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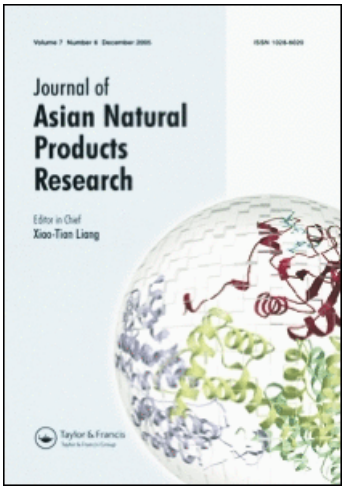

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Chemical constituents of *Cistanche sinensis*

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Chemical constituents of *Cistanche sinensis*

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One new phenylethanoid glycoside, cistansinenside A and one new oligosaccharide, cistansinensose A_1/A_2, were isolated from the stems of *Cistanche sinensis*, together with six known compounds. The structures of the new compounds were elucidated on the basis of spectral data.

Keywords: *Cistanche sinensis*; Orobanchaceae; Phenylethanoid glycoside; Oligosaccharide; Cistansinenside A; Cistansinensose A_1/A_2

1. Introduction

Herba Cistanches, the stems of *Cistanche* species (Orobanchaceae), is a tonic in traditional Chinese medicines and is used for the treatment of impotence, female infertility, morbid leukorrhea, profuse metrorrhagia, cold sensation in the loins and knees, and chronic constipation in the aged [1]. A number of constituents including phenylethanoid glycosides, iridoid glycosides and lignan glycosides have been isolated from the *Cistanche* plants [2]. Chemical analysis by HPLC showed that constituents of *Cistanche sinensis* were different from other *Cistanche* species [3]. To our knowledge, no phytochemical investigation on this plant has been reported. We have investigated the stems of *Cistanche sinensis* and isolated one new phenylethanoid glycoside, cistansinenside A (**1**) and one new oligosaccharide, cistansinensose A_1/A_2 (**2**) (figure 1), along with 2′-acetylacteoside (**3**) [4], jionoside D (**4**) [5], poliumoside (**5**) [6], 8-epiloganin (**6**) [7], geniposide (**7**) [8], galactitol (**8**) [9]. Compounds **3**–**8** were obtained from this plant for the first time. In this paper, we report the isolation and structural elucidation of these compounds.

2. Results and discussion

Cistansinenside A (**1**) was obtained as an amorphous light-yellowish powder. Its HRSI-MS exhibited a pseudomolecular ion $[M-H]^-$ at *m/z* 679.2227 (calcd. for $C_{32}H_{39}O_{16}$, 679.2243),

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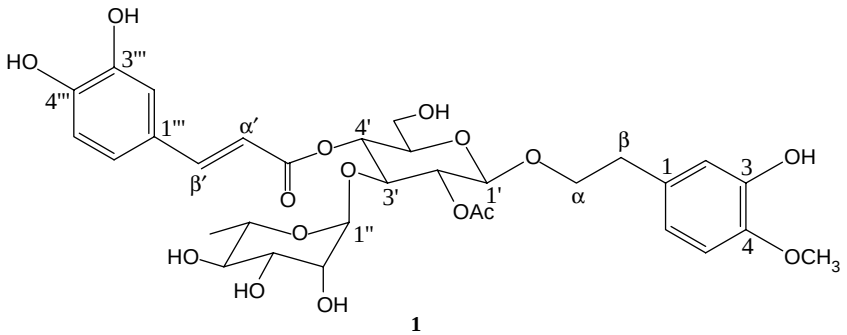

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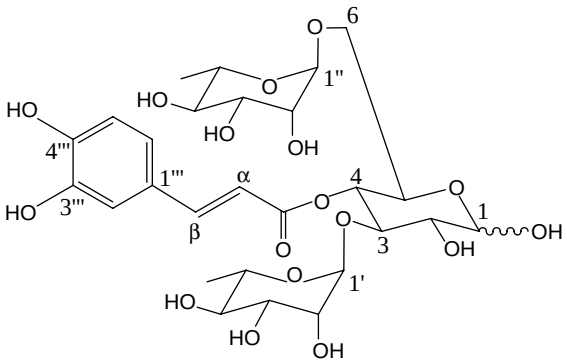

2a / 2b

Figure 1. Structures of compounds **1** and **2a/2b**.

