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NOVEL MARINE SPONGE DERIVED AMINO ACIDS 13. ADDITIONAL PSAMMAPLIN DERIVATIVES FROM *PSAMMAPLYSILLA PURPUREA*[a]

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ABSTRACT

An investigation of a sponge rich in psammaplin derivatives provides some insight into the biogenesis of this group of amino acid derivatives. Accompanying psammaplin A (**1**) were a new cysteine dimer, prepsammaplin A (**2**), and three new bromotyrosine-cysteine derivatives, psammaplins B (**3**), C (**4**), and D (**5**). Two other compounds were isolated including (3-bromo-4-hydroxy) benzaldehyde (**6**) and (3-bromo-4-hydroxyphenyl)-acetonitrile (**7**) which appear to be bromotyrosine derivatives. Psammaplin D (**5**) showed antimicrobial and mild tyrosine kinase inhibition activity.

INTRODUCTION

In recent years we have turned attention to the study of soft-bodied marine sponges most likely to be rich in nitrogen containing metabolites[1]. The investigation of taxa in the order Verongida (which lack spicules) seemed especially relevant. Bergquist has separated this group from the other orders that lack spicules (Dictyoceratida and Dendroceratida) because all its genera possess brominated tyrosine derivatives, they are devoid of terpene consitituents, and those studied ultrastructurally exhibited intricate histology.[2] The two species, *Pseudoceratina crassa* and *Psammaplysilla purpurea*, classified under the Verongida family Aplysinellidae illustrate these features. The few natural products known for the former include a simple yet highly modified tyrosine monomer, aeroplysinin I[3], and a more complex metabolite, ianthelline.[4] A similar small body of literature can be found for the latter and includes 2-hydroxy-3,5-dibromo-4-methoxyphenylacetamide[5], complex unsymmetrical tyrosine dimeric derivatives such as the psammaplysins[6], purealin[7] or the lipopurealins[8], and dimeric and tetrameric dipeptides including psammaplin A[9,10] and bisaprasin[10b].

The latter two compounds above present a unique biosynthetic pattern as they appear to be derived from union of bromotyrosine and cysteine. Our desire to more deeply understand this pattern stimulated a recollection of *P. purpurea* which yielded, in addition to psammaplin A (**1**), four new metabolites (**2** - **5**). These illustrate the biogenetic progression toward the psammaplin A framework because the simplest of this new group, prepsammaplin A (**2**), is a degraded cysteine dimer without a tyrosine unit while the others each contain a bromotyrosine linked to a modified cysteine.

RESULTS AND DISCUSSION

Prepsammaplin A (**2**), the only non-brominated compound isolated from this sponge, possessed a carbamate carbonyl as recognized by a strong IR band at 1724 cm^{-1} and a ^{13}C δ 154.2 (s). The $-SCH_2CH_2NHCO-$ group present in **1**[9] was identified in **2** by comparison of their ^1H, ^{13}C and ^1H-^1H COSY NMR spectral data. That **2** was a symmetrical dimer was

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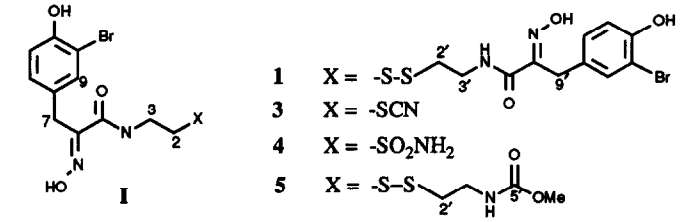
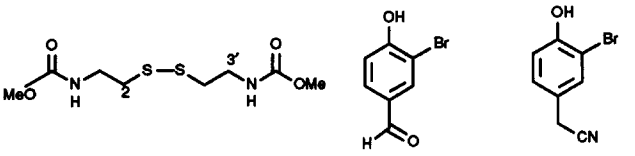
proved by comparing the ^{13}C APT[b] formula of C_4H_7 to the molecular formula of $C_8H_{16}N_2O_4S_2$ established by HRFABMS[b] (*m/z* 269.0643, M^++H, Δ0.4 mmu of calcd).

