An Alkaloid Protein Kinase C Inhibitor, Xestocyclamine A, from the Marine Sponge *Xestospongia* sp.[1]

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Enzymes, such as protein kinase C (PKC), are ubiquitously expressed by eukaryotes[2] and are an attractive target to guide discovery of new bioactive substances.[2c] The PKC signaling path constitutes a pivotal mechanism in the regulation of fundamental processes such as protein synthesis, gene expression, and cell proliferation. Consequently, the development of selective, nontoxic PKC inhibitors may provide treatments for cancer[3 a,b] or viruses.[3c] Only a few natural product PKC inhibitors have been discovered, and some of the most important are alkaloids such as the staurosporines,[4 a–c] chelerythrine,[4d] and balanol.[4e] We report below xestocyclamine A (**1**) as a new type of polycyclic alkaloid PKC inhibitor.

The work leading to the discovery of xestocyclamine A (**1**) began when the semipure extracts of *Xestospongia* sp., a soft, brown, massive sponge collected from the Milne Bay province of Papua, New Guinea,[5] exhibited 100% inhibition at 5 μg/mL against PKC β. The purification work was not straightforward, because regular or reversed-phase HPLC of the active fractions yielded impure mixtures. Fortunately, centrifugal partition chromatography (CPC) with a solvent system of *sec*-BuOH/H_2O (1:1) afforded pure **1**, whose ^1H NMR spectrum contained multiplets for 34 protons (CH and CH_2, no methyls) between δ 0.8 and 3.5 which were not well resolved, even at 500 MHz (Figure 1).

Three sets of ^1H NMR spectra were examined (Figure 1) because changing solvents from $CDCl_3$ to $CDCl_3$/DMSO-d_6 (9:1) to $CDCl_3$/pyridine-d_5 resolved overlapping vinylic proton peaks of **1**. An intense HREIMS M^+ = 396.3141 corresponded to a molecular formula of $C_{26}H_{40}N_2O$ (Δ0.1 mmu of that calculated), whose eight unsaturations were ascribed to three double bonds and five rings. The single heteroatom proton (HREIMS–APT

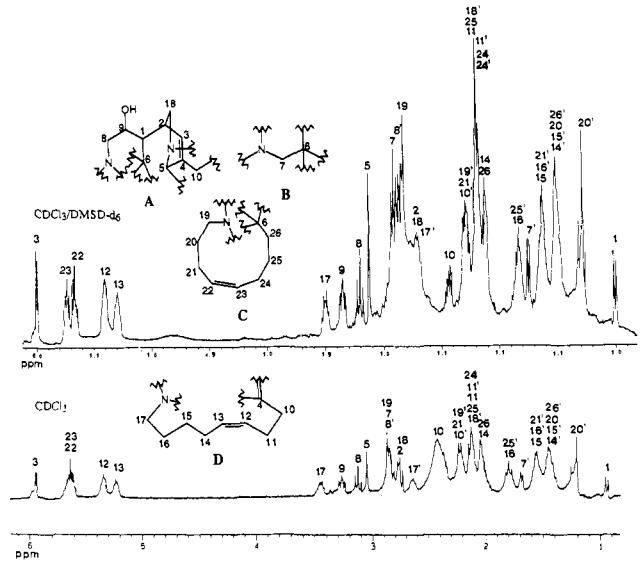

Figure 1. ^1H NMR of xestocyclamine (**1**) at 500 MHz.

proton count of $C_{26}H_{39}$)[6] was ascribed to a secondary alcohol recognized from $^{13}C/^1H$ NMR resonances at δ 65.56(d)/3.20(dt) which further suggested a -$CH_2CH(OH)CH$- moiety. These protons provided an important anchor point to begin further substructure analysis facilitated once ^1H–^1H COSY (Figure S3), TOCSY (Figure S4), ^1H–^{13}C COSY (Figure S2a), HMQC (Figure S2b), and HMBC (Figure S5) spectral data were in hand. Correlations were observed in the ^1H–^1H COSY (Figure S3, $CDCl_3$/pyridine-d_5) and TOCSY (Figure S4, $CDCl_3$/DMSO-d_6) NMR spectra from CH1 (δ 0.95) of this group to a second constellation consisting of δ 2.90-H2, 2.82/2.25-$H_2$18, 5.95-H3. A clear ^1H–^1H COSY correlation (Figure S3) was seen from H3 to H1 and to H2 and/or H18, but the multiplets of H2 (δ 2.90) and H18 (δ 2.82) badly overlapped in all solvents (Figure 1), so correlations to these protons were somewhat ambiguous. This difficulty was overcome by observing a TOCSY correlation (Figure S4) from H1 to H18′. Other relevant long-range COSY NMR correlations included those from H3 to δ 2.24-H10 (Figure S3) and HMBC (Figure S5, $CDCl_3$/DMSO-d_6 7:3) correlations from H5 to δ 141-C4 and δ 125-C3. These observations, coupled with the low-field sp^3 carbons at δ 51.04-C8, 49.30-C18, and 63.1-C5 (assigned as nitrogen-bearing), allowed the three-carbon fragment to be expanded to substructure **A**. Substructure **B** was proposed after the isolated diastereotopic proton pair at δ 2.87 (mult) and 1.67 (d, J = 9.5 Hz) and their attached carbon at δ 52.58 were assigned as $CH_2$7. The sharp AB multiplet for $H_2$7′ as well as its low-field $^1H/^{13}C$ NMR δ values indicated that this -CH_2- must be flanked by a tertiary N and the single sp^3 quaternary carbon present in **1**.

