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Lignin and the Formation of Wood[1]

By F. F. NORD and W. J. SCHUBERT[2]

Wood is a natural product of plant origin. But, of course, not all plants produce wood, or are, as we shall say, woody. What then distinguishes woody plants from those which are not woody? The two types of plants can be distinguished from one another on the basis of four criteria:

(1)—Woody plants are perennial, i.e., they may live for many years, and in the case of some timber-producing trees, such as the giant redwoods or Sequoia of California, for 1000 years or more.

(2)—Woody plants possess a stem that persists from one year to the next. In the case of a tree, this stem is what we call the trunk.

(3)—Woody plants are said to be 'vascular', i.e., to possess a highly specialized conducting system consisting of a 'xylem' and a 'phloem'. The xylem is in fact the wood of the mature tree, while the phloem, on the other hand, is the bark.

(4)—A typical woody plant exhibits 'secondary thickening', i.e., a means of thickening its stem by prolonged growth in its diameter occurring over a period of years. This thickening is achieved as a result of the changes occurring within the growing layer of the plant, or 'cambium', which is situated in between the last formed layer of xylem (or wood) and the last formed phloem (or inner bark). This process then annually produces new wood in between the layer of the previous year's wood and the bark of the tree.

Precisely because it is one of the very few raw materials which is continuously being regenerated annually by nature, and in such tremendous quantities, wood is potentially one of the most important natural sources of organic chemicals for industry. Indeed, millions of tons of wood are cut every year in all parts of the world, but of all of this, only about 10% at the very most is used for chemical purposes.

Of that small amount of wood which is so exploited, some of it is converted by the pulp industry into wood cellulose, some by the industry of wood saccharification into simple sugars (which in turn may be fermented into ethyl alcohol or other products), and finally, a very small amount is utilized by the wood distilling industry. Of these three, only the last makes use of the total wood. The saccharification industry uses only the carbohydrate components of the wood, which amount to approximately 65% of its weight, whereas the pulp industry uses even less, namely, only the cellulose, which amounts to about 50% of the weight of the wood.

Early History of Wood Chemistry

For many decades, wood was considered to be a uniform chemical compound. However, with the advent of the techniques of experimental organic chemistry over one hundred years ago, chemists gradually succeeded in separating wood into its individual components. The first serious attempt to study the composition of woody plants was made in 1838 by a French chemist, ANSELME PAYEN[3]. He treated wood alternately with nitric acid and caustic soda and then with ethyl alcohol and ethyl ether, and as a result he obtained a substance which was quite resistant to the action of these reagents and solvents. PAYEN called this substance 'cellulose'.

In the process of his isolation of cellulose, PAYEN observed that it was necessary to remove another substance from the wood, which substance had a higher percentage of carbon than did the cellulose. He first referred to this other material as 'la matière ligneuse véritable' (or, the true wood material), but he later designated it as 'la matière incrustante' (or, the incrusting material), because of his belief that in woody plants the cellulose was surrounded or impregnated with this material. PAYEN also attempted to isolate this incrusting material as such, but he was not entirely successful. However, by modifying his method of extraction, he did obtain a series of preparations which contained varying ratios of cellulose and the incrusting material, and he called these preparations 'lignose', 'lignon', 'lignireose' and 'lignin'. It is this latter term, lignin (which comes from the Latin word for wood, 'lignum'), which was later adapted in 1857 by SCHULZE[4]

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[1] Presented at the 4th International Congress of Biochemistry, Vienna 1958, in the Symposium *Biochemistry of Wood*.

[2] Department of Organic Chemistry and Enzymology, Fordham University, New York, Communication No. 356.

[3] A. PAYEN, Compt. Rend. *7*, 1052 (1838).

[4] F. SCHULZE, Chem. Centr. *28*, 321 (1857).

as the name for PAYEN's 'incrusting material', and this name has been retained and is still in use today.

As the result of a great deal of experimental work by many chemists over the years, the chemical properties of the cellulose portion of wood are quite thoroughly understood. Cellulose is an example of that group of carbohydrates known as the polysaccharides, and this signifies that it has a molecular size well above that of the average chemical compound. Only because of its tremendous size is it inconvenient to construct a structural formula for a complete cellulose molecule, but nevertheless, a small but representative portion of its structure may be illustrated as in Figure 1. Our knowledge of the chemical structure of the other principal component of wood, namely the lignin, has not kept pace with that of the cellulose, and as a result, it is still not possible to write with authority an equivalent structure for lignin.

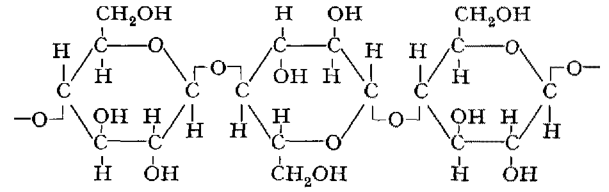

Fig. 1

Considering the tremendous amount of wood which goes to the pulp mills all over the world every day—in the U.S.A. alone, it averages over two billion cubic feet per year—and considering that about one-quarter of this is obtained as a lignin derivative of one form or another and is then discarded, it is not surprising that both the pulp and wood saccharification industries are constantly attempting to utilize this substance. While the latter has not yet found use for its waste lignin, except as a fuel, the pulp industry has had some success in applying a small part of its waste lignin in binders, adhesives, briquetting agents and synthetic rubber. A small amount of the lignin in sulfite waste liquor, furthermore, is converted into one high-grade chemical, namely vanillin, which is used as a synthetic flavoring agent.

