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The Structure and Biogenetic Origin of (-) Halicyclamine B from a *Xestospongia* Sponge

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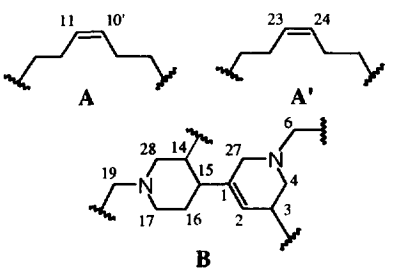
Abstract: A new tetracyclic diamine, (-) halicyclamine B (**1**), has been isolated from the sponge *Xestospongia sp.* and its structure was elucidated from NMR and X-ray crystallography data. Some biogenetic relationships are proposed between **1** and other polycyclic diamines recently reported from sponges. Copyright © 1996 Elsevier Science Ltd

Polycyclic alkaloids putatively formed[1] by the condensation of ammonia, acrolein and an unsaturated dialdehyde are of considerable current interest. During 1986 and 1987, (+) manzamine A[2a] (=keramamine A[2b]) and (+) manzamine B[2c] were described as the initial examples of polycyclic diamines condensed to a β-carboline. The structure of (+) kauluamine, a manzamine dimer, represents the most intricate example of this structural motif.[3] The remaining alkaloids of this class are devoid of the β-carboline moiety. There are pentacyclic diamine epimers, (-) papuamine[4a] and (-) haliclonadimanine,[4b] which we recently encountered as mixtures from *Haliclona* sponges. These compounds, subjects of recent total syntheses,[4c] have symmetry relationships that confound any simple explanations of the cyclization process responsible for their biosynthesis. Biogenetic precursors to the (+) manzamines have been isolated and fall into two categories. The most advanced includes the tetracyclic aldehydes, (+) ircinal A and (+) ircinal B,[5] while the other consists of pentacyclic $(CH_2)_n$ homologs, exemplified by (+) xestocyclamine B[6a] = (+) ingenamine,[6b] (+) keramphidin B[6c] and (+) ingamine A.[6d] We recently reported (-) halicyclamine A[7a] and Garson[7b] reported the haliclonacyclamines both of which constitute an additional biosynthetic shunt on this theme. Herein we report a new analog (-) halicyclamine B (**1**) and further outline stereostructural relationships between these two compounds and the manzamine precursors outlined above.

Specimens of *Xestospongia* sp. (1.0 kg, wet wt; coll. no. 94569 from Sangihe Islands, Indonesia) afforded a crude methanol extract (51.2 g). It was partitioned between aq. methanol (50%) and CH_2Cl_2 and the aq. methanol fraction (4.1 g) of which 2.3 g was purified by Sephadex LH20 chromatography (100% MeOH) followed by normal phase HPLC ($CHCl_3$/MeOH/NH_4OH 60:40:1) to give pure halicyclamine B (29.1 mg). The molecular formula of $C_{26}H_{42}N_2$ was established using mass spectrometry including both HRFABMS data in which an intense $[MH]^+$ at 383.3426 (Δ 0.0 mmu of calcd.) was observed along with the LRESIMS which included a peak at *m/z* 383.4 $[MH]^+$ and a base peak at *m/z* 192.2 $[M+2H]^{++}$. The APT MF $C_{26}H_{42}$ was calculated from ^{13}C NMR data (Table 1) where $17CH_2$, 8CH, and 1C were observed. Accounting for the seven degrees of unsaturation were three double bonds (δ 141.1 s, 132.4 d, 131.6 d, 131.4 d, 128.1 d, 120.7 d) which meant that four rings must be present containing two trisubstituted nitrogen atoms. The six sp^3 methylene carbons attached to N were observed from δ 48 - 58 (Table 1). The ^1H-^1H COSY NMR correlations to each of four vinyl protons supported substructures **A** and **A'**. A combination of ^1H-^1H COSY and HMBC correlations beginning with the vinyl CH2 (δ 120.7/5.81) justified substructure **B**. The specific steps in reaching this conclusion involved defining the first six-membered ring using HMBC correlations from δ 120.7 (C2) to δ 2.54 (H4), 2.40 (H4'), 3.15 (H27), and 1.75 (H15), HMBC correlations from δ 57.7 (C6) to 3.15 (H27) and 2.59 (H27'), and ^1H-



