ALTOHYRTINS B AND C AND 5-DESACETYLALTOHYRTIN A, POTENT CYTOTOXIC MACROLIDE CONGENERS OF ALTOHYRTIN A, FROM THE OKINAWAN MARINE SPONGE *HYRTIOS ALTUM*

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Following the characterization of altohyrtin A (**1**), altohyrtins B (**2**) and C (**3**) and 5-desacetylaltohyrtin A (**4**) have been isolated from the Okinawan marine sponge *Hyrtios altum* and their plane structures and parts of their relative configurations elucidated. Three congeneric macrolides (**2**, **3**, and **4**) exhibit extremely potent cytotoxicities similar to those of **1** against KB cells with IC_{50} values of 0.02, 0.4, and 0.3 ng/ml, respectively.

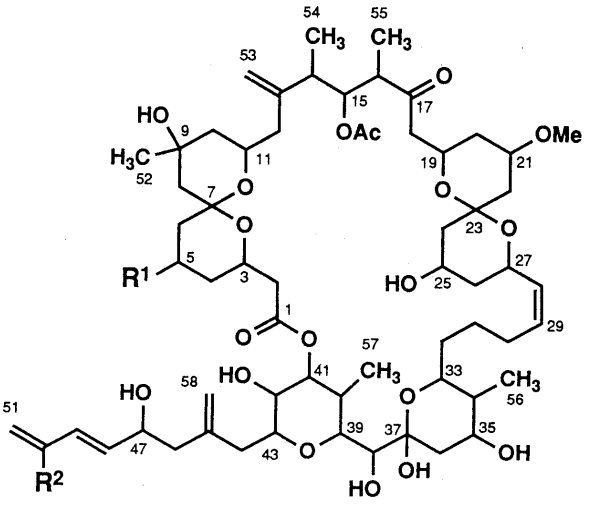
KEYWORDS marine sponge; *Hyrtios altum*; cytotoxic macrolide; altohyrtin B; altohyrtin C; 5-desacetylaltohyrtin A

During the course of our investigations in search of new biologically active substances from marine organisms,[1] we isolated a very potent cytotoxic macrolide named altohyrtin A (**1**) [IC_{50} 0.01 ng/ml (KB)] from the Okinawan marine sponge *Hyrtios altum* and characterized the structure.[2] Further investigation of this marine sponge has led to the isolation of three new related macrolides named altohyrtin B (**2**), altohyrtin C (**3**), and 5-desacetylaltohyrtin A (**4**). This paper describes the structure elucidation of these congeneric macrolides.

Repeated chromatography (SiO_2 and HPLC) of the AcOEt soluble portion (222g) of the acetone extract of the fresh titled sponge (112kg collected in July at Aragusuku-jima, Okinawa Prefecture) provided altohyrtin B (**2**)(0.5mg)(2.2×10^{-4}% from the AcOEt soluble portion), altohyrtin C (**3**)(0.5mg)(2.2×10^{-4} %), and 5-desacetylaltohyrtin A (**4**)(4.7mg)(2.1×10^{-3}%) together with altohyrtin A (**1**)(3.4×10^{-3}%).

Altohyrtin B (**2**) was obtained as an amorphous solid: $[\alpha]_D$ +45° (*c*=0.2, MeOH); UV λ_{max} (MeOH): 228 nm (ε=20000); IR (KBr): 3418, 2939, 1734 cm^{-1}. The FAB MS of **2** showed quasimolecular ions at *m/z* 1289 and 1291 $(M+Na)^+$, and the molecular formula has been shown as $C_{63}H_{95}O_{21}Br$ by HR-FAB MS and NMR analysis. The FAB MS of altohyrtin C (**3**)[3] gave a quasimolecular ion $(M+Li)^+$ peak at *m/z* 1195 [HR-FAB MS: $C_{63}H_{96}O_{21}Li$]. As shown in Table I, all proton signals in the NMR spectra of **2** and **3**, which were assigned on the basis of COSY experiment, closely resembled those of altohyrtin A (**1**). Above all, the 49-H, 50-H, and 51-H_2 signals in **3** were observed as (dd, *J*=10, 15 Hz), (ddd, *J*=10, 10, 17 Hz), and [(d, *J*=10 Hz) and (d, *J*=17 Hz)], while the 49-H and 51-H_2 signals in **1** and **2** were observed as (d, *J*=15 Hz) and (two s), respectively. Consequently, altohyrtin B and altohyrtin C have been

	R^1	R^2
altohyrtin A (**1**)	OAc	Cl
altohyrtin B (**2**)	OAc	Br
altohyrtin C (**3**)	OAc	H
5-desacetyl-altohyrtin A (**4**)	OH	Cl



elucidated to be a 50-bromo analog **2** and a deschloro analog **3** of altohyrtin A (**1**), respectively.