The properties of psammaplin A (**1**) (see Table 1), the major component of this sponge, were very useful in characterizing the other novel derivatives. The first new metabolite containing bromotyrosine, psammaplin B (**3**), was an oil. Its formula of $C_{12}H_{12}N_3O_3BrS$ was proposed by the LREIMS[b] M^+ cluster at *m/z* 357/359 and was verified by subsequent HRFABMS data for each isotope peak (see experimental). Diagnostic IR absorptions at 1678 and 1634 cm^{-1} and ^{13}C NMR signals at δ 153.2 and 164.5 pinpointed the oxime and amide groups, respectively. The APT formula for psammaplin A (**1**) of $C_{11}H_{11}$, corresponding to substructure **I**, was almost the same as the APT formula of $C_{12}H_{11}$ for psammaplin B (**3**), and all ^1H and ^{13}C NMR signals for this pair were the same excepting **3** showed the additional new signal at δ 112 (s) and shifted signals for C-2/H-2 at δ 34.1/3.10. Consequently, psammaplin B was comprised of substructure **I** plus an X which contained only one C atom. Subtracting the atoms of the fragment **I** from the molecular formula of **3** gave X = SCN. Also, the LRCIMS[b] spectral fragments included a facile loss of HCN (*m/z* 329/331) and HSCN (*m/z* 299/301), and the very weak IR band at 2157 cm^{-1} were characteristic of a thiocyanate group rather than an isothiocyanate group[11]. Finally, employing our previously described strategy involving ^{13}C NMR additivity effects[9] gave a calculated C-2 shift of δ 35.0 for X = -SCN, which is nearly identical to the observed C-2 shift at δ 34.1 in **3**.





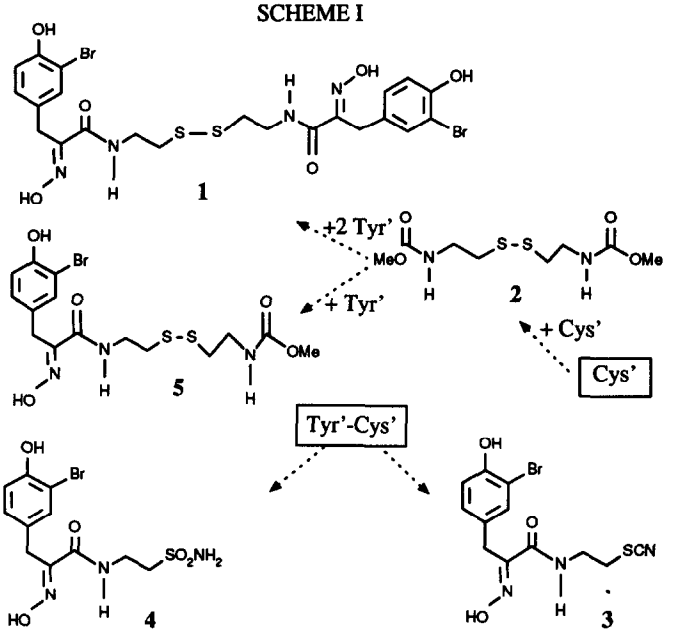
The next bromotyrosine was psammaplin C (**4**) whose formula of $C_{11}H_{14}N_3O_5BrS$ was established by HRFABMS data on the M^++H cluster (*m/z* 379.9913, 381.9891, Δ0.3, Δ0.4 mmu of calcd). The ^1H, ^{13}C NMR and ^1H-^{13}C COSY[b] data allowed all protons and carbons to be assigned (Table 1) and were consistent with substructure **I**. Once again, subtracting the atoms of **I** from the molecular formula of **4** gave an excess of SO_2NH_2 for the X substituent. Additional support for this array was the infrared absorption at 1343 cm^{-1}, the broad singlet at δ 5.49 (2H) in the ^1H NMR spectrum, and the agreement seen between the calculated C-2 shift of δ 53 for X = SO_2NH_2 versus the observed value of δ 54.5.

The last bromotyrosine, psammaplin D (**5**) was more complex as indicated by a formula of $C_{15}H_{20}N_3O_5BrS_2$ (HREIMS[b] *m/z* M^+ 465.0030, 467.0004, Δ0.2, Δ0.5 mmu of calcd). The ^{13}C and ^1H NMR spectra (Table 1) were again in accord with

b see experimental section for definition of acronyms.

substructure I and using the strategy employed above revealed the composition of X as $C_4H_8O_2NS_2$. The diagnostic ^{13}C shifts of two CH_2's at δ 39.0 (t) and 38.2 (t), indicated the S_2 as a disulfide with methylenes attached to either side of it. The presence of an additional carbamate function was indicated by the IR band at 1723 cm^{-1} and ^{13}C peak at δ 158.2. The S-CH_2 residue that was not part of substructure I, was identified by a ^1H NMR A_2B_2X pattern [δ 2.76 (t, H_2-2'), 3.33 (q, H_2-3'), and 5.79 (brt, NH), deduced by ^1H-^1H NMR COSY], indicating it was a part of a C_2H_4 group located between the carbamate and disulfide. Finally, fragments in the LREIMS spectrum at *m/z* 390/392 (M^+-NHCOOMe-H), 331/333 (M^+-SCH_2CH_2COOMe) and 299/301 (M^+-$SSCH_2CH_2NHCOOMe$) provided closing evidence in support of X as shown in structure **5**.

