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Studies on the Macrolides from Marine Dinoflagellate *Amphidinium* sp.: Structures of Amphidinolides R and S and a Succinate Feeding Experiment

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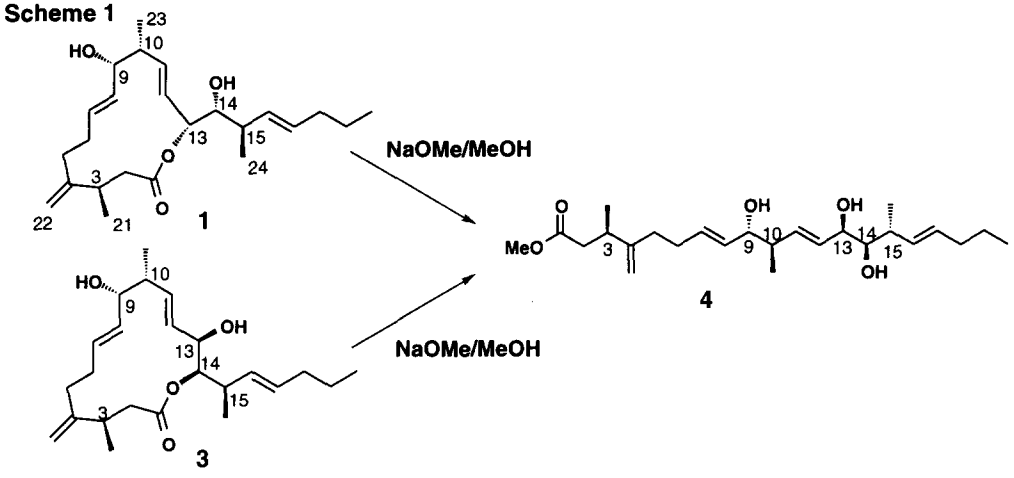
Abstract: Two new cytotoxic macrolides, amphidinolides R (**1**) and S (**2**), were isolated from the cultured marine dinoflagellate *Amphidinium* sp. and their structures including absolute configuration were elucidated on the basis of spectroscopic data as well as chemical experiments. In a feeding experiment of ^{13}C-labeled succinic acid into a culture of *Amphidinium* sp., no enrichment of the ^{13}C NMR signal intensity of any carbon of amphidinolide J (**3**), the most abundant macrolide in this dinoflagellate, was observed. © 1997 Elsevier Science Ltd.

During our continuing studies on bioactive substances from marine microalgae, we previously isolated a series of cytotoxic macrolides, named amphidinolides, possessing unique structural features from dinoflagellates of the genus *Amphidinium*.[1] We further investigated the constituents of this microalga (strain number, Y-5), which was a symbiont of Okinawan marine acoel flatworm of the genus *Amphiscolops*, and now isolated two new cytotoxic macrolides, amphidinolides R (**1**) and S (**2**). This paper deals with the isolation and structure elucidation of them possessing related structures to amphidinolide J (**3**), a 15-membered macrolide isolated from the same dinoflagellate.[2] Result of feeding experiments of ^{13}C-labeled succinic acid into the culture of the dinoflagellate *Amphidinium* sp. was also described here.

The harvested algal cells (1205 g, wet weight, from 4956 L of culture) were extracted with MeOH/toluene (3:1) and partitioned between toluene and water. The toluene-soluble fraction was subjected to a silica gel column ($CHCl_3$/MeOH, 95:5), and the macrolide-containing fractions were subsequently separated by reversed phase chromatography on ODS (80% MeOH) and gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1). Further purifications by HPLC using reversed and normal phase columns yielded amphidinolides R (**1**, 0.0005%, wet weight) and S (**2**, 0.0001%) together with the known macrolide, amphidinolide J (**3**, 0.003%).

