A COMPARATIVE STUDY OF THE HYDROLYTIC AND NON-HYDROLYTIC METHODS FOR THE ASSAY OF SOLANACEOUS DRUGS

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The results given by the hydrolytic and non-hydrolytic methods of determining the total alkaloidal content are in good agreement for belladonna, hyoscyamus and stramonium; the agreement is fair for *Duboisia leichhardtii* and *Datura sanguinea*, whilst for *Duboisia myoporoides* the non-hydrolytic method gives high values. An improved paper chromatographic system for the separation of the principal tropane alkaloids has been developed; the system was used to determine the individual alkaloidal content of the respective drugs and to provide the appropriate factor for converting titre to percentage of total alkaloid.

A potential source of error in the conventional assay of solanaceous drugs occurs in the final stage of the determination, in which the extract of the total alkaloids is directly titrated against standard acid after removal of the volatile bases by heating at 100°. Errors may arise from incomplete removal of volatile bases, the presence of non-volatile non-alkaloidal bases (for example, tropine and oscine), or the retention of traces of ammonia by the alkaloidal extract[1–7].

To obviate these possible sources of error Reimers proposed a modified method in which the alkaloidal extract is hydrolysed and the liberated carboxylic acids are extracted and titrated against standard alkali[8,9].

This method was subsequently incorporated in the International Pharmacopoeia as one of two alternative procedures for the assay of solanaceous vegetable drugs. Except for *Hyoscyamus muticus*[9] and stramonium[10], however, there is no literature on the results given by the two techniques, and accordingly a comparative study of the direct and hydrolytic methods was undertaken with drugs from commercial sources.

Preliminary Experiments

Loss on Heating of Alkaloids and Amino Alcohols at 100°

In the official assay it is specified that the extract of the bases be heated at 100° and weighed at intervals of 1 hour until two successive weighings do not differ by more than 1 mg. In view of the long periods of heating required by some extracts to reach constant weight, account had to be taken of the effect of prolonged heating of tropane bases at 100°. Information is given in the literature only for atropine and hyoscyamine, and is conflicting. Thus Hardy[11] and DeKay and Jordan[12] stated that atropine is non-volatile at 100°; on the other hand, Schousen[13] and Fricke and Kaufman[14] reported that prolonged heating of atropine and hyoscyamine at this temperature results in loss by decomposition or volatilisation.

Aliquots of standard solutions of the pure bases in chloroform were evaporated to dryness; the residues were heated at 100° for 0, 1, 2 and

4 hours respectively and titrated against standard acid. The respective average losses in weight, expressed in per cent per hour, were as follows:— Hyoscyamine, 0·05; hyoscine, 0·05; valeroidine, 0·15; tigloidine, 3·1; tropine, 2·6; oscine, 4·9.

Determination of Alkaloids by the Hydrolytic Method

A series of preliminary experiments was carried out to establish optimum conditions of hydrolysis, extraction and titration of acids.

Hydrolysis. In the method of Reimers and of the Ph.I. it is specified that the aqueous extract of the alkaloids, which measures not less than 50 ml., be hydrolysed by evaporating with 10 ml. of sodium hydroxide solution until only 10 ml. remains. Using a paper chromatographic procedure it was found, however, that under these conditions both hyoscyamine and hyoscine are completely hydrolysed in 2½ to 3½ minutes, and it is thus possible to reduce appreciably the time required for hydrolysis.

Extraction and titration of carboxylic acids. A systematic study of the variables involved in the hydrolytic method (optimum pH for extraction of carboxylic acids, number of extractions required, etc.,) showed that the procedure of Reimers and of the Ph.I. is satisfactory; the precaution was taken, however, of washing the chloroform-*iso*-propanol extracts with water to minimise the risk of traces of hydrochloric acid being carried over into the extract.

As a result of these experiments the following procedure was adopted: The aqueous solution of the alkaloid or alkaloidal extract was diluted to about 35 ml. with water, mixed with 10 ml. of 2N sodium hydroxide and evaporated for 15 minutes on the water bath. The solution was cooled, neutralised with dilute hydrochloric acid and 0·5 ml. of the acid added in excess. The acid liquid was extracted with four 25 ml. and two 20 ml. portions of a mixture of *iso*propanol (1 part) and chloroform (3 parts), each extract being washed with the same 15 ml. of water. The extracts were combined and the solvent removed by evaporation. The residue was dissolved in 15 ml. of warm water, cooled and titrated with 0·02N sodium hydroxide, using phenolphthalein as indicator.

Results of experiments using known amounts of tropic acid, together with comparative assay results for hyoscyamine sulphate and hyoscine hydrobromide by the hydrolytic and non-hydrolytic methods, are given in Table I. Experiments were also carried out using tropine base; it is seen that the presence of tropine causes no interference in the hydrolytic process.

