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Iridoids from *Pedicularis kansuensis* forma *albiflora*

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A new iridoid glycoside kansuenoside (**1**) and a new iridoid kansuenin (**2**), along with eight known compounds (**3**–**10**) were isolated from the whole plant of *Pedicularis kansuensis* forma *albiflora* Li. Their structures were elucidated by spectroscopic methods. Nine of them were assayed against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus.*

1. Introduction

The genus *Pedicularis* comprises about 329 species in China [1]. The roots and stems of many of them are used as Chinese folk medicines as cardiac tonics for the treatment of collapse, exhaustion and senility [2], and are usually called 'pseduo-ginseng' by local inhabitants. In continuation of our research on *Pedicularis* species [3–8], we report here the isolation and structural elucidation of two new iridoids, an iridoid glycoside kansuenoside (**1**), and an iridoid kansuenin (**2**), as well as eight known compounds (**3**–**10**) from the whole plant of *Pedicularis kansuensis* forma *albiflora*.

2. Investigations, results and discussion

From the 95% ethanol extract of the whole plant of *Pedicularis kansuensis* forma *albiflora*, a new iridoid glycoside kansuenoside (**1**) and a new iridoid kansuenin (**2**) were isolated, together with eight known iridoid glycosides: ixoroside (**3**) [9, 10], euphroside (**4**) [11], mussaenoside (**5**) [12], boschnaloside (**6**) [13], 7-deoxy-8-epi-loganic acid (**7**) [14], 8-epi-loganic acid (**8**) [15], aucubin (**9**) [16, 17], and geniposidic acid (**10**) [18]. The structures of the known compounds were identified by comparing their corresponding properties (FAB-MS, ^{1}H NMR and ^{13}C NMR) with the reported values in the literature.

Compound **1** was obtained as white amorphous powder, The UV spectrum showed λ_{max}^{MeOH} at 249 (0.65) nm, assigned to two conjugated double bonds, its IR spectrum showed the presence of hydroxyl (3464, 3200 cm^{-1}), double bond (1637 cm^{-1}) and C–O–C (1077, 1007 cm^{-1}), The quasi-molecular ion peaks at *m/z* 351 $[M + Li]^+$ and *m/z* 367 $[M + Na]^+$ in FAB-MS, suggesting the molecular formula to be $C_{16}H_{24}O_8$, which was supported by ^{13}C NMR and DEPT data. The ^{13}C NMR spectrum of compound **1** contained 16 signals of which 6 could be assigned to a β-glucopyranosyl moiety. The remaining ten signals corresponded to an iridoid aglucone with two double bonds present but with no further substitution in the cyclopentane ring. In fact, the signal pattern was somewhat similar to that reported for montinioside [19], except that no xylosyl moiety was present in **1**. This was consistent with the position of the signal for C-11 (δ 58.3) which is 9 ppm upfield compared to that of C-11 (δ 67.4) in montinioside. Also, the shift for C-10 in **1** was seen at δ 14.9 demonstrating that the methyl group is in the α-position [20] and this is different from montinioside which has a β-methyl substituent (δ 19.3). The relative

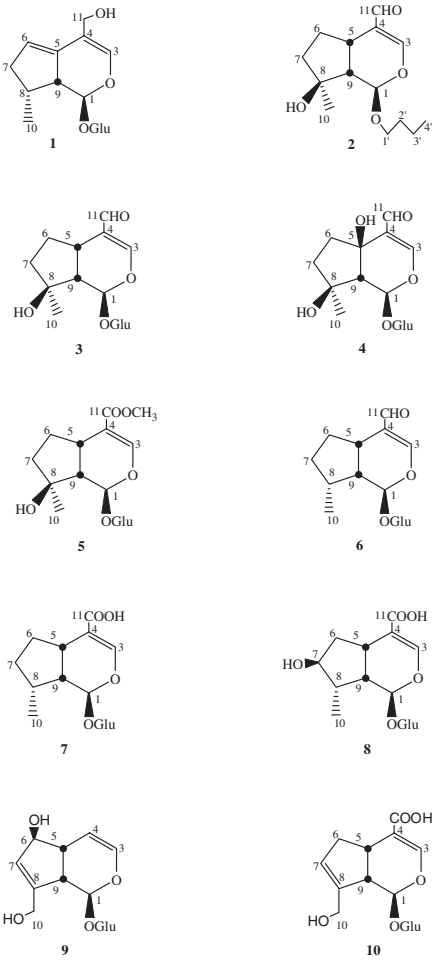

Table 1: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT data of 1 and 2 (δ_{ppm}, J_{Hz})

