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SHORT REPORTS

KOELZIOSIDE, AN IRIDOID DIGLYCOSIDE FROM *SCROPHULARIA KOELZII**

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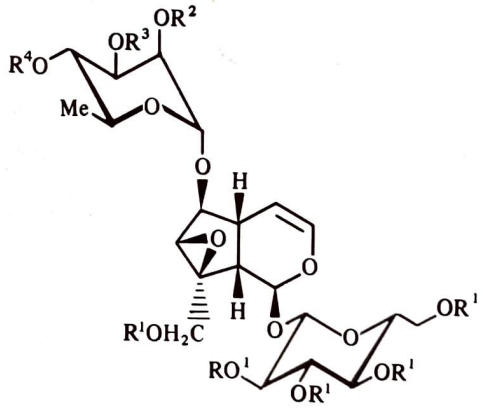
Key Word Index—*Scrophularia koelzii*; Scrophulariaceae; iridoid glycoside; 6-*O*-(tri-*O*-acyl)-α-L-rhamnopyranosyl catalpol; koelzioside.

Abstract—A new iridoid diglycoside named koelzioside has been isolated from *Scrophularia koelzii*. The structure of koelzioside was elucidated by chemical and spectral analysis as 6-*O*-(2″,3″-di-*O*-cinnamoyl-4″-*O*-acetyl)-α-L-rhamnopyranosyl catalpol.

INTRODUCTION

The genus *Scrophularia* is known for the presence of variety of iridoid glycosides [1, 2]. As part of a programme of screening Indian medicinal plants for a wide range of biological activity, the alcoholic extract of the whole plant of *Scrophularia koelzii* L., was found to show significant CNS depressant activity [3] which was shown to be localized in the chloroform-soluble fraction of the crude extractive. We report the isolation and characterization of a triacylrhamnopyranosyl catalpol derivative named keolzioside (**1**) from the active fraction. This is the first report on the chemical constituents of *S. koelzii*. To date more than 25 similar rhamnopyranosyl catalpol derivatives have been reported. Most of these iridoid diglycosides possess different degrees of acylation in the rhamnosyl moiety [4–6]. Recently two such rhamnosyl catalpol derivatives, scropoloside A and B (**2**), were reported from the roots of *Scrophularia scopolii* [7].



	R¹	R²	R³	R⁴
1	H	Cinn	Cinn	Ac
2	H	Ac	Cinn	Cinn
3	H	H	H	H
4	Ac	Cinn	Cinn	Ac

RESULTS AND DISCUSSION

Koelzioside (**1**) analysed for $C_{41}H_{46}O_{17}$, $[\alpha]_D$ −24° (MeOH). Its UV spectrum showed bands that are characteristic of an iridoid enol ether system and a cinnamoyl ester group(s). The IR spectrum confirmed the presence of ester groups and a non-conjugated enol ether system (see Experimental). The ^{1}H NMR spectrum of **1** was typical of catalpol monoglycoside showing signals for two sugar moieties along with those arising from acyl moieties and the aglycone part of catalpol. In addition to the signals due to 10 aromatic (δ 7.24–7.46) and four olefinic protons (δ 7.60, 7.56 and 6.52, 6.36; two AX systems, $J = 16$ Hz) arising from two *trans* cinnamoyl moieties; a three proton singlet of an acetoxy group was observed at δ 1.92. The signals of the anomeric protons appeared as doublets at δ 5.04 ($J = 1.7$ Hz; H-1 of α-L-rhamnose) and 4.67 ($J = 8$ Hz; H-1 of β-D-glucose). The signals of H-2, H-3 and H-4 of rhamnose were shifted downfield to δ 5.37, 5.31 and 5.14 respectively indicating acylation at these positions. The presence of two *trans*-cinnamoyl groups and an acetoxy group in the rhamnosyl catalpol was also evident from its ^{13}C NMR spectrum (Table 1).

Alkaline hydrolysis of koelzioside (**1**) yielded cinnamic acid and **3** as an amorphous compound analysing for $C_{21}H_{32}O_{14}$; $[\alpha]_D$ −148° (MeOH; *c* 2.1). The identity of **3** was confirmed as 6-*O*-α-L-rhamnopyranosyl catalpol [8] by its UV, IR, ^{1}H and ^{13}C NMR spectra (Table 1).

