THE CHEMISTRY OF LIGNIN

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I. INTRODUCTION

Quite early in the development of plant chemistry, it was recognized that in the woody ("lignified") portions of plants, such as stalks, stems, cobs, hulls, leaves, trunks of trees and shrubs, there is associated with the cellulose and the other carbohydrates a substance, or a group of closely related and possibly isomeric substances, which has been designated "lignin." Schulze (264) is generally credited with having introduced this term into chemical literature, although according to Czapek (30) it had previously been employed by de Candolle (31a).

Payen (223) was the first to attempt to bring about a separation of lignified materials into their component parts. By treating wood with nitric acid and potassium hydroxide he obtained a more or less pure cellulose, and pointed out that by this treatment a product richer in carbon than the residual cellulose had been removed. The material that could thus be separated from the wood by means of chemical reagents he designated "incrusting materials" ("matieres encrustants") and assumed that in lignified substances the cellulose was mechanically impregnated with this material. Payen's "incrustation hypothesis" was in the main supported by Schulze, who found that the incrustants, or, as he termed them, lignin, could be removed by protracted cold maceration with a mixture of nitric acid and potassium chlorate. The incrustation hypothesis has also been supported by König (175), Wislicenus (303), and more recently by Freudenberg (55). Other investigators, among them Erdmann (40), opposed Payen's views. Erdmann pointed out that whereas free cellulose dissolved readily in Schweitzer's reagent, wood substance did not, and he therefore assumed that the lignin was chemically combined with the cellulose. This view has been supported by several investigators, among them Lange (193), Hoppe-Seyler (141), Cross and Bevan (20), Grafe (86), Mehta (201), Phillips (227), and Klason (163).

There has been some speculation as to the manner in which the lignin is combined with the carbohydrate material. Some investigators (40, 193) have assumed that an ester-like union exists between an acidic group in the lignin and an hydroxyl of the carbohydrate, while others (141, 86, 201) are inclined to the view that there exists an ether-like linkage between the lignin and the cellulose or other carbohydrates. Holmberg (136) has suggested that the lignin in wood is combined with the carbohydrates in an acetal-like manner. The lignin in corn cobs seems to be dissimilarly combined with the carbohydrates, and it is assumed that part of it is combined possibly in the form of an ester, and the remainder is more firmly held, probably in the form of an ether-like combination (227).

During the past twenty years there has been a renewed interest in lignin. Besides the purely chemical investigations a great deal of work has been done in studying the microbiological transformations of this material, particularly as to its rôle in the formation of soil organic matter, peat and coal. Much work has also been done on the development of methods for the utilization of this very abundant plant material.

In this paper a review will be presented of the more important facts pertaining to the chemistry, the metabolism, and the microbiological decomposition of lignin.

II. FORMATION

There has been considerable speculation concerning the nature of the precursors of lignin. Cross and Bevan (24), König and Rump (180), and more recently Fuchs (77) have suggested that cellulose is the parent substance from which lignin is formed. The last-named investigator pointed out in support of his hypothesis that both lignin and cellulose showed double refraction in the polarizing microscope. Other investigators, such as Klason (158) and Rassow and Zschenderlein (251), have advanced the hypothesis that lignin is formed by the plant from pentoses or pentosans. The investigators last mentioned found that plant substances high in lignin were low in pentosans and vice versa. The possibility that soluble carbohydrates, pentoses, methylpentoses, and hexoses may be used by the plant in the formation of lignin has been suggested by Schrauth (263), Schmidt (259), v. Euler (41), Odén (212), and Jonas (147). Candlin and Schryver (**15**) have pointed out that lignified tissues contain lignin and hemicelluloses in relatively large amounts with only traces (if any) of pectins. Non-lignified tissues, on the other hand, contain relatively large amounts of pectin, small amounts of hemicelluloses, and no lignin. Ehrlich (38) has put forward the hypothesis that pectin is the precursor of lignin. He points out that the alcohol (70 per cent)-soluble fraction of hydropectin resembles lignin in many respects; it contains methoxyl to the extent of 11.6 per cent, and its percentage of carbon and hydrogen is of the same general order of magnitude as that recorded for lignin.

It has generally been assumed that the function of lignin in the plant is to give strength and rigidity to the cell wall. However, a too high lignin content tends to make the plant tissue rather brittle. Dadswell and Hawley (31) found that brash specimens of Douglas fir had a higher lignin content than tough specimens. Phillips, Davidson, and Weihe (234) observed that lodged wheat stalks contain a greater percentage of lignin than unlodged stalks.

III. COLOR REACTIONS

Certain reagents have for many years been used by botanists and plant physiologists in testing for lignin. Nearly one hundred years ago, Runge (257) found that pine wood when treated with phenol and hydrochloric acid assumed a greenish-blue coloration, and when it was treated with aniline sulfate, a yellow color was produced. Since then, many reagents have been introduced by chemists and botanists in testing for lignin. Organic and inorganic reagents have been employed. Among the former, the "phloroglucinol reagent," or "Wiesner's reagent," which gives a reddish-violet coloration with lignified material, has been widely used in testing for lignin. Other organic compounds, such as primary and secondary amines, have also been used in examining for lignin. The colors produced when lignified tissues are treated with phenols and with amines are given in tables 1 and 2, respectively.

In the case of the phenols the reaction is carried out by first moistening the plant material with dilute hydrochloric acid and then applying to it an aqueous solution of the phenol (if the phenol is insoluble in water, an alcoholic solution is employed). Grandmougin (87) recommends that a 0.1 molar solution of phenol in 60 per cent ethanol be used. With amino compounds the reaction is carried out by adding a 1 per cent solution of the base in dilute hydrochloric or sulfuric acid. However, with dimethyl-*p*-phenylenediamine the aqueous solution of the neutral sulfate is used.

TABLE 1

Color reactions given by phenols and lignified materials

PHENOL	COLORATION	INVESTIGATOR
Phenol	Greenish-blue	Runge (257, 87)
o-Cresol	Greenish-blue	Grandmougin (87)
m-Cresol	Blue	Grandmougin (87)
p-Cresol	Green	Grandmougin (87)
Thymol	Green	Czapek (29, 143)
Catechol	Blue	Wiesner (299)
Resorcinol	Blue	Wiesner (299)
Hydroquinone	Olive	Grandmougin (87)
Phloroglucinol	Violet-red	Wiesner (299)
Pyrogallol	Green	Wiesner (299)
1,2,3,5-Tetrahydroxybenzene	Green	Fuchs (74)
Orcinol	Dark red	Lippmann (196)
o-Nitrophenol	Yellowish olive	Grandmougin (87)
m-Nitrophenol	Yellowish olive	Grandmougin (87)
p-Nitrophenol	Yellowish olive	Grandmougin (87)
o-Aminophenol	Yellow	Grandmougin (87)
m-Aminophenol	Yellow	Grandmougin (87)
p-Aminophenol	Brownish-yellow	Grandmougin (87)
Lepidin	Red	Ihl (143)
α-Naphthol	Greenish-blue	Grandmougin (87)
β-Naphthol	Rose	Grandmougin (87)
1,2-Dihydroxynaphthalene	Light green	Fuchs (74)
1,4-Dihydroxynaphthalene	Flesh color	Fuchs (74)
1,5-Dihydroxynaphthalene	Dirty green	Fuchs (74)

The question whether the color reactions given by aromatic substances with lignified tissues can be attributed to some characteristic group of the lignin complex or to some minor constituent associated with the lignin has been a controversial subject for many years. Singer (275) considered that the coloration was possibly due to vanillin and coniferin. This was, however, challenged by Seliwanow (270). Nickel (209) made the rather interesting observation that wood behaves like an aldehyde towards Schiff's reagent (fuchsin and sulfur dioxide) and that wood treated with sodium bisulfite solution was no longer colored by the aniline sulfate reagent. Subsequently Czapek (28) isolated a substance which he called "hadromal" by digesting wood with stannous chloride solution and extracting the residue with benzene. From ligroin "hadromal" separated out as a brown crystalline substance melting at 70–80°C. "Hadromal" had phenolic and aldehydic properties and readily gave the various lignin color reactions.

TABLE 2

Color reactions given by amines and lignified materials

AMINE	COLORATION	INVESTIGATOR
Aniline	Yellow	Runge (257)
o-Toluidine	Yellow	Grandmougin (87)
m-Toluidine	Yellow	Grandmougin (87)
p-Toluidine	Yellow	Grandmougin (87)
o-Nitroaniline	Yellow	Grandmougin (87)
m-Nitroaniline	Orange	Grandmougin (87)
p-Nitroaniline	Orange	Bergé (7)
1-Methyl-2-amino-6-nitrobenzene	Yellow	Grandmougin (87)
o-Phenylenediamine	Orange-brown	Grandmougin (87)
m-Phenylenediamine	Yellow	Molisch (205)
p-Phenylenediamine	Orange-brown	Grandmougin (87)
Toluylenediamine (1-methyl-2,5-diaminobenzene)	Orange	Hegler (111)
Dimethyl-*p*-phenylenediamine	Red	Wurster (304)
p-Aminodiphenylamine	Bordeaux-brown	Grandmougin (87)
Benzidine	Orange	Schneider (260)
α-Naphthylamine	Orange-yellow	Nickel (210)
β-Naphthylamine	Orange-yellow	Nickel (210)
Diphenylamine	Golden orange	Ellram (39)
Pyrrole	Red	Ihl (143)
Indole	Cherry-red	Niggl (211)
Skatole	Cherry-red	Mattirolo (199)
Thalline	Orange	Hegler (110)
Carbazolo	Cherry-red	Mattirolo (199)

Grafe (86) considered the "hadromal" of Czapek a mixture of vanillin, methylfurfural, and catechol. However, since Grafe employed a different method for preparing "hadromal" (extraction of wood with 10 per cent hydrochloric acid or heating with water under pressure at 180°C. for 1 hour) than that described by Czapek, his results cannot be accepted as evidence against the homogeneity of "hadromal."

Crocker (19) and recently Hoffmeister (134) have confirmed Czapek's results. The last-named investigator, by employing essentially the Czapek method, succeeded in isolating "hadromal," and judging from the analysis and the chemical behavior of this substance considered it identical with coniferyl aldehyde. This conclusion has since been questioned by Pauly and Feuerstein (220). This leaves the question of the homogeneity and identity of "hadromal" unanswered, and accordingly a reinvestigation of this subject would be most desirable.

In summing up, it may be stated that the evidence thus far adduced indicates that most of the so-called lignin color reactions given by lignified tissues with aromatic compounds are due to an aldehydic substance possibly in the nature of "hadromal" (coniferyl aldehyde?), which is closely associated with the lignin complex.

Color reactions with inorganic reagents

Casparis (16) found that a solution of cobalt thiocyanate colored lignified tissue blue.

When lignified materials are chlorinated, as in the Cross and Bevan method for the determination of cellulose, and then treated with a dilute sodium sulfite solution, a pink to a purple-red coloration is produced (8). This is a very sensitive test and is apparently given by some characteristic group of the lignin complex.

Cross and Bevan (22) found that lignified tissues react with a ferric ferricyanide solution to form Prussian blue, which is attached to the fiber. Crocker (19) has shown that this test can be given by many reducing substances and is therefore too general in character and cannot be considered a "lignin reaction."

A reaction which has also been used in testing for lignin is the so-called Mäule (200) reaction. Lignified material when left in a 1 per cent potassium permanganate solution for 5 minutes, washed, treated with dilute hydrochloric acid, washed, and then immersed in an ammonia solution, acquires a deep red color. According to Browne (11a) the reaction appears to be due to a deposition of manganese dioxide on the wood. This reacts with the hydrochloric acid to produce chlorine, which forms the compound that turns red with alkalies. Chlorine water can be used in place of the potassium permanganate and hydrochloric acid, and any alkali or organic base can replace ammonia, but usually not with advantage (19).

de Lamarlière (32) found that gymnosperms did not give a red coloration by the Mäule reaction. This was confirmed by Schorger (261). Crocker (19) subjected to the Mäule reaction eighteen species of deciduous woods, including the interesting balsa wood, eighteen species of coniferous woods, and gingko wood. All the woods of deciduous angiosperms gave distinct red colors. All those of coniferous gymnosperm, and of the deciduous gymnosperm, *Gingko biloba*, gave only indefinite yellow or pale brown colors. Crocker's work was later extended by Sharma (272).

Combes (18) has described the following lignin reaction: The material is treated with a hypochlorite solution for 15 minutes, washed, heated on the water bath for 15 minutes with 1 g. of zinc oxide suspended in 30 cc. of water, washed, treated with hydrogen sulfide for 10 to 15 minutes, and finally treated with a few drops of sulfuric acid. A red coloration is produced which later changes to orange red, and finally to brown.

A lignin color reaction has been described by Podbrenznik (246), which depends on the action of hydrochloric acid and potassium chlorate. When warmed with this reagent, pine wood and lignin gave an orange color, which turned yellow on addition of ammonia.

Morquer (206) observed that lignin was stained bright red and cellulose violet by treating sections of plant tissue as follows: (1) Six minutes in an aqueous solution of sodium hypochlorite, and (2) 10 minutes in a 2 per cent solution of phloroglucinol in alcohol. The material is transferred to a watch glass and two drops of an iodine solution (0.5 g. of iodine, 1 g. of potassium iodide, 20 cc. of water) and eleven drops of freshly prepared hydriodic acid solution are added and allowed to react for 1 minute. The excess reagent is removed and one drop of glycerol that has been acidulated with hydriodic acid is added.

IV. QUANTITATIVE ESTIMATION

The various methods that have been described in the literature for the quantitative estimation of lignin may be classified as direct and indirect. In the direct methods, the lignin is separated from the other plant constituents and weighed as such. In the indirect methods either some characteristic group of lignin, such as the methoxyl group, is determined and by multiplication with a suitable factor the percentage of lignin is computed or advantage is taken either of the fact that lignin produces a coloration with certain reagents or of some other characteristic chemical property of lignin.

Indirect methods

1. Method of Schulze. The oldest indirect method for determination of lignin is that due to Schulze (264). Schulze oxidized lignified plant material with nitric acid and potassium chlorate and assumed that the loss in weight represented the lignin. This method is only of historical interest as the results obtained by it are much too high, for not only is the lignin completely oxidized by the drastic oxidizing reagent employed but the hemicelluloses and perhaps some of the cellulose are also destroyed.

2. Method of Benedikt and Bamberger (6). Benedikt and Bamberger determined the percentage of methoxyl in a large number of woods and showed that there was a close relationship between the percentage of methoxyl and the lignin content as determined by the method of Schulze (264). However, the method of Schulze is now known to give erroneous results owing to the fact that other constituents in the wood are determined along with the lignin. The method of Benedikt and Bamberger is based on the assumption that in lignified plant materials lignin is the only substance containing the methoxyl group and that the methoxyl content in lignin from various sources is the same. Neither of these assumptions is entirely true. This method for determining lignin is chiefly of historical interest and is hardly ever used now.

3. Method of Cross, Bevan, and Briggs (25). This method is based on a reaction between lignin and phloroglucinol and is carried out as follows: Two grams of finely ground and dry (100°C.) wood is treated with 40 cc. of phloroglucinol solution (2.5 g. of phloroglucinol in 500 cc. of hydrochloric acid, d. 1.06). The flask is stoppered, and the reaction mixture shaken from time to time and allowed to stand overnight. The reaction mixture is filtered and 10 cc. of the filtrate taken for titration. To this solution, 20 cc. of hydrochloric acid (d. 1.06) is added and the resulting solution warmed to 70°C. A furfural or formaldehyde solution (2 g. of furfural in 500 cc. of hydrochloric acid, d. 1.06, or 2 cc. of 40 per cent formaldehyde solution in 500 cc. of hydrochloric acid, d. 1.06) is then added from a burette in portions of 1 cc. After each addition of the aldehyde solution, the reaction mixture is allowed to stand for 2 minutes while the temperature is maintained at 70°C. A drop of the reaction mixture is placed on cheap newspaper, and if unreacted phloroglucinol is present, a red spot is obtained. Toward the end of the reaction, the aldehyde solution is added in portions of 0.25 cc., and the testing is done by placing a drop of the solution on the paper and holding it over the Bunsen flame (20 cm. away) for 1 minute. When the reaction is complete, no red spot on the paper is obtained. Ten cc. of the original phloroglucinol solution is titrated in a similar manner, and the amount absorbed by the lignocellulose obtained by difference. The results are expressed as per cent of the dry weight of lignocellulose taken for analysis.

A comparison was made by Venkastewaran (287) between the results obtained by this method and those given by the 72 per cent sulfuric acid method and the fuming hydrochloric acid method. The phloroglucinol absorption method was found to give much higher results than the other two methods. This the author believes is due to the absorption of phloroglucinol by the furfural-yielding groups.

The method of Cross, Bevan, and Briggs has been somewhat modified by Votoček and Potměšil (288), who determine the excess phloroglucinol gravimetrically by precipitation with furfural. Neumann (208) determines the excess phloroglucinol by titration with a solution of diazotized *p*-nitroaniline.

4. Method of Seidel (269). This method is based on the fact that when nitric acid is added to lignin, oxides of nitrogen are given off. For carrying out the determination a special apparatus is required, and the quantity of oxides of nitrogen produced is determined by titration with permanganate. By the use of an empirical factor, the percentage of lignin in the sample can be calculated.

5. Method of Waentig and Gierisch (290). This method is based on the fact that lignin takes up a considerable quantity of chlorine, and by determining the amount of chlorine absorbed it is possible to estimate the percentage of lignin. The weighed sample is placed in a special apparatus and a stream of dry chlorine gas passed through until no further increase in weight of the sample is noted. A stream of dry air is then passed through the apparatus to remove the excess chlorine. The per cent increase in weight corresponds to the "chlorine number." The "chlorine number" when divided by 1.4 gives the percentage of lignin in the sample. (The factor 1.4 is employed because lignin isolated from wood by the fuming hydrochloric acid method has a chlorine number of 140.)

