A Tetracyclic Diamine Alkaloid, Halicyclamine A, from the Marine Sponge *Haliclona* sp[1]

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Summary: Halicyclamine A, a tetracyclic, pentaunsaturated diamine alkaloid, was isolated from the marine sponge *Haliclona* sp. and its structure was determined by NMR and mass spectrometry. A suggestion is made as to its biogenetic origin.

Structurally diverse, but related, polycyclic alkaloids with two heterocyclic nitrogens and the absence of aliphatic methyls have been obtained from Haplosclerid sponges.[2,3] In both 1992[4] and 1993[2h] papers appeared clarifying the origin of some of their complex frameworks. The structure elucidation of many of these alkaloids has been extremely challenging. One major impediment is that slightly impure samples display extremely broad ^{1}H NMR resonances which are replaced by sharp but severely overlapping signals for purified compounds. The few crystalline members of this class have been effectively analyzed by X-ray crystallography,[2d,e,g] whereas structural work on many of the remaining compounds was arduous and required the extensive use of exotic NMR experiments.[2c,f,h,i,3] Described in this report is halicyclamine A, a new tetracyclic alkaloid which appears to be biogenetically related to other diamine metabolites of Haplosclerid sponges.

Work was begun on the crude extract of a soft, olive green, massive, tubular sponge, *Haliclona* sp.,[5] collected from Biak, Indonesia, since it showed activity against the enzyme target inosine monophosphate dehydrogenase (IMPDH)[6] at 1 μg/mL inhibition. Both the crude extract and solvent partition fractions contained halicyclamine A (1) accompanied by many homologous alkaloids. Eventually we discovered a successful purification strategy to yield 1 which began with a standard alkaloid extraction procedure followed by silica chromatography (MeOH/Et_3N fraction) and normal-phase HPLC (EtOAc:Et_3N 95:5).

The tasks of molecular formula and substructure analyses were begun in parallel. An intense HREIMS M^+ = 462.3975 supported the proposed molecular formula of $C_{32}H_{50}N_2$ (Δ 0.1 mmu of that calculated). Five double bonds were clearly visible by NMR so the additional unsaturations were ascribed to four rings[7] (Figures S1 and S2, supplementary material). The environment of the nitrogens, as two tertiary amines, was established by the correspondence between the APT formula $C_{32}H_{50}$ and the HREIMS molecular formula. The anchor point for further substructure analysis was the well-resolved ^{1}H NMR double bond resonances between δ 5.20 and δ 6.50. The ^{1}H—^{1}H COSY NMR spectra (Figure S3, supplementary material) justified the connectivities between C7—C12 and between C24—C29, respectively, with the double bond geometries based on the vicinal vinylic proton coupling constants. Expanding these respective fragments to those of substructures **A** and **B** was facilitated by C/H assignments made from an HMQC–TOCSY[8] NMR spectrum obtained with a mixing time of 0.05 s (Figure S5, supplementary material). Important correlations included those from C11 to H12/12′ and H13/13′, from C14 to H12/12′, from C28 to H29/29′, H30/30′, and $H_2$31, and from C32 to H30′ and $H_2$31. These enabled important new proton assignments to be unambiguously added to the

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(1) Novel Marine Sponge Alkaloids. 6. Part 5: ref 2h.

