On the Chemical Constituents of *Dipsacus asper*

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Bioassay-guided fractionation of 95% EtOH extract from the roots of *Dipsacus asper* lead to the isolation of some phenolic acids (caffeic acid, 2,6-dihydroxycinnamic acid, vanillic acid, 2′-*O*-caffeoyl-D-glucopyranoside ester, and caffeoylquinic acid) as the major active components, and five new iridoid glucoside dimers (1—5) and one new iridoid glucoside monomer (6), other known iridoid glycosides loganin, cantleyoside, triplostoside A, lisianthioside, 6′-*O*-β-D-apiofuranosyl sweroside, as well as triterpenoids oleanic acid and akebiasaponin D. The structures of new compounds 1—6 were determined as dipsanosides C (1), D (2), E (3), F (4), G (5), and 3′-*O*-β-D-glucopyranosyl sweroside (6) by spectroscopic, including 1D and 2D NMR techniques, and chemical methods.

Key words *Dipsacus asper*; dipsanoside C; dipsanoside D; dipsanoside E; dipsanoside F; dipsanoside G

Dipsacus asper WALL., a perennial plant distributed in mountainous regions of southwestern China, has been used in Chinese traditional folk medicines as antibacterial, anti-inflammatory, and anti-complement agents, and as growth stimulants of osseous cells as well.[1,2] In a recent report, the extract of *D. asper* was found to reduce the cognitive deficits and overexpression of β-amyloid protein induced by aluminum exposure.[3] Due to its numerous medicinal uses, extensive chemical investigations have been conducted on this plant.[4—8]

During the screening of plant extracts with cytotoxicity, the ethanolic extract of the roots of *D. asper* showed significant activity by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, so we began to investigate the chemical constituents responsible for its cytotoxicity. Bioassay-guided fractionation of the extract led to the isolation of main cytotoxic components, *e.g.*, caffeic acid, 2,6-dihydroxycinnamic acid, vanillic acid, 2′-*O*-caffeoyl-D-glucopyranoside ester, and caffeoylquinic acid, together with five new bis-iridoid glucosides dipsanosides C—G (**1**—**5**), one new iridoid glucoside 3′-*O*-β-D-glucopyranosyl sweroside (**6**), and the five known iridoid glucosides, loganin, cantleyoside, triplostoside A, lisianthioside, and 6′-*O*-β-D-apiofuranosyl sweroside as well as the triterpenoids oleanic acid and akebiasaponin D. All of the iridoidal glycoside monomers and dimers were found to be inactive in cytotoxic assays (see Table 4).

In a rapid communication, we reported the structure elucidation of two iridoid glycosides tetramers, the most complex iridoid glycosides hitherto known.[5] In this paper, we report the structural determination of the new iridoid glycosides **1**—**6** by various spectroscopic means, as well as the isolation and bioassay results of all above-mentioned compounds.

Results and Discussion

Many bis-iridoid glucosides have been isolated from Dipsacaceae, such as sylvestrosides I—IV from *D. sylvestris*,[9] and laciniatosides I—V from *D. laciniatus*.[10,11] Bis-iridoids have also been reported from *D. asperoides*,[12] *D. ferox*[13] and from *D. japonica*,[14] but none to date from *D. asper*. Bis-iridoids, composed of secologanin and loganin units, have so far been reported to have the same stereochemistry as the model compound cantleyoside, with a β-configuration at the C-1 position.

