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A Study of the Low-Molecular Weight Phenols Formed on the Hydrolysis of Aspenwood

Donald Albert Stanek

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A STUDY OF THE LOW-MOLECULAR WEIGHT PHENOLS FORMED ON THE HYDROLYSIS OF ASPENWOOD

A thesis submitted by

Donald Albert Stanek

B.S. 1953, Marquette University
M.S. 1955, Lawrence College

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INTRODUCTION

Heating wood with water under pressure has served as the first stage in commercial prehydrolysis-sulfate pulping for many years. In several instances investigators have noted that these aqueous hydrolysis liquors contained low-molecular weight aromatic materials as well as carbohydrate degradation products. No extended effort has yet been made to isolate and identify these aromatic units. The advent of chromatography techniques in the separation of complex mixtures of organic compounds was expected to aid materially in this work.

The primary goal of this investigation was the isolation and identification of the low molecular weight aromatic degradation products formed on the hydrolysis[1] of extracted aspen (Populus tremuloides) wood. The ultimate aim was an achievement of a greater understanding of the lignin complex as it occurs in wood.

[1] Hydrolysis is taken to mean the action of water at 170°C. unless otherwise stated.

HISTORICAL REVIEW

At the close of the last century, Klason (1) suggested that softwood lignin was in some way related to phenyl-propane units of the coniferyl type. He based this hypothesis upon the similarity between the reactions of the protolignin and coniferyl alcohol and its acid condensation products. Over the years, this idea has been substantiated by the work of Freudenberg (2), Harris (3), Hibbert (4), Phillips (40) and many other workers in the field. Recently, Goldschmid (5) analyzed the hydrolysis liquors obtained from western hemlock (Tsuga heterophylla) wood by means of paper chromatography and noted that these liquors contained several phenyl-propane derivatives. One of these materials he identified tentatively as coniferyl aldehyde. Earlier, Sohn and Lenel (6) studied the ether extract of pinewood hydrolysis liquors, and they found indications that coniferyl aldehyde was present. In no case, however, did Goldschmid or Sohn and Lenel unequivocally prove the identity of this aldehyde, since none of them isolated any crystalline products.

The work of Sohn and Lenel (6) and of Goldschmid (5) indicated that the noncarbohydrate portion of wood had been degraded through hydrolysis. This was supported by the work of several other investigators (7-10) who had noted that there was a decrease in the Klason lignin content of wood which had been hydrolyzed.

After aspenwood (Populus tremuloides) had been treated with water at elevated temperatures, Aronovsky and Gortner (7) noted that the lignin had been attacked or "depolymerized" since it was more readily extractable

with organic solvents or alkali. They also noted that a portion of the lignin became water soluble as a result of this hydrolytic attack. McGovern, Brown and Kraske (8) noted this latter effect when they studied the cooking of aspen chips (Populus tremuloides) with water and steam. Traynard and Eymery (9) found that 6.5% of the lignin of poplarwood was dissolved through the action of water at 140°C. for eight hours. As the pH of the hydrolyzing media was decreased, more lignin and wood were dissolved. Pepper and Haggermann (10) noted that when extracted aspenwood meal (Populus tremuloides) was subjected to the action of water at 180°C. for five hours, 27% of the wood dissolved. By extracting the resulting liquor with chloroform they were able to recover 3.9% of the original wood. The authors believed that this chloroform-soluble material had been cleaved from the lignin.

Pearl, Beyer, Johnson and Wilkinson (11) heated unextracted aspenwood (Populus tremuloides) under reflux with normal sodium hydroxide. Using paper chromatography, they were able to detect seven phenolic materials in the hydrolysis liquor: p-hydroxybenzaldehyde, vanillin, syringaldehyde, p-hydroxybenzoic acid, vanillic acid, syringic acid, and p-coumaric acid. Smith (12) treated the "isolated native lignin" of aspen (Populus tremula) with normal sodium hydroxide and obtained p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid. The last three compounds were identified by their R_f values on paper chromatograms. Smith (12) also found p-hydroxybenzoic acid, when thoroughly extracted aspenwood (Populus tremula) was treated with normal sodium hydroxide.

Aaltio and Roschier (13) extracted aspenwood (Populus tremula) repeatedly with neutral buffered water and butanol at 158°C. This caused the gradual solubilization of the wood, and after nine consecutive cooks, 62.5% of the wood and 92.5% of the Klason lignin had dissolved. The authors believed that the hydrolytic action of the water cleaved the bonds between lignin and carbohydrates, the former dissolving in the butanol, and the latter in the water, and that the presence of the buffer prevented the condensation of the lignin.

Richter (14, 15) studied the influence of hydrolysis pulping on the yield and Klason lignin content of a number of softwoods and hardwoods. He noted that little, if any, lignin was removed from the softwoods whereas up to 30% was removed from the hardwood. Kratzl and Silbernagel (16) treated beechwood and its acid-derived lignin products with water at 100-200°C. for various periods up to twenty hours. The woody residues from these treatments were then oxidized with alkaline nitrobenzene, and the yields of vanillin and syringaldehyde were determined. The yield of these aromatic aldehydes decreased as the temperature of hydrolysis was increased indicating that condensation and/or solution of part of the lignin had taken place.

McKenzie, McPherson and Stewart (17) subjected the Klason lignin from Eucalyptus regnans wood to successive treatments according to the Klason lignin procedure. A loss of material occurred after each successive treatment, and, after three such treatments, only 77% of the original material remained.

Goldschmid (5) studied the ether-soluble hydrolysis products formed on the treatment of alcohol-benzene extracted western hemlock wood (Tsuga heterophylla) with water at 175°C. Through the use of paper chromatography, color reactions and ultraviolet absorption data, he was able to detect five aromatic compounds: coniferyl aldehyde, coumaraldehyde, vanilloyl methyl ketone, guaiacyl acetone and vanillin. Repeated hydrolysis of the ether-extracted hydrolyzed wood yielded additional amounts of the same aromatic compounds.

Purves and Cabott (18) studied the effects of temperature and pH of the hydrolysis medium on the solution of isolated spruce periodate lignin. The percentage of lignin dissolved versus pH of the hydrolyzing medium for three temperatures is given in Figure 1. These authors hypothesized that the lignin was attacked and dissolved through the scission of aliphatic-aromatic ether bonds. Corey, Calhoun and Maas (19) believed that the heating of wood in aqueous solutions at elevated temperatures resulted in a physical agglomeration of the lignin present and, that therefore, there was a decrease in the surface area available for reaction.

