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Nankakurine A, a Novel $C_{16}N_2$-Type Alkaloid from *Lycopodium hamiltonii*

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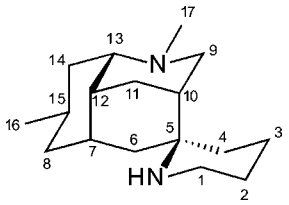
ABSTRACT



nankakurine A (**1**)

A novel, fused-tetracyclic *Lycopodium* alkaloid, nankakurine A (1), consisting of a cyclohexane ring and a 3-aza-bicyclo[3.3.1]nonane ring connected to a piperidine ring through a spiro carbon, was isolated from the club moss *Lycopodium hamiltonii*. The structure and relative stereochemistry were elucidated on the basis of spectroscopic data.

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 prompted the pursuit of the total synthesis[5] and SAR[6] studies of huperzine A. Recently, we 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

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

	δ_H	δ_C	HMBC
1	2.82 (2H, t, 5.1)	41.0	
2a	1.58 (1H, m)	26.3	1a
2b	1.53 (1H, m)		
3	1.57 (2H, m)	20.9	1
4	1.66 (2H, m)	34.6	
5		56.1	1a, 4, 6a, 7, 9b, 11a
6a	2.29 (1H, dd, 12.7, 12.7)	40.0	8
6b	1.64 (1H, m)		
7	1.85 (1H, m)	34.5	11
8a	1.49 (1H, m)	41.9	6a, 14b, 16
8b	1.20 (1H, ddd, 12.6, 12.6, 5.0)		
9a	3.00 (1H, ddd, 12.1, 2.4, 2.4)	58.5	11b, 17
9b	2.14 (1H, dd, 12.1, 2.8)		
10	1.81 (1H, m)	37.4	11b
11a	1.83 (1H, m)	32.5	
11b	1.53 (1H, m)		
12	1.53 (1H, m)	36.9	8b, 10, 11b, 14b
13	2.03 (1H, m)	65.1	9b, 11b, 14b, 17
14a	2.02 (1H, m)	40.0	8b, 16
14b	0.89 (1H, ddd, 12.1, 12.1, 2.1)		
15	1.95 (1H, m)	22.0	16, 8a
16	0.85 (3H, d, 6.6)	23.0	
17	2.12 (3H, s)	43.4	

himeradine A[13] from *L. chinense*, and cermizine A[14] from *L. cernuum*. During our continuing search for biogenetically interesting intermediates and new alkaloids with a novel skeleton from *Lycopodium* species, nankakurine A (**1**),[15] a novel alkaloid with an unprecedented caged system consisting of a cyclohexane ring and a 3-aza-bicyclo[3.3.1]nonane ring connected to a piperidine ring through a spiro carbon was isolated from the club moss *L. hamiltonii*. In this paper we describe the isolation and structure elucidation of **1**, which is a member of the $C_{16}N_2$ subclass.

The club moss *L. hamiltonii* 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), in which a fraction eluted with hexane/EtOAc (1:1) was purified by a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1) to afford nankakurine A (**1**, 1.6 mg, 0.003% yield) together with a known alkaloid, alopecuridine[16] (2.6 mg, 0.005%).

Nankakurine A {**1**, $[\alpha]^{21}_D$ +16° (*c* 0.4, MeOH)} showed the pseudomolecular ion peak at *m*/*z* 263 $(M + H)^+$ in the FABMS spectrum, and the molecular formula, $C_{17}H_{30}N_2$, was established by HRFABMS [*m*/*z* 263.2497, $(M + H)^+$, Δ +1.0 mmu]. IR absorptions implied the presence of an amine

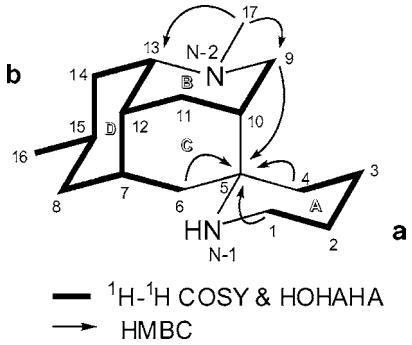

Figure 1. Selected two-dimensional NMR correlations for nankakurine A (**1**).

