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AMPHIDINOLIDE C3, A NEW CYTOTOXIC 25-MEMBERED MACROLIDE FROM MARINE DINOFLAGELLATE *AMPHIDINIUM* SP.

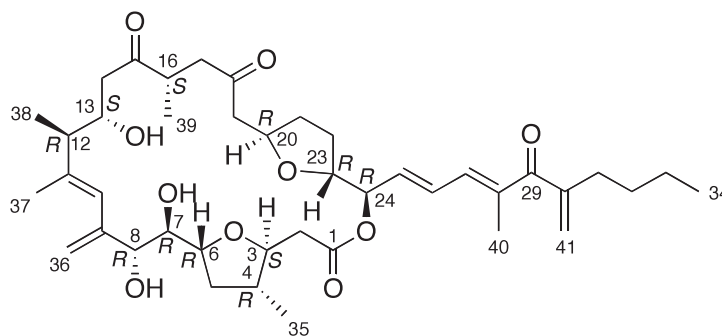
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Abstract - A new cytotoxic 25-membered macrolide, amphidinolide C3 (**1**), has been isolated from marine dinoflagellate *Amphidinium* sp. (Y-56 strain), and the structure of **1** was elucidated on the basis of spectroscopic data and chemical means.

INTRODUCTION

Marine dinoflagellates have been recognized as a source of novel bioactive substances with unique structures.¹⁻⁹ During our continuing search for structurally unique secondary metabolites from marine dinoflagellates *Amphidinium* sp, a series of cytotoxic macrolides, amphidinolides, as well as long chain polyhydroxy compounds have been isolated so far.¹⁻⁴ Further investigation of extracts of dinoflagellates *Amphidinium* spp. resulted in isolation of a new cytotoxic 25-membered macrolide, amphidinolide C3 (**1**), from the Y-56 strain of the dinoflagellate *Amphidinium* sp., which was separated from the inside cells of the marine acoel flatworms *Amphiscolops* sp. In this paper we describe the isolation and structure elucidation of amphidinolide C3 (**1**).



RESULTS AND DISCUSSION

The dinoflagellate *Amphidinium* sp. (Y-56 strain) was obtained from an acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa. The harvested cells of the cultured dinoflagellate (304 g, wet weight) obtained from 580 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 followed by a C₁₈ column and C₁₈ HPLC to afford amphidinolide C3 (**1**, 0.00006%) together with amphidinolide C (**2**, 0.0004%).¹⁰⁻¹³

The molecular formula of amphidinolide C3 (**1**) was revealed to be C₄₁H₆₀O₁₀ by HRFABMS [*m/z* 735.41075 (M + Na)⁺, +2.33 mmu]. The IR spectrum indicated the presence of hydroxy ($\nu_{\max}$ 3470 cm⁻¹) and carbonyl ($\nu_{\max}$ 1730 and 1710 cm⁻¹) functionalities, while UV absorptions at 237 nm (39000) and 280 nm (3200) suggested the presence of conjugated system. The ¹H and ¹³C NMR, and HMQC spectra of **1** disclosed the existence of three keto carbonyls, an ester carbonyl, four sp² quaternary carbons, four sp² methines, two sp² methylenes, eleven sp³ methines, ten sp³ methylenes, and six methyls, which were similar to those of amphidinolide C (**2**) except for the presence of a keto carbonyl carbon in place of an oxymethine carbon of amphidinolide C (**2**).

Table 1. ¹H and ¹³C NMR Data for Amphidinolide C3 (**1**) in C₆D₆.

