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Amphidinolide C2, New Macrolide from Marine Dinoflagellate *Amphidinium* Species

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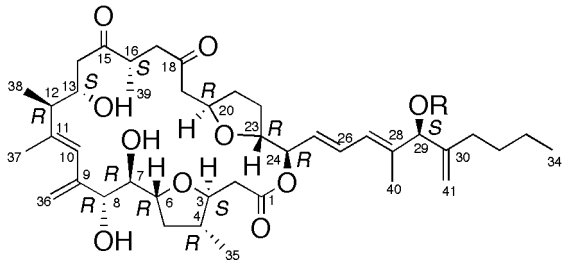
Abstract: A new cytotoxic 25-membered macrolide, amphidinolide C2 (**1**), has been isolated from marine dinoflagellate *Amphidinium* sp. (Y-71 strain), and the structure **1** was elucidated on the basis of spectroscopic data and chemical means.

Keywords: dinoflagellate; *Amphidinium* sp.; amphidinolide C2.

Introduction

Amphidinolides are a series of unique cytotoxic macrolides isolated from marine dinoflagellates *Amphidinium* species, which were separated from acoel flatworms *Amphiscolops* species [1,2]. Amphidinolide C (**2**) isolated previously from the Y-5 strain of *Amphidinium* sp. is a unique 25-membered macrolide having two tetrahydrofuran rings and vicinally-located one-carbon branches. [3] Recently, relatively large amounts of **2** have been isolated from three strains (Y-56, Y-59, and Y-71) of the genus *Amphidinium*, which were separated from the inside cells of the marine acoel flatworms *Amphiscolops* species. This allowed us to elucidate the absolute configurations at twelve chiral centers in **2**. [4] The biosynthetic origins of amphidinolide C (**2**) were investigated on the basis of ^{13}C NMR data of ^{13}C enriched samples obtained by feeding experiments with [1-^{13}C], [2-^{13}C], and [1,2-$^{13}C_2$] sodium acetates in cultures of a dinoflagellate *Amphidinium* species. [5] Amphidinolides F [6] and U [7] are considered to belong to the same group as amphidinolide C (**2**)

in the structural diversity of amphidinolides. In this paper we describe the isolation and structure elucidation of a new 25-membered macrolide, amphidinolide C2 (**1**), isolated from the Y-71 strain of the symbiotic dinoflagellate *Amphidinium* species.



1 : R = $COCH_3$
2 : R = H

Results and Discussion

The dinoflagellate *Amphidinium* sp. (Y-71 strain) was obtained from an acoel flatworm *Amphiscolops* sp. collected off Sunabe, Okinawa. The mass cultured algal cells (1200 g, wet weight) obtained from 1300 L of culture were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and water. The toluene extracts were subjected to silica gel and then successive C_{18} column chromatographies followed by C_{18} HPLC to afford amphidinolide C2 (**1**, 0.00015 %) together with known macrolides, amphidinolides B, [8-10] C (**2**), [3] T1, [11-13] and T2. [12]

Amphidinolide C2 (**1**) had the molecular formula of $C_{43}H_{64}O_{11}$ as revealed by HRFABMS [*m/z* 779.4323, $(M+Na)^+$, +2.3 mmu]. The IR spectrum indicated the presence of hydroxyl(s) (λ_{max} 3298 cm^{-1}) and carbonyl group(s) (λ_{max} 1738 and 1705 cm^{-1}). The UV spectrum showed the absorption at 230 nm (ε 20000) due to a conjugated diene chromophore. ^1H and ^{13}C NMR data disclosed the existence of two ketones, two ester carbonyls, four sp^2 quaternary carbons, four sp^2 methines, two sp^2 methylenes, twelve sp^3 methines (nine of them bearing an oxygen atom), ten sp^3 methylenes, and seven methyls (two of them attached to olefins), which were similar to those of amphidinolide C (**2**) except for the presence of additional methyl and ester carbonyl carbons. Interpretation of the ^1H-^1H COSY and TOCSY spectra revealed proton connectivities of the following partial structures: from H_2-2 to H_2-37, from H_3-38 to H_2-14, from H_3-39 to H_2-17, from H_2-19 to H_3-40, and from H-29 to H_3-34. Connections among five units were deduced from the following HMBC correlations; H_3-37/C-12, H_2-14/C-15, H_3-39/C-15, H_2-17/C-18, H_2-19/C-18, and H_3-40/C-29. Geometries of three internal olefins at C-10–C-11, C-25–C-26, and C-27–C-28 were assigned as all *E* on the basis of NOESY cross-peaks for H-10/H-12, H-24/H-26, H-25/H-27, H-26/H_3-40, H-27/H-29, and H-36b/H_3-37. HMBC cross-peaks from H-6/C-3 and H-20/H-23 suggested that two tetrahydrofuran rings were formed between C-3 and C-6 and between C-20 and C-23, and their relative

