EXTRACTION OF THE TOTAL ALKALOIDS FROM THE EPIGEAL PART OF Aconitum orientale

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The process of extracting the total alkaloids from Aconitum orientale with aqueous solutions of a number of electrolytes has been investigated. It has been established that it is most desirable to use 2% solutions of H_2SO_4 and 5% solutions of CH_3COOH. The optimum parameters of the process have been found, under which the degree of extraction of the total alkaloids averages 96%.

Plants of the genus Aconitum form a rich source of diterpene alkaloids, many of which possess a broad spectrum of pharmacological action [1-3].

The method of obtaining total alkaloids is based either on the classical procedure consisting in alkalinizing the raw material with a solution of sodium carbonate followed by extraction with chloroform, or in the extraction of the raw material with aqueous ethanol [4, 5].

We have made an attempt to select a cheaper and more readily available extractant. As the extractants we used water and aqueous solutions of a number of electrolytes [6-10]. The investigation was carried out by the "limited volume" method. The temperature was maintained with an accuracy of ±2°C. Stirring was carried out with a propeller-type mixer. Liquid samples of the extract were analyzed spectrophotometrically [11].

On single extraction, the majority of electrolytes possessed approximately the same extraction activity (Table 1), which did not enable the results of the experiments to be evaluated unambiguously. When a threefold extraction of the raw material was made, however, it was found (Table 1) that 2 and 3% solutions of sulfuric or hydrochloric acid and 5% solutions of acetic acid possessed the greatest extraction activity. A comparison with these results with those of experiments on the isolation of the total alkaloids by the classical method and the alcohol method [4, 5] showed that with respect to the passage of the total alkaloids into the extractant they were practically identical.

However, a comparison of the TLC chromatograms of the extracts obtained in the chloroform—methanol (6:1) or benzene—methanol (4:1) system (the adsorbent being KSK silica gel, with the deposition of 0.2 ml of a chloroform extract directly from the raw material and the same amount of a chloroform extract from the aqueous sulfuric acid extract obtained, at the same total amount of alkaloids in the sample) showed that in the second case the passage of nonalkaloid impurities into the chloroform solution was greatly suppressed.

The totality of the results obtained, after their statistical treatment (Table 2), permitted the recommendation as extractant of a 2% solution of H_2SO_4 or a 5% solution of acetic acid. Using just these extractants, we studied the influence of a number of technological parameters on the degree of passage of the total alkaloids into the extract.

In an investigation of the influence of the ratio of the phases, it was possible to obtain the best results at m_{sld}/m_{liq} = 1/8-1/10. A rise in this ratio led to a fall in extraction activity, and at a ratio of 1/5-1/6 a viscous mass difficult to stir was formed which complicated the hydrodynamic regime of the process and greatly lowered the degree of extraction. When the m_{sld}/m_{liq} ratio was lowered to less than 1/12, in addition to some increase in the degree of extraction, there was a considerable dilution of the solution which is technologically unjustified in view of the necessity for further concentration.

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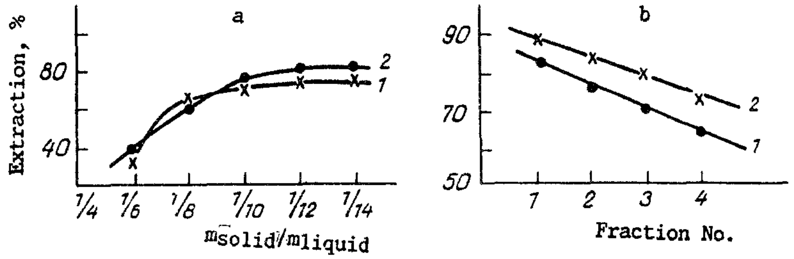

Fig. 1. Influence of the phase ratio (a) and of the size of the particles of raw material undergoing extraction (b) on the degree of passage of the total alkaloids into the extract (single extraction, conditions analogous to those described in Table 1): 1) 2% H_2SO_4; 2) 5% CH_3COOH.

