Calyxamines A and B, Novel Piperidine Alkaloids from the Caribbean Sea Sponge *Calyx podatypa*[1,2]

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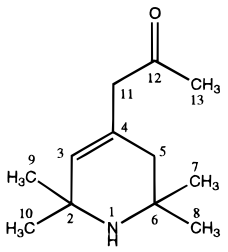
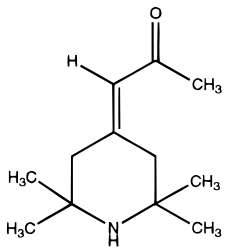
Calyxamines A (**1**) and B (**2**) are 2,2,4,6,6-pentasubstituted piperidine alkaloids possessing novel carbon skeletons isolated from the marine sponge *Calyx podatypa* collected in Puerto Rico. Their structures, after derivatization with trifluoroacetic acid, have been determined by a combination of X-ray and spectroscopic methods. A plausible biogenetic pathway to the calyxamines is suggested.

The Caribbean Sea sponge *Calyx podatypa* (Van Soest) (Phloeodictyidae) is known to be a source of a variety of sterols[4] and a series of six-membered heterocyclic compounds containing nitrogen, such as diketopiperazines[5] and 3-substituted *N*-methylpyridinium salts.[6] We report herein the isolation and structure elucidation of two novel 2,2,4,6,6-pentasubstituted piperidine alkaloids, calyxamines A (**1**) and B (**2**), from the marine sponge *C. podatypa* collected near Mona Island off the west coast of Puerto Rico. The purification and subsequent structure elucidation of the calyxamines were accomplished after derivatization with trifluoroacetic acid (TFAA), which produced the corresponding trifluoroacetate salts in crystalline form.



Calyxamine A (**1**)



Calyxamine B (**2**)

After prior extraction with several solvents, the MeOH extract of the sponge (927 g, dry wt) was filtered and concentrated in vacuo to yield a dark oily residue (see Experimental Section). The material obtained was subjected to TLC-guided fractionation using Sephadex LH-20 (1:1 $CHCl_3$−MeOH). The second of the three fractions collected was chromatographed over ODS Si gel (60% $CHCl_3$ in MeOH), reversed-phase HPLC (ODS Si gel, 9:8:0.02 $CH_3CN-H_2O-CF_3CO_2H$), and Si gel (60% $CHCl_3$ in Me_2CO), successively, to afford calyxamines A (**1**, 9.9 mg) and B (**2**, 13.3 mg) as the crystalline trifluoroacetate salts.

The structure of calyxamine A (**1**) was determined by a single-crystal X-ray experiment. Calyxamine A crystallized from $CHCl_3$−hexane mixtures as colorless needles with one molecule of $C_{12}H_{21}NO{\cdot}CF_3CO_2H$ in the asymmetric unit. A computer-generated perspective drawing of the final X-ray model is shown in Figure 1.

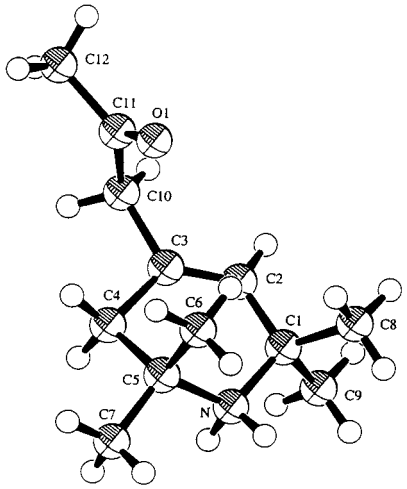


Figure 1. Computer-generated perspective drawing of the final X-ray model of the trifluoroacetate salt of calyxamine A (**1**). The trifluoroacetate anion has been omitted for clarity.

