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Phenylethanol glycosides from the seeds of *Aesculus chinensis* var. *chekiangensis*

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Abstract

Three new phenylethanol glycosides (**1**-**3**) and one known analogue (**4**) were isolated from the seeds of *Aesculus chinensis* Bge. var. *chekiangensis*. To the best of our knowledge, this represents the first isolation of phenylethanol glycosides from the genus of *Aesculus*, which enriched its chemical composition. Structure elucidations were performed via extensive NMR and HRESIMS data together with comparison with literature data. Thereafter, the isolated compounds were assayed for their neuroprotective activities against $CoCl_2$-induced cytotoxicity in PC12 cells and compound **3** exhibited moderate activity.

Keywords: *Aesculus chinensis* Bge. var. *chekiangensis* (Hu et Fang) Fang, Phenylethanol glycosides, Neuroprotective activity

Introduction

The genus *Aesculus*, which belongs to the family *Hippocastanaceae* contains about 30 species found worldwide. The dried seeds of *Aesculus chinensis* Bge. var. *chekiangensis* (Hu et Fang) Fang, *Aesculus chinensis* Bge and *Aesculus wilsonii* Rehd are commonly used to treat chest and abdomen pain, dysentery and ague [1, 2] in traditional Chinese medicine. Previous studies on the genus of *Aesculus* revealed the presences of diverse secondary metabolites such as triterpenoids [3–7], flavonoids [8, 9], coumarins [10] and steroids [11]. And a number of pharmacological studies have suggested that *A. chinensis* exhibited beneficial effects on antitumor [12], neuroprotective [13], anti-inflammatory [14] and cardio-protective activities [15]. Nevertheless, compared to other species of *Aesculus* genus, the chemical investigation of *Aesculus chinensis* Bge. var. *chekiangensis* (Hu et Fang) Fang is limited. Our interests in cytotoxic and neuroprotective components from *A. chinensis* Bge. var. *chekiangensis* (Hu et Fang) Fang have led to the isolation of numerous new ones [16, 17]. As a continuous search for structurally novel compounds with diverse bioactivities, three new phenylethanol glycosides (**1**-**3**) and one known analog (**4**) were obtained (Fig. 1), which represent the first examples of phenylethanol glycosides obtained from the genus of *Aesculus*. Herein, the isolation, structure identification and biological evaluation of **1**-**4** are described.

Methods

General experimental procedures

The chemicals and material were similar to our previous researches [16, 17].

Plant material

The plant was the same batch of medicinal material as our previous reports [16, 17].

Extraction and isolation

The extracted method was the same to our previous studies [16, 17]. Chopped, dried seeds of *A. chinensis* Bge. (8.8 kg) were extracted with 70% ethanol, then

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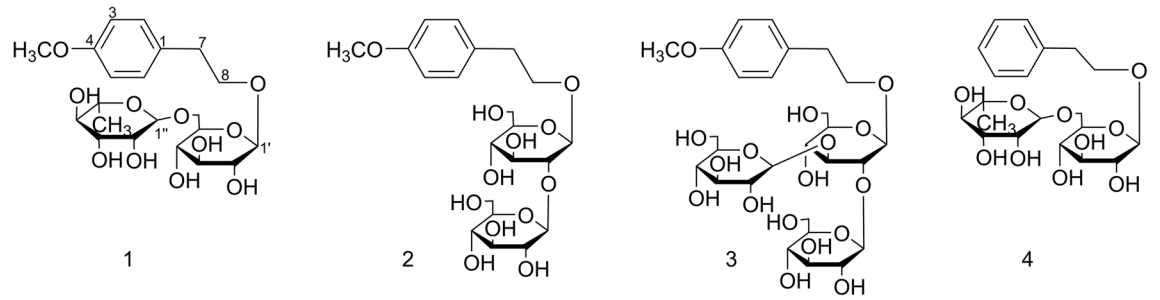

Fig. 1 The structures of isolated compounds

partitioned via D101 resin column eluting with a stepwise gradient of H_2O-EtOH.

The 60% EtOH-H_2O part was loaded onto a silica gel column using CH_2Cl_2/CH_3OH (100:1 → 1:1) to yield 4 fractions (A–D). Fraction A was further separated by RP C_{18} CC (MeOH–H_2O, from 0:100 to 100:0) to give four subfractions (A1–A4). Subfraction A2 was chromatographed over a Sephadex LH-20 column (MeOH) then RP-HPLC (MeOH–H_2O, 35:65, 3.0 mL/min) to give compounds **1** (11.0 mg) and **4** (20.0 mg). Subfraction A3 was further subdivided with an ODS RP-C18 column (MeOH/H_2O, 10:90 to 100: 0) to give seven subfractions (A3A–A3G). The subfraction A3G was applied to a Sephadex LH-20 column (MeOH), and then purified by recycling preparative HPLC with 40% MeOH/H_2O to yield compounds **2** (3.7 mg) and **3** (9.0 mg).

