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Flavonoids from the aerial parts of *Onobrychis montana* subsp. *scardica*

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Abstract: Rutin (**1**, main constituent) and two flavone *C*-glycosides, vitexin (**2**) and vitexin 2"-*O*-α-rhamnopyranoside (**3**) were isolated from the aerial parts of *Onobrychis montana* subsp. *scardica*. They were identified by ^{1}H-NMR, ^{13}C-NMR and UV–Vis spectroscopy (procedure with shift reagents), and high resolution ESI-MS. A relatively high content of **1** (5.27 mg/g of dry plant material), measured by HPLC, indicated *O. montana* subsp. *scardica* as a new natural source of this biologically active compound. The isolated flavonoid compounds might be of value as chemotaxonomic markers.

Keywords: *Onobrychis scardica*; Fabaceae; flavonoids.

INTRODUCTION

The Fabaceae family comprises about 12,000 species, divided into three subfamilies and 500 genera, widespread throughout the world. *Onobrychis montana* DC. subsp. *scardica* (Griseb) P. W. Ball (Synonim *Onobrychis scardica* (Griseb) Halácsy) is an endemic species growing in mountainous regions of the Balkan Peninsula.[1]

No previous phytochemical studies on any subspecies of *O. montana* have been reported. Chemical investigations of *Onobrychis* species revealed the presence of flavones, isoflavonoids,[2,3] flavonoid glycosides,[4–7] tannins,[8,9] cinnamic acid derivatives[10] and arylobenzofurans.[11,12]

The isolation, identification and quantification (HPLC) of three flavonoid glycosides as constituents of *O. montana* subsp. *scardica* are reported in this paper. The chemotaxonomic significance of the isolated compounds is also discussed.

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EXPERIMENTAL

General

The ^{1}H- (200 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded on a Varian Gemini 2000 spectrometer in DMSO-d_6. The UV spectra were measured on a Cintra 40 UV–Vis spectrometer. The high-resolution MS spectra were recorded on an Agilent 6210 LC ESI–MS TOF spectrometer.

Silica gel, 0.008 mm (Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) were used for preparative column chromatography (CC). Silica gel F-254 (Merck, Darmstadt, Germany) was used for analytical thin layer chromatography (TLC).

The HPLC separations were performed on a Hewlett-Packard Series 1100 equipped with a DAD model G1315B, bin pump model G1312A, autosampler model G1313A, and LiChrospher 100 RP-18e column (5 μm, 250×4 mm^2).

The HPLC standard of rutin was purchased from Roth, Germany. Acetonitrile and water were of HPLC grade and all other employed solvents were of analytical grade.

Plant material

The plant material was collected in July, 2006 on the mountain Bjelasica, Montenegro. A voucher specimen (144/06) was deposited in the Herbarium of the Biology Department, Faculty of Science, University of Montenegro, Podgorica.

Extraction and isolation

The air-dried, aerial parts of *O. montana* subsp. *scardica* (240 g) were extracted with 90 % methanol, twice (700 ml + 400 ml) at room temperature and 23 g of a crude extract was obtained after evaporation of the solvent. The extract was then partitioned between 300 ml H_2O and 200 ml chloroform. The aqueous layer was re-extracted with *n*-butanol to yield 5.5 g of residue after removal of the solvent. A part of the *n*-butanol extract (3 g) was subjected to isocratic silica gel flash chromatography (FC) with EtOAc/MeOH/H_2O/HCOOH 10/2/1/0.1, affording 51 fractions. The combined fractions 40–50 gave 950 mg of compound **1**, after evaporation of the solvent. Further purification of the combined fractions 18–19 on a Sephadex LH-20 column eluting with methanol yielded 2.0 mg of compound **2**. The combined fractions 25–30 after chromatography on a Sephadex LH-20 column with methanol yielded 3.0 mg of compound **3**.

HPLC analysis

HPLC analysis of the crude extract (90 % methanol) was realized using a linear gradient with two solvents (0.10 % H_3PO_4 in water as solvent A and acetonitrile as solvent B). The injection volume was 7 μl, and the elution at 1.0 ml min^{-1} with a gradient program (0–12 min: 8.0–10 % B, 12–14 min: 10–16 % B, 14–30 min: 16 % B, 30–36 min 16–36 % B, 36–42 min: 36–60 % B, 42–46 min: 60–100 % B). UV-Vis detection was performed at 254 and 320 nm. The extracts were dissolved in methanol and the quantification was based on the measured integration area, using the calibration equation of the corresponding standard. The concentrations used for the calibration were in range 0.2–2.0 mg ml^{-1}.

RESULTS AND DISCUSSION

The structures of the isolated compounds were established by spectrometric and chromatographic methods: UV with shift reagents (NaOMe, NaOAc, H_3BO_3, $AlCl_3$ and HCl), the NMR and HR–ESI–MS techniques; HPLC co-injection with

authentic samples and confirmed by comparison of the spectral data with those previously reported.[13,14] Compound **1** was identified as *O*-glycoside rutin, and compounds **2** and **3** as *C*-glycosides vitexin and vitexin 2"-*O*-α-rhamnopyranoside and vitexin, respectively.

