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Phormidinines A and B, novel 2-alkylpyridine alkaloids from the cyanobacterium *Phormidium* sp.

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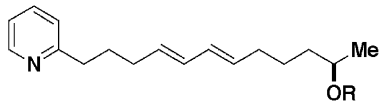
Abstract—Novel 2-alkylpyridine alkaloids, phormidinines A (**1**) and B (**2**), were isolated from cyanobacterium *Phormidium* sp. Their structures were determined by spectroscopic analysis. The absolute stereochemistry of **1** was established by the modified Mosher's method.

3-Alkylpyridine alkaloids are a well-known family of marine natural products. Although many 3-alkylpyridine alkaloids, such as navenones,[1] halitoxins,[2] niphatynes,[3] theonelladins,[4] ikimins,[5] xestamines,[6] niphatoxins,[7] niphatesines,[8] haminols,[9] cyclostellettamines,[10] and untenines[11] have been isolated from marine organisms, only one 2-alkylpyridine alkaloid, pulo'upone,[12] has been isolated from a marine mollusk. In our continuing search for new substances from marine organisms,[13–15] we investigated the constituents of the marine cyanobacterium *Phormidium* sp. collected at Bise, Okinawa, Japan, and isolated two new 2-alkylpyridine alkaloids, phormidinines A (**1**) and B (**2**). We report here the isolation and structural determination of **1** and **2**.



R=H Phormidinine A (**1**)
R=Ac Phormidinine B (**2**)

The marine mat-forming filamentous cyanobacterium *Phormidium* sp. (4.0 kg), collected at Bise, Okinawa, Japan in November 2003, was extracted with methanol (7.0 L) for 7 days. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation with column chromatography (silica gel, $CHCl_3$–MeOH; ODS silica gel, MeOH–H_2O) and reversed-phase HPLC (Develosil ODS-HG-5, MeOH–H_2O) to give phormidinines A (**1**) [2.3 mg] and B (**2**) [90 μg] as colorless oils.

The molecular formula of **1** was found to be $C_{17}H_{25}NO$ by ESIMS (*m/z* 260.2040, calcd for $C_{17}H_{26}NO$ $[M+H]^+$ 260.2014).[16] The NMR data for **1** are summarized in Table 1. The ^{13}C NMR spectrum and HMQC spectrum of **1** showed that there were nine unsaturated carbons, consisting of eight protonated sp^2 carbons (δ 149.5, 138.8, 133.3, 132.5, 132.3, 131.9, 124.7, and 122.8) and one non-protonated sp^2 carbon (δ 163.2), six saturated methylene carbons (δ 39.7, 38.2, 33.6, 33.2, 31.0, and 26.8), one methine carbon (δ 68.5) connected to an oxygen atom, and one methyl carbon (δ 23.5). The UV absorption at 262 nm (ε 3900) suggested a pyridine ring, which was confirmed by four aromatic ^1H NMR signals [δ 8.41 (1H, br d, J = 4.2 Hz, H-6), 7.74 (1H, ddd, 7.8, 7.4, 1.7 Hz, H-4), 7.29 (1H, d, 7.8 Hz, H-3), 7.23 (1H, dd, 7.4, 4.2 Hz, H-5)]. A detailed analysis of the COSY spectrum of **1** allowed three partial structures, C3–C6, C7–C11, and C12–C18, to be constructed, as shown in Figure 1. The connectivities of two partial structures, C3–C6 and C7–C11, were clarified by HMBC correlations: H3/C2, H7/C3, and H7/C2. Although no additional connectivities were obtained from the NMR analysis because of the overlap of the NMR signals of the diene unit, C11 and C12 in **1** should be connected

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Table 1. NMR data for **1** and **2** in CD_3OD

No.	Phormidinine A (**1**)		Phormidinine B (**2**)
	^1H[a,c]	^{13}C[b]	^1H[d]
2	163.2		
3	7.29 d (7.8)	124.7	7.30 d (7.9)
4	7.74 ddd (7.8, 7.4, 1.7)	138.8	7.76 ddd (7.9, 7.5, 1.8)
5	7.23 dd (7.4, 4.2)	122.8	7.23 dd (7.5, 5.0)
6	8.41 br d (4.2)	149.5	8.40 br d (5.0)
7	2.77 t (7.7)	38.2	2.77 t (7.7)
8	1.78 m 2H	31.0	1.78 m 2H
9	2.08 m 2H	33.2	2.10 m 2H
10	5.50 m	132.5*	5.55 m
11	6.00 m	131.9**	6.00 m
12	6.00 m	132.3**	6.00 m
13	5.50 m	133.3*	5.55 m
14	2.05 m 2H	33.6	2.06 m 2H
15a	1.45 m	26.8	1.53 m
15b	1.40 m		1.45 m
16	1.40 m 2H	39.7	1.43 m 2H
17	3.70 m	68.5	4.86 m
18	1.13 d (6.2) 3H	23.5	1.19 d (6.3) 3H
Ac			2.00 s

Assignments with the same superscript * and ** may be interchanged.
[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] Coupling constants (Hz) are in parentheses.
[d] Recorded at 600 MHz.

