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New lignan glycosides from the stems of *Securidaca inappendiculata* Hassk

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ABSTRACT

Three new lignan glycosides, (7*R*,8*S*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol-4-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (**1**), (7*S*,8*R*)-4-hydroxy-3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol-4-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (**2**) and (7*S*,7′*S*,8*R*,8′*R*)-4,4′-dihydroxy-3,5,3′,5′-tetramethoxy-7,9′:7′,9-diepoxylignane-4-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (**3**) were isolated from the stems of *Securidaca inappendiculata* Hassk. Their structures were elucidated on the basis of spectroscopic analyses, including 1D and 2D NMR, HRESIMS, CD and chemical evidence. The MTT assay showed that compounds **1-3** exhibited moderate hepatoprotective activities against acetaminophen-induced HepG2 cell injury at respective 10 μM concentrations (Bicyclol as positive contrast).

1. Introduction

Securidaca inappendiculata Hassk belongs to the genus *Securidaca* Linn. and grows mainly in tropical and subtropical areas. Previous phytochemical investigations have led to the isolation of xanthones, terpenes, steroids, lignans (Kang et al., 2009). This plant is a kind of ethnic medicine in China which is used to promote blood circulation for removing blood stasis. Modern pharmacological studies have proved that *Securidaca inappendiculata* Hassk possesses anti-tumor, anti-inflammatory and hepatoprotective activities (Yang et al., 2001; Zhang et al., 2005). At the current research, three new lignan glycosides (**1-3**) were obtained from the stems of *Securidaca inappendiculata* Hassk. Their structures were elucidated on the basis of spectroscopic data analyses (1D and 2D NMR, HRESIMS, CD) and chemical evidence. All the compounds were evaluated for their hepatoprotective activities against acetaminophen-induced HepG2 cell injury (Bicyclol as positive contrast). Herein, the isolation, structural identification and hepatoprotective activities of these compounds were displayed.

2. Results and discussions

Compound **1** was obtained as a yellow amorphous transparent solid. The HR-ESI-MS gave a pseudomolecular ion peak at *m/z* 707.2530 $[M + Na]^+$, corresponding to the molecular formula $C_{32}H_{44}O_{16}$ (calculated for $C_{32}H_{44}O_{16}Na$, 707.2527). In the ^{13}C-NMR spectrum of **1** (Table 1), 18 signals for 12 aromatic carbons at δ_C 149.4, 146.3, 145.9, 143.8, 136.1, 135.7, 129.2, 118.3, 116.9, 115.7, 112.9, 110.7, and 6 aliphatic carbons at δ_C 87.0, 63.5, 60.7, 54.0, 35.1, 32.0 were observed. All these signals with corresponding ^{1}H-NMR signals were similar to those of cedrusinin-4-*O*-*α*-L-rhamnopyranoside (He et al., 2012), which indicated that the aglycone of **1** was 4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol. Besides, signals at δ_H 4.99 (1H, d, J = 7.5 Hz) with δ_C 100.0 and δ_H 4.32 (1H, d, J = 7.5 Hz) with δ_C 103.6 suggested that **1** had two D-glucopyranosyl units which was proved by acid hydrolysis of **1**, and both of the D-glucopyranosyl units were of *β* configuration deduced by the coupling constants of two anomeric protons. The HMBC correlation from the anomeric proton signal of Glucose A moiety at δ_H 4.99 (d, J = 7.5 Hz) to the carbon signal at δ_C 146.3 (C-4) indicated that Glucose A moiety was attached at C-4. The carbon signals of Glucose A from C-1 to C-6 were δ_C 100.0, 73.4, 75.4, 80.2, 75.6, 60.4 respectively deduced by HSQC and ^{1}H-^{1}H COSY experiments. The terminal Glucose B moiety was speculated to attach at C-4-Glc A with an oxygen bridge from analysis of the Glucose B moiety at δ_H 4.32 (d, J = 7.5 Hz) to the carbon resonance at δ_C 80.2 (C-4-Glc A). The relative configuration of **1** was supposed to be *trans* by the coupling constant of H-7 (J = 5.5 Hz) (Wu et al., 2012). The CD spectrum of **1** showed a negative Cotton effect at 278 nm, which revealed its absolute configuration to be 7*R*,8*S* (Hideaki et al., 1996). Therefore, **1** was determined as (7*R*,8*S*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol-4-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (Figs. 1–4).

