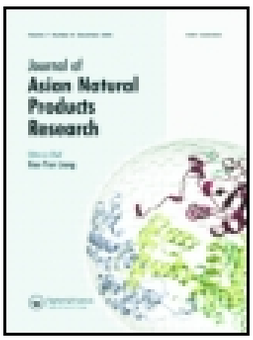

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Iridoid glycosides from the fruits of *Cornus officinalis*

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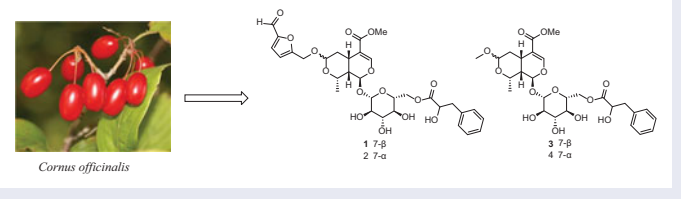
ABSTRACT

Four new iridoid glycosides named cornusphenosides A–D (1–4) were isolated from an ethanol extract of the fruits of *Cornus officinalis* (shan zhu yu). The structures of these compounds were elucidated on the basis of spectroscopic data (UV, IR, HRESIMS, and 1D and 2D NMR) and chemical evidence. The neuroprotective effects of compounds **1–4** were also assessed *in vitro*.

GRAPHICAL ABSTRACT



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KEYWORDS
Cornaceae; *Cornus officinalis*; iridoid glycosides; cornusphenosides A–D; isolation; structural elucidation

1. Introduction

The genus *Cornus* belongs to the family Cornaceae and consists of four species distributed mainly in Southern and Central Europe, Eastern Asia, and Eastern North America [1]. *Cornus officinalis* is the most widely used specie in this genus, and its fruits are known as "shan zhu yu" and used for treatment of various disorders in traditional Chinese medicine [2]. Modern pharmacology studies revealed that the extract and chemical constituents of *C. officinalis* are bioactive, and exhibit neuroprotective [3], anti-inflammatory [4], anti-diabetes [5], anti-oxidant [6], and cardiovascular system [7] activities. Previous phytochemical studies of this species have led to the isolation and identification of iridoid glycosides, flavonoids, terpenoids, and several other components [8–12]. Iridoid glycosides are an important type of constituent contributing to bioactivity, and have been revealed to possess potential anti-Alzheimer's disease activity [13,14]. As a part of our ongoing search for bioactive natural products, we found that a 40% ethanol elution fraction of the ethanol extract of

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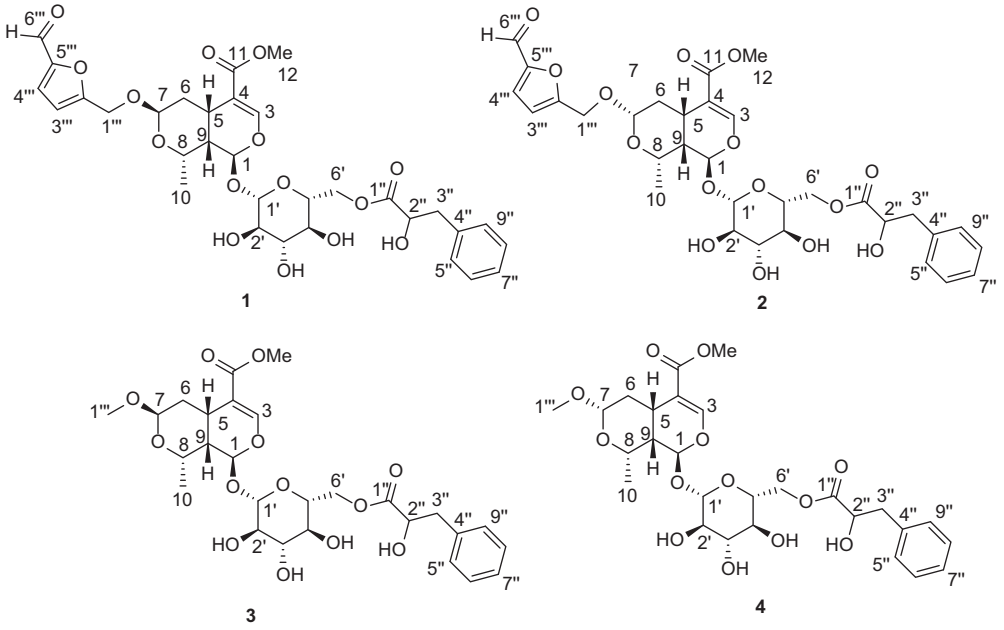

