JOC*Note*

Amphidinolide Y, a Novel 17-Membered Macrolide from Dinoflagellate *Amphidinium* sp.: Plausible Biogenetic Precursor of Amphidinolide X

Masashi Tsuda,† Naoko Izui,† Kazutaka Shimbo,† Masaaki Sato,† Eri Fukushi,‡ Jun Kawabata,‡ and Jun'ichi Kobayashi*,†

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

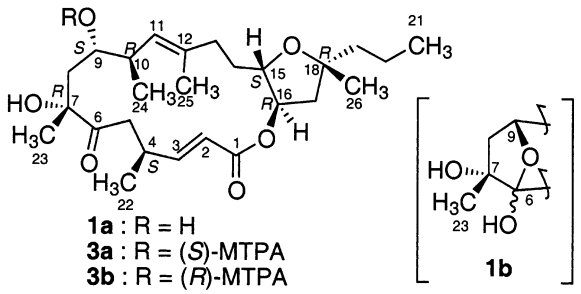
jkobay@pharm.hokudai.ac.jp

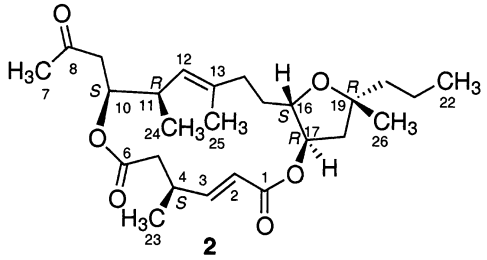
Received September 2, 2003

Abstract: A novel cytotoxic 17-membered macrolide, amphidinolide Y (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp., and it was elucidated to exist as a 9:1 equilibrium mixture of 6-keto- and 6(9)-hemiacetal forms (**1a** and **1b**, respectively) on the basis of 2D NMR data and chemical means. The feeding experiments with ^{13}C-labeled acetates suggested that amphidinolide Y (**1**) may be a biogenetic precursor of 16-membered macrodiolide, amphidinolide X (**2**).

In our continuing search for bioactive secondary metabolites from laboratory-cultured marine dinoflagellates,[1] a novel cytotoxic 16-membered macrodiolide, amphidinolide X (**2**), has been isolated from a dinoflagellate *Amphidinium* sp. (strain Y-42), which is a symbiont of Okinawan marine acoel flatworm *Amphiscolops* sp.[2] Amphidinolide X (**2**) is the first macrodiolide consisting of polyketide-derived diacid and diol units from natural sources. Our search for biosynthetic precursors of this unique macrodiolide resulted in the isolation of a novel 17-membered macrolide, designated amphidinolide Y (**1**), from the same strain. Here, we describe the isolation and structure elucidation of **1** and its labeling patterns with acetates.

The dinoflagellate *Amphidinium* sp. (strain Y-42) was separated from a marine flatworm *Amphiscolops* sp. collected off Sunabe, Okinawa. The dinoflagellate was mass cultured unialgally at 25 °C for 14 days in a seawater medium enriched with 1% ES supplement and ^{13}C-labeled $NaHCO_3$. The harvested algal cells (315 g, wet weight, from 500 L of culture) were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and water. The toluene-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH) followed by C_{18} HPLC (CH_3CN/H_2O) to afford amphidinolide Y (**1**, 0.0007%, wet weight) together with known





macrolides, amphidinolides G[3] (0.0008%), H[3] (0.0007%), W[4] (0.009%), and X[2] (**2**, 0.0004%). In the ^{13}C NMR spectrum, 10% enrichments for all carbon signals of **1** were observed.

