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PHENOLIC CONSTITUENTS OF PSAMMAPLYSILLA

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ABSTRACT: The cytotoxic extract of Psammaplysilla sp. collected from Tonga contains monobromo tyrosine derivatives, 3-bromo-4-hydroxyphenylacetonitrile (**1**) which is known, and psammaplin A (**2**) which is the first disulfide to be isolated from a sponge.

Several dibromotyrosine derivatives have been reported from the genus Psammaplysilla.[1] We began a study on a collection of Psammaplysilla sp. obtained in 1980 during our first expedition to the Kingdom of Tonga, because its CH_2Cl_2 crude extract was toxic to P388 mouse leukemia cells at 20 μg/ml. The major constituents from this sponge are monobromo tyrosine derivatives **1** and **2**, and the evidence in support of their structures are reported below.

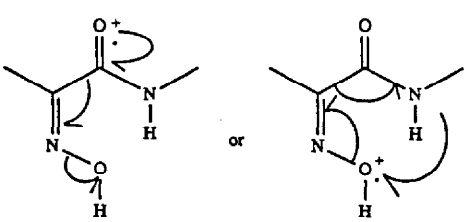
The sponge tissue was extracted with CH_2Cl_2 immediately after collection and the resultant crude oil (5.5 g) was redissolved in methanol and solvent partitioned (methanol (aq) versus: hexane, CCl_4, or CH_2Cl_2). Metabolites **1** and **2** were concentrated in both the CCl_4, and CH_2Cl_2 fractions, and the most abundant component **1**[2] (45 mg) eluted first by reverse phase HPLC (MeOH/H_2O, 40%) and **2** (19 mg) trailed behind. The methyl phenol ether of **1** was previously described in 1938[3], as a synthetic product, and **1** was first isolated in 1978 from Verongia aurea, but only its MS properties were described.[4]

The structure elucidation of psammaplin A **2** was troublesome because a misleading molecular formula of $C_{11}H_{13}O_3N_2BrS$ was initially implied by the highest HREIMS peak observed at m/z = 333.9790 (calc. = 333.9811 for $C_{11}H_{13}O_3N_2{}^{81}BrS$) and reinforced by the $C_{11}H_{11}$ count from APT ^{13}C and ^1H (MeCN-d_3) NMR spectra.[5] Several substructures were clearly evident including a disubstituted phenoxy (^{13}C δ = 153s, 131s, 110s), an isolated benzylic CH_2 (^1H δ = 3.74 with long range COSY ^1H-^1H correlation peaks from it to benzenoid protons H-2, H-5 & H-6), and a -NH-CH_2-CH_2-Z (^{13}C δ = 39.1t, 38.6t, ^1H δ = 3.46q J = 6.3 Hz - transformed to a triplet upon D_2O addition). The intense LREIMS peak at m/z = 211/213 (LRCIMS = 212/214) suggested a radical cation fragment of structure **1**. This is reminiscent of an EIMS fragmentation cleavage observed previously for several dibromotyrosines including: an aerothenin hydrolysis product which displays an intense EIMS fragment ion **i**[6], pentamethyl bastadin-1 which fragments to ion **iii**[7], and dibromo ianthelline which cleaves to ion **ii**.[8] The mechanism of this fragmentation process is uncertain as two alternatives are shown in Scheme 1. Thompson[6] suggested path **A**, whereas

path B represents a possibility which has analogy to a fragmentation cleavage proposed for hydrazones[9]. Based on this fragmentation behavior, the two C=X's (^{13}C NMR δ's = 153,s 164s) could be proposed as being part of a Ar-CH_2-C(=NOH)-C(=O)- subunit, and the oxime proton appeared as a singlet δ = 9.74 (MeCN-d_3). NMR data and LRFABMS data revealed that a sulfur was attached to C-11. In fact, an -SSR group could be suggested by comparing the observed C-11 shift of δ = 39 in **2** to that calculated as follows. An important initial model was CH_3-C(=O)NH-CH_2CH_2-Z whose δC = 14.6, Z = H. The calculated shifts at C for derivatives of this model were δ = 26, 33, and 40 for the respective series Z = SH, SC_2H_5, SSC_2H_5. These data were obtained by adding α increments we derived by computing δX - δH (ppm) based on experimental data for C_2H_5X- which were as follows: +11, X = SH; +18, X = SC_2H_5; +25, X = SSC_2H_5.[10] The best fit was Z = SSC_2H_5 which, along with the above data, indicated a dimeric disulfide structure as shown for **2**. The correct molecular formula was finally derived when we obtained LRFABMS data as an M^++H cluster was seen at 663/665/667/669 and Figure 1 shows the close agreement in m/z intensities that were observed versus that calculated for $C_{22}H_{25}N_4Br_2S_2$. In addition, structure **2** was fully consistent with the fragments observed in both the LRCIMS(CH_4) and LREIMS shown in the Figure 2. Adding chemical support for the phenol and oxime features was the treatment of **2** with DMF, K_2CO_3, MeI which afforded the tetramethoxy derivative **3** in 85% yield.[11] As expected, an intense mass spectral fragment peak was observed for $[3]^+$ fragmenting to ion **iv**.

