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Haterumalide B: A New Cytotoxic Macrolide from an Okinawan Ascidian *Lissoclinum* sp.

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Abstract: Haterumalide B (1), a new cytotoxic macloride, was isolated from an Okinawan ascidian *Lissoclinum* sp. and its structure of 1 determined by spectroscopic investigation. Haterumalide B completely inhibited the first cleavage of fertilized sea urchin eggs at a concentration of 0.01 μg/ml.
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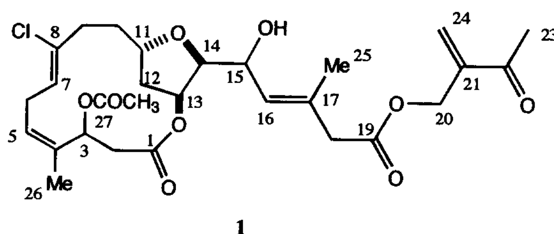
Key words: cytotoxin, macrolide, marine metabolite, NMR

Ascidians, one of the most interesting groups of marine invertebrates, have yielded a variety of marine natural products.[1,2] As part of our continuing chemical studies of Okinawan marine organisms, we examined the constituents of an ascidian *Lissoclinum* sp. whose crude organic extract displayed strong inhibition of the cell division of fertilized sea urchin eggs. Bioassay-guided fractionation of the extract led to the isolation of a new chlorinated macrolide, haterumalide B (1).

The encrusting brown ascidian (4.5 kg) was collected from dead corals in Hateruma Island, Okinawa and stored at -15° until extracted with acetone. The acetone extract was initially partitioned between ethyl acetate and water. The cytotoxic, EtOAc-soluble material was fractionated by silica-gel column chromatography with a gradient from hexanes to CH_2Cl_2 to MeOH. Active fractions were combined and separated by medium-pressure liquid chromatography (silica gel, $CHCl_3$-EtOAc, 1:1) and then by PTLC (silica gel, cyclohexane-dioxane-EtOAc, 3:1:1). Purification by HPTLC (silica gel 60 F_{254}, Merck) using $CHCl_3$-EtOAc (2:1) as the developing solvent gave haterumalide B (1, 1.5 mg).[3]



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The molecular formula, $C_{28}H_{37}ClO_9$, was established by HRFABMS [*m/z* 575.2021 (M^{+}+Na), Δ 0.3 mmu] and the ^{13}C NMR spectrum (Table 1). Analysis of the IR (ν_{max} 3436, 2926, 2855, 1738, 1732, 1714, 1682, 1372, 1295, 1230 cm^{-1}) and NMR data (Table 1) indicated the presence of three ester groups, a ketone, a hydroxyl group, three trisubstituted double bonds, a disubstituted double bond with a terminal methylene, and four methyls. Of the four methyls, two were associated with the double bonds, and the others were attached to the carbonyls, as indicated by their ^{1}H NMR chemical shifts. The presence of an exchangeable proton was confirmed by the fact that a ^{1}H NMR signal (δ 3.86, 1H, d, J= 5.5 Hz), which did not appear in $CDCl_3$, was observed in acetone-d_6 (Table 1). Four isolated spin systems (A-D)(Figure 1) could be assigned by ^{1}H-^{1}H COSY (Table 1) and HMQC experiments. HMBC correlations (Table 1) were used to assemble the partial structures. The HMBC correlations from methylene protons at C2 and the methine proton at C13 to the lactone (or ester) carbon C1 (δ 168.0) indicated that partial structure A was linked by an ester bond to C. The HMBC correlations from H3 to C4 and C5, from the methyl protons at C26 to C3 allowed for the attachment (C3-C4) of A to B. Of the methylene protons at C9 one showed a $^{2}J_{CH}$ coupling to C8 and the other a $^{3}J_{CH}$ coupling to C7. The second ester carbonyl (δ 170.7) was established at C19 based on correlations from the methylene protons at C20 to the carbonyl. The presence of a 2-methylene-3-oxobutyl group (C20-C24) in the molecule was confirmed by the HMBC correlations observed between the methyl protons at C23 and the carbonyl carbon C22 and between the terminal methylene protons at C24 and the same carbonyl carbon. The HMBC correlations from the methyl protons at C28 and the methine proton at C3 to the third ester carbonyl (δ 169.5) positioned an acetate group at C3. The presence of an ether bridge between C11 and C14 was confirmed by the HMBC correlation between C11 and H14. At the point of the structure elucidation, there were only two open ends on C8 and O15 of a partial structure comprised of all elements except for a chlorine and a hydrogen. Thus, the chlorine substituent was placed at C8 and the hydroxyl group at C15. The $^{3}J_{HH}$ coupling between the hydroxyl proton and the methine proton at C15 in acetone-d_6 supported the position of the hydroxyl group.

