

Sesquiterpene lactones. XXIX. Cnicin in species of the subgenus *Acrolophus* (Cass.) Dobrocz.

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Abstract

Cnicin was isolated from 18 species or subspecies of the subgenus *Acrolophus* (Cass.) Dobrocz.: *Centaurea apolepa* Moretti subsp. *apolepa*, *C. apolepa* Moretti subsp. *lunensis* (Fiori) Dostal, *C. arenaria* Bieb. ex Wild. subsp. *arenaria*, *C. arenaria* Bieb. ex Wild. subsp. *odessana* (Prodan) Dostal, *C. arenaria* Bieb ex Wild. subsp. *majorowii* (Dumbadze) Dostal, *C. calvescens* Pančić, *C. cineraria* L. subsp. *cineraria*, *C. cineraria* L. var. *circae* Somm., *C. diffusa* Lam. var. *brevispina* Boiss., *C. grisebachii* (Nyman) Form. subsp. *grisebachii*, *C. grisebachii* (Nyman) Form. subsp. *confusa* (Halácsy) Dostal, *C. leucophaea* Jordan subsp. *leucophaea*, *C. attica* Nyman subsp. *ossaea* (Halácsy) Dostal, *C. pallidior* Halácsy subsp. *palidior*, *C. pelia* DC., *C. rhenana* Boreau subsp. *savranica* (Klokow) Dostal, *C. tymphaea* Hausskn subsp. *tymphaea*, *C. tymphaea* Hausskn subsp. *brevispina* Hausskn (Dostal. Chromatographic analysis has shown appearance of cnicin in 10 investigated species or subspecies of subgenus *Acrolophus* (Cass.) Dobrocz.: *Centaurea attica* Nyman subsp. *drakiensis* (Freyn, Sint.) Dostal, *C. kartschiana* Scop., *C. aggregata* Fisch. et Mey, *C. cuneifolia* Sibth. SM. subsp. *pallida* (Friv.) Hayek, *C. exarata* Boiss. ex Cosson, *C. mantoudii* Georg., *C. orphanidea* Heldr. Sart. ex Boiss. subsp. *orphanidea*, *C. spinosa* L. subsp. *spinosa*, *C. transiens* Halácsy, *C. zuccariniana* DC.

Key words: subgenus *Acrolophus*, cnicin, Compositae, *Centaurea*.

INTRODUCTION

The subgenus *Acrolophus* (Cass.) Dobrocz. (*Centaurea* L., Compositae) groups 80-200 species of plants which are found most often in Europe, Northern Africa and Western Asia. Larger agglomerations of these plants can be seen in areas surrounding the Mediterranean and Black

Seas. The large difference in the number of species given here results from difficulties in making clear-cut internal divisions within this subgenus. Hence, some authors consider many species as subspecies. There are also suggestions that the species ranked in subg. *Acrolophus* (Cass.) Dobrocz. be grouped in larger taxonomic units. For example, Dostal in *Flora Europea* (1976) distinguishes 12 sections.

The proposed divisions, however, give rise to many doubts because they are based on changeable and not very distinct morphological traits of the plants. Dostal (1975) too, is aware of this and recommends further work on establishing the correct internal taxonomy of subg. *Acrolophus* (Cass.) Dobrocz. There are no major difficulties, however, in establishing the extent of subg. *Acrolophus* (Cass.) Dobrocz., which is considered a section by some authors (De Candolle 1837, Hoffmann 1894). This view was also expressed by Wagenitz in *Flora of Turkey* (1975) and *Flora Iranica* (1980) and by Georgiadis (1980) who took into consideration species found in Greece.

A different proposition was put forth recently by Holub (1972) and Dostal (1973). They suggest the further division of the genus *Centaurea* L. with the creation of a few new, independent species. One of them is to be the genus *Acosta* Adans, which will group together species presently classified in subg. *Acrolophus* (Cass.) Dobrocz.

Studies done so far have shown that plants from subg. *Acrolophus* (Cass.) Dobrocz. are characterized by the ability to synthesize cnicin (Fig. 1 — I), a sesquiterpene lactone from the germacranolide group. Cnicin was found in the majority of the studied plants classified as belonging to the above-mentioned subgenus. The only exceptions were *Centaurea crithinifolia* Vis. and *C. friderici* Vis. from the section *Paniculatae* (Hayek) Dostal, which accumulated salonitenolide (Fig. 1 — II) in their above-ground parts (Geppert et al. 1983).

