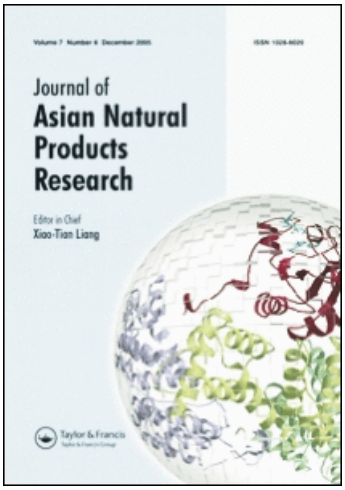

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 08 November 2010

To cite this Article He, Xiang-Hui , Yang, Wen-Zhi , Meng, A-Hui , He, Wen-Ni , Guo, De-An and Ye, Min(2010) 'Two new lignan glycosides from the seeds of *Cuscuta chinensis*', Journal of Asian Natural Products Research, 12: 11, 934 — 939
To link to this Article: DOI: 10.1080/10286020.2010.506434
URL: http://dx.doi.org/10.1080/10286020.2010.506434

Journal of Asian Natural Products Research
Vol. 12, No. 11, November 2010, 934–939

Two new lignan glycosides from the seeds of *Cuscuta chinensis*

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(*Received 23 April 2010; final version received 1 July 2010*)

Two new lignan glycosides, 2′-hydroxyl asarinin 2′-*O*-β-D-glucopyranoside (cuscutoside C, **1**) and 2′-hydroxyl asarinin 2′-*O*-β-D-apiofuranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside (cuscutoside D, **2**), were isolated from the seeds of *Cuscuta chinensis* Lam., along with six known compounds, 2′-hydroxyl asarinin 2′-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**), 2′-hydroxyl asarinin 2′-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside (cuscutoside A, **4**), kaempferol 3,7-di-*O*-β-D-glucopyranoside (**5**), 5-caffeoyl quinic acid (**6**), 4-caffeoyl quinic acid (**7**), and cinnamic acid (**8**). Their structures were elucidated on the basis of spectroscopic analyses including HR-ESI-MS, ESI-MS/MS, ^{1}H and ^{13}C NMR, HSQC, HMBC, and TOCSY.

Keywords: *Cuscuta chinensis* Lam.; lignan; cuscutoside C; cuscutoside D

1. Introduction

TuSiZi, which is derived from the seeds of *Cuscuta chinensis* Lam., is a commonly used traditional Chinese medicine (TCM). It was first recorded in the ancient Chinese herbal classic *ShenNong's Herbal* around 2000 years ago. TuSiZi was used as a tonic by TCM practitioners for the treatment of deficiency in kidney and liver. Modern researches have shown that it can improve sexual function, prevent and treat cardiovascular diseases, regulate the body's immune system, treat osteoporosis, and prevent senescence [1–9]. All these activities were hitherto considered to be due to flavonoids [10]. Recently, our phytochemical screening of *C. chinensis* by liquid chromatography coupled with mass spectrometry discovered a series of lignans and quinic acid derivatives [11]. Although a number of lignans had been isolated from this plant [12–14], many compounds remain to be unknown. In order to find out the relationship between bioactivities and chemical constituents, we conducted the phytochemical studies on the seeds of *C. chinensis*, particularly aiming to obtain new bioactive lignans. As a result, two new furofuran-type lignan glycosides, cuscutoside C (**1**) and cuscutoside D (**2**) (Figure 1), were isolated from an ethanol extract, together with two known lignans (2′-hydroxyl asarinin 2′-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**) [13] and 2′-hydroxyl asarinin 2′-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside or cuscutoside A (**4**) [12]), one flavonoid (kaempferol 3,7-di-*O*-β-D-glucopyranoside (**5**) [15]), two quinic acid derivatives (5-caffeoyl quinic acid (**6**) and 4-caffeoyl quinic acid (**7**) [16]), and one phenolic acid (cinnamic acid, **8**) [17]. Among them, **5**, **6**, **7**, and **8** were reported from this plant for the first time. The structures of **1** and **2** were elucidated on the basis of spectroscopic analyses including

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.506434
http://www.informaworld.com

