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Haliclonacyclamines A and B, Cytotoxic Alkaloids from the Tropical Marine Sponge *Haliclona* sp.

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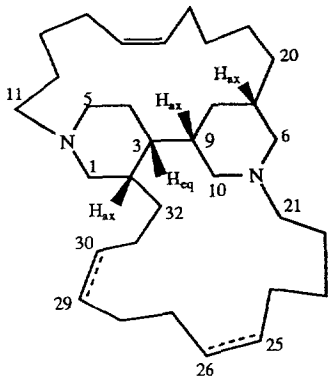
Abstract: The structures of haliclonacyclamines A (**1**) and B (**2**), and their methiodide salts (**3**) and (**4**), were investigated by 1D- and 2D-NMR experiments, notably DQFCOSY, HMBC, HMQC-HOHAHA, and HOHAHA. The relative stereochemistry and position of alkene substituents were determined by single crystal x-ray study at low temperature. The parent haliclonacyclamines show pronounced cytotoxic, antibacterial and antifungal activity. Copyright © 1996 Elsevier Science Ltd

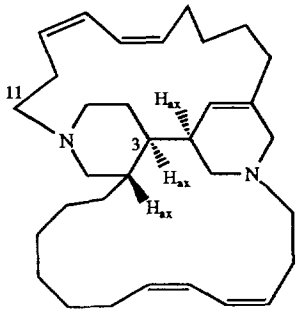
Haplosclerid sponges, notably those from the genera *Haliclona*, *Xestospongia* and *Amphimedon* spp, and dictyoceratid sponges of the genus *Ircinia* are a rich source of structurally-complex, cytotoxic alkaloids derived from 3-alkylpyridines or their reduction products[1-5]. In 1992, Baldwin and Whitehead[6] devised a retrobiosynthetic scheme for the complex manzamine alkaloids previously isolated from several sponge genera and postulated the intermediacy of *bis*-dihydropyridines related to the haliclamines.[1f] Alkaloids representative of the tetracyclic[3a,3d] and pentacyclic[2b-2g, 3c] intermediates identified by the Baldwin and Whitehead scheme have subsequently been found in several marine sponges. Halicyclamine A (**5**) recently reported by Crews *et al*[1g] represents a new tetracyclic alkaloid skeleton biogenetically related to the xestocyclamine/ingenamine class of alkaloids predicted by the Oxford group[2b-2g]. Literature reports that a Great Barrier Reef sponge *Haliclona* sp. (previously *Callyspongia* sp.) was bioactive were thus of interest to us, particularly as no chemical studies had been undertaken[7]. We therefore initiated a chemical investigation of the sponge and the structures of two new cytotoxic alkaloids isolated, haliclonacyclamine A (**1**) and haliclonacyclamine B (**2**), are reported below.

The olive-brown finger sponge *Haliclona* sp. grows on acroporid coral substrate at -10m to -15m on the Southern side of Heron Island, Great Barrier Reef. The sponge exudes mucus on collection and its surface is not fouled; crude organic extracts exhibited potent antibacterial and antifungal activity as well as an IC_{50} of 5μg/mL in the P_{388} mouse leukaemia assay. When the sponge tissue was examined by light microscopy, nematocysts (of length <20 μm) were detected as was an extracellular microalgal symbiont which morphologically resembles the dinoflagellate *Symbiodinium microadriaticum*. Specimens of *Haliclona* sp. (270 g) were collected by SCUBA and extracted with toluene/methanol (3:1). The aqueous methanol phase was further extracted with chloroform, and the combined organic extracts processed by reverse phase flash chromatography, then by normal phase HPLC using hexane/ethyl acetate/Et_3N 30:65:5 to give haliclonacyclamine A (**1**) and B (**2**), 68 mg and 17 mg respectively.

Haliclonacyclamine A, mp 149-150°, $[\alpha]_D$ -3.4°, gave the molecular formula $C_{32}H_{56}N_2$ by HREIMS. The ^{13}C NMR (**Table 1**) showed 8 methines, 4 of which were alkene, and 24 methylenes; there were no quaternary or methyl carbons. The presence of two double bonds required that the molecule was tetracyclic. The carbon resonances were matched to their respective protons by HMQC, and the geminal proton pairings crosschecked by



(1) Haliclonacyclamine-A, Δ = 25, 26
(2) Haliclonacyclamine-B, Δ = 29, 30
(3) Bis methyl iodide adduct of (1)
(4) Bis methyl iodide adduct of (2)
(6) 15,16,25,26-Tetrahydro-(1)



