Isolation of Psammaplin A 11′-Sulfate and Bisaprasin 11′-Sulfate from the Marine Sponge *Aplysinella rhax*

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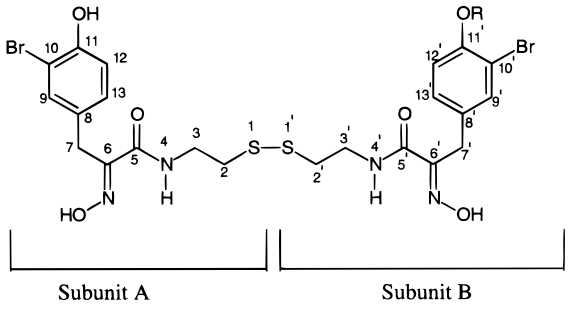
Psammaplin A 11′-sulfate (**3**) and bisaprasin 11′-sulfate (**4**) have been isolated from the marine sponge *Aplysinella rhax*, along with the known psammaplin A (**1**). Their structures were determined on the basis of their spectroscopic data. Compounds **1** and **3** inhibited [^{3}H]1,3-dipropyl-8-cyclopentylxanthine binding to rat-brain adenosine A_1 receptors.

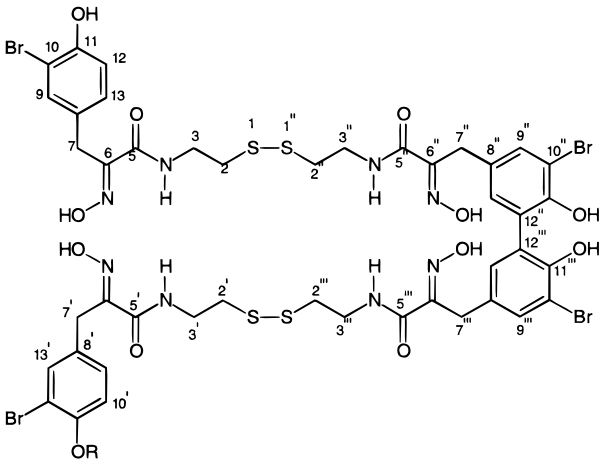
Psammaplin A (**1**), a symmetrical bromotyrosine disulfide, was described in 1987 by Arabshahi and Schmitz[1] from an unidentified marine sponge, by Quiñoà and Crews[2] from *Psammaplysilla* sp., and by Rodriguez, Akee, and Scheuer[3] from *Thorectopsamma xana*. A dimer of psammaplin A, bisaprasin (**2**), has also been reported.[3] In 1991, Jiménez and Crews isolated psammaplins A, B, C, and D and prepsammaplin A from *Psammaplysilla purpurea*.[4] Bisaprasin and psammaplins A and D have been reported to inhibit the growth of Gram-positive *Staphylococcus aureus*.[3,4] Psammaplin D also showed antimicrobial activity against the Gram-negative bacterium *Trichophyton mentagrophytes* and a mild in vitro activity against tyrosine kinase.[4] Studies on the chemical constituents of an *Aplysinella rhax* extract, which inhibited the binding of [^{3}H]1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) on adenosine A_1 receptors, led to the isolation of two new members of the psammaplin family, psammaplin A 11′-sulfate (**3**), bisaprasin 11′-sulfate (**4**), along with psammaplin A (**1**). In this paper we report the isolation and structure elucidation of **1**, **3**, and **4** by 1D and 2D NMR techniques and the inhibition of **1** and **3** on the binding of [^{3}H]DPCPX, an A_1 selective antagonist ligand, to rat-brain adenosine A_1 receptors.

The MeOH extract of the freeze-dried sponge was subjected to reversed-phase C_{18} flash column chromatography, followed by LH-20 gel permeation and reversed-phase HPLC to give psammaplin A (**1**), psammaplin A 11′-sulfate (**3**), and bisaprasin 11′-sulfate (**4**). The structure of **1** was confirmed by comparison of its ^{1}H and ^{13}C NMR data with published values.[1−3]

Comparison of ^{1}H NMR and ^{13}C NMR of **1** and **3** (Table 1) showed that **3** yielded signals corresponding to subunit A, another set of signals attributable to the partial structure from C-2′ to C-7′, and a pattern of signals for a trisubstituted aromatic ring. This suggested that the difference between **1** and **3** might only be the substitution group at the phenolic oxygen. (−)-HRESIMS showed a 1:2:1 triplet centered at *m/z* 764.8823 [M − H]$^-$, which supported a formula of $C_{22}H_{23}Br_2O_9N_4S_3Na$, corresponding to a sulfated psammaplin A. ^{1}H NMR data showed two oxime signals at δ 11.86 (1H, s) and 11.92 (1H, s), and one phenolic OH at δ 10.05 (1H, s), suggesting that C-11′ OH was sulfated. Comparison to the phenolic ring of subunit A, the location of the sulfate group was supported by the proton chemical shifts of H-9′, H-12′, and H-13′, which were



