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NOTE

A new phenylpropanoid glucoside from the aerial parts of *Lygodium japonicum*

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A new compound, 4-*O*-caffeoyl-D-glucopyranose (**1**), and a new natural product, 3-*O*-caffeoyl-D-glucopyranose (**2**), together with six known compounds (**3**–**8**) were isolated from the aerial parts of *Lygodium japonicum*. Their structures were elucidated on the basis of extensive spectroscopic data analyses. The oxygen radical absorbance capacity assay was applied to evaluate their antioxidative capacities *in vitro*, which revealed that **1**–**8** showed strong antioxidative properties.

Keywords: *Lygodium japonicum*; phenylpropanoid glucoside; antioxidative activity

1. Introduction

Lygodium japonicum (Thunb.) Sw, local name 'Hai-Jin-Sha', is a herbaceous perennial plant mainly distributed in the south area of China. It has been widely used for the treatment of pneumonia, dysentery, hepatitis, etc. [1,2]. The previous studies on the chemical constitutes of this plant led to the isolation of several flavones [3], sterosides [4], phenolic acids [5,6], and phenolic glycosides [7]. In our preliminary study, the water extract of the aerial parts of *L. japonicum* was found to exhibit significant antioxidative activity in the oxygen radical absorbance capacity (ORAC) assay. In order to find new and bioactive components, a further chemical investigation on the aerial parts of this plant was carried out, which resulted in the isolation of a new phenylpropanoid glucoside, 4-*O*-caffeoyl-D-glucopyranose (**1**), and a new natural product, 3-*O*-caffeoyl-D-glucopyranose (**2**) [8], together with six known compounds, 2-*O*-caffeoyl-D-glucopyranose (**3**) [9], 6-*O*-caffeoyl-D-glucopyranose (**4**) [10], 4-*O*-*p*-coumaroyl-D-glucopyranose (**5**) [11], 6-*O*-*p*-coumaroyl-D-glucopyranose (**6**) [10], caffeic acid (**7**) [12], and *p*-coumaric acid (**8**) [13] (Figure 1). Here, we report the isolation and structural elucidation of the new compounds and their antioxidative activities.

2. Results and discussion

The dried aerial parts of *L. japonicum* were extracted with water to give a residue, which was chromatographed on Diaion HP-20, ODS, Sephadex LH-20,

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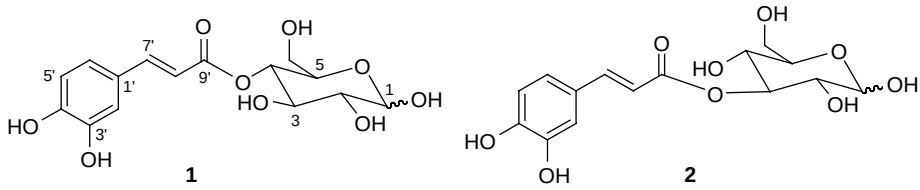

Figure 1. Chemical structures of **1** and **2**.

and HW-40 columns to afford a new phenylpropanoid glucoside (**1**), a new natural product (**2**), and six known compounds (**3**–**8**).

Compound **1,** obtained as amorphous powder, gave a molecular formula of $C_{15}H_{18}O_9$ by the analysis of the HR-ESI-MS data. The UV spectrum of **1** exhibited absorption maxima at 217, 243 (sh), 304, and 330 nm, suggesting the presence of a caffeoyl moiety [14]. The IR spectrum of **1** showed absorption bands for hydroxyl group (3381 cm^{-1}), ester carbonyl (1692 cm^{-1}), and aromatic ring (1602 and 1524 cm^{-1}).

