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Ingenamine, A Novel Pentacyclic Alkaloid From the Marine Sponge *Xestospongia ingens*

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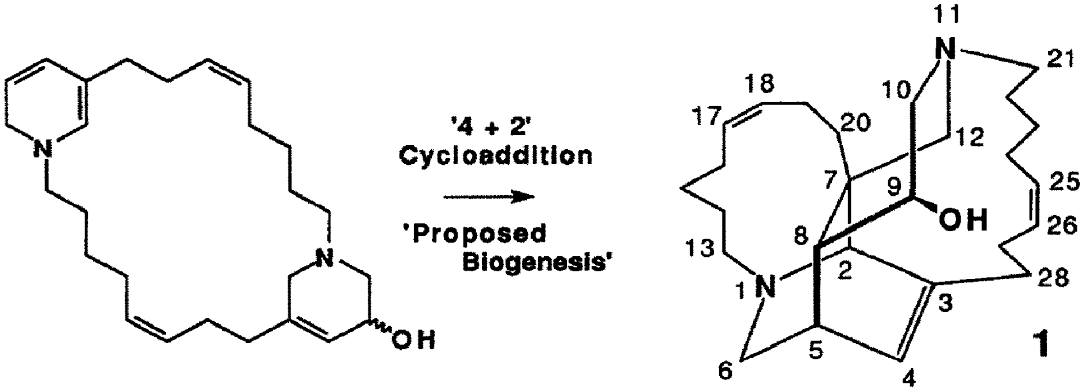
Abstract: Ingenamine (1), a novel cytotoxic pentacyclic alkaloid, has been isolated from the marine sponge *Xestospongia ingens* collected in Papua New Guinea. The structure of ingenamine has been solved by extensive spectrosopic analysis.

Marine sponges have been the source of an interesting group of nitrogenous metabolites that appear to be biosynthetically derived from 3-alkylpyridine or reduced 3-alkylpyridine units. The halitoxins[1] and a recently reported epidermal growth factor active constitutent from *Callyspongia fibrosa*[2] are high molecular weight cyclic oligomers (n>4) of 3-alkylpyridine monomers. It has been suggested that the haliclamines,[3] the sarains,[4] the xestospongins,[5] and the petrosins[6] are all biogenetically derived from macrocyclic dimers of fully or partially reduced 3-alkylpyridine monomers.[7] Baldwin and Whitehead[8] recently put forward a very elegant and insightful proposal which suggested that the manzamines[9] are also biogenetically derived from a *bis*-3-alkyldihydropyridine macrocycle. Interestingly, the Baldwin proposal suggested the possible occurrence of two new classes of alkaloids. Ircinal B,[10] subsequently isolated from the sponge *Ircinia* sp., represented the first example of one of the new classes of alkaloids predicted by the Baldwin proposal. As part of an ongoing search for biologically active metabolites in tropical marine sponges,[11] it was found that extracts of *Xestospongia ingens* (Thiele, 1989)[12] collected in Papua New Guinea showed in vitro cytotoxicity against murine leukemia P388. Bioassay guided fractionation of the extract led to the isolation of ingenamine (1), a novel cytotoxic pentacyclic alkaloid. Ingenamine (1) represents the first example of the second new class of alkaloids foreseen by the Baldwin proposal.[8]

Specimens of *X. ingens*, collected by hand using SCUBA on reefs (-15 to -20m) near Sek Point off Madang, Papua New Guinea, were frozen on site and transported to Vancouver over dry ice. Freshly thawed sponge was extracted exhaustively with methanol. Repeated fractionation of the methanol extract via Sephadex LH20 chromatography (EtOAc/MeOH/H_2O 40:10:4) gave pure ingenamine (1) (20 mg from 200 g wet wt. of sponge) as a white optically active ($[\alpha]_D$ +62°; MeOH, c 0.14) amorphous solid. Ingenamine (1) gave an intense ($M^+ + H$) ion in the CIHRMS at m/z 397.32058 appropriate for a molecular formula of $C_{26}H_{40}N_2O$ (ΔM 3.30ppm) requiring eight sites of unsaturation. The ^{13}C NMR spectrum of 1 (Table 1), which showed well resolved resonances for all twenty six carbon atoms (APT: 2 X C; 9 X CH; 15 X CH_2), contained six deshielded resonances that could be assigned to olefinic carbons. No additional unsaturated functional groups were apparent from the ^{13}C NMR data, indicating that ingenamine (1) was pentacyclic. A band at 3398 cm^{-1} in the IR spectrum and NMR resonances at δ 66.1 (CHOH: C9) and 3.35 (CHOH) ppm were assigned to a secondary alcohol, which accounted for the one proton not attached to carbon.



