ORGANIC CHEMISTRY OF BARK PHENOLIC ACID

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1957

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Date thesis is presented November 2, 1956

Typed by Norma Becker

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. E. F. Kurth for his aid and understanding direction.

He also wishes to express his appreciation to the Chemistry department for financial aid and to the Oregon Forest Products Laboratory for generous guest privileges extended to the author.

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INTRODUCTION

This study was undertaken with the purpose of learning more about the chemical nature of the "phenolic acid" present in the cork from the bark of white fir, Abies concolor, Lindl. and Gord. This substance is obtained by saponification of the extractive free cork and has similar properties to a number of preparations which have been isolated by similar procedures from various physical fractions of the barks of different trees. As a result of learning more of the chemical nature of this phenolic acid, further information will be available to aid in the establishment of a correlation among the structures of materials of this type isolated from various softwoods. In addition, this study will aid in determining if there is any relationship between the phenolic acid and the wood lignin. A better understanding of the chemistry of this material is also required to take advantage of this material as a source of organic chemicals since at present the greater portion of the bark is utilized only as a fuel.

The present status of bark chemistry makes it difficult to compare bark lignin or phenolic acid preparations. Conventional methods of wood analysis when applied to bark fail to provide clean separations. This

is true because the bark components are more complex than those of the wood, thus a Klason lignin determination applied to extractive free cork would include a considerable amount of extraneous material. Thus, while a number of bark lignin preparations have been isolated and reported (17, pp.39 and 41), differences in the methods of isolation make comparison difficult. In most cases nothing but yield and methoxyl content have been reported for these lignins.

Bark phenolic acids of comparable nature have been isolated from redwood bark, (Sequoia sempervirons (4, pp. 34-38 and 21, pp.759-764); Douglas fir, Pseudotsuga menziesii (18, pp.125-133); white spruce, Picea glauca (12, p.56) and white fir, Abies concolor (13, pp.1-14). Table I shows a comparison of the phenolic acids isolated from the barks of these trees, with the exception of white fir.

A complicating factor in the study of phenolic acids is the physical heterogeniety of the bark itself. Smith (18, p.127) isolated phenolic acid material from six physically different fractions of Douglas fir bark. The cork fraction is easily separable and contains the highest percentage of phenolic acid with the exception of the fines which make up a heterogeneous fraction. This and the availability of about one pound of the isolated material

was the basis for choosing white fir bark cork as a source of phenolic acid for this study.

TABLE I

CHARACTERISTICS OF PHENOLIC ACIDS FROM RED WOOD, DOUGLAS FIR AND SPRUCE BARKS

	Redwood			Douglas Fir	
	Bast Fiber	Dust	Spruce	Bast Fiber	Cork
Phenolic Hydroxyl %	7.8)	12.2	10.5	8.3	--
Aliphatic Hydroxyl %	2.1)			4.2	--
Methoxyl %	2.7	0.00	1.46	4.3	4.21
Carboxyl %	4.4	11.12	7.22	4.9-5.3	--
Vanillin From Nitrobenzene Oxidation %	---	---	0.00 0.50*	1.63	0.60

* From nitrobenzene oxidation of the methylated phenolic acid.

EXPERIMENTAL

SOURCE AND PREPARATION OF THE PHENOLIC ACID

The white fir bark for this study was obtained from Weyerhaeuser Timber Company in 1954. The procedure for the separation of the cork and isolation of the phenolic acid as described below was worked out by Hergert (13, pp.1-10). The bark was first hogged to pass a one-half inch screen and then further reduced to pass a ten mesh screen by means of a Reitz disintegrator. The ground bark was then suspended in hot water (60-85°C) with stirring for five minutes during which time the non cork fraction settled to the bottom. The cork was skimmed from the surface and air dried. The bark contained 51 percent cork as determined by this separation.

The cork thus obtained was then extracted with an azeotropic mixture of methyl-ethyl ketone and water (89 percent methyl-ethyl ketone) in a continuous extractor. This was followed by extraction with hot water to produce the extractive free cork. Analysis of the cork prepared in this manner indicated that it contained not more than three percent of the original extractives.

Extractive free cork was then dispersed in a solution of five percent sodium hydroxide in the ratio of one part cork to seven parts sodium hydroxide solution. Live steam was bubbled into the mixture for four hours. When

cool, the pH was adjusted to 2.0 and the mixture was allowed to settle overnight. The water insoluble material was then filtered from the acid solution and allowed to air dry. The dry residue was passed through a coffee mill, placed in Soxhlet extractors and exhaustively extracted with ether. When the ether extraction was complete, the solvent was changed to alcohol which extracted the phenolic acid. This treatment separated the extractive free cork into: material insoluble in five percent sodium hydroxide 32.1 percent, material soluble in water after saponification 16.4 percent, material soluble in ether 25.4 percent, and material soluble in alcohol 26.1 percent.

The phenolic acid was isolated from the alcohol by concentration of the extract to about 25 percent solids followed by precipitation into water. The phenolic acid was filtered, dried, taken up in alcohol and reprecipitated into water. A few drops of concentrated hydrochloric acid were added to coagulate the precipitate.

Samples for analytical work were extracted with ether in Soxhlet extractors and vacuum dried at 55°C over P_2O_5 in an Abderhalten dryer. This material was a red-brown powder with an elemental analysis of 60.22 percent carbon and 5.74 percent hydrogen. The material was soluble in ethanol, methanol, eight percent sodium bicarbonate, five percent sodium carbonate or one percent

sodium hydroxide. The material was also partially soluble in other organic solvents such as dioxane, acetone and pyridine.

CHROMATOGRAPHIC STUDY OF THE PHENOLIC ACID

In an attempt to determine if the phenolic acid were a pure material, an extensive chromatographic study was undertaken. Fifty-two solvent mixtures were used to develop the paper strips. To locate the position of the phenolic acid, the strips were sprayed with the ferric chloride-potassium ferrocyanide spray of Gardner (1, p.249). Results of this study are summarized in Table II.

Of all the solvent systems studied, mixtures of acetone, methyl isopropyl ketone, water and concentrated hydrochloric acid were the most useful. The mixture of butanol, pyridine and water also provided a well defined spot in contrast to most of the other solvent mixtures tried.

With the exception of the above named solvents, the results of the chromatographic study may be summarized by three types of result; (a) the solvent failed to move the phenolic acid, (b) the solvent produced a streak over the entire length of the development, and (c) the phenolic acid was carried along the solvent front.

Results of the first and third types mentioned above have been used by lignin chemists as evidence for the

TABLE II

CHROMATOGRAPHY OF THE PHENOLIC ACID

Solvent Components	Ratio v/v	Results
Butanol: concentrated ammonium hydroxide	4:1	Streak*
Acetic acid: water	6:4	Streak
Butanol: acetic acid: water	1:3:6	Streak
Butanol: acetic acid: water	4:1:5	Streak
Acetone: methyl-ethyl ketone: water	1:3:4	
Butanol: pyridine: saturated sodium chloride solution	1:1:2	Streak
Ligroin: benzene: methanol: water	50:5:1:50	Spot at R_f 0.00
Hexane saturated with 90% formic acid		Spot at R_f 0.00
Benzene saturated with 90% formic acid		Spot at R_f 0.00
Chloroform: methanol: hexane: water	2:1:7:5	Spot at R_f 0.00
Chloroform: acetic acid: water (aq layer)	3:4:3	Streak
Organic layer of the solvent above		Streak
Chloroform: acetic acid: water		Eight different ratios all produced streaks
Butanol: acetic acid: water	1:1:1	Spot at R_f 0.01-0.05
Butanol: acetic acid: water	4:1:1	Streak
Butanol: acetic acid: water	4:4:1	Streak
Butanol: acetic acid: water	4:2:1	Streak
Butanol: acetic acid: ethanol	1:1:1	Streak
Ethanol: acetic acid: chloroform	1:2:2	Streak
Ligroin: ethanol: formic acid	3:5:1	Spot at R_f 0.00
Ethanol layer of the above solvent		Streak

TABLE II (Continued)

Solvent Components	Ratio v/v	Results
Butanol: ethanol: concentrated ammonium hydroxide	50:50:1	Spot at R_f 0.00
Ethanol: concentrated ammonium hydroxide	99:1	Spot at R_f 0.00
Ethanol: concentrated hydrochloric acid	99:1	Spot at R_f 1.00
Butanol: ethanol: concentrated hydrochloric acid	50:50:1	Streak
Ligroin: ethanol: concentrated hydrochloric acid	1:50:1	Spot at R_f 1.00
Acetone: ethanol: concentrated hydrochloric acid: 3 methyl 2 butanone	1:1:1:1	Streak
Acetone: methyl isopropyl ketone: water	140:70:40	Streak
Acetone: methyl isopropyl ketone: water: concentrated hydrochloric acid	140:70:40:2	Spot at R_f 0.75-1.00
Acetone: water	1:5	Streak (faint) with most of the material at R_f 0.00
Acetone: concentrated hydrochloric acid: water	10:1:50	Streak
Methyl ethyl ketone: water	1:5	Spot at R_f 0.00
Acetone: methyl-isopropyl ketone: water: concentrated hydrochloric acid	60:40:40:1	Streak
Dioxane: acetone: concentrated hydrochloric acid	6:1:2:1	Spot at R_f 1.00
Acetone: methyl-isopropyl ketone: water: concentrated hydrochloric acid	7:4:2:1	Short streak from R_f 0.88-1.00

TABLE II (Continued)

Solvent Components	Ratio v/v	Results
Acetone: methyl-isopropyl ketone: water: concentrated hydrochloric acid	7:1:6:1	Spot at R_f 1.00
Acetone: methyl-isopropyl ketone: water: concentrated hydrochloric acid	5:2:2:1	Spot at R_f 1.00
Acetone: methyl-isopropyl ketone: water: concentrated hydrochloric acid	7:4:1:2	Spot at R_f 0.97
Butanol: pyridine: water	10:3:3	Faint spot at R_f 0.00 and well defined strong spot at R_f 0.89

* Unless further defined, the streak was from R_f 0.00-1.00

purity or homogeneity of lignin preparations (8, p.306). This is in agreement with the results from the solvent systems mentioned previously which indicated that the phenolic acid was pure. (Butanol, pyridine, water did show a very faint spot other than that for the main fraction of the phenolic acid, see Table II.) The streaking shown by the remainder of the solvent systems has been attributed to the ionization of compounds in the mixtures being chromatographed. Attempts to remedy this problem are usually made by the addition of an acid strong enough to prevent ionization. As may be noted from Table II, however, even the addition of hydrochloric acid did not prevent streaking in a large number of cases. In summary, the chromatography does indicate that this preparation is homogeneous, although the evidence is not completely satisfactory.

EXPLORATORY DEGRADATION REACTIONS

Since very little is known of the structure of phenolic acids, a series of exploratory degradations were carried out in order to find a reaction which would produce a significant yield of identifiable fragments.

Ethanolysis

In the study of wood lignin one of the more informative degradation reactions which have been employed is

ethanolysis. It seemed desirable to try this reaction since it is one of the few which yield phenyl propane type compounds rather than reducing the lignin to simple derivatives with a benzene nucleus. Therefore, this reaction was carried out on a sample of the bark phenolic acid according to Hibbert's procedure (14, p.1178). His isolation procedure applied to this reaction mixture yielded only a trace of ethanolysis products.

Nitration and Nitric Acid Oxidation

Nitric acid oxidation and nitration followed by nitric acid oxidation procedures were employed under a variety of conditions. The nitric acid oxidations were carried out by the addition of nitric acid to the phenolic acid, and by the addition of phenolic acid to solutions of nitric acid. Solutions from 25 to 47 percent nitric acid were used for these oxidations. Generally these oxidations produced amorphous materials which varied from a light yellow to a dark red-brown. All of the products isolated carbonized rather than melting when heated on the melting point block. Further oxidation produced oxalic acid as the only crystalline product. This product was isolated in yields as high as 25 percent based on the phenolic acid.

Nitration at -10°C in a mixture of concentrated sulfuric and nitric acids prior to nitric acid oxidation

did not improve the results. Methylation of the phenolic acid previous to treatment with the nitric acid retarded the oxidation, but it was still not possible to isolate any fragments of molecular size between the amorphous nitro-products and the oxalic acid. These results closely parallel those obtained in the treatment of lignin with nitric acid. There have been few instances where products other than oxalic acid, acetic acid, carbon dioxide and amorphous nitrolignins have been isolated from the nitric acid oxidation of lignin (3, Chap.12).