(5) Halicyclamine-A

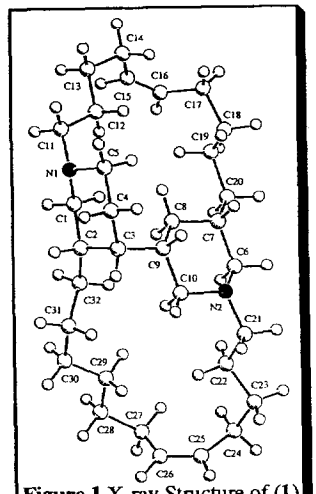


Figure 1 X-ray Structure of (1)

DQFCOSY (**Table 1**). The ^{1}H NMR spectrum was very congested, particularly in the δ 0.9-1.5 region; the well-resolved resonances at δ 3.08 and 2.98, assigned to protons adjacent to nitrogen, plus the four methine resonances at δ 1.56, 1.77, 1.84 and 1.90 were used for initial structure assembly. NMR evidence for the 3,5-disubstituted hexahydropyridine ring came from HMBC, DQFCOSY, HOHAHA and HMQC-HOHAHA connectivities. H6b† at δ 2.98 and its geminal partner at δ 2.49 showed COSY connectivities to H7 at δ 1.90, while C-7 at δ 41.0 showed an HMBC link to H8b at δ 1.97 and a methine at δ 1.56 assigned to H9. Correlations from H9 to H10a at δ 1.82 were evident in the DQFCOSY while HMBC linked C6 at δ 52.3 to H7, H20a and H21, C7 at δ 41.0 to H6b, C9 at δ 37.8 to H8b and H10a, and C10 at δ 60.3 to H21. Correlations between H6-H7, H8b-H9, and H9-H10 were evident in a HOHAHA experiment while a HMQC-HOHAHA showed links from C7 to H6 and H19 and from C9 to H10a. NMR support for the 3,4-disubstituted hexahydropyridine ring was provided initially by DQFCOSY correlations between H3 at δ 1.84 and H4b at δ 2.19, between H4b and the H5 resonances at δ 2.72 and 2.10, and by HMBC correlations for C5 at δ 59.3 to H1b, C2 at δ 34.1 to H3, C3 at δ 44.5 to H4a and to H2 at δ 1.77, C4 at δ 37.4 to H3 and H5, and C5 at δ 59.3 to H4a. A HOHAHA experiment also linked H1a to H2, not evident in the DQFCOSY, and a correlation between C2 and H4a was evident in an HMQC-HOHAHA experiment. The two rings A and B were linked on the basis of HMBC correlations between C3 and H9 and between C9 and H3. The absence of DQFCOSY cross peaks between H3 and H9, suggesting that the vicinal dihedral angle between them was close to 90°, enabled us to run a series of selective 1D HOHAHA experiments which finalised proton assignments of the two rings. As the mixing time was extended from 20.6 msec to 100 msec, magnetisation was propagated from H1 at δ 2.68 successively to H2, H3 etc to H5a at δ 2.10, and from H6b at δ 2.98 through to H10a at δ 1.82.

Other key methylenes were then located; correlations between C21 at δ 56.6 and H6 at δ 2.98, and by C11 at δ 45.4 to H5b identified the N termini while methylenes at C20 (δ 32.3) and C32 (δ 33.6) showed correlations to H7 and to H2 and H4a respectively. C32 also showed HMQC-HOHAHA correlations to H2. The remaining

† a and b denote upfield and downfield resonances respectively of a geminal pair.

Table 1. ^{1}H, ^{13}C NMR data and Long-Range ^{13}C-^{1}H Correlations for Haliclonacyclamine A (1)

Position	$\delta\,^{1}H^{a}$ (Multiplicity)^{b}	DQFCOSY	$\delta\,^{13}C^{c}$	Long-range ^{13}C-$^{1}H^{d}$
1	2.68 (m); 2.41(t, 10.8)	H2[e]	58.2t	H12a[f]
2	1.77 (m)	H32a	34.1d	H3, H4a
3	1.84 (t, 12.3)	H4b	44.5d	H2, H4a, H9, *H4*[g]
4	2.19 (m); 0.91 (m)	H3, H4, H5	37.4t	H2, H3, *H5*, *H12a*
5	2.72 (m); 2.10 (m)	H4b, H5	59.3t	H4a, H1b
6	2.98 (t, 12); 2.49 (m)	H6, H7, H7[e]	52.3t	H7, H20a, H21
7	1.90 (m)	H6,H20a,H6[e]	41.0d	H6b,H8b, H9b, *H6*, *H19*
8	1.97 (m); 1.7 (m)	H7	36.4t	H4a
9	1.56 (m)	H10a, H8b[e]	37.8d	H3, H4a, H8b, H10a, *H8b*, *H10a*
10	2.66 (m); 1.82 (t, 10.8)	H9, H10, H9[e]	60.3t	H4a, H21
11	3.08 (t, 10.8) 2.66 (m)	H12a	45.4t	H5b, H12b
12	1.96 (m); 1.38 (m)	H11a	26.8t	H5
18	2.05 (m); 1.38 (m)	H19	27.6t	H19, H20, *H20*
19	1.23 (m)	H20b	29.7t	H18b, H20, H23b
20	1.59 (m); 1.10 (m)	H7, H19	32.3t	H7, H19
21	2.82 (m)	H22	56.6t	H6, H22b, H23a
22	1.55 (m); 1.41 (m)	H21	20.4t	*H21*, *H23a*
23	2.06 (m); 1.29 (m)	H22b	27.4t	H21, H22, *H22*
31	1.92 (m)	H32b	26.2t	H30
32	1.37 (m); 0.91 (m)	H2	33.6t	H2, H4a

