Ingenamine G and Cyclostellettamines G−I, K, and L from the New Brazilian Species of Marine Sponge *Pachychalina* sp.

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The chemical investigation of the cytotoxic and antituberculosis active MeOH crude extract of the marine sponge *Pachychalina* sp. led to the isolation of six new nitrogenous metabolites, including ingenamine G (**1**), as well as a mixture of new cyclostellettamines G, H, I, K, and L (**10**−**14**) with the known cyclostellettamines A−F (**4**−**9**). Structural assignments of compound **1** were based on the analysis of MS and NMR data, while the structures of compounds **10**−**14** could be established by HPLC-MS/MS analysis. Ingenamine G displayed cytotoxic activity against HCT-8 (colon), B16 (leukemia), and MCF-7 (breast) cancer cell lines, antibacterial activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and four oxacilin-resistant *S. aureus* strains, and antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv.

Since the isolation of halitoxins by Schmitz's group in 1978 from sponges of the genus *Haliclona* (currently *Amphimedon*),[1] marine sponges in the order Haplosclerida have proven to be an exceptionally rich source of both alkylpyridine and alkylpiperidine alkaloids. Comprehensive reviews of this structural class of secondary metabolites have appeared in 1996,[2] 1997,[3] and 2000.[4,5] Not only are these alkaloids considered chemotaxonomic markers of Haplosclerid sponges,[2,3] but many of them present potent biological activities. Such is the case for the antituberculosis and antiviral manadomanzamines from *Acanthostrongylophora* sp.,[6] the cytotoxic and strongly antibiotic arenosclerins from *Arenosclera brasiliensis*,[7,8] the antimalarial manzamines,[9] and for xestospongins A, C, and D, araguspongine B, and demethylxestospongin B, a group of macrocyclic bis-1-oxaquinolizidines isolated from *Xestospongia* sp., which are potent blockers of IP_3-mediated Ca^{2+} release from endoplasmic reticulum vesicles of rabbit cerebellum.[10]

In our current program for the investigation of biologically active natural products from marine invertebrates,[11] we have recently prioritized the chemical investigation of the crude MeOH extract of a new species of marine sponge, *Pachychalina* sp. (Pinheiro, Berlinck, Hajdu, 2004), which displayed potent cytotoxic activity against HCT-8 colon cancer cells and B16 murine melanoma cancer cells, as well as antituberculosis activity against *Mycobacterium tuberculosis* H37Rv. A preliminary TLC analysis of the *Pachychalina* sp. MeOH extract indicated the presence of a very complex mixture of alkaloids. Herein we report the results of our initial efforts in identifying these alkaloids, which include the isolation of one new ingenamine-related alkaloid and five new cyclostelletamine derivatives.

Results and Discussion

The crude MeOH extract of *Pachychalina* sp. was subjected to a solvent−solvent partition with EtOAc and with n-BuOH. After evaporation, the EtOAc extract was dissolved in 9:1 MeOH−H_2O and partitioned with hexane. The 90% MeOH extract was evaporated, and the residue was dissolved in CH_2Cl_2−0.5 N HCl. The organic fraction was collected (named CH_2Cl_2-acid). The aqueous fraction was basified (1 N NaOH) and partitioned with CH_2Cl_2. This organic fraction was designed CH_2Cl_2-basic.

