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Ceratinamides A and B: New Antifouling Dibromotyrosine Derivatives from the Marine Sponge *Pseudoceratina purpurea*

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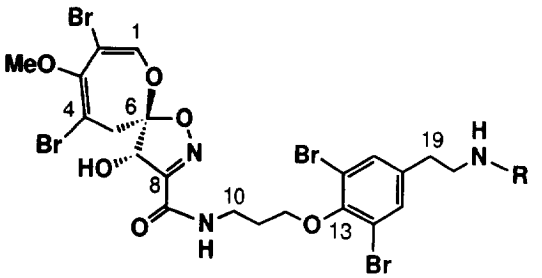
Abstract: Ceratinamides A and B (**1** and **2**), new bromotyrosine derivatives, have been isolated from the marine sponge *Pseudoceratina purpurea*, along with the known psammaplysins A (**3**) and E (**4**), ceratinamine (**5**), moloka'iamine (**6**), pseudoceratidine (**7**), and 4,5-dibromopyrrole-2-carbamide (**8**). Their structures were elucidated on the basis of spectral data. Compounds **1**-**7** inhibited larval settlement and metamorphosis of the barnacle *Balanus amphitrite* with ED_{50} values of 0.10-8.0 μg/mL, while compounds **3** and **8** induced larval metamorphosis of the ascidian *Halocynthia roretzi* at concentrations of 1.2 and 25 μg/mL, respectively. Compounds **5** and **6** also showed cytotoxicity against P388 murine leukemia cells with IC_{50} values of 3.4 and 2.1 μg/mL, respectively; **7** was antibacterial against *Flavobacterium marinotypicum*.

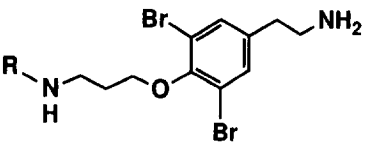
INTRODUCTION

Benthic organisms possess various defense systems against predators, larvae of other sessile organisms, and pathogenic microorganisms; hence their secondary metabolites might be potential nontoxic antifouling agents. During our ongoing program to discover antifouling substances in marine organisms, we found both antifouling[1a-1e] and metamorphosis-inducing[1f-1k] activities in the MeOH extract of the marine sponge *Pseudoceratina purpurea* collected off Hachijo-jima Isalnd, 300 km south of Tokyo. Bioassay-guided isolation resulted in isolation of antifouling bromotyrosine derivative, ceratinamine (**5**),[1e] and spermidine derivative, pseudoceratidine (**7**).[1c] Further examination of the MeOH extract led to the isolation of two new bromotyrosine derivatives named ceratinamides A and B (**1** and **2**), along with the known psammaplysins A (**3**)[2a] and E (**4**),[2b] moloka'iamine (**6**),[3] and 4,5-dibromopyrrole-2-carbamide (**8**).[4] These compounds showed various biological activities: antifouling activity (settlement and metamorphosis inhibitory activity) against the barnacle *Balanus amphitrite*, larval metamorphosis-inducing activity on the ascidian *Halocynthia roretzi*, cytotoxic activity against P388 murine leukemia cells, and antibacterial activity against *Flavobacterium marinotypicum*. This paper describes the isolation, structural elucidation, and biological activities of **1**-**8**.

RESULTS AND DISCUSSION

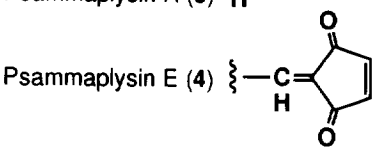
The ether soluble portion of the MeOH extract was sequentially fractionated by silica gel column chromatography ($MeOH/CHCl_3$), gel-filtration on Toyopearl HW-40 ($MeOH/CH_2Cl_2$), and reverse phase (C_{18}) HPLC (CH_3CN/H_2O/TFA) to afford ceratinamine[1e] (**5**, 2.7 mg, 1.2×10^{-3} % based on wet weight), ceratinamides A (**1**, 2.4 mg, 1.1×10^{-3} %), B (**2**, 11.1 mg, 5.0×10^{-3} %), moloka'iamine (**6**, 2.4 mg, 1.1×10^{-3} %), psammaplysins A (**3**, 13.5 mg, 3.1×10^{-2} %) and E (**4**, 5.4 mg, 2.4×10^{-3} %), pseudoceratidine[1c] (**7**, 12.2 mg, 2.1×10^{-4} %), and 4,5-dibromopyrrole-2-carbamide (**8**, 6.1 mg, 5.1×10^{-3} %). Compounds **3**, **4**, **6**, and **8** were readily identified by comparison of spectral data with those in the literature.[2-4]



