500. *The Chemistry of The Wood Cell Wall. Part I. The Delignification of Beech and Spruce Woods by Sodium Chlorite in Buffered Aqueous Solution.**

By (the late) W. G. CAMPBELL and I. R. C. MCDONALD.

In the delignification of European beech (*Fagus sylvatica*) and Sitka spruce (*Picea sitchensis*) by aqueous solutions of sodium chlorite, buffered at pH 6·3, at 50° and 70°, some 90% of the acid lignin can be removed from each wood without significant loss of polysaccharide, but attempts to delignify the wood residues further cause appreciable loss. Chemical analysis of some partly delignified residues, containing 5—90% of the original acid lignin, indicated that there was retained in the wood residues a modification of lignin which is not determined by the 72% sulphuric acid method. The total percentage of polysaccharide present in each wood is considerably less than $100-x$, where x is the acid lignin content of the original wood; therefore it may be inferred that x does not equal the total native lignin content of the wood. Any determination of " holocellulose " based on a yield equivalent to the formula $100-x$ is erroneous because even though the acid lignin content may be small there is also present a large proportion of " modified lignin."

ATTEMPTS to isolate the entire polysaccharide portion of the wood cell wall, Schmidt's " skeletal substance " (*Cellulosechem.*, 1931, **7**, 201; 1932, **8—9**, 129), or Ritter's " holocellulose " (*Ind. Eng. Chem.*, 1933, **25**, 1250), have been unsuccessful because the complete delignification of wood by any of the known methods is invariably accompanied by some loss of polysaccharides. Jayme (*Cellulosechem.*, 1942, **20**, 43) first suggested that, in the determination of " holocellulose " by means of acidified sodium chlorite, delignification should be arrested when the residue still contains a small amount of lignin—about 3% on an original wood basis. The residue is then weighed, the acid lignin determined, and the so-called lignin-free holocellulose calculated. When this procedure was used, the percentage of holocellulose was comparable with that given by the formula $100-x$, where x is the lignin content of the original wood as determined by the standard methods. While agreeing with Jayme (*loc. cit.*) that it was desirable to isolate a partly delignified residue and to correct for its small acid lignin content, Wise, Murphy, and D'Addieco (*Paper Trade J.*, 1946, **122**, *T.A.P.P.I. Sect.* 11), working with aspen, showed that the accuracy that had been claimed for summative analyses involving holocellulose determinations was fortuitous and arose through a compensation of errors; the holocellulose determination was therefore regarded as a method of isolating a substantially pure polysaccharide fraction of wood and not as an analytical tool.

In a series of experiments on beech wood under various delignifying processes, Müller (*Annalen*, 1947, **558**, 81) obtained crude residues with acid lignin contents varying from 1·4 to 6·7%. When corrected for their residual acid lignin content these yielded lignin-free holocelluloses of up to 90%, whereas the expected figure given by the formula $(100-x)$ was 78% on the original wood. Accordingly Müller concluded that the amount of " holocellulose " was not given correctly by the formula, but he did not suggest that the lignin determination was at fault. A similar result had been obtained earlier by Jayme and Finck (*Cellulosechem.*, 1944, **22**, 102) who isolated from spruce wood as much as 5% more than the theoretical yield of lignin-free holocelluloses. Thus the sum of the lignin-free holocellulose and the original acid lignin was as much as 112% in Müller's work on beech and 105% in Jayme and Finck's work on spruce woods.

The origin and composition of this apparently surplus material are uncertain. Jayme and Finck referred to it as " excess of carbohydrate," and suggested that it is a form of lignin that has been modified by oxidation in the acidified chlorite liquors so that it is not isolated as an insoluble residue by the Halse method. These investigations showed that wood

* This paper is a slight extension of that presented in abstract by (the late) Mr. W. G. Campbell at the XIIth International Congress of Pure and Applied Chemistry, New York, September, 1951.

residues treated with chlorite did not yield a greater proportion of reducing sugars on hydrolysis than did the original wood. Thus spruce wood yielded 69·5—73·9% of reducing sugars, while spruce residues treated with chlorite gave 63·2—70·5% of reducing sugar (as glucose). On the other hand, Müller found that beech wood gave 68% whereas beech residues treated with chlorite yielded on the average 72·9% of reducing sugars (as glucose), and concluded that a substantial part of the beech native lignin is converted during treatment with chlorite into a form in which it can be hydrolysed to reducing products. In subsequent papers, Müller (*Annalen*, 1947, **558**, 157, 164) stated that in beech wood a polysaccharide similar to an alkoxy-hexosan is an important unit of the native lignin structure.

