Two bromo compounds from the sponge

*Psammaplysilla purpurea*†

M Rama Rao, U Venkatesham & Y Venkateswarlu*
Natural Products Laboratory, Organic Chemistry Division-I
Indian Institute of Chemical Technology, Hyderabad 500 007, India

Received 13 May 1999; accepted (revised) 9 August 1999

Two bromo compounds, 3,5-dibromo-4-(3-dimethylaminopropoxy) phenethyl amine 1 and aplysamine-2 free base 2 have been isolated from the sponge *Psammaplysilla purpurea* and characterized through the interpretation of spectral data.

In continuation of our search for biologically active secondary metabolites from marine organisms,1,2,3 we have investigated the sponge *Psammaplysilla purpurea* (Carter) (Family Aplysinellidae), collected from the Mandapam coast, Tamil Nadu, India during October 1997. A literature survey revealed that the genus *Psammaplysilla* yielded several bromotyrosine-derived metabolites4,5 and macrocyclic bastadins.5

The 1:1 CH₂Cl₂: MeOH extract of the sponge *P. purpurea* was subjected to gel filtration (Sephadex LH-20) followed by silica gel chromatography eluting with chloroform through chloroform-methanol mixtures to methanol afforded compounds 1 and 2. Compound 1 was further purified as its acetate (Ac₂O/Py) 1a.

Compound 1a was obtained as an optically inactive brownish gummy substance, showed IR bands at 3300, 2750, 1650 and 1500 cm⁻¹ indicated the presence of an amide carbonyl group. The positive-ion FAB mass spectrum of compound 1a showed the molecular cluster ions (MH⁺) at m/z 421, 423 and 425 in 1:2:1 ratio indicated the presence of two bromine atoms and analyzed for the molecular formula C₂₃H₂₅Br₂N₃O₄ by high-resolution FAB mass spectrum.

The ¹H NMR of compound 1a showed the signals at δ 7.35 (2H, s), 5.80 (1H, brt), 4.05 (2H, t, J = 7.0 Hz), 3.40 (2H, q, J = 7.5 Hz), 2.85 (2H, t, J = 7.0 Hz), 2.75 (2H, t, J = 7.5 Hz), 2.50 (6H, s), 2.05 (2H, m) and 1.95 (3H, s). The ¹H-¹H decoupling experiments revealed that the methylene protons at δ 2.05 (2H, m) were coupled to the methylene signals at δ 2.85 (2H, t, J = 7.0 Hz) and δ 4.05 (2H, t, J = 7.0 Hz) and the methylene protons at δ 3.40 (2H, q, J = 7.5 Hz) were coupled to the signals at δ 2.75 (2H, t, J = 7.5 Hz) and the NH signal at δ 5.80 (1H, brt). The foregoing spectral data and a literature survey revealed that compound 1a was similar to 3,5-dibromo-4-(3-dimethylaminopropoxy) phenethyl amine salt,2 the two differing in their N,N-dimethyl group signal and the methylene attached to N,N-dimethyl group which resonated up field by ~0.50 and ~0.55 ppm respectively, suggested that compound 1a is a free base.7,8 Thus from the above findings, the structure of compound 1a was established as 3,5-dibromo-4-(3-dimethylaminopropoxy) phenethyl amine acetate.

Compound 2 was isolated as a brownish gummy substance, showed molecular cluster ions (MH⁺) at m/z 654, 652, 650 and 648 in 1:3:3:1 ratio indicated the presence of three bromine atoms by positive ion FABMS and analyzed for C₃₁H₂₅Br₂N₃O₄. The IR spectrum showed bands at 3350, 1655 and 1500 cm⁻¹ indicated the amide and oxime functionalities.

The ¹H NMR spectrum of compound 2 showed the characteristic pattern of a 1,2,4-trisubstituted benzene ring signals at δ 7.50 (1H, d, J = 1.5 Hz), 7.22 (1H, dd, J = 6.0 & 1.5 Hz) and 6.80 (1H, d, J = 7.0 Hz) and a two proton singlet at δ 7.35 ppm for a symmetrically 1,2,3,5-tetrasubstituted benzene ring. Further the ¹H NMR spectrum revealed the presence of five methylene groups at δ 4.05 (2H, t, J = 6.0 Hz), 3.45 (2H, t, J = 7.0 Hz), 2.82 (2H, t, J = 6.5 Hz), 2.75 (2H, t, J = 6.5 Hz) and 2.10 (2H, m), a methoxyl group at δ 3.65 (3H, s) and an amide carbonyl at δ 1655 cm⁻¹, and a NH at δ 7.50 ppm. The FAB mass spectrum of compound 2 showed the molecular cluster ions (MH⁺) at m/z 654, 652, 650 and 648 in 1:3:3:1 ratio indicated the presence of three bromine atoms by positive-ion FABMS and analyzed for C₃₁H₂₅Br₂N₃O₄. The IR spectrum showed bands at 3350, 1655 and 1500 cm⁻¹ indicated the amide and oxime functionalities.