Table 2. ^{1}H, ^{13}C NMR data and Long-Range ^{13}C-^{1}H Correlations for Haliclonacyclamine B (2)

Position	$\delta\,^{1}H^{a}$ (Multiplicity)^{b}	COSY	$\delta\,^{13}C^{c}$	Long-range ^{13}C-$^{1}H^{d}$
1	2.94 (t, 12.0); 2.51 (m)	H1, H2	52.3t	H2, H3[h], H11[h], *H2*[g]
2	2.00 (m)	H1, H32	40.0d	H1, H4, H1
3	1.83 (m)	H4[e]	34.2d	H4[h], *H2*, *H4*
4	2.07 (m); 1.75 (m)	H4, H5	35.8t	H2[h], H3[h], *H3*, *H5*
5	3.10 (t, 11.9); 2.69 (m)	H4	45.9t	*H4*, *H4*
6	2.75 (m); 2.27 (m)	H6, H7	59.6t	H7, H8, H10, H21, *H7*, *H8a*[f]
7	1.86 (m)	H6b, H8b, H20a	44.7d	H6a, H8a, H20a
8	2.19 (m); 0.89 (m)	H7, H8, H9	37.4t	H7, H9, *H6a*, *H7*
9	1.56 (m)	H8b, H10	37.8d	H8a, H10a, *H6*, *H7*
10	2.68 (m); 1.81 (m)	H9	59.3t	H6a, H9, H21, *H9*
11	2.82 (m)	H12b	57.0t	H1b, *H12*
12	1.55 (m); 1.41 (m)	H11	20.4t	H11, *H11*
15	5.38 (m)	H17	129.6d[i]	H17a, H19b, *H17*
16	5.38 (m)	H17	129.7d[i]	H17a, H19b, *H17*
17	1.76 (m); 2.18 (m)	H16, H17	26.0t	H19b, H15/H16, *H19b*
18	1.31 (m); 1.19 (m)	H18, H20[g]	25.9t	H15, H16, H19b, *H19*
19	1.38 (m); 1.29 (m)	H20	26.9t	H18b, *H18a*, *H20*
20	1.37 (m); 0.87 (m)	H7, H19, H20	33.7t	H19a, *H18a*, *H19*
21	2.61 (m)	H22	57.8t	H6, H10a, H22a, *H22b*, *H23a*
22	1.60 (m); 1.27 (m)	H21	21.4t	H21, *H10b*, *H23a*
23	1.38 (m); 1.30 (m)	H22	26.6t	H21
29	5.27 (m)	H30	130.2d	H31, *H2*
30	5.26 (m)	H29, H31[e], H32[e]	131.1d	H31a, H32b, *H2*
31	1.50 (m); 1.17 (m)	H30, H32	29.7t	H2, H4b, *H2*
32	1.27 (m); 1.47 (m)	H2, H32	32.5t	H30, *H2*

[a] 500 MHz; solution in $CDCl_3$ referenced to $CDCl_3$ at $^{1}H = \delta\,7.24$; [b] Coupling constants in Hz; [c] Inverse detection at 500 MHz (HMQC); solution in $CDCl_3$ referenced at $^{13}C = \delta\,77.0$; [d] Inverse detection at 500 MHz; correlations observed when $^{1}J\,^{13}C$-^{1}H = 135 Hz and long range $J\,^{13}C$-^{1}H = 4 Hz; [e] HOHAHA correlations; [f] a and b denote upfield and downfield resonances respectively of a geminal pair. [g] Correlation in italics are HMQC-HOHAHA correlations; [h] HMBC correlations for $J\,^{13}C$-^{1}H = 10 Hz; [i] assignments can be interchanged.

