XLVI. NOTE ON A NEW TANNASE FROM *ASPERGILLUS LUCHUENSIS*, INUI.

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PAULLINIA tannin¹ is hydrolysed by emulsin, dextrose and β -gambier-catechincarboxylic acid being produced [Nierenstein, 1922]. It was therefore surprising to find that tannase, which had been prepared by growing *Aspergillus Luchuensis*, Inui in a solution of gallotannin and which is known to hydrolyse gallotannin into dextrose and gallic acid [Rhind and Smith, 1922], had no effect on paullinia tannin. This suggested the possibility that the hydrolysing properties of tannase depended on the medium in which it had been produced by the fungus and that a catechutannin, such as paullinia tannin, required a tannase, which had been formed in a solution of a catechu- and not gallotannin. This has actually been found to be the case, since *Aspergillus Luchuensis* yields in a medium in which gallotannin had been replaced by catechutannin from cube-gambier (*Ourouporia Gambier*, Baill), a new tannase, which hydrolyses paullinia tannin, but which has no action on gallotannin. We have therefore to distinguish between these two kinds of tannase and it is proposed to refer to them as *gallotannase* and *catechutannase* respectively. In this connection reference must be made to what may in time prove a dangerous practice, which has recently been introduced by Freudenberg and Vollbrecht [1921]. These chemists prepare tannase by growing an unknown species of *Aspergillus* in the aqueous extract of ground myrobalams, the fruit of *Terminalia chebula*, Retz. Such a medium must obviously affect the general properties of the tannase and ought to be avoided.

Freudenberg's method [1920], in which gallotannin is replaced by an aqueous solution of the residues of cube-gambier, from which the catechin is removed by extraction with ethyl acetate, is used for the preparation of catechutannase. The catechutannase thus obtained resembles gallotannase in

¹ This catechutannin is present in the seeds of *Paullinia Cupana*, H.B. and K., which is a synonym of *P. sorbilis*, Mart. These seeds are used for the preparation of the paste known as "pasta guaraná" and the tannin is consequently also referred to as guarana tannin [compare, for example, Perkin and Everest, 1918]. Harvey [1921] and also Procter [1922] have wrongly assigned the name *P. sorbilis* to "guara," which contains a gallotannin and not a catechutannin, as "guara" signifies *Cupania americana*, L. For this information I am indebted to the authorities of Kew Gardens, who also inform me that in Cuba the names "guaraná" and "guara" are apparently used for *Cupania macrophylla*, A. Rich. and *Guarea trichiliodes*, L. respectively.

appearance and produces when added to a solution of paullinia tannin (0.5 g. catechutannase are used for 2 g. paullinia tannin in 150 cc. of water to which 5 cc. of chloroform are added and the mixture kept in a dark incubator at 23° for ten days), a bulky precipitate, which when filtered off crystallises from water in small, pointed needles, which melt at 252–253°, carbon dioxide being evolved. These are in every respect identical with the inactive β -gambier-catechincarboxylic acid, previously obtained by the action of emulsin. (Found: C = 57.2; H = 4.6. Calculated: C = 57.5; H = 4.2 %.) For further identification a small quantity of the acid was methylated with diazomethane, when the corresponding pentamethoxy methyl ester was obtained. It crystallises from light petroleum in long needles, which melt at 74°. This melting point is not depressed on admixture with the same substance (m.p. 74°) previously obtained by the action of diazomethane on the acid.

The filtrate from the crude β -gambier-catechincarboxylic acid, freed from unchanged paullinia tannin with the aid of lead acetate and hydrogen sulphide, contains dextrose, which is identified as the dextrosazone (m.p. and mixed m.p. 200–203°).

In conclusion it is interesting to note that catechutannase produces the identical β -gambier-catechincarboxylic acid when added to the aqueous extract of fat-free cocoa-beans or to a solution of catechutannin from cube-gambier. This indicates a very close relationship between these tannins which has so far not been suspected.

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