Figure 1. Structures of compounds **1**–**4**.

C. officinalis obtained over a macroporous resin showed potential anti-Alzheimer's disease activity *in vitro* and *in vivo* assays. Chemical and biological investigations of this fraction were performed, and the subfraction without morroniside and loganin still showed significant neuroprotective activity *in vitro* assay. In order to investigate additional minor iridoid glycosides with novel structures and neuroprotective activity from *C. officinalis*, bioassay-guided fractionation led to the isolation of four new minor iridoid glycosides cornusphenosides A–D (**1**–**4**) (Figure 1). Herein, the isolation and structure elucidation and biological assays of these iridoid glycosides have been described.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder with a specific rotation of $[\alpha]^{25}_{D}$ −118. The HRESIMS data showed a molecular ion peak at *m/z* 685.2097 $[M+Na]^+$, establishing the molecular formula as $C_{32}H_{38}O_{15}$. Its IR spectrum displayed absorption bands at 3431, 1741, 1704, and 1679 cm^{-1} attributed to the hydroxyl and carbonyl functionalities. The ^{1}H NMR spectrum of **1** exhibited one methyl group at δ_H 1.23, one methoxy group at δ_H 3.64, two methines at δ_H 1.75–1.77 and 2.96–3.01, two methylenes at δ_H 1.30–1.37, 1.76–1.80, 2.88, and 3.09, two oxygen-bearing methines at δ_H 4.22 and 4.36, one oxygen-bearing methylene at δ_H 4.43 and 4.49, two acetal protons at δ_H 5.79 and 4.70–4.72, seven olefinic protons at δ_H 7.15–7.17, 7.21, 6.50 and 7.30, one oxygen-bearing olefinic proton at δ_H 7.45, and one aldehyde group at δ_H 9.50. The ^{1}H NMR data of **1** also showed the presence of a *β*-glucopyranose moiety, the anomeric proton of which resonated at δ_H 4.76 (1H, d, J = 7.4 Hz, H-1′). Consistent with these observations, the ^{13}C NMR spectrum

