CXXVII. A BIOLOGICAL SYNTHESIS OF *m*-DIGALLIC ACID.

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WHATEVER position is assigned to the glucose residue it is now generally agreed that gallotannin is mainly a derivative of *m*-digallic acid [Schiff, 1871, 1872; Nierenstein, 1905, 1910; Fischer and Freudenberg, 1912, 1913, 1914; Fischer, Bergmann and Lipschitz, 1918; Herzig, 1923; Nierenstein, Spiers and Hatcher, 1925], which is thus the source from which gallic acid is produced on hydrolysis. In the case of tannase hydrolysis it was to be expected that the reaction would be reversible and this is now confirmed, since both *A. niger* and tannase produce *m*-digallic acid to a small extent from gallic acid. Gallotannin is of pathological origin, probably produced from gallic acid by the gall-forming larva, which by its tannase content [Nierenstein, 1930] hydrolyses and also synthesises gallotannin. This probably accounts for the close admixture of these two components and for the fact that so far no complete separation of gallotannin from gallic acid has been successfully carried out, a point investigated by other workers in this laboratory [Hooper, 1925; Nicholson and Rhind, 1924].

EXPERIMENTAL.

I. *General.* The cultivation of *A. niger* in gallic acid solution was carried out as already described and this also applies to the preparation of the tannase-pyrogallase mixture [Nicholson, Nierenstein, Pool and Price, 1931].

II. *Identification of m-digallic acid.* The solutions of gallic acid which had been acted on by *A. niger* or tannase were extracted with ether and the residue left on evaporation of the ether was crystallised from a large quantity of water (about 500 cc. for 2 g. of residue). Each fraction thus obtained was collected, dried and methylated with diazomethane in ethereal solution. Most fractions proved to be trimethylgallic acid methyl ester on crystallisation from methyl alcohol, but a small quantity (about 0.3 g. from 2 g. residue) was found to melt correctly (m.p. 127–128°, mixed m.p. with an authentic specimen 127–128°) for pentamethyl-*m*-digallic acid methyl ester after 3 to 5 crystallisations from methyl alcohol. ($C_{20}H_{22}O_9$ requires C = 59.1; H = 5.4. Found: C = 58.9; H = 5.5.)

REFERENCES.

- Fischer, Bergmann and Lipschitz (1918). *Ber. deutsch. chem. Ges.* **51**, 45.
—— and Freudenberg (1912). *Ber. deutsch. chem. Ges.* **45**, 915.
—— ——— (1913). *Ber. deutsch. chem. Ges.* **46**, 1116.
—— ——— (1914). *Ber. deutsch. chem. Ges.* **47**, 2485.
Herzig (1923). *Ber. deutsch. chem. Ges.* **56**, 221.
Hooper (1925). *Analyst*, **50**, 162.
Nicholson, Nierenstein, Pool and Price (1931). *Biochem. J.* **25**, 752.
—— and Rhind (1924). *Analyst*, **49**, 505.
Nierenstein (1905). *Ber. deutsch. chem. Ges.* **38**, 3641.
—— (1910). *Ber. deutsch. chem. Ges.* **43**, 628.
—— (1930). *Nature*, **125**, 348.
—— Spiers and Hatcher (1925). *J. Amer. Chem. Soc.* **47**, 846.
Schiff (1871). *Ber. deutsch. chem. Ges.* **4**, 231, 967.
—— (1872). *Liebig's Ann.* **163**, 209, 229.