compatible with the molecular formula $C_{32}H_{40}O_{16}$. The IR spectrum showed absorption bands typical of hydroxyls (3416 cm^{-1}), carbonyl (1720 cm^{-1}), α, β-unsaturated ester (1693, 1624 cm^{-1}), and aromatic rings (1599, 1510 cm^{-1}). The ^1H NMR of **1** (table 1) exhibited characteristic proton signals of an *E*-caffeoyl group [three aromatic protons at δ 7.03 (*d*, $J = 1.5$ Hz), 6.94 (*dd*, $J = 8.5$, 1.5 Hz) and 6.76 (*d*, $J = 8.5$ Hz) as an ABX system, two *trans* olefinic protons as an AB system at δ 7.59 (*d*, $J = 16.0$ Hz) and 6.26 (*d*, $J = 16.0$ Hz)] and a 3,4-dihydroxyphenylethanol moiety (three aromatic protons at δ 6.81, 6.67 and 6.63 as an ABX system, a multiplet at δ 2.72 due to a β-methylene and two non-equivalent protons at δ 4.09 and 3.66 of the side chain of the aglycone moiety). Additionally, two signals assignable to anomeric protons indicated the presence of two sugar moieties in **1**: a doublet at δ 4.35 ($J = 8.0$ Hz, H-1′ of β-D-glucose), a broad singlet at δ 4.79 (H-1″ of α-L-rhamnose). The ^{13}C NMR spectroscopic data (table 1) confirmed the diglycosidic sugar chain in **1**, exhibiting two anomeric carbon resonances at δ 101.7 and 103.3. These results were supported by the acidic hydrolysis of **1** yielding glucose and rhamnose. Furthermore, the ^1H and ^{13}C NMR spectroscopic data (table 1) showed the presence of a methoxyl group (δ_H 3.81, s; δ_C 56.5) and an acetyl group (δ_H 1.98, s; δ_C 20.9, 171.4). The complete assingnments of all proton and carbon data were based on the ^1H–H COSY, HMQC and HMBC experiments. All above data suggested that the structure of **1** is closely related to that of **3**, with the exception of a singlet at δ 3.81 (3H, s) due to a methoxyl group. The position of the methoxyl group was assigned to C-4 on the basis of HMBC correlations between C-4 (δ 147.5) and protons of the methoxyl group (δ 3.81). This was

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Table 1. ^{1}H and ^{13}C NMR data for compound **1** (500 MHz for ^{1}H and 125 MHz for ^{13}C, methanol-d_4)[a].

Position	δ_H	δ_C	*Position*	δ_H	δ_C
Aglycone			*Rhamnose*		
1		133.2	1″	4.79 (br*s*)	103.3
2	6.67 (*d*, 2.0)	117.1	2″	3.63 (*m*)	72.6
3		147.3	3″	3.52 (*m*)	71.6
4		147.5	4″	3.25 (*m*)	73.6
5	6.81 (*d*, 8.5)	112.8	5″	3.50 (*m*)	70.6
6	6.63 (*dd*, 8.5, 2.0)	121.1	6″	1.06 (*d*, 6.0)	18.5
α	3.66, 4.09 (*m*)	71.8	*Ester*		
β	2.72 (*m*)	36.3	1‴		127.4
OCH_3	3.81 (*s*)	56.5	2‴	7.03 (*d*, 1.5)	115.1
Glucose			3‴		147.0
1′	4.35 (*d*, 8.0)	101.7	4‴		150.3
2′	4.87 (*m*)	75.1	5‴	6.76 (*d*, 8.5)	116.5
3′	4.01 (*m*)	80.5	6‴	6.94 (*dd*, 8.5, 1.5)	123.3
4′	4.99 (*m*)	70.8	α′	6.26 (*d*, 16.0)	114.4
5′	3.75 (*m*)	76.2	β′	7.59 (*d*, 16.0)	148.2
6′	3.49, 3.67 (*m*)	62.2	CO		168.1
COCH_3		171.4			
CO*C*H_3	1.98 (*s*)	20.9			

[a] Assignments confirmed by ^{1}H–^{1}H COSY, HMQC and HMBC spectra.

