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NOVEL MARINE SPONGE AMINO ACIDS, 10.[1] XESTOAMINOLS FROM *XESTOSPONGIA* SP.

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ABSTRACT.—The study of the anthelminthic components from a Fiji sponge *Xestospongia* sp. has yielded new amino alcohols, xestoaminols A–C. Xestoaminol B, (2*S**)-aminotetradeca-11,13-dien-(3*R**)-ol [**2**], is isomeric to known *Xestospongia* products, (2*S*)-aminotetradeca-5,7-dien-(3*R*)-ol [**6**] and (2*S*)-aminotetradeca-5,7-dien-(3*S*)-ol [**7**], recently reported by Gulavita and Scheuer. Xestoaminols A [**1**] and C [**3**] are, respectively, the dihydro and tetrahydro derivatives of xestoaminol B. A combined nmr and molecular mechanics study on the oxazolidinone of xestoaminol A provided the basis for the relative stereochemistry assigned at C-2 and C-3 in xestoaminol A. This compound was extremely active in assays testing for action against parasites, microbes, and reverse transcriptase.

We have a continuing interest in the chemistry of red/orange Indo-Pacific sponges. The secondary metabolites from such taxa have included a rich array of anthelmintic nitrogen derivatives. These range from ketide-amino acids such as jasplakinolide (1,2) [=jaspamide (3)], the bengamides (4), and bengazoles (5), through alkaloids such as the plakinidines (6), to nitrogen-containing terpenes such as amino bisabolenes (7,8), isokalihinol F (9), and the isothiocyanato amorphenes (10). Two different species of a soft, orange *Xestospongia*, easily distinguished by a dissimilarity in their compressibility, were collected from a variety of habitats in Fiji. Species A, in shipboard tests of its aqueous and nonaqueous exudates, showed strong activity against Gram-negative bacteria. Similarly, species B yielded an organic extract that showed broad-spectrum activity against a parasite, microbes, and reverse transcriptase. The components of species A proved to be unstable mixtures of polyunsaturated branched lipid alcohols, whereas species B yielded tractable products consisting of amino alcohols and xestoaminols A [**1**], B [**2**], and C [**3**]. Xestoaminols A and C are, respectively, the dihydro and tetrahydro derivatives of xestoaminol B, which is isomeric to (2*S*)-aminotetradeca-5,7-dien-(3*R*)-ol [**6**] and (2*S*)-aminotetradeca-5,7-dien-(3*S*)-ol [**7**], just reported by Gulavita and Scheuer (11) from a Papua-New Guinea *Xestospongia* sp.

The xestoaminols A–C were efficiently isolated by a bioassay-guided fractionation. Thus, an *n*-BuOH fraction, generated by solvent partioning of the crude oil from species B, exhibited high in vitro anthelminthic and antimicrobial activity. Further chromatographic purification (Sephadex LH-20 followed by reversed-phase hplc) yielded all three metabolites.

The molecular formula, $C_{14}H_{29}NO$, for xestoaminol A [**1**], the major component, was deduced from hreims *m/z* 227.2251 (Δ −0.9 mmu of calcd). The termini of this monounsaturated acyclic molecule were proposed as CH_3-$CH(NH_2)$-$CH(OH)$-CH_2- and CH_3-CH_2-$CH{=}CH$-CH_2- by inspection of ^{13}C-^1H and ^1H-^1H- COSY nmr spectra. The *E* double bond geometry was established by comparing the ^{13}C-nmr shifts at C-10 and C-13 to those of (*E*)-3-octene (12) and the xestins (13) along with the observed $^3J_{11-12} = 15.3$ Hz. Treatment of xestoaminol A [**1**] with Ac_2O or with 1,1-carbonyl-di-imidazole (14) afforded a diacetate **4** or an oxazolidinone **5**. The relative cis H-2/3 stereochemis-

[1]For Part 9, see W.D. Inman, P. Crews, and R. McDowell, *J. Org. Chem.*, **54**, 2523 (1989).

