

SPECTROPHOTOMETRIC DETERMINATION OF LYCORINE
IN PLANT RAW MATERIAL AND A PREPARATION

A. D. Volodina, E. K. Dobronravova,
and T. T. Shakirov

UDC 547.944/945 + 543.422

The alkaloid lycorine, which is used in medicine in the form of the hydrochloride, is obtained from the leaves of *Ungernia severtzovii* B. Fedtsch. and *Ungernia trisphaera* Bge (family Arnyllidaceae) [1]. The methods of analysis described in the literature are suitable only for the determination of pure lycorine [2, 3].

TABLE 1. Determination of the Degree of Desorption of Lycorine

Amt. taken	Amt. found	Relative error, %	
mg		one determination	mean of two determinations
0,500	0,494	-1,20	-0,90
0,500	0,497	-0,60	
0,422	0,423	-0,23	-0,23
0,422	0,423	-0,23	
0,180	0,176	-2,22	-1,66
0,180	0,178	-1,11	
0,262	0,260	-0,76	-0,38
0,262	0,262	0	

TABLE 2. Results of an Analysis of Plant Raw Material

Sample	Repetition	Amt. of extract taken, mg	Lycorine cont.	Mean	Deviation from the mean
U. severtzovii	1970				
	1	0,5	0,248	0,254	±2,36
	2	0,3	0,260		
	1971				
	1	0,5	0,223	0,228	±2,19
	2	0,4	0,233		
U. trisphaera	1970				
	1	0,5	0,180	0,175	±2,85
	2	0,3	0,170		
	1971				
	1	0,5	0,225	0,217	±3,68
	2	0,4	0,209		

In the present paper we propose a method for the chromatographic determination of lycorine in plant raw material and for the spectrophotometric determination of the preparation in tablets.

The UV spectra of lycorine and its hydrochloride each have two maxima, in the 240 and 292 nm regions ($\log \epsilon$ 3.59, 3.67). We have made use of the maximum at a wavelength of 292 nm. Within the range of concentrations from 0.06 to 0.005 mg/ml the absorption of a solution of lycorine obeys the Bouguer-Lambert-Beer law. The epigeal parts of the species of plants mentioned contain a number of other alkaloids besides lycorine [4, 5]. The best separation of lycorine from the accompanying bases was achieved by the chromatography of a chloroform extract in a nonfixed layer of alumina (activity grade III) in the chloroform-ethanol-acetone (8:1:4) system. Under these conditions the R_f values of lycorine, galanthamine, hippeastrine, unguinorine, tazettine, pancreatine, and *dl*-narwedine are, respectively, 0.52, 0.90, 0.85, 0.25, 0.80, 0.26, and 0.90.

The completeness of the desorption of lycorine from the sorbent was established with a pure sample of the alkaloid. The optimum conditions for elution are steeping in ethanol for 24 h (98-100% desorption) (Table 1).

Lycorine is sparingly soluble in all organic solvents, and, therefore, exhaustive extraction from plant raw material requires 12-15 h. By chromatographing the extract we separated the lycorine from the accompanying alkaloids but possibly did not eliminate the other extractive substances. In order to check the influence of these substances on the absorption spectrum of lycorine, extracts with different amounts of them were analyzed (Table 2). It can be seen from Table 2 that different amounts of inert substances have no effect on the results of the determination of the alkaloid.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from *Khimiya Prirodnykh Soedinenii*, No. 6, pp. 761-764, November-December, 1972. Original article submitted March 28, 1972.

© 1974 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$15.00.

The lycorine in the two species of Ungernia was determined by the given method. To evaluate the accuracy of the method, the lycorine was determined in extracts from the plant raw material with the addition of a pure preparation to it. The relative error of three determinations did not exceed $\pm 6\%$.

The quantitative determination of lycorine hydrochloride in a powder was performed by nonaqueous titration [6] and in tablets by the spectrophotometric method at a wavelength of 292 nm. The relative error of the spectrophotometric determinations is $\pm 2\%$.

