

# Coumarins and Iridoids from *Crucianella graeca*, *Cruciata glabra*, *Cruciata laevipes* and *Cruciata pedemontana* (Rubiaceae)

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The coumarin and iridoid composition of *Crucianella graeca*, *Cruciata glabra*, *Cruciata laevipes* and *Cruciata pedemontana* has been studied. Daphnin and daphnetin glucoside dominated in *C. glabra* along with low concentrations of daphnetin, deacetylasperulosidic acid and scandoside. In *C. laevipes* and *C. pedemontana* were found the same coumarin glucosides along with six iridoid glucosides. In *Crucianella graeca* were found ten iridoid glucosides.

## Introduction

*Crucianella* L. and *Cruciata* Mill. (Rubiaceae) are represented each by three species in the Bulgarian flora (Ančev, 1976, 1979). They are morphologically well differentiated and all except *Cruciata glabra* (L.) Ehrend., do not suggest serious taxonomic problems. The coumarins, scopoletin, umbelliferone and cruciatin and the iridoid glucosides monotropein, asperuloside and aucubin, were found in some *Cruciata* species (Borisov, 1967, 1974; Borisov and Borisyuk, 1965; Borisov and Zoz, 1975; Plouvier, 1964; Ergun *et al.*, 1984). In *Crucianella* the presence of asperuloside was shown (Juillet *et al.*, 1938). In continuation of our studies on the iridoid glycosides in *Galium* L. (Handjieva *et al.*, 1996; Mitova *et al.*, 1996), in this paper we reported the coumarin and iridoid composition of three *Cruciata* species and one *Crucianella* species: *Cruciata laevipes* Opiz (= *Galium cruciata* (L.) Scop.), *C. pedemontana* (Bell.) Ehrend. (= *Valantia pedemontana* Bell.; = *Galium pedemontanum* (Bell.) All.), *C. glabra* (L.) Ehrend. (= *Galium verum* Scop.) and *Crucianella graeca* Boiss. with the purpose to throw additional light on the speciation and phylogenetic relationships within these species.

## Results and Discussion

The HPLC chromatograms showed low concentrations of iridoids and coumarins in all examined samples with the exception of *Cruciata glabra*.

Thirteen pure compounds (Fig. 1) were isolated and identified by <sup>1</sup>H and <sup>13</sup>C NMR spectra (Table I) and comparisons with authentic samples as the coumarins, daphnin (**1**), daphnetin glucoside (**2**) and daphnetin (**3**) (Jevers *et al.*, 1978) and the iridoids, deacetylasperulosidic acid (**4**), scandoside (**5**), asperuloside (**6**), asperulosidic acid (**7**), methyl ester of deacetylasperulosidic acid (**8**), daphylloside (**9**), geniposidic acid (**10**), 10-hydroxyloganin (**11**), deacetylasperuloside (**12**) and iridoid V3 (**13**) (Boros and Stermitz, 1990; El-Naggar & Beal, 1980).

From *C. glabra* were isolated in almost equal amounts in large concentrations as the main constituents, two aromatic compounds **1** and **2**, together with little deacetylasperulosidic acid (**4**) and scandoside (**5**). Compounds **1** and **2** by acid hydrolysis afforded identical products – glucose and the coumarin aglycone daphnetin (**3**), which we found in traces in the same taxon. Obviously, both glycosides appeared to be isomers, proved by their <sup>1</sup>H and <sup>13</sup>C NMR spectra, fully supporting the structures of the coumarin monoglucosides daphnin (**1**) and daphnetin glucoside (**2**) (Jewers and Zirvi, 1978). It is very likely that the unidentified coumarin monoglucoside, cruciatin, found in *Galium cruciata* (Borisov and Borisyuk, 1965) and *G. tauricum* (Borisov, 1967), was **1** or **2**.

*C. laevipes* and *C. pedemontana* showed similar HPLC chromatograms. Eight pure compounds were isolated in low concentrations. The main compound in both taxa was desacetylasperulosidic



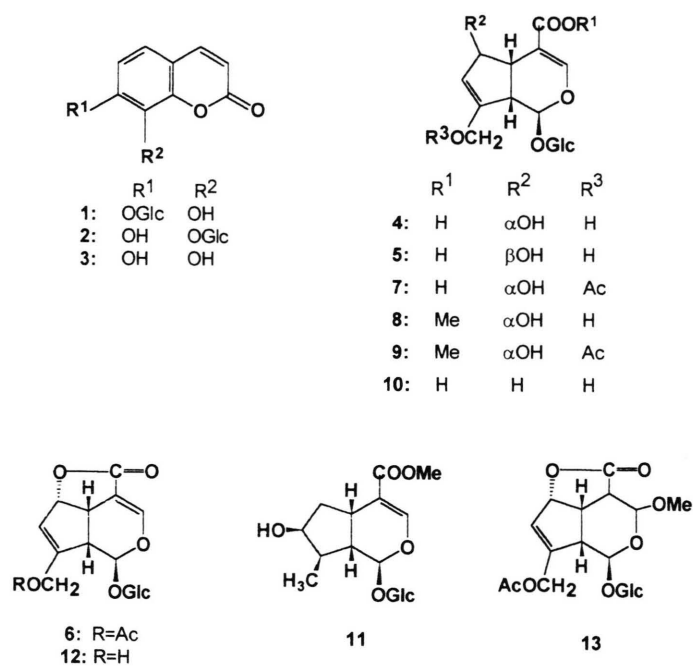
Glc:  $\beta$ -D-Glucopyranosyl

Fig. 1. Structures of the isolated coumarin and iridoid compounds.

