Arylethyl (=Phenylethanoid) Glycosides and Oligosaccharide from the Stem of *Cistanche tubulosa*

by **Peng-Fei Tu***, **Zhi-Hong Song**, **Hai-Ming Shi**, **Yong Jiang**, and **Yu-Ying Zhao**

Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center, No. 38 Xueyuan Road, Haidian District, Beijing 100083, P. R. China (phone/fax: +86-10-82802750; e-mail: pengfeitu@bjmu.edu.cn)

Three new analogues of the arylethyl (=phenylethanoid) glycoside echinacoside (**5**), namely cistantubulosides A (**1**), B_1/B_2 (**2a**/**2b**), and C_1/C_2 (**3a**/**3b**), and one new oligosaccharide, cistantubulose A_1/A_2 (**4a**/**4b**), were isolated from the stems of *Cistanche tubulosa* (SCHENK) R. WIGHT, together with five known compounds, *i.e.*, campneoside I, campneoside II (**6**), echinacoside (**5**), tubuloside, and cistanoside F. Among the compounds isolated, **2a**/**2b**, **3a**/**3b**, **4a**/**4b**, campneoside I, campneoside II (**6**), and cistanoside F each consisted of a pair of epimers. The structures of the new compounds were elucidated on the basis of spectral data.

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]. Recent pharmacological studies have shown that arylethyl (=phenylethanoid) glycosides from the genus *Cistanche* displayed neuroprotective activities [2][3]. A number of constituents including arylethyl (=phenylethanoid) glycosides, iridoid glycosides, and lignan glycosides have been isolated from the genus *Cistanche* [4]. There are four species and one variation of *Cistanche* plants found in China, and only the dried fleshy stems of *Cistanche deserticola* Y. C. MA has been recorded in the Chinese Pharmacopoeia (2000 edition). Due to the deficiency of natural resources, other species of the genus *Cistanche* are used as substitutes throughout the country. *Cistanche tubulosa* (SCHENK) R. WIGHT is widely distributed in the southern part of the Xinjiang Uigur Autonomous Region with an abundant yield. To exploit a new natural source for Herba Cistanches, we undertook a systematic chemical study of *C. tubulosa*. Analysis by HPLC showed that arylethyl glycosides are the major constituents of *C. tubulosa* (SCHENK) R. WIGHT [5]. We have previously reported on the constituents from this species [6][7]. In continuation of this research, we now describe the isolation from *C. tubulosa* of seven arylethyl glycosides, of which **1**–**3** are new, and of two oligosaccharides, of which **4** is new, and the structure elucidation of four new compounds (*Fig. 1*).

Results and Discussion. – Cistantubuloside A (**1**) was obtained as an amorphous light-yellowish powder. Its HR-SI-MS exhibited a pseudomolecular ion $[M-H]^-$ at m/z 769.2559, compatible with the molecular formula $C_{35}H_{46}O_{19}$. The IR spectrum showed absorption bands typical of OH groups (3397 cm^{-1}), an α,β-unsaturated ester

