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Sesquiterpene lactones. XXXVI. Sesquiterpene lactones in several subgenera of the genus *Centaurea* L.

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Abstract

The occurrence of both known and new sesquiterpene lactones was determined in six species classified in different subgenera of *Centaurea* L. Chlorojanerin, cynaropicrin and janerin were isolated from *C. phaeopappoides* Bordz. and *C. thracica* (Janka) Hayek. *C. marschalliana* Spreng. was found to contain acroptilin, chlorojanerin, cebellin D and janerin while *C. adjarica* Alb. had repin, acroptilin, chlorojanerin, centaurepensin, janerin, repidiolide, cebellin D, E, F and I. A new, hitherto underscribed cebellin J quaianolide was found in *C. bella* Trautv. and another germacranolide, 3α, 15-dihydroxycostunolide was found in *C. sphaerocephala* subsp. *lusitanica* (Boiss. et Reuter) Nyman.

Key words: sesquiterpene lactones, Centaurea, Compositae

INTRODUCTION

This study is a continuation of the search for sesquiterpene lactones in species of the subtribe *Centaureinae*. The results of the work completed to date indicate that this group of compounds has various compositions in individual genera, subgenera and sections, which can be made use of in their proper botanical classification (Buděšínský et al. 1984, Nowak et al. 1984, 1986a, 1986b, 1986c, 1986d, 1988).

MATERIAL AND METHODS

The dried and pulverized green parts of the following species were used in the study: *Centaurea bella* Trautv., *C. adjarica* Alb., *C. marschalliana* Spreng., *C. phaeopappoides* Bordz., *C. sphaerocephala* subsp. *lustanica* (Boiss. et Reuter) Nyman and *C. thracica* (Janka) Hayek. The plants were grown in the Garden of the Chair of Medicinal Plants of the Poznań Medical Academy (Poland) (Table 1). The species were identified on the basis of descriptions in Flora Europaea (1976) and Flora USSR (1961).

Table 1
Data on the studied species from the genus *Centaurea* L.

Species	Subgenus	Source of seeds	Amount of dry material (g)	Isolated sesquiterpene lactones
Centaurea adjarica Alb. syn. *C. koenigii* Sosn.	*Hyalinella* (Tzvel.) Tzvel.	Vienna (Austria)	135	repin, acroptilin, janerin centaurepensin, repdiolide, chlorojanerin cebellin D*, E, F, I
Centaurea bella. Trautv.	,,	Giessen (FRG)	1700	cebellin J
Centaurea marschalliana Spreng.	*Heterolophus* (Cass.) Dobrocz.	Lvov (USSR)	270	chlorojanerin, cebellin D*, acroptilin, janerin
Centaurea phaeopappoides Bordz.	*Odontolophopsis* Tzvel.	Erevan (USSR)	110	chorojanerin, cynaropicrin, janerin
Centaurea thracica (Janka) Hayek	*Microlophus* (Cass.) Dostal	Burgas (Bulgaria)	510	chlorojanerin, cynaropicrin, janerin
Centaurea sphaerocephala subsp. *lusitanica* (Boiss. et Reuter) Nyman	*Seridia* (Juss.) Czerp.	Coimbra (Portugal)	649	3α,15-dihydroxycostunolide

* A guaianolide obtained for the first time from *Centaurea imperialis* (Rustaiyan et al. 1984).

The "lactone fractions" according to Drożdż and Piotrowski (1973) were isolated from most of the above-mentioned plants. Only the guaianolide from *Centaurea bella* was obtained in a different way. The herb was subjected to extraction with methanol at room temperature. After the solvent was distilled off under reduced pressure the sediment was dissolved in H_2O. The aqueous extract was successively extracted with chloroform, ethyl acetate and a mixture of ethyl acetate and isobutanol. The ethyl acetate extract was used in the further studies.

ISOLATION AND IDENTIFICATION OF THE OBTAINED SESQUITERPENE LACTONES

The mixture of compounds was separated by column chromatography on silica gel (Kieselgel MN 100-200 mesh for the primary runs and then on Kieselgel 200-300 mesh, Serva). The separation was controlled by TLC on silica gel with sulfuric acid as the developer. The sesquiterpene lactones so obtained were identified by chromatographic methods and on the basis of spectral analyses (^{1}H-NMR, MS, CD, IR), with previously isolated lactones from the subtribe *Centaureinae* as standars. When a known sesquiterpene lactone was obtained in crystalline form, its melting point was checked in a mixture with the appropriate standard.

THE GUAIANOLIDES OF *CENTAUREA PHAEOPAPPOIDES*

The "lactone fraction" (0.65 g) was applied to a column packed with 40 g of silica gel. The mixture of the three compounds present in the fraction was separated with chloroform containing 10% acetone. Six fractions were obtained, of which two gave single spots and one required further purification. The remaining fractions, chromatographically unhomogeneous, were not analysed further due to their very small amounts.

