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AN IRIDOID DIGLYCOSIDE ISOLATED FROM *SCROPHULARIA SCORODONIA*

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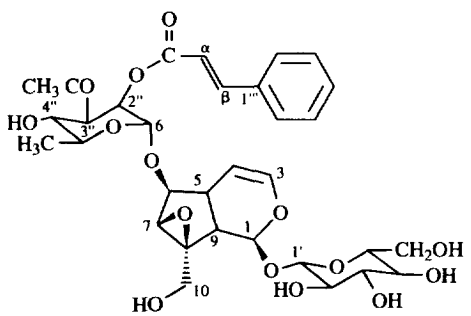
Key Word Index—*Scrophularia scorodonia*; Scrophulariaceae; iridoid diglycoside; 6-*O*-α-L-[3″-acetyl-2″-*trans*-cinnamoyl]-rhamnopyranosyl catalpol; scorodioside.

Abstract—A new iridoid diglycoside, scorodioside, has been isolated from *Scrophularia scorodonia*. The structure of this compound was elucidated by chemical and spectral analysis as 6-*O*-(3″-*O*-acetyl-2″-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosylcatalpol. In addition, 8-*O*-*cis*-cinnamoylharpagide has been isolated.

INTRODUCTION

The genus *Scrophularia* is known for the presence of variety of iridoid glycosides [1, 2]. In a continuation of our systematic phytochemical studies on Spanish medicinal plants, we have investigated *Scrophularia scorodonia* L., which is widespread in the south-west of Spain and in the north-west of Africa. In a previous study, four iridoids glycosides—harpagoside, bartsioside, aucuboside and 8-*O*-acetylharpagide—were isolated from the 80% methanol fraction of the aerial part of this plant [3].

In a further study on this fraction, a new iridoid diglycoside, scorodioside (**1**) was isolated together with the known iridoid 8-*O*-*cis* cinnamoylharpagide (**2**).



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RESULTS AND DISCUSSION

Scorodioside (**1**) was assigned the molecular formula $C_{32}H_{40}O_{16}$ (FAB-MS *m/z* 679 $[M - H]^-$). Its UV spectrum showed absorption bands characteristic of an iridoid enol ether system and a cinnamoyl chromophore. The ^{1}H NMR spectrum of **1** was typical of a catalpol monoglycoside showing signals for two sugar moieties along with those arising from acyl moieties and the genin part of catalpol. In addition to five aromatic (δ7.41–7.62) and two olefinic (δ6.59 and 7.73, AB system, J_{AB} = 16.0 Hz) protons arising from the *trans*-cinnamoyl moiety, one acetoxyl signal was observed at δ2.01, indicating the presence of one acetyl moiety and one *trans*-cinnamoyl moiety.

Two signals for anomeric protons appeared at δ4.76 (*d*, J = 7.9 Hz) and 5.05 (*d*, J = 1.6 Hz) indicating β-D-glucose and α-L-rhamnose as the sugar moieties. The locations of the two acyl groups were deduced from the fact that the ^{1}H NMR signals of H-2″ and H-3″ of rhamnose were shifted downfield to δ5.36 and 5.14, respectively.

A difference between the ^{1}H NMR spectra of compound **1** and that of catalpol was that the H-6 resonance was shifted downfield (δ4.05), indicating that the linkage involved the OH group at the C-6 carbon.

In the ^{13}C NMR spectrum, six typical signals for β-glucopyranose and six signals for a substituted α-rhamnopyranose were observed. The presence of one *trans*-cinnamoyl group and an acetoxy group in the rhamnopyranosyl catalpol was also evident. The remaining nine signals fitted very well with a 6-substituted 6-*O*-α-L-rhamnopyranosylcatalpol (**3**) [4] and suggested **1** to be this compound with one cinnamoyl and one acetyl unit linked to the rhamnosyl moiety. DEPT sequence allowed assignment of the carbon multiplicities.

The positions of the *trans*-cinnamoyl and acetoxy moieties were determined using (HMQC) and HMBC experiments. The HMQC sequence (^{1}H detected one bond

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heteronuclear multiple quantum coherence) established the connectivities between C-2″ (δ71.47) and H-2″ (δ5.36, *dd*, $J = 1.6/3.4$), C-3″ (δ73.18) and H-3″ (δ5.14, *dd*, $J = 3.4/9.9$). In the HMBC sequence spectrum, long-range connectivities ^3J were observed between $\underline{C}OCH_3$ (δ172.25) and H-3″ (δ5.14) and C-1‴ (δ167.51) and H-2″ (δ5.36). The results indicated clearly the locations of the *trans*-cinnamoyl and the acetoxy moieties.

