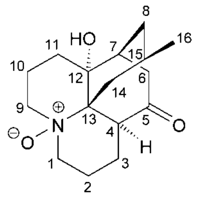
Lycopodine-Type *Lycopodium* Alkaloids from *Huperzia serrata*

by **Chang-Heng Tan** and **Da-Yuan Zhu***

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 ZuChongZhi Road, Shanghai 201203, P. R. China
(phone: +86-21-50806728; fax: +86-21-50807088; e-mail: dyzhu@mail.shcnc.ac.cn)

Eleven *Lycopodium* alkaloids with a lycopodine-type skeleton were isolated from the basic material of the whole plant of *Huperzia serrata* (Thunb.) Trev. (Huperziaceae). Among them, 12-epilycodoline *N*-oxide (=(12α,15*R*)-12-hydroxy-15-methyllycopodan-5-one *N*-oxide; **1**), 7-hydroxylycopodine (=(15*S*)-7-hydroxy-15-methyllycopodan-5-one; **2**), and 4,6α-dihydroxylycopodine (=(6α,15*R*)-4,6-dihydroxy-15-methyllycopodan-5-one; **3**) are new compounds. Their structures were identified spectroscopically, especially by means of 1D- and 2D-NMR.

Introduction. – Huperzine A [1–3], a *Lycopodium* alkaloid isolated from *Huperzia serrata* (Thunb.) Trev. (Huperziaceae), a traditional folk medicinal herb, is a potent acetylcholinesterase inhibitor, and has, thus, attracted, together with other new *Lycopodium* alkaloids, much attention among phytochemists [4–10]. Recently, we reported several novel *Lycopodium* alkaloids from *H. serrata* and related genus [11–15]. As part of our continuing phytochemical research, we have examined the mother liquor from a large-scale preparation (50 kg of *H. serrata*) of huperzine A, yielding eleven lycopodine-type *Lycopodium* alkaloids, three being new compounds: 12-epilycodoline *N*-oxide (**1**), 7-hydroxylycopodine (**2**), and 4,6α-dihydroxylycopodine (**3**), respectively. The other eight alkaloids were identified as lycopodine [16], lycodoline [16], 6α-hydroxylycopodine [5][17], 11α-hydroxylycopodine (lycoposerramine-M) [5], 6β-hydroxylycopodine (lycoposerramine-L) [5][18], 8,15-dehydro-6α-hydroxylycopodine (lycoposerramine-H) [5], lycoclavine [5][18], and acetyllycoclavine [5][18], respectively, on the basis of their physical and spectral data, and by comparison with those published in the literature. In this paper, we report the isolation and structure elucidation of the new alkaloids **1**–**3**.



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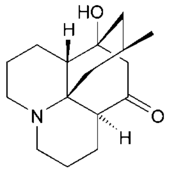

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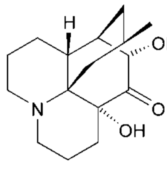

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Results and Discussion. – The lycopodine-type alkaloids, possessing a common rigid tetracyclic framework ($C_{16}N$) with an octahydro-2*H*-quinolizine partial structure, represent the largest group among *Lycopodium* alkaloids [19]. These compounds are generally found with a *cis*-oriented perhydroquinolizine ring system, with the exception of 12-epilycodoline [3g]. *Ayer* and co-workers [16] investigated the substituent and geometric effects on the ^{13}C-NMR shifts of lycopodine-type alkaloids, disclosing that δ(C(14)) and δ(C(4)) of the *trans*-oriented molecule were shifted by less and by more than *ca.* 10 ppm, respectively, relative to the *cis*-isomer [16].

Compound **1**, obtained as colorless prisms, showed a positive response with *Dragendorff*'s reagent. The HR-EI-MS signal at *m/z* 279.1844 gave rise to the empirical formula $C_{16}H_{25}NO_3$ (M^+, $C_{16}H_{25}NO_3^+$; calc. 279.1834). In the EI mass spectrum, the fragment peak at *m/z* 263 ($[M-16]^+$), due to the loss of an O-atom, suggested the presence of an *N*-oxide function; all other signals were nearly superimposable with those of lycodoline (=12β-hydroxylycopodine) and 12-epilycodoline [20], indicating a hydroxylated lycopodine *N*-oxide. The ^{13}C-NMR (DEPT) spectrum of **1** displayed 16 signals: one Me, nine CH_2, and three CH groups, and three quaternary C-atoms (*Table*). Its ^{1}H,^{1}H-COSY and HMQC spectra revealed three isolated spin systems, as shown in *Fig. 1*. In the HMBC spectrum, significant cross-peaks were observed between C(12) and CH_2(14), H_β–C(6), CH_2(8), H_β–C(10), and H_α–C(11); between C(13) and

