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Analytical Chemistry

2D NMR Studies of Bromotyrosine Alkaloid, Purpurealidin K from Marine Sponge *Psammaplysilla purpurea*

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Marine sponges of the order Verongida and related organisms are the richest source of bioactive bromotyrosine alkaloids. Purpurealidin K **(1)** along with purealidin Q **(2)**, aerophobin 2 **(3)** and aplysamine 2 **(4)** were isolated from methanol extract of marine sponge *Psammaplysilla purpurea* collected from Madapam, Tamil Nadu, India. The structure of natural product, purpurealidin K (1) was assigned by extensive ^{1}H, ^{13}C, DEPT, TOCSY, HSQC & HMBC NMR experiments and electrospray ionization-quadrupole time-of-flight (ESI-QTOF MS/MS) mass spectrometric measurements. For the first time, the complete set of spectroscopic data of compound **(1)** has been obtained. Moreover, compounds **(2-4)** were identified by using various spectroscopic data and their comparison with literature reports. These bromotyrosine derived metabolites were evaluated against multiple drug resistance bacterial cells and against clinical pathogens, wherein purpurealidin K **(1)** displayed moderate activities against *Methicillin Resistant Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*.

Introduction

Marine sponges belonging to order verongida has afforded series of bromotyrosine metabolites possessing antimicrobial, cytotoxic, anti-inflammatory activites etc.[1] These sponges has tremendous chemical diversity with several chemical modification occuring both in the side chain and the aromatic ring of the brominated tyrosine precursor, giving rise to a broad range of biosynthetically related compounds. Our earlier investigations on the sponge *Psammaplysilla purpurea* have resulted in the isolation of purpurealidins A–J.[2,3] These bromotyrosines, especially purpurealidins E, I and J served as an inspirational molecule to our collaborators. As consequences, 27 purpurealidin analogs were synthesised and explored for potassium channel Kv10.1 activity by Tytgat et al.[4] Potassium channel Kv10.1 represents as an interesting cancer target because of its ectopic expression in over 70% of human cancers.[5] Psammaplin-A with a disulfide bridge is another notable example of bromotyrosine alkaloids isolated from unidentified species of *Psammaplysilla*[6] possesses antibacterial activity against methicillin-resistant gram-positive *Staphylococcus aureus* that was almost comparable to ciprofloxacin, a quinolone antibiotic used in the US.[7] It also inhibited several enzymatic activities such as mycothiol-S-conjugate amidase and topoisomerase II.[8,9] This compound showed anticancer activity against several cancer cell lines and A549 lung xenograph mouse model.[10,11] Many natural products isolated from *P. purpurea* has shown series of activities like antimicrobial and mild tyrosine kinase inhibitory activity (psammaplin D),[12] cytotoxicity towards the human colon tumor cell HCT116 (psammaplysins A–C,[13] bastadins 2, 5, 7 & 12,[14] aplysamines 3–5[15]), inhibited enzymes topoisomerase II and dehydrofolate reductase (Bastadin 12), antibacterial activity (purpuramines A–I)[16] *etc.* Herein, we wish to report the isolation and structural elucidation of bromotyrosine alkaloids, purpurealidin K **(1)**, purealidin Q **(2)**, aerophobin 2 **(3)**[17] and aplysamine 2 **(4)**[18] (Figure 1) from the sponge *P. purpurea*. The structures of the compounds were established on the basis of NMR and ESI-MS spectroscopic data.

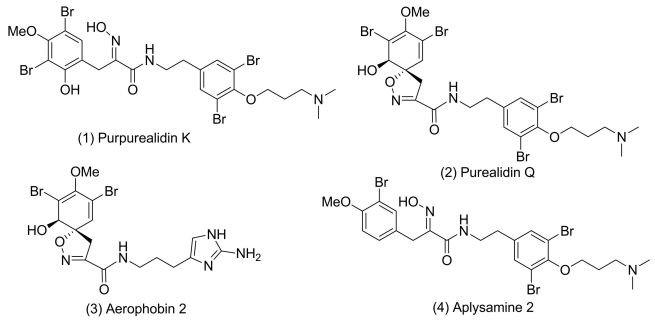

Figure 1. Structures of bromotyrosine alkaloids **(1-4)** from marine sponge *Psammaplysilla purpurea*.

