New Glycosides from *Cistanche salsa*

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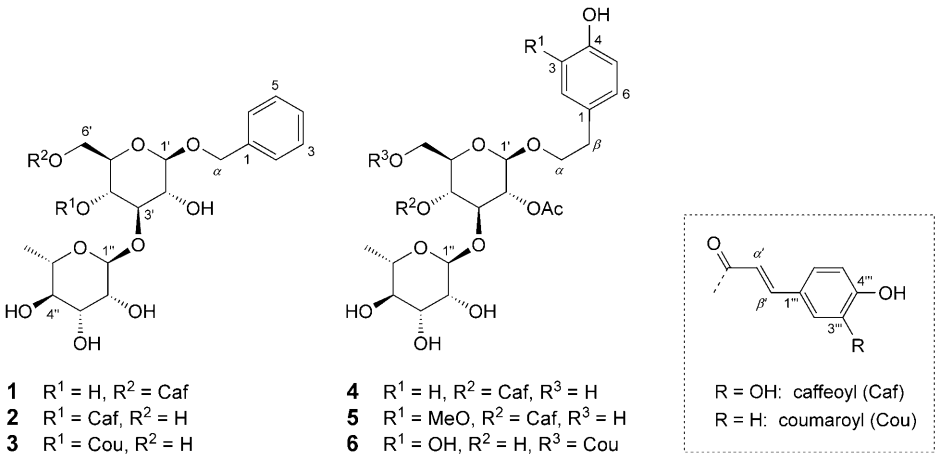
Six new glycosides, salsasides A–F (**1**–**6**, resp.), were isolated from the stems of *Cistanche salsa*, together with seven known glycosidic compounds. Their structures were elucidated by means of ester hydrolysis and chemical derivatization, in-depth NMR spectroscopic and mass spectrometric analyses, and by comparison with literature data of related compounds. The new glycosides are based on β-D-glucose (Glc) and α-L-rhamnose (Rha), carrying acetyl (Ac), benzyl (Bn), phenethyl, coumaroyl (Cou), and caffeoyl (Caf) substituents.

Introduction. – *Cistanche salsa* (C. A. MEY.) G. BECK, one of the species of *Herba Cistanche*, is a short parasitic Orobanchaceae plant native to Northwest China. As an important tonic in traditional Chinese medicine (TCM), the stems of *Herba Cistanche* have long been used by the Chinese and Japanese against kidney deficiency, female infertility, morbid leucorrhea, neurasthenin, and senile constipation due to colonic inertia, *etc.* [1]. Just as in other *Cistanche* plants, phenethyl-based glycosides are the main active constituents of *C. salsa*. These compounds were reported to have neuroprotective activity against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic toxicity in C57 mice [2–4]. There are only few phytochemical studies concerning *C. salsa*, the first ones dating back *ca.* 20 years [5–8]. In 1995, *Moriya et al.* [9][10] reported that the plant material of *C. salsa* had been identified falsely, when he investigated the sources of *Herba Cistanche* from a Japanese medical market [9][10]. Therefore, it is necessary to re-investigate the chemical constituents of *C. salsa*.

In the present work on *C. salsa*, we report the isolation and structure elucidation of six new glycosides, salsasides A–F (**1**–**6**), with different substituents, including acetyl (Ac), benzyl (Bn), caffeoyl (Caf), and coumaroyl (Cou) residues. Also isolated from the same extract were the following seven known glycosides: tubuloside B [11], acteoside [6], isoacteoside [11], 2′-acetylacteoside [7], echinacoside [6], cistanoside C [7], and cistanoside D [7].

Results and Discussion. – Compound **1** was isolated as an amorphous powder, and its molecular formula was deduced as $C_{28}H_{34}O_{13}$ by HR-ESI-MS (*m/z* 596.2348 ($[M+NH_4]^+$; calc. 596.2343). The ^{1}H-NMR data of **1** (*Table 1*) exhibited characteristic signals of an (*E*)-configured caffeoyl (Caf) group, with *ABX*-type aromatic signals at δ(H) 7.07 (br. *s*), 6.76 (br. *d*, $J=8.5$ Hz), and 7.03 (*d*, $J=8.5$ Hz), two olefinic H-