Compound **1** was isolated from the CH_2Cl_2-basic extract by a series of chromatographic separations as an optically active glassy solid. Its FABMS showed a quasi molecular ion peak at *m*/*z* 479, on which a high-resolution measurement (measd 479.40007; calcd 479.40014) indicated the formula $C_{32}H_{51}N_2O$ with nine degrees of unsaturation. Considering the four double bonds, indicated by analysis of the BBD and DEPT ^{13}C NMR spectra, the structure of **1** must have five rings. Additionally, analysis of the NMR data indicated the presence of three sp^3 methines (δ 62.1, 40.4, and 37.6), one sp^3 (δ 44.6) and one sp^2 (δ 144.5) quaternary carbons, and five typical nitrogen-substituted methylene resonances (δ 50.6, 51.2, 56.3, 58.0, 59.4), which suggested an ingenamine-type skeleton for **1**. Dereplication within the MARINLIT was strongly indicative that **1** belongs to the ingenamine/ingamine,[12] keramaphidin,[13] and xestocyclamine[14] class of alkaloids. This hypothesis was further supported by comparison of the ^{13}C NMR data of **1** with those of ingenamine F (**2**)[12c] and keramaphidin B (**3**),[12c,13] in particular chemical shifts of ^{13}C belonging to the tricyclic central core of these compounds (see Table 2 in the Supporting Information). Extensive analysis of NMR spectra including HSQC, HMBC, $^1H-^1H$ COSY, HSQC-TOCSY, and NOESY confirmed this assumption. In the COSY spectrum, we observed a vicinal $^1H-^1H$ correlation between H-4 and H-5 (δ 2.40, m), along with several $^1H-^1H$ long-range correlations between the two hydrogens of the CH_2-9 methylene (δ 1.79 and 1.30) and H-12a (δ 3.35) and H-20a (δ 1.82); between the CH_2-10 methylene signal at δ 3.15 and H-12a; and between both CH_2-12 hydrogens (δ 3.35 and 2.24) and both CH_2-21 hydrogens (δ 3.15 and 2.98). More informative were the long-range couplings detected in the HMBC spectra (6, 8, 12, and 15 Hz) and in the HSQC-TOCSY spectrum, which showed $^1H-^{13}C$ cou-

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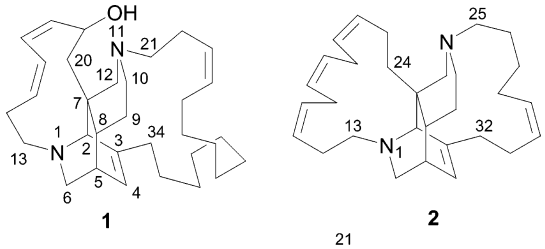
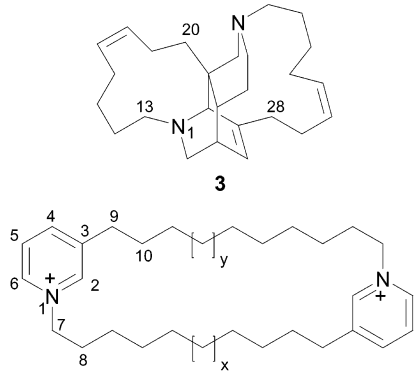
plings between H-2 (δ 2.95) and C-4 (δ 124.4); between H-4 (δ 6.09) and C-2 (δ 62.1), C-5 (δ 37.6), C-33 (δ 35.1), and C-34 (δ 37.9); between H-5 (δ 2.40) and C-7 (δ 44.6) and C-12 (δ 51.2); between H-6a (δ 3.10) and C-4 and C-5; between both CH_2-6 hydrogens and C-9 (δ 24.9); between H9a (δ 1.79) and C-7, C-10 (δ 50.6); between H9b (δ 1.30) and C-8 (δ 40.4) and C-12; between both CH_2-10 hydrogens and C-8; between CH_2-12 hydrogens and C-7, C-8, C-9, and C-10; between H-21b and C-12; and finally between H-34 and C-3 (δ 144.5). These data enabled us to define the "cage-like" central core of **1** as identical to that of **2** and **3**, including the relative stereochemistry, since the "central core" structure substituted by the two N-alkyl bridges supports only one relative stereochemistry. Therefore, structural differences must be present in the two N-alkyl bridges of **1**. Careful analysis of the NMR data indicated that the N-alkyl bridge connecting N-1 to C-7 consisted of eight carbons, in agreement with the structures of ingenamines A−D, ingamines,[12] keramaphidin B,[13] and xestocyclamine A.