BIOGENESIS AND CONSTITUTION OF LIGNIN

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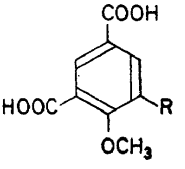
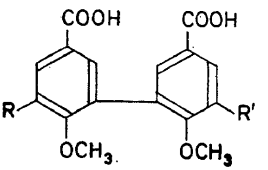
In recent years, the investigation of lignin has begun to take on a more definite form[1–7]; it is of value to present reviews, from time to time, expounding the latest position of research in this field. I take the liberty of placing the work done in our Heidelberg laboratory in the forefront of my discussion.

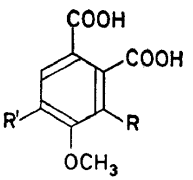
After achieving a biosynthesis of a lignin-like material by dehydrogenation of coniferyl alcohol, extensive efforts were devoted to the comparison of this synthetic product with milled wood lignin, isolated from conifers according to the Björkman procedure. The identity is as good as can be reasonably expected between high molecular weight substances, in which the sequence of their monomeric constituent units is not subject to a stringent pattern. One new proof of their identity is provided by the



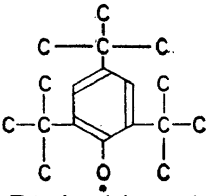
(I) R≡OCH_3: Isohemipinic acid
(VII) R≡H: 4-Methoxyisophthalic acid
(VIII) R≡COOH: Methoxytrimesic acid



(II) R,R′≡OCH_3: Dehydrodiveratric acid
(IX) R,R′≡H: Dehydrodianisic acid
(X) R≡H, R′≡OCH_3: 2,3,2′-Trimethoxydiphenyl-5,5′-dicarboxylic acid



(III) R≡H, R′≡OCH_3: Metahemipinic acid
(IV) R≡OCH_3, R′≡H: Hemipinic acid
(V) R,R′≡OCH_3: Trimethoxyphthalic acid
(VI) R,R′≡H: 4-Methoxyphthalic acid



(XI) Tri-t-butylphenoxyl radical

numerous methylated phenolic acids formed from both natural and synthetic lignins. The lignin is methylated, treated with hot alkali to hydrolyse ether linkages, re-methylated and oxidized. Apart from the veratric acid, isohemipinic acid (I), dehydrodiveratric acid (II), and metahemipinic acid (III) previously detected, degradation of natural lignin also yields hemipinic acid (IV), trimethylgallic acid, 3,4,5-trimethoxyphthalic acid (V), benzenepentacarboxylic acid, succinic acid and tricarballylic acid (propane-1,2,3-tricarboxylic acid). In addition, anisic acid, the related 4-methoxyphthalic acid (VI), 4-methoxyisophthalic acid (VII), methoxytrimesic acid (VIII),

dehydrodianisic acid (IX), and 2,3,2′-trimethoxydiphenyl-5,5′-dicarboxylic acid (X) are also found (24 in all).

If a dehydrogenation polymer formed from coniferyl alcohol is treated similarly, the acids cited above, from veratric to tricarballylic, are obtained in approximately the same relative proportions as from wood, with the exception of trimethoxyphthalic acid (V), the presence of which is dubious. If the synthetic lignin has been produced from 80 per cent coniferyl alcohol, 14 per cent *p*-coumaric and 6 per cent sinapic alcohol, the same acids are obtained together with anisic acid and its congeners (VI–X). The number and type of acids obtained from the natural and synthetic products are the same and, in our estimation, even the relative quantitative proportions are much the same.

Although these results primarily provide further evidence for the identity of the natural and biosynthetic preparations, the acids also allow further deductions regarding the constitution of lignin.

The occurence of trimethylgallic acid as a degradation product of a dehydrogenation polymer made from coniferyl alcohol alone is remarkable. Under equivalent degradation conditions, vanillic acid does not yield any gallic acid derivative. The trimethylgallic acid is formed from the dehydrogenation products of coniferyl alcohol. This conversion and the formation of benzene pentacarboxylic, tricarballylic and the phthalic acids will be discussed later. The same degradation products are obtained from both artificial and natural lignin—albeit in lower yield—when acetic anhydride plus a trace of perchloric acid is used to open the ether bonds instead of hot alkali.