1 R^1 = H, R^2 = Caf
2 R^1 = Caf, R^2 = H
3 R^1 = Cou, R^2 = H

4 R^1 = H, R^2 = Caf, R^3 = H
5 R^1 = MeO, R^2 = Caf, R^3 = H
6 R^1 = OH, R^2 = H, R^3 = Cou

atoms at δ(H) 7.51 (*d*, J = 16.0) and 6.34 (*d*, J = 16.0 Hz), and a Bn moiety with five aromatic resonances and two nonequivalent H-atoms at δ(H) 4.59, 4.77 (2*d*, J = 12.0 Hz each), which suggested that **1** was a Bn-substituted compound [12].

Total acid hydrolysis of **1** afforded rhamnose (Rha) and glucose (Glc). The NMR-data of **1** were similar to those of tubuloside B [11], except that the signals of a 3,4-dihydroxyphenylethanol unit were replaced by those of a Bn group. In the HMBC spectrum of **1**, correlations between the two nonequivalent H-atoms at δ(H) 4.59, 4.77 (α-CH_2 of aglycone) and δ(C) 101.7 (C(1′) of Glc), of both δ(H) 4.40 (br. *d*, J = 11.5 Hz, 1 H of CH_2(6′)) and 4.23 (*m*, 1 H of CH_2(6′)) with δ(C) 166.6 (C(α') of ester), and of δ(H) 5.04 (br. *s*, H–C(1″)) and δ(C) 80.8 (C(3′)) established the linkages between the aglycone, ester, and sugar moieties.

On the basis of the above spectroscopic evidence, in combination with 2D-NMR data, the structure of **1** was elucidated as benzyl 6-*O*-[(*E*)-3-(3,4-dihydroxyphenyl)-prop-2-enoyl]-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside[1]), and named *salsaside A*.

Compound **2** was isolated as an amorphous powder, and its molecular formula was determined as $C_{28}H_{34}O_{13}$ by HR-ESI-MS (*m*/*z* 596.2345 ($[M+NH_4]^+$)). The only difference between **1** and **2** was that the Caf moiety was attached at 4′-position in **2** instead at 6′-position. This was evident in the HMBC spectrum of **2**, where the signal at δ(H) 4.96 (*t*, J = 9.0 Hz, H–C(4′)) was correlated with δ(C) 167.3 (C=O of Caf), and further corroborated by comparison with the NMR data of 2′-acetylacteoside [4]. Thus, compound **2** was identified as benzyl 4-*O*-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside, and named *salsaside B*.

Salsaside C (**3**) was isolated as an amorphous powder, and its molecular formula was elucidated as $C_{28}H_{34}O_{12}$ by HR-ESI-MS (*m*/*z* 561.1958 ($[M-H]^-$; calc. 561.1972)). The NMR data of **3** were similar to those of **2**, except that the signals of the Caf group were replaced by those of an (*E*/*Z*)-coumaroyl (Cou) moiety (see

[1]) Rhamnopyranose corresponds to 6-deoxymannopyranose.

Table 1. *NMR Data of Compounds* **1**–**3**. At 500/125 MHz, resp., in (D_6)DMSO (**1**, **3**) and CD_3OD (**2**); δ in ppm, J in Hz. Arbitrary atom numbering. Assignments are based on COSY, HMBC, and HMQC experiments.

