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Isoprenoids from Rhaponticum carthamoides (Willd.) Iljin and Rhaponticum serratuloides (Georgi.) Bobr

Abstract. The article presents the results of a chemical study of Rhaponticum carthamoides (Willd.) Iljin. and Rhaponticum serratuloides (Georgi.) Bobr., which are of interest as sources of new adaptogenic, anabolic, and antiparasitic drugs; a method for complex chemical processing of the raw materials of the above-mentioned plants has been proposed. The structure of new sesquiterpene lactones raposerine, raserolide, 15-deacetylraposerine, and raserine has been isolated and established. The content of pharmacologically active ecdysterone in various organs of the studied Rhaponticum species is discussed according to the phenological phases of plant development. The antiparasitic, antiviral activity and cytotoxicity of the isolated compounds were determined.
Key words: Rhaponticum carthamoides (Willd.) Iljin., Rhaponticum serratuloides (Georgi.) Bobr., isoprenoids, sesquiterpene lactones, ecdysterone.

Introduction

Plants of the genus *Rhaponticum* Adans. (rhaponticum, leuzea) are promising sources of ecdysterone (20-hydroxyecdysone, 1) – a polyhydroxylated steroid that exhibits anabolic, adaptogenic, antiulcerogenic and other types of pharmacological activity and is the basis of the drug "Ecdisten", recommended as an anabolic agent. Ecdysterone does not exhibit the androgenic effect characteristic of anabolic steroids of the testosterone series, which makes it promising for use in medical practice [1]. For the industrial production of ecdysterone, the roots of the *Rhaponticum carthamoides* (Willd.) Iljin. plant and inflorescences *of Rhaponticum integrifolium* Winkl. are recommended as raw materials [2,3].

Previously, research on the chemistry and technology of ecdysteroids had not been carried out in Kazakhstan; considering the need to develop original domestic adaptogenic and anabolic drugs, research on plants from Central Kazakhstan that are promising as raw materials for the production of ecdysterone has been started.

As objects of study, *Rhaponticum serratuloides* (Georgi.) Bobr, common in the steppe zone of Kazakhstan, *Rhaponticum carthamoides* (Willd.) Iljin, introduced into cultivation at the collection site of the botanical garden of JSC International Research and Production Holding "Phytochemistry" were selected.

Materials and methods

Research methods were performed as follows:

Melting points were determined using a Boetius apparatus.

IR spectra were recorded on Thermo Nicolet Avatar-360ESP instrument in KBr tablets and chloroform, UV spectra were recorded on an Agilent instrument Cary 60, in ethanol and the stop-flow method on an Agilent 1260 microcolumn liquid chromatograph.

NMR spectra were recorded on a Jeol JNM-ECA500 spectrometer (operating frequency – 500.13 MHz for ^{1}H, 125.76 MHz for ^{13}C). Py-d_5 and a mixture of Py-d_5 – $CDCl_3$ ***1um serratulo***: 1 were used as solvents, tetramethylsilane (δ scale) as an internal standard. For recording two-dimensional spectra ^{1}H-^{1}H and ^{1}H-^{13}C COZY and ^{1}H-^{13}C COLOC (9Hz) standard programs from the Jeol company were used.

High-resolution mass spectra were recorded on a Finnigan MAT 8200 instrument using electron impact ionization with an energy of 70 eV.

Optical rotation was measured using a Polax 2L polarimeter at a wavelength of 580 nm.

Column chromatography was carried out on large silica gel, large porous and neutral aluminum oxide (IV degree of activity according to Brockman). For flash chromatography, silica gel "Armsorbsil" 100/160 and "Silpearl" (Czech Republic) 40/100 were used. For TLC, "Silufol -254" plates (Czech Republic) were used. Detection was carried out in UV light or using developers *A* and *B*.

Developers for TLC: *A* – solution of vanillin in sulfuric acid followed by heating at 100-110°C for 2-3 minutes, *B* – 1% solution of potassium permanganate; to detect substances, the plate was placed in the solution for 0.5-1 min, then washed with water.

HPLC of sesquiterpene lactones. Chromatography was carried out on an Agilent 1260 microcolumn liquid chromatograph. The column was 150 mm long, internal diameter 4.6 mm. Sorbent ZORBAX Eclipse XDB-C8, particle size 5 microns. Column temperature was 30 °C. The eluent was prepared by mixing MeOH and 0.05 M aqueous H_3PO_4. Eluent systems containing 40 and 50% methanol volumes were used. Detection of substances was carried out with a UV detector, operating wavelength 200 nm. The eluent flow rate was 0.5 ml/min. To record UV spectra, the eluent flow was stopped at the top of the chromatographic peak. The sample concentrations were about 1.2 mg/ml in MeOH; 1.4 μl of solution was applied to the column.

Sesquiterpene lactones of Rhaponticum serratuloides

Leaves of the *plant Rhaponticum serratuloides* (Georgi.) Beaver. were collected during the flowering phase in the vicinity of the village of Aynabulak, Zhanaarkinskyi district, Ulytau region, air-dried and crushed.

The raw material weighing 5.5 kg was extracted four times with a mixture of ethanol and chloroform with a ratio of 1:4 (in volume ratios) 17 l each at the boiling point of the solvent for 1 hour. The extracts were combined, filtered and evaporated on a rotary evaporator under vacuum at a temperature not exceeding 45 °C. 1 liter of thick dark green mass has been obtained.

The resulting residue was dissolved in 1.5 L of hot (60 °C) ethanol and diluted with 3 L of hot water (60 °C). The precipitate of nonpolar components (chlorophylls, lipids) that formed after cooling the solution was filtered off, the precipitate was similarly treated twice, and 1 and 0.5 L of ethanol were used. No sesquiterpene lactones were detected in the sediment by TLC method.

The combined filtrates were extracted with benzene six times, 2 L each. The combined benzene extracts were evaporated to dryness on a rotary evaporator under vacuum. The residue, 95 g, after distilling off the solvent was chromatographed on a silica gel column (2 kg).

Benzene – ethyl acetate systems were used as eluents, component ratio: 1) 40:1, 2) 25:1, 3) 20:1, 4) 10:1, 5) 7:1, 6) 5:1, 7) 4:1, 8) 3:1, 9) 2:1, 10) 1:1 and then benzene – acetone 11) 3:1, 12) 2:1, 13) 1:1, 14) ethyl acetate, ethyl acetate – ethanol: 15) 20:1, 16) 15:1. A number of fractions containing sesquiterpene lactones and related components were obtained.

Next, fractions of identical composition were combined and chromatographed on a flash column at a mass ratio of the chromatographed fraction and adsorbent of 1:50. The first 9 fractions contain lipids, colored non-crystalline components that were not further identified, and β-sitosterol (identified by TLC with a known sample). Fractions 9-11 contain loliolide as the main component. The last fractions (42 and further, see table 33) contain green and brown oils, in which no terpenoid and steroid substances were detected (TLC, developer *A)*.

Systems for flash chromatography: benzene – ethyl acetate: 1) 5:1, 2) 4:1, and petroleum ether – ethyl acetate: 3) 10:7, 4) 5:4, 5) 1:1, 6) 5: 6, 7) 5:7, 8) ethyl acetate.

The process was monitored by TLC, developer *B*.

For TLC the following solvent systems were used: petroleum ether – acetone: 1) 7:4, double elution 2) 5:3, double elution; 3) 2:1 three times elution.

The list of fractions and solvent systems for column and flash column chromatography is given in Table 1.

The lactones acroptilin (8) and raserolide (10) could not be separated chromatographically: a crystalline mixture was obtained, which gave one spot on TLC. Separation was carried out by fractional crystallization from system 1. Acroptilin is less soluble in this system and precipitates in the form of large cubic crystals; raserolide precipitates on the walls of the vessel in the form of small needle-shaped crystals.

