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Pyrinodemins E and F, new 3-alkylpyridine alkaloids from sponge *Amphimedon* sp.

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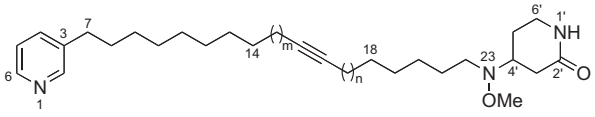
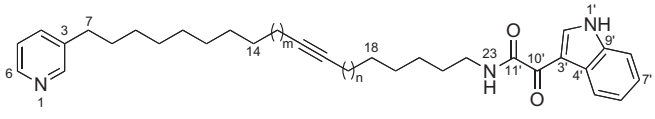
ABSTRACT

Two new 3-alkylpyridine alkaloids, pyrinodemins E (**1**) and F (**2**), were isolated from an Okinawan marine sponge *Amphimedon* sp. and the structures of **1** and **2** were elucidated on the basis of spectroscopic data. Pyrinodemins E (**1**) and F (**2**) were novel 3-alkylpyridine alkaloids possessing a 4-(methoxyamino)piperidinone moiety and an indol-3-glyoxylamide moiety, respectively. Pyrinodemin E (**1**) showed cytotoxicity against P388 and L1210 murine leukemia cells.

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] In our search for bioactive natural products from marine sponges, we previously isolated cytotoxic pyridine alkaloids from sponges of the genera *Theonella*,[4] *Niphates*,[5] *Amphimedon*,[6] and *Cribrochalina*.[7] Recently, we have investigated extracts of an Okinawan sponge *Amphimedon* sp. (SS-1236) and isolated two new 3-alkylpyridine alkaloids, pyrinodemins E (**1**) and F (**2**). Here we describe the isolation and structure elucidation of **1** and **2**.



pyrinodemin E (**1**)
(m + n = 1)



pyrinodemin F (**2**)
(m + n = 1)

The sponge *Amphimedon* sp. (SS-1236, 0.35 kg) collected at Zamami, Okinawa, was extracted with MeOH. EtOAc-soluble materials of the extract were purified by a silica gel column (*n*-hexane/EtOAc), C_{18} column (MeOH/H_2O), and C_{18} HPLC (MeOH/H_2O) to give pyrinodemins E (**1**, 0.00029%, wet weight)[8] and F (**2**, 0.000022%).[9]

Pyrinodemin E (**1**) was obtained as colorless oil. The ESIMS spectrum of **1** showed the pseudomolecular ion peak at *m*/*z* 442 $(M+H)^+$, and the molecular formula of **1** was revealed to be $C_{27}H_{43}N_3O_2$ by HRESIMS data [*m*/*z* 442.34238 $(M+H)^+$, Δ −0.97 mmu]. UV absorptions at 257 (ε 3200), 263 (3600), and 269 (2700) nm implied the presence of pyridine ring. IR absorptions indicated the existence of NH (3211 cm^{-1}) and amide carbonyl (1668 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra (Table 1) showed aromatic proton (H-2, δ_H 8.38; H-4, δ_H 7.69; H-5, δ_H 7.35; H-6, δ_H 8.35) and carbon (C-2, δ_C 150.8; C-3, δ_C 141.1; C-4, δ_C 139.0; C-5, δ_C 125.9; C-6, δ_C 148.3) signals, which were ascribed to a 3-substituted pyridine ring.

Inspection of the 2D NMR spectra of **1** disclosed three structural units **a**, **b**, and **c** (Fig. 1). ^1H–^1H COSY correlations for H-4/H-5, H-5/H-6, and H_2-7/H_2-8, and HMBC correlations for H-2/C-6, H-4/C-7, H_2-7/C-2, and H_2-7/C-3 indicated the existence of a 3-alkylpyridine moiety (**a**). HMBC correlations between two alkyne carbons (δ_C 81.8 and 81.7) and two methylene protons (δ_H 2.13, 4H) suggested the presence of an alkyl chain incorporating an acetylenic group (**b**). Partial structure **c** was elucidated as follows. Investigation of the ^1H–^1H COSY spectrum revealed connectivities of C-21 to C-22, C-3′ to C-6′, and N-1′ to C-6′. The HMBC correlation between H-3′ and an amido carbonyl carbon (C-2′, δ_C 175.2) indicated the

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Table 1
^{1}H and ^{13}C NMR data of pyrinodemins E (**1**) and F (**2**) in CD_3OD

