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# Iridoid Glycosides from *Linaria* Species#

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**ABSTRACT** - The iridoid composition of four *Linaria* species has been investigated. Two new iridoids along with known compounds were isolated and identified. The new iridoid glucoside, 7,8-*epi*-antirrinoside, found in *L. dalmatica*, is the first iridoid glucoside with an α-orientation of the 7,8-epoxide ring, while 6ß-hydroxyantirride, found in *L. genistifolia and L. peloponnesiaca*, appears as a second representative of the rare antirride iridoid type. Antirride was found for the first time in *L. simplex*. Structure elucidations were carried out mainly by spectral methods and molecular mechanics calculations. Chemosystematic relationships are also discussed.

## INTRODUCTION

The genus *Linaria* (Scrophulariaceae) comprises about 200 species of plant distributed in the moderate climatic zones of Europe and Asia. In folk medicine *Linaria* plants are used as laxative, spasmolitic, cholagogic and antiinflammatory drugs, for treatment of bladder catarrhs, haemorroids, skin rash, etc. Till now, fourteen iridoid glycosides have been isolated from eleven *Linaria* species[1-10]. In Bulgaria, eight *Linaria* species (*L. vulgaris, L. genistifolia, L. dalmatica, L. simplex, L. pelisseriana, L. chalepensis, L. arvensis and L. peloponnesiaca*) occur. In this work, we report the iridoid composition of *Linaria dalmatica* (L.) Mill., *L. genistifolia* (var. *genistifolia* and var. *euxina*) (L.) Mill., *L. simplex* (Wild.) and *L. peloponnesiaca* Bois & Heldr., as well as the structure elucidation of two new iridoids, 7,8-*epi*-antirrinoside (**1**) and 6ß-hydroxyantirride (**7**). *L. peloponnesiaca* has not been investigated for iridoids previously.

## RESULTS AND DISCUSSION

Separation of the methanol extract of *L. dalmatica* by sequential charcoal treatment, vacuum liquid chromatography (VLC) and semipreparative RP-HPLC yielded the new iridoid **1** along with the known compounds antirrinoside (**2**), linarioside (**3**), 5-O-glucosylantirinoside(**4**) and 5-O-allosylantirrinoside (**5**).[10]

Compound **1**, $[\alpha]_D^{20}$, has a molecular formula of $C_{15}H_{22}O_{10}$, the same as antirrinoside (**2**), as determined by $^{13}C$ NMR analysis and CIMS (isobutane). Acid hydrolysis of **1** afforded glucose. The $^1H$ and $^{13}C$ NMR data (Tables 1 and 2) support the structure of 7,8-*epi*-antirrinoside for **1**. The deshielded H-1 signal and its appearance as a broad singlet at δ 5.73 due to a small H-1/H-9 coupling as compared to δ 5.52 (d, $J$=6.5 Hz) for **2**, is in agreement with data for an α-oriented 7,8-epoxide ring[11]. The $^1H$ and $^{13}C$ NMR signals of Me-10 (δ 1.70, s; δ 27.7) are shifted to a lower field in comparison to those of antirrinoside (δ 1.50, s; δ 16.83).[5,12]

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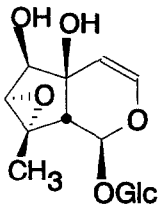
*#This work is dedicated to Prof. Carl Djerassi on the occasion of his seventieth birthday.*



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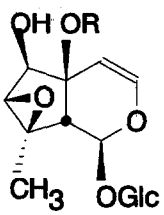
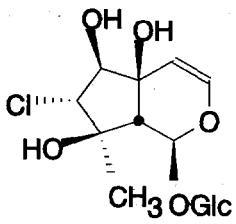


R

2 H

4 Glc

5 All



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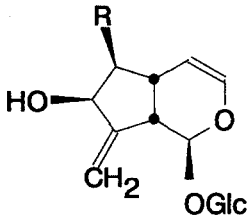

6 H

7 βOH

The signals of H-7 (δ 4.14, d) and H-9 (δ 2.71, bs) as well as C-7 (δ 81.2), C-8 (δ 73.8) and C-9 (δ 56.8) are also deshielded. The value of $J_{6,7}$ (4.4 Hz) favors of a ß-orientation of the 6-OH group. The existence of weak couplings H-9/H-1, H-9/H-4 and H-9/H-7 were proven by the presence of corresponding cross-peaks in the COSY spectrum of **1**. The latter served also for assignment of the glucosyl signals.

