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Artigo

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[1]. The species was recently found in Bulgarian flora[2]. 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[3].

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 μL min^{-1}. The MS and MS/MS data were obtained by using an API 365 tripe-quadroupole 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), Polyamid 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 to 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 deportment 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[4]. Fragmentation of aglycones provided characteristic ions for each family of flavonoids[5]. Isorhamnetin exhibits specific fragmentation with the loss of methyl radical, thus giving *m/z* 315 → *m/z* 300[6]. 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[7,8] (Figure 1).

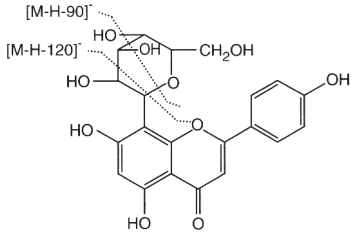

Figure 1. *Fragmentation of vitexin*

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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 (isoquercitrin), kaempferol, isorhamnerin, 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[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[10].

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

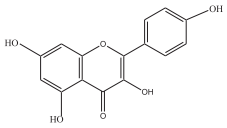
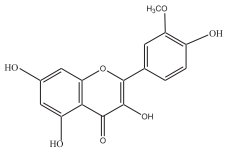
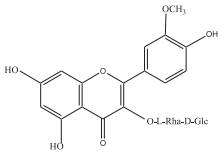
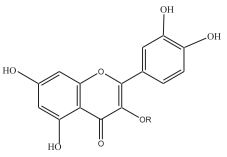
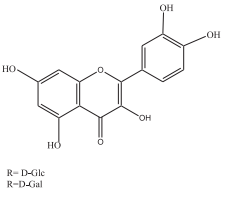
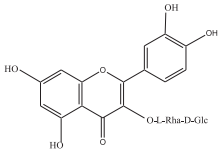
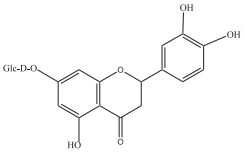
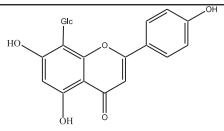
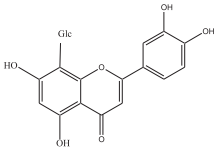
Flavonoids	Structure (m/z)	MS/MS ions (min)	t_r
Kaempferol		285/151	21.20
Isorhamnetin		315/300/151	21.50
Isorhamnetin -3-*O*-rutinoside		623/315	14.10
Quercetin		301/151	19.05
Quercetin-3-*O*-glucoside/ galactoside		463/301	13.93
Quercetin -3-*O*-rutinoside		609 / 301	13.54
Eriodyctiol -7-*O*-glucoside		449 /287	13.94

Table 2. Compounds identified by LC-MS/MS in the negative mode, compared with literature data

Flavonoids	Structure	MS/MS ions m/z (relative abundance, %)	t_r (min)
Vitexin		431 (43), 341(17), 311 (100), 269 (5)	12.80
Orientin		447 (46), 357 (38), 327 (100), 285 (6)	13.62

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**.**

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