Atom	**1**		**2**		**3**[a])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	137.7	–	138.0	–	139.0	–
H–C(2,6)	127.8	7.37 (br. *d*, *J* = 7.5)	128.2	7.45 (br. *d*, *J* = 7.5)	129.2	7.42 (br. *d*, *J* = 7.5)
H–C(3,5)	128.1	7.32 (br. *t*, *J* = 7.5)	128.3	7.36 (br. *t*, *J* = 7.5)	129.3	7.36 (br. *t*, *J* = 7.5)
H–C (4)	127.5	7.27 (br. *t*, *J* = 7.5)	127.8	7.31 (br. *t*, *J* = 7.5)	128.7	7.29 (br. *t*, *J* = 7.5)
α-CH_2	69.7	4.59 (*d*, *J* = 12.0), 4.77 (*d*, *J* = 12.0)	71.0	4.71 (*d*, *J* = 12.0), 4.98 (*d*, *J* = 12.0)	71.9	4.62 (*d*, *J* = 11.5), 4.87 (*d*, *J* = 11.5)
H–C(1′)	101.7	4.32 (*d*, *J* = 7.5)	102.2	4.47 (*d*, *J* = 8.0)	103.2	4.42 (*d*, *J* = 8.0), 4.40 (*d*, *J* = 7.5)
H–C(2′)	74.1	3.20 (*t*, *J* = 9.5)	75.2	3.49 (*t*, *J* = 9.0)	76.2	3.46 (*t*, *J* = 8.5)
H–C(3′)	80.8	3.40 (*t*, *J* = 9.5)	80.7	3.84 (*t*, *J* = 9.0)	80.8	3.81 (*t*, *J* = 9.0)
H–C(4′)	68.6	3.23 (*t*, *J* = 9.5)	69.7	4.96 (*t*, *J* = 9.0)	70.6	4.75 (*t*, *J* = 9.5), 4.71 (*t*, *J* = 9.5)
H–C(5′)	73.8	3.46–3.50	75.1	3.57–3.59	76.1	3.47–3.51
CH_2(6′)	63.4	4.40 (br. *d*, *J* = 11.5), 4.21–4.25	61.4	3.67 (br. *d*, *J* = 10.0), 3.57–3.61	62.4	3.39–3.43, 3.26–3.30
H–C(1″)	100.6	5.04 (br. *s*)	102.1	5.21 (br. *s*)	103.1	5.02 (br. *s*)
H–C(2″)	70.6	3.69 (br. *s*)	71.3	3.94 (*d*, *J* = 2.0)	72.3	3.68 (br. *s*)
H–C(3″)	70.6	3.46–3.54	71.1	3.57–3.61	72.0	3.30 (*dd*, *J* = 9.0, 3.0)
H–C(4″)	72.1	3.18 (*t*, *J* = 9.5)	72.8	3.32 (*t*, *J* = 9.5)	73.8	3.09 (*t*, *J* = 9.5)
H–C(5″)	68.1	3.89–3.01	69.4	3.57–3.61	70.4	3.40–3.45
Me(6″)	17.8	1.09 (*d*, *J* = 6.5)	17.5	1.11 (*d*, *J* = 6.0)	18.3	0.94 (*d*, *J* = 6.5), 1.03 (*d*, *J* = 6.0)
C(1‴)	125.5	–	126.7	–	127.1, 130.0	–
H–C(2‴)	114.9	7.07 (br. *s*)	114.2	7.08 (*d*, *J* = 1.5)	131.4, 134.3	7.53 (*d*, *J* = 9.0), 7.70 (*d*, *J* = 9.0)
C(3‴)	145.6	–	145.8	–	116.8, 115.8	6.80 (*d*, *J* = 9.0), 6.75 (*d*, *J* = 9.0)
C(4‴)	148.4	–	148.8	–	161.5, 160.4	–
H–C(5‴)	115.7	6.76 (br. *d*, *J* = 8.5)	115.5	6.81 (*d*, *J* = 8.0)	116.8, 115.8	6.80 (*d*, *J* = 9.0), 6.75 (*d*, *J* = 9.0)
H–C(6‴)	121.4	7.03 (*d*, *J* = 8.5)	122.2	6.98 (*dd*, *J* = 8.0, 1.5)	131.4, 134.3	7.53 (*d*, *J* = 9.0), 7.70 (*d*, *J* = 9.0)
C=O	166.6	–	167.3	–	168.3, 166.0	–
α'-C(H)=	113.9	6.34 (*d*, *J* = 16.0)	113.7	6.31 (*d*, *J* = 16.0)	114.7, 113.8	6.34 (*d*, *J* = 15.5), 5.70 (*d*, *J* = 12.5)
β'-C(H)=	145.3	7.51 (*d*, *J* = 16.0)	147.0	7.62 (*d*, *J* = 16.0)	147.6	7.55 (*d*, *J* = 15.5), 6.92 (*d*, *J* = 12.5)

[a]) Partly doubled signals due to (*E*/*Z*) mixture (see text).

