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TETRAHEDRON

The Haliclonacyclamines, Cytotoxic Tertiary Alkaloids from the Tropical Marine Sponge *Haliclona* sp.

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Abstract

2D-NMR spectroscopic data is reported for the haliclonacyclamines A - D (**1**)-(**4**) and for two bismethiodide adducts (**5**) and (**6**). The structures of two new alkaloids, haliclonacyclamines C (**3**) and D (**4**), which are the 15,16-dihydro analogues of the haliclonacyclamines A (**1**) and B (**2**) are described. Revised assignments deduced by 2D-INADEQUATE spectroscopy are presented for (**1**) and (**2**). The alkene substituent in the C_{12} spacer group of (**2**) and (**4**) is positioned between C27-C28 by NMR, and confirmed by x-ray structural analysis for (**2**). Metabolite (**3**) has a C25-C26 double bond.

Keywords: Alkaloids; NMR; Piperidines; Sponges.

INTRODUCTION

Cytotoxic alkaloids containing a 3-alkyl pyridine or piperidine motif are frequently isolated from marine sponges[1]. This bioactive class of metabolites can be subdivided into tricyclic[2], tetracyclic[3] or pentacyclic[4] alkaloids and into oxygen-containing alkaloids such as the ingenamines[4f,5], ingamine A[4b] and nakadomarin[6]. The manzamine class of alkaloids represents a complex structural variation in which a tetra- or pentacyclic system is bound to a β-carboline unit[1,7]. In early speculation on the biosynthesis of the saraines, petrosins and xestospongins, Cimino *et al.*[8] proposed a piperidine-containing macrocyclic intermediate. A mechanistic proposal for the biosynthesis of the manzamines, which involves tetracyclic and pentacyclic alkaloid intermediates, has

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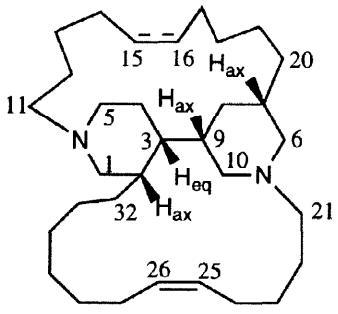
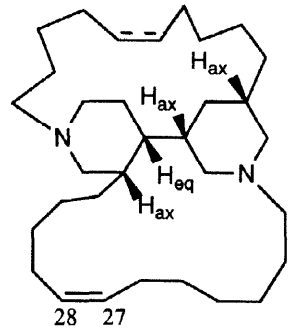
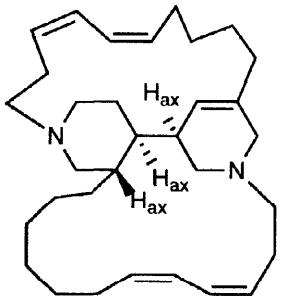
been fully elaborated by Baldwin and Whitehead[9]. Our research group recently described two cytotoxic alkaloids, the haliclonacyclamines A (**1**) and B (**2**), from the tropical marine sponge *Haliclona* sp. collected at Heron Island on the Great Barrier Reef[3c]. The haliclonacyclamines together with the halicyclamines A (**7**) and B (**8**) reported by Crews *et al.*[3a,3b] represent structural variants of the biosynthetic intermediates proposed by the Oxford group[9].We now report the structures of the haliclonacyclamines C (**3**) and D (**4**) which are 15,16-dihydro analogues of (**1**) and (**2**) respectively. Structural studies on the two new alkaloids have caused us to revise our original NMR assignments for (**1**) and (**2**) and to adjust the location of the C_{12} spacer group double bond in (**2**). X-ray details of (**2**) which confirm our structural revision are also presented.



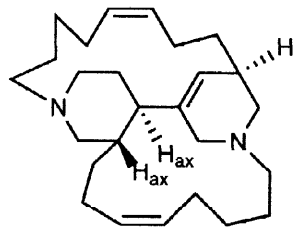
(**1**) Haliclonacyclamine A; Δ = 15,16
(**3**) Haliclonacyclamine C
(**6**) Bismethiodide adduct of (**3**)



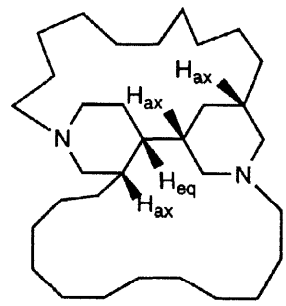
(**2**) Haliclonacyclamine B; Δ = 15,16
(**4**) Haliclonacyclamine D
(**5**) Bismethiodide adduct of (**2**)



(**7**) Halicyclamine A



(**8**) Halicyclamine B



(**9**)

Specimens of *Haliclona* sp. were collected at a depth of 12 m and were extracted into ethanol[3c]. The aqueous ethanol phase was further extracted with ethyl acetate, and the combined organic extracts then partitioned into 1M hydrochloric acid. The acid-soluble fraction was basified with NaOH to pH 10, then extracted into hexane to give an alkaloid fraction which was processed by NPHPLC using hexane/ethyl acetate/Et_3N 30:65:5, yielding haliclonacyclamines A (**1**), B (**2**), C (**3**) and D (**4**), 68 mg, 17 mg, 5 mg and 1 mg respectively.

NMR ANALYSIS OF HALICLONACYCLAMINES A (1) AND B (2)

Our comparison of the spectroscopic data for (**3**) and (**4**) with those of haliclonacyclamines A (**1**) and B (**2**) revealed some anomalies in the published NMR assignments for (**1**) and (**2**)[3c]. Consequently, we reinvestigated the ^{13}C assignments of (**1**) and (**2**) by 2D-INADEQUATE spectroscopy[10]. Interpretation of the spectrum for haliclonacyclamine A was facilitated by two secure assignments; C5 (45.4 ppm), which was the only -CH_2- next

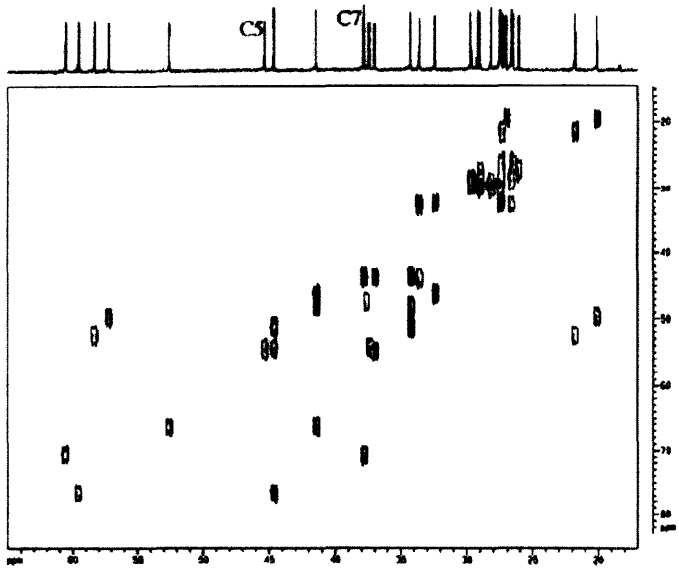


Figure 1. 2D-INADEQUATE spectrum of (**1**).

to nitrogen linked by a second -CH_2- to the methine position (C3), and C7 (37.8 ppm), which was identified as the only methine not adjacent to one of the three other methines (**Figure 1**). Starting from these two carbons, we were then able to assign unambiguously all of the remaining 30 carbons of (**1**) (**Table 1**).