The principal reason why a more complete utilization of lignin has not yet been achieved is precisely because our knowledge of its chemical structure is still so incomplete. Although over one hundred years have passed since its discovery by PAYEN, and although a tremendous amount of work has been done on this material, we still are unable to write a complete structural formula for this compound.

Due to our fragmentary knowledge of the structure of lignin, it is very difficult to define it. There is little doubt that lignin belongs to a class of compounds which are completely different from cellulose and the other polysaccharide carbohydrates. The portion of wood called 'lignin' can generally be separated from the other components only by particularly strong chemical reagents which undoubtedly have the effect of chemically modifying the lignin. This fact is one of the reasons why lignin preparations isolated by different methods vary so in their chemical compositions.

Furthermore, the term 'lignin' should not be regarded as the designation of one individual chemically defined compound, but rather, it should be thought of more as a collective term for a whole series of similar, very large compounds which are all closely related chemically to one another, perhaps in the same way as are certain other natural polymerization products, such as cellulose and starch, or perhaps even more closely, the proteins.

Unlike cellulose, lignin never occurs alone in nature, but it always seems to coexist with cellulose or some other carbohydrate. Although lignin appears to be physically associated with these carbohydrates, this does not imply that the lignin and carbohydrates form a chemical compound in wood, and indeed, it seems quite unlikely that this be the case.

As a result of the development of the wood-pulping industry, in which huge quantities of lignin are obtained as a by-product in the form of so-called 'sulfite' and 'black' liquors, it was natural that attempts be made to exploit this potentially valuable raw material. But such utilization requires a great deal of fundamental information on the chemical properties of the material, and so it was that in Sweden, around 1890, KLASON[5] (who is often referred to as the 'father of lignin chemistry') initiated the first intensive and extensive investigations of lignin by studying one of its common commercial by-products, namely, lignosulfonic acid.

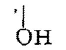

Fig. 2

Among the many contributions of KLASON to our knowledge of the chemistry of lignin, we may cite the following: he developed the first method for the quantitative determination of lignin in woody plants; he was the first to isolate lignin by applying sulfuric acid (a procedure which forms the basis for one method of lignin determination still in use today); he discovered

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[5] P. KLASON, Svensk Kem. Tid. 9, 135 (1897).

that if a simple alcohol, coniferyl alcohol (Figure 2), were treated under the same conditions as those used in the sulfite process of pulping wood, this alcohol was converted into a sulfonic acid possessing many properties which were very similar to those of lignosulfonic acid; but most important of all, KLASON was the first person to suggest that the parent structure of lignin might be a so-called 'phenyl-propane derivative of the coniferyl type' (Figure 3), a suggestion which is still widely accepted today.

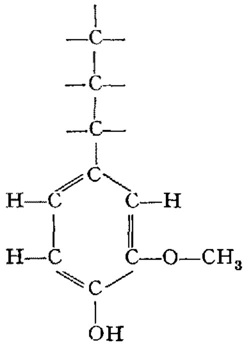

Fig. 3

The Isolation of Lignin

Much of the difficulty encountered by lignin chemists in their study of the chemistry of lignin can be traced to the fact that, for a long time, no method was known by which lignin could be isolated in its natural form. That is to say, no matter what method of isolation was attempted, the preparation obtained was not identical with the lignin as it had existed in the plant. The method of isolation itself had changed the lignin.

A solution to the problem of isolating lignin in its natural state therefore seemed to require a method of extraction from wood that would avoid the usual drastic treatments and instead employ some neutral, chemically inert solvent. The first chemist to be successful with this approach was BRAUNS[6], formerly of the Institute of Paper Chemistry at Appleton, Wis. In 1939, BRAUNS reported the extraction of some of the lignin from black spruce wood by thoroughly extracting very finely ground sawdust of this species with ethyl alcohol at room temperature[7]. This then became the first recorded isolation of a preparation which was identical with the lignin as it existed in the plant, and this was attributable to the extreme mildness of the conditions of the isolation. BRAUNS referred to his lignin as Native Black Spruce Lignin.

However, an important feature of BRAUNS' work was that he was able to isolate only 3% of the total content of the lignin of black spruce wood. The failure of the remaining 97% of the lignin present to be extracted by ethyl alcohol suggested either of two possibilities. (a) Perhaps the material which BRAUNS called Native Lignin was not really identical with the remainder of the lignin in the wood and happened to be more soluble in ethyl alcohol. Or perhaps, (b) the unextractable lignin was combined somehow with the cellulose of the wood, so that it was rendered less soluble in alcohol than that small amount of Native Lignin which presumably was not combined with the cellulose in any way whatever.