Chromatography

No combination of paper and developing solvent has been described in the literature for the separation of all the principal tropane alkaloids; in particular the separation of tigloidine from the other alkaloids has not been achieved[15]. Numerous combinations of paper and developing solvent were examined and it was found that a water-saturated mixture of *n*-butanol (3 vol.), *n*-butyl acetate (17 vol.) and glacial acetic acid (8 vol.) used in conjunction with 0·2M potassium chloride-treated papers

gave compact spots and afforded good separations of the amino alcohols and the principal tropane alkaloids, with the exception of hyoscyamine and *nor*hyoscyamine. The R_F values by the descending technique are as follows (bases run as salts). Oscine, 0·06; tropine, 0·09; belladonnine, 0·18; hyoscine, 0·25; hyoscyamine, 0·37; *nor*hyoscyamine 0·38; valeroidine, 0·46; tigloidine, 0·56; *apo*atropine, 0·67. The R_F values are independent of the nature of the anion of the alkaloid or amino alcohol.

TABLE I

RESULTS OF ASSAY BY HYDROLYTIC AND NON-HYDROLYTIC METHODS

Taken	Weight, g.	Method of assay	Per cent recovered
Blank (25 ml. water + 0·5 ml. dilute hydrochloric acid)		B	0
Tropine base	0·1075	C	63·0, 63·9*
	"	B	0
Tropic acid	0·05465	A	101·0, 101·9
	"	B	101·3, 101·7
	0·1070	A	99·4, 100·1
	"	B	99·8, 99·8
Hyoscyamine sulphate, anhyd.	0·1012	C	99·2, 99·5
	"	D	100·5, 100·8
	0·1960	C	97·5, 98·0
	"	D	98·4, 99·2
Hyoscine hydrobromide B.P.	0·1216	C	98·9, 99·3
	"	D	99·2, 99·8
	0·2984	C	99·6, 100·0
	"	D	100·8, 101·0

A Direct titration.
B Extraction from acid solution and titration.
C Chloroform extraction from alkaline solution and titration (non-hydrolytic method).
D Hydrolysis, extraction from acid solution and titration
* Low recovery due to solubility of tropine base in aqueous alkali.

The effect on the chromatograms of the presence of non-alkaloidal volatile bases in the extracts was also examined. The bases were obtained by adding an excess of sodium hydroxide solution to a concentrated extract of the total bases in dilute acid and steam-distilling into an excess of aqueous acid. The volatile bases from the official drugs gave only faint spots in the tropine position, whilst those from *Duboisia myoporoides* and *Duboisia leichhardtii* gave a number of relatively weak spots which did not interfere in the determination of the individual alkaloids.

COMPARATIVE ASSAY PROCESS

Determination of Total Alkaloids

The drug was reduced to No. 60 powder and 25 g. (100 g. in the case of hyoscyamus) extracted in two separate quantities each of 12·5 g. (50 g. in the case of hyoscyamus) using the B.P. 1958 method for Belladonna Herb; all quantities of solvent were increased by 25 per cent. The final chloroform extracts were mixed, evaporated to low bulk and diluted to exactly 50 ml. with chloroform. Two 20 ml. aliquots of this solution, each equivalent to 10 g. of sample (40 g. in the case of hyoscyamus) were taken for the determination of the total alkaloids.

Aliquot 1 (*"non-heated extract"*). The extract was evaporated to dryness and the alkaloids titrated with 0·05N sulphuric acid as in the pharmacopoeial assay. The solution was then diluted to about 35 ml.

with water, hydrolysed with 2N sodium hydroxide and the carboxylic acids extracted and titrated as described above.

Aliquot 2 (*"heated extract"*). The solution was evaporated to dryness in a tared dish, weighed and heated at 100° for 1 hour and re-weighed. The process was continued until two successive weighings did not differ by more than 1 mg. The total alkaloids were then determined as for Aliquot 1.

Determination of Individual Alkaloids and of Alkaloidal Factor

5 ml. of the chloroform extract was evaporated to dryness and the residues dissolved in the following volumes of 0·05N sulphuric acid. *Duboisia myoporoides*, 4 ml.; *Duboisia leichhardtii*, 6 ml.; other drugs, 2 ml. Volumes of this solution, ranging from 5 to 80 μl., and of aliquots of a standard solution of alkaloids were applied to the starting positions of

