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IRIDOIDS IN ROOTS OF *PEDICULARIS CHINENSIS*

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Key Word Index—*Pedicularis chinensis*; Scrophulariaceae; iridoid lactone; pedicularis-lactone; iridoid glucosides; 3β-butoxy-3,4-dihydroaucubin; 6-*O*-butyl-aucubin; 6-*O*-butyl-epiaucubin; bartsioside; aucubin.

Abstract—Four new iridoids, the glucosides 3β-butoxy-3,4-dihydroaucubin, 6-*O*-butyl-aucubin and 6-*O*-butyl-epiaucubin, together with pedicularis-lactone were isolated from roots of *pedicularis chinensis*. In addition, the known glucosides aucubin and bartsioside and a known iridoid lactone were also isolated. The compounds were identified mainly by spectral evidence.

INTRODUCTION

As an extension of our chemical and biological investigation of plants belonging to the genus *Pedicularis*, we now report the isolation and structural elucidation of four new compounds, pedicularis-lactone (**1**), 3β-butoxy-3,4-dihydroaucubin (**3**), 6-*O*-butyl-aucubin (**4**) and 6-*O*-butyl-epiaucubin (**5**) from the roots of *P. chinensis* [1]. In addition, we describe the isolation of three known iridoids, iridolactone (**2**), bartsioside (**6**) and aucubin (**7**).

RESULTS AND DISCUSSION

Iridolactone (**2**) [2, 3], bartsioside (**6**) [4, 5] and aucubin (**7**) [6–8] were identified by comparison of their spectral data (FAB and EI mass spectra, ^{1}H and ^{13}C NMR) with those published in the literature.

Compound (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{15}$ + 45.5° (MeOH, *c* 0.154). The EI mass spectrum of **1** showed a $[M]^+$ ion at *m/z* 184 and the $[M - H_2O]^+$ ion at *m/z* 166. The molecular formula of **1** was established as $C_9H_{12}O_4$ by the EI mass spectrum together with the ^{13}C NMR and DEPT data (Table 1). The NMR data (and molecular formula) for **1** showed that it was similar in structure to **2**, but was a γ-lactone (IR: 1734 cm^{-1}), and this suggested the overall structure given for **1**. Assuming the usual stereochemistry for iridoids, and that proven for **2** by correlation with aucubin [3], H-5 and H-9 were both in the β-position, and this necessitated that H-6 was also in the β-position in order for the two five-rings to contain a *cis*-junction. Taken together, this evidence gave the structure with the stereochemistry shown for **1**. This conclusion was confirmed by 2D ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY and ^{13}C–^{1}H COLOC spectra (Table 2) which were used to confirm the spectral assignments and connectivities. Thus the *cis*-arrangements between H-5/H-6 and H-5/H-9 were rendered probable by the similar coupling constants (7.3 and 7.5 Hz, respectively) and the attachment of the lactone oxygen from C-3 to C-6 was proved by a connectivity in ^{13}C–^{1}H COLOC between C-3 and H-6. We have named compound **1** pedicularis-lactone.

Compound **3** was obtained as an amorphous light-yellow powder, $[\alpha]_D^{15}$ − 26.2° (MeOH, *c* 0.275). It gave a positive coloration with Molish reagents. Its IR spectrum showed the presence of hydroxy (3334 cm^{-1}), double bond (1661 cm^{-1}) and C–O–C (1076, 1036 cm^{-1}). The ^{1}H NMR spectrum of **3** exhibited signals belonging to H-7 [δ5.53 (1H, *br s*)], H-1 [δ4.85 (1H, *d*, *J* = 5.8 Hz)], H-3 [δ4.73 (1H, *dd*, *J* = 9.0, 3.7 Hz)] and H-1′ [δ5.05 (1H, *d*, *J* = 7.8 Hz)] of an iridoid compound. The ^{13}C NMR spectrum showed that it had one butoxy group [δ14.3 (CH_3), 19.8 (CH_2), 32.1 (CH_2) and 70.3 (CH_2)]. The location of the butoxy group was determined to be at C-3 by comparison of ^{13}C NMR data with those of 3,4-dihydroaucubin [9] (C-3 and C-4 shifted downfield to δ99.1 (− 38.0) and 30.2 (− 5.7), respectively). The FAB mass spectrum gave ion peaks at *m/z* 427 $[M + Li]^+$ and 443 $[M + Na]^+$, which, together with its ^{13}C NMR and DEPT spectral data (Table 1) suggested the molecular formula to be $C_{19}H_{32}O_{10}$. In the ^{1}H NMR spectrum of **3**, the coupling constants of 9.0 ($J_{3,4a}$) and 3.7 Hz ($J_{3,4e}$) showed that H-3 is at the axial position. The chair conformation of A-ring in iridoid glycosides [10] determined that H-3 is at the α-configuration in compound **3**. The ^{13}C NMR spectrum proves the presence of a β-glucopyranosyl moiety. From the above results, com-