Amphidinolide Y (**1**) had the molecular formula of $C_{26}H_{42}O_6$ as revealed by HRESIMS [*m/z* 473.2875 $(M + Na)^+$, −0.4 mmu]. IR absorptions at 3450 and 1711 cm^{-1} were attributed to hydroxyl(s) and carbonyl group(s), respectively. ^1H and ^{13}C NMR data of **1** (Table 1) in $CDCl_3$ disclosed the existence of a ketone, an ester carbonyl, an sp^2 quaternary carbon, three sp^2 methines, two oxygenated sp^3 quaternary carbons, five sp^3 methines (three of which were oxygenated ones), seven sp^3 methylenes, and six methyl groups (three of which resonated as a singlet signal each due to connection to a quaternary carbon). In the ^1H NMR spectrum of **1**, a set of proton resonances were observed in a ratio of 9:1,[5] while the ^{13}C NMR spectrum showed some minor signals including a hemiacetal carbon (δ_C 104.56), indicating that **1** existed as a 9:1 equilibrium mixture of 6-keto and 6(9)-hemiacetal forms (**1a** and **1b**, respectively). Since four out of six unsaturations were accounted for, the 6-keto form (**1a**) of amphidinolide Y (**1**) was inferred to contain two rings. Interpretation of the $^1H-^1H$ COSY, TOCSY, and HMQC spectra revealed proton connectivities of the following units: (**a**) from H-2 to H_2-5 and H_3-22, (**b**) from H_2-8 to H-11 and H_3-24, (**c**) from H_2-13 to H_2-17, and (**d**) from H_2-19 to H_3-21 (Figure 1). ^1H and ^{13}C NMR data of three partial structures, **a**, **c**, and **d**, in **1a** were similar to those of the corresponding portions of amphidinolide X (**2**).

* To whom correspondence should be addressed. Phone: +81-11-706-4985. Fax: +81-11-706-4989.
† Graduate School of Pharmaceutical Sciences.
‡ Graduate School of Agriculture.

(1) Kobayashi, J.; Shimbo, K.; Sato, M.; Tsuda, M. *J. Org. Chem.* **2002**, *67*, 6585−6592 and references therein.
(2) Tsuda, M.; Izui, N.; Shimbo, K.; Sato, M.; Fukushi, E.; Kawabata, J.; Katsumata, K.; Horiguchi, T.; Kobayashi, J. *J. Org. Chem.* **2003**, *68*, 5339−5345.
(3) (a) Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221−5224. (b) Kobayashi, J.; Shimbo, K.; Sato, M.; Shiro, M.; Tsuda, M. *Org. Lett.* **2000** *2*, 2805−2807.
(4) Shimbo, K.; Tsuda, M.; Izui, N.; Kobayashi, J. *J. Org. Chem.* **2002**, *67*, 1020−1023.
(5) ^1H NMR chemical shifts of the 6(9)-hemiacetal form (**1b**) were assigned by the NOESY spectrum: δ_H 6.84 (H-3), 5.04 (H-11), 5.03 (H-16), 3.99 (H-15), 3.80 (H-9), 2.72 (H-4), 2.10 (H-8a), 1.96 (H-14a), 1.76 (H-5b), 1.60 (H-8b), 1.58 (H_3-25), 1.56 (H-5a), and 1.26 (H_3-23).

10.1021/jo035278z CCC: $25.00
Published on Web 10/23/2003

TABLE 1. ^{1}H and ^{13}C NMR Data of 6-Keto Form (1a) of Amphidinolide Y (1) in $CDCl_3$

position	δ_C	δ_H	(m, Hz)
1	165.81, s		
2	120.05, d	5.78	d, 15.6
3	153.56, d	6.59	dd, 9.5, 15.6
4	32.07, d	3.06	m
5a[a]	42.60, t	2.94	dd, 11.5, 17.8
5b[a]		2.38	dd, 2.1, 17.8
6	211.09, s		
7	77.26, s		
8a[a]	44.94, t	1.97	d, 14.5
8b[a]		1.76	dd, 9.0, 14.5
9	71.01, d	3.11	t, 9.0
10	39.23, d	2.25	m
11	128.61, d	4.86	m
12	138.21, s		
13	34.74, t	2.13[b]	m
14a[a]	33.97, t	1.86	m
14b[a]		1.48	m
15	79.99, d	3.92	dt, 4.1, 11.0
16	78.67, d	4.87	m
17a[a]	42.67, t	2.10	dd, 7.4, 14.3
17b[a]		1.76	dd, 2.4, 14.3
18	82.96, s		
19	44.85, t	1.47[b]	m
20	17.82, t	1.32[b]	m
21	14.55, t	0.91	t, 7.0
22	19.89, q	1.10[c]	d, 6.6
23	26.58, q	1.35[c]	s
24	16.84, q	0.87[c]	d, 6.5
25	17.51, q	1.70[c]	brs
26	25.74, q	1.23[c]	s

[a] a and b denote low-field and high-field resonances, respectively, of a geminal pair for C-5, C-8, C-14, and C-17. [b] 2H. [c] 3H.

