

## A New Dibromotyrosine-Derived Metabolite from the Sponge *Psammaphysilla purpurea*<sup>1</sup>

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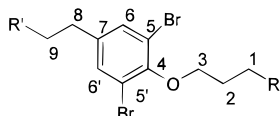
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A new dibromotyrosine-derived metabolite (**1a**) and the known compounds aerothionin, moloka'iamine diacetate (**2a**), and 2,6-dibromo 4-hydroxy-4-acetamido-1,1-dimethoxy-2,5-cyclohexadiene have been isolated from the sponge *Psammaphysilla purpurea* and characterized by spectral studies.

Sponges of the family Verongiidea have produced a series of antibiotics which may be considered as metabolites of dibromotyrosine.<sup>2</sup> In continuation of our search for biologically active secondary metabolites from marine organisms<sup>3</sup> we have investigated the sponge *Psammaphysilla purpurea* (Carter) (Verongiidea) collected off the coast of Mandapam during June 1996. A literature survey revealed that the genus *Psammaphysilla* has yielded several antibiotics, namely aerothionin,<sup>4</sup> fistularin,<sup>5</sup> the psammaphysins,<sup>6</sup> containing the spiro cyclohexadienyl oxazoline moiety, the purpuramines,<sup>7</sup> the purealidins A–F,<sup>8</sup> and macrocyclic bastadins composed of several bromotyrosine units.<sup>9</sup>

The 1:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH extract of the sponge *Psammaphysilla purpurea* was subjected to gel filtration (Sephadex LH-20, CH<sub>2</sub>Cl<sub>2</sub>:MeOH 1:1) followed by silica gel chromatography, eluting with chloroform through chloroform–methanol mixtures to methanol afforded the known compounds aerothionin,<sup>4</sup> 2,6-dibromo-4-hydroxy-4-acetamido-1,1-dimethoxy-2,5-cyclohexadiene,<sup>10</sup> 1-*O*-methyl glucose, and compounds **1** and **2**. Compounds **1** and **2** were obtained in 20% methanol–chloroform and were further purified as their acetates (Ac<sub>2</sub>O/Pyr), **1a** and **2a**, respectively. Compound **2a** was identified as moloka'iamine diacetate.<sup>6,11</sup>



1. R = N(CH<sub>3</sub>)<sub>2</sub>H<sup>+</sup>X<sup>-</sup>, R' = NH<sub>2</sub>
- 1a. R = N(CH<sub>3</sub>)<sub>2</sub>H<sup>+</sup>X<sup>-</sup>, R' = NHAc
2. R = R' = NH<sub>2</sub>
- 2a. R = R' = NHAc
3. R = N(CH<sub>3</sub>)<sub>2</sub>H<sup>+</sup>Cl<sup>-</sup>, R' = N(CH<sub>3</sub>)<sub>2</sub>H<sup>+</sup>Cl<sup>-</sup>

Compound **1a** was optically inactive, mp 148 °C, and showed molecular cluster ions (MH<sup>+</sup>) at *m/z* 421, 423, and 425 in the ratio of 1:2:1 by positive ion FABMS. The molecular formula C<sub>15</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>Br<sub>2</sub> of **1a** was confirmed by HRFABMS. The IR spectrum showed bands at 3328, 2700, 1680, 1580, and 1400 cm<sup>-1</sup>, indicating the presence of an amide carbonyl group. The <sup>1</sup>H NMR spectrum of **1a** displayed signals at δ 7.4 (2H, s, H<sub>2</sub>-6,6'), 4.12 (2H, t, *J* = 7.5 Hz, H<sub>2</sub>-3), 3.51 (2H, t, *J* = 7.5 Hz, H<sub>2</sub>-1), 3.35 (2H, t, *J* = 7.5 Hz, H<sub>2</sub>-9), 3.0 (6H, s, NMe<sub>2</sub>), 2.72 (2H, t, *J* = 7.5 Hz, H<sub>2</sub>-8), 2.38 (2H, m, H<sub>2</sub>-2), 2.01 (3H, s, NHAc). <sup>1</sup>H–<sup>1</sup>H

decoupling experiments revealed that the methylene protons at δ 2.38 were coupled to the signals at δ 4.12 and 3.51, and the methylene protons at δ 2.72 were coupled to protons at δ 3.35. The foregoing spectral data and a literature survey revealed that compound **1a** was similar to purealidin F (**3**), the two differing only in their N-substitution patterns. Further, the downfield signals at δ 3.0 (6H, s) (δ<sub>C</sub>: 43.7 q) and 3.51 (2H, t) (δ<sub>C</sub>: 56.99 t) suggested the presence of a dimethylammonium salt<sup>7</sup> in the alkyl ether chain of the molecule. However, no attempt was made to determine the nature of the counterion.

