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Two Bromotyrosine-Cysteine Derived Metabolites from a Sponge[1]

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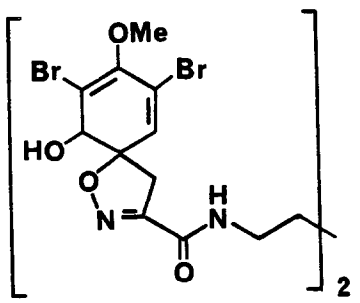
Summary. The structures of a dimeric and a tetrameric degraded dipeptide, derivable from bromotyrosine and cysteine, have been elucidated. Both compounds inhibit the growth of *Bacillus subtilis* and *Staphylococcus aureus*.

Bromotyrosine-derived secondary metabolites are characteristic of sponges of the order Verongida.[2] The compounds that have been encountered may be simple tyrosine degradation products, as e.g. aerothionin (1)[3] or they may be complex, as e.g. the psammaplysins (2),[4] which may have arisen from condensation of tyrosine with homoserine or its equivalent.



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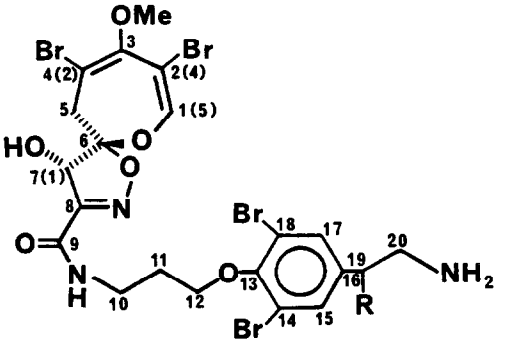
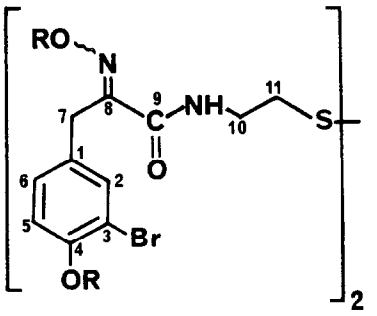


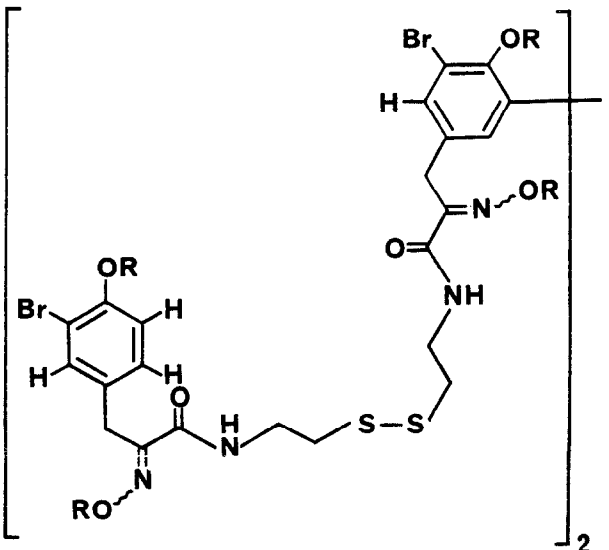
2 R = H, OH

We have now encountered two compounds from a sponge, tentatively identified as *Thorectopsamma xana*,[5] which appear to be degraded dipeptides derived from bromotyrosine and cysteine (or cystine). The thawed sponge (1.06 kg) was blended with ethanol (2x1L). The aqueous ethanolic extract after concentration was sequentially partitioned against hexane, chloroform, and butanol. The residue of the butanol extract upon trituration with ethyl acetate furnished a crude mixture (4.6 g) of compounds (3) and (5).[6] Sephadex LH-20 (methylene dichloride/methanol, 1:1) of part of the mixture (1.1 g) separated the constituents as a white foam (0.66 g, 0.27% of wet sponge). HPLC (Partisil M9, methylene dichloride/MeOH, 96:4) resulted in two pure compounds in a ratio of 15:1.



