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Nakinadine A, a novel bis-pyridine alkaloid with a β-amino acid moiety from sponge *Amphimedon* sp.

Takaaki Kubota,[a] Takami Nishi,[a] Eri Fukushi,[b] Jun Kawabata,[b] Jane Fromont[c] and Jun'ichi Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[b]*Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan*
[c]*Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia*

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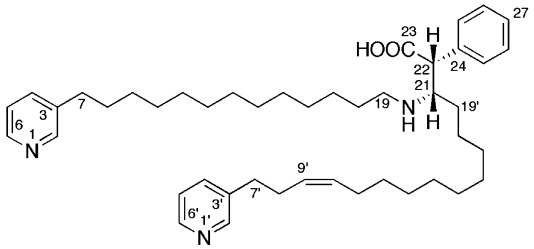
Abstract—A novel cytotoxic bis-3-alkylpyridine alkaloid with a β-amino acid moiety, nakinadine A (**1**), has been isolated from an Okinawan marine sponge *Amphimedon* sp., and the structure and stereochemistry were elucidated by spectroscopic data.

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 a 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] *Amphimedon*,[7] and *Cribrochalina*.[4,8] Here we describe the isolation and structure elucidation of a novel cytotoxic 3-alkylpyridine alkaloid, nakinadine A (**1**),[9] from a marine sponge *Amphimedon* sp. (SS-1059).



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Stereochemistry denotes relative one.

The sponge *Amphimedon* sp. (SS-1059) collected off Nakijin, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the MeOH extract were subjected to a silica gel column ($CHCl_3$/MeOH), in which a fraction eluted with $CHCl_3$/MeOH (9:1) was purified by an amino silica gel column ($CHCl_3$/MeOH) followed by C_{18} HPLC (MeOH/H_2O) to afford nakinadine A (**1**, 0.001%, wet weight).

Nakinadine A (**1**) was revealed to have the molecular formula, $C_{45}H_{67}N_3O_2$, by HRESIMS [*m*/*z* 682.5311 $(M+H)^+$, Δ +0.0 mmu]. Aromatic proton signals [H-2, H-2′, H-6, and H-6′, δ_H 8.44 (4H); H-4 and H-4′, δ_H 7.49 (2H); H-5 and H-5′, δ_H 7.19 (2H)] and [H-25 and H-29, δ_H 7.32 (2H); H-26 and H-28, δ_H 7.20 (2H); H-27, δ_H 7.15 (1H)] in the ^{1}H NMR spectrum and five pairs of sp^2 carbon signals [C-2 and C-2′, δ_C 149.6 (2C, d); C-3 and C-3′, δ_C 136.9 (2C, s); C-4 and C-4′, δ_C 135.5 (2C, d); C-5 and C-5′, δ_C 122.9 (2C, d); C-6 and C-6′, δ_C 146.8 (2C, d)] and four sp^2 carbon signals [C-24, δ_C 135.8 (s); C-25 and C-29, δ_C 129.4 (2C, d); C-26 and C-28, δ_C 128.2 (2C, d); C-27, δ_C 127.0 (d)] in the ^{13}C NMR spectrum suggested that **1** possessed two 3-alkylpyridine rings and a phenyl group. ^{13}C NMR data disclosed the presence of a carbonyl carbon (δ_C 176.0). IR absorptions at 3400–3000, 2900–2600, and 1725 cm^{-1} indicated the presence of carboxylic acid functionality. Treatment of **1** with TMS–CHN_2 gave the methyl ester of **1** [ESIMS (pos.) *m*/*z* 696 $(M+H)^+$]. Since the ^{1}H and ^{13}C NMR data of **1** [H-9′ and H-10′, δ_H 5.33 (2H); C-9′, δ_C 127.4 (d); C-10′, δ_C 131.1 (d)]

Keywords: Sponge; Alkaloid; Pyridine; β-Amino acid.
* Corresponding author. Tel.: +81 11 706 3239; fax: +81 11 706 4989; e-mail: jkobay@pharm.hokudai.ac.jp

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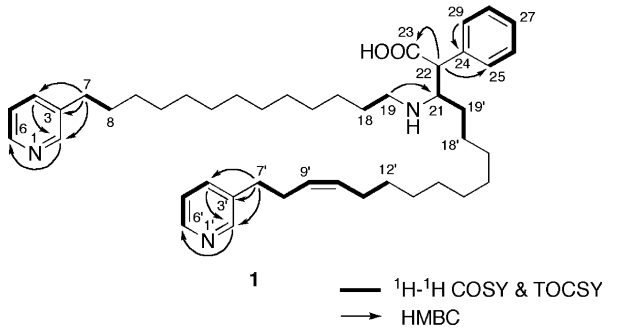


Figure 1. Selected 2D NMR correlations for nakinadine A (**1**).

indicated the presence of a disubstituted double bond, fourteen unsaturation numbers were accounted for.

The gross structure of **1** was elucidated by analyses of 2D NMR data in CD_3OD (Fig. 1). The ^1H–^1H COSY, TOCSY, and HMBC spectra revealed connectivities from two β-substituted pyridine rings to C-8 and C-12′. *Z*-Geometry of an olefin at C-9′ was assigned as *Z* from the chemical shifts of allylic carbons [C-8′, δ_C 28.4; C-11′, δ_C 26.9].[10] The ^1H–^1H COSY and TOCSY spectra of **1** revealed connectivities of C-18 to C-19, and C-21 to C-22 and C-19′, and C-18′ to C-19′. The ^1H and ^{13}C NMR data suggested that C-19 [δ_H 2.75 (1H) and 2.62 (1H); δ_C 44.9 (t)] and C-21 [δ_H 3.19 (1H); δ_C 59.5 (d)] were adjacent to a nitrogen atom. The connectivity of C-19 to C-21 through a nitrogen atom was implied by HMBC cross-peaks for H_2-19 to C-21. HMBC cross-peaks for H-22 to C-23 and C-25, and H-29 to C-24 indicated that a carboxy group and phenyl group were both attached to C-22.

