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PHLENUMDINES A−C, NEW *LYCOPODIUM* ALKALOIDS ISOLATED FROM *PHLEGMARIURUS NUMMULARIIFOLIUS*

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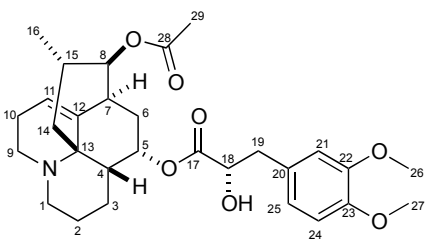
Abstract – Three new lycopodine-type alkaloids, phlenumdines A−C (**1**−**3**), have been isolated from the clubmoss *Phlegmariurus nummulariifolius* (Blume) Ching, and their structures were elucidated on the basis of spectroscopic data.

The Lycopodiaceae family[1] is in the major group of *Pteridophytes* (ferns and fern allies) that comprise16 genera: *Austrolycopodium*, *Diphasium*, *Lycopodiastrum*, *Lycopodium*, *Plananthus*, *Pseudolycopodium*, *Dendrolycopodium*, *Huperzia*, *Lycopodiella*, *Palhinhaea*, *Pseudodiphasium*, *Spinulum*, *Diphasiastrum*, *Lycopodiodes*, *Phlegmariurus*, and *Pseudolycopodiella*. Lycopodiaceae plants, also known as clubmosses, contain *Lycopodium* alkaloids,[2] a structurally diverse set of the plant constituents that possess unique heterocyclic ring systems such as $C_{16}N_1$, $C_{16}N_2$, and $C_{27}N_3$. The $C_{16}N_1$-type alkaloids are further



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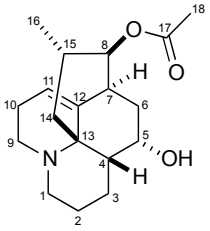

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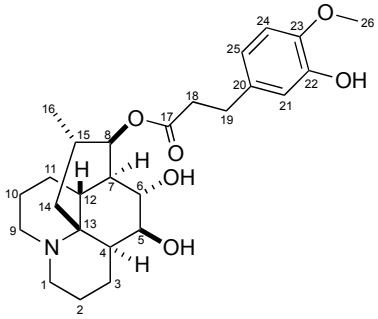

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divided into lycopodine-type, fawcettimine-type, phlegmariurine-type, etc. *Lycopodium* alkaloids are of great interest in biological,[3] biogenetic,[4] and chemical[5] fields. In our continuing efforts in seeking new *Lycopodium* alkaloids,[6] three new lycopodine-type alkaloids, phlenumdines A−C (**1**−**3**), were isolated from the clubmoss *Phlegmariurus nummulariifolius* (Blume) Ching along with known compounds, lycopodine,[7] lycodine,[8] and huperzine A.[5] The structures of **1**−**3** were elucidated on the basis of spectroscopic data.

Phlenumdine A (**1**) [$[\alpha]_D^{23}$ -21 (*c* 1.0, MeOH)] exhibited a protonated molecule at *m/z* 514 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{29}H_{39}NO_7$, was established by HRESIMS [*m/z* 514.2802, $(M+H)^+$, Δ–0.3 mmu]. ^1H and ^{13}C NMR data (Table 1) and the HSQC spectrum of **1** revealed 29 carbon signals due to two carbonyl carbons, four sp^2 quaternary carbons, one sp^3 quaternary carbon, four sp^2 methines, six sp^3 methines, eight sp^3 methylenes, and four sp^3 methyl groups. Of these, one sp^3 quaternary carbon (δ_C 58.2) and two sp^3 methylenes (δ_C 48.7; δ_H 2.82, and δ_C 46.0; δ_H 3.01 and 2.65) were concluded to be attached to a nitrogen atom.

The planar structure of **1** was elucidated by analysis of 2D NMR data including the ^1H-^1H COSY, TOCSY, HSQC, and HMBC spectra in CD_3OD. Five structural units **a** (C-1−C-4), **b** (C-5−C-8, C-8−C-15, and C-14−C-16), **c** (C-9−C-11), **d** (C-18−C-19), and **e** (C-24−C-25) were disclosed by ^1H-^1H COSY and TOCSY spectra of **1** (Figure 1). The connectivities of C-1, C-9, and C-13 through a nitrogen atom were revealed by HMBC collerations for H-1 (δ_H 2.82) and H-9b (δ_H 2.65) to C-13 (δ_C 58.2). HMBC cross-peaks of H-10a (δ_H 2.49) to C-12 (δ_C 139.3), H-11 (δ_H 5.57) to C-7 (δ_C 42.4), and H-7 (δ_H 2.75) to C-13 indicated that C-7 connected to C-11 (δ_C 120.0) and C-13 through C-12. Connectivity of C-4 and C-5 was revealed by an HMBC correlation for H-6b (δ_H 1.71) to C-4 (δ_C 47.4). HMBC cross-peaks of H_2-14 (δ_H 1.79 and 1.59) to C-4 and C-13 suggested that C-4 (δ_C 47.4) connected to C-14 (δ_C 42.1) through C-13. HMBC correlations for H-8 (δ_H 4.32) and H_3-29 (δ_H 2.06) to C-28 (δ_C 172.2)

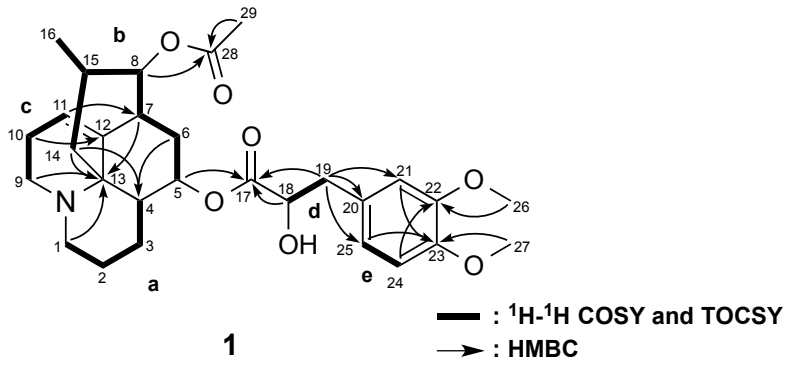


Figure 1. Selected 2D NMR correlations for phlenumdine A (**1**)

