The Journal of
Organic
Chemistry

VOLUME 58, NUMBER 10 MAY 7, 1993

Communications

Amphidinolide J: A Cytotoxic Macrolide from the Marine Dinoflagellate *Amphidinium* sp. Determination of the Absolute Stereochemistry

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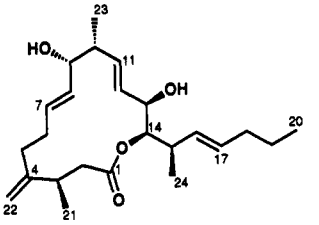
Received March 3, 1993

Summary: Amphidinolide J (1), a novel cytotoxic 15-membered macrolide, has been isolated from the cultured dinoflagellate *Amphidinium* sp., and its structure including absolute configurations was established by synthesis of the ozonolysis products.

Amphidinolides are a series of cytotoxic macrolides isolated from the laboratory-cultured dinoflagellates of the genus *Amphidinium*, which are symbionts of Okinawan marine flatworms *Amphiscolops* sp.[1] Here we describe isolation of a novel 15-membered macrolide, amphidinolide J (1), and determination of its structure including the



1

absolute stereochemistry on the basis of the synthesis of degradation products of 1 as optically active forms. Amphidinolide J (1) was cytotoxic against L1210 murine leukemia and KB human epidermoid carcinoma cells (IC_{50} 2.7 and 3.9 μg/mL, respectively).

The harvested cells of the cultured dinoflagellate *Amphidinium* sp.[2] (920 g, wet weight, from 3300 L of culture) were extracted with MeOH/toluene (3:1); the extracts were partitioned between toluene and water. The organic phase (33 g) was fractionated by a silica gel column ($CHCl_3$/MeOH (95:5)) followed by gel filtration on Sephadex LH-20 ($CHCl_3$/MeOH (1:1)). The cytotoxic fraction was further purified by reversed-phase HPLC (ODS, 88% MeOH) to yield amphidinolide J (1, 0.005% yield, wet weight): colorless oil; $[\alpha]^{26}_D$ +1.2° (*c* 0.7, MeOH); IR (KBr) 3410 and 1715 cm^{-1}; HRFABMS *m/z* 496.3610 (M + diethanolamine + H)$^+$ for $C_{28}H_{50}O_6N$, Δ −2.8 mmu, suggesting the molecular formula as $C_{24}H_{38}O_4$.

The planar structure of 1 was deduced by detailed analyses of its ^1H and ^{13}C NMR data[3] aided with 2D NMR experiments (^1H–^1H COSY, HSQC, HMBC, and NOESY). The ^1H–^1H COSY spectrum of 1 revealed proton connectivities from H_2-2 to the terminal methyl (H_3-20) throughout the molecule and the lactone carbonyl (C-1) showed HMBC correlations with H_2-2 and H-14, thereby leading to a gross structure of 1 consisting of a 15-membered lactone ring with three disubstituted *E*-olefins ($J_{7,8}$ = 15.0, $J_{11,12}$ = 15.8, and $J_{16,17}$ = 15.0 Hz).

Treatment of 1 with ozone (−78 °C, 1 min) followed by

(1) (a) Kobayashi, J. *J. Nat. Prod.* **1989**, *52*, 225–238. (b) Kobayashi, J.; Shigemori, H.; Ishibashi, M.; Yamasu, T.; Hirota, H.; Sasaki, T. *J. Org. Chem.* **1991**, *56*, 5221–5224 and references cited therein.

(2) From the dinoflagellate of this species amphidinolides A–D were previously isolated.[1a]

(3) Data: ^1H NMR (CD_3OD) δ 2.68 (dd, *J* = 15.8 and 12.5 Hz; 2), 2.24 (m; 2′), 2.52 (m; 3), 2.24 (m; 5), 1.85 (m; 5′), 2.10 (2H, m; 6), 5.33 (m; 7), 5.23 (dd, *J* = 15.0 and 8.0 Hz; 8), 3.86 (dd, *J* = 8.0 and 1.8 Hz; 9), 2.01 (m; 10), 5.49 (dd, *J* = 15.8 and 9.2 Hz; 11), 5.38 (m; 12), 4.19 (dd, *J* = 7.7 and 1.8 Hz; 13), 4.70 (dd, *J* = 10.3 and 1.8 Hz; 14), 2.54 (m; 15), 5.17 (dd, *J* = 15.0 and 8.8 Hz; 16), 5.42 (m; 17), 1.92 (2H, m; 18), 1.34 (2H, m; 19), 0.89 (3H, t, *J* = 7.0 Hz; 20), 1.02 (3H, d, *J* = 6.6 Hz; 21), 4.89 (s; 22), 4.46 (s; 22′), 1.09 (3H, d, *J* = 7.0 Hz; 23), and 1.05 (3H, d, *J* = 7.0 Hz; 24); ^{13}C NMR (CD_3OD) δ 173.4 (s; 1), 40.3 (t; 2), 35.4 (d; 3), 152.5 (s; 4), 36.6 (t; 5), 30.2 (t; 6), 131.1 (d; 7), 136.7 (d; 8), 79.4 (d; 9), 46.6 (d; 10), 133.5 (d; 11), 133.1 (d; 12), 72.4 (d; 13), 81.1 (d; 14), 39.8 (d; 15), 134.0 (d; 16), 132.1 (d; 17), 35.7 (t; 18), 23.8 (t; 19), 14.0 (q; 20), 22.3 (q; 21), 109.2 (t; 22), 18.8 (q; 23), and 17.4 (q; 24).

