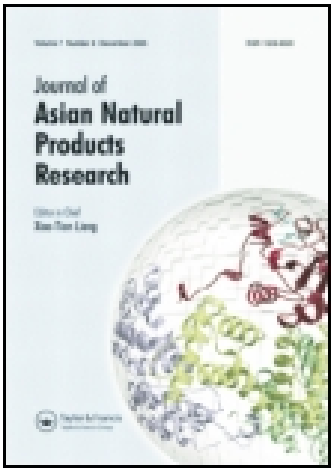

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New iridoid glycoside from Gratiola officinalis

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NOTE

New iridoid glycoside from *Gratiola officinalis*

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A new iridoid glycoside, 1β,6β-di-*O-trans*-cinnamoyl-9-*O*-β-D-glucopyranosyl-3-iridene-5β-ol (**1**), along with four known compounds loliolide (**2**), *β*-sitosterol-3-*O*-β-D-glucopyranoside (**3**), betulinic acid (**4**), and *β*-amyrin (**5**), was isolated from the aerial parts of *Gratiola officinalis* L. The structure of the new compound was deduced on the basis of 1D ^{1}H and ^{13}C NMR and 2D HMQC, HMBC, and COSY experiments, and mass spectrometric techniques (EI-MS and HR-EI-MS). The relative configuration of **1** was assigned by comparative analysis of the NMR spectral data with known analogs, together with NOESY experiments.

Keywords: Scrophulariaceae; *Gratiola officinalis*; iridoid

1. Introduction

Gratiola officinalis L., belonging to the family Scrophulariaceae, is a small herb which usually grows in wet habitats such as river valleys and has become much rarer in the last decades because of drainage [1,2]. In Europe, the aerial parts of this plant have been used for a wide variety of ailments, including menstrual disorders as well as skin and liver diseases [2]. For centuries, it was a popular medicinal plant and is still being used in homeopathic and in folk medicine as an anthelmintic [2]. The herb apparently tastes very bitter, and the bitter principles were thought to be cardenolides and glycosides of cucurbitacine [1]. Two groups of Russian and Austrian workers reported the presence of a number of flavon C-monoglycosides, cucurbitacine glycosides, and scutellarein derivatives in *G. officinalis* [3–6]. In the course of phytochemical investigations of medicinal plants from Pakistan and Turkey [7–11], we investigated *G. officinalis* and obtained one new iridoid glycoside, 1β,6β-di-*O-trans*-cinnamoyl-9-*O*-β-D-glucopyranosyl-3-iridene-5β-ol (**1**), along with four known compounds **2**–**5**.

2. Results and discussion

The water suspension of the crude extract, obtained from the aerial parts of the plant, was partitioned against *n*-hexane, dichloromethane (DCM), ethyl acetate, and *n*-butanol fractions. By repeated column chromatography of the DCM and ethyl acetate fractions, five compounds were obtained: 1β,6β-di-*O-trans*-cinnamoyl-9-*O*-β-D-glucopyranosyl-3-iridene-5β-ol (**1**), loliolide (**2**), β-sitosterol-3-*O*-β-D-glucopyranoside (**3**), betulinic acid (**4**), and

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β-amyrin (**5**). The known compounds **2**–**5** were identified on the basis of comparison with the published data [12–17].

The new compound **1** was isolated as a pale yellow gummy material from the ethyl acetate soluble fraction by repeated column chromatography over a silica gel column, and the structure was deduced on the basis of the spectroscopic methods. The IR spectrum of compound **1** showed absorption bands for hydroxyl and ester groups (3415 and 1708 cm^{-1}) and aromatic moiety. The UV spectrum showed absorption maxima at 203, 216, and 273 nm, characteristics of the iridoid-enol ether system with a cinnamoyl chromophore [18].