Compound **1**, white powder, $[\alpha]_D^{20}$ −34.8° (c=0.2095, MeOH), showed IR absorption bands due to the hydroxyl functions (3431 cm^{-1}) and α,β-unsaturated ester carbonyl groups (1701, 1637 cm^{-1}), as well as UV absorption maximum at 234.4 nm due to the α,β-unsaturated carbonyl groups. The molecular formula, $C_{36}H_{52}O_{21}$, was confirmed by high resolution (HR)-ESI-MS. Acid hydrolysis of **1** gave D-glucose, which was identified by HPLC and TLC analysis according to the reported procedure in the literature.[15] The ^{13}C- and ^{1}H-NMR spectroscopic data for compound **1** showed characteristic peaks of bis-iridoid glucosides. Unit A, a secoiridoid moiety, had proton signals almost identical to those of secologanic acid.[11] The significant differences were the low-field shift of H-7a (+0.83 ppm) and C-7a (+44.0 ppm), due to the acetal group in **1** instead of the hydroxyl group of secologanic acid. In the ^{1}H- and ^{13}C-NMR spectra of **1**, the existence of two methylenes [δ_H 3.72 (dd, J=5.0, 8.0 Hz, H-13a), 3.80 (dd, J=7.0, 8.0 Hz, H-13a); 3.50 (dd, J=5.0, 11.0 Hz, H-14a), 3.45 (dd, J=5.5, 11.0 Hz, H-14a); δ_C 68.1 (C-13a), 64.1 (C-14a)] and one methine [δ_H 4.02 (ddd, J=5.0, 5.5, 7.0 Hz, H-12a); δ_C 77.7 (C-12a)] suggested the presence of a 4-hydroxymethyl-1,3-dioxolane moiety, and this was further confirmed by long-range correlations from H-13a to both C-7a and C-14a, and H-14a to C-13a in HMBC spectrum. For unit B, the ^{13}C- and ^{1}H-NMR spectroscopic data were almost identical to those of loganin[15] except for downfield shifts of C-7b (+3.0 ppm) and H-7b, as a result of a deshielding effect due to esterification of the C-7b OH-group. Consequently, unit A and unit B are linked by an ester bond between C-7b and C-11a, this being confirmed by HMBC analysis. The stereochemistries of the seco-loganin and loganin portions in **1** were determined based on NOE observations and the coupling constants (see Table 1). For the 1,3-dioxolan part, on irradiation at H-7a, the enhancement of singals H-6a, H-12a, H-13a along with the magnitudes of the coupling constants $J_{12a,13a}$ (5.5, 7.0 Hz) indicated that H-12a should be axial. Thus, H-7a and H-12a should be in a *cis* relationship. Hence, the structure of **1**, named as dipsanoside C, was elucidated as shown in Fig. 1.

Compound **2**, white amorphous powder, had the same ele-

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Table 1. ^{1}H-NMR Spectroscopic Data for Compounds **1**—**4** in CD_3OD (500 MHz)

