APPLICATION OF THE CHUGAEV REACTION FOR THE QUANTITATIVE DETERMINATION OF ECDYSONES

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The specificity of the Chugaev reaction for a series of compounds of the sterol series and the possibility of its use for their identification and quantitative determination has been reported previously [1, 2]. On investigating the spectrometric characteristics of the products of the Chugaev reaction with ecdysones that we had isolated from Podocarpus andinus, Podocarpus macrophyllus (State Nikitskii Botanical Garden), Taxus baccata (Pechenezhinskoe Forestry, Ivano-Frankovo oblast) [3], and also kindly provided by Prof. N. K. Abubakirov [4-6] we found a characteristic λ_{max} 380 nm with a high molar coefficient of absorption E^{M} 4000-6500 for the products of the reaction with α-ecdysone (I); its 20-hydroxy derivative – ecdysterone (II); ecdysterone 25-acetate – viticosterone E (III); and cyasterone (IV), which differs from ecdysterone by the C_{24}-C_{25} fragment of the side chain, containing a γ-lactone grouping. The products of the reaction with 5β-hydroxyecdysterone – polypodin B (V) had a characteristic maximum in the 420 nm region (Fig. 1).

The introduction of a hydroxy group into position 20, and also the presence of a lactone ring in the side chain, obviously favored the appearance of additional chromophores, while, by analogy with the majority of sterols investigated [1], esterification does not affect the nature of the products of the Chugaev reaction. The introduction of a hydroxy group into the ring at C_5 causes the formation of a qualitatively new chromophore, displacing the main absorption maximum at 380 nm into the longer-wave region.

The Chugaev reaction was performed in chloroform solutions of the ecdysones (0.2-0.5 mg/ml) by the addition to one volume of the initial solution of two volumes of a 20% solution of zinc chloride in glacial acetic acid and one volume of acetyl chloride, followed by heating at 65°C for 10 min and 10-fold dilution of the initial solution with chloroform. The dependence of the intensity $\lambda_{max}^{CHCl_3}$ 380 nm on the concentrations of the ecdysones (I-IV) was linear (Fig. 2), obeying the Lambert–Beer law. Because of this, we used the Chugaev reaction for the quantitative determination of ecdysterone and viticosterone E in drawing up a balance of the chromatographic columns in the isolation of the ecdysones after thin-layer chromatography, and also for analysis of the amount of ecdysterone in plant raw material. The sensitivity of the Chugaev reaction calculated with respect to λ_{max} 380 nm is more than twice that of the Lieberman–Burchard reaction (E^{M}_{620} = 2400).

We originally analyzed a model mixture consisting of solutions of standard samples of ecdysterone and viticosterone E. The ecdysterone was well separated from the viticosterone E on silica gel in the chromatographic system usually used, chloroform–ethanol (4:1); the ecdysones were quite satisfactorily desorbed from the support. Below we give the results of a determination of a model mixture of ecdysterone and viticosterone E after chromatography (means of two determinations):

Replicate	Composition of mixture	Taken, mg	Found, mg	Found, %	Relative error, %
I	Ecdysterone	0,050	0,052	104,0	+4,00
	Viticosterone	0,050	0,048	96,0	−4,00
II	Ecdysterone	0,200	0,210	105,0	+5,00
	Viticosterone	0,100	0,100	100,0	0,00
III	Ecdysterone	0,100	0,095	95,0	−5,00
	Viticosterone	0,150	0,157	104,7	+4,67
IV	Ecdysterone	0,150	0,142	−94,7	−5,33
	Viticosterone	0,200	0,198	−99,0	−1,00

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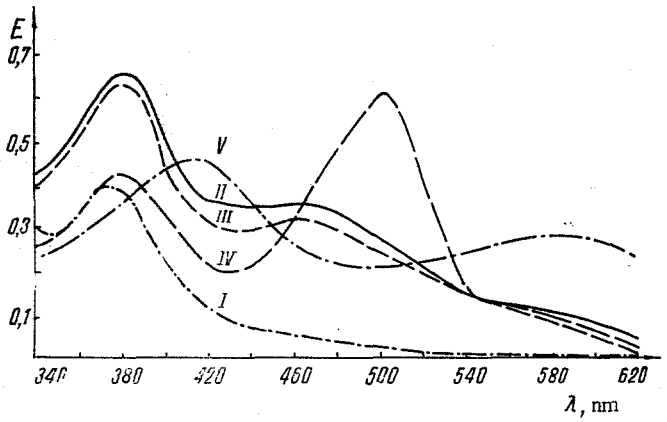

Fig. 1. Absorption spectra of the products of the Chugaev reaction with α-ecdysone (I), ecdysterone (II), viticosterone E (III), cyasterone (IV), and polypodin B (V).