stereochemistries were implied by analysis of NOESY correlations. The existence of the 25-membered macrocylcic ring was implied by HMBC correlations from H_2-2 and H-24 to C-1. HMBC correlations from the low-field oxymethine proton (δ_H 5.89, s) at C-29 and a single methyl proton (δ_H 1.74, s, C-43) to an ester carbonyl carbon (δ_C 168.91, C-42), suggesting that an acetoxy group was attached to C-29. Thus the gross structure of amphidinolide C2 (**1**) was elucidated to be the 29-*O*-acetyl form of amphidinolide C (**2**).

Amphidinolide C2 (**1**) was converted into its 7,8,13-*O*-trisacetate (**5**), while the tetrakisacetate of amphidinolide C (**2**) was also prepared (Scheme 1). The spectral data of **3** derived from **1** were identical to those at the tetrakisacetate prepared from **2**. Therefore, the absolute configurations of twelve chiral centers in **1** must be 3*S*, 4*R*, 6*R*, 7*R*, 8*R*, 12*R*, 13*S*, 16*S*, 20*R*, 23*R*, 24*R*, and 29*S*, the same as those previously determined for amphidinolide C (**2**).

Amphidinolide C2 (**1**) exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells (IC_{50} 0.8 and 3 μg/mL, respectively) in vitro, which were less potent than those of amphidinolide C (**2**).

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Experimental

General

The IR and UV spectra were taken on a FT/IR-5300 and a UV-1600PC spectrophotometers, respectively. NMR spectra were recorded on a Bruker AMX-600 spectrometer. Positive-mode FAB mass spectra were obtained on a JEOL JMS HX-110 using *p*-nitrobenzyl alcohol as a matrix.

Cultivation and Isolation

The dinoflagellate *Amphidinium* sp. (strain number Y-71) was isolated from the inside cells of the marine acoel flatworm *Amphiscolops* sp. collected off Sunabe, 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 (1200 g wet weight, from 1300 L of culture) were extracted with MeOH/toluene (3:1, 600 mL x 5). After addition of 1 M NaCl aq. (500 mL), the mixture was extracted with toluene (500 mL x 3). Parts (3.30 g) of the toluene-soluble materials (6.63 g) were subjected to silica gel ($CHCl_3$/MeOH, 98:2) and C_{18} column

chromatographies (MeOH/H_2O, 8:2) to give a fraction (31.5 mg) containing some macrolides. The fraction was separated by C_{18} HPLC (Develosil ODS-HG-5, Nomura Chemical Co. Ltd., 10 x 250 mm; eluent, CH_3CN/H_2O (65:35); flow rate, 2.5 mL/min; UV detection at 210 nm) to afford amphidinolides C2 (**1**, 1.8 mg, 0.00015 %, wet weight, t_R 23.0 min), B (0.0017 %), C (**2**, 0.0012 %), T1 (0.00092 %), and T2 (0.00013 %).

