Psammaplysin F, a New Bromotyrosine Derivative from a Sponge, *Aplysinella* sp.

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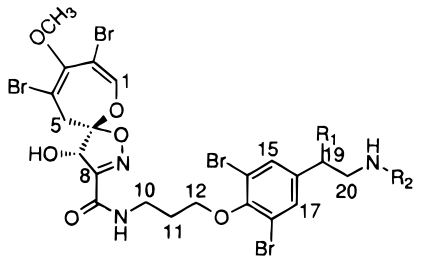
A new member of the psammaplysin family, psammaplysin F (**6**), has been isolated from an undescribed species of *Aplysinella* sponge, along with the four known psammaplysins A−C (**1**−**3**) and E (**5**). The structure of psammaplysin F was determined by spectral analysis.

Marine sponges of the order Verongida are noted for their production of bromotyrosine derivatives.[1] Psammaplysins A−E[2−4] (**1**−**5**), and ceratinamides A(**7**) and B(**8**)[5] are a unique, small family of such metabolites that feature an oxepin moiety proposed to arise from epoxidation and subsequent rearrangement of a bromotyrosine unit.[2] The psammaplysins have been reported to be toxic to different tumor cell lines[3,4] and psammaplysin D showed anti-HIV activity against the Haitian RF stain of HIV-1;[4] the ceratinamides display antifouling activity.[5] We report here the isolation of an additional member of the psammaplysin family, psammaplysin F (**6**), along with the known compounds, psammaplysins A−C (**1**−**3**) and E (**5**), from an *Aplysinella* sp. of sponge (order Verongida, family Aplysinellidae), from Chuuk, Federated States of Micronesia.

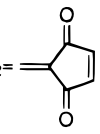


1 $R_1=R_2=H$ Psammaplysin A

2 $R_1=OH$, $R_2=H$ Psammaplysin B

3 $R_1=OH$, $R_2=Me$ Psammaplysin C

4 $R_1=H$, $R_2=CO(CH_2)_{11}CHMe_2$ Psammaplysin D



5 $R_1=H$, $R_2=$ Psammaplysin E

6 $R_1=H$, $R_2=Me$ Psammaplysin F

7 $R_1=H$, $R_2=CHO$ Ceratinamide A

8 $R_1=H$, $R_2=CO(CH_2)_{11}CHMe_2$ Ceratinamide B

The combined MeOH and MeOH−CH_2Cl_2 extracts of EtOH preserved sponges were partitioned between aqueous MeOH and various organic solvents (see Experimental Section). The mixture extracted by CH_2Cl_2 from a MeOH−H_2O (70:30) solution of the total extract was resolved by flash Si gel chromatography and HPLC using a Si gel or diol column to give psammaplysins A−C (**1**−**3**), E (**5**), and F (**6**). The structures of **1**−**3** and **5** were confirmed by comparison of their spectral data with published values. The ^{1}H- and ^{13}C-NMR data for **6** were nearly identical to that of **1**, except for the signals for the additional N-Me group (δ_H 2.44, 3H, δ_C 35.75). FABMS analysis revealed a cluster of ions at *m/z* 752, 750, 748, 746, and 744 consistent with the formula $C_{22}H_{25}O_6Br_4N_3$ as expected for **6**. ^{1}H-COSY data established the coupling sequences expected for **6**, HMQC data provided the assignment of the protonated carbon signals, and HMBC data (NMe to C-20 correlation) confirmed the location of the NMe group.

Experimental Section

General Experimental Procedures. NMR spectra (chemical shifts in ppm) were recorded on a Varian VXR-500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Mass spectra were recorded on a VG ZAB-E instrument. IR spectra were performed on a Bio Rad 3240-spc FT spectrometer; and optical rotations, on a Rudolph Autopol III automatic polarimeter.