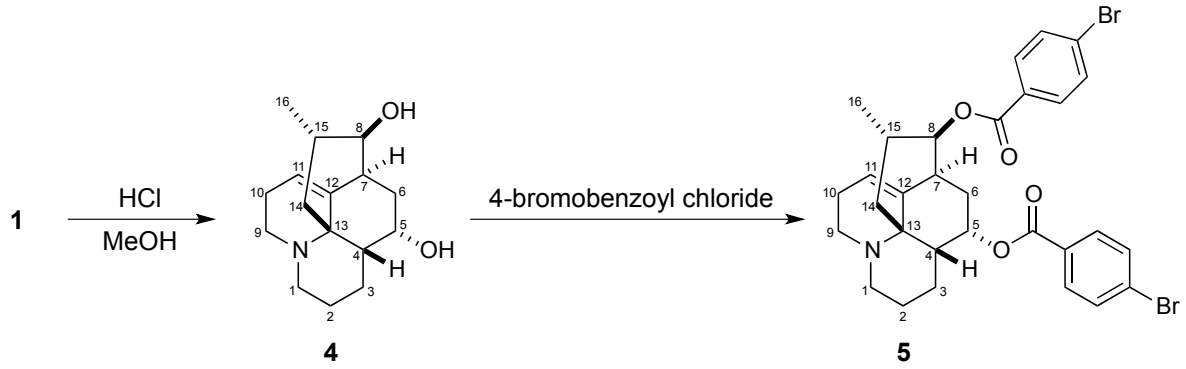
Table 1. ^{1}H and ^{13}C NMR Data of Phlenumdine A (**1**) in CD_3OD

Pos.	δ_H (ppm)	δ_C (ppm)	Pos.	δ_H (ppm)	δ_C (ppm)
1	2.82 (2H, m)	48.7	17		174.6
2a	1.80 (1H, m)	22.8	18	4.26 (1H, t 6.5 Hz)	74.0
2b	1.49 (1H, m)		19a	2.98 (1H, dd 14.0, 6.5 Hz)	41.1
3a	1.49 (1H, m)	22.5	19b	2.89 (1H, dd 14.0, 6.5 Hz)	
3b	1.29 (1H, m)		20		131.1
4	1.96 (1H, m)	47.4	21	6.84 (1H, d 2.0 Hz)	114.6
5	5.22 (1H, ddd 5.0, 5.0, 5.0 Hz)	73.1	22		150.2
6a	1.88 (1H, m)	28.5	23		149.3
6b	1.71 (1H, ddd 14.5, 8.5, 5.0 Hz)		24	6.85 (1H, d 8.5 Hz)	112.8
7	2.75 (1H, ddd 8.5, 5.0, 2.5 Hz)	42.4	25	6.77 (1H, dd 8.5, 2.0 Hz)	122.9
8	4.32 (1H, dd 10.5, 5.0 Hz)	83.0	26	3.81 (3H, s)	56.5
9a	3.01 (1H, m)	46.0	27	3.80 (3H, s)	56.5
9b	2.65 (1H, dd 13.0, 6.0 Hz)		28		172.2
10a	2.49 (1H, m)	23.9	29	2.06 (3H, s)	21.0
10b	1.93 (1H, m)				
11	5.57 (1H, brd 4.5 Hz)	120.0			
12		139.3			
13		58.2			
14a	1.79 (1H, m)	42.1			
14b	1.59 (1H, dd 12.0, 12.0 Hz)				
15	2.13 (1H, m)	32.2			
16	0.95 (3H, d 6.5 Hz)	19.6			

revealed that an acetoxy group connected to C-8 (δ_C 83.0). The presence of a 3-(3,4-dimethoxyphenyl)lactoyl group was disclosed by HMBC cross-peaks, as depicted in Figure 1. The position of a 3-(3,4-dimethoxyphenyl)lactoyl group was assigned to be C-5 by an HMBC correlation for H-5 (δ_H 5.22) to C-17 (δ_C 174.6). Thus, the planar structure of phlenumdine A was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from NOESY data (Figure 2). The chair form of the cyclohexane ring (C-7−C-8, C-12−C-15) and equatorial positions of the acetoxy group and methyl group at C-8 and C-15, respectively, were revealed from NOESY correlations for H-8/H-14b and H_3-16. The pseudo-chair conformation of the 1,2,3,6-tetrahydropyridine ring (C-9−C-13, N) was indicated by a NOESY cross-peak of H-9a/H-14b. β-orientations of H-4 and H-5 on the cyclohexane ring (C-4−C-7, C-12−C-13) were suggested by NOESY correlations for H-4/H-14a and H-5/H-15. The conformation of the piperidine ring (C-1−C-4, C-13, N) could not be assigned due to the overlapping signals of H-1a and H-1b. However, NOESY correlations for H-1b/H-9b and H-1a/H-10a in the NOESY spectrum of **4**

obtained by the acid methanolysis of **1** (Scheme 1) were observed, which indicated that NOESY cross-peaks of H-1/H-9b and H-1/H-10a in **1** were assigned to be H-1b/H-9b and H-1a/H-10a. These assignments implied that the piperidine ring was of chair-like form. Thus, the relative stereochemistry of phlenumdine A was elucidated as shown in Figure 2.