^{1}H COSY correlations from δ 5.81 (H2) to δ 2.20 (H3) and from δ 2.20 (H3) to δ 2.54 (H4) and δ 2.40 (H4'). Similar evidence for the second six membered ring was amassed from HMBC correlations from δ 49.5 (C17) to δ 1.90 (H16), to δ 1.75 (H16'), to 2.68 (H28), to δ 1.98 (H28') and to δ 1.75 (H15), and ^{1}H-^{1}H COSY correlations from δ 1.80 (H14) to δ 2.68 (H28) and to δ 1.98 (H28'). The only logical attachment position for the $CH_2$19 (δ 53.5) was to *N*18. The HMBC correlation from δ 141.1 (C1) to δ 1.75 (H15) allowed these two rings to be connected by the C1-C15.

The process of joining the remaining two undefined CH_2 groups to substrutures **A**, **A'**, **B** proved to be quite difficult. An HMQC-TOCSY NMR spectra was obtained at 500 MHz (Table 1) but the severe overlap of aliphatic resonances compromised the value of this data. The problem was that a broad multiplet at δ 2.2 consisted of nine protons, a broad multiplet at δ 1.5 contained six protons and complex multiplets between δ 1.9 - 2.1 had nine protons. Likewise, using NMR data it was impossible to definitively choose between the various possibilities for forming the two additional rings by interlacing the five substructural units defined

Table 1. NMR data ($CDCl_3$) of halicyclamine B (**1**) at 500/125 MHz.

Atom #	^{13}C	^{1}H (δ, mult., *J*, Hz)	COSY	HMBC	HMQC-TOCSY
1	141.1 s			H16-16',H27-27',H15	
2	120.7 d	5.81 bs	H3, H27-27', H15	H4, H27, H15	
3	35.0 t	2.20 m	H4-4', H2	H4', H2	H2, H4-4'
4	57.1 t	2.54 dd, *J*=11, 5.5	H3	H27-27', H2	H3
		2.40 dd, *J=11,4.0*	H3		
6	57.7 t	2.80 ddd, *J*=13.9, 9.5,4.5	H26-26'	H27-27'	H7-7'
		2.25 m	H26-26'		
7	22.9 t	1.59 m		H8	H6-6', H8
		1.44 m			
8	28.4 t	1.50 m	H9-9'	H9-9'	H10, H7-7', H9-9', H6-6'
9	25.2 t	2.10 m	H10,H8		H10, H11, H8, H7-7'
		2.21 m			
10	131.4 d	5.29 m	H11, H9-9'	H12, H7-7', H13-13', H11	H11, H9-9'
11	131.6 d	5.29 m	H10, H12-12'	H12, H13-13', H7, H10	H13-13', H10, H9-9'
12	24.9 t	2.20 m	H13-13', H11	H13-13', H10, H11	H10, H11, H13-13'
		2.10 m			
13	34.3 t	1.29 m	H14, H12-12'	H14, H15, H12	H11, H14, H28-28', H12-12'
14	36.8 d	1.80 m	H28-28', H13-13'	H15, H13-13', H16-16', H28	H28-28', H15
15	40.0 d	1.75 m	H3	H13-13', H2, H16-16', H28, H27	H14, H16-16', H17
16	22.5 t	1.90 m	H17-17', H14	H17-17', H14, H15	H17-17', H15
		1.75 m	H16-16'		
17	49.5 t	3.09 ddd, *J*=9.5, 8.0, 1.0	H16-16'	H28-28', H16-16', H15	H15, H16-16'
		2.25 m	H16		
19	53.5 t	2.45 m	H20-20'		H20-20'
20	25.4 t	1.49 m	H21-21', H19		H21-21', H22-22', H19
21	25.9 t	1.60 m		H20, H22	H23, H24
		1.30 m	H20-20'		
22	26.1 t	2.10 m	H23	H23, H24, H21	H23, H24
		1.90 m			
23	128.1 d	5.44 m	H24, H22-22'	H22-22', H25-25'	H24
24	132.4 d	5.38 m	H25-25', H23	H25-25'	H23
25	24.6 t	2.00 m	H24, H26-26'	H24, H23	
		2.22 m	H24, H26-26'		
26	36.1 t	1.61 m	H6-6'	H25-25', H4-4', H24-24'	H25-25', H3, H24
		1.45 m	H6-6'		
27	54.7 t	3.15 d, *J*=15.5	H2	H15, H4-4', H6-6', H2	H2
		2.56 d, *J*=15.5	H2		
28	55.4 t	2.68 dd, *J*=11.0, 6.0	H14	H17, H14	H13-13', H14, H15, H16'
		1.98 m	H14		