The all ^{1}H and ^{13}C signals of 5-desacetylaltohyrtin A (**4**)[4] [FAB MS: *m/z* 1203 $(M+Na)^+$, $C_{61}H_{93}O_{21}ClNa$] were assigned as given in Tables I and II on the bases of COSY, HMQC, and HMBC experiments of **4**. The C-4, 5, and 6 carbon signals in altohyrtin A (**1**) were observed with respective acylation shifts of $\Delta\delta$ -3.2, +2.6, and -2.4 ppm as compared with those of **4**. Thus, 5-desacetylaltohyrtin A (**4**) has been presumed to be a 5-desacetyl analog of **1**. In order to facilitate the location of free hydroxyl groups in **4**, we have taken the ^{13}C NMR spectra in CD_3OH and CD_3OD. The deuterium shifts of eight carbon signals due to C-5, 9, 25, 35, 37, 38, 42, and 47 were observed by 0.1-0.15 ppm while

Table I. ^{1}H NMR Data for Altohyrtins A (**1**), B (**2**), and C (**3**) and 5-Desacetylaltohyrtin A (**4**) (at 500MHz in d_6-DMSO, *J* Values in Hz)

Proton(s) at	1	2	3	4
2	2.50 (m)	2.53 (d-like, 11)	2.52 (d-like, 10)	2.58 (d-like, 10.5)
	2.70 (m)	2.74 (m)	2.75 (d-like, 9.5)	2.67 (m)
3	4.17 (t-like, 11.5)	4.17 (t-like, 11)	4.16 (m)	4.08 (m)
4	1.53 (m), 1.65 (m)	1.54(m), 1.65 (m)	1.54 (m), 1.65 (m)	1.47 (m), 1.56 (m)
5	4.90 (m)	4.90 (m)	4.90 (m)	3.85 (m)
6	1.62 (m)	1.67 (m)	1.63 (m)	1.53 (m)
	1.78 (d-like, 10.5)	1.78 (dd, 15.5, 10)	1.78 (d-like, 10)	1.65 (m)
8	1.52 (s), 1.55 (s)	1.52 (s), 1.55 (s)	1.52 (s), 1.55 (s)	1.52 (s), 1.55 (s)
10	1.21 (m)	1.22 (m)	1.24 (m)	1.32 (t-like, 12.5)
	1.55 (m)	1.54 (m)	1.53 (m)	1.60 (d-like, 12.5)
11	4.55 (t-like, 11)	4.55 (t-like, 10.5)	4.55 (t-like, 11)	4.57 (t-like, 11.5)
12	2.05 (m)	2.03 (m)	2.03 (m)	1.97 (m)
	2.25 (m)	2.24 (m)	2.24 (m)	2.36 (d-like, 13.5)
14	2.83 (q-like, 6.5)	2.84 (q-like, 6.5)	2.84 (q-like, 6)	2.83 (q-like, 7)
15	5.17 (d-like, 11)	5.17 (d-like, 10.5)	5.17 (d-like, 10.5)	5.21 (d-like, 12)
16	2.98 (dq, 11, 7)	2.98 (dq, 10.5, 7)	2.98 (dq, 10.5, 7)	2.98 (dq, 10.5, 7)
18	2.67 (d-like, 18)	2.68 (dd, 18, 3)	2.67 (m)	2.67 (d-like, 18)
	2.76 (dd, 18, 10)	2.78 (dd, 18, 10.5)	2.78 (dd, 18, 10.5)	2.79 (dd, 18, 10)
19	3.96 (t-like, 11.5)	3.95 (t-like, 10.5)	3.95 (t-like, 11.5)	3.95 (t-like, 11.5)
20	0.84 (m)	0.85 (d-like, 11)	0.85 (d-like, 11.5)	0.87 (d-like, 11)
	2.00 (m)	1.98 (m)	1.97 (m)	2.00 (m)
21	3.50 (m, Wh/2 = 24)	3.51 (m)	3.51 (m)	3.49 (m)
22	1.04 (m)	1.06 (m)	1.02 (m)	1.04 (m)
	1.98 (m)	1.98 (m)	1.97 (m)	1.96 (dd, 11.5, 7.5)
24	1.52 (m)	1.55 (m)	1.52 (m)	1.53 (d-like, 10.5)
	1.83 (m)	1.84 (m)	1.84 (m)	1.83 (dd, 10.5, 4.5)
25	3.60 (m, Wh/2= 12)	3.59 (m)	3.60 (m)	3.60 (m)
