METHOD OF OBTAINING ALLAPININE FROM THE EPIGEAL PART OF *Aconitum leucostomum*

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The industrial processes for obtaining allapinine (lappaconitine hydrobromide) from the epigeal part of *Aconitum leucostomum* have been studied. As a result, a rational method of obtaining allapinine from the plant raw material has been developed which consists in the extraction of the raw material with 80% ethanol and concentration to an aqueous residue, followed by extraction with chloroform, purification, the production of technical allapinine, and its recrystallization from methanol or from aqueous ethanol. The yield of allapinine satisfying the requirements of VFS 31-1667-86 amounts to 72-80% of its amount in the raw material.

Allapinine – the hydrobromide of the alkaloid lappaconitine – is an antiarrhythmic agent [1, 2] and has been approved for use in medical practice. The main source of lapaconitine is the epigeal part of the wild perennial herbaceous plant *Aconitum leucostomum* Worosch., which contains up to 0.5% of lappaconitine, depending on the period of collection [3, 4].

To develop a rational technology for obtaining allapinine we have studied the distribution of lappaconitine between chloroform and buffer solutions with various pH values [5, 6]. As a result of the experiments a $pH_{1/2}$ value of 3.0 was found and, consequently, lappaconitine is an alkaloid of medium basicity.

Several methods are known for isolating medium-basic alkaloids from plant raw material [6]. The use of methods where strong alkalinizing agents (NH_3, NaOH, KOH) are employed leads to a considerable fall in the yield of lappaconitine, since the alkaloid lappaconitine contains an ester group and is saponified, forming the amino alcohol lappaconine and acetylanthranilic acid [7].

Extraction of the raw material with aqueous solutions of acids using subsequent liquid-liquid extraction and alkalinization with soda (Na_2CO_3) likewise did not give a satisfactory result, since in this process a very stable emulsion was formed which was possible to separate in existing apparatus.

The aqueous-ethanolic extraction of the raw material followed by purification proved to be a rational and economically feasible method [8].

The processes involved in the extraction of alkaloids from raw material, the isolation of the alkaloids from the extracts, and the production of technical allapinine and its purification have been considered.

A preliminary study of the process of extracting the alkaloid from the raw material showed that the main factors affecting the process are: the degree of grinding of the raw material, X_1; the time of extraction, X_2; the concentration of ethanol, X_3; the ratio of the height of raw material in the extractor to its diameter (ℓ/d), X_4; and the temperature of the process, X_5. To determine the influence of these factors on the extraction process and to find the optimum conditions for its performance, an optimization of the process was carried out by the method of mathematical experimental planning [9].

The following levels of the factors and the intervals of their variation were selected: X_1 – 20 ± 10 mm; X_2 – 6 ± 3 h; X_3 – 60 ± 20%; X_4 – 4 ± 1; X_5 – 30 ± 10°C.

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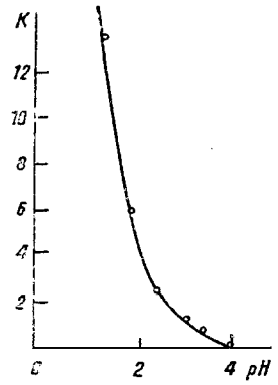

Fig. 1. Distribution of lappaconitine between chloroform and buffer solutions with various pH values.

After the performance of an experiment of the $Y = 2^{5-2}$ type, the following mathematical model of the process was obtained:

$$Y = 33.49 - 2.26\,X_1 + 9.23\,X_2 + 5.6\,X_3 + 5.67\,X_4 + 1.46\,X_5 - 0.09\,X_1X_2 - 0.85\,X_2X_3.$$

From the regression coefficients of the equation after the calculation of the confidence interval ($\Delta bi = \pm 0.58$) it was established that the main factors influencing the process are the degree of grinding of the raw material, the time of extraction, and the concentration of alcohol. A statistical analysis ($F_{calc} = 2.0 \leq F_{tab(2;8;0.5)} = 4.5$) of the results obtained showed that the mathematical model was adequate. After the performance of a steepest ascent, it was found that the optimum conditions for performing the process are: degree of grinding of the raw material, 12-16 mm; time of extraction, 10 h; concentration of alcohol, 80%; $\ell/d = 3$; temperature, 30°C. Under these conditions, in six extractions 94-96% of the total amount of alkaloids in the raw material is dissolved out.

