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THREE NEW BROMOTYROSINE-DERIVED METABOLITES OF THE SPONGE *PSAMMAPLYSILLA PURPUREA*[1]

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ABSTRACT.—Three new cytotoxic bromotyrosine-derived secondary metabolites, aplysamines 3 [**2**], 4 [**3**], and 5 [**4**], were isolated from the sponge *Psammaplysilla purpurea.*

Marine sponges of the order *Verongida* are often characterized by a wide range of bioactive bromotyrosine constituents (1,2). The large number of biosynthetically related compounds is due to chemical variations occurring in the side chain and/or aromatic ring of the tyrosine moiety. We now report the structures and in vitro bioactivity of aplysamines 3 [**2**], 4 [**3**], and 5 [**4**], which are related to aplysamine 2 [**1**].

A CH_2Cl_2-iPrOH (1:1) extract of the sponge *Psammaplysilla purpurea* Carter (family Aplysinellidae), collected by scuba on the south shore of Maui, Hawaii, at −40 m (sample ID 12-DD-91) displayed antimicrobial activity and cytotoxicity against human epidermoid carcinoma KB cells. Bioassay-guided fractionation of the extract resulted in the isolation of three new compounds **2**–**4**.

The hrfabms of the major metabolite, aplysamine 3 [**2**], indicated a molecular formula of $C_{21}H_{24}Br_3N_3O_4$. The ^{1}H-nmr spectrum of **2** (Table 1) showed the characteristic pattern of a 1,2,4-trisubstituted benzene (1H doublets at 7.42 and 6.88 and a doublet of doublets at 7.17 ppm) and a two-proton singlet at 7.43 ppm for a symmetrically tetrasubstituted benzene. Amide and oxime functionalities were indicated by ir absorptions at 3350, 1655, and 1620 cm^{-1} and confirmed by two ^{13}C-nmr signals at 165.8 and 153.0 ppm (Table 2). The upfield ^{13}C-nmr shift of C-7 (28.7 ppm) suggested *E* configuration of the oxime, as the corresponding value for a (*Z*)-oxime is >35 ppm (4). Comparison with the nmr data for aplysamine 2 [**1**] (3) (Tables 2 and 3, numbering system same as in **2**) showed that the dimethylammonium group in **1** was replaced by ammonium, which shifted the H_2-20 and H_2-19 signals to 3.29 and 2.19 ppm, the C-20 resonance upfield by 17.9 ppm and the C-19 signal downfield by 2.6 ppm. Treatment of **2** dissolved in CD_3OD with aqueous KOH and ^{1}H-nmr analysis of the resulting free amine confirmed that **2** was a salt. The ^{1}H-nmr signals of the methylenes α and β to the amino group were shifted upfield by 0.38 and 0.23 ppm. No attempt was made to determine the nature of the counter ion. The multiplicity of the shifted signals also proved the linkage mode of the two bromotyrosine-derived segments, in which the three-carbon chain C-18 to C-20 was bearing the terminal amine rather than the internal amide nitrogen. An alternative mode is represented by 14-debromoprearaplysillin I [**5**] (5).

The molecular formula of the second metabolite, aplysamine 4 [**3**], was $C_{21}H_{23}Br_4N_3O_4$ by hrfabms. ^{1}H- and ^{13}C-nmr spectra of **3** resembled those of **2** except for ring A signals (Tables 1 and 2), which unambiguously showed that the additional bromine atom was at C-4.

The hrfabms of the third metabolite, aplysamine 5 [**4**], indicated a molecular

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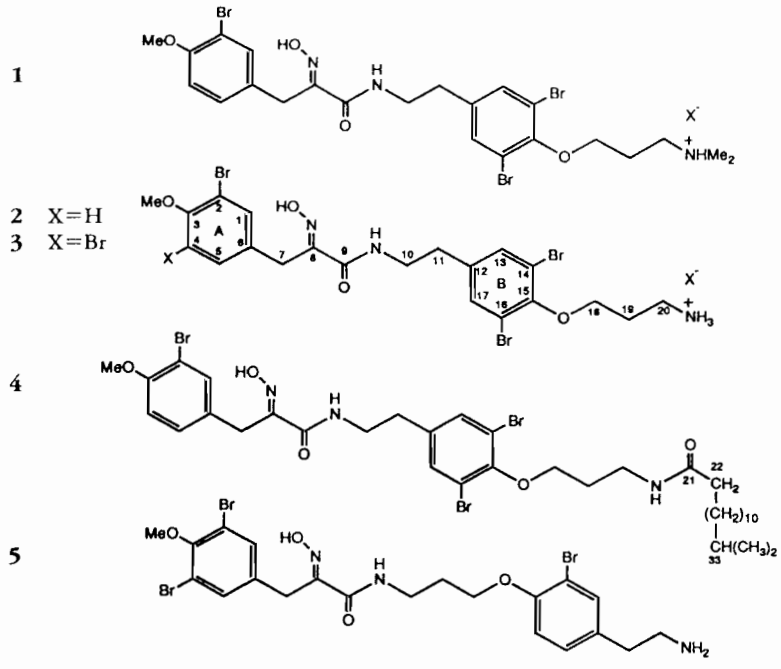

