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LIGNIN

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I. INTRODUCTION

The study of the structure of natural lignin and the conditions under which it is formed in the plant cell, and also the search for methods of utilising the enormous amount of lignin wastes from the modern paper and hydrolytic industries, are tasks of great scientific and economic importance.

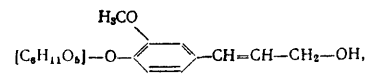
Lignin constitutes about 30% of the plant resources which are annually renewed on a hugh scale on the earth. It is the most important natural aromatic polymer, and is thus potentially a rich source of phenolic products, which are widely used in modern industrial organic synthesis.

During the past decade a great deal of research has been done on the chemistry and the biogenesis of lignin, which to a large extent enables us to understand and to explain the structure of this interesting natural polymer.

II. PATHWAYS OF LIGNIN BIOSYNTHESIS IN PLANTS

Study of plant evolution shows that lignin has a protective function, since the start of biosynthesis of lignin in plants is associated with the transition to life on land. Botanists consider that lignin first appeared in the Pteridophyta. No lignin is present in algae, liverworts, or mosses. It is contained in small amounts in horsetails, and definitely occurs in ferns[1].

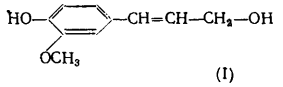
The process of lignification and lignin formation in the plant cell must be regarded as part of normal metabolism. Manskaya[1] showed that cambium and wood of the new annual layer of pine contain β-glucosidase and the oxidising enzymes peroxidase and polyphenol-oxidase. Coniferin (a glucoside)



found in the cambium of conifers, serves as a substrate

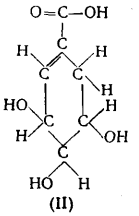
for oxidising enzymes. Under the action of the β-glucosidase it is split into glucose and coniferyl alcohol, the latter of which, by being oxidised and reduced, can serve as an intermediate link in the chain of reactions accompanying respiration of the cells. When the living cells die off in the course of lignification, the oxidising processes begin to predominate over the reducing processes, and the oxidation products are deposited in the form of lignin. Thus on Manskaya's hypothesis the formation of lignin is directly associated with breakdown of the respiratory apparatus in plant cells. When the cell wall, and in particular the region of the middle lamella, where the main amount of lignin is found, lignifies, the life of the cell and the activity of the enzymes cease.

The biosynthesis of lignin in plants has been investigated in detail during recent years by several workers[2-6]. A method which proved to be fruitful was to compare the principles underlying the synthesis of amino-acids by bacteria and of lignin monomers by the plant cell. Many protein amino-acids, *e.g.* phenylalanine and tyrosine, contain benzene nuclei. The experimental results now available show that coniferyl alcohol (I) (or its glucoside coniferin) is formed in the plant *via* the same intermediate stages as occur in the biosynthesis of phenylalanine or tyrosine in micro-organisms:



(I)

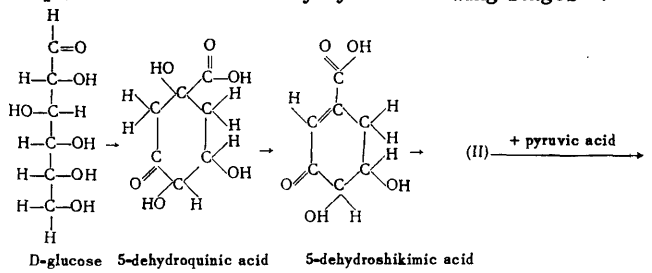
A protracted study of the metabolism of some mutants of *Aerobacter aerogenes* and *Escherichia coli* using radioactively labelled starting materials clarified the biosynthetic pathways of aromatic and in particular phenylpropane derivatives, which include phenylalanine and tyrosine as well as the lignin monomer, coniferyl alcohol[7,8]. It was shown that, starting from glucose, bacteria synthesise aromatic amino-acids in several stages. The organism first converts the glucose molecule through intermediate steps into the cyclic compound shikimic acid (II), a substance which is widely distributed in the vegetable kingdom[9]:



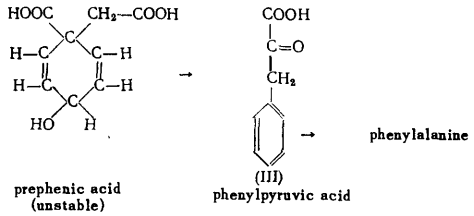
(II)

This latter compound, after a series of changes, passes into the aromatic compound hydroxyphenylpyruvic acid[10] and then into an aromatic precursor of proteins.

The biosynthesis of aromatic substances by bacteria can be represented schematically by the following stages[11]:



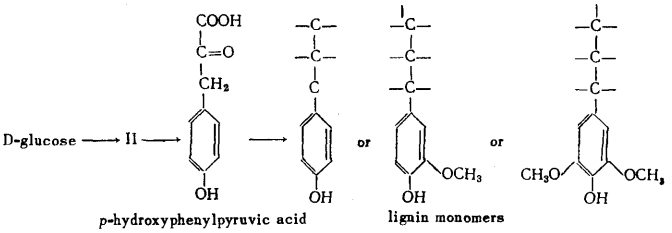
D-glucose 5-dehydroquinic acid 5-dehydroshikimic acid



prephenic acid (unstable) (III) phenylpyruvic acid

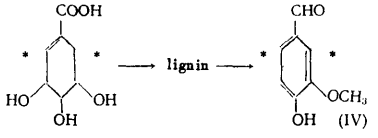
As can be seen from the scheme, the glucose molecule (the main product of photosynthesis) is converted into the cyclic compound 5-dehydroquinic acid[12], and then into 5-dehydroshikimic acid[13] and shikimic acid. This last acid can condense with pyruvic acid to form the unstable prephenic acid[14], which splits off carbon dioxide and water to yield (III). Phenylalanine can be formed from (III) by transamination[15].

The synthesis of aromatic lignin monomers by plants resembles the bacterial synthesis and is represented by the following sequence[11]:



p-hydroxyphenylpyruvic acid lignin monomers

The possibility of accomplishing such a stepwise aromatisation of shikimic acid has been demonstrated by the work of American biochemists[16-18]. Shikimic acid labelled in the 2,6-positions was introduced artificially through the leaves into the growing sugar cane. After six days of such feeding, the plant was cut, the lignin was separated, and from it vanillin (IV) was obtained by the usual methods. The cyclohexene ring of shikimic acid was shown[18] to pass directly into the aromatic nucleus of lignin, the radioactive carbon tracer (^{14}C) occupying the 2,6-positions in the vanillin:



(IV)

According to investigations by Brown and Neish[19], precursors in the formation of lignin can be also phenylalanine, cinnamic acid, and especially 4-hydroxy-3-methoxycinnamic (ferulic) acid, which is detected chromatographically in aqueous extracts of straw and wheat[20].

To obtain confirmation of these hypotheses, Freudenberg[21] introduced radioactively labelled ferulic acid or penylalanine into the wood of a young spruce. Structural

lignin was obtained by treating the radioactive region of the branches with alcohol in the presence of hydrochloric acid (by ethanolysis). After radioactively labelled phenylalanine had been introduced, it was possible to isolate from the wood of the spruce active lignin and also active coniferin[22]. These results show that plants possess all the enzymes which are necessary to convert an aromatic amino-acid into a ring-substituted carbinol. The hydroxylation of the nucleus, partial methylation of the phenolic hydroxyl, and reduction of the terminal carboxyl group, evidently occur at the same time as aromatisation, and are catalysed by enzymes of the phenolase type[23,24] or methyl-transferase[25]. The order in which the hydroxylation, methylation, and reduction reactions occur has not yet been confirmed experimentally.

When L-coniferin, obtained from L-glucose and radioactive coniferyl alcohol, was used, no radioactive lignin was obtained[26], which is perhaps explained by the absence of the enzyme L-glucosidase in plant tissue.

Freudenberg showed the presence of cellular β-glucosidase by using the synthetic glucoside indican, which is split by the β-glucosidase present in wood tissue into glucose and indoxyl. The latter is oxidised to indigo by the action of air. Application of indican to a wood section gave a blue coloration due to indigo[27].

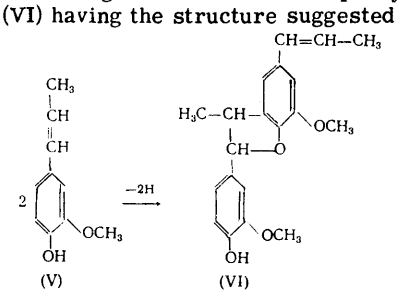
According to Freudenberg, coniferyl alcohol — coniferin aglucone — originates from an acid possessing a C_6-C_3 grouping, the precursors of which are shikimic and prephenic acids. During the period of growth, the cambium and the neighbouring cells contain a large amount of coniferin[28]. It diffuses into the newly formed cells, and is there hydrolysed by β-glucosidase into glucose and coniferyl alcohol. The latter, coming into contact with laccase and peroxidase, is transformed into lignin[27].

Thus lignin formation involves both enzyme systems, the presence of which in the lignification zone can be detected by means of colour reactions[29,30].

III. ARTIFICIAL PREPARATION OF LIGNIN BY ENZYMIC DEHYDROGENATION OF CONIFERYL ALCOHOL

Study of the course of the biochemical synthesis of lignin in plants *in vivo* proceeded in parallel with intensive investigation of the intermediate products formed by enzymic oxidation of coniferyl alcohol *in vitro*. The genetic relationship of coniferin to lignin was first suggested by Tiemann as early as 1875 [31], and later this idea was fruitfully applied by Klason[32].

The possibility of obtaining a product similar in properties to lignin by enzymic oxidation of aromatic monomers was pointed out by Erdtman[33]. He showed that the substance obtained by Cousin in 1908 [34] by the oxidation of isoeugenol (V) with fungal oxidases was the phenylcoumaran derivative (VI) having the structure suggested for lignin:



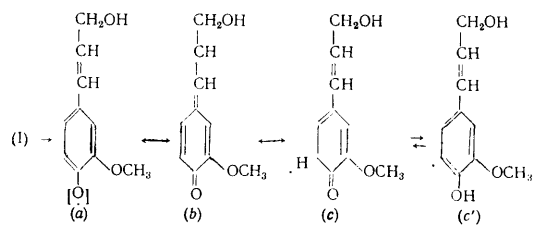
(V) (VI)

Study of this oxidising enzymic reaction made clear the principle underlying the formation of lignin, which on Erdtman's hypothesis[33] was to be regarded as a product of the dehydrogenation of guaiacylpropane derivatives having an oxidised side-chain.