SCHEME I



The characteristic ^{13}C δ's of the benzylic carbons were used to assign the stereochemistry of the oxime group in **3** - **5** as *E* and both groups in **1** as *E*. An important model was *E, Z* psammaplin A isolated by Schmitz[10a] in which ^{13}C δ's of the benzylic carbons varied diagnostically according to their geometry. The possibility was considered that **2** and **5** were artifacts derived by nucleophilic attack by MeOH on psammaplin A (**1**). This was ruled out when it was found that **1** was stable to overnight refluxing in acidic or basic methanol. Accompanying the psammaplins were (3-bromo-4-hydroxy)benzaldehyde (**6**) and (3-bromo-4-hydroxyphenyl)acetonitrile (**7**). These were identified by comparison of their spectroscopic data (IR, ^1H, ^{13}C NMR and MS) to those reported in the literature[12,13].

An overall biogenetic scheme can now be envisioned to rationalize the formation of the various compounds reported above and it is showed in Scheme I. A straightforward dimerization of a rearranged cysteine (Cys' = HS-CH_2CH_2-NHCOOMe) could generate **2**. Similarly, condensation of a functionalized tyrosine (Tyr' = the bromo oxime derivative) and the same rearranged cysteine (Cys') could generate **3** and **4**. Further condensation of cysteine dimer **2** with either one

or two tyrosine (Tyr') derivatives could rationalize the formation of respectively **5** and **1**. Interestingly, the X functional groups of **3** and **4** are unique and do not appear to have counterparts among any known marine sponge amino acid derivatives. Additionally, the only precedent of the X = thiocyanate group, as found in compound **3**, is the sesquiterpene thiocyanate isolated from *Trachyopsis aplysinoides*.[11]

Psammaplin D (**5**) showed potent antimicrobial activity at 100 ug/disk [with inhibition zone size in mm], against Gram-positive bacteria *Staphylococcus aureus* [4], Gram-negative bacteria *Trichophyton mentagrophytes* [13]. Psammaplyin D (**5**) and (3-bromo-4-hydroxy) benzaldehyde (**6**) showed a mild *in vitro* activity against tyrosine kinase, with a IC_{50} = 2800 uM and IC_{50} = 3200 uM respectively.

Table 1. ^{1}H and ^{13}C NMR spectral data in CD_3CN [Chemical shifts (multiplicity, coupling constants in Hz)] for psammaplins **1-5**

C	**1**		**2**		**3**		**4**		**5**	
2	38.6	2.77 (t, 6.6)	38.9	2.77 (t, 6.6)	34.1	3.10 (t, 6.6)	54.5	3.21 (t, 6.3)	39.0	2.79 (t, 6.6)
2'	"	"	"	"	-	-	-	-	38.2	2.76 (t, 6.3)
3	39.1	3.46 (q, 6.3)	40.7	3.35 (q, 6.3)	39.7	3.57 (q, 6.3)	35.1	3.65 (q, 6.3)	39.1	3.50 (q, 6.6)
3'	"	"	"	"	-	-	-	-	40.6	3.33 (q, 6.3)
NH-4		7.27 (brt)	5.77 (brs)			7.37 (brs)		7.47 (t, 5.4)		7.29 (t, 5.4)
NH-4'		"	"		-			-		5.82 (brs)
5	164.2		154.2		164.5		164.2		164.1	
5'	"		"		-		-		158.2	
6	153.4				153.4		153.2		153.4	
6'	"				-		-		-	
7	28.6	3.74 (s)			28.5	3.76 (s)	28.5	3.75 (s)	28.5	3.76 (s)
7'	"	"			-	-	-	-	-	-
8	130.9				130.9		130.8		130.9	
8'	"				-		-		-	
9	134.1	7.33 (d, 1.8)			134.1	7.36 (d,2.1)	134.0	7.35 (d, 1.8)	134.1	7.35(d, 2.1)
9'	"	"			-	-	-	-	-	-
10	110.0				109.1		109.9		109.9	
10'	"				-		-		-	
11	152.9				153.0		152.8		152.8	
11'	"				-		-		-	
12	117.2	6.79 (d, 8.1)			117.2	6.83 (d,8.4)	117.1	6.82 (d, 8.4)	117.1	6.82 (d, 8.4)
12'	"				-	-	-	-	-	
13	130.4	7.04 (dd, 1.8 & 8.4)			130.4 (dd,2.1&8.4)	7.08	130.4	7.07 (dd, 1.8 & 8.4)	130.4	7.07 (dd, 2.1&8.4)
13'	"	"			-	-	-	-		-
OMe			52.4	3.57 (s)					52.4	3.56 (s)
CN				111.9						
NH_2								5.49 (brs)		

