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Sesquiterpene lactones. XXXIV. Guaianolides in the genus *Leuzea* DC.

GERARD NOWAK*, MIROSLAV HOLUB**, MILOŠ BUDĚŠÍNSKÝ**

* Chair and Department of Medicinal Plants, Medical Academy, Mazowiecka 33, 60-623, Poznań, Poland

** Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingowo nam. 2, Prague, Czechoslovakia

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Abstract

Guaianolides were found in three species of the genus *Leuzea* DC. Chlorojanerin, cynaropicrin and janerin were isolated from *L. rhapontica* subg. *helenifolia* (Gren, Gordon) Holub and *L. rhaponticoides* Graells. From *L. carthamoides* (Willd.) DC., chlorojanerin, cynaropicrin, janerin, cebellin E and repdiolide were obtained.

Key words: *sesquiterpene lactones, Leuzea, Compositae, chemotaxonomy*

INTRODUCTION

There are many taxonomic groups in the subtribe *Centaureinae* Dumort. whose relationships have still not been precisely determined or studied. The subject of many discussions among botanists are species assigned to *Leuzea* DC. and *Rhaponticum auct.*

De Candolle (1937) takes *Leuzea* DC. and *Rhaponticum* DC. as separate species. Dittrich proposes in Flora Iranica (1980) that species classified in *Rhaponticum* Hill. be included in the genus *Stemmacantha* Cass.

The basic morphological difference between the two discussed groups of plants is considered to be the different structure of the pappus. In *Leuzea conifera* (L.) DC. it is soft, snow-white with long hairs and has a characteristic thick ring at its base. In *Rhaponticum scariosum* Lam., on the other hand, the pappus is stiffer, brown, smaller, brittle and although connected at its base, lacking a distinct ring. The differences are then clear, but they begin to be less so when these traits are examined in other species. And so, *Leuzea berariodes* Bettand has a pappus which is stiffer but also brittle. An almost identical

structure of the pappus is found in *Rhaponticum serratuloides* (Georgi) Bobrov.

A difference in the structure of the fruit is also detectable, but according to Holub (1973) does not suffice as the basis of forming separate taxonomic groups. In *Leuzea* the fruit is elongated at both ends and has indistinct ribs, while in the species assigned to *Rhaponticum*, it is rounded at both ends and has distinct ribbing. However, there are several species assigned to *Leuzea* which have fruit with a structure similar to those species which some botanists attribute to *Rhaponticum*. In turn, *R. acante* (L.) DC. has an elongated fruit, thus of the type which is found in *Leuzea* species.

The similarities between the discussed groups do not end here. *Leuzea* and *Rhaponticum* are considered to be primitive systematic units of the subtribe *Centaureinae*. This is supported by several common features found in them – morphological (large, single flower heads, dioecious flowers), palynmorphological ("Serratula"-type), karyological (x = 13). In addition, *Leuzea* and *Rhaponticum* have bracts with the same structure with long parchment appendages. This trait is very important in the classification of species in the subtribe of *Centaureinae*. For these reasons some botanists combine the species from *Leuzea* and *Rhaponticum* into one unified genus. The proper name of this united taxonomic unit should be *Leuzea* DC. (Holub 1973).

In the light of these botanical discussions, chemical studies of the plants from *Leuzea* DC., numbering about 25 species, seemed interesting. Since in the chemotaxonomy of the subtribe *Centaureinae*, sesquiterpene lactones play a prime role, their presence was initially checked for in three species.

MATERIALS AND METHODS

The material used in this study consisted of the dried and crumbled above-ground parts of: *Leuzea rhapontica* subsp. *helenifolia* (Gren, Gordon) Holub (syn. *Rhaponticum scariosum* subsp. *lyratum* (Bellardi/Hayek), *Leuzea rhaponticoides* Graells and *Leuzea carthamoides* (Willd.) DC (syn. *Rhaponticum carthamoides* Willd.), cultivated in the garden of the Chair of Medicinal Plants in Poznań (Poland) from seeds obtained from Karaganda, Minsk (USSR) and Barcelona (Spain). The "lactone fractions" were isolated from the green parts of these plants according to the method of Drożdż and Piotrowski (1973). IR analysis of the purified plant extracts revealed that sesquiterpene lectones were present in them. TLC studies in the presence of several standards obtained previously from species of the subtribe *Centaureinae*, suggested that in the studied plants from the genus *Leuzea* DC, known guaianolides such as: chlorojanerin, cynaropicrin, janerin, cebellin E and repdiolide may be present. This was confirmed by further detailed analyses (isolational and identificational) (Fig. 2). The chloroform extracts were separated on a column packed with silica gel (MN Kieselgel 100-200 mesh and Kieselgel 200-300 mesh, Serva). The separation was checked by thin-layer chromatography on silica gel. Concentra-