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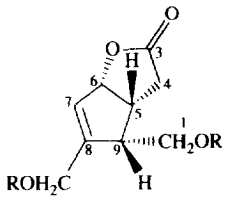

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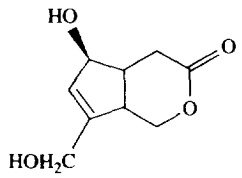

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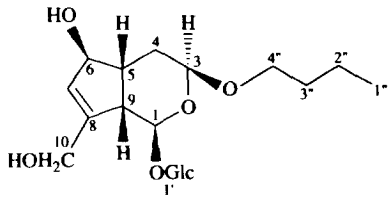

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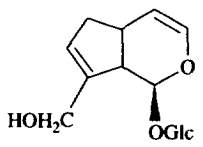

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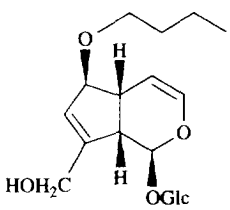

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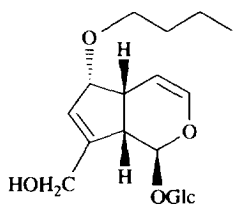

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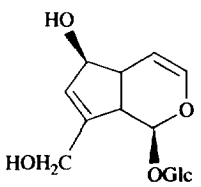

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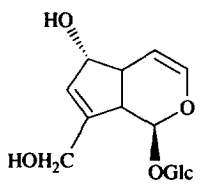

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Table 1. ^{13}C NMR spectral data of compounds 1–8 (100 MHz, in ppm)

C	1*†	2†	DEPT	3‡	4†	5†	6‡	7‡	8§	DEPT
C1	59.3	66.7	CH_2	98.6	94.8	95.2	97.0	96.3	99.1	CH
3	177.5	172.8	C	99.1	140.2	140.7	141.4	140.4	143.2	CH
4	29.8	33.5	CH_2	30.2¶	104.9	101.7	111.0	106.1	102.7	CH
5	38.6	43.4	CH	44.8	41.1	38.4	34.8	43.3	41.9	CH
6	87.3	81.4	CH	80.6	88.4	83.1	40.6¶	81.4	76.1	CH
7	122.3	129.2	CH	130.4	125.7	126.4	130.9	129.4	129.6	CH
8	154.0	145.7	C	147.5	148.2	148.6	143.7	147.6	150.6	C
9	48.4	42.9	CH	48.1	46.4	46.2	49.7	47.2	47.7	CH
10	58.9	58.5	CH_2	60.8	59.4	60.4	61.9	60.3	61.5	CH_2
GC										
1′				99.0	98.0	98.2	100.8	99.2	99.9	CH
2′				74.1	73.4	73.4	73.1	73.6	74.7	CH
3′				77.4	77.1	77.0	76.3	77.0	78.0	CH
4′				70.8	70.1	70.0	71.5	70.4	71.4	CH
5′				77.0	76.6	76.6	76.3	76.5	77.6	CH
6′				61.8	61.0	60.6	61.7	61.5	62.6	CH_2
Butyl										
1″				14.3	13.9	13.9				CH_3
2″				19.8	18.9	18.7				CH_2
3″				32.1	31.7	34.7				CH_2
4″				70.3	68.1	68.2				CH_2

*Assignment from 2D ^{13}C–^{1}H COSY experiments.
†In DMSO-d_6, TMS as int. standard.
‡In D_2O, DSS as int. standard.
§In CD_3OD, TMS as int. standard.
¶CH_2 signals in DEPT.

pound **3** was determined to be 3β-butoxy-3,4-dihydroaucubin.

