Three Bromotyrosine Derivatives, One Terminating in an Unprecedented Diketocyclopentenylidene Enamine†

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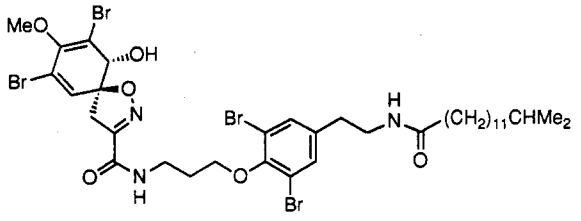
Bromotyrosine derivatives are characteristic constituents of marine sponges of the order Verongida.[1] In many of these compounds the bromotyrosine is rearranged to a distinctive spirocyclohexadieneisoxazoline system[2] as, e.g., in araplysillin-II (1).[3] Conversely, only three compounds



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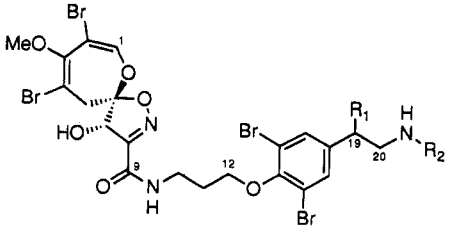
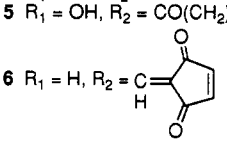
have so far been described, psammaplysins A, B, and C (2–4),[4,5] in which a bromotyrosine moiety is rearranged to a spirooxepinisoxazoline, presumably *via* a common arene oxide intermediate. Both types frequently exhibit antimicrobial and/or cytotoxic activities.[2] We now report reisolation of psammaplysin A (2) and two new members of the oxepin type, psammaplysin D (5) and E (6). In



2 $R_1 = R_2 = H$
3 $R_1 = OH, R_2 = H$
4 $R_1 = OH, R_2 = Me$
5 $R_1 = OH, R_2 = CO(CH_2)_{11}CHMe_2$



6 $R_1 = H, R_2 =$

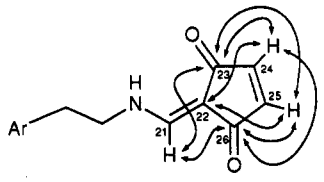
psammaplysin D (5), R_1 is hydroxy as in B or C and R_2 is an isopentadecanoyl residue, as in araplysillin-II (1).[3]

Psammaplysin E (6), the third constituent of *Aplysinella* sp. from Pingelap Atoll, Federated States of Micronesia, has as its R_2 constituent an unprecedented cyclopentenedione. Psammaplysin E (6) exhibits cytotoxicity against KB (human oral, epidermoid carcinoma) and LoVo (human colon, adrenocarcinoma) cells at 5 μg/mL and modest immunosuppressive activity (a potency of 40, IC_{50} 8.32E-01 for mixed lymphocyte reaction assay), while psammaplysin D (5) displays anti-HIV activity against the Haitian RF strain of HIV-I (51% inhibition at 0.1 μg/mL).

The nonpolar extract (5.6 g) of the lyophilized sponge was separated by vacuum flash chromatography, Sephadex LH-20, preparative TLC, and HPLC to yield pure psammaplysins D (5, 5.0 mg) and E (6, 19.8 mg). Previously known psammaplysin A (2) was obtained in the methanol eluent of a flash chromatography column as a colorless oil (23.4 mg) and identified by spectral comparison.[4]

Psammaplysin D (5), a colorless oil, had composition $C_{35}H_{51}Br_4N_3O_8$, established by HRFABMS data. ^1H and ^{13}C (Table I) NMR spectral data of 5 from C1 to C19 were identical with those of psammaplysin B (3). Distinctive spectral features of psammaplysin D (5) included a signal for C20, drastically shifted downfield to 47.9 ppm from 18.4 ppm in 3; an amide carbon signal at 174.8 ppm; a methyl carbon signal at 22.9 ppm and a six-proton doublet (J = 6.5 Hz) in the ^1H NMR spectrum, delineating an isopropyl terminus of the amide. The $C_{11}H_{22}$ difference between the molecular formula and the partial composition confirmed by NMR spectral data pointed to an isopentadecanoic acid amide, as previously encountered in araplysillin-II (1).[3] Comparison of the spectral data with those of 3 and 1 confirmed structure 5 for psammaplysin D (Table I and Experimental Section).

