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Halichlorine, an Inhibitor of VCAM-1 Induction from the Marine Sponge *Halichondria okadai* Kadota

Makoto Kuramoto, Chou Tong,† Kaoru Yamada,† Tatsuhiko Chiba, Yoshinori Hayashi, and Daisuke Uemura*

Department of Chemistry, Faculty of Science, Shizuoka University, Ohya, Shizuoka 422, Japan.
†Sagami Chemical Research Center, Nishi-Ohnuma 4-4-1, Sagamihara, Kanagawa 229, Japan.

Abstract: The novel alkaloid halichlorine was isolated from *Halichondria okadai* Kadota and its structure was elucidated by spectroscopic analysis. The relative stereochemistry was clarified by a detailed analysis of coupling constants and the NOESY spectrum. Halichlorine significantly inhibits VCAM-1 induction in cultured HUVE (human umbilical vein endothelial) cells.
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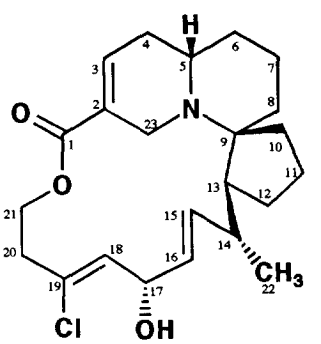
In our continuing search for biologically active substances from marine organisms,[1-4] we isolated a novel alkaloid, halichlorine (**1**), from the marine sponge *Halichondria okadai* Kadota.[5] This compound inhibits the induction of VCAM-1 (vascular cell adhesion molecule-1)[6] at IC_{50} 7 μg/ml. Drugs that block the induced expression of VCAM-1 may be useful for treating atherosclerosis, coronary artery diseases, angina and noncardiovascular inflammatory diseases.[7] We report here the isolation and structural elucidation of halichlorine.

The marine sponge *H. okadai* Kadota (collected at a mediolittoral zone in Kanagawa Prefecture in early April) was immersed in ethanol at room temperature. The ethanolic extract was filtered, concentrated under reduced pressure, and extracted with ethyl acetate. The ethyl acetate extract was partitioned between 70% aqueous methanol and hexane. The aqueous methanol layer was concentrated to give an oily material, which was separated by column chromatography on TSK G3000S polystyrene gel using a gradient elution of ethanol and water. The fraction eluted with 40% aqueous ethanol was subjected to column chromatography on ODS using a gradient elution of methanol and water. The fraction eluted with 25% aqueous methanol was purified by column chromatography on SiO_2 using a gradient elution of chloroform and methanol. The 5% methanol/chloroform eluate was finally purified by preparative TLC on SiO_2 with methanol/chloroform (V/V, 1:9) to give crystalline halichlorine (**1**) (70.8 mg, yield: 3.5×10^{-7}% from 200 kg of wet sponge).



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The molecular formula of halichlorine (**1**) ($[\alpha]_D$ + 240.7° (*c* 0.54, CH_3OH); m.p. 183.5-185.5 °C) was determined to be $C_{23}H_{32}ClNO_3$ by HR-EIMS data [*m/z* 405.2018 ($C_{23}H_{32}{}^{35}ClNO_3$), Δ -5.3 mmu] and EIMS data [*m/z* 405 (M^+), 407 (M^++2)].[8] Halichlorine exhibited IR (CS_2) absorption bands at 1715 cm^{-1} (carbonyl

group), 1655 cm^{-1} (double bond) and 3580 cm^{-1} (hydroxy group). The ^1H and ^{13}C NMR spectral data of halichlorine are shown in Table 1. An extensive NMR study (^1H NMR, ^{13}C NMR, ^1H-^1H COSY, ^{13}C-^1H COSY and DEPT) and a consideration of the molecular formula indicated that **1** has one methyl group, 10 methylenes, eight methines, four quaternary carbons, and one proton on a heteroatom.

