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## Pyrrolidinoindoline Alkaloids from *Selaginella moellendorffii*

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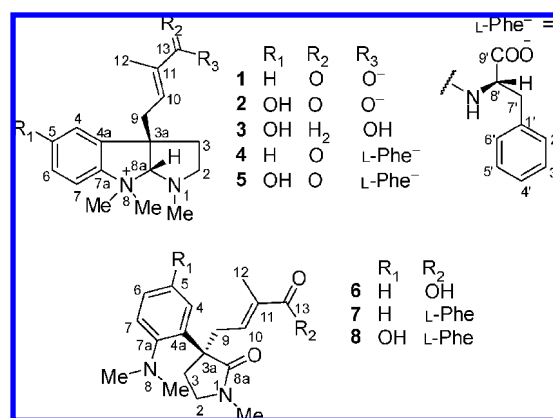
Eight new pyrrolidinoindoline alkaloids (**1**–**8**) were isolated from the whole plant of *Selaginella moellendorffii*. Their structures were determined by mass spectrometry, 1D and 2D NMR spectroscopy, and chemical interconversions. These alkaloids have a 3-carboxybut-2-enyl group at C-3a and two methyl groups at N-8. The possible biogenetic route from selaginellinic acid (**1**) to neoselaginellinic acid (**6**) was postulated and chemically mimicked. Tautomerization between **6** and **6a** was observed. Selected compounds were evaluated for antibacterial, cytotoxic, and acetylcholinesterase inhibitory activities.

Pyrrolidinoindoline alkaloids bearing a C-3a-substituted hexahydropyrrolo[2,3-*b*]indole ring system possess a variety of biological activities.<sup>1</sup> For example, (–)-physostigmine isolated from the seeds of *Physostigma venenosum* and (–)-de bromoflustramine B occurring in the cheilostome bryozoan of *Flustra foliacea* serve as inhibitors of cholinesterase (ChE),<sup>2</sup> while hodgekinsine identified from *Psychotria colorata* exhibits antinociceptive activity,<sup>3</sup> and (–)-pseudophrynaminol synthesized from tryptamine has antibacterial activity.<sup>4</sup>

*Selaginella moellendorffii* Hieron. (Selaginellaceae) is a perennial herb mainly distributed in the southern area of the Changjiang River in mainland China. It has a history of use in treating bleeding, gonorrhea, jaundice, and idiopathic thrombocytopenic purpura (ITP) in traditional Chinese folk medicine.<sup>5</sup> A coumarin,<sup>5a</sup> a lignanoside,<sup>5b</sup> flavone glycosides,<sup>5c</sup> and cytotoxic<sup>5d</sup> and antioxidative<sup>5e</sup> biflavones have been found in this plant. A positive reaction of the MeOH extract of *S. moellendorffii* with Dragendorff's reagent led us to further investigate this species. This study resulted in the isolation of a series of new pyrrolidinoindoline alkaloids (**1**–**8**) from the whole plant. These alkaloids are characterized by possessing a 3-carboxybut-2-enyl side chain at the 3a position and two *N*<sub>8</sub>-methyl groups. Compound **6** might be biogenetically derived from **1** and was biomimetically synthesized. Further, selected compounds (**1**, **4**, and **6**) were evaluated for activities against *Escherichia coli*, *Staphylococcus aureus*, human leukemia K562, and lung adenocarcinoma A549 cell lines, and acetylcholinesterase (AChE). This paper reports the structure elucidation of these new compounds as well as the results of chemical transformation and bioassays.

### Results and Discussion

Selaginellinic acid (**1**) was obtained as an optically active ( $[\alpha]_D^{23}$  –61.8), colorless, amorphous solid having the molecular formula C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>, as determined by HRESIMS (obsd  $[M + H]^+$  at *m/z* 301.1912, calcd 301.1916). The <sup>1</sup>H NMR spectra of **1** (Table 1) revealed the signals of four aryl protons [ $\delta_H$  7.68 (1H, d, *J* = 7.5 Hz) and 7.56–7.59 (3H, m)], one olefin proton [ $\delta_H$  6.33 (1H, br t, *J* = 7.5 Hz)], one aminoacetal proton [ $\delta_H$  5.75 (1H, s)], three



*N*-methyl groups [ $\delta_H$  3.34 (3H, s), 3.09 (3H, s), and 2.99 (3H, s)], and one methyl group [ $\delta_H$  1.90 (3H, br s)]. Comparison of NMR data of **1** with those of pseudophrynamine A and pseudophrynaminol<sup>6</sup> showed that **1** is a prenylated pyrrolidinoindoline alkaloid. The prenylated moiety was elucidated as a 3-carboxybut-2-enyl group and located at the C-3a position from the HMBC correlations of H<sub>3</sub>-12 to C-10, C-11, and C-13; H-10 to C-3a; and H<sub>2</sub>-9 to C-3, C-4a, C-8a, and C-11. The *E*-configuration of the double bond in this group, the same as that in tiglic acid, was confirmed by the ROESY correlation between H<sub>3</sub>-12 and H<sub>2</sub>-9. The three *N*-methyl groups were located at N-1, N-8, and N-8, from the HMBC correlations of N<sub>1</sub>-CH<sub>3</sub> to C-2 and C-8a and of N<sub>8</sub>-CH<sub>3</sub> to C-7a and C-8a, respectively. Thus, the planar structure of **1** was determined as 3a-[(2*E*)-3-carboxybut-2-enyl]-N<sub>1</sub>,N<sub>8</sub>,N<sub>8</sub>-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole. The presence of a carboxyl group and a quaternary nitrogen atom (N-8) in **1** led to the inference that this compound is an inner salt.