A resolution of this dilemma seemed to require a novel approach which would somehow avoid the many pitfalls of the previous experiments and yet permit a solution to this problem of the isolation of a natural lignin which would be identical with lignin *in situ*, or lignin as it exists in plants.

Ever since certain studies by FALCK[8], it is now quite generally agreed that in nature there are two different types of wood decay brought about by fungi. The two types are referred to as 'brown' and 'white' rots, and the difference between them is directly related to the chemical changes which the microorganisms effect on the chemical composition of the wood which is undergoing decay. In brown rot, the fungi[9] causing the rot selectively remove the carbohydrate components of the decaying wood, such carbohydrates as cellulose. But the lignin here remains completely unaffected, and the decaying wood gradually turns brown in color. On the other hand, in 'white' rot, the mold not only degrades the carbohydrates to a certain extent, but now, the lignin itself seems to be the main food supply of the organism. In decaying wood of this type, one can detect patches of a white substance which has been considered to be almost pure cellulose.

Some years ago, it occurred to the present authors that advantage might be taken of the selective ability of the 'brown rot' molds to remove the cellulose from wood, thereby presumably freeing additional amounts of the lignin present in association with it, for the purpose of eventually isolating greater amounts of chemically unchanged lignin from the partially decayed wood.

Briefly, the method of attacking this problem would be as follows. Sound uninfected wood would first be ground to a fine sawdust and subjected to sterilization, since even wood apparently free of decay might have present some cells or spores of wood-rotting fungi retained from its natural habitat. Then, under care-

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[6] F. E. BRAUNS, J. Amer. chem. Soc. *61*, 2120 (1939).

[7] The *acetone* extract described by K. FREUDENBERG and L. KNOF (Chem. Ber. *90*, 2857 (1957)) as well as the unpurified *benzene*-alcohol extract used by ERDTMAN and LEOPOLD (Svensk Kem. Tid. *63*, 195 (1951)) are complex mixtures of several fractions and are not comparable with the electrophoretically homogeneous (W. J. SCHUBERT, A. PASSANNANTE, G. DE STEVENS, M. BIER, and F. F. NORD, J. Amer. chem. Soc. *75*, 1869 (1953)) native and enzymically liberated lignins.

[8] R. FALCK, Ber. dtsch. botan. Ges. *44*, 652 (1927).

[9] Some of which, e.g., *Trametes pini*, produce lignin-like substances themselves (W. Y. SCHUBERT and F. F. NORD, J. Amer. chem. Soc. *72*, 5337 (1950)).

fully controlled conditions, this sterilized sawdust would be inoculated with the cells of a pure culture of some specific wood-destroying organism which is known to produce 'brown rot' in wood. The progress of the decay of this wood by the mold could then be followed by periodic chemical analyses of the decaying sawdust. Since the organism used would be of the 'brown rot' type, the percentage of the cellulose in the wood should gradually diminish, while the percent of lignin should concomitantly increase. This gradual increase in the relative content of lignin and the gradual removal of the interfering cellulose should then have the effect of rendering the lignin more soluble in ethyl alcohol than it was before the fungal decay, and consequently, should allow the removal of greater amounts of lignin by extraction with this solvent.

This approach was first attempted in the authors' laboratories at Fordham University by subjecting two different species of softwoods (white Scots pine and white fir) to the decaying effects of four different species of 'brown rot' fungi.

Sound white Scots pine wood, before microbial decay, yields about 3·2% of its lignin as Native Lignin by thoroughly extracting the sawdust of this wood with ethyl alcohol. But in one experiment of the type described above, after decaying this species of wood by a brown rot mold *Poria vaillantii* for 15 months, 22·7% of the lignin[10] was isolated by the method of alcohol extraction

Since it is as a result of the action of highly specific biochemical catalysts, the enzymes, which are formed by the cells of the growing fungus and which selectively digest the carbohydrates of the wood, thereby rendering the lignin more accessible to dissolution in ethyl alcohol, the authors have referred to their lignin obtained in this way as Enzymically Liberated Lignin[11].

Enzymically Liberated Lignin

In view of the dilemma posed by the isolation of BRAUNS' Native Lignin, referred to previously, the authors were curious to learn whether their enzymically liberated lignin was the same as, or different from, native lignin. Accordingly, they isolated lignin from white Scots pine wood by both methods, and made an exhaustive comparison of the two preparations.

This comparison revealed that both the native and enzymically liberated lignins were soluble in solvents such as methyl and ethyl alcohols, in dioxane, pyridine, glacial acetic acid and dilute sodium hydroxide solution, but both were insoluble in water, ethyl ether, benzene and petroleum ether. Both lignins reduced Fehling's solution, and both gave the same colorations with such typical lignin color reagents as phenols and amines.

Furthermore, the identity of the two lignins also extended to their elementary compositions: both contained approximately 64% carbon, 6·3% hydrogen and 14·5% of methoxyl groups. This similarity extended to certain derivatives of the two lignins (the acetates and phenylhydrazones), and also to their ultraviolet and infra-red absorption spectra. Consequently, it was concluded that as far as could be determined, the native and enzymically liberated lignins of white Scots pine wood were certainly very similar[11].

The authors were then intrigued by the possibility that the lignin isolated from white Scots pine wood might represent a uniform, standard chemical entity which could be constant for many different species of softwoods.