(2) The eight alkaloid types from Haplosclerida order sponges possessing these features are as follows: (a) The haliclamines (from *Haliclona* sp., family Chalinidae): Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hirota, H. *Tetrahedron Lett.* **1989**, *30*, 6891–6894. (b) The halitoxins (from *Haliclona rubens*, family Chalinidae, and *Callyspongia fibrosa*, family Callyspongiidae): Schmitz, F. J.; Hollenbeak, K. H.; Campbell, D. C. *J. Org. Chem.* **1978**, *43*, 3916–3922. Davies-Coleman, M. T.; Faulkner, D. J.; Dubowchik, G. M.; Roth, G. P.; Polson, C.; Fairchild, C. *J. Org. Chem.* **1993**, *58*, 5925–5930. (c) The saraines (from *Reniera sarai*, family Chalinidae): Cimino, G.; Mattia, C. A.; Mazzarella, L.; Puliti, R.; Scognamiglio, G.; Spinella, A.; Trivellone, E. *Tetrahedron* **1989**, *45*, 3863–3872. Cimino, G.; Fontana, A.; Madaio, A.; Scognamiglio, G.; Trivellone, E. *Magn. Reson. Chem.* **1991**, *29*, 327–322. (d) Petrosin (from *Petrosia seriata*, family Petrosiidae): Braekman, J. C.; Daloze, D.; Macedo de Abreu, P.; Piccinni-Leopardi, C.; Germain, G.; van Meerssche, M. *Tetrahedron Lett.* **1982**, *23*, 4277–4280. (e) The xestospongins (from *Xestospongia exigua*, family Petrosiidae): Nakagawa, N.; Endo, M.; Tanaka, N.; Gen-Pei, L. *Tetrahedron Lett.* **1984**, *25*, 3227–3230. (f) The papuamines (from *Haliclona* sp., family Chalinidae): Baker, B. J.; Scheuer, P. J.; Shoolery, J. N. *J.Am. Chem. Soc.* **1988**, *110*, 965–966. Fahy, E.; Molinski, T. F.; Harper, M. K.; Sullivan, B. W.; Faulkner, D. J. *Tetrahedron Lett.* **1988**, *29*, 3427–3428. (g) The manzamines = keramamine (from *Xestospongia* sp., family Petrosiidae, and *Pellina* sp., family Oceanapiidae): Ichiba, T.; Sakai, R.; Kohmoto, S.; Saucy, G.; Higa, T. *Tetrahedron Lett.* **1988**, *29*, 3083–3086. (h) Xestocyclamine A (from *Xestospongia* sp., family Petrosiidae): Rodríguez, J.; Peters, B. M.; Kurz, L.; Schatzman, R. C.; McCarley, D.; Lou, L.; Crews, P. *J. Am. Chem. Soc.* **1993**, *115*, 10436–10437. For revised structure see: Rodríguez, J.; Crews, P. *Tetrahedron Lett.*, in press. (i) Ingenamine (from *Xestospongia ingens*, family Petrosiidae) Kong, F.; Andersen, R. J.; Allen, T. M. *Tetrahedron Lett.* **1994**, *35*, 1643–1646.

(3) The manzamines plus a ninth alkaloid type of this group, the ircinals, come from a Dictyoceratida order sponge (*Ircinia* sp., family Thorectidae): Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J.Org. Chem.* **1992**, *57*, 2480–2483.

(4) Baldwin, J. E.; Whitehead, R. C. *Tetrahedron Lett.* **1992**, *33*, 2059–2062.

(5) An underwater photograph and a taxonomic description of this sponge is included as plate 1 in the supplementary material, and its identification (coll. no. 92510) as *Haliclona* sp. (order *Haplosclerida*, family *Haliclonidae*) is based on the characteristics described.

(6) For a discussion of the assay see: Jaspars, M.; Rali, T.; Laney, M.; Schatzman, R. C.; Diaz, M. C.; Schmitz, F. J.; Pordesimo, E. O.; Crews, P. *Tetrahedron* **1994**, in press.

