Novel Bromine-Containing Constituents of the Sponge *Psammaplysilla purpurea*[1]

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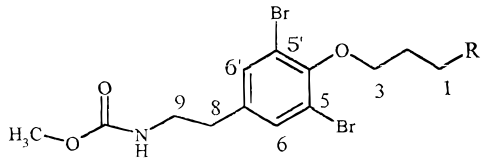
Two new dibromotyrosine-derived metabolites (**1** and **2**) and the known compounds bastadin-6, bastadin-16, purealidin P, purealidin G, and aplysamine-2 and its ammonium salt have been isolated from the sponge *Psammaplysilla purpurea*. Compounds **1** and **2** were characterized by interpretation of their spectral data.

A number of bromotyrosine-derived metabolites have been isolated from marine sponges, many of which belong to the order Verongiidae.[2,3] During the course of a search for bioactive secondary metabolites from marine organisms,[4] we have investigated the sponge *Psammaplysilla purpurea* Carter (Aplysinellidae), collected from the Mandapam coast in southern India during October 1997. A literature survey revealed that the genus *Psammaplysilla* has yielded several bromotyrosine-derived metabolites, namely, psammaplysins,[5] purpuramines,[6] and macrocyclic bastadins.[7]

The CH_2Cl_2−MeOH (1:1) extract of the freeze-dried sponge *Psammaplysilla purpurea* was partitioned between H_2O and EtOAc. The EtOAc-soluble portion was concentrated under reduced pressure and subjected to gel filtration (Sephadex LH-20) followed by Si gel column chromatography using hexane, hexane−EtOAc mixtures, and EtOAc as eluents, and yielded a new compound, **1**. The water-soluble portion was lyophilized and chromatographed over a Si gel column using $CHCl_3$, $CHCl_3$−MeOH mixtures, and MeOH as eluents, to afford the known compounds bastadin-6,[8] bastadin-16,[9] purealidin P,[10] aplysamine-2 and its ammonium salt,[11] purealidin G,[12] and the new compound **2**.



1 R = $N(CH_3)_2$

2 R = $N(CH_3)_2H^+X^-$

Compound **1** was obtained as an optically inactive brown solid, mp 160−162 °C. Its molecular formula was established as $C_{15}H_{22}Br_2N_2O_3$ by HRFABMS, which showed molecular cluster ion [MH^+] peaks at *m*/*z* 441, 439, and 437 in a 1:2:1 ratio, suggesting the presence of two bromine atoms. The IR bands at 3300, 2720, 1720, 1620, and 1480 cm^{-1} indicated the presence of an amide carbonyl. The ^1H NMR spectrum of compound **1** (see Table 1) displayed nine signals accounting for 22 protons. Its ^{13}C NMR spectrum (see Table 1) revealed the presence of ester signals at δ 156.8 (s) and 52.1 (q). $^1H-^1H$ decoupling NMR experiments revealed that the methylene protons at δ 3.40 (2H, q) were coupled to the benzylic protons at δ 2.65 (2H, t) and to an amide proton at δ 4.70 (1H, br t). Further, the methylene protons at δ 2.05 (2H, m) were coupled to the methylene protons at δ 4.05 (2H, t) connected to the phenoxy group and to the methylene protons at δ 2.75 (2H, t) bearing the dimethylamino group. The foregoing spectral data and a literature survey revealed that compound **1** is related to the 2,6-dibromotyrosine-derived metabolites isolated from the sponge *Psammaplysilla purpurea*,[12] and the methyl ester was considered as a carbamate, which could be attached either to the propyl ether side chain or to the ethyl side chain of the molecule. Rotem et al.[5] reported that mild alkaline hydrolysis of psammaplysin A afforded similar carbamates where the carbamate group was present in the alkyl ether side chain. From the $^1H-^1H$ decoupling NMR experiments performed on **1**, the carbamate group was assigned to the ethyl side chain and differed from the degraded products (carbamates) of psammaplysin A in the side-chain substitution patterns. On the basis of these findings, the structure of **1** was established as 3,5-dibromo-4-(3-dimethylaminopropoxy)phenethyl carbamic acid methyl ester.

