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XESTAMINES A, B, AND C, THREE NEW LONG-CHAIN METHOXYLAMINE PYRIDINES FROM THE SPONGE *XESTOSPONGIA WIEDENMAYERI*

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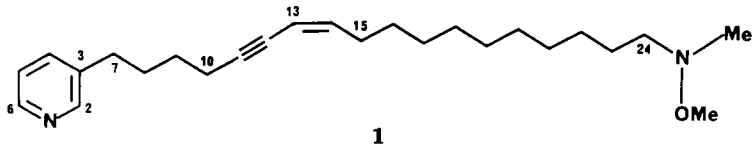
ABSTRACT.—Xestamines A [**1**], B [**2**], and C [**3**], which are pyridine alkaloids β-substituted by C_{18}, C_{14}, and C_{15} aliphatic methoxylamines, respectively, have been isolated from the marine sponge *Xestospongia wiedenmayeri*. The structures of these compounds are elucidated by a combination of spectral methods and chemical modification.

Marine sponges of the genus *Xestospongia* (Phylum Porifera, Class Demospongiae, Order Haplosclerida, Family Petrosiidae) have been known to contain a variety of unique alkaloids (1–4), terpenoids (5–7), terpenoid glycosides (8), pentacyclic quinones and hydroquinones (9–13), and straight-chain metabolites (14–19). We now report the isolation of three new alkaloids, xestamines A [**1**], B [**2**], and C [**3**], from our collection of *Xestospongia wiedenmayeri*, Van Soest (20). The xestamines, which all possess a long aliphatic chain terminated by a β-substituted pyridine and an unusual *N*-methyl-*N*-methoxylamine, are structurally related to the cytotoxic niphatynes A [**4**] and B from *Niphates* sp. (21); however, they were found inactive against P-388 cells in vitro.



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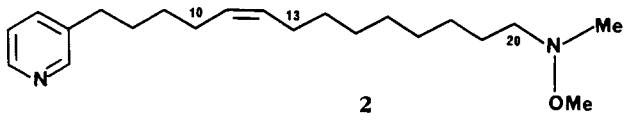

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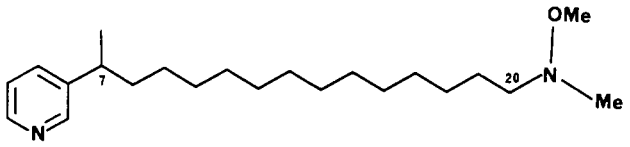

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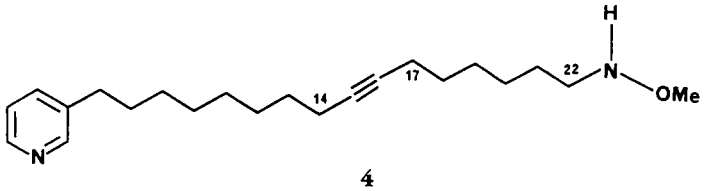

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Centrifugal countercurrent chromatography of the EtOAc extract followed by hplc yielded **1**, **2**, and **3**. The

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TABLE 1. ^{13}C- and ^{1}H-nmr Assignments for Xestamine A [**1**].[a]

Position	$^{13}C\delta$	$^{1}H\delta$	multiplicity, J (Hz)	Position	$^{13}C\delta$	$^{1}H\delta$	multiplicity, J (Hz)
2	149.8	8.38[b]	br s	15	29.9	2.20	br dt, 7.2
3	137.3	—	—	16	28.8	1.32	m
4	135.6	7.43[b]	br d, 7.7	17	29.5[c]		
5	123.1	7.13[b]	br dd, 7.7, 4.7	18	29.5[c]		
6	147.2	8.38[b]	br s	19	29.4[c]	1.20	br s (10H)
7	32.4	2.58	t, 7.6	20	29.3[c]		
8	30.0	1.70	m	21	29.1[c]		
9	28.1	1.52	m	22	27.3	1.25	m
10	19.2	2.32	dt, 1.9, 7.0	23	27.2	1.47	m
11	93.5	—	—	24	60.8	2.52	br t, 7.0
12	77.7	—	—	25-Me	45.1	2.49	s
13	109.1	5.35	br d, 10.6	25-OMe	59.9	3.44	s
14	142.7	5.75	dt, 10.6, 7.4				