Dehydrogenation of coniferyl alcohol occurs at the phenol group. This reaction is completely unspecific. Plants utilize laccase and peroxidase, and the process can be duplicated *in vitro* using these enzymes. However, dehydrogenation with cupric salts or manganese or lead dioxides produces the same result. Even Eugen Müller's blue 2,4,6-tri-t-butylphenoxyl radical (XI) effects the same operation. If the dehydrogenation is conducted so that approximately one hydrogen atom is abstracted per molecule of coniferyl alcohol, many new substances—at least forty in number—are formed; these are all phenols and can be distinguished by their coupling reaction using diazobenzene sulphonic acid. More than 20 of these substances have been isolated[7] and their constitutions elucidated, among them those which are formed in largest quantities.

From a knowledge of the oligomers of dehydrogenated coniferyl alcohol, it is not difficult to deduce that a radical R_α (XII) is first formed which is a mesomer of both R_β (XIII) and R_γ (XIV). These various radical forms saturate themselves mutually forming dimeric intermediates, such as dehydrodiconiferyl alcohol (XV), DL-pinoresinol and DL-epipinoresinol (XVI*a* and *b*), and *bis*-dehydroconiferyl alcohol (XVII). However, the most important dimeric product of coniferyl alcohol is the quinone methide (XIX) formed by combination of R_β with R_α. All the above dimeric transformation products of coniferyl alcohol are formed *via* quinone methides which can be stabilized by intramolecular prototropy. This cannot occur with (XIX). This substance is formed just like all the others under the dehydrogenation conditions (0·2 per cent aqueous coniferyl

alcohol solution at 20° and pH 5·5–6·0, mushroom laccase and oxygen (air), or peroxidase and hydrogen peroxide). It can be identified in 90 per cent dioxan by its characteristic quinone methide absorption using a Cary apparatus; here its half-life is about 1 hour. By addition of water, it is converted into guaiacylglycerol-β-coniferyl ether (XX), the most abundant intermediate of lignin formation. A monolactone corresponding pinoresinol (XVI) has also been isolated.

Aldehydes are also found among the intermediates mentioned above. Those isolated are coniferyl aldehyde itself and the aldehydes of substances (XV) and (XX). These aldehydes are also formed in the absence of oxygen using the blue aryloxy radical (XI); hence they are formed by hydrogen transfer to the aryloxy radicals, which in this case attack the carbinol hydroxyl of the cinnamyl alcohols.

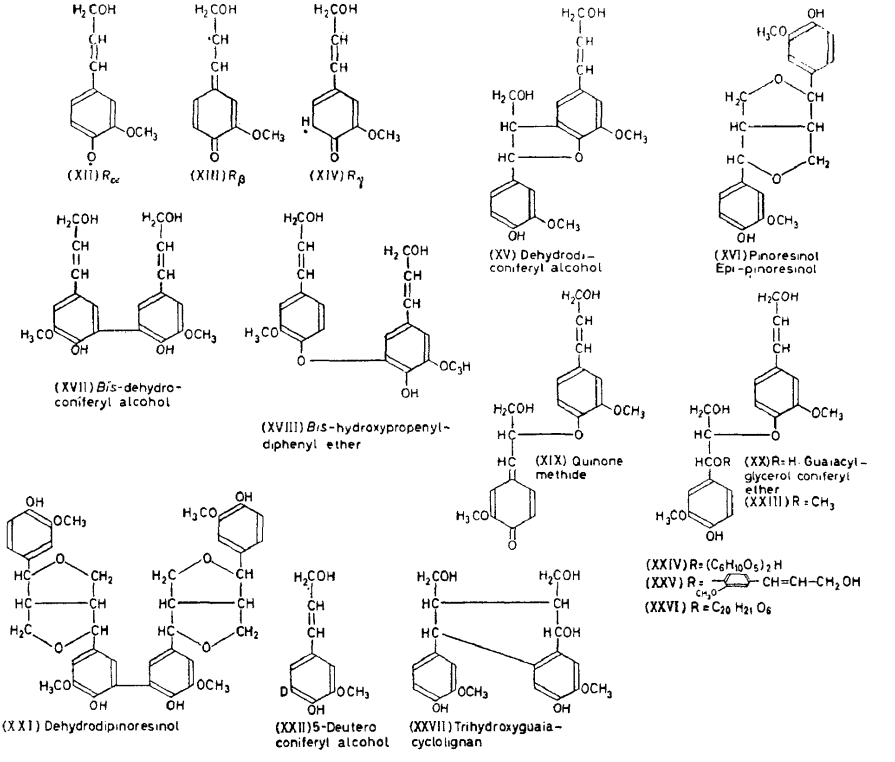
Furthermore, probably two other dimers are formed: the aryl ether (XVIII) and a cyclolignan for which we propose the preliminary formula (XXVII). These hypothetical dimers will be discussed below.

