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Ceratinadins A–C, new bromotyrosine alkaloids from an Okinawan marine sponge *Pseudoceratina* sp.

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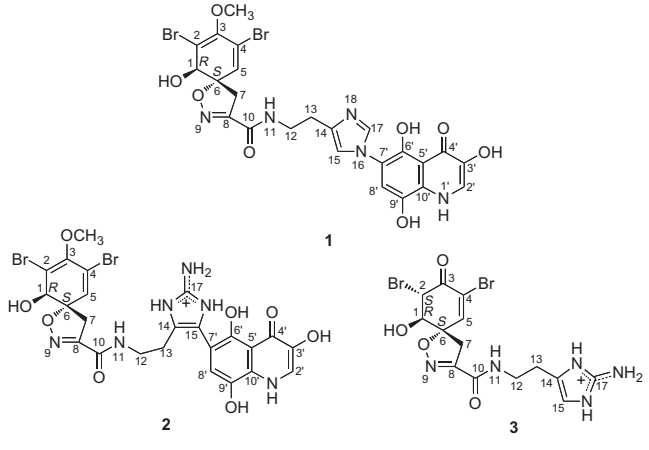
ABSTRACT

Three new bromotyrosine alkaloids, ceratinadins A–C (**1**–**3**), were isolated from an Okinawan marine sponge *Pseudoceratina* sp. and the structures of **1**–**3** were elucidated on the basis of spectroscopic data. Ceratinadin A (**1**) was a novel bromotyrosine alkaloid possessing an *N*-imidazolyl-quinolinone moiety. Ceratinadins A (**1**) and B (**2**) showed antifungal activity.

Marine sponges of the order Verongidae have been found to contain a number of bromotyrosine alkaloids.[1] In our search for bioactive substances from marine sponges, a series of bromotyrosine alkaloids have been isolated from a Verongid marine sponges such as *Pseudoceratina* (= *Psammaplysilla*) sp. and *Suberea* sp.[2–4] Recently, we have investigated extracts of an Okinawan sponge *Pseudoceratina* sp. (SS-214) and isolated three new bromotyrosine alkaloids, ceratinadins A–C (**1**–**3**). Here we describe the isolation and structure elucidation of **1**–**3**.



The sponge *Pseudoceratina* sp. (SS-214) collected off Ishigaki Island, Okinawa, was extracted with MeOH. *n*-BuOH-soluble materials of the extract were purified by a C_{18} column (MeOH/H_2O/TFA, 10:90:0 → 100:0:0.1), C_{18} MPLC (CH_3CN/H_2O/TFA, 30:70:0.1), and C_{18} HPLC (CH_3CN/H_2O/TFA, 25:75:0.1 or 30:70:0.1) to give ceratinadins A (**1**, 0.00018%, wet weight),[5] B (**2**, 0.00012%),[6] and C (**3**, 0.000022%),[7] together with known compounds, aerophobins-1 (0.00029%) and -2 (0.000030%),[8] aplysamine-1 (0.00047%),[9] purealidins E (0.00040%),[10] J (0.00015%),[2] and L (0.000014%),[2] fistularins-1 (0.000049%) and 2 (0.000037%),[11] and 11-*epi*-fistularin-3 (0.00025%).[12]