The work now described begins an investigation prompted by the apparent conflict between these previous workers.

It is considered that the only satisfactory definition of "lignin" is that employed by the early botanists, namely that, in an extractive free wood, lignin comprises the total non-polysaccharide portion or portions of the wood cell wall. The insoluble residue obtained by the normal analytical methods of lignin determination is advisedly termed acid lignin because it is not known what relation it bears to the native lignin or what proportion of the latter it represents.

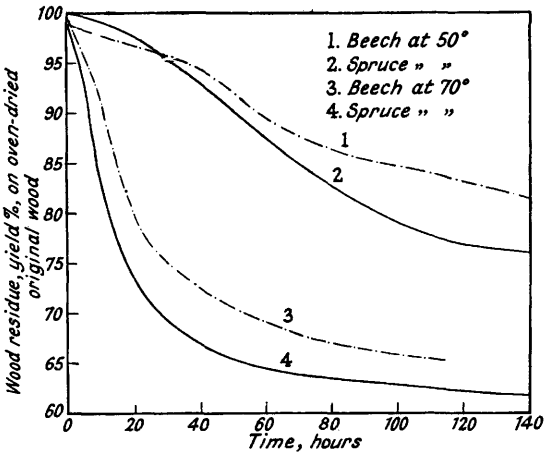


FIG. 1. *Rate of dissolution of wood substance in aqueous sodium chlorite buffered at* pH 6·3.

Figs. 1—5 show the results of the delignification experiments on beech and spruce woods, buffered sodium chlorite solutions (pH 6·3) being used at 50° and 70°. In Fig. 1 no allowance has been made for the small solubility of wood substance in the buffer solution alone.

In Fig. 1 the %-weight of the chlorite-treated wood residue is plotted against the time of delignification. Raising the temperature from 50° to 70° increases the rate of dissolution of both woods some six-fold. There is no indication from the form of the graphs at 70° that the theoretical holocellulose yield (75·5% for beech and 71·7% for spruce) has any kinetic significance.

Figs. 2 and 3 show that initially at each temperature the rate of removal of acid lignin is greater for spruce than for beech wood, but later the rates are similar. Although the original woods differ in their acid lignin contents, below an acid lignin content of about 10% the residual acid lignin content for both woods is approximately the same.

For each wood, results at 50° and 70° are identical when the amount of wood residue is plotted against the acid lignin isolable from it (Figs. 4 and 5). The straight line *aa'* on each figure is the ideal delignification graph which would result if the loss of wood substance were equal to the loss of acid lignin at all stages. Clearly the loss of acid lignin is greater than the weight of wood substance dissolved over the greater part of the range studied and this difference exhibits a maximum at 86·5% yield of wood residue for each wood. A similar conclusion was reached by Sen Gupta and Callow (*J. Textile Inst.*, 1951, **42**, T375) as a result of their experiments on the delignification of jute fibre by chlorine dioxide solutions

buffered at pH 4·7. At low acid lignin contents (1·5% for beech and 4% for spruce) the loss of wood substance is greater than the apparent loss of lignin; at this stage polysaccharide loss presumably becomes significant. Therefore, during the major part of the delignification reaction, that fraction of the native lignin which is recoverable from the normal wood as acid lignin has in part been modified or stabilised so that it is soluble in sulphuric acid. These figures also confirm the apparent surpluses observed by Jayme and Finck, and by Müller. The surplus is the difference between the graph and the ideal line *aa'*, measured in the direction of the ordinate. For example, a yield of 86·5% corresponded to an apparent

FIG. 2. *Rate of dissolution of acid lignin at* 50°.

