Tetrahedron Letters, Vol. 34, No. 17, pp. 2795-2798, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Altohyrtin A, a Potent Anti-tumor Macrolide from the Okinawan Marine Sponge *Hyrtios altum*

Motomasa Kobayashi,[a] Shunji Aoki,[a] Haruhiko Sakai,[a] Kazuyoshi Kawazoe,[a] Noriaki Kihara,[a] Takuma Sasaki,[b] and Isao Kitagawa,*[a]

Faculty of Pharmaceutical Sciences, Osaka University,[a] Yamada-oka 1-6, Suita, Osaka 565, Japan and Cancer Research Institute, Kanazawa University,[b] Takara-machi 13-1, Kanazawa, Ishikawa 920, Japan

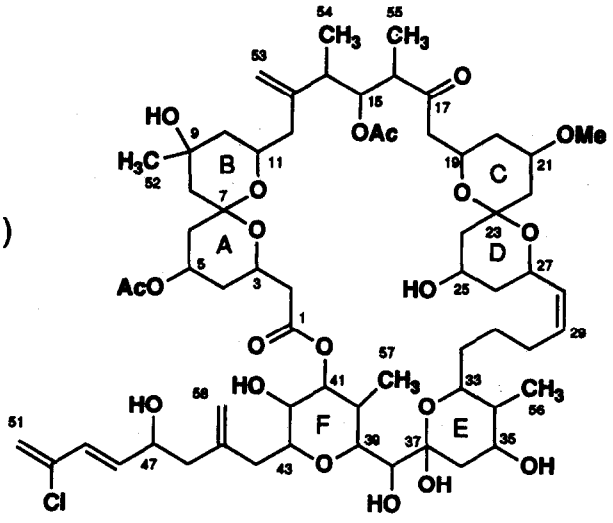
Abstract: Altohyrtin A (1) has been isolated from the Okinawan marine sponge *Hyrtios altum* and the plane structure and parts of its relative configurations elucidated. Altohyrtin A (1) exhibited extremely potent cytotoxicity against KB cells at IC_{50} 0.01 ng/ml.

In our continuing studies of searching for new bioactive substances from marine organisms,[1] we have isolated a very potent cytotoxic macrolide named altohyrtin A from the Okinawan marine sponge *Hyrtios altum*. This paper describes the structure elucidation.

An acetone extract of the titled fresh sponge (112 kg collected in July at Aragusuku-jima, Okinawa Prefecture), which exhibited a potent cytotoxic activity (IC_{50} 0.56 μg/ml) against KB cells, was subjected to bioassay-guided separation (cytotoxicities against KB and L1210 cells). The extract was partitioned into a water-AcOEt mixture to provide the cytotoxic AcOEt soluble portion (222 g). Repeated SiO_2 column chromatography of the AcOEt soluble portion (38 g) furnished fr.B (2.2 g)[IC_{50} 0.002 μg/ml (KB)]. The fr.B was found to demonstrate potent anti-tumor activity against P388 murine leukemia (mice, *i.p.*) : T/C 155% (10 mg/kg treated on days 1,5). Further repeated chromatography (SiO_2 and HPLC) of the fr.B (828 mg) provided altohyrtin A (1)(0.5 mg)(3.4×10^{-3}% from the AcOEt soluble portion) together with several congeners. Altohyrtin A (1) exhibited extremely potent cytotoxicities against KB (IC_{50} 0.01 ng/ml) and L1210 (IC_{50} 0.1 ng/ml) cell lines, respectively.

Altohyrtin A (1) was obtained as an amorphous solid: $[\alpha]_D$ +21.7° (*c*=1.2, MeOH); UV λ_{max} (MeOH): 227 nm (ε=19000)[2]; IR ($CHCl_3$): 3423, 2959, 1733 cm^{-1}. The FAB MS of 1 showed a quasi-molecular ion at *m/z* 1245 $(M+Na)^+$ with isotope ions in the ratio of 10:7:6 and the molecular formula was determined as $C_{63}H_{95}O_{21}Cl$ by HR-

altohyrtin A (1)



FABMS and NMR analysis. Ordinary acetylation (Ac_2O/pyridine, r.t.) of 1 furnished the triacetate [FABMS: *m/z* 1371 $(M+Na)^+$].

