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ORIGINAL ARTICLE

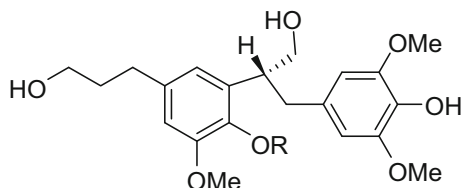# Neolignans from *Selaginella moellendorffii*

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Ren-Qiang Mei · Jun Yang · Yi Kong ·
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Abstract   Two new neolignans selaginellol (**1**) and selaginellol 4′-*O*-β-D-glucopyranoside (**2**), together with seven known compounds (**3–9**), were isolated from the whole plant of *Selaginella moellendorffii*. The structures of the new isolates were determined through spectroscopic data analysis. Compounds **1–9**, as well as compounds **10–18** previously isolated from the species, were measured for the activity against platelet aggregation induced by ADP or collagen. Three neoligans (**8**, **11**, and **12**), one flavanone (**14**), and one alkaloid (**16**) showed inhibitory activity against ADP- or collagen-induced platelet aggregation as compared with tirofiban. The dihydrobenzofuran neolignans (**8**, **11**, and **12**) are more potent than the benzofuran neolignan (**13**) and other types of neolignans (**1–7**). Glucosidation of the dihydrobenzofuran neolignans (**11** and **12**) is helpful for the activity.

Graphical Abstract   Two new neolignans selaginellol (**1**) and selaginellol 4′-*O*-β-D-glucopyranoside (**2**) were isolated from the whole plant of *Selaginella moellendorffii*. Several compounds from this plant showed the activity against platelet aggregation induced by ADP or collagen.

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1 Introduction

The family Selaginellaceae Willk. includes the single genus *Selaginella* Beauv. *Selaginella* is a nearly worldwide
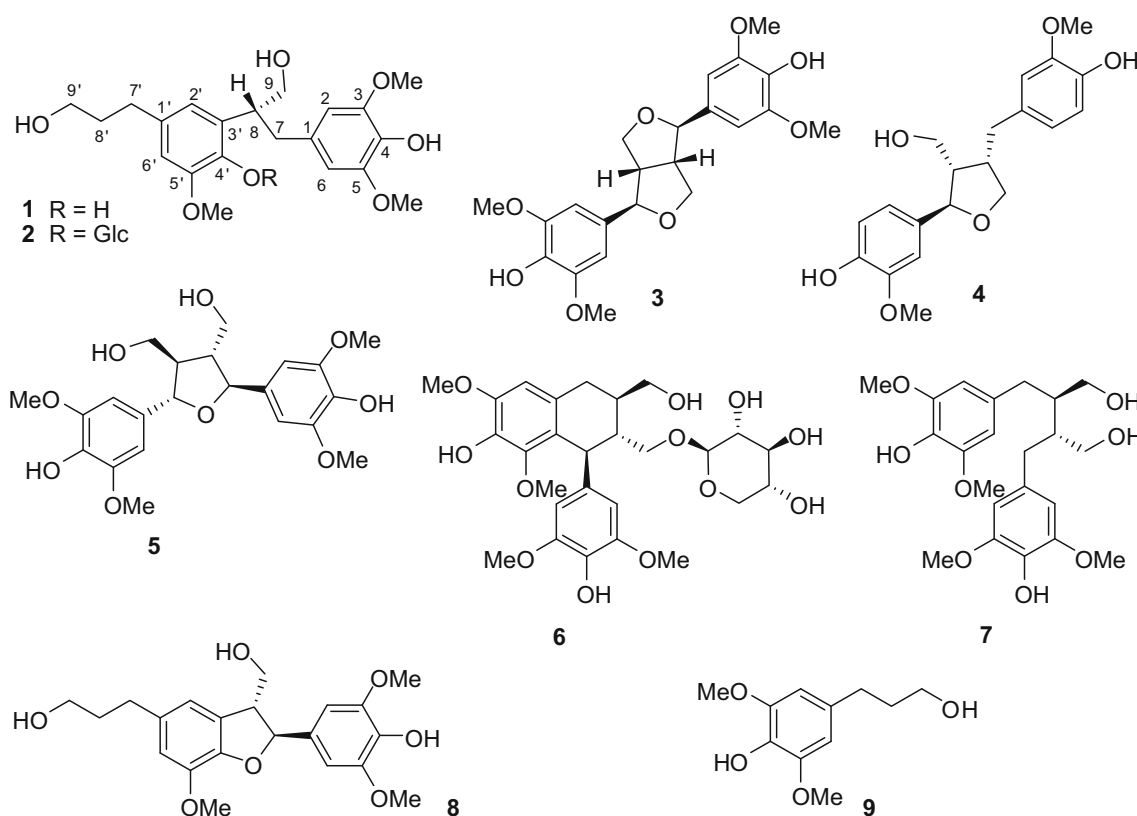
Fig. 1 The chemical structures of **1**–**9** from *Selaginella moellendorffii*