	1	**2**	**3**	**4**
1a	5.50 (d, 6.0)	5.49 (d, 5.5)	5.46 (d, 6.0)	5.49 (d, 6.0)
3a	7.38 (s)	7.38 (s)	7.38 (s)	7.39 (s)
5a	2.97 (br q, 5.5)	2.96 (br q, 6.0)	2.94 (ddd, 4.0, 6.0, 9.0)	2.94 (br q, 6.0)
6a	1.70 (dddd, 2.0, 4.0, 6.0, 14.0)	1.77 (ddd, 4.0, 6.0, 14.0)	1.68 (ddd, 6.0, 7.0, 14.0)	1.68 (ddd, 6.0, 6.0, 14.0)
	1.95 (ddd, 6.0, 6.0, 14.0)	1.90 (ddd, 6.0, 6.0, 14.0)	1.87 (ddd, 4.0, 6.5, 14.0)	1.93 (ddd, 6.0, 6.0, 14.0)
7a	4.93 (dd, 4.0, 6.0)	5.06 (dd, 4.0, 6.0)	4.50 (dd, 6.5, 7.0)	4.66 (q, 6.0)
8a	5.70 (ddd, 9.0, 10.5, 17.5)	5.70 (ddd, 9.0, 10.5, 17.5)	5.70 (ddd, 8.0, 10.5, 17.0)	5.71 (ddd, 8.0, 11.0, 17.0)
9a	2.67 (ddd, 5.5, 6.0, 9.0)	2.67 (ddd, 5.5, 6.0, 9.0)	2.62 (ddd, 6.0, 8.0, 9.0)	2.62 (br dd, 6.0, 8.0)
10a	5.24 (d, 17.5)	5.24 (d, 17.5)	5.24 (d, 17.0)	5.25 (d, 17.0)
	5.20 (d, 10.5)	5.20 (d, 10.5)	5.19 (d, 10.5)	5.19 (d, 11.0)
12a	4.02 (ddd, 5.0, 5.5 ,7.0)	4.06 (ddd, 3.0, 4.5, 11.5)	3.98 (ddd, 1.5, 5.0, 10.5)	3.79—3.88 (overlapped)
			3.19—3.32 (overlapped)	3.20—3.33 (overlapped)
13a	3.72 (dd, 5.0, 8.0)	3.59—3.85 (overlapped)	3.66 (dd, 5.0, 10.0)	3.60 (dd, 3.0, 5.5)
	3.80 (dd, 7.0, 8.0)	4.04 (dd, 4.5, 7.5)		
14a	3.50 (dd, 5.0, 11.0)	3.51—3.56 (overlapped)	4.02 (ddd, 1.5, 5.0, 10.5)	3.79—3.88 (overlapped)
	3.45 (dd, 5.5, 11.0)		3.19—3.32 (overlapped)	3.20—3.33 (overlapped)
1′a	4.63 (d, 8.0)	4.63 (d, 8.0)	4.63 (d, 8.0)	4.65 (d, 8.0)
2′a	3.14 (dd, 8.0, 9.0)	3.11—3.33	3.13—3.17 (dd, 6.5, 8.0)	3.12—3.16
3′a	3.31 (t, 8.5)	3.11—3.33	3.19—3.32	3.20—3.33
4′a	3.20—3.28	3.11—3.33	3.19—3.32	3.20—3.33
5′a	3.20—3.28	3.11—3.33	3.19—3.32	3.20—3.33
6′a	3.61 (dd, 5.5, 12.0)	3.59—3.85	3.59—3.68	3.59—3.64
	3.80 (br d, 11.5)	3.84 (d, 11.5)	3.83—3.86	3.81—3.88
1b	5.27 (d, 4.0)	5.27 (d, 4.0)	5.28 (d, 4.5)	5.25 (d, 4.0)
3b	7.36 (s)	7.36 (s)	7.35 (s)	7.36 (s)
5b	3.06 (br q, 8.0)	3.06 (br q, 8.0)	3.07 (q, 7.5)	3.08 (br ddd, 7.0, 7.5, 9.0)
6b	1.82 (ddd, 4.0, 8.0, 14.0)	1.70 (ddd, 5.0, 9.0, 14.0)	1.69 (ddd, 5.0, 7.5, 14.5)	1.69 (ddd, 5.0, 7.0, 14.0)
	2.25 (dd, 8.0, 14.0)	2.25 (br dd, 8.0, 14.0)	2.24 (br dd, 7.5, 14.5)	2.25 (dd, 7.5, 14.0)
7b	5.13 (dd, 4.0, 8.0)	5.12 (dd, 3.5, 5.0)	5.11 (dd, 5.0, 7.5)	5.14 (br s)
8b	2.03—2.09 (m)	2.03—2.13 (m)	2.03—2.06 (m)	2.08 (overlapped)
9b	2.12 (ddd, 4.0, 8.0, 9.0)	2.11 (ddd, 4.0, 8.0, 9.5)	2.18 (ddd, 4.5, 7.5, 9.0)	2.08 (overlapped
10b	1.01 (3H, d, 7.0)	1.02 (3H, d, 6.5)	1.02 (3H, d, 6.5)	1.01 (3H, d, 6.0)
MeO	3.64 (3H, s)	3.85 (3H, s)	3.63 (3H, s)	3.64 (3H, s)
1′b	4.60 (d, 8.0)	4.60 (d, 8.0)	4.60 (d, 8.0)	4.59 (d, 8.0)
2′b	3.14 (dd, 8.5, 8.0)	3.11—3.33	3.13—3.17 (dd, 6.5, 8.5)	3.12—3.16
3′b	3.31 (t, 8.5)	3.11—3.33	3.19—3.32	3.19—3.33
4′b	3.28—3.20	3.11—3.33	3.19—3.32	3.19—3.33
5′b	3.28—3.20	3.11—3.33	3.19—3.32	3.19—3.33
6′b	3.61 (dd, 5.5, 12.0)	3.51—3.56	3.59—3.68	3.59—3.64
	3.80 (br d, 11.5)		3.83—3.86	3.81—3.88

DEPT, HMQC, NOESY, and HMBC experiments resulted in the unambiguous assignments of all proton signals.