1			**2**		
Position	δ_H [mult., J (Hz)][a]	δ_C[b]	Position	δ_H [mult., J (Hz)][a]	δ_C[b]
1			1		
2	8.38 (br s)	150.8	2	8.43 (br s)	148.0
3		141.1	3		139.1
4	7.69 (d, 7.7)	139.0	4	7.61 (d, 7.9)	138.6
5	7.35 (dd, 7.7, 4.9)	125.9	5	7.29–7.34 (m)	123.9
6	8.35 (d, 4.9)	148.3	6	8.41 (d, 7.9)	145.3
7	2.66 (t, 7.6)	34.6	7	2.62 (t, 7.9)	33.0
8	1.65 (m)	33.0	8	1.62 (t, 7.9)	30.9
9–12 (9–13)	1.29–1.41 (m)	30.5–31.2	9–12 (9–13)	1.26–1.44 (m)	28.4–29.4
13, 18 (14, 19)	1.45 (m)	30.5–31.2	13,18 (14,19)	1.46 (m)	28.4–29.4
14, 17 (15, 18)	2.13 (m)	20.1	14,17 (15,18)	2.13 (m)	18.7
15, 16 (16, 17)		81.7, 81.8	15,16 (16,17)		80.0, 80.4
19, 20 (20)	1.29–1.41 (m)	30.5–31.2	19–20 (20)	1.26–1.44 (m)	28.4–29.4
21	1.63 (m)	28.8	21		
22	2.73 (m)	57.0	22	3.37 (dd, 13.5, 6.7)	39.2
1′	6.24 (br s)[c]		23	7.75 (br s)	
2′		175.2	1′	10.14 (br s)	
3′	2.49 (m)	35.8	2′	9.10 (d, 3.1)	137.7
	2.41 (m)	61.4	3′		113.2
4′	3.05 (m)		4′		126.8
5′	3.35 (m)	41.1	5′	8.40 (m)	122.4
	3.21 (m)		6′	7.29–7.34 (m)	123.9
6′	2.06 (m)	27.1	7′	7.29–7.34 (m)	123.2
	1.76 (m)		8′	7.47 (d, 7.4)	111.8
OMe	3.51 (s)	64.1	9′		135.9
			10′		180.8
			11′		162.5

[a] Recorded at 600 MHz.
[b] Recorded at 150 MHz.
[c] In $CDCl_3$.

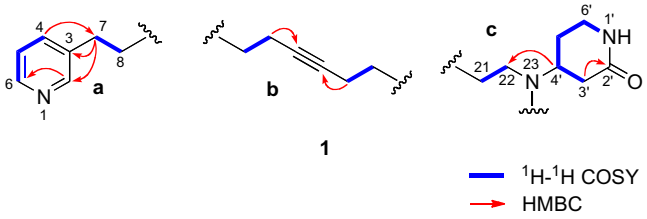


Figure 1. Selected 2D NMR correlations for partial structures **a**, **b**, and **c** of pyrinodemin E (**1**).

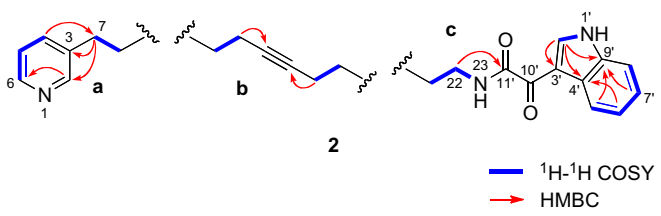


Figure 3. Selected 2D NMR correlations for partial structures **a**, **b**, and **c** of pyrinodemin F (**2**).

existence of a 2-piperidinone ring (N-1′, C-2′–C-6′). Connectivity of two N-bering carbons (C-22, δ_C 57.0; C-4′, δ_C 61.4) through N-23 was disclosed by an HMBC cross-peak of H-4′/C-22.

Analysis of fragmentation patterns of the EIMS spectrum of **1** revealed the existence of an *N*-OMe moiety and connectivities of three structural units **a**, **b**, and **c** (Fig. 2).

