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THREE NEW PHLEGMARINE-TYPE LYCOPODIUM ALKALOIDS, LYCOPOSERRAMINES–X, –Y AND –Z, HAVING A NITRONE RESIDUE, FROM *LYCOPODIUM SERRATUM*

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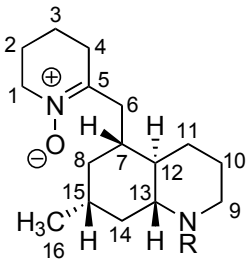
Abstract – Three new phlegmarine-type *Lycopodium* alkaloids, lycoposerramines-X (**2**), -Y (**3**), and -Z (**5**), having a nitrone residue were isolated from the club moss *Lycopodium serratum* Thunb. The structures of these new alkaloids, together with the stereostructures of the related alkaloids, huperzines J (**1**) and K (**4**), were elucidated by spectroscopic analyses.

INTRODUCTION

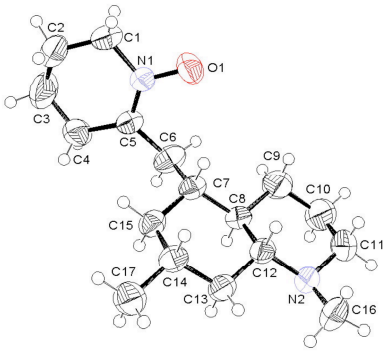
The genus *Lycopodium* is known as one of biological sources of structurally complex alkaloids including potent acetylcholine esterase inhibitors, and has been widely studied by many research groups.[2] In our continuing chemical study of *Lycopodium* plants,[3] we have isolated three new alkaloids, named lycoposerramines-X (**2**), -Y (**3**), and -Z (**5**), having a nitrone residue in the molecule. In this paper, we describe the structure elucidation of these new alkaloids as well as the determination of the hitherto ambiguous stereostructures of two known alkaloids, huperzine J (**1**) and K (**4**).[4]

RESULTS AND DISCUSSION

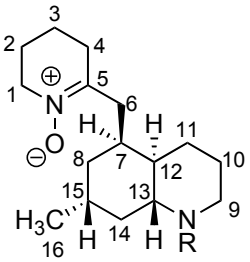
The crude alkaloidal fraction obtained by conventional procedure[3c] was purified by repeated column chromatography to afford three new alkaloids, lycoposerramines-X (**2**, 0.04% based on the crude base), -Y (**3**, 1.16%), and -Z (**5**, 0.04%), together with two known alkaloids, huperzine J (**1**, 2.50%) and huperzine K (**4**, 0.07%).



huperzine J (**1**): R=CH_3
lycoposerramine-X (**2**): R=H



ORTEP view of **1**



lycoposerramine-Y (**3**): R=CH_3
huperzine K (**4**): R=H

Figure **1**

Alkaloid (**1**) was obtained as colorless prisms ($C_{17}H_{30}N_2O$, mp. 120–123°C). Comparison of the spectroscopic data of **1** with those of huperzine J in the literature[4] revealed that our compound was the same as the phlegmarine-type *Lycopodium* alkaloid, huperzine J, isolated by a Shanghai group in 2000. However, they proposed the stereochemistry at the ring junction in the decahydroquinoline moiety in huperzine J to be a trans-fused form without any solid evidence, although they proved the relative stereochemistry at the C7, C13, and C15 positions by NOE experiments. X-Ray crystallographic analysis in the present study demonstrated the trans-fused bicyclic system in huperzine J (**1**).

