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Absolute Stereostructures of Altohyrtin A and Its Congeners, Potent Cytotoxic Macrolides from the Okinawan Marine Sponge *Hyrtios altum*

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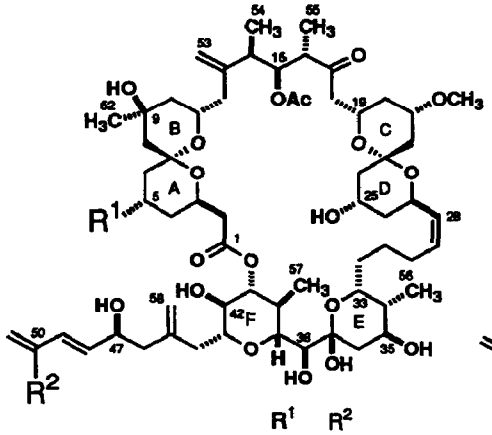
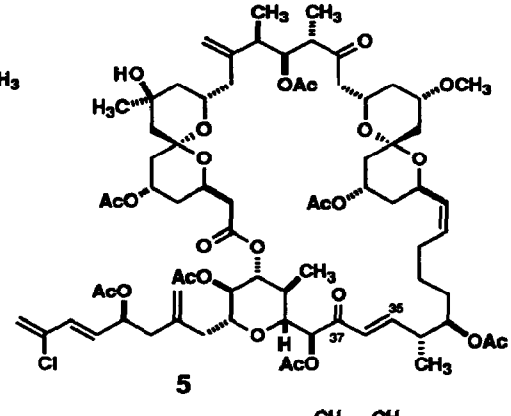
Abstract: The absolute stereostructures of altohyrtins A (1), B (2), C (3), and 5-desacetylaltohyrtin A (4), which were isolated from the Okinawan marine sponge *Hyrtios altum*, have been elucidated.

In our continuing studies of searching for new bioactive substances from marine organisms,[1] we have found potent cytotoxic [IC_{50} 0.01 - 0.3 ng/ml (KB)] macrolides named altohyrtins A (1), B (2), C (3), and 5-desacetylaltohyrtin A (4), and elucidated their plane structures along with parts of their relative configura-



		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



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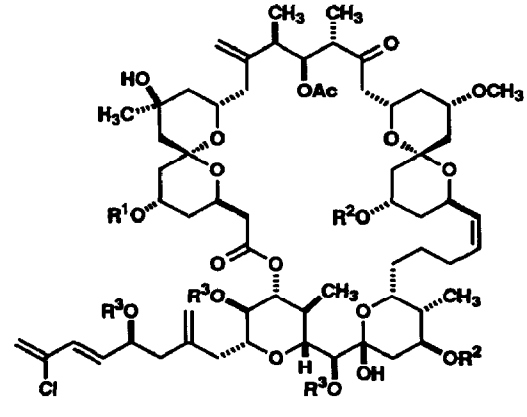
	R^1	R^2	R^3
6	*R*-(+)-MTPA	*R*-(+)-MTPA	*R*-(+)-MTPA
7	*S*-(-)-MTPA	*S*-(-)-MTPA	*S*-(-)-MTPA
8	Ac	H	-CO-  -Br



tions.[2,3] Recently, Pettit and his group independently reported the isolation of cytotoxic macrolides named spongistatins 1, 2, and 3 from a marine sponge *Spongia* sp. and the reported plane structures were similar to our **1**, **2**, and **4**, respectively.[4] On the other hand, Fusetani and his group reported the isolation of a 15-desacetyl analog of **1** (named cinachyrolide A) from a marine sponge *Cinachyra* sp. and elucidated the plane structure with parts of its relative configurations.[5] We report herein the absolute stereostructure elucidation of altohyrtins (**1** - **4**).