4-methoxy-phenylethanol-8-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (1)

Brown amorphous powder; [α]25 *D*–7.3 (*c* 0.10, MeOH); Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR): Table 1; HR-ESI–MS: *m/z* 505.1918 [M + COOH]$^-$ (calculated for $C_{22}H_{33}O_{13}$, 505.1921).

4-methoxy-phenylethanol-8-O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (2)

Brown amorphous powder; [α]25 *D*–11.2 (*c* 0.11, MeOH); ^{1}H-NMR and ^{13}C-NMR: Table 1; HR-ESI–MS: *m/z* 521.1870 [M + COOH]$^-$ (calculated for $C_{22}H_{33}O_{14}$, 521.1870).

4-methoxy-phenylethanol-8-O-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside (3)

Brown amorphous powder; [α]25 *D*–14.6 (*c* 0.10, MeOH); ^{1}H-NMR and ^{13}C-NMR: Table 1; HR-ESI–MS: *m/z* 683.2398 [M + COOH]$^-$ (calculated for $C_{28}H_{43}O_{19}$, 683.2399).

Hydrolysis and determination of absolute configuration of sugars

Compounds **1**–**3** (1.0 mg, respectively) was hydrolyzed with 2 M HCl (4.0 mL) at 90 °C for 2 h. Then the hydrolysed materials were disposed and tested by means of the procedure described in our previous work [16, 17].

Neuroprotective effect assay

Compounds **1**-**4** were assayed for their neuroprotective effects against $CoCl_2$-induced PC12 cell injury [18] by 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT) method with trolox as the positive control according to our previously reported procedure [16, 17]. PC12 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum as well as 100 U/mL penicillin/streptomycin and were incubated at 37 °C with 5% CO_2. PC12 cells were placed into a 96-well plate at a density of 2×10^4 cells/well and kept there for 24 h. Cells were incubated with test compounds and trolox (10 μM) for 2 h. To induce an oxidative stress, 1 mM $CoCl_2$ was added to the cells and incubated for 24 h. Then, the supernatant was changed with 100 μL MTT solution (5 mg/mL) for 2.5 h, the plate was vibrated, and the absorbance at 490 nm was measured using a microplate reader.

Cytotoxicity assay

Cell viability was determined with the MTT method [19, 20]. The human hepatocellular carcinomas cells (HepG2), the human colorectal carcinoma cells (HCT-116) and the human gastric carcinoma cells (MGC-803) were purchased from ATCC. HepG2, MGC-803 and HCT-116 were respectively cultured in DMEM and RPMI-1640 mediums, which were supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. HepG2, HCT-116, and MGC-803 cells (1×10^4) were seeded in 96-well tissue culture plates. Cells were treated in triplicate with five concentrations (50, 25, 12.5, 6.25 and 3.125 μM) of the tested compounds

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of 1–3 (δ in ppm, in DMSO-d_6)

