THE ESTIMATION OF *PARA*-AMINOSALICYLIC ACID

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THE methods which have been used so far in the assay of *p*-aminosalicylic acid are based on the estimation of amino, phenolic or carboxyl groups. Those involving bromination[1,2] and diazotisation[3,4,5] usually yield erroneous results owing to the presence, presumably, of *m*-aminophenol as an impurity, which is itself estimated by a modification of the latter method[6]. The spectrophotometric method[7] is believed to be accurate when highly sensitive instruments are used. However such instruments are not always available and with less sensitive ones considerable variations in results are seen.

p-Aminosalicylic acid has also been assayed by the estimation of the carboxylic group either by the formation of the insoluble silver salts[8] (Volhard's method) or by dry decarboxylation[9]. A combination of alkalimetric and bromination methods has also given consistent results[1]. On the other hand it is difficult to bring the acid and its calcium salt into solution to form the silver salt and dry decarboxylation cannot be applied to sodium and calcium salts. Further, in the case of *p*-aminosalicylic acid, it is difficult to identify the residue of *m*-aminophenol after dry decarboxylation.

In view of these facts a comparative study of the methods of assay was made and in the course of the work, a method based on the estimation of the labile carboxylic group and the amino group by combined decarboxylation and diazotisation was developed. The compounds assayed by the different methods were the acid and its sodium and calcium salts, the latter two in the form of their pharmaceutical preparations, i.e., granules and tablets. The *p*-aminosalicylic acid (sample "E") was purified by repeated crystallisations and the results are expressed on the assumption that this is pure as verified by its melting point and that of the derived *m*-aminophenol.

Methods

In the bromination and diazotisation methods, the techniques of Dino Coppini[2] and Pesez[5] were followed. The spectrophotometric method as described in "New and Nonofficial Remedies, 1951," was used, the instrument employed being a Spekker photometer.

Decarboxylation

Approximately 0·5 g. of the compound, accurately weighed, was placed in a reaction flask fitted with an intake for carbon dioxide-free air, a separating funnel and a reflux condenser to prevent steam passing over into a series of three absorption flasks, each containing 50 ml. of 0·2N

barium hydroxide solution containing 2 per cent. of barium chloride. Mild suction was applied and 100 ml. of N sulphuric acid was admitted slowly to the flask which was then gently heated. Heat and suction were then increased, and after 2 hours the excess of barium hydroxide was estimated by titration with hydrochloric acid using phenolphthalein as indicator. A blank was performed under similar conditions. Each ml. of 0·2N barium hydroxide is equivalent to 0·01531 g. of *p*-aminosalicylic acid; 0·02112 g. of sodium *p*-aminosalicylate (dihydrate); 0·01721 g. of calcium *p*-aminosalicylate (anhydrous).

The assay value was never less than 98 per cent.

Diazotisation

The solution left in the reaction flask was made up to 250 ml. Of this 100 ml. was pipetted out into a beaker and 10 ml. of concentrated hydrochloric acid was added. The solution was cooled in ice, and, the temperature being kept below 15° C., titrated against 0·1M sodium nitrite, starch potassium iodide paste being used as external indicator. The titration was considered to be complete when the end-point was reproduced after the titrated solution had been allowed to stand for 2 minutes. Each ml. of 0·1M sodium nitrite is equivalent to 0·01531 g. of *p*-aminosalicylic acid, 0·02112 g. of sodium *p*-aminosalicylate, or 0·01721 g. of calcium *p*-aminosalicylate.

The assay value obtained by this method lies between 98 and 102 per cent. With pure substances the assay values calculated from the decarboxylation process would be not less than 98 per cent. and those calculated from the diazotisation process would vary between 98 and 102 per cent. Table I shows that when the assay value by the decarboxylation process was about 93 per cent. that from the diazotisation process went up to 112 per cent. thus indicating that *m*-aminophenol was present in abnormal amounts.

The remaining 150 ml. of the solution was transferred to a separator and after making alkaline with ammonia, extracted 3 times with 15 ml. of freshly distilled ether. The ether solutions were mixed, washed with 5 ml. of water and the ether removed on a water bath. The residue after drying for half an hour in a steam oven had m.pt. 121 to 123° C. (*m*-aminophenol m.pt. about 122° C.).

Results and Conclusions

The results obtained by the different methods are presented in Table I. It is clear that the assay values obtained by bromination and diazotisation are subject to some variation, presumably due to the presence of *m*-aminophenol. Often with diazotisation the values are inconsistent and unusually higher. Similar variations are seen in the spectrophotometric method using the Spekker photometer which is probably due to the instrument not being sufficiently sensitive.

The results obtained by the combined decarboxylation and diazotisation method are more uniform. An additional check on the assay value is provided by the melting point of the product left after decarboxylation—

any variation indicating the presence of isomers of *p*-aminosalicylic acid or other impurities in the parent compound, because only *p*-aminosalicylic acid can, on decarboxylation, yield *m*-aminophenol.

The main disadvantage with the combined decarboxylation and diazotisation method described here is that the estimation takes about 5 hours

TABLE I

COMPARISON OF ASSAY RESULTS

	Sample	Bromination method per cent.	Diazotisation method per cent.	Spectrophotometric method per cent.	Proposed method: By decarboxylation per cent.	Proposed method: By estimation of *m*-aminophenol after decarboxylation per cent.	Proposed method: M.pt. of *m*-aminophenol after decarboxylation ° C.
p-Aminosalicylic acid	A	97·9	99·0	140·8	98·1	101·8	123
	B	98·2	99·2	81·3			
	C	98·9	99·5	80·6	96·4	102·0	122·5
	D		113·6	80·6	98·2	102·0	
	E		107·5	85·1	98·7		
			109·0		98·5		
	F				99·3	102·0	122
Sodium *p*-aminosalicylate	A	99·1	97·1	104·0	99·4	98·9	123
	B	99·1	100·7	100·2	98·8		
	C	98·5	105·7	96·5	101·4	102·0	122·5
	D	95·2	119·1	105·9	98·7	100·0	
	E	95·5	93·0	105·9	99·7	101·0	
				105·9	99·4		
				105·9	99·8		122
Calcium *p*-aminosalicylate and tablets	A	97·7	96·8	Could	95·3	99·9	123
	B	90·2	98·5	not be	99·5	99·0	
		87·4	94·5	used	100·2	100·7	123
	C	91·8	98·5		101·0	102·1	
	D	89·2	89·7		99·5		
			89·4		99·5	100·8	122
			89·4		99·1		
p-Aminosalicylic acid (granules) ..	A	103·8	116·0	Could	93·1	112·7	123
		98·1	105·3	not be	94·0	113·1	123
	B	98·7	110·4	used	92·9		122
		105·1	117·4		93·9		
	C	93·4	123·4		99·9	100·8	122
		101·6	119·4		99·8	102·0	

to complete but this is compensated for by its greater reliability and the ability to detect the presence of an excessive quantity of *m*-aminophenol in the parent compound.

SUMMARY

1. A comparative study of the existing methods of estimation of *p*-aminosalicylic acid and its compounds has been made and some of their defects pointed out.

2. A method based on both decarboxylation and diazotisation is proposed and its advantages over the existing methods pointed out by a comparative study.

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