Psammaplysenes C and D, Cytotoxic Alkaloids from *Psammoclemma* sp.

Malcolm S. Buchanan,[†] Anthony R. Carroll,[†] Rama Addepalli,[†] Vicky M. Avery,[†] John N. A. Hooper,[‡] and Ronald J. Quinn*,[†]

Eskitis Institute, Griffith University, Brisbane, Queensland 4111, Australia, and Queensland Centre for Biodiversity, Queensland Museum, South Brisbane, Queensland 4101, Australia

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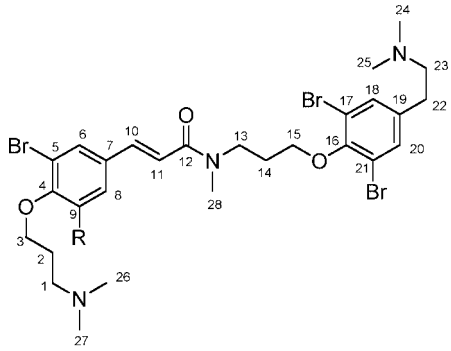
The sponge *Psammoclemma* sp. was investigated as part of our studies to discover $P2X_7$ receptor antagonists for the treatment of inflammatory disease. The biological activity of this extract was found to be due to the cytotoxicity of two new bromotyrosine alkaloids, psammaplysenes C (**1**) and D (**2**), and not $P2X_7$-specific activity. Their structures were determined by 1D and 2D NMR spectroscopy.

We have previously reported the structurally complex pyrrole-imidazole alkaloids stylissadines A and B, from *Stylissa flabellata*, as specific inhibitors of $P2X_7$.[1] Another extract that showed antagonism of the ion channel $P2X_7$, when a high-throughput screening procedure was performed against a library of extracts derived from plants and marine organisms, was from the marine sponge *Psammoclemma* sp. (Chondropsidae). The bioactive constituents isolated were the bromotyrosine alkaloids psammaplysenes C (**1**) and D (**2**). A wide variety of bromotyrosine-derived metabolites have been isolated from marine sponges ranging in complexity from simple monomeric metabolites, for example *N,N,N*-trimethyl-3,5-dibromotyrosine,[2] to more complex structures formed by combining two to four bromotyrosine-derived units through amide or ether bonds, such as the bastadins.[3] While the presence of bromotyrosines is characteristic of sponges from the order Verongida,[4,5] until now no bromotyrosine compounds have been reported from the genus *Psammoclemma* (order Poecilosclerida). Psammaplysenes A and B, isolated from a *Psammaplysilla* sp., are the closest bromotyrosine analogues to **1** and **2** and are described as specific inhibitors of FOXO1a nuclear export.[6] The isolation, structure elucidation, and biological activity of psammaplysenes C (**1**) and D (**2**) will be discussed here.



1 R = H
2 R= Br

Psammaplysene C (**1**) was assigned the molecular formula $C_{28}H_{38}Br_3N_3O_3$ by HRESIMS (*m/z* 351.5292 $[C_{28}H_{38}Br_3N_3O_3+2H]^{2+}$, calcd 351.5304). Furthermore, the isotopic pattern in the MS confirmed the presence of three bromine atoms in the compound. Resonances in the ^{13}C NMR spectrum (Table 1) indicated **1** contains 28 carbon atoms. The ^1H NMR spectrum (Table 1) contained signals for a 1,2,4-trisubstituted aromatic ring (δ_H 8.06, 7.67, 7.11), a symmetrical 1,2,4,6-tetrasubstituted aromatic ring (δ_H 7.64, 2H), a *trans* α,β-unsaturated carbonyl group {δ_H 7.39 (d, 15.6 Hz), 7.14 (m)} and five *N*-methyls {δ_H 3.20, 2.80 (6H), 2.84 (6H)}. There were also signals for eight methylenes {δ_H 3.24 (m, 2H); 2.16 (m, 2H); 4.17 (m, 2H); 3.60 (t, 7.0 Hz, 2H); 2.04 (quin., 7.0, 2H); 3.96 (t, 7.0); 2.95 (m, 2H); 3.30 (m, 2H)}. The gCOSY spectrum revealed $^1H-^1H$ coupling patterns for two $N-CH_2-CH_2-CH_2-O$ and one CH_2-CH_2-N unit. The different partial structures were then linked together by interpretation of correlations observed in a gHMBC spectrum. Thus, the *N*-methyl at δ_H 3.20 (H-28) showed correlations to δ_C 165.4 (C-12) and 44.8 (C-13), linking the α,β-unsaturated carbonyl group to one $N-CH_2-CH_2-CH_2-O$ unit. Correlations between δ_H 7.39 (H-10) and δ_C 131.8 (C-6) and 129.4 (C-8) linked the other end of the α,β-unsaturated carbonyl to the 1,2,4-trisubstituted aromatic ring. The $N-CH_2-CH_2-CH_2-O$ unit was also linked to the symmetrical 1,2,4,6-tetrasubstituted aromatic ring. This was deduced from observation of a gHMBC correlation between δ_H 3.96 (H-15) and δ_C 151.4 (C-16). The second $N-CH_2-CH_2-CH_2-O$ unit was also attached to the 1,2,4-trisubstituted aromatic ring, since a gHMBC correlation was observed between δ_H 4.17 (H-3) and δ_C 155.1 (C-4). Correlations between δ_H 2.95 (H-22) and δ_C 133.2 (C-18/-20) and 136.2 (C-19) indicated that the CH_2-CH_2-N unit is linked to the 1,2,4,6-tetrasubstituted aromatic ring. The positions of the four remaining *N*-methyls at 23-N and 1-N were confirmed by gHMBC correlations from the *N*-methyls at δ_H 2.80 (6H) to C-23 and from the *N*-methyls at 2.84 (6H) to C-1. Finally, some ^1H and ^{13}C NMR signals were either broadened or doubled (Table 1), indicating *trans*:*cis* isomerism about the amide bond. The *N*-methyl in the *trans* isomer (δ_H 3.20) was further downfield in the ^1H NMR spectrum than in the *cis* isomer (δ_H 2.97). This was due to anisotropic shielding from the amide carbonyl in the *cis* orientation. The NMR spectra indicated a 4:3 ratio of *trans* to *cis* isomers. Psammaplysene C was therefore assigned structure **1**.