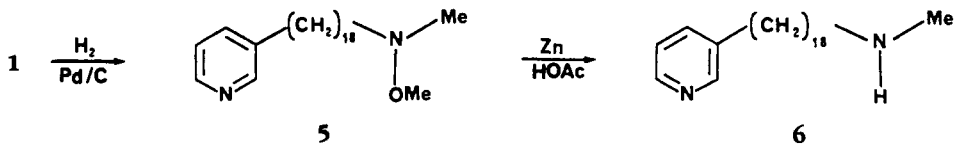
[a]Assignment based on the data from DEPT, COSY and HETCOSY experiments, recorded in $CDCl_3$, δ in ppm from TMS.

[b]δ (m, J) in CD_3OD: H-2, 8.38 (d, 1.8); H-4, 7.68 (dt, 7.7, 1.8); H-5, 7.34 (br dd, 7.7, 4.7); H-6, 8.35 (dd, 4.7, 1.8).

[c]Interchangeable.

molecular formula of **1** was established as $C_{25}H_{40}N_{20}O$ by high resolution fabms. The ^{1}H- and ^{13}C-nmr data, summarized in Table 1, showed the presence of a β-substituted pyridine, a disubstituted acetylene, a 1,2-disubstituted olefin, an *N*-methyl, a methoxyl, and 14 methylenes. The configuration of the double bond was assigned as *Z* by the coupling constant (10.6 Hz) between H-13 and H-14 and by the chemical shift value of C-15 (δ 29.9). The uv spectrum consisted of absorptions at 227 (ϵ 12,200) and 235 (shoulder) nm, indicative of a conjugated enyne chromophore, and absorptions at 257 (ϵ 2100), 263 (ϵ 2400) and 269 (ϵ 1600) nm, characteristic of a pyridine moiety (22). Catalytic hydrogenation of **1** yielded hexahydroxestamine A [**5**], confirming the enyne assignment (Scheme 1). The presence of a methoxyl group attached to a nitrogen was deduced from the lack of oxygen-bearing carbons in the ^{13}C-nmr spectrum in **1** and **5**. Treatment of **5** with zinc in HOAc resulted in the removal of the methoxyl group to yield demethoxyhexahydroxestamine A [**6**] (Scheme 1). The conversion of **5** to **6** was accompanied by the formation of an NH signal observed at δ 3.92 in the ^{1}H-nmr spectrum which was coupled to an *N*-methyl observed at δ 2.53 in **6**. This substantiated the *N*-methoxyl assignment and also illustrated the existence of an *N*-methyl-*N*-methoxylamine moiety in **1**. Finally, the H-H and C-H connectivity deduced from 2D COSY and HETCOSY (23) spectra, the latter as depicted in Figure 1, established the structure of xestamine A as **1**.

The molecular formulas of xestamines B and C were determined by high resolution fabms as $C_{21}H_{36}N_2O$ and $C_{22}H_{40}N_2O$, respectively. By the ^{1}H and ^{13}C analyses, both compounds appeared to possess the characteristic terminal β-substituted pyridine and *N*-



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SCHEME 1

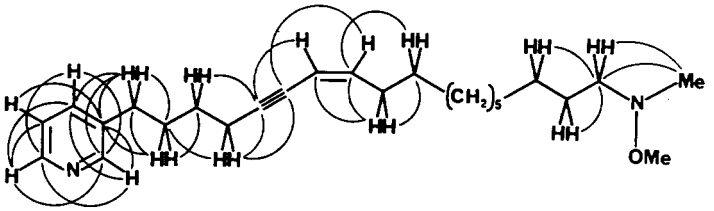

FIGURE 1. Long-range C-H correlation from HETCOSY.

