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Pyrinadine A, a novel pyridine alkaloid with an azoxy moiety from sponge *Cribrochalina* sp.

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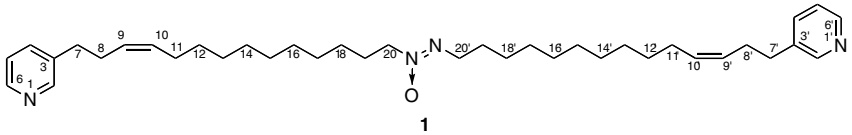
Abstract—A novel cytotoxic bis-3-alkylpyridine alkaloid with an azoxy moiety, pyrinadine A (**1**), has been isolated from an Okinawan marine sponge *Cribrochalina* sp. (SS-1115), and the structure was elucidated by spectroscopic data and chemical means.

Marine sponges are a rich source of bioactive secondary metabolites with unprecedented skeletons. A number of 3-alkylpyridine alkaloids have been isolated from marine sponges of several genera.[1] Most of them possess a long aliphatic chain with various nitrogen-containing terminus,[2] some of which have dimeric or polymeric structures of 3-alkylpyridine.[3] During our continuing search for bioactive substances from marine sponges,[4] we previously isolated cytotoxic pyridine alkaloids from sponges of the genera *Theonella*,[5] *Nyphates*,[6] and *Amphimedon*.[7] Here we describe the isolation and structure elucidation of a novel cytotoxic bis-3-alkylpyridine, pyrinadine A (**1**),[8] from the marine sponge *Cribrochalina* sp.

The sponge *Cribrochalina* sp. (SS-1115) collected off the Unten Port, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the MeOH extract were subjected to a silica gel column ($CHCl_3$/MeOH) followed by an amino silica gel column (hexane/EtOAc) and then reversed-phase HPLC (J'sphere ODS-L80, CH_3CN/H_2O) to afford pyrinadine A (**1**, 0.00011%, wet weight).

Pyrinadine A (**1**) was revealed to have the molecular formula, $C_{38}H_{60}N_4O$, by HRESIMS [*m*/*z* 589.4818 $(M+H)^+$, Δ −2.7 mmu]. The characteristic band at 1505 cm^{-1} in the IR spectrum suggested the presence of an azoxy group. Aromatic proton signals [H-2, H-2′, H-6, and H-6′, δ_H 8.50 (4H); H-4 and H-4′, δ_H 7.72 (2H); H-5 and H-5′, δ_H 7.40 (2H)] in the ^1H NMR spectrum suggested that **1** possessed two 3-alkylpyridine rings. The ^{13}C NMR spectrum revealed five pairs of sp^2 carbon signals [C-2 and C-2′, δ_C 147.0 (2C, d); C-3 and C-3′, δ_C 138.2 (2C, s); C-4 and C-4′, δ_C 138.7 (2C, d); C-5 and C-5′, δ_C 124.1 (2C, d); C-6 and C-6′, δ_C 144.6 (2C, d)] due to the two pyridine rings. The ^1H and ^{13}C NMR data [H-9 and H-9′, δ_H 5.33 (2H); H-10 and H-10′, δ_H 5.43 (2H); C-9 and C-9′, δ_C 127.0 (2C, d); C-10 and C-10′, δ_C 132.0 (2C, d)] indicated the presence of two disubstituted double bonds. Thus, nine unsaturation numbers were accounted for. The ^{13}C NMR spectrum showed a pair of sp^3 carbon signals due to methylenes (C-20, δ_C 69.7; C-20′, δ_C 52.1) at relatively lower field as compared with those of methylenes in long alkyl chains (δ_C 23–30). The chemical shifts of C-20 and C-20′ indicated that these carbons were adjacent to azoxy moiety, which was the remaining part (N_2O) derived from the NMR data and molecular formula. The position of the oxygen atom in the azoxy moiety



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Keywords: Sponge; Alkaloid; Pyridine; Azoxy.
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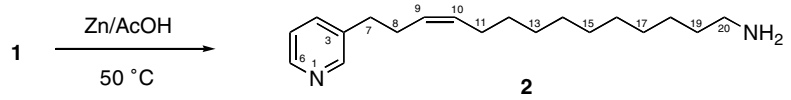

Figure 1. Reductive degradation of pyrinadine A (**1**) and the structure of its reductive product (**2**).