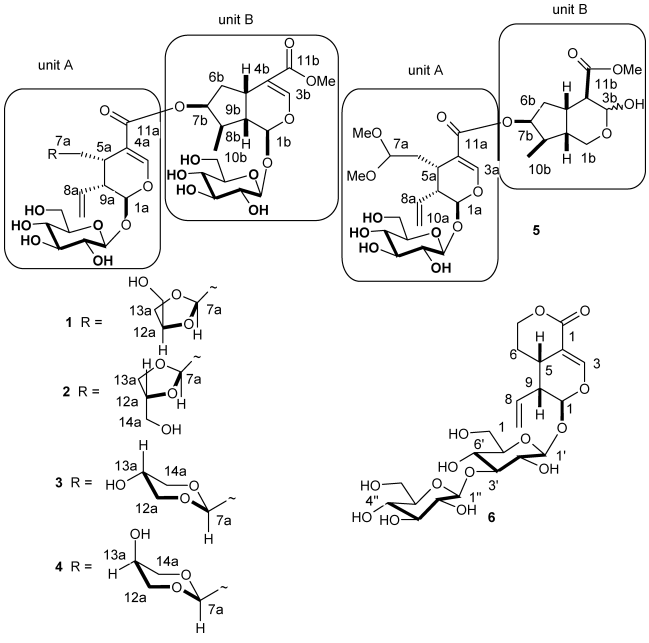

Fig. 1. Structures of Compounds **1**—**6**

mental composition and spectroscopic properties showing it to be an isomer of **1**. The NMR spectra of **2** (Tables 1, 3) were almost identical to those of **1** except for the 4-hydroxymethyl-1,3-dioxolane moiety. The NMR spectra also exhibited signals of one methine [δ_H 4.06 (ddd, J=3.0, 4.5, 11.5 Hz, H-12a), δ_C 77.4] and two methylenes [δ_H 3.59—3.85 (overlapped, H-13a), 4.04 (dd, J=4.5, 7.5 Hz, H-13a), 3.51—3.56 (2H, overlapped, H-14a), δ_C 68.0 and 63.4]. However, the observable NOEs between H-14a and H-7a along with the magnitudes of the coupling constants $J_{12a,13a}$ (3.0, 4.5 Hz) suggested the relationship between H-7a and H-12a was *trans*, and thus H-12a should be equatorial. As a result, compound **2**, named dipsanoside D, has the structure shown in Fig. 1.

Compound **3** was obtained as a white amorphous powder. The molecular formula of **3**, $C_{36}H_{52}O_{21}$, was the same as those of **1** and **2**. The ^{1}H- and ^{13}C-NMR spectra of **3** (see Tables 1, 3) were also similar to those of **1** and **2**, except for chemical shifts owing to a 7a-acetal function [δ_H 4.50 (dd, J=6.5, 7.0 Hz), δ_C 101.7], 13a-CH [δ_H 3.66 (dd, J=5.0, 10.0 Hz), δ_C 61.9], 12a,14a-CH_2 [δ_H 4.02 (ddd, J=1.5, 5.0, 10.5 Hz, H-14a), 3.98 (ddd, J=1.5, 5.0, 10.5 Hz, H-12a),

Table 2. ^{1}H-NMR Spectroscopic Data for Compounds **5** and **6** in CD_3OD (500 MHz)

No.	**5** (major)	No.	**5** (major)	No.	**6**
1a	5.45 (d, 5.5)	1b	3.80—3.88 (2H, br q, 11.0)	1	5.49 (d, 1.5)
3a	7.35 (s)	3b	4.66 (dd, 2.0, 6.5)	3	7.53 (s)
4a		4b	2.17 (dd, 6.5, 9.0)		
5a	3.13 (ddd, 2.0, 7.5, 8.0)	5b	2.43—2.48 (m)	5	3.05—3.11 (m)
6a	1.56—1.58 (m)	6b	1.75—1.82 (m)	6	1.61—1.74 (2H, m)
	1.92—1.96 (m)		2.10 (ddd, 4.5, 6.0, 7.0)		
7a	5.22 (dd, 4.0, 6.0)	7b	4.45 (br d, 4.5)	7	3.83 (ddd, 1.5, 2.5, 10.0)
					4.32 (ddd, 3.0, 9.0, 10.0)
8a	5.67 (ddd, 8.5, 10.0, 18.0)	8b	2.00 (m)	8	5.49 (ddd, 5.5, 10.5, 20.5)
9a	2.84 (br dd, 5.5, 8.5)	9b	2.61 (br d, 4.0)	9	2.65 (br dd, 5.5, 8.5)
10a	5.24 (d, 18.0)	10b	0.93 (d, 7.0)	10	5.25 (d, 20.5)
	2.10 (d, 10.0)				5.22 (d, 10.5)
MeO	3.25 (6H, s)	MeO	3.64 (3H, s)		
1′a	4.60 (d, 7.5)			1′	4.68 (d, 8.0)
2′a	3.08—3.31			2′	3.19—3.40
3′a	3.08—3.31			3′	4.38—4.41
4′a	3.08—3.31			4′	3.19—3.40
5′a	3.08—3.31			5′	3.19—3.40
6′a	3.58—3.64			6′	3.51—3.65
	3.08—3.31				3.51—3.65
				1″	4.51 (d, 8.0)
				2″	3.19—3.40
				3″	3.19—3.40
				4″	3.19—3.40
				5″	3.19—3.40
				6″	3.51—3.65
					3.51—3.65

3.19—3.32 (2H, overlapped, H-12a, H-14a), δ_C 72.6, 71.4]. By comparison with **1** and **2**, the large upfield shift for C-13a (−6.1 ppm), the smaller upfield shift for C-7a (−3.0 ppm), and the similar chemical shifts for C-12a and C-14a suggested that a 5-hydroxy-1,3-dioxane moiety in **3** took the place of 4-hydroxymethyl-1,3-dioxolane moiety in **1** and **2**. The HMBC spectrum gave additional evidence for this by showing long-range correlations from both H-12a and H-14a to C-7a. The orientation of H-13a was suggested to be axial by observation of the coupling constants $J_{12a,13a}$ (5.0, 10.0 Hz) and $J_{13a,14a}$ (5.0, 10.0 Hz), along with the NOESY correlations between H-7a and H-12a, H-7a and H-14a. Consequently, compound **3**, namely dipsanoside E, was elucidated as shown in Fig. 1.