Permanganate Oxidation

While oxidation of a phenolic material with potassium permanganate might not be expected to be a good degradation method, successful application has been made in the field of crystalline coloring matters isolated from plant materials (24, p.138). Since the bark contains materials of this nature, it is possible that the phenolic acid is related to these compounds. Therefore, it seemed that permanganate oxidation should be included in the exploratory phase.

Two different conditions of pH were investigated, oxidation in one percent sodium hydroxide solution and oxidation in solution just alkaline enough to dissolve the phenolic acid, that is, pH 8-9. No differences could be detected from these differences in pH.

The potassium permanganate was added portionwise, one gram at a time. The solutions were maintained at 60-70°C until they were decolorized. Then the solutions were filtered to remove the manganese dioxide. The filtrates were acidified and filtered again to remove the apparently unreacted phenolic acid and the filtrate was then extracted with ether. The unreacted phenolic acid was dried and extracted with ether also. This process was repeated until there was no precipitate when the oxidation mixture was acidified, which required five to seven grams of permanganate for each gram of phenolic acid. The ether extracts were combined, dried over sodium sulfate and evaporated to dryness. The maximum yield obtained was ten percent based on the phenolic acid. These oxidation products had no phenolic character as evidenced by negative tests with ferric chloride and bis diazotized benzidine reagents.

Evolution of carbon dioxide on acidification of the oxidation mixtures indicated that a portion of the phenolic acid had been completely oxidized with each gram of permanganate. There was also the production of a water soluble, ether insoluble fraction. Isolation was accomplished by evaporation of the aqueous solutions to dryness under vacuum, followed by treatment of the residue with alcohol. Traces of oxalic acid were isolated, but the water soluble fraction consisted of a dark red powder when dry. It was apparently made up of intermediate

molecular weight material, since it was of an infusible character. The aqueous solutions from which this substance was isolated indicated that it was quinoid in nature by the color changes which took place with changes in pH.

Alkaline Hydrolysis

The chromone structure is frequently found in natural products isolated from bark and wood. To determine if the phenolic acid contained this structure, samples were submitted to hydrolysis in solutions of 30-70 percent potassium hydroxide, this being a standard degradation for chromone compounds, splitting them into two fragments through a retrograde Claison condensation (9, p.258).

Both the methylated and the unmethylated phenolic acid samples were submitted to alkaline hydrolysis. A series of experiments were performed where the concentration of potassium hydroxide varied from 30-70 percent and the period of reflux varied from 30 minutes to four hours. This reaction failed to produce any detectable change in the phenolic acid. Acidification of these reaction mixtures precipitated a material of the same color and amorphous character as the starting material.

Alkaline Fusion

While alkaline fusion is quite drastic, it has been

frequently used in the study of tannins, lignin, crystalline coloring matters and other natural products. Thus it was included in the exploratory investigation.

An initial fusion indicated that the yield of simple phenols and phenolic acids was quite high, so further experiments were carried out to determine the best conditions for this reaction. Table III summarizes the conditions and yields obtained from these experiments.

TABLE III

ALKALINE FUSION OF THE PHENOLIC ACID

Phenolic Acid	Water	KOH	NaOH	Temp.	Time	Ether Soluble Yields	
						Phenols	Acids
gm	gm	gm	gm	°C	min.	%	%
5	5	25	25	270	25	18.0	40.0
10(a)	0	18	12	205-220	30	12.0	48.0
5(b)	2	20	13	200	10	12.0	46.0
20	0	33	22	200	60	23.0	30.0
10(c)	0	16.2	10.8	200	10	7.0	16.5
10(d)	10	33	22	200	30	6.1	18.8

(a) The phenolic acid was added when the temperature reached 170°C.

(b) The phenolic acid was added when the temperature reached 165°C. The temperature was then raised to 200°C within ten minutes and the reaction mixture was rapidly cooled.

(c) Same as (b) but the sample was Douglas fir cork phenolic acid.

(d) Sample was Douglas fir cork phenolic acid.

The fusion mixtures were poured into water while still warm, then neutralized with sulfuric acid (1:1). The solutions were made slightly basic and reacidified with carbon dioxide. Liquid-liquid extraction with ether over a period of 10-30 hours removed the phenols, then the pH was adjusted to 1.0 and the phenolic acids were extracted with ether in the liquid-liquid extraction apparatus. The crude yields reported in Table III were obtained by evaporation of aliquots of these extracts.

Chromatographic studies using, (a) carbonic acid (1, p.249), (b) butanol, acetic acid, water (4:1:5) (2, p.430) and (c) n-butanol, t-butanol, buffer pH 8.75 (4:4:1) (35, p.346) as the developing solvents showed the presence of phloroglucinol, catechol and protocatechuic acid in the mixture of fusion products of each of the runs shown in Table III. These compounds were checked by chromatographing known compounds adjacent to the unknowns and by the specific color produced when the sheets were sprayed with bis diazotized benzidine. This reagent gives an intense purple with phloroglucinol, a light brown with catechol and a yellow-orange with protocatechuic acid. The chromatograms also showed the presence of three or four more acids which were not identified. Comparison with para-hydroxybenzoic, vanillic, gallic and alpha resorcylic acids showed that none of these corresponded to the unknown acids from the fusion mixture. Fusions

three and five were carried out under conditions which Pearl (27, pp.376-377) found would not cause demethylation of vanillin. Since the same products were found under these conditions as under the more drastic conditions, they must be derived from the free phenolic nucleus and not the methoxyl containing nucleus of the original phenolic acid. Another point of interest is that the Douglas fir and white fir both yield the same products on alkaline fusion, however, the yield of ether soluble material is considerably greater in the case of the white fir.

Methods of purification tried in attempts to isolate the fusion products included cellulose column chromatography, ion exchange columns, neutral lead acetate precipitation and fractional precipitation. The cellulose column produced no noticable separation. Lead acetate precipitated all of the phenolic material including the tar and phloroglucinol rather than selecting the ortho-dihyroxy phenols. An anion exchange column of Duolite A-7 charged with sodium hydroxide removed a small amount of the tarry substance, however, acidification of the fractions collected from the column still contained black tar-like products. As a last resort, since most of the simple phenols are water soluble, the fusion products were taken up in boiling water and treated with activated charcoal. This was repeated until the solutions were

light yellow to colorless. The aqueous solutions were extracted with ether, dried over sodium sulfate and evaporated to dryness. After this treatment, the mixture of phloroglucinol and catechol was only one to two percent based on the phenolic acid, while the protocatechuic acid, along with oxalic acid amounted to four to six percent of the phenolic acid. The other phenolic acids detected in the chromatograms of the fusion products prior to the charcoal treatment had been removed. These acids must have been of a higher molecular weight since their water solubility was not great enough to keep them from being adsorbed by the charcoal. It is also possible that the yield of identified products was greater than indicated since the charcoal treatment may have removed or caused the degradation of these compounds.

Nitrobenzene Oxidation

High temperature nitrobenzene oxidation has been applied to the study of bark phenolic acids from spruce and Douglas fir. No vanillin or other products were identified from the oxidation of the spruce bark phenolic acid. Vanillin (2.60 percent), protocatechualdehyde (0.79 percent), syringaldehyde (trace) and para hydroxybenzaldehyde (trace) were the only products isolated from the nitrobenzene oxidation of the phenolic acid from Douglas fir bast fibers and only the first two of these

compounds (0.60 percent and 0.66 percent) were found in the oxidation mixture obtained from Douglas fir cork phenolic acid. The formation of a high yield of tarry material was also reported in the oxidation of Douglas fir phenolic acids. This might have been due to the high temperature employed for this reaction, so an atmospheric pressure nitrobenzene oxidation was carried out on the white fir cork phenolic acid.

The reaction was performed by dissolving five grams of the phenolic acid in 100 milliliters of five percent sodium hydroxide solution, adding 15 milliliters of nitrobenzene and refluxing the mixture vigorously for eight hours. The cooled mixture was treated in accordance with Pew's (34, p.2833) procedure for the separation of products from alkaline nitrobenzene oxidation as shown in Figure 1. All fractions except B solids were chromatographed on sheets of number one Whatman paper using butanol saturated with two percent ammonium hydroxide as the developing solvent. The chromatograms were observed under ultra violet light and then sprayed with 2,4 dinitro-phenylhydrazine and ferric chloride. The yields and number of compounds present in each fraction are shown in Table IV.

The compound responsible for the spot at R_f 0.41 in the C-bisulfite, C-sodium hydroxide and D-sodium hydroxide soluble fractions was vanillin. This was shown by

FIGURE 1

SEPARATION OF NITROBENZENE OXIDATION MIXTURE

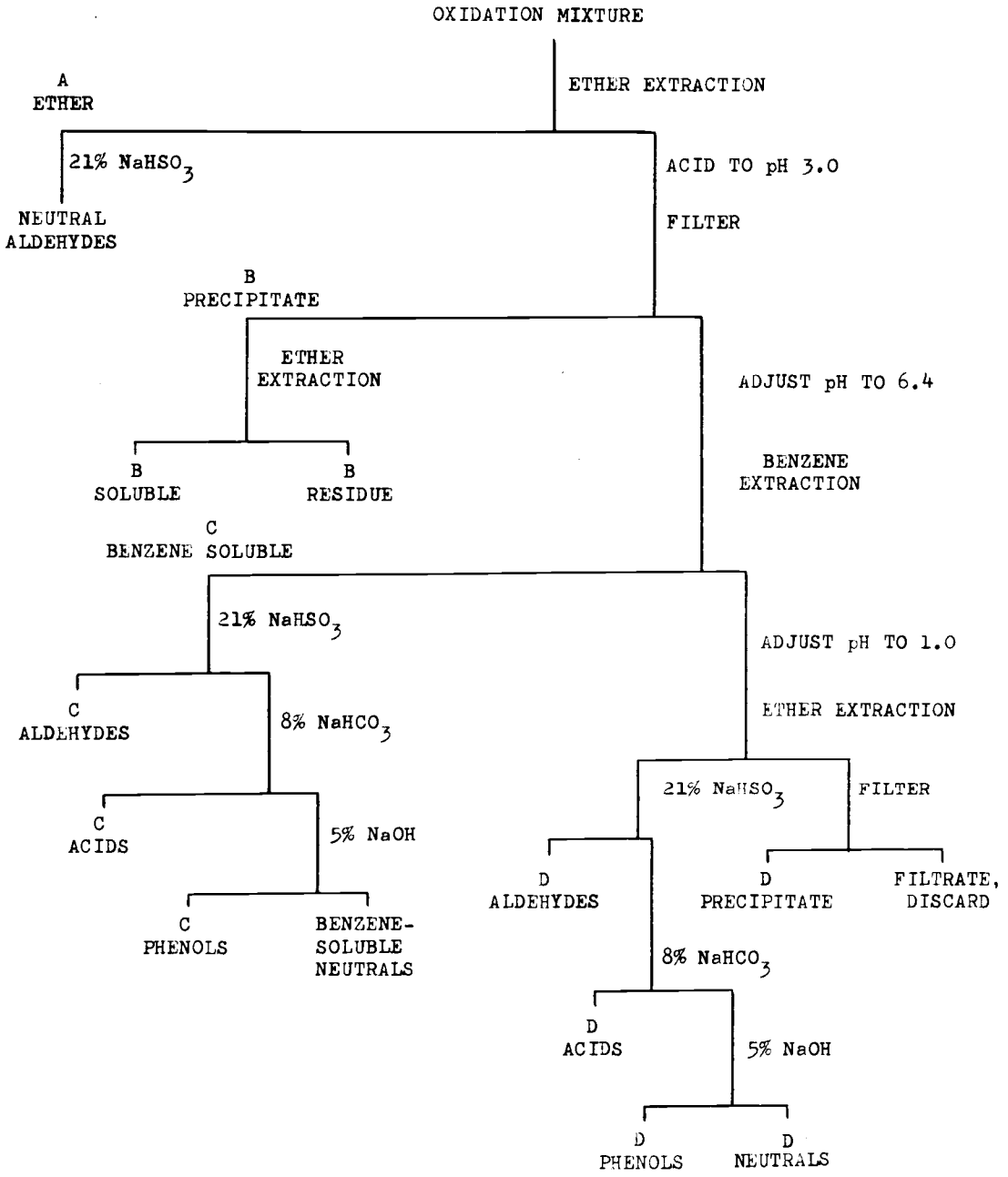

TABLE IV

PRODUCTS FROM ALKALINE NITROBENZENE OXIDATION OF PINE OLIC ACID

Fraction	Yield gm	Yield %	Chromatography R_f Values
A Bisulfite Soluble	0.00	0.00	-----
B Solids	1.30	26.00	-----
B Ether Soluble	0.50	10.00	0.00-0.03; 0.10
C Bisulfite Soluble	0.01	0.20	0.01; 0.04; 0.41
C Bicarbonate Soluble	0.01	0.20	0.02; 0.13
C Sodium Hydroxide Soluble	0.01	0.20	0.17; 0.18; 0.23; 0.42
C Neutral	0.00	---	-----
D Bisulfite Soluble	0.045	0.90	0.01; 0.02; 0.13-0.17
D Bicarbonate Soluble	0.01	0.20	0.02; 0.03; 0.05; 0.06; 0.13; 0.25
D Sodium Hydroxide Soluble	0.005	0.10	0.05; 0.12; 0.27; 0.41

comparison chromatography and later in this work by isolation and positive identification. The compound at R_f 0.02 and 0.13-0.17 also gave positive reactions with the 2,4 dinitro-phenylhydrazine reagent. These R_f values correspond with those reported by Pearl for 5-formyl vanillic acid and 5-carboxy vanillin respectively (28, p.2225).