The ^{1}H-NMR spectrum of the TFAA salt of calyxamine A (**1**) in $CDCl_3$ showed only six singlets integrating for 20 protons (Table 1). Five methyl groups (δ 1.46, 6H, Me-7 and Me-8; δ 1.48, 6H, Me-9 and Me-10; δ 2.17, 3H, Me-13), two methylenes (δ 2.21, 2H, H5; δ 3.14, 2H, H11), and one olefinic methine (δ 5.34, H3) accounted for all the ^{1}H-NMR signals. In the ^{13}C-NMR spectrum 12 signals were observed: five quartets (δ 26.4, 2 × C, Me-7 and Me-8; δ 27.7, 2 × C, Me-9 and Me-10; δ 29.6, Me-13; δ 117.2, CF_3CO_2H; δ 161.6, CF_3CO_2H), two triplets (δ 37.7, C5; δ 51.2, C11), one doublet (δ 128.0, C3), and four singlets (δ 55.1, C6; δ 55.9, C2; δ 127.8, C4; δ 205.7, C12). The HREIMS of calyxamine A failed to show a parent ion but did give an $[M-15]^+$ fragment ion at *m*/*z* 180.1389 ($C_{11}H_{18}NO$) as the highest mass peak in the mass spectrum, which, in light of the NMR data, could be attributed to the loss of a CH_3 group. The UV spectrum showed an absorbance maximum at λ_{max} 205 nm, confirming the absence of conjugation through the piperidine ring. These spectral data were in full support of the structure assigned earlier by X-ray analysis.

The TFAA salt of calyxamine B (**2**) was also obtained as a white crystalline solid whose structure was assigned by NMR using primarily selective INEPT and HMBC experiments ($CDCl_3$, see Table 1), and by

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Table 1. NMR Spectral Data for the Trifluoroacetate Salts of Calyxamines A (**1**) and B (**2**)

	calyxamine A		calyxamine B	
position	δ_H, mult, intrg[a]	δ_C (mult)[b]	δ_H, mult, intrg[a]	δ_C (mult)[b]
1	9.53, br s, 2H		9.20, br s, 2H	
2		55.9 (s)		58.8 (s)
3	5.34, s, 1H	128.0 (d)	3.02, s, 2H	37.8 (t)
4		127.8 (s)		148.7 (s)
5	2.21, s, 2H	37.7 (t)	2.33, s, 2H	46.1 (t)
6		55.1 (s)		58.2 (s)
Me−7	1.46, s, 3H[c]	26.4 (q)[c]	1.36, s, 3H[c]	27.2 (q)
Me−8	1.46, s, 3H[c]	26.4 (q)[c]	1.36, s, 3H[c]	27.2 (q)
Me−9	1.48, s, 3H[c]	27.7(q)[c]	1.37, s, 3H[c]	27.2 (q)
Me−10	1.48, s, 3H[c]	27.7(q)[c]	1.37, s, 3H[c]	27.2 (q)
11	3.14, s, 2H	51.2 (t)	6.14, s, 1H	126.9 (d)
12		205.7 (s)		198.2 (s)
Me−13	2.17, s, 3H	29.6 (q)	2.18, s, 3H	31.9 (q)
$CF_3CO_2^-$		117.2 (q)		117.1 (q)
$CF_3CO_2^-$		161.6 (q)		161.6 (q)

[a] The ^{1}H-NMR spectra were recorded at 300 MHz in $CDCl_3$ at 25 °C. Chemical shifts are given in δ units downfield from Me_4Si. Assignments were aided by ^{1}H−^{1}H COSY, RCT−COSY, TOCSY, and 2D PSNOESY experiments. [b] The ^{13}C-NMR spectra were recorded in $CDCl_3$ at 75 MHz. Number of attached protons were determined by APT experiments. Assignments were aided by selective INEPT, HETCOR, and HMBC experiments recorded in $CDCl_3$. [c] Labeled signals within a column may be interchanged.

comparison with calyxamine A (**1**). HREIMS measurements provided the molecular formula $C_{12}H_{21}NO$, suggesting three degrees of unsaturation in this molecule. Analysis of the NMR data revealed the presence of the same pentasubstituted piperidine ring found in **1** (see Table 1), while the UV absorbance observed [231 nm (ϵ 10 400)] was typical of an α,β-unsaturated carbonyl chromophore. This information along with a strong IR absorption at 1696 cm^{-1} suggested that the olefin in calyxamine B was exocyclic and conjugated to a ketone functionality. A combination of RCT−COSY, TOCSY, 2D NOESY, HMBC, and selective INEPT NMR experiments showed long-range ^{1}H−^{1}H and ^{1}H−^{13}C correlations consistent with the proposed structure for calyxamine B (**2**), which also allowed the assignment of nearly all the carbon and hydrogen atoms in the molecule.