Amphidinolide R (**1**) was isolated as a colorless oil and its molecular formula was determined as $C_{24}H_{38}O_4$ by HRFABMS [*m/z* 391.2834 $(M+H)^+$, Δ -1.4 mmu]. The IR spectrum suggested the presence of hydroxyl (3400 cm^{-1}) and ester (or lactone) (1720 cm^{-1}) groups, and its UV spectrum showed no characteristic absorptions. The ^1H and ^{13}C NMR spectral data of **1** (Table 1) were revealed to be similar to those of amphidinolide J (**3**),[2] containing one ester carbonyl, one exomethylene, six sp^2 methine carbons, three oxymethines, three unoxygenated methines, five sp^3 methylenes, and four methyl groups. The ^1H-^1H COSY spectrum of **1** showed correlations almost throughout the molecule (from H_2-2 to H_2-18 and from H_2-19 to H_3-20), and the connection between C-18 and C-19 was indicated from the HMBC cross-peak for H_3-20/C-18. From the ^1H-^1H COSY, HMQC, and HMBC spectral data of **1**, the positions of secondary methyls, exomethylenes and other olefins, and oxymethines of **1** were deduced to be parallel to those of

Scheme 1



amphidinolide J (**3**). Of the three oxymethine protons, the H-13 resonated particularly in the low-field (δ_H 5.44), implying that the ester oxygen on C-1 was connected to C-13. Thus, the structure of amphidinolide R was elucidated as **1** having a 14-membered lactone moiety with different location of the lactone linkage from that of **3**. Although attempts for acyl migration of **3** into **1** were unsuccessful by treatment with *p*-TsOH[3] or

Table 1. ^1H and ^{13}C NMR Data of Amphidinolides R (**1**), S (**2**), and J (**3**)[a] and Result of Incorporation Experiment with Disodium [$^{13}C_2$] Succinate

	1			**2**			**3**			ratio of relative
position	δ_H (C_6D_6)	δ_C (C_6D_6)	position	δ_H (C_6D_6)	δ_C (C_6D_6)	position	δ_H (C_6D_6)	δ_H (DMF-d_7)	δ_C (C_6D_6)	intensity[b]
1		169.7	1		171.2	1			171.6	0.79
2 (a)	2.31 dd	40.7	2 (a)	2.49 dd	40.4	2 (a)	2.52 dd	2.62 dd	39.9	1.10
(b)	2.19 dd		(b)	2.21 dd		(b)	2.21 dd	2.30 dd		
3	2.63 m	39.1	3	2.59 m	33.7	3	2.60 m	2.54 m	34.6	1.01
4		148.4	4		152.8	4			151.9	0.94
5 (a)	2.11 m	28.3	5 (a)	2.04 m	35.0	5 (a)	2.07 m	2.13 m	36.1	1.08
(b)	1.86 m		(b)	1.89 m		(b)	1.78 m	1.86 m		
6 (2H)	2.22 m	28.3	6 (a)	2.41 m	28.5	6 (a)	2.33 m	2.21 m	29.7	0.96
			(b)	1.97 m		(b)	2.07 m	2.08 m		
7	5.38 m	130.7	7	6.75 dt	146.4	7	5.15 ddd	5.27 m	130.8	1.18
8	5.29 dd	130.9	8	6.19 d	130.1	8	5.52 dd	5.27 m	136.5	1.36
9	4.05 br s	76.4	9		198.9	9	3.82 dd	3.86 br s	78.8	1.42
10	2.45 m	41.9	10	3.44 m	47.1	10	2.07 m	1.98 m	45.7	1.03
11	5.92 dd	138.3	11	5.38 dd	132.9	11	5.67 dd	5.52 dd	133.5	1.28
12	5.36 dd	125.0	12	5.53 dd	133.4	12	5.41 dd	5.39 dd	132.6	0.93
13	5.44 m	77.0	13	3.99 m	71.7	13	4.10 dd	4.21 td	72.6	1.10
14	3.47 br t	75.0	14	4.77 dd	79.1	14	4.94 dd	4.67 dd	79.9	1.33
15	2.39 m	39.7	15	2.73 m	39.1	15	2.77 m	2.66 m	39.5	1.10
16	5.57 dd	131.2	16	5.35 dd	132.2	16	5.34 dd	5.23 dd	133.6	0.93
17	5.46 m	132.3	17	5.48 dt	131.4	17	5.50 dt	5.44 dt	131.5	1.26
18 (2H)	1.95 m	35.0	18 (2H)	1.94 m	35.0	18 (2H)	1.91 m	1.93 m	35.3	1.33
19 (2H)	1.35 m	22.9	19 (2H)	1.36 m	22.9	19 (2H)	1.32 m	1.35 m	23.4	1.16
20 (3H)	0.89 t	13.8	20 (3H)	0.89 t	13.8	20 (3H)	0.84 t	0.89 t	14.2	1
21 (3H)	0.85 d	20.8	21 (3H)	0.89 d	21.9	21 (3H)	0.87 d	1.04 d	22.2	1.06
22 (a)	5.07 s	110.5	22 (a)	4.89 s	109.7	22 (a)	4.82 s	4.89 s	108.7	1.44
(b)	4.74 s		(b)	4.67 s		(b)	4.44 s	4.47 s		
23 (3H)	1.10 d	11.1	23 (3H)	1.30 d	16.3	23 (3H)	1.26 d	1.09 d	19.0	1.23
24 (3H)	1.18 d	18.2	24 (3H)	0.96 d	17.2	24 (3H)	0.96 d	1.08 d	17.5	1.26

