Ceratinamine: An Unprecedented Antifouling Cyanoformamide from the Marine Sponge *Pseudoceratina purpurea*

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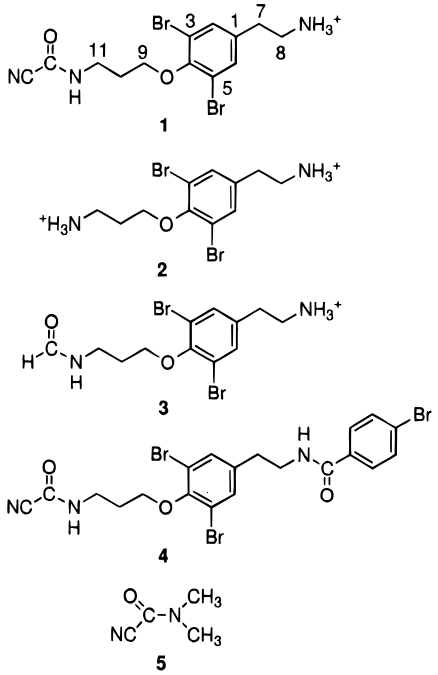
Soft-bodied benthic invertebrates are believed to have chemical defense against predators and overgrowth of other benthic organisms.[1] Therefore, metabolites of these invertebrates are potential "environmentally-friendly" antifouling agents.[2] During our ongoing search for antifouling substances in marine organisms[3] we found that the MeOH extract of the marine sponge *Pseudoceratina purpurea* collected off Hachijo-jima Island, 300 km south of Tokyo, was active against cyprids of the barnacle *Balanus amphitrite.* Bioassay-guided isolation afforded an unprecedented cyanoformyl derivative, ceratinamine (**1**), along with the known moloka'iamine (**2**).[4] This paper reports the isolation, structure elucidation, and biological activities of the new compound.



The ether-soluble portion of the MeOH extract was subsequently fractionated on silica gel (MeOH−$CHCl_3$) and Toyopearl HW-40 (MeOH), followed by reversed-phase (C_{18}) HPLC (50% CH_3CN−H_2O containing 0.01% TFA) to afford ceratinamine (**1**, 2.7 mg, 1.2×10^{-3}% wet weight)[5] and moloka'iamine (**2**, 2.4 mg, 1.1×10^{-3}%). The positive FAB mass spectrum exhibited $(M + H)^+$ ion peaks at *m*/*z* 404/406/408 (intensity, 1:2:1), indicating that **1** contained two bromine atoms. The ion peak at *m*/*z* 406 corresponded to a molecular formula of $C_{13}H_{15}Br_2N_3O_2$ (Δ −1.6 mmu). NMR data (Table 1) together with COSY and HMBC experiments were reminiscent of the known bromotyrosine metabolite, moloka'iamine (**2**).[4] The residual portion consisted of C_2NO which showed two characteristic carbon signals at δ 112.4 (s, CN) and 143.0 (s, CO), thus suggesting the presence of a cyanoformamide functionality, −NHCOCN. Connectivity of the cyanoformamide unit with C11 methylene was substantiated by an HMBC crosspeak between a methylene signal at δ 3.43 (H_2-11) and a carbonyl carbon at δ 143.0. Reduction of **1** with Co_2B/$NaBH_4$ afforded its *N*-formyl derivative **3**,[6,7] confirming the presence of the carbonyl carbon (δ 163.7). Although no cyanide absorption was observed in the IR spectrum of **1**, its *p*-bromobenzoyl derivative **4**[8] revealed a sharp absorption at 2230 cm^{-1}. Finally, the presence of a −NHCOCN unit was substantiated by a carbonyl signal at δ 144.4 for *N*,*N*-dimethylcyanoformamide (**5**).[9−11] Thus, the structure of **1** was unambiguously determined.

Table 1. ^1H and ^{13}C NMR Data for 1[a]

no.	^1H[b]			^{13}C[c]		HMBC
1				136.8	s	
2, 6	7.59 (2H)	s		133.2 (2C)	d	C2, C3, C4, C5, C6, C7
3, 5				117.5 (2C)	s	
4				151.1	s	
7	2.82 (2H)	t	7.2	31.4	t	C1, C2, C6, C8
8	3.07 (2H)	t	7.2	39.3	t	C1, C7
9	3.95 (2H)	t	5.7	70.6	t	C4, C10, C11
10	1.99 (2H)	tt	6.1, 5.7	28.6	t	C9, C11
11	3.43 (2H)	t	6.1	36.8	t	C9, C10, CO
CO				143.0	s	
CN				112.4	s	
NH	9.92 (1H)	br s				
NH_3	7.79 (3H)	br s				

[a] Data recorded in DMSO-d_6 at 500 MHz (^1H) and 125 MHz (^{13}C) at 27 °C. [b] Multiplicities and coupling constants in Hz are given. [c] Multiplicities were determined by an HMQC experiment.

