Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 4369–4371

Amphezonol A, a novel polyhydroxyl metabolite from marine dinoflagellate *Amphidinium* sp.

Takaaki Kubota,[a] Yusuke Sakuma,[a] Kazutaka Shimbo,[a] Masashi Tsuda,[a] Michiko Nakano,[b] Yasuhiro Uozumi[b] and Jun'ichi Kobayashi[a,*]

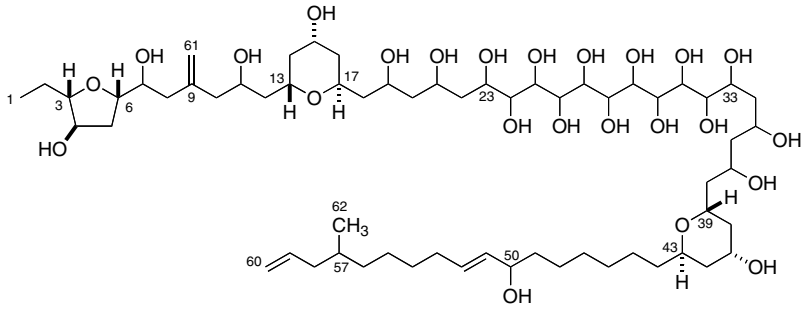
[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[b]*Institute for Molecular Science, Myodaiji, Okazaki 444-8787, Japan*

Received 24 March 2006; revised 15 April 2006; accepted 20 April 2006

Abstract—Amphezonol A (**1**), a novel polyhydroxyl linear carbon-chain metabolite, has been isolated from the cultured marine dinoflagellate *Amphidinium* sp., which was isolated from an Okinawan marine acoel flatworm *Amphiscolops* sp. The structure of **1** was elucidated by detailed analyses of 2D NMR spectra. Amphezonol A (**1**) possesses one tetrahydrofuran ring, two tetrahydropyran rings, and twenty-one hydroxyl groups on C_{60}-linear aliphatic chain with one *exo*-methylene and one methyl branch. Amphezonol A (**1**) exhibited a modest inhibitory activity against DNA polymerase α.

During our continuing search for structurally unique secondary metabolites from marine dinoflagellates, we have isolated a series of cytotoxic macrolides, amphidinolides, as well as long chain polyhydroxyl compounds from the dinoflagellate *Amphidinium* sp.[1] We previously investigated a strain of *Amphidinium* sp. (strain number Y-72), which was isolated from the inside cells of the Okinawan marine acoel flatworm *Amphiscolops* sp., and isolated amphidinolides G and H.[1a] Further investigation of extracts of the cultured dinoflagellate (Y-72) led to the isolation of a novel polyhydroxyl metabolite, amphezonol A (**1**),[2] possessing one tetrahydrofuran ring, two tetrahydropyran rings, and twenty-one hydroxyl groups on C_{60}-linear aliphatic chain with one *exo*-methylene and one methyl branches. In this letter, we describe the isolation and structure elucidation of amphezonol A (**1**).

The dinoflagellate was unialgally cultured at 25 °C for two weeks in seawater medium enriched with 1% ES supplement. The cultured algal cells were harvested by centrifugation and extracted with MeOH/toluene (3:1). The extract was partitioned between hexane and 1 M NaCl aq, and the aqueous phase was successively extracted with



Amphezonol A (**1**)

Keywords: Dinoflagellate; *Amphidinium* sp.; Polyhydroxyl metabolite; Amphezonol A.
* Corresponding author. Tel.: +81 11 706 3239; fax: +81 11 706 4989; e-mail: jkobay@pharm.hokudai.ac.jp

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.04.094

toluene. The toluene soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH) followed by purification with a C_{18} column (MeOH/H_2O) and then reversed-phase HPLC was performed (Luna phenylhexylsilyl, MeOH/H_2O/TFA, 72:28:0.05) to afford amphezonol A (**1**, 0.0038%, wet weight). FABMS of **1** showed the pseudomolecular ion peak at *m*/*z* 1266 $(M+Na)^+$, and its molecular formula, $C_{62}H_{114}O_{24}$, was established by HRFABMS [*m*/*z* 1265.7607 $(M+Na)^+$, $\Delta + 1.0$ mmu]. The IR spectrum indicated the presence of hydroxyl group (υ_{max} 3420 cm^{-1}). ^1H and ^{13}C NMR data (Table 1) revealed that **1** contained one sp^2 quaternary carbon, three sp^2 methines, two sp^2 methylenes, twenty-eight sp^3 methines, of which twenty-seven were oxymethines, twenty-six sp^3 methylenes, and two methyl groups. Since three out of six degree of unsaturation implied by the molecular formula were accounted for, **1** was inferred to possess three rings.

