ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 55, nr 4: 629-637
1986

Sesquiterpene lactones. XXXIII. Guaianolides in the subgenus *Psephellus* (Cass.) Schmalh., genus *Centaurea* L.

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(Received: December 17, 1985. Accepted: March 27, 1986)

Abstract

Sesquiterpene lactones were found to occur in all of the studied species of the subgenus *Psephellus* (Cass.) Schmalh. Differing compositions were found in the representatives of three sections. In *Centaurea declinata* MB. from the section *Leucophyllae* (Sosn.) Sosn., 15-deoxyrepin, linichlorin B and cynaropicrin were found. Linichlorin B dominated in *Centaurea hypoleuca* DC. from section *Hypoleucae* (Sosn.) Sosn., while in the species classified in section *Psephellus* Sosn., repin, acroptilin, jenerin, centaurepensin and, in some, also cynaropicrin, dominated.

Key words: *Psephellus*, *Centaurea*, *sesquiterpene lactones*, *Compositae*, *chemotaxonomy*

INTRODUCTION

The subgenus *Psephellus* (Cass.) Schmalh. is composed of approx. 35 species of plants belonging to the genus *Centaurea* L., found mainly in the Caucasus. *Centaurea dealbata* Willd. and several other, related species, are sometimes cultivated as decorative plants (Parey 1960). The plants classified as belonging to subg. *Psephellus* (Cass.) Schmalh. have a characteristic appearance. They are perennials with prostrate or erect stems up to 70 cm high. Their leaves are most often pinnate, with segments further divided, rarely smooth-edged. The heads are medium sized or large, flowers: pink, purple or blue. Their fruit pappus has bristles of various size.

In Flora Iranica (1980) and Flora of Turkey (1975), Wagenitz like Hoffman (1894), considers subg. *Psephellus* (Cass.) Schmalh. to be a section: *Centaurea* sect. *Psephellus* (Cass.) DC. While Dostal (1973) and Cassini (1817–1830) distinguish *Psephellus* Cass. as an independent genus.

The authors of Flora USSR (1961) divide the subg. *Psephellus* (Cass.) Schmalh. into three sections: *Leucophyllae* (Sosn.) Sosn., *Hypoleucae* (Sosn.) Sosn. and *Psephellus* Sosn. The trait distinguishing the species in the section *Psephellus* Sosn. is the presence of bracts completely covered by appendages. Whereas the main trait characterizing species from the section *Hypoleucae* (Sosn.) Sosn. is the different structure of the stem leaves.

The objective of this study was to examine the occurrence and structure of sesquiterpene lactones in species assigned to the three sections of subg. *Psephellus* (Cass.) Schmalh.

MATERIAL AND METHODS

Dried plants of 11 species belonging to the subgenus *Psephellus* (Cass.) Schmalh. cultivated in the garden of the Chair of Medicinal Plants of the Medical Academy in Poznań (Poland) were used for study. The identification of the plants was based on Flora USSR (1961) and Flora of Turkey (1975). A method previously developed (Drożdż and Piotrowski 1973) was used to isolate the "lactone fractions" – purified chloroform extracts containing sesquiterpene lactones from dried, above-ground plant parts. Data on the studied plants is presented in Table 1.

CHROMATOGRAPHIC ANALYSIS

A preliminary examination of the sesquiterpene lactone composition of the studied species of *Centaurea* subg. *Psephellus* (Cass.) Schmalh. was done by thin-layer chromatography on plastic plates coated with silica gel, and compared with standards – lactones found in other *Centaurea* L. species. The compounds separated most favorably in the ethyl acetate--hexane-chloroform-benzene 3:1:1:0.5 (v/v/v/v) system (Fig. 1). On the basis of these experiments it was already possible to have an idea on which compounds might be present in the studied plants. The following lactones were expected to be isolated: centaurepensin, repin, acroptilin, linichlorin B, cynaropicrin, janerin. In addition, a spot was visible on the chromatograms that did not coincide with any of the standards. Also, evident differences were observed in the composition of compounds in species assigned to the three sections of subg. *Psephellus* (Cass.) Schmalh.