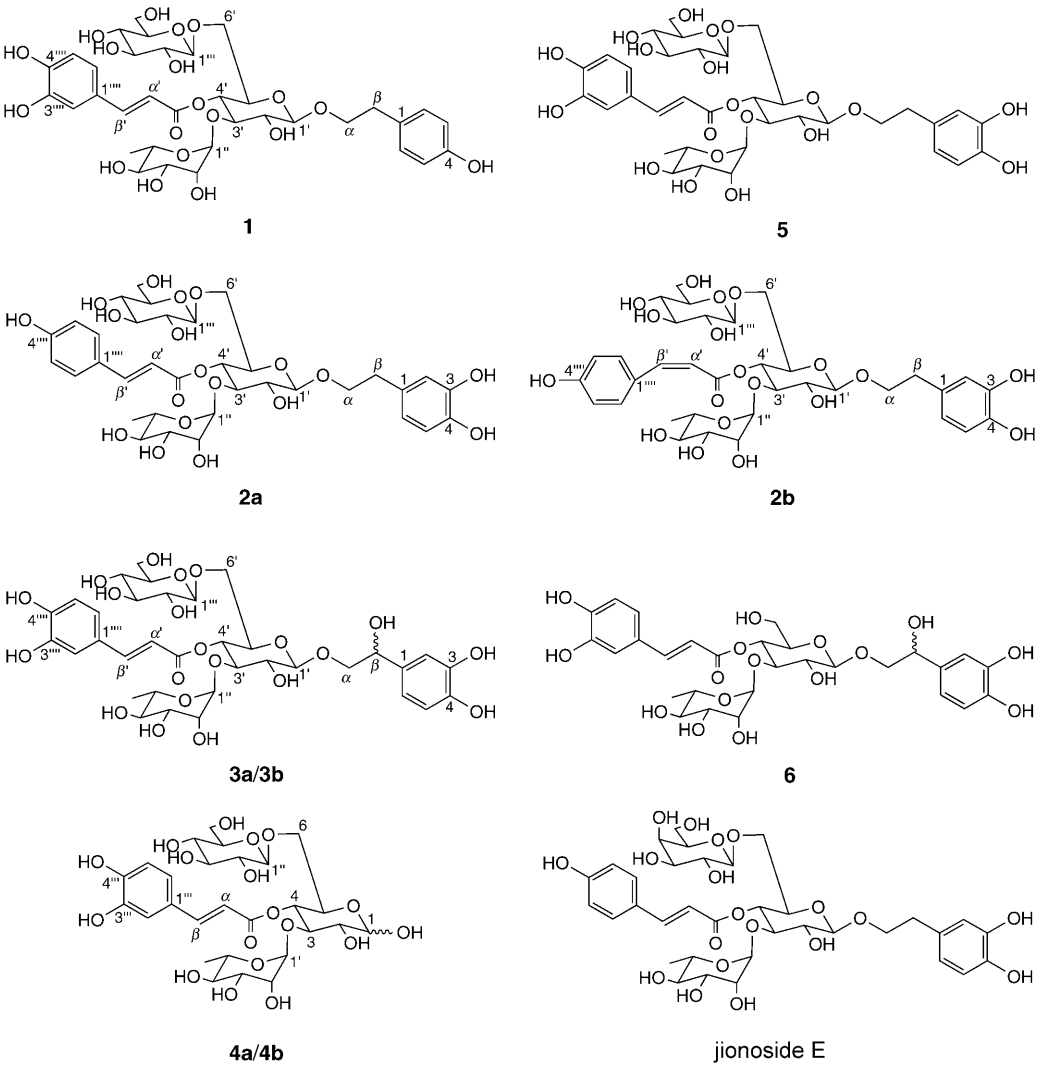

Fig. 1. *Structures of compounds* **1**, **2a**/**2b**, **3a**/**3b**, **4a**/**4b**, **5**, **6** *and* jionoside E

(1692, 1630 cm^{-1}), and aromatic rings (1596, 1512 cm^{-1}). On the basis of spectral evidence, mainly 2D-NMR, the structure of compound **1** was elucidated as 2-(4-hydroxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[β-D-glucopyranosyl-(1 → 6)]-4-*O*-[(*E*)-caffeoyl]-β-D-glucopyranoside, named cistantubuloside A.

The ^{1}H-NMR of **1** (*Table 1*) exhibited characteristic proton signals of an (*E*)-caffeoyl group, *i.e.*, three aromatic protons at δ 7.03 (*d*, *J* = 1.3 Hz), 6.99 (*dd*, *J* = 8.1, 1.3 Hz), and 6.76 (*d*, *J* = 8.1 Hz) as an *ABX* system and two *trans*-positioned olefinic protons as an *AX* system at δ 7.47 (*d*, *J* = 15.9 Hz) and 6.21 (*d*, *J* = 15.9 Hz), and a (4-hydroxyphenyl)ethyl moiety, *i.e.*, a pair of aromatic protons at δ 7.06 and 6.67 as an *AA′XX′* system, a broad *t* at δ 2.76 due to $CH_2(\beta)$ and two nonequivalent protons at

Table 1. *^{1}H-NMR Data for Compounds* **1** *and* **2a**/**2b**[a]). δ in ppm, J in Hz.