Table 1 – Chromatographic separation of *Rhaponticum serratuloides* sesquiterpene lactones

No. of factions	Solvent system for column chromatic graphy	Faction composition	Solvent system for flash chromatography	Individual components
1	2	3	4	5
12, 13 14-17	6 7	Centaurepensin, acroptilin (8), minor cynaropicrin (2) and raserolide (10)	1 2	centaurepensin crystalline mixture of acroptilin and raserolide
18-23	8	Acroptilin (8), Raserolide (10), Raposerine (9)	3.4 4.5	crystalline mixture of acroptilin and raserolide raposerine
24-28	8-10	Cynaropicrin (2), raposerine (9), minor acroptilin (8) and raserolide (10)	4.5	cynaropicrin, raposerine
29-32	10.11	15-Deacetylraposerine (11), minor raposerine (9)	5	raposerine, 15-Deacetylraposerine
33.34	12, 13	15-Deacetylraposerine (11)	6	15-Deacetylraposerine
35-39	14-16	15-Deacetylraposerine (11), 15-deacetyl-2α-hydroxyraserolide	6, 7.8	15-deacetylraposerine, 15-deacetyl-2α-hydroxyraserolide
40-41	16	15-deacetyl- 2α-hydroxyraserolide	7 8	15-deacetyl-2α-hydroxyraserolide

(2'S*)*-3β,4α-dihydroxy-8α-O-[2'-hydroxy-2'-methyl-3'-chloropropionyl]-15-chloro-1α,5α,6β,7α(H)-guai-10(14),11(13)-diene-6(12)-olide (centaurepensin) (7)

Compound **(7):** m.p. 221-223 °C (petroleum ether-acetone = 2:1)

$[\alpha]_{580}^{23}$ + 135.1° (*c* 0.73; tetrahydrofuran).

UV: λ_{max}: 196, 216 nm (shoulder)

TLC: system 1 R_f 0.39, system 2 R_f 0.46.

^{1}H NMR spectra (Py-d_5, 500 MHz, δ, ppm, *J,* Hz) **(7):** 3.97 (1H, d.d.d., J_1 =11, J_2 =8, J_3 =8, H-1), 1.80 (1H, d.d., J_1 =14.5, J_2 =8, H-2a), 2.79 (1H, d.d.d., J_1 =14.5, J_2 =11, J_3 =6, H-2b), 4.65 (1H, br.d., *J* = 6, <1.5, H-3), 2.63 (1H, d.d., J_1 =8, J_2 =11, H-5), 5.32 (1H, d.d., J_1 =11, J_2 =9, H-6), 3.19 (1H, d.d.d.d., J_1 =9, J_2 =6.5, J_3 =3,5, J_4 =3,0, H-7), 5.39 (1H, d.d.d., J_1 =6.5, J_2 =5, J_3 =1, H-8), 2.59 (1H, d., *J* =14.5, H-9a), 2.94 (1H, d.d., J_1 =14.5, J_2 =5, H-9b), 5.75 (1H, d., *J* =3.0, H-13a),6.18 (1H, d., *J* =3.5, H-13b), 5.07 (1H, br.d., *J* =1.5, H-14a), 5.12 (1H, d., *J* ~1.5, H-14b), 4.34 (1H, d., *J* =11.5, H-15a), 4.78 (1H, d., *J* =11.5, H-15b), 4.00 (1H, d., *J* =11.5, H-18a), 4.10 (1H, d., *J* =11.5, H-18b), 1.70 (1H, s., H-19).

^{13}C NMR spectra (Py-d_5, 125 MHz, δ, ppm) **(7):** 48.55 d. (C-1), 40.29 t. (C-2), 76.32 d. (C-3), 85.32 s. (C-4), 59.39 d. (C-5), 77.46 d. (C-6), 46.50 d. (C-7), 75.42 d. (C-8), 35.06 t. (C-9), 144.57 s. (C-10), 138.87 s. (C-11), 169.03 s. (C-12), 121.05 t. (C-13), 117.06 t. (C-14), 51.23 t. (C-15), 173.45 s. (C-16), 75.38 s. (C-17), 52.19 t. (C-18), 24.29 q. (C-19).

The following cross-peaks are observed in the two-dimensional COLOC spectrum, the presence of which was used to assign the indicated signals of carbon atoms: C(10)/H(8), H(9a), H(9b); C(11)/H(6), H(7), H(8), H(13a); C(12)/H(13a), H(13 b).

Crystal cell parameters (E): a = 10.47(1), b = 9.25(1), c = 11.49(2); p = 113.1(1).

The yield (**7**) was 0.008% (hereinafter the yields are given based on air-dry raw materials).

(2'S*)*-3β-dihydroxy-8α-O-[2'-hydroxy-2'-methyl-3'-chloropropionyl]-15-chloro-1α,5α,6β,7α(H)-guai-10(14),11(13)-diene-6(12)-olide (acroptilin) (8)

Compound **(8)**: m.p. 195-198 °C (petroleum ether-acetone = 2:1)

$[\alpha]_{580}^{23}$ + 110.7 ° (*c* 0.62; methanol).

UV: λ_{max}: 196, 216 nm (shoulder).

TLC: system 1 R_f 0.26, system 2 R_f 0.34.

IR spectrum (KBr), ν, cm^{-1}: 3450 (OH), 2950, 1640 (C=CH_2), 1750 (C=O γ-lactone), 1275 (C-O, ester), 1180 (C-O).

^{1}H NMR spectrum (Py-d_5, 500 MHz, δ, ppm, *J,* Hz): 3.28 (d.d.d., H-1, 1H, *J* 11, 8, 8, H-1), 2.10 (d.d.d.d, 1H, *J* 14.5, 8, 1.5, H-2a), 2.36 (d.d.d., 1H, *J*

14.5, 11, 6, H-2b), 4.23 (br.d.d., 1H, *J* 6, ~1.5, H-3), 2.19 (d.d., 1H, *J* 8, 11, H-5), 4.94 (d.d., *J* 11, 11.9, H-6), 3.10 (d.d.d.d., 1H, *J* 9, 6.5, 3.5, 3, 11-7), 5.32 (d.d.d., 1H, *J* 6.5, 5, ~ 1.5, H-8), 2.47 (d.d., 1H, *J* 14.5, 1.5, H-9a), 2.85 (d.d., 1H, *J* 14.5, 5, H-9b), 5.75 (d., 1H, *J* 3, H-13a), 6.15 (d. 1H, *J* 3.5, H-13b), 5.08 (br.d., 1H, *J* 1.5, H-14a), 5.10 (br.d., 1H, J~l , H-14b), 3.20 (d., 1H, *J* 5, H-15a), 3.40 (d., 1H, *J* 5, H-15b), 3.95 (d., 1H, *J* 11.5, H-18a), 4.06 (d., 1H, *J* 11.5, H18b), 1.65 (s., 3H, H-19).

^{13}C NMR spectra (Py-d_5, 125 MHz, δ, ppm) **(8):** 46.05 d. (C-1), 38.90 t. (C-2), 75.26 d. (C-3), 69.05 s. (C-4), 53.22 d. (C-5), 77.25 d. (C-6), 47.58 d. (C-7), 75.16 d. (C-8), 36.08 t. (C-9), 142.80 s. (C-10), 138.53 s. (C-11), 168.98 s. (C-12), 121.26 t. (C-13), 117.98 t. (C-14), 48.89 t. (C-15), 173.33 s. (C-16), 75.27 s. (C-17), 52.02 t. (C-18), 24.19 q. (C-19).

Yield (8) was 0.004%

(2'***S***)-15-O-acetyl-3β,4α-dihydroxy-8α-O-[2'-hydroxy-2'-methyl-3'-chloropropionyl]-1α,5α,6β,7α(H)-guai-10(14),11(13)-diene-6(12)-olide (raposerine) (9)

Compound **(9)**: m.p. 191-193 °C (petroleum ether-acetone = 2:1)

$[\alpha]_{580}^{23}$ + 214 ° (*c* 0.35; methanol).

