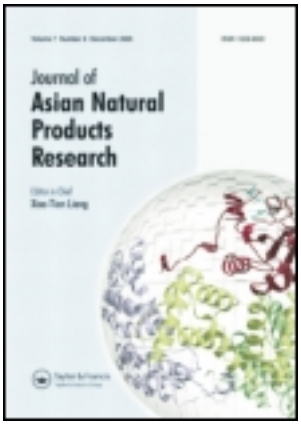

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Selaginellin M, a new selaginellin derivative from *Selaginella pulvinata*

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NOTE

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A new selaginellin derivative, selaginellin M (**1**), together with one known compound, selaginellin E (**2**), was isolated from *Selaginella pulvinata*. The structure of the new compound was elucidated and named as (R,S)-4-((4′-hydroxy-4-((2-hydroxyethoxy) methyl))-3-((4-hydroxyphenyl)ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene)-cyclohexa-2,5-dienone on the basis of the spectroscopic data including UV, IR, 1D, and 2D NMR as well as HR-ESI-MS analysis.

Keywords: selaginella; *Selaginella pulvinata*; selaginellin M; selaginellin E

1. Introduction

Selaginella pulvinata (Hook. et Grev.) Maxim. (Selaginellaceae), a perennial herb widely distributed in China, is one of the two qualified species documented in Chinese Pharmacopoeia [1]. Pharmacological investigation shows that they possess a variety of biological properties such as anti-inflammatory, antibacterial, antivirus, immunity stimulating, antitumor, analgesic, antispasmodic, and so on [2–4]. A number of compounds, such as flavones, lignans, sesquilignans, glucosides, and anthraquinones, have been isolated from genus *Selaginella*. Recently, selaginellins A–L, new alkynyl phenols with unusual carbon skeleton, have been isolated from *Spiranthes sinensis* [5], *Selaginella tamariscina* [6], and *S. pulvinata* [7–11], respectively. In our continuation study on chemical constituents of the genus *Selaginella*, herein, we report the isolation and structural elucidation of a new selaginellin derivative, selaginellin M (**1**), named as (R,S)-4-((4′-hydroxy-4-((2-hydroxyethoxy)methyl))-3-((4-hydroxyphenyl)ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene)cyclohexa-2,5-dienone (**1**) (Figure 1), together with one known compound, selaginellin E (**2**).

2. Results and discussion

The 75% EtOH extract of the whole herbs of *S. pulvinata* was chromatographed over polyamide resin, silica gel, HW-40C, HW-40F and prep.-HPLC to yield compounds **1** and **2**.

Compound **1** was obtained as a red powder. ESI-MS gave the quasi-molecular ion peak at *m/z* 580.2 $[M + Na + H]^+$ and its molecular formula was deduced as $C_{36}H_{28}O_6$ by HR-ESI-MS at *m/z* 557.1955 $[M + H]^+$ with 23 degrees of unsaturation. The UV spectrum of **1** showed the absorption maxima at 278 and 433 nm, the characteristic values of a selaginellin

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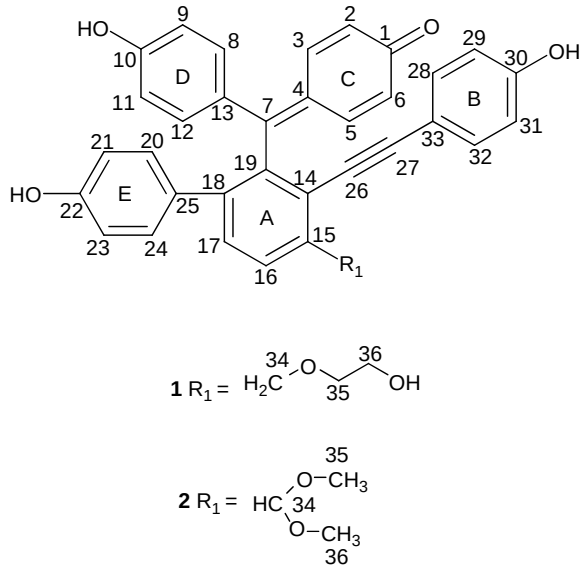

Figure 1. Structures of compounds **1** and **2**.

chromophore [5–11]. The IR spectrum of **1** showed absorption bands for hydroxyl (3434 cm^{-1}), alkynyl (2203 cm^{-1}), conjugated carbonyl (1654 cm^{-1}), and aromatic ring (1608 1509 cm^{-1}).

