INFRASPECIFIC CHEMICAL TAXA OF *ARCTOSTAPHYLOS UVA-URSI* (L.) SPRENG FROM ROMANIA

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Two infraspecific chemical taxa were identified for *Arctostaphylos uva-ursi* (L.) Spreng from Romania based on the presence or the absence of methyl-arbutoside in the two populations where the species vegetates. Thus, in the population from the Apuseni Mountains, the leaves contain arbutoside and methyl-arbutoside, while the leaves of the population from the Oriental Carpathian Mountains (Bucovina) contain only arbutoside.

A different phytogeographical origin of the two populations in Romania can be evaluated, as a correlation with the distribution of the two taxa in Europe. These data confirmed the hypothesis from 1958 of Prof. Borza, that the two populations of *Arctostaphylos* from Romania had a different origin.

Key words: *Arctostaphylos uva-ursi,* the Apuseni Mountains.

INTRODUCTION

Infraspecific chemical taxa were initially defined as chemical varieties of the same species, which appear depending on their geographical areal, but can differ also genetically, their development being a phenotypical and chemical expression of genotype, in the same way as for other morphological or structural differences. For many species, both qualitative and quantitative chemical differences were identified [1, 2, 3].

Identification of these chemical taxa was initially done by analysis of secondary metabolism products, which can be considered third messengers, their synthesis being determined by specific enzymes, which are second messengers, whose synthesis is encoded by nucleic acids (first messengers). If the analysis of the sequences of amino acids from proteins or the nucleotides from nucleic acids is expensive and requires high performance instruments, secondary metabolism products can be analyzed using more accessible and simple techniques. The importance of their analysis results also from the fact that many of them are active principles in medicinal plants, having a practical value, along with a theoretical and scientific one [3].

Arctostaphylos uva-ursi (L.) Spreng (Ericaceae), also known as bearberry, represents an important element in the Romanian flora both phytogeographically and geobotanically (Borza, Flora). In Romania the species exists only in two stations (Fig. 1), on small and isolated areas, and it is considered an endangered species, protected by law, its harvesting for medicinal purposes being prohibited. The two stations are in the Apuseni Mountains at Scărița-Belioara (Alba district) and in the northern Oriental Carpathian Mountains at Răchitiș Hill (Suceava district) [4, 5, 6].

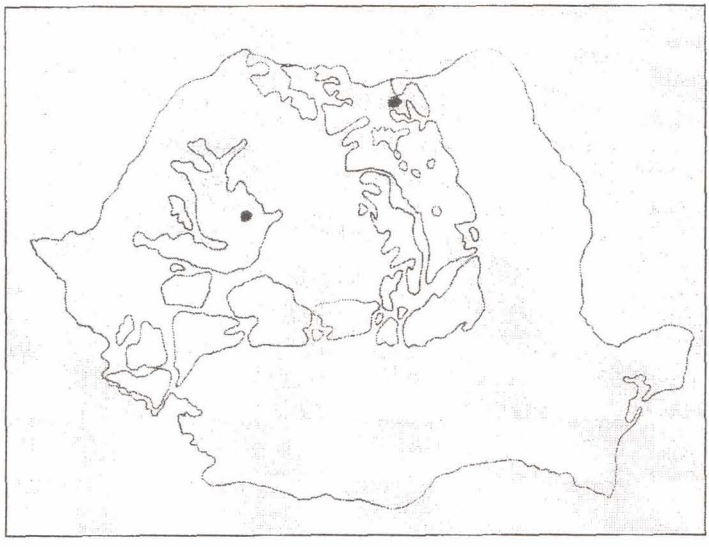

Fig. 1 – *Arctostaphylos uva-ursi* in Romania.

The isolated status of the two stations from Romania raised questions regarding their origin. Based on compared ecological and phytocenological studies, A. Borza [4] launched the hypothesis of a different geographic origin of the plants from the two stations: those from the Apuseni Mountains would have a southern, balcanic origin, while those from the Oriental Carpathian Mountains (Bucovina) had a northern, borealic origin. Also, morphologically, the leaves from the two stations present different characteristics [4, 7].

On the other hand, *A. uva-ursi* is an important medicinal plant, its leaves (*Uvae-ursi folium*) containing a phenolic heteroside-arbutoside, which has hydroquinone as an aglycon. Due to the presence of arbutoside, the leaves have renal antiseptic properties and are indicated for the treatment of nephritis, cystitis and pielonephritis [10]. The leaves are officinal in the Pharmacopoeias of many countries, a large number of pharmaceutical products containing standardized leaf extracts, existing already in therapeutical use [11, 12].

