Pikuroside: A Novel Iridoid from *Picrorhiza kurroa*

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A new iridoid, pikuroside (**1**), was isolated from the roots of *Picrorhiza kurroa*, together with three known iridoids, picroside I (**2**), picroside II (**3**), and 6-feruloyl catalpol (**4**). The structure of **1** was established by interpretation and full assignments of NMR spectral data. Pikuroside (**1**) had no antiinflammatory activity, although the crude extract and picroside II (**3**) demonstrated moderate activity.

Picrorhiza kurroa Royle ex Benth (Scrophulariaceae) grows in the northwestern Himalayan region and is utilized in India for treatment of jaundice, indigestion, common fever, acute viral hepatitis, and bronchial asthma.[1] Pharmacological studies have revealed hepatoprotection,[2] antiinflammation,[3,4] anti-asthma,[5] immunostimulation,[6] and free radical scavenging activities.[7] From the roots of the plant, acetophenones,[4,8] iridoid glycosides,[9] triterpenoids,[10,11] and benzoic acid derivatives[12] have been isolated. In our search for antiinflammatory substances from traditional herbs, an aqueous methanol extract from the roots of *P. kurroa* yielded pikuroside (**1**) and three known iridoid glycosides: picroside I (**2**),[13] picroside II (**3**),[14] and 6-feruloyl catalpol (**4**)[9] (Figure 1).

Results and Discussion

The molecular formula of pikuroside (**1**) was determined by HREIMS to be $C_{23}H_{30}O_{14}$, which required nine degrees of unsaturation. The UV spectrum in MeOH showed three absorption maxima at 221.0, 263.0, and 292.5 nm. The IR spectrum of **1** disclosed one carbonyl band at 1689 cm^{-1}. Its ^{13}C NMR spectrum (Table 1) displayed 23 carbon signals and clearly indicated the presence of a vanillic acid ester and a β-glucosylide moiety, which were confirmed by ^1H NMR spectroscopy and COSYPS, HMQC, and HMBC experiments. Construction of the iridoid skeleton started with the carbon at 91.5 ppm (C-1). The shift was that of an acetal carbon consistent with the attached proton (H-1) at δ 5.52. The point of attachment for the β-glucosylide unit, C-1, was confirmed by HMBC correlations between C-1 and H-1′ (δ 4.53 d) and also between C-1′ (δ 97.2) and H-1. The α-positioned C−H bond at C-1 was established by its small coupling (J = 2.1 Hz) with only one proton at δ 2.45 (H-9) indicating a β H-9, where the dihedral angle was close to 60°. In addition to the coupling with H-1, H-9 had one additional large coupling (9.9 Hz) indicating a dihedral angle near 0° with the adjacent proton at H-5 (δ 2.12). This demonstrated that the stereochemistry of the catalpol ring fusion was cis. Since there were no other vicinal couplings to H-9, it was concluded that one of the remaining carbons attached to C-9, i.e., C-8 (δ 78.4) was quaternary.

The acetal linkage between C-10 and C-3 was established in two ways. First, the COSYPS spectrum showed an obvious coupling between H-7 and the proton at δ 3.42 on C-10 and also a coupling between H-9 and the proton at δ 3.87 on C-10. Although the coupling constants were very small, they were consistent with W couplings seen in rigid bicyclic systems. A molecular model showed almost perfect W arrangements for the coupled protons described above, provided that H-7 is β-positioned, thus establishing the stereochemistry at C-7. The small couplings between H-3 and the protons at δ 1.94 and 2.35 on C-4 were consistent with H-3 occupying an equatorial-type position.[15] Finally, the weak 3-bond correlation between H-3 and C-10 in the HMBC spectrum confirmed the 3,10-acetal linkage. Therefore, the OH group at C-8 had to be β to allow the 3,10-acetal α-linkage. The unambiguous 3-bond correlation between H-6 and the carbonyl carbon C-7″ in the HMBC spectrum confirmed ester β-linkage at C-6 that was consistent with the small coupling between H-6 and H-5, since the corresponding dihedral angle was near 120°. The large coupling between H-6 and H-7 (7.3 Hz) corresponded to a dihedral angle of approximately 160°, which was in excellent agreement with molecular models. Similar iridoids

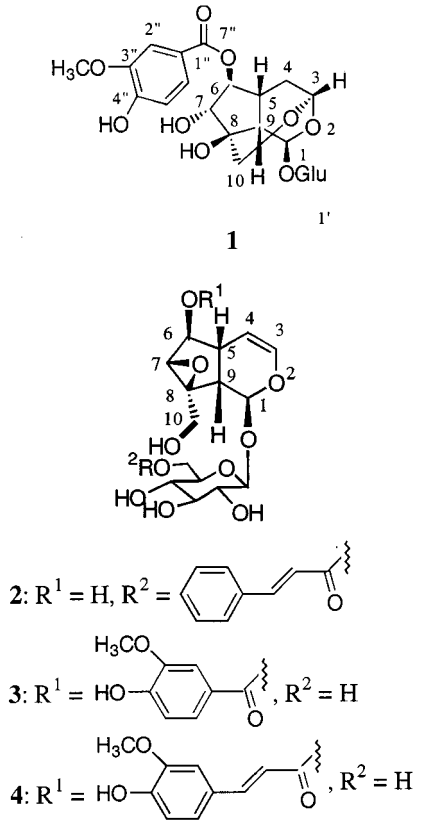

Figure 1. Structures of **1**, **2**, **3**, and **4**.

