608. *The Chemistry of the Wood Cell Wall. Part II.* The Isolation of Beech and Spruce Acid-soluble and Modified Lignins.*†

By (the late) W. G. Campbell and I. R. C. McDonald.

From the sulphuric acid lignin hydrolysates of beech and spruce woods, beech and spruce acid-soluble lignins have been isolated by a new method. Similarly, beech and spruce modified lignins have been prepared from the sulphuric acid lignin hydrolysates of the corresponding " holocelluloses." Therefore the total lignin content of beech or spruce wood or of their holocelluloses cannot be estimated by the 72% sulphuric acid method. The methoxyl and the carbon contents of these substances are considerably lower than those of the sulphuric acid lignins from the same woods.

The previous paper in this series * drew attention to the presence in partly delignified beech- and spruce-wood residues (" holocelluloses ") of a form of lignin which was not isolable by the usual method of lignin determination. This material was called " modified lignin " because it was considered to be a form of lignin so modified during the delignification of wood by buffered aqueous sodium chlorite that it was no longer isolated as an insoluble residue by the normal 72% sulphuric acid method for determination of lignin. It was further shown that the true total polysaccharide content of either wood, as a percentage, was less than the value $100 - x$ (where x is the percentage of acid lignin) for the probable reason that a portion of the native lignin present in these woods is not recoverable as acid lignin. This suggested that a fraction of native lignin is " acid soluble."

Various authors (Hägglund, *Papierfabrikant*, 1925, **23**, 406; *Chem. Abs.*, 1925, **19**, 3015; Bamford and Campbell, *Biochem. J.*, 1936, **30**, 419) have remarked on the difficulty of filtering the sulphuric acid lignin precipitate from wood and have observed that subsequent flocculation occurs in the filtered lignin hydrolysates unless very fine filters are used. Migita and Kawamura (*J. Agric. Chem. Soc. Japan*, 1944, **20**, 348, 507) reported that the acid lignin fraction of wood could be fractionally precipitated if the acid strength was reduced stepwise from 72 to 3%. A further precipitate was formed when the filtered 3% mixture was boiled, but these authors did not suggest that an additional lignin fraction still remained in solution. The acid lignin hydrolysates from beech- or spruce-wood are known to give ultra-violet absorption spectra regarded as characteristic of lignin and also, in the case of beech-wood, a pronounced Maüle colour reaction. It has been observed that similar hydrolysates from the lignin determinations on beech- and spruce-wood residues, which have been partly delignified by buffered sodium chlorite solution (" holocelluloses "), also have these properties. Von Wacek and Schroth (*Holz als Roh-und Werk-stoff*, 1951, **9**, No. 1, 7; *Das Papier*, 1950, **4**, 410; *Monatsh.*, 1950, **81**, 1151) have reported the presence of a soluble form of lignin (which was called protolignin I, after Jayme) in the acid lignin filtrates of spruce wood and also in alkaline extracts of *Entandrophragma utile* wood. These authors state that they could not isolate this soluble lignin from the acid lignin hydrolysates but have isolated it from the alkaline wood extracts. Although some 5·5% of it has been isolated from spruce wood, methoxyl balance was used to calculate the amount of this material in the tropical hardwood.

In experiments in this laboratory it was observed that the ultra-violet absorption spectrum of the acid lignin hydrolysates of beech wood had a definite maximum at 277·5, a minimum at 255, and inflexions at 320 and 225 mμ (Fig. 1). If this acid solution was passed through an ion-exchange resin column (Zeo-Karb 215) the resulting acid solution showed greatly reduced absorption throughout the entire spectrum. The maximum, the minimum, and the inflexion at 225 mμ are, however, still present (Fig. 1). The original solution gave a pronounced Maüle reaction (chlorine and sodium sulphite) whereas after passing through the column the solution gave a very weak pink colour with

---

* Part I, *J.*, 1952, 2644.

† This paper was in part presented by the late W. G. Campbell at the Lignin Session of the International Pulp and Paper Symposium, Appleton, Wisconsin, September 1951.

these reagents. Absorption curves similar to those in Fig. 1 have also been obtained for the sulphuric acid lignin hydrolysate from spruce wood before and after passage through an ion-exchange column.

From these observations a simple method has been devised by which the materials responsible for the major part of the absorption of the original sulphuric acid lignin hydrolysates of beech or spruce woods have been isolated as buff to pale brown powders or as brown to red resinous products. These materials are not polysaccharides or mixtures of sulphuric acid lignins and polysaccharides. Therefore, because these materials were also soluble in 3% sulphuric acid, under the conditions prevailing in the 72% sulphuric acid method, the names " beech and spruce acid-soluble lignin " have been assigned to them. These materials, when isolated in the powder form, are labile compounds, which are easily converted by warming into resinous products. In the case of beech this conversion is evidently a polymerisation because both the resinous and the powder form give the same analytical figures. Beech acid-soluble lignin has been isolated from the lignin hydrolysates in yield equivalent to 7·4% by weight of the original wood and spruce acid soluble lignin in 5·8% yield calculated on a similar basis.