New alkaloid (**2**), named lycoposerramine-X, was obtained as a colorless amorphous powder and its molecular formula was established to be $C_{16}H_{28}N_2O$ by HRFAB-MS analysis. The ^{1}H NMR spectrum strongly resembled that of **1** except for the absence of a signal for an *N*-methyl group. The ^{13}C NMR data indicated the presence of two sp^3 amino methylenes, one sp^3 amino methine, eight sp^3 methylenes, three sp^3 methines, one sp^3 secondary methyl group, and a unique sp^2 carbon signal at δ_C 148.5 ascribable to the nitrone carbon as in **1**. All the carbons were assigned on the basis of ^{1}H-^{1}H COSY, HMQC and HMBC spectra, enabling us to determine the structure of lycoposerramine-X to be a phlegmarine-type alkaloid like **1**. Elucidation of the stereochemistry at the four chiral centers (C7, C12, C13, and C15) in **2** by NOE experiments was difficult because of the severe overlapping of the concerned signals in the ^{1}H NMR spectrum. However, by comparing the chemical shifts of the carbons in **1** and **2** (see Table), particularly those of C7, C10, C12, C13, and C15, which may be affected by the stereochemistry at the chiral centers [a detailed discussion is given for alkaloids (**3**) and (**5**)], lycoposerramine-X was found to have the same stereostructure at the four chiral centers as **1**. Evidently, due to the absence of an *N*-methyl group in **2**, the signals at C9 and C13 exhibited an upfield shift compared with those in **1**. Thus, we proposed that lycoposerramine-X (**2**) is an *N*-demethyl derivative of huperzine J (**1**).

Table. ^{13}C NMR data for **1**, **2**, **3**, **4** and **5**

position	1	2	3	4	5
1	58.4	58.3	58.3	58.3	58.3
2	23.3	23.2	23.3	23.2	23.2
3	19.0	18.9	19.0	18.9	18.8
4	30.4	30.5[a]	29.9	30.1	30.0
5	148.6	148.5	148.5	148.2	149.0
6	36.1	35.6	35.8	35.2	35.9
7	37.8	37.4	33.0	32.6	29.6
8	40.8	41.0	37.7	37.8	41.1
9	57.7	46.3[b]	57.7	46.0	47.4
10	25.7	26.0	25.4	25.6	20.3
11	28.6	28.1	28.5	27.8	26.4[a]
12	46.4	46.6[b]	47.0	47.2	40.6
13	68.2	60.7	63.4	56.0	56.6
14	39.0	41.2	35.7	37.8	40.6
15	30.7	30.6[a]	27.3	27.2	26.5[a]
16	22.8	22.4	19.5	18.9	22.5
N-CH_3	43.1		42.8		

[a, b] interchangeable

The second new alkaloid, named lycoposerramine-Y (**3**), was obtained as a colorless amorphous powder. The molecular formula was established by HRFAB-MS analysis to be $C_{17}H_{30}N_2O$. Comparison of its ^{1}H and ^{13}C NMR spectra, which exhibited signals characteristic of the tetrahydropyridine *N*-oxide moiety [δ_C 148.5 (C5), δ_H 3.73 (H1) and 2.32 (H4)], with those of **1** suggested that the two alkaloids are stereoisomers. The ^{13}C NMR signals of C13 and C15 in **3** were shifted upfield by 4.8 ppm and 3.4 ppm, respectively, compared with those of **1**. These phenomena can be interpreted in terms of the γ-gauche (steric compression) effect[5] when the side chain (C6) assumed the β-axial configuration, as shown in Figure 2. Therefore, lycoposerramine-Y (**3**) was concluded to be the 7-epimer of huperzine J (**1**).

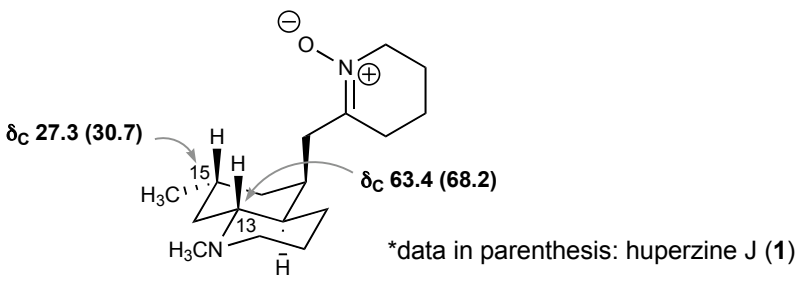
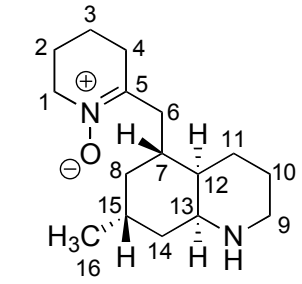