26	1.43 (m), 1.53 (m)	1.43 (m), 1.54 (m)	1.41 (m), 1.52 (m)	1.42 (m), 1.53 (m)
27	4.90 (m)	4.90 (m)	4.90 (m)	4.89 (td-like, 7, 4)
28	5.35 (d-like, 11)	5.34 (d-like, 11.5)	5.35 (d-like, 11)	5.34 (d-like, 11)
29	5.36 (m)	5.35 (m)	5.36 (m)	5.36 (m)
30	2.05 (m), 2.08 (m)	2.03 (m), 2.08 (m)	2.03 (m), 2.09 (m)	2.02 (m), 2.05 (m)
31	1.12 (m)	1.11 (d-like, 9.5)	1.12 (m)	1.12 (m)
	1.65 (m)	1.64 (m)	1.66 (m)	1.65 (m)
32	1.12 (m), 1.28 (m)	1.12 (m), 1.28 (m)	1.13 (m), 1.26 (m)	1.12 (m), 1.26 (m)
33	4.05 (d-like, 10.5)	4.05 (d-like, 11)	4.05 (d-like, 9.5)	4.05 (d-like, 10)
34	1.43 (m)	1.43 (m)	1.43 (m)	1.43 (m)
35	3.87 (m)	3.87 (m)	3.85 (m)	3.85 (m)
36	1.55 (m), 2.24 (m)	1.52 (m), 2.24 (m)	1.54 (m), 2.22 (m)	1.55 (m), 2.23 (m)
38	3.28 (d, 7)	3.28 (m)	3.28 (d, 8)	3.25 (d, 8)
39	3.66 (d, 10.5)	3.66 (m)	3.60 (d-like, 10)	3.67 (d, 10)
40	1.84 (m)	1.84 (m)	1.85 (m)	1.87 (m)
41	4.68 (t-like, 10)	4.67 (dd, 10.5, 9.5)	4.68 (t-like, 10)	4.71 (m)
42	3.05 (ddd, 10.5, 10, 6)	3.04 (t-like, 10.5)	3.04 (ddd, 10, 10, 6)	3.05 (ddd, 10, 9, 6)
43	3.37 (t-like, 10.5)	3.37 (td, 10.5, 2)	3.36 (t-like, 10)	3.39 (t-like, 9)
44	2.05 (m)	2.03 (m)	1.98 (m)	2.00 (m)
	2.73 (m)	2.72 (m)	2.72 (m)	2.72 (dd, 14, 3)
46	2.12 (m)	2.12 (dd, 6, 13)	2.08 (m)	2.12 (dd, 14, 6.5)
	2.24 (d-like, 13)	2.24 (d-like, 13)	2.25 (d-like, 13.5)	2.25 (d-like, 14)
47	4.26 (dd-like, 13, 6)	4.27 (m)	4.16 (m)	4.25 (m)
48	6.07 (dd, 15, 6)	6.02 (dd, 14.5, 5.5)	5.72 (dd, 15, 6)	6.07 (dd, 15, 5.5)
49	6.40 (d, 15)	6.31 (d, 14.5)	6.16 (dd, 15, 10)	6.40 (dd, 15, 1)
50	-	-	6.30 (ddd, 17, 10, 10)	-
51	5.36 (s)	5.61 (s)	5.01 (d, 10)	5.36 (s)
	5.54 (s)	5.97 (s)	5.14 (d, 17)	5.54 (s)
52	1.03 (s)	1.02 (s)	1.02 (s)	1.03 (s)
53	4.75 (s), 4.80 (s)	4.75 (s), 4.85 (s)	4.82 (s), 4.82 (s)	4.72 (s), 4.72 (s)
54	0.93 (d, 6.5)	0.93 (d, 6.5)	0.93 (d, 7)	0.91 (d, 7)
55	1.09 (d, 7)	1.09 (d, 7)	1.09 (d, 7)	1.09 (d, 7)
56	0.81 (d, 7)	0.81 (d, 7)	0.81 (d, 7)	0.81 (d, 7)
57	0.73 (d, 6.5)	0.73 (d, 6.5)	0.73 (d, 6.5)	0.73 (d, 6.5)
58	4.82 (s), 4.85 (s)	4.80 (s), 4.82 (s)	5.25 (s), 5.27 (s)	4.82 (s), 4.85 (s)
5-Ac	1.94 (s)	1.94 (s)	1.93 (s)	-
15-Ac	1.82 (s)	1.82 (s)	1.82 (s)	1.81 (s)
21-OMe	3.21 (s)	3.21 (s)	3.21 (s)	3.21 (s)
5-OH	-	-	-	3.72 (d, 10.5)
9-OH	3.93 (s)	3.93 (s)	3.93 (s)	3.89 (s)
25-OH	4.10 (d, 7)	4.10 (d, 7.5)	4.10 (d, 7.5)	4.10 (d, 7)
35-OH	4.28 (d, 10.5)	4.28 (d, 10)	4.28 (d, 10)	4.25 (d, 10)
37-OH	4.74 (s)	4.74 (s)	4.74 (s)	4.74 (d, 2)
38-OH	4.63 (d, 7)	4.64 (br. s)	4.61 (d, 8.5)	4.63 (d, 8)
42-OH	5.25 (d, 6)	5.26 (br. s)	5.35 (br. s)	5.26 (d, 6)
47-OH	4.94 (d, 6)	4.95 (d, 6)	4.80 (d, 5)	4.94 (d, 5.5)