No.	^{1}H(α/β)		^{13}C (DEPT)	
	1	**2**	**1**	**2**
1	5.07 (d, 10.0)	4.95 (d, 4.4)	97.8 (CH)	100.2 (CH)
3	6.50 (s)	7.15 (s)	141.8 (CH)	160.7 (CH)
4	–	–	114.4 (C)	124.1 (C)
5	–	3.20 (m)	133.4 (C)	29.7 (CH)
6	5.53 (brs)	2.34 (m)/1.75 (m)	118.6 (CH)	28.8 (CH_2)
7	1.92 (m)/2.48 (m)	1.72 (m)/1.58 (m)	40.6 (CH_2)	40.5 (CH_2)
8	2.42 (m)	–	32.8 (CH)	79.9 (C)
9	2.58 (dd, 10.0, 6.5)	2.21 (dd, 4.4, 8.5)	48.6 (CH)	51.3 (CH)
10	0.84 (d, 6.8)	1.34 (s)	14.8 (CH_3)	24.8 (CH_3)
11	4.02 (brs)	9.25 (s)	58.3 (CH_2)	190.4 (CH)
1′	4.57 (d, 7.8)	3.87 (m)	98.8 (CH)	69.3 (CH_2)
2′	2.90–3.37	1.45 (m)	73.2 (CH)	31.5 (CH_2)
3′	2.90–3.37	1.38 (m)	76.7 (CH)	19.2 (CH_2)
4′	2.90–3.37	0.93 (t, 7.2)	70.1 (CH)	13.8 (CH_3)
5′	2.90–3.37	–	77.2 (CH)	–
6′	3.65 (d, 11.2)	–	61.3 (CH_2)	–

DMSO-d_6 as solvent for **1** and $CDCl_3$ as solvent for **2**

stereochemistry of **1** was also determined by NOESY experiments. Thus H-8 (δ 2.42) showed correlations with H-9 and the methyl group (δ 0.84) with H-1. Furthermore, no correlations were seen between H-1 (δ 5.07) and H-9, or between the methyl group (δ 0.84) and H-9. This proved the formula shown for **1**, and we have named the compound kasuenoside.

Compound **2**, colorless needle. Its EI-MS exhibited a molecular ion peak at *m/z* 254. The molecular formula was deduced as $C_{14}H_{22}O_4$ by its MS, ^{13}C NMR and DEPT spectra. The ^{13}C NMR spectra of compound **2** (Table 1) were similar to those of the known compound ixoroside [9] except for the presence of an *n*-butyl group instead of the β-D-glucopyranosyl at C-1 in ixoroside. The ^{13}C NMR data δ_C 51.3 (C-9) suggested the −OH at C-8 was β-oriented [21], through the NOESY experiment, the correlations of δ_H 4.95 (H-1) with δ_H 1.34 (H-10) and δ_H 3.20 (H-5) with δ_H 2.21 (H-9), and there were no correlations between δ_H 4.95 (H-1) and δ_H 2.21 (H-9), suggested that the H-1 and the $-CH_3$ at C-8 were α, the H-5 and H-9 were β-orientation. Thus, the structure of compound **2** was confirmed, and named kansuenin.

Compounds **3**–**10** were elucidated by spectroscopic methods. Nine compounds (**1**, **3**–**10**) were screened for antibacterial activity, the results are given in Table 2.

The preliminary results indicated that most of the compounds were active against the bacteria tested. Compound **8** and compounds **3**, **6**, **9** exhibited strong antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*.