While the yield from the high temperature alkaline nitrobenzene oxidation of Douglas fir cork phenolic acid was not high, it was much better than the yield from the

oxidation described above. Therefore, a high temperature nitrobenzene oxidation of the white fir cork phenolic acid was carried out and analyzed qualitatively for comparison with Douglas fir cork phenolic acid oxidation products. Table V summarizes the conditions and yields obtained from three runs carried out under slightly different conditions.

TABLE V

ALKALINE NITROBENZENE OXIDATION OF PHENOLIC ACID AT 160°C

Phenolic Acid	Nitro-benzene	Temp.	Time	Water	Sodium Hydroxide	Ether Soluble Yield
gm	cc	°C	hr	cc	gm	%
5	12	160	3	60	6.5	28.0
5	6	160	3	120	12	30.0
5	15	160	3	120	4.8	38.0

The ether soluble oxidation products were chromatographed using butanol saturated with two percent ammonium hydroxide and a solvent made up of benzene, ligroin, (d. 0.67-0.69), methanol and water (v/v 50:50:1:50) (22, p.454). The developed chromatograms were inspected under ultra violet light and then sprayed with 2,4 dinitophenylhydrazine and bis diazotized benzidine reagents. By means of comparison with knowns, the compounds which gave positive reactions with 2,4 dinitro-phenylhydrazine were vanillin, para hydroxybenzaldehyde and

protocatechualdehyde. Bis diazotized benzidine gave a positive color reaction for vanillic acid, although the spot was quite weak. This spray reagent also showed the presence of four other phenolic components, however, the spots were quite weak again. In addition, there were five other spots observed under ultra violet light. Neither of the spray reagents produced any reaction with these spots. This must mean that they were not due to phenolic compounds since the bis diazotized benzidine is a very powerful spray reagent for the detection of the hydroxyl activated benzene nucleus.

An attempt to isolate the aldehydes for melting point determinations and derivative formation was unsuccessful due to the low yield of these products.

It should be pointed out that the yields indicated in Table V are misleading. During the attempts to isolate the aldehydes, it was discovered that about one-third of the ether soluble material was composed of para-hydroxy azobenzene, one of the alkali soluble nitrobenzene reduction products. This compound was isolated by extraction of the oxidation products with boiling hexane. As the hexane cooled, a bright orange precipitate formed. After recrystallization this material melted at 145-148°C (literature value 150-151°C) (34, p.2833). The extraction removed most of this compound which reduced the yield of oxidation product to 10-25 percent based on the phenolic acid.

Mercuric Oxide Oxidation

Pearl, in his investigations of basic calcium lignosulfonates, found that mercuric oxide was capable of producing yields of 54-60 percent of low molecular weight degradation products (26, p. 2197). The products he obtained were similar to those produced by alkaline nitrobenzene, with the difference that most of the vanillin type nucleus was isolated as vanillic acid rather than vanillin. The high yield from this oxidation indicated that it might be a better oxidant than the nitrobenzene, and it was decided to include this reaction in the exploratory experiments.

Pearl's procedure for the oxidation and treatment of the oxidation mixture, with slight modification, was followed as described below. To a solution of five grams of phenolic acid and 30 grams of sodium hydroxide in 200 grams of water, were added 37.5 grams of mercuric oxide. The mixture was heated over a steam bath for 48 hours, then refluxed for five hours. The cooled mixture was saturated with sulfur dioxide. With continued addition of sulfur dioxide, the mixture was heated to boiling to break up mercury derivatives of the oxidation products. After boiling for five minutes, the mixture was cooled and extracted continuously with ether for 12 hours. The ether solution was labeled fraction A. The sulfur dioxide saturated aqueous solution was filtered to remove the

mercuric oxide reduction products which included a small amount of free mercury. The filtrate was acidified to pH 1.0 with sulfuric acid (1:1) and placed on a hot water bath with a stream of carbon dioxide passing through the solution to remove the sulfur dioxide. The sulfur dioxide free solution was extracted continuously with ether for ten hours. The ether solution was labeled fraction B.

Fractions A and B were further separated into the acidic, phenolic, aldehyde and neutral fractions by standard procedures. The yields of these fractions are shown in Table VI.

TABLE VI

YIELD OF MERCURIC OXIDE OXIDATION PRODUCTS

Fraction A	27.52%
Bicarbonate Soluble	26.10%
Sodium Hydroxide Soluble	1.42%
Neutral	Trace
Fraction B	12.58%
Bisulfite Soluble	8.57%
Bicarbonate Soluble	2.13%
Sodium Hydroxide Soluble	1.88%
Neutral	Trace
Total Yield	40.00%

Composition of the fractions listed in Table VI was studied by chromatography. The developing solvent was butanol saturated with two percent ammonium hydroxide. Detection of components on the developed sheets was done

in the same manner as in the case of the nitrobenzene oxidation described above. Results of the chromatography are shown in Table VII.

The most intense spot on each of the chromatograms was due to the material which remained at R_f 0.00. This is characteristic for very polar or higher molecular weight compounds such as 5 carboxy vanillic acid and dehyrodivanillic acids in the case of this particular solvent. The solubility characteristics of these fractions lead to the conclusion that high molecular weight is the reason for the high concentration of material in these fractions that remain at R_f 0.00.

All fractions which gave evidence for the presence of vanillin were extracted with boiling hexane (Skelly B) and chloroform. The solutions were combined and evaporated to dryness. A light tan crystalline residue of 59 milligrams was recovered. The melting point range for this material was 69-73°C. A chromatogram gave spots corresponding to vanillin and p-hydroxybenzaldehyde, the spot due to vanillin being of much greater intensity than that from p-hydroxybenzaldehyde. Sublimation at atmospheric pressure and 100°C raised the melting point to 75-78°C. There was still a faint spot for p-hydroxybenzaldehyde on a paper chromatogram of the sublimate. Thirty-eight milligrams of sublimate were collected, at which time the residue in the sublimation chamber was quite dark. This

TABLE VII

CHROMATOGRAPHY OF MICROBIC OXIDE OXIDATION PRODUCTS

	Rf	U.V.	BDB	2,4 D	Compound
Fraction A					
Bicarbonate	0.00	Dark	Red	-	
Soluble	0.01	Orange	Red	-	
	0.02	Blue	Red	Yellow-Orange	5-formylvanillic Acid?
	0.04	Yellow	Brown	-	
	0.06	Blue	Red	-	
	0.08	Blue-Green	Orange-Brown	-	Vanillic Acid
	0.11	Blue	Violet	-	
	0.14	Invisible	Pink	-	
Fraction A					
Sodium Hydroxide	0.00	Dark	Red	Orange	
Soluble	0.05	Invisible	-	Orange	
	0.33	Blue	Red-Brown	Red	Vanillin
	0.43	Invisible	Invisible	Red-Orange	p-hydroxybenzaldehyde
Fraction B					
Bicarbonate	0.00	Dark	Red	Orange	
Soluble	0.02	Blue	Red	Red	5-formylvanillic Acid?
	0.05	Yellow-Green	Red	-	
	0.07	Invisible	Orange-Brown	-	
	0.09	Blue	Violet	-	

TABLE VII (Continued)

	Rf	U.V.	BDB	2,4 D	Compound
	0.33	Blue	Red-Brown	Red	Vanillin
	0.42	Invisible	Invisible	Orange	p-hydroxybenzaldehyde
Fraction B					
Bisulfite	0.00	Dark	Red	Orange	
Soluble	0.02	Green	Invisible	Red	5-formylvanillic Acid?
	0.07	Blue	Orange	Invisible	
	0.35	Blue	Invisible	Red	Vanillin
Fraction B					
Sodium Hydroxide	0.04	Invisible	Pink	Invisible	
Soluble	0.35	Invisible	Invisible	Red	Vanillin

residue still had a strong odor of vanillin, however, higher temperature produced a very small amount of orange oily sublimate.

The overall yield of this reaction was the highest obtained in the exploratory degradations carried out at this time. The oxidation mixture was also the most interesting from the standpoint of the number of components it contained. While it was felt that this reaction deserved further study, the most suitable apparatus for a large scale oxidation was constructed of metal. Therefore an investigation of cupric oxide was carried out before deciding which method should be used in a large scale oxidation.

Cupric Oxide Oxidation of the Phenolic Acid

Pearl has recently published a series of reports on the use of cupric oxide in the oxidation of calcium lignosulfonates (29, 30 and 33). His work also includes studies on the oxidation of model compounds which would be valuable in the interpretation of results from the use of this oxidant. Pearl has also made an extensive study of the variables involved using fermented spent sulfite waste liquor as experimental material (30). The ratio of reactants which were optimum for the production of vanillin were arbitrarily chosen for the oxidation of the phenolic acid.

The oxidation mixture, consisting of ten grams of phenolic acid, 30 grams of sodium hydroxide, 90 grams of cupric oxide and 450 grams of water was placed in the bomb from a hydrogenation apparatus, American Instrument Company model 406-801 DA., equipped with heating jacket and shaking mechanism. The bomb was placed in the heating jacket and raised to 170°C over a period of one hour. The reaction, or the continued effect of the heating jacket, carried the temperature up to 190°C. After 30 minutes the temperature dropped to 170°C. The temperature was then maintained at 170-180°C for another 2.5 hours. The mixture required three hours to cool to room temperature. When cool, the copper containing residue was removed by filtration and washed thoroughly with hot water. The dry residue weighed 66.0 grams. There was no evidence of any copper in the solution, so the cupric oxide must have been completely reduced to the cuprous state.

The filtrate and washings were saturated with sulfur dioxide and continuously extracted with ether for 15 hours. The sulfur dioxide saturated solution was then acidified with sulfuric acid (1:1) to pH 1.0 and placed on a hot water bath with carbon dioxide bubbling through the mixture to remove the sulfur dioxide. After removal of the sulfur dioxide, ether extraction was continued for 30 hours. These ether solutions were further separated into bisulfite soluble, bicarbonate soluble, sodium

hydroxide soluble and neutral fractions by standard procedures. The yields of these products are shown in Table VIII.

TABLE VIII

YIELD OF COPPER OXIDE OXIDATION PRODUCTS

Sulfur Dioxide Acidification	
Bisulfite Soluble	0.7%*
Bicarbonate Soluble	7.4%
Sodium Hydroxide Soluble	3.0%
Neutrals	Trace
Sulfuric Acid Acidification	
Bisulfite Soluble	35.0%
Bicarbonate Soluble	10.9%
Sodium Hydroxide Soluble	2.0%
Neutrals	0.2%
Total Yield of Ether Soluble	59.2%

* Probably due to incomplete saturation of the original oxidation mixture with sulfur dioxide.

Each of these fractions were chromatographed in the same way as the mercuric oxide oxidation products, with the exception that these fractions were run in triplicate and ferric chloride was used as a spray reagent in addition to the bis diazotized benzidine and 2,4 dinitrophenylhydrazine. Results of chromatographic examination are shown in Table IX.

