CCLXXVI. THE CHLORINE-SODIUM SULPHITE COLOUR REACTION OF WOODY TISSUES

II. THE BEARING OF THE COLOUR REACTION ON THE CONSTITUTION OF HARDWOOD LIGNIN

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IN a previous communication from this Laboratory [Campbell *et al.* 1937] it was shown that the well known colour reaction given by woody tissues with sodium sulphite following chlorination was not characteristic of lignin only but could also be given by certain tannins. It was concluded that the reaction was probably specific for phenolic compounds containing the 1:2:3-trihydroxybenzene nucleus but, although such a nucleus is known to occur in the gallotannins, there is no evidence that it occurs as such in native lignin. Great interest is therefore attached to the isolation by Freudenberg [1938] of substances containing a modified 1:2:3-trihydroxybenzene nucleus such as syringic acid and also to the identification by Leger & Hibbert [1938] of pyrogallol 1:3-dimethyl ether among the degradation products of lignin. It was considered that, if any of these substances could be shown to give the characteristic colour reaction with chlorine and sodium sulphite, additional proof would be forthcoming for the occurrence in native lignin of a modified trihydroxybenzene nucleus as a recurring unit for the reason that, during the delignification of hardwoods by the Cross and Bevan procedure, the coloration persists until the last traces of lignin have been removed. This would, of course, involve the assumption that lignin is composed for the most part of aromatic units. Until the publication of the results of Harris *et al.* [1938] such an assumption could not be maintained with any certainty. In their study of the products of the hydrogenation and hydrogenolysis of hardwood lignin these authors have confirmed and extended the work of Moldavskii and Vainshtein [1935] and proved beyond doubt that the greater part of lignin is aromatic in character. In the present study chlorine and sodium sulphite have been applied to a further range of substances, particular attention being paid to products obtained by Freudenberg [1938] in view of their importance as likely building units in lignin.

EXPERIMENTAL

The following substances were exposed to gaseous chlorine followed by sodium sulphite in the manner previously described [Campbell *et al.* 1937] with the results shown in Table I.

The faint reaction of pure 1:2:3-trimethoxybenzene is probably associated with the low solubility of this compound in water and the consequent difficulty in securing its optimal reaction with chlorine. It was observed that of all the substances so far tested gallic, trimethylgallic and syringic acids gave colorations

Table I. *Colour reactions of various phenols and their derivatives with chlorine and sodium sulphite*

Substance	Colour reaction
Guaiacol	Nil
Vanillin	Reddish brown
Veratrole	Nil
Eugenol	Very faintly positive
Gallic acid	Strongly positive
1:2:3-Trimethoxybenzene	Faintly positive
Trimethylgallic acid	Strongly positive
Syringic acid	Strongly positive

which were most reminiscent of the deep magenta colour given by hardwoods and isolated hardwood lignin.

In certain cases it was found that a magenta coloration could be produced by reagents other than chlorine and sodium sulphite. For instance it was given by gallic and syringic acids on adding a trace of FeCl₃ in the presence of either Na₂SO₃ or NaHCO₃. Gallic acid solutions also give the coloration (1) when aerated in the presence of NaHCO₃ and Na₂SO₃, (2) with a trace of K₂CrO₄ and Na₂SO₃, (3) with a trace of CuSO₄ and Na₂SO₃.

In contradistinction to gallic acid, in which the three OH groups are free, and to syringic acid, which has one free OH group, it was found that trimethylgallic acid, which has no free OH groups, could only give the colour reaction with Na₂SO₃ after previous chlorination. The same has been found to be true of hardwood lignin and, in view of the possible significance of this analogy, the behaviour of pure trimethylgallic acid on chlorination was investigated in detail as follows.

10 g. of the acid were dispersed in 15 ml. of warm water and exposed to a slow stream of chlorine for 30 min. The mixture was cooled and the solid matter (11 g.) was separated by filtration and extracted with light petroleum (B.P. 40–60°). The crystalline substance which settled out from the petrol and was recrystallized from CCl₄ consisted of colourless needles (M.P. 120°) and was soluble in EtOH, Me₂CO, CHCl₃, C₆H₆ and CCl₄. The substance dissolved in NaHCO₃ with the evolution of CO₂ and its equiv. wt. by titration was 270. (C₆Cl₂(OCH₃)₃.COOH requires equiv. wt. 281; C₆HCl(OCH₃)₃.COOH requires equiv. wt. 247.5.) No coloration was given by either FeCl₃ or FeFe(CN)₆, indicating that the substance contained no phenolic OH groups. It therefore probably consisted of a mixture of mono- and di-chlorotrimethylgallic acids. Contrary to expectation this chlorinated compound, which represented by far the greater part of the original trimethylgallic acid, gave no coloration with sodium sulphite. It was found, however, that the clear aqueous filtrate obtained after the original chlorination did give the coloration. A test for phenols in this filtrate gave a positive result. Repeated chlorination of the already chlorinated trimethylgallic acid gave rise to further small amounts of a phenol which gave an intense magenta coloration with sodium sulphite.

DISCUSSION

It is now realized that the chlorine : sodium sulphite colour reaction is not specific for compounds containing the 1:2:3-trihydroxybenzene nucleus itself since it can also be given by substances in which this nucleus has been modified by substitution. It is worthy of note, however, that so long as at least one OH group remains unsubstituted the colour reaction can be produced by oxidizing agents other than chlorine and weak alkalis other than sodium sulphite. When,

on the other hand, all three OH groups are alkylated chlorination is a necessary antecedent to the production of the magenta coloration with sodium sulphite. In this latter case the major reaction product is a chlorinated derivative of the starting material, but the colour reaction is given by traces of a phenolic compound which is also produced during chlorination. In the case of trimethylgallic acid it must be concluded that chlorine has acted as a demethylating, as well as an oxidizing agent. So far as is known chlorination must always be applied to hardwood lignin before the characteristic coloration is given in the presence of sodium sulphite. By analogy it is therefore strongly suggested that lignin contains a modified pyrogallol nucleus as a recurring unit. This unit may be given the general formula (I).

Formula (I)

A magenta colour reaction in the presence of sodium sulphite could be given by such a unit if it were transformed into syringic acid (formula II) or gallic acid (formula III).

Formula II
syringic acid Formula III
gallic acid

In the light of the degradation studies of Freudenberg [1938], Leger & Hibbert [1938] and suggestions in the literature by Klason [1930], Wacek [1930] and others, it is reasonable to assume that R_1 and R_2 in formula (I) are OCH_3 groups and that side chains occur at positions 1 and 4. These side chains could form linkages between essentially similar methylated pyrogallol nuclei.

SUMMARY

1. Continuation of previous work [Campbell *et al.* 1937] indicates that the chlorine : sodium sulphite colour reaction of hardwoods is not specific for compounds containing the 1:2:3-trihydroxybenzene nucleus itself, since it can also be given by substances in which this nucleus is modified by certain substituents.

2. Provided that at least one OH group of a pyrogallol derivative is free the colour reaction is given by oxidizing agents other than chlorine in conjunction with weak alkalis other than Na_2SO_3 .

3. If all three OH groups are methylated chlorination is a necessary antecedent to the production of the coloration in the presence of sodium sulphite.

4. The major product of the chlorination of trimethylgallic acid is a crystalline chlorinated derivative of the starting material which gives no coloration with Na₂SO₃. The coloration is given by a secondary product of chlorination which is shown to be a phenol.

5. The analogy with respect to this colour reaction between certain gallic acid derivatives and hardwood lignin is discussed, and it is suggested that hardwood lignin contains a recurring unit of formula

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