The 2D-INADEQUATE data (**Figure 2**) for haliclonacyclamine B (**2**) suggested that the signals at 59.2 and 37.7 ppm, previously assigned to C10 and C9, should instead be assigned to C6 and C7 respectively. The revised assignments for the core of (**2**) then matched those determined for (**1**). This minor revision for (**2**) was also confirmed by analysis of HMBC and HMQC-TOCSY spectra (**Table 2**). However an anomaly in the published structure of haliclonacyclamine B (**2**), which was noticed when its NMR data were compared with that of the new dihydro analogue haliclonacyclamine D (**4**), was that the double bond position in the C_{12} spacer group of (**2**) could not be between C29 and C30. In our original work on haliclonacyclamine B preliminary x-ray crystallographic data confirmed all the structural features of (**2**) other than the position of the C_{12} spacer group double bond[3c,11]. After many attempts, crystals of haliclonacyclamine B (**2**) of sufficient size and quality for an improved x-ray structure analysis were obtained from EtOAc/hexane/Et_3N. The structure was solved by direct methods[12,13] and difference-Fourier techniques. Most of the molecule was clearly defined, but atoms C29 and C30 were each observed to be split over two sites. Only one combination of these sites gave rise to sensible

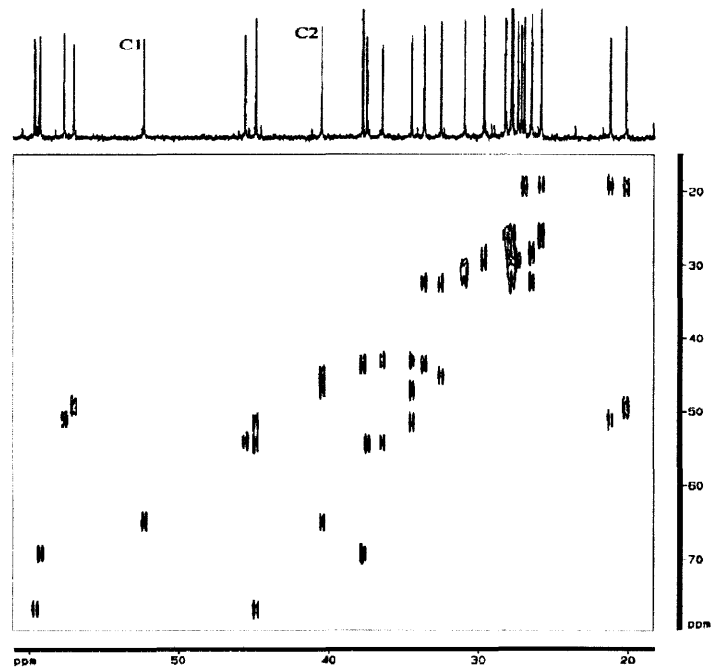


Figure 2. 2D-INADEQUATE spectrum of (**2**)

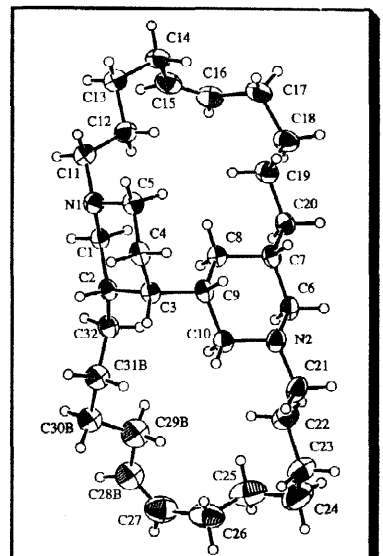


Figure 3. X-ray structure of (**2**); one conformation of the region between C28-C31 is shown.

connectivities, corresponding to two conformations, A and B, for this section of the chain. The disorder was subsequently extended to C28 and C31. Restraints were imposed on distances and angles involving disordered atoms during least-squares refinement[14-16]. Interatomic distances, angles and dihedral angles indicated double bonds of *E* stereochemistry at the C15-C16 and C27-C28 positions (**Figure 3**). The NMR data for the C_{12} spacer group of (**2**) were then carefully reviewed. In the 500 MHz ^{1}H NMR spectrum of (**2**), multiplets at δ 5.27 and 5.38 were assigned to the alkene of the C_{10} and C_{12} spacer group double bonds respectively; for example, the proton resonances at δ 5.27 showed an HMQC-TOCSY to C11 at 57.0 ppm and to C20 at 33.7 ppm while the δ 5.38 multiplet showed an HMQC-TOCSY correlation to C32 at 32.5 ppm. The H27/28 multiplet at δ 5.38 was then traced by DQFCOSY and TOCSY through four methylenes to the methine proton H2 at δ 1.84 consistent

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

Position	δ ^{13}C[a]	δ ^{1}H[b]	COSY[c]	HMBC[d]	^{13}C-^{13}C
1	52.3t	2.88, 2.28	H1, H2	H3, H5a[e], H11, H32a	C2
2	41.0d	1.74	*H1*	H1b, H3, H4a	C1, C3, C32
3	34.1d	1.71	*H4*	H1, H4a, H5a, H9, H10b, H32	C2, C4, C9
4	36.4t	1.81, 1.65	H4, *H3*	H5	C3, C5
5	45.4t	2.99, 2.45	H5, H4b, *H4a*	H1a, H11	C4
6	60.3t	2.58, 1.74	H6, *H7, H8a*	H8, H10	C7
7	37.8d	1.45	H8a	H6b, H8, H9, H10a	C6, C20
8	37.4t	2.26, 0.85	H8, H7, H9	H4, H9, H10b, H21b	C11
9	44.5d	1.72	H10a	H4, H8a, H10	C3, C8, C10
10	59.3t	2.67, 2.02	H10, H9	H6a, H8, H9, H21	C9
11	56.6t	2.73	H12, *H15*	H1b, H12	C12
12	20.4t	1.55, 1.41	H11	H11	C11, C13
13	27.0t	1.34		H11, H12a. H14	C12
14	27.2t	2.08, 1.31	H15	H11, H13, H15	C15
15	131.0d	5.24	*H11*, H14	H14a	C14
16	130.3d	5.24	H17a, H17b, *H19a*	H17a, H17b	C17
17	26.2t	2.15, 1.88	H16	H16, H18	C16, C18
18	29.0t	1.38		H17a, H17b	C17, C19
19	26.8t	2.10, 1.04	*H16*	H18, H20b	C18, C20
20	33.6t	1.37, 0.87	*H16*	H7, H17a, H18	C7, C19
21	58.2t	2.60, 2.35	H22	H6a, H10a, H22, H23a	C22
22	21.8t	1.39		H21a, H21b, H23b	C21, C23
23	27.5t	2.06, 1.29		H21a, H21b	C24, C22
24	26.7t	1.92	H25	H23a, H25a	C25, C23
25	129.9d	5.24	H21b, H24	H23b, H24a	C24
26	131.3d	5.24	H27a, H27b	H27a, H27b, H28	C27
27	27.6t	2.05, 1.38	H26	H26, H28, H29, H30a	C26
28	29.2t	1.30		H26, H27a, H27b, H29, H30b, H31	C29
29	28.2t	1.29		H27b, H28, H30b	C28, (C30)[f]
30	29.7t	1.47, 1.17		H27a, H28, H31	C31, (C29)
31	27.55t	1.22		H29, H30b	C30, C32
32	32.3t	1.59, 1.05		H1	C2, C31

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; ^{13}C = 77.0 ppm.