Difference NOE experiments also support the structure and stereochemistry of **1**. Thus, irridation of Me-10 enhanced the signals of H-9, H-7 and H-1. The latter enhancement is compatible with a conformation having H-1 in an equatorial position.[11]

In order to prove the suggested relative stereochemistry at C-7 and C-8, molecular analogues of **1** and **2** (designated as **1a** and **2a**), in which the glucosyl residue was replaced by a methyl group were subjected to molecular mechanics energy minimization, using the standard force field MM2 of Allinger[13]. The minimum-energy conformation of **1a** is shown on Figure 1. The results from the calculations show a qualitative agreement between the experimental $J_{1,9}$-values (Table 1) and those calculated according to the Haasnoot equation[14]: a high value for **2a** (calc. 10.0 Hz, dihedral angle 171°) and a low value for **1a** (1.6 Hz, 66°). This corresponds to conformations with an axial orientation of H-1 in **2** and equatorial orientation in **1**, supported also by the NOE measurements for **1**.

The CIMS (isobutane) spectrum of **1** is also in agreement with the proposed structure. It is almost identical with that of antirrinoside (**2**) (see Experimental). Both spectra contain a base peak at *m/z* 165 [AglH-2x$H_2O$]$^+$. The elimination of water from MH$^+$ [**1**: *m/z* 345 (8); **2**: *m/z* 345 (5)] and from AglH$^+$ [**1**: *m/z 183* (75); **2**: *m/z* 183 (55)] was more intensive for **1**. Differences in the relative intensities of the fragments at *m/z* 149 [AglH-2x$H_2$O-O]]$^+$ and *m/z* 137 [AglH-2$H_2$O-CO]$^+$ were also observed.

Hence, **1** is 7,8-*epi*-antirrinoside. This is the first isolation of an iridoid glucoside with α-orientation of the 7,8-epoxide ring. Till now only synthetic transformation products of iridoids with a 7,8 α-epoxide substituent were described[11].

From *L. simplex* we isolated five compounds identified by spectral methods as the known iridoids **2**-**6**[10]. Compound **6** was identified by spectral methods as antirride[4,6] found for the first time in *L. simplex*. Only partial $^1$H-NMR data (60 MHz[6] and 100 MHz[4]) have been published for **6**, whereas complete $^{13}$C-NMR assignments were available for solutions in $CD_3OD$[15] and $D_2O$[16]. The present $^1$H-NMR data (400 MHz, Table 1) are in good agreement with the previous results[4,6], proving the assignments of H-1/H-10 as given in [6] rather than in [4]. The proton coupling pattern was confirmed by the H,H-COSY spectrum, which in addition to the couplings specified, exhibited cross-peaks for the following couplings: H-3/H-1, H-10/H-7, H-10/H-9, H-9/H-5 and H-2'/H-3'. The coupling constants in the H-5, H-6a, H-6b, H-7-fragment were obtained with the help of selective decoupling and computer spectral

