THE SPECTROPHOTOMETRIC DETERMINATION OF PTERYXIN

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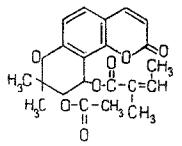
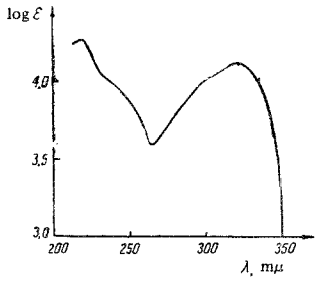
Pteryxin, 3'-acetyl-4'-angeloyl-2', 2'-dimethylpyrano-5', 6':7, 8-coumarin, possesses a spasmolytic action with a myotropic and neurotropic nature



The pharmacological study and clinical trials of pteryxin have now been completed. In this connection, the necessity has arisen for the development of a method for the quantitative determination of pteryxin in plant raw material—the roots of Libanotis condensata (L) Grants and in the crystalline powder.

The roots of the plant contain, besides pteryxin, a number of other natural coumarins [1–3]. In the method developed for the determination of pteryxin, a stage has been introduced in which the coumarin derivatives are separated by thin-layer chromatography. The separation was carried out on a plate with a nonfixed layer of KSK silica gel, GOST [State Standard] 3956/54, using n-hexane–benzene–methanol (5 : 4 : 1) as the mobile phase. In UV light, pteryxin possesses a violet fluorescence; R_f 0.47 (figure). The UV spectrum of pteryxin has a strong absorption band with a molar absorption coefficient of 12931 at 322 mμ, which is characteristic for 7, 8-dihydropyranocoumarins, with a specific absorption coefficient of 335 ± 1.13 (mean of 25 independent determinations). This opens up the possibility of the development of a spectrophotometric method of determining pteryxin.



UV spectrum of pteryxin in 96% ethanol.

In the range of working concentrations, the absorption of solutions of pteryxin obeys the Lambert-Beer law.

Experiments have been carried out on the determination of pteryxin in the crystalline powder (Table 1). As follows from Table 1, the maximum relative error in this case does not exceed ±1.7%.

Table 1

Pteryxin taken, mg	Pteryxin found, mg (mean of two determinations)	Error	
		Absolute, mg	Relative, %
0.930	0.940	+0.010	+1.06
0.979	0.962	−0.017	−1.63
1.003	0.998	−0.005	−0.50
1.050	1.035	−0.015	−1.43
1.044	1.041	−0.003	−0.21

To determine the completeness of the elution of the pteryxin from the silica gel, experiments were carried out on the chromatography and subsequent determination of pteryxin (Table 2). As can be seen from Table 2, the

relative error of a single determination is between 0.84 and 3.72% and is negative, which is due to the incomplete removal of the pteryxin from the silica gel. The mean error of three determinations for three samples was 2.37–3.05, which permits a mean correction factor of 1.030 to be introduced into the formula for calculation.

Table 2

Number in sequence	Amount taken (mg)	Amount found (mg)	Relative error, % (one determination)	Relative error, % (mean of three)
1	0.02604	0.02537	−2.57	−2.37
2		0.02582	−0.84	
3		0.02507	−3.72	
1	0.02991	0.02895	−3.20	−3.05
2		0.02925	−2.26	
3		0.02880	−3.71	
1	0.03489	0.03403	−2.49	−2.90
2		0.03389	−2.90	
3		0.03373	−3.33	

The results of the determination of pteryxin in the roots of the plant in duplicate are given in Table 3. The deviation from the mean of two independent determinations does not exceed 1.38%, which shows the satisfactory reproducibility of the method.

Table 3

Number in sequence	Pteryxin content (%)	Mean (%)	Deviation from the mean (%)
1	2.520	2.535	−0.59
2	2.550		+0.59
1	0.215	0.217	+1.36
2	0.220		−1.36
1	1.100	1.085	+1.38
2	1.070		−1.38

To determine the accuracy of the method, experiments were carried out with additions of pure pteryxin to the extract from the roots of L. condensata. The mean relative error of three determinations did not exceed 1.3% (Table 4).

Table 4

Pteryxin added (mg)	Nominal amount of pteryxin (mg)	Pteryxin found (mean of three determinations) (mg)	Error: Absolute, mg	Error: Relative, %
—	25.340	25.340	—	—
0.672	26.012	25.873	0.139	0.530
1.494	26.834	26.491	0.343	1.280
4.093	29.433	29.102	0.321	1.090

EXPERIMENTAL

Determination of pteryxin in a powder. The work was carried out with a chromatographically pure sample of pteryxin with mp 80–81° C. Pteryxin (1 mg, accurately weighed) was dissolved in ethanol in a 25-ml measuring flask, and the solution was made up to the mark with ethanol (solution A); 1 ml of solution A was diluted with 5 ml of ethanol, and the optical density of the resulting solution was determined on an SF-4A spectrophotometer in a 1-cm cell at a wavelength of 322 mμ. The percentage content of pteryxin was calculated from the formula

$$X = \frac{1000 \cdot v \cdot n \cdot D_{322}}{(D^{1\%}_{1cm})_{322} \cdot p \cdot l},$$

where v is the volume of solution A;

n is the dilution factor;

p is the weight of pteryxin, mg; and
l is the thickness of the cell, cm.

Determination of pteryxin in the roots of Libanotis condensata (L) Grants. The ground roots with a particle size of 1–2 mm (1 g, accurately weighed) were covered with 30 ml of methanol and the mixture was boiled for 15–20 min. The contents of the flask were cooled to 20° C and the solution was filtered through a paper filter, after which 15 ml of the methanolic extract was evaporated to dryness. The dry residue was dissolved in 5 ml of chloroform and 0.01–0.02 ml of the resulting solution was deposited on a chromatogram and was chromatographed for 25–35 min in a thin layer of KSK silica gel (2 g of silica gel in 7 ml of isopropanol on a plate) in the n-hexane–benzene–methanol (5 : 4 : 1) system. The chromatogram was examined in UV light and the spot of pteryxin with R_f 0.47 was marked (in UV light pteryxin possesses a violet fluorescence). The section of silica gel marked was transferred quantitatively to a ground stoppered flask having a capacity of 15–20 ml, 15 ml of ethanol was added, and the flask was heated in the water bath at 40–50° C for 20–25 min. Then the contents of the flask were cooled to room temperature, 5 ml of the solution was taken with a pipet, and this was filtered through a dense filter paper into a cell 1 cm thick. The optical density of the resulting solution was determined at a wavelength of 322 mμ against the eluate from an equal amount of silica gel from the same plate. The percentage of pteryxin was calculated from the following formula:

$$X = \frac{1{,}030 \cdot v_1 \cdot v_3 \cdot D_{322}}{v_2 \cdot p \cdot \left(D_{1\,\text{cm}}^{1\%}\right)_{322}},$$

where v is the volume of the extract;
v_2 is the volume of the extract deposited on the chromatogram;
v_3 is the volume of the eluate;
p is the weight of the raw material, g; and
1.030 is a correction factor.

CONCLUSIONS

A spectrophotometric method for the determination of pteryxin in a crystalline powder and in the roots of Libanotis condensata (L) Grants has been proposed.

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