Results and discussion

Compound **(1)** was obtained as white amorphous solid. The UV spectrum showed λ_{max} at 284 and 277 nm. The IR absorptions at 3395 and 1650 cm^{-1} implied the presence of NH/OH and secondary amide carbonyl group respectively. ^{1}H and ^{13}C NMR (S5 in supp. Info.) shows some similarities with purealidin Q.

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The intense singlet at δ_H 2.50 (6H) and δ_C 44.1 were assigned to the methyl group of dimethyl amine group (Table 1). In addition to N–Me, the peaks at δ_H 3.88 (3H, s) and δ_C 60.4 (CH_3) were evident for -OMe. The triplets at δ_H 3.12 (δ_C 40.0, C-10) and 2.62 (δ_C 33.8, C-11) were attributed to 1,2-disubstituted ethane moiety. While, signals at δ_H 4.05 (t, H-18,), 2.12 (m, H-19), 2.97 (t, H-20) were attributed to 1,3-disubstituted propane moiety. This was also confirmed by TOCSY experiment (S6 in supp. Info.) which showed cross peaks H10/H11 for 1,2-disubstituted ethane moiety and H18/H19, H19/H20 for 1,3-disubstituted propane moiety. High field Sp^2 at δ_C 108.1, 106.6, 118.1 can be considered for carbons bearing bromine atoms. On comparison with ^{1}H and ^{13}CNMR of purealidin Q (S8 in supp.info.), absence of signals for spiroisoxazole quaternary carbon and proton signal at 4.33(t, δ_C 73.8) ring indicated opening of the spiroisooxazole ring to form an oxime moiety. These formed tetra substituted aromatic ring with only one ^{1}H signal observed at δ_H 7.60 (s, δ_C 134.1) which showed HMBC connections (S7 in supp. Info.) with δ_C 120.9 (C1), 153.8 (C2), 108.1 (C3), 149.7 (C4), 106.6 (C5) and methylene δ_C 24.7 (C7). The methylene at δ_H 3.86 (s, δ_C 24.7) is connected to δ_C 120.9 (C1), 153.8 (C2), 134.1(C6) of the aromatic ring, δ_C 153.2 (C8) oxime carbon and δ_C 165.3 (C9) amide carbonyl. The careful examination of the TOCSY and HMBC connectivities revealed that the part structure from C1 to C9 is matching with the reported compound purpuramine M.[19]

Table 1. ^{1}H, ^{13}C, TOCSY and HMBC NMR data for purpurealidin K (**1**) recorded in $CDCl_3$

Carbon No.	^{13}CNMR δ, ppm	^{1}HNMR δ, ppm	TOCSY	HMBC
1	120.9, C			
2	153.8,C			
3	108.1, C			
4	149.7, C			
5	106.6, C			
6	134.1, CH	7.60, s		C1, C2, C3, C4, C5, C7
7	24.7, CH_2	3.86, s		C1, C2, C6, C8, C9
8	153.2, C			
9	165.3, C			
10	40.0, CH_2	3.12(2H,t, J = 12.2,6.6 Hz)	H11, H13	C9, C13/17
11	33.8,CH_2	2.62(2H,t, J = 12.6,6.8 Hz)	H10, H17	C10, C12, C13/17,
12	137.9, C			
13,17	132.7,CH	7.17(2H,s)		C11, C14, C15
14,16	118.1, C			
15	150.8, C			
18	70.7, CH_2	4.05(2H,t, J = 12.0,5.6 Hz)	H19	C19, C20, C15
19	26.8, CH_2	2.12(2H,m)	H18,H20	C18, C20
20	56.0, CH_2	2.97(2H,t, J = 5.6 Hz)	H19	C18, C20
-OMe	60.4, CH_3	3.88(3H,s)		C3
-N(Me)$_2$	44.1, CH_3	2.50(6H,s)		C20
NH		7.40(1H,s)		

