SPECTROPHOTOMETRIC DETERMINATION OF ASPIDIN

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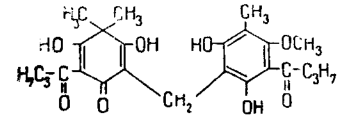
The ferns growing in the USSR have not been subjected to a deep chemical study until recently, especially in regard to the phloroglucides that they contain. A biological investigation of some of the phloroglucinol derivatives that we have isolated from ferns, for example, aspidin



has shown that they possess a high biological activity.

In the present paper we give experimental facts on the development of a method for the quantitative determination of aspidin in various species of fern, in particular Dryopteris spinulosa (M) (toothed woodfern), Dr. phegopteris (L) (narrow beechfern), and Dr. fragrans (L) Schott.

The rhizomes of ferns contain a number of other natural phloroglucides besides aspidin [1, 2], and therefore a stage of the chromatographic separation of the phloroglucinol substances was introduced, this being carried out by TLC in a fixed layer of silica gel G (Merck) with chloroform–n-hexane (1 : 1) as the mobile phase. Aspidin has an R_f value of about 0.8 and shows yellow fluorescence in UV light. The long-wave band in the UV spectrum (absorption maximum 345 nm) that is characteristic for phloroglucides (Fig.1) is the most convenient for spectrophotometric determination. The specific absorption coefficient is 404 ± 1.5% (average of 20 independent determinations), which opens up the possibility of the development of a spectrophotometric method for determining aspidin. In the range of working concentrations, the absorption of solutions of aspidin obeys the Lambert–Beer law.

We have carried out experiments on the determination of aspidin in a crystalline powder (Table 1). It can be seen from Table 1 that the maximum relative error in this case does not exceed ± 1.5%.

To determine the completeness of the elution of aspidin from silica gel, experiments were carried out using chromatography and the subsequent determination of the aspidin (Table 2). It follows from Table 2 that the relative error of one determination is between 0.49 and 3.62% and is negative. This is due to the incomplete removal of the aspidin from the silica gel. The main error of three determinations for five samples was 1.23-2.69%, which permits a correction factor of 1.023 to be introduced into the formula for calculation.

The results of the determination of aspidin in the rhizomes are given in Table 3. The deviation from the mean of two determinations does not exceed 1.72%, which shows that the reproducibility of the method is satisfactory.

To determine the accuracy of the method, experiments were carried out with additions of pure aspidin to an extract of fern rhizomes. The mean relative error of three determinations did not exceed 2.05% (Table 4).

EXPERIMENTAL

Determination of Aspidin in a Powder. The work was carried out with a chromatographically pure sample of aspidin having mp 122-123°C (ethanol). One milligram (accurately weighed) of aspidin was

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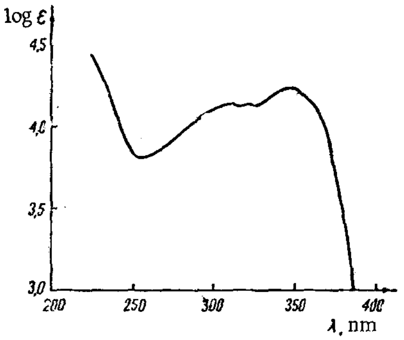

Fig. 1. UV spectrum of aspidin.

TABLE 1

Aspidin		Error	
taken, mg	found (ave. of 2 detns.)	absolute, mg	relative, %
1,035	1,021	—0,114	—1,35
1,070	1,062	—0,008	—0,75
0,930	0,928	—0,002	—0,22
1,120	1,116	—0,004	—0,36
0,960	0,954	—0,006	—0,63
0,990	1,002	+0,012	+1,21
1,050	1,065	+0,015	+1,43
0,980	0,991	+0,011	+1,12
0,990	0,987	—0,003	—0,30
1,170	1,176	+0,006	+0,51