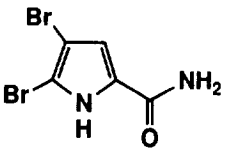


Ceratinamine (**5**) R = COCN
Moloka'iamine (**6**) R = H

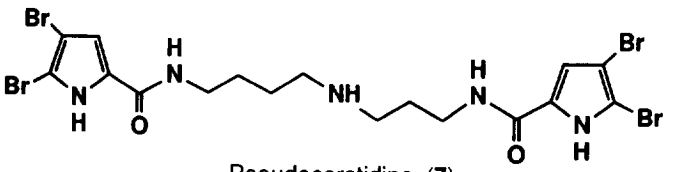
Ceratinamide A (**1**) R = CHO
Ceratinamide B (**2**) R = $CO(CH_2)_{11}CHMe_2$
Psammaplysin A (**3**) R = H



Psammaplysin E (**4**)



4,5-Dibromopyrrole
-2-carbamide (**8**)



Pseudoceratidine (**7**)

The ^1H and ^{13}C NMR spectra of ceratinamide A (**1**) were almost superimposable on those of psammaplysin A (**3**),[2a] except for signals of an aldehyde [δ_H 7.80 (1H, s, H-21) and δ_C 163.8 (d, C21)], which was supported by a molecular fornula of $C_{22}H_{23}Br_4N_3O_7$ corresponding to **5**+CO as determined by HRFABMS [*m/z* 783.8152, Δ +2.6 mmu, $(M+Na)^+$ for $C_{22}H_{23}{}^{79}Br_2{}^{81}Br_2N_3O_7Na$]. The aldehyde would likely be linked to the terminal amine, which was verified by HMBC corss peaks, δ 7.80/δ 40.0 (t, C20) and δ 3.44 (2H, t, *J*=7.1 Hz, H_2-20)/δ 163.8. Thus, **1** was *N*-formylpsammaplysin A.

Ceratinamide B (**2**) had a molecular formula of $C_{35}H_{51}Br_4N_3O_7$, one oxygen less than psammaplysin D.[2b] The ^1H and ^{13}C NMR spectra of **2** were superimposable on those of psammaplysin D, except for the absence of an oxymethine. A 19-deoxyl structure was secured by a methylene signal in the ^1H NMR spectrum [δ 2.72 (2H, t, *J*=6.8 Hz, H_2-19)] and 2D NMR data including an HMBC experiment, thus **2** was 19-deoxypsammaplysin D.

Ceratinamides A and B (**1** and **2**), psammaplysins A and E (**3** and **4**), ceratinamine (**5**) and moloka'iamine (**6**), and psudoceratidine (**7**) showed antifouling activity (settlement and metamorphosis inhibitory activity) against cyprid larvae of the barnacle *Balanus amphitrite* with ED_{50} values ranging from 0.10 to 8.0 μg/mL as summarized in Table 1. Ceratinamide A (**1**) and psammaplysin A (**3**) were particularly potent. Compounds **2**, **3**, **5**, and **7** were lethal to the larvae at a concentration of 30 μg/mL, while others were not toxic at this concentration. Thus, **1** is a promising antifouling agent. Interestingly, psammaplysin A (**3**) and 4,5-dibromopyrrole-2-carbamide (**8**) induced larval metamorphosis of the ascidian *Halocynthia roretzi*. Psammaplysin E (**4**), ceratinamine (**5**), and moloka'iamine (**6**) also exhibited potent cytotoxicity against P388 murine leukemia cells, while psammaplysin A (**3**) and pseudoceratidine (**7**) were antibacterial against *Flavobacterium marinotypicum*.

Table 1. Biological Activities of Compounds **1**-**8**

Compounds	Metamorphosis inducing activity on ascidian *Halocynthia roretzi*, ED_{100} (μg/mL)	Antifouling activity against barnacle *Balanus amphitrite*, ED_{50} (μg/mL)	Antibacterial activity against *Flavobacterium marinotypicum*, halo (mm)[a]	Cytotoxic activity against P388 cell, IC_{50} (μg/mL)
Ceratinamide A (**1**)	-	0.10	-	>10
Ceratinamide B (**2**)	-	2.4	-	>10
Psammaplysin A (**3**)	1.2	0.27	10	>10
Psammaplysin E (**4**)	-	4.8	-	2.1
Ceratinamine (**5**)	-	5.0	-	3.4
Moloka'iamine (**6**)	-	4.3	-	2.1
Pseudoceratidine (**7**)	-	8.0	15	>10
4,5-Dibromopyrrole -2-carbamide (**8**)	25	>30	-	>10

[a]Each 10 μg of sample was put on a disk.