Compound **2** was obtained as a yellow amorphous solid. The HR-ESI-MS gave a pseudomolecular ion peak at *m/z* 737.2641 $[M + Na]^+$, corresponding to the molecular formula $C_{33}H_{46}O_{17}$ (calculated for $C_{33}H_{46}O_{17}Na$, 737.2633). The ^{13}C-NMR spectrum of **2** (Table 1) was very similar to that of **1**. By comparing the 1D and 2D NMR spectra of **1**

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Table. 1
NMR of compounds **1-3**. (500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR, DMSO-d_6).

Position	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	136.1		137.8		137.8	
2	110.7	6.99 (br s, 1 H)	104.5	6.70 (br s, 1 H)	104.6	6.66 (br s, 1 H)
3	149.4		153.1		153.1	
4	146.3		134.3		134.0	
5	115.7	7.08 (d, J = 8.0 Hz, 1 H)	153.1		153.1	
6	118.3	6.86 (br d, J = 8.0 Hz, 1 H)	104.5	6.70 (br s, 1 H)	104.6	6.66 (br s, 1 H)
7	87.0	5.49 (d, J = 5.5 Hz, 1 H)	87.2	5.47 (dd, J = 6.5, 2.5 Hz, 1 H)	85.5	4.67 (d, J = 4.0 Hz, 1 H)
8	54.0	3.42 (m, 1 H)	53.7	3.47 (m, 1 H)	54.1	3.07 (m, 1 H)
9	63.5	3.61 (m, 1 H)	63.5	3.64 (m, 1 H)	71.7	3.80 (m, 1 H)
		3.72 (m, 1 H)		3.74 (m, 1 H)		4.18 (t, J = 6.8 Hz, 1 H)
1'	135.7		135.8		131.8	
2'	116.9	6.70 (br s, 1 H)	116.9	6.70 (br s, 1 H)	104.1	6.60 (br s, 1 H)
3'	129.2		129.3		148.4	
4'	145.9		145.9		135.3	
5'	143.8		143.9		148.4	
6'	112.9	6.70 (br s, 1 H)	113.0	6.70 (br s, 1 H)	104.1	6.60 (br s, 1 H)
7'	32.0	2.54 (m, 2 H)	32.0	2.54 (m, 2 H)	85.8	4.62 (d, J = 4.4 Hz, 1 H)
8'	35.1	1.69 (m, 2 H)	35.2	1.70 (m, 2 H)	54.1	3.07 (m, 1 H)
9'	60.7	3.42 (m, 2 H)	60.6	3.42 (m, 2 H)	71.6	3.80 (m, 1 H)
						4.18 (t, J = 6.8 Hz, 1 H)
3-OCH_3	56.1	3.76 (s, 3 H)	56.9	3.75 (s, 3 H)	56.9	3.76 (s, 3 H)
3'-OCH_3					56.5	3.75 (s, 3 H)
5-OCH_3			56.9	3.75 (s, 3 H)	56.9	3.76 (s, 3 H)
5'-OCH_3	56.1	3.78 (s, 3 H)	56.2	3.78 (s, 3 H)	56.5	3.75 (s, 3 H)
Glucose A						
1	100.0	4.99 (d, J = 7.5 Hz, 1 H)	102.7	4.97 (d, J = 7.5 Hz, 1 H)	102.8	4.94 (dd, J = 7.2, 1.6 Hz, 1 H)
2	73.4	3.34 (m, 1 H)	74.2	3.31 (m, 1 H)	74.3	3.29 (m, 1 H)
3	75.4	3.46 (m, 1 H)	75.2	3.40 (m, 1 H)	75.3	3.38 (m, 1 H)
4	80.2	3.44 (m, 1 H)	80.7	3.46 (m, 1 H)	80.9	3.44 (m, 1 H)
5	75.6	3.46 (m, 1 H)	75.4	3.24 (m, 1 H)	75.5	3.24 (m, 1 H)
6	60.4	3.64 (m, 2 H)	60.8	3.59 (m, 1 H)	60.9	3.58 (m,1 H)
				3.64 (m, 1 H)		3.64 (m, 1 H)
Glucose B						
1	103.6	4.32 (d, J = 7.5 Hz, 1 H)	103.5	4.30 (d, J = 8.0 Hz, 1 H)	103.6	4.29 (d, J = 7.6 Hz, 1 H)
2	73.7	3.04 (m, 1 H)	73.7	3.02 (m, 1 H)	73.8	2.99 (m, 1 H)
3	76.9	3.19 (m, 1 H)	77.0	3.19 (m, 1 H)	76.9	3.17 (m, 1 H)
4	70.5	3.09 (m, 1 H)	70.6	3.08 (m, 1 H)	70.5	3.05 (m, 1 H)
5	77.2	3.21 (m, 1 H)	77.3	3.21 (m, 1 H)	77.3	3.20 (m, 1 H)
6	61.5	3.44 (m, 1 H)	61.5	3.43 (m, 1 H)	61.5	3.42 (m, 1 H)
		3.72 (m, 1 H)		3.71 (m, 1 H)		3.71 (m, 1 H)

