Two New Bromotyrosine-Derived Metabolites from the Sponge *Psammaplysilla purpurea*

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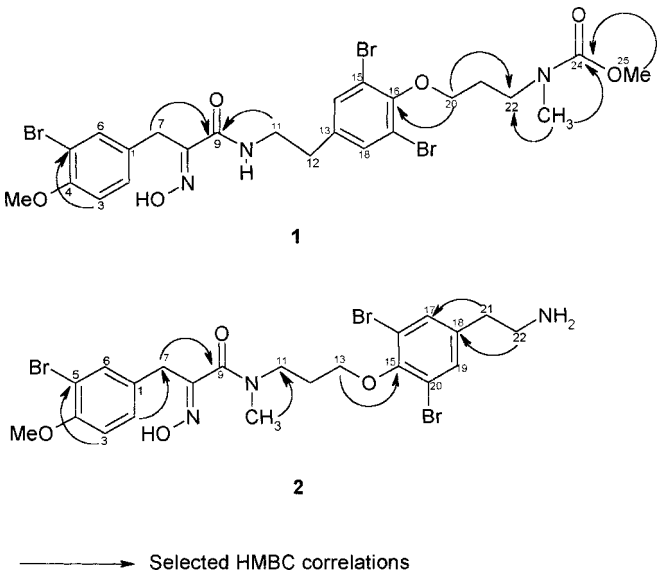
Two new bromotyrosine-derived metabolites (1, 2) have been isolated along with the known compounds 3,5-dibromo-4-methoxyphenylacetonitrile, 3-bromo-4-methoxyphenylacetonitrile, 3-bromo-4-hydroxyphenylacetonitrile, 1-hydroxyuracil, 1-methoxyhemibastadin 2, purpuramine H and a steroid 5α,8α-epidioxycholest-6-en-3β-ol from the sponge *Psammaplysilla purpurea*. Compounds 1 and 2 were characterized by interpretation of their spectral data. The antibacterial activity of these compounds is summarized.

Key words sponge; *Psammaplysilla purpurea*; purpuramine; antibacterial activity

Sponges of the family Verongiidea have produced a series of antibiotics, which may be considered as metabolites of halogenated tyrosine metabolites.[1,2] During the course of our search for biologically active compounds from the marine organisms, we have investigated the sponge *Psammaplysilla purpurea* Carter (Aplysinellidae), collected from the Mandapam coast in Southern India during February 2002. A literature survey revealed that the genus *Psammaplysilla* has yielded several bromotyrosine-derived metabolites, namely psammaplysins,[3] purpuramines,[4] macrocyclic bastadins and hemibastadins.[5,6]

The CH_2Cl_2–MeOH (1 : 1) extract of the sponge *Psammaplysilla purpurea* was partitioned between water and EtOAc. The EtOAc soluble portion was concentrated under reduced pressure and subjected to gel filtration (Sephadex LH-20) followed by silica gel column chromatography eluting with a step gradient of hexane–EtOAc mixtures, to MeOH, to afford known compounds 3,5-dibromo-4-methoxyphenyl acetonitrile, 3-bromo-4-methoxyphenylacetonitrile,[7] 3-bromo-4-hydroxyphenyl acetonitrile,[8] 1-hydroxyuracil,[9] 1-methoxyhemibastadin 2,[10] purpuramine H[4] and a steroid 5α,8α-epidioxycholest-6-en-3β-ol,[11] and two new compounds, **1** and **2**. Compounds **1** and **2** were obtained as a mixture that resisted separation by Sephadex LH-20 partition chromatography or normal silica gel chromatography using a variety of solvent systems. The mixture was then rechromatographed on a reversed-phase (C_{18}) HPLC column using 20% aqueous MeOH as eluent, where upon two new compounds purpuramine K (**1**) and L (**2**) were isolated, and the isolated compounds were tested for antibacterial activity against *Staphylococcus*, *Bacillus* (gram positive), *Chromobaterium*, *Klebsiella* and *Pseudomonas* (gram negative).



