of the catalytic current: the higher the pK_a values the greater the catalytic current. Consequently, by arranging the alkaloids in order of increasing catalytic current we shall obtain information on the relative basicities of this series of compounds.

EXPERIMENTAL

The polarographic measurements were performed in an electrolyzer with an internal anode on a LP-55A polarograph; characteristics of the capillary at $h_{Hg} = 45$ cm: $m = 0.76$ mg · sec^{-1}, $t = 3.5$ sec in 1 N KCl. The temperature of the determinations was 25 ± 0.5°C.

SUMMARY

1. The polarographic behavior of six benzoisoquinoline alkaloids has been investigated. In the reduction of thalfine, thalsimine, and thalsimidine the products of the electrode reactions are the corresponding tetrahydro derivatives.

2. The size of the catalytic current can be used for a comparative evaluation of the basicities of this series of alkaloids.

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METHOD FOR THE QUANTITATIVE DETERMINATION OF PROTOPINE IN Fumaria vaillantii

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The alkaloid protopine [1] isolated from epigeal part of Fumaria vaillantii Loisel. (family Papaveraceae) is pharmacologically active [2, 3]. In the present paper we give the results of a polarographic investigation of protopine and propose a method for its quantitative determination in plant raw material.

Polarographic waves have been obtained previously [4] for protopine, but they were unsuitable for analytical purposes. We have detected distinct diffusion waves of this alkaloid with $E_{1/2} = -1.80$ to -1.85 V in 0.1 N $(C_2H_5)_4NOH$ in 70% dioxane. A linear relationship was found between the current and the concentration of protopine in the solution in the range from $2.9 \cdot 10^{-3}$ to $2.9 \cdot 10^{-4}$ M.

In the range of working concentrations, the polarographic behaviors of the alkaloid and of its hydrochloride are similar. This circumstance has been used for the quantitative determination of protopine by the method of standard solutions, with the introduction into the formula for calculation of a correction factor equal to the ratio of the molecular weights of the alkaloid and its salt. In order to determine the mechanism of the

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electrode process the number of electrons participating in the the reaction was determined by polarographic microcoulometry [5] and found to be two, and the product obtained as the result of prolonged electrolysis at a controlled potential was investigated. When this product was chromatographed in a fixed layer of silica gel in the chloroform−acetone (8 : 2) system, a spot with R_f 0.65 corresponding to dihydroprotopine was found. Consequently, it is the carbonyl group of protopine that undergoes polarographic reduction. Dihydroprotopine [6] has no diffusion wave, which confirms the mechanism suggested above.

In the case of protopine in buffer solutions at pH 6-11 a two-electron diffusion wave corresponding to the reduction of the carbonyl group was observed. At $\mathrm{pH} < 6$, the diffusion wave diminishes, which is due to the conversion of the polarographically active carbonyl group into a carbinol group under the action of acids [7].

From the epigeal part of *Fumaria vaillantii* more than 10 alkaloids, in addition to protopine, have been isolated [8]. To separate protopine from the accompanying alkaloids we used thin-layer chromatography in a nonfixed layer of neutral alumina (activity grade II). On elution with chloroform 98-100% desorption was achieved. The accuracy of the method was checked by the analysis of model mixtures of alkaloids and extracts with the addition of the pure base. The relative error of the determinations did not exceed ±5%. Using the method developed, we have determined the amounts of protopine in plant raw material collected in the fruit-bearing period in the Tashkent oblast in 1972-1976: 0.14, 0.12, 0.19, 0.07, and 0.13% of the weight of the dry raw material, respectively.

EXPERIMENTAL

A LP-55A polarograph was used. The characteristics of the capillary at $h_{Hg} = 45$ cm were: $m = 0.76$ $\mathrm{mg \cdot sec^{-1}}$, $t = 3.5$ sec in 1 N KCl. An electrolyzer with an internal anode was used, and the temperature of the determinations was 25 ± 0.5°C.

Analysis of the Raw Material. About 20 g (weighed with an accuracy of 0.01 g) of comminuted air-dry raw material was moistened with 20 ml of 5% sodium carbonate solution, the mixture was stirred, and the alkaloids were exhaustively extracted with chloroform. The chloroform extract was concentrated to 10-15 ml and was transferred quantitatively to a 25-ml measuring flask, and the volume was made up to the mark with chloroform. On the starting line of a plate (13 × 18 cm) with a nonfixed layer of alumina (particle size 0.25-0.1 mm, layer thickness 1.5 mm) we deposited 1 ml of the chloroform extract, and chromatography was carried out in the benzene−chloform−acetone (10 : 10 : 6) system. A plate with a "marker" (0.5 ml of a 0.1% ethanolic solution of protopine hydrochloride) was run under the same conditions, and the spots were revealed in the moist state with the Dragendorff reagent. The section of the sorbent corresponding to the protopine spot with R_f 0.6 ± 0.1 was eluted with 50 ml of chloroform in a Schott No. 4 funnel. The eluate was evaporated to dryness, and the residue was dissolved in 2.5 ml of 0.1 N $(C_2H_5)_4NOH$ in 70% dioxane and polarographed in the range from −1.0 to −2.5 V. A standard solution of protopine hydrochloride containing 0.5 mg/ml was polarographed under the same conditions. The amount of protopine x calculated on the dry raw material was found from the formula (%):

$$x = \frac{10 \cdot 0{,}906 \cdot C_{\mathrm{st}} \cdot H_x \cdot V_1 \cdot V_2}{p \cdot H_{\mathrm{st}} \cdot V_3 (100 - h)},$$

where H_{st} and H_s are the heights of the waves of the solutions of the standard sample and that undergoing investigation, mm; C_{st} is the concentration of the solution of the standard sample, mg/ml; V_1 is the volume of the chloroform extract, ml; V_2 is the volume of the solution in the electrolyzer, ml; V_3 is the volume of the chloroform extract deposited on the chromatogram, ml; p is the weight of the raw material, g; h is the moisture content of the raw material, %; and 0.906 is a calculation factor equal to the ratio of the molecular weights of the alkaloid and its hydrochloride (353.32/389.82).

SUMMARY

In an investigation of the polarographic behavior of protopine against a background of 0.1 N $(C_2H_5)_4NOH$ in 70% dioxane, it was found that the product of the electrode reaction is dihydroprotopine. A chromatopolarographic method has been proposed for determining protopine in *Fumaria vaillantii* Loisl.

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