THE PARTITION CHROMATOGRAPHY OF ALKALOIDS

PART IV. THE ASSAY OF SOLANACEOUS DRUGS

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CHEMICAL methods for the standardisation of solanaceous drugs have hitherto usually involved determination of the total alkaloids by a suitable adaptation[1] of the classical Stas-Otto process. Useful improvements have been effected by chromatographic adsorption of the total alkaloids on an alumina column,[2] by precipitation of their silicotungstates[3] and, for tropates, by application of the Vitali-Morin colour test[4] or direct determination of the tropic acid liberated by hydrolysis.[5] For the separate determination of hyoscine and hyoscyamine, differences in their dissociation constants and solubilities[6] and in the solubilities of their picrates in chloroform[7] have been exploited. Chromatographic separation of the bases has been achieved by means of a silica column[8] and of the hydrochlorides by means of partition columns.[9]

It is known that hyoscine and hyoscyamine exhibit qualitative differences in their pharmacological actions, particularly on the central nervous system, and that important variations in the relative proportions of these alkaloids within a given species are frequently encountered.[10,11,12] Accordingly it was considered worth while to adapt the partition chromatographic procedure described in Parts I and II[13,14] to the routine determination of hyoscine and hyoscyamine in solanaceous drugs. Factors influencing the quantitative separation of the alkaloids and the extraction of the drug have been examined and an assay process based on these observations has been applied to a range of samples of solanaceous drugs.

SEPARATION OF HYOSCINE AND HYOSCYAMINE

As an extension of the experiments recorded in Part I,[13] a systematic study was made of the variables which appeared most likely to operate in the separation of hyoscine and hyoscyamine by elution development of a partition column. The arbitrary, standard partition chromatographic system adopted for comparison consisted of a column of 10 g. of kieselguhr ("Hyflo Super-cel"), mixed with 3·2 ml. of 0·25 M phosphate buffer of *p*H 7·0, packed[15] in a tube 1·7 cm. in internal diameter; a mixture of 14·6 mg. of hyoscyamine and 5·8 mg. of hyoscine dissolved in 2 ml. of ether was chosen as the standard load and the ether eluate was collected in fractions of 2·5 ml. The experimental technique employed in following the course of fractionation and the method of presentation of the results were the same as those described in Part I.

The results of experiments concerned with systematic variations in the rate of flow of the eluting solvent, in the quantity of buffer distributed on the kieselguhr, in the load of alkaloids, in the concentration of the buffer and in its *p*H value and in the dimensions of the column are

summarised in Tables I, II, III, IV, V and VI. A buffer containing disodium hydrogen phosphate and citric acid was employed for *p*H values below 5·8 (Table V). Results obtained with the arbitrarily chosen standard system are printed in italics.

TABLE I

VARIATION IN THE RATE OF FLOW OF THE ELUANT

Rate of flow of eluant ml./minute	Hyoscine 1·8 mg./ml. of buffer		Hyscyamine 4·6 mg./ml. of buffer	
	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.
2·5	*3*	*17·5*	*29*	*210*
6·6	3	17·5	26	210
13·0	3	17·5	21	210

Column, 10 g. of kieselguhr, 3·2 ml. of 0·25 M phosphate buffer, *p*H 7·0; eluant, ether; eluate fractions, 2·5 ml.

TABLE II

VARIATION IN THE QUANTITY OF BUFFER

0·25 M phosphate buffer *p*H 7·0 ml.	Hyoscine			Hyoscyamine		
	Hyoscine per ml. of buffer mg.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.	Hyoscyamine per ml. of buffer mg.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.
0·0	0	–	—	‡	3	37·5
1·0	5·7	3	70*	14·4	—	—
2·0	2·9	3	175†	7·3	11	—
2·5	2·5	3	17·5	6·2	16	180
3·0	1·9	3	17·5	5·0	18	195
3·2	*1·8*	*3*	*17·5*	*4·6*	*29*	*210*
3·5	1·6	3	17·5	4·2	37	215
4·0	1·5	3	17·5	3·8	42	275
5·0	1·1	3	17·5	2·9	50	285

* Volume required to elute both alkaloids without separation.
† Volume required to elute both alkaloids with partial separation.
‡ 15 mg. on plain kieselguhr.
Column, 10 g. of kieselguhr; eluant, ether; eluate fractions, 2·5 ml.