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	130.5		130.7		130.6	
2	129.9	7.17 (1H, d, J = 8.5 Hz)	130.0	7.17 (1H, d, J = 8.7 Hz)	130.0	7.18 (1H, d, J = 8.7 Hz)
3	113.7	6.83 (1H, d, J = 8.5 Hz)	113.6	6.83 (1H, d, J = 8.7 Hz)	113.6	6.82 (1H, d, J = 8.7 Hz)
4	157.6		157.6		157.7	
5	113.7	6.83 (1H, d, J = 8.5 Hz)	113.6	6.83 (1H, d, J = 8.7 Hz)	113.6	6.82 (1H, d, J = 8.7 Hz)
6	129.9	7.17 (1H, d, J = 8.5 Hz)	130.0	7.17 (1H, d, J = 8.7 Hz)	130.0	7.18 (1H, d, J = 8.7 Hz)
7	34.8	2.78 (2H, dt, J = 8.2, 6.3 Hz)	34.7	2.78 (2H, dt, J = 8.2, 6.4 Hz)	34.6	2.78 (2H, dt, J = 8.4, 6.6 Hz)
8	69.9	3.83 (1H, dt, J = 8.2, 6.3 Hz) 3.62 (1H, overlap)	69.8	3.89 (1H, dt, J = 8.2, 6.4 Hz) 3.60 (1H, overlap)	69.9	3.92 (1H, dt, J = 8.4, 6.6 Hz) 3.60 (1H, overlap)
4-OCH_3	55.0	3.71 (3H, s)	55.0	3.71 (3H, s)	54.9	3.71 (3H, s)
1′	103.0	4.17 (1H, d, J = 7.7 Hz)	101.3	4.33 (1H, d, J = 7.7 Hz)	101.6	4.39 (1H, d, J = 7.5 Hz)
2′	73.4	2.94 (1H, t, J = 8.5 Hz)	82.2	3.23 (1H, dd, J = 9.1, 7.7 Hz)	79.6	3.44 (1H, m)
3′	76.7	3.13 (1H, t, J = 8.9 Hz)	76.7	3.10 (1H, m)	86.2	3.49 (1H, t, J = 8.8 Hz)
4′	70.2	2.99 (1H, t, J = 9.1 Hz)	69.8	3.10 (1H, m)	68.4	3.19 (1H, m)
5′	75.4	3.26 (1H, m)	76.1	3.36 (1H, m)	76.1	3.18 (1H, m)
6′	67.0	3.81 (1H, dd, J = 11.2, 2.0 Hz) 3.42 (1H, dd, J = 11.2, 6.0 Hz)	60.9	3.65 (1H, m) 3.42 (1H, dd, J = 11.2, 5.2 Hz)	61.2	3.65 (1H, m) 3.47 (1H, m)
1″	100.8	4.59 (1H, d, J = 1.2 Hz)	104.1	4.41 (1H, d, J = 7.8 Hz)	102.8	4.55 (1H, d, J = 8.0 Hz)
2″	70.5	3.60 (1H, m)	74.9	3.00 (1H, dd, J = 8.4, 7.8 Hz)	74.5	2.94 (1H, dd, J = 8.5, 5.9 Hz)
3″	70.7	3.42 (1H, m)	77.1	3.08 (1H, m)	76.4	3.19 (1H, m)
4″	72.0	3.17 (1H, t, J = 9.1 Hz)	69.9	3.10 (1H, m)	70.0	3.62 (1H, m)
5″	68.4	3.45 (1H, m)	76.1	3.16 (1H, m)	76.2	3.16 (1H, m)
6″	18.0	1.12 (3H, d, J = 6.2 Hz)	61.0	3.65 (1H, m) 3.49 (1H, dd, J = 11.2, 5.5 Hz)	61.0	3.67 (1H, m) 3.39 (1H, dd, J = 11.8, 5.9 Hz)
1‴					103.4	4.38 (1H, d, J = 7.9 Hz)
2‴					73.7	3.04 (1H, d, J = 8.7 Hz)
3‴					76.9	3.07 (1H, m)
4‴					70.1	3.06 (1H, m)
5‴					76.8	3.05 (1H, m)
6‴					60.7	3.69 (1H, m) 3.45 (1H, m)

for 24 h, with 5-fluorouracil (5-FU) as positive control. Subsequently, 100 μL of MTT (5 mg/mL) was added and the cells were incubated for additional 2.5 h. Thereafter, the supernatant was discarded and 0.15 ml of DMSO was added to each well, then the plate was mixed on a microshaker for 10 min and read on a microplate reader at 490 nm.

Results and discussion

Compound **1** was obtained as brown amorphous powder with a molecular formula of $C_{21}H_{32}O_{11}$ deduced from its HR-ESI-MS spectrum (*m/z* 505.1918 $[M+COOH]^-$, calcd. for $C_{22}H_{33}O_{13}$, 505.1921). The ^{1}H-NMR spectrum of compound **1** exhibited signals characteristic for a 1, 4-disubstituted benzene ring [δ_H 7.17 (2H, d, J = 8.5 Hz, H-2, 6), 6.83 (2H, d, J = 8.5 Hz, H-3, 5)], an ethoxy moiety [δ_H 2.78 (2H, dt, J = 8.2, 6.3 Hz), 3.83 (1H, dt, J = 8.2, 6.3 Hz)] as well as a *O*-methyl at δ_H 3.71 (3H, s) (Table 1). The heteronuclear multiple bond correlations (HMBC) (Fig. 2) of H-2 (δ_H 7.17) to C-4 (δ_C 157.6), C-6 (δ_C 129.9), C-7 (δ_C 34.8); H-3 (δ_H 6.83) to C-1 (δ_C 130.5), C-5 (δ_C 113.7); H-8 (δ_H 3.83) to C-1 (δ_C 130.5) and OCH_3 (δ_H 3.71) to C-4 (δ_C 157.6) indicated **1** contains a 4-methoxyphenylethanol moiety.