TABLE II

RESULTS OF ASSAY OF SOLANACEOUS VEGETABLE DRUGS

Drug	Time of heating extract at 100°	Total alkaloids				Individual alkaloids				Factor M
		By direct titration		Hydrolytic method						
		Extract not heated	Extract heated	Extract not heated	Extract heated	Hyoscyamine M.W. = 289·4	Hyoscine 303·3	Valeroidine 241·3	Tigloidine 223·3	
Belladonna herb	1 hr.	0·497	0·486	0·500	0·494	0·45				289·4
Hyoscyamus ..	2 hrs.	0·071	0·053	0·052	0·048	0·021	0·025			296·9
Stramonium ..	1 hr.	0·265	0·260	0·257	0·251	0·13	0·11			295·7
Datura sanguinea ..	1 hr.	0·331	0·304	0·284	0·267	0·05	0·23			300·8
Duboisia myoporoides sample No. 1	8 hrs.	1·68	1·17	0·988	0·913	0·24	0·37	0·15	0·1	279·4
Duboisia myoporoides sample No. 2	10 hrs.	2·34	1·64	1·43	1·11	0·18	0·61	0·35	0·1	277·3
Duboisia leichhardtii ..	10 hrs.	3·36	3·03	3·16	2·94	2·15	0·37			291·4

Whatman's No. 1 filter paper which had previously been impregnated with a 0·2M aqueous potassium chloride solution and blotted between sheets of filter paper[16]. The chromatograms were developed in the downward direction, dried at room temperature for 5 hours and immersed in an aqueous tartaric acid solution of potassium iodobismuthate[17]; the individual alkaloids were then estimated by the technique of matching of spots[18].

The results of the chromatographic examination were used to compute the factor M for converting titre to percentage of total alkaloid. $M = \frac{303{\cdot}3\,a + 289{\cdot}4\,b + 241{\cdot}3\,c + 223{\cdot}3\,d}{a + b + c + d}$ where a, b, c and d are the percentages in the drug of hyoscine, hyoscyamine, valeroidine and tigloidine, respectively. The results of the determinations are given in Table II.

DISCUSSION

The figures in columns 4 and 5 of Table II show that for the official drugs the results given by the hydrolytic and non-hydrolytic methods

are in good agreement. With *Datura sanguinea* and *Duboisia leichhardtii* the agreement is fair, whilst for *Duboisia myoporoides*, even after 8 to 10 hours' heating, the direct titrimetric method gives high results, and this drug should therefore be assayed by the hydrolytic method. It is recommended that the hydrolytic process be used also for *Duboisia leichhardtii* to avoid the need for an unduly long period of heating of the alkaloidal extract. The same remark probably applies to Indian belladonna, which has an exceptionally high content of volatile bases[19] and the extract of which may have to be heated at 100° for periods of up to 6 hours to reach constant weight[20]. Unfortunately no sample of this drug was available to investigate this point.

The discrepancy between the "non-heated" and "heated" hydrolytic values for *Duboisia myoporoides* and *Duboisia leichhardtii* (columns 5 and 6) is most probably due to the presence of small quantities of esters that are slightly volatile at 100°, for example tigloidine base in *Duboisia myoporoides* (*v. supra*), and *iso*butyryltropeine and *d*-α-methylbutyryltropeine in *Duboisia leichhardtii*[21,22].

In the paper chromatographic examination the error in the determination of the individual alkaloids amounted to 10 to 15 per cent; this error, however, had only a small effect on the accuracy of the factor M.

No *apo*atropine was found in any of the drugs that were examined, and only the sample of hyoscyamus gave a spot in the belladonnine position on the chromatograms, but no attempt was made to confirm the identity of this spot.

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DISCUSSION

The paper was presented by MR. R. E. A. DREY.

THE CHAIRMAN. It appears that the hydrolytic method is of more general application than the non-hydrolytic process.

DR. W. MITCHELL (London). Was the *Duboisia myoporides* botanically identified? He thought that true duboisia did not contain any atropine and was surprised to hear that it contained hyoscyamine. Were the lower results with duboisia by the hydrolytic method due to the minor alkaloids, which were less readily hydrolysed than hyoscyamine and which are contained in all species of duboisia, not being completely hydrolysed? Tigloidine but not valeroidine could be extracted with chloroform from hydrobromic acid solution.

MR. C. A. JOHNSON (Nottingham). The suggested method would no doubt be more satisfactory for Indian belladonna than the old B.P. process. Had assays been carried out on belladonna root which might contain a high proportion of volatile bases?

MR. DREY replied. The duboisia was of commercial grade which he thought was genuine, but it may have been contaminated with some similar material. Using a paper chromatographic procedure he had found that hydrolysis of hyoscine and hyoscyamine was complete in 2½ minutes, but 15 minutes had been allowed for the hydrolysis. Account had to be taken of the possibility that extraction of tigloidine hydrobromide with chloroform might not be complete. Belladonna root had not been included among the drugs examined.