methyl-*N*-methoxyl amine moieties, analogous to **1**. A C_{14} straight chain (C-7–C-20) containing a double bond was found in **2**. The proton homonuclear correlations from a COSY spectrum of **2** yielded the connectivity from C-2 to C-13. The two overlapped olefinic proton resonances at δ 5.30 and 5.35 are sufficiently distinct in the COSY spectrum to place the double bond between C-11 and C-12. The chemical shifts of C-10 (δ 29.5) and C-13 (δ 29.7) led to the assignment of *Z* configuration for the double bond. A fully saturated C_{15} straight chain containing a branched methyl group was suggested for **3** by the nmr data. The observation of a 7.1 Hz coupling between the methyl at δ 1.27 and the methine at δ 2.75 in the ^{1}H-nmr spectrum, and the detection of a long-range coupling between the same methine and the two aromatic protons, H-2 and H-4, from a COSY experiment, indicated that the methyl was attached to C-7. On the basis of the above data, xestamines B and C are represented by structures **2** and **3**, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were determined on a Perkin-Elmer 1310 spectrophotometer as KBr disks. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM 360 spectrometer. Chemical shifts are reported as δ values in ppm relative to TMS (solvent peaks used as references). Uv spectra were recorded on a Perkin Elmer Lambda 3 uv/vis spectrophotometer. Mass spectra were recorded on Finnigan-MAT CH5 and 731 mass spectrometers. Centrifugal countercurrent chromatography was performed on Ito multi-layer coil separator-extractor (P.C. Inc.). Hplc was performed on 7μ 250 × 10 mm Dynamax C-18, Hibar NH_2, and Si columns by a Bio-Rad hplc pump 1330, equipped with a Bio-Rad refractive index monitor and an ISCO V absorbance detector. All solvents are hplc grade and chemicals are reagent grade.

COLLECTION AND EXTRACTION OF SPONGE.—The sponge, *X. wiedenmayeri*, was collected by scuba diving on June 16, 1985, from the southwestern tip of Acklin Island, Bahamas at a depth of 21 to 27 m on a fore reef slope. A taxonomic voucher specimen is deposited at Harbor Branch Oceanographic Institution, Indian River Coastal Zone Museum, catalog number 003:00049 (DBMR #16-VI-85-3-16). The sponge (75 g wet wt) was stored frozen and extracted with MeOH-$CHCl_3$ (2:1) (200 ml × 3). The extract was concentrated to an aqueous suspension in vacuo at 40°, followed by EtOAc extraction (70 ml × 3) to afford a dark brown oil (662 mg).

XESTAMINE A [**1**].—The EtOAc extract (600 mg) was fractionated by centrifugal countercurrent chromatography using heptane-CH_2Cl_2-MeCN (10:3:7) (lower phase stationary). Hplc of fraction 5 with a combination of Si gel [heptane-EtOAc (1:1)] and reversed-phase C_{18} columns (MeOH) yielded xestamine A [**1**] (51 mg, 0.075% of wet wt): colorless oil; hrfabms $[MH]^+$ 385.3215 (calcd for $C_{25}H_{40}N_2O$ + H, Δ 0.4 mmu); lreims (rel. %) $[M]^+$ 384 (5), $[M-OMe]^+$ 353 (100), 339 (3), 327 (22), 325 (5), 313 (5), 310 (4), 301 (9), 299 (4), 287 (4), 240 (1), 226 (3), 212 (6), 198 (4), 184 (5), 178 (6), 165 (5), 156 (4), 149 (11), 134 (6), 120 (14), 106 (35), 93 (48), 74 (94); ir (film) 3020, 2930, 2855, 2210 (weak), 1573, 1478, 1462, 1441, 1422, 1049, 1025, 794, 740, 715 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1.