TABLE III

VARIATION IN THE LOAD OF ALKALOIDS

Weight of kieselguhr g.	Volume of buffer ml.	Hyoscine			Hyoscyamine		
		Hyoscine per ml. of buffer mg.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.	Hyoscyamine per ml. of buffer mg.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.
15·6	5·0	1·2	4	20·0	3·2	66	275
12·5	4·0	1·6	3	20·0	3·9	41	250
10·0	*3·2*	*1·8*	*3*	*17·5*	*4·6*	*29*	*210*
10·0	3·2	0·9	3	17·5	2·6	55	195
10·0	3·2	2·5	3	17·5	6·6	25	230
10·0	3·2	3·3	3	15·0	9·0	12	240
10·0	3·2	4·2	3	250*	11·6	—	—
9·4	3·0	2·1	3	17·5	5·2	26	205
7·8	2·5	2·6	3	17·5	6·4	17	180
6·3	2·0	3·1	3	15·0	7·5	9	140
3·1	1·0	6·2	3	30·0*	15·6	—	—

* Volume required to elute both alkaloids without separation.
Column, 0·25 M phosphate buffer, *p*H 7·0; eluant, ether; eluate fractions, 2·5 ml.

TABLE IV

VARIATION IN THE CONCENTRATION OF THE BUFFER

Molarity of buffer solution *p*H 7·0	Hyoscine 1·8 mg./ml. of buffer		Hyoscyamine 4·6 mg./ml. of buffer	
	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.
0·5	3	17·5	90	240
0·25	*3*	*17·5*	*28*	*210*
0·125	3	17·5	17	215
0·063	3	255†	15	—
0·032	3	50*	—	—

* Volume required to elute both alkaloids without separation.
† Volume required to elute both alkaloids with partial separation.
Column, 10 g. of kieselguhr, 3·2 ml. of phosphate buffer; eluant, ether; eluate fractions, 2·5 ml.

TABLE V

VARIATION IN THE *p*H VALUE OF THE BUFFER

*p*H value of buffer	Hyoscine 1·8 mg./ml. of buffer		Hyoscyamine 4·6 mg./ml. of buffer	
	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.
7·7	3	75*	—	—
7·2	3	200†	13	—
7·0	*3*	*17·5*	*28*	*210*
6·8	3	17·5	59	200
6·3	8	22·5	103	30§
5·8	21	47·5	105	75§
5·8‡	23	50	106	55§
5·5‡	56	80	117	100§
5·0‡	104	27·5§	163	210§

* Volume required to elute both alkaloids without separation.
† Volume required to elute both alkaloids with partial separation.
‡ Citrate buffer.
§ Volume of chloroform required for elution after 100 eluate fractions with ether.
Column, 10 g. of kieselguhr, 3·2 ml. of phosphate or citrate buffer; eluant, ether; eluate fractions, 2·5 ml.

TABLE VI

VARIATION IN THE DIMENSIONS OF THE COLUMN

Length of column cm.	Diameter of column cm.	Hyoscine 1·8 mg./ml. of buffer		Hyoscyamine 4·6 mg./ml. of buffer	
		Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.	Position of maximum on elution curve. Eluate fraction number	Volume of eluant for complete elution ml.
33·2	1·0	3	12·5	22	165
15·5	1·4	3	17·5	26	200
11·7	*1·7*	*3*	*17·5*	*28*	*210*
9·0	2·0	3	17·5	32	210
4·1	2·9	3	22·5	39	270

Column, 10 g. of kieselguhr, 3·2 ml. of phosphate buffer, *p*H 7·0; eluant, ether; eluate fractions, 2·5 ml.

The quality of kieselguhr employed as carrier for the buffer has been found to be of great importance. Of 6 commercial samples examined, only 2 grades proved satisfactory, namely "Hyflo Super-cel" and "Celite

No. 545."* With columns prepared from other varieties, the alkaloids were strongly adsorbed and their elution with ether or chloroform was either very slow or impossible. Attempts to improve such varieties by washing with acid, elutriation or calcination were unsuccessful. The following simple, empirical test, based on the adsorptive capacity of kieselguhr for hyoscyamine, served to distinguish between satisfactory and unsatisfactory grades:—6 ml. of 0·004 per cent. solution of hyoscyamine in water is shaken with 1 g. of the kieselguhr; 5 ml. of solvent ether is added and the mixture is again shaken for 1 minute. The ether layer is allowed to separate and 2 ml. is transferred to 7 ml. of water containing 2 drops of solution of bromocresol green adjusted to the transition colour (*p*H 4·4). On shaking, the aqueous layer turns blue with satisfactory grades of kieselguhr; with unsatisfactory grades there is no colour change.