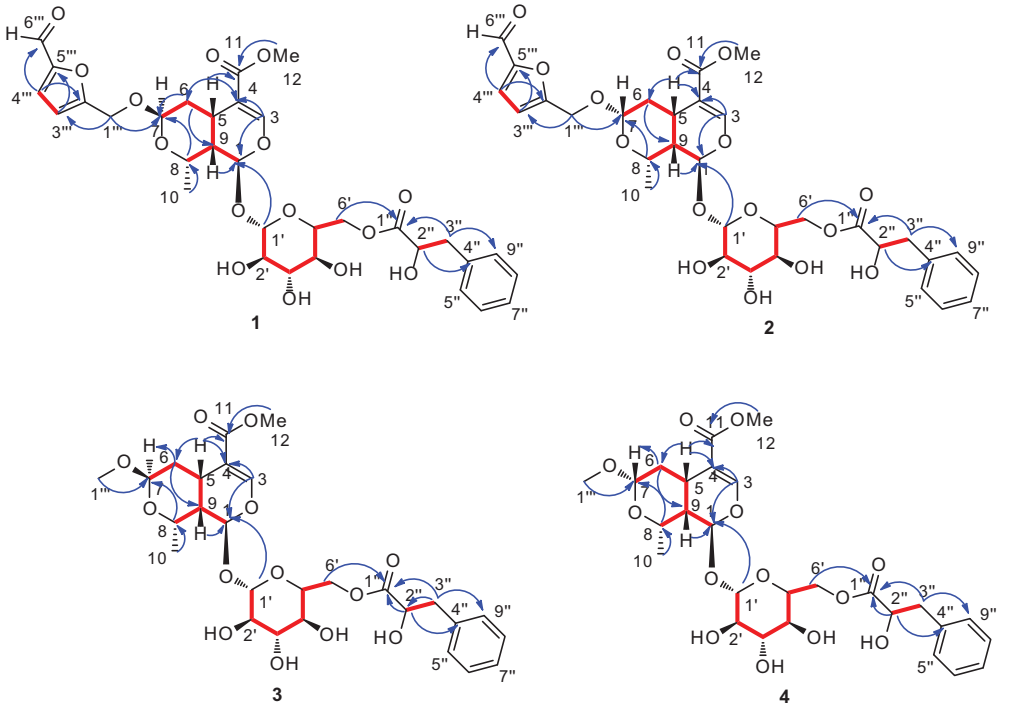

Figure 2. Key HMBC (⌒) and ^{1}H-^{1}H COSY (—) correlations of compounds **1**–**4**.

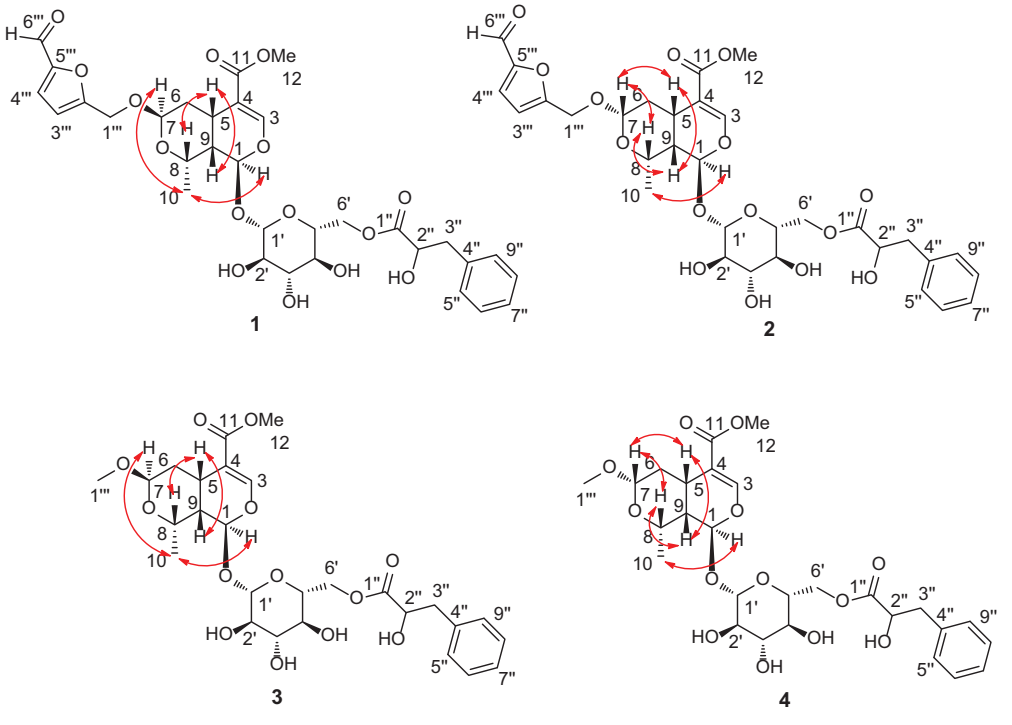

Figure 3. Key NOESY (⌒) correlations of compounds **1**–**4**.

showed 32 carbon resonances, including 1 methyl, 1 methoxyl, 2 methylenes, 2 methines, 1 oxygenated methylene, 2 oxygenated methines, 3 acetal carbons, 12 olefinic carbons (δ_C 111.8, 154.5, 138.9, 130.3, 130.3, 129.4, 129.4, 127.8, 159.8, 112.7, 124.4, 154.1), three carbonyl carbons (δ_C 168.6, 175.0, and 179.4), and resonances for a glucopyranosyl group.