The structure of amphezonol A (**1**) was elucidated by extensive 2D NMR experiments. The ^1H–^1H COSY and HOHAHA spectra of **1** revealed connectivities of five partial structures, C-1–C-8, C-10–C-23, C-33–C-44, C-49–C-53, and C-56–C-60 and C-57–C-62 as shown in Figure 1. HMBC correlations of H_2-61 to C-8, C-9, and C-10 and H-8–C-10 implied that an *exo*-methylene (C-61, δ_C 115.4) at C-9 were connected to C-8 and C-10 via C-9 (δ_C 145.9). Connections between C-23–C-33, C-44–C-49, and C-56–C-60 were deduced from correlations obtained from the HSQC-TOCSY and INADEQUATE spectra.

The disubstituted double bond at C-51 was indicated to have an *E* geometry by the ^1H–^1H coupling constant ($J_{51,52} = 15$ Hz). The presence of a tetrahydrofuran and two tetrahydropyran rings were deduced from deuterium-induced shift analysis of the oxymethine carbon signals in the ^{13}C NMR spectra of **1**, observed in CD_3OD and CD_3OH, respectively, although HMBC correlations through each ether linkage were not observed. Six oxymethine signals for C-3 (δ_C 77.6), C-6 (δ_C 74.7), C-13 (δ_C 68.9), C-17 (δ_C 71.7), C-39 (δ_C 65.3), and C-43 (δ_C 69.6) did not show deuterium-induced shifts, suggesting that C-3 and C-6, C-13 and C-17, and C-39 and C-43 were connected to each other through an ether linkage, respectively. Relative stereochemistries of a tetrahydrofuran ring (C-3–C-6) and two tetrahydropyran rings (C-13–C-17 and C-39–C-43) in **1** were elucidated on the basis of ROESY correlations of **1** (Fig. 2). Thus, the structure of amphezonol A was assigned as **1**.

Amphezonol A (**1**) was a novel polyhydroxyl metabolite consisting of a C_{60}-linear aliphatic chain with a tetrahydrofuran ring, two tetrahydropyran rings, twenty-one hydroxyl groups, one *exo*-methylene, and one methyl group. Although some linear long chain polyhydroxyl compounds such as amphidinols,[3] luteophanols,[4]

Table 1. ^1H (920 MHz) and ^{13}C NMR (230 MHz) data of amphezonol A (**1**) in CD_3OD/C_5D_5N (2:1)

Position	δ_H		δ_C	Position	δ_H		δ_C
1	1.01[b]		11.5	32	3.98		85.7
2	1.53	1.74	27.0	33	4.00		73.9
3	3.55		77.6	34	1.83	2.18	41.0
4	3.91		75.4	35	4.26		77.1
5	1.91	2.17	36.6	36	2.37[a]		42.9
6	4.03		74.7	37	4.59		73.6
7	3.94		73.9	38	1.77	1.85	39.4
8	2.44	2.56	41.1	39	4.30		65.3
9			145.9	40	1.84	2.38	36.9
10	2.35	2.38	46.4	41	4.02		73.5
11	4.18		70.5	42	1.73	1.80	41.4
12	1.65	1.71	45.8	43	4.05		69.6
13	4.15		68.9	44	1.48	1.56	40.0
14	1.53	1.61	45.2	45	1.36	1.48	27.3
15	3.68		75.4	46	1.26[a]		31.1
16	1.36	1.59	33.3	47	1.26[a]		31.2
17	3.31		71.7	48	1.35	1.41	27.1
18	1.54	2.07	34.5	49	1.50	1.59	39.3
19	3.37		82.2	50	4.10		73.8
20	1.77	2.22	41.7	51	5.55		135.6
21	4.21		70.8	52	5.66		132.0
22	1.74	1.87	44.0	53	1.99		33.6
23	4.44		72.1	54	1.25	1.32	30.8
24	4.43		73.0	55	1.19	1.23	28.3
25	3.93		83.4	56	1.02	1.23	37.8
26	4.33		72.8	57	1.39		34.1
27	4.47		74.5	58	1.79	1.98	42.7
28	4.73		83.7	59	5.73		139.0
29	4.47		79.3	60	4.95		116.6
30	4.60		81.0	61	4.99	5.03	115.4
31	4.60		78.0	62	0.80[b]		20.4

[a] 2H.
[b] 3H.

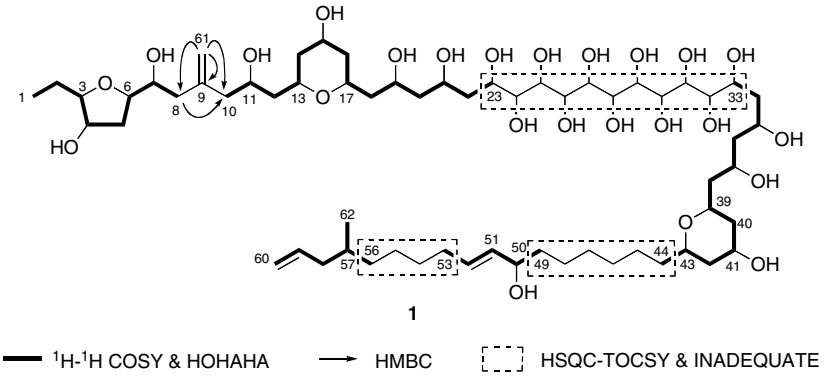


Figure 1. Selected 2D NMR correlations for amphezonol A (**1**).