The HPLC analysis, carried out immediately after the extraction, revealed **1** as the dominant compound, and **2** and **3** as minor constituents (Fig. 1). According to quantitative HPLC, the content of **1** was estimated to be 55 mg/g of extract, or 5.27 mg/g of dry plant material. Thus, *O. montana* subsp. *scardica* could be regarded as a new source of **1**, known for a variety of biological activities, such as prevention of diabetes and anti-oxidant properties.[15]

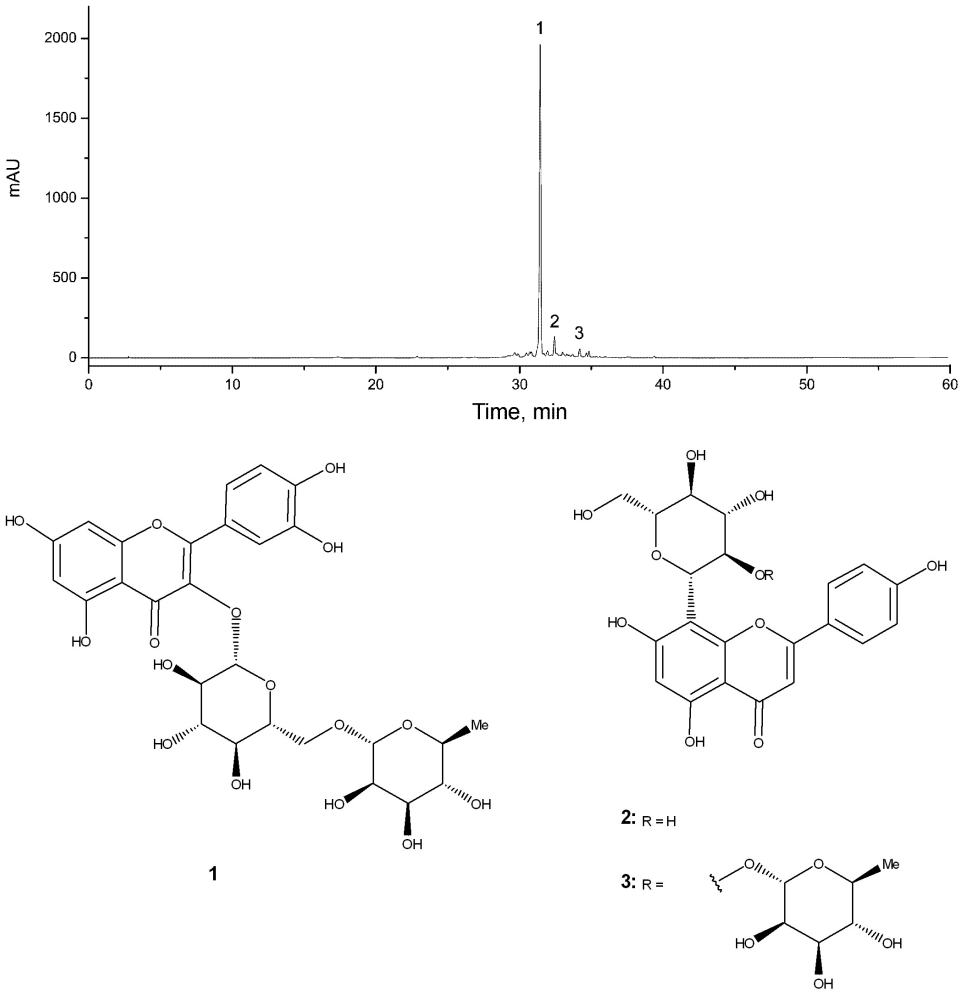

Fig. 1. HPLC profile of MeOH extract of *Onobrychis montana* subsp. *scardica*. Compounds: **1** rutin, **2** vitexin, **3** vitexin 2"-*O*-α-rhamnopyranoside.

The isolation of rutin from *O. montana* subsp. *scardica* conforms to the pattern of flavonoid distribution in several species of the *Onobrychis* genus, such as *O. viciifolia*,[9] *O. cornuta*, *O. echidna*, *O. ferganica*, *O. grandis*, *O. amoena*, *O. chorassanica*, *O. seravschanica,*[7], *O. biebersteinii,*[6] *O. bobrovi,*[5] *O. tanaitica*[16] and *O. pulchella*.[4] The minor constituent, *C*-glycoside vitexin, was detected previously in only one member of the genus, *i.e.*, *O. adans* from Georgia.[17] This is the first report of vitexin 2''-*O*-α-rhamnopyranoside in this family.

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И З В О Д

ФЛАВОНОИДИ ИЗ НАДЗЕМНИХ ДЕЛОВА *Onobrychis montana* subsp. *scardica*

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Из надземних делова *Onobrychis montana* subsp. *scardica* изоловани су рутин (**1**, главни састојак) и два флавоноидна *C*-гликозида, витексин (**2**) и његов 2''-*O*-α-рамнопиранозид (**3**). Њихова структура је одређена применом ^{1}H-NMR, ^{13}C-NMR и UV спектроскопије (процедура са реагенсима UV-померања) и масене спектрометрије високог разлагања (HR-ESI-MS). Релативно висок садржај рутина (5.27 mg/g сувог материјала) одређен помоћу течне хроматографије (HPLC), указује на *O. montana* subsp. *scardica* као нов природни извор овог биолошки активног једињења. Изолована једињења могу бити и од вредности као хемотаксономски маркери.

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