LIGNINSULPHONIC ACIDS FROM BIRCH PERIODATE LIGNIN

A Thesis

by

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Also to my husband for his never-failing encouragement and support, and without whom this work would not have been undertaken.

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GENERAL INTRODUCTION

Of the wood used by the pulp industry, only about 50% is accounted for as the cellulose isolated from the pulping process. The other 50% is dissolved by the cooking liquors which in consequence contain carbohydrates and much lignin. In the past these liquors have been mostly discharged as waste, but nowadays much research is going into methods of recovering these waste materials and using them industrially. A small amount of the recovered lignin finds use in the manufacture of vanillin and some is also used in polymers as an extender, in tanning materials, in adhesives, in oil well drilling muds, as a road binder, and as a briquetting agent - uses which reflect the role of lignin in Nature where it is laid down on the cell walls and provides mechanical support for the plant tissues. Further uses of lignin depend largely on the elucidation of its structure and especially that of the soluble ligninsulphonic acids in the waste liquors.

Originally, it was chiefly softwoods that were used by the pulp industry, but since the coniferous forests have been excessively depleted it is becoming increasingly important today to find successful methods for pulping hardwoods.

Hardwoods possess shorter fibres than softwoods and are thus less suitable for paper-making, although they have found use in admixture with softwoods for the manufacture of newsprint, and also in the manufacture of rayon pulp where fibre length is unimportant. However, it is now realised that certain hardwoods can yield pulps of value to other sectors of the paper industry,

especially when pulped with sodium base liquor. Birch seems to be the best hardwood to use as it yields the strongest paper. It also possesses large amounts of extractables, the type and amount varying with "seasoning". Experiments indicate that from green wood some of these extractables would be useful as detergents, and possibly some would be of use in connection with polymers. These possibilities make the use of birch green wood in pulp attractive.

White birch is very widespread in Canada, growing on fertile slopes and banks of streams and lake shores from the Atlantic to the Pacific, and ranging northward to the Arctic Circle. It normally attains a height of 70-80 feet and a diameter of 2-3 feet; although west of the Rocky Mountains specimens occur 120 feet high and 3-4 feet in diameter. It is a short-lived species which reproduces vigorously. Since it can grow in rich or sandy soils and has spreading roots, it is often found springing up in abundance after forest fires. Thus it is very useful for providing pulpwood fairly quickly from burned forests.

This research is a study of the formation of ligninsulphonic acids from White birch (Betula papyrifera).

HISTORICAL INTRODUCTION

In 1857 Schulze (1) treated wood with potassium chlorate and nitric acid, obtaining a residue which he regarded as the substance named cellulose by Payen (2) and believed to be the substance in the cell wall which determined its structure. The incrusting material which was removed by the oxidising agents he called lignin; but it was Fremy (3) who first differentiated between two substances in the incrusting material. One of these he called "cuticle ligneuse", corresponding more or less to present-day lignin, and the other he called "substance incrustante", roughly corresponding to today's hemicellulose. The definition of lignin has gradually narrowed until it is generally held that pure lignin is a high-molecular, three-dimensional polymer which must not contain any residues which are capable of conversion into sugar. Present-day knowledge indicates that lignin is formed from oxygenated phenylpropane units. This idea is based on the results of many experiments, especially on oxidations, hydrogenations, and ethanolyses.

The formation and properties of lignin have been thoroughly reviewed (4,5,6) and therefore only those aspects pertinent to the present study will be discussed.

The Carbohydrate-Lignin Bond

Since aquatic plants, which are supported by the buoyancy of the water in which they grow, possess little or no lignin, botanists have long regarded lignin as the constituent giving

mechanical support to land plants. Cellulose in the land plant, for example, can be carefully dissolved away leaving a complete framework of lignin (7); and lignin, too, is not present in very young cells. Much work on the distribution of lignin in the wood has been carried out by Lange (8), who has shown that the concentration of lignin is highest in the compound middle lamella, and that it decreases continuously within the cell wall from the middle lamella toward the lumen. Measurements on the dichroism of softwood lignin in ultraviolet light showed that the lignin is deposited to some extent in a preferred direction which might indicate the existence of some type of bond between the lignin and other components of the fibre.

More recently (9) the same investigator has reviewed the morphology of hardwood fibres. In this case lignin seems to be isotropically deposited in the middle lamella, which contains more than 70% of the total, and there is no reason to believe that differences should exist in the general structure of the middle lamella of hard- and soft-woods. The primary wall is highly lignified, while the secondary wall of hardwoods is less lignified than that of softwoods. Of the three layers of the secondary wall the outer layer is strongly lignified while the middle layer seems less lignified for hardwoods than for softwoods. The inner layer of the birchwood secondary wall is stated to be free from lignin. This does not seem to be the case for softwoods.

Since the two chief constituents of wood, carbohydrate and

lignin, can be separated by treatment with certain chemicals, many chemists have supported the "mechanical incrustation theory" of Payen (10). Products isolated by such chemical methods appear to possess the original structure of the plant membranes when examined under the microscope. The idea that lignification is simply the deposition of lignin substances in a cellular framework, which consists essentially of cellulose, has long been held by Freudenberg (7,11,12,13). Such deposition would leave the cellulose structure completely intact, while considerable condensation of the lignin with itself probably occurs simultaneously, giving rise to three-dimensional lignin molecules. The resulting mesh would be so permeated with cellulose that if the cellulose was dissolved the remaining lignin should show the form and morphology of the original cell wall as was achieved by Freudenberg (7).

This concept of lignification weakens one of the chief reasons for believing that a definite bond exists between carbohydrate and lignin, namely, that cellulose cannot be dissolved out of wood with customary solvents for isolated cellulose. The intimate network of lignin would be expected to hinder the diffusion of cellulose in such treatments, so that the cellulose could only be completely dissolved after the removal of lignin.

There is, however, a large volume of evidence supporting the existence of a chemical bond, such as Lange's measurement of the dichroism of the cell-wall lignin (8) and the fact that many chemical reactions undergone by isolated lignin do not occur with wood itself. Evidence both for and against such a

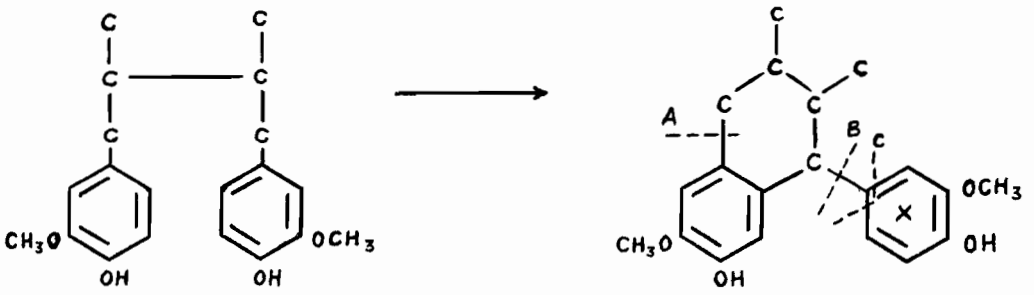
bond has been reviewed (4,5,6) and seems very much in favour. In most cases, both physical and chemical methods of investigation assume such a bond to explain failure to achieve complete separation of the two components. This assumption has always been open to the objection that the failure could equally well be explained by adsorption or by an entanglement of the carbohydrate chains within the solid three-dimensional lignin polymer. However, recently Brownell and West (14) have been able to indicate the existence of such a bond by precipitation of a solution of hydroxyethylated ball-milled wood. Careful fractionation with different solvents failed to give a fraction in which the lignin was completely separated from the carbohydrate. In this case, since both the lignin and the carbohydrate were in solution, the argument for adsorption or entanglement was considerably weaker, and the failure to separate the lignin and carbohydrate therefore strengthened the evidence for a lignin-carbohydrate chemical bond.

At present, emphasis is not on whether such a bond exists, but on which carbohydrate component is linked with the lignin. Much evidence favours a linkage between lignin and hemicellulose, possibly the xylan (15). This problem is an important one, since if such a bond is broken on the isolation of lignin, then one cannot expect isolated lignin to react in a similar manner to lignin in the wood. Therefore results obtained from reactions of isolated lignins can only be cautiously applied to lignin <u>in situ</u>.

Periodate Lignin

The problem of the carbohydrate-lignin bond is not the only one associated with applying results from isolated lignins to lignin *in situ*. It is well known that lignin undergoes considerable change during many isolation procedures, either by interaction with the extracting agent or by condensation by heat or acid. To isolate a lignin that represents as nearly as possible the lignin in the cells, strict attention must be paid to these factors and a procedure used which involves the mildest of conditions. Such a procedure is that of Ritchie and Purves (16) which involves oxidation of the carbohydrates present in woodmeal with periodic acid and solution of the resulting dialdehydes in dilute sodium hydroxide. The treatments are carried out in alternation at room temperature.

That periodate lignin does not undergo much condensation is shown by the low yield (~ 1%) of benzenepolycarboxylic acids on drastic oxidation with alkaline permanganate (17). It is assumed that such acids arise in a manner analogous to their formation



(I) (II)

from lignans (I), such as laricіresinol, under the influence of heat, acids, or alkali, the penta- and 1,2,4,5-tetra-carboxylic acids arising by oxidation of ring X in the cyclised structure (II) at either B or C.

Work on the permanganate oxidation of coal (18,19) suggests that lignin in situ may in fact contain no such condensed units and that these only become evident on isolation. Such an argument is in opposition to the lignification theory already discussed (see p.5) and again supports the idea that normal cellulose solvents are unable to remove that substance from wood because it is linked in a chemical bond with lignin. Bone and Groocock's work (Table 1) shows that a gradual condensation occurs during the geological development of coal, and since lignin is possibly a fundamental substance in coal formation, the above conclusion can be logically inferred. Many lignins isolated

TABLE 1 - Permanganate oxidation of coals.

	Anthracite	Soft coal	Peat
% benzenepolycarboxylic acids formed	50	39-46	10-25

by other methods give large yields of polycarboxylic acids, so obviously periodate lignin is preferable from the point of view of the amount of condensation it undergoes.

Unfortunately, other changes also occur during the preparation. It has been shown (20) that some substituted phenols, possibly related to lignin, are oxidised by periodic

acid under the conditions previously considered specific for $\alpha\beta$-glycols, α-hydroxyketones, $\alpha\beta$-diketones, and certain β-carbonyl compounds (21,22,23). This reaction has been examined in detail by Adler (24). Various substituted guaiacol compounds were treated with sodium periodate and, provided the phenolic group was not substituted, a rapid oxidative demethylation occurred, methanol being liberated in a yield of about 0.9 moles per mole of guaiacol. The probable mechanism of the reaction is shown in Fig. 1.

It is evident that such a reaction could occur on guaiacyl units situated at the end of lignin chains. This may partly account for the colour of the isolated lignin, a light brown, since quinone groups are known to be chromophores; and it also accounts for the observation (25) that periodate lignin contains more carbonyl groups than other isolated lignins. The carbonyl groups are not necessarily all formed in the above manner, as there is also another possibility for degradation by periodate. Since the structure of the side chains in lignin is far from established, some of the units may possess the glycolic or ketonic groupings that are normally oxidised by periodate to carbonyl products. In spite of the belief that periodate lignin is really an oxy-lignin, it seems to be a good model substance for degradative studies of lignin because its degree of condensation is low.

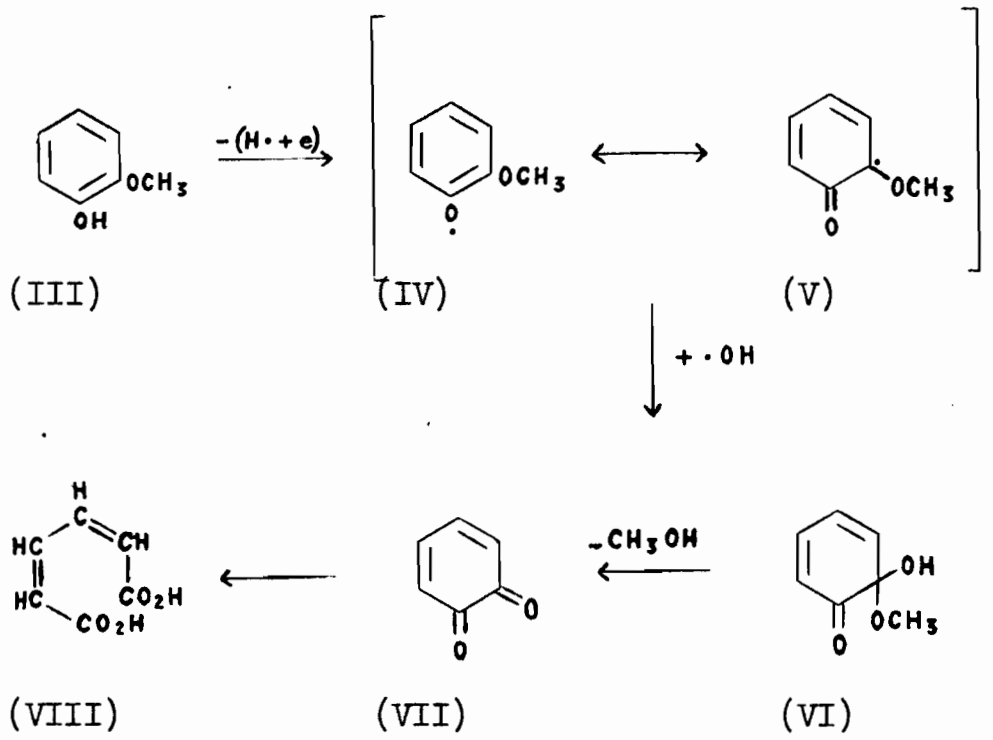
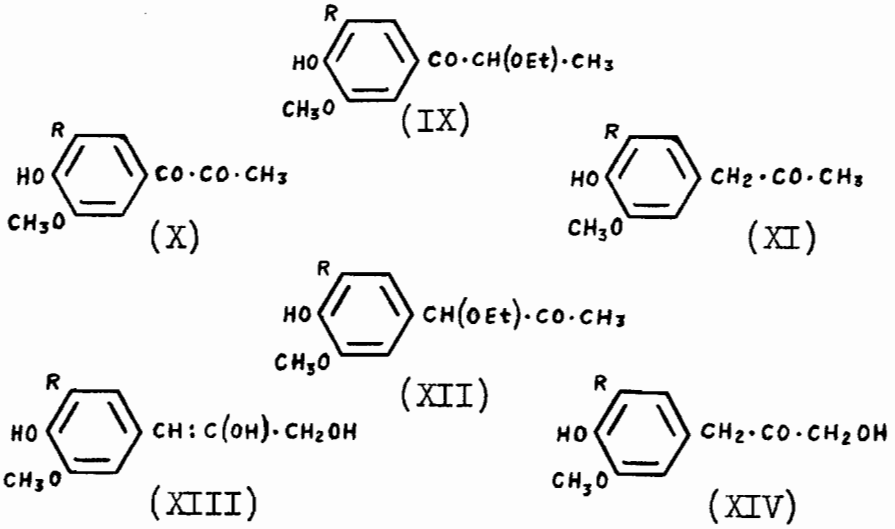


FIG. 1. Mechanism of oxidative demethylation of guaiacyl compounds.

Structure of Lignin Monomers

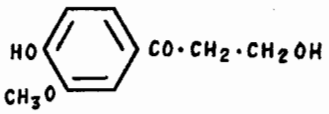
By comparative periodate treatments of various lignin derivatives and model compounds, Ishikawa (26) concluded that the non-benzenoid portion of the lignin molecule contains αβ-glycol and hydroxyketone groupings. However, Lindgren and Saeden (27) obtained no iodine on treatment of the di(toluene-p-sulphonate) of lignin with sodium iodide in acetone, nor were they able to detect any formation of formaldehyde during periodate oxidation. Both should be produced from an α-glycol grouping.

However, there is much evidence for the presence of hydroxyketone groupings in the side chain. Compounds such as the ketones (IX - XIV) isolated from the ethanolysis products of lignin by Hibbert and co-workers (e.g. 28) have been shown to undergo rearrangement very rapidly (29), a property which might explain the variability in position of the ketol group (-COCH(OH)-). Wacek (30) has suggested the probable existence of an "aldol" configuration in the side chain, which would render it a derivative of the coniferyl class postulated by Hibbert. Wacek bases



Where, R = H for softwoods
R = H or OMe for hardwoods

his ideas mainly on results with the sulphonation and alkaline hydrolysis of lignin model compounds. "Aldols" with carbonyl and hydroxyl groups in the 1 and 3 positions are readily converted by loss of water into systems of conjugated double bonds, all of which are readily sulphonatable. Hence the ready exchangeability of the terminal alcoholic hydroxyl in compounds such as (XV). This grouping is of special interest in view of the predominance of 3-hexylpropan-1-ol derivatives in products of



(XV)

hydrogenation under high pressures. The aldol type compound can thus be regarded as a system of conjugated double bonds, one of which is "masked".

The presence of such an aldol grouping is also demonstrated by the action of hot alkali, which is specific for such a grouping in yielding aldehydes by a reverse aldol condensation. Vanillin and acetaldehyde have been isolated from such degradations (31,32); the same decomposition products have also been obtained from coniferyl aldehyde, thus giving support to Freudenberg's idea of coniferyl aldehyde as a structural unit of lignin. The presence of such a unit in lignin is also suggested by certain colour reactions (33).

Cinnamaldehyde or cinnamyl alcohol groupings have been suggested as units in the side chain because of a strong red colour reaction attributed to the formation of p-dimethylamino-

anilides (34), and of studies of the ultraviolet absorption spectra of lignins (35). The amount of such groupings has been estimated as one per fifty phenylpropane monomers. This estimate is supported by a spectroscopic examination of hydrogenated lignin products (36) which indicates 0.03-0.04 cinnamaldehyde units per methoxyl group, and also 0.03 ethylenic groups which presumably are present as cinnamyl alcohol units.

Free hydroxyl groups are found in the lignin molecule, and since isolated native lignin can be methylated to various degrees by different methylating agents, different types of hydroxyl must be present, some phenolic, some aliphatic. There are also small amounts of tertiary hydroxyl groups which cannot be methylated but can be acetylated (37).

Several reactions demonstrate the presence of an hydroxyl group in the α-position to the benzene rings, the most important being oxidation with alkaline nitrobenzene to give phenolic aldehydes. The formation of vanillin from model compounds has been shown to be greatly favoured by the presence of a free phenolic hydroxyl group in the p- position (30) since methylation of such a group considerably decreases the yield of vanillin. Thus the mechanism of formation of vanillin in this case was considered different from that of alkaline hydrolysis where it was found to be independent of the presence of such a free phenolic hydroxyl, and to be governed only by the presence of specific side-chain configurations. However, later workers (38,39) found that veratraldehyde gave quite a lot of vanillin

both on treatment with alkaline nitrobenzene or alkali alone at atmospheric (or higher) pressure. Thus phenolic ethers can also give vanillin. Gierer (40) estimated, by determination of the blue dye formed from such units with quinone monochloroimide, that the p-hydroxybenzyl alcohol unit occurs in spruce lignin at every 7-8 units. Again, if the phenolic and/or the alcoholic hydroxyl group is "blocked" no colour is formed, with quinone monochloroimide, indicating that the same grouping is involved in this reaction as in oxidation to vanillin.

Much work has been carried out on the carbonyl and aldehyde groups in lignin (5,6) and Adler (41) has reviewed the recent attention given by the Swedish school to the quantitative determination of these groups. The presence of at least 0.2 carbonyl groups per methoxyl group has been demonstrated by oximation of Björkman lignin, by infrared studies,and by the increase in hydroxyl content taking place on reduction of the lignin with sodium borohydride, this reagent being used with such mild conditions that only ethylenic and carbonyl groups in the side-chain are reduced and not the aromatic ring.

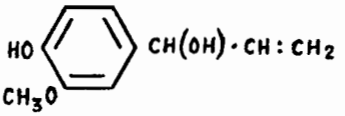
The carbonyl groups in lignin may include (a) aldehyde groups in structures of the coniferyl aldehyde type, (b) keto-groups conjugated with the aromatic ring, and (c) isolated keto-groups (in the β-position of the propane side-chain). By spectrochemical examination of the hydrogenated lignin products using the $\Delta\varepsilon$-method of Aulin-Erdtman (42), lignin was found to contain less than 0.01 phenolic and 0.03 non-phenolic

coniferyl aldehyde groups, and less than 0.01 phenolic and 0.06 non-phenolic aryl-α-ketone groups, making a total of about 0.1 conjugated carbonyl groups per methoxyl, or 50% of the total amount of carbonyl. Therefore, by difference, there must be about 0.1 β-keto groups which could not be made visible by this method.

The $\Delta\varepsilon$-method was developed to determine ionisable phenolic groups from the ultraviolet spectra of spruce lignin preparations. The effect of the phenolic groups on the spectrum was obscured due to the fact that the spectrum derived mainly from completely etherified lignin elements, and in this method the absorption of the non-ionisable elements was eliminated. A $\Delta\varepsilon$- curve was obtained by subtracting, wavelength by wavelength, the "molar" extinction coefficient, ε, (as calculated, for e.g. per OCH_3 group or per aromatic nucleus) of the lignin derivative in acid, neutral, or slightly alkaline solution, from that of the unionized material in a strongly alkaline solution. Provided the two solutions contained the same solvent, the absorption of the non-ionisable chromophores was then practically the same in both cases and was eliminated. The resulting $\Delta\varepsilon$- curve was characteristic of the ionisable (phenolic) elements only and could be used to obtain quantitative data about these elements by comparison with $\Delta\varepsilon$-curves for model phenols. The applicability of the $\Delta\varepsilon$-method is not restricted to ionisation processes. Any modification of the absorption properties of a chromophore by a suitable reaction, e.g.

hydrogenation or dehydrogenation, may give a convenient $\Delta\varepsilon$-curve.

The presence of double bonds in the side-chain has been strongly disputed since normal addition reactions give contradictory results (5,6). The addition of iodine and maleic anhydride to lignin has been explained (43) by assuming the presence in lignin of an isoconiferyl alcohol group with a reversed aldol allyl alcoholic group (XVI). The existence of



(XVI)

aliphatic double bonds, consistent with a coniferyl alcohol structure in lignin, has also been claimed by Kyurshner and Gostomskii (44) and Adler (41) reports the presence of 0.03 ring-conjugated double bonds as a result of his hydrogenation-studies.

Although both carbonyl and ethylenic groups, especially the latter, are only present in small amounts in the lignin molecule, Adler considers that it is important to establish them quantitatively, since they may play an important part in the sulphonation of lignin.

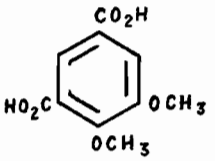
Hägglund (4) states that carboxyl groups are not present in spruce lignin, but small amounts of acidic groups have been reported as a result of potentiometric titrations (e.g.45, 46); these may be largely phenolic hydroxyl groups. However,

bands in the 1720 $cm.^{-1}$ region of the infrared spectra of lignins have been identified as carboxylic CO vibrations (47). Comparisons with the spectra of models indicates that the absorption of the carboxyl groups lies midway between those of aliphatic and aromatic models. On esterification, the interpretation seems to be in favour of the aliphatic type. No definite conclusions can be drawn about the origin and nature of these groups. They are present only in very small amounts as the physical state of the substance has hardly any effect on the band - unlike normal carboxylic acids where the concentration is great enough for association to cause strong effects. Spectroscopic evidence has also been claimed for the existence of ester groups (48).

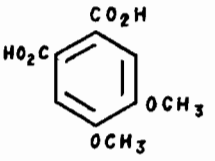
The structure of the side-chain therefore appears to be quite complex, although much evidence favours Freudenberg's view of lignin being made up of β-hydroxyconiferyl aldehyde units. The known ease of rearrangement of the related aldol compounds leads one to wonder whether perhaps the complexity of the side-chains is an artifact of the isolation procedure, and whether in the plant the lignin molecule has a more or less uniform side-chain structure. Overend has commented (49) "Most polymeric natural products such as cellulose, starch, proteins, and fats, are built up according to an orderly pattern, and it is perhaps not out of place to speculate whether lignin also possesses an orderly structure".

On the other hand, the problem of the substituents in the aromatic ring is fairly well settled, a good indication of its form having been obtained from the nature of the early degradation

products obtained from lignin. Isolation of vanillin from both soft- and hard-woods and syringaldehyde from hardwoods showed the position of the methoxyl groups and of the aliphatic side-chain in the molecule, and also indicated the existence of an ether linkage through the phenolic group. For hardwoods the ratic of syringaldehyde to vanillin was normally about 3:1. The isolation of isohemipinic acid (XVII) (50,51,52) after wood or isolated lignin was heated with 70% potassium hydroxide and subsequently methylated and oxidised with permanganate indicated that at least some of the guaiacyl units carry carbon-carbon linked substituents in the 5-position. Freudenberg estimated that about 45-50% of the phenylpropane units are condensed by such linkages (53,54). This particular condensation is not possible for syringyl units, where OCH_3 is in the 5-position.



(XVII)
isohemipinic
acid



(XVIII)
metahemipinic
acid

Richtzenhain (55) studied the same reaction and in addition to isohemipinic acid isolated small amounts of metahemipinic acid (XVIII); hence there are also lignin units in which condensation occurred at the 6-position rather than the 5-position. As such a unit could only be obtained by treatment of isolated lignin, and not of the wood itself, Richtzenhain assumed that

such condensation had occurred during the isolation procedure.

Since the phenylpropane unit has been undoubtedly proved to be the basic one in lignin, the chief interest in lignin now is the question of the mode of combination of these units in the lignin macromolecule.

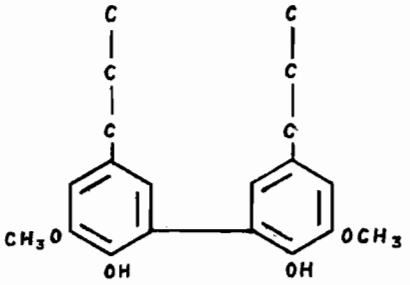
Dimeric Units in Softwood Lignin

Much experimental work indicates the presence of two types of arylpropane units in conifer lignin. One is characterised by an unsubstituted 5-position ("uncondensed" unit) with a free or etherified phenolic hydroxyl group. The other ("condensed" unit) has the 5-position substituted by a carbon atom, either of another aromatic ring (diphenyl structure) or of the side-chain of an adjacent arylpropane unit. The number of "uncondensed" units with a free phenolic hydroxylic group has been determined to be 0.15-0.18 per methoxyl group in lignin (56). This determination depended on the oxidation of the uncondensed units to o-benzoquinone structures with Fremy's salt ($(KSO_3)_2NO$, potassium nitrosodisulphonate)followed by spectrophotometric measurement of the quinone grouping. Since Björkman lignin has been shown by treatment with periodate to contain about 0.30 phenolic hydroxyl groups per methoxyl group, 50-60% of the total phenolic guaiacylpropane units are "uncondensed" and 40-50% are "condensed". These findings can be taken as a basis for the classification of the types of units probably present in lignin.

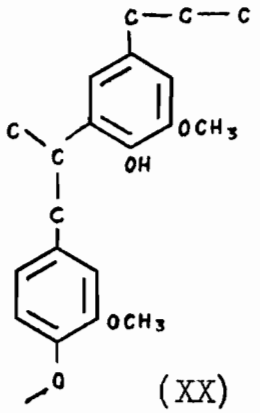
1. Units with free phenolic groups.

The condensed phenolic units may consist of at least two

structural types, namely the 5,5′-diguaiacyl elements (XIX) and the C_6-nucleus-coupled dimeric elements (XX).