**Table 1.** $^1$H NMR spectral data of compounds **1**, **2**, **6** and **7**

| H | 1[a] | 2[b] | 6[c] | 7[c] |
|---|---|---|---|---|
| 1 | 5.73 *bs* | 5.52 *d* | 5.54 *d* | 5.60 *d* |
| (1.6;66°)[d] | | $J_{1,9}$=6.2 (10.0;171°) | $J_{1,9}$=2.5(2.2;59°) | $J_{1,9}$=2.3(2.3;61°) |
| 3 | 6.34 *d* | 6.48 *d* | 6.19 *dd* | 6.25 *dd* |
| | $J_{3,4}$=6.6 | $J_{3,4}$=6.3 | $J_{3,4}$=6.3,$J_{3,5}$=1 | $J_{3,4}$=6.3,$J_{3,5}$=1.9 |
| 4 | 5.15 *bd* | 4.98 *d* | 4.88 *dd* | 4.94 *dd* |
| | $J_{3,4}$=6.4 | *J*=6.3 | $J_{3,4}$=6.3,$J_{4,5}$=1.1 | $J_{3,4}$=6.3,$J_{4,5}$=1.7 |
| 5 | | | 2.83 *m* | 2.77 *dd* $J_{4,5}$=1.6;$J_{5,9}$=7.8(6.7; 38°);$J_{5,6\alpha}$=1(1.5;74°) |
| 6 | 4.00 *d* | 4.09 *d* | 2.03 *m* | 4.00 *bd* |
| | $J_{6,7}$=4.4 | *J*=1.7 | $J_{5,6\alpha}$=2.0(1.4;74°) $J_{6\alpha,7}$=6.9(7.4;37°) 1.73 *m* $J_{5,6\beta}$=6.8(5.3;45°) $J_{6\beta,7}$=9.5(9.5;158°) | $J_{6\alpha,7}$=4.2(4.2;42°) |
| 7 | 4.14 *d* | 3.61 *d* | 4.61 *m* | 4.64 *m* |
| | $J_{6,7}$=4.4 | $J_{6,7}$=1.7 | | |
| 9 | 2.71 *bs* | 2.52 *d* | 3.07 *m* | 3.22 *m* |
| 10 | 1.70 *s* | 1.50 *s* | 5.30 *m*, 5.34 *m* | 5.42 *m* |
| 1' | 4.72 *d* | 4.78 *d* | 4.76 *d* | 4.78 *d* |
| | $J_{1'2'}$=8.3 | $J_{1'2'}$=8.2 | $J_{1'2'}$=8.0 | $J_{1'2'}$=8.3 |
| 2' | 3.27 *t* | 3.35-3.50 | 3.29 *m* | 3.31 *m* |
| | *J*=8.5 | | | |
| 3' | 3.43 *m* | 3.35-3.50 | 3.48 *m* | 3.35-3.50 *m* |
| 4' | 3.36 *t* | 3.35-3.50 | 3.38 *m* | 3.35-3.50 *m* |
| | *J*=8.5 | | | |
| 5' | 3.46 *m* | 3.35-3.50 | 3.48 *m* | 3.35-3.50 *m* |
| 6'a | 3.68 *dd* | 3.74 *dd* | 3.71 *dd* | 3.74 *dd* |
| | $J_{5'6'a}$=5.6 | $J_{5'6'a}$=5.1 | $J_{5',6'a}$=6.0; | $J_{5'6'}$=5.6; |
| | $J_{6'a,6'b}$=12.3 | $J_{6'a,6'b}$=12.3 | $J_{6'a,6'b}$=12.4 | $J_{6'a,6'b}$=12.3 |
| 6'b | 3.88 *dd* | 3.93 *dd* | 3.91 *dd* | 3.95 *dd* |
| | $J_{5',6'b}$=1.8 | $J_{5'6'b}$=2.4 | $J_{5'6'b}$=2.0 | $J_{5',6'b}$=1.6 |
| | $J_{6'a,6'b}$=12.4 | $J_{6'a,6'b}$=12.3 | $J_{6'a,6'b}$=12.3 | $J_{6'a,6'b}$=12.3 |

[a]250 MHz. [b]Ref.5. [c]400 MHz. [d]In parentheses are the *J*-values calculated on the basis of the Haasnoot equation and the corresponding dihedral angles of the minimum energy conformation obtained from the molecular mechanics calculations (see text).

