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CHROMATO-SPECTROPHOTOMETRIC METHOD OF DETERMINING ECDYSTERONE IN PLANT RAW MATERIAL

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UDC 543.544.4+543.420.62+581.192+547.92.02

Ecdysones are natural polyhydroxysteroid compounds possessing the activity of hormones for the moulting and metamorphosis of arthropods. Ecdysterone (β-ecdysone) is found most frequently in the vegetable kingdom [1, 2]; this possesses an anabolic effect [3].

In connection with the search for the most profitable source of ecdysterone the necessity has arisen for the development of a method for its quantitative determination in plant raw material. The qualitative identification of ecdysterone is carried out with the aid of TLC, GLC, mass spectrometry, and other physicochemical methods [4]. For quantitative purposes, at the present time fluorimetry in sulfuric acid [5], thin-layer chromatography with subsequent analysis by the photochemical method [6, 7], and gas–liquid chromatography of the trimethylsilyl derivatives of ecdysterone [8, 9] are used.

We have developed a chromato-spectrophotometric method of determining ecdysterone in the epigeal part and in the roots of *Rhaponticum orientale* Soscov and *Rhaponticum integrifolium* C. Winkl (family Compositae), which were known to contain cedysones from previous investigations [10, 11].

The UV spectrum of ecdysterone [12] has the absorption maximum ($\pi \rightarrow \pi^*$ transition) λ_{max}^{MeOH} 242 nm (log ε 4.07; $E_{1\,cm}^{1\%}$ = 255 ± 4.5) because of the presence of a Δ^7-6-keto grouping in the molecule [13]. The intensity of this extremum permits it to be used as an ana-

TABLE 1. Statistical Treatment of the Results of the Chromato-spectrophotometric Determination of Ecdysterone in the Roots of *Rhaponticum orientale* (P = 0.95; n = 6)

Found, % (x)	$(x_{av}-x)$ %	$(x_{av}-x)^2 \cdot 10^{-6}$ %	Metrological characteristics
0,141 0,157	0,008 −0,008	64 64	$S=\sqrt{\frac{\Sigma (x_{av}-x)^2}{n-1}}$
0,151 0,146	−0,002 0,003	4 9	$S=\sqrt{\frac{226\cdot 10^{-6}}{5}}=\pm 0{,}0067\%$
0,156 0,143	−0,007 0,006	49 36	$\varepsilon_\alpha=\pm\frac{S\cdot t_{\alpha n}}{\sqrt{n}}$
			$\varepsilon_\alpha=\frac{0{,}0067\cdot 2{,}271}{\sqrt{6}}=\pm 0{,}0069\%$
x_{av}=0,149		$(x_{av}-x)^2$=226	x=0,149±0,0069 %

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 737-740, November-December, 1978. Original article submitted June 30, 1978.

0009-3130/78/1406-0627$07.50

TABLE 2. Results of Experiments with Additions of Ecdysterone to an Extract of the Epigeal Part of *Rhaponticum orientale*

Plant organ	Taken for analysis	Standard ecdysterone added	Amount of ecdysterone after the addition		Relative error, %
			calculated	found	
	mg				
Leaves	0,118	0,082	0,200	0,200	—1,99
	0,118	0,093	0.211	0,220	+4,20
	0,118	0,093	0.211	0,206	—2,23
Stems	0,116	0,093	0,209	0,214	+2,54
	0,116	0,093	0.209	0.214	+2,54
Inflorescences	0.295	0,093	0,388	0,405	+3,36
	0.295	0,093	0,388	0,400	+3,10

lytical band. In the region of working concentrations (c = 0.005-0.025 mg/ml) the absorption of ecdysterone solutions obeys the Beer law.

In the development of the method it was necessary to solve two main problems: to find the conditions for the exhaustive extraction of ecdysterone from the plant raw material and to select the optimum conditions for its chromatographic separation from the accompanying substances. As the experiments showed, ecdysterone is most completely extracted by methanol. As the sorbents we used silica gel L5/40 μ, alumina (Brockmann activity II), and a mixture of silica gel with alumina — alusil (pH of a 10% aqueous suspension ∿7).