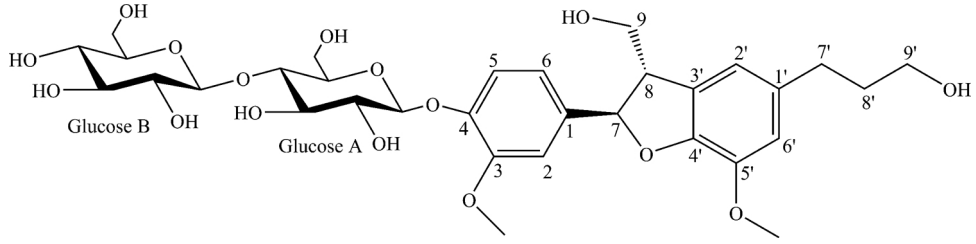

Fig. 1. The structure of compound **1**.

with those of **2**, it could be deduced that **2** possessed one more methoxyl at C-5 than **1**. Acid hydrolysis of **2** suggested two D-glucopyranosyl units and same as **1**, both of the D-glucopyranosyl units were of β configuration, the Glucose A moiety was attached at C-4, the Glucose B moiety was attached at C-4-Glc A. The relative configuration of **2** was supposed to be *trans* by the coupling constant of H-7 (J = 6.5 Hz). The CD spectrum of **2** showed a positive Cotton effect at 278 nm, which revealed its absolute configuration to be 7*S*,8*R*. Therefore, **2** was determined as (7*S*,8*R*)-4-hydroxy-3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol-4-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (Figs. 2 and 5).

Compound **3** was obtained as a white amorphous powder. The HR-ESI-MS gave a pseudomolecular ion peak at m/z 765.2538 $[M + Na]^+$, corresponding to the molecular formula $C_{34}H_{46}O_{18}$ (calculated for $C_{34}H_{46}O_{18}Na$, 765.2576). In the NMR spectra of **3** (Table 1), the signals at δ_H 6.66 (s, 2 H), δ_C 137.8, 104.6, 104.6, 153.1, 153.1, 134.0 and δ_H 6.60 (s, 2 H), δ_C 131.8, 104.1, 104.1, 148.4, 148.4, 135.3 indicated the presence of two 1, 3, 4, 5-tetrasubstituted aromatic rings. The signals at δ_C 85.5, 54.1, 71.7, 85.8, 54.1, 71.6 suggested the presence of a 2, 6-diaryltetrahydrofuran ring system. In the ^{1}H-NMR spectrum, four aromatic methoxyl signals at δ_H 3.75 (s, 6 H) and 3.76 (s, 6 H) were observed. All these spectroscopic data of **3** was similar to that of syringaresinol. In addition, two anomeric protons and corresponding carbons signals at δ_H 4.94 (dd, J = 7.2, 1.6 Hz, 1 H), δ_C 102.8 and δ_H 4.29 (d, J = 7.6 Hz, 1 H), δ_C 103.6 showed the presence of two D-glucopyranosyl units which was identified by acid hydrolysis of **3**, and both of the D-glucopyranosyl units were of β configuration deduced by the coupling constants of two anomeric protons. The HMBC correlation