[a] *J*(H/H) in Hz. **1**: 2a/2b=14.4, 2a/3=11.9, 2b/3=4.8, 3/21(3H)=7.0, 7/8=15.8, 8/9=6.6, 10/11=5.0, 10/23(3H)=6.9, 11/12=15.8, 12/13=7.5, 13/14=4.2, 14/15=4.2, 15/16=8.6, 15/24(3H)=6.9, 16/17=15.3, 17/18=6.8, and 19/20=7.4; **2**: 2a/2b=16.0, 2a/3=11.1, 2b/3=3.4, 3/21(3H)=7.1, 6(2H)/7=6.9, 7/8=16.0, 10/11=8.6, 10/23(3H)=6.7, 11/12=15.7, 12/13=7.0, 13/14=1.3, 14/15=9.1, 15/16=8.9, 15/24(3H)=6.7, 16/17=15.3, 17/18=6.7, and 19/20=7.2; **3** (C_6D_6): 2a/2b=15.9, 2a/3=12.4, 2b/3=2.9, 3/21(3H)=6.8, 6a/7=10.3, 6b/7=4.4, 7/8=15.0, 8/9=8.8, 9/10=1.5, 10/11=9.2, 10/23(3H)=7.0, 11/12=15.8, 12/13=8.1, 13/14=1.8, 14/15=9.5, 15/16=8.1, 15/24(3H)=7.0, 16/17=15.4, 17/18=7.0, 18/19=7.3, and 19/20=7.3; **3** (DMF-d_7): 2a/2b=15.7, 2a/3=12.4, 2b/3=2.6, 3/21(3H)=6.8, 8/9=6.0, 9/10=1.8, 10/11=9.5, 10/23(3H)=7.2, 11/12=15.5, 12/13=8.2, 13/13(OH)=8.2, 13/14=1.7, 14/15=10.0, 15/16=8.8, 15/24(3H)=6.6, 16/17=15.3, 17/18=6.8, 18/19=7.4, and 19/20=7.4.

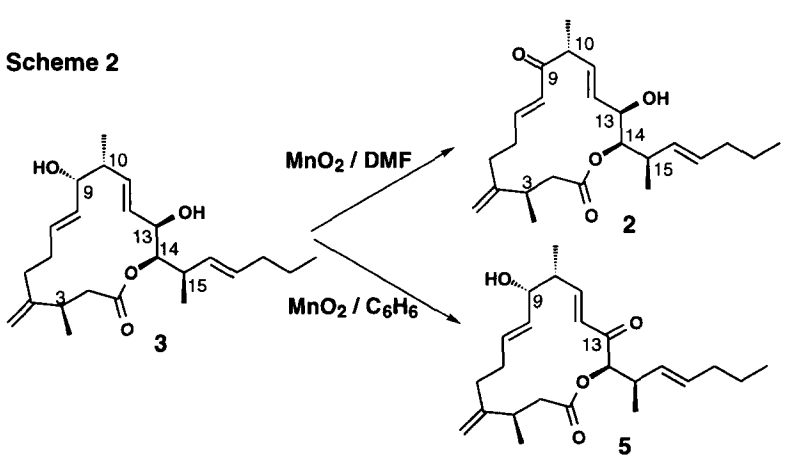
[b] Ratio of ^{13}C NMR signal intensity of **3** fed with disodium [$^{13}C_2$] succinate over natural abundance; ratio normalized to that of C-20 signal

