QUANTITATIVE DETERMINATION OF NARWEDINE IN *Ungernia victoris* AND *U. Severtzovii*

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The alkaloid narwedine, isolated from the epigeal part of *Ungernia victoris* [1] and *U. severtzovii* [2], family Amaryllidaceae, possesses valuable pharmacological properties [3].

In the present paper we propose a polarographic method for the quantitative determination of this alkaloid in plant raw materials. A polarographic study of the alkaloids of *U. victoris* in aqueous ethanol in the presence of 0.1 N $(C_2H_5)_4NOH$ showed that under these conditions they all possess catalytic hydrogen waves [4]. However, in addition to a catalytic wave, narwedine also has a diffusion wave with $E_{1/2} = -1.60$ V (SCE). A linear relationship between the magnitude of the current and the concentration of the alkaloid in solution is observed at concentrations from $1.5 \cdot 10^{-2}$ to $3.5 \cdot 10^{-4}$ M.

It was established by the method of least squares [5] that the calibration graphs describe an equation of the form $y = a + bx$, and the method does not include a systematic error. Below we give the results of a calculation of the parameters of the linear calibration graph for narwedine:

	x_i	y_i	x^2_i	$x \cdot y_i$	y^2_i	$(y-y)^2$
$\sum$	7.9	17.596	15.17	33.589	76.639	0.0243

statistical data: $a = 3.9 \cdot 10^{-2}$; $b = 2.2$; $S_y^2 = 4.8 \cdot 10^{-2}$; $S_a = 1.7 \cdot 10^{-2}$; $t_a = 0.2$; $p = 0.830$; $b_{corr} = 0.221$.

The concentration of alkaloids in the raw material was determined by the method of standard solutions [6]. For the latter we used narwedine hydrobromide, since the polarographic indices of the alkaloid and its salt are identical (in the range of working concentrations). In this case we used in the calculation formula a correction factor equal to the ratio of the molecular weights of the alkaloid and its salts. On adding an organic solvent (ethanol) to the solution, the catalytic activity of the alkaloids of *U. victoris* fell, and in 80% ethanol in 0.1 N $(C_2H_5)_4NOH$ only the diffusion wave of narwedine remained on the polarograms. This fact was taken into account in the quantitative determination of narwedine directly in the total alkaloids from *U. victoris*.

In the epigeal part of *U. severtzovii*, in addition to the alkaloids present in *U. victoris*, we isolated ungerine, hippeastrine, ungminorine, and tazettine [2]. Of these, ungerine and hippeastrine also form diffusion waves under the conditions described above with $E_{1/2}$ values close to that of narwedine. Consequently, for the quantitative determination of narwedine in this raw material we used a chromatopolarographic method.

Narwedine was separated from the accompanying bases by thin-layer chromatography in a nonfixed layer of alumina (activity grade III) in the chloroform–methanol–acetone (8:1:4) system, the R_f value for narwedine being 0.8 ± 0.05. Elution with chloroform achieved 98-100% desorption. The accuracy of the method was checked by analyzing model mixtures of alkaloids (Table 1) and extracts with the addition of the pure base. The relative error

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TABLE 1. Results of Determinations of Narwedine in Model Mixtures

Amount taken, mg			Narwedine found		Statistical data
narwedine	hippeastrine	ungerine	mg	%	
1,05	1,40	1,20	0,990	96,28	$\bar{x} = 95{,}36\%$
0,59	0,75	1,15	0,505	95,28	$S^2 = 1{,}64$
					$S_{\bar{x}} = 0{,}57$
0,40	0,45	0,30	0,376	94,00	$\alpha = 0{,}95$
0,86	0,20	0,25	0,827	96,21	$t_{\alpha \cdot k} = 2{,}906$
					$\varepsilon_a = 1{,}31$
0,60	1,01	0,80	0,582	97,03	$P_{rel} = \pm 1{,}37\%$

of the determination did not exceed ±6.0%. The amounts of narwedine in plant raw material were determined by the method developed: in *U. victoris* (1971, 1972, and 1973 crops) 0.01, 0.01, and 0.008%, respectively; in *U. severtzovii* (1971, 1972, and 1973 crops) 0.025, 0.020, and 0.028, respectively.

EXPERIMENTAL

The work was performed in an LP-55 polarograph. The characteristics of the capillary at h_{Hg} = 50 cm were m = 0.42 mg·sec^{-1}, t = 4.9 sec in 1 N KCl. An electrolyzer with an internal anode was used; oxygen was purged with electrolytic hydrogen; the temperature of the determinations was 25 ± 0.5°C.

Determination of Narwedine in *U. victoris.* The combined alkaloids [7] necessary for analysis were obtained from 50 g of the plant raw material; they were dissolved in 2 ml of ethanol, 0.5 ml of 0.5 N $(C_2H_5)_4NOH$ was added, and polarography was carried out with a cathodic polarization of the dropping electrode of from −1.0 to −2.0 V. The solution of a standard sample of narwedine hydrobromide with a concentration of 0.5 mg/ml was polarographed under the same conditions. The heights of the waves obtained with $E_{1/2}$ = −1.35 to −1.45 V were found. The narwedine content (x, %) calculated on the dry raw material was found from the formula

$$x = \frac{0{,}779 \cdot 25 \cdot C_{st} \cdot H_x}{H_{st} \cdot p\,(100 - h)},$$

where C_{st} is the concentration of the solution of the standard sample, mg/ml; H_{st} is the height of the wave of the standard substance, mm; H_x is the height of the wave of the substance being determined, mm; P is the weight of the raw material, g; h is the moisture content of the raw material, %; and 0.779 is a calculation factor equal to the ratio of the molecular weights of the alkaloid and its hydrobromide (285.35/366.27).

Determination of Narwedine in *U. severtzovii.* The combined alkaloids obtained from 50 g of the plant were dissolved in 2 ml of chloroform and the solution was filtered. A continuous band of 0.5-1.0 ml of the filtrate was deposited on a plate (13 × 18 cm) and adjacent to it was placed a "marker" — 0.1 ml of an ethanolic solution of narwedine hydrobromide with a concentration of 0.5 mg/ml. Chromatography was performed in the system indicated, and the spots were revealed in UV light. The narwedine was eluted with 100 ml of chloroform into a Schott No. 3 funnel, the eluate was evaporated to dryness, the residue was dissolved in 2 ml of ethanol, 0.5 ml of 0.5 N $(C_2H_5)_4NOH$ was added, and polarography was performed as described above. The amount of narwedine in the raw material was calculated from the formula given taking the additional dilution into account.

SUMMARY

A polarographic method for the quantitative determination of narwedine in *Ungernia victoris* and a chromatopolarographic method for its determination in *U. severtzovii* have been proposed.

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