Compounds **4** and **5** were obtained as a 2:1 mixture. The IR spectrum showed the presence of hydroxy (3331 cm^{-1}), double bond (1660 cm^{-1}) and C–O–C (1077, 1050 cm^{-1}). The ion peaks at m/z 409 $[M + Li]^+$ and 425 $[M + Na]^+$ in the FAB-mass spectrum suggested the molecular formula to be $C_{19}H_{30}O_9$, which was confirmed by the ^1H NMR, ^{13}C NMR and DEPT data. Most NMR signals in the spectrum of the mixture were doubled but due to the different amounts of **4** and **5**, it was possible to pick the separate signals for the two

Table 2. The cross peaks in 2D ^{13}C–^{1}H COSY, ^{1}H–^{1}H COSY and ^{13}C–^{1}H COLOC of compound **1**

^{13}C–^{1}H COSY		^{1}H–^{1}H COSY		^{13}C–^{1}H COLOC	
C-1 (59.3)	H_2-1 (3.42, 3.52)	H_2-1	H-9	C-3	H_2-4, H-6
C-3 (177.5)		H-9	H-5, H-7,	C-8	H-9, H-7,
C-4 (29.8)	H_2-4 (2.55)		H_2-1		H_2-10
C-5 (38.6)	H-5 (3.15)	H-5	H-9, H-6,	C-7	H_2-10
C-6 (87.3)	H-6 (5.31)		H_2-4	C-5	H_2-1, H-9,
C-7 (122.3)	H-7 (5.69)	H-6	H-7, H-5,		H-7
C-8 (154.0)			H_2-10		
C-9 (48.4)	H-9 (2.81)	H-7	H-6, H-9,		
C-10 (58.9)	H_2-10 (3.95, 4.05)		H_2-10		
		H_2-10	H-7, H-6		
		H_2-4	H-5		

compounds (see Table 1 and Experimental). It was evident from the spectra that both **4** and **5** contained an *n*-butyl moiety, and that both were β-glucopyranosides very similar to aucubin. Thus, when comparing the NMR spectra of **4** with those of aucubin **7** both H-6 and C-6 showed significant downfield shifts (0.3 and 7.0 ppm, respectively), proving the position of the butyl group at the C-6 oxygen. Furthermore, the differences between **5** and **4** (− 5.3 (C-6), − 2.7 (C-5), − 3.2 (C-4), + 0.5 (C-3) and + 0.4 (C-1)) showed that the butyl group in compound **5** was in the α-position at C-6 [11–13]. Moreover, the difference between compound **5** and 6-epi-aucubin (**8**) [14] (+ 7.0 ppm (C-6)) was identical with that of compound **4** and aucubin, which further confirmed the above results. Thus, compound **5** was determined to be 6-*O*-butyl-epiaucubin and compound **4** to be 6-*O*-butyl-aucubin. They are an epimeric iridoidic pair.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively, in FT mode.

Plant material. Pedicularis chinensis Maxim was collected in Zhang County, Gansu province in August 1989. It was identified by Prof. Zhang Guoliang of Lanzhou University. A voucher specimen (933101) has been preserved in the herbarium of the authors' Institute.

Extraction. Dried the roots of plant (3.5 kg) were extracted with MeOH under reflux (3 × 2 l). After concn of the combined extracts, hot water was added and the water-insol. material removed by filtration through Celite. The filtrate was extracted successively with $CHCl_3$ (1.5 l), $MeCO_2Et$ (2.5 l) and *n*-BuOH (2.5 l). The *n*-BuOH portion was evapd to obtain a crude syrup, which was chromatographed over silica gel eluting with $CHCl_3$–MeOH (12:1) followed by increasing concns of MeOH; four frs were collected.