Scheme 1. Chemical derivatization of phlenumdine A (**1**) to **4** and **5**

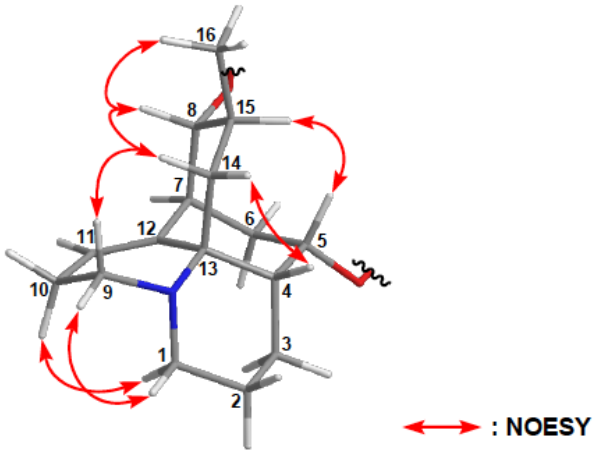
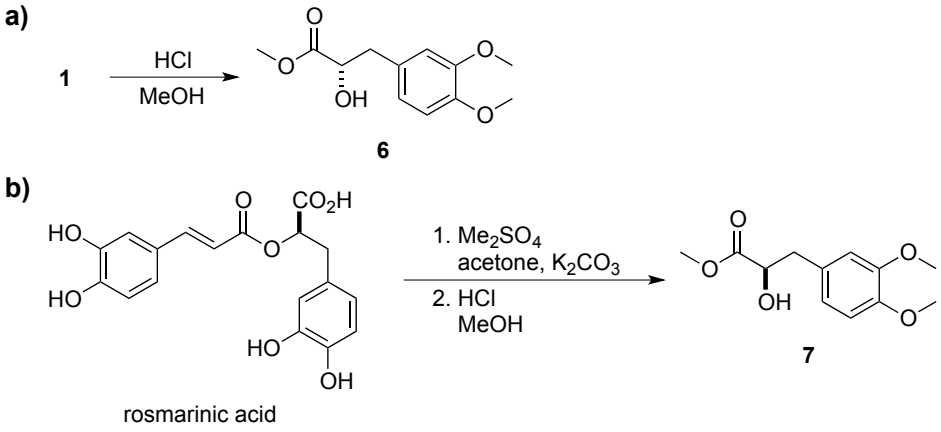


Figure 2. Selected NOESY correlations for phlenumdine A (**1**)

The absolute stereochemistry of **1** was elucidated by the circular dichroism (CD) exciton chirality method.[9] **4**, derived from **1**, was treated with 4-bromobenzoyl chloride to yield the bis-acylated product **5** (Scheme 1). The CD spectrum of **5** showed a negative bisignate Cotton effect centered at 239 nm, which suggested that the absolute configuration at C-5 and C-8 was assigned as 5*S* and 8*R*. On the other hand, methanolysis of **1** with HCl gave **6**, whose spectroscopic data was identical with those of **7** derived from rosmarinic acid (Scheme 2). However the optical rotation of **6** [$[\alpha]_D^{23}$ -10 (*c* 0.23, $CHCl_3$)] was opposite in sign to that of **7** [$[\alpha]_D^{23}$ +11 (*c* 0.23, $CHCl_3$)], which indicated that the stereochemistry of C-18 of **1** was 18*S*. Thus, the absolute configuration of **1** was established as 4*R*, 5*S*, 7*S*, 8*R*, 13*S*, 15*S*, and 18*S*.



Scheme 2. Chemical derivatizations of (a) phlenumdine A (**1**) to **6** and (b) rosmarinic acid to **7**

Phlenumdine B (**2**) [$[\alpha]_D^{21}$ -61 (*c* 0.22, MeOH)] showed a protonated molecule at *m/z* 306 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{18}H_{27}NO_3$, was established by HRESIMS [*m/z* 306.2085, $(M+H)^+$, Δ+1.6 mmu]. ^{1}H and ^{13}C NMR data (Table 2) and the HSQC spectrum of **2** revealed 18 carbon signals due to one carbonyl carbon, one sp^2 quaternary carbon, one sp^3 quaternary carbon, one sp^2 methine, five sp^3 methines, seven sp^3 methylenes, and two sp^3 methyl groups. Among them, one quaternary carbon (δ_C 55.4) and two sp^3 methylenes (δ_C 46.9; δ_H 2.76, and δ_C 44.6; δ_H 2.68 and 2.52) were attributed to those attached to a nitrogen atom.

The planar structure of **2** was elucidated by analysis of 2D NMR data including the ^{1}H-^{1}H COSY, TOCSY, HSQC, and HMBC spectra in $CDCl_3$. Three structural units **a** (C-1−C-4), **b** (C-5−C-8, C-8−C-15, and C-14−C-16), **c** (C-9−C-11) were disclosed by ^{1}H-^{1}H COSY and TOCSY spectra of **2** (Figure 3). The planar structure of phlenumdine B was indicated to be **2** by HMBC correlations for H-1 (δ_H 2.76) and H-9b (δ_H 2.52) to C-13 (δ_C 55.4), H-10a (δ_H 2.32) to C-12 (δ_C 140.6), H-11 (δ_H 5.70) to C-7 (δ_C 40.1) and C-13, H-3b (δ_H 1.39) and H-14a (δ_H 1.62) to C-13, and H-8 (δ_H 4.36) and H_3-18 (δ_H 2.06) to C-17 (δ_C170.5) (Figure 3). The planar structure and the stereochemistry of **2** was confirmed by chemical correlation with **1**. The spectral data of desacetyl derivative obtained by the methanolysis of **2** was identical with **4** from **1**. Thus the absolute configuration of **2** was assigned as 4*R*, 5*S*, 7*S*, 8*R*, 13*S*, and 15*S*.

Furthermore, we confirmed the absolute configuration of **2** by the modified Mosher's method.[10] With respect to (*S*)- and (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters of **2**, Δδ values (δ_S-δ_R) of for H_2-1, H_2-2, H_2-3, H-4, H-6a, H_2-9, and H-14a showed positive values, whereas those of H-6b, H-7, H-8, H_2-10, H-11, and H-15 were negative, suggesting that C-5 possessed *S*-configuration (Figure 4). These results supported the absolute configuration of **2** assigned by the chemical correlation with **1**.