$Ti(O\text{-}i\text{-}Pr)_4$,[4] treatment of each of **1** and **3** with sodium methoxide in methanol yielded an identical linear methyl ester (**4**, Scheme 1) on the basis of comparison of TLC, HPLC, and ^1H NMR as well as the sign of optical rotation. The evidence for the stereochemistry of six chiral centers (3*R*, 9*R*, 10*R*, 13*R*, 14*R*, 15*R*) in **1** was thus provided since the absolute stereochemistry of amphidinolide J (**3**) was already known.[2]

Amphidinolide S (**2**), also isolated as a colorless oil, had the molecular formula of $C_{24}H_{36}O_4$ as established by HRFABMS [*m/z* 389.2686 $(M+H)^+$, Δ -0.6 mmu], and showed a UV absorption (λ_{max} 224 nm) assignable to an α,β-unsaturated ketone. The ^1H and ^{13}C NMR data (Table 1) indicated that amphidinolide S (**2**) was also structurally related to amphidinolide J (**3**), containing the corresponding functionalities such as one exomethylene, three disubstituted olefins, and four methyl groups. It was also suggested that amphidinolide S (**2**) possesses a ketone group and one less oxymethine carbon than **3**. Interpretation of the ^1H-^1H COSY, HMQC, and HMBC spectral data of **2** revealed that the C-9 hydroxyl group in **3** was replaced by the ketone in **2** to form a conjugated enone, and structure of other part of molecule of **2** was parallel to that of amphidinolide J (**3**). This finding was further corroborated by the following experiments (Scheme 2). Treatment of amphidinolide J (**3**) with manganese dioxide in DMF at room temperature for 18 h afforded an oxidation product, which was identified to be amphidinolide S (**2**) by TLC, HPLC, and ^1H NMR analysis, and both were dextrorotatory. Thus, amphidinolide S (**2**) was revealed to be 9-dehydro derivative of amphidinolide J (**3**), possessing the same absolute configuration as amphidinolide R (**1**). Interestingly, when the MnO_2 oxidation was carried out in benzene solution, 13-keto derivative (**5**) was produced exclusively on the basis of HPLC analysis. The MnO_2 oxidation in DMF did not afford 13-keto derivative (**5**), and no 9,13-diketo derivative was detected in the oxidation products in either DMF or benzene solution. The ^1H NMR of amphidinolide J (**3**) in DMF-*d*7 was recorded and was compared with that in C_6D_6 solution (Table 1). The proton-proton coupling constants in both solutions were almost comparative; however, a small difference was observed only between H-8 and H-9 (in DMF-*d*7: $J_{8,9}$=6.0 Hz; in C_6D_6: $J_{8,9}$=8.8 Hz). Thus, a small difference in the conformation around the C-9 hydroxyl group of **3** in the two solvents might have resulted in the selective oxidation of C-9 or C-13 hydroxyl group depending on the solvent.

Amphidinolides R (**1**) and S (**2**) are two new cytotoxic 14- and 15-membered macrolides, respectively, possessing related structures to that of amphidinolide J (**3**).[2] Their structures including absolute

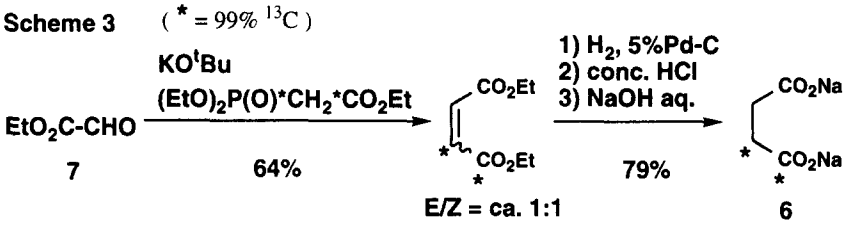
Scheme 2



stereochemistry were established on the basis of chemical derivatization experiments. Compounds **1** and **2** showed cytotoxicity against murine lymphoma L1210 (IC_{50}, 1.4 and 4.0 μg/mL) and human epidermoid carcinoma KB cells (IC_{50}, 0.67 and 6.5 μg/mL) in vitro, respectively.

