Madangamine A, a Novel Cytotoxic Alkaloid from the Marine Sponge *Xestospongia ingens*

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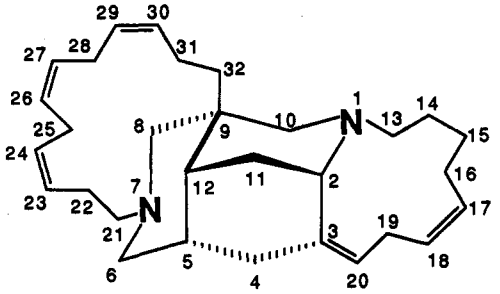
A growing number of complex polycyclic alkaloids, representing a wide variety of skeletal types, that are apparently related to each other through a common biogenetic origin from oligomeric macrocycles composed of 3-alkylpyridine or partially reduced 3-alkylpyridine monomers, have recently been isolated from marine sponges. Examples include the halitoxins,[1] the haliclamines,[2] the saraines 1-3,[3] the xestospongins,[4] the petrosins,[5] saraine A,[6] manzamine B,[7] ircinal B,[8] and xestocyclamine A.[9] The relationship of the haliclamines, the xestospongins, the petrosins, and the saraines to a putative bis-3-alkylpyridine biogenetic precursor was obvious from inspection of their structures;[10] however, the "provenance" of the manzamines was not as immediately apparent.[7] An elegant proposal put forth by Whitehead and Baldwin[11] suggested that the manzamines also arise from a bis-3-alkyldihydropyridine precursor. Furthermore, their proposal anticipated the occurrence of two new classes of alkaloids corresponding to the pentacyclic and tetracyclic intermediates in their biogenetic scheme. The subsequent discovery of ircinal B,[8] the first example of an alkaloid corresponding to the Whitehead and Baldwin tetracyclic intermediate, supported their proposed biogenesis of the manzamines. Recently, we reported the isolation of ingenamine[12] and the ingamines[13] from *Xestospongia ingens*.[14] Ingenamine represented the first example of an alkaloid corresponding to the Whitehead and Baldwin pentacyclic intermediate. Ongoing bioassay-guided investigations of *X. ingens* extracts have now resulted in the isolation of the novel cytotoxic alkaloid madangamine A (**1**). The structure of **1**, the first example of a new class of pentacyclic alkaloids that also appear to arise biogenetically from a partially reduced bis-3-alkylpyridine precursor, is reported below.



Madangamine A (1)

Fractionation of the hexanes-soluble fraction of the crude methanol extract of *X. ingens* by silica gel flash chromatography (gradient elution: EtOAc/hexanes 1:9 to EtOAc/hexanes 1:1) and normal-phase HPLC (EtOAc/hexanes/*i*-Pr_2NH_2 98.4:1.5:0.1) gave pure madangamine A (**1**).[15] A parent ion at *m/z* 432.3503 in the EIHRMS of **1** was appropriate for a molecular formula of $C_{30}H_{44}N_2$, requiring 10 sites of unsaturation. The ^{13}C/APT NMR spectrum of **1** (16 × CH_2, 12 × CH, 2 × C) showed that all 44 hydrogen atoms were attached to carbon and revealed that the molecule contained 10 sp^2 hybridized carbons, assigned to five olefins.