Accordingly, a similar comparison was also made of the native and enzymically liberated lignins of another species of softwood, namely white fir. From the results of this comparison, it appeared that the two lignins of this species were also identical, and moreover, that both were also very similar to the lignins of the white Scots pine. Hence, it appeared that these lignins might very well be characteristic for several species of softwoods[11].

If the reader reflects upon the experiments previously described, he may reasonably come to the conclusion that these investigations do not permit the unequivocal statement that our enzymically liberated lignin did not consist to some extent of the native lignin which already existed in the sound wood prior to the fungal decay, for, because of its solubility in ethyl alcohol, the native lignin of the wood had to be present in the alcoholic extract of the decayed wood, together with the lignin liberated by the activities of the wood-rotting mold. A clear-cut separation of these two lignin fractions was therefore required before their identity could be unequivocally established.

It appeared to the authors that this ambiguity could easily be clarified by first extracting the sawdust of sound wood with ethyl alcohol very thoroughly until the native lignin was completely removed. This 'native lignin-free' wood could then be subjected to the rotting effects of a fungus, and, after a suitable period of decay, the wood residue could be re-extracted with ethyl alcohol. This extract then could of necessity contain only that lignin which was liberated from its association in the wood by the fungal decay, and would, of course, have to be completely free of native lignin.

The species of wood employed for this experiment was white Scots pine, and the 'brown rot' fungus employed to effect the decay was *Poria vaillantii*.

Accordingly, the native lignin was completely removed from a sample of sawdust of this species by thoroughly extracting it with ethyl alcohol until a color test on the extract revealed that further alcoholic

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[10] The claim of B. LEHMANN (in the discussion to our paper presented at the 4th Internat. Congress of Biochem. *2*, 205 (1959)) that our yields amount to 2–5% is misleading.

[11] W. J. SCHUBERT and F. F. NORD, J. Amer. chem. Soc. *72*, 977 (1950).

extraction was removing no additional lignin. The native lignin-free wood was then subjected to decay by *P. vaillantii* for fifteen months. At the conclusion of this period, the decayed sawdust was then thoroughly re-extracted with ethyl alcohol at room temperature. As a result of the 'lignin enrichment' of the wood, the yield of alcohol-soluble lignin isolated from the *P. vaillantii* decayed white Scots pine wood increased from 3·2% to 22·7% of the total lignin. Thus, after a prolonged period of decay by a 'brown rot' wood-destroying mold, there was a substantial increase in the yield of alcohol-extractable lignin obtainable from decayed wood.

It was then of importance to determine whether this lignin, liberated from its association in the wood *exclusively* by the enzymic activity of the fungus, was really identical with, or different from, the native lignin from sound wood of this species. Consequently, another complete comparison was made of the lignin liberated from white Scots pine wood exclusively by *P. vaillantii*, with the native lignin of this species.

This comparison also involved the chemical compositions, solubilities, reducing ability, color reactions and ultraviolet and infra-red absorption spectra of the lignins, as well as a comparison of their acetate and phenylhydrazone derivatives. From the results of this comparison, it was concluded that the lignins liberated from the wood exclusively by the enzymic activity of the fungus and the native lignin of white Scots pine wood were indeed identical. It appeared, therefore, that the properties of the native lignin of white Scots pine wood are characteristic for the *total* lignin content of this species of wood[12].

In addition to the above experiments on the typical softwood, white Scots pine, similar investigations were also performed on the hardwoods[13], oak, birch and maple, on Japanese kiri wood, and on bagasse[14], which is the supporting fiber of the sugar cane plant. The analytical comparisons of these lignin preparations revealed certain divergencies among the lignins of the various species studied, but they also revealed the identity of the native lignin of any individual species with the enzymically liberated lignin of that same species[15].

Since the enzymically liberated lignins were found in all cases studied to be identical with the native lignins of the same species with regard to all their properties which could be compared, it now appears that all of the lignin of each species is a uniform chemical substance, or group of substances, the greater part of which, i.e., the 'extra-native' lignin, is associated with the cellulose in such a way as to make impossible, or at least very difficult, its extraction from the sound wood by inert solvents, such as ethyl alcohol[16].

The Nature of Lignin

It is really premature at the present time even to attempt to suggest a formula for lignin. However, on the basis of certain experimental results, it is possible to indicate certain structural features which a formula for lignin must accomodate.

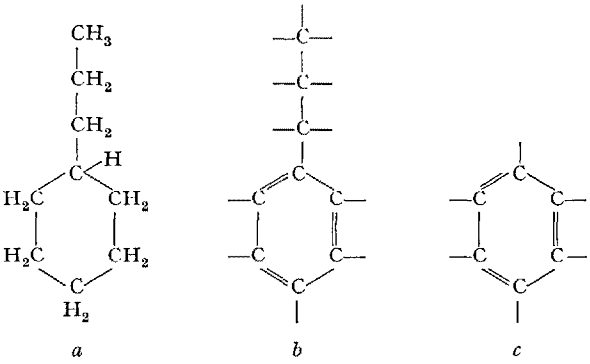

Fig. 4

As a result of hydrogenation experiments on lignin, up to 50% of 'cyclohexyl-propane' type derivatives can be obtained (Figure 4a). This shows that a large part of the lignin structure is composed of 'phenyl-propane' type skeletons (Figure 4b), since there is no doubt that the cyclohexyl rings of the products resulted from the hydrogenation of benzene rings (Figure 4c) in lignin.