Chlorojanerin (I): the green color on control chromatograms of the dominating compound in fraction two (0.22 g) and its R_f value suggested the presence of chlorojanerin. It was obtained in pure form after rechromatography on 15 g of silica gel in a system of hexane: chloroform: ethyl acetate (1:1:1) (V/V/V). The subfractions containing a chromatographically homogeneous compound were crystallized in a mixture of chloroform-ethyl acetate. A compound in needle form (6.8 mg) with a m.p. of 176-179°C was obtained. On the basis of chromatographic and spectral analyses (^{1}H-NMR, IR) it was concluded that this was chlorojanerin (I) (Fig. 1).

Cynaropicrin (II): fraction four contained a chromatographically homogeneous compound which stained blue on the control chromatograms. After the solvents were distilled off, 25 mg of an oily substance remained. Identification on the basis of chromatography and spectral analysis (^{1}H-NMR and IR) showed it to be cynaropicrin (II) (Fig. 1).

Janerin (III): a compound staining brown on control chromatograms was contained in the chromatographically homogeneous fraction seven. It had an identical R_f value and color as the standard, which indicated that this was janerin. After the solvent was distilled off, 20 mg of an amorphous substance were obtained, which on the basis of chromatographic and spectral analyses was identified as janerin (III) (Fig. 1).

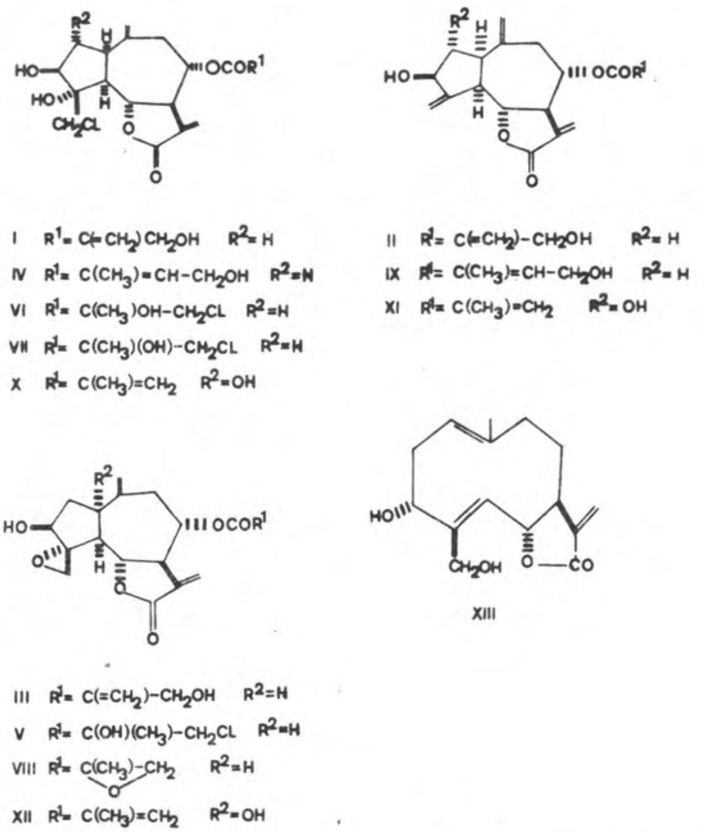

Fig. 1. I – chlorojanerin, II – cynaropicrin, III – janerin, IV – cebellin D, V – acroptilin, VI – cebellin J, VII – centaurepensin, VIII – repin, IX – cebellin E, X – cebellin F, XI – repdiolide, XII – cebellin I, XIII – 3α,15-dihydroxycostunolide

THE GUAIANOLIDES OF *CENTAUREA THRACICA*

The "lactone fraction" (1 g) was separated on a column of silica gel in a chloroform-acetone solvent system. Six fractions were obtained which were shown to contain identical guaianolides as *C. phaeopappoides*. They were obtained and identified in the manner described above in the following amounts: 4.5 mg chlorojanerin (I), 210 mg cynaropicrin (II) and 22 mg janerin (III).

THE GUAIANOLIDES OF *CENTAUREA MARSCHALLIANA*

The "lactone fraction" (1.5 g) was separated on 80 g of silica gel using chloroform with 30% ethyl acetate. Ten fractions were obtained of which the majority contained two spots and required further purification.

Chlorojanerin (I) and **cebellin D (IV)**: two compounds in fraction three (72 mg) were separated on small-particle silica gel in a mixture of hexane-chloroform-ethyl acetate (1:1:1:1) (V/V/V/V). Two subfractions were obtained containing chromatographically homogeneous substances. The first subfraction crystallized from a mixture of chloroform-ethyl ether. A white substance in needle form (8.3 mg) with a m.p. of 176-179°C and shown to be chromatographically and spectrally identical with chlorojanerin (I) was obtained. In the third subfraction, 12.6 mg of an amorphous substance was present. It was chromatographically identified as cebellin D (IV). The spectral data (IR, ^{1}H-NMR) supported this conclusion.