The molecular ion was established by FAB-MS (-) at *m/z* 623, corresponding to the formula, $C_{33}H_{35}O_{12}$, for the deprotonated ion. The molecular formula of compound **1**, $C_{33}H_{36}O_{12}$, was further confirmed through HR-FAB-MS showing the quasimolecular ion at *m/z* 623.2187 $[M-1]^+$. The other prominent ions in the EI-MS were observed at *m/z* 316, 154, 137, and 85, which further supported the presence of an iridoid skeleton with hydroxyl, glucosyl, and the cinnamoyl moieties (Figure 1).

The ^{1}H NMR spectrum of **1** also indicated the presence of an iridoid skeleton [19] with cinnamoyl and glucosyl moieties. It showed the signals for H-1 at δ 5.18 as a broad singlet and the methylene protons (H-7) resonating at δ 2.79 as a multiplet. Another doublet at δ 1.09 (3H, $J = 6.0$ Hz) was assigned to the methyl group, H-10. The presence of two olefinic methine protons (H-3 and H-4) was also indicated by two doublets at δ 6.43 (1H, d, $J = 6.6$ Hz) and 5.73 (1H, d, $J = 6.6$ Hz), respectively, in the ^{1}H NMR spectrum. The two cinnamoyl moieties were inferred from the signals of 10 aromatic protons at δ 7.05–6.55, whereas the *trans* olefinic protons at δ 7.58 and 6.26 appeared as doublets with coupling constant 15.9 Hz. The anomeric proton of the sugar moiety at δ 4.37 as a doublet ($J = 7.2$ Hz) indicated the β-linked sugar. The remaining protons of the sugar moiety resonated between δ 4.09 and 3.52. Furthermore, the methylenic carbon C-6′ at δ 62.4 and four methine carbons (C-2′ to C-5′) at δ 76.1–70.4 also supported the presence of β-linked sugar in compound **1**.

On acid hydrolysis with 10% H_2SO_4, compound **1** gave the aglycone along with two cinnamic acid molecules and a sugar moiety that was identified as D-glucose by Co-TLC with the authentic sample.

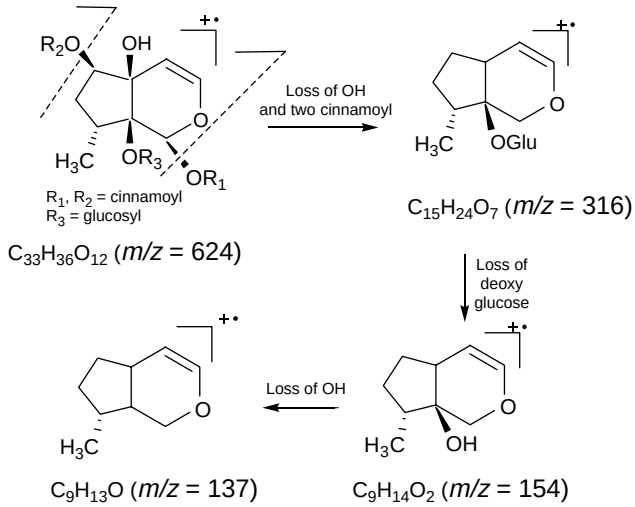

Figure 1. Key mass fragmentations of compound **1**.

The ^{13}C NMR spectrum (BB and DEPT) also supported the presence of two cinnamoyl groups. The signals for two ester carbonyl carbons (C-9″, C-9‴) were resonated at δ 168.3, whereas those for olefinic and aromatic carbons skeleton were observed in the downfield region (δ 148.0–116.3) with double intensity of the aromatic carbon signals (Table 1).

The direct one-bond $^{1}H-^{13}C$ connectivities were inferred from the HMQC spectrum, whereas the COSY and HMBC interactions (Figure 2) helped in constructing the structure of compound **1** by assigning the position of the individual groups on the iridoid skeleton. The anomeric proton (H-1′) showed HMBC interactions with C-9 (δ 81.7), C-2′ (δ 72.3), and C-5′ (δ 76.1), indicating the attachment of the sugar moiety at C-9 position. Similarly, H-1 and H-6 showed interactions with C-9″ (δ 168.3) and C-9‴ (δ 168.3), respectively. On the basis of these observations, the attachments of the two cinnamoyl groups were assigned at C-1 and C-6 positions of the iridoid nucleus.