[14] In the case of these previously reported compounds, this chain contained either one double bond at C-16 or at C-17 or three nonconjugated insaturations at C-15, C-18, and C-21 within the structures of ingenamines E and F.[12c] However, compound **3** showed two conjugated double bonds at C-15 and C-17, with a carbinol group at C-19. Analysis of the NMR data clearly indicated that the methylene CH_2-13 (δ 58.0) was vicinally coupled to the methylene CH_2-14 (δ 26.5), which was in turn at the allylic position to CH-15 (δ 132.6). The conjugated system was clearly observed through $^1H-^1H$ COSY and long-range HMBC and HSQC-TOCSY couplings from CH-15 to CH-18 (δ 134.5) through CH-16 (δ 126.7) and CH-17 (δ 125.6). The *Z*,*Z* stereochemistry was assigned to the C-15, C-17 unsaturated system on the basis of the coupling constants measured between H-15 and H-16 (J = 9.5 Hz) and between H-17 and H-18 (J = 9.0 Hz), as well as on the C-14 (δ 26.5) and C-19 (δ 67.3) high-field chemical shifts. The carbinol hydrogen methine CHOH-19 (δ 4.78) showed $^1H-^1H$ couplings with both CH-18 (δ 5.37) and CH_2-20 (δ 1.82 and 1.46), as well as $^1H-^{13}C$ long-range correlations with carbons C-16 and C-17. Finally, the methylene CH_2-20 showed key $^1H-^1H$ and $^1H-^{13}C$ correlations with CH_2-19, C-7, and CH_2-9. The remaining N-alkyl bridge consisted of 14 carbons, two of which participate in a double bond. The position of the unsaturation was unambiguously established by analysis of the $^1H-^1H$ COSY spectrum, which showed a long-range $^1H-^1H$ coupling between CH_2-12 and CH_2-21, as well as vicinal $^1H-^1H$ couplings between CH_2-21 and CH_2-22, between CH_2-22 and CH-23, and finally between CH-23 and CH-24. The position of this double bond was also confirmed by analysis of the HMBC and HSQC-TOCSY spectra, which displayed long-range couplings between C-12 and the hydrogens of CH_2-21, between C-21 and the hydrogens of CH_2-22, CH-23, and CH-24, between C-22 and CH_2-21, CH-23, and CH-24, between C-23 and CH_2-22 and CH_2-25, and finally between C-24 and CH_2-22 and CH_2-25. The *Z* stereochemistry was assigned to this double bond on the basis of the H-23/H-24 coupling constant (J = 10.3 Hz) and the ^{13}C shielded chemical shifts of both CH_2-22 (δ 22.5) and CH_2-25 (δ 27.5). The ^1H and ^{13}C assignments of the remaining methylene chain were largely based on a detailed scrutiny of $^1H-^{13}C$ long-range couplings observed in HMBC and HSQC-TOCSY spectra. Both carbons C-33 and C-34 were shown to be long-range coupled to H-4, establishing the attachment point of the second N-alkyl chain to the C-3/C-4 double bond. We proposed the name ingenamine G for compound **1**.

