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Preparation and properties of lactic acid lignin

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PREPARATION AND PROPERTIES OF LACTIC ACID LIGNIN

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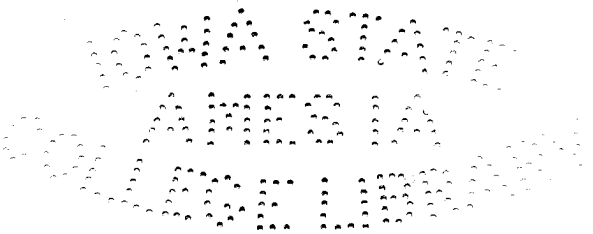

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INTRODUCTION

Evidence indicating that unchanged natural lignin can be isolated from plant tissue by the action of organic or other solvents is conspicuous only by its absence. Thus any attempt to determine the structure of lignin must consider the solvent as the first reactant of the series used in studying the structural problem. This necessitates an investigation of the reaction between natural lignin (or the lignin's precursors) and the solvent before valid data regarding the structure of lignin can be obtained.

Hawley and Harris (1) found that mild pyrolysis of Cross and Bevan Cellulose produces methoxyl free "synthetic lignin", water and a small amount of carbon dioxide. It was demonstrated by Lowry (2) that lignin may, on the basis of elementary analysis, be regarded as intermediate between cellulose and coal. Evidence indicating that solvent extraction can be regarded as a mild type of pyrolysis has been presented (2).

A study of the reaction between natural and isolated (caustic alkali) cornstalk lignins and lactic acid is reported in this thesis. The data obtained are correlated with similar but less extensive data for a series of simple organic acids.

HISTORICAL

The literature on lignin has been reviewed by Phillips (3), Norman (4), Freudenberg (5) and Hibbert (6). Unfortunately the action between lignin and organic solvents is not completely covered in these reviews.

The historical section of this thesis is limited to a review of the literature covering the action between lignin and alcohols, ethers and acids. Lactic acid has an alcoholic hydroxyl group and may act as an alcohol or as an acid. α-Methoxypropionic acid may function as either an ether or as an acid. On the other hand the remaining acids (formic, acetic and propionic) used in this investigation contain no functional groups other than the carboxyl group.

Klason (7) reported, in 1893, that nearly half of the lignin is dissolved when spruce wood is cooked with methyl alcohol containing hydrogen chloride. The soluble lignin contains 20% methoxyl; this indicates that methylation occurs when lignin is treated with methyl alcoholic hydrogen chloride. It is thought that the alcohol insoluble lignin remaining in the wood is condensed by the mineral acid. The acid catalyzed condensation of lignin in plant tissue forming alcohol insoluble lignin has recently been observed by Hibbert and co-workers (8, 9).

Extensive investigations using ethylene glycol (10, 11, 12, 13), ethylene glycol monomethyl ether (11, 12, 14, 15, 16), glycerol (11, 17), glycerol α-monochlorohydrin (18), n-amyl alcohol (17), iso-amyl alcohol (17, 19, 20), n-butyl alcohol (17, 21, 22, 23, 24, 25, 26, 27, 28), iso-butyl alcohol (17, 19), n-propyl alcohol (17), iso-propyl alcohol (17), ethyl alcohol (8, 9, 17, 20, 29, 30, 31, 32, 33, 34, 35, 36), methyl alcohol (7, 17, 37, 38, 39, 40), and benzyl alcohol (41) have lead to the conclusion that alcohols extract lignin from plant tissues in varying amounts depending on the tissue used and on the conditions. In general the presence of water, mineral acid or caustic alkali[1] catalyzes the extraction. It is also agreed that the isolated lignin is combined with the alkyl group of the alcohol. Hägglund and Urban (19, 20) believe that a carbonyl group of the lignin forms a semi-acetal with the extracting alcohol. A similar mechanism was proposed by Charbonnier (28) who believes that an acetal is formed between a tautomeric hydroxyl (42) of the lignin and the alcohol. Another and more probable explanation was proposed by the Swedish investigator Holmberg (43) who

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[1] Charbonnier (28) reported that a condensation product of butanol with lignin is obtained only when a catalyst such as hydrochloric acid is present during the extraction. He also believes that alkali acts as the active pulping agent rather than as a catalyst in "alkali catalyzed" butanol extractions. On the other hand Bailey (27) found that a lignin-butanol condensation occurs in the absence of mineral acid catalysts.

believes that the reaction:

$$Li\cdot O\cdot R + HOR' = Li\cdot O\cdot R' + HOR$$

takes place between the lignin and the extracting alcohol. It is left undecided whether R consists of hydrogen, a carbohydrate group, another lignin group or even another part of the same lignin molecule.

Lignin extracted by alcoholysis is usually recovered by pouring the alcoholic solution into a large volume of water; this causes part of the lignin to precipitate. The failure to obtain a quantitative yield of precipitated lignin led to a series of investigations of the water soluble products formed by the ethanolysis of lignin. This work has been adequately reviewed by Hibbert (6); it indicates that lignin is, in part, composed of phenylpropane building units.

The ethanolamines and morpholine-ethanol constitute an interesting group of lignin solvents because they contain alcoholic hydroxyl groups and also have basic properties. Thus, as lignin solvents, they may function as alcohols catalyzed by their own alkalinity or as organic bases. Triethanolamine was studied by Gibbs (42) who inferred that its properties as a lignin solvent are due to the alcoholic character of the base. Reid and co-workers (45, 46) found that monoethanolamine renders lignin acid soluble; they also reported that this base effects partial demethylation of native and isolated (sodium hydroxide) lignin. The action of the ethanolamines and morpholine-ethanol on cornstalk

lignin has been reported from this laboratory (47). It was found that the amount of lignin extracted depends upon the basic strength of the extracting liquors for both the aqueous and anhydrous nitrogen bases. The addition of caustic alkali to aqueous solutions of the bases increases the amount of lignin extracted; this is apparently due to increased alkalinity of the extracting liquor rather than to catalytic action by the alkali. In no case was the recovery of extracted lignin quantitative. This shows that the anhydrous bases and their aqueous solutions render all or part of the lignin acid soluble. The bases form nitrogenous compounds with lignin. The mechanism through which the ethanolamines extract and combine with lignin is not known. It is probable that they extract lignin by virtue of their alkalinity and then degrade the dissolved lignin by alcoholysis.

Dioxane catalyzed by hydrogen chloride has been used to remove lignin from wood (48, 49, 50). Hilpert and Wisselinck (51) investigated this reaction. They reported that the general belief that an organic solvent such as dioxane acts on lignin only as a solvent and the mineral acid as a catalyst is false. According to these investigators xylose or fructose when treated with dioxane containing hydrogen chloride form water insoluble "lignins" which are similar to those obtained from wood tissue. The "sugar lignins" contain no methoxyl and slightly less hydrogen than do wood lignins produced by the same treatment. Nikitin and co-workers (52, 53) reported

that the compositions of dioxane and hydrochloric acid (Willstätter) lignins from wood are almost identical. These results were interpreted as meaning that lignin is present in wood as a special component which is isolated by the dioxane method in a form very similar to native lignin. The Russian investigators also claim that the mild conditions under which the dioxane lignin was obtained precluded the possibility of its formation by carbohydrate resinification. It may be significant that Hilpert recovers his dioxane lignin by precipitation into water while Nikitin precipitates his into ether. Nikitin's contention that dioxane lignin is obtained in a form very similar to native lignin does not appear well founded, because it implies a similar form for hydrochloric acid lignin. F. E. Brauns (54) reported that purified dioxane, even in the presence of hydrochloric acid, has no pulping effect. If this finding is confirmed the pulping action attributed to dioxane is undoubtedly due to the presence of alcohols which exist as impurities in commercial grades of dioxane.

A patent covering the pulping of wood with 85% acetic acid catalyzed by 0.3% sulfuric acid was obtained by Pauly (55). Friedrick (56) used glacial acetic acid containing hydrogen chloride to extract lignin. The acetic acid lignin was precipitated by the addition of water. Alkaline hydrolysis showed that this lignin is an ester of lignin and acetic acid. It was reported (57) that acetic acid lignin is a

phenolic ester of lignin and acetic acid. The presence of phenol glucosides or phenol glycuronides and their acetates was established by hydrolysis of acetic acid lignin; this hydrolysis liberates carbohydrate material, acetic acid and lignin having a higher phenolic hydroxyl content than the unhydrolyzed lignin.