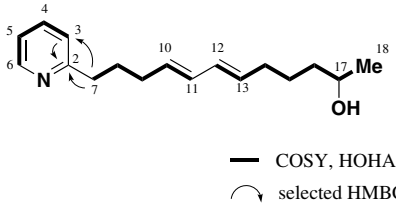
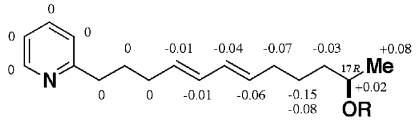


Figure 1. Structure of phormidinine (**1**), based on 2D NMR correlations.

considering of its molecular formula and degree of unsaturation. This was confirmed by a comparison of the chemical shifts of C10–C13 and H10–H13 with those of typical conjugated dienes.[17] Consequently, the entire carbon chain was assembled as shown in Figure 1, and the chemical shifts of all protons and carbons were assigned as shown in Table 1. The two double bonds were both determined to be in the *E*-configuration, based on the ^{13}C chemical shift values of allylic methylenes (δ 33.2 and 33.6).[17] This result was also supported by the ^1H NMR spectrum in which the magnitude of $^3J_{H10-11}$ was 13.9 Hz and that of $^3J_{H12-13}$ was 13.9 Hz as measured in C_6D_6.[18] Thus, the gross structure of phormidinine A (**1**) was determined to be as shown in Figure 1.

The absolute stereochemistry of **1** was determined by the modified Mosher's method.[19] Treatment of **1** with (*R*)- and (*S*)-MTPACl gave (*S*)- and (*R*)-MTPA esters **3** and **4**, respectively. The ^1H NMR signals of these two MTPA esters **3** and **4** were assigned on the basis of the COSY spectra, and the $\Delta\delta$ values ($\delta_S-\delta_R$, ppm) were then calculated. The results, shown in Figure 2, established that the absolute stereochemistry of C17 is *R*.



3; R=(*S*)-MTPA
4; R=(*R*)-MTPA

Figure 2. $\Delta\delta$ values ($\delta_S-\delta_R$) for the MTPA esters **3** and **4** in parts per million.

The molecular formula of **2** was found to be $C_{19}H_{27}NO_2$ by ESIMS (*m*/*z* 302.2093, calcd for $C_{19}H_{28}NO_2$ $[M+H]^+$ 302.2120), indicating that **2** is an acetyl derivative of **1**.[20] In both normal and reversed-phase chromatography, **2** was much less polar than **1**. A detailed analysis of the COSY spectra of **2** led to three partial structures, C3–C6, C7–C11, and C12–C18, the same as in **1**. The chemical shifts and coupling constants of protons of **2** closely resembled those of **1**, except for the downfield shift in the chemical shift of H17. This result and its chromatographic behavior suggested that **2** should be the acetate of **1**. To confirm the structure of **2**, **1** was acetylated, and the NMR spectra and CD data of acetate of **1**[21] were found to be identical to those of phormidinine B (**2**). Thus, the absolute stereochemistry of phormidinine B (**2**) was determined to be as shown in formula **2**.

In conclusion, two novel 2-alkylpyridine alkaloids, phormidinines A (**1**) and B (**2**), were isolated from marine cyanobacterium *Phormidium* sp. Their structures were determined based on 2D NMR spectra and the modified Mosher's method. Although many 3-alkylpyridines has been isolated from marine sources, 2-alkylpyridines from natural sources are rare, and only one 2-alkylpyridine alkaloid, pulo'upone,[12] has been previously isolated from a marine mollusk. In a previous investigation of the constituents of marine cyanobacterium *Phormidium* sp., a novel bromine-containing phormidolide[22] was isolated. In addition, Schirmer and co-workers reported that the five freshwater *Phormidium* species produce anti-tumor compounds and neurotoxins.[23] Thus, the five freshwater *Phormidium* species should be considered in environmental risk assessment but as well, as a source of therapeutic agents.

The biosynthesis of phormidinines is interesting. A structurally related compound, haminol-2 (**5**), has been isolated from the Mediterranean cephalaspidean mollusk *Haminoea orbignyana* (Fig. 3).[9] Fontana's group reported the biogenesis of haminol-2 in the marine mollusk *H. orbignyana*. Their feeding experiments proved that the pyridine ring and C7 originated from nicotinic acid, that is, they observed the incorporation of intact molecule of the precursor.[24] One plausible biogenetic

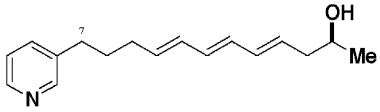


Figure 3. Structure of haminol-2 (**5**).

pathway for phormidinine A (**1**) is that the pyridine ring and C7 originate in picolinic acid rather than nicotinic acid. Another plausible biogenetic pathway is that **1** is synthesized by the formation of a pyridine ring from a linear C17 chain. Further investigations of their biogenesis and biological activities are in progress.

Acknowledgements

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