Although the absolute stereochemistry of ingenamine G (**1**) herein isolated from *Pachychalina* sp. was not established, the similarity of both the specific rotation and NMR data observed for the ^1H and ^{13}C at the central tricyclic core strongly suggests that it has the same absolute stereochemistry as ingenamine F (**2**). We have not attempted to establish the absolute stereochemistry of **1**, due to previous negative results on derivatizing with MTPA a similar conjugated allylic alcohol function in the arenosclerins.[7] Ingenamine G (**1**) displayed cytotoxic activity against HCT-8 (colon), B16 (leukemia), and MCF-7 (breast) cancer cell lines at the level of 8.6, 9.8, and 11.3 μg/mL, respectively, and antibacterial activity against *Staphylococcus aureus* (ATCC 25923) at 105 μg/mL, *Escherichia coli* (ATCC 25922) at 75 μg/mL, and four oxacilin-resistant *S. aureus* strains, two of which at concentrations between 10 and 50 μg/mL, as well as antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv at 8 μg/mL.

Chromatographic separation of the n-BuOH crude extract (see Experimental Section) led to the isolation of two fractions containing 3-alkylpyridinium alkaloids. ^1H NMR analysis of these fractions showed the very characteristic pattern observed for these compounds, which includes four aromatic hydrogen signals at δ 9.17 (s, H-2), 9.05 (d, 7.1 Hz, H-6), 8.45 (d, J = 7.1 Hz, H-4), and 8.09 (t, J = 7.1 Hz, H-5) as well as aliphatic hydrogen signals at δ 4.63 (t, J = 6.7 Hz, CH_2-7), 1.89 (m, CH_2-8), 2.80 (t, 6.7 Hz, CH_2-9), and 1.63 (m, CH_2-10) and a broad singlet between δ 1.14 and 1.21 (methylene chain).[15,16] Typical ^{13}C resonances of 3-alkylpyridine compounds were also observed in the HSQC spectrum. Although a preparative HPLC purification of these fractions lead to a single HPLC peak, further HPLC-MS/MS (ESI-TOF, API-CID) analyses of these fractions revealed the presence of an alkaloid mixture of the known cyclostellettamines A−F (**4**−**9**), together with the new cyclostellettamines G (**10**), H (**11**), I (**12**), K (**13**), and L (**14**). The known cyclostellettamines A−F (**4**−**9**) consist



1 **2** **3**



4 x = 3, y = 3
5 x = 3, y = 4
6 x = 4, y = 4
7 x = 3, y = 5
8 x = 4, y = 5
9 x = 5, y = 5
10 x = 2, y = 3
11 x = 1, y = 3
12 x = 1, y = 4
13 x = 1, y = 5
14 x = 2, y = 5

