

THE ADSORPTION ANALYSIS OF ALKALOIDS

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IN a previous communication¹ it was shown that tropa alkaloids are quantitatively taken up by the synthetic zeolite, decalco, and that they are fully desorbed by moderately strong solutions of acids or salts. In this paper it is proved that the same applies to a number of other alkaloids, viz. to morphine hydrochloride, codeine phosphate, papaverine hydrochloride, berberine sulphate, hydrastinine hydrochloride, neostigmine bromide, *d*-tubocuraine chloride and *dl*- α -(4-hydroxyphenyl)- β -methylaminoethanol tartrate.

As with the tropa alkaloids both tertiary and quaternary salts behave similarly, and it is believed that the sorption and desorption processes are generally valid for salts of nitrogenous bases, thus offering a convenient method of isolating them.

EXPERIMENTAL

General procedure. Pour an aqueous solution of the alkaloidal salt on a column of activated decalco. Here 0.7 to 5 mg. of the substance and 0.25 to 0.6 g. of decalco have been used (5 to 20 micro-equivalents per g. of decalco) in a tube 4 to 5 mm. wide. Wash with water *ad libitum* and elute the base in the column with a sufficient quantity of 0.2N hydrochloric, sulphuric or acetic acid or with 25 per cent. potassium chloride or sodium chloride solution. (Further details are given in our previous paper¹.) Determine the substance in the eluate by a suitable method.

If not otherwise stated, the final estimation of the alkaloid was performed in the following way. The eluate was diluted to 50.0 ml. with the eluant acid or, when salt had been used as the eluant, with water and hydrochloric acid to make the solutions 0.2N in hydrochloric acid. In this solution the amount of the alkaloid was determined by measuring the extinction at the maximum of the absorption curve in a Hilger spectrophotometer, model Uvispek.

As blank values are often obtained when the assays are performed at wavelengths below 300 $m\mu$ the following device was used. The same amount of alkaloid as in the test was put into a 50-ml. flask. Into this flask the same amount of eluant as in the test was allowed to flow through a blank column and the resultant mixture diluted as in the test. The extinction of this solution was considered to be the quantity of alkaloid taken. This method is correct only if Beer's law is valid, which was ascertained in each case.

It should be pointed out that elution with acids will dissolve iron from the decalco if too large eluates are taken. Thus, with 0.4 g. of decalco, iron will appear after about 10 ml. of eluate, when 0.2N hydrochloric or sulphuric acid is used as eluant, and after about 20 ml. when 0.2N

acetic acid is used. More decalso delays the break-through of iron, and the reverse.

If the spectrophotometric assay is performed at wavelengths below 300 $m\mu$, mere traces of iron will render the analysis impossible. As will be seen from the results in those cases where iron might have been eluted, the measurements are made at high wavelengths where the interference is negligible.

Results. The results for codeine phosphate, papaverine hydrochloride, hydrastinine hydrochloride, *d*-tubocurarine chloride and *dl*- α -(4-hydroxyphenyl)- β -methylaminoethanol tartrate are given in Tables I, II, III, IV and V.

TABLE I

CODEINE PHOSPHATE

Sample: Ph. Sv. Ed. XI. Assayed at 284.0 $m\mu$

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with						
		Water 10 to 30 ml.	0.2N hydrochloric acid		0.2N acetic acid		25 per cent. potassium chloride	
			5 ml.	10 ml.	5 ml.	10 ml.	5 ml.	10 ml.
5	0.6	0	99.1	100.7	79.1	99.4	97.3	98.6
		0	99.4	101.4	79.9	99.9	98.3	102.2

TABLE II

PAPAVERINE HYDROCHLORIDE

Sample: Ph. Sv. Ed. XI. Assayed at 308.5 $m\mu$

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with					
		Water 10 to 30 ml.	0.2N hydrochloric acid		0.2N acetic acid		
			10 ml.	15 ml.	First 15 ml.	Second 15 ml.	Total
1.2	0.6	0		100.1	97.7	2.9	100.6
		0		100.9	98.3	3.1	101.4
1.8	0.6	0	98.8		98.6	1.0	99.6
		0	97.8		98.3	0.0	98.3

TABLE III

HYDRASTININE HYDROCHLORIDE

Sample: A product from Merck, Germany, recrystallised 3 times from ethanol and ether. Assayed at 306.5 and 364.0 $m\mu$. The recoveries are means of values obtained at the two wavelengths. The elution could be followed in ultra-violet light

Quantity of alkaloid g.	Decalso g.	Percentage recovery after elution with						
		Water 10 to 30 ml.	0.2N hydrochloric acid			0.2N acetic acid		
			First 10 ml.	Second 10 ml.	Total	First 10 ml.	Second 10 ml.	Total
0.7	0.4	0	99.5	0	99.5	100.3	0.6	100.9
		0	97.0	2.4	99.4	95.9	0.6	97.5
		0	98.3	2.0	100.3			
	0.6	0	97.9	4.8	102.7			
		0	100.0	3.0	103.0			
		0	97.4	0.8	98.2			

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TABLE IV

d-TUBOCURARINE CHLORIDE

U.S.P. XIV sample. Assayed at 280.5 m μ

Quantity of alkaloid mg.	Decalso g.	Water 10 to 30 ml.	Percentage recovery after elution with										
			0.2N hydrochloric acid			0.2N acetic acid				0.2N sulphuric acid			
			5 ml.	First 5 ml.	Second 5 ml.	Third 5 ml.	Total	First 15 ml.	Second 15 ml.	Total	First 5 ml.	Second 5 ml.	Total
2.0	0.4	0	98.5					93.9	5.9	99.8			
		0	95.9					94.1	5.9	100.0			
		0	97.1										
		0	100.7										
		0	98.6*										
		0	100.0*										
4.0	0.4	0		27.4	45.6	18.6	91.6				92.0	7.0	99.0
				35.9	39.6	14.9	90.4				92.3	5.0	97.3

* Second eluate nil.