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confirmed by NOE correlations between the methoxyl group and H-5. Finally, HMBC experiment permitted the further confirmation of all the relevant interfragmental connectivities (figure 2). Thus, cross-peaks were observed between α-C atom (δ 71.8) of the phenylethanol moiety and the H-1′ (δ 4.35) of the glucose, C-3′ (δ 80.5) of the glucose and H-1″ (δ 4.79) of the rhamnose, the carbonyl carbon (δ 168.1) of the caffeoyl moiety and H-4′ (δ 4.99) of the glucose, the carbonyl carbon (δ 171.4) of the acetyl group and H-2′ (δ 4.87) of the glucose. Therefore, the structure of **1** was established as β-(3-hydroxy-4-methoxyphenyl)ethyl-*O*-(2-*O*-acetyl)-α-L-rhamnopyranosyl-(1 → 3)-4-*O*-*E*-caffeoyl-β-D-glucopyranoside, named Cistansinenside A.

Cistansinensose A_1/A_2 (**2a/2b**) was obtained as an amorphous light-yellowish powder with a molecular formula of $C_{27}H_{38}O_{17}$, as determined by data from negative-ion HRSI-MS, showing an $[M-H]^-$ ion at *m/z* 633.2022 (calcd. for $C_{27}H_{37}O_{17}$, 633.2036) and ^{13}C NMR. The IR spectrum showed absorption bands typical of hydroxyls (3411 cm^{-1}), α, β-unsaturated ester (1689, 1632 cm^{-1}), and aromatic rings (1602, 1514 cm^{-1}). The ^{1}H NMR

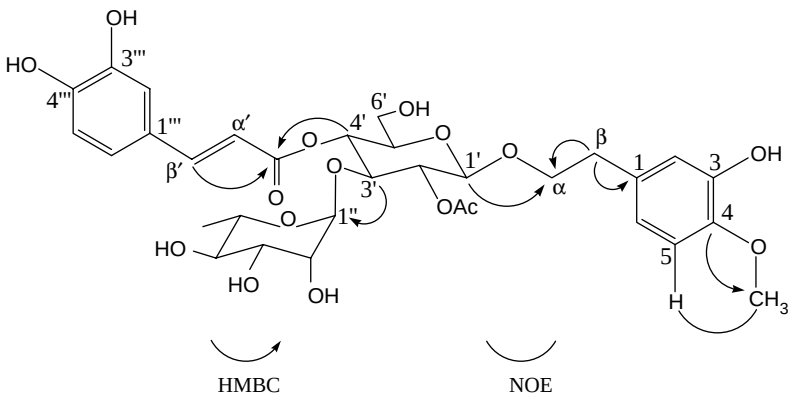

Figure 2. Selected HMBC and NOE correlations for Cistansinenside A (**1**).

Table 2. ^{1}H and ^{13}C NMR data for compound **2a/2b** (500 MHz for ^{1}H and 125 MHz for ^{13}C, DMSO-d_6)[a].

Position	δ_H	δ_C	*Position*	δ_H	δ_C
Glucose			*Rhamnose′*		
1	4.96 (brs)/4.46 (*d*, 7.0)	92.4/96.5	1″	4.44 (*s*)/4.46 (*s*)	100.1/100.3
2	3.39 (*m*)/3.11 (*m*)	72.1/75.8	2″	3.58 (*m*)	70.6
3	3.90 (*m*)/3.70 (*m*)	75.8/78.8	3″	3.38 (*m*)	70.2
4	4.72 (*m*)/4.68 (*m*)	69.0/69.1	4″	3.13 (*m*)	71.7
5	3.98 (*m*)/3.62 (*m*)	68.6/72.8	5″	3.29 (*m*)	68.4
6	3.24, 3.49 (*m*)/ 3.23, 3.48 (*m*)	65.6/65.7	6″	1.07 (*d*, 6.0)	17.8
Rhamnose			*Ester*		
1′	5.00 (*s*)/5.03 (*s*)	101.2/101.0	1‴		125.5/125.6
2′	3.68 (*m*)/3.65 (*m*)	70.6	2‴	7.03 (*d*, 1.5)	114.7
3′	3.27 (*m*)	70.5	3‴		145.6
4′	3.10 (*m*)	71.8/71.9	4‴		148.5/148.6
5′	3.35 (*m*)	68.7/68.8	5‴	6.76 (*d*, 7.5)	115.8
6′	0.96 (*d*, 6.0)	18.2	6‴	6.98 (*dd*, 7.5, 1.5)	121.6
			α	6.21 (*d*, 16.0)	113.3/113.5
			β	7.48 (*d*, 16.0)	145.6/145.7
			CO		165.7

[a] Assignments confirmed by $^{1}H-^{1}H$ COSY, HMQC and HMBC spectra.

