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SENEPODINE F, A NEW $C_{22}N_2$ ALKALOID FROM *LYCOPODIUM CHINENSE*

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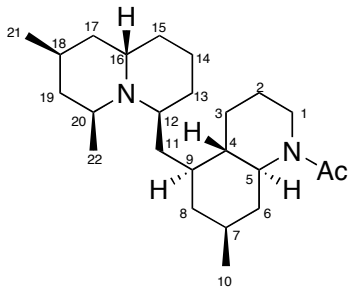
Abstract – A new $C_{22}N_2$ *Lycopodium* alkaloid, senepodine F (**1**), consisting of a decahydroquinoline and a quinolizidine ring has been isolated together with senepodines A (**2**) and E (**3**) from the club moss *Lycopodium chinense*. The relative stereochemistry of **1** was determined by NOESY correlations for a deacetylated derivative.

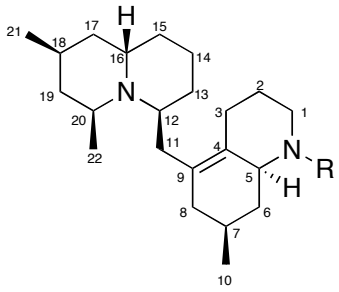
INTRODUCTION

Lycopodium alkaloids[1] with unique heterocyclic frameworks of $C_{11}N$, $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$ types have attracted great interest from biogenetic[1,2] and biological[3] points of view. A common feature in all *Lycopodium* alkaloids is a polycyclic carbon skeleton with varying levels of oxidation. These unique skeletons have also been challenging targets for total synthesis.[4] Among them, huperzine A is a highly specific and potent inhibitor of acetylcholinesterase (AChE).[3] The inherent inhibition of AChE has promoted the pursuit of the total synthesis[5] and SAR[6] studies of huperzine A. Recently we have isolated new types of alkaloids such as sieboldine A[7] from *Lycopodium sieboldii*, serratezomine A[8] from *L. serratum* var. *serratum*, complanadine A[9] and lyconadin A[10] from *L. complanatum*, senepodine A,[11] lyconesidine A,[12] and himeradine A[13] from *L. chinense*, cermizine A[14] from *L. cernuum*, and nankakurine A[15] from *L. hamiltonii*. Further investigation on extracts of *L. chinense* (Lycopodiaceae) resulted in the isolation of a new $C_{22}N_2$ alkaloid, senepodine F (**1**), as well as known related alkaloids, senepodines including senepodines A (**2**) and E (**3**),[11] lyconesidines,[12] and himeradine A.[13] This paper describes the isolation and structure elucidation of **1**.

This paper is dedicated to Dr. Pierre Potier on the occasion of his 70th birthday.



1



2: R=Me
3: R=Ac

RESULTS AND DISCUSSION

The club moss *L. chinense* was extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 0:1, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with hexane/EtOAc (3:2) was purified by a silica gel column ($CHCl_3$/MeOH → $CHCl_3$/MeOH/TFA) and then C_{18} HPLC to afford senepodine F (**1**, 0.01%), as well as known related alkaloids, senepodines including senepodines A (**2**, 0.003%) and E (**3**, 0.01%),[11] lyconesidines,[12] and himeradine A.[13]

Senepodine F (**1**) was shown to have the molecular formula of $C_{24}H_{42}N_2O$ by HRFABMS spectrum [*m/z* 375.3352, $(M+H)^+$, Δ -2.3 mmu]. The IR and NMR spectra were indicative of the presence of an *N*-acetyl group (1639 cm^{-1}; δ_H 2.09; δ_C 171.8). The presence of an amide carbonyl carbon [C-23, δ_C 171.8 (s)] was elucidated by HMBC correlations for H-1 and H-5 to C-23 through a nitrogen atom. ^1H and ^{13}C NMR spectral data of **1** were analogous to those of senepodine E (**3**),[11b] although two quaternary carbons at C-4 and C-9 lacking for **1** were observed for **3**. Thus, the structure of senepodine F was presumed to be the dihydro form at C-4 and C-9 of senepodine E (**3**). Alkaloids with *N*-acetyl group may produce *cis*/*trans* conformers which interconvert at a rate slow enough to give the broad signals. Since most of ^1H and ^{13}C NMR spectral signals of **1** were broadened as well as those of **3**, further connections could not be clarified by the NMR spectral analysis of **1**. Hydrolysis of **1** with 1N HCl afforded deacetylsenepodine F (**4**), which gave well-resolved sharp signals in ^1H and ^{13}C NMR spectra.