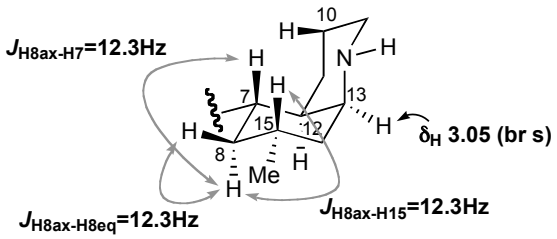
Figure **2**. Stereostructure of lycoposerramine-Y (**3**)

Alkaloid (**4**) was obtained as a colorless amorphous powder and its spectroscopic data were completely identical with those of huperzine K (**4**) in the literature.[4] However, the four chiral centers at C7, C12, C13, and C15 in **4** were not elucidated. Except for the absence of the signal due to an *N*-methyl group, the ^{1}H NMR data of huperzine K (**4**) were very similar to those of lycoposerramine-Y (**3**). Further, in analogy with the relationship of the chemical shifts of the carbons in huperzine J (**1**) and lyocoposerramine-X (**2**), the ^{13}C NMR signals of C9 and C13 in **4** were observed in the higher field region than those of **3**, whereas the other signals of these two compounds appeared at almost the same positions. These data suggested that the hitherto unknown stereochemistry in huperzine K (**4**) is the same as that of lycoposerramine-Y (**3**).

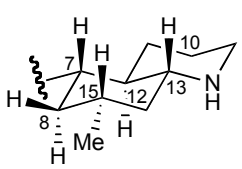
The third new alkaloid named lycoposerramine-Z (**5**) was obtained as a colorless amorphous powder. HRFAB-MS analysis gave the molecular formula $C_{16}H_{28}N_2O$. ^{13}C NMR data indicated the presence of two sp^3 amino methylenes, one sp^3 amino methine, eight sp^3 methylenes, three sp^3 methines, one sp^3 secondary methyl group, and a characteristic sp^2 carbon signal at δ_C 149.0 ascribable to the nitrone carbon, similar to the alkaloids mentioned above. This information as well as the ^{1}H NMR data indicated that lycoposerramine-Z (**5**) is the stereoisomer of **2** or **4**. Lycoposerramine-Z (**5**) was deduced to be the stereoisomer of lycoposerramine-X (**2**) on the basis of the relative configuration at position 13, as follows (Figure 3). The proton signal at position 13 was observed as broad singlet, which indicated that this proton was equatorially oriented in the cyclohexane ring system composed of C7-C8 and C12-C15. Further, one of the two proton signals at position 8 was split ddd whose *J* values were respectively 12.3 Hz, which implied that this proton was axially oriented and the protons at positions 7 and 15 also occupied axial positions. Comparison the ^{13}C NMR data of **2** with those of **5** (see Table) revealed certain differences in the tetrahydroquinoline moiety. The upfield shifts of C7, C15 and C13 signals in **5** could be interpreted by the γ-gauche (steric compression) effect[5] with axial protons on C7 and C15 and an axially oriented nitrogen atom. Furthermore, the same discussion was applicable to the upfield shifts of C10 and C12 signals (interaction between axial proton on C10 and the carbon-7, which occupies an axial position



lycoposerramine-Z (**5**)



lycoposerramine-Z (**5**)



lycoposerramine-X (**2**)

Figure **3**

on C12). These analyses of ^{1}H coupling constants and ^{13}C chemical shifts allowed us to elucidate the stereostructure of lycoposerramine-Z (**5**), i.e., it is an epimer of lycoposerramine-X (**2**) at position 13.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM A-500 and ECP-600 spectrometers with tetramethylsilane as the internal standard. EI and FAB-MS spectra were measured on JEOL JMS BU20 and JEOL JMS AX-500, respectively. HRFAB-MS spectra were obtained on a JEOL HX-110 spectrometer. CD spectra were measured on a JASCO J-729WI spectrometer.