Ceratinadin A (**1**) was obtained as an optical active {$[\alpha]_D^{20}$ +51 (*c* 0.45, MeOH)} brown amorphous solid. The ESIMS spectrum of **1** showed the pseudomolecular ion peaks at *m/z* 666, 668, 670 [$(M+H)^+$, (1:2:1)], indicating the presence of two bromine atoms, and the molecular formula of **1** was revealed to be $C_{24}H_{21}N_5O_8{}^{79}Br_2$ by HRESIMS data [*m/z* 665.98296 $(M+H)^+$, Δ −0.30 mmu]. The IR absorptions indicated the existence of OH and/or NH (3610, 3580, and 3130 cm^{-1}) and carbonyl (1670 cm^{-1}) functionalities. The ^1H and ^{13}C NMR data (Table 1) disclosed that **1** consisted of 2 carbonyls, 11 sp^2 quaternary carbons, 1 sp^3 quaternary carbon, 5 sp^2 methines, 1 sp^3 methine, 3 sp^3 methylenes, and 1 methoxy. Comparison of the ^1H and ^{13}C NMR chemical shifts of C-1–C-8 for **1** with those reported for a known bromotyrosine alkaloid such as purealidin J[2] suggested that **1** possessed a 2,4-dibromo-1-hydroxy-3-methoxy-spiro-cyclohexadienylisoxazole unit (C-1–C-8, N-9, and 6-O), which was confirmed by the ^1H–^{15}N HMBC correlation for H-7/N-9 in addition to ^1H–^{13}C HMBC correlations (Fig. 1). The existence of a trihydroxy quinolinone ring (N-1′, C-2′–C-10′) was

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deduced from the ^{1}H and ^{13}C NMR chemical shifts for C-2′–C-10′ of **1**,[4] which was supported from correlations observed in the ^{1}H–^{1}H COSY and HMBC spectra of **1** (Fig. 1). Interpretation of the ^{1}H–^{1}H COSY and HMQC spectra of **1** disclosed a segment N-11 to C-13. ^{1}H–^{13}C HMBC correlations for H_2-7/C-10, H-11/C-10, and H_2-12/C-10 indicated that C-8 and N-11 were connected through an amide bond. The remaining part of **1** consisting of $C_3H_2N_2$ unit was deduced to be an imidazole ring from the ^{1}H–^{15}N HMBC correlation for H-15/N-16 as well as ^{1}H–^{13}C HMBC correlations (Fig. 1). ^{1}H–^{13}C HMBC correlations for H_2-13/C-14 and H_2-13/C-15 indicated the linkage between C-13 and C-14. The ^{1}H–^{15}N HMBC correlation for H-8′/N-16 disclosed the connection between N-16 and C-7′. ROESY correlations for H-15/H-8′ and H-17/H-8′ also supported the connection. The relative stereochemistry of a spiro ring of **1** was assigned as 1,6-*trans* by comparison of ^{1}H NMR data of **1** with those of known 1,6-*trans* and 1,6-*cis* 2,4-dibromo-1-hydroxy-3-methoxy-spiro-cyclohexadienylisoxazoles.[13] The absolute stereochemistry of **1** was elucidated to be 1*R* and 6*S*, since the pattern of the CD spectrum of **1** was coincident with that of purealidin J[2]. Thus, the structure of ceratinadin A was concluded to be **1**.

Ceratinadin B (**2**) was obtained as an optical active {$[\alpha]_D^{20}$ +55 (*c* 0.25, MeOH)} brown amorphous solid. The molecular formula of **2** was established as $C_{24}H_{22}N_6O_8{}^{79}Br_2$ by HRESIMS data [*m/z* 680.99386 $(M+H)^+$, Δ −0.18 mmu]. The ^{1}H and ^{13}C NMR data (Table 1) were almost coincident with those of a known bromotyrosine alkaloid, compound 1.[14] The detailed analysis of spectral data of **2** including 2D NMR data (Fig. 2) disclosed that the structure of ceratinadin B (**2**) was the same as compound **1** including the relative stereochemistry. However, the CD spectrum of **2** showed opposite pattern to that of compound **1**, suggesting that ceratinadin B (**2**) was the enantiomer of compound **1**. Thus, the absolute stereochemistry of **2** was elucidated to be 1*R* and 6*S*.