During the past decade Freudenberg and his collaborators have studied the possibility of preparing lignin *in vitro*. When air is passed through a dilute (< 0.5%) aqueous solution of coniferyl alcohol in the presence of fungal laccase at 20° and pH 5.5–6.5, a brownish-grey deposit is formed. Up to 80–90% of the coniferyl alcohol is used up in this process. The fungal laccase can be replaced by cambium laccase, horseradish peroxidase, or dilute solutions of hydrogen peroxide[35]. The time required for the formation of lignin varies from a few minutes to many hours depending on the concentration of the enzymes. Some of the products of low molecular weight can be extracted from the precipitate with butanol, and the residue ("dehydropolymer") represents a biosynthetic lignin formed by enzymic dehydrogenation of coniferyl alcohol.

Physical and chemical studies of biosynthetic and natural lignin preparations suggested that they were quite similar[36-40]. Investigations with labelled coniferyl alcohol *in vitro* and *in vivo* confirmed that coniferyl alcohol is utilised in the biosynthesis of lignin[20,41-43]. The preparation of dimeric products by the enzymic dehydrogenation of coniferyl alcohol *in vitro* and their chromatographic detection in the cambial sap must be regarded as the most important result[44]. Study of the structure of the dimeric products makes it possible to judge the nature of the bonds between the phenylpropane monomers in lignin. It must be stressed, however, that it is impossible to apply with complete success conclusions derived from *in vitro* experiments to processes occurring in the living cell[45]. It is still true that "no natural compound has been structurally elucidated by following its biosynthesis, nor is it likely that this will be accomplished, especially in so complex a group of ill defined compounds as the lignins"[45].

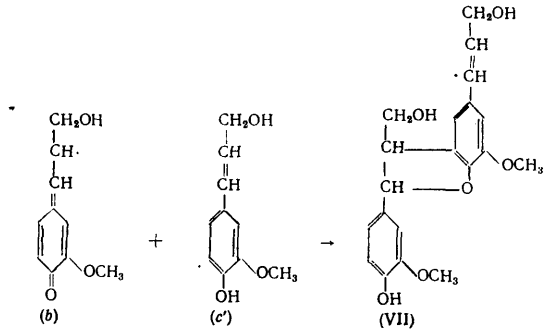
The "oxidase" system (mainly laccase)[46-48] takes part in the enzymic dehydrogenation of coniferyl alcohol and the formation of lignin dehydropolymer. According to Freudenberg[49], the formation of lignin in spruce wood begins with the dehydrogenation of coniferyl alcohol, and the radical which is formed (*a*) can be represented in several mesomeric and tautomeric forms (*b*), (*c*), (*c'*):



As a result of spontaneous dimerisation, these mesomeric radicals are stabilised, forming dimeric intermediate products, the secondary structural units of lignin. Four of them, the structures of which have been elucidated, form about 80% of all these products. It has proved possible to isolate the following intermediates[26,29,49-51]:

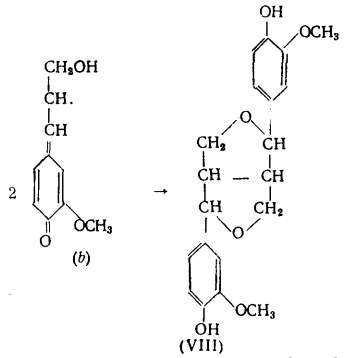
A. Dehydrodiconiferyl alcohol (VII), corresponding to about 20% of the initial coniferyl alcohol. Its formation can be explained by condensation of the tautomeric

radical (*c'*) with a quinomethane of the type of (*b*), the phenolic hydroxyl groups undergoing ring-closure with formation of a benzenoid structure:



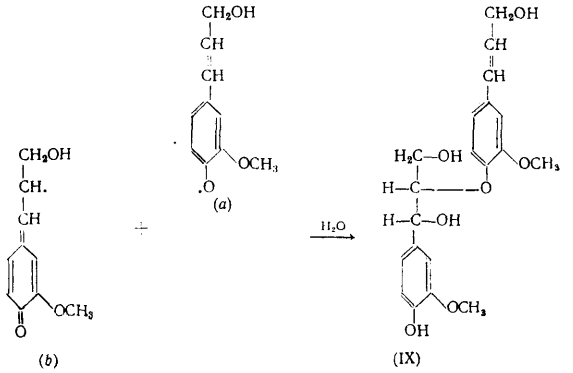
(*b*) (*c'*) (VII)

B. DL-Pinoresinol (VIII), corresponding to 20% of the initial coniferyl alcohol. It is formed from two quinomethyl radicals (*b*):



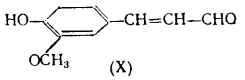
(*b*) (VIII)

C. The β-ether of guaiacylglycerol and coniferyl alcohol (IX), corresponding to 30% or more of the initial coniferyl alcohol. It is formed from (*b*) and (*a*) with subsequent addition of water:



(*b*) (*a*) (IX)

D. Coniferaldehyde (X) (1% yield)

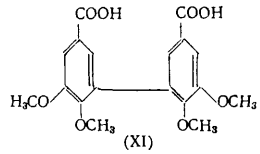


(X)

E. Dehydrodiconiferyl aldehyde, 4%.

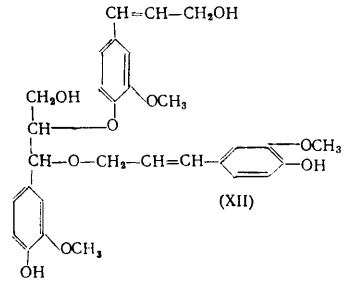
F. Aldehyde corresponding to (IX), 3%.

G. Dehydrodipinoresinol, a crystalline diphenyl derivative formed from two molecules of (VIII) with the elimination of two hydrogen atoms, 4%. Dehydrodiveratric acid (XI) can be obtained from this, after methylation and oxidation; the link between the two molecules is thus in the 5,5'-positions[27].



(XI)

H. A diether formed by two molecules of coniferyl alcohol with guaiacylglycerol (XII), 5%.

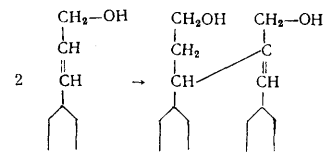


(XII)

I. A diether formed by coniferyl alcohol (β) and dehydrodiconiferyl alcohol (γ) with α-guaiacylglycerol, analogous to (XII).

The maximum yield of each substance occurs at different stages in the formation of lignin[27], and it is therefore unallowable to add up the above percentages. The continued action of enzymes leads to the occurrence of further dehydrogenation and condensation processes, with the formation of polymeric lignin[49]. The initial radical, dehydrogenated coniferyl alcohol, is optically inactive. Therefore all dimeric intermediate products are also inactive, as are both native and biosynthetic lignins.

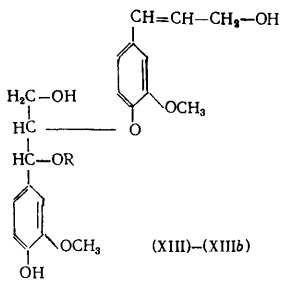
The above forms of combination between different radicals do not exhaust the variety of possible links. Some time ago it was shown spectrophotometrically[52] that diphenyl links probably occur in dehydropolymers and in native lignin. It is possible that α–β bonds are formed between two side-chains, and these may arise as a result of dimerisation of coniferyl alcohol or of a coniferaldehyde group[50]:



The possibility of links between α–α' carbon atoms is likewise not excluded[53,54]. No direct proof of the existence of quinomethyl radicals is yet available. This is evidently due to their short life. Spectrophotometric measurements have shown that their half-life under the experimental conditions was about 60 sec.

If the enzymic dehydrogenation of coniferyl alcohol is carried out in water containing 30% of methanol, the methyl ether (XIIIa) is formed. In the presence of much sorbitol or of a sugar, an adduct is formed between the dimeric

structural unit and the polyhydroxy compound (XIII*b*):



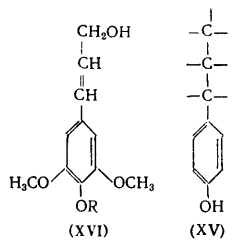
(XIII)–(XIII*b*)

where for (XIII) R = H, for (XIII*a*) R = CH_3, and for (XIII*b*) R = $C_{12}H_{21}O_{10}$.

Direct spectrophotometric measurements[55], colour reactions, and the formation of the methyl ether and the adduct with the sugar (XIII*b*) may serve as a basis for accepting the existence, even if for only a short period, of quinomethyl radicals during the enzymic dehydrogenation of coniferyl alcohol. The formation of a chemical bond with a sugar molecule is important also for an understanding of the nature of the possible links between the aromatic units in lignin and carbohydrates.

The enzymic dehydrogenation of coniferyl alcohol does not lead to its quantitative conversion into biosynthetic lignin. This is explained by the fact that the lignin itself undergoes degradation as a result of the action of the laccase or peroxidase enzymes[35]. This process is probably similar to the humification of lignin and phenolic substrates in the soil. It should also be noted that wood-degrading fungi contain, predominantly in the mycelia, laccase and peroxidase, the enzymic action of which leads to the breakdown of lignin[27]. Consequently, both the formation and the decomposition of lignin can evidently be caused by the same enzymes.

Although coniferyl alcohol may be regarded as the original structural unit for lignin from conifer wood (spruce), the lignin from deciduous species contains also sinapyl alcohol (XIV), while the lignin of annual plants contains *p*-hydroxyphenol residues (XV):



(XVI) (XV)

where for (XIV) R = H and for (XVI) R = $C_6H_{11}O_5$.

Sinapyl alcohol, which is found in the wood of deciduous trees in the form of the glucoside syringin (XVI), does not form lignin. The action of laccase and peroxidase on sinapyl alcohol leads solely to the formation of an 80–90% yield of syringoresinol (the 5,5'-dimethoxy derivative of pinoresinol)[35,56].

Freudenberg believes that in this case intermediates containing phenyl ether links, *e.g.* as in (VII) or (IX), cannot be formed owing to steric hindrance[27]. This to some extent explains the experiments *in vitro*, because the lignin of deciduous wood necessarily includes coniferyl alcohol, apart from sinapyl alcohol, as a structural unit.

Study of the degradation products of lignins obtained from different kinds of plants has shown that each of them is built up from monomers of several types in varying proportions. Therefore the term "lignin" refers not to a single compound but to a group of closely related products[11]. The great variety of combinations arising among the separate structural units leads to the formation, not of an individual substance of definite constitution, lignin, but to a group of "lignin substances"[57].