Further, triplet at δ_H 3.12 (H10) of the 2-aminoethyl moiety showed HMBC connection with the amine carbonyl δ_C 165.3 (C9) and with δ_C 132.7 (C13,17) of the second aromatic ring. While, triplet at δ_H 2.62 (H11) showed connections with the quaternary carbon δ_C 137.9 (C12) and δ_C 132.7 (C13,17) bearing bromine atoms of the aromatic ring. Considering the intensities of the ^{13}CNMR at δ_C 132.7 (C13,17) and 118.1 (C14,16) were ascribed for two carbons each of the second aromatic ring. The HMBC showed δ_H 7.17 (2H, s) is connected to C11 of 2-aminoethyl moiety and C14, C15 of the same aromatic rings. The tripet at δ_H 4.05(H18) of 1,3-disubstituted propane moiety showed HMBC connection with quaternary carbon δ_C 150.8 (C15) of the second aromatic ring. The N–Me proton δ_H 2.50 showed connection with C20 of the propane group. This fully confirmed the structure of the new compound purpurealidin K (**1**). The key HMBC and TOCSY connections are shown in the Figure 2. It is worth to note that compound **1** is reported as a synthetic derivative by Harburn *et. al.* (2005).[20] Surprisingly, our previous knowledge on elucidation of series of bromotyrosine alkaloids[2,3] suggested that, its reported ^{13}CNMR by Harburn *et. al.* is not convincing with the proposed structure. Moreover, later in 2008, Ma *et. al.* isolated this compound from marine sponge *Pseudoceratina sp.* and presented it as 'ring A rearranged purealidin Q, but, did not give any spectroscopic data.[21] This motivated us to give the complete characterization of purpurealidin K (**1**) as a natural product with 2D NMR (HSQC, TOCSY & HMBC) and ESI-MS/MS data for the first time.

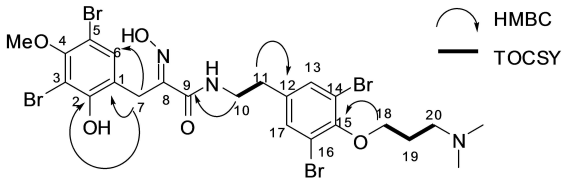

Figure 2. Key TOCSY and HMBC NMR correlations for purpurealidin K (**1**)

The ESI-MS with pseudomolecular ion peak at *m/z* 741.8, 743.8, 745.8, 747.8, 749.8 in the ratio 1:4:6:4:1, was indicative of the presence of four bromine atoms in the molecule and established the molecular formula as $C_{23}H_{27}Br_4N_3O_5$ (Figure 3A) In the ESI-MS, the molecular ion at *m/z* 745.8 was subjected to CID at collision energy of 40 V. The fragment ions observed is given in Figure 3B. The side chain, *N,N*-dimethylpropyl amine gives three fragment ions at *m/z* 86, 71 and 58. The dominant fragment ions were observed after cleavage at amide carbonyl at *m/z* 379, 381 and 383. It is for second aromatic ring with intact ethylamine and *N,N*-dimethylpropyl amine chain. Further loss of side chain *N,N*-dimethylpropyl amine chain gives peaks at *m/z* 293, 295, 297. The cleavage at oxime carbon and amide carbonyl gives fragment cluster at *m/z* 405, 407, 409. The small cluster at *m/z* 277, 279, 281 is for the first aromatic ring with methyl and with the loss of hydroxyl.

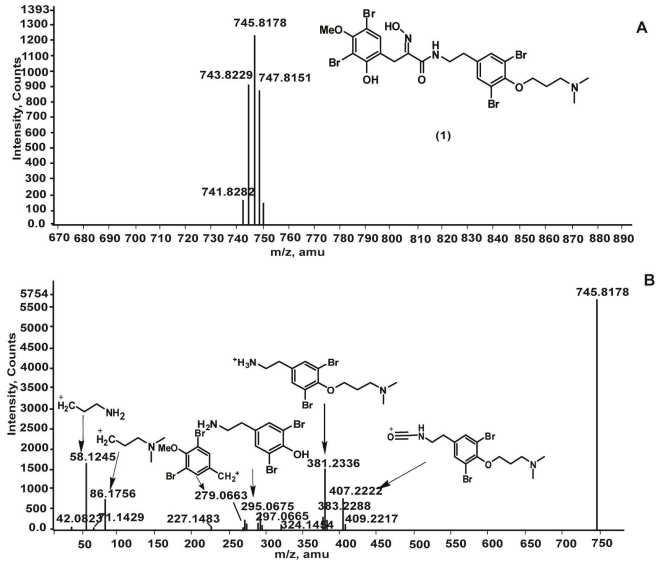

Figure 3. ESI-MS for purpurealidin K; A) Cluster of molecular ion peaks; B) MS/MS at *m/z* 745.8