The ¹H NMR spectrum of compound 2 showed the characteristic pattern of a 1,2,4-trisubstituted benzene ring signals at δ 7.50 (1H, d, J = 1.5 Hz), 7.22 (1H, dd, J = 6.0 & 1.5 Hz) and 6.80 (1H, d, J = 7.0 Hz) and a two proton singlet at δ 7.35 ppm for a symmetrically 1,2,3,5-tetrasubstituted benzene ring. Further the ¹H NMR spectrum revealed the presence of five methylene groups at δ 4.05 (2H, t, J = 6.0 Hz), 3.45 (2H, t, J = 7.0 Hz), 2.82 (2H, t, J = 6.5 Hz), 2.75 (2H, t, J = 6.5 Hz) and 2.10 (2H, m), a methoxyl group at δ 3.65 (3H, s) and an amide carbonyl at δ 1655 cm⁻¹, and a NH at δ 7.50 ppm. The FAB mass spectrum of compound 2 showed the molecular cluster ions (MH⁺) at m/z 654, 652, 650 and 648 in 1:3:3:1 ratio indicated the presence of three bromine atoms by positive-ion FABMS and analyzed for C₃₁H₂₅Br₂N₃O₄. The IR spectrum showed bands at 3350, 1655 and 1500 cm⁻¹ indicated the amide and oxime functionalities.

The ¹H NMR spectrum of compound 2 showed the characteristic pattern of a 1,2,4-trisubstituted benzene ring signals at δ 7.50 (1H, d, J = 1.5 Hz), 7.22 (1H, dd, J = 6.0 & 1.5 Hz) and 6.80 (1H, d, J = 7.0 Hz) and a two proton singlet at δ 7.35 ppm for a symmetrically 1,2,3,5-tetrasubstituted benzene ring. Further the ¹H NMR spectrum revealed the presence of five methylene groups at δ 4.05 (2H, t, J = 6.0 Hz), 3.45 (2H, t, J = 7.0 Hz), 2.82 (2H, t, J = 6.5 Hz), 2.75 (2H, t, J = 6.5 Hz) and 2.10 (2H, m), a methoxyl group at δ 3.65 (3H, s) and an amide carbonyl at δ 1655 cm⁻¹, and a NH at δ 7.50 ppm. The FAB mass spectrum of compound 2 showed the molecular cluster ions (MH⁺) at m/z 654, 652, 650 and 648 in 1:3:3:1 ratio indicated the presence of three bromine atoms by positive-ion FABMS and analyzed for C₃₁H₂₅Br₂N₃O₄. The IR spectrum showed bands at 3350, 1655 and 1500 cm⁻¹ indicated the amide and oxime functionalities.

The ¹H NMR spectrum of compound 2 showed the characteristic pattern of a 1,2,4-trisubstituted benzene ring signals at δ 7.50 (1H, d, J = 1.5 Hz), 7.22 (1H, dd, J = 6.0 & 1.5 Hz) and 6.80 (1H, d, J = 7.0 Hz) and a two proton singlet at δ 7.35 ppm for a symmetrically 1,2,3,5-tetrasubstituted benzene ring. Further the ¹H NMR spectrum revealed the presence of five methylene groups at δ 4.05 (2H, t, J = 6.0 Hz), 3.45 (2H, t, J = 7.0 Hz), 2.82 (2H, t, J = 6.5 Hz), 2.75 (2H, t, J = 6.5 Hz) and 2.10 (2H, m), a methoxyl group at δ 3.65 (3H, s) and an amide carbonyl at δ 1655 cm⁻¹, and a NH at δ 7.50 ppm. The FAB mass spectrum of compound 2 showed the molecular cluster ions (MH⁺) at m/z 654, 652, 650 and 648 in 1:3:3:1 ratio indicated the presence of three bromine atoms by positive-ion FABMS and analyzed for C₃₁H₂₅Br₂N₃O₄. The IR spectrum showed bands at 3350, 1655 and 1500 cm⁻¹ indicated the amide and oxime functionalities.
3.84 (3H, s), an uncoupled methylene signal at δ 3.65 (2H, s) and a signal at δ 2.45 (6H, s) indicated the presence of an N, N-dimethyl group.

1H-1H decoupling experiments revealed that the methylene signals at δ 2.10 were coupled to the protons at δ 4.05 and 2.82 and the methylene protons at δ 3.5 were coupled to the methylene protons at δ 3.45. The 13C NMR spectrum revealed the presence of oxime and amide group signals at δ 152.9 and 165.8 respectively. The foregoing spectral data and a literature survey revealed that compound 2 was found to be identical with that of aplysamine-2,7 except for the N,N-dimethyl signal at δ 2.45 (6H, s) which resonated up field by ~ 0.45 ppm suggesting that compound 2 is a free base8. Thus from the above findings the structure of compound 2 was established as aplysamine-2 free base.