Table I.  $^{13}\text{C}$  NMR data of compounds **1–3** in DMSO and **4–13** in  $\text{D}_2\text{O}$  at 62.8 MHz.

| C    | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1    |       |       |       | 99.8  | 98.0  | 93.0  | 99.6  | 99.8  | 99.6  | 97.2  | 98.2  | 93.7  | 97.3  |
| 2    | 160.3 | 160.3 | 160.5 |       |       |       |       |       |       |       |       |       |       |
| 3    | 112.3 | 113.6 | 111.3 | 151.7 | 149.3 | 150.0 | 155.5 | 155.8 | 155.7 | 150.4 | 152.1 | 150.8 | 96.2  |
| 4    | 144.9 | 144.9 | 145.2 | 112.5 | 115.8 | 104.7 | 110.3 | 107.5 | 107.3 | 11.5  | 112.2 | 105.7 | 45.5  |
| 5    | 118.4 | 124.2 | 118.9 | 42.5  | 45.8  | 36.0  | 41.5  | 41.2  | 40.8  | 35.3  | 32.6  | 36.7  | 36.4  |
| 6    | 113.6 | 111.7 | 112.6 | 75.3  | 81.8  | 86.3  | 74.9  | 74.9  | 74.8  | 39.0  | 41.4  | 87.5  | 88.5  |
| 7    | 148.4 | 153.5 | 149.8 | 129.6 | 129.4 | 128.2 | 131.7 | 129.6 | 131.7 | 130.0 | 72.5  | 125.6 | 127.2 |
| 8    | 134.2 | 131.2 | 132.2 | 150.2 | 147.0 | 142.2 | 144.8 | 150.0 | 144.7 | 142.0 | 48.9  | 148.1 | 148.7 |
| 9    | 142.8 | 144.9 | 143.8 | 45.6  | 48.8  | 43.5  | 45.3  | 45.1  | 45.2  | 50.0  | 42.2  | 44.0  | 43.7  |
| 10   | 114.6 | 112.2 | 112.1 | 61.1  | 60.6  | 60.8  | 63.7  | 60.9  | 63.7  | 60.6  | 61.8  | 59.5  | 62.5  |
| 11   |       |       |       | 172.1 | 171.1 | 172.0 | 174.7 | 170.6 | 174.7 | 173.0 | 168.6 | 174.5 | 174.4 |
| 1'   | 101.9 | 103.9 |       | 101.1 | 99.6  | 98.6  | 100.8 | 101.4 | 101.0 | 99.5  | 99.7  | 99.4  | 98.6  |
| 2'   | 73.4  | 74.0  |       | 73.7  | 73.7  | 72.6  | 73.5  | 73.6  | 73.5  | 73.6  | 73.9  | 73.4  | 73.5  |
| 3'   | 75.9  | 77.4  |       | 76.6  | 76.6  | 75.5  | 76.4  | 76.5  | 76.4  | 76.5  | 77.1  | 76.3  | 76.4  |
| 4'   | 69.9  | 69.7  |       | 70.4  | 70.4  | 69.6  | 70.2  | 70.3  | 70.2  | 70.4  | 70.7  | 70.4  | 70.3  |
| 5'   | 77.5  | 76.4  |       | 77.0  | 77.1  | 76.4  | 76.8  | 77.0  | 76.9  | 77.5  | 77.4  | 77.2  | 76.7  |
| 6'   | 60.8  | 60.9  |       | 61.5  | 61.5  | 61.5  | 61.5  | 61.5  | 61.5  | 61.5  | 61.5  | 61.5  | 61.5  |
| MeCO |       |       |       |       |       | 20.3  | 21.0  |       | 21.0  |       |       |       | 21.0  |
| MeCO |       |       |       |       |       | 170.3 | 172.0 |       | 170.4 |       |       |       | 172.0 |
| OMe  |       |       |       |       |       |       |       |       |       |       |       | 52.6  |       |

For compounds **4–13** the shift for C-6' was arbitrary set as  $\delta$  61.5.

acid (**4**) along with the iridoids **5–9** and the coumarins **1** and **2**. In *C. laevipes* additionally were found traces of **10**. The coumarin and iridoid profile of *C. laevipes* and *C. pedemontana* gives no possibility to distinct both taxa but allows to distinguish them from *C. glabra*.