(3300 cm^{-1}) functionality. Analysis of the ^{1}H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of one sp^3 quaternary carbon, five sp^3 methines, nine sp^3 methylenes, and two methyl groups. Among them, two sp^3 methylene (δ_C 41.0; δ_H 2.82; δ_C 58.5; δ_H 2.14 and 3.00), one sp^3 methine (δ_C 65.1; δ_H 2.03), and one sp^3 quaternary carbon (δ_C 56.1) were ascribed to those bearing a nitrogen atom.

The gross structure of **1** was deduced from extensive analyses of the two-dimensional NMR 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 two partial structures **a** (C-1 ∼ C-4) and **b** (C-6 to C-8, C-9 ∼ C-16, C-7 to C-12, and C-8 to C-15) as shown in Figure 1. HMBC correlations were observed for H-1 and H-4 to C-5 (δ_C 56.1), suggesting that C-1 and C-4 were connected to each other through C-5 and a nitrogen atom to form a piperidine ring (ring A). The connectivities of C-17 to C-9 and C-13 through a nitrogen atom were implied by HMBC correlations for H_3-17 to C-9 (δ_C 58.5) and C-13 (δ_C 65.1). HMBC cross-peaks for H-9b and H-6a to C-5 (δ_C 56.1) indicated that a 3-aza-bicyclo[3.3.1]nonane ring (rings B and C) was connected to a piperidine ring (ring A) through a spiro carbon (C-5). ^{1}H−^{1}H correlations observed in the ^{1}H−^{1}H COSY and HOHAHA spectra indicated that a cyclohexane ring (ring D) with a methyl at C-15 was connected to the 3-aza-bicyclo-[3.3.1]nonane ring at C-7, C-12, and C-13.

Thus, the gross structure of nankakurine A was elucidated to be **1** possessing an unprecedented caged skeleton consisting of a cyclohexane ring (C-7 ∼ C-8 and C-12 ∼ C-15) with a methyl group at C-15 and a 3-aza-bicyclo[3.3.1]-nonane ring (C-5 ∼ C-7, C-9 ∼ C-13, and N-2) with a *N*-methyl group (C-17) connected to a piperidine ring (N-1 and C-1 ∼ C-5) through a spiro carbon at C-5.

The relative stereochemistry of **1** was elucidated by NOESY correlations and $^3J_{H-H}$ couplings as depicted in the computer-generated three-dimensional drawing (Figure 2). Conformations of the piperidine ring (N-1, C-1 ∼ C-5), the bicyclo[3.3.1]nonane ring (C-5 ∼ C-7, C-9 ∼ C-13, and N-2), and the cyclohexane ring (C-7, C-8, and C-12 ∼ C-15), in which all of the six-membered rings took chair forms,

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(15) Nankakurine A (**1**): colorless solid; $[\alpha]^{21}_D$ +16° (*c* 0.4, MeOH); IR (neat) ν_{max} 3300, 2920, 2765, 1450, 1340, 1270, 1110, and 1050 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); FABMS *m*/*z* 263 $(M + H)^+$; HRFABMS *m*/*z* 263.2497 (M + H; calcd for $C_{17}H_{31}N_2$, 263.2487).

