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Amphidinolide X, a Novel 16-Membered Macrodiolide from Dinoflagellate *Amphidinium* sp.

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A novel cytotoxic 16-membered macrodiolide, amphidinolide X (**1**), has been isolated from a marine dinoflagellate *Amphidinium* sp. (strain Y-42). The gross structure of **1** was elucidated on the basis of spectroscopic data including one-bond and long-range $^{13}C-^{13}C$ correlations. The relative and absolute stereochemistries were determined by combined analyses of NOESY data and $^{1}H-^{1}H$ and $^{1}H-^{13}C$ coupling constants of **1** and NMR data of the degradation products. Amphidinolide X (**1**) is the first macrodiolide consisting of polyketide-derived diacid and diol units from natural sources. The biosynthetic origins of **1** were investigated by means of feeding experiments with ^{13}C-labeled acetates.

Introduction

Marine microorganisms have been proven to produce a variety of chemically unique and biologically interesting secondary metabolites[1] such as long-chain polyketides and polyethers from marine dinoflagellates.[2] We have isolated 25 macrolides, named amphidinolides, from extracts of marine dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine acoel flatworms, *Amphiscolops* spp.[3,4] These macrolides have a variety of backbone skeletons and different sizes of macrocyclic lactone rings (12- to 29-membered rings), and more than half (16) of the amphidinolides have odd-numbered macrocyclic lactone rings. Most of the amphidinolides contain vicinally located one-carbon branches and at least one *exo*-methylene unit, and some of them exhibit potent cytotoxicity and antitumor activity. Therefore, amphidinolides have attracted great interest as challenging targets for total synthesis[5] and biosynthetic studies.[2]

In our continuing search for bioactive secondary metabolites from laboratory-cultured marine dinoflagellates,[4,6] we encountered an unprecedented 16-membered macrodiolide, amphidinolide X (**1**), consisting of polyketide-derived diacid and diol units from the dinoflagellate *Amphidinium* sp. (strain Y-42). The structure was elucidated on the basis of spectroscopic data including one-bond and long-range $^{13}C-^{13}C$ correlations. The relative and absolute stereochemistries were determined by a combination of analysis of NOESY data together with $^{1}H-^{1}H$ and $^{1}H-^{13}C$ coupling constants of **1** and NMR data of the degradation products. The biosynthetic origins of **1** were investigated by means of feeding experiments with ^{13}C-labeled acetates. This paper describes the isolation, structure elucidation, and biosynthesis of **1**.

Results and Discussion

Isolation of Amphidinolide X (1). The dinoflagellate *Amphidinium* sp. (strain Y-42)[4] was mass cultured unialgally at 25 °C for 14 days in a seawater medium enriched with 1% Provasoli's Ert-Schereiber (ES) supplement. The harvested algal cells (363 g, wet weight, from 686 L of culture) were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene

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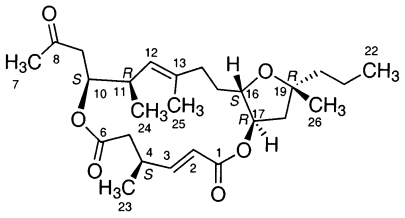
TABLE 1. ^{1}H and ^{13}C NMR Data for Amphidinolide X (1) in $CDCl_3$.

position	δ_C	δ_H (m, Hz)	HMBC (H)	LR $^{13}C-^{13}C$
1	165.74 s		2, 3,	2, 4, 16, 17
2	120.16 d	5.79 d, 15.8	3	3, 23
3	153.15 d	7.12 dd, 7.2, 15.8	5a, 5b, 23	4
4	33.02 d	2.79 m	2, 3, 5a, 5b, 23	3
5	41.41 t	2.58 dd, 3.7, 13.4	23	2
		2.41 dd, 6.3, 13.4		
6	170.69 s		5a, 5b	5, 10
7	30.35 q	2.14[b] s		
8	205.52 s		7, 9a, 9b	7, 9
9	47.10 t	2.69 dd, 6.0, 16.5	7, 10	10
		2.57 dd, 8.2, 16.5		
10	74.23 d	5.21 m	9a, 9b, 24	9
11	35.47 d	2.69 m	9a, 9b, 12, 24	10, 12
12	125.99 d	4.95 d, 10.3	10, 11, 14a, 14b, 24, 25	11
13	135.47 s		11, 14a, 14b, 25	14, 25
14	35.31 t	2.18 m	12, 25	12, 15
		2.11 brt, 9.4		
15	30.39 t	1.95 tt, 2.9, 13.4		16
		1.54 m		
16	80.54 d	3.97 dt, 11.1, 3.6	18a	15, 17
17	78.41 d	5.19 m	15a, 18b	18
18	43.49 t	2.16 m		17
		1.75 dd, 2.4, 13.8		
19	82.92 s		17, 18a, 18b, 20, 26	18, 20, 26
20	44.17 t	1.50[a] m	18a, 18b, 21, 22	
21	17.81 t	1.34[a] m	20, 22	
22	14.61 q	0.92[b] t, 7.4	21	
23	17.48 q	1.14[b] d, 6.8	3, 5a	
24	18.09 q	0.92[b] d, 6.8	11, 12	
25	15.37 q	1.55[b] s	12, 14b	
26	24.52 q	1.30[b] s	18a, 18b	

[a] 2H. [b] 3H.

and water. The toluene-soluble materials were chromatographed on a silica gel column ($CHCl_3$/MeOH) to give a lipophilic fraction including a small amount of macrolides. To remove free fatty acids, the fraction was treated with trimethylsilyl diazomethane and then purified by silica gel column chromatography to give a macrolide-rich fraction, which was subjected to C_{18} column chromatography followed by C_{18} HPLC (CH_3CN/H_2O) to afford amphidinolide X (**1**, 0.001%, wet weight) together with known macrolides, amphidinolides G[7] (0.0008%), H[7] (0.0007%), and W (0.0012%).[4a] The ^{13}C-labeled sample of **1** was obtained from the algal cells cultured in a medium enriched with ^{13}C-labeled sodium acetate.