Compound **1**. Fr. 2 was purified by prep. TLC eluting with $CHCl_3$–MeOH–water (40:8:1) (20 mg). $[\alpha]_D^{15}$ + 45.5° (MeOH, *c* 0.154); IR ν_{max}^{MeOH} (cm^{-1}): 3322 (OH), 2947 (C–H), 1734 (*r*-lactone), 1669, 1450 (C=C), 1115, 1028 (C–O–C), 660; ^{1}H NMR (DMSO-d_6, TMS) δ: 2.55 (2H, *dddd*, *J* = 12.5, 5.7 Hz, *J* = 12.5, 8.7 Hz, CH_2-4), 2.81 (1H, *m*, H-9), 3.15 (1H, *m*, *J* = 7.3, 7.5, 8.7, 5.7 Hz, H-5), 3.52, 3.42 (each 1H, *dd*, *J* = 11.0, 4.1 Hz, *J* = 11.0, 6.4 Hz, CH_2-1), 4.05, 3.95 (each 1H, *dd*, *J*, = 15.5, 1.5 Hz, CH_2-10), 4.94, 4.64 (*br s*, 1, 10-OH), 5.31 (1H, *br d*, $J_{6,5}$ = 7.3 Hz, $J_{6,7}$ = 1.5 Hz, H-6), 5.69 (1H, *br s*, *J* = 1.5 Hz, H-7) (assignment from 2D ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY experiments); ^{13}C NMR: see Table 1. EI-MS (70 ev) *m/z*: 184 $[M]^+$, 166 $[M - H_2O]^+$, 154 $[M + H - CH_2OH]^+$, 136 $[154 - H_2O]^+$, 124 $[M - 2CH_2OH]^+$, 108, 95, 91, 79 $[M - 2CH_2OH - CO_2]^+$, 67, 53.

Peracetate **1a**. Compound **1** (5 mg) was acetylated with Ac_2O (0.5 ml) and dry pyridine (0.5 ml) at room temp. overnight. The residue was extracted with Et_2O. The Et_2O was evapd. and residue was purified by prep. TLC eluting with petrol–EtOAc (2:1) to obtain **1a**. EIMS *m/z*: 268 $[M]^+$, 208 $[M - HOAc]^+$, 166 $[M - HOAc - CH_2CO]^+$, 148 $[M - 2HOAc]^+$, 135, 121, 78.

Compound **3**. Fr. 1 was purified by CC on silica gel eluting with $CHCl_3$–MeOH (8:1) (15 mg). $[\alpha]_D^{15}$ − 26.2° (MeOH, *c* 0.275); IR ν_{max}^{MeOH} (cm^{-1}): 3334, 2991, 1661, 1446, 1240, 1076, 1036, 633; ^{1}H NMR (D_2O, DSS) δ: 0.66 (3H, *t*, H-1″), 1.10 (2H, *m*, H-2″), 1.35 (2H, *m*, H-3″), 1.75 (2H, *m*, H-4), 2.28 (1H, *m*, H-9), 2.63 (1H, *m*, H-5), 3.00–4.25 (*m*, H of sugar), 4.00 (2H, *t*, H-4″), 4.50 (2H, *br d*, *J* = 15.5, 1.5 Hz, H-10), 4.73 (1H, *dd*, *J* = 9.0, 3.7 Hz, H-3), 4.85 (1H, *d*, *J* = 5.8 Hz, H-1), 4.88 (1H, *d*, *J* = 6.0 Hz, H-6), 5.05 (1H, *d*, *J* = 7.8 Hz, H-1 of glc), 5.53 (1H, *br s*, H-7). ^{13}C NMR: see Table 1. FAB–MS (S-Gly) *m/z*: 427 $[M + Li]^-$, 443 $[M + Na]^+$, 258 $[M - glc]^+$.

Compounds **4** *and* **5**. Fr. 3 was purified by CC on silica gel eluting with $CHCl_3$–MeOH (6:1) (40 mg) to yield an amorphous powder. On TLC (silica gel GF_{254}) eluting with $CHCl_3$–MeOH–H_2O (4:1:0.1), this mixture showed only one clear red spot with 5% H_2SO_4–EtOH (R_f: 0.6). Furthermore, this mixture was not separated by HPLC and TLC with any solvent systems. IR ν_{max}^{MeOH} (cm^{-1}): 3331, 2958, 2930, 1660, 1377, 1226, 1076, 1049, 699, 586. ^{1}H NMR (DMSO-d_6, TMS) δ: compound **4**: 0.85 (3H, *t*, H-1″), 1.30 (2H, *m*, H-2″), 1.45 (2H, *m*, H-3″), 2.64 (1H, *m*, H-5), 2.76 (1H, *dd*, *J* = 5.0, 6.8 Hz, H-9),