Table 2. ^{1}H and ^{13}C NMR Data of Phlenumdine B (**2**) in $CDCl_3$

Pos.	δ_H (ppm)	δ_C (ppm)
1	2.76 (2H, m)	46.9
2a	1.92 (1H, m)	21.2
2b	1.52 (1H, m)	
3a	2.05 (1H, m)	22.1
3b	1.39 (1H, m)	
4	1.77 (1H, m)	48.3
5	3.75 (1H, brs)	69.8
6a	1.92 (1H, m)	31.5
6b	1.80 (1H, ddd 15.0, 3.5, 3.5 Hz)	
7	2.92 (1H, m)	40.1
8	4.36 (1H, dd 10.5, 5.0 Hz)	81.2
9a	2.68 (1H, ddd 11.0, 11.0, 3.5 Hz)	44.6
9b	2.52 (1H, dd 11.0, 6.0 Hz)	
10a	2.32 (1H, m)	25.3
10b	1.89 (1H, m)	
11	5.70 (1H, d 4.5 Hz)	120.2
12		140.6
13		55.4
14a	1.62 (1H, dd 12.0, 3.5 Hz)	37.4
14b	1.37 (1H, m)	
15	1.98 (1H, m)	29.3
16	0.93 (3H, d 6.0 Hz)	18.4
17		170.5
18	2.06 (3H, s)	21.2

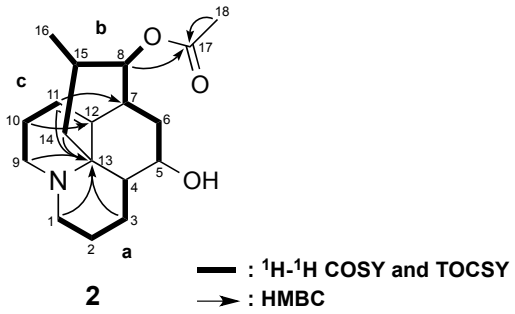


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Figure 3. Selected 2D NMR correlations for phlenumdine B (**2**)

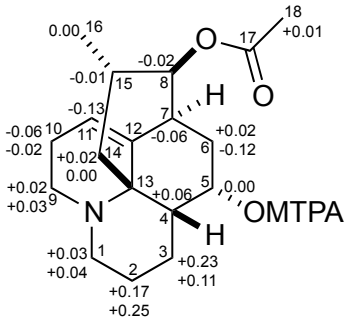

Figure 4. $\Delta\delta$ Values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters of phlenumdine B (**2**)

Phlenumdine C (**3**) [$[\alpha]_D^{22}$ +11 (*c* 1.0, MeOH)] showed a protonated molecule at *m/z* 460 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{26}H_{37}NO_6$, was established by HRESIMS [*m/z* 460.2699, $(M+H)^+$, Δ0.0 mmu]. ^1H and ^{13}C NMR data (Table 3) and the HSQC spectrum of **3** revealed 26 carbon signals due to one carbonyl carbon, three sp^2 quaternary carbons, one sp^3 quaternary carbon, three sp^2 methines, seven sp^3 methines, nine sp^3 methylenes, and two sp^3 methyl groups. Among them, one quaternary carbon (δ_C 62.9) and two sp^3 methylenes (δ_C 47.5; δ_H 3.72 and 3.11, and δ_C 47.0; δ_H 3.53 and 3.25) were attributed to those attached to a nitrogen atom. One carbonyl carbon (δ_C 172.7), two sp^2 quaternary carbons (δ_C 148.1 and δ_C 147.1) and three sp^3 methines (δ_C 79.6; δ_H 5.03, δ_C 73.4; δ_H 4.46, and δ_C 71.6; δ_H 4.61) were attributed to those attached to an oxygen atom.

The planar structure of **3** was elucidated by analysis of 2D NMR data including the ^1H-^1H COSY, TOCSY, HSQC, and HMBC spectra in C_5D_5N. Four structural units **a** (C-1−C-8, C-8/C-15, and C-14−C-16), **b** (C-9−C-12), **c** (C-18−C-19), and **d** (C-24−C-25) were disclosed by ^1H-^1H COSY and TOCSY spectra of **3** (Figure 5). Connectivities of C-1, C-9, and C-13 through a nitrogen atom were revealed by HMBC correlations for H-1b (δ_H 3.11) and H-9b (δ_H 3.25) to C-13 (δ_C 62.9). HMBC cross-peaks of H-6 (δ_H 4.61) to C-12 (δ_C 42.2) and H-7 (δ_H 2.73) to C-13 suggested that C-7 (δ_C 46.7) connected to C-13through C-12. HMBC correlations for H-5 (δ_H 4.46) and H-14b (δ_H 1.95) to C-13 revealed connectivities of C-4 and C-14 through C-13. The presence of a dihydroisoferuloyloxy group was disclosed by HMBC cross-peaks depicted in Figure 5. Position of a dihydroisoferuloyloxy group was assigned to be C-8 by an HMBC correlation for H-8 (δ_H 5.03) to C-17 (δ_C 172.7). Thus, the planar structure of phlenumdine C was elucidated to be **3** (Figure 5).

Table 3. ^{1}H and ^{13}C NMR Data of Phlenumdine C (**3**) in C_5D_5N

Pos.	δ_H (ppm)	δ_C (ppm)	Pos.	δ_H (ppm)	δ_C (ppm)
1a	3.72 (1H, m)	47.5	17		172.7
1b	3.11 (1H, dd 14.0, 4.0 Hz)		18a	2.64 (1H, m)	36.4
2a	1.88 (1H, m)	19.0	18b	2.56 (1H, m)	
2b	1.63 (1H, m)		19a	2.91 (1H, m)	30.7
3a	2.24 (1H, m)	21.5	19b	2.88 (2H, m)	
3b	1.62 (1H, m)		20		134.2
4	2.89 (1H, m)	30.6	21	7.10 (1H, d 2.0 Hz)	116.7
5	4.46 (1H, d 5.5 Hz)	73.4	22		148.1
6	4.61 (1H, brs)	71.6	23		147.1
7	2.73 (1H, d 5.0 Hz)	46.7	24	6.91 (1H, d 8.0 Hz)	112.5
8	5.03 (1H, dd 11.0, 5.0 Hz)	79.6	25	6.72 (1H, dd 8.0, 2.0 Hz)	119.1
9a	3.53 (1H, ddd 13.0, 13.0, 3.0 Hz)	47.0	26	3.71 (3H, s)	55.9
9b	3.25 (1H, brd 13.0 Hz)				
10a	2.05 (1H, m)	23.9			
10b	1.66 (1H, m)				
11a	2.58 (1H, m)	24.6			
11b	1.40 (1H, brd 14.5 Hz)				
12	2.18 (1H, brd 13.0 Hz)	42.2			
13		62.9			
14a	2.82 (1H, m)	37.5			
14b	1.95 (1H, dd 13.0, 13.0 Hz)				
15	3.72 (1H, m)	30.0			
16	0.99 (3H, d 6.5 Hz)	19.8			