1

A $C_9H_{12}O$ fragment, that included C2 to C6 and C8 to C10 and contained the secondary alcohol functionality and a trisubstituted alkene, was routinely identified from the COSY, HMQC, and HMBC data (Table 1) collected for 1. COSY correlations were observed between H4 (δ 6.01) and H5 (δ 2.75), H8, (δ 0.87), H2 (δ 3.30), H28 (δ 2.41) and H28' (δ 2.36); between H5 (δ 2.75) and H6 (δ 2.89), H6' (δ 1.76) and H8 (δ 0.87); between H8 (δ 0.87) and H9 (δ 3.35); and between H9 (δ 3.35) and H10 (δ 3.00) and H10' (δ 2.92). HMBC correlations were observed between H4 (δ 6.01) and C2 (δ 63.8), C5 (δ 34.8), C8 (δ 51.6) and C28 (δ 37.2); between H5 (δ 2.75) and C3 (δ 143.0); between H6 (δ 2.89) and C4 (δ 125.8), C5 (δ 34.8) and C8 (δ 51.6); between H8 (δ 0.87) and C5 (δ 34.8); between H9 (δ 3.35) and C5 (δ 34.8); and between H10 (δ 3.00) and C8 (δ 51.6).The aliphatic quaternary carbon at δ 45.2 (C7), that was indicated by the APT experiment, could be positioned between C2 and C8 on this fragment by a series of HMBC correlations. Thus, H8 (δ 0.87) and H2 (δ 3.30) both showed two bond HMBC correlations to δ 45.2 (C7). In addition, H2 (δ 3.30) was correlated to C8 (δ 51.6) via a three bond coupling. A Network of HMBC correlations identified C12 and C20 as the remaining two substituents attached to the quaternary carbon. A pair of geminal methylene protons at δ 3.10 (H12) and 2.22 (H12') that were attached to a carbon at δ 50.8 (C12) showed two bond HMBC correlations to the quaternary carbon (δ 45.2: C7) and three bond correlations to a methylene carbon at δ 41.6 (C20). In addition, H12 (δ 3.10) showed a three bond correlation to C8 (δ 51.6) and H12' (δ2.22) showed a three bond correlation to C2 (δ 63.8). The protons (δ 1.93 and 1.71: H20, H20') attached to the methylene carbon at δ 41.6 (C20) in turn showed two bond HMBC correlations to the quaternary carbon (δ 45.2: C7). Furthermore, H20 (δ1.93) showed a three bond correlation to C12 (δ 50.8), H20' (δ 1.71) showed a three bond correlation to C2 (δ 63.8), and H8 (δ 0.87) showed a three bond correlation to δ 41.6 (C20).

Since the single proton in 1 not attached to carbon could be assigned to an alcohol, it was apparent that the two nitrogen atoms were not directly bonded to hydrogen atoms. COSY and HMBC data provided evidence

Table 1: NMR data for ingenamine (1). Recorded in MeOH-d_4 at 500 MHz (^{1}H).

Carbon no.	δ ^{13}C	δ ^{1}H	^{a} COSY Correlations	^{b} HMBC Correlations	^{c} NOEs
2	63.8	3.30, bs	H4	H28, H28’ H4, H6, H12’, H20’, H13	H13, H13’, H28, H28’
3	143.0			H28, H28’, H2, H5	
4	125.8	6.01, d(6.4)	H2, H8,H5, H28, H28’	H28, H28’, H8, H2, H6, H5	H5, H28
5	34.8	2.75, m	H4, H8, H6, H6’	H6’, H9, H6, H4, H8	H8, H4, H6’
6	53.8	2.89, dd(9.3, 1.8)	H6’, H5	H13, H13’, H2	H6’, H8
6’		1.76, dd(9.3, 2.4)	H6, H5		H6, H13’, H4, H5
7	45.2			H2, H19, H12, H12’, H20, H20’, H5, H8	
8	51.6	0.87, dd(10.5, 1.8)	H9, H4, H5	H6’, H2, H12, H10, H20, H6, H4	H6, H5, H20, H20’, H10
9	66.1	3.35, ddd(10.5, 12.1, 4.8)	H10, H10’, H8	H10, H10’, H8	H4, H5, H12’
10	52.5	3.00, dd(12.1, 4.8)	H10’, H9	H12	H10’
10’		2.92, t(12.1)	H10, H9		H10
12	50.8	3.10, d(12.3)	H12’	H20, H21’, H2	H12’
12’		2.22, bd(12.3)	H12		H12, H9
13	55.1	3.02, td(12.7, 4.9)	H13’, H14, H14’	H2, H6’	H13’, H2
13’		2.24, m	H13, H14, H14’		H13
14	26.9	1.53	H14’, H13, H13’, H15, H15’	H13	
14’		1.31	H14, H13, H13’, H15, H15’		
15	27.4	1.62	H15’ H14, H14’	H113’, H16	
15’		1.48	H15, H14, H14’, H16		
16	23.8	2.41	H16’, H17, H15’	H15, H18	
16’		1.62	H16, H17		
17	133.3	5.70, dt(10.3, 5.3)	H18, H16, H16’	H16, H16’, H19, H19’	
18	130.2	5.65, dt(10.3, 5.8)	H17, H19, H19’	H16, H16’, H19, H19’, H20	
19	21.2	2.40	H19’, H18, H20, H20’	H17, H18, H20, H20’	
19’		1.82, m	H19, H18, H20, H20’		
20	41.6	1.93	H20’,H19, H19’	H12, H12’, H19, H19’, H8	H8, H20’, H12
20’		1.71, m	H20, H19, H19’		H20, H8
21	56.7	3.49, m	H21’, H22, H22’	H12’, H10, H10’	
21’		2.88	H21, H22, H22’		
22	20.2	1.92	H22’, H21, H21’	H21, H21’	
22’		1.54	H22, H21, H21’		
23	26.04	1.53	H24, H24’	H25	
24	26.26	2.31	H24’, H23, H25	H22, H25, H26	
24’		2.07	H24, H23, H25		
25	132.6	5.32, bt(10.5)	H26, H24, H24’	H26, H27, H24	
26	133.7	5.45, bt(10.5)	H25, H27, H27’	H25, H28, H28’, H27	
27	26.3	2.37	H27’, H26	H28, H28’, H25, H26	
27’		2.15	H27, H26, H28		
28	37.2	2.41	H28’, H27’, H4	H27, H2, H4, H26	H2, H4
28’		2.36	H28, H4		