Semmler and Pringsheim (271) applied this method to the determination of lignin in straws and fodders and found that the results agreed reasonably well with those obtained by direct methods. However, in the case of straw which had been treated by Beckmann's method, the analytical procedure of Waentig and Gierisch gave results which were decidedly high.

6. Method of Mehta (201). Taking advantage of the fact that a solution of phosphotungstic and phosphomolybdic acids in phosphoric acid is a very sensitive reagent for detecting minute amounts of aromatic substances containing hydroxyl groups, Mehta has developed a micro method for the estimation of lignin. The reagent is prepared in accordance with the directions of Folin and Denis (53). A standard solution of lignin in dilute sodium hydroxide is prepared so that 1 cc. equals 1 mg. of lignin. Known volumes (1.0 to 0.1 cc.) of the standard lignin solution are accurately measured out into Nessler cylinders and to each is added 2.5 cc. of the phosphotungstic-phosphomolybdic reagent and after 5 minutes 12.5 cc. of a saturated solution of sodium carbonate. The volume is made up to 100 cc., and the colors are matched with that obtained under similar conditions from an unknown amount of lignin.

Direct methods

The direct methods for the quantitative estimation of lignin may be divided into two classes: (1) Those that depend upon dissolving cellulose and other carbohydrates and leaving the lignin as an insoluble residue; and (2) those that dissolve out the lignin and thus separate it from the cellulose and other carbohydrate material. As examples of the first class may be mentioned the 72 per cent sulfuric acid and the fuming hydrochloric acid methods, whereas the method of Mehta (201) is an example of the second class.

The methods of class 1 are most commonly employed for the quantitative estimation of lignin. It must be pointed out, however, that these methods are not altogether accurate. In fact, any method based on weighing a "residue" cannot be considered satisfactory. Among the objectionable features of these methods may be mentioned (1) the possibility of incomplete hydrolysis of the carbohydrates, (2) the formation of reversion products from the carbohydrates, and (3) contamination of the lignin residue with nitrogenous complexes. In the determination of lignin in such materials as corn cobs, corn stalks, hay, bran, hulls, and similar agricultural materials, the "lignin" residue obtained always contains a substantial amount of nitrogen.

1. Sulfuric acid method. In 1883 Flechsig (52) showed that 72 per cent sulfuric acid will hydrolyze cellulose in the cold. This fact has been applied by Klason (154) and by Ost and Wilkening (214) to the quantitative estimation of lignin. König and Rump (181) subjected the plant material to a preliminary extraction with an alcohol–benzene solution and then hydrolyzed it with 72 per cent sulfuric acid until a sample of the product when treated with iodine and sulfuric acid showed no blue coloration when observed through the microscope. Schwalbe and Becker (265) subjected flax and hemp chaff to a preliminary hydrolysis with hydrochloric acid (d. 1.19) and extraction with hot water before treatment with 72 per cent sulfuric acid. The sulfuric acid method in slightly modified form has been used by Becker (3), Dore (34), Mahood and Cable (197), Paloheimo (215), Venkateswaran (287), and Müller and Hermann (207). According to von Euler (42), several corrections must be applied when the sulfuric acid method is used, in order to obtain "rational" lignin values. To correct for the acetic acid split off from the lignin, the per cent of crude lignin must be increased by 2 per cent. To this must be added the percentage of alcoholic extractives (found after the wood has been given a preliminary extraction with benzene), which von Euler believes properly belongs to the lignin fraction. From the final results 5.7 per cent is deducted for the sulfuric acid that cannot be removed from the lignin by washing. The errors, however, balance, so that the lignin content of Norway spruce as determined by Becker's method is approximately correct. Thus, Norway spruce when analyzed by Becker's method gave 30.5 per cent of lignin. The alcohol extractives amounted to 3.5 per cent. The "rational" lignin value is then $30.5 + 2 + 3.5 - 5.7 = 30.3$ per cent.

Klason (166) has modified his original procedure and recommends that 64 or 66 per cent sulfuric acid be used in place of the 72 per cent acid. To

1 g. of ground wood which has been extracted with ether and dried at 100°C., 50 cc. of 66 per cent sulfuric acid is added, and the mixture is stirred with a glass rod until the gelatinization is complete. The following day the mixture is again stirred and allowed to stand for another day. It is then diluted with water, and the lignin is filtered in an alundum crucible and washed with water until the filtrate is free from sulfuric acid. Fifty cc. of 0.5 per cent hydrochloric acid is then added, and the mixture digested on the steam bath for 12 hours. The hydrochloric acid is then removed by filtration and the lignin washed with water until free from sulfuric acid. The lignin is first dried at 75°C. and for one-half hour at 100°C., then weighed, ashed, and reweighed. The weight of lignin is obtained by difference.

The U. S. Forest Products Laboratory method is described by Bray (11) as follows: Two grams of air-dry wood, sawdust, or shredded pulp is weighed into a tared alundum crucible (porosity R.A. 98) contained in a glass-stoppered weighing bottle. The crucible and its contents are dried to constant weight at 105°C. and weighed. The material is then extracted with ether in a Soxhlet for three to four hours. (In the case of pulps the extraction with ether is not necessary.) The dry material is transferred from the crucible to a weighing bottle and treated with 40 cc. of 72 per cent (±0.1) by weight of sulfuric acid, the strength of which is determined by titration against standard alkali. The hydrolysis is allowed to proceed for 16 hours at room temperature with frequent stirring and thorough mixing of the acid with the sample at the beginning of the operation. The resulting product is transferred to a 2-liter Erlenmeyer flask and diluted to 1570 cc. with distilled water (which makes the concentration of the sulfuric acid 3 per cent). It is then covered with a watch glass and boiled for 2 hours, distilled water being added from time to time to keep the volume constant. The lignin is filtered off on the tared alundum crucible used in the beginning of the determination, washed thoroughly with hot distilled water (500 cc.), dried at 105°C., and weighed.

Ritter, Seborg, and Mitchell (252) proposed the following modification of the above method: (1) The residue after the extraction with alcohol-benzene should be extracted with hot water for 3 hours; (2) the concentrated acid-wood mixture should be kept in a bath at 20°C., and 2 hours should be allowed for solution of the carbohydrates in the 72 per cent sulfuric acid; (3) the diluted mixture is hydrolyzed for 4 hours. Their analytical procedure is as follows:

> "Approximately 2 grams of air-dried sawdust (60 to 80, or 80 to 100 mesh) are weighed in a tared alundum crucible. The crucible and its contents are dried to constant weight at 105°C., cooled, and weighed. The material is then extracted for 4 hours in a Soxhlet apparatus with a minimum boiling solution of alcohol-benzene.

The solvent is removed by suction; the residue is washed with alcohol by suction to remove the benzene, and then extracted with 400 cc. of hot water in a water bath for 3 hours, filtered, washed with hot water, then with alcohol, and finally dried. Washing the residue with alcohol aids in the removal of the sawdust from the crucible after drying.) The dried residue is transferred to a glass-stoppered weighing bottle, and is well mixed with 25 cc. of 72 per cent sulfuric acid at 20°C., and maintained at that temperature for 2 hours. The resulting mixture is transferred to an Erlenmeyer flask, diluted with water to make a 3 per cent acid solution, and then boiled for 4 hours under a reflux condenser. The hydrolyzed residue is filtered on a tared alundum crucible, washed free of acid by means of hot water, dried, and weighed. The lignin content is calculated on the basis of the oven-dry sample.

"In case a correction for ash is desired, transfer the lignin residue to a tared platinum dish and ash the usual way.

"In determination of lignin in chemical pulps, 40 cc. of 72 per cent sulfuric acid should be used for dissolving the carbohydrates present in a 2-gram sample. The water extraction may be omitted with all chemical pulps and the alcohol benzene may be omitted with alkaline-cooked pulps."

Ross and Hill (255) add formaldehyde solution to the 72 per cent sulfuric acid–wood mixture. The time required for the hydrolysis of the carbohydrates is thereby considerably reduced. Wise and Fairbrother (302) found that in the case of hardwoods, the U. S. Forest Products Laboratory method invariably gave higher lignin than the Ross and Hill method, but if the material is previously extracted with hot water and alcohol–benzene solution, the results are in approximate agreement. In the case of coniferous woods, however, the Ross and Hill method gives the higher values.

Waksman and Stevens (295) hydrolyze the residue remaining after extracting the plant material successively with ether, cold and hot water, alcohol and dilute acid, with 80 per cent sulfuric acid. From the weight of the crude lignin the weight of ash and crude protein is deducted.

Peterson, Walde, and Hixon (224a) have called attention to the fact that in determining lignin by the 72 per cent sulfuric acid method, the temperature of the acid should be below 15°C., as otherwise too high values for lignin are obtained. They recommend that the temperature of the sulfuric acid should be between 4° and 15°C.

Cohen and Dadswell (17) found that the usual 72 per cent sulfuric acid method failed to give satisfactory results when applied to wood of the Eucalyptus family. By first digesting the wood with 0.125 *N* sodium hydroxide at 98–100°C. for 80 minutes, the interfering substances could be removed. The lignin could then be determined in the usual way. It seems quite likely, however, that the preliminary digestion with alkali removes part of the lignin along with other substances (107).

2. Fuming hydrochloric acid method. In 1913 Willstätter and Zechmeister (301) recorded the observation that fuming hydrochloric acid (d. 1.212 to 1.223 at 15°C.) will completely hydrolyze cellulose in the cold. This principle has been applied by Krull (184) to the determination of lignin. Five grams of wood moistened with 15 cc. of water is placed in a Claisen flask, cooled with ice, and saturated with hydrogen chloride gas, generated by dropping concentrated sulfuric acid into concentrated hydrochloric acid. After standing twenty to twenty-four hours most of the hydrochloric acid is removed by heating the reaction mixture to 70°C. at 14 to 19 mm. pressure. It is then diluted with water, boiled for 8 hours under a reflux condenser, and the lignin filtered off.

König and Becker (179) use 6 cc. of water to 1 g. of wood to secure greater fluidity and then saturate this with hydrogen chloride gas. Dore (34) uses ordinary concentrated hydrochloric acid instead of water and then passes a stream of hydrogen chloride gas through the concentrated hydrochloric acid containing the sample in suspension.

Schwalbe and Becker (266) add 60 cc. of fuming hydrochloric acid (d. 1.21) to 1 g. of wood which has been previously extracted with alcohol-benzene solution and allow the mixture to stand in a cool place for 24 hours. The product is then filtered off, washed with water, dried, weighed, and ashed, and the crucible is reweighed.

Semmler and Pringsheim (271) applied the fuming hydrochloric acid method to the determination of lignin in straw and feedstuffs. The sample was mixed with the acid and allowed to remain in the ice chest for two days. It was then diluted with water, and filtered, and the precipitate again hydrolyzed with the fuming hydrochloric acid.

Heuser and Skiöldebrand (124) treated the purified wood with fuming hydrochloric acid (d. 1.212) and allowed it to stand for several days in a cool place. The product was then filtered, and washed with water, and the precipitate again treated with fuming hydrochloric acid. The hydrolysis with the strong acid was continued until a sample of the acid filtrate no longer gave a test for reducing sugars with Fehling's solution.

A modification of the hydrochloric acid method has been introduced by Wenzl (298), who used a mixture of phosphorus pentoxide and concentrated hydrochloric acid (30 g. of phosphorus pentoxide to 100 cc. of concentrated hydrochloric acid) to remove the polysaccharides.

Schwalbe (268) used a mixture of hydrochloric acid (d. 1.07) and 72 per cent sulfuric acid for hydrolysis of the cellulose and the other carbohydrates associated with it.

Paloheimo (217) has made a critical study of the fuming hydrochloric acid method as applied to the determination of lignin in feedstuffs. The method is subject to two possible errors: (1) incomplete hydrolysis of proteins; (2) formation of "reversion products" or humus-like material when the hydrolyzed product is diluted with water before filtration. To eliminate the second error, Paloheimo has devised a special apparatus in which the strong acid is in contact with the plant material for only a short time and the acid removed by filtration. The disturbing influence of the substances yielding "reversion products" may also be eliminated by giving the plant material a preliminary treatment for half an hour with 1.25 per cent potassium hydroxide solution. The results obtained by the method of Paloheimo are very much lower than those obtained by the other methods. In determining the lignin content of hay, straw, stalks, and similar agricultural materials, it is undoubtedly good practice to determine the percentage of nitrogen in the crude lignin and to make the proper deductions. The pre-treatment of the plant material with potassium hydroxide solution is, however, of doubtful value, as some lignin is unquestionably removed by this treatment, thus giving low values for lignin.

A detailed description of the fuming hydrochloric acid method for determination of lignin has been given by Phillips (233). The crude lignin obtained is analyzed for its percentage of nitrogen and ash. The weight of lignin in the sample is computed as follows: Weight of lignin equals weight of crude lignin minus weight of ash, minus weight of nitrogen, times 6.25. In this procedure the presumption is, of course, made that the nitrogenous residue is in the nature of a protein. While this is perhaps not entirely justified, in view of the present knowledge of the chemistry of lignin, it is possibly the best procedure to follow.

Billington, Simmonds, and Baird (9) made a study of four methods for determination of lignin, namely, the Forest Products Laboratory Method (197, 11), the modified Forest Products Laboratory method (252), the Ross and Potter method (255), and the method of Willstätter and Zechmeister (301). They conclude that the Willstätter and Zechmeister method possesses the greatest inherent accuracy. The modified Forest Products Laboratory method is slightly less accurate than the method of Willstätter and Zechmeister, but has the advantage over the latter in facility of operation and speed. The Ross and Potter method gave in every case a lignin fraction having the lowest percentage of methoxyl, whereas the lignin isolated by the method of Willstätter and Zechmeister contained the highest percentage of methoxyl.

3. Method of König and Rump (182). In this method the hydrolysis of the cellulose and the other carbohydrates associated with it is brought about by heating the sample for 5 hours with 1 per cent hydrochloric acid under a pressure of 5 to 6 atmospheres. The lignin residue obtained is tested for cellulose with the iodine–sulfuric acid reagent, and if a positive reaction is obtained the heating with hydrochloric acid is repeated.

4. Alkali method—method of Mehta (201). This method is based on the fact that the lignin fraction in a lignified plant material can be removed by heating the latter with alkali under pressure. Five grams of the sample and 100 cc. of 4 per cent sodium hydroxide solution are heated for 1 hour under a pressure of 10 atmospheres. The alkaline solution is then filtered and made up to a definite volume, and an aliquot is acidified with hydrochloric acid. The lignin precipitate is filtered off, washed with water, and redissolved in boiling 95 per cent ethanol. The alcoholic solution is filtered, and the filtrate evaporated to dryness, dried, and weighed. The lignin values obtained by this method are considerably lower than those obtained either by the fuming hydrochloric acid or the 72 per cent sulfuric acid methods, owing probably to the partial degradation of the lignin by the sodium hydroxide solution heated to a comparatively high temperature.

V. ISOLATION

Whatever method for the isolation of lignin is used, it is important that the finely ground plant material be first freed from various "extractives," such as fatty substances, resins, and volatile oils. This is generally accomplished by extracting the lignified material with ether and ethanol or acetone and ethanol, but preferably with a 1:2 alcohol–benzene solution. This may be followed by an extraction with cold and hot water. Some investigators, such as Friederich and Diwald (70), give the plant material a preliminary treatment with 5 per cent sodium hydroxide solution in order to remove gummy substances. Freudenberg, Zocher, and Dürr (65) remove the gums from the wood with sodium hydroxide solution and then reflux the residual material with 1 per cent sulfuric acid. When the material is degummed with sodium hydroxide solution, there is, however, the danger of losing some of the lignin because of its solubility in this reagent.

It may be stated at the very outset that there is as yet no method known by which lignin can be isolated unchanged. Whatever method is employed, a lignin preparation is obtained which is no longer identical with the natural lignin—the claims of some investigators to the contrary notwithstanding. In the methods described below for the isolation of lignin, preparations are obtained that are more or less related to the lignin as it occurs naturally in the plant. This should not be taken as implying that all methods are of equal value from the standpoint of one who is interested in studying the chemistry of lignin. Some methods are more drastic than others and yield therefore considerably degraded lignin preparations.

The various methods described in the literature for the isolation of lignin may be conveniently divided into two classes: (1) Those that depend on the removal, by hydrolysis, of the cellulose and other components, leaving the lignin as an insoluble residue, and (2) those that depend on the removal of lignin from the cellulose and the other substances with which it is associated.

Methods of class 1

1. Sulfuric acid method. For the isolation of lignin by this method, 72 per cent sulfuric acid is generally employed, although lower concentrations of acid (64, 66, and 70 per cent) have also been used. Although it has long been known that strong sulfuric acid will dissolve cellulose at ordinary temperatures, this acid was first made use of by Klason for the isolation of lignin from wood.

2. Method of Klason (154). For every gram of wood, 10 cc. of 70 to 72 per cent sulfuric acid is added. The reaction mixture is stirred and allowed to stand until a sample of the product no longer gives a test for cellulose. The product is then poured in water, filtered, and washed with cold and with hot water.

The method described above has since been modified by Klason (167). The ground and sifted wood is first extracted with ether and then dried at 100°C. For every gram of wood, 50 cc. of 66 per cent sulfuric acid is employed, and the mixture stirred until gelatinization is complete. It is then allowed to stand for 48 hours with frequent stirring. The reaction product is diluted with water, filtered, and washed with water. To remove the combined sulfuric acid, the reaction product is heated on the steam bath for 12 hours with 0.5 per cent hydrochloric acid. The product is washed with water until free of acid and dried.