Compound **4**, white amorphous powder, also showed IR and UV spectra typical for an enol ether system conjugated to a carbonyl group (UV 232 nm; IR 3388, 1693, 1631 cm^{-1}). Its molecular formula, $C_{36}H_{52}O_{21}$, is also the same as those of **1**, **2**, and **3**. In fact, its NMR spectra were almost identical to those of **3**, except for differences in the 5-hydroxy-1,3-dioxane moiety. Namely, H-7a, C-7a, and C-13a were shifted downfield to 4.66, 102.3, and 65.0, and H-12a and H-14a upfield to 3.79—3.88 by comparison with **3**. Moreover, the coupling constants $J_{12a,13a}$ (3.0, 5.5 Hz) and $J_{14a,13a}$ (3.0, 5.5 Hz), along with NOE correlations between H-12a and H-7a, H-14a and H-7a, suggested that H-13a should be equatorial. Consequently, the structure of **4**, named dipsanoside F, was elucidated as shown in Fig. 1.

Compound **5** was obtained as a white amorphous powder. Its molecular formula, $C_{29}H_{44}O_{15}$, was established by high resolution (HR)-ESI-MS. The IR spectrum indicated the presence of hydroxyl (3417 cm^{-1}), ester (1732 cm^{-1}), and α,β-conjugated ester (1699, 1633 cm^{-1}) groups in **5**. Acid hydrolysis of **5** gave D-glucose, and suggested the presence of one glucose moiety. The ^{1}H- and ^{13}C-NMR spectroscopic signals of **5** were almost identical to those of laciniatoside I,[15] except for presence of resonances [δ_H 5.22 (dd, J=4.0, 6.0 Hz, H-7a), 3.25 (6H, s), δ_C 104.3, 52.8, 52.3] attributed to the dimethoxy moiety, instead of the signal for an aldehyde group in laciniatoside I. In the HMBC spectrum, both protons for two methoxyl groups showed long range correlations with C-7a confirming this connectivity. Similar to laciniatoside I, compound **5** occurs as a mixture of two isomers. Two sets of signals for unit B were observed in the NMR spectra of **5**, although with so significantly different intensity ratios that the minor ones could be ignored. Accordingly the structure of dipsanoside G (**5**) was elucidated as shown in Fig. 1.

Compound **6** was isolated as a white amorphous powder. Its molecular formula was determined as $C_{22}H_{32}O_{14}$ by (HR)-FAB-MS. Acid hydrolysis of **6** gave D-glucose. The ^{1}H-NMR spectrum (Table 2) of **6** was essentially in agreement with that of sweroside[10]; however, two anomeric proton signals [δ_H 4.68 (d, J=8.0 Hz, H-1′), 4.51 (d, J=8.0 Hz, H-1″)] were recognized. The ^{13}C-NMR spectrum (Table 3) of the aglycone part of **6** showed a close similarity to the aglycone of sweroside,[10] except that two sets of additional signals, corresponding to β-D-glucopyranosyl groups were present. The downfield shift of C-3′ and smaller upfield shifts of C-2′ and C-4′ of **6** were ascribed to glycosidation of the hydroxyl group at C-3′. This was confirmed by observation of a long-range correlation from H-1″ (δ_H 4.51) to C-3′ (δ_C 87.3) in the HMBC spectrum. Based on this evidence, the structure of **6** was determined to be 3′-*O*-β-D-glucopyranosyl sweroside.

It is well known that the skeleton of iridoids are not very stable, and with stringent extraction conditions, it is possible

they are isolated in artifact form. In order to confirm that compounds **1**—**6** are not artifacts, EtOH extracts of *D. asper* were prepared with cold EtOH and with boiling EtOH, respectively. Compounds **1**—**6** could be detected by HPLC in both extracts. Since cantleyoside is the major constituent isolated from this plant, and compounds **1**—**5** may be obtained as the adducts of cantleyoside from the structural point of view, so it is necessary to exclude the possibility of these adducts being artifacts. After these compounds were treated in boiling EtOH and MeOH for 4 h, as well as in the glycerine for 2 d, no derivatives were detected by HPLC. It was also important to mention that no glycerine was used in the experiment, and glycerine was not isolated from the title plant. Therefore, we concluded that all of the compounds are not artifacts formed during extraction and isolation.