All the dimeric products of coniferyl alcohol cited above are phenols, which themselves form radicals, either by repeated dehydrogenation or radical transfer, and which can combine either with monomeric radicals to give trimers or with each other giving tetramers. All these substances and their derivatives are true lignification intermediates and give valid information concerning the constitution of the natural material, since the latter is identical with synthetic lignin. The starting materials—*p*-hydroxycinnamyl alcohols—are the same for both the synthetic and natural products. Hence the mode of formation—which can be investigated using the synthetic material—is the same for the natural product. This is confirmed by the following experiment. Trunks of spruce felled at the end of May during the growth season were immediately stripped of their bark, and the cambial layers exposed on both trunk and bark were washed down with formalin solution to inactivate the enzymes. The sap contained coniferin and very small quantities of coniferyl alcohol, *p*-gluco-coumar alcohol and the main intermediates also found in the *in vitro* lignification process.

Growth beyond the dimers is confirmed by substance (XXI), dehydrodipinoresinol, also one of the intermediates. Undoubtedly, substances (XV) and (XX) can also " dimerize " similarly through a diphenyl linkage, and (XV), (XVI*a*), and (XX) can form mixed diphenyl derivatives. Recently, 5 new trimeric dehydrogenation products were isolated. The substances isolated so far represented more than 80 per cent by weight of the sum total of the intermediates.

From the discussion so far, formation of larger entities by progressive dehydrogenation can be envisaged. This is the first lignin growth principle.

It soon becomes apparent to anyone attempting to construct a scheme for the massive, branched lignin molecule with the aid of progressive dehydrogenation alone that this will give unsatisfactory results. On the average, each coniferyl alcohol unit would have to lose two atoms of hydrogen to form a bifunctional intermediate. A glance at the units etherified at the phenol group in the dimeric building stones (XV) and (XX) reveals that many side arms incapable of further dehydrogenation are formed. The elementary composition of lignin suggests that, on conversion into lignin,



the coniferyl alcohol loses approximately two hydrogen atoms, simultaneously incorporating 0·3–0·5 molecules of water. However, not every carbon or oxygen atom deprived of its hydrogen takes part in a linking process. For example, if a primary alcohol group becomes aldehydic, two hydrogen atoms are removed without effecting a condensation. The number of hydrogen atoms dislodged without producing a condensation can be estimated at 0·2–0·3 hydrogen atom per unit. Accordingly, the number of hydrogen atoms whose displacement leads to new bonding is reduced to 1·7–1·8 atoms per unit. This implies that, apart from progressive dehydrogenation, some other growth principle associated with no loss of hydrogen, and hence based on a simple addition, must occur to some extent in lignification.

We have found examples of such a second growth principle centred upon the unstable quinone methide (XIX). It has already been stated that this is capable of adding on water. If the enzymatic or chemical dehydrogenation of coniferyl alcohol is carried out in a solution containing 30 per cent methanol, a new substance occurs in quantities which can be isolated, alongside the numerous known dehydrogenation products. This was separated and identified as the methyl ether (XXIII) of guaiacylglycerol-β-coniferyl ether. Hence, despite the excessive amount of water present, here the methanol adds onto the quinone methide. The ether can be split by very dilute acids into methanol and substance (XX). If the dehydrogenation of

coniferyl alcohol is carried out in a concentrated solution of sucrose, the latter behaves just like the methanol. Two combinations of cane sugar with lignin units occur, the more abundant being an adduct (XXIV) of cane sugar onto the quinone methide (XIX). This is not a glycoside but an ether of the sucrose with the carbinol group of compound (XX). This product can also be hydrolysed easily, giving (XX), glucose and fructose. It is a phenol, and just like any other, can take part in further lignification processes. We believe that this reveals the principle of the bonding of lignin onto the polysaccharides of wood and thus provides the mechanism whereby lignin is grafted onto polysaccharides. Alcohols which, unlike cinnamic alcohols, are not unsaturated (*e.g.* substances (XV) and (XX)) occur in sufficient amounts among the dimeric lignin building stones. It can be assumed that in all probability, such primary alcohols add onto the quinone methide (XIX) in the same way as methanol does in (XXIII). We have not yet succeeded in adding the primary carbinol of cinnamyl alcohols onto the quinone methide (XIX).