Pepper and his co-workers (20) studied the degradation products formed on the alkaline hydrogenation of aspenwood (Populus tremuloides) and were able to isolate and identify three compounds: 4-hydroxy-3, 5-dimethoxyphenylethane, 4-hydroxy-3-methoxyphenylethane and 4-hydroxy-3-methoxyphenylethanol. The fact that these three products were of the phenylethane type and the fact that phenyl-propane derivatives were

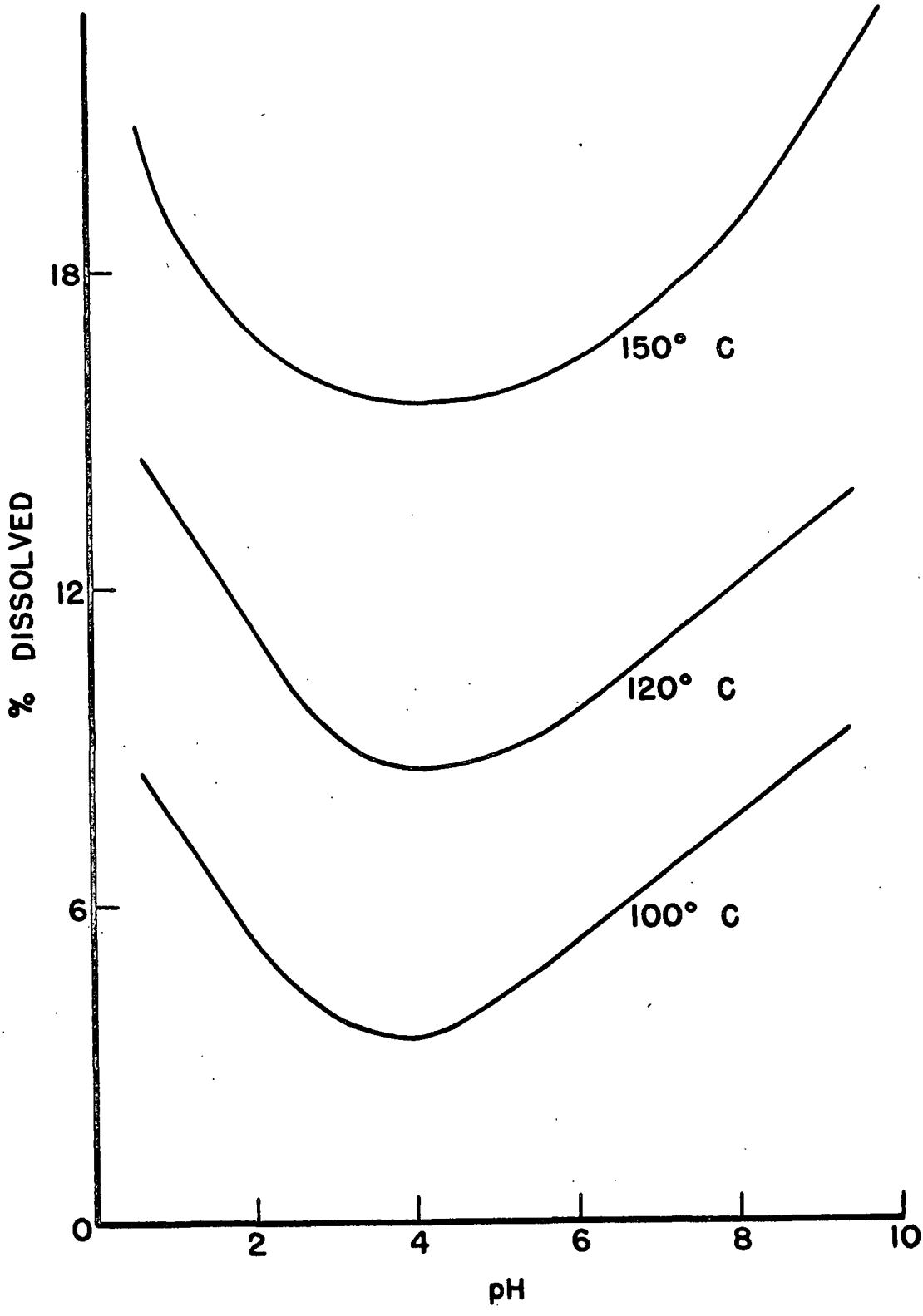


Figure 1. Solubility of Periodic Acid Spruce Lignin When Heated for 4 Hours at the Temperatures and pH Indicated.

isolated from a similar hydrogenation of maplewood under acid conditions suggested to the authors a β-γ linkage in the protolignin of aspen which was susceptible to cleavage under alkaline but not acidic conditions. As indicated previously by Pepper and Hibbert (21), the isolation of 4-hydroxy-3, 5-dimethoxyphenylethanol as a lignin degradation product provides some evidence for a carbon-oxygen linkage through the β-carbon atom of the alkyl side chain. The authors hypothesized that the oxygen functions as part of an enolic system which, under alkaline conditions, was stabilized in a form favorable to cleavage of the double bond.

Kavanagh and Pepper (22) determined the conditions of time and temperature necessary to obtain the maximum yields of vanillin and syringaldehyde from aspenwood (Populus tremuloides) by alkaline nitrobenzene oxidation. The maximum yield of each aldehyde was obtained at a temperature of 170-180°C., suggesting that these aldehydes were being freed from the same type of linkages in the wood. Oxidations carried out under similar conditions on a glycoside and the methyl ethers of syringaldehyde and vanillin indicated that the phenolic alkyl ether linkages were unlikely in aspen protolignin, but that glycosidic-type bonds were possible.

SUMMARY OF THE LITERATURE

When wood is subjected to the action of water at 160-180°C. both the carbohydrate and noncarbohydrate portion or lignin is attacked and degraded. A part of the lignin is rendered water soluble, and that portion remaining in the wood can be extracted more readily then normally

with organic solvents or alkali. In general, it appears that the lignin of hardwoods is attacked and degraded more readily than is the lignin of softwoods. There is some evidence to indicate that coniferyl aldehyde and several other phenyl-propane derivatives are freed through the hydrolytic degradation of a softwood.

PRESENTATION OF THE PROBLEM

The immediate objective of this research was the isolation and unequivocal identification of the monomeric phenolic materials freed by the hydrolysis of aspenwood (Populus tremuloides) that had been pre-extracted with n-propanol and water. The various techniques of chromatography were used in separating the complex mixtures of lignin degradation products present in these hydrolysis liquors. In its broader sense, this study was to give an insight into the chemical structure of aspen lignin.

EXPERIMENTAL SECTION

WOOD PREPARATION

Four bolts were taken from an aspen tree (Populus tremuloides) cut near Keshena, Wisconsin. The tree was thirty-five years old and had a growth rate of 4.0 rings per inch. The logs were hand-peeled and then converted into sawdust by means of a circular saw. The moisture content of the sawdust was 53.7%.

n-PROPANOL EXTRACTION

Twenty and a half pounds (ovendry basis) of this sawdust were extracted at room temperature in a 20-gallon stainless steel extractor with 85 liters of 70% n-propanol adjusted to the moisture content of the sawdust. Each day the extract was drained from the bottom of the extractor and fresh alcohol was added to the top. This extract was concentrated under reduced pressure, and the recovered alcohol was re-used. In all, 270 liters of extract were obtained from the extractor. When the extraction was completed, the extractor was allowed to drain, and the sawdust, still fairly wet with propanol, was placed in polyethylene bags and stored in the cold room at 40°C.

WATER EXTRACTION

Prior to the hydrolysis, the alcohol present in the sawdust was removed by soaking the sawdust in distilled water at room temperature at 3.0% consistency, filtering the wood and then washing it with more distilled water. Solids determinations were run on aliquots of each extract to determine the percentage of water-soluble material.

HYDROLYSIS REACTIONS

Eight hydrolysis reactions were carried out; two of these (numbers five and six) were alkaline in nature and will be described later. In hydrolyses numbers 1, 2, and 4, 200 grams (ovendry basis) of sawdust were processed with water in a one-gallon stainless steel autoclave, whereas in hydrolysis number 7, 15 grams (ovendry basis) of sawdust were processed in a 420-ml. stainless steel bomb. In each instance, these autoclaves were heated by immersion in a wax bath. In Cooks 3 and 8, one thousand grams (ovendry) of sawdust were hydrolyzed using a 34-liter, gas-fired, stainless steel, rotary autoclave. The conditions that were used in these six hydrolyses are given in Table I.