The multiplicities of carbons were determined through the DEPT experiments, whereas the relative stereochemistry of compound **1** was deduced on the basis of the NOESY interactions. The $^{1}H-^{1}H$ NOESY correlations of Me-10

Table 1. ^{1}H and ^{13}C NMR spectral data for compound **1**.

C. No.	^{13}C (δ)	^{1}H (δ)	Multiplicity	HMBC correlation
1	103.1	5.18, br s	CH	9, 9″
3	146.2	6.43, d, $J = 6.6$ Hz	CH	4
4	104.2	5.73, d, $J = 6.6$ Hz	CH	3, 5
5	76.2		C	
6	70.6	4.91, m	CH	5, 9, 9‴
7	36.5	2.79, m	CH_2	
8	49.3	3.27, m	CH	9, 10
9	81.7		C	
10	18.5	1.09, d, $J = 6.0$ Hz	CH_3	8
1′	104.2	4.37, d, $J = 7.2$ Hz	CH	9, 2′
2′	72.3		CH	
3′	73.8	3.90–3.52	CH	
4′	70.4	Overlap H-2′ to H-5′	CH	
5′	76.1		CH	
6′	62.4	4.09, m; 3.98, m	CH_2	
1″	131.5		C	
2″	121.3	7.05, dd, $J = 7.6$, 2.0 Hz	CH	
3″	123.2	6.75, dd, $J = 7.6$, 6.9 Hz	CH	
4″	127.5	6.55, t, $J = 6.9$ Hz	CH	
5″	123.2	6.75, dd, $J = 7.6$, 6.9 Hz	CH	
6″	121.3	7.05, dd, $J = 7.6$, 2.0 Hz	CH	
7″	148.0	7.58, d, $J = 15.9$ Hz	CH	1″, 8″
8″	116.3	6.26, d, $J = 15.9$ Hz	CH	9″, 7″
9″	168.3		C	
1‴	131.5		C	
2‴	121.3	7.01, dd, $J = 7.6$, 2.0 Hz	CH	
3‴	123.2	6.82, dd, $J = 7.6$, 6.9 Hz	CH	
4‴	127.5	6.55, t, $J = 6.9$ Hz	CH	
5‴	123.2	6.82, dd, $J = 7.6$, 6.9 Hz	CH	
6‴	121.3	7.01, dd, $J = 7.6$, 2.0 Hz	CH	
7‴	148.0	7.58, d, $J = 15.9$ Hz	CH	1‴, 8‴
8‴	117.1	6.26, d, $J = 15.9$ Hz	CH	9‴, 7‴
9‴	168.3		C	

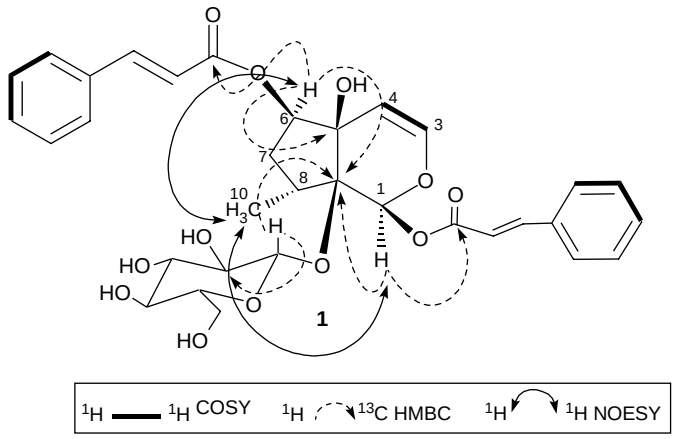

Figure 2. Key COSY, HMBC, and NOESY interactions for compound **1**.

with H-1 and H-6 were helpful in assignment of the relative stereochemistry of C-1 and C-6, whereas the remaining positions were confirmed on the basis of biogenetic considerations and by comparing the NMR spectral data with the reported data for iridoids [20,21]. Compound **1** was closely related to the known compound, harpagoside, earlier reported from *Harpagophytum procumbens* [20,21], with the difference of the number of cinnamoyl moieties and the position of the sugar group in the skeleton.