Spectral Data

Amphidinolide C2 (**1**): colorless oil; UV (MeOH) λ_{max} 230 (ε 20000); IR (KBr) ν_{max} 3298, 1738, 1705 cm^{-1}; ^{1}H-NMR (600 MHz, benzene-d_6) δ 0.72 (3H, d, *J* = 6.4 Hz, H_3-35), 0.86 (3H, t, *J* = 7.4 Hz, H_3-34), 1.00 (6H, d, *J* =7.0 Hz, H_3-38/H_3-39), 1.13 (1H, m, H-21), 1.24 (2H, m, H_2-33), 1.36 (1H, m, H-22), 1.45 (1H, m, H-5), 1.53 (1H, m, H-4), 1.56 (3H, m, H-22/H_2-32), 1.72 (3H, br s, H_3-40), 1.74 (3H, s, H_3-43), 1.75 (1H, m, H-21), 1.77 (3H, br s, H_3-37), 1.86 (1H, m, H-5), 1.95 (1H, m, H-31), 1.99 (1H, m, H-31), 2.17 (2H, m, H-2/H-17), 2.29 (1H, m, H-12), 2.35 (2H, m, H-14/H-19), 2.50 (1H, dd, *J* = 8.2, 14.9 Hz, H-2), 2.59 (1H, dd, *J* = 9.5, 15.7 Hz, H-14), 2.75 (1H, dd, *J* = 9.5, 15.7 Hz, H-19), 3.00 (1H, dd, *J* = 8.5, 17.6 Hz, H-2), 3.14 (1H, m, H-16), 3.62 (1H, br t, *J* = 4.5 Hz, H-7), 3.89 (1H, dt, *J* = 2.5, 9.4 Hz, H-3), 4.00 (1H,m, H-23), 4.09 (1H, m, H-6), 4.10 (1H, m, H-13), 4.30 (1H, m, H-20), 4.32 (1H, d, *J* = 4.5 Hz, H-8), 4.97 (1H, s, H-41), 5.00 (1H, s, H-36), 5.19 (2H, s, H-36/H-41), 5.47 (1H, t, *J* = 8.0 Hz, H-24), 5.64 (1H, dd, *J* = 8.0, 15.1 Hz, H-25), 5.89 (1H, s, H-29), 6.30 (1H, s, H-10),6.30 (1H, d, J = 10.9 Hz, H-27), 6.74 (1H, dd, *J* = 10.9, 15.1 Hz, H-26); ^{13}C NMR (150 MHz, benzene-d_6) δ 13.06 (C-40), 14.07 (C-34), 15.09 (C-37), 15.39 (C-38), 15.71 (C-35), 16.27 (C-39), 20.59 (C-43), 22.64 (C-33), 28.40 (C-22), 30.10 (C-32), 32.18 (C-21), 32.38 (C-31), 37.06 (C-5), 39.13 (C-14), 40.12 (C-4), 42.57 (C-16), 45.83 (C-19), 46.25 (C-17), 48.72 (C-2), 49.38 (C-12), 71.03 (C-13), 75.60 (C-20), 76.64 (C-7), 77.13 (C-24), 77.67 (C-8), 79.28 (C-6), 80.02 (C-23), 80.34 (C-29), 81.73 (C-3), 111.36 (C-41), 115.27 (C-36), 125.42 (C-10), 127.46 (C-27), 129.44 (C-26), 130.46 (C-25), 136.67 (C-28), 140.35 (C-9), 146.07 (C-11), 146.15 (C-30), 168.91 (C-42), 171.13 (C-1), 207.38 (C-18), 213.27 (C-15); (+)-FABMS *m/z* 779 $(M+Na)^+$; (+)-HRFABMS *m/z* 779.4323 ($C_{43}H_{64}O_{11}Na$ requies $(M+Na)^+$, 779.4300).