(7) Halicyclamine (1): $[\alpha]_D$ −7.3 (*c* 0.72, CH_2Cl_2); IR (film) ν_{max}(cm^{-1}) 2919, 2848, 1355, 1264; LREIMS, positive ion, *m/z* (relative intensity) 462 (M^+, 85), 447 (24), 244 (57), 231 (17), 216 (26), 164 (24), 136 (24), 79 (79); HREIMS M^+ 462.3975 = $C_{32}H_{50}N_2$ (Δ 0.1 mmu of calcd), fragment ions 244.2069 = $C_{17}H_{26}N$ (Δ 0.4 mmu of calcd), 216.1760 = $C_{15}H_{22}N$ (Δ 0.7 mmu of calcd), 164.1447 = $C_{11}H_{18}N$ (Δ 0.7 mmu of calcd), 136.1134 = $C_9H_{14}N$ (Δ 0.8 mmu of calcd); HMQC data 125/500 MHz ($CDCl_3$) δ 134.7 s (C1); 132.8 d, 5.36 m (CH11); 131.6 d, 5.62 dt, *J* = 15.0, 7.0 Hz (CH25); 131.6 d, 5.51 dt, *J* = 9.5, 5.5 Hz (CH8); 129.8 d, 5.29 dt, *J* = 10.5, 5.5 Hz (CH28); 128.8 d, 5.89 t, *J* = 11.0 Hz (CH27); 127.4 d, 6.46 dd, *J* = 14.5, 11.5 Hz (CH26); 124.2 d, 6.32 t, *J* = 11.0 Hz (CH10); 123.6 d, 6.17 t, *J* = 11.0 Hz (CH9); 119.4 d, 5.38 s (CH2); 56.9 t, 2.70 m/1.72 m ($CH_2$34); 55.8 t, 3.05 dt, *J* = 14.5, 5.0 Hz/2.99 m ($CH_2$6); 55.5 t, 2.72 d, *J* = 11.0 Hz/2.62 d, *J* = 11.0 Hz ($CH_2$4); 54.1 t, 2.63 d, *J* = 10.5 Hz/2.36 m ($CH_2$23); 53.8 t, 2.73 d, *J* = 10.5 Hz/2.36 m ($CH_2$21); 53.5 t, 3.16 d, *J* = 15.5 Hz/2.75 d, *J* = 15.5 Hz ($CH_2$33); 43.2 d, 2.24 m (CH3); 40.9 d, 1.03 m (CH19); 35.7 d, 1.42 m (CH18); 32.9 t, 1.25 m/0.71 m ($CH_2$17); 32.7 t, 2.14 m/1.74 m ($CH_2$32); 32.2 t, 2.14 m ($CH_2$24); 31.7 t, 1.67 m/1.43 m ($CH_2$20); 28.9 t, 1.09 m ($CH_2$15); 28.7 t, 1.32 m/1.17 m ($CH_2$30); 28.4 t, 2.36 m/1.87 m ($CH_2$29); 28.0 t, 1.42 m/1.32 m ($CH_2$13); 26.4 t, 1.11 m ($CH_2$14); 26.2 t, 1.11 m/0.89 m ($CH_2$16); 26.0 t, 2.23 m/2.03 m ($CH_2$12); 25.6 t, 1.53 m ($CH_2$31); 25.2 t, 2.72 m/2.10 m ($CH_2$7). For additional NMR data see Figures S1–S5 (supplementary material).

(8) Lerner, L; Bax, A. *J. Magn. Reson.* **1986**, *69*, 375–380.