Gymnosperm lignin (spruce, pine, etc.) is, according to Freudenberg, a dehydropolymer of coniferyl alcohol. The lignin of angiosperms (beech, etc.) is formed by the dehydropolymerisation of coniferyl and sinapyl alcohols. Monocotyledonous plants (bamboo, etc.) contain also a substantial amount of *p*-hydroxyphenyl structural units[58].

IV. ISOLATION OF LIGNIN AND LIGNIN–CARBOHYDRATE BONDS

The separation of unchanged natural lignin ("protolignin") from plant tissue has unfortunately not yet been achieved. All existing methods require the application of chemical, mechanical, or fermentation procedures to the plant tissue for the separation. However, Brauns has shown[59,60] that is is possible to remove 2–3% of the total amount of lignin by extracting wood with cold ethanol. It was found that after numerous reprecipitations the preparation behaved in many respects as protolignin, exhibited typical lignin reactions, and gave derivatives identical with those obtained directly from wood. Chromatographic analysis of the products of hydrolysis of native beech lignin (according to Brauns) showed that it contains no combined carbohydrates[61]. However, it differs substantially from the main part of the lignin, *e.g.* in the content of free phenolic hydroxyls. By extracting spruce wood with cold acetone or alcohol, it is possible to obtain substances of low molecular weight of the type of lignans, to which the soluble lignin obtained by Brauns[37] evidently belongs. Therefore the lignin isolated by Brauns' method can hardly be considered any longer to be representative of the lignin in wood[62].

The task of isolating natural lignin from wood touches directly on the problem of lignin–carbohydrate bonds, the discovery of the nature of which has been the aim of numerous investigations, summarised in the work of Merewther, Lindgren, and others[63-66]. The insolubility of lignin in tissues can be explained in three ways: it is chemically combined with some component of the cell wall, it has a high molecular weight and therefore has a low solubility, or it is "encrusted" with carbohydrates to such an extent that it cannot be extracted by organic solvents.

The "encrustation" theory was shown to be untenable by the work of Brauns and Seiler[67], who ground up finely divided wood meal with water to such an extent that part of it passed into colloidal solution, from which the wood was separated in the form of a fine powder. It could be completely dissolved by cuprammonium hydroxide solution, and reprecipitated with acid. Nevertheless, lignin could not be extracted from such wood powder, either with ethanol or with dioxan. Brauns' lack of success can be explained by the swelling of the wood in water and the possible formation of additional hydrogen bonds between carbohydrates and lignin.

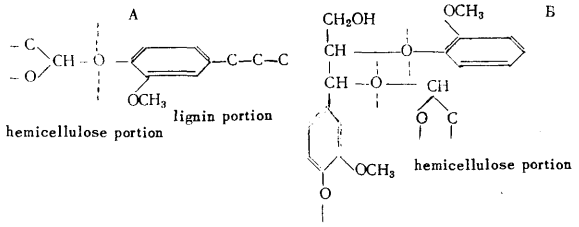
The second possible cause is also improbable, since lignin can be partly extracted from wood by ethanol in the

presence of very small amounts of hydrochloric acid, which are insufficient to bring about the depolymerisation of lignin.

The most probable cause is the presence of hydrolysable lignin–carbohydrate bonds in wood. Heating wood with water, chlorination, ethanolysis, acetylation, and boiling with sulphite and sulphate led to the separation of compounds containing no free carbohydrates but liberating lignin and sugar on hydrolysis. Since such products were separated under greatly varying conditions, it can be assumed that part of the wood exists in the form of a lignin–carbohydrate complex[68-71]. Xylan has been frequently identified as the principal carbohydrate linked with the lignin[72].

According to the results of Aaltio and Roshier[73], wood contains two lignin–carbohydrate complexes, one in the middle lamella, where the greatest amount of lignin is concentrated, and the other (with a very different ratio of lignin to hemicellulose) in the secondary wall.

Considerable scientific and technological interest attaches to the nature of the bonds existing between the lignin and the carbohydrate components of vegetable tissue. Among the many types which have been suggested, the following are selected as the most probable — (A) phenyl β-glucosides and (B) benzyl ethers:



Phenyl β-glucoside structures (A) are abundant in woody tissue (coniferin), and such links are evidently very probable for some of the lignin–carbohydrate complexes.

Study of the ultra-violet absorption of fractions of acetylxylolignin showed the presence of phenyl glucoside bonds in the lignin of wheat straw[74]. As with all glucoside structures, phenyl β-glucosides are hydrolysed by acids. It was shown also that such links, in contrast to alkyl glucosides, are relatively easily broken also by alkalis[71,75]. The possibility of the existence of phenyl β-glucoside links was shown also with a model substance[76].

It is quite probable that some lignin–carbohydrate links are also benzyl ethers (B). In contrast to ordinary ethers, the oxygen bonds here are more difficult to break down with acid[77]. The presence of benzyl ether groups in lignin has now been proved by numerous experiments. The reactive alcoholic hydroxyl attached to the α carbon atom of the propane side-chain of the structural unit of lignin may be the position for the formation of a chemical bond with carbohydrates in the biosynthesis of lignin. This is convincingly shown by the later papers of Freudenberg (see XIII*b*)[55].

Thus there now exists hardly any doubt as to the presence in wood (and in other plant tissue) of a lignin–hemicellulose complex. The chemical nature of the lignin–carbohydrate bonds varies. The most probable are phenyl β-glucoside structures and benzyl ethers.

During recent years attempts have been made to isolate lignin from wood without the use of acid or alkaline catalysts. An interesting method was suggested by Asplund[77a], treatment of the wood with steam at 170°–180° for 1–1½ min. In this case softening of the middle lamella is accompanied by breakdown of the bonds and liberation of the lignin. No data are yet available as to the nature of the resulting preparation; it can merely be assumed that lignin which has been subjected to such a strong, though short-lived, thermal treatment will differ from the natural, native lignin.

Experimental work has shown that high yields of lignin can be extracted from well ground wood by neutral solvents. Recently this has been most successfully accomplished by Björkman[78] and by Pew[79]. The latter ground the wood in a dry state, and observed that the carbohydrates themselves underwent strong cleavage during the grinding. In order to shorten this process, Björkman ground the wood in the form of a suspension in toluene. The resulting wood meal did not swell and proved to be less soluble in alkali than Pew's material.

Wood ground by Björkman's method is extracted first with aqueous dioxan and then either with an aqueous solution of acetic acid or with dimethylformamide. Both fractions contain about half of the lignin present in the wood. The dioxan-soluble lignin contained only a few per cent of carbohydrates (mainly hemicelluloses). The first fraction was termed by Björkman "milled wood lignin" and represents about 30% of the wood lignin. The second fraction, obtained from the dimethylformamide extract, was termed the "lignin–carbohydrate complex"; it contained about one part of lignin associated with 3–4 parts of carbohydrates. Electrophoretic studies[65] have shown that this "complex" consists of two fractions with different mobilities, a pure hemicellulose fraction and a lignin–hemicellulose fraction, in which the weight ratio of the two components is approximately 1 : 1.

Björkman's lignin cannot be regarded in any way as wood protolignin, since mechanical treatment can produce breakdown of the chemical links between the parts of the lignin complex. However, the use of comparatively mild conditions to separate the product in quite a good yield led to the acceptance of this preparation as the most suitable material for studying the structure of lignin.

During the past 10–12 years biochemical methods have also been used to separate lignin[80,81]. In the enzymic breakdown of wood by the so-called "brown rot" fungus, the latter destroys the cellulose and other carbohydrates, setting free the lignin, which becomes accessible for extraction with neutral solvents.

On the basis of his biochemical investigations, Nord[81] concludes that lignin is not combined with other components of wood. This contradicts the now proven facts of the existence of lignin–hemicellulose complexes in wood. Biochemical lignin, obtained by the fermentative action of "brown rot" fungi, can hardly be identical with natural lignin. As Freudenberg[27] has shown, wood-degrading fungi contain, predominantly in the mycelium, laccase and peroxidase, the enzymic action of which leads to rupture of the links between carbohydrates and lignin and also to destruction of the latter.

An investigation made not long ago of the properties of lignin obtained by Schubert and Nord's[81] enzymic degradation of spruce wood with the fungus *Lenzites saepiaria* showed that it differed sharply from natural lignin (especially with respect to the methoxyl content). Therefore the native character of enzymically separated lignin is now denied[82].

Other methods for extracting lignin in the presence of catalysts (acid or alkaline) lead to its interaction with the

solvent used for extraction[83]. It has been proved that an alkoxyl group adds on to lignin when it is obtained by extraction with ethanol in the presence of hydrochloric acid[84], a phenolic group during extraction with phenol[85], and a mercaptoacetic acid group in mercaptoacetyl lignin[86].

The alkoxy groups in the lignin derivatives obtained by condensation of the corresponding alcohol with lignin can be split off again by means of acid[87]. However, this requires the use of severe methods, which may change the nature of the lignin.

A new method for preparing ethanol-lignin[88] consists in treating spruce wood meal with a mixture of alcohol and chloroform (1 : 4) in the presence of 0.2 *N* hydrochloric acid at 60°. In this way it is possible to separate 80% of the lignin in the wood, 20% of it being converted into an ether-soluble oil.

A good yield of lignin can be obtained by the action on wood of benzyl alcohol containing 1–2% of hydrochloric acid[89]. In view of the fact that benzyloxy groups are comparatively readily cleaved forming toluene on reduction with hydrogen in the presence of palladium under mild conditions, Brauns[90] proposed this method for obtaining lignin free from benzyl radicals. To what extent it would be possible to remove benzyloxy groups without changing the lignin is difficult to assess. If this could be fully achieved, then it would be possible to obtain in this way a product closely similar to natural lignin. Hydrolytic methods of splitting carbohydrate – lignin bonds, which make it possible to obtain different lignin preparations (Willstätter's hydrochloric-acid lignin[91], "cuprammonium lignin"[92], or "periodate lignin"[93]), result in a marked change in the properties (*e.g.* the lignin loses its solubility in organic solvents). At the present time these methods, and the products obtained by means of them, cannot be regarded as entirely suitable for studying the structure of lignin.

V. ANALYTICAL DATA[44]

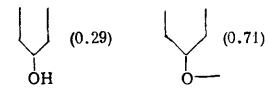
As already pointed out, the lignin isolated by Björkman[78] is closely similar in properties and structure to natural lignin. Therefore the analytical data cited below are based on Björkman's lignin obtained from conifer species (spruce) or on "guaiacyl lignin"[94].