Formic acid has been used as a solvent for lignin (58). The work of Staudinger and Dreher (59) shows that formic acid will extract lignin from spruce without the use of mineral acid. The lignin obtained resembles dioxane lignin in its properties. Cotton is attacked slowly by formic acid and in 10 hours at 100° C. the cotton molecules are degraded about one half. On boiling several days part of the cotton dissolves and evaporation leaves a methoxyl free residue. It was reported (60) that the digestion of quantitative filter paper with 92% formic acid forms a light brown solution; a precipitate resembling lignin is formed when this solution is added to a large amount of water. A series of investigations by Hibbert and co-workers (61, 62, 63) show that hot formic acid demethylates lignin and anhydrous formic acid gives a higher yield of recovered lignin than is given by 93% formic acid. Formylation takes place when lignin is extracted with anhydrous formic acid. Spencer and Wright (57) state that formic acid lignin and acetic acid lignin contain chemically bound carbohydrate material. Since they gave no details showing how their products were purified to

remove coprecipitated carbohydrate material such as that reported by Sarkar (60) this claim must remain open to further investigation.

It was reported by Hillmer (64) that lignin is soluble in monochloroacetic acid. Nikitin and Rudneva (65) investigated the action of hot anhydrous monochloroacetic acid catalyzed by hydrogen chloride on Willstätter lignin. They found that the isolated lignin is almost completely soluble in acid. Precipitation into ether gives an easily hydrolyzable ester containing 9% chlorine and 13% methoxyl. Under the same conditions the reaction between monochloroacetic acid and pine pulp results in the solution of two thirds of the esterified lignin and part of the carbohydrates. Precipitation of the product into ether and removal of the carbohydrate by acid hydrolysis gives a regenerated lignin with a composition similar to that of Willstätter lignin. The action of hot 80 - 85% monochloroacetic acid on wood meal and straw was studied by Schütz (66). He reported that alpha cellulose is left as a fibrous residue, the lignin and hydrolyzed hemicellulose go into solution. No mineral acid was added but some hydrochloric acid is formed during the reaction. It was found that anhydrous chloroacetic acid produces considerable decomposition when used as a pulping agent. The extracted lignin was recovered by precipitation into a large amount of water. The recovered lignin is alkali soluble, sodium carbonate insoluble and it contains no

chlorine and no carboxyl groups.

Holmberg (43, 67, 68) investigated the action of thioglycollic acid on lignin. He proposed the following mechanism:

$$Li\cdot O\cdot R + HSCH_2COOH = Li\cdot S\cdot CH_2COOH + HOR$$

R may be a hydrogen, a carbohydrate group, another lignin group or part of the same lignin molecule. The result of a study on thioglycollic acid lignin was recently reported by Ahlm and Brauns (69). They attempted to prove the presence of carboxyl groups on the thioglycollic acid lignin by methylating an analyzed sample with diazomethane, analyzing for sulfur and methoxyl, hydrolyzing with sodium carbonate and repeating the analysis. Although some sulfur is lost during the hydrolysis the results tend to support the view that part of the acid groups add according to the method of Holmberg (43).

Hillmer (64) found that lignin is slightly soluble in 50% lactic acid. Lactic acid was later used as an extractant for lignin by Hibbert and Phillips (18) and by Gibbs (44). These investigators implied that lactic acid's solvent action is due to the presence of an alcoholic hydroxyl group. They presented no evidence to substantiate this implication other than the fact that mineral acid or iodine (which catalyze the extraction of lignin by alcohols) catalyze the extraction of lignin by lactic acid. On the other hand

Schütz (66) said, in a foot note, that di-, and trichloroacetic acids and some hydroxy acids act on lignin in a manner similar to that of monochloroacetic acid.

EXPERIMENTAL

Preliminary and Orientation Experiments

Fifty g. of air dried ground cornstalks were boiled with 600 ml. of 85% lactic acid for twelve hours. The sample was then diluted with an equal volume of hot 85% lactic acid, filtered and thoroughly washed with hot lactic acid. The extracted lignin was precipitated by pouring the cooled filtrate into 5 l. of distilled water. The precipitated lignin was filtered, washed and air dried; a yield of 8.3 g. was obtained. The recovered lignin was analyzed for methoxyl (70) after drying over phosphoric anhydride in a vacuum desiccator.

A sample of lactic acid lignin was heated at 100° C. for 4½ hours in a solution of 5% sodium hydroxide. The saponified lignin was precipitated with dilute sulfuric acid, filtered, washed, dried and analyzed as above. Another sample was treated at room temperature for 24 hours with alcoholic ammonia; after precipitation with acid this sample was washed, dried and analyzed.

The iodine oxidation number of lactic acid lignin was determined by a modification of the method of Walde and Hixon (71). A sample of lignin weighing 100 mg. was

dissolved in 25 ml. of 4% sodium hydroxide, 15 ml. of 0.2N iodine-potassium iodide solution was added and the reaction mixture allowed to stand for 4 hours at room temperature. The solution was acidified with 15 ml. of concentrated hydrochloric acid, diluted to 200 ml. and the excess iodine determined by back titration with 0.1N sodium thiosulfate. The number of ml. of 0.2N iodine-potassium iodide solution used per 100 mg. of lignin is reported as the iodine oxidation number. The iodine oxidation value for lactic acid lignin is 10.70.

Forty g. of ground air dried cornstalks were boiled for twelve hours with 400 ml. of 92% formic acid. The sample was treated by the method used in isolating lactic acid lignin. A yield of 6.7 g. formic acid lignin was obtained.

A sample of formic acid lignin was analyzed for methoxyl; another sample was saponified with 5% alkali, recovered and analyzed. The iodine oxidation number of formic acid lignin was found to be 12.52.

Since it is known that some hydroxy compounds demethylate lignin (47) it was decided to test the action of lactic acid on anisole. Anisole was prepared from phenol and methyl sulfate (72). A 3-necked 1 l. flask was provided with a separatory funnel, a reflux condensor and a mechanical stirrer. A solution of phenol (0.17 mol)

and 7 g. sodium hydroxide in 70 ml. water was placed in the flask and the solution was cooled to -10°C. and stirred. Methyl sulfate (0.34 mol) was added gradually from the separatory funnel. After the methyl sulfate was added the reaction flask was heated on a boiling water

Table 1. Methoxyl Content of Lignins

Type of Lignin	% Methoxyl
Lactic Acid Lignin	8.9
Lactic Acid Lignin Saponified with 5% Sodium Hydroxide	8.5
Lactic Acid Lignin Treated with Alcoholic Ammonia	8.4
Formic Acid Lignin	9.5
Formic Acid Lignin Saponified with 5% Sodium Hydroxide	9.9
Sulfuric Acid Lignin	12.5 [1]

[1] This value was given by Fisher and Bower, J. Am. Chem. Soc., 63, 1881 (1941).

bath; in about 1/2 hour a further amount of phenol (0.17 mols) and 7 g. of sodium hydroxide in 70 ml. of water was added. The mixture was refluxed for 12 hours and cooled. The anisole was extracted with ether, washed with water, dried over anhydrous calcium sulfate (Drierite), and distilled (151 - 152°C.). A yield of 64.7% (0.22 mols) was obtained.

Five g. of anisole were added to 50 ml. of 85% lactic acid in a 200 ml. flask provided with a reflux condensor. Another sample similar to the first but containing 1 ml. of concentrated hydrochloric acid was prepared. These samples were refluxed for 6 hours, cooled and slowly added to 600 ml. beakers containing 40 g. of sodium hydroxide in 250 ml. of water. The mixtures were extracted with ether, the ether solutions were washed with water and dried over anhydrous calcium sulfate (Drierite). The ether was distilled off and the anisole was then distilled (150 - 153°C.); a yield of 4.20 g. was obtained from the first sample and a yield of 3.96 g. was obtained from the second sample (catalyzed by hydrochloric acid). The alkali soluble portions were neutralized with hydrochloric acid and saturated with sodium hydrogen carbonate. The sodium hydrogen carbonate solutions were extracted with ether; the ether solutions were washed with water and dried with anhydrous sodium sulfate, then the ether was removed by distillation. The first sample left no residue, and the second sample left 100 mg. of sirupy liquid that gave no color change with iron (III) chloride.

Although it was found that lactic acid does not demethylate anisole, caution must be employed in making such an assumption regarding the action of lactic acid on lignin. Accordingly it was decided to prepare a sample of

lactic acid lignin from cornstalks that had been freed of pentosans by acid hydrolysis (73). Three kg. of air dried ground cornstalks were placed in containers and 40 l. of 0.1N hydrochloric acid were added. The mixture was autoclaved for 3 hours at 20 pounds pressure, cooled, filtered and washed free of chlorides. A yield of 1850 g. of air dried acid hydrolyzed cornstalks was obtained. Five g. of acid hydrolyzed cornstalks were boiled for 8 hours with 60 ml. of 85% lactic acid. The lactic acid lignin was recovered and analyzed by the procedures given above. A yield of 1.18 g. lignin containing 13.5% methoxyl was obtained. This indicates that lactic acid lignin made from cornstalks containing pentosans is contaminated with carbohydrate material. Accordingly all further lignin preparations reported in this thesis were made from acid hydrolyzed cornstalks.[1]

A simplification of the cold 72% sulfuric acid method for lignin (74) was tested and found to give satisfactory results.