Table 2: Antibacterial activity of compounds

Compd.	*B. subtilis*	*E. coli*	*Streptococcus*
1	+	++	++
3	++	++	+++
4	+	++	++
5	+	++	++
6	++	++	+++
7	+	++	++
8	+	+++	++
9	++	++	+++
10	–	++	++
H_20	–	–	–
Chloramphenicol	+++	+++	+++

Zone diameter of growth inhibition: <10 mm (–), 10–12 mm (+), 13–15 mm (++) and 16–20 mm (+ + +)

3. Experimental

3.1. Apparatus

Optical rotations: Perkin-Elmer 341 Polarimeter; IR: Nicolet 170SX FT-IR instrument; EI-MS: HP 5988A GC/MS instrument; FAB-MS: VG-ZAB-HS mass spectrometer (at 70 eV); ^{1}H NMR (400.13 MHz), ^{13}C NMR (100.16 MHz), ^{1}H, ^{1}H NOESY: Bruker AM-400 FT-NMR spectrometer; silica gel (200–300 mesh) for CC and silica GF254 for TLC were supplied by the Qingdao Marine Chemical factory.

3.2. Plant material

Pedicularis kansuensis forma *albiflora* Li was collected in the Gansu province of China, in August 1999. It was identified by Prof. Guo-Liang Zhang from the Department of biology of Lanzhou University. A voucher specimen (No. 990801) is deposited in the herbarium of our institute.

3.3. Extraction and isolation

The air-dried whole plant of *Pedicularis kansuensis* forma *albiflora* Li (2.3 kg) were powdered and extracted three times (7 days per time) at room temperature with 95% ethanol. The extract was concentrated under reduced pressure to yield residue (393 g), which was diluted with hot water and the water-insoluble material removed by filtration through Celite. The filtrate was extracted with EtOAc and *n*-BuOH. The *n*-BuOH portion (144 g) was chromatographed over a silica gel column (eluted with EtOAc : MeOH from 30 : 1 to 1 : 1), five fractions were collected according to TLC analysis. Fraction 1 (16 g) was chromatographed over a silica gel column (eluted with petroleum ether-Me_2CO from 30 : 1 to 1 : 1) to obtain **2** (3 mg); Fraction 2 (52 g) was isolated over a silica gel column (eluted with $CHCl_3$: MeOH 20 : 1 to 1 : 1) to get **1** (40 mg), **6** (5 mg) and **7** (28 mg) respectively. Fraction 3 (23 g) was subjected to a silica gel column (eluted with $CHCl_3-CH_3OH$ from 20 : 1 to 1 : 1) to obtain **3** (30 mg), **4** (18 mg), **5** (27 mg), **8** (19 mg). Fraction 4 (43 g) was applied to a silica gel column (eluted with $CHCl_3$–MeOH from 20 : 1 to 1 : 1) to afford **9** (37 mg) and **10** (21 mg).

3.3.1. Kansuenoside (**1**)

White amorphous powder. $[\alpha]_D^{24}$ −10 (c, 1.44, CH_3OH); IR (ν_{max}^{KBr}, cm^{-1}): 3464, 3200 (OH), 1637 (C=C), 1077, 1007 (C–O–C); FAB-MS *m/z* (3 NBA): 351.2 $[M + Li]^+$, 367.2 $[M + Na]^+$; ^{1}H NMR, ^{13}C NMR and DEPT data see Table 1.

3.3.2. Kansuenin (**2**)

Colorless needle. $[\alpha]_D^{23}$ −62 (c, 0.23, CH_3OH); EI-MS *m/z* (int.) 254 (1.5), 220 (3.8), 177 (42.8), 148 (61.1), 131 (49.5), 105 (79.3), 91 (89.4), 77 (28.2), 43 (100.0); ^{1}H NMR, ^{13}C NMR and DEPT data see Table 1.

3.3.3. Ixoroside (**3**)

White amorphous powder. FAB-MS *m/z*: 367.1 $[M + Li]^+$, 383.1 $[M + Na]^+$; ^{1}H NMR δ ppm (CD_3OD, 400 MHz): 5.52 (1 H, d, 4 Hz, H-1), 7.26 (1 H, s, H-3), 1.20 (3 H, s, H-10), 9.08 (1 H, s, H-11); ^{13}C NMR and DEPT δ ppm (CD_3OD, 100 MHz): 96.6 (CH, C-1), 163.2 (CH, C-3), 126.0 (C, C-4), 30.1 (CH, C-5), 29.7 (CH_2, C-6), 41.0 (CH_2, C-7), 80.3 (C, C-8), 52.2 (CH, C-9), 24.6 (CH_3, C-10), 193.1 (CH, C-11), 100.0 (CH, C-1′), 74.7 (CH, C-2′), 78.0 (CH, C-3′), 71.7 (CH, C-4′), 78.5 (CH, C-5′), 63.0 (CH_2, C-6′).