1

Structure Elucidation of Amphidinolide X (1). Amphidinolide X (**1**) showed molecular ion peaks at *m*/*z* 449 [(M + H)$^+$] and 471 [(M + Na)$^+$] in the ESIMS spectrum, and the molecular formula $C_{26}H_{40}O_6$ was established by HRESIMS [*m*/*z* 471.2701, (M + Na)$^+$, Δ −2.2 mmu]. The broad IR absorption at 1718 cm^{-1} was attributed to carbonyl group(s). The UV absorption at 209 nm (ε 6800) was suggestive of the presence of an enone chromophore. ^{1}H and ^{13}C NMR data (Table 1) disclosed the existence of a ketone, two ester carbonyls, an sp^2 quaternary carbon, three sp^2 methines, an sp^3 quaternary carbon, five sp^3 methines including three oxygenated ones, seven sp^3 methylenes, and six methyl groups, three of which resonated as singlet signals due to connection to quaternary carbon(s). Since five out of seven unsaturations were accounted for, amphidinolide X (**1**) was inferred to possess two rings.

Detailed analyses of the ^{1}H−^{1}H COSY, TOCSY, and HSQC spectra revealed the following proton−proton networks; (**a**) from H-2 to H_2-5 and H_3-23, (**b**) from H_2-9 to H-10, (**c**) from H-11 to H-12 and H_3-24, (**d**) from H_2-14 to H_2-18, and (**e**) from H_2-20 to H_3-22 (Figure 1a). The connection between partial structures **b** and **c** was revealed by the HMBC correlation for H_3-24 (δ_H 0.92) to C-10 (δ_C 74.23), though the proton−proton connectivity for H-10−H-11 was not assigned unambiguously by the ^{1}H−^{1}H COSY and TOCSY spectra due to its small coupling constant [J(H-10/H-11) = <1 Hz]. HMBC correlations were observed for H_2-9 (δ_H 2.69 and 2.57) and H_3-7 (δ_H 2.14, s) to C-8 (δ_C 205.52), implying the presence of a methyl ketone group at C-9. HMBC correlations for H-12 (δ_H 4.95)/C-14 (δ_C 35.31), H_2-14 (δ_H 2.18 and 2.11)/C-13, and H_3-25 (δ_H 1.55)/C-13 suggested that the partial structure **c** and an olefinic methyl (C-25) were attached to an sp^2 quaternary carbon (C-13) connected to C-14 in the partial structure **d**. *E*-geometry for the trisubstituted double bond at C-12−C-13 was assigned by NOESY correlations for H-11 (δ_H 2.69)/H_3-25 and H-12/H_2-14. The connection between partial structures **d** and **e** through an oxygenated sp^3 quaternary carbon (C-19) was deduced

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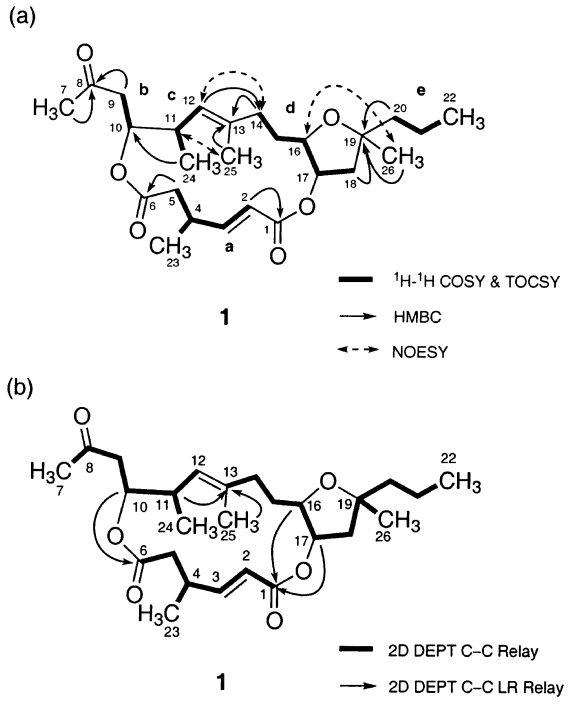

FIGURE 1. Selected 2D (a) $^1H-^1H$ and $^1H-^{13}C$ and (b) $^{13}C-^{13}C$ correlations for amphidinolide X (**1**).

from $^1H-^{13}C$ long-range correlations for H_2-18 (δ_H 2.16 and 1.75)/C-19 (δ_C 82.92) and H_2-20 (δ_H 1.50, 2H)/C-19. A singlet methyl signal (H_3-26, δ_H 1.30) was correlated to C-19, indicating the presence of a methyl group (C-26) on C-19. The existence of a tetrahydrofuran ring at C-16−C-19 was implied from the NOESY correlation for H-16/H_3-26. For the partial structure **a**, the relatively large *J*(H-2/H-3) value (15.8 Hz) was suggestive of the *E*-geometry for the C-2−C-3 double bond. H-2 (δ_H 5.79) and H_2-5 (δ_H 2.58 and 2.41) showed HMBC correlations to two ester carbonyl carbons at δ_C 165.74 (C-1) and δ_C 170.69 (C-6), respectively. Although the relatively lower field sp^3 proton resonances at C-10 (δ_H 5.21) and C-17 (δ_H 5.19) suggested that these carbons were involved in two ester linkages to C-1 or C-6, no HMBC correlations for H-10 or H-17 to ester carbonyls were observed. To elucidate the positions of the two ester linkages, 2D DEPT C−C Relay[8] and DEPT C−C Long-Range (LR) Relay[9] experiments were employed, using the ^{13}C-labeled sample of **1**. Analyses of the DEPT C−C Relay spectrum revealed three carbon−carbon networks as shown in Figure 1b. Two-bond $^{13}C-^{13}C$ correlations for C-10 (δ_C 74.23)/C-6 and C-17 (δ_C 78.41)/C-1 and three-bond correlation for C-16 (δ_C 80.54)/C-1 were observed in the 2D DEPT C−C LR Relay spectrum, indicating that two ester linkages existed between C-1 and C-17 and between C-6 and C-10. Therefore, the gross structure of amphidinolide X was elucidated to be **1**.