3.00–3.50 (*m*, H of sugar), 4.35 (2H, *t*, H-4″), 4.47 (1H, *d*, $J = 7.8$ Hz, H-1′ of glc), 4.83 (1H, *dd*, $J = 6.0$, 5.6 Hz, H-4), 4.90 (1H, *d*, $J = 6.0$ Hz, H-6), 4.95 (1H, *d*, $J = 5.0$ Hz, H-1), 5.74 (1H, *br s*, H-7), 6.28 (1H, *dd*, $J = 6.0$, 1.5 Hz, H-3). Compound **5**: 0.85 (3H, *t*, H-1″), 1.32 (2H, *m*, H-2″), 1.35 (2H, *m*, H-3″), 2.87 (1H, *dd*, $J = 4.7$, 6.7 Hz, H-9), 4.28 (2H, *t*, H-4″), 4.46 (1H, *d*, $J = 7.8$ Hz, H-1′ of glc), 4.85 (1H, *dd*, $J = 6.0$, 3.5 Hz, H-4), 4.93 (1H, *d*, $J = 6.7$ Hz, H-6), 5.00 (1H, *d*, $J = 4.7$ Hz, H-1), 5.78 (1H, *br s*, *H*-7), 6.33 (1*H*, *dd*, $J = 6.0$, 1.5 Hz, H-3). ^{13}C NMR: see Table 1. FAB–MS (S-Gly) *m/z*: 409 $[M + Li]^+$, 425 $[M + Na]^+$.

Compound **2**. Fr. 2 was purified by prep. TLC eluting with $CHCl_3$–MeOH–water (40:8:1) to yield an amorphous powder (25 mg). IR ν_{max}^{KBr} (cm^{-1}): 3400 (OH), 1725, 1240 (δ-lactone). ^{1}H NMR (DMSO-d_6, TMS) δ: 2.42, 2.78 (each 1H, *dd*, $J = 14.9$, 4.1 Hz, $J = 14.9$, 7.5 Hz, resp. CH_2-4), 2.50 (1H, *m*, H-5), 3.15 (1H, *m*, H-9), 3.95, 4.02 (each 1H, *dd*, $J = 14.8$, 1.1 Hz, 14.8, 0.9 Hz, resp. CH_2-10), 4.16, 4.28 (each 1H, *dd*, $J = 11.8$, 3.7 Hz, $J = 11.8$, 4.1 Hz, resp. CH_2-1), 5.03 (1H, *dd*, $J = 5.4$, 1.5 Hz, H-6), 5.58 (1H, *br s*, H-7). ^{13}C NMR: see Table 1. FAB–MS (S-Gly) *m/z*: 185 $[M + H]^+$, 166 $[M - H_2O]^+$. Spectral data of **2** were identical to those published for iridolactone [2–3].

Compound **6**. Fr. 3 was purified by CC on silica gel eluting with $CHCl_3$–MeOH (6:1) to yield amorphous powder (30 mg). mp 118–120°. ^{1}H NMR (D_2O, DSS) δ: 2.95 (1H, *m*, H-5), 4.78 (1H, *d*, $J = 8.0$ Hz, H-1′ of glc), 4.95 (1H, *dt*, H-4), 5.34 (1H, *d*, $J = 3.5$ Hz, H-1), 5.56 (1H, *br s*, H-7), 6.28 (1H, *d*, $J = 6.0$ Hz, H-3). ^{13}C NMR: see Table 1. FAB–MS (S-Gly) *m/z*: 337 $[M + Li]^+$, 353 $[M + Na]^+$. Analytical data of **6** were identical to those reported for bartsioside [4–5].

Compound **7**. Fr. 4 was recrystallized with MeOH (500 mg). IR ν_{max}^{KBr} (cm^{-1}): 3339 (OH), 1700, 1652 (double bonds), 1350 (CH_2), 1055 (C–O–C). ^{1}H NMR (D_2O, DSS) δ: 4.30 (2H, *br d*, $J = 15.5$ Hz, CH_2-10), 4.60 (1H, *d*, $J = 5.8$ Hz, H-6), 4.83 (1H, *d*, $J = 8.0$ Hz, H-1′ of glc), 5.12 (1H, *dd*, H-4), 5.23 (1H, *d*, $J = 5.0$ Hz, H-1), 5.87 (1H, *br d*, H-7), 6.32 (1H, *d*, $J = 6.2$ Hz, H-3). ^{13}C NMR: see Table 1. FAB–MS (S-Gly) *m/z*: 353 $[M + Li]^+$, 369 $[M + Na]^+$. Identified as aucubin by direct comparison with an authentic sample.

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