Elementary analysis of the Björkman lignin shows that it contains 63.84% C, 6.04% H, 29.68% O, and 15.75% OCH_3.[78] If account is taken of the fact that this lignin contains 1.9% of a sugar, then the appropriate correction (it being assumed for simplicity that the sugar consists of equal proportions of hexosans and pentosans) yields the following calculated formula for the phenylpropane or C_9 unit:

$$C_9H_{8.83}O_{2.37}(OCH_3)_{0.96} \quad (M = 184.7).$$

It can be seen from this formula that 0.96 OCH_3 group is associated with 2.37 oxygen atoms, or one methoxy group with 2.43 oxygen atoms. The distribution of oxygen not combined as methoxyl is of considerable interest for an understanding of the nature of the functional groups present in the phenylpropane structural unit of lignin.

Of the 2.37 oxygen atoms present in the C_9 monomer, one may be the phenolic oxygen of the guaiacyl nucleus. According to the data of Swedish investigators, however, only 0.29 oxygen atom occurs in the form of a free phenolic hydroxyl[95,96]. Hence the difference, 0.71 oxygen atom, must be present in blocked phenolic groups, evidently as the oxygen of alkyl aryl ethers:



The difference between the total oxygen and the oxygen forming part of phenolic hydroxyls (2.37 - 1 = 1.37 atoms) occurs as aliphatic hydroxyls, and as carbonyl and ether groups.

Acetylation of lignin shows the presence of 1.15 hydroxy groups[78], of which 0.29 represents phenolic hydroxyl. Hence the difference, 0.86, represents aliphatic hydroxy groups (primary, secondary, or tertiary).

The content of carbonyl groups in Björkman's lignin was determined volumetrically using potassium borohydride[97], which gave 0.41–0.48 carbonyl group for every methoxy group. Investigations recently carried out with model carbonyl compounds similar in structure to lignin have shown that the hydroxylamine method gives results which are too low, whereas use of borohydride yields values which are close to the theoretical content of carbonyl groups in the products investigated[97].

If the content of carbonyl groups in lignin is taken as 0.41 (this value requires verification), the remaining aliphatic oxygen (0.51 - 0.41 = 0.10) can be ascribed to alkyl–alkyl ether bridges, since carboxy groups and diphenyl ether links have not been found in spruce lignin.

On the basis of this oxygen balance, the formula for Björkman lignin can be written as $C_9H_{7.68}$(phenolic OH)$_{0.29}$ · · (aliphatic OH)$_{0.86}$(carbonyl O)$_{0.41}$(alkyl aryl ether O)$_{0.71}$ · · (dialkyl ether O)$_{0.10}$$(OCH_3)_{0.96}$.

The molecular weight of the Björkman "milled wood lignin" is about 11 000, which corresponds roughly with 60 phenylpropane units.

VI. THE STRUCTURE OF LIGNIN

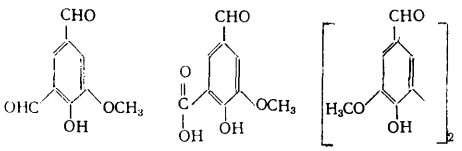
The phenylpropane structural unit of lignin substances (guaiacylopropane unit for spruce lignin) possesses an aromatic nucleus which has been methoxylated and hydroxylated, as well as a propane side-chain carrying various oxygen-containing functional groups.

Consideration of the structure of wood lignin can suitably start from an account of the principles underlying substitution in aromatic nuclei and of the ways in which they can condense together. The methods of hydrolytic cleavage utilised to study many plant polymers (*e.g.* cellulose, starch) are unsuitable for application to lignin. The most valuable have proved to be oxidative cleavage in alkaline medium, cleavage by hydrogenation, and also cleavage with metallic sodium or potassium in liquid ammonia[98,99].

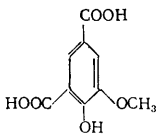
1. Oxidative degradation with alkali and nitrobenzene[100–102]. By this method, vanillin is obtained from spruce lignin, and mixtures of vanillin and syringaldehyde from deciduous lignin. Lignin from straw[103] yields also *p*-hydroxybenzaldehyde on oxidation, this compound being formed in small quantities from lignins of hard woods and soft woods too[104,105].

Spruce lignin has been found[106] to contain also derivatives of vanillin substituted in the *ortho* position relative to

the phenolic hydroxyl:

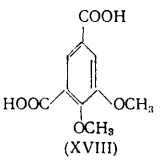


Oxidation of ligninsulphonates obtained by boiling aspen wood with sulphite leads to the separation[107] of appreciable amounts of 5-carboxyvanillic acid (XVII):



(XVII)

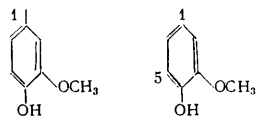
2. Oxidative cleavage of wood meal or of isolated lignin with permanganate, after preliminary methylation with diazomethane, involves the formation of veratric and isohemipinic (XVIII) acids in yields of 4.9% and 0.9% respectively based on the lignin:



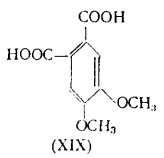
(XVIII)

If lignin is first heated with 70% caustic potash at 170°, then methylated with dimethyl sulphate, and finally oxidised with permanganate, the yield of isohemipinic acid can be approximately doubled[108,110,111].

Experimental data on the nitrobenzene and permanganate oxidation indicate the existence in natural lignin of guaiacyl radicals substituted in the 1- and 1,5-positions:



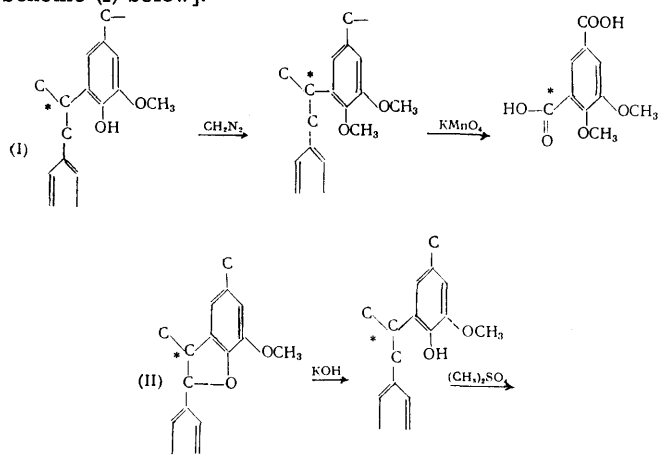
Richtzenhain[109] separated 1.3% of metahemipinic acid (XIX), in addition to isohemipinic acid, by the permanganate oxidation of isolated methylated "alcohol-lignin".



(XIX)

However, he was unable to prove the presence of metahemipinic acid in protolignin by oxidation of wood meal[112]. This acid evidently originates in condensation processes occurring in the lignin macromolecule during its separation with alcohol in the presence of acid catalysts.

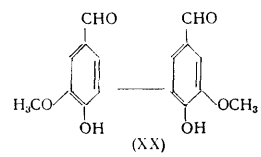
The formation of isohemipinic acid can be explained by the presence of *ortho*-substituted phenolic nuclei [see scheme (I) below].



The increase in the yield of veratric and isohemipinic acids after severe alkali treatment of lignin (before methylation and permanganate oxidation) can be explained either by the presence of etherified phenolic hydroxyls, which are hydrolysed by the action of alkali, or by the existence of a structure similar to that occurring in dehydrodiconiferyl alcohol. Scheme (II) shows that the action of strong caustic alkali can cause rupture of the oxygen link and formation of the product capable of yielding additional isohemipinic acid.

Freudenberg[113] synthesised artificial lignin from coniferyl alcohol labelled in the β-position of the side-chain with radioactive carbon (indicated by an asterisk in the scheme). In both cases of permanganate oxidation (*i.e.* with and without alkali treatment) the resulting isohemipinic acid was radioactive. It is thus proved that the β carbon atom is linked to the nucleus of the neighbouring guaiacyl unit.

In the above two condensation schemes, the aromatic structural units are linked *via* the side-chain. Special interest attaches to condensation with the formation of a diphenyl structure. Pew[114] was able some time ago to isolate dehydrodivanillin (XX) from lignin after oxidation with nitrobenzene and alkali. Under the conditions used, numerous model substances not containing diphenyl links were unable to form such a product.

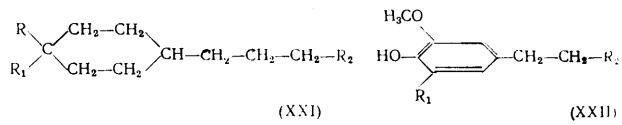


(XX)

The yield of XX was several per cent, so that it can be concluded that diphenyl links of this type are quite abundant in lignin.

The presence of diphenyl structures in natural lignin is supported also by spectrochemical investigations[52]. Thus aromatic nuclei can be linked either by means of an ether bond or by C–C bonds. Leopold[115] suggested that these two types of bonds occur in roughly equal proportions in the lignin molecule. Later investigations show that the number of units linked by C–C bonds ("condensed units") is evidently less and constitutes 30–40%[44] of the total number of links.

3. Hydrogenolysis of lignin preparations. This leads to the production of considerable quantities of derivatives of propylcyclohexane and of substituted phenols (XXI), (XXII) [116-120].



(XXI) (XXII)

1. $R = R_1 = R_2 = H$
2. $R = H$; $R_1 = OH$; $R_2 = OH$
3. $R_1 = R_2 = H$; $R = OH$, etc.

1. $R_1 = H$; $R_2 = OH$
2. $R_1 = OCH_3$; $R_2 = H$
3. $R_1 = OCH_3$; $R_2 = OH$

Identification of the products of hydrogenation and hydrogenolysis showed the presence of one free or etherified hydroxyl group in the γ- and β-positions of the side-chain of the phenylpropane structural unit of lignin [121].

The hydrogenation of sulphate lignin under pressure demonstrated [122] that water-soluble and ether-soluble phenolic compounds predominate among the reaction products, cyclohexanol, catechol, and guaiacol being isolated in addition to other aromatic substances. Results of the hydrogenation of lignin, yielding up to 50% of cyclohexylpropane derivatives, show that a great proportion of lignin is composed of phenylpropane derivatives, since there can be no doubt that the cyclohexyl ring is formed by hydrogenation of benzene nuclei.

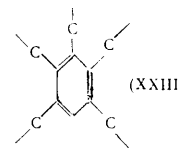
4. An important contribution to the uncovering of the aromatic structure of lignin was made by Shorygina and her collaborators. By treating cuprammonium lignin with metallic sodium in liquid ammonia at -33° nine times, they were able [123,124] to separate dihydroeugenol from the fraction of low molecular weight, and from aspen lignin they also separated 1-(4-hydroxy-3,5-dimethoxyphenyl)propane [125].