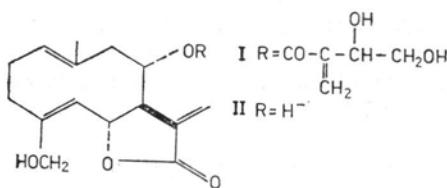


Fig. 1. Sesquiterpene lactones found in subgenus *Acrolophus* (Cass.) Dobrocz.
I — cnicin, II — salonitenolide

The finding of cnicin is not limited to the subg. *Acrolophus* (Cass.) Dobrocz. It was first found in *Cnicus benedictus* L. subtribus *Centaureinae*, next in representatives of a few subgenera of the genus *Centaurea* L.

It was interesting to find that some species, e. g. *Centaurea calcitrapa* L. from subg. *Calcitrapa* (Heister ex Fabr.) Hayek, and *Centaurea eriophora* L. from subg. *Solstictaria* (Hill) Dobrocz. accumulated cnicin or salonitenolide (Geppert et al. 1983).

The study presented here, which covered a relatively large number of representatives of the studied taxon, was aimed at determining if the presence of cnicin can be used as a chemotaxonomic characteristic of subg. *Acrolophus* (Cass.) Dobrocz. At the same time it was intended to obtain more information on the presence of sesquiterpene lactones in the studied plants.

MATERIAL AND METHODS

Twenty-eight species or subspecies of *Centaurea* L., representatives of 10 sections of the subgenus *Acrolophus* (Cass.) Dobrocz. were studied. The names of the plants and their classification into sections are given according to Flora Europea (1976).

Centaurea aggregata Fisch. et Mey. which is not listed in Flora Europea was identified on the basis of the description in Flora USSR (1961) and included in the section *Aggregatae* Dumb.

Dried, above-ground parts of plants cultivated in the gardens of the Department of Medicinal Plants of the Medical Academy in Poznań (Poland) were used in these studies. Seeds were collected from plants growing in their natural habitat in Greece by one of the authors (Th. G.). Some of the seeds were obtained from different botanical gardens (Table 1).

The presence of cnicin was studied using methods described in a previous paper (Geppert et al. 1983): "lactone fractions" were isolated from dry, powdered, above-ground parts of plants. The composition of the "lactone fractions" was monitored by thin-layer chromatography on silica gel plates. The chromatograms of the studied fractions as well as of standard substances (cnicin and other lactones found in species of the genus *Centaurea* L.) were developed by selective staining (Drożdż and Błoszyk 1978).

It was attempted to isolate cnicin by the method already described (Drożdż 1966) from 18 species or subspecies which we had in sufficient amounts.

Identification was done by comparing the Rf values, melting points and IR spectra of the isolated compounds with those of the standard cnicin.

Table 1

Data on the classified as belonging to subgenus *Acrolophus* (Cass.) Dobrocz. tested for cnicin

No.	Species	Catalogue No.	Section	Place of origin	Cnicin found
1	2	3	4	5	6
1	<i>Centaurea aggregata</i> Fisch. et May	2/78	<i>Aggregatae</i>	USSR (Erevan)	+
2	<i>Centaurea apolepta</i> Moretti subsp. <i>apolepta</i>	483/81	<i>Apolepidae</i>	Italy (Pisa)	++
3	<i>Centaurea apolepta</i> Moretti subsp. <i>lunensis</i> (Fiori) Dostal	655/82-N	"	Italy (Genova)	++
4	<i>Centaurea arenaria</i> Bieb. ex Wild. subsp. <i>arenaria</i>	505/80	<i>Arenariae</i> (Hayek) Dostal	Hungary (Debreczen)	++
5	<i>Centaurea arenaria</i> Bieb. ex Wild. subsp. <i>odessana</i> (Prodan) Dostal	564/81	"	USSR (Odessa)	++
6	<i>Centaurea arenaria</i> Bieb. ex Wild. subsp. <i>majorowii</i> (Dumbadze) Dostal	31/82	"	GDR (Muhlhausen)	++
7	<i>Centaurea kartschiana</i> Scop.	4/78	"	Italy (Trieste)	+
8	<i>Centaurea grisebachii</i> (Nyman) Form. subsp. <i>grisebachii</i>	558/79-N	"	Greece (Joannina)	++
9	<i>Centaurea grisebachii</i> (Nyman) Form. subsp. <i>confusa</i> (Halácsy) Dostal syn. <i>C. confusa</i> Halácsy	525/80-N	"	Greece (Thesaloniki)	++
10	<i>Centaurea attica</i> Nyman subsp. <i>ossaea</i> (Halácsy) Dostal syn. <i>C. ossaea</i> Halácsy	542/79-N	"	Greece (Mt. Ossa)	++
11	<i>Centaurea attica</i> Nyman subsp. <i>drakiensis</i> (Freyn, Sint.) Dostal syn. <i>C. drakiensis</i> (Freyn, Sint.)	543/79	<i>Arenariae</i> (Hayek) Dostal	Greece (Protaria Volos)	+
12	<i>Centaurea transiens</i> Halácsy	528/80-N	"	Greece (Olimpos)	+
13	<i>Centaurea calvescens</i> Pancíć	206/78	<i>Maculosae</i> (Hayek) Dostal	FRG (Frankfurt)	++
14	<i>Centaurea exarata</i> Boiss. ex Cosson.	18/82	"	Spain (Madrid)	+
15	<i>Centaurea rhenana</i> Boreau subsp. <i>savranica</i> (Klokow) Dostal syn. <i>C. savranica</i> (Klokow) Dostal	526/80-N	"	Greece (Ajos-Ilias)	++