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spectrum (table 2) showed the presence of one pair of ABX system protons at δ 7.03 (2H, *d*, $J = 1.5$ Hz), 6.98 (2H, *dd*, $J = 7.5$, 1.5 Hz), 6.76 (2H, *d*, $J = 7.5$ Hz), one pair *trans* olefinic protons at δ 7.48 (2H, *d*, $J = 16.0$ Hz), 6.21 (2H, *d*, $J = 16.0$ Hz). Furthermore, two glucose anomeric protons [δ 4.96 (1H, brs), 4.46 (1H, *d*, $J = 7.0$ Hz)], four rhamnose anomeric protons [δ 5.03, 5.00, 4.46, 4.44 (1H, each, brs)] and two pairs of overlapped secondary methyl groups of rhamnose [δ 1.07 (6H, *d*, $J = 6.0$ Hz), 0.96 (6H, *d*, $J = 6.0$ Hz)] can be observed. Meanwhile, the ^{13}C NMR spectrum (table 2) showed the presence of two pairs chemical shifts for some carbons (such as C-1–C-6 of glucose moiety and C-1‴, C-4‴, C-α, C-β of ester). The above spectroscopic data, together with two adjacent peaks in HPLC suggested that **2a/2b** might be a pair of anomeric epimers at C-1 of the glucose (α/β) moiety with a ratio of 1:1. The two epimers (**2a/2b**) were isolated by further preparative HPLC, but it was not possible to separate one from the other due to its interconversion. This phenomenon has already been described in cistanoside F [10]. Comparison of ^{1}H and ^{13}C NMR spectral data with those of cistanoside F revealed that the aglycon of the two compounds were identical, indicating that compound **2a/2b** was a caffeoyl trisaccharide glycoside with one glucose and two rhamnose units. Finally, the HMBC experiment permitted the confirmation of all the relevant interfragmental connectivities. Thus, cross-peaks were observed between C-1′ (δ 101.2, 101.0) of the rhamnose and H-3 (δ 3.90, 3.70) of the glucose, C-1″ (δ 100.3, 100.1) of the rhamnose′ and H-6 (δ 3.24, 3.49; 3.23, 3.48) of the glucose, the carbonyl carbon (δ 165.7) of the caffeoyl moiety and H-4 (δ 4.72, 4.68) of the glucose. Consequently, **2a/2b** was assigned as α-L-rhamnopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 6)]-(4-*O*-*E*-caffeoyl)-D- glucopyranoside, named Cistansinensose A_1/A_2.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a PERKIN-ELMER 243B digital polarimeter. UV spectra were measured with a SHIMADZU spectrometer. IR spectra were determined using

a NICOLET AVATAR 360 FTIR spectrometer. NMR spectra were recorded in methanol-d_4 or DMSO-d_6 using a Bruker AM-500 spectrometer with tetramethylsilane as internal standard. The HR SI-MS were measured on a Bruker APEX II FTCR spectrometer in negative ion mode. Preparative HPLC was performed on a Waters model 600 instrument (Waters ODS, 7.8 i.d. × 300 mm, Waters CO. Ltd, America, flow rate, 2.5 ml/min, detected at UV 330 nm). GC analysis was carried out on an Angilent 6890N gas chromatograph using a HP-5 capillary column (28m × 0.32 mm i.d.); detection, FID; detector temperature, 260°C; column temperature, 180°C; carrier gas, N_2; flow rate, 40 mL/min. Diaion HP-20 (Mitsubishi Chemical Industries), Silica gel (Qingdao Ocean Chemical Corporation, Qingdao, China), ODS (100–200 mesh, Fuji Sylisia Chemical, Ltd, Aichi, Japan) were used for column chromatography.

3.2 Plant material

Stems of *Cistanche sinensis* G. Beck were collected in May 1997 at Yanchi, Ningxia Hui autonomous region, People's Republic of China. The identification of the plant was performed by one of authors (Prof. Peng-fei Tu). A voucher specimen (No. 19970509) is kept in the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

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3.3 Extraction and isolation