CONCLUSION

Marine sponges of the order Verongida are a rich source of bromotyrosine-derived metabolites, some of which are cytotoxic,[2, 4] antibacterial,[2, 5] *etc*. This, however, is the first report of antifouling activity against barnacle larvae and larval metamorphosis-inducing activity in ascidian. Interestingly, psammaplysin A (**3**) showed both metamorphosis-inducing activity on the ascidian larvae (ED_{100} of 1.2 μg/mL) and settlement and metamorphosis inhibitory activity against the barnacle larvae (ED_{50} of 0.27 μg/mL). Although **3** and **7** showed moderate antibacterial activity against *Flavobacterium marinotypicum*, **1-8** were inactive against *Alteromonus macleodii, Pseudomonas nautica, Vibrio alginolyticum, Bacillus marinus, Penicillium chrysogenum, Candida albicans*, and *Mortierella ramanniana* at 10 μg/disk, suggesting that antifouling activity are not associated with antibacterial or antifungal activity.

Aerothionin and homoaerothionin,[6a] which contain a spiro[4.5]oxazadecane skeleton and are biogenetically related to the spiro[4.6]dioxazundecane skeleton in ceratinamides A and B (**1** and **2**) and in the psammaplysins,[2] are reportedly secreted from the sponge *Aplysina fistularis*.[6b, 6c] Hence the compounds reported here may also play defensive roles against settling of benthic organisms.

EXPERIMENTAL

General.

Optical rotations were determined with a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Hitachi U-2000 spectrometer in EtOH. Infrared sepctra were measured on a JASCO IR-700 spectrometer. NMR spectra were recorded on a Bruker ARX-500 NMR spectrometer at 27 °C in MeOH-d_4 or chloroform-*d*. Residual CHD_2OD (3.30 ppm), CD_3OD (49.0 ppm), $CHCl_3$ (7.26 ppm), and $CDCl_3$ (77.0 ppm) signals were used as internal standard. FAB mass spectra were measured on a JEOL SX-102 mass spectrometer.

Antifouling assay.

Cyprid larvae were obtained by the method previously reported.[7] Samples dissolved in EtOH were applied into each well of 24-well polystyrene plates. After removal of the solvent, 2 mL of 80 % seawater and six one-day-old cyprid larvae were added to each well, and the plates were incubated for 48 h at 25 °C in the dark. The number of settled and metamorphosed larvae was counted under a microscope.

Metamorphosis-inducing assay.[1g]

Fifteen newly hatched larvae and 4 mL of artificial seawater (460 mM NaCl, 10.1 mM KCl, 9.2 mM $CaCl_2$, 35.9 mM $MgCl_2 \cdot 6H_2O$, 17.5 mM $MgSO_4 \cdot 7H_2O$, 10 mM Tris-HCl, pH 8.2) were added to each well of 12-well polystyrene plates. The sample to be assayed was added to the larvae in DMSO solution (10 μL), and to the control group 10 μL of DMSO was added. The plates were incubated for 10 h at 13.2 °C in the dark. The number of settled and metamorphosed larvae was counted under a microscope. No larvae in the control group underwent metamorphosis.

Antibacterial and antifungal assay.

Antibacterial and antifungal activities were tested by the paper disk method. Paper disk (thick, 8 mm, Toyo Roshi Kaisha, Ltd, Tokyo) impregnated with 10 μg of sample was placed on an agar plate containing bacterium or fungus, and the plate was incubated for 24 h at 28 °C.

Isolation of 1-8.