While the amino acid lysine is primarily involved in the biogenesis of many piperidine alkaloids, certain piperidine derivatives do not come from lysine at all.[7] A likely biosynthetic pathway to the calyxamines, for instance, probably involves the condensation of NH_3 with four molecules of Me_2CO (or their respective biosynthetic equivalents). The in vivo pathways may involve Schiff base formation, subsequent reactions of the Mannich type, an aldol condensation at some stage, and dehydration to yield the novel carbon skeleton of the calyxamines.[8] The simplest method for the preparation of calyxamines A and B in the laboratory probably would require the condensation of Me_2CO with NH_3 in the presence of an acid catalyst. In 1976, Sosnovsky and Konieczny reported an improved method for the formation of triacetonamine (2,2,6,6-tetramethyl-4-piperidone) using this general methodology.[9] In addition to this product (formed in 85% yield), diacetone alcohol, diacetone amine, mesityl oxide, and acetonin (2,2,4,4,6-pentamethylpiperidine) were the only other products detected by gas chromatography. In view of their results, we tried to synthesize **1** and **2** directly from an aldol-type condensation between 2,2,6,6-tetramethyl-4-piperidone and Me_2CO in the presence of trace amounts of TFAA. After several attempts under a variety of reaction conditions, we did not detect **1** or **2** based on TLC analyses. On the other hand, these results constitute circumstantial evidence that the calyxamines are true natural products and not artifacts formed during our isolation procedures. We cannot, however, discount the possibility that calyxamine B may arise from the acid-induced isomerization of calyxamine A (**1**). Unfortunately, the determination of the bioactivity of **1** and **2** was thwarted due to lack of material.

Experimental Section

General Experimental Procedures. For general experimental procedures, see Rodríguez and Soto.[10] Me_2CO, TFAA, and 2,2,6,6-tetramethyl-4-piperidone were purchased from Aldrich Chemical Co., Ltd.

Animal Material. *C. podatypa* (class Demospongiae, order Haplosclerida, family Phloeodictyidae)[11] is a dark reddish-brown to black, soft sponge of irregular mass and smooth-textured surface with scattered excurrent openings that form volcano-like projections. It inhabits reefs, especially small patch reefs in exposed areas. Maroon sponge zoanthids may be found growing on the surface. A voucher specimen (no. MI-030) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras campus.

Collection and Extraction. Minced and freeze-dried specimens of *C. podatypa* (927 g) collected near Mona Island, Puerto Rico, in 1992, were extracted exhaustively with $CHCl_3$−MeOH (1:1) (5 × 1L). After filtration the crude extract was evaporated under vacuum to yield a residue (62 g) that was suspended in H_2O and then partitioned with *n*-hexane (4 × 400 mL), $CHCl_3$ (4 × 400 mL), and finally *n*-BuOH (4 × 400 mL). Concentration of the aqueous phase in vacuo at 65 °C followed by high vacuum storage at 25 °C for 2 days left a solid residue that was taken up with dry MeOH. After desalting through vacuum filtration, the MeOH filtrate was concentrated to yield a dark oily residue (38 g). Subsequent TLC-guided fractionation by size exclusion column chromatography over Sephadex LH-20 using 1:1 $CHCl_3$−MeOH as eluent produced three fractions. The second fraction (6.1 g) was chromatographed successively over ODS Si gel (60% $CHCl_3$ in MeOH), reversed-phase HPLC (ODS Si gel, 9:8:0.02 $CH_3CN-H_2O-CF_3CO_2H$), and Si gel (60% $CHCl_3$ in Me_2CO) to give 9.9 mg (1.07×10^{-3}% based on dry wt of the sponge) of calyxamine A (**1**) and 13.3 mg (1.43×10^{-3}% based on dry wt of the sponge) of calyxamine B (**2**) as their trifluoroacetate salts.