The relative configuration of **1** was deduced as *cis* by the ROESY correlation of H-8a to H<sub>2</sub>-9. The absolute configuration of C-3a was determined as *S* by transformation of **1** to noselaginellinic acid (**6**, Scheme 2), for which the configuration was suggested by comparing its CD data with those of known compounds (see the structure elucidation paragraph for **6** below).

Compound **2** was assigned the molecular formula C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> by HRESIMS. By comparing the MS and NMR (Tables 1 and 3) data of **2** with those of **1**, compound **2** was inferred as being a derivative of selaginellinic acid (**1**) substituted by a hydroxy group, which was located at C-5 by the presence of an aromatic ABX coupling system [ $\delta_H$  7.45 (d, *J* = 8.8 Hz, H-7), 6.89 (dd, *J* = 8.8

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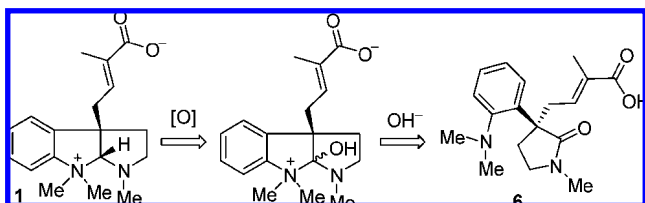
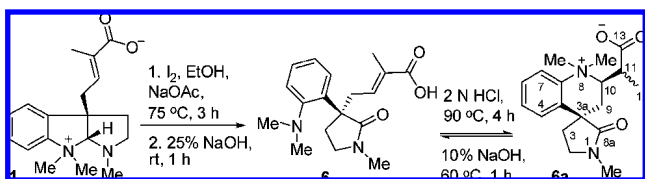
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**Table 1.**  $^1\text{H}$  NMR Data of Compounds **1–3** and **6a** ( $\delta$  in ppm,  $J$  in Hz)

position	<b>1</b> <sup>a</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>c</sup>	<b>6a</b> <sup>b</sup>
2	3.58, ddd (9.0, 9.0, 4.0) 3.40, ddd (9.0, 9.0, 9.0)	3.55, ddd (8.6, 8.6, 3.6) 3.31, overlapped	3.41, m 3.21, ddd (8.5, 8.5, 8.5)	3.65, 2H, m
3	2.40, ddd (13.5, 9.0, 4.0) 2.15, ddd (13.5, 9.0, 9.0)	2.34, ddd (13.5, 8.6, 3.6) 2.13, ddd (13.5, 8.6, 8.6)	2.18, m 1.91, ddd (13.5, 8.5, 8.5)	2.43, 2H, m
4	7.57, m <sup>d</sup>	6.81, d (2.4)	6.75, d (2.5)	7.24, m
5	7.59, m			7.54, m
6	7.56, m <sup>d</sup>	6.89, dd (8.8, 2.4)	6.79, dd (8.5, 2.5)	7.57, m
7	7.68, d (7.5)	7.45, d (8.8)	7.31, d (8.5)	8.02, m
8a	5.75, s	5.62, s	5.46, s	
9	2.89, 2H, m	2.87, 2H, m	2.56, 2H, m	2.71, dd (16.0, 13.0) 2.34, d (16.0)
10	6.33, br t (7.5)	6.62, br t (7.6)	5.15, br t (7.6)	4.04, d (13.0)
11				3.68, m
12	1.90, 3H, br s	1.90, 3H, br s	1.55, 3H, br s	1.47, 3H, d (7.2)
13			3.79, 2H, s	
$N_1$ -CH <sub>3</sub>	3.09, 3H, s	3.02, 3H, s	2.88, 3H, s	3.01, 3H, s
$N_8$ -CH <sub>3</sub>	3.34, 3H, s 2.99, 3H, s	3.25, 3H, s 2.92, 3H, s	3.09, 3H, s 2.77, 3H, s	3.80, 3H, s 3.58, 3H, s

<sup>a</sup> Measured in  $\text{CD}_3\text{OD}$  at 500 MHz. <sup>b</sup> In  $\text{CD}_3\text{OD}$  at 400 MHz. <sup>c</sup> In acetone- $d_6$  at 500 MHz. <sup>d</sup> Assignments with the same superscript are interchangeable.