(XIX)



(XX)

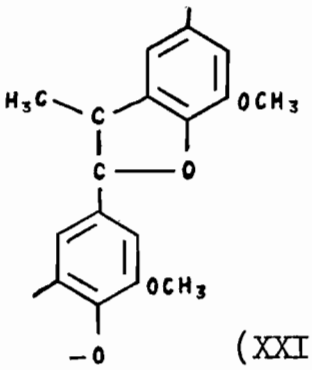
Qualitative evidence has been obtained for both types and quantitative data for (XIX). Aulin-Erdtman's $\Delta\varepsilon$-method (57, 58) showed about 0.06 such groups per methoxyl group in Brauns' native lignin, and a similar value has been determined for Björkman lignin (36). If all the diphenyl elements are assumed to be diphenolic as in (XIX) they would account for 0.12 condensed phenolic units, and only a very small number of units of type (XX), at a maximum 0.02 units, would be present. Recently it has been claimed (59) that coniferous lignin may contain 25% or more of diphenyl-linked units.

Uncondensed free phenolic groups are, in all probability, at the end of lignin chains, and linked to the next unit by means of an ether linkage between their side-chains and the phenolic group of the next unit. Since 15-18% of these units are present, the lignin molecule is possibly highly branched.

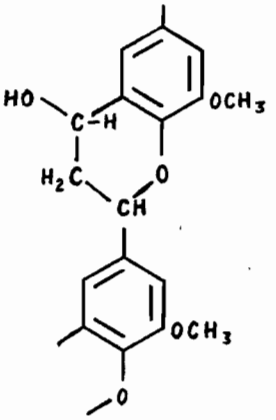
2. Units with etherified phenolic groups.

In the case of condensed units, linkage between the

phenolic group of one unit and the side-chain of the next could occur through one of two of the carbon atoms in the side-chain, the third carbon atom being linked to the 5-position of the aromatic ring. By analogy with other naturally occurring systems which are derived from phenylpropane units, it has been suggested (60) that the benzofuran (or phenylcoumaran) system (XXI) is more probable than the benzopyran system (XXII).



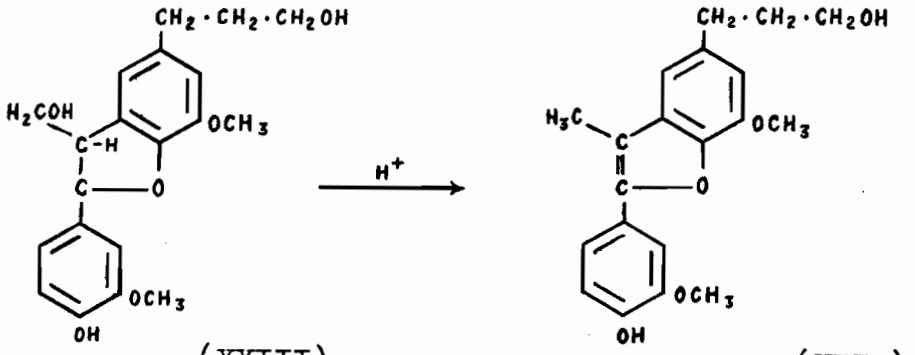
(XXI)



(XXII)

This type of unit (XXI) was first postulated by Freudenberg (61) and its existence is supported by biosynthetic results (62,63) and by the isolation of isohemipinic acid (50,51,52) after treatment of the wood with alkali followed by methylation and oxidation. The criticism (64, 65) that such units may be formed during the alkali treatment by condensation between phenolic nuclei and side-chains led Richtzenhain to suggest that Freudenberg's estimate (53) that about 50% of the phenylpropane units are thus condensed is probably too high. But Freudenberg still claims his value is correct (54).

Quantitative determinations of this type of dimeric unit in lignin have been made (66). Phenylcoumarans (e.g. XXIII) can be converted in about 90% yield into phenylcoumarones (e.g. XXIV) by treatment with 0.2N hydrogen chloride in methanol. Spectroscopic determination of the



(XXIII)
dihydrodehydroconiferyl alcohol

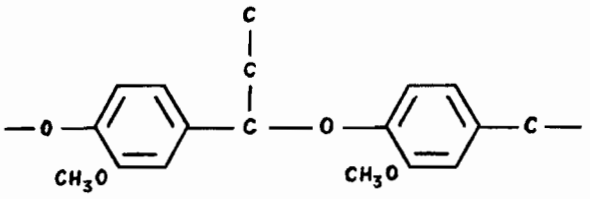
(XXIV)

phenylcoumarone indicated the presence of 0.11 dimeric elements of this type per methoxyl group. Diazomethane methylation reduced the number of phenylcoumarone elements formed on acidolysis by about 0.03 units. This decrease probably represented the amount of open chain dimeric elements (XX), which are also linked in the 5-position through the β-carbon atoms, and which are also converted into phenylcoumarone elements on acidolysis. By difference, the number of phenylcoumaran elements in Björkman lignin was found to be 0.08 per methoxyl group. Since these elements consist of two phenylpropane units they constitute about 16% of the lignin molecule.

In the case of uncondensed units, the ether linkage could be formed between the phenolic group and any one of the three side-chain carbon atoms.

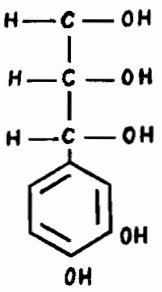
(a) Etherification through the α-carbon atom.

This simple benzyl ether type of linkage (XXV) would account for the ketones (IX-XIV) obtained by Hibbert, and

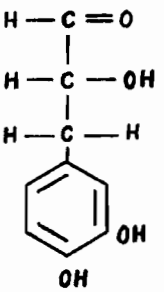


(XXV)

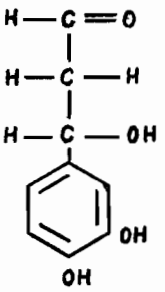
also agrees well with Freudenberg's suggestion (67) that pyrocatechol derivatives (XXVI-XXIX) could give lignin after



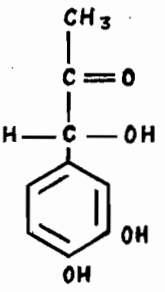
(XXVI)



(XXVII)



(XXVIII)



(XXIX)

condensation and methylation. From a biogenetic point of view these substances are equivalent to the Hibbert compounds. Such a unit, and also several other types, would contribute to the vanillin which lignin yields on oxidation with nitrobenzene.

However, it has not been possible to demonstrate the presence of any appreciable amounts of non-cyclic benzyl aryl

ether groups in lignin (41). This negative result is of special interest because Freudenberg (68) found that trimeric and oligomeric products containing benzyl aryl ether groups were formed in the oxidative polymerisation of coniferyl alcohol, which he regarded as the biogenetic route to lignin.

(b) Etherification through the β-carbon atom.

Such is the arylglycerol β-aryl ether linkage first predicted by Erdtman (69) and supported by the isolation of guaiacylglycerol β-coniferyl ether in Freudenberg's biosynthetic mixture (70).

Model compounds of this type were found to be hydrolysed by alcoholic or aqueous acid to products among which were ketones similar to or identical with the Hibbert ketones, containing a terminal C-methyl group in the side-chain. Kuhn-Roth determinations gave no evidence for the presence of C-methyl groups in lignin itself. When Björkman lignin was heated for several hours with 0.2 N hydrogen chloride in dioxan-water, about 0.3 phenolic hydroxyl groups were liberated together with a similar number of C-methyl groups. This result indicated that about 25% of all guaiacylpropane units in lignin were arylglycerol units which were connected by a β-aryl ether linkage to the adjacent monomer, and therefore up to 50% of the lignin may consist of dimeric elements of this type (71).

(c) Etherification through the γ-carbon atom.

This possibility seems to have been discounted. Such a

structure has not been detected in lignin and it seems very improbable that it occurs (72).

There is one other type of grouping to be considered. This is the lignan-type which could have the phenolic groups either free or etherified. The existence of this was suggested by Richtzenhain's isolation (55) of metahemipinic acid from the oxidised products of methylated wood and lignin, and also by the isolation of DL-pinoresinol from Freudenberg's biosynthetic mixture (73). The cyclisation of lignans to tetrahydro-naphthalene derivatives and the possible analogy in isolated lignins has already been mentioned (p. 7). Oxidation of (II) at point A would explain the formation of metahemipinic acid. On the basis of the yields of metahemipinic acid obtained, Richtzenhain estimated the amount of lignan or isolignan groups to be only about 5% of all lignin units.

Although the various dimeric elements may "overlap" to some extent, one or both of the two phenylpropane moieties of a dimeric element also forming part of a following one, the above quantitative results, obtained chiefly by the Swedish school, almost completely account for the structural elements of the average lignin molecule (see Fig. 2). Absolute proof of the existence of these dimeric units, however, still awaits their isolation from lignin degradation products.

Dimeric Units in Hardwood Lignin

No quantitative examination of the type made by Adler on a softwood has been carried out on a hardwood lignin. Present

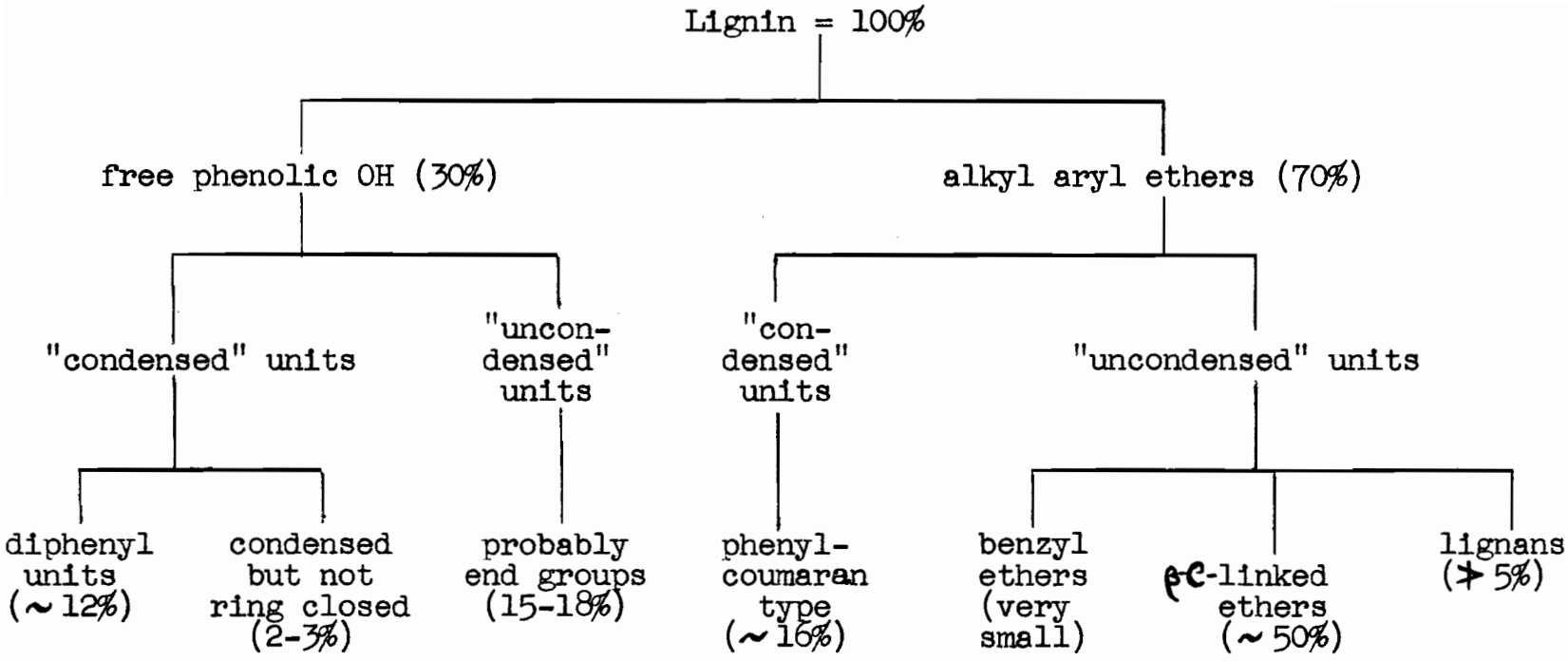


FIG. 2. Distribution of various types of phenylpropane units in softwood lignin.

knowledge indicates that the only difference between monomeric softwood and hardwood lignin units is the occurrence of an extra methoxyl group in some of the latter. It is possible that the side-chain could also vary somewhat in hardwoods but there is no evidence on this point. On account of the extra methoxyl group in the 5-position, condensed dimeric units of the type (XIX), (XX), and (XXI) are not possible for syringyl units - unless their formation involves demethylation, in which case they will in effect be dimeric softwood units. All other types of dimeric units considered for softwood lignin would seem equally possible for hardwood lignin.

Heterogeneity of Lignin

By analogy with other high-molecular weight, naturally-occurring or synthetic compounds, it is possible that lignin is a mixture (74). Many natural polymeric compounds are known but none in a strictly homogeneous form. They may differ in molecular size, in molecular shape, or in chemical composition, such as straight-chain amylose and branched-chain amylopectin in starch; such variations having a marked influence on the properties of the material. A pre-requisite for their study is normally an investigation of their degree of homogeneity, so that any reactions they undergo can be correctly assigned either to a uniform chemical type or to a mixture.

It therefore seems very surprising that only about 1940 were such studies seriously commenced on lignin. By this time

the reactions of lignin had yielded a great amount of information, the utility of which might be questionable if lignin was not a homogeneous substance. Unfortunately lignin in situ is insoluble in all chemically inert liquids and no way of examining its homogeneity has been found. Therefore such studies have been made on soluble isolated lignin, but the solubility of such lignin almost certainly implies that it has been chemically changed during isolation.

A fractionation of formic acid spruce lignin from dioxan by means of water (75) separated four fractions with differing solubilities and methoxyl content, but which showed only small variations in viscosity and none in molecular weight.

Butanol lignin was subjected to a series of extractions and cross-extractions by Bailey (76) who failed to find any definite evidence either for or against heterogeneity. He also attempted fractionation by means of molecular distillation and found that in this case fractions were obtained with methoxyl contents varying from that of the original lignin. Although lignin preparations could be distilled in this way, lignin could not be distilled from wood itself. Hence isolation changes the lignin or lignin-carbohydrate bonds exist in wood. The change in methoxyl content apparently indicated that an extremely complex mixture of compounds was present, and it was felt that the compounds in one preparation may bear little or no relation to those present in another. Similarly, the compounds present in lignin preparations may bear little or no

relation to those present in the wood itself, and such compounds may in fact be formed from the protolignin during isolation. Therefore it is very difficult to draw definite conclusions as to the homogeneity of the protolignin itself from this work.

The same author later (77) extracted wood with butanol and chromatographed the products. His claim of evidence for heterogeneity has been discredited by Hess (78) who felt that the degree of heterogeneity found is not surprising since the preparations were not rigorously purified. However, other isolated lignins (79) have given fractions of varying methoxyl content, and were presumably heterogeneous.

The heterogeneity of isolated "native" spruce lignin was studied in detail by Hess (78), fractionation being carried out by precipitation with benzene from dioxan solution. Fifty-four fractions were obtained,which were recombined to give eleven major fractions exhibiting differences in molecular weight as determined by both osmotic and viscosity methods. Their methoxyl, carbon, and hydrogen contents also showed that they differed in composition, as did their spectra.

Analysis of the ultraviolet absorption spectra indicated that the contributing structural configurations were repeated among the fractions. Although the repeating units leading to higher molecular weights did not appear to be identical, it was thought that the relatively constant band positions observed

in the overall spectra might mean that the basic chromophoric structure was similar, and that differences observed among the fractions were due to differences in substitution on this basic structure.

The infrared spectra also indicated that the fractions contained essentially the same structural elements, and there were no marked changes to be observed in the bands present in unfractionated as compared with fractionated lignin. Although no new bands appeared in the spectra of the fractions, a change in the bands did seem to occur in the carbonyl region.

This work supported a hypothesis developed by Jones (80) who visualised lignin as composed of two or more structurally dissimilar fractions, differences possibly being due to the position of the carbonyl groups. The varying chromophoric property of the group might explain the observed variation in colour - the higher molecular weight fractions being more deeply coloured.

Considerable evidence suggests that hardwood lignin consists of at least two types of lignin. Two fractions were isolated from an extraction of yellow birch with boiling 95% formic acid (81). The methoxyl contents of the fractions led Hibbert to call one the "guaiacyl fractions" and the other the "syringyl fraction". It is felt, however (82), that partial demethylation had occurred in the formic acid.

A little later (83) a fractionation of crude maple ethanol lignin gave a series of fractions which differed from one another

with respect to reduced viscosities, elementary analysis, and methoxyl content.

Using certain procedures in which lignin is isolated by solution from the wood, lignins have been obtained from hardwoods which seem to resemble those from softwoods. Samples of "sound" wood and of the same wood after enzymic decay have been extracted by Nord and Schubert (84) and products obtained which are identical with native spruce lignin (Table 2), those of oak and birch having methoxyl contents of about 14.8% as compared with about 21% for the protolignin. This low methoxyl value is the same as that of the native spruce lignin isolated by Brauns (85). Brauns also found a similar methoxyl content for the native lignin from Western hemlock.

TABLE 2 - Methoxyl contents of native and enzymically-liberated lignins. (Data of Nord and co-workers.)

Wood	% OMe in native lignin	% OMe in enzymically-liberated lignin
White Scots pine	14.85	14.5
Oak	14.8	14.6
Birch	14.9	14.8
Maple	17.4	17.8
Bagasse	15.3	15.4

The low methoxyl values for the lignins from oak and birch were in agreement with the fact that no syringaldehyde was obtained from them on oxidation with nitrobenzene. Nord took this as evidence for the existence of a "guaiacyl hardwood lignin" in these woods of the type proposed by Hibbert (81).

However, later experiments (86) indicated the presence of small amounts of syringaldehyde in the nitrobenzene oxidation products.

The native lignins of Eucalyptus regnans (87), aspen (88), and maple (86) all had higher methoxyl values, as also had the enzymically liberated lignin from maple. Both native and enzymically-liberated maple lignins also gave higher yields of syringaldehyde on oxidation than those from oak and birch, and the infrared spectrum of native aspen lignin was shown (80) to be quite different from that of native spruce lignin. Thus differences in the types of lignin present in hardwoods exist, depending on species. Unfortunately nitrobenzene oxidations on the other hardwood native and enzymically-liberated lignins apparently have not been made.

Nord concluded that the soluble native lignin of coniferous woods may be representative of the total lignin in the wood, but that the native lignin of hardwoods may not. Desmet, as a result of fractionation (89) and electrophoretic (90) experiments, considered that native poplarwood lignin was a highly complicated mixture of closely related substances. There is still controversy, however, between Brauns and Freudenberg as to whether native lignin is really a lignin or should be classified as part of the soluble resins in wood (91).

Aaltio and Roschier (92) found evidence for two types of lignin in aspenwood when they repeatedly extracted it with a

neutral buffered mixture of butanol and water under pressure at 158°C. Analyses showed that there was a constant ratio between the amounts of lignin and pentosans dissolved. In cooks 2-5 this ratio was about 0.85 and in cooks 7-9 about 0.64. The change in the ratio was comparatively rapid and occurred at the sixth cook. With the change in this ratio, there was also a decrease in the methoxyl content of the lignin from 20-21% to about 15%. Hence a syringyl or mixed syringyl and guaiacyl type lignin might have been dissolved preferentially in this solvent system, leaving a purely guaiacyl type to be extracted after the other kind was completely removed. Oxidations of these fractions with alkaline nitrobenzene might have been very interesting and would have thrown light on this point. The authors assumed the existence in aspenwood of two different complexes between pentosans and lignin, the character of the lignin differing in the complexes.

Simultaneously with the change in the lignin:pentosan ratio and in the methoxyl content of the lignin, a distinct defibration of the wood was observed. Thus the compound middle lamella dissolved at this stage, and a connection between the complexes and the cell anatomy was indicated. The complex of ratio 0.85 thus belonged to the middle lamella, dissolving during the first cooks, and the complex of ratio 0.64 was a constituent of the secondary lamella. Thus the lignin of the middle lamella possibly consisted of a syringyl or mixed syringyl-guaiacyl type lignin and that of the secondary lamella of a purely guaiacyl

type lignin. This conclusion seems hard to reconcile with Lange's observation that there was no reason to believe that differences exist in the general structure of the middle lamella of hard- and soft-woods. Logically the above observations imply that in softwoods the middle lamella should not be so strongly lignified since softwood lignin does not possess syringyl units.

The fact that considerable quantities of cellulose were dissolved by the neutral butanol-water at the same time as the complex of ratio 0.85 again suggested the possibility of a carbohydrate-lignin bond, possibly one involving the pure guaiacyl-type lignin. This idea agrees with the greater ease with which lignin may be removed from aspenwood than from sprucewood using dioxan-water for extraction (93). In the case of aspen, an inflexion appeared in the rate of lignin removal which was not shown by spruce. The more rapidly extracted two-thirds of aspen lignin contained a greater amount of syringyl-type units than the more slowly released remainder, which might have been a purely guaiacyl-type lignin bonded to some other wood constituent.

Aaltio and Roschier attributed the divergent result of the first cook (lignin : pentosan, 1.35) to the presence of a "free" lignin in the middle lamella. They assumed that this part is easily soluble during the first cooking thus changing the ratio in favour of the lignin.

Further evidence for two types of lignin has recently

been presented (94). On isolation of aspenwood lignin by extraction with hydrochloric acid of varying concentrations and dioxan:water (9:1) as solvent, it was found that about 75% was easily recoverable at lower acid concentrations, but that higher initial acid concentrations were required for the remainder. As in Aaltio and Roschier's work the easily recoverable lignin was thought to come from the middle lamella and on oxidation with alkaline nitrobenzene yielded syringaldehyde and vanillin in a ratio of 2.5:1. The remainder of the lignin, suggested as being cell-wall lignin, yielded the aldehydes in a ratio of 1:1. A difference in the two types of lignin isolated was also shown by the infrared spectra. Thus while there would seem to be at least two types of lignin, they are not differentiated simply as one being composed only of guaiacyl units and one of either only syringyl or a mixture of syringyl and guaiacyl. More likely, they are both formed of mixed units, with the ratio of these units, and presumably their distribution, differing.

No fraction has been isolated composed only of syringyl units, and such a fraction seems unlikely in view of Freudenberg's finding (95) that whereas a lignin-like product can be obtained by biosynthesis from an equimolar mixture of coniferyl alcohol and sinapyl alcohol, little or no product results from such a treatment of sinapyl alcohol alone.

The existence of at least two different types of lignin in hardwoods, differing in the ratio of syringyl to guaiacyl

units, thus seems well-founded, but caution is still needed in interpreting the results of degradation studies since the heterogeneity exhibited by isolated spruce-wood lignin with respect to molecular weight, etc. may also apply to the main types of isolated hardwood lignin. Also heterogeneity exhibited by isolated lignins need not necessarily be shown by lignin in situ.

Sulphonation of Softwood Lignin

This reaction, the basic one in the sulphite methods for the preparation of cellulose in the pulp and paper industry, has been thoroughly reviewed (4,5,6,96,97,98). It was first recognised by Tilghman (99) when he discovered that lignin could be extracted from wood by hot aqueous sulphurous acid or its salts, preferably the latter since they neutralised the acids produced during the cook. Both Eckman and Mitscherlich (100) also produced pulps by the sulphite process at about this time, but not until nearly thirty years later was it recognised that lignin reacted with the sulphite to form a soluble ligninsulphonic acid (101). Although the bisulphite ion is usually considered to be the entity that sulphonates the lignin, recently it has been suggested that sulphur dioxide is the sulphonating agent (102).

The mechanism of formation of ligninsulphonic acids from sprucewood was investigated by Hägglund (103), by Maass (104), and by Freudenberg (105). Hägglund cooked sprucewood with

sodium bisulphite liquor and concluded that a two-stage process was involved because sulphonated wood could be withdrawn from the cooking liquor, and the insoluble lignin-sulphonic acids could be extracted in a subsequent, separate operation by an acidic solution, even though this contained no sulphite. He considered that in the first stage the lignin was rapidly sulphonated to give a "solid ligninsulphonic acid", while in the second stage the solid ligninsulphonic acid was rendered soluble by hydrolysis. Whether the insolubility of the stage I acid was the result of a chemical linkage between the lignin and other wood constituents, or was due to a high degree of polymerisation of the lignin molecule, was not known. It has also been suggested (106) that the insolubility was due to the great buffer capacity of the strong bisulphite solution which prevented the development of the acidity which was necessary for the second, hydrolytic stage of the cook.

Kullgren (107) provided further support for Hägglund's two-stage theory when he found that sulphonated wood from a calcium base cook could be dissolved by heating it in distilled water if the calcium ions were exchanged with dilute acids to give the free stage I acid.

Hägglund (108) assumed that in delignification the first stage was the more rapid and that consequently the rate of delignification was determined by the rate of hydrolysis. Maass (104) found that the rate of delignification was dependent upon the product of the hydrogen ion concentration and the

bisulphite ions, and that sulphonation was probably the dominant rate-determining reaction. It was later shown (109) that the pH of the reaction governed which stage was rate-determining.

According to Maass, lignin may be dissolved in two ways: firstly, the introduction of sulphonic acid groups may confer hydrophilic properties on lignin, thus allowing it to be more easily peptised in a medium of suitable ionic concentration. The second possibility is that depolymerisation or rupture of the bonds between lignin building units may occur, the greater the degree of depolymerisation or sulphonation, the more rapidly would the lignin be peptised. Delignification approximated to a first order kinetic reaction, and from the slope of the linear part of a plot of the logarithm of the lignin content of the starting material as a function of reaction time, the activation energy for delignification of sprucewood was determined as 20,200 cal./gm. mole. Support for the peptisation theory of sulphonation has also been obtained from the sulphonation of periodate lignin (110), since a correlation between the fractional rate of solution and $[\eta]$ was observed for fourteen fractions obtained when periodate lignin was sulphonated in stages by a series of short bisulphite cooks.

Arguments for both one-stage and two-stage processes have been presented (111) for the delignification reactions of

technical sulphite cooks.

It was also found by Hägglund (112) that hydrochloric acid spruce lignin was completely dissolved within ten hours in a "normal" bisulphite cook at 135°; but when this lignin was sulphonated at pH 5-6 the major part of the lignin remained undissolved but could be dissolved on heating with acidic solutions as in the case of sprucewood itself. Since the hydrochloric acid lignin was free of carbohydrates the dissolution of the solid ligninsulphonic acid must have been due to cleavage of the lignin molecule. The rate of dissolution of the sprucewood in this two-stage process was much lower than that of the stage I acid from the hydrochloric acid lignin; and while the latter went quantitatively into solution the former never dissolved completely.

In competition with sulphonation, lignin heated to high temperatures at low pH condenses to products of high molecular weight which cannot be sulphonated further and therefore any such lignin is not dissolved.

The work of Cabott (97) and of Brickman (98) led to a milder method of cooking which did not cause too much autocondensation of the lignin and gave a completely soluble ligninsulphonic acid. Periodate lignin was heated in 9% aqueous sodium bisulphite solution at 100° for 72 hours, the insoluble sodium salt (in about 80% yield) was isolated and the sodium replaced with hydrogen by means of an acid wash.

The insoluble ligninsulphonic acid so obtained was digested in distilled water at 135° for two hours at a concentration of about 2%, which was sufficient to give a pH just above 2. The complete solubility of the solid stage I ligninsulphonic acid during hydrolysis was taken by Cabott as direct experimental support for the two-stage theory of Hägglund and Kullgren.

