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# Pseudoceratidine: A New Antifouling Spermidine Derivative from the Marine Sponge *Pseudoceratina purpurea*

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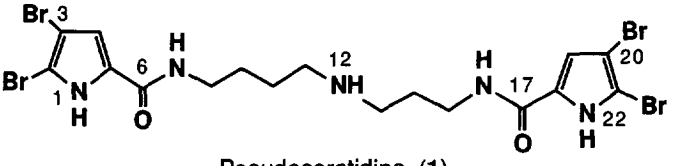
***Abstract:*** A new spermidine with two 4,5-dibromopyrrole-2-carbamyl units, pseudoceratidine (**1**), has been isolated from the marine sponge *Pseudoceratina purpurea* as an antifouling compound. Its structure was elucidated on the basis of spectral data. Compound **1** inhibited larval settlement and metamorphosis of the barnacle *Balanus amphitrite* with an $ED_{50}$ value of 8.0 μg/mL.

Since sessile marine organisms are widely believed to possess chemical defense systems against predators, larvae of other sessile organisms, and pathogenic microorganisms, their secondary metabolites might be potential nontoxic antifouling agents.[1] In the course of our search for antifouling compounds from Japanese marine invertebrates, we have isolated from the marine sponge *Acanthella cavernosa* five diterpenes[2] which inhibit larval settlement and metamorphosis of the barnacle *Balanus amphitrite*. Subsequently, we found the antifouling activity against *B. amphitrite* in the MeOH extract of the marine sponge *Pseudoceratina purpurea* collected off Hachijo-jima Isalnd, Japan. Bioassay-guided isolation afforded a new spermidine derivative containing two 4,5-dibromopyrrole-2-carbonyl units, pseudoceratidine (**1**). This paper describes the isolation, structural elucidation, and biological activities of this new compound.

The ether soluble portion of the MeOH extract of the sponge was successively fractionated by silica gel column chromatography (MeOH-$CHCl_3$), gel-filtration on Toyopearl HW-40 (MeOH), and reverse phase HPLC ($CH_3CN$-$H_2O$-TFA) to afford pseudoceratidine (**1**)[3] (yield: 2.1 x $10^{-4}$ % wet weight).

Pseudoceratidine (**1**) had a molecular formula of $C_{17}H_{21}Br_4N_5O_2$ as determined by the HRFAB mass spectrum [*m/z* 647.8467, Δ +0.1 mmu $(M+H)^+$ for $C_{17}H_{22}{}^{79}Br_2{}^{81}Br_2N_5O_2$]. The $^1H$ NMR spectrum in DMSO-$d_6$ exhibited seven methylene groups [δ 1.52, 1.58, 1.78, 3.21, and 3.26 (each 2H, $H_2$-9, 10, 14, 8, and 15, respectively) and 2.90 (4H, $H_2$-11 and 13)], two aromatic protons [δ 6.91 (2H, s, H-4 and 19)], and five NH protons [δ 8.15 (t, *J*=5.5 Hz, H-7), 8.25 (t, *J*=5.5 Hz, H-16)], 8.36 (br. s, H-12), and 12.65 (2H,




Pseudoceratidine (**1**)

br. s, H-1 and 22)]. Interpretation of the COSY spectrum led to two alkyl amine units, N(7)H$(CH_2)_4$ and N(16)H$(CH_2)_3$. The remaining portion, $C_{10}H_5Br_4N_3O_2$ contained four pairs of quaternary carbons [δ 97.7/97.8 (C3, C20), 104.4/104.7 (C2, C21), 127.9/128.2 (C5, C18), and 158.9 (C6)/159.1 (C17)] and a pair of $sp^2$ doublet carbons [δ 112.5/112.7 (C4, C19)], reminiscent of two 4,5-dibromopyrrole-2-carbamyl units, which was supported by HMBC cross peaks, H-4/C-2 and C-5, H-7/C6, and H-19/C18 and C21, as well as by NOE cross peaks, H-4/H-7 and H-16/H-19. Consequently, the two alkyl amine units, N7~C11 and C13~N16, and the remaining NH were incorporated into a spermidine unit, thereby constructing the gross structure of **1** as shown.

Pseudoceratidine (**1**) inhibited larval settlement and metamorphosis of the barnacle *Balanus amphitrite* with an $ED_{50}$ value of 8.0 μg/mL, while it was lethal to larvae at concentrations greater than 30 μg/mL.[4] Bromopyrrole derivatives including oroidin[5] and sceptorin[6] have been isolated from several marine sponges, some of which were reported to have weak antifouling activity.

Although spermidine is widely distributed in both plants and animals, only a few spermidine derivatives have been isolated from marine organisms so far: spermidine monoamides from soft corals *Sinularia brongersmai*[7a] and *Sinularia* sp.,[7b] polycyclic guanidine alkaloides from marine sponge *Ptilocaulis spiculifer*,[7c] and a steroid from the shark *Squalus acanthius*.[7d, 7e] These compounds showed antimicrobial, antifungal, or cytotoxic activities. This is the first example of an antifouling spermidine derivative.

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**References and Notes**

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3. 1: Colorless solid. IR $\nu_{max}$ (film) 3250 and 1620 $cm^{-1}$. UV $\lambda_{max}$ (EtOH) 208 (sh, ε 13300) and 275 nm (18400). $^1H$ NMR (DMSO-$d_6$) δ 1.52 (2H, tt, *J*=7.5 and 6.5 Hz, $H_2$-9), 1.58 (2H, tt, *J*=7.5 and 6.5 Hz, $H_2$-10), 1.78 (2H, tt, *J*=7.5 and 6.2 Hz, $H_2$-14), 2.90 (4H, t, *J*=7.5 Hz, $H_2$-11 and $H_2$-13), 3.21 (2H, dt, *J*=5.5 and 6.5 Hz, $H_2$-8), 3.26 (2H, dt, *J*=5.5 and 6.2 Hz, $H_2$-15), 6.91 (2H, s, H-4 and H-19), 8.15 (1H, t, *J*=5.5 Hz, H-7), 8.25 (1H, t, *J*=5.5 Hz, H-16), and 8.36 (1H, br. s, H-12), and 12.65 (2H, br. s, H-1 and H-22). $^{13}C$ NMR (DMSO-$d_6$) δ 23.1 (t, C10), 26.1 (t, C9), 26.2 (t, C14), 35.7 (t, C15), 37.8 (t, C8), 44.8 (t, C11), 46.6 (t, C13), 97.7 (s, C3[a]), 97.8 (s, C20[a]), 104.4 (s, C2[b]), 104.7 (s, C21[b]), 112.5 (d, C4[c]), 112.7 (d, C19[c]), 127.9 (s, C5[d]), 128.2 (s, C18[d]), 158.9 (s, C6), and 159.1 (s, C17) ([a-d]May be interchangeable). HMBC correlations: H-4/C-2 and C-5; H-7/C6; H-19/C18 and C21. FABMS (positive, glycerol matrix) *m/z* 666/668/670/672/674 $(M+Na)^+$, 644/646/648/650/652 $(M+H)^+$, and 566/568/570/572 $(M+H-Br)^+$. HRFABMS (positive, glycerol matrix) *m/z* 647.8467 (calcd for $C_{17}H_{22}{}^{79}Br_2{}^{81}Br_2N_5O_2$, Δ +0.1 mmu).
4. The bioassay procedure will be reported somewhere else. $CuSO_4$: $EC_{50}$ 0.15 μg/mL; $LD_{50}$ 0.40 μg/mL.
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