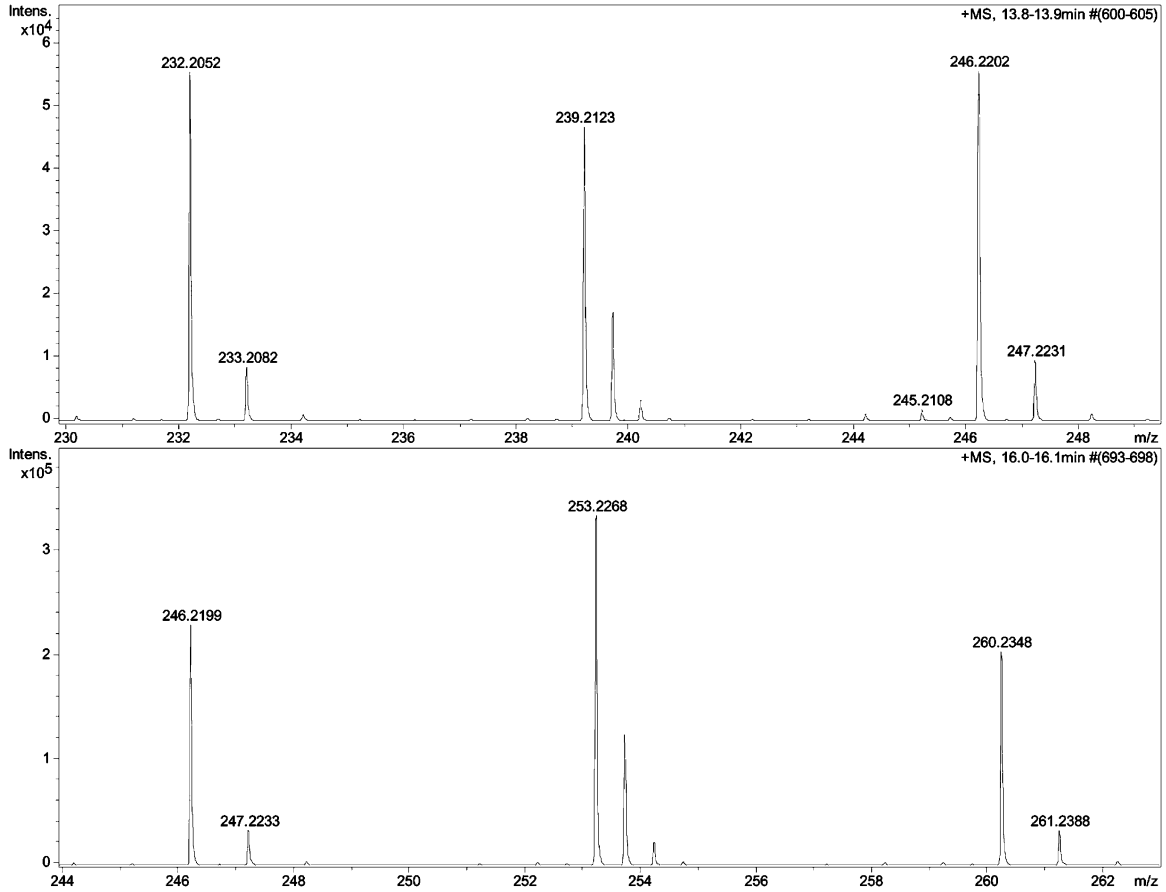


Figure 1. ESI-API-CID-mass spectrum of cyclostellettamine H (**11**, top) and cyclostellettamine B (**5**, bottom). The peak at *m*/*z* 239 is the doubly charged intact molecule ion M^{2+}, which is proven by the mass difference of isotope peaks of one-half mass units. The other singly charged peaks at *m*/*z* 232 and *m*/*z* 246 are the main fragments of cyclostellettamine H (**11**).

of alkyl chains with 12−14 carbon atoms each (C_x or C_y); the sum of both alkyl chains is therefore 24−28 ($\sum[C_x + C_y]$). The HPLC-MS/MS analysis showed five new cyclostellettamines with $\sum[C_x + C_y] = 22$, 23 (two different ones), 24, and 25. Dimeric pyridinium compounds undergo a mass spectral cleavage that leads to a ring opening (Hofmann fragmentation). Therefore, usually the singly charged molecular ion $[M - H]^+$ is observed at one mass unit less than its actual molecular weight. In our investigations only the doubly charged molecular ion M^{2+} was detected. This information together with the NMR chemical shifts proves unambigiously the cyclostellettamine core. The distribution of the carbon atoms between the two alkyl chains ($C_x + C_y$) required information about the MS fragments (HR-MS/MS analysis). Under API-CID conditions the two singly charged main fragments (probably due to two Hofmann fragmentations that split the molecules into halves) could be observed and used for the differentiation of the previously known cyclostellettamines (see Figure 1). The main fragments were identified on the basis of their intensity in the MS/MS spectrum (under API-CID conditions). Therefore, the new cyclostellettamines with $\sum[C_x + C_y] =$ 22 and 23 were identified as cyclostellettamines G (**10**, 11 + 12),[17] H (**11**, 10 + 12), and I (**12**, 10 + 13). The sum $\sum[C_x + C_y] = 24$ and 25 was already known for cyclostellettamines A (**4**) and B (**5**). However, the information from the two main fragments indicated the occurrence of two further new derivatives, cyclostellettamines K (**13**, 10 + 14) and L (**14**, 11 + 14). We suggest that the name cyclostelletamine J should be given to the 11 + 13 analogue, which has not been isolated yet.

Up to the present, the marine sponge *Pachychalina* sp. contain an interesting secondary metabolism profile. Although alkylpyridinium and alkylpiperidine alkaloids are commonly found within sponges of the order Haplosclerida, they are rarely found simultaneously. To the best of our knowledge, only the Mediterranean marine sponge *Reniera sarai* possesses halitoxins[18] and alkylpiperidine alkaloids (sarains and isosarains).[19] However, since bis-alkylpyridine alkaloids are currently considered to be the biogenetic precursors of their respective alkylpiperidine counterparts, as originally suggested by Cimino[19b] as well as by Baldwin and Whitehead,[20] it is not surprising to have isolated both alkaloid classes within a single species of marine sponge. It is possible that the co-occurrence of both alkylpyridines and alkylpiperidines within sponges of the order Haplosclerida is more common than it has been reported. However, since alkylpyridine alkaloids are considered as nuisance compounds that interfere in various bioassays,[11,21] they are not usually investigated. We are currently identifying minor alkaloids of the marine sponge *Pachychalina* sp.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at 29 °C. IR spectra (film on Si plate) were recorded on a FT-IR Bomem MB102 infrared spectrometer. The NMR spectra were recorded either on a Bruker ARX400 9.4 T instrument, operating at 400.35 MHz for ^{1}H and 100.10 MHz for ^{13}C channels, respectively, or on a Bruker DRX500 11.7 T, operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, respectively. All NMR spectra were obtained at 25 °C using TMS as internal