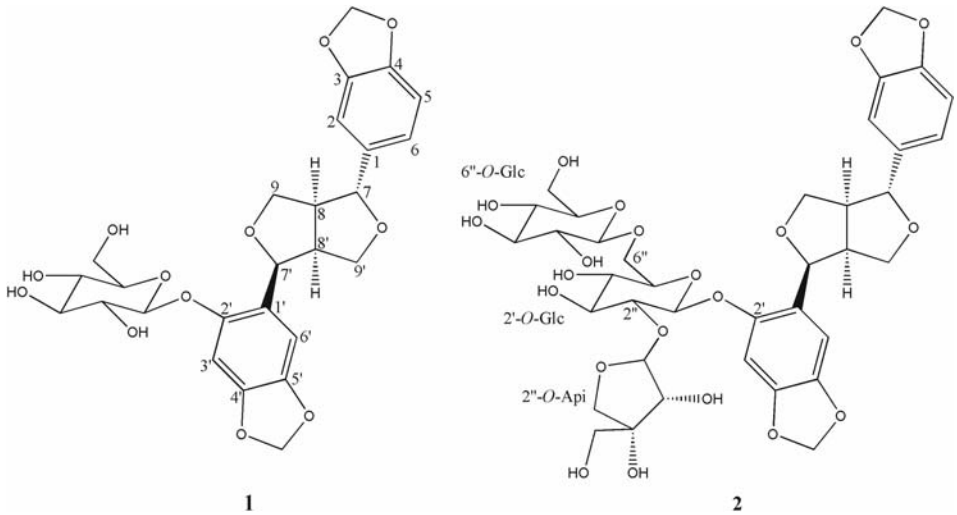

Figure 1. Structures of cuscutoside C (**1**) and D (**2**).

HR-ESI-MS, ESI-MS/MS, ^{1}H and ^{13}C NMR, HSQC, HMBC, and TOCSY.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The HR-ESI-MS spectrum showed the $[M + Na]^{+}$ ion at *m/z* 555.1481, thus its molecular formula was established as $C_{26}H_{28}O_{12}$. In the negative ESI-MS/MS spectrum, the $[M - H]^{-}$ ion at *m/z* 531 yielded a product ion at *m/z* 369 by losing 162 Da, suggesting that compound **1** contained one hexosyl residue. The ^{1}H NMR spectrum showed signals due to five aromatic protons in the region of δ_H 6.81–6.91, signals due to four methylenedioxy protons in the region of δ_H 5.90–6.10, and signals due to protons of ditetrahydrofurofuran (δ_H 2.87 (m), 3.81 (dd, $J = 3.4$, 9.0 Hz), 4.01 (dd, $J = 4.0$, 9.1 Hz), 4.14 (dd, $J = 6.4$, 6.6 Hz), 4.57 (d, $J = 5.2$ Hz), 5.08 (d, $J = 4.2$ Hz)). These signals were very similar to those of 2′-hydroxyl asarinin-type furofuran lignans [12,13]. Indeed, several 2′-hydroxyl asarinin glycosides had been reported from the seeds of *C. chinensis* [12,13]. Thus, the aglycone of compound **1** was deduced to be 2′-hydroxyl asarinin. The ^{13}C NMR spectrum also showed signals due to 2′-hydroxyl asarinin (Table 1). In addition, signals (δ_C 60.8, 69.9, 76.7, 77.1, 73.4, 101.9) due to a hexosyl residue were observed. By comparing with reported NMR spectral data [12] and by acid hydrolysis–TLC analysis, the hexose was identified as glucose. The coupling constant of the anomeric proton at δ_H 4.71 (d, $J = 7.3$ Hz) revealed that the glucosyl residue should be oriented in the β-configuration. The location of the sugar moiety was deduced to be at C-2′ (δ_C 148.8), according to the HMBC correlation between H-1″ (δ_H 4.71) of Glc and C-2′ of the aglycone (Figure 2). Based on the above analyses, the structure of **1** was elucidated as 2′-hydroxyl asarinin 2′-*O*-β-D-glucopyranoside, and was named cuscutoside C.