Furthermore, alkaline hydrolysis of **1**, yielded compound **3**. Based on these data, the structure of **1** was determined to be 6-*O*-[(3″-*O*-acetyl-2″-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosyl]-catalpol.

To date several acylated 6-*O*-α-L-rhamnopyranosyl-catalpols have been reported. Rhamnopyranosylcatalpol was first isolated from *Scrophularia nodosa* [4]. Diacyl-rhamnopyranosylcatalpols have been reported from *Verbascum sinuatum* [5, 6] and triacyl derivates from *S. scopolii* [7], *S. koelzii* [8], *S. spicata* [9], *S. ilwensis* [10] and *S. korainensis* [11]. In particular, scrospioside **B** (6-*O*-α-L-[2″-acetyl-3″-*trans*-cinnamoyl]-rhamnopyranosyl-catalpol) from *S. spicata* is not very different from scorodioside (**1**). The differences are the locations of the *trans*-cinnamoyl and the acetoxy residues.

Compound **2**, $C_{24}H_{30}O_{11}$, was obtained as an amorphous powder. When compared with harpagide, C-8 was shifted in the ^{13}C NMR spectrum of **2** from δ78.1 to 88.7. A downfield shift was also observed for the resonance of Me-10 in the ^{1}H NMR spectrum. These data, which are in good agreement with those found for 8-*O*-*cis*-cinnamoylharpagide isolated from *Rogeria adenophylla* [12], indicated that OH-8 was esterified by the cinnamoyl residue. In the ^{1}H NMR spectrum of **2**, the olefinic protons H-α and H-β appeared as two doublets at δ6.95 and 5.96, respectively, with a coupling constant of 12.6 Hz. This demonstrated the *cis* configuration of the cinnamoyl moiety which also accounts for the UV spectrum of **2** [λ_{max} 271 nm (log ε 3.97)].

EXPERIMENTAL

General. Prep. MPLC: Supercosil RP-18 column (26–40 μm; i.d. 4.6 × 15 mm); TLC: silica gel G-60 UV 254 plates (Merck) with $CHCl_3$–MeOH–H_2O (80:20:1). The spots were visualized by spraying with vanillin sulphuric reagent (vanillin 1% and sulphuric acid 1% in alcohol 1.1). FAB-MS was in a 10-10H Nermag mass spectrometer in the negative ion mode. ^{1}H and ^{13}C NMR: 400 and 400 MHz, respectively, CD_3OD, TMS as int. standard. Resonance multiplicities for ^{13}C NMR were established via the aquisition of DEPT spectra obtained for proton pulse P = 3M/4 (CH and CH_3 differentiated from CH_2). Standard Bruker pulse sequences were used for both direct and long-range heteronuclear correlation experiments. For other experimental detail see Faure *et al.* [13].

Plant material. Scrophularia scorodonia was collected in May 1992 from Jaen (Spain) and identified by Dra C. Bartolomé Esteban (Department of Vegetal Biology, Faculty of Sciences, Alcalá de Henares, Madrid, Spain). A voucher specimen (L. MF 92) is deposited at the Pharmacognosy Department of the University of Alcalá de Henares.

Extraction and isolation. Powdered aerial parts (1 kg) were extracted at room temp with 80% MeOH. The 80% MeOH extract (134 g) was fractionated on a polyamide column (MN SC 6; 1 kg) and eluted succesively by H_2O, 50% MeOH, 75% MeOH and MeOH. 25 fractions were collected (I–XXV). Fraction VII (2 g) was submitted to MPLC on RP-18; elution with MeOH–H_2O (1:1) gave **1** (61.3 mg). Compound **2** (10 mg) was isolated by the same technique from fraction V (440 mg) with MeOH–H_2O (11:9).

Scorodioside (**1**). Amorphous powder. UV λ_{max}^{MeOH} nm (log ε): 205 (4.02), 218 sh (4.17), 282 (4.46); FAB-MS *m/z*: 679 $[M - H]^-$, 517 $[(M - H) - 162]^-$; ^{1}H NMR (400 Mz, CD_3OD). δ1.33 (3H, *d*, $J = 6.2$ Hz, H-6″), 2.01 (3H, *s*, OMeCO), 2.47 (1H, *m*, H-5), 2.57 (1H, *dd*, $J = 9.6/7.7$ Hz, H-9), 3.24–3.39 (4H, H-2′, H-3′, H-4′, H-5′), 3.61 (1H, *dd*, $J = 12/5.6$ Hz, H-6′A), 3.65 (1H, *br s*, H-7), 3.80 (1H, *d*, $J = 13.2$ Hz, H-10A), 3.90 (1H, *dd*,