A detailed analysis of the COSY, HMQC, HMBC, and NOE data (see supplementary material) for **1** identified the constitution and relative configurations of the central tricyclic core (N1 to C12) and four additional carbons attached at N1, C3, N7, and C9. Thus, significant COSY and long-range COSY (LR) correlations were observed between H2 (δ 3.69) and $H11_{ax}$ (δ 2.40)/$H11_{eq}$ (δ 1.27); between $H11_{ax}$/$H11_{eq}$ and H12 (δ 1.14); between H12 and H5 (δ 1.70); between H5 and $H4_{ax}$ (δ 3.07), $H4_{eq}$ (δ 2.21), $H6_{ax}$ (δ 2.11, LR) and $H6_{eq}$ (δ 2.31, LR); between $H6_{eq}$ and $H8_{eq}$ (δ 2.72, LR); between H20 (δ 5.16) and $H4_{ax}$ (δ 3.07, LR); and between H32 (δ 1.00) and $H10_{ax}$ (δ 3.10, LR). HMBC correlations were observed between H2 (δ 3.69) and C4 (δ 38.3), C10 (δ 52.4), C11 (δ 32.4), and C12 (δ 39.1); between $H8_{eq}$ (δ 2.72)/ $H8_{ax}$ (δ 1.38) and C6 (δ 61.6), C9 (δ 37.0), C10 (δ 52.4), and C12 (δ 39.1); between $H11_{eq}$ (δ 1.27) and C5 (δ 37.1); between H20 (δ 5.16) and C2 (δ 51.8) and C4 (δ 38.3); between H32 (δ 1.00) and C8 (δ 59.3), C9 (δ 37.0), C10 (δ 52.4), and C12 (δ 39.1); between $H6_{eq}$ (δ 2.31) and C21 (δ 57.7); between $H8_{ax}$ (δ 1.38) and C21 (δ 57.7); between $H10_{eq}$ (δ 2.45) and C13 (δ 55.6); and between H13 (δ 2.81) and C2 (δ 51.8). The chemical shifts of the C2 (δ 51.8), C10 (δ 52.4), and C13 (δ 55.6) resonances indicated that these three carbons were attached to nitrogen, and the H2/C10, $H10_{eq}$/C13, and H13/C2 HMBC correlations indicated that all three carbons were attached to the same tertiary amine nitrogen atom. Irradiation of H2 (δ 3.69) induced a NOE in H13′ (δ 2.63), supporting the attachment of C13 to N1. A

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(14) Specimens of *X. ingens* (200 g dry wt) were collected by hand using SCUBA on reefs at depths of −15 to −20 m near Sek Point off Madang, Papua, New Guinea. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. The sponge was identified by Dr. R. van Soest. A voucher sample (ZMA 10701) is deposited at the Zoologisch Museum, University of Amsterdam.

(15) Madangamine A (**1**): colorless glass (50 mg from 200 g dry wt of sponge); $[\alpha]_D$ + 319° (*c* 1.0, EtOAc); EIHRMS M^+, *m/z* 432.3503 ($C_{30}H_{44}N_2$ ΔM − 0.1 mmu); NMR (500-MHz, benzene-d_6) (**C**no., δ^1H ($\delta^{13}C$)) ***C2***, 3.69, br s (51.8); ***C3*** (139.3); ***C4***, $2.21_{(eq)}$, dd, *J* = 16.5, 7.7 Hz, $3.07_{(ax)}$, t, *J* = 16.5 Hz (38.3); ***C5***, 1.70 (37.1); ***C6***, $2.11_{(ax)}$, dd, *J* = 3.1, 10.8 Hz, $2.31_{(eq)}$, bd, *J* = 10.8 Hz (61.6); ***C8***, $1.38_{(ax)}$, d, *J* = 11.2 Hz, $2.72_{(eq)}$, d, *J* = 11.2 Hz (59.3); ***C9*** (37.0); ***C10***, $2.45_{(eq)}$, d, *J* = 12.0 Hz, $3.10_{(ax)}$, d, *J* = 12.0 Hz (52.4); ***C11***, $1.27_{(eq)}$, dt, *J* = 12.4, 2.6 Hz, $2.40_{(ax)}$, dt, *J* = 12.4, 3.4 Hz (32.4); ***C12***, 1.14, br s (39.1); ***C13***, 2.81, ddd, *J* = 13.5, 11.9, 5.8 Hz, 2.63, br t, *J* = 13.5 Hz (55.6); ***C14***, 1.42, 1.81 (23.2); ***C15***, 1.19, 1.19 (25.4); ***C16***, 2.24, 1.80 (25.8); ***C17***, 5.38 (129); ***C18***, 5.43, td, *J* = 10.7, 4.1 Hz (129); ***C19***, 2.32, dd, *J* = 13.3, 3.0 Hz, 3.34, dt, *J* = 13.3, 11.3 Hz (26.8); ***C20***, 5.16, dt, *J* = 11.5, 2.8 Hz (121.8); ***C21***, 2.14, ddd, *J* = 11, 5.5, 3.3 Hz, 2.47, td, *J* = 11.0, 5.9 Hz (57.7); ***C22***, 1.93, 2.40 (25.5); ***C23***, 5.36 (129.2); ***C24***, 5.36 (128); ***C25***, 2.60, br d, *J* = 16.9 Hz, 3.07 (26.6); ***C26***, 5.35 (127.5); ***C27***, 5.35 (128.3); ***C28***, 2.56, br d, *J* = 16.5 Hz, 3.12 (26.7); ***C29***, 5.31 (125.9); ***C30***, 5.55, m (132.8); ***C31***, 1.93, 2.25 (22.7); ***C32***, 1.00, dd, *J* = 13.0, 7.2 Hz, 2.50, ddd, *J* = 13.0, 10.5, 3.0 Hz (36.1).