[b] 500 MHz; solution in $CDCl_3$ referenced at ^{1}H = δ 7.25.

[c] Correlations in italics are HOHAHA correlations.

[d] Inverse detection at 500 MHz; correlations observed when $^1J_{13C-1H}$ = 135 Hz and long range $^nJ_{13C-1H}$ = 8Hz.

[e] a and b denote upfield and downfield resonances respectively of a geminal pair.

[f] Weak correlations in brackets

with a C27-C28 double bond. C29 (at 28.0 ppm) showed HMBC correlations to H28 and H30, while C30 (at 31.1 ppm) showed correlations to H28, H29 and H31. C31 and C32 both showed long range correlations to H1 while C2 showed HMQC-TOCSY correlations to H28. The revised NMR assignments for C27-C31 were entirely consistent with the 2D-INADEQUATE spectrum of (**2**) (**Figure 2**). The remaining carbons, from C21 to C26, were then assigned by COSY, HMBC, HMQC-TOCSY and INADEQUATE data. The assignments for the C_{10} spacer group also required revision, particularly C15-C19, as shown in **Table 2.**

NMR data with improved signal-to-noise were then acquired for the bismethiodide adduct (**5**) of haliclonacyclamine B in acetone-d_6; these data, when analysed, supported the revised double bond position. In

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

Position	$\delta\ ^{13}C^a$	$\delta\ ^1H^b$	COSY[c]	HMBC[d,e]	^{13}C-^{13}C
1	52.3t	2.86 (t, 11 Hz), 2.38	H1, H2, *H5*, *H29b*[f], *H30*, *H31b*, *H32*	H3, H5, H11, *H2*, *H30b*, *H32b*	C2
2	40.0d	1.84	H1, H32b, *H27*, *H28*, *H29b*, *H30a*	H1, H4a, H9, H32, *H28*, *H30*	C1, C3, C32
3	34.2d	1.76	H2, H4b, *H1b*, *H7*, *H8*	H1, H5, H10b, H30a, H32b, *H1*, *H4*	C2, C4, C9
4	35.8t	1.90, 1.69	H3, H4, H5	H3, H5	C3, C5
5	45.9d	3.03 (t, 11Hz), 2.59	H4, H5, *H1*	H1, H3, H4b, *H4a*	C4
6	59.3d	2.63, 1.69	H6, H7, *H8*, *H10*, *H19a*, *H20a*	H8a, H19a, H20a, H21, H22a, *H7*, *H10*	C7
7	37.8d	1.49	H6, H8, H20a, *H10*	H3, H8a, H10, H19a, *H9*, *H20a*	C6, C20
8	37.4t	2.22, 0.84	H7, H8, H9, *H6*, *H10b*, *H19a*	H3, H9, H10, H20	C9
9	44.7d	1.78	H10, *H7*	H1, H4, H8, H10, H20, *H6*, *H7*	C3, C8, C10
10	59.6t	2.70, 2.22	H9, H10, *H2*, *H6b*, *H8a*	H7b, H8a, H21, *H6b*	C9
11	57.0t	2.75	H12, *H13*, *H14*, *H15*	H2, H12, H13a, *H14*, *H15*	C12
12	20.4t	1.50, 1.39	H11, *H14*	H11, H13a, H14, *H15*	C11, C13
13	26.9t	1.32, 1.25	H14, *H11*, *H15*	H11, H12a, H14, H15	C12, C14
14	27.2t	2.12, 1.35	H13, H15, *H11*, *H12*	H13, H15, *H11*, *H17*, *H19a*	C13, C15
15	130.1d	5.27	H14, *H11*, *H13*	H11, H14	C14, C16
16	130.7d	5.27	H17, *H8a*, *H18a*	H18, *H17*, *H19a*, *H20a*	C15, C17
17	27.6t	2.03	H16, H18, *H8b*, *H19a*, *H20a*	H16, H18, *H19a*, *H20a*	C16, C18
18	29.7t	1.47, 1.16	H17, H19a *H20a*	H16, H17, H19a, H20a, *H6*, *H10a*	C17, C19
19	26.6t	1.29, 1.05	H18a, H20, *H6b*, *H17*, *H8b*	H17, H20, *H16*	C18, C20
20	33.7t	1.34, 0.86	H7, H19, H20, *H6*, *H17*, *H18b*	H7, H8a, H9, *H5b*, *H6b*, *H16*, *H19a*	C8, C19
21	57.8t	2.57	H22, *H23a*, *H24*,	H6, H10, H12, *H22*, *H23*, *H27*	C22
22	21.4t	1.58, 1.25	H21, H22, H23	H21, H23, H25, *H27*	C21, C23
23	25.9t	1.29, 1.16	H22, *H21*	H22b, H24, *H26*	C22, C24
24	27.8t[e]	1.39, 1.28	*H21*	H25, H27, *H30a*	C23, C25
25	28.4t	1.39, 1.25	H26	H24, H26, *H22b*	C24, C26
26	26.0t	2.18, 1.77	H25, H27	H27, H25b, *H23*	C25, C27
27	129.5d	5.38	H26, *H25a*	H2, H26	C26, C28
28	129.7d	5.38	H29, *H2*, *H30a*, *H32b*	H2, H29b, *H1*, *H30a*, *H32*	C27, C29
29	28.0t	2.15, 1.84	H28, H30, *H1*, *H32a*	H28, H30, H32, *H2*	C28, C30
30	31.1t	1.42, 1.09	H29, H31, *H2*, *H28*,	H28, H29, H31, *H1*	C29, C31
31	27.9t[e]	1.39, 1.28	H30a	H28, H32, *H1*, *H30a*	C30, C32
32	32.5t	1.47, 1.25	H2, H32, *H29*, *H30a*	H1, H31, *H28*, *H29*	C2, C31

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; ^{13}C = 77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced at ^1H = δ 7.25.
[c] Correlations in italics are TOCSY correlations.
[d] Inverse detection at 500 MHz; correlations observed when $^1J_{13C\text{-}1H}$ = 135 Hz and long range $^nJ_{13C\text{-}1H}$ = 8Hz.
[e] Correlations in italics are HMQC-TOCSY correlations.
[f] a and b denote upfield and downfield resonances respectively of a geminal pair.
[g] Assignments may be interchanged

particular, C32 (31.4 ppm) was easily identified by the COSY correlation of its associated protons at δ 1.78 to H2 at δ 2.54. The alkene resonance associated with H28 (δ 5.40) exhibited COSY correlations to signals at δ 2.31 and 1.90 which were assigned as H29 since the associated carbon signal at 28.2 ppm showed HMQC-TOCSY correlations to H32. Carbons C30 (31.1 ppm) and C31 (27.9 ppm) were then assigned by use of HMBC and HMQC-TOCSY data, and the data cross-checked by inspection of DQFCOSY data (**Table 3**). These revisions to our published NMR data for (**1**) and (**2**)[3c] indicate the considerable challenges in assigning haliclonacyclamine structures by NMR methods alone.