TABLE I

CONDITIONS USED IN THE HYDROLYSIS REACTIONS

Cook Number	Time to Max. Temp., min.	Time at Max. Temp., min.	Weight of O.D. Wood, g.	Maximun Temp., °C.	Water to Wood Ratio
1	90	60	200	170	20:1
2	55	60	200	170	20:1
3	90	90	1000	170	20:1
4	60	60	200	170	20:1
7	105	60	15	170	20:1
8	60	60	1000	170	20:1

The unscreened propanol and water-extracted sawdust was used as the starting material in Cooks 1, 2, 3 and 8. Cook 4 was a repeated hydrolysis of the ether-extracted hydrolyzed wood from Cook 3. In Cook 7, the wood hydrolyzed was the residual sawdust from Cook 6 which had been an alkaline hydrolysis.

PROCESSING OF THE HYDROLYSIS LIQUORS

After each cook was complete, the autoclave was relieved to atmospheric pressure and cooled. The mixture was filtered, and the residual wood was washed with several portions of distilled water. The liquor and washings were then extracted thoroughly with ether. These ether extracts were combined and concentrated to a small volume. In this work none of the ether solutions were taken to dryness, but rather solids determinations were made on aliquots to determine the amount of ether-soluble material.

FRACTIONATION OF THE ETHER-SOLUBLE MATERIAL OF THE HYDROLYSIS LIQUORS

The concentrated ether solution was extracted successively with 21% sodium bisulfite, saturated sodium bicarbonate and 6% sodium hydroxide solutions. The material remaining in the ether after these three extractions was designated as the "neutral fraction". Each of the aqueous solutions was then acidified with dilute sulfuric acid. The acidified sodium bicarbonate and sodium hydroxide solutions were re-extracted with ether to yield the sodium bicarbonate-soluble (B) and sodium hydroxide-soluble (C) fractions, respectively. Nitrogen was passed into the acidified bisulfite solution to displace the dissolved sulfur dioxide. This solution was then extracted with ether to yield the sodium bisulfite-soluble fraction (A). In order to insure the complete removal of sulfur dioxide, the aqueous solution was heated to boiling, cooled and again extracted with ether. This additional ether extract was added to fraction A. Each of these ether solutions was then concentrated to a small volume,

and the respective solids contents were determined on aliquots. These ether solutions were then ready for chromatographic investigations.

ALKALINE HYDROLYSIS

Cooks 5 and 6 were alkaline hydrolyses of 100 grams (ovendry basis) of wood. The wood used for Cook 5 was the ether-extracted hydrolyzed sawdust from Cook 5. Cook 6 was an alkaline hydrolysis of the unscreened, propanol and water-extracted aspen sawdust. In each case, the wood was heated for eight hours under reflux with stirring in three liters of normal sodium hydroxide. The mixture was cooled and filtered, and the residual sawdust was washed with water. The liquor and water washings were combined, acidified with dilute sulfuric acid and extracted thoroughly with ether. The amount of ether-soluble material was obtained by determining solids on an aliquot. The contents of the ether solution was then determined by means of paper chromatography.

KLASON LIGNIN DETERMINATIONS

The Klason lignin content of the wood and various pulps was determined using Institute Method 13.

EXPERIMENTAL DATA

EXTRACTION DATA

The 70% n-propanol extraction removed 3.4% of the wood, and the water extraction removed 0.3% of the wood, making the total percentage extracted 3.7%.

INVESTIGATION OF THE MATERIAL IN THE WATER EXTRACT

A nine-liter sample of the water extract from the sawdust of Cook 8 was concentrated to a small volume under reduced pressure. No monosaccharides were detected in this extract when it was analyzed by means of paper chromatography using 10:3:3 butanol-pyridine-water (BPW) as the developer and p-anisidine as the indicating spray. After this extract was heated under reflux with normal sulfuric acid, xylose and galactose were detected. No phenolic material could be found in this extract either before or after the acid treatment.

HYDROLYSIS DATA

The data obtained from the various hydrolyses are shown in Table II.

TABLE II

HYDROLYSIS DATA

Cook No.	Type[1]	Time at 170°C., min.	Yield (based on wood), %	Weight of O.D. Wood Used, (g.)	Yield Ether Soluble, % (wood basis)	Remarks
1	H.	60	79.0	200	1.5	
2	H.	60	79.6	200	2.4	
3	H.	90	67.5	1000	2.3	
4	H.	60	95.0	200	1.6	Recook of wood from Cook three
5	A.H.	--	----	100	2.2	Recook of wood from Cook four
6	A.H.	--	71.5	100	1.6	
7	H.	60	58.5	15	1.1	Recook of wood from cook six
8	H.	60	81.0	1000	1.7	

[1] H. -- hydrolysis
A.H. -- alkaline hydrolysis

INVESTIGATION OF THE ETHER-EXTRACTED HYDROLYSIS LIQUOR

A nine-liter sample of the ether-extracted liquor from Cook 8 was concentrated to a small volume under reduced pressure and tested for the presence of carbohydrates by means of paper chromatography. Using BPW as the developer and p-anisidine as the indicating spray, rhamnose, xylose, arabinose, galactose and several oligosaccharides were detected.

Further investigations indicated that this extracted liquor did not contain any free phenolic material. In an attempt to free any combined phenols present, a portion of this liquor was heated under reflux with normal sodium hydroxide, and another portion was oxidized with alkaline nitrobenzene. In neither case were phenols detected.

FRACTIONATION OF THE ETHER-SOLUBLE HYDROLYSIS PRODUCTS

The amounts of ether-soluble material extractable with 21% sodium bisulfite, saturated sodium bicarbonate and 6% sodium hydroxide solutions are given in Table III.

TABLE III

AMOUNTS EXTRACTED BY VARIOUS REAGENTS FROM THE ETHER-SOLUBLE HYDROLYSIS PRODUCTS

Hydrolyses from cook no.	1	2	3	8
Ether extract, % of wood	1.5	2.4	2.2	1.8
Bisulfite soluble, %[1]	24	11	13	15
Bicarbonate soluble, %[1]	15	11	16	15
Hydroxide soluble, %[1]	30	13	11	16
Neutrals, %[1]	20	47	36	22

[1] Percentage of ether extract

INVESTIGATION OF THE SODIUM BISULFITE-SOLUBLE FRACTION

PAPER CHROMATOGRAPHY

Several unsuccessful attempts to separate the components of Fraction A were made employing the paper chromatographic developers used by Goldschmid (5). In each instance, the developed chromatogram was streaked from the origin to the solvent front with no separation taking place. However, successful separations of the materials in Fraction A were made on paper chromatograms using butanol saturated with 2% aqueous ammonia (BA) (23) and n-butyl ether saturated with water (BW) (24) as developers. Each of these fractions was spotted in replicate on Whatman No. 1 paper and developed by the descending method. The locations of the spots on the developed

chromatograms were determined by visual and ultraviolet examination directly and after exposure to ammonia vapors, and by spraying with 2,4-dinitrophenylhydrazine (2,4-D.), phloroglucinol (PHL.), diazotized p-nitroaniline (DNP.) and by use of Mäule test reagents. The R_f values and color reactions of the materials present in the Fraction A of Cooks 1, 2, 3 and 8 are given in Table IV.