Deacetylsenepodine F (**4**) showed the pseudomolecular ion peak at *m/z* 333 $(M+H)^+$ in the FABMS spectrum, and the molecular formula, $C_{22}H_{40}N_2$, was established by HRFABMS spectrum [*m/z* 333.3279, $(M+H)^+$, Δ + 0.9 mmu]. Analysis of the ^1H and ^{13}C NMR spectral data (Table 1) and the HMQC spectrum

of **4** revealed the presence of eight sp^3 methines, eleven sp^3 methylenes, and three sp^3 methyl groups. Among them, one sp^3 methylene and four sp^3 methines were ascribed to those bearing a nitrogen atom.

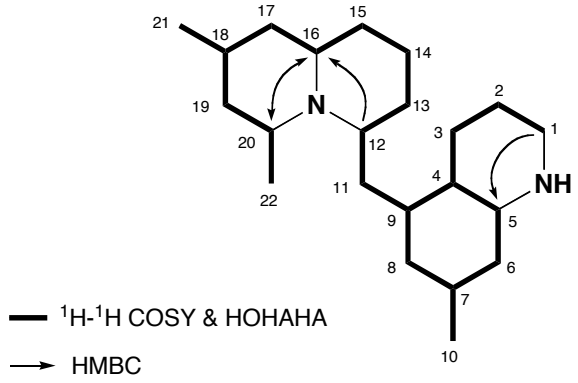


Figure 1. Selected 2D NMR Correlations for deacetylsenepodine F

The gross structure of **4** was deduced from extensive analyses of the two-dimensional NMR spectral data, including the ^{1}H-^{1}H COSY, HOHAHA, HMQC, and HMBC spectra in CD_3OD (Figure 1). The ^{1}H-^{1}H COSY and HOHAHA spectra in CD_3OD revealed connectivities of all the carbon atoms for deacetylsenepodine F (**4**). The connectivity of C-1 and C-5 through a nitrogen atom was implied by an HMBC correlation for H_2-1 to C-5. HMBC cross-peaks for H-12 and H-20 to C-16, and H-16 to C-20 indicated that C-12, C-16, and C-20 were connected to each other through a nitrogen atom. Thus, the gross structure of deacetylsenepodine F was elucidated to be **4** possessing a decahydroquinoline ring (C-1 ~ C-9 and N) with a methyl group at C-7 and a quinolizidine ring (C-12 ~ C-20 and N) with two methyl groups at C-18 and C-20 through a methylene carbon at C-11.

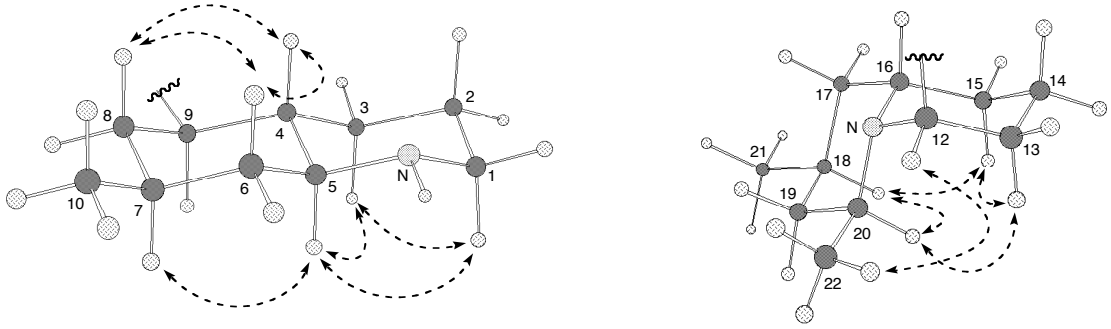


Figure 2. Selected NOESY Correlations and Relative Stereochemistry for deacetylsenepodine F (**4**).