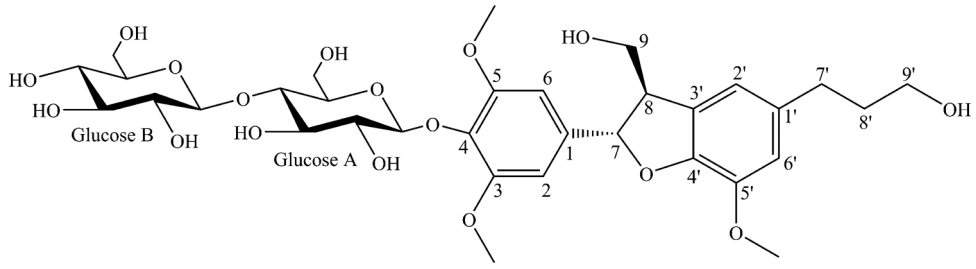

Fig. 2. The structure of compound **2**.

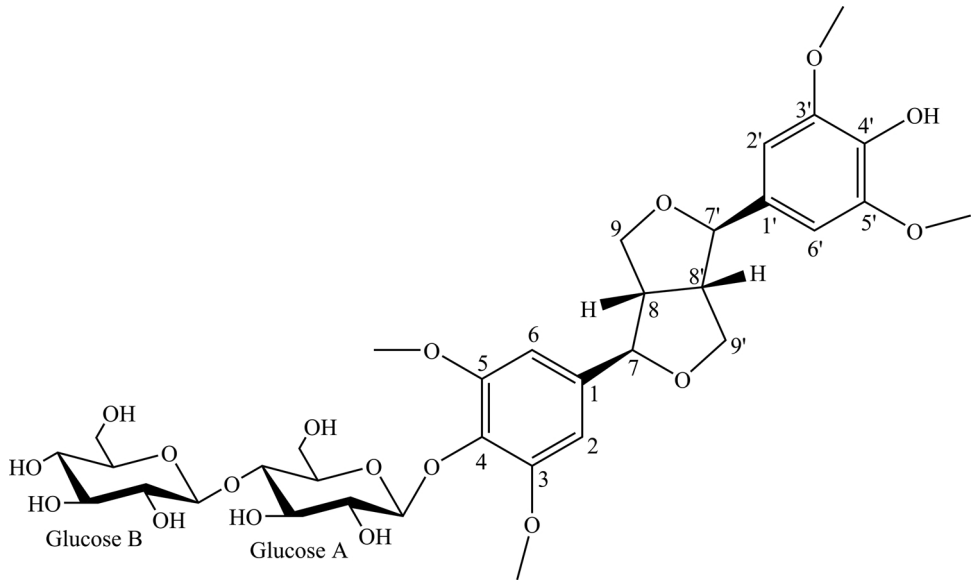

Fig. 3. The structure of compound **3**.

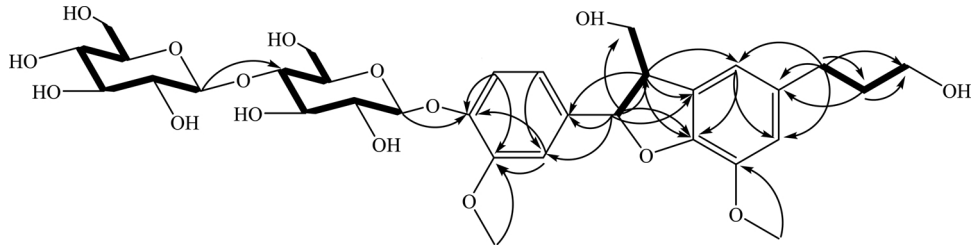

Fig. 4. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1** (—^{1}H-^{1}H COSY →HMBC).