Table 2. : Antimicrobial activities of compound **(1–4)** against clinical isolate at concentration of 50 µg/disc

Sr. no	Pathogenic Bacteria	1	2	3	4	[a]Std
	BACTERIAL STRAINS					
1	*Escherichia coli*	++	+++	++	+++	+++
2	*Pseudomonas aeruginosa*	++	++	++	-	+++
3	*Staphylococcus aureus*	++	+++	++	+++	+++
4	*Shigella flexineri*	-	-	-	-	+++
5	*Salmonella typhi*	++	++	+++	++	+++
6	*Vibrio cholera*	-	-	-	-	+++
7	*Klebsiella pneumonia*	-	-	-	-	+++
	MULTI DRUG RESISTANT					
8	*Acinetobacter sp.*	-	-	-	-	+++
9	*Methicillin Resistant Staphylococcus aureus (MRSA)*	++	-	++	++	+++

Zone of inhibition in mm; 0 mm: no activity (-); 1–5 mm: weak activity (+), 6–10 mm: good activity (++); 11–16 mm: significant activity (+++); data given are the mean of three replicates. [a]Gram positive bacteria (Standard: Penicillin)

Compound **2** (purealidin Q) was obtained as colourless oil. HRMS showed isotopic cluster ion peaks at *m/z* 741.8, 743.8, 745.8, 747.8, 749.8 in the ratio 1:4:6:4:1, indicating the presence of four bromine atoms in the molecule and established the molecular formula as $C_{23}H_{27}Br_4N_3O_5$. It was identified by comparison with the spectral data (UV, IR, 1D and 2D NMR) as previously described from the Okinawan marine sponge *Psammaplysilla purea* and our earlier investigation from *P. purpurea*. (^{1}H, ^{13}C NMR spectra for compound **2**, **3** & **4** are available in supplementary information).

The mass spectrum of the aerophobin 2 **(3)** revealed characteristic isotope peaks for $[M+H]^+$ pseudomolecular ion at *m/z* 504, 506, 508 (in the ratio 1:2:1) for molecular formula indicating the presence of three bromine atoms in the molecule. The aerophobin 2 contains a dibromospirocyclohexadien-onyldihydroisoxazole moiety of the type found in purealidin Q **(2)** as also confirmed by ^{1}H signals at δ 6.44, 4.09 and ^{13}C NMR signals δ147.9, 112.7, 121.4, 91.9, 74.4. The methylene protons displayed signals at δ1.90 (m), 1.75 (t), 2.58 (t) for propyl moiety and doublet doublet at 3.82 (dd), 2.76 (dd) for dihydroisoxazole moiety. The data is corresponding with the reported literature.[17]

^{1}H NMR spectrum of aplysamine 2 **(4)** showed the characteristic pattern of a 1,2,4-trisubstituted benzene (lH doublets at δ7.33 and 6.79 and a doublet of doublets at δ7.25) and a two-proton singlet at δ7.53 for a symmetrically tetrasubstituted benzene. Amide and oxime functionalities were indicated by IR absorptions at λ_{max} 3352, 1656, and 1620 cm^{-1} and confirmed by ^{13}C NMR signals at δ163.6 and 154.3. ^{1}H singlet at δ2.81 is for the dimethylamine group. The other connectivities were established by 2D NMR experiments and by comparison with the literature.[15]

The compounds exhibited moderate to high activities against clinical isolates under study (Table 2). Most importantly, all the compounds showed activities against *E. coli, P. aeroginosa, S. aureus, S. typhi* and *MRSA*. Among these, purealidin Q **(2)** and aplysamine 2 **(4)** showed significant activities against *E. coli* and *S. aureus* while, aerophobin 2 **(3)** showed against *S. typhi*.

Conclusion

This paper describes the isolation of purpurealidin K, purealidin Q, aerophobin 2 and aplysamine 2 from the methanolic extract of sponge *P. purpurea* collected from Mandapam, Tamil nadu. The complete structural elucidation of purpurealidin K using 2D NMR and MS/MS experiments are highlights of this work. The other compounds were identified by comparing the ^{1}H and ^{13}C NMR data with the literature value. The antibacterial bioassays of compounds **1–4** showed moderate to significant activities against the bacterial pathogens tested, wherein, purpurealidin K showed moderate activities against *MRSA*, *E. coli*, *P. aeruginosa*.