Table 1

Data on the studied plants from the subgenus *Psephellus* (Cass.) Schmalh.

No.	Species	Cataloque number	Source of seeds	Amount of dried material, g	Yield of "lactone fraction", %	Isolated sesquiterpene lactones
	A. Section *Leucophyllae* (Sosn.) Sosn.					
1	*Centaurea declinata* MB. syn. *Psephellus declinatus* (MB.) C. Koch	191/77	Mińsk (USSR)	450	0.26	15-deoxyrepin$^+$, linichlorin B$^+$, cynaropicrin
2	*Centaurea leucophylla* MB. syn. *Psephellus leucophyllus* (MB.) CAM.	21/80	Cluj (Rumania)	450	0.23	15-deoxyrepin$^+$, linichlorin B$^+$, cynaropicrin
	B. Section *Hypoleucae* (Sosn.) Sosn.					
3	*Centaurea hypoleuca* DC.	210/85	Cluj (Rumania)	400	0.20	15-deoxyrepin, linichlorin B$^+$, cynaropicrin
	C. Section *Psephellus* Sosn.					
4	*Centaurea carthalinica* (Sosn.) Sosn. syn. *Psephellus carthalinicus* Sosn.	39/76	Tigru-Mures (Rumania)	370	0.52	15-deoxyrepin, linchlorin B, cynaropicrin$^+$, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$
5	*Centaurea colchica* (Sosn.) Sosn. syn. *Psephellus colchicus* Sosn., *P. dealbatus* var. *humilior* Alb.	19/83	Keil (FRG)	310	0.38	15-deoxyrepin, linichlorin B, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$
6	*Centaurea dealbata* Willd. syn. *Psephellus dealbatus* (Willd.) Boiss., *P. calocephalus* Cass.	29/74	Pallanza (Italy)	520	0.48	15-deoxyrepin, linichlorin B, cynaropicrin$^+$, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin
7	*Centaurea exsurgens* Sosn. syn. *Psephellus daghestanicus* Sosn.	523/78	Samöens (France)	320	0.37	15-deoxyrepin, linichlorin B, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$
8	*Centaurea karabaghensis* (Sosn.) Sosn. syn. *Psephellus karabaghensis* Sosn.	214/81	Erewań (USSR)	490	0.45	15-deoxyrepin, linichlorin B, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$
9	*Centaurea somchetica* (Sosn.) Sosn. syn. *Psephellus somcheticus* Sosn., *P. dealbatus* var. *simplicicaulis* Bordz.	429/78	Vácrátót (Hungary)	530	0.40	15-deoxyrepin, linichlorin B, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$
10	*Centaurea taochia* (Sosn.) Sosn. *syn. Psephellus taochius* Sosn.	266 77	Vacratot (Hungary)	420	0.52	15-deoxyrepin, linichlorin B, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$
11	*Centaurea zangezuri* (Sosn.) Sosn. syn. *Psephellus zangezuri* Sosn.	32/77	Bruksela (Belgium)	350	0.35	15-deoxyrepin, linichlorin B, cynaropicrin$^+$, centaurepensin$^+$, repin$^+$, acroptilin$^+$, janerin$^+$

Legend: + — dominating sesquiterpene lactones.

In the species from sections *Leucophyllae* (Sosn.) Sosn. and *Hypoleucae* (Sosn.) Sosn., spots of varying intensity, turning dark blue after being sprayed with concentrated sulfuric acid (identified with linichlorin B and