The HMBC correlations from H-1 to C-3, C-5, and C-8; from H-3 to C-11 and C-5; from H-6 to C-4 and C-7; from H-7 and C-5, C-8; from H-1′ to C-1; together with their chemical shifts, revealed the presence of an iridoid glycoside moiety (Figure 2). These correlations and chemical shifts were similar to those previously reported for 7β-morroniside [15]. The evident differences were that **1** showed resonances due to an additional 5-hydroxymethyl furfural group and a phenyllactic acid group. In the HMBC spectrum, correlations from H-4‴ to C-2‴ and C-6‴; and from H-3‴ to C-5‴ and C-1‴ revealed the presence of the 5-hydroxymethyl furfural moiety in **1**, and correlations from H-1‴a/1‴b to the carbon at δ_C 97.9 indicated that the 5-hydroxymethyl furfural moiety was presented at C-7 of the 7β-morroniside moiety. In addition, the HMBC correlations from H-2″ to C-1″, C-3″, and C-4″; from H-3″ to C-1″, C-5″, and C-9″, together with the chemical shifts showed the presence of a phenyllactic acid moiety which was connected to the Glc-6′. This was further confirmed by the HMBC correlations of H-6′a/6′b with the ester carbonyl at δ_C 175.0.

The relative stereochemistry of **1** was determined by the NOESY spectrum and coupling constants (Figure 3). In the NOESY spectrum, correlations from H-1 to H-10 and H-6α, from H-7 to H-6α indicated that H-1, H-6α, H-7 and H-10 were on the same side in α orientation; correlations from H-5 to H-6β, H-8, and H-9 confirmed that H-5, H-6β, H-8, and H-9, in turn, on the other side in β oriented. This was also substantiated based on biogenetic grounds in that nearly all iridoids found in nature have a configuration of 5β and 9β, and the coupling constant of H-1 and H-9 ($J = 9.2$ Hz) indicated that the iridoid moiety was *cis*-fused, thus H-1 should be α oriented. In addition, the β configuration at C-7 was also determined by comparison of the data to those of the known compound, 7β-morroniside [15]. Acid hydrolysis of **1** afforded D-glucose which was determined by GC-MS of methanolysate and silylated derivatives, and the β-anomeric configuration of the glucosyl unit was judged from its large coupling constant ($J = 7.4$ Hz). Analysis of the HSQC, ^{1}H-^{1}H COSY, HMBC, and NOESY spectra led to the complete assignments of the proton and carbon signals in compound **1** (Table 1). On the basis of the above data, cornusphenoside A was characterized as shown.

Compound **2** was found to have the same molecular formula, $C_{32}H_{38}O_{15}$, as established by its HRESIMS (*m/z* 685.2096 $[M + Na]^+$). The IR spectrum of **2** showed characteristic absorption bands for the hydroxyl (3424 cm^{-1}) and carbonyl (1739, 1678 cm^{-1}) groups. The NMR spectroscopic data of **2** were also very similar to those of **1** except for the downfield shifts of C-5 (δ_C 31.7), C-6 (δ_C 35.5), C-7 (δ_C 102.5) and C-8 (δ_C 74.0). These changes suggested that the iridoid glycoside skeleton of **2** was 7α-morroniside [15] instead of 7β-morroniside, which was confirmed by the NOESY correlations of H-7 with H-5 and H-8β. Thus, the spectroscopic data of **2**

Table 1. ^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **1**–**4**.