Table 1. *^{1}H- and ^{13}C-NMR Data of the Novel* Lycopodium *Alkaloids* **1**–**3**. At 400 (^{1}H) and 100 MHz (^{13}C), in $CDCl_3$ (**1**) or CD_3OD (**2** and **3**); δ in ppm, *J* in Hz.

Position	^{1}H			^{13}C		
	1	**2**	**3**	**1**	**2**	**3**
H_α–C(1)	2.89 (*dd*, *J* = 13.3, 4.5)	3.15 (*dt*, *J* = 13.1, 2.1)	3.97 (*dt*, *J* = 14.0, 5.4)	63.2	49.0	48.7
H_β–C(1)	3.58 (*td*, *J* = 13.7, 4.9)	3.78 (*td*, *J* = 13.2, 2.0)	3.68 (*td*, *J* = 14.0, 4.1)			
H_α–C(2)	1.81 (*qt*, *J* = 14.1, 4.6)	1.61 (*qt*, *J* = 13.2, 5.0)	2.30 (*qt*, *J* = 14.4, 5.2)	21.4	18.9	16.4
H_β–C(2)	1.90 (br. *d*, *J* = 14.3)	2.11 (br. *d*, *J* = 13.2)	1.77 (br. *d*, *J* = 14.8)			
H_α–C(3)	2.16 (br. *d*, *J* = 15.5)	1.94 (*qt*, *J* = 13.2, 4.2)	1.95 (br. *d*, *J* = 13.3)	17.8	19.1	26.1
H_β–C(3)	1.64 (*qd*, *J* = 15.5, 4.7)	1.88 (br. *d*, *J* = 13.2)	1.87 (*td*, *J* = 14.3, 4.5)			
H–C(4)[a])	2.84 (*dd*, *J* = 12.0, 3.1)	3.26 (*dd*, *J* = 13.2, 3.0)	–	49.9	44.1	78.9
C(5)	–	–	–	207.0	206.8	205.9
H_α–C(6)	2.59 (*dd*, *J* = 17.0, 6.1)	2.72 (*d*, *J* = 15.9)	–	44.1	51.0	79.5
H_β–C(6)	2.38 (*dd*, *J* = 17.0, 1.3)	2.37 (*dd*, *J* = 15.9, 1.3)	3.98 (br. *s*)			
H–C(7)[b])	2.04 (br. *s*)	–	2.33 (br. *s*)	41.2	72.5	44.2
H_{exo}–C(8)	2.07 (*td*, *J* = 13.1, 3.5)	1.33 (*td*, *J* = 12.1, 1.9)	1.30 (*td*, *J* = 12.6, 4.2)	35.2	50.9	40.8
H_{endo}–C(8)	1.29 (*dt*, *J* = 13.4, 1.9)	1.84 (*dd*, *J* = 13.4, 3.6)	1.74 (*dd*, *J* = 12.6, 2.2)			
H_α–C(9)	3.01 (*dt*, *J* = 12.7, 4.8)	3.70 (*td*, *J* = 13.8, 4.5)	4.40 (*ddd*, *J* = 13.3, 11.9, 3.8)	59.7	49.5	52.8
H_β–C(9)	4.03 (*td*, *J* = 12.7, 3.5)	3.00 (*dd*, *J* = 13.7, 4.8)	2.98 (br. *d*, *J* = 12.8)			
H_α–C(10)	2.97 (*q*, *J* = 13.5, 4.5)	2.11 (br. *d*, *J* = 13.5)	1.98 (br. *d*, *J* = 14.5)	16.4	24.2	25.4
H_β–C(10)	1.74 (br. *d*, *J* = 14.1)	1.93 (*qt*, *J* = 13.5, 4.6)	1.87 (*m*)			
H_α–C(11)	1.61 (*dd*, *J* = 13.4, 5.0)	1.78 (*qd*, *J* = 12.8, 3.3)	2.87 (*qd*, *J* = 13.4, 4.3)	29.6	18.8	26.9
H_β–C(11)	2.21 (*td*, *J* = 13.4, 4.4)	2.14 (br. *d*, *J* = 13.0)	1.56 (*dq*, *J* = 13.3, 3.0)			
H–C(12)[c])	–	1.96 (*dd*, *J* = 13.4, 3.6)	1.86 (*dd*, *J* = 9.9, 4.4)	71.0	51.8	46.4
C(13)	–	–	–	72.8	66.3	65.7
H_{exo}–C(14)	2.72 (*t*, *J* = 13.2)	1.28 (*t*, *J* = 12.2)	1.18 (*t*, *J* = 12.7)	29.6	40.6	44.0
H_{endo}–C(14)	1.86 (*dd*, *J* = 13.0, 5.6)	2.68 (*dd*, *J* = 12.2, 3.0)	2.54 (*dd*, *J* = 13.5, 3.8)			
H–C(15)	1.31 (*m*)	1.39 (*m*)	1.33 (*m*)	24.7	26.6	27.8
Me(16)	0.92 (*d*, *J* = 6.2)	0.95 (*d*, *J* = 5.7)	0.84 (*d*, *J* = 5.6)	22.5	22.6	23.4