formula of $C_{36}H_{52}Br_3N_3O_5$. The ir spectrum displayed two strong bands in the amide region at 1660 and 1645 cm^{-1}. The ^1H-nmr spectrum of **4** exhibited the same characteristic resonances as **2** (Table 1), except for a 2H triplet (H-20) that was shifted downfield (3.41 ppm). In addition, the spectrum showed signals typical for a terminal iso-branched saturated fatty acid chain: protons α and β to an acyl group (2.17 and 1.60 ppm), a 6H doublet of two methyls (0.86 ppm), a multiplet of a methine (1.51 ppm), and two signals for methylenes (1.28 ppm, 16H and 1.16 ppm, 2H). These data, when compared with those for **2**, suggested that

TABLE 1. ^1H-nmr Data for Compounds **1**–**4** (CD_3OD, 500 MHz, ppm, *J* in Hz).

Proton	Compound			
	1*	2	3	4
H-1	7.43, 1H, d, 2.2	7.42, 1H, d, 2.0	7.47, 2H, s, (1 and 5)	7.43, 1H, d, 2.0
H-4	6.88, 1H, d, 8.5	6.88, 1H, d, 8.5		6.88, 1H, d, 8.5
H-5	7.17, 1H, dd, 2.2, 8.5	7.17, 1H, dd, 2.0, 8.5	7.47, 2H, s (1 and 5)	7.14, 1H, dd, 2.0, 8.5
H-7	3.79, 2H, s	3.79, 2H, s	3.81, 2H, s	3.79, 2H, s
H-10	3.41, 2H, t, 7.0	3.42, 2H, t, 7.3	3.43, 2H, t, 7.2	3.43, 2H, t, 7.2
H-11	2.73, 2H, t, 7.0	2.74, 2H, t, 7.3	2.75, 2H, t, 7.2	2.73, 2H, t, 7.2
H-13 and H-17	7.42, 2H, s	7.43, 2H, s	7.44, 2H, s	7.41, 2H, s
H-18	4.05, 2H, t, 5.5	4.08, 2H, t, 5.7	4.06, 2H, t, 5.8	3.99, 2H, t, 6.2
H-19	2.25, 2H, tt, 5.5, 7.6	2.19, 2H, tt, 5.7, 7.5	2.18, 2H, tt, 5.8, 7.8	2.03, 2H, tt, 6.2, 6.5
H-20	3.46, 2H, t, 7.6	3.29, 2H, t, 7.5	3.29, 2H, t, 7.8	3.41, 2H, t, 6.5
MeO	3.81, 3H, s	3.82, 3H, s	3.81, 3H, s	3.82, 3H, s
$^+NHMe_2$	2.93, 6H, s			
H-22				2.17, 2H, t, 7.3
H-23				1.60, 2H, m
H-34, -35				0.86, 6H, d, 6.5
H-33				1.51, 1H, m
H-24–H-32				1.28, 16H, m
				1.16, 2H, m

TABLE 2. ^{13}C-nmr Data for Compounds **1**–**3** (CD_3OD, 125 MHz, ppm).

Carbon	Compound		
	1[a]	**2**	**3**
C-1	134.7	134.7	134.5
C-2	130.3	131.8	118.6
C-3	155.8	155.9	152.1
C-4	112.1	113.2	118.6
C-5	131.7	130.4	134.5
C-6	113.1	112.2	137.4
C-7	28.7	28.7	28.8
C-8	152.9	153.0	152.2
C-9	165.8	165.8	165.5
C-10	41.3	41.3	41.3
C-11	35.2	35.2	35.2
C-12	140.3	140.3	140.3
C-13	134.4	134.4	134.4
C-14	118.7	118.7	118.7
C-15	152.1	152.2	152.1
C-16	118.7	118.7	118.7
C-17	134.4	134.4	134.4
C-18	71.7	71.6	71.6
C-19	26.4	29.0	29.0
C-20	56.9	39.0	39.0
MeO	56.7	56.7	61.0
$^{+}NHMe_2$	43.7		

[a]Recorded at 100 MHz; data are from Xynas and Capon (3).

aplysamine 5 [**4**] was an aplysamine 3 derivative, in which the terminal amine was acylated with 13-methyltetradecanoic acid.

