XLVI. NOTE ON THE SUPPOSED PRODUCTION OF MACLURIN FROM ACACATECHIN.

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HAZELTON AND NIERENSTEIN [1924] state that Nierenstein has established the constitution (I) for acacatechin both analytically and synthetically. On the other hand Freudenberg has advanced evidence tending to show that the substance is a mixture of stereoisomerides, namely, *l*-epicatechin, *l*-catechin and *r*-catechin (all corresponding to (IV)). Hazelton and Nierenstein do not accept the findings of Freudenberg, because they observed that maclurin (III) was formed in 86 % yield when *Penicillium solitum* was cultured in a solution of acacatechin containing 5 % sucrose. The production of maclurin from acacatechin is in agreement with the constitution ascribed to acacatechin by Nierenstein and the suggested mechanism of the decomposition was the following:

The leuco-maclurin (II) and glycol were not isolated. It should be noted that the formation of maclurin from acacatechin does not necessarily exclude the Freudenberg formulation (IV) for the latter substance, and Robinson [1928] has suggested that the course of the degradation might involve a double benzil-benzilic acid transformation of the type supposed by some to occur in the formation of citric acid from glucose, as shown on p. 389.

Hazelton and Nierenstein had noticed that a specific mould developed in dilute solutions of catechin and that a coloured precipitate was produced. The mould was identified as *Penicillium solitum* and the precipitate as maclurin. They therefore made a 0.72 % solution of acacatechin in water containing 5 % sucrose, inoculated it with *Penicillium solitum* and kept it in an

incubator at 22°. The first sign of growth became visible after 7 days and after several weeks the solid was collected and afforded pure maclurin after extraction with boiling water and purification through the lead salt.

An attempt to repeat these experiments has now been made, but with negative results. Controls using a dilute solution of glucose in pure water containing small amount of inorganic salts were instituted. Although it must be assumed that the normal technique was adopted, Hazelton and Nierenstein do not specifically state that they sterilised their solutions; therefore experiments with both sterilised and unsterilised solutions were made. Different flasks were kept at 22° and 28° in order to determine how the growth of the mould was influenced by the temperature. These variations of the original experiments were carried out with solutions of Gambier catechin rather than with the less accessible acacatechin, and from them favourable conditions for growth were ascertained. A solution of acacatechin was then made and kept under those conditions, and another solution was made and kept under the conditions described in Hazelton and Nierenstein's paper, *i.e.* in 5 % aqueous sucrose solution at 22°.

A yellowish brown solid was obtained from the acacatechin solutions, but it could not be recrystallised from hot water, or dilute acetic acid, or purified through the lead salt, nor did it give a precipitate with bromine in aqueous solution, as did an authentic specimen of maclurin (Schuchardt). It was concluded therefore that the solid was not maclurin.

EXPERIMENTAL.

The catechin employed in most experiments was Gambier catechin (Merck). The *Penicillium solitum* was kindly supplied by Dr Thom of the Laboratory of the Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, to whom the author is greatly indebted. In view of the sequel it ought to be mentioned that strains of *Penicillium* cannot always

be characterised with great accuracy and Dr Thom stated that the specimen was very close to, and probably identical with, *Penicillium solitum*.

The following solutions were made up to 500 cc. and were kept at the temperatures indicated:

					Temp. of incubator
(A)	Reeder's solution*	0.72 % of catechin	Inoculated	Sterilised	22°
(B)	"	"	"	"	28°
(C)	"	"	Not inoculated	"	22°
(D)	"	"	"	"	28°
(E)	"	No catechin	Inoculated	Unsterilised	22°
(F)	"	"	"	Sterilised	28°
(G)	"	"	Not inoculated	"	22°
(H)	"	"	"	Unsterilised	28°
(I)	5 % aqueous sucrose	0.72 % of catechin	Inoculated	Sterilised	22°
(J)	"	"	"	Unsterilised	28°
(K)	"	"	Not inoculated	"	22°
(L)	"	"	"	Sterilised	28°
(M)	"	No catechin	Inoculated	Unsterilised	22°
(N)	"	"	"	Sterilised	28°
(O)	"	"	Not inoculated	Unsterilised	22°
(P)	"	"	"	Sterilised	28°

* Reeder's solution contains: ammonium sulphate, 0.3 %; magnesium sulphate, 0.07 %; potassium dihydrogen phosphate, 0.10 %; dipotassium hydrogen phosphate, 0.016 %; sodium chloride, 0.05 %; calcium nitrate, 0.04 %; glucose, 5 %.

The solutions were placed in Pasteur flasks, which were plugged with cotton-wool. To ensure sterilisation these flasks were heated at 100° for 30 minutes on 3 successive days; they were then placed in incubators. In all flasks which had been inoculated with *Penicillium solitum* growth appeared after 8 days, and a yellowish brown precipitate was produced; this did not increase after 8 weeks (found in material dried at 120°: C, 47.5; H, 6.3 %). No growth appeared in the flasks which had been sterilised and not inoculated (C, D, G, L, P), and a very small growth in the flasks which were not inoculated and unsterilised (H, K, O). No coloured precipitate was formed in any flask which did not contain catechin. On the whole growth was more abundant in the Reeder's solution than in the sucrose, and was favoured by the higher temperature—thus flask (B) contained the most solid. After 8 weeks the solid was filtered off from all the solutions, and, as some of the solid adhered to the mould, this was extracted with boiling water.

A brown solution was obtained, but no solid separated on cooling; after concentration to a small bulk a colloidal solution was obtained from which no solid could be salted out. No success attended the attempts to purify this material; maclurin which crystallises from hot water with facility could not be isolated, nor was its presence indicated by the general reactions of the material.

A further set of experiments was carried out with acacatechin. A solution (500 cc.) containing 0.72 % of acacatechin and 5 % of sucrose in water was inoculated with the mould and kept at 22°. Reeder's solution (500 cc.) containing 0.72 % of acacatechin was sterilised, inoculated and then kept at 28°. The precipitate obtained was similar to that produced from Gambier catechin but had a purer yellow colour.

After extraction with boiling water, nothing separated on cooling, and the solution was concentrated to obtain the amorphous product. According to Hazelton and Nierenstein the material may be purified through the lead salt [Hoesch and von Zarzecki, 1917] and accordingly this method was tried without success. A specimen of maclurin supplied by the firm of Schuchardt could be recrystallised from hot water and dilute acetic acid, and had the properties attributed to the substance in the literature.

SUMMARY.

Under the conditions above, and which differ in no obvious respect from those described by Hazelton and Nierenstein, no evidence of the transformation of Gambier catechin or acacatechin into maclurin could be obtained.

The author wishes to thank Dr Thom for supplying the *Penicillium solitum* and Prof. Drummond for his kind interest in the investigation.

REFERENCES.

- Hazelton and Nierenstein (1924). *J. Amer. Chem. Soc.* **46**, 2100.
Robinson (1928). *Chem. Age*, **19**, 337.
Hoesch and von Zarzecki (1917). *Ber. deutsch. chem. Ges.* **50**, 467.