TABLE IV

R_f VALUES AND COLOR REACTIONS OF THE COMPONENTS IN FRACTION A OF COOKS ONE, TWO, THREE AND EIGHT

Developer: n-butyl ether-water

Sprays	R_f	.17[a]	.33[b]	.44[c]	.62[d]	.74[e]	.82[f]
2,4-D.		+	+	+	+	+	+
PHL.		+	Red	+	Red	-	Red
DNP.		-	-	+	+	+	+
Mäule		+	+	-	-	-	-
UV light		L	D	L	D		D
Visible light		Y		Y			Y

Developer: butanol-2% aqueous ammonia

Sprays	R_f	.36[b]	.40[f]	.44[d]	.52[a]	.60[c]	.81[e]
2,4-D.		+	+	+	+	+	+
PHL.		Red	Red	Red	+	+	-
Mäule		+	-	-	+	-	-
DNP.		+	+	+	-	+	+
UV light		D	D	D	L	L	
Visible light			Y		Y	Y	

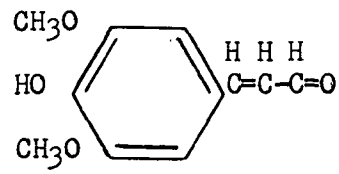
a, b, c, d, e, f, - refer to the compound present in Fraction A

D - dark fluorescence
L - light fluorescence Y - yellow when exposed to ammonia

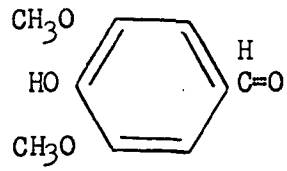
Compound a gave a positive Mäule test indicating syringyl activity, a 2,4-dinitrophenylhydrazone indicating a carbonyl group and a positive

phloroglucinol reaction indicating an aldehyde of the cinnamyl type. By means of paper chromatography, a small amount of this aldehyde was obtained for ultraviolet studies. In 95% ethanol, this compound showed an ultraviolet absorption maximum at 346 mμ, and after treatment with alkali as described by Lemon (25) this peak moved to 442 mμ. An authentic sample of sinapaldehyde (26) gave the same respective color reactions, the same ultraviolet absorption maxima in neutral and alkaline solutions, and had the same R_f values in BA and BW as did compound a.

Compound b gave a positive Mäule test and a 2,4-dinitrophenylhydrazone indicating a syringyl aldehyde. This product gave the same color reactions and had the same R_f values in BA and BW as did an authentic sample of syringaldehyde (26).



sinapaldehyde

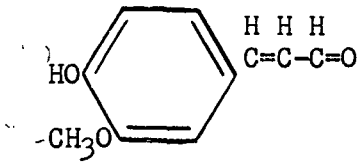


syringaldehyde

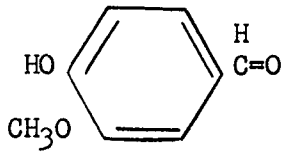
Compound c gave a negative Mäule test, a positive phloroglucinol test, a color with diazotized p-nitroaniline and a 2,4-dinitrophenylhydrazone. These tests are indicative of a nonsyringyl phenolic aldehyde with a cinnamyl configuration. An ultraviolet absorption spectrum of this compound in 95% ethanol showed a peak at 342 mμ, and after a Lemon shift, this maximum moved to 420 mμ. An authentic sample of coniferyl aldehyde (26) showed the same ultraviolet absorption maxima in neutral and alkaline solutions as compound c. When compound c was chromatographed on paper

using BA and BW as developers, it had the same R_f values and gave the same color reactions as did the authentic coniferyl aldehyde.

Compound d formed a 2,4-dinitrophenylhydrazone, gave a negative Mäule test and a gray color with diazotized p-nitroaniline. These tests are indicative of a nonsyringyl phenolic aldehyde. When this compound was chromatographed on paper using BA and BW as developers, it had the same R_f values and the same color reactions as did vanillin.



coniferyl aldehyde

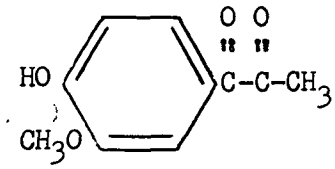


vanillin

Compound e gave a yellow, 2,4-dinitrophenylhydrazone, a negative Mäule test, a negative phloroglucinol test and an orange color with diazotized p-nitroaniline. These tests indicate the presence of a nonsyringyl phenolic carbonyl compound. The ultraviolet absorption spectrum of this material in 95% ethanol showed no maximum although there were breaks in the curve at 250 mμ and 280 mμ. The identity of this material is still unknown.

Compound f gave a red color with phloroglucinol, a yellow 2,4-dinitrophenylhydrazone, a color with diazotized p-nitroaniline, a yellow color with ammonia, and a dark fluorescence in ultraviolet light. An ultraviolet absorption spectrum of this material in 95% ethanol showed a peak at 320 mμ and after a Lemon shift, this peak moved to 370 mμ. These ultra-

violet data, color reactions, and R_f values in BA and BW are identical with those of an authentic sample of vanilloyl methyl ketone (26).



vanilloyl methyl ketone

Although these color reactions, R_f values and ultraviolet absorption data are indicative of each of the compounds present, they do not constitute positive proof. To prove unequivocally the presence of these materials, it was necessary to isolate crystalline products and compare the properties of these products with authentic samples.

COLUMN CHROMATOGRAPHY

Preliminary investigations of Fractions A from Cooks 1 and 2 indicated the acid-washed Magnesol with petroleum ether (65-110°C.)-ethanol (50:1) as the developer separated some of the materials present. This technique was used previously by Pearl and Dickey (27) for the separation of vanillin and syringaldehyde.

The general procedure used in this type of chromatography follows. The solution containing the products to be separated was evaporated to dryness and the resulting solids were taken up in benzene or chloroform. This solution was adsorbed on acid-washed Magnesol and the chromatogram was developed with petroleum ether-ethanol (50:1). The amount of developer

used depended on the size of the column; for example, when using a 260 mm. by 45 mm. column, 1000 to 1100 ml. of developer were used. The developed chromatogram was extruded, examined in visible and ultraviolet light and streaked with 2,4-dinitrophenylhydrazine, phloroglucinol and occasionally with Mäule test reagents. Using these tests as a guide, the column was cut into sections and each section was eluted with redistilled acetone. The contents of each zone was then monitored by means of paper chromatography using BA or BW as developers. Through the use of this monitoring technique, sections containing the same compounds could be combined and rechromatographed.

Cook One

Fraction A of Cook 1 was chromatographed on a column of acid-washed Magnesol 150 mm. in length and 20 mm. wide, and developed with 100 ml. of petroleum ether-ethanol (50:1). Seven bands were obtained and each was eluted with acetone. The fourth band, which was 27 mm. from the top and 20 mm. in length, contained compound b only. Upon evaporation of the acetone, brown crystals formed. This material was decolorized with a small amount of carbon and recrystallized from water. The light tan crystals obtained melted at 109-110°C.[1] and did not depress the melting point of a mixture with syringaldehyde (26). The other products present were in amounts too small to isolate.

[1] All melting points are uncorrected.

Cook Two

Fraction A from Cook 2 was handled in the same manner as Fraction A from Cook 1. However, no other crystalline material could be isolated.

Cook Three

Fraction A of Cook 3 contained 2.8 g. of material. This entire fraction was chromatographed on an acid-washed Magnesol column 40 mm. wide and 230 mm. in length. The developed chromatogram was cut into sections. Two of these, the one from the top and one from the bottom of the column, contained nothing. Of the five remaining zones, each section contained at least two of the compounds present in the original fraction. Several attempts were made to isolate crystalline vanillin from section four which was 15 mm. in length and 110 mm. from the top of the column. These attempts failed, and in each case, only sirups were obtained.

