

Lectures on
Soil Organic Matter

by

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March, April, May 1959

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ZENTRALBÜCHEREI

94/660

Foreword

To my friends and the friends of the subject.

In this manuscript are some unpublished results and therefore only for friends and not to use for publication.

I would enjoy having any comments on this material.

W. Flaig

Acknowledgments

This manuscript came about as a result of the kind invitation of Prof. Dr. W. H. Pierre, Head, Department of Agronomy, to give lectures about soil biochemistry. I am very thankful for this opportunity.

I would like to express my best thanks also to my colleague, Prof. Dr. Lloyd Frederick, who stood by me helpfully at all times during the writing of these lectures in the English language and I appreciate his suggestions during our many discussions.

Without the help of his co-workers, Messrs. McIntosh Sims, Horton, Brown, and of the secretaries, Mrs. McLaughlin, Misses Sansgaard and Zart, it would not have been possible to mimeograph the lectures. Also to these, many thanks.

June, 1959

W. Flaig

SOIL ORGANIC MATTER

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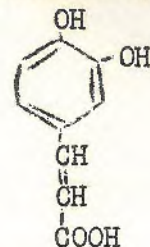
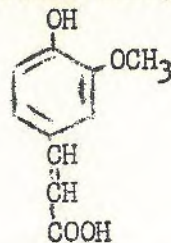
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Some Facts of Lignin Chemistry

Another important group of substances are phenol derivatives which can play a role during humification. I will restrict myself to the kind of phenolic compounds in the plants which have some chemical relationship to lignin or its building blocks on the one hand and the model substances of precursors of humic substances studied by us on the other.

In more recent times some interesting investigations had been made to determine phenolic compounds in different, especially in agricultural, plants. Herrmann gives a summary about the amount of phenolic compounds, especially partially methylated phenolic acids. (Herrmann, K: Z. Lebensmittel-Unters, u. Forsch. 106, 341 (1957) Über Oxydationsformate und phenolische Substrate in Gemüse und Obst. II. Über den Gehalt des Gemüses an Peroxydase und o-Polyphenoloxydase. Z. Lebensmittel-Unters, u. Forsch. 106, 451-454 (1957) Über Oxyzimtsäuren mit Ausnahme der Kaffeesäure und der Chlorogensäuren. Pharmazie 13, 266-276 (1958) Über Oxyzimtsäuren in Getreideblättern. Naturwiss, 45, 111-112 (1958, Plant a media 6, 35-39 (1958).

I will give only some examples for coumaric acid, ferulic acid, caffeic acid.



coumaric acid ferulic acid caffeic acid

<i>Rheum rhabarbarum</i> L. (Rhabarbar) leaf	+	+	((+))
<i>Beta vulgaris</i> L. ssp. <i>vulgaris</i> sugarbeet leaf	((+))	+	
root		(+)	
<i>Spinacia oleraceae</i> L. spinach	(+)	+	
<i>Brassica oleracea</i> Species (cabbage)	(+)	(+)	(+)
<i>Raphanus sativus</i> L. raddish leaf	(+)	+	+
root		((+))	((+))
<i>Phaseolus vulgaris</i> L. (Bohne) bean leaf	((+))	+	(+)
fruit	((+))	(+)	(+)
<i>Pisum sativum</i> L. pea leaf	(+)	+	(+)
<i>Trifolium pratense</i> L. red clover flower	+		
<i>Vicia faba</i> L. var <i>megalosperma</i> bean leaf	(+)	+	(+)
<i>Lycopersicon esculentum</i> tomato fruit	+	+	+
<i>Avena sativa</i> L. (oat) leaf	(+)	+	((+))
<i>Hordeum vulgare</i> barley leaf	(+)	+	((+))
straw	+	+	
<i>Secale cereale</i> L. rye leaf	(+)	+	((+))
straw	+	+	
<i>Triticum aestivum</i> L. wheat leaf	(+)	+	((+))
straw	+	+	
<i>Zea mays</i> L. maize leaf	+	+	(+)

In another paper by E. C. Bate-Smith, (Sc. Proc. Roy. Dublin Soc. 27, 165(1956)), data for investigations of 467 species of dicotyledons are given. 66% contain caffeic-, 48% coumaric, 26% sinapinic- and 33% ferulic acid. 3, 4, 5-trihydroxy-cinnamic acid had never been found (compare E. C. Bate-Smith, Colloquium on "Hydroxy-aromatic acids" Cambridge 1956, ref. Chem. and Ind., 478, 1956), although gallic acid is found very often.

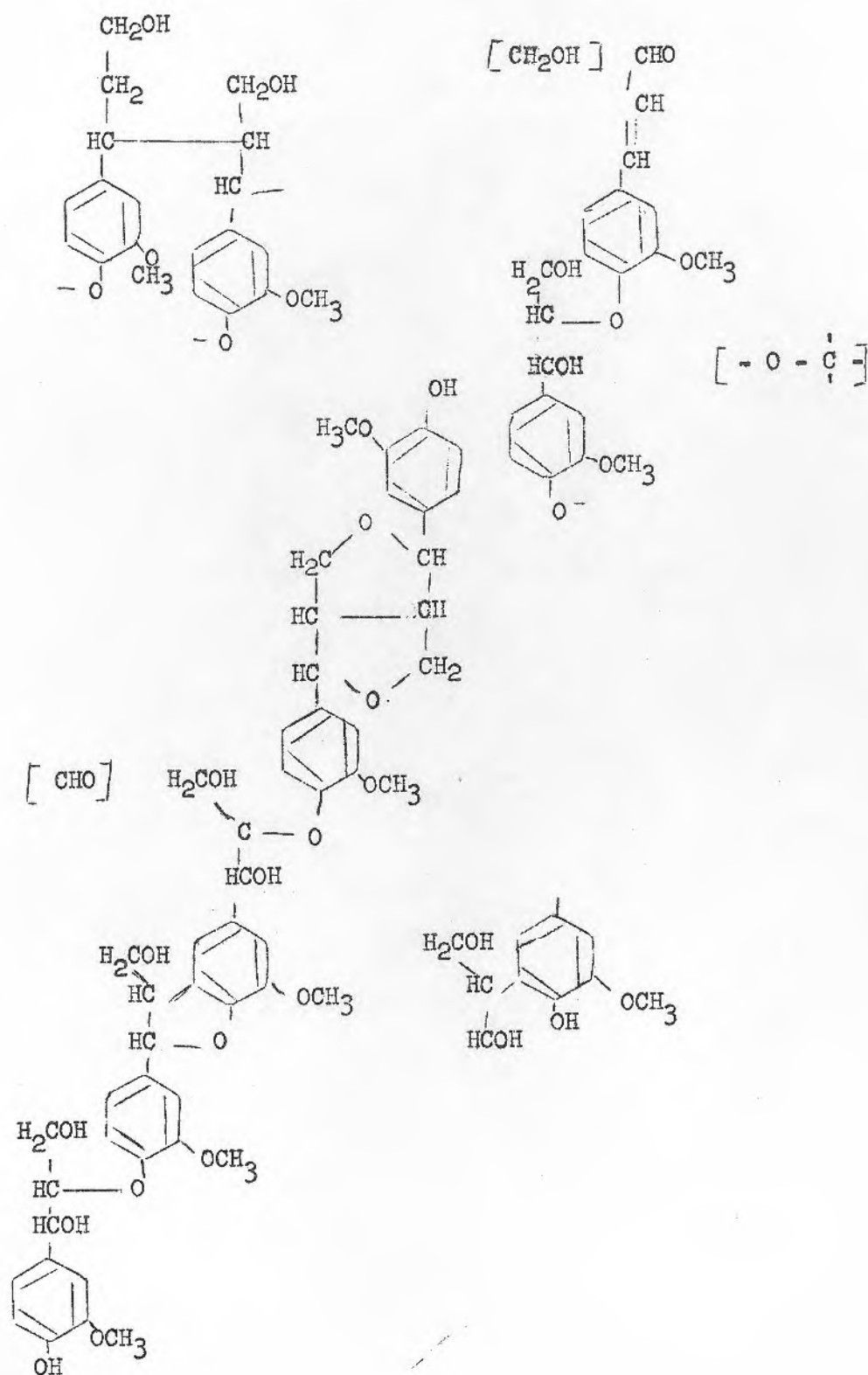
Börner (Börner.: Untersuchungen über phenolische Verbindungen aus Getreidestroh und Getreiderückständen. Naturwiss, 42, 583 (1955); Der papierchromatographische Nachweis von Ferulasäure in wässrigen Extrakten von Getreidestroh und Getreiderückständen. Naturwiss, 43, 129-130 (1956); Die Abgabe organischer Verbindungen aus den Karyopsen, Wurzeln und Ernterückständen von Roggen (*Secale cereale* L.), Weizen (*Triticum aestivum* L.) und Gerste (*Hordeum vulgare* L.) und ihre Bedeutung bei der gegenseitigen Beeinflussung der höheren Pflanzen. Beitr. Biol. Pflanzen 33, 33 (1956). made extensive studies to determine phenolic compounds in different kinds of straw. He found in straw of rye, wheat, barley, stubbles, respectively, 4-hydroxy-benzoic acid, vanillinic acid (4-hydroxy-3-methoxy benzoic acid), p-coumaric acid, 4-hydroxy cinnamic acid and ferulic acid (4-hydroxy-3-methoxy-cinnamic acid. To give some ideas about the content of phenolic acids in the different kinds of straw, I will mention that in each case 25 grams of straw cut into small pieces has been leached with 10 fold amount of distilled water at 15° during 24 hours. The extract was acidified and extracted with ether. The ether was almost completely evaporated. In 25 ml of the extract between 825 and 1000 micrograms p-hydroxy-cinnamic acid, 800 to 1300 µg ferulic acid, 300 µg p-hydroxy-benzoic acid and nearly 800 µg vanillinic acid were found. This seems to be a very little amount, but if the stubble has lengths of 10 cm., in average 2800 kg of dry weight or 2032 kg organic substance/ha is incorporated into soil with every harvest. This means, that from straw with a content of 18% of lignin in average for instance 80 g, p-hydroxycinnamic acid can be leached by water into the soil. If the entire amount of the acid would be distributed in depths of 20 cm/ha, there would be a concentration of 0.2×10^{-6} moles. According to our experience, such kind of lignin fragments have no more an effect on plant growth in the concentration. But in the environment of the stubble, the concentration can be higher and influence plant growth. Indeed experiments are known, which support this idea. These phenolic compounds are, however, not very stable and decompose more or less rapidly.