If an acid such as very dilute acetic acid is present during the dehydrogenation of coniferyl alcohol, a labile acetate can be identified in the mixture of primary products. If phenols are present, *e.g.* phenol, guaiacol or symmetrical pyrogallol dimethyl ether, adducts of these phenols onto the quinone methide (XIX) are detectable on paper chromatograms. This process yields $\beta\gamma$-*bis*-aryl ethers of guaiacylglycerol. The γ-aryl ether linkage is very easily hydrolysed. Such $\beta\gamma$-*bis*-aryl ethers of guaiacylglycerol also occur among the normal conversion products of dehydrogenated coniferyl alcohol; for example, the substance designated as guaiacylglycerol-*bis*-coniferyl (ether XXV). Here coniferyl alcohol acts as phenol and adds onto the quinone methide (XIX). Dilute acid splits the compound into coniferyl alcohol and substance (XX). A second example of this group occurs in substance (XXVI), which is an adduct of preformed substance (XV) onto the quinone methide (XIX). Both are hydrolysed within a few days, even in aqueous solution and disappear rapidly from the bulk of the dehydrogenation products of coniferyl alcohol on continued dehydrogenation. We do not doubt that a portion of these products are incorporated directly into the lignin but we believe that they are still reactive after inclusion and may undergo rearrangement. I have only spoken so far of *one* quinone methide, substance (XIX), because this has been identified. However, it is obvious that other analogous quinone methides occur as intermediates. Addition onto quinone methides is the second growth principle of a lignin macromolecule of any considerable size. A third principle recognized recently is the polymerization of the quinone methides.

The discussion so far is based on isolated intermediates of lignin synthesis of known constitution.

Numerous other intermediates have not yet been isolated and identified and may harbour some surprises. Two types can be forecast with a high degree of certainty, even if not yet isolated. One of them is represented by formula (XVIII). The corresponding trimethoxydiphenylether dicarboxylic acid has been isolated. As mentioned above, when treated with strong alkali, methylated and oxidized, the dehydrogenation product of coniferyl alcohol

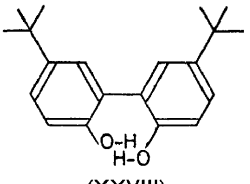
yields trimethylgallic acid among other acids. A dimer (XVIII), originating from R_α and R_γ, would explain its formation. The other dimer is the substance from which benzenepentacarboxylic, tricarballylic and the *o*-phthalic acids are derived. It must be of the isolignan type; it might be better to term these cyclolignans[5]. (XXVII) shows a trihydroxyguaiacyclolignan which might occur as an intermediate. Its formation can be explained as entailing the double quinone methide formed by combination of two R_β radicals; after addition of two moles of water, this could condense intramolecularly to give (XXVII). Such cyclolignans are tetralin derivatives which can be aromatized and which yield benzenepentacarboxylic acid on oxidation. Tricarballylic acid can also be portioned off and the *o*-phthalic acids can be explained in the same fashion. It is, therefore, permissible to include such a cyclolignan in a lignin structure scheme containing a sufficiently large number of units.

So far, I have stressed the synthesis of lignin and the intermediates in this synthesis. Besides oxidation reference has been made to analytical results, such as the hydrogen balance. Recently we have studied the participation of carbon atom-5 of the coniferyl alcohol in the dehydrogenation.

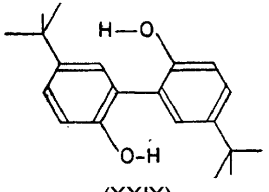
The 5-position in coniferyl alcohol, *i.e.* the unsubstituted position next to the phenolic hydroxyl, is involved in three different functions in the intermediates mentioned above: in substance (XV) it is linked by dehydrogenative condensation directly to the β-carbon atom of the side chain; in substance (XVIII) by etherification with one phenol oxygen; and in (XVII) and (XXI) in diphenyl bonds. The number of units reacting in position 5 can be measured by converting 5-deuteroconiferyl alcohol (XXII) into its dehydrogenation polymer. This was done in both ordinary and heavy water. Normal coniferyl alcohol was also dehydrogenated in heavy water and absorbed a few per cent of deuterium in the process. The results of these experiments indicate that about 45 per cent of all coniferyl alcohol units react in the 5-position during lignification.

Analysis in lignin chemistry will now be discussed, albeit incompletely for the sake of brevity, as the data are extensive and have been gleaned from the work of many laboratories, particularly Swedish.

It is only since it became possible to isolate a considerable proportion of the lignin from wood without chemical interference, as Björkman's milled wood lignin, that an analytical investigation of lignin became feasible.



