Bromotyrosine Alkaloids from the Sponge *Pseudoceratina verrucosa*

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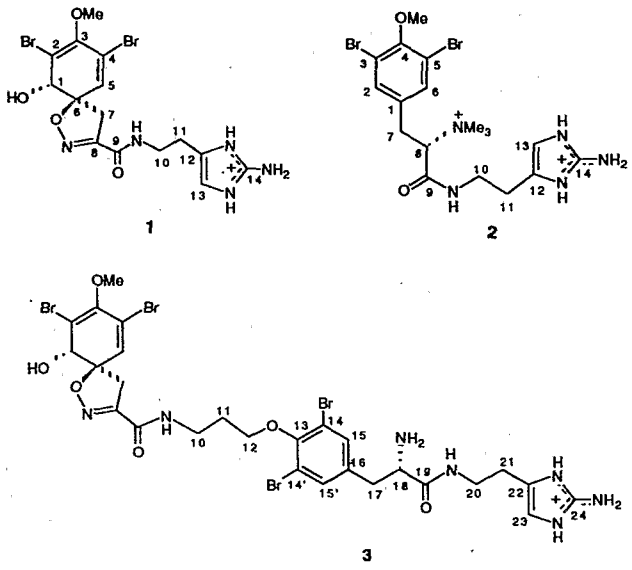
Three new bromotyrosine alkaloids, pseudoceratinines A (**1**), B (**2**), and C (**3**), have been isolated from two specimens of the sponge *Pseudoceratina verrucosa*. In addition, the known alkaloids aplysamine-1 (**4**), aplysamine-2 (**5**), purealin (**6**), and purealidins A (**7**) and B (**8**) were found in both specimens. The structures and absolute configurations of the new products were elucidated by spectral methods.

Bromotyrosine-derived alkaloids are well known in marine Verongida sponges. We report here the isolation and structural elucidation of three new bromotyrosines, pseudoceratinines A (**1**), B (**2**), and C (**3**), from two specimens of the sponge *Pseudoceratina verrucosa* Bergquist (order Verongida, family Aplysinellidae) collected off the coast of two different New Caledonian islands. Compounds **1** and **2** were isolated from a specimen collected at Ile Longue (Chesterfield Archipelago), whereas compounds **2** and **3** were found in a specimen collected at Ile Walpole. In addition, the known alkaloids aplysamine-1 (**4**),[2] aplysamine-2 (**5**),[2] purealin (**6**),[3] and purealidins A (**7**)[4] and B (**8**)[5] have been isolated from both specimens.

The MeOH extract of each specimen was partitioned between CH_2Cl_2 and H_2O, and the aqueous layer was subsequently extracted with *n*-BuOH. Repeated Si gel column chromatography of the CH_2Cl_2 extracts with CH_2Cl_2/MeOH mixtures yielded aplysamine-2 (**5**). The butanol extracts were first chromatographed on a Sephadex LH-20 column with CH_2Cl_2/MeOH (1:1) and further on a Si gel column with EtOAc/butanone containing increasing amounts of HCO_2H/H_2O (5:3:0.5:0.5 to 5:3:1.5:1.5) to give compounds **1**, **2**, and **4**–**8** from the specimen from Ile Longue and compounds **2**–**9** from the specimen from Ile Walpole. The new alkaloids **1**–**3** were converted into their hydrochloride salts on an Amberlite IR-45 anion exchange column.

Pseudoceratinine A (**1**) showed an isotopic cluster of MH^+ ions in the ratio 1:2:1, consistent with two bromine atoms, in the FABMS at *m/z* 490, 492, and 494. The molecular formula $C_{15}H_{18}Br_2N_5O_4$ was determined by HRFABMS (*m/z* 489.9725, MH^+, Δ 0.2). The ^{1}H-NMR spectrum exhibited the characteristic signals of a spirocyclohexadiene–isoxazole ring at δ 4.08 (1H, s, H-1), 6.40 (1H, s, H-5) and 3.09, 3.75 (two d, 1H each, $J = 18$ Hz). This partial structure was further supported by the ^{13}C resonances for carbons 1–9 (Table 1). The remaining part of **1** consisted of a 2-aminohistamine residue as suggested, in conjunction with the elemental composition of the molecule, by the two triplets at δ 3.51 and 2.76 (2H each), by the singlet at δ 6.55 observed in the ^{1}H-NMR spectrum, and by analysis of the ^{13}C-NMR spectrum (Table 1). Thus, pseudoceratinine A was assigned structure **1**, which differs from the previously reported aerophobin-2[6] only by the lack of one methylene in the central chain.