One-gram samples of ground cornstalks were weighed and placed in 50 ml. beakers within a desiccator. The desiccator was placed in an ice box. When the samples acquired the temperature of the ice box, 15 ml. of cold

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1. It was found by Peterson, Walde, and Hixon (74) that extraction with alcohol-benzene mixture is not necessary before preparing lignin from cornstalks because cornstalks contain very little extractable material.

72% sulfuric acid were added to each sample. The samples were stirred until all lumps disappeared; then they were left, with occasional stirring, for 18 hours.

At the expiration of 18 hours, the samples were removed from the ice box, each was made up to 600 ml. with distilled water, heated to boiling, and kept above 85°C. on a steam plate for 2 hours (the volume was kept constant). The samples were removed from the steam plate and allowed to stand until the flocculent material settled. Each sample was then filtered through a prepared Gooch crucible, washed with hot water until free from sulfate, dried at 105°C., cooled in a desiccator, and weighed. The weighed crucibles were ignited in a muffle furnace, cooled in a desiccator, and weighed. The loss in weight was taken as the lignin.

Table 2. Comparison of Methods

Material	% Lignin by Simplified Method	% Lignin by Peterson, Walde and Hixon Method
Air Dried Cornstalks	16.3	16.4
Air Dried HCl Hydrolyzed Cornstalks	31.5	31.2
Air Dried Cornstalks Extracted with 50% Monoethanolamine	2.6	2.5
Air Dried HCl Hydrolyzed Cornstalks Extracted with 85% Lactic Acid	23.3	23.5

Action of Lactic Acid on Cornstalks

Five-gram samples of air dried, acid hydrolyzed, ground cornstalks were digested with 60 ml. portions of aqueous solutions of lactic acid for varying lengths of time at elevated temperatures. Some of the extractions were catalyzed by the addition of 1 ml. of concentrated hydrochloric acid to the extraction liquors. Each sample was then diluted with an equal volume of hot lactic acid solution, filtered and thoroughly washed with hot lactic acid solution. The pulp was then added to 1 l. of boiling water, allowed to stand over night, filtered, washed with boiling water until the filtrate was free of acid and air dried. The dried pulp was weighed and analyzed for lignin by the previously described simplification of the cold sulfuric acid method (74). This is a general procedure and was used when investigating the action of α-methoxypropionic acid, propionic acid, acetic acid and formic acid on cornstalks.

A 25 g. sample of air dried, acid hydrolyzed, ground cornstalks was extracted for 4 hours at 112°C. with 300 ml. of 85% lactic acid. At the completion of the digestion the sample was treated by the method described above. A yield of 19.68 g. pulp containing 17.3% lignin was obtained. A 15 g. sample of air dried pulp from the first extraction was digested for 4 hours at 112°C. with 180 ml. of 85%

lactic acid. A yield of 14.06 g. of pulp analyzing 13.6% lignin was obtained. Ten grams of pulp from the second extraction was extracted for 4 hours with 120 ml. of 85% lactic acid; this produced 9.71 g. of pulp containing 11.9% lignin. Finally a 5 g. sample of pulp from the third extraction was subjected to a fourth extraction. A yield of 4.87 g. pulp containing 10.0% lignin was obtained.

Recovery of lactic acid lignin.

Each filtrate from the lactic acid extractions of cornstalks was cooled and slowly poured into 1.5 l. of distilled water;[1] the water was stirred continuously while the acid solution was added. This caused the lignins to precipitate. The precipitated lignin was filtered and washed until the filtrate was free of acid. The recovered lignin was dried over phosphoric anhydride in a vacuum desiccator. Certain samples of the dry lactic acid lignin were analyzed for methoxyl. This method was used to recover lignin extracted from cornstalks by the other organic acids used in this investigation.

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1. The lignin solutions from 5 g. samples of cornstalks were added to 1.5 l. of water; the solutions from larger samples of stalks were added to proportionally larger amounts of water.

Table 3.

Five Grams Cornstalks Extracted with 85% Lactic Acid

Time, Hours	Temp., °C.	Weight of Pulp, g.	Lignin Removed, %
1	92	4.13	38.6
2	92	4.07	43.2
4	92	4.10	50.1
8	92	4.06	54.4
0.5	112	4.23	37.5
1	112	3.97	50.3
2	112	3.90	58.4
4	112	3.75	62.1
8	112	3.73	69.3
12	112	3.64	72.3
16	112	3.61	75.4
16[a]	112	3.09	84.2
1	122	3.74	63.0
2	122	3.64	68.2
4	122	3.95	76.4
8	122	3.41	85.6
1	126	3.68	65.2
2	126	3.58	71.8
4	126	3.48	78.9
8	126	3.34	86.0

[a] Catalyzed by the addition of 1 ml. concentrated hydrochloric acid.

Table 4.

Five Grams of Cornstalks Extracted 4 Hours with 60 ml. of Aqueous Lactic Acid at 112° C.

Strength of Acid, %	Weight of Pulp, g.	Lignin Removed, %
85	3.75	62.1
74.5	3.89	53.2
44	4.56	20.0
44 [a]	4.36	35.5

[a] Catalyzed by the addition of 1 ml. concentrated hydrochloric acid and digested only one hour.

Table 5.

Five Grams of Cornstalks Extracted 4 Hours with 60 ml. of Propionic Acid and Propionic Acid Solutions.

Strength of Acid, %	Temp., °C.	Weight of Pulp, g.	Lignin Removed, %
100	112	4.30	29.9
86	112	4.05	45.3
72.4	105	4.01	44.1
72.4 [b]	105	3.07	79.4

[b] Catalyzed by the addition of 1 ml. concentrated hydrochloric acid.

Preparation of Sodium Hydroxide Lignin

Sodium hydroxide lignin was prepared by a modification of the method used by Bower (75). One kilogram of air dried, acid hydrolyzed, ground cornstalks was placed in a 30 l. battery jar and 15 l. of 4% aqueous alkali were added to it. The mixture was allowed to stand 15 hours with a weight keeping the stalk immersed. The insoluble pulp was removed from the alkaline liquor by filtering through cheesecloth in a stoneware filter and washed with 2 l. of water.

The black liquor was placed in a 30 l. battery jar and the lignin precipitated from the mechanically-stirred solution by an excess of concentrated hydrochloric acid. After settling the supernatant liquid was decanted to 5 l. The lignin precipitate was filtered with suction over Whatman No. 50 quantitative filter paper, washed free of acid with distilled water and air dried.

The lignin was dissolved in a dilute alkaline solution, 1 l. of 3% aqueous sodium hydroxide per 100 g. of lignin. The solution was heated to 60° C. and filtered to remove any residual cellulose. The filtered solution was acidified, filtered, washed free of acid and dried.

Action of Lactic Acid on Sodium Hydroxide Lignin.

Three grams of sodium hydroxide lignin were digested for 4 hours at 112° C. with 75 ml. of 85% lactic acid. The solution was cooled and filtered. The lignin was recovered by pouring the solution into distilled water.[1] The precipitated lignin was filtered, washed, dried in a vacuum desiccator over phosphoric anhydride and analyzed for methoxyl (70). A 1 g. sample of sodium hydroxide lignin was digested for 4 hours at 112° C. with 25 ml. of 74.5% lactic acid. The cooled solution was filtered, and the lignin was recovered by pouring the solution into 350 ml. of distilled water.[1]

Preparation of α-Methoxypropionic Acid

α- Methoxypropionic acid was prepared according to a modification of the method of Purdie and Irvine (76). A mixture of methyl lactate (3 mols) and methyl iodide (5 mols) was added gradually from a separatory funnel to silver oxide (4 mols) in a 3-necked 5 l. flask. The flask

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[1] The lignin solution from 3 g. of sodium hydroxide lignin was added to 1 l. of water; the solution from 1 g. of alkali lignin was added to 350 ml. of water.

was provided with a mechanical stirrer and a reflux condensor. The reaction was moderated by occasional cooling and completed by heating for 3 hours on a water bath. The product was diluted with dry ether, filtered and the ether and excess methyl iodide distilled off. The ester was distilled (132 - 136° C.), washed with cold 0.5% aqueous potassium hydroxide, washed free of alkali, dried with anhydrous calcium sulfate (Drierite), distilled under reduced pressure (44 - 46° C. at 20 - 22 mm.) and saponified by heating with 10% aqueous potassium hydroxide. On completion of the hydrolysis the liquid was neutralized with dilute sulfuric acid, concentrated to a syrup, acidified, extracted with ether and dried over anhydrous sodium sulfate. The ether was removed by distillation and the acid was purified by distillation under reduced pressure (96 - 98° C. at 11 - 12 mm.). A yield of 2.4 mols (80%) α-methoxypropionic acid was obtained.

Methyl lactate for use in the synthesis of α-methoxypropionic acid was prepared by the method proposed by Clemmensen and Heitmann (77). Six mols of lactic acid that had been dried by heating on a steam plate for 24 hours were dissolved in 10 mols of anhydrous methyl alcohol and boiled with 300 g. of anhydrous copper sulfate[1] for 24 hours.

