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N O T E

The HPLC determination of the content of magnoflorine in *Epimedium alpinum* L.

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Abstract: The aporphine alkaloid magnoflorine, known as a constituent of Asian *Epimedium* species, was isolated for the first time from the wild growing European species, *Epimedium alpinum* L. Identification was done by comparison with literature data. The HPLC method was applied for the determination of the content of the alkaloid in methanolic extracts of plant material. The underground part of *E. alpinum* contains 1–2 % of magnoflorine, while its content in methanolic extracts was 9.2–11.8 %. The aerial part of the plant contains less alkaloid (0.06–0.12 %), while the magnoflorine content in methanolic extracts of the aerial part was 0.4–0.8 %. Probably, the high content of magnoflorine in the methanolic extracts of underground parts of *E. alpinum* influences its dose-dependent cytotoxic activity (300 μg/ml; 39 % survival of cells in an experimental *K562* cell culture).

Keywords: *Epimedium alpinum*, magnoflorine, HPLC determination.

INTRODUCTION

In traditional medicine of East Asian countries, aerial parts (leaves or *herba*) of several *Epimedium* species (*E. grandiflorum*, *E. koreanum*, *E. sagittatum*, *E. pubescens* and other) are used in the treatment of renal illness, impotence, infertility in women, rheuma, asthma, coronary illness, hypertension, *etc.*[1] According to recent data, over 60 flavonoid glycosides (most of them derivatives of 8-C-prenyl-kaempherol), around 30 flavonoid aglycones and up to 50 other glycosides, known as icarisides, have been isolated from different *Epimedium* species.[1] The other pharmacologically active constituents of *Epimedium* species are phytosterols, fatty acids, polysaccharides[1] and aporphine type alkaloids (mainly magnoflorine).[2,3]

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The genus *Epimedium* is represented by 2 species in Europe (*E. pubigerum* Morr. et Decne and *Epimedium alpinum* L.). In the flora of Serbia and Montenegro, only the latter species is present. *E. alpinum* (Berberidaceae), Alpine Barrenwort, has not been chemically and pharmacologically investigated. In folk medicine, the leaf is used as a diaphoretic and in breast illness.[2] The only data about the chemical constituents of this species suggest the presence of alkaloids in the underground parts.[4]

RESULTS AND DISCUSSION

This paper reports for the first time the isolation of the aporphine alkaloid magnoflorine from the underground parts of *E. alpinum*, the wild growing *Epimedium* species in Europe. Characterization of magnoflorine ($C_{20}H_{24}O_4N^+$, M_r 342.41) was made by ^{1}H-NMR, ^{13}C-NMR and MS spectroscopy and identification was made by comparison with literature data.[5] This alkaloid is a well known constituent of other *Epimedium* species (*E. pubigerum*,[2] *E. cremeum*, *E. grandiflorum*, *E. rugosum*[3]). It was presumed that the aerial parts of *Epimedium* species were without alkaloids.[3] However, Slavik and co-workers[6] isolated magnoflorine (as the iodide, 0.14 %) from the underground parts of the decorative species *E. versicolor* and detected a trace of this alkaloid in the aerial parts. Chen and co-workers[7] confirmed by HPLC that magnoflorine was present in all parts of *E. koreanum*, mostly in the rhizome.

TABLE I. The magnoflorine content (%) in the *E. alpinum* extracts and plant materials, determined by the HPLC method

Plant material	Solvent	Mass ratio drug/extract	Content of magnoflorine in the extracts/%	Calculated content of magnoflorine in the plant material/%
Underground part Divčibare, Aug. 1999	MeOH	8.13 : 1	9.17	1.13
Underground part Divčibare, June 2001	MeOH	5.64 : 1	11.82	2.09
Aerial part Divčibare, Aug. 1999	MeOH	6.96 : 1	0.85	0.12
Aerial part Divčibare, June 2001	MeOH	7.97 : 1	0.46	0.06
Aerial part Divčibare, June 2001	H_2O	5.26 : 1	0.40	0.07

Qualitative and quantitative analysis of *E. alpinum* from Serbia indicated the presence of alkaloids in the underground parts, as well as in the aerial parts. Hitherto, there were no data about the quantity of alkaloids in *E. alpinum*. A spectroscopic method (official method for the quantification of boldine in *Boldo folium*)[8] was used for alkaloid quantification in plant material and extracts of *E. alpinum*.[9] After isolation of magnoflorine, which is the main alkaloid in *E. aplinum*, it was possible to apply the HPLC method for the quantitative determination of the alkaloid content in

extracts of underground and aerial parts of *E. alpinum*, using isolated magnoflorine as the reference substance. Methanolic extracts of underground parts contained 9.2–11.8 % of magnoflorine, while the content of this alkaloid was few time smaller in the extracts of the aerial parts (0.4–0.8 %) (Table I). When the results were calculated on plant material, the alkaloid content in the underground parts was much higher, (1–2 %), than in the aerial parts of *E. alpinum* (0.06–0.12 %). The HPLC method is more sutable for the quantitative analysis of alkaloids of *E. alpinum* than the spectroscopic method,[8] because of the difference in the molecular weight of boldine and magnoflorine, as well as because of the difference in the solubility of magnoflorine (as quaternary base is highly water-soluble) and boldine.