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similar chemical shift and HMBC correlation analysis showed that C6 (δ 61.6), C8 (δ 59.3), and C21 (δ57.7) were attached to the second tertiary amine nitrogen. The HMBC correlations observed between $H8_{ax}/H8_{eq}$ and C9, C10, C12, and C32 and between H32 and C8, C9, C10, and C12 situated the quaternary carbon C9 (δ 37.0) between C8, C10, C12, and C32.

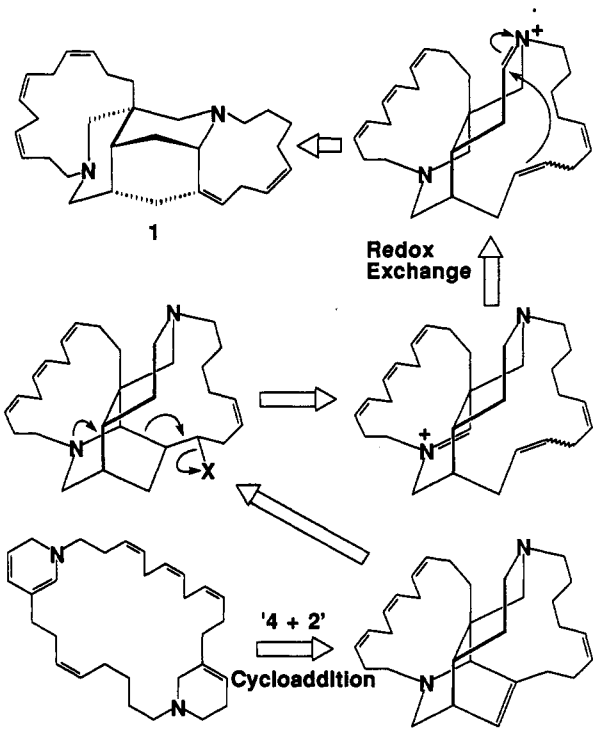
The LR-COSY correlation observed between H32 (δ 1.00) and $H10_{ax}$ (δ 3.10) was assigned to *W* coupling, indicating that the piperidine ring containing N1 was in a chair conformation and that C32 was axial. A NOE observed between H32 (δ 1.00) and H12 (δ 1.14) showed that the second piperidine ring was cis-fused to the first. Irradiation of H12 (δ 1.14) induced NOEs in $H6_{ax}$ (δ 2.11), $H8_{ax}$ (δ 1.38), and H5 (δ 1.70), and irradiation of $H6_{ax}$ induced NOEs in H5, H12, and $H8_{ax}$, indicating that the piperidine ring containing N7 was also in a chair conformation. The cis fusion of the two piperidine rings, both residing in chair conformations, further required that C3 be attached in the axial orientation at C2 and that C4 be attached in the axial orientation at C5, resulting in a slightly flattened chair conformation for the carbocyclic ring.