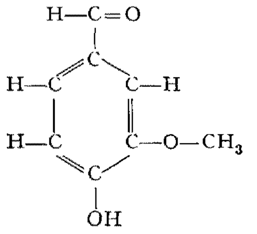

Fig. 5

That these benzene rings do occur in lignin is also indicated by its elementary analysis, which reveals a high degree of unsaturation (which is characteristic of compounds containing benzene rings) and also by the fact that on mild oxidation, up to 25% of an aromatic compound, vanillin (Figure 5), has been obtained. The formation of vanillin from lignin further indicates that, in at least a part of the lignin structure, these benzene rings contain methoxyl groups in the position of the ring *meta* to the three carbon side chain and a hydroxyl group in the position *para* to the three carbon side chain.

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[12] W. J. SCHUBERT and F. F. NORD, J. Amer. chem. Soc. *72*, 3835 (1950).

[13] S. F. KUDZIN and F. F. NORD, J. Amer. chem. Soc. *73*, 690, 4619 (1951).

[14] G. DE STEVENS and F. F. NORD, J. Amer. chem. Soc. *73*, 4622 (1951); *74*, 3326 (1952); *75*, 305 (1953).

[15] F. F. NORD and W. J. SCHUBERT, Holzforsch. *5*, 1 (1951).

[16] W. J. SCHUBERT, Holz als Roh- und Werkstoff *12*, 373 (1954).

Furthermore, there is little doubt that lignin is a high polymer which is formed to a great extent (perhaps entirely) by some kind of self-addition of these phenylpropane units. Just how these units are joined to one another, whether they are joined according to one single pattern, or in different ways, are all questions whose answers are still unknown.

Before a valid structural formula for lignin can be evolved, we must first know the structures of all the lignin 'building stones', and then we must characterize the 'building units', i.e., the dimerized and trimerized building stones, in order to learn the mode of combination of the building stones, one to another, with the sum total forming the very large lignin molecule.

How Lignin Is Formed

During the growth of woody plants, the carbohydrates are formed first. Then the formation of lignin begins, the process we call 'lignification'. In this process, the spaces in between the cellulose fibers are gradually filled in with lignin, or perhaps, some of the cellulose already present is converted somehow into lignin.

The purpose of this 'lignification' is really twofold. It cements and anchors the cellulose fibers together, and at the same time, it stiffens them and protects them from chemical and physical attack. Thus, the completely lignified fiber no longer plays an active role in the life of the plant, but it serves mainly as a supporting medium.

Although the complete picture of the chemical mechanism for the biogenesis of lignin is still unknown, there is little doubt that it originates ultimately from the carbohydrates which are formed first, from atmospheric carbon dioxide, by the process of photosynthesis. The 'lignification problem' then consists of the elucidation of the identity of the ultimate carbohydrate forerunner of lignin, together with an understanding of those enzymic mechanisms and intermediate compounds which are formed when this carbohydrate precursor is eventually transformed into the final product, namely, lignin.

With regard to the identity of the carbohydrate from which lignin may be ultimately derived, many suggestions have been advanced in the past as to its nature[17]. As the result of recent studies (F. F. Nord, W. J. Schubert, and S. N. Acerbo (in press) 1959), employing the radioactive tracer technique, it has been established that it is the most common of all naturally occurring sugars, glucose.

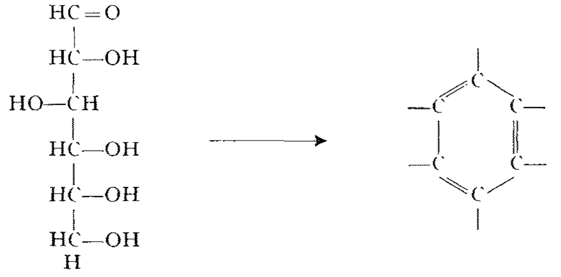
However, regardless of its identity, the carbohydrate forerunner must somehow be transformed into the very complex polymer, lignin. This cannot occur by a direct conversion, but must proceed by way of the continued self-addition, or polymerization, of some simpler, or monomeric, unit or units, which are referred to as the 'primary building stones' of lignin. The complete dissimilarity in chemical nature between carbohydrates, such as glucose, on the one hand, and an aromatic monomer on the other, implies an extended series of far-reaching enzymic reactions in order to effect this profound chemical change.

The complexity of the structure of lignin practically excludes the possibility of the existence of one simple 'building unit' for lignin in the sense in which glucose or cellobiose are considered the building units of cellulose. For lignin formation, it is necessary to postulate the existence of somewhat more complex, or 'dimeric secondary building stones'.

The formation of lignin itself finally may be either the direct condensation, or polymerization, of these secondary building stones as such, or else, there could conceivably be additional modifications before the final polymerization takes place, resulting in that very complex compound we call lignin.

