Haliclorensin, a Novel Diamino Alkaloid from the Marine Sponge *Haliclona tulearensis*

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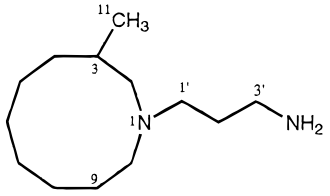
Haliclorensin (**1**), a novel diamino alkaloid possessing an azacyclodecane ring, has been isolated from the sponge *Haliclona tulearensis*. Its structure was elucidated on the basis of spectroscopic data, as well as by comparison with γ-amino azacycloalkanes.

A number of interesting biologically active compounds have been reported from the marine sponge genus *Haliclona*. The examples reported from this genus include the cytotoxic haliclonacyclamines;[1] the antifungal pentacyclic alkaloid, papuamine;[2] haliclonadiamine;[3] the antimicrobial cytotoxic alkaloids, haliclamines;[4] and the manzamines.[5,6]

In our search for biologically active substances from marine organisms,[7] an extract from the orange marine sponge *Haliclona tulearensis* exhibited strong cytotoxicity against P-388 mouse leukemia cells (IC_{50} = 0.1 mg/mL). The sponge was collected by scuba at Sodwana Bay, Durban, South Africa, at a depth of 15 m; MeOH−EtOAc (1:1) extraction of the freeze-dried sponge yielded an extract that was partitioned between different solvents.[8] The *n*-BuOH- and $CHCl_3$-soluble materials were separately subjected to RP-18 chromatography, eluting with a MeOH−H_2O gradient. Further purification of fractions containing **1** on a Sephadex LH-20 column, eluting with MeOH, afforded pure haliclorensin (**1**, 0.55% dry wt).



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Haliclorensin (**1**), $[\alpha]_D = -2.2°$, was obtained as a colorless oil. The ^{13}C-NMR and DEPT experiments (Table 1) disclosed one methine, 11 methylenes, and one methyl. The ^{13}C chemical shifts of C-2 (δ_c 48.5), C-10 (δ_c 42.4), C-1′ (δ_c 41.3), and C-3′ (δ_c 41.2) indicated that each one of these four carbons was proximate to a nitrogen atom. Evident from the proton NMR spectrum was a $>NCH_2CH(CH_3)$ group (δ 2.67 dd, 2.88 m, H-2a,-2b; 1.91 m, H-3 and 0.92 d, CH_3-11). COSY and TOCSY analysis of the ^{1}H-NMR spectra (Table 1) revealed two spin systems: $>N(CH_2)_3NH_2$ and $>NCH_2CH(CH_3)-(CH_2)_7N<$. HMBC cross peaks (Table 1) confirmed these assignments and suggested the connectivity between the two spin systems through the tertiary nitrogen as shown in the structure. The positive FABMS of **1** showed a pseudomolecular ion peak at *m*/*z* 213, and HREIMS established its molecular formula as $C_{13}H_{28}N_2$, in full agreement with structure **1**. Also, in full agreement were the carbon−carbon bond cleavages, α,β to the N-atoms, in the mass spectrum, yielding the ions at *m*/*z* 44 ($C_2H_6N^+$, 100%), 183 ($M^+ - CH_2{=}NH$, 19%), and 170 ($M^+ - C_2H_4N$, 13%).

Interesting to note is the strong dependence of the NMR spectra of **1** on the acidity of the measured sample and, therefore, also on the presence of other accompanying polar compounds. This behavior created difficulties during the purification process. Although protonation of a nitrogen atom, in acidic media, causes a pH-dependent downfield shift of the α-proton signals,[9] the carbon resonances of **1** were shifted upfield at an unpredictable rate. The complexity of the situation is demonstrated by the comparisons of the δ_c values of **1** and several model compounds (**2**−**5**),[10] summarized in Table 2.

The comparison of the δ_c values of compounds **1**−**4** with the resonances of **5** (the only mono amino compound) point clearly to the influence of the primary γ-amino group, an influence that might suggest a strong hydrogen bond between the two nitrogen atoms, in the neutral state, which affects the carbon resonances.

Keramaphidin C, 6Z-azacycloundecene, was the first reported marine azamacrocycle,[11] and it is suggested to be a precursor of manzamine C, which incorporates this ring in its structure.[11] The azamacrocycle in manzamine C is attached to a second nitrogen atom through a three-methylene unit, which is suggested to be derived from acrolein or its bio-precursor. A similar biogenetic route to the one suggested for manzamine C[11] may also be suggested for haliclorensin (**1**), with the tryptophan being replaced by ammonia or its bio-precursor.

