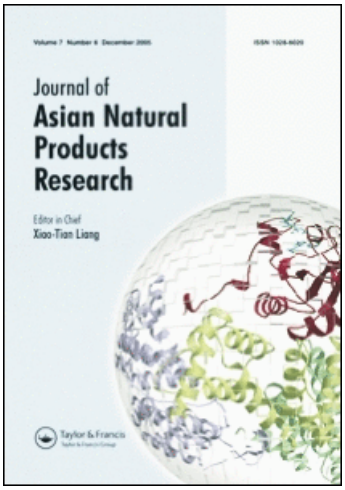

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Two new iridoid glucosides from *Picrorhiza scrophulariiflora*

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Two new iridoid glucosides with 3,4-dihydrocatalpol skeleton, piscrosides A (**1**) and B (**2**) together with nine known iridoid glucosides and three known cucurbitacin glucosides, were isolated from the stems of *Picrorhiza scrophulariiflora*. Their structures were established by MS, ^{1}H NMR, ^{13}C NMR and 2D NMR methods (including HSQC, HMBC and NOESY experiments).

Keywords: *Picrorhiza scrophulariiflora*; Scrophulariaceae; Iridoid glucoside; Piscrosides A and B

1. Introduction

The genus *Picrorhiza* is widely distributed in China, Nepal, and India, and contains two species, *P. scrophulariiflora* Pennell and *P. kurrooa* Royle ex Benth (Scrophulariaceae). The plant, *P. kurrooa,* was a traditional medicine used in India for treatment of jaundice, indigestion, common fever, acute viral hepatitis, and bronchial asthma [1]. Previous pharmacological studies for this species have revealed hepatoprotective [2], immunostimulative [3], anti-asthma [4], neuritogenic [5], anti-inflammatory [6] and scavenging free radical activities [7]. The other species, *P. scrophulariiflora*, is a traditional Chinese medicine, which also has hepatoprotective, choleretic, immunostimulative and anti-inflammatory activities [8,9]. To date there are several chemical constituents reported from *P. scrophulariiflora*, except for our work dealing with some new compounds including three phenyl glycosides and two phenylethyl glycosides [10]. During our systematic chemical investigation**,** two new iridoid glucosides, piscrosides A (**1**) and B (**2**), were isolated from the stems of *P. scrophulariiflora*, together with nine known iridoid glucosides, picroside I (**3**), picroside II (**4**), picroside III (**5**), picroside IV (**6**), 6-*O-trans*-feruloylcatalpol (**7**), minecoside (**8**), verminoside (**9**), catalposide (**10**), and aucubin (**11**) [11,12] (figure 1). Additionally, the stems of the plant afforded only three cucurbitacin glucosides, 2β-glucopyranosyloxy-3,16,20,22-tetrahydroxy-9-methyl-19-norlanosta-5,24-diene (**12**) [13], 2β-glucopyranosyloxy-3,16,20,22-tetrahydroxy-19-norlanosta-5,23-diene-22-ol (**13**) [14],

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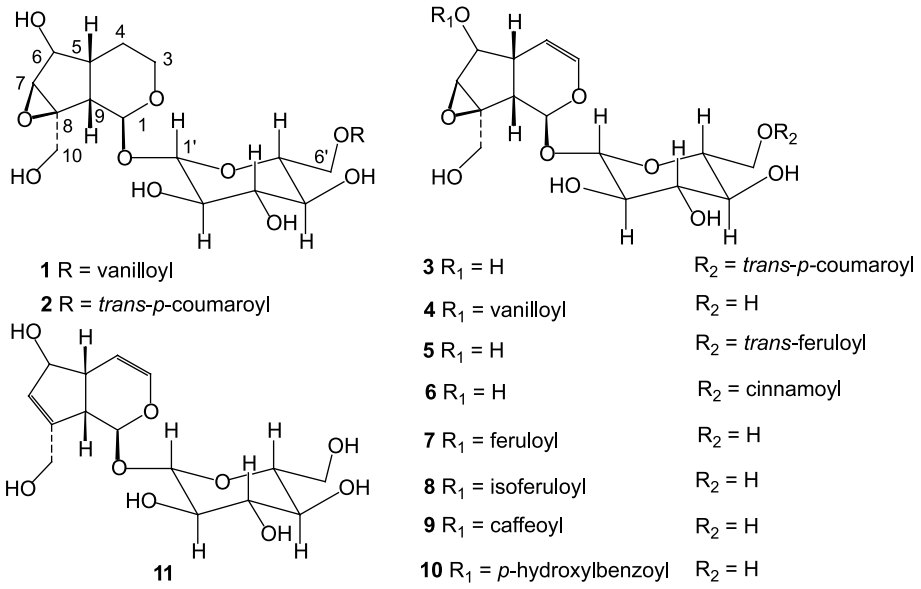