One of the most important phenolic compounds in plants is lignin. I will give a short summary about formation and chemistry of lignin. Since the radioactive tracer methods have been introduced, the chemistry of lignin made enormous progresses. With these studies it has been possible to give a formula of lignin with which one tries to combine the present knowledge.

Formula of Lignin

In this formula, the structural information are collected which at present appears to be experimentally supported. The figure can help to review the results of different authors. This formula was summarized by Adler, E.: (Newer views of lignin formation. Tappi: 40, 2949301 (1957)). Seven guaiacylpropane building blocks can be distinguished. Some of the propane side-chains represent a glycerol structure with an oxygen atom at each carbon atom. Others are substituted with only one or two oxygens. The linkages of the different building blocks vary. The guaiacylglycerol elements are combined by glycerol - β -aryl ether bonds. Another type of ether linkage is the phenylcoumarin system, which involved a benzylaryl ether linkage as well as a carbon-carbon bond between the β -C-atom of the side-chain and the aromatic nucleus of the adjacent building blocks. It has also been found

STRUCTURE SCHEME OF LIGNIN

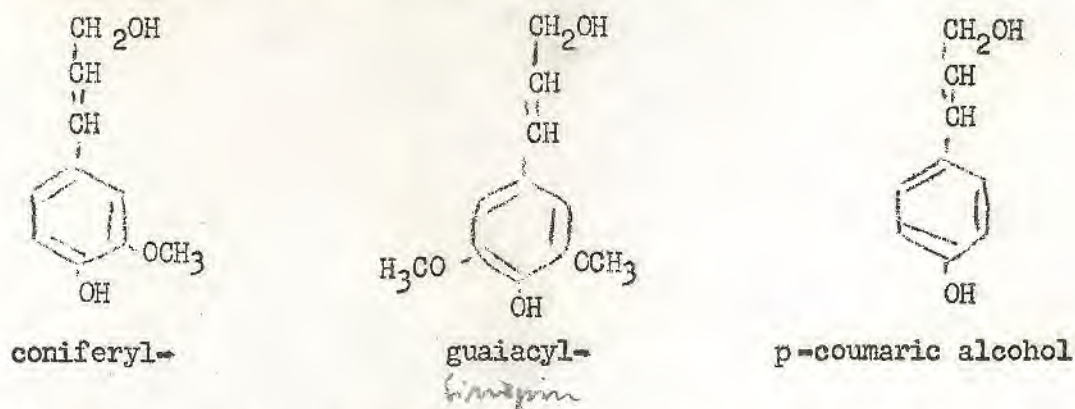


that there exists similar condensations between side-chain and nucleus without ring closure. Another kind of linkage is the pinoresinol grouping. In this case two monomers are joined together by aliphatic ether bridges as well as C-C bond between two β -carbon atoms. The last possibility is a C-C bond between two nuclei, forming a diphenyl linkage. Some physical properties of lignin can be explained by a branching condensation in which phenolic groups and β -hydroxyl groups of the guaiacylglycerol unit take part by etherification. Only about one third of the phenolic hydroxide groups are free.

It is especially interesting from the biogenetic point of view to study the C_9 -units. The precursors of lignin reflect the structure of C_9 -units. Coniferyl alcohol is the first one of these which had been found. The coniferyl aldehyde residue is responsible for several color reactions of lignin. There are only very small amounts of coniferyl aldehyde in the final lignin molecule.

The coniferyl alcohol has been found in the cambial sap as a glucoside. It is suggested that coniferyl alcohol polymerises with the unsaturated endgroups like styrene.

We will now discuss the question of how lignin is synthesized in nature. A large amount of experimental evidence is given by different authors. Coniferous lignin is formed from coniferyl alcohol by dehydrogenative polymerisation with a simultaneous uptake of the elements of water. In other monocotyledons and dicotyledons another type of lignin has been found. A second building block, the guaiacyl alcohol, is copolymerized. This type of lignin is called guaiacyl-syringyl-lignin. p-coumaric-alcohol is a third precursor for grasses and mosses.