genus of about 700 species, with 72 of them in China and more than 20 species used in traditional Chinese medicine [1, 2]. Several *Selaginella* species including *S. delicatula* (Desv. ex Poir.) Alston, *S. moellendorffii* Hieron., *S. nipponica* Franch. & Sav., *S. sanguinolenta* (L.) Spring, *S. stauntoniana* Spring, and *S. tamariscina* (P. Beauv.) Spring are used in promotion of blood circulation (Huoxue in Chinese) [1]. Traditional Chinese medicines with the functions of "Huoxue" and/or "Huayu" (removing blood stasis) are claimed to be useful in antiplatelet therapies and the treatment of thrombotic diseases [3, 4]. Previously, a pyrrolidinoindoline alkaloid selaginellic acid with antiplatelet activity was found from the whole plant of *S. moellendorffii* [5, 6]. This result prompted us to further investigate the plant which led to the isolation of nine compounds (**1**–**9**, Fig. 1) including two new neolignans (**1** and **2**). Compounds **1**–**9**, as well as those (**10**–**18**) previously isolated from the plant [5, 7, 8], were evaluated for antiplatelet activity. The structural elucidation of the new compounds and the bioassay results are reported.

2 Results and Discussion

The HRESIMS analysis of selaginellol (**1**) gave an $[M+Na]^+$ ion at *m/z* 415.1729 appropriate for a molecular formula of $C_{21}H_{28}O_7$ requiring eight sites of unsaturation. The IR absorption signals revealed the presence of hydroxy (3428 cm^{-1}) and aromatic (1614, 1518, 1496, and 1461 cm^{-1}) groups. The ^1H NMR data of **1** (Table 1) exhibited three methoxy groups [δ_H 3.82 (3H, s) and 3.70 (6H, s)], and two 1,2,3,5-tetrasubstituted benzene rings [δ_H 6.30 (2H, s); 6.63 (d, *J* = 1.6 Hz) and 6.47 (d, *J* = 1.6 Hz)]. The ^{13}C NMR data of **1** (Table 1) showed the signals for three methoxy groups (δ_C 56.5 × 2 and 56.4), two phenyl rings, five methylenes including two oxygenated ones (δ_C 65.9, 62.2, 37.9, 35.8, and 32.8), and one methine (δ_C 45.4). According to above NMR signal characteristics [8], compound **1** might be a neolignan.