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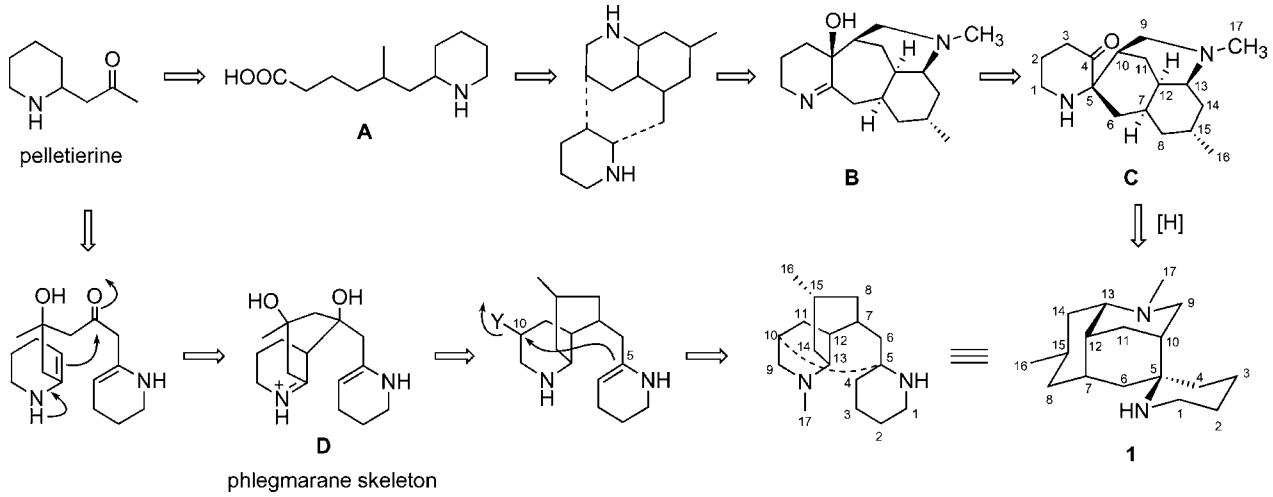
Scheme 1. Plausible Biogenetic Pathway for Nankakurine A (**1**)



were deduced from NOESY correlations as shown in Figure 2. Small ^3J coupling constants between H-12 and H-13 and between H-12 and H-7 indicated that the cyclohexane ring (ring D) was cis-fused with the 3-aza-bicyclo[3.3.1]nonane system (rings B and C). The NOESY correlation of H-15 to H-6a led to assignment of the stereochemistry at C-15 as shown in Figure 2. Stereochemistry of the spiro carbon at C-5 was elucidated by the NOESY correlations of H-1b and H-11a, and H-10. Thus, the relative stereochemistry of **1** was assigned as shown in Figure 2.

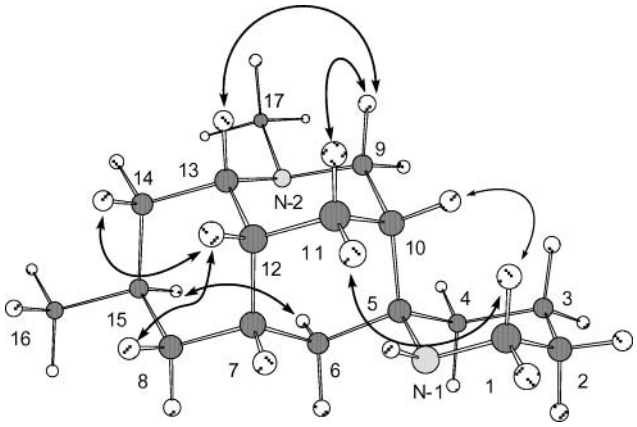


Figure 2. Selected NOESY correlations and relative stereochemistry for nankakurine A (**1**).

A plausible biogenetic pathway for nankakurine A (**1**) is proposed in Scheme 1. Nankakurine A (**1**) might be generated from a piperideine unit and a decahydroquinoline unit derived from an intermediate **A** via intermediates **B** and **C**. This path is close to part of the biogenesis for lucidine B[17] and oxolucidine B[18] proposed by Ayer et al., and the skeleton of **1** corresponds to a part of spirolucidine.[18] On the other hand, an alternative pathway for **1** through a phlegmarane skeleton (**D**), followed by the bond formation between C-5 and C-10 is also possible.

Nankakurine A (**1**) exhibited cytotoxicity against human epidermoid carcinoma KB cells (IC_{50}, 3.1 μg/mL) in vitro.

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Supporting Information Available: One- and two-dimensional NMR spectra for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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