The two anomeric protons at δ 4.17 (1H, d, J = 7.7 Hz), 4.59 (1H, d, J = 1.2 Hz) correlated with carbons at δ 103.0 and 100.8 in heteronuclear single quantum coherence (HSQC) spectrum, respectively, indicated a disaccharide residue. Acid hydrolysis of **1** liberated D-glucose and L-rhamnose, which were identified by HPLC analysis after derivatization [21, 22]. The β-orientation of the glucopyranosyl unit was deduced from the coupling

constant (J=7.7 Hz, H-1′). The α- anomeric configuration of rhamnose was determined from the absence of nuclear overhauser effect spectroscopy (NOESY) correlations between protons H-1 and H-3/H-5. The β-D-glucose was attached to the 4-methoxy-phenylethanol nucleus at C-8, evidenced by the HMBC correlation between H-1′ (δ_H 4.17) to C-8 (δ_C 69.9). In addition, the downfield chemical shift of C-6′ (δ_C 67.0) of the glucose coupled with the cross peak of H-1″ (δ_H 4.59) to C-6′ (δ_C 67.0) in HMBC spectrum suggesting the α-L-rhamnose was linked to C-6′. Based on these data, compound **1** was concluded to be 4-methoxy-phenylethanol-8-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside.

The elemental formula of compound **2** was confirmed to be $C_{21}H_{32}O_{12}$ with one oxygen more than that of **1** according to the $[M+COOH]^-$ ion peak at *m/z* 521.1870 in its HRESIMS spectrum. The ^1H and ^{13}C NMR data of **2** revealed a close resemblance to **1** except for the corresponding signals to the two sugar units. Careful analysis of the NMR data and the acid hydrolysis results affirmed the existence of two β-D-glucose groups in **2** instead of one β-D-glucose and one α-L-rhamnose in **1**. HMBC correlations from H-1′ (δ_H 4.33) to C-8 (δ_C 69.8) and H-1″ (δ_H 4.41) to C-2′ (δ_C 82.8) revealed the position and sequences of the sugar moiety in **2** as shown in Fig. 2. Hence, compound **2** was assigned as 4-methoxy-phenylethanol-8-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside.

Compound **3** was also acquired as a brown solid with the molecular formula of $C_{27}H_{42}O_{17}$ (*m/z* 683.2398 $[M+COOH]^-$; calcd. for $C_{28}H_{43}O_{19}$, 683.2399), which is 162 mass units more than that of **2**. The NMR data of **3** were closely resemble to those of **2**, indicating the same aglycone with the difference of an additional hexose moiety. D-glucose was afforded from **3** via the same procedure as before and the β configuration was inferred from the large coupling constants: [δ_H 4.39 (1H, d, J=7.5 Hz, H-1′), 4.55 (1H, d, J=8.0 Hz, H-1″), 4.38 (1H, d, J=7.9 Hz, H-1‴)]. HMBC correlations from H-1′ (δ_H 4.39) to C-8 (δ_C 69.9) supported the attachment of the sugar units to C-8. The sequence of the sugar chain was further established by the long correlations of H-1″ (δ_H 4.55) and C-2′ (δ_C 79.6), H-1‴ (δ_H 4.38) and C-3′ (δ_C 86.2) (Fig. 2). Consequently, compound **3** was assigned as 4-methoxy-phenylethanol-8-*O*-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside.

The other known one, phenylethanol-8-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**4**) were also obtained and identified by NMR analysis and comparison with literature data [23].

All compounds (**1**–**4**) were tested in three human cancer cell lines, HepG2, HCT-116 and MGC-803, using 5-FU as the positive control. However, they did not show obvious cytotoxicity (IC_{50} > 50 μM).

The neuroprotective effects of **1**–**4** were also evaluated in $CoCl_2$-induced PC12 cell damage [24] by MTT assay. According to the references [25, 26] and our study, the positive control, trolox, exhibited statistically significant neuroprotective effect at 10 μM (Fig. 3). Therefore, the concentration of 10 μM was selected for the cytotoxic and neuroprotective evaluation of these compounds. First, the cytotoxic activity of compounds **1**–**4** against PC12 cell line was tested and none of them showed cytotoxicity at 10 μM (Additional file 1: Fig. S16). Subsequently, 10 μM compounds were bioassayed for their neuroprotective properties. And according to Fig. 3, compound **3** exhibited moderate activities against $CoCl_2$-induced PC12 cell injury.