Psammaplysene D (**2**) was assigned the molecular formula $C_{28}H_{37}Br_4N_3O_3$ by HRESIMS (*m/z* 390.4827 $[C_{28}H_{37}Br_4N_3O_3+2H]^{2+}$, calcd 390.4857). The isotopic pattern in the MS confirmed the presence of four bromine atoms for this compound. Thus, compound **2** had an additional bromine atom compared with **1**. The ^1H NMR spectrum (Table 1) clearly showed that **2** differs from **1** by the replacement of the trisubstituted aromatic ring with a symmetrical tetrasubstituted ring. Thus, **2** has two symmetrical 1,2,4,6-tetrasubstituted aromatic rings (δ_H 7.64, 2H and 8.10, 2H), compared with **1**, which had one 1,2,4-trisubstituted aromatic ring (δ_H 8.06, 7.67, 7.11) and one symmetrical 1,2,4,6-tetrasubstituted aromatic ring (δ_H 7.64, 2H). Psammaplysene D was also isolated as a 4:3 mixture of *trans*:*cis* isomers and was assigned structure **2**.

The $P2X_7$ biological screen, described previously,[1,7] was set up to identify specific antagonists of the $P2X_7$ receptor (ligand gated ion channel). However, psammaplysenes C (**1**) and D (**2**) showed

* To whom correspondence should be addressed. Tel: +61 7 3735 6006. Fax: +61 7 3735 6001. E-mail: r.quinn@griffith.edu.au.
[†] Griffith University.
[‡] Queensland Museum.

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Table 1. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR Data for Psammaplysenes C (**1**) and D (**2**) in DMSO-d_6[a]

	1		**2**	
position	δ_C	δ_H (mult., *J* Hz)	δ_C	δ_H (mult., *J* Hz)
1	54.2 CH_2	3.24 (m, 2H)	54.2 CH_2	3.36 (m, 2H)
2	23.7 CH_2	2.16 (m, 2H)	24.8 CH_2	2.19 (m, 2H)
3	66.1 CH_2	4.17 (m, 2H)	70.4 CH_2	4.04 (m, 2H)
4	155.1 qC		152.5 qC	
5	111.6 qC		117.9 qC	
6	131.8/131.6 CH	8.06 (s)/8.00 (s)	132.1/132.0 CH	8.10/8.04 (s)
7	129.7 qC		134.8 qC	
8	129.4 CH	7.67 (d, 8.0)/7.64 (m)	132.1/132.0 CH	8.10/8.04 (s)
9	113.6 CH	7.11 (m)	117.9 qC	
10	139.4 CH	7.39/7.42 (d, 15.6)	137.7 CH	7.37/7.41 (d, 15.6)
11	117.8/117.5 CH	7.14 (m)	121.1/120.8 CH	7.28/7.27 (d, 15.6)
12	165.4 qC		165.0 qC	
13	44.8/45.9 CH_2	3.60/3.78 (t, 7.0, 2H)	44.8/45.9 CH_2	3.60/3.78 (t, 7.0, 2H)
14	27.6/29.4 CH_2	2.04/2.10 (quin., 7.0, 2H)	27.5/29.4 CH_2	2.05/2.10 (quin., 7.0, 2H)
15	71.5/71.0 CH_2	3.96/4.03 (t, 7.0, 2H)	71.4/71.0 CH_2	3.95/4.02 (t, 7.0, 2H)
16	151.4/151.5 qC		151.4/151.5 qC	
17	117.5 qC		117.55/117.49 qC	
18	133.2 CH	7.64 (s)	133.2 CH	7.64 (s)
19	136.2 qC		136.3 qC	
20	133.2 CH	7.64 (s)	133.2 CH	7.64 (s)
21	117.54/117.49 qC		117.55/117.49 qC	
22	28.2 CH_2	2.95 (m, 2H)	28.2 CH_2	2.94 (m, 2H)
23	56.7 CH_2	3.30 (m, 2H)	56.8 CH_2	3.29 (m, 2H)
24	42.23 CH_3	2.80 (s, 3H)	42.25 CH_3	2.80 (s, 3H)
25	42.23 CH_3	2.80 (s, 3H)	42.25 CH_3	2.80 (s, 3H)
26	42.32 CH_3	2.84/2.83 (s, 3H)	42.28 CH_3	2.85/2.84 (s, 3H)
27	42.32 CH_3	2.84/2.83 (s, 3H)	42.28 CH_3	2.85/2.84 (s, 3H)
28	35.2/33.6 CH_3	3.20/2.97 (s)	35.2/33.7 CH_3	3.21/2.97 (s)

