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ANTIMICROBIAL N-METHYLPYRIDINIUM SALTS RELATED TO
THE XESTAMINES FROM THE CARIBBEAN SPONGE
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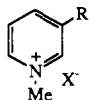
ABSTRACT.—Two antimicrobial fractions were obtained from the sponge *Calyx podatypa* from the Bahamas. The less polar fraction contained the known compounds xestamines A [1] and B [2] and three new metabolites called xestamines D [3], E [4], and F [5]. The more polar fraction consisted of a mixture of xestamines G [6] and H [7].

Cytotoxic alkaloids containing a 3-substituted pyridine ring system have been reported from several sponges. These include the halitoxins from *Haliclona* spp. (1), the niphatynes, which are methoxylamines related to the xestamines, from a Fijian species of *Niphates* (2), and the xestamines 1 and 2 from a Caribbean species of *Xestospongia* (3). Further examples of 3-substituted pyridines have recently been reported from an unidentified Micronesian sponge (4). In this paper we report the isolation and identification of two new 3-substituted pyridines, xestamines D [3] and E [4], together with a series of N-methyl salts, xestamines F [5], G [6], and H [7], from *Calyx podatypa* de Laubenfels (Haplosclerada) collected at Jamaica Bay, Acklins Island, Bahamas at a depth of 28 m.

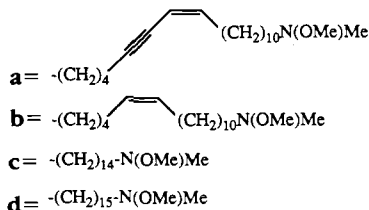
Candida albicans and Gram positive bacteria. The antimicrobial activity in the aqueous fraction could be extracted into MeOH by lyophilization and trituration of the residue. The EtOAc extract was chromatographed on Sephadex LH-20 using MeOH-CH₂Cl₂ (1:1) as eluent, and final separation was achieved by using centrifugal counter current chromatography (cccc) [heptane-MeCN-CH₂Cl₂ (50:35:15)] to obtain xestamine A [1] (0.17% dry wt), xestamine B [2] (0.06% dry wt), a 2:1 mixture of xestamines D [3] and E [4] (0.08% dry wt), and xestamine F [5] (0.06% dry wt). Chromatography of the MeOH-soluble material on Sephadex LH-20 using MeOH as eluent resulted in the isolation of a mixture of xestamine G [6] and xestamine H [7] (0.12% dry wt). All compounds were isolated as oils.



1 R = a
2 R = b
3 R = c
4 R = d



5 R = a
6 R = c
7 R = d



The MeOH extract of the sponge *Calyx podatypa* was partitioned between EtOAc and H₂O. Both fractions showed strong antimicrobial activity against

Xestamines A [1] and B [2] were identified by comparison of spectral data with literature values (3). The ca. 2:1 mixture of xestamines D [3] and E [4] could only be separated by gc. GC-MS analysis indicated that 3 and 4 were homologues with molecular formulae of C₂₁H₃₈N₂O and C₂₂H₄₀N₂O,

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respectively. The ^1H -nmr spectrum contained signals at δ 8.43 (br s, 2H), 7.52 (d, 1H, $J = 6.8$ Hz) and 7.21 (m, 1H), due to the 3-substituted pyridine ring, and at 3.55 (s, 3H, OMe), 2.62 (t, 2H, $J = 7$ Hz, ArCH₂), 2.60 (s, 3H, NMe), and a broad signal at ca. 1.20–1.60 due to the methylene groups in the alkyl chains. These data, together with the ^{13}C -nmr data, allowed the structural assignments of xestamines D [3] and E [4].

The molecular formula of xestamine F [5], C₂₆H₄₃N₂O, was established by hrfabms. It is assumed that the counter ion in the natural product is chloride. The ^1H -nmr spectrum of 5 was very similar to that of xestamine A [1] (3), except for the addition of an *N*-methyl signal at δ 4.40 (s, 3H) and the downfield shift of the aromatic signals to 8.84 (br s, 1H), 8.75 (br d, 1H, $J = 6.8$ Hz), 8.43 (br d, 1H, $J = 7.8$ Hz) and 7.99 (br t, 1H, $J = 7$ Hz), which indicated that xestamine F [5] was the *N*-methyl pyridinium salt of xestamine A [1]. Reaction of xestamine A [1] with MeI (neat) gave xestamine F [5] as the iodide salt.

