio (labeled/unlabeled) ^b			assignment	positrn	δ_{C}	intensity ratio (labeled/unlabeled) ^b		
		$[1-^{13}\text{C}]$ -acetate	$[2-^{13}\text{C}]$ -acetate					$[1-^{13}\text{C}]$ -acetate	$[2-^{13}\text{C}]$ -acetate	
1	168.67 s	1.19	1.60	m	17	40.87 t	2.17	1.57	c	
2	127.89 s	1.28	1.70	m	18	67.50 d	1.03	1.99	m	
3	141.02 d	2.06	1.20	c	19	45.18 t	2.36	1.43	c	
4	26.97 t	0.99	2.09	m	20	212.18 s	0.96	1.76	m	
5	30.89 t	2.25	1.19	c	21	77.69 d	1.14	1.88	m	
6	135.65 d	1.08	2.59	m	22	75.41 d	1.18	2.05	m	
7	128.64 d	2.38	1.10	c	23	32.98 d	2.06	1.02	c	
8	60.34 d	1	2.19	m	24	33.46 t	1.18	2.39	m	
9	59.50 d	2.10	1	c	25	73.42 d	2.52	1.07	c	
10	39.76 t	1.09	2.93	m	26	66.12 t	1.21	3.07	m	
11	29.10 d	2.15	1.05	c	27	12.56 q	1.23	2.27	m	
12	47.14 t	1.03	2.29	m	28	17.98 q	1.19	2.57	m	
13	144.15 s	2.32	0.96	c	29	114.72 t	1.10	2.79	m	
14	126.10 d	0.93	2.03	m	30	13.18 q	1.16	2.11	m	
15	141.70 s	1.43	1.54	m	31	20.29 q	1.41	2.07	m	
16	40.75 d	1.23	1.50	m	32	12.56 q	1.35	2.89	m	

^a The ^{13}C NMR spectra were recorded in CDCl_3 solution at 125 MHz with sweep width of 35700 Hz using 'zgpg30'. Number of scans were 8000. ^b Intensity of each peak in the labeled **2** divided by that of the corresponding signal in the unlabelled **2**, normalized to give a ratio of 1 for unenriched peak (C-8 for $[1-^{13}\text{C}]$ -acetate labeling and C-9 for $[2-^{13}\text{C}]$ -acetate labeling). ^c c denotes the carbon derived from C-1 of acetate, while m indicates the carbon derived from C-2 of acetate.

for 22 carbons (C-1, C-2, C-4, C-6, C-8, C-10, C-12, C-14, C-15, C-16, C-18, C-20, C-21, C-22, C-24, C-26, C-27, C-28, C-29, C-30, C-31, and C-32). Thus, all 32 carbon signals contained in **2** were shown to be labeled by acetates, although isotope enrichments of C-15 and C-16 by C-2 of acetate were relatively low. In the ^{13}C NMR spectra of **2** obtained from feeding experiments with $[1-^{13}\text{C}]$ sodium propionate and $[\text{methyl-}^{13}\text{C}]$ -L-methionine, appreciable enrichment of any carbon was not observed. The one-bond ^{13}C - ^{13}C coupling constants ($^1J_{\text{CC}}$)⁷ as well as ^{13}C - ^{13}C correlations observed in the INADEQUATE spectra of **2** labeled with $[1,2-^{13}\text{C}_2]$ 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-17/C-18, C-19/C-20, C-23/C-24, and C-25/C-26 (Fig. 1). These results suggested that three parts from C-3 to C-14, from C-17 to C-20, and from C-23 to C-26 were likely to be classical polyketide chains derived from six, two, and two acetate units, respectively, while three irregular labeling patterns (**m-m**) derived only from C-2 of acetates were observed for C-1/C-2, C-15/C-16, and C-21/C-22. The C_1 branches of C-27, C-28, C-29, C-30, C-31, C-32 were all derived from C-2 of acetates, in which the carbonyl carbons were lost. On the other hand, amphidinolide G (**1**) also showed the same incorporation patterns of acetates as those of amphidinolide H (**2**).⁸

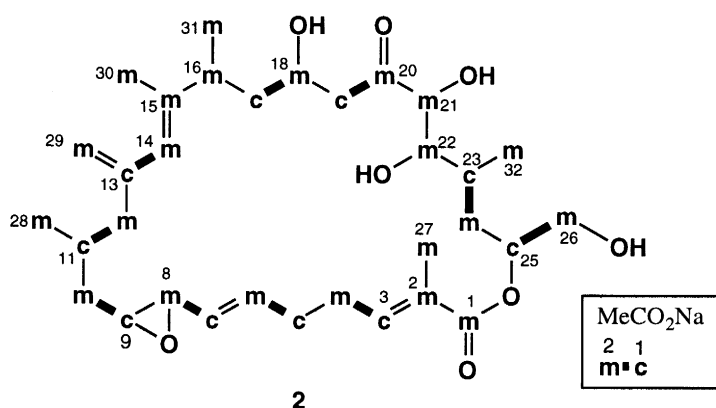


Fig. 1. Labeling patterns of amphidinolide H (**2**) resulting from feeding experiments with ^{13}C -labeled acetates

The ^{13}C NMR spectrum of **2** labeled with $[2-^{13}\text{C}]$ sodium pyruvate displayed significant enrichments (6%) for all carbon signals with double satellite signals. On the other hand, in the ^{13}C NMR spectrum of **2** labeled with $[2-^{13}\text{C}]$ sodium acetate under the same schedule as that of the pyruvate feeding, predominant enrichments (8%) were observed for 22 carbons described above, although double satellite signals (2%) for all carbons due to scrambled label were observed. These results indicated that pyruvate was not incorporated via acetate, but incorporated indirectly after conversion into oxaloacetate and then passage through TCA cycle.