Table 3.
^{1}H, ^{13}C NMR Data and Long-Range ^{13}C-^{1}H Correlations for Haliclonacyclamine B Bismethiodide (**5**).

Position	δ $^{13}C^{a}$	δ $^{1}H^{b}$	COSY	HMBC[c,d]
1	60.0t	3.65, 3.24	H1, H2, H33	H3, H11, H33, *H2*, *H31a*[e], *H32*
2	32.4d	2.54	H1, H3, H32a	H8, H10a, *H1b*, *H9*, *H10b*
3	33.13d	2.17	H2, H4	H1, H2, *H5*, *H8b*
4	30.3t	2.50, 2.35	H3, H4, H5b	
5	56.0t	4.06 (t, 15Hz), 3.28	H4, H5	H2, H3, H11, H33
6	64.4t	3.35	H7	H8a, H20a, H21, *H7*, *H8b*, *H9*
7	35.4d	2.53	H6, H8	H1b, H3, H5a
8	33.9t	2.38, 1.64	H7, H8, H9	H3, H10, H19, *H1b*, *H5*, *H7*, *H20*
9	37.9d	2.91	H8, H10	H4, H8a, H10b, *H3*, *H7*, *H10a*
10	63.9t	3.88, 3.30	H9, H10	H3, H8a, H21, H34, *H9*
11	68.8t	3.71	H12	H1, H12, H33
12	22.5t	1.97, 1.90	H11	H11, H14, H33, *H13*, *H15*
13	26.6t	1.62, 1.50	H14a	H12, H15, *H33*
14	27.1t	2.31, 2.22	H13b, H15	H15, *H18*
15	131.1d	5.32	H14	H14, H17
16	132.0d	5.32	H17	H14, H17
17	27.9t	2.13, 2.07	H16, H18a	H16, H19
18	30.1t	1.55, 1.18	H17b, H19a	
19	26.0t	1.49, 1.36	H18a, H20a	H20a, *H8*, *H16*, *H18*
20	33.11t	1.44, 1.13	H7, H19a	H8a, *H7*, *H8b*, *H19*
21	68.8t	3.70	H22	H22, H34
22	21.9t	1.96, 1.89	H21, H23b	H23b, H25b, H34, *H23*, *H26*
23	24.8t	1.61, 1.37	H22a	H24, H25, H34
24	28.7t	1.43, 1.33		H25, H26, *H34*
25	28.0t	1.54, 1.45	H26a	H24a, H26a
26	26.6t	2.29, 1.80	H25a, H27	H25, H27, *H23b*, *H24a*
27	130.3d	5.40	H26	H24, H26, H29
28	130.7d	5.40	H29	H26, H29, H32
29	28.2t	2.31, 1.90	H28, H30a	H28, *H32*
30	31.5t	1.50, 1.29	H29a	H29, H31, *H1b*, *H28*
31	28.3t	1.60, 1.54	H32a	H2, H28, *H1b*, *H32*
32	31.4t	1.78	H2, H31a	H4a, H30, H31, *H2*, *H9*
33	50.8q	3.45	H1b, H5b	H1, H5
34	48.9q	3.39		H6, H21

[a] Inverse detection at 500 MHz (geHSQC); solution in d_6-acetone; ^{13}C = 29.8 ppm.
[b] 500 MHz; solution in acetone-d_6 referenced at ^{1}H = 2.05 ppm.
[c] Inverse detection at 500 MHz; correlations observed for $^{1}J_{13C\text{-}1H}$ = 135 Hz and long range $^{n}J_{13C\text{-}1H}$ = 8Hz.
[d] Correlations in italics are HMQC-TOCSY correlations
[e] a and b denote upfield and downfield resonances respectively of a geminal pair.

NEW HALICLONACYCLAMINE METABOLITES

The first new metabolite, haliclonacyclamine C, $[\alpha]_D$ +4.8°, gave the molecular formula $C_{32}H_{58}N_2$ by HREIMS, and was therefore a dihydro analogue of the previously isolated haliclonacyclamines. Hydrogenation of (**3**) gave a saturated product (**9**), $[\alpha]_D$ +12.7°, with the same sign, and similar magnitude, of optical rotation as that of the hydrogenation product from (**1**) and (**2**) (+23.9°)[3c]. This suggested all three haliclonacyclamines may have identical ring stereochemistry. Structure determination of haliclonacyclamine C (**3**) by NMR was complicated by the congestion in the high field region of its proton and ^{13}C NMR spectra. With secure structural details for (**1**) and (**2**) in hand, the NMR analysis of haliclonacyclamine C (**3**) proceeded identically to the *bis*-hexahydropyridine core and the methylene termini C11, C20, C21 and C32 of the two linear bridges (**Table 4**). A NOESY correlation between H7 and H9 was consistent with the stereochemistry shown.

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

Position	$^{13}C^a$	$^1H^{b,c}$	COSY[d]	HMBC[e,f]
1	53.4t	2.75, 2.44	H2	H3, H5b[£], H11, H32a, *H2*
2	41.1d	1.82	H1b[g], *H1a*	H1, H3, H4a, H9, H32a
3	34.9d	1.78	H4b, *H4a, H5*	H1, H2, H4, H5a, H32a, *H5b*
4	36.2t	1.93, 1.70	H3, H5	H3, H9, H32a, *H5*
5	46.7t	2.95, 2.63	H4, *H3*	H1a, H3, H4b, H11, *H4a*
6	60.5t	2.61, 1.83	*H8a*	H8, H10, H21b, *H7, H20b*
7	37.6d	1.48	H6, H8, *H9*	H6, H8, H20a
8	38.0t	2.36, 0.92	H7, H9, *H6, H10*	H3, H6, H10, *H9*
9	45.0d	1.82	H8a, *H8b, H10*	H3, H4, H8a, H10, *H8b*
10	59.4t	2.73, 2.11	H9, *H8*	H6, H7, H8, H21, *H9*
11	56.8t	2.79, 2.70	H12	H1b, H12
12	21.3t	1.52	H11	H11
13	26.3t			H11, H12
14	27.7t			H12, *H11*
18	25.8t			H20a
19	27.9t			*H20a*
20	34.1t	1.35, 0.93	H6a	H6a, H8a
21	58.4t	2.65, 2.39	H6a, H10a, H22, *H24, H25*	H6a, H10a
22	22.1t	1.46	H23a, H21	H21
23	27.5t	1.35, 1.25	H22, H24a	H21a, *H21b, H24, H25*
24	26.7t	2.12, 1.96	H23a, H25, *H21*	H25, *H21*
25	130.0d	5.27	H24, *H21*	H24b, H27
26	131.2d	5.27	H27	H27
27	26.1t	2.17, 1.91	H26, H28b	H24, H26
28	29.1t	1.42, 1.32	H27	H27, H26, H32b
29	28.3t	1.38, 1.31		H27, *H32*
30	29.3t	1.30, 1.22		H27b, H32a, *H32b*
31	27.8t	1.37, 1.19		H32
32	32.7t	1.61, 1.11		H1

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; ^{13}C = 77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced at ^1H = δ 7.25.
[c] Coupling constants in Hz.
[d] Correlations in italics are TOCSY correlations.
[e] Inverse detection at 500 MHz; correlations observed for $^1J_{13C\text{-}1H}$ = 135 Hz and long range $^nJ_{13C\text{-}1H}$ = 8Hz.
[f] Correlations in italics are HMQC-TOCSY correlations.
[g] a and b denote upfield and downfield resonances respectively of a geminal pair.
[h] These assignments may be interchanged.