Table 1 continued

1	2	3	4	5	6
16	<i>Centaurea cineraria</i> L. subsp. <i>cineraria</i>	9/82	<i>Pannophyllum</i> Hayek	Italy (Trieste)	++
17	<i>Centaurea cineraria</i> L. var. <i>circae</i> Somm.	647/82	„	Italy (Pisa)	++
18	<i>Centaurea cuneifolia</i> Sibth. SM. subsp. <i>pallida</i> (Friv.) Hayek	547/79-N	„	Greece (Meteora)	+
19	<i>Centaurea pallidior</i> Halácsy subsp. <i>pallidior</i> syn. <i>C. affinis</i> subsp. <i>pallidior</i> (Halácsy) Hayek	561/79-N	„	Greece (Mt. Vardousia)	++
20	<i>Centaurea diffusa</i> Lam. var. <i>brevispina</i> Boiss. syn. <i>C. bovina</i> Velen.	524/80-N	<i>Cylindraceae</i> (Hayek) Dostal	Greece (Kavalla)	++
21	<i>Centaurea tymphaea</i> Hausskn subsp. <i>tymphaea</i>	550/79-N	„	Greece (Klinon-Ambelia)	++
22	<i>Centaurea tymphaea</i> Hausskn subsp. <i>brevispina</i> Hausskn (Dostal) syn. <i>C. brevispina</i> Hausskn (Dostal)	522/80-N	<i>Cylindraceae</i> (Hayek) Dostal	Greece (Domocos)	++
23	<i>Centaurea orphanidea</i> Heldr. Sart. ex Boiss. subsp. <i>orphanidea</i>	551/79-N	„	Greece (Mt. Pateras)	+
24	<i>Centaurea mantoudii</i> Georg.*	556/79-N	<i>Albiflorae</i> Dostal	Greece (Mantoudii)	+
25	<i>Centaurea pelia</i> DC.	529/79	„	Greece (Volos)	++
26	<i>Centaurea leucophaea</i> Jordan subsp. <i>leucophaea</i>	479/81-N	<i>Paniculatae</i> (Hayek) Dostal	Italy (Pisa)	++
27	<i>Centaurea spinosa</i> L. subsp. <i>spinosa</i>	539/79-N	<i>Dumulosae</i> (Hayek) Dostal	Greece (Saunion)	+
28	<i>Centaurea zuccariniana</i> DC.	549/79-N	<i>Pseudoplumosae</i> (Hayek) Dostal	Greece (Meteora)	+

si N—seeds collected from plants in their natural habitat, +—cnicin found by chromatography, ++—cnicin crystals isolated, *—species does not figure in Flora Europea, described by Georgiadis (1980).

RESULTS AND DISCUSSION

Chromatographic controls showed the presence of cnicin in all of the 28 species or subspecies from subg. *Acrolophus* (Cass.) Dobrocz. (Table 1). A spot with the same Rf value and color as that of standard cnicin was seen on developed chromatograms. There also were other colored spots on the chromatograms. This indicated that other sesquiterpene lactones could also be found in the studied plants.

Attempts to isolate cnicin from the above-ground parts of 18 species or subspecies of plants were successful. In each case a crystalline compound, unequivocally identified as cnicin (melting point, identical IR spectra and Rf values) was obtained.

Representatives of 10 sections of subg. *Acrolophus* (Cass.) Dobrocz. were studied. They were characterized by the ability to synthesize cnicin, which accumulated in the above-ground parts of the plant.