The mixture of xestamine G [6], C₂₂H₄₁N₂O, and xestamine H [7], C₂₃H₄₃N₂O, gave two hrfabms peaks at m/z 349.3227 and 363.3366, indicating that the mixture might consist of *N*-methyl pyridinium salts of xestamines D [3] and E [4], respectively. The possibility that the peak at m/z 349 represents a fragment ion resulting from loss of CH₂ from the molecular ion at m/z 363 can be discounted because the hrfabms spectrum of xestamine F [5] shows no such fragmentation. The ^1H - and ^{13}C -nmr spectral data are completely in accord with the structural assignments.

Comparison of the antimicrobial properties of the xestamines using a standard 7-mm disk assay revealed that the *N*-methyl pyridinium salts were ca. 100 times more active than the unsubstituted pyridines (5: *Staphylococcus aureus*, 9 mm at 5 $\mu\text{g}/\text{ml}$, *Bacillus subtilis*,

8 mm at 5 $\mu\text{g}/\text{ml}$, *C. albicans*, 10 mm at 10 $\mu\text{g}/\text{ml}$; 6,7: *S. aureus*, 11 mm at 5 $\mu\text{g}/\text{ml}$, *B. subtilis*, 8 mm at 5 $\mu\text{g}/\text{ml}$, *C. albicans*, 13 mm at 5 $\mu\text{g}/\text{ml}$, 3,4: *S. aureus*, 8 mm at 200 $\mu\text{g}/\text{ml}$, *B. subtilis*, 8 mm at 200 $\mu\text{g}/\text{ml}$, *C. albicans*, inactive at 200 $\mu\text{g}/\text{ml}$). In contrast, the mixture of xestamines G and H (LD₅₀ 3×10^{-5} M) was ca. 100 times less active than the mixture of xestamines D and E (LD₅₀ $< 1 \times 10^{-6}$ M) in the brine shrimp cytotoxicity assay (5).

EXPERIMENTAL

COLLECTION, EXTRACTION AND ISOLATION PROCEDURES.—The sponge *C. podatypa* (234 g dry wt, collection #88-242, SIO Benthic Invertebrate collection #P1109) was collected by hand using SCUBA (–28 m) at Jamaica Bay, Acklins Island, Bahamas. The sponge was immediately frozen and was stored at –20° until it was extracted with MeOH. The MeOH extract was concentrated in vacuo to obtain an aqueous suspension that was partitioned between EtOAc and H₂O. Both fractions showed strong antimicrobial activity against *C. albicans* and Gram positive bacteria. The aqueous fraction was lyophilized, and the residue was triturated with MeOH to obtain an antimicrobial MeOH-soluble fraction. The EtOAc-soluble material (3.3 g) was chromatographed on Sephadex LH-20 using MeOH-CH₂Cl₂ (1:1) as eluent, and final separation was achieved by using (ccc) [heptane-MeCN-CH₂Cl₂ (50:35:15)] to obtain xestamine A [1] (390 mg, 0.17% dry wt), xestamine B [2] (150 mg, 0.06% dry wt), a 2:1 mixture of xestamines D [3] and E [4] (196 mg, 0.08% dry wt), and xestamine F [5] (150 mg, 0.06% dry wt). Chromatography of the MeOH-soluble material on Sephadex LH-20 using MeOH as eluent resulted in the isolation of a mixture of xestamine G [6] and xestamine H [7] (280 mg, 0.12% dry wt).