Psammaplysin E (6), a bright yellow oil, had composition $C_{27}H_{25}Br_4O_8$. ^{13}C and ^1H NMR spectral data of 6 were superimposable on those of psammaplysin A (2)[4] except for three additional olefinic protons and six sp^2 carbons (Table II). The structure of this $C_6H_3O_2$ fragment (a) could be established by HMBC measurements. Most



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commonly encountered end groups among the Verongida bromotyrosine derivatives are amino acid derivatives; occasionally, as in araplysillin-II (1), we find acetate-derived moieties. The unique cyclopentenedione in psammaplysin E (6) conceivably might have its origin in a sugar. Apparently, this molecular entity has not previously been encountered in compounds from natural sources, but is a reasonably familiar synthetic product. Arylidenecyclopentenediones, e.g., have found use as radiosensitizers in biochemical research.[6]

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(1) Bergquist, P. R.; Wells, R. J. In *Marine Natural Products—Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic: New York, 1983; Vol. 5, pp 1–50.

(2) Ireland, C. R.; Molinski, T. F.; Roll, D. M.; Zabriskie, T. M.; McKee, T. M.; Swersey, J. C.; Foster, M. P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer: Berlin, 1989; Vol. 3, pp 1–46.

(3) Longeon, A.; Guyot, M.; Vacelet, J. *Experientia* **1990**, *46*, 548–550.

(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) Copp, B. R.; Ireland, C. M.; Barrows, L. R. *J. Nat. Prod.* **1992**, *55*, 822–823.

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Table I. ^{13}C NMR Data (C1–C20) of Psammaplysins D (5) and B (3)

carbon	D (5)[a]	B (3)[b,4]
1	146.4	146.7
2	103.6	104.3
3	149.3	149.8
4	104.1	104.5
5	37.5	38.2
6	119.9	120.8
7	80.2	80.4
8	158.5	158.7
9	159.1	160.6
10	37.5	38.0
11	30.4	30.5
12	72.0	72.1
13	152.6	153.6
14, 18	118.4	119.1
15, 17	131.4	131.5
16	143.6	143.2
19	72.3	72.7
20	47.9	18.4
OMe	59.0	59.3

[a] In acetone-d_6. [b] In methanol-d_4.

Table II. ^{13}C NMR Data of Psammaplysins E (6) and A (2)[4]

carbon	E[a]	E[b]	A[b]
1	146.3	147.3	146.3
2	103.5	104.3	103.9
3	149.3	147.3	149.4
4	104.1	104.5	104.3
5	37.4	38.8	37.9
6	119.9	120.8	120.4
7	80.1	80.8	80.2
8	158.4	158.8	158.2
9	159.1	160.7	160.2
10	37.9	38.0	38.2
11	30.4	30.5	29.9
12	72.0	72.1	71.9
13	152.5	153.1	153.0
14, 18	118.6	119.2	119.1
15, 17	134.3	134.3	134.1
16	138.6	135.2	136.9
19	36.4	35.9	32.9
20	51.2	51.9	41.5
21	149.8	146.3	
22	99.4	99.4	
23	197.4	198.5	
24	142.6	142.6	
25	142.4	142.6	
26	194.1	196.6	
OMe	60.0	59.8	59.3

[a] In acetone-d_6. [b] In methanol-d_4.