Table 1). The presence of an (*E*/*Z*)-mixture was indicated by split (2.38 : 1) NMR resonances as well as by peak doubling in the HPLC chromatogram, a phenomenon reported before in some phenethyl glycosides [13][14]. In the HMBC spectrum of **3**, correlations between the two nonequivalent aglycone α-CH_2 resonances at δ(H)

4.62, 4.87 (2*d*, *J* = 11.5 Hz each) and δ(C) 103.2 (C(1′)), between δ(H) 4.75/4.71 (*t*, *J* = 7.5 Hz, H−C(4′)) and δ(C) 168.3/166.0 (C=O of Cou), and between δ(H) 5.02 (br. *s*, H−C(1″)) and δ(C) 80.8 (C(3′)) established the linkages between the aglycone, the ester, and the sugar moieties. Thus, the structure of compound **3** was elucidated as benzyl 4-*O*-[(*E*/*Z*)-3-(4-hydroxyphenyl)prop-2-enoyl]-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside, and named *salsaside C*.

Salsaside D (**4**) was obtained as an amorphous powder, and its molecular formula was determined as $C_{31}H_{38}O_{15}$ by HR-ESI-MS (*m*/*z* 668.2563 ($[M+NH_4]^+$; calc. 668.2554). The ^{1}H-NMR spectrum of **4** (*Table 2*) exhibited signals characteristic of an (*E*)-Caf group and of a (4-hydroxyphenyl)ethoxy group [δ(H) 2.65 (*m*, 2 H), 3.55 (*m*, 1 H), 3.90 (*m*, 1 H), 6.64 (*d*, *J* = 8.0 Hz, 2 H), 6.97 (*d*, *J* = 8.0 Hz, 2 H)]. Total acid hydrolysis of **4** afforded Rha and Glc. The fragment ion peak observed in the negative FAB mass spectrum at *m*/*z* 43, and the NMR signals at δ(H) 1.95 (*s*, 3 H), and at δ(C) 169.3 and 20.7, indicated the presence of an Ac group. The ^{1}H- and ^{13}C-NMR data were very similar to those of syringalide A 3′-α-L-rhamnopyranoside, except for an additional Ac signal [15]. In the HMBC spectrum of **4**, the signal at δ(H) 4.68 (*t*, *J* = 9.0 Hz, H−C(2′)) was correlated with δ(C) 169.3 (C=O of Ac), which indicated that the Ac moiety was linked to C(2) of Glc. Thus, the structure of compound **4** was elucidated as 2-(4-hydroxyphenyl)ethyl 2-*O*-acetyl-4-*O*-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside, and named *salsaside D*.

Compound **5** was isolated as an amorphous powder, and its molecular formula was determined as $C_{32}H_{40}O_{16}$ by HR-ESI-MS (*m*/*z* 679.2228 ($[M-H]^-$; calc. 679.2238). The ^{1}H- and ^{13}C-NMR data of **5** (*Table 2*) were very similar to those of **4**, except for the signals of the phenethyl moiety. In the ^{1}H-NMR spectrum of **5**, there was an *AMX* system [δ(H) 6.63 (*d*, *J* = 8.0 Hz, 1 H), 6.69 (*d*, *J* = 8.0 Hz, 1 H), 6.77 (br. *s*, 1 H)] and a MeO signal at δ(H) 3.85 (*s*, 3 H). In the HMBC spectrum, the MeO signal was correlated with δ(C) 148.7 (C(3)), which, in turn, correlated with δ(H) 6.69 (*d*, *J* = 8.0 Hz, H−C(5)) and 6.77 (br. *s*, H−C(2)). Therefore, the MeO substituent was at C(3) of the phenethyl moiety, as corroborated by comparison with literature data [7]. Hence, the structure of compound **5** was elucidated as 2-(4-hydroxy-3-methoxyphenyl)ethyl 2-*O*-acetyl-4-*O*-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside, and named *salsaside E*.