In the ^1H NMR spectrum of **1** (Table 1), there were two *trans*-olefinic proton signals at δ_H 7.58 (1H, d, $J = 16.0$ Hz, H-7′) and 6.29 (1H, d, $J = 16.0$ Hz, H-8′), and three aromatic proton signals at δ_H 7.05 (1H, br s, H-2′), 6.77 (1H, d, $J = 8.0$ Hz, H-5′), and 6.95 (1H, br d, $J = 8.0$ Hz, H-6′). Meanwhile, nine carbon signals at δ_C 168.7 (C-9′), 149.6 (C-4′), 147.5 (C-7′), 146.8 (C-3′) 127.7 (C-1′), 123.0 (C-6′), 116.5 (C-5′), 115.2 (C-2′), and 114.8 (C-8′) were observed in the ^{13}C NMR spectrum of **1** (Table 1). The pieces of evidence mentioned above indicated the presence of a caffeoyl moiety. In addition, there were two anomeric proton signals at δ_H 5.16 (1/2H, d, $J = 3.6$ Hz, H-1α) and 4.55 (1/2H, d, $J = 8.0$ Hz, H-1β) in the ^1H NMR spectrum and two sets of carbon signals at δ_C 93.8 (C-1α), 73.8 (C-2α), 72.9 (C-3α), 72.6 (C-4α), 71.2 (C-5α), and 62.5 (C-6α); and 98.3 (C-1β), 76.4 (C-2β), 76.2 (C-5β), 75.8 (C-3β), 72.6 (C-4β), and 62.6 (C-6β) in the ^{13}C NMR spectrum, which suggested the presence of glucose moieties by comparing with those of 4-*O*-*p*-coumaroyl-D-glucose [11]. The location of caffeoyl moiety was determined to be at the C-4 position of the glucose moiety from the chemical shift values in the ^{13}C NMR spectrum. The signals assignable to the C-4α and C-4β positions of the glucose moities were each downfield shifted by +2.2 and +2.3 ppm, respectively, as compared with those of unsubstituted α-D-glucose and β-D-glucose [15]. This is further confirmed by the HMBC correlations from the proton at δ_H 4.80 (H-4α and H-4β) to the carbon at δ_C 168.7 (C-9′; Figure 2). Hydrolyzed with 5% hydrochloric acid, compound **1** yielded a glucose residue. The absolute configuration of the glucose was demonstrated to be D configuration by gas chromatographic analysis using the method of Hara et al. with slight modification [16,17]. Therefore, compound **1** was characterized as 4-*O*-caffeoyl-D-glucopyranose.

Compound **2** was obtained as amorphous powder with a molecular formula of $C_{15}H_{18}O_9$ by HR-ESI-MS data. The NMR spectral data of **2** were similar to those of **1** (Table 1), showing the presence of a caffeoyl moiety and a glucose moiety. The location of the caffeoyl moiety was concluded to be at the C-3 position of the glucose moiety from the observations of the downfield shift of the C-3α (+3.6) and the C-3β (+2.7) in the ^{13}C NMR spectrum, as compared with those of unsubstituted α/β-D-glucose [15], which was further confirmed by the HMBC correlations from the proton at δ_H 5.32 (H-3α), 5.03 (H-3β) to the carbon at δ_C

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for **1** and **2** (CD_3OD, *J* in Hz).[a]

	1				**2**			
	δ_H		δ_C		δ_H		δ_C	
No.	α	β	α	β	α	β	α	β
1	5.16 (1/2H, d, 3.6)	4.55 (1/2H, d, 8.0)	93.8	98.3	5.17 (1/2H, br s)	4.60 (1/2H, d, 8.0)	94.0	98.2
2	3.47 (1/2H, m)	3.25 (1/2H, m)	73.8	76.4	3.56 (1/2H, m)	3.33 (1/2H, m)	72.3	74.7
3	3.91 (1/2H, t, 9.2)	3.62 (1/2H, m)	72.9	75.8	5.32 (1/2H, t, 9.2)	5.03 (1/2H, t, 9.2)	77.0	79.1
4	4.80 (1/2H, o[b])	4.80 (1/2H, o[b])	72.6	72.6	3.54 (1/2H, m)	3.55 (1/2H, m)	69.9	70.0
5	3.99 (1/2H, m)	3.54 (1/2H, m)	71.2	76.2	3.88 (1/2H, m)	3.39 (1/2H, m)	72.9	77.8
6	3.55 (1H, m)	3.58 (1H, m)	62.5	62.6	3.75 (1H, m)	3.72 (1H, m)	62.4	62.6
1′			127.7				127.8	
2′	7.05 (1H, br s)		115.2		7.05 (1H, br s)		115.2	
3′			146.8				146.8	
4′			149.6				149.5	
5′	6.77 (1H, d, 8.0)		116.5		6.77 (1H, d, 8.0)		116.5	
6′	6.95 (1H, br d, 8.0)		123.0		6.94 (1H, br d, 8.0)		122.9	
7′	7.58 (1H, d, 16.0)		147.5		7.58 (1H, d, 16.0)		146.9	
8′	6.29 (1H, d, 16.0)		114.8		6.32 (1H, d, 16.0)		115.6	
9′			168.7				169.5	

[a] Assignments were established by DEPT-135, $^{1}H-^{1}H$ COSY, HSQC, and HMBC experiments.
[b] 'o' refers to peak overlapped with other signals.