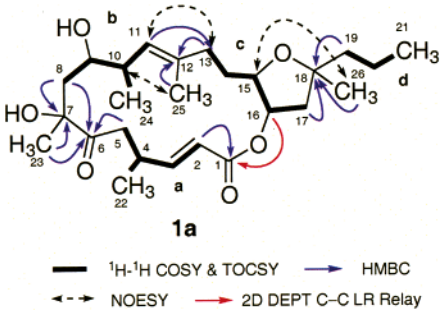


FIGURE 1. Selected 2D (a) ^{1}H−^{1}H and ^{1}H−^{13}C and (b) ^{13}C−^{13}C correlations for amphidinolide X (**1**).

The geometry of the disubstituted olefin (C-2-C-3) in **a** was assigned as *E* by the ^{1}H−^{1}H coupling constant [*J*(H-2/H-3) 15.6 Hz]. Connections among partial structures **a** and **b** through a ketone carbonyl (C-6; δ_C 211.09) and an oxygenated quaternary carbon (C-7; δ_C 77.26) were assigned by HMBC correlations of H_2-5 (δ_H 2.94 and 2.38)/C-6,H-8a (δ_H 1.97)/C-6, H-8a/C-7, H_3-23 (δ_H 1.35, 3H, s)/C-6, and H_3-23/C-7. HMBC correlations for H_2-13 (δ_H 2.13, 2H)/C-12 (δ_C 138.21), H_3-25 (δ_H 1.70)/C-12, and H-11 (δ_H 4.86)/C-13 (δ_C 34.74) suggested the presence of a trisubstituted double bond at C-11−C-12, which was assigned as *E*-geometry by NOESY cross-peaks for H-10 (δ_H 2.25)/H_3-25 and H-11 (δ_H 4.86)/H_2-13. Connectivities among C-17, C-19, and C-26 through C-18 were deduced from HMBC correlations of H-17a (δ_H 2.10)/C-18 (δ_C 82.96), H_2-19 (δ_H 1.47, 2H)/C-18, and H_3-26 (δ_H 1.23)/C-18. The existence of an ether linkage between C-15 and C-18 was implied by the NOESY correlation for H-15/H_3-26, thereby constructing a tetrahydrofuran ring with *syn*-relation for H-15/C-26 (Figure 2). The ester carbonyl (C-1) was shown to be adjacent to C-2 by an HMBC correlation of H-2 (δ_H 5.78) to C-1 (δ_C 165.81). The relatively lower-field resonance of H-16 (δ_H 4.87) suggested that C-16 was involved in the ester linkage with C-1. Although no HMBC correlation for H-16 to C-1 was observed, a two-bond correlation for C-16 (δ_C 78.67)/C-1 was observed in the 2D DEPT C−C LR Relay spectrum,[6] indicating that the ester linkage existed between C-1 and C-16. Thus, the gross structure of the 6-keto form of amphidinolide Y (**1**) was elucidated to be **1a**.

