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Secoiridoid glycosides from the fruits of *Cornus officinalis*

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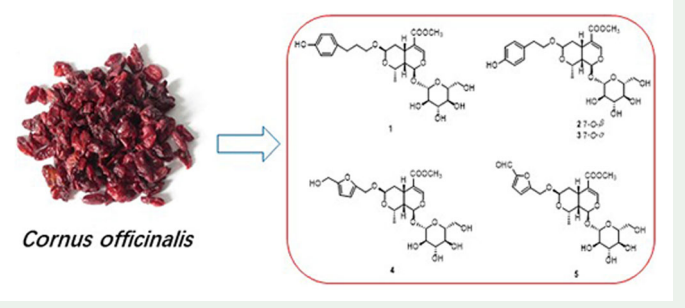
ABSTRACT

Five new secoiridoid glycosides, cornusphenosides E-I (**1**–**5**), were isolated and characterized from an active fraction of ethanol extract of the fruits of *Cornus officinalis*. Their structures were determined by extensive spectroscopic data analysis, including 2 D NMR and HRESIMS experiments. In the preliminary assay, compound **5** (when evaluated at 10 μM) showed the neuroprotective effect against H_2O_2-induced SH-SY5Y cell damage.



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Cornus officinalis; secoiridoid glycosides; neuroprotective activity

1. Introduction

The fruits of *Cornus officinalis*, named "Shan Zhu Yu" in Chinese, are important traditional Chinese medicines used for dementia, lumbar debility, and diabetes (Chinese Pharmacopoeia Commission 2015; Williams et al. 2011). They are among the most common ingredients of formulations used for nourishing the liver and kidneys (Chinese Pharmacopoeia Commission 2015). Considerable chemical and pharmacological studies have been reported, along with the isolation of more than a hundred compounds from various extracts of *C. officinalis* (He et al. 2012; Xie et al. 2012; Jang et al. 2014; Ma et al. 2014; Park et al. 2016; Kang et al. 2018; Ye et al. 2020). Among the reported chemical constituents, iridoids were recognized as main active

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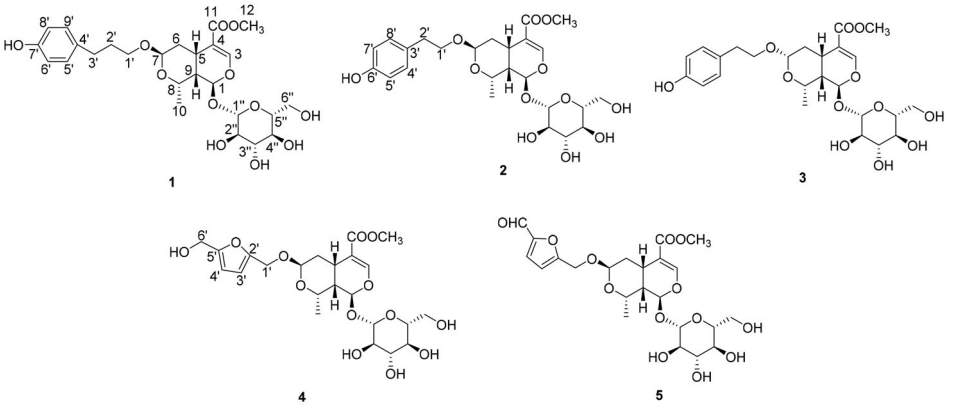

Figure 1. Structures of compounds **1**–**5.**

components, especially the iridoid glycosides which had significant effects on the neurodegenerative diseases (Yao et al. 2009; Ye et al. 2017; Huang et al. 2018). As a part of our program to systematically study the chemical diversity of medicinal/edible homologous resources and their biological effects, an ethanol extract of the fruits of *C. officinalis* was investigated. In the previous study, we found that the 40% ethanol elution fraction of the ethanol extract of *C. officinalis*, obtained over a microporous resin, showed potential neuroprotective activities. In order to investigate minor iridoids with novel structures and neuroprotective activities, a series of chromatography methods were used to firstly remove morroniside and loganin which were the main iridoid glycosides with neuroprotective activity, and then the four sub-fractions (F1-F4) were obtained. Through the neuroprotective activity screening *in vitro*, the sub-fractions F2∼F4 still exhibited the strong neuroprotective activity. Seven novel iridoids were isolated from sub-fraction F2, one of which had a moderate neuroprotective activity (Wang et al. 2018; Ji et al. 2019). To further clarify the neuroprotective active compounds of *C. officinalis*, a continuation on another active sub-fraction F3 has resulted in the structure characterization of five new secoiridoid glycosides (**1**–**5**) (Figure 1). Herein, we report the isolation, structure elucidation, and neuroprotective activities of these compounds.