Extraction of Alkaloids from the Crude Drug

The choice of a base for the liberation of the alkaloids in the drug and of a solvent for their extraction was examined with samples of *Datura stramonium*, *Atropa belladonna* and *Hyoscyamus niger* which had been assayed for total alkaloids by the Pharmacopœial method. The alkaloids extracted under a given set of conditions were determined after chromatographic separation on a column consisting of 10 g. of kieselguhr on which was distributed 3·2 ml. of 0·25 M phosphate buffer of *p*H 6·0. In a lengthy series of experiments, which do not merit detailed description, it was found that a number of features could interfere with the convenient operation of a chromatographic assay of hyoscine and hyoscyamine.

Liberation of the alkaloids with ammonia was unsuitable, since its removal, prior to chromatographic separation of the alkaloids, necessitated heating the residue obtained by evaporation of the percolate. Calcium hydroxide was found to be entirely satisfactory for this purpose; the volume of solvent required for complete extraction was less than when ammonia was used, and the amount of colouring matter extracted was reduced.

The large amounts of non-alkaloidal extractive matter obtained when mixtures of ether and ethanol were employed for extraction rendered such solvents useless. Ether alone was found to comply with all requirements except for the volume needed for complete extraction. By appropriate modification of the extraction technique, details of which are given below, this volume could be reduced. The turbid percolate obtained with certain samples of *A. belladonna* and *H. niger* could be clarified by filtration through No. 42 Whatman filter-paper. Failure to remove the material responsible for this turbidity resulted in a greatly reduced rate of flow of the mobile phase through the chromatographic column.

Chromatography of the Total Alkaloids of Solanaceous Drugs

Two problems were involved in the chromatography of the alkaloids extracted from solanaceous drugs, namely, separation of the hyoscine

* Messrs. Johns-Manville Co. Ltd., Artillery House, Artillery Road, London, S.W.1.

and hyoscyamine from colouring matter and, in some samples of these drugs, from other weakly basic material. In systematic experiments on these problems, the residue left after evaporation of the percolate was dissolved in the solvent selected as eluant, the solution was transferred to the column and the eluate from the column was collected in fractions and titrated in the usual way. Sufficiently sharp separation of the alkaloids from the colouring matter could not be achieved with ether, light petroleum or benzene as eluting solvents. Elution first with carbon tetrachloride, then with ether and finally with chloroform afforded satisfactory results with a buffer of *p*H 5·9 to 6·2 distributed on the column. With carbon tetrachloride most of the colouring matter was eluted together with the weakly basic material present in certain samples; hyoscine was then quantitatively eluted with ether and finally hyoscyamine was eluted with chloroform. The mixture of solvents issuing from the column at the change over of solvents from carbon tetrachloride to ether usually contained no alkaloid. From these observations, it became clear that the eluate could be collected in three main fractions.

Proposed Method of Assay

Moisten the drug, in moderately fine powder, with water (Note 1) and set aside overnight in a well-closed container. Triturate with 1 g. of calcium hydroxide until a uniform mixture is produced; transfer the mixture to a 100-ml. cylindrical separator, plugged with cotton wool, and complete the transference, using in all about 50 ml. of solvent ether. Close the separator securely and shake continuously for 1 hour. Allow the solid to settle, drain off the supernatant liquid, compress the marc and percolate with solvent ether until complete extraction of the alkaloids is effected (Note 2). If the percolate is turbid, filter through a No. 42 Whatman filter-paper. Remove the ether and dissolve the residue in 2 ml. of carbon tetrachloride.

