POLAROGRAPHIC DETERMINATION OF CYTISINE IN THERMOPSIS ALTERNIFLORA

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The alkaloid cytisine, which is used in medicine in reflex cessations of respiration and in operations [1] is obtained industrially from the seeds of Thermopsis lanceolate with a yield of about 0.8% [2].

In a preceding communication [3], a technology of the isolation of cytisine from the epigeal part of T. alterniflora Rgl. with a yield of about 1.2% has been proposed.

The known gravimetric methods of determining the content of cytisine in plant raw material are time- and labor-consuming [4, 5]. The method recommended in GF X [Russian State Pharmacopeia, X-th Edition] is suitable only for a pure preparation [6].

In this paper we propose a method for determining the amount of cytisine in plant raw material which consists in the extraction of the total alkaloids from the plant with the subsequent direct polarographic determination of the cytisine in the mixture.

As a preliminary, we studied the polarographic behavior of cytisine and pachycarpine isolated from T. alterniflora [7]. In view of the fact that the qualitative composition of this mixture of alkaloids may be the object of a further study, we also investigated the behavior of the alkaloids found in other species of thermopsis [8].

Polarography was carried out in aqueous and aqueous ethanolic solutions in the presence of 0.1 N $(C_2H_5)_4NOH$. All the alkaloids are polarographically active in an aqueous medium, and the polarographic indices were found for equimolar concentrations of them (Table 1).

Table 1. Polarographic Indices of the Alkaloids of Thermopsis in an Aqueous Medium 0.1 N with Respect to $(C_2H_5)_4NOH$ (C_{alk} 1.00 mM/l)

Alkaloid	i'	i''	$E'_{1/2}$	$E''_{1/2}$
	μA		V	
Cytisine	—	44.54	—	−2.20
N-Methyl-cytisine	2.93	3.66	−1.90	−2.12
Thermopsine	3.98	2.30	−1.90	−2.20
Pachycarpine	—	0.90	—	−1.94

The results of a study of the nature of the currents obtained have shown that for all the alkaloids the first wave is a diffusion wave and the second is a catalytic hydrogen wave. Under these conditions cytisine forms only one catalytic wave, with a characteristic hump-like form and a limiting current exceeding by a factor of 10–20 the catalytic currents of the other alkaloids. The heights of the catalytic waves obtained are increased somewhat when the height of the mercury column above the dropping electrode is reduced and undergoes scarcely no change when the temperature is raised (temperature coefficient 0.3% per 1° C); the nature of the current strength–concentration curve shows the adsorption of the catalyst on the surface of the electrode.

The introduction of an organic solvent (ethanol) into the solution under investigation leads to a fall in the catalytic currents (Table 2). As can be seen from the table, at a concentration of ethanol greater than 30% cytisine exhibits a first wave analogous to the first wave of N-methylcytisine and thermopsine, while in 80% ethanol only cytisine retains its catalytic activity. We made use of the latter circumstance for the quantitative determination of cytisine in the presence of the accompanying alkaloids. The analysis of synthetic mixtures of alkaloids with different contents of the possible components on a support of 0.1 N $(C_2H_5)_4NOH$ in 80% ethanol showed that the first wave with $E_{1/2}$ of −1.75 to −1.85 V corresponds to the combined cytisine, N-methylcytisine, and thermopsine, and the second

wave with $E_{1/2}$ of −2.10 to −2.20 V corresponds to the amount of cytisine (Table 3). The height of the cytisine wave was measured as the height of the maximum [9]. The concentration of alkaloid was determined by the method of standard solutions [10]. A direct proportionality between the height of the wave and the concentration of cytisine is observed between 5×10^{-4} and 2.50×10^{-3} mole/l. The objectivity of the polarographic method was checked by an analysis of extracts with the pure material added to them. The relative error of the determination did not exceed ±5%. We have found that the total alkaloids from T. alterniflora collected in the budding period contain 57–59% of cytisine (1.0–1.90% of cytisine on the weight of the absolutely dry raw material).

Table 2. Polarographic Indices of Thermopsis Alkaloids in Aqueous Ethanol 0.1 N with Respect to $(C_2H_5)_4NOH$ (concentration of the alkaloids 2.50 mM/l)

