Tetrahedron Letters,Vol.30,No.49,pp 6891-6894,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

HALICLAMINES A AND B, CYTOTOXIC MACROCYCLIC ALKALOIDS FROM A SPONGE OF THE GENUS HALICLONA[1]

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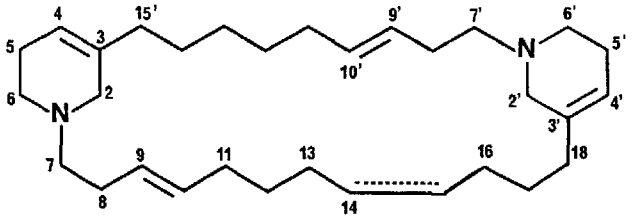
Abstract: Two cytotoxic alkaloids, haliclamines A and B, have been isolated from a sponge Haliclona sp., and their structures were elucidated mainly by spectroscopic analyses including extensive 2D NMR experiments.

Marine sponges of the family Haliclonidae have proved to be a rich source of nitrogen-containing metabolites with various biological activities,[2] e.g. halitoxin,[3] xestospongins,[4] sarains,[5,6,7] papuamines,[8] and haliclonadiamine.[9] In the course of our studies on bioactive metabolites from Japanese marine invertebrates, we collected a sponge of the genus Haliclona in Hiburi-jima Island (-10 to -15 m), the Uwa Sea, 600 km southwest of Tokyo, whose lipophilic extract showed marked activity both in antifungal and starfish egg assays. We have isolated from this sponge two new cytotoxic alkaloids, haliclamines A (1) and B (2), which consist of two tetrahydropyridines linked through C_9 and C_{12} alkyl chains. In this paper we describe the isolation and structure elucidation of these alkaloids.



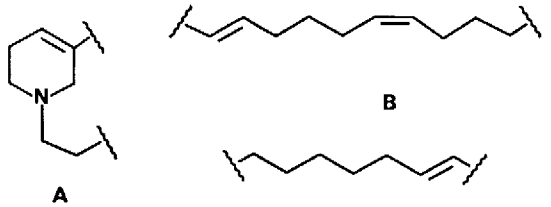
1

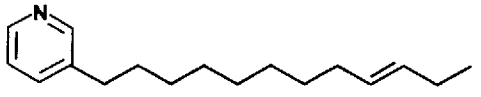
2: Δ^{14}

The ether-soluble materials of the ethanol extracts of the frozen sponge (1 kg) were subjected to vacuum flash chromatography on Kieselgel 60H (E. Merck) with $CHCl_3$/MeOH. The active fractions eluted with $CHCl_3$/MeOH (19 : 1) were successively fractionated on Sephadex LH-20 with n-hexane/$CHCl_3$/MeOH

(2 : 1 : 1). Antifungal fractions thus obtained were finally purified by HPLC on Capcell Pak C_{18} (Shiseido Co., Ltd.) with 95 % aq MeOH containing 50 mM $HCOONH_4$ (pH 9.0) to obtain haliclamines A (213.6 mg) and B (142.7 mg), each as a colorless oil. All purification stages were monitored by bioautography on Kieselgel 60 TLC (E. Merck) ($CHCl_3$/MeOH 9 : 1) using the fungus Mortierella ramannianus.