In the preceding paper,[3] we presumed that altohyrtins B (**2**) and C (**3**) and 5-desacetylaltohyrtin A (**4**) possess the same relative stereostructure as that of altohyrtin A (**1**) on the basis of comparisons in detail of chemical shifts and coupling patterns in their ^{1}H NMR spectra. Furthermore, treatment of altohyrtin A (**1**) and 5-desacetylaltohyrtin A (**4**) with Ac_2O-pyridine and DMAP furnished an identical heptaacetate **5**,[6] which has a 35-en-37-one structure presumably formed through opening of the hemiketal at C-37 and dehydration of the 35-hydroxyl moiety. Thus, it has been confirmed that 5-desacetylaltohyrtin A (**4**) possesses the same stereostructure including the C-5 configuration as that of altohyrtin A (**1**).

The absolute stereostructures of altohyrtins (**1**-**4**) have been elucidated in the following manner. Firstly, we have examined in detail the NOESY spectrum of both altohyrtin A (**1**) and 5-desacetylaltohyrtin A (**4**).[2,3] As shown in Fig. 1, the NOESY correlations (in d_6-DMSO) in **4** have led us to figure out four partial relative stereostructures [C_2-C_{16}; C_{18}-C_{27}; C_{33}-C_{44}; C_{45}-C_{47}].[7]

Secondly, we have applied the modified MTPA method[8] to determine the absolute stereostructures of altohyrtins (**1**-**4**). Treatment of **4** with R-(+)- or S-(-)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) and DCC, DMAP in CH_2Cl_2 at room temperature furnished the hexa-MTPA ester (**6**[9] or **7**[10]), respectively. The all proton signals of both **6** and **7** were assigned as given in Table I on the bases of COSY, HOHAHA, and HMQC experiments. The comparisons in detail of chemical shifts of all proton

Table I. ^{1}H NMR Data for R-(+)-hexaMTPA ester (**6**) and S-(-)-hexaMTPA ester (**7**) (at 500 MHz in d_6-DMSO, *J* Value in Hz)

Proton(s) at	**6**	**7**	Proton(s) at	**6**	**7**
2	2.50 (m), 2.61 (m)	2.15 (m), 2.47 (m)	33	3.88 (t-like, 9)	4.12 (t-like, 10)
3	4.15 (m)	4.02 (m)	34	1.40 (m)	1.70 (m)
4	1.58 (m), 2.00 (m)	1.64 (m), 1.85 (m)	35	4.92 (m)	4.92 (m)
5	5.28 (m)	5.38 (m)	36	1.73 (m), 1.74 (m)	1.75 (m), 1.90 (m)
6	1.80 (m), 1.85 (m)	1.85 (m), 190 (m)	38	4.98 (s)	4.70 (s)
8	1.45 (s), 1.48 (s)	1.55 (s), 1.64 (s)	39	3.94 (d, 11)	3.90 (d, 11)
10	1.10 (m), 1.60 (m)	1.15 (m), 1.65 (m)	40	1.94 (m)	1.50 (m)
11	3.95 (m)	4.05 (m)	41	4.90 (m)	4.92 (m)
12	1.69 (m), 2.00 (m)	1.72 (m), 2.10 (m)	42	4.96 (m)	4.70 (m)
14	2.40 (q-like, 7)	2.45 (m)	43	3.73 (t-like, 10)	3.80 (td, 10.5, 2)
15	4.97 (m)	4.85 (t-like, 5)	44	1.83 (m), 2.02 (m)	1.93 (m), 1.97 (m)
16	2.94 (m)	2.80 (t-like, 5)	46	2.05 (m), 2.24 (m)	2.23 (m), 2.23 (m)
18	2.61 (m), 2.71 (m)	2.45 (m), 2.50 (m)	47	5.59 (dd-like, 12, 6)	5.63 (dd-like, 12, 6)
19	3.57 (t-like, 11)	3.70 (m)	48	5.78 (dd, 15, 6)	6.01 (dd, 15, 6)
20	0.63 (m), 1.91 (m)	0.78 (m), 2.06 (m)	49	6.20 (d, 15)	6.50 (d, 15)
21	3.42 (m)	3.50 (m)	51	5.45 (s), 5.50 (s)	5.55 (s), 5.61 (s)
22	1.02 (m), 1.97 (m)	1.05 (m), 1.98 (m)	52	1.05 (s)	1.05 (s)
24	1.75 (m), 2.27 (m)	1.75 (m), 2.38 (m)	53	4.70 (s), 4.75 (s)	4.75 (s), 4.78 (s)
25	5.28 (m)	5.34 (m)	54	0.89 (d, 7)	0.75 (d, 7)
26	1.67 (m), 1.79 (m)	1.60 (m), 1.73 (m)	55	0.91 (d, 7)	0.95 (d, 7)
27	5.05 (td-like, 10, 4)	4.75 (m)	56	0.24 (d, 7)	0.70 (d, 7)
28	5.45 (m)	5.35 (m)	57	0.95 (d, 6.5)	0.78 (d, 7)
29	5.40 (m)	5.40 (m)	58	4.65 (s), 4.82 (s)	4.70 (s), 4.70 (s)
30	1.90 (m), 1.90 (m)	1.85 (m), 1.85 (m)	15-Ac	1.78 (s)	1.78 (s)
31	2.00 (m), 2.00 (m)	1.80 (m), 1.85 (m)	21-OMe	3.29 (s)	3.29 (s)
32	1.05 (m), 1.20 (m)	1.18 (m), 1.30 (m)			