0022-3263/94/1959-3253$04.50/0

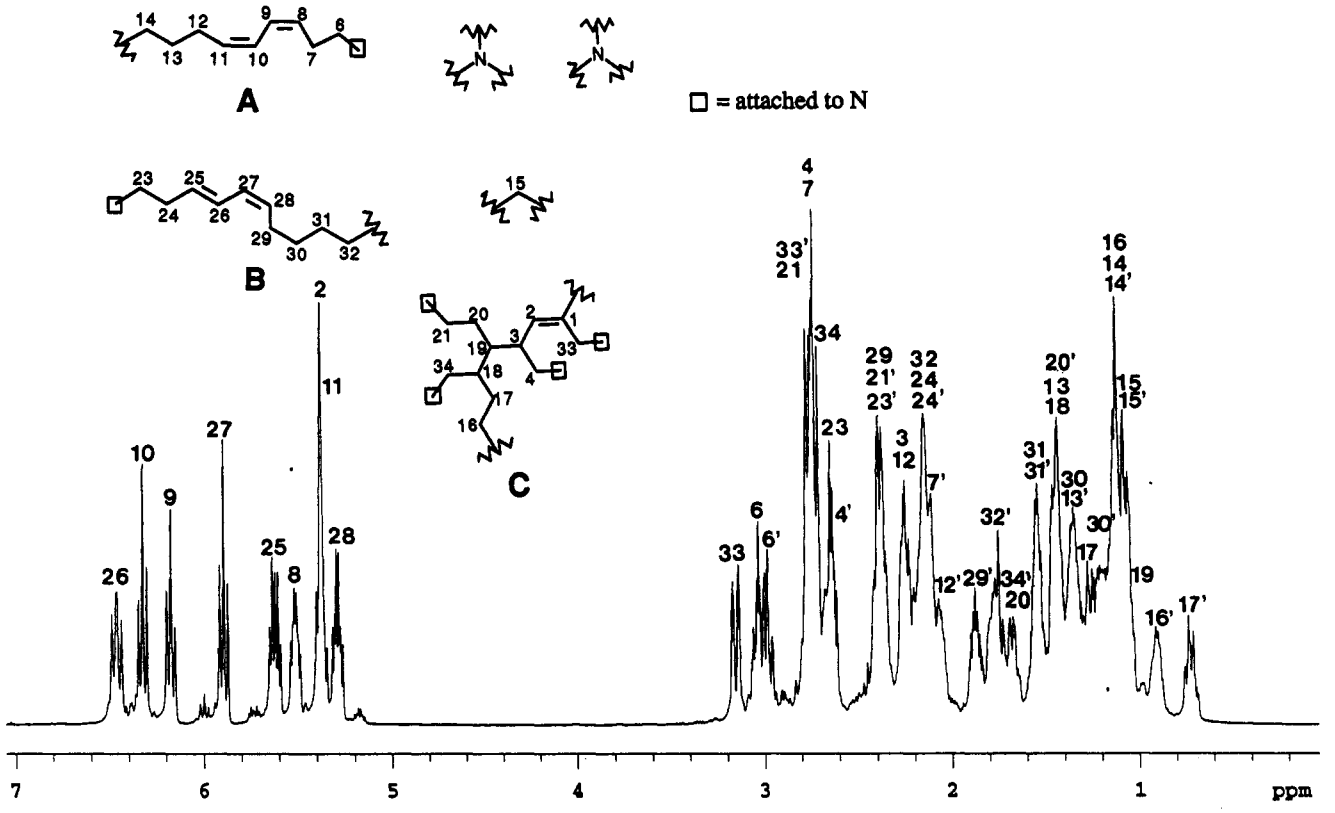


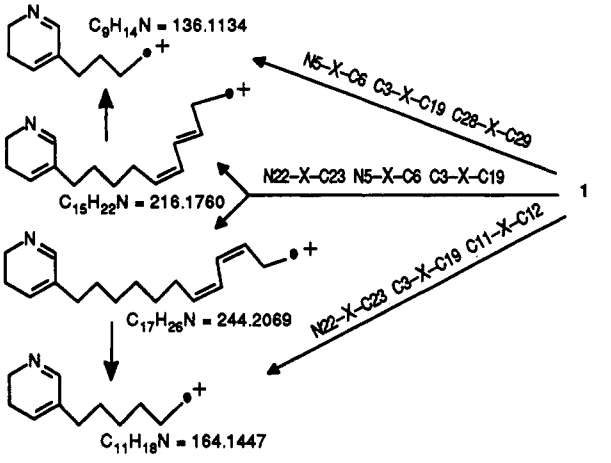
Figure 1. ^1H NMR of halicyclamine (1) in $CDCl_3$ at 500 MHz.