The ^1H–^1H COSY correlations (Fig. 2) exhibited two partial structures from C-7 to C-9 and C-7′ to C-9′. Based on the HMBC correlations (Fig. 2) from H-2 and H-6 to C-4, H_2-7 to C-2 and C-6, H-8 to C-1, H_2-8′ to C-1′, H_2-7′ to C-2′ and C-6′, H-2′ and H-6′ to C-4′, 3-OMe to C-3, 5-OMe to C-5, and 5′-OMe to C-5′, two phenylpropanoid moieties, namely 4-(3-hydroxypropyl)-2,6-dimethoxyphenol and 4-(3-hydroxypropyl)-2-methoxyphenol, were confirmed. The two fragments were linked through C-8-C-3′ by the HMBC correlations from H_2-7 and H_2-9 to C-3′ as well as H-8 to C-2′ and H-2′ to C-8. Therefore, the relative configuration of **1** was elucidated as 3,5,5′-trimethoxy-8,3′-neoligna-4,4′,9,9′-tetraol. The absolute configuration of

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** and **2** in CD$_3$OD (δ in ppm, J in Hz)

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1		132.9 (C)		132.5 (C)
2,6	6.30 (s)	107.3 (CH)	6.28 (s)	107.0 (CH)
3,5		148.7 (C)		148.6 (C)
4		134.2 (C)		134.1 (C)
7	3.00 (dd, 13.4, 5.7)	37.9 (CH$_2$)	3.01 (dd, 14.0, 5.0)	39.6 (CH$_2$)
	2.88 (dd, 13.4, 9.4)		2.71 (dd, 14.0, 10.0)	
8	3.42 (m)	45.4 (CH)	3.96 (m)	42.7 (CH)
9	3.76 (m)	65.9 (CH$_2$)	3.76 (dd, 10.6, 4.8)	67.1 (CH$_2$)
			3.67 (dd, 10.6, 7.9)	
1′		133.7 (C)		140.4 (C)
2′	6.47 (d, 1.6)	122.0 (CH)	6.73 (d, 1.8)	120.2 (CH)
3′		129.3 (C)		138.5 (C)
4′		143.7 (C)		143.5 (C)
5′		148.7 (C)		153.2 (C)
6′	6.63 (d, 1.6)	110.6 (CH)	6.72 (d, 1.8)	111.6 (CH)
7′	2.53 (t, 7.5)	32.8 (CH$_2$)	2.64 (t, 7.6)	33.1 (CH$_2$)
8′	1.74 (m)	35.8 (CH$_2$)	1.82 (m)	35.7 (CH$_2$)
9′	3.51 (t, 6.4)	62.2 (CH$_2$)	3.57 (t, 6.2)	62.2 (CH$_2$)
1″			4.57 (d, 7.5)	105.6 (CH)
2″			3.44 (m)	75.9 (CH)
3″			3.39 (m)	77.8 (CH)
4″			3.38 (m)	71.1 (CH)
5″			3.11 (m)	78.0 (CH)
6″			3.79 (overlapped)	62.4 (CH$_2$)
			3.70 (overlapped)	
3,5-OMe	3.70 (s)	56.5 (CH$_3$)	3.70 (s)	56.5 (CH$_3$)
5′-OMe	3.82 (s)	56.4 (CH$_3$)	3.80 (s)	56.3 (CH$_3$)
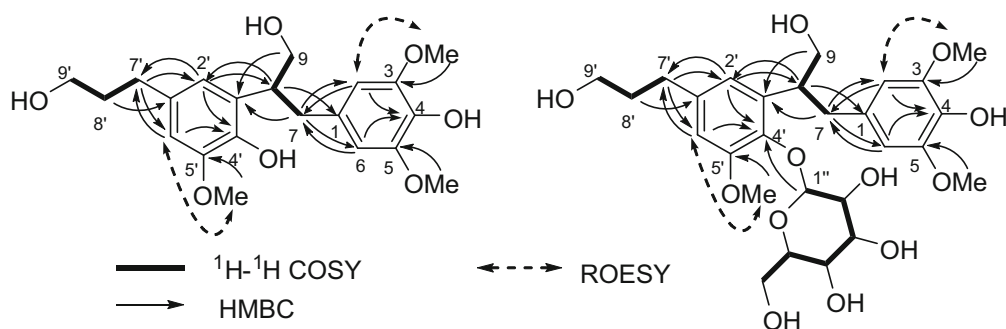
Fig. 2 Key 2D NMR correlations of **1** and **2**

selaginellol (**1**) was elucidated as (8R)-3,5,5′-trimethoxy-8,3′-neoligna-4,4′,9,9′-tetraol by comparing its electronic circular dichroism (ECD) spectrum [$\Delta\varepsilon$ −0.12 (273)] with that of a known analogue secodihydrodehydrodiconiferyl alcohol tetraacetate [9].