In the alkene region, a two proton resonance centred at δ 5.27 could be linked by 2D-TOCSY to the H21 protons at δ 2.65 and 2.39, thus positioning the sole double bond in the C_{12} spacer group rather than in the C_{10} spacer group. H21 gave HMBC or HMQC-TOCSY correlations to three non-core carbons at 22.1, 27.5 and 26.7 ppm, suggesting that there were at least three bonds between position 21 and the double bond. H21 was coupled by DQFCOSY to a signal at δ 1.46 (H22), which was linked to the carbon at 22.1 ppm by geHSQC. The alkene hydrogens were COSY coupled to proton signals at δ 1.21, 2.17, 1.96 and 1.91, of which the signals δ 2.12 and 1.96 correlated to the carbon at 26.7 ppm which could be assigned to C24 since it showed HMQC-TOCSY correlations to H21. The H24 signals also showed TOCSY correlations to H21. The carbon signal at 27.5 ppm gave geHMBC or HMQC-TOCSY correlations to H21 and H24, and was therefore C23. No other carbon signals gave correlations to H21, H22 or H23, therefore a chain of five carbons from C21 at 58.4 ppm to to C25 at 130.0 ppm had been identified. Additional support for the double bond position was obtained by analysis of NMR data

Table 5.
^{1}H, ^{13}C NMR Data and Long-Range ^{13}C-^{1}H Correlations for Haliclonacyclamine C Bismethiodide (**6**).

Position	δ $^{13}C^{a}$	δ $^{1}H^{b}$	COSY[c]	HMBC[d, e]
1	61.1t	3.49, 3.21	H2	H34, H3 *H2*
2	36.2d	2.30	H1, *H3*	H1, H9 *H3*
3	33.4d	2.07	*H4, H2*	H9 *H4*
4	29.6t	2.36, 2.27	*H3*	H9, *H3, H5*
5	57.8t	3.85, 3.27	H4, *H3*	H11, H34, *H4, H3*
6	64.6t	3.20	H7	H33, H7, H21 *H8*
7	32.2d	2.37	H6, *H8a*[f]	H6
8	57.2t	2.34, 1.48	H9, *H10*	H10, *H9, H7, H6*
9	37.8d	2.66	H10, *H8*	H10a[f] *H10b*
10	63.4t	3.48, 3.14	H9, *H8*	H33, H3, *H9, H8*
11	69.3t	3.42	H12	H34, H12, H1a
12	21.9t	1.94	H11	*H11*
13	24.8t	1.56, 1.49		*H11, H12*
19	29.9t	1.42		*H7*
20	33.6t	1.47, 1.28		*H6*
21	69.5t	3.49. 3.36	H22, *H24*	H6, H10, H33, H22
22	21.6t	1.83	H21, *H23, H24*	H21
23	27.0t	2.20	*H21, H22, H24*	H22
24	26.81t	2.28	H25, *H21, H23*	H25
25	130.0d	5.41	H24	H23b, H24
26	132.7d	5.41	H27	H24, H27
27	26.73t	2.05	H26, H28b	H26
28	26.77t	2.28, 1.64	H27, H29, *H26*	H26
29	29.6t	1.50	H28b	H28 *H27, H26*
30	28.5t	1.40		*H28*
31	29.0t	1.53		H32
32	33.6t	1.66, 1.41	*H2*	H2
33	48.4q	3.22		H1, H11
34	49.3q	3.25		H6, H10, H21

[a] Inverse detection at 500 MHz (geHSQC); solution in 1:1 $CDCl_3$:CD_3OD; ^{13}C = 77.0 ppm.
[b] 500 MHz; solution in 1:1 $CDCl_3$:CD_3OD referenced at ^{1}H = δ 7.25.
[c] Correlations in italics are TOCSY correlations.
[d] Inverse detection at 500 MHz; correlations observed for $^{1}J_{13C\text{-}1H}$ = 135 Hz and long range $^{n}J_{13C\text{-}1H}$ = 8Hz.
[e] Correlations in italics are HMQC-TOCSY correlations.
[f] a and b denote upfield and downfield resonances respectively of a geminal pair.
[g] These assignments may be interchanged.

for the bismethiodide salt (**6**) of haliclonacyclamine C (**Table 5**). Assignment of the bicyclic core proceeded in an analogous fashion to that of haliclonacyclamine C with the two methyl signals at 48.4 and 49.3 ppm assigned to C33 and C34 on the basis of HMBC correlations to their respective N-methylenes. The H21 protons showed DQFCOSY correlations to a signal at δ 1.83, assigned to H22. An HMBC correlation was seen from H22 to a carbon at 27.0 ppm which could be assigned to either C23 or C24. The corresponding protons, at δ 2.20, were coupled to H21, H22 and a signal at δ 2.28 assigned to H24 because it was COSY coupled to the alkene signal. Therefore the signal at 27.0 ppm was C23. HMBC and HMQC-TOCSY correlations for the C21-C26 region were entirely consistent with this analysis. The ^{13}C NMR data for (**3**) and (**6**) suggested an *E* double bond.

Haliclonacyclamine C showed potent biological activity. An IC_{50} value of 0.7 μg/ml was obtained in a P_{388} assay, and the compound displayed antibacterial activity towards *Bacillus subtilis* and was strongly antifungal against *Candida albicans* and *Trichophyton mentagrophytes*.

The second new metabolite, haliclonacyclamine D (**4**), $[\alpha]_D$ +16.1°, had a molecular formula of $C_{32}H_{58}N_2$ by HREIMS. The ^{13}C spectrum confirmed the presence of two alkene carbons indicating a second dihydro analogue of (**1**) and (**2**). Preliminary examination of the NMR data (**Table 6**) suggested that (**4**) had a structure similar to that of (**2**) with no double bond at C15-C16. The bicyclic core was assigned as previously described for the other haliclonacyclamines. The four terminal methylenes of the spacer group were then assigned as follows;

Table 6.
^1H, ^{13}C NMR Data and Long-Range ^{13}C-^1H Correlations for Haliclonacyclamine D (**4**).

