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# AN ACYLATED ISORHAMNETIN GLYCOSIDE FROM *HERNIARIA FONTANESII*

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**Key Word Index**—*Herniaria fontanesii*; Caryophyllaceae; ( − )-catechin; isorhamnetin 3-robinobioside; isorhamnetin 3-*O*-β-[3‴-feruloyl-α-rhamnosyl (1 → 6) galactoside].

**Abstract**—A new flavonol derivative, isorhamnetin 3-[3‴-feruloylrhamnosyl(1 → 6)galactoside], was isolated from the aerial parts of *Herniaria fontanesii* together with isorhamnetin 3-robinobioside and ( − ) catechin.

## INTRODUCTION

The flavonoids of *Herniaria* species, which have a widespread distribution in the Mediterranean area, have been until now little studied [1–4]. In a previous investigation of the secondary metabolites of *Herniaria fontanesii* J. Gray, we isolated a triterpenoid saponin (herniaria saponin A) [5] and now report the characterization of a new flavonoid derivative from the aerial parts of the same plant, in addition to two known flavonoids.

## RESULTS AND DISCUSSION

The dried aerial parts of *H. fontanesii* were consecutively extracted with dichloromethane and methanol. The dried alcoholic extract, suspended in water was extracted with dichloromethane, then ethyl acetate. The latter phase was concentrated and then purified by a combination of flash chromatography and preparative TLC on silica gel. Three compounds (**1**, **2** and **3**) were isolated. Compounds **1** and **2** were rapidly identified by comparison of their spectral data with the literature as ( − )-catechin and isorhamnetin 3-robinobioside, respectively [6, 7]. Methanolysis of **2** and **3** afforded the same sugar residues, identified as galactose (Gal) and rhamnose (Rha), by GC of their trimethylsilylated methylglycosides [8]. The FAB-mass spectrum of **3** showed a quasimolecular ion at *m/z* 823 corresponding to $[M + Na]^+$ and indicating the molecular formula $C_{38}H_{40}O_{19}$. Thus, **3** was considered to be an isorhamnetin 3-robinobioside substituted with a ferulic acid residue. The $^1$H NMR spectrum of **3** showed the expected signals in the aromatic region; the *E*-vinylic protons appeared as two doublets at δ6.50 and 7.75 ($J$ = 16 Hz) and the anomeric protons at δ5.32 ($J$ = 7.8 Hz) and 4.65 ($J$ = 1.6 Hz) were assigned to a β-D-Gal-$H_1$ and a α-L-Rha-$H_1$ [9], respectively. The complete assignment of the proton and carbon resonances (Table 1) was achieved using homo- and hetero-nuclear 1D and 2D NMR techniques. More particularly, the HMBC [10] spectrum displayed a cross-peak between C3 and β-D-Gal-$H_1$, confirming that this sugar residue was attached to the isorhamnetin nucleus, the deshielding of Gal-C6 (δ67.3) established that this position was involved in the interglycosidic linkage and attribution of the signal at δ5.1 (*dd*, $J$ = 9.6 and 3.1 Hz) to Rha-H3 from the COSY spectrum demonstrated that this position was acylated by the feruloyl moiety. Finally, the location on C-3 of the methoxyl groups of each benzene ring was deduced from the NOESY spectrum, which displayed a correlation peak between the methoxy at δ4.06 and H2′ and between the methoxyl at δ3.98 and Fer-H2.

## EXPERIMENTAL

NMR spectra were recorded on Bruker AM-400 WB or AC-300 P spectrometers; chemical shifts are given in ppm in relation to the solvent peak. FAB-MS were recorded on Kratos MS 80 or concept II. HH instruments.