Section 5 from this column contained compounds d, e and f. In an attempt to isolate crystalline vanillin from this section, its contents were streaked across the top of several sheets of Whatman No. 1 paper and developed with BW. The separated compounds were detected by examining the developed chromatogram under ultraviolet light. The zone containing the vanillin was cut from the paper and eluted with 95% ethanol. Attempts were made at crystallizing the eluted material from petroleum ether (65-110°C.) and from water, but each failed. However, evaporation of the water produced brown flakes which were recrystallized

from high boiling petroleum ether. The resulting off-white spheres melted at 79-80°C., and when mixed with vanillin, did not depress its melting point.

The attempted isolation of crystalline products from the other section failed.

Cook Eight (See Diagram One)

The entire Fraction A from Cook 8 was chromatographed on a column of acid-washed Magnesol 45 mm. wide and 240 mm. in length and developed with 1000 ml. of petroleum ether-ethanol (50:1). The developed chromatogram was divided into six sections and each was eluted with redistilled acetone. The contents of each section were monitored using paper chromatography with BA as the developer. With this monitoring technique as a guide, sections were combined and rechromatographed several times on acid-washed Magnesol using the procedure described previously.

Isolation and Identification of Vanillin

Column 2-zone 4 contained one product which appeared to be compound d. Evaporation of the mother liquor gave brown flakes which were recrystallized from high boiling petroleum ether. The resulting yellow crystals melted at 76-8°C. This material was further purified by vacuum sublimation to yield white needles which melted at 79-80°C. and did not depress a mixed melting point with vanillin.

Isolation and Identification of Sinapaldehyde

Column 6-zones 2 and 3 each contained two acids and an aldehyde. From its chromatographic behavior and color reactions, this aldehyde appeared to be compound a. The two zones were combined and evaporated to dryness. The acids were separated from the phenolic aldehyde

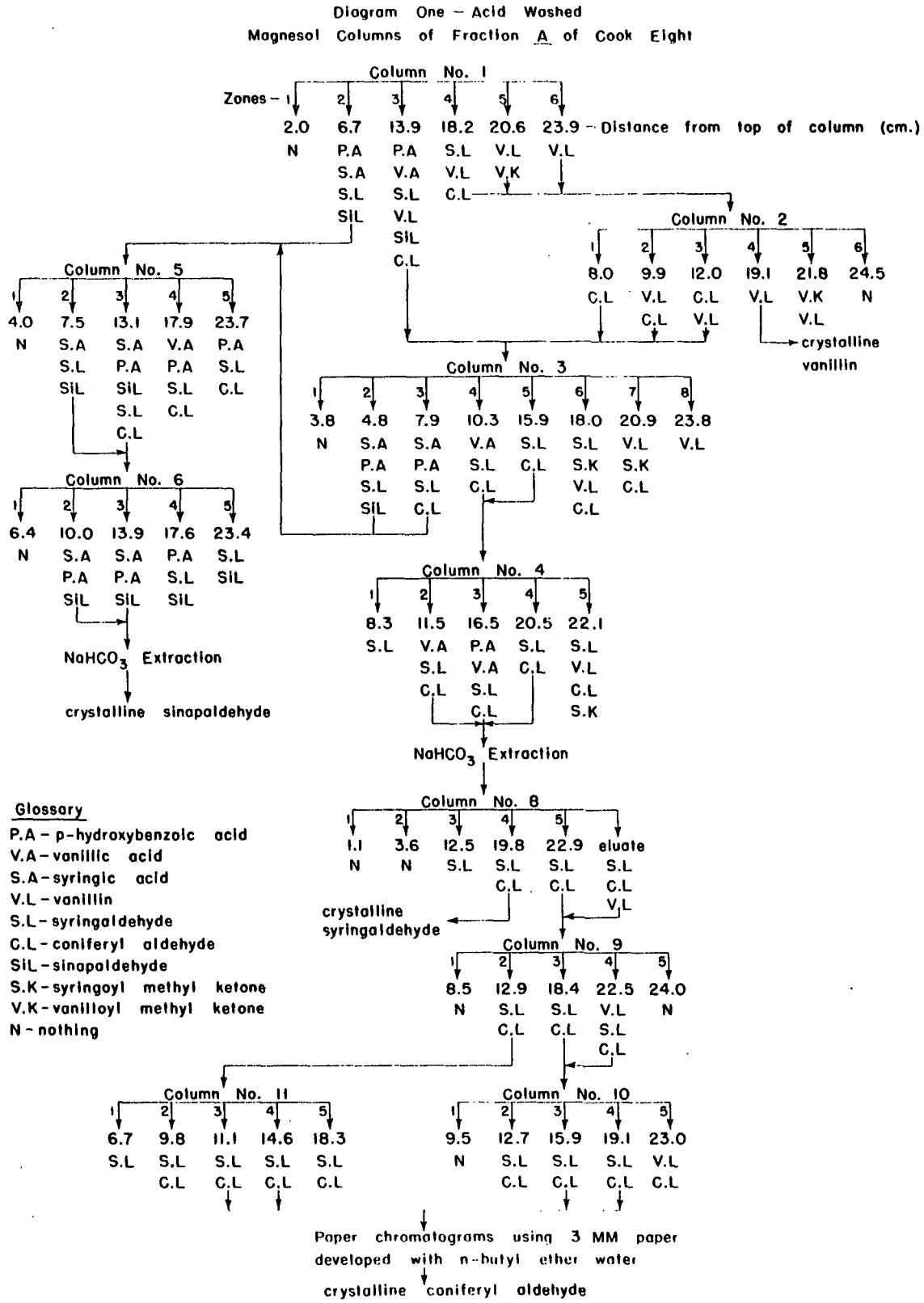


Figure 2. Acid-Washed Magnesol Columns of Fraction A of Cook Eight

by taking up the solids in 3% sodium hydroxide and then bubbling in carbon dioxide to form a sodium bicarbonate solution, which was then extracted with chloroform to remove the phenolic aldehyde while the acids remained soluble in the aqueous phase as sodium salts. The chloroform solution was taken to dryness under reduced pressure and the resulting solids were taken up in benzene. After several unsuccessful attempts had been made to isolate a crystalline material from the benzene solution failed, the latter was evaporated, and the resulting solids were taken up in chloroform. Tan crystalline spheres were obtained from this chloroform solution by the addition of several small amounts of petroleum ether (65-110°C.) followed by cooling. These spheres melted at 106-7°C. and did not depress the melting point when mixed with authentic sinapaldehyde (26).

Isolation and Identification of Syringaldehyde

Column 8-zone 4 contained only material which appeared to be compound b. Upon evaporation of the mother liquor, brown needles formed which were recrystallized from chloroform and then from water. The resulting light tan needles melted at 109-10°C. and did not depress a mixed melting point with syringaldehyde (26), but did depress a mixed melting point with the isolated sinapaldehyde.