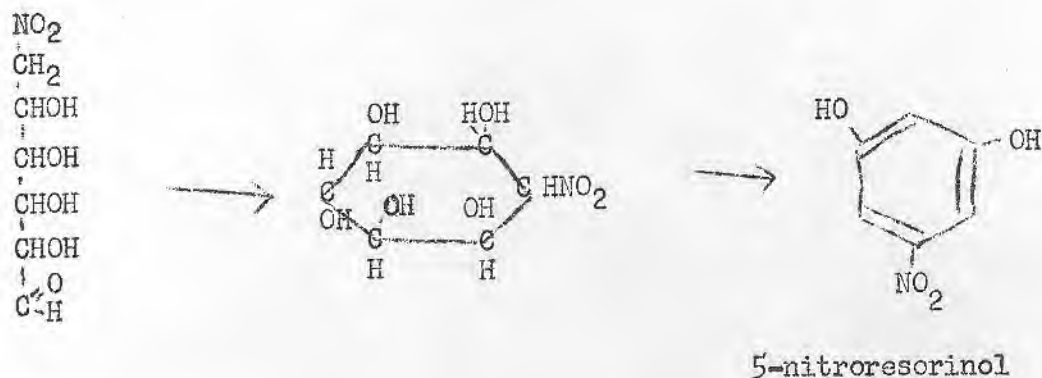


As the first step of formation of lignin the reactions for the synthesis of lignin precursors in the plants must be studied; the second step are the reactions for their condensation to lignin.

The first is interesting for our consideration not only in the case of humification of plant residues, of the formation of humic substances, respectively, but also for the explanation of the participation of microorganisms during humification. It is one of the most plausible ways to explain the formation of the humic like substances in their cultures in absence of lignin. Later on we will discuss more details.

With respect to the bio-synthesis of phenylalanine the formation of the C_6 - C_3 -compounds has been successfully demonstrated in the recent years.

Older theories suggested that glucose would be closed to a ring by aldolcondensation and the formed cyclcohexite, dehydrated to an aromatic compound. In vitro it has never been possible to prove this reaction. Only with a derivative 6-Nitro-6-deoxy-d-glucose H.O.L. Fischer and co-worker succeeded in the formation of ring compound.

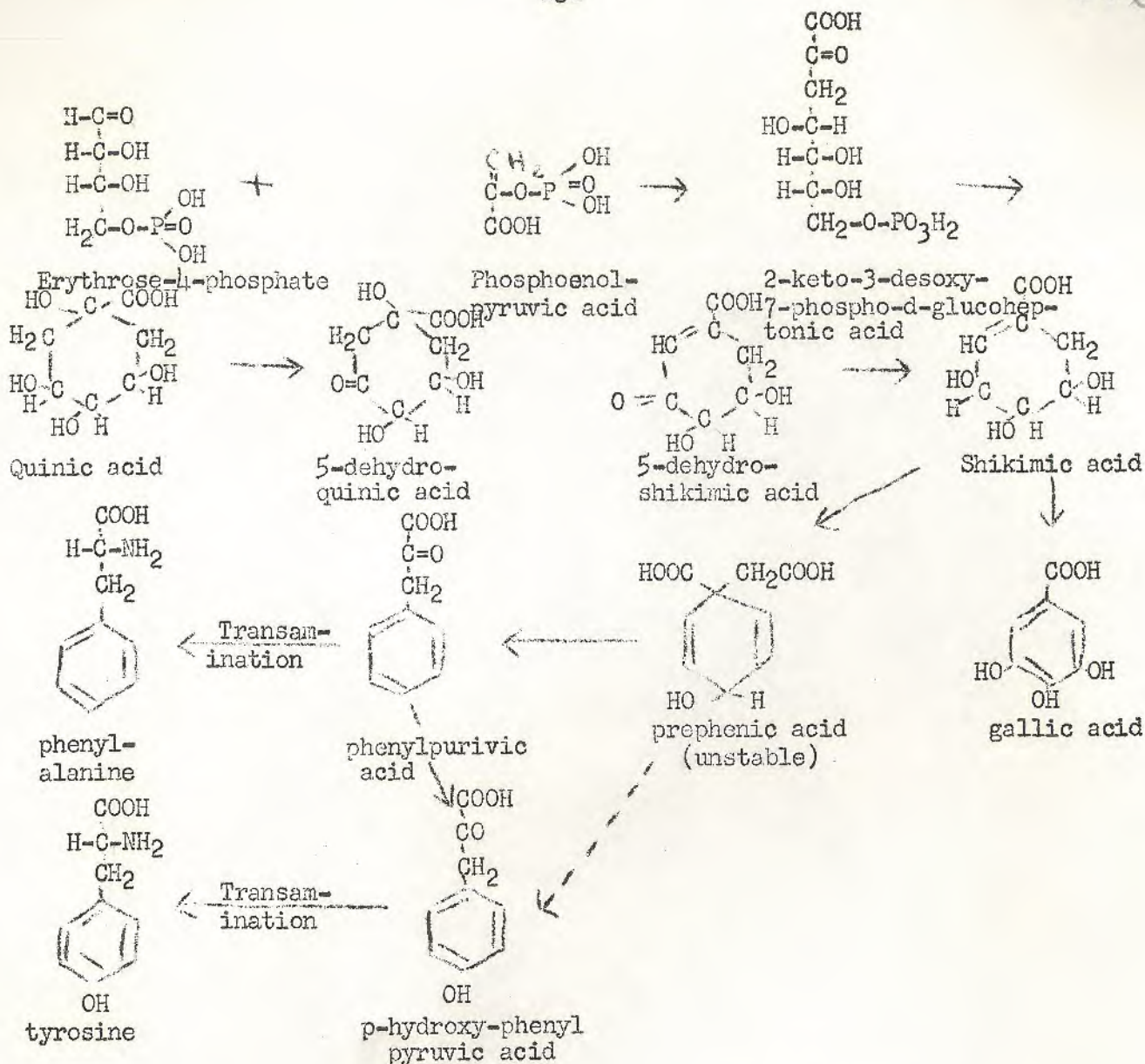


M. Grosheintz u. H.O.L. Fischer, *J. Amer. Chem. Soc.* 1479-1484 (1948).

F. Weygand^{1/} had been able to show with labeled inositol that no aromatic compounds could be formed in plants by this cyclcohexite. This way is therefore not possible for the formation of aromatic compounds in the plants although it seems to be successful by the relatively high content of inositol in the plant.

But there is another way to aromatic compounds which also comes from glucose as initial material but is not absolutely elucidated. During glycolysis glucose decomposes into C₄- and C₃- fragments, which form a C₇ compound. (Srinivasan, P. R., M. Katagiri u. D. B. Sprinson: Enzymic synthesis of shikimic acid from D-erythrose-4-phosphate and phosphoenolpyruvate. *J. Am. Chem. Soc.* 77, 4943 (1955); Brown, S. A. u. A. C. Neish: Shikimic acid as a precursor in lignin biosynthesis. *Nature* 175, 688 (1955)).

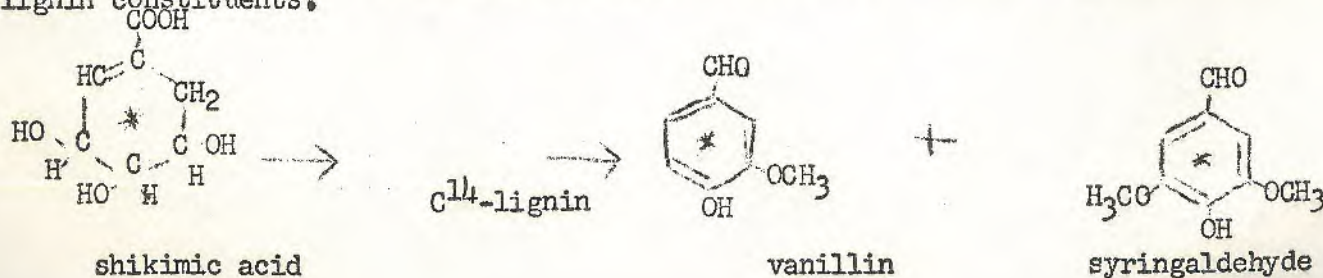
^{1/} F. Weygand and E. Schulze: Synthese von meso-inosit-C₁₄. *f. Naturforsch.* 116, 370-374. (1956).



