ISOLATION OF ECDYSTERONE

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The kinetics of the extraction of ecdysterone from the roots with rhizomes of *Rhaponticum carthamoides* has been studied. The results of investigations on the selection of the optimum solvent for extracting ecdysterone from the raw material, for the process of sorption purification, and for the recrystallization of the ecdysterone are given.

We have previously [1] reported the isolation of ecdysterone from the roots with rhizomes of the perennial herbaceous plant *Rhaponticum carthamoides* (Willd) Iljin.

Pharmacological investigations performed in the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR [2-6] have permitted the creation from ecdysterone of a new medicinal preparation with an anabolic and tonic action which is undergoing wide clinical trials.

We have investigated the isolation of ecdysterone in the various stages of the technological cycle. The choice of a selective solvent is extremely important in the extraction of the drug from the plant raw material.

According to the figures for the solubility of ecdysterone [1], methyl and 70% ethyl alcohols have an advantage with respect to dissolving capacity over other solvents. We have made a comparative extraction of the roots with rhizomes of *Rh. carthamoides* with ethyl alcohol in various concentrations and with methyl alcohol.

The extraction of ecdysterone with methyl alcohol and 70% ethyl alcohol gave almost identical results:

Extractant	Sum of the ecdysteroids and extractive substances		Yield of ecdysterone	
	g	%	g	%
Methyl alcohol	120.0	12.0	0.56	0.056
95% ethyl alcohol	72.1	7.2	0.16	0.016
90% ethyl alcohol	91.0	9.1	0.20	0.020
80% ethyl alcohol	109.0	10.9	0.45	0.045
70% ethyl alcohol	118.0	11.8	0.54	0.054

To determine the length of the process we studied the kinetics of the extraction of ecdysterone. The change in the concentration of ecdysterone with time was analyzed (Fig. 1).

To achieve equilibrium concentration on the first contact of the phases required 5 h. The phase equilibrium on the second contact was achieved after 3 h, on the third contact after 2 h, and on the fourth and fifth contacts after 1 h (see Fig. 1).

The extraction curves consist of typical isotherms tending to equilibrium. With a decrease in the amount of extractive substances in the raw material the relative rate of extraction in the ecdysteroids rose. On fivefold extraction for 12 h, the degree of extraction amounted to 90%.

The concentrated extract after the elimination of the organic component was an aqueous solution containing flavanoids, glycosides, inulin, potassium oxalate, salts of phosphoric acid, gums, and ecdysteroids [7-10]. A considerable part of the impurities of low polarity was eliminated by treatment with chloroform. On fivefold extraction of the aqueous solution with chloroform, the bulk of the accompanying substances of hydrophobic nature was eliminated,

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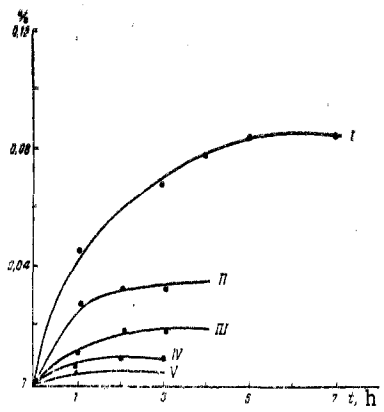

Fig. 1. Kinetics of the extraction of ecdysterone.

and the ecdysterone remained in the aqueous solution. After the treatment of the aqueous solution with chloroform, other fairly fairly hydrophilic impurities remained in it besides the ecdysterone.

We have investigated the dynamics of the extraction of the ecdysteroids from an aqueous solution with various mixtures of solvents:

Extracting Mixture	Extracted after 10 extractions %
Chloroform–ethyl alcohol (1:1)	77.65
Chloroform–ethyl alcohol (2:1)	48.0
Chloroform–isopropyl alcohol (1:1)	97.5
Chloroform–isopropyl alcohol (2:1)	88.5
Chloroform–propyl alcohol (1:1)	94.5
Chloroform–n-butyl alcohol (1:1)	95.0
Benzene–isopropyl alcohol (1:1)	25.0

The best extractants are mixtures of chloroform with propyl, isopropyl, and n-butyl alcohols in ratios of 1:1 (Fig. 2).

From economic considerations, we used isopropyl alcohol for the technology developed.

Other ecdysteroids and impurities were extracted from an aqueous solution of ecdysterone by a mixture of chloroform with isopropyl alcohol. The sum of the ecdysteroids was purified by a sorption method on chromatographic alumina. Methanol, ethanol, chloroform, isopropyl alcohol, ethyl acetate, and acetone were used as eluents. The presence of ecdysterone in the eluates was determined qualitatively by thin-layer chromatography on Silufol plates in the chloroform–methanol (4:1) system, the visualizing agent being a 1% solution of vanillin in concentrated sulfuric acid. The best result was given by elution with a mixture of chloroform and methanol in a ratio of 2:1. Having determined the most effective eluent we investigated various ratios of the sum of the ecdysteroids and the sorbent. The results of the experiments are given below:

Amount of solvent, g	Amount of total ecdysteroids, g	Yield of purified extractive substances with the eluate		Amount of ecdysterone in the eluate	
		g	%	g	%
150	30	12	40	—	—
180	30	9	30	—	—
210	30	8	27	—	—
240	30	6	20	2	6,6
270	30	4,5	15	1,8	6
300	30	3,1	10,3	1,8	6
330	30	3,2	10,4	1,8	6
360	30	3,1	10.3	1,8	6

The optimum sorption of impurities was achieved at ratios of the total material to sorbent of 1:10–1:12.

