FLAVONOIDS IN *Astragalus corniculatus*

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Nine flavonoids were identified in aerial parts of *Astragalus corniculatus* Bieb. (Fabaceae) by liquid chromatography coupled with ionspray mass spectrometry in the tandem mode (LC/MS/MS) with negative ion detection. Vitexin, orientin and eriodictyol-7-O-glucoside are obtained for the first time in genus *Astragalus* L, and isorhamnetin-3-O-glucoside in the species.

Keywords: Fabaceae; *Astragalus corniculatus*; flavonoids.

INTRODUCTION

*Astragalus corniculatus* Bieb. (Fabaceae) is a perennial herbaceous plant, distributed in Moldova, South Ukraine and Southeastern Romania. The species was recently found in Bulgarian flora. Our earlier investigations of the ethyl acetate extract of this species, containing flavonoids, resulted in a low acute oral toxicity and a remarkable antihypoxic activity, especially in a model of circulatory hypoxia.

The aim of this study is to determine the flavonoids in the ethyl acetate extract of *A. corniculatus* using liquid chromatography coupled with ionspray mass spectrometry in tandem mode (LC/MS/MS).

EXPERIMENTAL

General procedures

LC analysis was performed on a Agilent 1100, Model G1312A O (Hewlet Packard, Palo Alto, CA, USA). An Aqua C18 125 A (150 x 3.0 mm i.d., 5 mL) (Phenomenex, Torrance, CA, USA) was used. Gradient elution was carried out with water - 0.1% formic acid and water – acetonitrile - 0.1% formic acid at a constant flow rate of 400 $\mu$L min$^{-1}$. The MS and MS/MS data were obtained by using an API 365 tripe-quadropole mass spectrometer (Perkin-Elmer Sciex, Concord, ON, Canada). All the analyses were performed using a Turbo Ionspray source. The operating parameters as follows: capillary voltage – 3500 V, nebolizer gas (N$_2$; 10 arbitrary units), curtain gas (N$_2$; 8 arbitrary units), draying gas (N$_2$; 7000 cm$^3$/min$^{-1}$), collision gas (N$_2$; 5 arbitrary units), focusing potential – 240 V and entrance potential 10 V. The collision energy (CE) and declustering potential (DP) were optimized for each standard. Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60 F$_{254}$, Merck, Germany). The spots were visualized by spraying with 1% methanolic solution of diphenylboric acid aminoethyl ester (NST). Column chromatography (CC) was performed on cellulose (Watman, Germany), Polyamide S (Riedel-de Haën, Germany) and Sephadex LH-20 (Pharmacia, Sweden).

Plant material

*Astragalus corniculatus* herbs were collected in July 1999 near the town of Svishtov, Bulgaria and identified by Dr. D. Pavlova. A voucher specimen has been deposited in the Herbarium of the Faculty of Biology, Sofia University, Bulgaria (SO95265).

Extraction and obtained of purified flavonoid fractions

Air-dried plant material (800 g) was powdered and extracted with 50% EtOH under reflux. After the removal of ethanol *in vacuo* the aqueous residue was consecutively treated with CHCl$_3$ and EtOAc. The EtOAc extract was evaporated to dryness to give a solid residue (14 g), which was submitted to column chromatography on cellulose, using 0-95% EtOH linear gradient. Further purification of combined fractions (TLC analysis) was achieved by rechromatography over Polyamid and Sephadex LH-20. Four main purified flavonoid fractions were obtained and analysed by LC/MS/MS.

RESULTS AND DISCUSSION

Solvent partition and repeated column chromatography over cellulose, Polyamide S and Sephadex LH-20 of the ethyl acetate extract was submitted to fractionation, and the flavonoid fractions were analysed by LC/MS/MS. Standard solutions of 12 flavonoids were studied in the negative ion mode using MS/MS product ion scans (multiple reactions monitoring (MRM). For flavonol and flavanon O-glycosides, the spectra present both the deprotonation molecule [M-H]$^-$ of the glycosides and the ion corresponding to the deprotonated aglycone [A-H]$^-$.

The latter ion is formed by loss of the sugar residue from the glycosides of flavones. Fragmentation of aglycones provided characteristic ions for each family of flavonoids. Isorhamnetin exhibits specific fragmentation with the loss of methyl radical, thus giving $m/z$ 315$	o$m/z 300. Hyperoside and isoquercetin have the same molecular mass and were identified together. Different fragmentation patterns were observed in MS/MS experiments for flavone-C-glycosides. Losses of 120 and 90 u were observed, corresponding to cross-ring cleavages in the sugar unit of Biologicals.
After MRM analysis of flavonoid fractions, nine flavonoids were identified as quercetin, quercetin-3-O-rutinoside (rutin), quercetin-3-O-galactoside (hyperoside), quercetin-3-O-glucoside (isoorientin), kaempferol, isorhamnetin, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-rutinoside and eriodictyol-7-O-glucoside (Table 1).

Apigenin-8-C-glucoside (vitexin) and luteolin-8-C-glucoside (orientin) were identified on the bases of the product ion spectrum and comparison with literature data\textsuperscript{9}. Vitexin shows the ions at \( m/z \) 431 (deprotoned molecule), \( m/z \) 341 (loss of 90 u) and \( m/z \) 311 (loss of 120 u) as characteristic ions in the MS/MS mode (Table 2). The product ion spectra of the ion \( m/z \) 447 of orientin differ in relative abundance of the \( m/z \) 357 (loss of 90 u) and \( m/z \) 327 (loss of 120 u) ions (Table 2). Moreover, the ion spectra of the ion at \( m/z \) 429 in the spectrum of isoorientin, was not present in that of orientin and for isovitexin – the ion at \( m/z \) 353 wasn’t absent in the spectrum of vitexin\textsuperscript{10}.

**CONCLUSIONS**

LC/MS/MS analysis of ethyl acetate fractions from the aerial parts of *Astragalus corniculatus* led to the identification of nine flavonoids. Vitexin, orientin and eriodictyol-7-O-glucoside are identified for the first time in genus *Astragalus* L, and isorhamnetin-3-O-glucoside – in the species *Astragalus*.

**ACKNOWLEDGEMENTS**

This work was partially supported by EU Socrates-Erasmus programme (Bilateral collaboration between Medical University, Sofia, Bulgaria and Fachhochschule Halle – Anhalt, Köthen, Germany).

**REFERENCES**


**Table 1.** Compounds identified by LC-MS/MS in the negative mode, compared with a standard

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Structure</th>
<th>MS/MS ions (m/z)</th>
<th>( t_r ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td><img src="image" alt="Kaempferol" /></td>
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<tr>
<td>Isorhamnetin</td>
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<td>Isorhamnetin-3-O-rutinoside</td>
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<td>Quercetin</td>
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<td>Quercetin-3-O-glucoside/</td>
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<td>463/301</td>
<td>13.93</td>
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<tr>
<td>Quercetin-3-O-rutinoside</td>
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<td>Eriodyctiol-7-O-glucoside</td>
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<td>449/287</td>
<td>13.94</td>
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**Table 2.** Compounds identified by LC-MS/MS in the negative mode, compared with literature data

<table>
<thead>
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<th>Flavonoids</th>
<th>Structure</th>
<th>MS/MS ions (m/z)</th>
<th>( t_r ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitexin</td>
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<td>431 (43), 341(17), 311 (100), 269 (5)</td>
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<tr>
<td>Orientin</td>
<td><img src="image" alt="Orientin" /></td>
<td>447 (46), 357 (38), 327 (100), 285 (6)</td>
<td>13.62</td>
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</tbody>
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