Extraction with ether of the acid portion of the decomposition products of lignin yielded hydrated eugenol, *i.e.* 1-(4-hydroxy-3-methoxyphenyl)propan-2-ol. In the case of cuprammonium lignin, a total of about 28% of monomeric aromatic substances was obtained [126].

The formation of dihydroeugenol (as a result of hydrogenation of phenols) was confirmed also by the cleavage of spruce wood with metallic sodium under the same conditions [125]. Chromatographic analysis of the phenols obtained [127] showed that their composition was quite complicated. In addition to the previously observed dihydroeugenol and 1-(4-hydroxy-3-methoxyphenyl)propan-2-ol, the cleavage products contained 1-(4-hydroxy-3-methoxyphenyl)propan-1(and 3)-ols.

The *p*-hydroxybenzyl alcohol grouping, which is extremely important for an understanding of many of the reactions of native lignin (see below), is usually detected by indirect methods. Employment of the decomposition of lignin with sodium enabled Shorygina and her collaborators for the first time to demonstrate the presence of this group directly. The possibility of a far reaching cleavage of lignin with metallic sodium into monomers shows that the aromatic components are linked to one another mainly by C–O–C bonds, which, of course, does not exclude the presence of a certain number of C–C bonds between the main units in lignin isolated from wood [128]. The cleavage of the phenyl ether bonds of lignin by the action of alkali metals in liquid ammonia was confirmed also by Freudenberg's work [129].

Kratzl [98] included, among the criteria which he suggested for distinguishing lignin from other substances in wood, its ability to yield benzenepolycarboxylic acids on oxidation with permanganate [130]. Although the yield of such polycarboxylic acids (especially of the pentacarboxylic acid) obtained from native lignin is low (0.14% according to Cabott's data) [131], the possibility of obtaining them is an argument in support of the existence in protolignin of a small number of systems of the type (XXIII):



(XXIII)

As a result of condensation processes occurring during alkaline or acid treatment of lignin preparations, the yield of polycarboxylic acids obtained during oxidation with alkaline permanganate is sharply increased [131,132].

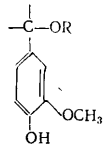
VII. REACTIVE GROUPS IN LIGNIN

Among the reactive groups in lignin, the three carbon atoms of the propane side-chain, which can carry various functional groups, are usually included. These are responsible for the majority of the typical reactions of lignin–sulphonation by sulphite solution at different pH values, alkylation by alcohol, and reaction with mercaptoacetic acid [44]. The phenolic hydroxy (free or etherified) situated *para* to the propane side-chain is also an important reactive group of lignin, governing many properties of native and technical lignins (*e.g.* the ability to dissolve in alkalis, form salts, etc.).

During recent years there has been considerable interest in the determination of the content of phenolic hydroxyl groups in lignin. Their amount forms one of the criteria of identity between artificial lignin preparations and native lignin [133]. Several different methods for determining phenolic hydroxyls in lignin have been published [134-137]. Among the suggested methods the most important is the spectrochemical method, which has been developed and thoroughly investigated by Aulin-Erdtmann [138]. The spectra of phenols undergo bathochromic shifts when they are measured in the ionised condition, *i.e.* in an alkaline medium. If the shift for an appropriate model is compared with the spectrum of lignin, an estimate can be obtained of the number of free phenolic hydroxyls in the preparation under investigation. The content of phenolic groups fluctuates depending on the lignin preparation tested and on the method of determination. Therefore the number of phenolic hydroxyls per phenylpropane unit indicated above (0.29 OH/OCH_3) must be regarded as approximate. The complexity of the lignin molecule and steric factors result in several cases in some of the phenolic hydroxyls not being recorded, even by an optical method [133]. The three-carbon propane side-chain of the aromatic structural unit of lignin is a most important functional group, and it is mainly by means of this group that the phenylpropane units are linked together. The propane side-chain is also the location of the most important reactions, the most characteristic of lignin. The α carbon atom of the propane chain is especially important.

Lignin contains several types of functional groups with different reactivities with respect to sulphonation. However, the hydroxylated α carbon atom (benzyl atom), in either the free or the etherified form (XXIV), is evidently

responsible for sulphonation and for reaction with methanol and hydrochloric acid and with mercaptoacetic acid[139].



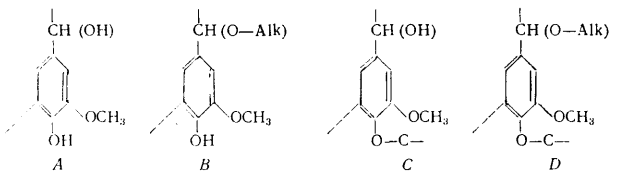
(XXIV)

1. R = H; 2. R = alkyl

The main reaction in the sulphite process for obtaining cellulose is based on the conversion of lignin into water-soluble ligninsulphonic acid. The process by which wood is delignified during boiling with sulphite is still not very clear[140]; according to Hägglund[141], it occurs in two stages: in the first stage, lignin is sulphonated in the solid state without dissolution, and in the second stage the action of hydrogen ions produces hydrolytic liberation of lignin-sulphonic acids and their passage into solution with further sulphonation to a content of about 0.5 SO_3H group per methoxyl. Hägglund's hypothesis of a two-stage sulphonation of lignin was confirmed by boiling periodate lignin[142] with bisulphite.

From an investigation of numerous model substances, Lindgren[143,144] worked out a very probable picture of the sulphonation of the various groups which are evidently present in lignin. He imagined four possible structural variants having different reactivities with respect to sulphonation.

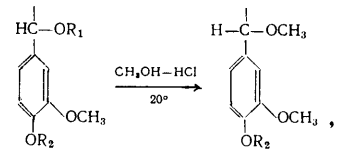
Rapid sulphonation of lignin, which is observed even in neutral sulphite solution, occurs at the expense of the phenolic benzyl alcohol A and its ethers B, the aliphatic hydroxyl or ether group being replaced by SO_3H.



A grouping which reacts slowly in neutral medium but quite rapidly with acid sulphite solution is represented by C, *i.e.* guaiacylcarbinols etherified at the phenolic hydroxyls. Structural elements which react only with acid sulphite solutions (group D) are guaiacylcarbinols etherified both at the phenolic and at the alcoholic hydroxyls. It is quite possible, however, that some of the sulpho groups are combined also with other carbon atoms (not only with the benzyl carbons) of the lignin complex.

It is very significant that during sulphite treatment (as during mild alkylation) no additional formation of free phenolic hydroxyls is observed[95,96,138]. This means that the α carbon atom is linked either to a free hydroxyl or, if it is etherified, to an alkyl but not an aryl radical.

The presence of benzyl alcohol and benzyl ether groups was shown also by the reaction with alcohol in the presence of small amounts of mineral acid. By the action of 0.5% methanolic hydrochloric acid on Brauns' lignin at room temperature, up to 50% of methoxyl (based on its original content in the lignin) can be introduced[145]. Methylation for 72 h enabled up to an additional 70% of methoxyl to be introduced into Björkman's lignin.

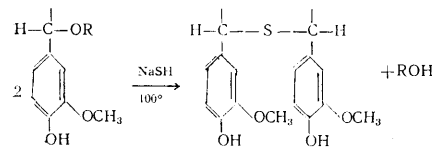


where R_1 = H or C – alkyl; R_2 = H or C – alkyl.

Brauns[89] put forward the hypothesis that such alkylation leads to acetalisation of the carbonyl group or that an existing acetal group is transalkylated. If, however, all the carbonyl groups in lignin are reduced with sodium tetrahydroborate, and the lignin is then again treated with methyl alcohol in the presence of hydrochloric acid, it is methylated just as before reduction. In view of the absence of carboxyl groups in native lignin, the only process which remains probable is alkylation of the benzyl group. Examination of the reaction with methanol in the presence of hydrochloric acid of numerous model substances of the type of benzyl alcohol or benzyl ethers showed that the alcohol or ether groups were replaced by methoxyl, the rate of reaction being different for each class and depending on the nature of the radicals in the side-chain[145].

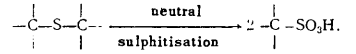
The presence of a benzyl alcohol or an alkyl benzyl ether group in lignin received additional support from a study of sulphide formation in model substances and in lignin during the boiling of cellulose with sulphate[146-148].

It was shown that *p*-hydroxybenzyl alcohol and *p*-hydroxybenzyl ether react with a neutral solution of sodium hydrogen sulphide at 100° to form the corresponding dibenzyl sulphide[146,147].

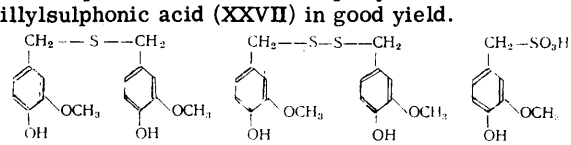


Sulphide formation occurs under similar mild conditions also with Brauns' and Björkman's lignins[149,150].

Neutral sulphite treatment of thiolignin preparations and model sulphides results in the formation of ligninsulphonic acids containing the whole of the sulphur in the form of sulphonic acid groups which can be titrated potentiometrically. The number of such groups corresponds exactly with the quantity to be expected if such sulphite treatment converts one sulphide group into two sulphonic acid groups:



Thus under conditions of the neutral sulphite treatment of thiolignin there is a complete replacement of sulphide groups by sulphonic acid groups[148]. A similar exchange has been shown in model experiments. For example, divanillyl sulphide (XXV) and divanillyl disulphide (XXVI) on neutral sulphite treatment are rapidly converted into vanillylsulphonic acid (XXVII) in good yield.



(XXV) (XXVI) (XXVII)

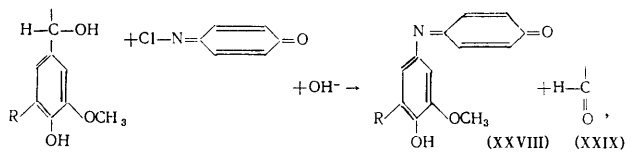
Etherification of the phenolic hydroxyl group completely blocks reaction with sulphite under mild conditions.

If thiolignin preparations are heated with a mixture of acids (HI, HCl, and H_3PO_2)[151], they give up a large part of their combined sulphur in the form of H_2S, which can be determined iodometrically. This cleavage reaction is to some extent specific to *p*-hydroxy- and *p*-alkoxy-benzyl sulphide and disulphide[152]. An analogous cleavage is observed also with ligninsulphonic acids.

On the basis of the above there are sufficient grounds for accepting the hypothesis[153] that the reactions of sulphonation and sulphide formation occur to a considerable extent at the same reactive groups in lignin.