The above spectral studies thus fully supported the structure of 1β,6β-di-*O*-*trans*-cinnamoyl-9-*O*-β-D-glucopyranosyl-3-iridene-5β-ol for compound **1** as a new constituent from *G. officinalis*.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO DIP 360 polarimeter (Easton, MD, USA). IR spectra were recorded on a Bruker VECTOR 22 spectrophotometer. EI-MS and HR-EI-MS were recorded on mass spectrometers JEOL JMS HX 110 (Akishima, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers operating at 300 MHz (75 MHz for ^{13}C). The chemical shifts values are reported in ppm (δ) units and the coupling constants (J) are given in Hz.

For TLC, pre-coated aluminum sheets (silica gel 60F-254, E. Merck) were used. Visualization of the TLC plates was achieved under UV at 254 and 366 nm and by spraying with the ceric sulfate reagent. MeOH:DCM 1–5% was used as solvent system.

3.2 Plant material

The plant *G. officinalis* L. (Scrophulariaceae) was collected from Swat (Pakistan) in 2003 and was identified by Mr Mehboob ur Rahman, Assistant Professor, Department of Botany, Govt Jehanzeb Post Graduate College, Saidu Sharif, Swat, K.P.K., Pakistan. A voucher specimen (OG-14) has been deposited at the herbarium of the department.

3.3 Extraction and isolation

The air dried and powdered aerial parts (*G. officinalis* 7 kg) were extracted exhaustively with 80% ethanol–water at room temperature to get 400 g of the crude extract. The residue was suspended in water and partitioned in *n*-hexane (10 g), DCM (25 g), ethyl acetate (20 g), and *n*-butanol (120 g) extracts. The ethyl

acetate and the DCM extracts were subjected to further fractionation and purification. On repeated silica gel column chromatography using 1% MeOH:DCM, the ethyl acetate extract yielded compounds **1** (4.7 mg) and **2** (7.5 mg). The DCM extract was subjected to column chromatography on silica gel by using various polarity solvent systems which gave 16 sub-fractions.

The semi-pure fractions 7–12 were again loaded on silica gel and eluted with MeOH:DCM. Compounds **3** (12 mg) and **4** (3.5 mg) were purified from fraction 12 by eluting with MeOH:DCM (2:98) and (3:97), respectively, and compound **5** (4 mg) was purified by repeated silica gel column chromatography eluted with MeOH:DCM (5:95).

*3.3.1 1β,6β-di-O-Trans-cinnamoyl-9-O-β-D-glucopyranosyl-3-iridene-5β-ol (**1**)*

Brown gummy solid: $[\alpha]_D^{30} + 79$ (*c* 0.05, CH_3OH); UV (MeOH) λ_{max} (log ε): 203 (3.94), 216 (3.74), 273 (3.13) nm; IR (KBr): ν_{max} 3415, 2922, 1705, 1635, 1450, 1395 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): see Table 1; ^{13}C NMR (CD_3OD, 75 MHz): see Table 1; FAB-MS: *m/z* 623 $[M-1]^+$; HR-FAB-MS: *m/z* 623.2187 $[M-1]^+$ (calcd for $C_{33}H_{35}O_{12}$, 623.2181).

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The authors are grateful to Mr Mehboob ur Rahman, Assistantt Professor, Department of Botany, Govt Jehanzeb Postgraduate College, Saidu Sharif, Swat, K.P.K., Pakistan, for help in the collection and identification of the plant and to the Higher Education Commission (Pakistan) for providing financial support.

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