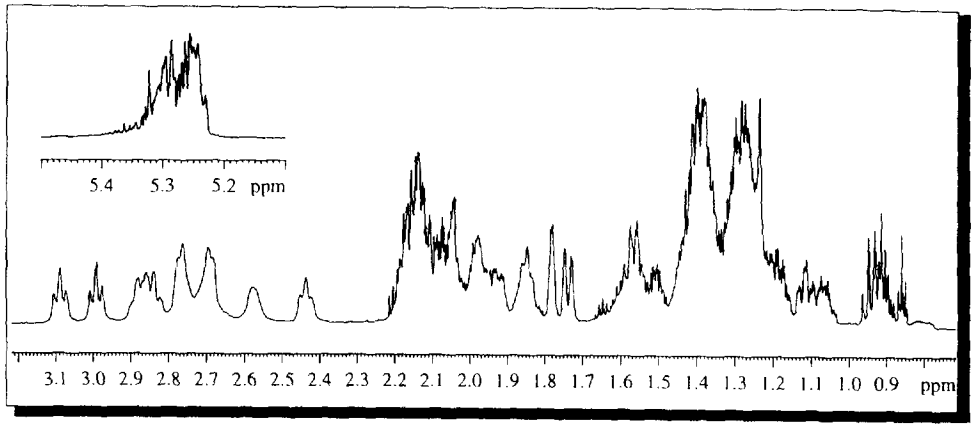


Figure 2 750 MHz ^{1}H NMR Spectrum of Haliclonacyclamine A (**1**) in $CDCl_3$

portion of haliclonacyclamine A consisted of two aliphatic chains containing the alkene groups and terminating in C11, C20, C21 and C32. Spectral crowding in the methylene and alkene regions of the ^{1}H NMR was not improved by change of NMR solvent, or even at the very high field strength of 750 MHz (**Figure 2**). It was not therefore possible to extend the NMR analysis to determine the individual lengths of the two aliphatic chains, or to determine the position of their double bonds. All ^{13}C resonances above 30 ppm were already assigned, therefore the four allylic carbons, irrespective of their positions, had chemical shift below 30 ppm, consistent with Z stereochemistry at the double bonds[2d]. All the complex alkaloids previously reported from *Haliclona, Xestospongia* and *Amphimedon* spp. have *Z* double bond stereochemistry in their linking bridges[1-3].

Relative stereochemistry could only be partially deduced by NMR. A 2D NOESY experiment confirmed that C7 and C9 were on the same face of the molecule, but the relative stereochemistry of the other ring could not be deduced. We therefore resorted to X-ray study to sort out these structural features. Small needles crystallised from ethyl acetate/hexane/triethylamine were suitable for a low temperature X-ray study which confirmed that the linear bridges were C_{10} and C_{12} respectively, that the double bonds, located between C15-C16, and between C25-C26, were both Z, and the ring stereochemistry was as shown in **Figure 1**. A crystalline methiodide derivative (**3**) of haliclonacyclamine A was prepared, but the crystals did not diffract X-rays and an absolute configuration could not therefore be deduced.

Haliclonacyclamine B (**2**), mp 145-146°, $[\alpha]_D$ +3.4°, was an isomer of (**1**) by HREIMS, and the NMR analysis proceeded identically to the *bis*-hexahydropyridine core and the methylene termini C11, C20, C21 and C32 of the two linear bridges (**Table 2**). Hydrogenation of either (**1**) and (**2**) gave a single tetrahydroproduct (**6**), each with identical $[\alpha]_D$, which suggested (**1**) and (**2**) had identical ring stereochemistry and length of bridging groups. The alkene region of the ^{1}H NMR of (**2**) was better resolved than for (**1**); a two proton resonance centred at δ 5.38 could be traced by 2D-NMR through four methylenes to the methine proton H7 at δ 1.86 , thus positioning a double bond at C15-C16, the same position as in (**1**). Notably correlations from H15/16 were to resonances at δ 2.18 and 1.76, assigned to H17. C17 in turn showed HMQC-HOHAHA correlations to H19 at δ 1.38, while C18 at δ 25.9 showed HMBC correlations to the alkene protons and HMQC-HOHAHA

correlations to H19 at δ 1.38 and 1.29. Further correlations linked C19 through C20 to H7. The other double bond was then located at C29-C30 as follows: H30 at δ 5.26 showed HOHAHA correlations to H31 at δ1.50 and H32 at δ1.27. HMBC correlations were evident between H30 and C32 at δ 32.5. Additional support for the double bond positions was obtained by analysis of NMR data for the methiodide salt (**4**) of haliclonacyclamine B. DQFCOSY correlations between H30-H31 and H31-H32 as well as HMBC correlations between C1-H32 and C29-H32 were detected. DQFCOSY correlations linked H16 through four methylenes to H7 (**Table 3**).