2. Results and discussion

Compound **1** was obtained as an amorphous powder with a specific rotation of $[\alpha]-35.5$ (0.03, MeOH). Its molecular formula, $C_{26}H_{36}O_{12}$, was deduced by the HRESIMS (*m/z*: 539.21326 $[M\text{-}H]^-$). The IR spectrum showed absorption bands for hydroxy (3392 cm^{-1}) and carbonyl (1706 cm^{-1}) groups. The ^1H and ^{13}C NMR data (Table S1) of **1** displayed the signal characteristic of an iridoid glycoside, which were similar to those reported for 7β-morroniside (Ma et al. 2014). The only evident difference was that **1** showed the resonances due to an additional *p*-hydroxyphenyl propanol group. The 1 D NMR data exhibited an AA′BB′ signal pattern [(δ_H 6.69 (2H, d, $J=8.5$ Hz, H-6′, 8′) and δ_H 7.02 (2H, d, $J=8.5$ Hz, H-5′, 9′)], two methylene signals at δ_H 1.89 (2H, m, H-2′) and δ_H 2.63 (2H, t, $J=7.5$ Hz, H-3′), and one oxygen carbon signal at

δ_C 67.6. ^{1}H-^{1}H COSY correlations of H-1′a and H-1′b/H-2′/H-3′ together with the HMBC correlations of H-3′ with C-4′/C-5′/C-9′ revealed the presence of a *p*-hydroxyphenyl propanol group (Figure S1). The C-6 upfield shift (δ_C 33.9 from δ_C 34.2) and the C-7 downfield shift (δ_C 98.4 from δ_C 92.3) suggested the attachment of the *p*-hydroxyphenyl propanol group to the C-7 carbon. The assumption was confirmed by the HMBC correlation of H-7 (δ_H 4.81) with C-1′ (δ_C 95.6) of the *p*-hydroxyphenyl propanol group.

The relative configuration of **1** was established by the analysis of the NOESY spectrum (Figure S2). In the NOESY spectrum, correlations between CH_3-10 and H-1/H-7α indicated that H-1, H-7 and CH_3-10 were both α-oriented. Moreover, NOESY correlations between H-8β and H-5/H-9 confirmed that H-5, H-8 and H-9 were all β-oriented. The acid hydrolysis of **1** afforded D-glucose, which was identified using GC analysis after the chiral derivatization. The β-anomeric configuration for the glucosyl unit was judged from its large $^3J_{H1'', H2''}$ coupling constant ($J = 7.9$ Hz). Analysis of the ^{1}H-^{1}H COSY, HSQC and HMBC spectra led to the complete assignments of the proton and carbon signals in compound **1** (Table S1). For convenience purposes, compound **1** was named as cornusphenoside E.

Compound **2** gave a molecular formula of $C_{25}H_{34}O_{12}$ according to its HRESIMS (*m/z*: 525.19775 [M-H]$^-$). Comparison of the spectroscopic data of **2** with those of **1** showed they were almost superimposable on those of the iridoid skeleton, but the *p*-hydroxyphenyl propanol group at C-7 in **1** was replaced by a *p*-hydroxyphenyl ethanol group in **2**. In the HMBC spectrum (Figure S1), the correlation from H-7 (δ_H 4.82) to C-1′ (δ_C 69.5) suggested the *p*-hydroxyphenyl propanol group attaching to C-7 of the 7β-morroniside moiety. Thus, compound **2** was named as cornusphenoside F.

Compound **3** was assigned a molecular formula of $C_{25}H_{34}O_{12}$ from its HRESIMS (*m/z*: 525.19775 [M-H]$^-$). It showed similar UV, IR, and NMR data to **2**, suggesting their structural resemblance. Comparing the NMR data between **2** and **3**, the downfield shifts of C-5, C-6, C-7, and C-8 (δ_C 31.8 from δ_C 28.0, δ_C 35.7 from δ_C 33.9, δ_C 103.5 from δ_C 98.1, δ_C 74.1 from δ_C 66.3;) revealed that 7β-morroniside moiety in **2** was replaced by 7α-morroniside moiety in **3**. This discrepancy was confirmed by NOESY correlations of H-7 and H-5/H-8β (Figure S2). Hence, cornusphenoside G was characterized as **3**.

