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# Cylindradines A and B: Novel Bromopyrrole Alkaloids from the Marine Sponge *Axinella cylindratus*

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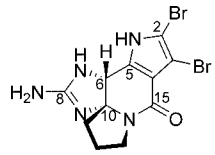
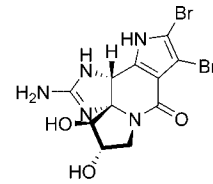
## ABSTRACT



Cylindradine A (**1**)



Cylindradine B (**2**)

**The novel alkaloids cylindradines A and B were isolated from *Axinella cylindratus*, and their structures were elucidated by spectroscopic analyses. Stereochemistries of these compounds were determined by X-ray analysis. Cylindradines showed moderate inhibitory activity against the murine leukemia cell line P388.**

Over the past 30 years a number of oroidin-class alkaloids have been isolated from marine sponges.[1] The phakellin family[2] of compounds (dibromophakellin (**6**),[3] dibromoisophakellin,[4] and dibromophakellastatin[5]) is considered to be a metabolic member of the oroidin-class alkaloids. This phakellin family has attracted interest due to the intriguing tetracyclic framework, various biological activities, and biogenetic pathway. Kitagawa[6] and Van Soest[7] reported that proline, ornithine, and guanidine were plausible precursors of 2-aminoimidazolinone (**3**) and bromopyrrole moieties (**4**). Further metabolic pathways from oroidin-class alkaloids to dibromophakellin were proposed by Al-Mourabit as shown in Figure 1.[1,8] Biosynthetic study using a cell culture system of sponge and labeled amino acids was also reported by Kerr.[9] Ornithine and proline had been used in the biomimetic synthesis of dibromophakellin by Büchi[10] and the enantioselective biomimetic synthesis of the phakellin family by Romo.[11] In our ongoing research for biologically active compounds from marine organisms,[12] we isolated novel key compounds, cylindradines A (**1**) and B (**2**), from the marine

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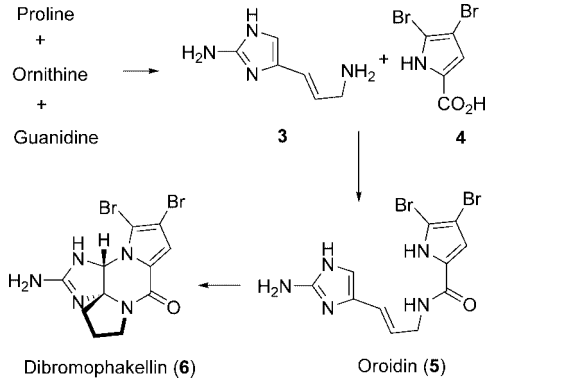
**Table 1.** NMR Spectral Data of Cylindradines A (**1**) and B (**2**)[a]

| cylindradine A | | | cylindradine B | | |
|---|---|---|---|---|---|
| position | $^1$H (mult, *J* in Hz) | $^{13}$C | position | $^1$H (mult, *J* in Hz) | $^{13}$C |
| 1 NH | 12.9 (1H, s) | | 1 NH | 12.9 (1H, s) | |
| 2 | | 104.5[d] | 2 | | 104.7[d] |
| 3 | | 95.4[d] | 3 | | 95.4[d] |
| 4 | | 111.5 | 4 | | 111.2 |
| 5 | | 133.0 | 5 | | 132.5 |
| 6 | 5.28 (1H, s) | 53.5 | 6 | 5.34 (1H, s) | 49.5 |
| 7 NH | *b* | | 7 NH | *c* | |
| 8 | | 156.7 | 8 | | 156.7 |
| 9 NH | *b* | | 9 NH | *c* | |
| 10 | | 83.0 | 10 | | 85.4 |
| 11 | 2.17−2.24 (2H, m) | 39.2 | 11 | 3.90 (1H, d, 4.4) | 78.4 |
| | | | OH | 6.23 (1H, d, 4.4) | |
| 12 | 1.99−2.06 (2H, m) | 19.0 | 12 | 4.14 (2H, d, 4.6) | 71.3 |
| | | | OH | 6.02 (1H, br.s) | |
| 13 | 3.38−3.57 (2H, m) | 44.3 | 13 | 3.42 (1H, d, 13.2) | 52.2 |
| | | | | 3.72 (1H, d, 4.6, 13.2) | |
| 14 N | | | 14 N | | |
| 15 | | 157.8 | 15 | | 158.5 |
| 16 $NH_2$ | *b* | | 16 $NH_2$ | *c* | |

[a] Spectra were recorded in DMSO-$d_6$. Multiplicity was determined by the DEPT experiment. [b] $D_2O$ exchangeable protons were observed at 8.20, 8.58, and 9.78 ppm. [c] $D_2O$ exchangeable protons were observed at 7.67, 8.22, and 8.69 ppm. [d] Exchangeable assignment.

sponge *Axinella cylindratus*, which suggested the metabolic pathway from oroidin (**5**)[13] to the phakellin family members.