[a]) C(4) for **3**. [b]) C(7) for **2**. [c]) C(12) for **1**.

H_α–C(9), H_α–C(1), H–C(4), CH_2(14), H–C(7), H_α–C(11), and Me(16); and between C(5) and H–C(4), CH_2(6), H–C(7), and H_β–C(3), confirming the connectivity of **1**.

The relative configuration of **1** was found to be based on a hydrated *cis*-quinolizine moiety, according to the diagnostic chemical shifts of C(14) (δ(C) 29.6) and C(4) (49.9) [16]. This was further confirmed by conduct-effect and γ-gauche-effect analysis of the substituent. Compared with 12-epilycodoline [16], there was an increase in the chemical shifts for C(1), C(9), and C(13) ($\Delta\delta = 15.2$, 11.0, and 10.3 ppm, resp.), and a decrease for C(2), C(4), and C(10) ($\Delta\delta = -3.8$, -2.4, and -4.6 ppm, resp.). The positive $\Delta\delta$ values were attributed to the deshielding effect of the *N*-oxide function, and the negative ones to the γ-gauche effect from the substituent (*Fig. 1*). In addition, the $\Delta\delta$(C(14)) value of $+4.1$ was probably due to a mild distortion of the molecular skeleton. From the above data, the structure of **1** was deduced as 12-epilycodoline *N*-oxide (=(12α,15*R*)-12-hydroxy-15-methyllycopodan-5-one *N*-oxide)¹).

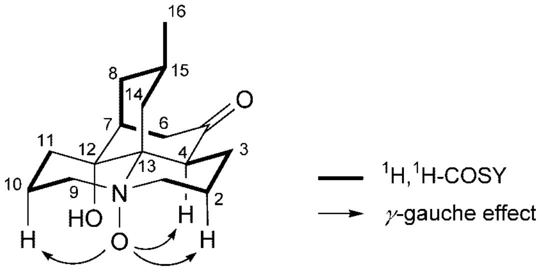

Fig. 1. *¹H,¹H-COSY Connectivities and key γ-gauche effects of* **1**

The EI-MS and NMR spectral data of compound **2** supported the empirical molecular formula $C_{16}H_{25}NO_2$, as confirmed by the HR-EI-MS molecular-ion peak at *m/z* 263.1863 (M^+, $C_{16}H_{25}NO_2^+$; calc. 263.1885). The diagnostic EI-MS fragments at *m/z* 246 ($[M-17]^+$), 220 ($[M-43]^+$), and 206 (base peak, $[M-57]^+$) indicated **2** to be a hydroxylated lycopodine [21]. Its ^{13}C-NMR spectrum (*Table*) displayed 16 resonances: one Me, eight CH_2, and four CH groups, and three quaternary C-atoms (C_q). The most important difference between the ^{13}C-NMR data of **2** and those of lycopodine was a C_q signal at δ(C) 72.5 instead of a CH at δ(C) 36.0 (C(7) of lycopodine), suggesting the OH group to be located at C(7). Such a structure was consistent with the results of ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Fig. 2,a*). Hydroxylation at C(7) in lycopodine is expected to increase the chemical shifts of C(6), C(8), and C(12) ($\Delta\delta =$ 8.2, 9.4, and 9.1, resp.) due to deshielding effects, and to decrease the chemical shift of C(11) ($\Delta\delta = -4.8$) due to the γ-gauche effect, but have little effect on the remaining C-atoms [16][22]. The above data, thus, led to the assignment of **2** as 7-hydroxylycopodine (=(15*S*)-7-hydroxy-15-methyllycopodan-5-one).

