Pergamon

PII: S0031-9422(96)00328-7

*Phytochemistry*, Vol. 43, No. 3, pp. 625–628, 1996

0031-9422/96 $15.00 + 0.00

# IRIDOID GLUCOSIDES FROM *GALIUM ALBUM* AND *G. LOVCENSE*

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(*Received in revised form* 27 *March* 1996)

**Key Word Index**—*Galium album* ssp. *album*; *G. album* ssp. *pychnotrichum*; *G. lovcense*; Rubiaceae; iridoids; 7-*O*-acetyl-10-acetoxyloganin; 7$\beta$-hydroxy-11-methyl forsythide.

**Abstract**—The iridoid composition of *Galium album* ssp. *album*, *G. album* ssp. *pychnotrichum* and *G. lovcense* was studied. Eleven known iridoids were isolated and identified. Two new iridoids, 7-*O*-acetyl-10-acetoxyloganin and 7$\beta$-hydroxy-11-methyl forsythide, were isolated from *G. lovcense*, and their structures elucidated. Copyright © 1996 Published by Elsevier Science Ltd

## INTRODUCTION

The *Galium mollugo* group comprises four species, i.e. *G. mollugo* L. (2n = 22) distributed in most of Europe, the Balkan endemic *G. lovcense* Urumov (= *G. heldreichii* ssp. *protopychnotrichum* (Ehrend. and Krendl) Ančev) (2n = 22), *G. heldreichii* Halacsy (2n = 22) (Crete, Greece and W. Anatolia) and the extremely polymorphic tetraploid *G. album* Mill. (2n = 44) found all over Europe [1]. In most phytochemical studies the name '*Galium mollugo*' has been used for the widespread narrowleaved *G. album* ssp. *album*. In *G. album* (reported as '*G. album* = *G. mollugo*') more than 10 iridoids were identified [2–5]. No data about the iridoid composition of *G. lovcense* have been published. In continuation of our studies on iridoids from *Galium* plants, in this paper we report on the iridoid glucoside composition of *G. album* ssp. *album* (two populations), *G. album* ssp. *pychnotrichum* (two populations) and *G. lovecense* (one population with two samples, collected in different years).

## RESULTS AND DISCUSSION

The methanol extracts of the examined *Galium* samples have the same qualitative iridoid composition, but the quantities of the individual iridoids as monitored by TLC and HPLC differ significantly. The samples of the different taxa showed no distinction as regards collection site (samples 1 and 2 of *G. album* ssp. *album* and samples 3 and 4 of *G. album* ssp. *pychnotrichum*) and collection year (samples 5 and 6 of *G. lovecense*). The extracts were worked up as described in the Experimental. Eleven known iridoids, secogalioside (**1**), asperuloside (**2**), deacetylasperulosidic acid (**3**), scandoside (**4**), monotropein (**5**), asperulosidic acid (**6**), geniposidic acid (**7**), 10-hydroxyloganin (**8**), 10-hydroxymorroniside (7$\alpha$ and 7$\beta$ isomers) (**9**), V3 (**10**) and daphylloside (**11**) were isolated and identified by spectral methods [6–7] and comparison with authentic samples along with two new iridoids **12** and **13** (*G. album* ssp. *album*: **1–6**, **8–11**; *G. album* ssp. *pychnotrichum*: **1–11**; *G. lovcense*: **1–13**).

Compound **12** was obtained as an amorphous powder. The UV spectrum (230 nm) indicated the presence of a conjugated enol–ether system. The $^1$H NMR spectrum is very similar to that of 10-hydroxy loganin (**8**) [8–9] except for the signals arising from two acetyl groups ($\delta$ 2.01 and 2.03) and deshielding of the H-7 ($\delta$ 5.31) and H-10 ($\delta$ 4.16 and 4.26) signals. Thus, a structure for a diacetoxy derivative of **8** was suggested. The decoupling experiments, as well as the $^{13}$C NMR (Table 1) and H–C COSY assignments confirmed the structure of 7-*O*-acetyl-10-acetoxyloganin (**12**). Acetylation afforded a hexaacetate **12a**, whose $^1$H and $^{13}$C NMR spectra (Table 1), were identical with those of the hexaacetate of 10-hydroxy loganin [9].

