HETEROCYCLES, Vol. 82, No. 1, 2010, pp. 333 - 338. © The Japan Institute of Heterocyclic Chemistry
Received, 5th January, 2010, Accepted, 9th February, 2010, Published online, 10th February, 2010
DOI: 10.3987/COM-10-S(E)3

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

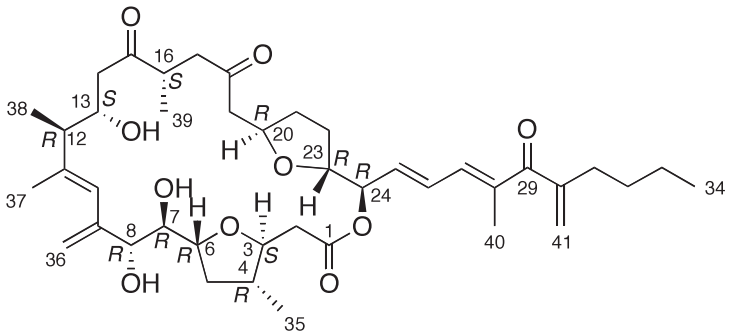
Takaaki Kubota,[a] Akiko Suzuki,[a] Mika Yamada,[a] Shigeyuki Baba,[b] and Jun'ichi Kobayashi[a,*]

[a]Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, [b]Tropical Biosphere Research Center, University of the Ryukyus, Taketomi, Okinawa, 907-01541, Japan. E-mail: jkobay@pharm.hokudai.ac.jp

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**).



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 C_{18} column and C_{18} 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 $C_{41}H_{60}O_{10}$ by HRFABMS [*m/z* 735.41075 $(M + Na)^+$, +2.33 mmu]. The IR spectrum indicated the presence of hydroxy (ν_{max} 3470 cm^{-1}) and carbonyl (ν_{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 ^{13}C NMR, and HMQC spectra of **1** disclosed the existence of three keto carbonyls, an ester carbonyl, four sp^2 quaternary carbons, four sp^2 methines, two sp^2 methylenes, eleven sp^3 methines, ten sp^3 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 ^{13}C NMR Data for Amphidinolide C3 (**1**) in C_6D_6.

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 ^{1}H-^{1}H 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 C17 and C-39), **d** (C-19 to C-27), and **e** (C-31 to C-34), as shown in Figure 1. ^{1}H-^{1}H COSY cross-peaks of H-36 to C-10 and H_3-37 to C-10 due to allyl couplings, and HMBC correlations for H_2-36/C-8, H_3-37/C-11, H_3-38/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 H_3-39 to C-15, H_2-17 to C-18, and H_2-19 to C-18. Two cross-peaks due to allyl coupling of H-27 to H_3-40 and H_2-31 to H-41 observed in the ^{1}H-^{1}H COSY spectrum, and HMBC cross-peaks of H_3-40 to C-28, H_3-40 to C-29, H-41 to C-29, and H_2-31 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)/H_3-37 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 ^{1}H-^{1}H coupling constant ($^{3}J_{H\text{-}25/H\text{-}26}$ = 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/H_3-40. Thus, the gross structure of amphidinolide C3 was elucidated to be **1** (Figure 1).

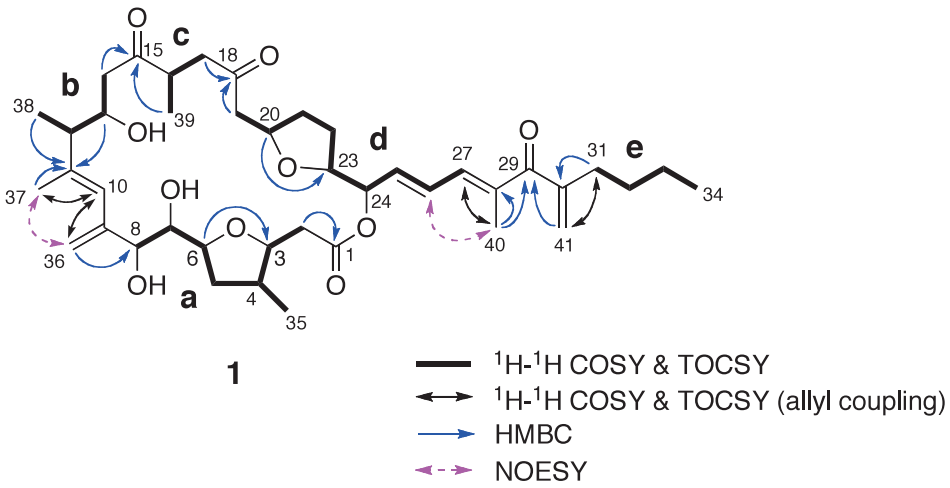


Figure 1. Selected 2D NMR correlations for amphidinolide C3 (**1**).