Found (%): C, 55.03; H, 6.21; Cl, 7.65. $C_{21}H_{27}O_9Cl$.

Calculated (%): C, 54.96; H, 5.91; Cl, 7.72.

UV: λ_{max}: 196, 216 nm (shoulder).

TLC: system 1 R_f 0.07, system 2 R_f 0.24.

IR spectrum (chloroform), ν, cm^{-1}: 3540 (OH), 3095, 1640 (C=CH$_2$) , 1765 (C=O γ-lactone), 1715 (C=O, ester), 1215, 1140, 1060 (C-O);

IR spectrum (KBr), ν, cm^{-1}: 1775 (C=O γ-lactone), 1737 (C=O, ester), 1715 (C=O, ester)

Mass spectrum, *m/z* (I_{rel} *(%)):* 429 (6), 427 (19), 260 (30), 247 (19), 243 (24), 242 (23), 229 (30) , 203 (23), 175 (26), 43 (100).

Found: *m/z:* 427.11711 [M-CH$_3$0]$^+$. $C_{20}H_{24}O_8Cl$.

Calculated: 427.11596.

^{1}H NMR spectra (Py-d_5, 500 MHz, δ, ppm, *J,* Hz) **(9):** 3.86 (1H, d.d.d., J_1 =11, J_2 =8, J_3 =8, H-1), 1.81 (1H, d.d., J_1 =14.5, J_2 =8, H-2a), 2.76 (1H, d.d.d., J_1 =14.5, J_2 =11, J_3 =6, H-2b), 4.57 (1H, br.d., *J* = 6, <1.5, H-3), 2.66 (1H, d.d., J_1 =8, J_2 =11, H-5), 5.24 (1H, d.d., J_1 =11, J_2 =9, H-6), 3.17 (1H, d.d.d.d., J_1 =9, J_2 =6.5, J_3 =3,5, J_4 =3,0, H-7), 5.35 (1H, d.d.d., J_1 =6.5, J_2 =5, J_3 =1.5, H-8), 2.54 (1H, d., *J* =14.5, H-9a), 2.92 (1H, d.d., J_1 =14.5, J_2 =5, H-9b), 5.74 (1H, d., *J* =3.0, H-13a),6.14 (1H, d., *J* =3.5, H-13b), 5.04 (1H, s., H-14a), 5.11 (1H, br.d., *J* ~1.5, H-14b), 5.04 (1H, s., H-15a), 5.04 (1H, s., H-15b), 3.97 (1H, d., *J* =11.5, H-18a), 4.07 (1H, d., *J* =11.5, H-18b), 1.67 (1H, s., H-19), 1.81(1H, s., H-21).

^{13}C NMR spectra (Py-d_5, 125 MHz, δ, ppm) **(9):** 47.60 d. (C-1), 40.10 t. (C-2), 77.01 d. (C-3), 84.26 s. (C-4), 58.32 d. (C-5), 77.46 d. (C-6), 46.76 d. (C-7), 75.47 d. (C-8), 35.50 t. (C-9), 144.48 s. (C-10), 139.06 s. (C-11), 169.20 s. (C-12), 121.00 t. (C-13), 117.02 t. (C-14), 67.50 t. (C-15), 173.44 s. (C-16), 75.37 s. (C-17), 52.17 t. (C-18), 24.28 q. (C-19), 171.13 s. (C-20), 20.85 q. (C-21).

The yield **(9)** was 0.03%.

15-O-acetyl-3β,4α-dihydroxy-8α-O-[methacryloyl]-1α,5α,6β,7α(H)-guai-10(14),11(13)-diene-6(12)-olide (raserolide) (10)

Compound **(10)**: m.p. 153-155 °C (petroleum ether-acetone = 2:1)

$[\alpha]_{580}^{23}$ + 126° (*c* 0.44; chloroform).

UV: λ_{max}: 198-208 nm (plateau)

TLC: system 1 R_f 0.24, system 2 R_f 0.30.

IR spectrum ($CHC1_3$): ν, cm^{-1}: 3500 (broad) (OH), 3095, 1640, 1060 (C=CH$_2$), 1765 (C=O γ-lactone), 1715 (C=O , ester), 1215, 1140 (C-O).

Mass spectrum, *M/Z* (I_{rel} *(%)):* 375 (19), 333 (2), 259 (15), 69 (100).

Found: *M/Z:* 375.14589, [M-CH$_3$0]$^+$. $C_{20}H_{23}O_7$. Calculated: 375.1443. Found: *M/Z:* 333.13655, [M-CH$_2$OAc]$^+$. $C_{18}H_{21}O_6$. Calculated: 333.13380.

^{1}H NMR spectra (Py-d_5, 500 MHz, δ, ppm, *J,* Hz) **(10):** 3.81 (1H, d.d.d., J_1 =11, J_2 =8, J_3 =8, H-1), 1.78 (1H, d.d., J_1 =14.5, J_2 =8, H-2a), 2.71 (1H, d.d.d., J_1 =14.5, J_2 =11, J_3 =6, H-2b), 4.53 (1H, br.d., *J* = 6, <1.5, H-3), 2.64 (1H, d.d., J_1 =8, J_2 =11, H-5), 5.20 (1H, d.d., J_1 =11, J_2 =9, H-6), 3.16 (1H, d.d.d.d., J_1 =9, J_2 =6.5, J_3 =3,5, J_4 =3,0, H-7), 5.23 (1H, d.d.d., J_1 =6.5, J_2 =5, J_3 =1.5, H-8), 2.46 (1H, d., *J* =14.5, H-9a), 2.87 (1H, d.d., J_1 =14.5, J_2 =5, H-9b), 5.46 (1H, d., *J* =3.0, H-13a),6.09 (1H, d., *J* =3.5, H-13b), 5.01 (1H, s., H-14a), 5.05 (1H, br.d., *J* ~1.0, H-14b), 5.01 (1H, s., H-15a), 5.01 (1H, s., H-15b), 5.52 (1H, br.d., *J* =1.5, H-18a), 6.16 (1H, br.d., *J* ~1.0, H-18b), 1.88 (1H, s., H-19), 1.78(1H, s., H-21).

^{13}C NMR spectra (Py-d_5, 125 MHz, δ, ppm) **(10):** 47.47 d. (C-1), 39.96 t. (C-2), 77.06 d. (C-3), 84.16 s. (C-4), 58.27 d. (C-5), 77.57 d. (C-6), 46.74 d. (C-7), 74.57 d. (C-8), 35.75 t. (C-9), 144.62 s. (C-10), 139.06 s. (C-11), 169.20 s. (C-12), 120.68 t. (C-13), 116.55 t. (C-14), 67.46 t. (C-15), 166.52 s. (C-16), 136.80 s. (C-17), 126.30 t. (C-18), 18.26 q. (C-19), 171.14 s. (C-20), 20.86 q. (C-21).

The yield **(10)** was 0.010%.

(2'S*)*-3β,4α,15-trihydroxy-8α-O-[2'-hydroxy-2'-methyl-3'-chloropropionyl]-1α,5α,6β,7a(H)-guai-10(14),11(13)-diene-6(12)-olide (15-deacetylraposerine) (11)

Compound **(11):** m.p. 153-155 °C (chloroform-petroleum ether = 1:1)

$[\alpha]_{580}^{23}$ + 48.8 ° (*c* 0.66; acetone).

UV: λ_{max}: 196, 216 nm (shoulder).

IR spectrum (KBr), ν, cm^{-1}: 1766 (C=O γ-lactone), 1739 (C=O), 1668, 1643 (C=C) 1274, 1226, 1158, 1113, 1065, 754 (C-Cl).