The relative stereochemistry of **4** was elucidated by NOESY correlations and $^3J_{H\text{-}H}$ couplings (Figure 2). The H-5, H-7, H-12, H-18, and H-20 were assigned as all □-orientations and H-4 and H-16 as □-

orientations by NOESY cross-peaks as shown in Figure 2. The junction of the two piperidine rings with chair-forms in the quinolizidine ring (C-12 ~ C-20 and N) was elucidated to be *cis* by NOESY correlations of H-13/H-20, H-13/H-15, H-18/H-20, and H-15/H-18. On the other hand, both junctions at C-4 and C-9, and C-4 and C-5 of decahydroquinoline ring (C-1 ~ C-9 and N) were assigned to be *trans* by ^{3}J proton coupling constants of H-8b [δ_H 0.83 (1H, ddd, 11.8, 11.8, 11.8)] and H-6b [δ_H 1.09 (1H, ddd, 11.8, 11.8, 11.8)], and NOESY correlations of H-4/H-6 and H-4/H-8. Thus, the partial relative stereochemistry of **4** was assigned as shown in Figure 2.

Further investigation of structurally interesting alkaloids and biosynthetic intermediates to clarify the biogenetic pathway is in progress in our laboratory.

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Deacetylsenepodine F (**4**) in CD_3OD at 300K

	δ_H	δ_C	HMBC (^{1}H)
1a	2.92 (1H, ddd, 12.8, 12.8, 2.5)	45.5	2a, 3b
1b	3.32 (1H, m)		
2a	1.72 (1H, m)	23.7	1a, 3b
2b	1.93 (1H, m)		
3a	1.22 (1H, m)	27.3	1b, 2b, 4, 5
3b	2.16 (1H, m)		
4	1.21 (1H, m)	45.2	2a, 3b, 6, 11b
5	2.87 (1H, m)	60.7	1, 3b, 6
6a	1.90 (1H, m)	39.0	8a, 10
6b	1.09 (1H, ddd, 11.8, 11.8, 11.8)		
7	1.59 (1H, m)	31.5	6, 8, 10
8a	1.72 (1H, m)	42.3	6a, 10
8b	0.83 (1H, ddd, 11.8, 11.8, 11.8)		
9	1.30 (1H, m)	40.2	8b, 11b
10	0.95 (3H, d, 6.5)	22.2	
11a	1.72 (1H, m)	33.5	12, 13a
11b	1.99 (1H, m)		
12	3.67 (1H, m)	57.3	11b, 14a
13a	1.89 (1H, m)	21.7	15a
13b	1.60 (1H, m)		
14a	1.73 (1H, m)	18.1	12, 13a
14b	1.80 (1H, m)		
15a	2.12 (1H, m)	24.3	17a
15b	1.54 (1H, m)		
16	3.72 (1H, brd, 13.4)	55.2	12, 14a, 15a, 20
17a	1.60 (1H, m)	37.8	19b, 21
17b	1.75 (1H, m)		
18	1.94 (1H, m)	25.0	16, 17a, 19a, 21
19a	1.40 (1H, m)	41.6	21, 22
19b	1.87 (1H, m)		
20	3.86 (1H, m)	52.9	16, 19a, 22
21	0.90 (3H, d, 6.3)	21.6	
22	1.33 (3H, d, 6.2)	17.9	19a