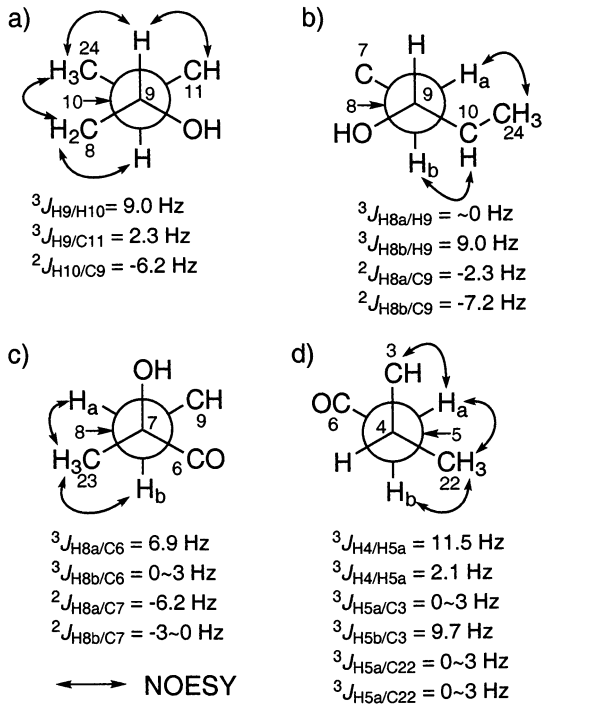


FIGURE 2. Rotation models for (a) C-9−C-10, (b) C-8−C-9, (c) C-7−C-8, and (d) C-4−C-5 bonds in the 6-keto form (**1a**) of amphidinolide Y (**1**).

The relative stereochemistry of the tetrahydrofuran ring in **1a** was deduced to be H-15/H-16-anti and H-15/C-26-syn, which were the same as those of the corresponding portion in **2**, from NOESY correlations as shown in Figure 2. The relative configurations at C-4, C-7, C-9, and C-10 of **1a** were elucidated on the basis of the *J*-based configuration analysis,[7] and long-range ^{13}C-^{1}H coupling constants obtained from the HETLOC[8] and *J*IMPEACH-MBC[9] spectra. The relative configuration for C-9−C-10 bond was assigned as erythro by *J*(H-9/H-10) (9.0 Hz), *J*(C-11/H-9) (2.3 Hz), and *J*(C-9/H-10) (−6.2 Hz) values as well as NOESY correlations for H-8a/H_3-24, H-8b/H-10, H-9/H-11, and H-9/H_3-24 (Figure 2a).

(6) Fukushi, E.; Kawabata, J. Symposium Papers. In *43rd Symposium on The Chemistry of Natural Products*; Osaka, 2001, pp 341−346.
(7) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(8) (a) Otting, G.; Wüthrich, K. *Quart. Rev. Biophys.* **1990**, *23*, 39−96. (b) Wollborn, U.; Leibfritz, D. *J. Magn. Reson.* **1992**, *98*, 142−146. (c) Kurz, M.; Schmieder, P.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1329−1331.
(9) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Martin, G. E.; Krishnamurthy, V. V. *Magn. Reson. Chem.* **2001**, *39*, 127−132.

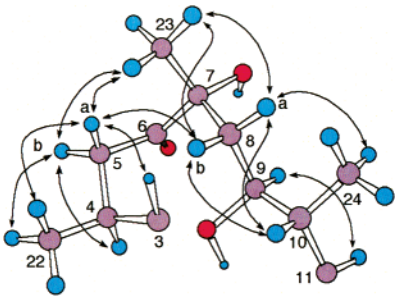


FIGURE 3. NOESY correlations for the C-3−C-11 portion in the 6-keto form (**1a**) of amphidinolide Y (**1**).

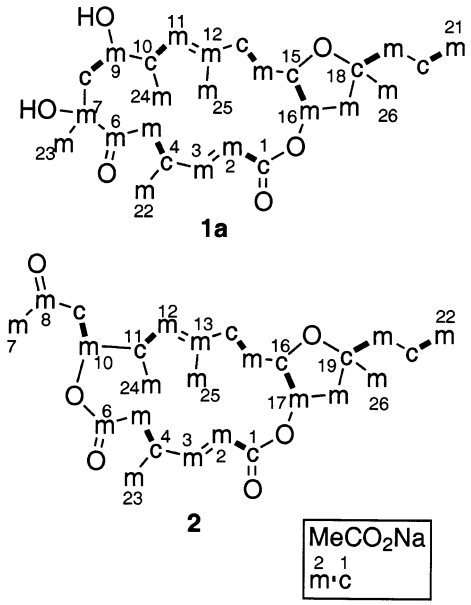


FIGURE 4. Labeling patterns of the 6-keto form (**1a**) of amphidinolide Y (**1**) and amphidinolide X (**2**) resulting from feeding experiments with ^{13}C-labeled acetates.