Position	δ $^{13}C^a$	δ $^1H^b$	COSY[c]	HMBC[d,e]
1	53.0t	2.83, 2.54	H1, H2	H3, H11a, *H32*
2	40.0d	1.96	H1b, H32b	H4a, H30a, H32
3	34.5d	1.85	H4a[f]	*H4, H5*
4	35.3t	2.07, 1.76	H3, H4, H5	H3, *H5*
5	47.1t	3.04, 2.75	H5, H4, *H3*	H3, H11, *H4*
6	59.1t	2.69, 1.89	H6, H7, *H8a, H20a*	H8a, H21, *H7, H20*
7	37.5d	1.52	H6a, H7, H8a, H20a	H8a, H20a, H6a, *H9*
8	37.9t	2.37, 0.91	H7, H8, H9	H7, H6a, *H9, H10*
9	45.1d	1.92	H8a, H10a	H3, H8a, *H8b*
10	59.2t	1.96	H9	H8, H21
11	56.6t	2.88, 2.77	H12	H7, *H12*
12	21.3t	1.55	H11	*H11*
20	34.0t	1.36, 0.93	H7, H20	H7, *H8a*
21	57.8t	2.63	H22	H5a, H22
22	21.4t	1.62, 1.29	H21	*H21*
26	26.0t	2.21, 1.76	H26, H27	H27
27	129.65d	5.40	H26, *H29*	H26
28	129.70d	5.40	H29, *H26*	H29, *H30a, H32b*
29	27.9t	1.87, 2.16	H28, H29, H30	H28, H32, *H26, H31a*
30	31.2t	1.45, 1.13	H29, H30, H31a	H31, *H28, H30a ,H31b, H32b*
31	27.75-27.85t[g]	1.42, 1.32	H30a	
32	32.8t	1.50, 1.34	H2	H30a, *H29, H30a, H1b*

[a] Inverse detection at 500 MHz (geHSQC); solution in $CDCl_3$; ^{13}C = 77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced at ^1H = 7.25 ppm.
[c] Correlations in italics are TOCSY correlations.
[d] Inverse detection at 500 MHz; correlations observed for $^1J_{13C\text{-}1H}$ = 135 Hz and long range $^nJ_{13C\text{-}1H}$ = 8Hz.
[e] Correlations in italics are HMQC-TOCSY correlations.
[f] a and b denote upfield and downfield resonances respectively of a geminal pair.
[g] one of four carbons in this chemical shift range.

HMBC correlations from C21 at 57.8 ppm to H6 and by C11 at 56.6 ppm to H5 identified the N-methylene termini while COSY correlations from H2 to H32b at δ 1.50 and from H7 to H20a at δ 0.93 allowed the assignment of C32 and C20 respectively. The carbon signals at 21.4 and 21.3 ppm were assigned as C22 and C12 respectively on the basis of chemical shift and COSY correlations to C21 and C11. The two allylic carbons at 27.9 ppm (C29) and at 26.0 ppm (C26) were identified by COSY correlations from their respective protons at δ 2.16/1.87 and at δ 2.21/1.76 to the alkene signal at δ 5.40. The protons at δ 2.16 and 1.87 were assigned to H29 since they were COSY coupled to a signal at δ 1.13 (H30), itself linked to a carbon at 31.2 ppm. The carbons at 27.9 and 31.2 ppm were assigned to C29 and C30 since both exhibited long-range C-H correlations to H32 confirming their close proximity to the 2,3-disubstituted ring. H30 at δ 1.13 had a COSY correlation to a group of carbon signals between 27.75 and 27.85 ppm. It was impossible to determine exactly which one of these four carbon signals corresponded to C31. Nevertheless a minimum of four carbon centres had been located between

Table 7.
^{1}H and ^{13}C NMR Data for Haliclonacyclamines A - D.

Position	Haliclonacyclamine A (1)	Haliclonacyclamine B (2)	Haliclonacyclamine C (3)	Haliclonacyclamine D (4)
1	52.3t[a] (2.88[b], 2.28)	52.3t (2.86, 2.38)	53.4t (2.75, 2.44)	53.0t (2.83, 2.54)
2	41.0d (1.74)	40.0d (1.84)	41.1d (1.82)	40.0d (1.96)
3	34.1d (1.71)	34.2d (1.76)	34.9d (1.78)	34.5d (1.85)
4	36.4t (1.81, 1.65)	35.8t (1.90, 1.69)	36.2 (1.93, 1.70)	35.3t (2.07, 1.76)
5	45.4d (2.99, 2.45)	45.9d (3.03, 2.59)	46.7 (2.95, 2.63)	47.1t (3.04, 2.75)
6	60.3t (2.58, 1.74)	59.3d (2.63, 1.69)	60.5t (2.61, 1.83)	59.1t (2.69, 1.89)
7	37.8d (1.45)	37.8d (1.49)	37.6 (1.48)	37.5d (1.52)
8	37.4t (2.26, 0.85)	37.4t (2.22, 0.84)	38.0t (2.36, 0.92)	37.9t (2.37, 0.91)
9	44.5d (1.72)	44.7d (1.78)	45.0d (1.82)	45.1d (1.92)
10	59.3t (2.67, 2.02)	59.6t (2.70, 2.22)	59.4t (2.73, 2.11)	59.2t (1.96)
11	56.6t (2.73)	57.0t (2.75)	56.8t (2.79, 2.70)	56.6t (2.88, 2.77)
12	20.4t (1.55, 1.41)	20.4t (1.50, 1.39)	21.3t (1.52)	21.3t (1.52)
13	27.0t (1.34)	26.9t (1.32, 1.25)	26.3t [c]	[d]
14	27.2t (2.08, 1.31)	27.2t (2.12, 1.35)	27.7t [c]	[d]
15	131.0d (5.24)	130.1d (5.27)	[d]	[d]
16	130.3d (5.24)	130.7d (5.27)	[d]	[d]
17	26.2t (2.15, 1.88)	27.6t (2.03)	[d]	[d]
18	29.0t (1.38)	29.7t (1.47, 1.16)	25.8t [c]	[d]
19	26.8t (2.10, 1.04)	26.6t (1.29, 1.05)	27.9t [c]	[d]
20	33.6t (1.37, 0.87)	33.7t (1.34, 0.86)	34.1t (0.93)	34.0t (1.36, 0.93)
21	58.2t (2.60, 2.35)	57.8t (2.57)	58.4t (2.65, 2.39)	57.8t (2.63)
22	21.8 (1.39)	21.4t (1.58, 1.25)	22.1t[c]	21.4t (1.62, 1.29)
23	27.5t (2.06, 1.29)	25.9t (1.29, 1.16)	27.5t[c]	[d]
24	26.7t (1.92)	27.8t[e] (1.39, 1.28)	26.7t (2.12, 1.96)	[d]
25	129.9d (5.24)	28.4t (1.39, 1.25)	130.0d (5.27)	[d]
26	131.3d (5.24)	26.0t (2.18, 1.77)	131.2d (5.27)	26.0t (2.21, 1.76
27	27.6t (2.05, 1.38)	129.5d (5.38)	26.1t (2.17, 1.19)	129.65d (5.40)
28	29.2t (1.30)	129.7d (5.38)	29.1t[c]	129.70d (5.40)
29	28.2t (1.29)	28.0t (2.15, 1.84)	28.3t[c]	27.9t (1.87, 2.16)
30	29.7t (1.47, 1.17)	31.1t (1.42, 1.09)	29.3t[c]	31.2t (1.45, 1.13)
31	27.55t (1.22)	27.9t[e] (1.39, 1.28)	27.8t[c]	27.8t[f] (1.42, 1.32)
32	32.3t (1.59, 1.05)	32.5t (1.47, 1.25)	32.7t (1.61, 1.11)	32.8t (1.50, 1.34)

[a] Inverse detection at 500 MHz; solution in $CDCl_3$ referenced at ^{13}C = 77.0 ppm.
[b] 500 MHz; solution in $CDCl_3$ referenced at ^{1}H = δ 7.25.
[c] proton resonances not assigned.
[d] carbon and proton resonances not assigned.
[e] assignments may be interchanged.
[f] one of four carbons between 27.75-27.85 ppm.