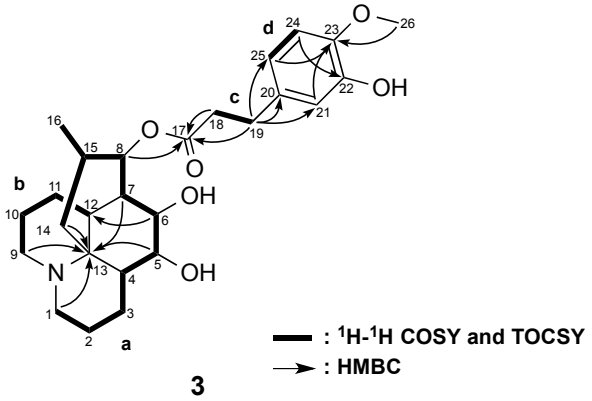

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Figure 5. Selected 2D NMR correlations for phlenumdine C (**3**)

The relative stereochemistry of **3** was deduced from NOESY data and ^{3}J coupling constants (Figure 6). The chair form of the cyclohexane ring (C-7−C-8, C-12−C-15), equatorial positions of the dihydroisoferuloyloxy group and methyl group at C-8 and C-15, respectively, and a *trans*-fused ring junction between the cyclohexane ring and the piperidine ring (C-9−C-13 and N) were revealed from NOESY correlations for H-12/H-8 and H-14b and $^{3}J_{\text{H-14b/H-15}}$ value (13.0 Hz). The chair forms of the piperidine ring (C-9−C-13 and N) and the cyclohexane ring (C-4−C-7 and C-12−C-13) were indicated by a NOESY correlation for H-4/H-11a. A NOESY cross-peak of H-1a/H-14a suggested that the piperidine ring (C-1−C-4, C-13, and N) was also chair form. The β- and α-orientations for hydroxy groups at C-5 and C-6, respectively, were indicated by NOESY correlations for H-4/H-5 and H-6/H-15. Thus the relative stereochemistry of **3** was elucidated as shown in Figure 6.

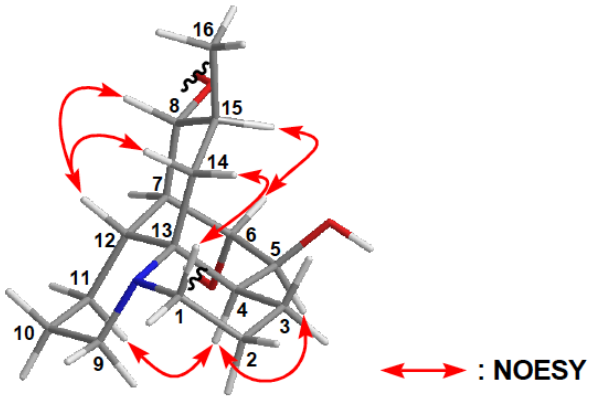

Figure 6. Selected NOESY correlations for phlenumdine C (**3**)

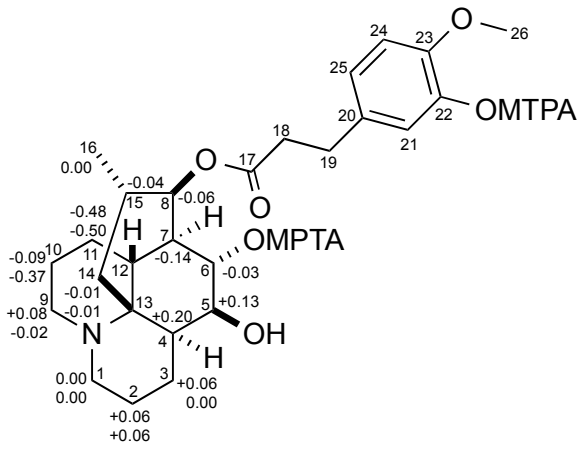

Figure 7. $\Delta\delta$ Values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters of phlenumdine C (**3**)

The absolute configuration at C-6 of phlenumdine C (**3**) was elucidated by the modified Mosher's method[10] for the (*S*)- and (*R*)- MTPA esters of **3**. $\Delta\delta$ Values (δ_S-δ_R) for H_2-2, H-3a, H-4, H-5, and H-9a showed positive values, while those of H-7, H-8, H-9b, H_2-10, H_2-11, H_2-14, and H-15 were negative,

suggesting that C-6 possessed *S*-configuration (Figure 7). Thus the absolute configuration of **3** was assigned as 4*S*, 5*S*, 6*S*, 7*R*, 8*R*, 12*R*, 13*R*, and 15*S*.

In this study, phlenumdines A−C (**1**−**3**), three new lycopodine-type *Lycopodium* alkaloids, were isolated from *P. nummulariifolius*. The structures were elucidated using of spectroscopic data and chemical correlation. There are three ring systems as represented by lycopodine[7] (normal-type), 12-*epi*-lycodoline[11] (12-*epi*-type), and lycopodatine C[12] (4-*epi*-type) in lycopodine-type alkaloids. **1** and **2** form a 4-*epi*-type ring system. Although over a hundred of lycopodine-type species in *Lycopodium* alkaloids have been reported so far, the ring systems of nearly all were the normal-type. While 12-*epi*-type and 4-*epi*-type ring systems have only four and one examples, respectively.[11-13] **1** and **2** are second and third examples of 4-*epi*-type ring system and rare in lycopodine-type alkaloids.