	1		**2a**/**2b**
Aglycone:		*Aglycone:*	
H–C(2)	7.06 (*d*, *J* = 8.4)	H–C(2)	6.64 (*d*, *J* = 1.5)
H–C(3)	6.67 (*d*, *J* = 8.4)		
H–C(5)	6.67 (*d*, *J* = 8.4)	H–C(5)	6.63 (*d*, *J* = 8.0)
H–C(6)	7.06 (*d*, *J* = 8.4)	H–C(6)	6.51 (*dd*, *J* = 8.0, 1.5)
$CH_2(\alpha)$	3.88–3.94 (*m*), 3.61–3.66 (*m*)	$CH_2(\alpha)$	3.85–3.93 (*m*), 3.59–3.66 (*m*)
$CH_2(\beta)$	2.76 (br. *t*, *J* = 7.4)	$CH_2(\beta)$	2.67–2.72 (*m*)
Inner glucose:		*Inner glucose:*	
H–C(1′)	4.37 (*d*, *J* = 7.8)	H–C(1′)	4.37 (*d*, *J* = 7.8)/4.36 (*d*, *J* = 7.8)
H–C(2′)	3.21 (*t*, *J* = 8.4)	H–C(2′)	3.19–3.25 (*m*)/3.22–3.29 (*m*)
H–C(3′)	3.68–3.75 (*m*)	H–C(3′)	3.68–3.75 (*m*)/3.64–3.72 (*m*)
H–C(4′)	4.68–4.72 (*m*)	H–C(4′)	4.68–4.76 (*m*)/4.60–4.66 (*m*)
H–C(5′)	3.70–3.76 (*m*)	H–C(5′)	3.70–3.77 (*m*)/3.68–3.75 (*m*)
CH_2(6′)	3.46–3.53 (*m*), 3.61–3.71 (*m*)	CH_2(6′)	3.46–3.54 (*m*), 3.62–3.71 (*m*)
Rhamnose:		*Rhamnose:*	
H–C(1″)	5.03 (br. *s*)	H–C(1″)	5.03 (br. *s*)
H–C(2″)	3.65–3.72 (*m*)	H–C(2″)	3.65–3.71 (*m*)
H–C(3″)	3.24–3.32 (*m*)	H–C(3″)	3.26–3.33 (*m*)
H–C(4″)	3.08–3.14 (*m*)	H–C(4″)	3.06–3.13 (*m*)
H–C(5″)	3.34–3.41 (*m*)	H–C(5″)	3.32–3.38 (*m*)/3.36–3.43 (*m*)
Me(6″)	0.96 (*d*, *J* = 6.1)	Me(6″)	0.95 (*d*, *J* = 6.1)/1.04 (*d*, *J* = 6.1)
Outer glucose:		*Outer glucose:*	
H–C(1‴)	4.18 (*d*, *J* = 7.7)	H–C(1‴)	4.16 (*d*, *J* = 7.6)/4.17 (*d*, *J* = 7.6)
H–C(2‴)	2.94 (*t*, *J* = 8.2)	H–C(2‴)	2.91–2.96 (*m*)
H–C(3‴)	3.07–3.13 (*m*)	H–C(3‴)	3.04–3.12 (*m*)
H–C(4‴)	2.98–3.05 (*m*)	H–C(4‴)	3.00–3.08 (*m*)
H–C(5‴)	2.96–3.03 (*m*)	H–C(5‴)	2.99–3.06 (*m*)
CH_2(6‴)	3.35–3.43 (*m*), 3.57–3.66 (*m*)	CH_2(6‴)	3.35–3.44 (*m*), 3.59–3.68 (*m*)
Ester:		*Ester:*	
H–C(2⁗)	7.03 (*d*, *J* = 1.3)	H–C(2⁗)	7.53 (*d*, *J* = 8.5)/7.69 (*d*, *J* = 8.6)
		H–C(3⁗)	6.80 (*d*, *J* = 8.5)/6.76 (*d*, *J* = 8.6)
H–C(5⁗)	6.76 (*d*, *J* = 8.1)	H–C(5⁗)	6.80 (*d*, *J* = 8.5)/6.76 (*d*, *J* = 8.6)
H–C(6⁗)	6.99 (*dd*, *J* = 8.1, 1.3)	H–C(6⁗)	7.53 (*d*, *J* = 8.5)/7.69 (*d*, *J* = 8.6)
H–C(α')	6.21 (*d*, *J* = 15.9)	H–C(α')	6.35 (*d*, *J* = 15.9)/5.71 (*d*, *J* = 12.8)
H–C(β')	7.47 (*d*, *J* = 15.9)	H–C(β')	7.56 (*d*, *J* = 15.9)/6.94 (*d*, *J* = 12.8)

[a]) Recorded in (D_6)DMSO, at 400 MHz.

δ 3.88–3.94 and 3.61–3.66 of the side chain of the aglycone moiety. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** (*Tables 1* and *2*) with those of echinacoside (**5**) suggested that their structures are closely related and contain the same molecular subunits, except for the arylethyl moiety. The HMBC experiment permitted the confirmation of all the relevant interfragmental connectivities of **1** (*Fig. 2*). HMBC Correlations between δ(H) 4.37 (*d*, *J* = 7.8 Hz, inner Glc H–C(1′)) and δ(C) 70.1 (Agly C(α)) and δ(H) 4.68–4.72 (inner Glc H–C(4′)) and δ(C) 166.0 (ester C=O) established linkages between the aglycon, ester, and the suger moieties. A cross-peak in the HMBC plot between δ(H) 5.03 (br. *s*, Rha H–C(1″)) and δ(C) 78.9 (inner Glc C(3′)) indicated that the rhamnose unit was linked to the inner glucose

Table 2. *^{13}C-NMR Data for Compounds* **1**, **2a**/**2b**, **3a**/**3b**, *and* **4a**/**4b**. δ in ppm.