*Extraction and isolation.* Aerial parts of *H. fontanesii* were collected in Oujda (Morocco) in spring 1993 and authenticated by Drs Kahouadji and Bentatou from the Department of Botany, University Mohamed V. Rabat, Morocco. A voucher specimen (Ch. Sauvage 8918) has

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Table 1. $^{13}C$ and $^{1}H$ NMR data for compound **3**

| Position | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 2 | 158.7 | |
| 3 | 135.4 | |
| 4 | 179.5 | |
| 5 | 163.3 | |
| 6 | 99.9 | 6.23, *d*, *J* = 2.8 Hz |
| 7 | 166.1 | |
| 8 | 94.9 | 6.48, *d*, *J* = 2.8 Hz |
| 9 | 158.5 | |
| 10 | 105.5 | |
| 1′ | 123.8 | |
| 2′ | 114.6 | 8.10, *d*, *J* = 2.0 Hz |
| 3′ | 148.4 | |
| 4′ | 150.8 | |
| 5′ | 116.0 | 7.00, *d*, *J* = 8.5 Hz |
| 6′ | 124.0 | 7.65, *dd*, *J* = 8.5, 2.0 Hz |
| $OCH_3$ | 56.9 | 4.06, *s* |
| Gal-1 | 104.9 | 5.32, *d*, *J* = 7.8 Hz |
| Gal-2 | 73.1 | 3.95, *m** |
| Gal-3 | 75.3 | 3.68, *m** |
| Gal-4 | 69.9 | 3.84, *m** |
| Gal-5 | 75.3 | 3.90, *m** |
| Gal-6 | 67.3 | 3.56 and 3.68, *m** |
| Rha-1 | 101.8 | 4.65, *d*, *J* = 1.6 Hz |
| Rha-2 | 73.1 | 3.92, *m** |
| Rha-3 | 75.0 | 5.10, *dd*, *J* = 9.6, 3.1 Hz |
| Rha-4 | 71.3 | 3.64, *m** |
| Rha-5 | 69.9 | 3.76, *m** |
| Rha-6 | 18.0 | 1.32, *d*, *J* = 6.1 Hz |
| Fer-1 | 127.8 | |
| Fer-2 | 111.8 | 7.29, *d*, *J* = 1.9 Hz |
| Fer-3 | 149.3 | |
| Fer-4 | 150.6 | |
| Fer-5 | 116.5 | 6.85, *d*, *J* = 8.2 Hz |
| Fer-6 | 122.7 | 7.18, *dd*, *J* = 8.8, 1.9 Hz |
| Fer-7 | 146.9 | 7.75, *d*, *J* = 16.0 Hz |
| Fer-8 | 115.7 | 6.50, *d*, *J* = 16.0 Hz |
| Fer-9 | 168.6 | |
| $OCH_3$ | 56.3 | 3.98, *s* |

*Exact determination of the multiplicity precluded by the overlapping of the signals.

been deposited in the RAB-Herbarium of the Scientific Institute of Rabat. The dried aerial parts of *H. fontanesii* (250 g) were extracted at room temp. with $CH_2Cl_2$ then with MeOH. The MeOH extract (15 g) was suspended in $H_2O$ and extracted successively with $CH_2Cl_2$ and EtOAc. Concn of the EtOAc layer gave 3.7 g of residue, an aliquot (0.5 g) of which was subjected to CC. Final purification, achieved by prep. TLC using EtOAc–MeOH–$H_2O$ (100:17:13) as eluent afforded **1** (16 mg), **2** (5 mg) and **3** (5 mg).

*Molar carbohydrate composition.* Monosaccharides were analysed by GC of their trimethysilylated methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 hr, 80°) and trimethylsilylation [8].

*Compound* **3**. Amorphous powder, $[\alpha]_D^{19}$ −62° (MeOH; *c* 0.08). Positive FAB-MS *m/z* 823 $[M + Na]^+$. UV $\lambda_{max}$ nm: MeOH. 250, 268, 298sh, 328; + NaOMe, 268, 310sh, 389; + $AlCl_3$, 272, 296sh, 400; + $AlCl_3$ + HCl, 272, 296sh, 328, 400, + NaOAc, 276, 296sh, 324, 376sh; + NaOAc + $H_3BO_3$, 250, 268, 296sh, 328. $^{1}H$ and $^{13}C$ NMR data: Table 1.

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