Table 3. ^{1}H, ^{13}C NMR data and Long-Range ^{13}C-^{1}H Correlations for Bismethiodide Adduct (**4**)

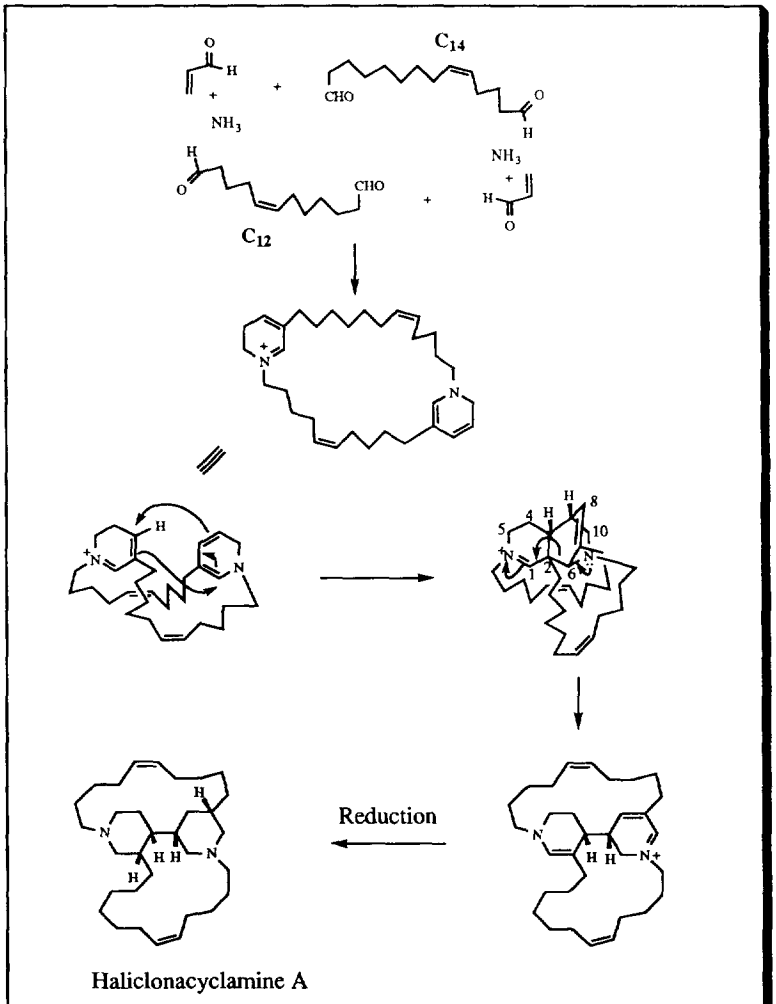
Position	$\delta\,^{1}H^{a}$ (Multiplicity)^{b}	COSY	$\delta\,^{13}C^{c}$	Long-range ^{13}C-$^{1}H^{d}$
1	3.43 (m)	-	69.7t	H5a[e], H32, H33
2	2.04 (m)	H3, H32	33.3d	H3
3	2.38 (m)	H2, H4a	35.9d	H1
4	2.43 (m); 2.11 (m)	H3, H5b	30.3t	H12
5	3.85 (t, 14.0); 3.15 (m)	H5, H4b	55.8t	H4b, H2, *H2*;[f] *H4*
6	3.16 (m); 3.08 (m)	H6, H7	64.9t	H10a, H8, H34, *H7, H8a*
7	2.23 (m)	H6a, H20	32.8d	H6a
8	2.18 (m); 1.43 (m)	H9	34.3t	H6a, H10b
9	2.49 (m)	H8a, H10b	39.1d	H8a
10	3.51 (m); 3.11 (m)	H9, H10	64.1t	H9, H34, *H7, H8a, H9*
11	3.48 (m); 3.02 (m)	-	60.3t	H2, H33, *H13a*
12	1.27 (m)	H13b	31.8t	H11b
13	2.19 (m); 2.13 (m)	H12	28.1t[g]	H15/H16
14	2.19 (m); 2.13 (m)	H15	28.2t[g]	H15/H16
15	5.35 (m)	H14b	132.5d[g]	H16
16	5.33 (m)	H17	131.2d[g]	H15
17	2.34 (m); 2.27 (m)	H16, H17, H18b	27.3t	H15/H16
18	1.43 (m); 1.25 (m)	H17b, H18	26.5t	H15/H16, H17b
19	1.56 (m); 1.36 (m)	H18b	25.4t	H20a
20	1.60 (m); 1.42 (m)	H7, H19a, H20	31.8t	*H18*
21	3.4 (m)	H22	69.7t	H22a, H23, H6b, *H23a, H22b, H24*
22	1.87 (m); 1.76 (m)	H21H23a	23.0t	*H24*
23	1.37 (m); 1.45 (m)	H22b	29.1t	H21, H24a
24	2.34 (m); 2.27 (m)	-	27.3t	*H22*
25	2.28 (m); 1.93 (m)	H26	28.6t	*H23*
26	2.00 (m)	H25b	26.7t	H27
27	1.86 (m)	H28b	26.9t	H28b
28	2.28 (m); 1.93 (m)	H29, H27	28.5t	H29
29	5.43 (m)	H28	130.9d	H28a, H31a
30	5.46 (m)	H31	130.5d	H28, H31, H32
31	1.95 (m); 1.82 (m)	H30, H32	22.3t	H1
32	1.49 (m)	H31a	30.2t	H5b
33	3.21 (s)	-	50.7q	H1, H11
34	3.15 (s)	-	48.2q	H6a, H10, H21