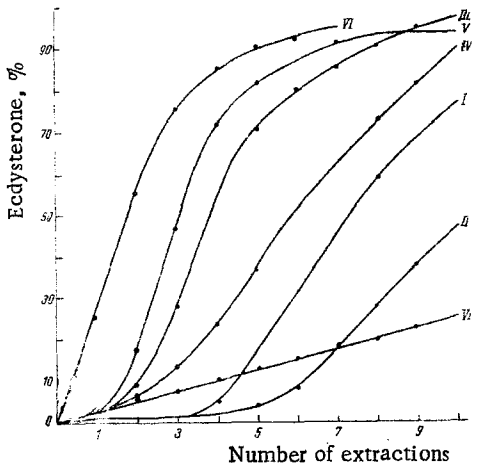

Fig. 2. Dynamics of the extraction of ecdysterone from aqueous solution: I) chloroform–ethanol (1:1); II) the same (2:1); III) chloroform–isopropanol (1:1); IV) the same (2:1); V) chloroform–isopropanol (1:1); VI) chloroform–butanol (1:1); VII) benzene–isopropanol (1:1).

After chromatographic purification, the amount of ecdysterone in the technical product was 70-75%. To obtain pharmapoeial ecdysterone we investigated the conditions of recrystallization from various mixtures of solvents:

System	Yield of ecdysterone		
	g	% on the weight of the techn. product	% on the weight of the raw material
Methanol–acetone (1:9)	3.5	45.2	0.058
Methanol–ethyl acetate (1:9)	4.7	61.1	0.078
Ethanol–ethyl acetate (1:9)	4.3	56.1	0.071
Ethanol–acetone (1:9)	4.5	58.9	0.075
Isopropyl alcohol–acetone (1:9)	4.1	53.5	0.065
Isopropyl alcohol	4.3	56.1	0.071

Double recrystallization from a mixture of methanol (ethanol) with ethyl acetate (acetone) made it possible to obtain crystals of ecdysterone with a purity of 95% and above.

EXPERIMENTAL

Selection of a Solvent for the Extraction of Ecdysterone from the Raw Material. Comparative extraction was performed by using samples of the roots with rhizomes of *Rh. carthamoides* weighing 1 kg and containing 0.16% of ecdysterone. This was extracted seven times. The further operations were carried out by the procedure described above.

A Study of the Kinetics of the Extraction Process. To establish the phase equilibrium at the first contact of the phases, 1 kg of comminuted roots with rhizomes was charged into each of seven ten-liter extractors, 70% ethyl alcohol being used as the extractant. In the first extractor the time of extraction was 1 h, in the second 2 h, in the third, 3 h, in the fourth 4 h, in the fifth 5 h, in the sixth 6 h, and in the seventh 7 h. After expiration of the given times, the extracts were decanted off and evaporated, and the amount of ecdysterone was determined. To establish the phase equilibrium in the second contact of the phases the experiments were performed under the following conditions: 1 kg of roots with rhizomes of

Rh. carthamoides was extracted in each of five extractors for 5 h (the time necessary for the establishment of the phase equilibrium in the first contact). The extracts were decanted off and the residues were covered with fresh portions of solvent. The extract was decanted off from the first extractor after 0.5 h, from the second after 1 h, from the third after 2 h, from the fourth after 3 h, and from the fifth after 4 h, and the amounts of ecdysterone were determined.

To find the time of extraction at the third contact of the phases, 1 kg of the *Rh. carthamoides* roots was extracted for 5 h, the extract was poured off, fresh solvent was added, and the raw material was extracted for another 3 h, after which the time necessary to achieve phase equilibrium in the third contact was established. The phase equilibrium in the fourth and fifth contacts were determined in the same way.

Determination of the Dynamics of the Extraction of Ecdysterone from an Aqueous Solution. The ecdysterone was extracted from solutions of 2 g of ecdysterone in 200 ml of water containing 2.5% of methanol in various mixtures under identical conditions (10 × 50 ml). The extracts were evaporated and dried to constant weight, and the amount of ecdysterone extracted with each portion of solvent was determined from the dry residue.

Adsorption Purification of the Total Ecdysteroids. Different amounts of alumina and, in each case, 30 g of a powder of the combined ecdysteroid were charged into columns with a diameter of 50 mm. They were eluted with chloroform–methanol (2:1) until the eluate gave a negative reaction for ecdysterone. The eluate was evaporated to dryness and the residue was crystallized from the methanol–ethyl acetate system.

Choice of the Optimum Conditions for the Recrystallization of Ecdysterone. The sum of the ecdysteroids obtained from 36 kg of *Rh. carthamoides* roots (60 g), after chromatographic purification, containing 73% of ecdysterone, was separated into six parts and they were crystallized from different solvents.

SUMMARY

1. The optimum solvent for the extraction of ecdysterone from raw material has been selected.

2. The kinetics of the extraction of ecdysterone from the roots of *Rhapontieum carthamoides* has been studied.

3. The dynamics of the extraction of ecdysterone from aqueous solution has been investigated.

4. The optimum ratio of the sum of the ecdysteroids and the sorbent in the chromatographic purification of ecdysterone has been determined.

5. The process of recrystallizing ecdysterone has been studied.

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