**Scheme 1.** Possible Metabolic Pathway to Dibromophakellin[1,8]




The ramal sponge *A. cylindratus* (8.2 kg) was collected at the Seto inland sea near Sada Cape in Ehime prefecture. The methanolic extract was filtered, concentrated under reduced pressure, and extracted with ethyl acetate. The ethyl-acetate extract was partitioned with 90% aqueous methanol and hexane. The methanol layer was concentrated to give an oily material, which was separated by column chromatography on ODS using a gradient elution with methanol and $H_2O$. The 40% methanol/$H_2O$ eluate was subjected to Sephadex LH-20 chromatography, eluting with methanol to afford a mixture of cylindradines. Finally, the mixture was purified by reversed-phase HPLC using 20% acetonitrile/$H_2O$ (0.1% TFA) to give **1** (59.2 mg, $6.3 \times 10^{-5}$ %) and **2** (16.1 mg, $2.0 \times 10^{-6}$ %) as colorless glassy materials.[14] These compounds were positive to ninhydrin and Dragendorff tests. Cylindradines **1** and **2** exhibited moderate toxicity against murine leukemia cell line P388 at $IC_{50}$ values of 7.9 and 33 $\mu$g/mL, respectively.[15] From other fractions of ODS column chromatography, various dibromopyrrole compounds were obtained, and the structures were assigned as 4,5-dibromopyrrole-2-carboxylic acid (**4**), 4,5-dibromopyrrole-2-carboxamide, ethyl 4,5-dibromopyrrole-2-carboxylate, and methyl 4,5-dibromopyrrole-2-carboxylate.[16] However, no pyrrole-3-carbonyl compound was isolated at all.

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(14) Cylindradine A (**1**): colorless glass; $[\alpha]_D$ −75.3° (*c* 0.10, MeOH); FABMS *m/z* = 338, 390, 392 (intensity ratio = 1:2:1); IR (KBr) 3400−3100, 1697, 1637 cm$^{-1}$; UV $\lambda_{max}$ ($CH_3OH$) 244 nm ($\varepsilon$ 4779), 270 nm ($\varepsilon$ 1503); $^1$H NMR ($CD_3OD$) $\delta$ 2.06−2.15 (2H, m, H12), 2.23−2.41 (2H, m, H11), 3.54−3.75 (2H, m, H13), 5.25 (1H, s, H6); $^{13}$C NMR ($CD_3OD$) $\delta$ 21.3 (C12), 41.7 (C11), 46.6 (C13), 56.5 (C6), 85.9 (C10), 98.3 (C3), 107.3 (C2), 113.9 (C4), 134.6 (C5), 159.4 (C8), 161.6 (C15). Cylindradine B (**2**): colorless glass; $[\alpha]_D$ +59.1° (*c* 1.49, MeOH); FABMS *m/z* = 420, 422, 424 (intensity ratio = 1:2:1); IR (KBr) 3500−3000, 1689, 1655, 1630 cm$^{-1}$; UV $\lambda_{max}$ ($CH_3OH$) 242 nm ($\varepsilon$ 3166), 275 nm ($\varepsilon$ 1266); 1H NMR ($CD_3OD$) $\delta$ 3.63 (1H, d, *J* = 12.5 Hz, H13a), 3.87 (1H, dd, *J* = 4.6, 12.5 Hz, H13b), 4.05(1H, s, H11), 4.28 (1H, d, *J* = 4.6 Hz, H12), 5.48 (1H, s, H6); $^{13}$C NMR ($CD_3OD$) $\delta$ 52.0 (C6), 54.6 (C13), 74.0 (C12), 80.5 (C11), 88.1 (C10), 98.2 (C3), 107.1 (C2), 113.6 (C4), 134.1 (C5), 159.0 (C8), 162.1 (C15).