1 R1=R2=H
4 R1=R2=Ac
2
3
5
6
7

try in the oxazolidinone **5** was assigned by comparing the measured $^3J_{2-3} = 7.5$ Hz (obtained while spin-decoupling at Me-1) to values obtained from molecular mechanics calculations on the oxazolidinone **5**. The best match was proved by the calculated J's of the cis isomer, which were $^3J_{2-3} = 7.0$–7.8 Hz (at dihedral angles of 2–22°), whereas the trans isomer had $^3J_{2-3} = 4.1$–5.1 (at angles of 118–124°). These results can be used to propose *2S**, *3R** stereochemistry for xestoaminol A [**1**].

The two other metabolites, xestoaminols B [**2**] and C [**3**], were recognized as, respectively, the bis-unsaturated and saturated analogues of xestoaminol A [**1**]. In particular, a terminal diene was revealed by the APT ^{13}C- and ^1H-nmr spectra of xestoaminol B [**2**] ($C_{14}H_{27}NO$ established by hreims *m/z* 225.2086, Δ 0.0 mmu of calcd). Alternatively, the fully saturated nature of xestoaminol C [**3**] was evident from its molecular formula of $C_{14}H_{31}NO$ (established by hreims *m/z* 229.2395, Δ −0.3 mmu of calcd). The analogous shifts at C-2 and C-3, in the series xestoaminols A–C, were the basis for the erythro stereochemistry at C-2/C-3 shown for the latter compounds, and the C-10 shift of xestoaminol B [**2**] and $^3J_{11-12} = 15.0$ Hz were indicative of the *E*-11/12 stereochemistry.

All of the xestoaminols were evaluated against a helminth model *Nippostrongylus brasiliensis*, but only xestoaminol A [**1**] was active (with 100% reduction of viability and motility and 90% reduction of casts).[2] Likewise only xestoaminol A [**1**] showed potent antimicrobial activity [at 100 μg/disk with inhibition zone size in mm] against Gram-positive bacteria [*Staphylococcus aureus* (3), *Streptococcus pyogenes* (4)], Gram-negative bacteria [*Escherichia coli* (7)], and fungi [*Candida albicans* (7) and *Trichophyton mentagrophytes* (13)]. Alternatively, both xestoaminols A [**1**] and C [**3**] were active in assay[3] against reverse transcriptase as they exhibited, respectively, 97% and 95% inhibition at 1 mg/ml. Finally, an *S* stereochemistry can be inferred at C-2 in the xestoaminols A–C as they fit the biogenesis proposed by Gulavita and Scheuer (11) for **6** and **7** in which unbranched C-11 fatty acids are united with L-alanine.

EXPERIMENTAL

Nmr spectra were recorded on a JOEL FX-100 PFT spectrometer (99.5 MHz for ^1H and 25.0 MHz for ^{13}C) or on a GN-300 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C). Low resolution ms data were obtained on a Finnigan 4000 (6000 LS7 computer system), while high resolution mass spectra were obtained from the University of California, Berkeley. Hplc was done using a Regis 10μ-ODS or 10μ Si gel column (25 × 1.0 cm). All solvents were distilled and dried for hplc and were spectral grade for spectroscopy. Rotations were measured on a Perkin-Elmer 141 polarimeter.

TWO-DIMENSIONAL NMR PROCEDURES.—Standard pulse sequences were used for the ^1H-^1H COSY, ^1H-^{13}C COSY, and long range ^1H-^{13}C COSY experiments (15,16). Regular homo and hetero COSY as well as long range experiments were performed on xestoaminol A [**1**] and its oxazolidinone **5**.