Smith (113) modified this two-stage reaction still further. He found that a soluble stage II ligninsulphonic acid could be obtained by carrying out both stages at steam-bath temperature. Sulphonation for 20 hours, followed by either cooking the salt of the solid stage I acid thus formed in a buffer solution at pH2, or by liberating the free acid by an acid wash and autohydrolysing it at 2% solids concentration for 12-15 hours, caused the solution of about 90% of the lignin. He greatly simplified the experimental procedure when he discovered that the sealed tubes used by the earlier workers were unnecessary and that a more convenient cook at atmospheric pressure could be used with a stronger sodium bisulphite solution (16% instead of 9%) so that it still acted as a buffer at pH5.2 over the period of the cook despite loss of some sulphur dioxide.

A significantly slower rate of delignification in all types of sulphite cook has been observed (114) in cooking at low temperatures, especially below 120°. This retardation may be due to a difference in mechanism, but the possibility

of a physical difference must not be overlooked, since it is known that lignin does undergo physical changes at about this temperature.

Studies on the adhesive and thermoplastic properties of various types of lignin (115) have shown that lignin bonds to itself and other substances when heated, and that the presence of water lowers the bonding temperature to about 100°. This behaviour is thought to be due to the inherent polyelectrolyte nature of lignin, and the presumption is that any chemical process which produces ionisable hydrophilic groups will also enhance the polyelectrolyte properties of the lignin. If the process is sufficiently hydrophilic, the lignin becomes soluble. If degradation is small the lignin may remain insoluble as a water-swollen polyelectrolyte gel with an adhesive capacity. If this softening in water occurs at about 100°, then in sulphonation by aqueous sodium bisulphite the lignin will be in this semi-molten state both in the middle lamella and between the fibrils of the secondary wall. Therefore the softening of lignin may play an important role in sulphite pulping, as has been suggested by Björkman and Person (116).

For many years sulphonation has been assumed to occur in the side-chain of the phenylpropane units. This view arose because no ligninsulphonic acids have ever been degraded to compounds containing the sulphonic group in the aromatic ring, in spite of the known stability of aromatic sulphonic acids.

There is, however, no definite evidence for the isolation of aliphatic fragments bearing sulphonic acid groups. Andrus (117) to be sure, has claimed the isolation of the salt of a sulphonic acid to which he has attributed the structure calcium 1-(4-hydroxy-3-methoxyphenyl)prop-2-ene-1-sulphonate. He synthesised this substance and found it to be similar to the compound he isolated. This result is in keeping with most theories, based mainly on the sulphonation of model compounds, which assume that sulphonation occurs at the carbon atom alpha to the aromatic ring (e.g. 118,119,120); but Adler (121) has suggested that the α-hydroxyl group may not be as important as originally thought.

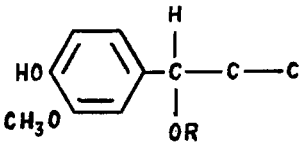
On considering the possibly complex nature of the side-chains of the phenylpropane units, it is not surprising that the actual mechanism of sulphonation is still far from being understood. Some of the theories put forward were reviewed by Brickman (98). Sulphonation could, and probably does, occur in several different ways; either by replacement of hydroxyl groups or by addition to carbonyl or ethylenic groups. That there are at least two types of sulphonic acid groups combined with lignin was shown by Mason and co-workers (122) by means of exchange reactions with ^{35}S.

The sulphonatable groups in lignin were classified by Erdtman and by Hägglund (69,108,123) as "A-groups" which can be sulphonated in acidic, neutral, or weakly alkaline media,

and "B-groups" which can be sulphonated only in acid sulphite solutions. Later (124) "A-groups" were divided into "X-groups" which are very rapidly sulphonated and "Z-groups" which are more slowly sulphonated in neutral sulphite solution. Possible models for these groups were reviewed by Lindgren (125) and will be merely summarised here.

X-groups. These are probably alkyl p-hydroxybenzyl ether groups or p-hydroxybenzyl alcohol groups, since the only model substances which react as rapidly as the X-groups with neutral sulphite solutions are phenol-activated benzyl alcohols (e.g. vanillyl alcohol, o-vanillyl alcohol) or phenol-activated benzyl ethers (e.g. pinoresinol).

i.e. X-groups are of the type

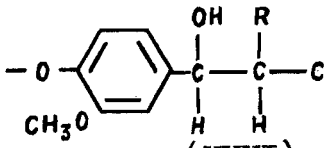


R = H or alkyl

(XXX)

Mikawa (126) has found about 0.12 X-groups per methoxyl group.

Z-groups. Model experiments indicate these to be of the type:-



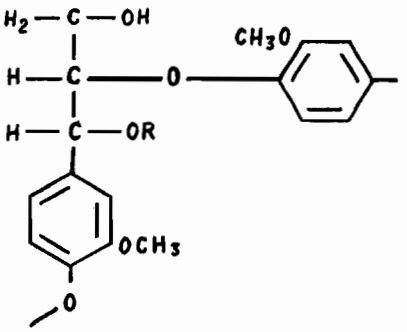
R is a small substituent

(XXXI)

When the benzyl alcohol group is blocked the appropriate substance is sulphonated too slowly to be a model for a Z-group.

Mikawa (127) found 0.12 Z-groups per methoxyl group.

B-groups. Sulphonation of model compounds indicates that these may be alkoxybenzyl ether groupings. These are probably the groups assumed by Holmberg (128) to link the lignin units to carbohydrate molecules or to other lignin units. Leopold (129) has shown that B-groups on treatment with acids are hydrolysed to B′-groups which, unlike the B-groups themselves, can be sulphonated by neutral sulphite solutions. Holmberg's theory that the sulphonatable groups are benzyl alcohols or benzyl ethers has been so supported by work on model compounds that it is now more or less accepted. According to Lindgren (125) this theory leads to the recognition of B′-groups as benzyl alcohols. The close analogy that exists between the sulphonation of the β-guaiacyl ether of veratryl glycerol and the B′-groups favours the claim that a large fraction of lignin is built up of arylglycerol derivatives (see p. 24). B and B′ groups are thus of the type:-



B R=alkyl

B′ R=H

(XXXII)

Mikawa (130) postulates that the total amount of sulphonatable groups in spruce lignin is about 0.6 per methoxyl

group. By reactions with thioglycollic acid 0.3-0.35 per methoxyl group are shown to be B-groups.

Sulphonation of Hardwood Lignin

Although many papers have been published on the delignification of hardwoods by the bisulphite process, in general most of them deal with technical rather than chemical aspects. This presumably is due to the practical experience that hardwoods cook as well or more easily than softwoods (e.g. 131,132).

The first sulphite cook of a hardwood seems to have been made by Heuser (133) who obtained a low yield of a badly burned pulp from beech. A comprehensive study of the cooking of hardwoods was made by Wells and Rue (134) who found that several species, including the birches, could be readily cooked by the sulphite method. Hägglund and Urban (135) showed that the pulping mechanism of beech was similar to that involved in the cooking of spruce.

Most early papers (136,137,138,139,140,141,142) dealing with the effect of operating conditions in the mill on the yield of easily bleachable pulp, stressed the importance of a slow rise in temperature for the hardwood cooks. Since birch was shown to be no less readily penetrated by the cooking liquor than spruce (142), the reason for "shiviness" (i.e. incompletely separated fibre bundles) in many of the pulps obtained was attributed to the inability of the denser hardwoods to hold a large volume of liquor. If insufficient base

was present to neutralise the "wood acids" as well as the sulphonic acids formed, a burnt cook occurred.

A difference in the pulping of various hardwoods was noted by Dorland (131), the rate being equal to or faster than that for spruce, depending on the species. Yorston (96) found a difference between the cooking of birch and aspen, birch requiring longer cooking, stronger liquor, or higher maximum temperatures than aspen to give well-delignified pulps free from shives. The slower cooking of birch he attributed to the higher density of that wood.

Yorston also showed that the rates of delignification of birch and spruce woods were about equal, but as birch contained less lignin than spruce it could be cooked to pulps of a given lignin content in a shorter time. Jensen (143) reported that birchwood cooked faster to the same bleachability than spruce and pine, and this was supported by the work of Björkqvist (144) and of Hägglund (145), the latter finding that birch lignin was dissolved more rapidly than spruce lignin by sulphite solutions of initial pH5, probably because the pH decreased to 3.7 in the experiment with birch but only to 4.3 in the case of spruce. However, Richter and Pancoast (132) showed that the sulphonation of birch with sulphur dioxide solutions at low temperatures was much slower than that of hemlock, spruce, or Douglas fir, in agreement with Kitao (146) who found that in the case

of sulphonation with acid sulphite solutions the sulphonation of hardwood lignin was always slower than that of softwood lignin.

Husband (147) found that for any fixed spent liquor pH value between 4 and 6.5 more lignin could be removed by the application of 15% rather than 5% sulphur dioxide. This result could not be attained by increasing the amount of sulphite or of sulphite:bisulphite mixture because the ratio of sulphite base to the wood acids was increased simultaneously with the increase in the ratio of SO_2 to the wood. When the ratio of sodium base to SO_2 in the applied chemicals was reduced to that in sodium bisulphite the cooking liquors became buffered at about pH 3.4 to 3.5 during the digestion. Under these conditions the removal of lignin was conditioned by the duration of the cook, temperature, and the concentration of the bisulphite. Husband pointed out that sulphonation itself should not affect the pH of the liquor, since each sulphonic group formed from a bisulphite ion binds its own sodium ion. But the acetic acid liberated from the hemicelluloses tended to make the liquor attain a more acid pH, and if insufficient bisulphite was present to neutralise these acids a burnt cook resulted, presumably by condensations due to the acid which rendered the lignin insoluble. The fact that acetyl groups were not present in softwoods explained the difference in the final pH of birch and spruce sulphite liquors observed by Hägglund. In the present study, using an isolated lignin,

there is no possibility for the production of acids in this way and therefore according to Husband's hypothesis the pH of the cook should be the same at the end as at the beginning.

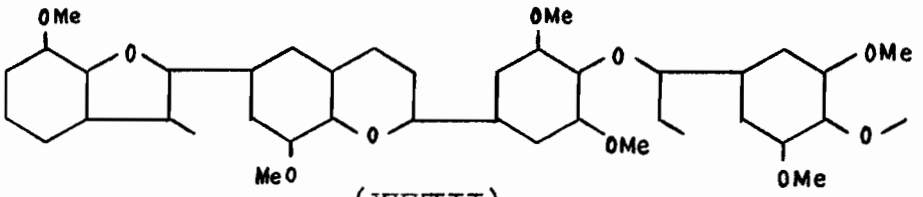
Although hardwoods seem to pulp more easily than softwoods, it was noted (132) that less sulphur was introduced into the hardwoods by sulphonation than into the softwoods. This suggested that hardwood lignin either required less sulphonation to reach the soluble stage, or that the sulphonated lignin was more easily hydrolysed to a soluble form than the coniferous lignins. A low sulphur content was observed in the sulphonated products of birch, the S:OMe ratio of which was 0.1 compared with a ratio of 0.3 for softwoods cooked under the same conditions. As birch contained about 1.5 methoxyl groups per phenylpropane monomer, the above ratio for neutral cooks implied that it contained about 0.15 "A" groups per monomer, or considerably less than the figure for spruce lignin.

Hägglund (145) found that only a small amount of birch lignin was extracted by neutral sulphite solutions and that to dissolve the bulk of the lignin acid solutions had to be used. Since this was also the case for spruce, it was concluded that the bulk of birch lignin is bound by the same alkoxybenzyl alkyl ether linkages as the major part of spruce lignin. However, Hotin Den (148) found that in cooks of Manchurian "Sirakamba" birch the lignin dissolved easily in solutions of low acidity but with difficulty in those of high acidity. Miura and Maeno (149) noted that hardwoods were

digested best by long cooking with a liquor of low total sulphur dioxide content.

A "critical" temperature was observed for hardwood cooks (150) similar to that described for softwoods (see p. 40), a perceptible reaction between sodium sulphite and lignin beginning at about 120° and the rate increasing in geometric progression, doubling for about every 10° increase.

Larsson (151) realised that in the syringyl units no condensation to a coumaran or chroman type of compound could occur as in the case of guaiacyl units, and postulated a structure for hardwood lignin (XXXIII) based on this idea.



(XXXIII)

Assuming that sulphonation of lignin took place by splitting of ether linkages, as postulated by Freudenberg (51), with simultaneous formation of new hydroxyl groups, scission in the hardwood lignin should give a sulphite liquor containing a greater amount of low molecular weight sulphonic acids. Larsson separated the ligninsulphonic acids in the waste liquor of an experimental aspen cook into three fractions by extraction with bis(p-dimethylaminophenylmethane), brucine, or strychnine, and precipitated the acids with ethanol. He found no evidence for the enrichment of the ethanol fraction by syringyl elements as would be expected if the hardwood lignin was a mixture of a

guaiacyl-type and a syringyl-type lignin, and he therefore assumed that aspen lignin was composed of alternating syringyl and guaiacyl building stones in a ratio of approximately 1:1.

However, this result is contradictory to that of Stone (152) who determined the amounts of vanillin and syringaldehyde formed on oxidation of the liquor obtained from a sulphite cook of aspenwood (Fig. 3). He found that in the first 60 minutes only vanillin could be obtained from the liquor on oxidation. Syringaldehyde only appeared after about 90 minutes. Although Stone actually showed the relationship between oxidation products and time, by making use of comparisons between time and temperature given in his paper, it is possible to estimate that no syringaldehyde appeared until a temperature of above 100° was reached. This was taken as evidence for two types of lignin with sulphonation occurring preferentially in the guaiacyl type. Although the abundant evidence for both theories of the structure of hardwood lignin has already been discussed, these results serve to illustrate the problems involved in lignin chemistry, two very conflicting results being obtained from the same species of hardwood, in this case aspen.

Most later work on the sulphonation of hardwoods was primarily aimed at finding technical conditions which gave the maximum brightness, strength, or yield of pulp. No comprehensive investigation of the mechanism of the reaction on an isolated hardwood lignin appears to have been attempted.

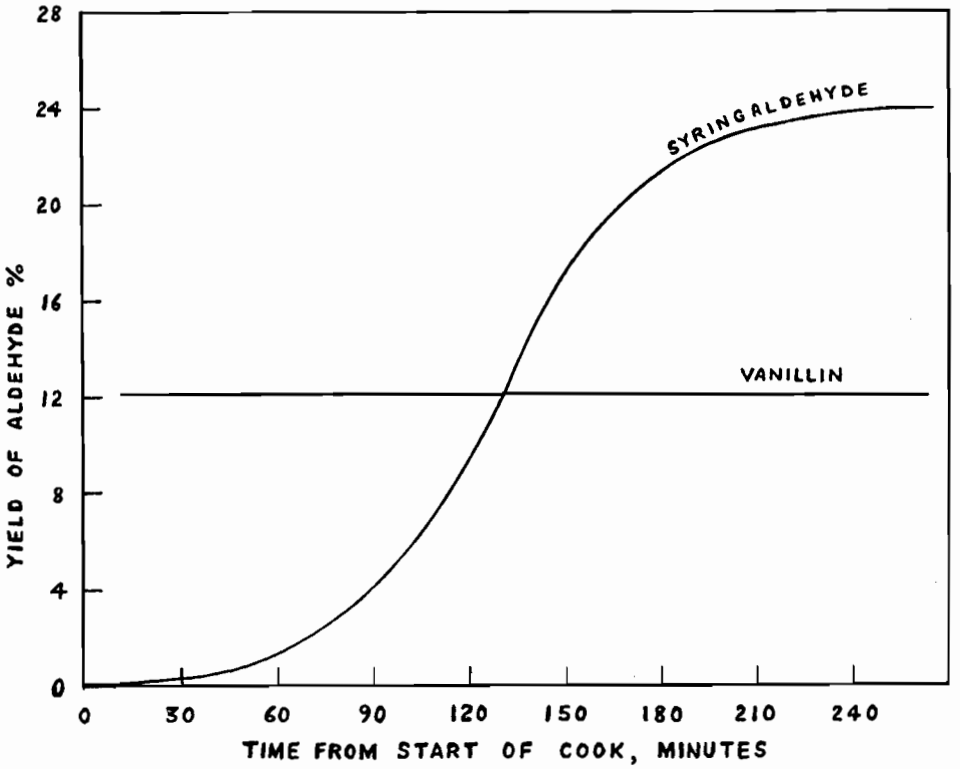


FIG. 3. Aldehyde yield from alkaline nitrobenzene oxidation of liquors from neutral sulphite cook of aspen. (After Stone.)

RESULTS AND DISCUSSION

Preparation of Birch Periodate Lignin

Four preparations of birch periodate lignin were carried out, and their analyses were compared with similar preparations made by other workers (Table 3).

The lower yields obtained in the present work, as compared with those of Ritchie and Purves (16) were attributable to a much larger scale of operation and probably, as suggested by Smith (113), to loss of fines during the syphoning procedure. The periodate lignin was obtained as a light brown, friable substance, retaining much of the morphological structure of wood as reported by Ritchie and Purves. This observation was in contrast to more recent descriptions of spruce periodate lignin as a hard resinous substance showing no wood structure after the final drying. Since the analytical data agreed well, the first two preparations were thoroughly mixed and the average data used in future work.

During the fourth preparation, a study was made of the removal of carbohydrate material from the wood. A plot of the percentage of Klason lignin in the insoluble residue at the end of each oxidation-hydrolysis cycle (Fig. 4) showed that the first three cycles removed large amounts of carbohydrate, but that very little more was removed in further cycles. Ritchie and Purves (16) found a sharp decrease in

TABLE 3. Comparison of birch periodate lignins.

Worker	% Klason lignin in wood	% Yield		Periodate lignin				
		Wood basis	Klason lignin basis	% Klason lignin	% Ash	% Moisture	% OMe	% Hollocellulose
Ritchie and Purves[a]	20.0	21.8	85.4	78.5	1.08	-	21.4	-
Lim Lee[b]	-	-	-	78.5	1.05	2.3	21.5	-
Bradley (1)	20.0	11.2	56.2	86.3	0.51	5.2	19.8	trace
(2)	"	9.7	48.7	86.1	0.63	4.0	19.9	"
(3)	"	11.5	57.5	84.0	0.34	6.7	20.6	"
(4)[c]	"	-	-	83.8	1.08	7.1	21.5	"

a) Ref 16.
b) Personal communication.
c) With the technical assistance of Mr. Shafqut Hussein

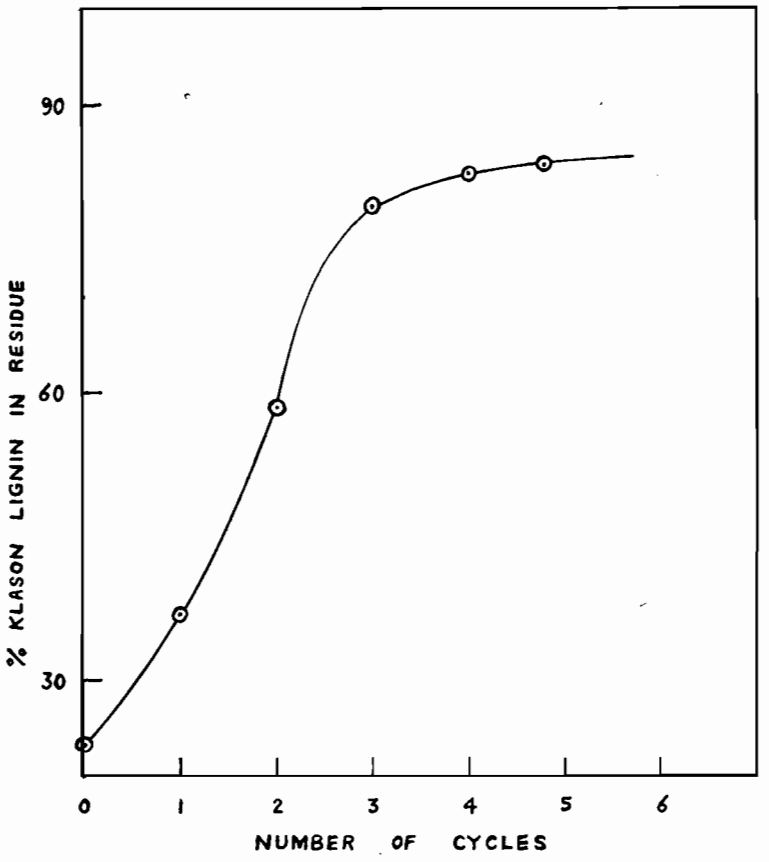


FIG. 4. Preparation of birch periodate lignin.

the amount of periodate consumed by birchwood after the third cycle was complete, and a similar but definitely less pronounced decrease occurred in periodate consumption by sprucewood. The rate of consumption, however, was greater for birch, indicating that the lignin was more readily obtained from this wood. Various workers (143,144,145) had also shown that lignin was more readily removed from birchwood than from sprucewood by the sulphite or the alkaline pulping process (153).

Although it would seem that the extraction of lignin was virtually at an end after five cycles, the percentage of Klason lignin in the preparation (84%) was much less than that (about 97%) in spruce periodate lignin after the same number of cycles. This observation, which was originally made by Ritchie and Purves (16), suggested that more of the hardwood lignin dissolved in the 72% sulphuric acid used in the Klason determination.

Sulphonation and Hydrolysis

Since a survey of the literature indicated that hardwoods pulped in a similar manner to softwoods in the sulphite process, preliminary sulphonations of birch periodate lignin employed the conditions found optimum by Smith (113) for the two-stage sulphonation of spruce lignin. The lignin was heated in 16% aqueous sodium bisulphite at 98° for 50 hours followed by hydrolysis at pH2 and 98° for 20 hours.

The progress of two such runs (Figs. 5,6) was compared (Fig.7)

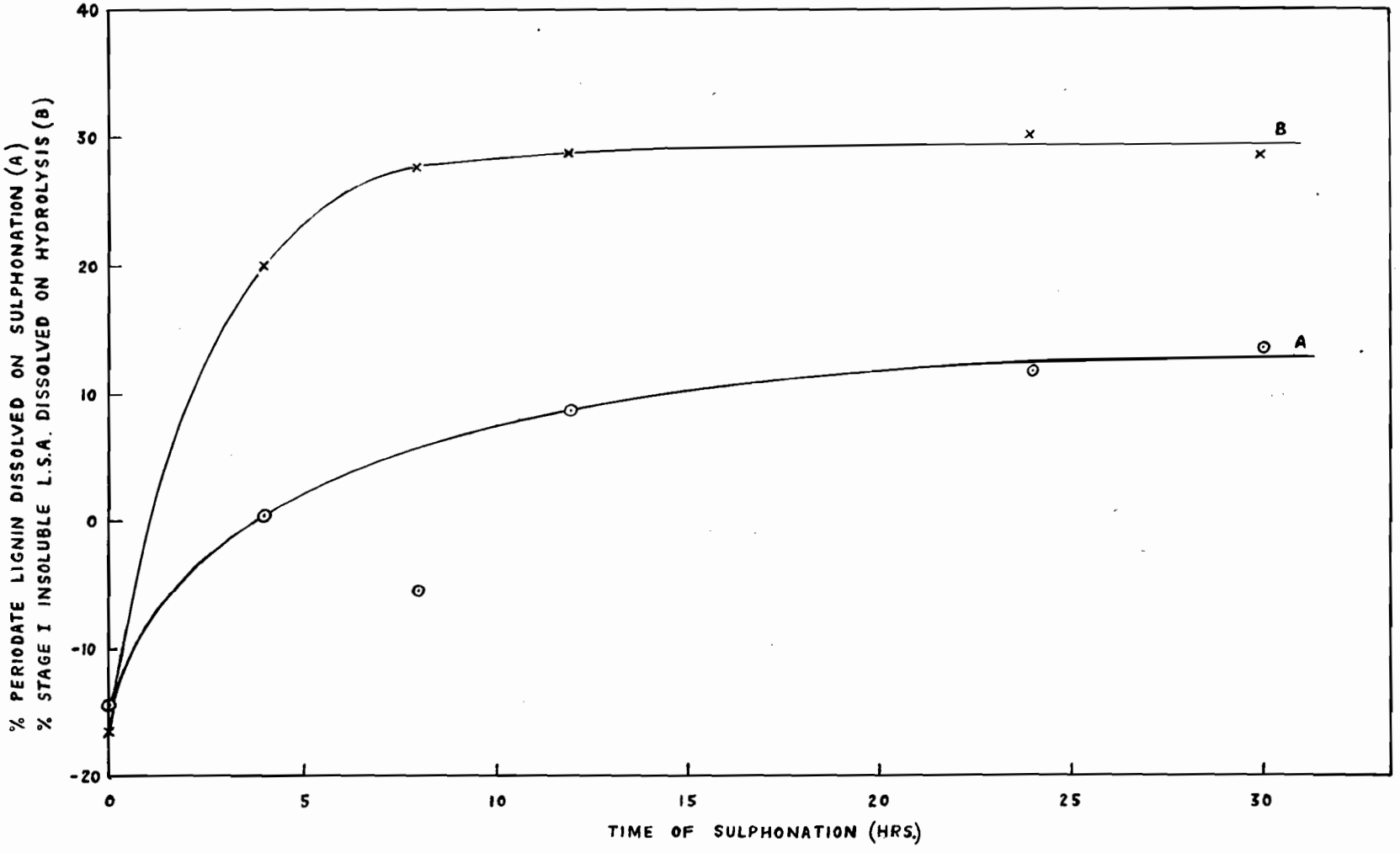


FIG. 5. Trial sulphonation at 96° using Lim Lee's birch periodate lignin.