	1[a])	**2a**/**2b**[a])	**3a**/**3b**[b])		**4a**/**4b**[c])
Aglycone:					
C(1)	128.5	129.2	133.6/133.5		
C(2)	129.7	116.3	114.7/114.8		
C(3)	115.0	144.9	146.3/146.2		
C(4)	155.6	143.5	146.1/146.0		
C(5)	115.0	115.5	116.2		
C(6)	129.7	119.5	119.0/119.1		
C(α)	70.1	70.2	76.9/77.5		
C(β)	34.7	35.0	74.1/73.9		
Inner glucose:				*Inner glucose:*	
C(1′)	102.1	102.1	104.6/104.5	C(1)	94.2/98.3
C(2′)	74.4	74.4/74.3	76.3/76.1	C(2)	74.7/77.4
C(3′)	78.9	78.9	81.3/81.4	C(3)	79.1/81.6
C(4′)	69.1	69.1	70.5/70.6	C(4)	70.8/70.9
C(5′)	73.1	73.1/73.0	74.7/74.8	C(5)	69.9/74.7
C(6′)	68.0	68.0/68.1	69.4/69.5	C(6)	69.6/69.7
Rhamnose:				*Rhamnose:*	
C(1″)	101.2	101.2	103.0	C(1′)	103.1/103.0
C(2″)	70.4	70.5	72.3	C(2′)	72.4
C(3″)	70.3	70.3	72.1	C(3′)	72.1
C(4″)	71.6	71.6/71.7	73.8	C(4′)	73.8
C(5″)	68.7	68.7	70.5	C(5′)	70.5/70.4
C(6″)	18.1	18.1/18.0	18.4	C(6′)	18.6
Outer glucose:				*Outer glucose:*	
C(1‴)	103.3	103.3	104.7/104.6	C(1″)	104.6/104.5
C(2‴)	73.4	73.4	75.1/75.1	C(2″)	75.2
C(3‴)	76.5	76.6	77.8/77.7	C(3″)	77.8
C(4‴)	69.9	69.9	71.4	C(4″)	71.5
C(5‴)	76.8	76.8	77.9	C(5″)	77.9
C(6‴)	61.0	60.9	62.6	C(6″)	62.7
Ester:				*Ester:*	
C(1⁗)	125.3	125.0/125.1	127.6	C(1‴)	127.7
C(2⁗)	114.7	130.3/133.0	115.3	C(2‴)	115.4
C(3⁗)	145.6	115.8/114.8	146.8	C(3‴)	146.9
C(4⁗)	148.8	160.0/159.1	149.9	C(4‴)	149.9
C(5⁗)	115.8	115.8/114.8	116.5	C(5‴)	116.6
C(6⁗)	121.5	130.3/133.0	123.3	C(6‴)	123.3
C(α′)	113.2	113.5/114.3	114.6	C(α)	115.0/114.9
C(β′)	145.9	145.4	148.3	C(β)	148.1
C(C=O)	166.0	166.1/164.9	168.5/168.4	C(C=O)	168.5/168.6

[a]) Recorded in (D_6)DMSO, at 100 MHz. [b]) Recorded in CD_3OD, at 150 MHz. [c]) Recorded in CD_3OD, at 100 MHz.

unit by a (1 → 3) linkage. Moreover, another correlation between δ(H) 4.18 (*d*, *J* = 7.7 Hz, outer Glc H–C(1‴)) and δ(C) 68.0 (inner Glc C(6′)) suggested that the two glucose units were connected by a (1 → 6) linkage.

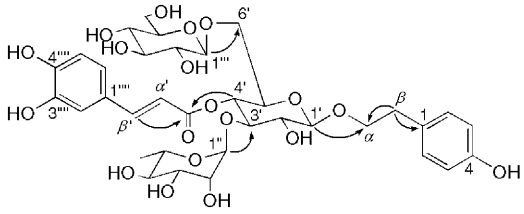

Fig. 2. *Selected HMBC correlations* (H → C) *of Cistantubuloside A* (**1**)

Cistantubuloside B_1/B_2 (**2a**/**2b**) was obtained as an amorphous light-yellowish powder with a molecular formula of $C_{35}H_{46}O_{19}$, as determined by data from the negative-ion HR-SI-MS showing an $[M-H]^-$ ion at *m*/*z* 769.2562 and from the ^{13}C-NMR spectrum. The IR spectrum showed absorption bands typical of OH groups (3416 cm^{-1}), an α,β-unsaturated ester (1690, 1630 cm^{-1}), and aromatic rings (1600, 1510 cm^{-1}). Comparison of the ^{1}H- and ^{13}C-NMR data of **2a**/**2b** (*Tables 1* and *2*) with those of jionoside E [8] suggested that the structure of **2a**/**2b** is similar to that of jionoside E (see *Fig. 1*) except that the signals of the galactose unit in jionoside E were replaced by the signals of a glucose unit in **2a**/**2b**. All spectroscopic data, together with two adjacent peaks in HPLC suggested that **2a**/**2b** might be a pair of *cis*/*trans* isomers with a ratio of 4.2 : 1. The two isomers **2a**/**2b** were isolated by further prep. HPLC, but it was not possible to separate them due to their interconversion. This phenomenon has already been described for other arylethyl glycosides [9][10]. Consequently, **2a**/**2b** was identified as 2-(3,4-dihydroxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[β-D-glucopyranosyl-(1 → 6)]-4-*O*-[(*E*/*Z*)-*p*-coumaroyl]-β-D-glucopyranoside, named cistantubuloside B_1/B_2.