Dried powdered stems of *C. sinensis* (3.1 kg) were extracted with 95% EtOH(12L × 3) under reflux. The EtOH extract was suspended in H_2O and successively extracted with petroleum ether, EtOAc and *n*-BuOH, to yield petroleum ether (32.0 g), EtOAc(209.0 g), *n*-BuOH(126.5 g), and H_2O extracts(250.0 g), respectively. The EtOAc extract(160.0 g) was subjected to silica-gel CC, eluting with $CHCl_3$–MeOH system to give Frs. 1–10. The fr. 3 (1.0 g) was subjected to Sephadex LH-20 CC eluting with MeOH-H_2O(1:1), then purified by PTLC [MeOH-H_2O (5:1)] to give geniposide (**7**) (150 mg). The fr. 5 (12.0 g) was subjected to Sephadex LH-20 CC eluting with MeOH-H_2O(1:1), then purified by HPLC [MeOH-H_2O (45:55)] to give cistansinenside A (**1**) (6.0 mg). The fr. 6 (5.0 g) was subjected to Sephadex LH-20 CC eluting with MeOH-H_2O(3:7), then purified by PTLC [MeOH-H_2O (5:1)] to give 8-epiloganin (**6**) (100 mg). The fr. 7 (8.0 g) was subjected to polyamide CC with MeOH-H_2O(4:6) as eluent and purified by Sephadex LH-20 CC with MeOH-H_2O(2:3) to yield 2′-acetylacteoside (**3**) (150 mg) and jionoside D (**4**) (50 mg). The fr. 10(10.0 g) was chromatographied on polyamide CC with MeOH-H_2O (4:6) and purified by Sephadex LH-20 CC with MeOH-H_2O(2:3) to provide poliumoside (**5**) (150 mg). The H_2O extract (150 g) was subjected to Diaion HP-20 and eluted with H_2O, 10% EtOH, 30% EtOH, 50% EtOH and 70% EtOH, successively. The fraction eluted with 10% EtOH was subjected to Sephadex LH-20 CC with MeOH–H_2O(3:7) to give six subfractions (Subfr. 1–6). The subfr. 5 was subjected to prep. HPLC [MeOH–H_2O (18:82)] to afford cistansinensose A_1/A_2 (**2**) (100 mg). The fraction eluted with H_2O was concentrated and recrystallized to yield galactitol (**8**) (1.0 g).

3.3.1 Cistansinenside A. (**1**), amorphous light-yellowish powder; $[\alpha]_D^{20} - 32.2$ (*c* 0.1, MeOH); UV (MeOH)λ_{max} 330, 212 nm; IR (KBr) v_{max} 3416, 2927, 1720, 1693, 1624, 1599, 1510, 1039, 810 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) see table 1; ^{13}C NMR

(methanol-d_4, 125 MHz) see table 1. HR SI-MS *m/z* 679.2227 $[M-H]^-$ (calcd for $C_{32}H_{39}O_{16}$, 679.2243).

3.3.2 Cistansinensose A_1/A_2. (**2a/2b**), amorphous light-yellowish powder; $[\alpha]_D^{20}$ −25.2 (*c* 0.1, MeOH); UV (MeOH)λ_{max} 328, 210 nm; IR (KBr) v_{max} 3411, 2919, 1689, 1632, 1602, 1514, 1446, 1046, 811 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see table 2; ^{13}C NMR (DMSO-d_6, 125 MHz) see table 2. HR SI-MS *m/z* 633.2022 $[M-H]^-$ (calcd for $C_{27}H_{37}O_{17}$, 633.2036).

3.4 Acid hydrolysis of compounds 1 and 2a/2b

Each compound (3 mg) was heated in 3 ml of 10% HCl-dioxane (1:1) at 80°C for 4 h. After the dioxane was removed, the solution was extracted with EtOAc (3 ml × 3) to yield the sugar and the aglycone, respectively.

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3.5 Sugar analyses by TLC and GC

The sugar components in the aqueous layer left after hydrolysis were analyzed using silica gel TLC by comparison with the standard sugars. The solvent system was $CHCl_3$–MeOH–H_2O (8:5:1), and spots were visualized by spraying with 95% EtOH–H_2SO_4-anisaldehyde (9:0.5:0.5, v/v), then heated at 120°C for 10 min. For sugars of **1** and **2a/2b**, the R_f of glucose and rhamnose in TLC was 0.30 and 0.50, respectively. The results were confirmed by GC analyses of methyl sugar peracetates. The aqueous layer was evaporated, dissolved in anhydrous pyridine (100 μl), 0.1M L-cysteine methyl ester hydrochloride (200 μl) was added, and the mixture was warmed at 60°C for 1 h. The trimethysilylation reagent HMDS-TMCS (hexamethyldisilazane-trimethylchloro- silane-pyridine, 2:1:10) (Acros Organics, Belgium) was added and warmed at 60°C for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. The retention times of D-glucose (t_R, 12.45 min) and L-rhamnose (t_R, 5.32 min) were confirmed with those of authentic standards [11].

Acknowledgements

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