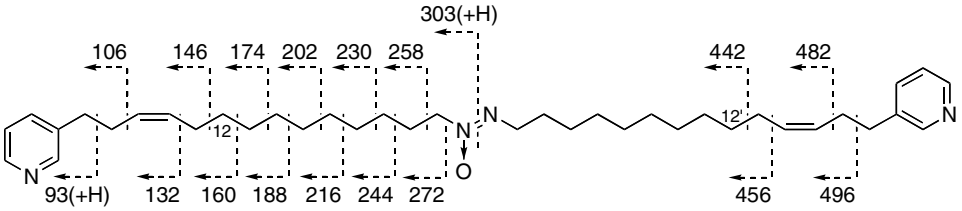

Figure 2. Fragmentation pattern of pyrinadine A (**1**) in ESI MS/MS [parent ion; *m/z* 589 $(M+H)^+$].

was elucidated to be on the C-20 side on the basis of the ^1H and ^{13}C NMR chemical shifts of the C-20 and C-20′.[9] The geometry of the azoxy moiety was deduced to be *Z* from the UV absorption maximum (213 nm) of **1**, since those of the *Z*- and *E*-azoxy compounds have been observed in the range of 220 ± 3 and 230 ± 3 nm, respectively.[10]

The ^1H–^1H COSY, HOHAHA, and HMBC spectra revealed the connectivity from two β-substituted pyridine rings to C-12 and C-12′. *Z*-Geometry of two olefins at C-9 and C-9′ was assigned from the chemical shifts of allylic carbons [C-8 and C-8′, δ_C 28.4 (2C); C-11 and C-11′, δ_C 27.2 (2C)].[11] Pyrinadine A (**1**) was treated with zinc/acetic acid (Fig. 1),[12] to give 3-alkylpyridine **2**, which was generated by cleavage at the azoxy moiety of **1**. The molecular formulae of **2** ($C_{19}H_{32}N_2$) was revealed from HRESIMS [**2**, *m/z* 289.2650 $(M+H)^+$, Δ +0.6 mmu], and ninhydrin test indicated that **2** had an amino group. Analysis of the ESI MS/MS spectrum of **1** revealed connectivities from C-12 and C-12′ to the azoxy moiety (Fig. 2). Thus, the structure of pyrinadine A was concluded to be **1**.

Pyrinadine A (**1**) is the first pyridine alkaloid with an azoxy moiety from natural origins. Pyrinadine A (**1**) showed cytotoxicity against L1210 murine leukemia (IC_{50}, 2 μg/mL) and KB human epidermoid carcinoma cells (IC_{50}, 1 μg/mL) in vitro.

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8. *Pyrinadine A* (**1**). A colorless oil; UV (MeOH) λ_{max} 213 (ε 10,600), 251 (3600), 257 (4200), 262 (4700), and 269 (3700) nm; IR (KBr) ν_{max} 2924, 2853, and 1505 cm^{-1}; ^1H ($CDCl_3$) δ 8.50 (4H, m), 7.72 (2H, m), 7.40 (2H, m), 5.43 (m), 5.33 (m), 4.14 (2H, t, $J = 7.3$ Hz), 3.40 (2H, t, $J = 7.2$ Hz), 2.73 (4H, t, $J = 7.6$ Hz), 2.38 (4H, dt, $J = 7.6$ and 7.2 Hz), 1.95 (2H, m), 1.95 (2H, m), 1.90 (4H, m), 1.69 (2H, m), 1.0–1.4 (28H, m); ^{13}C NMR ($CDCl_3$) δ 147.0 (2C, d), 144.6 (2C, d), 138.7 (2C, s), 132.0 (2C, d), 127.0 (2C, d), 124.1 (2C, d), 69.7 (t), 52.1 (t), 33.0 (2C, t), 28.4 (2C, t), 27.2 (2C, t), 26–30 (16C, t); ESIMS (pos.) *m/z* 589 $(M+H)^+$; HRESIMS *m/z* 589.4818 $(M+H)^+$, Δ −2.7 mmu.
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