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SERRALONGAMINE A, A NEW *LYCOPODIUM* ALKALOID FROM *LYCOPODIUM SERRATUM* VAR. *LONGIPETIOLATUM*

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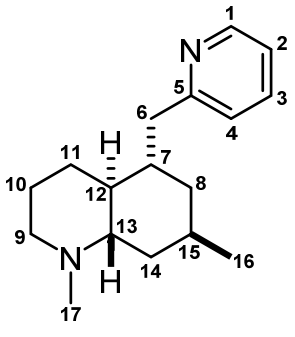
Abstract – A new *Lycopodium* alkaloid, serralongamine A (**1**), has been isolated from the club moss *Lycopodium serratum* var. *longipetiolatum*, and the structure was elucidated on the basis of spectroscopic data.

Club moss (Lycopodiaceae) are known to be a rich source of *Lycopodium* alkaloids[1] possessing unique heterocyclic ring system such as $C_{16}N_1$, $C_{16}N_2$, and $C_{27}N_3$, which have attracted great interest from biogenetic,[2] synthetic,[3] and biological[4] points of view. In our continuing efforts to find new *Lycopodium* alkaloids,[5] a new phlegmarine-type alkaloid, serralongamine A (**1**), was isolated from the club moss *Lycopodium serratum* var. *longipetiolatum*. In this paper, we describe the isolation and structure elucidation of **1**.



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The club moss *L. serratum* var. *longipetiolatum* collected in Taiwan, was extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with saturated Na_2CO_3, were partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (*n*-hexane/EtOAc, then $CHCl_3$/MeOH), followed by a silica gel column ($CHCl_3$/MeOH, then $CHCl_3$/MeOH/H_2O/TFA). The fraction eluted with $CHCl_3$/MeOH/H_2O/TFA

(6/4/1/0.01) was purified by C_{18} HPLC (MeOH/H_2O/TFA, 14/86/0.1) to afford serralongamine A (**1**, 0.0008% yield), huperzine A,[4] huperzine B,[4] and lycoposerramine-V.[6]

Table 1. ^1H and ^{13}C NMR Data of Serralongamine A (**1**) in CD_3OD

Position	δ_H (ppm)	δ_C (ppm)
1	8.73 (1H, d 5.7 Hz)	143.8
2	7.85 (1H, dd 6.9, 5.7 Hz)	125.9
3	8.44 (1H, dd 8.0, 6.9 Hz)	146.6
4	7.88 (1H, d 8.0 Hz)	128.8
5		158.1
6a	3.51 (1H, dd 13.6, 4.2 Hz)	38.5
6b	2.66 (1H, dd 13.6, 10.9 Hz)	
7	2.04 (1H, m)	37.5
8a	1.38 (1H, m)	36.8
8b	1.15 (1H, brd 13.4 Hz)	
9a	3.54 (1H, m)	57.4
9b	3.12 (1H, ddd 13.4, 13.4, 2.4 Hz)	
10a	2.04 (1H, m)	24.1
10b	1.86 (1H, m)	
11a	2.24 (1H, m)	27.2
11b	1.41 (1H, m)	
12	1.48 (1H, m)	46.4
13	3.15 (1H, m)	66.0
14a	2.16 (1H, brd 12.8 Hz)	33.9
14b	1.60 (1H, ddd 12.8, 12.8, 4.6 Hz)	
15	2.22 (1H, m)	28.1
16	0.96 (3H, d 6.9 Hz)	18.3
17	2.87 (3H, s)	41.4

Serralongamine A {**1**, $[\alpha]_D^{18}$ -9.1 (*c* 0.6, MeOH)} was revealed to have the molecular formula, $C_{17}H_{26}N_2$, by HRESIMS data [*m/z* 259.2176, $[M+H]^+$, Δ +0.2 mmu]. The ^1H, ^{13}C NMR (Table 1), and HMQC spectra of **1** showed signals due to one sp^2 quaternary carbon, four sp^2 methines, four sp^3 methines, six sp^3 methylenes, and two methyls. Among them, one sp^3 methine (δ_C 66.0), one sp^3 methylene (δ_C 57.4), and one methyl carbon (δ_C 41.4) were ascribed to those bearing a nitrogen atom. Also one sp^2 methine (δ_C 143.8) and one sp^2 quaternary carbon (δ_C 158.1) were assigned to those bearing the other nitrogen atom.