The aqueous alcoholic extracts of the alkaloids that were obtained were concentrated in a vacuum-evaporating apparatus until the alcohol had been eliminated completely (aqueous residue). As is known, chlorophyll and other resinous substances present in plant raw material are readily soluble in organic solvents, including ethanol. After the ethanol had been distilled off they floated to the surface of the aqueous residue and could be removed by filtration or by decanting the extract.

Chloroform was used to obtain the alkaloid from the evaporated extract; with 4-5 contacts of the phases at pH 8.5-9.0 (alkalinization with Na_2CO_3), the alkaloids had passed into the chloroform exhaustively. The alkaloids were extracted from the chloroform solution with 5% sulfuric acid, hydrophobic impurities were removed by washing with chloroform, the acid solution was made alkaline with soda to pH 8.5-9, and the alkaloids were reextracted with chloroform and, after evaporation, the total alkaloids were obtained.

The total alkaloids obtained included more than ten alkaloids [TLC on silica gel in the chloroform–benzene–95% ethanol–ammonia (40:40:10:0.2) system] the amount of lappaconitine ranging between 40 and 60%. The allapanine was obtained from the combined alkaloids by the following schemes:

1. Preparation of the hydrobromide of lappaconitine in an ethanolic medium, and recrystallization from methanol.

2. Precipitation of the lappaconitine with ethanol, the preparation of the hydrobromide, washing with chloroform, and recrystallization from methanol.

3. Precipitation of the lappaconitine with ethanol, preparation of the hydrobromide, washing with chloroform, and crystallization from aqueous ethanol.

High yields of allapinine were obtained by the second and third methods, in which a single recrystallization of the technical lappaconitine hydrobromide (allapinine) was sufficient, satisfying the demands of the temporary pharmacopeial document (VFS 42-1667-86), which were drawn up for samples of allapinine obtained by the method described above. A check on the reproducibility of the method on the pilot plant of the Institute showed that the yield of allapinine was 72-80% of its amount in the raw material.

In this way we drew up experimental-industrial rules for the production of allapine which were introduced in 1987 in the experimental factory of the Institute of the Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences.

EXPERIMENTAL

The $pH_{1/2}$ value of lappaconitine was determined by the method of [5] and is shown in the form of a graph of K = f(pH) (Fig. 1) from which the required value was found.

Production of Allapinine. An extractor was charged with 80 kg of the comminuted epigeal part of Aconitum leucostomum (containing 0.24% of lappaconitine on the air-dry weight of the raw material), and this was covered with 80% ethanol in a ratio of 1:3.5 (280 liters) and allowed to steep for 10 h. The alcoholic extract of alkaloids was decanted off. The 140 liters of extract that were obtained were concentrated. A second extraction was made with 140 liters of 80% ethanol. Another four extractions were made similarly. The extracts obtained were concentrated in a vacuum evaporating apparatus to a residue with 8.0-11.0% of the initial volume. About 70 liters of extract were obtained.

The extract was made alkaline with soda to pH 8.5-9.0, and the alkaloids were extracted with chloroform (4 × 25 liters). The chloroform extracts were combined and the alkaloids were reextracted with 5% sulfuric acid solution (5 × 5 liters). To eliminate impurities of nonbasic nature, the solution of the alkaloids was washed with chloroform (2 × 10 liters). The washed acid solution of the alkaloids was made alkaline with soda to pH 9, and the alkaloids were extracted with chloroform. The chloroform solution of alkaloids so obtained was evaporated, giving 472 g of total alkaloids, or 0.59% on the air-dry weight of the raw material.

The total alkaloids (472 g) were treated with 2 liters of ethanol, and the mixture was left for 12 h. The precipitate of technical lappaconitine that had deposited was filtered off, washed with ethanol, and dried (173 g). To obtain allapinine (the hydrobromide of lappaconitine), the technical lappaconitine was treated in ethanol with a 5% aqueous ethanolic solution of hydrobromic acid. The technical allapinine obtained was washed with chloroform and recrystallized from methanol.

The yield was 139 g or 0.17% on the air-dry weight of the raw material.

CONCLUSIONS

A method has been developed for obtaining allapinine by aqueous alcoholic extraction with a yield of 72% of the amount of the corresponding alkaloid in the raw material.

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