Pseudoceratinine B (**2**) displayed an isotopic cluster of MH^+ ions at *m/z* 502, 504, and 506 (ratio 1:3:1) in the FABMS. Accurate mass measurement confirmed a formula $C_{18}H_{26}Br_2N_5O_2$ (*m/z* 502.0432, MH^+, Δ −2.1). The ^{1}H-NMR spectrum showed two singlets at δ 3.82 (3H) and 3.32 (9H) corresponding to an MeO and to a Me_3N^+ group, respectively. Two additional singlets were observed at δ 7.55 (2H) and 6.42 (1H). The signal at δ 7.55 was assigned to a 1,2,4,6-tetrasubstituted aryl ring, while the signal at δ 6.42 was assigned to the heterocyclic proton of a 2-aminoimidazole. This assignment was confirmed by characteristic ^{13}C signals (Table 1), which corresponded to the aromatic carbons of an *O*-methyl-3,5-dibromotyrosine moiety and to a 2-aminoimidazole ring, respectively (Table 1). In the NMR spectra, the signals of the *CH*$_2$*CH*N*Me*$_3$*CO* group of the bromotyrosine residue were observed at δ_H 3.30 (2H, m, H-7) and 4.25 (1H, dd, H-8) and δ_C 32.0 (C-7), 75.8 (C-8), and 166.2 (C-9). The 2-aminoimidazole was part of an aminohistamine moiety showing the usual resonances for the CH_2CH_2CO group (Table 1). Thus, pseudoceratinine B was assigned structure **2**, which was further supported by HMBC cross peaks H-7/C-1,C-6,C-8,C-9 and H-8/C-7,C-9, and Me_3.

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Table 1. ^{13}C- (75 MHz) and ^{1}H-NMR (400 MHz) Data[a] for Pseudoceratines A (**1**), B (**2**), and C (**3**)

	1		**2**		**3**			
position	δ C	δ H (*J* Hz)	δ C	δ H (*J* Hz)	position	δ C	δ H (*J* Hz)	HMBC
1	75.6	4.08 s	134.0		1	75.4	4.10 s	3, 4, 5, 6
2	123.1		135.2	7.55 s	2	122.7		
3	149.0		118.9		3	149.3		
4	114.5		154.8		4	114.1		1, 2, 3, 4, 7
5	132.6	6.40 s	118.9		5	132.3	6.40 s	
6	92.9		132.5	7.55 s	6	92.5		
7	39.5	3.75 d (18)	32.0	3.30 m	7	40.2	3.78, d (18)	1, 5, 6, 8
		3.09 d (18)					3.10, d (18)	1, 5, 6, 8
8	155.6		75.8	4.25 dd (12, 4)	8	155.3		
9	162.1		166.2		9	161.1		
					10	38.3	3.60, t (7)	10,12
					11	30.6	2.12 m	10,11
					12	72.3	4.02 t (7)	13
					13	154.1		
					14,14′	119.2	7.52 s	13, 14, 16, 17
					15,15′	135.0		
					16	136.0		
					17	38.0	3.07 m	16, 18, 19
					18	56.0	4.07 m	16, 17, 19
					19	170.1		
10	40.5	3.51 t (7)	38.7	3.30 m	20	39.3	3.45 m	19, 21, 22
11	26.1	2.76 t (7)	25.2	2.45 m	21	25.7	2.65 m	20, 21, 22
12	126.3		125.3		22	125.9		
13	111.3	6.55 s	110.5	6.42 s	23	110.6	6.55 s	22, 24
14	149.7		148.5		24	149.9		
MeO	60.8	3.72 s	61.0	3.82 s	MeO	60.4	3.72 s	
Me_3N			53.2	3.32 s				
NH-9		8.75 br s		9.20 t (6)	NH-9		8.60 t (6)	
					NH-19		8.70 t (6)	
NH-12[b]		11.70 s		11.70 s	NH-22		11.80 s	
NH-13[b]		12.30 s		12.25 s	NH-23		12.35 s	
NH_2-14		7.45 s		7.25 br s	NH_2-24		7.35 br s	
OH-1		6.55 br s			OH-1		6.50 br s	
					NH_2-18		4.18 br	