The ^1H NMR spectrum of **1** (Table 1) showed 18 aromatic protons at δ 7.75 (d, 1H, $J = 8.0$ Hz), 7.36 (d, 1H, $J = 8.0$ Hz), 7.06 (d, 4H, $J = 8.5$ Hz), 6.91 (d, 2H, $J = 8.0$ Hz), 6.75 (d, 2H, $J = 8.0$ Hz), 6.61 (d, 2H, $J = 8.0$ Hz), 6.54 (d, 2H, $J = 8.0$ Hz), and 6.40 (d, 4H, $J = 8.5$ Hz), and 3 methylene groups at δ 4.98 (s, 2H), 3.55 (t, 2H, $J = 5.0$ Hz), and 3.47 (t, 2H, $J = 5.0$ Hz), which was confirmed by the DEPT experiment. As we previously reported, extensive delocalization takes place in rings C and D caused rings C and D chemical equivalent. Thus, the signals at δ 7.06 (d, 4H, $J = 8.5$ Hz) and δ 6.40 (d, 4H, $J = 8.5$ Hz) indicated two equivalent para-substituted benzene rings and should be eight aromatic protons of rings C and D, which was confirmed by the HMBC (Figure 2) correlations of δ_H 6.40 (H-2, 6, 9, 11)/δ_C 129.5 (C-4, 13) and δ_H 7.06 (H-3, 5, 8, 12)/δ_C 161.4 (C-1, 7, 10) and $^1H-^1H$ correlation spectroscopy ($^1H-^1H$ COSY). The two doublets at δ 6.91 (d, 2H, $J = 8.0$ Hz) and δ 6.61 (d, 2H, $J = 8.0$ Hz) appearing as AA′BB′ type suggested the presence of a para-substituted benzene ring (ring B) which was confirmed by $^1H-^1H$ COSY and long-range correlations between H-28/32 at δ 6.91 and C-30 at δ_C 158.3. Another para-substituted phenol (ring E) was indicated by the two doublets at δ 6.75 (d, 2H, $J = 8.0$ Hz) and δ 6.54 (d, 2H, $J = 8.0$ Hz). And the two doublets at δ 7.75 (d, 1H, $J = 8.0$ Hz) and 7.36 (d, 1H, $J = 8.0$ Hz) in ^1H NMR spectrum suggested a 1,2,3,4-tetrasubstituted benzene ring (ring A). The ^{13}C NMR spectrum (Table 1) of **1** contained 23 signals, which could be classified using the DEPT spectrum as 8 methines, 3 methylenes and 12 quaternary carbons, revealing the presence of symmetrical units and overlapped signals in the structure. The ^{13}C NMR spectrum disclosed the presence of an acetylene bond (δ_C 84.4 and δ_C 99.0), which was connected with ring B as evidenced in HMBC correlations (H-28, H-32/C-27) (Figure 1). The correlation between H-35 at δ_H 3.55 and H-36 at δ_H 3.47 revealed in

Table 1. ^{1}H (500 and 400 MHz) and ^{13}C NMR (125 and 100 MHz) spectral data of compounds **1** and **2** in DMSO-d_6[a] (δ in ppm, J in Hz).

	1		**2**	
Position	δ_H (multility, J in Hz)	δ_C	δ_H (multility, J in Hz)	δ_C
1		161.4		158.2
2/6	6.40 (2H, d, 8.5)	121.8	6.46 (2H, d, 8.4)	121.4
3/5	7.06 (2H, d, 8.5)	137.3	7.03 (2H, d, 8.4)	136.0
4		129.5		128.7
7		161.4		158.2
8/12	7.06 (2H, d, 8.5)	137.3	7.03 (2H, d, 8.4)	136.0
9/11	6.40 (2H, d, 8.5)	121.8	6.46 (2H, d, 8.4)	121.4
10		161.4		158.2
13		129.5		128.7
14		123.5		122.7
15		138.2		137.8
16	7.75 (1H, d, 8.0)	129.1	7.65 (1H, d, 8.0)	126.6
17	7.36 (1H, d, 8.0)	130.1	7.35 (1H, d, 8.0)	129.2
18		141.9		142.1
19		140.8		140.5
20/24	6.75 (1H, d, 8.0)	129.9	6.81 (2H, d, 8.8)	129.3
21/23	6.54 (1H, d, 8.0)	115.0	6.55 (2H, d, 8.8)	114.6
22		156.5		156.5
25		130.9		130.2
26		84.4		83.7
27		99.0		98.3
28/32	6.91 (1H, d, 8.0)	133.2	7.00 (2H, d, 8.8)	132.7
29/31	6.61 (1H, d, 8.0)	115.9	6.67 (2H, d, 8.8)	115.5
30		158.3		158.7
33		112.5		111.9
34	4.98 (2H, s)	69.6	5.73 (1H, s)	102.4
35	3.55 (2H, t, 5.0)	72.3	3.42 (3H, s)	54.2
36	3.47 (2H, t, 5.0)	60.5	3.42 (3H, s)	54.2

[a] NMR spectra of compound **1** were recorded on Varian (Palo Alto, California, USA) INOVA-500 spectrometers, and NMR spectra of compound **2** were recorded on Varian INOVA-400 spectrometers.

the $^{1}H-^{1}H$ COSY spectrum and the HMBC correlation between H-34 at δ_H 4.98 (s, 1H) and C-35 at δ_C 72.3 indicated the presence of $-CH_2OCH_2CH_2OH$ group. HMBC correlations of H-34/C-14, C-16 revealed that $-CH_2OCH_2CH_2OH$ group should be located at C-15 (δ_C 138.2), which was also confirmed by nuclear Overhauser effect spectroscopy (NOESY) (Figure 3). HMBC correlations of H-20, H-24/C-18, and H-17/C-25 showed that ring E should be connected with A ring at C-18 (δ_C 141.9). The correlation between H-34 and H-28 in the NOESY spectrum suggested the connection between C-26 (C≡C) and C-14. The delocalization unit of rings C and D must be connected with A-ring at C-19 (δ_C 140.8). Accordingly, the structure of **1** was characterized as (R,S)-4-((4′-hydroxy-4-((2-hydroxyethoxy)methyl))-3-((4-hydroxyphenyl)ethynyl)biphenyl-2-yl)(4-hydroxyphenyl)methylene)cyclohexa-2,5-dienone on the basis of the above evidence.