Table II. ^{13}C NMR Data for Altohyrtins A (1) and 5-Desacetylaltohyrtin A (4) at 125 MHz in d_6-DMSO

Carbon at	1	4	Carbon at	1	4	Carbon at	1	4
1	171.5 (s)	171.9 (s)	23	98.4 (s)	98.4 (s)	45	143.1 (s)	143.0 (s)
2	38.2 (t)	38.2 (t)	24	34.0 (t)	33.9 (t)	46	42.7 (t)	42.6 (t)
3	60.4 (d)	60.7 (d)	25	69.5 (d)	69.5 (d)	47	68.8 (d)	68.8 (d)
4	33.1 (t)	36.3 (t)	26	38.2 (t)	38.0 (t)	48	138.6 (d)	138.6 (d)
5	65.8 (d)	63.2 (d)	27	59.5 (d)	59.4 (d)	49	125.5 (d)	125.4 (d)
6	36.9 (t)	39.3 (t)	28	130.6 (d)	130.6 (d)	50	137.5 (s)	137.5 (s)
7	97.5 (s)	99.7 (s)	29	132.5 (d)	132.5 (d)	51	116.0 (t)	115.8 (t)
8	45.8 (t)	45.0 (t)	30	26.6 (t)	26.7 (t)	52	29.8 (q)	29.6 (q)
9	67.7 (s)	67.2 (s)	31	25.9 (t)	25.7 (t)	53	113.3 (t)	112.6 (t)
10	44.1 (t)	43.6 (t)	32	31.3 (t)	31.6 (t)	54	11.5 (q)	11.1 (q)
11	63.1 (d)	63.8 (d)	33	65.6 (d)	65.9 (d)	55	12.7 (q)	12.6 (q)
12	42.7 (t)	42.6 (t)	34	46.2 (d)	46.1 (d)	56	11.2 (q)	11.1 (q)
13	146.8 (s)	148.3 (s)	35	62.4 (d)	62.4 (d)	57	11.9 (q)	11.9 (q)
14	36.0 (d)	35.9 (d)	36	34.9 (t)	35.1 (t)	58	114.6 (t)	114.5 (t)
15	72.9 (d)	72.7 (d)	37	97.6 (s)	97.4 (s)	5-Ac	20.7 (q)	-
16	46.1 (d)	46.2 (d)	38	71.6 (d)	71.5 (d)		170.2 (s)	-
17	210.8 (s)	210.5 (s)	39	80.2 (d)	80.1 (d)	15-Ac	20.2 (q)	20.4 (q)
18	49.8 (t)	49.6 (t)	40	33.6 (d)	33.4 (d)		168.4 (s)	168.3 (s)
19	64.4 (d)	64.4 (d)	41	78.7 (d)	78.9 (d)	21-OMe	54.2 (q)	54.7 (q)
20	36.9 (t)	36.7 (t)	42	71.4 (d)	71.4 (d)			
21	72.5 (d)	72.4 (d)	43	78.1 (d)	78.0 (d)			
22	42.7 (t)	42.6 (t)	44	38.4 (t)	38.6 (t)			