The hydroxyl groups from glucose disappear at first and others are again introduced into phenylpyruvic acid or phenyl alanine for formation of tyrosine and hydroxy phenyl-pyruvic acid. The introduction of phenol groups in aromatic systems must be discussed later on.

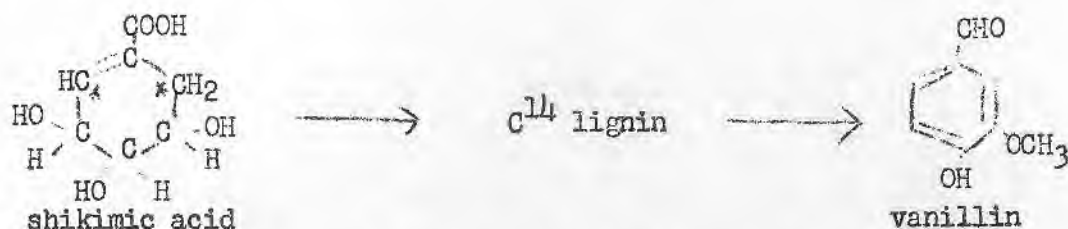
Most of these reactions which I mentioned have been studied with microorganisms but shikimic acid is also found in numerous plants, therefore a transformation in lignin components would be of interest.

Brown, Stewart, A., and Neish, A. G. ((1955) Shikimic acid as a precursor in lignin biosynthesis. Nature 175, 688-689) studied at first the biogenesis of lignin constituents.



They gave labeled shikimic to young wheat and maple plants and isolated the lignin from these plants after 24 or 48 hours. After oxidation of the lignin with nitrobenzene in alkali, they identified remarkably highly active vanillin and syringaldehyde. This means that shikimic acid has been converted into the aromatic lignin compounds.

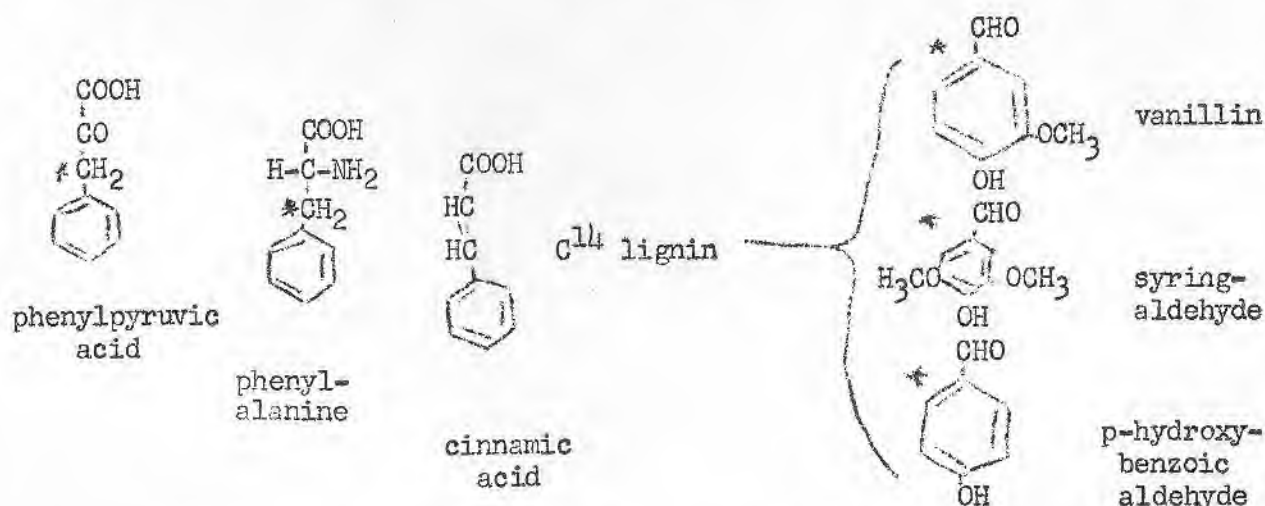
Eberhardt and Schubert (Eberhardt, G., and Schubert, W. J.: Evidence for the meditation of shikimic acid in the biogenesis of lignin building stones, *J. Am. Chem. Soc.* 78, 2832-2835 (1956)) made a similar experiment with labeled shikimic acid in 2,6-position in sugar cane and found after isolation of vanillin, that this is also radioactive in 2,6-position.



This fact is important in so far as it is proved that the hydroaromatic system, shikimic acid, is converted in the aromatic one of lignin, vanillin, respectively, without a change of the skeleton of the ring system.

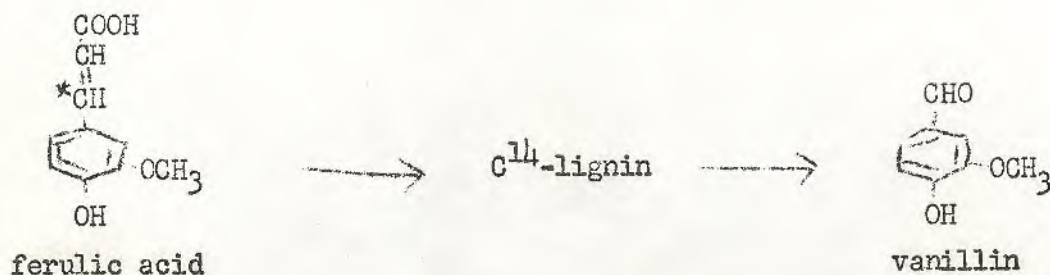
The first aromatic compound formed from shikimic acid is without a phenolic hydroxyl group. Therefore it must be elucidated, if known aromatic systems derived from shikimic acid can serve as intermediates of lignin precursors or lignin itself.

Brown, Stewart A., and Neish, A. G. et al. (Studies of lignin biosynthesis using isotopic carbon. *Can. J. Biochem. and Physiol.* 33, 948-962 (1955) 34, 769-778, (1956)) labeled phenylpyruvic acid, phenylalanine and cinnamic acid in the side chain.



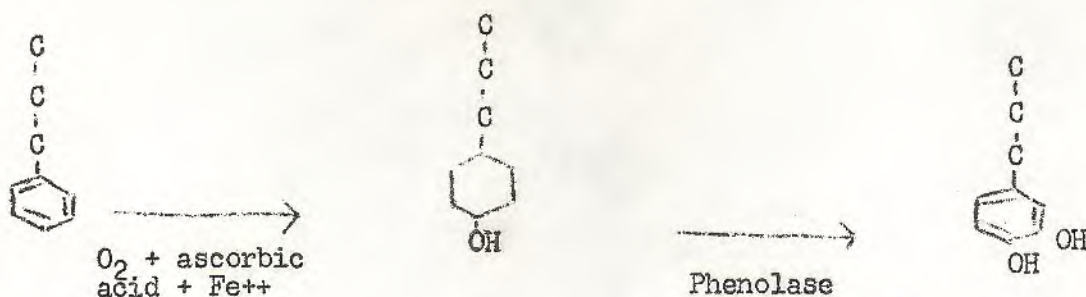
All three compounds are converted in wheat plants into lignin; phenylpyruvic acid is converted in a smaller extent. After isolation of lignin and its oxidation with nitrobenzene in alkali, vanillin, syringaldehyde and p-hydroxy benzaldehyde could be identified.

In graminaceae there exists three types of building blocks. These experiments show that by oxidation aromatic systems could convert into the different types of hydroxy-compounds, 2 of them partially methylated.