The compounds without question marks, listed in Table IX, were labeled from results of running comparison compounds adjacent to the mixture. Compounds followed by a

TABLE IX

CHROMATOGRAPHY OF COPPER OXIDE OXIDATION PRODUCTS

Rf	U.V.	2,4 D	FeCl3	BDB	Compound
Sulfur Dioxide Acidification					
Bisulfite Soluble					
0.00	Dark	--	Black	Red	
0.02	Yellow	--	--	Red	
0.05	Blue	--	--	Pink	
0.07	Yellow-Green	--	--	Yellow	
0.43	Blue	Red	Green-Violet	Red-brown	Vanillin
0.52	Invisible	Orange	--	--	p-hydroxybenzaldehyde
Bicarbonate Soluble					
0.00	Dark	--	Black	Red	
0.02	Yellow	--	Red-Brown	Brown	
0.05	Green	--	Gray-Green	Pink	
0.07	Yellow	--	--	Red	
0.10	--	--	--	Brown	Vanillic Acid
0.12	--	--	--	Yellow	p-hydroxybenzoic Acid
0.20	Blue	--	Gray-Green	Orange-Brown	
0.25	Blue	--	--	Pink	
Sodium Hydroxide Soluble					
0.07	--	--	Gray	Orange-Yellow	
0.15	Brown	Red	Green	--	Protocatechualdehyde?
0.25	--	--	Brown	--	

TABLE IX (Continued)

Rf	U.V.	2,4 D	FeCl3	BDB	Compound
Sodium Hydroxide Soluble (Continued)					
0.44	Blue	Red	Violet	Red-Brown	Vanillin
0.54	Blue	Orange	--	--	p-hydroxybenzaldehyde
0.60	--	--	--	Red-Brown	
0.67	Blue	--	Red	Red-Brown	Acetovanillone?
Sulfuric Acid Acidification					
Bisulfite Soluble					
0.00	Dark	Orange	Black	Red	
0.01	Yellow	Gray	Gray	--	
0.03	Blue	--	--	--	
0.04	Blue	--	--	--	
0.36	Blue	Orange	--	--	Syringaldehyde?
0.43	--	Red	--	Red-Brown	Vanillin
Bicarbonate Soluble					
Same as the sulfur dioxide bicarbonate soluble fraction.					
Sodium Hydroxide Soluble					
0.14	Blue	--	--	--	
0.42	Blue	Red	Violet	Red-Brown	Vanillin
0.52	--	Orange	--	Red-Brown	p-hydroxybenzaldehyde

question mark were indicated by Rf values reported in the literature (28, p.2225).

Further chromatography with ligroin, n-butyl ether, water (6:1:1) (37, p.772) was carried out on the fractions containing aldehydes. A known mixture of vanillin and p-hydroxybenzaldehyde was used for comparison. Both of these aldehydes were present in the two sodium hydroxide soluble fractions and in the bisulfite soluble fraction from the sulfur dioxide acidification. The bisulfite soluble fraction from sulfuric acid acidification gave a very weak spot corresponding to vanillin, and a strong spot for another aldehyde.

Extraction of the hydroxide soluble fractions with boiling hexane removed 0.150 gram which remained as light tan crystals on evaporation of the hexane. The crystals melted over the range of 63-73°C. Chromatography showed that the material was a mixture of vanillin and p-hydroxybenzaldehyde. An attempt to separate the mixture by sublimation was not successful. Two batches of pale yellow crystals were collected amounting to 95 and 46 milligrams. Both melted over a range between 63 and 73°C. The vanillin in the sublimate was determined by the method of Stone and Blundell (37, p.773), which involves separation by chromatography, elution of the vanillin and determination of the vanillin by spectrophotometric means. The vanillin in these two fractions by this determination was 58.1 and 23

milligrams respectively. The p-hydroxybenzaldehyde by difference was 36.9 and 23 milligrams respectively.

The hydroxide soluble residue from hexane extraction was a dark red sticky material. There was still evidence for traces of vanillin and p-hydroxybenzaldehyde. However, it could not be extracted with hexane. Boiling benzene removed 80 milligrams of a yellow-orange sticky material which could not be crystallized. When taken up in water and treated with an excess of 2,4 dinitro phenylhydrazine reagent, a precipitate formed. After recrystallization twice from alcohol-water, the hydrazone melted at 267-270°C. This corresponds to the melting point for the 2,4 dinitro phenylhydrazone of vanillin (36, p.230). Twenty-four milligrams of the hydrazone were collected. This increased the vanillin content of the hydroxide soluble fraction by ten milligrams.

The bisulfite fraction from sulfur dioxide acidification was also extracted with hexane. Evaporation of the hexane left an oily film. This was taken up in water and treated with 2,4 dinitro phenylhydrazine as described above. The precipitate amounted to 25 milligrams which corresponds to 10.3 milligrams of vanillin, however this hydrazone was due to both vanillin and p-hydroxybenzaldehyde.

The residue from the hexane extraction was a dark red. Attempts to isolate further compounds from this

residue or the residue from the hydroxide soluble fraction were unsuccessful.

Concentration of the bisulfite fraction from sulfuric acid acidification caused the precipitation of needle shaped crystals. The crystals were removed by filtration and washed with a small amount of cold ether. This residue removed by filtration was a mixture of needles and a red gummy substance. A sample of the needles was separated and heated on the melting point block, however, they sublimed completely before the melting point was reached. To separate this compound from the red material, the mixture was submitted to sublimation at 100°C. Three tenths of a gram of colorless crystals were collected with a melting point of 186-188°C. Treatment with concentrated sulfuric acid decomposed this compound with evolution of a gas, and a mixed melting point determination with oxalic acid caused no depression in the melting point.

The red sticky portion of the mixture had changed to a red-brown powder during the sublimation. A test of this powder showed that it was infusible and it was discarded.

The filtrate from the separation of the oxalic acid was taken to dryness and successively extracted with benzene, chloroform and anhydrous ether. The residue was taken up in acetone. These solvents removed 0.02 gram, 0.20 gram, 1.45 grams and 1.35 grams respectively.

Evaporation of the benzene left a semisolid, from which 16 milligrams of the two aldehydes reported above were isolated by sublimation. No attempt was made to determine the yield of the individual compounds.

The remaining three solutions were examined chromatographically with butanol saturated with two percent ammonium hydroxide as the developing solvent. The acetone fraction showed a single spot at R_f0.00, while the other two fractions showed very weak spots at R_f 0.01, 0.03 and 0.28 in addition to the strong spot at R_f 0.00. These spots correspond to the reported R_f values of 5-formyl vanillic acid (28, p.2225), vanilloyl formic acid (shown later) and 5-carboxy vanillin (28, p.2225).

From previous experience with the paper chromatograms, spots as weak as those observed above indicate the presence of only trace amounts of these compounds.

In an attempt to separate these aldehydes from the mixture, the three fractions were recombined, evaporated to a black sticky residue and taken up in butanol-two percent ammonia. The solution was slurried with a small amount of cellulose powder and applied to the top of an 18x300 millimeter cellulose column. Solvent was added to the column until the colored material reached the bottom. The column was then extruded and arbitrarily separated into seven fractions. The cellulose was eluted with acetone-concentrated hydrochloric acid (v/v 10:1).

Examination of the eluates by paper strip chromatography showed little or no separation. Several attempts were made to crystallize the component causing the spot at R_f 0.00, however all precipitates that were obtained were infusible or softened and carbonized when heated. Apparently the R_f 0.00 compounds were a mixture with molecular weights between that of the original phenolic acid and the simple benzene derivatives.

The bicarbonate soluble fractions from both the sulfur dioxide and sulfuric acid acidifications were combined, evaporated to dryness, taken up in alcohol and treated three times with activated carbon without appreciably decreasing the color of the solution. The solution was again evaporated to dryness and extracted successively with hot benzene and chloroform. These solvents removed only trace quantities.

The residue was then taken up in butanol-two percent ammonia, slurried with cellulose, applied to the top of a cellulose column 18x300 millimeters and developed with the above solvent. The development was continued until nine fractions had been collected. The column was then extruded and eluted to make fraction number 10. Fractions 1, 2 and 3 did not contain any of the components from the original mixture. Fractions 4 and 5 contained most of the material which moved beyond R_f 0.00. These fractions were of the same composition, producing identical paper strip

chromatograms. Fractions 6-9 were composed of the material causing the spot at R_f 0.00 and a small amount of the compound producing a spot at R_f 0.08 on the paper strip chromatograms. The eluate from the cellulose gave only one spot at R_f 0.00 on the paper strip chromatogram.

Fractions 4 and 5 were recombined (0.69 gram) and placed on a fresh cellulose column. Eleven fractions were collected from solvent flow, with the eluate from the extruded cellulose making up fraction 12. The yields and results of paper chromatographic examination of these fractions are shown in Table X.

TABLE X

PAPER CHROMATOGRAPHIC EXAMINATION OF FRACTIONS FROM COLUMN CHROMATOGRAPHY OF ACIDIC FRACTION

Fraction	Yield gm	R_f Values For The Components of These Fractions
1, 2 and 3	Trace	----
4	0.02	0.00, 0.13
5	0.07	0.00, 0.06, 0.13, 0.23, 0.40
6	0.09	0.00, 0.06, 0.08, 0.13, 0.23, 0.40
7	0.11	0.00, 0.02, 0.08, 0.12, 0.16, 0.24, 0.40
8	0.09	0.00, 0.02, 0.08, 0.12
9	0.03	0.00, 0.06, 0.24
10 and 11	Trace	----
12	0.20	0.00

The table shows that cellulose powder columns are not as efficient as paper strips in the separation of complex mixtures. Pearl has found that the efficiency of cellulose columns as compared to paper is dependent on the mixture

(31, p.174). He has obtained good separations in some cases, whereas in others, his results are parallel with the results described above.

Because these fractions were complex and present in insufficient amount for fractional crystallization, a large scale separation was attempted by means of paper strip chromatography. Sheets of number one Whatman filter paper 18x150 centimeters were streaked along the narrow edge with fractions 7 and 8. The sheets were then developed with butanol-two percent ammonia. Narrow strips were cut from the developed sheets and sprayed with bis diazotized benzidine in order to locate the position of the bands. The narrow strip was then held next to the edge of the main sheet and the bands were marked. The mark was extended across the sheet with the aid of ultra violet light, which caused the bands to show up. The R_f values, color under ultra violet light and the color produced by bis diazotized benzidine for these bands are tabulated in Table XI.

This table lists more components than the original bicarbonate fraction contained. This is explained by the higher concentration of material on the chromatogram which will allow detection of components present in very small amounts. It also has the advantage over the original mixture in that a greater portion of the R_f 0.00 material

TABLE XI

RESULTS OF STREAK CHROMATOGRAPHY OF THE BICARBONATE SOLUBLE PRODUCTS FROM COPPER OXIDE OXIDATION

R_f	Ultra Violet Color	Bis Diazotized Benzidine
0.00	Dark	Red
0.02	Orange	Red-Brown
0.04	Yellow	Pink
0.06	Violet	Red
0.11	Green-Blue	Orange-Brown
0.16	Bright Blue	Violet
0.18	White	Pink
0.20	Blue	Orange
0.25	Blue	Pink
0.35	Green	Orange

had been removed, resulting in a higher concentration of the mobile compounds.

It should also be pointed out that the R_f values were not reproducible although the bands were always present in the same order. As an example, the compound responsible for the band at R_f 0.06 in the above table varied from sheet to sheet over an R_f range of 0.05. This caused no trouble, since all of the compounds had the same variation and it was easy to locate corresponding bands on the different sheets from the ultra violet fluorescence, and the color and intensity of the bands when sprayed with bis diazotized benzidine. This variation makes any identification by R_f values alone impossible. For identification purposes, authentic compounds should be added to the mixture and run as a separate spot adjacent to the mixture

when there is this variation of R_f values. Following this procedure, the bands at R_f 0.11 and 0.20 were identified tentatively as vanillic and p-hydroxybenzoic acids.

The bands indicated in Table XI were cut from the sheets. After the entire amount of fractions 7 and 8 had been separated in this fashion, corresponding bands from the different sheets were placed in solution of concentrated ammonium hydroxide, alcohol, water (v/v 40:30:60) and boiled for five minutes, filtered to remove the paper which was thoroughly washed with hot water. The filtrates were evaporated over in hot water bath to remove most of the alcohol; then the solutions were acidified with dilute sulfuric acid and extracted with ether. The ether solutions were dried and the ether was removed by distillation. In each case there was only a few milligrams of residue. Most of the fraction must have remained in the band at the point of application. This band was not extracted, having been discarded when the sheets were cut up.