According to the HREIMS ion at *m/z* 554.2365 [M]$^+$ (calcd for C$_{27}$H$_{38}$O$_{12}$, 554.2363), the molecular formula of compound **2** was determined as C$_{27}$H$_{38}$O$_{12}$ with nine degrees of unsaturation. The IR absorption signals showed the presence of hydroxy (3425 cm^{-1}) and aromatic (1614,

1518, and 1461 cm^{-1}) groups. The NMR data (Table 1) of **2** were very similar to those of **1**, except that more signals for a β-glucopyranosyl moiety [δ_H 4.57 (d, $J = 7.5$ Hz); δ_C 105.6, 75.9, 77.8, 71.1, 78.0, and 62.4] were observed. As demonstrated in the ^1H–^1H COSY, HMBC and ROESY correlations (Fig. 2), compound **2** was determined to be the β-glucopyranoside of selaginellol (**1**). The HMBC correlation from H-1″ to C-4′ indicated that the β-glucopyranosyl part was located at C-4′. According to our previously acidic hydrolysis of rel-(7R,8S)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol 4-O-β-D-glucopyranoside (**11**) [8], the sugar in the plant is D-glucose. The absolute configuration of the aglycone was elucidated to be the same as that of selaginellol (**1**) by comparison of its ECD spectrum [$\Delta\varepsilon$ −0.37 (273)] with that of **1**. Therefore, compound **2** is selaginellol 4′-O-β-D-glucopyranoside.

The known compounds were determined as (−)-syringaresinol (**3**) [10], (−)-lariciresinol (**4**) [11], 7S,7′S,8R,8′R-icariol A$_2$ (**5**) [12], lyoniside (**6**) [13], (−)-8,8′-bisdihydrosiringenin (**7**) [14], (7S,8R)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol (**8**) [8], and dihydrosinapyl alcohol (**9**) [15], by comparing their NMR data (for all known compounds) and optical rotation values (for the neolignans) with those reported in the literature.

All of these compounds (**1**–**9**), along with those previously isolated from the plant, including (7S,8R)-4,9-dihydroxy-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-9′-oic acid methyl ester (**10**) [8], rel-(7R,8S)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol 4-O-β-D-glucopyranoside (**11**) [8], rel-(7R,8S)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol 9-O-β-D-glucopyranoside (**12**) [8], 3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolign-7-ene-4,9,9′-triol 9-O-β-D-glucopyranoside (**13**) [8], 5-carboxymethyl-7,4′-dihydroxyflavanone 7-O-β-D-glucopyranoside (**14**) [7], N-selaginelloyl-L-phenylalanine (**15**) [5], paucine 3′-O-β-D-glucopyranoside (**16**) [8], paucine (**17**) [8], and N^1-cis-p-coumaroylagmatine (**18**) [8], were evaluated for the inhibitory activity against platelet aggregation induced by ADP or collagen. As shown in Table 2, compounds **8**, **11**, **12**, **14**, and **16** showed potential inhibitory activity against ADP-induced platelet aggregation with