Table 1. ^{1}H, ^{13}C, COSY, HSQC-TOCSY, and HMBC NMR Data Recorded in MeOH-d_4 for Ingenamine G (**1**)

position	δ ^{13}C[a]	δ ^{1}H (mult, J in Hz)	COSY[c]	HSQC-TOCSY[a,c]	HMBC[b,c]
CH-2	62.1	2.95		H17	H4
C-3	144.5				H33ab, H34
CH-4	124.4	6.09 (m)	H5	H2, H5	H6b
CH-5	37.6	2.40 (m)	H4	H6b	H4
CH_2-6	56.3	3.10 (m); 1.96 (m)			
C-7	44.6				H5, H9a, H12ab, H20
CH-8	40.4	1.28 (m)		H9b, H10	H10, H12
CH_2-9	24.9	1.79 (m); 1.30 (m)	H12a, H20	H6, H12a	H12, H20b
CH_2-10	50.6	3.15 (m)	H12a	H9a, H12a	H12a
CH_2-12	51.2	3.35 (m); 2.24 (m)	H21ab		H5, H9b, H21b
CH_2-13	58.0	2.72 (m); 2.33 (m)		H14a, H15	
CH_2-14	26.5	2.47 (m); 2.07 (m)	H15	H13a	H16
CH-15	132.6	5.64 (m, 9.5, 5.4)	H16		H17
CH-16	126.7	6.37 (q, 10.6)	H15	H15, H17, H19	H17, H18
CH-17	125.6	6.33 (q, 10.6)		H18	H15, H19
CH-18	134.5	5.37 (m, 9.0, 5.6)	H17	H20b	H15, H16, H20a
CH-19	67.3	4.78 (ddd, 3.9, 5.5, 9.7)	H18, H20a	H17	H16, H17
CH_2-20	36.4	1.82 (m); 1.46 (m)	H9, H19	H20a	H19
CH_2-21	59.4	3.15 (m, 4.3, 8.1); 2.98 (m, 4.3, 8.3)	H12ab	H22ab, H23, H24	H22ab, H23
CH_2-22	22.5	2.65 (m); 2.27 (m)	H21ab, H23, H24	H21ab, H22ab, H23	H21ab, H23, H24
CH-23	124.4	5.33(m)	H24		H22ab, H25ab
CH-24	134.5	5.72 (dd, 8.2, 10.3)	H25a		H22ab, H25ab
CH_2-25	27.5	2.24 (m); 1.96 (m)	H24	H23, H24	H21b, H23, H24, H26
CH_2-26	28.9	1.40 (m)	H25b		H25b, H9
CH_2-27	28.2[d]	1.24 (m)			
CH_2-28	27.9[d]	1.24 (m)		H29	
CH_2-29	26.2	1.02 (m)	H30	H26	H33b
CH_2-30	27.7[d]	1.28 (m)		H29	H30, H33ab
CH_2-31	27.5	1.44(m)	H33a	H30, H32, H33a	H32, H33b, H34b
CH_2-32	25.2	1.59 (m)	H33ab	H30, H31, H33a	H31, H33ab
CH_2-33	35.1	2.15 (m); 2.05 (m)		H31, H32	H4, H32
CH_2-34	37.9	2.05 (m); 1.36 (m)		H4, H29, H30	H30

[a] Assignments by inverse detection at 400 MHz (HSQC). [b]Inverse detection at 400 MHz, for $^{n}J_{^{13}C-^{1}H} = 8.3$. [c]a and b denote downfield and upfield resonances respectively of a geminal pair. [d] Assignments may be reversed.