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[1] The copper sulfate was dehydrated by heating in an oven at 110° C. for 24 hours and then heating at 150° C. for 4 hours.

The product was filtered, the copper sulfate was washed with anhydrous methyl alcohol and the excess methyl alcohol was removed from the filtrate by distillation. The methyl lactate was purified by distillation under reduced pressure (58 - 60° C. at 19 - 20 mm.). A yield of 3.5 mols (58.4%) was obtained.

The methyl iodide used in preparing α-methoxypropionic acid was made from potassium iodide and methyl sulfate (78). A 5 l. flask was provided with a mechanical stirrer, a separatory funnel, a thermometer and a fractionating column leading to a condensor set downward for distillation. A solution of potassium iodide (8 mols) in 800 ml. of water was placed in the flask, 120 g. of calcium carbonate were added and the mixture was heated to 60 - 65° C. with stirring. The temperature was maintained at 60 - 65° C. and methyl sulfate (8.4 mols) was added gradually from the separatory funnel at such a rate that methyl iodide distilled briskly. After the methyl sulfate was added the temperature was increased to 68 - 72° C. for an hour to complete the distillation of methyl iodide. The methyl iodide was separated from a small amount of water, dried with anhydrous calcium chloride and decanted into a dry distilling flask. A few crystals of solid potassium iodide were added and the material was distilled (40 - 41° C.). A yield of 7.3 mols (91.2%) was obtained.

Action of α-Methoxypropionic Acid on Cornstalks

A 5 g. sample of air dried, acid hydrolyzed ground cornstalks was digested for 4 hours at 112° C. with 60 ml. of 85% α-methoxypropionic acid. The reaction product was treated by the previously described procedure. A yield of 3.64 g. pulp analyzing 16.7% lignin was obtained.

Recovery of α-methoxypropionic acid lignin.

The lignin extracted from cornstalks by α-methoxypropionic acid was recovered by pouring the acid solution into water and following the procedure given previously.

Action of α-Methoxypropionic Acid on Sodium Hydroxide Lignin.

A sample of sodium hydroxide lignin weighing 1 g. was digested for 4 hours at 112° C. with 25 ml. of 85% α-methoxypropionic acid, cooled, filtered and precipitated by pouring into 350 ml. of distilled water. The recovered lignin weighed 0.75 g. and analyzed 17.0% methoxyl.

Action of Propionic Acid on Cornstalks

The general procedure previously described was used to investigate the action of propionic acid and aqueous solutions of propionic acid on 5 g. samples of cornstalks.

Recovery of propionic acid lignin.

The previously described procedure was used to recover the lignin in each of the filtrates obtained from the propionic acid extractions of cornstalks.

Action of Propionic Acid on Sodium Hydroxide Lignin.

One-gram samples of sodium hydroxide lignin were digested with 25 ml. portions of propionic acid for 4 hours at elevated temperatures. Both the anhydrous acid[1] and an aqueous solution of the acid were used. The dissolved lignin was recovered by the usual procedure.

Action of Acetic Acid on Cornstalks.

The action of acetic acid and its aqueous solutions on cornstalks was investigated through the use of the general procedure described above.

Recovery of acetic acid lignin.

The lignin extracted from cornstalks by acetic acid was recovered by pouring the acid liquor from each extraction into water, filtering and washing the precipitated lignin.

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1 Only part of the sodium hydroxide lignin was soluble in hot anhydrous propionic acid.

Action of Acetic Acid on Sodium Hydroxide Lignin

The previously described method was used to investigate the action of both glacial acetic acid[1] and a 66.4% aqueous solution of acetic acid on sodium hydroxide lignin.

Action of Formic Acid on Cornstalks

Studies of the action of formic acid and its aqueous solutions on cornstalks were made using the general method described above.

Recovery of formic acid lignin.

Formic acid lignin was recovered from each of the acid liquor filtrates from the formic acid extractions of cornstalk samples. The formic acid lignin was recovered by the method used to recover lactic acid lignin.

Action of Formic Acid on Sodium Hydroxide Lignin

The action of anhydrous formic acid and a 60.4% aqueous solution[2] of the acid on sodium hydroxide lignin was investigated. The previously described method was used in this investigation.

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1 A small amount of the sodium hydroxide lignin was insoluble in glacial acetic acid.

2 Sodium hydroxide lignin was almost completely insoluble in 60.4% formic acid.

Table 6.

Five Grams of Cornstalks Digested 4 Hours with Acetic Acid and Acetic Acid Solutions.

Strength of Acid, %	Temp., °C.	Weight of Pulp, g.	Lignin Removed, %
100	112	4.22	39.6
100[a]	112	3.44	74.5
100[b]	112	3.80	46.5
85	105	3.90	54.7
85[a]	105	3.04	89.8
66.4	103	3.97	41.7
66.4[a]	103	3.31	72.7

[a] Catalyzed by the addition of 1 ml. of concentrated hydrochloric acid.

[b] Catalyzed by the addition of 1 ml. of constant boiling hydroiodic acid.

Table 7.

Five Grams of Cornstalks Digested 4 Hours at 100°C. with Formic Acid and Formic Acid Solutions.

Strength of Acid, %	Weight of Pulp, g.	Lignin Removed, %
100	3.07	78.3
100[a]	2.58	79.6
85	3.20	79.0
85[a]	2.73	75.3
60.4	3.97	40.3
60.4[a]	3.83	37.4
5	-[b]	-

[a] Catalyzed by the addition of 1 ml. of concentrated hydrochloric acid.

[b] The pulp was not recovered. No lignin was recovered when the acid liquor was added to water.

Table 8.

Maple Wood Digested at 165° C. with Ethanol, Ethanol Solutions and Water.[a]

Strength of Ethanol, %	Time, Hours	Original Lignin Remaining in Pulp, %
100	1	89.8
100	4	83.7
100	10	74.7
100[b]	1	28.3
100[b]	4	17.1
100[b]	10	12.8
50	1	72.0
50	4	24.6
50	10	14.0
50[b]	1	4.4
50[b]	4	4.7
50[b]	10	26.4[c]
0[d]	1	76.5
0[d]	4	96.0[c]
0[d]	10	101.0[c]

a The data in this table were given by Hewson, McCarthy and Hibbert, J. Am. Chem. Soc., 63, 3045 (1941).

b Catalyzed by the addition of 0.1% HCl.

c The observed redeposition of lignin was assumed to be due to condensation-polymerization changes.

d These extractions were made using water containing no alcohol.

Table 9. Analysis of Acid Lignins

Acid	Temp., °C. of Extraction	Time, Hours of Extraction	CH_3O, %
85% Lactic	92	4	10.7
	92	8	11.6
	112	0.5	12.0
	112	1	12.0
	112	2	13.6
	112	4	13.5[a]
	112	4	12.3[b]
	112	4	13.3[c]
	112	4	15.0[c]
	112	4	12.0[c]
	112	8	12.4
	112	16	12.7
	112	32	12.3[d]
	112	1	12.4[e]
	122	4	12.3
	122	8	14.3
	126	4	12.0
	126	8	13.5
74.7% Lactic	112	4	12.6
85% α-Methoxy-propionic	112	4	12.3
100% Propionic	112	4	10.2
85% Propionic	112	4	8.4
72.4% Propionic	105	4	12.5
100% Acetic	112	4	10.2
	112	4	11.5[e]
66.4% Acetic	103	4	13.6
100% Formic	100	4	11.7
60.4% Formic	100	4	13.4

[a] This extraction was made using 5 g. of cornstalks and 60 ml. of 85% lactic acid.

[b] This extraction was made using 25 g. of cornstalk and 600 ml. of 85% lactic acid.

[c] This extraction was made on the pulp from the preceding extraction.

[d] The temperature dropped below 112° C. during the night.

[e] This extraction was catalyzed by the addition of 1 ml. of concentrated hydrochloric acid to the reaction mixture.

Table 10.

Sodium Hydroxide Lignin Digested 4 Hours with Organic Acids

Acid Used	Temp. °C.	Lignin Recovered, %	CH_3O, % in Recovered Lignin[a]
85% Lactic Acid	112	85	15.0
74.7% Lactic Acid	112	91	15.1
85% α-Methoxy-propionic Acid	112	93	17.0
100% Propionic Acid	112	28[b]	16.1
72.4% Propionic Acid	105	84	15.2
100% Acetic Acid	112	74[c]	17.2
66.4% Acetic Acid	103	84	16.0
100% Formic Acid	100	78	16.2
60.4% Formic Acid	100	-[d]	-

a Untreated sodium hydroxide lignin analyzes 15.4% methoxyl.
b Only part of the sodium hydroxide lignin was soluble in hot anhydrous propionic acid.
c A small amount of the sodium hydroxide lignin was insoluble in hot glacial acetic acid.
d Sodium hydroxide lignin was almost completely insoluble in hot 60.4% formic acid.