IDENTIFICATION.—The sponge (collection no. 87126) *Xestospongia* sp. (Family Petrosiidae, Order Nepheliospongida, voucher specimens and

[2]These results were provided by Dr. Tom Matthews (Syntex Research, Palo Alto, CA) and his staff. Compounds were tested in the parasite assay at 50 μg/ml against the fourth larval stage of *N. brasiliensis*. Parameters examined are reduction of motility, viability, and the ability of the larvae to molt to the adult (cast formation). Positive activity is scored when cast formation is reduced 40% or more, or when viability and motility together are reduced 40% or more. Levamisole (ca. 5.0 μg/ml) is the positive control and it shows 100% reduction of casts, viability, and motility. Compounds were tested in the antimicrobial screens at 100 μg/disk, and positive controls at 10 μg/disk [inhibition zone size in mm] are: gentamicin; *S. aureus* [11], *S. pyogenes* [10], *E. coli* [10] and myconazole; *C. albicans* [10], *T. mentagrophytes* [11].

[3]These results were also provided by Dr. Tom Matthews (Syntex Research, Palo Alto, CA) and his staff.

underwater photo available from P.C.) was identified by Ms. M.C. Diaz (UCSC, Institute of Marine Sciences). This organism can be regularly collected from turbid waters in Fiji or Tonga. Our voucher specimen no. 87126 was carefully examined and displays the following characteristics. Color apricot-orange alive, tan in spirit; shape thick-encrusting; consistency crumbly and compressible; surface smooth but very irregular; ectosome thick detachable layer, usually comes with part of choanosome when detached, more unispicular than polyspicular, isodictyal reticulations; spicules fusiform oxea (170–200) × (2–5) μ; association calcareous skeleton within the sponge. It appears to be an atypical *Xestospongia* and cannot be identified to the species level.

ISOLATION PROCEDURES.—The sponge (4.5 kg) was preserved for a short period before extraction in MeOH (twice) followed by concentration of the crude extract to yield 26.8 g of a crude oil. This was successively partitioned between aqueous MeOH (percent adjusted to produce a biphase solution) and this series: hexanes (0.83 g), CCl_4 (1.5 g), CH_2Cl_2 (2.6 g), and *n*-BuOH (3.0 g) (17). The bioassay of these fractions showed that the *n*-BuOH was the most active (against *N. brasiliensis* at 50 μg/ml, % reductions were: cast 90, viability 100, and motility 100). It was chromatographed on Sephadex LH-20 (MeOH) followed by reversed-phase hplc [MeOH-H_2O (65:35)] to give semipure xestoaminols A–C in the ratio 14:1:2. These were separately rechromatographed on reversed-phase hplc [with MeOH-H_2O (45:55) and a flow of 2 ml/min] to give the three products **1** (Rt = 40 min), **2** (Rt = 30 min), and **3** (Rt = 57 min).

XESTOAMINOL A [**1**].—(2*S**)-Aminotetradeca-11-en-(3*R**)-ol: oil; $[\alpha]_D$ +7.7° (*c* = 0.26, MeOH); ir (neat) 3360, 2931, 1607, 1507, 1460, 1000 cm^{-1}; hreims *m/z* 227.2251 (calcd for $C_{14}H_{29}NO$, 227.2242); lreims *m/z* (rel. int.) $[M]^+$ 227 (28), $[M-H_2O]^+$ 208 (3), 149 (5), 95 (21), 74 (100), 70 (56); lrcims (isobutane) *m/z* (rel. int.) $[M+H]^+$ 228 (100), $[M-H_2O]^+$ 210 (48); ^1H nmr ($CDCl_3$) δ 7.86 (2H, br s, NH_2), 5.30–5.39 (2H, m, H-11, H-12), 3.95 (1H, m, H-3), 3.39 (1H, m, H-2), 1.94 (4H, m, H_2-10, H_2-13), 1.41 (2H, m, H-4), 1.28 (10H, m, H_2-5 to H_2-9), 1.20 (3H, d, *J* = 6.6 Hz, Me-1), 0.92 (3H, t, *J* = 7.5 Hz, Me-14); ^1H nmr (CD_3OD) δ 5.40 and 5.38 (1H each, dt, *J* = 15.3, 5.1, 5.1 Hz, H-11, H-12), 3.68 (1H, m, H-3), 3.25 (1H, m, H-2), 1.96 (4H, m, H_2-10, H_2-13), 1.41 (2H, m, H_2-4), 1.19 (3H, d, *J* = 6.6 Hz, Me-1), 0.93 (3H, t, *J* = 7.5 Hz, Me-14); ^{13}C nmr ($CDCl_3$) δ 132.0 (d, C-12), 129.2 (d, C-11), 70.6 (d, C-3), 51.9 (d, C-2), 33.2 (t, C-4), 32.7 (t, C-10), 29.8 / 29.3 / 26.2 (t, C-5 to C-9), 25.7 (t, C-13), 14.1 (q, Me-14), 11.9 (q, Me-1).