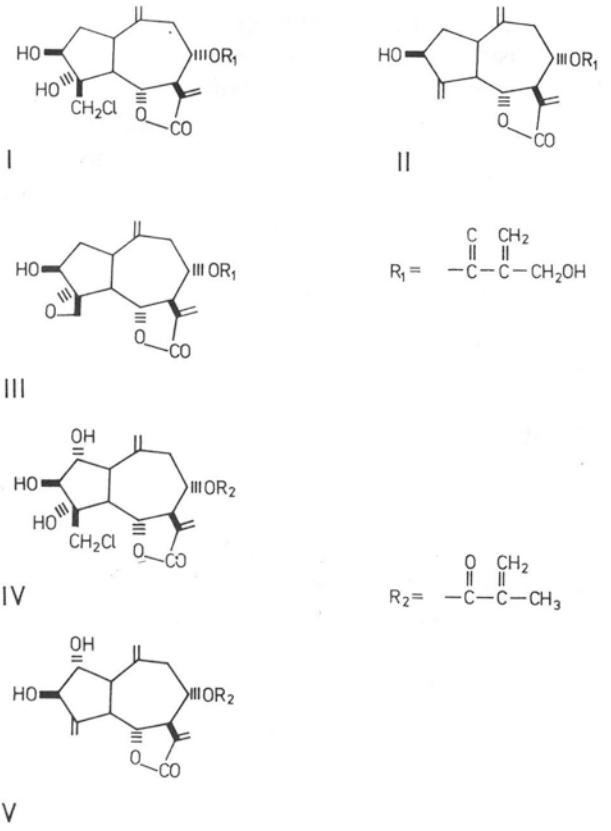

Fig. 1. The guaianolides occurring in the studied species of the genus *Leuzea* DC, I – chlorojanerin, II – cynaropicrin, III – janerin, IV – cebellin E, V – repdiolide

ted sulfuric acid was used as the developer. The indification of the obtained compounds was carried out by chromatographic and spectrographic methods (IR, MS, ^{1}H-NMR), comparing the data with standard sesquiterpene lactones.

ISOLATION OF CHLOROJANERIN (I)

A chloroform extract was obtained from 570 g of dried *Leuzea carthamoides*, and was (1.3 g) separated on a column packed with 80 g of silica gel. Lactones were eluted with a mixture of chloroform with the addition of 40% ethyl acetate, gradually increasing the polarity of the system. Seven fractions were collected. The fourth fraction (0.52 g) contained a compound which stained green on control chromatograms and showed a small degree of impurities. They were removed through successive chromatography on a column packed with 25 g of silica gel (Serva), eluting with a mixture of hexane-chloroform-ethyl acetate (1:1:1). One subfraction was obtained, which

was composed of 25 tubes, each containing 5 cm^3 of a solution of a purified compound. After distilling off the solvents, it was found that it was chromatographically uniform, and crystallization was undertaken. The studied compound was dissolved in 1 cm^3 of chloroform and about 2 cm^3 ethyl ether were added. After cooling, tiny white needles were formed, which were filtered off and washed with ethyl ether. Thirteen mg of crystals with a melting point of 181-183°C were obtained. They were initially identified by chromatographic methods. The color and Rf value suggested that it might be chlorojanerin (I). This was confirmed by spectral analysis (IR, ^{1}H-NMR).

This guaianolide was similarly obtained and identified from *Leuzea rhapontica* subsp. *helenifolia* (410 g of dry material, 1 g "lactone fraction", 28 mg chlorojanerin) and *Leuzea rhaponticoides* (620 g dry material, 1.3 g "lactone fraction", 10 mg chlorojanerin).

ISOLATION OF CYNAROPICRIN (II)

The initial check of the "lactone fractions" of the three studied species against standard guaianolides suggested that cynaropicrin may be present in them. This observation was confirmed by detailed analyses.

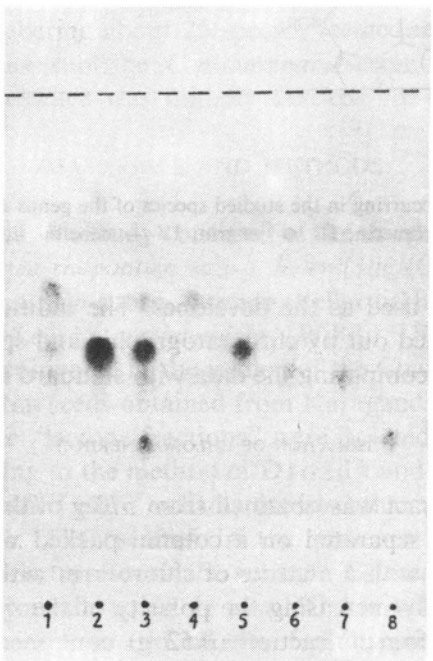

Fig. 2. A chromatogram of the "lactone fractions" from species of the genus *Leuzea* DC, and the guaianolides occurring in them. 1 – "lactone fraction" of *L. rhapontica* subsp. *helenifolia*, 2 – "lactone fraction" of *L. rhaponticoides*, 3 – "lactone fraction" of *L. carthamoides*, 4 – chlorojanerin, 5 – cynaropicrin, 6 – cebellin E, 7 – janerin, 8 – repdiolide.