Although aplysamine 2 [**1**] was reported (3) inactive in several tests against Gram-positive and Gram-negative bacteria, both aplysamines 3 and 4 showed mild activity against *Staphylococcus aureus* at 100 μg/disk (10 mm inhibition zone). In a similar test with a Gram-negative bacterium, *Escherichia coli*, no activity was observed. Analogous results were reported (5) for 14-debromoprearaplysillin I [**5**]. Aplysamines **2**, **3**, and **4** were also found active in mouse lymphoid neoplasm (P388), human lung carcinoma (A549), human colon adenocarcinoma (HT-29), and human oral epidermoid carcinoma (KB) assays (Table 3). Compounds **2**, **3**, and **4** were also tested in Haitian RF strain of HIV-1 assay but showed no antiviral inhibition at a level of 1 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were measured on a Perkin-Elmer 1420 spectrometer. Uv spectra were determined in MeOH on a Hewlett Packard 8452A spectrophotometer. ^{1}H-nmr spectra were recorded at 500 MHz and ^{13}C-nmr spectra at 125 MHz on a General Electric GE W-500 spectrometer. Mass spectra were obtained with a VG 70/SE mass spectrometer. All solvents were distilled prior to use. The yield of each compound is based on the weight of the initial extract.

BIOLOGICAL MATERIAL.—The sample was collected by scuba at South Kihei, Maui, Hawaii, at −40 m in June 1991. The yellow sponge formed a thin encrustation on a rock surface and turned deep violet after collection. The sample closely corresponds to *Psammaplysilla purpurea* (Demospongiae, Verongida, Aplysinellidae) as previously described (6,7). A voucher specimen is on deposit at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (catalog number 003:00828).

Table 3. Bioassay Data (IC_{50}) for Compounds **2**–**4**.

Compound	IC_{50} (μg/ml)			
	P-388	A549	HT-29	KB
Aplysamine 3 [**2**]	1	2	2	5
Aplysamine 4 [**3**] ...	2.5	2.5	2.5	5
Aplysamine 5 [**4**]	10	2.5	2.5	2

BIOASSAYS.—Cytotoxicity tests against P388, A 549, and HT-29 were performed by PharmaMar S.A. Madrid, Spain (11/25/92); KB and antimicrobial by Faith Caplan, University of Hawaii (10/16/92); HIV antiviral inhibition by PharmaMar S.A. Cambridge, Massachusetts (11/12/92).

ISOLATION.—The sample was frozen on collection and lypophilized to yield 23.3 g of dry mass, which was extracted thrice with CH_2Cl_2/iPrOH, followed by removal of the solvent in vacuo. The residue (290 mg) was partitioned using H_2O-MeOH-hexane (1:9:10). The lower phase was subjected to reversed-phase RP-18 flash chromatography.

Aplysamines 3 [**2**] *and 4* [**3**].—The fraction (150 mg) eluted with MeCN-H_2O (7:3) was subjected to RP-18 hplc in MeCN-H_2O (8:2) to yield a mixture (108 mg) of **2** and **3**. Repetition of hplc on RP-18 using MeCN-iPrOH (9:1) afforded two compounds. Aplysamine 3 [**2**] (20 mg, 6.9%): white, semicrystalline solid; hrfabms *m/z* $[M+H]^+$ 619.9380 (calcd for $C_{21}H_{25}{}^{79}Br_3N_3O_4$, 619.9395); uv λ max 214 (ε 17000), 222 (ε 16700), 280 nm (ε 3100); ir (KBr) ν max 3350, 2940, 1655, 1620, 1530, 1490, 1455 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. Aplysamine 4 [**3**] (3 mg, 1.0%): white, semicrystalline solid; hrfabms *m/z* $[M+H]^+$ 699.8480 (calcd for $C_{21}H_{24}{}^{79}Br_2{}^{81}BrN_3O_4$, 699.8480); uv λ max 218 (ε 17600), 222 (ε 17400), 274 nm (ε 2100); ir (KBr) ν max 3400, 2990, 1670, 1630, 1530, 1460 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Aplysamine 5 [**4**].—The fraction (12 mg) eluted with MeCN was purified by hplc on RP-18 in MeOH and afforded **4** (0.5 mg, 0.2%): colorless gum; hrfabms *m/z* $[M+H]^+$ 844.1459 (calcd for $C_{36}H_{53}{}^{79}Br_3N_3O_5$, 844.1535); uv λ max 214 (ε 18500), 222 (ε 17200), 282 nm (ε 2700); ir ($CHCl_3$) ν max 3400, 2910, 1660, 1645, 1490, 1445 cm^{-1}; ^1H nmr see Table 1. The sample was too small to record a carbon spectrum.

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LITERATURE CITED

1. P.R. Bergquist and R.J. Wells, in: "Marine Natural Products: Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Academic Press, New York, 1985, Vol. 5, p. 17.
2. H.C. Krebs, *Fortschr. Chem. Org. Naturst.*, **49**, 151 (1986).
3. R. Xynas and R.J. Capon, *Aust. J. Chem.*, **42**, 1427 (1989).
4. L. Arabshahi and F.J. Schmitz, *J. Org. Chem.*, **52**, 3584 (1987).
5. D.M. James, H.B. Kunze, and D.J. Faulkner, *J. Nat. Prod.*, **54**, 1137 (1991).
6. P.R. Bergquist, *Pac. Sci.*, **19**, 135 (1965).
7. M. Kelly-Borges and P.R. Bergquist, *Indo-Malayan Zoology*, **5**, 121 (1988).

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