Acroptilin (V): the fifth fraction from the column chromatography exhibited a tendency to crystallize from the solvent system in the column. After the crystals were filtered off and washed with chloroform it was found that they were chromatographically homogeneous. Co-chromatography showed that this may be acroptilin. These data were confirmed by spectral analysis (IR, ^{1}H-NMR). Twelve mg of crystals with a m.p. of 195-197°C were identified as acroptilin (V) (Fig. 1).

Janerin (III): fraction nine was chromatographically pure and contained a compound which chromatographically identified with janerin. After the solvents were distilled off, 65 mg of an amorphous substance were left, whose spectral data confirmed the structure of janerin (III) (Fig. 1).

A GUAIANOLIDE FROM *CENTAUREA BELLA*

Cebellin J (VI): the ethyl acetate extract (2.5 g) was separated on silica gel in a mixture of ethyl acetate with 17% chloroform. Thirteen fractions were obtained. In one of them a compound dominated which was further purified on small paticle silica gel in a mixture of hexane-chloroform-ethyl acetate (1:1:5) (V/V/V). An amorphous substance (2.5 g) was obtained. Its empirical formula was determined to be $C_{19}H_{25}ClO_8$, and was named cebellin J (VI). Its structure was determined on the basis of spectral analyses: ^{1}H-NMR (200 MHz; $CDCl_3$): H(1): 3.46 bq (9.0; 9.0; 9.0); H(2): 2.51 ddd (6.5; 10.5; 14.8); H(2'): 1.62 ddd (3.0; 8.5; 14.8); H(3): 4.16 bd H(5): 2.31 bt (10.0; 10.0); H(6): 4.65 dd (9.3; 10.4); H(7): 3.15 tt (3.2; 3.2; 9.3; 9.3); H(8): 5.19 ddd (3.4; 5.2; 9.8); H(9): 2.69 bdd (5.0; 14.8); H(9): 2.40 bdd (3.2; 14.8); H(13): 5.62 d (3.0); H(13) 6.24 d (3.4); H(14): 5.06 bd (1.6); H(14): 5.17 bd (1.6); H(15): 3.60 d (11.1); H(15): 3.82 d (11.1); H(17): 1.13 d (6.1), IR (cm^{-1}): 3435 (hydroxyl). 1765 (γ-lactone), 1745, 1700 (ester), 1657 (double bond). Mass (m/z): 385 ($M-CH_2OH$), 349 ($M-CH_2OH-HCl$), 332 ($M-CH_2OH-HCl-OH$), 278 (M-ester), 260 ($M-ester-H_2O$), 247 ($M-CH_2OH$-ester), 229 ($M-CH_2OH$-ester-H_2O), CD spectrum (nm, $\Delta\varepsilon$): 237, -0.5: 208, $+6.3$.

THE GUAIANOLIDES OF *CENTAUREA ADJARICA*

The "lactone fraction" (0.98 g) was applied to a column of silica gel (60 g) which was eluted with a mixture of chloroform with 12% ethyl acetate. The fractionation was controlled by TLC using a hexane-chloroform-benzene-ethyl acetate system (1:1:0.5:3). The eluates were collected in 15 fractions on the basis of their chromatographic patterns.

Centaurepsin (VII): fraction two crystallized from the eluant. Crystals (13.2 mg) with a m.p. of 218-220°C were obtained and identified on the basis of comparative chromatography with a standard, IR spectra and determination of the m.p. in a mixture with a standard.

Repin (VIII): the solvents were distilled off from fraction three, the sediment was dissolved in chloroform and after ethyl acetate was added, crystals with a m.p. of 154-156°C were obtained and identified by means of co-chromatography, IR spectra and determination of m.p. in a mixture with a standard.

Chlorojanerin (I): fraction four (150 mg) was purified by TLC (hexane-chloroform-benzene-ethyl acetate 1:1:0.5:3). A crystalline substance (3.1 mg) with a m.p. of 181-183°C was obtained. This guaianolide was identified similarly as the two previous compounds from *C. adjarica.*

Cebellin D (IV): fraction five (250 mg) was rechromatographed on 15 g of silica gel using a hexane-chloroform-ethyl acetate (1:1:2) (V/V/V) system. From the first subfraction, 2.9 mg of an amorphous substance were obtained and identified on the basis of cochromatography and IR spectra.

Cebellin E (IX): as the result of rechromatography of fraction five, 4.2 mg of an amorphous substance were obtained and identified on the basis of TLC and spectral analyses (IR, ^{1}H-NMR).