Table 1. ^1H and ^{13}C NMR Spectral Data (δ/ppm) for Compounds **1**[a] and **2**[b]

	^1H (mult.) (Hz)		^{13}C (mult.)	
position	**1**	**2**	**1**	**2**
1	2.75 t (7.0)	3.50 t (7.0)	56.0 t	57.1 t
2	2.05 m	2.30 m	27.1 t	26.4 t
3	4.05 t (6.5)	4.05 t (6.5)	71.1 t	71.1 t
4			151.6 s	152.1 s
5,5′			118.2 s	118.7 s
6,6′	7.35 s	7.38 s	132.9 d	134.3 d
7			137.6 s	140.4 s
8	2.65 t (7.0)	2.70 t (7.0)	35.0 t	34.9 t
9	3.40 q (6.8)	3.20 t (6.5)	41.9 t	42.8 t
NH	4.70 brt			
$N(CH_3)_2$	2.32 s	3.00 s	44.4 q	44.0 q
CO			156.8 s	159.5 s
OCH_3	3.70 s	3.65 s	52.1 q	52.5 q

[a] $CDCl_3$. [b] CD_3OD.

Compound **2** was isolated as an optically inactive white solid, mp 170−173 °C. Its molecular formula was established as $C_{15}H_{22}Br_2N_2O_3$ by HRFABMS, which showed molecular cluster ion [MH^+] peaks at *m*/*z* 441, 439, and 437 in a 1:2:1 ratio. The NMR data appear in Table 1. A careful examination of the ^1H NMR spectral data of compound **2** revealed that they were similar to those of compound **1**, except that the dimethylamino methyls resonated (δ 3.00, 6H, s) further downfield by 0.68 ppm as

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compared to those of compound **1** (Table 1) and suggested that compound **2** is the dimethylammonium salt of **1**. Thus, the structure of **2** was established as 3,5-dibromo-4-(3-dimethylaminopropoxy)phenethyl carbamic acid methyl ester salt. No attempt was made to determine the nature of the counterion in compound **2**.

The known compounds bastadin-6,[8] bastadin-16,[9] purealidin P,[10] aplysamine-2 and its ammonium salt,[11] and purealidin G[12] were characterized by comparing their spectral data with those reported in the literature. Previously, bastadins-2, -5, -7, and -12 were reported from the sponge *P. purpurea*.[7] This is the first report of bastadin-6 and -16 from this species.

Experimental Section

General Experimental Procedures. ^{1}H (200 MHz) and ^{13}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 the coupling constants (*J*) are expressed in Hertz. The IR spectra were recorded on a Perkin−Elmer 240C instrument. The mass spectra were recorded on a VG Autospec-M instrument. Melting points were measured on a Buchi-510 apparatus, and optical rotations were measured on a JASCO DIP-370 polarimeter.

Biological Material. The sponge *Psammaplysilla purpurea* Carter (Aplysinellidae) (IIC-275) 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 (IIC− 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 workup. The MeOH was decanted, the sponge freeze-dried (1.5 kg dry wt), and extracted with CH_2Cl_2−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 (40 g) was further partitioned between H_2O and EtOAc. The EtOAc-soluble portion was concentrated in vacuo, and the concentrate (25 g) was subjected to gel filtration (Sephadex LH-20), followed by Si gel column chromatography using hexane, hexane−EtOAc mixtures, and EtOAc to yield compound **1.** The H_2O-soluble portion was lyophilized (15 g) and chromatographed over a Si gel column using $CHCl_3$ and MeOH as eluents. The fraction containing 10% MeOH in $CHCl_3$ yielded bastadin-6 (18 mg)[8] and bastadin-16 (18 mg).[9] The fraction with 20% MeOH in $CHCl_3$ afforded compound **2** and purealidin G.[12] The fraction with 25% MeOH in $CHCl_3$ yielded the known compounds purealidin P (100 mg),[10] aplysamine-2 (30 mg), and its ammonium salt (25 mg).[11]

Compound 1: obtained as an optically inactive brown solid (20 mg); mp 160−162 °C; IR (KBr) ν_{max} 3300, 2720, 1720, 1620, 1480 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m/z* 437, 439 and 441 [MH$^+$], in a 1:2:1 ratio; HRFABMS *m/z* 437.0083 (calcd for $C_{15}H_{23}Br_2N_2O_3$ *m/z* 437.0075).

Compound 2: obtained as an optically inactive white solid (20 mg); mp 170−173 °C; IR (KBr) ν_{max} 3350, 2700, 1725, 1610 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m/z* 437, 439 and 441 [MH$^+$], in a 1:2:1 ratio; HRFABMS *m/z* 437.0090 (calcd for $C_{15}H_{23}Br_2N_2O_3$ *m/z* 437.0075).

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