The studies have shown cnicin to be found in: *Centaurea apolepa* Moretti subsp. *apolepa*, *C. apolepa* Moretti subsp. *lunensis* (Fiori) Dostal, *C. arenaria* Bieb. ex Wild. subsp. *arenaria*, *C. arenaria* Bieb. ex Wild. subsp. *odessana* (Prodan) Dostal, *C. arenaria* Bieb. ex Wild. subsp. *majorowii* (Dumbadze) Dostal, *C. calvescens* Pančić, *C. cineraria* L. subsp. *cineraria*, *C. cineraria* L. var. *circae* Somm., *C. diffusa* Lam. var. *brevispina* Boiss., *C. grisebachii* (Nyman) Form. subsp. *grisebachii*, *C. grisebachii* (Nyman) Form. subsp. *confusa* (Halácsy) Dostal, *C. leucophaea* Jordan subsp. *leucophaea*, *C. attica* Nyman subsp. *ossaea* (Halácsy) Dostal, *C. pallidior* Halácsy subsp. *pallidior*, *C. pelia* DC., *C. rhenana* Boreau subsp. *savranica* (Klokow) Dostal, *C. tymphaea* Hausskn subsp. *tymphaea*, *C. tymphaea* Hausskn subsp. *brevispina* Hausskn (Dostal).

The results of chromatographic analysis have shown that cnicin may also be found in: *Centaurea attica* Nyman subsp. *drakiensis* (Freyn, Sint) Dostal, *C. kartschiana* Scop., *C. aggregata* Fisch. et Mey, *C. cuneifolia* Sibth. SM. subsp. *pallida* (Friv.) Hayek, *C. exarata* Boiss. ex Cosson., *C. mantoudii* Georg., *C. orphanidea* Heldr. Sart. ex Boiss. subsp. *orphanidea*, *C. spinosa* L. subsp. *spinosa*, *C. transiens* Halácsy, *C. zuccari-niana* DC.

Further studies are needed, especially on the representatives of the section *Paniculatae* (Hayek) Dostal, where species synthesizing salonitenolide (*C. crithinifolia* Vis., *C. friderici* Vis. and *C. paniculata* L.) have been found, in order to determine if they synthesize cnicin in addition to salonitenolide. It is also indicated to undertake further studies to identify the sesquiterpene lactones accompanying cnicin in the species of plants classified in the subgenus *Acrolophus* (Cass.) Dobrocz.

On the basis of the results of the studies presented here and in earlier works (Geppert et al. 1983), it can be accepted that a chemotaxonomic trait of plants belonging to subg. *Acrolophus* (Cass.) Dobrocz. is the ability to synthesize cnicin I, a sesquiterpene lactone from the germacranolide group. Cnicin was found in almost all of the studied to date plants from subg. *Acrolophus* (Cass.) Dobrocz., which is a significant percent of the plants grouped in it.

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*Laktony seskwiterpenowe. XXIX. Występowanie knicyny w gatunkach podrodzaju *Acrolophus* (Cass.) Dobrocz.*

Streszczenie

Knicynę wyizolowano z 18 gatunków lub podgatunków subgenus *Acrolophus* (Cass.) Dobrocz.: *Centaurea apolepa* Moretti subsp. *apolepa*, *C. apolepa* Moretti subsp. *lunensis* (Fiori) Dostal, *C. arenaria* Bieb. ex Wild. subsp. *arenaria*, *C. arenaria* Bieb. ex Wild. subsp. *majorowii* (Dumbadze) Dostal, *C. calvescens* Pančić, *C. cineraria* L. subsp. *cineraria* L. var. *circae* Somm., *C. diffusa* Lam. var. *brevispina* Boiss., *C. grisebachii* (Nyman) Form. subsp. *grisebachii*, *C. grisebachii* (Nyman) Form. subsp. *confusa* (Halácsy) Dostal, *C. leucophaea* Jordan subsp. *leucophaea*, *C. attica* Nyman subsp. *ossaea* (Halácsy) Dostal, *C. pallidior* Halácsy subsp. *pallidior*, *C. pelia* DC., *C. rhenana* Boreau subsp. *savranica* (Klokow) Dostal, *C. tymphaea* Hausskn subsp. *tymphaea*, *C. tymphaea* Hausskn subsp. *brevispina* Hausskn (Dostal). Na drodze chromatograficznej wykazano występowanie knicyny w 10 badanych gatunkach lub podgatunkach z subg. *Acrolophus* (Cass.) Dobrocz.: *Centaurea attica* Nyman subsp. *drakiensis* (Freyn, Sint.) Dostal, *C. kartschiana* Scop., *C. aggregata* Fisch. et Mey, *C. cuneifolia* Sibth. SM. subsp. *pallida* (Friv.) Hayek), *C. exarata* Boiss. ex Cosson., *C. mantoudii* Georg., *C. orphanidea* Heldr. Sart. ex Boiss. subsp. *orphanidea*, *C. spinosa* L. subsp. *spinosa*, *C. transiens* Halácsy, *C. zuccari-niana* DC.