Mix intimately 3·2 ml. of phosphate buffer (Note 3) with 10 g. of kieselguhr (Note 4). Compress a plug of cotton wool into a glass tube, 1·7 cm. in internal diameter and about 35 cm. length, fitted with a tap. Pour about 30 ml. of carbon tetrachloride into the tube and introduce about 3 g. of the kieselguhr mixed with buffer. Agitate the suspension by rapid vertical strokes of a perforated plunger and then by slow strokes gradually compress the solid; continue the packing using successive quantities of 1 to 2 g. of the remainder of the kieselguhr mixed with buffer; pack 1 g. of kieselguhr and a plug of cotton wool on top of the column. Allow the supernatant carbon tetrachloride to drain from the column and transfer the carbon tetrachloride solution of the residue obtained by evaporation of the percolate to the top of the column; complete the transference using successive quantities each of 1 ml. of carbon tetrachloride, allowing each to flow into the column before adding the next. Develop the column with carbon tetrachloride at a rate of about 3 ml. per minute until the liquid leaving the column just becomes pale yellow (Note 5). Continue the development with solvent ether and reject the first portion of the eluate if it is free from basic material (Note 6);

collect the subsequently issuing eluate in a cylindrical vessel until no more base is eluted from the column (Note 7). Add 5 ml. of water and titrate (Note 8) with 0·005N sulphuric acid, using solution of bromocresol green as indicator. Each ml. of 0·005 N sulphuric acid is equivalent to 0·00152 g. of hyoscine. Continue the development with chloroform until no further base is eluted (Note 9). Evaporate the chloroform solution to about 5 ml. (Note 10), add 10 ml. of solvent ether and 5 ml. of water and titrate with 0·005 N sulphuric acid, using solution of bromocresol green as indicator. Each ml. of 0·005 N sulphuric acid is equivalent to 0·00145 g. of hyoscyamine.

Notes 1. The quantities of drug and water are as follows: belladonna herb and root, stramonium 5 g.; 3 ml.; hyoscyamus 10 g.; 6 ml.

2. About 250 ml. of percolate is required; 0·05 N iodine is employed in testing for complete extraction.

3. The phosphate buffer (*p*H 5·9 to 6·2) is made by mixing 25 ml. of M potassium dihydrogen phosphate, 3·0 to 4·1 ml. of carbonate-free N sodium hydroxide and diluting with freshly boiled and cooled water to 100 ml.

4. "Hyflo Super-cel" or "Celite No. 545" are suitable grades.

5. About 30 ml. of carbon tetrachloride is required. Prolonged development may cause loss of hyoscine. Basic material eluted in the first, dark green eluate is not hyoscine.

6. It is preferable to reject as much as possible of the mixture of carbon tetrachloride and ether issuing from the column at the change over of solvents, since the presence of carbon tetrachloride reduces the sharpness of the end-point in the titration of the ether solution of hyoscine. Test portions of the eluate are collected and shaken with diluted solution of bromocresol green which has been adjusted to its transition tint; the eluate is collected as soon as basic material is detected. Occasionally elution of the hyoscine begins before all the carbon tetrachloride has been displaced.

7. About 100 ml. of eluate is collected. Solution of bromocresol green is used in testing for completion of the elution.

8. The two layers are thoroughly agitated with a glass stirrer during the titration. This titration is most conveniently carried out in a cylindrical vessel.

9. About 100 ml. of chloroform is required.

10. Chloroform interferes with the observation of the end-point unless most of it is removed. A pale brown colour in the chloroform causes no difficulty, since the end-point is observed in the aqueous layer.

The results obtained with this process are summarised in Table VII. Indian belladonna root contains an alkaloid additional to hyoscine which is eluted from the column with ether. The process described above is therefore not applicable to this drug unless the eluate is collected in small fractions and those corresponding to hyoscine and hyoscyamine are titrated separately.

TABLE VII

ALKALOIDS IN SOLANACEOUS PLANTS

Sample	Total alkaloids calculated as hyoscyamine by Pharmacopœial assay per cent.	Hyoscine per cent.	Hyoscyamine per cent.	Equivalent of total alkaloids calculated as hyoscyamine per cent.	Hyoscine/hyoscyamine ratio
Atropa belladonna					
1. English herb	0·48	0	0·47	0·47	—
		0	0·51	0·51	
2. Sample 1 plus 0·056 per cent. of hyoscine	—	0·052	0·051	—	—
3. English herb	0·43	0	0·45	0·45	—
		0	0·43	0·43	
4. Sample 3 plus 0·084 per cent. of hyoscine	—	0·078	0·43	—	—
5. Indian leaf	0·21	0	0·22	0·22	—
		0.	0·22	0·22	
6. European root ..	0·62	0·02	0·58	0·60	0·035
		0·02	0·59	0·61	
Datura stramonium					
7. Herb	0·26	0·07	0·20	0·27	0·30
		0·07	0·21	0·28	
8. Herb	0·25	0·05	0·20	0·25	0·25
		0·05	0·19	0·24	
9. Herb	0·37	0·11	0·28	0·37	0·38
		0·10	0·28	0·36	
Datura tatula					
10. Herb	0·43	0·27	0·17	0·43	1·55
		0·29	0·18	0·44	
11. Herb	0·27	0·09	0·19	0·28	0·45
		0·08	0·19	0·27	
12. Herb	0·15	0·05	0·15	0·20	0·32
		0·04	0·13	0·17	
Hyoscyamus niger					
13. Herb	0·04	0·03	0·02	0·05	1·5
		0·03	0·02	0·05	
14. Herb	0·05	0·01	0·05	0·06	0·2
15. Herb	0·05	0·02	0·04	0·06	0·5