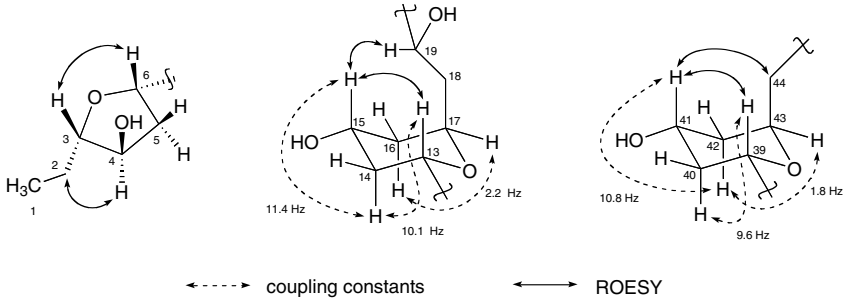


Figure 2. Selected ROESY correlations and relative stereochemistry for a tetrahydrofuran and two tetrahydropyran rings in amphezonol A (**1**).

lingshuiols,[5] karatungiols,[6] and colopsinols,[7] have been isolated from the dinoflagellate *Amphidinium* sp., the successive hydroxylated moiety of the carbon chain (C-23–C-33) is characteristic of **1**. Amphezonol A (**1**) exhibited a modest inhibitory activity against DNA polymerase α (IC_{50} 15 μM). Further investigations on the stereochemistry of **1** are currently carried out.

Acknowledgments

The authors thank Ms. S. Oka and Ms. M. Kiuchi, Center for Instrumental Analysis, Hokkaido University, for measurements of FABMS. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References and notes

1. (a) Kobayashi, J.; Tsuda, M. *Nat. Prod. Rep.* **2004**, *21*, 77–93; (b) Kubota, T.; Sakuma, Y.; Tsuda, M.; Kobayashi, J. *Mar. Drugs* **2004**, *3*, 83–87; (c) Tsuda, M.; Kariya, Y.; Iwamoto, R.; Fukushi, E.; Kawabata, J.; Kobayashi, J. *Mar. Drugs* **2005**, *3*, 1–8.
2. *Amphezonol A* (**1**). A colorless amorphous solid; IR (KBr) ν_{max} 3420 cm^{-1}; FABMS (pos.) *m/z* 1265 $(M+Na)^+$; HRFABMS *m/z* 1265.7607 $(M+Na)^+$, Δ +1.0 mmu.
3. (a) Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9859–9861; (b) Paul, G. K.; Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 6279–6282; (c) Paul, G. K.; Matsumori, N.; Konoki, K.; Murata, M.; Tachibana, K. *J. Mar. Biotechnol.* **1997**, *5*, 124–128; (d) Echigoya, R.; Rhodes, L.; Oshima, Y.; Satake, M. *Harmful Algae* **2005**, *4*, 383–389; (e) Morsy, M.; Matsuoka, S.; Houdai, T.; Matsumori, N.; Adachi, S.; Murata, M.; Iwashita, T.; Fujita, T. *Tetrahedron* **2005**, *61*, 8606–8610.
4. (a) Doi, Y.; Ishibashi, M.; Nakamichi, H.; Kosaka, T.; Ishikawa, T.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3820–3823; (b) Kubota, T.; Tsuda, M.; Doi, Y.; Takahashi, A.; Nakamichi, H.; Ishibashi, M.; Fukushi, E.; Kawabata, J.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 14455–14464; (c) Kubota, T.; Takahashi, A.; Tsuda, M.; Kobayashi, J. *Mar. Drugs* **2005**, *3*, 113–118.
5. (a) Huang, X.; Zhao, D.; Guo, Y.; Wu, H.; Lin, L.; Wang, Z.; Ding, J.; Lin, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3117–3120; (b) Huang, X.; Zhao, D.; Guo, Y.; Wu, H.; Trivellone, E.; Cimino, G. *Tetrahedron Lett.* **2004**, *45*, 5501–5504.
6. Washida, K.; Koyama, T.; Yamada, K.; Kita, M.; Uemura, D. *Tetrahedron Lett.* **2006**, *47*, 2521–2525.
7. (a) Kobayashi, J.; Kubota, T.; Takahashi, M.; Ishibashi, M.; Tsuda, M.; Naoki, H. *J. Org. Chem.* **1999**, *64*, 1478–1482; (b) Kubota, T.; Tsuda, M.; Takahashi, M.; Ishibashi, M.; Naoki, H.; Kobayashi, J. *J. Chem. Soc., Perkin Trans. 1* **1999**, *64*, 3483–3487; (c) Kubota, T.; Tsuda, M.; Takahashi, M.; Ishibashi, M.; Oka, S.; Kobayashi, J. *Chem. Pharm. Bull.* **2000**, *48*, 1447–1451.