[a] Chemical shifts are given for both isomers (*trans*/*cis*) when distinguishable signals were identified.

44% and 72%, respectively, cytotoxicity after 90 min incubation. When psammaplysenes C (**1**) and D (**2**) were incubated for 21 h, they both had a cytotoxicity IC_{50} of 7 μM. The bioactivity of **1** and **2** in both the $P2X_7$ and hemolysin specificity assays was therefore attributed to cytotoxicity against the pre-monocytic cell line THP-1, on which the $P2X_7$ receptor was expressed.

Experimental Section

General Experimental Procedures. Water was Millipore Milli-Q PF filtered, while all other solvents used were Lab-Scan HPLC grade. Trifluoroacetic acid (TFA) was Fluka spectroscopic grade. A Betasil C_{18} 5 μm column (21.2 mm × 150 mm i.d.) was used for semipreparative HPLC. A Waters 600 pump fitted with a 996 photodiode array detector and a 717 plus Autosampler was used for the semipreparative separations. The C_{18} was 04K-4348 Sepra C_{18} end-capped silica. FTIR and UV spectra were recorded on a Bruker Tensor 27 FTIR spectrophotometer and an Agilent 8453 UV/vis spectrophotometer, respectively. NMR spectra were recorded at 30 °C on Varian INOVA 500 and 600 MHz NMR spectrometers. Samples were dissolved in DMSO-d_6 (residual ^{1}H δ 2.50 and ^{13}C δ 39.5 ppm). Multiplicity was determined by DEPT (s = C, d = CH, t = CH_2 , q = CH_3). Standard parameters were used for the 2D experiments, which included gradient COSY, HSQC ($^1J_{CH}$ = 140 Hz), and HMBC ($^nJ_{CH}$ = 8.3 Hz). HRESIMS were measured on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer, fitted with an Apollo API source. A WALLAC Victor II was used as the spectrophotometric reader. Assay materials: EDTA, glucose, and BzATP were obtained from Sigma Aldrich. KCl and HEPES were obtained from BDH. Sytox Orange was purchased from Molecular Devices. RPMI media with Glutamax was purchased from Invitrogen. White 384 optiplates were obtained from Perkin-Elmer Life Sciences. THP-1 cells were from AstraZeneca.

Animal Material. The sponge sample *Psammoclemma* sp. 1542 (phylum Porifera, class Demospongiae, order Poecilosclerida, family Chondropsidae) was collected by scuba diving at a depth of 16 m from caves at the base of the cliff Bommie Bay, northeast side of Lizard Island, Queensland, Australia, in April 1994. A voucher sample, QMG304271, was lodged at the Queensland Museum, South Brisbane, Queensland, Australia. A small, single specimen was collected, approximately 5 cm in diameter, with irregularly subspherical growth form, massively encrusting, growing on the cave walls of the base of a rock cliff. There was a single, large osculum on the apex of the sponge, approximately 20 mm in diameter. The surface of the sponge was slightly conulose, silt covered, with a distinctly fleshy appearance, opaque and smooth to touch, and when preserved the ectosome collapsed, producing a prominently and regularly conulose surface. When alive, the color was pale yellow, mottled olive yellow-green on the ship deck, turning olive brown in ethanol. The texture was harsh and brittle due to the incorporation of a large amount of detritus into the skeleton. It had a membranous ectosome, stretched across a thin layer of sand grains, without any fibers or native mineral skeleton at the surface. The choanosomal skeleton was packed with large sand grains bound tightly with collagenous spongin. It was probably enclosed in spongin fibers. However, these were fully packed and could not easily be distinguished, with fibers forming vaguely ascending tracts extending from the deeper regions of the choanosome to the surface. The collagen was very heavy with red-brown pigmentation throughout. Both megascleres and microscleres were absent. It is most likely that this is a new species; however at present its identification is ambiguous. As for the other 27 species described so far in the genus worldwide, it lacks most of the pivotal taxonomic characters (such as mineral spicules, and its fibers were totally cored by foreign sand grains) that are generally used to classify poriferans.