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(1) Pawlik, J. R. *Chem. Rev.* **1993**, *93*, 1911−1922.

(2) Clare, A. S.; Rittschof, D.; Gerhart, D. J.; Maki, J. S. *Invert. Reprod. Dev.* **1992**, *22*, 67−76.

(3) Previous papers: (a) Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1995**, *36*, 8637−8640. (b) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 1439−1440. (c) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Nat. Prod.*, in press. (d) Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359−2368.

(4) Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 6565−6569.

(5) **1**: colorless solid; IR ν_{max} (film) 3200, 3040, 2950, 1680, 1450, 1200, and 1130 cm^{-1}; UV λ_{max} (EtOH) 207 (ϵ 37800), 220 (sh, 12600), and 276 nm (850); ^1H and ^{13}C NMR (DMSO-d_6) see Table 1; FABMS (positive, glycerol matrix) *m*/*z* 326/328 $(M + 2H - Br)^+$, 404/406/408 $(M + H)^+$, and 496/498/500 $(M + H + \text{glycerol})^+$; HRFABMS (positive, glycerol matrix) *m*/*z* 405.9572 (calcd for $C_{13}H_{16}{}^{79}Br^{81}BrN_3O_2$, Δ −1.6 mmu).

(6) Heinzman, S. W.; Ganem, B. *J. Am. Chem. Soc.* **1982**, *104*, 6801−6802.

(7) Cobalt(II) chloride anhydrous (548 mg) was treated with sodium borohydride (802 mg) in MeOH (20 mL) for 20 min. Then, deposited cobalt boride was afforded by filtration.[6] Pseudoceramine (**1**, 2.0 mg) was dissolved in 200 μL of MeOH, and the solution was stirred in the presence of cobalt boride (0.64 mg) and sodium borohydride (0.92 mg) at room temperature for 14 h. After addition of $CHCl_3$ (500 μL), the reaction mixture was applied to the silica gel column (0.5 × 1 cm). The product was eluted with $CHCl_3$−MeOH−H_2O (7:3:0.5), followed by purification with reversed-phase (C_{18}) HPLC (30% CH_3CN−H_2O containing 0.01% TFA) to afford **3** (0.5 mg). ^{13}C chemical shifts were detected and assigned by the HMQC and HMBC spectra. **3**: colorless solid; ^1H NMR (MeOH-d_4) δ 2.06 (2H, tt, J = 6.1 and 5.7 Hz, H_2-10), 2.86 (2H, t, J = 7.2 Hz, H_2-7), 3.11 (2H, t, J = 7.2 Hz, H_2-8), 3.50 (2H, t, J = 6.1 Hz, H_2-11), 4.05 (2H, t, J = 5.7 Hz, H_2-9), 7.52 (2H, s, H-2 and H-6), and 8.06 (1H, s, CHO); ^{13}C NMR (MeOH-d_4) δ 30.7 (t, C10), 33.0 (t, C7), 36.2 (t, C11), 41.2 (t, C8), 72.2 (t, C9), 119.0 (2C, s, C3 and C5), 134.1 (2C, d, C2 and C6), 137.0 (s, C1), 153.6 (s, C4), and 163.7 (s, CO); FABMS (positive, glycerol matrix) *m*/*z* 301/303 $(M + 2H - Br)^+$, 379/381/383 $(M + H)^+$, and 471/473/475 $(M + H + \text{glycerol})^+$; HRFABMS (positive, glycerol matrix) *m*/*z* 380.9629 (calcd for $C_{12}H_{17}{}^{79}Br^{81}BrN_2O_2$, Δ −0.8 mmu).