Freudenberg, K. (Zwischenprodukte bei der Bildung von Lignin: Chem. Ber. 88, 617-625 (1956) there are other references) found the interesting fact that ferulic acid added to spruce resulted in a lignin fraction, from which only vanillin could be isolated. Even with high amounts of ferulic acid vanillin was always the radioactive reaction product of a mixture of aldehydes. It seems that the syringyl type is not formed, if the performed guaiacyl type is added. There must be special reasons or reactions which are unknown. Later on we will hear that ferulic acid differs also in its physiological effect on the growth of organisms.

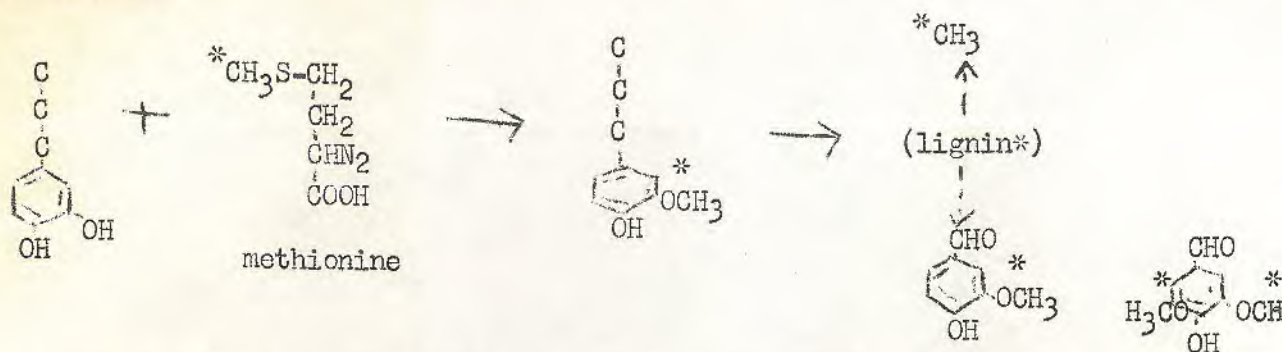
In the plants must occur a hydroxylation of the benzene nucleus. There may be different possibilities, for instance it has been demonstrated by Udenfriend and co-workers (Ascorbic acid in aromatic hydroxylation. I. A model system for aromatic hydroxylation. J. Biol. Chem. 208, 731-739 (1954)) that the system oxygen-ascorbic acid-ferrous ion is able to introduce a hydroxyl group into substituted benzene rings.



This reaction has been made only in vitro but may be considered to occur also in the plants.

The introduction of further hydroxyl groups in the phenols is mentioned in different papers of which we will discuss several later. In the case of lignin, Mason showed that the enzyme complex phenolase is able to introduce a second hydroxyl group to the pyrocatechol type. The phenolase is a copper protein widely distributed in plants as the catalyst to transfer the molecular oxygen. The reaction to form pyrogallol compounds are not well known.

The next step must be the methylation of the polyphenols to the guaiacyl and syringyl structure. Byerrum and co-workers labeled sulphur-bound methyl group of methionine.

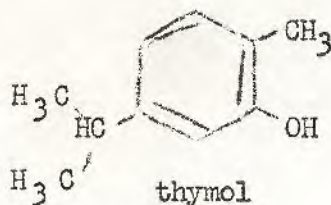


(Bylrrum, R. H., Flokstra, S. H., Dewey, L. J., Ball, Ch. D.: Incorporation of formate and the methyl group of methionine into methoxyl groups of lignin. *J. Biol. Chem.* 210, 633-643 (1954)) They could show in experiments with barley that the methyl group is transferred to the aromatic portion of the lignin. They isolated the lignin and oxidized with nitrobenzene in alkali. The isolated aromatic aldehydes had been radioactive as well as the methyl group which had been split off the lignin with hydrogen iodide.

Further experiments which concern the side-chain will not be recorded because they are not important for our considerations of humification. I will only say that it has been proved, that also the side-chain in the labeled precursors can be found in the isolated lignin. It had also been possible to find the Hibbert ketones.

For our discussion it is more important to refer to the reactions which belong to the second step of formation of lignin, the condensation reactions. Siegel has found eugenol in the presence of hydrogen peroxide was converted by pea roots into a polymeric product which was deposited in the tissue of the roots. This product was similar to lignin in several physical and chemical respects. It gave the lignin color reactions, for instance, with phloroglucinol-hydrochloric acid.

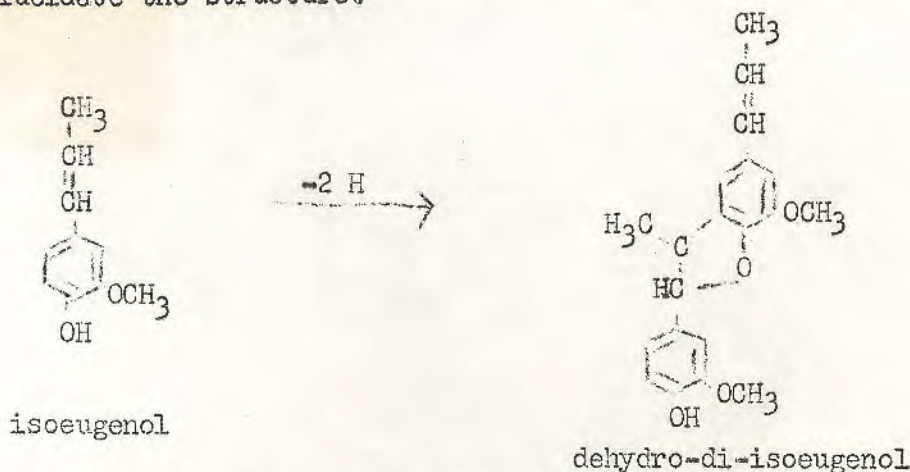
Together with other experiments one can suggest that eugenol undergoes oxidative polymerization after oxidation. In this connection we will remark that thymol polymerizes also in bean tissue to a lignin like product, although thymol does not possess a lignin structure.



I mention this fact because later on I will speak about some experiments with thymolhydroquinone or thymolquinone comparable with lignin fragments or with a precursor of humic acids.