All of the products from this separation were sticky semisolid materials. There was not sufficient material for further separation, however when they were spotted on paper and rechromatographed, a spot was observed on the sheet which corresponded to each of the bands. There was also a faint spot for the compounds of the next higher and the next lower R_f, indicating that there was some

overlapping in the bands. There was insufficient material remaining for further work on these fractions.

In summary, copper oxide oxidation produced a yield of 0.81 percent vanillin, 0.60 percent para-hydroxy benzaldehyde, 0.26 percent of a mixture of these two compounds and 3.0 percent oxalic acid as the identified compounds. Evidence for the acids corresponding to the two aldehydes mentioned above was quite strong, but positive identification was not achieved. For the comparison of R_f values alone, which is subject to question, the presence of protocatechualdehyde, syringaldehyde and acetovanillone were also indicated in trace quantities. The acid fraction from the oxidation mixture was shown to contain nine compounds along with a considerable amount of amorphous material.

LARGE SCALE COPPER OXIDE OXIDATION

From examination of the various exploratory degradation reactions, mercuric oxide and copper oxide oxidation appeared to give the best yields, and the most interesting mixture of products. While these products formed a rather complex mixture, it was believed that if a sufficient amount could be obtained, a separation procedure could be worked out. There was little basis on which to choose between the copper or mercuric oxide from the standpoint

of the type of oxidation products. Chromatograms of the oxidation products from these two reactions were nearly identical. Equipment available for the reaction favored the copper oxide, so this reagent was chosen for the large scale reaction.

A total of 100 grams of the phenolic acid was oxidized in 25 gram batches. Four runs were made using fresh material, and a fifth run was made with the "unoxidized" material from the first four runs.

The reaction was carried out by dissolving 25 grams of phenolic acid in a solution of 55 grams of sodium hydroxide in 800 milliliters of water. This solution was placed in the bomb described in the exploratory copper oxide oxidation. One-hundred fifty grams of copper oxide were added and the bomb was placed in the heating jacket. The temperature was carefully raised to 170°C, and maintained as close as possible to this temperature for three hours. The cooled reaction mixture was worked up as described previously. The ether extractions from the oxidation mixture were accumulated until the five oxidations had been completed. Then these ether solutions were separated into bisulfite, bicarbonate, sodium hydroxide and neutral fractions, a further separation into water soluble and water insoluble fractions was carried out to reduce the amount of dark red amorphous material causing the R_f 0.00 spot found on the chromatograms in the exploratory

oxidation. The yields of these fractions along with results of chromatographic examination are shown in Table XII.

The yield obtained from the large scale oxidation was slightly over half of that obtained from the exploratory reaction. This is probably due to the smaller ratio of copper oxide to phenolic acid and the better temperature control. The ratio of oxidant to phenolic acid was decreased to prevent the formation of oxalic acid, while the temperature was raised more slowly in order that 170°C was not exceeded since this was the temperature which Pearl had found optimum for the production of vanillin.

Bicarbonate Soluble-Water Soluble Fraction

A sample of 4.0 grams of the acid fraction from the sulfur dioxide acidification was taken up in acetone and diluted with an equal volume of water. This solution was passed through a column containing Duolite A-7 anion exchange resin which had been charged with sodium bicarbonate. The column was washed with a solution of acetone-water (v/v 1:1), then four percent bicarbonate solution and finally with a two percent solution of sodium hydroxide. A total of eleven fractions were collected. Table XIII contains the results of this separation.

TABLE XII

YIELDS AND CHROMATOGRAPHY OF THE OXIDATION PRODUCTS FROM COPPER OXIDE OXIDATION

Fraction	Yield	Rf	U.V.	2,4 D	BDB	
Ether Soluble From SO_2 Acidification	15.35					
$NaHCO_3$-H_2O Soluble	8.80	0.00	Dark	Brown	Red	
		0.02	Yellow	--	Red-Brown)	
		0.04	White	--	Red-Brown)	Streak
		0.05-6	Blue	Red	Red-Brown)	
		0.07	White	--	Red Brown)	
		0.08	Blue	--	Pink	
		0.11	Blue	--	Red	
		0.13	Blue	--	Brown	
		0.14	Blue	--	Violet	
		0.17	Blue	--	Yellow	
		0.22	--	--	Orange	
		0.25	--	--	Pink	
$NaHCO_3$ Soluble H_2O Insoluble	5.50	0.00	Dark	--	Red	
		0.04-7	Yellow	--	Pink	
		0.09	Blue	--	Pink	
		0.11	Blue	--	Brown	
		0.16	Blue	--	Violet	
		0.25	--	--	Pink	
NaOH Soluble	1.05	0.00-14	Tan	--	Peach	
		0.41	Blue	Red	Red-Brown	
		0.52	Blue	Orange	Red-Brown	
		0.88	White	--	Red	
Ether Soluble From H_2SO_4 Acidification	11.92					
$NaHSO_3$ Soluble H_2O Soluble	6.20	0.00	Dark	Orange	Red	
		0.04	Violet	Yellow	Pink	
		0.08	Yellow-Green	?	--	
		0.10	Yellow	--	--	
		0.42	Blue	Red	Red-Brown	
		0.52	--	Orange	Red-Brown	

TABLE XII (Continued)

Fraction	Yield	Rf	U.V.	2,4 D	EDB
H_2O Insoluble					
	2.30	0.00	Dark	Orange	Red
$NaHCO_3$-H_2O Soluble					
	1.44	0.00	Dark	Orange	Red
		0.04	Yellow	Orange	Pink
		0.06	Green	--	Pink
		0.08	Blue	--	Red
		0.11	Blue	--	Brown
		0.15	Blue	--	Violet
H_2O Insoluble					
	1.06	0.00	Dark	Orange	Red
NaOH Soluble					
	9.92	0.16	*	Red	Pink-Red)
		0.24		--	Pink-Red) Streak
		0.42		Red	Pink-Red)
		0.50		Orange	--
		0.87		--	Pink
Total Yield					
	27.27				

*This fraction appeared as a streak under ultra violet light.

TABLE XIII

FRACTIONS COLLECTED FROM TREATMENT OF ACIDS ON DUOLITE A-7 ANION EXCHANGE COLUMN

Fraction	Solvent	Recovered Acids gm
1	Acetone-Water	0.05
2	Bicarbonate	1.52
3	Bicarbonate	0.31
4	Bicarbonate	0.10
5	Bicarbonate	0.07
6	Bicarbonate	0.22
7	Sodium Hydroxide	0.15
8	Sodium Hydroxide	0.46
9	Sodium Hydroxide	0.56
10	Sodium Hydroxide	0.16
11	Sodium Hydroxide	0.06

With the exception of fraction 1, all fractions were acidified and extracted with ether. Fraction 1 was heated over a steam cone to remove the acetone and extracted with ether. The ether solutions were dried and distilled to dryness. Chromatographic examination revealed that most of the oxidation products with R_f values greater than 0.00 were contained in fractions 2 and 3. Fraction 1 was discarded, while fractions 4 through 6 were combined and labeled 4'. Fractions 7 through 11 were also combined and labeled 5'. These fractions were then dried for 24 hours in a vacuum desiccator. This was followed by extraction with anhydrous ether which did not dissolve the entire residue. The ether insoluble portions were combined to form 2.12 grams. This substance was a dark red-brown powder. When this material was heated on the melting

point block it softened, turned black and carbonized. The anhydrous ether dissolved 0.75 gram, 0.16 gram, 0.20 gram and 0.38 gram from fractions 2, 3, 4' and 5' respectively.

Attempts to crystallize these fractions from ether-hexane, ethyl acetate, alcohol-benzene, acetone-water and water failed. The use of ethyl acetate-chloroform mixtures caused most of the remaining material, which gave rise to the spot at R_f 0.00, to separate as a dark red oil. The oil was removed by decantation and the procedure was repeated until the combined acids weighed 1.0 gram.

The complex nature of this mixture precluded the use of fractional crystallization, and no solvent could be found with enough selective action to improve the separation, so the streak method of chromatography described earlier was employed to isolate the individual acids of this mixture. The procedure was the same as described in the discussion of the acid fraction of the exploratory copper oxide oxidation. One modification on this procedure was the use of Whatman number 3 rather than the number 1 paper. This is a thicker paper and will handle larger samples. A streak containing approximately 0.05 gram could be applied on a sheet six inches wide. More than this caused uneven movement and serious overlapping of the bands. A summary of this chromatographic separation is shown in Table XIV.

TABLE XIV

CHROMATOGRAPHIC SEPARATION OF THE ACIDS FROM COPPER OXIDE OXIDATION

Band	Rf	U.V.	BDB	Yield gm
0	0.00-0.10	---*	---	
1	0.11	Blue	Red	0.06
2	0.13	---	Pink	0.02
3	0.14	Blue	Brown	0.15
3a	0.18	Blue	Violet	0.07
4	0.20	Blue	Yellow	0.05
5	0.23	Blue	Orange	0.03
6	0.27	---	Pink	0.02
7	0.30	Yellow	---	Trace

*The first band was actually a number of narrow bands which had not travelled far enough for good separation.

Band 0 was set aside to be eluted and rechromatographed with another solvent. The remainder of the bands were cut from the sheets and eluted by a modified Dent procedure (7, p.245). This involved placing one end of the strips between two microscope slides. The slides were then placed in a petri dish with one end on the bottom of the dish and the other extending out over the edge of the dish. The petri dish was set on a support in a covered container and a beaker was placed under the free end of the paper strip. The petri dish was filled with alcohol which travelled by capillary action between the microscope slide to the end of the paper and down the strip into the beaker. Elution was continued until the strip gave a negative reaction to bis diazotized benzidine reagent.

The eluates were concentrated and stored in small flasks until the entire acid fraction had been separated and eluted, then the fractions were taken to dryness to determine the yields.

The compounds isolated from bands 3 and 4 were of a crystalline nature, while the residues from the remainder of the bands was a tacky semisolid. The crude residue from band 3 melted at 201-204°C with sublimation. The sublimate was collected on a watch glass over the melting point block. The sublimate, heated in a sealed tube melted at 206-208°C, which corresponds to the melting point for vanillic acid. A mixed melting point with an authentic sample of vanillic acid was undepressed. The ultra violet spectra was identical with that of vanillic acid and chromatography of the sample mixed with and adjacent to a sample of authentic vanillic acid gave further evidence that the compound isolated from band 3 was vanillic acid.

The material from band 4, when heated on the melting point block also sublimed, forming needles on a watch glass which had been placed on the block. The sublimate, in a sealed capillary, melted at 190-195°C. Chromatography of the sublimate mixed with and adjacent to an authentic sample of p-hydroxy benzoic acid produced a strong spot identical with that of p-hydroxy benzoic acid along with a faint spot for compound 3a. Not only were the R_f values

identical, but the color with bis diazotized benzidine (a bright yellow which is slow to form) were the same for the authentic sample and the compound from band 4. The remainder of the fraction was sublimed and resublimed raising the melting point to 200-205°C, while p-hydroxy benzoic acid melts at 215°C. Further attempt to prove the identity of this compound was delayed until the remainder of the acid fraction was separated.

While chromatographic examination of the material isolated from band 1 revealed that it was quite pure, it was not possible to obtain a melting point. A sample, heated on the melting point block, started melting at about 60°C, but was not completely liquid until the temperature reached 200°C. Between these temperatures, the compound changed from a light yellow to a dark red-orange color. A small deposit of sublimate was collected from this melting point determination. The sublimate was a light yellow semisolid which was shown to be identical with the original material by means of a paper chromatogram. The ultra violet spectra were measured, both on the original material and the sublimate. The spectra had a broad weak absorption band centered at 306mu and strong absorption corresponding to the benzene nucleus. All spectra reported in this paper were measured with a Beckman automatic recording spectrophotometer, Model DK-2.

Attempts to crystallize this fraction from ether-hexane and ether-benzene mixtures failed. The continued treatment of this material caused it to become darker. A chromatogram was run to recheck the purity and two spots were observed at R_f 0.00 and 0.80 in addition to the original spot. Further work on this material was postponed until the remainder of the acid fraction was separated.

Material from band 2 was similar in appearance to that of band 1. It also reacted the same in an attempt to determine the melting point. Again the spectra of the original and the sublimate were identical. These spectra were only slightly different from the spectra of the band 1 compound, the only difference being the location of the broad weak absorption band at 283 mu.