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Table 1. ^{13}C NMR spectral data of compounds **1**, **3** and **4** (100.57 MHz)

C	**1** (CD_3OD)	**1** ($CDCl_3$)	**4** ($CDCl_3$)	**3** (CD_3OD)	Catalpol*
Aglycone					
1	95.11	(94.52)	94.15	95.12	95.33
3	142.47	(141.21)	140.99	142.17	141.78
4	103.18	(102.65)	102.32	103.64	104.03
5	37.13	(35.82)	35.36	37.25	39.10
6	84.91	(83.58)	83.53	83.56	79.58
7	59.43	(58.68)	57.96	59.34	62.55
8	66.53	(65.16)	62.20	66.51	66.23
9	43.23	(42.00)	41.64	43.24	43.60
10	61.40	(61.14)	62.01	61.42	61.60
β-D-Glucose					
1′	99.68	(98.74)	96.45	99.71	99.74
2′	74.77	(73.19)	70.53	74.82	74.82
3′	78.58	(77.09)	72.46	78.57	78.54
4′	71.71	(70.28)	68.18	71.72	71.74
5′	77.61	(76.31)	72.14	77.65	77.70
6′	62.91	(61.20)	62.24	62.91	62.90
α-L-Rhamnose					
1″	97.77	(96.55)	96.54	100.32	
2″	71.38	(70.28)	70.17	72.24	
3″	70.84	(69.25)	68.95	72.24	
4″	72.38	(71.22)	71.13	73.81	
5″	68.03	(66.89)	66.88	70.19	
6″	17.74	(17.44)	17.31	18.01	
Acyl moieties					
trans-Cinnamoyl-I					
1‴	135.43	(134.14)	134.03		
2‴,6‴	130.04	(128.89)	129.84		
3‴,5‴	129.50	(128.36)	128.70		
4‴	131.82	(130.55)	130.52		
Cα	147.74	(146.31)	146.22		
Cβ	117.92	(117.15)	117.03		
CO	167.32	(165.97)	165.77		
trans-Cinnamoyl-II					
1⁗	135.35	(134.14)	134.03		
2⁗,6⁗	129.98	(128.77)	128.82		
3⁗,5⁗	129.35	(128.36)	128.22		
4⁗	131.71	(130.55)	130.35		
Cα′	147.37	(145.92)	145.84		
Cβ′	117.84	(117.15)	117.03		
CO	167.14	(165.72)	165.50		
OCOMe	171.87	(170.12)	170.48 × 2, 170.05, 169.91, 169.10, 168.88		
OCOMe	20.74	(20.82)	22.54, 20.48 × 3, 20.43 × 2		

*Data taken from ref. [9].

Assignment of the protons in the rhamnopyranosyl moiety of koelzioside (**1**) was confirmed by decoupling difference experiments. In **1** decoupling of the signals at δ5.14 caused a collapse in the signals at δ5.31 and 3.96, the most upfield of all the rhamnose sugar protons (H-5″). Thus the signal at δ5.14 was assigned to H-4″ and that at δ5.31 to H-3″. Further decoupling experiments gave the unambiguous assignments of the various sugar protons. The signals at δ5.04 and 5.37 were assigned to H-1″ and H-2″ respectively.

The position of the cinnamoyl and the acetyl moieties in **1** was determined by comparing the carbonyl multiplets in the proton coupled ^{13}C NMR spectrum with those obtained in the selective low power proton decoupled ^{13}C NMR spectrum [7]. It was found that decoupling of the protons at δ5.37 (H-2″) and 5.31 (H-3″) caused the collapse of the carbonyl multiplets at δ167.32 and 167.14 and that iradiation at δ5.14 (H-4″) caused the collapse of the carbonyl signal at δ171.87. This confirmed the attachment of acetyl group at C-4″ and that of cinnamoyl units at position 2″ and 3″ of the rhamnopyranosyl moiety. Thus the structure of koelzioside was established as 6-*O*-(2″,3″-di-*O*-cinnamoyl-4″-*O*-acetyl)-α-L-rhamnopyranosyl catalpol (**1**). A similar mono-rhamnosyl catalpol

named scropolioside-B (**2**) had been reported from *S. scopolli*. It differs from koelzioside (**1**) in the substitution pattern of the cinnamoyl and acetyl groups in the rhamnose moiety (Table 1). Koelzioside (**1**) was devoid of any CNS activity but showed immunostimulant action.

EXPERIMENTAL

Mp: uncorr; IR: KBr; UV: MeOH–$CHCl_3$; ^1H and ^{13}C NMR: $CDCl_3$ and/or MeOH-d_4 using 90 and/or 400 MHz, with TMS as int. standard. CC: silica gel (60–120 mesh) (BDH); Flash chromatography: EF-10 (EYELA) A.S.C. silica gel (250–400 mesh); TLC: silica gel G (BDH). The spots were visualized by spraying with 1% $Ce(SO_4)_2$ in 1 M H_2SO_4 or vanillin perchloric acid reagent [vanillin 2.5% and perchloric acid 2.5% in alcohol (1:1)]. PC: Whatmann paper No. 1. Standard D-glucose and L-rhamnose (both Sigma) were used. Descending method with (a) BuOH–HOAc–H_2O (4:1:5; upper phase), spray aniline hydrogen phthalate and (b) BuOH–EtOH–H_2O (5:1:4; upper phase), spray anisidine hydrochloride.

