POLAROGRAPHIC DETERMINATION OF GALANTHAMINE IN UNGERNIA VICTORIS

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At present, the alkaloid galanthamine, which is used in medicine in the form of the hydrobromide [1], is isolated from the epigeal part of Ungernia victoris Vved. (family Amaryllidaceae).

To determine the content of galanthamine in the plant raw material according to MRTU [Interrepublic Technical Standard] 42 No. 2912–62, a gravimetric method is used which consists of extracting the combined alkaloids from the raw material, subsequent precipitation of the alkaloid with hydrobromic acid, and the isolation and weighing of the hydrobromide. This method (which essentially repeats the industrial method on the micro scale) is time-consuming and laborious.

Methods described in the literature [2–6] are suitable only for the pure material. We propose the polarographic determination of galanthamine in a chloroform extract from the plant material. As a preliminary step, the polarographic behavior of galanthamine and the alkaloids accompanying it, lycorine, pancratine, hordenine, and *dl*-narwedine [7, 8], were studied. Polarography was carried out in aqueous and ethanolic aqueous media in the presence of $(C_2H_5)_4NI$ and $(C_2H_5)_4NOH$. Well-defined waves were observed on a support of 0.01 N $(C_2H_5)_4NOH$.

The polarographic indices of the alkaloids of Ungernia obtained under these conditions [aqueous medium, 0.01 N $(C_2H_5)_4NOH$, $c_{alk} = 0.4$ mm/*l*] are given below:

Alkaloid	i, μa	$E_{1/2}$, V
Galanthamine	8.58	−2.05
Lycorine	0.27	−2.05
Pancratine	0.23	−2.05
Hordenine	8.45	−2.45
dl-Narwedine	5.56	−2.00

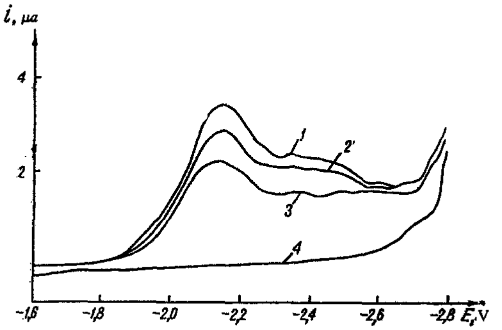
Lycorine and *dl*-narwedine are practically insoluble in water and therefore they were polarographed in 40% ethanol. The catalytic nature of the waves formed was established. The heights of the waves do not depend on the height of the mercury column above the dropping electrode, and with a decrease in the concentration of the supporting electrolyte from 0.2 to 0.01 N they increase ten- to fifteen-fold; the temperature coefficient is 3% per degree. An organic solvent (ethanol) reduces the catalytic currents and in 60% ethanol the alkaloids exhibit no polarographic activity. Direct proportionality between the height of the wave and the concentration of galanthamine is preserved in the range from $2 \cdot 10^{-4}$ to $2 \cdot 10^{-5}$ M. For analytical purposes, the maximum current of the wave (figure), which is proportional to the limiting current [9], was measured; the concentration of alkaloid was determined by the method of standard solutions [10].

The polarographic behaviors of the Ungernia alkaloids are similar, and therefore in mixtures it is possible to determine only their total amount.

The galanthamine was separated from the accompanying alkaloids by thin-layer chromatography in a nonfixed layer of alumina (Brockmann activity grade III) in a chloroform–methanol (9.75 : 0.25) system. Galanthamine, lycorine, pancratine, hordenine, and *dl*-narwedine have the following R_f values, respectively: 0.63, 0.1, 0.07, 0.1, and 0.01. On elution with chloroform, 95–98% desorption of the galanthamine is achieved.