Results and Discussion

Purpuramine K (**1**) was obtained as an optically inactive white solid (yield 0.058% on dry wt basis), mp 190—195 °C. Its molecular formula was established as $C_{24}H_{28}N_3O_6Br_3$ by high resolution (HR)-FAB-MS, which showed molecular cluster ion $(M+H)^+$ peaks at *m/z* 692, 694, 696 and 698 in 1 : 2 : 2 : 1 ratio suggesting the presence of 3 bromine atoms. The IR spectrum showed bands at 3404, 2926, 1675 and 1494 cm^{-1} which indicated the presence of hydroxyl and amide groups. Its ^{1}H-NMR spectrum (Table 1) displayed four aromatic signals ascribable to the presence of two benzene rings *viz.* a 1,3,4-trisubstituted benzene δ 7.12 (1H, dd, 2-H), δ 6.97 (1H, d, 3-H) and δ 7.38 (1H, d, 6-H) and a 1,3,4,5 tetra substituted benzene with a peak at δ 7.46 (2H, s, 14,18-H). Further its ^{1}H-NMR spectrum displayed signals for six methylene groups at δ 3.91 (2H, t, 20-H), δ 3.72 (2H, s, 7-H), δ 3.38 (2H, t, 22-H), δ 3.35 (2H, t, 11-H), δ 2.73 (2H, t, 12-H) and δ 2.03 (2H, m, 21-H); an aromatic methoxyl group at δ 3.79 (3H, s), an ester methyl at δ 3.57 (3H, s), a N–Me at δ 2.83 (3H, s) and D_2O exchangeable protons at δ 7.99 (1H, t, NH) and at δ 11.82 (1H, br s, N–OH). The foregoing spectral data revealed that compound **1** belongs to the purpuramine A—I[4] class. The linear connectivity of the methylene groups was established by the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum. In the ^{1}H–^{1}H COSY spectrum the signal at δ 2.03 showed correlations with an oxygen bearing methylene at δ 3.91 and with a methylene at δ 3.38, and two mutually coupled methylene signals at δ 2.73 and δ 3.35. From the study of its ^{1}H- and ^{13}C-NMR spectra, the three methyl signals at δ 3.79, δ 3.57 and δ 2.83 were assigned to an aromatic methoxyl [δ_C 56.2 (q)], to an ester

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Table 1. ^{13}C- (125 MHz), ^{1}H-NMR (500 MHz) and HMBC (500 MHz) Data for Compounds **1**[a] and **2**[b]

Position	Compound **1**			Compound **2**		
	δ_C (mult.)	δ_H	HMBC	δ_C (mult.)	δ_H	HMBC
1	130.4 (s)			131.7 (s)		
2	129.1 (d)	7.12 (1H, dd, *J*=2.0, 8.0)	C4, C6, C7	130.4 (d)	7.02 (1H, dd, *J*=2.0, 8.0)	C6, C7
3	112.6 (d)	6.97 (1H, d, *J*=8.0)	C1, C5	113.2 (d)	6.78 (1H, d, *J*=8.0)	C1, C5
4	153.8 (s)			155.7 (s)		
5	110.2 (s)			112.5 (s)		
6	133.0 (d)	7.38 (1H, d, *J*=2.0)	C2, C4, C5	135.0 (d)	7.44 (1H, d, *J*=2.0)	C4, C7
7	27.8 (t)	3.72 (2H, s)	C2, C6, C8, C9	28.1 (t)	3.76 (2H, s)	C1, C2, C6, C8, C9
8	151.8 (s)			152.7 (s)		
9	163.2 (s)			165.5 (s)		
11	41.1 (t)	3.35 (2H, t, *J*=7.0)	C9, C12, C13	48.7 (t)	3.15 (2H, t, *J*=7.2)	C9, C12, C13, N–CH_3
12	34.0 (t)	2.73 (2H, t, *J*=7.0)	C11, C13, C14, C18	29.0 (t)	2.15 (2H, tt, *J*=7.2, 6.0)	C11, C13
13	139.0 (s)			71.5 (t)	4.08 (2H, t, *J*=6.0)	C11, C12
14	132.9 (d)	7.46 (2H, s)	C12, C15, C16	—		
15	117.2 (s)			152.2 (s)		
16	150.7 (s)			118.9 (s)		
17	117.2 (s)			134.3 (d)	7.36 (2H, s)	C15, C16, C19, C21
18	132.9 (d)	7.46 (2H, s)	C12, C16, C17	139.9 (s)		
19	—			134.3 (d)	7.36 (2H, s)	C15, C17, C20, C21
20	71.0 (t)	3.91 (2H, t, *J*=6.0)	C16, C21, C22	118.9 (s)		
21	28.0 (t)	2.03 (2H, m)	C20, C22	35.5 (t)	2.72 (2H, t, *J*=7.8)	C17, C18, C19, C22
22	40.6 (t)	3.38 (2H, t, *J*=6.8)	C20, C21, C24, N–Me	41.5 (t)	3.45 (2H, t, *J*=7.8)	C18, C21
24	156.5 (s)			—		
25	52.3 (q)	3.57 (3H, s)	C24	—		
NH		7.99 (1H, t, *J*=6.0)		—		
N–Me	41.4 (q)	2.83 (3H, s)	C22, C24	34.4 (q)	2.60 (3H, s)	C11
OMe	56.2 (q)	3.79 (3H, s)	C4	57.0 (q)	3.78 (3H, s)	C4