The ^{13}C NMR spectra of altohyrtin A (1) disclosed the presence of 63 carbons (Table I), whose characteristics were defined by DEPT experiments. Four partial structures (fragment A: C-1 ~ C-6 and C-38 ~ C-51, fragment B: C-7 ~ C-14, fragment C: C-15 ~ C-23, fragment D: C-24 ~ C-37) were elucidated by the detailed analysis of ^1H-^1H COSY, HMQC, HOHAHA, and HMBC spectra[3,4]. The locations of hydroxyl groups were determined by deuterium shift observed in the ^{13}C NMR spectra of 1 taken in CD_3OH and CD_3OD. The seven carbons at C-9 (δc 71.1), 25 (δc 65.9), 35 (δc 72.8), 37 (δc 100.0), 38 (δc 74.2), 42 (δc 74.4), and C-47 (δc 71.8) were shifted to high-field by 0.1 ppm while other oxygenated carbons unchanged. Furthermore, the locations of hydroxyl groups were also confirmed by ^1H-^1H COSY spectrum taken in d_6-DMSO,[5] which revealed the correlations between several hydroxyl protons and hydroxymethine protons [25-H and 25-OH (δ 4.10, d, *J*=7Hz); 35-H and 35-OH (δ 4.28, d, *J*=10.5); 38-H and 38-OH (δ 4.63, d, *J*=7Hz); 42-H and 42-OH (δ 5.25, d, *J*=6Hz); 47-H and 47-OH (δ 4.94, d, *J*=6Hz)].

Each ring structure and the connectivity of those partial structures were figured out mainly on the basis of following HMBC correlations measured in d_5-pyridine. 1) Macrolide structure: cross peaks between C-1 carbon and H_2-2, H-3, H-41, 2) adjacency of rings A and B: cross peaks between C-7 and H-2; C-7 (δc 100.3 in CD_3OD) and H-3 (δ 4.35 in CD_3OD); C-6 and H-8; C-7 and H_2-8, 3) connection of fragments B (C-7~C-14) and C (C-15~C-23): cross peaks between C-13 (δc 149.4 in CD_3OD), C-54 (δc 12.9 in CD_3OD) and H-15 (δ 5.32 in CD_3OD); C-15 (δc 76.1 in CD_3OD)

Table I. ^{13}C (125MHz) and ^{1}H (500MHz) NMR Spectral Data for Altohyrtin A in d_5-pyridine.

