Genistifolin and Other Iridoid Glucosides from *Linaria genistifolia* (L.) Mill.

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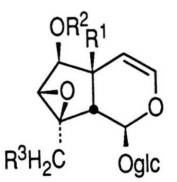
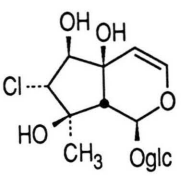
Iridoids, *Linaria*

The new iridoid genistifolin and the known antirrinoside and linarioside have been isolated from aerial parts of *Linaria genistifolia* (Scrophulariaceae). TLC data indicate the presence of 6-deacetylgenistifolin.

Introduction

Recently, we reported the isolation of iridoids from *Linaria vulgaris* [1]. As a part of our phytochemical and chemotaxonomic investigations on iridoids, we report the isolation of the new iridoid glucoside, genistifolin (**1**), from the MeOH extract of fresh aerial parts of *L. genistifolia,* as well as the known antirrinoside (**2**) and linarioside (**3**). The iridoid composition of *L. genistifolia* has not been investigated till now.





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	R^1	R^2	R^3
1	H	Ac	H
2	OH	H	H
4	H	H	OH

Results and Discussion

Separation of the methanol extract of *L. genistifolia* by sequential charcoal treatment, vacuum liquid chromatography (VLC) on silica gel and HPLC on a reversed phase (see Experimental), yielded iridoids **1, 2** and **3.** The main compound **2** and compound **3** were identified as antirrinoside and linarioside, respectively, by ^{1}H and ^{13}C NMR spectra in comparison with literature data and reference samples [2–4].

Compound **1** is a novel iridoid named genistifolin. After acetylation a pentaacetate was obtained. Acid hydrolysis of **1** yielded glucose. The ^{1}H and ^{13}C NMR spectra of **1** supported an iridoid structure closely related to that of catalpol (**4**) [5, 6] (Tables I and II). The singlet at 2.15 ppm as well as the signals at 13.32 ppm and 173.38 ppm, respectively, showed the presence of an acetoxy group in the molecule. Its position in the aglycone was confirmed with eliminations of 42 and 60 u from some aglycone fragments and the unchanged glucose

Table I. ^{1}H NMR data of iridoids **1, 4** and **5.**

H	**1** [D_2O]	**4*** [D_2O]	**5** [D_2O]
1	5.15 d (8.8)	5.02 d (9.8)	5.09 d (9.1)
3	6.40 dd (5.8, 1.5)	6.33 dd (6, 1.7)	6.40 dd (5.6, 1.5)
4	5.01 dd (6.0, 4.0)	5.08 dd (6, 4.6)	5.15 dd (5.5, 4.6)
5	2.68 m	2.25 m	2.31 m
6	5.05 dd (6.4, 1.0)	4.00 dd (8.1, 1.0)	4.05 dd (8.2, 1.0)
7	3.44 bs	3.56 bs	3.53 s
9	2.53 dd (8.8, 7.8)	2.58 dd (9.8, 7.7)	2.47 dd (9.1, 7.8)
10	1.57 s	4.21 d (13.2) 3.70 d (13.2)	1.56 s
1′	4.88 d (7.8)	4.81 d (8.0)	4.80 d (8.0)
6′A	3.92 dd (12.4, 1.5)	3.84 bd	3.92 dd (12.3, 2.0)
6′B	3.76 dd (12.4, 5.1)	3.66 dd (12.3, 5.5)	
Ac	2.15 s		

* 360 MHz; ref. [5].

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Table II. ^{13}C NMR data of iridoids **1** and **4.**

C	**1** [D_2O]	**4*** [D_2O]
1	95.41	95.33
3	141.77	141.78
4	102.98	104.03
5	36.46	39.10
6	81.83	79.58
7	63.54	62.55
8	65.63	66.23
9	44.99	43.60
10	17.19	61.60
1′	99.21	99.74
2′	73.65	74.82
3′	76.62	78.54[a]
4′	70.48	71.74
5′	77.03	77.70[a]
6′	61.50	62.90
MeCO	13.32	
CO	173.38	

* Ref. [6].

fragments in the CI/MS spectrum (see Experimental) and the glucose signals in the ^{13}C NMR spectrum. The signal (dd at 4.00 ppm) corresponding to H-6 in catalpol (**4**) appeared in **1** as a dd signal at 5.05 ppm indicating acetylation at C-6. The singlet at 1.57 ppm showed the presence of a methyl group instead of the 10-CH_2OH group in catalpol (d, 4.21 ppm). The proposed structure **1** was supported also with the DCI spectrum of the silylated iridoid (see Experimental) and the key difference signals in the ^{13}C NMR spectrum for C-6 (81.83 ppm), MeCO (13.32 and 173.38 ppm) and 10-Me (17.19 ppm).

Deacetylation of **1** gave 6-deacetylgenistifolin (**5**) identified by ^1H NMR (Table I). This iridoid not found in nature till now could be expected to be present in *L. genistifolia* from a biogenetic point of view. The TLC data indicated the presence of **5** as a minor component in the MeOH fraction obtained after charcoal treatment of the water-soluble part of the methanolic plant extract. The low amount of the latter prevented its isolation in a pure state.