The optimum separation of ecdysterone from the accompanying substances takes place in a thin layer of silica gel—alumina—gypsum (3:2:0.16) in the chloroform—methanol—acetone (6:2:1) system. Ecdysterone was revealed on the chromatograms with a 1% solution of vanillin in sulfuric acid, the sensitivity of the analysis being 4 μg.

The ecdysterone was eluted from the sorbent with methanol. The completeness of desorption of the ecdysterone was checked by the chromatography of known amounts of a standard sample of it: followed by spectrophotometry of the eluate at 242 nm. Desorption takes place satisfactorily with an error of ±1.13% at the 95% probability level. The sensitivity of the quantitative method is 60 μg.

The UV spectrum of ecdysterone eluted from a plate was identical with that of a standard sample of the material. To check the reproducibility and correctness of the method, the amount of ecdysterone in one sample of raw material was determined in sextuplicate (Table 1).

Experiments with additions of pure ecdysterone (Table 2) showed that the error of the determination does not exceed ±4.5%.

EXPERIMENTAL

Analysis of the *Rh. orientale* Roots. About 10 g (to an accuracy of 0.01 g) of *Rh. orientale* roots comminuted and sieved through a sieve with apertures having a diameter of 0.5 mm was extracted with methanol in a Sokhlet apparatus for 8 h. The extract was concentrated in a rotary evaporator (at 40°C) to 25 ml. Plates with a fixed layer of alusil and having dimensions of 18 × 24 cm were divided into four parallel bands. In each of two of them was deposited 0.15-0.20 ml of the extract obtained and in the third a solution of a standard sample of ecdysterone, and the fourth band served as the background (blank experiment) for spectrophotometry. Chromatography was carried out in the chloroform—methanol—acetone (6:2:1) system. The plates were dried at room temperature and one of the bands with the solution under investigation and the band with the solution of the standard sample were treated with a 1% solution of vanillin in sulfuric acid. The zones of localization of the ecdysterone at the level of appearance of the spots were marked on the remaining part of the plate and they were quantitatively transferred into flasks with ground-glass stoppers. The ecdystrone was eluted from the sorbent with 15 ml of methanol with continuous shaking for 4 h. The optical densities of the eluates filtered through filter paper (Blue Band) were measured on an SF-16 spectrophotometer at 242 nm in cells with a layer thickness of 1 cm against the eluate of the blank experiment. The percentage of ecdysterone (x) referred to the absolutely dry raw material was calculated from the formula

$$x = \frac{C_{st} \cdot D_x V_1 \cdot V_3 \cdot 100 \cdot 100}{D_{st} \cdot V_2 \cdot a\,(100 - h) \cdot 1000},$$

TABLE 3. Results of a Determination of Ecdysterone in Various Samples of Raw Material

Species of plant and collection site	Part of the plant	Amount of ecdysterone, %, on the absolutely dry raw material		Maximum deviation from the mean
		material	found	
Rhaponticum orientale Khakass Autonomous Oblast, Shirinskii region	Inflorescences	0,282	0,276	±1,990
	Beginning of fruit bearing	0,271		
	Stems	0,258		
		0,260	0,259	±0,386
	Leaves	0,560		
		0,576	0,568	±1,408
	Roots	0,140		
		0,145	0,142	±1,754
Rhaponticum integrifolium, Osh oblast	Inflorescences	0,610	0,600	±1,660
		0,590		
	Stems	0,097	0,100	±3,482
		0,104		
	Leaves	0,097	0,095	±2,634
		0,092		
	Roots	0,300	0,304	±1,312
		0,308		

where D_x is the optical density of the solution under investigation; D_{st} is the optical density of the solution of the standard sample; C_{st} is the concentration of the solution of the standard sample, mg/ml; V_1 is the volume of eluate, ml; V_2 is the volume of solution under investigation deposited on the chromatogram, ml; V_3 is the volume of extractant, ml; a is the weight of the roots, g; and h is the amount of moisture in the roots, %.

Analysis of the epigeal part of the *Rh. orientale* was carried out similarly to the determination of the ecdysterone in the roots, and the results are given in Table 3.

SUMMARY

A chromato-spectrophotometric method is proposed for determining ecdysterone in the epigeal part of the roots of *Rhaponticum orientale* and *Rh. integrifolium*, the limit of detection of ecdysterone being 60 μg.

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