**Table 2.** $^{13}C$ NMR spectral data of compounds **1**, **2**, **6** and **7** ($D_2O$)

| C | 1[a] | 2[b] | 6[c] | 7[c] |
|---|---|---|---|---|
| 1 | 93.5 *d* | 94.96 | 95.7 *d* | 96.0 |
| 3 | 141.2 *d* | 142.84 | 139.4 *d* | 140.5 *d* |
| 4 | 107.8 *d* | 107.04 | 108.3 *d* | 104.5 *d* |
| 5 | 68.6 *s* | 74.21 | 30.8 *d* | 35.7 *d* |
| 6 | 77.4 *d* | 76.71 | 39.0 *t* | 74.5 *d* |
| 7 | 81.2 *d* | 66.22 | 73.3 *d* | 76.0 *d*[d] |
| 8 | 73.8 *s* | 64.22 | 152.2 *s* | 149.3 *s* |
| 9 | 56.8 *d* | 52.04 | 44.0 *d* | 42.0 *d* |
| 10 | 27.7 *q* | 16.83 | 115.5 *t* | 112.7 *t* |
| 1' | 98.9 *d* | 99.19 | 98.8 *d* | 98.7 *d* |
| 2' | 73.4 *d* | 73.39 | 73.3 *d* | 73.2 *d* |
| 3' | 77.2 *d* | 76.36 | 76.2 *d*[d] | 76.1 *d*[d] |
| 4' | 70.5 *d* | 70.38 | 70.2 *d* | 70.2 *d* |
| 5' | 76.2 *d* | 76.98 | 76.8 *d*[d] | 76.8 *d*[d] |
| 6' | 61.5 *t* | 61.50 | 61.3 *t* | 61.3 *t* |

[a]62.9 MHz. [b]Ref. 12. [c]100.6 MHz. [d]Values in the column are interchangeable.

simulation. The $^{13}C$-NMR parameters obtained by us for **6** are in very good agreement with the published data[15,16], especially for the same solvent (Table 2).

In *L. genistifolia* (var. *genistifolia* and var. *euxina)* and *L. peloponnesiaca* we found compounds **2**, **4**, **5** and **7**, whereas compound **3** was found only in *L. genistifolia*. The two varieties of *L. genistifolia* studied differed in the. amounts of **2** and **7**.

**7a** **1a**

**Figure 1.** Minimum-energy conformations of **1a** and **7a**

The structure and relative stereochemistry of the hitherto unknown compound **7** was determined as 6ß-hydroxyantirride on the basis of spectral studies and molecular mechanics calculations. Such calculations were applied for the first time to an iridoid structure. The $^1H$ and $^{13}C$ NMR parameters of **7** are presented in Tables 1 and 2, resp. The proton spectral analysis was facilitated by selective decoupling experiments. In order to establish the relative stereochemistry at C-6 and C-7, molecular mechanics calculations were also performed for the antirride (**6**) and 6ß-hydroxyantirride (**7**) analogues with OMe instead of OGlc residue (**6a** and **7a**). On the basis of the dihedral angles

measured from the minimum-energy conformations of the diastereoisomers of **6a** and **7a** with all possible combinations of α- and ß-orientations of the substituents at C-6 and C-7, the corresponding vicinal coupling constants were calculated using the equation of Haasnoot *et al.*[14] The results indicated that a good agreement between the calculated and experimental vicinal *J*-values in the five-membered ring (Table 1) was obtained only for the stereostructures corresponding to antirride (**6**) and 6ß-hydroxyantirride (**7**). All other diastereoisomers show very significant deviations for some or all of the $J_{5,6}$ and $J_{6,7}$ values: *e.g.*, for the diastereoisomers of **7a** with 6α-OH group the calculated values of $J_{5,6}$ are 5.7 Hz (experimental value *ca.* 1 Hz), whereas for the 7-*epi*-antirride analogue (with 7α-OH) one of the calculated $J_{6,7}$ is 1.4 Hz, *i.e.* much lower than any of the experimental values (Table 1). In this way, the calculations not only established the stereochemistry of the new compound **7**, but also confirmed that of **6**.