Compound **2** was obtained as a white amorphous powder. Based on the HR-ESI-MS data, its molecular formula was deduced as $C_{37}H_{46}O_{21}$. The ^{1}H and ^{13}C NMR spectral data indicated that **2** possessed the same 2′-hydroxyl asarinin aglycone as **1**. The only difference was the sugar moiety. In the negative ESI-MS/MS spectrum, an $[M - H]^{-}$ ion at *m/z* 825 and

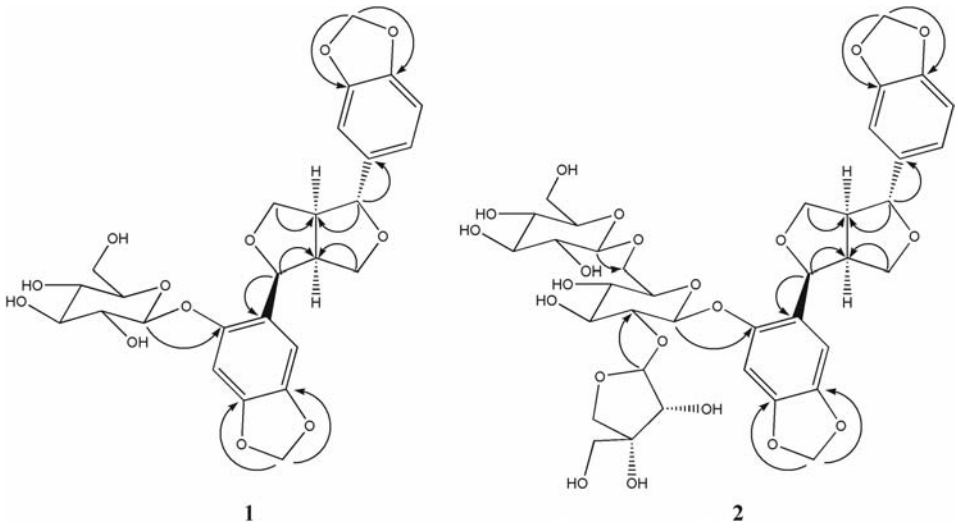

Figure 2. Key HMBC correlations of cuscutoside C (**1**) and D (**2**).

fragment ions $[M - H - hexose]^-$ at *m/z* 663, $[M - H - hexose - pentose]^-$ at *m/z* 531, and $[M - H - 2hexose - pentose]^-$ at *m/z* 369 were observed. It suggested that the sugar moiety of **2** should contain two hexosyl residues and one pentosyl residue. The exact sugar types and sequence were determined by detailed analyses of the 1D and 2D NMR spectra. Comparison of the carbon chemical shifts with reported ^{13}C NMR spectral data [12,13] and TLC analysis after acid hydrolysis allowed the identification of sugar types as Glc and Api. Proton signals due to the sugar residues were assigned according to HSQC and TOCSY spectra (Table 1). In the HMBC spectrum, correlations between H-1″ (δ_H 5.00) and C-2′ (δ_C 148.1), between H-1‴ (δ_H 4.15) and C-6″ (δ_C 69.0), and between H-1⁗ (δ_H 5.35) and C-2″ (δ_C 78.8) indicated the attachment positions of sugars to sugars and sugars to the aglycone (Figure 2). From the *J* values of H-1″ (δ_H 5.00, d, $J = 8.0$ Hz), H-1‴ (δ_H 4.15, d, $J = 8.0$ Hz), and H-1⁗ (δ_H 5.35, d, $J = 4.0$ Hz), the β-configurations of Glc and Api were suggested [18]. From the above evidences, the structure of **2** was elucidated as 2′-hydroxyl asarinin 2′-*O*-β-D-apiofuranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside, and was named cuscutoside D.

The structures of known compounds **3**–**8** were identified by comparing with the literature data.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a Rudolph Research Analytical Autopol III automatic polarimeter. Melting point were measured with an uncorrected X-4A microscope melting point apparatus (CBIO Bioscience & Technologies Co. Ltd, Beijing, China). UV spectra were recorded on a TU1901 UV–vis spectrophotometer (Beijing Purkinje General Instrument Co. Ltd, Beijing, China). IR spectra were recorded on a Nicolet NEXUS-470 spectrometer (Madison, WI, USA). 1D and 2D NMR spectra were obtained on a Bruker Avance III 400 spectrometer. HR-ESI-MS spectra were obtained on a Bruker Apex IV FT-MS spectrometer (Billerica, MA, USA). ESI-MS/MS spectra were recorded on a Finnigan LCQ Advantage ion trap mass

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data for compounds **1** and **2** (in DMSO-d_6, δ in ppm, J in Hz).