Materials and Methods

The ^1H and ^{13}C NMR spectra were measured on a Bruker 250 MHz spectrometer. CI/MS and DCI/MS were recorded with JEOL JMS D-300. The HPLC separations were achieved on a Perkin-Elmer chromatograph with a RP-18 column (Whatman ODS-3 10 μm, i.d. 4.60 × 250 mm) and mobile phase MeOH–H_2O.

Plant material

Aerial parts of *Linaria genistifolia* were collected near Makotsevo in August 1990. A voucher specimens SOM 150734 is deposited in the Institute of Botany with Botanical Garden, Bulgarian Academy of Sciences, Sofia.

Isolation

130 g fresh aerial parts of *L. genistifolia* were two fold extracted with MeOH. The alcoholic concentrate was dissolved in H_2O and consistently extracted with Et_2O and EtOAc. The water-soluble part (9 g) was chromatographed on a charcoal column (90 g) eluted with 1.5 l portions of H_2O, 10% MeOH, MeOH, MeOH–Me_2CO (1:1) and MeOH–$Cl(CH_2)_2Cl$ (1:2), respectively. Part (0.7 g) of the MeOH fraction (1.8 g) was separated on a VLC silica gel column (72 g). Eight fractions were collected after elution with $CHCl_3$–MeOH mixtures (6:1 to 1:3, 50 ml each). Fr. 6 (213 mg) consisted of pure **2.**

The MeOH–Me_2CO fraction (102 mg) was separated by VLC on silica gel (10 g) with $CHCl_3$–MeOH mixtures (10:1 to 1:1, 10 fractions, 25 ml each). Fr. 7–8 (34 mg) were separated by HPLC with mobile phase MeOH–H_2O (40:60) to give pure **2** (19 mg) and **3** (10 mg). Fr. 4 (24 mg) purified by HPLC with mobile phase MeOH–H_2O (45:55) yielded pure **1** (8 mg).

The MeOH–$Cl(CH_2)_2Cl$ fraction (420 mg) was separated by VLC on silica gel (70 g) and eluted with $CHCl_3$–MeOH mixtures (10:1 to 5:1, 100 ml each). Six fractions were collected. Fr. 4 (63 mg) and fr. 5 (47 mg) contained crude **1.** Further purification of fr. 4 by HPLC with a mobile phase MeOH–H_2O (45:55) yielded pure **1** (8.3 mg).

Genistifolin (**1**)

Amorphous solid. $C_{17}H_{24}O_{10}$. $[\alpha]_D^{20}$ −80.16° (*c* = 0.6, MeOH). UV: λ_{max} (MeOH) nm: 209; CI/MS: *m/z* (rel. int.): 227 $[AH]^+$ (25), 213 $[AH-15]^+$· (95), 209 $[AH-18]^+$ (10), 195 $[AH-15-18]^+$· (41), 185 $[AH-42]^+$ (10), 171 $[AH-15-42]^+$· (21), 167 $[AH-60]^+$ (48), 149 $[AH-17-60]^+$· (62), 151 $[AH-15-60]^+$· (39), glucose − 163 (100), 145 (52), 127 (55); DCI/MS ($C_{17}H_{20}O_6$ $(OTMS)_4$): 299

$[AH+72]^{+\cdot}$ (11), 257 $[AH+72-42]^+$ (75), 227 $[AH]^+$ (8), 186 $[AH-42]^+$ (100), glucose – 451 (10), 379 (8), 361 (12), 204 (15). ^1H and ^{13}C NMR data given in Tables I and II.

Acetylation of **1**

Compound **1** (3 mg) was treated with pyridine–Ac_2O in the usual manner to give genistifolin pentaacetate. ^1H NMR ($CDCl_3$): δ 2.05–2.15 (5 Ac).

Acid hydrolysis of **1**

Compound **1** (5 mg) was refluxed with 0.5 ml 2 N HCl for 1 h. After neutralization, glucose was identified in the water phase (TLC).

Deacetylation of **1**. *6-Deacetylgenistifolin* (**5**)

Methanolic NaOMe (0.1 M, 0.1 ml) was added to a solution of **1** (4 mg) and the mixture was refluxed for 10 min. After cooling the solution was neutralized and concentrated under vacuum. The residue was purified by prep. TLC ($CHCl_3$–MeOH–H_2O, 60:22:4) to give **5**. DCI/MS ($C_{15}H_{17}O_4(OTMS_5)$): 707 $[MH]^+$ (3), 617 $[MH-90]^+$ (2), 527 $[MH-2\times90]$ (3), 329 $[AH+72]^{+\cdot}$ (25), 314 $[AH+72-15]^+$ (75), 257 $[AH]^+$ (17), glucose – 451 (18), 379 (100), 361 (35), 271 (16). ^1H NMR given in Table I.

Antirrinoside (**2**)

^1H and ^{13}C NMR as reported in ref. [2, 3].

Linarioside (**3**)

^1H and ^{13}C NMR as reported in ref. [4].

Acknowledgements

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