^1H–^1H COSY NMR spectra which then unveiled assignments in support of subunit **A**, via correlations from H12—H13′ and H7′ to H6/6′ with C6 being attached to nitrogen, and subunit **B**, via correlations from H29′—H30 and from $H_2$31 to H32 and also a correlation from nitrogen bearing C23 to $H_2$24 being corroborated both by a correlation from C24 to H23′ and an HMBC (Figure S4, supplementary material) correlation from C24 to H23/23′.

The analysis of substructure **C** began by observing unequivocal ^1H–^1H COSY correlations between proton sets H21′—H20, H21—H20′, H20/20″—H19, H16′—H17′, H18–H17′. When this information was combined with HMBC correlations from C18 to H34/34′, H19/19′, and H20/20′, it defined the entire C16–C21/C34 subunit. Nitrogen-bearing C33 showed a low-field diastereotopic AB pattern (δ 3.16, 2.75; J = 15.5 Hz) indicating it was connected to the sole quaternary carbon, C1. This was confirmed by observing an HMBC correlation from C1 to H33/33′. ^1H–^1H COSY correlations from H3 to H4/4′and H33, and from H2 to H33/33′ when combined with HMBC correlations from C2 to H19, H3, H4, and H33/33′, completed subunit **C**. Remaining unassigned at this juncture was $CH_2$15.

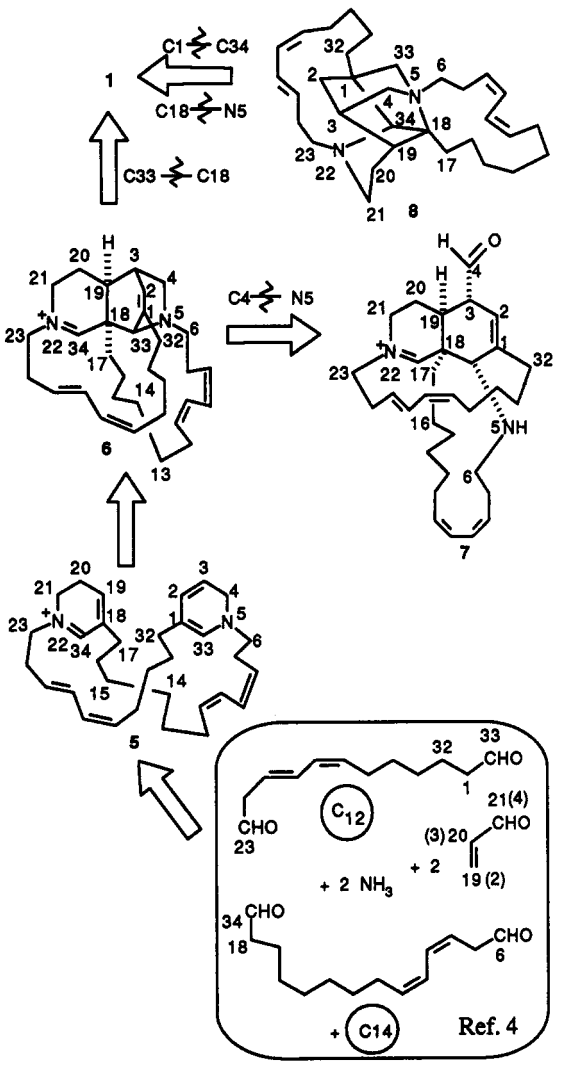
Merging of the substructures was accomplished next. The HMBC NMR correlations from C1 to H32/32′ and from C2 to H32/32′ justified linking substructures **B** and **C**. It was then clear that the unconnected aliphatic ends of **A** and **B/C** must be joined by the remaining $CH_2$15 group. This could be verified by the MS fragmentation pattern, which shows two major fragments at m/z 244.2069 = $C_{17}H_{26}N$ (Δ 0.4 mmu of calculated), attributed to C6—C21/C34/N22 and m/z 216.1760 = $C_{15}H_{22}N$ (Δ 0.7 mmu of calculated), attributed to C23—C33/C1—C4/N5 (Scheme 1). Smaller diagnostic mass fragment ions could also be observed as outlined in Scheme 1.