Noteworthy is the sodium channel-blocker activity as well as inhibition of leishmania parasites of a whole series of compounds incorporating the N-(γ-aminopropyl) azacycloalkane moiety in their structure.[12,13] The primary amino group of **1** is expected to form a Schiff base readily and thus to result in analogous bioactive compounds. Work is ongoing in tracing the more polar compounds responsible for the cytotoxicity of the sponge extract.

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Table 1. NMR Data of Haliclorensin (**1**) (500 MHz, in DMSO-d_6)

C#	δ_c (mult)	δ_H (mult, J in Hz)	COSY & TOCSY	HMBC (H→C)
2	48.5 (t)	2a: 2.67 (dd, J = 12.8,6.9)	2b, 3, 11	3, 4, 11, 1′
		2b:2.88 (m)	2a, 3, 11	3, 4, 11, 1′
3	26.5 (d)	1.91 (m)	2a, 2b, 11	2, 4, 5, 11
4	30.3 (t)	4a: 1.11 (m)	4b	2, 3, 6, 11
		4b: 1.52 (m)	4a	2, 3, 6, 11
5	22.6 (t)	1.30−1.42		6, 7
6	24.3 (t)	6a: 1.24 (m)		8
		6b: 1.42 (m)		8
7	24.1 (t)	7a: 1.24 (m)		8, 9
		7b: 1.42 (m)		8, 9
8	22.3 (t)	1.30−1.42 (m)		6, 7
9	21.5 (t)	9a: 1.50 (m)	9b, 10a, 10b	7, 10
		9b: 1.70 (m)	9a, 10a, 10b	7, 8, 10
10	42.4 (t)	10a: 2.84 (m)	9a, 9b, 10b	8, 1′
		10b: 2.96 (m)	9a, 9b, 10a	9, 1′
11	17.7 (q)	0.92 (d, J = 6.5)	3, 5, 4a, 4b	2, 3, 4
1′	41.3 (t)	1′a: 2.94 (m)	1′b, 2′a, 2′b	2′, 3′, 2
		1′b: 3.09 (m)	1′a, 2′a, 2′b	2′, 3′, 2
2′	19.2 (t)	2′a: 1.94 (m)	2′b, 1′a, 1′b	1′, 3′
		2′b: 2.12 (quin, J = 7.0)	2′a, 1′a, 1′b	1′, 3′
3′	41.2 (t)	3.03 (m)	2′a, 2′b, 1′a, 1′b	1′, 2′

Table 2. ^{13}C-NMR Data of Haliclorensin (**1**) and Model Compounds **2**−**5**[a,b]

no.	**1**	**2**	**3**	**4**	**5**[c]
1′	48.1	49.7	57.0	56.8	55.7
2′	22.1	24.1	24.3	30.2	19.5[e]
3′	46.7	47.1	47.7	39.6	21.4[e]
2	53.1	58.2	54.3	44.7	52.1
10	50.7				
1′	41.9 (6.2)[d]	49.7 (0)	54.0 (3.0)	55.1 (1.7)	56.1 (−0.4)
2′	20.6 (1.5)	22.9 (1.2)	22.3 (2.0)	22.9 (7.3)	19.8[e] (−0.3)
3′	41.3 (5.4)	37.3 (9.8)	37.3 (10.4)	37.1 (2.5)	21.6[e] (−0.2)
2	49.6 (3.5)	52.7 (5.5)	54.0 (0.3)	43.4 (1.3)	52.4 (−0.3)
10	44.1 (6.6)				

[a] **2**: N-(3-Aminopropyl)azacyclotridecane; **3**: N-(3-aminopropyl)piperidine; **4**: 3-dimethyaminopropylamine; **5**: N-butylpiperidine. [b] Taken in D_2O with dioxane as an internal standard for calibration. [c] Measured in neutral and acidic DMSO-d_6. [d] TFA added, values in parentheses are $\Delta\delta_c$ values between neutral and acidic measurements. [e] Exchangeable.

Experimental Section

General Experimental Procedures. Mass spectra (low resolution and high resolution) were recorded on a Fisons, Autospec Q instrument. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. Optical rotation was measured on a Perkin−Elmer model 141 polarimeter using a 1-cm microcell.