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
Aglycone				
C(1)		135.5		135.5
CH(2)	6.91 (s)	106.5	6.92 (s)	106.5
C(3)		146.5		146.5
C(4)		147.4		146.5
CH(5)	6.85 (overlapped)	107.9	6.86 (d, $J = 8.0$)	107.9
CH(6)	6.84 (overlapped)	119.3	6.83 (d, $J = 8.0$)	119.3
CH(7)	4.57 (d, $J = 5.2$)	84.3	4.54 (d, $J = 4.0$)	84.6
CH(8)	2.87 (m)	54.0	2.86 (m)	54.1
CH_2(9)	4.14 (dd, $J = 6.6$, 6.4) (H*a*)	70.8	4.12 (m) (H*a*)	70.7
	3.81 (dd, $J = 9.0$, 3.4) (H*e*)		3.80 (m) (H*e*)	
C(1′)		124.5		123.7
C(2′)		148.8		148.1
CH(3′)	6.84 (s)	98.4	6.92 (s)	97.7
C(4′)		146.4		146.5
C(5′)		141.7		141.5
CH(6′)	6.81 (s)	104.6	6.78 (s)	104.6
CH(7′)	5.08 (d, $J = 4.2$)	81.1	5.05 (d, $J = 4.0$)	80.5
CH(8′)	2.87 (m)	54.0	2.86 (m)	54.0
CH_2(9′)	4.14 (dd, $J = 6.6$, 6.4) (H*a*)	72.4	4.19 (m) (H*a*)	72.5
	4.01 (dd, $J = 9.1$, 4.0) (H*e*)		3.95 (m) (H*e*)	
O—CH_2—O	5.99 (s), 5.95 (d, $J = 11.4$)	100.8	5.94 (d, $J = 8.0$), 5.99 (s)	100.8
		101.0		100.9
2′-O-Glc				
CH(1″)	4.71 (d, $J = 7.3$)	101.9	5.00 (d, $J = 8.0$)	98.4
CH(2″)	3.21 (m)	73.4	3.47 (m)	78.8
CH(3″)	3.29 (m)	77.1	3.01 (m)	77.0
CH(4″)	3.14 (m)	69.9	3.17 (m)	70.1
CH(5″)	3.21 (m)	76.7	3.68 (m)	75.1
CH_2(6″)	3.43 (m), 3.72 (d, $J = 11.3$)	60.8	3.60 (d, $J = 4.0$), 3.94 (d, $J = 4.0$)	69.0
6″-O-Glc				
CH(1‴)			4.15 (d, $J = 8.0$)	103.5
CH(2‴)			2.95 (m)	73.5
CH(3‴)			3.79 (m)	76.7
CH(4‴)			3.02 (m)	70.1
CH(5‴)			3.10 (m)	76.9
CH_2(6‴)			3.64 (d, $J = 8.0$), 3.40 (m)	61.1
2″-O-Api				
CH(1⁗)			5.35 (d, $J = 4.0$)	109.2
CH(2⁗)			3.80 (m)	76.2
CH(3⁗)			3.47 (m)	78.0
CH_2(4⁗)			3.31 (d, $J = 8.0$)	63.4
CH_2(5⁗)			3.57 (m), 3.86 (m)	73.5

spectrometer (ThermoFinnigan, San Jose, CA, USA). Semi-preparative HPLC was conducted on a LabAlliance Series 1500 pump equipped with a LabAlliance Model 500 variable wavelength detector and an Agilent Zorbax SB-C18 semi-preparative column (9.4 × 250 mm, 5 μm). Sephadex LH-20 (GE Healthcare Bio-Sciences

Corporation, formerly Amersham Pharmacia Biotechnology Incorporation, NJ, USA), polyamide (80–100 mesh; Linjiang Reagent Chemical Corporation, Jiangsu, China), and ODS (YMC Company, Ltd, Kyoto, Japan) were used for column chromatography (CC). TLC were performed on silica gel plates (Branch of Qingdao Haiyang Chemical Plant, Qingdao, China) and polyamide sheets (Taizhou Si-Jia Biochemical Plastic Company, Zhejiang, China) with spots examined in the UV light at 254 nm.