reference. Low-resolution mass spectra were recorded on a VG-7070 mass spectrometer operating at a nominal accelerating voltage of 70 eV. High-resolution mass spectra were recorded at a nominal resolution of 5000 or 10000 as appropriate. All spectra were obtained using EI, FAB, or CI ionization techniques using perfluorokerosene, 3-nitrobenzyl alcohol, or poly(ethylene glycol) as the internal standard. LOBAR Lichroprep (Merck) separations were performed with size B (310 × 25 mm) columns. Solvents used for extraction and flash chromatography were glass distilled prior to use. HPLC-grade solvents were utilized without further purification in LOBAR and HPLC separations. TLC analyses were performed with plastic-backed Si gel TLC sheets, eluting with different mixtures of MeOH in CH_2Cl_2. Plates were visualized by spraying with Dragendorff reagent. HPLC separations were performed either with a Waters quaternary pump 600, double beam UV detector 2487, and data module 746 or with a Waters autosampler 717, Waters 600 pump, and Waters 2996 photodiode array detector monitored by Waters Millenium 32. For HPLC-MS/MS (API-CID) analysis, fractions were dissolved in 2-propanol. The samples were injected into a HPLC System 1100 Series (Agilent) equipped with a photodiode array detector (Agilent) and a microTOF LC mass spectrometer (Bruker Daltonik). The detection with the DAD was performed at a wavelength of 260 nm. Mass spectra were acquired with an ESI source (Bruker Daltonik). The following ESI inlet conditions were applied: dry gas temperature 180 °C; dry gas flow 10 L/min; nebulizer pressure 1.5 bar; capillary voltage 4500 V. For fragmentation the voltage of the Capillary Exit was set to 150% of the normal voltage and the voltage of Skimmer 1 was set to 133% of the normal voltage.

Animal Material. The sponge *Pachychalina* sp. was collected in Ilha do Pai (Father's Island), Niterói, Rio de Janeiro (22°59.205′ S−43°05.252′ W), on May 9, 2000, at 10−15 m depth, and immediately immersed in EtOH. The whole material was shipped to the Instituto de Química de São Carlos, Universidade de São Paulo. Voucher specimens are deposited at the Museu Nacional (MNRJ 3098 and 3099).

Extraction and Isolation. The sponge (2.0 kg) was separated from the EtOH extract, blended in MeOH, and left overnight. After filtration of the MeOH extract, the solid material was re-extracted with MeOH. Both EtOH and MeOH extracts were pooled and evaporated until 500 mL of an aqueous suspension was obtained. The H_2O phase was partitioned with EtOAc. Then 25% NH_4OH was added to the H_2O phase until pH ~10, and the alkaline phase was partitioned with n-BuOH to yield 5.1 g of a brown gum after evaporation.

The EtOAc extract was evaporated, dissolved in 9:1 MeOH−H_2O, and partitioned with hexanes (5.9 g of a light brown gum). The aqueous MeOH extract was subsequently evaporated, and the gummy material was solubilized in CH_2Cl_2−0.5 N HCl. The CH_2Cl_2 fraction was collected and named CH_2Cl_2-acid (5.03 g of a light greenish gum). NaOH (1 N) was added to the aqueous fraction, which was partitioned with CH_2Cl_2. The fraction was named CH_2Cl_2-basic (0.87 g of a yellow gum). All extracts were analyzed by TLC and revealed the presence of alkaloids (Dragendorff).

The CH_2Cl_2-basic extract was fractionated by chromatography on a cyanopropyl-bonded Sep Pak column (10 g) with a gradient of MeOH in CH_2Cl_2 to give two fractions. The first fraction was separated by chromatography on a silica gel Sep Pak column (10 g) with a gradient of MeOH in CH_2Cl_2, to give four fractions. The second fraction was separated by chromatography on a silica gel Sep Pak column (10 g) with a gradient of 1:1 acetonitrile−MeOH in CH_2Cl_2, yielding seven fractions. The fourth one was shown to be pure by ^{1}H and ^{13}C NMR, giving 55.7 mg of compound **1** (0.0028% wet weight).

The n-BuOH extract was fractionated by chromatography on an amino-bonded Sep Pak column (10 g) with a gradient of MeOH in CH_2Cl_2. Five fractions were obtained. The second and the fourth fractions were subjected to identical separations by chromatography on Sephadex LH20 (1:1 CH_2Cl_2−MeOH). The second and third fractions of each of these last separations were shown to be identical by TLC. Therefore, these four

fractions were all pooled and further separated by chromatography on a cyanopropyl-bonded LOBAR column, with a gradient of MeOH in CH_2Cl_2. The first, fourth, and fifth fractions obtained from this last separation were purified by HPLC (column: C_{18} μBondapak, 7.8 × 300 mm, 10 μm 125 Å; eluents: 9:1 acetonitrile−H_2O or 7:3 MeOH−H_2O). The two fractions obtained, AmNL1FN5a (3.0 mg) and AmNL4a (3.0 mg), were further analyzed by HPLC-MS/MS. For the HPLC-MS separation, a XTerra RP-18 column (3.0 × 150 mm, 3.5 μm, Waters) was used. Separation was achieved by applying a 30 min gradient from 20% acetonitrile/80% formic acid (0.1% in water) to 80% acetonitrile/20% formic acid (0.1% in water), then isocratic conditions during 15 min. Total analysis time was 45 min with a flow rate of 0.4 mL/min and an oven temperature of 30 °C.