C2 and the alkene carbon C28, hence it was likely the double bond was located no closer to C32 than C27-C28. Analysis of the HMBC, HMQC-TOCSY and DQFCOSY data for the C21-C25 region could not be completed because of uncertainties about the carbon assignments for C23-C25. Haliclonacyclamine D (**4**) may thus be analogous to (**2**) in the same way as (**3**) is to (**1**). Comparison of the proton and carbon chemical shifts of (**2**) and (**4**) shows good agreement in the C26-C32 region which further supports the proposed structure of (**4**); likewise the data for (**1**) and (**3**) match well in the C21-C32 region (**Table 7**). There was insufficient material to compare relative stereochemistry by preparation of the hydrogenated analogue (**9**), but NOESY data suggested the ring stereochemistry at C7 and C9 was identical to haliclonacyclamines A-C, (**1**)-(**3**). There was insufficient of (**4**) to derivatise or submit for biological testing.

From a biological perspective *Haliclona* sp. is an intriguing sponge since the sponge tissue contains dinoflagellates and nematocysts in addition to major cell types such as spongocytes, choanocytes and archaeocytes. Electron microscopic inspection shows the dinoflagellates are morphologically similar to *Symbiodinium microadriaticum*, the common symbiont of corals, and are intracellular rather than free-living within the sponge tissue. Our research has further shown that the haliclonacyclamines are stored within the sponge cells rather than within the dinoflagellate[17]. It is not clear whether the associated dinoflagellates are a genuine symbiont, or whether they are taken up from coral as the nematocysts likely are. Specimens of *Haliclona* sp. are frequently found growing on coral substrates and appear to kill coral. Ecological studies to determine explicitly the role of the haliclonacyclamines in coral tissue necrosis are in progress in our laboratory.

EXPERIMENTAL

Isolation of Metabolites

Sponge samples were collected using SCUBA at about 12 m depth at Heron Island on the Great Barrier Reef. For a brief description of the sponge and the dinoflagellate symbiont, see references 3c and 17. A voucher sample of *Haliclona* sp. (G304086) is held at the Queensland Museum, Brisbane.

The frozen sponge (250 g, wet weight) was cut into pieces and left in ethanol (600 mL) at room temperature for 4 hours. The ethanol solution was filtered through a plug of cotton wool and the solvent removed by rotary evaporation. The resultant extract was partitioned between ethyl acetate and water. The organic extract was concentrated under vacuum to give a dark brownish green tar (1.1 g). A portion of the crude extract (600 mg) was dissolved in ethyl acetate (750 mL) and extracted into 1M hydrochloric acid (3 x 250 mL). The aqueous extract was made alkaline (pH 10) by the addition of 2M sodium hydroxide solution and extracted into hexane (3 x 350 mL). The combined hexane extracts were dried over anhydrous magnesium sulphate (50 g) and concentrated under vacuum to give a bright yellow oil which showed activity in antibacterial, antifungal and P_{388} assays. The bioactive fraction was further purified by normal phase HPLC using EtOAc/Hex/Et_3N (30:65:5 or 80:15:5) to give haliclonacyclamines A (**1**, 68 mg, 0.025 %) B (**2**, 17 mg, 0.006 %), C (**3**, 5 mg, 0.002%) and D (**3**, 1 mg, 0.0004%).

Haliclonacyclamine A (1)

see reference 3c; ^1H and ^{13}C NMR, see **Table 1**.

Haliclonacyclamine B (2)

see reference 3c; ^{1}H and ^{13}C NMR, see Table 2.

Haliclonacyclamine C (3)

colourless gum; $[\alpha]_D$ + 4.8° (c, 0.30, CH_2Cl_2); IR (film) 1633 cm^{-1}; LREIMS (m/z, relative intensity) 470 (M^+, 100), 235 (15); HREIMS M^+ m/z 470.4596 ($C_{32}H_{58}N_2$ ΔM -0.0002 mmu); ^{1}H NMR ($CDCl_3$; 500 MHz), δ 0.85-0.93 (2H, m), 1.11 (1H, br t), 1.14-1.48 (27H, m), 1.86-2.37 (15H, m), 2.36 (1H, m), 2.42 (1H, m), 2.54 (1H, m), 2.85-2.79 (7H, m), 2.97 (1H, br t), 5.27 (2H, m); ^{13}C NMR ($CDCl_3$; 125 MHz): 131.2, 130.0, 60.5, 59.4, 58.4, 56.8, 53.4, 46.7, 45.0, 41.1, 38.0, 37.6, 36.2, 34.9, 34.1, 32.7, 29.3, 29.1, 28.3, 27.9, 27.8, 27.7, 27.5, 27.0, 26.9, 26.7, 26.3, 26.1, 25.8, 25.5, 22.1 and 21.3 ppm.

Haliclonacyclamine D (4)

colourless gum; $[\alpha]_D$ + 16.1° (c, 0.30, CH_2Cl_2); IR (film) 1633 cm^{-1}; LREIMS (m/z, relative intensity) 470 (M^+, 100), 235 (15); HREIMS M^+ m/z 470.4601 ($C_{32}H_{58}N_2$ ΔM -0007 mmu 470.4594); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88-0.98 (2H, m), 1.13 (1H, m), 1.65-1.18 (32H, m), 1.78-1.71 (2H, m), 2.01-1.82 (5H, m), 2.25-2.04 (2H, m), 2.41-2.28 (2H, m), 2.54 (1H, m), 2.92-2.61 (8H, m), 3.04 (1H, t, 12Hz), 5.40 (2H, m); ^{13}C NMR ($CDCl_3$; 500 MHz), 129.7, 129.65, 59.2, 59.1, 57.8, 56.6, 53.0, 47.1, 45.1, 40.0, 37.9, 37.5, 35.3, 34.5, 34.0, 32.8, 31.2, 28.3, 27.9, 27.9, 27.8(4C), 26.9, 26.7, 26.2, 26.0, 25.8, 25.5(2C), 21.4 and 21.3 ppm.