[a] 500 MHz; solution in CD_3OD referenced to CD_3OD at $^{1}H = \delta\,3.3$; [b] Coupling constants in Hz; [c] Inverse detection at 500 MHz (HMQC); solution in CD_3OD referenced to CD_3OD at $^{13}C = \delta\,49.0$; [d] Inverse detection at 500 MHz; correlations observed when $^{1}J\,^{13}C$-^{1}H = 135 Hz and long range $J\,^{13}C$-^{1}H optimized for 8Hz; [e] a and b denote upfield and downfield resonances respectively of a geminal pair; [f] correlations in italics are HMQC-HOHAHA correlations; [g] assignments can be interchanged

Although the carbon skeleton had been determined for (**2**), and evidence obtained in support of (**1**) and (**2**) sharing the same relative stereochemistry, the opposite sign of $[\alpha]_D$ for (**1**) and (**2**) was of concern. Crystals of (**2**) were submitted for X-ray, but an incomplete analysis was obtained. Disorder in the crystal lattice prevented determination of the position of the double bond in the C_{12} linking group, however the C_{10} chain double bond was confirmed as being between C15 and C16. The relative stereochemistry of (**2**) was clearly identical to that of (**1**). In their work on the related halicyclamine A (**5**),[1g] Crews *et al* determined H2, H3 and H9 to be axial from

coupling constant magnitudes. Our X-ray work clearly revealed that for both (**1**) and (**2**) H3 was equatorial, while H2, H7 and H9 were all axial.

Scheme 1 outlines a proposed biosynthesis for (**1**). Two acrolein molecules combine with a C_{12} and C_{14} monounsaturated dialdehyde and two ammonias to generate a partially reduced *bis*-3-alkylpyridine macrocycle (**4**) which undergoes Diels-Alder cyclisation, setting up a *cis* relationship between H3 and H9. Intramolecular rearrangement then cleaves the C2-C6 bond and produces the haliclonacyclamine skeleton. Reduction of the C6-N and C1-C2 double bonds generated in the rearrangement and the C7-C8 bond produced by Diels-Alder cyclisation provides (**1**). Interestingly enzymatic reduction occurs on one face of the molecule only and discriminates between the central double bonds and those in the linking groups. Reversing the orientation of the C_{14} dialdehyde produces (**2**).



Scheme 1 Proposed Biosynthetic Pathway for Haliclonacyclamine A (**1**)

Haliclonacyclamines A and B showed potent biological activity. IC_{50} values of 0.8 and 0.6 μg/ml, respectively, were obtained in a P_{388} assay and both compounds were strongly antibacterial and antifungal. Neither compound showed activity against protein kinase C. The organic and aqueous extracts of *Haliclona* sp. contain further examples of complex alkaloid metabolites whose structures will be described in a future manuscript.

EXPERIMENTAL

Isolation of Metabolites

The olive-brown sponge *Haliclona* sp. was collected by hand at about 12 m depth at Heron Island on the Great Barrier Reef. The sponge grows as erect fingers from an encrusting base. The exterior tissue is dull olive-brown in colour and the interior pale olive-brown. Sponge fibres are neither laminated nor cored, while the major spicule type is an oxea (mean length 35.8 μm) slightly recurved in the midline. The sponge tissue is soft, compressible and easily torn, and the sponge produces copious mucus on collection. The ectosome contains nematocysts and a symbiont morphologically similar to the dinoflagellate *Symbiodinium microadriaticum*. A voucher sample G304086 is held at the Queensland Museum, Brisbane. The frozen sponge (270g, wet weight) was cut into pieces and left in methanol/toluene (600 mL, 1:3, v/v) for 48 hrs at -20°C. The solution was filtered and a solution of $NaNO_3$ (120 mL, 1M) was added. The aqueous phase was extracted exhaustively with $CHCl_3$ and the organic phase concentrated *in vacuo* to give a crude extract. A portion of the crude organic extract (600 mg) was purified by C-18 flash column using step gradient elution with H_2O-MeOH through to 100% EtOAc, CH_2Cl_2, and hexane yielding nine fractions, five of which were active in antibacterial, antifungal and P_{388} assays. The bioactive fractions were further purified by normal phase HPLC using EtOAc/Hex/Et_3N (30:65:5% or 80:15:5%) to give Haliclonacyclamine A (**1**, 68 mg, 0.025 %) and B (**2**, 17 mg, 0.006 %).