Haliclamine B (**2**)[10] had a molecular formula of $C_{31}H_{50}N_2$, which was established by HREIMS (m/z 450.3998, Δ +2.7 mmu). The ^1H and ^{13}C NMR spectra contained no methyl signal, but 21 methylenes and 10 sp^2 carbons, thereby suggesting the macrocyclic nature of haliclamine B. The NMR spectra also revealed a number of broad and overlapping signals, which indicated that **2** had a symmetrical or nearly symmetrical structure. The presence of two moieties of partial structure **A** was readily implied by homo- and heteronuclear COSY and HMBC[11] spectra in $CDCl_3$ [δ_H 2.85 (H-2,2'), 5.41 (H-4,4'), 2.14 (H-5,5'), 2.50(H-6,6'), 2.46 (H-7,7'), and 1.93 (H-18,15')], which were consisted with the 1,3-disubstituted-3,4-dehydropiperidine moiety contained in sarains[5,6] and isosarain-1.[7] Partial structures **B** and **C** were also deduced by extensive NMR experiments including COSY, HMBC, and NOESY[12] experiments in various solvents, which overcame broadening of signals due to the almost symmetrical structure. The connectivities of partial structures **A**, **B** and **C** were also established by HMBC experiments in C_6D_6, which led to the gross structure **2**. This was also supported by the HREIMS spectrum: prominent peaks at m/z 244.2030 ($C_{17}H_{26}N$, Δ 1.6 mmu) and 204.1748 ($C_{14}H_{22}N$, Δ -0.3 mmu), resulting from fission of both N-C bonds between N-1 and C-7 and between N-1' and C-7'.





Δ^9- and $\Delta^{9'}$-Double bonds had E-configuration as judged by the large coupling constants (J = 15 Hz) which were obtained by a decoupling experiment in C_6D_6 and by chemical shift values of C-8 (δ 30.6) and C-11 (δ 31.7) [C-8' (δ 30.7) and C-11'(δ 31.8)]. On the other hand, the Z-geometry of Δ^{14}-double bond was evidenced by a coupling constant (J = 10 Hz) obtained by a decoupling experiment and by chemical shift values of C-13 (δ 26.2) and C-16 (δ 27.0).

Haliclamine A (**1**),[13] $C_{31}H_{52}N_2$ (m/z 452.4114, Δ -1.3 mmu), was a dihydroderivative of **2** which was apparent from ^1H and ^{13}C NMR spectra as well

Table 1: $^{13}C^{a}$ and $^{1}H^{b}$ NMR Data for Haliclamines A and B

	haliclamine A (1)				haliclamine B (2)		
	^{13}C ($CDCl_3$)	^{13}C (C_6D_6)	^{1}H ($CDCl_3$)	^{1}H (C_6D_6)	^{13}C ($CDCl_3$)	^{1}H ($CDCl_3$)	^{1}H (C_6D_6)
2	55.1[c]	55.6[c]	2.95	2.90[c]	55.5[c]	2.85	2.90
3	135.7[g]	136.4			136.0		
4	118.9	119.4[d]	5.45	5.45	118.8[d]	5.41	5.49
5	25.1	26.1[n]	2.18	2.12	25.8	2.14	2.14
6	49.8	50.2	2.60	2.47	50.0[e]	2.50	2.48
7	57.9[h]	58.4[h]	2.54	2.49	58.6	2.46	2.49
8	30.3[f]	30.8	2.29	2.31	30.6[f]	2.24	2.30
9	128.1[i]	129.1	5.45	5.60	128.5	5.41	5.58
10	132.0[j]	131.5[j]	5.45	5.52	131.3	5.43	5.49
11	32.1[k]	32.7[k]	2.00	2.06	31.7	2.00	2.09
12	28.9[l]	29.4[l]	1.34	1.40	29.3	1.41	1.46
13	28.4	28.8[o]	1.27	1.30	26.2	2.00	2.05
14	28.8	29.4[o]	1.27	1.30	130.0	5.34	5.52
15	28.8	29.3[o]	1.27	1.30	129.7	5.34	5.52
16	29.1	29.7[o]	1.27	1.30	27.0	2.00	2.09
17	27.4[m]	29.5[m]	1.38[m]	1.38	27.7	1.45	1.51
18	35.3	35.7[p]	1.94	1.93[p]	34.8	1.93	1.97
2'	54.9[c]	55.4[c]	2.96	2.94[c]	56.1[c]	2.85	2.89
3'	135.6[g]	136.4			136.1		
4'	118.9	119.3[d]	5.45	5.45	119.1[d]	5.41	5.49
5'	25.1	26.0[n]	2.18	2.12	25.8	2.14	2.14
6'	49.8	50.2	2.60	2.47	50.3[e]	2.50	2.48
7'	58.6[h]	58.3[h]	2.54	2.49	58.6	2.46	2.49
8'	30.2[f]	30.8	2.29	2.31	30.7[f]	2.24	2.30
9'	128.0[i]	129.1	5.45	5.60	128.5	5.41	5.58
10'	131.9[j]	131.4[j]	5.45	5.52	131.4	5.43	5.49
11'	32.0[k]	32.6[k]	2.00	2.01	31.8	2.00	2.05
12'	29.0[l]	28.1[l]	1.34	1.38	28.5	1.36	1.40
13'	28.1	28.7[o]	1.27	1.30	27.5	1.25	1.32
14'	27.7[m]	28.3[m]	1.38[m]	1.40	27.2	1.36	1.40
15'	35.3	35.6[p]	1.94	1.95[p]	35.4	1.93	1.97