Paloheimo (218) employed 70 per cent sulfuric acid for the isolation of lignin.

A modification of the sulfuric acid method has recently been proposed by Sherrard and Harris (273). They hydrolyze the lignified material at 10°C. with 70 per cent sulfuric acid. Dry sawdust previously extracted with 1:2 alcohol–benzene solution and with water is added (in the proportion of 10 cc. of acid to 1 g. of wood) to 70 per cent sulfuric acid previously cooled to 10°C. The reaction mixture is shaken until gelatinization occurs (about three minutes) and again placed in the cooling bath. After about one hour, when the mixture begins to thin, it is again shaken for a few minutes to insure contact of the acid with all the sawdust. It is then allowed to stand for 15 hours in the cooling bath at 10°C. It is diluted with water (37 to 38 volumes), and well stirred, and the mixture is boiled under a reflux condenser for 4 hours. The product is filtered and washed with hot water.

3. Fuming hydrochloric acid method. This method was first proposed by Willstätter and Zechmeister (301). These investigators found that hydrochloric acid of 42 to 43 per cent strength readily dissolved cellulose, leaving the lignin as an insoluble residue. Lignin prepared by this method, or its modifications, is frequently referred to in the literature as "Willstätter lignin." The fuming hydrochloric acid (d. 1.212 to 1.223 at 15°C.) required for this method may be prepared as follows (187, 233): To 500 g. of sodium chloride contained in a liter Pyrex distilling flask provided with a ground glass stopper, a cold solution of 250 cc. of water in 450 cc. of concentrated sulfuric acid is added. The side tube of the distilling flask is connected to a glass tube which passes through a sulfuric acid wash bottle. The outlet tube of the sulfuric acid wash bottle is connected to another glass tube which is immersed in a flask containing 3 liters of concentrated hydrochloric acid. The flask containing the hydrochloric acid is surrounded with crushed ice. The distilling flask is heated with a small flame, and the hydrogen chloride gas is passed into the acid solution until it attains the specific gravity of 1.212 to 1.223 at 15°C. The fuming hydrochloric acid thus prepared is stored in a refrigerating room.

According to Schwalbe and Ekenstam (267), in the isolation of lignin by the Willstätter method from both sound and rotted wood, the residual lignin is liable to contain cellulose, owing to the rapid swelling action of the highly concentrated acid and the consequent prevention of diffusion of the acid to the interior of the wood. This difficulty may be overcome by treating the wood with relatively dilute acid (25 per cent) and gradually increasing the concentration of the acid to 42 per cent. Under these conditions, the lignin is more rapidly and completely freed from cellulose, and the product obtained is more easily filtered.

a. Method of Willstätter and Kalb (300). The following is a description of the procedure followed by Willstätter and Kalb for the isolation of lignin by the fuming hydrochloric acid method: Pine sawdust is successively extracted with water, 80 per cent acetone, and pure acetone, and finally dried. Two hundred grams of this pretreated sawdust is added, with stirring, to 4 liters of fuming hydrochloric acid, and the mixture is allowed to remain at room temperature for 4 hours. To this, 1300 g. of ice is added in small portions and allowed to stand for 18 hours. Finally, 1300 cc. of water is added, and the mixture is then filtered. The lignin precipitate is washed with dilute hydrochloric acid and then with water. It is finally boiled with water, and the acid in the aqueous solution carefully neutralized with soda. A light brown lignin preparation is obtained. Yield, 26 to 28 per cent of the weight of the starting material.

A modification of this method is given by Kürschner (187). The method, however, is rather troublesome to carry out and difficulty is experienced in obtaining a lignin preparation entirely free from cellulose.

b. Method of Kalb and Lieser (148). This method is a modification of that of Willstätter and Kalb. One hundred grams of dry wood flour is added to 2 liters of hydrochloric acid (d. 1.222 at 0°C.) and cooled to 1°C. to 5°C. The reaction mixture is well stirred and allowed to stand for 2 hours. To this, 650 cc. of water or ice is added, and the mixture allowed to remain at room temperature for 18 hours more. It is then diluted with another 650 cc. of water, and the lignin is filtered off and washed successively with 2 liters of 1:1 hydrochloric acid, water, very dilute soda solution, and hot water. Yield, 25 per cent of the weight of the starting material.

Kalb and Lieser (148) have described another modification of the fuming hydrochloric acid method. The extracted wood is first hydrolyzed with fuming hydrochloric acid for 2.5 hours at 0°C. The product is poured into ice, filtered, and washed with water and with dilute ammonia. It is then treated with a cuprammonium solution (prepared by adding copper carbonate to 25 per cent ammonia solution), and the mixture is allowed to stand overnight and then shaken for 4 hours in a shaking machine. The product is filtered off and washed with dilute hydrochloric acid, water, and dilute ammonia solution.

Bremer (12) failed to obtain a carbohydrate-free lignin preparation by treating pine wood flour with 2 liters of 42 per cent hydrochloric acid at 0°C. for 24 hours. It was only after five successive treatments with the 42 per cent acid that it was possible to obtain a preparation which was free from carbohydrates.

A modification of the hydrochloric acid method has been proposed by Kucher (185). He employs a mixture of hydrochloric acid (d. 1.18) and sulfuric acid (d. 1.84) for the hydrolysis of the cellulose.

4. Method of Urban (286). Urban avoids the use of fuming hydrochloric acid and strong sulfuric acid for the hydrolysis of the polysaccharides. Twenty grams of extracted wood is treated with a mixture consisting of 1350 cc. of hydrochloric acid (d. 1.18) and 450 cc. of phosphoric acid (d. 1.7) and allowed to remain for fifty to sixty hours at 20°C. The product is filtered, washed with a fresh portion of the hydrochloric–phosphoric acid mixture, then successively with concentrated hydrochloric acid, dilute hydrochloric acid, and hot water, and finally extracted with acetone and again washed with water.

Freudenberg and Harder (58) have used the method described above for the isolation of lignin. The wood was, however, first degummed with 5 per cent sodium hydroxide solution according to the method of Friedrich and Diwald (68). This preliminary treatment of the wood with alkali unquestionably results in the partial removal of the lignin along with the gums.

5. Method of Freudenberg and coworkers (61). In this method the lignin is isolated by alternately hydrolyzing wood with boiling 1 per cent sulfuric acid and cuprammonium solution. However, it must be pointed out that the latter reagent not only dissolves cellulose but also to some extent lignin (285), so that the yield by this method is somewhat lower. Ground and extracted (alcohol-benzene solution) pine wood is digested in the cold with 5 per cent sodium hydroxide solution for 24 hours. The alkali is then poured off and the residue similarly treated with a fresh quantity of 5 per cent sodium hydroxide solution. The product is filtered, washed with water, dilute acetic acid, and again with water. To remove pentosans and hemicelluloses, it is boiled with 1 per cent sulfuric acid. The product is shaken in a shaking machine for 12 hours with Schweitzer's reagent (prepared by adding an excess of copper turnings to 25 per cent ammonia, cooling to 0°C., and passing through the solution a mixture of air and ammonia gas for 8 hours). The reaction mixture is centrifuged and the precipitate washed with Schweitzer's solution, concentrated ammonia, water, dilute hydrochloric acid, and again with water. The operation of boiling with 1 per cent sulfuric acid and treating with Schweitzer's solution is repreated three times.

Using the method described above, Freudenberg obtained a lignin preparation which was light brown in color and contained 16 per cent methoxyl. When this was treated for 5 hours with a solution consisting of 3 volumes of hydrochloric acid (d. 1.19) and 1 volume of syrupy phosphoric acid, a product was obtained which was darker in color, but the methoxyl content of the preparation had increased to 17 per cent.

Methods of class 2

1. Sulfite method. This method for the removal of lignin is of considerable industrial importance, and has been the basis of the preparation of wood pulp for many years. The delignification is brought about by heating wood with acid sulfites under pressure. By this method lignin is obtained not in the free state but in the form of water-soluble sulfonic acids. A description of these acids will be give elsewhere.

2. Alkali method. This method of isolation of lignin has been used by a number of investigators. Both aqueous and alcoholic sodium hydroxide solutions have been employed. The ease with which lignin can be obtained by this method depends somewhat on the character of the lignified plant material. In case of cereal straws and corn cobs, for example, lignin fractions can be obtained by treatment with alcoholic or aqueous sodium hydroxide solutions in the cold, whereas in the case of wood a more drastic treatment is required.

Methods of Beckmann, Liesche, and Lehmann (4). (1) Aqueous sodium hydroxide.—Rye straw cut in small pieces (1 part) is treated with 8 parts of 1.5 per cent aqueous sodium hydroxide solution and allowed to remain at room temperature for two days. The product is filtered and the straw pressed. To the alkaline filtrate sufficient hydrochloric acid is added so that there is 2 to 2.5 per cent free acid per liter, and the solution is boiled for 5 to 10 minutes. The product is filtered and washed with water. Yield, 9.5 per cent of the weight of dry straw.

(2) Alcoholic sodium hydroxide.—In this method the pentosans and hexosans are removed with alcohol. The extraction of the lignin is carried out as in (1) except that an alcoholic sodium hydroxide solution (prepared by dissolving 20 g. of sodium hydroxide in 400 cc. of water and adding 600 cc. of 96 per cent ethanol) is used. The alkaline–alcoholic extract is neutralized with hydrochloric acid, and most of the alcohol is distilled off under reduced pressure. The residual solution is made acid with hydrochloric acid, and the lignin filtered off and washed with water. Beckmann, Liesche, and Lehmann, using this method for the isolation of lignin from winter rye straw, obtained a yield of 5.2 per cent, which could be increased to 7 per cent by subjecting the straw to three successive extractions with the alcoholic sodium hydroxide solution.

Employing a method similar to that above described, Phillips (226) isolated a lignin fraction from corn cobs in 3.49 per cent yield. The product was a light yellow amorphous powder.

Powell and Whittaker (241) isolated lignin from flax shoves by heating it with 10 per cent sodium hydroxide solution under pressure. The yield was 20 to 22 per cent of the weight of the flax shoves taken. These investigators have also applied this method to the isolation of lignin from wood. In the case of wood, however, a somewhat higher temperature was employed, 140–160°C. (243).

Mehta (201) isolated lignin in 15.6 to 17.1 per cent yields by heating wood with 4 per cent sodium hydroxide solution under 8 atmospheres pressure for 1 to 1.5 hours. A similar method has been employed by Dorée and Barton-Wright (35). The lignin fraction which they isolated was designated "metalignin." The product was found to be soluble in organic solvents.

Phillips (227) isolated four lignin fractions from corn cobs by "fractional extraction." The corn cobs, previously extracted with an alcohol–benzene solution, were subjected to extraction with a 2 per cent alcoholic sodium hydroxide solution until a test sample upon removal of the alcohol and subsequent acidulation no longer gave any precipitate of lignin (fraction A). The residual material was successively extracted with a 2 per cent aqueous sodium hydroxide solution at 100°C. until the extract was free from lignin (fraction B). The residue from this treatment was similarly extracted with a 2 per cent aqueous sodium hydroxide solution at 135°C. (fraction C) and finally with a 4 per cent sodium hydroxide solution at 180°C. The total yield (calculated on the corn cobs treated) amounted to 9.0 per cent and was distributed among the four fractions as follows: (A) 4.4 per cent; (B) 3.7 per cent; (C) 0.84 per cent; and (D) 0.14 per cent. The cellulose residue obtained from the final treatment was entirely free from lignin.

By a similar procedure two lignin fractions were isolated from oat hulls (229).

3. Separation of lignin by alcoholysis. The isolation of lignin by heating wood with various hydroxylic compounds in the presence of catalysts has come into use during the past few years. Grüss (89) in 1920 recorded the observation that lignin could be isolated from wood by heating the latter with alcohol and hydrochloric acid. This reaction has been applied by Friedrich and Diwald (70), Friedrich and Brüda (67), Hägglund and Rosenqvist (104), Hägglund and Urban (106), Fuchs (80), Campbell (14), Hibbert and Rowley (130), Hibbert and Marion (127), Hibbert and Phillips (129), Rassow and Gabriel (247), Rassow and Lüde (248), Fuchs and Daur (82), and Kleinert and Tayenthal (170). In place of ethanol other hydroxy compounds have been used, such as butyl and amyl alcohols (106), ethylene glycol (130, 127, 247, 248), the monomethyl ether of ethylene glycol (80, 127), and glycerol α-monochlorohydrin (129). In all cases a product is obtained containing the alkyl or aryl group, as the case may be, in combination with the lignin. The mechanism of the reaction between the hydroxy compound and the lignin is not clear. It has been suggested by Hägglund (106) that a product in the nature of an acetal is formed. However, the evidence available as to the acetal character of the lignin–alcohol complex seems to be inconclusive.

a. Method of Friedrich and Diwald (70). Finely ground pine wood which had been extracted with alcohol–benzene solution and degummed with 5 per cent sodium hydroxide solution is treated with an equal weight of hydrochloric acid (1 volume of hydrochloric acid, d. 1.17, and 1 volume of water), thoroughly mixed and allowed to stand for 48 hours. To the reaction mixture ten times its weight of 96 per cent ethanol is added, and then it is refluxed for eight to ten hours. The reaction product is filtered, and the dark brown filtrate concentrated to one-third its original volume. The concentrated filtrate is poured into ten times its volume of water to which a little hydrochloric acid has been added. The precipitated product is purified by redissolving in alcohol and reprecipitating. The freshly prepared material is soluble in alcohol, acetone, acetic acid, ethyl acetate, and in sodium hydroxide solution. It gives a strong reaction with the phloroglucinol–hydrochloric acid reagent. This lignin preparation has been designated by Friedrich and Diwald as "primary" lignin. When heated with hydriodic acid, as in the Zeisel determination of alkoxyl groups, it yields ethyl iodide and methyl iodide, the ethyl iodide arising from the ethyl alcohol used in the isolation of this lignin fraction (104).

b. Amyl-lignin (method of Hägglund and Urban) (106). Forty grams of ground pine wood which has been extracted with acetone is treated with 800 cc. of isoamyl alcohol and 50 cc. of 37 per cent hydrochloric acid and refluxed for 45 minutes. The reaction product is filtered, and the filtrate freed from hydrochloric acid and sugars by extraction with water. It is then concentrated under reduced pressure. The residue is dissolved in warm dilute sodium hydroxide solution, and filtered, and the filtrate acidified with hydrochloric acid. The precipitate is filtered, washed with water, and redissolved in glacial acetic acid and reprecipitated with water.

c. "Isobutyl-lignin" has similarly been prepared by Hägglund and Urban (106).

d. "Methylglycol-lignin" (Fuchs (80)). Four grams of wood flour, 60 cc. of the monomethyl ether of ethylene glycol, and 1 cc. of concentrated hydrochloric acid are heated together under a reflux condenser for 24 hours. The reaction product is filtered, and the "methylglycol-lignin" precipitated by the addition of water.

Details for the isolation of lignin with ethylene glycol (so-called glycol-lignin) are given by Hibbert and Rowley (130), Hibbert and Marion (127), and by Rassow and Gabriel (247).

4. Extraction of lignin with phenols. The fact that lignin can be extracted from wood with phenols was first noted by Bühler (13). As in the case of the extraction of lignin with alcoholic compounds, the phenols also combine chemically with the lignin, yielding the so-called phenol lignin. According to Kalb and Schoeller (149), "phenol-lignin" may be prepared by heating pine sawdust with phenol and hydrochloric acid for 1 hour at 90°C. The reaction product is filtered, and the filtrate steam-distilled to remove the excess phenol, whereupon the lignin preparation remains in the distilling flask.

Among others who have described the preparation of "phenol-lignin" may be mentioned Legeler (194), Hillmer (132), and Wedekind, Engel, Storch, and Tauber (296).

5. Other methods. Karrer and Widmer (151) found that wood, straw, and similar materials, when treated for several hours with acetyl bromide, dissolve completely. This solution, when poured on ice, yields a precipitate from which a bromo derivative of lignin may be separated.

Friedrich (66) obtained a lignin preparation by treating wood meal with a mixture of 1 volume of glacial acetic acid and 0.03 volume of concentrated hydrochloric acid at 100°C. The reaction product was filtered, and the lignin preparation obtained by the addition of water to the filtrate. The product was hydrolyzed with alkali hydroxide to remove combined acetic acid.

A rather interesting method for the separation of a lignin fraction from wood has recently been reported by Küster and Daur (192). They treated extracted beechwood flour with diazotized sulfanilic acid in the presence of sodium hydroxide solution. The dissolved lignin was precipitated with β-naphthylamine hydrochloride or with *p*-bromoaniline hydrochloride. Küster and Daur believe that this method of isolating lignin causes the least decomposition in the lignin molecule. An objectionable feature of the method, however, is the fact that part of the cellulose is also dissolved by the diazobenzenesulfonic acid treatment.

TABLE 3

Elementary composition of various lignin preparations

SOURCE	METHOD OF ISOLATION	CARBON	HYDROGEN	INVESTIGATOR
		per cent	*per cent*	
Flax	A	63.9	5.8	Powell and Whittaker (241)
Larch	A	63.8	5.2	Powell and Whittaker (243)
Pine	A	63.4	5.6	Powell and Whittaker (243)
Spruce	A	64.0	5.5	Powell and Whittaker (243)
Ash	A	63.2	5.6	Powell and Whittaker (243)
Birch	A	63.2	5.5	Powell and Whittaker (243)
Poplar	A	63.3	5.8	Powell and Whittaker (243)
Corn cobs	A	62.5	5.0	Phillips (227)
Oat hulls	A	64.4	5.2	Phillips (229)
Rye straw	B	63.0	5.6	Beckmann, Liesche, and Lehmann (4)
Corn cobs	B	61.1	5.8	Phillips (226)
Spruce	C	62.4	6.4	Heuser, Schmitt, and Gunkel (122)
Spruce	C	64.0	5.3	Hägglund (94)
Spruce	D	63.9	5.3	Klason (165)
Spruce	E	63.9	6.0	Urban (286)
Wood	E	64.7	5.5	Freudenberg and Harder (58)
Spruce	F	63.6	5.7	Freudenberg, Zocher, and Dürr (65)

A, aqueous sodium hydroxide method.
B, alcoholic sodium hydroxide method.
C, fuming hydrochloric acid method.
D, sulfuric acid method.
E, method of Urban.
F, Freudenberg method.