XESTAMINE B [**2**].—Fractions 3 (47 mg) and 4 (78 mg) were combined and chromatographed sequentially on hplc reversed-phase C_{18} (MeOH), Si gel [heptane-EtOAc (2:1)], and amino columns [heptane-EtOAc (5:2)] to give pure xestamines B [**2**] (0.0059%) and C [**3**] (0.0073%). Xestamine B [**2**]: colorless oil; hrfabms $[MH]^+$ 333.2916 (calcd for $C_{21}H_{36}N_2O$ + H, Δ 1.0 mmu); lreims (rel. %) $[M]^+$ 332 (0.5), 315 (3), $[M-OMe]^+$ 301 (100), 289 (17), 275 (7), 272 (5), 258 (7), 246 (2), 244 (2), 232 (1), 230 (1),

216 (1), 202 (1), 188 (2), 176 (1), 174 (1), 162 (1), 148 (2), 134 (2), 120 (6), 106 (16), 93 (19), 74 (44); uv λ max (MeOH) 205 (ε 9700), 257 (ε 4300), 262 (ε 4900), 269 (ε 3700); ir (KBr) 3005 (sh), 2925, 2855, 1572, 1475 (sh), 1460, 1440, 1422, 1370, 1048, 1026, 795, 724 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.45 (2H, br s, H-2, H-6), 7.57 (1H, br d, J = 7.7 Hz, H-4), 7.27 (1H, dd, J = 7.7, 4.8 Hz, H-5), 5.30 (1H, m, H-12), 5.35 (1H, m, H-11), 3.51 (3H, s, 21-OMe), 2.62 (2H, t, J = 7.8 Hz, H-7), 2.58 (2H, br t, J = 7.6 Hz, H-20), 2.55 (3H, s, 21-Me), 2.04 (2H, td, J = 7.0, 7.0 Hz, H-10), 1.99 (2H, br td, J = 6.6, 6.6 Hz, H-13), 1.63 (2H, m, H-8), 1.52 (2H, m, H-19), 1.38 (2H, m, H-9), 1.31 (4H, br s, H-14, H-18), 1.27 (6H, br s, H-15–H-17); ^{13}C nmr ($CDCl_3$) δ 148.5 (d, C-2), 145.9 (d, C-6), 138.6 (s, C-3), 137.1 (d, C-4), 130.5 (d, C-12), 129.1 (d, C-11), 123.7 (d, C-5), 60.9 (t, C-20), 60.1 (q, 21-OMe), 45.2 (q, 21-Me), 32.9 (t, C-7), 30.6 (t, C-8), 29.7 (t, C-13), 29.5 (t × 2, C-10), 29.3 (t), 29.2 (t), 27.4 (t), 27.2 (t × 2, C-9, C-19), 26.9 (t).

XESTAMINE C [**3**].—Colorless oil $[\alpha]^{20}D$ −12.9° (c = 0.34, MeOH); hrfabms $[MH]^+$ 349.3217 (calcd for $C_{22}H_{40}N_2O$ + H, Δ 0.2 mmu); lreims (rel. %) $[M]^+$ 348 (0.1), 331 (1), $[M-OMe]^+$ 317 (100), 301 (4), 288 (4), 286 (2), 274 (6), 260 (2), 246 (1), 232 (1), 218 (1), 204 (1), 190 (1), 176 (1), 162 (1), 148 (1), 134 (3), 120 (12), 106 (23), 92 (4), 74 (30); uv λ max (MeOH) 205 (ε 5200), 256 (ε 2700), 262 (ε 3100), 269 (ε 2300) nm; ir (KBr) 3010 (sh), 2920, 2850, 1573, 1460, 1422, 1372 (br), 1318, 1048, 1023, 808, 717 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.48 (2H, br s, H-2, H-6), 7.61 (1H, br d, J = 7.9 Hz, H-4), 7.32 (1H, br dd, J = 7.9, 5.0 Hz, H-5), 3.53 (3H, s, 21-OMe), 2.75 (1H, tq, J = 7.1, 7.1 Hz, H-7), 2.61 (2H, br t, J = 7.5 Hz, H-20), 2.57 (3H, s, 21-Me), 1.58 (2H, m, H-8), 1.55 (2H, m, H-19), 1.27 (3H, d, J = 7.1 Hz, 7-Me), 1.35–1.15 (20H, br s, H-9–H-18); ^{13}C nmr ($CDCl_3$) δ 147.6 (d), 145.8 (d), 143.8 (s), 135.7 (d), 123.9 (d), 61.0 (t), 60.0 (q), 45.2 (q), 38.1 (t), 37.7 (d), 29.6 (t × 8), 27.5 (t), 27.4 (t), 27.3 (t), 21.9 (t).