Compound **6** was isolated as an amorphous powder, and its molecular formula was determined as $C_{31}H_{38}O_{15}$ by HR-ESI-MS (*m*/*z* 668.2543 ($[M+NH_4]^+$; calc. 668.2554)). The ^{1}H-NMR spectrum of **6** exhibited signals characteristic of an (*E*)-Cou group [δ(H) 6.35 and 7.46 (2*d*, *J* = 16.0 Hz each, 1 H each), 6.69 (*d*, *J* = 7.5 Hz, 2 H), 7.45 (*d*, *J* = 7.5 Hz, 2 H)], of a (3,4-dihydroxyphenyl)ethoxy moiety [δ(H) 6.31 (br. *d*, *J* = 8.0 Hz, 1 H), 6.46 (br. *s*, 1 H), 6.49 (br. *d*, *J* = 8.0 Hz, 1 H), 2.49 (*m*, CH_2), 3.41 and 3.72 (2*m*, 1 H each)], and of two anomeric resonances [δ(H) 4.45 (*d*, *J* = 8.5 Hz, H−C(1′)), 4.55 (br. *s*, H−C(1″))]. Like in **4** and **5**, there was also an AcO group present in **6** [δ(H) 1.88 (*s*, 3 H); δ(C) 169.2, 20.6], which was at C(2′) of the Glc moiety, as determined by HMBC experiments. In the HMBC spectrum, correlations were observed between the signals of CH_2(6′) and δ(C) 166.6 (C=O of Cou), between δ(H) 3.41, 3.72 (2*m*, α-CH_2 of aglycone) and δ(C) 99.5 (C(1′)), and between δ(H) 4.55 (br. *s*, H−C(1″)) and δ(C) 68.8 (C(3′)), from which all linkages were established. Thus, the structure of compound **6** was settled as 2-(3,4-dihydroxyphenyl)ethyl 2-*O*-acetyl-6-*O*-[(*E*)-3-(4-

Table 2. *NMR Data of Compounds* **4**–**6**. At 500/125 MHz, resp., in (D_6)DMSO (**4**, **6**) and CD_3OD (**5**); δ in ppm, J in Hz. Arbitrary atom numbering. Assignments are based on COSY, HMBC, and HMQC experiments.

Atom	**4**		**5**		**6**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	128.9	–	131.8	–	129.3	–
H–C(2)	129.9	6.97 (*d*, *J* = 8.0)	113.9	6.77 (br. *s*)	116.3	6.46 (br. *s*)
H–C(3)	115.1	6.64 (*d*, *J* = 8.0)	148.7	–	145.0	–
H–C(4)	155.7	–	145.8	–	143.6	–
H–C(5)	115.1	6.64 (*d*, *J* = 8.0)	116.0	6.69 (*d*, *J* = 8.0)	115.4	6.49 (br. *d*, *J* = 8.0)
H–C(6)	129.9	6.97 (*d*, *J* = 8.0)	122.3	6.63 (*d*, *J* = 8.0)	119.5	6.31 (br. *d*, *J* = 8.0)
α-CH_2	70.0	3.51–3.59, 3.89–3.92	71.9	4.08–4.12, 3.50–3.54	69.9	3.39–3.43, 3.69–3.73
β-CH_2	34.5	2.64–2.66	36.6	2.74–2.78	34.8	2.44–2.51
3-MeO	–	–	56.3	3.85 (*s*)	–	–
H–C(1′)	99.3	4.60 (*d*, *J* = 7.5)	101.8	4.53 (*d*, *J* = 8.0)	99.5	4.45 (*d*, *J* = 8.5)
H–C(2′)	73.6	4.68 (*t*, *J* = 9.0)	75.1	4.84 (*t*, *J* = 9.0)	73.3	4.55 (*t*, *J* = 9.0)
H–C(3′)	78.2	3.95 (*t*, *J* = 9.5)	80.5	4.00 (*t*, *J* = 9.0)	79.5	3.52 (*t*, *J* = 9.0)
H–C(4′)	69.0	4.80 (*t*, *J* = 9.5)	70.6	4.98 (*t*, *J* = 9.0)	68.8	3.25 (*t*, *J* = 9.0)
H–C(5′)	74.6	3.51–3.68	76.1	3.56–3.62	73.8	3.45–3.49
CH_2(6′)	60.5	3.31–3.35, 3.36–3.42	62.2	3.60–3.64, 3.51–3.55	63.1	4.29 (*d*, *J* = 11.5), 4.10–4.15
*Me*CO	20.7	1.95 (*s*)	20.8	1.92 (*s*)	20.6	1.88 (*s*)
Me*C*O	169.3	–	171.3	–	169.2	–
H–C(1″)	102.1	4.59 (br. *s*)	103.3	4.79 (*s*)	101.3	4.55 (br. *s*)
H–C(2″)	70.9	3.35 (br. *s*)	72.6	3.60–3.68	70. 5	3.29 (br. *s*)
H–C(3″)	70.2	3.20 (br. *d*, *J* = 10.0)	71.9	3.60–3.68	70. 7	3.39 (br. *d*, *J* = 10.5)
H–C(4″)	71.5	3.06 (*t*, *J* = 9.5)	73.6	3.25 (*t*, *J* = 9.5)	71.9	3.08 (*t*, *J* = 9.0)
H–C(5″)	69.4	3.26–3.34	70.8	3.53 (br. *d*)	68.7	3.67–3.72
Me(6″)	18.3	0.90 (*d*, *J* = 6.0)	18.5	1.06 (*d*, *J* = 6.0)	17.8	1.00 (*d*, *J* = 6.0)
C(1‴)	125.5	–	127.6	–	125.1	–
H–C(2‴)	114.7	7.02 (br. *s*)	115.2	7.03 (br. *s*)	130.4	7.45 (*d*, *J* = 7.5)
C(3‴)	145.7	–	146.7	–	115.8	6.69 (*d*, *J* = 7.5)
C(4‴)	148.8	–	149.6	–	160.0	–
H–C(5‴)	115.9	6.75 (*d*, *J* = 7.0)	116.5	6.77 (br. *d*, *J* = 7.5)	115.8	6.69 (*d*, *J* = 7.5)
H–C(6‴)	121.7	6.96 (*d*, *J* = 7.0)	123.2	6.94 (br. *d*, *J* = 7.5)	130.4	7.45 (*d*, *J* = 7.5)
COO	165.8	–	168.1	–	166.6	–
α'-CH=	113.4	6.21 (*d*, *J* = 16.0)	114.5	6.26 (*d*, *J* = 16.0)	114.0	6.35 (*d*, *J* = 16.0)
β'-CH=	146.1	7.46 (*d*, *J* = 16.0)	148.2	7.59 (*d*, *J* = 16.0)	145.0	7.46 (*d*, *J* = 16.0)