[a] Measurements were taken in CD_4O, except for OH and NH, which were taken in DMSO-d_6. Assignments in CD_4O were based on 2D experiments for compounds **2** and **3**. [b] Respective values for NH-12 and NH-13 are from Nakamura *et al.*[3]

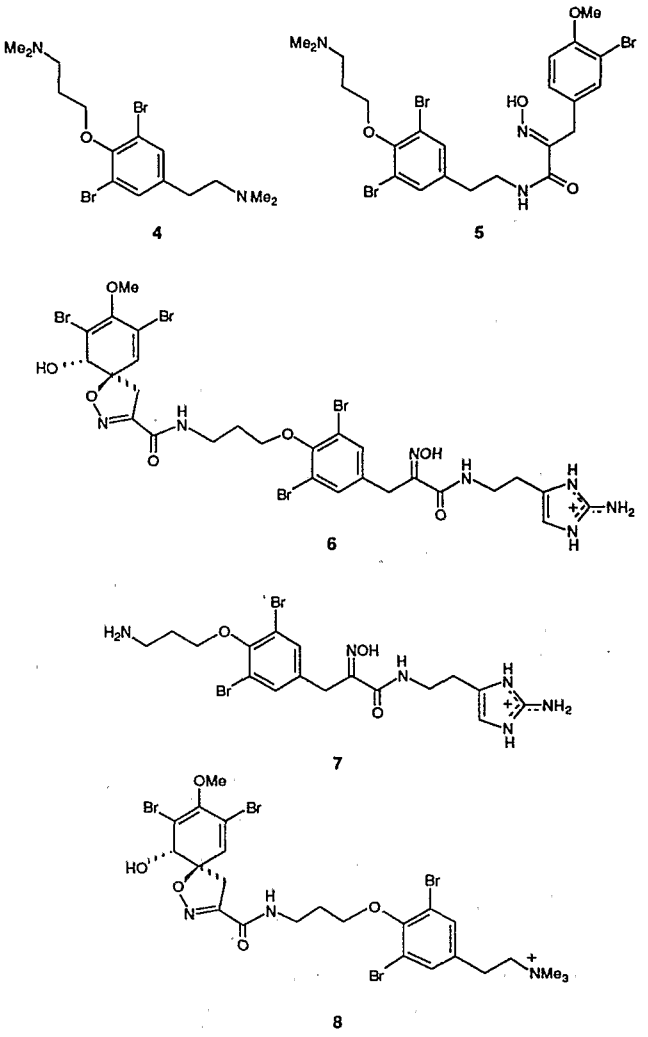
Pseudoceratinine C (**3**) exhibited an isotopic cluster of five MH^+ ions separated by 2 amu (ratio 1:4:6:4:1) centered at MH^+ 870 in the FABMS, indicating the presence of four bromine atoms. The molecular formula $C_{27}H_{32}Br_4N_7O_6$ was established by HRFABMS (*m/z* 869.9088, MH^+, Δ −1.9). Comparison of the NMR data of **3** (Table 1) with those of the known purealin **6**[3] showed similar signals for the C_1−C_{16} moiety and the aminohistamine residue (C_{19}−C_{24}), respectively. The only difference between the two alkaloids was an 18-$CHNH_2$ group in **3** instead of the 18-C=NOH group in **6**. In the ^{1}H-NMR spectrum, the NH_2 signal was observed as a broad singlet at δ_H 4.18. The CH-18 group appeared at δ_C 56.0 and δ_H 4.07 and was coupled in the COSY with CH_2-17 (δ_C 38.0 and δ_H 3.07). The HBMC experiment (Table 1), which displayed correlations from H-17 and H-18 to C-16 and C-19, further supported these assignments.

The absolute configuration at C-6 of compounds **1**−**3** was established as illustrated on the basis of the CD spectra, which showed negative Cotton effects near 250 and 290 nm.[3] To our knowledge, there is only one report of a bromotyrosine alkaloid containing a nonoxidized amino group. However, the absolute configuration of the α carbon has not been determined.[7] The stereochemistry of an aromatic amino acid such as tyrosine can be deduced from the Cotton effect observed in the region of 220 nm.[8] Both pseudoceratinines B (**2**) and C (**3**) exhibited positive Cotton effects at 212 and 215 nm, respectively, corresponding to an *S*-configuration of the tyrosine residue. The Me_3 group did not affect the CD results, inasmuch as **10**, which was obtained by treatment of 3,5-dibromo-L-tyrosine (**9**)[9] with MeI,[10] also exhibited a positive Cotton effect in the same region. Acid hydrolysis (HCl 6 N, 24 h, reflux) of pseudoceratinine B (**2**) did not yield *O*-methyl-3,5-dibromo-NMe_3-L-tyrosine (**10**), probably because of steric hindrance.