Mass spectrum (*EI*, 70 eV), *(M/Z, I_{rel}* (%)): 387 $[M\text{-}CH_2OM]^+$ (^{37}Cl)](18), 385 $[M\text{-}CH_2OH]^+$(^{35}Cl)] (54), 265(59), 247(87), 229(71), 201(52), 175(91), 93(100). Found *(m/z) :* 385.10581, calculated for $C_{18}H_{22}O_7Cl$: 385.10539.

TLC: system 2 R_f 0.14

^{1}H NMR spectra (Py-d_5, 500 MHz, δ, ppm, *J,* Hz) **(11):** 3.74 (1H, d.d.d., J_1 =11, J_2 =8, J_3 =8, H-1), 1.82 (1H, d.d.d.d., J_1 =14.5, J_2 =9.5, J_3 =7,5, J_4 =2, H-2a), 2.66 (1H, d.d.d., J_1 =14.5, J_2 =11.5, J_3 =6, H-2b), 4.63 (1H, br.d., *J* = 6, <1.5, H-3), 2.57 (1H, d.d., J_1 =9.5, J_2 =10.5, H-5), 5.23 (1H, d.d., J_1 =11, J_2 =9, H-6), 3.12 (1H, d.d.d.d., J_1 =9, J_2 =6.5, J_3 =3,5, J_4 =3,0, H-7), 5.29 (1H, d.d.d., J_1 =7.5, J_2 =5, J_3 =2.5, H-8), 2.47 (1H, d.d., J_1 =15.0, J_2 =1.5, H-9a), 2.93 (1H, d.d., J_1 =14.5, J_2 =5, H-9b), 5.73 (1H, d., *J* =3.0, H-13a), 6.11 (1H, d., *J* =3.5, H-13b), 4.99 (1H, br.d., *J* =1.5 H-14a), 5.0 (1H, br.d., *J* =1.5, H-14b), 4.39 (1H, d., *J* =11.5 H-15a), 4.69 (1H, d., *J* =11.5, H-15b), 3.93 (1H, br.d., *J* =11.5, H-18a), 4.04 (1H, d., *J* =11.5, H-18b), 1.63 (1H, s., H-19).

^{13}C NMR spectra (Py-d_5, 125 MHz, δ, ppm) **(11):** 47.07 d. (C-1), 39.84 d. (C-2), 77.07 d. (C-3), 85.77 s. (C-4), 57.74 d. (C-5), 77.89 d. (C-6), 47.00 d. (C-7), 75.57 d. (C-8), 36.22 t. (C-9), 144.61 s. (C-10), 138.91 s. (C-11), 169.36 s. (C-12), 121.09 t. (C-13), 116.65 t. (C-14), 63.92 t. (C-15), 173.45 s. (C-16), 75.38 s. (C-17), 52.17 t. (C-18), 24.31 q. (C-19).

The yield (**11**) was 0.015%.

2α,3β,4α,15-tetrahydroxy-8α-O-[methacryloyl]-1α,5α,6β,7a(H)guai-10(14), 11(13)-diene-6(12)-olide (15-deacetyl-2α-hydroxyraserolide; raserine) (12)

Compound **(12)**: m.p. 168-171 °C (chloroform-methanol = 3:1)

$[\alpha]_D^{20}$+110.9° *(c* 0.99; acetone).

UV: λ_{max}: 198-208 nm (plateau)

IR spectrum (chloroform), ν, cm^{-1}: 1766 (γ-lactone), 1715 (C=O), 1632, (C= C), 1145, 1051, 910, 855.

Mass spectrum (*EI*, 70 eV), *(m/z, I_{rel}* (%)): 362$[M\text{-}H_2O]^+$(3), 349 $[M\text{-}CH_2OH]^+$ (5), 235 (10), 217(10), 175 (7), 69 (100). Found *(m/z):* 349.12884, calculated for $C_{19}H_{21}O_7$: 349.12872 .

TLC: system 3 R_f 0.2

^{1}H NMR spectra (Py-d_5, 500 MHz, δ, ppm, *J,* Hz) **(12):** 3.43 (1H, d.d., J_1 =11.5, J_2 =8, H-1), 4.74 (1H, d.d., J_1 =6.5, J_2 =8, H-2), 4.69 (1H, d., *J* = 6.5, H-3), 2.95 (1H, d.d., J_1 =11.5, J_2 =11.5, H-5), 5.05 (1H, d.d., J_1 =11, J_2 =9, H-6), 3.18 (1H, d.d.d.d., J_1 =9, J_2 =6.5, J_3 =3,5, J_4 =3,0, H-7), 5.17 (1H, d.d.d., J_1 =10, J_2 =5, J_3 =5, H-8), 2.33 (1H, d.d., J_1 =14.0, J_2 =5, H-9a), 2.93 (1H, d.d., J_1 =14.0, J_2 =5, H-9b), 5.33 (1H, d., *J* =3.0, H-13a), 6.12 (1H, d., *J* =3.5, H-13b), 5.00 (1H, br.d., *J* =2, H-14a), 5.30 (1H, br.d., *J* =1.2, H-14b), 4.49 (1H, d., *J* =11.0, H-15a), 4.54 (1H, d., *J* =11.0, H-15b), 5.51 (1H, d.d., J_1 =1.5, J_2 =1.5, H-18a), 6.14 (1H, d., J_1 =1.5, J_2 =1.0, H-18b), 1.86 (1H, s., H-19).

^{13}C NMR spectra (Py-d_5, 125 MHz, δ, ppm) **(12):** 52.46 d. (C-1), 78.36 d. (C-2), 84.46 d. (C-3), 80.97 s. (C-4), 54.79 d. (C-5), 77.70 d. (C-6), 47.84 d. (C-7), 74.45 d. (C-8), 39.59 t. (C-9), 141.41 s. (C-10), 138.22 s. (C-11), 169.27 s. (C-12), 121.01 t. (C-13), 116.81 t. (C-14), 63.63 t. (C-15), 166.01 s. (C-16), 136.32 s. (C-17), 126.04 t. (C-18), 17.90 q. (C-19).

The yield **(12)** was 0.002%.

Sesquiterpene lactones **of *Rhaponticum carthamoides***

Leaves of *Rhaponticum carthamoides* (Willd.) Iljin plants were collected during the flowering phase in the botanical garden of JSC "IRPH "Phytochemistry" (Karaganda), air-dried and crushed.

A diagram of the process for isolating sesquiterpene lactones is shown in Figure 1. The raw material weighing 0.8 kg was extracted four times with a mixture of ethanol and chloroform with a ratio of 1:4 (in volume ratios), 7 l each at the boiling point of the solvent for 1 hour. The extracts were combined, filtered and evaporated on a rotary evaporator under vacuum at a temperature not exceeding 45 °C. 0.3 liters of thick dark green mass was isolated .

The resulting residue was dissolved in 0.5 L of hot (60 °C) ethanol and diluted with 1 L of hot water (60 °C). The precipitate of nonpolar components (chlorophylls, lipids) that formed after cooling the solution was filtered off, the precipitate was similarly treated twice, and 0.5 and 0.25 L of ethanol were used. No sesquiterpene lactones were detected in the sediment by TLC .

The combined filtrates were extracted with benzene six times, 0.5 L each. The combined benzene extracts were evaporated to dryness on a rotary evaporator under vacuum. The residue after distilling

off the solvent, 18 g, was chromatographed on a silica gel column (500 g).

Benzene – ethyl acetate systems were used as eluents with the component ratio: 1) 5:1, 2) 4:1, 3) 3:1, 4) 2:1, 5) 1:1, 6) ethyl acetate, 7) ethyl acetate – ethanol 10:1. A number of fractions containing sesquiterpene lactones and related components were obtained.