**Scheme 1.** Biogenetic Pathway for Neoselaginellollic Acid (**6**)**Scheme 2.** Chemical Transformation from **1** to **6** and Tautomerization between **6** and **6a**

and 2.4 Hz, H-6), and 6.81 (d,  $J$  = 2.4 Hz, H-4)] in **2** and the HMBC correlations of H-7 to C-5 and H-4 to C-3a. Therefore, compound **2** was elucidated as 5-hydroxyselaginellollic acid.

The molecular formula of **3** was determined as  $\text{C}_{18}\text{H}_{27}\text{N}_2\text{O}_2$  by HRESIMS. The NMR spectra of **3** were very similar to those of pseudophrynaminol. Comparison of their MS and NMR data revealed that **3** has one more hydroxy group and two more  $N$ -methyl groups than the latter.<sup>6</sup> As in **2**, the hydroxy group was located at C-5 and the two  $N$ -methyl groups at N-8. On the basis of these findings, **3** was elucidated as 5-hydroxy- $N_8,N_8$ -dimethylpseudophrynaminol.

Compound **4** was found to have a molecular formula of  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_3$ , according to HRESIMS. Detailed analysis of the MS and NMR data of **4** showed that this compound is comprised of two moieties, a selaginellollic acid (**1**) unit and a phenylalanine unit, which are joined through an amide bond formed by the carboxyl group of the selaginellollic acid and the amino group of the phenylalanine, and supported by the HMBC correlation of H-8' [ $\delta_{\text{H}}$  4.68 (dd,  $J$  = 9.5 and 4.5 Hz)] to C-13 [ $\delta_{\text{C}}$  171.3 (qC)]. The configuration of phenylalanine was determined as L by acidic hydrolysis of **4** to give a product having a negative specific rotation. Thus, the structure of **4** was determined as  $N$ -selaginellolyl-L-phenylalanine.

Compound **5** gave the molecular formula  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_4$  according to HRESIMS. By comparing the MS and NMR data of **5** with those of **4**, it was found that the former has an additional hydroxy group. As in **2** and **3**, the hydroxy group was also located at C-5. Accordingly,

compound **5** was elucidated as  $N$ -(5-hydroxyselaginellolyl)-L-phenylalanine.

Neoselaginellollic acid (**6**) was assigned the molecular formula  $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_3$ , as determined by HRESIMS. Similar to selaginellollic acid (**1**), **6** has a disubstituted phenyl ring, a 3-carboxybut-2-enyl group, and three  $N$ -methyl groups. The difference between **1** and **6** is that the signals of the aminoacetal proton and carbon disappeared in the NMR data of **6** and were replaced by the signal for a lactam carbonyl group [ $\delta_{\text{C}}$  178.5 (qC, C-8a)]. This difference signifies that C-8a is oxidized and the N-8–C-8a bond is broken in the structure of **6**. This was supported by the HMBC correlations of  $N_1$ -CH<sub>3</sub> to C-8a and  $N_8$ -CH<sub>3</sub> to C-7a. Compound **6** is also structurally similar to a known compound, chimonamidine,<sup>7</sup> except that the latter has a hydroxy group at C-3a rather than a 3-carboxybut-2-enyl group and has only one methyl at N-8 rather than having two. The C-3a substituent of **6** was suggested as having an  $S$ -configuration by comparing its CD data [ $\Delta\epsilon$  –2.00 (218), –1.86 (198)] with those of (–)- and (+)-chimonamidine. (–)-Chimonamidine exhibited negative Cotton effects [ $\Delta\epsilon$  –2.9 (224) and –27 (201)] at 224 and 201 nm, while (+)-chimonamidine has positive values [ $\Delta\epsilon$  +3.1 (226) and +24.4 (202)] at 226 and 202 nm.<sup>7</sup>

Compounds **7** and **8** were assigned the molecular formulas of  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_4$  and  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_5$ , respectively, as determined by HRESIMS. By comparing the MS and NMR data of **7** and **8** with those of **4–6**, both **7** and **8** were assigned with two units, a neoselaginellolyl or 5-hydroxynneoselaginellolyl group and a phenylalanine residue. On the basis of their 2D NMR correlations, the two compounds were elucidated as  $N$ -neoselaginellolyl-L-phenylalanine and  $N$ -(5-hydroxynneoselaginellolyl)-L-phenylalanine, respectively.

A plausible biogenetic pathway for compound **6** is proposed as shown in Scheme 1. To substantiate the proposal, **1** was oxidized with  $\text{I}_2$  in the presence of NaOAc in refluxing EtOH followed by basification with NaOH in  $\text{H}_2\text{O}$  to give **6** (Scheme 2).<sup>8</sup> On the basis of the biogenetic relationship between selaginellollic acid (**1**) and neoselaginellollic acid (**6**), we suggested that the latter and its derivatives (**7** and **8**) as well as the chimonamidines should be regarded as pyrrolidinoindoline alkaloids.