The $^{13}$C NMR spectrum of compound **13** was somewhat similar to that of 11-methyl forsythide (**14**) [10] except for the presence of a signal belonging to an oxygenated carbon at $\delta$ 73.6. The $^1$H NMR data supported this, and selective decoupling data and NOE experiments confirmed the hydroxy group to be at the 7$\beta$-position. The shift of the methyl ester group ($\delta$ 52.7) showed it to reside at C-11 as in **14** since methylation afforded a dimethyl ester with signals at $\delta$ 52.7 and 53.4, the latter being typical for the C-10 position [10]. Thus, the structure of 7$\beta$-hydroxy-11-methyl forsythide (**13**) was established for this compound.

In all of the samples examined we found sec-

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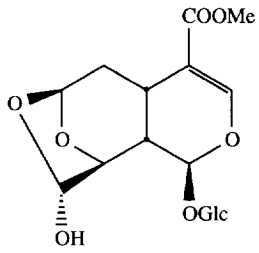


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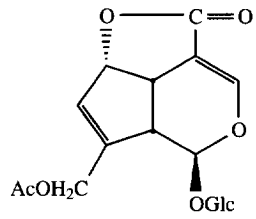


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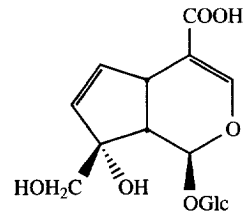


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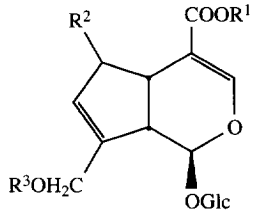
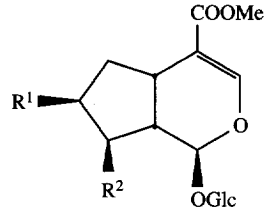
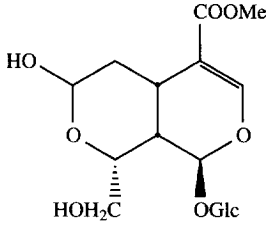



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **3:** | H | αOH | H |
| **4:** | H | βOH | H |
| **6:** | H | αOH | Ac |
| **7:** | H | H | H |
| **11:** | Me | αOH | Ac |




| | $R^1$ | $R^2$ |
|---|---|---|
| **8:** | OH | $CH_2OH$ |
| **12:** | OAc | $CH_2OAc$ |
| **13:** | OH | COOH |
| **14:** | H | COOH |

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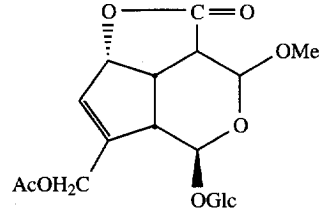


**10**

ogalioside, which confirms previous results [2, 5]. Thus this iridoid appears to be characteristic for the *G. mollugo* group. According to our unpublished results, secogalioside is not found in more than 20 *Galium* species belonging to other *Galium* groups. *G. album* ssp. *pychnotrichum* and *G. lovcense* could be easily distinguished from *G. album* ssp. *album* by the HPLC fingerprint chromatograms. Both taxa showed similar iridoid compositions and a high content of secogalioside, which was the main constituent (the highest content found in *G. album* ssp. *pychnotrichum*), while in the third taxon examined *G. album* ssp. *album* secogalioside, asperuloside, deacetylasperulosidic acid, scandoside and monotropein were found in similar concentrations. Evidently, the biosynthesis in *G. album* ssp. *pychnotrichum* and *G. lovcense* proceeds predominantly in the direction of the subroute through loganin toward secogalioside and to a lesser extent through geniposidic acid toward asperuloside type iridoids, while in *G. album* ssp. *album* both subroutes are equally represented.

## EXPERIMENTAL

*General procedures.* NMR: 250.1 MHz for $^1H$ and 62.9 MHz for $^{13}C$, solvents $D_2O$, $CD_3OD$, $CDCl_3$, int. standard TSPA, TMS. The $^1H$ spectra were measured with solvent presaturation. The DEPT sequence was used for $^{13}C$ multiplet selection. The H,H-COSY-45 spectrum was measured with standard Bruker software.