MIXTURE OF XESTAMINE D [3] AND XESTAMINE E [4].—Oil: ir (CHCl₃) 2918, 2842, 1571, 1460, 1046, 710 cm⁻¹; uv (MeOH) 269 nm (ϵ 1800), 262 (2500), 257 (2200), 226 (8000); ^1H nmr (CDCl₃) δ 8.43 (br s, 2H), 7.52 (d, 1H, $J = 6.8$ Hz), 7.21 (m, 1H), 3.55 (s, 3H), 2.62 (m, 2H), 2.60 (s, 3H); ^{13}C nmr (CDCl₃) δ 149.8 (d), 147.2 (d), 138.0 (s), 135.6 (d), 123.2 (d), 60.9 (t), 59.9 (q), 45.1 (q), 38.0 (t), 36.8 (t), 36.4 (t), 33.2 (t), 31.0 (t), 29.8 (t), 29.5 (3-4t), 29.0 (t), 27.3 (t), 27.2 (t), 26.9 (t); hreims 3 m/z [M – OMe]⁺ 303.2801 (C₂₀H₃₅N₂ requires 303.2800), 4 m/z [M – OMe]⁺ 317.2960 (C₂₁H₃₇N₂ requires 317.2957).

XESTAMINE F [5].—Oil: ir (film) 2960,

2822, 1632, 1503, 1460, 1045 cm^{-1} ; ^1H nmr (MeOH- d_4) δ 8.84 (br s, 1H), 8.75 (d, 1H, $J = 6.8$ Hz), 8.43 (d, 1H, $J = 7.8$ Hz), 7.99 (t, 1H, $J = 7$ Hz), 5.80 (dt, 1H, $J = 10.6, 7.4$ Hz), 5.40 (br d, 1H, $J = 10.6$ Hz), 4.40 (s, 3H), 3.49 (s, 3H), 2.87 (t, 2H, $J = 7.0$ Hz), 2.59 (t, 2H, $J = 7.0$ Hz), 2.54 (s, 3H), 2.30 (m, 4H); ^{13}C nmr (MeOH- d_4) δ 144.2 (2d), 144.0 (2d), 143.0 (s), 128.6 (d), 110.7 (d), 95.2 (s), 78.5 (s), 61.8 (t), 60.1 (q), 48.8 (q), 45.4 (q), 33.5 (t), 31.5 (t), 30.9 (t), 29.8 (6t), 28.4 (t), 28.2 (t), 27.9 (t), 20.0 (t); hrfabms m/z 399.3399 ($\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}$ requires 399.3375).

MIXTURE OF XESTAMINE G [6] AND XESTAMINE H [7].—Oil: ir (film) 2960, 2822, 1632, 1503, 1460 cm^{-1} ; ^1H nmr (MeOH- d_4) δ 8.86 (br s, 1H), 8.76 (d, 1H, $J = 6.5$ Hz), 8.43 (d, 1H, $J = 8.0$ Hz), 8.00 (t, 1H, $J = 7$ Hz), 4.42 (s, 3H), 3.49 (s, 3H), 2.87 (t, 2H, $J = 7.0$ Hz), 2.59 (t, 2H, $J = 7.0$ Hz), 2.54 (s, 3H); ^{13}C nmr (MeOH- d_4) δ 146.1 (2d), 145.2 (s), 144.1 (d), 128.6 (d), 61.7 (t), 60.1 (q), 48.8 (q), 45.4 (q), 38.1 (t), 33.8 (t), 33.5 (t), 31.5 (t), 29.8 (7-8t), 28.1 (t), 28.0 (t), 27.9 (t); hrfabms m/z 349.3227 ($\text{C}_{22}\text{H}_{41}\text{N}_2\text{O}$ requires 349.3219), **7** m/z 363.3366 ($\text{C}_{23}\text{H}_{43}\text{N}_2\text{O}$ requires 363.3375).

CONVERSION OF XESTAMINE A [1] INTO XESTAMINE F [5].—A solution of xestamine A [1] (10 mg) in excess MeI (1 ml) was stirred at 25° for 2 h. The excess MeI was evaporated to obtain a

quantitative yield of the iodide salt of xestamine F [5], which gave ^1H -nmr spectrum identical to that of the natural product, which is assumed to be the chloride salt.

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