3.3.4. Euphroside (**4**)

White amorphous powder. FAB-MS *m/z*: 383.3 $[M + Li]^+$, 399.3 $[M + Na]^+$; ^{1}H NMR δ ppm (D_2O, 400 MHz): 5.92 (1 H, brs, H-1), 7.36 (1 H, s, H-3), 1.04 (3 H, s, H-10), 9.04 (1 H, s, H-11), 4.69 (1 H, d, 6.8 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (D_2O, 100 MHz): 95.9 (CH, C-1), 164.7 (CH, C-3), 125.1 (C, C-4), 71.4 (C, C-5), 37.2 (CH_2, C-6), 39.7 (CH_2, C-7), 79.4 (C, C-8), 60.8 (CH, C-9), 23.4 (CH_3, C-10), 194.9 (CH, C-11), 99.8 (CH, C-1′), 73.6 (CH, C-2′), 76.5 (CH, C-3′), 70.8 (CH, C-4′), 77.5 (CH, C-5′), 61.9 (CH_2, C-6′).

3.3.5. Mussaenoside (**5**)

White amorphous powder. FAB-MS *m/z*: 397.4 $[M + Li]^+$, 413.3 $[M + Na]^+$; ^{1}H NMR δ ppm (DMSO-d_6, 400 MHz): 5.30 (1 H, d, 4 Hz, H-1), 7.28 (1 H, s, H-3), 1.17 (3 H, s, H-10), 3.69 (3 H, s, OMe), 4.60 (1 H, d, 7.6 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (DMSO-d_6, 100 MHz): 93.4 (CH, C-1), 149.8 (CH, C-3), 112.0 (C, C-4), 30.4 (CH, C-5), 29.4 (CH_2, C-6), 39.5 (CH_2, C-7), 78.2 (C, C-8), 48.6 (CH, C-9), 24.4 (CH_3, C-10), 167.8 (C, C-11), 50.5 (CH_3, OMe), 98.0 (CH, C-1′), 73.1 (CH, C-2′), 76.7 (CH, C-3′), 70.1 (CH, C-4′), 77.2 (CH, C-5′), 61.2 (CH_2, C-6′).

3.3.6. Boschnaloside (**6**)

White amorphous powder. FAB-MS *m/z*: 351.2 $[M + Li]^+$, 367.2 $[M + Na]^+$; ^{1}H NMR δ ppm (CD_3OD, 400 MHz): 5.53 (1 H, d, 3.6 Hz, H-1), 7.27 (1 H, s, H-3), 0.98 (3 H, d, 6.2 Hz, H-10), 9.07 (1 H, s, H-11), 4.60 (1 H, d, 7.6 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (CD_3OD, 100 MHz): 97.5 (CH, C-1), 164.2 (CH, C-3), 126.3 (C, C-4), 31.3 (CH, C-5), 30.8 (CH_2, C-6), 33.6 (CH_2, C-7), 44.1 (CH, C-8), 37.1 (CH, C-9), 16.6 (CH_3, C-10), 193.0 (CH, C-11), 99.9 (CH, C-1′), 74.8 (CH, C-2′), 78.0 (CH, C-3′), 71.7 (CH, C-4′), 78.5 (CH, C-5′), 63.0 (CH_2, C-6′).

3.3.7. 7-Deoxy-8-epi-loganic acid (**7**)

White amorphous powder. ^{1}H NMR δ ppm (CD_3OD, 400 MHz): 5.45 (1 H, d, 4.8 Hz, H-1), 7.43 (1 H, s, H-3), 1.09 (3 H, d, 6.4 Hz, H-10), 4.70 (1 H, d, 7.6 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (CD_3OD, 100 MHz): 96.1 (CH, C-1), 152.8 (CH, C-3), 113.4 (C, C-4), 33.2 (CH, C-5), 32.3 (CH_2, C-6), 33.2 (CH_2, C-7), 34.6 (CH, C-8), 44.4 (CH, C-9), 16.7 (CH_3, C-10), 171.0 (C, C-11), 99.7 (CH, C-1′), 74.8 (CH, C-2′), 78.0 (CH, C-3′), 71.7 (CH, C-4′), 78.3 (CH, C-5′), 62.9 (CH_2, C-6′).