The plant was collected from Lahul and Spiti district, Himachal Pradesh, India and identified by Dr B. N. Mehrotra of our Botany Division. A voucher specimen is preserved in the Herbarium of our Institute. The shed-dried aerial parts (12 kg) were extracted with 90% EtOH. The total alcoholic extract concentrate was successively fractionated with hexane, $CHCl_3$ and BuOH. The $CHCl_3$ fraction (57.0 g) was chromatographed over silica gel G with a stepwise increase of MeOH content in $CHCl_3$ (2, 5, 10, 15, 20, 25, 30, 50, 100%). The residue of the 15–25% MeOH as eluent (16.2 g) was subjected to CC over silica gel with a C_6H_6–EtOAc (5–50%) gradient and finally eluted with EtOAc. Frs eluted with C_6H_6–EtOAc (50%) and EtOAc (100%) yielded iridoid glycoside-containing fractions which on flash chromatography over silica gel (250–400 mesh) using $CHCl_3$–MeOH (6 to 7%) as eluent afforded koelzioside (**1**) (1.2 g) as an amorphous, TLC single spot, product.

6-O-α-L-(2″,3″-O-trans-*cinnamoyl*-4″-O-*acetyl*)*Rhamnopyranosyl catalpol* (**1**). Amorphous powder, $[\alpha]_D$ −24° (MeOH; *c* 0.42); UV λ_{max}^{MeOH} nm (log ε): 205 (4.02), 222 sh (4.17), 278 (4.46); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1725 (acetyl), 1715 (ester), 1660 (C=C), 1630 (C=C, cinnamoyl), 1575, 1490, 1450 (aromatic region), 1220, 1202, 1160, 1060, 940, 770; ^1H NMR (400 MHz, CD_3OD); δ 1.13 (3H, *d*, *J* = 6 Hz, H-6″), 1.92 (3H, *s*, OMeCO), 2.40 (1H, *m*, H-5), 2.48 (1H, *dd*, *J* = 9.3/7.6 Hz, H-9), 3.12–3.40 (4H, H-2′, H-3′, H-4′, H-5′), 3.51 (1H, *dd*, *J* = 12.4/6.0 Hz, H-6′A), 3.57 (1H, *br s*, H-7), 3.70 (1H, *d*, *J* = 13.2 Hz, H-10 A), 3.93 (1H, *dd*, *J* = 12.4/1.7, H-6′B), 3.96 (1H, *dq*, *J* = 10/6 Hz, H-5″), 3.99 (1H, *dd*, *J* = 8.9/0.9 Hz, H-6), 4.05 (1H, *d*, *J* = 13.2 Hz, H-10B), 4.67 (1H, *d*, *J* = 8 Hz, H-1′), 4.95 (1H, *dd*, *J* = 3.4/6 Hz, H-4), 4.96 (1H, *d*, *J* = 9.5 Hz, H-1), 5.04 (1H, *d*, *J* = 1.7 Hz, H-1″), 5.14 (1H, *dt*, *J* = 10 Hz, H-4″), 5.31 (1H, *dd*, *J* = 3.4/10 Hz, H-3″), 5.37 (1H, *dd*, *J* = 1.7/3.4 Hz, H-2″), 6.28 (1H, *dd*, *J* = 6.1/1.7 Hz, H-3), 6.36 (1H, *d*, *J* = 16 Hz, H-β′), 6.52 (1H, *d*, *J* = 16 Hz, H-β), 7.24 (3H, *m*, H-3‴, H-4‴, H-5‴), 7.27 (2H, *m*, H-2‴, H-6‴), 7.34 (3H, *m*, H-3‴, H-4‴, H-5‴), 7.46 (2H, *m*, H-2‴, H-6‴), 7.56 (1H, *d*, *J* = 16 Hz, H-α′), 7.60 (1H, *d*, *J* = 16 Hz, H-α); ^{13}C NMR: Table 1. (Found: C, 60.41; H, 5.61% $C_{41}H_{46}O_{17}$ requires; C, 60.74; H, 5.67%.)

Alkaline hydrolysis of compound **1**. Compound **1** (200 mg) was hydrolysed with 10 ml 0.5 M NaOH at 20°. The reaction was followed by TLC (silica gel, precoated, $CHCl_3$–MeOH, 6:1). After disappearance of the starting material, the reaction mixt. was neutralized with methanolic HCl. The resulting mixt. was chromatographed over silica gel G using $CHCl_3$–MeOH (50:1, 7:3), as eluent to give an acid and **3** respectively. The acid on methylation was identified as methylcinnamate (^1H NMR, MS).

