Chemical investigation of the Marine sponge
Axinella tenuidigitata

Natural Product Laboratory, Organic Chemistry Division-I
Toxicology Laboratory, Biology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, India

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Four pyrrole-based alkaloids 1-4 and a A-norsteroid 5 have been isolated from the sponge Axinella tenuidigitata and have been characterized from their spectral studies. Compounds 1-4 exhibited mild toxicity against brine shrimp (Artemia salina)

In continuation of our search for bioactive metabolites from marine organisms1, we have investigated the marine sponge Axinella tenuidigitata (Dendy) collected from the Mandapam coast during October 1997. Sponges of the genus Axinella yielded various types of secondary metabolites2 viz. pyrrole and guanidine based alkaloids3, halistatins4, axinasstatins5, sodowanone triterpenes6, nitrogenous sesquiterpenes7, A-norsteroids8, unusual pregnone steroids9 etc. A variety of pharmacological activities displayed by these metabolites greatly increased their importance. We report herein the isolation and characterization of pyrrole-based alkaloids and their cytotoxicity against brine shrimp (Artemia salina) and a rare A-norsteroid with D-ring unsaturation.

The 1:1 dichloromethane-methanol extract of the sponge was partitioned between ethyl acetate and water. The ethyl acetate extract was subjected to Sephadex LH-20 followed by silica gel column chromatography, when compounds 1-5 were obtained.

Compound 1 was obtained as solid, m.p. 268°C (Lit10, 269°C), analyzed for C19H16N2O, from its mass spectral data [m/z 164 (M+)]. Its IR spectrum showed bands at 3250 (-NH), 1665 and 1650 cm⁻¹. The ¹H NMR spectrum showed signals at δ 7.03 (d, J=2.5 Hz, 1H), 6.89 (d, J=2.5 Hz, 1H), 3.55 (m, 2H) and 2.85 (m, 2H). The foregoing spectral data and a literature survey revealed that the compound 1 was a pyrrole derivative aldisin, which was previously isolated from the sponges Hymeniacidon aldis10.

Compound 2 was obtained as solid, m.p. 243°C (Lit10, 243°C), its EIMS showed molecular ion peaks at m/z 244 and 242 in 1:1 ratio indicated the presence of one bromine atom and was analyzed for C19H16O2Br. Its IR spectrum showed bands at 3270 (br), 1670 and 1650 cm⁻¹. The ¹H NMR spectrum showed signals at δ 6.69 (s, 1H), 2.85 (m, 2H) and 3.57 (m, 2H). A literature survey revealed that the foregoing spectral data is reminiscent to that of bromoaldis from the sponges Hymeniacidon aldis10, Lissodendoryx sp10, Psuedaxinysa cantherella11.

Compound 3 was obtained as white solid, m.p. 156°C (Lit10, 158°C), and its mass spectrum showed peaks at m/z 205, 203 in 1:1 ratio, indicated the presence of one bromine atom and analyzed for C19H16NO2Br. Its IR spectrum showed bands at 3285 and 1700 cm⁻¹. The ¹H NMR spectrum showed signals at δ 6.95 (1H, br s), 6.88 (1H, br s) and 3.84 (s, 3H). A literature survey and from above spectral characteristics revealed that compound 3 is in good agreement in all respects with 4-bromo-pyrrole-2-carboxylic acid methyl ester, isolated from the sponges Lissodendoryx sp10 and Axinella sp12.

Compound 4 was obtained as solid, m.p. 159°C (Lit10, 159°C), and its EIMS showed peaks at m/z 281, 283, 285 in 1:2:1 ratio indicated the presence of two bromine atoms, and analyzed for C19H16O2Br2. The ¹H NMR spectrum showed signals δ 6.89 (s, 1H) and 3.85 (s, 3H). A literature survey reveals that the
The sterol 5 was obtained as a mixture, hence the sterol fraction was acetylated using Ac₂O/Py and the acetylated steroid mixture was subjected to AgNO₃ impregnated silica gel column chromatography yielded compound 5a and a mixture of other well known A-norsteroids from this genus.¹²

Compound 5a was obtained as white solid, m.p. 104°, analyzed for C₂₉H₄₆O₂ from its mass spectral data [m/z 442 (M⁺)]. Its IR spectrum showed bands at 1735 and 1620 cm⁻¹ for acetoxy and unsaturation functionalities. The ¹H NMR spectrum showed signals for the presence of six methyls at δ 0.74 (3H, s), 0.77 (3H, s), 0.87 (6H, d, J=7 Hz), 0.88 (3H, d, J=6.8 Hz) and 0.90 (3H, d, J=6.8 Hz) and an acetyl methyl at δ 2.04. And further the downfield spectrum showed signals at δ 3.93 (dd, J=6.8, 2.04). And further the downfield spectrum revealed that the compound Sa is 3-acetoxy methyl A-norergostane-15-ene-3α,5β-ol, previously isolated from the sponge Phakellia aruncus.¹⁴ The stereochemistry at C-24 was fixed as 24R based on the ¹H NMR values of 26,27-methyl signals which appeared at δ 0.87 (3H, d, J=6.8 Hz) and 0.88 (3H, d, J=6.8 Hz). For compound 5 the ¹³C NMR and stereochemistry at C-24 were not reported previously.