(**1**) R = H Psammaplin A
(**3**) R = SO_3Na Psammaplin A 11'-sulfate



(**2**) R = H Bisaprasin
(**4**) R = SO_3Na Bisaprasin 11'-sulfate

further downfield (H-9′, 7.32, $\Delta\delta$ +0.07 ppm; H-12′, 7.41, $\Delta\delta$ +0.61 ppm; H-13′, 7.07, $\Delta\delta$ +0.10 ppm) and the carbon chemical shifts, which showed upfield shifts for carbon *ipso* to OSO_3^- group (C-11′, 148.9 ppm, $\Delta\delta$ −3.4 ppm) and downfield shifts for carbons *ortho* and *para* to C-11′ (C-10′, 113.8 ppm, $\Delta\delta$ +5.0 ppm; C-12′, 121.3 ppm, $\Delta\delta$ +5.2 ppm; C-8′, 132.8 ppm, $\Delta\delta$ +4.0 ppm).[5] The oxime groups in **3** were assigned *E* geometries on the basis of ^{13}C NMR shifts of 27.7 ppm for benzylic carbon atoms C-7 and C-7′.[1] Thus, the sulfated psammaplin A was assigned structure **3**.

Compound **4** was isolated as a minor component and had a molecular formula of $C_{44}H_{44}Br_4N_8O_{15}S_5Na$. This was

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Table 1. ^{1}H and ^{13}C NMR Data of Psammaplin A 11′-Sulfate (**3**)

position[a]	^{13}C[b] δ	^{1}H[c] δ (mult., J in Hz)	HMBC[c] (C no.)
2	36.9	2.77 (t, 6.9)	3
3	38.2	3.40 (m)	2, 5
4-NH		8.08 (t, 6.0)	
5	163.2		
6	151.8		
6-NOH		11.86 (s)	
7	27.7	3.65 (s)	5, 6, 8, 9, 13
8	128.8		
9	132.8	7.25 (d, 2.4)	7, 11, 13
10	108.8		
11	152.3		
12	116.1	6.80 (d, 8.4)	8, 10
13	129.1	6.97 (dd, 8.4, 2,4)	7, 9, 11
11-OH		10.05 (br s)	
2′	37.0	2.77 (t, 6.9)	3′
3′	38.2	3.40 (m)	2′, 5′
4′-NH		8.10 (t, 6.0)	
5′	163.2		
6′	151.5		
6′-NOH		11.92 (s)	
7′	27.9	3.71 (s)	5′, 6′, 8′, 9′, 13′
8′	132.8		
9′	132.4	7.32 (d, 2.4)	7′, 11′, 13′
10′	113.8		
11′	148.9		
12′	121.3	7.41 (d, 8.4)	8′, 10′
13′	128.5	7.07 (dd, 8.4, 2.4)	7′, 9′, 11′

[a] Numbering for structure of **3** is identical to that used for psammaplins B−D.[4] [b] Spectra were recorded in DMSO-d_6, 150 MHz. [c] Spectrum was recorded in DMSO-d_6, 600 MHz.

established by a (−)-HRESIMS spectrum, which showed a 1:4:6:4:1 quintet for the molecular ion centered at m/z 1426.8114 [M − H]$^-$. ^{1}H NMR, ^{13}C NMR, and HMQC data (Table 2) showed that **4** consisted of subunits A and B, and allowed the identification of another two −S−CH_2−CH_2−NH−C(=O)−C(=NOH)−CH_2− moieties and two sets of aromatic proton resonances (1H, br s, δ 7.17; 1H, br s, δ 7.00) belonging to 1,2,3,5-tetrasubstituted benzene rings. Although these moieties were not identical, they had superimposable chemical shifts due to the distance of the asymmetric substituted groups at C-11 and C-11′. The ^{13}C NMR spectrum acquired in DMSO-d_6 did not show the quaternary carbons at the positions C-8″ (or 8‴), C-10″ (or 10‴), C-11″ (or 11‴), and C-12″ (or 12‴). Also, the ^{1}H NMR spectrum in DMSO-d_6, with overlapping signals between H-13 and H-13″ (or H-13‴), did not allow resolution of the linkage between the two phenyl groups. However, the linkage was established using an HMBC experiment acquired in MeOH-d_4. HMBC correlations were observed from H-7″ to C-8″, C-9″, and C-13″; H-9″ to C-7″, C-10″, C-11″, and C-13″; and H-13″ to C-7″, C-9″, C-11″, and C-12‴. The ^{13}C chemical shifts of the aryl rings (C-8″−C-13″ or C-8‴−C-13‴) were consistent with those reported in **2**,[3] bastadin-3,[6] and 10-sulfatobastadin-3.[7] In addition, ^{1}H COSY analysis provided the coupling sequences from the benzylic protons at H-7″ (2H, s, δ 3.84) to the *ortho* aryl ring protons at H-9″ (1H, s, δ 7.35) and H-13″ (1H, s, δ 7.12), and between H-9″ and H-13″. The foregoing data