Compound **4** was isolated as an amorphous powder, and its molecular formula was established as $C_{24}H_{33}O_{15}$ on the basis of HRESIMS (*m/z*: 561.18207 [M + HCOO]$^-$). The ^{1}H NMR and ^{13}C NMR spectra of **4** (Table 1) resembled of those of **1**; however, the *p*-hydroxyphenyl propanol group was replaced by a 2, 5-dihydroxymethyl furan moiety in **4**. The 1 D NMR data showed an olefinic signal coupling pattern at δ_H 6.27 (1H, d, $J = 3.1$ Hz, H-3′) and 6.33 (1H, d, $J = 3.1$ Hz, H-4′), two methylene signals at δ_H 4.47 (1H, d, $J = 12.9$ Hz), (1H, d, $J = 12.9$ Hz) and 4.50 (2H, s, H-6′); and an furan group (δ_C 109.2, 111.3, 156.4, 152.7). The HMBC correlation of H-1′ with C-3′ and H-6′ with C-4′ revealed the presence of a 2, 5-dihydroxymethyl furan moiety (Figure S1). Furthermore, the HMBC correlation from H-7 to C-1′ verified the location of the furan group at C-7 of the 7β-morroniside moiety. From these data, the structure of compound **4**, named as cornusphenoside H, was characterized as shown in Figure 1.

Compound **5** gave a molecular formula of $C_{24}H_{31}O_{15}$ as established by HRESIMS (*m/z*: 559.16595 [M + HCOO]$^-$). The NMR spectroscopic data of **5** were very similar to those of **4** except that an aldehyde group [δ_H 9.57 (1H, s, H-6′); δ_C 179.5] was attached at C-

5′ instead of a hydroxymethyl group, which indicated the presence of the 5-hydroxymethyl furfural moiety in **5**. Furthermore, a HMBC correlation of H-7 with C-1′ suggested the 5-hydroxymethyl furfural moiety attaching to C-7 of the 7β-morroniside moiety (Figure S1). Accordingly, cornusphenoside I was characterized as **5**.

The effect of compounds **1**-**5** on H_2O_2-induced SH-SY5Y cells damage was determined by MTT assay. The results showed that only compound **5** exhibited the significant neuroprotective effect (Table S2).

According to the literature, iridoid glycosides were recognized as the main neuroprotective active components, and the main compound morroniside has the most significant neuroprotective activity (Wang et al. 2009; Jeong et al. 2012; Ji et al. 2019). Compounds **1**-**5** were the analogues of morronisides, which had different substituents at C-7 position. However, the neuroprotective activities of compound **1**-**5** were different from morronisides, only compound **5** showed neuroprotective activity, which indicated that the substitution of C-7 might reduce or eliminate their neuroprotective activity. Compound **5** has the same 5-hydroxymethylfural substitution as known compounds cornusphenosides A and B, isolated and identified in our previous research, but cornusphenosides A and B showed inactive on H_2O_2-induced SH-SY5Y cells damage, which indicated that the substitution of glucoside also had effects on the neuroprotective activities (Wang et al. 2018).

3. Experimental

3.1. General experimental procedures

The 1 D and 2 D NMR spectra were acquired in CD_3OD with TMS internal standard on Varian 500 MHz and Bruker AV500-III spectrometers (Bruker Corporation, Billerica, Massachusetts, USA). HR-ESIMS was performed using a Thermo QE Plus orbitrap mass spectrometer (Thermo, Waltham, MA, USA). IR spectra were recorded on a Nicolet IS5 spectrometer by FT-IR microscope transmission method (Nicolet Instrument, Inc., Madison, Wisconsin, USA). CD spectra were recorded on a JASCO J-810 circular dichroism spectrometer (JASCO Corporation, Tokyo, Japan). Column chromatography was performed with silica gel (160-200 mesh, Qingdao Marin Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Medium pressure liquid chromatography experiments were performed using a Buchi Pump Mooute C-650 pump (Buchi Corporation, Flawil, Switzerland). HPLC experiments were performed using an instrument consisting of a Waters e2695 pump, and a Waters 2998 dual-wave-length absorbance detector (Waters Corporation, Milford, Massachusetts, USA) with a Sunfire C18 preparative column (Waters, 5 μm, 250 $\times$ 19 mm, *i.d.*).

3.2. Plant material

The fruits of *C. officinalis* were purchased from Tong-Ren-Tang Company in Beijing, and authenticated by Professor Wen Wang (Xuanwu Hospital of the Capital Medical University, China). A voucher specimen (20090305) has been deposited at the Beijing Union University, Beijing Key Laboratory of Bioactive Substances and Functional Foods, Beijing Union University, Beijing, China.