(XXVIII)



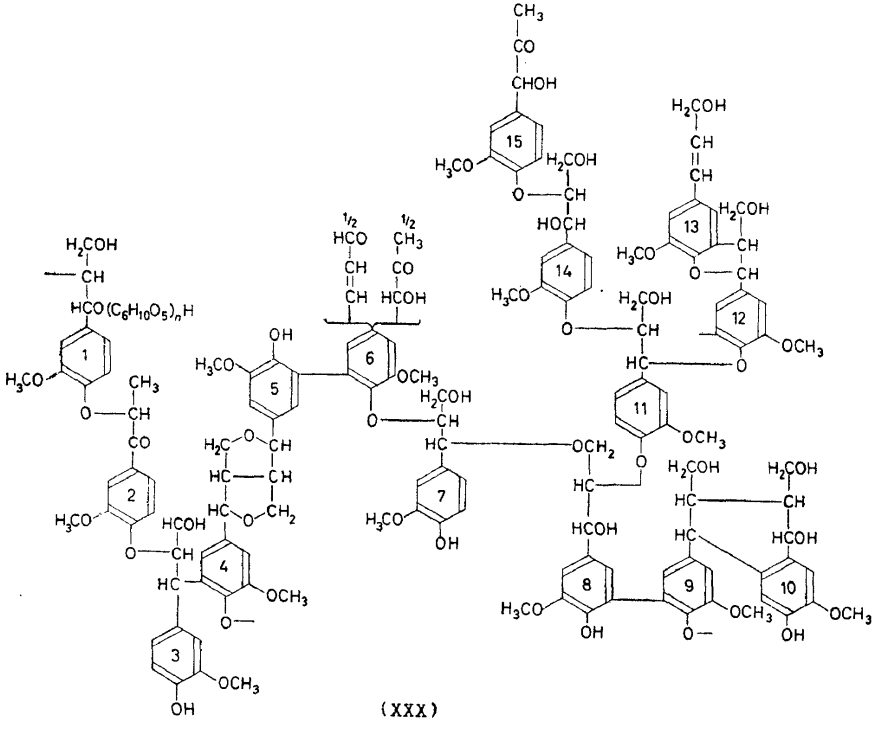
(XXIX)

This comprises aromatic and aliphatic hydroxyl, keto and aldehyde groups, alkyl and aryl ether groups, free, unsubstituted guaiacyl groups, *etc.* Both chemical and optical methods have been used. The principal advances in this field are due to Adler, Marton and their collaborators[4]. In most cases, the chemical and optical assay methods were tested on simple model

compounds and then applied to lignin. However, here, the influence of unsurveyable topochemical and conformational effects makes analysis more complicated. For example, diphenyls bearing two *o,o'*-hydroxyls or one hydroxyl and an ether function will behave differently if the oxygen atoms can approach each other as in (XXVIII) or when, after rotation, they lie on opposite sides of the diphenyl axis as in (XXIX). In a small molecule, one definite conformation will be preferred, but in the massive lignin structure, the conformation of such a bond is determined by quite different factors. Even crosslinking may be possible.

If reactions and kinetic studies carried out on small molecules be applied to a macromolecule containing the corresponding groups, it is to be expected that side reactions occurring with different groups in the larger molecule can scarcely be avoided, and that the products of such reactions may remain occluded within the molecular framework. Optical methods, in particular, may lose some of their precision in this way. However, evaluated with caution, comparative measurements using models provide very valuable data.

About a year ago, a first attempt was made by the author and Sidhu[6] to construct a tentative scheme illustrating the constitution of lignin and comprising 15 coniferyl units. The time is ripe for such attempts to be earnestly considered. They illustrate the concepts recognized so far and, as has already become evident, they form a suitable basis for discussions. Such a draft is made up of the intermediates identified from studies of lignin



(XXX)

synthesis. The growth pathways disclosed above have formed the basis for the combination of the individual building stones. Any such draft must be tailored to fit the number and type of hydroxyls, the number of condensations in the 5-position, the number and type of ether bonds, carbonyl groups, olefinic linkages, and much more.