Experimental Section

Kiesel Gel 60 H was used for vacuum flash chromatographies. Analytical TLC separations were performed on precoated HPTLC plates: silica gel 60 F254, RP-18 F254s, or CN F254s. Preparative TLC separations were performed on silica gel GF, 20 cm × 20 cm, 1-mm thickness. Gel permeation chromatography was carried out by Sephadex LH-20 column. Ultracarb 30 ODS was used for reversed-phase HPLC separations. YMC-Pack CN and Li-chrosorb Si 60 were used for normal-phase HPLC.

Isolation. A sponge was collected from a vertical coral wall at 15 m depth, at Pingelap Atoll, Micronesia, on June 22, 1990. The sponge is a new species of *Aplysinella* (Porifera, Demospongiae, Verongida, Aplysinellidae). A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, FL (catalog no. 003:00829). The freeze-dried specimen (470 g) was thoroughly extracted with ethanol, and the solvent was removed in vacuo. The resulting residue was partitioned with EtOAc/hexane/MeOH/water (7:4:4:3) to furnish 5.6 g of nonpolar extract (upper layer) and 17.0 g of polar extract (lower layer).

The nonpolar extract was separated by a vacuum flash chromatography into nine fractions (combination of hexane/EtOAc/MeOH). The fraction (800 mg) eluted with hexane/EtOAc (1:1) was further separated on Sephadex LH 20 (2.5 cm × 100 cm, MeOH/CH_2Cl_2 (2:1)), silica gel flash chromatography, preparative TLC, and silica gel HPLC to yield crude **6** and 5.0 mg of pure **5**. Final purification of **6** was achieved by CN and ODS HPLC to yield 19.8 mg (pure) as a bright yellow oil.

Previously reported psammaplysin A (**2**)[4] was obtained from MeOH eluent of a vacuum flash chromatography of the nonpolar extract as a colorless oil (23.4 mg).

Psammaplysin D (5): colorless oil; $[\alpha]^{18}_D$ −71.4° (acetone, *c* 2.8); UV (MeOH) λ_{max} 210 nm (ϵ 50 900), 224 (sh, ϵ 27 600), 258 (sh, ϵ 10 400); IR ($CHCl_3$) ν_{max} 3430 cm^{-1}, 3390, 2910, 2830, 1660, 1570, 1445, 1135, 1105, 1030, 980, 950, 890; ^{13}C NMR (125 MHz, acetone-d_6): δ 174.8 (C21), 159.1 (C9), 158.5 (C8), 152.6 (C13), 149.3 (C3), 146.4 (C1), 143.6 (C16), 131.4 (C15 and C17), 119.9 (C6), 118.4 (C14 and C18), 104.1 (C4), 103.6 (C2), 80.2 (C7), 72.3 (C19), 72.0 (C12), 59.0 (OMe), 47.9 (C20), 39.7 (C22), 37.5 (C5), 37.5 (C10), 36.5, 30.4 (C11), 28.6, 28.1, 27.8, 26.6, 22.9 (C34 and C35); ^1H NMR (500 MHz, acetone-d_6) δ 7.84 (NH, t, J = 5.8 Hz), 7.60 (H15 and H17, 2H, d, J = 0.5 Hz), 7.26 (NH, 1H, brt, J = 5.3 Hz), 7.17 (H1, 1H, s), 5.99 (OH, 1H, d, J = 7.0 Hz), 5.19 (OH, 1H, dd, J = 4.5 Hz), 5.07 (H7, 1H, d, J = 7.0 Hz), 4.78 (H19, 1H, q, J = 5.3 Hz), 4.10 (H12, 2H, t, J = 6.3 Hz), 3.64 (H10, 2H, q, J = 6.5 Hz), 3.64 (OMe, 3H, s), 3.49 (H20, 1H, dt, J = 13.8, 5.1 Hz), 3.45 (H5, 1H, d, J = 16.0 Hz), 3.37 (H20, 1 H, dt, J = 14.0, 6.0 Hz), 3.13 (H5, 1H, d, J = 16.5 Hz), 2.15 (H11, 2H, quintet, J = 6.0 Hz); HRFABMS observed m/z 974.0468 (M + H), $C_{36}H_{52}{}^{79}Br_2{}^{81}Br_2N_3O_8$ requires m/z 974.0451 (Δ 1.7 mmu).

