ON THE RÔLE OF PHENOLS, TANNIC ACIDS, AND OXYBENZOIC ACIDS IN CORK FORMATION

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Tannins, phenols and oxybenzoic acids are very generally present in plant tissues. Among the more commonly occurring phenols are phloroglucinol, resorcinol, and hydroquinone; of oxybenzoic acids, gallic, salicylic and protocatechuic are commonly found. Some of these substances are widely distributed particularly tannins and phloroglucinol (1). If present in considerable quantity they generally act as poisons, but in some plants, for instance, *Loxopterygium Lorenzii*, Griesb., *Eucalyptus occidentalis*, Endl., *Caesalpinia coriaria*, Willd., and *C. brevifolia*, Baill., tannins are present in very large proportions. Pfeffer (2) has suggested that these substances being contained within the vacuole of the cell, fail to exercise a poisonous influence, not being capable of penetrating the protoplasmic boundary of the vacuole. When plants are fed with glucosides an injurious influence is often exerted owing to the liberation of the oxyaromatic radicles. It has been supposed that tannins may serve as reserve food materials, but this is still very doubtful. This question will be re-opened in another paper dealing with the function of tannic acids in the plant economy.

It is generally believed that tannins and other aromatic compounds play an important part in the lignification of the wood elements. Very little is known, however, with certainty, as to the function of these bodies in plants.

When tannins, phenols and oxybenzoic acids are treated with formaldehyde and a little hydrochloric acid, condensation products are precipitated. These have been studied by Bayer (3), Kleeberg (4),

Caro (5), Möhlau and Kahl (6), Nierenstein (7), Stiasny (8), and others. They are mixtures of diphenylmethaneoxybenzoic and aurinoxycarbonic acids. Stiasny in particular has studied the condensation products of formaldehyde with various tannic acids. All these products are powders, varying in colour from white through pale yellow to reddish brown and deep brown.

In experimenting with these condensation products we found that they could be produced from gallic acid, pyrogallol, proto-catechuic acid, resorcinol, phloroglucinol, salicin, tannic acid, and quebrachotannic acid. All the previous workers seem to have used hydrochloric acid, but we have found that similar substances are precipitated by using phosphoric, acetic, and formic acids, and more slowly by passing carbon dioxide through the mixture of formaldehyde and the phenol, tannic or oxybenzoic acid. A study of the reactions of these bodies has shown a remarkable similarity to those given by cork. Thus, they are insoluble in copper ammonium hydroxide (Schweizer's reagent) and in concentrated sulphuric acid, but dissolve readily in strong potassium hydroxide, giving a yellow or deep red-brown solution.

This suggested that in the formation of cork similar condensation products may play an important part. It is known that the required phenols, tannins and oxybenzoic acids are present in the plant and there is but little doubt that formaldehyde is also present; indeed, in some cases it is quite possible to demonstrate it.

An examination of a number of living plants has been made in order to trace the distribution of the tannic acid, phenols and oxybenzoic acids in the stem. The usually adopted method of testing for tannins in plants is by the use of ferric chloride, which gives a blue-black or green-black colour; with ferric chloride gallic acid gives a similar colouration. Potassium bichromate is also used and is said to give a dark brown colouration with tannic acids. These methods were employed by us. In addition, it was found that a most useful reagent for microchemical work is potassium cyanide. This gives a clear pink colour in the presence of gallic acid. Young (9) has used this reagent with success in chemical analysis, but we believe that it

has not generally been used in microchemical testing. It is also of great interest to note that contrary to the generally accepted belief that a brown colour is given by the action of potassium bichromate on tannic acid in microchemical work, we find that while gallic acid gives this reaction immediately, tannic acid fails to do so, or only develops a brown colour slowly.

Other reagents that have been used are gold chloride and ammonia, but for microchemical testing they have been less useful than potassium cyanide and ferric chloride.

The following plants have been examined :—

Sambucus nigra, L.

Potassium cyanide. A little red colouration in the cells in the immediate neighbourhood of the cork. (Fig. 1).

Ferric chloride. A little blue-black in the same positions.

Potassium bichromate. A little browning in the cork.

Ribes Grossularia, L. This plant has deeply seated cork.

Potassium cyanide. A red colour in the cells both outside and inside the cork. The walls of the cells bounding the cork layer internally also gave a strong red colour. (Fig. 2).

Ferric chloride. A tinge of blue-black in the same positions.

Potassium bichromate. A rich brown colour.

Acer Pseudoplatanus, L. This plant has deeply seated cork.

Potassium cyanide. Well developed red colour on both sides of the cork and in the external cortex. (Fig. 3).

Ferric chloride. Blue-black colour in the same positions. (Fig. 4).

Potassium bichromate. A deep brown colour.

Fuchsia. In this plant the cork cells are of two kinds, large and small, alternating with one another in a radial direction.

Potassium cyanide. Red colour in the cells internal to the cork and in the small cells of the cork itself ; also a little in the cells external to the cork. (Fig. 5).

Ferric chloride. Well developed blue-black colour in the same positions. (Fig. 6).

Potassium bichromate. Extensive browning.

Ilex Aquifolium, L.

No cork present.

Potassium cyanide. No red colour.

Ferric chloride. No blue-black colour.

Potassium bichromate. No brown colour.

Ulmus campestris, L.

Potassium cyanide. Much red colour outside the cork.

Ferric chloride. Much blue-black colour outside the cork.

Potassium bichromate. No colouration.