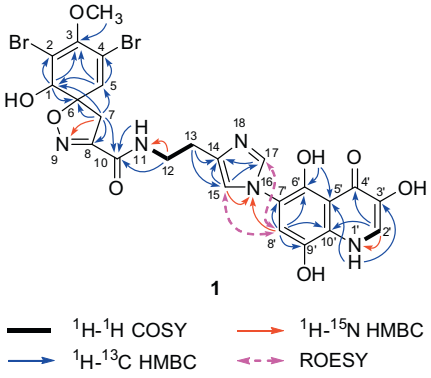

Figure 1. Selected 2D NMR correlations for ceratinadin A (**1**).

Ceratinadin C (**3**) was obtained as an optical active {$[\alpha]_D^{20}$ +14 (*c* 0.15, MeOH)} colorless amorphous solid. The ESIMS spectrum of **3**

Table 1
NMR data of ceratinadins A (**1**) and B (**2**) in DMSO-d_6[a]

Position	**1**		Position	**2**	
	δ_H [mult., *J* (Hz)][b]	δ_C[c] or δ_N[d]		δ_H [mult., *J* (Hz)][b]	δ_C[c]
1	3.93 (1H, br s)	73.5	1	3.86 (1H, d, 6.8)	73.5
1-OH	6.37 (1H, br s)		1-OH	6.34 (1H, d, 7.8)	
2		113.1	2		113.0
3		147.1	3		147.1
3-OMe	3.65 (1H, s)	59.6	3-OMe	3.62 (1H, s)	59.6
4		120.8	4		120.8
5	6.55 (1H, s)	131.2	5	6.83 (1H, s)	131.2
6		90.3	6		90.2
7a	3.20 (1H, d, 18.0)	39.3	7a	3.07 (1H, d, 18.0)	39.3
7b	3.63 (1H, d, 18.0)		7b	3.54 (1H, d, 18.0)	
8		154.4	8		154.2
9		−116.7	10		158.8
10		159.1	11	8.56 (1H, br s)	
11	8.62 (1H, br s)	114.6	12	3.49 (2H, m)	38.0
12	3.51 (2H, m)	37.9	13	2.71 (2H, m)	24.1
13	2.92 (2H, m)	24.6	14		120.3
14		132.0	15		118.7
15	7.77(1H,s)	119.5	16	12.00 (1H, br s)	
16		178.2	17		146.5
17	9.26 (1H, s)	135.9	17-NH_2	7.29 (2H, br s)	
18		22.1	18	12.24 (1H, br s)	
1′	12.02 (1H, br s)	130.0	1′	11.77 (1H, br s)	
2′	7.75 (1H, d, 4.6)	125.0	2′	7.64 (1H, s)	124.1
3′		140.2	3′		139.9
4′		173.2	3′-OH	8.94 (1H, s)	
5′		112.5	4′		173.2
6′		145.2	5′	6.51 (1H, s)	112.5
6′-OH	14.69 (1H, s)		6′		149.5
7′		109.1	6′-OH	14.34 (1H, s)	
8′	7.05 (1H, s)	109.8	7′		103.2
9′-OH	10.90 (1H, s)		8′	6.83 (1H, s)	114.0
10′		129.1	9′		137.2
			9′-OH	10.24 (1H, s)	
			10′		129.0

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 (2.49 and 39.5 ppm, respectively). ^{15}N NMR chemical shifts are referenced to the formamide (−267.5 ppm).
[b] 600 MHz.
[c] 150 MHz.
[d] 50 MHz.

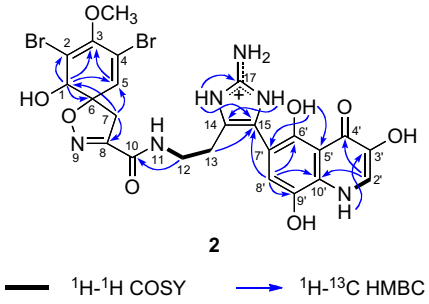

Figure 2. Selected 2D NMR correlations for ceratinadin B (**2**).