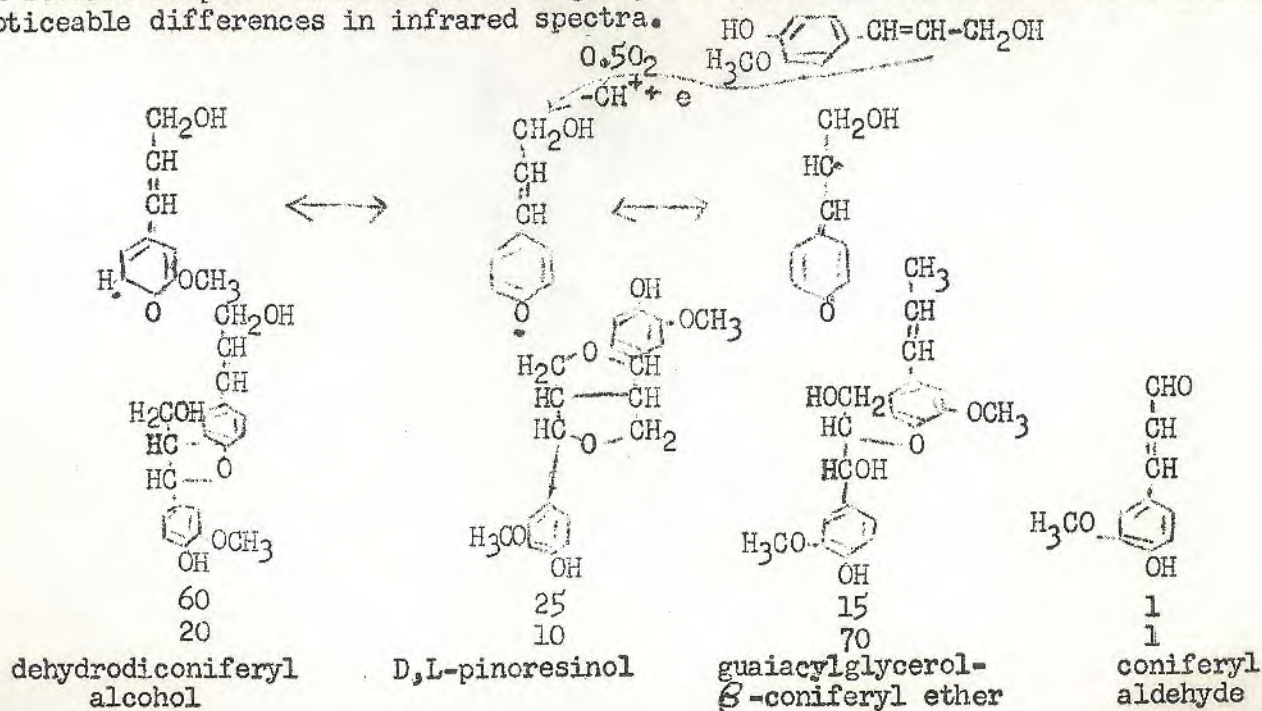
For the condensation reactions some special remarks must be made. Freudenberg, K. (Lignin in Rahmen der polymeren Naturstoffe, Angew. Chem. 68, 84 (1956)) showed that the three types of precursors are as glucosides in plants, especially coniferyl alcohol as coniferin and syringyl alcohol and syringen. They are hydrolyzed by a cell-bound β -glucosidase in about 10 cell rows close to the cambium. The formed coniferyl alcohol, for instance, is then polymerized to lignin in adjacent cell rows by a phenol oxidase which had been also identified.

Some facts about the mechanisms of the polymerization are also interesting for the chemistry of humic acids. Erdtman, H. (Dehydrierungen in der Coniferylreihe. Ann. 503, 283-294 (1933)) investigated p-hydroxyphenylpropane compounds which are unsaturated in the middle of the side chain. He could show that by oxidation reactions coupling occurred not only in the position ortho to the phenolic hydroxyl but also at the β -carbon atom of the side chain. He could isolate dehydro-diisoeugenol and elucidate the structure.



In recent time Cousin and Herissey got the same compound by oxidation of isoeugenol by action of a mushroom oxidase.

In the last 10 years Freudenberg studied the condensation reactions of lignin precursors such as especially coniferyl alcohol. He treated coniferyl alcohol with air in the presence of mushroom oxidases and obtained an amorphous powder which in several respects was similar to lignin, for instance there had not been found noticeable differences in infrared spectra.



He gave also information regarding the mechanism of this process. Insofar as it had been possible to isolate from the reaction mixture at an intermediary stage, four well defined products viz. dehydrodiconiferyl alcohol, D,L-pinoresinol, guaiacylglycerol- β -coniferyl ether, and coniferyl aldehyde.

The three dimeric products yielded also amorphous polymers by enzymatic oxidation.

The mechanism of the initial reactions has been formulated by Freudenberg as follows: "the oxidizing enzyme catalyzes the removal of a proton plus an electron from the phenolic hydroxyl group, thus yielding a mesomeric radical". Stabilization of the radical is brought about by dimerization, which, due to the existence of various reactive centers, can proceed in different ways, resulting in either nucleus- β -coupling, $\beta + \beta$ coupling, or aroxyl- β -coupling. Secondary reactions such as cyclization or addition of water, terminate the reactions.

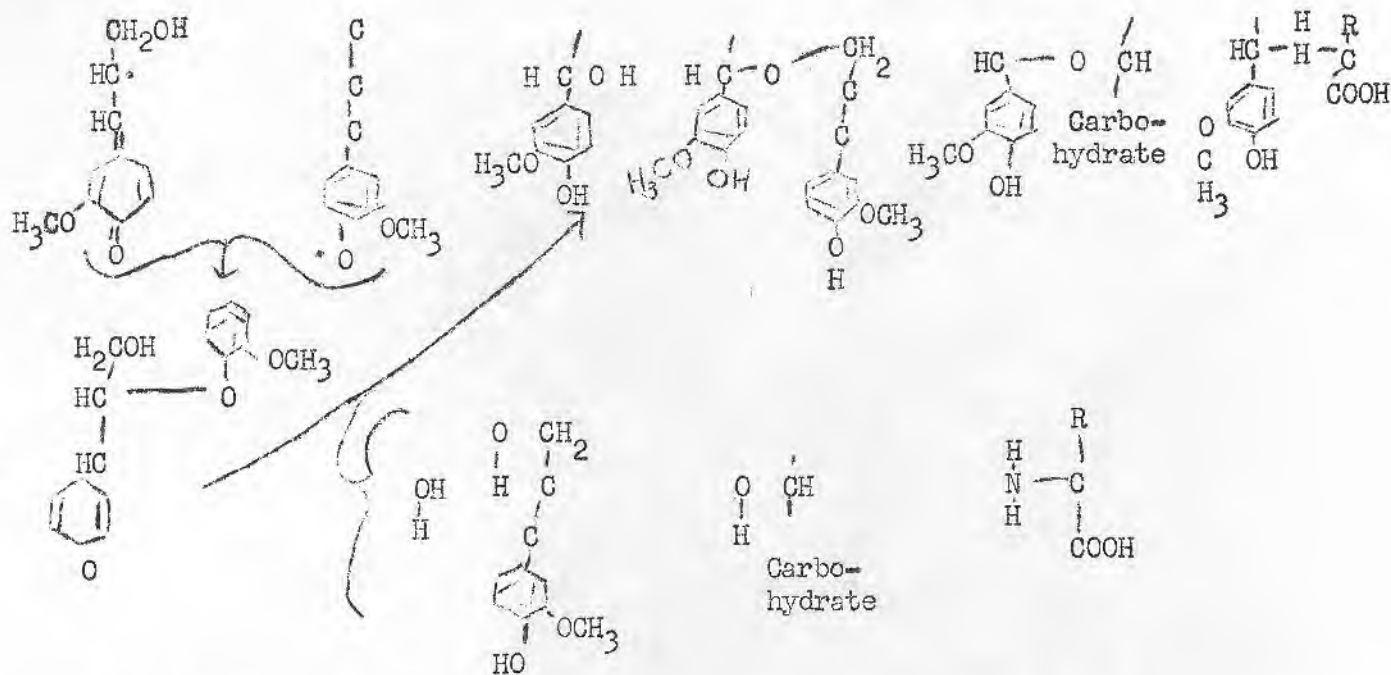
Something must be said about the nature of the enzyme or enzymes, with which the condensation of coniferyl alcohol was made. This was a crude mushroom preparation and can contain polyphenol oxidation and peroxidase. Some authors had also determined lactase in this crude preparation of enzymes. It must be emphasized that the enzymatic reaction in vitro may give results which differ from those attained in the living cell. Freudenberg investigated the influence of experimental conditions on the kind and yield of the different condensation products. He reported that, if the solution of coniferyl alcohol and mushroom enzymes were mixed at once and then treated with air, the yield of the dehydrodiconiferyl alcohol amounted to 60% of the total mixtures of dimers. Guaiacyl glycerol- β -coniferyl ether becomes the major product with a yield of 70% if one adds coniferyl alcohol dropwise to the enzyme. The second procedure forms products which are closer to the various structures in lignin.

Some results of investigations of the linkage of the three dimeric products must be given in addition. Pew showed by oxidative degradation and by the spectrophotometric investigations of Aulin-Erdtman that there is a possibility of linkage between the aromatic part.