EXPERIMENTAL

Optical rotation was recorded on a JASCO P-2100 polarimeter. UV spectrum was recorded on a Shimadzu UV-1280 spectrophotometer. IR spectrum was recorded on a Shimadzu IR Affinity-1 spectrometer. NMR spectra were recorded on an Agilent Varian VNS500 spectrometer. Chemical shifts (ppm) were referenced to the residual solvent peaks (δ_H 3.31 and δ_C 49.0 for CD_3OD; δ_H 7.26 and δ_C 77.0 for $CDCl_3$; δ_H 7.21 and δ_C 135.5 for C_5D_5N). Positive-mode ESITOFMS was obtained on a JEOL JMS-T100LP AccuTOF LC-plus 4G spectrometer using a sample dissolved in MeOH.

Plant Material

Phlegmariurus nummulariifolius (Blume) Ching was purchased at a flower market in Bangkok, Thailand. The botanical identification was made by Dr. Santi Watthana (School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand) and Dr. Kazumi Fujikawa (Kochi Prefectural Makino Botanical Garden, Kochi, Japan). Voucher specimens (no. 20130801) are stored in Nagoya City University.

Extraction and Isolation

The aerial part of *Phlegmariurus nummulariifolius* (Blume) Ching (740 g, wet weight) was extracted with methanol (MeOH). The extract was partitioned between ethyl acetate (EtOAc) and 3% tartaric acid. Water-soluble materials, adjusted to pH 10 with saturated sodium carbonate (Na_2CO_3) aqueous, were extracted with chloroform ($CHCl_3$). $CHCl_3$-soluble materials were subject to an amino silica gel column (*n*-hexane/EtOAc, 1:0 → 1:1 and then $CHCl_3$/MeOH, 1:0 → 1:1). A fraction eluted with *n*-hexane/EtOAc (4:1 and 1:1) was purified by silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1) to obtain phlenumdine A (**1**, 35.0 mg). The fraction eluted from the amino silica gel column with *n*-hexane/EtOAc (4:1) was separated

with silica gel column ($CHCl_3$/MeOH, 1:0 → 4:1) to afford phlenumdine B (**2**, 3.4 mg). The fraction eluted from the amino silica gel column with $CHCl_3$/MeOH (50:1 and 30:1) was purified by silica gel column ($CHCl_3$/MeOH/H_2O/TFA, 1:0:0:0 → 6:4:1:0 → 6:4:1:0.01) to obtain phlenumdine C (**3**, 6.2 mg).

Phlenumdine A (1): colorless amorphous solid; $[\alpha]_D^{23}$ -21 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 280 (ε 2820), 230 (8256) and 202 (36974) nm; IR (ATR) ν_{max} 3360, 2931, 1732, and 1458 cm^{-1}; ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1; ESIMS *m/z* 514 $[M+H]^+$; HRESIMS *m/z* 514.2802 $[M+H]^+$ (calcd for $C_{29}H_{40}NO_7$, 514.2805).

Phlenumdine B (2): colorless amorphous solid; $[\alpha]_D^{21}$ -61 (*c* 0.22, MeOH); IR (ATR) ν_{max} 3356, 2931, 1732, and 1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2; ESIMS *m/z* 306 $[M+H]^+$; HRESIMS *m/z* 306.2085 $[M+H]^+$ (calcd for $C_{18}H_{28}NO_3$, 306.2069).

Phlenumdine C (3): colorless amorphous solid; $[\alpha]_D^{22}$ +11 (*c* 1.0, MeOH); UV (MeOH) λ_{max} 280 (ε 1789), 260 (1422) and 201 (10734) nm; IR (ATR) ν_{max} 3363, 2924, 1732, and 1458 cm^{-1}; ^{1}H-NMR (C_5D_5N, 500 MHz) and ^{13}C-NMR (C_5D_5N, 125 MHz), see Table 3; ESIMS *m/z* 460 $[M+H]^+$; HRESIMS *m/z* 460.2699 $[M+H]^+$ (calcd for $C_{26}H_{38}NO_6$, 460.2699).

6 and 4 derived from phlenumdine A (1): Phlenumdine A (**1**, 10.7 mg) was treated with 6 N hydrochloric acid (HCl) aqueous (1 mL) in MeOH (1 mL) at room temperature for 48 h. H_2O (1 mL) was added to the reaction mixture. The HCl solution was partitioned with EtOAc (2 mL × 4) to obtain **6** (3.5 mg). $[\alpha]_D^{23}$ -10 (*c* 0.23, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ6.80 (1H, d 8.5 Hz), 6.75 (1H, brs), 6.74 (1H, d 8.5 Hz), 4.44 (1H, dd 7.0, 4.5 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.78 (3H, s), 3.08 (1H, dd 14.0, 4.5 Hz), 2.92 (1H, dd 14.0, 7.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ174.5, 148.8, 148.0, 128.7, 121.5, 112.6, 111.1, 71.4, 55.8, 55.8, 52.5, 40.1; EIMS *m/z* 240 $[M]^+$; HREIMS *m/z* 240.0994 $[M]^+$ (calcd for $C_{12}H_{16}O_5$, 240.0998). The aqueous layer was adjusted to pH 9 with 28% ammonia and extracted with EtOAc (2 mL × 4). The EtOAc layer was subjected to an amino silica gel column (MeOH) to obtain **4** (4.7 mg). $[\alpha]_D^{21}$ -136 (*c* 0.07, MeOH); ^{1}H-NMR (CD_3OD) δ5.57 (1H, d 5.0 Hz, H-11), 4.36 (1H, ddd 9.0, 5.0, 5.0 Hz, H-5), 3.18 (1H, m, H-9a), 3.16 (1H, dd 9.5, 5.0 Hz, H-8), 2.87 (1H, ddd 11.5, 11.5, 5.5 Hz, H-1a), 2.75 (1H, dd 11.5, 5.0 Hz, H-1b), 2.62 (1H, dd 13.5, 6.0 Hz, H-9b), 2.53 (1H, m, H-7), 2.49 (1H, m, H-10a), 2.05 (1H, ddd 13.5, 5.0, 2.5 Hz, H-6a), 1.89 (1H, m, H-15), 1.83 (1H, m, H-4), 1.82 (1H, m, H-2a), 1.80 (1H, m, H-3a), 1.80 (1H, m, H-10b), 1.75 (1H, m, H-14a), 1.66 (1H, dd 11.5, 11.5 Hz, H-14b), 1.60 (1H, ddd 13.5, 9.0, 6.5 Hz, H-6b), 1.52 (1H, m, H-2b), 1.34 (1H, dddd 13.0, 13.0, 13.0, 2.5 Hz, H-3b), 1.02 (3H, d 6.0 Hz, H-16); ESITOFMS *m/z* 264 $(M+H)^+$; HRESITOFMS *m/z* 264.1970 (M+H; calcd for $C_{16}H_{26}NO_2$, 264.1964).