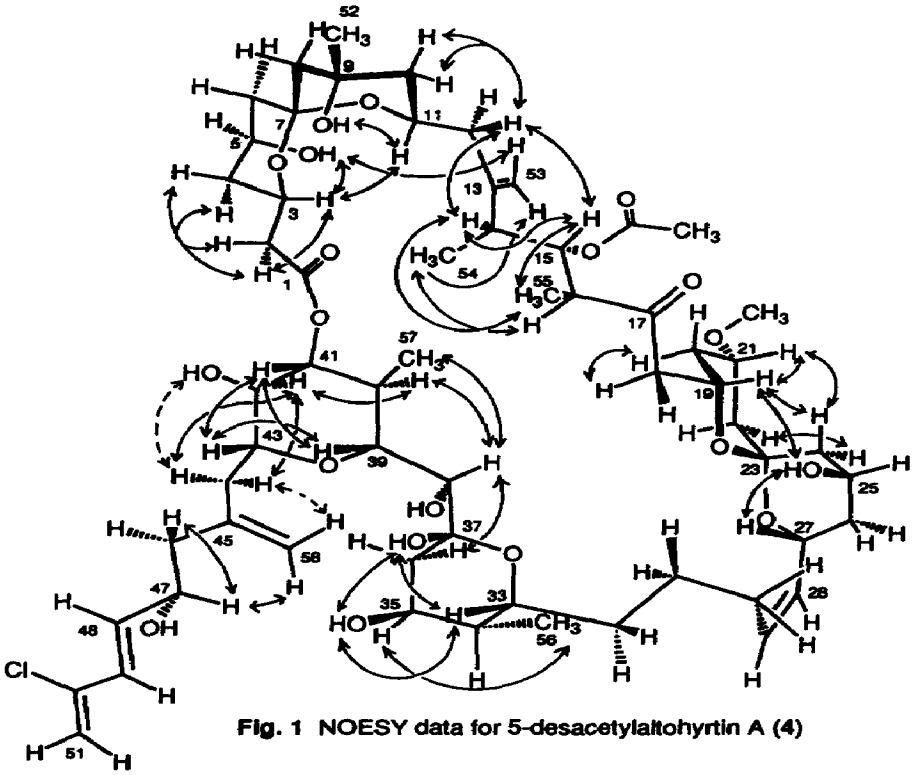


Fig. 1 NOESY data for 5-desacetylaltohyrtin A (4)