Alkali Saponification of Lignins

A number of acid lignins, sodium hydroxide lignin treated with the acids and untreated sodium hydroxide lignin were subjected to saponification with caustic alkali. Samples of lignin weighing 150 mg. were dissolved in 20 ml. of 10% sodium hydroxide solution, heated to boiling, placed on a

steam plate and kept above 90° C. for 2 hours. At the end of this time the samples were removed from the steam plate, cooled, filtered and diluted to 200 ml. The lignin was precipitated by the addition of a slight excess of hydrochloric acid; the precipitated lignin was filtered, washed until the filtrate was free of chloride, dried, weighed and analyzed for methoxyl.

Acid Hydrolysis of Lignins

Several samples of lactic acid lignin, sodium hydroxide lignin treated with lactic acid and untreated sodium hydroxide lignin were hydrolyzed with dilute hydrochloric acid (1 volume of concentrated acid to 4 volumes of water). Lignin samples weighing 250 mg. were covered with 25 ml. of dilute hydrochloric acid, heated to boiling and kept above 90° C. on a steam plate for 2 hours. The samples were then cooled, filtered and washed until the filtrate was free of chloride. After drying the acid hydrolyzed lignins were analyzed for methoxyl (70).

Methylation of Lignins

One gram of lactic acid lignin analyzing 12.3% methoxyl was methylated with diazomethane by a modification of the method used by Morgal (79). The lignin sample was

Table 11.

Alkali Saponification of Lignins

Type of Lignin	CH_3O, % Before Saponification	Lignin Recovered, % After Saponification	CH_3O, % After Saponification
Lactic Acid Lignin, Prepared by Boiling Stalks with 85% Lactic Acid for 8 hours.	13.5	78	15.7
Lactic Acid Lignin, Prepared by Digesting Stalks with 85% Lactic Acid for 4 Hours at 112° C.	12.3	79	14.7
Lactic Acid Lignin, Prepared by Digesting Stalks with 74.7% Lactic Acid for 4 Hours at 112° C.	12.6	73	14.9
Lactic Acid Lignin, Prepared by Digesting Stalks with 85% Lactic Acid (Catalyzed by 1 ml. Concentrated Hydrochloric Acid) for 1 Hour at 112° C.	12.4	77	12.3
Sodium Hydroxide Lignin	15.4	83	16.7
Sodium Hydroxide Lignin Digested 4 Hours at 112° C. with 85% Lactic Acid	15.0	82	16.4
Sodium Hydroxide Lignin Digested 4 Hours at 112° C. with 74.7% Lactic Acid	15.1	74	16.1

Table 11. (continued)

Type of Lignin	CH_3O, % Before Saponification	Lignin Recovered, % After Saponification	CH_3O, % After Saponification
α-Methoxypropionic Acid Lignin	12.3	70	14.3
Sodium Hydroxide Lignin Digested with α-Methoxypropionic Acid	17.0	78	15.5
Propionic Acid Lignin, Prepared by Digesting Stalks 4 Hours at 112° C. with 100% Propionic Acid	10.2	76	14.5
Propionic Acid Lignin, Prepared by Digesting Stalks 4 Hours at 105° C. with 72.4% Propionic Acid.	12.5	71	12.5
Sodium Hydroxide Lignin, Digested 4 Hours at 112° C. with 100% Propionic Acid	16.1	75	16.3
Sodium Hydroxide Lignin, Digested 4 Hours at 105° C. with 72.4% Propionic Acid	15.2	74	16.1
Acetic Acid Lignin, Prepared by Digesting Stalks 4 Hours at 112° C. with 100% Acetic Acid	10.2	76	15.4
Acetic Acid Lignin, Prepared by Digesting Stalks 4 Hours at 103° C. with 66.4% Acetic Acid	13.6	72	14.3
Sodium Hydroxide Lignin Treated 4 Hours at 112° C. with 100% Acetic Acid	17.2	77	15.8
Sodium Hydroxide Lignin Treated 4 Hours at 103° C. with 66.4% Acetic Acid	16.0	71	14.7

Table 11. (continued)

Type of Lignin	CH_3O, % Before Saponification	Lignin Recovered, % After Saponification	CH_3O, % After Saponification
Formic Acid Lignin, Prepared by Treating Stalks 4 Hours at 100° C. with 100% Formic Acid	11.7	74	14.4
Formic Acid Lignin, Prepared by Treating Stalks 4 Hours at 100° C. with 80.4% Formic Acid	13.4	73	14.9
Sodium Hydroxide Lignin, Digested 4 Hours at 100° C. with 100% Formic Acid	16.2	83	15.9

passed through a 100 mesh sieve, dried in a vacuum desiccator over phosphoric anhydride, placed in a 150 ml. Erlenmeyer flask and an ether solution of diazomethane prepared from 2 ml. of nitrosomethylurethane (80) poured over it and allowed to stand overnight. The ether was filtered off and the lignin was washed with low boiling petroleum ether (Skelly A). The lignin was then subjected to another methylation similar to the above but using only 1 ml. of nitrosomethylurethane to generate the diazomethane. The twice methylated lactic acid lignin was methylated a third time, dried in vacuum and analyzed for methoxyl (70).

A 1 g. sample of sodium hydroxide lignin was subjected to three methylations using the procedure given above. The dried methylated lignin was analyzed for methoxyl (70).

A 1 g. sample of the sodium hydroxide lignin that had been digested with 85% lactic acid for 4 hours at 112° C. was methylated three times with diazomethane by the method described above. The methylated lignin was dried and analyzed for methoxyl (70).

Hydrolysis of the Methylated Lignins

The diazomethane methylated lignins were hydrolyzed by a modification of the method of Ahlm and Brauns (69). The methylated lignins were dissolved in 15 ml. of dioxane, 0.5 g. of sodium carbonate in 5 ml. of water was added (this caused part of the lignin to precipitate).

The dioxane solution was heated on a water bath at 75° C. for an hour; the precipitated lignin gradually dissolved with the evolution of carbon dioxide. Another 0.5 g. of sodium carbonate in 10 ml. of water was added and part of the dioxane was removed with reduced pressure. The solution was heated at 75° C. for another hour. The dioxane was removed by heating *in vacuo*; the lignin was kept in solution by constantly adding water. The dioxane free solution was filtered and heated at 75° C. for another hour. After cooling the lignin was precipitated by the addition of a

Table 12.

Acid Hydrolysis of Lignins

Type of Lignin	CH_3O, % Before Hydrolysis	Lignin Recovered, % After Hydrolysis	CH_3O, % After Hydrolysis
Lactic Acid Lignin, Prepared by Boiling Stalks 8 Hours with 85% Lactic Acid	13.5	91	13.6
Lactic Acid Lignin, Prepared by Digesting Stalks 4 Hours at 112° C. with 85% Lactic Acid	12.3	93	13.1
Lactic Acid Lignin, Prepared by Digesting Stalks 1 Hour at 112° C. with 85% Lactic Acid (Catalyzed by the Addition of 1 ml. Concentrated Hydrochloric Acid)	12.4	82	13.1
Sodium Hydroxide Lignin	15.4	88	16.6
Sodium Hydroxide Lignin Digested 4 Hours at 112° C. with 85% Lactic Acid	15.0	89	16.0

Table 13.

Methylation and Saponification of Lignins

Type of Lignin	CH_3O, % Before Methylation	CH_3O, % After Methylation	Lignin Recovered, % After Saponification	CH_3O, % After Saponification of Methylated Lignin
Lactic Acid Lignin, Prepared by Treating Stalks with 85% Lactic Acid at 112° C. for 4 Hours	12.3	22.4	80	17.5
Sodium Hydroxide Lignin, Treated 4 Hours at 112° C. with 85% Lactic Acid	15.0	23.6	85	18.1
Sodium Hydroxide Lignin	15.4	24.8	83	23.5

slight excess of hydrochloric acid. The precipitated lignin was filtered, washed until the filtrate was free of chloride and air dried. The dried hydrolyzed lignin was dissolved in 10 ml. of dioxane and precipitated by pouring into 500 ml. of dry ether. The precipitated lignin was filtered, washed with low boiling petroleum ether (Skelly A), dried in vacuum over phosphoric anhydride and analyzed for methoxyl (70).

Color Test

None of the lignins prepared gave a color change when treated with Wiesner's reagent (phloroglucinol-HCl).

DISCUSSION OF RESULTS

The data plotted in Fig. 1 indicate that during the extraction of lignin from cornstalks with a large excess of lactic acid two major changes occur, namely a rapid initial process and a slow time-determining first order reaction. This is almost identical to the mechanism proposed by Hibbert and co-workers (81) to explain the reactions involved in the chlorination of lignin. It is observed that the straight lines obtained by plotting the logarithm of the lignin extracted against time (for a series of extractions at varying temperatures) are nearly parallel. This indicates that the temperature coefficient for the slow part of the reaction is negligible; accordingly nearly all temperature effect is evidenced in the rapid initial reaction. From these data it is not possible to determine whether lactic acid extracts lignin by organic solvent action or by reacting chemically with the lignin in plant tissues.

