CHROMATOPOLAROGRAPHIC DETERMINATION OF THE AMOUNT OF CYTISINE IN *Thermopsis alterniflora*

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We have previously reported a polarographic determination of cytisine in the combined alkaloids obtained from plant raw material [1]. However, this simple and rapid method cannot be used in many cases since on working at the limit of the negative range of potentials ($E_{1/2}$ of the second, catalytic, wave of cytisine is −2.25 V relative to the potential of the mercury pool) careful purification of the solvent and of the supporting electrolyte is necessary.

In view of this, we have developed a chromatopolarographic determination based on the use of the first, diffusion, wave with $E_{1/2}$ = −2.00 to −2.10 V on a support of 0.1 N $(C_2H_5)_4NOH$ in 80% ethanol. Direct proportionality between the diffsuion currents and the concentration of alkaloid is observed in the range from 0.1 to 1.0 mg/ml.

In addition, we studied the alteramine isolated from Thermopsis alterniflora [2], which also possesses a diffusion wave with $E_{1/2}$ = −2.05 to −2.10 V and a hydrogen catalytic wave with $E_{1/2}$ = −2.20 V. All the alkaloids of thermopsis showed diffusion waves with similar values of $E_{1/2}$, and therefore to separate the cytisine from the accompanying bases we used thin-layer chromatography in a fixed layer of silica gel in the chloroform–acetone–methanol–ammonia (30 :25 :8 :1) system. The R_f values for cytisine, N-methylcytisine, thermopsine, pachycarpine, and alteramine are 0.30, 0.50, 0.70, 0.11, and 0.90, respectively. Elution with ethanol gives 98-100% desorption. The accuracy of the method was checked by analysis of model mixtures and of extracts with the addition of the individual alkaloid. The relative error of the determinations is ±5%.

The isolation of the combined alkaloids from the raw material and the polarographic determination was performed as described previously [1], with the difference that we used the indices of the first cytisine wave for calculation. The combined alkaloids were dissolved in 5 ml of ethanol; 0.2-0.5 ml of the solution was chromatographed on a plate (13 × 18 cm) in the system mentioned above. A cytisine marker was deposited on the same plate and this was revealed with the Dragendorff reagent. The section of the sorbent with the cytisine was eluted with 50 ml of ethanol in a Schott No. 4 funnel, the eluate was evaporated to dryness, the residue was dissolved in 2 ml of ethanol, 0.5 ml of 0.5 N $(C_2H_5)_4$ NOH was added, and polarography was performed. The concentration was calculated by the method of standard solutions; as the latter we used a solution of cytisine with C = 0.2-0.4 mg/ml. The cytisine content (x, %) referred to the dry raw material was calculated from the formula

$$x = \frac{40 \cdot H_x \cdot C_{st} \cdot v_1 \cdot v_3}{p \cdot H_{st} (100 - h) \cdot v_2},$$

where p is the weight of the sample of raw material, g; h is the moisture content of the raw material, %; h_x and H_{st} are the heights of the waves of the substances being determined and of the standard substance, mm; C_{st} is the concentration of the solution of the standard sample, mg/ml; v_1 is the volume of ethanol in which the combined alkaloids were dissolved, ml; v_2 is the volume of the solution of the combined alkaloids deposited on the chromatogram, ml; and v_3 is the volume of the solution in the electrolyzer, ml.

The dynamics of the accumulation of cytosine in Thermopsis alterniflora is being studied by this method.

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PROTAMINES

THE PEPTIDES FROM THE THERMOLYSIN HYDROLYSIS OF STURINE B

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In a preceding paper, we gave the results of a study of the partial amino-acid sequence of sturine B [1]. To determine its complete sequence it was necessary to determine the number of arginine residues in blocks. For this purpose we have performed the hydrolysis of sturine B with thermolysin produced by the firm "Sigma" from Bacillus thermoproteolyticus Rokko.

Hydrolysis was performed in 0.02 M tris-hydrochloride buffer, pH 8.0, containing 0.005 M $CaCl_2$ (40°C, 10 h) at an enzyme–substrate ratio of 1:75 (by weight). The resulting mixture of peptides was separated by ion-exchange chromatography on a 1×25 cm column of carboxymethyl-Sephadex G-25 equilibrated with 0.05 M phosphate buffer, pH 6.15. For elution we used a step-exponential NaCl gradient. The peptides were purified additionally on Bio-Gel P2 (column 1 × 150 cm; elution with 0.02 N HCl).

The structures of the majority of the peptides were established on the basis of their amino-acid compositions and the determination of their N-terminal amino acids by the dinitrophenylation [2] and dansylation [3] methods and of their C-terminal amino acids with carboxypeptidases A and B [1] (Table 1).

From a trypsin hydrolyzate of sturine B [1] the peptide TI Ser-Ser-Arg-Pro-Glx-Arg has been isolated. The results of a comparison of the structure of this peptide with those on the amino-acid composition and the N- and C-terminal amino acids of peptide TmV, TmVI-1, and TmVI-2 made it possible to determine the primary structures of these peptides.

Because of the presence in the sturine B molecule of the sequences Arg-Ser↓-Ser↓ and Arg-His-Gly, in which thermolysin hydrolysis affects all the bonds shown by arrows, the overlapping peptides TmIV-2, TmIV-1, TmV, TmVI-1, TmVI-2, TmIII, and TmI were obtained. A comparison of these results with those

TABLE 1

Peptide	Amino-acid composition (in residues)	Amino acid		Structure of the peptides
		N-terminal	C-terminal	
Tm1	Arg1, 9, Gly1, 0	Gly	Arg	Gly-Arg_2
Tm2	Arg 3, 8, Gly1, 0	Gly	Arg	Gly-Arg_4
Tm	His 0,9, Arg1, 1,9, Gly 1,0	His	Arg	His-Gly-Arg_2
TmIV-1	Arg 4,7, Ser 0,9, Ala 1,0	Ala	Ser	Ala-Arg_5-Ser
TmIV-2	Arg 4,8, Ala, 1,0	Ala	Arg	Ala-Arg_5
TmV	Arg 6,6, Ser 1, 8, Pro 1,0; Glx 1,0	Ser	Arg	Ser-Ser-Arg-Pro-Glx-Arg_6
TmVI-1	His 0,9, Arg 6,8, Ser 1,7 Pro 1,0, Glx 1,0	Ser	His	Ser-Ser-Arg-Pro-Glx-Arg_6-His
TmVI-2	His 0,9, Arg 7,0, Ser 1,1 Pro 1,0, Glx 1,0	Ser	His	Ser-Arg-Pro-Glx-Arg_6-His

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