The presence of a benzyl alcohol group in lignin was confirmed also by Gierer's investigations[154]. He found that the action of benzoquinone-*N*-chlorimine on wood or isolated natural lignin forms a blue dye, guaiacylindophenol, identical with the product obtained from free guaiacol and the chlorimine. This reaction may arise from the presence of phenolic guaiacylcarbinol groups in lignin, the alcoholic substituent being split off as aldehyde under the conditions of the coupling reaction (in faintly alkaline solution) with the formation of the corresponding indophenol[155].

By treatment of spruce lignin it was possible to isolate "guaiacylindophenol" (XXVIII), and aspen lignin yielded in addition "syringylindophenol" (XXIX)[154].

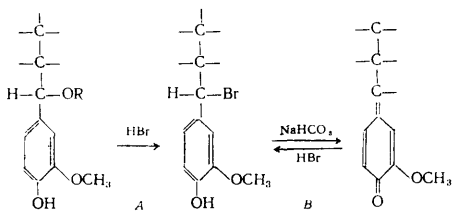


(XXVIII) (XXIX)

where for (XXVIII) R = H, and for (XXIX) R = OCH_3.

The reaction does not take place in the absence of phenolic and alcoholic hydroxyl in the free state. Quantitative measurements showed that in Brauns' lignin approximately every seventh, and in Björkman's lignin every fourteenth, phenylpropane unit has the guaiacylcarbinol structure[154].

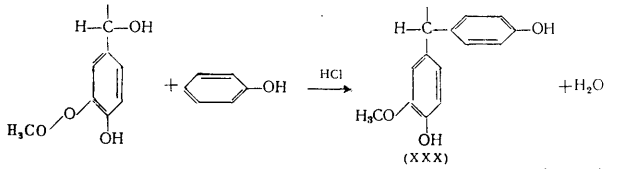
Apart from the colour reaction with the quinoneimine, other reactions were found which demonstrated the presence in lignin of *p*-hydroxybenzyl alcohol and *p*-hydroxybenzyl ether groups. It has been known for a comparatively long time[156] that both *p*-hydroxybenzyl alcohol and *p*-hydroxybenzyl ether are very readily converted by the action of HBr into *p*-hydroxybenzyl bromide (reaction *A*), which reacts in ethereal or chloroform solution with an aqueous solution of sodium bicarbonate to form a *p*-quinomethane (reaction *B*):



The occurrence of quinomethane reactions (*A* and *B*) in solution is readily observed from the change in the absorption curve. Adler and Stenemur[157] worked out a technique for carrying out "quinomethane" reactions with various model substances for lignin, and showed that a dioxan–chloroform solution of Brauns' lignin, which originally had a single maximum in the absorption curve, gave a new maximum after reactions *A* and *B*.

Shorygina's discovery by chromatography[127] of the "benzyl alcohol" group, the indophenol and quinomethane reaction, and also the results of studies on the model reactions of sulphonation, sulphide formation, and alkylation with methanol containing hydrogen chloride — all these important investigations demonstrate convincingly the presence of *p*-hydroxybenzyl alcohol (or ether) groups in native lignin.

The condensation of lignin with phenol in the presence of a small amount of hydrochloric acid may also to some extent be adduced as evidence of the presence of a benzyl carbon atom. Reaction between lignin and phenol leads to the formation of phenol-lignin, the phenol reacting with hydroxyls in the lignin situated *para* or *ortho* to the phenolic hydroxyl[158,159].

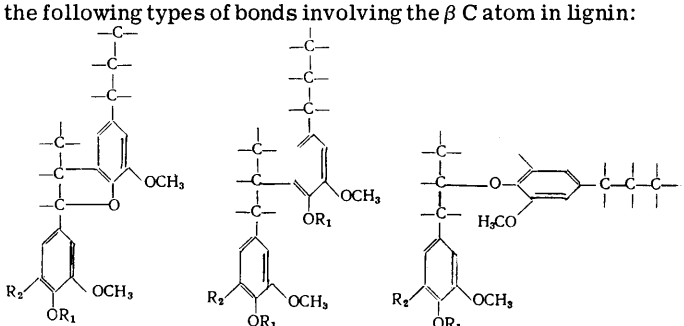


(XXX)

The formation of a diphenylmethane structure (XXX) was confirmed experimentally not long ago[160]. As Ziegler[155] has shown, benzoquinone-*N*-chloroimine splits off the carbinol group from *p*-hydroxybenzyl alcohol, and removes the methylene bridge from *p*-hydroxydiphenylmethane. Therefore compounds having the diphenylmethane structure (XXX) may be expected to yield two indophenols — (1) a derivative of phenol itself and (2) a "guaiacylindophenol" of the type of (XXVIII). After reaction of Brauns' and Björkman's lignins with 2,6-xylenol and subsequent treatment with the quinonechlorimine in faintly alkaline solution, it was possible to separate and identify both indophenols by means of paper chromatography[160].

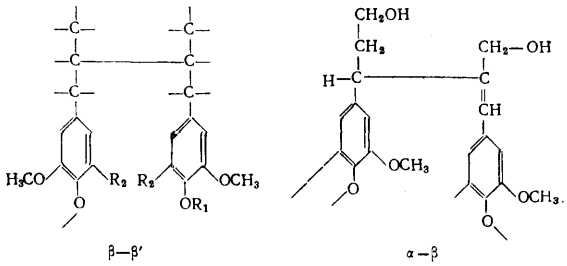
No quantitative assessment of the reaction between phenol and the "benzyl group" in lignin has yet been made. It is quite probable that the condensation of phenol with hydroxy groups occurs also at the β or γ carbon atoms of the propane side-chain. The possibility of condensation with glycol groups in the side-chain[158] is not excluded, although the proportion of these in the lignin macromolecule appears to be small[161].

In the main dimeric products (VII)–(IX) and (XII), obtained by Freudenberg in the enzymic dehydrogenation of coniferyl alcohol and the formation of "biochemical lignin", the links between the guaiacyl(or syringyl)propane units necessarily involve the β carbon atom of the side-chain. Study of the dehydrogenation of coniferyl alcohol and of several model compounds[77,162,163] suggests the existence of the following types of bonds involving the β C atom in lignin:



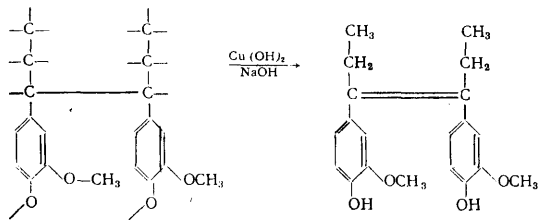
A. phenylcoumaran B. β-aryl C. β-aryl ether

Apart from these three main types there is the possibility of the existence of bonds between $\beta-\beta'$ and $\alpha-\beta$ carbon atoms. Formation of the latter was shown recently in the dimerisation of cinnamyl alcohol[164].



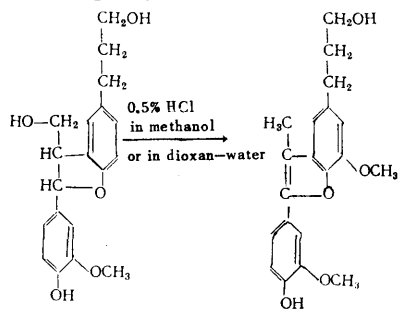
β—β′ α—β

Pearl and Beuer[53] suggested the existence also of $\alpha-\alpha'$ carbon bonds in lignin, since oxidation of ligninsulphonic acids with alkaline copper hydroxide leads to the formation of a small quantity of stilbene derivatives:



The phenylcoumaran structure (A) was considered in the early 1930's to be characteristic of lignin[33,34] and investigated in detail in connection with the sulphite reaction[165], but has now been shown to be present in comparatively small proportions in the lignin molecule. Dehydrodiconiferyl alcohol, having the phenylcoumaran structure, is formed in amounts up to 20% of the original coniferyl alcohol during the biosynthesis of lignin. Adler[166] employed a spectrophotometric method to determine the abundance of the phenylcoumaran structure.

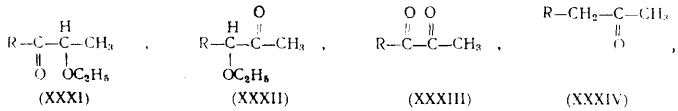
The action of $CH_3OH-HCl$ or of HCl in aqueous dioxan on dihydrodehydrodiconiferyl alcohol or its methyl ether was found to convert the saturated phenylcoumaran system into the unsaturated phenylcoumarone system:



Phenylcoumaran has a typical guaiacylpropane absorption spectrum with a maximum at about 282 mμ. The phenylcoumarone obtained exhibits both a sharp increase in absorption and a bathochromic shift at the maximum to about 310 mμ. Björkman's lignin treated with a $CH_3OH-HCl$ mixture for 24 h showed an increase in absorption. It was found by comparison with the phenylcoumarone maximum obtained on models that lignin does not contain more than 5–7% of the phenylcoumaran structure[154]. Freudenberg, employing acetolytic ring cleavage (acetic anhydride–acetic acid–perchloric acid), also came to the conclusion that native lignin contains comparatively few phenylcoumaran rings.

The possibility of the existence of both phenylcoumaran (type A) and β-aryl (type B) structures is demonstrated by the formation of isohemipinic acid, as noted previously in the section concerned with the oxidative cleavage of native lignin with potassium permanganate. The β-aryl ether structure (type C) is of considerable interest and importance for an understanding of the nature of the links between the individual monomers. In the biosynthesis of lignin Freudenberg separated from among the dimeric products of guaiacylglycerol β-coniferyl ether, having a structure of the β-aryl ether type, with a yield of more than 30%.

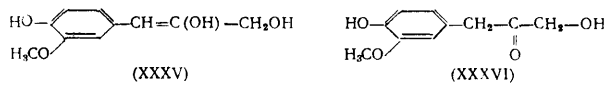
Investigations carried out in the 1940's by Hibbert and his coworkers[167-171] showed that treatment of wood meal (or lignin preparations separated under mild conditions) with ethanol in the presence of HCl results in the formation, together with ethanol-lignin, of four ketones:



(XXXI) (XXXII) (XXXIII) (XXXIV)

where R = 4-hydroxy-3-methoxyphenyl for conifer lignin or R = 4-hydroxy-3,5-dimethoxyphenyl for lignin from deciduous species.

Hibbert considered that native conifer lignin is formed from the extremely reactive β-hydroxyconiferyl alcohol (XXXV), which is the enolic form of the keto-alcohol (XXXVI).