[a] Correlated to proton resonance in δ ^{1}H column. [b] Correlated to carbon resonance in δ ^{13}C column. [c] Observed as difference NOEs and as ROESY correlations. Resonance in δ ^{1}H column was irradiated in difference NOE experiments.

that both nitrogens were present as tertiary amines and that they were attached to the carbon atoms at C6/C2/C13 and C10/C12/C21, respectively. The COSY data (Table 1) suggested that the remaining substitutents on C2, C6, C10 and C12 had to be non-protonated atoms and the chemical shifts of the carbons (δ

C2, 63.8; C6, 53.8; C10, 52.5; C12, 50.8) were consistent with attachment to nitrogen. HMBC correlations between H2 (δ 3.30) and C6 (δ 53.8) and between H6 (δ 2.89) and C2 (δ 63.8) were consistent with a nitrogen bridge between C2 and C6. Further HMBC correlations between H6' (δ 1.76) and a methylene carbon at δ 55.1 (C13), and between one of the protons (δ 3.02: H13) attached to the carbon at δ 55.1 and C2 (δ 63.8) and C6 (δ 53.8) identified the methylene carbon (C13) as the third substituent of the teriary amine. Similarly, HMBC correlations between H12 (δ 3.10) and C10 (δ 52.5) provided evidence for a nitrogen bridge between C10 and C12, and correlations between a methylene carbon at δ 56.7 (C21) and H10, H10' (δ 3.00 and 2.92) and H12' (δ 2.22) identified this methylene carbon as the third substituent of the second tertiary amine. Location of the two tertiary amines completed the structure of the tricyclic central core of 1 complete with methylene appendages at N1, C3, C7, and N11. Difference NOEs observed between H9 (δ 3.35) and H12' (δ 2.22) showed that the C7 to C12 piperidine ring was in a boat conformation with H9 and H12' occupying flagpole orientations. Difference NOEs observed between H9 (δ 3.35) and H4 (δ 6.01), between H8 (δ 0.87) and H6 (δ 2.89), and between H8 and H20 (δ 1.93) required that the relative configurations at C2, C5, C7 and C8 be those shown in 1. A COSY correlation observed between the H4 (δ 6.01) and H8 (δ 0.87) resonances, that was attributed to W coupling, was consistent with the proposed relative configurations.

The remaining portion of 1 had to account for eight aliphatic methylene and four olefinic methine carbons. Detailed analyis of the COSY, HMQC and HMBC data (Table 1) showed that these atoms comprised two linear six carbon chains (C14 to C19 and C22 to C27), each containing an alkene. Furthermore, the NMR data revealed that the six carbon chains linked the C21 and C28, and the C13 and C20 methylene appendages, respectively, as shown in 1. Comparison of the NMR data for ingenamine (1) with that reported for the ircinals[10] and the haliclamines[3] confirmed the Z geometry for the $\Delta^{17,18}$ and $\Delta^{25,26}$ alkenes.

Ingenamine (1) represents a new class of cytotoxic alkaloid (in vitro murine leukemia P388: ED$_{50}$ = 1 µg/mL). Its biosynthesis can be formally envisaged to arise from an intramolecular '4 + 2' cycloaddition reaction as shown. The discovery of ingenamine (1) provides further support for Baldwin and Whitehead's proposal for the biosynthesis of the manzamines.[8]

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12. Identified by R. van Soest. A voucher sample (ZMA 10701) is deposited at the Zoologisch Museum, University of Amsterdam.

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