The exact position of a triple bond in **1** was elucidated as described below. Pyrinodemin E (**1**) was hydrogenated with Lindlar

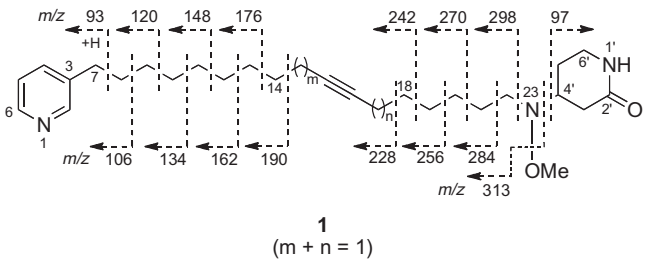
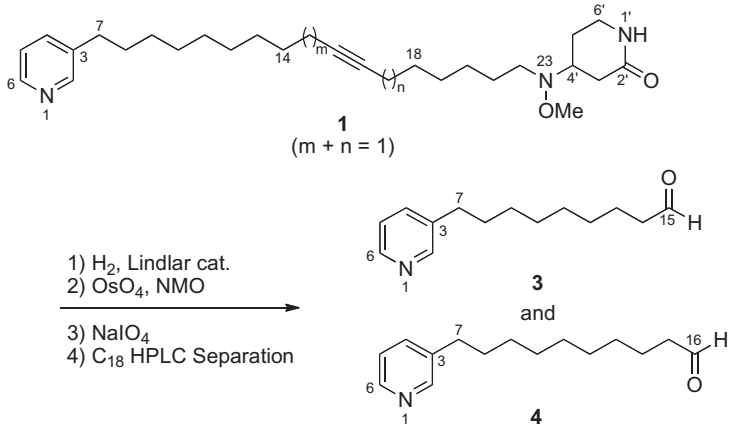


Figure 2. Fragmentation patterns observed in EIMS spectrum of pyrinodemin E (**1**) [precursor ion, m/z 442 $(M+H)^+$].

catalyst, and the resulting double bond was cleaved by treatment with OsO_4 and $NaIO_4$ (Scheme 1). Reaction mixture was separated by C_{18} HPLC to give aldehydes **3**[10] and **4**[11] (1:1), indicating that pyrinodemin E (**1**) was the mixture of two regioisomers, pyrinodemins E1 and E2, possessing a triple bond at C-15/C-16 and C-16/C-17, respectively. Thus, the gloss structure of pyrinodemin E was elucidated to be **1**. The optical rotation[8] suggested that **1** was a racemate.

Pyrinodemin F (**2**) was obtained as colorless oil. The molecular formula of **2** was revealed to be $C_{31}H_{39}N_3O_2$ by HRESIMS data [m/z 486.31242 $(M+H)^+$, Δ −0.18 mmu]. IR absorptions indicated the existence of NH (3324 cm^{-1}) and carbonyl (1674 and 1621 cm^{-1}) functionalities. In addition to the signals due to a 3-substituted pyridine ring, aromatic proton (H-1′, δ_H 10.14; H-2′, δ_H 9.10; H-5′, δ_H 8.40; H-6′, δ_H 7.29–7.34; H-7′, δ_H 7.29–7.35;; H-8′, δ_H 7.47) and carbon (C-2′, δ_C 137.7; C-3′, δ_C 113.2; C-4′, δ_C 126.8; C-5′, δ_C 122.4; C-6′, δ_C 123.9; C-7′, δ_C 123.2; C-8′, δ_C 111.8; C-9′, δ_C 135.9) signals were observed in the ^{1}H and ^{13}C NMR spectra of **2**, indicating that **2** was 3-alkylpyridine alkaloid possessing an additional aromatic ring (Table 1).

^{1}H–^{1}H COSY and HMBC data of **2** revealed the existence of a 3-alkylpyridine moiety (**a**) and an alkyne chain moiety (**b**) (Fig. 3).



Scheme 1. Chemical degradation of pyrinodemin E (**1**).

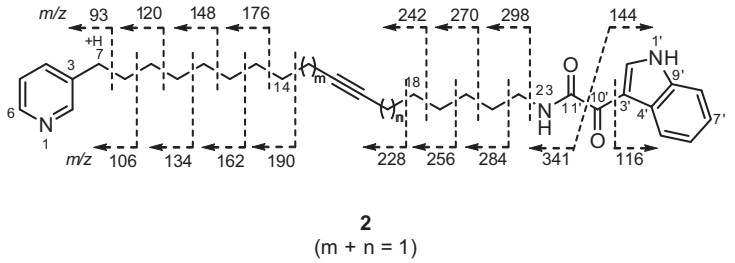


Figure 4. Fragmentation patterns observed in ESIMS/MS spectrum of pyrinodemin F (**2**) [precursor ion, *m*/*z* 486 $(M+H)^+$].