The substance which stained dark blue on the control chromatograms was easy to isolate on 100-200 mesh silica gel by choosing only those test tubes which contained a chromatographically uniform substance. It was eluted from the column with a mixture of chloroform+40% ethyl acetate. An oily, colorless substance remained after the solvents had been distilled off. Its chromatographically determined identity was confirmed by spectral data (IR, ^{1}H-NMR). The substance obtained was shown to be cynaropicrin (II). It occurred in dominating amounts in *Leuzea rhaponticoides* (840 mg) and *Leuzea carthamoides* (210 mg), in a smaller amount (19 mg) in *Leuzea rhapontica.*

ISOLATION OF JANERIN (III)

The control chromatograms of the "lactone fractions" of the three studied species indicated the presence of a brown-staining substance with an identical Pf value and color as the standard janerin. It was isolated by column chromatography on silica gel using chloroform with 50% ethyl acetate, and then purified on a column with small-particle silica gel, using a system of chloroform+30% acetone. An amorphous substance was obtained, whose spectral properties corresponded with those of janerin (III). It was obtained in the following amounts from *Leuzea carthamoides* – 13 mg, *L. rhapontica* subsp. *helenifolia* – 21 mg and *L. rhaponticoides* – 11 mg.

ISOLATION OF CEBELLIN E (IV) AND REPDIOLIDE (V)

A fraction giving two spots was obtained from the chloroform extract of *Leuzea carthamoides* after column chromatography using chloroform with 50% ethyl acetate. One of the spots stained light green, the other black. This fraction (0.43 g) was separated on a column packed with 25 g of silica gel and eluted with a mixture of chloroform: ethyl acetate: hexane (1:2:1), giving three subfractions. The first and third contained chromatographically uniform compounds, while the second, a mixture of the two, was not separated further due to its minute amount.

The solvents of the first subfraction were distilled off and the remnant was dissolved in 1 cm^3 of chloroform and about 2 cm^3 of ethyl ether were added. After cooling, 8.5 mg of a crystalline compound were obtained, whose melting point equalled 204-206°C, and which was identified by spectral (IR, ^{1}H-NMR and MS) and chromatographic methods. On the basis of these analyses it was found that the studied compound was cebellin E (IV).

The third subfraction contained an amorphous compound (15.4 mg) which chromatographically and spectrally corresponded to repdiolide (V).

RESULTS AND DISCUSSION

Sesquiterpene lactones occur in plants from the genus *Leuzea* DC. From the three studied species, 5 of this type of guaianolide derivatives were isolated

and known to occur in other species of the subtribe *Centaureinae* Dumort. They are: chlorojanerin (I), a compound called cebellin C, found among over ten-odd guaianolides of *Centaurea bella* Trautv. (Nowak et al. 1986a) and first obtained from *Centaurea janeri* Graells (Gonzales et al. 1977), cynaropicrin (II) – a guaianolide commonly occurring in species from the genus *Centaurea* L. (Nowak et al. 1986b), isolated for the first time from *Cynara scolymus* L. (Samek et al. 1971), janerin (III) first found in *Centaurea janeri* Graells (Gonzales et al. 1977). The three guaianolides named above occur in various amounts (Fig. 2) in *Leuzea rhapontica* subsp. *helenifolia* (Gren, Gordon) Holub, *L. rhaponticoides* Graells and *L. carthamoides* (Willd.) DC. Two other sesquiterpene lactones were isolated only from *L. carthamoides* (Willd.) DC, They are: repdiolide (V), first found in *Acroptilon repens* L. (Stevens 1982) and cebellin E (IV), first isolated from *Centaurea bella* Trautv. (Nowak et al. 1986a).

It can therefore be stated that the sesquiterpene lactone composition of the three studied species is similar, although not identical. Somewhat different compounds occur in *L. carthamoides* (Willd) DC., a species which is assigned by some botanists to the genus *Rhaponticum*. The other species, however, which is also sometimes assigned to this taxonomic group as *Rhaponticum scariosum* subsp. *lyratum* (Bellardi) Hayek (in this study, *Leuzea rhapontica* subsp. *helenifolia* (Gren, Gordon/Holub)), has a qualitatively identical composition as *Leuzea rhaponticoides* Graells, a species always assigned to the genus *Leuzea* DC.

Further phytochemical studies of other species in this taxonomic group will make it possible to draw more general chemotaxonomic conclusions.

Acknowledgement

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Laktony seskwiterpenowe. XXXIV. Gwajanolidy w rodzaju leuzea DC

Streszczenie

W trzech gatunkach rodzaju *Leuzea* DC. stwierdzono obecność gwajanolidów. Z *L. rhapontica* subg. *helenifolia* (Gren, Gordon) Holub i *L. rhaponticoides* Graells wyizolowano chlorojanerynę, cynaropikrynę i janerynę. Z *L. carthamoides* (Willd.) DC. otrzymano chlorojanerynę, cynaropikrynę, janerynę, cebellinę E i repdiolid.