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¹) Systematic name: (1*S*,8a*R*,9*S*,11*R*,12a*S*)-dodecahydro-8a-hydroxy-11-methyl-1,9-ethanopyrido[2,1-*j*]quinolin-14-one 5-oxide.

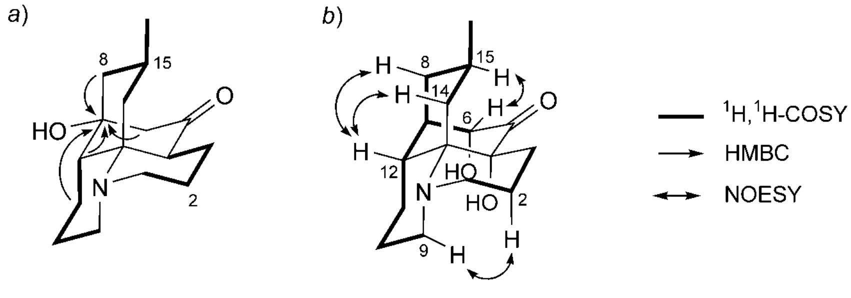

Fig. 2. a) *^{1}H,^{1}H-COSY and Key HMBC correlations of* **2**, *and* b) *^{1}H,^{1}H-COSY and NOESY correlations of* **3**

The molecular formula of **3** was elucidated as $C_{16}H_{25}NO_3$, as deduced from the HR-EI-MS molecular-ion peak at *m/z* 279.1852 (M^+, $C_{16}H_{25}NO_3^+$; calc. 279.1834). Its IR spectrum was similar to that of lycopodine, except for two more bands at 3427 and 3248 cm^{-1}, indicating **3** to be a lycopodine-type alkaloid with two OH groups. The above assumption was supported by the presences of EI-MS fragment peaks at *m/z* 262 ($[M-17]^+$) and 244 ($[M-17-18]^+$). The OH groups were established by a ^{1}H,^{1}H-COSY experiment to be located at C(4) and C(6), respectively, of the lycopodane skeleton (*Fig. 2,b*), as confirmed by HMBC cross-peaks between H–C(7) and both C(6) and C(5), and between CH$_2$(3) and both C(4) and C(5). To H–C(6) was assigned the β-configuration based on significant NOESY interactions, as shown in *Fig. 2,b*. Also, the *cis*-quinolizine partial structure was corrobarated this way. Accordingly, **3** was identified as 4,6α-dihydroxylycopodine (=(6α,15*R*)-4,6-dihydroxy-15-methyllycopodan-5-one).

Experimental Part

General. Column chromatography (CC): silica gel (200–300 or 400 mesh; *Qingdao Haiyang, Co.*, China). M.p.: *Fisher-John* apparatus; uncorrected. Optical rotation: *Perkin-Elmer-241-MC* polarimeter. Circular Dichroism (CD): *Jasco J-715* spectropolarimeter; in deg cm^2 dmol^{-1}. IR Spectra: *Nicolet-Magna-750-FTIR* spectrophotometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker-AM-400* instrument (400/100 MHz); in CDCl$_3$ or CD$_3$OD; δ in ppm rel. to SiMe$_4$, *J* in Hz. EI- and HR-EI-MS (70 eV): *MAT*-95 mass spectrometer; in *m/z* (rel. int. in %).

Plant Material. The fresh whole plant of *Huperzia serrata* (THUNB.) TREV. (Huperziaceae) was collected in Xianju County, Zhejiang Province, P. R. China, in September 2002. The plant was identified by Dr. *X.-Q. Ma.* A voucher specimen (No. 02–98) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, The Chinese Academy of Sciences.