hydroxyphenyl)prop-2-enoyl]-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside, and named *salsaside F.*

In compounds **1**–**6**, the configuration at the anomeric center of the Glc residue was deduced to be β from J values of 7.5–8.5 Hz. In the case of the Rha residues, the α-anomeric configuration was derived by comparison of the pertinent ^{13}C-NMR data with those given in the literature [6]. The absolute configurations of the sugars, D-Glc and L-Rha, were determined by GC analysis of chiral derivatives (see *Exper. Part*) in comparison with standard monosaccharides [16].

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Experimental Part

General. Silica gel (200–300 mesh; *Qing Dao Hai Yang Chemical Group, Co.*), *Sephadex LH-20* (*Pharmacia*), *D101* resin (*Tianjin Chemical, Co.*), and *ODS* (100–200 mesh; *Fuji Sylisia Chemical, Ltd.*) were used for column chromatography (CC). Prep. HPLC was performed on a *Waters-600* instrument, using an *RP-C_{18}* column (10×250 mm i.d.; *Alltech*) at a flow rate of 2.5 ml/min (UV detection at 330 nm). GC Analysis was carried out on an *Agilent-6890N* gas chromatograph, using an *HP-5* capillary column (28 m×0.32 mm i.d.), an FID detector at 260°, and a column temp. of 180°, with N_2 carrier gas and flow rate of 40 ml/min. UV Spectra were recorded on a *Shimadzu* spectrometer; λ_{max} (log ε) in nm. Optical rotations were determined on a *Perkin-Elmer 243B* digital polarimeter. IR Spectra were recorded on a *Nicolet Avatar-360* FT-IR spectrometer; in cm^{-1}. NMR Spectra were recorded in CD_3OD or (D_6)DMSO on a *Bruker AM-500* spectrometer; δ in ppm rel. to Me_4Si, *J* in Hz. FAB- and HR-ESI mass spectra were recorded on *KYKY-ZHP-5* and *Bruker APEX* mass spectrometers, resp.