IC$_{50}$ values of 80.84, 35.76, 42.47, 27.70, and 59.19 μM, respectively, as compared with the positive control tirofiban (IC$_{50} = 25.32$ μM). Compounds **8**, **11**, **12**, and **14** also showed the activity against collagen-induced platelet aggregation with IC$_{50}$ values of 146.70, 31.17, 24.57, and 26.25 μM, respectively, as compared with the positive control tirofiban (IC$_{50} = 148.20$ μM). The dihydrobenzofuran neolignans (**8**, **11**, and **12**) are more potent than the benzofuran neolignan (**13**) and other types of neolignans (**1**–**7**). Glucosidation of the dihydrobenzofuran neolignans (**11** and **12**) is helpful for the activity as compared the bioassay result of **11** and **12** with that of **8**.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were recorded using a JASCO P-1020 Polarimeter (Jasco Corp., Tokyo, Japan). UV spectra were taken on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). ECD spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics Ltd., Leatherhead, UK). IR spectra were measured on a Bruker Tensor 27 FTIR Spectrometer (Bruker Corp., Ettlingen, Germany) with KBr disks. ^1H and ^{13}C NMR spectra were collected on Bruker Avance 400, DRX-500 or Avance III-600 spectrometers (Bruker Bio-Spin GmbH, Rheinstetten, Germany) with TMS as an internal standard. ESIMS and HRESIMS analyses were carried out on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). HREIMS were carried out on a Waters AutoSpec Premier p776 spectrometer (Waters, Millford, MA, USA). Silica gel G (80–100 and 300–400 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, China), C$_{18}$ silica gel (40–75 μm, Fuji Silysia Chemical Ltd., Aichi, Japan), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and D$_{101}$ macroporous resin (Qingdao Marine Chemical Ltd., Qingdao, China) were used for column chromatography, and silica gel GF$_{254}$ (Qingdao Meigao Chemical Co., Ltd.) was used for preparative TLC as precoated plates. TLC spots were visualized under UV light at 254 nm and by dipping into 5 % H$_2$SO$_4$ in alcohol followed by heating. Semipreparative HPLC was performed on an Agilent 1200 series pump (Agilent Technologies, Santa Clara, USA) equipped with a diode array detector and an Agilent Zorbax SB-C$_{18}$ column (5.0 μm, ϕ 9.4 × 250 mm).

3.2 Plant Material

The whole plant of *S. moellendorffii* was collected from Jingxi County of Guangxi Zhuang Autonomous Region in

Table 2 The effect of compounds on rabbit platelet aggregation induced by ADP (10 μM) or collagen (2.5 μg/mL)

Compound	ADP (IC$_{50}$ μM)	Collagen (IC$_{50}$ μM)
8	80.84	146.70
11	35.76	31.17
12	42.47	24.57
14	27.70	26.25
16	59.19	>200
Tirofiban (positive control)	25.32	148.20

2008. A voucher specimen (No. JX0801) was identified by one of the authors (Chun-Lin Long) and deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried, powdered *S. moellendorffii* plants (15 kg) were exhaustively extracted with MeOH (45 L × 3) at 60 °C. The solvent was removed to give a residue (0.89 kg). The crude extract was subjected to chromatography on a D_{101} macroporous resin column eluted successively with H_2O, 35 % EtOH, and 95 % EtOH to give three portions (I–III), respectively. Portion II (398 g) was subjected to column chromatography (silica gel G; $CHCl_3$/ MeOH, 1:0 → 0:1, v/v) to yield six fractions (A–F). Fr. A was subjected to column chromatography (silica gel G; petroleum ether/EtOAc, 15:1 → 0:1, v/v) to yield four fractions (A1–A4). Fr. A1 was purified by column chromatography (silica gel G; $CHCl_3$-acetone, 15:1, v/v) to obtain **9**. Fr. A2 was chromatographed on a Sephadex LH–20 column (MeOH) to give subfractions A2–1 and A2–2. Subfraction A2–1 was subjected to chromatography on a silica gel G column ($CHCl_3$-acetone, 20:1, v/v) and then further purified by semi-preparative HPLC (MeCN/H_2O, 30:70, v/v) to yield **4** (14.9 mg, t_R = 13.099 min). Subfraction A2-2 was purified by preparative TLC ($CHCl_3$/ MeOH, 10:1, v/v) to obtain **3** (24.6 mg). Fr. A3 was chromatographed over a C_{18} silica gel column (MeOH/ H_2O, 30:70, v/v), a Sephadex LH-20 column (MeOH), and a silica gel G column ($CHCl_3$/MeOH/H_2O, 50:1:0.25), and purified by semi-preparative HPLC (MeOH/H_2O, 40:60, v/v) to obtain **5** (15.5 mg, t_R = 5.864 min). Fr. A4 was chromatographed on a Sephadex LH-20 column (MeOH), a C_{18} silica gel (MeOH/H_2O, 50:50, v/v), and a silica gel G column ($CHCl_3$/MeOH, 60:1, v/v), and purified by semi-preparative HPLC (MeCN/H_2O, 30:70, v/v) to yield **7** (2.0 mg, t_R = 7.716 min), **8** (2.0 mg, t_R = 8.917 min) and **1** (4.0 mg, t_R = 13.652 min). Fr. D was chromatographed on a C_{18} silica gel column (MeOH/H_2O, 30:70, v/v), a Sephadex LH-20 column (MeOH), and a silica gel G column ($CHCl_3$/MeOH/H_2O, 100:10:0.5, v/v), and purified by semi-preparative HPLC (MeOH/H_2O, 40:60, v/v) to yield **2** (7.8 mg, t_R = 12.138 min) and **6** (4.4 mg, t_R = 18.734 min).