Pure arbutoside is also used as a skin-bleaching agent in cosmetology [13].

In Romania, the species is considered endangered, being replaced with cowberry leaves (*Vaccinium vitis-idaea*) [14]. An alternative solution would be the import of the product from the countries where it is abundant. Knowing the existence of two chemical taxa for this species in Europe, we proposed a study of the two Romanian populations. Thus, in Europe, plants of northern origin (Russia, Scandinavia, Poland) contain only arbutoside, while those of southern origin (Balkans, Alps) contain arbutoside and also methyl-arbutoside, even in high concentrations. The existence of these taxa was confirmed by Hegnauer [1], Tetenyi [2] and Wagner [8] (Fig. 2).

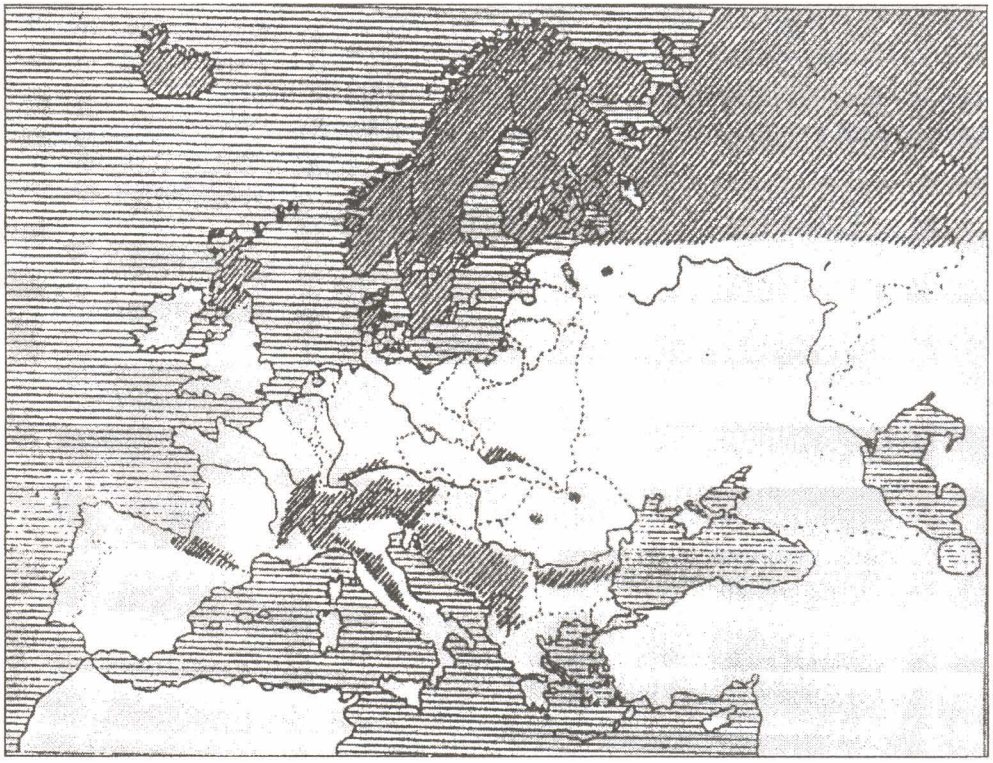

Fig. 2 – *Arctostaphylos uva-ursi* in Europe (after Madaus, 1938, modified).

In a first step, methyl-hydroquinone was put in evidence as a hydrolysis product, along with hydroquinone in the leaves from the Apuseni Mountains, but only hydroquinone in the plants from Bucovina [7], thus indirectly showing the presence of methyl-arbutoside in the plants from the first region. In order to confirm the presence of methyl-arbutoside, a high performance liquid chromatography (HPLC) analysis was necessary, because in thin layer chromatography (TLC), methyl-arbutoside (MA) could not be identified with the reagents for arbutoside (A) due to the blocking of both phenolic groups [3, 7].

MATERIAL AND METHODS

Vegetal material was harvested from Scărița-Belioara station (the Apuseni Mountains) in July 2001 and from Răchitișul Mare Hill in the northern Oriental Carpathian Mountains (Suceava district) in August 2001. The leaves were separated from the stems, dried at room temperature and then ground to a fine powder (sieve no. VI, FRX). The vegetal material was processed according to the

technique of Horhammer and Wagner [11], Thieme and Winkler [12] and Stahl [9]. Thus, 0.5 grams of leaf powder are extracted with 50 ml methanol:water (1:1) on boiling water for 30 minutes. Then, 0.5 grams lead acetate 5% are added and agitated until a yellow precipitate is formed, which is filtered. The filtered solution is diluted with methanol:water (1:1) and samples are taken.