XESTOAMINOL B [**2**].—(2*S**)-Aminotetradeca-11,13-dien-(3*R**)-ol: oil; $[\alpha]_D$ +6.0° (*c* = 0.06, MeOH); uv λ max (MeOH) 222 nm (log ε = 5.5); ir (neat) 3389, 2924, 1602, 1207, 1000 cm^{-1}; hreims *m/z* 225.2086 (calcd for $C_{14}H_{27}NO$, 225.2086); lreims *m/z* (rel. int.) $[M]^+$ 225 (8), 211 (7), 109 (17), 95 (37), 81 (65), 67 (100); lrcims (isobutane) *m/z* (rel. int.) $[M+H]^+$ 226 (100), 208 (20); ^1H nmr (CD_3OD) δ 6.21 (1H, dt, *J* = 16.8, 10.2, 10.2 Hz, H-13), 5.95 (1H, dd, *J* = 14.7, 10.5 Hz, H-12), 5.63 (1H, dt, *J* = 15.0, 7.0, 7.0 Hz, H-11), 4.97 (1H, d, *J* = 17.1, H-14), 4.80 (1H, H-14 under MeOH), 3.63 (1H, m, H-3), 3.23 (1H, m, H-2), 2.05 (2H, m, H_2-10), 1.41 (2H, m, H_2-4), 1.27 (10H, m, H_2-5 to H_2-9), 1.14 (3H, d, *J* = 6.0 Hz, Me-1); ^{13}C nmr (CD_3OD) δ 139.7 (d, C-13), 137.2 (d, C-12), 133.5 (d, C-11), 116.0 (t, C-14), 72.7 (d, C-3), 53.7 (d, C-2), 35.1 (t, C-4), 34.6 / 31.7 / 31.6 / 31.4 / 31.3 (t, C-5 to C-9), 28.0 (t, C-10), 13.2 (q, Me-1).

XESTOAMINOL C [**3**].—(2*S**)-Aminotetradeca-(3*R**)-ol: oil; $[\alpha]_D$ +7.0° (*c* = 0.14, MeOH); ir (neat) 3366, 3061, 2932, 1608, 1461; hreims *m/z* 229.2395 (calcd for $C_{14}H_{31}NO$, 229.2398); lrcims (isobutane) *m/z* (rel. int.) $[M+H]^+$ 230 (100), 212 (18); ^1H nmr ($CDCl_3$) δ 7.88 (2H, br s, NH_2), 3.95 (1H, m, H-3), 3.33 (1H, m, H-2), 1.15 (3H, d, *J* = 6.0 Hz, Me-1), 0.86 (3H, t, *J* = 6.9 Hz, Me-14); ^{13}C nmr ($CDCl_3$) δ 70.6 (d, C-3), 51.9 (d, C-2), 33.2 (t, C-4), 32.0 (t, C-12), 29.8 / 29.5 (t, C-6 to C-11), 26.2 (t, C-5), 22.8 (t, C-13), 15.0 (q, Me-14), 11.9 (q, Me-1).