3.3. Extraction and isolation

The dried fruits (10 kg) of *C. officinalis* were extracted with 50% EtOH (100 L × 3) at room temperature and the combined extracts were concentrated under reduced pressure to dryness. The residue was suspended in H_2O and applied to a Diaion HP-20 column that was eluted using a stepwise gradient of EtOH-H_2O (0:100, 20:80, 40:60, 70:30, and 95:5, *v/v*), to afford five fractions. After removing the solvent, the EtOH-H_2O (40:60, *v/v*) eluate (500 g) was separated subsequently by silica gel chromatography eluting with $CHCl_3$-MeOH (15:1 to 8:1, *v/v*) to afford four major fractions (F1∼F4) based on TLC analysis. F3 was chromatographed on a reversed-phase C18 silica column, eluted with an EtOH-H_2O (5:95-100:0, *v/v*) gradient, to give seven fractions (F3-1∼F3-7). Fraction F3-6 was separated subsequently by a reversed-phase C18 silica flash column eluting with MeOH-H_2O (20:80-100:0, *v/v*) gradient, to give six fractions (F3-6-1-F3-6-6). Fraction F3-6-2 was chromatographed over Sephadex LH-20 and eluted with $CHCl_3$-MeOH (2:1, *v/v*) as the mobile phase to give two subfractions F3-6-2-1 and F3-6-2-2. Fraction F3-6-2-2 was purified by preparative reversed-phase HPLC, eluting with acetonitrile-H_2O (35:65, 18 ml/min), to afford **1** (60 mg, t_R = 45.0 min). Fraction F3-6-2-1 was purified by preparative reversed-phase HPLC, eluting with actonitrile-H_2O (30:70, 18 ml/min) to afford **2** (30 mg, t_R = 39.0 min) and **3** (25 mg, t_R = 43.4 min). Further separation of fraction F3-5 by reversed-phase C18 silica flash column, eluting with MeOH-H_2O (15:85-100:0, *v/v*) gradient, which was purified by Sephadex LH-20, eluting with $CHCl_3$-MeOH (2:1, *v/v*) to afford subfractions F3-5-1∼F3-5-4. Subfraction F3-5-2 and F3-5-3 were purified by preparative reversed-phase HPLC, eluting with MeOH-H_2O (36:64, 18 ml/min) to afford **4** (20 mg, t_R = 33.2 min) and with acetonitrile-H_2O (20:80, 18 ml/min) to afford **5** (50 mg, t_R = 38.5 min).

3.4. Physical and spectroscopic data of compounds 1–5

Compound 1: White amorphous powder, $[\alpha]_D^{25}$ − 35.5 (0.03, MeOH); UV (*c* 0.08, C_2H_3N) λ_{max} (log ε) 242 (3.41) nm; CD (MeOH) 206 (Δε −5.98), 240 (Δε −33.5) nm; IR v_{max}3,392, 1,706 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C (methanol-d_4, 125 MHz) spectral data, see Table S1; (-)-HRESIMS *m/z* 539.21326 $[M-H]^-$ (calcd for $C_{26}H_{35}O_{12}$, 539.21326).

Compound 2: White amorphous powder, $[\alpha]_D^{25}$ − 45.3 (0.03, MeOH); UV (*c*, 0.06, C_2H_3N) λ_{max} (log ε) 240 (3.40) nm; CD (MeOH) 222 (Δε −19.58), 254 (Δε −3.05) nm; IR v_{max} 3,394, 2,939, 1,705 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C (methanol-d_4, 125 MHz) spectral data, see Table S1; (-)-HRESIMS *m/z* 525.19775 $[M-H]^-$ (calcd for $C_{25}H_{34}O_{12}$, 525.19775).

Compound 3: White amorphous powder, $[\alpha]_D^{25}$ − 66.6 (0.02, MeOH); UV (*c* 0.06 C_2H_3N) λ_{max} (log ε) 240 (3.41) nm; CD (MeOH) 206 (Δε −5.98), 240 (Δε −33.5) nm; IR v_{max} 3,394, 2,942, 1,701 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C (methanol-d_4, 125 MHz) spectral data, see Table S1; (-)-HRESIMS *m/z* 525.19775 $[M-H]^-$ (cacld for $C_{25}H_{34}O_{12}$, 525.19775).

Compound 4: White amorphous powder, $[\alpha]_D^{25}$ − 29.2 (0.03, MeOH); UV (*c* 0.03 MeOH) λ_{max} (log ε) 252 (3.60) nm; CD (MeOH) 229 (Δε −21.02) nm; IR v_{max}3,400, 2,917, 1,704 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C (methanol-d_4, 125 MHz)

spectral data, see Table S1; (-)-HRESIMS *m/z* 561.18207 $[M+HCOO]^-$ (cacld for $C_{24}H_{33}O_{15}$, 561.18229).

Compound 5: White amorphous powder, $[\alpha]_D^{25}-33.5$ (0.02, MeOH); UV (c 0.04 C_2H_3N) λ_{max} (log ε) 240 (3.80) nm, 280 (4.04) nm; CD (MeOH) 232 (Δε −6.33), 286 (Δε + 1.64) nm; IR v_{max} 3,408, 2,912, 1,678, 1,640 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C (methanol-d_4, 125 MHz) spectral data, see Table S1; (-)-HRESIMS *m/z* 559.16595 $[M+HCOO]^-$ (cacld for $C_{24}H_{31}O_{15}$, 559.16580).

Disclosure statement

No potential conflict of interest was reported by the authors.

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