VI. ELEMENTARY COMPOSITION

The elementary composition of the lignin complex varies somewhat with the source and with the method employed for its isolation. A few of the many results on the elementary analysis of lignin found in the literature are given in table 3, from which it will be observed that, considering the fact that the various investigators were never absolutely certain as to the purity of their preparation, the agreement is fairly good.

VII. CONSTITUENT GROUPS

The presence of the methoxyl group (—OCH_3) has been definitely established in the lignin preparations isolated from various sources and by different methods. The methoxyl groups of lignin are not readily split out, from which it is inferred that they are attached in the form of an ether linkage rather than an ester linkage as in the case of the pectins. The absence of other alkoxyl groups, such as ethoxyl (—OC_2H_5), has been definitely established in the case of spruce (105) and oat hull lignin (230).

According to Freudenberg (56), the methoxyl groups present in lignin are attached to aromatic nuclei. He studied the rate of removal of the methoxyl groups of lignin with hydriodic acid and found that this corresponded approximately to that of vanillin. In the case of an aliphatic substance like 3-methylglucose, 96 per cent of the total methoxyl was split out in 20 minutes, whereas in vanillin and lignin a much longer reaction period was required to accomplish this.

TABLE 4

Percentage of methoxyl in various lignin preparations

SOURCE	METHOD OF ISOLATION	METHOXYL	INVESTIGATOR
		per cent	
Spruce	A	14.47	Klason (156)
Fir	A	13.95	König and Rump (176)
Fir	A	16.40	König (174)
Sugar maple	A	21.00	Sherrard and Harris (273)
Spruce	B	14.39	Hägglund (90)
Spruce	B	13.10	Fischer and Schrader (47)
Spruce	B	11.60	Kürschner (186)
Flax	C	14.90	Powell and Whittaker (241)
Winter rye straw	D	14.85	Beckmann, Liesche, and Lehmann (4)
Corn cobs	D	12.10	Phillips (226)
Corn cobs	C	14.30	Phillips (228)
Oat hulls	C	15.80	Phillips (229)
Oat hulls	D	15.61	Phillips (229)

A, sulfuric acid method.
B, fuming hydrochloric acid method.
C, aqueous sodium hydroxide method.
D, alcoholic sodium hydroxide method.

The methoxyl content of lignin varies somewhat with the source and the method employed for the isolation of the lignin. Phillips (227) found that the methoxyl content of lignin from corn cobs varied with the temperature employed for the isolation of the lignin. In table 4 are given a few of the many methoxyl determinations recorded in the literature.

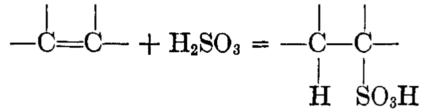
The presence of hydroxyl groups in lignin is indicated by the fact that it can be acetylated and alkylated. Alcoholic as well as phenolic hydroxyl groups seem to be present. Powell and Whittaker (241) acetylated lignin isolated from flax shoves by the aqueous sodium hydroxide method, and found that the acetylated product was insoluble in cold sodium hydroxide solution, thus indicating that the acidic character of their lignin preparations was due to phenolic hydroxyl groups. Freudenberg (65) contends that in the lignin isolated by his method no free phenolic hydroxyl groups are present.

The fact that wood and similar lignified materials when distilled with dilute mineral acids afford formic and acetic acids has caused several investigators to assume that lignin contains acetyl and formyl groups (26). However, the presence of either acetyl or formyl groups in any of the lignin preparations has not been established, and from the evidence thus far presented no definite conclusion can be drawn as to their presence or absence in natural lignin.

The evidence as to the presence of carbonyl groups in lignin preparations is rather inconclusive. Powell and Whittaker (241) found that their lignin preparation reduced Fehling's solution and concluded from this that an aldehydic group was present. However, it must be remembered that substances other than aldehydes can reduce Fehling's solution, so that the claim of Powell and Whittaker must be accepted with considerable reserve. Friedrich and Diwald (69) obtained condensation products of an indefinite character of "primary lignin" with phenylhydrazine and semicarbazide. According to Hägglund (102), no free carbonyl groups are present in Willstätter lignin, although treatment of this material with acid in the presence of phenols and aromatic amines discloses their presence.

Freudenberg (59) found that lignin isolated from wood by Urban's method when distilled with 12 per cent hydrochloric acid yields formaldehyde (0.7 per cent of weight of lignin). This investigator has accordingly advanced the hypothesis that the formaldehyde arises from a methylene dioxide group ($—O—CH_2—O—$) present in the lignin molecule, as compounds containing this group are known to yield formaldehyde when they are distilled with hydrochloric acid. Phillips and Goss (236) isolated lignin from corn cobs, oat hulls, and spruce wood by the fuming hydrochloric acid method, by the alcoholic and aqueous sodium hydroxide methods, and by Freudenberg's modification of Urban's method. These lignin preparations were distilled with 12 per cent hydrochloric acid. Formaldehyde was found in an appreciable quantity only in the distillate from the lignin isolated from oat hulls by the Freudenberg and Harder modification of Urban's method and in the distillate from the lignin from spruce wood prepared by the same method. The percentage of formaldehyde obtained by these investigators from the lignin of spruce wood was in substantial agreement with that recorded by Freudenberg and Harder (59). However, if the theory of Freudenberg were to be accepted that the formaldehyde arises from a methylene dioxide group present in the lignin molecule, the conclusion would have to be drawn that this group is present only in lignin isolated from certain plant materials. Furthermore, it is rather difficult to conceive how such a mild treatment as that employed in the isolation of lignin by the alcoholic sodium hydroxide method could bring about the removal of such a comparatively stable group as the methylene dioxide group. It seems questionable, therefore, whether the formaldehyde that is obtained in the hydrolysis of certain lignin preparations can be considered as a fission product of a methylene dioxide group. Recently, Freudenberg (64) presented evidence of a rather indirect character as to the presence of the methylene dioxide group in lignin. Altogether, however, the presence of this group in lignin must be regarded with some uncertainty.

The presence of the ethylenic bond in lignin has not been definitely established. To account for the formation of rather stable ligninsulfonic acids by the interaction of lignified material with sulfurous acid and bisulfites, Klason (159) assumed that an ethylenic bond is present in lignin and that the formation of the stable sulfonic acid takes place in this fashion:



$$-\overset{|}{C}=\overset{|}{C}- + H_2SO_3 = -\underset{\underset{H}{|}}{\overset{|}{C}}-\underset{\underset{SO_3H}{|}}{\overset{|}{C}}-$$

Data on the halogenation of lignin are rather inconclusive. According to Freudenberg (56), when lignin prepared by his method is brominated, substitution takes place but no addition. On the other hand, Hibbert (131) working with "glycol-lignin" found that addition of bromine as well as substitution took place.

VIII. ACYLATION

Pringsheim and Magnus (245) acetylated Willstätter lignin with acetic anhydride and pyridine. The acetyl content of the product varied considerably with the source of the lignin, as is seen from table 5.

A systematic study of the acetylation of Willstätter lignin from pine wood was made by Heuser (114). He employed five acetylation methods. Products containing the highest percentages of acetyl were obtained when acetic anhydride and pyridine or acetyl chloride were used as the acetylating agents.

By heating alkali lignin from flax shoves with acetic anhydride in the presence of a few drops of concentrated sulfuric acid, Powell and Whittaker (241) obtained a product which was insoluble in sodium hydroxide solution and which contained 20.4 per cent of acetyl.

Phillips (226) acetylated the lignin fraction (isolated from corn cobs by the alcoholic sodium hydroxide method) with acetic anhydride in the presence of sulfuric acid and obtained a product which contained 17.3 per cent acetyl.

The two lignin fractions isolated from oat hulls by the alcoholic and the aqueous sodium hydroxide methods gave acetyl derivatives which assayed 16.8 and 16.2 per cent acetyl, respectively (229).

Fuchs and Horn (85) acetylated Willstätter lignin with acetic anhydride containing sulfuric acid and obtained an acetylated lignin with 19.2 per cent acetyl. Elementary analysis, coupled with the determination of methoxyl and acetyl, indicated the presence of four methoxyl and four acetyl groups in a compound represented by the formula $C_{50}H_{52}O_{20}$. Lignin as it occurs naturally in wood can be acetylated so that the product appears to contain three acetyl groups in excess of those present in acetylated Willstätter lignin. It appears, therefore, that isolated lignin must be so altered by the hydrochloric acid treatment that its activity toward acetylation is diminished.

TABLE 5

Acetyl content of acetylated Willstätter lignin

SOURCE OF LIGNIN	ACETIC ACID
	per cent
Pine wood	19.85
White beech	37.85
Straw	27.20

The acetylation of wood with the subsequent separation of the acetylated lignin fraction has been reported by Suida and Titsch (278, 279) and by Friese (73).

Beckmann, Liesche, and Lehmann (4) prepared a benzoyl derivative of alkali lignin from winter rye straw by treating the lignin with benzoyl chloride and pyridine. Results of the analysis of the benzoylated product for carbon and hydrogen indicated the presence of four benzoyl groups. The same investigators also prepared *p*-bromobenzoyl and *p*-nitrobenzoyl derivatives.

A benzoyl derivative of "primary lignin" has been prepared by Friedrich and Diwald (70).

A *p*-toluenesulfonyl derivative of lignin isolated from pine wood by the method of Urban (286) has been prepared by Freudenberg and Hess (62). Five grams of lignin was suspended in 250 cc. of 2 *N* sodium hydroxide solution, stirred mechanically, and 50 g. of *p*-toluenesulfonyl chloride in 200 cc. of benzene was added at room temperature in the course of ten hours.

The reaction product was filtered off, and the above operation repeated. Yield, 8.5 g. The product contained 7.0 per cent of sulfur.

Benzoyl derivatives of ligninsulfonic acid have been prepared by Klason (155) and by Dorée and Hall (36).

IX. ALKYLATION

Lignin can be readily alkylated by the usual alkylating reagents. Heuser and coworkers (122) methylated lignin, isolated from wood by the fuming hydrochloric acid method, by suspending 5 g. of lignin in 100 cc. of 10 per cent sodium hydroxide solution and adding gradually 30 g. of dimethyl sulfate and maintaining the temperature at 60°C. The original methoxyl content was thereby increased from 14.65 per cent to 20.73 per cent. By repeated methylation, a product was finally obtained containing 26.29 per cent of methoxyl. Lignin which had previously been heated with sodium hydroxide in an autoclave at 170°C. yielded a product which contained 24.69 per cent of methoxyl. Another sample of lignin containing 14.15 per cent of methoxyl gave on repeated methylation a product with 26.05 per cent of methoxyl (120).

Holmberg (137) methylated with dimethyl sulfate and alkali two fractions of alkali lignin which he designated as α and γ alkali lignin. The methoxyl percentage of the α-fraction was increased from 15.45 to 23.41 and that of the γ-fraction was increased from 14.6 to 23.16 per cent.

The lignin fraction isolated by Powell and Whittaker (241) from flax shoves was readily methylated with dimethyl sulfate and 3 per cent sodium hydroxide solution. The methylated product (25.6 per cent methoxyl), unlike the original lignin, was no longer soluble in alkali and therefore, presumably, no longer contained free phenolic hydroxyl groups (71).

Urban (286) methylated lignin with dimethyl sulfate and 45 per cent potassium hydroxide at 80–100°C. After fifteen successive methylations a product was obtained containing 28.23 per cent of methoxyl. However, if the temperature during the methylation was controlled so that it did not exceed 25°C., a product was obtained after two successive methylations which contained 32.4 per cent of methoxyl.

Lignin prepared by Urban's method was treated with an ether solution of diazomethane, and after two days the percentage of methoxyl increased from 15.5 to 19.8 per cent (63). Fuchs and Horn (84), employing a different technic, obtained similar results. They treated pine wood with diazomethane and obtained a product which contained about 16 per cent of methoxyl and from which a lignin with 19.7 per cent of methoxyl could be isolated. Since the original lignin isolated by the treatment with hydrochloric acid contained 15.4 per cent of methoxyl, it follows that one hydroxyl group susceptible to methylation with diazomethane is present in a molecule having a molecular weight of about 800. The methylated lignin exhibited double refraction.

Klason (162) methylated calcium ligninsulfonate. Heuser and Samuelsen (120) methylated ligninsulfonic acid by adding 10 g. of the acid to 200 g. of 10 per cent sodium hydroxide solution and treating with 65 g. of dimethyl sulfate. The methoxyl content of the product amounted to 20.67 per cent and this could be increased to 25.43 per cent by five successive methylations. The product after the sixth methylation had the same methoxyl percentage as that from the fifth, thus indicating that 25.43 is the maximum percentage of methoxyl that can be introduced in ligninsulfonic acid, at least under the conditions employed by the above investigators. The methylated product was light yellow in color, but when exposed to the air it became darker in color. It was insoluble in water, ether, petroleum ether, and mineral acids, and very slightly soluble in ethanol.

X. HALOGENATION

The chlorination of the lignin complex was first studied by Cross and Bevan (21). By the chlorination of jute they obtained a substance, represented by the formula $C_{19}H_{18}Cl_4O_9$ (26.8 per cent chlorine), which they designated "lignone chloride." This substance dissolved in alkaline solutions and in glacial acetic acid and alcohol. "Lignone chloride" was believed to be closely "allied to mairogallol and leucogallol."

Heuser and Sieber (123) in similar manner studied the action of chlorine on spruce wood. After two hours the wood absorbed 31 per cent of its weight of chlorine. The chlorinated product was extracted with absolute alcohol and a product was obtained which contained 47 per cent of carbon, 4.6 per cent of hydrogen, and 22.7 per cent of chlorine. The percentage of chlorine was therefore considerably less than that recorded by Cross and Bevan for their "lignone chloride." Heuser and Sieber were unable to obtain the chloropyrogallol derivatives from chlorinated lignin as claimed by Cross and Bevan.

Hägglund (92) chlorinated Willstätter lignin at 0°C. and obtained a product which contained 46 per cent of chlorine. The sublimation of this product afforded no chloroquinone nor any trichloropyrogallol.

According to Jonas (145), if Willstätter lignin is chlorinated under carefully controlled temperature conditions, the same chlorolignin derivatives are always obtained.

A rather drastic method of halogenation has been employed by Tropsch (284). He refluxed Willstätter lignin with antimony pentachloride containing a small amount of iodine and obtained perchloroethane and hexachlorobenzene.

"Primary lignin" in acetic acid solution was brominated by Friedrich and Diwald (70). A product was obtained which was not homogeneous, and methoxyl determinations made on this material indicated that considerable degradation of the "primary lignin" had taken place.

Powell and Whittaker (241) passed a stream of dry chlorine through a suspension of alkali lignin in carbon tetrachloride. They obtained a brick-red solid, soluble in alkalies, which contained 35.1 per cent of chlorine. Phillips (226), working in a similar manner with alkali lignin from corn cobs, isolated a chlorinated lignin product which contained 32.1 per cent of chlorine.

Alkali lignin from straw was treated by Paschke (219) with sulfuryl chloride at room temperature and a pale brown amorphous product, $C_{37}H_{42}S_3Cl_3O_{12}$, was obtained. When the halogenation was performed at 100°C. in a sealed tube, a product was obtained which contained 38.2 per cent of chlorine and was free from sulfur.

Fuchs and Horn (83) treated acetylated wood with a solution of bromine in carbon tetrachloride in the presence of iodine as a catalyst. Hydrolysis of the bromoacetylated wood with acid afforded a lignin preparation having 11 per cent of bromine.

Fuchs (79) treated a suspension of 2 g. of dry Willstätter lignin in 25 cc. of carbon tetrachloride with a 4 per cent solution of bromine in the same solvent and with a small crystal of iodine as a catalyst, and refluxed the mixture for 20 minutes. A product was obtained which contained 11.4 per cent of bromine. Part of the bromine could be removed by boiling with a 5 per cent sodium acetate solution.

Chlorolignins produced in the chlorination process from woody fibers were found to have a mean content of chlorine of 30 per cent, if extracted by an organic solvent. Lime precipitated chlorolignin from soda solution, hence the disadvantages attached to the use of lime and bleaching powder in processes for cellulose production (289).

Freudenberg, Belz, and Niemann (56) treated lignin (isolated from spruce wood by the Freudenberg method) with a solution of bromine in hydrobromic acid and found that under these conditions substitution took place but no addition. Polymerized coniferyl alcohol behaved similarly, thus indicating that neither substance contained a double bond. However, Hibbert and Sankey (131), working with glycol-lignin, found that on the assumption of a molecular weight of 400 for lignin, the bromine absorbed indicated 1 to 1.5 ethylenic bonds in this substance. Whether this difference in results can be attributed to the fact that different lignin preparations were used by Freudenberg and by Hibbert is not known. It is evident, however, that a critical study of the halogenation of lignin will be necessary before any conclusion can be drawn regarding the presence or absence of ethylenic bonds in this substance.