5 derived from 4: A solution of **4** (3.5 mg) and dichloromethane (CH_2Cl_2) (200 μL) was prepared. To this, 4-bromobenzoyl chloride (30 mg), triethylamine (20 μL), and *N*,*N*-dimethylaminopyridine (0.5 mg) were added. The reaction mixture was stirred at room temperature for 3 h and partitioned between water (1 mL) and EtOAc (1 mL × 4) under basic conditions. The EtOAc layer was subject to a silica gel column ($CHCl_3$/MeOH/H_2O/TFA, 1:0:0:0 → 6:4:1:0.01). A fraction eluted with $CHCl_3$/MeOH (50:1) was purified by C_{18} HPLC (COSMOSIL Packed Column 5C_{18}-AR-II, 4.6 mm I.D. × 250 mm, solvent MeCN/H_2O/TFA, 53/47/0.1, flow rate 0.6 mL/min) to yield **5** (1.2 mg). UV (MeOH) λ_{max} 245 (ε 14259) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 234 (+1.1), 239 (0.0) and 252 (−10.7) nm; ^{1}H-NMR ($CDCl_3$) δ7.96 (2H), 7.85 (2H), 7.62 (2H), 7.60 (2H), 6.10 (1H), 5.95 (1H), 4.75 (1H), 3.71 (1H), 3.56 (1H), 3.33 (1H), 3.21-3.13 (2H), 2.84 (1H), 2.74-2.59 (3H), 2.38 (1H), 2.24 (1H), 2.09 (1H), 2.05-1.96 (2H), 1.90 (1H), 1.82 (1H), 1.45 (1H), 1.10 (3H); ESITOFMS *m/z* 628, 630, 632 [1:2:1, $(M+H)^+$]; HRESITOFMS *m/z* 630.0689 [$(M+H)^+$, calcd for $C_{30}H_{32}NO_4{}^{79}Br^{81}Br$, 630.0678].

7 derived from rosmarinic acid: A solution of rosmarinic acid (Sigma-Aldrich) (99.8 mg) and acetone (7 mL) was prepared, and dimethyl sulfate (263 μL) and potassium carbonate (384 mg) were added. The reaction mixture was stirred at reflux for 15 h and partitioned between saturated aqueous NH_4Cl (10 mL) and Et_2O (10 mL × 3) to obtain the Et_2O extracts (200 mg). The Et_2O layer (27.2 mg) was treated with 6 N hydrochloric acid (HCl) aqueous (500 μL) in MeOH (500 μL) at room temperature for 46 h. H_2O (500 μL) was added to the reaction mixture. The HCl solution was partitioned with Et_2O (1 mL × 5), then water layer was partitioned with EtOAc (1 mL × 4). The Et_2O layer and the EtOAc layer were subject to silica gel column (hexane/EtOAc, 1:0 → 0:1) to give **7** (0.45 mg). $[\alpha]_D^{23}$ +11 (*c* 0.23, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ6.80 (1H, d 8.5 Hz), 6.75 (1H, brs), 6.74 (1H, d 8.5 Hz), 4.44 (1H, dd 7.0, 4.0 Hz), 3.87 (3H, s), 3.86 (3H, s), 3.78 (3H, s), 3.08 (1H, dd 14.0, 4.0 Hz), 2.92 (1H, dd 14.0, 7.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ174.5, 148.8, 148.0, 128.7, 121.5, 112.7, 111.1, 71.4, 55.8, 55.8, 52.5, 40.1; EIMS *m/z* 240 $[M]^+$; HREIMS *m/z* 240.0996 $[M]^+$ (calcd for $C_{12}H_{16}O_5$, 240.0998).

4 derived from phlenumdine B (2): Phlenumdine B (**2**, 0.3 mg) was treated with 6 N HCl aqueous (100 μL) in MeOH (100 μL) at room temperature for 46 h. H_2O (600 μL) was added to the reaction mixture. The HCl solution was partitioned with EtOAc (700 μL × 3), and the aqueous layer was adjusted to pH 9 with 28% aqueous ammonia and extracted with EtOAc (700 μL × 3). The EtOAc layer was subjected to an amino silica gel column (MeOH) to obtain **4** (0.1 mg). $[\alpha]_D^{21}$ -80 (*c* 0.07, MeOH); ^{1}H-NMR (CD_3OD) δ5.57 (1H, d 5.0 Hz, H-11), 4.36 (1H, ddd 9.0, 5.0, 5.0 Hz, H-5), 3.18 (1H, m, H-9a), 3.16 (1H, m, H-8), 2.86 (1H, ddd 11.5, 11.5, 5.0 Hz, H-1a), 2.74 (1H, dd 11.1, 4.9 Hz, H-1b), 2.62 (1H, dd 13.5, 6.0 Hz, H-9b), 2.53 (1H, m, H-7), 2.49 (1H, m, H-10a), 2.05 (1H, ddd 13.8, 5.1, 2.2 Hz, H-6a), 1.89 (1H, m,

H-15), 1.86-1.72 (5H, H-2a, H-3a, H-4, H-10b, H-14a), 1.65 (1H, dd 12.0, 12.0 Hz, H-14b), 1.60 (1H, ddd 13.8, 9.0, 7.0 Hz, H-6b), 1.52 (1H, m, H-2b), 1.02 (3H, d 6.0 Hz, H-16); ESITOFMS *m/z* 264 $(M+H)^+$; HRESITOFMS *m/z* 264.1972 (M+H; calcd for $C_{16}H_{26}NO_2$, 264.1964).