TABLE 2

Repetition	Taken	Found	Relative error, %	
	mg		one determination	mean of three
1		0,01039	—3,62	
2	0,01078	0,01064	—1,30	—2,53
3		0,01049	—2,69	
1		0,01609	—0,49	
2	0,01617	0,01584	—2,04	—1,23
3		0,01598	—1,17	
1		0,01931	—2,67	
2	0,01984	0,01918	—3,33	—2,49
3		0,01955	—1,46	
1		0,02425	—2,18	
2	0,02480	0,02413	—2,70	—2,69
3		0,02401	—3,18	
1		0,02908	—2,28	
2	0,02976	0,02921	—1,85	—2,41
3		0,02884	—3,09	

TABLE 3

Sample	Aspidin content	Mean	Deviation from mean
	%		
D. spinulosum (M) (Caucasus, 1969)	0,68	0,670	+1,38
	0,66		—1,38
D. spinulosum (Podmoskov'e, 1970)	0,870	0,855	+1,72
	0,840		—1,72
D. phegopteris (L.) (1968)	0,800	0,810	—1,23
	0,820		+1,23
D. spinulosum (Podmoskov'e, 1964)	0,680	0,685	—0,73
	0,690		+0,73
D. fragrans (L.) Schott. (Far East, 1969).	0,780	0,770	+1,28
	0,760		—1,28

TABLE 4

Aspidin			Error	
added	nominal total	found (mean of three determinations)	absolute, mg	relative, %
mg				
—	14,620	14,620	—	—
0,499	15,119	14,896	0,213	1,41
0,994	15,618	15,297	0,321	2,05
2,738	17,358	17,053	0,305	1,76

dissolved in methanol in a 25-ml measuring flask, and the solution was made up to the mark (solution A). To 1 ml of solution A was added 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 345 nm.

The percentage content of aspidin was calculated from the formula

$$X\,\% = \frac{1000 \cdot V \cdot n \cdot D_{345}}{\left(D^{1\%}_{1\,\text{cm}}\right)_{345} \cdot P \cdot l},$$

where V is the volume of solution A, ml;

n is the dilution factor;

P is the weight of aspidin, mg; and

l is the length of the cell, cm.

Determination of Aspidin in Fern Rhizomes. To 1 g (accurately weighed) of comminuted rhizomes with a particle size of 1-2 mm was added 20 ml of chloroform, the mixture was shaken for 5 min and left to steep for 12 h, and then the solution was filtered through a paper filter. A 10-ml sample of the chloroform extract was evaporated to dryness, and the dry residue was dissolved in 5 ml of chloroform. A 0.01-0.03-ml sample of the solution obtained was deposited on a plate of silica gel G (2 g of silica gel and 7 ml of nitrate-phosphate buffer, pH 6), and was chromatographed for 1 h in the chloroform–hexane (1 : 1) system.

The chromatogram was observed in UV light, and the spot of aspidin with R_f 0.8, possessing a yellow fluorescence, was marked out. This section of the silica gel was transferred quantitatively into a 15-20-ml flask with a ground-in stopper, 5 ml of ethanol was added, and the mixture was boiled in the flask on the water bath under reflux for 10 min. The contents of the flask were cooled to room temperature, and 4 ml of the solution was taken with a pipette and 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 345 nm against the eluate from an equal amount of silica gel from the same plate.

The percentage content of aspidin was calculated from the following formula:

$$X = \frac{1{,}023 \cdot V_1 \cdot V_3 \cdot D_{345}}{V_2 \cdot P \cdot \left(D^{1\%}_{1\,\mathrm{cm}}\right)_{345}},$$

where V_1 is the volume of the extract, ml;

V_2 is the volume of the extract deposited on the chromatogram, ml;

V_3 is the volume of eluate, ml;

P is the weight of raw materal, g;

1.023 is the correction factor.

SUMMARY

A spectrophotometric method is proposed for determining the content of aspidin in a crystalline powder and in the rhizomes of the ferns Dryopteris spinulosa (M), D. phegopteris (L.), and D. fragrans (L.) Schott.

LITERATURE CITED

1. M. V. Schantz and C. Widen, J. Scient. Pharmac., 35, No. 3, 197-200, 1967.
2. A. Penttila and J. Sundman, Nordisk Medicin, 67, No. 14, 439-447, 1962.