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# A Convenient Synthesis of Pseudoceratidine and Three Analogs for Biological Evaluation.

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**Abstract:** The recently isolated marine natural product pseudoceratidine (1) has been synthesized from 2-trichloroacetylpyrrole. Bromination in the 4- and 5-position followed by nucleophilic displacement of the trichloromethyl group with spermidine gave 1 in 79 % yield. The procedure is general and can easily be adopted to the preparation of other derivatives. This was demonstrated by the synthesis of a 5,5'-didebromo derivative (2) and two analogs (3-4). The compounds 1-4 have been tested for antibacterial activity and the results compared to a previous study. Also activity against the marine brine shrimp *Artemia salina* is reported. © 1997, Elsevier Science Ltd. All rights reserved.

Antifouling agents are widely used as additives to paints for hulls of ships, cooling systems of power plants, and other marine facilities. Today most antifouling paint is based on organostannanes, which apart from being lethal towards invertebrate marine organisms as intended, are also suspected to be poisonous to higher vertebrates. Therefore, at present many resources are allocated to discover alternative antifouling agents devoid of such side effects.

Recently a new natural compound, pseudoceratidine (1), was isolated from the marine sponge *Pseudoceratina purpurea*.<sup>1</sup> Pseudoceratidine exhibits a significant antifouling activity against *Balanus amphitrite* larvae and might be a useful alternative for the stannanes.



Pseudoceratidine (1)

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The structural simplicity of pseudoceratidine has prompted us and others<sup>2</sup> to synthesize a series of analogs in order to locate the functionalities responsible for the biological activity. The antifouling activity was tested against *Artemia salina*. Since bacteria are the first organisms in the fouling community the compounds were also tested against 5 different bacterial species.

### Synthesis:

Apart from being a spermidine derivative, pseudoceratidine, along with a range of other marine natural products like oroidin<sup>3</sup> and dispacamid,<sup>4</sup> contains the 4,5-dibromopyrrole-2-carbamoyl unit. This moiety can easily be obtained from commercially available 2-trichloroacetyl pyrrole<sup>5</sup> by dibromination. Thus, 2-trichloroacetylpyrrole was dissolved in chloroform, cooled to  $0^{\circ}$ , and 2 equivalents of bromine were added (Scheme 1). After 1 h at  $0^{\circ}$ , 4,5-dibromo-2-trichloroacetylpyrrole (**6**) was isolated in good yield (70 % after recrystallisation from ethanol/water). Subsequently **6** was added to a solution of spermidine in tetrahydrofuran and left at room temperature for 48 h. After standard work-up including flash chromatography<sup>6</sup> a 79 % yield of **1** was obtained as a white solid. The reaction takes place preferentially at the primary amino groups, leaving the secondary amine free. Similarly, **3** and **4** were obtained in 82% and 63 % yield from *N*, *N*-bis(3-aminopropyl)amine and triethylenetetramine, respectively. A 5,5'-didebromo derivative (**2**) was obtained from 4-bromo-2-trichloroacetyl pyrrole<sup>7</sup> (**5**) and spermidine in 87 % yield.





Pseudoceratidine (1) exhibited spectral data identical to those previously reported<sup>1</sup> with the sole exception, that the two aromatic protons on the two chemically very similar pyrrole rings

were observed as distinct singlets at 6.77 ppm and 6.82 ppm.<sup>8</sup> The other analogs had spectral data in agreement with the assigned structures.<sup>8</sup>

#### Biological activity:

The effect on bacterial growth<sup>9</sup> of compounds **1-4** was tested on two Gram-positive strains, *Staphylococcus aureus* and *Listeria monocytogenes*, and three Gram-negative strains *Pseudomonas aeruginosa, Escherichia coli* and *Serratia liquefaciens* (Table 1).

	Temperature, °C	Media <sup>a)</sup>	MIC (µg/ml) of compounds 1-4			
Bacterial species			1	2	3	4
Staphylococcus aureus	37	TSB	5	100	10	50
Listeria monocytogenes	37	TSB	5	250	10	50
Pseudomonas aeruginos	sa 37	TSB	250	>500	100	>500 <sup>c</sup> )
Escherichia coli	37	TSB	50	250	50	100
Serratia liquefaciens	25	LB	>500	>500	>500 <sup>b</sup> )	>500°)

 Table 1
 Minimal inhibitory concentration (MIC)<sup>9</sup> of compound 1-4 against 5 bacteria.

a) Culture conditions: S. aureus, L. monocytogenes, Ps. aeruginosa and E. coli were pre-cultured in Tryptone Soy Broth, TSB (Oxoid CM 129) at 37 $^{\circ}$ C for 24 hours. S. liquefaciens was pre-cultured in LB Broth, Miller (Difco 0446-17-3) for 24 hours at 25  $^{\circ}$ C.