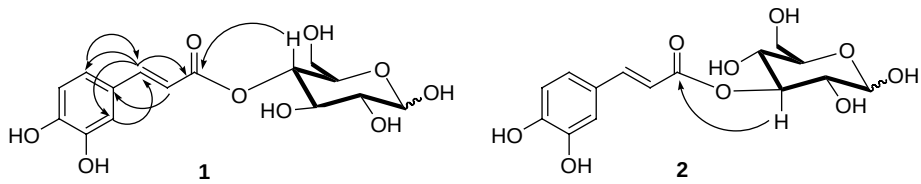

Figure 2. Key HMBC (→) and COSY (—) correlations of **1** and **2**.

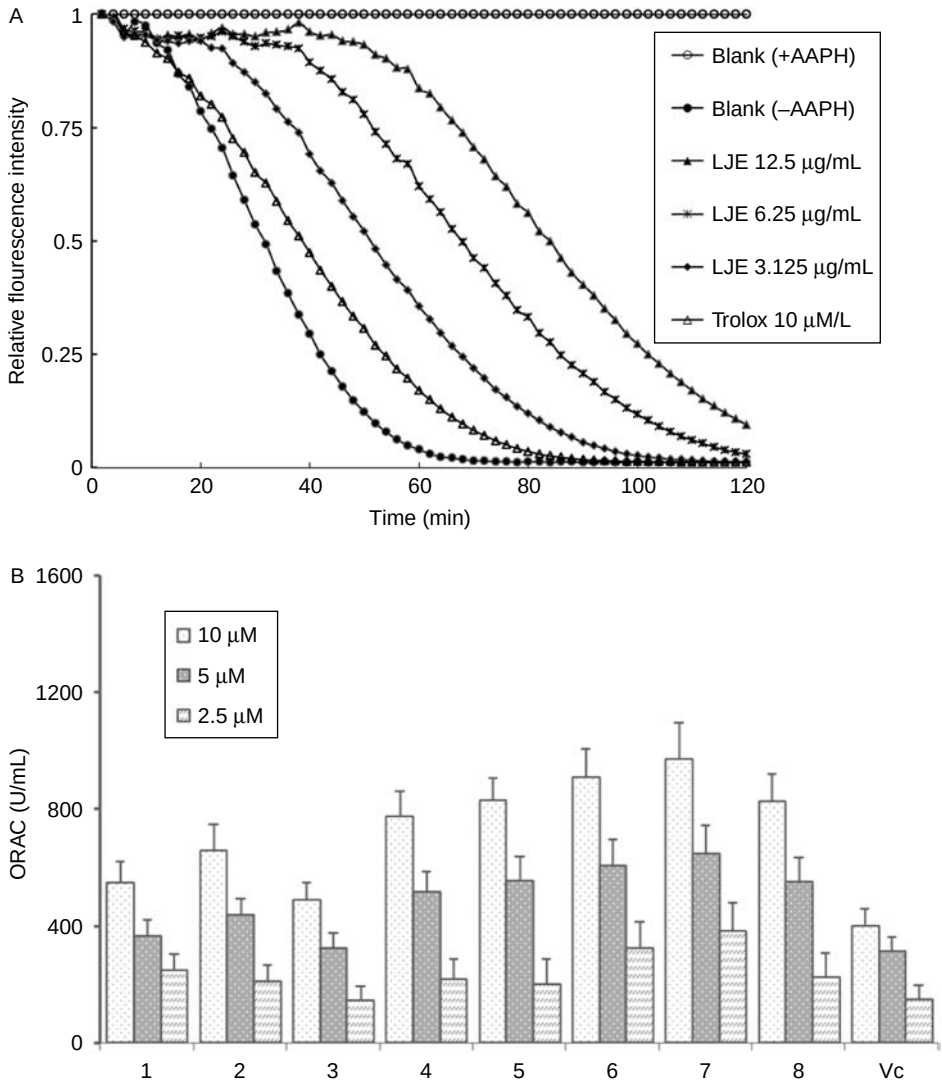

Figure 3. Curves of fluorescence decay induced by AAPH in the presence of *L. japonicum* extract (A) and compounds isolated (B) at different concentrations. Trolox, a water-soluble vitamin E analog, was used as a control standard. The antioxidative activity of a sample was expressed as the net area under the curve. V_c was used as a positive control in Figure 3(B). One ORAC unit is calculated as $U = K(S_{\text{Sample}} - S_{+\text{AAPH}})/(S_{\text{Trolox}} - S_{+\text{AAPH}})$, K: samples multiple of dilution and S: area below the fluorescence decay curve. Data are expressed as means of three experiments.

169.5 (C-9′; Figure 2). Thus, compound **2** was identified as 3-*O*-caffeoyl-D-glucopyranose [8].

The antioxidative properties of *L. japonicum* extract and compounds **1**–**8** were determined by the ORAC assay as described previously [18]. The antioxidative activity of a sample was expressed as the net area under the curve. Trolox was used as the control standard (10 μM). Figure 3(A),(B) shows the working curves of fluorescein oxidation used as an index of resistance time for the oxidative reaction, which clearly illustrated that *L. japonicum* extract and compounds **1**–**8** displayed potent ORACs in a dose-dependent manner (as shown in Figure 3(B)).