Next, fractions identical in composition were combined and chromatographed on a flash column at a mass ratio of the chromatographed fraction and adsorbent of 1 : 50. The first 11 fractions contain lipids, colored non-crystalline components that were not further identified, and β-sitosterol (identified by TLC with a known sample). The last fractions contain green and brown oils, in which substances of a terpenoid and steroid nature (TLC, developer *A*) were not detected.

Systems for flash chromatography: petroleum ether – ethyl acetate: 1) 10:7, 2)5:4, 3) 1:1

The process was monitored by TLC, developer *B.* Recrystallization of the isolated components was carried out from the following solvent systems: 1) petroleum ether – acetone 2 : 1; 2) petroleum ether – chloroform 1 : 1;

The list of fractions and solvent systems for column and flash column chromatography is presented in Table 2.

Table 2 – Chromatographic separation of *Rhaponticum carthamoides* sesquiterpene lactones

No. factions	Solvent system for column chromatic graphies	Faction composition	Solvent system for flash chromatography	Individual components
12, 13 14-17	6 7	Chlorojanerin (**3**)	1	chlorojanerin
18-23	8	Chlorojanerin (**3**), Cynaropicrin (**2**)	1 2	chlorojanerin cynaropicrin
24-28	8-10	Cynaropicrin (**2**)	2	cynaropicrin

3 β , 4 α - d i h y d r o x y - 8 α - O - [4 ' - hydroxymethacryloyl]-15-chloro-1α,5α,6β,7α(H)-guai-10(14),11(13)-diene-6(12)-olide (chlorojanerin) (3)

Compound **(3)**: m.p. 160-162 °C (chloroform – petroleum ether)

$[\alpha]_{580}^{23}$ + 116.3 ° (*c* 0.946; acetone).

^{1}H NMR spectra ($CDCl_3$, 500 MHz, δ, ppm, *J,* Hz) **(3):** 3.59 (1H, d.d.d., J_1 =9, J_2 =9, J_3 =10.5, H-1), 1.58 (1H, d.d., J_1 =14.0, J_2 =8, H-2a), 2.52 (1H, d.d.d., J_1 =15.0, J_2 =11.5, J_3 =6.5, H-2b), 4.16 (1H, br.d., *J* = 6.5, H-3), 2.31 (1H, d.d., J_1 =9, J_2 =11, H-5), 4.72 (1H, d.d., J_1 =11.5, J_2 =9.5, H-6), 3.15 (1H, d.d.d.d., J_1 =9, J_2 =6.5, J_3 =3,5, J_4 =3,0, H-7), 5.35 (1H, d.d.d., J_1 =7.5, J_2 =5.5, J_3 =2.0, H-8), 2.65 (1H, d.d., J_1 =15.5, J_2 =5, H-9a), 2.43 (1H, d., J_1 =15.5, H-9b), 5.59 (1H, d., *J* =3.5, H-13a),6.19 (1H, d., *J* =3.5, H-13b), 4.81 (1H, br.d., *J* =1.5, H-14a), 5.12 (1H, br.d., *J* =1.5, H-14b), 3.94 (1H, d., *J* =12, H-15a), 4.32 (1H, d., *J* =12, H-15b), 5.94 (1H, br.d.d., J_1 =1, J_2 =1.5, H-18a), 6.32 (1H, br.d., *J* =1, H-18b), 4.37 (1H, br.s., H-19).

^{13}C NMR spectra ($CDCl_3$, 125 MHz, δ, ppm) **(3):** 47.01 d. (C-1), 37.69 t. (C-2), 77.15 d. (C-3), 84.42 s. (C-4), 57.45 d. (C-5), 76.01 d. (C-6), 46.38 d. (C-7), 74.02 d. (C-8), 35.02 t. (C-9), 142.1 s. (C-10), 139.16 s. (C-11), 168.51 s. (C-12), 122.69 t. (C-13), 117.90 t. (C-14), 49.79 t. (C-15), 165.25 s. (C-16), 136.70 s. (C-17), 126.68 t. (C-18), 62.23 t. (C-19).

The yield was 0.01%.

3β,-dihydroxy-8α-O-[4'-hydroxymethacryloyl]-1α,5α,6β,7α(H)-guai-4(15),10(14),11(13)-triene-6(12)-olide (2) (cynaropicrin) (2)

Compound (**2**): a transparent yellowish oil, decomposes in air to form a cheesy precipitate insoluble in chloroform and ethyl acetate. Identified by TLC by comparison with a known sample isolated from *Chartolepis intermedia* Boiss. Yield (2) was 0.04%.

^{1}H NMR spectra (125 MHz, $CDCl_3$, δ, ppm, J/Hz) **(2)**: 2.96 (1H, d.d., J= 8.1, 7.5 Hz, H-1), 2.22 (1H, m, H-2a), 1.74 (1H, m, H-2b), 4.55 (1H, t.t.t., J= 7.2, 1.9, 1.9 Hz, H-3), 2.84 (1H, d.d., J= 10.4, 9.0 Hz, H-5), 4.24 (1H, d.d., J= 11.0 9.1 Hz, H-6), 3.20-3.16 (1H, m, H-7), 5.14-5.13 (1H, m, H-8), 2.40 (1H, d.d., J= 14.5, 3.8 Hz, H-9a), 2.72 (1H, d.d., J= 14.5, 5.1 Hz, H-9b), 6.22 (1H, d, J= 3.4 Hz, H-13a), 5.61 (1H, d, J= 3.0 Hz, H-9b), 5.14 (1H, d, J= 0.9 Hz,

H-14a), 4.93 (1H, d, J= 1.0 Hz, H-14b), 5.49 (1H, t, J= 1.8 Hz, H-15a), 5.36 (1H, t, J= 1.5 Hz, H-15b), 6.32 (1H, d, J= 1.2 Hz, H-3a'), 5.95 (1H, d.d., J= 0.9 Hz, H-3b'), 4.37 (2H, s, H-4').

^{13}C NMR spectra (125 MHz, $CDCl_3$) **(2)**: 45.42 d. (C-1), 39.16 t. (C-2), 73.85 d. (C-3), 152.29 d. (C-4), 51.49 d. (C-5), 78.55 d. (C-6), 47.68 d. (C-7), 74.43 t. (C-8), 37.15 t. (C-9), 141.84 s. (C-10), 137.38 s. (C-11), 169.13 s. (C-12), 122.50 t. (C-13), 117.12 t. (C-14), 112.82 t. (C-15), 165.39 s. (C-1'), 139.36 q. (C-2'), 126.80 s. (C-3'), 60.50 t. (C-4')

Isolation and identification of ecdysterone from the aerial parts of *Rhaponticum serratuloides* and *Rhaponticum carthamoides.*

Leaves of *Rhaponticum serratuloides* and *Rhaponticum carthamoides* were extracted and processed according to previously described methods [4,5], respectively, obtaining in each case the sum of the polar components in an aqueous-alcoholic solution. Next, the ecdysteroid fraction was obtained by extraction of an aqueous-alcohol solution with a mixture of chloroform and isopropanol (eight times extraction with 0.5 l of extractant in the case of *Rhaponticum serratuloides* and six times with 0.3 l in the case of *Rhaponticum carthamoides*).

After evaporation of the extractant, an ecdysteroid fraction of *Rhaponticum serratuloides* weighing 95 g was obtained in the form of a brown resinous residue. Next, the ecdysteroid fraction was chromatographed on aluminum oxide (II degree of activity according to Brockmann), the mass of the sorbent was 950 g. By eluting the column with a chloroform-ethanol mixture 6:1, fractions containing (1) were obtained. Individual component with m.p. 240-242 °C was obtained by double recrystallization from a mixture of ethyl acetate – ethanol 10:1. The yield was 0.015%.