Friedrich and Pelikan (72) found that when spruce wood lignin is brominated a loss of methoxyl takes place to the extent of 30 per cent of the original methoxyl content. Since all the methoxyl groups could not be removed by the bromination, these investigators assumed that the methoxyl groups in lignin are not similarly combined. The maximum amount of bromine taken up by lignin was 38.7 per cent, of which 28 per cent was firmly bound. When brominated lignin was alkylated with diazomethane, 28 per cent of the bromine was split out.

XI. NITRATION

Lignin is nitrated very readily. Tropsch and Schellenberg (282) obtained an amorphous nitro substance by treating lignin with 5 *N* nitric acid. The readiness with which lignin is nitrated, these investigators believe, indicates that it has a phenolic constitution.

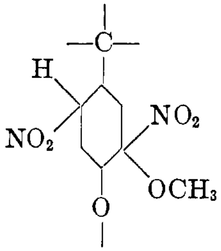
Nitrolignin was prepared (50) by treating 150 g. of Willstätter lignin, with cooling, with 1 liter of 5 *N* nitric acid. The reaction mixture was then warmed until the product became uniformly orange colored. The product was purified by dissolving in alcohol, cooling the solution with ice and salt, and precipitating the nitrolignin by passing in dry hydrogen chloride gas. From 20 g. of the crude material only 11.5 g. of purified nitrolignin was obtained. It had the composition $C_{42}H_{37}N_3O_{24}$. The percentage of nitrate nitrogen in this preparation, as determined by the method of Silberrad, Phillips, and Merriman (274), was found to be 44. Acetylation yielded a product containing 15.5 per cent of acetyl. Reduction with tin and hydrochloric acid afforded a brown polymerized product which contained 3.27 per cent of nitrogen as against 4.34 per cent in the original nitrolignin.

Powell and Whittaker (242) obtained a nitrolignin by adding portionwise 15 g. of lignin (isolated from flax shoves by the alkali method) to a solution consisting of equal parts of sulfuric and nitric acids cooled to −5°C. The reaction mixture was poured into water, and the nitro product separated out as an amorphous red precipitate which was soluble in alcohol and acetone. It was readily acetylated, and a product containing 18.9 per cent of acetyl was obtained.

Lignin from various sources when nitrated with 5 *N* nitric acid was found to yield compounds of a similar character. According to Fuchs (78) they are isonitrosoketohydroxylcarboxylic acids of high molecular weight and have a cyclic structure.

Lignin was found to react very rapidly with gaseous nitrogen dioxide (258). After a short period the initial absorption was succeeded by evolution of nitric oxide resulting from a secondary oxidation. With methylated lignin only the initial rapid absorption was observed. During the reaction the lignin became partly demethylated, with the production of methanol.

These observations were explained on the assumption that substitution had occurred. Under favorable conditions the ratio of nitrogen introduced to the original aromatic methoxyl approached 1:1. Double linkings were not involved to an important extent, since nitrolignin and nitromethyllignin could be brominated and bromomethyllignin could be nitrated. Loss of methoxyl from lignin or methyllignin was considered due to addition of nitrogen dioxide yielding compound I, which in part lost nitrous acid to give compound II and in part eliminated methyl nitrite, giving a quinol derivative passing into the nitrophenol III (57).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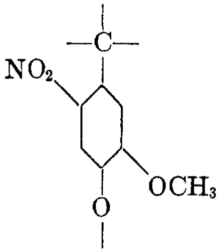
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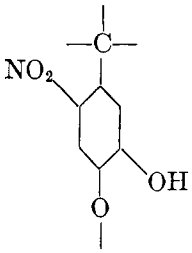

III

Glycol-lignin (obtained by digesting wood which had been extracted with an alcohol–benzene solution with ethylene glycol at 110°C. in the presence of a catalyst, such as iodine or hydrochloric acid), was readily nitrated by adding it to a mixture consisting of 4 parts of acetic anhydride and 1 part of fuming nitric acid (d. 1.49) maintained at temperatures below 0°C. Acetylation occurred simultaneously. The nitrated lignin contained 8.65 per cent of total nitrogen, 3.18 per cent of ester nitrogen (NO_3), 2.57 per cent of combined nitro (NO_2) and nitroso (NO) nitrogen. The character of the remaining nitrogen (2.89 per cent) was not determined. The nitrated lignin could be methylated and could also be reduced with sodium amalgam in alkaline solution. The resulting reduction product could be diazotized, and the latter when coupled with β-naphtholdisulfonic acid afforded a product which dyed silk and wool a brownish color when mordanted with tannic acid (128).

Kürschner (190) nitrated lignin with an alcoholic solution of nitric acid (1 volume of nitric acid and 4 volumes of alcohol). The nitrated product was crystalline. This, however, requires confirmation.

Methylated lignin (27.2 per cent methoxyl), prepared from lignin isolated from corn cobs by the alkali method, when treated with 5 *N* nitric acid afforded an orange-colored amorphous product which contained 5.25 per cent of nitrogen, of which 29.1 per cent was present in the form of the nitro group. The ease of nitration, together with the fact that the C:H ratio of the nitration product of methylated lignin was nearly 1:1, indicated the aromatic character of the product (237).

Phelps (225) obtained a yellow dyestuff by the treatment of ligninsulfonic acid with nitric acid.

Nitro derivatives of ligninsulfonic acid from sulfite liquor were prepared by Oman (213) by the use of 50 per cent nitric acid at 30–40°C., or with mixed acid (1 part by weight of nitric acid and 1.5 parts by weight of concentrated sulfuric acid) at 30°C.

The nitration of ligninsulfonic acid has been reported by Dorée and Hall (37). One part of ligninsulfonic acid was heated on the water bath for 4 hours with 20 parts of 5 per cent nitric acid. The reaction product was dialyzed, and on evaporation a light orange-colored powder was obtained in 60 per cent yield. It was free from sulfur, and its composition could be represented by the formula $C_{25}H_{27}O_{17}N_2(OCH_3)$. When the nitration product was distilled with $N/2$ sodium hydroxide, 29 per cent of the nitrogen was evolved in the form of ammonia. The nitration product on reduction with zinc and hydrochloric acid yielded a nitrogen-free ketonic substance.

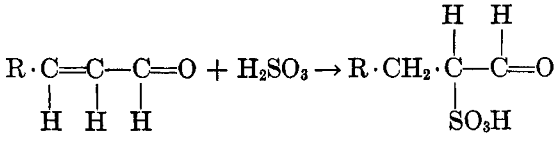
XII. SULFONATION (LIGNINSULFONIC ACIDS)

When wood and similar lignified materials are heated with a solution of sulfurous acid and acid sulfites, as in the sulfite process for pulping wood, the lignin goes into solution, leaving the cellulose in a more or less pure condition. The solution containing the lignin (waste sulfite liquor from pulp mills) has been the subject of numerous investigations. No attempt will be made here to review the extensive chemical and patent literature on the utilization of this industrial waste material. The résumé presented here includes only the important papers dealing with the chemistry of the lignin derivatives in the sulfite liquor.

Lindsey and Tollens (195) were the first to demonstrate that the lignin in waste sulfite liquor was present in the form of a calcium salt of a sulfonic acid, although this had previously been suggested by Pedersen (224). With lead acetate they obtained a precipitate from which the free acid could be obtained as an amorphous product. Analytical results agreed with the formula $C_{26}H_{30}SO_{12}$. This formula, according to Lindsey and Tollens, represented merely the average composition of the product and was not intended to indicate that they were dealing with a definite chemical compound. Later Streeb (276), working in Tollens' laboratory, found that the ligninsulfonic acids could be desulfonated by treatment with alkali.

Hönig and Spitzer (140) attempted to obtain definite fractions from the ligninsulfonic acids. The ligninsulfonic acids were precipitated from waste sulfite liquor with sulfuric acid, and the free sulfonic acids converted into their barium salts, which were then fractionally reprecipitated with alcohol. From the analytical data obtained from the several fractions of their barium salts, it was concluded that the ligninsulfonic acids were mixtures and not uniform substances. This has been confirmed by Melander (204) and by other investigators (171). Melander precipitated the ligninsulfonic acid from sulfite liquor with sodium chloride and found that only a portion of the total lignin derivatives present were thus precipitated. That fraction of the ligninsulfonic acids which could be precipitated with sodium chloride he designated α-lignin-S-acid, and that which could not thus be precipitated he designated β-lignin-S-acid. The former apparently differed materially from the α-ligninsulfonic acid of Klason.

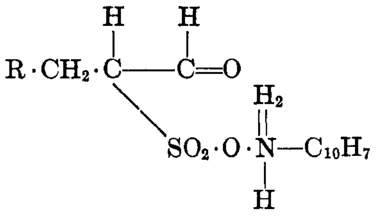
Klason (157) employed calcium chloride instead of sodium chloride for the precipitation of the ligninsulfonic acids, because the excess precipitant could be removed with alcohol. The fraction precipitated with this reagent Klason named α-ligninsulfonic acid, and that portion which remained in solution he designated β-ligninsulfonic acid. Subsequently it was found that β-naphthylamine hydrochloride was a much more desirable precipitant for α-ligninsulfonic acid (161). When the β-naphthylamine precipitate was warmed with dilute alkali it was found that only part of the β-naphthylamine could be split out. This, according to Klason, could be explained on the assumption that the lignin-fraction yielding α-ligninsulfonic acid contained the acrolein grouping. The first step in the reaction of the lignin with sulfurous acid, according to Klason, consisted in adding the elements of this acid as indicated in equation 1.



$$R\cdot\underset{H}{C}=\underset{H}{C}-\underset{H}{C}=O + H_2SO_3 \rightarrow R\cdot CH_2\cdot\overset{H}{\underset{SO_3H}{C}}-\overset{H}{C}=O \qquad (1)$$

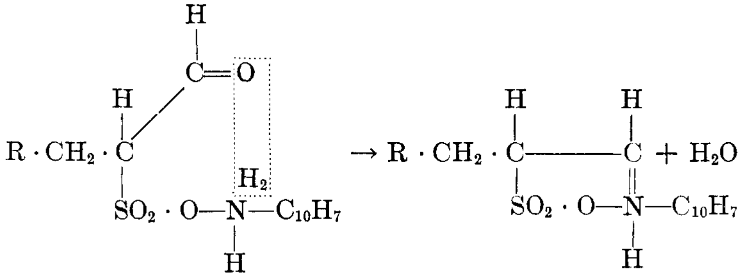
A

When the sulfonic acid (A) was treated with β-naphthylamine at first the normal salt (B) separated out as a white precipitate. A secondary reaction



B

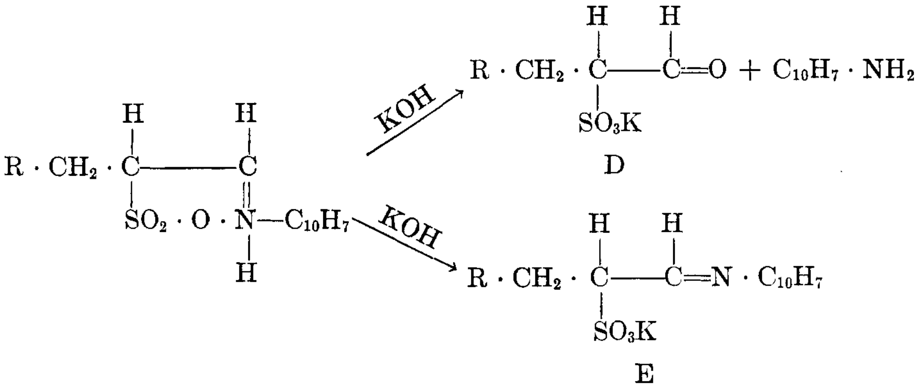
then took place, and the yellow inner salt (C) was formed in a manner indicated in equation 2. When C was treated with alkali the reaction



(2)

C

proceeded in two ways as indicated below (equation 3).



(3)

Of the total ligninsulfonic acids in sulfite liquor approximately two-thirds was found to be in the form of the α-acid and one-third in the form of the β-acid. According to Klason β-ligninsulfonic acid contains an acrylic acid group.

Lignin, according to Klason, combines with four molecules of sulfur dioxide, only two of which are present in the form of the sulfonic acid. The third is more loosely combined, and although not titratable with iodine, gradually splits off as sulfuric acid on standing in contact with air. The fourth molecule is the most weakly combined and can be titrated with iodine.

Hintikka (133) questions whether an acrolein group is present in lignin which combines with sulfurous acid to form a soluble sulfonic acid.

Dorée and Hall (36) isolated a ligninsulfonic acid from the liquor obtained by heating spruce wood with 7 per cent sulfurous acid at 100–110°C., which resembled the α-ligninsulfonic acid of Klason. They were, however, unable to obtain evidence as to the formation of an inner salt of the type C and considered the β-naphthylamine precipitate a simple salt of the base with the sulfonic acid.

The evidence which Klason has presented as to the presence of acrolein and acrylic groups in α- and β-lignin, respectively, is rather fragmentary and incomplete, although some of the work of Hägglund (96) tends to support Klason's ideas. Klason's analytical data were obtained, of course, on amorphous material, and it cannot accordingly be stated definitely whether the α- and β-ligninsulfonic acids are really homogeneous substances. However, Klason's speculations on the structure of lignin and the mechanism of the reaction between lignin and sulfurous acid and bisulfites have stimulated research on this phase of lignin chemistry.

Hägglund (95) heated wood with a solution of sulfurous acid and bisulfite and at various intervals some of the liquor was withdrawn, and precipitated with β-naphthylamine hydrochloride. His analytical results are given in table 6, from which it will be observed that the first three precipitates had substantially the same elementary composition. When heating was continued longer than seventeen hours the composition of the product changed considerably, as is evident from the increase in the percentage of sulfur (table 6).

TABLE 6

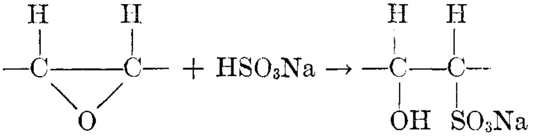
Composition of β-naphthylamine precipitate from sulfite liquor

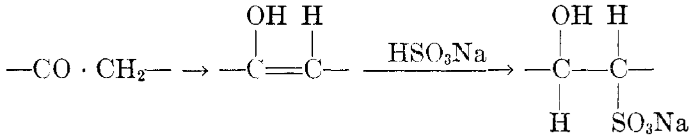
COLOR OF LIQUOR	HEATING PERIOD	CARBON	HYDROGEN	SULFUR	NITROGEN
	hours	*per cent*	*per cent*	*per cent*	*per cent*
Light yellow	13	63.0	5.3	5.2	2.3
Yellow	15	63.8	5.3	5.1	2.2
Brown	17	62.8	5.0	5.3	2.25
Dark brown	18	63.6	5.4	5.8	—
Black	20	63.0	5.2	6.2	—

The solubility of Willstätter lignin in bisulfite solution varies considerably with the procedure followed in isolating the lignin. The longer the lignin remains in contact with the fuming hydrochloric acid, the more difficult it is to dissolve it in bisulfite solution. The sulfonation proceeds in two stages. In the first stage, an insoluble ligninsulfonic acid is formed, which in the presence of the cooking liquor is converted into soluble ligninsulfonic acids. The rate of dissolution of the lignin is directly related to the pH of the cooking liquor, so that in the presence of sulfurous acid the sulfonation of the lignin takes place faster than when sodium bisulfite solution alone is employed (101).

According to Hägglund (97) β-lignin is not preëxistent in pine wood, but β-ligninsulfonic acid is produced from the α-acid during the treatment with bisulfites at high temperatures. It is suggested that the diminution in the methoxyl content in the passage from the α- to the β-acid is due to an oxidizing action, since the methoxyl content of the α-acid was found to fall from 9.77 per cent to 6.14 per cent after treatment with 2 per cent hydrogen peroxide solution for 12 hours at ordinary temperature.

There is at present some disagreement among investigators as to the mechanism of the reaction between lignin and sulfurous acid and bisulfites. In order to account for the fact that fairly stable ligninsulfonic acids are formed, Klason (152) has assumed, as already stated, that the sulfurous acid is added at an ethylenic bond in the lignin molecule. Freudenberg (64), however, has recently come out in favor of the view that the sulfonic acid group is substituted in a benzene ring. According to Hägglund (100), when lignin is sulfonated there is formed one hydroxyl group for every unit of the lignin molecule containing two methoxyl and two hydroxyl groups. This, according to Hägglund, can best be explained on the assumption that an oxygen linkage between two carbon atoms is ruptured as indicated below (equation 4), or a ketone methylene group is rearranged to its enolic form, which then reacts with sulfurous acid or bisulfites as indicated in equation 5.


(4)



$$-CO\cdot CH_2- \rightarrow -\overset{\overset{OH}{|}}{C}=\overset{\overset{H}{|}}{C}- \xrightarrow{HSO_3Na} -\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}-\underset{\underset{SO_3Na}{|}}{\overset{\overset{H}{|}}{C}}- \qquad (5)$$

XIII. OXIDATION

The published results on the oxidation of lignin are rather disappointing in so far as throwing any light on the structure of this substance is concerned. When lignin is subjected to oxidation even under mild conditions, complete disruption of the molecule takes place and simple degradation products are obtained. König (173) ozonized Willstätter lignin suspended in water and in glacial acetic acid. Formic and oxalic acids were the oxidation products identified. Similar results were obtained when barium ligninsulfonate and ligninsulfonic acids were ozonized (36). The oxidation of lignin with hydrogen peroxide in neutral and in alkaline solutions as well as the oxidation with potassium permanganate yielded simple organic acids, such as formic, acetic, oxalic, succinic, and malonic acids. Among those who have studied the oxidation of lignin and have obtained the above oxidation products may be mentioned König (174), König and Rump (183), Anderzén and Holmberg (1), Hägglund and Björkman (99), Urban (286), and Rassow and Zickmann (250).