TABLE V

dl- α -(4-HYDROXYPHENYL)- β -METHYLAMINOETHANOL TARTRATE

Sample: Oxedrini tartras, Ph. Sv. Ed. XI. Assayed at 273.5 m μ

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with			
		Water 10 to 30 ml.	0.2N hydrochloric acid 5 ml.	0.2N sulphuric acid 5 ml.	0.2N acetic acid 5 ml.
4	0.4	0	99.1	98.8	99.8
		0	99.8	99.8	100.2
		0	100.5		
		0	99.3		

Morphine hydrochloride. A sample complying with the Swedish Pharmacopœia, Ed. XI, was used. The morphine was assayed photometrically by a modified nitrosation method^{2,3}. As the intensity of the colour developed varied with the concentration of salts, acids, etc., in the reaction mixture, a new standard curve had to be made for each case. To get conditions identical in the standard curve solutions and in the eluates, decalso blank columns were eluted with the specified amount of a certain eluant, a known amount of morphine was added to the eluate and the determination was performed in the following way. The solution was mixed with 0.2 g. of sodium citrate* and sufficient water and hydrochloric acid to make about 20 ml. of solution approximately 0.5N in hydrochloric acid. 8.0 ml. of a 1 per cent. sodium nitrite solution was added and after exactly 5 minutes, 10 ml. of 5N ammonia and water to 50.0 ml. The extinction was measured at 470 m μ in 1-cm. cells. For results see Table VI.

Berberine sulphate. Preliminary experiments gave somewhat low recoveries and paper chromatography performed by Rutter's method⁴, with isobutanol saturated with 0.2N acetic acid as the developer and ultra-violet light to visualise the process, revealed that the substance

* The citrate is necessary to keep dissolved aluminium in solution after the addition of ammonia.

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TABLE VI
MORPHINE HYDROCHLORIDE

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with							
		Water 10 to 30 ml.	0.2N hydrochloric acid 5 ml.	0.2N acetic acid			25 per cent. sodium chloride		
				5 ml.	10 ml.	15 ml.	First 5 ml.	Second 5 ml.	Total
4	0.5	0	101.3	86.4	95.5	99.8	90.2	10.1	100.3
4	0.5	0	101.3	83.5	95.0	100.4	90.9	8.6	99.5
4	0.5	0	100.2						
4	0.5	0	97.9						

contained an impurity with an R_f value about half as great as that of the berberine itself. Therefore, the sample was recrystallised 3 times from ethanol and ether and was then chromatographically pure. It was easy to follow the elution process visually, as berberine exhibits a strong fluorescence in ultra-violet light.

TABLE VII
BERBERINE SULPHATE
Sample: See text. Assayed at 421.0 $m\mu$

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with							
		Water 10 to 30 ml.	0.2N sulphuric acid				0.2N acetic acid		
			First 15 ml.	Second 15 ml.	Total	20 ml.	25 ml.	20 ml.	25 ml.
1.5	0.6	0	96.4	4.0	100.4				99.9
		0	96.4	1.5	97.9				99.4
2.1	0.6	0				97.7*		95.7*	
		0				98.0*		100.7*	
1.5	0.25	0					99.9		99.3

* Second eluate nil.

TABLE VIII
STEPWISE ELUTION OF BERBERINE SULPHATE

Quantity of alkaloid mg.	Decalso g.	Elution with	Percentage recovery			
			First 5 ml.	Second 5 ml.	Third 5 ml.	Total
1.2	0.25	0.2N Sulphuric acid	88.8	9.2	1.9	99.9
		0.2N Acetic acid	75.7	24.6	1.9	102.2

TABLE IX
NEOSTIGMINE BROMIDE

Quantity of alkaloid mg.	Decalso g.	Percentage recovery after elution with		
		Water 10 to 30 ml.	0.2N hydrochloric acid 5 ml.	0.2N acetic acid 10 ml.
2.5	0.4	0	100.5	100.0
		0	100.7	99.6
		0	98.5	
		0	101.4	

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Neostigmine bromide. A sample of prostigmine bromide, of U.S.P. XIV quality, was used. For the assay the substance was hydrolysed with 1N sodium hydroxide on the water bath for 15 minutes. Experiments gave 95 per cent. hydrolysis after 5 minutes, 100 per cent. after 10 minutes, and 100 per cent. after 30 minutes. The product was assayed in 0.2N sodium hydroxide at 293.5 $m\mu$. (Cf. Mørch⁵ and Spross⁶.)

DISCUSSION

The results show that all substances tested were recovered quantitatively by the proposed procedure. There appears to be no significant difference between the eluability of tertiary and quaternary bases. Generally speaking, the bases are more easily eluted by the strong acids than by acetic acid, although the difference is very slight for some alkaloids.

Of the compounds tested berberine was by far the most difficult one to elute. In this case hydrochloric acid is not suitable, as the chloride is very slightly soluble in hydrochloric acid. Even with 30 ml. of 0.2N hydrochloric acid only 90 per cent. of the base was eluted from 0.6 g. of decalzo.

It may be mentioned that narcotine hydrochloride could not be recovered quantitatively from decalzo. The reason was proved to be that the alkaloid was partly decomposed in the column. It was, however, completely adsorbed by the decalzo.

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