Aromatization

Biochemists have long wondered about the mechanism by which plants are able to accomplish the quite remarkable feat of synthesizing 'benzenoid compounds' from non-benzenoid forerunners (Figure 6). The opportunity for an experimental approach to this problem arose from the isolation of certain very specialized strains of microorganisms which require a mixture of aromatic compounds in order to carry on their normal metabolic activities. Biochemical investigations have resulted in the elucidation of the following (incomplete) scheme (Figure 7) for the origin of two typical aromatic amino acids, phenylalanine and tyrosine[18].



Glucose, a non-benzenoid precursor

A benzenoid product

Fig. 6

The probability of the origin of the aromatic plant polymer, lignin, from carbohydrate as its ultimate source then posed the intriguing possibility of a relationship between the scheme of aromatic amino acid biogenesis and lignification.

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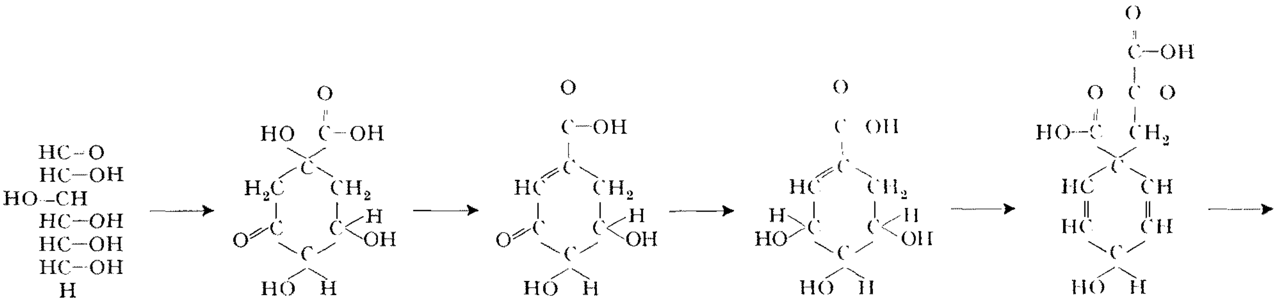
[17] F. F. Nord and J. C. Vitucci, Adv. Enzymol. *8*, 253 (1948).

[18] B. D. Davis, Adv. Enzymol. *16*, 247 (1955).



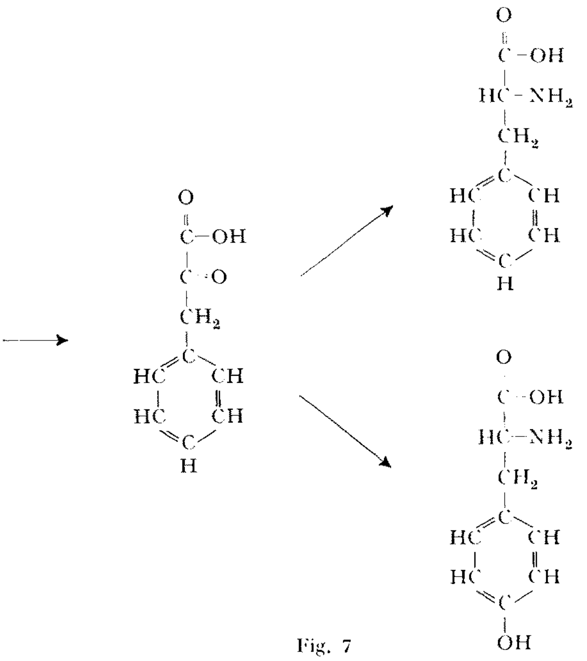


Fig. 7

Lignification in Sugar Cane Plants

Since the biochemical conversion of carbohydrates into aromatic amino acids proceeds by way of the intermediate formation of certain organic acids (shikimic acid and p-hydroxyphenylpyruvic acid), it can be reasoned that these same acids might likewise play an important part in the lignification process as well. This has been demonstrated to be the case as the result of certain investigations utilizing the radioactive tracer technique in the authors' laboratories at Fordham University[19].

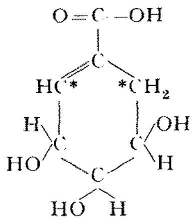


Fig. 8

This was accomplished by first preparing samples of specifically labelled shikimic acid containing the radioactive isotope, carbon -14, in the two indicated positions, as in Figure 8.

This specifically labelled sample of shikimic acid was then 'force-fed' through the leaves of a growing sugar cane plant (Figure 9). Sugar cane plants, like trees, form much lignin.