a) DMSO-d_6. *b*) CD_3OD.

methyl of a carbamate[12] [δ_C 156.5 (s), δ 52.3 (q)] and to a methyl on nitrogen bearing carbamate ester [δ_C 41.4 (q)] respectively. The structure of compound **1** was established by the study of heteronuclear multiple bond connectivity (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) correlations. In its HMBC spectrum the C-22 methylene protons at δ 3.38 (2H, t, *J*=6.8 Hz) showed correlations with C-20, C-24 and to a *N*-methyl at δ_C 41.4 (q) respectively. Further, the *N*-methyl at δ_H 2.83 (δ_C 41.4) showed correlation with carbamate carbonyl at δ_C 156.5 (s). The C-11 methylene protons at δ 3.35 (2H, t, *J*=7 Hz) showed correlation with C-9 and C-13 carbons. In the NOESY spectrum, the benzylic methylene signal at δ 2.73 (2H, t, *J*=7 Hz, 12-H) showed correlation with two aromatic protons at δ 7.46 (2H, s, 14,18-H), the methoxyl signal at δ 3.79 (3H, s) showed correlation with an aromatic proton at δ 6.97 (1H, d, *J*=8 Hz, 3-H), the methyl signal at δ 2.83 (3H, s) showed correlation with a methylene group at δ 3.38 (2H, t, *J*=6.8 Hz, 22-H) connected to methyl carbamate. The foregoing spectral data suggested that compound **1** is methyl carbamate of purpuramine I. Thus the structure of purpuramine K was established as **1**.