Preparation of derivatives

Bismethiodide of haliclonacyclamine B (5)

Haliclonacyclamine B (30 mg, 0.06 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 24 hours the solvent was removed and the crude product was crystallized from MeOH/EtOAc (3:1) yielding yellow needles of the methiodide adduct (**5**); mp 170-175° (decomp.); LREIMS (m/z, relative intensity) (M^+-CH_3I) 610 (43), 468 (100), 234 (100); HREIMS (M^+-CH_3I) m/z 610.3708 ($C_{33}H_{59}N_2$ ΔM -0.84 mmu), ESMS [$(M+H-CH_3I)^+$, relative intensity] 611.4 (93), 249.8 (100); ^{1}H (acetone-d_6; 500 MHz) δ 1.13-1.20 (2H, m), 1.22-1.70 (18H, m), 1.72-1.82 (2H, m), 1.83-2.10 (6H, m), 2.15-2.25 (3H, m), 2.27-2.40 (5H, m), 2.42-2.59 (3H, m), 2.91 (1H, m), 3.28-3.48 (11H, m), 3.6-3.73 (5H, m), 3.88 (1H, m), 4.06 (1H, t, 15Hz), 5.32 (2H, m), 5.40 (2H, m); ^{13}C NMR (acetone-d_6; 500MHz) 132.0, 131.1, 130.7, 130.3, 68.84, 68.83, 64.4, 63.9, 60.0, 56.0, 50.8, 48.9, 37.9, 35.4, 33.9, 33.1 (2C), 32.4, 31.5, 31.4, 30.3, 30.1, 28.7, 28.3, 28.2, 28.0, 27.9, 27.1, 26.6 (2C), 26.0, 24.8, 22.5 and 21.9 ppm.

Bismethiodide of haliclonacyclamine C (6)

Haliclonacyclamine C (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 24 hours the solvent was removed and the crude product was crystallized from MeOH/EtOAc (3:1) yielding yellow needles of the methiodide adduct (**6**); mp 196-197° (decomp.); ESMS m/z $(M+H-CH_3I)^+$ 613 (67), 306 (100); ^{1}H NMR (1:1 CD_3OD:$CDCl_3$; 500 MHz) δ 1.45-1.54 (27H, m), 1.56-1.98 (6H, m), 2.01-2.48 (12H, m), 2.65 (1H, br t), 3.21 (2H, m), 3.25 (3H, s), 3.22 (3H, s), 3.33-3.51 (7H, m), 3.85 (1H, m), 5.41 (2H, m); ^{13}C NMR (500 MHz, 1:1 CD_3OD:$CDCl_3$) : 132.7, 130.0, 69.5, 69.3, 64.6, 63.4, 61.1, 57.2, 49.3, 48.4, 37.8, 36.2, 34.4, 33.6, 33.4, 32.2, 31.6, 29.9, 29.6 (2C), 29.0 (2C), 28.5, 28.1, 27.7, 27.1, 27.0, 26.8 (2C), 26.7, 25.8, 24.8, 21.9 and 21.6 ppm.

15,16-Dihydrohaliclonacyclamine C (9)

Haliclonacyclamine C (5 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 hydrogenated product which was then passed through a pad of celite prior to purification on normal phase HPLC (EtOAc/Hex/Et_3N; 80:15:5) yielding a compound identical to tetrahydrohaliclonacyclamine A (2.8 mg, 55.8%)[3c]; $[\alpha]_D$ +12.7° (c, 0.28, 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); ^{1}H 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 (2C), 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.

NMR experiments

All 1D and 2D NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer, using $CDCl_3$, MeOH-d_4 or acetone-d_6 as solvents, referenced at δ 7.25/77.0 ppm or δ 3.3/49.0 ppm for ^{1}H/^{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 π/2 shifted sine-squared bell window function was applied in both dimensions before Fourier transformation. The HMBC and the phase sensitive HMQC/geHSQC spectra were acquired with 64 and 24 transients respectively. The evolution delay was set for $^nJ_{CH}$ of 4 and 8 Hz (geHMBC) and $^1J_{CH}$ of 135 Hz (HMQC or geHSQC). 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-TOCSY spectrum was acquired with either 64 or 48 transients per increment using a spin-lock mixing time of 20.6 ms. All the 2D TOCSY experiments were acquired with 16 or 32 transients per increment using mixing times of 20.6 and 100 ms. The selective 1D TOCSY 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 TOCSY experiments were carried out using a MLEV17 spin-lock pulse applied with RF field strength of

$\gamma B1$= 11.4 kHz. The 2D-INADEQUATE experiment was acquired using 128 scans per increment and with a 2K by 2K matrix and using a 90_C = 12.2 μsec pulse.

X-ray structure determination:

A crystal of approximate dimensions 0.22 × 0.10 × 0.06mm was cleaved from a colourless needle of $C_{32}H_{56}N_2$ and mounted on a Rigaku AFC6R diffractometer equipped with a graphite monochromator and using copper radiation from a rotating anode generator. The crystal was orthorhombic, space group $P2_12_12_1$, a = 11.482(1) Å, b = 15.449(1) Å, c = 16.876(1) Å, V = 2993.5(5) Å^3, Z = 4, D_{calc} = 1.040 g cm^{-3}. Accurate lattice parameters were determined by least-squares analysis of the setting angles of 25 reflections with 77.96 < 2θ < 93.84° (λ(Cu Kα$_1$) = 1.5406Å). Intensity data were collected for reflections $+h,+k,+l$ with 2θ ≤ 120° using ω-2θ scans of width (1.2 + 0.3tanθ)° in ω at a rate of 16° min^{-1} in ω. The weak reflections (I < 10.0σ(I)) were rescanned (maximum of 4 scans) and the counts were accumulated to ensure good counting statistics. Stationary background counts were recorded on each side of the reflection. The ratio of peak counting time to background counting time was 2:1. The intensities of 2518 reflections were collected. Three representative reflections were measured after every 150 reflections to monitor orientation and decomposition. Over the course of data collection the standards decreased by 3%. A linear correction factor was applied to the data to account for this phenomenon. The linear absorption coefficient, μ, for Cu-Kα radiation is 4.1 cm^{-1}. A semi-empirical absorption correction, derived from azimuthal scans of several reflections, was applied which resulted in transmission factors ranging from 0.93 to 0.98. Details of the structure solution and refinement are given in the text. Anisotropic displacement factors were used for non-hydrogen atoms of full occupancy and isotropic displacement factors were used for all other atoms. Hydrogen atoms were placed at calculated positions and included in the structure factor calculations; their coordinates were not refined but were recalculated regularly. Refinement was continued until all shift/error ratios were < 0.03.

Least-squares refinement was performed using full-matrix methods minimising the function $\Sigma w(|F_O| - |F_C|)^2$ where $w = [\sigma^2(F) + 0.0009 \times F^2]^{-1}$ to a final R = 0.046. Maximum and minimum heights in a final difference map were 0.18(3) and -0.18(3) e Å^{-3}, respectively. The absolute configuration has not been determined in this study, but has been assigned to match that of haliclonacyclamine A. Data reduction was perfomed with use of teXsan[13] computer software and refinement computations were performed with XTAL3.4[14]; atomic scattering factors for neutral atoms and real and imaginary dispersion terms were taken from *International Tables for X-ray Crystallography*[18]. **Figure 3** shows the molecule with one conformation of the region between C28-C31, and selected atom labelling. Additional crystallographic details are available and the data have been deposited in the Cambridge Crystallographic Data Centre.

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