Table 2. ^{1}H, ^{13}C, and HMBC NMR Data of Bisaprasin 11′-Sulfate (**4**)

position[a]	^{13}C[b] δ	^{1}H[c] δ (mult., J in Hz)	^{13}C[d*] δ	^{1}H[d] δ (mult., J in Hz)	HMBC[d] (C no.)
2	36.9	2.80 (m)	38.5	2.78 (t, 6.6)	3
3	38.2	3.41 (m)	39.7	3.50 (t, 6.6)	2, 5
4-NH		8.05 (t, 6.0)			
5	163.2		167.0		
6	151.8		153.1		
6-NOH		11.86 (s)			
7	27.7	3.68 (s)	28.7	3.79 (s)	5, 6, 8, 9, 13
8	128.8		130.5		
9	132.8	7.29 (d, 2.0)	134.9	7.35 (br s)	7, 10, 11, 13
10	108.8		110.4		
11	152.3		153.9		
11-OH		10.05 (br s)			
12	116.2	6.81 (d, 8.4)	117.2	6.76 (d, 8.4)	8, 10, 11
13	129.1	7.00 (dd, 8.4, 2.0)	130.8	7.06 (dd, 8.4, 2.4)	7, 9, 11
2′	37.0	2.80 (m)	38.5	2.81 (t, 6.6)	3′
3′	38.2	3.41 (m)	39.7	3.54 (t, 6.6)	2′, 5′
4′-NH		8.10 (t, 6)			
5′	163.3		165.8		
6′	151.5		152.6		
6′-NOH		11.92 (s)			
7′	27.9	3.74 (s)	29.1	3.86 (s)	5′, 6′, 8′, 9′, 13′
8′	132.8		135.9		
9′	132.4	7.35 (d, 2.0)	134.9	7.49 (d, 2.4)	7′, 10′, 11′, 13′
10′	113.8		116.6		
11′	148.9		149.8		
12′	121.3	7.44 (d, 8.4)	123.3	7.48 (d, 8.4)	8′, 10′, 11′
13′	128.5	7.10 (dd, 8.4, 2.0)	130.1	7.21 (dd, 8.4, 2.4)	7′, 9′, 11′
2″, 2‴	36.9	2.80 (m)	38.5	2.79 (t, 6.6)	3″
3″, 3‴	38.1	3.41 (m)	39.7	3.52 (t, 6.6)	2″, 5″
4″-NH, 4‴-NH		8.06 (t, 6)			
5″, 5‴	163.4		166.1		
6″, 6‴	151.8		153.5		
6″-NOH		11.76 (s)			
6‴-NOH		11.75 (s)			
7″, 7‴	27.8	3.70 (s)	28.7	3.84 (s)	5″, 6″, 8″, 9″, 13″
8″, 8‴	*e*		128.2		
9″, 9‴	130.4	7.17 (s)	133.5	7.35 (br s)	7″, 10″, 11″, 13″
10″, 10‴	*e*		114.5		
11″, 11‴	*e*		154.5		
11″-OH, 11‴-OH		10.05 (br s)			
12″, 12‴	*e*		130.5		
13″, 13‴	130.0	7.00 (s)	132.4	7.12 (br s)	7″, 9″, 11″, 12‴

[a] Numbering for structure of **3** is identical to that used for psammaplins B−D.[4] [b] Spectrum was recorded in DMSO-d_6, 150 MHz. [c] Spectrum was recorded in DMSO-d_6, 400 MHz. [d] Spectra were recorded in MeOH-d_4, 600 MHz. [d] *Assignments were based on HMQC and HMBC experiments (recorded in MeOH-d_4, 600 MHz). [e] Signals were not seen with acquisition time = 72 h and d_1 = 5 s.

secured the complete substitution patterns around the phenyl rings and the linkage at C-12″ (130.5 ppm) and C-12‴ (130.5 ppm). The four oximes in **4** were all present as *E* isomers.[1] Thus, **4** is a phenyl dimer of **1** and **3** and was assigned as bisaprasin 11′-sulfate.