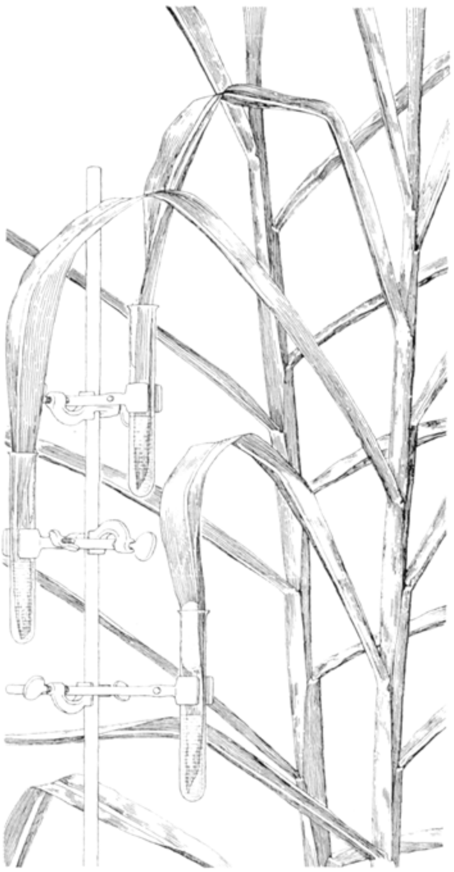


Fig. 9

After several days of this 'feeding', the plants were cut down and their lignin isolated by a standard method. This lignin was then treated with a mild oxidizing reagent and was thereby converted into vanillin (Figure 5). An examination of this vanillin revealed that it contained the radioactivity of the C^{14} which was originally contained in the 'fed' shikimic acid. This, of course, meant that the sugar cane plant had in fact converted the shikimic acid into lignin.

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[19] G. Eberhardt and F. F. Nord, Arch. Biochem. Biophys. 55, 578 (1955).

But even more important, it was possible to take this radioactive vanillin and degrade its aromatic ring in such a way as to selectively isolate the two carbon-atoms of the ring of this compound corresponding in their relative positions to the two 'tagged' positions of the original shikimic acid. An examination of these carbon atoms in the form of tribromopicrin (CBr_3NO_2), revealed that these two positions had indeed still retained the original radioactivity. This meant that the six-membered ring of shikimic acid was converted directly into the six-membered rings of the lignin polymer (as represented by vanillin) without any randomization of the carbon atoms[20].

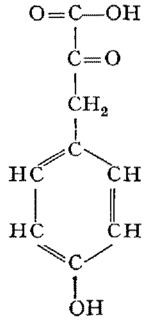


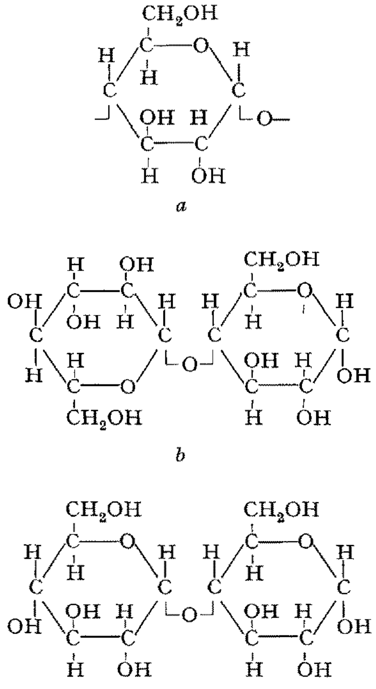
Fig. 10

This is excellent evidence for the fact that shikimic acid is an intermediate in the aromatization stage of the lignification process. That is, during the course of the conversion of carbohydrates into lignin by a sugar cane plant, shikimic acid is first formed, and then is consumed by further reactions of the process which result in the formation of lignin.

Similarly, it has also been demonstrated[21] at Fordham University that another acid, p-hydroxyphenylpyruvic acid (Figure 10), is likewise converted into lignin by the sugar cane plant, and hence, that this acid as well is to be regarded as one of the natural precursors of lignin, in the same sense as is shikimic acid. Of course, since it already is aromatic, p-hydroxyphenylpyruvic acid must be formed at a later stage in the lignification process than is shikimic acid, i.e., shikimic acid ultimately is converted into p-hydroxyphenylpyruvic acid, and not *vice versa*, before the latter is converted into lignin.

Lignin Building Stones

The use of the term 'lignin building stones' is based on the assumption that the lignin molecule, like cellulose and starch, is a long chain polymer composed of simple units which, in turn, are linked to one another in some way to form the next more complex pattern, or 'lignin building unit'. This concept is very similar to the use of 'glucose anhydride' (Figure 11a) as a building stone for cellulose and starch: the combination of two glucose anhydride groups can form either cellobiose (Figure 11b) which is the building unit of cellulose, or maltose (Figure 11c) which is the building unit of starch.



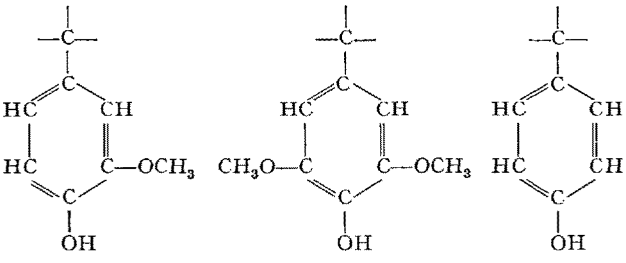
a

b

c

Fig. 11

The building stone of lignin possesses a phenylpropane carbon structure (Figure 4b), and *at least* four of these are linked together into a 'lignin building unit'. Then, a series of lignin building units, joined together, makes up the whole lignin molecule.



a b c

Fig. 12

Unlike the building stones of cellulose and starch which are uniform, lignin building stones, although having the same basic phenylpropane carbon structure, may be of at least three types, the vanillyl (Figure 12a), syringyl (Figure 12b), or p-hydroxyphenylmethyl (Figure 12c) structure.