atom	^{13}C (mult.)	^{1}H(mult., J(Hz))	atom	^{13}C (mult.)	^{1}H(mult., J(Hz))
1	172.2 (s)		31	26.7 (t)	1.87 (m)
2	40.2 (t)	2.61 (m)			1.46 (m)
		2.47 (d-like, 11.5)	32	32.5 (t)	1.63 (m)
3	62.2 (d)	4.54 (t-like, 11.5)			1.56 (m)
4	34.3 (t)	1.76 (t-like, 13.5)	33	66.7 (d)	4.61 (d-like, 8)
		1.44 (br. s)	34	39.1 (d)	1.87 (m)
5	66.5 (d)	5.14 (br.s)	35	71.2 (d)	3.99 (d-like, 3)
6	37.9 (t)	2.07 (d-like, 11.5)	36	34.3 (t)	2.59 (m)
		1.60 (d-like, 11.5)			1.95 (d-like, 14)
7	98.4 (s)		37	99.4 (s)	
8	46.5 (t)	1.70 (d, 13.5)	38	73.2 (d)	3.80 (s)
		1.41 (d, 13.5)	39	81.5 (d)	4.17 (d, 10.5)
9	68.5 (s)		40	37.3 (d)	2.55 (m)
10	44.9 (t)	1.67 (m)	41	80.7 (d)	5.25 (dd, 11, 9.5)
		1.33 (m)	42	73.3 (d)	3.57 (t-like, 9.5)
11	64.2 (d)	4.69 (t-like, 11)	43	79.2 (d)	3.90 (t-like, 9.5)
12	44.0 (t)	2.67 (dd, 13.5, 11)	44	40.1 (t)	3.32 (d-like, 13)
		2.44 (m)			2.21 (m)
13	148.0 (s)		45	143.9 (s)	
14	36.4 (d)	3.23 (qd, 8, 3)	46	44.2 (t)	2.73 (dd, 13.5, 7)
15	74.9 (d)	5.73 (d-like, 10)			2.51 (m)
16	47.7 (d)	3.26 (m)	47	70.0 (d)	4.75 (t-like, 5.5)
17	211.8 (s)		48	139.9 (d)	6.54 (dd, 15, 5.5)
18	51.2 (t)	3.03 (dd, 15, 10)	49	126.4 (d)	6.72 (d, 15)
		2.78 (d-like, 15)	50	139.1 (s)	
19	65.6 (d)	4.26 (t-like, 10)	51	115.4 (t)	5.44 (s)
20	37.4 (t)	2.00 (m)			5.39 (s)
		1.17 (m)	52	30.6 (q)	1.17 (s)
21	73.7 (d)	3.50 (tt, 11.5, 3)	53	114.2 (t)	5.22 (br. s)
22	44.0 (t)	2.25 (dd, 10, 3)			5.12 (br. s)
		1.59 (m)	54	12.0 (q)	1.35 (d, 8)
23	99.5 (s)		55	13.1 (q)	1.33 (d, 7.5)
24	34.9 (t)	2.41 (dd, 12, 8)	56	11.4 (q)	0.87 (d, 7)
		1.60 (d-like, 12)	57	12.7 (q)	1.06 (d, 6.5)
25	63.8 (d)	4.20 (br. s)	58	115.8 (t)	5.11 (s)
26	39.0 (t)	1.95 (d-like, 14)			4.87 (s)
		1.72 (m)	5-Ac	170.6 (s)	
27	61.3 (d)	5.48 (d-like, 10)		21.5 (q)	2.17 (s)
28	131.5 (d)	5.69 (d-like, 10)	15-Ac	169.2 (s)	
29	132.5 (d)	5.64 (m)		20.7 (q)	2.03 (s)
30	27.5 (t)	2.41 (m)	21-OMe	55.3 (q)	3.29 (s)
		2.31 (dd-like, 8, 4)			

and H-54 (δ 1.05 in CD_3OD), 4) 15-acetate: cross peaks between C-15 and acetoxymethyl protons (δ 2.03); acetoxycarbonyl carbon (δc 169.2) and H-15, 5) adjacency of rings C and D: cross peaks between C-23 (δc 101.0 in CD_3OD) and H-18 (δ 2.74 in CD_3OD); C-21 and methoxyl protons; C-23 and H-27; C-24 and H-22, 6) connection of fragments D and A: a cross peak between C-37 and H-38, 7) ring F: cross peak between C-43 and H-38.

In Fig. 1, NOESY correlations (in d_6-DMSO)[5] in each partial structure are shown, thus confirming the above-mentioned ring structures and the connectivity of the partial structures and also leading to the partial relative stereostructures.

Based on the accumulated above evidence, the plane structure, with some partial relative configurations, of altohyrtin A has been elucidated shown as **1**.[6] Altohyrtin A (1) is a new class of anti-tumor marine macrolide and the stereostructure is now under investigation.