EXPERIMENTAL SECTION

NMR spectra were recorded at 99.5 MHz for ^{1}H and 25.0 MHz for ^{13}C or at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Low resolution mass spectrometry data were obtained at U.C. Santa Cruz, low resolution mass spectra were obtained from U.C. Berkeley. High performance liquid chromatography (HPLC) was done using a Regis 10μ-ODS or 10μ silica gel column (25 x 1.0 cm). All solvents were distilled and dried for HPLC and were spectral grade for spectroscopy. The acronyms used in this report are as follows: APT - Attached Proton Test[14], LREIMS - Low Resolution Electron Impact Mass Spectrometry; HREIMS - High Resolution Electron Impact Mass Spectrometry; HRFABMS - High Resolution Fast Atom Bombardment Mass Spectrometry; COSY - Correlated Spectroscopy.

Two-dimensional Nmr Procedures. Standard pulse sequences were used for the ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, long range ^{1}H-^{13}C COSY experiments.[15] Regular homo and hetero COSY as well as long range experiments were performed on **1** and **4**.

Isolation Procedures. The sponge (0.4 Kg) was extracted in methanol (twice) followed by concentration of the crude extract to yield 27.74 g of a crude oil. It was successively partitioned between aqueous methanol and the series: hexanes (3.81 g), CCl_4 (1.04 g), CH_2Cl_2 (2.34 g) and BuOH (0.50 g).[16] The pharmacological screening of these fractions showed that the CH_2Cl_2 and BuOH were the most active. Chromatographic purification of the CH_2Cl_2 fraction over a silica gel column gave a mixture which was separated by reverse phase HPLC (MeOH/H_2O 2:3) yielding compounds **1** (400 mg), **2** (6mg), **3** (4 mg), **5** (4 mg), **6** (2 mg), **7** (3 mg). The BuOH fraction was chromatographed on Sephadex LH-20 (Methanol) to give a mixture of two products which were separated by reverse phase HPLC as below to yield **1** (40 mg) and **4** (10 mg).

Presammaplin A (2): oil; IR (neat) 3630, 3547, 2960, 1724, 1630, 1525, 1255, 1052 cm^{-1}; HRFABMS (positive), *m/z* 269.0626, M^+, $C_8H_{17}N_2O_4S_2$, Δ0.4 mmu of calcd]; LREIMS, *m/z* (relative intensity) 268 M^+ (18), 193 (60), 134 M^+-$SCH_2CH_2NHCOOMe$ (60), 102 M^+-$SSCH_2CH_2NHCOOMe$ (100); LRCIMS (isobutane), *m/z* (relative intensity) 269 M^++H (100), 237 (18), 197 (5), 134 (80), 102 (8); ^{1}H NMR (CD_3CN) and ^{13}C NMR (CD_3CN), see Table 1.

Psammaplin B (3): oil; UV (MeOH) λ_{max} 280 nm (ε 5450); IR (neat) 3627, 3541, 2257, 2157, 1678, 1634, 1442, 1378, 1040 cm^{-1}; HRFABMS (positive), *m/z* 357.9856 $C_{12}H_{13}N_3O_3{}^{79}BrS$, Δ0.5 mmu of calcd, 359.9834 $C_{12}H_{13}N_3O_3{}^{81}BrS$, Δ0.8 mmu of calcd; LREIMS, *m/z* (relative intensity) 357/359 M^+(3), 328/330 M^+-HCN-2H (5), 298/300 M^+-HSCN (5), 281/283 (7), 211/213 C_8H_6NOBr (100), 59 HSCN (30); LRCIMS (isobutane), *m/z* (relative intensity) 358/360 M^++H (21), 329/331 M^+-HCN-H (29), 299/301 M^+-HSCN+H (35), 283/285 (25); ^{1}H NMR (CD_3CN) and ^{13}C NMR (CD_3CN), see Table 1.