The gross structure of **1** was elucidated by analysis of 2D NMR data including the ^1H-^1H COSY, HMQC, and HMBC spectra (Figure 1). The ^1H-^1H COSY spectrum disclosed three structural units **a** (C-1 to C-4), **b** (C-6 to C-8), and **c** (C-9 to C-16). Connectivities of C-9, C-13, and C-17 through N-9 were revealed by HMBC cross-peaks of H_3-17 (δ_H 2.87) to C-9 (δ_C 57.4) and C-13 (δ_C 66.0). An HMBC cross-peak of H-3 (δ_H 8.44) to C-5 (δ_C 158.1) revealed that C-4 connected with C-5. An HMBC correlation for H-1 (δ_H 8.73)

to C-5 suggested the connectivity between C-1 and C-5 via N-1 to form the monosubstituted pyridine ring (C-1 to C-5, N-1). HMBC cross-peaks of H-6a, b (δ_H 3.51, 2.66) to C-4 (δ_C 128.8) and C-5 revealed that C-6 was attached to C-5. HMBC correlations for H-16 (δ_H 0.96) to C-8 (δ_C 36.8) suggested that C-8 connected with C-15. The connectivity of C-7 and C-12 was revealed by HMBC cross-peaks of H-6b and H-8a, b (δ_H 1.38, 1.15) to C-12 (δ_C 46.4). Thus, the gross structure of serralongamine A was elucidated to be **1** (Figure 1).

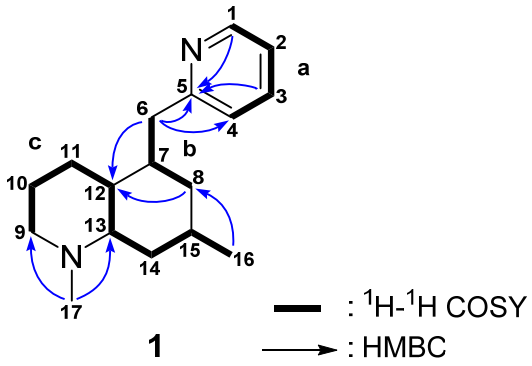

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Figure 1. Selected 2D NMR correlations for serralongamine A (**1**)

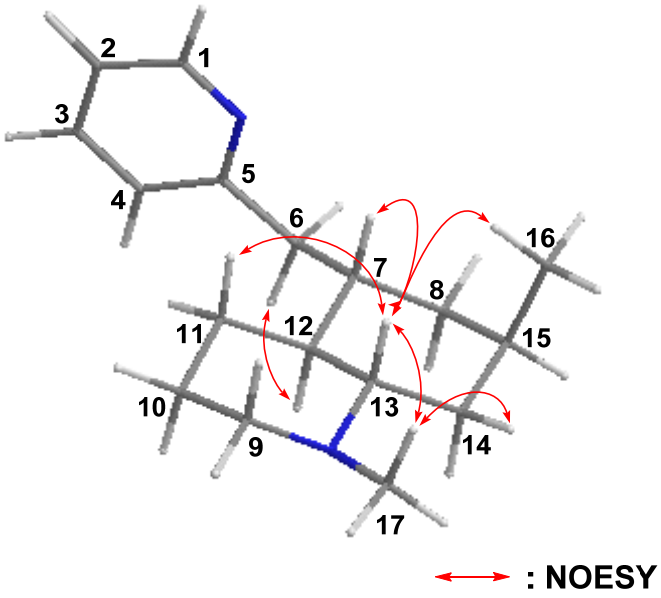
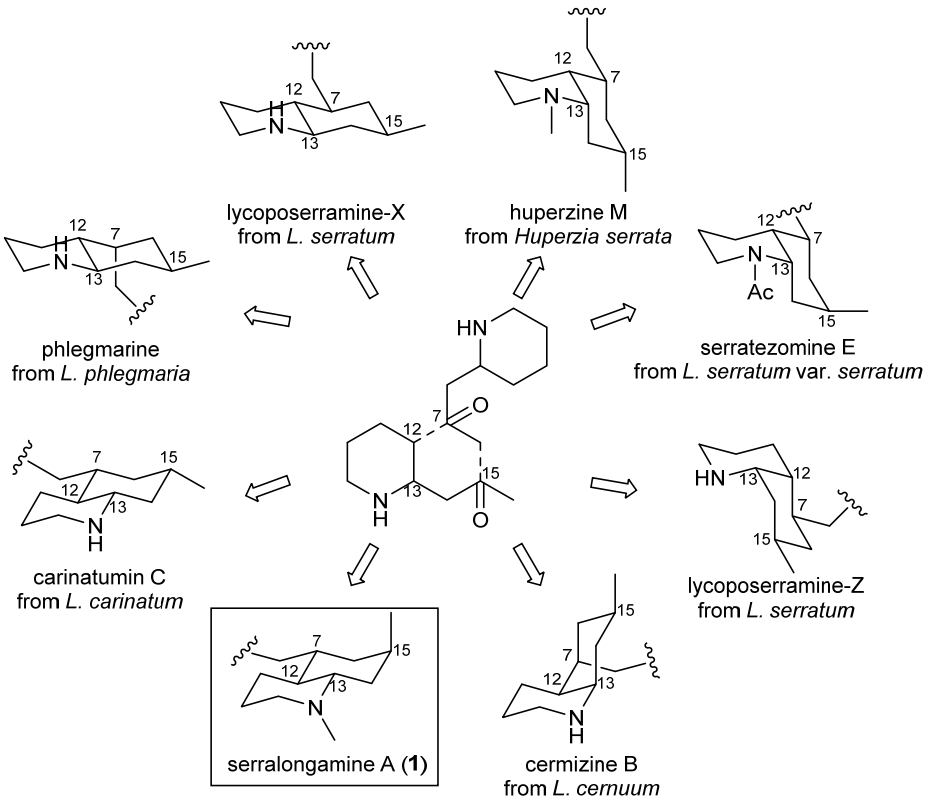