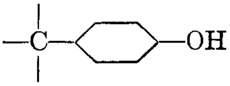
Fischer and Tropsch (49) heated lignin isolated by the fuming hydrochloric acid method with 10 *N* potassium hydroxide solution at 300°C., and obtained adipic acid. However, when the above experiment was conducted at 250°C. instead of at 300°C., oxalic and succinic acids were obtained but no adipic acid.

Fischer, Schrader, and Friedrich (48) subjected lignin to what these investigators designated as "pressure oxidation," which consisted essentially in heating lignin with 1.25 *N* sodium hydroxide solution in an autoclave at 200°C. under 55 atmospheres pressure, and obtained in addition to some simple aliphatic acids, such as formic, acetic, oxalic, succinic, and fumaric acids, small quantities of aromatic acids, such as benzoic (0.6 per cent), phthalic (trace), isophthalic (0.05 per cent), trimellitic (0.5 per cent), hemimellitic (0.53 per cent), prehnitic (0.12 per cent), pyromellitic (0.28 per cent), benzene pentacarboxylic (1.65 per cent), and mellitic (0.38 per cent) acids. However, in view of the rather drastic method of oxidation employed by Fischer and coworkers, it seems doubtful whether their results can be interpreted as definitely indicating that an aromatic nucleus is present in the lignin molecule.

Previous methylation of the lignin and the subsequent oxidation with potassium permanganate failed to alter the course of the reaction, oxalic acid being the only oxidation product obtained (120). The same reaction product was obtained when lignin was oxidized with hydrogen peroxide (119) and with nitrogen tetroxide (258). Drastic oxidation of lignin with nitric acid afforded oxalic and mellitic acids (142).

In a patent application, Pauly and Feuerstein (221) claimed that lignin or lignin-containing substances when oxidized with ozone in glacial acetic acid yield vanillin. Other oxidizing agents which may be employed are chromic acid in glacial acetic acid, potassium permanganate, and activated oxygen. This work requires confirmation.

Phillips and Goss (237) found that alkali lignin from corn cobs when oxidized with ozone yielded oxalic acid. However, when the lignin was first methylated and then oxidized with 5 *N* nitric acid or with ozone, anisic acid was obtained. When lignin was ethylated and then oxidized, *p*-ethoxybenzoic acid was obtained. This indicates that the group



is in all probability present in alkali lignin from corn cobs and that the methyl group of the anisic acid represented one which had been introduced by the methylation process and not one which was naturally present in lignin. In the oxidation of methylated lignin with the 5 *N* nitric acid, there was obtained, in addition to the anisic acid, an orange-colored amorphous product containing 5.25 per cent of nitrogen, of which 29.1 per cent was present in the form of the nitro group. The ease of nitration, together with the fact that the C:H ratio of the nitrated methyllignin was found to be nearly 1:1, tended to indicate the presence of an aromatic nucleus in the alkali lignin from corn cobs.

XIV. REDUCTION

Reduction with hydriodic acid and phosphorus

Willstätter and Kalb (300) heated lignin isolated by the fuming hydrochloric acid method with hydriodic acid and red phosphorus at 250°C. The reaction product consisted of a mixture of hydrocarbons together with some oxygen-containing substances of an acidic nature. The hydrocarbons were resolved into an acetone-soluble or liquid fraction and an acetone-insoluble or solid fraction. The former contained 88.14 per cent of carbon and 11.85 per cent of hydrogen. The molecular weight ranged from 167 for the lowest liquid fraction to 842 for the highest solid fraction. No definite compound was separated. It was established that *n*-hexyl iodide was not an intermediate product. Mixtures of hydrocarbons of the same general composition were obtained by the reduction of cellulose, glucose, and other carbohydrates. This, according to Willstätter and Kalb, indicates that a close structural relationship exists between lignin and the carbohydrates. In view of the rather drastic method of reduction employed, however, it seems rather doubtful whether any such definite conclusion is justified.

Catalytic hydrogenation under pressure

Fierz-David and Hannig (44) subjected Willstätter lignin to catalytic hydrogenation under a pressure of 250 atmospheres, nickel being used as the catalyst. The distillation products from 500 g. of lignin are given in table 7. The tar contained 39.0 g. of phenols and 21.5 g. of neutral substances. In the absence of the nickel catalyst practically no hydrogenation took place even at 300 atmospheres pressure.

Bowen and Nash (10) gradually heated lignin to about 450°C. with hydrogen under pressure (maximum up to 240 atmospheres) in the presence of catalysts, such as alumina and nickel oxide. Liquid and gaseous products were formed, but the oils obtained were not fully saturated, owing probably to the deposition of small quantities of coke on the catalyst. The oil contained phenolic substances.

Zinc dust distillation

Karrer and Bodding-Wiger (150) heated Willstätter lignin with zinc dust in an atmosphere of hydrogen. None of the fractions was oxygen-free, nor did they appear to be homogeneous. From one fraction a crystalline substance melting at 210–212°C. was separated, but the identity of it was not established.

Phillips (232) distilled alkali lignin isolated from corn cobs with zinc dust in an atmosphere of hydrogen at a temperature not exceeding 400°C. The aqueous distillate contained catechol. An oil was obtained equal to 16 per cent of the weight of the lignin used. In the phenolic fraction of the oil, guaiacol and 1-*n*-propyl-3-methoxy-4-hydroxybenzene were identified. The "neutral" fraction of the oil when oxidized with potassium permanganate yielded anisic acid. The isolation of these degradation products of lignin indicates that there are in the lignin molecule, in addition to other possible units, at least two aromatic nuclei or those which can be readily converted into aromatic nuclei. One nucleus has a side chain having at least three carbon atoms in addition to a methoxyl group and a hydroxyl group in the positions meta and para, respectively, to the side chain. The other nucleus has a side chain and a methoxyl substituent para to the side chain.

TABLE 7

Products from catalytic hydrogenation of lignin

PRODUCTS	QUANTITY
	grams
Combustible residue	78.0
Aqueous distillate	251.0
Tar	89.0
Ash, and gaseous products	88.0

XV. HYDROLYSIS

The demethoxylation of lignin by heating it with dilute acids under pressure has been reported by Heuser (121) and coworkers. Ten grams of lignin was heated for 3.5 hours in a sealed tube at 150–160°C. with 100 cc. of 5 per cent hydrochloric acid. The lignin lost practically none of its methoxyl by this treatment. However, when this operation was repeated for three times at a temperature of 170–180°C. complete demethoxylation resulted. In addition, deep-seated changes in the composition of the lignin took place, for the demethoxylated product could only be partly methylated with dimethyl sulfate. After three successive treatments with this methylating agent a dark product was obtained which contained only 5.79 per cent of methoxyl.

Holmberg and Wintzell (137) and Beckmann, Liesche, and Lehmann (5) heated lignin with hydriodic acid and obtained dark colored amorphous products soluble in alkali. The last-named investigators heated lignin, which had been isolated from winter-rye straw by the alcoholic sodium hydroxide method, with hydriodic acid (d. 1.9) in glacial acetic acid solution for 4 hours. The product was completely demethoxylated and contained chemically bound iodine.

Hägglund (93) hydrolyzed lignin which he prepared from spruce by his method (hydrolysis of the wood for 15 minutes with 45 per cent hydrochloric acid at 0°C.) with fresh portions of boiling 3 per cent hydrochloric acid until the acid extract gave no test for sugar with Fehling's solution. In this operation 33.7 per cent of the starting material was lost, and the acid extract contained 15.8 per cent of sugar. With *p*-bromophenylhydrazine a hydrazone melting at 155°C. was obtained. The sugar was therefore, in all probability, arabinose. Hägglund considered the carbohydrate as part of the structural arrangement of the lignin molecule. This seems doubtful, and as Heuser (113) has pointed out, the presence of pentosans or pentoses in lignin preparations can be ascribed to incomplete hydrolysis of the lignified plant material. In this connection, it may be pointed out that the lignin isolated from wood with alkali by Powell and Whittaker (243) and the lignin fraction isolated by Phillips (226) from corn cobs were entirely free from furfural-yielding substances.

Hägglund and Björkman (98) and later Hägglund and Rosenqvist (104) distilled Willstätter lignin with 12 per cent hydrochloric acid and obtained a distillate which gave a precipitate with phloroglucinol, barbituric acid, and thiobarbituric acid. The substance present in the distillate was not definitely identified, but was shown to be neither furfural, methylfurfural, nor hydroxymethylfulfural. This substance was subsequently identified by Freudenberg and Harder (58) as formaldehyde. The significance of the identification of formaldehyde as a fission-product of lignin from the standpoint of the presence of the methylene dioxide group in lignin is discussed elsewhere.

Aronovsky and Gortner (2) found when wood was heated with water under pressure that although the total quantity of lignin as determined by the 72 per cent sulfuric acid method remained practically constant, part of the lignin was altered so as to be soluble in alcohol, indicating that possibly depolymerization had taken place.

When barium ligninsulfonate was refluxed for 4 to 6 hours with a saturated solution of barium hydroxide, a portion of the lignin derivatives dissolved. From this solution a light yellow amorphous product was obtained which in many of its properties resembled tannins. It gave a greenish coloration with ferric chloride solution, and was precipitated with solutions of lead acetate, quinine hydrochloride, and gelatin. It was furthermore absorbed by hide powder and had an astringent taste. The composition of the free acid could be represented by the formula $C_{18}H_{32}O_{10}S$. On fusion with potassium hydroxide protocatechuic acid was obtained (139).

XVI. FUSION WITH ALKALIES

The fusion of lignin with alkali has been undertaken by several investigators. The reaction products in nearly all cases have been protocatechuic acid, catechol, oxalic, and other simple aliphatic acids. Vanillic acid has also been reported as one of the degradation products. In addition to the above substances, there is always obtained a dark, amorphous, humin-like degradation product generally referred to as "lignic acid." Below 240°C., the main product of the fusion is "lignic acid."

Fischer and Tropsch (49) fused Willstätter lignin with potassium hydroxide at 240–300°C. There was obtained 35.5 per cent of humin-like material and 14.9 per cent of ether-soluble substances. In the latter, protocatechuic acid was identified. There was also isolated a crystalline substance which did not melt below 260°C. and which gave a green coloration with ferric chloride. The identity of this substance was not established.

Among others who have identified protocatechuic acid in the alkaline melt of lignin may be mentioned Klason (160), Hägglund (91), and Hägglund and Malm (103).

Heuser and coworkers have made extensive studies of the fusion of lignin with alkali. It was first reported that protocatechuic acid, but no oxalic acid, was formed by the fusion of carbohydrate-free lignin with potassium hydroxide at a maximum temperature of 270°C. (117). It was later (125) found, however, that a 20 per cent yield of oxalic acid could be obtained when 4 parts of lignin and 50 parts of potassium hydroxide were heated for 40 minutes at 280°C.

Heuser and Winsvold (126) fused lignin with 10 parts by weight of potassium hydroxide and 2 to 3 parts of water at 240–290°C. for ½ to 1½ hours. Various substantial amounts of "lignic acid" were recovered from the product of the fusion. The aromatic products obtained consisted of protocatechuic acid (16 to 19 per cent) and catechol (1 to 3 per cent). When the fusion was conducted in contact with air, secondary oxidation took place, with the formation of oxalic acid (up to 20 per cent). However, when the fusion was carried out in an atmosphere of hydrogen or nitrogen, the formation of oxalic acid was practically suppressed and the yield of catechol increased to 9 per cent. Iron appears to have considerable influence on the course of the reaction. The fusion of lignin with alkali in an iron crucible out of contact with air increased the yield of catechol up to 23 per cent, but the presence of protocatechuic acid could no longer be detected. Protocatechuic acid appears to be the primary product of the reaction; the catechol is formed from it by the elimination of carbon dioxide. This finds confirmation in the fact that when Heuser and Winsvold fused pure protocatechuic acid with potassium hydroxide in the presence of air, the yields of catechol and oxalic acid were 2.5 and 20 per cent, respectively. When the fusion was conducted in an atmosphere of hydrogen, no oxalic acid was obtained, and the yield of catechol increased to 19 per cent and could be increased to 26 per cent when iron was present in the melt.

The fact that substantial amounts of aromatic substances are formed in the alkali fusion of lignin is seen by Heuser and Winsvold as additional evidence in favor of the hypothesis that lignin contains a benzenoid nucleus. Under similar conditions, cellulose yields only negligible quantities of aromatic derivatives.

When iron powder is present in the alkali fusion mixture, the yield of aromatic substances obtainable from lignin is increased, and the elimination of carbon dioxide from protocatechuic acid is promoted, but not to such a large extent as when the fusion is performed in an iron crucible. There is only a slightly lower yield when the fusion is carried out at 240–260°C. instead of 270–280°C. Fusion in an atmosphere of carbon dioxide exerts a protective influence in suppressing the formation of oxalic acid, but not so completely as hydrogen. Carbon dioxide also moderates the total reaction and more "lignic acid" is obtained. Carbon dioxide is produced to the extent of 27 to 28 per cent of the weight of the lignin when the fusion is carried out in a nickel crucible in the presence of air. Since the decomposition of protocatechuic acid into catechol is only promoted in the presence of an iron catalyst in an atmosphere of hydrogen, the carbon dioxide formed under normal conditions is the result of oxidation of other products of the reaction (116).

The fusion of 3 parts of lignin with the 20 parts of potassium hydroxide, 20 parts of water and 12 parts of zinc dust at 250°C. for 1½ hours afforded a yield of protocatechuic acid which varied from 7 to 15 per cent (250).

The methylation of lignin and subsequent fusion of the methylated product with potassium hydroxide at 270–280°C. had little effect on the character and yield of aromatic substances obtained (286).

Freudenberg, Harder, and Markert (60) obtained only a 4.8 per cent yield of protocatechuic acid (isolated as its dimethyl ether—veratric acid) when technical lignin was fused with potassium hydroxide at 260–270°C. in an atmosphere of nitrogen. When pure protocatechuic acid was subjected to fusion with potassium hydroxide under similar conditions, about one-half of the acid was lost in this treatment. Because of this, Freudenberg, Harder, and Markert feel justified in stating that the yield of protocatechuic acid obtained is only one-half of that actually formed and probably amounts to from 9 to 10 per cent.

Beckmann, Liesche, and Lehmann (5) have shown that the yields of phenols and protocatechuic acid obtainable in the alkali fusion of lignin from straw depend considerably on the method used for isolating the lignin, as can be seen from table 8.

Holmberg and Wintzell (137) isolated a lignin fraction from so-called black liquor (a waste product obtained in the delignification of wood by the sodium hydroxide process) and subjected this to fusion with potassium hydroxide. They obtained protocatechuic acid together with some oxalic and acetic acids.

TABLE 8

Products of the alkali fusion of lignin from straw

PRODUCTS	METHOD USED FOR ISOLATING LIGNIN		
	Cold NaOH	Reflux with NaOH	NaOH in CH_3OH
Protocatechuic acid	6.0 to 7.6 per cent	7.0 to 8.0 per cent	15.3 per cent
Phenols	2.2 to 3.3 per cent	0.6 to 1.5 per cent	45.0 per cent

The alkali fusion of the so-called ligninsulfonic acid obtainable from waste sulfite liquor has received the attention of several investigators. Melander (203) obtained catechol, protocatechuic acid, vanillic acid, acetic acid, and traces of higher fatty acids. The yield of catechol amounted to 10 per cent of the organic portion of the starting material.

Hönig and Fuchs (138) subjected to alkali fusions the fractions of barium ligninsulfonate prepared according to the method of Hönig and Spitzer (140). Two grams of barium lignin sulfonate, 8 g. of potassium hydroxide, and 8 cc. of water were heated in a nickel crucible at 240–250°C. The temperature was gradually raised to 300°C. and kept at this point not longer than 10 minutes. The yield of protocatechuic acid varied from 13 to 19 per cent, based on the organic portion of the starting material. Although a positive test for catechol with ferric chloride was obtained, this compound was, however, not actually isolated.

XVII. DRY DISTILLATION

Hägglund (90) distilled lignin isolated from pine wood by the hydrochloric acid method of Willstätter and Zechmeister and obtained 45 per cent carbon residue, 9.6 per cent oil, 0.10 per cent acetone, 0.67 per cent methanol, and 0.64 per cent acetic acid.

Fischer and Schrader (47) distilled Willstätter lignin and obtained 13.2 per cent aqueous distillate, 12.5 per cent oil, 57.2 per cent carbon residue, and 17.0 per cent gas (obtained by difference). It was found that 16.4 per cent of the oil dissolved in sodium carbonate and 33.9 per cent in sodium hydroxide solutions.

Tropsch (283) destructively distilled commercial Willstätter lignin under reduced pressure (1 to 2 mm.) and obtained 14.2 per cent aqueous distillate, 10.2 per cent oil, and 53.8 per cent carbon residue. The oil contained 37.5 per cent phenolic compounds. Results of a similar character were obtained by Fischer and Tropsch (51).