Relative Stereochemistry of Amphidinolide X (1). The relative stereochemistry of the C-10−C-11 bond was elucidated by *J*-based configuration analysis[10] (Figure 2). However, ^nJ(C−H) values around the C-10−C-11 bond

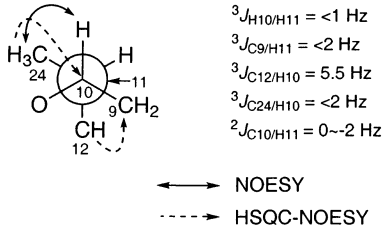

FIGURE 2. Rotation model for the C-10−C-11 bond of amphidinolide X (**1**).

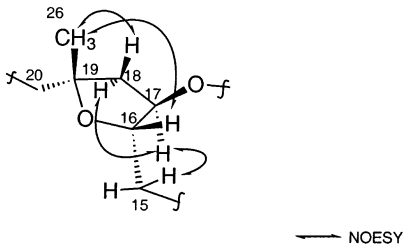

FIGURE 3. NOESY correlations and relative stereochemistry for the tetrahydrofuran ring in amphidinolide X (**1**).

were not obtained from the HETLOC[11] spectrum, since the *J*(H-10,H-11) value was quite small. Hence, the *J*-IMPEACH-MBC[12] experiment was employed to obtain absolute values of ^nJ(C−H) values, and signs of coupling constants were determined by analysis of the phase-sensitive HMBC[13] spectrum. Furthermore, NOE data were obtained from the HSQC-NOESY[14] spectrum in addition to the NOESY spectrum, since signals due to H-9a and H-11 were overlapped. The ^3J(H-10,H-11) value (<1 Hz) indicated that H-10 was gauche to H-11. The relatively large ^3J(C-12,H-10) (5.5 Hz) based on the value [scaling factor 44 × *J*(C−H) = 246.5 Hz] observed in the *J*-IMPEACH-MBC spectrum suggested that H-10 was anti to C-12, while the small ^3J(C-24,H-10) value (<2 Hz) indicated that H-10 was gauche to C-24. The anti-relation for H-11 and 10-O and the gauche-relation for H-11 and C-9 were deduced from ^2J(C-10,H-11) (0 to ca. −2 Hz) and ^3J(C-9,H-11) (<2 Hz) values, respectively. Therefore, the relative stereochemistry of the C-10−C-11 bond was concluded to be *erythro*. This was supported by the NOESY correlation for H-10/H_3-24 and HSQC-NOESY correlations for H_3-24/C-10 and H-12/C-9. The relative stereochemistry of three chiral centers (C-16, C-17, and C-19) in the tetrahydrofuran ring was elucidated to be anti for H-16 and H-17 and syn for H-16 and C-26 on the basis of NOESY correlations for H-15b (δ_H 1.54)/ H-17, H-16/H_3-26, H-17/H-18a (δ_H 2.16), and H-18b (δ_H 1.75)/ H_3-26 (Figure 3).

Absolute Configurations of Amphidinolide X (1). Absolute configurations at six chiral centers were exam-

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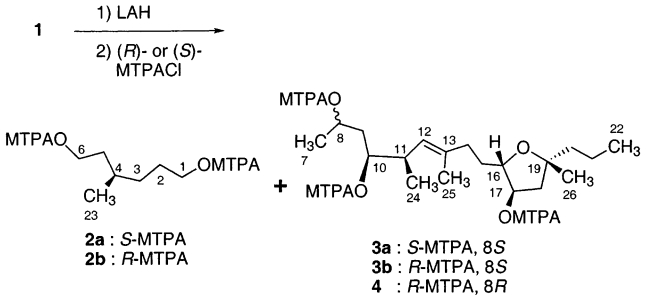
SCHEME 1



ined by NMR data of degradation products of **1** as follows. Two ester carbonyls at C-1 and C-6 of **1** were reduced with $LiAlH_4$ and then the reduction products were treated with (*R*)-(−)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl). HPLC separation furnished the 1,6-bis-(*S*)-MTPA ester of the C-1−C-6 segment (**2a**) and the 8,10,17-tris-(*S*)-MTPA ester of the (8*S*)-C-7−C-22 segment (**3a**) (Scheme 1), though the 8*R*-isomer of **3a** was not obtained. On the other hand, the 1,6-bis-(*R*)-MTPA ester of the C-1−C-6 segment (**2b**) and the 8,10,-17-tris-(*R*)-MTPA esters of the (8*S*)- and (8*R*)-C-7−C-22 segments (**3b** and **4**, respectively) were obtained by the same procedure as described above. Their structures were assigned on the basis of the $^1H-^1H$ COSY spectra and FABMS data. The C-1−C-6 segments (**2a** and **2b**) were obtained as a 2,3-dihydro form, probably due to reduction of the C-2−C-3 double bond by $LiAlH_4$. In the ^1H NMR spectrum of **3b**, methylene protons at C-9 appeared as separated signals at δ_H 1.98 and 1.67, while those of **4** were observed as overlapped 2H signals at δ_H (1.77), indicating that the relative stereochemistry of H-8 and H-10 in **3b** and **4** was syn and anti, respectively.[15] Proton signal patterns of H_2-9 (δ_H 1.96 and 1.70) for **3a**, which were similar to those for **3b**, implied a syn-relation for H-8 and H-10.