The remaining portion of **1**, consisting of eight aliphatic methylene and eight olefinic methine carbons, had to form a pair of linear bridges between the C13, C20, C21, and C32 carbons attached to N1, C3, N7, and C9, respectively, to complete the final two rings required by the unsaturation number. Detailed analysis of the COSY, HOHAHA, HMQC, and HMBC data supported an eight-carbon bridge spanning N1 and C3 as shown. COSY correlations were observed between H20 (δ 5.16) and H19 (δ 2.32)/H19′ (δ 3.34); between H19/H19′ and H18 (δ 5.43); between H18 and H17 (δ 5.38); between H17 and H16 (δ 1.80)/H16′ (δ 2.24); between H16/H16′ and H15/H15′ (both δ 1.19); between H15/H15′ and H14 (δ 1.42)/ H14′ (δ 1.81); and between H14/H14′ and H13 (δ 2.81)/H13′ (δ 2.63). The HOHAHA and HMBC data supported the connectivity indicated by the COSY data. For example, H14 (δ 1.42) showed HOHAHA correlations to H13/H13′ (δ 2.81/2.63), H14′ (δ 1.81), H15/H15′ (δ 1.19), H16/H16′ (δ 1.80/2.24), and H17 (δ 5.38); H19′ (δ 3.34) showed HOHAHA correlations to H20 (δ 5.16), H19 (δ 2.32), H18 (δ 5.43), H17 (δ 5.38), H16 (δ1.80), and H15/H15′ (δ 1.19); and both H18 (δ 5.43) and H14 (δ 1.42) showed HMBC correlations to C16 (δ 25.8). A NOE observed between H2 (δ 3.69) and H19′ (δ 3.34) established the *Z* configuration for the $\Delta^{3,20}$ olefin, and the ^{13}C NMR shifts of C19 (δ 26.8) and C16 (δ 25.8) were consistent with the *Z* configuration for the Δ^{17} olefin.[16] The C14 (δ 23.2) and C16 (δ 25.8) NMR resonances were both very broad, suggestive of a slow interconversion of low-energy conformers in this part of the molecule.

The remaining N7-to-C9 bridge had to account for six aliphatic methylene carbons and six olefinic methine carbons. Absence of a UV chromophore in **1** indicated the lack of conjugation between the three olefins, and the combined COSY and HMQC data showed that there were two aliphatic methylene carbons between both N7 and C9 and the first olefinic carbon (C23 and C30) in each end of the bridge, resulting in the methylene-interrupted triene substructure shown. The HOHAHA and HMBC data were completely consistent with the indicated constitution of the N7-to-C9 bridge, and the ^{13}C NMR shifts of the allylic carbons at C22 (δ 25.5), C25 (δ 26.6), C28 (δ 26.7), and C31 (δ 22.7) indicated that all three olefins had the *Z* configuration.[16]

(16) ^{13}C NMR data reported for the ircinals[8] and the haliclamines[2] indicates that in this series of compounds, *Z* alkenes have allylic carbons at δ < 27 and *E* alkenes have allylic carbons at δ >30.

Scheme 1



Madangamine A (**1**) represents the first example of a new class of pentacyclic alkaloids. Scheme 1 outlines a proposed biogenesis for **1** that proceeds through a partially reduced bis-3-alkylpyridine macrocycle, that by analogy with the Whitehead and Baldwin proposal for the biogenesis of the manzamines,[11] undergoes the biological equivalent of a "4 + 2" cycloaddition reaction to generate an intermediate related to the ingenamine class of alkaloids.[12,13] The ingenamine-type intermediate can then undergo rearrangement as shown to generate the madangamine A (**1**) skeleton. Madangamine A (**1**) showed in vitro cytotoxicity against murine leukemia P388 (ED_{50} 0.93 μg/mL) and human lung A549 (ED_{50} 14 μg/mL), brain U373 (ED_{50} 5.1 μg/mL), and breast MCF-7 (ED_{50} 5.7 μg/mL) cancer cell lines.

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Supplementary Material Available: 1D and 2D COSY, HMQC, HMBC, and HOHAHA NMR, and EIHRMS spectra for madangamine A (17 pages). This material is contained in many 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.