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured using a JASCO P-1030 automatic digital polarimeter (JASCO, Tokyo, Japan). UV spectra were measured on a JASCO V-550 UV–vis spectrophotometer. IR spectra were recorded on a JASCO FT-IR-400 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz for ^{1}H; Bruker BioSpin, Fallanden, Switzerland). ESI-MS data were recorded on a Finnigan LCQ Advantage MAX mass spectrometer (Thermo Electron, Lowell, MA, USA). HR-ESI-MS data were determined by an Agilent 6210 LC/MSD TOF mass spectrometer (Agilent, Pala Alto, CA, USA). HPLC analysis was carried out on a Dionex HPLC system equipped with a Dionex P-680 quaternary pump, a PDA-100 diodearray detector, a TCC-100 oven, and an ASP-100 autosampling system (Dionex, Sunnyvale, CA, USA) using a reversed-phase (RP) C_{18} column (XB-C18, 5 μm, 4.6 × 250 mm; Welch Materials, Inc., Shanghai, China). Preparative HPLC was carried out on a Varian instrument equipped with UV detectors (Varian, Salt Lake City, UT, USA) and a RP C_{18} column (XB-C18, 5 μm, 21.2 × 250 mm; Welch Materials, Inc., Shanghai, China). Column chromatography (CC) was carried out using Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), ODS (50 μm, YMC, Kyoto, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and Toyopearl HW-40C (Tosoh, Tokyo, Japan). Thin-layer chromatography was carried out using precoated silica gel plates (silica gel GF_{254}, Yantai Chemical Industry Research Institute, Yantai, China).

3.2 Plant material

The plant material was generously provided by Guangzhou Wanglaoji Pharmaceutical Co. (Guangzhou, China), which was then identified as *L. japonicum* (Thunb.) Sw. by Prof. Guang-Xiong Zhou, College of Pharmacy, Jinan University. A voucher specimen (2006WZY0044) is deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, China.