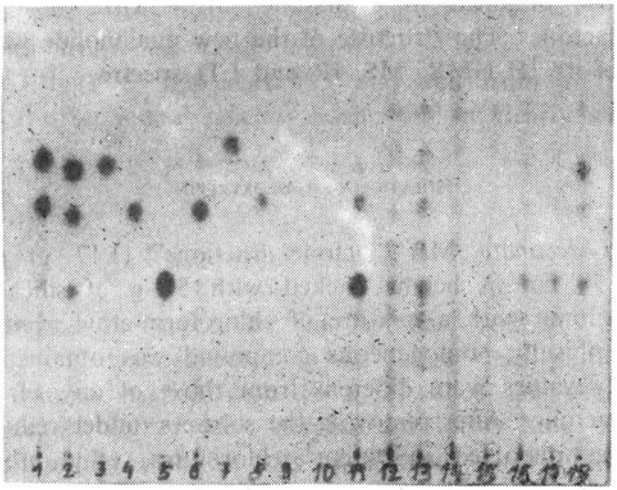

Fig. 1. A chromatographic check of the lactone fractions from the species of the subgenus *Psephellus* (Cass.) Schmalh. in comparison with isolated lactones: 1 — "lactone fractions" of *Centaurea declinata* MB., 2 — "lactone fraction" from *C. leucophylla* MB. 3 — 15-deoxyrepin, 4 — linichlorin B, 5 — cynaropicrin, 6 — "lactone fraction" from *C. hypoleuca* DC., 7 — centaurepensin, 8 — repin, 9 — acroptilin, 10 — janerin, 11 — "lactone fraction" from *C. dealbata* Willd., 12 — "lactone fraction" from *C. carthalinica* (Sosn.) Sosn., 13 — "lactone fraction" from *C. zangezuri* (Sosn.) Sosn., 14 — "lactone fraction" from *C. karabaghensis* (Sosn.) Sosn., 15 — "lactone fraction" from *C. somchetica* (Sosn.) Sosn., 16 — "lactone fraction" from *C. exsurgens* Sosn., 17 — "lactone fraction" from *C. colchica* (Sosn.) Sosn., 18 — "lactone fraction" from *C. taochia* (Sosn.) Sosn.

cynaropicrin) were seen, while in the species from section *Psephellus* Sosn., substances staining brown (similarly as repin, acroptilin and janerin) dominated.

ISOLATION OF SESQUITERPENE LACTONES

Chromatographically homogeneous compounds were isolated by separating the "lactone fractions" by column chromatography on Machery Nagel silica gel (MN-Kieselgel 100–200 mesh) suspended in chloroform. The column was eluted with a mixture of chloroform-ethyl acetate 5:1 (v/v), then with chloroform mixed with increasing amounts of ethyl acetate. Some of the fractions were rechromatographed on fine-particle silica gel from Serva (Kieselgel 200–300 mesh) using ethyl ether or a mixture of chloroform-acetone 3:1 (v/v) as eluents.

IDENTIFICATION OF THE ISOLATED COMPOUNDS

The isolated sesquiterpene lactones were identified using chromatographic methods and by comparison of their IR, ^{1}H-NMR spectra and melting points (of those obtained in crystalline form) with those of standard sesquiterpene lactones. The structure of the new guaianolide was determined on the basis of its ^{1}H-NMR, MS, IR and CD spectra.

ISOLATION OF 15-DEOXYREPIN

Centaurea declinata MB "lactone fractions" (1.17 g) were applied to a column 70 cm in height packed with 50 g of silica gel. During washing the column with a mixture of chloroform-ethyl acetate 5:1 (v/v), a chromatographically homogeneous compound was obtained in the first fraction. Its R_f values were different from those of any of the standard sesquiterpene lactones. After removing the solvents (under reduced pressure), the residue was dissolved in 1 cm^3 chloroform, ethyl ether was then added until the solution became cloudy (approx. 2 cm^3). After cooling, a substance precipitated, was filtered off and rinsed with chloroform. Seventy-two mg of crystals were obtained, whose melting point was 140°–141°C.

MS (m/z): 346 (M), 244 (M-102), 236, 226 (M-102-18), 85 ($C_3H_5OCO^+$), 57 ($C_3H_5O^+$);

IR (cm^{-1}): 3475 (OH), 1750 (γ-lactone), 1734 (ester), 1655 (double bond).