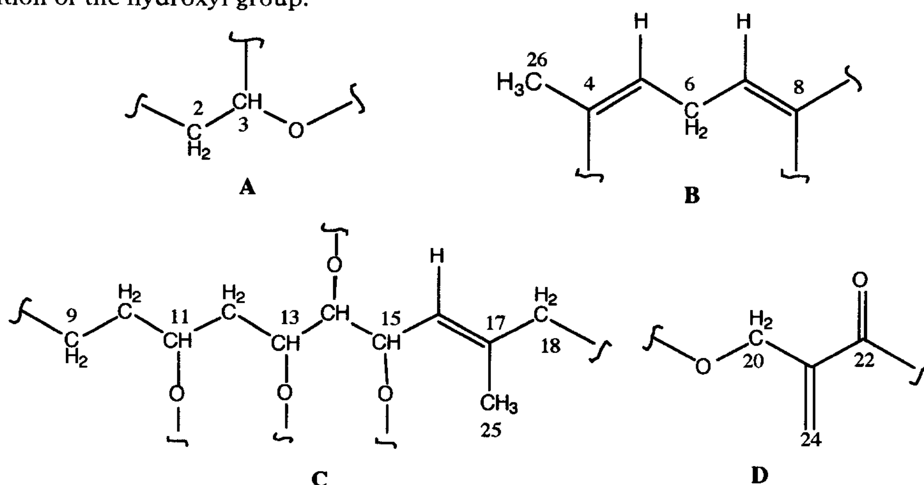

Table 1 NMR Data for Haterumalide B[a]

C#	δ_C mult.	δ_H	mult., J (Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC (8 Hz)[b]
1	168.0 s				
2a	38.0 t	2.70	dd, 5.2, 11.3	2b, 3	C1, C3, C4
2b		2.73	dd,11.0, 11.3	2a, 3	C1, C3, C4
3	67.4 d	5.71	dd, 5.2, 11.3	2a, 2b	C1, C2, C4, C5, C26,27
4	133.2 s				
5	129.8 d	5.62	m	6a, 6b, 26-CH_3	C3, C26
6a	26.7 t	2.47	m	5, 6b, 7	C5, C7
6b		3.46	m	5, 6a, 7	
7	125.6 d	5.20	br d, 8.5	6a, 6b	
8	132.0 s				
9a	34.8 t	2.10	m	9b, 10a, 10b	C7
9b		2.44	m	9a, 10a, 10b	C8
10a	28.0 t	1.34	m	9a, 9b, 10b, 11	C11
10b		2.45	m	9a, 9b, 10a, 11	C11
11	76.8 d	3.89	tt , 3.4, 12.0	10a, 10b, 12a, 12b	
12a	37.8 t	1.41	dt, 3.4, 12.0	11, 12b, 13	C10, C11
12b		2.07	dd, 3.4, 12.0	11, 12a	C13, C14
13	75.8 d	5.32	t, 3.4	12a, 14	C11[d], C14[d]
14	82.7 d	3.89	dd, 3.4, 7.9	13, 15	C11[d], C13[d], C15, C16
15	66.4 d	4.59	t, 7.9	14, 16	C13, C14, C16, C17
		(4.56	dt, 5.5, 8.0)[c]		
16	129.6 d	5.43	d, 7.9	15, 18, 25-CH_3	C14, C18, C25
17	134.3 s				
18	44.9 t	3.11	br s	6	C16, C17, C19, C25
19	170.7 s				
20	62.3 t	4.80	br s	24	C19, C21
21	143.2 s				
22	98.0 s				
23	25.9 q	2.37	s		C22
24a	126.8 t	6.01	br s	20	C20, C22
24b		6.17	s		C20, C22
25	17.4 q	1.86	br s	16	C16, C17, C18
26	18.3 q	1.87	br s	5	C3, C4, C5
27	69.5 s				
28	21.1 q	2.05	s		C27
OH		(3.86	d, 5.5)[c]		