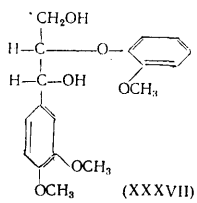
(XXXV) (XXXVI)

The extremely reactive side-chains of the propylphenol units of lignin undergo intramolecular rearrangements[172] according to the scheme:

$$R-CH_2-CO-CH_2OH \rightleftarrows R-CH=C(OH)-CH_2OH \rightleftarrows$$
$$\rightleftarrows R-CH(OH)-C(OH)=CH_2 \rightleftarrows R-CH(OH)-CO-CH_3 \rightleftarrows$$
$$\rightleftarrows R-C(OH)=C(OH)-CH_3 \rightleftarrows R-CO-CH(OH)-CH_3,$$

where R = 4-hydroxy-3-methoxy(or 3,5-dimethoxy)phenyl. As a result of such allylic and keto-enol transitions, etherification with ethanol in the presence of HCl may give rise to the compounds (XXXI) and (XXXII), and disproportionation of R.CH(OH).CO.CH_3 may yield (XXXIII) and (XXXIV).

Adler[166] studied veratrylglycerol β-guaiacyl ether (XXXVII) as a model substance containing a β-aryl ether structure:

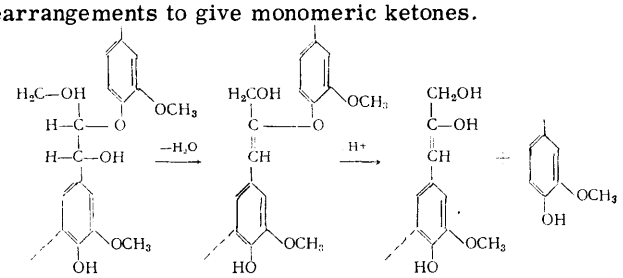


(XXXVII)

This product on treatment with sulphite solutions or with cold methanol in the presence of HCl was converted into

sulphonic acid derivatives or gave a methyl ether at the α carbon atom. But the most important fact was that, like lignin, it gave Hibbert ketones on ethanolysis. The products obtained by the ethanolysis of lignin by Hibbert's method (see above) are characterised by a considerable content of $\geqslant C(CH_3)$ groups. On heating the model substance (XXXVII) with 0.2 *N* hydrogen chloride in aqueous dioxan (1 : 9) (acidolysis), guaiacol was liberated, *i.e.* the proportion of free phenolic hydroxyls increased, and the reaction products formed were shown chromatographically to include ketones also containing $\geqslant C(CH_3)$ groups.

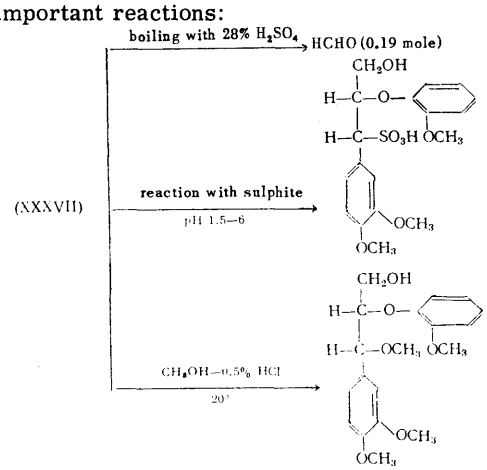
In order to determine the abundance of the β-aryl ether structure in lignin, Adler[166] compared the behaviour of the model substance (XXXVII) and lignin preparations in acid hydrolysis. Two preparations were used, dioxan–hydrochloric acid lignin[173] and Björkman's lignin[78]. In both cases acid hydrolysis gave an ether-soluble oily fraction containing monomeric "Hibbert ketones" in addition to a solid substance insoluble both in water and in ether. The solid substances were characterised by an increased content of free phenolic hydroxyls and $\geqslant C(CH_3)$ groups. Adler supposes that the guaiacylglycerol β-aryl ether structures (XXXVIII) in lignin undergo acid hydrolysis. Apparently dehydration occurs first, leading to the enol aryl ether (XXXIX), which is sensitive to acid hydrolysis. This reaction exposes a new phenolic hydroxyl in addition to the hydroxyconiferyl alcohol. The latter undergoes Hibbert rearrangements to give monomeric ketones.



(XXXVIII) (XXXIX)

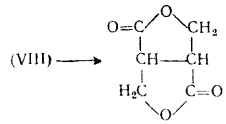
Comparison of model substances with lignin preparations led Adler[166] to the conclusion that at least 25–33% of all phenylpropane units in spruce lignin are guaiacylglycerol units having a β-aryl ether linkage. Such a conclusion is in agreement with the results obtained by Freudenberg in the biosynthesis of lignin[49] by the enzymic dehydrogenation of coniferyl alcohol.

The synthetic model substance, veratrylglycerol β-guaiacyl ether (XXXVII), proposed by Adler, behaves like lignin in three important reactions:



According to this scheme, the compound (XXXVII) yields formaldehyde on being boiled with 28% H_2SO_4, is sulphonated by acid sulphite solutions, and forms a methyl ether with CH_3OH and HCl. In contrast to lignin, however, this product does not react with sodium hydrosulphide under mild conditions[146–150]. This fact again shows that the use of model substances to ascertain the structure of lignin, and the conclusions thus obtained, can have only a relative value, since no single model can reproduce the whole variety of properties which Nature has piled into the lignin macromolecule.

To determine the presence in native lignin of a lignan structure, *i.e.* of bonds between the β carbon atoms of the propane side-chains [this structure is represented by pinoresinol (VIII) among the dimeric products of biosynthesis], Erdtman's cleavage reaction[175] was employed[174]. If pinoresinol is methylated, brominated, and then oxidised, the dilactone of bishydroxymethylsuccinic acid should be obtained.



However, it has not yet proved possible to separate this lactone from native lignin.

Structures of lignan type are apparently present in lignin, since natural lignans are akin to lignin in many respects. Freudenberg found up to 20% of pinoresinol among the dimeric products of biosynthesis. Its content in wood protolignin requires more exact determination.

The third (γ) carbon atom of the propane side-chain displays its properties in the reactions of hydrogenation, ethanolysis, and of hot concentrated mineral acid. The cyclohexylpropanols obtained by the hydrogenation of lignin preparations contain a primary alcoholic hydroxyl attached to the γ carbon atom[116–120]; during ethanolysis $-\overset{|}{C}-\overset{|}{C}-CH_3$ groups are formed. On treatment with sulphuric acid, formaldehyde is obtained as a result of secondary reactions, although not in a quantitative yield[174,176].

In order to prove that the formaldehyde was formed from the γ carbon atom, Freudenberg[177] synthesised biolignin from coniferyl alcohol having a labelled terminal carbon, and showed that the resulting product was radioactive and was, in fact, formed from the γ carbon atom. It can thus be supposed that the γ carbon atoms of the propane side-chain are either free or bear an oxygen-containing functional group, *i.e.* are linked to hydroxyl groups which are freed by chemical treatment (possibly etherified alkyls or aryls). Lindgren and Mikawa[178] were able to detect by means of a colour reaction the presence of a small proportion of cinnamyl alcohol structural groups in spruce lignin.

The great activity of the cinnamyl alcohol structure makes it unlikely that it is present in lignin which has been isolated, even if native lignin contains it. Therefore the colour reactions were carried out on wood sections about 20 μ thick which had been extracted. After the colour reaction had been obtained (treatment with a solution of toluene-*p*-sulphonyl chloride in pyridine, followed by careful rinsing and treatment with a solution of *p*-nitrosodimethylaniline and potassium cyanide in aqueous alcohol), the light absorption of these sections was measured on a Beckman spectrophotometer.

A double bond conjugated to the nucleus (*i.e.* the structure of the side-chain in coniferyl alcohol and coniferyl and sinapyl aldehydes) is a structural element of lignin which appears to be present in small amount. A small quantity (1%) of coniferyl aldehyde was found by Freudenberg[49] among the dimeric products of biosynthesis.

Native lignin from spruce wood gives a red colour with phloroglucinol (the Wiesner reaction). Adler[179,180] explains it by the reaction of the latter with coniferyl aldehyde, which he was the first to separate (in small yield) from wood. These conclusions were confirmed also by Kratzl[181] and Pew[182]. The proportion of coniferyl aldehyde groups in lignin is very low, about 0.1%[183], *i.e.* one for every 35 phenylpropane units (in Brauns' lignin) or for every 40–60 units in ligninsulphonic acid[179,180].

Although the structure of the phenylpropane units in lignin, and to a considerable extent the way in which they are linked, have become clear, the character of the side-chain requires further investigation in many respects.

In view of fresh work on the determination of carbonyl groups in lignin by the tetrahydroborate method[97], according to which every two phenylpropane structural units are accompanied by almost one carbonyl group (0.41–0.48 CO/OCH_3), it is necessary to re-examine both the distribution of oxygen in the side-chain and existing ideas about the structure of the latter. Therefore the "problem of the side-chains" is one of the most important in the chemistry of lignin[184]. The constitution of the side-chains of lignin is obscure and is difficult to determine because of their tendency to undergo rearrangements and changes in the positions of the substituents depending on the method employed to isolate the lignin. Following the work of Gierer[97], however, great attention has been paid to the role of carbonyl groups in the side-chain, and in future investigations the model substances will probably include the carbonyl group as an essential part of their propane side-chain. Therefore the schematic structures for lignin published[44] during recent years, in which the propane side-chains are practically devoid of carbonyl groups (as followed from earlier investigations) are inaccurate.

During recent years numerous studies have been made of the physical properties of the lignin macromolecule[185]. The molecular weights of soluble lignin and lignin derivatives have been investigated by various methods[186,187]. The values obtained varied within the limits 300–14 000. The molecular size is probably affected by hydrolytic degradation during the isolation of the lignin and by condensation into larger molecules. For lignins which are soluble in organic solvents the molecular weights vary within the limits 800–10 000. A high molecular weight is found for ligninsulphonic acids (20 000–100 000), which is explained by condensation processes occurring (owing to the acidity of the medium) during the initial stage of sulphonation.

Results obtained from diffusion measurements show that lignin exists as a tangled spiral[188]. An X-ray investigation carried out by Becherer[189] refuted Jodl's view[190] of a crystallite structure for lignin: it is a typical amorphous substance having a three-dimensional structure.

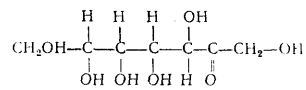
Fundamental investigation of the colloidal nature and lyophilic properties of lignin were carried out by Odintsov[191]. On the basis of a large amount of experimental data he discovered the laws governing the swelling of the plant cell in different media and the part played by lignin in these processes, which are important for an understanding of the mechanism of the hydrolysis of wood.