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The molecular formula of cylindradine A (**1**) ($[\alpha]_D$ −75.3° (*c* 0.12, MeOH)) was determined to be $C_{11}H_{11}Br_2N_5O$ by HR-FABMS data [*m/z* 389.9389 (Δ +0.1 mmu, $C_{11}H_{12}{}^{79}Br^{81}BrN_5O$)]. Cylindradine A (**1**) exhibited IR (KBr) absorption bands at 1697 (carbonyl group), 1637 (N═C bond), and 3415−3101 (amino group) $cm^{-1}$. The $^1H$ and $^{13}C$ NMR spectral data of **1** are shown in Table 1. Extensive NMR experiments ($^1H$ NMR, $^{13}C$ NMR, $^1H-^1H$ COSY, $^{13}C-^1H$ COSY, and DEPT) and consideration of the molecular formula indicated that **1** has three methylenes, one methine, seven quaternary carbons, and four protons on heteroatoms.

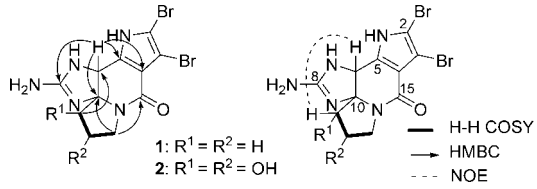


**Figure 1.** Planar structures of cylindradines A (**1**) and B (**2**)

The pyrrolidine ring moiety of **1** was revealed by observation of cross peaks in the $^1H-^1H$ COSY (H11−H13) and the HMBC (H11/C10 ($\delta_C$ 84.4 ppm) and H13/C10) spectra. Furthermore, **1** displayed an amide carbonyl (C15, $\delta_C$ 157.8 ppm), a guanidinium carbon (C8, $\delta_C$ 156.5 ppm), and a quaternary carbon (C10) characteristic of pyrrole-imidazole families involving dibromophakellin, dibromoisophakellin, and cantharelline.[3b,4] Therefore, the structural assignment was carried out both by detailed analysis of the 2D NMR spectra and by comparison of the chemical shifts between **1** and the reported pyrrole-imidazole alkaloids. The location of these quaternary carbons (C8, C10, and C15) were verified by the HMBC cross peaks, H6/(C8, C10, and C11) and H13/C15, as shown in Figure 1. Although an absorption maximum at 270 nm (log ε 3.2) in the UV spectrum was indicative of the presence of the pyrrole ring,[3a,4,5] carbon chemical shifts in the dibromopyrrole moiety of **1** disaccorded with those of phakellins: **1** showed absorption peaks at $\delta_C$ 95.4, 104.5, 111.5, and 133.0 ppm in DMSO-$d_6$, while the resonance peaks were found at $\delta_C$ 101.6, 106.0, 114.4, and 124.8 ppm and at $\delta_C$ 96.5, 108.6, 122.6, and 122.8 ppm in dibromophakellin[3b] and dibromoisophakellin,[4] respectively. These data made the isolated compound distinct from the structurally related phakellins. Finally, the HMBC cross peaks (H6/C4 and H6/C5) and chemical shifts[17] at C4 and C5 indicated the planar structure of cylindradine A (**1**), as shown in Figure 1. The *cis* fusion of the imidazoline moiety at C6 and C10 positions was suggested by the NOE correlation between H6 and H11.

The molecular formula of cylindradine B (**2**) ($[\alpha]_D$ +59.1° (*c* 1.73, MeOH)) was determined to be $C_{11}H_{11}Br_2N_5O_3$ by HR-FABMS data [*m/z* 423.9266 ($C_{11}H_{12}{}^{81}Br_2N_5O_3$, Δ +3.0 mmu)]. The $^1H$ and $^{13}C$ NMR spectral data of **2** are shown in Table 1. The $^1H$ and $^{13}C$ NMR spectral data of **2** (Table 1) closely resembled those of **1** except for C11 and C12 positions bearing hydroxyl groups ($\delta_{C11}$ 78.5 and $\delta_{C12}$ 78.2). The HMBC correlation of **2** was very similar to that of **1** (Figure 1). Furthermore, the NOE correlation between H6 and H11 was also observed. Therefore, the structure of **2** was thought as shown in Figure 1.