Residues from the isolation of bands 3a, 5 and 6 were all mixtures. Bands 5 and 6 were mixtures of the same two components, there having been a considerable overlap of these bands on the chromatograms. Band 3a contained a mixture of vanillic acid, p-hydroxy benzoic acid and a small amount of a third compound. These fractions were set aside to be further purified when the remainder of the acid fraction was isolated.

To provide further material for the identification of the compounds responsible for bands 1, 2, 3a, 5 and 6,

the bicarbonate-water soluble fractions from the sulfur dioxide and sulfuric acid acidifications were combined.

In the separation of aldehydes from oxidation of lignin, an activated cellulose powder has been found effective in column chromatography (20, p.44). Therefore, one more attempt was made to separate these acids on a cellulose column of 40x400 millimeters, filled with Whatman standard cellulose powder which had been boiled for five minutes in five percent nitric acid (5, p.180). The cellulose was thoroughly washed with hot water followed by an acetone wash. The air dry powder was then suspended in butanol-two percent ammonium hydroxide, allowed to stand overnight, filtered and resuspended in the same solvent. The slurry was poured into the column and allowed to settle for 48 hours to obtain even packing.

In paper strip chromatography, the sheets are equilibrated in an atmosphere of two percent ammonium hydroxide vapor before the developing solvent is added. Since this treatment provides an excess of ammonia, it would seem that all of the acids spotted on a sheet would be in the form of their ammonium salts before the addition of the developing solvent. To duplicate this condition, the remaining bicarbonate-soluble, water-soluble acid mixture from both the sulfur dioxide and sulfuric acid acidifications were combined, taken up in butanol saturated with

two percent ammonium hydroxide and ammonia gas was passed through the solution until it was saturated. The solution was then applied to the column and developed until no more of the acids were removed by further development. Six fractions were collected. The cellulose was removed from the column and eluted with one percent sodium hydroxide to make fraction 7.

Paper chromatography revealed that the separation was somewhat improved by the use of oxidized cellulose, however the separation was still not satisfactory. Fraction 7 contained 51.7 percent of the acid mixture and exhibited only faint spots for compounds 1 and 2 as described above, the remainder of the components of this fraction being concentrated at R_f values less than 0.10. Fractions 1 through 6 from the cellulose column were recombined and separated by the "streak" technique on sheets of number 3 Whatman paper. A wooden tank was constructed whereby nine sheets 10x20 inches could be developed at one run. With this set up approximately one gram of the acid mixture could be chromatographed per run.

This system required a more rapid method for the elution of the bands cut from these sheets, therefore as the bands were cut from the sheets they were placed in Soxhlet extractors and eluted with ethanol by continuous extraction for six hours. The ethanol was removed under

vacuum. Purity of the residues was checked by paper chromatography. Results showed that there had been some overlapping of the bands and each of the compounds was rechromatographed by the streak technique. When the bands were cut out, care was taken to cut the sheets so that any overlap would not be included. The yield from elution of the twice chromatographed material is shown in Table XV.

TABLE XV

YIELD OF ACIDS SEPARATED BY STREAK CHROMATOGRAPHY

Band	Yield gm
1	0.31
2	0.09
3	0.94
3a	0.08
4	0.12
5	0.24
6	
7	Trace

Vanillic acid, compound number 3, was obtained as pale yellow crystals. Recrystallization from water gave nearly white crystals which melted at 206-208°C. A mixed melting point with authentic vanillic acid was not depressed.

Acid number 4 was also crystalline, but of a pale tan color. Sublimation at 2.0 millimeters mercury and 160-170°C produced a pale yellow sublimate. Recrystallization from benzene-ligroin yielded 15 milligrams of white crystals. Evaporation of the mother liquor left a residue

of 14 milligrams of sticky yellow semicrystalline material. The crystals melted at 212-214°C which corresponds well with 215°C, the literature value for the melting point of p-hydroxybenzoic acid. A mixed melting point with p-hydroxybenzoic acid showed no depression. The ultra violet absorption spectra measured in 95 percent ethanol was identical with that of p-hydroxybenzoic acid.

From the work described previously, it appeared that the acid from band 1 would sublime. While the major portion of the acid had decomposed on the melting point block during this test, it was felt that high vacuum would decrease the temperature required for sublimation so that decomposition would not be serious. Thus the residue was taken up in ether and transferred to a vacuum sublimation apparatus. The ether was evaporated and the pressure was reduced to 1.0 millimeters of mercury. The temperature was slowly raised to 100°C and held there for six hours. A small amount of white deposit slowly collected on the cold finger, while the residue gradually darkened until at the end of six hours it was black. The cold finger was removed and a tan mixture of oil and crystals weighing 18 milligrams were obtained. Sublimation was continued as the temperature was slowly raised to 260°C. After six hours more, an additional 12 milligrams of a tan wax-like sublimate was removed. The residue from sublimation was

a black, insoluble infusible solid. Most of acid 1 had decomposed, being more heat sensitive than suspected.

Attempts to obtain a melting point on the sublimate were unsatisfactory. As the temperature was raised, the sample started to melt at 60°C, then gradually darkened until between 160 and 180°C the last traces of solid material melted.

Paper strip chromatograms of the sublimate showed the presence of only a very faint spot at R_f 0.00 in addition to the original spot for this material. Therefore, the action on the melting point block can be explained by the heat sensitivity of this compound, or by the fact that it is a mixture which is not separated by this solvent system. This latter reason is unlikely since other solvent systems were employed to check the purity of this compound and in every case only one spot appeared on the chromatogram. The apparent instability of this compound would also help explain the large amount of amorphous material in the acid fraction which caused the spot at R_f 0.00 on paper chromatograms.

A sample of 18.1 milligrams was titrated with the aid of a semi-micro burrete and pH meter. A plot of the change in pH against the amount of sodium hydroxide added gave a sharp end point and the calculated value of 288 for the neutralization equivalent.

The remainder of the sample was used to measure the ultra violet spectra which was identical with those obtained earlier. The spectra is shown in Figure II.

The acidic material from band 2 was sublimed at 1.0 millimeters mercury and 150°C to yield 31 milligrams of pale yellow wax-like solid. On the melting point block a sample softened at 120-130°C, commenced to sublime at 160°C and sublimed rapidly at 190°C, leaving a black residue at 205°C. The sublimate was collected and placed on the melting point block. The twice sublimed acid softened at 170°C and completely sublimed from the block when the temperature reached 220°C. Again the sublimate was collected. Heated in a sealed capillary, it appeared to soften above 170°C but no liquid appeared until the temperature reached 217-221°C. The melt was dark, whereas the sublimate had been colorless. On cooling the melt resolidified at 180-190°C to a tan solid which melted between 190 and 200°C when reheated. The ultra violet absorption spectrum of this compound is shown in Figure II. Every attempt to isolate a pure compound from band 3a failed. Repeated streak chromatography caused further separation of vanillic acid and p-hydroxybenzoic acid. Observation of the sheets under ultra violet light did show a narrow band of bright blue fluorescence which was not characteristic of either of these acids. The narrow width of this band indicated that the compound producing it

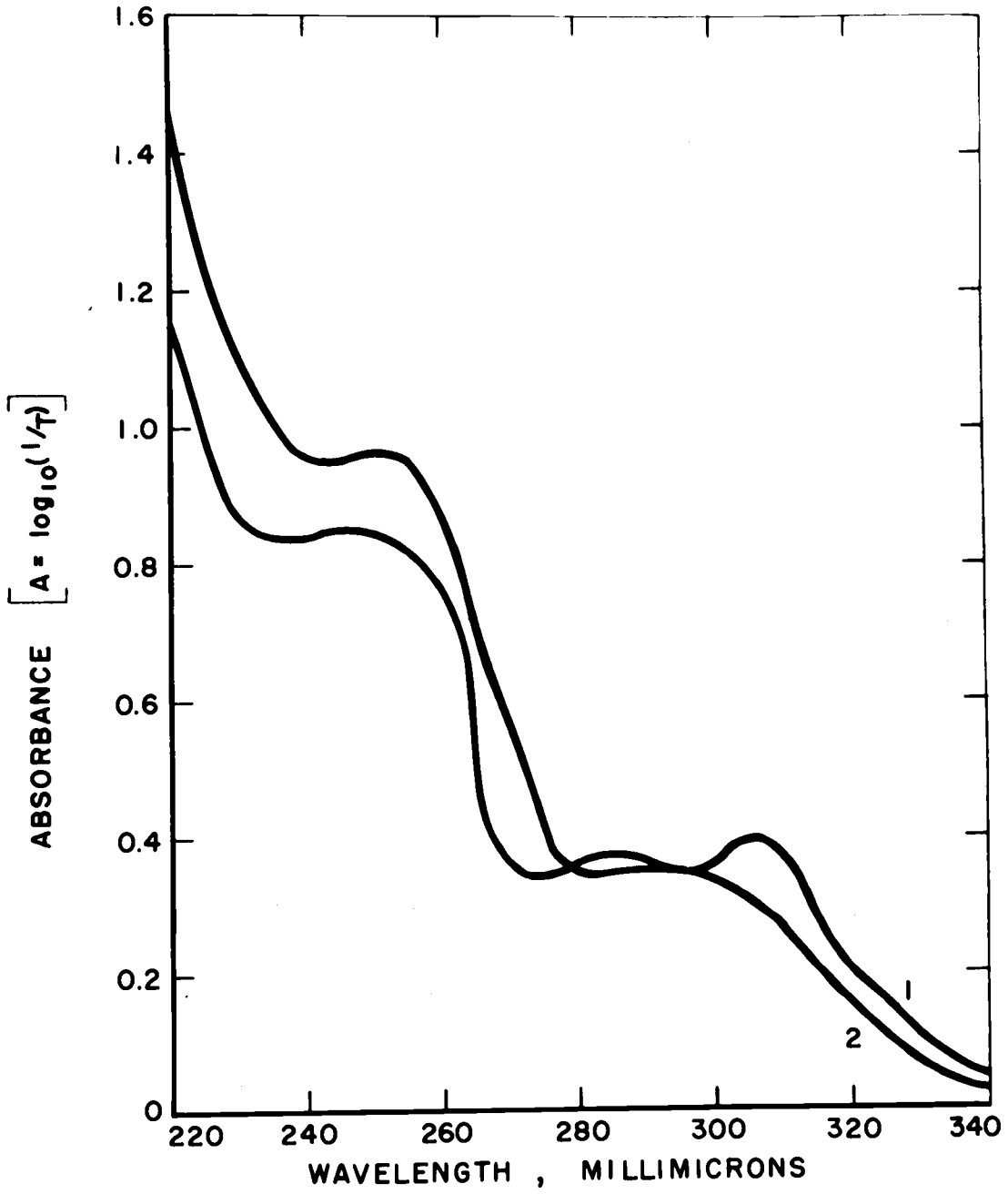

FIGURE 2. ULTRAVIOLET ABSORPTION SPECTRA OF UNIDENTIFIED ACIDS 1 AND 2.

was present in small quantity and attempts to isolate this material were continued until there was an insufficient amount left for further work.

Compounds labeled 5 and 6 overlapped on the chromatograms so that it was impossible to cut the sheet into bands that contained pure compounds. It was possible to cut a narrow strip from the sheets which corresponded to acid 6 which gave only one spot when eluted and rechromatographed. Evaporation of the eluate resulted in a brown sticky residue. The compound could not be crystallized from benzene, acetone-chloroform, ether-hexane or alcohol-water. Since only a few milligrams could be obtained from the lower edge of the band, the only additional characterization carried out on this material was the measurement of the ultra violet spectrum. The spectrum showed an absorption shoulder at 310 mu and a maximum at 284 mu. This is similar to the spectra of vanillin and 5-carboxy vanillin.

Attempts to separate the remainder of compound 6 from the mixture of 5 and 6 by selective solvent action failed. Other chromatographic solvent systems were investigated to find one producing a separation of these compounds. Results of this investigation were disappointing. Solvents were found which would move the compounds to higher Rf values but in no case was the separation an improvement over butanol saturated with two percent ammonium

hydroxide. Solvents which produced a separation equivalent to that of the butanol-ammonia mixture were; butanol, dimethyl formamide, water (2:1:1), butanol, pyridine, water (10:3:3), butanol, acetic acid, water (4:1:5), butanol, isopropyl alcohol, two percent ammonium hydroxide (2:4:1) and papers buffered with phosphate-borate solutions at various pH values, developed with water saturated butanol. Butanol, pyridine, water (10:3:3) produced a slightly better separation than butanol saturated with two percent ammonium hydroxide, but trailing of the compounds in this solvent made it impractical for the streak method of separation.