	psammaplysin D (5)	araplysillin-II (1)[3]
H22	2.14, t, J = 7.0 Hz	2.12, m
H23-32	1.28, brs	1.25, s
H33	1.55, m	1.54, m
H34 and H35	0.85, d, J = 6.5 Hz	0.87, d

Psammaplysin E (6): bright yellow oil; $[\alpha]^{18}_D$ −80.3° (acetone, *c* 0.3); UV (MeOH) λ_{max} 208 nm (ϵ 53 400), 224 (sh, ϵ 33 300), 298 (ϵ 25 000); IR ($CHCl_3$) ν_{max} 3380 cm^{-1}, 2910, 1700, 1640, 1610, 1445, 1145, 1105, 1035, 985, 950, 880; ^{13}C NMR data shown in Table II; ^1H NMR (300 MHz, acetone-d_6) δ 8.64 (NH, brs), 7.86 (OH, brt, J = 5.4 Hz), 7.55 (H15 and H17, 2H, s), 7.34 (H21, 1H, d, J = 14.4 Hz), 7.17 (H1, 1H, s), 6.73 (H25, 1H, d, J = 6.3 Hz), 6.64 (H24, 1H, d, J = 6.3 Hz), 6.02 (NH, d, J = 7.2 Hz), 5.07 (H7, 1H, d, J = 7.2 Hz), 4.08 (H12, 2H, t, J = 6.2 Hz), 3.75 (H20, 2H, q, J = 7.0 Hz), 3.63 (H10, 2H, q, J = 6.6 Hz), 3.63 (OMe, 3H, s), 3.44 (H5, 1H, d, J = 16.2 Hz), 3.13 (H5, 1H, d, J = 16.2 Hz), 3.01 (H19, 2H, t, J = 7.2 Hz), 2.14 (H11, 2H, quintet, J = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.4 (C23), 194.1 (C26), 158.9 (C9), 156.0 (C8), 151.8 (C13), 148.7 (C21 and C3), 145.4 (C1), 142.2 (C24), 142.1 (C25), 135.9 (C16), 133.0 (C15 and C17), 121.8 (C6), 118.5 (C14 and C18), 105.2 (C4), 103.4 (C2), 99.4 (C22), 79.3 (C7), 71.0 (C12), 59.1 (OMe), 50.6 ((C20), 37.1 (C10), 37.1 (C5), 36.0 (C19), 29.2 (C11); ^1H NMR (500 MHz, $CDCl_3$) δ 8.41 (NH, 1H, quintet, J = 6.5 Hz), 7.30 (H15 and H17, 2H, s), 7.23 (NH, 1H, t, J = 6.5 Hz), 7.20 (H21, 1H, d, J = 14.0 Hz), 7.01 (H1, 1H, s), 6.76 (H25, 1H, d, J = 6.0 Hz), 6.70 (H24, 1H, d, J = 6.0 Hz), 5.14 (H7, 1H, d, J = 4.5 Hz), 4.25 (OH, 1H, d, J = 5.0 Hz), 4.08 (H12, 2H, dt, J = 1.8, 5.6 Hz), 3.71 (H10, 2H, dt, J = 6.2, 6.5 Hz), 3.54 (H20, 2H, q, J = 6.7 Hz), 2.83 (H19, 2H, t, J = 7.0 Hz), 2.11 (H11, 2H, quintet, J = 6.0 Hz); HRFABMS observed m/z 993.8563 (M + H + $C_4H_{10}O_2S_2$), $C_{31}H_{36}{}^{79}Br_2{}^{81}Br_2N_3O_{10}S_2$ requires m/z 993.8538 (Δ 2.5 mmu); LRFABMS m/z 994.4 (M + H + $C_4H_{10}O_2S_2$), 840.0 (M + H).

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(6) Koike, H.; Hori, H.; Inayama, S.; Terada, H. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1066–1074.