3.3.1 Selaginellol (1)

Pale yellow oil (MeOH); $[\alpha]_D^{24}$ −50.4 (*c* 0.40, MeOH); UV (CH_3OH) λ_{max} (log ε) 280 (3.31), 228 (3.98) nm; ECD Δε (*c* 0.010, MeOH) −0.12 (273), −3.84 (214), +3.63 (197); IR (KBr) ν_{max} 3428, 1614, 1518, 1496, 1461, 1431, 1289,

1217, 1114 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ion ESIMS *m/z* 415 [M+Na]$^+$; positive ion HRESIMS *m/z* 415.1729 [M+Na]$^+$ (calcd for $C_{21}H_{28}O_7Na^+$, 415.1727).

3.3.2 Selaginellol 4′-O-β-D-glucopyranoside (2)

Pale yellow solid (MeOH); $[\alpha]_D^{22}$ −57.8 (*c* 0.26,MeOH); UV (CH_3OH) λ_{max} (log ε) 274 (3.80) nm; ECD Δε (*c* 0.011, MeOH) −0.37 (273), −6.17 (210), +3.20 (200); IR (KBr) ν_{max} 3425, 1615, 1518, 1461, 1428, 1325, 1216, 1113, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ion ESIMS *m/z* 577 [M+Na]$^+$; HREIMS *m/z* 554.2365 [M]$^+$ (calcd for $C_{27}H_{38}O_{12}$, 554.2363).

3.4 In Vitro Platelet Aggregation Assay

In vitro platelet aggregation was conducted using the turbidimetric method with a minor modification [16, 17]. Briefly, blood was withdrawn from the carotid artery of New Zealand rabbits,and anticoagulated with 3.8 % sodium citrate (1:9 citrate/blood, v/v) and centrifuged for 15 min at 950 rpm to prepare platelet-rich plasma (PRP) or 10 min at 3000 rpm to obtain platelet-poor plasma (PPP). The platelet concentration was adjusted to 3×10^8 platelets/mL. PRP in 270 μL was preincubated at 37 °C for 5 min in the cuvette with 20 μL of sample or vehicle (saline), and then platelet aggregation was induced by 10 μL ADP (10 μM) or collagen (2.5 μg/mL). The maximum platelet aggregation rate was determined within 5 min with continuous stirring at 37 °C using four-channel aggregometer (Beijing Steellex Science Instrument Company, China).

For each compound, five concentrations were chosen and a percentage inhibition-concentration curve was derived. From this curve the IC_{50} value was calculated as the concentration of inhibitor causing a 50 % inhibition of the aggregation using SPSS software.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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