Supporting Information Summary

This segment contains the experimental Section and copies of NMR spectral data for compounds 1–4.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

[1] J. Peng, J. Li, M. T. Hamann, *Alkaloids Chem. Biol.* **2005**, *61*, 59–262.
[2] S. Tilvi, C. Rodrigues, C. G. Naik, P. S. Parameswaran, S. Wahidulla, *Tetrahedron* **2004**, *60*, 10207–10215.
[3] S. Tilvi, L. D'Souza, *Eur. J. Mass spectrum.* **2012**, *18*, 333–343.
[4] L. Moreels, C. Bhat, M. Voráčová, S. Peigneur, H. Goovaerts, E. Mäki-Lohiluoma, F. Zahed, L. A. Pardo, J. Yli-Kauhaluoma, P. Kiuru, J. Tytgat, *PLoS One* **2017**, *12*, e0188811.
[5] L. A. Pardo, D. Gomez-Varela, F. Major, K. Sansuk, R. Leurs, B. R. Downie, L. F. Tietze, W. Stuhmer, *Curr. Med. Chem.* **2012**, *19*, 675–682.
[6] E. Quinoa, P. Crews, *Tetrahedron Lett.* **1987**, *28*, 3229–3232.
[7] D. Kim, I. S. Lee, J. H. Jung, S. I. Yang, *Arch. Pharm. Res.* **1999**, *22*, 25–29.
[8] D. Kim, I. S. Lee, J. H. Jung, C. O. Lee, S. U. Choi, *Anticancer Res.* **1999**, *19*, 4085–4090.
[9] G. M. Nicholas, L. L. Eckman, S. Ray, R. O. Hughes, J. A. Pfefferkorn, S. Barluenga, K. C. Nicolaou, C. A. Bewley, *Bioorg. Med. Chem. Lett.* **2003**, *12*, 2487–2490.
[10] Y. Park, Y. Liu, J. Hong, C. O. Lee, H. Cho, D. K. Kim, K. S. Im, J. H. Jung, *J. Nat. Prod.* **2003**, *66*, 1495–1498.
[11] I. C. Pina, J. T. Gautschi, G. Y. Wang, M. L. Sanders, F. J. Schmitz, D. France, S. Cornell-Kennon, L. C. Sambucetti, S. W. Remiszewski, L. B. Perez, K. W. Bair, P. Crews, *J. Org. Chem.* **2003**, *68*, 3866–3873.
[12] C. Jimenez, P. Crews, *Tetrahedron* **1991**, *47*, 2097–2102.
[13] B. R. Copp, C. M. Ireland, L. R. Borrows, *J. Nat. Prod.* **1992**, *56*, 822–823.
[14] J. R. Carney, P. J. Scheur, M. Kelly-Borges, *J. Nat. Prod.* **1993**, *56*, 153–157.
[15] J. Jurek, W. Y. Yoshida, P. J. Scheuer, M. Kelly-Borges , *J. Nat. Prod.* **1993**, *56*, 1609–1612.
[16] H . Yagi, S. Matsunaga, N. Fusetani, *Tetrahedron* **1993**, *49*, 3749–3754.
[17] G. Cimino, S. D. Rosa, S. D. Stefano, R. Self, G. Sodano, *Tetrahedron Lett.* **1983**, *24*, 3029–3032.
[18] R. Xynas, R. J. Capon, *Aust. J. Chem.* **1989**, *42*, 1427–1433.
[19] J. Dai, S. M. Parrish, W. Y. Yoshida, M. L. R. Yip, J. Turkson, M. Kelly, P. Williams, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 499–504.
[20] J. J. Harburn, N. P. Rath, C. D. Spilling, *J. Org. Chem.* **2005**, *70*, 6398–6403.
[21] K. Ma, Y. Yang, Z. Deng, N. J. de Voogd, P. Proksch, W. Lin, *Chem. Biodiver.* **2008**, *5*, 1313–1320.
[22] J. Kobayashi, K. Honma, T. Sasaki, M. Tsuda, *Chem. Pharma. Bull.* **1995**, *43*, 403–407.

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