From analyses of rotation models for the C-8−C-9 and C-7−C-8 bonds (Figures 2b and 2c, respectively), the relative configuration of the 1,3-diol at C-7 and C-9 was assigned as a syn-relation (Figure 3). For the C-4−C-5 bond (Figure 2d), relations between C-6 and C-22 and between H-5b and C-3 were elucidated to be both anti. Since NOESY correlations were observed for H_2-5/H_3-23 and H-5a/H-8a, conformation of the C-4−C-10 portion was elucidated as shown in Figure 4, in which the relative configurations at C-4, C-9, and C-10 of **1a** were the same as those of C-4, C-10, and C-11 in amphidinolide X (**2**).[2]

To determine the absolute stereochemistry of amphidinolide Y (**1**), application of modified Mosher's method[10] for C-9 and oxidative cleavage of the C-6−C-7 bond were carried out as follows. $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) obtained from ^{1}H NMR data of 9-(*S*)- and 9-(*R*)-MTPA esters (**3a** and **3b**, respectively) showed negative values for H-4 (−0.02), H_2-5 (both −0.02), H_2-8 (−0.02 and −0.11), and H_3-23 (−0.01), while those of H-10 (+0.07), H-11 (+0.06), and H_3-24 (+0.14) were positive, suggesting that C-9 possessed *S*-configuration. Amphidinolide Y (**1**) was treated with lead tetraacetate to afford amphidinolide X (**2**), of which the spectral data including the CD spectrum [λ_{ext} 224 nm ($\Delta\epsilon$ −7.9)] were the same as those of natural specimen of **2** [λ_{ext} 225 nm ($\Delta\epsilon$ −8.7)]. Therefore, the absolute configurations at seven chiral centers were assigned as 4*S*, 7*R*, 9*S*, 10*R*, 15*S*, 16*R*, and 18*R*.

Feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates were carried out as reported previously,[11] and ^{13}C-labeled amphidinolide Y (**1**) was separated by the same procedure as described before. The ^{13}C NMR spectra ($CDCl_3$ and C_6D_6) of **1** derived from [1-^{13}C] sodium acetate showed significant enrichment of 8 carbons (C-1, C-4, C-8, C-10, C-13, C-15, C-18, and C-20). On the other hand, enrichment by [2-^{13}C] sodium acetate was observed for 18 carbons (C-2, C-3, C-5, C-6, C-7, C-9, C-11, C-12, C-14, C-16, C-17, C-19, C-21, C-22, C-23, C-24, C-25, and C-26), though intensity ratios for C-6, C-7, and C-12 were less than those of others. One-bond C−C couplings for C-1/C-2 (J_{CC}, 73 Hz), C-4/C-5 (J_{CC}; 32 Hz), C-8/C-9 (41 Hz), C-10/C-11 (44 Hz), C-13/C-14 (35 Hz), C-15/C-16 (40 Hz), C-18/C-19 (J_{CC}, 40 Hz), and C-20/C-21 (J_{CC}, 35 Hz) revealed the incorporation of eight intact acetates, suggesting the presence of two acetates from C-1 to C-2 and from C-4 to C-5 and three diketides from C-8 to C-11, from C-13 to C-16, and from C-18 to C-21. The C_1 branches at C-22, C-23, C-24, C-25, and C-26 were all derived from C-2 of acetates, in which the carbonyl carbons were lost. All 26 carbon signals contained in the 6-keto form (**1a**) of amphidinolide Y (**1**) were shown to be labeled by acetates (Figure 4). These incorporation patterns suggested that **1a** was generated from three diketide units, two acetate units, three isolated "**m**" units from C-2 of acetates, a "**m**−**m**" unit, and five branched C_1 units from C-2 of acetates. The labeling patterns at C-1−C-6 and C-23−C-7−C-21 parts in **1a** corresponded to those at diacid and diol units in amphidinolide X (**2**), indicating that **2** might be generated from **1a** through oxidative cleavage at the C-6−C-7 position of the minor 6(9)-hemiacetal form (**1b**).

The 6-keto form (**1a**) of amphidinolide Y (**1**) is a new 17-membered macrolide possessing a tetrahydrofuran ring, five branched methyls, a ketone, and two hydroxyl groups, and **1b** is 6(9)-hemiacetal isomer of **1a**. Amphidinolide Y (**1**) is considered to be a biogenetic precursor of amphidinolide X (**2**). Amphidinolide Y (**1**) exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro with IC_{50} values of 0.8 and 8.0 μg/mL, respectively.