Partial structure **c** including an indol-3-glyoxylamide moiety was elucidated by comparison of the ^{13}C NMR data with those of known compounds as well as analysis of 2D NMR data. The ^1H–^1H COSY spectrum revealed the connectivities of C-21 to C-22 and C-22 to N-23. The HMBC correlation for H_2-22/C-11′ revealed an amide linkage between N-23 and C-11′. Connectivities of N-1′ to C-2′ and C-5′ to C-8′ disclosed from the ^1H–^1H COSY spectrum and HMBC cross-peaks of H-2′/C-3′, H-2′/C-4′, H-2′/C-9′, H-5′/C-9′, H-6′/C-4′, and H-7′/C-9′ implied the existence of a 3′-substituted indole ring (N-1′, C-2′–C-9′). ^{13}C chemical shifts for C-3′, C-10′, and C-11′ of **2** (δ_C 113.2, 180.8, and 162.5, respectively) were close to those for the corresponding position of polyandrocarpamide A[12] (δ_C 114.0, 183.3, and 165.8, respectively), indicating that C-3′ and C-11′ were connected via C-10′.

Connections of structural units **a**, **b**, and **c** were elucidated on the basis of fragmentation patterns of the ESIMS/MS spectrum of **2** (Fig. 4). Pyrinodemin F (**2**) was assigned as a mixture of two regioisomers, pyrinodemins F1 and F2, that possesses a triple bond at C-15/C-16 and C-16/C-17, respectively. Thus, the structure of pyrinodemin F was elucidated to be **2**.

Pyrinodemin E (**1**) was a new 3-alkylpyridine alkaloid possessing a 4-(methoxyamino)piperidinone moiety, while pyrinodemin F (**2**) was a new 3-alkylpyridine alkaloid possessing an indol-3-glyoxylamide moiety. Pyrinodemin E (**1**) showed cytotoxicity against P388 and L1210 murine leukemia cells (IC_{50}, 5.7 and 8.8 μg/mL, respectively) in vitro, while pyrinodemin F (**2**) did not show such activity.

Acknowledgments

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8. *Pyrinodemin E* (**1**): colorless oil; $[\alpha]_D^{21} \simeq 0$ (*c* 0.28, MeOH); UV (MeOH) λ_{max} 257 (ε 3200), 263 (3600), and 269 (2700) nm; IR (neat) ν_{max} 3211, 2930, 2855, 2362, and 1668 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS *m*/*z* 442 $(M+H)^+$; HRESIMS *m*/*z* 442.34238 [$(M+H)^+$, Δ −0.97 mmu], calcd for $C_{27}H_{44}N_3O_2$, 442.34335.
9. *Pyrinodemin F* (**2**): colorless oil; UV (MeOH) λ_{max} 257 (ε 6700), 263 (6700), 269 (6100), and 324 (3700) nm; IR (neat) ν_{max} 3324, 2931, 2856, 1674, 1621, 1494, 1434, 1240, 1131 and 748 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS *m*/*z* 486

(M+H)$^+$; HRESIMS m/z 486.31242 [(M+H)$^+$, Δ −0.18 mmu], calcd for $C_{31}H_{40}N_3O_2$, 486.31260.

10. *Compound* **3**: colorless oil; ^{1}H NMR ($CDCl_3$) δ: 9.77 (s, CHO), 8.59 (br s, H-2), 8.58 (d, 5.7, H-6), 8.22 (d, 8.1, H-4), 7.83 (dd, 5.4, 7.9, H-5), 2.84 (t, 7.8, H_2-7), 2.44 (m, H_2-14), 1.70 (m, H_2-8), 1.35–1.26 (m, H_2-9 ∼ H_2-13); APCIMS m/z 220 (M+H)$^+$; HRAPCIMS m/z 220.16973 [(M+H)$^+$, Δ −0.41 mmu], calcd for $C_{14}H_{22}N_1O_1$, 220.17014.
11. *Compound* **4**: colorless oil; ^{1}H NMR ($CDCl_3$) δ: 9.77 (s, CHO), 8.59 (br s, H-2), 8.58 (d, 5.7, H-6), 8.22 (d, 8.1, H-4), 7.83 (dd, 5.4, 7.9, H-5), 2.84 (t, 7.8, H_2-7), 2.44 (m, H_2-15), 1.70 (m, H_2-8), 1.35–1.26 (m, H_2-9 ∼ H_2-14); APCIMS m/z 234 (M+H)$^+$; HRAPCIMS m/z 234.18539 [(M+H)$^+$, Δ −0.40 mmu], calcd for $C_{15}H_{24}N_1O_1$, 234.18579.
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