DIACETYL XESTOAMINOL A [**4**].—Xestoaminol A (50 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) to give diacetyl xestoaminol A [**4**] (40 mg): oil; $[\alpha]_D$ +15.0° (*c* = 0.06, MeOH); ir (neat) 3339, 2930, 2857, 1740, 1719, 1642, 1428, 1373, 1240 cm^{-1}; lreims *m/z* (rel. int.) $[M]^+$ 311 (2), 251 (8), 192 (14), 129 (35), 85 (100); lrcims (isobutane) *m/z* (rel. int.) $[M+H]^+$ 312 (25), 286 (48), 242 (29), 227 (100), 197 (35), 86 (34); ^1H nmr ($CDCl_3$) δ 12.13 (1H, br s, NH), 5.26 (2H, m, H-11, H-12), 4.75 (1H, m, H-3), 4.02 (1H, m, H-2), 1.95 (3H, s, Ac), 1.90 (4H, m, H_2-10, H_2-13), 1.84 (3H, s, Ac), 1.38 (2H, m, H-4), 1.11 (10H, m, H_2-5 to H_2-9), 0.97 (3H, d, *J* = 6.9 Hz, Me-1), 0.80 (3H, t, *J* = 7.5 Hz, Me-14); ^1H nmr (CD_3OD) δ 5.28–5.45 (2H, m, H-11, H-12), 4.90 (1H, m, H-3), 4.06 (1H, m, H-2), 2.02 (3H, s, Ac), 1.96 (4H, m, H_2-10, H_2-13), 1.88 (3H, m, Ac), 1.51 (2H, m, H_2-4), 1.28 (10H, m, H_2-5 to H_2-9), 1.07 (3H, d, *J* = 6.9 Hz, Me-1), 0.94 (3H, t, *J* = 7.5 Hz, Me-14); ^{13}C nmr ($CDCl_3$) δ 171.4 (s, Ac), 170.0 (s, Ac), 131.8 (d, C-12), 129.2 (d, C-11), 76.3 (d, C-3), 47.4 (d, C-2), 32.5 (t, C-10), 30.9 (t, C-4), 29.5 / 29.3 / 29.0 (t, C-5 to C-9), 25.5 (t, C-

13), 23.2 (q, Ac), 21.2 (q, Ac), 14.8 (q, C-14), 13.9 (q, C-1).

OXAZOLIDINONE XESTOAMINOL A [**5**].—Xestoaminol A (45 mg) was reacted with 1,1-carbonyl-bis-imidazole (110 mg) in refluxing C_6H_6 for 6 h. The solution was washed with H_2O, dried over Na_2SO_4, concentrated under reduced pressure, and chromatographed by normal phase hplc [hexane-EtOAc (3:2)], yielding 26 mg of the oxazolidinone derivative: oil, $[\alpha]_D$ +25.8° (c = 0.20, MeOH); ir (neat) 3267, 2929, 2856, 1749, 1398, 1232, 1102, 969 cm^{-1}; lreims *m/z* (rel. int.) $[M]^+$ 253 (10), 207 (7), 192 (5), 88 (100); lrcims (isobutane) *m/z* (rel. int.) $[M+H]^+$ 254 (100), 207 (70), 192 (10), 88 (100); ^1H nmr ($CDCl_3$) δ 5.88 (1H, br s, exchanged with D_2O, NH), 5.40–5.29 (2H, m, H-11 to H-12), 4.52 (1H, m, H-3 but transformed into a dd by spin decoupling at δ 1.12), 3.86 (1H, dq, J = 6.6, 7.5 Hz, H-2), 1.96 (4H, m, H_2-10 and H_2-13), 1.7–1.5 (1H, m, H_2-4), 1.26 (10H, m, H_2-5 to H_2-9), 1.12 (3H, d, J = 6.6 Hz, Me-1), 0.93 (3H, t, J = 7.2 Hz, Me-14); ^{13}C nmr ($CDCl_3$) δ 160.0 (s, CO), 132.0 (d, C-12), 129.3 (d, C-11), 80.3 (d, C-3), 51.2 (d, C-2), 32.6 (t, C-10), 29.6 (t, C-4), 29.4 / 29.2 / 29.0 (t, C-5 to C-9), 25.9 (t, C-13), 15.9 (q, Me-1), 14.1 (q, Me-14).

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