It is worthwhile to note that the alkaloids **4**−**8**, which have been found previously in two different species [i.e., aplysamine-1 (**4**) and aplysamine-2 (**5**) in *Aplysina* sp. and purealin (**6**) and purealidins A (**7**) and B (**8**) in *Psammaplysilla* (= *Pseudoceratina*) *purea*] were in this case all isolated from a sponge belonging to another species. The specimens of *Pseudoceratina verrucosa* studied here were shown to have a somewhat different metabolic composition, for pseudoceratinine A (**1**) was isolated only from one specimen and pseudoceratinine C (**3**) only from the other. However, this often occurs for specimens coming from different areas and has been observed previously for a species belonging to the same genus, *Pseudoceratina crassa*.[11]

Experimental Section

General Experimental Procedures. Optical rotations at 20 °C were determined on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Shimadzu UV-1616 UV−vis spectrophotometer. CD spectra were recorded on a Jobin-Yvon Mark 5; IR on Nicolet 205 FT-IR spectrometer; FABMS on a Kratos MS 80; HR-FABMS on a VG-Zab-Seq spectrometer; and NMR on Bruker AC 300 (^{1}H and ^{13}C spectra) and AM 400 (2D spectra) spectrometers. UV spectra were recorded in



MeOH. Column chromatography was performed using Si gel Merck H60.

Animal Material. The samples of *Pseudoceratina verrucosa* Bergquist (order Verongida, family Aplysinellidae) were collected at Ile Longue (Chesterfield Archipelago, 15 m depth, sponge I) and at Ile Walpole (8–10 m depth, sponge II) as part of the CNRS-ORSTOM program "Substances Marines d'Intérêt Biologique" (SMIB). Samples of the sponges I (ref R1576) and II (ref R 1593) were identified by P. R. Bergquist and C. Lévi and are preserved at ORSTOM, Nouméa, New Caledonia.

Extraction and Purification. The freeze-dried animal materal (225 g of sponge I and 200 g of sponge II) was extracted with 80% EtOH (3 × 2 L) at room temperature. After filtration, each solution was concentrated *in vacuo* to an aqueous suspension, which was extracted with CH_2Cl_2. The organic extract (4.8 g for I, 4.2 g for II) was subjected to Si gel column chromatography with CH_2Cl_2 containing increasing concentrations of MeOH (+ minor amounts of H_2O for the fraction eluted with CH_2Cl_2 containing more than 10% of MeOH). The fractions eluted with CH_2Cl_2/MeOH/H_2O (80:20:1) were further purified by column chromatography on Si gel, yielding aplysamine-2 (**5**) [eluent CH_2Cl_2/MeOH (95:5)] in yields of 30 mg from sponge I and 14 mg from sponge II. The aqueous layer (from the CH_2Cl_2 extract of each sponge) was extracted with *n*-BuOH. The organic layers were evaporated to give a crude residue (4.8 g for I; 4.2 g for II). The *n*-BuOH extracts (0.5 g for I, 0.75 g for II) were chromatographed on Sephadex LH 20 with CH_2Cl_2/MeOH (1:1), discarding the first and the last deep-colored fractions. The middle fractions were further chromatographed on Si gel using EtOAc/butanone containing increasing amounts of HCO_2H/H_2O (5:3:0.5:0.5 to 5:3:1.5:1.5). Sponge I yielded purealin A (**6**) (10 mg, eluent 5:3:0.5:0.5), purealidin B (**8**) (9 mg, 5:3:0.5:0.5), purealidin A (**7**) (11 mg, 5:3:1:1), pseudoceratinine B (**2**) (120 mg, 5:3:1:1), pseudoceratinine A (**1**) (26 mg, 5:3:1:1), and aplysamine-1 (**4**) (11 mg, 5:3:1.5:1.5). Sponge II yielded purealin A (**6**) (22 mg, eluent 5:3:0.5:0.5), purealidin B (**8**) (14 mg, 5:3:0.5:0.5), pseudoceratinine C (**3**) (45 mg, 5:3:1:1), purealidin A (**7**) (11 mg, 5:3:1:1), pseudoceratinine B (**2**) (120 mg, 5:3:1.5:1.5), and aplysamine-1 (**4**) (11 mg, 5:3:1.5:1.5). The MeOH solutions of the three alkaloids pseudoceratinins A (**1**), B (**2**), and C (**3**) were passed through a small column of Amberlite IR-45 (Cl^-) to yield the hydrochloride salts. Pseudoceratinine C HCl (**3**) was further purified on a C-18 Sep-Pak column (Waters) using MeOH/H_2O as eluent.