Sponge samples (570 g) were collected by hand using scuba at a depth of 3 m off Hachijo-jima Island. The sample was identified as *Pseudoceratina purpurea* by Dr. Rob van Soest. A voucher specimen (ZMA POR. 11020) was deposited at the Institute for Systematics and Population Biology of University of Amsterdam. The frozen sponge (570 g) was extracted with MeOH (1 L x 3). The extract was cocentrated under reduced pressure and partitioned between H_2O and ether (0.8 L x 3). The fractions were monitored by antifouling activity against barnacle larvae, metamorphosis-inducing activity on ascidian larvae, and antibacterial activity against *Flavobacterium marinotypicum*: the ether fraction (4.31 g) was more active than the aqueous fraction (32.13 g). Part (1.69 g) of the ether fraction was subjected to silica gel column chromatography (Wakogel C-300, 2.2 x 40 cm, Wako Purechemical Industries, Ltd.) using $CHCl_3$~50 % MeOH/$CHCl_3$. The active 10 % MeOH/$CHCl_3$ fraction (0.39 g) was purified by silica gel column chromatography (2.2 x 40 cm) using acetone-hexane (1:1) followed by gel-filtration on Toyopearl HW-40 (2.2 x 40 cm, Tosoh Corporation) with MeOH/CH_2Cl_2 (1:1) to afford ceratinamides A (**1**, 2.4 mg, 1.1 x 10^{-3} % wet weight) and B (**2**, 11.1 mg, 5.0 x 10^{-3} %) and psammaplysin E (**4**, 5.4 mg, 2.4 x 10^{-3} %). The active 20 % MeOH/$CHCl_3$ eluate from the first column (0.70 g) was purified by gel-filtration on Toyopearl HW-40 (2.2 x 40 cm) with MeOH/CH_2Cl_2 (1:1) and HPLC (Develosil ODS-HG-5, 5 μM, 10 x 250 mm, Nomura Chemical, Co., Ltd.) using 50 % CH_3CN/H_2O (0.01 % TFA) to afford psammaplysin A (**3**, 13.5 mg, 3.1 x 10^{-2} %), ceratinamine (**5**, 2.7 mg, 1.2 x 10^{-3} %), moloka'iamine (**6**, 2.4 mg, 1.1 x 10^{-3} %), pseudoceratidine (**7**, 12.2 mg, 2.1 x 10^{-4} %), and 4,5-dibromopyrrole-2-carbamide (**8**, 6.1 mg, 5.1 x 10^{-3} %).

1: colorless solid. $[\alpha]_D^{24}$ -89.7° (*c* 0.146, MeOH). IR ν_{max} (film) 3300, 2930, 1660, 1530, 1450, 1380, 1260, 1110, and 760 cm^{-1}. UV λ_{max} (EtOH) 207 (ε 64200), 218 (sh, 28900), and 255 nm (sh, 11000). ^1H NMR (MeOH-d_4) δ 2.13 (2H, tt, *J*=6.0 and 6.8 Hz, H_2-11), 2.77 (2H, t, *J*=7.1 Hz, H_2-19), 3.07 (1H, d, *J*=16.1 Hz, H-5), 3.39 (1H, d, *J*=16.1 Hz, H-5), 3.44 (2H, t, *J*=7.1 Hz, H_2-20), 3.62 (2H, t, *J*=6.8 Hz, H_2-10), 3.65 (3H, s, OMe), 4.07 (2H, t, *J*=6.0 Hz, H_2-12), 4.99 (1H, s, H-7), 7.14 (1H, s, H-1), 7.48 (2H, s, H-15 and H-17), and 7.80 (1H, s, H-21). ^{13}C NMR (MeOH-d_4) δ 30.6 (t, C11), 35.0 (t, C19), 38.0 (t, C10), 38.3 (t, C5), 40.0 (t, C20), 59.3 (q, OMe), 72.1 (t, C12), 80.4 (d, C7), 104.4 (s, C2), 104.6 (s, C4), 119.0 (2C, s, C14 and C18), 120.9 (s, C6), 134.3 (2C, d, C15 and C17), 139.7 (s, C16), 146.8 (d, C1), 149.9 (s, C3), 152.9 (s, C13), 158.8 (s, C8), 160.7 (s, C9), and 163.8 (d, C21). FABMS (positive, glycerol matrix) *m/z* 758/760/762/764/766 $(M+H)^+$ and 780/782/784/786/788 $(M+Na)^+$. HRFABMS (positive, glycerol matrix) *m/z* 783.8152 (calcd for $C_{22}H_{23}{}^{79}Br_2{}^{81}Br_2N_3O_7Na$, Δ +2.6 mmu).