No	1		2		3		4	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	5.79 d (9.2)	95.9	5.74 d (9.2)	96.2	5.79 d (9.2)	96.2	5.72 d (9.2)	96.3
3	7.45 s	154.5	7.47 s	154.5	7.47 s	154.5	7.47 s	154.5
4	–	111.8	–	111.0	–	111.9	–	111.1
5	2.96–3.01 m	27.6	2.74–2.77 m	31.7	2.93–2.98 m	27.9	2.73–2.77 m	31.8
6a	1.30–1.37 m	33.5	1.09–1.17 m	35.5	1.27–1.31 m	33.7	1.03–1.11 m	35.5
6b	1.76–1.80 m	–	1.89–1.93 m	–	1.72–1.76 m	–	1.87–1.91 m	–
7	4.70–4.72 m	97.9	4.57–4.63 m	102.5	4.48–4.50 m	99.0	4.40 o	104.3
8	4.22 o	66.6	3.83–3.90 m	74.0	4.16–4.18 m	66.1	3.83–3.85 m	73.9
9	1.75–1.77 m	40.3	1.73–1.76 m	40.3	1.74–1.76 m	40.5	1.71–1.75 m	40.3
10	1.23 d (6.9)	19.6	1.35 d (6.8)	19.7	1.26 d (7.0)	19.7	1.33 d (6.8)	19.7
11	–	168.6	–	168.5	–	168.6	–	168.6
12	3.64 s	51.8	3.64 s	51.8	3.65 s	51.2	3.67 s	51.8
1′	4.76 d (7.4)	100.4	4.75 d (7.9)	100.3	4.75 d (7.9)	100.2	4.73 d (7.9)	100.1
2′	3.22 dd (7.4, 8.2)	74.9	3.21 dd (8.0, 9.1)	74.9	3.22 o	74.9	3.20 dd (9.1, 7.9)	74.9
3′	3.34–3.39 m	77.8	3.34–3.38 m	77.8	3.35–3.38 m	77.8	3.34 d (9.1)	77.8
4′	3.29 o	71.9	3.29 o	71.7	3.30 o	71.8	3.30 d (6.0)	71.9
5′	3.47–3.51 m	75.2	3.41–3.44 m	75.4	3.46–3.48 m	75.4	3.39–3.43 m	75.2
6′a	4.20 o	65.1	4.17 dd (6.5, 11.8)	65.1	4.20 dd (6.7, 11.8)	65.1	4.26 dd (6.4, 11.8)	65.0
6′b	4.49–4.53 m	–	4.57 dd (2.7, 11.8)	–	4.54 dd (2.3, 11.8)	–	4.49 dd (2.2, 11.8)	–
1″	–	175.0	–	175.1	–	175.1	–	175.2
2″	4.36 dd (4.3, 5.6)	73.0	4.38 dd (4.8, 7.9)	73.0	4.37 dd (4.4, 8.2)	73.0	4.37 o	72.9
3″a	2.88 dd (13.8, 5.6)	41.3	2.89 dd (13.8, 7.9)	41.8	2.88 dd (8.2, 13.9)	41.8	2.89 dd (7.9, 13.8)	41.8
3″b	3.09 dd (13.8, 4.3)	–	3.02 dd (13.8, 4.8)	–	3.08 dd (4.4, 13.9)	–	3.03 dd (4.8, 13.8)	–
4″	–	138.9	–	138.7	–	138.8	–	138.7
5″	7.21 o	130.3	7.20 o	130.7	7.22 o	130.6	7.21 o	130.8
6″	7.21 o	129.4	7.20 o	129.3	7.22 o	129.3	7.21 o	129.3
7″	7.15–7.17 m	127.8	7.12–7.17 m	127.5	7.15–7.18 m	127.6	7.13–7.17 m	127.7
8″	7.21 o	129.4	7.20 o	129.3	7.22 o	129.3	7.21 o	129.3
9″	7.21 o	130.3	7.20 o	130.7	7.22 o	130.6	7.21 o	130.8
1‴a	4.43 d (13.5)	61.7	4.58 d (13.5)	63.0	3.20 s	54.8	3.36 s	56.6
1‴b	4.49 d (13.5)	–	4.70 d (13.5)	–				
2‴	–	159.8	–	159.8				
3‴	6.50 d (3.5)	112.7	6.55 d (3.6)	112.7				
4‴	7.30 d (3.5)	124.4	7.31 d (3.6)	124.1				
5‴	–	154.1	–	154.1				
6‴	9.50 s	179.4	9.50 s	179.6				