Experimental Section

Cultivation and Isolation. The dinoflagellate *Amphidinium* sp. (strain no. Y-42) was separated from the inside cells of the marine acoel flatworm *Amphiscolops* sp., which was collected off Sunabe, Okinawa. The dinoflagellate was unialgally cultured at 25 °C for 2 weeks in seawater medium enriched with 1% ES supplement and ^{13}C-lableled sodium bicarbonate (100 mg/L). The harvested cells (315 g, wet weight, from 500 L of culture) were extracted with MeOH/toluene (3:1, 1 L × 3). After addition of 1 M NaCl aq (1 L), the mixture was extracted with toluene (1 L × 3). Parts (1.27 g) of the toluene-soluble fractions (3.78 g) were subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) and a Sep-Pak cartridge C_{18} (MeOH/H_2O, 8:2) followed by C_{18} HPLC

(10) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

(11) Tsuda, M.; Izui, N.; Sato, M.; Kobayashi, J. *Chem. Pharm. Bull.* **2002**, *50*, 976−977.

[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 Y (**1**, 2.3 mg, 0.0007%, wet weight, t_R 14 ∼ 16 min) and together with amphidinolides G (0.0008%), H (0.0007%), W (0.009%), and X (**2**, 0.004%).

Amphidinolide Y (1): a colorless oil; $[\alpha]^{17}_D$ −33° (*c* 1.0, $CHCl_3$); UV (EtOH) λ_{max} 208 nm (ϵ 12 400); IR (neat) ν_{max} 3450, 2928, and 1711 cm^{-1}; FABMS *m*/*z* 433 $(M - H_2O + H)^+$ and 451 $(M + H)^+$; ESIMS *m*/*z* 451 $(M + H)^+$ and 473 $(M + Na)^+$; HRESIMS *m*/*z* 473.2875 [calcd for $C_{26}H_{42}O_6Na$ $(M + Na)^+$, 473.2879].

(*S*)-MTPA Ester (3a) of Amphidinolide Y (1). To a CH_2Cl_2 solution (50 μL) of amphidinolide Y (**1**, 0.18 mg) was added 4-(dimethylamino)pyridine (50 μg), triethylamine (5 μL), and (*R*)-(−)-MTPACl (1.5 μL) at 4 °C, and stirring was continued for 3 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (5 μL) and evaporation of solvent, the residue was subjected to C_{18} HPLC (TSK-gel ODS-100S, Tosoh Co., Ltd., 4.6 × 250 mm; eluent CH_3CN/H_2O, 95:5; flow rate, 1.0 mL/min; UV detection at 230 nm) to give 9-(*S*)-MTPA ester (**3a**, 0.1 mg, t_R 12.6 min) of amphidinolide Y (**1**) as colorless oil: ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, *J* = 7.0 Hz, H_3-21), 0.96 (3H, d, *J* = 6.5 Hz, H_3-24), 1.15 (3H, d, *J* = 6.6 Hz, H_3-22), 1.20 (3H, s, H_3-26), 1.30 (2H, m, H_2-20), 1.36 (3H, s, H_3-23), 1.40 (1H, m, H-8), 1.45−1.54 (3H, m, H-14 and H_2-19), 1.55 (1H, m, H-8), 1.66 (3H, s, H_3-25), 1.74 (1H, dd, *J* = 2.0, 14.2 Hz, H-17), 1.81 (1H, m, H-14), 2.12 (2H, m, H_2-13), 2.14 (1H, dd, *J* = 7.8, 14.2 Hz, H-17), 2.51 (1H, m, H-5), 2.55 (1H, m, H-5), 2.56 (1H, m, H-10), 3.00 (1H, m, H-4), 3.51 (3H, s, OCH_3), 3.85 (1H, m, H-15), 4.95 (1H, brt, *J* = 4.0 Hz, H-16), 5.13 (1H, brd, *J* = 8.0 Hz, H-9), 5.50 (1H, d, *J* = 8.5 Hz, H-11), 5.72 (1H, d, *J* = 15.6 Hz, H-2), 6.52 (1H, dd, *J* = 9.3, 15.6 Hz, H-3), 7.39 (3H, m, Ph), and 7.62 (2H, m, Ph); FABMS *m*/*z* 667 $(M + H)^+$; HRFABMS *m*/*z* 667.3435 [calcd for $C_{34}H_{50}O_8F_3$ $(M + H)^+$, 667.3458].