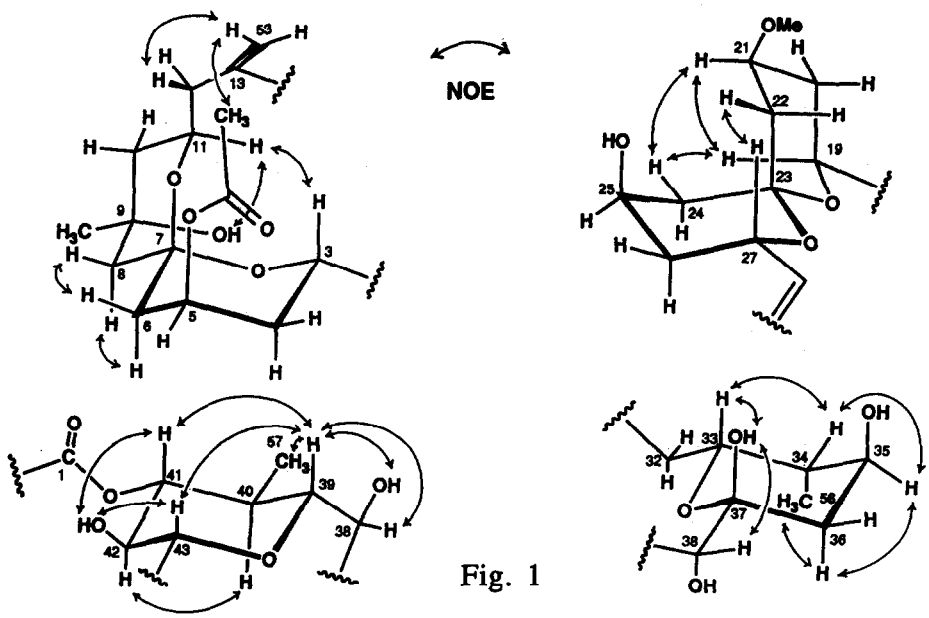


Fig. 1

Acknowledgement The authors are grateful to Prof. N. Fusetani, the Univ. of Tokyo, for helpful discussion and invaluable information.[6] Thanks are due to Research Laboratory of Fujisawa Pharmaceutical Co., Ltd. for NMR measurement. The authors are also grateful to the Ministry of Education, Science, and Culture of Japan for financial support.

References and Notes

1) Part XXX: M. Kobayashi, K. Kawazoe, T. Katori, and I. Kitagawa, *Chem. Pharm. Bull.*, **40**, 1773 (1992), and preceding papers.

2) This UV absorption was assignable to a 1,3-butadiene unit substituted with an alkyl and a chlorine (R-CH=CH-C(Cl)=CH_2) which were supported by 2D NMR analysis.

3) 2D NMR were recorded on a JEOL GX-500 or a Bruker AMX500 NMR spectrometer in d_5-pyridine, CD_3OD, or d_6-DMSO and were mainly discussed on the data obtained by d_5-pyridine measurement.

4) The geometry of Δ^{28} and Δ^{48} double bonds were assigned *Z* and *E* respectively on the basis of the coupling constants ($J_{28,29}$=10.5 Hz, $J_{48,49}$=15 Hz).

5) δ (*J*=Hz) in d_6-DMSO: H-3 (4.17, t-like, *J*=11.5), 5-Ac (1.94, s), H_a-6 (1.62, m), H_b-6 (1.78, d-like, *J*=10.5), H_a-8 (1.52, s), H_b-8 (1.55, s), OH-9 (3.93, s), H-11 (4.55, t-like, *J*=11), H_a-12 (2.05, m), H_a-53 (4.80, s), H-19 (3.96, t-like, *J*=11.5), H-21 (3.50, m), H-22eq (1.98, m), H-24eq (1.52, m), H-25 (3.60, m, changed to $W^h/_2$= 7.2 Hz upon irradiation of 25-OH), H-27 (4.90, m), H-33 (4.05, d-like, *J*=10.5), H_3-56 (0.81, d, *J*=7), H-34 (1.43, m), H_3-56 (0.81, d, *J*=7), H-35 (3.87, m), H_a-36 (1.55, m), OH-37 (4.74, s), H-38 (3.28, d, *J*=7), H-39 (3.66, d, *J*=10.5), H-40 (1.84, m), H_3-57 (0.73, d, *J*=6.5), H-41 (4.68, t-like, *J*=10), H-42 (3.05, ddd, *J*=10.5, 10, 6), H-43 (3.37, t-like, *J*=10.5), H-47 (4.26, dd-like, *J*=13, 6).

6) Prof. N. Fusetani and his group have recently isolated a potent cytotoxic macrolide named cinachyrolide A from a marine sponge of *Cinachyra* sp. collected at Hachijo-jima, Japan, and elucidated the plane structure with the same basic skeleton as **1** (a personal communication).

(Received in Japan 12 February 1993)