The second draft shown here (XXX) is an adaptation of the first[6], incorporating the results of the intervening year, particularly the results of the oxidative degradation, studies on the intermediates, and analytical data. Other alterations involving the side chains are unimportant. Naturally, an illustration of this type is necessarily imperfect. Björkman lignin and dehydrogenation polymers have molecular weights of the order 8,000 and, hence, consist of more than 40 units. Lignin in spruce wood and sufficiently dehydrogenated synthetic lignins, have even higher molecular weights. Thus, it is impossible to include all possible forms in a scheme with only 15 units. Moreover, as stated above, many intermediates are still unidentified and may encompass forms completely unaccounted for by both the synthetic and analytical modes of approach. Such a tentative scheme is, therefore, only an approximation based on the status of current knowledge, and future embodiments will have to be adapted step by step to conform with new information. This was in fact done when, thanks to a useful symbiosis of synthesis and analysis, *Draft 2* (XXX) was evolved from *Draft 1*. Thus, as shown, the second draft is also only intended as a stepping stone. This concept will now be discussed in detail.

Unit 1 has been formed from one half of the quinone methide intermediate (XIX); a polysaccharide has been added to this. After loss of its phenolic hydrogen, the aryloxy radical of unit 1 combines with a radical R_β (XIII), which is converted into a β-substituted coniferyl alcohol and undergoes an allylic rearrangement. After dehydrogenation of 2 to its aryloxy radical, unit 3 is added in its R_β form. The linkage between the quinone methide and the unit 4 involves one of the addition reactions[7]. It is feasible that the quinone methide at 3 reacts directly with carbon atom-5 of the dehydrogenated unit 4. The phenol group of unit 4 is etherified forming a branching point. Units 4 and 5 both pertain to a pinoresinol molecule which is joined by a diphenyl bond through carbon-5 of unit 5 to the corresponding position in unit 6. The side chain in unit 6 represents half a cinnamic aldehyde, half a ketol. The conformation of the diphenyl bond linking units 5 and 6 is represented in such a way that the phenol hydroxyl of 5 and the ether group of 6 assume a type of *trans* configuration (*cf.* XXIX). The bulk of the entangled aggregates 1–5 and 6–15 prevents normal free rotation. This completes the chain from 1 to 6. The discussion will now be continued starting from unit 15.

Together, 15 and 14 correspond to substance (XX) with the difference that the cinnamyl alcohol group in 15 has undergone rearrangement and oxidation. After removal of its phenolic hydrogen, unit 14 combines with an R_β radical, which first forms a quinone methide as unit 11, onto which the phenol group of substance (XV) is added in the form of units 12 and 13. The 5-position in unit 12 is also condensed. Unit 11 loses its phenolic hydrogen and adds on an R_β radical as unit 8, which then adds on water giving it initially three hydroxyl groups. Formation of a diphenyl bond

then links it up to the cyclolignan composed of 9 and 10. The formation of the oligomer 8 through 15 is now complete.

The entities 1–6 and 8–15 now combine as follows. Unit 6 loses its phenolic hydrogen either by direct dehydrogenation or radical transfer; an R_β radical couples with the aryloxy radical forming unit 7, at present still in the form of a quinone methide. This quinone methide can combine with the primary hydroxyl of unit 8. This completes the whole chain from 1 to 15. It can be seen how larger, prefabricated sections of the lignin chain are welded together by the bifunctional role of the R_β radical.

The units 3 and 11 have a similar function. Unit 11 forms one of the branching points; branching also occurs at units 4, 8, and 12. The bond between units 3 and 4, and the side chains with β-carbonyl groups (in units 6 and 15), are still only hypothetical. All other types of bonds between units, and the various modifications of the coniferyl monomer, are based on experimental results. However, the sequence of the constituent units and their relative proportions are arbitrary. Dimers are included in the forms of pinoresinol (XVI), dehydrodiconiferyl alcohol (XV) and the cyclolignan (XXVII) respectively.

The construction of this scheme is based on an idealized lignin, composed entirely of coniferyl units and of composition:

$$C_9H_7O_2(H_2O)_{0\cdot37}(OCH_3)$$

In comparison with coniferyl alcohol, $C_9H_9O_2(OCH_3)$, this artificial lignin has lost two atoms of hydrogen and gained 0·37 molecule of water. In reality, in a formula entailing 15 units, about 2·1 units of *p*-coumaryl alcohol and 0·9 unit of sinapic alcohol should be entailed with 12 of coniferyl alcohol. The composition of this polymer would be $C_9H_{7\cdot08}O_2(H_2O)_{0\cdot37}(OCH_3)_{0\cdot92}$ and hence would agree favourably with that of conifer lignin.