7,8,13-O-Trisacetate (**3**) *of Amphidinolide C2* (**1**): Amphidinolide C2 (**1**, 0.2 mg) was treated with acetic anhydride and pyridine (both 100 μL) at room temperature for 17 h. After evaporation of the solvent, the residue was subjected to a silica gel column (hexane/acetone, 2:1) to afford 7,8,13-*O*-triacetate (**3**, 0.2 mg) of amphidinolide C2. The spectroscopic data for compound **3** were identical to those of reported 7,8,13,29-*O*-tetrakisacetate of amphidinolide C (**2**). [3]

Reference and Notes

1. Tsuda, M.; Izui, N.; Shimbo, K.; Sato, M.; Fukushi, E.; Kawabata, J.; Kobayashi, J. Amphidinolide Y, a novel 17-membered macrolide from dinoflagellate *Amphidinium* sp.; plausible biogenetic precursor of amphidinolide X. *J. Org. Chem.* **2003**, *68*, 9109-9112.
2. Kobayashi, J.; Tsuda, M. Amphidinolides, bioactive macrolides from symbiotic marine dinoflagellates, *Nat. Prod. Rep.* **2004**, *21*, 77-93.
3. Kobayashi, J.; Ishibashi, M.; Walchli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. Amphidinolide C, the first 25-membered macrocyclic lactone with potent antineoplastic activity from the cultured dinoflagellate *Amphidinium* sp. *J. Am. Chem. Soc.* **1988**, *110*, 490-494.
4. Kubota, T.; Tsuda, M.; Kobayashi, J. Absolute stereochemistry of amphidinolide C. *Org. Lett* **2001**, *3*, 1363-1366.
5. Kubota, T.; Tsuda, M.; Kobayashi, J. Biosynthetic study of amphidinolide C. *Tetrahedron* **2001**, *57*, 5975-5977.
6. Kobayashi, J.; Tsuda, M.; Ishibashi, M.; Shigemori, H.; Yamasu, T.; Hirota, H. Sasaki, T. Amphidinolide F, a new cytotoxic macrolide from the marine dinoflagellate *Amphidinium* sp. *J. Antibiot.* **1991**, *44*, 1259-1261.
7. Tsuda, M.; Endo, T.; Kobayashi, J. Amphidinolide U, novel 20-membered macrolide from marine dinoflagellate *Amphidinium* sp. *Tetrahedron* **1999**, *55*, 14565-14570.
8. Ishibashi, M.; Ohizumi, Y.; Hamashima, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. Amphidinolide-B, a novel macrolide with potent antineoplastic activity from the marine dinoflagellate *Amphidinium* sp. *J. Chem. Soc., Chem. Commun.* **1987**, 1127-1129.
9. Bauer, I.; Maranda, L.; Shimizu, Y.; Peterson, R. W.; Cornell, L.; Steiner, J. R.; Clardy, J. The structures of amphidinolide B isomers: strongly cytotoxic macrolides produced by a free-swimming dinoflagellate *Amphidinium* sp. *J. Am. Chem. Soc.* **1994**, *116*, 2657-2658.
10. Ishibashi, M.; Ishiyama, H.; Kobayashi, J. Absolute stereochemistry of amphidinolide B. *Tetrahedron Lett.* **1994**, *35*, 8241-8242.
11. Tsuda, M.; Endo, T.; Kobayashi, J. Amphidinolide T, novel 19-membered macrolide from marine dinoflagellate *Amphidinium* sp. *J. Org. Chem.* **2000**, *65*, 1349-1352.
12. Kobayashi, J.; Kubota, T.; Endo, T.; Tsuda, M. Amphidinolides T2, T3, and T4, new 19-menbered macrolides from dinoflagellate *Amphidinium* sp. and biosynthesis of amphidinolide T1 *J. Org. Chem.* **2001**, *66*, 134-142.
13. Kubota, T.; Endo. T.; Tsuda, M.; Shiro, M.; Kobayashi, J. Amphidinolide T5, a new 19-membered macrolide from a dinoflagellate and X-ray structure of amphidinolide T1 *Tetrahedron* **2001**, *57*, 6175-6179.

Sample Availability: Samples are available from the authors.