Animal Material. The sponges were collected from Chuuk Atoll, Federated States of Micronesia, in August 1993, at Pis Island (collection 5T93), and in August 1995, at Northeast Pass (collection 18T95). They formed irregular, thick encrustations with erect broad branches. The surface was softly conulose and fleshy to the touch, the sponge compressible, flexible, and easily torn. The color in life was dull pink with a beige interior, turning deep brown in EtOH preservative. Fibers are sparce, dendritic, knotted, and composed of a granular pith with a very thin patchy bark. Sandy detritus is incorporated into the center of each fiber. The sponges represent a new species of aplysinellid-verongid sponge, probably most closely related to *Aplysinella strongylata* Bergquist (order Verongida, family Aplysinellidae). Although the genus *Aplysinella* is not well known, it is the genus of choice for this sponge as the diagnosis defines the sponge as having fibers with both bark and pith components, the later being dominant,[6] as is the case for the sponge described here. *Pseudoceratina* (and synonym *Psammaplysilla*) is reserved for sponges whose fibers contain pith components only; and *Suberea*, sponges whose fibers are dominated by the bark component.[7] These specimens are almost identical to that

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described previously.[4] A voucher specimen has been deposited at the Natural History Museum, London, UK (BMNH 1996.10.9.1).

Extraction and Isolation. Specimens from the 1993 and 1995 collections were extracted separately three times with MeOH and then twice with 1:1 MeOH−CH_2Cl_2 (dry wt after extraction 20 g and 290 g, respectively). The extracts from each collection were combined and concentrated *in vacuo*. Each concentrated extract was dissolved in CH_3OH-H_2O (9:1) and the solution extracted with hexane. The aqueous MeOH layer was diluted to 7:3 with MeOH−H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 fraction (0.61 g) from 5T93 was chromatographed on Si gel using a step gradient, from 5 to 8% CH_3OH in CH_2Cl_2. The fraction containing psammaplysins A and F was resolved through SiO_2 HPLC ($CH_3OH-CHCl_3$−conc. NH_4OH, 20:80:0.1) to give psammaplysin A (**1**), 7.8 mg, and psammaplysin F (**6**), 10.6 mg. The fraction containing psammaplysin E was further purified through Si gel chromatography (EtOAc−hexane, 2.5:1), then SiO_2 HPLC ($CH_2Cl_2-CH_3OH-NH_4OH$, 90:10:0.1) to give psammaplysin E (**5**), 2.6 mg. A portion (8 g) of the CH_2Cl_2 extract (22 g) of 18T95 was likewise chromatographed over Si gel using CH_2Cl_2 with increasing amounts of MeOH as eluent (13 fractions). HPLC of fraction 7 on a diol column using MeOH−$CHCl_3$−conc. NH_4OH (98:2:0.2) as eluent yielded psammaplysins C (**3**) (20 mg) and F (**6**) (17 mg). Psammaplysin B (**2**) (15 mg) was obtained from the ninth fraction of the Si gel chromatography using the same diol HPLC column and eluting solvent as above.

Psammaplysin F (6): $[\alpha]_D$ −62.3 (*c* 1.2, MeOH−CH_2Cl_2); IR (neat) 3450, 3300, 1660 (s), 1620, 1590, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34 (2 H, s, H-15, H-17), 7.26 (1 H, s, −CONH−), 7.0 (1 H, s, H-1), 5.03 (1 H, s, H-7), 4.1 (1 H, s, −OH), 4.05 (2 H, t, J = 5.5 Hz, H-12), 3.67 (3 H, s, $-OCH_3$), 3.68 (3 H, m, H-10, −NH−), 3.38 (1 H, d, J = 16 Hz, H-5), 3.09 (1 H, d, J = 16 Hz, H-5′), 2.82 (2 H, t, J = 7 Hz, H-20), 2.73 (2 H, t, J = 7 Hz, H-19), 2.44 (3 H, s, $-NCH_3$), 2.08 (2 H, m, H-11); ^{13}C NMR ($CDCl_3$) δ 158.8 (C-9), 156.7 (C-8), 151.0 (C-13), 148.5 (C-3), 145.4 (C-1), 138.6 (C-16), 132.8 (C-15, C-17), 121.3 (C-6), 118.0 (C-14, C-18), 104.6 (C-4), 103.5 (C-2), 78.8 (C-7), 70.8 (C-12), 59.0 (OMe), 52.1 (C-20), 37.1 (C-5), 37.0 (C-10), 35.8 (NCH_3) 34.3 (C-19), 29.3 (C-11); FABMS $[M + H]^+$ 752 (22), 750 (57), 748 (100), 746 (71), 744 (23), 393 (26).

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