Conclusion

In this paper, three new phenylethanol glycosides (**1**-**3**) and one known compound (**4**) were obtained from the seeds of *A. chinensis* Bge. var. *chekiangensis*., which represents the first isolation of phenylethanol glycosides from the genus of *Aesculus*. The findings also

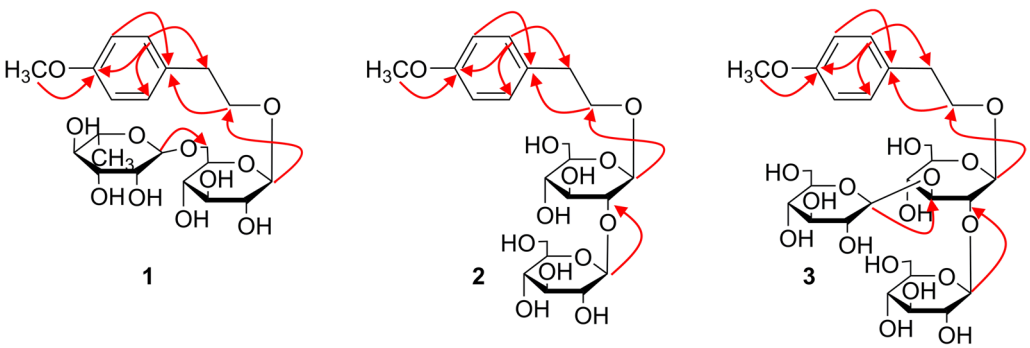

Fig. 2 Selected HMBC (H → C) correlations of compounds **1-3**

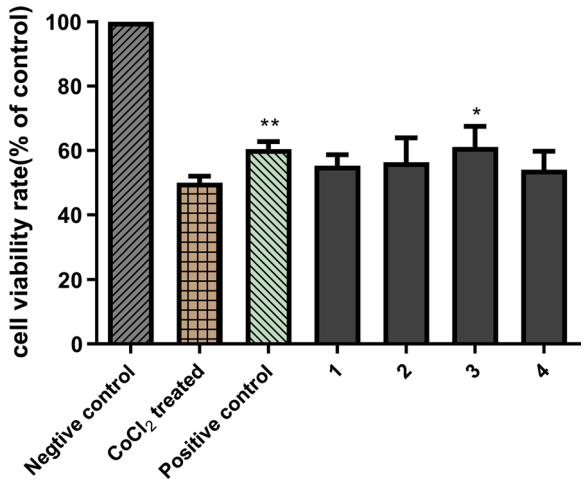

Fig. 3 Neuroprotective activities of compounds **1-4** (10 μM) against $CoCl_2$-induced cell death in PC12 cells. The data (cell viability, measured by MTT assay) are expressed as mean ± SD. Three independent experiments were performed. Trolox was used as the positive control at 10 μM. Compared with $CoCl_2$ treated group, $^{*}P < 0.05$, $^{**}P < 0.01$

provided more insights into the chemotaxonomy of the *Aesculus* genus. Besides, the neuroprotective activities of the phenylethanol glycosides were also evaluated and compound **3** exhibited statistically significant neuroprotective activity.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13065-020-00685-3.

Additional file 1: HR-ESI-MS, 1D- and 2D-NMR spectra of compounds **1**–**3** (**Figures S1**–**S15**), cytotoxic activities of compounds **1**–**4** on PC12 cells at 10 μM (**Figure S16**).

Abbreviations

DMSO-d_6: Deuterated dimethyl sulfoxide; ^{1}H-NMR: Proton nuclear magnetic resonance; ^{13}C-NMR: Carbon-13 nuclear magnetic resonance; HMBC: Heteronuclear multiple bond correlation; HSQC: Heteronuclear single quantum coherence; NOESY: Nuclear overhauser effect spectroscopy; HepG2: The human hepatocellular carcinomas cells; HCT-116: The human colorectal carcinoma cells; MGC-803: The human gastric carcinoma cells; MTT: 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide; 5-FU: 5-Fluorouracil.

Authors' contributions

NZ conceived and designed the experiments. NZ and DL were responsible for the isolation and elucidation the structures. NZ tested cytotoxicity and neuroprotective effects of the compounds. NZ interpreted the data and wrote the paper. DL, SW, SC, XF and KW revised the manuscript. LD and FQ were the project leaders organizing and guiding the experiment. All authors read and approved the final manuscript.

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Availability of data and materials

All other datasets generated for this study are included in the article and Additional file 1.

Competing interests

No potential conflict of interest was reported by authors.

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