Magnoflorine has chemotaxonomic importance as a characteristic alkaloid for the families of *Polycarpicae*.[6,10] From the pharmacological aspect, it is interesting as neuromuscular blocking agent[11] (AChR blocking agent),[12] lipoxygenase inhibitor,[12,13] as well as for its cytotoxic,[14] immunosupressive[15] and antimicrobial[16] activities. According to the results of a preliminary investigation on cytotoxic activity, a methanolic extract of the underground parts of *E. alpinum* showed strong activity at a concentration of 300 μg/ml against *K562* cell line of malignantly-transformed cells.[9] Also, the same extract showed immunomodulatory activity *in vitro* and *in vivo* (unpublished results). It is quite possible that these activities could be explained by the rather high content of magnoflorine in methanolic extracts of the underground parts of *E. alpinum* (9.2–11.8 %).

EXPERIMENTAL

Plant material

Plant material (underground parts = root and rhizome; aerial parts = leaves) was collected on Divčibare (mountain Maljen, western Serbia) in August 1999. and in June 2001. After collection, the plant material was dried in thin layers, in the shade, by air circulation and powdered prior to extraction.

Isolation and characterization of magnoflorine

The methanolic extract of the underground parts was dissolved in hot distilled water and extracted with diethyl ether, ethyl acetate and *n*-butanol. The aqueous phase was alkalinized with NH_4OH to pH 10 and extracted with chloroform (extraction of tertiary alkaloids). The alkaline aqueous phase was acidified with HCl to pH 3 and treated with an aqueous solution of picric acid (1 %), in order to precipitate quaternary alkaloids. After 24 h, the precipitate was dissolved in MeOH, treated with activated charcoal and filtered over Celite. The methanolic solution was passed through an ion-exchange resin (Amberlite IRA 400 OH^-) column, evaporated and further purified by preparative chromatography on silica gel (mobile phase: MeOH–H_2O–25 % NH_3, 15:3:1) to obtain magnoflorine (R_f 0.3, blue fluorescent band at 365 nm, red after treatment with Dragendorf's reagent). The ^{1}H-NMR (200 Mz, CD_3OD) and ^{13}C-NMR (50 MHz, CD_3OD) spectra were recorded on a Varian Gemini – 2000 instrument. The mass spectrum was recorded on a Finnigan MAT 8230 instrument.

Preparation of extracts for quantitative analysis

Methanolic extract: Powdered plant material (aerial parts 574 g; underground parts 474 g) was extracted by maceration at room temperature, with frequent shaking, first with $CHCl_3$ (2 × 2.5 l), during 3 days, then with MeOH (2 × 2.5 l), during 7 days. Aqueous extract of the aerial parts: Powdered plant material (108 g) was extracted with distilled water (1300 ml) at 100 ºC for 1 h. The extracts were evaporated to dryness under vacuum at 40 ºC.

Analytical HPLC

HPLC determination was performed on a Perkin Elmer instrument with a UV/VIS LC 290 Spectrophotometric Detector and LC 250 Pump on a Bakerbond BDC column (250 mm × 4.6 mm; 5 μm), using methanol–glacial acetic acid–triethanolamine–water (30 : 1 : 0.5 : 68.5), pH 5 as the mobile phase. Other conditions: temperature – ambient, flow rate – 1 ml/min, paper rate – 10 mm/min, sensitivity – 0.2 mAu, injection volume – 5 μl, detection at 270 nm. The reference solution was prepared by dissolving 16.7 mg of magnoflorine in 10 ml of methanol.

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ИЗВОД

HPLC ОДРЕЂИВАЊЕ САДРЖАЈА МАГНОФЛОРИНА У ПРЕВОЛЦУ, *Epimedium alpinum* L.

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Апорфински алкалоид магнофлорин, познат као састојак других врста рода *Epimedium*, по први пут је изолован из европске врсте *Epimedium alpinum* L. и идентификован поређењем са литературним подацима. Ово једињење је коришћено као стандард у HPLC анализи метанолних екстраката *E. alpinum*. Садржај магнофлорина је знатно виши у екстрактима подземних органа (9,2–11,8 %, што одговара садржају од 1–2 % магнофлорина у биљном материјалу), него у екстрактима надземних органа преволца (0,4–0,8 %, што одговара садржају од 0,06–0,12 % магнофлорина у биљном материјалу). Сасвим је вероватно да је потврђена цитотоксична активност метанолног екстракта подземних органа (300 μg/ml; 39 % преживелих ћелија *K562* културе малигно-трансформисаних ћелија), као и имуномодулаторна активност, повезана са високом концентрацијом овог алкалоида у испитиваном екстракту.

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