DISCUSSION

The ideal operation of a partition chromatographic system as a true partition process requires perfect transfer of the solutes between the two phases, constant partition ratios and complete equilibration during the transfer. In practical systems these conditions do not normally exist and the operating conditions must be determined empirically. Hyoscine and hyoscyamine differ sufficiently in those physical properties which are exploited in their partition chromatographic separation to permit considerable latitude in the operating conditions.

The results recorded in Table I show that wide variation in the rate of flow of developing solvent is permissible, although the rate of equilibration of the solute depends mainly on the time and area of contact of the phases and the concentration gradient between the phases.[16,17] From the data recorded in Tables II and III, it is apparent that satisfactory separations are possible over a considerable range of buffer-kieselguhr ratios and alkaloid-buffer ratios at *p*H 7. The degree of separation increases as the alkaloid-buffer ratio decreases. The position of the maximum of the hyoscine peak on the elution curve remains unaltered since the partition coefficient of hyoscine under the conditions studied is overwhelmingly in favour of the mobile phase (see Fig. 4).

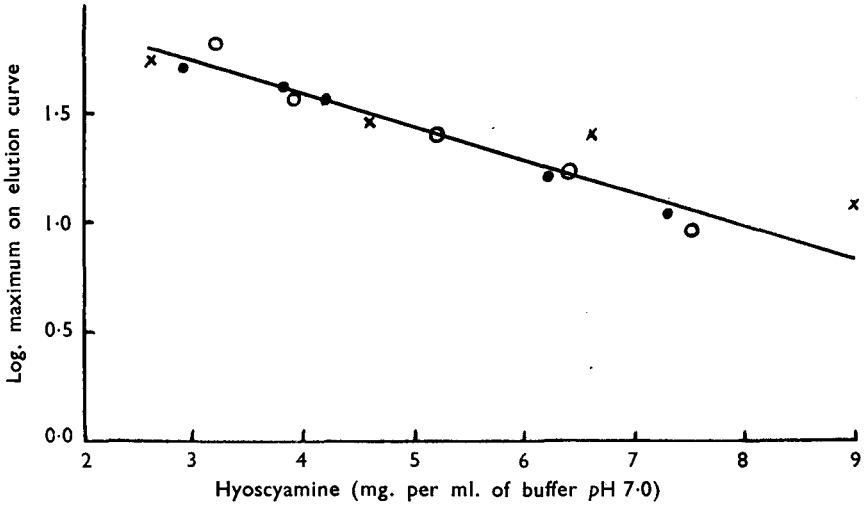

FIG. 1. Elution of hyoscyamine from columns with varying loads of alkaloid, sizes of the column and buffer-kieselguhr ratios.

×—variable load of alkaloid
○—variable size of column
●—variable buffer-kieselguhr ratio

Variation in the buffer-alkaloid ratio is one of the chief factors determining the position of the maximum of the hyoscyamine peak; this is demonstrated in Figure 1, from which it is clear that the buffer-alkaloid ratio is approximately linearly related to the logarithm of the position of the maximum of the hyoscyamine peak on the elution curve for differing buffer-kieselguhr ratios, column sizes and absolute amounts of alkaloid.

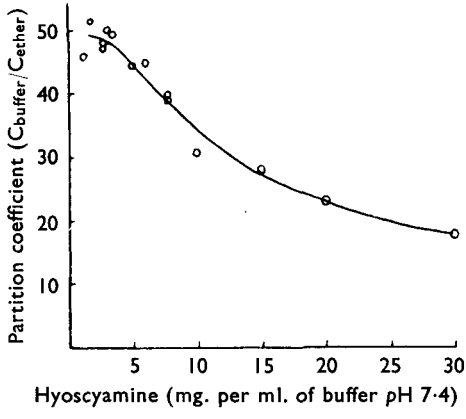

FIG. 2. Variation of partition coefficient of hyoscyamine with concentration.