We also noted that compound **6** is unstable. Signals for an impurity were observed in its NMR spectra (see the Supporting Information), although it was purified repeatedly. The possible reason is that a nucleophilic addition reaction may occur between the nucleophilic atom of N-8 and the partially positive atom of C-10, especially under acidic conditions. It was found that **6** and its tautomer **6a** interconverted easily. As shown in Scheme 2, compound **6** could be transformed to this major product, **6a**, under acidic conditions, and in turn, **6a** was converted to **6** under basic

**Table 2.**  $^1\text{H}$  NMR Data of Compounds **4**–**8** ( $\delta$  in ppm,  $J$  in Hz)

position	<b>4</b> <sup>a</sup>	<b>5</b> <sup>a</sup>	<b>6</b> <sup>a</sup>	<b>7</b> <sup>a</sup>	<b>8</b> <sup>a</sup>
2	3.53, ddd (9.0, 9.0, 3.5) 3.26, overlapped	3.52, ddd (9.0, 9.0, 3.0) 3.23, m	3.68, m 3.60, m	3.58, 2H, m	3.55, 2H, m
3	2.34, ddd (13.5, 9.0, 3.5) 2.10, ddd (13.5, 9.0, 9.0)	2.28, m 2.08, ddd (13.9, 9.0, 9.0)	2.72, 2H, m	2.68, 2H, m	2.64, m 2.56, m
4	7.49, dd (7.5, 1.5)	6.78, d (2.0)	7.59, m <sup>b</sup>	7.54, m	6.81, br s
5	7.56, m		7.54, m <sup>b</sup>	7.50, m	
6	7.54, m	6.89, dd (9.0, 2.0)	7.60, m <sup>b</sup>	7.57, m	6.94, br d (9.0)
7	7.65, dd (7.5, 1.5)	7.44, d (9.0)	7.83, m	7.77, br d (7.5)	7.57, d (9.0)
8a	5.67, s	5.51, s			
9	2.85, 2H, d (8.0)	2.78, 2H, d (7.5)	2.84, 2H, m	2.77, 2H, m	2.71, 2H, m
10	6.02, br t (8.0)	6.02, br t (7.5)	6.49, br t (7.5)	6.03, br t (7.5)	6.05, br t (7.5)
12	1.84, 3H, br s	1.84, 3H, br s	1.68, 3H, br s	1.53, 3H, br s	1.59, 3H, br s
2', 6'	7.17, 2H, m	7.17, 2H, m		7.22, 2H, d (7.2)	7.20, 2H, d (7.5)
3', 5'	7.21, 2H, m	7.21, 2H, m		7.29, 2H, t (7.2)	7.28, 2H, t (7.5)
4'	7.18, m	7.18, m		7.23, t (7.2)	7.21, m
7'	3.26, overlapped 3.00, overlapped	3.25, m 3.00, m		3.25, dd (14.5, 5.0) 2.99, overlapped	3.23, dd (14.0, 4.3) 2.97, overlapped
8'	4.68, dd (9.5, 4.5)	4.67, dd (9.5, 4.5)		4.65, dd (10.0, 5.0)	4.65, dd (10.0, 4.3)
N <sub>1</sub> -CH <sub>3</sub>	2.99, 3H, s	2.95, 3H, s	3.00, 3H, s	2.98, 3H, s	2.96, 3H, s
N <sub>8</sub> -CH <sub>3</sub>	3.25, 3H, s 2.94, 3H, s	3.20, 3H, s 2.89, 3H, s	3.29, 3H, s 3.25, 3H, s	3.17, 3H, s 3.05, 3H, s	3.11, 3H, s 3.01, 3H, s

<sup>a</sup> Measured in CD<sub>3</sub>OD at 500 MHz. <sup>b</sup> Assignments with the same superscript are interchangeable.**Table 3.**  $^{13}\text{C}$  NMR Data of Compounds **1**–**8** and **6a** ( $\delta$  in ppm)