### Experimental Section

**General Experimental Procedures.** The <sup>1</sup>H NMR (200 MHz) and <sup>13</sup>C 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 coupling constants (*J*) are expressed in hertz. UV and IR spectra were recorded on Shimadzu and Perkin-Elmer 240-C instruments. The mass spectra were recorded on a VG AUTOSPEC-M instrument.

**Animal Material.** The sponge *Psammaphysilla purpurea* (Carter) (Verongiidea) (IIC-224) was collected at Mandapam coast in the Gulf of Mannar during June 1996, and a voucher specimen (IIC-224) is on deposit at the National Institute of Oceanography, Goa, India.

**Extraction and Isolation.** The freshly collected specimen (500 g dry weight after extraction) was extracted with CH<sub>2</sub>Cl<sub>2</sub>–MeOH (1:1, 3 × 1.5 L) at room temperature. The combined extract was filtered, and the solvent was removed under reduced pressure. The crude extract (30 g) was subjected to gel filtration chromatography (Sephadex LH-20, 1:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH) followed by silica gel using chloroform through chloroform–methanol mixture to methanol.

The 5% methanol in chloroform fraction afforded aerothionin<sup>4</sup> (50 mg). The 10% methanol in chloroform elute afforded 2,6-dibromo-4-hydroxy-4-acetamido-1,1-dimethoxy-2,5-cyclohexadiene<sup>10</sup> (1 g). The 20% methanol in chloroform fraction afforded compounds **1** and **2**, respectively. The fractions containing **1** and **2** were acetylated and characterized as acetates **1a** and **2a**. Compound **2a** was identified as moloka'iamine diacetate (**2a**) (20 mg).<sup>6</sup>

**Acetylation of Compound 1.** A solution of compound **1** (100 mg) in acetic anhydride (2 mL) and pyridine (0.5 mL) was allowed to stand overnight at room temperature. The contents were poured into ice cold water and extracted with ethyl acetate, and the organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure followed by silica gel chromatography to obtain compound **1a** (100 mg).

Compound **1a**: Obtained as a colorless crystalline compound, mp 148 °C; IR (KBr) ν<sub>max</sub> 3328, 2700, 1680, 1580, 1480 cm<sup>-1</sup>; UV (MeOH, HCl salt) λ<sub>max</sub> 280 (ε 944), 275 (950), 223 (5769);

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$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 200 MHz)  $\delta$  7.4 (2H, s,  $\text{H}_{2-6,6'}$ ), 4.12 (2H, t,  $J = 7.5$  Hz,  $\text{H}_{2-3}$ ), 3.51 (2H, t,  $J = 7.5$  Hz,  $\text{H}_{2-1}$ ), 3.35 (2H, t,  $J = 7.5$  Hz,  $\text{H}_{2-9}$ ), 3.0 (6H, s,  $\text{NMe}_2$ ), 2.72 (2H, t,  $J = 7.5$  Hz,  $\text{H}_{2-8}$ ), 2.38 (2H, m,  $\text{H}_{2-2}$ ), 2.01 (3H, s,  $\text{NHAc}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 50 MHz)  $\delta$  173.25 (s), 152.17 (s), 140.37 (s), 134.29 (2C, d), 118.74 (2C, d), 71.12 (t), 56.99 (t), 43.74 (2C, q), 41.44 (t), 35.12 (t), 26.38 (t), 22.56 (q); positive FABMS  $m/z$  421, 423, 425 (1:2:1); HRFABMS found  $m/z$  421.010534, calcd for  $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_2\text{Br}_2$  ( $\text{M}^+ + 1$ ) 421.012625.

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