Cistantubuloside C_1/C_2 (**3a**/**3b**) was obtained as an amorphous light-yellowish powder with a molecular formula $C_{35}H_{46}O_{21}$, as determined by data from the negative-ion HR-SI-MS showing an $[M-H]^-$ ion at *m*/*z* 801.2478, and from the ^{13}C-NMR spectrum. The IR spectrum showed absorption bands typical of OH groups (3370 cm^{-1}), an α,β-unsaturated ester (1690, 1630 cm^{-1}), and aromatic rings (1600, 1514 cm^{-1}). The NMR spectra (*Tables 2* and *3*) revealed doubling of several peaks demonstrating that **3a**/**3b** is an epimer mixture due to the configuration at C(β) of the aglycone, similar to the epimer mixture found for campneoside II (**6**) [11]. The ratio of the epimers was deduced to be 1.14 : 1 from the intensities of the proton signals in the ^{1}H-NMR. However, as seen from the spectra **3a**/**3b** contains an additional β-D-glucopyranosyl unit like that found in **1**. Also the HMBC experiment displayed a correlation between δ(H) 4.26 and 4.29 (*d*, $J = 7.7$ Hz, outer Glc H–C(1‴)) and δ(C) 69.4 and 69.5 (inner Glc C(6′)). On the basis of the above results, **3a**/**3b** was assigned as (2*R*)/(2*S*)-2-(3,4-dihydroxyphenyl)-2-hydroxyethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[β-D-glucopyranosyl-(1 → 6)]-4-*O*-[(*E*)-caffeoyl]-β-D-glucopyranoside, named cistantubuloside C_1/C_2.

Cistantubulose A_1/A_2 (**4a**/**4b**) was obtained as an amorphous light-yellowish powder with a molecular formula $C_{27}H_{38}O_{18}$, as determined by data from the positive-ion HR-FAB-MS, showing an $[M+Na]^+$ ion at *m*/*z* 673.1950, and from the ^{13}C-NMR spectrum. The IR spectrum showed absorption bands typical of OH groups (3408 cm^{-1}), an α,β-unsaturated ester (1692, 1630 cm^{-1}), and aromatic rings (1600, 1491 cm^{-1}). The NMR spectra (*Tables 2* and *3*) exhibited several splitted peaks; and two adjacent peaks appeared in the HPLC, suggesting that **4a**/**4b** is a pair of anomers due to the configu-

Table 3. *^{1}H-NMR Data for Compounds* **3a**/**3b** *and* **4a**/**4b**. δ in ppm, J in Hz.

	3a/**3b**[a])		**4a**/**4b**[b])
Aglycone:			
H–C(2)	6.84 (d, J = 1.6)/6.85 (d, J = 1.6)		
H–C(5)	6.73 (d, 8.1)		
H–C(6)	6.71 (dd, J = 8.1, 1.6)		
$CH_2(\alpha)$	3.55–3.62 (m), 3.90–3.96 (m)/ 3.76–3.83 (m), 3.81–3.87 (m)		
H–C(β)	4.73–4.76 (m)/4.72–4.74 (m)		
Inner glucose:		*Inner glucose:*	
H–C(1′)	4.43 (d, J = 8.0)/4.44 (d, J = 8.0)	H–C(1)	5.10 (d, J = 3.2)/4.56 (d, J = 7.9)
H–C(2′)	3.43–3.48 (m)	H–C(2)	3.53–3.58 (m)/3.31–3.35 (m)
H–C(3′)	3.81–3.86 (m)	H–C(3)	4.02–4.06 (m)/3.78–3.83 (m)
H–C(4′)	5.01–5.04 (m)/4.97–5.00 (m)	H–C(4)	5.01–5.05 (m)/4.96–5.00 (m)
H–C(5′)	3.76–3.83 (m)	H–C(5)	4.18–4.23 (m)/3.75–3.81 (m)
CH_2(6′)	3.60–3.63 (m), 3.91–3.94 (m)	CH_2(6)	3.57–3.63 (m), 3.91–3.95 (m)/ 3.58–3.63 (m), 3.93–3.96 (m)
Rhamnose:		*Rhamnose:*	
H–C(1″)	5.20 (br. s)/5.19 (br. s)	H–C(1′)	5.12 (br. s)/5.18 (br. s)
H–C(2″)	3.90–3.94 (m)	H–C(2′)	3.89–3.93 (m)
H–C(3″)	3.53–3.60 (m)	H–C(3′)	3.56–3.62 (m)
H–C(4″)	3.24–3.32 (m)	H–C(4′)	3.24–3.31 (m)
H–C(5″)	3.52–3.57 (m)	H–C(5′)	3.53–3.56 (m)
Me(6″)	1.08 (d, J = 6.2)	Me(6′)	1.08 (d, J = 6.0)
Outer glucose:		*Outer glucose:*	
H–C(1‴)	4.26 (d, J = 7.7)/4.29 (d, J = 7.7)	H–C(1″)	4.25 (d, J = 7.2)/4.26 (d, J = 7.2)
H–C(2‴)	3.16–3.20 (m)	H–C(2″)	3.15–3.19 (m)
H–C(3‴)	3.30–3.35 (m)	H–C(3″)	3.32–3.35 (m)
H–C(4‴)	3.22–3.30 (m)	H–C(4″)	3.21–3.28 (m)
H–C(5‴)	3.18–3.25 (m)	H–C(5″)	3.20–3.26 (m)
CH_2(6‴)	3.58–3.63 (m), 3.77–3.85 (m)	CH_2(6″)	3.60–3.64 (m), 3.76–3.82 (m)
Ester:		*Ester:*	
H–C(2⁗)	7.05 (d, J = 1.5)	H–C(2‴)	7.06 (d, J = 1.6)
H–C(5⁗)	6.77 (d, J = 8.2)	H–C(5‴)	6.78 (d, J = 8.0)
H–C(6⁗)	6.96 (dd, J = 8.2, 1.5)	H–C(6‴)	6.97 (dd, J = 8.0, 1.6)
H–C(α′)	6.27 (d, J = 15.8)	H–C(α)	6.29 (d, J = 15.9)/6.28 (d, J = 15.9)
H–C(β′)	7.59 (d, J = 15.8)	H–C(β)	7.59 (d, J = 15.9)