Table 1. ^1H and ^{13}C NMR Spectral Data of Halichlorine (**1**).[a]

position	^1H		J (Hz)	^{13}C (mult.)[b]	HMBC	position	^1H		J (Hz)	^{13}C (mult.)[b]	HMBC
1				167.6 (s)		12 H12'	1.74	m		24.6 (t)	
2				128.4 (s)		H12'	1.75	m			
3	7.03	dddd	5.3, 2.6, 2.0, 1.5	139.2 (d)	C1, C2, C4	13	1.73	br. s		51.9 (d)	
4 H4	1.98	ddddd	19.5, 5.3, 3.5, 1.5, 0.5	33.5 (t)	C3, C5	14	2.73	qdd	7.0, 8.5, 1.0	33.7 (d)	C9, C12, C13, C15, C22
H4'	2.64	ddddd	19.5, 6.5, 4.0, 2.6, 2.0			15	5.76	ddd	15.5, 8.5, 1.5	139.0 (d)	C13, C14, C16, C17, C22
5	3.18	dddd	13.0, 6.5, 2.0, 1.5	51.8 (d)	C4, C6, C9, C23	16	5.35	ddd	15.5, 4.5, 1.0	128.3 (d)	C14, C15, C17
6 H6a	1.50	dddd	13.0, 13.0, 13.0, 4.5	24.9 (t)	C4, C5, C7, C8	17	5.03	ddd	7.8, 4.5, 1.5	69.6 (d)	C15, C16, C18, C19
H6b	1.13	dddd	13.0, 5.0, 4.5, 2.0			18	5.57	ddd	7.8, 1.1	133.0 (d)	C17, C19, C20
7 H7a	1.71	ddddd	9.5, 7.0, 5.0, 5.0, 4.5	22.1 (t)	C6, C8, C9	19				129.7 (s)	
H7b	1.78	ddddd	13.0, 9.5, 4.5, 4.0, 2.0			20 H20a	2.56	dddd	14.5, 3.0, 2.5, 1.1	38.7 (t)	C18, C19, C21
8 H8a	1.29	ddd	11.0, 5.0, 2.0	27.1 (t)	C6, C7, C9	H20b	2.87	ddd	14.5, 12.5, 4.5		
H8b	1.64	ddd	11.0, 7.0, 4.0			21 H21a	4.62	ddd	12.5, 11.5, 3.0	62.3 (t)	C1, C19, C20
9				70.8 (s)		H20b	3.98	ddd	11.5, 4.5, 2.5		
10 H10a	2.18	ddd	12.0, 7.0, 4.0	32.1 (t)	C9, C13	22	1.01	d	7.0	18.1 (q)	C13, C14, C15
H10b	1.43	ddd	12.0, 10.0, 3.0			23 H23	3.21	dddd	17.5, 2.0, 1.5, 0.5	41.9 (t)	C2, C3, C5
11 H11	1.66	m		22.4 (t)		H23'	3.45	dddd	17.5, 4.0, 3.5, 2.0		
H11'	1.68	m									

[a] Spectra were recorded in CD_3OD on a JEOL JNM-GSX400 and a GE GN-500 NMR spectrometers.
[b] Multiplicity was determined by a DEPT experiment.