For HPLC analysis the following were used:

Laboratory instruments:

– pump: Jasco PH-980

– column: Merck Hibar Lichrospher 100 RP 18, 250 × 4.6 mm, 5μm with a C18 precolumn

– injector: Rheodyne 7725 I

– UV detector: Pharmacia LKB-VWM 2141

Experimental conditions:

– length wave: 280 nm

– mobile phase: methanol:water (8:92), 1.5 ml/minute flow rate

– injected volume: 20 μl

– temperature: 20 °C ± 2 °C

Solvents and substances:

– p-arbutin (Fluka) 0.1% in methanol

– hydroquinone (Schuchardt) 0.1% in methanol

– methanol for chromatography (Merck)

– pure water (Milipore).

RESULTS AND DISCUSSION

In our experimental conditions, arbutoside is separated at retention time $t_R = 3.1$ minutes, hydroquinone at $t_R = 4.4$ minutes and methyl-arbutoside at $t_R = 18.2$ minutes. Other two unidentified peaks appear at $t_R = 16$ minutes and $t_R = 23.1$ minutes. Identification of methyl-arbutoside was performed after Sticher *et al.* method [14]. Linearity domain for arbutoside is between 300 and 5 600 μg/ml, using 7 different concentrations in this domain, each concentration being analyzed 3 times on the same day and in different days. The mean variance coefficient was 1.8% for the same day and 2.8% for different days. Detection limit was 7.5 μg/ml, three times the height of background noise.

Linearity domain for hydroquinone is between 40 and 450 μg/ml, using 7 different concentrations in this domain, each concentration being analyzed 3 times on the same day and different days. The mean variance coefficient was 0.9% for the same days and 1.75% for different days. Detection limit was 1.32 μg/ml, three times the height of background noise. The calibration curve equations were:

Area = 725c-37514r = 0.99782 for arbutoside,

Area = 2046c-77599r = 0.99971 for hydroquinone.

In Scărița-Belioara sample we identified and quantitatively determined arbutoside (4 782 µg/ml), free hydroquinone (112.4 µg/ml) and methyl-arbutoside (579 µg/ml, reported to arbutoside) (Fig. 3).