[a]) Recorded in CD_3OD, at 600 MHz. [b]) Recorded in CD_3OD, at 400 MHz.

ration at C(1) of the glucose moiety, with a ratio of 1 : 1, like that found for cistanoside F (= 4-*O*-[(*E*)-caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-D-glucopyranoside) [12]. Comparison of the ^{1}H- and ^{13}C-NMR data of **4a**/**4b** with those of cistanoside F revealed a downfield shift of C(6) of the glucose moiety (+7.1 ppm), indicating that **4a**/**4b** has a glucose unit attached to C(6) of the inner glucose. The HMBC experiment also displayed a correlation between δ(H) 4.25/4.26 (d, J = 7.2 Hz, outer Glc H–C(1″)) and δ(C) 69.6/69.7 (inner Glc C(6)). Therefore, **4a**/**4b** was assigned as *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-

[β-D-glucopyranosyl-(1 → 6)]-4-*O*-[(*E*)-caffeoyl]-D-glucopyranoside, named cistantubulose A_1/A_2.

The five known compounds were identified by comparison of their spectral data with literature values as campneoside I (=2-(3,4-dihydroxyphenyl-2-methoxyethyl 4-*O*-[(*E*)-caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside) [11], campneoside II (=2-(3,4-dihydroxyphenyl)-2-hydroxyethyl 4-*O*-[(*E*)-caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside; **6**) [11], echinacoside (=2-(3,4-dihydroxyphenyl)-ethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[β-D-glucopyranosyl-(1 → 6)]-2-*O*-acetyl-4-*O*-[(*E*)-caffeoyl]-β-D-glucopyranoside) [13], tubuloside A (=2-(3,4-dihydroxyphenyl)-ethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-[β-D-glucopyranosyl-(1 → 6)]-2-*O*-acetyl-4-*O*-[(*E*)-caffeoyl]-β-D-glucopyranoside) [13], and cistanoside F (=4-*O*-[(*E*)-caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-D-glucopyranoside) [12]. It is well known that arylethyl glycosides are the characteristic and bioactive constituents of the *Cistanche* genus plants. Up to now, we have isolated eleven known arylethyl glycosides from *C. tubulosa*, which also have been found in *C. deserticola*, except for campneoside I and campneoside II (**6**). Our present study provides a powerful evidence for the rationality of using *C. tubulosa* as a new natural source of Herba Cistanches. Due to our systematic work, *C. tubulosa* has been approved to be listed in the Chinese Pharmacopoeia (2005 edition). Compounds isolated from *C. tubulosa* include six pairs of epimers, and this is rarely reported from other species of the *Cistanche* genus.

Experimental Part

General. TLC: silica gel GF_{254} plates from *Qing Dao Hai Yang Chemical Group Co. Column chromatography (CC): Diaion HP-20* (*Mitsubishi Chemical Industries*), *ODS* (100–200 mesh; *Fuji Sylisia Chemical, Ltd.,* Aichi, Japan). Prep. HPLC: *Waters-600* instrument; *Waters ODS* (7.8 i. d.×300 mm; *Waters Co. Ltd.*, USA, flow rate 2.5 ml/min, detection at UV 330 nm; *Waters* column *Prep. Nova-Pak HR* C_{18} (7.8×300 mm). GC: *Agilent-6890N* gas chromatograph; *HP-5* capillary column (28 m×0.32 mm i.d.); detection by FID; detector temp. 260°; column temp. 180°; carrier gas N_2; flow rate 40 ml/min. $[\alpha]_D$: *Perkin-Elmer-243B* digital polarimeter. UV Spectra: $\lambda_{max}(\log \varepsilon)$ in nm. IR spectra: *Nicolet-Avatar-360* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-ARX-400* and *-DMX-600* spectrometer; in CD_3OD or $(D_6)DMSO$; $SiMe_4$ as internal standard. MS: *Bruker-APEX-II-FTCR* spectrometer in the negative-ion mode (HR-SI); *AutoSpec-Ultima-TOF* spectrometer (HR-FAB); in *m*/*z*.