Isolation and Identification of Coniferyl Aldehyde

Column 5-zones 4 and 5 and column 4-zones 2, 3 and 4 contained compounds b, c and two acids. The contents of the five zones were combined and the phenolic aldehydes were separated from the acids as previously described. After three unsuccessful attempts had been made to separate the remaining two aldehydes on acid-washed Magnesol, a paper chromatographic separ-

ation was tried using Whatman 3 MM paper with BW as the developer. It was found that the aldehydes could be separated in this manner and, therefore, the contents of column 10-zones 3 and 4 and column 11-zones 3 and 4 were concentrated to a small volume and streaked across the top of four sheets of 3 MM paper. The desired aldehyde, compound c, was detected on the developed chromatogram by its unique yellowish-green fluorescence in ultraviolet light. This material was eluted from the paper using chloroform which was then concentrated to dryness under reduced pressure. The solid material obtained was taken up in sufficient benzene to give a clear solution. On standing, a yellow crystalline solid formed. For further purification, this material was again taken up in benzene, and small amounts of petroleum ether (65-110°C.) were added. Upon cooling of this mixture, a brown solid formed which was filtered off. More petroleum ether was added, and upon cooling, yellow flakes were formed. This product melted at 78-80°C. and did not depress a mixed melting point with coniferyl aldehyde (26), but did depress a mixed melting point with the isolated vanillin.

Further Efforts to Prove the Presence of Vanilloyl Methyl Ketone

Column 2-zone 5 contained at least two materials, compounds d and f. Paper chromatograms of this zone indicated that compound f was present in larger amounts than compound d. After efforts to crystallize the former failed, an attempt was made to form its 2,4-dinitrophenylhydrazone. An orange solid was obtained in a small amount which melted 30°C. below the reported melting point of this derivative of vanilloyl methyl ketone. An infrared spectrum of the solution of column 2-zone 5 showed little similarity to that of vanilloyl methyl ketone indicating that this solution was perhaps a complex mixture of more than just two materials.

THE SODIUM BICARBONATE-SOLUBLE FRACTION

PAPER CHROMATOGRAPHY

Orienting paper chromatograms of this fraction indicated that benzene saturated with formic acid (BeF) (28), BPW and toluene-acetic acid-water (4:1:5) (TAW) (29) were satisfactory developers. The developed chromatograms were examined under ultraviolet light, and sprayed with bis-diazotized benzidine (BDB), diazotized p-nitroaniline (DPN) and Mäule test reagents. The R_f and R_{va} values and color reactions of the materials present in fraction B of Cooks 1, 2, 3 and 8 are given in Table V.

TABLE V

R_f AND R_{va} VALUES AND COLOR REACTIONS OF THE MATERIALS PRESENT IN FRACTION B OF COOKS' ONE, TWO, THREE AND EIGHT

Developer: Benzene-formic acid

Sprays	R_{va}	.20[g]	.80[h]	1.00[i]
BDB		gold	orange	grey
DPN		yellow	orange	yellow
Mäule		-	+	-
UV light		D		

R_{va} - reference vanillic acid

Developer: BPW

Sprays	R_f	.33[h]	.43[i]	.57[g]
BDB		orange	grey	gold
DPN		orange	yellow	yellow
Mäule		+	-	-
UV light				D

g, h, and i refer to the compounds present in Fraction B.

When fraction B was chromatographed in BA, compounds g, h and i had R_f values of .09, .11 and .13, respectively. The low R_f values in this developer together with solubility in sodium bicarbonate are indicative of carboxylic acids.

Compound g gave a golden color with BDB, a yellow color with DPN, a negative Mäule test and dark spots where the fluorescence was quenched in ultraviolet light. These tests were indicative of a nonsyringyl phenolic carboxylic acid. Para-hydroxybenzoic acid gave the same color reactions and had the same R_f and R_{va} values in BPW and BeF as did compound g.

Compound h gave an orange color with both BDB and DPN and a positive Mäule test. These tests were indicative of a syringyl-carboxylic acid. Syringic acid gave the identical color reactions and had the same R_f and R_{va} values in BPW and BeF as did compound h.

Compound i gave a gray color with BDB, a yellow color with DPN and a negative Mäule test. These tests indicated the presence of a nonsyringyl carboxylic acid. Vanillic acid gave the same color reactions and had the same R_f and R_{va} in BPW and BeF as did compound i.

THE SEPARATION OF COMPOUNDS g, h AND i USING THE CRAIG MACHINE

To facilitate the separation of these three materials on a large scale, a 60-tube Craig-Post countercurrent distribution machine was used. Bray, White and Thorpe (30) suggested benzene-acetic acid-water (2:2:1) as a developer for the separation of various phenolic carboxylic acids on paper. The upper and lower phases of this mixture were used in the Craig machine

in an attempt to separate compounds g, h and i. Fraction B from Cook 3 was evaporated to dryness and taken up in the lower phase of this mixture and added to the first tube of the Craig machine. After one hundred transfers were made, every fifth tube was monitored by means of paper chromatography using BeF as the developer. Tubes 3-19 contained compound g, tubes 20-23 contained all three compounds, tubes 24-31 contained compounds h and i and tubes 32-36 contained the latter compound only. Tubes 37-60 contained none of these materials.

Isolation and Identification of Vanillic Acid

The contents of tubes 32-36 were evaporated to dryness and taken up in ether. This ether solution was diluted with petroleum ether (37-70°C.) to a decided turbidity and the mixture was cooled. The clear supernatant solution was decanted off and the procedure was repeated. This mixture was then evaporated to dryness yielding a tan product which was recrystallized from chloroform. The resulting yellow flakes melted at 204-5°C. Recrystallization from water gave off white crystals which melted at 205-6°C. and which did not depress a mixed melting point with an authentic sample of vanillic acid.

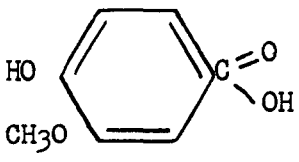
Isolation and Identification of Syringic Acid

The solution from tubes 24-31 was evaporated to dryness and the residue taken up in acetone. This acetone solution was streaked across the top of several sheets of paper (Whatman No. 1) and developed with BeF. The separated materials were detected by spraying a strip cut from the side of

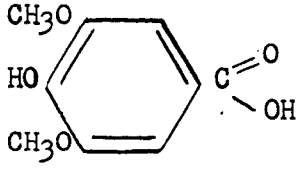
the developed chromatogram with DPN. The two compounds h and i were then eluted with 95% ethanol. When the ethanol was evaporated from the solution containing compound h, yellow crystals were formed. These crystals melted at 198-200°C. Recrystallization from water yielded light gray needles which melted at 202-3°C. and which did not depress the melting point of authentic syringic acid.

Isolation and Identification of p-Hydroxybenzoic Acid

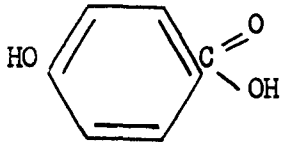
The solution from tubes 3-19 was evaporated to dryness and the residue was taken up in hot water. Upon cooling, brown crystals formed which were purified further by vacuum sublimation at 180°C. The resulting white needles melted at 213-14°C. and did not depress a mixed melting point with p-hydroxybenzoic acid.



vanillic acid



syringic acid



p-hydroxybenzoic acid

THE SODIUM HYDROXIDE-SOLUBLE FRACTION

Orienting paper chromatograms using BA as a developer indicated that some of the material present had been identified in Fractions A and B. However, the bulk of the material present in Fraction C had an R_f value of 0.80 or above in this developer. Two unsuccessful attempts were made at separating these materials using acid-washed Magnesol.

THE NEUTRAL FRACTION

When this fraction was chromatographed on paper using BA and BeF as developers, no materials could be detected using any of the sprays listed above.

THE ETHER EXTRACT OF THE HYDROLYZED WOOD

The air-dried hydrolyzed sawdust from Cook 3 was extracted with ether in a Soxhlet. The ether-soluble material constituted 1.9% of the hydrolyzed wood. Paper chromatograms of this extract developed with BA indicated the presence of p-hydroxybenzoic acid, syringaldehyde, vanillin, coniferyl aldehyde and sinapaldehyde.