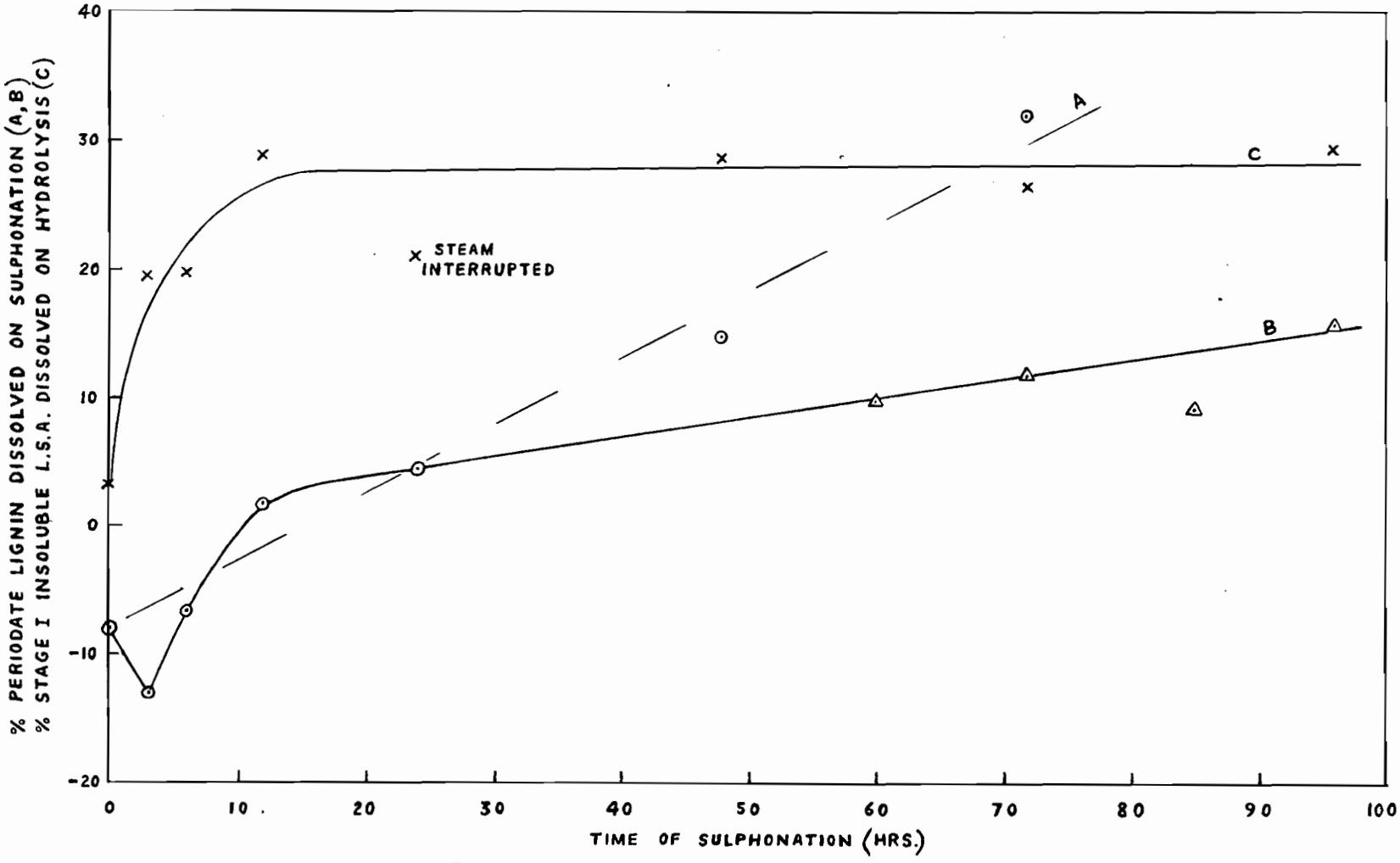


FIG. 6. Sulphonation at 96° using newly-prepared birch periodate lignin. (A - plot using original values; B - plot using values (△) obtained by withdrawing aliquots from a large volume of liquor after longer times.)

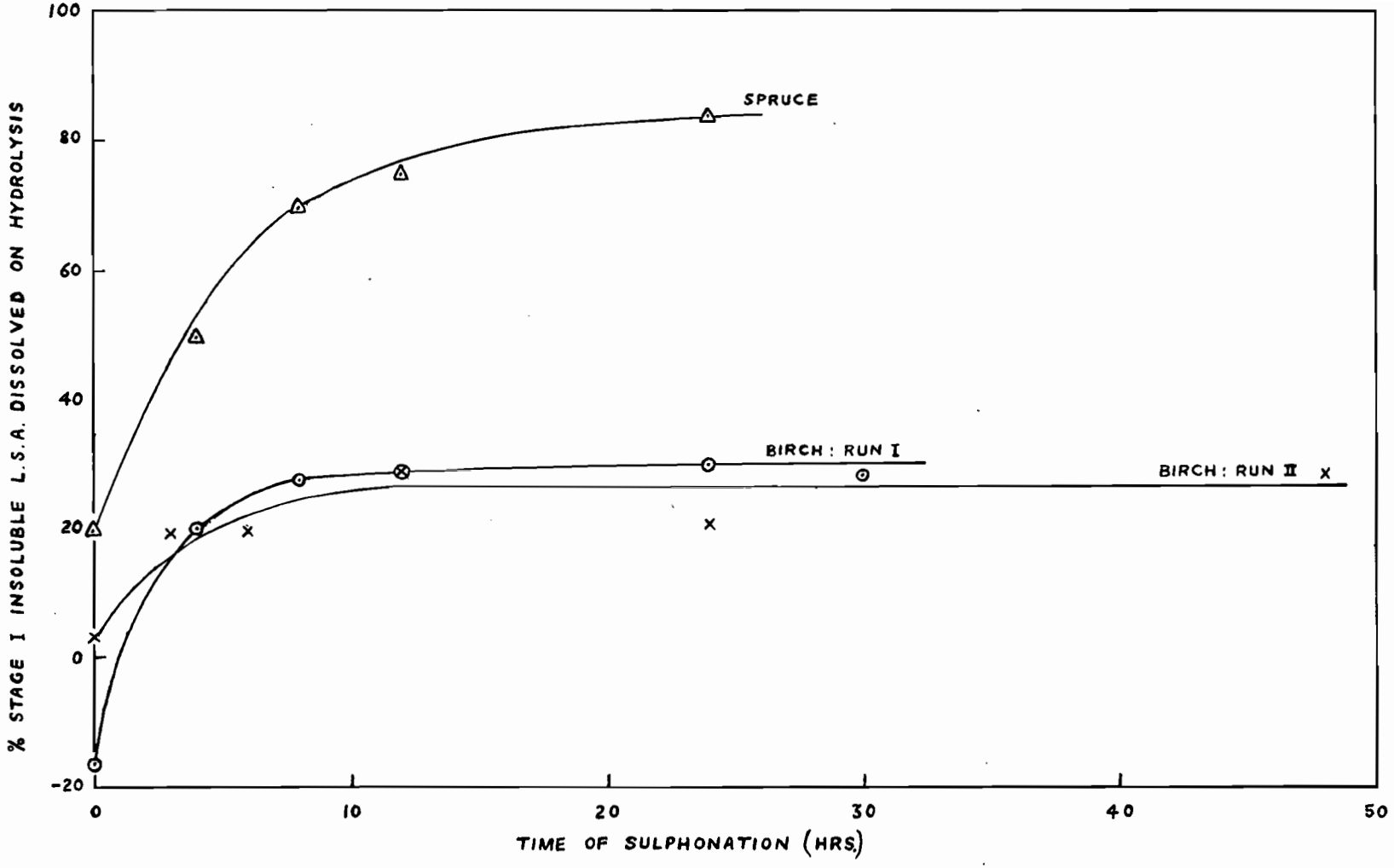


FIG. 7. Comparison of dissolution of birch and spruce periodate lignins using D.M. Smith's conditions.

with the plot of corresponding results for spruce periodate lignin obtained by Smith. In the second run (Fig. 6) the time of sulphonation was extended to see whether more lignin could be dissolved by longer sulphonation. The apparent increase in the solubility after longer sulphonation times might have originated in the inadequacies of the sampling procedure, since aliquots were eventually being withdrawn from only a small amount of liquor. To check this possibility, a cook was carried out in which aliquots were withdrawn only after 60 hours of sulphonation. These determinations showed that the sampling technique was at fault for longer sulphonations, and that the plots could be extrapolated back to zero solubility. Hence the negative values obtained at low sulphonation times perhaps arose from a similar error, although a rapid acquisition of sulphonic acid groups prior to solution would also give a negative value to the percentage dissolved. Cabott (97) found that this was the correct explanation for the negative values initially observed for the corresponding dissolution of spruce periodate lignin.

In any event, the dissolution of birch periodate lignin in the first twelve hours of sulphonation was much greater than that of the product from spruce under the same circumstances, as noted in the case of the woods themselves (see p. 46). As predicted in the introduction (p. 48) the pH (about 5) of the cooking liquor was the same at the end of the cook as at the beginning.

The plots (Fig. 7) for the hydrolyses of birch and spruce stage I ligninsulphonic acids differed greatly. For birch it seemed impossible to dissolve more than about 30% of the stage I acid using Smith's conditions. Under these conditions about 80-85% of the stage I acid from spruce dissolved in 24 hours.

Sulphur determinations carried out during the second cook (Fig. 8) showed that a maximum of just over 2.1% was introduced in the conditions used. Since the corresponding methoxyl content of the birch solid stage I ligninsulphonic acid was 20.1%, the S:OMe ratio was roughly 0.1, the value found by Hägglund (145) when birchwood was sulphonated at pH 4-7. Kitao (154) also found this ratio for both birch and beech when sulphonation was carried out at neutrality. The corresponding ratio for spruce under these conditons was 0.3. If acid sulphite solutions were used (146), a ratio of 0.2 was soon reached for birch and beech as compared with 0.5 for softwoods, and these ratios continued to increase with time without showing the maximum characteristic of the neutral cooks.

It was interesting that the S:OMe ratio was the same for the periodate lignin as for the wood, as it was known that considerable oxidative demethylation occurred in the preparation of the periodate lignin. That the same ratio was obtained seemed to indicate that with removal of each methoxyl group one site of sulphonation was also destroyed, perhaps because the oxidation removed sulphonatable guaiacyl end-groups.

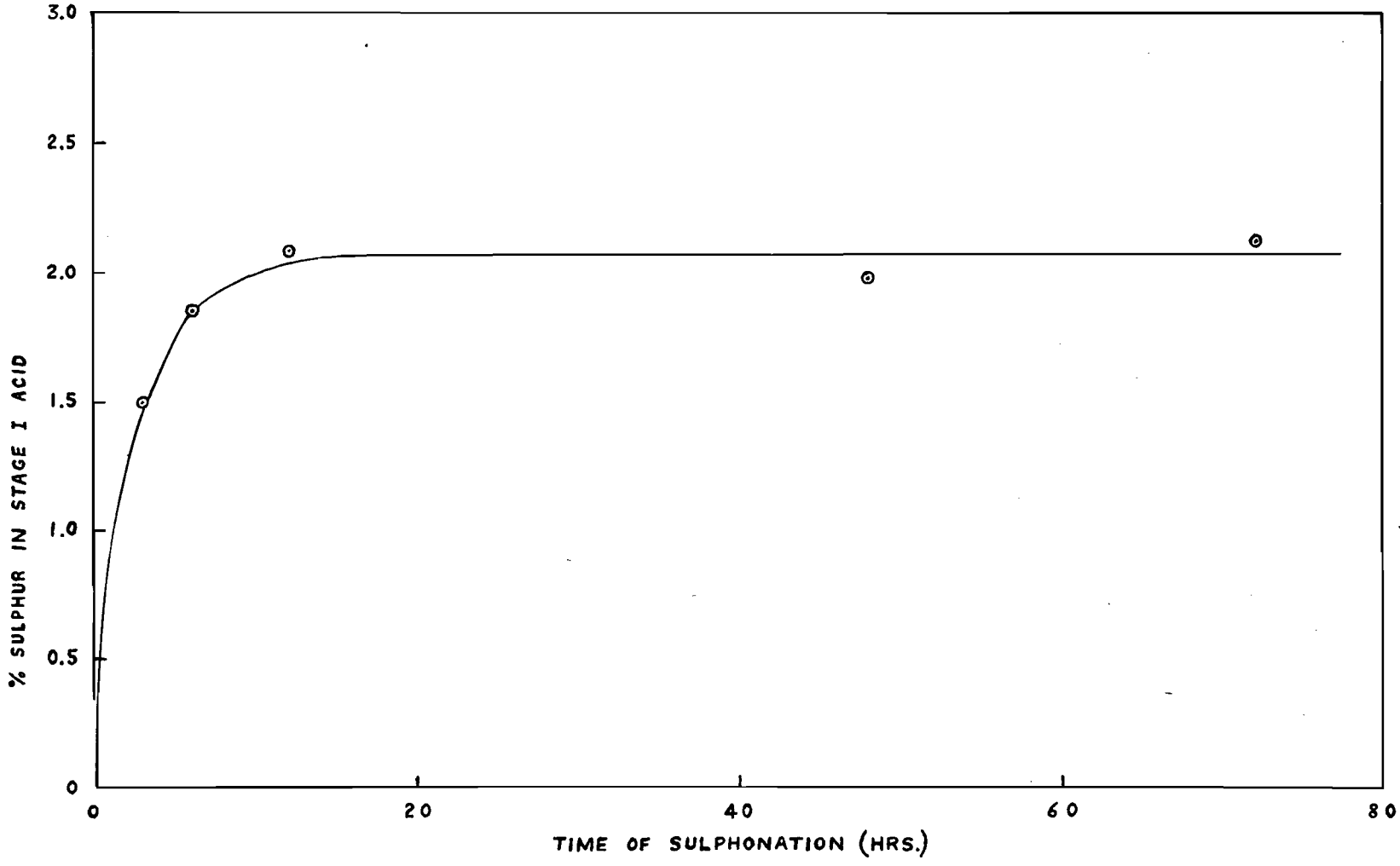


FIG. 8. Percentage of sulphur introduced during sulphonation at 96° using 16% aqueous $NaHSO_3$.

The above work made it evident that the conditions used by Smith to obtain good yields of the stage I ligninsulphonic acids from spruce could not be used for birch. Since the hydrolysis plot closely resembled that of the sulphur determinations, perhaps the 30% limit in solubility reflected a failure to substitute enough sulphonic acid groups during the cook to render the lignin soluble on hydrolysis. To test whether an increase in concentration of the bisulphite solution would increase the solubility of the lignin, two cooks were carried out in sealed tubes at 96° for 24 hours, one using 16% and the other 8% aqueous sodium bisulphite; hydrolysis in both cases was for 24 hours at 96°, in a buffer at pH3. Twelve per cent. of the lignin dissolved in the 16% bisulphite and a further 15% during hydrolysis. In the case of the 8% solution, 13% dissolved in each reaction. Thus within the experimental error expected in these cooks, altering the concentration of the cooking liquor had no effect on the solubility of the lignin, despite the fact that more lignin could be removed from wood by application of 15% rather than 5% sulphur dioxide at a pH of 4-6.5 (147).

Samples taken from each sulphonation were very carefully de-ashed, thoroughly washed, and suspended in distilled water. In each case the pH of the slurry was difficult to measure accurately but was between 3 and 4 rather than about 2, as it was for a spruce stage I ligninsulphonic acid. If, as most workers believe, the sulphonic group entered at the α-carbon

atom of the phenylpropane unit, it is possible that the extra methoxyl group in the benzene ring could cause the acidic hydrogen atom to be held a little more tightly than in a softwood because of the inductive effect. Although it seems improbable that a large difference could be caused by the presence of an extra methoxyl group, it might explain why Hotin Den (148) observed that the lignin from Manchurian "Sira-kamba" birch dissolved easily in sulphite solutions of low acidity but with difficulty in those of high acidity, since higher concentrations of hydrogen ions would oppose ionisation. It might also be the reason why only 30% of the birch stage I ligninsulphonic acid dissolved under conditions giving 80-85% solution of the spruce stage I acid. However, in the latter case a low sulphonic acid content, possibly due to steric effects, seems more plausible.

A series of cooks was carried out to determine the effect of pH on the hydrolysis of two stage I ligninsulphonic acids, both obtained by sulphonation of birch periodate lignin at 96° for 96 hours, with a 16% aqueous solution of sodium bisulphite. The results of the first run (Fig. 9) indicated that dissolution was favoured by pH3 rather than by pH2 as for spruce, and was in agreement with the finding that the pH of the free birch stage I ligninsulphonic acid was also about 3. In the second run, it was found that hydrolysis at pH1 also led to increased solubility, the actual percentage dissolved at pH1 and 3 being almost the same. The absolute values obtained in the second run were all lower than

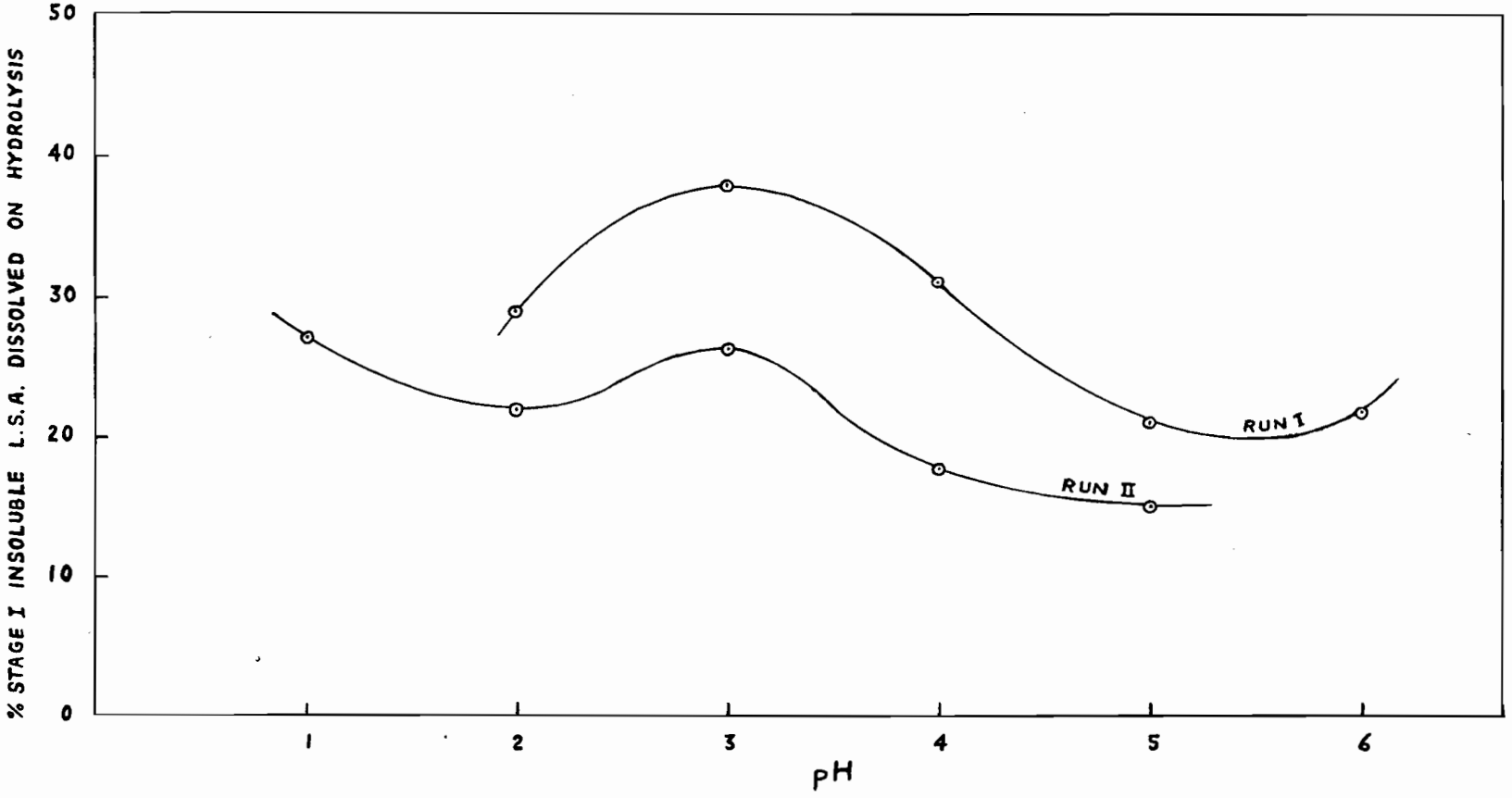


FIG. 9. Effect of pH on the hydrolysis of the stage I ligninsulphonic acid from birch periodate lignin.

in the first case, either because the stage I ligninsulphonic acids used were from different samples, or because hydrolyses were carried out in sealed tubes instead of in an open vessel with stirring. No explanation is offered for the two maxima in the second curve. On the basis of these results, later hydrolyses were at pH3, since at pH1 the possibility of causing intramolecular condensations in the product was probably greater.

The effect of time on the hydrolysis (Fig. 10) indicated that most of the reaction had occurred in 24 hours, although for quantitative purposes more extended hydrolysis would be desirable. Previous work on softwood stage I ligninsulphonic acids showed that over 90% was hydrolysed in this time. The temperature of hydrolysis had very little effect on the extent of dissolution, since stage I birch acids prepared at 96° dissolved to the same extent when hydrolysed at 96° and at 105° (about 25% in each case).

Attention was next directed toward the conditions for sulphonation, and the effect of increasing the temperature from 96° to 105° was studied. It was no longer possible to carry out the reaction under atmospheric pressure and the cooks were therefore made in sealed tubes immersed in a thermostatically controlled diethylene glycol bath. Hydrolysis was at pH3 and 96° for 24 hours, also in sealed tubes. Sulphonation and hydrolysis plots (Fig. 11) showed good agreement, although in the second run the hydrolyses of the stage I acids obtained by

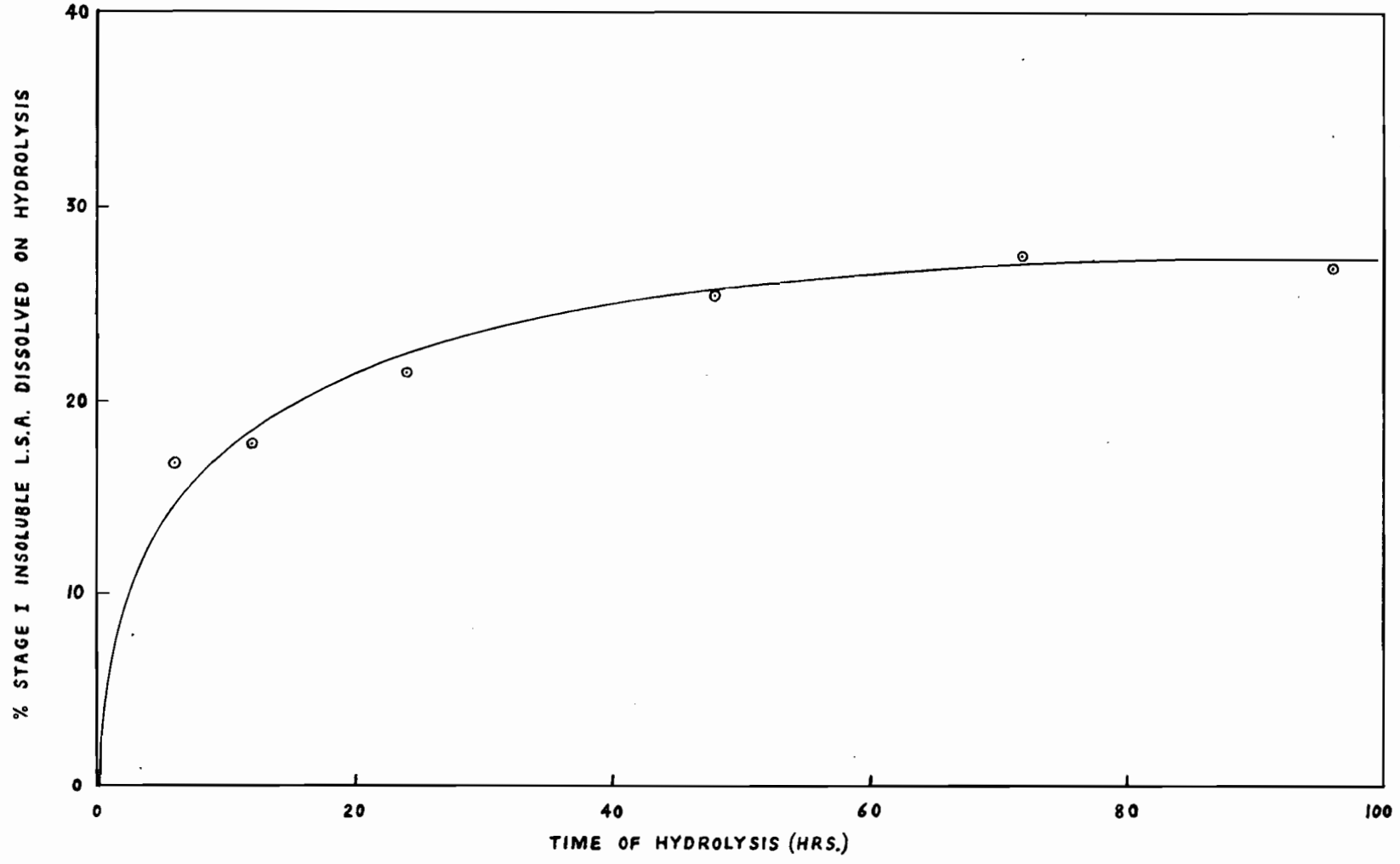


FIG. 10. Effect of time on the hydrolysis of the stage I ligninsulphonic acid from birch periodate lignin.

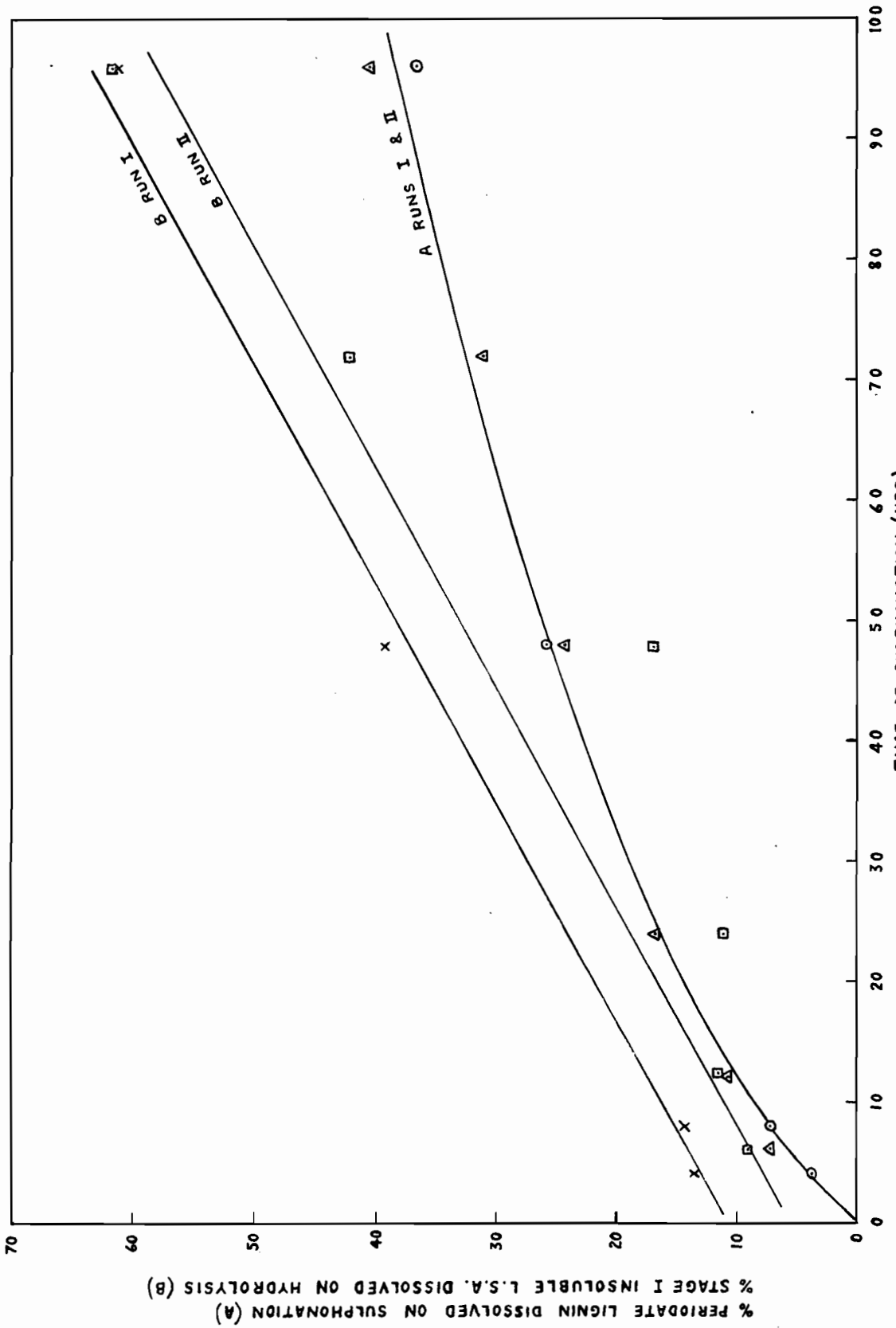


FIG. 11. Sulphonation of birch periodate lignin at 105°.