CATALYTIC HYDROGENATION OF **1**.—A solution of **1** (10 mg) in 2 ml of absolute EtOH was treated with 10 mg of 10% Pd/C under H_2 at room temperature for 4 h. After filtration and removal of solvent, the crude gummy product was chromatographed by hplc with an NH_2 column using heptane-EtOAc (3:1) giving pure hexahydroxestamine A [**5**] (4 mg): colorless oil; hrfabms $[MH]^+$ 391.3678 (calcd $C_{25}H_{46}N_2O$ + H, Δ 1.0 mmu); lreims (rel. %) $[M]^+$ 390 (1), $[M-OMe]^+$ 359 (100), 343 (4), 331 (27), 317 (8), 316 (7), 302 (5), 288 (3), 274 (3), 260 (3), 246 (3), 232 (3), 218 (4), 204 (4), 190 (3), 176 (3), 162 (5), 148 (5), 134 (5), 120 (14), 119 (11), 106 (43), 93 (41), 74 (61), 70 (68), 64 (95); uv λ max (MeOH) 205 (ε 5300), 257 (ε 2700), 263 (ε 3100), 269 (ε 2200) nm; ^1H nmr ($CDCl_3$) δ 8.42 (2H, br s), 7.50 (1H, br d, J = 7.7 Hz), 7.21 (1H, dd, J = 7.7, 5.0 Hz), 3.50 (3H, s), 2.58 (4H, m), 2.54 (3H, s), 1.59 (2H, m), 1.51 (2H, m), 1.28 (8H, br s), 1.23 (20H, br s); ^{13}C nmr ($CDCl_3$) δ 149.5 (d), 146.7 (d), 138.2 (s), 136.2 (d), 123.4 (d), 61.0 (t), 60.0 (q), 45.2 (q), 33.0 (t), 31.1 (t), 29.6 (t × 10), 29.5 (t), 29.4 (t), 29.1 (t), 27.4 (t), 27.3 (t).

DEMETHOXYLATION OF **5**.—A solution of **5** (2.5 mg) in 1 ml of 85% aqueous HOAc was treated with 5 mg of zinc dust at 50° for 5 h. After filtration and elimination of solvent, the oily product was partitioned between $CHCl_3$ and 0.1 N KOH aqueous solution. Demethoxylhexahydroxestamine A [**6**] (2.2 mg) was obtained from the $CHCl_3$ phase: colorless oil; hreims $[M]^+$ 360.3487 (calcd for $C_{24}H_{44}N_2$, Δ 0.7 mmu); lreims (rel. %) $[M]^+$ 360 (17), 359 (22), 345 (13), 330 (17), 318 (14), 317 (58), 316 (21), 302 (10), 288 (10), 274 (18), 260 (11), 246 (9), 232 (10), 218 (11), 204 (9), 190 (8), 176 (8), 162 (13), 148 (10), 134 (6), 120 (14), 106 (95), 93 (100); ^1H nmr ($CDCl_3$) δ 8.42 (2H, br s), 7.47 (1H, br d, J = 7.8 Hz), 7.18 (1H, br dd, J = 7.8, 4.8 Hz), 3.92 (1H, br, D_2O exchangeable), 2.76 (2H, br t, J = 7.4 Hz), 2.58 (2H, t, J = 7.5 Hz), 2.53 (3H, br s, sharpened by D_2O exchange), 1.66 (2H, m), 1.59 (2H, m), 1.28 (8H, br s), 1.24 (20H, br s).

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