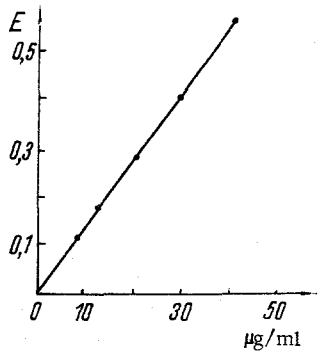

Fig. 2. Dependence of the intensity of absorption of the products of the Chugaev reaction with ecdysterone at 380 nm on its concentration in the photometered solution.

Then we determined the amount of ecdysterone in the inflorescences of Serratula xeranthemoides. The raw material was collected at Askaniya-Nova, Kherson oblast. The objectivity of the method was checked in experiments with the addition of pure ecdysterone to the extract. No viticosterone could be detected in the crude extract without the addition of the purified material.

The amount of ecdysterone in an extract of the inflorescences of Serratula xeranthemoides with the addition of a standard sample and without it were as follows (means of five determinations):

Replicate	Amount of ecdysterone in 0.4 ml of the initial extract, mg	Added, mg	Calculated, mg	Found, mg	Relative error, %
I	0.130	0.000	0.130	0.130	0.00
II	0.130	0.050	0.180	0.175	−2.78
III	0.130	0.100	0.230	0.230	0.00
IV	0.130	0.150	0.280	0.285	+1.79
V	0.130	0.200	0.330	0.315	−4.54

The amount of ecdysterone in inflorescences of Serratula xeranthemoides collected in July 1974, was 0.26-0.31% on the weight of the air-dry raw material in various batches.

EXPERIMENTAL

The Chugaev reaction was performed in the following way. To 0.5 ml of a chloroform solution of an ecdysone (0.15-0.5 mg/ml) was added 1 ml of a 20% solution of zinc chloride in glacial acetic acid and 0.5 ml of acetyl chloride. The mixture was heated on the water bath at 65°C for 6 min. After cooling, the volume of

the liquid was made up with chloroform to 5 ml. Photometry was carried out in a 1-cm cell on a Specord or SF-4 spectrophotometer. The curves of the dependence of the optical density on the concentrations of solutions of analytical samples of ecdysterone and viticosterone E were obtained at λ_{max} 380 nm (see Fig. 2).

The amounts of ecdysterone and viticosterone E in the mixture were determined by their chromatographic separation in a thin layer of LS 5/40 μ silica gel including 13% gypsum. A 13 × 18 cm glass plate was coated with 7.5 g of silica gel in 20 ml of distilled water. The plate was dried at room temperature and divided into four parallel bands, one of which served as a background in photometry while on the others were deposited in the form of spots or continuous lines various amounts of ethanolic solutions of the ecdysones as described by Genkina et al. [7, 8]. The amount of ecdysones deposited in a sample was usually 50-500 μg. After drying, the plate with the deposited substances was chromatographed in the chloroform–ethanol system for 2 h.

The zones for elution were marked out from their fluorescence in UV light or from the results of spraying the edges of the bands with concentrated H_2SO_4. The revealed zones containing the ecdysones and corresponding zones of pure silica gel of equal weight were transferred to centrifuge tubes and were eluted four times with ethanol. The eluates were combined and evaporated in vacuum. The residue was dissolved in 0.5-1.0 ml of chloroform. The background in photometry was a sample obtained similarly from an equal weight of pure silica gel. The concentrations of the ecdysones (μg/ml) in the photometered solution were found from a calibration curve plotted for a standard solution.

The amounts of ecdysterone in the inflorescences of Serratula xeranthamoides were determined by the preliminary hot extraction of the comminuted air-dry inflorescences (5-6 g) with methanol (50-60 ml) for a day. Samples of this extract amounting to 0.4-0.6 ml, or smaller volumes of a concentrated extract (0.1 ml) were deposited on 2-3 bands of a plate with a thin layer of LS 5/40 or LSL_{254} silica gel with a luminescent indicator. The first band serves as background, and standard samples of the ecdysterone were deposited on the second band.

Chromatography, elution, and quantitative determination were performed similarly to the determination in the standard mixture. The accuracy of collection of the zones containing the ecdysones was collected by the subsequent treatment with the vanillin–sulfuric acid revealing agent of the remaining parts of the chromatogram.

SUMMARY

The spectrophotometric characteristics of the products of the Chugaev reaction with ecdysones have been studied. Methods have been proposed for determination of ecdysterone and viticosterone E in an artificial mixture and of ecdysterone in extracts of plant raw material by thin-layer chromatography followed by the Chugaev reaction.

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