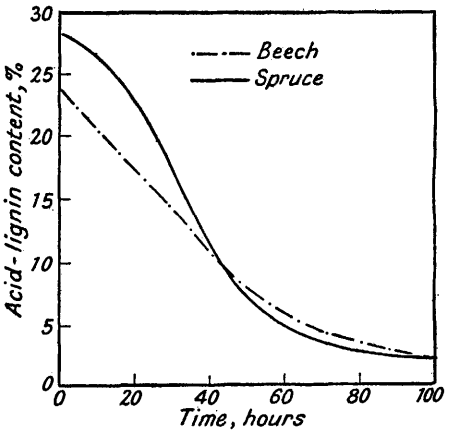


FIG. 3. *Rate of dissolution of acid lignin at* 70°.

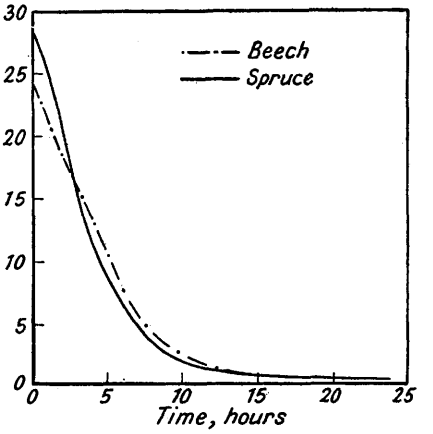


FIG. 4. *Beech at* 50° *and* 70°.

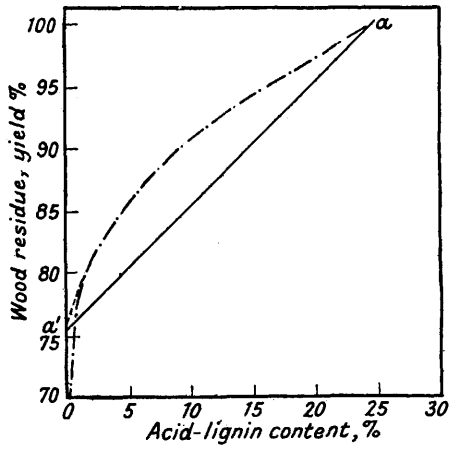
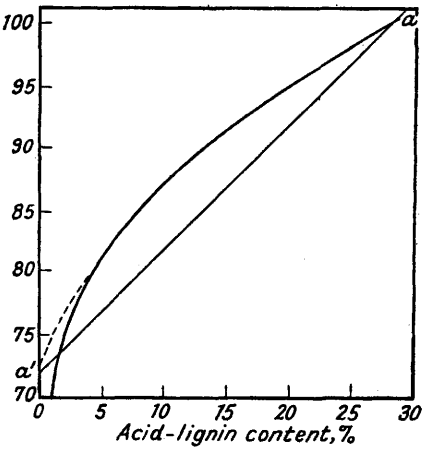


FIG. 5. *Spruce at* 50° *and* 70°.



lignin loss of 19·0% and the sum of holocellulose yield and apparent lignin loss is 105·5%; this was the maximum apparent surplus found. The nature of the apparent surplus material cannot be decided on the basis of these delignification experiments alone, because the possibility that it is " excess of carbohydrate " remains.

To determine the nature of this material large samples of partly delignified wood were prepared from beech and spruce woods and analysed. Table 1 presents the results of these analyses, together with similar determinations on samples of the original woods used throughout this investigation. All results have been calculated as percentages of the oven-dried wood.

Table 1 shows that the loss of acid lignin from the wood residues is accompanied by an increasing loss of recognisable polysaccharides, and that this loss is not confined to any single

	Beech				Spruce				
	Original wood	After delignification at 50° for 46 h.	71 h.	151 h.	Original wood	After delignification at 50° for 40·5 h.	65 h.	142 h.	At 70° for 23 hr.

TABLE 1.