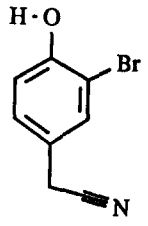
Psammaplin A showed an <u>in vitro</u> IC_{50} = 0.3 μg/ml against P388 cells while 3-bromo-4-hydroxyphenylacetonitrile was inactive at concentrations of less than 5 μg/ml.[12] It is tempting to imagine that the biosynthesis of psammaplin A, the first disulfide containing metabolite to be isolated from a marine sponge, involves a condensation of a didecarboxylated cystine with two monobromotyrosine units. There are only two other known marine animal metabolites with a disulfide substructure. These include the recently revised structure of citorellamine[13] from the tunicate <u>Polycitroella mariea</u> which has a similar -N-C_2H_4-S-S-C_2H_4-N- array to that of psammaplin A, and ulithiacyclamide from the tunicate <u>Lissoclinum pamide</u> which contains a -N-CH(C-)-CH_2-S-S-CH_2-CH(C-)-N- moiety[14].

Scheme 1. Cleavage fragmentation mechanisms

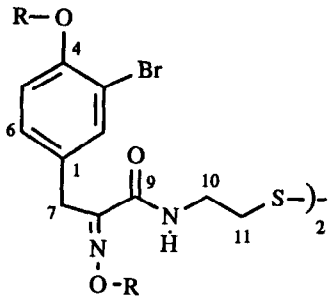


A B

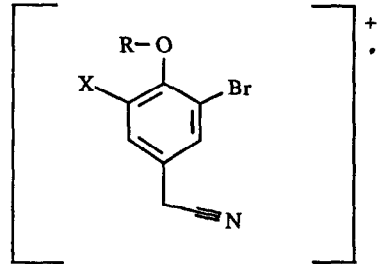
Acknowledgement. Partial research support to PC was from NOAA, National Sea Grant College Program, Department of Commerce, University of California project number R/MP-41. A grant to PC from the University Research Expeditions Program supported our field work in Tonga. We thank Dr. Julie Leary (UCB Mass Spectrometry Lab) for the HREIMS data.



1

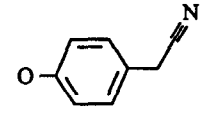


2 R = H 3 R = Me



i X= Br, R = H

ii X= Br, R = CH_3

iii X=  R = H

iv X = H, R = CH_3

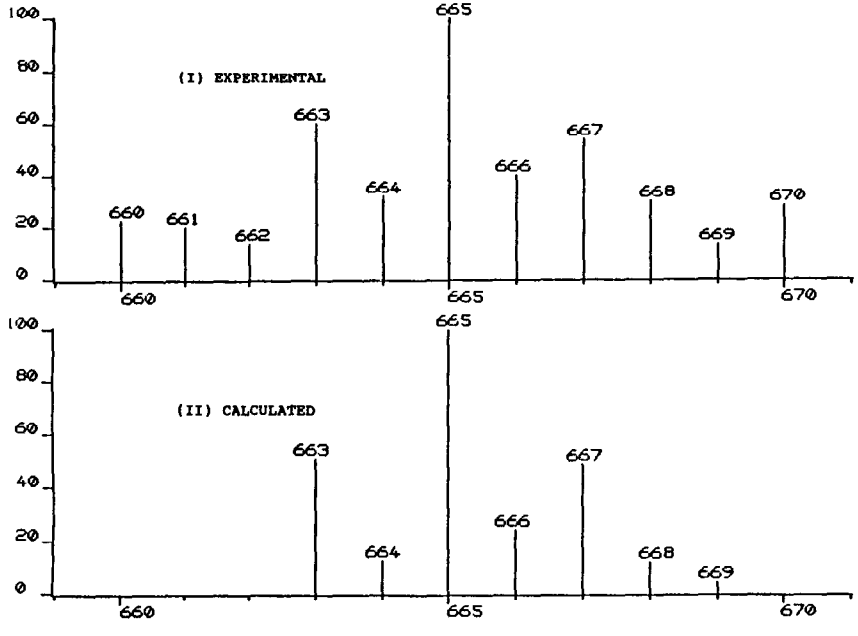


Figure 1. LRFABMS of psammaplin A (2): I, experimental molecular ion region II, calculated for $C_{22}H_{25}O_6N_4Br_2S_2$

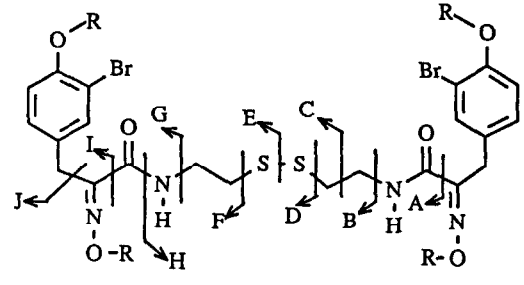


Figure 2. Fragmentation of 2 (R = H) & 3 (R = Me)
[letters = fragments of masses in notes 5 & 11]

References

1. Psammaplysilla purea (Okinawa): (a) Wu, H.; Nakaumara, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. Experientia **1986**, 42, 855. and refs within. P purpurea (Palau) (b) 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. P purpurea (Red Sea) (c) Rotmen, M.; Carmely, S.; Kashman Y. Tetrahedron **1983**, 39, 667. P purpurea (Enewetak) (d) Chang, C. W. J.; Weinheimer, A. J. Tetrahedron Lett. **1977**, 4005.