3.3.8. 8-Epi-loganic acid (**8**)

White amorphous powder. ^{1}H NMR δ ppm (DMSO-d_6, 400 MHz): 5.36 (1 H, d, 4 Hz, H-1), 7.28 (1 H, s, H-3), 0.92 (3 H, d, 7.2 Hz, H-10), 4.46 (1 H, d, 8 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (DMSO-d_6, 100 MHz): 94.2 (CH, C-1), 150.2 (CH, C-3), 112.6 (C, C-4), 29.6 (CH, C-5), 40.1 (CH, C-6), 76.7 (CH, C-7), 41.1 (CH, C-8), 43.5 (CH, C-9), 13.9 (CH_3, C-10), 167.9 (C, C-11), 98.1 (CH, C-1′), 73.2 (CH, C-2′), 76.7 (CH, C-3′), 70.1 (CH, C-4′), 77.3 (CH, C-5′), 61.3 (CH_2, C-6′).

3.3.9. Aucubin (**9**)

White amorphous powder. $[\alpha]_D^{23}$ −149 (c, 1.99, H_2O); HRESI-MS: 369.1162 $[M + Na]^+$; ^{1}H NMR δ ppm (D_2O, 400 MHz): 5.11 (1 H, d, 5.0 Hz, H-1), 6.13 (1 H, d, 5.9 Hz, H-3), 4.95 (1 H, dd, 6.0, 7.2 Hz, H-4), 2.62 (1 H, m, H-5), 4.38 (1 H, brs, H-6), 5.67 (1 H, brs, H-7), 2.98 (1 H, bt, 6.0, 5.6 Hz, H-9), 4.14 (2 H, brd, 15.3, H-10), 4.62 (1 H, d, 8.0 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (D_2O, 100 MHz): 96.4 (CH, C-1), 140.6 (CH, C-3), 106.3 (CH, C-4), 43.4 (CH, C-5), 81.6 (CH, C-6), 129.5 (CH, C-7), 148.0 (C, C-8), 47.4 (CH, C-9), 60.6 (CH_2, C-10), 99.4 (CH, C-1′), 73.8 (CH, C-2′), 76.7 (CH, C-3′), 70.6 (CH, C-4′), 77.3 (CH, C-5′), 61.7 (CH_2, C-6′).

3.3.10. Geniposidic acid (**10**)

White amorphous powder. FAB-MS: 381.2 $[M + Li]^+$, 397.2 $[M + Na]^+$; ^{1}H NMR δ ppm (D_2O, 400 MHz): 5.20 (1 H, d, 6.4 Hz, H-1), 7.39 (1 H, s, H-3), 5.74 (1 H, brs, H-7), 4.70 (1 H, d, 9.6 Hz, H-1′); ^{13}C NMR and DEPT δ ppm (D_2O, 100 MHz): 98.0 (CH, C-1), 153.3 (CH, C-3), 113.8 (C, C-4), 35.4 (CH, C-5), 40.9 (CH_2, C-6), 130.4 (CH, C-7), 142.5 (C, C-8), 47.2 (CH, C-9), 61.9 (CH_2, C-10), 173.2 (C, C-11), 100.1 (CH, C-1′), 74.0 (CH, C-2′), 76.9 (CH, C-3′), 70.8 (CH, C-4′), 77.5 (CH, C-5′), 61.0 (CH_2, C-6′).

3.4. Antibacterial assays

The antibacterial screening was carried out employing the cup-plate method. Chloramphenicol was used as a positive control. Three strains bacteria, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of beef broth, the three bacteria were cultured in agar medium dishes respectively, six cups (8 × 10 mm) were put onto the dishes, and each tested compound (0.2 ml of 100 μg/ml) was respectively added into the cups under aseptic conditions. Then the dishes were cultured at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each test was performed twice.

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