Position	δ_{H}	δ_{C}		Position	δ_{H}	δ_{C}	
1	-	172.40	s	21a	1.82 (1H, nd)	32.15	t
2a	2.62 (1H, dd, 15.6, 9.5)	39.00	t	21b	1.37 (1H, nd)		
2b	2.38 (1H, nd)			22a	1.56 (1H, nd)	30.15	t
3	3.89 (1H, t, 9.6)	81.73	d	22b	1.30 (1H, nd)		
4	1.54 (1H, nd)	40.11	d	23	3.97 (1H, q, 7.0)	79.87	d
5a	1.86 (1H, nd)	36.98	t	24	5.45 (1H, t, 7.0)	76.64	d
5b	1.43 (1H, nd)			25	5.79 (1H, dd, 14.9, 7.3)	134.97	d
6	4.09 (1H, nd)	79.26	d	26	6.75 (1H, dd, 14.9, 11.4)	137.78	d
7	3.62 (1H, brt, 5.4)	76.64	d	27	6.91 (1H, d, 10.8)	129.97	d
8	4.31 (1H, brs)	77.73	d	28	-	138.35	s
9	-	145.93	s	29	-	199.13	s
10	6.31 (1H, brs)	125.43	d	30	-	148.96	s
11	-	143.58	s	31	2.47 (2H, nd)	33.04	t
12	2.29 (1H, quint, 7.3)	49.31	d	32	1.43 (2H, nd)	30.59	t
13	4.09 (1H, nd)	71.07	d	33	1.30 (2H, nd)	22.73	t
14a	2.76 (1H, dd, 15.9, 8.9)	45.85	t	34	0.87 (3H, t, 7.3)	13.98	q
14b	2.43 (1H, nd)			35	0.71 (3H, d, 6.7)	15.32	q
15	-	213.24	s	36a	5.17 (1H, s)	115.43	t
16	3.09 (1H, m)	42.48	d	36b	5.00 (1H, s)		
17a	2.97 (1H, dd, 17.5, 8.9)	46.31	t	37	1.77 (3H, s)	14.96	q
17b	2.08 (1H, dd, 17.5, 4.5)			38	0.96 (3H, d, 7.3)	15.73	q
18	-	208.03	s	39	0.98 (3H, d, 7.3)	16.17	q
19a	2.51 (1H, nd)	48.59	t	40	2.02 (3H, s)	12.93	q
19b	2.17 (1H, m)			41a	5.33 (1H, s)	120.89	t
20	4.23 (1H, m)	75.59	d	41b	5.30 (1H, s)		

nd : not determined by overlapping

The ^1H - ^1H COSY and TOCSY spectra of **1** revealed connectivities of five partial structures, **a** (C-2 to C-8 and C-4 to C-35), **b** (C-12 to C-14 and C-38), **c** (C-16 to C-17 and C-39), **d** (C-19 to C-27), and **e** (C-31 to C-34), as shown in Figure 1. ^1H - ^1H COSY cross-peaks of H-36 to C-10 and H₃-37 to C-10 due to allyl couplings, and HMBC correlations for H₂-36/C-8, H₃-37/C-11, H₃-38/C-11, and H-13/C-11 suggested that C-8 and C-12 were connected through a diene moiety (C-9 to C-11, C-9 to C-36, and C-11 to C-37). Connectivities of C-14 and C-16 to C-15, and C-17 and C-19 to C-18 were implied by HMBC cross-peaks of H₃-39 to C-15, H₂-17 to C-18, and H₂-19 to C-18. Two cross-peaks due to allyl coupling of H-27 to H₃-40 and H₂-31 to H-41 observed in the ^1H - ^1H COSY spectrum, and HMBC cross-peaks of H₃-40 to C-28, H₃-40 to C-29, H-41 to C-29, and H₂-31 to C-30 disclosed the linkages of C-27, C-29, and C-40 via C-28, and C-29, C-31, and C-41 via C-30. The presence of two tetrahydrofuran rings (C-3 to C-6 and C-20 to C-23) were deduced from HMBC cross-peaks of H-6 to C-3 and H-20 to C-23, respectively. The HMBC correlation for H-2 to an ester carbonyl carbon (C-1, δ 172.40) and the chemical shifts for H-24 (δ 5.45) and C-24 (δ 76.64) suggested that C-2 and C-24 were connected through an ester linkage. The NOESY correlation for H-36b (δ 5.00)/H₃-37 indicated that the conjugated diene C-36-C-9-C-10-C-11 was *s-cis* form. *E*-geometry of the C-25-C-26 double bond was deduced from the ^1H - ^1H coupling constant ($^3J_{\text{H-25/H-26}} = 15.6$ Hz), while that of the C-27-C-28 double bond was assigned as *E* on the basis of a NOESY cross-peak of H-26/H₃-40. Thus, the gross structure of amphidinolide C3 was elucidated to be **1** (Figure 1).