Figure 2. Selected NOESY correlations and relative stereochemistry for serralongamine A (**1**)

The NOESY spectrum of **1** showed cross-peaks as shown in Figure 2. NOESY correlations for H-13/H-7 and H-13/H_3-16 revealed that a cyclohexane ring (C-7-C-8 and C-12-C-15) was chair form and C-6 and C-16 were in an equatorial and an axial position of the cyclohexane ring, respectively. The chair form of the piperidine ring (C-9-C-13, N-9) was implied from NOESY cross-peaks of H-13/H-11b, H-17/H-13, and

H-17/H-14a, which indicated a trans-fused ring junction between the piperidine ring and the cyclohexane ring (C-7-C-8 and C-12-C-15). Thus, the relative stereochemistry of serralongamine A (**1**) was elucidated to be shown in Figure 2.

Serralongamine A (**1**) is a new phlegmarine-type *Lycopodium* alkaloid possessing a monosubstituted pyridine ring and a *trans* decahydroquinoline ring. There are many variations of decahydroquinoline ring system, stereochemistry of C-7, C-12, C-13, and C-15, in phlegmarine-type alkaloids isolated from *Lycopodium* spp., such as phlegmarine,[7] carinatumin C,[8] cermizine B,[9] lycoposerramine-X and Z,[10] huperzine M,[11] and serratezomine E.[12] The stereochemistry of the decahydroquinoline moiety of **1** is rare in phlegmarine-type alkaloids (Scheme 1). Serralongamine A (**1**) did not show acetylcholinesterase inhibitory activity[13] (IC_{50} > 100 μM).



Scheme 1. Decahydroquinoline ring diversity of phlegmarine-type alkaloids biosynthesized by the condensation of two pelletierine molecules

EXPERIMENTAL

Optical rotation was recorded on a JASCO P-1020 polarimeter. UV spectrum was recorded on a HITACHI U-1800 spectrophotometer. IR spectrum was recorded on a JASCO FT/IR-4100 spectrometer. NMR spectra were recorded on a JEOL JNM-ECX500 spectrometer using 3.0 mm microcells (Shigemi Co., Ltd.).

Chemical shifts (ppm) in CD_3OD are reported using residual CD_2HOD and CD_3OD (δ_H 3.31 and δ_C 49.0, respectively) as internal references. Positive-mode ESITOFMS was obtained on a Xevo G2-S QTof spectrometer (Waters Co., Ltd.) using a samle dissolved in MeOH.

Plant Material

The club moss *Lycopodium serratum* var. *longipetiolatum* was collected at Miaoli County in Taiwan. The botanical identification was made by Dr. Y.-C. Chen, Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University. A voucher specimen has been deposited in the herbarium of China Medical University.

Extraction and Isolation

The club moss *Lycopodium serratum* var. *longipetiolatum* (190 g, dry weight) was crushed and extracted with MeOH. The MeOH extract was treated with 3% tartaric acid (pH 3) and then partitioned with EtOAc. The aqueous layer was treated with Na_2CO_3 (aq) to pH 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction. The alkaloidal fraction was subjected to an amino silica gel column (*n*-hexane/EtOAc, then $CHCl_3$/MeOH), in which a fraction eluted with *n*-hexane/EtOAc (10:1) was purified by silica gel columns ($CHCl_3$/MeOH, 1:0 to 1:1 and then $CHCl_3$/MeOH/H_2O/TFA, 6:4:1:0 to 6:4:1:0.01). The fraction eluted with $CHCl_3$/MeOH/H_2O/TFA (6:4:1:0.01) was further purified by C_{18} HPLC (CAPCELL PAK C18 AQ (SHISEIDO), 5 μm, 10 mm I.D. x 250 mm, solvent MeOH/H_2O/TFA, 14:86:0.1, flow rate 2.5 ml/min, detection 254 nm) to afford serralongamine A (**1**, 0.0008% yield), huperzine A (0.06% yield),[4] huperzine B (0.03% yield),[4] and lycoposerramine-V (0.001% yield).[6]

Serralongamine A (1): A colorless amorphous solid; $[\alpha]_D^{18}$ -9.1 (*c* 0.6, MeOH); UV (MeOH) λ_{max} 261 (ε 1828); IR (KBr) ν_{max} 2928, 1594, and 1476 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESITOFMS *m/z* 259 $[M+H]^+$; HRESITOFMS *m/z* 259.2176 $[M+H]^+$; calcd for $C_{17}H_{27}N_2$, 259.2174).