Psammaplin A (**1**) and psammaplin A 11′-sulfate (**3**) inhibited [^{3}H]DPCPX binding to rat-brain adenosine A_1 receptors with IC_{50} of 20 μM and 90 μM, respectively. Bisaprasin 11′-sulfate (**4**) did not show the inhibition up to the concentration of 2.2 mM.

Experimental Section

General Experimental Procedures. Solvents used were Omnisolv MeOH and milli-Q filtered water. Sephadex LH-20 (400 mm × 40 mm i.d.) (Pharmacia Biotech) was used for gel permeation chromatography. The flash column (150 mm × 40 mm i.d.) was packed with Davisil C_{18} (30−40 μm). A Rainin C_{18} (3 μm, 50 mm × 4.6 mm i.d.) column was used for semipreparative chromatography. A Waters 600 pump equipped with a 996 PDA detector was used for semipreparative HPLC separations. NMR spectra were recorded on a Varian Inova 600 MHz NMR and 400 MHz spectrometers with ^{1}H and ^{13}C chemical shifts referenced to the solvent peak δ 2.50 and 39.5 ppm (DMSO-d_6) and δ 3.31 and 49.1 ppm (MeOH-d_4). LRESMS was recorded on a single quadruple VG platform II mass spectrometer with MassLynx Version 1 used for data acquisition, and HRESIMS were measured on a Bruker BioAPEX 47e mass spectrometer.

Sponge Material. The sponge was collected by hand via scuba diving (−24 m) (21.59.5′S 152.28.6′E) at Gannet Cay, southside reef, fore-reef slope, Swain Reefs, Queensland, Australia. It was identified as *Aplysinella rhax* de Laubenfels, 1954 (phylum Porifera, class Demospongiae, order Verongida, family Aplysinidae). Voucher specimen QMG305403 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. Freeze-dried *A. rhax* (60 g) was extracted exhaustively with MeOH. After evaporation of the solvent, the crude extract (7 g) was purified through a flash C_{18} column with H_2O, H_2O−MeOH (1:1), and MeOH. The MeOH fraction was further separated on a Sephadex column LH-20, using MeOH as the eluent at a rate of 2 mL/min. The fractions were combined on the basis of TLC. The last-eluting fraction contained a mixture of compounds **1**, **3**, and **4**. This fraction was concentrated and chromatographed on reversed-phase HPLC, gradient from H_2O−MeOH (2:8) to H_2O−MeOH (1:1) in 15 min and from H_2O−MeOH (1:1) to H_2O−MeOH (3:7) for a further 25 min to give psammaplin A (**1**) (200 mg, 0.33% dry wt) (24 min), psammaplin A 11′-sulfate (**3**) (3 mg, 0.006% dry wt) (13 min), and bisaprasin 11′-sulfate (**4**) (1 mg, 0.001% dry wt) (18 min).

Receptor Binding Assays. Binding of **1**, **3**, and **4** to rat-brain A_1 receptors were performed as described previously.[8] Data were analyzed using a nonlinear, least-squares regression program (Prism 2.0) to determine IC_{50} values.

Psammaplin A (1): white powder; (−)-LRESMS *m*/*z* 663 [M − H, $C_{22}H_{23}Br_2N_4O_6S_2$]; ^{1}H and ^{13}C NMR data were identical with published data.[1−3]

Psammaplin A 11′-sulfate (3): oil; UV (MeOH) λ_{max} nm (ϵ) 206 (33 000), 283 (3000), 324 (750); IR (film) 3386, 1660, 1531, 1486, 1425, 1272, 1230, 1060, 1037, 1014, 951, 826, 708 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6) data, see Table 1; (−)-HRESIMS *m*/*z* 762.8840 (calcd for M − H, $C_{22}H_{22}{}^{79}Br_2N_4O_9S_3$-Na, 762.8819).

Bisaprasin 11′-sulfate (4): oil; UV (MeOH) λ_{max} nm (ϵ) 203 (83 000), 283 (7900), 324 (5300); IR (film) 3403, 1656, 1529, 1483, 1418, 1272, 1226, 986 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6 and MeOH-d_4) data, see Table 2; (−)-HRESIMS *m*/*z* 1422.8142 (calcd for M − H, $C_{44}H_{43}{}^{79}Br_4N_8O_{15}S_5Na$, 1422.8166).

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