*Crucianella graeca*, in comparison with the examined *Cruciata* taxa, contained the relatively larger iridoid concentrations and no coumarins. Both samples, collected in different years, showed similar results. Asperuloside (**6**) appeared to be the main iridoid, accompanied by **5**, **4**, **11** and **10** and traces of **7–9**, **12** and **13**.

All isolated compounds are new for the genera *Cruciata* and *Crucianella* with the exception of asperuloside.

## Materials and Methods

<sup>1</sup>H NMR: 250 MHz; <sup>13</sup>C NMR: 62.9 MHz. ISCO-HPLC system with UV detector V4 variable and pumps model 2350 with connected columns Whatman ODS-3 (250x4.6, 10 µm) and Lichrospher RP-18 (250x4.6 mm, 5 µm) was used.

### Plant material

Above ground parts of *Crucianella graeca* (Pirin mountain, 600 m, A9264), *Cruciata glabra* (Rila mountain, 1200 m, A9517), *C. laevipes* (Osogovo mountain, 1100 m, A9219) and *C. pedemontana* (Veliko Tirnov, 350 m, A954) were collected in flowering. The plant material was identified by Dr. M. Anchev, Institute of Botany, Bulgarian Academy of Sciences, Sofia. Voucher specimens are deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia (SOM).

### Isolation

*Cruciata glabra*. Dried aerial parts (33 g) were extracted with MeOH (x 2) with MeOH. After the evaporation of the solvent *in vacuo* the residue (8 g) was partitioned between water and chloroform and the water layer (6 g dry residue) was separated on a charcoal column (31 g) with H<sub>2</sub>O (350 ml) and with 175 ml portions of 5% MeOH, 30% MeOH, 50% MeOH, MeOH, MeOH-Me<sub>2</sub>CO (1:1 v/v) and MeOH-CHCl<sub>3</sub> (1:1 v/v). The combined MeOH and 50% MeOH fractions (0.6 g) were chromatographed on silica gel (37 g) successively

with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (60:15:4, 60:22:4; 67 fractions of 20 ml each) and MeOH (100 ml) to give **1** (fr.17–22, 70 mg), **2** (fr.12–13, 47 mg), **3** (fr.6, 5 mg). The MeOH fr. (51 mg) after separation on a Lobar C18 column yielded **4** (5 mg) and **5** (4 mg). From the MeOH-Me<sub>2</sub>CO and MeOH-CHCl<sub>3</sub> fractions (0.9 g) after purification on silica gel with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O were obtained pure **1** (fr.63–80, 145 mg) and **2** (fr.42–58, 58 mg).

Dried aerial parts from *Crucianella graeca* (97 g), *Cruciata laevipes* (359 g) and *Cruciata pedemontana* (40 g) and were extracted with MeOH. The MeOH extracts (9 g, 47 g, 6 g, respectively) were processed as shown above. *Crucianella graeca*: The MeOH fr. obtained after charcoal treatment (0.3 g) was purified on silica gel (20 g) with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (60:15:4, 60:22:4; 67 fractions of 20 ml each) and 100 ml MeOH to give pure **13** (fr.7, 5 mg), **9** (fr.8, 9.6 mg), **6** and **9** (fr.9–11, 44 mg), **8**, **11** and **12** (fr.16–18, 24 mg), **10** (fr.23–29, 15 mg), **7** (fr. 46–60, 22 mg), a mixture of **4** and **5** (MeOH fr., 59 mg). The latter was additionally separated on a Lobar C18 column to afford pure **4** (15 mg) and **5** (4 mg). The MeOH-Me<sub>2</sub>CO fr. (0.2 g) separated on silica gel (9 g) with the above mentioned solvents yielded **6** (fr.4–5, 39 mg). *C. laevipes*: The MeOH fr. obtained after charcoal treatment was purified on silica gel with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O to give **1**, **2**, **4–10**. *C. pedemontana*: The MeOH fr. obtained after charcoal treatment was purified on silica gel with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O to yield **1**, **2**, **4–9**.

### HPLC analysis

Dried ground aerial parts (0.4 g) were extracted with MeOH (2x6 ml). After concentration and addition of water (3 ml), extraction with CHCl<sub>3</sub> (3x2 ml) was carried out. The water layer was treated with neutral aluminium oxide (1 g). After filtration and washing with 3 ml H<sub>2</sub>O and 3 ml H<sub>2</sub>O-MeOH (1:1), the combined filtrates were concentrated and dissolved in 2 ml MeOH-H<sub>2</sub>O (1:1). 10 µl samples were injected. Gradient elution was used – pump A: H<sub>2</sub>O-MeOH (95:5) and H<sub>3</sub>PO<sub>4</sub> (15 µl/100 ml mobile phase) and pump B: MeOH. The substances were detected at 233 nm. The flow-rate was 0.8 ml/min. R<sub>t</sub>: compound **4** – 16.6 min, **12** – 20.1 min, **5** – 22.7 min, **8** – 28.1 min, **10** – 28.9 min, **11** – 32.9 min, **6** – 35.8 min, **1** – 36.8 min, **7** – 37.1 min, **2** – 41.3 min, **9** – 48.1 min.

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