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Pyrinodemin A, a Cytotoxic Pyridine Alkaloid with an Isoxazolidine Moiety from Sponge *Amphimedon* sp.

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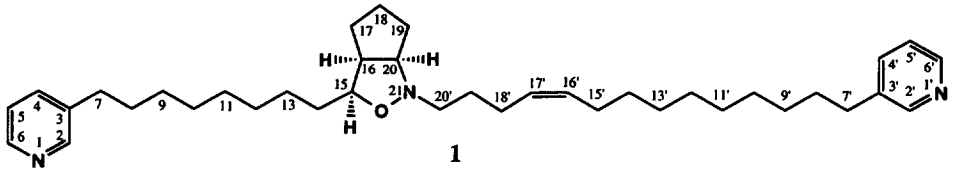
Abstract; A novel cytotoxic pyridine alkaloid, pyrinodemin A (**1**), with a unique *cis*-cyclopent[*c*]isoxazolidine moiety has been isolated from the Okinawan marine sponge *Amphimedon* sp., and the structure was elucidated from 2D NMR data and EIMS fragmentation.

keywords: sponge; alkaloid; pyridine; isoxazolidine

During our search for bioactive substances from marine organisms,[1] we isolated a novel cytotoxic pyridine alkaloid, pyrinodemin A (**1**), possessing a unique bicyclic ring system from the marine sponge *Amphimedon* sp. Here we describe the isolation and structure elucidation of **1**.

EtOAc-soluble materials of the MeOH extract of the sponge *Amphimedon* sp. (SS-955) collected off Nakijin, Okinawa, were subjected to silica gel columns ($CHCl_3$/MeOH and hexane/EtOAc) followed by reversed-phase HPLC on 6-(phenyl)hexylsilyl (MeOH/H_2O) to afford pyrinodemin A (**1**, 0.00011 %, wet weight) as colorless oil.

Pyrinodemin A[2] {**1**, $[\alpha]_D^{25}$ -9° (*c* 1.0, $CHCl_3$)} was revealed to have the molecular formula, $C_{38}H_{59}N_3O$, by HRFABMS [*m/z* 574.4742 $(M+H)^+$, Δ +0.6 mmu]. UV absorption at 264 nm (ε 6300)[3] as well as aromatic proton signals [H-2 and H-2', δ_H 8.42 (2H); H-4 and H-4', δ_H 7.48 (2H); H-5 and H-5' δ_H 7.19 (2H); H-6 and H-6', δ_H 8.44 (2H)] in the ^1H NMR spectrum suggested **1** to possess two 3-alkyl-substituted pyridine rings. The ^1H and ^{13}C signals at δ_H 5.34 (2H) and δ_C 129.3 (2C) indicated the presence of the disubstituted double bond. Since nine degrees out of eleven unsaturation numbers were accounted for, it was implied that **1** had two more rings. The ^{13}C NMR spectrum showed sp^3 carbon signals due to three methines (C-15, δ_C 77.2; C-16, δ_C 49.3; C-20, δ_C 72.1) and one methylene (C-20', δ_C 57.3) in relatively lower field as well as methylenes in long alkyl chains (δ_C 26 ~ 34). The chemical shifts of C-15, C-20, and C-20' indicated that these carbons were adjacent to a nitrogen or an oxygen atom. The ^1H-^1H COSY spectrum revealed connectivities among three methine protons at C-15 (δ_H 4.05), C-16 (δ_H 2.82) and C-20 (δ_H 3.46). The presence of a cyclopentane ring was implied by HSQC-TOCSY correlations from H-15 to C-17 (δ_C 26.5), from H-16 to C-18 (δ_C 26.3), from



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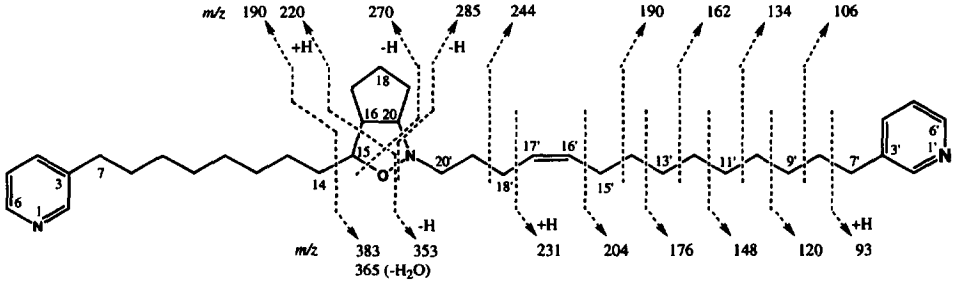