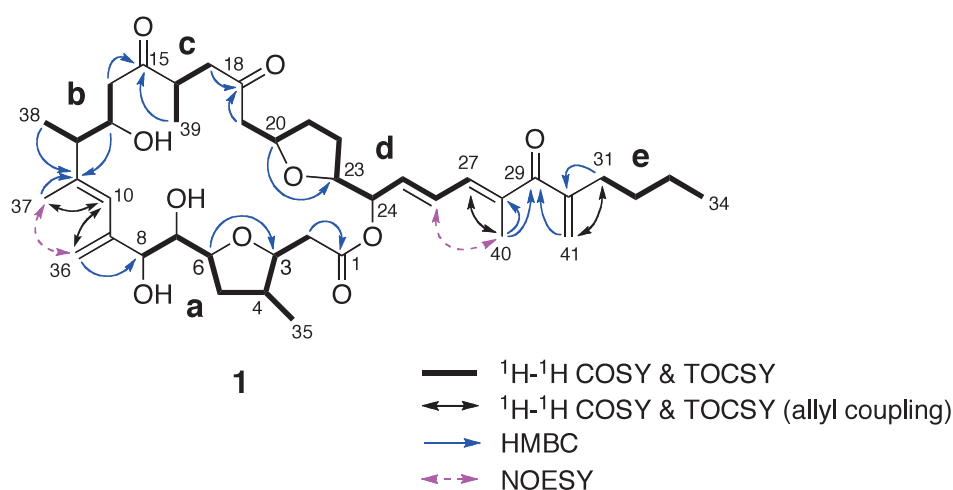


Figure 1. Selected 2D NMR correlations for amphidinolide C3 (**1**).

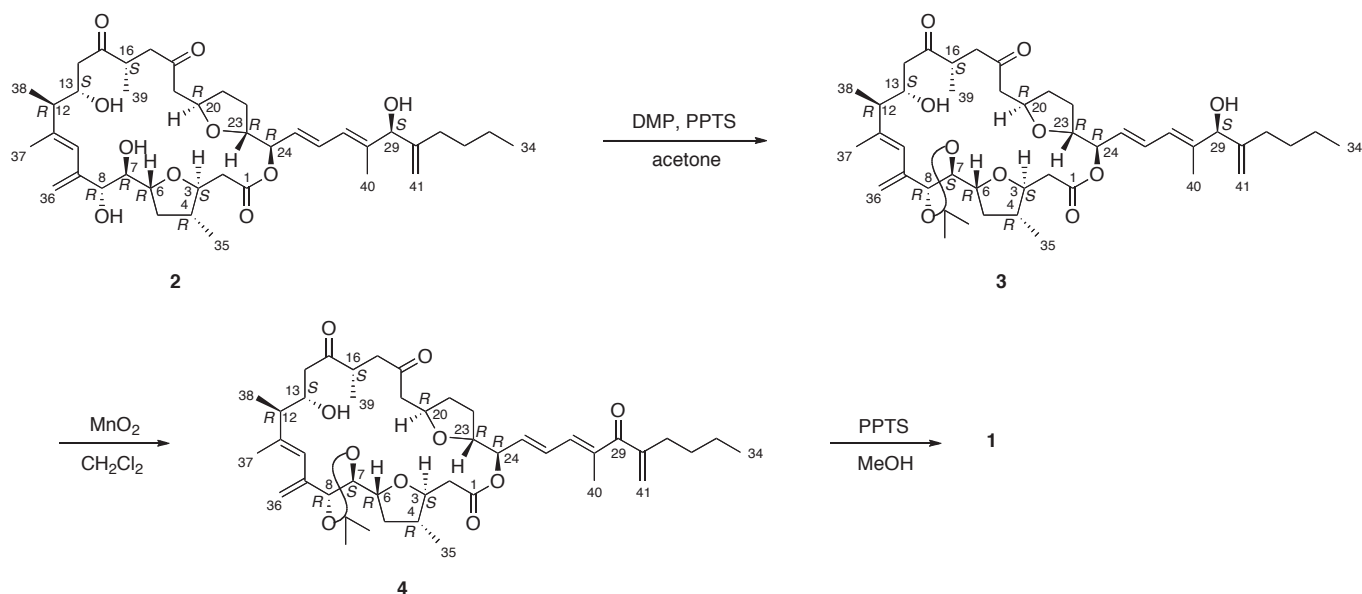


Figure 2. Conversion of amphidinolide C (2) into amphidinolide C3 (1).

The absolute stereochemistry of amphidinolide C3 (1) was elucidated by chemical correlation with amphidinolide C (2) as follows (Figure 2). Amphidinolide C (2) was treated with 2,2-dimethoxypropane (DMP) and pyridinium *p*-toluenesulfonate (PPTS) to afford 7,8-*O*-isopropylidene derivative 3 of amphidinolide C (2). Selective oxidation of the allylic alcohol at C-29 in 7,8-*O*-isopropylidene derivative 3 with manganese dioxide gave 7,8-*O*-isopropylidene 29-keto derivative 4 of amphidinolide C (2). Deprotection of acetonide group at C-7 and C-8 of 7,8-*O*-isopropylidene 29-keto derivative 4 afforded 29-keto derivative of amphidinolide C (2), whose NMR data and $[\alpha]_D$ value $\{[\alpha]_D^{22} -32.7$ (*c* 0.20, CHCl₃) $\}$ were coincident with those of natural amphidinolide C3 (1). Thus, the absolute stereochemistry of amphidinolide C3 was concluded to be 1.