The resulting ecdysteroid fraction of *Rhaponticum carthamoides* leaves weighing 18 g was chromatographed as described on 200 g of aluminum oxide. After recrystallization from the system ethyl acetate – ethanol 10:1, crystals of substance (1) with m.p. 240-242 °C were obtained. The yield (1) was 0.07%.

Identification of the isolated components was carried out by direct comparison with sample (1) isolated from the roots of *Rhaponticum carthamoides*.

Isolation and identification of ecdysterone from roots with rhizomes of *Rhaponticum serratuloides* (Georgi.) Bobr.

Roots with rhizomes of *Rhaponticum serratuloides* (Georgi.) Bobr. were collected during the flowering phase in the vicinity of the village of Aynabulak, Zhanaarkinskyi district, Ulytau region. The raw materials were dried in air and crushed.

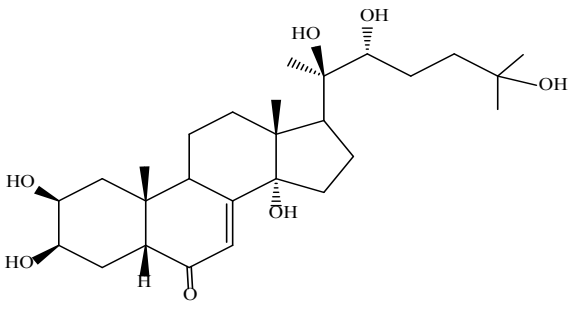
Extraction of raw materials and isolation of individual components. The crushed air-dry raw material, weighing 8 kg, was extracted four times with 45 liters of 70% aqueous ethanol at the boiling point of the solvent for 1-1.5 hours. The extract was cooled, drained and evaporated on a rotary evaporator at a temperature not exceeding 45-50 °C to a volume of 2 l. 1.5 L of ethanol and 1.5 L of water were added to the resulting extract. Next, the aqueous-alcohol solution was extracted sequentially with petroleum ether (6x4 l) and m-butanol (8x4 l). Butanol extracts were evaporated on a rotary evaporator. We obtained 120 g of a thick brown resinous mass (ecdysteroid fraction).

The ecdysteroid fraction was purified from phenolic components by chromatography on aluminum oxide (2.5 kg). The column was eluted with a system of chloroform – 96% ethanol 6:1.

Fractions containing components and more polar (1) according to TLC data were combined. Individual components were obtained by repeated rechromatography on silica gel. Columns were eluted in all cases with chloroform-ethanol mixtures with a gradient increase in polarity of 40:1 – 10:1. A homogeneous fraction containing the mixture (yield of sum 0.005%) was subsequently isolated by chromatography (TLC).

Results and discussion

Using the HPLC method, the quantitative content of ecdysterone (**1**) was determined in various organs of the studied plants depending on the phase of the growing season (Table 3).



(1)

As can be seen from the presented data, the content of ecdysterone (1) in the aerial part of *Rhaponticum carthamoides* reaches 0.60 % in the regrowth phase; during the growth process it decreases to 0.05% (death phase). In the roots of *Rhaponticum*

carthamoides, depending on the phenophase, the content of ecdysterone (1) is relatively stable (0.18-0.30 %), and reaches a maximum in the dying phase (October). In the roots of *Rhaponticum serratuloides* ecdysterone (1) accumulates in amounts from 0.12 (phase of death of the aerial part) to 0.10% (phase of spring growth). In the above-ground part of the specified plant, the maximum amount of ecdysterone (1) was noted in the regrowth phase (0.12 %) – during the development of shoots it decreases (0.11 in the budding and flowering phase and 0.02 % in the fruiting phase).

Table 3 – Ecdysterone content (**1**) in various organs of *Rhaponticum carthamoides*, *Rhaponticum serratuloides* depending on the phenological phase (% of the weight of absolutely dry raw materials)

Phenophase	Roots	Leaves	Stems	Inflorescences/ Buds
Rhaponticum carthamoides				
Regrowth	0.20	0.60	0.12	-
Budding	0.18	0.58	0.06	0.49
Bloom	0.21	0.49	0.04	0.44
Fruiting	0.24	0.34	0.01	-
Dieback of the aboveground part	0.30	0.05	0.01	-
Rhaponticum serratuloides				
Regrowth	0.10	0.12	0.06	-
Budding	0.09	0.11	0.04	0.12
Bloom	0.10	0.11	0.02	0.07
Fruiting	0.11	0.05	-	-
Dieback of the aboveground part	0.12	0.02	-	-

Thus, the aerial parts of *Rhaponticum carthamoides,* cultivated under conditions of Central Kazakhstan, are distinguished by a high content of ecdysterone (1) and can be used as a raw material for its production, as well as for the production of ecdysteroid containing drugs.

In view of the complexity of the technology for isolating ecdysteroids from plant extracts, it is of interest to develop herbal preparations based on the studied plants, which, along with ecdysteroids, contain biologically active compounds of other classes. Studying the composition and biological activity of the latter was one of the objectives of this study.

Of the studied plants *Rhaponticum serratuloides* is the most adapted to the arid conditions of Central Kazakhstan and therefore, despite the fact that this plant contains a relatively small amount of ecdysterone, it was also of interest to study it for the content of biologically active components of other classes in order to predict the biological activity of herbal preparations based on these raw materials.

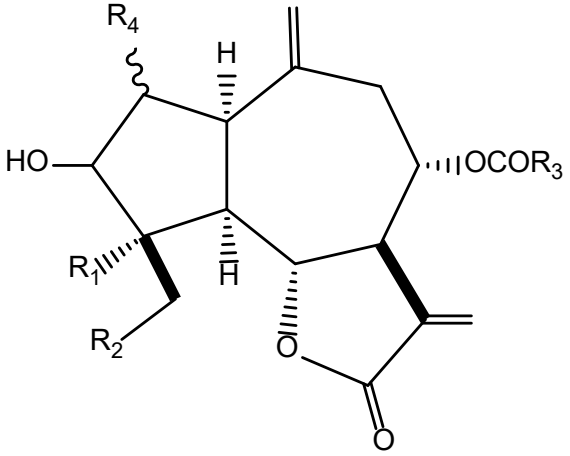
Subsequent studies consisted of searching for the most optimal methods for separating various groups of biologically active compounds of the studied plants, and developing methods for complex chemical processing of raw materials.

It is known that the aerial parts of plants of the genus *Rhaponticum* contain sesquiterpene lactones of the guaian type. In particular, the composition of sesquiterpene lactones from *Rhaponticum carthamoides*, *Rhaponticum scariosum* subsp. *lyratum* (Bellardi/Hayek) and *Rhaponticum serratuloides*, introduced into Poland, was studied, and it was found that these species contain cynaropicrin (2). *Rhaponticum scariosum* subsp. *lyratum* and *Rhaponticum carthamoides* also contain chlorojanerin (3) and janerin (4); moreover, in *Rhaponticum carthamoides,* more polar lactones repdiolide (5) and cebellin E (6) were found [6-9].

Cynaropicrin has been shown to have pronounced cytostatic and antiprotozoal activity. The presence of (2) and structurally similar lactones is associated with the presence of pronounced antiparasitic (antigiardiasis and antiopisthorchiasis) activity of extracts of various species of the genus *Saussurea* DC. [10].

Thus, it was of interest to study the composition of the sesquiterpene lactones of *Rhaponticum serratuloides* and *Rhaponticum carthamoides* with the aim of creating total herbal preparations with antiparasitic action based on the aerial parts of these plants.

Study of the composition of sesquiterpene lactones from *Rhaponticum serratuloides* showed the presence of 2 in the plant, as well as known guaianolides centaurepensin (7), acroptilin (8), which were not previously found in this plant [4], and four crystalline lactones, which turned out to be new. These compounds were named raposerine (9), raserolide (10), 15-deacetylraposerine (11) and raserine (12) [11,12].