Data presented in Fig. 2 show the effect of the presence of hydrochloric acid on the extraction of lignin by a series of organic acids. These data also show that 85% aqueous solutions of formic, acetic and propionic acids extract more lignin than do the anhydrous acids. The fact

that aqueous solutions of formic acid containing hydrochloric acid remove slightly less lignin than do hydrogen chloride free aqueous solutions of formic acid may be due to condensation-polymerization changes such as those shown in Table 8. The fact that hydrochloric acid has a slightly positive catalytic effect on the extraction of lignin from cornstalks by anhydrous formic acid tends to confirm this opinion.

The data of Hibbert and co-workers (9) presented in Table 8 show that effects similar to those discussed above are observed when plant tissue is extracted with alcohol. These workers attributed the catalytic effects produced by water and hydrogen chloride to a hydrolytic influence of the hydrogen ions present in the alcoholic reagent. They (9) found that acidic substances are formed when maple wood is extracted with pure ethanol. The organic acids formed probably exert a hydrolytic influence similar to but less intense than that of hydrogen chloride. Confirmation of this view is found in the fact that formic and acetic acids catalyze the pulping of plant tissue by alcohols (82).

It seems possible that a hydrolytic effect may play an important role in the extraction of lignin by both alcohols and organic acids.

Methoxyl analyses of lignin extracted from cornstalks by lactic acid indicate that fractionation takes place during the extraction. The fractions first removed have a

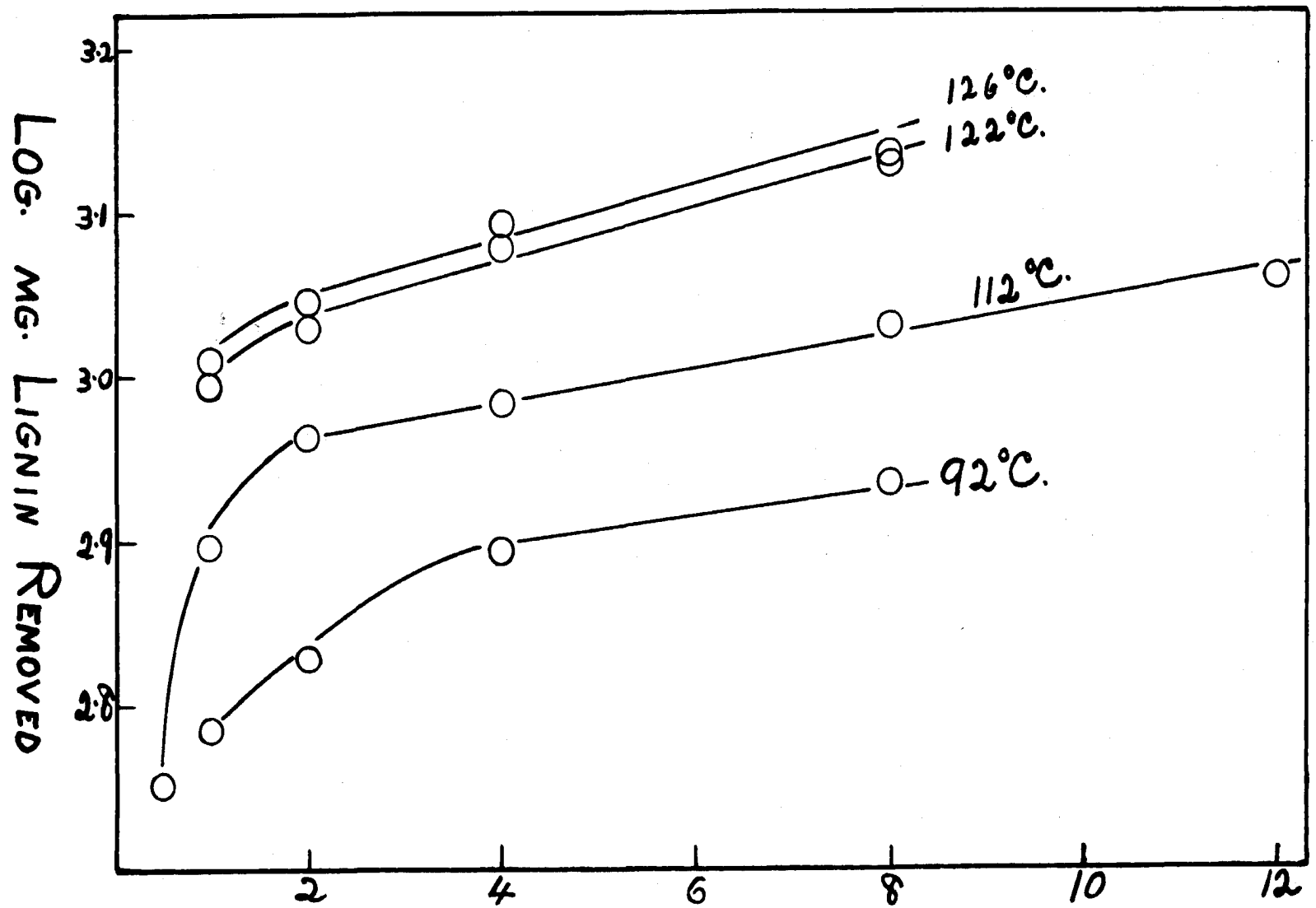

FIG. 1 LIGNIN EXTRACTED WITH LACTIC ACID.

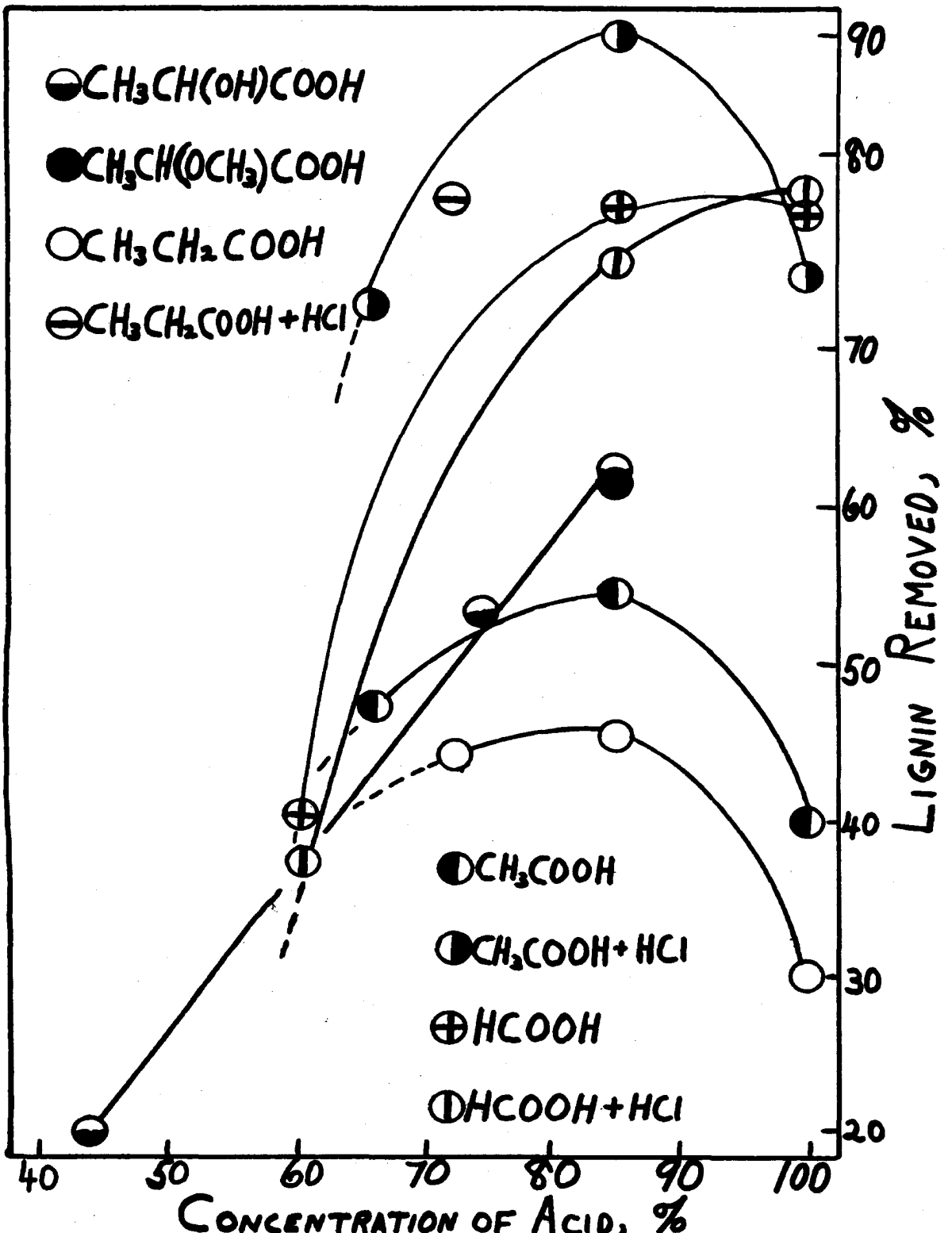

FIG. 2 LIGNIN EXTRACTED WITH ACIDS

lower methoxyl content than do the intermediate fractions. The intermediate fractions contain more methoxyl groups than the final fractions.