One open question is the presence of C-methyl groups at the ends of side chains. It is possible that these are formed mainly from cinnamyl alcohols during the estimation procedure; however, they are also found to a small extent when a cold chromic acid treatment precedes the determination. Adler has kindly called our attention to the absence of a guaiacylcarbinol in *Draft 1*. This can be included easily as a (XX)-type substance, but in a scheme entailing only 15 units, all requirements cannot be satisfied. The same applies to the too high proportion of free to etherified hydroxyls.

Investigation of beechwood lignin has indicated that the proportion of syringyl to vanillyl components is in the ratio of 11 : 12; otherwise, there is no significant difference between the condensing principles involved in spruce or beech lignin. Judging by their methoxyl content, deciduous wood lignins never contain more than 50 per cent sinapic component. A dehydrogenation polymer cannot be made from sinapic alcohol alone. If coniferyl alcohol is added, even with an excess of the sinapic component, its content in the biosynthetic lignin cannot be increased. Although it is plausible that inclusion of sinapic alcohol alters the sequence and form of the units, it is indeed remarkable that, in our formula, there is space available on 8 out of 15 units (or 53 per cent) for inclusion of a second methoxyl

group; as in beechwood lignin, sinapic alcohol constitutes less than 50 per cent of the monomers, it seems that coniferyl alcohol occupies some positions which could be held by sinapic alcohol.

All intermediates, as well as synthetic and natural lignins, are optically inactive, despite the presence of numerous asymmetric carbon atoms. This is so because no asymmetric form is produced by the simple abstraction of the phenolic hydrogen, and all the subsequent processes occur automatically without the participation of enzymes. Since optically active lignans, particularly (+)-pinoresinol, occur in resinous extrusions from spruce, some different type of enzymatic mechanism must be involved in resin metabolism.

It is worthwhile comparing lignin with other macromolecules. In the limiting case of linear polymerization, the chain is lengthened by unitary addition of monomeric units. All the units are identical and the bonding is of a uniform type. Lignin is just the opposite from this. Apart from the fact that three different *p*-hydroxycinnamyl alcohols react together in admixture, hardly a single unit is the same as another although the majority originate from the same monomer, as can be seen from the formula. Initially, oligomers of great diversity are formed, including even some bound to polysaccharides.

The foundations for constructing such a formula are given by the type and quantity of the intermediates occuring in the lignin biosynthesis, together with the constructional principles deduced. Furthermore, limitations are set by the need to conform to the type and number of functional groups, such as hydroxyl, ether linkages, *etc.* In constructing a formula representation based on these considerations, further limitations are encountered as only a few forms are compatible with the previous ones. The freedom with which one can proceed is so restricted that it is not at all easy to draw up a scheme which satisfies all the numerous conditions, and it is surprising how similar various drafts starting from different points become in the end.

With no other known substance is a knowledge of the constitution so dependent upon the clarification of its mode of genesis as with lignin. All the experiments and considerations reported above depend upon the assumption that in the biosynthesis of lignin, *p*-hydroxycinnamyl alcohols, principally coniferyl alcohol, are the ultimate monomolecular C_6–C_3 compounds, and only after their generation in the plant does polymerization to larger molecules occur. Another opinion occasionally expressed is that the characteristic features of these starting materials, such as the methoxyl or primary alcohol groups, are formed at a later period, after commencement of the condensation. This conjecture is invalidated by everything that has been ascertained about the formation of lignin from *p*-hydroxycinnamyl alcohols.

It is another question as to whether other extraneous phenols, which can also be dehydrogenated by laccase or peroxidase and which may be present in the cambium and surrounding tissues, may be copolymerized into the lignin. For this reason, we have examined " cambial sap " closely, trying to detect foreign phenols or their glycosides, but have found no indication of their occurrence in significant amounts. If they are not

present, obviously they are not incorporated. The presence of hydroxymatairesinol[8] is of particular interest. This occurs in the acetone-soluble fraction of spruce wood in the remarkable quantity of 0·3 per cent of the wood, corresponding to about 1 per cent of the lignin content. It is a precursor of the conidendrin found in spent sulphite liquor. In " cambial sap " as we prepared it, hydroxymatairesinol occurs in noticeable quantities. Perhaps it originates from another vessel system, *e.g.* the resin ducts. Hydroxymatairesinol is optically active, in contrast to the lignin intermediates. This indicates that it does not belong to the lignification system. On the other hand, the sap contains small but readily identifiable amounts of coniferyl alcohol and the most abundant dimeric lignification intermediates. Besides large quantities of various sugars, there are considerable amounts of coniferin in the sap, but so far the glycoside of sinapic alcohol (syringin) has not yet been observed in cambial sap from spruce. However, a small amount of the glycoside of *p*-coumaric alcohol has been found accompanying coniferin.