Wood residue	—	92·9	87·4	79·5	—	93·4	87·8	74·5	71·6
Acid-insoluble lignin	24·5	14·0	7·2	1·6	28·3	19·0	10·4	2·2	1·6
Methoxyl in lignin	4·9	2·5	1·2	—	4·3	2·3	1·1	—	—
Methoxyl in wood residue	6·2	4·1	3·1	1·8	4·9	2·8	1·9	1·3	1·1
Lignin-free holocellulose	75·5	78·9	80·2	77·9	71·7	74·4	77·4	72·3	70·0
Cross and Bevan cellulose	58·1	57·6	56·5	54·4	61·2	58·2	57·2	57·2	54·0
Pentosan in Cross and Bevan cellulose	18·0	16·8	16·6	15·8	4·6	4·3	4·2	4·4	3·4
α-Cellulose	44·6	44·0	43·8	41·2	44·0	43·1	43·6	42·7	40·1
Pentosan in α-cellulose	5·4	5·4	4·9	3·4	nil	nil	nil	nil	nil
α-Cellulose (pentosan free)	39·2	38·6	38·9	37·8	44·0	43·1	43·6	42·7	40·1
Total pentosan	27·8	27·0	27·3	25·7	10·7	8·9	8·8	7·7	6·4
Decrease in α-cellulose	—	0·6	0·3	1·4	—	0·9	0·4	1·3	3·9
Decrease in total pentosan	—	0·8	0·5	2·1	—	1·8	1·9	3·0	4·3
Loss in total recognisable polysaccharide	—	1·4	0·8	3·5	—	2·7	2·3	4·3	8·2

TABLE 2. *Yield, %, of reducing sugars as glucose*

(Standard cotton linters, 104·0; filter paper, Whatman, no. 1, 101·0.)

α-Cellulose, that basis	104·6	99·0	102·7	105·1	101·2	103·5	99·8	101·2	—
Cross and Bevan cellulose, that basis	100·0	93·5	96·4	96·9	92·4	94·8	94·6	90·8	—
Wood residue, original-wood basis	68·1	67·4	67·8	63·4	64·6	63·1	63·8	57·8	57·7
Total polysaccharide *	65·5	64·8	65·2	61·0	62·1	60·7	61·3	55·6	55·4
Decrease in total polysaccharide *	—	0·7	0·3	4·5	—	1·4	0·8	6·5	6·7
Decrease in α-cellulose + pentosan	—	1·4	0·8	3·5	—	2·7	2·3	4·3	8·2

* Total polysaccharide content equals total reducing sugars × 100/104.

TABLE 3.

Apparent acid-lignin loss	—	10·5	17·3	22·9	—	9·3	17·9	26·1	26·7
Total polysaccharide loss	—	0·7	0·3	4·5	—	1·4	0·8	6·5	6·7
Total apparent loss	—	11·2	17·6	27·4	—	10·7	18·7	32·6	33·4
Measured weight loss	—	7·1	12·6	20·5	—	6·6	12·2	25·5	28·4
"Modified lignin," by difference	—	4·1	5·0	6·9	—	4·1	6·5	7·1	5·0

Proximate analysis of original woods

	Beech	Spruce		Beech	Spruce
Acid lignin	24·5	28·3	Total components determined	91·8	92·9
Total polysaccharide	65·5	62·1	Undetermined material	8·2	7·1
Alcohol–benzene extractives	1·8	2·5			

polysaccharide fraction. The calculations for spruce wood are complicated by the fact that hexosans, such as mannan, which are soluble in 17·5% sodium hydroxide were not separately determined.

To determine whether, by the action of buffered aqueous sodium chlorite on native lignin, a material is formed which is hydrolysed to reducing sugars, but has not been isolated as a recognisable polysaccharide, the total reducing sugars present in the lignin hydrolysates were estimated, by use of a method involving titration of standard alkaline potassium ferricyanide solution.