24 and 48 hours of sulphonation, appeared to have resulted in a much lower solubility than they should. The only explanation available was that an un-notified interruption of power caused a temporary decrease in temperature.

Much more of the lignin dissolved during sulphonation at 105° than at 96°. In both runs over 60% of the stage I lignin-sulphonic acid dissolved on hydrolysis of samples which had been sulphonated for 96 hours. One determination made after eight days showed that no less than 75% of the lignin had gone into solution. Hence it should be possible to dissolve birch periodate lignin completely at this temperature provided sulphonation was continued long enough. But increase in the length of sulphonation diminished the yield of insoluble stage I ligninsulphonic acid and hence the yield of the soluble stage II acid obtained by hydrolysis was also less.

Since it was now obvious that the optimum conditions for the dissolution of birch periodate lignin were not the same as those previously found for the product from spruce, the feasibility of carrying out a statistically planned series of investigations was considered. The help of Mr. K. Vroom, Chairman of the Technical Services Department of the Pulp and Paper Research Institute of Canada, was enlisted, and the following series of experimental cooks was carried out in accord with his suggestions, to obtain the maximum amount of information from a minimum amount of experimentation.

Statistical Treatment of the Sulphonation of Birch Periodate Lignin

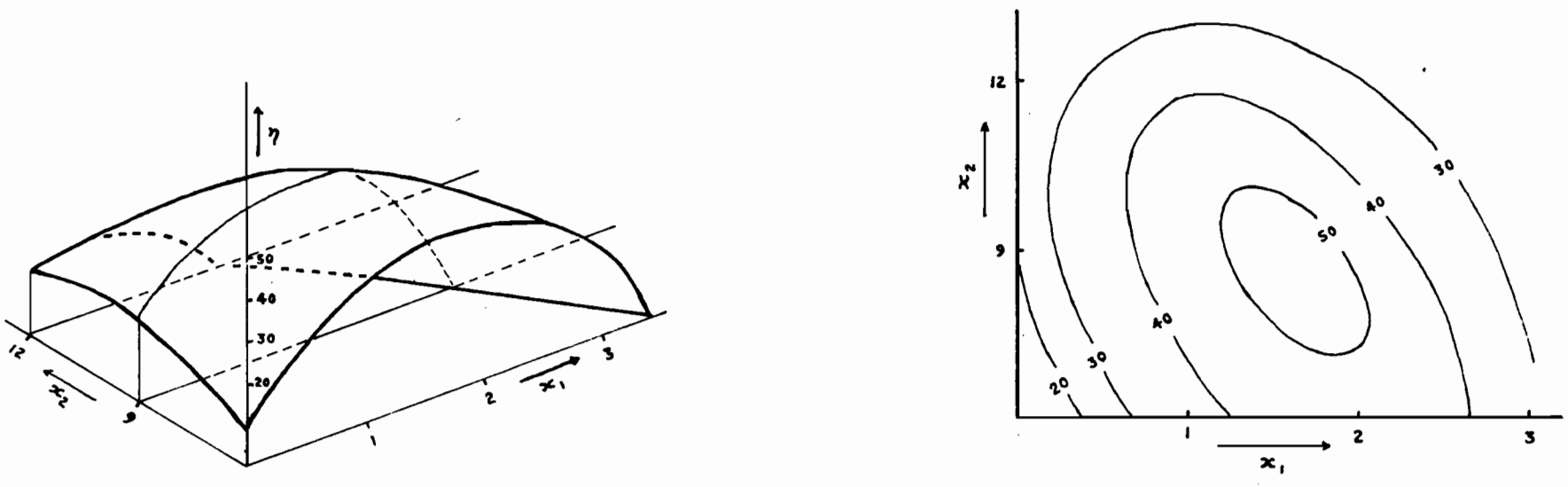
The treatment was based on a practical exposition (155) of a paper by Box and Wilson (156). If η was the true level of response, its value could be found by determining the values of the constants in the polynomial

$$\eta = \phi(x_1, x_2, \ldots\ldots\ldots\ldots\ldots x_n)$$

where $x_1 \ldots\ldots\ldots x_n$ were the independent variables. The lines of maximum slope in the multidimensional surface defined by this function indicated the significant variables and the directions in which they should be changed to move toward optimum conditions. When only two variables affected the response, a visual representation of the results could be given either by a response surface or by a contour diagram (Fig. 12). The effect of each variable at any particular point on the surface was given by the slope of the plane at that point. This idea could be developed for any number of variables, although a visual representation became more difficult as the number increased. However, the effect of each variable at any particular point was still the slope of the plane in the direction of this variable.

In simple mathematical terms, the true response would be represented by a polynomial of the type

$$\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots + \beta_n x_n + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \ldots\ldots$$
$$\ldots\ldots + \beta_{nn} x_n^2 + \beta_{111} x_1^3 + \ldots\ldots$$
$$+ \text{mixed terms.}$$



(a) Response surface.

(b) Contour diagram.

FIG. 12. Visual representation of the relation between response and factor levels (for two variable factors).

In regions far removed from the stationary point, small changes in the variables would probably show a large change in the response, and the terms of power 2 and higher could be neglected. Thus

$$\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots \beta_n x_n$$

This was the equation to a plane of slope $\beta_1, \beta_2, \ldots \beta_n$ in the direction of variable 1, variable 2, ...variable n. Near a stationary point terms of higher degree would have to be included to provide a reasonable approximation to the response surface, and then by mathematical manipulation the true stationary point could be calculated.

The general procedure was, therefore, first to define a region in the response field and to determine the effect of the variables on the response function at this point. If the effect of these variables was large, a new region was defined moving "higher up" in the direction of the factor which had the largest effect (i.e.slope) and changing the other variables in proportion. For a small effect further terms had to be added to the polynomial to get a better fit of the equation to the surface and then the stationary point was calculated.

It was first assumed that Hägglund's two-stage theory held for the dissolution of birch periodate lignin, and the object was to find the optimum conditions for each stage. The possible variables were:-

(a) in sulphonation: temperature, time, concentration and pH,

(b) in hydrolysis: temperature, time, pH.

There were two possibilities for the response function, which could either be the percentage of sulphur introduced during sulphonation, or the percentage of the insoluble stage I ligninsulphonic acid which dissolved during hydrolysis. In view of their low values (about 2%), sulphur determinations proved to be insufficiently accurate to allow of their use as a response function; the percentage dissolution of the stage I acid was used, because fairly good reproducibility of results was obtainable.

Since a considerable amount of experimentation had already been carried out on the hydrolysis, the initial conditions for this reaction were those already found to give the best results, namely, temperature 105°, time 24 hours, and pH3. If necessary, these values could be refined after determining the optimum conditions for sulphonation. Cabott's work (97) had already indicated that sulphonations should be carried out at about pH5 to prevent side effects, and a change in the concentration of the bisulphite liquor had already been shown to have little or no effect on the dissolution. Therefore, the initial experiments were concerned only with the effect of time and temperature on sulphonation. To this end, cooks were carried out (Table 4) at 95° and at 110° for 10 hours and 20 hours, followed in all cases by hydrolysis at pH3 and 105° for 24 hours.

Since both time and temperature had large effects on the amount of lignin dissolved, these conditions were far removed from the region where maximum solubility could be achieved.

TABLE 4. Statistical study: results of determinations in first factor space.

Trial	Factors		% dissolution	
	Time (hr.)	Temp. (°C)	during sulphonation	during hydrolysis
1	10	95	6.6	23.5
2	20	95	11.2	27.5
3	10	110	15.2	28.3
4	20	110	22.5	37.1

A second region in the factor space was investigated with temperatures increased to 120° and 135° and times increased to 20 hours and 30 hours (Table 5).

TABLE 5. Statistical study: results of determinations in second factor space.

Trial	Factors		% dissolution	
	Time (hr.)	Temp. (°C)	during sulphonation	during hydrolysis
5	20	120	40.7	64
6	30	120	64.1	93
7	20	135	99	-
8	30	135	99	-

At 120°, there was an increase in dissolution on hydrolysis as compared with the lower-temperature cooks, but this was accompanied by a considerable increase in the solubility during

sulphonation, thus decreasing the yield of the insoluble stage I acid. Complete dissolution of the lignin occurred at 135° during sulphonation itself, thus breaking down the statistical treatment as far as considering the dissolution of lignin as a two-stage process was concerned. The results indicated that it was possible to prepare an hydrolysable, insoluble, stage I ligninsulphonic acid from only a small fraction of birch periodate lignin. Thus Hägglund's two-stage theory did not seem to apply to a major part of the birch lignin in the way it had to spruce periodate lignin.

For the sake of comparison, a sample of spruce periodate lignin prepared by Mr. Das was similarly cooked at 135° and was also found to be completely soluble during sulphonation alone. Thus, at a temperature similar to those employed in commercial cooks, both softwood and hardwood lignin gave similar results, in accord with many observations that hardwoods pulped as easily as softwoods in technical cooks. However, at lower temperatures the two lignins did not react in the same way. Sulphonation was a necessary requisite for dissolution, because it was found that only 25% of the birch periodate lignin dissolved by cooking in the hydrolysis buffer at 135°. Also, the stage I birch ligninsulphonic acid from a sulphonation using Smith's conditions failed to dissolve completely in a similar treatment, suggesting that further sulphonation was necessary.

The Introduction summarises work on the sulphonation of lignin model compounds (p. 43), but all these reactions seem to have been carried out at a temperature of about 135°. It was possible that at this temperature some units were sulphonated which remained inert at lower temperatures, whereas others reacted readily at lower temperatures also. This assumption could well explain the difference in behaviour between spruce and birch periodate lignins during sulphonation, and might also give valuable clues as to the structure of the two lignins. Those interested in the sulphonation of model compounds might profitably carry out some further experiments at lower temperatures, especially to discover whether or not the presence of an extra methoxyl group in syringyl-type compounds introduces any marked stereochemical effects at these temperatures.

It was decided to restrict the statistical study to the sulphonation itself, in an attempt to determine the minimum temperature and time needed to dissolve the lignin completely. The results which had already been obtained from the statistical program were analysed by Mr. Vroom and an equation derived for the percentage of lignin dissolved during sulphonation, which was now taken as the response function. By substituting certain values for the percentage dissolved, contour lines were constructed showing combinations of time and temperature which would give the same amount of dissolution (Fig. 13).

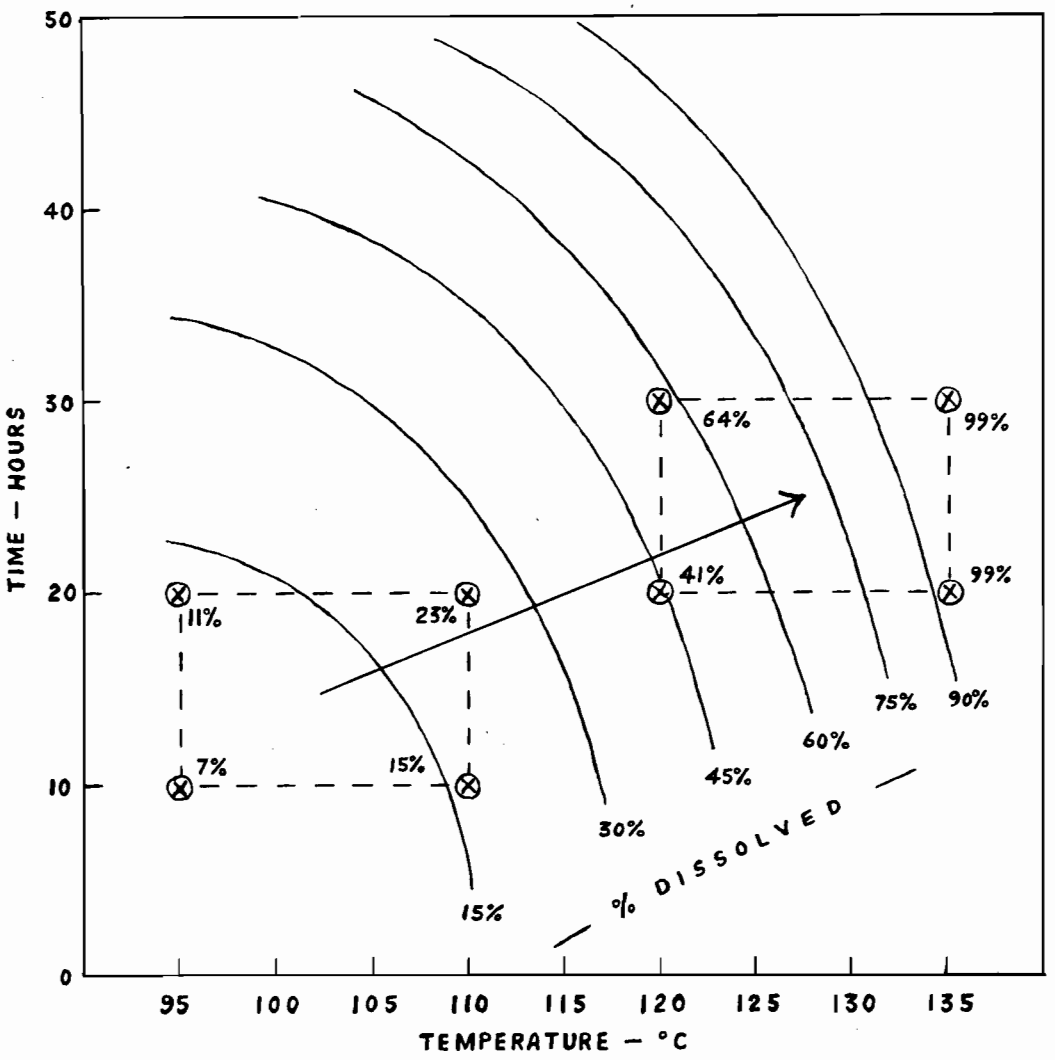


FIG. 13. Contour diagram composed of data from Tables 4 and 5.

This diagram indicated that a further region in the factor space, bounded by times of 5 hours and 15 hours, and temperatures of 130° and 140°, might profitably be studied. The results of these cooks (Table 6) were combined with the earlier results and once again a contour diagram was constructed (Fig. 14). An outline of the mathematics involved in these calculations is

TABLE 6. Statistical study: results of determinations in third factor space.

Trial	Factors		% dissolution during sulphonation
	Time (hr.)	Temp.(°C)	
9	5	130	29.7
10	15	130	56.9
11	5	140	49.4
12	15	140	97.8

given in Appendix B. This time, the shape of the contour lines was greatly altered and it now appeared that rather than the stationary region being a maximum or minimax (saddle-point), we were dealing with a ridge. When this kind of response is found to hold, there is not a unique optimum set of conditions for the reaction being studied, but a whole series of conditions producing nearly equal response. Thus the two variables, temperature and time, in the sulphonation of birch periodate lignin were mutually inter-related, the direction of the ridge indicating the amount by which the temperature should be increased to compensate for a

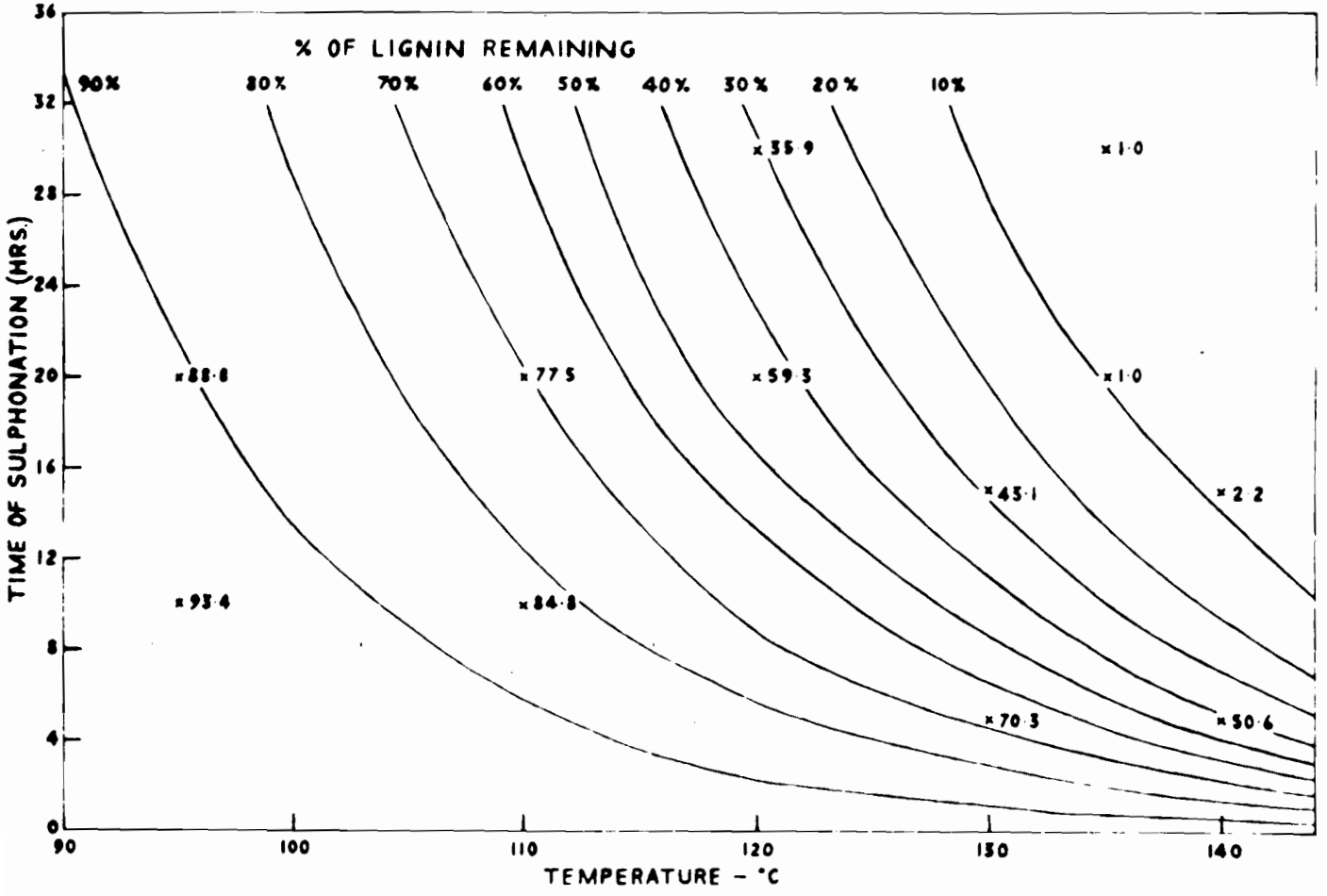


FIG. 14. Contour diagram composed of data from Tables 4, 5, and 6.

given decrease in the time of reaction.

These contour diagrams showed the weakness of the one-factor-at-a-time method of experimentation. Any series of trials involving variations of temperature with percentage of lignin remaining undissolved, keeping time constant, taken with a similar series of trials of variations with time keeping temperature constant, would lead to a maximum in the contour line corresponding to zero per cent. remaining. Thus with the first series of experiments carried out "optimum conditions" would be found and the interdependence of time and temperature would be hidden.

This brought the statistical experiments to an end. However, some further calculations carried out by the Statistical Department of the Pulp and Paper Research Institute are worth mentioning. In connection with the development of an equation for constructing the final contour diagram, it was necessary to find out whether a linear relationship existed between the percentage of the lignin remaining undissolved and the time of dissolution. Perhaps not too much emphasis should be placed on the linear relationship indicated (Fig. 15) because only a few points were involved. The experimental errors in the determinations of the amount of lignin remaining at the end of a cook were also considerable, a variation of 1% between duplicate experiments being considered good.

The slopes of these lines were calculated to obtain the rate of the reaction, k, and a plot of ln k against the

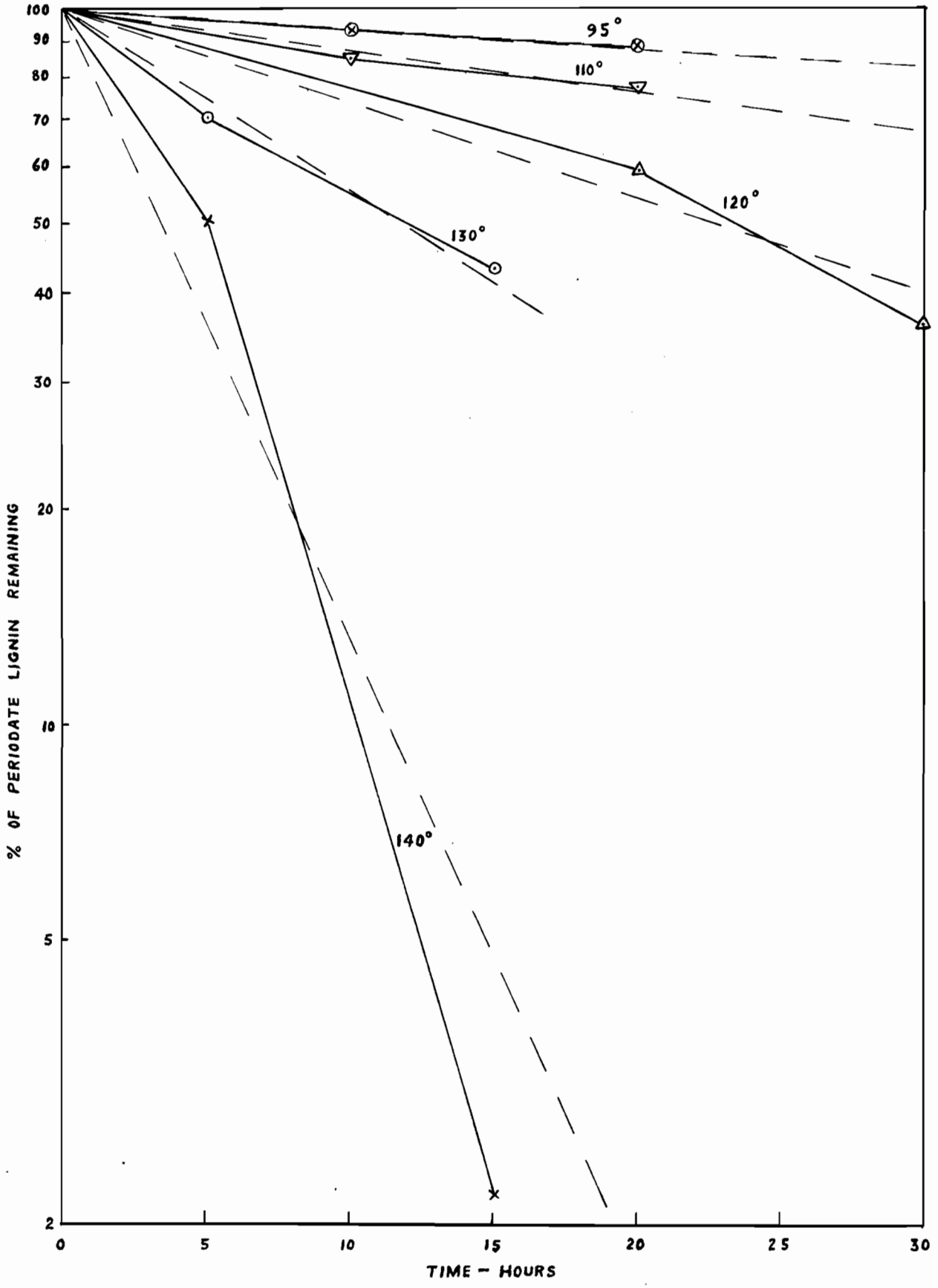


FIG. 15. Plot of the percentage of lignin remaining undissolved and time of dissolution.

reciprocal of the temperature was then made (Fig. 16). The three middle points were very nicely on a straight line, while the position of the point corresponding to the 95° plot could be altered considerably by small changes in the slope. Since this slope was difficult to measure, the point could conceivably lie on a straight line with the others. The point for the 140° plot could also be brought more nearly into line with the others if it was assumed that there was an error in the point plotted in Fig. 15 for 15 hours at this temperature. A slight error in the value of the percentage of lignin remaining undissolved under these conditions would be magnified disproportionately because the plot was logarithmic.

Since a straight-line relationship probably held between ln k and $^{1}/_{T}$, the Arrhenius equation was valid for this reaction. From the slope of this line the activation energy was calculated to be 23,890 cal./gm. mole. This value compared favourably with that of 20,200 cal./gm. mole found by Calhoun, Yorston, and Maass (104) for sprucewood. Hence the statement that hardwoods pulped in a similar manner to softwoods under technical conditions (131,132) received still more support.

A plot of the variation with temperature of the amount of lignin dissolving during a 24 hour cook (Fig. 17) showed very definitely that above 100° there was a change in the mechanism involved. This change in mechanism was also shown

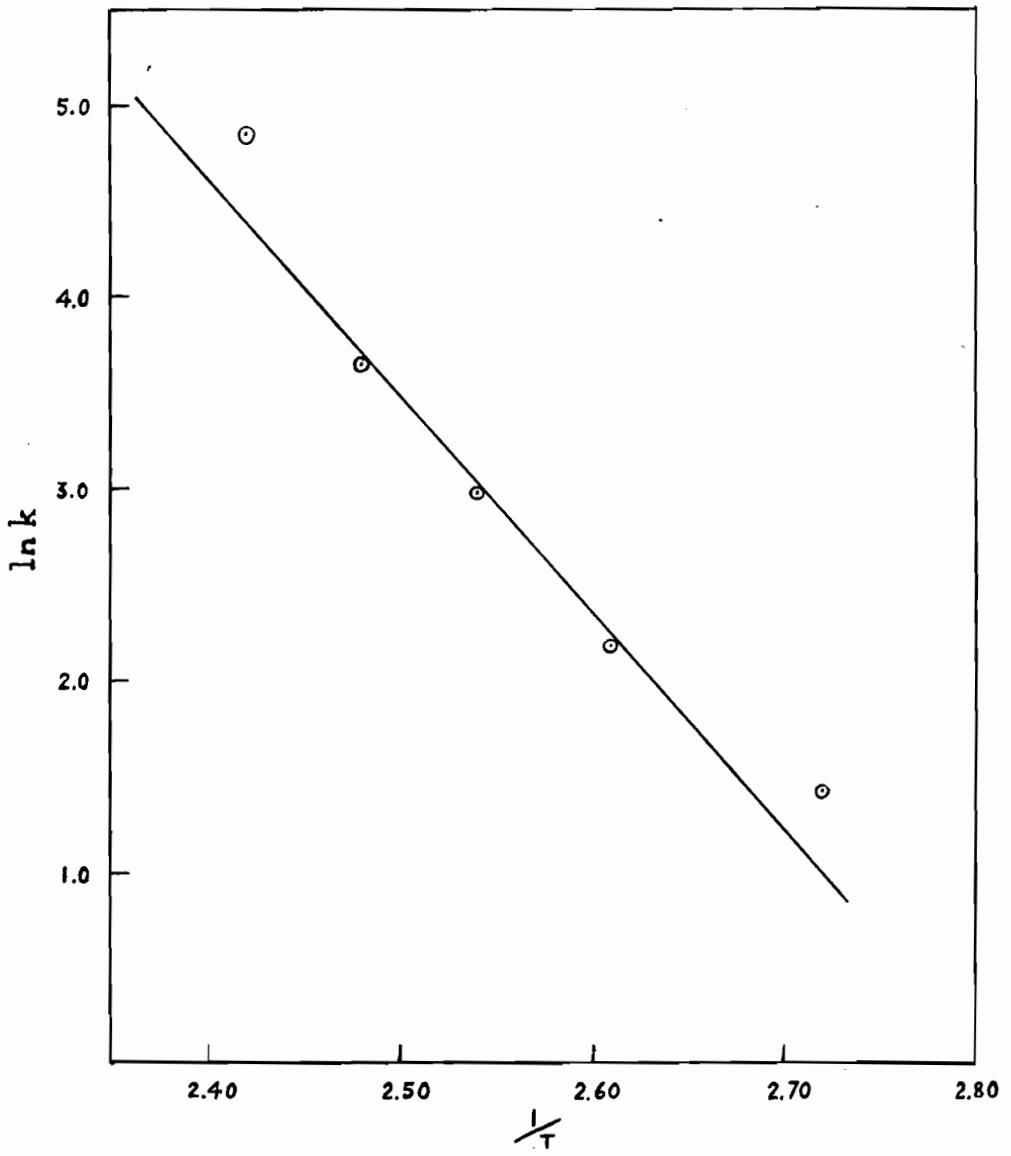


FIG. 16. Dissolution of birch periodate lignin: plot of ln k against the reciprocal of the temperature.