The sections of the sheets from Rf 0.00 to 0.10 in the separation of the acid fractions described above were eluted to be chromatographed in a solvent other than butanol-ammonia. However, after investigation of the solvents listed above, this fraction was streaked on sheets of number 3 Whatman paper and developed for three days with butanol-ammonia. When the dried sheets were sprayed with bis-diazotized benzidine, seven incompletely separated bands were observed, all of which appeared to be of nearly equal intensity. Because of the time requirement, this method of separation was considered impractical. Solvent fractionation of this mixture also failed and no further work was carried out on this material.

Bisulfite Soluble Oxidation Products

The bisulfite soluble-water soluble material was divided into two equal portions of 2.85 grams. One portion was taken up in alcohol-water and placed in the freezer to cause crystallization. The second portion was fractionated by dissolving the mixture in ethyl acetate and adding chloroform until the solution became cloudy. The solution was then cooled overnight while a dark heavy tar separated. The solution was removed by decantation and both fractions were analyzed chromatographically. The tar contained only material which would not move with butanol-ammonia, while the solution contained three aldehydes. This process was repeated eight times. A total of 1.70 grams of the tar was removed. The remaining 1.1 grams of soluble aldehydes now showed the presence of five aldehydes when chromatographed.

Chromatograms of the original mixture had a short streak between R_f 0.00 and R_f 0.10 which reacted weakly positive when sprayed with 2,4 dinitrophenyl hydrazine. As the tar was removed by this fractionation procedure, the aldehydes making up this streak became separate spots and increased in intensity when sprayed with 2,4 dinitrophenyl hydrazine. The movement of these aldehydes must have been inhibited by the tar, since as will be shown later, these aldehydes were present in as great a yield as the p-hydroxybenzaldehyde which gave a strong positive spot on the initial chromatograms.

The 1.1 grams of aldehydes were separated by streaking approximately 0.1 gram samples on 11 sheets of Whatman number 3 paper, of size 10x18 inches. Bands cut from the developed sheets were eluted by extraction with one percent sodium hydroxide followed by acidification and extraction with ether. Table XVI lists the yields obtained from this separation.

TABLE XVI

YIELD OF ALDEHYDES FROM COPPER OXIDE OXIDATION

Aldehyde	Yield	
	Gram	Percent of Phenolic Acid
1	0.19	0.38
2	0.11	0.22
3	0.15	0.30
4	0.30	0.60
5	0.09	0.18

Aldehydes 1, 2 and 3 were soluble in bicarbonate solutions, while aldehydes 4 and 5 were not. Chromatographic examination of the isolated aldehydes with butanol saturated with two percent ammonium hydroxide and butanol, pyridine, water (10:3:3) indicated that the three acidic aldehydes were pure, while the aldehydes 4 and 5 contained traces of aldehyde 2. These latter two fractions were purified by extraction with bicarbonate solution.

Aldehyde 4, the major component was identified as vanillin based on chromatography with comparison samples, melting point determination, formation of the 2,4

dinitrophenylhydrazone and measurement of the ultra violet absorption spectra.

Aldehyde 5 was identified as p-hydroxybenzaldehyde through chromatography with an authentic sample for comparison. The identity was confirmed by measurement of the ultra violet absorption curve which was identical with that of the authentic compound. Aldehyde 4 melted at 112-114°C as compared with the literature value of 115°C for p-hydroxybenzaldehyde. The 2,4 dinitrophenylhydrazone melted at 277-279°C with decomposition which corresponds to 280°C, the literature value for the melting point of this derivative of p-hydroxybenzaldehyde.

Aldehyde 3 gave a violet color with five percent aqueous ferric chloride solution. The melting point for this compound was 258-260°C which corresponds with the melting point reported for 5-carboxyvanillin (32, p.4266). The ultra violet absorption spectra measured in 95 percent ethanol was the same as that reported for 5-carboxyvanillin. While the Rf value was not constant from run to run, it was in the range reported for 5-carboxyvanillin in the solvents listed above (28, p.2225). An authentic sample of 5-carboxyvanillin was obtained through the generosity of Dr. J. C. Pew. The mixed melting point was undepressed and comparison chromatograms were identical.

Aldehyde 2 could not be obtained in a crystalline

form from ether-ligroin. It was quite soluble in water and alcohol, but only moderately soluble in benzene. The benzene solutions precipitated a heavy oil rather than a crystalline compound however.

A small amount of this aldehyde dissolved in ether was transferred to a melting point coverglass and the ether was evaporated. The residue was heated to 160-180°C. A sublimate appeared on a watch glass which had been placed over the melting point block. The sublimate gave a positive reaction with 2,4 dinitrophenylhydrazine and a red-brown color with five percent ferric chloride solution. Both of these color tests were characteristic of the original fraction indicating that the aldehyde had not decomposed during the sublimation. This procedure was repeated until enough sublimate had collected for a melting point determination. The melting point was 126-129°C. The ultra violet absorption spectra measured in 95 percent ethanol showed maxima at 230.5, 282 and 310 mu.

Recently, in the study of the alkaline cleavage products from lignosulfonates, vanilloylformic acid has been reported (10). The reported melting point for this compound was 132-133°C. An authentic sample of this compound was obtained from Dr. D. W. Glennie. This sample, chromatographed adjacent to aldehyde 2 with three different solvent systems, (a) butanol-two percent ammonium hydroxide,

(b) butanol, pyridine, water (10:3:3) and (c) n-heptane, butanol, acetic acid, water (8:7:4:1) (11), gave a spot at the same R_f as aldehyde 2. Vanilloylformic acid also produced a red-brown color with five percent ferric chloride reagent. A 2,4 dinitrophenylhydrazone formed from the remainder of aldehyde 2 turned dark above 190°C, softened and evolved a gas at 206-207°C and completely melted at 230-235°C. This corresponds with the description of this derivative of vanilloylformic acid (10, p.2411).

Aldehyde 1 from the copper oxide oxidation still contained some dark colored material after isolation. Solution in bicarbonate, followed by ether extraction, acidification and re-extraction of the aldehyde with ether did not improve the color of the product.

Attempts to crystallize the aldehyde from ether-ligroin, alcohol-benzene and alcohol-water mixtures failed. The aldehyde gave a blue violet color with five percent ferric chloride in ethanol, but chromatograms developed with butanol-ammonia showed a brown-black color for this aldehyde when sprayed with ferric chloride.

The R_f value for this compound was 0.02 in butanol saturated with two percent ammonium hydroxide. This value and the color reactions of this compound correspond with those reported for 5-formylvanillic acid (32, p.4266). In order to determine if aldehyde 1 were 5-formylvanillic acid, this compound was synthesized by the Reimer-Tieman

reaction on vanillic acid (38, p.1281). The synthetic compound was chromatographed adjacent to aldehyde 1 on sheets of Whatman number 1 paper developed with butanol saturated with two percent ammonium hydroxide and heptane, butanol, acetic acid, water (8:7:4:1). Results showed that aldehyde 1 was not 5-formylvanillic acid. The ultra violet spectrum of this compound measured in 95 percent ethanol showed an absorption shoulder at 320 mu with continued increasing absorption as the wavelength decreased. No further work was carried out on this fraction.

Sodium Hydroxide Soluble

The original chromatographic examination of this fraction showed the presence of vanillin and p-hydroxybenzaldehyde. From visual estimation of the spot intensity, these two aldehydes made up the major portion of this fraction. The compound causing the spot at R_f 0.88 either reacted weakly with the spray reagent or was present in trace amounts only. The third aldehyde listed in Table XII at R_f 0.16 appeared to be protocatechualdehyde from comparison chromatograms, however it seems unlikely that this aldehyde would survive copper oxide oxidation. This fraction had been stored in the freezing compartment of a refrigerator for three months while the bicarbonate and bisulfite soluble products were being analyzed. During this period, the solution became very dark red and

chromatographic examination revealed only two spots, these for vanillin and p-hydroxybenzaldehyde. The third compound giving a positive reaction with 2,4 dinitrophenylhydrazine had apparently decomposed on standing. This is a further indication that this might have been protocatechualdehyde since it is known to be unstable in solution.

For the quantitative determination of vanillin and p-hydroxybenzaldehyde, the mixture was taken up in acetone and diluted in a volumetric flask to 50 milliliters. A two milliliter aliquot was pipetted into a beaker and concentrated to one-half milliliter. This solution was streaked on eight sheets of Whatman number 3 paper, developed with butanol saturated with two percent ammonium hydroxide and the bands corresponding to these aldehydes were cut from the sheets and eluted in Sohxlet extractors with 95 percent ethanol. The ethanol was removed under reduced pressure. The residues were taken up with hot water and treated with an excess of 2,4 dinitrophenylhydrazine solution. The precipitates were digested for three hours on a steam cone, filtered and dried at 105°C. The hydrazones were weighed and the yields of the two aldehydes were calculated. From this determination there was 0.266 gram vanillin and 0.091 gram p-hydroxybenzaldehyde in the sodium hydroxide soluble fraction from the copper oxide oxidation.

Summary of the Copper Oxide Oxidation

The yield of oxidation products from cupric oxide oxidation of the phenolic acid seems to be quite good at first glance. However, it appears that the major portion of these oxidation products are either the original phenolic acid that is only partially degraded or products which were oxidized to quinone structures, followed by recondensation. Quinone formation was indicated by the color changes that took place when any of the oxidation mixtures were subjected to a change in pH. A positive reaction of the material that appeared at R_f 0.00 on all of the chromatograms when sprayed with 2,4 dinitrophenylhydrazine favors the idea of quinone formation.

The oxidation products obtained from the copper oxide oxidation are a complex mixture, particularly the acid components. The compounds making up this mixture must be closely related in structure because of the difficulty in separating this mixture by chromatography. Their molecular weight, solubility and structure must be quite similar, or the separation on the paper strips would have been more satisfactory.

This similarity is quite strikingly shown by the components of the aldehyde mixture. This suggests that the phenolic acid is a homogeneous material, however, the yield of identified oxidation products is too low for this to

be more than a tentative conclusion.

From the oxidation products which were identified, it would appear that the phenolic acid is similar in structure to wood lignin, the main difference being in the methoxyl content and the presence of carboxyl groups in the phenolic acid. The low yield would again make this a tentative conclusion, however the low methoxyl content and high phenolic hydroxyl content would help to explain why the same oxidizing agent when applied to both of these materials would produce a higher yield from lignin.

It is interesting to note that while nearly all of the compounds identified from the oxidation of the phenolic acid were stabilized by methoxyl groups, a substantial portion of the methoxyl containing nuclei were not recovered among the identified products. The methoxyl content of 3.56 percent would have led to a yield of 17.0 percent, based on total recovery of the methoxyl content of the phenolic acid in the form of vanillin.

Although some of the methoxyl was probably isolated in the unidentified oxidation products, it is likely that a portion of the methoxyl is attached to a non-vanillin type nucleus of less stability to copper oxide than vanillin.

Table XVII summarizes the results of the copper oxide oxidation.

The combined yield of vanillic acid and vanillin from this oxidation is quite similar to the yield of vanillin

TABLE XVII

YIELDS OF OXIDATION PRODUCTS FROM COPPER OXIDE OXIDATION OF THE PHENOLIC ACID

Product	Percent Yield Based on Phenolic Acid
Vanillin	0.87
P-hydroxybenzaldehyde	0.27
5-carboxyvanillin	0.30
Vanilloylformic Acid	0.22
Unknown Aldehyde	0.38
Vanillic Acid	1.09
P-hydroxybenzoic Acid	0.17
Unknown Acid 1	0.37
Unknown Acid 2	0.11
Unknown Acid 3a	Trace
Unknown Acids 5 and 6	0.29

obtained by nitrobenzene oxidation of Douglas fir bast fiber phenolic acid, but is considerably higher than the yield of vanillin obtained by nitrobenzene oxidation of Douglas fir cork phenolic acid (See Table 1). Parahydroxybenzaldehyde was not reported among the oxidation products from the nitrobenzene oxidation of Douglas fir cork phenolic acid. Traces of this compound were found in the oxidation mixture from the bast fibers, while 0.27 percent was obtained from the copper oxide oxidation of white fir cork phenolic acid.