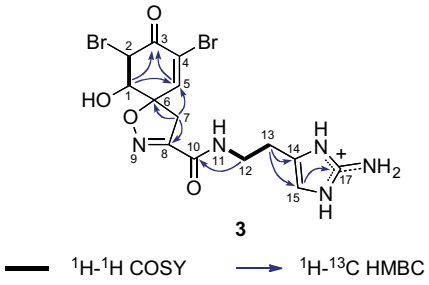

Figure 3. Selected 2D NMR correlations for ceratinadin C (**3**).

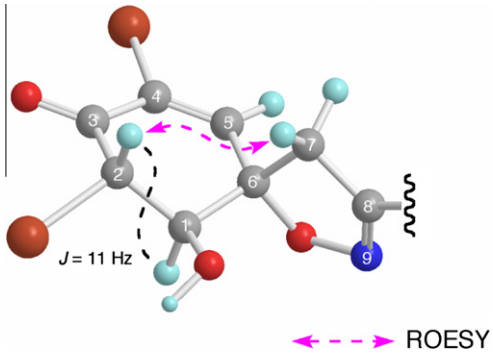

Figure 4. Relative stereochemistry for a spiro ring of ceratinadin C (**3**).

showed the pseudomolecular ion peaks at *m/z* 476, 478, 480 [$(M+H)^+$, (1:2:1)], indicating the presence of two bromine atoms, and the molecular formula of **3** was revealed to be $C_{14}H_{15}N_5O_4{}^{79}Br_2$ by HRESIMS data [*m/z* 475.95636 $(M+H)^+$, Δ −0.61 mmu]. The IR absorptions indicated the existence of OH and/or NH (3610, 3580, and 3300 cm^{-1}) and carbonyl (1670 cm^{-1}) functionalities. The ^1H and ^{13}C NMR data (Table 2) were close to those of purealidin J[2], except for signals due to 2,4-dibromo-3-methoxycyclohexa-2,4-dienol moiety. Inspection of the 2D NMR data (Fig. 3) of **3** disclosed that **3** possessed 2,6-dibromo-5- hydroxycyclohex-2-enone moiety in place of 2,4-dibromo-3-methoxycyclohexa-2,4-dienol moiety of purealidin J. The relative stereochemistry for a spiro ring of **3** was deduced from the ROESY correlation for H-2/H-7 and *J* value (11 Hz) between H-1 and H-2 as shown in Figure 4. The absolute stereochemistry of **3** was elucidated as follows. Treatment of purealidin J with TFA at room temperature for 1 h followed by C_{18} HPLC separation (CH_3CN/H_2O/TFA, 20:80:0.1) gave a reaction product, the structure of which was the same as **3** including the relative stereochemistry (Fig. 5).[15] The CD spectrum of ceratinadin C (**3**) was coincident with that of the reaction product obtained from purealidin J. Therefore, the absolute stereochemistry of **3** was elucidated to be 1*R*, 2*S*, and 6*S*. Thus, the structure of ceratinadin C was concluded to be **3**.[16]

Table 2
NMR data of ceratinadin C (**3**) in DMSO-d_6[a]

Position	δ_H [mult., *J* (Hz)][b]	δ_C[c]
1	4.18 (1H, br d, 9.8)	73.3
2	5.17 (1H, br d, 11.0)	57.6
3		183.8
4		121.3
5	7.64 (1H, s)	146.9
6		90.5
7a	3.10 (1H, d, 18.0)	37.9
7b	3.63 (1H, d, 18.0)	
8		153.8
10		158.9
11	8.62 (1H, br s)	
12	3.38 (2H, m)	37.3
13	2.62 (2H, m)	24.2
14		124.2
15	6.60 (1H, s)	109.4
16[d]	12.13 (1H, br s)	
17		146.9
17-NH_2	7.38 (2H, s)	
18[d]	11.71 (1H, br s)	

[a] ^1H and ^{13}C chemical shifts are referenced to the DMSO-d_6 (2.49 and 39.5 ppm, respectively).
[b] 600 MHz.
[c] 150 MHz.
[d] Exchangeable.