It was observed that lignin extracted with 85% propionic acid has a lower methoxyl content than lignin extracted with the anhydrous acid. It was also found that lignin extracted with anhydrous propionic acid contains less methoxyl than does lignin extracted with 72.4% propionic acid. This is partly due to fractionation and also to esterification of lignin extracted with the anhydrous acid.

Alkali saponification studies indicate that anhydrous propionic, acetic and formic acids form esters with the lignins that they extract. Apparently esterification plays no important role in the extraction of lignin by these acids because maximum extractions of lignin are obtained under conditions that do not favor esterification. Alkali saponification studies confirm the opinion that little esterification takes place when lignin is extracted with aqueous solutions of lactic, α-methoxypropionic, propionic, acetic and formic acids.

In most cases the action of the organic acids on sodium hydroxide lignin is not clearly revealed by the data available. In all the reactions investigated there is evidence of fractionation. The increase in methoxyl content of sodium hydroxide lignin when it is treated with α-methoxypropionic acid is probably due to esterification of the lignin.

The fact that the methoxyl content of the treated lignin is decreased by alkali saponification tends to confirm this conclusion, but the possibility that the decrease in methoxyl content is due to fractionation rather than to hydrolysis is not eliminated. The action of most of the acids on isolated lignin is not the same as their action on the natural lignin of plant tissues. Acid hydrolysis of lactic acid lignins, sodium hydroxide lignin and sodium hydroxide lignin treated with lactic acid indicates that the lactic acid does not form an acetal with lignin.

Figure 3 summarizes the results obtained by the diazomethane methylation of lactic acid lignin, sodium hydroxide lignin treated with lactic acid and sodium hydroxide lignin. The fact that sodium carbonate saponification of the methylated lignins decreased the methoxyl content of lactic acid lignin and sodium hydroxide lignin treated with lactic acid several percent more than it decreased the methoxyl content of sodium hydroxide lignin indicates that lactic acid adds free carboxyl groups to lignin. An indication that lactic acid reacts with sodium hydroxide lignin through phenolic groups of the lignin is found in the fact that methylated sodium hydroxide lignin contains a larger percentage of methoxyl than does methylated sodium hydroxide lignin that has been digested with lactic acid before methylation. From these data it appears that lactic acid may combine with both isolated and natural lignin according to

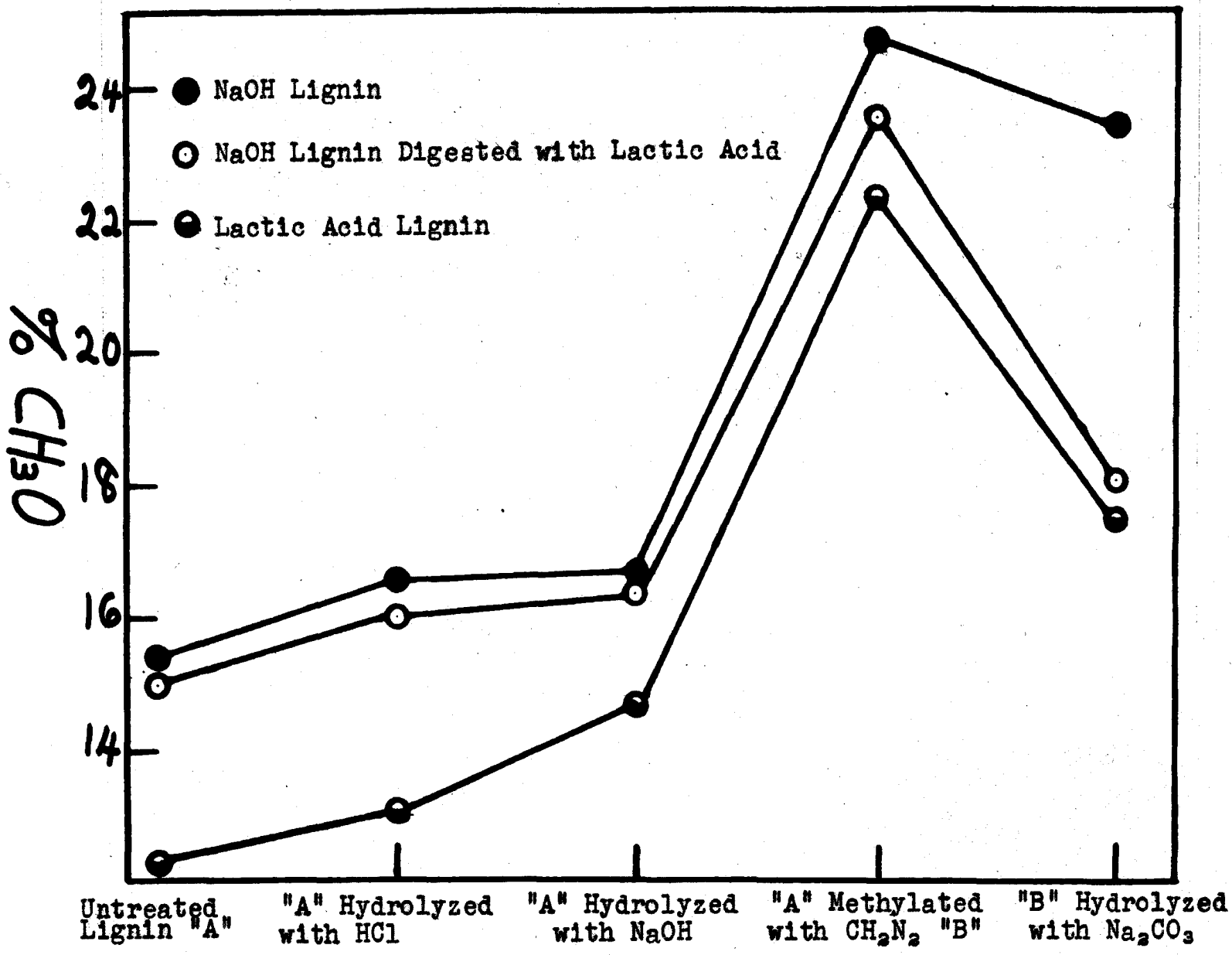

Fig.3. Effect of Various Treatments on the Methoxyl Content of Lignin.

the mechanism proposed by Holmberg (43) to explain the action between alcohols and lignins. The following reaction illustrates the mechanism involved:

$$\text{LiOR} + \text{HO}\underset{\text{CH}_3}{\overset{\text{COOH}}{\text{CH}}} = \text{LiO}\underset{\text{CH}_3}{\overset{\text{COOH}}{\text{CH}}} + \text{ROH}$$

In the case of natural lignin it is undecided whether R consists of a hydrogen, a carbohydrate group, another lignin group or even another part of the same lignin molecule. In isolated lignin R is probably the hydrogen of a phenolic hydroxyl.

The data do not indicate whether the combination of lactic acid with lignin plays an important role in the isolation of lignin or whether this combination is merely a side reaction of little or no importance in the extraction of lignin from plant tissues. Furthermore the evidence does not show whether the combination takes place before the lignin is extracted or whether it occurs after the lignin is in solution.

Ahlm and Brauns (69) reported that Wiesner's reagent gives a positive test with isolated spruce lignin. Since the test is no longer positive after lignin is methylated with diazomethane they attributed the test to the presence of a hydroxyl group. The absence of a positive Wiesner's test in all samples of acid and alkali lignin prepared from cornstalks indicates that the group responsible for this test is not present in isolated cornstalk lignin.

SUMMARY AND CONCLUSIONS

1. When lignin is extracted from cornstalks by a large excess of lactic acid two major changes occur, namely a rapid initial process and a slow time-determining first order reaction. The temperature coefficient of the slow part of the reaction is negligible.
2. A hydrolytic effect appears to play an important role in the extraction of lignin from plant tissue by both alcohols and organic acids.
3. Evidence indicating that aqueous solutions of formic acid containing hydrochloric acid may cause lignin in plant tissue to undergo condensation-polymerization reactions is presented.
4. Fractionation takes place when lignin is extracted from plant tissue by organic acids.
5. Anhydrous organic acids form esters with the lignin that they extract. Esterification plays no important role in the extraction of lignin from plant tissue by aqueous solutions of organic acids.
6. In most cases the action of organic acids on isolated (caustic alkali) lignin is not the same as their action on natural lignin.
7. Lactic acid adds free carboxyl groups to both natural

and isolated lignin. It is possible that this acid combines with lignin according to the mechanism Holmberg (43) proposed to explain the action between alcohols and lignin.