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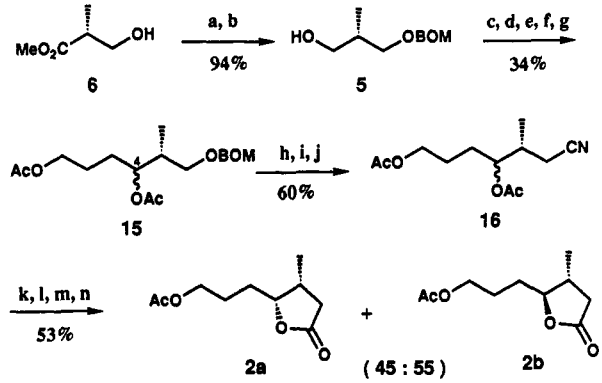
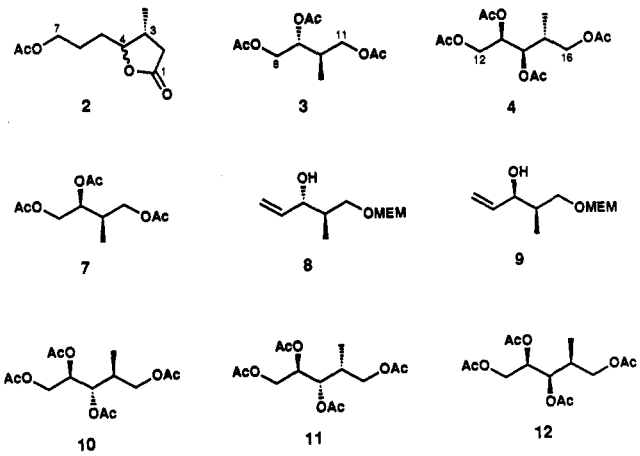
Scheme I. Synthesis of the C-1–C-7 Fragment 2[a]



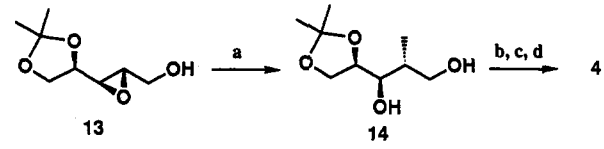
[a] Key: (a) BOMCl, i-Pr_2NEt, CH_2Cl_2, rt, 44 h; (b) $LiAlH_4$, Et_2O, rt, 30 min; (c) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C, 30 min, then Et_3N, 0 °C, 30 min; (d) $CH_2{=}CHCH_2CH_2MgBr$, ether, 50 °C, 40 min; (e) O_3, MeOH, −78 °C, 2.5 h; (f) $NaBH_4$, MeOH, 0 °C, 1 h; (g) Ac_2O, pyridine, rt, 12 h; (h) H_2, Raney Ni (W-2), EtOH, rt, 48 h; (i) TsCl, pyridine, rt, 44 h; (j) NaCN, DMSO, 85–90 °C, 2 h; (k) NaOH, H_2O_2, EtOH, 65 °C, 1.5 h, then 90 °C, 7 h; (l) 2 M HCl, rt; (m) Ac_2O, pyridine, rt, 11 h; (n) HPLC separation.

