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METHOD FOR THE QUANTITATIVE DETERMINATION OF LAPPACONITINE IN THE EPIGEAL PART OF Aconitum orientale

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The alkaloids of some species of monkshood exhibit specific pharmacological activity [1]. A new antiarrhythmic drug based on the alkaloid lappaconitine has been created [2, 3]. In connection with this, we have developed a method for the quantitative determination of this alkaloid in the epigeal part of Caucasian monkshood growing in the Borzhomi region of Georgia [4] which consists in its chromatographic separation from accompanying alkaloids in a fixed layer of type KSK silica gel in the solvent system benzene–chloroform–ethanol–ammonia (40:40:10:0.1) and its spectrophotometric determination in the eluates. The desorption of the alkaloid by 95% ethanol is approximately 100%.

The analysis of the raw material was carried out in the following way. The comminuted air-dry raw material (20 g) was wetted (20 ml) with a 5% solution of sodium carbonate and the mixture was stirred and was left for 1 h, after which the raw material was exhaustively extracted with chloroform in a Soxhlet apparatus until the reaction with tungstosilicic acid was negative. The extract was concentrated in 20-25 ml and the total alkaloids were obtained in the usual way. The sulfuric acid extracts were brought to pH 8 with sodium carbonate. The chloroform extract was dried with anhydrous sodium sulfate and was filtered through a paper filter which was then washed with chloroform (3 × 3 ml). The chloroform was evaporated to dryness and the residue was dried to constant weight at 70°C and was dissolved in 20 ml of 95% ethanol. An 18 × 24 cm plate with a fixed layer of silica gel (particle size 0.16 mm) was separated into three equal parts. On the first band was deposited 0.2 ml of a 0.1% ethanolic solution of lappaconitine hydrobromide; and on the second, 0.2 ml of a 1% solution of the total ethanolic material. The third band was left as control. Chromatography was conducted by the ascending method in the system given above.

After drying in the air, the plate was examined in ultraviolet light. Sections of the sorbent from the three bands at the R_f level of lappaconitine were transferred into flasks, and treated with 10 ml of 95% ethanol; the flasks were shaken for 10-15 min and were left for 16-18 h. After another five-minute shaking, the ethanolic solutions were filtered through a Schott No. 4 funnel. The eluates were measured spectrophotometrically at a wavelength of 308 nm in comparison with the control.

The lappaconitine content, X, was calculated by means of the formula

$$X\% = \frac{C_{st} \cdot D_x \cdot 200 \cdot K}{D_{st} \cdot P \cdot V_1 \cdot (100 - h)},$$

where C_{st} is the concentration of the ethanolic solution of the standard sample, mg/ml; D_{st} is the optical density of the standard sample; D_x is the optical density of the sample under investigation; V_1 is the volume of the ethanolic solution of the total material deposited on the chromatogram, ml; h is the loss in mass on the drying of the raw material, %; P is the weight of the sample of raw material, g; and K is recalculation factor derived from the molecular masses of lappaconitine and its salt, equal to 0.89.

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The amount of lappaconitine in the epigeal part of the new material collected in 1985 was determined by the method developed. The results of a statistical treatment showed that the relative error of the method is about ±7%:

f	$\bar{X}$, %	S^2	S	P	$t_{p,f}$	ΔX	E, %	E_f, %
5	0,202	$36 \cdot 10^{-6}$	$6 \cdot 10^{-3}$	95	2,57	±0,015	±6,21	±2,53

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DYNAMICS OF THE ALKALOID CONTENT OF Lilium martagon

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The present communication gives the results of investigations of the dynamics of the accumulation of the total alkaloids in Lilium martagon growing in the high-mountain part of the South Urals (Malyi Yaman-Tau range), where there are large thickets of this plant [1-3].

Samples of L. martagon were extracted with ethanol, and the alkaloids were isolated with chloroform by a known procedure [4].

The results obtained (Table 1) showed that the greatest accumulations of alkaloids are confined to the early vegetation period, and in the withering period these indices decreased more than twofold. We may note that in our case the total amount of the alkaloids in the flowering period was less than in plants from Buryatia [4].

When chloroform extract was concentrated, a mixture of crystals deposited in the separation of which on a column of silica gel the first 40 ml of chloroform–methanol (10:0.5) eluates yielded a base with mp 118-119°C (acetone), identical in R_f and melting point of a mixture with an authentic sample of lilidine [4, 5]. On the separation of the mother solution from the crystals on a column of alumina a chloroform–methanol (10:0.5) eluate yield an additional amount of lilidine.

TABLE 1

Developmental period	Time of collection (1989)	Amount of total alkaloids, %	
		in the epigeal part	in the bulbs
Beginning of the vegetation period	20 April	—	0,358
	30 April	0,160	0,346
Appearance of shoots (1-2 cm long)	18 May	0,121	0,247
Growth of the stem to 20 m	30 June	0,117	0,207
Flowering	20 August	0,082	0,159
Ripening of the seeds	1 September	0,063	0,150
Beginning of weathering			

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