Extraction and Isolation. The material was ground (2.6 g) and extracted sequentially with *n*-hexane, CH_2Cl_2−MeOH (4:1), and finally MeOH. The CH_2Cl_2−MeOH (4:1) and MeOH extracts were combined (265 mg). The sample was preadsorbed on C_{18} and loaded into a refillable preparative guard column (10 mm × 30 mm i.d.) in line with the semipreparative C_{18} HPLC column. The following solvent conditions were used: H_2O−1% TFA isocratic for 5 min (flow: 5 to 10 mL/min), then to MeOH−1% TFA in 45 min, then isocratic for 15 min; 60 fractions were collected. Fraction 35 (39 mg) and fractions 36 and 37 (35 mg) were purified further by C_{18} HPLC. Fraction 35: H_2O−1% TFA to H_2O−1% TFA; MeOH−1% TFA (2:3) in 5 min, followed by isocratic for 15 min, then to MeOH−1% TFA in 5 min, and finally isocratic for 5 min. Psammaplysene C (**1**) (20.3 mg, 0.78% dry wt) was collected, with a retention time of 14.4 min. Fractions 36 and 37: H_2O−1% TFA to H_2O−1% TFA; MeOH−1% TFA (2:3) in 5 min, followed by isocratic for 15 min, then to MeOH−1% TFA in 5 min, and finally isocratic for 5 min. Psammaplysene D (**2**) (11.7 mg, 0.45% dry wt) was collected, with a retention time of 16.3 min. Compounds **1** and **2** were isolated as their trifluoroacetate salts.

Psammaplysene C (1), (2*E*)-3-{3-bromo-4-[3-(dimethylamino) propoxy]phenyl}-*N*-(3-{2,6-dibromo-4-[2-(dimethylamino)ethyl]phenoxy}propyl)-*N*-methylacrylamide: amorphous solid; UV (MeOH) λ_{max} (log ε) 206 (4.87), 218 sh (4.61), 294 (4.36), 312 sh (4.30) nm; IR ν_{max} (film) 3415, 1678, 1596, 1269, 1202, 1142 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; positive-HRESIMS *m/z* 351.5292 $[C_{28}H_{38}{}^{79}Br_3N_3O_3+2H]^{2+}$ (calcd 351.5304).

Psammaplysene D (2), (2*E*)-*N*-(3-{2,6-dibromo-4-[2-(dimethylamino)ethyl]phenoxy}propyl)-3-{3,5-dibromo-4-[3-(dimethylamino)propoxy]phenyl}-*N*-methylacrylamide: amorphous solid; UV (MeOH) λ_{max} (log ε) 206 (4.83), 219 sh (4.59), 286 (4.37) nm; IR ν_{max} (film) 3414, 1679, 1602, 1250, 1202, 1125 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; positive-HRESIMS *m/z* 390.4827 $[C_{28}H_{37}{}^{79}Br_4N_3O_3+2H]^{2+}$ (calcd 390.4857).

Bioassays. The principles, procedures, and methods for the $P2X_7$, hemolysin specificity, and Alamar Blue cytotoxicity assays have been described in detail previously.[1]

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Supporting Information Available: Photographs of the investigated sponge are available free of charge via the Internet at http://pubs.acs.org/jnp.

References and Notes

(1) Buchanan, M. S.; Carroll, A. R.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Quinn, R. J. *J. Org. Chem.* **2007**, *72*, 2309–2317.
(2) Albrizio, S.; Ciminiello, P.; Fattorusso, E.; Magno, S. *Tetrahedron* **1994**, *50*, 783–788.
(3) Miao, S.; Andersen, R. J. *J. Nat. Prod.* **1990**, *53*, 1441–1446.
(4) Yagi, H.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 3749–3754.
(5) Tilvi, S.; Rodrigues, C.; Naik, C. G.; Parameswaran, P. S.; Wahidhulla, S. *Tetrahedron* **2004**, *60*, 10207–10215.
(6) Schroeder, F. C.; Kau, T. R.; Silver, P. A.; Clardy, J. *J. Nat. Prod.* **2005**, *68*, 574–576.
(7) Buchanan, M. S.; Carroll, A. R.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Quinn, R. J. *J. Nat. Prod.* **2007**,in press.

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