position	<b>1</b> <sup>a</sup>	<b>2</b> <sup>a</sup>	<b>3</b> <sup>b</sup>	<b>4</b> <sup>a</sup>	<b>5</b> <sup>a</sup>	<b>6</b> <sup>a</sup>	<b>7</b> <sup>c</sup>	<b>8</b> <sup>a</sup>	<b>6a</b> <sup>c</sup>
2	56.0	56.0	55.0	56.1	56.0	48.3	48.3	48.2	47.2
3	38.9	38.6	37.6	38.6	38.6	33.9	34.2	34.1	35.7
3a	57.7	57.5	56.7	57.5	57.4	57.4	57.6	57.3	50.0
4	126.3	111.5	111.0	126.2	111.5	132.0 <sup>d</sup>	132.0	117.8	131.2
4a	140.4	141.5	141.2	140.2	141.4	133.9	135.7	137.1	134.7
5	132.4	161.4	158.6	132.5	161.4	132.4 <sup>d</sup>	132.4	160.3	132.1
6	131.2	118.2	117.1	131.3	118.2	131.5 <sup>d</sup>	131.4	118.0	131.2
7	120.6	121.4	120.8	120.7	121.4	123.5	123.3	124.6	123.1
7a	146.2	137.3	137.0	146.0	137.3	142.8	142.8	134.3	146.4
8a	124.0	122.5	123.1	124.1	122.4	178.5	178.3	178.2	177.1
9	36.2	36.4	34.2	35.9	35.6	40.4	40.5	40.5	29.0
10	131.7	136.7	119.4	131.2	131.3	135.2	129.9	130.1	74.2
11	137.5	133.0	139.4	135.7	135.7	135.5	136.8	136.7	39.2
12	14.0	13.0	13.7	13.2	13.3	12.7	12.8	12.8	17.9
13	174.9	170.8	67.1	171.3	171.4	170.4	171.1	171.1	174.8
1'				138.7	138.6		138.8	138.9	
2', 6'				130.2	130.1		130.3	130.3	
3', 5'				129.4	129.4		129.6	129.6	
4'				127.8	127.8		127.8	127.8	
7'				37.8	37.8		37.9	37.9	
8'				55.1	55.2		55.2	55.3	
9'				174.6	174.7		174.6	174.6	
N <sub>1</sub> -CH <sub>3</sub>	39.2	39.1	38.6	39.0	39.1	31.2	31.2	31.2	30.6
N <sub>8</sub> -CH <sub>3</sub>	53.0	53.5	52.7	53.0	53.5	47.1	48.3	48.4	55.6
	48.6	49.0	48.1	48.4	49.1	48.3	47.0	47.2	53.1

<sup>a</sup> Measured in CD<sub>3</sub>OD at 100 MHz. <sup>b</sup> In acetone-*d*<sub>6</sub> at 125 MHz. <sup>c</sup> In CD<sub>3</sub>OD at 125 MHz. <sup>d</sup> Assignments with the same superscript are interchangeable.

conditions. The structure of **6a** was confirmed by 1D and 2D NMR spectra, except for the configuration of C-11.

Compounds **1**, **4**, and **6** were tested for biological activity against two bacteria, *Escherichia coli* and *Staphylococcus aureus*, two cancer cell lines, K562 and A549, and acetylcholinesterase (AChE). However, none of these compounds showed inhibitory effects up to the 200  $\mu\text{g/mL}$  dose used.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a JASCO DIP-370 automatic digital polarimeter. UV spectra were recorded on a Shimadzu double-beam 210A spectrometer. CD spectra were recorded on a JASCO J-715 spectropolarimeter. IR spectra were recorded on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. MS were measured on a VG Auto Spec-3000 mass spectrometer. Column chromatography was performed over silica gel G (80–100 and 300–400 mesh), silica gel H (10–40  $\mu\text{m}$ ), D<sub>101</sub> resin (Qingdao Marine Chemical Ltd.,

Qingdao, People's Republic of China), and Sephadex LH-20 (40–70  $\mu\text{m}$ ; Amersham Pharmacia Biotech AB, Uppsala, Sweden). TLC was conducted on precoated silica gel plates GF<sub>254</sub> (Qingdao). HPLC separations were performed using an Agilent 1200 series pump equipped with a diode array detector and a semipreparative Zorbax SB-C<sub>18</sub> (5  $\mu\text{m}$ ,  $\phi$  9.4  $\times$  250 mm) column. L-Phenylalanine (L-Phe) was purchased from Shanghai Xinxing Chemical Reagent Institute (Shanghai, People's Republic of China), and trifluoroacetic acid (TFA) from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, People's Republic of China).

**Plant Material.** *Selaginella moellendorffii* was collected from Jingxi County of Guangxi Zhuang Autonomous Region, People's Republic of China, in June 2008. The plant was identified by Dr. Guang-Wan Hu (Kunming Institute of Botany, Chinese Academy of Sciences), and a voucher specimen (No. JX0801) was deposited at the Laboratory of Ethnobotany, Kunming Institute of Botany.

**Extraction and Isolation.** The air-dried whole plant of *S. moellendorffii* (6 kg) was exhaustively extracted with MeOH. The solvent was evaporated under reduced pressure, and the remaining residue (780 g) was partitioned into three fractions, CHCl<sub>3</sub> (A, 32 g), Me<sub>2</sub>CO (B, 154 g), and MeOH (C, 350 g), by silica gel column chromatography. Fraction C was separated into two further fractions, H<sub>2</sub>O (C<sub>1</sub>, 270 g) and MeOH (C<sub>2</sub>, 71 g), by D<sub>101</sub> resin column chromatography. Fraction C<sub>2</sub>, which was alkaloid positive by Dragendorff's reagent, was fractionated by C<sub>18</sub> column chromatography (MeOH–H<sub>2</sub>O, 5:95 to 95:5) into three major alkaloid positive fractions, 20% MeOH (C<sub>21</sub>, 2.0 g), 30% MeOH (C<sub>22</sub>, 0.5 g), and 50% MeOH (C<sub>23</sub>, 1.5 g).