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Table 1. ^{13}C and ^{1}H NMR Assignments (Recorded in DMSO-d_6, 100 MHz/400 MHz) for Pikuroside (**1**)

position	δ_C	δ_H	COSY	HMBC (H→C)
1	91.5	5.52 (1H, d, J = 2.1 Hz)	9	3, 5, 1′
3	94.1	5.28 (1H, br d, J = 2.7 Hz)	4a, 4b	1, 5, 10
4a	33.9	1.94 (1H, dd, J = 13.5, 2.7 Hz)	4b, 3	6
4b		2.35 (1H, br dd, J = 13.5, 8.6 Hz)	4a, 5, 3	6
5	32.0	2.12 (1H, ddd, J = 9.9, 8.6, 2.6 Hz)	9, 4b, 6	3, 6, 8
6	86.9	4.76 (1H, dd, J = 7.3, 2.6 Hz)	7, 5	4, 7, 7″
7	80.3	4.19 (1H, br d, J = 7.3 Hz)[a]	6, 10a	6, 8, 10
8	78.4			
9	46.3	2.45 (1H, br dd, J = 9.9, 2.1 Hz)[a]	5, 1, 10b	4, 5, 8, 10
10a	60.1	3.42 (1H, br d, J = 11.9 Hz)[a]	10b, 7	7
10b		3.87 (1H, br d, J = 11.9 Hz)[a]	10a, 9	7
1′	97.2	4.53 (1H, d, J = 8.0 Hz)	2′	1
2′	73.4	2.94 (1H, dd, J = 8.9, 8.0 Hz)	3′, 1′	1′, 3′
3′	76.7	3.20 (1H, dd, J = 8.9, 8.9 Hz)	2′, 4′	2′, 4′
4′	70.4	3.06 (1H, dd, J = 9.3, 8.9 Hz)	5′, 3′	2′, 3′, 5′
5′	77.3	3.16 (1H, ddd, J = 9.3, 5.8, 1.8 Hz)	4′, 6′a, 6′b	
6′a	61.3	3.46 (1H, dd, J = 11.9, 5.8 Hz)	6′b, 5	
6′b		3.70 (1H, dd, J = 11.9, 1.8 Hz)	6′a, 5	
1″	121.2			
2″	113.0	7.49 (1H, d, J = 2.0 Hz)	6″	4″, 6″, 7″
3″	147.8			
4″	151.8			
5″	115.5	6.91 (1H, d, J = 8.3 Hz)	6″	1″, 3″, 4″
6″	124.1	7.53 (1H, dd, J = 8.3, 2.0 Hz)	5″, 2″	2″, 4″
7″	166.7			
OCH_3	56.1	3.83 (3H, s)		3″

[a] Broadening is due to W coupling which is indicated by COSY spectrum correlation.

with a rigid three-ring skeleton have been isolated from *Catalpa bignonioides*,[15] the Chinese herb, *Rehmannia glutinosa*[16] and also obtained through bromination and dehydration of the catalposide extracted from the roots of *P. kurroa*.[17]

The antiinflammatory activities of *P. kurroa* crude extract and purified compounds were compared in the croton oil mouse ear-swelling assay.[18] The crude extract and picroside II (**3**) showed 65% and 46%, respectively, inhibition of mouse ear swelling at 6 h, while compounds **1**, **2**, and **4** showed slight stimulation of inflammation, suggesting increased blood flow or infiltration of cells. Compound **2** appeared to account for most of the antiinflammatory activity in the aqueous methanol extract.

Experimental Section

General Experimental Procedures. Column chromatography, silica gel (32−63 mesh), Selecto Scientific; reverse phase column chromatography, Amberchrom CG-161cd resin (80−160 μm), Tosohaas; preparative HPLC, Rainin SD-1 pump, UV-1 detector, and Hewlett-Packard integrator, using a Rainin C-18 column (8 μm, 41.4 × 250 mm). Optical rotation was analyzed on a Rudolph Autopol III polarimeter. IR spectra were recorded on a Midac Prospect-IR spectrometer. UV spectra were recorded on a Hitachi U-3000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 400 NMR spectrometer operating at 399.970 and 100.582 MHz, respectively, using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference. MS spectra were recorded on a VG-7070E MS spectrometer.

Plant Material. *P. kurroa* was collected in Sankri, Vikosnagar province, India, by Zandu Pharmaceutical Works Ltd., Mumbai, India, and identified by Dr. J. M. Pathak. The voucher specimen was deposited at Univera Pharmaceuticals, Inc.