The full identification of two C_9 substructures **C** and **D** was not straightforward due to the severe crowding of upfield proton resonances shown in Figure 1. Tracing the ^1H–^1H COSY (Figure

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(1) Part five in the series Novel Marine Sponge Alkaloids. For part four, see: Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P. *Tetrahedron* **1993**, *49*, 329.

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(5) A photograph of this sponge is included as Plate 1 in the supplementary material, and its identification (coll. nos. 90144 and 91157) as *Xestospongia* sp. (order Haplosclerida, family Petrosiidae) is based on characteristics described in Plate 1.

(6) Xestocyclamine (**1**): $[\alpha]_D$ - 13.5 (*c*, 0.019, MeOH); IR (film) ν_{max} 3470, 2980, 1650, 1450, 1205; LREIMS, positive ion, *m/z* (relative intensity) 396 (M^+, 65), 379 (20), 365 (30), 353 (20), 267 (45), 242 (10), 217 (25), 188 (42), 146 (25), 93 (100); HREIMS 396.3141 = $C_{26}H_{40}N_2O$ (Δ0.1 mmu of calcd); ^{13}C–^1H COSY NMR data 125/500 MHz ($CDCl_3$/DMSO-d_6 9:1) δ 140.99 s (C4); 132.21 d, 5.32 m (CH12); 131.57 d, 5.23 m (CH13); 131.57 d, 5.55 dt, J = 11.0 and 5.0 Hz (CH22); 129.69 d, 5.57 m (CH23); 125.27 d, 5.95 d, J = 7.0 Hz (CH3); 65.56 d, 3.20 dt, J = 8.4 and 4.1 Hz (CH9); 63.10 d, 3.05 s (CH5); 55.88 t, 3.42 m/2.65 m ($CH_2$17); 54.12 t, 2.75 m/2.12 m ($CH_2$19); 52.58 t, 2.87 m/1.67 d, J = 9.5 Hz ($CH_2$7); 51.04 t, 3.10 t, J = 11.2 Hz/2.85 m ($CH_2$8); 49.38 d, 0.95 dd, J = 9.3 and 0.8 Hz (CH1); 49.30 t, 2.82 m/2.25 m ($CH_2$18); 44.03 s (C6); 40.26 t , 2.17 m/1.80 m ($CH_2$25); 36.15 t, 2.38 m/2.24 m ($CH_2$10); 33.41 d, 2.90 m; 26.35 t, 1.80 m/1.67 m ($CH_2$15); 26.28 t, 1.50 m/1.28 m ($CH_2$20); 25.78 t, 2.20 m ($CH_2$11); 25.50 t, 2.10 m/1.53 m ($CH_2$26); 25.24 t, 2.10 m/1.53 m ($CH_2$14); 22.78 t, 1.79 m/1.53 m ($CH_2$21); 20.20 t, 2.20 m ($CH_2$24); 19.36 t, 1.80 m/1.65 m ($CH_2$16). For additional NMR data, see Figures S1–S6.