$NaBH_4$ reduction and acetylation[4] afforded degradation products (**2**–**4**),[5] corresponding to C-1–C-7,[6] C-8–C-11, and C-12–C-16 moieties of **1**, respectively. For unambiguous



determination of the absolute configurations of six chiral centers of **1**, the fragments (**2**–**4**) together with their all possible diastereomers were prepared in optically active forms. The C-1–C-7 fragment (**2**) was synthesized as shown in Scheme I, starting with monoprotected 2(*S*)-methylpropane-1,3-diol **5**, which was readily supplied from (−)-methyl 3-hydroxy-2(*R*)-methylpropionate (**6**). The Grignard addition to the corresponding aldehyde from **5** afforded the diastereomeric mixture at C-4[7] in the ratio of 45:55, which was separated in the final step by silica HPLC.[6] The 3,4-syn (**2a**) and 3,4-anti (**2b**) isomers thus obtained were completely identical with those from natural specimens including the sign of optical rotations [synthetic: **2a**, $[\alpha]_D$ +17° (*c* 1.0, $CHCl_3$); **2b**, $[\alpha]_D$ −22° (*c* 1.0, $CHCl_3$); natural: **2a**, $[\alpha]_D$ +17° (*c* 0.06, $CHCl_3$); **2b**, $[\alpha]_D$ −34° (*c* 0.2, $CHCl_3$)] to establish the 3*R*-configuration for **1**. The C-8–C-11 fragment **3** and its syn-isomer **7** were readily prepared [(1) reductive ozonolysis, (2) deprotection, and (3) acetylation] from allyl alcohols **8** and **9** (respectively), which were obtained from **6** via modifications of literature procedures.[8] The spectral data of the C-8–C-11 fragment obtained by degradation of **1** were indistinguishable from those of the anti-isomer **3**, and their optical data [synthetic, $[\alpha]_D$ +5.0° (*c* 1.0, $CHCl_3$); natural, $[\alpha]_D$ +2.8° (*c* 0.22, $CHCl_3$)] revealed the 9*R*,10*R*-configurations for **1**. Preparations of the C-12–C-16 fragment **4** and its diastereomers **10**–**12** were achieved by applying Kishi's methods for pentose synthesis[9] (Scheme II). The epoxy alcohol **13**, obtained from D-glyceraldehyde acetonide,[9] was treated with dimethyl cuprate to give 1,3-diol **14** together with undesired 1,2-diol in the ratio of 1:1, which was separated in the final step by silica HPLC (hexane/EtOAc (2:1)). The diastereomers **10**–**12** were also obtained by similar procedures from the corresponding epoxy alcohols.[9] The C-12–C-16 fragment derived from **1** was identical with the syn–anti isomer **4** including the sign of optical rotation [synthetic, $[\alpha]_D$ +41° (*c* 1.0, $CHCl_3$); natural, $[\alpha]_D$ +44° (*c* 0.23, $CHCl_3$)], thus determining the 13*R*,14*R*,15*R*-configurations for **1**. From these results the structure of amphidinolide J was firmly established as **1** including the absolute stereochemistry of the six chiral centers.

Scheme II. Synthesis of the C-12–C-16 Fragment 4[a]



[a] Key: (a) CuI (12 equiv), MeLi (24 equiv), Et_2O, −40 °C, 4 h, then −23 °C, 30 min; (b) 1 N HCl, THF, rt, 25 h; (c) Ac_2O, pyridine, rt, 11 h; (d) HPLC separation.

Amphidinolide J (**1**) bears a novel molecular constitution and substitution pattern compared with those of previously obtained macrolides from marine dinoflagellates and is a very rare example of microalgal metabolites whose absolute configurations have been defined by chemical means.

Acknowledgment. We thank Prof. T. Sasaki (Kanazawa University) for the cytotoxicity test. This work was partly supported by a Grant-in-Aid from the Tokyo Biochemical Research Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Supplementary Material Available: Experimental procedures, compound characterization, and 2D NMR spectra of compound **1** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(4) Barchi, J. J., Jr.; Moore, R. E.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 8193–8197.

(5) All new substances described here afforded satisfactory spectroscopic data including HRMS (see supplementary material).

(6) For the C-1–C-7 fragment, 3,4-syn (**2a**) and 3,4-anti (**2b**) isomers were concurrently generated and were able to be separated by silica HPLC (hexane/EtOAc (2:1)). The structures of **2a** and **2b** were assigned based on the spectral comparison with the data of *cis*- and *trans*-whisky lactones: Günther, C.; Mosandl, A. *Liebigs Ann. Chem.* **1986**, 2112–2122.

(7) The numberings of the carbons of all compounds described here corresponded to those of the parent compound, amphidinolide J (**1**).

(8) Williams, D. R.; Jass, P. A.; Allan Tse, H.-L.; Gaston, R. D. *J. Am. Chem. Soc.* **1990**, *112*, 4552–4554. We gratefully thank Dr. N. Nakajima and Prof. O. Yonemitsu for the generous gift of allyl alcohols **8** and **9**.

(9) Minami, N.; Ko, S. S.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 1109–1111.