EXPERIMENTAL

Analysis of the Raw Material. An accurately weighed 10-g sample of the air-dry comminuted leaves of Ungernia was moistened with 10 ml of 5% ammonia solution and exhaustively extracted with chloroform in a Soxhlet apparatus. The extract was concentrated to a volume of 20-30 ml and quantitatively transferred into a 50-ml measuring flask and made up to the mark with chloroform; 0.3-0.5 ml of the chloroform extract was deposited on a plate (13 × 18 cm) as a continuous band, a marker (lycorine) being placed adjacent to it. Chromatography was performed in the system given above, and the marker was revealed in the moist state by means of Dragendorff's reagent as modified by Munier [7]. The section of the sorbent corresponding to the lycorine spot in the region with R_f 0.50 ± 0.05 was transferred to a 50-ml flask, covered with 10 ml of ethanol, and left for a day. Then the solution was filtered off through a Schott No. 4 funnel, and the optical density of the filtrate was measured on an SF-4 spectrophotometer at a wavelength of 292 nm in a cell with a layer thickness of 1 cm. An ethanolic solution obtained by the elution of an equal amount of alumina from the same plate was used as the comparison solution. The standard solution was the eluate obtained from the chromatography of 0.5 ml of an ethanolic solution of lycorine (c 0.5 mg/ml), the mp of this standard material being 265°C. The amount of lycorine (x, %) on the dry raw material was calculated from the formula

$$x = \frac{10 \cdot D_1 \cdot c_0 \cdot V_1 \cdot V_2}{D_0 \cdot p \cdot V_3 (100 - h)},$$

where D_1 and D_0 are the optical densities of the solution under investigation and the solution of the standard sample, respectively; c_0 is the concentration of the solution of the standard sample, mg/ml; V_1 is the volume of the extract, ml; V_2 is the volume of ethanol taken for elution, ml; V_3 is the volume of the extract deposited on the chromatogram, ml; p is the weight of raw material, g; and h is the moisture content, %.

Analysis of a Preparation. About 1.25 g (accurately weighed) of the powdered tablets was shaken with 15 ml of water in a 25-ml measuring flask for 5-10 min, and then the solution was made up to the mark with water and was filtered. The optical density of the filtrate at a wavelength of 292 nm was measured in a cell with a layer thickness of 1 cm. Water was used as the comparison solution. In parallel the optical density of a solution of a standard sample containing 0.05 mg of lycorine hydrochloride was measured. The sample of hydrochloride used satisfied the requirements of MRTU (Interrepublican Technical Specification) 42, No. 3909-70. The amount of lycorine hydrochloride (x, g) in one tablet was calculated from the formula

$$x = \frac{D_1 \cdot c_0 \cdot b \cdot V}{D_0 \cdot a \cdot 1000},$$

where D_1 is the optical density of the solution under investigation; D_0 is the optical density of the solution of the standard sample; c_0 is the concentration of the solution of the standard sample, mg/ml; a is the weight of the sample of tablet material, g; b is the mean weight of a tablet, g; and V is the dilution, ml.

SUMMARY

A chromatophotometric method for determining lycorine in the epigeal parts of Ungernia severtzovii and U. trisphaera and a spectrophotometric method for determining the preparation in tablets have been developed.

LITERATURE CITED

1. T. Sadikov and T. T. Shakirov, *Khim. Prirodn. Soedin.*, 134 (1972).
2. T. Kanieska and B. Borkowski, *Dissert. Pharmac. Pharmacol. PAN*, 20, No. 1, 111 (1968).
3. A. Kolusheva and A. V"lkova, *Farmatsiya (Sofia)*, 16, No. 5, 45 (1966).
4. L. S. Smirnova, Kh. A. Abduazimov, and S. Yu. Yunusov, *Khim. Prirodn. Soedin.*, 322 (1965).

5. Kh. B. Allayarov, Kh. A. Abduazimov, and S. Yu. Yunusov, Dokl. Akad. Nauk UzSSR, No. 7, 50 (1962).
6. A. P. Kreshkov, L. N. Bykova, and N. A. Kazaryan, Acid-Base Titration in Nonaqueous Solutions [in Russian], Moscow (1967), p. 79.
7. N. A. Izmailov, A. K. Franke, and I. S. Simon, Med. Prom. SSSR, 11, 36 (1959).