Table 1. $^{13}C$ NMR spectral data of compounds **8**, **12**, **12a**, **13** and **14**

| C | **8** ($D_2O$)* | **12** ($D_2O$) | **12a** ($CDCl_3$) | **13** ($D_2O$) | **14** ($D_2O$)† |
|---|---|---|---|---|---|
| 1 | 98.2 | 97.8 | 94.0 | 97.8 | 97.7 |
| 3 | 152.1 | 152.9 | 148.9 | 152.1 | 153.2 |
| 4 | 112.2 | 112.0 | 111.9 | 113.4 | 111.8 |
| 5 | 32.6 | 32.2 | 29.5 | 30.8 | 34.9 |
| 6 | 41.4 | 39.1 | 37.8 | 41.4 | 32.4 |
| 7 | 72.5 | 76.1 | 73.3 | 73.6 | 29.0 |
| 8 | 48.9 | 42.0 | 42.0 | 55.0 | 45.9 |
| 9 | 42.2 | 44.0 | 41.6 | 42.2 | 44.5 |
| 10 | 61.8 | 63.8 | 60.9 | 180.0 | 180.4 |
| 11 | 168.6 | 170.4 | 166.1 | 170.9 | 170.8 |
| 1′ | 99.7 | 99.7 | 95.2 | 99.6 | 99.9 |
| 2′ | 73.9 | 73.5 | 69.8 | 73.5 | 73.5 |
| 3′ | 77.1 | 76.5 | 71.6 | 76.4 | 76.5 |
| 4′ | 70.7 | 70.3 | 67.4 | 70.4 | 70.4 |
| 5′ | 77.4 | 77.0 | 71.6 | 77.1 | 77.2 |
| 6′ | 61.5 | 61.5 | 61.5 | 61.5 | 61.5 |
| MeCO | | 21.1, 21.3 | 20.3, 20.0, 19.9, 19.8, 19.4, 19.0 | | |
| MeCO | | 174.5, 174.8 | 170.0, 169.8, 168.3, 170.2, 169.4, 169.1 | | |
| OMe | 50.8 | 52.6 | 50.6 | 52.7 | 52.7 |

The shift for C-6′ was arbitrary set as 61.5.
*Data from ref. [9].
†Data from ref. [10].

The NOE experiments were performed in the difference mode with a preirradiation time of 5 s.

*Plant material.* Aerial parts of *G. album* ssp. *album* (sample 1: Znepole region, 22.07.1992, A9286; sample 2: Osogovo Mt, 3.07.1994, A9480), *G. album* ssp. *pychnotrichum* (sample 3: Chepun Mt, 12.06.1992, A9240; sample 4: Balkan Mt, Vitinja, 2.07.1995, A95120), *G. lovcense* (sample 5: Konjavska Mt, 3.06.1992, A9214; sample 6: Konjavska Mt, 3.06.1993, A9311) were collected during flowering. Voucher specimens were identified by Dr M. Anchev and deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia (SOM).

*Isolation of iridoids.* Dried ground aerial parts of *G. album* ssp. *album* (189 g), *G. album* ssp. *pychnotrichum* (365 g) and *G. lovcense* (91 g) were extracted twice with MeOH. After concn the residues were dissolved in water and extracted with $CHCl_3$. The water-soluble parts were treated with charcoal and eluted with $H_2O$, 5% MeOH, 30% MeOH, 50% MeOH, MeOH, MeOH–$Me_2CO$ (1:1) and MeOH–$Cl(CH_2)_2Cl$ (1:2). The obtained frs, after sepn by ascending DCCC with $CHCl_3$–MeOH–$H_2O$ (43:37:20) and repeated CC on silica gel with $CHCl_3$–MeOH–$H_2O$ (60:15:4; 60:22:4; 6:4:1) and $CHCl_3$–MeOH–$H_2O$–HCOOH (375:120:5:1), LPLC and HPLC with RP-18 columns with MeOH–$H_2O$ mixtures, yielded the following iridoids: from *G. album* ssp. *album*—compounds **1–6**, **8–11**; from *G. album* ssp. *pychnotrichum*—compounds **1–11**; from *G. lovcense*—compounds **1–13**.