^{1}HNMR data (δ) were measured in methanol-d_4 at 500 MHz and ^{13}C NMR data (δ) were measured in methanol-d_4 at 125 MHz for **1**–**4**. Coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY experiments.
o: overlapped.

indicated that it is an epimer of **1** and the configuration of H-7 is β-oriented. Hence, the structure of **2** (cornusphenoside B) was established as shown.

Compound **3** was isolated as a white amorphous powder, and its molecular formula was established as $C_{27}H_{36}O_{13}$ on the basis of HRESIMS (*m/z* 591.2045 $[M+Na]^+$). Its IR spectrum showed the absorption characteristic of hydroxyl (3441 cm^{-1}) and carboxyl (1739, 1708 cm^{-1}) groups. The ^{13}C NMR spectrum showed 27 carbon signals including six for a glucopyranosyl unit, nine for a phenyllactic acid group, and the remaining 12 for an iridoid skeleton. Comparison of the spectroscopic data of **3** with those of **1** showed that they were almost superimposable on those of the iridoid glycoside skeleton and phenyllactic acid group, but the 5-hydroxymethyl furfural group at C-7 in **1** was replaced by a methoxy moiety in **3**. In the HMBC spectrum, the correlation from H-1‴ to C-7 confirmed the assignment of the methoxy moiety to C-7 of the iridoid glycoside group. NOESY experiments indicated that the relative stereochemistry of **3** was the same as that of **1**. The structure of this compound was confirmed by detailed analysis of the 2D NMR data including HSQC, ^1H-^1H COSY, HMBC, and NOESY spectra. Accordingly, cornusphenoside C was characterized as **3**.

Compound **4** shared the same molecular formula $C_{27}H_{36}O_{13}$ as **3** with HRESIMS ion at *m/z* 591.2045 $[M+Na]^+$ and required 10° of unsaturation. It exhibited similar UV, IR, and NMR data to **3**, suggesting their structural resemblance. However, a comparison of NMR data revealed that 7β-O-methyl-morroniside in **3** was replaced by 7α-O-methyl-morroniside in **4**. This discrepancy was confirmed by NOESY correlation of H-7 with H-8β. With the exception of this difference, other 1D NMR data of **4** and **3** were matched. Thus compound **4** was named as cornusphenoside D.

Compounds **1–4** were tested for their neuroprotective effects against hydrogen peroxide (H_2O_2)-induced cell injury in SH-SY5Y cells. However, at 10 μM, all exhibited no effect on H_2O_2-induced neurotoxicity.

In conclusion, four new iridoid glucosides, cornusphenosides A–D, were isolated from the fruit of *C. officinalis* and evaluated for their neuroprotective activities. Four new compounds all have a phenyllactic acid group in sugar moiety; additionally, cornusphenosides A and B also have a rare furan ring in iridoid skeleton. Experiments were taken to prove that **1–4** are genuine natural products.

