XX. THE FORMATION OF ELLAGIC ACID FROM GALLOYL-GLYCINE BY *PENICILLIUM*.

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Kraemer [1900], who investigated the galls formed by *Cynips aciculata* on *Quercus cocinea*, found that during the chrysalis stage gallic acid was produced and that as the imago developed the gallic acid gave place to tannic acid. These observations gain in interest if viewed in the light of the recent investigations of v. Stockert and Zellner [1914], who have proved the presence of nitrogenous products in a number of galls caused both by insects and fungi¹, and also of the results of Nierenstein [1914] who has isolated galloyl-leucine

from the galls of *Quercus aegilops*, which are caused by *Cynips calcis*. These investigations suggest, namely, the possible formation of the digallic acid nuclei in tannic acid by way of some nitrogenous product of the galloyl-leucine type which serves as food-stuff for the growing parasites and at the same time leads to the formation of digallic acid, the presence of which in the tannic acid molecule has been established by Nierenstein [1910, 1 and 1912, 1] and which has been synthesised by Emil Fischer and K. Freudenberg [1913], so that the identification of this substance, if experimentally formed, was possible. The conditions selected as a preliminary to other investigations in progress were inquiries into the action of *Penicillium* on galloyl-glycine, which is easily prepared by the usual methods of Emil Fischer. There was, of course, the possibility, especially in the light of the beautiful investigations

¹ With reference to gall formation see E. T. Connold [1908, 1909].

of Lewis Knudson [1913] on the action of fungi on tannic acid, that the digallic acid, if formed, would at once be decomposed into gallic acid; however, the selected conditions lead not to digallic acid, but to ellagic acid, which is a stable oxidation product of digallic acid and is frequently formed from the digallic acid nuclei of several tannins, both *in vivo* and *in vitro* [Nierenstein, 1910, 2]. It is quite possible that digallic acid is formed and subsequently converted into ellagic acid:

However, if one considers the above-mentioned investigations of Knudson and the pronounced oxidative properties of *Penicillium* [Herzog and Meir, 1908, 1909; Meir, 1909] it is far more likely that the elimination of the glycine radical and the ellagic acid formation through oxidation and anhydration [Nierenstein, 1912, 2] take place simultaneously:

This interpretation assumes that the glycine radical is eliminated by hydrolysis, which is hypothetical, as the present investigations have only established the formation of ellagic acid and nothing is at present known as to the fate of the glycine part.

In this connection reference is made to C. L. Berthollet [1809] and also to Merklin and Woehler [1845], who have found ellagic acid in the benzoar-stones of Persian goats, which suggests that galloyl-glycine (trihydroxy-hippuric acid) is, perhaps, also formed in the animal organism, especially as ellagic acid has also been found by Woehler [1848] in the bladder of *Castoreum canadense*.

It must, however, be remembered that gallic acid on administration is recovered either as free gallic acid [Stockmann, 1886, 1898; Baumann and Herter, 1877; Harnack, 1898] or in combination with sulphuric acid [Rost, 1898], but never in combination with glycine [Heffter, 1905].

EXPERIMENTAL.

Tricarbomethoxygalloyl-glycine.

Five grams glycine in 75 cc. water and 3.5 g. potassium hydroxide are mechanically shaken for three hours with 26.8 g. tricarbomethoxygalloyl chloride [Emil Fischer, 1908] dissolved in ether, which is added in three portions and cooled before adding. The solution is then left for two to three hours in a cooling mixture and acidified with carefully cooled hydrochloric acid after separation from the ether. The product thus obtained crystallises from alcohol in prismatic needles, which melt at 202°–204° with decomposition and evolution of carbon dioxide. The substance is also soluble in acetone and ethyl acetate. The yield is 94 %.