Pictet and Gaulis (240) distilled technical Willstätter lignin under reduced pressure. The temperature varied from 350°C. to 390°C., and the pressure from 5 to 25 mm. The lignin yielded 15 per cent of its weight in oil, 21 per cent aqueous distillate, and 52 per cent carbon residue. No examination of the aqueous distillate was made. The oil was extracted with 10 per cent sodium hydroxide solution, in which 89 per cent dissolved. In the alkali-soluble fraction eugenol was identified. The alkali-insoluble fraction was separated by means of liquid sulfur dioxide into saturated and unsaturated hydrocarbons. They consisted entirely of hydroaromatic hydrocarbons. The saturated hydrocarbons gave five fractions having approximately the following compositions: 235–240°C., $C_{13}H_{26}$; 260–270°C., $C_{14}H_{26}$; 270–280°C., $C_{16}H_{30}$; 315–320°C., $C_{24}H_{44}$; above 320°C., $C_{30}H_{60}$. In the last fraction melene, a hydrocarbon found in some coal tars and in Galician petroleum, was identified. The unsaturated hydrocarbons gave three fractions, 200–210°C., $C_{11}H_{16}$; 230–240°C., $C_{12}H_{16}$; 250–260°C., $C_{13}H_{16}$. Of these, the first appeared to be identical with a coal tar fraction. The last fraction gave a tetrabromo derivative, $C_{13}H_{12}Br_4$, which melted at 193°C. and appeared to be related to hexahydrofluorene of coal tar. Pictet and Gaulis believe that their results indicate a relationship between lignin tar and coal tar, and to that extent support the hypothesis of Fischer and Schrader (46) that coal originated not from the cellulose of the plants but rather from the lignin. The isolation of eugenol they believe confirms the view that lignin contains the grouping of coniferyl alcohol. The results also indicate the existence of a hydroaromatic ring in lignin. It must be remembered, however, that the degradation products referred to were obtained by subjecting lignin to a very drastic treatment and results obtained by such methods must necessarily be interpreted with considerable reserve.

Kürschner (189) subjected lignin to what he calls sublimation, but it is not sublimation in the ordinary sense of the term but rather a thermal degradation of lignin. Small quantities of lignin were heated to 200°C. in a special apparatus, and crystals of vanillic acid were obtained.

Heuser and Brötz (115) distilled lignin isolated by the fuming hydrochloric acid method from pine and aspen following the method used by one of the authors in a previous investigation (124), and the results obtained were respectively as follows: carbonized residue, 45.6 and 44.3 per cent; tar, 13.3 and 14.2 per cent; aqueous distillate, 29.7 and 30.5 per cent; acetic acid, 1.26 and 1.28 per cent; methanol, 0.82 and 0.86 per cent; acetone, 0.18 and 0.21 per cent.

Dry distillation of a mixture of technical Willstätter lignin and silver powder at 280–300°C. gave about 10 per cent of distillate consisting of an aqueous and a tarry layer (75). The former contained catechol to the extent of 0.5 per cent of the original lignin, a little guaiacol, an organic acid, and 5-hydroxymethylfurfural. In the tarry distillate eugenol and melene were identified. Similar results were obtained by treating technical Willstätter lignin with superheated steam at 280–300°C. Consideration of the yield of 5-hydroxymethylfurfural, Fuchs believes, indicates the presence of a hexal but not a hexose complex in lignin (76).

Szelenyi and Gömöry (280) distilled lignin isolated from beech, oak, and birch. The yields of aqueous distillates and tar were about the same in all cases, namely, 19 and 3 to 4 per cent, respectively. Of the total percentage of methoxyl in oak lignin and beechwood lignin, about 10 per cent was accounted for in the distillate, whereas in the case of birch lignin, only 7.9 per cent was thus accounted for.

Phillips (228) made a study of the dry distillation of lignin from corn cobs isolated by three different methods, namely, the alkali method, the fuming hydrochloric acid method of Willstätter and Zechmeister, and the 72 per cent sulfuric acid method. From the alkali lignin the following results were obtained: aqueous distillate, 15.5 per cent; water-insoluble oily distillate, 17.7 per cent; carbon residue, 52.2 per cent; gas (by difference), 13.5 per cent; acetone, 0.13 per cent; methanol, 1.9 per cent; acids (calculated as acetic acid), 0.05 per cent. The oily distillate analyzed as follows (the results are expressed as per cent by weight of the oil): acids, 8.6 per cent; phenols, 84.0 per cent; neutral substances, 7.2 per cent. In the case of the Willstätter lignin and the lignin prepared by the 72 per cent sulfuric acid method, the percentage of aqueous distillate was about the same as that obtained from alkali lignin, whereas the oily distillate was only about one-half. The percentages of carbon residue obtained from the Willstätter lignin and from the 72 per cent sulfuric acid lignin were considerably greater than that obtained from alkali lignin. The percentages of acetone and acetic acid obtained were considerably greater in the case of the Willstätter and the 72 per cent sulfuric acid lignin than in the alkali lignin, whereas the percentage of methanol was less. This higher percentage of methanol is probably due in part to the greater methoxyl content of alkali lignin as compared with that of Willstätter and 72 per cent sulfuric acid lignin. The low yield of acetic acid obtained from alkali lignin is explained by the fact that in preparing lignin by this method deacetylation occurs.

In a subsequent paper Phillips and Goss (235) reported the results of a systematic chemical examination of both the aqueous and oily distillates obtained when alkali lignin from corn cobs was subjected to dry distillation at a reduced atmosphere (25 mm.) of carbon dioxide. The following results (calculated on the basis of dry lignin) were obtained: aqueous distillate, 11.7 per cent; oily distillate, 28.3 per cent; carbonized residue, 50.5 per cent; gas (by difference), 9.3 per cent. The aqueous distillate contained acetic acid, acetone, and methanol, in amounts of (calculated on the basis of dry lignin) 0.30, 0.10, and 0.65 per cent, respectively. Catechol was identified in the aqueous distillate. In the oil the following compounds were identified: acetic acid, catechol, phenol, *o*-cresol, guaiacol, creosol, 1-vinyl-3-methoxy-4-hydroxybenzene, 1-*n*-propyl-3-methoxy-4-hydroxybenzene, and a dimethoxycarboxylic acid, $C_8H_6O_2 \cdot (OCH_3)_2$. The steam-volatile "neutral" fraction of the oil, when oxidized with potassium permanganate, yielded anisic acid. In the non-steam-volatile "neutral"

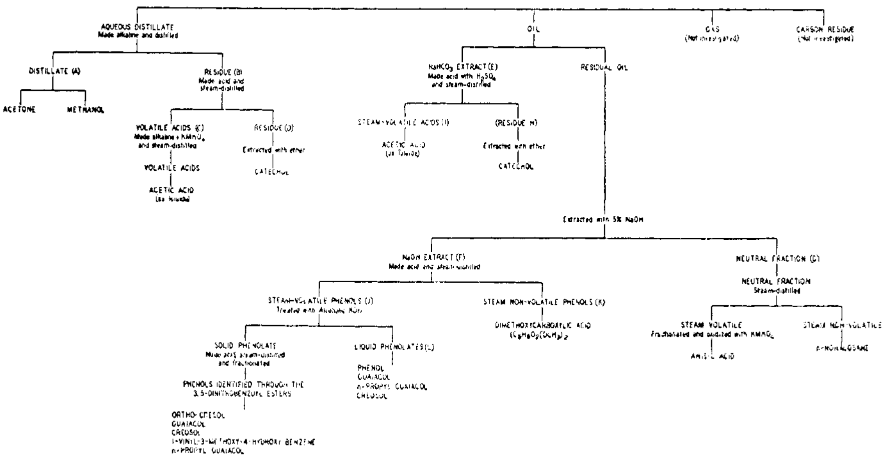
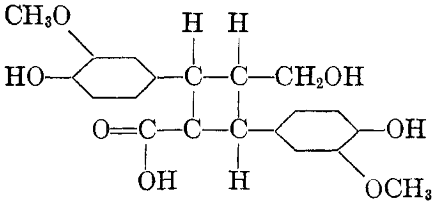


FIG. 1. PRODUCTS OBTAINED IN THE DRY DISTILLATION OF LIGNIN IN A REDUCED ATMOSPHERE OF CARBON DIOXIDE

fraction of the oil, *n*-nonacosane was identified. The results of this investigation are given schematically in figure 1.

XVIII. THEORIES CONCERNING THE CONSTITUTION OF LIGNIN

Considerable difference of opinion prevails among investigators of the chemistry of lignin as to whether this substance belongs to the aliphatic, aromatic, hydroaromatic, or heterocyclic series. Willstätter and Kalb (300), as a result of their work on the reduction of lignin with hydriodic acid and red phosphorus, have concluded that lignin is structurally related to the carbohydrates. They based their conclusion on the fact that similar reduction products were obtained from lignin and from certain carbohydrates. However, none of the products they obtained was homogeneous. They were unable to identify any definite chemical substance. Jonas (146) and Marcusson (198) contend that lignin is not aromatic but is made up of furane nuclei, but very little definite experimental evidence has been presented in support of this. However, the evidence obtained from the experiments on the alkali fusion, zinc dust distillation, dry distillation, "sublimation," oxidation, halogenation, nitration, and metabolism of lignin, when taken all together, indicates that lignin contains either an aromatic nucleus or one which is readily converted into an aromatic nucleus (hydroaromatic). In this connection it may be pointed out that Holmberg (135) isolated a crystalline substance from waste sulfite liquor (a by-product from the delignification of wood by the sulfite process) in 0.1 to 0.2 per cent yield. This substance was found to be phenolic and contained two methoxyl groups, two hydroxyl groups that could either be acetylated or methylated, and a lactone group. On the basis of his experiments, Holmberg assigned either formula I or formula II to the hydroxy acid from which the lactone is derived. According to him, it may be considered as the inner anhydride of diguaiacoltetramethylenecarbinol



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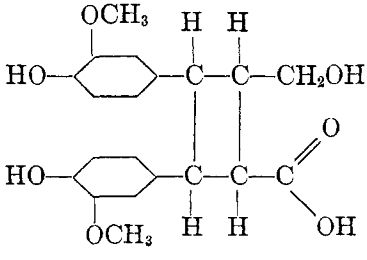
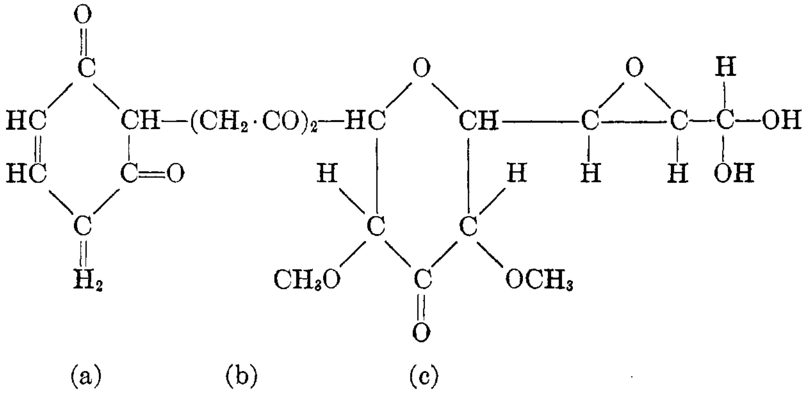


II

carboxylic acid. It may be stated, however, that the connection between this lactone and the original lignin in the wood has not yet been definitely established.

The literature concerning the structure of lignin is now rather extensive and quite a number of constitutional formulas have been proposed. It may be stated at the very outset that all these formulas are more or less speculative in character, although evidence of a fragmentary character can be mustered for their support. The fact that at frequent intervals the proponents of the constitutional formulas have themselves found it necessary to change them is in itself an indication of the very insecure foundation upon which they rest. However, since these speculative and tentative constitutional formulas have stimulated research on lignin, a brief résumé of the literature concerning them is here presented.

Cross and Bevan (23), as a result of their work on "lignone chloride," have proposed the following constitutional formula (III) for lignin:

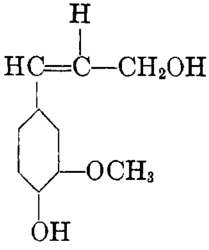


III

According to Cross and Bevan, chlorination takes place at the ethylenic bond of group (a), from which the derivatives of pyrogallol are subsequently obtained. However, it must be pointed out that attempts by other investigators to isolate these derivatives have always resulted in failure. The occurrence of the hydropyrone group (c) is based on the fact that acetone and maltole were obtained at higher temperatures. Inasmuch as these fission products can be obtained from substances other than those having a hydropyrone nucleus, this evidence is not valid. The presence of the group (b) is assumed because on hydrolysis, oxidation, and dry distillation, acetic acid is obtained. In view of the fact that this acid can be obtained from numerous organic substances, this evidence is not convincing.

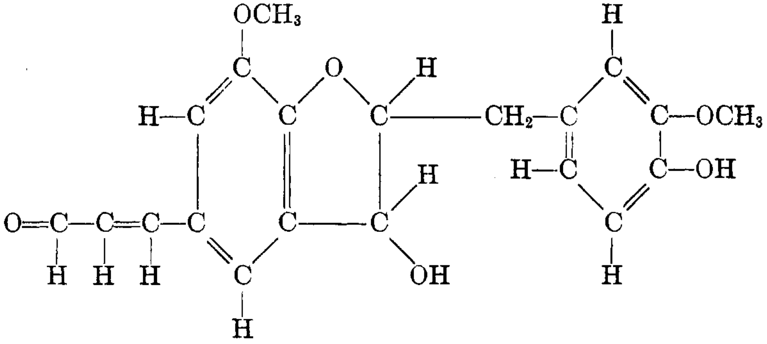
A formula for lignin has been proposed by Green (88), but as it is no longer in harmony with our present knowledge of the chemistry of lignin, it need not be presented here.

Klason was the first to suggest an aromatic structure for lignin. According to his original conception (153), lignin is a condensation product of coniferyl alcohol (IV) and oxyconiferyl alcohol. In view of the fact that

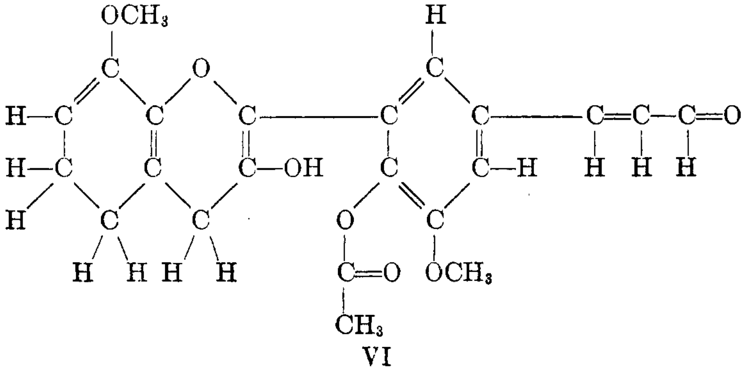


IV

he was subsequently able to isolate two lignosulfonic acids, one of which could be precipitated with calcium chloride and one which could not, Klason was led to the conclusion that there were two lignins. The lignin, the sulfonic acid of which was precipitated with calcium chloride, was designated α-lignin, and the other β-lignin. α-Lignin was considered a condensation product derived from coniferyl aldehyde and the following possible formulas (V and VI) were proposed for it (164):

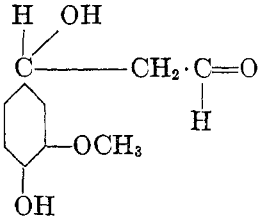


V



VI

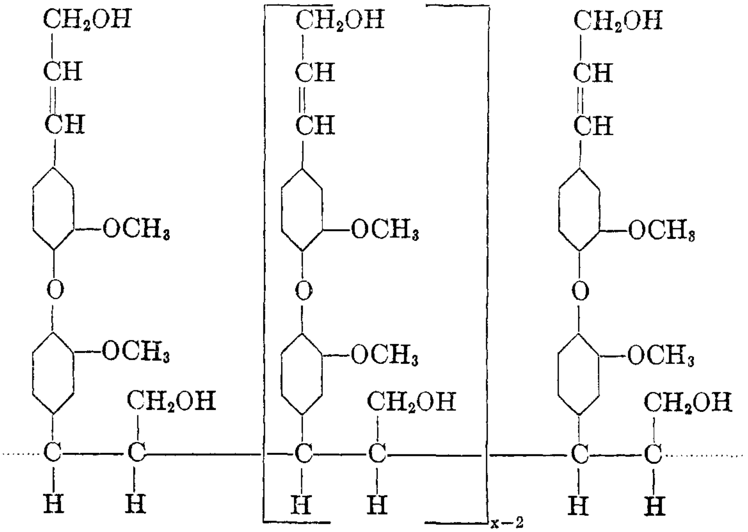
In subsequent publications (168) Klason found it necessary to alter considerably his conception as to the structure of lignin, and in a more recent article (169) he expresses himself in favor of the view that lignin contains the grouping represented by formula VII:



VII

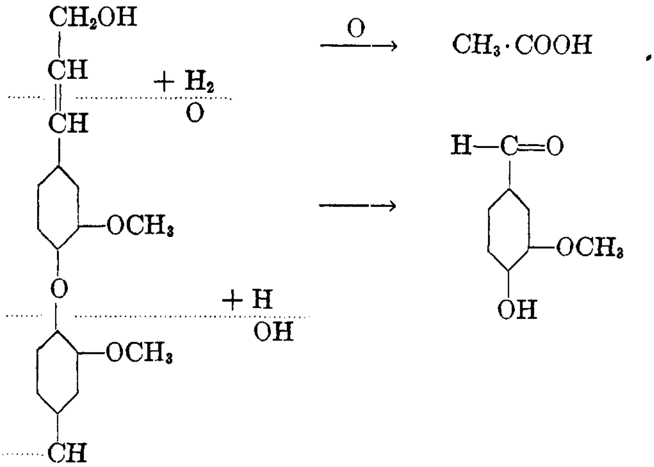
Whether lignin contains a grouping related to coniferyl alcohol or aldehyde has not been definitely established, although the results of experiments on dry distillation and zinc dust distillation of lignin would tend to support this hypothesis.