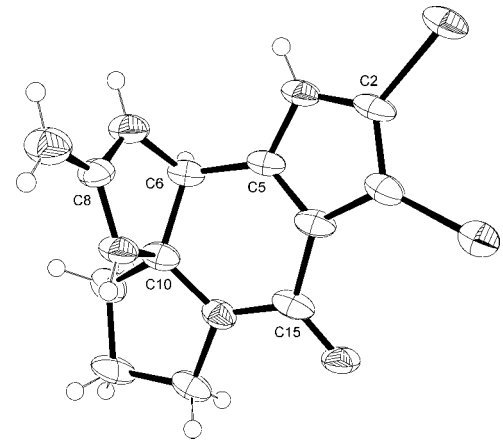


**Figure 2.** Ortep drawing of protonated forms of cylindradine A (**1**). Trifluoroacetate and methanol are omitted for clarity.

The proposed structures of cylindradines **1** and **2** have a β-carbamoylpyrrole skeleton, which is rare in the marine pyrrole alkaloid family.[18] In order to confirm the structures, we decided to do X-ray analysis. Fortunately, diffusion of EtOAc into methanol solutions of **1** and **2** containing TFA gave suitable crystals for the X-ray analysis.[19] Cylindradine A (**1**) crystallized as a guanidinium salt containing trifluoroacetate as the counteranion and methanol as a cocrystallizing solvent. The crystal composition was $\mathbf{1}{+}H^+{\cdot}CF_3CO_2^-{\cdot}CH_3OH$. The Ortep drawing of $\mathbf{1}{+}H^+$ without trifluoroacetate and methanol is shown in Figure 2. To our surprise, the space group of **1** was centro-symmetric *P*-1 and the relative stereochemistry for two stereogenic centers was assigned as 6*S** and 10*R**, although the isolated material showed the fairly negative $[\alpha]_D$ value mentioned above.

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(19) Crystallographical data: Cylindradine A (**1**): crystal formula, $C_{11}H_{12}Br_2N_5O^+{\cdot}CF_3CO_2^-{\cdot}CH_3OH$; 0.30 × 0.15 × 0.02 $mm^3$; triclinic, space group *P*-1; $a$ = 8.422(2), $b$ = 9.324 (4), $c$ = 13.223(6) Å, α = 70.298(18)°, $\beta$ = 85.55(2)°, $\gamma$ = 75.751(19)°, $V$ = 947.5(8) $Å^3$; Mo Kα, $T$ = 150 K, $Z$ = 2, $\rho_{calcd}$ = 1.876 g $cm^{-3}$, $\mu$ = 4.338 $mm^{-1}$, $F(000)$ = 528, 13492 measured, 4316 unique, 2563 observed [$I > 2\sigma(I)$]; $R_1$ = 0.0790 [$I > 2\sigma(I)$], $wR_2$ = 0.2370 (all); GOF = 1.006. CCDC-702691. Cylindradine B (**2**): crystal formula, $C_{11}H_{12}Br_2N_5O_3^+{\cdot}CF_3CO_2^-{\cdot}H_2O$; 0.27 × 0.10 × 0.07 $mm^3$; orthorhombic, space group $P2_12_12$; $a$ = 12.830(5), $b$ = 38.570(14), $c$ = 7.609 (3) Å, $V$ = 3765(2) $Å^3$; Mo Kα, $T$ = 150 K, $Z$ = 8, $\rho_{calcd}$ = 1.952 g $cm^{-1}$, $\mu$ = 4.378 $mm^{-1}$, $F(000)$ = 2176, 51677 measured, 8605 unique, 8007 observed [$I > 2\sigma(I)$]; $R_1$ = 0.0687 [$I > 2\sigma(I)$], $wR_2$ = 0.1700 (all); GOF = 1.062. CCDC-702692. Structures of cylindradines in CIF format have been deposited in CCDC.

Therefore, we doubted that the isolated material of **1** was a heterogeneous mixture of enantiomers and the racemic pairs would favorably crystallize from the solvent system. In fact, the mother liquor of **1** indicated a larger $[\alpha]_D$ value of −141.7° (*c* 0.12, MeOH). This observation clearly suggested that the isolated material of **1** was the heterogeneous mixture of enantiomers. The chiral HPLC analysis (CHIRALCEL OD-R, 90% aqueous methanol containing 0.01 M $NaClO_4$) of the isolated material and the mother liquor of **1** revealed the enantiomeric ratios as 29/71 and 13/87, respectively (Figure 3). Therefore, the e.e. values of these samples were calculated as 42% and 74%, respectively, and the $[\alpha]_D$ values of these pure enantiomers were roughly estimated to be ±180°.