Cynaropicrin (2)	$R_1+R_2= \Delta^{4,15}$; $R_3= C(CH_2OH)=CH_2$; $R_4 = H$
Chlorojanerin (3)	$R_1 = OH$; $R_2 = Cl$; $R_3 = C(CH_2OH)=CH_2$; $R_4 = H$
Janerin (4)	$R_1 =R_2 = OH$; $R_3 = C(CH_2OH)=CH_2$; $R_4 = H$
Repdiolide (5)	$R_1+R_2= \Delta^{4,15}$; $R_3= C(CH_3)=CH_2$; $R_4 = \alpha$-OH
Cebellin E (6)	$R_1 = OH$; $R_2 = Cl$; $R_3= C(CH_3)=CH_2$; $R_4 = \beta$-OH
Centaurepensin (7)	$R_1 = OH$; $R_2 = Cl$; $R_3 = (17\ S)C(OH)(CH_2Cl)CH_3$; $R_4 = H$
Acroptilin (8)	$R_1+R_2 =$ -O- CH_2-; $R_3 = (17\ S)C(OH)(CH_2C1)CH_3$; $R_4 = H$
Raposerine (9)	$R_1 = OH$; $R_2 = OAc$; $R_3 = (17\ S)C(OH)(CH_2C1)CH_3$; $R_4 = H$
Raserolide (10)	$R_1 = OH$; $R_2 = OAc$; $R_3= C(CH_3)=CH_2$; $R_4 = H$
15-Deacetylraposerine (11)	$R_1 =OH$;$R_2 = OH$; $R_3 = (17\ S)C(OH)(CH_2C1)CH_3$; $R_4 = H$
Raserine (12)	$R_1 = OH$; $R_2 = OH$; $R_3= C(CH_3)=CH_2$; $R_4 = \alpha$-OH

Previously, the lactone, to which structure (11) is proposed, was isolated as an oil from the plant *Centaurea bella* by a group of Czech and Polish researchers and was named cebellin J. However, the configuration of the asymmetric C-17 center in this compound was not determined [13].

When acetylation of the mother liquor from the crystallization of raposerine (9) followed by chromatographic separation of the acetylation products oily lactone diacetate (5) was isolated [14].

Thus, discovered a significant difference has been discovered in the composition of lactones in the aerial parts of *Rhaponticum serratuloides*, cultivated in Poland and collected from nature in Central Kazakhstan: in the first case (2) is the main component (no other lactones were detected by TLC and not isolated), in the second – the plant contains 7 lactones and at the same time content (2) does not prevail (Table 4).

Considering the discovered differences in the compositions of sesquiterpene lactones of *Rhaponticum serratuloides* collected in various habitats, a study of these components was conducted in the aerial part of *Rhaponticum carthamoides*, introduced into cultivation in Central Kazakhstan. In this case, lactones (2) and (3) were detected, the presence of which was also established in plants cultivated in Poland.

Table 4 – Sesquiterpene lactones of *Rhaponticum serratuloides* and *Rhaponticum carthamoides*

Name	Yield, % by weight of air-dried raw materials
Rhaponticum serratuloides	
Raposerine (**9**)	0.030
15-Deacetylraposerine (**11**)	0.015
Cynaropicrin (**2**)	0.013
Raserolide (**10**)	0.010
Centaurepensin (**7**)	0.008
Acroptilin (**8**)	0.004
Raserine (**12**)	0.002
Rhaponticum carthamoides	
Cynaropicrin (**2**)	0.100
Chlorojanerin (**3**)	0.040

Based on our research, a technology for complex chemical processing of the aerial parts of *Rhaponticum serratuloides* and *Rhaponticum carthamoides* has been proposed, including the release of sesquiterpene lactones, ecdysteroids, and polyphenolic compounds (Figure 1).

Compared to the ecdysteroids and polyphenolic compounds present in the plant extract isolated with 70% ethanol, sesquiterpene lactones are less polar substances. In this regard, the separation of these compounds from the sum of extractive substances was carried out at the stage of liquid-liquid extraction.

To purify ecdysteroids from polyphenolic compounds close to them in polarity, as a rule, the method of column chromatography on a sorbent with basic properties (aluminum oxide, polyamide) is used. However, the technology for such purification, given the high cost of sorbents, eluents and the duration of the process, is quite expensive. In addition, the desorption of polyphenic compounds from the main sorbents is an extremely labor-intensive process.

A method for separating ecdysteroids and polyphenolic compounds at the stage of liquid-liquid extraction has been proposed by converting the latter into water-soluble phenolates, based on the method described in [15].

Thus, the aerial parts of *Rhaponticum serratuloides* and *Rhaponticum carthamoides* are of interest as sources of sesquiterpene lactones with antiparasitic and antitumor effects; aerial part of *Rhaponticum carthamoides* is a promising source of ecdysterone. The fraction of polyphenolic compounds is of particular interest, since the latter often exhibit antioxidant and hepatoprotective activity.

Anabolic activity of *Rhaponticum carthamoides* and *Rhaponticum serratuloides* root extracts were studied along with the amount of ecdysteroids from these plants. Tests were carried out on female outbred white rats, the magnitude of anabolic activity was assessed by the integral indicator of body weight gain in laboratory animals, as well as by the nature of the effect of drugs on motor activity and behavioral reactions. It was found that all studied samples exhibited anabolic activity at the level of the reference drug, methandrostenolone.

It is known that ecdysterone (1) exhibits antiulcerogenic activity. In this regard, the gastroprotective effect of *Rhaponticum serratuloides* root extract has been studied when modeling psycho-emotional stress in rats caused by their twelve-hour fixation. It was found that the extract exhibits a pronounced gastroprotective effect.

A study was carried out of the effect of sesquiterpene lactones centaurepensine (7) and raposerine (9) on the reproduction and infectivity of influenza virus (strain A/FPV/Rostock/34) and Newcastle disease virus (strain La Sota). It was determined that both (7) and (9) exhibit antiviral activity comparable to that of a widely used commercial drug “Remantadine”, which is effective against the influenza A virus.

Antifungal activity of acroptilin (8), cynaropicrin (2), raposerine (9) and raserolide (10), as well as the sum of sesquiterpene lactones with accompanying components of *Rhaponticum carthamoides*, obtained according to the scheme shown in Fig. 1, was determined *in vitro* against *Aspergillus niger*, *Candida albicans*, *Trychophyton rubrum*, *Trychophyton mentagrophytes* and *Microsporum canis.* Nystatin and nitrofungin were used as comparison drugs. The lactone fraction from *Rhaponticum carthamoides* showed relatively pronounced antifungal activity, samples of these lactones showed somewhat low activity, (9) turned out to be inactive against all the studied strains. The activity of all studied samples is inferior to the activity of commercial drugs nystatin and nitrofungin.