above. Fortuitously, very small, clear crystals formed during the slow evaporation of a methanol solution of (-) **1** and these were immediately subjected to X-ray crystallography. Eventually, a refined structure was obtained to firmly justify the atom arrangements and relative stereochemical features shown in **1**. Interestingly, the X-ray structure[8] (Figure 1) shows the six-membered rings lie in a perpendicular arrangement relative to the two loops defined by the methylene groups. NOESY data was obtained showing that the conformation of **1** in solution and in the crystalline state are parallel.

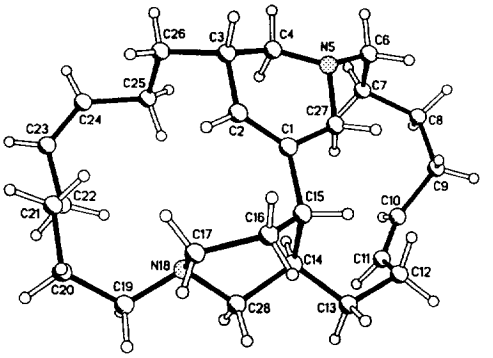
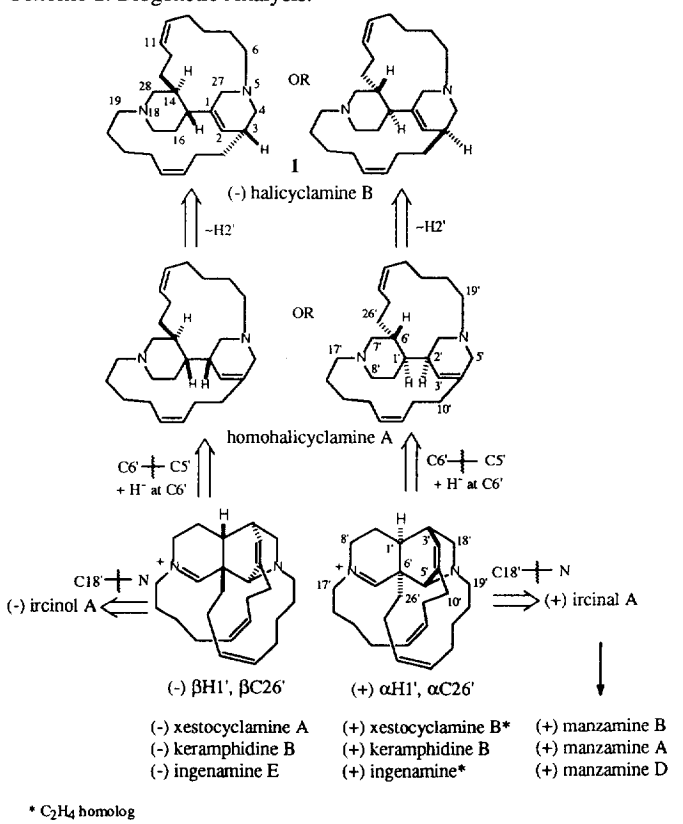


Figure 1. Computer Generated Drawing of X-ray Model of **1**.