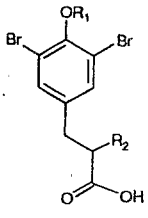
Pseudoceratinine A (**1**): $[\alpha]_D$ −158° (MeOH, *c* 1); UV λ max nm 220 (log ϵ 4.47) and 284 (log ϵ 4.08); IR ν max (KBr) 3400, 1680, 1543, 1437, 1381, 1250 cm^{-1}; CD (MeOH) λ ext 248 ($\Delta\epsilon$ − 9.66) and 290 ($\Delta\epsilon$ − 8.48); FABMS *m/z* 490, 492, 494 (MH^+); NMR see Table 1.

Pseudoceratinine B (**2**): $[\alpha]_D$ +17° (MeOH, *c* 1); UV 285 (log ϵ 3.17) and 330 (log ϵ 2.69); IR ν max (KBr) 3406, 1680, 1556, 1475, 1262 cm^{-1}; CD (H_2O) λ ext 212 ($\Delta\epsilon$ + 12.6); FABMS *m/z* 502, 504, 506 (MH^+); NMR see Table 1.

Pseudoceratinine C (**3**): $[\alpha]_D$ −10° (MeOH, *c* 1); UV 220 (log ϵ 4.54) and 228 (log ϵ 4.11); IR ν max (KBr) 3375, 1680, 1543, 1456, 1387, 1250 cm^{-1}; FABMS *m/z* 866, 868, 870, 872, 874 (MH^+); CD (H_2O) λ ext 215 ($\Delta\epsilon$ + 17.4), 255 ($\Delta\epsilon$ − 1.91) and 293 ($\Delta\epsilon$ − 2.44); NMR see Table 1.

3,5-Dibromo-L-tyrosine HBr (**9**):[9] CD (H_2O) λ ext 210 ($\Delta\epsilon$ + 15.3); ^1H NMR (MeOH) δ 7.45 (2H, s, H-4 and H-6), δ 4.25 (1H, dd, J = 5.5, 7; H-8), δ 3.15 (2H, ddd, J = 15, 5.5, 7; H-8).

***O*-Methyl-3,5-dibromo-NMe$_3$-L-tyrosine (10).** A stirred solution of 3,5-dibromotyrosine (0.17 g) in MeOH (10 mL) was treated with HCO_3K (0.50 g) and MeI (0.5 mL), and stirring was continued overnight. The solution was neutralized with concentrated HCl and evaporated to dryness. MeOH was added, and the MeOH-soluble material was passed through an Amberlite IR-120 column (2 mL). The column was eluted with 5% NH_4OH. The alkaline solution was evaporated and the residue (35 mg) was chromatographed on Si gel yielding **10** (15 mg) (eluent CH_2Cl_2/MeOH/H_2O 80:20:1); $[\alpha]_D$ +30° (MeOH, *c* 0.8) (lit.[10] $[\alpha]_D$ −8.3°), probably the



9 R_1 = H, R_2 = NH_2
10 R_1 = Me, R_2 = $\overset{+}{N}Me_3$

R-isomer]; FABMS *m/z* 394, 396, 398 (MH^+); CD (H_2O) λ ext 215 ($\Delta\epsilon$ + 7.10); ^1H NMR,[11] δ 3.75 (1H, dd, J = 12, 3.5; H-8, splitting pattern similar to that of H-8 of **2**, see Table 1).

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Note Added in Proof. During the preparation of this paper, the isolation from the sponge *Psammaplysilla purea* and the structure elucidation of compound **1**, though of reverse absolute configuration, named purealidin J, have been published: Kobayashi, J.; Honma, K.; Sasaki, T.; Tsuda, M. *Chem. Pharm. Bull.* **1995**, *50*, 403−407.

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