Figure 1. The structures of **1**–**11**.

and 25-aceloxy-2β-glucopyranosyloxy-3,16,20-trihydroxy-19-norlanosta-5,23-diene-22-ol (**14**) [14]. The known compounds were identified by comparisons of spectroscopic data with those of reported ones.

2. Results and discussion

Piscroside A (**1**) was obtained as an amorphous powder. The positive ESI-MS showed a pseudomolecular ion $[M + Na]^+$ at *m/z* 537 and the negative ESI-MS exhibited the ions $[M - H]^-$ at *m/z* 513. All these data were compatible with the molecular formula $C_{23}H_{30}O_{13}$, which was also confirmed by HRFAB-MS at *m/z* $[M - H]^-$ 513.1571. The IR spectrum showed absorption bands at 3419 (br OH), 1702 (C═O), and 1605, 1516, 1430 (aromatic ring) cm^{-1}. In the ^1H NMR spectrum, **1** showed three aromatic protons [δ 7.44 (*d*, $J = 1.8$ Hz), 7.46 (*dd*, $J = 1.8$, 8.4 Hz), 6.87 (*d*, $J = 8.4$ Hz)], one phenolic hydroxyl proton (δ 9.95, s) and a methoxy protons (δ 3.81, s), due to the vanilloyl ester part of the molecule. Besides these, **1** resembled those of catalpol, except for the absence of the olefinic signals attributable to H-3 and H-4. The signals corresponding to this double bond were also absent from the ^{13}C NMR spectrum of **1,** where two signals at δ 61.7 and 23.1 suggest that it has the structure of 3,4-dihydrocatalpol unit. In the HMBC spectrum, the correlations of H-5 (δ 1.80) and H-1 (δ 4.46) with C-3 (δ 61.7), H-6 (δ 3.77), H-5 (δ 1.80) and H-9 (δ 2.04) with C-4 (δ 23.1) were observed. Additionally, the clear correlations, H_2-6′ (δ 4.32) with C═O (δ 166.1), enabled us to assign the position of the vanilloyl group to C-6′ of glucosyl group. The relative configurations of C-1, C-5, C-6, C-7, C-8, and C-9 were deduced from the results of the NOESY spectrum (figure 2). In the ESI-MS^n analysis, MS^1 gave a pseudomolecular ion at *m/z* 513 $[M - H]^-$, MS^2 gave a fragment ion at *m/z* 311 $[M - H-202]^-$, and MS^3 gave the fragment ion at *m/z* 167 $[M - H-202-144]^-$, which

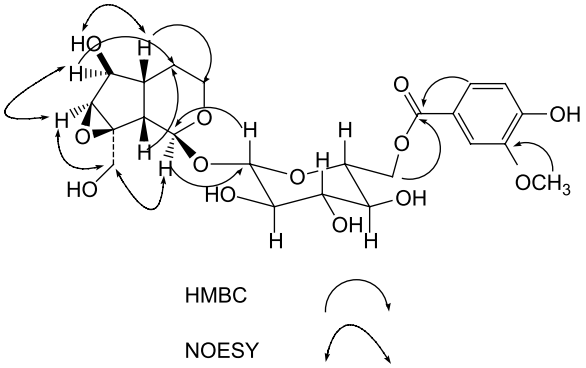

Figure 2. The key correlations of HMBC and NOESY of **1**.

corresponds to the sequential loss of an iridoid unit and a glucosyl group. Thus, **1** was unambiguously identified as 6′-*O*-vanilloyl-3,4-dihydrocatalpol, named piscroside A.