Psammaplin C (4): oil; UV (MeOH) λ_{max} 282 nm (ε 2200); IR (neat) 3628, 3540, 3362, 2248, 1678, 1634, 1377, 1343, 1152, 1039, 918 cm^{-1}; HRFABMS (positive), *m/z* 379.9913 $C_{11}H_{15}N_3O_5Br^{79}S$, Δ0.3 mmu of calcd, 381.9891 $C_{11}H_{15}N_3O_5Br^{81}S$, Δ0.4 mmu of calcd; LREIMS, *m/z* (relative intensity) 379/381 M^+ (4), 363/365 M^+-NH_2 (2), 211/213 C_8H_6NOBr (100); LRCIMS (isobutane), *m/z* (relative intensity) 380/382 M^++H (32), 364/366 M^+-NH_2+H (20), 301/303 M^+-SO_2NH_2+2H (15); ^{1}H NMR (CD_3CN) and ^{13}C NMR (CD_3CN), see Table 1.

Psammaplin D (5): oil; UV (MeOH) λ_{max} 280 (ε 17733) ; IR (neat) 3521, 3372, 1723, 1674, 1634, 1525, 1420, 1256, 984 cm^{-1}; HREIMS, *m/z* 465.0030 $C_{15}H_{20}N_3O_5{}^{79}BrS_2$, Δ0.2 mmu of calcd, 467.0004 $C_{15}H_{20}N_3O_5{}^{81}BrS_2$, Δ0.5 mmu of calcd; LREIMS, *m/z* (relative intensity) 465/467 M^+ (5), 390/392 M^+-NHCOOMe-H (1) 331/333 M^+-$SCH_2CH_2NHCOOMe$ (22), 299/301 M^+-$SSCH_2CH_2NHCOOMe$ (37), 226/230 (40), 211/213 C_8H_6NOBr (31); LRCIMS (isobutane), *m/z* (relative intensity) 466/468 M^++H (10), 375/377 (3), 333/335 (8), 299/301 (35); ^{1}H NMR (CD_3CN) and ^{13}C NMR (CD_3CN), see Table 1.

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REFERENCES

1. Recent papers in this series include: **#10** Jiménez, C.; Crews, P. *J. Nat. Prod.*, **1990**, *53*, 978. **#11** Adamczeski, M.; Quiñoá, E.; Crews, P. *J. Org. Chem.* **1990**, *55*, 240. **#12** Jiménez, C.; Quiñoá, E.; Adamczeski, M.; Hunter, L. M.; Crews, P. *J. Org. Chem.* in press.

2. (a) Bergquist, P. R. *Sponges*; Hutchinson: London 1978. (b) Bergquist, P.R.; Wells, R. J. *Marine Natural Products*; Scheuer, P. J., Ed., Academic Press: New York, 1983; Vol V, pp 17-21.

3. It is important to note that synonyms for *P. crassa* are *Ianthella ardis* and *Aiolochroia crassa*. For a summary of sources of aeroplysinin see: Makarieva, T. N.; Stonik, V. A.; Alcolado, P.; Elyakov, Y. B. *Comp. Biochem. Physiol.* **1981**, *68B*, 481.

4. Litaudon, M.; Guyot, M. *Tetrahedron Lett.* **1986**, *27*, 4455.

5. Chang, C. W. J.; Weinheimer, A. J. *Tetrahedron Lett.* **1977**, 4005

6. Roll, D. M.; Chang, C. W. J.; Scheuer, P. J.; Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 2916.

7. Nakamura, H.; Wu, H.; Kobayashi, J.; Nakamura, Y.; Ohizumi, Y. *Tetrahedron Lett.* **1985**, *26*, 4517.

8. Wu, H.; Nakamura, H.; Kobayashi, J.; Hirata, Y. *Experientia* **1986**, *42*, 855.

9. Quiñoá, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 3229.

10. (a) Arabshahi, L.; Schmitz, F. J. *J. Org. Chem.* **1987**, *52*, 3584. (b) Rodriguez, A. D.; Akee, R. K.; Scheuer P. J. *Tetrahedron Lett.* **1987**, *28*, 4989.

11. He, H.; Faulkner, J. D.; Shumsky, J. S.; Hong, K.; Clardy, J., *J. Org. Chem.* **1989**, *54*, 2511.

12. Suzuki, M; Kowata, N; Kurosawa, E. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2099.

13. Naik, R. G.; Wheeler, T. S. *J. Chem. Soc.* **1938**, 1780.

14. Patt, S. L.; Shoolery, J. N., *J. Mag. Reson.* **1982**, *42*, 535.

15. For reviews see: (a) Kessler, H.; Gehrke, M.; Griesinger, C. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 490. (b) Schoolery, J. N. *J. Nat. Prod.* **1984**, *47*, 226.

16. Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178.