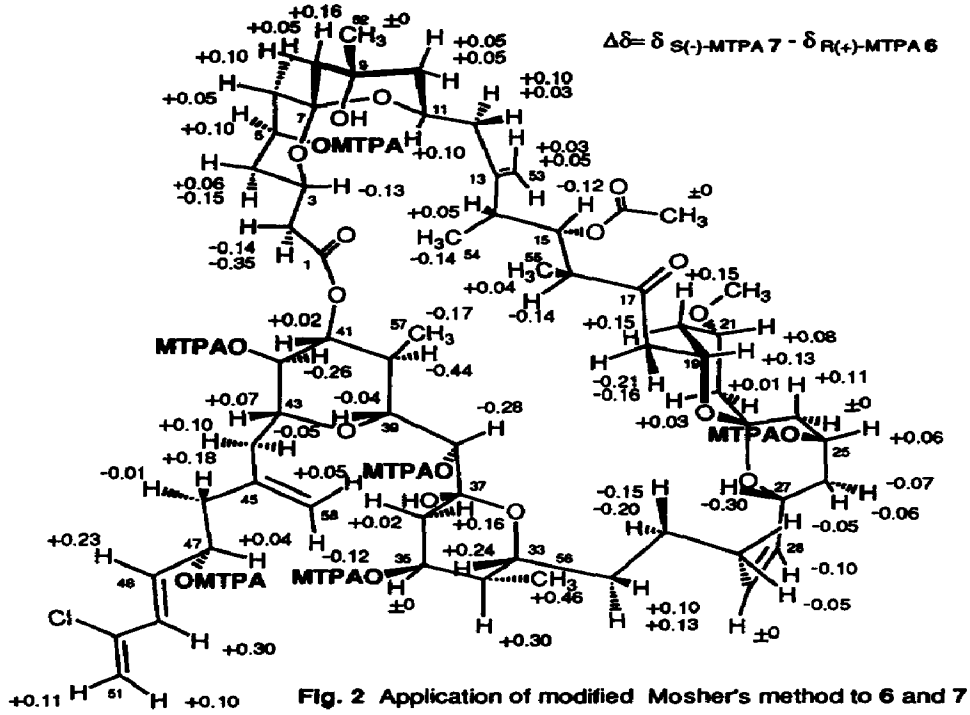


Fig. 2 Application of modified Mosher's method to 6 and 7

signals of **6** and **7** have shown the absolute configurations to be 5S, 25S, 35S, 38S, 42R, and 47S as depicted in Fig. 2. Thus, the C_{38} MTPA residue was found to face the opposite direction as compared to both C_{35} and C_{42} MTPA residues. So that, significant $\Delta\delta$ values were observed for the signals of H_3-56 and H-40. It is noteworthy to mention that the reversed shifts ($\Delta\delta$) were observed for the signals of H-15, H-16, H_2-18, and H_3-54 due to their spacial location behind the C_{25} MTPA residue.

Thirdly, we have applied the circular dichroism (CD) exciton chirality method[11] to the tri-*p*-bromobenzoate **8**[12], which was prepared from **1** by treatment with *p*-bromobenzoic acid, DCC, and DMAP. **8** showed a split CD maxima ($\Delta\varepsilon$ +44.5 at 244 nm, $\Delta\varepsilon$ -15.5 at 230 nm), which arose from the exciton coupling between the 47-*p*-bromobenzoate and the 48,50-diene chromophores. So that, the absolute configuration at C_{47} of **8** and consequently of **1** has been determined as S. The result thus obtained is consistent with that obtained by the above MTPA method.

The above-mentioned evidence has led us to propose the absolute stereostructures of altohyrtins (1-4) as shown. We are currently engaged in synthetic and molecular dynamics study of altohyrtins in order to acquire more informations on the structure-bioactivity relations.

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References and Notes

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