Purpuramine L (**2**) was obtained as an optically inactive white solid (yield 0.08% on dry wt basis), mp 175—178 °C. Its molecular formula was established as $C_{22}H_{26}N_3O_4Br_3$ by HR-FAB-MS, which showed molecular cluster ion $(M+H)^+$ peaks at *m*/*z* 633, 635, 637 and 639 in 1 : 2 : 2 : 1 ratio suggesting the presence of 3 bromine atoms. The IR spectrum showed bands at 3350, 1670, 1200, 1137 and 720 cm^{-1}, which indicated the presence of hydroxyl and amide groups. The ^{1}H-NMR spectrum of compound **2** displayed 11 signals for 26 protons and its ^{13}C-NMR spectrum showed the presence of N–Me and O–Me [δ_H 2.60 (δ_C 34.4); δ_H 3.78 (δ_C 57.0)]. The ^{1}H- and ^{13}C-NMR spectra of compound **2** closely resemble those of purpuramines A—I.[4] Its ^{1}H-NMR spectrum displayed four aromatic signals due to two benzene rings *viz.*, a 1, 3, 4 tri substituted benzene [δ 7.02 (1H, dd, 2-H), 6.78 (1H, d, 3-H), 7.44 (1H, d, 6-H)] and a 1, 3, 4, 5 tetra substituted benzene [δ 7.36 (2H, s, 17, 19-H)]. Further, its ^{1}H-NMR displayed signals for six methylenes (Table 1). The linear connectivity of these methylene groups was established by following ^{1}H–^{1}H COSY spectral data. A methylene group proton signal at δ 2.15 (2H, tt, 12-H) showed correlation with a methylene bearing an oxygen atom at δ 4.08 (2H, t, 13-H) and also with a methylene bearing amide nitrogen at δ 3.15 (2H, t, 11-H). There were also, two mutually coupled methylene group signals at δ 3.45 (2H, t, 22-H) and δ 2.72 (2H, t, 21-H). Of the two methyl groups present in the molecule, one was assigned to an aromatic methoxyl group at C-4 [δ 3.78 (3H, s)]. The other methyl group which resonated at δ 2.60 (3H, s) was placed on the amide nitrogen of the molecule due to its NOESY correlations with the methylene protons at δ 3.15 (2H, t, H-11) and by consideration of its ^{13}C-NMR chemical shift.[4] The ^{1}H- and ^{13}C-NMR values of the N–Me group in the propyl ether side chain of purpuramine G and I were reported at δ_H 2.76 (3H, s), δ_C 27.7(q) and δ_H 2.76 (3H, s), δ_C 27.7 (q) respectively.[4] However in the case of compound **2** its ^{13}C signal appeared [δ_C 34.4 (q)] substantially further downfield due to its presence on amide nitrogen.[16] The foregoing spectral data was further corroborated by its HMBC correlations. In the HMBC spectrum of **2**, the C-11 methylene protons at δ 3.15 (2H, t, *J*=7.2 Hz) showed correlations with C-9, C-12, C-13 and N–Me. The C-21 methylene protons at δ 2.72 (2H, t, *J*=7.8 Hz) showed corre-

Table 2. Antibacterial Activity of Compounds **1** and **2**

	Name of the organism	Concentration (μg/disk)[a]						Kanamycin[b] (30 μg/disk)
		1			**2**			
		25	50	100	25	50	100	
Gram positive	*S. aureus*	10	10	12	10	14	16	10
	B. subtilis	8	10	12	10	14	16	18
	B. sphaericus	—	9	11	7	12	14	20
Gram negative	*C. violaceum*	7	8	11	10	13	15	17
	K. aerogenes	10	11	13	7	10	12	15
	P. aeruginosa	10	12	14	12	16	18	27

a) Values represent zones inhibition in mm/dia. *b*) Positive control.

lation with C-17, C-18, C-19 and C-22 respectively. From the foregoing spectral data the structure of purpuramine L was established as **2**.

The upfield ^{13}C-NMR chemical shifts of C-7 in compounds **1** and **2** [δ_C 27.8 (t) and δ_C 28.1 (t)] suggests an *E*-configuration for the oxime[13] (the corresponding in the case of a (*Z*) oxime is >35 ppm[14]).

Antibacterial Activity The antibacterial activity was assayed by disk susceptibility tests according to the NCCLS (Wayne, 1997),[15] Inocula were adjusted to 0.5 McFarland turbidity. Excess moisture was allowed to absorb for 10 min. before applying dried disks containing the compound. Compounds were dissolved in sterile dimethyl sulfoxide (DMSO), which did not influence the growth of bacteria. Kanamycin was used as a positive control according to the standard method. The test plates were incubated at 37 °C and zones of inhibition were recorded after 24 h. Purpuramines K (**1**) and L (**2**) were tested (Table 2) against gram positive bacteria *Staphylococcus aureus* (ATCC #9144), *Bacillus subtilis* (ATCC #6051), *Bacillus sphaericus* (ATCC #14577) and gram negative bacteria *Chromobaterium violaceum* (ATCC #12472), *Klebsiella aerogenes* (ATCC #15380), *Pseudomonas aeruginosa* (ATCC #25619).