The minimum-energy stereostructure of **7a** (Fig.1) indicates that the conformation of the six-membered ring is close to a half-chair with an axially oriented substituent at C-1, which also corresponds to the experimental $J_{1,9}$ values of 2.3-2.5 Hz (Table 1). The conformation of the five-membered ring resembles that of a twisted envelope, with C-6 above and C-7 below the plane of the other three atoms. Judging from the close values of the corresponding dihedral angles in **6a** and **7a**, the favoured conformations of compounds **6** and **7** should be very similar.

According to Flora Europaea[17] *L. genistifolia* and *L. dalmatica* are combined in the species *L. genistifolia* subdivided into ssp. *dalmatica*, ssp. *genistifolia* (var. *genistifolia*, var. *euxina*) and ssp. *sofiana* ( (Vel.) (*L. concolor* auct.). Our previous studies showed that *L. genistifolia* contained the iridoid glucoside genistifolin[9] characteristic only for this species and no flavonoids while flavonoids and now the unusual iridoid 7,8-*epi*-antirrinoside were found *in L. dalmatica*. This clearly indicates the sole position of *L. genistifolia* and absence of similarity between *L. dalmatica* and *L. genistifolia*. On the other hand both varieties of *L. genistifolia* (var. *genistifolia* and var. *euxina*) could easily be distinguished chemically by the decreased amount of antirrinoside (**2**) and increased amount of 6ß-hydroxyantirride (**7**) in the latter.

## EXPERIMENTAL

**GENERAL PROCEDURES**. Silica gel (type 60; Merck) was used for column chromatography (0.063-0.2 mm) and VLC (15 μm). Aluminium-backed plates coated with silica gel 60 $F_{254}$ 0.2 mm thick (Merck) were used for TLC. HPLC was carried out with a Perkin Elmer 2/2 using a Whatman ODS-3 column (250x9.4 mm, 10 μm, reversed phase). The NMR spectra were obtained on Bruker spectrometers at 400.1 or 250.1 MHz for $^1H$ and 100.6 or 62.9 MHz for $^{13}C$, solvent $D_2O$, internal standard acetone (2.20 ppm for $^1H$ and 30.2 ppm for $^{13}C$). The DEPT sequence was used for $^{13}C$ multiplet selection. The H,H-COSY-45 spectra were measured with standard Bruker software. Mass spectra were recorded on JEOL JMS D-300. CIMS (isobutane): ion source temp. 160°.

**PLANT MATERIAL.** *L. dalmatica, L.simplex, L.genistifolia* var. *genistifolia, L. genistifolia* var. *euxina* and *L. peloponnesiaca were* collected in flower in 1991 from Vitosha, Kozhuh mountain, Makotsevo, Sozopol and Dragoman, respectively. Voucher specimens SOM 150736, 150732, 151013, 151018 and 151020 were deposited in the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

**ISOLATION.** Fresh aerial parts of *L. dalmatica* (85 g) and dried aerial parts of *L. simplex* (60 g), *L.genistifolia* var.*genistifolia* (103 g), *L. genistifolia* var. *euxina* (50 g) and *L. peloponnesiaca* (50 g) were extracted twice with MeOH. After evaporation of the solvent under vacuum the residue was dissolved in water and extracted with $Et_2O$ and EtOAc. Half of the water soluble part (3.2, 2.9, 9.0, 3.0 and 2.6 g, respectively) was treated with charcoal and washed with $H_2O$, 10% MeOH, MeOH-$Me_2CO$ (1:1) and MeOH-$Cl(CH_2)_2Cl$ (1:2).

*L. dalmatica*

The MeOH-$Cl(CH_2)_2Cl$ fraction (140 mg) was separated by VLC on silica gel (14 g) with $CHCl_3$-MeOH (10:1 to 1:1, 10 fractions, 50 ml each). Fractions 7-8 (90 mg) were purified on silica gel with $CHCl_3$-MeOH (8:1 to 1:1, 8 fractions, 30 ml each). Fractions 5-7 (65 mg) were successively purified by HPLC on RP-18 (MeOH-$H_2O$ 20:80) to give pure **2** (3 mg), **3** (7 mg) and **1** (7 mg).

