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

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IN a recent paper by Cohen & Harris [1937] it is shown that the lignin of maple wood is, to a limited extent, soluble in hot dilute sulphuric acid and also in hot water. That a fraction of lignin is soluble in water has been suspected for many years and recently it has been definitely suggested by Paloheimo [1929]. The solubility of lignin in dilute acids is discussed by Schorger [1926, p. 92] and Hawley & Wise [1926, p. 70], and only recently it has been shown that the lignin of beech wood is soluble in hot 10 % sulphuric acid [Bamford & Campbell, 1936]. The study of the action of dilute sulphuric acid and of hot water on the chemical composition of various woods has been in progress in this Laboratory during the past few years. Results so far obtained indicate that treatment of oak sapwood with water at 98° for periods over 100 hr. results in the solution or decomposition of approximately 20 % of the apparent lignin, while continuous treatment of oak heartwood with 1 % sulphuric acid under the same conditions for 17 days causes the solution or decomposition of some 34 % of the apparent lignin. The object of the present communication is to record some recent observations concerning the pink coloration which develops after sodium sulphite is added to hardwoods or their extracts after chlorination and treatment with sulphurous acid. Cohen & Harris [1937] have used this well-known but little studied colour reaction as a confirmatory test for the presence of lignin in the aqueous and acid extracts of maple wood but, as the following results will show, little reliance can be placed upon their conclusions.

EXPERIMENTAL

Small amounts of the substances named in Table I which had been prepared in this Laboratory at various times were moistened with water and exposed to gaseous chlorine in diffuse sunlight for 5 min. Sodium sulphite solution was then added in slight excess after adding a few drops of water saturated with sulphur dioxide. All the substances gave a positive pink colour reaction.

A few drops of cold, saturated chlorine water were added to approximately 25 ml. of each of the extracts in Table II. After shaking for 5 min. a few drops of water saturated with sulphur dioxide were added. Anhydrous sodium sulphite was added in sufficient excess to neutralize the acids present and in every case a positive pink coloration was observed.

The striking conclusion which emerges from the foregoing qualitative experiments is that the structure of a number of hardwoods is permeated with a body or bodies which give an extremely sensitive chlorine-sodium sulphite reaction. We have evidence that such a body is also present in softwoods such as Sitka

Table I. *Partial list of wood products which give a positive chlorine-sodium sulphite reaction*

Substance	Source
Oak sapwood starch	Campbell [1935, 1]
Water-soluble polysaccharide from oak sapwood related to starch	Campbell [1935, 2]
Lignin-like residue remaining after acid hydrolysis of oak sapwood starch	Campbell [1935, 1]
Hemicellulose A from oak sapwood	O'Dwyer [1934]
Hemicellulose B from oak sapwood	O'Dwyer [1934]
Water-insoluble fraction of hemicellulose A from oak sapwood	O'Dwyer [1937]
Fraction of oak sapwood hemicellulose A which is insoluble in H ₂ O and not hydrolysed by takadiastase	O'Dwyer [1937]
Water-soluble fraction of oak sapwood hemicellulose A	O'Dwyer [1937]
Hemicellulose A from oak sapwood which had previously been extracted with water at 98° for 105 hr.	O'Dwyer [1937]
Hemicellulose A from oak heartwood	O'Dwyer [1934]
Lignin-like residue from hemicellulose A of oak heartwood	O'Dwyer [1934]
Impure starch from oak leaves	Campbell [1935, 2]
Exuded "kino" from jarrah wood	Campbell & Bryant [1937]

Table II. *Partial list of wood extracts which give a positive chlorine-sodium sulphite reaction*

- * Hot- and cold-water extracts of oak sapwood, maple, beech and ash woods
- † Alcohol-benzene (1 : 2) extracts of oak, maple, beech and ash woods
- † Filtrate from a lignin determination on oak sapwood, using the method of Bamford & Campbell [1936]
- * Hot-water extract of lignin from oak heartwood which latter had previously been treated with 1% H₂SO₄ at 98° for 17 days
- * Hot-water extract of lignin isolated from oak, ash and beech woods by the above method
- * Hot-water extract of beech wood lignin. Wood pre-treated with hot 5% H₂SO₄ in 2 min. periods [Bamford & Campbell, 1936]
- * Cold-water extract of lignin from oak sapwood, beech and ash woods
- * Cold-water extract from oak sapwood which latter had previously been extracted with water at 98° for 141 hr.

* Extracts obtained by shaking a small quantity of the material with water in a test-tube for a few minutes and filtering through a Whatman No. 50 filter paper.

† Extracts first evaporated to dryness and dispersed in cold water.

spruce and Douglas fir. Since we have found that purified wood cellulose (filter-paper) and Cross and Bevan cellulose from beech wood give no such colour reaction it is obvious that the body in question is destroyed or removed during the ordinary processes used in the isolation of cellulose. That it is capable of surviving the somewhat drastic conditions which prevail during the isolation of lignin by the sulphuric acid process is proved by the fact that a positive chlorine-sodium sulphite reaction is given by an aqueous extract of isolated lignin. The claim of Cohen & Harris [1937] that the filtrate from a lignin determination does not give any form of colour reaction with chlorine and sodium sulphite cannot be sustained. It is, of course, well known that the reaction will only develop in a neutral or slightly alkaline system.