CD: 258 nm, $\Delta\varepsilon$ — 0.4; 226 nm, $\Delta\varepsilon$ — 0.2; 205 nm, $\Delta\varepsilon$ +18.6 (last reading).

This is a new guaianolide called 15-deoxyrepin (I). The determination of its structure is described in a separate publication (Budesinsky et al. 1986).

15-Deoxyrepin was isolated in a similar way from all of the remaining studied species from subg. *Psephellus* (Cass.) Schmalh. in the following amounts: *Centaurea leucophylla* MB. — 67 mg, *C. hypoleuca* DC. — 4 mg, *C. dealbata* Willd. — 9 mg, *C. zangezuri* (Sosn.) Sosn. — 5 mg., *C. carthalinica* (Sosn.) Sosn. — 5 mg, *C. karabaghensis* (Sosn.) Sosn. — 6 mg, *C. somchetica* (Sosn.) Sosn. — 7 mg, *C. colchica* (Sosn.) Sosn. — 5 mg, *C. exsurgens* Sosn. — 4 mg, *C. taochia* (Sosn.) Sosn. — 19 mg.

ISOLATION OF LINICHLORIN B (II)

The third fraction of the chromatographic separation of the "lactone fractions" from the herb *Centaurea declinata* MB. contained a compound which, chromatographically, identified with linichlorin B. The residue obtained

after removing the solvents by distillation, was subjected to crystallization in an identical manner as in the case of 15-deoxyrepin. Crystalline needles, 50 mg, with a melting point of 144–145°C were identified as linichlorin B (II).

This guaianolide was isolated in a similar way from the remaining studied species from the subg. *Psephellus* (Cass.) Schmalh.: *Centaurea leucophylla* (53 mg), *C. hypoleuca* (45 mg) and from all of the studied species from the section *Psephellus* Sosn., but in clearly smaller amounts (5–12 mg).

ISOLATION OF CYNAROPICRIN (III)

The sixth fraction (separation of lactones from *C. declinata* MB.), where cynaropicrin was expected to be found, was purified with ethyl ether on a column packed with 18 g of silica gel. After evaporation of the solvents, 21 mg of an amorphous, colorless and chromatographically pure compound with an oily consistency were obtained from the second subfraction. It was identified as cynaropicrin (III).

This quaianolide was obtained in a similar manner from: *Centaurea leucophylla* (23 mg), *C. hypoleuca* (12 mg), *C. dealbata* (89 mg), *C. carthalinica* (45 mg) and *C. zangezuri* (50 mg).

ISOLATION OF CENTAUREPENSIN (IV) AND REPIN (V)

The chloroform extract ("lactone fractions") from *Centaurea dealbata* Willd. (2.49 g) was applied to a column 80 cm in length packed with 70 g of silica gel. By eluting this column with a mixture of chloroform-ethyl acetate 5:1 (v/v), two compounds were obtained in the second fraction. The characteristic green and brown spots on the control chromatograms suggested the presence of centaurepensin and repin. This fraction was concentrated to a small volume and, after cooling, a precipitate formed which was then rinsed with chloroform and recrystallized from an ethyl acetate-chloroform mixture. Thirty-seven mg of crystals with a melting point of 218–220°C, identified as centaurepensin (IV) were obtained. This lactone was isolated in a similar way from all of the studied species from the section *Psephellus* Sosn.: *Centaurea carthalinica* (34 mg), *C. zangezuri* (39 mg), *C. karabaghensis* (28 mg), *C. somchetica* (30 mg), *C. colchica* (25 mg), *C. exsurgens* (29 mg) and *C. taochia* (43 mg).