b) Growth delayed by concentrations  $\geq 100 \ \mu g/ml$ 

c) Growth delayed by concentrations  $\geq$  50 µg/ml and almost totally inhibited by 500 µg/ml

As can be seen from the table pseudoceratidine (1) is, in general, more active than 2. This clearly indicates, that the 5-bromo atom on the pyrrole-2-carbamoyl unit is important for the biological activity. Only small changes in biological activity are observed for 3 where the chain length between the two heterocycles is shortened by one atom. Analog 4 has the same distance between the pyrrole carbamoyl units as pseudoceratidine. Nevertheless, it is 10 fold less active against the two Gram-positive bacteria *S. aureus* and *L. monocytogenes* indicating, that the position of nitrogen in the spacer is of importance. In a similar study recently published by J. A. Ponasik *et. al.*<sup>2</sup> it was demonstrated, that analogs lacking either of the two 4,5-dibromopyrrole-2-carbamoyl units significantly reduced the bacteriostatic activity, and that a discrimination between the two units exist. This is in agreement with the diminished activity observed for compound 3. In general, the same sequence of antibacterial activity for compound 1-4 was observed as in Ponasik's study, i.e. *S. aureus* > *E. coli* > *P. aeruginosa*.

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Compounds 1-3 have also been tested for activity against the marine nauplii of the brine shrimp *Artemia salina* (Figure 1). While pseudoceratidine was lethal to the organisms, 5,5'-didebromo-pseudoceratidine and analog 3 showed almost no activity. The fact, that analog 3 behaves different when exposed to either bacteria or *Artemia* suggests that a different mode of action is operative in the two classes of organisms.



Figure 1. Concentration-response curve depicting the effect of pseudoceratidine (1), 5,5'-didebromopseudoceratidine (2) and the symmetric analog 3 on the brine shrimp Artemia salina. The shrimps were exposed to the compounds (1-3) for 24 h at room temperature in artificial sea water (460 mM NaCl, 10.1 mM KCl, 9.2 mM CaCl<sub>2</sub>,35.9 mM MgCl<sub>2</sub>• 6H<sub>2</sub>O, 17.5 mM MgSO<sub>4</sub>•7H<sub>2</sub>O, 10 mM Tris-HCl, pH 8.2) and the amount of dead animals counted. The sharp bend observed for pseudoceratidine (1) at doses higher than 20 mg/L reflects a lack of solubility in sea water at high concentration. For the control values between 1-2 % were recorded.

In conclusion, the 5'-bromo atoms on the pyrrole-carbamoyl units, as well as the length and composition of the inter-pyrrole spacer, are important for the activity of pseudoceratidine.

#### **References and notes:**

- \* To whom correspondance should be addressed.
- 1. Tsukamoto, S.; Kato, H.; Hirota, H. and Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 1439-1440.
- 2. Ponasik, J. A.; Kassab, D. J. and Ganem, B. Tetrahedron Lett. 1996, 37, 6041-6044.
- (a) Walker, R. P. and Faulkner, D. J. J. Am. Chem. Soc. 1981, 103, 6772-6773.
   (b) Garcia, E.E.; Benjamin, L.E. and Fryer, R.I. J. Chem. Soc., Chem. Commun. 1973, 78-79. (c) Forenza, S.; Minale, L. and Ricco, R. J. Chem. Soc., Chem. Commun. 1971, 1129-1130.

- 4. Cafieri, F.; Fattorusso, E.; Mangoni, A. and Taglialatela-Scafati, O. *Tetrahedron Lett* **1996**, *37*, 3587-3590.
- 5. Aldrich-Chemie GmbH & Co. KG, Riedstrasse 2, D-89555 Steinheim, Germany.
- 6. Silica G60, 10 % triethyl amine / 20 % methanol / 70 % chloroform.  $R_f = 0.4$ . After evaporation of solvent an oil was obtained. This was dissolved in a minimum amount of ethyl acetate and added to cold hexane to precipitable the pure compound.
- 7. Bélanger, P. Tetrahedron Lett. 1979, 27, 2505-2508.
- 8. <u>Pseudoceratidine:(1):</u>

Yield: 79 %. FABMS (positiv, m-NBA matrix): m/z 644/646/648/650/652 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR (DMSO- $d_6$ ): 1.50 ppm (m, 4H, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-); 1.67 (t, 2H, J = 7 Hz, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>- ); 2.68 (t, 4H, J = 7 Hz, -CH<sub>2</sub>NHCH<sub>2</sub>-); 3.22 (m, 4H, -CONHCH<sub>2</sub>-); 6.77 (s, 1H, H<sup>3</sup>-pyrrole); 6.82 (s, 1H, H<sup>3</sup>-pyrrole); 8.00 (t, 1H, J = 7 Hz,-CONHCH<sub>2</sub>-); 8.02 (t, 1H, J = 7Hz,-CONHCH<sub>2</sub>-).

<sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): 25.37 ppm; 26.94; 28.25; 36.19; 38.14; 45.96; 48.00: 96.30;
96.85; 105.53; 106.45; 112.34; 112.44; 129.14; 129.37; 159.63; 160.42.

5,5'-Didebromopseudoceratidine(2):

Yield: 87 %. FABMS (positiv, m-NBA matrix): m/z 488/490/492 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR (DMSO- $d_6$ ): 1.46 ppm (m, 4H, -CH<sub>2</sub>C<u>H<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-); 1.62</u> (t, 2H, J = 7 Hz, -CH<sub>2</sub>C<u>H<sub>2</sub>CH<sub>2</sub>-); 2.52</u> (m, 4H, -C<u>H<sub>2</sub>NHCH<sub>2</sub>-); 3.21</u> (dt, 2H, J = 7 Hz and J = 6 Hz, -CONHC<u>H<sub>2</sub>-); 3.36</u> (dt, 2H, J = 7 Hz and J = 6 Hz, -CONHC<u>H<sub>2</sub>-); 6.80</u> (d, 1H, J = 1Hz, H<sup>3</sup>-pyrrole); 6.82 (d, 1H, J = 1 Hz, H<sup>3</sup>'-pyrrole); 6.94 (d, 1H, J = 1Hz, H<sup>5</sup>-pyrrole): 6.95 (d, 1H, J = 1Hz, H<sup>5</sup>'-pyrrole); 8.06 (t, 1H, J = 6 Hz, -CON<u>H</u>CH<sub>2</sub>-); 8.12 (t, 1H, J = 1 Hz, -CON<u>H</u>CH<sub>2</sub>-).

<sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): 26.80 ppm.; 27.26; 29.37; 36.88; 38.50; 46.87; 48.95; 94.90;
111.22; 111.28; 120.91; 121.07; 127.09; 127.14; 159.51; 159.58 (the two pyrrolic C<sup>4</sup>-carbons collapse at 94.90 ppm.).

<u>Analog 3:</u>

Yield: 82 %. FABMS (positiv, m-NBA matrix): m/z 630/632/634/636/638 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR (DMSO- $d_6$  / CDCl<sub>3</sub>): 1.72 ppm (tt, 4H, J = 6 Hz and J = 7 Hz, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-); 2.74 (t, 4H, J = 7 Hz, -CH<sub>2</sub>NHCH<sub>2</sub>-); 3.28 (dt, 4H, J = 6 Hz and J = 6 Hz, -CONHCH<sub>2</sub>-); 6.82 (s, 2H, H<sup>3-</sup> and H<sup>3</sup>-pyrrole); 8.12 (t, 2H, J = 6 Hz, -CONHCH<sub>2</sub>-). <sup>13</sup>C-NMR (DMSO- $d_6$  / CDCl<sub>3</sub>): 27.60 ppm.; 36.08; 45.73; 97.65; 104.32; 112.69; 128.01; 159.30.

# <u>Analog 4:</u>

Yield: 63 %. FABMS (positiv, m-NBA matrix): m/z 645/647/649/651/653 (M+H)<sup>+</sup>. <sup>1</sup>H-NMR (DMSO- $d_6$ ): 2.67 ppm (m, 8H, -C<u>H</u><sub>2</sub>NHC<u>H</u><sub>2</sub>C<u>H</u><sub>2</sub>NHC<u>H</u><sub>2</sub>-); 3.29 (dt, 4H, J = 6 Hz and J = 6 Hz, -CONHC<u>H</u><sub>2</sub>-); 6.82 (s, 2H, H<sup>3</sup>- and H<sup>3</sup>'-pyrrole); 8.02 (t, 2H, J = 6 Hz, -CON<u>H</u>CH<sub>2</sub>-). <sup>13</sup>C-NMR (DMSO- $d_6$ ): 40.58 ppm.; 47.81; 48.57; 96.71; 105.91; 112.59; 129.16;

- 160.09.
- 9. Growth experiments: Stock solutions were made by diluting compounds 1-3 in 96 % ethanol to 100 mg/ml and compound 4 (less soluble) to 50 mg/ml. Appropriate amounts were added to TSB or LB Broth to give 0, 5, 10, 50, 100, 250 and 500 µg/ml. The pre-cultures were diluted and inoculated to a starting cell density of approximately 10<sup>5</sup> cfu/ml. Growth was measured as absorbance at 600 nm and all combinations were performed as triplicate. Minimal inhibitory concentration (MIC) was determined as the lowest concentration not allowing growth for a 4-day period.

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