a. Recorded at 125 MHz

b. Recorded at 500 MHz

c - p Assignments may be interchanged within the columns.

as the EIMS spectrum. Homo- and heteronuclear COSY,HMBC and NOESY data led to assign structure **1**.

Chemical confirmation of the tetrahydropyridine systems in the molecule were made by dehydrogenation of **1**. A small portion of **1** (10 mg) was heated with an excess amount of 5 % Pd-C in p-cymene under a N_2 atmosphere. After usual work-up followed by purification by reversed-phase HPLC on C_{18} (95 % aq MeOH), 0.4 mg of a pyridine **3** was obtained. The ^1H and ^{13}C NMR spectra of **3** clearly showed the presence of a 3-substituted pyridine [δ_C 149.9 d, 147.1 d, 135.9 d, 128,2 s, and 123.2 d; δ_H 8.40 (s), 8.40 (dd J = 5, 1 Hz), 7.48 (dt J = 7, 1 Hz), and 7.20 (dd J = 5, 8 Hz)]. This was also supported by EIMS fragment ions[m/z 245 (m^+), 230, 216, 202, 190, 188, 176, 174, 162, 148, 134, 120, 106 (base peak), and 93]. Thus, haliclamines A and B are tetrahydropyridines 1 and 2.

Haliclamines A and B not only inhibited cell division of fertilized sea urchin (Hemicentrotus pulcherrimus) eggs at 5 and 10 μg / mL, respectively, but also growth of L1210 (IC_{50} 1.5 and 0.9 μg/mL, respectively) and P388 (0.75 and 0.39) leukemia cells. As predicted by Cimino et al,[5] haliclamines are most likely biogenetic precursors of xestospongins, sarains, and even halitoxins.

Acknowldgments. We thank Professor Paul J. Scheuer of the University of Hawaii for reading this manuscript. We are also grateful to Dr. Y. Watanabe of the Ochanomizu University for identification of the sponge specimen, to Dr. Y. Shida of the Tokyo College of Pharmacy for measurements of HREIMS, and to Dr. Y. Numazaki of the Central Research Labolatories of Yamanouchi Pharmaceutical Co., Ltd. for cytotoxity tests. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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10. Haliclamine A (**1**): no UV absorption above 220 nm; IR (film) 2990, 1470, 1440, 970 cm^{-1}; EIMS m/z 450 (M^+), 244, 204, 190, 150, 136, 122, 110, 109, 96, 81, 79, 67 (base peak), 55; ^1H and ^{13}C NMR in Table 1.
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13. Haliclamine B (**2**): no UV absorption above 220 nm; IR (film) 2900, 2850, 2800, 1470, 1440, 970 cm^{-1}; EIMS m/z 452 (M^+, base peak), 437, 341, 264, 246 ($C_{17}H_{28}N$), 204 ($C_{14}H_{22}N$), 192, 150, 136, 122, 110, 96, 81, 66, 54; ^1H and ^{13}C NMR in Table 1.

(Received in Japan 5 September 1989)