8. Isolated cornstalk lignin does not give a color change when treated with Wiesner's reagent.

LITERATURE CITED

(1) Hawley and Harris, Ind. Eng. Chem., 24, 873 (1932).

(2) Lowry, Ind. Eng. Chem., 26, 133 (1934).

(3) Phillips, Chem. Rev., 14, 103 (1934).

(4) Norman, "The Biochemistry of Cellulose, the Polyuronides, Lignin &c.," Oxford University Press, London, England, 1937, p. 151.

(5) Freudenberg, Ann. Rev. Biochem., 8, 81 (1939).

(6) Hibbert, Paper Trade J., 113, No. 4, 35 (1941).

(7) Klason, Tek. Tid. Kemi Metallurgi, 23, 11 (1893). Original not seen; cited in Freudenberg, Ann. Rev. Biochem., 8, 103 (1939).

(8) Hewson, McCarthy and Hibbert, J. Am. Chem. Soc., 63, 3041 (1941).

(9) Hewson, McCarthy and Hibbert, J. Am. Chem. Soc., 63, 3045 (1941).

(10) Hibbert, Pulp Paper Mag. Can., International No., 125 (1928). Original not seen; abstracted in Chemical Abstracts, 22, 4802 (1928).

(11) Hibbert and Rowley, Can. J. Research, 2, 357 (1930).

(12) Hibbert and Marion, Can. J. Research, 2, 364 (1930).

(13) Gray, King, Brauns and Hibbert, Can. J. Research, 13B, 35 (1935).

(14) Fuchs, Ber. 62, 2125 (1929).

(15) Fuchs and Daur, Cellulosechem., 12, 103 (1931).

(16) Fuchs, J. Am. Chem. Soc., 58, 673 (1936).

(17) Aronovsky and Gortner, Ind. Eng. Chem., 28, 1270 (1936).

(18) Hibbert and Phillips, Can. J. Research, 3, 65 (1930).

(19) Hägglund and Urban, Cellulosechem., 8, 68 (1927).

(20) Hägglund and Urban, Cellulosechem., 9, 49 (1928).

(21) McMillen, Gortner, Schmitz and Bailey, Ind. Eng. Chem., 30, 1407 (1938).

(22) Bailey, Paper Trade J., 110, No. 1, 29 (1940).

(23) Bailey, Paper Trade J., 110, No. 2, 29 (1940).

(24) Bailey, Paper Trade J., 111, No. 6, 27 (1940).

(25) Bailey, Paper Trade J., 111, No. 7, 27 (1940).

(26) Bailey, Paper Trade J., 111, No. 9, 86 (1940).

(27) Bailey, J. Am. Chem. Soc., 64, 22 (1942).

(28) Charbonnier, Paper Trade J., 114, No. 11, 31 (1942).

(29) Grüss, Ber. deut. bot. Ges., 38, 361 (1920).

(30) Grüss, Ber. deut. bot. Ges., 41, 48 (1923).

(31) Holmberg and Runius, Svensk Kem. Tids, 37, 189 (1925). Original not seen; abstracted in Chemical Abstracts, 20, 774 (1926).

(32) Friedrich and Diwald, Monatsh., 46, 31 (1925).

(33) Müller, U. S. Patent, 1,816,394 (1931). Original not seen; abstracted in Chemical Abstracts, 25, 5561 (1931).

(34) Goombridge and Mellers, U. S. Patent, 2,022,664 (1935). Original not seen; abstracted in Chemical Abstracts, 30, 862 (1936).

(35) Brauns, J. Am. Chem. Soc., 61, 2120 (1939).

(36) Brauns, Paper Trade J., 108, No. 1, 42 (1939).

(37) Brauns and Hibbert, Can. J. Research, 13B, 28 (1935).

(38) King and Hibbert, Can. J. Research, 14B, 12 (1936).

(39) Mackinney and Hibbert, Can. J. Research, 14B, 55 (1936).

(40) Compton, Greig and Hibbert, Can. J. Research, 14B, 115 (1936).

(41) Friedman and McCully, Paper Trade J., 107, No. 26, 28 (1938).

(42) Friedrich, Z. Physiol. Chem., 168, 50 (1927).

(43) Holmberg, Papier-Fabr. 36, Tech-Wiss. Tl. 218 (1938).

(44) Gibbs, Can. J. Research, 12, 715 (1935).

(45) Reid, Nelson and Aronovsky, Ind. Eng. Chem., Anal. Ed. 12, 255 (1940).

(46) Reid, Dryden and Aronovsky, Paper Trade J., 113, No. 7, 27 (1941).

(47) Fisher and Bower, J. Am. Chem. Soc. 63, 1881 (1941).

(48) Engel and Wedekind, German Patent, 581,806 (1933). Original not seen; abstracted in Chemical Abstracts, 28, 1186 (1934).

(49) Dreyfus, U. S. Patent, 2,022,654 (1935). Original not seen; abstracted in Chemical Abstracts, 30, 861 (1936).

(50) Wedekind, Ber., 68, 2363 (1935).

(51) Hilpert and Wisselinck, Ber. 69, 680 (1936).

(52) Nikitin and Orlova, Ber., 69, 2434 (1936).

(53) Nikitin, Avidon and Orlova, Ber., 69, 2439 (1936).

(54) Brauns, Private Communication to Charbonnier. Original not seen; cited by Charbonnier, Paper Trade J., 114, No. 11, 33 (1942).

(55) Pauly, German Patent, 309,551 (1916). Original not seen; cited by Schwalbe, "Die Chemie der Hölzer", Gebrüder Borntraeger, Berlin, Germany, 1938, p. 176.

(56) Friedrich, Z. Physiol. Chem., 176, 127 (1928).

(57) Spencer and Wright, J. Am. Chem. Soc., 63, 2017 (1941).

(58) Freudenberg, Janson, Knopf and Haag, Ber., 69, 1415 (1936).

(59) Staudinger and Dreher, Ber., 69, 1729 (1936).

(60) Sarkar, Science and Culture, 2, 411 (1937). Original not seen; abstracted in Chemical Abstracts, 31, 3689 (1937).

(61) Wright and Hibbert, J. Am. Chem. Soc., 59, 125 (1937).

(62) Bell, Cramer, Wright and Hibbert, Ber., 71, 746 (1938).

(63) Lieff, Wright and Hibbert, J. Am. Chem. Soc., 61, 1477 (1939).

(64) Hillmer, Cellulosechem., 6, 169 (1925).

(65) Nikitin and Rudneva, J. Applied Chem. (U.S.S.R.) 10, 1915 (1937).

(66) Schütz, Cellulosechem. 18, 76 (1940).

(67) Holmberg, Svensk Pappers-Tid., 33, 679 (1930). Original not seen; abstracted in Chemical Abstracts, 24, 6007 (1930).

(68) Berg and Holmberg, Svensk Kem. Tids., 47, 257 (1935). Original not seen; abstracted in Chemical Abstracts 30, 2370 (1936).

(69) Ahlm and Braune, J. Am. Chem. Soc., 61, 277 (1939).

(70) Johns, "Laboratory Manual of Microchemistry", Burgess Publishing Co., Minneapolis, Minn., 1941, p. 42.

(71) Walde and Hixon, J. Am. Chem. Soc., 56, 2656 (1934).

(72) Hickinbottom, "Reactions of Organic Compounds", Longmans, Green and Co., London, England, 1936, p. 75.

(73) Morgal, "Fractionation of Oxidized Cornstalk Lignin", Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library, 1937, p. 19.

(74) Peterson, Walde and Hixon, Ind. Eng. Chem., Anal. Ed., 4, 216 (1932).

(75) Bower, "The Oxidation of Methylated Lignin", Unpublished M. S. Thesis, Ames, Iowa, Iowa State College Library, 1941, p. 12.

(76) Purdie and Irvine, J. Chem. Soc. 75, 483 (1899).

(77) Clemmensen and Heitmann, Amer. Chem. J., 42, 319 (1909).

(78) Hartman in Fuson, "Organic Syntheses", John Wiley & Sons, Inc., New York, N. Y., 1938, Vol. 18, p. 88.

(79) Morgal, "Fractionation of Oxidized Cornstalk Lignin", Unpublished Ph. D. Thesis, Ames, Iowa, Iowa State College Library, 1937, p. 28.

(80) Hickinbottom, "Reactions of Organic Compounds", Longmans, Green and Co., London, England, 1936, p. 351.

(81) White, Swartz, Peniston, Schwartz, McCarthy and Hibbert, Paper Trade J. 113, No. 24, 33 (1941).

(82) Erbring and Geinitz, Kolloid-Z., 84, 215 (1938).