The so-called colour reactions for lignin have long been a subject of discussion, which has been well summarized by Schorger [1926, p. 122] and Hawley & Wise [1926, p. 49]. The latter authors have concluded that certain of the colour reactions may be given by natural substances quite apart from the components of wood and should on this account be interpreted with the greatest caution. Schorger [1926, p. 83] has observed that the chlorine derivatives of pyrogallol

and "lignone chloride" give identical colour reactions with sodium sulphite and ferric chloride. The chlorine-sodium sulphite test was therefore applied to a number of phenolic bodies which happened to be available, with the results shown in Table III.

Table III. *Colour reactions of various phenols with chlorine and sodium sulphite*

Substance	Colour reaction
Pyrogallol (B.D.H. preparation)	Pink as with hardwoods
Methyl gallate (B.D.H. preparation)	Pink as with hardwoods
Tannic acid (<i>m</i> -digallic acid, B.D.H. preparation)	Pink as with hardwoods
Phloroglucinol (AnalaR)	Green
Resorcinol (B.D.H. preparation)	Nil
Quinol	Nil
Catechol	Nil
Guaiacum resin	Nil

It therefore appears that the chlorine-sodium sulphite reaction is specific for phenolic bodies containing a 1:2:3-trihydroxybenzene nucleus, and the suggestion presents itself that such bodies are actually present in hardwoods and are responsible for the well-known colour reaction given by such woods with chlorine and sodium sulphite. The presence of phenols in various wood products was confirmed by the ferric ferri cyanide test of Cross and Bevan and Crocker [Schorger, 1926, p. 122], positive blue colour reactions being given by

Oak heartwood
Cold-water extract of oak sapwood
Cold-water extract of Sitka spruce
Cold-water extract of East African camphor
Filtrate from lignin determination on oak sapwood
Cold- and hot-water extracts of lignin from beech and Douglas fir woods

A few experiments revealed that the above colour reactions for phenols are extremely sensitive. By means of chlorine water, sulphurous acid and sodium sulphite it was possible to detect 1 part of tannic acid (B.D.H. preparation) in 1 million parts of water, whilst by means of ferric ferri cyanide 1 part of tannic acid in 10 million parts of water could readily be detected. Of the two tests only the former is specific for the 1:2:3-trihydroxybenzene nucleus.

A final experiment was carried out in order to determine whether the colour reaction given by a naturally occurring trihydric phenol of the type mentioned above is capable of surviving the conditions employed in a standard lignin determination. 1 g. of tannic acid (B.D.H. preparation) was incorporated with 25 ml. of 72 % H_2SO_4 and maintained at 10° for 5 hr. Thereafter the sulphuric acid was diluted to 3 % concentration and the system was boiled for 2 hr. The tannic acid passed into solution in the dilute acid, but no precipitate was formed even after standing in the cold for 3 days. The cold solution gave a strongly positive colour reaction with chlorine and sodium sulphite.

DISCUSSION

There can now be little doubt that if the well-known colour reaction given by woods with chlorine and sodium sulphite is to be accepted as a typical lignin reaction, any method involving sulphuric acid for the analytical determination of lignin is reduced to an absurdity. The colour reaction in question has been shown to be so extremely sensitive as to show that even isolated sulphuric acid-lignin from various woods is partially dissolved during a few minutes' shaking with cold water.

The above experimental evidence is in favour of the view that certain phenolic bodies containing a definite structural unit, probably the 1:2:3-trihydroxybenzene nucleus, permeate the structure of certain hardwoods and softwoods. Such bodies are soluble in water and alcohol-benzene and are capable, in part at least, of surviving the drastic conditions employed in the isolation of lignin by the sulphuric acid method. The extent to which such bodies occur in isolated lignin has not yet been assessed, but a much fuller knowledge of them should obviously be of far reaching importance in lignin chemistry. The results of this preliminary investigation strongly suggest that the substances in question are either tannins or some form of lignin itself, and their fuller investigation is in progress, since the lists of wood products in the foregoing tables, which give the chlorine-sodium sulphite reaction, are by no means exhaustive.

SUMMARY

1. The well-known colour reaction given by woody tissues with chlorine and sodium sulphite has been studied in a series of qualitative experiments.
2. The reaction is given by cold and hot aqueous extracts of wood, by the acid filtrate from lignin determinations and even by cold aqueous extracts of isolated sulphuric acid-lignin itself.
3. The reaction is probably specific for phenolic bodies containing the 1:2:3-trihydroxybenzene nucleus, such as pyrogallol, tannic acid, etc.
4. It is suggested that phenolic bodies, which may be either tannins or even a form of lignin itself, permeate the structure of certain hardwoods and softwoods and are capable of surviving, in part at least, the conditions employed in the isolation of lignin by means of concentrated sulphuric acid.

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