With the structure firmly established our attention shifted to first evaluation of the biological activity properties and then to an analysis of the biogenetic pathway responsible for the formation of **1**. Extremely weak but selective antimicrobial activity was observed for halicyclamine B (**1**) as it showed growth inhibition of 20% and 50% at 200 μg/disk against *E. coli* and *B. subtilis*, respectively. No effect was seen against *Candida albicans*. Moderate solid tumor selective cytotoxicity was observed for **1** in the Valeriote-Corbett[9] model anticancer *in vitro* screen in that solutions of 600 μg/disk exhibited 170 and 200 zone units, respectively, in the murine mouse tumor lines (PO3, M17) with L1210 as the reference point.

Scheme 1. Biogenetic Analysis.[11]



We previously outlined[7] how halicyclamine A fits into the biosynthetic cascade proposed by Baldwin.[1] Ammonia, acrolein and dialdehyde (two equivalents each) supposedly condense, rearrange and eventually form the manzamine framework. Different insights, however, may be derived from biomimetic synthetic approaches executed by Marazano and Das to halicyclamine A.[10] The later steps of the Baldwin type pathway seem appropriate for **1**, but there are some interesting new considerations. Shown in Scheme 1[11] is that **1** contains the same two OHC-$(CH_2)_8$CHO moieties present in many other compounds, including the ten manzamines reported to date; the (+) ircinals, the (-) ircinols, (-) xestocyclamine A, and the (+) and (-) keramphidin[2] B. Recently,

Kobayashi[6c] offered comments about an approximate relationship between the absolute stereochemistry at C1'-C6' and the *d/l* optical rotation for manzamine analogs (Scheme 1) in that *d* correlates to 1'α, 6'α and *l* correlates to 1'β, 6'β.[11b] The C1', C6' stereochemistry has been unambiguously defined for (+) manzamine A, (+) B, (+) D, (+) ircinal A and (-) ircinol A.[2,12] Interestingly, the latter two have enantiomeric configurations. Also, the optical antipods have been obtained for keramphidin B. We envision that halicyclamine B arises from a hypothetical analog of halicyclamine A[7] (shown in Scheme 1 as homohalicyclamine A) via a 1,3 sigmatropic shift. Thus, in view of the preceding discussion, it would appear that the halicyclicamines are related to one or the other of the xestocyclamine optical forms (shown in Scheme 1). However, additional data must be obtained to specify the absolute nature of this relationship.

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8. Optical rotation of compound **1** $[\alpha]_D$ = -143.5 (*c* = .63). Crystals of **1** are monoclinic ($P2_1$) with lattice parameters *a* = 6.005(1), *b* = 21.812(3), *c* = 9.085(1) Å, β = 103.86(10)°; Z = 2. Intensities were measured with θ/2θ scans from a crystal of size 0.40 x 0.20 x 0.15 mm. The strucutre was determined by direct methods and refined on F^2 by full-matrix least -squares method from 1510 unique reflections ($2\theta_{max}$ = 112°, R_{int} = 0.022). Isotropic hydrogen atoms were refined with a riding model, all other atoms were assigned anisotropic displacement parameters. The final conventional R = 4.90% on F values of 1433 reflections having $F_o > 4\sigma(F_o)$. Coordinates have been deposited.
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11. (a) The αH1' and αC26' stereochemistry shown in Scheme 1 has been implied for the following: (+) manzamine J: Ref. 6c; 6-hydroxymanzamine A and (+) 3,4,-dihydromanzamine A: Kobayashi, J.; Tsuda, M.; Kawasaki, N. *J. Nat. Prod.* **1994**, 57, 1737-1740. (b) The same pattern of *d,l* rotation *vs* H1' and C26' stereochemistry in Scheme 1 has been assumed to apply to the (+) and (-) forms of keramphidin B,[6c] respectively. If this is true then the (+) xestocyclamine B[6a] = (+) ingenamine[6b] probably have opposite C1',C6' (Scheme 1) configurations *vs.* (-) xestocyclamine A.[6a] (c) The stereochemistry assigned to the various (+) and (-) forms of ingenamines B - D[6b] does not appear to fit this preceeding relationship.
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