3.3 Extraction and isolation

The dried aerial parts of *L. japonicum* (6.5 kg) were refluxed twice with water for 2 h each time. After filtration, the filtrate was evaporated to dryness under vacuum. The dried extract (758.3 g) was chromatographed over Diaion HP-20 column and eluted with water and 30%, 50%, and 95% (V/V) EtOH–H_2O, successively, to afford four fractions (Fractions A–D). Fraction B (30% EtOH–H_2O eluent, 88.2 g) was chromatographed over ODS column and eluted with CH_3OH–H_2O (1:9 to 1:0) to afford four subfractions (B1–B4). Subfraction B1 (10% CH_3OH–H_2O eluent, 16.8 g) was further subjected to Toyopearl HW-40 column using 30% CH_3OH–H_2O as eluents, and then purified by preparative HPLC with 10% CH_3OH–H_2O to afford compounds **1** (t_R 42.3 min, 52.2 mg), **2** (t_R 28.5 min, 28.6 mg), and **3** (t_R 33.0 min,

6.0 mg). Subfraction B2 (10% CH_3OH-H_2O eluent, 4.4 g) was chromatographed over Toyopearl HW-40 column, using CH_3OH-H_2O (2:8 to 1:0) as mobile phase to afford four subfractions (B2A–B2D). B2B (40% CH_3OH-H_2O eluent) was then purified by preparative HPLC with 23% CH_3OH-H_2O to give compounds **4** (t_R 22.3 min, 9.0 mg) and **6** (t_R 31.8 min, 50.0 mg). Compound **5** (24.7 mg) was recrystallized in CH_3OH-H_2O (3:7) from subfraction B2C (60% CH_3OH-H_2O eluent). Subfraction B3 (30% CH_3OH-H_2O eluent, 3.4 g) was applied to Toyopearl HW-40 CC, using 40% CH_3OH-H_2O as eluents, and then subjected to Sephadex LH 20 CC, eluted with 50% CH_3OH-H_2O to yield compound **7** (320.0 mg). Fraction C (50% $EtOH-H_2O$ eluent, 80.0 g) was chromatographed over ODS CC, eluted with CH_3OH-H_2O (1:9 to 1:0) to afford three subfractions (C1–C3). Subfraction C2 (50% CH_3OH-H_2O eluent, 18.0 g) was further subjected to Toyopearl HW-40 column using 30% CH_3OH-H_2O as eluents, and then purified by Sephadex LH 20 CC with 50% CH_3OH-H_2O to afford compound **8** (135.0 mg).

3.3.1 4-O-Caffeoyl-D-glucopyranose (1)

Amorphous powder, $[\alpha]_D^{25} + 14.9$ ($c = 0.6$, CH_3OH), UV (MeOH) λ_{max} (log ϵ): 217 (3.97), 243 (sh, 3.81), 304 (3.80), and 330 (3.99) nm; IR (KBr) ν_{max}: 3381, 1692, 1602, and 1524 cm^{-1}; for ^1H and ^{13}C NMR spectral data see Table 1; ESI-MS: *m/z* 365 $[M + Na]^+$, 707 $[2M + Na]^+$; HR-ESI-MS: *m/z* 365.0816 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_9Na$, 365.0843).

3.3.2 3-O-Caffeoyl-D-glucopyranose (2)

Amorphous powder, UV (MeOH) λ_{max} (log ϵ): 218 (4.06), 246 (sh, 3.87), 296 (sh, 3.89), and 328 (3.95) nm; IR (KBr) ν_{max}: 3403, 1701, 1603, and 1522 cm^{-1}; for ^1H and ^{13}C NMR spectral data see Table 1; ESI-MS: *m/z* 365 $[M + Na]^+$, 707 $[2M + Na]^+$; HR-ESI-MS: *m/z* 365.0807 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_9Na$, 365.0843).

3.4 Measurement of antioxidative capacities

The antioxidative properties of *L. japonicum* extract and compounds **1**–**8** were determined by the ORAC assay as described previously [18]. Purities of compounds **1**–**8** were greater than 98% as measured by HPLC analyses. The compounds were dissolved in the potassium phosphate buffer before use. Trolox, purchased from Wako Pure Chemical Industries (Osaka, Japan), was used as a control standard. Peroxyl radicals were generated at a controlled rate by thermal decomposition of 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH). The 180-μl assay solution contained 20 μl of samples and 20 μl of 75-mM potassium phosphate buffer (pH 7.4) with 140 μl of APPH solution (final concentration 12.8 mmol/liter). The reaction was started by adding 20 μl of fluorescein (final concentration 63 mmol/liter) to the assay solution. Decay of the fluorescein signal was determined at 485/527 nm in a GENios Lueifcrase microplate reader (TECAN, Mannedorf, Switzerland) at 37°C.

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