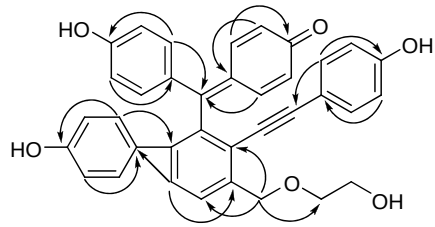

Figure 2. Key HMBC correlations of compound **1**.

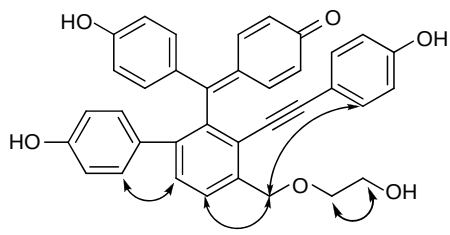

Figure 3. Key NOE correlations of compound **1**.

The full assignments of ^{1}H and ^{13}C NMR spectra were accomplished with the aid of DEPT, HSQC, HMBC, $^{1}H-^{1}H$ COSY, and NOESY experiments (Figures 2 and 3).

Compound **2** was identified as selaginellin E by 1D and 2D NMR spectroscopic analysis as well as by the comparison of its physical and spectral data with the reported values in the literature [5–11].

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a UV Probe-2450 spectrometer. IR spectra were obtained using KBr disks on an AVATAR 360FT-IR spectrophotometer (Nicolet Instrument Corporation, Madison, WI, USA). ESI-MS were registered on an LCQ-Advantage mass spectrometer. HR-ESI-MS were recorded on a Micromass Zabspec (Micromass UK Ltd, Manchester, UK), HR-MS spectrometer, and JMS-T100 CS. NMR spectra, including ^{1}H and ^{13}C NMR, COSY, NOESY, HMQC, and HMBC experiments, were recorded on a Varian Unity INOVA-400 and INOVA − 500 MHz spectrometer with tetramethylsilane as an internal standard. Polyamide (30–60 mesh; 200–300 mesh; China National Medicine Corporation Ltd, Shanghai, China), HW-40C, and HW-40F (TOYOPEARL TOSOH, Tokyo, Japan) were used for column chromatography (CC), and silica gel GF-254 (Qingdao Marine Chemical Factory, Qingdao, China) was used for TLC. Pre-HPLC experiments were carried out on a preparative YMC-Pack ODS-A column (15 μm, 250 × 30 mm, YMC, Kyoto, Japan).

3.2 Plant material

Herbs of *S. pulvinata* were collected in Hunan Province, China, and identified by Associate Prof. Jin-Ping Li (Central South University, Changsha, China). A voucher specimen has been deposited in School of Pharmaceutical Sciences, Central South University (No. JB-001).

3.3 Extraction and isolation

The air-dried whole herbs of *S. pulvinata* (10 kg) were extracted two times with 75% EtOH, and the extract (1 kg) obtained by concentrating solvent under reduced pressure was chromatographed over polyamide resin (60–100 mesh) column with EtOH–H_2O gradient elution (0, 40, 70, and 95%). The 70% EtOH portion was subjected to silica gel CC eluting with $CHCl_3$–MeOH (in gradient) to obtain Fr. 83–116. Fr. 83–116 were fractionated via polyamide resin (200–300 mesh) column with EtOH–H_2O gradient elution (50, 60, and 95%) to give Fr. 36–56. Fr. 36–56 were subjected to HW-40C with 60% MeOH–H_2O isocratic elution to obtain Fr. 2–5 (500 mg) and Fr. 19–24 (400 mg). Fr. 19–24 were further purified by preparative HPLC (70% aqueous MeOH, 30 ml/min) to yield compound **1** (30 mg, $t_R = 40.0$ min). Fr. 2–5 were separated by HW-40F gel permeation chromatography with 60% MeOH–H_2O isocratic elution to yield compound **2** (51 mg).

3.3.1 Selaginellin M (1)

A red powder. UV (MeOH) λ_{max} (nm): 278, 433. IR (KBr) ν_{max} (cm^{-1}): 3434, 2910, 2203, 1654, 1608, 1509. ^{1}H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z*: 580.2 $[M + Na + H]^+$. HR-ESI-MS *m/z*: 557.1955 $[M + H]^+$ (calcd for $C_{36}H_{29}O_6$, 557.1964).

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