Plant Material. The stems of *Cistanche salsa* were collected from Yanchi, Ningxia Hui Autonomous Region, China, in April. The plant was identified by Prof. *Peng-Fei Tu*, School of Pharmaceutical Sciences, Peking University. A voucher specimen was deposited at the Herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation. The dried stems of *C. salsa* (8.0 kg) were extracted with 75% aq. EtOH (80 l) at r.t. by percolation. The solvent was removed, the residue was suspended in H_2O (4 l), and extracted with petroleum ether (PE; 12 l), AcOEt (12 l), and BuOH (12 l) to afford, after solvent removal, 100 g of PE-, 99 g of AcOEt-, and 100 g of BuOH-soluble extract, resp. Part of the AcOEt-soluble extract (90 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 0:1 → 1:2) to afford 75 fractions (Fr.). *Fr. 51–53* (6.0 g) were combined (= *Fr. A*) and rechromatographed (*Sephadex LH-20*; MeOH/H_2O 1:1) to afford eleven subfractions (*Fr. A1–A11*). *Fr. A6* and *Fr. A7* were combined (2.5 g; *Fr. B*) and resubjected to CC (*ODS*; MeOH/H_2O 1:9–5:5) to provide 35 further fractions (*Fr. B1–B35*). *Fr. B16–B25* were combined (0.5 g; *Fr. C*) and rechromatographed (*Sephadex LH-20*; then prep. HPLC, MeCN/MeOH/H_2O 10:18:75) to provide tubuloside B [11] (55 mg). *Fr. B26–B32* (0.35 g) were combined (0.35 g, *Fr. D*) and rechromatographed (*Sephadex LH-20*; 20% aq. MeOH) to yield seven fractions (*Fr. D1–D7*). *Fr. D1* (70 mg) was purified by prep. HPLC (MeCN/MeOH/H_2O 10:26:72) to afford **2** (23 mg), **3** (8 mg), and cistanoside C [7] (12 mg). *Fr. D3* (45 mg) was purified by prep. HPLC (MeCN/MeOH/H_2O 10:20:70) to provide **6** (22 mg). *Fr. D4* (36 mg) was purified by prep. HPLC (MeCN/MeOH/H_2O 9:18:73) to provide **5** (20 mg). *Fr. D5* (55 mg) was purified by prep. HPLC (MeCN/MeOH/H_2O 10:24:66) to afford **1** (28 mg). *Fr. D7* (40 mg) was purified by prep. HPLC (MeCN/MeOH/H_2O 10:16:74) to provide **4** (18 mg). The original *Fr. 54* and *Fr. 55* were combined and purified by repeated CC (*Sephadex LH-20*) to afford acteoside [6] (0.1 g) and 2′-acetylacteoside (8.9 mg) [10]. *Fr. 56–58* were combined and purified by repeated CC (*Sephadex LH-20* and *ODS*) to furnish isoacteoside (25 mg) [11] and cistanoside D (32 mg) [7]. *Fr. 59–64* were combined and purified by repeated CC (*Sephadex LH-20*) and prep. HPLC (MeCN/MeOH/H_2O 10:15:84) to afford echinacoside (33 mg) [6].

Salsaside A (= *Benzyl 6*-O-*[(*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl]-3*-O-*α*-L-*rhamnopyranosyl-β*-D-*glucopyranoside*; **1**). Amorphous powder. UV (MeOH): 328 (3.50). $[\alpha]_D^{20} = -35.6$ ($c = 0.1$, MeOH). IR (KBr): 3421, 1690, 1628, 1605, 1520. ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS: 577 ($[M-H]^-$). HR-ESI-MS: 596.2348 ($[M+NH_4]^+$, $C_{28}H_{38}NO_{13}^+$; calc. 596.2343).

Salsaside B (= *Benzyl 4*-O-*[(*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl]-3*-O-*α*-L-*rhamnopyranosyl-β*-D-*glucopyranoside*; **2**). Amorphous powder. UV (MeOH): 332 (3.20). $[\alpha]_D^{20} = -35.6$ ($c = 0.1$, MeOH). IR (KBr): 3411, 1692, 1630, 1600, 1514. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 596.2345 ($[M+NH_4]^+$, $C_{28}H_{38}NO_{13}^+$; calc. 596.2343).