TABLE 1. Influence of the Extractant on the Passage of the Total Alkaloids into the Extract (s/ℓ = 1:10; d_3 – 3.0-6.0; t – 20°C; n_{rev} = 60 rpm; extraction time – 3 h)

Extract (solution)		Degree of extraction, % of the amount in the raw material on extraction	
		once	thrice.
1% H_2SO_4		58,2	89,5
2% H_2SO_4		69,3	92,9
5% CH_3COOH		77,7	91,6
2% Na_2CO_3		69,8	79,8
2% NaOH		69,2	71,3
2% NaOH		52,3	
2% NaOH+	10% NaCl		88,3
	1% HCl	55,3	88,9
	2% HCl	67,1	99,8
	3% H_2SO_4	70,1	93,3
	5% H_2SO_4	70,5	93,8

TABLE 2. Metrological Characterization of the Extraction of the Raw Material with a 2% Solution of H_2SO_4 and a 5% Solution of CH_3COOH

Extractant	n	$\bar{x}$	S^2	S	P	$t(p,f)$	X	
2% solution of H_2SO_4	10	92,93	0,796	0,892	95	2,26	2,015	2,18
5% solution of CH_3COOH	10	91,62	0,760	0,874	95	2,26	1,97	2,15

The results obtained are shown in Fig. 1a. The influence of the particle size of the raw material was studied on material sieved into four fractions: 1) 0.5-1 mm, 2) 2-4 mm, 3) 3-6 mm, 4) 8-12 mm. A preliminary analysis of all the fractions showed that the amounts of alkaloids in them were practically identical.

It appears most desirable to use fraction 3 (Fig. 1b), since the use of material with finer grains (fractions 1 and 2) causes certain difficulties at the stage of separating the solid and liquid phases after the performance of the extraction process. An increase in the size of the particles is connected with the fall in the degree of extraction.

An investigation of the kinetics of extraction showed the influence of the rate of stirring (in the range of 260-200 rpm) on the rate of extraction. The time of extraction was changed within certain limits (Fig. 2a). On passing to fraction 4, this influence was slight.

The influence of the temperature factor was studied at 20-60°C. The temperature was not raised higher, since preliminary experiments had shown the possibility of a decomposition of the alkaloid. Additional spots colored by the Dragendorff reagent appeared on chromatograms of the extract.

The results obtained permitted the conclusion that with a rise in the temperature there was a certain increase in the degree of extraction and also an improvement in the kinetics of the indices (Figs. 2b and 3a).

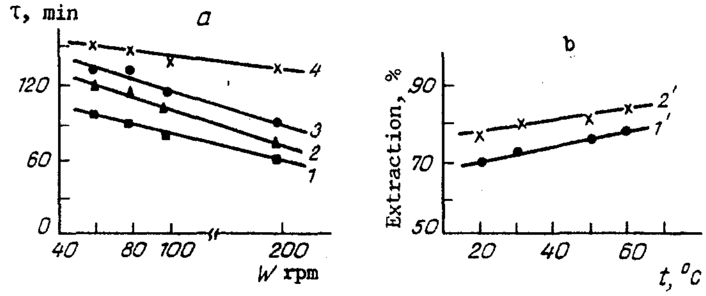

Fig. 2. Influence of the rate of stirring on the time of establishing equilibrium (a) and of the temperature of the process (b) on the passage of the total alkaloids into the extract (single extraction, conditions analogous to those described in Table 1): particle diameter: 1) 8-12 mm; 2) 3-6 mm; 3) 2-4 mm; 4) 0.5-1 mm; 1') 2% H_2SO_4; 2') 5% CH_3COOH.