The following experiment with tracers illustrates once more the ability of coniferyl alcohol and phenylalanine to form lignin in spruce. By treatment with hot concentrated mineral acids, a synthetic lignin produced from coniferyl alcohol labelled at the end of its side chain yields formaldehyde which is radioactive, and which therefore originates from the α-carbon atom. Identically labelled coniferin produces in spruce a radioactive lignin which also yields radioactive formaldehyde.

Coniferyl alcohol labelled at the central carbon atom of the side chain gives a dehydrogenation polymer from which radioactive isohemipinic acid is obtained, *e.g.* from substance (XV). Isohemipinic acid derived from radioactive lignin obtained after administration of β-^{14}C-phenylalanine to spruce was also radioactive.

An experiment with radioactive glucovanillin demonstrated the fact that phenols which do not belong to the *p*-hydroxycinnamyl alcohol class can be incorporated into lignin. Introduced by our shoot immersion procedure, the radioactivity of the glucovanillin distributed itself in the tiny spruce stem mainly in the lignin directly above the shoot. However, when the radioactive lignin was extirpated and degraded according to Hibbert to give the well known group of C_6–C_3 ketones, these proved to be almost inactive. This means that only a small amount of the vanillin from the glucovanillin is built up to a C_6–C_3 compound corresponding to a lignification monomer which would be capable of yielding the ketones. The major part of the vanillin is incorporated into lignin in some form other than C_6–C_3. Similar observations were made by Kratzl[1] when he applied *p*-creosol to spruce. A small part of it was transformed into C_6–C_3 lignin units.

The *p*-hydroxycinnamyl alcohols are the main intermediates in the biogenesis of lignin. As mentioned above, coniferyl alcohol occurs in minute amounts in cambial sap. However, the majority is present as the glucoside coniferin. I am inclined to believe that the coniferyl alcohol can be converted directly into lignin together with the accompanying small amounts of *p*-coumaric and sinapic alcohols, and that only an excess of coniferyl alcohol is stored in reserve as coniferin. The aglycone of radioactive D-coniferin is transformed very readily in spruce into genuine lignin

which yields radioactive Hibbert ketones. By contrast, radioactive coniferin synthesized from L-glucose is not metabolized. The explanation is due to the presence, in spruce, of a tissue bound β-glucosidase in the layers of young cells undergoing lignification. It can, therefore, be concluded that both free *p*-hydroxycinnamyl alcohols, and their D-glucosides are suitable lignin progenitors.

Thus, the problem of lignification is divided into two main sections: the biochemical formation of *p*-hydroxycinnamyl alcohols, and their conversion into lignin. The pathway followed in converting *p*-hydroxycinnamyl alcohols into lignin has been discussed above. That from glucose to the *p*-hydroxycinnamyl alcohols has been largely explored by the work of many authors. This route leads *via* shikimic and prephenic acids to phenylpyruvic acid, which is in biochemical equilibrium with phenyl-lactic acid, cinnamic acid and phenylalanine. One or more of these acids is converted into the *p*-hydroxycinnamyl alcohols in several reaction stages, about whose sequence little is known. The results so far obtained are somewhat inconclusive since the various authors have experimented with different kinds of plants, and the plants behaved differently. This is illustrated by the fact that *p*-hydroxyphenylpyruvic acid, for example, is converted into lignin readily in Gramineae but hardly at all in spruce.

What is the fate of lignin when dehydrogenases attack it further? If coniferyl alcohol is shaken in extremely dilute aqueous solution with air or oxygen and laccase at pH 5·5–6·0, oxygen is absorbed as long as the laccase remains active. After the addition of fresh enzyme, the oxygen consumption proceeds further, even after the coniferyl alcohol has been converted into lignin. Björkman lignin brought into extremely fine suspension by dissolution in acetone and reprecipitation with water is also oxidized by laccase and air, some of the lignin going into solution in the process. Some also assumes humin-like character. The reaction leading to lignin, therefore, destroys it again on proceeding further. This does not occur in the healthy plant since the enzyme activity ceases on completion of lignification. However, if dehydrogenating enzymes come into contact with the wood from external sources, such as is the case with wood rotting fungi, the lignin is attacked. The laccase in the mycelia of common mushrooms, which are not lignified, enables them to degrade lignin and phenols in general for their own assimilation.

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