Collection and Isolation Procedures. The sponge *Haliclona tulearensis* (class Demospongiae, order Haplosclerida, family Chalinidae, genus Haliclona) was collected in Sodwana Bay, South Africa, by scuba at a depth of 15 m during September 1995. The sponge was described as a fine, "muddy" orange laminate sponge with large oscula on its ridges. A voucher sample is deposited in the Zoological Department at Tel Aviv University (TASA 121).

After collection, the sponge was immediately frozen at −25 °C. The freeze-dried sponge (3.63 g) was then extracted with EtOAc−MeOH (1:1) to give a brown gum (0.38 g). The crude gum was divided among different solvents.[8] The $CHCl_3$ fraction and the *n*-BuOH fraction (each separately) were chromatographed on RP-18 column several times, eluted with a MeOH−H_2O gradient, and then on a Sephadex LH-20 column, eluted with MeOH−$CHCl_3$ (1:1), to afford **1** (20 mg, 0.55% dry wt).

Haliclorensin (1): an oil; R_f = 0.62 (MeOH−$CHCl_3$; 3:2), visualized with I_2; $[\alpha]_D$ −2.2° (*c* 1.3, MeOH); NMR data in Table 1; EIMS *m/z* (%) $[M]^+$ 212 (35), 183 (22), 170 (13), 168 (10), 154 (30), 112 (33), 99 (84), 85 (39), 70 (69), 56 (41), 44 (100); HREIMS *m/z* 212.2250 (calcd for $C_{13}H_{28}N_2$, 212.2252).

Synthesis of *N*-(3-Aminopropyl)azacyclotridecane (2). Azacyclotridecane (92 mg, 0.5 mmol) and an equimolar amount of acrylonitrile (33 μL) were dissolved in dry MeOH (6 mL), and the mixture was stirred at room temperature for 4 h.[13] The solvent was removed under reduced pressure, and the residue was chromatographed on Sephadex LH-20. The purified product [*N*-(3-propionitrile)azacyclotridecane] (75 mg, 0.31 mmol) was dissolved in MeOH (20 mL) (saturated with NH_3) and was hydrogenated over Raney Ni at room temperature at 3 atm, for 1 h. The catalyst was removed by filtration, and the residue was purified by Sephadex LH-20 column to give **2** (65 mg, 0.27 mmol, 85% yield).

Compound 2: an oil; R_f = 0.59 (MeOH−$CHCl_3$; 3:2), visualized with I_2; ^{1}H NMR (D_2O + dioxane + TFA; 100:0.1:0.1; 360 MHz) (pH = 2.5) δ 3.22 (4H, m), 3.12 (4H, m), 1.78 (4H, m), 1.39−1.48 (18H, m); ^{13}C NMR (D_2O + dioxane + TFA; 100:0.1:0.1; 90 MHz) (pH = 2.5) δ 52.7 (t, C-2/13), 49.7 (t, C-1′), 37.3 (t, C-3′), 26.0, 25.2, 25.0, 24.5, 21.6 (10 methylenes, C-3÷C-12), 22.9 (t, C-2′); FABMS; *m/z* (%) $[MH]^+$ 241 (100), 196 ($M^+ - C_2H_6N$, 10), 184 (14).

Synthesis of N-(3-Aminopropyl)piperidine (3). Synthesis of **3** was done following the same procedure as for **2**, starting from piperidine.

Compound 3: an oil, R_f = 0.45 (MeOH−$CHCl_3$; 3:2), visualized with I_2; 1H NMR (DMSO-d_6, 500 MHz) δ 2.80 (2H, d, J = 11.6 Hz, H-2a/6a), 3.33 (2H, d, J = 11.6 Hz, H-2b/6b), 1.74 (2H, m, H-3a/5a), 1.78 (2H, m, H-3b/5b), 1.33 (1H, m, H-4a), 1.64 (1H, m, H-4b), 3.08 (2H, m, H-1′), 2.02 (2H, br q, J = 7.1 Hz, H-2′), 2.84 (2H, br t, H-3′); ^{13}C NMR (DMSO-d_6 + traces of TFA, 90 MHz) δ 51.5 (t, C-2/6), 21.7 (t, C-3/5), 20.9 (t, C-4), 52.4 (t, C-1′),

20.9 (t, C-2′), 35.9 (t, C-3′); EIMS; *m*/*z* (%) $[M]^+$ 142 (60), 100 ($M^+ - C_2H_4N$, 15), 44 ($C_2H_6N^+$, 100).

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