**7,8-*epi*-Antirrinoside (1).** $[\alpha]_D^{20}$ -113.21 (c=0.28, MeOH). CIMS (isobutane) *m/z*(rel.int.): 345 $[MH-18]^+$(8), 327 $[MH-18-18]^+$(6), 183 $[AglH-H_2O]^+$(75), 165 $[AglH-2xH_2O]^+$(100), 149 $[AglH-2H_2O-O]^+$(11), 137 $[AglH-2xH_2O-CO]^+$(12); Glc: 163(35), 145(82), 127(41). $^1H$ NMR ($D_2O$, 250 MHz) and $^{13}C$ NMR ($D_2O$, 62.9 MHz) are given in Tables 1 and 2.

**Antirrinoside (2).** CIMS (isobutane) *m/z* (rel.int.): 345(5), 327(5), 183(55), 165(100), 149(7), !37(15); Glc: 163(21), 145(25), 127(20).

**Acid hydrolysis of 1**. Compound **1** was refluxed with 0.5 ml 0.5 M $H_2SO_4$ for 1 hr. After neutralization glucose was identified in the water phase (TLC).

*L. simplex*

The MeOH-$Me_2CO$ fraction (417 mg) was purified by VLC on silica gel (40 g) and $CHCl_3$-MeOH (10:1 to 1:1; 10 fr, 100 ml each). Fraction 7 was additionally purified by preparative TLC on silica gel with $CHCl_3$-MeOH (3:1) to give **2** (28 mg) and **6** (13 mg). Fraction 10 (53 mg) after HPLC purification with MeOH-$H_2O$ (10:90) gave **3** (43 mg).

**Antirride (6)**. CIMS (isobutane) *m/z*(rel.int.): 331 $[MH]^+$(11), 313 $[MH-18]^+$(32), 294 $[MH-2x18]^+$(32), 169 $[MH-162]^+$(13), 151 $[MH-162-18]^+$(100), 133 $[MH-162-2x18]^+$(70). $^1H$ NMR (400.1 MHz, $D_2O$) and $^{13}C$ NMR (100.6 MHz, $D_2O$) given in Tables 1 and 2.

*L. genistifolia* var. *genistifolia*

Part (1.1 g) of the MeOH fraction (1.8 g) was purified on silica gel (90 g) with $CHCl_3$-MeOH-$H_2O$ (30:11:2) (30 fractions, 60 ml each). Fraction 6 (30 mg) contained pure *7*.

**6ß-Hydroxyantirride (7)**

$[\alpha]_D^{20}$-85.7° (MeOH). CIMS (isobutane) *m/z*(rel.int.): 347 $[MH]^+$(5), 329 $[MH-18]^+$(50), 311 $[MH-2x18]^+$(45), 185 $[MH-162]^+$(20), 167 $[MH-162-18]^+$(100), 149 $[MH-162-2x18]^+$(22); Glc: 163 (20), 145 (10), 127 (8). $^1H$ NMR (400.1 MHz, $D_2O$) and $^{13}C$ NMR data (100.6 MHz, $D_2O$) are given in Tables 1 and 2.

**Acid hydrolysis of 7.** Compound *7* was refluxed with 0.5 ml 0.5 M $H_2SO_4$ for 1 hr. After neutralization glucose was identified in the water phase (TLC).

*L. genistifolia* var. *euxina*

The same way as above. After purification the MeOH fraction yielded **7** (20 mg).

*L. peloponnesiaca*

The MeOH fr (480 mg) was purified by column chromatography on silica gel (48 g) and mobile phase $CHCl_3$-MeOH-$H_2O$ (30:11:2) (16 fractions, 130 ml each). Fractions 3-5 (30 mg) contained pure **2**. Fraction 6 (60 mg) additionally purified by column chromatography on silica gel (6 g) with the same mobile phase gave pure **7** (10 mg). Fractions 7-8 (31 mg) contained **4** and fractions 9-12 (23 mg) - pure **5** (23 mg).

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