In order to isolate larger amounts of these products, a cellulose column was wet packed and prewet with BA. The concentrated ether extract from the hydrolyzed wood of Cook 3 was carefully placed on the top of this column and the chromatogram was developed with BA. As the materials moved down the column, two distinct yellow bands formed each of which was taken off separately. The contents of each of the zones was concentrated to a small volume and acidified. These concentrated solutions were monitored by means of paper chromatography and each was found to contain at least three materials, indicating that no real separation had occurred.

The hydrolyzed wood from Cook 4 was extracted with ether in a Soxhlet extractor and 0.3% of it was found to be ether-soluble. When this extract was analyzed using paper chromatography, it was found to contain the same compounds as did the ether extract of the hydrolyzed wood from Cook 3.

INVESTIGATION OF THE LIQUORS OF COOKS FOUR AND SEVEN

The ether extract of the hydrolysis liquors from Cooks 4 and 7 were not fractionated. The entire ether extract was analyzed using paper chromatography.

Cook 4 involved a repeated hydrolysis of the extracted hydrolyzed wood from Cook 3. The ether-soluble portion of this liquor contained p-hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, syringaldehyde, coniferyl aldehyde and sinapaldehyde.

Cook 7 was a hydrolysis of the air-dried alkaline-hydrolyzed sawdust from Cook 6. In the ether extract from the liquor of Cook 7, seven compounds were identified by their color reactions and R_f values in BA and BW and R_{va} values in BeF developers. These compounds were p-hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, syringaldehyde, coniferyl aldehyde and sinapaldehyde.

INVESTIGATION OF THE ALKALINE HYDROLYSIS LIQUORS

COOK FIVE

Cook 5 was an alkaline hydrolysis of the ether-extracted hydrolyzed sawdust from Cook 4. In the ether extract of the acidified hydrolysis liquor, vanillin, syringaldehyde, p-hydroxybenzoic acid, vanillic acid and syringic acid were detected by their color reactions and R_f and R_{va} values in BA and BeF developers, respectively.

COOK SIX

Cook 6 was an alkaline hydrolysis of the n-propanol and water-extracted aspen sawdust. In the ether extract of the acidified hydrolysis liquor the same five compounds were detected as those found in Cook 5.

QUANTITATIVE DETERMINATION OF THE ACIDS FREED BY COOKS FIVE AND SIX

A method devised by Pearl, et al. (11) was used for the quantitative determination of each of the acids present in Cooks 5 and 6 (see Tables VI and VII). A known quantity of the ether extract was streaked on the top of a sheet of paper which was then developed with BeF. The bands containing the separated acids were located by spraying a strip cut from the developed chromatogram with DPN. Each of the bands was cut from the paper and eluted with 95% ethanol. The ethanol extracts were made alkaline, diluted to a known volume, and their optical densities determined at a prescribed wavelength. Using these optical densities and a standard calibration curve of milligrams of acid per liter versus optical densities, the amount in the extract could be determined.

TABLE VI

ACIDS PRESENT IN THE LIQUOR OF COOK SIX

Acid	Wave-length, mμ	Yield	
		Milligrams	Per cent of Wood
p-Hydroxybenzoic acid	278	560.0	0.56
Vanillic acid	300	69.5	0.07
Syringic acid	298	96.5	0.10

TABLE VII

ACIDS PRESENT IN THE LIQUOR OF COOK SEVEN

Acid	Yield	
	Milligrams	Per Cent of Wood
p-Hydroxybenzoic acid	4.9	0.03
Vanillic acid	4.5	0.03
Syringic acid	4.4	0.03

QUANTITATIVE DETERMINATION OF THE ACIDS AND ALDEHYDES PRESENT IN THE LIQUOR OF COOK EIGHT

For the determination of the three acids present in Cook 8 the previously described method was used (see Table VIII). Vanillin and syringaldehyde were determined by the spectrophotometric method of Lemon (25) after elution from paper chromatograms by the procedure described by Stone and Blundell (24).

TABLE VIII

ACIDS AND ALDEHYDES PRESENT IN THE LIQUOR FROM COOK EIGHT

Compounds	Yield	
	Milligrams	Per Cent of Wood
p-Hydroxybenzoic acid	1711.0	0.17
Vanillic acid	300.0	0.03
Syringic acid	300.0	0.03
Vanillin	105.0	0.01
Syringaldehyde	355.0	0.03

LOSS IN KLASON LIGNIN DUE TO HYDROLYSIS

Klason lignin was determined on the original extracted wood, and on the residual wood from Cooks 3 and 4 (see Table IX).

TABLE IX

LOSS IN KLASON LIGNIN DUE TO HYDROLYSIS

Cook	Yield (based on wood charged)	Ether-soluble from Liquor (based on orig. wood), %	Ether-soluble from Wood (based on orig. wood), %	Klason Lignin (based on residue), %	Klason Lignin (based on orig. wood) %
Wood	—	—	—	18.6	18.6
Three	67.5	2.3	1.2	22.3	15.0
Four	95.0	0.7	0.2	20.0	12.7
Five	—	1.4	—	--	--

These three cooks were successive treatments of the same wood. Cooks 3 and 4 were hydrolyses and Cook 5 was an alkaline hydrolysis.

CHROMATOGRAPHIC INVESTIGATION OF THE PREHYDROLYSIS PULPING LIQUORS FROM BLACK GUM WOOD

Bernardin (31) in investigating the nature of the carbohydrate degradation induced by the prehydrolysis pulping of black gum wood (Nyssa sylvatica) furnished the writer with a portion of one of his liquors which was obtained under the following conditions:

Maximum temperature - 160°C.
Time at maximum temperature - 60 minutes
Water to wood ratio - 18.7:1
Yield of the fibrous residue - 77.0%

The original wood had not been extracted with organic solvents prior to hydrolysis. This liquor was extracted with ether and 1.0% (based on the wood) was found to be ether-soluble. By means of paper chromatography using BA, BW and BeF as developers, seven phenolic materials were detected in this extract and tentatively identified as vanillic acid, syringic acid, vanillin, syringaldehyde, coniferyl aldehyde, sinapaldehyde, and vanilloyl methyl ketone. It is of interest to point out that no p-hydroxybenzoic acid was detected in this extract.

ELECTROPHORETIC STUDIES

Three unsuccessful attempts were made to separate vanillin, syringaldehyde, p-hydroxybenzoic acid, vanillic acid and syringic acid using continuous paper electrophoresis. The apparatus of Grassman and Hannig (32) as constructed by Bender and Hobein and commonly known as the Elphor Va model was used. The conditions of each run are given in Table X.

TABLE X

CONDITIONS USED IN THE ELECTROPHORETIC STUDIES

Type of Buffer	Ionic Strength of Buffer	pH of Buffer	Applied Voltage
0.02M Disodium phosphate	0.06	8.3	200
0.00175M Citric acid and 0.0165M disodium phosphate	0.01	7.2	500
0.01M Acetic acid and 0.01M sodium acetate	0.02	4.6	800

It should be pointed out that whereas in each case the aldehydes were separated from the individual acids, however, there was only a slight separation of the acids and aldehydes from each other. The path of these compounds was determined by examining the developed chromatogram in ultraviolet light and then by spraying with DPN.