Though several bromotyrosine alkaloids possessing a 6-(4-imidazolyl)-4-quinolinone moiety were reported so far,[4,14] ceratinadins A (**1**) was the first bromotyrosine alkaloid possessing a 6-(*N*-imidazolyl)-4-quinolinone moiety. Ceratinadins A (**1**) and B (**2**) showed antifungal activity against *Cryptococcus neoformans* (MIC, 4 and 8 μg/mL, respectively) and *Candida albicans* (MIC, 2 and 4 μg/mL, respectively), while ceratinadin C (**3**) did not show such activity (MIC, >32 μg/mL).

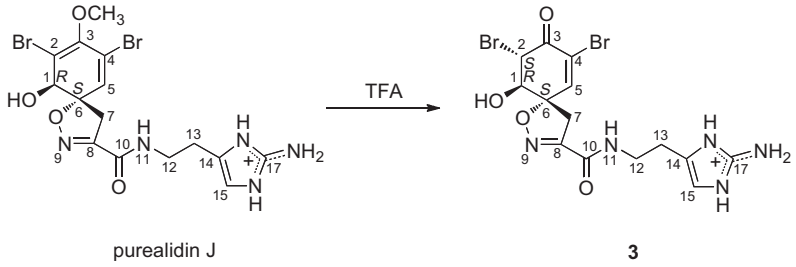

Figure 5. Chemical conversion of purealidin J into ceratinadin C (**3**).

Acknowledgments

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5. Ceratinadin A (**1**): brown amorphous solid; $[\alpha]_D^{20}$ +51 (*c* 0.45, MeOH); UV (MeOH) λ_{max} 244 (ε 23,400), 331 (5800), 377 (3700) nm; CD (MeOH) λ_{ext} 247 ($\Delta\varepsilon$ +4.5), 288 (+4.6) nm; IR (film) ν_{max} 3610, 3580, 3130 (br), 1670, 1600, 1550, 1460, 1200, 1150, 670 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS *m/z* 666, 668, 670 [$(M+H)^+$, 1:2:1]; HRESIMS *m/z* 665.98296 [$(M+H)^+$, Δ −0.30 mmu], calcd for $C_{24}H_{22}N_5O_8{}^{79}Br_2$, 665.98326.
6. Ceratinadin B (**2**): brown amorphous solid; $[\alpha]_D^{20}$ +55 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 239 (ε 26,400), 282 (13,200), 344 (5300) nm; CD (MeOH) λ_{ext} 250 ($\Delta\varepsilon$ +3.35), 289 (+2.73) nm; IR (film) ν_{max} 3610, 3580, 3370 (br), 1680, 1450, 1200, 1140, 670 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); ESIMS *m/z* 681, 683, 685 [$(M+H)^+$, 1:2:1]; HRESIMS *m/z* 680.99386 [$(M+H)^+$, Δ −0.18 mmu], calcd for $C_{24}H_{23}N_6O_8{}^{79}Br_2$, 680.99404.
7. Ceratinadin C (**3**): colorless amorphous solid; $[\alpha]_D^{20}$ +14 (*c* 0.15, MeOH); UV (MeOH) λ_{max} 257 (ε 4800) nm; CD (MeOH) 234 ($\Delta\varepsilon$ +2.84), 266 (+1.20) nm; IR (film) ν_{max} 3610, 3580, 3300 (br), 1670, 1600, 1540, 1430, 1270, 1200, 1130, 1020, 1000, 920, 840, 800, 720, 670 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 2); ESIMS *m/z* 476, 478, 480 [$(M+H)^+$, 1:2:1]; HRESIMS *m/z* 475.95636 [$(M+H)^+$, Δ −0.61 mmu], calcd for $C_{14}H_{16}N_5O_4{}^{79}Br_2$, 475.95697.
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16. It cannot be denied that ceratinadin C (**3**) is an artifact generated during the purification process.