Extraction and Purification. The air-dried and finely powdered root of *P. kurroa* (500 g) was percolated with 80% MeOH at room temperature for 4 h. The crude extract (199.5 g) was concentrated *in vacuo*, and a portion (33.0 g) was fractionated by reverse phase column chromatography, eluted with 40% MeOH and 60% MeOH to generate two fractions, 13.5 and 14.5 g, respectively. The first fraction was further purified on a silica gel column by elution with $CHCl_3$−MeOH (4:1). Further purification by reverse phase HPLC eluting with MeOH−H_2O (4:6) to yield pikuroside (**1**, 167 mg, 0.20%). The second fraction was further purified on a silica gel column (eluent: $CHCl_3$−MeOH 8:1) followed by reverse phase HPLC elution with MeOH−H_2O (1:1) to yield picroside I (**2**, 647 mg, 0.78%), picroside II (**3**, 571 mg, 0.69%), and 6-feruloyl catalpol (**4**, 413 mg, 0.50%).

Pikuroside (1): amorphous solid; mp 187−189 °C; $[\alpha]^{21}_D$ −31.5° (*c*, 0.036, MeOH); UV (MeOH) λ_{max} 221.0, 263.0, 292.5 nm; IR (KBr) ν_{max} 3333, 2942, 2360, 2338, 1688, 1598, 1515, 1454, 1428, 1285, 1221, 1150, 1073, 1006 cm^{-1}; positive FABMS *m/z* 531 $[M + H]^+$; HRFABMS *m/z* 529.1547 $[M - H]^-$ (calcd for $C_{23}H_{29}O_{14}$, 529.1557 $[M - H]^-$); ^{1}H and ^{13}C NMR, see Table 1.

Mouse Ear-Swelling Assay. Male BalbC mice, 10−12 weeks of age and murine pathogen free (B&K Universal, Inc.), were randomly chosen and housed five to a cage. All mice were allowed free access to Harlan Lab Chow LM 485 and water. Topical activity was measured by the method of Carlson.[18] Croton oil (irritant), hydrocortisone (positive control), and test samples were dissolved in the same vehicle (pyridine−H_2O−Et_2O 4:1:5 solution) at concentrations of 250 μg/10 μL (25 mg/mL), 200 μg/10 μL (20 mg/mL), and 200 μg/10 μL (20 mg/mL), respectively. Mouse ear-swelling was induced by applying 10 μL of croton oil to the outside/front pinna of the mouse's ears. After 30 min of croton oil treatment, each test material was applied to the same location. Measurement was made before application of croton oil and at 6 and 24 h after the application of treatment solutions.

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References and Notes

(1) Bhavan, B. V. *Selected Medicinal Plants of India*; Tata Press: Bombay, India, 1992; pp 238−240.
(2) Dhawan, B. N. *Med. Chem. Res.* **1995**, *5*, 595−605.
(3) Engels, F.; Renirie, B. F.;'T Hart, B. A.; Labadie, R. P.; Nijkamp, F. P. *FEBS* **1992**, *305*, 254−256.

(4) Wagner, H.; Dorsch, W.; Stuppner, H.; Antus, S. U.S. Patent 5,481,-043, 1996.
(5) Dorsch, W.; Stuppner, H.; Wagner, H.; Gropp, M.; Demoulin, S.; Ring, J. *Int. Arch. Allergy Appl. Immunol.* **1991**, *95*, 128−133.
(6) Puri, A.; Saxena, R. P.; Sumati; Guru, P. Y.; Kulshreshtha, D. K.; Saxena, K. C.; Dhawan, B. N. *Planta Med.* **1992**, *58*, 528−532.
(7) Chander, R.; Kapoor, N. K.; Dhawan, B. N. *Biochem. Pharm.* **1992**, *44*, 180−183.
(8) Stuppner, H.; Wagner, H. *Sci. Pharm.* **1992**, *60*, 73−85.
(9) Stuppner, H.; Wagner, H. *Planta Med.* **1989**, *55*, 467−469.
(10) Stuppner, H.; Müller, E. P. Wagner, H. *Phytochemistry* **1991**, *30*, 305−310.
(11) Stuppner, H.; Müller, E. P. *Phytochemistry* **1993**, *33*, 1139−1145.
(12) Basu, K.; Dasgupta, B.; Ghosal, S. *J. Org. Chem.* **1970**, *35*, 3159−3161.
(13) Kitagawa, I.; Hino, K.; Nishimura, T.; Mukai, E.; Yosioka, I. *Tetrahedron Lett.* **1969**, *43*, 3837−3840.
(14) Weinges, V. K.; Kloss, P.; Henkels, W.-D. *Liebigs Ann. Chem.* **1972**, *759*, 173−182.
(15) Iwagawa, T.; Hamada, T.; Kurogi, S.; Hase, T.; Okubo, T.; Kim, M. *Phytochemistry* **1991**, *30*, 4057−4060.
(16) Yoshikawa, M.; Fukuda, Y.; Taniyama, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1986**, *34*, 1403−1406.
(17) Weinges, K.; Schick, H.; Neuberger, K.; Ziegler, H. J.; Lichtenthäler, J.; Irngartinger, H. *Liebigs Ann. Chem.* **1989**, 1113−1122.
(18) Carlson, R. P.; O'Neil-Davis, L.; Chang, J.; Lewis, J. A. *Agents Actions* **1985**, *17*, 197−204.

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