DISCUSSION

Hardwood lignin is believed to be composed of a mixture of combined phenyl-propane derivatives of the sinapyl and coniferyl types. The cleavage of these derivatives from a hardwood by a relatively mild treatment and their identification should add greatly to the knowledge of the chemical structure of hardwood lignin, even though the degradation products represent only a small part of the total wood.

Goldschmid (5) analyzed the prehydrolysis liquors of western hemlock wood by means of paper chromatography. He tentatively identified several "lignin degradation products" in the ether-soluble portion of these liquors by their R_f values, color reactions and ultraviolet absorption spectra. In no case, however, did Goldschmid (5) prove unequivocally the identity of these extracted phenols.

This hydrolysis treatment, being relatively mild, appears to be an elegant method for freeing these "lignin monomeric units" while the various methods of chromatography offered means of isolating these freed materials.

In the present work, it was found that when aspen sawdust was hydrolyzed, a part of the noncarbohydrates was degraded and found in the liquor as monomeric phenols. Seven crystalline compounds were isolated from the ether-soluble portion of the hydrolysis liquors and identified by their color reactions, R_f values, melting points and mixed melting points with authentic samples. These compounds were p-hydroxybenzoic acid, vanillic

acid, syringic acid, vanillin, syringaldehyde, coniferyl aldehyde and sinapaldehyde. Vanilloyl methyl ketone was tentatively identified by its R_f values, color reactions and ultraviolet absorption maxima in neutral and alkaline solutions.

The isolation of coniferyl aldehyde and sinapaldehyde from these hydrolysis liquors is in agreement with the hypothesis that hardwood lignin is built up of these units. It appears that this is the first time that sinapaldehyde has been isolated from wood.

The origin of these degradation products is unknown. Since they all are aromatic in nature they were either associated with the extraneous material or with the lignin. There would obviously be some overlapping in attempting to assign material to either of these groups since both are defined in rather vague terms. In this realm of definition, it is interesting to point out that the so-called "isolated native lignin" is actually an extractive.

Considering the origin of the p-hydroxybenzoic acid, Smith (12) obtained this compound from "isolated native aspen lignin" by caustic hydrolysis. Pearl (33) has found free p-hydroxybenzoic acid in the extractives of aspenwood. With this evidence in mind, p-hydroxybenzoic acid should be classed as an extractive of aspenwood. On the other hand, Pearl (33) has also detected p-hydroxybenzoic acid in the Klason lignin filtrate from extractive-free aspenwood. The finding of this compound in this filtrate indicates that it was probably a part of the cell wall, since prior to the Klason lignin determination the wood was thoroughly

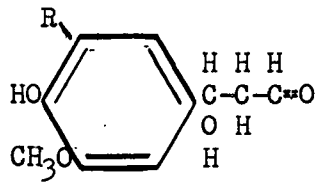
extracted to remove any interfering extraneous materials. This observation that p-hydroxybenzoic acid may be a part of the cell wall was substantiated by the present work, since successive hydrolyses of aspenwood yielded more of this same compound. It should be pointed out that the original aspenwood was extracted prior to hydrolysis with 70% n-propanol and water in order to remove as much of the extraneous material as possible. It follows, therefore, that some of the p-hydroxybenzoic acid present in aspenwood is combined in the cell wall and possibly with the lignin.

Reasoning in the same manner, the six other compounds freed on successive hydrolyses of aspenwood would be assumed to be part of the cell wall rather than the extractives. Since they were aromatic in nature and contained methoxyl groups and since three of these materials were phenylpropane derivatives, it follows that they were probably derived from the lignin of the wood.

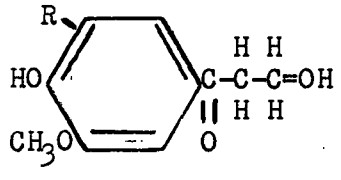
Considering the origin of the monomeric phenols in the light of other data, there was a 3.6% decrease in the Klason lignin as a result of hydrolysis. By extracting the hydrolysis liquor and air-dried residual wood from cook three, 3.6% (based on the original wood) was found to be ether soluble. This balance presents the possibility that the Klason lignin was being converted quantitatively into ether-soluble material through hydrolysis. This statement must be qualified since Pearl (33) has detected several free phenols in the Klason lignin filtrate from aspenwood. However, it would seem that a part of the ether-soluble

material was derived from the same material which yields the Klason lignin. The Klason lignin and "protolignin" are by no means the same, however; the former is derived entirely from the latter in a changed form. It follows from this that a part of the ether-soluble hydrolysis products was derived from the protolignin, since part of the ether-soluble products were derived from the material which yields the Klason lignin which originally was a product of the protolignin. In other words, a portion of the ether-soluble hydrolysis product had its origin in the original lignin of aspenwood.

Since the caustic hydrolysis of aspenwood yielded vanillin and syringaldehyde and since Kratzl and Wacek (34) noted that vanillin was obtained upon the caustic hydrolysis of "aldol" guaiacyl lignin model compounds, it follows that perhaps aspen lignin may contain this "aldol" structure. "Aldol" refers to a class of compounds of which 3-hydroxy-1-butanol is the parent. The "aldol" configurations used by Kratzl and Wacek (34) are shown below.



Type A



Type B

R= -H or
-OCH3

Aspen lignin might well contain the "aldol" structure since sinapaldehyde and coniferyl aldehyde were found in the hydrolysis liquors from this wood. These two aldehydes are the dehydrated form of "aldol" type A.

An important question remains unanswered: How were these aromatic materials linked in the wood? Presumably the coniferyl aldehyde and sinapaldehyde might have been the end units in the lignin structure as has been suggested by Adler (35). The location of the remaining materials is unknown. However, when the above question is answered, lignin chemistry will be advanced another step toward its ultimate goal, the complete elucidation of this complex system.

SUMMARY

When n-propanol-extracted aspen sawdust was treated with water at 170°C., a part of the wood was broken down into phenolic "lignin-type monomers". From the ether-soluble portion of the hydrolysis liquors, seven crystalline products were isolated and identified: p-hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, syringaldehyde, coniferyl aldehyde and sinapaldehyde. Vanilloyl methyl ketone was tentatively identified by its R_f values, color reactions and ultraviolet absorption maxima in neutral and alkaline solutions, but this was not an unequivocal identification. By successive hydrolyses of the same wood, more of these same compounds could be obtained. Para-hydroxybenzoic acid was present in much greater amounts than were any of the other materials. These materials probably have their origin in the lignin of the wood.

GLOSSARY

1. Hydrolysis - action of water at 170-5°C.

2. Alkaline Hydrolysis - action of normal sodium hydroxide when heated under reflux

3. Paper Chromatographic Developers

   A. Butanol saturated with 2% aqueous ammonia - BA (23)
   B. Benzene saturated with formic acid - BeF (28)
   C. n-Butyl ether saturated with water - BW (24)
   D. Toluene-acetic acid-water (4:1:5) - TAW (29)
   E. Butanol-pyridine-water (10:3:3) - BPW

4. Spray Reagents

   A. 2,4-Dinitrophenylhydrazine - 2,4-D. (36)
   B. Phloroglucinol reagent - PHL. (5)
   C. Mäule[1] (37)
   D. Diazotized p-nitroaniline - DPN (30)
   E. Bis-diazotized benzidine - BDB (38)
   F. p-Anisidine - pA (39)

[1]Mäule test procedure - The dried chromatogram was placed in an atmosphere of chlorine for 10 minutes and then sprayed with a 10% solution of sodium sulfite. A positive test is a cerise coloration.

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