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[20] I. EBERHARDT and W. J. SCHUBERT, J. Amer. chem. Soc. *78*, 2835 (1956).

[21] W. J. SCHUBERT, S. N. ACERBO, and F. F. NORD, J. Amer. chem. Soc. *79*, 251 (1957); Naturwiss. *44*, 35 (1957); J. Amer. chem. Soc. *80*, 1990 (1958).

Furthermore, intermediate between the most simple aromatic monomers and the final, highly complex, polymerized lignin, it is possible that some preliminary stage of lignification could occur. In this stage, a dimerization or trimerization has already occurred, and the final product, lignin, is formed as a result of an eventual polymerization of these intermediary 'secondary building stones'.

Our present knowledge of the process of lignification may then be expressed as follows: by the process of photosynthesis, the carbon dioxide of the atmosphere is converted by plants into glucose. The straight chain of carbon atoms of the sugar molecule is then cyclized into shikimic acid. This compound is then aromatized into compounds of the type of p-hydroxyphenylpyruvic acid. These latter compounds then become the first, or 'primary', lignin building stones. They then undergo dimerization, or other changes, and are thereby converted into the 'secondary' lignin building stones. These latter then eventually undergo the final polymerization which results in the enigmatic substance we call lignin[22].

Additional insight into the mechanism of lignification may be had from a study of the metabolism of a species of wood-destroying mold, namely, *Lentinus lepideus*. This fungus has the capacity to produce methyl p-methoxycinnamate from glucose-containing media[23]. The parallel between the two biochemical processes is apparent from the similarity of the structure of this aromatic ester with the structures of the lignin building stones[19],[20]. Thus, the biogenesis of this ester has likewise been found to proceed by a pathway involving shikimic acid[24].

Recently, it has been found that methyl p-coumarate may be regarded as an intermediate in the metabolism of methyl p-methoxy cinnamate by *L. lepideus*, and also possibly in the biosynthesis of that compound[25]. It is therefore possible that, in the first step of the metabolism of methyl p-methoxycinnamate, this compound may be demethylated to methyl p-coumarate, and then, this latter compound may be further oxidized by a phenolase enzyme, possibly tyrosinase[26]. Such methylation and demethylation of phenolic compounds could have an important bearing on the mechanism of the biogenesis of lignin, and also on the metabolism of that substance by wood-destroying fungi.

Of course, this outline does not represent a complete scheme, since there are still some gaps in it which must be filled in. However, what is of transcendent importance is that now, finally, with the availability of present-day enzymological techniques, and even more recently, of the radioactive tracer technique, biochemists can now look forward with confidence to a complete clarification of the mechanism of this important natural process which occurs in certain plants and makes them 'woody', which process has mystified plant chemists now for over a century.

Zusammenfassung

Lignin kann in seiner ursprünglichen Form durch Abbau von Holz mit Hilfe von Zellulose-zerstörenden Pilzen und darauffolgender Extraktion mit kaltem Alkohol isoliert werden. Die Verbindung stellt enzymatisch freigesetztes Lignin dar. Im verholzenden Gewebe entsteht das Lignin aus photosynthetisch gebildeter Glukose über Shikimisäure und *p*-Hydroxyphenyl-Brenztraubensäure.

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[22] W. J. SCHUBERT and F. F. NORD, Adv. Enzymol. *18*, 349 (1957).

[23] F. F. NORD and J. C. VITUCCI, Arch. Biochem. *14*, 243 (1947); *15*, 465 (1947).

[24] H. SHIMAZONO, W. J. SCHUBERT, and F. F. NORD, J. Amer. chem. Soc. *80*, 1992 (1958).

[25] H. SHIMAZONO and F. F. NORD, Arch. Biochem. Biophys. *78*, 263 (1958).

[26] H. SHIMAZONO, Arch. Biochem. Biophys. *83* (in press), 1959.

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Structure of Albizziine [L(—)-2-Amino-3-ureidopropionic Acid], an Amino Acid from Higher Plants *(Mimosaceae)*

Albizziine is a new plant amino acid, recognized by GMELIN *et al.*[1] as a constituent of several species belonging to the family *Mimosaceae*. Its elementary composition, $C_4H_9N_3O_3$, infra-red spectrum and colour reactions with ninhydrin and Ehrlich's reagent[1], together with its degradation upon acid hydrolysis to carbon dioxide, ammonia and partially racemized L-2, 3-diaminopropionic acid[2] strongly suggest the structure (I) or (II) for albizziine. Evidence is now available in this laboratory in support of the expression (I), which is formally analogous to L-citrulline.

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[1] R. GMELIN, G. STRAUSS, and G. HASENMAIER, Z. Naturforsch. *13b*, 252 (1958).

[2] R. GMELIN, G. STRAUSS, and G. HASENMAIER, Hoppe-Seyler's Z. *314*, 28 (1959).