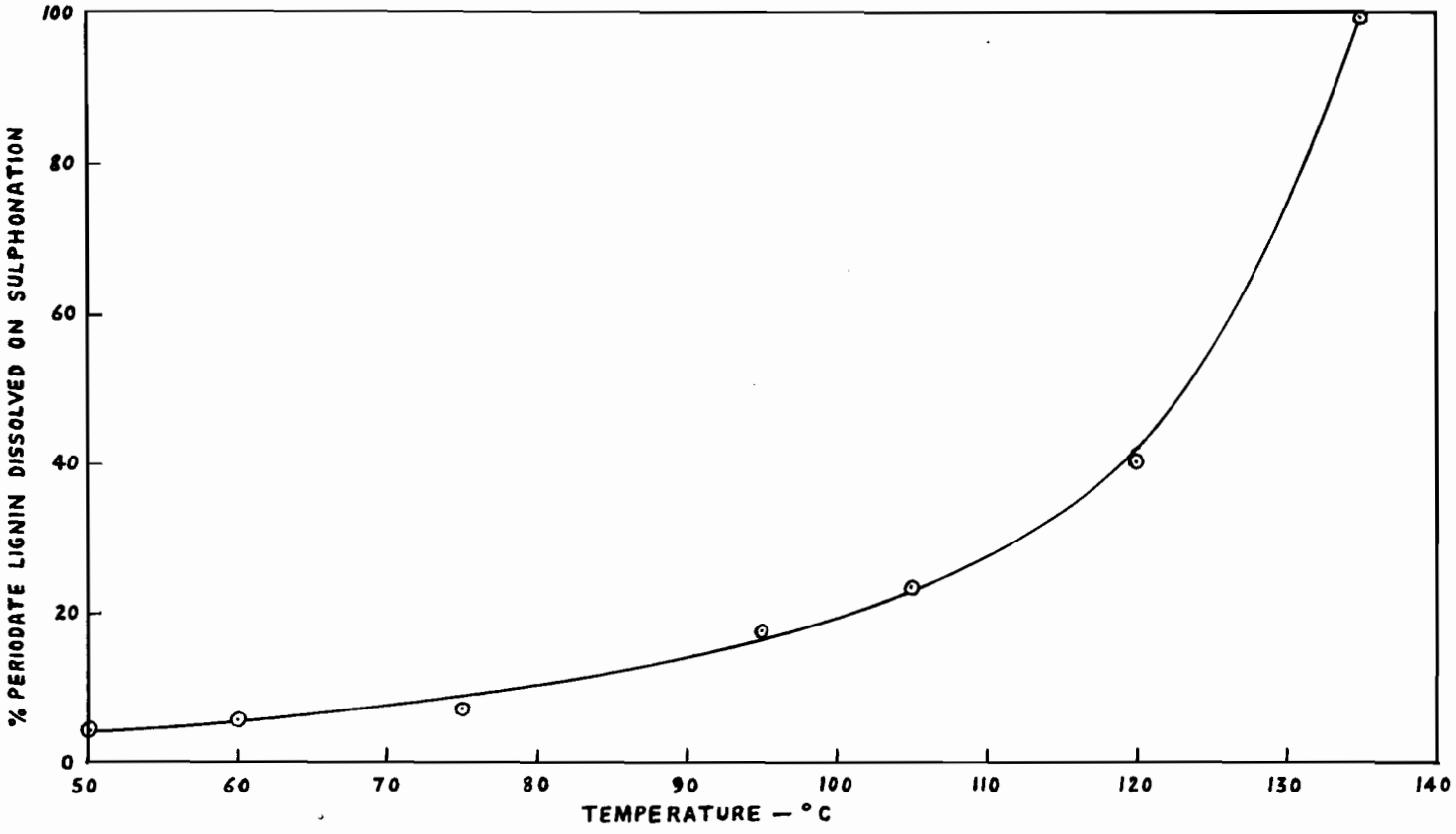


FIG. 17. Dissolution of birch periodate lignin: variation of amount dissolved against temperature of sulphonation.

very clearly on the contour diagram (Fig. 13), the contours being widely spaced until about 100°, above which they tend to crowd together to give almost a "cliff". A similar change in mechanism has already been noted in the Introduction in the case of sulphite cooks of both softwoods and hardwoods. This change may be due to a chemical reaction with a different type of lignin unit, but the possibility of a physical difference must not be overlooked as it has been shown that lignin undergoes physical change at about this temperature.

Interpretation of Sulphonation Data

The results of the sulphonation studies indicated that only a small amount of birch periodate lignin seemed to follow Hägglund's two-stage mechanism of dissolution as it was interpreted by Cabott. However, these results need not necessarily mean that this theory is not true. As originally defined (103), the theory referred to the dissolution of lignin from wood, not to the dissolution of an isolated lignin, and it was not known whether the insolubility of the solid lignin-sulphonic acid was a result of a chemical linkage between the lignin and other wood constituents or was due to a high degree of polymerisation in the lignin molecule. The Historical Introduction has shown that there is much indecisive evidence for the existence of such a linkage and thus Hägglund's two-stage theory is easily accounted for as initial sulphonation of the lignin followed by cleavage of the lignin-carbohydrate

bond to release soluble ligninsulphonic acids. The results of sulphonating spruce periodate lignin (97,98,113) could be explained by two different mechanisms depending on temperature. The apparent two-stage process at lower temperatures could be due to substitution of an hydroxyl group by a sulphonic group followed by hydrolysis of a particular linkage, while the solubility upon sulphonation alone at higher temperatures could be due to rupture of the molecule simultaneously with entry of the sulphonic group. The side-chain structure of hardwood lignin units might differ from those of softwood units in such a way as to prevent dissolution of the lignin by hydrolysis at low temperatures. These ideas are highly speculative, and obviously a quantitative study of the side-chain of hardwood lignin, similar to that carried out by Adler (41) on softwood lignin would be of great value.

In trying to explain the sulphonation data, the chief thing to consider seemed to be the fact that only about 30% of the birch periodate lignin dissolved under conditions (100° or less) which dissolved spruce periodate lignin almost completely. Since softwoods on oxidation with nitrobenzene or cupric hydroxide gave only vanillin, and hardwoods usually gave a mixture of syringaldehyde and vanillin in a ratio of about 3:1, perhaps the 30% or so of birch periodate lignin which dissolved was composed of guaiacyl units yielding exclusively vanillin on oxidation. This possibility was not so improbable in the light of the considerable speculation as

to whether or not hardwood lignin was a mixture of lignins.

A somewhat similar explanation for the difference in sulphonation of birch and spruce periodate lignins could be reached by assuming that the difference in reactivity was because hardwood lignin contained less of the hydrolysable linkage than the softwood lignin. As already discussed, the only linkage that hardwood lignin was likely to contain less of was the carbon-oxygen linkage of the furan type, since the 5-position in many of the aromatic rings was blocked by an extra methoxyl group. Therefore, perhaps, dissolution in softwood and in 30% of hardwood lignin took place principally by the sulphonation of such units. This could occur in two steps as first postulated by Freudenberg (37, 51), but it has been noted (157) that the resulting differences to be expected in the spectrum of lignin before and after sulphonation, have not been found. Also, Adler's low value of 16% for the amount of furan type dimers in lignin (66) seemed against such a mechanism, although this value does not necessarily apply to hardwoods. In all events, the present work has shown that there definitely are certain linkages in hardwood lignin which are more difficult to cleave than those in softwood lignin. Such linkages may need more drastic conditions to cleave them or dissolution at a higher temperature could be due merely to a physical process.

The Historical Introduction showed how little was definitely known concerning the mechanism of the sulphite

cook, even in the case of softwoods like spruce, which have been intensively studied. Since the differences in sulphonation at 100° or less observed between birch and spruce periodate lignins reflected differences between two chemical structures, both of which were largely unknown, a study was made of structural differences that could be assessed at the present time. The hope was that eventually these differences might be found of relevance to the problem of sulphonation. The assessable differences included the maximum number of hydroxyl groups that might be replaced by sulphonation, the distribution of the syringyl units possessed by birch but not by spruce lignins, and the benzenepolycarboxylic acids produced by drastic oxidation.

Methylation Studies

As Adler and Hernestam showed (23), free phenolic groups were readily oxidised by aqueous periodate and their survival in a periodate lignin was not to be expected; the observed slow increase in methoxyl content when spruce periodate lignin was methylated with diazomethane (97) perhaps reflected the presence of benzyl alcohol units. In the present work, all of the hydroxyl groups in birch periodate lignin were methylated with dimethyl sulphate and alkali, the methoxyl content becoming virtually constant at 26.1% after four methylations. If birch periodate lignin was assumed to possess a methoxyl-free base molecular weight of 770, like the soluble methanol lignin from black spruce examined by

Brauns (85), then we could make use of the formula

$$\% \text{ OMe} = \frac{3100x}{770+14x}$$

where x was the number of moles of methoxyl groups in the base molecular weight. Since the original methoxyl content of the periodate lignin was 21.5%, methylation brought about an increase of 0.96 mole of methoxyl group. Spruce periodate lignin on methylation with dimethyl sulphate (97) showed an increase of 1.7 moles. This would therefore indicate the presence of more hydroxyl groups capable of methylation in spruce than in birch, and presumably more opportunity for sulphonation.

This inference was consistent with Aaltio and Roshier's view (92) that only pure guaiacyl-type lignin units bonded to carbohydrate. On isolation, owing to cleavage of a possible ether linkage, a free hydroxyl group, or an oxidised form, might be present in guaiacyl units but not in syringyl-type units.

On subjecting methylated birch periodate lignin to sulphonation at 96° for 20 hours, 8.54% dissolved. A further 11.5% dissolved on hydrolysis of the sulphonated product at 96° and pH2 for 24 hours; that is, the extent of solution was less than half that of unmethylated birch lignin under the same conditions. Therefore methylation blocked sulphonation in just over half the previously soluble material. When sulphonation was carried out at 135°, only 29.5% of the

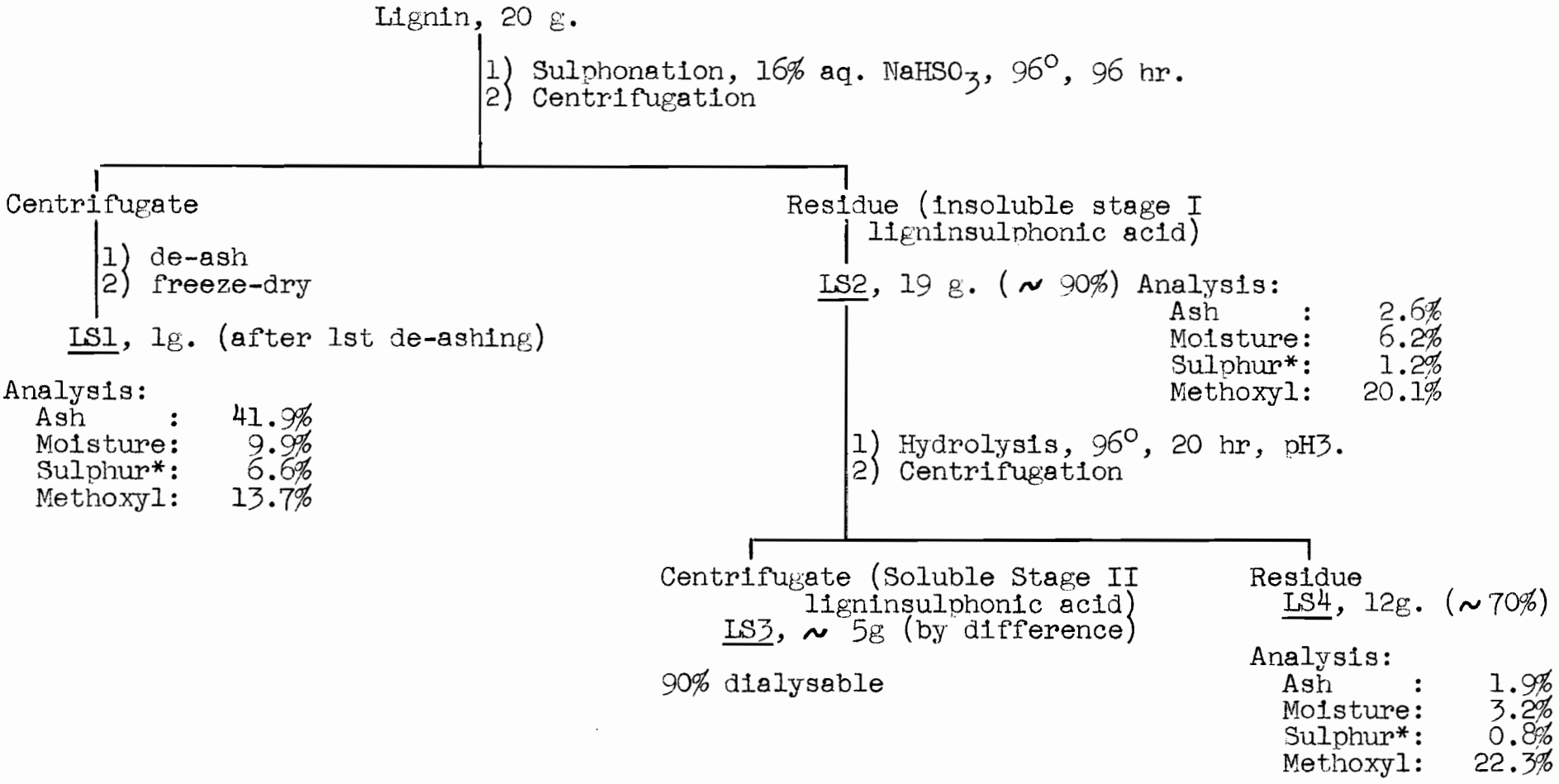
methylated lignin dissolved, compared with total dissolution of the unmethylated lignin. This value was very similar to that obtained by Freudenberg (52), who found that about 30% of a fully methylated spruce cuoxam lignin (29% OMe) dissolved in bisulphite at 135°. Hägglund and Richtzenhain (158) found that about 20% of a methylated spruce hydrochloric acid lignin was soluble in an acid bisulphite cook at 135°, but that only about 10% dissolved during a neutral cook. They concluded that methylation prevented the first-stage of sulphonation in neutral solution. This hydrochloric acid lignin, however, unlike periodate lignin, could not be completely dissolved by a neutral cook at 135°.

A direct comparison with the effect of methylation on the sulphonation of spruce periodate lignin was unfortunately not available. It did seem, however, that differences existed between lignins from birch and from spruce as far as methylation was concerned.

Large-scale Sulphonation

Since sulphonation at 100° possibly distinguished between different units in birch periodate lignin, a larger-scale sulphonation was carried out from which samples were isolated at various stages for degradative studies. The sulphonation and hydrolysis were in open vessels at 98° according to Smith's conditions (113), the products being separated as indicated in Fig. 18.

Sample LS1 contained much sodium bisulphite and soluble fragments which had been cleaved from the lignin during sulphonation.



* Sulphur determinations made by the Analytical Dept., Pulp and Paper Research Institute of Canada (using the "Leco" combustion method).

FIG. 18. Flow-sheet for first large-scale sulphonation of birch periodate lignin.

These soluble fragments were isolated as a light brown powder after de-ashing and freeze-drying the resulting solution. Although this fraction was de-ashed twice, it was not possible to lower the ash content below 41.9%. This indicated either incomplete de-ashing or that the fraction contained a large amount of silica, possibly from the stirrer used in the sulphonation vessel. It was not possible to de-ash and freeze-dry a third time because of the small size of the fraction. This high ash content made the analytical data of the fraction of little value; but the low methoxyl content might be due to a preponderance of guaiacyl units.

An infrared spectrum of LS1, made by Dr. H.I. Bolker of the Pulp and Paper Research Institute, in connection with another project, indicated that most of the main lignin bands were still present although they were of low intensity and rather diffuse (159). This fraction therefore seemed highly degraded while the other fractions had more typical spectra. On the other hand the weakening of the bands might have been caused by the high ash content.

The methoxyl content of sample LS2 agreed fairly well with corresponding values for samples in the initial sulphonation experiments, but the value for the sulphur, 1.2%, was lower than that of previous samples (S~2.1%).

The solids content of sample LS3 was determined by evaporating an aliquot to dryness on a rotary evaporator; another aliquot was examined in the same way after being

dialysed for several days against changes of distilled water. The results indicated that about 90% of the solids were dialysable, and therefore dialysis could not be used to purify this fraction from the buffer salt. It has been noted (160) that readily dialysable ligninsulphonic acids probably were of molecular weight not more than 2000, and therefore it might be possible to isolate units still containing the sulphonic group from this fraction. Since the buffer ions present increased the difficulty of isolation, another sample (LS3′) was prepared by isolating the free insoluble stage I ligninsulphonic acid and autohydrolysing it in distilled water.

Dialysis of the hydrolysate showed that 75% of the solids passed through the cellophane membrane. After neutralisation with dilute barium hydroxide, 70% was dialysable. Thus in each case there was slightly less dialysable material than in the first hydrolysate. The discrepancy might have been due to molecular association in the case of the free acid and to the greater size of the barium ion as compared with the sodium ion. By freeze-drying the hydrolysate, LS3′ was isolated as a very fluffy, light brown material (ash, 14.6%; moisture, 3.7%; OMe, 19.8%; S, 3.09%).

Attempts were made to chromatograph an aqueous solution of fraction LS3′ in solvent systems previously used for phenols, since if sulphonation or hydrolysis occurred by cleavage of a phenyl ether bond phenolic products would be expected. Neither butanol: 2% aqueous ammonia nor butanol:pyridine:water effected

any separation. A solvent system butanol:ethanol:water, which had been used successfully for the separation of various aminonaphthalenesulphonic acids (161) caused two spots, of Rf 0.51 and 0.77, to separate. Both these spots gave a blue colouration with a ferric chloride-ferricyanide spray and were presumably phenolic. With Fast Red Salt G G the spot at 0.77 was visible before spraying with sodium carbonate but not after, while the other spot was not visible prior to spraying with sodium carbonate but afterwards was a pale grey-green spot. Neither substance gave a colouration with 2,4-dinitrophenyl-hydrazine.

A large-scale chromatographic separation of the spot of Rf 0.51 from substance LS3′ was carried out using the system butanol:ethanol:water (3:1:1) as described in the Experimental Part. Three grams of LS3′ yielded about 70 mg. of a brown, hydroscopic substance (X). An infrared spectrum of this substance was found by Dr. Bolker (159) to resemble closely that of the entire fraction LS3′, the main difference being that LS3′ clearly had a singlet band near 1600 $cm.^{-1}$, but substance X had a doublet. Some lignins show singlets and some doublets at this frequency, and it has been suggested (162) that the lower frequency band is due to structures conjugated with the benzene rings of the lignin. There appears to be some contribution from alpha carbonyl, but not enough to account for the high intensity of the band. Kolboe and Ellefsen (162) suggested that diphenyl groups might be involved.

Substance X has a weak absorption band at 1043 $cm.^{-1}$. This band has been observed in other ligninsulphonic acids (159) and has been taken to be the strongest of the bands due to the sulphonic acid group. Other, weaker sulphonic acid bands at 655 $cm.^{-1}$ and 531-534 $cm.^{-1}$ were absent from X, LS1, LS2, LS3′, and LS4. Thus further evidence is provided for a difference in the behaviour in sulphonation of soft- and hard-woods, the latter being only slightly sulphonated.

Although the absorption at 1043 $cm.^{-1}$ suggested that substance X was a low-molecular weight ligninsulphonic acid, a sulphur determination carried out by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y., showed only 2.24% to be present, that is, far less than would be expected for a uniformly substituted low-molecular weight ligninsulphonic acid. For this reason, and because of the small yield, it was decided that further investigation of fraction X would have to be deferred.

The residue left after sulphonation and hydrolysis, LS4, had a higher methoxyl content than the original birch periodate lignin and might have had a larger proportion of syringyl-type units. But Brickman (98) noticed that the residues from cooks of spruce periodate lignin also had high methoxyl values and considered that a portion of the lignin that was low in methoxyl was preferentially solubilised during sulphonation. On the other hand, a separation of the components in spent sulphite liquor was recently carried out by means of ion

exclusion and gel filtration (163) giving a series of fractions differing in molecular weights but with identical ultraviolet spectra and methoxyl contents.

Qualitative Oxidations with Alkaline Nitrobenzene

The oxidation mixtures from birchwood and birch periodate lignin were chromatographed in the butanol:2% aqueous ammonia system and the products detected by spraying with 2,4-dinitrophenylhydrazine (Table 7). Since direct spotting of the oxidation mixture resulted in much streaking and indistinct spots, presumably because of interference by nitrobenzene and its products, the larger-scale oxidation (p. 131) was used in these experiments, in which nitrobenzene and its products had been removed by extraction with ether.

The chromatograms showed the presence of the expected products, vanillin and syringaldehyde, as well as the related acids, from birchwood as found by Pearl (164). Birch periodate lignin gave similar products. The pink spot (Rf 0.84) was later found to be an impurity in the nitrobenzene used. The orange-brown spot (Rf 0.91) which was visible prior to spraying as a bright yellow spot, exhibited colour changes according to the pH of its environment. These changes indicated that it might be o- or p-nitrophenol, substances which were known to be formed when nitrobenzene was heated with sodium hydroxide (165). The spot did in fact appear in the products from a control experiment omitting the lignin sample. However, authentic samples of the nitrophenols did not exhibit chromatographic behaviour similar to that of the unknown substance.

Since the spot obviously did not arise from the lignin, it was not further investigated. This spot was observed by Stone (166) who also considered it to be a by-product of the nitrobenzene.

When chromatographed in butanol:pyridine:water (10:3:3) the oxidation products from birchwood (Table 8) again resembled those obtained by Pearl.

Chromatography of the products from the oxidation of the residue from the large-scale bisulphite cook (LS4) gave contradictory results. Using the butanol:pyridine:water system to develop the chromatogram no spot was obtained which corresponded to vanillin, thus apparently supporting the idea that sulphonation had caused preferential dissolution of a guaiacyl type lignin, leaving a syringyl type. However, chromatography using butanol:2% aqueous ammonia indicated a faint spot corresponding to vanillin, and chromatography in the system petroleum ether (b.p. 100-120°):n-butyl ether: water (6:1:1) showed a definite spot for vanillin. Using the last-mentioned system, vanillin and syringaldehyde were detected in the oxidation products from LS1, LS2, and LS3' also. Therefore the presence of a completely guaiacyl type of lignin was not supported, and it appeared that the differences in sulphonation shown by the hardwood lignin at various temperatures were probably due to lignins containing different ratios of syringyl and guaiacyl units.

Qualitative oxidations with nitrobenzene were also

TABLE 7. Rf values of products from oxidation of birch periodate lignin with alkaline nitrobenzene. (Developed in butanol: 2% aqueous ammonia; spray, 2,4-dinitrophenylhydrazine.)

Rf values	0.00	0.04	0.08	0.18	0.44	0.59	0.79	0.84	0.91
Authentic syringaldehyde					brown +++				
Authentic vanillin						orange-brown +++			
Birchwood					brown +++	orange-brown +++	yellow-brown +++	pink +++	orange-brown +++
Birch periodate lignin	brown +	yellow ++	pinkish brown +	light brown +	brown +++	orange-brown +++		pink +++	orange-brown +++

TABLE 8. Rf values of products from oxidation of birch periodate lignin with alkaline nitrobenzene. (Developed in butanol: pyridine: water (10:3:3); spray, 2,4-dinitrophenylhydrazine.)

Rf values	0.00	0.16	0.23	0.27	0.40	0.62	0.78	0.82	0.89
Authentic syringaldehyde							brown +++		
Authentic vanillin								orange-brown +++	
Birch periodate lignin	pinkish-brown +	light brown +	yellow brown +	yellow brown +	yellow brown +	yellow brown +	brown +++	orange-brown ++	light brown +

+++ indicates strong colouration; ++, medium; +, weak.

carried out on the soluble products obtained by cooking samples of birch periodate lignin for 24 hours in 16% aqueous sodium bisulphite at various temperatures, and also on the soluble products from the residues of such a cook formed on hydrolysis at 105° in a buffer solution at pH3 for 24 hours. The oxidation products were developed in the petroleum ether: n-butyl ether:water system and the chromatograms sprayed with 2,4-dinitrophenylhydrazine. Neither syringaldehyde nor vanillin was detectable in the products of the liquor from a sulphonation at 60°, or of the hydrolysate obtained from the residue of this cook. However, when sulphonation was carried out at 75°, syringaldehyde and vanillin were obtained from the oxidation products of both the sulphonation liquor and the hydrolysate. This observation provided further evidence that birch periodate lignin did not contain a pure guaiacyl type lignin such as had been suggested by the work of Stone (152) on the sulphonation of aspenwood. The results in fact agreed with those of Marth (167) who found that the yield of syringaldehyde was never zero, although it was low at the beginning of a cook.

Quantitative Oxidations with Alkaline Nitrobenzene

The qualitative oxidations supported the view that hardwood lignin was possibly composed of lignins containing different ratios of guaiacyl to syringyl units. Therefore a series of quantitative oxidations was carried out on the samples obtained from the large-scale sulphonation of birch

periodate lignin. The method was based on that of Stone and Blundell (168), but considerable difficulty was at first encountered with the reproducibility of the results. Early determinations on birchwood itself gave syringaldehyde to vanillin ratios varying about unity, as compared with values of 2.6 to 3.0 determined for various hardwoods by earlier workers (169,170,171,172). Pearl (173) obtained a ratio of 2.6 for the aldehydes present in the extract from birch treated with boiling 1N sodium hydroxide.

Both Leopold (174) and Pepper (172) had determined the conditions which gave optimum yields of both syringaldehyde and vanillin to be 180° for 2 hours and 170-180° for 2½ hours respectively. When a temperature of 180° was employed for 2½ hours for the oxidation of birchwood, a slight increase in the ratio of the aldehydes was observed but the value was still nowhere near 2.6. Pepper also showed that a further increase in the temperature caused a decrease in the syringaldehyde:vanillin ratio. This was also observed by Krätzl (175).

Earlier workers employed bombs of stainless steel rather than nickel, but oxidations carried out with stainless steel bombs, kindly loaned by Dr. Towers, of the Department of Botany, gave ratios which were almost identical with those obtained under the same conditions using the nickel bombs. Neither was improvement obtained in the ratio by extracting the oxidation mixture with ether (see p.131); and the low ratio could not be ascribed to any of the reasons noted by

Towers (171) as causing a decrease in the yield of the aldehydes, as these factors had been accounted for.