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S3) and TOCSY (Figure S4) NMR correlations between the vinyl and aliphatic protons firmly established just limited elements of **C** and **D** including (a) two separate -H_2CCH=$CHCH_2$- residues [supported by four vinyl to aliphatic correlations]; (b) a -H_2CCH_2- group [based on correlations from H19 to H20′]; and (c) a -$H_2CCH_2CH_2$- array [assigned via correlations from H17 to H16 and H15]. Fortunately, these substructures could be expanded once a HMQC-TOCSY (Figures S6a and S6b, $CDCl_3$/DMSO-d_6) spectrum was obtained. The complete subunit **C** was drawn by observing several sets of HMQC-TOCSY correlations—from δ 20.2-C24 to H23, H25/25′, from δ 25.50-C26 to H25′, from δ 22.78-C21 to H22, H20, and from δ 22.68-C20 to H19. Parallel arguments generated the complete fragment **D** via correlations traced from δ 132.21-C12 to H13, H11, from δ 36.15-C10 to H-11, from δ 19.36-C16 to H15/15′, H17/17′, and from δ 25.24-C14 to H15.

Isomeric pentacyclics **1** and **2** were envisioned as plausible complete structures. These were derived by first merging common atoms C4 and C6 in substructures **A–D**. Next, connecting C5 to C6, justified from an enhancement observed at C6 during selective INEPT irradiation at H5, provided a tricyclic structure which could then be closed to either **1** or **2**. Each of these frameworks is biosynthetically related to the manzamine-type alkaloids (e.g., **3**).[7] The manzamines are now considered to arise from the union of tryptophan and aldehydes such as **4**,[7a] and the latter can be formed from the acyclic building blocks outlined in Scheme I. Both **2** and **5** have identical frameworks, and each could come from intramolecular cyclization of **7**.[7a] Similarly, **1** could be derived from the hypothetical pentacycle **6** formed by cycloaddition of **8a** to **8b**. Structure **2** was ruled out by an HMBC correlation (Figure S5, $CDCl_3$/DMSO-d_6 7:3) observed from H7′ to δ 54-C19 (accompanied by a correlation from H7′ to δ 50-C1). The structural description of xestocyclamine A was concluded by proposing the stereochemistry shown for **1** based on ROESY correlations between H1 and $H_2$25 and key proton couplings J_{1-9} = 9.3 Hz and J_{1-2} = 0.8 Hz.

Xestocyclamine A (**1**) has a skeleton without prior precedent and provides an important parallel to an intermediate proposed in the biosynthesis of manzamines. It is moderately potent against PKC ε (IC_{50} = 4 μg/mL) and also exhibits activity in a whole cell IL-1 release assay with an IC_{50} of 1 μM. This action appears to be selective, as **1** is inactive against other cancer-relevant targets, including PTK and IMPDH. Finally, **1** (NSC 666048), at doses as high as 100 μM, does not show *in vitro* growth inhibition effects against cancer cells in the NCI's disease oriented screening program.

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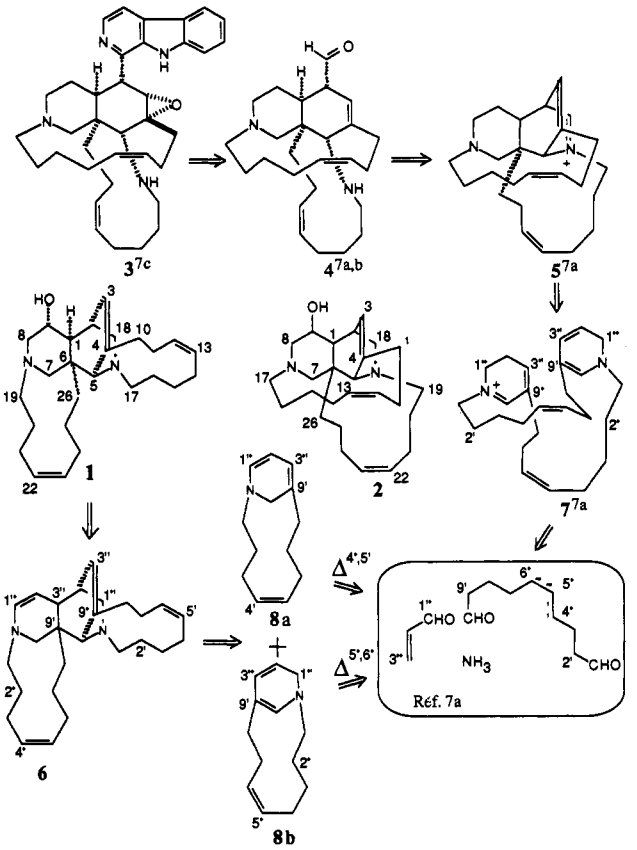
Scheme I. Biosynthetic Analysis



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Supplementary Material Available: Plate 1 plus Figures S1–S6 (9 pages). This material is contained in many libraries on microfiche; it can also be ordered from the ACS. Ordering information is given on any current masthead page.