EXPERIMENTAL

General Experimental Procedures. ^{1}H and 2D NMR spectra were recorded on a 600 MHz spectrometer at 300K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. NMR sample of senepodine F (**1**) was prepared by dissolving 1.0 mg in 30 mL of CD_3OD in 2.5 mm micro cells (Shigemi Co. Ltd.) and chemical shifts were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments. COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1 K data points for each of 256 t_1 increments. NOESY and HOHAHA spectra in the phase sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HMQC spectra in the phase sensitive mode and HMBC spectra, a total of 256 increments of 1 K data points were collected. For HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long-range C-H coupling. Zero-filling to 1 K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. FABMS was measured by using glycerol as a matrix.

Plant Material. The club moss *Lycopodium chinense* was collected at Kiyosato in Hokkaido in 2001. The botanical identification was made by Mr. N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen has been deposited in the herbarium of Hokkaido University.

Extraction and Isolation. The club moss (2 kg) of *L. chinense* was extracted with MeOH (10 L x 3) for 1 week at 20°C. The MeOH extract (146 g) was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, after being adjusted at pH 10 with sat. Na_2CO_3, were partitioned with $CHCl_3$. $CHCl_3$ soluble materials (2.4 g) were subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 0:1, and then $CHCl_3$/MeOH, 1:0 → 0:1). The fraction eluted with hexane/EtOAc (3:2) was separated by a silica gel column ($CHCl_3$/MeOH, 10:1 → $CHCl_3$/MeOH/TFA, 1:1:0.1) and then C_{18} HPLC (Mightysil RP-18 GP 250-20, 5mm, Kanto Chemical Co., 10 x 250 mm; eluent, 35% CH_3CN/0.1% TFA; flow rate, 2 mL/min; UV detection at 205 nm) to afford senepodines F (**1**, 0.01%), A (**2**, 0.003%), and E (**3**, 0.01%) together with known related alkaloids, senepodines,[11] lyconesidines,[12] and himeradine A.[13]

Senepodine F (1). colorless solid; $[\alpha]_D^{23}$ –35° (*c* 0.5, MeOH); IR (neat) ν_{max} 2922, 2862, 1639, and 1435 cm^{-1}; ^{1}H NMR (CD_3OD) δ: 0.95 (3H, d, 6.2), 1.36 (3H, d, 6.3), 2.09 (3H, s), 3.19 (1H, br t, 13.2), 3.69 (3H, m), 3.83 (1H, br d, 12.9), 3.92 (1H, m), 4.79 (1H, ddd, 12.7, 4.3, 4.3); ^{13}C NMR (CD_3OD) δ: 18.0, 18.1, 21.6, 22.6, 24.5, 25.0, 26.1, 27.2, 27.8, 33.0, 33.1, 33.2, 38.1, 38.7, 41.6, 41.9, 43.0, 53.0, 55.5, 56.9, 171.8. FABMS *m/z* 375 $(M+H)^+$; HRFABMS *m/z* 375.3352 (M+H; calcd for $C_{24}H_{43}N_2$, 375.3375).

Hydrolysis of Senepodine F (1). Senepodine F (**1**, 8 mg) in 1N HCl (2 mL) was allowed to stand at 100 °C for 1 day. After evaporation of solvent, the residue was applied to C_{18} (Phenomenex LUNA

C18(2), 5mm, Shimadzu, 10 x 250 mm; eluent, 25% CH_3CN/0.1% TFA; frow rate, 2 mL/min) to give deacetylsenepodine F (**4**, 2.0 mg), colorless solid; $[a]_D^{23}$ +21° (*c* 0.5, MeOH); IR (neat) ν_{max} 3276, 2921, 2860, 1452, and 1115 cm^{-1}; FABMS *m/z* 333 $(M+H)^+$; HRFABMS *m/z* 333.3279 (M+H; calcd for $C_{22}H_{41}N_2$, 333.3270).

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