Janerin (III): fraction six contained a chromatographically homogeneous substance (50.3 mg) which was identified by TLC and IR.

Cebellin F (X): fraction eight contained 3.9 mg of an amorphous substance which was identified by TLC and spectral analyses.

Repdiolide (XI): was obtained from fraction ten. By preparative chromatography, 10.2 mg were obtained and identified by TLC and spectral analyses.

Cebellin I (XII): the solvents were distilled off from fraction thirteen, next chloroform and then ethyl ether were added to the sediment. Crystals (49.5 mg) with a m.p. of 137-139°C were obtained. This compound was identified on the basis of comparison of IR spectra with a standard, cochromatography and determination of m.p. in a mixture with a standard.

A GERMACRANOLIDE FROM *CENTAUREA SPHAEROCEPHALA* SUBSP. *LUSITANICA*

The "lactone fraction" (1.3 g) was separated on a column of silica gel (80 g) eluated with chloroform containing 16% acetone. As a result, in one fraction a chromatographically homogeneous compound which crystallized from the eluating mixture, was obtained. After the crystals were filtered off and washed with chloroform. 46.6 mg of the compound (XIII) (Fig. 1) with an empirical formula of $C_{15}H_{20}O_4$ and a m.p. of 180°C were obtained. After its structure was determined, it was called 3α,15-dihydroxycostunolide. ^{1}H-NMR (200 MHz; $CDCl_3+CD_3OD$): H(1): 5.14 m (9.4; 7.2; 1.4/3x): H(3): 4.16 dd (10.7; 2.0); H(5): 4.69 dm (10.0; 0.8/3x): H(6): 4.88 dd (10.1; 8.8); H(7): 2.65 m (9.8; 10.7; 3.5; 3.3; 1.6); H(8): 1.88 dt (10.7; 14.6); H(13): 6.28 d (3.5); H(13'): 5.64 d (3.3); H(14): 1.39 d (1.4); H(15): 4.25 bd (13.8; 1.2); H(15'): 4.02 bd (13.8; 0.5). IR (cm^{-1}): 3300 (hydroxyl), 1754 (γ-lactone), 1660 (double bond). Mass spectrum (m/z): 264 (M), 246 (M-18). CD spectrum (nmΔε): 259, −1.8: 218, +31.1.

A SUMMARY OF THE RESULTS

The conducted studies have shown that the same guaianolides occur in the examined species of the subgenus *Microlophus* (Cass.) Dostal (*C. thracica* (Janka) Hayek), *Odontolophopsis* Tzvel. (*C. phaeopappoides* Bordz.): cynaropicrin (II), janerin (III) and chlorojanerin (I). Only quantitative differences were noted. There was significantly more cynaropicrin in *C. thracica* than in *C. phaeopappoides*. Similar sesquiterpene lactones were found in *C. marschalliana* from the subg. *Heterolophus* (Cass.) Dobrocz. The presence of chlorojanerin (I) and janerin (III) was found here, while cynaropicrin was not. In its place were acroptilin (V) and cebellin D (IV).

The occurrence of guaianolides in the subgenus *Hyalinella* (Tzvel.) Tzvel. was confirmed. The following were obtained from *C. adjarica* Alb: centaurepsin (VII), repine (VIII), chlorojanerin (I), cebellin D (IV), cebellin F (X), janerin (XIII), cebellin E (IX), repdiolide (XI) and cebellin I (XII). Another new guaianolide, cebellin J (VI), was isolated and identified from *C. bella* Trautv.

It was demonstrated that alongside cnicin (Geppert et al. 1983), the germacranolide 3α,15-dihydroxycostunolide (XIII) is present in *C. sphaerocephala* subsp. *lusitanica* (Boiss. et Reuter) Nyman.

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Laktony seskwiterpenowe. XXXVI. Laktony seskwiterpenowe w kilku podrodzajach rodzaju Centauera L.

Streszczenie

Stwierdzono występowanie znanych i nowych laktonów seskwiterpenowych w sześciu gatunkach zaliczanych do różnych podrodzajów *Centaurea* L. Z *C. phaeopappoides* Bordz. i *C. thracica* (Janka) Hayek wyizolowano chlorojanerynę, cynaropikrynę i janerynę, w *C. marschalliana* Spreng. znaleziono akroptylinę, chlorojanerynę, cebellinę D i janerynę, a w *C. adjarica* Alb. – repinę, akroptylinę, chlorojanerynę, centauropensynę, janerynę, raptdiolid, cebelliny D, E, F i I. W *C. bella* Trautv. znaleziono inny, nie opisany w literaturze gwajanolid – cebellinę J, a w *C. spherocephala* subsp. *lusitanica* (Boiss. et Reuter) Nyman kolejny germakranolid – 3α,15-dihydroksykostunolid.