The decrease in the total volume of ether required to elute the hyoscyamine for increasing alkaloid-buffer ratios recorded in Table III indicates that the partition coefficient of hyoscyamine between the two phases is not constant. Direct determination of the partition coefficient over a range of concentrations confirmed this (Fig. 2). Experimental difficulties prevented the accurate determination of the partition coefficient at very low concentrations of the alkaloid but some indication was obtained that the value approaches a constant with decreasing concentration. This change in partition

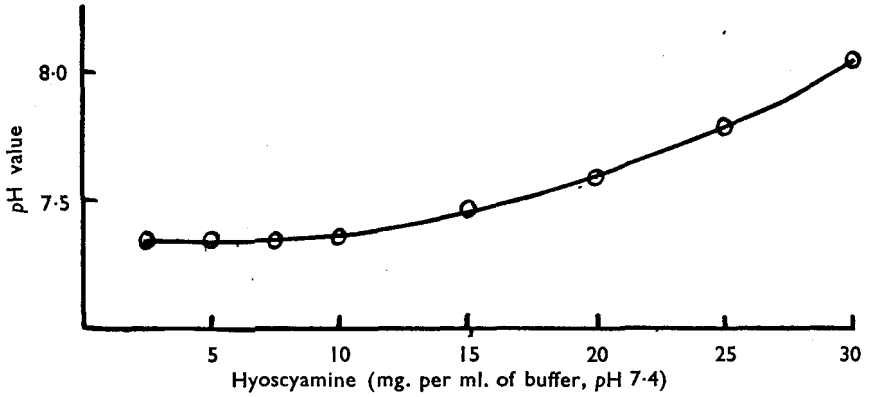

FIG. 3. Variation of *p*H value of buffer with concentration of hyoscyamine.

coefficient is in part accounted for by the relatively low buffering capacity of even 0·25 M buffer. From the curve illustrated in Figure 3, it is apparent that with concentrations of alkaloid in excess of 10 mg./ml. of buffer, the *p*H value of the buffer is changed. Evidence on the relationship of the partition coefficient to the *p*H value of the buffer is summarised in Figure 4. Buffer capacity is approximately proportional to the molarity of the buffer; the results recorded in Table IV therefore afford evidence of the effect of buffer capacity on the fractionation of the alkaloids. The shape of the elution curve for hyoscyamine is in agreement with these observations; as elution proceeds, it becomes progressively slower as the partition coefficient of the alkaloid increases in favour of the aqueous phase.

That the *p*H of the buffer has an important effect on the separation of hyoscine and hyoscyamine is shown by the results given in Table V; at *p*H 6·8 to 7·0 the alkaloids can be separated by fractional elution with ether, at *p*H 5·5 to 6·3 hyoscine can be eluted with ether and hyoscyamine with chloroform and at *p*H 5·0 the separation can be effected by

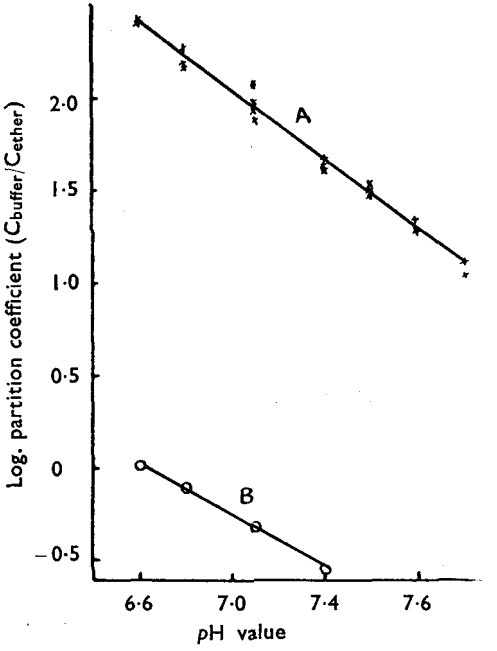

FIG. 4. Variation of partition coefficient of hyoscine and hyoscyamine with *p*H value of buffer.