If the hydrolysis of polysaccharides proceeded quantitatively pure cellulose would yield 111·1% of glucose and xylan would yield 113·6% of xylose, but under the conditions of hydrolysis employed cellulose (cotton linters) yielded some 104% of reducing sugars. Table 2 shows that α-cellulose from beech, which contained 12% of its own weight of pentosan, gave about 103% of reducing sugars while spruce α-cellulose gave some 102%; it is assumed therefore that the overall method is 94% efficient and, since it was carefully controlled, particularly the hydrolysis, this factor should be common to all the determinations. Any sample, such as Cross and Bevan cellulose, which did not yield about 104% of reducing sugars is considered not to be a pure polysaccharide. Since the yield of reducing sugars from beech α-cellulose, containing a proportion of pentosan, is similar to that from cotton cellulose, it is assumed that little difference exists between the yields of sugars from pentosans and from cellulose. It has been shown that, after pure xylose has been subjected to the hydrolytic process, there is no measurable decrease in the amount of xylose present, as determined by titration. The reaction between reducing sugars and alkaline potassium ferricyanide is not stoicheiometric, and the sugar factors for the standard potassium ferricyanide solution were the same whether glucose or xylose was used for the standardisation. Therefore the total polysaccharide content of a wood hydrolysate can be calculated by multiplying the percentage of reducing sugars obtained from it, and estimated in this way, by 100/104, and not by the theoretical factor 100/111·1. The accuracy of this method is assessed at ±1%; this is considered reasonable for sugar solutions of concentration as low as 0·1%.

Table 2 confirms the previous observation that as delignification proceeds there is a progressive loss, and not a gain, of total polysaccharides. This loss only becomes appreciable when about 95% in the case of beech, and 90% in the case of spruce, of the acid lignin has been removed. The chlorite delignification liquors give reducing reactions indicative of polysaccharides, and Jayme and Hanke (*Cellulosechem.*, 1943, **21**, 127) and Bublitz (*T.A.P.P.I.* 1951, **34**, 427) have reported the isolation of polysaccharides from the chlorite liquors of spruce wood. A comparison between the total polysaccharide loss, as measured by titration, and the sum of the individual losses sustained by the α-cellulose and the pentosan fraction indicates only fair agreement, but this is sufficient to prove that there is no polysaccharide present that is not detected by the traditional methods. Hence the "apparent surplus" detected previously is not a polysaccharide, and must therefore by definition be considered a lignin; the term "modified lignin" is here introduced to describe this material. It is defined as a product which is formed from a portion of the native lignin in wood by the action of sodium chlorite and has thereby been modified or stabilised in a form in which it is no longer recoverable as an insoluble residue by the 72%-sulphuric acid method of determination.

The total polysaccharide contents of beech (65·5%) and spruce (62·1%) are considerably less than those indicated by the formula $100-x$, but for beech the sum of the pentosan-free α-cellulose and the total pentosan is in close agreement with the total polysaccharide value. A similar summation is not possible for spruce in the absence of a separate mannan determination. It would appear that the total polysaccharide content of beech wood, and possibly also of spruce wood, may be determined by calculation from the total reducing sugars in the manner indicated. By using these total polysaccharide values it is now possible to estimate the amount of modified lignin present in the partly delignified samples. This amount is represented by the difference between the sum of the total apparent lignin loss and the total polysaccharide loss, and the actual measured loss.

A summation of the known components of beech and spruce wood is made in Table 3.

This indicates that a significant proportion of the wood is not accounted for in a summation which involves acid lignin, total polysaccharides, and alcohol–benzene extractives. There is little direct evidence of the nature of this missing material (8·2% for beech wood and 7·1% for spruce wood), beyond the fact that it is not a polysaccharide. Unless very extensive demethylation occurs by the action of 72% sulphuric acid on wood, or unless the proportion of methoxylated glucuronic acid residues in the hemicellulose fraction is considerably greater than present evidence suggests, this material must contain methoxyl groups. The acid hydrolysate solutions from lignin determinations on beech and spruce woods are known to exhibit ultra-violet absorption spectra considered to be characteristic of lignin, and also, in the case of beech, to give the well-known Mäule reaction.

It is concluded therefore that at least part of the missing material has lignin-like properties, and it is suggested that use of 72% sulphuric acid is not an effective method for estimating all the non-polysaccharide portion of the wood cell wall (*i.e.* by definition, its lignin content). This view is supported by von Wacek [*Holz als Roh und Werkstoff*, 1951, **9**, (1), 7], whose work on the determination of lignin in the tropical hardwood *Entandrophragma utile* was published while this work was in progress. A proportion of the native lignin was stated to be soluble under the conditions of the lignin determination; and termed, after Jayme, protolignin I.

The nature and isolation of this acid-soluble lignin, which is inferred to be present in the lignin hydrolysates of normal wood, and of modified lignin form the subject of a further paper now in preparation.