Amphidinolide C3 (1) exhibited cytotoxicity against murine lymphoma P388 and L1210, and human epidermoid carcinoma KB cells (IC₅₀ 2.9, 7.6, and 10.0 μg/mL, respectively) in vitro, which were less potent than those of amphidinolides C (2) and C2.¹⁴

EXPERIMENTAL

General Experimental Procedures.

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. ¹H and ¹³C NMR spectra were recorded on a Bruker AMX-500 or AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd.) for C₆D₆. The 7.20 and 128.0 ppm resonances of residual C₆D₆ were used as internal references for ¹H and ¹³C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

Cultivation and Isolation.

The dinoflagellate *Amphidinium* sp. (strain number Y-56) was isolated from the inside cells of a marine acoel flatworm *Amphiscolops* sp. collected off Zanpa, Okinawa. The dinoflagellate was unialgally cultured at 25 °C for two weeks in a seawater medium enriched with 1% ES supplement. The harvested cells of the cultured dinoflagellate (304 g wet weight, from 580 L of culture) were extracted with MeOH/toluene (3:1, 600 mL x 3). After addition of 1 M NaCl aq. (500 mL), the mixture was extracted with toluene (500 mL x 3). The toluene soluble fractions were evaporated under reduced pressure to give a residue (2.55 g), which was subjected to a silica gel column (Wakogel C-300, Wako Pure Chemical Industries, Ltd., CHCl₃/MeOH) and a C18 column (Cosmosil 140C18-PREP, nacalai tesque, MeOH/H₂O) followed by C₁₈ HPLC [YMC Pack Pro C₁₈, 5 mm, YMC Co., Ltd., 10 x 250 mm; eluent, CH₃CN/H₂O (70:30); flow rate, 3.0 mL/min; UV detection at 210 nm] to afford amphidinolide C3 (**1**, 0.2 mg, 0.00006%, *t_R* 38.0 min) together with amphidinolide C (**2**, 1.3 mg, 0.0004%, *t_R* 25.0 min).

Amphidinolide C3 (1): colorless amorphous solid; $[\alpha]_D^{22}$ -31.5 (*c* 0.20, CHCl₃); UV (MeOH) $\lambda_{\max}$ 204 (ϵ 82000), 237 (39000), and 280 nm (3200); IR $\nu_{\max}$ 3470, 1730, 1710 and 1630 cm⁻¹; ESIMS (pos.) *m/z* 735 [M + Na]⁺; HRESIMS (pos.) *m/z* 735.41075 [(M + Na)⁺, calcd for C₄₁H₆₀O₁₀Na, 735.40842].

Amphidinolide C3 (1) derived from amphidinolide C (2). Amphidinolide C (**2**, 1.0 mg) was dissolved in acetone (30 μ L) and treated with 2,2-dimethoxypropane (10 μ L) and PPTS (0.24 mg) at room temperature for 1 h. After addition of triethylamine (0.24 μ L), the reaction mixture was concentrated and purified by a silica gel column (*n*-hexane/acetone, 3:1) to afford 7,8-*O*-isopropylidene derivative (**3**, 0.9 mg) of amphidinolide C (**2**). To a solution of 7,8-*O*-isopropylidene derivative (**3**, 0.9 mg) of amphidinolide C (**2**) in CH₂Cl₂ (300 μ L) was added MnO₂ (1.8 mg). The resultant mixture was stirred at room temperature for 48 h. The mixture was filtered through a cotton plug, and the filtrate was concentrated to afford 7,8-*O*-isopropylidene 29-keto derivative (**4**, 0.7 mg) of amphidinolide C (**2**). The 7,8-*O*-isopropylidene 29-keto derivative (**4**, 0.6 mg) of amphidinolide C (**2**) was dissolved in MeOH (300 mL) and treated with PPTS (0.23 mg) at 55 °C for 3 h. After addition of triethylamine (0.23 μ L), the reaction mixture was concentrated and purified by a silica gel column (*n*-hexane/acetone, 3:1 to 3:2) to afford amphidinolide C3 (**1**, 0.6 mg).