Fig. 1. Fragmentation Pattern of Pyrinodemin A (**1**) in EIMS [parent ion; *m/z* 573 (M^+)]

H-16 to C-19 (δ_C 33.6), and from H-20 to C-18. Connection between C-20 and C-20' through a nitrogen atom (N-21) was assigned by the NOESY correlations from H-20 to H_2-20' (δ_H 2.84 and 2.59) and the chemical shifts of C-20 and C-20'. Considering the unsaturation number and the chemical shift of C-15, **1** was suggested to contain an isoxazolidine ring. The EIMS spectrum of **1** showed an intense fragment ion peak at *m/z* 270 [*m/z* 270.2219, $(C_{19}H_{28}N)^+$, Δ -0.2 mmu]. This fragment ion might be generated from **1** through Hofmann-like elimination in the isoxazolidine ring.[4] The position of the olefin was implied to be at C-16' and C-17' from the fragmentation pattern (Fig.1). Fragment ions at *m/z* 220 [*m/z* 220.1694, $(C_{14}H_{22}NO)^+$, Δ -0.7 mmu] and 285 [*m/z* 285.2320, $(C_{19}H_{29}N_2)^+$, Δ -1.0 mmu] suggested that the two alkyl chains from C-7 to C-14 and from C-7' to C-20' consisted of C_8 and C_{14} units, respectively. *Z*-Geometry of the olefin at C-16' was based on the chemical shifts of allylic carbons (both C-15' and C-18', δ_C 27.1).[5] *Cis*-Ring junction of the bicyclic system was deduced from the NOESY correlation of H-16/H-20. NOESY correlations of H-15/H-16 and H-15/H-20 indicated that the relative stereochemistry of H-15 and H-16 was *cis*. Thus the structure of pyrinodemin A was concluded to be **1**.

Pyrinodemin A (**1**) is a unique *bis*-3-alkylpyridine alkaloid with a *cis*-cyclopent[*c*]isoxazolidine moiety and exhibited potent cytotoxicity against murine leukemia L1210 (IC_{50}, 0.058 μg/mL) and KB epidermoid carcinoma cells (IC_{50}, 0.5 μg/mL) *in vitro*.

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References and Notes

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2 **1**: colorless oil; $[\alpha]_D^{25}$ -9° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 264 nm (ε 6300); IR (neat) ν_{max} 1575 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2 ~ 1.3 (18H), 1.33 (4H, m, H_2-9 and H_2-9'), 1.40 (1H, m, H-17), 1.42 (1H, m, H-18), 1.44 (1H, m, H-14), 1.53 (2H, m, H_2-19'), 1.55 (1H, m, H-14), 1.60 (4H, m, H_2-8 and H_2-8'), 1.64 (1H, m, H-17), 1.65 (1H, m, H-19), 1.66 (1H, m, H-18), 1.76 (1H, m, H-19), 2.03 (4H, m, H_2-15' and H_2-18'), 2.59 (1H, m, H-20'), 2.60 (4H, t, *J* = 7.6 Hz, H_2-7 and H_2-7'), 2.82 (1H, m, H-16), 2.84 (1H, m, H-20'), 3.46 (1H, m, H-20), 4.05 (1H, m, H-15), 5.34 (2H, m, H-16' and H-17'), 7.19 (2H, brt, *J* = 7.0 Hz, H-5 and H-5'), 7.48 (2H, d, *J* = 7.5 Hz, H-4 and H-4'), 8.42 (2H, brs, H-2 and H-2'), and 8.44 (2H, m, H-6 and H-6'); ^{13}C NMR ($CDCl_3$) δ 26.3 (t), 26.5 (t), 27.1 (2C, t), 27.9 (t), 28.9 (2C, t), 29.2 (2C, t), 29.4 ~ 29.8 (8C, t), 31.2 (2C, t), 33.0 (2C, t), 33.6 (t), 49.3 (d), 57.3 (t), 72.1 (d), 77.2 (d), 122.5 (2C, d), 129.3 (2C, d), 135.4 (2C, d), 137.5 (2C, s), 146.5 (2C, d), and 150.0 (2C, d); FABMS *m/z* 574 $(M+H)^+$; HRFABMS *m/z* 574.4742 $(M+H)^+$, calcd for $C_{38}H_{60}N_3O$, 574.4736.

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