3 R = H
4 R = Me

The major constituent, psammaplin A (**3**) is a colorless semicrystalline solid, mp 67-75°C, optically inactive, with UV maxima at 217 (64,600) and 291 (10,000) nm. The IR spectrum (KBr) had broad absorption at 3500-3200 cm^{-1} in addition to bands at 1670, 1550, 1500, 1430, 1200, and 980 cm^{-1}. The ^{13}C and ^{1}H NMR spectra[7] revealed a compound possessing eight sp^2 and three sp^3 carbons, three exchangeable and three aromatic protons in addition to two vicinal methylenes with chemical shifts at 3.41 and 2.79 and one isolated methylene at δ 3.67. The EI mass spectrum produced no molecular ion but several fragments contained diagnostic bromine doublets. The HREI mass spectrum of the tetramethyl ether (**4**)[8] established a molecular formula of $C_{26}H_{32}{}^{79}Br_2N_4O_6S_2$ (*m/z* 718.0129; calcd 718.0129) for **4** and hence $C_{22}H_{24}Br_2N_4O_6S_2$ for the parent compound (**3**). These data argued for a symmetrical bromotyrosine-derived



5 R = H
6 R = Me

compound. The chemical shift of the 2H singlet at δ 3.67 was reminiscent of the bastadins,[9] in which the amino nitrogens are oxidized to oximes (δ 3.76, 3.65, DMSO-d_6). The two contiguous methylenes (δ 3.41, 2.79; 39.54, 38.48) were placed between nitrogen and sulfur based on their chemical shifts.[10] This assignment was fully confirmed by CSCM and INEPT experiments. The disulfide function was reduced to thiol,[11] which was recognized by its odor and by a spot test,[12] which is based on the acceleration by thiol of the usually slow sodium azide-iodine reaction.

The minor component, bisaprasin[13] (5) was obtained as a colorless foam, $[\alpha]_D^{24°}$ 0° (c 1, MeOH). Its close structural relationship to psammaplin A (3) was seen from UV, IR, and NMR data.[14] Its dimeric nature was recognized by the HRFAB mass spectrum of its octamethyl ether (6)[15] which has a composition of $C_{52}H_{62}{}^{79}Br_4N_8O_{12}S_4$ (calcd 1435.0181, found *m/z* 1435.0181) or $C_{44}H_{46}Br_4N_8O_{12}S_4$ for bisaprasin (5). That the dimer is a biphenyl derivative rather than a diphenyl ether follows from formation of an octamethyl ether and from comparison of the aromatic proton NMR data with those of the bastadins,[16] some of which are bromotyrosine-based diphenyl ethers.

Both psammaplyn A (3) and bisaprasin (5) inhibit the growth of *Bacillus subtilis* and *Staphylococcus aureus*.[17]

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References and Notes

(1) Presented at the 193rd National ACS Meeting, Denver, CO, April 5-10, 1987, Abstract ORGN.61.

(2) Bergquist, P. R.; Wells, R. J. in *Marine Natural Products*, Vol. 5 (Scheuer, P. J. Ed.) Academic Press, New York, 1983, pp 1-50.

(3) Fattorusso, E.; Minale, L.; Sodano, G.; Moody, K.; Thomson, R. H. *J. Chem. Soc. Chem. Commun.* **1970**, 752-3.

(4) 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*, 2916-2920.

(5) The sponge was collected and tentatively identified by Dr. P. Karuso in September, 1986 in Apra Harbor, Guam at -3 m. It is a dusky purple encrusting sponge with mustard yellow interior and a conulose surface.

(6) After completion of this work Professor Crews informed us that he had also isolated 3 as a minor constituent from a sponge, *Psammaplysilla* sp.; a report of his work was in press (Quiñoà, E.; Crews, P. *Tetrahedron Lett.*). These authors did not isolate 5. Their and our spectral data (IR, MS, ^1H and ^{13}C NMR) are in full agreement.

(7) ^{13}C NMR spectrum of 3 (MeOH-d_4): δ 165.78 s C-9, 153.59 and 153.07 s C-4 and C-8, 134.39 d C-2, 130.55 s C-3, 130.35 d C-6, 116.99 d C-5, 110.45 s C-1, 39.54 t C-10, 38.48 t C-11, 28.67 t C-7; ^1H NMR (DMSO-d_6): δ 11.90 and 10.02 (1H s each, exchangeable, oxime and phenol), 8.05 (1H t, J = 6 Hz, exchangeable, NH), 7.29 (1H d, J = 1.6 Hz H-2), 7.00 (1H dd, J = 8.3, 1.6 Hz, H-6), 6.83 (1H d, J = 8.3 Hz, H-5), 3.67 (2H s, H_2-7), 3.41 (2H dt, J = 7, 6 Hz, H_2-10), 2.79 (2H t, J = 7 Hz, H_2-11).