Table 3. ^{13}C-NMR Spectroscopic Data for Compounds **1**—**6** in CD_3OD (125 MHz)

No.	1	2	3	4	5	6
1a	97.7	97.7	97.7	97.7	97.8	98.1
3a	153.5	153.4	153.3	153.3	153.2	154.0
4a	113.5	113.5	113.6	113.4	112.1	105.2
5a	30.0	30.0	30.0	32.5	29.3	28.5
6a	35.2	32.3	35.7	36.0	44.3	25.9
7a	104.8	104.5	101.7	102.3	104.3	69.7
8a	135.8	135.8	135.9	135.9	135.8	133.3
9a	45.4	45.4	45.5	45.5	44.3	43.8
10a	119.8	119.8	119.7	119.6	119.8	120.9
11a	168.4	168.4	168.4	168.4	168.3	168.5
MeO					52.8, 52.3	
12a	77.7	77.4	72.6	72.5		
13a	68.1	68.0	61.9	65.0		
14a	64.1	63.4	71.4	72.3		
1′a	100.2	100.2	100.2	100.3	100.1	99.5
2′a	74.7	74.7	74.7	74.7	74.6	74.1
3′a	78.0	78.0	78.0	78.4	78.0	87.3
4′a	71.6	71.6	71.5	71.6	71.6	70.0
5′a	78.4	78.4	78.4	78.4	78.4	77.8
6′a	62.8	62.8	62.7	62.8	62.8	62.5
1b	97.2	97.3	97.2	97.4	64.8	
3b	152.3	152.3	152.2	152.4	97.1	
4b	111.8	111.9	112.0	112.2	54.0	
5b	32.3	35.2	32.1	29.7	39.9	
6b	40.4	40.4	40.5	40.4	38.2	
7b	78.6	78.6	78.6	78.0	78.1	
8b	40.9	40.9	40.9	41.0	38.7	
9b	47.0	47.1	46.9	47.1	43.8	
10b	13.5	13.5	13.4	13.7	12.4	
11b	169.3	169.3	169.4	169.4	175.1	
MeO	51.7	51.7	51.7	51.7	53.7	
1′b	100.1	100.1	100.1	100.2		106.0
2′b	74.7	74.7	74.6	74.7		75.5
3′b	78.0	78.0	78.0	78.4		78.1
4′b	71.6	71.6	71.4	71.6		71.6
5′b	78.4	78.4	78.3	78.4		78.2
6′b	62.7	62.8	62.6	62.8		62.6

DEPT, HMQC, and HMBC experiments resulted in the unambiguous assignments of all carbon signals.

Chemotaxonomic Significance of *D. asper* Bis-iridoids Bis-iridoids, found as natural products in a large number of plant families, can be classified into three subtypes: iridoid/iridoid and secoiridoid/secoiridoid homodimers, and secoiridoid/iridoid heterodimers. The bis-iridoids isolated from *Dipsacus* (the *Dipsacus* bis-iridoids), including *D. ferox*, *D. laciniatus*, *D. japonicus*, *D. sylvestris*, and *D. asperoides*, were found to possess a secoiridoid/iridoid subtype skeleton consisting of secologanic acid condensed to the 7-OH of loganin or loganin-like iridoids. None of bis-iridoid glucosides have been reported prior to our studies on chemical constituents on *D. asper*, and our results demonstrated close relationship of *D. asper* to other *Dipsacus* species in terms of phytochemical substances. The genus *Dipsacus* contains 20 species, among of which 9 species have been explored to some extents, and the *Dipsacus* bis-iridoids were found in the 6 species after thorough chemical studies. Hence, *Dipsacus* bis-iridoids can be regarded as the chemotaxonomic markers of the *Dipsacus* genus.

Concluding Remarks Bioassay-guided fractionation led to the identification of simple derivatives of caffeic acid and cinnamic acid as the cytotoxic compounds in *D. asper*. Meanwhile, some novel and known iridoid glycosides were also isolated during this process. Although it was a little disappointing to find that the iridoid glycosides are non-cytotoxic, their discovery in *D. asper* added new pieces of evidences for their significance in chemotaxonomy of the *Dipsacus* genus.

Experimental

General Experimental Procedures Optical rotations were recorded on a PE-343 polarimeter. Mass spectra were obtained on an Autospec-Ultima ETOF. ESI-MS measurements were carried out at an Agilent 1100 series LC/MSD Trap SL mass spectrometer. IR spectra were recorded on a Nicolet-Impact 400 IR spectrometer with KBr disk, whereas UV spectra were acquired on an HP 8453 spectrophotometer. NMR experiments were performed on an INOVA-500 spectrometer using TMS as internal standard. Silica gel for column chromatography and silica gel GF_{254} for TLC were obtained from Qingdao Marine Chemical Company, China. RpC-18 (40—60 μm) silica gel and macroporous resin D_{101} were purchased from Fuji Silysica Chemical Ltd. Size-exclusion chromatography was performed using Sigma Lipophilic Sephadex LH-20. Analytical HPLC was carried out on an Agilent 1100 series. Preparative HPLC was carried out on an Shimadzu LC-9A using an ODS-column (Nihon Waters Ltd.).