Extraction and Isolation. A part (25 g) of the mother liquor from a large-scale isolation of huperzine A (50 kg of dry *H. serrata*), as described in [1], was subjected to CC (1.5 kg of SiO$_2$; CH$_2$Cl$_2$/MeOH 100:0 → 100:10, 100:20, 100:50 gradient), eluting until the eluent became colorless: 13 fractions (Fr.). The CH$_2$Cl$_2$ fraction (*Fr. 1*) was further purified by CC (SiO$_2$; CH$_2$Cl$_2$/MeOH 100:0 → 100:10): eleven fractions (*Fr. 1.1–Fr. 1.11*). From *Fr. 1.1*, crude needles were obtained, which, after purification by CC (SiO$_2$; petroleum ether/acetone 2:1), led to acetyllycoclavine (8 mg). Repeated CC (SiO$_2$; CH$_2$Cl$_2$/MeOH 25:1) of *Fr. 1.3* afforded lycoclavine (7 mg). *Fr. 8* yielded a solid mixture of lycopodine and 6β-hydroxylycopodine, which could be separated by CC (SiO$_2$; CH$_2$Cl$_2$/MeOH 20:1), and recrystallization to afford the pure compounds (54 and 11 mg, resp.). Compounds **3** (6 mg), lycodoline (24 mg), 6α-hydroxylycopodine (21 mg), and 8,15-dehydro-6α-hydroxylycopodine (22 mg) were obtained from the remaining *Fr. 8* after repeated CC (SiO$_2$; CH$_2$Cl$_2$/MeOH 15:1). *Fr. 10* furnished prismatic crystals of **1** (780 mg). The remaining *Fr. 10* was combined with *Fr. 11*, and this mixture was purified by CC (SiO$_2$; CHCl$_3$/MeOH 12.5:1) to afford, after recrystallization, compound **2** (18 mg) and 11α-hydroxylycopodine (12 mg).

*(12α,15*R*)-12-Hydroxy-15-methyllycopodan-5-one* N-*Oxide* (=*12-Epilycodoline* N-*Oxide*; **1**). Colorless prisms. M.p. 238–240° (acetone). $[\alpha]_D^{25} = -21.2$ ($c = 1.33$, $CHCl_3$). CD ($c = 2.7$ mM, MeOH): $[\theta]_{285} = +1800$. IR (KBr): 3518, 2946, 1693, 1626, 1458, 1416, 1319, 1146, 912. ^1H- and ^{13}C-NMR: see the *Table*. EI-MS: 279 (M^+, 6), 263 (30), 262 (100), 251 (10), 246 (9), 244 (14), 235 (16), 234 (81), 220 (6), 190 (18), 177 (43), 159 (23). HR-EI-MS: 279.1844 (M^+, $C_{16}H_{25}NO_3^+$; calc. 279.1834).

*(15*S*)-7-Hydroxy-15-methyllycopodan-5-one* (=*7-Hydroxylycopodine*; **2**). Amorphous powder. M.p. 250–252°. $[\alpha]_D^{25} = -29.2$ ($c = 0.367$, MeOH). CD ($c = 3.3$ mM, MeOH): $[\theta]_{287} = [\theta]_{294} = -3200$. IR (KBr): 3485, 3415, 2958, 2916, 1699, 1458, 1317, 1118, 1024. ^1H- and ^{13}C-NMR: see the *Table*. EI-MS: 263 (100, M^+), 248 (21), 246 (86), 232 (33), 220 (25), 206 (100), 204 (14), 192 (16), 180 (24), 162 (28), 152 (48), 123 (22). HR-EI-MS: 263.1863 (M^+, $C_{16}H_{25}NO_2^+$; calc. 263.1885).

*(6α,15*R*)-4,6-Dihydroxy-15-methyllycopodan-5-one* (*4,6α-Dihydroxylycopodine*; **3**). Amorphous powder. M.p. >300° (dec.). $[\alpha]_D^{25} = -56.8$ ($c = 0.158$, MeOH). CD ($c = 1.4$ mM, MeOH): $[\theta]_{287} = -105000$, $[\theta]_{292} = -106000$. IR (KBr): 3427, 3248, 2931, 2607, 1722, 1456, 1385, 1111, 1049, 984. ^1H- and ^{13}C-NMR: see the *Table*. EI-MS: 279 (60, M^+), 262 (14), 244 (7), 222 (47), 204 (42), 194 (10), 176 (24), 164 (100), 150 (21), 122 (14). HR-EI-MS: 279.1852 (M^+, $C_{16}H_{25}NO_3^+$; calc. 279.1834).

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