Calyxamine A (1)·CF_3CO_2H: crystalline white solid; IR (cast) 3423, 3059, 2917, 2848, 1720, 1691, 1679, 1314, 1282, 1201, 1175, 1133, 1026, 801, 720 cm^{-1}; UV (CH_3OH) λ_{max} 205 (ϵ 1000) nm; ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m/z* $[M - 15]^+$ 180.1389 (100) (calcd for $C_{11}H_{18}NO$, 180.1388), $[M - CH_3COCH_2]^+$ 138.1282 (14) (calcd for $C_9H_{16}N$, 138.1283), $[M - CH_3-CH_3COCH_3]^+$ 122.0974 (16) (calcd for $C_8H_{12}N$, 122.0970), $[H_2N{=}C(CH_3)_2]^+$ 58.0615 (13) (calcd for C_3H_8N, 58.0657).

X-ray Structure Determination. Crystallization of the TFAA salt of **1** from $CHCl_3$−hexane mixtures at room temperature yielded clear prisms of marginal quality. X-ray diffraction data were collected on a

Siemens SMART CCD system at 23 ± 1 °C to a maximum 2θ of 54°, using Mo Kα radiation (λ = 0.710 73 Å). The structure, which was solved by direct methods and completed by successive Fourier calculations, was refined by full-matrix least-squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. HO8 was refined with a fixed isotropic thermal parameter and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. The diffraction data were weak and exhibited wide mosaic spread, limiting the quality of the refinement, which converged to R = 0.087 and Rw = 0.144 for 1963 observed reflections [$I > 2\sigma(I)$], of a total of 3513 unique measured intensities. The final difference map had a maximum peak of 0.49 e^- $Å^{-3}$ in the vicinity of the trifluoroacetate and was otherwise featureless. All calculations were performed with the NRCVAX package of crystallographic programs.[12] Scattering factors were taken from the *International Tables for X-ray Crystallography*.[13,14]

Calyxamine B (2)·CF_3CO_2H: crystalline white solid; IR (cast) 3377, 3051, 2990, 2922, 2850, 1696, 1684, 1635, 1489, 1428, 1392, 1241, 1205, 1150, 1135, 830, 800, 719 cm^{-1}; UV (CH_3OH) λ_{max} 231 (ϵ 10 400) nm; ^{1}H-NMR ($CDCl_3$, 300 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) (see Table 1); HREIMS *m/z* [M^+] 195.1625 (31) (calcd for $C_{12}H_{21}NO$, 195.1623), [$M - CH_3$]$^+$ 180.1388 (56) (calcd for $C_{11}H_{18}NO$, 180.1388), [$M - C_4H_{10}N$]$^+$ 123.0798 (76) (calcd for $C_8H_{11}O$, 123.0809), [$(CH_3)_2C{=}N{=}C(CH_3)_2$]$^+$ 98.0966 (28) (calcd for $C_6H_{12}N$, 98.0969), [$M - C_4H_{10}N - CO$]$^+$ 95.0857 (21) (calcd for C_7H_{11}, 95.0860), [$(CH_3)_2C{=}NH_2$]$^+$ 58.0606 (100) (calcd for C_3H_8N, 58.0657).

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References and Notes

(1) Taken in part from the Ph.D. Dissertation of Cóbar, O. M., University of Puerto Rico, 1996.
(2) Presented in part at the 20th Senior Technical Meeting of the American Chemical Society, Puerto Rico Section, Tropimar Beach Club and Convention Center, Isla Verde, Puerto Rico, Nov. 8−9, 1996.
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(14) Hydrogen coordinates, thermal parameters, and bond distances and angles have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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