3. Experimental

3.1. General experimental procedures

IR spectra were recorded on a Nicolet 5700 spectrometer by FT-IR microscope transmission method (Nicolet Instrument, Inc., Madison, Wisconsin, USA). CD spectra and UV data were recorded on a JASCO J-810 circular dichroism spectrometer (JASCO Corporation, Tokyo, Japan). HRESIMS was performed using an Agilent 1100 series LC/MSD ion trap mass spectrometer (Agilent, Santa Clara, California, USA). The 1D- and 2D-NMR spectra were acquired in CD_3OD with TMS as internal standard on Varian 500 MHz and Bruker AV500-III spetrometers (Bruker Corporation, Billerica, Massachusetts, USA). 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). HPLC experiments were performed with an instrument consisting of a Waters 600 pump, and a Waters 2996 dual-wavelength absorbance detector (Waters Corporation, Milford, Massachusetts, USA) with a Sunfire (Waters, 250 × 10 mm *i.d.*) preparative column packed with C18 (5 μm) (Alltech Associates, Inc., Bannockburn, Illinois, USA). TLC was carried out with glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 8% H_2SO_4 in 95% EtOH followed by heating.

3.2. Plant material

The fruits of *Cornus officinalis* were purchased from Tong-Ren-Tang Company in Beijing, and authenticated by Professor Wen Wang (Xuanwu Hospital of 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, 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. 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) 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 (F_1–F_4) based on TLC analysis. Fraction F_2 was chromatographed on a reversed-phase C18 silica column, eluted with an EtOH-H_2O (5:95–100:0) gradient, to give three subfractions $F_{2\text{-}1}$–$F_{2\text{-}3}$. Fraction $F_{2\text{-}3}$ (128.3 g) was subjected to a column of silica gel, eluting with $CHCl_3$–MeOH (20:1–3:1, v/v), to give six subfractions $F_{2\text{-}3\text{-}1}$–$F_{2\text{-}3\text{-}6}$. Fraction $F_{2\text{-}3\text{-}2}$ (10.1g) was chromatographed over Sephadex LH-20 and eluted with $CHCl_3$–MeOH (2:1) as the mobile phase to give four subfractions $F_{2\text{-}3\text{-}1\text{-}1}$–$F_{2\text{-}3\text{-}1\text{-}4}$. Fraction $F_{2\text{-}3\text{-}1\text{-}1}$ (2.7 g) was separated using a reversed-phase C18 silica gel column, by eluting with a MeOH-H_2O (30:70–100:0) gradient, to yield subfractions $F_{2\text{-}3\text{-}1\text{-}1\text{-}1}$–$F_{2\text{-}3\text{-}1\text{-}1\text{-}7}$. Fraction $F_{2\text{-}3\text{-}1\text{-}1\text{-}6}$ (76 mg) was purified by preparative reversed-phase HPLC, eluting with MeOH–H_2O (58:42, 2.5 ml/min) to afford **1** (9.3 mg, $t_R = 16$ min) and **3** (5.1 mg, $t_R = 20$ min). Further separation of fractions $F_{2\text{-}3\text{-}1\text{-}1\text{-}7}$ (103 mg) by Sephadex LH-20 gel, eluting with MeOH, which was purified by preparative reversed–phase HPLC, eluting with MeOH-H_2O (58:42, 2.5 ml/min) to afford **2** (4.3 mg, $t_R = 40$ min), and **4** (7.8 mg, $t_R = 44$ min).

3.3.1. Cornusphenoside A

White amorphous powder, $[\alpha]^{25}_{D}$−118 (*c* 0.08, CH_3OH); UV λ_{max} (MeOH, log ε): 235 (4.75), 280 (4.20) nm; CD (*c* 3.7×10^{-4}, MeOH) $\Delta\varepsilon_{224\,nm}$ −5.64; IR ν_{max} 3431, 2912, 1741, 1704, 1679, 1522, 1440 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 1; (+)-HRESIMS *m/z*: 685.2097 $[M + Na]^+$ (calcd for $C_{32}H_{38}O_{15}Na$, 685.2103).