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REFERENCES

1. For reviews of the *Lycopodium* alkaloids, see: Y. Hirasawa, J. Kobayashi, and H. Morita, *Heterocycles,* 2009, **77**, 679; J. Kobayashi and H. Morita, 'Alkaloids: The *Lycopodium* Alkaloids,' Vol. 61, ed. by G. A. Cordell, Academic Press, New York, 2005, pp. 1-57; X. Ma and D. R. Gang, *Nat. Prod. Rep.*, 2004, **21**, 752.
2. H. Luo, Y. Li, C. Sun, Q. Wu, J. Song, Y. Sun, A. Steinmetz, and S. Chen, *BMC Plant Biol.*, 2010, **10**, 209; H. Luo, C. Sun, Y. Li, Q. Wu, J. Song, D. Wang, X. Jia, R. Li, and S. Chen, *Physiol. Plant.*, 2010, **139**, 1; T. Hemscheidt and I. D. Spenser, *J. Am. Chem. Soc.*, 1996, **118**, 1799; T. Hemscheidt and I. D. Spenser, *J. Am. Chem. Soc.*, 1993, **115**, 3020.
3. R. Ding, J.-G. Fu, G.-Q. Xu, B.-F. Sun, and G.-Q. Lin, *J. Org. Chem.*, 2014, **79**, 240; X. Cheng and S. P. Waters, *Org. Lett.*, 2013, **15**, 4226; T. Nishimura, A. K. Unni, S. Yokoshima, and T. Fukuyama, *J. Am. Chem. Soc.*, 2013, **135**, 3243; J. N. Newton, D. F. Fischer, and R. Sarpong, *Angew. Chem. Int. Ed.*, 2013, **52**, 1726; J. A. Pigza, J.-S. Han, S. Chandra, D. Mutnick, M. Pink, and J. N. Johnston, *J. Org. Chem.*, 2013, **78**, 822.
4. J. S. Liu, Y. L. Zhu, C. M. Yu, Y. Z. Zhou, Y. Y. Han, F. W. Wu, and B. F. Qi, *Can. J. Chem.*, 1986, **64**, 837; X. Ma, C. Tan, D. Zhu, D. R. Gang, and P. J. Xiao, *J. Ethnopharmacol.*, 2007, **113**, 15.
5. K. Ishiuch, T. Kubota, H. Ishiyama, S. Hayashi, T. Shibata, and J. Kobayashi, *Tetrahedron Lett.*, 2011, **52**, 289; K. Ishiuchi, T. Kubota, H. Ishiyama, S. Hayashi, T. Shibata, K. Mori, Y. Obara, N. Nakahata, and J. Kobayashi, *Bioorg. Med. Chem.*, 2011, **19**, 749; K. Ishiuchi, T. Kubota, S. Hayashi, T. Shibata, and J. Kobayashi, *Tetrahedron Lett.*, 2009, **50**, 6534; K. Ishiuchi, T. Kubota, S. Hayashi, T. Shibata, and J. Kobayashi, *Tetrahedron Lett.*, 2009, **50**, 4221.
6. T. Shigeyama, K. Katakawa, N. Kogure, M. Kitajima, and H. Takayama, *Org. Lett.*, 2007, **9**, 4069.
7. L. NyemBo, A. Goffin, C. Hootele, and J.-C. Braekman, *Can. J. Chem.*, 1978, **56**, 851; A. Leniewski, J. Szychowski, and D. B. MacLean, *Can. J. Chem.*, 1981, **59**, 2479.
8. C. Y. Choo, Y. Hirasawa, C. Karimata, K. Koyama, M. Sekiguchi, J. Kobayashi, and H. Morita, *Bioorg. Med. Chem.*, 2007, **15**, 1703.
9. H. Morita, Y. Hirasawa, T. Shinzato, and J. Kobayashi, *Tetrahedron*, 2004, **60**, 7015.
10. K. Katakawa, M. Kitajima, K. Yamaguchi, and H. Takayama, *Heterocycles*, 2006, **69**, 223.
11. W.-Y. Gao, Y.-M. Li, S.-H. Jiang, and D.-Y. Zhu, *Helv. Chim. Acta*, 2008, **91**, 1031.
12. T. Kubota, H. Yahata, S. Yamamoto, S. Hayashi, T. Shibata, and J. Kobayashi, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3577.
13. G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, *Biochem. Pharmacol.*, 1961, **7**, 88.