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Himeradine A, a Novel $C_{27}N_3$-Type Alkaloid from *Lycopodium chinense*

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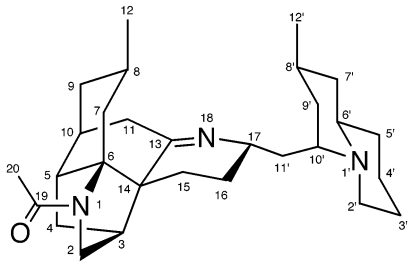
Abstract: A novel $C_{27}N_3$-type *Lycopodium* alkaloid consisting of a fastigiatine-type skeleton ($C_{16}N_2$) and a quinolizidine moiety ($C_{11}N$), himeradine A (**1**), has been isolated from the club moss *Lycopodium chinense*, and the structure and relative stereochemistry were elucidated on the basis of spectroscopic data.

Lycopodium alkaloids[1] with unique heterocyclic frameworks of $C_{16}N$, $C_{16}N_2$, and $C_{27}N_3$ types have attracted great interest from biogenetic[1,2] and biological[3] points of view. These unique skeletons have also been challenging targets for total synthesis.[4] Recently we have isolated serratezomines A, B, and C[5] with seco-serratinine-type, serratinine-type, and lycodoline-type skeletons, respectively, from the club moss *Lycopodium serratum* var. *serratum*, complanadine A[6] with a lycodine-dimeric skeleton and lyconadin A[7] consisting of a fused pentacyclic ring system from *L. complanatum*, and senepodine A[8] and lyconesidines A−C[9] from *L. chinense*. Further investigation of the extracts of *L. chinense* resulted in the isolation of himeradine A (**1**), a novel $C_{27}N_3$-type alkaloid consisting of a fastigiatine-type skeleton ($C_{16}N_2$) and a quinolizidine moiety ($C_{11}N$). In this paper we describe the isolation and structure elucidation of **1**.

Isolation of Himeradine A (1). 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 at pH 10 with sat. 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), in which a fraction eluted with hexane/EtOAc (3:2) was purified by a silica gel column ($CHCl_3$/MeOH/EtOAc, 10:1:0.5) followed by C_{18} HPLC (26% CH_3CN/0.1% TFA) to afford himeradine A (**1**, 2.0 mg, 0.001% yield) as TFA salts together with known related alkaloids, lucidine B[10] (**2**, 0.001%), lyconesidines A[9] (0.002%), B[9] (0.005%), and C[9] (0.003%), lycodoline[11] (0.001%), and senepodine A[8] (0.003%).



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Structure of Himeradine A (1). Himeradine A (**1**) showed the pseudomolecular ion peak at *m*/*z* 452 $(M + H)^+$ in the FABMS spectrum, and the molecular formula, $C_{29}H_{45}N_3O$, was established by HRFABMS [*m*/*z* 452.3647, $(M + H)^+$, Δ +0.6 mmu]. IR absorptions implied the presence of amide carbonyl and/or imine (1640 cm^{-1}) functionalities. Analysis of ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of two sp^2 and two sp^3 quaternary carbons, eight sp^3 methines, fourteen sp^3 methylenes, and three methyl groups. Among them, two sp^3 methylene (δ_C 57.5; δ_H 3.34 and 3.80; δ_C 52.9; δ_H 3.19 and 3.33), three sp^3 methines (δ_C 56.6; δ_H 4.09; δ_C 58.4; δ_H 3.24; δ_C 60.0; δ_H 3.77), and one sp^3 quaternary carbon (δ_C 76.1) were ascribed to those bearing a nitrogen. The remaining carbons were assigned as one amide carbonyl carbon (δ_c 174.3) and one sp^2 iminium carbon[12] (δ_C 196.7).

The gross structure was deduced from extensive analyses of the 2D NMR data of **1** including the $^1H-^1H$ COSY, HOHAHA, HMQC, HMBC, and HMQC−HOHAHA spectra in CD_3OD and pyridine-d_5 (Figure 1). The HMBC correlation (Table 1) for H-11′ to C-10′ (δ_C 35.5) gave rise to the connectivity between C-10′ and C-11′. The connectivity between C-3 and C-15 through C-14 was

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TABLE 1. ^{1}H and ^{13}C NMR Data of Himeradine A (1) in CD_3OD at 300 K