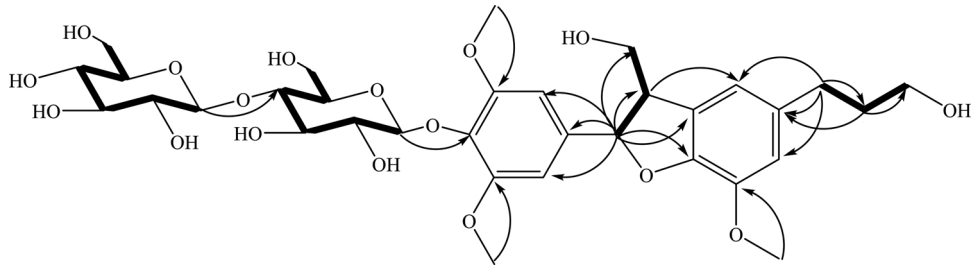

Fig. 5. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **2** (—^{1}H-^{1}H COSY →HMBC).

from the anomeric proton signal of Glucose A moiety at δ_H 4.94 (dd, J = 7.2, 1.6 Hz, 1 H) to the carbon signal at δ_C 134.0 (C-4) indicated that Glucose A was attached at C-4. The carbon signals of Glucose A from C-1 to C-6 were δ_C 102.8, 74.3, 75.3, 80.9, 75.5, 60.9 respectively deduced by HSQC and ^{1}H-^{1}H COSY experiments. The terminal Glucose B moiety was speculated to attach at C-4-Glc A with an oxygen bridge from analysis of the Glucose B moiety at δ_H 4.29 (d, J = 7.6 Hz) to the carbon resonance at δ_C 80.9 (C-4-Glc A). The relative configuration of **3** was deduced by ROESY spectrum. First, natural bistetrahydrofurans exist in the form of *cis*. Second, no NOE effect could be seen between H-

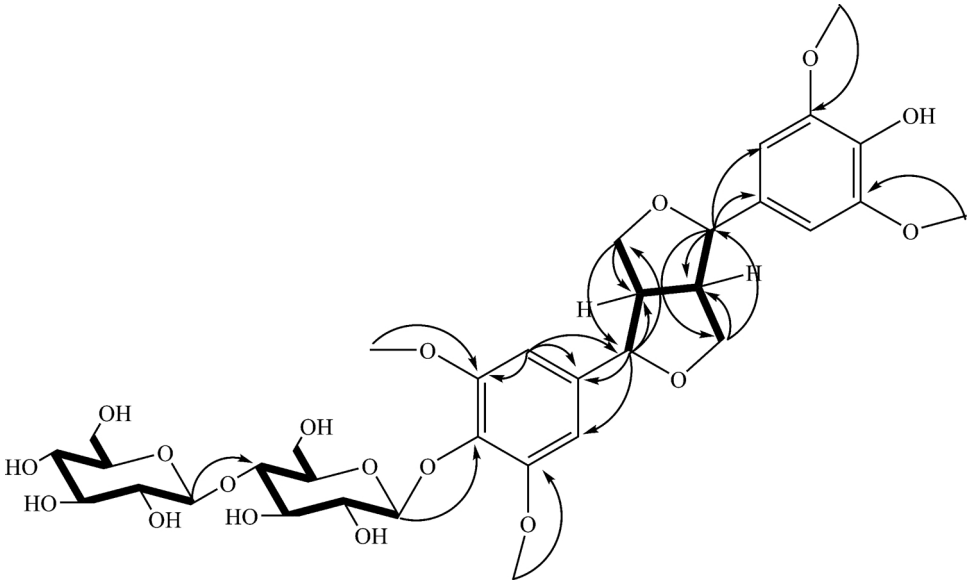

Fig. 6. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **3** (—^{1}H-^{1}H COSY →HMBC).

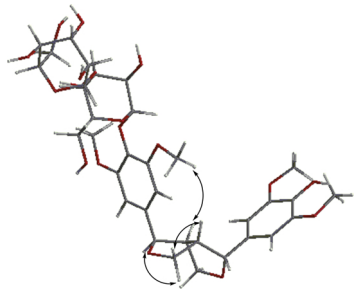

Fig. 7. Key ROESY correlations of compound **3** (↔ROESY).

7 and H-8, H-7′ and H-8′. The CD spectrum of **3** showed a positive Cotton effect at 274 nm, which was same as (-)-syringaresinol (Liu et al., 2013). Therefore, **3** was determined as (7*S*,7′*S*,8*R*,8′*R*)-4,4′-dihydroxy-3,5,3′,5′-tetramethoxy-7,9′:7′,9-diepoxylignane-4-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (Figs. 3, 6 and 7).