3.2 Plant material

The seeds of *C. Chinensis* Lam. were collected in Beijing in fall 2000 and were identified by Dr Min Ye. A voucher specimen (No. 20000901) has been deposited at the Pharmacognosy Biotechnology Laboratory, School of Pharmaceutical Sciences, Peking University, China.

3.3 Extraction and isolation

Air-dried seeds of *C. Chinensis* (5 kg) were pulverized and sieved (40 mesh), defatted with petroleum ether under reflux, and then extracted with 95% ethanol under reflux for three times (2 h for each time). The extract was concentrated in a rotary vacuum evaporator to give 600 g of crude residue. The residue was dispersed in hot water and then extracted with $CHCl_3$. The water layer afforded 500 g of the residue which was subjected to a polyamide column and eluted with aqueous ethanol (0% → 40%, v/v) to give eight fractions (Fr.1–8). Fr.7 (7.5 g) was subjected to an ODS CC (eluted with gradient aqueous methanol, 10% → 100%, v/v) and yielded 10 fractions (Fr.7.1–7.10). Fr.7.8 was then subjected to semi-preparative HPLC and eluted with aqueous methanol (45%, v/v, 2 ml/min) to afford **2** (35 mg), **3** (140 mg), and **4** (36 mg). Fr.7.5 was subjected to Sephadex LH-20 CC and eluted with methanol to afford **5** (30 mg). Fr.7.2 was subjected to semi-preparative HPLC and eluted with aqueous acetonitrile (10%, v/v, 2 ml/min) to afford **6** (6 mg) and **7** (20 mg). Fr.5 (12 g) was also subjected to ODS CC (eluted with gradient aqueous methanol, 10% → 100%, v/v) to give six fractions (Fr.5.1–5.6). Fr.5.6 was then applied to semi-preparative HPLC and eluted with aqueous acetonitrile (33%, v/v, 2 ml/min) to give **1** (60 mg) and **8** (5 mg).

3.3.1 Cuscutoside C (*1*)

White amorphous powder. mp 112–113°C. $[\alpha]_D^{20}$ −73.5 ($c = 0.067$, MeOH). UV (MeOH) λ_{max} (nm): 291.0, 237.0. IR (KBr) ν_{max} (cm^{-1}): 3383, 2883, 1630, 1503, 1485, 1440, 1400, 1347, 1249, 1180, 1074, 1036, 931, 864, 810, 717, 559. ^1H and ^{13}C NMR spectral data: see Table 1. HR-ESI-MS: *m/z* 555.1481 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_{12}Na$, 555.1473).

3.3.2 Cuscutoside D (*2*)

White amorphous powder. mp 153–154°C. $[\alpha]_D^{20}$ −99.0 ($c = 0.067$, MeOH). UV (MeOH) λ_{max} (nm): 291.5, 237.0. IR (KBr) ν_{max} (cm^{-1}): 3394, 2884, 1705, 1632, 1503, 1485, 1440, 1249, 1179, 1068, 1037, 931, 875, 813, 717, 560. ^1H and ^{13}C NMR spectral data: see Table 1. HR-ESI-MS: *m/z* 849.2417 $[M + Na]^+$ (calcd for $C_{37}H_{46}O_{21}Na$, 849.2424).

3.4 Acid hydrolysis of 1 and 2

Compounds **1** (3 mg) and **2** (5 mg) were heated in 2 ml of 2 M CF_3COOH at 90°C for 12 h, respectively. The reaction mixture was then diluted with 2 ml H_2O and extracted with $CHCl_3$ (4 ml × 3). The water layer was evaporated to dryness and then dissolved with 1 ml of MeOH. The solutions were then subjected to silica gel TLC together with authentic D-Glc and D-Api samples, and developed using *n*-BuOH–Me_2CO–H_2O (4:5:1) as the solvent. The R_f values of D-Glc and D-Api were 0.63 and 0.80, respectively.

Acknowledgement

We are grateful for the financial support from the Peking University Health Science Center (No. 985-2-119-121).

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