After separating centaurepensin, the filtrate was condensed, dissolved in chloroform and ether added. After cooling, a crystalline substance was obtained which was filtered off, washed with ethyl ether and recrystal-

lized from a mixture of chloroform-ethyl ether. A chromatographically homogeneous substance (167 mg) in the form of white flakes with a melting point of 154–156°C was obtained. It was identified as repin (V). This guaianolide was obtained in a similar manner from all of the studied species from the section *Psephellus* Sosn.: *Centaurea carthalinica* (76 mg), *C. zangezuri* (59 mg), *C. karabaghensis* (95 mg), *C. somchetica* (56 mg), *C. colchica* (45 mg), *C. exsurgens* (48 mg) and *C. taochia* (67 mg).

ISOLATION OF ACROPTILIN (VI)

The fifth fraction of the chromatographic separation of the "lactone fractions" obtained from *Centaurea dealbata* Willd. (eluted with a mixture of chloroform-ethyl acetate 3:1) gave a spot on the control chromatograms with an identical R_f value and color as the standard acroptilin. After concentrating the eluate under reduced pressure, a crystalline substance precipitated. It was recrystallized from ethyl acetate and chloroform, obtaining in this way, 72 mg of a compound with a melting point of 195–197°C and identified as acroptilin (VI). This guaianolide was obtained in the same way from all of the studied species from the section *Psephellus* Sosn.: *Centaurea carthalinica* (45 mg), *C. zangezuri* (50 mg), *C. karabaghensis* (76 mg), *C. somchetica* (53 mg), *C. colchica* (39 mg), *C. exsurgens* (48 mg) and *C. taochia* (69 mg).

ISOLATION OF JANERIN (VII)

The tenth fraction obtained during the chromatographic separation of the "lactone fractions" from *Centaurea dealbata* Willd. (chloroform-ethyl acetate 1:1), in which control chromatography suggested the presence of janerin, was purified with a chloroform-acetone 3:1 (v/v) mixture, using 18 g of silica gel. From the second subfraction, 30 mg of an amorphous, chromatographically homogeneous compound, identified as janerin (VII), were obtained. In a similar way, this compound was obtained from: *Centaurea carthalinica* (26 mg), *C. zangezuri* (37 mg), *C. karabaghensis* (29 mg), *C. somchetica* (32 mg), *C. colchica* (25 mg) *C. exsurgens* (26 mg) and *C. taochia* (30 mg).

DISCUSSION

The presence of sesquiterpene lactones was found in all of the studied species belonging to the subg. *Psephellus* (Cass.) Schmalh. Chromatographic checks revealed that in the plants assigned in Flora USSR (1961) to

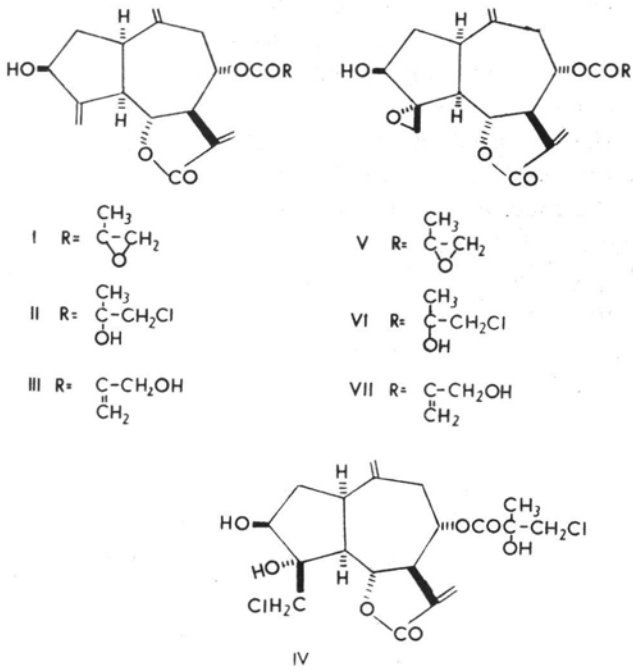

Fig. 2. Sesquiterpene lactones isolated from species of the subgenus *Psephellus* (Cass.) Schmalh. I — 15-deoxyrepin, II — linichlorin B, III — cynaropicrin, IV — centaurepensin, V — repin, VI — acroptilin, VII — janerin