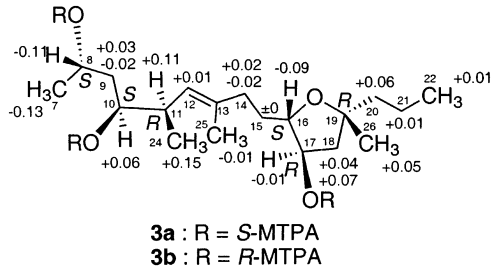
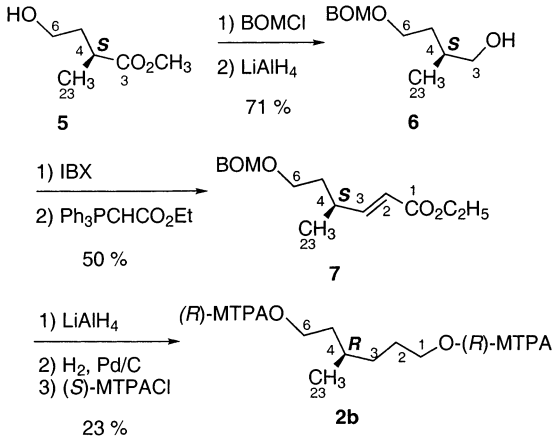


FIGURE 4. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the 8,10,17-tris-(*S*)- and (*R*)-MTPA esters (**3a** and **3b**, respectively) of the (8*S*)-C-7−C-22 segment of amphidinolide X (**1**).

The absolute configuration at C-17 was determined by application of the modified Mosher's method[16] for **3a** and **3b** (Figure 4). The $\Delta\delta$ value ($\delta_S - \delta_R$) of H-16 (−0.09) showed the negative values, while those of H_2-18 (+0.04 and +0.07), H_2-20 (2H, +0.06), and H_3-26 (+0.05) were positive, thus suggesting the 17*R*-configuration. Positive

SCHEME 2



$\Delta\delta$ values of H-11 (+0.11) and H_3-24 (+0.15) and a negative $\Delta\delta$ value of one of H_2-9 (−0.02, +0.03) were interpreted as the 10*S*-configuration, although chemical shifts of H_2-9 were affected by both of the MTPA esters at C-8 and C-10. A negative $\Delta\delta$ sign for H-8 (−0.11) and a positive one for H-10 (+0.06) corresponded to typical $\Delta\delta$ patterns for diesters of *S*,*S*-1,3-diol with chiral anisotopic reagents reported by Riguera and co-workers,[17] thus indicating 8*S*- and 10*S*-configurations. Therefore, the absolute configurations at C-10, C-11, C-16, C-17, and C-19 were concluded to be *S*, *R*, *S*, *R*, and *R*, respectively.

To elucidate the absolute configuration at C-4, the C-1−C-6 segment was prepared from methyl (2*S*)-4-hydroxy-2-methylbutanoate[18] (**5**), which was synthesized from dimethyl (*S*)-(−)-methylsuccinate through regioselective hydrolysis catalyzed by porcine pancreatic lipase[18] and reduction of the carboxyl group with borane-dimethyl sulfide.[19] Compound **5** was treated with benzyloxymethyl chloride (BOMCl), and then the ester carbonyl was reduced with $LiAlH_4$ to give the BOM alcohol (**6**), which was subjected to oxidation with *o*-iodoxybenzoic acid (IBX)[20] and C_2-elongation through a Wittig reaction to afford the unsaturated ester (**7**). Reduction of the unsaturated ester (**7**) followed by catalytic hydrogenation gave the (4*R*)-C-1−C-6 segment, which was converted into the 1,6-bis-(*R*)-MTPA ester of the C-1−C-6 segment (**2b**).

The ^1H NMR spectra of the 1,6-bis-(*S*)- and (*R*)-MTPA ester (**2a** and **2b**, respectively) of the C-1−C-6 segment obtained from natural amphidinolide X (**1**) were compared with those of the 1,6-bis-(*R*)-MTPA ester (**2b**) of the synthetic C-1−C-6 segment (Figure 5). Though **2a** and **2b** showed very similar NMR profiles, significant differences were observed for signals due to methylene protons at C-1 (**2a**, δ_H 4.33 and 4.22; **2b**, δ_H 4.29 and 4.24) and C-6 (**2a**, δ_H 4.31, 2H; **2b**, δ_H 4.35 and 4.28). The ^1H NMR data for the synthetic (*R*)-MTPA ester (**2b**) were identical with those for the 1,6-bis-(*R*)-MTPA esters (**2b**) derived from the natural specimen, respectively, thus

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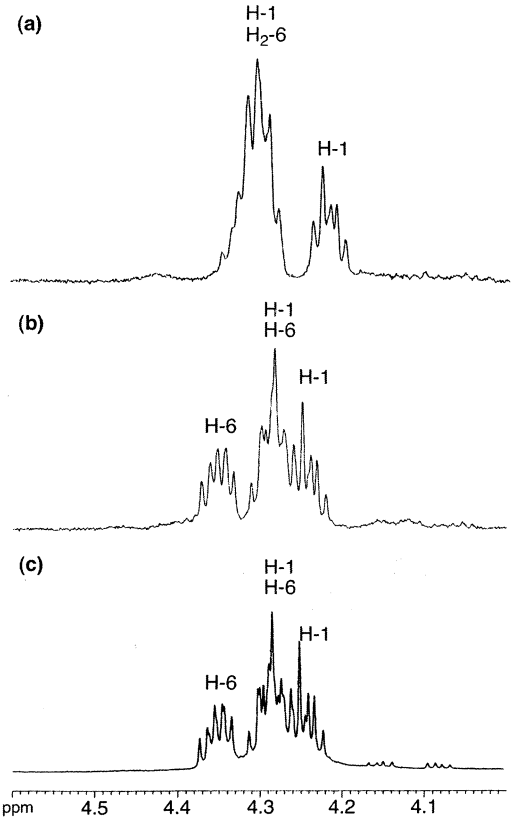

FIGURE 5. ^{1}H NMR spectra (partial) of (a) 1,6-bis-(*S*)- and (b) 1,6-bis-(*R*)-MTPA esters (**2a** and **2b**, respectively) of the C-1−C-6 segment derived from amphidinolide X (**1**) and (c) the 1,6-bis-(*R*)-MTPA ester (**2b**) of the synthetic C-1−C-6 segment.

indicating the 4*R*-configuration for the C-1−C-6 segment. Therefore, the absolute configurations at six chiral centers in amphidinolide X (**1**) were elucidated to be 4*S*, 10*S*, 11*R*, 16*S*, 17*R*, and 19*R*.