Haliclonacyclamine A (1): White needles, mp 149-150°C; $[\alpha]_D$ -3.4° (c, 1.21, CH_2Cl_2); IR (film) 1633 cm^{-1}; LREIMS (m/z, relative intensity) 468 (M^+,100), 234 (12.8); HREIMS M^+ m/z 468.4463 ($C_{32}H_{56}N_2$ ΔM 1.99 mmu), 234.2225 ($C_{16}H_{28}N$ ΔM 0.3 mmu); ^1H and ^{13}C NMR ($CDCl_3$; 500 MHz), see **Table 1**.

Haliclonacyclamine B (2): White needles, mp 145-146°C; $[\alpha]_D$ + 3.4° (c, 0.55, CH_2Cl_2); IR (film) 1633 cm^{-1}; LREIMS (m/z, relative intensity) 468 (M^+,100), 234 (14.5); HREIMS M^+ m/z 468.4475 ($C_{32}H_{56}N_2$ ΔM -3.44 mmu), 234.2234 ($C_{16}H_{28}N$ ΔM 1.28 mmu); ^1H and ^{13}C NMR ($CDCl_3$; 500 MHz), see **Table 2**.

Preparation of derivatives

15,16,25,26-Tetrahydrohaliclonacyclamine A: Haliclonacyclamine A (26 mg) was dissolved in MeOH (20 mL), and 10% Pd/C (3 mg) was added. The mixture was left stirring under an atmosphere of H_2 (60 psi, 90 hrs), then filtered and the solvent removed to obtain a crude tetrahydroproduct which was then passed through a pad of celite prior to purification on normal phase HPLC (EtOAc/Hex/Et_3N; 80:15:5%) yielding tetrahydrohaliclonacyclamine A (19.5 mg, 75 %); $[\alpha]_D$ +23.9° (c, 0.45, CH_2Cl_2); LREIMS (m/z, relative intensity) 472 (M^+, 100); HREIMS M^+ m/z 472.4754 ($C_{32}H_{60}N_2$ ΔM -0.25 mmu); ^1H NMR ($CDCl_3$; 500 MHz) δ 0.89-0.94 (2H, m), 1.15-1.46 (31H, m), 1.54-1.65 (5H, m), 1.78-2.08 (8H, m), 2.13-2.20 (1H, m), 2.26-2.30 (2H, m), 2.53-2.67 (2H, m), 2.75-3.00 (8H, m), 3.06 (1H, m); ^{13}C NMR ($CDCl_3$; 500 MHz) 59.5, 59.2, 57.7, 55.8, 53.4, 52.0, 46.7, 44.0, 39.5, 37.4, 36.9, 34.3, 34.1, 33.8, 32.9, 29.2, 27.8, 27.5, 27.5, 27.3, 27.1, 26.8, 26.7, 26.6, 26.1, 26.0, 25.6, 25.5, 25.3, 23.4, 21.5 and 20.9 ppm; the same procedure was

used for haliclonacyclamine B (15 mg) yielding 11 mg (73%) of tetrahydroproduct $[\alpha]_D$ +24.9° (c, 0.45, CH_2Cl_2), identical by ^1H, ^{13}C NMR and by MS analysis with tetrahydrohaliclonacyclamine A.

Bismethiodide of haliclonacyclamine A (3): Haliclonacyclamine A (4.7 mg, 0.01 mmol) was dissolved in diethyl ether (2 mL) and treated with methyl iodide (2 mL) and the resulting cloudy solution was left in the fridge. After four days the solvent was removed and the crude product was crystallized from MeOH/EtOAc (3:1) yielding yellow needles of the methiodide adduct (**3**); mp 196-197° (decomp.); LREIMS (m/z, relative intensity) 610 (M^+-CH_3I, 43), 468 (100), 234 (100); HREIMS M^+ m/z 610.3708 ($C_{33}H_{59}N_2$ ΔM -1.68 mmu), electrospray [(M+H)$^+$, relative intensity] 611.4 (93), 249.8 (100); ^1H NMR (MeOH-d_4; 500 MHz) δ 0.87 (2H, m), 1.15-1.65 (25H, m), 1.72-1.90 (6H, m), 1.96-2.48 (12H, m), 3.02 (2H, m), 3.15 (3H, s), 3.20 (3H, s), 3.33-3.53 (4H, m), 3.63 (1H, m), 3.84 (1H, m), 5.33 (4H, m); ^{13}C NMR (MeOH-d_4; 500 MHz) 132.9, 132.6, 131.2, 130.7, 69 8, 69.6, 64.9, 64.1, 60.3, 56.0, 50.8, 48.7, 38.6, 36.5, 34.4, 33.6, 33.3, 33.1, 31.8, 30.5, 30.3, 30.2, 30.0, 29.6, 28.9, 28.3, 27.4, 27.3, 27.3, 27.1, 26.9, 26.8, 22.9, and 22.2 ppm.