[a] NMR spectra were recorded in $CDCl_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C, except where specifically noted. [b] ^{1}H-^{13}C HMBC in CDCl3 at 400 MHz. [c] Signals obtained in acetone-d_6 at 500 MHz. [d] ^{1}H-^{13}C HMBC (5 Hz) in $CDCl_3$ at 500 MHz.

The stereochemistry of haterumalide B was elucidated on the basis of difference nOe and NOESY experiments. The NOESY correlations H3/H6a, H5/$H_3$26, H7/H9a, H15/$H_3$25 revealed that the stereochemistry of three trisubstituted double bonds was 4Z, 7Z, and 16E. The relative stereochemistry of the tetrahydrofurane ring moiety was deduced from the NOESY correlations (H11/H12b, H12a/H12b, H12a/H13, H13/H14) and the ^{1}H-^{1}H coupling constants ($J_{11,12a}$ = 3.5 Hz, $J_{11,12b}$ = 12.0 Hz, $J_{12a,12b}$ = 12.0 Hz, $J_{12a,13}$ = 0 Hz, $J_{12b,13}$ = 4.0 Hz, $J_{13,14}$ = 4.2 Hz) for the tetrahydrofurane ring protons, which are analogous to those[4] for the protons of the similarly substituted tetrahydrofurane rings in fijianolide A (isolauliamide)[5,6] and pectenotoxin-1.[7] The configuration of C3 and C15 remained

to be determined.[8]

Although more than 200 marine macrolides have been recorded in the two decades since the first isolation of the aplysiatoxins,[9,10] halogenated marine macrolides are rarely found.[9-13] Haterumalide B, a chlorinated 14-membered ring macrolide, completely inhibited the first cleavage of fertilized sea urchin eggs at a concentration of 0.01 μg/ml.

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3. **1**: colorless oil; $[\alpha]^{23}_D$= -0.002° (c 0.08 , $CHCl_3$); UV (MeOH): λ_{max} 206 nm (ε=12000); FDMS: *m/z* (relative intensity) 554 [$(M+2)^+$, 10] , 552 (M^+, 12), 537 [$(M+2+H)^+$-H_2O, 20}, 535 [$(M+H)^+$-H_2O, 60), 377 (7), 475 (19), 211 [M^+-$C_{17}H_{22}ClO_5$, $(M+2)^+$-$C_{17}H_{22}ClO_5$, 100] ; for ^{1}H NMR and ^{13}C NMR Data, see Table 1.
4. Fijianolide A: $J_{11,12a}$ = 5.1 Hz, $J_{11,12b}$ = 10.6 Hz, $J_{12a,12b}$ =15.0 Hz, $J_{12a,13}$ = 0.7 Hz, $J_{12b,13}$ = 3.9 Hz, $J_{13,14}$ = 4.2 Hz. Pectenotoxin-1: δ_H 4.50 (dd, J = 6, 11 Hz, H11), 5.48 (ddd, J = 1, 3, 4 Hz, H13), 4.73 (ddd, J = 2, 3, 4 Hz, H14). The proton numbers of haterumalide B were given to the corresponding protons in fijianolide A and pecnotoxin-1. There were no ^{1}H NMR data for the methylene protons at C-12 in pecnotoxin-1.
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