Helianthus sp.

A young stem with no cork formation was used.

Potassium cyanide. No colour.

Ferric chloride. No colour.

Potassium bichromate. No colour.

Cheiranthus Cheiri, L.

Potassium cyanide. Some red colour in the neighbourhood of the cork.

Ferric chloride. A little blue-black colour particularly outside the cork.

Potassium bichromate. A brown colour.

Ribes rubrum, L.

Potassium cyanide. Deep red colouration in the cortex and inside the cork.

Ferric chloride. A little blue-black in neighbourhood of the cork.

Potassium bichromate. Brown colour in the same positions.

In the examination of the plants described, in every case where cork was found, a colouration of the cells in the immediate vicinity was shown in the presence of potassium cyanide or ferric chloride, and usually also with potassium bichromate, indicating the presence of gallic or tannic acids. In the two plants which showed no reaction with ferric chloride, or potassium cyanide, namely, the Sunflower and the Holly, no cork was present.

CHEMICAL EXAMINATION OF THE CORK

Cork shavings, probably the produce of *Quercus Suber*, L., were extracted with water. The aqueous solution was tested with ferric chloride and with potassium cyanide and gave a blue-black with the former, and a red with the latter reagent. The aqueous solution was acidified with sulphuric acid and then extracted with ether. After distilling off the ether, small crystals of gallic acid were obtained which melted at $236-237^{\circ}$ C., the melting point of gallic acid being given as 240° C. The presence of gallic acid in the cork was thus demonstrated. Chevreul (10) in 1807 and 1815, stated that he had obtained gallic acid by extracting cork with water, and his statement is thus confirmed.

The cork was next extracted with alcohol and the extract was not examined. The remaining cork was then extracted for two hours with a 30 per cent. solution of potassium hydroxide on a steam bath. It was then filtered through glass-wool. The extract was neutralised with sulphuric acid, when a brownish yellow precipitate was formed. This was distilled over zinc dust and diphenylmethane was obtained, small crystals of which from chloroform gave a melting point 25.5° C. The powder was also distilled in coal gas with the result that pyrogallol was formed. By fusion with alkalis, protocathechuic acid was produced and another substance, of which no crystals could be obtained.

The condensation products of gallic acid with formaldehyde have been thoroughly studied by Möhlau and Kahl (6), who have shown them to consist of a mixture of four different substances one of which is hexaoxydiphenylmethanedicarbonic acid. This has the following constitution :—

On reduction this should give diphenylmethane :—

To prove this the condensation product of gallic acid with formaldehyde was distilled with zinc dust and diphenylmethane resulted, the crystals from chloroform having a melting point of 25°C.

The fact that the cork examined by us yields diphenylmethane strongly supports our belief that condensation products of a similar nature to those produced artificially by the action of formaldehyde on the phenols, tannic acid and oxybenzoic acids play a part in cork formation. For these condensation products the name *phellemic acids* is suggested. The name phellonic acids which was first chosen, has been used previously by Kügler (11), Thoms (12), Hientz (13), Gilson (14), and v. Schmidt (15), for a substance extracted from cork by chloroform. For this phellonic acid v. Schmidt has suggested the constitution :—

Evidently this could not yield diphenylmethane and hence it is not related to our phellemic acids.

The facts related above have led us to attribute to tannic acids, phenols, gallic acid and other oxybenzoic acids an important rôle in the formation of cork. The presence of substances of this class has been clearly shown in close association with the cork wherever this tissue was found. It has been shown that substances giving cork reactions are precipitated from tannic acids, phenols and oxybenzoic acids by means of formaldehyde in presence of various acids, and products having the same mother substance as these condensation products have been obtained from cork itself.

Our theory then is that the tannic and gallic acids, and substances of this nature are acted upon in the plant by formaldehyde and acids, and are precipitated in the walls of the cork cells.

SUMMARY

1. Condensation products which give reactions similar to those given by cork (insolubility in copper ammonium hydroxide and in concentrated sulphuric acid; solubility in potassium hydroxide) are precipitated by the action of hydrochloric, phosphoric, acetic, and formic acids, and of carbon dioxide on a mixture of formaldehyde and a phenol, tannic acid, or oxybenzoic acid.

2. The condensation product of gallic acid yields diphenylmethane when reduced with zinc dust.

3. Tannic and oxybenzoic acids are present in the plant in close association with the cork.

4. From cork itself was extracted gallic acid and a substance resembling the condensation products prepared artificially. This last substance when reduced with zinc dust yielded diphenylmethane.

5. Probably the condensation products of formaldehyde with tannic and oxybenzoic acids are formed in the plant and precipitated at an early stage in the cell walls of the cork.

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- (15) v. Schmidt, *Monatsb. Chem.*, Bd. XXV (1904), pp. 277, 302.

EXPLANATION OF PLATE

- Fig. 1. Transverse section of stem of *Sambucus nigra*, L., treated with potassium cyanide.
- Fig. 2. Transverse section of stem of *Ribes Grossularia*, L., treated with potassium cyanide.
- Fig. 3. Transverse section of stem of *Acer Pseudoplatanus*, L., treated with potassium cyanide.
- Fig. 4. Transverse section of stem of *Acer Pseudoplatanus*, L., treated with ferric chloride.
- Fig. 5. Transverse section of stem of *Fuchsia* treated with potassium cyanide.
- Fig. 6. Transverse section of stem of *Fuchsia* treated with ferric chloride.