Compound **2** is highly active against *S. aureus*, *B. subtilis* and *C. violaceum*, moderately active against *B. sphaericus*, *K. aerogenes*, and *P. aeruginosa*. Compound **1** is moderately active against all the organisms. Interestingly, gram positive bacteria are highly susceptible to compound **2** when compared to the positive control kanamycin.

Experimental

General Optical rotations were measured on a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Shimadzu-240 and Perkin-Elmer 240-C instruments, respectively. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Varian Gemini 500 MHz spectrometer using tetramethyl silane (TMS) as internal standard. Chemical shifts are reported in parts per million and coupling constants (*J*) are expressed in Hertz. Mass spectra were recorded on a VG Auto Spec-M instrument. Preparative scale HPLC was performed using a Supelcosil C_{18} column (60 Å, 12 μm, 25 cm×21.2 mm).

Animal Material The sponge *Psammaplysilla purpurea* was collected from the Mandapam coast in the Gulf of Mannar, Tamilnadu, India, during February 2002. A voucher specimen (IIC-441) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and Isolation The freshly collected sponge specimens were soaked in MeOH at the site of collection and kept in MeOH until workup. The sponge *Psammaplysilla purpurea* (1.5 kg) was extracted with 1 : 1 CH_2Cl_2–MeOH (3×3 l) at room temperature. The combined extracts including the initial methanol extract were filtered, and the solvent was removed under reduced pressure to give a predominantly aqueous suspension, which was partitioned between water and EtOAc. The EtOAc soluble portion was again concentrated under reduced pressure to give a dark brown gummy mass (10 g). This crude extract (10 g) was subjected to gel filtration chromatography (Sephadex LH-20, 1 : 1 CH_2Cl_2–MeOH, 47 mm×820 mm) collecting 30 fractions (25 ml each) followed by silica gel chromatography of selected fractions using a step gradient of hexane to hexane–ethyl acetate mixtures to MeOH, to yield 3,5-dibromo-4-methoxyphenylacetonitrile (40 mg), 3-bromo-4-methoxyphenylacetonitrile (60 mg), 3-bromo-4-hydroxyphenylacetonitrile (100 mg), 1-hydroxyuracil (30 mg), 5α,8α-epidioxycholest-6-ene-3β-ol (150 mg), compound **1** (7 mg) and compound **2** (10 mg). The fractions eluted with hexane–EtOAc (40 : 60) which could not be purified by silica gel chromatography are combined and purified on a preparative reversed-phase (C_{18}) HPLC column [MeOH–H_2O (80 : 20)], (flow rate of 5 ml/min) to give compounds **1** and **2**.

Purpuramine K (**1**): Optically inactive white solid (7 mg), mp 190—195 °C; IR (KBr) ν_{max} 3404, 2926, 1675 and 1494 cm^{-1}; UV (MeOH) λ_{max} (log ε) 217.8 (3.65), 280.2 (4.78); for ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 1; positive FAB-MS *m/z* 692, 694, 696 and 698 $(M+H)^+$ in a 1 : 2 : 2 : 1 ratio; positive HR-FAB-MS *m/z* observed 691.9726 $[M+H]^+$ (Calcd for $C_{24}H_{28}N_3O_6{}^{79}Br_3$ *m/z* 694.2150, Δ−2.2 mmu).

Purpuramine L (**2**): Optically inactive white solid (10 mg), mp 175—178 °C; IR (KBr) ν_{max} 3350, 1670, 1200, 1137 and 720 cm^{-1}; UV (MeOH) λ_{max} (log ε) 214.5 (5.12), 280.0 (4.02), for ^{1}H-NMR (500 MHz, CD_3OD), ^{13}C-NMR (125 MHz, CD_3OD) see Table 1; positive FAB-MS *m/z* 633, 635, 637 and 639 $(M+H)^+$ in a 1 : 2 : 2 : 1 ratio; positive HR-FAB-MS *m/z* observed 632.9650 $[M+H]^+$ (Calcd for $C_{22}H_{26}N_3O_4{}^{79}Br_3$ *m/z* 635.7964, Δ−2.8 mmu).

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