Biosynthesis of Amphidinolide X (1). The feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates with the dinoflagellate *Amphidinium* sp. (strain Y-42) were carried out under the following conditions: the dinoflagellate was cultured in 50 L of 1% ES-containing seawater treated with penicillin-streptomycin. Each labeled precursor (610 μM) was added to the culture solution in one portion 10 days after inoculation, and then the culture was harvested by centrifugation after 14 days. By the same separation procedure as described above, ^{13}C-labeled amphidinolide X (**1**) was obtained. ^{13}C-Enrichments derived from labeled precursors were estimated as ca. 5% on the basis of the relative intensity of satellite peaks in the ^{1}H NMR spectra. Isotopic incorporation results of **1** are presented in Table 2. In the ^{13}C NMR spectrum of **1** enriched by [1-^{13}C] sodium acetate, significant enrichments of 8 out of 26 carbons were observed as follows: C-1, C-4, C-9, C-11, C-14, C-16, C-19, and C-21. Enrichments by [2-^{13}C] sodium acetate were observed for the remaining eighteen carbons (C-2, C-3, C-5, C-6, C-7, C-8, C-10, C-12, C-13, C-15, C-17, C-18, C-20, C-22, C-23, C-24, C-25, and C-26). Judging from *J*(C−C) values as well as INADEQUATE correlations, eight acetate units were directly incorporated for C-1−C-2 (75 Hz), C-4−C-5 (32 Hz), C-9−C-10 (40 Hz), C-11−C-12 (44 Hz), C-14−C-15 (34 Hz), C-16−C-17 (39 Hz), C-19−C-20 (39 Hz), and C-21−C-22 (34 Hz) as illustrated in Figure 6.

TABLE 2. Isotope Incorporation Results Based on ^{13}C NMR Data for Amphidinolide X (1) in $CDCl_3$

position	[1-^{13}C]-acetate[a]	[2-^{13}C]-acetate[a]	assignment c or m[b]
1	1.61	1.10	c
2	0.83	2.01	m
3	1	3.22	m
4	1.56	1	c
5	0.93	2.45	m
6	0.98	2.54	m
7	0.68	1.91	m
8	0.91	2.41	m
9	2.02	1.17	c
10	0.81	2.51	m
11	1.59	1.07	c
12	0.89	2.44	m
13	1.05	1.92	m
14	1.67	1.23	c
15	1.03	3.76	m
16	1.70	1.50	c
17	0.78	2.05	m
18	0.91	2.39	m
19	1.58	1.02	c
20	0.76	2.95	m
21	1.87	1.20	c
22	0.90	2.91	m
23	0.73	2.31	m
24	0.74	2.52	m
25	0.79	2.08	m
26	0.91	2.50	m

[a] Intensity ratios of each peak in the labeled **1** divided by that of the corresponding signal in the unlabeled **1**, respectively, normalized to give a ratio of 1 for the unenriched peak (C-3 for [1-^{13}C]-acetate labeling and C-4 for [2-^{13}C]-acetate labeling). [b] The "c" denotes the carbon derived from the C-1 of acetate, while the "m" indicates the carbon derived from the C-2 of acetate.

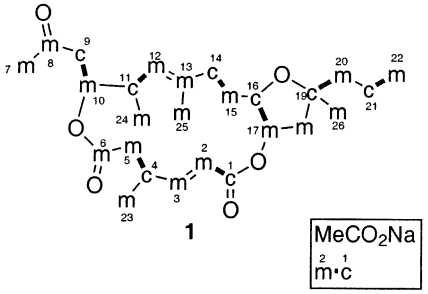

FIGURE 6. Labeling patterns of amphidinolide X (**1**) resulting from feeding experiments with ^{13}C-labeled acetates.

The incorporation patterns for the C-7−C-22 part were revealed to be "**m**−**m**−**c**−**m**−**c**(−**m**)−**m**−**m**(−**m**)−**c**−**m**−**c**−**m**−**m**−**c**(−**m**)−**m**−**c**−**m**", in which three diacetate units, five isolated C_1 units (C-13, C-18, C-24, C-25, and C-26) from the methyl carbon (C-2) of acetates, and a "**m**−**m**" unit (C-7−C-8) derived only from C-2 of acetates were included. Thus, the C-7−C-22 part is suggested to be a nonsuccessive mixed polyketide. A unique labeling pattern, "**c**−**m**−**m**−**c**", for the tetrahydrofuran ring portion in **1** was observed, while those of amphidinolides C[21] and T1,[22] okadaic acid,[23] and goniodomin A[24] were "**m**−**c**−**m**−**m**", "**m**−**c**−**m**−**c**", "**m**−**c**−**m**−**c**", and "**c**−**m**−**c**−**m**", respectively.

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Conclusion

To our knowledge, all macrodiolides isolated from marine and terrestrial sources consist of two molecules[24] of homogeneous or heterogeneous hydroxy fatty acids,[25] while amphidinolide X (**1**) is the first macrodiolide natural product consisting of polyketide-derived diacid and diol units. Furthermore, it is noted that amphidinolide X (**1**) is the second example of amphidinolides having no exomethylene.[4a] Amphidinolide X (**1**) exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro with IC_{50} values of 0.6 and 7.5 μg/mL, respectively.