Fraction C<sub>21</sub> was submitted to chromatography on a silica gel column (CHCl<sub>3</sub>–Et<sub>2</sub>NH, 1:1; CHCl<sub>3</sub>–MeOH–Et<sub>2</sub>NH, 90:30:1) to afford **6** (100 mg), which was purified by LH-20 column chromatography (MeOH) and semipreparative HPLC [MeOH–H<sub>2</sub>O (containing 0.05% TFA), 35:65], and **1** (200 mg), which was purified by LH-20 column chromatography (MeOH). Fraction C<sub>22</sub> was separated by silica gel column chromatography (Me<sub>2</sub>CO–H<sub>2</sub>O, 10:1 and 5:1) to yield **3** (13.0 mg), which was purified by HPLC [MeCN–H<sub>2</sub>O (0.05% TFA), 20:80], and **2** (8.3 mg), which was purified by HPLC [MeOH–H<sub>2</sub>O (0.05% TFA), 35:65]. Fraction C<sub>23</sub> was chromatographed over a silica gel (CHCl<sub>3</sub>–MeOH–Et<sub>2</sub>NH, 90:30:1) and a Sephadex LH-20 column (MeOH) and by HPLC [MeOH–H<sub>2</sub>O (0.05% TFA), 45:55] to give **8** (2.5 mg), **7** (14.0 mg), **5** (11.0 mg), and **4** (23.8 mg).

**Selaginellinic acid (1):** colorless, amorphous solid (MeOH);  $[\alpha]_{\text{D}}^{25}$  –61.8 (c 0.51, H<sub>2</sub>O); UV (H<sub>2</sub>O)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 260 (3.40), 205 (4.11) nm; CD (c 0.060, MeOH)  $\Delta\epsilon$  –2.91 (205), –6.19 (194); IR (KBr)  $\nu_{\text{max}}$  3428, 1718, 1695, 1677, 1460  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR, see Tables 1 and 3; ESIMS  $m/z$  301 [M + H]<sup>+</sup>; HRESIMS  $m/z$  301.1912 [M + H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>25</sub>N<sub>2</sub>O<sub>2</sub>, 301.1916).

**5-Hydroxyselaginellinic acid (2):** colorless, amorphous solid (MeOH);  $[\alpha]_{\text{D}}^{25}$  –25.0 (c 0.36, H<sub>2</sub>O); UV (H<sub>2</sub>O)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 274 (3.07), 257 (3.02) nm; CD (c 0.060, MeOH)  $\Delta\epsilon$  +1.87 (211), 0 (204), –6.93 (194); IR (KBr)  $\nu_{\text{max}}$  3432, 1687, 1605, 1476, 1460  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR,



see Tables 1 and 3; ESIMS  $m/z$  317  $[M + H]^+$ ; HRESIMS  $m/z$  317.1860  $[M + H]^+$  (calcd for  $C_{18}H_{25}N_2O_3$ , 317.1865).

**5-Hydroxy- $N_8,N_8$ -dimethylpseudophrynaminol (3):** colorless, amorphous solid (MeOH);  $[\alpha]_D^{25}$   $-17.0$  ( $c$  0.24,  $H_2O$ ); UV ( $H_2O$ )  $\lambda_{max}$  (log  $\epsilon$ ) 274.0 (3.06), 195.4 (4.14) nm; CD ( $c$  0.055, MeOH)  $\Delta\epsilon$   $-1.25$  (224), 0 (209),  $+1.52$  (199); IR (KBr)  $\nu_{max}$  3424, 1679, 1606, 1460  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR, see Tables 1 and 3; ESIMS  $m/z$  303  $[M]^+$ ; HRESIMS  $m/z$  303.2071  $[M]^+$  (calcd for  $C_{18}H_{27}N_2O_2$ , 303.2072).

***N*-Selaginelloyl-L-phenylalanine (4):** colorless, amorphous solid (MeOH);  $[\alpha]_D^{25}$   $-37.8$  ( $c$  0.48,  $H_2O$ ); UV ( $H_2O$ )  $\lambda_{max}$  (log  $\epsilon$ ) 205 (4.08) nm; CD ( $c$  0.070, MeOH)  $\Delta\epsilon$   $+0.22$  (221), 0 (217),  $-1.16$  (205),  $-3.34$  (193); IR (KBr)  $\nu_{max}$  3428, 1684, 1631, 1532, 1457  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR, see Tables 2 and 3; ESIMS  $m/z$  448  $[M + H]^+$ ; HRESIMS  $m/z$  448.2585  $[M + H]^+$  (calcd for  $C_{27}H_{34}N_3O_3$ , 448.2600).