Plant material

The club moss *Lycopodium serratum* Thunb. was collected from Boso Peninsula, Chiba Prefecture and identified by Mr. Tamotsu Nose, a member of the Botanical Society of Chiba Prefecture, Japan. A voucher specimen was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Chiba University.

Extraction and separation

The air-dried club moss (1.45 kg) was extracted with MeOH (7.7 L) four times and filtered. The combined filtrates were concentrated under reduced pressure to give a crude extract (336 g), which was then dissolved in 2% tartaric acid and filtered. The aqueous layer was extracted with petroleum ether, alkalinized with $NaHCO_3$ (pH 10), and exhaustively extracted with 5% MeOH-$CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude alkaloidal fraction (3.32 g). A portion of the crude base (3.18 g) was roughly separated by silica gel flash column chromatography using a $CHCl_3$ ~ 30% MeOH/$CHCl_3$ gradient, 30% MeOH in $CHCl_3$ (saturated with NH_4OH), then MeOH. The MeOH eluate was rechromatographed over SiO_2 using a gradient of 100% AcOEt to MeOH:AcOEt:NH_4OH=25:75:2.5 to afford lycoposerramines-X (**2**), -Y (**3**) and -Z (**5**) together with two known alkaloids, huperzines J (**1**) and K (**4**).

Huperzine J (1): colorless prisms, mp 120–123℃ (crystallized from AcOEt–*n*-hexane); ^{1}H NMR ($CDCl_3$) δ_H 3.78 (2H, dd, *J*=6.0, 6.0, H_2-1), 2.86 (1H, dd, *J*=3.8, 12.9, H-6), 2.82 (1H, br d, *J*=11.5, H-9), 2.38 (2H, dd, *J*=6.3, 6.3, H_2-4), 2.23 (3H, s, *N*-$\underline{CH_3}$), 2.11 (1H, dd, *J*=10.4, 13.2, H-6), 2.03 (2H, m, H-9, 14), 1.99 (1H, m, H-11), 1.91 (2H, m, H_2-2), 1.76 (1H, m, H-7), 1.72 (2H, m, H_2-3), 1.60 (2H, m, H_2-10), 1.54 (1H, ddd, *J*=3.4, 8.5, 11.8, H-13), 1.46 (1H, m, H-8), 1.42 (1H, m, H-15), 0.96 (2H, m, H-11, 12), 0.88 (3H, d, *J*=6.3, H_3-16), 0.79 (1H, m, H-14), 0.77 (1H, m, H-8); ^{13}C NMR (see Table); EI-MS (%) *m/z* 278 (M^+, 17.6), 261 (30.1), 166 (100); CD (0.61 mM, MeOH, 25℃) nm ($\Delta\varepsilon$) 277 (0), 255 (-1.1), 245 (0), 229 (+2.3), 210 (0)

Crystal data: Data were acquired with a Bruker SMART 1000 CCD diffractometer Mo-Kα radiation (λ=0.71073), graphite monochromated, monoclinic, $C_{17}H_{30}N_2O$ (MW 278.43), space group $P2_1$ with *a*=6.968 (2) Å, *b*=7.433 (3) Å, *c*=16.146 (6) Å, *V*=836.2 (5) Å^3, *Z*=2, and D_{calc}=1.106 g/cm^3. The final *R* value was 0.0613 for 2049 reflections (I>2σ(I)).

Lycoposerramine-X (2): colorless amorphous powder; ^{1}H NMR ($CDCl_3$) δ_H 3.80 (2H, dd, *J*=6.4, 6.4, H_2-1), 3.12 (1H, br d, *J*=9.8, H-9), 2.89 (1H, dd like, *J*=4.0, 12.8, H-6), 2.66 (1H, ddd, *J*=3.1, 12.2, 12.2, H-9), 2.41 (2H, dd, *J*=6.1, 6.1, H_2-4), 2.33 (1H, m, H-13), 2.09 (2H, m, H-6, 11), 1.94 (2H, m, H_2-2), 1.80 (1H, m, H-7), 1.75 (4H, m, H-3, 3, 10, 14), 1.58 (1H, m, H-10), 1.52 (2H, m, H-8, 15), 1.09 (1H, m, H-11), 1.02 (1H, m, H-14), 0.97 (1H, m, H-12), 0.89 (3H, d, *J*=6.4, H_3-16), 0.82 (1H, m, H-8); ^{13}C NMR (see Table); FAB-MS (NBA) *m/z* 265 $(MH)^+$; HRFAB-MS (NBA/PEG) *m/z* 265.2292 [$(MH)^+$ calcd. for $C_{16}H_{29}N_2O$ 265.2280]; CD (0.49 mM, MeOH, 25℃) nm (Δε): 280 (0), 258 (-0.6), 246 (0), 231 (+1.1), 206 (0)