A—Hyoscyamine
B—Hyoscine

fractional elution with chloroform. An explanation of the dependence of the process of fractionation on the *p*H of the buffer is afforded by a consideration of the relationship of this *p*H value and the partition coefficients of the alkaloids illustrated in Figure 4. Between *p*H 6·6 and 8·0, the partition coefficient of hyoscine changes but little with change in *p*H and favours the ether phase, whereas for hyoscyamine the value is very sensitive to changes in the *p*H of the aqueous phase.

Within the range of column sizes examined (Table VI), no significant effect on the ease of fractionation of the alkaloid mixture was observed. With long narrow columns, development is more even but greater pressure is required to obtain an adequate rate of flow of solvent.

Adsorption of the solute on the carrier of the stationary phase usually hinders fractionation.[18] Since it is not possible to predict adsorptive affinities, the suitability of any particular carrier must be found experimentally.

The application of the foregoing results to an assay of solanaceous drugs is based on earlier observations that in belladonna, stramonium and hyoscyamus, alkaloids other than hyoscine and hyoscyamine occur in only minor proportions[13,14,19]; atropine is determined as hyoscyamine. By the use of three solvents, carbon tetrachloride to separate colouring matter, ether to recover hyoscine and chloroform to recover hyoscyamine, the procedure is considerably simplified. The weak, non-volatile bases found in some samples of belladonna and hyoscyamus are eliminated in the course of purification of the alkaloids in the Pharmacopœial assay; in the present process, they are eluted from the column with the colouring matter and do not interfere with the determination of the alkaloids. The evidence presented in Table VII shows that by partition chromatographic determination of hyoscine and hyoscyamine results are obtained which are in satisfactory agreement with the results of parallel Pharmacopœial assays for total alkaloids.

The very significant variations found in the proportions of the two alkaloids even within a single species supplement other observations[10,11,12] and support the view that standardisation of solanaceous drugs for the individual alkaloids is desirable.

We are greatly indebted to Mr. J. L. Forsdike for the supply of a number of standardised samples of solanaceous drugs.

Summary

1. Factors influencing the separation of hyoscine and hyoscyamine by elution development of a partition chromatographic column have been studied.

2. A simple assay for hyoscine and hyoscyamine of belladonna, stramonium and hyoscyamus is described.

This communication is abstracted mainly from a thesis submitted by one of us (W.C.E.) in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of London.

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DISCUSSION

The paper was presented by DR. M. W. PARTRIDGE.

DR. J. M. ROWSON (London) said that he fully supported the authors' final plea that solanaceous drugs should be standardised on the individual alkaloids. He was convinced that they should be standardised on their hyoscine content as well as on total alkaloids. He asked the authors for further comment on sample 10 of *D. tatula* which had a very large hyoscyamine content as compared with the others.

DR. M. MITCHELL (London) said he liked the use of lime in eliminating alkaloids, and he could see the point in using carbon tetrachloride because hyoscine and atropine are nearly insoluble in that solvent. He was interested to notice that, in the sample of European belladonna root which was examined, the amount of hyoscine was 0·02 per cent. and of total alkaloids 0·60 per cent., which was a ratio of 0·035. In 1948 the authors found 0·09 per cent. in a sample of drug showing 0·48 per cent. of total alkaloids, which was a ratio of 0·2, or some six times as much. It confirmed that there was a big variation in the species. It might be desirable to standardise the hyoscine content, but what should the standard be?

MR. J. E. CARLESS (Manchester) observed that the authors' results confirmed his own work using buffered filter paper strips. It indicated that the buffered filter paper technique might be a useful guide towards working on a larger scale. Had the authors any comments to make on the use of powdered cellulose, which could be otained in pure form, as opposed to the use of kieselguhr?

DR. M. W. PARTRIDGE, in reply, said that the values recorded for *D. tatula* were those found and the specimen was regarded as authentic. The result confirmed the suggestion that standardisation of the individual alkaloids was desirable. There was an extensive variation in the alkaloidal content of most of the solanaceous drugs, and there were quite important

variations throughout the life cycle. The chemist or pharmacist was not in a position to say what was the best type of standardisation to be adopted; that should be settled by those who used the drugs. He agreed that the *p*H was important. There was little doubt that powdered cellulose would work reasonably well. In earlier work with the alkaloids of pomegranate they had found that powdered cellulose and many of the usual inert supporters were buffers having a strong adsorptive capacity for alkaloids and vitiated partition chromatography.