	δ_H	δ_C	HMBC (^{1}H)		δ_H	δ_C	HMBC (^{1}H)
1				17	4.09 (1H, m)	56.6	11a′
2a	3.34 (1H, m)	57.5	4a	18			
2b	3.80 (1H, m)			19		174.3	2a[a], 20
3	2.70 (1H, brs)	45.4	15a	20	2.02 (3H, s)	24.4	
4a	1.73 (1H, m)	31.6	2	1′			
4b	1.97 (1H, m)			2a′	3.19 (1H, m)	52.9	
5	2.57 (1H, brs)	43.0	3, 7a, 9b, 11a[a]	2b′	3.33 (1H, m)		
6		76.1	7, 10	3a′	1.82 (1H, m)	24.6	4b′, 5b′
7a	1.78 (1H, m)	35.0	9b, 12	3b′	1.93 (1H, m)		
7b	3.01 (1H, brt, 12.9)			4a′	1.54 (1H, m)	22.9	
8	1.26 (1H, m)	27.7	7b, 9a, 10, 12	4b′	1.82 (1H, m)		
9a	1.30 (1H, m)	40.7	7a, 11a[a], 12	5a′	1.55 (1H, m)	32.5	7b′
9b	1.65 (1H, m)			5b′	1.93 (1H, m)		
10	2.35 (1H, brs)	35.5	4a, 9a, 11a[a]	6′	3.24 (1H, m)	58.4	5a′, 7a′
11a	2.35 (1H, m)[a]	39.0[a]		7a′	1.19 (1H, brq, 13.2)	40.5	9b′, 12′
11b	2.79 (1H, m)[a]			7b′	1.90 (1H, m)		
12	0.96 (3H, d, 4.6)	22.8		8′	1.92 (1H, m)	24.2	7a′, 12′
13		196.7	3[a], 11a[a]	9a′	1.65 (1H, m)	37.0	7b′, 12′
14		57.1	2a, 7b, 11a[a]	9b′	1.95 (1H, m)		
15a	1.80 (1H, m)	20.6	16a	10′	3.77 (1H, m)	60.0	11a′
15b	1.96 (1H, m)			11a′	2.10 (1H, m)	31.1	9a′
16a	1.75 (1H, m)	26.0	15a	11b′	2.49 (1H, brd, 11.8)		
16b	2.29 (1H, m)			12′	0.98 (3H, d, 5.9)	21.5	

[a] In pyridine-d_5.

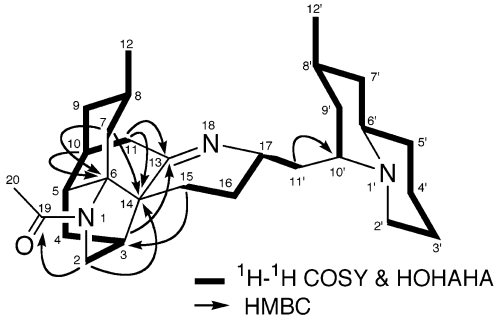

FIGURE 1. Selected 2D NMR correlations for himeradine A (**1**).

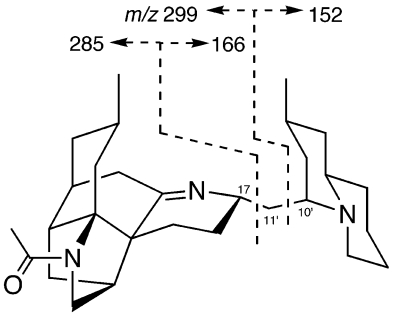

FIGURE 2. Fragmentation patterns observed in positive ion FABMS/MS spectrum of himeradine A (**1**) (precursor ion *m*/*z* 452).

elucidated by HMBC cross-peaks for H-2a (δ_H 3.34) to C-14 (δ_C 57.1) and H-15a (δ_H 1.80) to C-3 (δ_C 45.4). HMBC correlations for H-10 (δ_H 2.35) and H_2-7 (δ_H 1.78 and 3.01) to C-6 (δ_C 76.1), and H-7b (δ_H 3.01) to C-14 revealed connectivities among C-5, C-7, and C-14 through C-6. The connectivity between C-11 and C-14 through an iminium carbon (C-13) was implied by long-range correlations for H-11a (δ_H 2.35) to C-13 (δ_C 196.7) and C-14, and H-3 (δ_H 2.70) to C-13. The HMBC cross-peak for H-2a to C-19 (δ_C 174.3) indicated that an acetyl group was attached to N-1. Further evidence supporting the proposed structure of **1** was provided by tandem mass spectrometry through examination of the collision-induced dissociation (CID) mass spectrum of the $(M + H)^+$ ion. The positive ion FABMS/MS spectrum of **1** showed product ion peaks generated by fissions at the bonds between C-10′ and C-11′, and between C-11′ and C-17 (Figure 2). Thus, the gross structure of himeradine A was elucidated to be **1** possessing a fused-pentacyclic fastigiatine-type ring system[13] consisting of a tetrahydropyridine ring (N-18, C-13 to C-17), a bicyclo[3.3.1]nonane ring (C-5 to C-11, C-13, and C-14) with a methyl group at C-8, and a 2-azabicyclo-[2.2.1]heptane ring (N-1, C-2 to C-6, and C-14) with an acetyl group at N-1, which was further connected through C-11′ to a quinolizidine ring (N-1′ and C-2′ to C-10′) with a methyl group at C-8′.