Experimental Section

Material. The dinoflagellate *Amphidinium* sp. (strain number Y-42) was separated from the inside cells of the marine acoel flatworm *Amphiscolops* sp., which was collected off Sunabe, Okinawa. The culture was maintained in IMK medium (Nippon Seiyaku, Osaka, Japan) at 20 °C under an illumination of about 50 μmol photons·m^{-2}·s^{-1} with a 16:8 light:dark cycle. Total genomic DNA was extracted from the cultured cells, according to the method published by Horiguchi et al.[26] The small subunit ribosomal RNA gene (SSU rDNA) was amplified by using the primer pairs described previously,[26] and both coding and noncoding strands were sequenced with use of a DNA Sequencer. The DNA sequence was compared with those of the SSU rDNA in the databases by using BLAST SEARCH, and the SSU rDNA of *Amphidinium belauense* (accession No. L13719), which was originally described as a symbiont of a flatworm, *Haplodiscus* sp.,[27] was found to be the closest relative (>99% identity). The sequence data of this strain Y42 have been submitted to the DDBJ/EMBL/GenBank under accession No. AB107845.

Cultivation and Isolation. The dinoflagellate was unialgally cultured at 25 °C for two weeks in seawater medium enriched with 1% ES supplement, 16 h light and 8 h dark. The harvested cells (363 g, wet weight, from 686 L of culture) were extracted with MeOH/toluene (3:1, 3 × 400 mL). After addition of 1 M aq NaCl (500 L), the mixture was extracted with toluene (3 × 400 mL). The toluene-soluble fractions (3.79 g) were subjected to a silica gel column ($CHCl_3$/MeOH, 98:2) to afford a macrolide-containing fraction (765 mg). Treatment of this fraction with a 2 M solution of $TMSCHN_2$ in hexane (1 mL) and then silica gel column chromatography (hexane/EtOAc, 2:1) afforded a crude macrolide fraction, which was separated with a Sep-Pak cartridge C_{18} (MeOH/H_2O, 8:2) followed 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 230 nm] to give amphidinolide X (**1**, 3.6 mg, 0.001%, wet weight, t_R = 17.0 min) together with amphidinolide W (0.0012%, t_R = 15.4 min). Amphidinolides G (0.0008%) and H (0.0007%) have been isolated from another fraction.

Amphidinolide X (1): colorless oil; $[\alpha]^{17}_D$ −12° (*c* 1.0, $CHCl_3$); UV (EtOH) λ_{max} 209 nm (ϵ 6800); IR (neat) ν_{max} 2921 and 1718 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m*/*z* 449 $(M + H)^+$ and 471 $(M + Na)^+$; HRESIMS *m*/*z* 471.2701 [calcd for $C_{26}H_{40}O_6Na$ $(M + Na)^+$, 471.2723].

Reduction of Amphidinolide X (1) with $LiAlH_4$. Amphidinolide X (**1**, 1.0 mg) was dissolved in THF (120 μL) and treated with $LiAlH_4$ (1.7 mg, 14 μmol) at 4 °C for 4 h. To the reaction mixture was added 1 M phosphate buffer (100 μL) and the solution was then extracted with EtOAc (3 × 200 μL×). The organic phase was evaporated in vacuo to afford a mixture of reduction products of **1** (0.9 mg). To a solution of the mixture (0.4 mg) in a 1% DMAP solution in CH_2Cl_2 (50 μL) were added Et_3N (5 μL) and (*R*)-(−)-MTPACl (1.5 μL), and the mixture was stirred at 4 °C for 15 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (5 μL), the solvent was evaporated in vacuo. The residue was passed through a silica gel column (hexane/acetone, 5:1) and purified by C_{18} HPLC (Wakosil-II 5C18 RS, Wako Pure Chemical Ind., Ltd., 4.6 × 250 mm; eluent CH_3CN/H_2O, 92:8; flow rate, 1.0 mL/min; UV detection at 230 nm) to give the 1,6-bis-(*S*)-MTPA ester of the C-1−C-6 segment (**2a**, 0.08 mg, t_R = 6.4 min) and the 8,10,17-tris-(*S*)-MTPA ester of the (8*S*)-C-7−C-22 segment (**3a**, 0.12 mg, t_R = 24.8 min). The 1,6-bis-(*R*)-MTPA ester of the C-1−C-6 segment (**2b**) and the 8,10,17-tris-(*R*)-MTPA esters of the (8*S*)- and (8*R*)-C-7−C-22 segments (**3b** and **4**, respectively) were prepared from the another batch (0.4 mg) of reductive products through the same procedure as described above.

1,6-Bis-(*S*)-MTPA ester of the C-1−C-6 segment (2a): colorless oil; ^1H NMR ($CDCl_3$) δ 0.87 (3H, d, J = 6.0 Hz, H_3-23), 1.13 (1H, m, H-3), 1.30 (1H, m, H-3), 1.47 (1H, m, H-4), 1.61 (2H, m, H_2-2), 1.67 (2H, m, H_2-5), 3.53 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 4.24 (1H, m, H-1), 4.29 (1H, m, H-1), 4.33 (1H, m, H-6), 4.38 (1H, m, H-6), 7.36−7.42 (6H, m, Ph), and 7.46−7.55 (4H, m, Ph); FABMS *m*/*z* 565 $(M + H)^+$; HRFABMS *m*/*z* 565.2001 [calcd for $C_{27}H_{31}O_6F_6$ $(M + H)^+$, 565.2025].

1,6-Bis-(*R*)-MTPA ester of the C-1−C-6 segment (2b): colorless oil; ^1H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 6.0 Hz, H_3-23), 1.14 (1H, m, H-3), 1.30 (1H, m, H-3), 1.46 (1H, m, H-4), 1.61 (2H, m, H_2-2), 1.63 (2H, m, H_2-5), 3.539 (3H, s, OCH_3), 3.543 (3H, s, OCH_3), 4.22 (1H, m, H-1), 4.25 (1H, m, H-6), 4.27 (1H, m, H-1), 4.32 (1H, m, H-6), 7.35−7.42 (6H, m, Ph), and 7.47−7.55 (4H, m, Ph); FABMS *m*/*z* 565 $(M + H)^+$; HRFABMS *m*/*z* 565.2003 [calcd for $C_{27}H_{31}O_6F_6$ $(M + H)^+$, 565.2025].