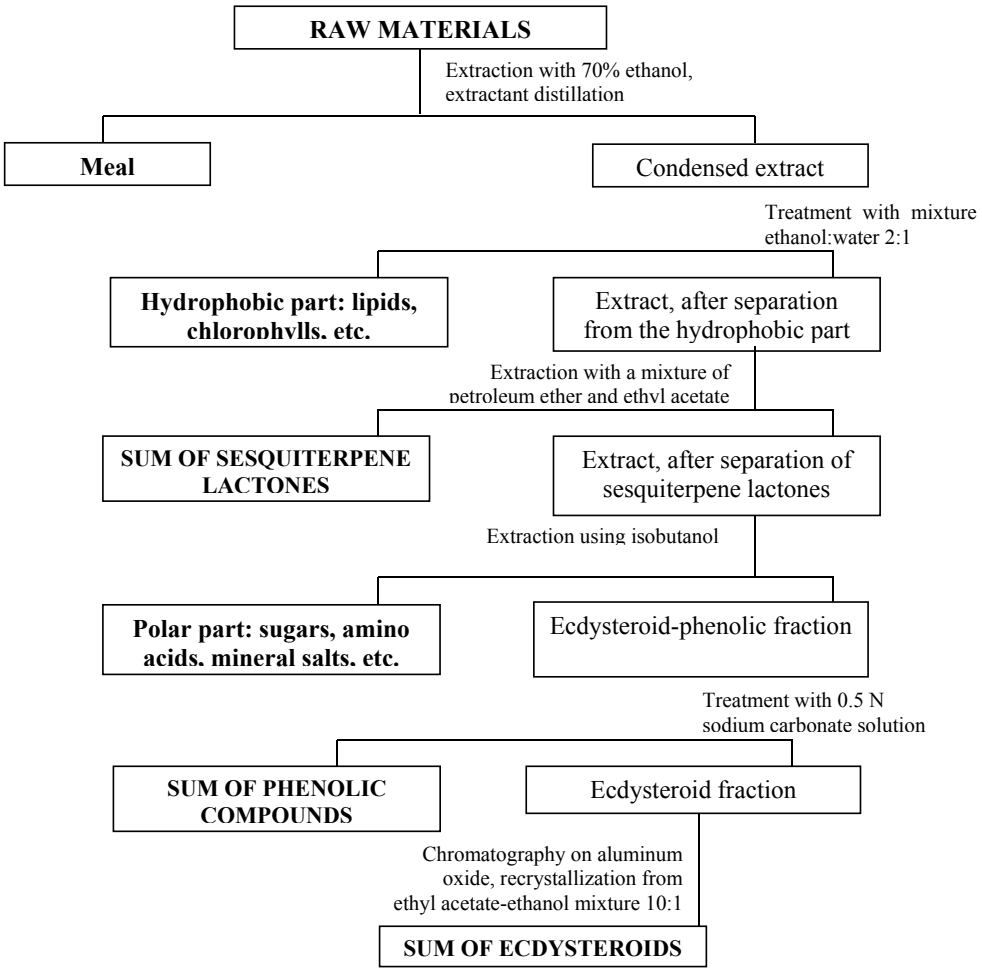


Figure 1 – Scheme of complex chemical processing of raw materials from the aerial parts of *Rhaponticum carthamoides* and *Rhaponticum serratuloides*

Thus, it has been established that the roots and aerial parts of the plant *Rhaponticum serratuloides* contain ecdysterone, and, in addition, the aerial part of this plant is a promising source of sesquiterpene lactones with cytostatic, antiviral and antiparasitic activity. The aerial part of *Rhaponticum carthamoides,* which also contains sesquiterpene lactones that have antiparasitic and cytostatic activity, is of significant interest in terms of ecdysterone release.

Conclusion

Plants of the genus *Rhaponticum* Adans. (Rhaponticum, Leuzea, tribe of the family *Asteraceae* L.) are sources of valuable biologically active substances, primarily ecdysones and sesquiterpene lactones, as well as polyacetylenes and flavonoids.

All studied plants of this genus contained ecdysterone, which is used as a substance in the tonic drug “Ecdisten”. The roots with rhizomes of *Rhaponticum carthamoides*, as well as the inflorescences of *Rhaponticum integrifolium*, are industrially important raw materials for the production of ecdysterone. The presence of ecdysterone and accompanying ecdysteroids necessitated intensive study of the ecdysteroid composition of various representatives of this genus. However, the composition of ecdysteroids in some species of the genus remains unknown.

At the same time, the composition of other components of plants of the genus *Rhaponticum* Adans. remain little studied or not studied at all. Thus, in the literature there is only isolated information about the composition of such valuable components of plants of the genus *Rhaponticum* as sesquiterpene lactones. The composition of sesquiterpene lactones of this genus, growing in Central Asia, has not been studied.

Therefore, the search and study of biologically active isoprenoids among plants of the genus

Rhaponticum growing in the territory of Central Kazakhstan or introduced into culture in this territory, with the prospect of large-scale production of raw materials for the production of practically valuable drugs, is an urgent task. The second problem that arises when processing plant raw materials is the problem of its rational use, that is, complex processing with the fullest possible use of all valuable components.

A comprehensive study of the biologically active components of *Rhaponticum serratuloides* (Georgi.) Bobr. and *Rhaponticum carthamoides* (Willd.) Iljin allowed to establish that all organs of *Rhaponticum serratuloides* contain ecdysterone as the main ecdysteroid, the yield of which from the aerial part is 0.015%, and from the roots 0.014% based on air-dried raw materials.

The minor ecdysteroid amarasterone A and two new ecdysteroids, 25-epi-amarasterone and 25-deoxy-24-hydroxyethylideneecdysterone (rasersterone), were isolated from the roots of *Rhaponticum serratuloides*, the structure of which was determined by NMR spectroscopy methods, including two-dimensional COLOC and COSY. β-sitosterol, which has depyrogenized and anti-sclerotic effect, and its glycoside, a possible raw material for the production of polyoxysteroids, were obtained as accompanying components in the isolation of ecdysterone.

A number of sesquiterpene lactones have been isolated from the leaves of *Rhaponticum serratuloides*, including the well-known cynaropicrin, centaurepensin, acroptilin and new ones named raposerine, raserolide, raserine and 15-deacetylraposerine. Details of the structure of these sesquiterpene lactones were established on the basis of data from the ^{1}H and ^{1}C NMR spectra, including using the methods of two-dimensional NMR spectroscopy ^{1}H -^{1}H and ^{1}H – ^{13}C COSY, ^{1}H-^{13}C COLOC and X-ray diffraction. To prove the structure of the isolated compounds, chemical modification of sesquiterpene lactones was carried out. Thus, the spatial structure of the new sesquiterpene lactones raposerine and 15-deacetylraposerine was established by obtaining acetates of these compounds, which turned out to be identical substances.

For the isolated lactones, the optimal parameters for analysis by HPLC on a reverse-phase sorbent were determined, which made it possible to select the conditions for separating a mixture of lactones.

Ecdysterone was isolated from the leaves and roots of the introduced *Rhaponticum carthamoides*. From the roots of *Rhaponticum carthamoides*, an industrial raw material for the production of ecdysterone, the yield was 0.05%, which corresponds to industrial value and allows us to consider the raw materials produced in Central Kazakhstan as a promising source of ecdysterone. The yield from the leaves of *Rhaponticum carthamoides* was 0.07%, which allows us to recommend the leaves of this plant as a new potential source of ecdysterone.

In addition to ecdysterone, the well-known sesquiterpene lactones cynaropicrin and chlorojanerin have also been isolated from the leaves of *Rhaponticum carthamoides*.

A method has been developed and processes for complex chemical processing have been optimized for the most promising in terms of production of ecdysterone-containing drugs – leaves of *Rhaponticum carthamoides*, which consists in the joint release of ecdysteroids, sesquiterpene lactones and phenolic components.

A study of the antiviral activity of the sesquiterpene lactones centaurepensin and raposerine isolated from the leaves of *Rhaponticum serratuloides* showed that both compounds can effectively inhibit the process of reproduction of the influenza virus (strain A/FPV/Rostock/34) and the virus that causes Newcastle disease (strain La Sota), in addition, both centaurepensin and raposerine reduce the infectivity of the above viruses.

The results obtained during the research allows recommending:

− the aerial part of *Rhaponticum carthamoides*, introduced in Central Kazakhstan, as a raw material for the production of ecdysterone and the release of sesquiterpene lactones;

− roots of *Rhaponticum carthamoides*, introduced in Central Kazakhstan as a high-quality raw material for the production of ecdysterone;

− roots and aerial parts of *Rhaponticum serratuloides* as additional raw materials for the production of ecdysterone;

− aerial part of *Rhaponticum serratuloides* for the production of ecdysterone and sesquiterpene lactones with antiviral, cytotoxic, antiprotozoal activity.

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