Piscroside B (**2**) was also obtained as amorphous powder. Based on the HRFAB-MS, the pseudomolecular ion peak at *m/z* 509.1615 [M − H]$^-$, the molecular formula of **2** was determined as $C_{24}H_{30}O_{12}$. Comparison of the ^{1}H NMR and ^{13}C NMR data of **2** with those of **1** indicated the existence of the same skeleton of 3,4-dihydrocatalpol. The ^{1}H NMR spectrum of **2** showed the presence of a *p*-coumaroyl group, which was confirmed by the four aromatic proton signals between δ 6.79 and 7.57 for an AA′BB′ systems [δ 7.57 (2H, *d*, $J = 8.5$ Hz), 6.79 (2H, *d*, $J = 8.5$ Hz)], a *trans*-double bond proton [δ 7.56 (1H, *d*, $J = 15.9$ Hz), 6.40 (1H, *d*, $J = 15.9$ Hz)], and an aromatic hydroxyl proton [δ 10.02 (1H, *s*)]. All further essential spectral data of **2** were in full agreement with those of **1**. Thus, compound **2** was determined to be 6′-*O*-*p*-coumaroyl-3,4-dihydrocatalpol, named piscroside B.

Only two iridoid glucosides containing a 3,4-dihydrocatalpol unit have been reported so far since the isolation of the first one in 1979 [15,16]. In addition, the previous and this investigation showed that the two species, *P. scrophulariiflora* and *P. kurrooa,* are similar in the chemical constituents, both mainly containing iridoid glucosides. However, the difference is that cucurbitacin glucosides are far more abundant in the species of *P. kurrooa* than that of *P. scrophulariiflora*.

3. Experimental

3.1 General experimental procedures

IR spectra were recorded on Perkin–Elmer Spectrum One FT-IR spectrometer. NMR spectra were recorded on Bruker AV-600 spectrometer using tetramethylsilane (TMS) as an internal standard. Optical rotations were measured with PE-341 polarimeter. VG AutoSpec-3000 spectrometer was used to record HRFAB-MS and Finnigan LCQ^{DECA} to record ESI-MS and tandem MS spectra. Sephadex LH-20 (Pharmacia), silica gel (200–300 mesh, Qingdao Marine Chemical Group Co.), Lobar LiChroprep RP-18 (40–63 μm, Merck), Lobar LiChroprep Si-60 (40–63 μm, Merck) were used for column chromatography.

3.2 Plant material

The dried stems of *Picrorhiza scrophulariiflora* were collected in Tibet of China, and identified by Prof Zuo-Cheng Zhao. A voucher specimen is deposited in the Herbarium of Chengdu Institute of Biology, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried stems of *P. scrophulariiflora* (6 kg) were extracted with EtOH at 60°C for 3 times, each for 8 h. The concentrated extract was suspended in H_2O and partitioned successively with petroleum ether, chloroform and EtOAc/EtOH (9:1) mixture. The EtOAc/EtOH fraction (50 g) was subjected to column chromatography over silica gel eluted with $CHCl_3/CH_3OH$ (40:1–2:1) to obtain six fractions. Fraction I (6 g) was separated by silica gel column chromatography with $CHCl_3/CH_3OH$ gradient system (20:1–10:1) to give compound **14**. Fraction II (10 g) was chromatographed on ODS column eluting with MeOH/H_2O (1:1) to afford two crude compounds, which were purified by silica gel column chromatography eluting with $CHCl_3$/MeOH to give **3** (2.1 g) and **12** (100 mg), respectively. Fraction III (2 g) was chromatographed on a Lobar LiChroprep Si-60 column using $CHCl_3/CH_3OH$ (10:1) to

Table 1. ^{1}H NMR and ^{13}C NMR data for compounds **1** and **2**.

