6. F. Zollo, E. Finamore, and L. Minale, Gazz. Chim. Ital., 115, 303 (1985).
7. R. Riccio, M. Iorizzi, O. Squillace-Greco, L. Minale, D. Laurent, and Y. Barbin, Gazz. Chim. Ital., 115, 505 (1985).
8. S. A. Avilov, A. I. Kalinovskii, and V. A. Stonik, Khim. Prir. Soedin, No. 1, 53, (1990).

ANALYSIS OF GLYCOSIDES OF Allochruza gypsophiloides IN THE PREPARATION "ALLOKHROZID" BY CHROMATOSPECTROPHOTOMETRY

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The possibility has been shown of using a readily available method for estimating the level of the main glycoside of the organs of the plant soapwort – acanthophylloside B – in commercial products used in the food and medical industries, and also in samples of the preparation Allokhrozid. The amount of the individual glycoside was calculated in relation to a standard sample of acanthophylloside B.

Medicinal forms based on plants containing triterpene glycosides are manufactured by the medical industry for the treatment and prevention of a number of diseases of man and animals. The most popular include medicinal forms obtained from ginseng, Manchurian aralia, and eleutherococcus – plants of the family Araliaceae. Many plants of the family Caryophillaceae form the raw material for obtaining soap substitutes and in the production of shampoos, effervescent beverages and halvas, and also in industry. The rising demand for these plants as a raw material for the industrial manufacture of such products requires the obligatory standardization of the initial raw material and the monitoring of the production steps and of the final product [1-4]. In the data bank on methods of monitoring the products on the basis of triterpene derivatives, in the main, two methods of preparing the material for analysis are known. The first is the hydrolysis of the glycosides, followed by the determination of the products so obtained – mainly aglycons and their degradation products. The second is the direct determination of the glycosides using some reactions or other.

The first method is not always suitable in view of the ambiguity (nonreproducibility) of the results of the analysis. This is due to a number of factors: the lability of the aglycon, the poor solubility of the initial preparation or of the products of its incomplete hydrolysis, and their inhomogeneity, which is connected with the complex structure of the carbohydrate chains of the glycosides.

When the second method is chosen, it is possible to determine both the total amount of glycosides and also the amounts of the individual components from the intensities of the coloration of the derivatization products of the glycosides. Their color reactions with H_2SO_4 must be included among the known reactions used for the quantitative estimation of triterpene glycosides. A method of photometric determination is based on this reaction. The reliability of the method depends largely on the degree to which the product being analyzed has been freed from impurities. Various forms and methods of chromatography are used to separate the glycosides to be determined from accompanying substances, including those close in structure.

The aim of the present work was to study the possibility of using the spectrophotometric method for analyzing the preparation Allokhrozid, obtained from the roots of Allochruza gypsophiloides (synonym – Acanthophyllum gypsophiloides). The method is based on the

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 0009-3130/93/2902-0210$12.50

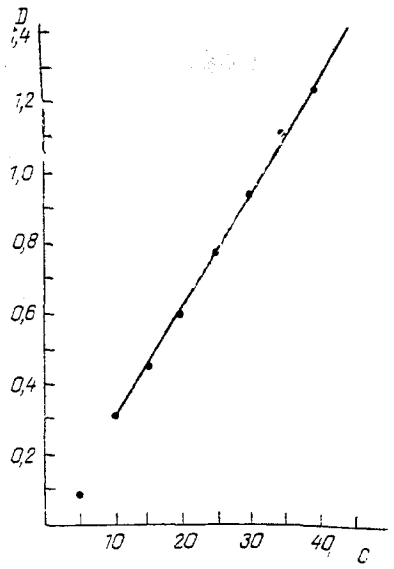

Fig. 1. Dependence of the optical density on the concentration of acanthophylloside B (μg/ml).

TABLE 1

Sample	X, %	S	$S_{\bar{x}}$	$t_{a,k}$	E_a
Laboratory 08. 1991	37,15	3,23	1,32	2,57	9,13
" 07. 1991	51,13	3,62	1,62	2.78	4,02
" 07. 1991	40,00	0,42	0,21	3,18	1,72
" 1983	28,46	2,12	0,71	2,36	5,86
Factory 1985	35,54	1,22	0,14	2,36	2,71
Gypsophylla Rgl. saponins (Czechoslovskia)	27,49	1,93	0,86	2,78	8,75
Merck (FRG) saponins, batch 12256	41,89	0,83	0,48	4,30	4,95
Merck (FRG) saponins, batch 19061799	42,29	0,23	0,13	4,30	1,34

derivatization with sulfuric acid of the main glycoside in the total triterpene compounds present in the preparation. The reaction is carried out directly on a plate with a fixed layer of silica gel, after the chromatography of total product in a solvent system, by spraying it with a sulfuric acid solution and then heating it. We have investigated the stability of the optical density of the derivative of acanthophylloside B obtained and its dependence on the concentration of acid used, the temperature and time regime of the thermostating of the chromatogram, and the desorption of the preparation. This showed that the minimum concentration of substance reliably detectable through the derivative under the optimum conditions developed is 0.005 mg/ml. Observance of the Beer–Lambert law, i.e., a linear dependence of the optical density on the concentration of acanthophylloside B, was observed in the concentration range of 10-40 μg/ml. The error of a single determination at a confidence level of 95% did not exceed ±5.5%.