Lycoposerramine-Y (3): colorless amorphous powder; ^{1}H NMR ($CDCl_3$) δ_H 3.73 (2H, ddd, *J*=1.2, 6.2, 6.2, H_2-1), 2.82 (1H, br d, *J*=11.0, H-9), 2.72 (1H, dd, *J*=3.7, 13.4, H-6), 2.32 (2H, dd, *J*=6.1, 6.1, H_2-4), 2.23 (1H, dd, *J*=10.1, 13.4, H-6), 2.18 (3H, s, *N*-$\underline{CH_3}$), 2.03 (2H, m, H-9, 15), 1.94 (1H, m, H-11), 1.86 (3H, m, H_2-3, H-7), 1.83 (1H, m, H-14), 1.73 (1H, ddd, *J*=3.1, 9.5, 12.5, H-13), 1.67 (2H, m, H_2-2), 1.59 (2H, m, H_2-10), 1.33 (1H, m, H-8), 1.31 (1H, m , H-14), 1.25 (1H, m, H-8), 0.99 (1H, m, H-12), 0.93 (3H, d, *J*=7.3, H_3-16), 0.92 (1H, m, H-11); ^{13}C NMR (see Table); EI-MS (%) *m/z* 278 (M^+, 15.2), 261 (67.7), 166 (100), 164 (79.4); HRFAB-MS (NBA/PEG) *m/z* 279.2427 $[(MH)^+$ calcd. for $C_{17}H_{31}N_2O$ 279.2436]; CD (0.90 mM, MeOH, 25℃) nm (Δε) 280 (0), 253 (+0.6), 245 (0), 229 (-1.3), 204 (0)

Lycoposerramine-Z (5): colorless amorphous powder; ^{1}H-NMR ($CDCl_3$) δ_H 3.82 (2H, ddd like, *J*=5.3, 5.3, 5.3, H_2-1), 3.21 (1H, br d, *J*=11.3, H-9), 3.05 (1H, br s, H-13), 2.76 (1H, ddd, *J*=3.2, 12.4, 12.4, H-9), 2.72 (1H, br d, *J*=12.2, H-6), 2.55 (1H, m, H-7), 2.46 (2H, m, H_2-4), 2.26 (1H, dd, *J*=10.4, 13.1, H-6), 2.06 (1H, br d, *J*=15.6, H-11), 1.95 (2H, m, H_2-2), 1.76 (5H, m, H-3, 3, 10, 14, 15), 1.58 (1H, br d, *J*=13.1, H-8), 1.47 (1H, m, H-11), 1.42 (1H, m, H-10), 1.40 (1H, m, H-12), 1.22 (1H, ddd, *J*=3.8, 13.9, 13.9, H-14), 0.85 (3H, d, *J*=6.1, H_3-16), 0.79 (1H, ddd, *J*=12.3, 12.3, 12.3, H-8); ^{13}C NMR (see Table); FAB-MS (NBA): *m/z* 265 $(MH)^+$; HRFAB-MS (NBA/PEG) *m/z* 265.2292 $[(MH)^+$ calcd. for $C_{16}H_{29}N_2O$ 265.2280]; CD (0.43 mM, MeOH, 25℃) nm (Δε) 277 (0), 258 (-0.6), 248 (0), 231 (+1.7), 207 (0).

ACKNOWLEDGEMENTS

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1. Present address: Faculty of Pharmaceutical Sciences, International University of Health and Welfare.

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