Ingenamine G (1): glassy solid; $[\alpha]^{29}_D$ −59.2 (*c* 0.050, MeOH); UV (MeOH) λ_{max} 230 (ϵ 2500) 283 (ϵ 625) nm; IR (film on a Si plate) ν_{max} 3390 (OH), 2929 (CH), 2858 (CH), 2554 (Bohlmann), 1660, 1447, 1023 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz), see Table 1; ^{13}C NMR (MeOH-d_4, 100 MHz), see Table 1; positive HRFABMS *m/z* 479.40007 $[M + H]^+$ (calcd for $C_{32}H_{52}N_2O$ 479.40014).

Cyclostellettamine G (10): UV (HPLC-DAD) λ_{max} 267 nm; positive ESI-MS *m/z* 239.2126 M^{2+} (calcd for $C_{33}H_{54}N_2$ 239.2138), Δm = 5.1 ppm. Results for the fragmentation: (a) *m/z* 232.2052 $[M - C_{17}H_{28}N]^+$ (calcd for $C_{16}H_{26}N$ 232.2060), Δm = 3.2; (b) *m/z* 246.2202 $[M - C_{16}H_{26}N]^+$ (calcd for $C_{17}H_{28}N$ 246.2216) Δm = 5.9.

Cyclostellettamine H (11): UV (HPLC-DAD) λ_{max} 267 nm; positive ESI-MS *m/z* 232.2051 M^{2+} (calcd for $C_{32}H_{52}N_2$ 232.2060), Δm = 3.6 ppm. Results for the fragmentation: (a) *m/z* 218.1905 $[M - C_{17}H_{28}N]^+$ (calcd for $C_{15}H_{24}N$ 218.1903), Δm = 0.7; (b) *m/z* 246.2216 $[M - C_{15}H_{24}N]^+$ (calcd for $C_{17}H_{28}N$ 246.2216), Δm = 6.7.

Cyclostellettamine I (12): UV (HPLC-DAD) λ_{max} 267 nm; positive ESI-MS *m/z* 239.2127 M^{2+} (calcd for $C_{33}H_{54}N_2$ 239.2138), Δm = 4.5 ppm. Results for the fragmentation: (a) *m/z* 218.1909 $[M - C_{18}H_{30}N]^+$ (calcd for $C_{15}H_{24}N$ 218.1903), Δm = 2.7; (b) *m/z* 260.2349 $[M - C_{15}H_{24}N]^+$ (calcd for $C_{18}H_{30}N$ 260.2373) Δm = 9.0.

Cyclostellettamine K (13): UV (HPLC-DAD) λ_{max} 267 nm; positive ESI-MS *m/z* 246.2198 M^{2+} (calcd for $C_{34}H_{56}N_2$ 246.2216), Δm = 7.2 ppm. Results for the fragmentation: (a) *m/z* 218.1907 $[M - C_{19}H_{32}N]^+$ (calcd for $C_{15}H_{24}N$ 218.1903), Δm = 1.5; (b) *m/z* 274.2499 $[M - C_{15}H_{24}N]^+$ (calcd for $C_{19}H_{32}N$ 274.2529) Δm = 11.2.

Cyclostellettamine L (14): UV (HPLC-DAD) λ_{max} 267 nm; positive ESI-MS *m/z* 253.2271 M^{2+} (calcd. for $C_{35}H_{58}N_2$ 253.2295), Δm = 9.3 ppm. Results for the fragmentation: (a) *m/z* 232.2057 $[M - C_{19}H_{32}N]^+$ (calcd for $C_{16}H_{26}N$ 232.2060), Δm = 1.0; (b) *m/z* 274.2507 $[M - C_{16}H_{26}N]^+$ (calcd for $C_{19}H_{32}N$ 274.2529) Δm = 8.0.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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