Since in plants of the Caryophillaceae family the overwhelming majority of the glycosides contain gypsogenin as their aglycon and the carbohydrate chains include nine and more sugar units, the method developed has been used for the evaluation of commercial preparations produced by a number of foreigns firms and containing triterpene glycosides close in chemical structure to acanthophylloside B [6].

The results of the analysis of several samples of various batches of Allokhrozid obtained under laboratory conditions and in the experimental factory of the Institute of Chemistry of Plant Substances, the Uzbekistan Republic Academy of Sciences, and also of the commercial preparations, are given in Table 1. In the evaluation of the 07.1991 laboratory sample and the Czech sample, we employed the method of adding an internal standard, as which we used the sample of acanthophylloside B mentioned below.

EXPERIMENTAL

To develop the procedure for quantitative determination we used, as standard, acanthophylloside B with mp 240-242°C; $[\alpha]_D^{20}$ +40.3° (c 1.2; H_2O) isolated from a methanolic extract of Acanthophyllum gypsophiloides roots. Thin-layer chromatography was conducted on type KSK silica gel (<80 μm) containing 10% of gypsum in the solvent system chloroform–methanol–water (100:85:25). Optical densities were determined on an SF-4A spectrophotometer at a wavelength of 280 nm.

Procedure for Quantitative Determination. On a chromatographic plate with dimensions of 13 × 18 cm, 0.1 ml of a solution (0.005 g/ml) of one of the samples under investigation was deposited by means of a micropipette on the starting line in a band 3 cm long (first band), and a standard solution of acanthophylloside B was deposited in a similar band (third band), while the second band was left free and was used as a control. The plate with the deposited samples was dried in the air for 15-20 min and was placed in a chamber with the above-mentioned mobile phase that had previously been saturated with its vapors and was chromatographed by the ascending method. When the solvent front had reached the edge of the plate, it was removed and was dried in a drying chest at 100°C for 10 min. Then it was sprayed with an 8% solution of H_2SO_4 and was heated in the drying chest at 110-115°C for 35-45 min (until red-violet spots had appeared). The zones at the level of the acanthophylloside B were cut out, extracted with 5 ml of ethanol (95%), with shaking for 10 min, and filtered through a glass filter (110/40). The optical densities of the filtrates were measured on a spectrophotometer in a cell with a layer thickness of 10 mm. The solution from the control band of the plate was used as the comparison solution. The amount of acanthophylloside B in the sample (as a percentage) was calculated from the formula:

$$X = \frac{D \cdot 0{,}0002 \cdot V \cdot 100}{D_0 \cdot A \cdot V_{al}}$$

where D and D_0 are the optical densities of the solution of the substance under investigation and the standard solution, respectively;
0.0002 is the amount of acanthophylloside B in 0.1 ml of the standard solution, g;
V is the initial volume of the solution of the sample, ml;
V_{al} is the volume of sample solution deposited on the chromatogram, ml; and
A is the weight of the sample, g.

Preparation of the Solutions of the Samples and of the Acanthophylloside B Standard. The dissolution of 0.0100 g of acanthophylloside B in 5 ml of 80% ethanol gave a solution 0.1 ml of which contained 0.0002 g of acanthophylloside B. Solutions of the samples to be analyzed were prepared by dissolving 0.0100 g of substance in 2 ml of 80% ethanol.

LITERATURE CITED

1. T. N. Poletaeva, in: The Cultivation of Medicinal Plants [in Russian], BNII SÉNTI Moscow (1991), No. 1, p. 42.
2. I. A. Shreter, in: The Cultivation of Medicinal Plants [in Russian], BNII SÉNTI, Moscow (1991), No. 2, p. 19.
3. P. K. Kintya, S. A. Burtseva, L. P. Koval'chuk, N. E. Mashchenko, and V. A. Bobenko, Khim.-farm. Zh., No. 1, 95 (1982).
4. V. K. Kolkhir and S. Ya. Sokolov, Khim.-farm. Zh., No. 5, 20-25 (1982).
5. Zh. M. Putieva, L. G. Mzhel'skaya, T. T. Gorovits, E. S. Kondratenko, and N. K. Abubakirov, Khim. Prir. Soedin., No. 6, 806-809 (1977).
6. E. S. Kondratenko, Zh. M. Putieva, and N. K. Abubakirov, Khim. Prir. Soedin., No. 4, 417-483 (1981).