other signals due to oxygenated carbons were unchanged. Consequently, the plane structure of 5-desacetylaltohyrtin A has been determined as **4**.

Next, the NOESY correlations of 5-desacetylaltohyrtin A (**4**) have led to the partial relative stereostructures depicted in Fig. 1, which are quite similar to those of altohyrtin A (**1**).[2] Furthermore, the partial relative stereostructures of altohyrtins B (**2**) and C (**3**) have been presumed also to be identical with those of **1** as based on the detailed comparison of chemical shifts and coupling patterns in their ^1H NMR spectra shown in Table I.

Altohyrtins B (**2**) and C (**3**) and 5-desacetylaltohyrtin A (**4**) exhibit extremely potent cytotoxicities against KB (IC_{50} 0.02, 0.4, and 0.3 ng/ml) and L1210 (IC_{50} 0.03, 1.3, and 2.3 ng/ml) cell lines, respectively.

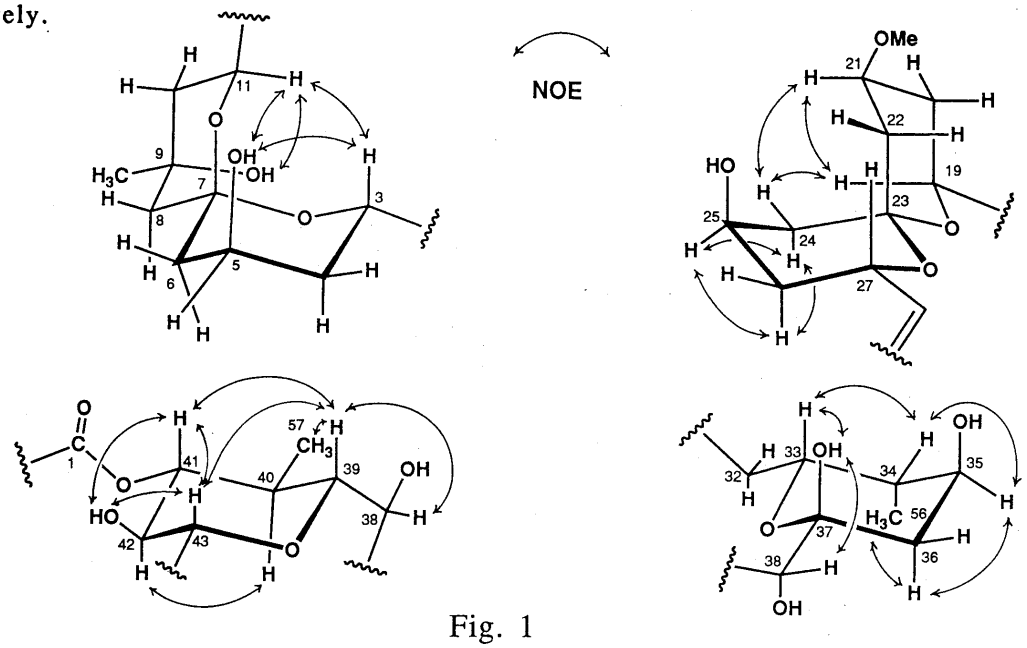


Fig. 1

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2) M. Kobayashi, S. Aoki, H. Sakai, K. Kawazoe, N. Kihara, T. Sasaki, and I. Kitagawa, *Tetrahedron Lett.*, in press.
3) **3**: $[\alpha]_D$ +31° (c=0.3, MeOH); UV (MeOH): 226 nm (ε=17000); IR (KBr): 3420, 2937, 1736 cm^{-1}.
4) **4**: $[\alpha]_D$ +19° (c=1.1, MeOH); UV (MeOH): 227 nm (ε=20000); IR (KBr): 3416, 2929, 1736 cm^{-1}.

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