Stereochemistry of Himeradine A (1). The relative stereochemistry of **1** was elucidated by NOESY correlations and $^3J_{H-H}$ coupling as depicted in the computer-generated 3D drawing (Figure 3).[14,15] Conformations of the quinolizidine ring (N-1′, C-2′ to C-10′) and the bicyclo-[3.3.1]nonane ring (C-5 to C-11, C-13, and C-14), in which all the six-membered rings took chair forms, were deduced from NOESY correlations as shown in Figure 3, except for the stereochemistry at C-17 and C-10′. Large ^3J coupling constant (12.9 Hz) between H-8 and H-7b indicated the configuration of the methyl group at C-8 as shown in Figure 3. NOESY correlations for H-17/H-10′, H-11′a/H-8′ and H-6′, and H-11′b/H-2′ were observed for free base of **1** (Figure 4), indicating that H-17 and H-10′ were both β configurations. The large vicinal

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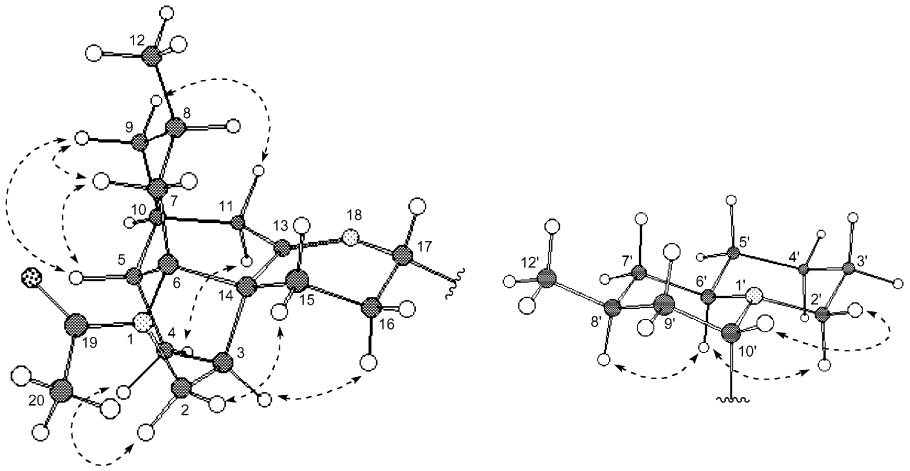
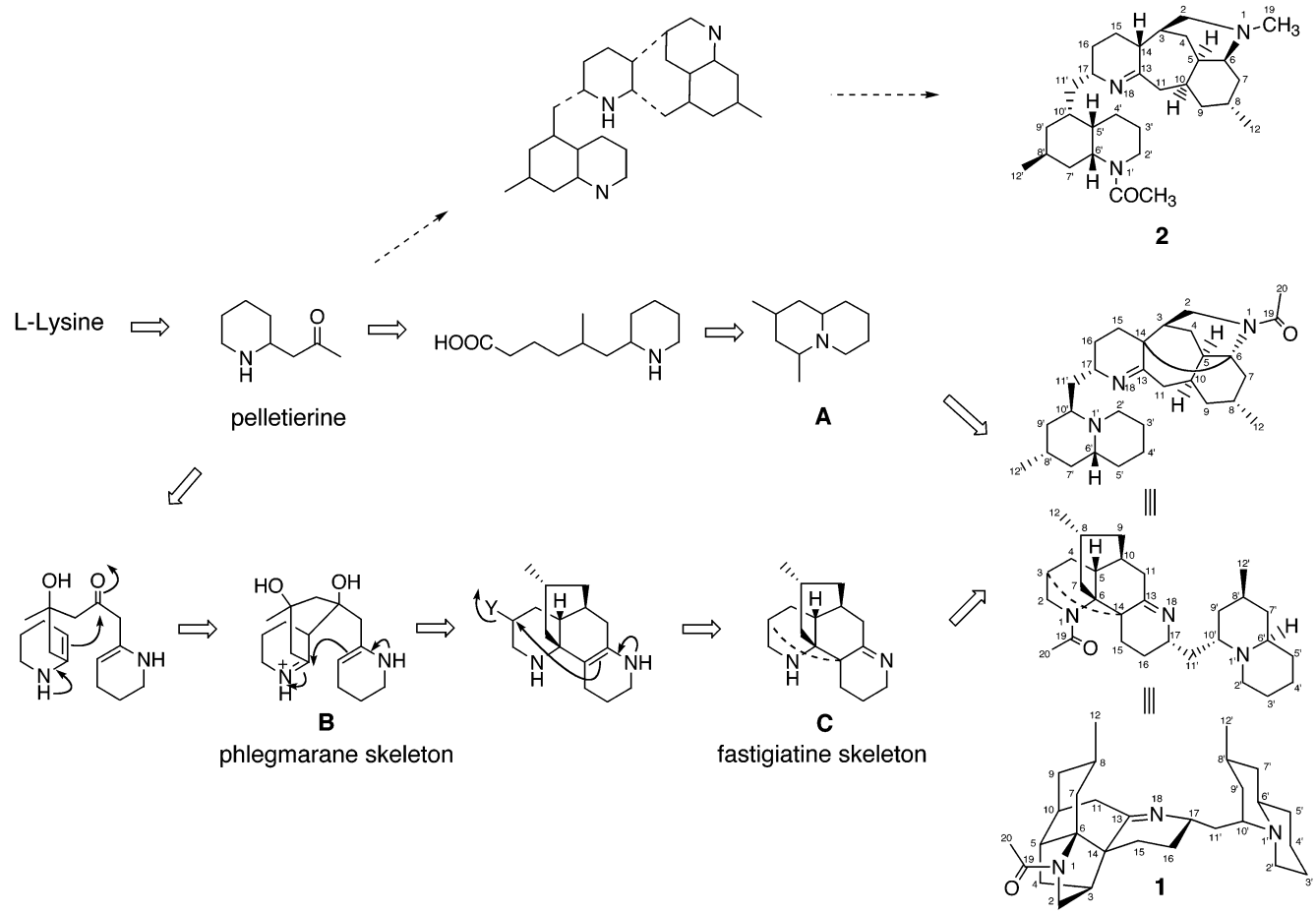