(*R*)-MTPA Ester (3b) of Amphidinolide Y (1). Amphidinolide Y (**1**, 0.44 mg) was treated with 4-(dimethylamino)-pyridine (50 μg), triethylamine (5 μL), and (*S*)-(+)-MTPACl (1.5 μL) by the same procedure as described above to afford the 9-(*R*)-MTPA ester (**3b**, 0.1 mg, t_R 12.4 min) of amphidinolide Y (**1**) as colorless oil: ^1H NMR ($CDCl_3$) δ 0.82 (3H, d, *J* = 6.5 Hz, H_3-24), 0.89 (3H, t, *J* = 7.0 Hz, H_3-21), 1.15 (3H, d, *J* = 6.6 Hz, H_3-22), 1.23 (3H, s, H_3-26), 1.30 (2H, m, H_2-20), 1.37 (3H, s, H_3-23), 1.40−1.54 (4H, m, H-14, H-8, and H_2-19), 1.57 (1H, m, H-8), 1.61 (3H, s, H_3-25), 1.72 (1H, dd, *J* = 2.0,14.2 Hz, H-17), 1.76 (1H, m, H-14), 2.06 (2H, m, H_2-13), 2.12 (1H, dd, *J* = 7.8, 14.2 Hz, H-17), 2.49 (1H, m, H-10), 2.53 (1H, m, H-5), 2.57 (1H, m, H-5), 3.02 (1H, m, H-4), 3.53 (3H, s, OCH_3), 3.82 (1H, m, H-15), 4.94 (1H, brt, *J* = 4.0 Hz, H-16), 5.06 (1H, brd, *J* = 8.0 Hz, H-9), 5.44 (1H, d, *J* = 8.5 Hz, H-11), 5.72 (1H, d, *J* = 15.6 Hz. H-2), 6.53 (1H, dd, *J* = 9.3, 15.6 Hz, H-3), 7.38 (3H, m, Ph), and 7.60 (2H, m, Ph); FABMS *m*/*z* 667 $(M + H)^+$; HRFABMS *m*/*z* 667.3467 [calcd for $C_{34}H_{50}O_8F_3$ $(M + H)^+$, 667.3458].

Oxidation of Amphidinolide Y (1) with Lead Tetraacetate. Amphidinolide Y (**1**, 0.2 mg) was treated with lead tetraacetate (0.5 mg) in EtOAc (50 μL) at room temperature for 15 h. After filtration of insoluble materials, the solvent was evaporated to afford amphidinolide X (**2**, 0.1 mg) as a colorless oil: CD (MeOH) λ_{ext} 224 nm ($\Delta\epsilon$ −7.9); FABMS *m*/*z* 449 $(M + H)^+$; HRFABMS *m*/*z* 449.2918 [calcd for $C_{26}H_{41}O_6$ $(M + H)^+$, 449.2903]. ^1H NMR data of **2** were consistent with those of natural specimen.

Feeding Experiments with ^{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 days after inoculation, and then the culture was harvested by centrifugation after 14 days to obtain cells of the dinoflagellate (70 g as an average, wet weight). Extraction and isolation of amphidinolide Y (**1**) from the harvested cells were carried out through the same procedure as described above. The ^{13}C-labeled amphidinolide Y (**1**) was obtained in 0.001% yield as an average from wet weight of the cells.

Acknowledgment. We thank Prof. T. Yamasu, University of the Ryukyus, and Prof. M. Ishibashi, Chiba University, for help with dinoflagellate collection, Dr. T. Horiguchi and Mr. K. Katsumata, Hokkaido University, for 16S rDNA analysis, and Ms. S. Oka and Miss M. Kiuchi, Center for Instrumental Analysis, Hokkaido University, for ESIMS and FABMS measurements. This work was partly supported by a Grant-in-Aid from the Takeda Science Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Supporting Information Available: General method, isotope incorporation results, spectral data of **1**, and an overlay of ^1H NMR spectra of **2** derived from **1** and the natural specimen. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035278Z