Compounds 1-4 have been tested for their cytotoxicity against brine shrimp (Artemia salina) assay and showed moderate cytotoxicity. LC₅₀±SE (ppm) for compounds 1, 2 and 4 were 17.49±1.15, 38.32±3.19, 67.03±2.88 and 10.95±0.80 respectively. This is the first chemical examination report of the sponge Axinella tendulgidata.

**Experimental Section**

**General procedure.** ¹H NMR (200 MHz) and ¹³C NMR (50 MHz) spectra were recorded on Varian Gemini 200 MHz Spectrometer using TMS as internal standard. Chemical shifts are reported in ppm and coupling constants (J) are expressed in Hz. Optical rotations were measured with JASCO DIP-370 polarimeter. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. UV spectra on Shimadzu spectrophotometer and mass spectra on Finnigan MAT 1020 instrument.

**Collection, Extraction and Isolation.** The sponge Axinella tendulgidata (II-296) specimens were collected from Mandapam coast (N 17°, E 83°), Tamil Nadu, India, during October 1997 by skin diving at a depth of 30 feet. The voucher specimen was deposited at National Institute of Oceanography, Goa, India. The specimens were preserved in MeOH until work-up. After the removal of initial MeOH, the sponge (200 g dry wt) was freeze dried and extracted with MeOH-CH₂Cl₂ (1:1) (3×1 litre). The combined extract was concentrated under reduced pressure, and the crude concentrate was partitioned between EtOAc and H₂O. Concentration of the organic layer resulted in gummy crude extract (19 g), which was subjected to Si gel (100-200 mesh) column chromatography, eluting with hexane, hexane/EtOAc mixtures to EtOAc to give compounds 1 (40 mg), 2 (110 mg), 3 (20 mg), 4 (60 mg) and 5 (30 mg).

**Compound 1 was obtained as solid, m.p. 268°; [α]D=6° (c 0.12, MeOH); UV (MeOH) λ max (ε): 295 (6770), 245 (8350) and 216 (19,100); IR (KBr): 3450, 3250, 1665, 1650 cm⁻¹; ¹H NMR (200 MHz, CD₃OD-CDCl₃): δ 7.03 (d, J=2.5 Hz, 1H), 6.89 (d, J=2.5 Hz, 1H), 3.55 (m, 2H), 2.85 (m, 2H); EIMS m/z: 164 (M⁺) (72), 136 (21), 107 (58), 93 (100), 79 (38).

**Compound 2 was obtained as solid, m.p. 243°; [α]D=5° (c 0.5, MeOH); UV (MeOH) λ max (ε): 303 (8130), 244 (12,100) and 220 (29, 600); IR (KBr): 3270, 1670, 1650, 1460, 1365, 828, 680 cm⁻¹; ¹H NMR (200 MHz, CD₃OD-CDCl₃): δ 6.69 (s, 1H), 2.85 (m, 2H), 3.57 (m, 2H); EIMS m/z: 244 (78), 242 (M⁺) (78), 216 (30), 214 (29), 187 (22), 185 (20), 173 (34), 171 (34), 159 (15), 157 (15), 92 (92), 78 (54), 64 (100).

**Compound 3 was obtained as solid, m.p. 156°; UV (MeOH) λ max (ε): 271 (22,300), 228 (17,250), 206 (17,850); IR (KBr): 3285, 1700, 1550, 1440, 1390 cm⁻¹; ¹H NMR (200 MHz, CDCl₃): δ 6.95 (br s, 1H), 6.88 (br s, 1H), 3.84 (s, 3H); EIMS m/z: 205 (96), 203 (M⁺) (96), 173 (100), 171 (100), 163 (36), 146 (10), 144 (12).

**Compound 4 was obtained as solid, m.p. 159°; UV (MeOH) λ max (ε): 270 (22,350), 228 (17,250), 205 (17,860); IR (KBr): 3440, 3225, 1700, 1550, 1440, 1395 cm⁻¹; ¹H NMR (200 MHz, CDCl₃): δ 6.89 (s, 1H), 3.85 (2, 3H); EIMS m/z: 285 (15), 283 (30), 281 (M⁺) (15), 253 (38), 251 (78), 249 (37), 224 (8), 197 (10), 146 (12), 144 (12).
Compound 5a was obtained as solid, m.p. 104°; IR (KBr): 2950, 1735, 1620, 960, 685 cm⁻¹; ¹H NMR (200 MHz, CDCl₃): δ 5.83 (m, 1H), 5.74 (m, 1H), 4.10 (dd, J=10.8, 6.8 Hz, 1H), 3.94 (dd, J=10.6, 8.8 Hz, 1H), 2.34 (m, 2H), 2.04 (s, 3H), 0.90 (d, J=6.8 Hz, 3H), 0.88 (d, J=6.8 Hz, 3H), 0.87 (d, J=7 Hz, 6H), 0.77 (s, 3H), 0.74 (s, 3H); ¹³C NMR (50 MHz, CDCl₃): δ 171.3, 133.6, 131.2, 68.3, 62.2, 61.5, 55.8, 52.4, 49.8, 44.1, 39.3, 38.7, 38.3, 37.4, 36.4, 32.6, 32.6, 32.1, 27.9, 27.2, 24.1, 22.8, 22.7, 22.6, 20.9, 18.5, 14.4, 12.7; EIMS m/z: δ 442 (M⁺) (10), 382 (7), 330 (60), 315 (10), 257 (28), 215 (22), 161 (20), 147 (31), 95 (65), 43 (100).

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