It was suggested by Dr. Kleinert of the Pulp and Paper Research Institute of Canada that the inability to obtain the value determined by the earlier workers might be due to differences in the chromatography paper employed. He had demonstrated (176) that present-day chromatographic papers contained peroxidic groups which interfered with the quantitative determination of xylose and arabinose by partially oxidising these sugars. He overcame this interference by first reducing the chromatography papers with sodium borohydride. Sheets of chromatography paper were therefore reduced by this method and used in the quantitative determination of vanillin and syringaldehyde. But this treatment produced no marked change in the syringyl to vanillin ratio and peroxides were therefore not the source of error.

Since the error might be connected with the duration of the oxidation, a series of oxidations was carried out at 180^{o} for varying times. A plot of the ratio of the two aldehydes against time (Fig. 19) showed that when the nickel bomb was used (plot A) it was still not possible to obtain a ratio of 2.6, although the ratio had obviously been greatly increased by prolonged heating. Since there might have been slight leakage of syringaldehyde from the bombs, the experiment was repeated using stainless steel bombs kindly loaned by Dr. Stone of the Pulp and Paper Research Institute of Canada. In this

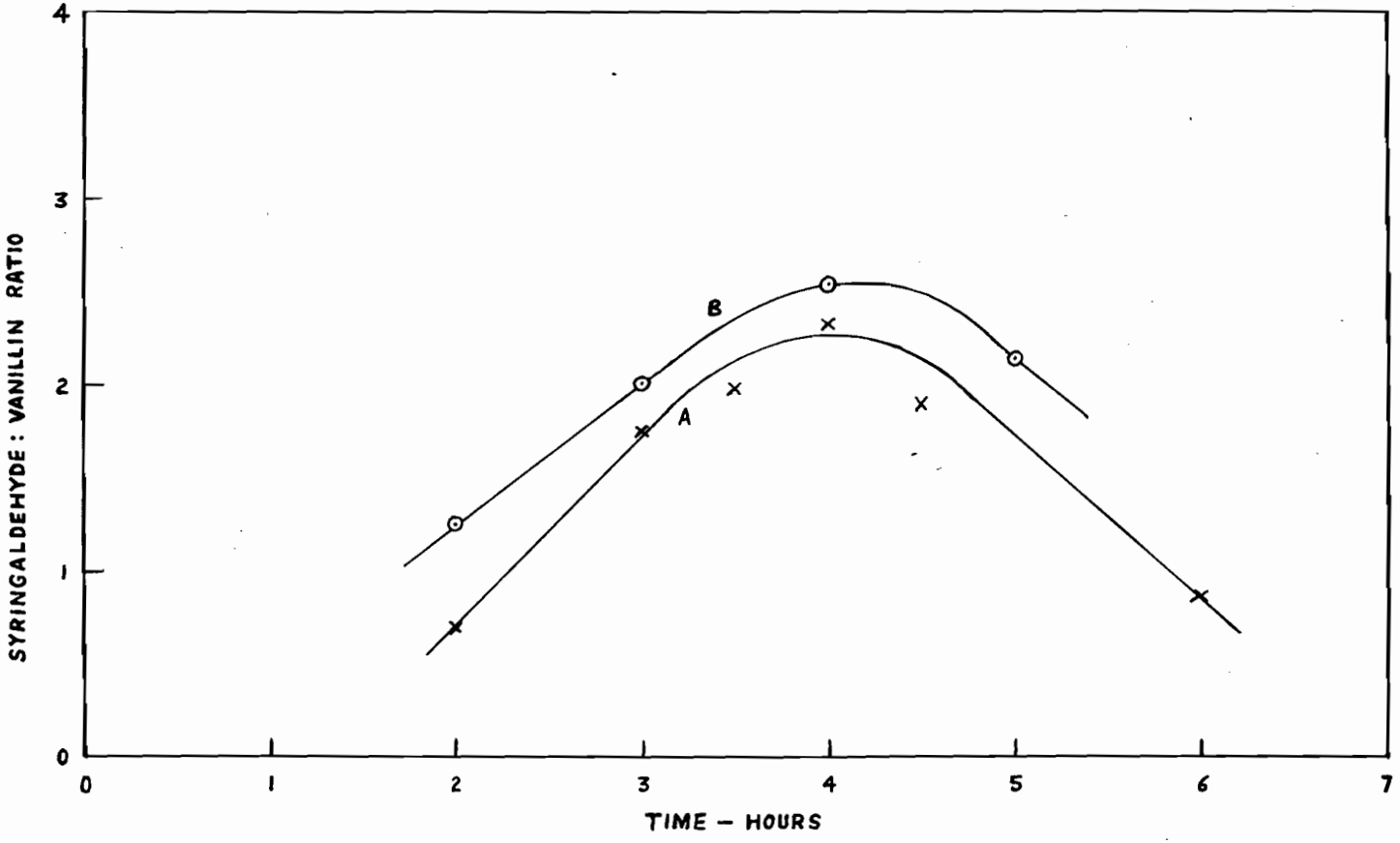


FIG. 19. Variation in syringaldehyde:vanillin ratio against length of oxidation with alkaline nitrobenzene at 180°.
(A - plot using nickel bomb; B - plot using steel bomb.)

case (Fig. 19, plot B), a definite maximum was obtained at a time of just over 4 hours, showing that in comparison with experiments carried out in a glycol bath there was a considerable time lag in the heating of the bombs in the air-oven.

Therefore all further quantitative oxidations were carried out at 180° for just over 4 hours with the results shown in Table 9.

TABLE 9. Ratio of syringaldehyde to vanillin obtained on alkaline nitrobenzene oxidation of various samples.

	Ratio, syringaldehyde/vanillin
Birchwood	2.6
Birch periodate lignin	2.3
LS1	1.1
LS2	2.3
LS3′	1.5
LS4	2.4

The lower ratio for birch periodate lignin might indicate that more end groups in the hardwood lignin molecule were syringyl units than guaiacyl units. It has already been mentioned that end units bearing free phenolic hydroxyl groups would be destroyed during the preparation of periodate lignin.

The syringaldehyde:vanillin ratios of samples LS1, LS2, LS3′, and LS4 indicated that during sulphonation at 98° a lignin having a roughly equal number of guaiacyl and syringyl units was dissolved, leaving a lignin which had a higher ratio of syringyl units to guaiacyl. Oxidation of birch spent sulphite

liquor by Pearl and Beyer (177) gave weight ratios for syringaldehyde and vanillin of 1.89 when cupric oxide was used as oxidising agent, and 0.83 when silver oxide was used. The oxidising agent therefore had a profound effect on the amount of aldehydes produced and therefore, perhaps, not too much importance should be placed on absolute values of the ratios obtained. However, for the present comparative purposes, when the same oxidising agent was used for several samples, the results were of considerable interest.

The ratios for samples LS1 and LS3′ compared favourably with a value of about unity obtained by Pepper (94) for the aldehydes produced from the 25% of aspen lignin that he found was less easily dissolved in dioxan:water (9:1). For the more readily dissolved lignin, he obtained a ratio of syringaldehyde:vanillin of about 2.5. He suggested that this more easily dissolved lignin came from the middle lamella, and that the other was cell-wall lignin. Therefore, one might speculate that in sulphonation it was the cell-wall lignin which dissolved first, while the lignin from the middle lamella required higher temperatures to bring about solution.

Naturally, this speculation is open to much criticism. It has already been shown that contradictory results could be obtained from experiments on the same species of wood (p. 50) so that conclusions drawn from comparisons of experiments on different types of wood must necessarily be held in doubt. Nevertheless, the present results on birch periodate lignin

were in accord with those of Marth (167) who studied the lignin dissolved from cooks of aspenwood. He found, by microscopic examinations, that the liquors which were removed during the early stages of the cook, and which had a high guaiacyl content, probably originated mainly in the cell-wall and were not necessarily sulphonated. In the later stages of the cook the more highly sulphonated middle lamella, having a higher syringyl content, was removed.

The present results therefore provide further support for the idea that guaiacyl and syringyl units are not homogeneously distributed across the cell-wall and that the ratio of syringyl units to guaiacyl units may be greater in the middle lamella lignin, which is sulphonated first but does not dissolve first.

Oxidations with Iodic Acid and Alkaline Potassium Permanganate

As discussed in the Historical Introduction, drastic oxidation with alkaline potassium permanganate afforded an indication of the amount of lignan-type units in spruce lignin, because these types were probably the precursors of the benzenepolycarboxylic acids isolated. Oxidation with iodic acid in hot concentrated sulphuric acid gave similar information about the same lignin, which obviously would be condensed to Klason lignin.

First, a comparison was made between the products from iodic acid oxidations under Eisenbraun's conditions, (178), 180-200°, of birchwood and sprucewood, the resulting

TABLE 10. Rf values of products from iodic acid oxidations[a].

a) Temperature 180-200°

Birchwood	Sprucewood
Rf = 0.000?	0.000?
0.017	0.017
0.059	0.059

b) Temperature close to 180°

Birchwood	Sprucewood
1.Rf = 0.00	0.00
2. 0.05	0.05
3. 0.10	0.11
4. 0.22	0.23
5. 0.41	0.41
6. 0.85	0.85

c) Temperature close to 180°

Birch periodate lignin	Spruce periodate lignin
1.Rf = 0.00	0.00
2. 0.04	0.04
3. 0.10	0.10
4. -	0.17
5. 0.20	0.20
6. 0.36	0.35
7. -	0.76

d) Temperature close to 180°

Birchwood	Birch periodate lignin
1.Rf = 0.00	0.00
2. 0.05	0.04
3. 0.10	0.09
4. 0.20	0.20
5. 0.36	0.36
6. 0.77	-

[a] methyl isobutyl ketone:formic acid:water (10:1:1), and a bromophenol blue spray

benzenepolycarboxylic acids being separated by paper chromatography. The results (Table 10a) indicated no difference between the two types of wood. In a confirmatory experiment, the temperature of the reaction was maintained at or a little below 180°. Chromatography of the products separated six distinct spots (Table 10b), one of which remained on the starting line and was probably mellitic acid.

From the Rf values of pure benzenepolycarboxylic acids (Table 11) spot no. 2 in Table 10b was probably the pentacarboxylic acid, no. 3 the 1,2,3,4-tetracarboxylic acid, and no. 4 the 1,2,4,5-tetra-isomer. This seems to be the first time that the 1,2,3,4-isomer has been detected in such an oxidation of wood or lignin and confirmation of this observation is needed. The other two spots were possibly those of benzenetricarboxylic acids.

TABLE 11. Rf values of benzenepolycarboxylic acids (after Eisenbraun (178)).

Mellitic (benzenehexacarboxylic)	0
Benzenepentacarboxylic	0.035
Benzene-1,2,3,4-tetracarboxylic	0.12
" -1,2,4,5- "	0.22
" -1,2,3,5- "	0.24

These results indicated the necessity for maintaining strict temperature control in this oxidation, since obviously at higher temperatures some of the acids formed were liable to be destroyed. Dlouhy and Kleinert (179) studied the reaction between 180° and 220° and found that oxidation was a function of temperature and time. P.P.R.I.C. testing procedure C-O-2 recommended that the temperature of the reaction flask should not be raised much beyond 170°. When this was done, two spots appeared on the chromatogram for spruce periodate lignin (kindly donated by Mr. Das) which were not present for birch periodate lignin (Table 10c). The spot of Rf 0.76 was probably the spot at Rf 0.85 from both spruce- and birch-wood, but the spot at Rf 0.17 was not identified.

A comparison of the oxidations of birchwood and birch periodate lignin, keeping the temperature just below 180°, is shown in Table 10d. One of the tricarboxylic acids (?) seemed to be absent from the products of oxidation of birch periodate lignin, in spite of the fact that both the birchwood and the lignin would be highly condensed by the concentrated sulphuric acid at 180° present during the oxidation.

On similar oxidation and chromatography of the products, sample LS4 gave the same number of spots as birch periodate lignin itself. Hence dissolution during sulphonation probably did not occur by means of any lignan-type units in the lignin.

The above results showed a slight alteration in the units yielding benzenepolycarboxylic acids in the isolated periodate

lignin as compared with the wood, and also a slight difference between the periodate lignins isolated from sprucewood and birchwood. In view of the possible condensations caused by the drastic acid conditions used in the oxidation, it is difficult to place much importance on the slight differences. Spruce lignin might show more condensation in the iodic acid - sulphuric acid than birch lignin, since in birch the 5-position was blocked by a methoxyl group in many units, thus cutting down the possibilities for condensation. However, one would expect this difference to exist in the woods also, and such was not found in the present study.

For the sake of comparison, a sample of birch periodate lignin was oxidised with alkaline potassium permanganate under conditions not expected to produce further condensation of the lignin. Chromatography of the products separated two spots, a faint one which remained on the base line and was presumably mellitic acid, and a stronger one with an Rf value of 0.03 which was probably benzenepentacarboxylic acid. There was perhaps a very weak spot at Rf 0.05. Possibly the same result as this would have been obtained if the iodic acid oxidation of birch periodate lignin had been carried out at 180-200° and not below 180°. No definite spot was detectable for the tetracarboxylic acid found by Jain (180) from spruce periodate lignin, which also failed to yield any hexacarboxylic acid. This result would tend to indicate that although both oxidations gave rise to benzenepolycarboxylic acids, they did not necessarily yield identical products.

There has been some disagreement in the results obtained by permanganate oxidation of lignins. Systematic errors in the work of Read and Purves (17), Cabott and Purves (181), and Chudakov (182) have been indicated by Zarubin and Tishchenko (183). Chudakov oxidised Scholler's lignin (a technical sulphuric acid lignin obtained in the wood saccharification process with 0.4% sulphuric acid at 170°) and obtained only tetra- and tri-acids. The pentacarboxylic acid obtained in large amounts by the other workers was not detected. Zarubin suggested that this discrepancy might be due to the use of non-extracted materials, but it has been stated (184) that both Read and Cabott used wood very thoroughly extracted with solvents. Thus Zarubin was at least partly wrong in his surmise.

While these oxidations indicated that dissolution during sulphonation probably did not discriminate any lignan-type units in lignin, it did not seem feasible to draw any definite conclusions from the differences observed until a more thorough investigation of the actual reactions involved had been carried out, especially in relation to the effect of temperature on the yield and nature of the products. When this information becomes available, useful data might be obtained from a very careful quantitative study of the two woods, of the periodate lignins, and of the ligninsulphonic acids obtained from them.

EXPERIMENTAL

Throughout this thesis, data reported were the mean of at least two concordant determinations.

Analytical Methods

Klason lignin was determined by the standard procedure for lignin in wood (185), as the percentage of lignin which was not dissolved by 72% sulphuric acid. Although most workers used 72% sulphuric acid in this determination, it was shown (186) that hardwood lignins dissolved partially in acid of this strength. For more reliable values, the concentration of sulphuric acid giving the lowest lignin yield with the highest methoxyl content should be determined for each hardwood.

Determinations of moisture were made on accurately weighed samples of approximately 300mg. The sample was placed in a stoppered container and heated in the oven at 105° overnight with the stopper removed. The container was then re-stoppered before removal from the oven, cooled in a desiccator, and weighed to determine the loss in moisture.

The standard procedure (187) was used for the determination of holocellulose. This method was based on the exhaustive extraction of a 300mg. sample with ethanol-monoethanolamine alternated with a series of brief chlorinations. This method proved very laborious because of the very

slow filtration of ethanol and water washes. Possibly, during the reaction, part of the sample had been converted into a colloidal state which clogged the sintered-glass filter.

Ash was determined by heating to constant weight, in a micromuffle furnace at 750°, accurately weighed samples, 10-20mg., of known moisture content.

The method of Viebock and Schwappach (188) as modified by Peniston and Hibbert (189) was used for the determination of methoxyl, employing the apparatus of Clark (190). Sample size was approximately 15mg.

Sulphur was determined by the Parr micro-bomb method (191), employing 20-100mg. of sample depending on the sulphur content expected. The barium sulphate formed during oxidation in the bomb, was collected, ignited, and weighed in a Neuberger crucible.

Analysis of sodium paraperiodate was made using a modification of the Fleury-Lange method (192). The paraperiodate was treated with potassium iodide in a solution buffered with borax.

$$Na_3H_2IO_6 + 2KI + H_2O \longrightarrow 2KOH + 2NaOH + NaIO_3 + I_2$$
$$I_2 + H_3AsO_3 + H_2O \longrightarrow H_3AsO_4 + 2HI$$

Since iodates did not liberate iodine from iodides in neutral or basic solution, therefore only the periodate reacted, giving two equivalents of iodine, which in turn oxidised one mole of arsenite to arsenate and were thus determined. If x ml. of arsenite were required to neutralise

the iodine, then the weight of periodate in the aliquot was,

$Na_3H_2IO_6(g.) = \frac{x}{2000} \times 0.1 \times 294.$

Paper Chromatography (193)

1. Descending method.

Sheets of Whatman No. 1 paper were used. A pencil line was ruled about 7 cm. from, and parallel to, one end. Then test solutions were applied from a micropipette to points 3 cm. apart on this line, and in such a way that the wetted spots would not exceed 1 cm. in diameter. When the spots were dry, the sheets were allowed to equilibrate with the solvent vapour in tightly covered chromatography tanks, which were previously saturated with the vapour of the appropriate solvent mixture for 24 hours. Several hours later the ends of the chromatograms were dipped into the solvent trays and the tank was closed tightly. When the solvent had run downward about 35 cm., the paper sheets were removed and dried, and mobile substances were detected with a suitable spray reagent.

2. Ascending method.

In this method a pencil line was drawn on the chromatography paper an inch from, and parallel to, one end. After application of the test spots, the sides of the paper were stapled together to form a cylinder which was equilibrated with the solvent system vapour in the previously saturated chromatography tanks. The cylinder was then placed upright

in the tightly-covered tank, in a petrie dish containing the solvent system, which came about ½ in. up the paper. The chromatogram was developed until the solvent front had nearly reached the top of the paper. The paper cylinder was then removed and dried; the staples were removed, and the mobile substances detected with a suitable spray reagent.

3. Reduction of chromatography paper.

The method of Kleinert and co-workers was used (176). The sheets of paper were rolled and placed into wide graduated cylinders, so that no excessive bending of the sheets took place. The cylinders were then filled with a 0.2 M solution of sodium borohydride, and the reduction was allowed to proceed for 24 hours. After this treatment, the sheets were washed in the cylinders with distilled water until acid-free. Then the washed sheets were dried at room temperature in a forced draft cabinet.

Under this treatment, Whatman No. 1 paper disintegrated and therefore No. 3 paper had to be used. Even this showed signs of blistering from the action of the reducing solution.

4. Solvent systems.

The components of each solvent system were thoroughly shaken and then allowed to stand overnight. The appropriate layer was then placed in the chromatography trays.

5. Composition of detecting reagents.

a) Ferric chloride-potassium ferricyanide (194): Water, 300ml.,

1% ferric chloride, 100ml., and 1% potassium ferricyanide, 100ml., prepared freshly as required. Phenolic zones turned blue but were not permanent. If the paper was washed with very dilute hydrochloric acid immediately after spraying, fairly permanent spots were obtained.

b) 2,4-Dinitrophenylhydrazine (195): A 0.4% solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid. Aldehydes and ketones turned brown, orange,or pink, on a pale yellow background. The colour was fairly permanent.

c) Bromophenol blue (196): Bromophenol blue, 50mg., was dissolved in 5ml. of distilled water and neutralised with sodium hydroxide to pH7. The volume was then made up to 100ml. with ethanol. Acidic zones immediately turned yellow against a blue background but the spots were not permanent. Prior to spraying chromatograms run in acidic solvents, drying overnight at 105^{o} was necessary.

d) Fast Red Salt GG (commercial name for the stabilised diazo-salt of p-nitroaniline) (197): A 0.05% aqueous solution of the salt was sprayed on the paper immediately after exposure of the paper to ammonia vapour. The paper was allowed to dry in the air, and the colour of any spots was recorded. After 30 minutes the paper was sprayed with a saturated solution of sodium carbonate in water and allowed to dry. The colour of the spots was again recorded. Acidic, ketonic, and aldehydic compounds give variously coloured spots.

Preparations

1. Trisodium paraperiodate.

The procedure of Hill (198) was used. Water, 3.5l., was heated to about 40-50° in a beaker, and 400g. of sodium iodate was slowly added with stirring. When all the iodate had dissolved, pure chlorine gas was vigorously bubbled through the solution, and 350g. of solid sodium hydroxide (Analytical Grade) was slowly added. The temperature was gradually raised to the boiling point and the passage of chlorine was continued until no more paraperiodate was precipitated and the solution became yellow. Heating was then stopped, but chlorine was passed for a further 5 minutes before its inlet pipe was disconnected. The white precipitate was removed by filtration through a sintered-glass funnel after the solution had cooled to room temperature. The product was washed with cold water until the filtrate was neutral and was oven-dried at 105°. Two batches of paraperiodate were prepared with yields of 467.3g. (78.7%) and 513.7g. (86.5%) respectively.

The paraperiodate was analysed by the procedure described on p. 111.

Purity found: batch I, 99.3%; batch II, 98.0%.

A solution of sodium paraperiodate, of approximately 5% concentration, was prepared by slowly suspending 200g. in 3l. of water, stirring continuously, and then adding glacial acetic acid until the solution became clear (pH 3.6 - 4.2). The

stirring was continued for a further 5 minutes, and then the solution was filtered through a sintered-glass funnel to remove any undissolved paraperiodate, and any silica originating from the glassware used in the preparation of the paraperiodate.

2. Birch periodate lignin.

A modified version of Ritchie and Purves' method (16) was used. The birch woodmeal, 60-80 mesh fineness, had previously been extracted with ethanol, benzene, and water.

The solution of sodium paraperiodate, about 3l., was placed in a large, glazed, stone crock, and, with slow stirring, 400g. of woodmeal was slowly added. The crock was covered with cardboard to exclude light and dust, and stirring was continued for 24 hours. Then the suspension was filtered through a sintered-glass funnel under suction and the filtrate was put aside for subsequent recovery of paraperiodate. The residual woodmeal on the funnel was washed with cold water until free from acid.

Distilled water, 17l., was then run into the crock and 68g. of sodium hydroxide (Analytical Grade) was added with stirring. The oxidised woodmeal was suspended in this solution and stirring was continued for 24 hours before the suspension was allowed to settle for several hours and the supernatant liquid decanted. The residue was repeatedly washed by filling the crock with distilled water, stirring, allowing it to settle,

and syphoning off the supernatant water. This sequence was continued until the pH of the wash water was about 8. The solution was then acidified to pH4 with glacial acetic acid, well stirred, and allowed to settle overnight. After another decantation the residual woodmeal was recovered on a sintered-glass funnel. The oxidation-hydrolysis cycle was repeated four or five times, until the residual periodate lignin showed a Klason lignin content greater than 78%.

The periodate lignin was finally washed with distilled water and dried in the air. Yields and analyses were given in Table 3.

3. Recovery of sodium paraperiodate.

Sodium hydroxide pellets were added to the hot filtrate from oxidised woodmeal until the filtrate became strongly alkaline, a large quantity being required. Chlorine was then bubbled into the boiling solution at a brisk rate for about 15-20 minutes after the first appearance of a precipitate of sodium paraperiodate. The suspension was kept strongly alkaline throughout by the addition of more sodium hydroxide. The suspension was cooled and filtered, and the precipitate was washed on a Buchner funnel three or four times with cold water and dried. The purity of the sodium paraperiodate was checked against an arsenite solution as previously described.

If insufficient chlorine was passed, colourless crystals

of sodium acetate formed on cooling, while with a great excess of chlorine sodium chloride separated.

4. Standard buffer solutions.

Buffer solutions of pH1-6 were prepared according to the directions of Clark and Lubs (199) from 0.2N potassium chloride, 0.1M potassium hydrogen phthalate, 0.1N and 0.2N hydrochloric acid, and 0.1N sodium hydroxide. The acid was standardised against borax and the purity of the potassium hydrogen phthalate was determined by titration against standard alkali (found to be 100%).

The composition of the buffer solutions of pH3-6 inclusive is shown in Table 12. A buffer solution of pH2 was prepared from 25ml. of 0.2N potassium chloride plus 5.3ml. of 0.2N hydrochloric acid; that of pH1 from 25ml. of 0.2N potassium chloride and 47.5ml. of 0.2N hydrochloric acid; both were made up to 100ml. with distilled water.

TABLE 12. Composition of buffer mixtures of pH3-6.

pH	0.1M potassium hydrogen phthalate (ml.)	0.1N hydrochloric acid (ml.)	0.1N sodium hydroxide (ml.)	
3	50	20.32	-	+ water to give 100ml.
4	50	-	3.70	
5	50	-	23.85	
6	50	-	45.45	

The pH of each buffer mixture was measured on a Beckman Glass Electrode pH meter (Model H2) which had previously been

calibrated against an M/20 solution of potassium hydrogen phthalate. This salt had a pH of 4.00 at room temperature.

5. Standard potassium iodate in concentrated sulphuric acid.

Kleinert's method (200) was used. About 6g. of Analytical Grade potassium iodate, moistened with a few drops of distilled water, was placed in a flask and Analytical Grade concentrated sulphuric acid was added to make about 100ml. of the mixture. Heat was supplied carefully while stirring with a thermometer, whereupon the potassium iodate dissolved. The solution was heated at 180-200° for 30 minutes, thus oxidising any organic impurities and stabilising the solution as regards its oxidation equivalent. The cooled solution was kept in a bottle with a ground glass stopper.

Sulphonation and Hydrolysis

1. Preliminary experiments.

The conditions used were those selected by Smith (113) as optimum for the sulphonation and hydrolysis of spruce periodate lignin at atmospheric pressure.

Sulphonation was carried out in a three-necked flask carrying a condenser, stirrer, and thermometer. About 5g. of finely powdered birch periodate lignin, accurately weighed, was heated on a steam bath at 96-98° with a solution containing 40g. of sodium bisulphite in 250ml. of water. Aliquots, 20ml., of the well-stirred mixture were withdrawn

after various time intervals and centrifuged. The residue in the centrifuge tube was washed with distilled water and recentrifuged five times, and then dried in vacuo over phosphorus pentoxide at 40°. From the weight of the residue the yield of insoluble stage I ligninsulphonic acid was determined. These stage I acids were then separately treated overnight with 15ml. of a buffer solution at pH2, and then heated on a water bath at 96-98°, for 24 hours, in an apparatus similar to that used for sulphonation. The weight of residue was determined after it had been centrifuged, washed, and dried in vacuo over phosphorus pentoxide at 40°. The difference between this weight and the weight of the insoluble stage I ligninsulphonic acid gave the weight of the soluble stage II acid.

The results from a run using a sample of birch periodate lignin prepared by Lim Lee (Fig. 5) agreed well with those from a sample prepared in the present work (Fig. 6). In each case no more than 30% of the stage I ligninsulphonic acid could be hydrolysed to a soluble stage II acid.

The initial and final pH of the bisulphite liquor from the second cook were both 5, as measured with a Beckman Glass Electrode pH meter (Model H2) which had previously been calibrated. Thus the pH had not changed during the cook.

A sample of the solid stage I ligninsulphonic acid was twice de-ashed with 1N hydrochloric acid and thoroughly washed. In an attempt to measure the pH of this free stage I acid in

aqueous suspension, it proved difficult to obtain a definite reading. The needle of the pH meter varied between pH3 and 4, whereas the free stage I acid from spruce periodate lignin had a pH of 2.

