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QUANTITATIVE DETERMINATION OF THE ALKALOIDS OF *Anabasis aphylla* BY THIN-LAYER CHROMATOGRAPHY

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In connection with the proposal of a new method for the separate isolation of the alkaloids of *Anabasis aphylla* [1] with the subsequent production of pachycarpine from the high-boiling fraction of the alkaloids [2], the necessity has arisen for the development of a method for the quantitative determination of the main alkaloids in the raw material, the intermediates, and the final products of this process.

Several methods exist for the quantitative determination of anabasine in the raw material and in the products of anabasine production [3]. According to the well-known standard method (factory method) [4], the anabasine is precipitated in the form of a complex mercury salt and, after decomposition with hydrochloric acid, the alkaloid is titrated with tungstosilicic acid. When the anabasine is precipitated in the form of the mercury complex, some other accompanying alkaloids (aphylline, aphyllidine, anabasamine) may also be precipitated, which is responsible for the high results of the standard method.

The present paper gives a method for the quantitative determination of the main alkaloids of Anabasis by means of thin-layer chromatography. The chromatography of the alkaloids has been performed in the acetone–water (100:8) (1) and the ether–chloroform (100:70) (2) systems in a non-fixed layer of alumina (activity grade II according to Brockmann). Af-

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TABLE 1. Molar Ratio of Alkaloids and Tungstosilica Acid

Alkaloids	Amount of the alkaloid			Amount of tungstosilicic acid consumed in titration, ml		Amount of alkaloids (g) corresponding to 1 ml of 0.01 M tungstosilicic acid	Molar ratio of base and tungstosilicic acid
	in 100 ml of 0.1 N HCl, mg	taken for titration		calculated	found (mean of two determinations)		
		mg	mg				
Aphylline	248	1	2,48	0,25	0,28	0,00992	1:4
		2	4,96	0,50	0,49		
		3	7,44	0,75	0,78		
		4	9,92	1,00	1,03		
Aphyllidine	249	1	2,49	0,25	0,27	0,00984	1:4
		2	4,98	0,50	0,52		
		3	7,47	0,75	0,78		
		4	9,96	1,00	1,03		
Anabasamine	253	1	2,53	0,50	0,52	0,00506	1:2
		2	5,06	1,00	1,03		
		3	7,59	1,50	1,52		
		4	10,12	2,00	2,03		
Lupinine	170	1	1,7	0,33	0,36	0,00507	1:3
		2	3,4	0,66	0,68		
		3	5,1	1,00	0,97		
		4	6,8	1,33	1,35		
Pachycarpine	230	1	2,3	0,50	0,52	0,00460	1:2
		2	4,6	1,00	1,03		
		3	6,9	1,50	1,48		
		4	9,2	2,00	2,02		
Anabasine	162	1	1,62	0,50	0,51	0,00324	1:2
		2	3,24	1,00	1,02		

TABLE 2. Results of an Investigation of Artificial Mixtures of Alkaloids

Alkaloid	Amount of the alkaloid		R_f	Consumption of tungstosilicic acid (mean of 2 determinations), ml	Amount of alkaloid in the synthetic mixture, %	
	in 100 ml of methanol, mg	deposited on chromatogram, ml			calc.	found
Aphylline	246	2,0	0,60	0,52	0,246	0,252
Aphyllidine	243	—	0,74	0,53	0,243	0,252
Lupinine	171	—	0,10	0,71	0,171	0,173
Anabasamine	250	—	0,40	1,03	0,250	0,258
Anabasine	164	—	0,63	0,97	0,164	0,166

ter drying, the chromatograms were revealed in iodine vapor. Under these conditions, the alkaloids had the following R_f values: lupinine 0.40, anabasine 0.63, anabasamine 0.78, aphylline 0.80, aphyllidine 0.82, and pachycarpine 0.21 (in system 1), and lupinine 0.10, anabasine 0.16, anabasamine 0.40, aphylline 0.60, and aphyllidine 0.74 (in system 2). Because of the great difference in the R_f values, for the quantitative determination of anabasine, lupinine, and pachycarpine we used system 1, and for aphylline, aphyllidine, and anabasamine system 2. After the elution of the spots of the individual alkaloids from the absorbent with 0.1 N hydrochloric acid, the amounts of the substances were determined by titration with a 0.01 M solution of tungstosilicic acid.

The corresponding molar ratios of the individual alkaloids and tungstosilicic acid were determined by the titration of standard solutions of the basis with tungstosilicic acid (Table 1).

To check the reliability of the method, we determined the amounts of alkaloids present in synthetic mixtures (Table 2) and also in natural solutions with the addition to the raw material and to anabasine sulfate of a known amount of the pure base (Table 3).

The results of a quantitative determination of the alkaloids in the raw material, in anabasine sulfate, and in the mixture of the high-boiling fractions of the bases are given in Table 4.