The proton-proton coupling network in the ^1H-NMR spectrum of this compound could not be readily assigned due to the presence of quaternary carbons and overlapping of the signals at δ 1.64-1.75 ppm. Therefore, structural assignment was carried out by detailed analysis of the ^1H-^1H COSY and HMBC spectra, which gave structural units **a-d**, as shown in Figure 1. The proton connectivities of H3-H8 were revealed by crosspeaks in the ^1H-^1H COSY spectrum. On the other hand, carbon connectivities of C2 (δ_C 128.4), C3 and C23 were deduced by HMBC crosspeaks: H3/C2 and H23/C2. Furthermore, allylic couplings were observed between H23a,b and H3, and homoallylic couplings were observed between H4 and H23, respectively, in the ^1H-^1H COSY spectrum. These data suggested partial structure **a**. The location of quaternary carbon C19 (δ_C 129.7) between C18 and C20 was verified by the HMBC crosspeaks, H17/C19, H18/C19, and H20a and H20b/C19, and allylic coupling between H18 and H20a. A NOESY crosspeak was also observed between H18 and H20b. Therefore, the geometry of the double bond (C18-C19) was defined as shown in **b**. When halichlorine was acetylated with acetic anhydride/pyridine, the corresponding monoacetate was obtained. In the ^1H-NMR spectrum of the monoaceate, H17 (**1**: δ_H 5.03, δ_C 69.6) showed a clear downfield shift: δ 6.05 (-OCO$\underline{CH_3}$: δ_H 2.05). This result suggested the presence of a hydroxy group at C17.[9] The chemical shift of C21 (δ_H 3.98,

4.62, coupled with H20, δ_C 62.3) indicates that C21 was linked to an ether or ester oxygen. The geometry of the double bond (C15-C16) was determined to be *E* based on the large coupling constant of H15/H16 (15.5 Hz). Although a ^{1}H-^{1}H COSY crosspeak was observed between H13 and H14, the signal of H13 (δ 1.73) was observed as a broad singlet. And HMBC crosspeaks were observed for H14/C13, H15/C13 and H22/C13, thus indicating direct connectivity between C13 and C14. Proton-proton networks from H10-H13 were revealed by the ^{1}H-^{1}H COSY crosspeaks.

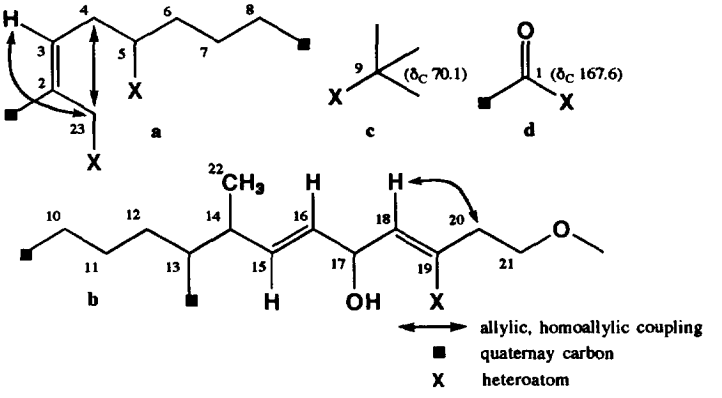

Figure 1. Partial structures of halichlorine.

Further extensive HMBC experiments revealed the connectivities of units **a-d**. The HMBC crosspeaks (H5/C23 and H23/C5) and the ^{13}C chemical shift (C5; δ_C 51.8, C23; δ_C 41.9) indicated the presence of a piperazine ring. Insertion of the quarternary carbon (C9; δ_C 70.8) between C8 and C10 was suggested by HMBC crosspeaks for H7/C9, H8/C9, and H10/C9. Furthermore, the ^{13}C chemical shift of C9 and the HMBC crosspeaks (H14/C9) corresponding to a three bond correlation verified the presence of an azabicyclo[4.4.0] ring consisting of C2-C9, C23 and a nitrogen atom, and of a [5.6]-spiro ring composed of C5-C13 and a nitrogen atom. Furthermore, HMBC crosspeaks for H3/C1, H21/C1, and H23/C1 revealed the presence of an α,β-unsaturated carbonyl and a 15-membered lactone. Finally, the chlorine atom could be at C19 (δ 129.7). This assignment was reasonable based on the ^{13}C NMR chemical shift,[10] and only this position remained for a chlorine atom among the 5 possible positions (X) in Figure 1. The double bond (C18-C19) was determined to be *E*-geometry based on an NOE between H18 and H20. Thus the planar structure of halichlorine was assigned to be **1**.