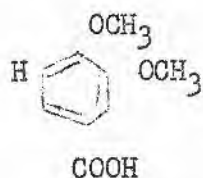
The formation of the structure can be explained by a dimerization of the quinonic form of the initial radical with an active center in the 5-position of the nucleus. This type of reaction is very common in the case of dimerization of phenols by oxidation. Later on we have the occasion to discuss more about this point.

In this connection it must be mentioned that the first step of polymerization of humic acids is in the case of coniferyl alcohol the intermediate comparable with p-quinone-methide. This is condensed with different groups of the side-chain.

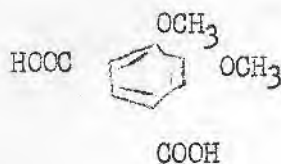


With the elements of water another molecule of coniferyl alcohol and carbohydrates can be added on this radical form. In our case it may be important that also amino acids can be added.

In the case of fragments of lignin after oxidation, it must be mentioned that Richtzenhain (H. Richtzenhain, Svensk. Papperstidn. 35, 644 (1950)) after oxidation and following methylation found veratric acid and several percent isohemipinic acid.



Veratric acid



Isohemipinic acid

This is a proof that two phenyl propane units are connected with a C-C-bond. Besides the decomposition of aromatic products there are many other aliphatic compounds as formaldehyde, acetaldehyde, formic acid, acetic acid, oxalic acid and others. This cannot help to elucidate the structure of the lignin molecules.

(H. Richtzenhain, Ber. Dtsch. Chem. Ges. 75, 269 (1942), K. Kratzl, Mg. Chem. 80, 314 (1949)).

Now some remarks about the place of the lignin in the plant. Lignin may be called the encrusting material of the plant cell walls. The lignification of the cell wall is accompanied by a change in the function of the cell. The cell dies and the lignified structures become abundant in the conducting and the supporting tissues.

S 1a 43

Recent electron microscopic studies on the structure of plant fiber have shown that an intermicellar system of cellulose exists with two capillary systems merging into one another. The fine capillaries of the order 10 Å can be penetrated by water and molecular solutions. The coarser capillaries are also submicroscopic capillaries and contain other cell wall substances such as wax, cutin or lignin. They must be considered as channels by which the living protoplasm received after the formation of the micro fibriles. (Frey-Wyssling, A., 1953: Submicroscopic morphology of protoplasm. Elsevier Publishing Co. Inc., New York. 411 pp.) It is not distributed equally through whole structure of the cell. The largest amount is in the middle lamella up to 75%.

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Ritter determined that, on the average, 25% of the lignin is in the cell wall. (Ritter, G. J.: Paper Ind. 16, 178 (1934)). The distribution of lignin in the cell therefore led to the opinion that lignin is a cementing substance which holds the cells together.

Freudenberg compared the composition (structure) of the cell wall with a building principle of reinforced concrete.

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The iron bar is comparable with the micelle of polysaccharides, cellulose, and the concrete with lignin. In this connection it is important to know if lignin and carbohydrate in the cell wall are only a mechanical mixture or exist a chemical linkage between the substances. The opinions about this point are divided. Decomposition experiments with enzymes such as new chemical experiments speak for a chemical linkage. (Ploetz, Th.: - B. 73, 57, 61, 74, 790 (1940) - Freudenberg, K. u. Th. Ploetz: Holz als Rog- u. Werkstoff 3, 105 (1940) - Richtzenhain, H.: Uber die Frage der Lignin-Kohlenhydrat-Bindung im Holz. Z. f. Pflanzenernahr., Dung., Bodenkunde, 69 (114) 25-32 (1955)).

Richtzenhain succeeded in isolation of lignin-carbohydrate complexes with a ratio 1:1 by the extraction of methylated wood with formic acid.

Also other recent work shows that there is a chemical combination of lignin with carbohydrates, especially with pentose. (I. Kawamura und. T. Higuchi, J. Soc. Textile und Cellulose Ind. Japan 9, 157 (1953) 9, 454 (1953) 10, 15 (1954). P. H. Traynard und. A. M. Ayroud, Bull. Soc. Chim. Fr. 1954, 345). Bjorkman (A. Bjorkman, Svensk. Papperstidn. 59, 477 (1956)) succeeded in recent time to extract and isolate a complex of lignin and carbohydrate with dimethylformamide out of very fine milled spruce wood. After hydrolysis the same sugars could be identified which are also in the hemicellulose.

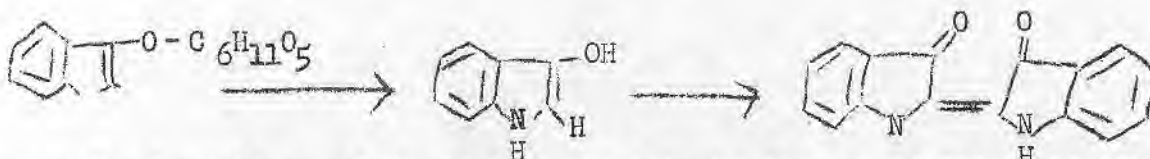
This consideration must have been made because later on we must discuss the decomposition of lignin in the presence of cellulose. In some cases it seems that lignin is decomposed only in the presence of carbohydrates as a carbon source by microorganisms. Another fact is understandable by this model of Freudenberg. Lignin has high exchange capacity according to the very large surface. It adsorbs up to 14% K₂O of its own weight. (Scholler, H.: - Chem. Ztg. 63, 737 (1939)). We must speak about the change of the sorption capacity of lignin during rotting later on. Some details about the formation of lignin in the plants must also be mentioned.

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Scheme of a cross-section through
a coniferous sprout (1 year old)

Part of a microscopic cross section
through the phloem, cambium and xylem
of a living sprout of pinus.

Continuing his work, Freudenberg investigated the formation of lignin in coniferous plants. He infiltrated radioactive coniferin in a sprout of pinus. With cross-sections he could show that the labeled coniferin had been in the cambium sap. The lignified cells have been colored with phloroglucine. The effect of the glucosidase he could show by the infiltration of indican.



The β-glucosidase split off indican; the phenolase oxidized it to indigo. With this method Freudenberg succeeded in showing that near the cambium tissue there is a β-glucosidase which split off coniferin in coniferyl alcohol and glucose. In the same space is also the phenoloxidase which polymerizes coniferyl alcohol into lignin.

The left drawing is a schematic one. The right one is a microscopic section which shows the same results.

Lignin of deciduous trees differ from that of needle trees. In the deciduous trees, in addition to coniferyl alcohol, sinapin alcohol occurs. In this compound both places which are adjacent to the OH group are occupied by methoxyl groups. For this reason the rate of condensation is lowered. It may be that this fact causes the higher tendency of peptisation of deciduous tree lignin.

	N in %	OCH ₃ in %
Lignin in hard wood	0	20.0
Lignin in soft wood	0.2-0.3	15
Lignin in graminaceae	1.2-1.6	10
Lignin in leguminous plants	2.9-3.4	5

The different content of methoxyl groups of coniferyl alcohol and sinapin alcohol explains why the needle tree lignin has on the average, 15% -OCH₃ and deciduous tree lignin 20%. The lignin content is 25-30% in needle trees and 18-25% in deciduous trees. On this table the different kinds of wood are divided in hard wood and in soft wood. The hard wood trees contain more lignin than the soft wood.

It can be also observed that in the part of the trees which are exposed to mechanical forces the lignin content is higher. In other plants, there is generally not the same relationship and also not the same composition of lignin in all parts of the plants. Generally it is to be said that in all plants and in all their parts the content of lignin and of methoxyl of lignin increases with age. In grasses and in leguminous plants the lignin content is 10% and 5%, respectively.