Analysis of the ESIMS/MS spectrum of **1** revealed connectivities from C-8 to C-18 and from C-12′ to C-18′ (Fig. 2). These fragmentation patterns also supported the proposed structure of nakinadine A (**1**).

The relative stereochemistry of **1** was deduced from NOESY correlations and *J*-values[11] as shown in Figure 3. A *gauche* relation for H-21 and H-22 was deduced from the NOESY cross-peak for H-21/H-22 and $^3J_{H\text{-}21/H\text{-}22}$ (2 Hz). The NOESY cross-peaks for H-21/H-25 and H-21/H-26 suggested a *gauche* relation for H-21 and a phenyl group. A large coupling constant ($^3J_{H\text{-}22/C\text{-}19'} = 8$ Hz), which was obtained from HETLOC spectrum of **1**, indicated an *anti* relation for H-22 and C-19′ (Fig. 3).

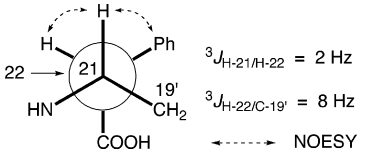
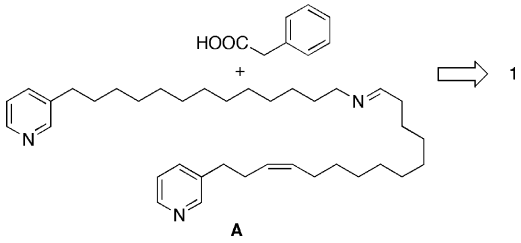


Figure 3. Rotation model for C-21–C-22 of nakinadine A (**1**).



Scheme 1. Plausible biogenetic path of nakinadine A (**1**).

Nakinadine A (**1**) is the first bis-3-alkylpyridine alkaloid with a β-amino acid moiety.[12] Biogenetically, nakinadine A (**1**) might be generated from an intermediate **A** and phenylacetic acid through Mannich-type reaction (Scheme 1).[13] Nakinadine A (**1**) showed cytotoxicity against L1210 murine leukemia (IC_{50}, 1.3 μg/mL) and KB human epidermoid carcinoma cells (IC_{50}, 2.5 μg/mL) in vitro.

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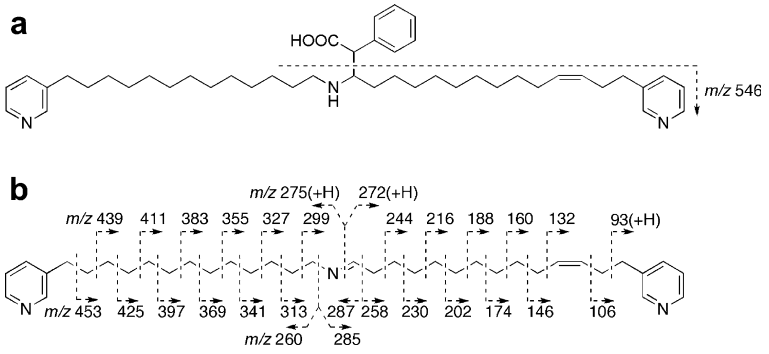

Figure 2. Fragmentation pattern of nakinadine A (**1**) in ESI MS/MS. [parent ion; (a) at *m*/*z* 682 $(M+H)^+$, (b) at *m*/*z* 546 $(M-C_8H_9O_2+H)^+$].

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9. *Nakinadine A* (**1**): A colorless oil; $[\alpha]_D^{23}$ −3 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 257 (ε 3700), 263 (4100), and 269 (3600) nm; IR (film) ν_{max} 3200 (br), 2850 (br), and 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (4H, m), 7.49 (2H, m), 7.32 (2H, m), 7.20 (2H, m), 7.19 (2H, m), 7.15 (m), 5.33 (2H, m), 3.75 (br s), 3.19 (br s), 2.75 (m), 2.62 (3H, m), 2.56 (2H, t, $J = 6.7$ Hz), 2.33 (2H, m), 1.89 (2H, m), 1.57 (2H, m), 1.54 (2H, m), 1.42 (2H, m), 1.43 (2H, m), 1.0–1.4 (34H, m); ^{13}C NMR ($CDCl_3$) δ 176.0 (s), 149.6 (2C, d), 146.8 (2C, d), 136.9 (2C, s), 135.8 (s), 135.5 (2C, s), 131.1 (s), 129.4 (2C, s), 128.2 (2C, s), 127.4 (s), 127.0 (s), 122.9 (2C, d), 59.5 (s), 51.9 (s), 44.9 (s), 32.7 (2C, d), 30.8 (s), 28.5–30.0 (16C, m), 28.4 (s), 26.9 (2C, s), 26.6 (s); ESIMS (pos.) *m*/*z* 682 $(M+H)^+$; HRESIMS *m*/*z* 682.5311 $(M+H)^+$, Δ −0.0 mmu.
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