In vitro hepatoprotective activities of the three compounds were evaluated by MTT assay. The results (Table 2) suggested that all of the three compounds showed moderate hepatoprotective activities compared with bicyclol.

Table. 2
Hepatoprotective activities of compounds **1-3**.

Group[a]	Relative protection (%)[b]
Control	100.00 ± 4.28
Model	0.00 ± 1.63***
Bicyclol	14.00 ± 1.83###
1	11.37 ± 4.12##
2	9.31 ± 4.31##
3	8.64 ± 6.73#

[a] Acetaminophen (8 mM)-induced cells as the model group; Bicyclol as the positive contrast; The compounds were tested at 10 μM.
[b] Results are expressed as the means ± SD (n = 4).
*** $p < 0.001$ (vs control group).
$p < 0.001$.
$p < 0.01$.
# $p < 0.05$ (vs model group).

3. Experimental

3.1. General experimental procedures

^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz) and 2D NMR spectra were obtained on Bruker AV-500 with TMS as internal reference. HR-ESI-MS were acquired in the positive ion mode on a Q-TOF MS (Triple TOF 5600+, AB Sciex, USA) and Q-TOF LC/MS (Agilent Technologies, 6530, Accurate-Mass). UV spectra were recorded on a UV-VIS recording spectrophotometer (UV-2401PC, Shimadzu). IR spectra were measured on a Nicolet iS5 spectrometer. CD spectra were obtained on a J-810 Circular Dichroism spectrapolarimeter (JASCO, Japan). Optical rotations were acquired on Autopol III automatic polarimeter (Rudolph Research Analytical). Suger analyses were performed on HPLC (Waters Alliance e2695) using NH_2 column (5 μm, 4.6 mm × 250 mm, Hypersil) equipped with ELSD (Alltech 2000ES). Semi-preparative HPLC experiments were performed on a Shimadzu LC-6AD equipped with a SPD-20A detector (Shimadzu, Japan) using a SunFire C18 OBD Prep Column (5 μm, 10 mm × 250 mm, Waters, USA). Bicyclol (LOT:101044-201001) and acetaminophen (LOT: 100018-201610) were obtained from National Institutes for Food and Drug Control. Column chromatography silica gel (SiO_2; 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China); Macroporous resin (Yuanye Bio.); Sephadex LH-20 (Pharmacia, GE). All other chemicals were of analytical reagent grade.

3.2. Plant material

The stems of *Securidaca inappendiculata* Hassk were collected in December 2014 at Wenshan, Yunnan Province, China and identified by Professor Jianwei Chen (College of Pharmacy, Nanjing University of Chinese medicine, China). A voucher specimen of the plant (NO. CYT20141224) was deposited in the Herbarium Center, Nanjing University of Chinese Medicine, China.

3.3. Extraction and isolation

The dried stems of *Securidaca inappendiculata* Hassk (20 kg) were extracted with 95% EtOH (90 L × 3, 2 h each) under condition of reflux. The EtOH extract (2688 g) was evaporated to dryness under reduced pressure. This extract was mixed with silica gel (100–200 mesh, 2.5 kg) and heated to dryness. Then it was partitioned with petroleum

ether, CH_2Cl_2, EtOAc and MeOH respectively. The CH_2Cl_2 fraction (140 g), EtOAc fraction (277 g) and MeOH fraction (1261 g) are obtained after the solution was evaporated. Part of the MeOH fraction (1125 g) was subjected to column chromatography using macroporous resin with gradient mixtures of EtOH/H_2O (10%, 30%, 50%, 70%, 95%, fraction A–E). Fracton B (105 g) was later separated on a silica gel column with a gradient elution of CH_2Cl_2/MeOH (100:1˜0:1) to afford Fr.B1-Fr.B45. Fr.B14 was divided into twenty six parts (Fr.B14a-Fr.B14z) by MPLC using C18, eluted by a increasing gradient of MeOH/H_2O (20%˜60%). The twenty-first part (Fr.B14 u) and the twenty-second part (Fr.B14v) were purified using semi-preparative HPLC to afford **1** (90 mg, 35% MeOH/H_2O, t_R = 13 min, flow rate = 6.5 mL/min) and **2** (80 mg, 37% MeOH/H_2O, t_R = 15 min, flow rate = 5 mL/min) respectively. Fr.B9 was isolated into twenty-five parts (Fr.B9a-Fr.B9y) using MPLC, eluted by a increasing gradient of MeOH/H_2O (10%˜50%). The twenty-first part (Fr.B9u) was subjected to column chromatography over Sephadex LH-20 (50% MeOH/H_2O) to yield **3** (32 mg).