Table 1. ^{13}C NMR data of compounds **1** and **3** (CD_3OD)

C	1	3*
Aglycone		
1	95.15	95.14
3	142.35	142.18
4	103.36	103.21
5	37.20	37.29
6	84.38	83.51
7	59.37	59.31
8	66.55	66.56
9	43.28	43.22
10	61.45	61.44
Glucose		
1′	99.71	100.24
2′	74.83	74.82
3′	77.68	77.63
4′	71.78	71.74
5′	78.64	78.61
6′	62.96	62.89
Rhamnose		
1″	97.76	99.69
2″	71.47†	72.33
3″	73.18	72.2
4″	71.45†	73.84
5″	70.28	70.17
6″	17.98	17.97
Cinnamoyl		
CO	167.51	
β	147.34	
α	118.15	
1‴	135.56	
2‴, 6‴	130.09	
3‴, 5‴	129.41	
4‴	131.78	
$\underline{C}OCH_3$	172.25	
$CO\underline{C}H_3$	20.87	

* Data taken from Ref. [7].

† ^{13}C shifts may be reversed.

J = 12/1.9 Hz, H-6′B), 3.87 (1H, *dq*, J = 9.6/6.2 Hz, H-5″), 4.05 (1H, *dd*, J = 8.5/0.9 Hz, H-6), 4.14 (1H, *d*, J = 13.2 Hz, H-10B), 4.76 (1H, *d*, J = 7.9 Hz, H-1′), 5.09 (1H, *dd*, J = 4.7/6 Hz, H-4), 5.08 (1H, *d*, J = 9.6 Hz, H-1), 5.05 (1H, *d*, J = 1.6 Hz, H-1″), 3.61 (1H, *dt*, J = 9.9/9.6 Hz, H-4″), 5.14 (1H, *dd*, J = 3.4/9.9 Hz, H-3″), 5.36 (1H, *dd*, J = 1.6/3.4 Hz, H-2″), 6.38 (1H, *dd*, J = 6/1.7 Hz, H-3), 6.59 (1H, *d*, J = 16 Hz, H-α), 7.41 (3H, *m*, H-3‴, H-4‴, H-5‴), 7.62 (2H, *m*, H-2‴, H-6‴), 7.73 (1H, *d*, J = 16 Hz, H-β); ^{13}C NMR: Table 1.

Alkaline hydrolysis of compound **1**. A soln of **1** (10 mg) in 5% methanolic KOH (2 ml) was kept at room temp for 2 hr. The mixture was neutralized with 1M HCl and filtered. The filtrate was evaporated to dryness *in vacuo*, and the residue, compound **3**, was identified by TLC comparison with an authentic samples (silica gel, $CHCl_3$–MeOH, 6:5:3).

8-O-cis-*Cinnamoylharpagide* (**2**). Amorphous powder. UV λ_{max}^{MeOH} nm (log ε): 271 (3.97); ^{1}H NMR (CD_3OD): δ7.54 (2H, *m*, H-2″, H-6″), 7.33 (3H, *m*, H-3″, H-4″, H-5″), 6.95 (1H, *d*, J = 12.6 Hz, H-β), 6.36 (1H, *d*, J = 6.4 Hz, H-3), 6.08 (1H, *d*, J = 1.1 Hz, H-1), 5.96 (1H, *d*, J = 12.6 Hz, H-α), 4.90 (1H, *dd*, J = 6.4/1.6 Hz, H-4), 4.55 (1H, *d*, J = 7.8 Hz, H-1′), 3.70 (1H, *m*, H-6), 2.85 (1H, *br s*, H-9), 2.12 (1H, *br d*, J = 15.0 Hz, H-7β), 1.95 (1H, *dd*, J = 15/4.5 Hz, H-7α), 1.48 (3H, *s*, H8-10); ^{13}C NMR: δ94.38 (C-1), 143.68 (C-3), 107.14 (C-4), 73.09 (C-5), 77.58 (C-6), 45.98 (C-7), 88.74 (C-8), 55.66 (C-9), 22.45 (C-10), 99.71 (C-1′), 74.56 (C-2′), 78.15 (C-3′), 71.74 (C-4′), 77.78 (C-5′), 62.82 (C-6′), 168.14 (C =O), 122.03 (C-α), 143.07 (C-β), 136.41 (C-1″), 130.67 (C-2″, C-6″), 129.18 (C-3″, C-5″), 129.93 (C-4″).

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