Plant Material The roots of *Dipsacus asper* WALL. were collected from Dali, Yunnan Province of China, in October 2001. It was identified by Professor Guangming Liu, Dali Medical College. The authenticated sample of the plant was deposited at the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences (No. 79), Beijing.

Extraction and Isolation Dried roots (2.0 kg) of *D. asper* were powdered and extracted three times with boiling ethanol in round-bottom flask (10 l) using an electric jacket (10 l, 2200 W) as a heater. The combined

Table 4. The IC_{50} (μg/ml) Values of Active Compounds from the Butanol Soluble Portion

Compounds	A549	Bel7402	BGC-823	HCT-8	A2780
2,6-Dihydroxycinnamic acid	3.883	7.346	4.321	—	—
Vanillic acid	—	6.437	—	5.218	7.395
2′-*O*-Caffeoyl-D-glucopyranoside ester	5.663	5.545	6.432	5.700	6.380
Caffeoylquinic acid	5.713	5.586	6.204	5.370	6.679
FU	0.177	0.542	0.695	0.670	0.569

The symbol "—" represents inactivity.

ethanol extracts were concentrated *in vacuo* to furnish a dark brown residue (390 g), which was suspended in H_2O and partitioned in turn with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH extract was evaporated under reduced pressure to yield a residue (360 g). The latter was separated by column chromatography on macroporous resin D_{101} with a EtOH : H_2O (0 : 100, 20 : 80, 40 : 60, 60 : 40, 80 : 20, 100 : 0) gradient system to give five fractions (XD-B-1—5), and their cytotoxic activities were tested (see Table 4). The active fraction XD-B-3 (30 g) was applied to a Sephadex LH-20 column using MeOH : H_2O (80 : 20) to yield six subfractions (XD-B-3-1—6). The active subfraction XD-B-3-2 (10 g) was further separated by CC over Rp-18 using a MeOH : H_2O (10 : 90, 30 : 70, 40 : 60, 60 : 40, 0 : 100) gradient system to give six subfractions (XD-B-3-2-1—6). Active subfraction XD-B-3-2-3 (3.6 g), containing iridoid glucosides, was subjected to a series of purification steps using Sephadex LH-20 (MeOH : H_2O, 80 : 20) and Rp-18 (CH_3CN : H_2O, 20 : 80) CC, preparative TLC ($CHCl_3$: MeOH : H_2O, 75 : 25 : 10), and finally purified by preparative reversed phase HPLC on a C-18 column using CH_3CN : H_2O (20 : 80) as mobile phase to afford **1** (20 mg), **2** (10 mg), **3** (8 mg), **4** (8 mg), **5** (6 mg), **6** (6 mg), loganin (200 mg), cantleyoside (500 mg), triplostoside A (300 mg), lisianthioside (100 mg), 6′-*O*-*β*-D-apiofuranosyl sweroside (10 mg), caffeic acid (50 mg), 2,6-dihydroxycinnamic acid (7 mg), vanillic acid (5 mg), 2′-*O*-caffeoyl-D-glucopyranoside ester (10 mg), caffeoylquinic acid (15 mg), oleanic acid (6 mg), and akebiasaponin D (50 mg).

Dipsanoside C (**1**): White amorphous powder; $[\alpha]_D^{20}$ −34.8° (*c*=0.209, MeOH); UV λ_{max}^{MeOH} nm: 234.4; IR ν_{max}^{KBr} cm^{-1}: 3431, 1701, 1637, 1292, 1074; The ^1H- and ^{13}C-NMR spectroscopic data, see Tables 1 and 3, respectively; ESI-MS *m*/*z* 843.3 $[M+Na]^+$; HR-ESI-MS *m*/*z* 843.2903 (Calcd for $C_{36}H_{52}O_{21}Na$, 843.2899).

Dipsanoside D (**2**): White amorphous powder; $[\alpha]_D^{20}$ −36.6° (*c*=0.260, MeOH); UV λ_{max}^{MeOH} nm: 236.4; IR ν_{max}^{KBr} cm^{-1}: 3408, 1697, 1633, 1570, 1290, 1074, 1018; The ^1H- and ^{13}C-NMR spectroscopic data, see Tables 1 and 3, respectively; ESI-MS *m*/*z* 843.2 $[M+Na]^+$; HR-ESI-MS *m*/*z* 843.2827 (Calcd for $C_{36}H_{52}O_{21}Na$, 843.2899).