Plant Material. The stems of *Cistanche tubulosa* (SCHENK) R. WIGHT were collected in September 1996 at Hetian, Xinjiang Uigur Autonomous Region, People's Republic of China. The identification of the plant was performed by Prof. *Peng-Fei Tu*, School of Pharmaceutical Sciences, Peking University Health Science Center. A voucher specimen (No. 19960912) is kept in the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation. The stems (36.0 kg) of *C. tubulosa* were extracted four times with hot 95% EtOH, for 2 h each time. After evaporation, the residue (11.8 kg) was suspended in H_2O and defatted with petroleum ether. The aq. layer was further extracted successively with AcOEt and BuOH: AcOEt extract (547 g) and BuOH extract (4.0 kg). The BuOH extract (500 g) was subjected to CC (*Diaion HP-20*, H_2O, then 10%, 30%, 50%, and 70% EtOH, successively). The fraction eluted with 10% EtOH was subjected to CC (*ODS*, MeOH/H_2O 1:9 → 1:1): *Fr. 1.1–1.10* with R_f 0.05, 0.08, 0.10, 0.15, 0.22, 0.35, 0.44, 0.50, 0.55 and 0.61 on TLC ($CHCl_3$/MeOH/H_2O 7:3:1; yellow spots after spraying with 5% H_2SO_4 soln.). *Fr. 1.7* was subjected to prep. HPLC (MeOH/H_2O 35:65): *campneoside I* (12 mg; t_R 12.1 min), *campneoside II* (**6**; 27 mg; t_R 13.3 min). The fraction eluted with 30% EtOH was subjected to CC (*ODS*, MeOH/H_2O 2.5:7.5 → 7.5:2.5): *Fr. 2.1–2.16* with R_f 0.07, 0.12, 0.20, 0.32, 0.35, 0.35, 0.41, 0.42, 0.45, 0.50, 0.55, 0.55, 0.58, 0.61, 0.65, and 0.72 on TLC ($CHCl_3$/MeOH/H_2O 7:3:1; yellow spots after

spraying with 5% H_2SO_4 soln.). *Fr. 2.4* was subjected to prep. HPLC (MeOH/H_2O 30:70) *echinacoside* (**5**); 50 mg; t_R 17.5 min) and *tubuloside A* (16 mg; t_R 19.4 min). *Fr. 2.7* was subjected to prep. HPLC (MeOH/H_2O 32:68): **1** (12 mg; t_R 8.9 min) and **2a**/**2b** (15 mg; t_R 10.4/14.6 min). The fraction eluted with H_2O was subjected to CC (*ODS*, MeOH/H_2O 2:8 → 7:3): *Fr. 3.1–3.7* with R_f 0.21, 0.25, 0.30, 0.41, 0.45, 0.48, and 0.53 on TLC ($CHCl_3$/MeOH/H_2O 6:4:1; yellow spots after spraying with 5% H_2SO_4 soln.). *Fr. 3.5* was subjected to prep. HPLC (MeOH/H_2O 30:70): **3a**/**3b** (11 mg; t_R 12.5 min). *Fr. 3.3* was subjected to prep. HPLC (MeOH/H_2O 20:80): *cistanoside F* (7.2 mg; t_R 10.5 and 11.1 min) and **4a**/**4b** (8.0 mg; t_R 6.1 and 7.4 min).

Cistantubuloside A (=*2-(4-Hydroxyphenyl)ethyl* O-*6-Deoxy-α-*L*-mannopyranosyl-(1 → 3)*-O-*[β-*D*-glucopyranosyl-(1 → 6)]-β-*D*-glucopyranoside 4-[(2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoate]*; **1**): Amorphous light-yellowish powder. $[\alpha]_D^{20} = -52.1$ ($c = 0.1$, MeOH). UV (MeOH): 328 (3.50). IR (KBr): 3397, 2922, 1692, 1630, 1596, 1512, 1070, 1041. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Tables 1* and *2*. HR-SI-MS: 769.2559 ($[M-H]^-$, $C_{35}H_{45}O_{19}^-$; calc. 769.2555).

Cistantubuloside B_1/B_2 (=*2-(3,4-Dihydroxyphenyl)ethyl* O-*6-Deoxy-α-*L*-mannopyranosyl-(1 → 3)*-O-*[β-*D*-glucopyranosyl-(1 → 6)]-β-*D*-glucopyranoside 4-[(2*E*)/(2*Z*)-3-(4-Hydroxyphenyl)prop-2-enoate]*; **2a**/**2b**): Amorphous light-yellowish powder. $[\alpha]_D^{20} = -57.6$ ($c = 0.1$, MeOH). UV (MeOH): 315 (3.12). IR (KBr): 3416, 2923, 1690, 1630, 1600, 1510, 1070, 1044. ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO: *Tables 1* and *2*. HR-SI-MS: 769.2562 ($[M-H]^-$, $C_{35}H_{45}O_{19}^-$; calc. 769.2555).