Experimental Section

General. 1H NMR (200 MHz) and 13C NMR (50 MHz) spectra were recorded on a Varian Gemini 200 MHz spectrometer using TMS as internal standard. Chemical shifts are reported in parts per million and the coupling constants (J) are expressed in Hz. The IR spectra were recorded on a Perkin-Elmer 240-C instrument. The mass spectra were recorded on a VG Autospec-M instrument. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Animal material. The sponge Psammaplysilla purpurea (Carter) (Family Aplysinellidae) was collected from the Mandapam coast of the Gulf of Mannar (N 9°18', E 79°08') in southern India during October 1997. A voucher specimen (HIC-275) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and isolation. The freshly collected sponge Psammaplysilla purpurea was soaked in MeOH until being worked-up. The MeOH was decanted, the sponge mass freeze-dried (1.5 Kg dry wt), and extracted with CH2Cl2-MeOH (1:1, 3×1.5 L). The combined extract was concentrated under reduced pressure to obtain a brownish gum (40 g). The crude brownish gum was further partitioned between H2O and EtOAc. The water-soluble portion was lyophilized (15 g) and chromatographed over a silica gel column using CHCl3 and MeOH as eluents. The 20% MeOH in CHCl3 fraction afforded compound 1 and the 25 % MeOH in CHCl3 fraction yielded compound 2. Compound 1 was further acetylated (Ac2O/Py) and purified as its acetate 1a.

Compound 1a. It was obtained as an optically inactive brownish gummy substance, IR (KBr): 3300, 2922, 1650 and 1580 cm⁻¹; 1H NMR (CDCl3, 200 MHz): δ 7.35 (2H, s, H2-6, 6'), 5.80 (1H, brt, NH), 4.05 (2H, t, J = 7.0 Hz, H2-3), 3.40 (2H, t, J = 7.5 Hz, H2-9), 2.85 (2H, t, J = 7.0 Hz, H2-1), 2.75 (2H, t, J = 7.5 Hz, H2-8), 2.50 (6H, s, NMe2), 2.05 (2H, m, H2-2) and 1.95 (3H, s, Ac); 13C NMR (CDCl3, 50 MHz): δ 173.25 (s), 152.17 (s), 143.29 (d, 2C), 118.74 (s, 2C), 71.12 (t), 56.99 (t), 43.74 (q, 2C), 41.74 (t), 35.12 (t), 26.38 (t) and 22.56 (q); positive ion FABMS: m/z 425, 423 and 421 (M^+1) in 1:2:1 ration; positive HRFABMS found m/z 421.0105 (M^+1), Calcd for C14H22N2O2Br2 421.0126.

Compound 2. It was isolated as an optically inactive brownish gummy substance, IR (KBr): 3427, 2922, 1632, 1455, 1256 and 1053 cm⁻¹; 1H NMR (CD2OD, 200 MHz): δ 7.50 (1H, d, J = 1.5 Hz, H-2), 7.35 (2H, s, H2-13, 17), 7.22 (1H, dd, J = 6.0 & 1.5 Hz, H-6), 6.80 (1H, d, J = 7.0 Hz, H-5), 4.05 (2H, t, J = 6.0 Hz, H2-18), 3.84 (3H, s, OMe), 3.65 (2H, s, H2-7), 3.45 (2H, t, J = 7.0 Hz, H2-10), 2.82 (2H, t, J = 6.5 Hz, H2-20), 2.75 (2H, t, J = 6.5 Hz, H2-11), 2.45 (6H, s, NMe2) and 2.10 (2H, t, J = 6.0 Hz, H2-19); 13C NMR (CD2OD, 50 MHz): δ 166.0 (s), 156.0 (s), 152.9 (s), 152.5 (s), 139.8 (s), 134.7 (d), 134.3 (d, 2C), 131.7 (d), 130.2 (s), 118.79 (s, 2C), 113.18 (s), 112.13 (d), 72.12 (t), 57.32 (t), 56.69 (q), 44.8 (q, 2C), 41.2 (t), 35.1 (t), 28.67 (t) and 28.1 (t); positive FABMS: m/z 654, 652, 650 and 648 (M^+1) in 1:3:3:1 ratio; Anal. Found: C, 42.476; H, 4.36; N, 6.451; Br, 36.848. Calcd for C14H22N2O2Br2: C, 42.483; H, 4.341; N, 6.435; Br, 36.863%.

Acknowledgements

We are thankful to Dr P A Thomas, Central Marine Fisheries Research Institute, Thiruvananthapuram, India, for identifying the sponge; the Department of Ocean Development for financial assistance, the Director, Indian Institute of Chemical Technology and Dr J S Yadav for their constant encouragement, and to UGC and CSIR for providing fellowships to MRR and UV respectively.

References