FIG. 1.

1, *Original sulphuric acid lignin hydrolysate.*
2, *Solution, as above, after passage through Zeo-Karb* 215.

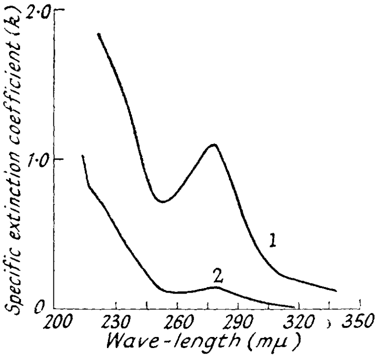
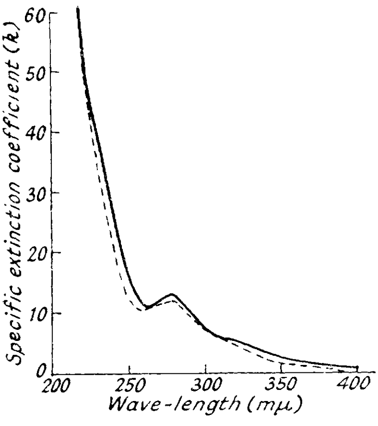
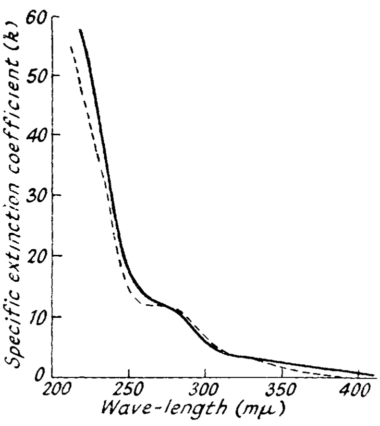


FIG. 2.



——— *Beech acid-soluble lignin.*
– – – – – *Beech modified lignin.*

FIG. 3.



——— *Spruce acid-soluble lignin.*
– – – – – *Spruce modified lignin.*

Similarly, beech modified lignin has been isolated from the acid lignin hydrolysates of samples which have been partly delignified with sodium chlorite. Wood residues obtained in yields of 92·9, 87·4, and 71·9% gave respectively 14·0, 7·2, and 0·7% of sulphuric acid lignin and 8·7, 10·0, and 5·0% of modified lignin.

In view of the difficulties in determining whether a lignin preparation is homogeneous, analyses should be examined with caution (see p. 3183). Tentative formulæ have been calculated as follows, on the assumption that the materials are in fact homogeneous: beech acid-soluble lignin, $[C_{40}H_{43}O_{20}(OMe)_5]_n$; beech modified lignin, $[C_{40}H_{43}O_{20}(OMe)_5,2HCl]_n$. Similar formulæ are not possible for the spruce acid-soluble and modified lignins on the results so far obtained.

Ultra-violet Absorption Spectra of Acid-soluble and Modified Lignins.—The ultra-violet absorption spectra of beech acid-soluble and modified lignins and spruce acid-soluble and modified lignins are shown in Figs. 2 and 3. As the molecular weights were not known the specific extinction coefficients, k (concn. calc. as g./l.), have been plotted against the wave-length in mμ. In either figure there is little difference between the spectrum of the acid-soluble lignin and the modified lignin, indicating that the addition of Cl_2 or HCl has not affected the main conjugating system. While in the case of the beech samples (Fig. 2) a distinct peak is noticeable at 280 mμ, in addition to inflexions at 320 and 225 mμ, the spruce samples do not show this definite peak—the inflexion at 320 mμ is, however, present, but that at 225 mμ was not detected. The specific extinction coefficients over the entire spectra are approximately the same for all the substances, suggesting that the same conjugating system may be present in beech and spruce acid-soluble and modified lignins. Further, these spectra are similar to the spectra usually assigned to lignin preparations of higher carbon and methoxyl contents.

EXPERIMENTAL

Samples of European beech (*Fagus sylvatica*) and Sitka spruce (*Picea sitchensis*), taken from the batches prepared at the commencement of this series, were exhaustively extracted with alcohol–benzene (1 : 2), thoroughly washed with warm water, and air-dried to a moisture content of about 12%. For the lignin determination some 50 g. were oven-dried at 105°, then cooled in a desiccator. To 20 g. of this material, 125 ml. of 72% (w/w) sulphuric acid at 10° were added and the slurry was stirred and placed in a desiccator at $10° \pm 0{\cdot}5°$ for 16 hours (5 hours are sufficient for beech wood or 6 hours for spruce, but 16 hours, *i.e.*, overnight, were convenient). The mixture was then diluted with 4·750 l. of water, and the resultant 3% acid solution was gently boiled for 2 hours under reflux and filtered through a 17 G.4 sintered-glass filter to remove all sulphuric acid lignin. The volume of the filtrate and washings was measured and its ultra-violet absorption spectrum and total reducing sugar content determined.