We previously reported a biosynthetic study of amphidinolide J (**3**) by feeding experiments with ^{13}C-labeled sodium acetate precursors to show that all carbons in **3** were derived from acetates and the labeling pattern could not be accounted for by the classical polyketide pathway.[5] We proposed that the unusual labeling patterns of **3** were derived from participation of dicarboxylic acids[6]; *e.g.*, the C-1/C-2/C-3/C-21 unit labeled as "c-m-m-m"[7] may come from α-ketoglutarate while the C-10/C-11/C-12 unit labeled as "c-m-m" may be derived from succinate.[5] Wright and coworkers, however, recently described that the labeling pattern of **3** was explained by an oxidation and carbon deletion process in the polyketide chain which was proposed to proceed through flavin monooxygenase-mediated oxidation and a Favorski-type rearrangement.[8] Hence we tried feeding experiment of ^{13}C-labeled succinate into the dinoflagellate *Amphidinium* sp., especially amphidinolide J (**3**), the most predominant macrolide in this dinoflagellate. Disodium [1,2-$^{13}C_2$] succinate (**6**) was prepared from ethyl glyoxylate (**7**) and triethyl phosphonoacetate-$^{13}C_2$ as shown in Scheme 3. The ^{13}C-labeled succinate was fed to the alga (370 μM) in one portion 10 days after inoculation, then 2 days later the culture was harvested. Amphidinolide J (**3**) was isolated from the extract of the harvested cells by improved procedures,[5] and the ^{13}C NMR of **3** obtained by this experiment showed no enhancement of the signal intensities of any carbons as shown in Table 1. Particularly, increase in signal intensities for the C-10/C-11/C-12 and C-1/C-2/C-3/C-21 unit as well as observation of satellites due to the ^{13}C-^{13}C coupling for these units had been expected, but no appreciable difference in the ^{13}C NMR spectrum was detected. Thus, incorporation of succinate into amphidinolide J (**3**) was not observed in this experiment. Evidence for accounting for the unusual labeling pattern of acetates therefore remains unprovided.

Scheme 3 (* = 99% ^{13}C)



EXPERIMENTAL

General methods. Optical rotations were determined on a JASCO DIP-370 digital polarimeter and IR spectra were taken on a JASCO FT/IR-230 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and/or AMX-600 spectrometers. FAB mass spectra were obtained on a JEOL HX-110 spectrometer.

Isolation. The harvested cells of the cultured dinoflagellate *Amphidinium* sp. (1205 g, wet weight, from 4956 L of culture) were extracted with MeOH/toluene (3:1; 1 L x 3). After addition of 1 M NaCl (1.5 L), the mixture was extracted with toluene (500 mL x 4). The toluene-soluble fraction was evaporated under reduced pressure to give a residue (44 g), which was partially (26.7 g) subjected to a flash column

chromatography on silica gel (4.5 x 40 cm) eluted with $CHCl_3$/MeOH (95:5). The fraction eluting from 330 to 615 mL (4.1 g) was then partially (1.0 g) separated by flash chromatography on ODS (YMC-GEL ODS 60 A, I-40/60, 20 x 70 mm; 80% MeOH), and subsequently separated by gel filtration on Sephadex LH-20 (4.0 x 100 cm; $CHCl_3$/MeOH, 1:1) to give a macrolide-containing fraction (0.43 g). This fraction was further purified by reversed-phase chromatography (CPO-HS-221-20, Kusano Kagakukikai Co., 22 x 100 mm; flow rate, 2.5 mL/min; eluant, 60% CH_3CN), followed by reversed-phase HPLC (Develosil ODS-5, Nomura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; detection, RI (refractive index) and UV at 220 nm; eluant, 73% CH_3CN) to afford amphidinolide R (**1**, 1.1 mg, t_R 15.0 min) together with amphidinolide J (**3**, 6.8 mg, t_R 15.9 min). The fraction of this HPLC (1.7 mg, t_R 20.2 min) was subsequently separated by normal phase HPLC (YMC-Pack SIL-06, 4.6 x 250 mm; flow rate, 1.0 mL/min; eluant, hexane/2-propanol, 95:5) to give amphidinolide S (**2**, 0.2 mg, t_R 6.4 min).