Position	***1***		***2***	
	^1H*	^{13}C†	^1H*	^{13}C†
1	4.46 d (8.7)	96.5 d	4.51 d (8.7)	96.6 d
3α	3.74 m	61.7 t	3.75 m	61.7 t
3β	3.19‡		3.21‡	
4α	1.44 d (13.5)	23.1 t	1.45 d (13.5)	23.1 t
4β	1.58 m		1.59 m	
5	1.80 dd (13.5, 8.1)	37.2 d	1.83 dd (13.5, 8.1)	37.2 d
6	3.77 dd (9.0, 6.6)	71.6 d	3.79 dd (9.0, 6.6)	71.6 d
7	3.18 d (9.0)	60.9 d	3.19 d (9.0)	61.0 d
8		65.2 s		65.3 s
9	2.04 t (8.0)	42.4 d	2.06 t (8.0)	42.5 d
10a	3.41 dd (13.2, 7.3)	59.7 t	3.46 dd (13.2, 7.3)	59.7 t
10b	3.81‡		3.84 dd (13.2, 7.3)	
1′	4.55 d (7.8)	98.3 d	4.54 d (7.8)	98.4 d
2′	3.02dd (7.8, 5.0)	74.0 d	3.03 dd (7.8, 5.0)	73.9 d
3′	3.21‡	76.9 d	3.22‡	76.9 d
4′	3.20‡	70.9 d	3.23‡	70.7 d
5′	3.47 m	74.6 d	3.40 m	74.5 d
6′a	4.44 br d (12.0)	64.1 t	4.34 br d (12.0)	65.3 t
6′b	4.32 dd (12.0, 6.3)		4.25 dd (12.0, 6.0)	
1″		121.3 s		125.7 s
2″	7.44 d (1.8)	113.3 d	7.57 d (8.5)	131.0 d
3″		148.0 s	6.79 d (8.5)	116.5 d
4″		152.2 s		160.5 s
5″	6.87 d (8.4)	115.8 d	6.79 d (8.5)	116.5 d
6″	7.46 dd (8.4, 1.8)	124.1 d	7.57 d (8.5)	131.0 d
OCH_3	3.81 s	56.3 q		
CO		166.1 s		167.2 s
α			6.40 d (15.9)	114.7 d
β			7.56 d (15.9)	145.5 d

* 600 MHz, DMSO-d_6; chemical shifts in ppm relative to TMS; coupling constant (J) in Hz.
† 150 MHz, DMSO-d_6; multiplicity was established from DEPT data.
‡ Overlapped.

give two fractions; one fraction was purified by repeatedly silica gel to obtain compound **6** (35 mg). The other fraction was submitted to column chromatography (RP-18) with MeOH/H_2O (1:1) to yield compounds **5** (20 mg) and **13** (17 mg), respectively. Compounds **4** (3.5 g), **7** (15 mg), and **8** (150 mg) were obtained from fraction IV (20 g) by a Lobar LiChroprep RP-18 column. Fraction V (5 g) was repeatedly chromatographed on ODS gel (eluted with $H_2O \rightarrow$ 70% MeOH), Lobar LiChroprep RP-18 (eluted with $H_2O \rightarrow$ 60% MeOH), Lobar LiChroprep Si-60 [eluted with $CHCl_3 \rightarrow CHCl_3$/MeOH (4:1)] and Sephadex LH-20 (eluted with MeOH) to give compounds **1** (15 mg), **2** (5 mg), **9** (14 mg) and **10** (8 mg), respectively. Compound **11** (15 mg) was obtained from the last fraction (4.5 g) by chromatography on silica gel column.

3.4 Identification

Piscroside A (**1**): White amorphous powder. $[\alpha]_D^{25}$ −59.8 (*c* 0.40, MeOH). IR (KBr): v_{max} 3419, 2925, 1702, 1605, 1516, 1430, 1286, 1221, 1070, 763 cm^{-1}. ESI-MS: *m/z* 513 $[M - H]^-$, 549 $[M + Cl]^-$, 537 $[M + Na]^+$. HRFAB-MS: *m/z* 513.1571 $[M - H]^-$ (calcd for $C_{23}H_{30}O_{13}$, 513.1608). ^1H NMR and ^{13}C NMR: see table 1.

Piscroside B (**2**): White amorphous powder. $[\alpha]_D^{25}$ −60.1 (*c* 0.10, MeOH). IR (KBr): v_{max} 3380, 2918, 1661, 1610, 1580, 1511, 1280, 1241, 1066, 974, 772 cm^{-1}. ESI-MS: *m/z* 509 $[M - H]^-$, 533 $[M + Na]^+$. HRFAB-MS: *m/z* 509.1615 $[M - H]^-$ (calcd for $C_{24}H_{30}O_{12}$, 509.1659). ^1H NMR and ^{13}C NMR: see table 1.

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