PURIFICATION OF THE COMBINED DIGLYCOSIDES OF THE ROOTS OF Apocynum androsaeimifolium BY ADSORPTION CHROMATOGRAPHY

M. S. Khagi, M.-R. I. Shamsutdinov, and T. T. Shakirov

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Adsorption molecular chromatography is one of the methods widely used for obtaining cardiac glycosides [1]. This method is also used for the purification of a technical product after its isolation from a solution of the combined glycosides and for separating it into individual substances. The method has been successfully applied by N. K. Abubakirov et al., in the isolation of K-strophanthin-β and the apobioside [2, 3].

The present communication gives the results of a chromatographic purification on alumina of the combined glycosides extracted from the roots of Apocynum androsaeimifolium.

The adsorbent was alumina (Brockmann activity grade III); the eluents were methanol, ethanol, and isopropanol in different combinations with chloroform. The results of the experiments showed that of the eluents considered the highest degree of desorption of the diglycosides is obtained with a mixture of ethanol and chloroform (1:3).

To determine the possibilities of isolating a fraction of an eluate containing the minimum amount of contaminating substances, the dynamics of the desorption of the total diglycosides and of the contaminating substances were studied (Fig. 1).

The desorption process can be separated into several characteristic stages. Zone A (see Fig. 1) relates to the first stage. It is characterized by the fact that the eluate contains only inert substances. The following stage of desorption (zone B) begins with the "breakthrough" of the diglycosides and ends with a fall in the desorption of the inert substances. In this stage 42% of the diglycosides is desorbed; in this fraction, their purity if 66%. Of the greatest interest is the third stage of desorption (zone C). From this fraction 38% of the diglycosides with a purity in the eluate of 91.5% is eluted. And, finally, the last stage (zone D) gives an eluate containing 6.4% of diglycosides with a purity of 36%. This stage completes the desorption of the diglycosides. Thus, a study of the dynamics of desorption has shown that 42% of the total diglycosides can be brought to a purity of 66%, and 38.0% to a purity of 91.5%, starting with an initial technical product containing 40.7% of diglycosides.

EXPERIMENTAL

Desorption of Diglycosides and Inert Substances. The amounts of diglycosides and inert substances desorbed from active alumina by various eluents were determined in 13 columns (d = 40.0 mm, h = 150 mm). Each column was charged with 70 g of alumina and with a technical product containing 0.45 g of diglycosides and 0.83 g of inert substances. The columns were eluted with methanol, ethanol, and isopropanol in various combinations with chloroform [methanol–chloroform, isopropanol–chloroform (1:9), (1:5), (1:3), (1:2), and (1:1.5); ethanol–chloroform (1:9), (1:5), and (1:3)] at the rate of 120 liters/h · m^2. Desorption of the glycosides was observed in experiments where the eluates used were mixtures of methanol and chloroform (1:2) and (1:1.5) and ethanol and chloroform (1:3). The greatest desorption of the combined diglycosides was found in the latter case. Elution was performed until the Raymond reaction of the eluates for glycosides

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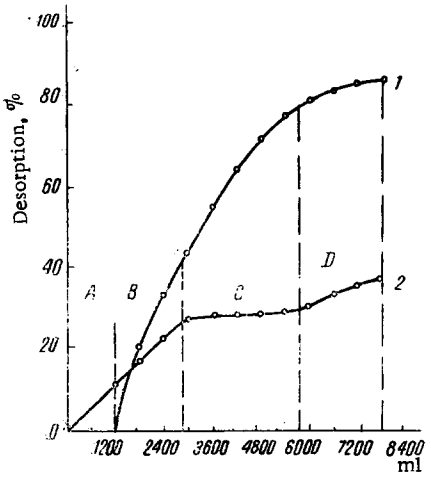

Fig. 1. Dynamics of the desorption of diglycosides and inert substances from alumina [eluent: ethanol–chloroform (1:3)]. 1) Desorption of the diglycosides; 2) desorption of the inert substances.

was negative. The amount of glycosides in the eluates was determined by a known method [4] and that of inert substances from the difference between the weight of the dry residue (after the evaporation of the eluate to dryness) and the amount of diglycosides found by analysis.

In the other experiments, no diglycosides were eluted even when a 400-fold amount of eluent (on the weight of the diglycosides on the alumina) was passed through the column. In these experiments, no further desorption was performed.

Investigation of the Dynamics of the Desorption of the Total Diglycosides and Inert Substances. The technical product (8.85 g, containing 3.6 g of diglycosides) was deposited on a column (d = 40.0 mm, h = 480 mm) containing 427 g of alumina. Elution was performed with a mixture of ethanol and chloroform (1:3) at the rate of 120 liters/h · m^2. The diglycosides in the eluate were detected by Raymond's qualitative reaction for glycosides.

The fraction of eluate before the breakthrough of diglycosides was evaporated to dryness and the residue was weighed, amounting to 0.57 g, or 11.4%, of inert substances (as a percentage of their amount in the initial technical product). The volume of this fraction of eluate was 1200 ml.

The subsequent eluates were collected in 600-ml fractions until the reaction for glycosides was negative. The amounts of diglycosides and inert substances were determined in each fraction (see Fig. 1).

In desorption from alumina, 86.4% of diglycosides and 37.1% of inert substances (on their amounts in the initial technical product) were eluted.

The diglycosides remaining on the alumina were eluted with 70% ethanol. This gave 13.2% of diglycosides and 54.3% of inert substances (calculated on the initial technical product).

SUMMARY

1. Of the eluents studied, ethanol–chloroform (1:3) possessed the highest power of desorbing the diglycosides from alumina.

2. The results of an investigation of the dynamics of the desorption of the diglycosides and the inert substances with ethanol–chloroform (1:3) showed the possibility of obtaining 42% of the total diglycosides with a purity of 66%, and 38% of the total diglycosides with a purity of 91.5%, using a technical product containing 40.7% of diglycosides.

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