Amphidinolide R (1): Colorless oil; $[\alpha]_D^{20}$ +23° (*c* 0.53, MeOH); IR (film) ν_{max} 3400 and 1720 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS (positive, matrix: glycerol) *m/z* 391 $(M+H)^+$; HRFABMS *m/z* 391.2834, Calcd for $C_{24}H_{39}O_4$: (M+H) 391.2848.

Amphidinolide S (2): Colorless oil; $[\alpha]_D^{20}$ +5° (*c* 0.17, MeOH); UV (MeOH) λ_{max} 224 nm (ε 6300); IR (film) ν_{max} 3450 and 1730 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS (positive, matrix: glycerol) *m/z* 389 $(M+H)^+$; HRFABMS *m/z* 389.2686, Calcd for $C_{24}H_{37}O_4$: (M+H) 389.2692.

Methyl Ester (4). A solution of amphidinolide J (**3**, 0.5 mg) in methanol (0.4 mL) was treated with a 28% sodium methoxide-methanol solution (20 μL) at room temperature for 15 h. After addition of water (1 mL), the mixture was extracted with EtOAc (1 mL x 4), and the EtOAc layer was dried over $MgSO_4$ and evaporated under reduced pressure to give a residue, which was purified with HPLC (Develosil ODS-5, Nomura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; eluant, 70% CH_3CN) to afford the methyl ester (**4**, 0.3 mg, t_R 16.8 min): colorless oil; $[\alpha]_D^{23}$ -75° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3440 and 1740 cm^{-1}; ^1H NMR (C_6D_6) δ 0.97 (3H, t, *J*=7.2 Hz), 1.01 (3H, d, *J*=7.1 Hz), 1.08 (3H, d, *J*=6.8 Hz), 1.17 (3H, d, *J*=7.0 Hz), 3.27 (1H, m), 3.39 (3H, s), 3.83 (1H, m), 4.07 (1H, m), 4.80 (1H, s), 4.84 (1H, s), 5.50 (2H, m), 5.59 (3H, m), and 5.83 (1H, dd, *J*=15.5 and 7.8 Hz); FABMS *m/z* 423 $(M+H)^+$; HRFABMS *m/z* 423.3098, Calcd for $C_{25}H_{43}O_5$: (M+H) 423.3110. Amphidinolide R (**1**, 0.5 mg) was also subjected to methanolysis by the same procedures as above to give the methyl ester (**4**, 0.4 mg), which was identified by comparison of ^1H NMR, TLC (silica gel; hexane/acetone, 3:1, Rf 0.31), HPLC (the same condition as above), and the sign of optical rotation.

MnO_2 Oxidation of 3 in DMF. Amphidinolide J (**3**, 1.5 mg) dissolved in DMF (0.4 mL) was treated with MnO_2 (100 mg) under argon atmosphere at rt for 18 h. After addition of ether (2 mL), insoluble material was removed by filtration, and the filtrate was concentrated *in vacuo* to give a residue, which was separated by HPLC (Develosil ODS-5, Nomura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; eluant, 73% CH_3CN) to afford amphidinolide S (**2**, 0.1 mg, t_R 20.2 min) together with starting amphidinolide J (**3**, 0.1 mg, t_R 15.9 min). Amphidinolide S (**2**) was identified by ^1H NMR, TLC (silica gel; hexane/acetone, 3:1, Rf 0.60), HPLC (the same condition as above), and the sign of optical rotation.