Dipsanoside E (**3**): White amorphous powders; $[\alpha]_D^{20}$ −28.3° (*c*=0.198, MeOH); UV λ_{max}^{MeOH} nm: 234.0; IR ν_{max}^{KBr} cm^{-1}: 3446, 1695, 1635, 1624, 1074; The ^1H- and ^{13}C-NMR spectroscopic data, see Tables 1 and 3, respectively; ESI-MS *m*/*z* 843.3 $[M+Na]^+$; HR-ESI-MS *m*/*z* 843.2904 (Calcd for $C_{36}H_{52}O_{21}Na$, 843.2899).

Dipsanoside F (**4**): White amorphous powder; $[\alpha]_D^{20}$ −10.2° (*c*=0.885, MeOH); UV λ_{max}^{MeOH} nm: 235.2; IR ν_{max}^{KBr} cm^{-1}: 3388, 1693, 1631, 1074; The ^1H- and ^{13}C-NMR spectroscopic data, see Tables 1 and 3, respectively; ESI-MS *m*/*z* 843.3 $[M+Na]^+$; HR-ESI-MS *m*/*z* 843.2909 (Calcd for $C_{36}H_{52}O_{21}Na$, 843.2899).

Dipsanoside G (**5**): White amouphous powder; $[\alpha]_D^{20}$ −3.4° (*c*=1.277, MeOH); UV λ_{max}^{MeOH} nm: 235.0; IR ν_{max}^{KBr} cm^{-1}: 3417, 1732, 1699, 1633, 1385, 1074; The ^1H- and ^{13}C-NMR spectroscopic data, see Tables 2 and 3, respectively; ESI-MS *m*/*z* 655.5 $[M+Na]^+$; HR-ESI-MS *m*/*z* 655.2602 (Calcd for $C_{29}H_{44}O_{15}Na$, 655.2578).

3′-*O*-*β*-D-Glucopyranosyl sweroside (**6**): White amorphous powder; UV λ_{max}^{MeOH} nm: 242.6, 206.8; IR ν_{max}^{KBr} cm^{-1}: 3404, 1697, 1616, 1072, 987; The ^1H- and ^{13}C-NMR spectroscopic data, see Tables 2 and 3, respectively; FAB-MS *m*/*z* 543.1 $[M+Na]^+$; HR-FAB-MS *m*/*z* 543.1725 (Calcd for $C_{22}H_{32}O_{14}Na$, 543.1690).

Identification of Sugars According to the reported procedures in the literature,[15] the identification of the sugar was determined by comparison of the retention times of derivatives of sugars obtained upon the hydrolysis mixture with those of standard samples using HPLC, which were performed with an Inertsil sil-100A column (250×4.6 mm, 5 μm, Dikma) eluting with *n*-hexane/ethanol (95 : 5); flow rate 1.2 ml/min; detection at 230 nm, 0.04 aufs. The retention times of the derivatives of the sugars were as follows: D-glucose 40.0 min, L-glucose 39.0 min.

Detection of Compounds 1—5 Dried roots of *D. asper* (11 g) were powdered and divided into two equal sections. One section (5.1 g) was extracted with 95% EtOH at room temperature and gave the extract A (1.683 g), while the other was extracted with boiling EtOH and afforded extract B (1.628 g). Both ethanol extracts were suspended in water and partitioned in turn with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The *n*-BuOH solubles were evaporated under reduced pressure to yield residue A-4 (0.6696 g) and B-4 (1.1921 g), respectively. A-4 and B-4 were redissolved in MeOH (2 ml), respectively, and then detected by HPLC (Dima-ZY1104 ODS C-18, 150×4.6 mm; eluent, 20% CH_3CN; flow rate, 1.0 ml/min; detection UV at 240 nm). Compounds **1**—**5** were eluted at 9.47 min, 10.63 min, 11.36 min, 8.34 min, and 29.98 min, respectively.

Cytotoxicity Assay Cytotoxic activities of those compounds were evaluated using five human tumor cell lines including lung carcinoma A549, hepatoma Bel7402, gastric carcinoma BGC-823, colon cancer HCT-8, and ovary cancer A2780, all of which were obtained from the American Type Culture Collection (ATCC). Fluorouracil (FU) was used as a positive control, with cells continuously treated with samples for 96 h. The supernatant was doffed off and 0.1 ml MTT (0.4 mg/ml in RPMI 1640) was added after each well had been carefully washed with RPMI 1640. The cell viabilities were measured with an MTT assay procedure.[16]

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