The method was checked by analyzing model mixtures of alkaloids (table) and extracts with the addition of the pure material. The relative error of the determinations was ±5%. We have applied the method developed to the analysis of plant raw material. The results of the analysis were compared with the results obtained by the gravimetric method. The figures found for galanthamine by the polarographic and the gravimetric methods (%) were:

in raw material gathered in: 1967) 0.109 and 0.095, 1968) 0.095 and 0.080, 1969) 0.078 and 0.070, respectively. Some loss of material is possible in the isolation of galanthamine by the gravimetric method, and therefore the polarographic method is preferable.



Polarographic curves of galanthamine on a support of 0.01 N $(C_2H_5)_4$ NOH: 1) 0.19 mM/l, 2) 0.16 mM/l; 3) 0.12 mM/l; 4) support.

EXPERIMENTAL

The work was carried out on an LR-55 polarograph. The characteristics of the capillary at h_{HG} 50 cm were: m 1.03 mg · sec^{-1}, t 4.0 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.

Results of the Determination of Galanthamine in Model Mixtures of Alkaloids (I–III) on a Support of 0.01 N $(C_2H_5)_4$ NOH

Composition of the mixture	I			II			III		
	taken, mg/ml	found, mg/ml	% rel. error	taken, mg/ml	found, mg/ml	% rel. error	taken, mg/ml	found, mg/ml	% rel. error
Galanthamine	0,042	0,041	–2,38	0,049	0,047	–4,25	0,059	0,058	–1,69
Lycorine	0,032	—	—	0,028	—	—	0,058	—	—
dl-Narwedine	0,038	—	—	0,012	—	—	0,055	—	—
Pancratine	0,028	—	—	0,031	—	—	0,046	—	—
Hordenine	0,032	—	—	0,048	—	—	0,043	—	—

Preparation of the extract. About 10 g (accurately weighed) of the air-dried raw material previously moistened with 10 ml of 8% ammonia solution was exhaustively extracted with chloroform in a Soxhlet apparatus. The extract was concentrated to a volume of 15–20 ml, quantitatively transferred to a 50-ml volumetric flask and made up to the mark with chloroform.

Chromatopolarographic determination. The chloroform extract (0.5–1 ml) was deposited on a plate (13 × 18 cm) in the form of a continuous band together with a marker (galanthamine). Chromatography was carried out in the system given above; the marker was revealed in the moist state with Dragendorff's reagent. The galanthamine was eluted in a Schott No. 3 funnel with 50 ml of chloroform. The eluate was evaporated to dryness, and the residue was dissolved in 2.5 ml of 0.01 N aqueous $(C_2H_5)_4$ NOH and polarographed at a cathodic polarization of the dropping electrode of −1.6 to −2.4 V. A standard solution of galanthamine containing 0.03–0.04 mg/ml was polarographed under the same conditions. The galanthamine was the eluate obtained in the chromatography of 0.5–1.0 ml of a solution of galanthamine with a concentration of 0.20 mg/ml; a chromatographically homogeneous sample with mp 127–129° C was used. The height of the wave obtained with $E_{1/2}$ = −2.00 to −2.10 V was determined. The percentage of galanthamine calculated on the absolutely dry raw material was determined from the formula

$$X = \frac{10 \cdot C_{st} \cdot H_x \cdot V \cdot b}{H_{st} \cdot p \cdot (100 - h) \cdot a},$$

where p is the weight of the raw material, g;
H_x is the height of the wave of the solution under investigation, mm;
H_{st} is the height of the wave of the solution of the standard sample, mm;
C_{st} is the amount of galanthamine in 1 ml of standard solution, mg;

h is the moisture content of the raw material, %;
V is the volume of the chloroform extract, ml;
a is the volume of the chloroform extract deposited on the plate, ml;
b is the volume of the solution in the electrolyzer, ml.

CONCLUSIONS

1. The polarographic behavior of galanthamine, lycorine, pancratine, hordenine, and *dl*-narwedine on a support of 0.01 N $(C_2H_5)_4NOH$ has been studied.

2. A chromatopolarographic method for determining the amount of galanthamine in a chloroform extract from plant raw material has been developed.

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