These incorporation patterns suggested that amphidinolides G (**1**) and H (**2**) were generated from three successive polyketide chains and three unusual '**m-m**' units derived only from C-2 of acetates. Furthermore, it was noted that six oxygenated carbons, C-1, C-18, C-20, C-21, C-22, and C-26, were not derived from the C-1 carbonyl but from the C-2 methyl of acetates. The previous biosynthetic studies of a 15-membered macrolide, amphidinolide J, has also revealed that it was generated through non-successive mixed polyketides.⁹ The vicinally located one-carbon branches [C-15(C-30)-C-16(C-31)] of **1** and **2** were labeled as **m(m)-m(m)**, while the labeling pattern of the corresponding part [C-3(C-21)-C-4(C-22)] in amphidinolide J was **m(m)-c(m)**.⁹ Although the unusual labeling patterns of **1** and **2** may be explained by a mechanism proposed for generation of the non-successive polyketide chains of okadaic

acid and DTX-4,¹⁰ experimental evidences seem to be essential for its application. Further biosynthetic studies using other stable isotope-labeled precursors and different culture conditions are now in progress.

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References

1. Ishibashi, M.; Kobayashi, J. *Heterocycles* **1997**, *44*, 543–572.
2. (a) Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1127–1129. (b) Bauer, I.; Maranda, L.; Shimizu, Y.; Peterson, R. W.; Cornell, L.; Steiner, J. R.; Clardy, J. *J. Amer. Chem. Soc.* **1994**, *116*, 2657–2658.
3. Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *J. Nat. Prod.* **1989**, *52*, 1036–1041.
4. Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221–5224.
5. Tsuda, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1994**, *59*, 3734–3737.
6. Kobayashi, J.; Kubota, T.; Takahashi, M.; Ishibashi, M.; Tsuda, M.; Naoki, H. *J. Org. Chem.* **1999**, *64*, 1478–1482.
7. ¹³C/¹³C coupling constants (J_{CC}/Hz) for amphidinolide H (**2**): 43.4 (C-3/C-4), 42.1 (C-5/C-6), 56.8 (C-7/C-8), 44.3 (C-9/C-10), 35.5 (C-11/C-12), 53.3 (C-13/C-14), 40.4 (C-17/C-18), 39.2 (C-19/C-20), 34.2 (C-23/C-24) and 40.6 (C-25/C-26).
8. Intensity ratio ([1-¹³C]/[2-¹³C]-acetate) for amphidinolide G (**1**): C-1 (1.16/2.02), C-2 (0.52/1.41), C-3 (2.06/0.94), C-4 (0.88/1.91), C-5 (2.08/1.02), C-6 (1.02/2.51), C-7 (2.25/1.23), C-8 (1/2.33), C-9 (1.92/1), C-10 (1.09/2.96), C-11 (2.00/1.11), C-12 (1.00/2.23), C-13 (1.49/0.77), C-14 (0.88/1.75), C-15 (0.93/1.43), C-16 (1.14/1.63), C-17 (1.74/1.33), C-18 (1.18/1.89), C-19 (1.67/1.15), C-20 (0.79/1.52), C-21 (0.95/1.66), C-22 (0.94/2.08), C-23 (1.76/0.97), C-24 (1.08/2.68), C-25 (2.02/1.14), C-26 (1.05/2.44), C-27 (1.05/2.31), C-28 (1.14/2.57), C-29 (1.06/2.57), C-30 (1.11/1.98), C-31 (0.96/1.98), and C-32 (1.04/2.81); ¹³C/¹³C coupling constants (J_{CC}/Hz) for **1**: 42.9 (C-3/C-4), 43.5 (C-5/C-6), 57.2 (C-7/C-8), 43.2 (C-9/C-10), 34.5 (C-11/C-12), 52.9 (C-13/C-14), 39.2 (C-17/C-18), 40.2 (C-19/C-20), 34.9 (C-23/C-24) and 41.2 (C-25/C-26).
9. Kobayashi, J.; Takahashi, M.; Ishibashi, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1639–1640. (b) Ishibashi, M.; Takahashi, M.; Kobayashi, J. *Tetrahedron* **1997**, *53*, 7827–832.
10. Wright, J. L. C.; Hu, T.; McLachlan, J. L.; Needaham, J.; Walter, J. A. *J. Amer. Chem. Soc.* **1996**, *118*, 8757–8758.