*Isolation of* **12** *and* **13** *from G. lovcense.* The MeOH–$Me_2CO$ (1.9 g) and MeOH–$Cl(CH_2)_2Cl$ (0.6 g) frs obtained after charcoal treatment were sepd by ascending DCCC with $CHCl_3$–MeOH–$H_2O$ (43:37:20). Removal of the stationary phase as 100-ml frs, afforded pure **12** (fr. 3, 42 mg). Fr. 50% MeOH (1 g) obtained after charcoal treatment was sepd on a silica gel column with $CHCl_3$–MeOH–$H_2O$ (6:4:1). Fr. 19–23 (123 mg) additionally purified on a Lobar RP 18 column with 10% MeOH yielded pure **13** (fr. 28–41; 26 mg).

7-O-*Acetyl*-10-*acetoxyloganin* (**12**). $[\alpha]_D^{20}$ −29.14° ($CHCl_3$). $^1H$ NMR ($CD_3OD$): $\delta$ 5.19 (1H, d, $J$ = 6.7 Hz, H-1), 7.50 (1H, *d*, $J$ = 2.7 Hz, H-3), 3.11 (1H, *m*, H-5), 2.32 (1H, *dddd*, $J$ = 1.7, 7.0 and 13.9 Hz, H-6), 1.72 (1H, *m*, H-6), 5.31 (1H, *ddd*, $J$ = 1.7 and 3.8 Hz, H-7), 2.13 (1H, *m*, H-9), 4.16 (1H, *dd*, $J$ = 5.9 and 10 Hz, H-10), 4.26 (1H, *dd*, $J$ = 8.9 and 10 Hz, H-10), 3.70 (3H, *s*, 11-OMe), 4.67 (2H, *d*, $J$ = 7.8 Hz, H-1′), 3.65 (1H, *dd*, $J$ = 5.8 and 11.9 Hz, H-6′), 3.90 (1H, *dd*, $J$ = 1.9 and 11.9 Hz, H-6′), 2.01 (3H, *s*, Ac), 2.03 (3H, *s*, Ac); $^{13}C$ NMR: Table 1. (Found: C, 52.08; H 6.62. $C_{21}H_{30}O_{13}$ requires: C, 51.43; H, 6.17%.)

10-*Hydroxyloganin hexaacetate* (**12a**). Acetylation of **12** afforded **12a**. $^{13}C$ NMR: Table 1 [9].

7*β*-*Hydroxy*-11-*methyl forsythide* (**13**). $[\alpha]_D^{20}$ −45.59° (MeOH). $^1H$ NMR ($D_2O$): $\delta$ 5.31 (1H, *d*, $J$ = 3.7 Hz, H-1), 7.47 (1H, *bs*, H-3), 3.09 (1H, *q*, $J$ = 7.5 Hz, H-5), 1.64 (1H, *m*, H-6*α*), 2.18 (1H, *dddd*, $J$ = 7.8 and 14.5 Hz, H-6*β*), 4.36 (1H, *dt*, $J$ = 4.5 Hz, H-7), 2.62 (1H, *dd*, $J$ = 4.6 and 10 Hz, H-8), 2.68 (1H, *dddd*, $J$ = 3.7 and 10 Hz, H-9), 3.67 (3H, *s*, 11-OMe), 4.70 (2H, *d*, $J$ = 7.8 Hz, H-1′), 3.61 (1H, *dd*, $J$ = 5.5 and 12.4 Hz, H-6′), 3.84 (1H, *dd*, $J$ = 1.7 and 12.4 Hz, H-6′); $^{13}C$ NMR: Table 1. (Found: C, 49.08; H 6.35. $C_{17}H_{24}O_{12}$ requires: C, 48.57; H, 5.76%.)

*HPLC analysis.* Dried ground aerial parts (0.4 g) were extracted with MeOH (2 × 6 ml). After concn and addition of water (3 ml), extraction with $CHCl_3$ (3 × 2 ml) was carried out. The water layer was treated with

neutral aluminium oxide (1 g). After filtration and washing with 3 ml $H_2O$ and 3 ml $H_2O$–MeOH (1:1), the combined filtrates were concd and dissolved in 2 ml MeOH–$H_2O$ (1:1). 10 $\mu$l samples from the above-mentioned extracts were injected. Gradient elution was used – pump A: $H_2O$–MeOH (19:1) and $H_3PO_4$ (15 $\mu$l per 100 ml mobile phase) and pump B: MeOH. The substances were detected at 233 nm. The flow-rate was 0.8 ml $min^{-1}$.

*Acknowledgement*—This project was completed with the financial support of the National Foundation of Scientific Research in Bulgaria, which is gratefully acknowledged.

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