Thus, the proposed method permits the simultaneous quantitative determination of the amount of anabasine and of the accompanying alkaloids — lupinine, aphylline, aphyllidine, and anabasamine — in the raw material and the anabasine sulfate, and also of pachycarpine in

TABLE 3. Results of the Quantitative Determination of Anabasine in the Raw Material and in Anabasine Sulfate with the Addition of Anabasine

Raw material	Taken for analysis, g	Anabasine content, %	Pure anabasine added, (g) to		Anabasine content (%) after addition to				Experimental error, %
			raw material	anabasine sulfate	raw material		anabasine sulfate		
					calc.	found (mean of two determinations)	calc.	found (mean of two determinations)	
№ 1	4	1,56	0,0317		2,35	2,43			+3,4
№ 2	4	1,62	0,0234		2,21	2,14			−3,1
№ 3	4	1,79	0,0228		2,36	2,44			+3,3
Anabasine sulfate	1,55	34,4		0,2449			40,9	42,1	+2,8
	1,55	34,4		0,0803			36,9	38,2	+3,4
	1,55	34,4		0,1289			38,3	39,1	+2,3

TABLE 4. Amounts of Alkaloids in the Raw Material in Anabasine Sulfate, and in a Mixture of High-Boiling Fractions after Hydrogenation (%)

Sample	Anabasine	Lupinine	Aphylline	Aphyllidine	Anabasamine	Pachycarpine
	mean of two determinations					
Raw material						
№ 1	1,44	0,22	0,27	0,21	0,047	—
№ 2	1,61	0,30	0,34	0,27	0,060	—
№ 3	1,58	0,27	0,36	0,28	0,078	—
№ 4	1,78	0,24	0,38	0,19	0,065	—
№ 5	1,63	0,26	0,29	0,22	—	—
Anabasine sulfate						
№ 1	27,8	5,70	5,62	3,70	2,45	—
№ 2	29,2	6,2	5,55	3,35	2,21	—
№ 3	33,1	6,4	4,88	3,95	1,98	—
№ 4	29,8	5,8	5,01	4,10	2,01	—
№ 5	30,1	6,2	4,98	3,78	1,89	—
Mixture of the high-boiling fractions after the hydrogenation	—	—	44,4	—	—	52,2
	—	—	45,4	—	—	50,9
	—	—	45,6*	—	—	50,6

*The molecular weights of aphylline and aphyllidine differ by only two units, and therefore these bases were titrated together.

the products of the hydrogenation of the high-boiling alkaloids of *Anabasis aphylla*. This method can be used in laboratory analyses and also for determining these alkaloids in industry. The experimental error in titration with tungstosilicic acid does not exceed ±3.5%.

EXPERIMENTAL

A. Determination of the Amounts of Alkaloids in the Raw Material. Two samples of *Anabasis* of 4 g each were made alkaline with 25% caustic soda solution (4 ml) and were covered with 30 ml of chloroform, and the mixture was left for the extraction of the alkaloids for 15 h. Then half of each of the two samples (filtered) of chloroform extract (15 ml each) was concentrated (to 7.5 ml for the determination of the anabasine and to 5 ml for the determination of lupinine, aphylline, aphyllidine, and anabasamine).

Of the concentrated extracts, 0.5-ml portions (for the determination of the amount of the anabasine) and 1-ml portions (for the determination of the amounts of lupinine, aphylline, aphyllidine, and anabasamine) were deposited in the form of continuous lines on chromatograms (40 × 8 cm) with a thin layer of absorbent (0.8 mm) having a particle size of 0.14 mm. Chromatography was carried on for 60-70 min. The solvent was evaporated and the alkaloids were revealed in iodine vapor. The revealed alkaloid spots were outlined and the chromatograms were left in the air at room temperature until the iodine had disappeared (8-10 min). The collected alkaloid spots were eluted with 0.1 N hydrochloric acid (3-5 × 1 ml) and the eluate was titrated with a 0.01 M solution of tungstosilicic acid.

The amounts of alkaloids in the raw material were calculated from the formula (the discrepancy of parallel experiments amounted to 0.01-0.05%):

$$\% x = a \cdot b \cdot c \cdot 100 \cdot f/d \cdot e$$

where a is the mean amount of tungstosilicic acid used in titration, ml; b is the amount of chloroform taken for extraction, ml; c is the amount of alkaloids corresponding to 1 ml of 0.01 M tungstosilicic acid, g; f is the normality of the tungstosilicic acid; d is the weight of the sample, g; and e is the amount of chloroform extract taken for chromatography, allowing for evaporation (concentration, ml).

B. Determination of the Alkaloids in Anabasine Sulfate. Anabasine. An accurately weighed sample of 1.55 g of anabasine sulfate and 0.6 g of caustic potash were dissolved in 50 ml of methanol (in a measuring flask), and 0.15-ml portions of this solution were deposited on a chromatogram. The other operations were performed by the method described above as in case A. The percentage of anabasine was calculated by means of the formula given where b is the amount of methanol, ml, and e is the amount of methanol taken for chromatography, ml.

Accompanying Alkaloids. An accurately weighed sample of 3.29 g of anabasine sulfate was dissolved in 10 ml of distilled water, the solution was made alkaline to pH 12 with caustic sode (30%), and the alkaloids were extracted with chloroform until the reaction with Dragendorff's reagent was negative. After drying over Na_2SO_4, the solution was filtered and the solvent was evaporated to 5 ml. From this, 1-ml samples were deposited on chromatograms.

The remaining operations were as in case A. The percentage of alkaloids was calculated by the formula given above, where b is the amount of chloroform extract after evaporation, ml (5 ml), and e is the chloroform extract taken for chromatography, ml.

C. Determination of Aphylline, Aphyllidine, Anabasamine, and Pachycarpine in a Mixture of the Hydrogenation Products of the High-Boiling Alkaloids. Samples (1 ml) of a solution in 10 ml of chloroform of 1.60 g of the mixture after hydrogenation were deposited on chromatograms. The remaining operations were as in case A. The percentage contents of the individual alkaloids were calculated from the formula given above.

SUMMARY

A chromatographic method has been developed for the quantitative determination of the alkaloids of *Anabasis aphylla* — anabasine, aphylline, lupinine, aphyllidine, and anabasamine — and also pachycarpine in the mixture of reduction products of the high-boiling fraction of alkaloids, in a thin layer of alumina.

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