8,10,17-Tris-(*S*)-MTPA ester of the (8*S*)-C-7−C-22 segment (3a): colorless oil; ^1H NMR ($CDCl_3$) δ 0.91 (3H, d, J = 6.2 Hz, H_3-24), 0.92 (3H, t, J = 7.3 Hz, H_3-22), 1.15 (3H, s, H_3-26), 1.19 (3H, d, J = 6.2 Hz, H_3-7), 1.34 (2H, m, H_2-21), 1.53 (2H, m, H_2-20), 1.60 (2H, m, H_2-15), 1.60 (3H, s, H_3-25), 1.70 (1H, m, H-9), 1.80 (1H, dd, J = 2.5 and 14.0 Hz, H-18), 1.96 (1H, m, H-9), 1.97 (1H, m, H-14), 2.05 (1H, m, H-14), 2.18 (1H, dd, J = 7.7 and 14.0 Hz, H-18), 2.74 (1H, m, H-11), 3.52 (6H, s, 2 × OCH_3), 3.53 (3H, s, OCH_3), 3.88 (1H, m, H-16), 4.93 (1H, m, H-12), 4.96 (1H, m, H-8), 5.01 (1H, m, H-10), 5.09 (2H, m, H-17), 7.36−7.42 (9H, m, Ph), and 7.46−7.55 (6H, m, Ph); FABMS *m*/*z* 977 $(M + H)^+$ and 999 $(M + Na)^+$; HRFABMS *m*/*z* 977.3887 [calcd for $C_{49}H_{58}O_{10}F_9$ $(M + H)^+$, 977.3886].

8,10,17-Tris-(*R*)-MTPA ester of the (8*S*)-C-7−C-22 segment (3b): colorless oil; ^1H NMR ($CDCl_3$) δ 0.76 (3H, d, J = 6.8 Hz, H_3-24), 0.91 (3H, t, J = 7.3 Hz, H_3-22), 1.10 (3H, s, H_3-26), 1.32 (3H, d, J = 6.2 Hz, H_3-7), 1.33 (2H, m, H_2-21), 1.47 (2H, m, H_2-20), 1.60 (2H, m, H_2-15), 1.61 (3H, s, H_3-25), 1.67 (1H, m, H-9), 1.73 (1H, dd, J = 2.4 and 14.1 Hz, H-18), 1.98 (1H, m, H-9), 1.99 (1H, m, H-14), 2.03 (1H, m, H-14), 2.14 (1H, dd, J = 7.6 and 14.1 Hz, H-18), 2.63 (1H, m, H-11), 3.53 (6H, s, 2 × OCH_3×), 3.54 (3H, s, OCH_3), 3.97 (1H, m, H-16),

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4.92 (1H, d, J = 9.5 Hz, H-12), 4.96 (1H, m, H-10), 5.07 (1H, m, H-8), 5.09 (2H, m, H-17), 7.35−7.42 (9H, m, Ph), and 7.45−7.54 (6H, m, Ph); ESIMS m/z 999 $(M + Na)^+$; HRESIMS m/z 999.3713 [calcd for $C_{49}H_{57}O_{10}F_9Na$ $(M + Na)^+$, 999.3704].

8,10,17-Tris-(*R*)-MTPA ester of the (8*R*)-C-7−C-22 segment (4): colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.78 (3H, d, J = 6.9 Hz, H_3-24), 0.91 (3H, t, J = 7.3 Hz, H_3-22), 1.10 (3H, s, H_3-26), 1.24 (3H, d, J = 6.2 Hz, H_3-7), 1.31 (2H, m, H_2-21), 1.48 (2H, m, H_2-20), 1.57 (2H, m, H_2-15), 1.59 (3H, s, H_3-25), 1.72 (1H, dd, J = 2.5 and 14.0 Hz, H-18), 1.77 (2H, m, H_2-9), 1.97 (1H, m, H-14), 2.02 (1H, m, H-14), 2.13 (1H, dd, J = 7.3 and 14.0 Hz, H-18), 2.75 (1H, m, H-11), 3.52 (6H, s, 2 × OCH_3), 3.53 (3H, s, OCH_3), 3.95 (1H, m, H-16), 4.86 (1H, d, J = 8.8 Hz, H-12), 5.00 (1H, m, H-8), 5.02 (1H, m, H-10), 5.08 (2H, m, H-17), 7.35−7.41 (9H, m, Ph), and 7.46−7.53 (6H, m, Ph); ESIMS m/z 999 $(M + Na)^+$; HRESIMS m/z 999.3713 [calcd for $C_{49}H_{57}O_{10}F_9Na$ $(M + Na)^+$, 999.3704].