Kürschner (188) has advanced the hypothesis that lignin is a polymer of the glucoside coniferin, which Tiemann and Haarmann (281) have shown yields glucose and coniferyl alcohol upon hydrolysis. In support of this hypothesis, he points out the fact that he was able to obtain fairly large yields of vanillic acid in his "sublimation" experiments, and indicates further that coniferin and spruce lignin show an analogous behavior in several chemical reactions, such as action with sulfurous acid and bisulfites, fusion with alkalies, and solubility in trichloroacetic acid. In a recent article Kürschner (191) suggested the following as a possible structural formula for lignin (VIII):



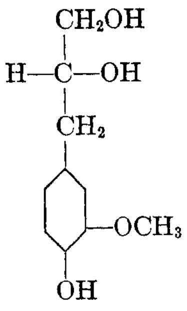
VIII

A compound of this formula could yield vanillin through a process of oxidation and hydrolysis, the vanillic acid resulting as a secondary oxidation product of the vanillin.

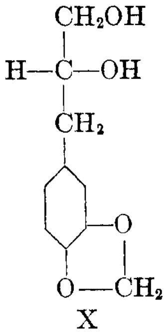


The fact that lignin does not behave like a true unsaturated compound would speak against the Kürschner conception of the structure of lignin. Further, when lignin is heated with hydriodic acid as in the Zeisel determination of alkoxyl groups, methyl iodide only is given off. This has been definitely established in the case of spruce lignin (105). If lignin were a derivative of coniferyl alcohol, one ought to obtain a mixture of methyl iodide and ethyl iodide upon heating it with hydriodic acid. Tiemann and Haarmann (281) have shown that coniferyl alcohol when heated with hydriodic acid gives both methyl iodide and ethyl iodide, the latter resulting from a degradation of the propylene side chain. In the Kürschner formula the various benzene nuclei are connected through oxygen in etherlike combination. A compound having such a structure ought to yield fairly simple derivatives of benzene when heated with hydriodic or hydrochloric acid. No such derivatives have been obtained from spruce lignin when treated in this manner.

A somewhat related theory has been put forth by Freudenberg (54). This investigator considers that the following two groups (IX and X) are the basic units from which lignin may be considered as derived. He assumes that the primary hydroxyl is joined through an ether linkage with one of the phenol hydroxyls of the next molecule and a condensation product represented by formula XI is formed. The material represented

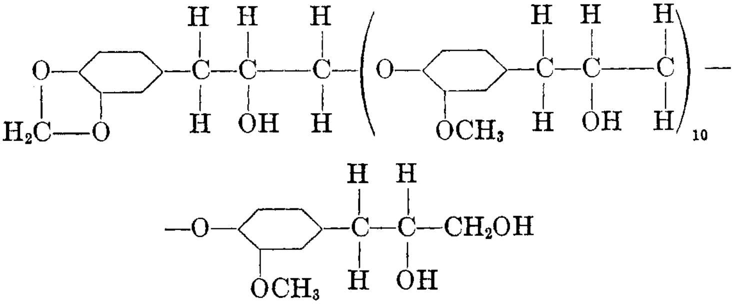


IX

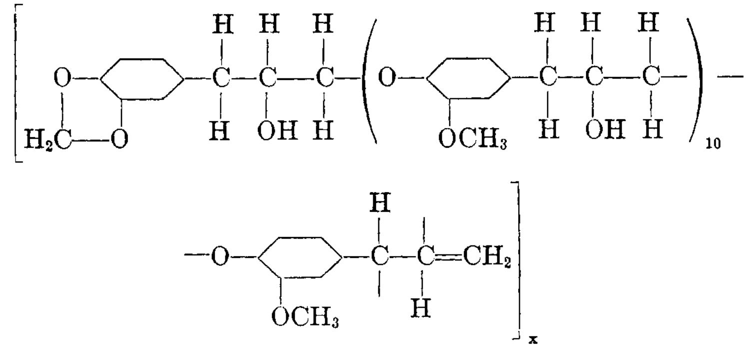


X

by formula XI Freudenberg calls "primary lignin." By the polymerization of "primary lignin" (XI) "secondary lignin" (XII) results.



XI

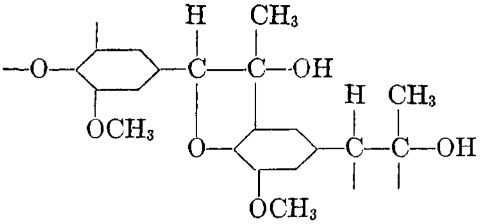


XII

This is formed either by condensation in the wood after the cells are dead or during the process of isolation in the laboratory. It is the parent substance of almost all lignin derivatives, particularly of ligninsulfonic acid. The latter substance is a result of partial elimination of water and addition of H—SO_3H to the resulting double bond.

According to Freudenberg, the formulas XI and XII fulfill the following essential conditions which experiment requires: The formation of "primary lignin" in physiological synthesis from definite structural units; post mortem condensation of "primary lignin" to the three-dimensional "secondary lignin" of enormous molecular weight; the absence of phenolic hydroxyls; the presence of aromatic methoxyl, dioxymethylene, and secondary hydroxyl groups in the proportions indicated by the formula; the completely amorphous condition; the permutoid nature of the material, shown by the stoichiometric relations upon methylation or acetylation of the carbinol, or upon bromination, nitration, or mercuration of the nucleus. The refractive index, 1.61, also corresponds to this conception.

In a recent publication, Freudenberg (64) appears to have abandoned his original idea that the nuclei are joined together through an ether linkage, and suggests that union may take place as indicated in formula XIII:

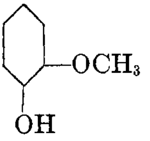


XIII

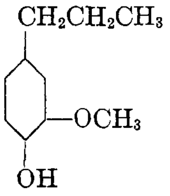
The ligninsulfonic acids are formed through a substitution in the benzene ring and do not result from the addition of the elements of H_2SO_3 at a double bond.

Although the formulas for lignin proposed by Freudenberg are still to a considerable extent speculative, yet it must be stated that he has mustered more facts in support of his ideas than any other investigator who has put forward a tentative constitutional formula for lignin. Freudenberg's ingenious speculations ought to prove useful and serve as stimuli to those engaged in the chemical investigations on lignin.

Phillips (231) isolated guaiacol (XIV) and *n*-propylguaiacol (XV) from the oil obtained in the distillation of lignin with zinc dust in an atmosphere of hydrogen, and has accordingly suggested that in all probability the two



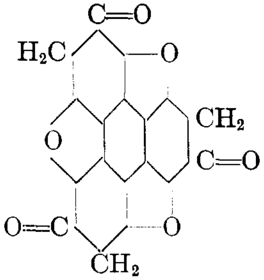
XIV



XV

fundamental units in the structure of lignin are guaiacol and *n*-propylguaiacol, the latter having the hydrogen atoms in the *n*-propyl side chain substituted by hydroxyl groups. It may be pointed out in this connection that the ultra-violet absorption spectra of lignin and lignin derivatives, as determined by Herzog, Hillmer, and others (112), indicate a benzenoid structure for lignin. The absorption spectra of lignin preparations resemble those of coniferyl alcohol and related compounds.

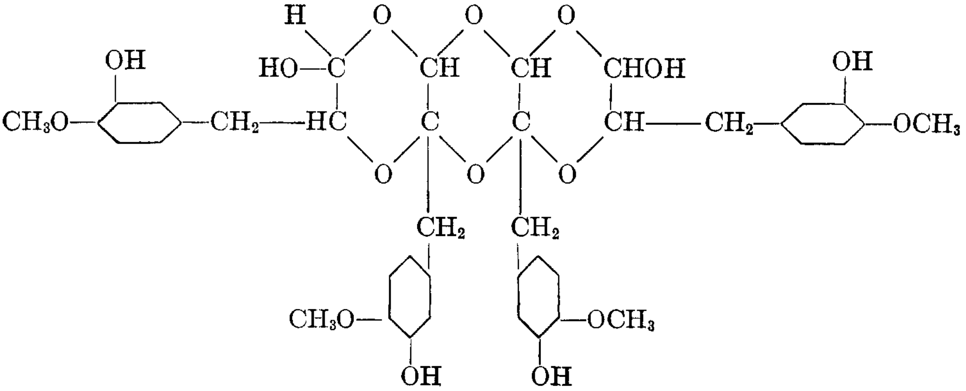
Schrauth (263) has proposed a constitutional formula for lignin. This formula (XVI) is admittedly speculative.



XVI

It is suggested that the fundamental unit of the lignin molecule is formed by the condensation of three molecules of 5-hydroxymethylfurfural, which is itself produced by the internal condensation of carbohydrates. Units so formed consist of a compact condensed ring system, of which three of the outer rings are furane nuclei and the other three outer rings and the central ring benzene nuclei.

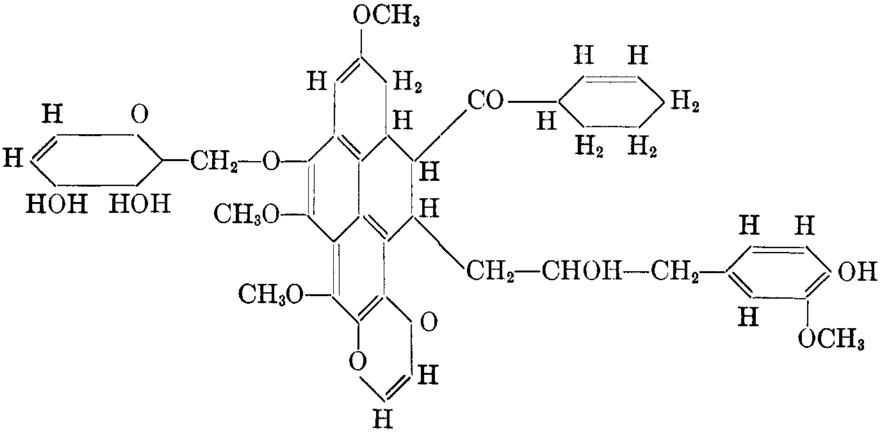
According to Pavolini (222), lignin is a tetramer of special aromatic hydroxyaldehydes, as indicated below (formula XVII):



XVII

This formula is not in harmony with several experimental facts, particularly with the results obtained on heating lignin with hydriodic acid, and with those obtained on the distillation of lignin with zinc dust in an atmosphere of hydrogen.

An hypothetical structural formula (XVIII) for lignin, which is intended to account for several facts concerning its chemistry, has also been proposed by Fuchs (81).



XVIII

XIX. METABOLISM

Although many of the materials of plant origin that are used as food by man and animals contain lignin in substantial amounts, it is rather surprising that such a common food ingredient has not been studied and subjected to metabolism experiments to a greater extent than that found recorded in the literature.

Several investigators, among them Meissner and Shepard (202), Harten (108), Weiske (297), and more recently König (177), have suggested that lignin probably furnishes the aromatic group involved in the formation of hippuric acid, but they have presented no experimental evidence in support of this claim. The last-named investigator, working with Dietrich (33) in 1871, as well as Stutzer (277), has claimed, however, that lignin is not digested in the animal body. König and Becker (178) found that the lignin of wheat bran was not digested by rabbits, whereas free lignin was digested by these animals to the extent of 12.8 per cent. According to Paloheimo (216), lignin reappears quantitatively in the feces. Rosgozinski and Starzewska (253) experimenting with sheep found that the lignin of oat straw and that prepared by Beckmann's method were not digested by these animals. Rubner (256) noted a loss of lignin in experiments conducted with human beings as well as with dogs. Phillips, Weihe, Jones, and Csonka (238) and later Csonka, Phillips, and Jones (27) reported that lignin prepared from corn cobs by the alkali method, when fed to cows or dogs, caused an increase in the benzoic acid (hippuric acid) eliminated in the urine. In table 9 are presented some of the data obtained in their experiments in which a dog was used as the experimental animal.

TABLE 9

Lignin feeding experiments with dog showing effect on hippuric acid (benzoic acid) elimination

MEAT FED DAILY	LIGNIN ADDED TO MEAT DIET	TOTAL BENZOIC ACID ELIMINATED DAILY
grams	*grams*	*grams*
150	None	0.357
150	25	0.648
150	25	0.879
150	25	0.676
150	25	0.795
150	25	0.810
150	None	0.359
150	None	0.339
None	None	0.207
None	25	0.705

TABLE 10

Methoxyl group balance in metabolism experiments

EXPERIMENTAL ANIMAL	FORE PERIOD DAILY METHOXYL			EXPERIMENTAL PERIOD				
	Intake in food	Output in feces	Loss	Methoxyl in lignin added	Total methoxyl output in feces	Increase over normal output due to lignin	Loss of methoxyl in lignin added	
	grams	*grams*	*per cent*	*grams*	*grams*	*grams*	*grams*	*per cent*
Dog	None	None	—	13.0	10.4	10.4	2.6	20.0
Dog	None	None	—	13.48	11.67	11.67	1.81	13.3
Cow	249.5	77.8	68.8	210.80	522.20	133.30	77.50	36.7

In two lignin feeding experiments with cows the average daily output of benzoic acid increased from 32.34 to 45.08 and from 62.12 to 64.85 g. The total quantity of methoxyl in the lignin fed and in the feces was also determined, and the results showed a distinct loss of this group, as can be seen from table 10.

Results of digestion experiments conducted in vitro using the fresh material from a cow's stomach and also the stomach juice indicated that demethoxylation of lignin had taken place. This apparently was not brought about by bacteria, but rather by some other agent, possibly an enzyme present in the gastric juice of the animal.

It may be concluded from the above that the results of the more recent lignin metabolism experiments indicate that this substance is, at least in part, broken down by the digestive processes of the animal body.

XX. MICROBIOLOGICAL DECOMPOSITION

There appears to be a decided difference, in so far as the action of fungi and bacteria are concerned, between lignin as it occurs naturally in plants and free or isolated lignin. Willstätter lignin or lignin isolated by the 72 per cent sulfuric acid method appears to be very resistant to the action of soil microörganisms and to the wood-destroying fungi *Trametes pini* and *Polystictus hirsutus* (262). Natural lignin under aerobic conditions can be decomposed by soil microörganisms, at least in part, although the rate of decomposition is generally less than that of the cellulose and hemicelluloses (239a).

According to Waksman and Gerretsen (292), the decomposition of the lignin of straw is affected considerably by temperature. At 7°C. the loss of lignin was negligible, whereas at 37°C. over 30 per cent of the lignin was removed in 3½ months and 50 to 60 per cent in 9 months.

Waksman and Nissen (294) found that the cultivated mushroom (*Agaricus campestris*) when grown on horse manure reduced the lignin content of this material from 20.8 per cent to 14.8 per cent in 47 days. The reduction of the lignin in the composted manure accounted for nearly 80 per cent of the total reduction in the weight of the original material.

Waksman and Tenney (239b and c) found that under anaerobic conditions the lignin of alfalfa was preserved practically quantitatively. Because of the relative resistance of the lignin of plant materials to microbial attack under certain natural soil conditions, Waksman and coworkers (291) have concluded that lignin contributes largely, though not exclusively, to the formation of humus. Fischer (45) has suggested that peat and coal originated largely from lignin. According to this theory, under the anaerobic conditions prevailing in peat bogs, the other plant constituents, such as cellulose and hemicellulose, are decomposed into gaseous and water-soluble products, whereas the lignin, being more resistant, accumulates. Subsequently, the methoxyl groups of the lignin are removed by hydrolysis, yielding humin-like bodies which enter into the formation of coal.

Rose and Lisse (254) made a chemical examination of a sound sample of Douglas fir heart and also of one partly rotted and of one almost completely rotted. They found that the percentage of cellulose and pentosans decreased with the advance in the decomposition of the wood, whereas the alkali-soluble fraction and the percentage of methoxyl increased. They concluded from their results that lignin is far more resistant to decomposition than cellulose, although no direct lignin determinations were made and no attempt was made to identify the fungi responsible for the decay. It has been known for some time that wood parasitic fungi, such as *Trametes pini*, *Polystictus hirsutus*, and *Polyporus annosus*, preferentially, though not exclusively, attack lignin, producing what is known as "white rot," whereas the organisms producing "brown rot" attack lignin only to a very limited extent (109).

Falck and Haag (43) conclude from their studies that in the microbiological decomposition of plant materials two distinct processes take place, namely, "destruction" and "corrosion." The effect of "destruction" is to decompose the cellulose and pentosans, the lignin being very little affected. "Corrosion," on the other hand, causes principally the decomposition of the lignin. "Destruction" may follow "corrosion," but not vice versa.

Studies of the microbiological decomposition of lignin or lignified materials have thus far afforded no degradation product that would help to throw light on the constitution of lignin. Pringsheim and Fuchs (244),. after adding the necessary inorganic salts, inoculated alkali lignin with forest soil. The product obtained differed from the original material in containing up to one-half of its weight of substances soluble in alcohol, although the original lignin was practically insoluble in alcohol. The alcohol-soluble fraction was found to have a smaller percentage of methoxyl but a greater percentage of carbon than the original lignin. The fraction insoluble in alcohol showed some decrease in the percentage of methoxyl, but the percentage of carbon was not affected. No substance of a definite character was isolated.

Jensen (144) and also Waksman and Iyer (293) found that lignin has a depressing effect upon the decomposition of protein as measured by the evolution of carbon dioxide and the formation of ammonia. Alkali lignin (5 parts) was dissolved in sodium hydroxide solution, and 1 part of casein dissolved in the same solvent added. Acid was added until the pH was 7.0. The precipitate, designated as "humus nucleus" or "ligno-protein" complex, was found to be resistant to the action of microörganisms. The depressive effect on the decomposition of proteins by lignin was found to be due not to any toxic action of the lignin but to the fact that as a result of the interaction of the lignin and the protein a "ligno-protein" complex was formed which was resistant to microbial attack.

Many of the contradictory statements found in the literature on the microbiological decomposition of lignin and lignified materials can be attributed to the fact that our present methods for the quantitative determination of lignin are faulty, and more definite and conclusive information must wait the development of more exact analytical methods.

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