Sulphur determinations were made on the soluble stage I ligninsulphonic acids obtained during the second run (Fig. 8).

2. Effect of pH on the hydrolysis of the stage I lignin-sulphonic acid.

About 5g. of lignin and a solution containing 40g. of sodium bisulphite in 250ml. of water were heated under reflux for 96 hours on a steam bath at 96°, using the same apparatus as in the previous experiment. The product was recovered on the centrifuge, washed five times with distilled water and dried *in vacuo* over phosphorus pentoxide at 40°.

Accurately weighed samples, about 0.3g., of the sulphonated lignin were allowed to stand overnight in 15ml. of the buffer solution. The mixture was then heated in the same apparatus as before for 24 hours, centrifuged, washed, dried, and weighed. A plot was made of the pH against the corresponding percentage of insoluble stage I ligninsulphonic acid dissolved on hydrolysis (Fig. 9).

In a second experiment, hydrolysis of the stage I acids was carried out in sealed glass tubes immersed in a small diethylene glycol bath thermostatically controlled at 96°. The tubes were opened using the method described by Brickman (98), and the contents transferred to centrifuge tubes and

then treated as above. In both runs it was found that more of the solid stage I ligninsulphonic acid dissolved at pH3 than at pH2.

3. Effect of time on the hydrolysis of the stage I ligninsulphonic acid.

A solid stage I ligninsulphonic acid was prepared from 5g. of birch periodate lignin as previously described.

Samples of the stage I acid, 0.3g., together with 15ml. of buffer solution at pH3, were sealed in glass tubes and allowed to stand overnight. The tubes were heated in the diethylene glycol bath at 96° for varying times and the contents then centrifuged, washed, dried, and weighed as previously described.

Hydrolysis was found to be almost complete after 24 hours (Fig. 10).

4. Effect of increased temperature on the hydrolysis of the stage I ligninsulphonic acid.

Samples of birch periodate lignin, 0.3g., were cooked with aqueous 16% sodium bisulphite in sealed tubes for 96 hours at 96°. The stage I acids were isolated as before and hydrolysed in sealed tubes with a buffer mixture at pH3 for 24 hours at 105°.

There was no increase in dissolution of the stage I acid with the increase in temperature. (% dissolved: about 27%.)

5. Effect of increased temperature on sulphonation.

Samples of birch periodate lignin, 0.3g., and 15ml. of aqueous 16% sodium bisulphite solution, were sealed in glass tubes and immersed in a diethylene glycol bath thermostatically controlled at 105°. Tubes were removed after given time intervals, opened, and their contents centrifuged, washed, dried, and weighed.

Hydrolysis of these stage I acids was carried out for 24 hours at 96° in sealed tubes with 15ml. of a buffer solution at pH3. The percentage of dissolved stage I ligninsulphonic acid was obtained as previously and plotted as a function of time (Fig. 11). The plot showed that an increase in the temperature of sulphonation caused an increase in the amount of stage I acid dissolved during hydrolysis.

6. Effect of concentration of the bisulphite solution on the dissolution of lignin.

Samples of birch periodate lignin, 0.3g., together with 15ml. of aqueous 16% or 8% sodium bisulphite, were heated for 24 hours in sealed tubes at 96°. Hydrolysis, also in sealed tubes, was at 96° for 24 hours in a buffer solution at pH3.

No significant difference was observed in the amount of lignin dissolved in each case. (16% solution: 12% dissolved during sulphonation and 15% during hydrolysis;

8% solution: 13% dissolved in each reaction.)

7. Statistical experiments.

Sulphonation and hydrolysis were carried out in glass tubes as in the previous experiments, but these glass tubes were placed inside steel bombs similar to those described by Cabott (97). Before screwing on the caps of the steel bombs a little water was introduced to give a back pressure. The bombs were then rotated for a given time at the desired temperature in a large steam-heated diethylene glycol bath at the Pointe Claire Laboratories of the Pulp and Paper Research Institute. Opening of the glass liners, centrifugation, and washing of the residue was exactly as described for the lower-temperature cooks.

In each case sulphonation involved 0.3g. samples of periodate lignin, and 15ml. of aqueous 16% sodium bisulphite. Hydrolyses of the residues were carried out in 15ml. of buffer solution at pH3.

8. Sulphonation of methylated samples.

The procedure used was that described for the statistical experiments at high temperatures, using 0.3g. samples. For low-temperature sulphonations the steel bombs were not used and the reaction was carried out in the small electrically-heated diethylene glycol bath.

9. First large-scale sulphonation and examination of the fractions obtained.

About 20g. of birch periodate lignin was suspended in 1l. of aqueous 16% sodium bisulphite contained in a three-necked flask fitted with a mechanical stirrer, a thermometer, and a condenser. The mixture was heated on a steam-bath kept near 96° for 96 hours, after which it was centrifuged, and the supernatant liquid, together with the first washing of the residue, was stored in the cold room (LS1). The residue was washed five times with distilled water and centrifuged each time. Finally, the residue was dried to constant weight. All but a small sample (LS2) of the dried residue was then suspended overnight in a buffer solution at pH2 (solid to liquid ratio of 1:50); the suspension was then heated on a steam-bath near 96° for 24 hours, and finally centrifuged. The supernatant liquor containing the soluble stage II ligninsulphonic acid was stored in the cold room together with the first washing of the residue (LS3). The residue was washed with distilled water and centrifuged five times. It was then dried to constant weight (LS4).

After concentration, the bisulphite solution, LS1, was acidified with sulphuric acid to pH1, whereupon a white flocculent mass of sodium sulphate precipitated with evolution of sulphur dioxide. On filtration, a bright yellow solution was obtained, to which sodium hydroxide was added to bring the pH to about 7. Considerable darkening

of the solution occurred as the neutral point was reached. Some sodium sulphate separated from this solution overnight, but the greater part was obtained as a copious white precipitate on the addition of two volumes of alcohol. After filtration and washing, the bright yellow filtrate was evaporated to small volume under reduced pressure in a rotary evaporator and was then freeze-dried. Considerable darkening of the solution occurred on concentration. Since the cream-coloured residue contained a very high percentage of ash (85.4%) it was redissolved in a small amount of water and the precipitation with ethanol was repeated. This time a light-brown residue was obtained.

Found: ash, 41.9%; moisture, 9.9%; sulphur, 6.6%; methoxyl, 13.7%.

The amount was too small to permit of further attempts at de-ashing.

Fraction LS2 was analysed.

Found: ash, 2.6%; moisture, 6.2%; sulphur, 1.2%; methoxyl, 20.1%.

Since about 90% of the total solids in the liquor, LS3, proved to be dialysable through cellophane, the inorganic matter it contained could not be separated by this technique.

Fraction LS4 was analysed.

Found: ash, 1.9%; moisture, 3.2%; sulphur, 0.8%; methoxyl, 22.3%.

10. Second large-scale sulphonation.

The aim of this experiment was to prepare a hydrolysate without the interference of the inorganic ions present in LS3, and to try to isolate low-molecular weight compounds from this hydrolysate.

Ten grams of birch periodate lignin was suspended in 500ml. of aqueous 16% sodium bisulphite and heated on a steam-bath for 96 hours near 98°. Centrifugation and washing were carried out as in the first large-scale sulphonation. The residue was then de-ashed twice with normal sulphuric acid to liberate the free stage I ligninsulphonic acid, and again thoroughly washed. Autohydrolysis of this product was in 500ml. of distilled water kept near 98° for 24 hours. After centrifugation and washing, the hydrolysate and first washing (LS3′) were evaporated somewhat on a rotary evaporator. The solution was then carefully neutralised with 2% barium hydroxide solution, evaporated to small volume on a rotary evaporator, and then freeze-dried to give a much expanded, light brown material. (Yield: 5g. Found: ash, 14.6%; moisture, 3.7%; sulphur, 3.1%; methoxyl, 19.8%.)

Prior to neutralisation one aliquot of the solution was evaporated to dryness to determine the solids content, and another was dialysed against frequent changes of distilled water and then evaporated to dryness. The result indicated that 75% of the free acid was dialysable. This determination was repeated after neutralisation with barium

hydroxide, and 70% of the barium salt was found to be dialysable.

An aqueous solution of LS3′ was subjected to chromatography, using the descending technique, in butanol: 2% aqueous ammonia, in butanol:pyridine:water (10:3:3), and in butanol:ethanol:water (3:1:1) for 12-16 hours. The first two systems caused no movement of phenolic substances but the last-mentioned system produced two spots (Rf 0.51 and 0.77) which gave a blue colouration with ferric chloride-potassium ferricyanide, and were presumably phenolic. The spot of Rf 0.77 was of low intensity and diffuse. This spot was subjected to a few colour reactions with specific spray reagents, but no attempt was made to separate the compound.

Examination of the spot of Rf 0.51 obtained from LS3′

Three grams of LS3′ were shaken with distilled water. Only about half of the material went into solution, indicating that LS3′ had in some way been changed by drying, since it was obtained from aqueous solution. The aqueous extract was streaked across thirteen large Whatman No. 1 chromatography papers and developed in the system butanol:ethanol: water (3:1:1) for 16 hours, using the descending technique. After drying the papers, trial strips were cut from the edges of each chromatogram and sprayed with ferric chloride-potassium ferricyanide to detect the position of the spot of Rf 0.51. The areas of the papers containing this spot were

then cut from the chromatograms and the unknown substance was eluted from the cut-out strips with aqueous ethanol (1:1). Ethanol alone eluted the spot too slowly to be of practical use. The eluant was evaporated to dryness on a rotary evaporator and the residue was taken up in a small amount of distilled water and freeze-dried. The resulting compound, "X", was tan-coloured. (Yield, about 70mg., i.e. about 2% of the fraction LS3'.) A little of the substance was dissolved in distilled water, spotted on a chromatogram, and developed in the butanol:ethanol:water system to test its homogeneity. The bulk of the substance moved as a spot of Rf 0.53, but there was a very faint spot at Rf 0.17, suggesting that compound X had undergone a little change during its isolation. Samples of the compound were analysed for sulphur, and an infrared spectrum was run.

Methylation with Dimethyl Sulphate

The methylation was based on a general procedure outlined by Vogel (201), depending on the reaction

$$ROH + (CH_3)_2SO_4 + NaOH \longrightarrow ROCH_3 + CH_3NaSO_4 + H_2O$$

where R is an aliphatic or aromatic system.

The sample, 5g., was placed in a stout-walled flask and 50ml. of distilled water was added. The contents of the flask were agitated vigorously by means of a mechanical stirrer. Caustic soda solution, 0.2N, was added dropwise from a burette until 5ml. were released, at which time a 30%

solution of dimethyl sulphate in dioxan was added at an equal rate until 50ml. of each solution had been added. A small amount of heat was applied to the flask at the commencement of the reaction. Agitation was continued for 30 minutes and the product was then recovered on a filter, washed, and dried in vacuo over phosphorus pentoxide.

The procedure was repeated until the methoxyl content showed no further increase (Table 13).

TABLE 13. Methoxyl content of methylated lignin

Cycle	% OMe
1	23.7
2	24.8
3	26.03
4	26.05

Oxidations with Alkaline Nitrobenzene

1. Qualitative oxidation of solid samples.

The micromethod of Stone and Blundell (168) as modified by Towers (171) was used, with the exception that heating was carried out in an air-oven at 175° as described by Jain (180).

Woodmeal, 40mg., or lignin, 10mg., with 0.06ml. of freshly-distilled nitrobenzene and 1ml. of 2N sodium hydroxide, was sealed in a small nickel bomb and heated in an air-oven at 175° for 3 hours. During this time the bomb was shaken

vigorously two or three times. The bomb was then rapidly cooled, centrifuged for 10 minutes, and its contents directly spotted on to Whatman No. 1 chromatography paper. The paper, with the dried spots, was held for a minute over a beaker containing boiling glacial acetic acid to liberate the free phenolic groups. Translucence of the spots was taken as indication of complete acidification. After the spots were allowed to dry for 10 minutes, the paper was placed in the chromatograph chamber containing either the system butanol: 2% aqueous ammonia, or butanol:pyridine:water (10:3:3), or petroleum ether (b.p. 100-120°):n-butyl ether:water (6:1:1). The paper was equilibrated overnight, the chromatogram was run for 15-16 hours, and was then sprayed with ferric chloride-potassium ferricyanide or 2,4-dinitrophenylhydrazine. In the first two systems the chromatograms were developed by the descending technique, and in the last-mentioned by the ascending technique.

In some cases, when nitrobenzene and its products caused much streaking on the chromatogram, a larger-scale oxidation was used, the oxidation products being extracted by the method of Pearl (173). Lignin, 200mg., and 4ml. of freshly distilled nitrobenzene were sealed with 4ml. of 2N sodium hydroxide in a nickel bomb and heated at 175° for 3 hours. The bomb was then rapidly cooled and aromatic products in the contents were repeatedly extracted with small amounts of ether until the

ether layer was no longer coloured. Aqueous sulphuric acid, 10%, was then added, drop by drop, to the aqueous layer until it reacted acid to Congo red paper. The acidic aqueous layer was then shaken vigorously with ether to remove aldehydic and acidic oxidation products. The ether extract was dried over anhydrous sodium sulphate and the solvent evaporated. The residue was then taken up in a little alcohol and spotted on a chromatogram which was run and sprayed in the usual manner.

At first, considerable difficulty was experienced with the petroleum ether:n-butyl ether:water system. Sometimes the spots refused to move at all and at other times incomplete separation occurred with much streaking. No reason for this behaviour could be found, and when the system did eventually work properly, no alterations in the procedure had been made. Since the initial, unsuccessful, experiments were made during very hot weather while the later, successful, ones were made during the winter, the humidity of the air or the temperature perhaps affected the system. To obtain good separation of vanillin and syringaldehyde, it was most important that the air in the chromatography tank was thoroughly saturated with the aqueous phase of the system. Also, complete acidification of the spots was essential.

2. Qualitative oxidation of liquid samples.

The method was that used by Stone (152). One millilitre of concentrated liquor from a bisulphite cook was made up to

be 2N in sodium hydroxide by the addition of 0.5ml. of 6N alkali. Freshly-distilled nitrobenzene, 0.15ml., was added and the bomb heated at 160° for 2½ hours, with vigorous shaking two or three times during this period. The oxidation mixture was then chromatographed as usual.

3. Quantitative oxidation of solid samples.

Vanillin and syringaldehyde were determined by the spectrophotometric method of Lemon (202) after separation on paper chromatograms using the procedure of Stone and Blundell (168).

A known amount of the oxidation mixture was spotted on Whatman No. 1 paper (5 spots, each of 0.01ml. volume). In addition, a sixth spot was placed about 1 inch away from the others. After development in petroleum ether:n-butyl ether:water (6:1:1) by the ascending technique, the lane of the sixth spot was cut out and the positions of vanillin and syringaldehyde were detected by spraying with 2,4-dinitrophenylhydrazine. The bands containing the aldehydes were cut from the unsprayed paper and eluted with 25ml. volumes of absolute ethanol in micro-Soxhlet extractors for 2½ hours. The cooled ethanol extracts were treated with 4ml. of 0.2% alcoholic potassium hydroxide, and the volume adjusted to 50ml. with absolute ethanol in a volumetric flask. The optical densities of the alkaline ethanolic solutions were then determined using a Beckman Model DU

spectrophotometer, with a tungsten light source, at the wavelength of maximum absorption; 352 mμ for vanillin and 368 mμ for syringaldehyde. Conversion of the density to the concentration in milligrams per litre was by the factors 5.33 for vanillin and 6.76 for syringaldehyde (173). From these values the weight ratio of syringaldehyde to vanillin was found.

Initial determinations on birchwood itself gave varying syringaldehyde to vanillin ratios of about unity, as compared with values of 2.6 to 3 determined by earlier workers. The alterations made in the conditions of the reaction to increase the ratio of the aldehydes to 2.6 has already been discussed (p.99). They were such that the oxidations of birch periodate lignin and the fractions obtained from the large-scale sulphonation of the lignin, were carried out at 180° for just over 4 hours.

Oxidations with Iodic Acid

The details were taken from Eisenbraun's thesis (178).

One gram of sample was placed in an open 50ml. flask and was mixed with 10ml. of a properly prepared, saturated solution of potassium iodate in concentrated sulphuric acid (p. 119). A violent reaction started with evolution of iodine. Through a funnel, 10g. of powdered potassium iodate was added in small portions. Strong fumes of iodine were again evolved and much heat produced. The temperature was

held at 180-200° for 40 minutes and nitrogen was bubbled through the solution. An additional 10ml. of concentrated sulphuric acid was added, and about 10 minutes later 50mg. of paraformaldehyde, and the mixture was heated again for 20 minutes, to destroy excess iodic acid. The solution was then cooled and 25ml. of distilled water carefully added.

After being boiled to expel iodine, the solution was cooled, made up to 60ml. with distilled water, and then continuously extracted with ether for 24 hours. The ether extract was evaporated to dryness several times with water on a rotary vacuum evaporator. Finally, the residue was taken up in 8ml. of distilled water and one or two drops of this solution were spotted on the chromatography paper.

The benzenepolycarboxylic acids formed were developed by a descending method using a solvent composed of isobutyl methyl ketone:formic acid:water (10:1:1), the aqueous layer being used for conditioning the paper overnight and the organic phase as the developer. The chromatogram was run for 12 hours, dried in the air overnight, and then placed in an oven at 105° to remove traces of formic acid which interfered with the bromophenol blue spray.

The effect on this reaction of changing the temperature slightly has been noted in the Discussion (p. 107).

Oxidation with Alkaline Potassium Permanganate.

Birch periodate lignin was oxidised with alkaline

potassium permanganate according to the method of Read and Purves (17). Details of the procedure were taken from Jain's thesis (180).

Eighteen grams of birch periodate lignin in 2.9l. of 1% potassium hydroxide were heated on a water-bath at 75-80° and oxidised by the gradual addition of powdered potassium permanganate. Additions were continued over a period of 2 days until the solution remained pink. Then 10-15ml. of ethanol was added to the cold alkaline solution to decompose any residual permanganate. The manganese dioxide formed was separated and washed and the solution and combined washings were reduced under vacuum to 4 litres. After acidification to pH2.6, the solution was further evaporated to about 700ml. and the pH brought to 10.5 by addition of dilute potassium hydroxide. The barium salts of the polycarboxylic acids and oxalic acid were then precipitated by adding a hot solution of 10% barium chloride to the hot liquid. The precipitate was digested in its mother liquor for 30 minutes, recovered on the centrifuge, and washed twice with a little dilute barium hydroxide to replace any entrained potassium ions by barium ions.

The dry barium salts were extracted with boiling 1N hydrochloric acid and the extract treated with the exact amount of 1N sulphuric acid to precipitate barium as barium sulphate, which was removed on the centrifuge. The liquor was evaporated to small volume and, after removal of crystalline

oxalic acid dihydrate, was evaporated to dryness. The residue was taken up in 20ml. of concentrated nitric acid, and boiled for 2 hours to destroy any residual oxalic acid. On evaporation of the solution to dryness a small amount of a yellow crystalline solid was obtained which, by paper partition chromatography in isobutyl methyl ketone:formic acid:water, was found to contain mellitic acid and benzenepentacarboxylic acid.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. Four batches of birch periodate lignin were prepared which compared favourably with similar preparations made by earlier workers.

2. A sample of birch periodate lignin was subjected to a bisulphite cook using the conditions determined by Smith to be optimum for the sulphonation of spruce periodate lignin, namely sulphonation with aqueous 16% sodium bisulphite at 98°, followed by hydrolysis at 98° at pH2 for 24 hours. Only 30% of the birch periodate lignin dissolved compared with almost complete dissolution of spruce periodate lignin.

3. Sulphur determinations showed that a maximum of 2.1% was introduced under the conditions employed. This corresponds to a S:OMe ratio of about 0.1, in agreement with similar findings by other workers.

   The amount of sulphur introduced was considerably less than that introduced into spruce periodate lignin under similar conditions ($\sim$ 3.5%), and the sulphur plot closely resembled the hydrolysis plot, indicating that a minimum sulphur content was necessary for dissolution as had previously been shown to be the case for spruce periodate lignin.

4. The effect, on the rates of sulphonation and hydrolysis, of changing such variables as time, temperature, concentration and pH was studied. A pH of 3 gave a slightly greater dissolution during hydrolysis than a pH of 2, and a suspension of the free stage I birch ligninsulphonic acid had a pH of 3 compared with a pH of 2 for the corresponding acid from spruce periodate lignin. The only appreciable increase in the dissolution of lignin occurred on increasing the temperature of sulphonation.

5. A statistical treatment was applied to the sulphonation and hydrolysis of birch periodate lignin. The results indicated that the solution of birch periodate lignin was not a two-stage process, but depended entirely on the temperature and time of the cook. Thus Hagglund's two-stage theory of sulphonation apparently did not hold for birch periodate lignin.

6. From the statistical data, a value for the activation energy of 23,890 cal./gm.mole was calculated, as compared with 20,200 cal./gm.mole for spruce wood, indicating that under technical conditions near 140^{o} the two woods pulped similarly. This was in agreement with many such claims made in the technical literature.

7. Methylation with dimethyl sulphate showed that birch

periodate lignin contained fewer hydroxyl groups than did spruce periodate lignin. There was an increase in methoxyl of 0.96 mole per base molecular weight for birch, compared with an increase of 1.7 moles for spruce. As in the case of spruce, methylation blocked sulphonation, e.g. at 135° only 30% of the methylated birch periodate lignin dissolved as compared with complete dissolution of the unmethylated lignin.

8. A large-scale sulphonation of birch periodate lignin was carried out in 16% aqueous bisulphite at 98° (Smith's conditions), and attempts were made to isolate a low-molecular weight fragment containing a sulphonic acid group. A "soluble stage II ligninsulphonic acid" was chromatographed in butanol:ethanol:water (3:1:1), a system which had been used to separate naphthalenesulphonic acids. This system separated 70mg. of a compound, X, from 20g. of birch periodate lignin. An infrared spectrum indicated that X was probably a low-molecular compound containing a small amount of sulphonic groups and possibly exhibiting some form of conjugation. The sulphur content (2.24%) suggested that this fraction was of little significance.

9. Qualitative oxidations with alkaline nitrobenzene yielded no evidence of vanillin or syringaldehyde from the soluble products of sulphonations at temperatures below 75°. At

75°, both vanillin and syringaldehyde were obtained from the oxidation mixture, indicating that birch periodate lignin did not contain a purely guaiacyl component.

10. Quantitative oxidations on the products of a large-scale sulphonation at 98° (employing Smith's conditions) indicated that a lignin having a roughly equal number of guaiacyl and syringyl units was dissolved, leaving a lignin which had a higher ratio of syringyl to guaiacyl units. Other workers suggested that the lignin in aspen which was more readily dissolved in a bisulphite cook was situated in the cell-wall, and was high in guaiacyl units; the middle lamella lignin was only dissolved in the later stages and had a higher syringyl content. The present results for birch periodate lignin were in agreement, and gave further support for the idea that the guaiacyl and syringyl units were not homogeneously distributed across the cell-wall in hardwoods.

11. Oxidations of birch periodate lignin with iodic acid and alkaline potassium permanganate gave results that in general were qualitatively similar to those obtained for spruce periodate lignin and for the woods themselves. Thus dissolution during sulphonation probably did not discriminate any lignan-type units in lignin.

APPENDIX A

Data from sulphonation and hydrolysis experiments used in plotting Figures

Fig. 5

Time of sulphonation (hrs.)	0	4	8	12	24	30
% dissolved during sulphonation	-14.2	0.6	-5.3	8.9	11.9	13.7
% dissolved during hydrolysis	-16.3	20.2	27.9	29.0	30.3	28.8

Fig. 6

Time of sulphonation (hrs.)	0	3	6	12	24	48	72	60*	72*	85*	96*
% dissolved during sulphonation	-8.0	-13.0	-6.5	1.7	4.5	14.7	31.8	9.8	11.6	9.2	15.5
% dissolved during hydrolysis	3.2	19.5	19.8	28.9	21.0	28.6	26.3	-	-	-	29.2

*Separate experiment with no aliquots withdrawn until sulphonation had proceeded for 60 hours (see P. 59).

Fig. 8

Time of sulphonation (hrs.)	3	6	12	48	72
% S in stage I acid	1.50	1.87	2.08	1.98	2.12

Fig. 9

	run 1					run 2				
pH of hydrolysis liquor	2	3	4	5	6	1	2	3	4	5
% stage I acid dissolved	28.8	37.8	31.0	21.0	21.7	27.3	22.0	26.4	17.8	15.0

Fig. 10

Time of hydrolysis (hrs.)	6	12	24	48	72	96
% stage I acid dissolved	16.9	17.9	21.6	25.5	27.5	26.9

	run 1				run 2					
Fig. 11 Time of sulphonation (hrs.)	4	8	48	96	6	12	24	48	72	96
% dissolved during sulphonation	3.8	7.3	25.8	36.6	(8.4 (6.1	(9.9 (11.6	(16.1 (17.5	(23.9 (24.8	(30.7 (31.6	(39.5 (41.4
% dissolved during hydrolysis	13.6	14.3	39.1	61.1	(8.2 (10.0	(11.5 (11.5	*(9.9 *(12.6	(16.7 (17.3	(40.1 (44.4	(58.5 (64.6

The duplicate values given for run 2 in Fig. 11 illustrate the errors involved in these experiments.

*These tubes were cooked together and the apparently low percentages dissolved presumably reflect a failure in the heating system for a short period.

APPENDIX B

Outline of Calculations Based on the Statistical Data

On completion of the twelve experiments to locate conditions which gave complete dissolution of birch periodate lignin in aqueous sodium bisulphite, an attempt was made to relate lignin removal to cooking temperature and time over the range of conditions covered experimentally.

A possible functional mathematical model was proposed, based on the work of Maass and co-workers (104).

Where R = % lignin remaining

t = time in hours

T = temperature in ^{o}A

then, for a first order reaction

$$\ln R = a - kt \quad \ldots\ldots\ldots\ldots (1)$$

where k = reaction rate.

From the Arrhenius equation

$$k = b\, e^{-E/GT} \quad \ldots\ldots\ldots\ldots (2)$$

Where E = activation energy in cal./gm. mole

G = gas constant = 1.985.

Substituting (2) in (1)

$$\ln R = a - t\, b\, e^{-E/GT} \quad \ldots\ldots\ldots\ldots (3)$$

From equation (1), when t = 0

$\ln R = a$ and R = 100

Therefore a = 4.60517

Equation (3) can be transformed into:

$$\ln \left(\frac{4.60519 - \ln R}{t} \right) = \ln b - \frac{E}{G} \cdot \frac{1}{T} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

which is a simple linear equation of the form:

$$Y = c + dX$$

where $Y = \ln \left(\frac{4.60517 - \ln R}{t} \right)$

$c = \ln b$

$d = -\frac{E}{G} = -\frac{E}{1.985}$

$X = \frac{1}{T}$

Then by the method of least squares, equation (4) can be solved to give values for ln b and E which minimize the variance of the experimental points about this relationship.

This procedure was followed and a value for E (the activation energy) of 23,890 cal./gm. mole was obtained. The value for ln b was 27.385. Equation (4) therefore becomes:

$$\ln \left(\frac{4.60517 - \ln R}{t} \right) = 27.385 - 12036. \frac{1}{T} \quad \ldots\ldots\ldots (5)$$

Equation (5) can be represented graphically as on p. 78, where the expected lignin remaining is shown for any combination of time and temperature.

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