3.3.2. Cornusphenoside B

White amorphous powder, $[\alpha]^{25}_{D}-28$ (*c* 0.02, CH_3OH); UV λ_{max} (MeOH, log ε): 235 (4.10), 280 (3.92) nm; CD (*c* 3.1×10^{-4}, MeOH) $\Delta\varepsilon_{228\,nm}$ −5.61; IR ν_{max} 3424, 2938, 1739, 1678, 1641, 1440 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 1; (+)-HRESIMS *m/z*: 685.2096 $[M+Na]^+$ (calcd for $C_{32}H_{38}O_{15}Na$, 685.2103).

3.3.3. Cornusphenoside C

White amorphous powder, $[\alpha]^{25}_{D}-97.6$ (*c* 0.05, CH_3OH); UV λ_{max} (MeOH, log ε): 238 (4.36) nm; CD (*c* 2.2×10^{-4}, MeOH) $\Delta\varepsilon_{225\,nm}$ −3.03; IR ν_{max} 3441, 2940, 1739, 1708, 1639, 1440 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) spectral data, see Table 1; (+)-HRESIMS *m/z*: 591.2045 $[M+Na]^+$ (calcd for $C_{27}H_{36}O_{13}Na$, 591.2048).

3.3.4. Cornusphenoside D

White amorphous powder, $[\alpha]^{25}_{D}-68$ (*c* 0.03, CH_3OH); UV λ_{max} (MeOH, log ε): 238 (4.82) nm; CD (*c* 4.2×10^{-4}, MeOH) $\Delta\varepsilon_{227\,nm}$ −9.81; IR ν_{max} 3418, 2938, 1704, 1702, 1640, 1441 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125MHz) spectral data, see Table 1; (+)-HRESIMS *m/z*: 591.2045 $[M+Na]^+$ (calcd for $C_{27}H_{36}O_{13}Na$, 591.2048).

3.4. Acid hydrolysis of compounds 1–4

Each compound (1 mg) was individually refluxed in 2 M HCl (2.0 ml) at 80 °C for 1.5 h. The reaction mixture was extracted with EtOAc, and H_2O phase evaporated under vacuum, diluted repeatedly with H_2O, and evaporated *in vacuo* to furnish a neutral residue. The residue was then dissolved in anhydrous pyridine (1.0 ml), to which 1 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h and after evaporation *in vacuo* to dryness, 0.1 ml of N-trimethylsilylimidazole was added. After stirring the mixture another 2 h at 60 °C, it was partitioned between *n*-hexane and H_2O (1.0 ml each), and then the *n*-hexane extract was analyzed by GC under the following conditions: capillary column, HP-5 (30 m, 0.25 mm, with a 0.25 μm film; Dikma); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature, 160 °C, then raised to 280 °C at 5 °C/min, final temperature maintained for 10 min; carrier gas, N_2. From the acid hydrolysate of each compound, D-glucose was confirmed by comparison of the retention time of its derivative with that of authentic sugar derivatized in a similar way, which showed a retention time of 20.55 min.

3.5. Neuroprotective activity assay

Human neuroblastoma SH-SY5Y cells were cultured in 100 mm dishes and grown in DMEM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2. Cells were cultured in 96-well plates at a density of 1×10^4 cells/well in 200 μl for 24 h. Cells were incubated with test

compounds (10 μM) for 12 h. To induce an oxidative stress, 100 μM H_2O_2 freshly prepared was added to the cells and incubated at 37 °C for 1.5 h. Cell viability was determined colourimetrically using MTT assay. Then 20 μl of the MTT solution (5 mg/ml) was add into each well. After incubation for 4h at 37 °C, the cells were finally lysed with 150 μl of DMSO. The absorbance was read at 490 nm with a micro-plate reader. The value for cell viability was expressed as the percentage of the control value.

Disclosure statement

There are no potential conflicts of interest to declare.

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