***N*-(5-Hydroxyselaginelloyl)-L-phenylalanine (5):** colorless, amorphous solid (MeOH);  $[\alpha]_D^{25}$   $-67.5$  ( $c$  0.20,  $H_2O$ ); UV ( $H_2O$ )  $\lambda_{max}$  (log  $\epsilon$ ) 268 (3.46) nm; CD ( $c$  0.057, MeOH)  $\Delta\epsilon$   $+1.81$  (214), 0 (203),  $-2.22$  (197); IR (KBr)  $\nu_{max}$  3379, 1674, 1628, 1604, 1500, 1457  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR, see Tables 2 and 3; ESIMS  $m/z$  464  $[M + H]^+$ ; HRESIMS  $m/z$  464.2553  $[M + H]^+$  (calcd for  $C_{27}H_{34}N_3O_4$ , 464.2549).

**Neoselaginellol acid (6):** colorless, amorphous solid (MeOH);  $[\alpha]_D^{25}$   $-17.8$  ( $c$  2.58, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 206 (3.93) nm; CD ( $c$  0.060, MeOH)  $\Delta\epsilon$   $-2.00$  (218),  $-1.86$  (198); IR (KBr)  $\nu_{max}$  3435, 1686, 1650, 1619, 1471  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR, see Tables 2 and 3; ESIMS  $m/z$  317  $[M + H]^+$ ; HRESIMS  $m/z$  317.1870  $[M + H]^+$  (calcd for  $C_{18}H_{25}N_2O_3$ , 317.1865).

***N*-Neoselaginelloyl-L-phenylalanine (7):** colorless, amorphous solid (MeOH);  $[\alpha]_D^{25}$   $-19.3$  ( $c$  0.28, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 207 (4.10) nm; CD ( $c$  0.060, MeOH)  $\Delta\epsilon$   $-0.70$  (216), 0 (209),  $+5.29$  (193); IR (KBr)  $\nu_{max}$  3411, 3399, 1733, 1646, 1617, 1528, 1497, 1454  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR, see Tables 2 and 3; ESIMS  $m/z$  464  $[M + H]^+$ ; HRESIMS  $m/z$  464.2536  $[M + H]^+$  (calcd for  $C_{27}H_{34}N_3O_4$ , 464.2549).

***N*-(5-Hydroxyneloselaginelloyl)-L-phenylalanine (8):** colorless, amorphous solid (MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 281 (3.22), 204 (4.39) nm; IR (KBr)  $\nu_{max}$  3430, 1676, 1501, 1454  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR, see Tables 2 and 3; ESIMS  $m/z$  480  $[M + H]^+$ ; HRESIMS  $m/z$  480.2490  $[M + H]^+$  (calcd for  $C_{27}H_{34}N_3O_5$ , 480.2498).

**Acid Hydrolysis of 4.** Compound 4 (10 mg, 0.0224 mmol) was dissolved in 2 mL of 5.7 N HCl and hydrolyzed (6 h) at 110 °C. The acidic solution was evaporated in vacuo to dryness. About 5 mL of  $H_2O$  was added to the reaction mixture and then evaporated in vacuo to dryness. This step was repeated three times. Finally, the reaction mixture was separated by HPLC [MeOH– $H_2O$  (0.05% TFA), 30:70] to yield L-phenylalanine (1.2 mg, 0.0073 mmol, 33%). Analytical data:  $[\alpha]_D^{17}$   $-22.2$  ( $c$  0.06,  $H_2O$ ); HPLC retention time was identical with that of authentic L-phenylalanine.

**Transformation of 1 to 6.**  $I_2$  (11 mg, 0.043 mmol) and NaOAc (3.9 mg, 0.048 mmol, 1.1 equiv) were added to a stirred solution of compound 1 (13 mg, 0.043 mmol) in EtOH (1 mL) at 75 °C. After 3 h, the reaction mixture was concentrated. Then, 25% NaOH (0.5 mL, 3.12 mmol) was added and stirred for 1 h at room temperature. This was followed by the addition of 2 N HCl (1.6 mL, 3.20 mmol) into the reaction mixture, which was then concentrated and separated by HPLC [MeOH– $H_2O$  (0.05% TFA), 35:65] to yield 6 (2.4 mg, 0.0076 mmol, 18%). Analytical data:  $[\alpha]_D^{18}$   $-45.8$  ( $c$  0.12, MeOH);  $^1H$  NMR spectrum (see the Supporting Information) and HPLC retention time were identical to those of natural neoselaginellol acid isolated from the plant.

**Tautomerization of 6 to 6a.** Compound 6 (80 mg, 0.253 mmol) was dissolved in 2 mL of 2 N HCl and stirred for 4 h at 90 °C. Following this procedure, the acidic solution was concentrated and separated by HPLC [MeCN– $H_2O$  (0.05% TFA), 10:90] to result in the purification of 6a (30 mg, 0.095 mmol, 38%). Analytical data: colorless, amorphous solid (MeOH);  $[\alpha]_D^{18}$   $+5.4$  ( $c$  0.37, MeOH); UV ( $H_2O$ )  $\lambda_{max}$  (log  $\epsilon$ ) 262 (2.58), 205 (3.75) nm; CD ( $c$  0.057, MeOH)  $\Delta\epsilon$   $+0.53$  (229), 0 (224),  $-1.93$  (215),  $-7.34$  (197);  $^1H$  and  $^{13}C$  NMR, see Tables 1 and 3; ESIMS  $m/z$  317  $[M + H]^+$ .