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This is the first report of a cyanoformamide metabolite in natural products, although *N,N*-dimethylcyanoformamide (**5**)[9] was detected in apples, oranges, and tomatoes as a degradation metabolite of a pesticide. Ceratinamine (**1**) and moloka'iamine (**2**) were not only antifouling against *B. amphitrite* cyprids with EC_{50} values of 5.0 and 4.3 μg/mL, respectively, but also cytotoxic against P388 murine leukemia cells with IC_{50} values of 3.4 and 2.1 μg/mL, respectively. However, **1** and **2** did not show antifungal or antibacterial activity against *Candida albicans*, *Penicillium chrysogenum*, *Mortierella ramanniana*, *Pseudomonas nautica* (IAM 12929), *Alteromonas macleodii* (IAM 12920), *Vibrio alginolyticus* (ATCC 17749), *Flavobacterium marinotypicum* (ATCC 12960), or *Bacillus marinus* (ATCC 29841) at 10 μg/disk.

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Supporting Information Available: 1D and 2D NMR spectra and IR spectrum for compound **1** and IR spectrum for compound **4** (8 pages).

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(8) Ceratinamine (**1**, 1.0 mg) was dissolved in 200 μL of pyridine, and the solution was stirred in the presence of *p*-bromobenzoyl chloride (0.65 mg) and *p*-(dimethylamino)pyridine (0.15 mg) at room temperature for 17 h. The reaction mixture was freed from solvents and purified on silica gel with 1% MeOH−$CHCl_3$ to afford the *p*-bromobenzoylamide (**4**, 0.8 mg). **4**: colorless solid: IR ν_{max} (film) 3280, 3055, 2920, 2230, 1690, 1640, 1550, 1535, 1450, 1255, and 1210 cm^{-1}; UV λ_{max} (EtOH) 207 (ϵ 96800), 241 (sh, 16700), 280 (sh, 2200), and 295 nm (sh, 1100). The ^1H and ^{13}C NMR spectra exhibited a pair of signals for C1−C(8)NH due to the presence of newly-formed *s-cis*- and *s-trans*-amides in a 1:1 ratio. Proton signals of the *s-cis*-amide were shielded by a ring current effect of the *p*-bromobenzoyl ring. ^{13}C chemical shifts were detected and assigned by the HMQC and HMBC spectra. **4**: ^1H NMR ($CDCl_3$, italicized chemical shifts were for *s-cis*-amide) δ 2.11 (2H, tt, J = 6.1 and 5.7 Hz, H_2-10), 2.82 (2H, t, J =7.2 Hz, H_2-7), *2.86* (2H, t, J = 7.1 Hz, H_2-7), 3.58 (2H, q, J = 7.2 Hz, H_2-8), *3.65* (2H, q, J = 7.1 Hz, H_2-8), 3.70 (2H, q, J = 6.1 Hz, H_2-11), 4.12 (2H, t, J = 5.7 Hz, H_2-9), 6.09 (1H, br s, C(8)NH), *6.31* (1H, br s, C(8)NH), 6.98 (1H, br s, C(11)NH), 7.36 (2H, s, H-2 and 6), *7.39* (2H, s, H-2 and 6), and 7.57 (4H, s, aromatic protons of *p*-bromobenzoyl ring); ^{13}C NMR ($CDCl_3$, italicized chemical shifts were for *s-cis*-amide) δ 27.9 (C10), 33.2 (C7), *34.1* (C7), 38.8 (C11), 40.3 (C8), *40.8* (C8), 72.0 (C9), *117.6* (C3 and C5), 118.0 (C3 and C5), 128.0, 131.7, 132.8 (C2 and C6), 136.6 (C1), *138.1* (C1), 142.8 (CNCONH), *150.8* (C4), 151.3 (C4), and 166.6 (NHCO); FABMS (positive, glycerol matrix) m/z 430/432 (M + 3H − 2Br)$^+$, 508/510/512 (M + 2H − Br)$^+$, 586/588/590/592 (M + H)$^+$, and 678/680/682/684 (M + H + glycerol)$^+$.

(9) Harvey, J., Jr.; Han, J. C-Y.; Reiser, R. W. *J. Agric. Food Chem.* **1978**, *26*, 529−536.

(10) Rafik, C.; Abboud, J-L. M.; Guiheneuf, G. *J. Org. Chem.* **1983**, *48*, 4761−4763.

(11) *N,N*-Dimethylcyanoformamide (**5**) was prepared from *N,N*-dimethylcarbamoyl chloride according to Rafik *et al.*[10] **5**: volatile oil; IR ν_{max} (film) 2230, 1680, 1490, 1440, 1400, 1260, 1140, 1060, 950, 890, 720, 670, 580, and 510 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.90 and 3.17 (each 3H, s, NMe); ^{13}C NMR (DMSO-d_6) δ 33.9 (NMe), 37.5 (NMe), 111.2 (CN), and 144.4 (CO).