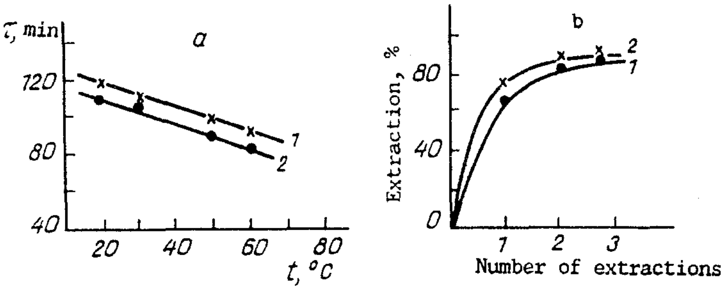

Fig. 3. Influence of the temperature of the process on the time of establishing a state of equilibrium in the passage of the total alkaloids into the extract (single extraction, conditions of the process analogous to those described in Table 1) (a) and the degree of passage of the total alkaloids into the extract as a function of the stage of extraction (b): 1) 2% H_2SO_4; 2) 5% CH_3COOH.

TABLE 3. Dependence of the Concentration of Total Alkaloids in the Extract on the Number of the Reactor in the Case of Single Extractions

Number of the reactor	Concentration of total alkaloids in the extract, %	
	2% solution H_2SO_4	5% solution of CH_3COOH
1	0,049	0,052
2	0,069	0,073
3	0,080	0,085
4	0,085	0,088
5	0,088	0,088

TABLE 4. Extraction in a Battery System (extractant: 5% CH_3COOH)

Number of the reactor	Concentration (%) in exract No.			
	1	2	3	4
1	0,049	0,069	0,089	0,085
2	0,014	0,040	0,054	0,061
3	0,004	0,009	0,030	0,042
4	0,002	0,005	0,008	0,008

The average concentration of an extract with respect to the total alkaloids was 0.04-0.05%. As one of the means of raising the concentration, let us consider the conditions of a process of multiple extraction of the raw material in a battery system.

At the optimum parameters of the process ($s:\ell = 1:10$, $d_3 = 3.0-6.0$ mm; $n = 100$ rpm; $t = 20°C$), three extractions of the raw material with 2% aqueous sulfuric acid in one diffusor led to a situation in which (Fig. 3b) with each successive extraction the amount of total alkaloids in the extract fell sharply. While the first extract, as the most concentrated, could be used for the further isolation of the total alkaloid, the subsequent extracts were so dilute that their use was undesirable. Even making use of the possibility of decreasing the volume of extractant in the subsequent stages of the process (in the first extraction, $s:\ell = 1/10$ and in the second and third, 1/6-1/8) did not enable the concentration of the extracts to be raised to a useful level.

Performing the extraction process in a system consisting of 4-5 reactors enabled the concentration of the extract to be almost doubled (Table 3). It is obvious that it is possible to be limited to four extractors. The optimum number of extractions in each diffusor must also be considered to be four (Table 4), after which the tail reactor is replaced by one charged with fresh raw material. It must be mentioned that the use of the battery system permits an increase in the passage of the total alkaloids into the extract to an average of 96% and enables extraction to be carried out at room temperature.

On the basis of the experiments performed it is possible to recommend the following optimum regime of the process: extraction in a four-battery system with four extractions from each reactor; particle size of the raw material 3-6 mm, temperature of the process 20-25°C, $s/\ell = 1/10$; $n_{rev} = 100$ rpm; average time of reaction in each reactor for the first extract 110-120 min, for the second 90-95, and for the third and fourth 70-75 min. The extract obtained is made alkaline with sodium carbonate to pH 8.5-9.0, and the total alkaloids are extracted with chloroform until the reaction is negative.

On an average, at a phase ratio $V_{org}/V_{aq} = 1/3$, three extractions were necessary, after which the total alkaloids were extracted with 5% sulfuric acid. The acid extracts were washed with chloroform and made alkaline to pH 8.5-9. The alkaloids were extracted with chloroform. After the chloroform had been distilled off, the total alkaloids were obtained with a yield averaging about 85-90% of the amount in the raw material.

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