(8) Prepared from 3 (21 mg) in 15 mL DMF with MeI (200 μL) and K_2CO_3 (230 mg), 25°C, 19 h, stirring. Purified by HPLC (Partisil M9 10/50, EtOAc/hexane, 3:1) to a colorless solid (4); ^1H NMR ($CDCl_3$): δ 7.43 (1H d, J = 1.6 Hz, H-2), 7.18 (1H dd, J = 8.3, 1.6 Hz, H-6), 7.05 (1H brt, J = 6 Hz, exchangeable, NH), 6.76 (1H d, J = 8.3 Hz, H-3), 4.00 (3H s, methoximino), 3.82 (3H s, phenoxymethyl), 3.79 (2H s, H_2-7), 3.60 (2H dt, J = 7, 6 Hz, H_2-10), 2.80 (2H t, J = 7 Hz, H_2-11).

(9) Kazlauskas, R.; Lidgard, R. O.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1980**, 21, 2277-80.

(10) Pretsch, E.; Clerc, T.; Seibl, J.; Simon W. *Tabellen zur Strukturaufklarung organischer Verbindungen*; Second Ed.; Springer:Berlin, 1981; pp H155, H110, C210.

(11) Aprasin (3) in EtOH (1N, 1 mL), 6 mL EtOH, 2 mL HCl in 5% aq EtOH, 10 mg Raney Ni, heated to 100°C/1 min, then cooled.

(12) Feigl, F. *Spot Tests in Organic Analysis*; Seventh Ed., Elsevier:Amsterdam, 1966; pp 219-20, 224-5.

(13) The name is derived from Apra Harbor, Guam, where the sponge was collected.

(14) Bisaprasin (5): UV (MeOH): λ_{max} 212 (110,000), 290 (14,100); IR (KBr): υ_{max} 3400-3100 br, 2900, 1670, 1570, 1450, 1200, 1025 cm^{-1}. ^1H NMR (DMSO-d_6): δ 8.08 (1H t, J = 6 Hz, exchangeable), 8.05 (1H t, J = 6 Hz, exchangeable), 7.29 (1H d, J = 2 Hz), 7.00 (1H dd, J = 8.3, 2 Hz), 6.83 (1H d, J = 8.3 Hz), 6.73 (1H d, J = 1.9 Hz), 6.65 (1H d, J = 1.9 Hz), 3.67 (2H s), 3.61 (2H s), 3.44 (2H dt, J = 7, 6 Hz), 3.41 (2H dt, J = 7, 6 Hz), 2.81 (2H t, J = 7 Hz), 2.79 (2H t, J = 7 Hz); ^{13}C NMR (MeOH-d_4): δ 166.05 s, 165.83 s, 154.74 s, 153.60 s, 153.56 s, 153.07 s, 134.43 d, 133.08 d, 132.12 d, 130.61 s, 130.36 d (2), 128.04 s, 117.03 d, 114.44 s, 110.47 s, 39.69 t, 39.57 t, 38.53 t (2), 28.88 t, 28.70 t.

(15) Prepared and purified identically as was 4 from 3; ^1H NMR ($CDCl_3$): δ 7.53 (1H d, J = 2 Hz), 7.45 (1H d, J = 2 Hz), 7.20 (1H dd, J = 8.3, 2 Hz), 7.07 (2H brm, J = 6 Hz, exchangeable), 6.98 (1H d, J = 2 Hz), 6.77 (1H d, J = 8.3 Hz), 4.02 (3H s), 4.00 (3H s), 3.84 (6H s), 3.82 (2H s), 3.61 (4H q, J = 7, 6 Hz), 3.52 (2H s), 2.80 (4H t, J = 7 Hz).

(16) Kazlauskas, R.; Lidgard, R.O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1981**, 34, 765-86.

(17) When tested against *Pseudomonas aeruginosa*, both compounds appear to promote rather than inhibit the growth of bacteria.

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