2. **1**. Mp (MeOH) = 117 - 118°. HREIMS: 210.9639/212.9609 requires C_8H_6NOBr. NMR (MeCN-d_3): 125.1 (C-1), 133.5 (C-2), 7.45 d = 1.8 Hz (H-2), 110.5 (C-3), 154.1 (C-4), 117.7 (C-5), 6.94 d = 8.4 Hz (H-5), 129.6 (C-6), 7.17 dd = 8.4, 1.8 Hz (H-6), 22.6 (C-7), 3.71 s (H-7), 119.5 (C-8). UV (MeOH) λ_{max} 220, 280, 288sh. IR (neat) 3600 - 3200, 2950, 2250, 1610, 1510 cm^{-1}.

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

4. Goo, Y. M.; Rinehart, K. L.; Jr. In "Drugs and Food from the Sea", Kaul, P. N.,; Sindermann, C. J. Eds.; The University of Oklahoma Press: Norman, OK, 1978; pp107-115.

5. **2**. LRFABMS (glycerol): 663/665/667/669 requires $C_{22}H_{25}O_6N_4O_6Br_2S_2$. LRCIMS ($CH_4$) m/z (% intensity) [fragment, see figure]: 391/393 (2) [B], 375/377 (5) [C-2H], 361/363 (3) [D-2H], 333/335 (55) [E+2H], 273/275 (10) [G+2H], 230/232 (8) [H+2H], 212/214 (78) [1+H], 185/187 (100) [J], 132 (70) [1-Br]. NMR (MeCN-d_3): 130.9 (C-1), 134.1 (C-2), 7.33 d = 1.8 Hz (H-2), 110.0 (C-3), 152.9 (C-4), 117.2 (C-5), 6.79 d = 8.1 Hz (H-5), 130.4 (C-6), 7.04 dd = 8.1, 1.8 Hz (H-6), 28.6 (C-7), 3.74 s (H-7,7'), 153.4 (C-8), 164.2 (C-9), 39.1 (C-10), 3.46 q = 6.3 (H-10,10'), 38.6 (C-11), 2.77 t = 6.6 (H-11,11'), 9.74 bs (NOH), 7.27 bt (NH) [changes upon addition of D_2O - 3.46 t = 6.3 Hz (H-10,10'), peaks disappear at 9.74 & 7.27]. UV (MeOH) λ_{max} 220, 280, 286sh. IR (neat) 3600 - 2800, 1670, 1550, 1440 cm^{-1}.

6. Moody, K.; Thompson, R. H.; Fattorusso, E.; Minale, L.; Sodano, G. J.C.S. Pk-I **1972**, 18.

7. (a) Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J. Tetrahedron Lett. **1980**, 21, 2277. (b) Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. Aust. J. Chem. **1981**, 34, 765.

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

9. Budzikiewciz, H.; Djerassi, C.; Williams, D. H. In "Mass Spectrometry of Organic Compounds", Holden-Day, Inc: San Francisco, CA, 1967, p384.

10. Spectra from the Catalog of ^{13}C NMR Spectra, Sadtler Research Laboratories, 1982, Philadelphia, PA. served as a source for these data.

11. **3**. LRFABMS (glycerol): 719/721/723/725 requires $C_{26}H_{33}O_6N_4O_6Br_2S_2$. LRCIMS ($CH_4$), m/z (% intensity) [fragment, see figure]: 462/464 (2) [A], 389/391 (4) [D-2H], 361/363 (100) [E+2H], 327/329 (5) [F], 297/299 (4) [G-2H], 284/286 (5) [H], 254/256 (3) [I-2H], 226/228 (20) [iv+H], 199/201 (80) [J]. ^1H NMR (MeCN-d_3): 7.40 d = 1.8 Hz (H-2), 6.89 d = 8.4 Hz (H-5), 7.17 dd = 8.4, 1.8 Hz (H-6), 3.76 s (H-7,7'), 3.48 q = 6.6 Hz (H-10,10'), 2.80 t = 6.6 Hz (H-11,11'), 3.80, s, 3H (OMe), 3.95 s, 3H (OMe), 7.33 bt (NH).

12. We thank Dr. Jake Clement of SeaPharm for this data.

13. Moriarty, R. M.; Roll, D. M.; Ku, Y-K; Nelson, C.; Ireland, C. I. Tetrahedron Lett. **1987**, 28, 749.

14. Wasylyk, J. M.; Biskupiak, J. E.; Costello, C. E.; and Ireland, C. I. J. Org. Chem. **1983**, 48, 4445.

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