Cistantubuloside C_1/C_2 (=*(2*R*)/(2*S*)-2-(3,4-Dihydroxyphenyl)-2-hydroxyethyl* O-*6-Deoxy-α-*L*-mannopyranosyl-(1 → 3)*-O-*[β-*D*-glucopyranosyl-(1 → 6)]-β-*D*-glucopyranoside 4-[(2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoate]*; **3a**/**3b**): Amorphous light-yellowish powder. $[\alpha]_D^{20} = -12.2$ ($c = 0.1$, MeOH). UV (MeOH): 332 (3.20). IR (KBr): 3370, 2932, 1690, 1630, 1600, 1514, 1070, 1046. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 2* and *3*. HR-SI-MS: 801.2478 ($[M-H]^-$, $C_{35}H_{45}O_{21}^-$; calc. 801.2453).

Cistantubuloses A_1/A_2 (=O-*6-Deoxy-α-*L*-mannopyranosyl-(1 → 3)*-O-*[β-*D*-glucopyranosyl-(1 → 6)]-*D*-glucopyranoside 4-[(2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoate*; **4a**/**4b**): Amorphous light-yellowish powder. $[\alpha]_D^{20} = -22.5$ ($c = 0.1$, MeOH). UV (MeOH): 329 (2.86). IR (KBr): 3408, 2918, 1692, 1630, 1600, 1491, 1070, 1042. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 2* and *3*. HR-FAB-MS: 673.1950 ($[M+Na]^+$, $C_{27}H_{38}NaO_{18}^+$; calc. 673.1956).

Acid Hydrolysis and Sugar Analysis. Compound **1**, **2a**/**2b**, **3a**/**3b**, or **4a**/**4b** (3 mg each) was heated in 10% HCl soln./dioxane 1:1 (3 ml) at 80° for 4 h. After the dioxane was removed, the soln. was extracted with AcOEt (3 × 3 ml) to yield the sugar and the aglycone components. The sugar components in the aq. phase were analyzed by TLC ($CHCl_3$/MeOH/H_2O 8:5:1) comparison with the standard sugars. The spots were visualized by spraying with 95% EtOH/H_2SO_4/anisaldehyde 9:0.5:0.5, (*v*/*v*) followed by heating at 120° for 10 min: R_f 0.30 for glucose and 0.50 for rhanmnose from **1**–**4**.

The TLC results were confirmed by GC analyses of methyl sugar peracetates: The aq. phase was evaporated, the residue dissolved in anh. pyridine (100 µl), 0.1M L-cysteine methyl ester hydrochloride (200 µl) added, and the mixture warmed at 60° for 1 h. The trimethysilylation reagent hexamethyldisilazane (HMDS)/chlorotrimethylsilane/pyridine 2:1:10 (*Acros Organics*, Belgium) was added and the mixture warmed at 60° for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification: D-glucose derivative (t_R 12.45 min) and L-rhamnose derivative (t_R 5.32 min) from **1**–**4**.

REFERENCES

[1] Jiangsu New Medical College Edita, 'Dictionary of Chinese Materia Medica', Shanghai Science and Technology Press, 1977, p. 895.
[2] X.-C. Geng, L.-W. Song, X.-P. Pu, P.-F. Tu, *Biol. Pharm. Bull.* **2004**, *27*, 797.
[3] X.-F. Tian, X.-P. Pu, *J. Ethnopharmacol.* **2005**, *97*, 59.
[4] L. Lei, Z.-H. Song, P.-F. Tu, *Zhong Cao Yao* **2003**, *34*, 473.
[5] P.-F. Tu, B. Wang, T. Deyama, Z.-G. Zhang, Z.-C. Lou, *Acta Pharm. Sin.* **1997**, *32*, 294.
[6] Z.-H. Song, S.-H. Mo, Y. Chen, P.-F. Tu, Y.-Y. Zhao, J.-H. Zheng, *Zhong Cao Yao* **2000**, *25*, 728.
[7] Z.-H. Song, P.-F. Tu, Y.-Y. Zhao, J.-H. Zheng, *Zhong Cao Yao* **2000**, *31*, 808.
[8] H. Sasaki, H. Nishimura, M.-S. Chin, H. Mitsuhashi, *Phytochemistry* **1989**, *28*, 875.
[9] T. Miyase, R. Yamamoto, A. Ueno, *Phytochemistry* **1996**, *43*, 475.

[10] Z.-J. Jia, J.-J. Gao, Z.-M. Liu, *Indian J. Chem., Sect. B* **1994**, *33*, 460.
[11] Y. Imakura, X. Shigeru, S. Kobayashi, A. Mima, *Phytochemistry* **1985**, *24*, 139.
[12] H. Kobayashi, H. Karasawa, T. Miyase, S. Fukushima, *Chem. Pharm. Bull.* **1985**, *33*, 1452.
[13] H. Kobayashi, H. Oguchi, N. Takizawa, T. Miyase, A. Veno, K. Usmanghani, M. Ahmad, *Chem. Pharm. Bull.* **1987**, *35*, 3309.

Received January 13, 2006