Concentration of ethanol, %	Cytisine				N-Methylcytisine				Thermopsine				Pachycarpine			
	i′	i″	$E'_{1/2}$	$E''_{1/2}$	i′	i″	$E'_{1/2}$	$E''_{1/2}$	i′	i″	$E'_{1/2}$	$E''_{1/2}$	i′	i″	$E'_{1/2}$	$E''_{1/2}$
	μA		V		μA		V		μA		V		μA		V	
—	—	111.35	—	−2.20	7.34	9.17	−1.88	−2.10	9.96	5.75	−1.90	−2.25	—	2.12	—	−1.94
20	—	70.75	—	−2.21	7.07	4.72	−1.90	−2.23	6.81	—	−1.91	—	—	—	—	—
40	5.90	53.70	−1.85	−2.22	7.07	—	−1.92	—	6.07	—	−1.90	—	—	—	—	—
60	5.25	32.75	−1.88	−2.24	7.34	—	−1.91	—	5.24	—	−1.89	—	—	—	—	—
80	5.90	22.28	−1.86	−2.25	7.34	—	−1.92	—	6.03	—	−1.90	—	—	—	—	—

EXPERIMENTAL

The work was carried out on a LP-55A polarograph. Characteristics of the capillary at h_{Hg} = 55 cm: m = 2.175 mg·sec^{-1}, t = 2.0 sec in 1 N KCl. An electrolyzer with an internal anode was used; oxygen was eliminated by purging with electrolytic hydrogen; the temperature of the determinations was 25 ± 0.5° C. The supporting electrolyte was 0.1 N $(C_2H_5)_4NOH$ in 80% ethanol.

Table 3. Results of the Determination of Cytisine in Synthetic Mixtures on a Support of 0.1 N $(C_2H_5)_4NOH$ in 80% Ethanol

Composition of the mixture	No. 1			No. 2			No. 3			No. 4		
	taken	found		taken	found		taken	found		taken	found	
	mg/ml		% rel. error	mg/ml		% rel. error	mg/ml		% rel. error	mg/ml		% rel. error
Cytisine	0.210	0.200	−4.76	0.180	0.187	+3.89	0.334	0.338	+1.33	0.224	0.227	+1.34
N-Methylcytisine	0.172	—	—	0.344	—	—	0.190	—	—	0.101	—	—
Thermopsine	0.203	—	—	0.400	—	—	0.229	—	—	0.122	—	—
Pachycarpine	0.302	—	—	0.606	—	—	0.682	—	—	0.182	—	—

Preparation of a solution of a standard sample. Cytisine satisfying the requirements of GF X was used as the standard sample; 50–100 mg (accurately weighed) was dissolved in 96% ethanol in a 50-ml measuring flask, and 5 ml of the resulting solution was diluted with the supporting electrolyte in a 25-ml measuring flask. The standard solution contained 0.2–0.4 mg/ml of cytidine.

The combined alkaloids were obtained by the usual method [4] from 10 g of air-dry raw material, which was covered with 400 ml of chloroform and extracted for 2 hr. To isolate the total alkaloids, 100 ml of the chloroform extract was taken.

Polarographic determination. The total alkaloids obtained were dissolved in 5–10 ml of 96% ethanol and the solution was transferred quantitatively to a 50-ml measuring flask and made up to the mark with ethanol. 5 ml of the ethanolic solution of the total alkaloids was placed in a 25-ml measuring flask and made up to the mark with the supporting electrolyte. The solution so prepared contained 0.2–0.4 mg/ml of total alkaloids. An aliquot of this solution (3–5 ml) was transferred into the electrolyzer, hydrogen was passed for 10–15 min, and polarography was carried out with cathodic polarization of the dropping electrode in the range from −1.5 to −2.5 V. With the same galvanometer shunt an aliquot (3–5 ml) of the standard solution was polarographed. The heights of the waves obtained (maxima) with $E_{1/2}$ = −2.10 to −2.20 V were determined and the cytisine content was calculated from the formula given below. The alcoholic solutions of the total alkaloids and of the standard cytisine were diluted with the supporting electrolyte immediately before polarography.

Calculation of the results. The content of cytisine (X %) referred to the absolutely dry raw material was

calculated from the formula

$$X = \frac{10 H_x \cdot C_{st} \cdot m \cdot v \cdot w}{H_{st} P (100 - h) \cdot n \cdot a},$$

where P is the weight of the raw material, g;

h—moisture content of the raw material, %;

H_x—height of the wave of the substance being determined, mm;

H_{st}—height of the wave of the standard substance, mm;

C_{st}—concentration of the standard substance, mg/ml;

m—total volume of chloroform extract, ml;

n—volume of the chloroform extract taken to obtain the total alkaloids, ml;

v—volume of the measuring flask in which the combined alkaloids were dissolved, ml;

a—volume of the ethanolic solution of the combined alkaloids taken for dilution with the supporting electrolyte, ml; and

w—volume of the measuring flask in which the dilution of the ethanolic solution of the combined alkaloid with the supporting electrolyte was carried out, ml.

CONCLUSIONS

A polarographic method has been developed for the determination of the content of cytisine in the combined alkaloids from Thermopsis alterniflora Rgl.

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