Experimental

Samples of both European beech (*Fagus sylvatica*) and Sitka spruce (*Picea sitchensis*) woods were air-dried to a moisture content of about 12%, and then separately converted into sawdust; and the fraction which passed a 20 mesh screen but not a 60 mesh screen was retained as the sample. Proximate analyses were carried out by methods standard in this laboratory; in cases where these methods differ from those in more general use they are fully described.

Delignification Procedure.—The buffered aqueous solution of sodium chlorite (pH 6·3) was prepared by mixing 50 ml. of a solution containing 37 g. of commercial sodium chlorite (85% by weight of sodium chlorite, the remainder being substantially sodium carbonate) in a litre of distilled water with 50 ml. of one containing 19 g. of hydrated disodium hydrogen phosphate ($Na_2HPO_4,12H_2O$) and 100 ml. of N-orthophosphoric acid per litre. The buffered solution, at reaction temperature, was added to 2 g. of sawdust of known moisture content. One series of flasks were maintained in a thermostatically controlled oil-bath at 50° ± 0·25°, the other series at 70° ± 0·25°, and individual flasks were removed at intervals. The wood residues were filtered off on tared alundum crucibles (porosity RA 98) and washed with water till free from chlorite, followed by alcohol and ether, and finally oven-dried to constant weight. The larger preparations were carried out with 2 l. of buffered chlorite solution and 40 g. of wood of known moisture content.

Determination of Lignin Content.—Bamford and Campbell's method (*Biochem. J.*, 1936, **30**, 419) was used.

Determination of Cross and Bevan Cellulose.—The usual method, involving repeated chlorination of a cooled extractive-free moist wood sample, followed by treatment with hot sodium sulphite, was used. Beech and spruce woods required six chlorinations, and the partly delignified samples required three or four chlorinations.

Determination of α-Cellulose.—α-Cellulose was determined by the method described by Schorger (" The Chemistry of Cellulose and Wood," McGraw Hill, New York, 1926, p. 539) involving a cold treatment of Cross and Bevan cellulose with 17·5% wt./wt. sodium hydroxide solution for 30 minutes.

Determination of Total Polysaccharide Content.—The volume of the filtrate and washings from the normal lignin determination was measured and an aliquot neutralised with anhydrous sodium carbonate. This solution, containing 0·1% of reducing sugars, was used to titrate a stirred boiling alkaline solution of potassium ferricyanide containing 10 ml. of 10% aqueous sodium hydroxide, 20 ml. of 1% aqueous potassium ferricyanide, and 150 ml. of distilled water. The course of the reaction was followed potentiometrically by using two platinum electrodes and a polarising current. The approximate titration was determined in a preliminary experiment, and in the accurate determination about 90% of this titre was added at once to the boiling

alkaline solution. Boiling was continued for 2 minutes then the beaker was transferred to the titration apparatus. The remaining few ml. of titrant were added as quickly as possible to the stirred solution. The total titration time should not exceed 4 minutes. This modification of Hagedorn and Jensen's method (" Sugar Analysis," Browne and Zerban, 3rd Edn., Chapman and Hall, London, 1941, p. 872) was suggested by Podlubnaya and Bukharov (*J. Analyt. Chem., U.S.S.R.*, 1948, **3**, 131), but it is not necessary to carry out the titration in an atmosphere of nitrogen as they suggested. Further, the reaction, which is of a normal oxidation–reduction type, could be followed potentiometrically by the use of two platinum electrodes and a polarising current just as efficiently as and more simply than by using the electrodes suggested by Podlubnaya and Bukharov. The glucose and xylose equivalent of 20 ml. of the potassium ferricyanide solution was determined separately for each series of experiments. For any one series these equivalents were the same. It was found that 20 ml. of a 1% solution of AnalaR potassium ferricyanide was equivalent to 0·0235 g. of pure anhydrous glucose ($[\alpha]_D$ +52·3°). A factor of 0·0219 g. has been reported (Browne and Zerban, *op. cit.*, p. 873).

By appropriate calculation the sugar equivalent, usually expressed as glucose, of any unknown hydrolysate was determined and the value converted into total polysaccharide by means of the factor 100/104, as already described.

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