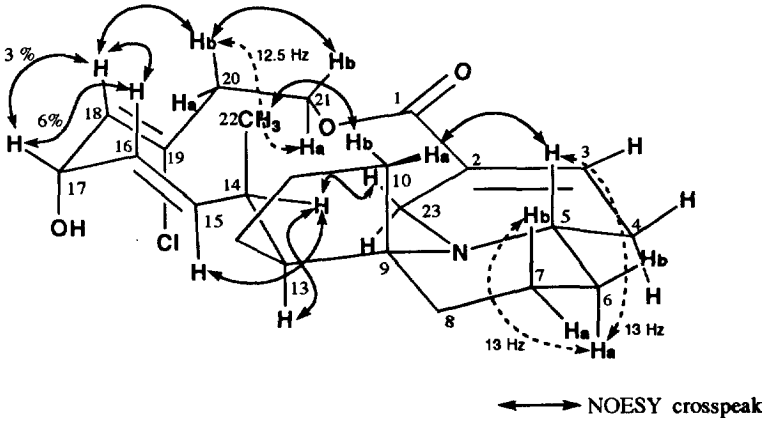

Figure 2. Proposed relative stereochemistry of halichlorine.

The relative stereochemistry of **1** (Figure 2) was clarified mainly by coupling constants and NOESY spectral data. H5, H6a and H7b were assigned to be axial based on the relatively larger coupling constants ($J_{5,6a}$= 13.0 Hz, $J_{6a,7b}$= 13.0 Hz). The NOESY crosspeaks (H5/H10a, H10b/H22) indicated the stereochemistry of the [5.6]-spiro ring. The stereochemistry and conformation of C14-C21 were established by the NOESY crosspeaks (H14/H23, H14/H15, H16/H17, H18/H17, and H18/H20b) and by the large vicinal coupling constants (12.5 Hz) between H20b and H21a. The IR absorption band (3580, 1070 cm^{-1}, CS_2)[11] indicated an intramolecular hydrogen bond between the hydroxy group and chlorine. Therefore, the configuration at C17 can be reasonably assigned as shown in Figure 2. The Bohlmann band (2800-2950 cm^{-1})[12] in the IR spectrum suggests the conformation around the tertiary amine functionality.

Interestingly, halichlorine is closely related to pinnaic acid.[13] Therefore, each carbon atom has been tentatively numbered under biogenetic consideration of formation of the N-C23 bond. Further studies on the detailed chemistry of halichlorine, including its absolute configuration, biogenetic pathway and structure-activity relationships, are currently underway in our laboratory.

Acknowledgments

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9. A downfield shift of H17 was also observed in the ^{1}H-NMR spectrum of the *p*-bromobenzoyl ester of halichlorine. The ester was obtained by treating **1** with *p*-bromobenzoyl chloride/pyridine at 40 °C for 12 h. Since the resulting ester did not dissolve in methanol, the ^{1}H-NMR spectrum was measured in C_6D_6. The ^{1}H NMR spectral data of H15-H18 of **1** and *p*-bromobenzoate are as follows: ^{1}H NMR of **1**, δ 5.86 (1H, ddd, J= 15.4, 9.2, 1.5 Hz, H15), 5.45 (1H, ddd, J= 15.4, 4.4, 1.0 Hz, H16), 5.10 (1H, ddd, J= 7.8, 4.4, 1.5 Hz, H17), 5.43 (1H, dd, J= 7.8, 1.1 Hz, H18); ^{1}H NMR of *p*-bromobenzoate, δ 6.16 (1H, ddd, J= 16.9, 7.7, 1.5 Hz, H15), 5.72 (1H, ddd, J= 16.9, 5.1, 1.0 Hz, H16), 6.69 (1H, ddd, J= 8.1, 5.1, 1.5 Hz, H17), 5.65 (1H, dd, J= 8.1, 1.1 Hz, H18).
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