Bismethiodide of haliclonacyclamine B (4): Haliclonacyclamine A (5.5 mg, 0.012mmol) was dissolved in diethyl ether (3 mL) and treated with methyl iodide (2 mL) and the resulting cloudy solution was left in the fridge. After four days the solvent was removed and the crude product was crystallized from MeOH/EtOAc (3:1) yielding yellow needles of the methiodide adduct (**4**);.mp 170-175° (decomp.); LREIMS (m/z, relative intensity) 610 (M^+-CH_3I, 100), 468 (100), 234 (12); HREIMS M^+ m/z 610.3722 ($C_{33}H_{59}N_2$ ΔM -0.84 mmu), electro-spray [(M+H)$^+$, relative intensity] 611.4 (93), 249.8 (100); ^1H and ^{13}C NMR (MeOH-d_4; 500 MHz) see Table 3; ^{13}C (d_5-pyridine, 500 MHz) 131.7, 130.5, 130.3, 129.8, 68.5, 68.4, 68.4, 64.9, 63.6, 59.4, 55.4, 54.1, 50.3, 37.5, 35.0, 33.8, 32.6, 32.1, 31.2, 31.0, 29.4, 29.3, 28.3, 27.9, 27.7, 27.5, 27.4, 26.7, 26.3, 26.2, 25.6, 24.6, 22.1 and 21.6 ppm.

NMR experiments

NMR spectra were recorded on a Bruker AMX 500 MHz spectrometer, using $CDCl_3$ or MeOH-d_4 as the solvent, referenced at δ 7.24/77.0 ppm or δ 3.3/49.0 ppm for $^1H/^{13}C$ respectively. All 2D spectra were acquired using 1K x 256 complex data matrix which was zero filled once in each dimension and a $\pi/2$ shifted sine-squared bell window function was applied in both dimensions before Fourier transformation. The HMBC and the phase sensitive HMQC spectra were acquired with 64 and 24 transients respectively. The evolution delay was set for $^nJ_{CH}$ of 4 and 10 Hz (HMBC) and $^1J_{CH}$ of 135 Hz (HMQC). The DQFCOSY and the NOESY spectra were acquired with 16 or 24 transients per increment. A mixing time of 800ms was used in the NOESY experiments.The HMQC-HOHAHA spectrum was acquired with either 64 or 48 transients per increment using a spin-lock mixing time of 20.6 ms. All the 2D HOHAHA experiments were acquired with 16 or 32 transients per increment using mixing times of 20.6 and 100 ms. The selective 1D HOHAHA experiments incorporating a Z-filter were carried out with a total of 256 transients using mixing times of 20.6 and 100ms. A 90° 100ms self-refocussing e-burp1 pulse was used for selective excitation. All HOHAHA experiments were carried out using a MLEV17 spin-lock pulse applied with RF field strength of $\gamma B1 = 11.4$ kHz.

X-ray structure determination:

All measurements were made at -60°C on a Rigaku AFC6R diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.54178 Å) and a 12kW rotating anode generator. The crystal was orthorhombic, space group $P2_12_12_1$ (#19), a = 11.428(2) Å, b = 15.732(1) Å, c = 16.484(2) Å, V = 2963.7(5) Å^3, Z = 4, D_{calc} =

1.051 g cm^{-3}, μ = 4.4 cm^{-1}. The intensities of a total of 2532 reflections with $2\theta < 120°$ were collected using θ-2θ scans. No absorption or decomposition corrections were required. After correction for Lorentz and polarisation effects, 2057 reflections with $I > 3.0\sigma(I)$ were judged observed and used in structure solution and refinement. The structure was solved by direct methods (SHELXS-86)[8] and refined using the teXsan program package[9] to a final R = 0.038. The non-hydrogen atoms were refined with anisotropic displacement factors while hydrogen atoms were included at calculated positions, but not refined. The absolute configuration of the molecule was not determined, so that shown in (**1**) was selected arbitrarily. Additional crystallographic details are available and the data have been deposited in the Cambridge Crystallographic Data Centre.

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