*3.3.1. (7R,8S)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol-4-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**1**)*

Yellow solid (MeOH); $[\alpha]_D^{25}$ 65° (c 0.1, MeOH); $[\theta]_{278}$ -1.31; UV (MeOH) λ_{max} (logε) 279 (3.54) nm; IR (KBr) ν_{max} 3382, 2929, 1605, 1514, 1452, 1421, 1325, 1266, 1213 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); (+)-HRESIMS *m/z* 707.2530 $[M + Na]^+$ (calculated for $C_{32}H_{44}O_{16}Na$ at *m/z* 707.2527).

*3.3.2. (7S,8R)-4-hydroxy-3,5,5′-trimethoxy-4′,7-epoxy-8,3′-neoligna-9,9′-diol-4-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**2**)*

Yellow solid (MeOH); $[\alpha]_D^{25}$ -65.3° (c 0.1, MeOH); $[\theta]_{278}$ +1.29; UV (MeOH) λ_{max} (logε) 280 (3.37) nm; IR (KBr) ν_{max} 3423, 2908, 1597, 1499, 1464, 1425, 1333, 1215, 1077 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); (+)-HRESIMS *m/z* 737.2641 $[M + Na]^+$ (calculated for $C_{33}H_{46}O_{17}Na$ at *m/z* 737.2633).

*3.3.3. (7S,7′S,8R,8′R)-4,4′-dihydroxy-3,5,3′,5′-tetramethoxy-7,9′:7′,9-diepoxylignane-4-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (**3**)*

White powder (MeOH); $[\alpha]_D^{25}$ -16.7° (c 0.1, MeOH); $[\theta]_{274}$ +4.94; UV (MeOH) λ_{max} (logε) 307 (2.76) nm; IR (KBr) ν_{max} 3421, 2915, 1595, 1509, 1463, 1425, 1330, 1234 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); (+)-HRESIMS *m/z* 765.2538 $[M + Na]^+$ (calculated for $C_{34}H_{46}O_{18}Na$ at *m/z* 765.2576).

3.4. Hepatoprotective effect

In vitro hepatoprotective activities of three compounds were evaluated by the MTT assay. HepG2 cells were cultured in DMEM media with 10% fetal calf serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere of 5% CO_2. The cells were seeded in 96-well culture plates at a density of 1×10^4 per well with 200 μL culture media. After incubation for 24 h, the culture supernatant was discarded, fresh media (100 μL) containing test samples (20 μM) were added and the cells were cultured for 2 h. Then, fresh media (100 μL) containing APAP (16 mM) were added to the cultured cells. The culture supernatant was discarded, and 100 μL of the 0.5 mg/mL MTT was added to the cells and maintained for 4 h after culturing for 48 h. The formazan was dissolved in DMSO (150 μL) after removal of the MTT solution. The optical density (OD) of the formazan solution was determined using a microplate reader at a wavelength of 570 nm. Bicyclol was used as the positive contrast.

*3.5. Acid hydrolysis of **1-3***

Compounds **1-3** (2 mg each) were heated with 2 mol/L TFA (2 mL) for 3 h at 90 °C. The mixture was cooled and partitioned between CH_2Cl_2 (2 mL) and H_2O for 3 times. The aqueous layer was evaporated under reduced pressure to 500 μL. Then the samples were analysed on HPLC (85% CH_3CN/H_2O, flow rate = 1 mL/min) equipped with ELSD using NH_2 Column. At last, compare the retention time with those of rhamnose, xylose, arabinose, mannose, glucose, galactose.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.03.011.

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