6-O-α-L-*Rhamnopyranosyl catalpol* (**3**). Powder, $[\alpha]_D$ −148° (MeOH; *c* 2.1): UV λ_{max}^{MeOH}: <210 nm; IR ν_{max}^{KBr} cm^{-1}: 3480, 1665, 1050, 940, 850, 785; ^1H NMR (MeOH-d_6: 400 MHz); δ 1.27 (3H, *d*, *J* = 6 Hz, H-6″), 2.45 (1H, *m*, H-5), 2.60 (1H, *t* like, *J* = 8 Hz, H-9), 6.36 (1H, *dd*, *J* = 6 Hz, H-3); ^{13}C NMR: Table 1. (Found: C, 46.11; H, 6.14, $C_{21}H_{32}O_{14} \cdot 2H_2O$; requires: C, 46.32; H, 6.61%.)

Compound **3** (56 mg) dissolved in 0.5 M H_2SO_4 (1 ml), was refluxed for 2 hr. Black degradation products were removed by filtration. The aq. hydrolysate was neutralized with $BaCO_3$ and subjected to PC (co-chromatography) in solvent systems (a) and (b). This revealed the presence of D-glucose (R_f 0.18, 0.42) and L-rhamnose (R_f 0.40, 0.7).

Methyl cinnamate. Needles ($CHCl_3$), mp 128°. IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1690 (C=O), 1640, 1460, 1422 (aromatic), 1320, 1298, 1236, 980 and 935; ^1H NMR ($CDCl_3$: 90 MHz); δ 6.34 (1H, *d*, *J* = 16 Hz; H-α), 7.30–7.45 (5H, *m*, H-2, H-3, H-4, H-5, H-6), 7.70 (1H, *d*, *J* = 16 Hz, H-β), EIMS *m/z*: 162 $[M]^+$, 148, 131, 120, 113, 103, 102, 91, 83, 76, 70, 57.

Compound **1** *hexaacetate* (**4**). Compound **1** (50 mg) was treated with a mixt. of Ac_2O and pyridine. Usual work-up, gave a powder (62 mg), $[\alpha]_D$ −42° ($CHCl_3$; *c* 0.24); UV λ_{max}^{MeOH} (log ε): 218 (4.14), 278 (4.36) nm; IR ν_{max}^{KBr} cm^{-1}: 1750 (acetyl), 1635, 1500, 1420, 1365, 1280, 1185, 1040, 950 and 880; ^1H NMR (400 MHz, $CDCl_3$); δ 1.29 (3H, *d*, *J* = 6 Hz, H-6″), 1.99, 2.03, 2.04, 2.05, 2.11, 2.14 (each, 3H, *s*, OMeCO), 2.54 (1H, *m*, H-5), 2.64 (1H, *dd*, *J* = 9.3/7.6 Hz, H-9), 3.84 (1H, *br s*, H-7), 3.96 (1H, *dd*, *J* = 8.9/0.9 Hz, H-6), 3.97 (1H, *d*, *J* = 12.8 Hz, H-10A), 3.98 (1H, *dd*, *J* = 10.1/1.7/6.2 Hz, H-5′), 4.04 (1H, *dq*, *J* = 10/6 Hz, H-5″), 4.16 (1H, *dd*, *J* = 6.2/12.4 Hz, H-6′A), 4.38 (1H, *dd*, *J* = 12.4/1.7 Hz, H-6′B), 4.78 (1H, *d*, *J* = 9.7 Hz, H-1), 4.79 (1H, *d*, *J* = 8/9.5 Hz, H-2′), 4.83 (1H, *d*, *J* = 12.8 Hz, H-10B), 4.96 (1H, *d*, *J* = 8 Hz, H-1′), 5.07 (1H, *d*, *J* = 1.7 Hz, H-1″), 5.08 (1H, *dd*, *J* = 3.4/6 Hz, H-4), 5.14 (1H, *dd*, *J* = 8.8/10 Hz, H-4′), 5.18 (1H, *d*, *J* = 9.5/8.8 Hz, H-3′), 5.22 (1H, *dd*, *J* = 1.7/3.4 Hz, H-2″), 5.34 (1H, *dt*, *J* = 10 Hz, H-4″), 5.53 (1H, *dd*, *J* = 3.4/10 Hz, H-3″), 6.33 (1H, *dd*, *J* = 6/1.7 Hz, H-3), 6.36 (1H, *d*, *J* = 16 Hz, H-β′), 6.59 (1H, *d*, *J* = 16 Hz, H-β), 7.34 (6H, *m*, H-3‴, H-3⁗, H-4‴, H-4⁗, H-5‴, H-5⁗), 7.57 (4H, *m*, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.66 (1H, *d*, *J* = 16 Hz, H-α′), 7.74 (1H, *d*, *J* = 16 Hz, H-α); ^{13}C NMR: see Table 1. (Found: C, 61.89; H, 5.57%, $C_{53}H_{58}O_{23}$, requires: C, 62.35, H, 5.68%.)

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