MnO_2 Oxidation of 3 in Benzene. Amphidinolide J (**3**, 1.1 mg) dissolved in benzene (0.25 mL) was treated with MnO_2 (100 mg) under argon atmosphere at rt for 18 h. After addition of ether (2 mL), insoluble material was removed by filtration, and the filtrate was concentrated *in vacuo* to give a residue, which was separated by HPLC (Develosil ODS-5, Nomura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; eluant,

73% CH_3CN) to give 13-dehydroamphidinolide J (**5**, 0.1 mg, t_R 40.0 min) together with starting amphidinolide J (**3**, 0.1 mg, t_R 15.9 min). 13-Dehydroamphidinolide J (**5**): colorless oil; UV (MeOH) λ_{max} 239 nm (ε 8000); IR (film) ν_{max} 3450, 1740, and 1690 cm^{-1}; ^1H NMR (C_6D_6) δ 7.29 (1H, m), 6.31 (1H, d, *J*=15.5 Hz), 5.55 (1H, dd, *J*=15.2 and 7.6 Hz), 5.48 (1H, d, *J*=15.2 and 6.4 Hz), 5.25 (1H, ddd, *J*=15.5, 9.3, and 1.3 Hz), 5.15 (1H, d, *J*=15.5, 7.5, and 5.6 Hz), 4.96 (1H, d, *J*=4.6 Hz), 4.87 (1H, s), 4.50 (1H, s), 3.78 (1H, m), 2.93 (1H, m), 1.17 (3H, d, *J*=6.9 Hz), 1.09 (3H, d, *J*=6.9 Hz), 0.87 (3H, d, *J*=6.7 Hz), and 0.86 (3H, d, *J*=7.3 Hz); FABMS (positive, matrix: glycerol) *m/z* 389 $(M+H)^+$; HRFABMS *m/z* 389.2690, Calcd for $C_{24}H_{37}O_4$: (M+H) 389.2692.

Preparation of Disodium [$^{13}C_2$] Succinate (6). Ethyl glycolate (**7**, monomer) was prepared by distilllation under reduced pressure (80 °C/153 mmHg) in the presence of 85% phosphoric acid (0.1% w/w) from its polymer form (Tokyo Kasei Kogyo Co. Ltd., G0264). A solution of triethyl phosphonoacetate-$^{13}C_2$ (99 atom % ^{13}C, Aldrich 28384-3; 5 g) in THF (30 mL) was added to a suspension of potassium *tert*-butoxide (2.02 g) in THF (40 mL), and the mixture was stirred at room temperature for 40 min. The reaction mixture was cooled at - 60 °C, and the soultion of ethyl glycolate (**7**, monomer, 3.06 g) obtained as above in THF (30 mL) was added. The mixture was stirred for 2 h at - 40 °C and then 30 min at - 20 °C. After addition of brine (50 mL), the mixture was extracted with EtOAc (50 mL x 3), dried over $MgSO_4$, and evaporated under reduced pressure to give a residue, which was purified with silica gel column chromatography (hexane/EtOAx, 40:1) to give a 1:1 mixture of [1,2-$^{13}C_2$] diethyl maleate and [1,2-$^{13}C_2$] diethyl fumarate (2.50 g, 64% yield based on ^{13}C-labeled reagent). The 1:1 *E/Z* mixture (2.50 g) in ethanol (50 mL) was treated under hydrogen atmosphere in the presence of 5% Pd-C (210 mg) at room temperature for 6 h. After filtration of the catalyst through celite, the filtrate was evaporated under reduced pressure to give [1,2-$^{13}C_2$] diethyl succinate, which was hydrolyzed by treatment with conc. hydrochloric acid (5 mL) at 40 °C for 4 h. Evaporation of the mixture in vacuo afforded [1,2-$^{13}C_2$] succinic acid, which was neutralized with 3 M NaOH aqueous solution, and the solution was lyophilized to give disodium [1,2-$^{13}C_2$] succinate (1.87 g, 79% in 3 steps).

Incubation with Disodium [$^{13}C_2$] Succinate (6). Culturing was carried out by essentially the same conditions as those described previously.[9] On day 10 after inoculation, the labeled succinate (370 μM) was added, and the culture was harvested on day 12. The harvested algal cells (28.4 g, wet weight, from 80 L of culture) was extracted and purified by the procedures described previously[5] to give amphidinolide J (**3**, 0.6 mg).

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