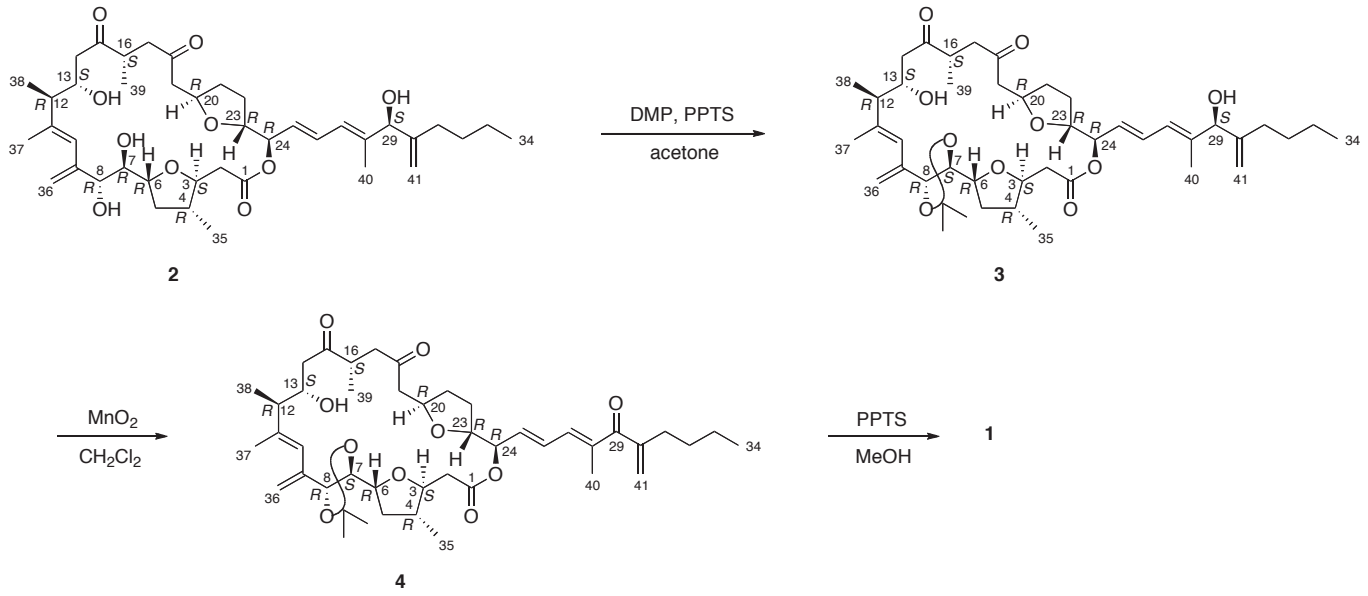


Figure 2. Conversion of amphidinolide C (**2**) into amphidinolide C3 (**1**).

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, $CHCl_3$)} 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 (IC_{50} 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 ^{13}C NMR spectra were recorded on a Bruker AMX-500 or AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd.) for C_6D_6. The 7.20 and 128.0 ppm resonances of residual C_6D_6 were used as internal references for ^1H and ^{13}C 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 ^{o}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., $CHCl_3$/MeOH) and a C18 column (Cosmosil 140C18-PREP, nacalai tesque, MeOH/H_2O) followed by C_{18} HPLC [YMC Pack Pro C_{18}, 5 mm, YMC Co., Ltd., 10 x 250 mm; eluent, CH_3CN/H_2O (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, $CHCl_3$); UV (MeOH) λ_{max} 204 (ε 82000), 237 (39000), and 280 nm (3200); IR ν_{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 $C_{41}H_{60}O_{10}Na$, 735.40842].

Amphidinolide C3 (1) derived from amphidinolide C (2). Amphidinolide C (**2**, 1.0 mg) was dissolved in acetone (30 μL) and treated with 2,2-dimethoxypropane (10 μL) and PPTS (0.24 mg) at room temperature for 1 h. After addition of triethylamine (0.24 μL), 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 CH_2Cl_2 (300 μL) was added MnO_2 (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 ^{o}C for 3 h. After addition of triethylamine (0.23 μL), 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,).

ACKNOWLEDGMENTS

We thank Dr. E. Fukushi, Graduate School of Agriculture, Hokkaido University, for measurement of HMBC spectrum of **1**, and Ms. S. Oka, Equipment Management Center, Hokkaido University, for measurements of ESIMS. This work was supported by Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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. Wälchli, 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.