(*R*)- and (*S*)-MTPA esters of phlenumdine B (2): A solution of **2** (0.15 mg) and CH_2Cl_2 (50 μL) was prepared, and the following compounds were added: (*R*)-MTPACl (1.1 μL), triethylamine (1.4 μL), and *N*, *N*-dimethylaminopyridine (28 μg). The reaction mixture was stirred at room temperature for 3 h and partitioned between water (650 μL) and EtOAc (700 μL × 3) under basic conditions. The EtOAc layer was subject to silica gel column ($CHCl_3$/MeOH/H_2O/TFA, 1:0:0:0 → 6:4:1:0.01) to give the (*S*)-MTPA ester of **2** (0.2 mg). The (*R*)-MTPA ester of **2** was prepared similarly.

(*S*)-MTPA ester of phlenumdine B (2): ^{1}H-NMR ($CDCl_3$) δ5.84 (1H, H-5), 5.69 (1H, H-11), 4.43 (1H, H-8), 3.59 (1H, H-9a), 3.31 (1H, H-1a), 3.08 (1H, H-9b), 3.07 (1H, H-1b), 2.95 (1H, H-7), 2.82 (1H, H-14a), 2.66 (1H, H-4), 2.43 (1H, H-10a), 2.34 (1H, H-10b), 2.31 (1H, H-15), 2.16 (1H, 2a), 2.14 (1H, H-6a), 2.13 (3H, H-18), 1.96 (1H, H-3a), 1.90 (1H, H-14b), 1.74 (1H, H-2b), 1.70 (1H, H-6b), 1.32 (1H, H-3b), 1.02 (3H, H-16); ESITOFMS *m/z* 522 $(M+H)^+$; HRESITOFMS *m/z* 522.2476 (M+H; calcd for $C_{28}H_{35}NO_5F_3$, 522.2467).

(*R*)-MTPA ester of phlenumdine B (2): ^{1}H-NMR ($CDCl_3$) δ5.84 (1H, H-5), 5.81 (1H, H-11), 4.45 (1H, H-8), 3.57 (1H, H-9a), 3.27 (1H, H-1a), 3.05 (1H, H-9b), 3.03 (1H, H-1b), 3.01 (1H, H-7), 2.80 (1H, H-14a), 2.60 (1H, H-4), 2.49 (1H, H-10a), 2.36 (1H, H-10b), 2.31 (1H, H-15), 2.12 (1H, H-6a), 2.12 (3H, H-18), 1.99 (1H, H-2a), 1.90 (1H, H-14b), 1.82 (1H, H-6b), 1.73 (1H, H-3a), 1.49 (1H, H-2b), 1.21 (1H, H-3b), 1.02 (3H, H-16); ESITOFMS *m/z* 522 $(M+H)^+$; HRESITOFMS *m/z* 522.2476 (M+H; calcd for $C_{28}H_{35}NO_5F_3$, 522.2467).

(*R*)- and (*S*)-MTPA esters of phlenumdine C (3): A solution of **3** (0.5 mg) and CH_2Cl_2 (160 μL) was prepared, and (*R*)-MTPACl (2.5 μL), triethylamine (5.2 μL), and *N*,*N*-dimethylaminopyridine (63 μg) were added. The reaction mixture was stirred at room temperature for 5.5 h and partitioned between water (650 μL) and EtOAc (650 μL × 3) under basic conditions. The EtOAc layer was subject to C_{18}-HPLC (MeCN/H_2O/TFA, 62:38:0.1) to give the (*S*)-MTPAester of **3** (0.1 mg). The (*R*)-MTPA ester of **3** was prepared similarly.

(*S*)-MTPA ester of phlenumdine C (3): ^{1}H-NMR ($CDCl_3$) δ7.11 (1H, H-25), 6.94 (1H, H-21), 6.90 (1H, H-24), 5.01 (1H, H-6), 4.55 (1H, H-8), 3.90 (1H, H-5), 3.78 (1H, H-26), 3.49 (1H, H-1a), 3.25 (1H, H-9a), 3.15 (1H, H-9b), 3.03 (1H, H-1b), 2.95 (2H, H-19), 2.76 (2H, H-18), 2.74 (1H, H-15), 2.44 (1H, H-4),

2.42 (1H, H-14a), 2.07 (1H, H-7), 1.98 (1H, H-3a), 1.89 (1H, H-10a), 1.89 (1H, H-2a), 1.64 (1H, H-14b), 1.57 (1H, 2b), 1.57 (1H, 3b), 1.25 (1H, H-10b), 0.90 (3H, H-16), 0.89 (1H, H-11a), 0.59 (1H, H-11b); ESITOFMS *m/z* 892 $(M+H)^+$; HRESITOFMS *m/z* 892.3519 (M+H; calcd for $C_{46}H_{52}NO_{10}F_6$, 892.3495).

(*R*)-MTPA ester of phlenumdine C (3): ^{1}H-NMR ($CDCl_3$) δ7.08 (1H, H-25), 6.89 (1H, H-21), 6.89 (1H, H-24), 5.03 (1H, H-6), 4.61 (1H, H-8), 3.79 (1H, H-26), 3.77 (1H, H-5), 3.49 (1H, H-1a), 3.17 (1H, H-9a), 3.17 (1H, H-9b), 3.03 (1H, H-1b), 2.91 (2H, H-19), 2.78 (1H, H-15), 2.70 (2H, H-18), 2.43 (1H, H-14a), 2.24 (1H, H-4), 2.21 (1H, H-7), 1.98 (1H, H-10a), 1.91 (1H, H-3a), 1.83 (1H, H-2a), 1.65 (1H, H-14b), 1.62 (1H, H-10b), 1.57 (1H, H-3b), 1.51 (1H, H-2b), 1.37 (1H, H-11a), 1.08 (1H, H-11b), 0.90 (3H, H-16); ESITOFMS *m/z* 892 $(M+H)^+$; HRESITOFMS *m/z* 892.3523 (M+H; calcd for $C_{46}H_{52}NO_{10}F_6$, 892.3495).

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