Scheme 1. Analysis of HREIMS Fragments



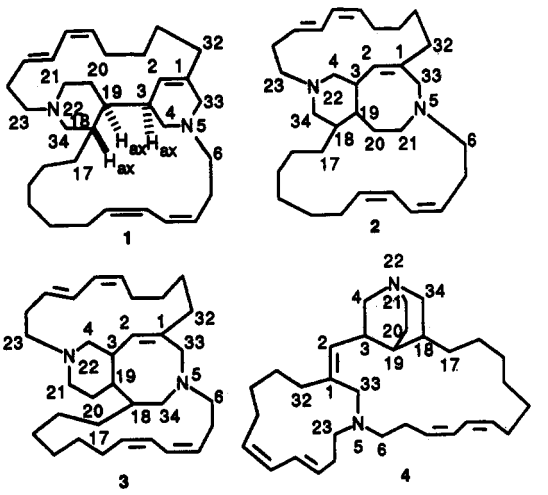
Determining the exact disposition of the groups about the tertiary amine nitrogens was not straightforward. Unfortunately, only one useful ^1H–^1H COSY long-range correlation, between H6 and H33′, could be observed. The four alternatives which now remained for the CH_2 attached to N5 were C4, C21, C23, or C34, and structures 1–4 represent the possibility for each case. Eventually we observed diagnostic HMBC data which favored structure 1, and these included correlations between atom sets C33—H6/6′, C4—H6/6′, and C4—H33/33′. A final important HMBC correlation was that from C21 to H34′. The structural description of 1 was concluded by using 2D NMR J-resolved data to establish the relative orientation between H18 and H19. A coupling constant of 0 Hz between H2 and H3 showed H3 to be axial. The relative stereochemistry between H3 and H19 was assigned based on the biogenetic argument discussed below. A coupling constant of 7.5 Hz between $H34_{ax}$ and H18 established

Scheme 2. Biosynthetic Analysis



H18 as axial and the $J_{H18-H19}$ = 8.0 Hz suggested H18 and H19 to be in a diaxial conformation. The observed coupling constants closely matched those found by molecular modeling using the MMX forcefield in PCMOD 4.0 with H18 and H19 diaxial (calcd $J_{H34ax-H18}$ = 8.0 Hz, $J_{H18-H19}$ = 5.7 Hz), and these calculated J's were different for the other diastereomers.

Halicyclamine (1) appears to be closely related to a hypothetical haliclamine[2a,4] such as **5**. However, there is no easy way to explain the biogenetic cleavage of the C3 to C19 bond in **1** which is needed to set up this relationship. A more plausable pathway, incorporating principles proposed to rationalize the biosyntheses of the manzamines[4] and xestocyclamine A,[2h] involves the reductive



coupling of C_{12} and C_{14} dialdehydes with two acroleins and two ammonias to give **5** (Scheme 2). A subsequent Diels–Alder cyclization of **5** leads to the xestocyclamine/ingenamine[2h,i] type structure **6** which has a cis relationship between H3 and H19. A fragmentation to cause scission of the C18—C33 bond in **6** and formation of a C18 —C34 double bond gives an intermediate structure which, after biosynthetic reduction, yields halicyclamine A (**1**). Also, an oxidative cleavage of C4 —N5 in **6** would give the manzamines' and ircinals' bicyclic [4.4.0] core as in **7**. Cleavage of the C1—C34 and C18—N5 bonds in a hexacyclic saraine A–C-type structure **8** again gives halicyclamine A. The isolation of **1** significantly expands the scope of the biosynthetic processes which generate both the manzamines and xestocyclamine. In addition, we believe pentacyclic structures such as **6** are central to the biosynthesis of other members of this class including saraines 1–3, the petrosins, and the xestospongins. We will outline this along with the additional structures and bioactivity properties of the halicyclamines in a future paper.

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Supplementary Material Available: Plate 1 plus Figures S1–S5 (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.