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REFERENCES AND NOTES

1. (a) J. Kobayashi and M. Tsuda, *Nat. Prod. Rep.*, 2004, **21**, 77. (b) J. Kobayashi and T. Kubota, *J. Nat. Prod.*, 2007, **70**, 451. (c) J. Kobayashi, *J. Antibiot.*, 2008, **61**, 271.
2. (a) Y. Doi, M. Ishibashi, H. Nakamichi, T. Kosaka, T. Ishikawa, and J. Kobayashi, *J. Org. Chem.*, 1997, **62**, 3820. (b) T. Kubota, M. Tsuda, Y. Doi, A. Takahashi, H. Nakamichi, M. Ishibashi, E. Fukushi, J. Kawabata, and J. Kobayashi, *Tetrahedron*, 1998, **54**, 14455. (c) T. Kubota, A. Takahashi, M. Tsuda, and J. Kobayashi, *Mar. Drugs*, 2005, **3**, 113.
3. (a) J. Kobayashi, T. Kubota, M. Takahashi, M. Ishibashi, M. Tsuda, and H. Naoki, *J. Org. Chem.*, 1999, **64**, 1478. (b) T. Kubota, M. Tsuda, M. Takahashi, M. Ishibashi, H. Naoki, and J. Kobayashi, *J. Chem. Soc., Perkin Trans. 1*, 1999, **64**, 3483. (c) T. Kubota, M. Tsuda, M. Takahashi, M. Ishibashi, S. Oka, and J. Kobayashi, *Chem. Pharm. Bull.*, 2000, **48**, 1447.
4. T. Kubota, Y. Sakuma, K. Shimbo, M. Tsuda, M. Nakano, Y. Uozumi, and J. Kobayashi, *Tetrahedron Lett.*, 2006, **47**, 4369.
5. I. Bauer, L. Maranda, K. A. Young, Y. Shimizu, and S. Huang, *Tetrahedron Lett.* 1995, **36**, 991.
6. (a) M. Satake, M. Murata, T. Yasumoto, T. Fujita, and H. Naoki, *J. Am. Chem. Soc.*, 1991, **113**, 9859. (b) G. K. Paul, N. Matsumori, M. Murata, and K. Tachibana, *Tetrahedron Lett.*, 1995, **36**, 6279. (c) G. K. Paul, N. Matsumori, K. Konoki, M. Murata, and K. Tachibana, *J. Mar. Biotechnol.*, 1997, **5**, 124. (d) R. Echigoya, L. Rhodes, Y. Oshima, and M. Satake, *Harmful Algae*, 2005, **4**, 383. (e) N. Morsy, S. Matsuoka, T. Houdai, N. Matsumori, S. Adachi, M. Murata, T. Iwashita, and T. Fujita, *Tetrahedron*, 2005, **61**, 8606. (f) N. Morsy, T. Houdai, S. Matsuoka, N. Matsumori, S. Adachi, T. Oishi, M. Murata, T. Iwashita, and T. Fujita, *Bioorg. Med. Chem.*, 2006, **14**, 6548.
7. (a) X. Huang, D. Zhao, Y. Guo, H. Wu, L. Lin, Z. Wang, J. Ding, and Y. Lin, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 3117. (b) X. Huang, D. Zhao, Y. Guo, H. Wu, E. Trivellone, and G. Cimino, *Tetrahedron Lett.*, 2004, **45**, 5501.
8. K. Washida, T. Koyama, K. Yamada, M. Kita, and D. Uemura, *Tetrahedron Lett.*, 2006, **47**, 2521.
9. S. J. Huang, C. M. Kuo, Y. C. Lin, Y. M. Chen, and C. K. Lu, *Tetrahedron Lett.*, 2009, **50**, 2512.
10. J. Kobayashi, M. Ishibashi, M. R. Wälcchli, H. Nakamura, Y. Hirata, T. Sasaki, and Y. Ohizumi, *J. Am. Chem. Soc.*, 1988, **110**, 490.
11. T. Kubota, M. Tsuda, and J. Kobayashi, *Org. Lett.*, 2001, **3**, 1363.
12. T. Kubota, M. Tsuda, and J. Kobayashi, *Tetrahedron*, 2003, **59**, 1613.
13. T. Kubota, M. Tsuda, and J. Kobayashi, *Tetrahedron*, 2001, **57**, 5975.
14. T. Kubota, Y. Sakuma, M. Tsuda, and J. Kobayashi, *Mar. Drugs*, 2004, **2**, 83.