If one isolates lignin, in every case more or less nitrogen compounds are included. This increases from hard wood to leguminous plants. It is not sure if this is an effect of the isolation or the nitrogen content belongs to these different kinds of lignin. A possible reaction of lignin with amino acids has been mentioned before.

The increase of lignin with age shall be shown, for instance, by the results of the following table. (Beckmann, E., O. Liesche u. F. Lehmann: - B. Z. 139, 491 (1923) - Phillips, H. u. M. J. Goss: - J. Agr. Res. 51, 301 (1935)).

Lignin content and lignin composition depending
on the age of the plants

Winter rye	192 days	13.03% lignin with 3.03% OCH ₃
	274 days	20.49% lignin with 13.42% OCH ₃
Barley	7 days	1.71% lignin with - OCH ₃
	14 days	1.98% lignin with 2.03% OCH ₃
	86 days	8.36% lignin with 15.18% OCH ₃

For the characterization of lignin it is necessary to give some physical data. Form and size of the molecular lignin is not yet exactly ascertained. The average molecular weight are 1000 to 10000. It depends upon the kind of isolation. They are mostly determined to be between 3000 and 4000.

By recent investigations with x-rays lignin is a three dimensional cross-linked substance. (Becherer und. G. Voiglander-Tetzner, Naturwiss. 42, 577 (1955)). The largest part of lignin is not soluble in water and in organic solvents if it remains unchanged. Lignins which are isolated in a soluble form and therefore more or less decomposed have low specific activities. It is suggested that they probably consist of 5 to 6 units of phenylpropane. (S. M. Siegel, Quart. Rev. Biol. 31, 1 (1956)). Grelen (N. Gralen, J. Colloid Sci. 1, 453 (1946)) isolated spruce lignin with thioglycolic acid and determined the molecular weight with the Svedberg-Ultracentrifuge to be 6000 to 7000. After treating the wood with hot hydrochloric acid, the molecular weight increases to approximately 34,000. The osmometric values have been 3800 to 4500. (S. M. Siegel, Quart. Rev. Biol. 31, 1 (1956)). The "molecular weights" determined by the cryoscopic and ebullioscopic methods such as calculated by the empirical formula are mostly between 800 and 900. It may be suggested that this value is the weight of one unit; the lignin molecule is a multiple of this.

Colloid chemical studies of lignin showed that the surface area of one gram lignin is 180 sq. meters. In comparison the values are 10.35 for vermiculite, 15.5 for montmorillonite, 22.2 for diatomaceous earth and 1700 for cocconut shell charcoal. (Cassidy, H., Adsorption and chromatography. Technique of organic chemistry, Vol. V. Interscience Publishers Inc., New York. 360 pp. (1951)).

To give an idea about the efficiency of the different methods to isolate lignin and the different composition resulting from these, the elementary analyses are mentioned, especially for wheat straw which is more interesting for our considerations about humification than wood.

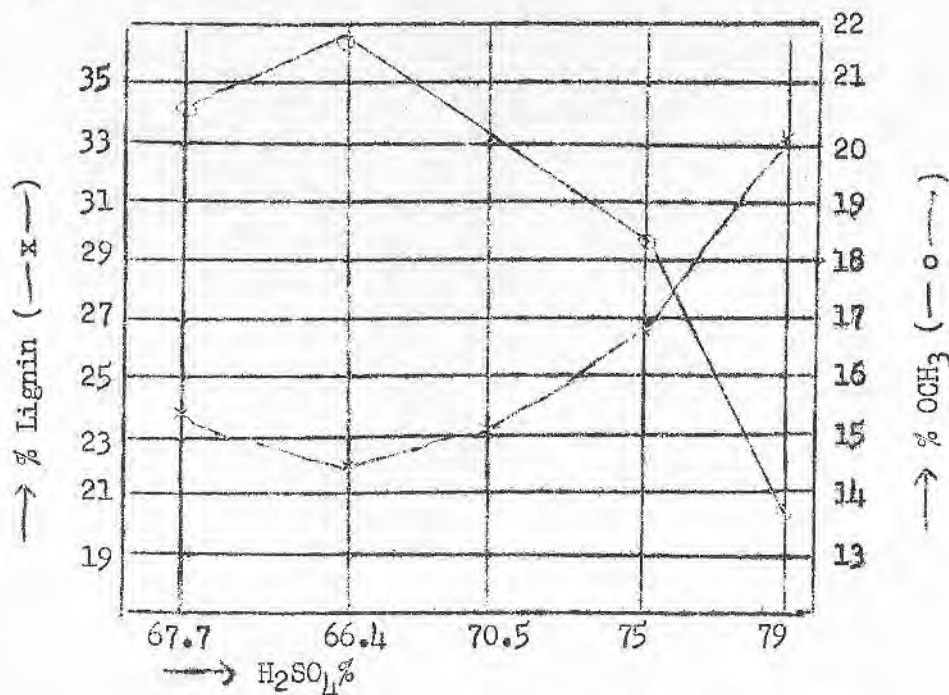
Elementary composition of different lignins in percent*)

	C	H	O	N	S	OCH ₃	Ash
Isolated with Sulfuric acid following Klason	58.59	5.60	31.37	0.54	3.9	15.33	14.22
Isolated following Brauns	60.87	6.11	32.58	0.44		14.29	0.42
Isolated following Bjorkman	60.68	5.79	33.11	0.42		16.76	1.11

*) W. Flaig, U. Schobinger and H. Deuel: Umwandlung von Lignin in Huminsauren bei der Verrottung von Weizenstroh. In press.

About these different methods of isolation some remarks must be made. Using sulfuric acid, lignin can be isolated nearly quantitatively. Adams and Castagne (G. A. Adams and A. E. Castagne, Canad. J. Res. Sect. B, 27, 915 (1949)) modified this method to determine the lignin content of different kinds of straw. This method has given better results than 42% hydrochloric acid. They got with 72% sulfuric acid a minimum of lignin with a maximum of methoxyl. This is according to Freudenberg and Ploetz the criterium of a useful method for determination of lignin. (K. Freudenberg and Th. Ploetz. Ber. Dtsch. Chem. Ges. 73, 754 (1940)).

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Yield of Lignin and %OCH₃ of Sulfuric Acid Lignin of Red Beech

In this case the yields and methoxyl content of lignin isolated with sulfuric acid of red beech are shown. The useful concentration of sulfuric acid is in this case 66.4%. The concentration of sulfuric acid must be found out in every case. There can be two kinds of errors: on the one hand, that a part of lignin is dissolved; on the other, that carbohydrates are dissolved or humified.

Seborg and Mitchell (G. J. Ritter, R. M. Seborg and R. L. Mitchell, Ind. Engng. Chem. Analyt. Edit, 4, 202 (1932)) modified this method also for the determination of lignin in different plant materials.

The method of Brauns gives only a small amount of the part of lignin in straw, approximately 0.1 to 0.2%.

The new method of Bjorkman has been investigated for straw in more detail by us. We were interested to see how a small amount of hydrochloric acid in the aqueous dioxane changes the composition of the isolated lignin.

Conditions of extraction	C	H	N	OCH ₃	Ash	in %
Room temperature	60.68	5.79	0.42	16.76	1.11	
60 - 70° C	60.46	5.72	0.42	16.70	4.03	
80° C + HCl	61.08	5.97	0.19	16.86	0.76	

By variation of temperature and with and without hydrochloric acid the differences are not varied remarkably. It seems that most of the hydrolyzed nitrogen is in the form of amino acids. The rest of the nitrogen must be fixed rather strongly in the molecule.