**Tautomerization of 6a to 6.** Compound 6a (20 mg, 0.063 mmol) was dissolved in 2 mL of 10% NaOH (5 mmol) and stirred for 1 h at 60 °C. Then, 2 N HCl (2.5 mL, 5 mmol) was added to the reaction mixture and concentrated and separated by HPLC [MeOH– $H_2O$  (0.05% TFA), 35:65] to give 6 (17.2 mg, 0.0544 mmol, 86%). Analytical data: HPLC retention time identical to that of natural neoselaginellol acid isolated from the plant.

**Biological Testing.** The activity of compounds 1, 4, and 6, at the concentrations of 2, 20, and 200  $\mu g/mL$ , against *E. coli* and *Staphylococcus aureus* were measured by the microdilution assay with gentamicin as positive control (MIC = 0.10  $\mu M$ ),<sup>9</sup> K562 cells by the MTT method with adriamycin as positive control (IC<sub>50</sub> = 0.32  $\mu M$ ),<sup>10</sup> A549 cells by the SRB method with 5-fluorouracil as positive control (IC<sub>50</sub> = 20.8  $\mu M$ ),<sup>11</sup> and AChE by Ellman's method with tacrine as positive control (IC<sub>50</sub> = 0.20  $\mu M$ ),<sup>12</sup> respectively.

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**Supporting Information Available:** 1D and 2D NMR spectra for compounds 1–8 and 6a. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- (1) Huang, A.; Kodanko, J. J.; Overman, L. E. *J. Am. Chem. Soc.* **2004**, *126*, 14043–14053.
- (2) (a) Yu, Q. S.; Holloway, H. W.; Flippen-Anderson, J. L.; Hoffman, B.; Brossi, A.; Greig, N. H. *J. Med. Chem.* **2001**, *44*, 4062–4071. (b) Houghton, P. J.; Ren, Y. H.; Howes, M. J. *Nat. Prod. Rep.* **2006**, *23*, 181–199. (c) Rivera-Becerril, E.; Joseph-Nathan, P.; Perez-Alvarez, V. M.; Morales-Rios, M. S. *J. Med. Chem.* **2008**, *51*, 5271–5284.
- (3) Amador, T. A.; Verotta, L.; Nunes, D. S.; Elisabetsky, E. *Planta Med.* **2000**, *66*, 770–772.
- (4) Dix, A. V.; Meseck, C. M.; Lowe, A. J.; Mitchell, M. O. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2522–2524.
- (5) (a) Chen, D. Z.; Yu, J. G. *Zhongcaoyao* **1986**, *17*, 4. (b) Zheng, X. K.; Li, K. K.; Wang, Y. Z.; Feng, W. S. *Chin. Chem. Lett.* **2008**, *19*, 79–81. (c) Zhu, T. M.; Chen, K. L.; Zhou, W. B. *Chin. Chem. Lett.* **2008**, *19*, 1456–1458. (d) Sun, C. M.; Syu, M. J.; Huang, Y. T.; Chen, C. C.; Ou, J. C. *J. Nat. Prod.* **1997**, *60*, 382–384. (e) Shi, S. Y.; Zhou, H. H.; Zhang, Y. P.; Huang, K. L. *Chromatographia* **2008**, *68*, 173–178.
- (6) Spande, T. F.; Edwards, M. W.; Pannell, L. K.; Daly, J. W.; Erspamer, V.; Melchiorri, P. *J. Org. Chem.* **1988**, *53*, 1222–1226.
- (7) Takayama, H.; Matsuda, Y.; Masubuchi, K.; Ishida, A.; Kitajima, M.; Aimi, N. *Tetrahedron* **2004**, *60*, 893–900.
- (8) (a) Shirasaka, T.; Takuma, Y.; Shimpuku, T.; Imaki, N. *J. Org. Chem.* **1990**, *55*, 3767–3771. (b) Nakagawa, M.; Maruyama, T.; Hirakoso, K.; Hino, T. *Tetrahedron Lett.* **1980**, *21*, 4839–4842. (c) Nakagawa, M.; Kato, S.; Fukazawa, H.; Hasegawa, Y.; Miyazawa, J.; Hino, T. *Tetrahedron Lett.* **1985**, *26*, 5871–5874.
- (9) Xu, S. Y.; Bian, R. L.; Chen, X. *Pharmacological Experiment Methodology*, 3rd ed.; People's Medical Publishing House: Beijing, 2002; pp 1647–1719.
- (10) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
- (11) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokes, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.
- (12) (a) Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88–95. (b) Rhee, I. K.; Appels, N.; Hofte, B.; Karabatak, B.; Erkelens, C.; Stark, L. M.; Flippin, L. A.; Verpoorte, R. *Biol. Pharm. Bull.* **2004**, *27*, 1804–1809.

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