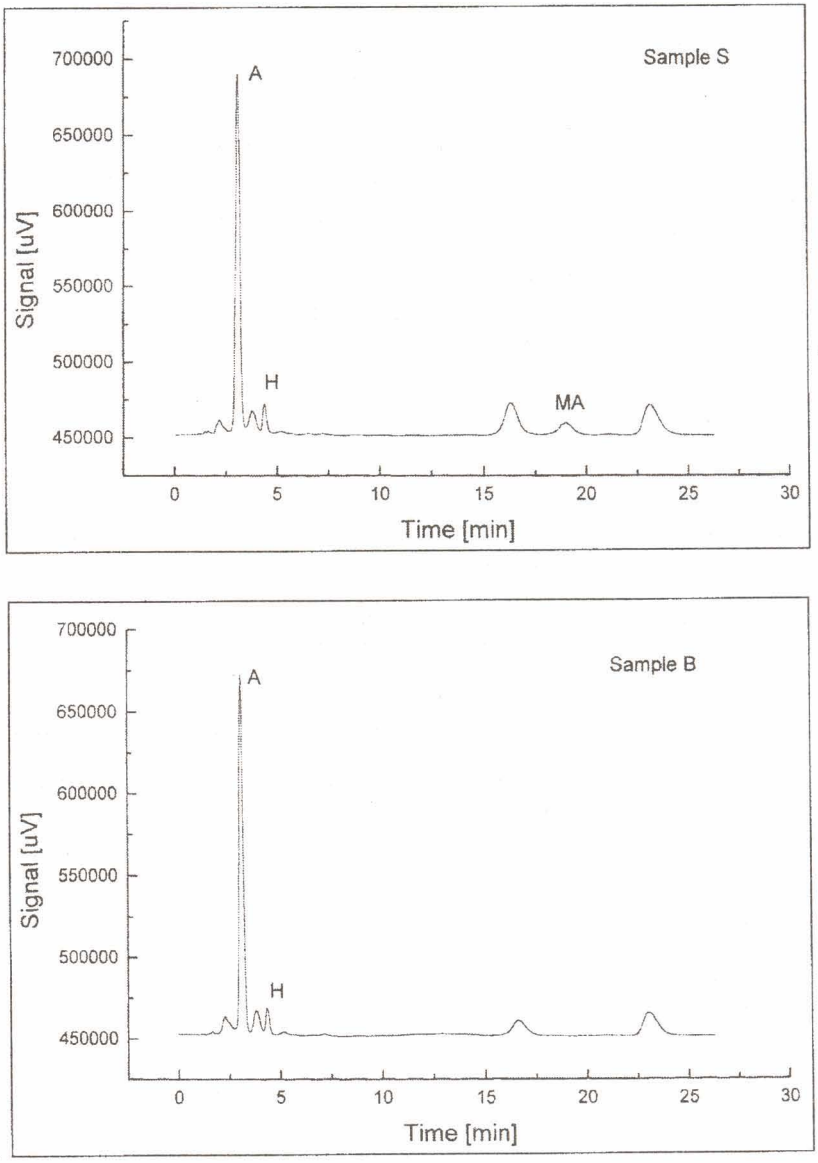

Fig. 3 – HPLC chromatograms for the phenolic compounds of *Arctostaphylos uva-ursi*:
Sample S – the sample from Scărița-Belioara;
Sample B – the sample from Bucovina;
A – arbutin; MA – methyl-arbutin; H – hydroquinone.

In Bucovina sample we identified and quantitatively determined arbutoside (4 199 μg/ml) and free hydroquinone (93.5 μg/ml). Methyl-arbutoside was not present in this sample.

HPLC analysis performed upon leaf extracts of *A. uva-ursi* from the two Romanian experimental stations revealed the presence of methyl-arbutoside only in the plants from the Apuseni Mountains. This analysis confirmed Prof. A. Borza's hypothesis, in 1958, referring to the different origin of the two Romanian populations. The presence of methyl-arbutoside in the Apuseni Mountains population allowed the establishment of its southern origin, while the lack of methyl-arbutoside in Bucovina population confirmed its northern origin (Fig. 2).

HPLC analysis performed for arbutoside and methyl-arbutoside confirmed also prior analysis by TLC of the aglycons of these phenolic heterosides, hydroquinone and methyl-hydroquinone [7].

These data confirmed the presence in Romania of two infraspecific chemical taxa of *Arctostaphylos uva-ursi*, conclusion based also on the very old age of these populations Ice Age relicts.

REFERENCES

1. R. Hegnauer, *Chemotaxonomie der Pflanzen*, vol IV, Birkhauser Verl, Basel-Stuttgart, 1966, 61.
2. P. Tetenyi, *Infraspecific Chemical Taxa of Medicinal Plants*, Akád. Kiádo, Budapest, 1970, 156.
3. M. Tămaş, *Rev. Med. (Tg. Mureş)*, **17**(2), 202–207, 1971.
4. A. Borza, *Lucr. Conf. Naţionale de Farmacie*, Bucureşti, 411–417, 1958.
5. ***, *Flora R.P.R.*, Vol. VII, Ed. Academiei, Bucureşti, 1960, 121–122.
6. T. Ştefureac, I. Cristureanu, I. Sihota, *Ocrot. Nat.*, **8**, 219–230, 1964.
7. M. Tămaş, *Ocrot. Nat. Med. Înconj.*, **40**(1–2), 55–60, 1996.
8. H. Wagner, *Arzneidrogen und Ihre Inhaltsstoffe*, Bd. 2, Wissenschaft Verl., Stuttgart, 1999, 303–304.
9. E. Stahl, W. Schild, *Pharmazeutische Biologie. Drogen Analyse* II, G. Fischer Verlag, Stuttgart – New York, 1981, 90–85.
10. G. Madaus, *Lehrbuch der Biologischen Heilmittel*, Bd. II, W. Thieme Verlag, Leipzig, 1938, 2759.
11. L. Horhammer, H. Wagner, *Dtsch. Apoth. Zeitung*, **103**(1), 1–4, 1963.
12. H. Thieme, H-J. Winkler, *Die Pharmazie*, **26**, 235–243, 1971.
13. J. Arend, H. Warzecha, J. Stockigt: *Phyotchemistry*, **53**, 187–193, 2000.
14. O. Sticher, F. Soldati, D. Lehmann, *Planta Medica*, **35**, 253–261, 1979.
15. M. Tămaş, V. Hodişan, *Clujul Medical*, **54**(4), 350–353, 1981.

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