In addition to the usual analysis an hydroxyl estimation by the method of Daniel and Nierenstein [1911] was also made. As in other cases, where the hydroxyls were in the meta-position to the carboxyl groups [Daniel and Nierenstein, 1911; Nierenstein, 1912, 3], nearly 2 % more carbon dioxide was formed than required by theory. This increase is derived from the carboxyl group, which is partly eliminated in these cases by the pyridine [see Bredig and Fajans, 1910; Nierenstein, 1912, 4].

Analysis. 0.2577 g. : 8.3 cc. N₂ at 19° and 767 mm.
 0.1850 g. : 0.2816 g. CO₂; 0.0565 g. H₂O¹
 0.2462 g. : 0.0708 g. CO₂; (Daniel and Nierenstein)

Calculated for C₁₈H₁₅O₁₃N

N	3.74 %	3.49 %
C	42.49 %	42.76 %
H	3.42 %	3.69 %
CO ₂	28.86 %	26.92 %

Galloyl-glycine

Five g. tricarbomethoxygalloyl-glycine suspended in water are treated on the water-bath at about 40° with 50 cc. of a 10 % solution of pyridine

¹ The combustion was carried out in oxygen [see Francis and Nierenstein, 1911].

and water. The evolution of carbon dioxide is finished in about two hours, when the solid is crystallised from absolute alcohol. It is obtained in cubes, which melt at 282° – 283° , the yield being 97–98 %. Galloyl-glycine gives like galloyl-leucine [Nierenstein, 1914] only the iron chloride reaction, but not the potassium cyanide reaction for gallic acid [Sidney Young, 1883].

Analysis. 0.2399 g. : 13.3 cc. N_2 at 18° and 755 mm.
0.1758 g. : 0.3057 g. CO_2 ; 0.0572 g. H_2O

Calculated for $C_9H_9O_6N$

N	6.36 %	6.23 %
C	47.32 %	47.59 %
H	3.64 %	3.89 %

Three g. galloyl-glycine were hydrolysed with dilute hydrochloric acid (10 %) in a sealed tube and the solution extracted with ether. The ethereal part gave on evaporation gallic acid, which had the correct melting point and gave all the specific reactions for gallic acid. The aqueous solution was benzoylated; the hippuric acid obtained melted at 185° – 186° and caused no depression when a mixed melting point with hippuric acid was carried out.

Formation of ellagic acid from galloyl-glycine.

Five g. galloyl-glycine and 2 g. sodium bicarbonate are dissolved in 250 cc. of water and the solution saturated with carbon dioxide. The sterilised solution is then inoculated with *Penicillium* (*spec.* ?) grown in a solution of tannic acid, which contains free sugar [see Geake and Nierenstein, 1914]. The solution becomes cloudy after standing in an incubator for 32 hours at 42° and in about 78 hours a precipitate begins to be formed. After an incubation of 22 days, the *Penicillium* is carefully removed and the precipitate collected and washed with dilute hydrochloric acid. The product gives the specific Griessmayer reaction for ellagic acid [Nierenstein, 1909] and crystallises from pyridine in prismatic needles, which contain pyridine [see Perkin and Nierenstein, 1905]. The yields of three preparations were 83 %, 87 % and 84.5 %.

For analysis the product was treated with alcohol and dried at 160° .

Analysis 0.1946 g. : 0.3958 g. CO_2 ; 0.0395 g. H_2O

Calculated for $C_{14}H_6O_8$

C	55.50 %	55.62 %
H	2.27 %	1.98 %

The acetyllagic acid prepared according to Perkin and Nierenstein [1905] crystallised from alcohol in glistening, colourless, flat needles, which

commenced to sinter at about 331° and melted at 341° – 344° , which agrees with the melting point given by Perkin and Nierenstein.

Analysis. 0.1780 g. : 0.3679 g. CO_2 ; 0.0489 H_2O

Calculated for $\text{C}_{14}\text{H}_2\text{O}_8(\text{CO}-\text{CH}_3)_4$

C 56.37 %	56.17 %
H 3.06 %	2.98 %

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