Isolation of Acid-soluble Lignin.—An aliquot portion (3 l.) of the foregoing solution was passed, at a rate of about 3 l. an hour, through a column ($1\frac{1}{2} \times 18$ in.) containing 1 lb. of Zeo-Karb 215 which had been previously treated with 5% hydrochloric acid, distilled water (until neutral), 95% ethyl alcohol (about 3 l.; during this treatment the column was left 24 hours full of alcohol), and finally 2—3 l. of distilled water until no alcohol remained on the column. The ultra-violet absorption spectrum of the eluate, compared with the original spectrum, showed a decrease of some 90% in absorption intensity at 277·5 mμ although there was no great change in absorption at 225 mμ. Total reducing sugar determinations (W. G. Campbell and I. R. C. McDonald) indicated that no reducing sugars had been retained on the column together with the acid-soluble material. The column was then washed with 5% hydrochloric acid, distilled water until neutral, and finally 95% ethyl alcohol. Some material was immediately eluted with alcohol (total solids in a yield of 2·2% of the original wood in the case of beech and 1·0% in the case of spruce) but the greater part was eluted only after the alcohol had been in contact with the column for 16 hours (yield, 5·2% for beech and 4·8% for spruce). The total recovered material on an original wood basis was thus 7·4% for beech and 5·8% for spruce. These total solid residues were, however, resinous materials insoluble in acids, alkalis, or organic solvents.

To prepare the powder forms these aqueous-alcohol eluates were distilled in a vacuum at low temperature ($\not> 30°$) and the residue dried by azeotropic distillation with benzene or ethyl methyl ketone. From either wood an amber–brown resinous material resulted which was extremely soluble in alcohol. This was dissolved in the minimum quantity of alcohol or dioxan at room temperature (warming causes immediate polymerisation) and added to a large excess of dry ether. A buff to pale brown precipitate formed immediately in the case of beech acid-soluble lignin and a pink to pale brown precipitate in the case of spruce. A proportion of the material, however, dissolved in the alcohol–ether and was recovered by concentration of this solution and reprecipitation. These precipitates can be easily filtered off and on careful vacuum-drying are isolated as pale brown powders, readily soluble in 85% alcohol. Friction converts either powder into transparent amber-coloured wax-like resins which are both brittle and soft. Warming converts these substances into the resinous forms which are virtually insoluble in organic solvents, acids, or alkalis.

The same technique of isolation was applied to the lignin hydrolysates of partly delignified beech and spruce wood residues. From the lignin hydrolysate of a beech wood residue (yield, 71·9% containing 0·7% of sulphuric acid lignin), 5% of beech modified lignin was isolated as a resin. From the lignin hydrolysate of a spruce wood residue (93·4% yield containing 19·0% of sulphuric acid lignin), 6·1% of spruce modified lignin was isolated as a resin. Beech modified lignin was also recovered as a pink powder, and spruce modified lignin as a buff-coloured powder. Modified lignins are not as labile as acid-soluble lignins, but resinify when heated.

Analyses, together with those of the sulphuric acid lignins, are reported in the Table.

	Beech				Spruce			
	C, %	H, %	OMe, %	Cl, %	C, %	H, %	OMe, %	Cl, %
Acid lignin from wood	60·4	5·5	20·0	0	63·6	5·7	14·9	0
Acid-sol. lignin (powder)	54·0	5·7	16·4	0	46·3	5·5	3·0	0
,, ,, (resin)	53·9	5·8	—	0	54·0	5·3	—	0
Modified lignin	50·4	5·7	14·6	6·5	48·7	5·4	—	5·1
$C_{40}H_{43}O_{20}(OMe)_5,2HCl$ requires ...	50·4	5·6	14·6	6·6				

Ultra-violet Spectra.—The ultra-violet absorption spectra of the acid hydrolysates were determined in 1-cm. quartz cells in a Unicam SP 500 quartz spectrophotometer. It was necessary to dilute a normal hydrolysate 5 times to a concentration of approx. 0·04% of wood substance. The acid-soluble or modified lignins were dissolved in 80% ethyl alcohol (*c*, 3·00 mg. per 100 ml. of solution), and the spectra measured in 1-cm. quartz cells.

This paper was written after the death of Mr. W. G. Campbell. Thanks are offered to Dr. R. H. Farmer of this Laboratory who kindly assisted in its preparation. The work forms part of the current research programme of the Forest Products Research Board and is offered for publication by permission of the Department of Scientific and Industrial Research.

SECTION OF CHEMISTRY, FOREST PRODUCTS RESEARCH LABORATORY,
PRINCES RISBOROUGH, AYLESBURY, BUCKS.
[*Received, April* 16*th*, 1952.]