Salsaside C (= *4*-O-*[(*E/*Z*)-3-(4-Hydroxyphenyl)prop-2-enoyl]-3*-O-*α*-L-*rhamnopyranosyl-β*-D-*glucopyranoside*; **3**). Amorphous powder. UV (MeOH): 315 (3.23), 225 (1.80). IR (KBr): 3432, 1689, 1628, 1609, 1519. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 561.1958 ($[M-H]^-$, $C_{28}H_{33}O_{12}^-$; calc. 561.1972).

Salsaside D (=*2-(4-Hydroxyphenyl)ethyl 2*-O-*Acetyl-4*-O-*[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-3*-O-*α*-L-*rhamnopyranosyl-β*-D-*glucopyranoside*; **4**). Amorphous powder. UV (MeOH): 328 (3.40). $[\alpha]_D^{20} = -58.3$ ($c = 0.1$, MeOH). IR (KBr): 3397, 1720, 1692, 1630, 1596, 1512. ^{1}H- and ^{13}C-NMR: see *Table 2*. FAB-MS: 649 ($[M-H]^-$), 443 ($[M-H-Ac-Caf]^-$). HR-ESI-MS: 668.2563 ($[M+NH_4]^+$, $C_{31}H_{42}NO_{15}^+$; calc. 668.2554).

Salsaside E (=*2-(4-Hydroxy-3-methoxyphenyl)ethyl 2*-O-*Acetyl-4*-O-*[(*E*)-3-(3,4-dihydroxyphenyl)-prop-2-enoyl]-3*-O-*α*-L-*rhamnopyranosyl-β*-D-*glucopyranoside*; **5**). Amorphous powder. UV (MeOH): 330 (3.21). $[\alpha]_D^{20} = -33.3$ ($c = 0.1$, MeOH). IR (KBr): 3370, 1721, 1690, 1630, 1600, 1514. ^{1}H- and ^{13}C-NMR: see *Table 2*. FAB-MS: 679 ($[M-H]^-$), 533 $[M-H-Rha]^-$). HR-ESI-MS: 679.2228 ($[M-H]^-$, $C_{32}H_{39}O_{16}^-$; calc. 679.2238).

Salsaside F (=*2-(3,4-Dihydroxyphenyl)ethyl 2*-O-*Acetyl-6*-O-*[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-3*-O-*α*-L-*rhamnopyranosyl-β*-D-*glucopyranoside*; **6**). Amorphous powder. UV (MeOH): 315 (3.28). $[\alpha]_D^{20} = -38.5$ ($c = 0.1$, MeOH). IR (KBr): 3416, 1725, 1690, 1630, 1600, 1510. ^{1}H- and ^{13}C-NMR: see *Table 2*. FAB-MS: 649 ($[M-H]^-$). HR-ESI-MS: 668.2543 ($[M+NH_4]^+$, $C_{31}H_{42}NO_{15}^+$; calc. 668.2554).

Acid Hydrolysis and Determination of Absolute Sugar Configuration. The compound (3 mg) was placed in a sealed tube and hydrolyzed with 2N aq. CF_3COOH (5 ml) by heating on a water bath for 3 h [16]. After cooling down, the mixture was diluted with H_2O (15 ml), and extracted with $CHCl_2$ (3×5 ml). The aq. layer was repeatedly evaporated to dryness with MeOH, until neutral. The sugars were identified by co-TLC with authentic samples, eluting with BuOH/AcOH/H_2O 4:2:1, and detected by spraying with anisaldehyde/H_2SO_4, followed by heating. The R_f values of glucose (Glc) and rhamnose (Rha) were 0.54 and 0.69, resp.

The abs. sugar configurations were determined as follows. To a soln. of the sugar residue in pyridine (60 µl), obtained from the hydrolysis, were added L-cysteine methyl ester hydrochloride and hexamethyldisilazine/Me_3SiCl 3:1. The mixture was stirred at 60° for 30 min. The resulting precipitate was removed by centrifugation, and the supernatant was concentrated and partitioned between hexane and H_2O. The org. layer was then analyzed by GC. By comparison with standard monosaccharides, D-Glc (t_R 12.45 min) and L-Rha (5.32 min) were identified for **1**–**6**.

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