(2*S*)-4-Benzyloxymethyloxy-2-methylbutan-1-ol (6). To a solution of methyl (2*S*)-4-hydroxy-2-methylbutyrate (434.6 mg, 3.29 mmol) in CH_2Cl_2 (1.5 mL) were added diisopropylethylamine (920 μL, 6.58 mmol) and BOMCl (688 μL, 4.94 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 3.5 h. After addition of 1 N aqueous HCl, the mixture was extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and H_2O, dried with $MgSO_4$, and evaporated to afford the crude BOM ether (1028 mg). To a solution of the crude BOM ether (1028 mg) in ether (2 mL) was added a suspension of $LiAlH_4$ (140 mg, 3.68 mmol) in ether (2 mL) at 0 °C, and stirring was continued for 30 min. After addition of H_2O, 15% aqueous NaOH, and then H_2O and filtration of insoluble materials, the filtrate was evaporated, and then subjected to silica gel column chromatography (hexane/EtOAc, 90:10 → 60:40) to afford alcohol **6** (519 mg, 2.32 mmol, 71% by two steps) asa colorless oil: $[\alpha]^{24}_D$ −4° (*c* 2.0, $CHCl_3$); IR (neat) ν_{max} 3425, 3031, 2929, 2875, 1456, 1381, 1109, and 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, d, J = 6.8 Hz), 1.53 (1H, m), 1.71 (1H, m), 1.82 (1H, m), 3.45−3.54 (2H, m), 3.65 (1H, m), 3.71 (1H, m), 4.61 (2H, s), 4.76 (2H, s), 7.30 (1H, m), and 7.32−7.39 (5H, m); ^{13}C NMR ($CDCl_3$) δ 16.96, 33.58, 33.69, 66.24, 68.08, 69.54, 94.65, 127.72, 127.86 (2C), 128.44 (2C), and 137.77; FABMS m/z 225 $(M + H)^+$; HRFABMS m/z 225.1471 [calcd for $C_{13}H_{21}O_3$ $(M + H)^+$, 225.1491].

Ethyl (4*S*)-6-Benzyloxymethyloxy-4-methyl-2-hexenoate (7). A solution of alcohol **6** (118.5 mg, 530 μmol) in THF (1.5 mL) was treated with IBX (225 mg, 795 μmol) in DMSO (3 mL) at room temperature for 3.5 h. After addition of H_2O and filtration of precipitates, the filtrate was extracted with ether. The extract was washed with brine, dried with Na_2SO_4, and evaporated to afford a crude aldehyde (75.6 mg). To a solution of the aldehyde (76.0 mg) in benzene (2.5 mL) was added (ethoxycarbonylmethylene)triphenylphosphorane (300 mg, 900 μmol), and stirring was continued at room temperature for 14 h. After evaporation of the solvent, the residue was subjected to silica gel column chromatography (hexane/EtOAc, 9:1) to afford compound **7** (77.0 mg, 264 mmol, 50% by two steps) as a colorless oil; $[\alpha]^{24}_D$ +21° (*c* 2.0, $CHCl_3$); IR (neat) ν_{max} 3030, 2932, 2875, 1718, 1651, 1455, 1371, 1271, 1217, 1181, 1112, and 1051 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.09 (3H, d, J = 6.8 Hz), 1.28 (3H, t, J = 7.2 Hz), 1.68 (2H, m), 2.52 (1H, m), 3.58 (2H, m), 4.18 (2H, q, J = 7.2 Hz), 4.59 (2H, s), 4.74 (2H, s), 5.81 (1H, d, J = 15.5 Hz), 6.87 (1H, dd, J = 8.0 and 15.5 Hz), 7.30 (1H, m), and 7.32−7.39 (5H, m); ^{13}C NMR ($CDCl_3$) δ 14.26, 19.41, 33.43, 35.74, 60.21, 65.67, 69.45, 94.72, 120.13, 127.69, 127.86 (2C), 128.43 (2C), 137.85, 153.62, and 166.70; FABMS m/z 293 $(M + H)^+$ and 315 $(M + Na)^+$; HRFABMS m/z 293.1750 [calcd for $C_{17}H_{25}O_4$ $(M + H)^+$, 293.1752].

Synthetic 1,6-Bis-(*R*)-MTPA Ester of the C-1−C-6 Segment (2b). To the solution of unsaturated ester (39.2 mg, 134 μmol) in ether (2 mL) was added $LiAlH_4$ (10 mg, 263 μmol) at 0 °C, and stirring was continued for 3 h. After addition of H_2O and then 15% aqueous NaOH, the insoluble materials were filtered, and the filtrate was evaporated to afford an crude alcohol (26.8 mg, 107 μmol, 80%). To a solution of the alcohol (12.6 mg, 50.4 μmol) in MeOH (0.5 mL) was added 10% Pd/C (5 mg), and stirring was continued under H_2 atmosphere at room temperature for 14 h. After filtration of insoluble materials, evaporation of the solvent gave the crude C-1−C-6 segment (5.6 mg, 47.5 μmol, 94%). To a solution of the segment (1.4 mg, 11.9 μmol) in CH_2Cl_2 (100 μL) were added DMAP (0.1 mg), Et_3N (10 μL), and (*S*)-(+)-MTPACl (7 μL), and stirring was continued at room temperature for 14 h. After addition of *N*,*N*-dimethyl-1,3-propanediamine (10 μL) and evaporation of the solvent, a fourth of the residue was subjected to C_{18} HPLC (Wakosil-II 5C18 RS, Wako Pure Chemical Ind., Ltd., 4.6 × 250 mm; eluent CH_3CN/H_2O, 92:8; flow rate, 1.0 mL/min; UV detection at 230 nm) to give the 1,6-bis-(*R*)-MTPA ester of the C-1−C-6 segment (**2b**, 0.5 mg, 0.89 μmol, 30%, t_R = 6.0 min) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 6.0 Hz, H_3-23), 1.14 (1H, m, H-3), 1.30 (1H, m, H-3), 1.46 (1H, m, H-4), 1.61 (2H, m, H_2-2), 1.63 (2H, m, H_2-5), 3.539 (3H, s, OCH_3), 3.543 (3H, s, OCH_3), 4.22 (1H, m, H-1), 4.25 (1H, m, H-6), 4.27 (1H, m, H-1), 4.32 (1H, m, H-6), 7.35−7.42 (6H, m, Ph), and 7.47−7.55 (4H, m, Ph); FABMS m/z 565 $(M + H)^+$; HRFABMS m/z 565.2012 [calcd for $C_{27}H_{31}O_6F_6$ $(M + H)^+$, 565.2025].

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 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 X (**1**) from the harvested cells were carried out through the same procedure as described above. The ^{13}C-labeled amphidinolide X (**1**) was obtained in 0.001% yield as an average from the wet weight of the cells.

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Supporting Information Available: Spectral data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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