2: colorless solid. $[\alpha]_D^{24}$ -53.5° (*c* 0.263, acetone). IR ν_{max} (film) 3320, 2920, 2850, 1650, 1540, 1450, 1250, and 1120 cm^{-1}. UV λ_{max} (EtOH) 207 (ε 51100), 219 (sh, 22700), and 255 nm (sh, 9000). ^1H NMR ($CDCl_3$) δ 0.83 and 0.85 (each 3H, t, *J*=6.7 Hz, H_3-34 and H_3-35), 1.2-1.3 (19H, H_{19}-24~33), 1.59 (2H, m, H_2-23), 2.08 (2H, tt, *J*=6.2 and 5.6 Hz, H_2-11), 2.11 (2H, t, *J*=7.7 Hz, H_2-22), 2.72 (2H, t, *J*=6.8 Hz, H_2-19), 3.10 and 3.35 (each 1H, d, *J*=16.0, H-5), 3.43 (2H, dt, *J*=6.4 and 6.8 Hz, H_2-20), 3.67 (3H, s, OMe), 3.71 (2H, dt, *J*=6.4 and 6.2 Hz, H_2-10), 3.77 (1H, br.s, OH), 4.07 (2H, t, *J*=5.6 Hz, H_2-12), 5.11 (1H, s, H-7), 5.46 (1H, br.s, NHC(21)O), 7.00 (1H, s, H-1), 7.18 (1H, br.s, NHC(9)O), and 7.32 (2H, s, H-15 and H-17). ^{13}C NMR ($CDCl_3$) δ 22.6 (2C, q, C34 and C35), 25.8 (t, C23), 29.2 (t, C11), 29.3-29.7 (10C, C24~C33), 34.5 (t, C19), 36.8 (t, C22), 37.3 (t, C10), 40.3 (t, C20), 59.1 (q, OMe), 71.0 (t, C12), 79.4 (d, C7), 103.4 (s, C4), 105.4 (s, C2), 118.1 (2C, s, C14 and C18), 122.1 (s, C6), 132.9 (2C, d, C15 ad C17), 138.1 (s, C16), 145.4 (d, C1),148.7 (s, C3), 151.3 (s, C13), 155.8 (s, C8), 159.0 (s, C9), and 173.3 (s, C21). FABMS (positive, NBA matrix) *m/z* 954/956/958/960/962 $(M+H)^+$ and 976/977/980/982/984 $(M+Na)^+$. HRFABMS (positive, NBA matrix) *m/z* 980.0320 (calcd for $C_{36}H_{51}{}^{79}Br_2{}^{81}Br_2N_3O_7Na$, Δ -0.4 mmu).

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REFERENCES AND NOTES

1. a) Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1995**, *36*, 8637-8640.
   b) Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359-2368.
   c) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 1439-1440.
   d) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Nat. Prod.* in press.
   e) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Org. Chem.* in press.
   f) Tsukamoto, S.; Hirota, H.; Kato, H.; Fusetani, N. *Tetrahedron Lett.* **1993**, *34*, 4819-4822.
   g) Tsukamoto, S.; Hirota, H.; Kato, H.; Fusetani, N. *Experientia* **1994**, *50*, 680-683.
   h) Tsukamoto, S.; Hirota, H.; Kato, H.; Fusetani, N. *Fisheries Sci.* **1994**, *60*, 319-321.
   i) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 5873-5874.
   j) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1994**, *50*, 13583-13592.
   k) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6687-6694.
2. a) Roll, D. M.; Chang, C. W. J.; Scheuer, P. J.; G. A. Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 2916-2920.
   b) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 4149-4150.
   c) Copp, B. R.; Ireland, C. M.; Barrows, L. R. *J. Nat. Prod.*, **1992**, *55*, 822-823.
3. Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 6565-6569.
4. Forenza, S; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129-1130.
   8: ^1H NMR (DMSO-d_6) δ 6.80 (1H, s, H-2), 12.74 (2H, br. s, NH_2), and 12.94 (1H, br.s, NH). ^{13}C NMR (DMSO-d_6) δ 98.6 (s, C4), 106.5 (s, C5), 116.6 (d, C3), 125.1 (s, C2), and 160.2 (s, CO).
5. Yagi, H.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 3749-3754.
6. a) Moody, K.; Thompson, R. H.; Fattorusso, E.; Minale, L.; Sodano, G. *J. Chem. Soc. Perkin Trans. 1* **1972**, 18-24.
   b) Thompson, J. E. *Mar. Biol.* **1985**, *88*, 23-26.
   c) Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *Mar. Biol.* **1985**, *88*, 27-32.
7. Yamamoto, H.; Tachibana, A.; Matsumura, K.; Fusetani, N. *Zool. Sci.* **1995**, *12*, 391-396.

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