I. NOTE ON TANNASE.

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SINCE the middle of last century, this enzyme has been the subject of many investigations, but it is notable that no method has been described whereby its hydrolysing power may be estimated. The investigation described in this paper was undertaken at the suggestion of Dr Nierenstein, since it was thought that some such method might be of assistance to other work at present in progress in this laboratory.

This enzyme was prepared from *Aspergillus Luchuensis* Inui.¹ by a slight modification of Freudenberg's method [1913]. It was used without further purification, a 1 % solution in distilled water being employed in each experiment.

The gallotannin used was Schering's leviss. puriss. which had been further purified according to Fischer's method [1912]. An approximately 0.3 % solution in distilled water was prepared, and the exact amount of gallotannin present estimated by Nierenstein's caseinogen method [1911] as modified by Spiers [1914].

The hydrolyses were carried out in 250 cc. Erlenmeyer flasks, 100 cc. of gallotannin solution, to which various quantities of tannase solution were added, being employed in each experiment. During the reaction the flasks were plugged with cotton wool, and toluene was added to the reaction mixture to prevent the growth of fungi or bacteria, the whole being kept in a dark incubator at 23°. The amount of gallotannin present in the reaction mixture was estimated immediately after the tannase solution had been added and again after the hydrolysis had proceeded for a certain time.

The details of the estimation are as follows. A solution of approximately 0.1 % KMnO_4 was prepared and standardised against pure ammonium oxalate of known strength; 20 cc. of a solution of 0.5 % of indigo carmine in dilute sulphuric acid (A-R reagent, British Drug Houses), being used as indicator. As the indigo carmine reacts with potassium permanganate, it is at first necessary to determine the quantity of the latter used by the indicator solution. To this end 20 cc. of this solution were placed in a large porcelain basin, 750 cc. of distilled water added, and titrated with the KMnO_4 solution. The end-point is reached when a faint pink colour can be seen round the edge of the solution. This titration gives the amount of KMnO_4 solution taken up by the indicator, which must be allowed for in all calculations. The gallo-

¹ We are indebted to Mr F. W. Mason of the Bureau of Biotechnology at Leeds for a pure culture of this fungus.

tannin present is estimated as follows: 25 cc. of the reaction mixture are withdrawn and 4 cc. of this solution placed in a porcelain dish together with 20 cc. of indigo carmine solution and 750 cc. of distilled water. The whole is then titrated with permanganate solution until the end-point as previously described is obtained. This titration represents both the tannin and non-tannins present. The remainder of the solution is shaken for 15 minutes with 1 g. of fat-free caseinogen and then passed through a barium sulphate filter. This process is repeated twice. When all gallotannin is removed [cf. Nierenstein, 1911], 4 cc. of this filtrate are then titrated as before. The difference between the two titration readings represents the amount of gallotannin present. The actual quantity may be calculated by means of Spier's ammonium oxalate gallotannin ratio, viz. 1 g. of ammonium oxalate is equivalent to 0.4648 g. of gallotannin.

In estimating the amount of gallotannin present after hydrolysis, the procedure is the same as that just described except that it is necessary first to filter off the precipitate of gallic acid formed.

The following table gives the results obtained.

In each case three titrations were made, the mean of these being recorded; also as a check each worker carried out an independent investigation.

Sample	Age in days	Cc. of 1 % tannase solution added to 100 cc. of gallotannin solution	Duration of hydrolysis in hours	Percentage of gallotannin hydrolysed
1	112	10.0	23.75	14.70 14.40
2	2	5.0	19.50	10.52 11.02
2	2	5.0	45.00	16.60 15.70
3	2	5.0	19.50	13.48 13.25
4	2	7.5	19.50	13.00 12.17
4	2	7.5	45.00	18.25 17.30
5	2	10.0	19.50	15.00 15.35
5	2	10.0	45.00	20.00 20.51

From the results given in the above table it is evident that (1) this method represents a moderately accurate procedure for estimating the hydrolysing power of any sample of tannase; (2) the bulk of the hydrolysis takes place during the first 24 hours.

It is proposed by Dr Nierenstein to continue this work at a later date, but since the authors are unable to pursue this research at present, it was thought advisable to publish this preliminary note.

REFERENCES.

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