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This review of the most important papers published during the past twelve years on the nature, structure, and properties of lignin enables some preliminary conclusions and generalisations. These conclusions, of course, do not pretend to cover exhaustively the extensive and varied results, which are sometimes contradictory and controversial. But it must be emphasised that the aromatic nature of the main part of the lignin "polymolecule"* is not open to doubt.

Aromatic structural units exist in native lignin and are formed as a result of complex oxidation–reduction processes occurring in the living plant cell and involving the multifunctional enzymic system demonstrated by numerous investigators.

Fresh light was thrown on the complex problems of the assimilation of carbon dioxide by the living cell, with the formation of carbohydrates and the further conversion of the latter into aromatic products, by the elucidation of the role of phosphorus compounds of sedoheptulose (XL) in the photochemical conversion of carbon dioxide into carbohydrates in living plants[192].



(XL)

Sedoheptulose 1,7-diphosphate undergoes cyclisation under the influence of enzymes with subsequent conversion into aromatic monomers of lignin, as had been shown schematically earlier (see the biosynthesis of aromatic substances by bacteria).

It is difficult to say anything about the order of priority in which carbohydrates and cyclohexene precursors of lignin are formed, but there is no doubt about their genetic relationship, and the chemical links between the lignin which is formed and the carbohydrates in the plant cell are obvious.

In the course of phylogenetic evolution plants have acquired strength and rigidity, in which lignin has played an essential part. Cotton fibres, consisting of pure cellulose, differ fundamentally from wood fibres, the cell walls of which are lignified. Electron microscopy has shown that in these cells plaits of cellulose are embedded in an amorphous mass of lignin and hemicellulose, one of the most frequently observed substances of this group being xylan[192].

The presence of a lignin–hemicellulose complex in wood has been proved, and, it seems, can be used to explain the difficulty of isolating native aromatic lignin unchanged from plant tissue.

Lignin is not uniformly distributed in the plant cell. Preparations separated by the Björkman method from spruce wood contain some fractions which differ not only in their content of carbohydrate residues but apparently also in the degree of condensation of the aromatic lignin itself which forms part of the complex. This is proved by the fact that ligninsulphonic acids differing in molecular weight are obtained, and is due to the lignification of the cell itself taking place gradually, over a period of time.

* By "polymolecule" Freudenberg meant a molecule formed by the repetitition, not necessarily regular, of a monomer unit.

The links between the lignin and the carbohydrates are not all of the same type. Apart from the readily hydrolysable phenyl β-glucoside and the more difficultly cleaved benzyl ether links, it is possible that stable C–C bonds exist between lignin and carbohydrates. An indirect proof of this can be found in the work of Kratzl and coworkers[193] showing that methanol introduced into lignin can form some stable C–C bonds in addition to benzyl alcohol groups.

Freudenberg's extremely convincing and effective investigations on the biosynthesis of lignin based on coniferyl alcohol have revealed important regularities underlying the condensation of aromatic monomers. In his latest work, published not long ago[194], he obtained a new class of carbohydrate compounds, ethers of sugars of lignin structural units, by enzymic dehydrogenation of coniferyl alcohol in the presence of sugars. Previously Siegel[195] had adduced data on the participation of cellulose in the oxidative polymerisation of eugenol during the formation of lignin from it. The above work shows that the formation of lignin from aromatic monomers in the plant cell necessarily involves carbohydrates, which were ignored in Freudenberg's previous investigations. In the light of Freudenberg's latest experimental results[194], the conclusions of Traynard[196], that the carbohydrate components forming part of the lignin molecule are not linked by the polyose chain, appear quite probable.

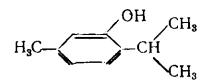
Coniferyl alcohol is by no means the only substance to be regarded as an aromatic monomer in the formation of lignin materials. Lignins have been obtained based on other phenylpropane monomers — ferulic acid, cinnamic acid, and eugenol.

The possibility of obtaining coniferin from phenylalanine[22] is an important argument in support of the probable similarity of the starting materials involved in the formation of protein and the walls of the living plant cell. The presence of a small quantity of nitrogen in the composition of lignin can now be explained by the possible participation of phenylalanine or tyrosine in the biosynthesis of coniferin and then of lignin in the cell.

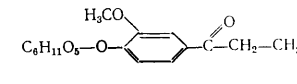
The formation of lignin materials based on phenylpropane monomers not containing methoxyls (cinnamic acid, phenylalanine) indicates that methoxylation is evidently the last stage in the formation of the aromatic precursors of lignin.

Non-methoxylated aromatic nuclei have been detected even in "finished" lignin. Thus Smith[197] isolated from aspen lignin 6.9% of hydroxybenzoic acid, combined, in his opinion, by means of ester links with aliphatic hydroxyls of the side-chain of the lignin molecule. In view of the great reactivity of the hydroxyl group attached to an α carbon atom, it is quite probable that not only carbohydrate residues (adducts with sugar, prepared by Freudenberg, or coniferyl alcohol radicals) but also aromatic monomers can be linked to it.

The enzymic system for the lignification of plants is by no means universal, but is strictly specific. When labelled vanillin is introduced into growing tissue, no radioactive lignin is formed. Thus vanillin is not assimilated in the biosynthesis of lignin. It is characteristic that all labelled aromatic monomers tested as precursors of lignin by introduction (implantation) in growing tissue which gave the corresponding lignin preparations had a propane side-chain. However, not every phenylpropane unit can be assimilated in biosynthesis. When delignified tissue from spruce bark is grown on thymol (XLI) or on propionylguaiacylglucose (XLII), the colour reaction with phloroglucinol characteristic of lignin is not observed; if the same tissues are then cultured on media containing coniferin, the test is shown distinctly[198].



(XLI)



(XLII)

Biochemical investigations of lignification should lead to accurate definition of the enzymic systems involved with a delimitation of their functions.

The order of the bonds between the aromatic precursors of lignin is not uniform. This is explained by the presence of highly reactive functional side groups and positions in the phenolic nucleus possessing great potentialities for condensation. In contrast to other natural and synthetic polymers, the lignin "polymolecule" possesses an irregular structure[49]. However, this irregularity is not equivalent to chaos or randomness in the principles underlying the condensation of the monomers. Lignin, like other natural polymers, has its own structural order. It is more complicated and more difficult to discern.

The principle of the simulation of lignin reactions, which has found wide application in the chemistry of lignin during the past 12–15 years, and has revealed many peculiarities of lignin, is nevertheless of limited value. A critical approach is necessary in attempting to use analogy to transfer to lignin the results from the models.

The lignin "polymolecule", isolated by Björkman's method, possesses a molecular weight of 11 000–12 000. Its colloidal properties and steric factors considerably restrict the applicability of the usual methods, even including optical methods, for determining the functional groups (*e.g.* phenolic hydroxyls, carbonyls). All this requires the development of new methods and reactions specific to the main structural units.

Future work on the chemistry of lignin will probably involve, as the main problem, the elucidation of the nature of the non-aromatic groups, in particular of the propane side-chain. Its lability and tendency to undergo rearrangement are responsible for the variety of oxygen-containing functional groups attached to the carbon atoms of the side-chains. Intramolecular changes in them were shown by the investigations of Hibbert[167-171] (see above). According to Hibbert's scheme, oxygen may be present in the side-chain in the form of carbonyl or of alcoholic or enolic hydroxyls. To postulate any one definite structure for the propane side-chain, seems to be unjustified, since that would be contrary to its extraordinary variability.

It is quite reasonable to assume that the considerable divergences in the analytical data (in the determination of carbonyl groups, phenolic and enolic groups) have their origin in the nature of the propane side-chains, which change depending on the experimental conditions.

It is important to note that the three carbon atoms of the side-chain are the location of the majority of the condensation processes leading to the formation of the lignin "polymolecule", the irregularity of the structure of which is to a considerable extent explained by the nature of the side-chains. They are the points of formation not only of ether links but also of carbon–carbon bonds between the chains and with aryl radicals (α–α'-, α–β-, β-aryl bonds, etc.).

The side-chains are responsible also for additional condensation processes occurring in the lignin "polymolecule" during many technically important methods of processing wood (in the hydrolysis of wood, in the cellulose–paper industry).

The presence and the proportion of double bonds in the side-chains have not yet been completely ascertained. Measurement of the nuclear magnetic resonance in artificial lignin preparations has not been able to show the presence of double bonds. Wacek considers that their content is below 0.1 per phenylpropane unit[62].

The aromatic framework of lignin is built up from three benzene nuclei which are hydroxylated and methoxylated to differing extents (4-hydroxy-, 4-hydroxy-3-methoxy-, and 4-hydroxy-3,5-dimethoxy-phenyl nuclei). They occur in different proportions, but the guaiacyl (4-hydroxy-3--methoxy) grouping predominates, especially in the lignin of conifer wood.

As a result of the orienting influence of the phenolic hydroxyl, the aromatic nuclei in lignins are reactive mainly at the 5- and to some extent at the 6-positions. The formation of biphenyl structures and addition to a β carbon atom of a side-chain of a neighbouring unit both occur at the 5-position of the nucleus. The phenolic hydroxyls govern the formation of phenyl ether and glucoside links.

On the basis of the investigations of Freudenberg and Adler, it may be assumed that the predominant form of the linkage of phenylpropane units in native lignin is the β-aryl ether link, as in guaiacylglycerol β-coniferyl ether (IX).

At the present time there are still insufficient grounds for representing the lignin "polymolecule" in terms of the ordinary symbolism of organic chemistry, but individual details, fragments of the overall picture, still with many blank patches, are being accumulated. In view of the great progress made in the study of lignin during recent years, it seems likely that the elucidation of its structure is approaching its final solution.

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DIFFERENTIAL CAPACITY AND STRUCTURE OF THE ELECTRICAL DOUBLE LAYER AT THE MERCURY/ELECTROLYTE INTERFACE

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1. INTRODUCTION

It is well known that an electrical double layer appears at the interface between two phases, one of which contains ions or polar molecules; it is formed because the electrochemical potentials in the two phases (*e.g.* metal and solution) are unequal. In general, one needs to consider the difference in chemical potential for the ions in the metal and in the solution, the influence of any external electric field, and also the specific adsorption of ions or polar molecules on the metal surface.

If any of the components of a system comprising an electrode immersed in an electrolyte is present in both phases at finite concentrations, an electrochemical reaction may be made to proceed in either direction by an externally applied potential. It is usually assumed that equilibrium in the electrical double layer is not thereby disturbed, and that the passage of current through the electrode/solution interface affects the double layer structure only in so far as it alters the surface concentration of ions. Levich has discussed[1,2] the non-equilibrium double