different sections, different sesquiterpene lactones dominated (Fig. 1). Isolation of these compounds entirely supported the results of the preliminary chromatographic tests. It was possible to isolate and identify the sesquiterpene lactones occurring in the studied plants (Table 1). Mainly, they were the same guaianolides found in other species of the genus *Centaurea* L. Centaurepensin (IV), repin (V), acroptilin (VI) or janerin (VII) have been shown to occur in: *Centaurea hyrcania* Bornm. (Evstratova et al. 1969), *C. linifolia* L., *C. hyssopifolia* Vahl., *C. nigra*, *C. janeri* Graells (Gonzales et al. 1977), *Acroptilon repens* (L.) DC. (Evstratova et al. 1967, Rustaiyan et al. 1981), *C. bella* Trautv. (Geppert et al. 1983, Nowak et al. 1986a), *C. solstitialis* L. (Merrill and Stevens 1985), *Chartolepis biebersteinii* Jaub. et Spach., *Ch. glastifolia* (L.) Cass., *Ch. pterocaula* (Trautv.) Czer. (Nowak et al. 1986b). Linichlorin B (II) has been found in *Centaurea linifolia* L. (Gonzales et al. 1977) and *C. kotschyi* Boiss. (Oksuz and Putun 1983). *Cynaropicrin* (III) occurs in many species from the subtribe *Centaureinae* (Nowak et al. 1986c).

A new compound, 15-deoxyrepin (I), was isolated from all of the studied species from the subg. *Psephellus* (Cass.) Schmalh.

It was found that in *Centaurea declinata* MB. and *C. leucophylla* MB.

from the section *Leucophyllae* (Sosn.) Sosn., the following sesquiterpene lactones occurred: 15-deoxyrepin (I), linichlorin B (II) and cynaropicrin (III). In *C. hypoleuca* DC., the only tested representative of section *Hypoleuca* (Sosn.) Sosn., linichlorin B (II) dominated, while 15-deoxyrepin (I) and cynaropicrin (III) occurred in much smaller amounts. Whereas, in all of the studied species from the section *Psephellus* Sosn., a greater amount of sesquiterpene lactones occurred. The following compounds were dominated: repin (V), acroptilin (VI), jenerin (VII) and centaurepensin (IV) — *Centaurea dealbata* Willd., *C. carthalinica* (Sosn.) Sosn., *C. zangezuri* (Sosn.) Sosn., *C. karabaghensis* (Sosn.) Sosn., *C. somchetica* (Sosn.) Sosn., *C. colchica* (Sosn.) Sosn. *C. exsurgens* Sosn., *C. taochia* (Sosn.) Sosn., and, in some species also cynaropicrin (III) — *C. dealbata* Willd., *C. carthalinica* (Sosn.) Sosn. and *C. zangezuri* (Sosn.) Sosn.

Acknowledgment

This study was financed through the interdepartmental problem (PMR-I.12.1.5.3.) and CPBP 01.13.2.20.

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Laktony sesquiterpenowe. XXXIII. Gwajanolidy w podrodzaju Psephellus (Cass.) Schmalh. rodzaju Centaurea L.

Streszczenie

Stwierdzono, że u wszystkich badanych gatunków subg. *Psephellus* (Cass.) Schmalh. występują laktony seskwiterpenowe. Zaobserwowano różny skład laktonów u przedstawicieli trzech sekcji. U *Centaurea declinata* MB. i *Centaurea leucophylla* MB. z sekcji *Leucophyllae* (Sosn.) Sosn. występowały: 15-dezoksyrepina, linichloryna B i cynaropikryna. U *Centaurea hypoleuca* DC. z sekcji *Hypoleucae* (Sosn.) dominowała linichlorina B, a u gatunków zaliczanych do sekcji *Psephellus* Sosn. dominowały: repina, akroptylina, janeryna, centaurepensyna, a u niektórych także cynaropikryna.