FIGURE 3. Selected NOESY correlations (dotted arrows) and relative stereochemistry for himeradine A (**1**).

SCHEME 1



coupling constant (11.8 Hz) for H-11′b/H-17 and H-11′b/H-10′ indicated that the two heterocyclic ring systems were almost fixed through C-11′. The conformational space for **1** searched by Monte Carlo simulation[14,15] followed by minimization was consistent with the NOESY data and proton vicinal coupling constants. Thus, the relative stereochemistry of **1** was assigned as shown in Figure 4.

Plausible Biogenesis of Himeradine A (1). A plausible biogenetic pathway for himeradine A (**1**) is proposed as shown in Scheme 1. Himeradine A (**1**) might be generated from a quinolizidine unit (**A**) and fastigiatine skeleton (**C**), through phlegmarine skeleton (**B**) derived from L-lysine via pelletierine. The fastigiatine skeleton (**C**) has been found only in the structure of fastigiatine isolated from *L. fastigiatum*.[13] Ayer's proposal[16] for lucidine B (**2**) is also showed in Scheme 1, in

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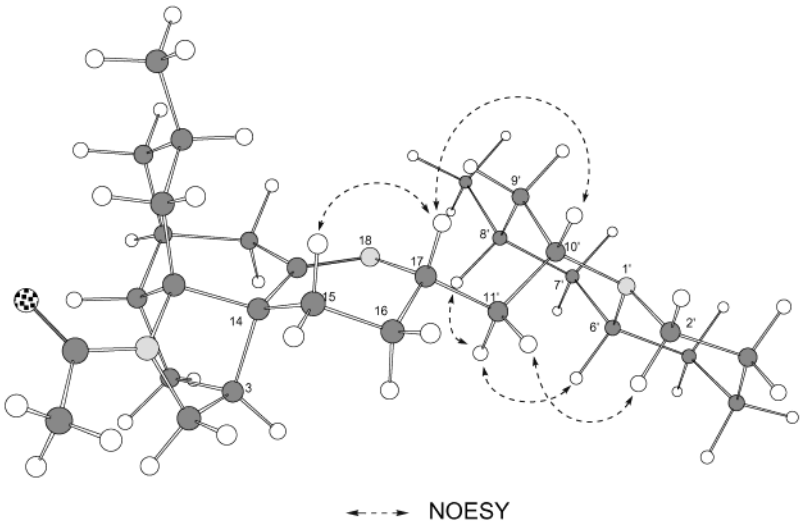

FIGURE 4. Stable conformer analyzed by Monte Carlo simulation followed by minimization and selected NOESY correlations for himeradine A (**1**).

which **2** is generated from two (enantiomeric) $C_{11}N$ units and a piperidine ring. In the present study, isolation of himeradine A (**1**) and lucidine B (**2**) from the same plant suggests that **2** might be also derived from $C_{16}N_2$ and $C_{11}N$ units such as **1**.

Bioactivity of Himeradine A (1). Himeradine A (**1**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 10 μg/mL) in vitro.

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Supporting Information Available: Experimental procedures, 1D and 2D NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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