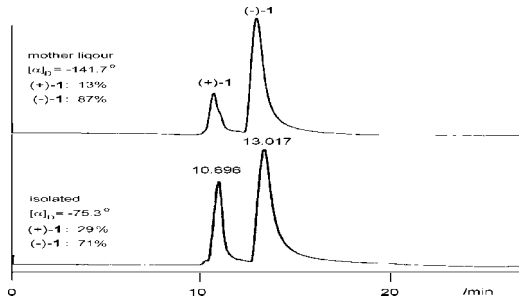


**Figure 3.** HPLC chromatograms of the isolated (lower) and mother-liquor (upper) samples (CHIRALCELL OD-R, 4.6 × 250 mm, 0.1 M aq $NaClO_4$/MeOH = 1/9, flow rate = 0.3 mL/min, detector UV 270 nm)

Cylindradine B (**2**) crystallized as a guanidinium trifluoroacetate with one molecule of water as a cocrystallizing solvent. Although the crystal composition was **2**+$H^+$•$CF_3CO_2^-$•$H_2O$, two crystallographically independent molecules of **2**+$H^+$•$CF_3CO_2^-$ and three solvent water molecules, two of which had a half-occupancy, were found in an asymmetric unit. The water molecules with a half-population were on and near the special axis (0.5, 0.5, *z*). The Ortep drawing of **2**+$H^+$ is shown in Figure 4. Contrary to the crystal of **1**, the space group of this crystal was chiral $P2_12_12$, and the absolute stereochemistry of **2** was unambiguously determined by Flack parameter value of −0.017(15),[20] which was due to the pronounced anomalous dispersion effect induced by bromine atoms. The absolute stereochemistries for four chiral centers of **2** were assigned as 6*R*, 10*R*, 11*S*, and 12*S*. The absolute stereochemistry of the ring junctures was the same as that of dibromophakellin.[21] In the case of **2**, the obtained material was enantiomerically pure, because the mother liquor showed the same $[\alpha]_D$ value.

The oroidin class of alkaloids is defined by the characteristic brominated 2-carbamoylpyrrole and 2-aminoimidazolidine moieties, but cylindradines consist of a brominated 3-carbamoylpyrrole unit. As 4,5-dibromopyrrole-3-carboxylic acid or its derivative was not found yet despite all of our efforts for searching dibromo derivatives in the extract, we think that the 3-carbamoylpyrrole moiety in cylindradines is not the starter unit but a rearrangement process caused by an intramolecular *ipso* substitution[10] that gives the cylindradine skeleton in their biogenesis.

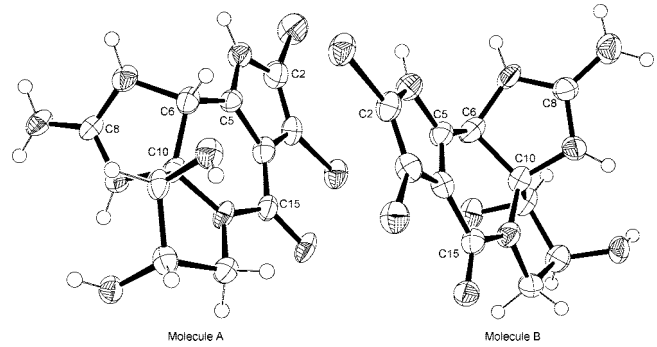


**Figure 4.** Ortep drawing of protonated forms of cylindradine B (**2**). Trifluoroacetate and water are omitted for clarity.

In summary, we have elucidated the structures of cylindradines A (**1**) and B (**2**) isolated from *A. cylindratus*. Cylindradines had moderate toxicity toward P388. These compounds were elucidated to have a 3-carbamoylpyrrole skeleton, and the relative configuration of **1** and the absolute stereochemistry of **2** were unambiguously determined by spectroscopic and X-ray analyses. Further studies on cylindradines including structure elucidation of their analogues, the biogenetic pathway, and structure−activity relationships are under way in our laboratory.

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**Supporting Information Available:** Detailed experimental procedures and characterization data (including $^1H$ and $^{13}C$ NMR spectra) for cylindradines. This material is available free of charge via the Internet at http://pubs.acs.org.

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