Protocatechualdehyde, isolated from the oxidation mixtures of both Douglas fir phenolic acid fractions, was not isolated from the copper oxide oxidation although there was fragmentary evidence for its presence.

No other compounds were identified from the nitrobenzene oxidation of the Douglas fir phenolic acids although all of the compounds isolated from the copper oxide oxidation of white fir with the exception of vanilloylformic acid have been isolated from nitrobenzene oxidations of lignin and are stable to this oxidant under the usual reaction conditions. Either a basic difference in the structures of these phenolic acids, or the small yield of these products could account for their not being isolated from Douglas fir phenolic acids. As pointed out earlier though, alkaline fusion gave slightly different yields, but chromatograms of the fusion products from the cork phenolic acids of both white and Douglas fir were identical.

Comparison of white fir cork phenolic acid with redwood phenolic acid is not possible since there has been neither copper oxide nor nitrobenzene oxidation reported for this material. Spruce bark phenolic acid also shows a definite difference from the white fir cork phenolic acid. No vanillin is obtained from spruce bark phenolic acid when it is oxidized with alkaline nitrobenzene. None of the products from this oxidation were identified. Even the fully methylated preparation yields only 0.50 percent vanillin on oxidation with this reagent.

METHYLATION OF THE PHENOLIC ACID

In order to determine the number and type of hydroxyl groups in the phenolic acid, methylation was carried out with diazomethane, dimethyl sulfate separately and dimethyl sulfate, followed by diazomethane. While there has been criticism of this method in the determination of the hydroxyl content of natural products (15, pp.209-214), most of the substitute methods offered to replace methylation are more limited or do not provide any assurance of better accuracy. One of these, based on differences between the ultra violet absorption curve of the ionized and unionized sample (23, pp.1423-1427) was tried on the phenolic acid without success (24).

In the first attempt to methylate the phenolic acid, dimethyl sulfate was added to a solution of this material in 30 percent sodium hydroxide at such a rate that the solution was heated to the reflux temperature. This caused a copius black precipitate to form. This precipitate was insoluble in ten percent sodium hydroxide at 100°C and in acetone and ethanol at room temperature. When the methylation was carried out at 40°C or lower, the methylated products were easily soluble in acetone and ethanol. It therefore appears that dimethyl sulfate at elevated temperature will cause the phenolic acid to condense. As a further test, two grams of the phenolic acid were dissolved in

methanol, one-half gram of p-toluene sulfonic acid was added and the solution was allowed to stand overnight on a steam bath. This treatment caused most of the phenolic acid to deposit as a black insoluble precipitate. Some methylation might have occurred, however this would not cause the phenolic acid to become insoluble in methanol. Therefore, it was concluded that heating in the presence of acids would condense the phenolic acid.

For the above reason, methylation was carried out at room temperature. The phenolic acid was dissolved in a solution of five percent sodium hydroxide and dimethyl sulfate and 30 percent sodium hydroxide were added at such a rate that the pH was maintained above 8.0 and the temperature below 35-40°C. Methylation was interrupted when ten milliliters of dimethyl sulfate had been added for each gram of phenolic acid. The solution was acidified and filtered. This procedure was repeated until 30 milliliters of dimethyl sulfate had been added for each gram of sample. During the addition of the last portion of dimethyl sulfate, the major portion of the phenolic acid became insoluble in sodium hydroxide. This insoluble methylated fraction was not the same as that obtained from high temperature methylation in that it was a light color and was soluble in acetone. This insoluble fraction was a tan color and amounted to 80-90 percent of the methylated

product. The sodium hydroxide soluble methylated product was slightly darker and made up 10-20 percent of the methylated product. Remethylation of these products would not increase the methoxyl content, nor could the soluble fraction be insolubilized, even by methylation at 100°C.

The production of two fractions by methylation may mean that the original phenolic acid is actually two closely related polymers. It is also possible to interpret these results in terms of molecular weight differences. Methylation as described above will not methylate carboxylic hydroxyl groups. Therefore, if the polymer were of high molecular weight there might not be sufficient solubilizing power from the carboxyl group to make the polymer soluble, whereas the lower molecular weight fraction could still be soluble in alkaline solution.

For comparison purposes only, a small sample of Douglas fir cork phenolic acid was methylated under these same conditions. This material also produced sodium hydroxide insoluble and sodium hydroxide soluble fractions.

Samples from both the alkali soluble and insoluble products from the dimethyl sulfate methylation were further methylated in diazomethane. The samples were dissolved in methanol and treated with an excess of diazomethane dissolved in ether. The solutions were allowed to stand in the refrigerator for one week with occasional shaking.

At the end of this time a drop of acetic acid indicated an excess of diazomethane. Both of the solutions contained precipitates which were removed by filtration. The filtrates were evaporated to dryness to recover the soluble portion of the methylated products. All of these preparations were dissolved in acetone and precipitated into water. Table XVIII shows the yield of the soluble and insoluble methylation products from the treatment with diazomethane.

TABLE XVIII

DIAZOMETHANE METHYLATION OF PHENOLIC ACID PREVIOUSLY METHYLATED WITH DIMETHYL SULFATE

NaOH insoluble from dimethyl sulfate methylation	
Product insoluble in CH_2N_2 methylation medium	73.8%
Product soluble in CH_2N_2 methylation medium	26.2%
NaOH soluble from dimethyl sulfate methylation	
Product insoluble in CH_2N_2 methylation medium	50.0%
Product soluble in CH_2N_2 methylation medium	50.0%

The third phase of the methylation study involved the methylation of phenolic acid with diazomethane alone. The methylation was carried out as described above. In this case there was only one fraction, that which was insoluble in the methylation medium. This product was also purified by dissolving it in acetone and precipitating it into water.

All methylated products were dried in a vacuum oven at 50°C for 12 hours before methoxyl determinations were

performed. Results of methoxyl determinations are shown in Table XIX.

TABLE XIX

METHOXYL CONTENT OF METHYLATED PHENOLIC ACID

	Percent Methoxyl
Phenolic Acid	3.56
Dimethyl Sulfate Methylation	
A-Alkali Soluble Fraction	19.10
B-Alkali insoluble fraction	22.24
Methylation With Diazomethane	
Phenolic Acid	23.54
Fraction A Above	
Soluble in CH_2N_2 Medium	27.34
Insoluble in CH_2N_2 Medium	28.18
Fraction B Above	
Soluble in CH_2N_2 Medium	26.03
Insoluble in CH_2N_2 Medium	26.69

Based on the assumption that there is one methoxyl group for each basic polymeric unit, the molecular weight of this unit is 872. This is similar to the value reported for the basic polymeric unit of Douglas fir bast fiber phenolic acid (19, p.18) and is in the range of 840-900 reported for the basic unit of lignin (3, p.190).

An analysis of the methoxyl values, showing the number of different functional groups is given in Table XX. For this analysis, the different fractions obtained from the dimethyl sulfate methylation are considered as two different phenolic acids.

TABLE XX

HYDROXYL GROUPS IN PHENOLIC ACID

Hydroxyl Groups	Phenolic Acid Producing NaOH Soluble Product with $(CH_3)_2SO_4$	Phenolic Acid Producing NaOH Insoluble Product with $(CH_3)_2SO_4$
Alcoholic	1.86-2.80	1.22-1.55
Phenolic	2.25-3.19	5.17-5.50
Carboxylic	4.13-5.07	1.82-2.15

TABLE XXI

FUNCTIONAL GROUPS IN THE PHENOLIC ACID BEFORE METHYLATION

Functional Group	Phenolic Acid Producing NaOH Soluble Product With $(CH_3)_2SO_4$	Phenolic Acid Producing NaOH Insoluble Product With $(CH_3)_2SO_4$
	Percent by Weight	Percent by Weight
Methoxyl	3.56	3.56
Carboxyl	21.9-26.2	9.4-11.1
Phenolic Hydroxyl	4.4-6.2	10.1-10.7
Alcoholic Hydroxyl	3.6-4.1	2.4-3.0

The functional group content of the white fir cork phenolic acid which produces the alkali insoluble product with dimethyl sulfate is quite similar to the phenolic acid derived from spruce bark. It is also comparable to the redwood and Douglas fir bast fiber preparations with the exception of the carboxyl content which is much higher in the white fir phenolic acid.

The results shown in Tables XX and XXI clearly show that the difference between the two fractions of the phenolic acid is more than a difference in the degree of polymerization. The minor fraction has a carboxyl group for approximately every unit of molecular weight of 190. In contrast to this, the major fraction has a carboxyl group for every molecular weight of 450. If both the alcoholic and phenolic hydroxyl content were reduced proportionally to the increase in carboxyl of the minor fraction, an explanation could be provided to account for the minor fraction in terms of a homogeneous phenolic acid.

The isolation procedure described earlier involves passing steam through a caustic dispersion of the bark. It is possible that carboxyl groups could be introduced during this step in the isolation, thereby producing a phenolic acid of variable carboxyl content. This could account for the minor fraction of different phenolic material which was produced by methylation with the dimethyl sulfate. One difficulty with this explanation is the alcoholic hydroxyl content of this minor fraction. It would be lower, rather than equivalent to or higher than that of the low carboxyl content phenolic acid to be compatable with this explanation. Therefore, this evidence leads to the conclusion that this preparation is a mixture of two closely related phenolic acids.

It is interesting to note that a determination of the neutralization equivalent prior to the methylation study gave values of 448 and 452 for the equivalent weight. This corresponds to a molecular weight of 896-902 for a basic unit with two carboxyl groups. This is in fair agreement with the value obtained from the methoxyl content. The value from either of these determinations can only be approximate since the material is a mixture.

NITROBENZENE OXIDATION OF METHYLATED SAMPLES OF THE PHENOLIC ACID

To obtain higher yields and to determine if the phenolic acid is unmethylated wood lignin, alkaline nitrobenzene oxidations were carried out on methylated samples of the phenolic acid. Nitrobenzene was used rather than copper oxide because the aldehyde group is stable to this oxidant. In the analysis of the oxidation products, aldehydes would be preferable to work with from the standpoint of detection on chromatograms. A comparison of the vanillin yield would also be desirable in attempts to show a relationship between the methylated phenolic acid and wood lignin.

Samples for the oxidation were methylated with dimethyl sulfate and sodium hydroxide in aqueous medium, or with dimethyl sulfate and potassium carbonate in acetone. Five exploratory oxidations were carried out with different

reactant ratios. The products were separated according to Pew's procedure as shown earlier in Figure 1. Table XXII summarizes the results of these experiments.

Fraction A from this separation (not shown on Table XXII) contains the neutral oxidation products and the nitrobenzene reduction products. This fraction was tested for veratraldehyde by extraction with bisulfite. Traces of a compound giving a positive reaction with 2,4 dinitrophenylhydrazine reagent were detected, but no compound could be isolated. This is not surprising, since oxidation of veratraldehyde with nitrobenzene at 180°C has been reported to produce 31 percent vanillin, and no recovery of unchanged veratraldehyde (16, p.28). Pew, in the oxidation of lignin model compounds has recovered nearly equal amounts of veratraldehyde and vanillin from fully methylated models, while Brauns has found that methylated lignin yields only small amounts of veratraldehyde, the major product being vanillin.

Table XXII shows a total recovery of over 100 percent based on the original sample. This was due to the formation and isolation in fraction B of p-hydroxy azobenzene along with p-phenyl azobenzoic acid in fraction D. The yield of these compounds was greater at higher concentration of sodium hydroxide. The oxidation mixture containing the lowest concentration of sodium hydroxide produced a

TABLE XXII

SMALL SCALE OXIDATION OF METHYLATED PHENOLIC ACID

Ratio of Phenolic Acid, $C_6H_5NO_2$ and NaOH *	Temp. °C	Reference	Percent Yield B Solid	B Ether Soluble	C	D Solid	D Ether Soluble
1:8:4.8	160	34, p.2832	28.0	14.0	4.0	10.0	46.0
1:3.6:1.3	160	6, p.473	32.0	12.0	2.0	4.0	48.3
1:4:0.8	180	---	38.0	9.0	2.4	2.0	36.2
1:4:6	180	---	38.2	42.6	4.0	2.0	51.4
1:4:4 **	180	20, p.44	40.0	13.4	2.6	1.2	42.0

* All samples were five grams, methylated in acetone to a methoxyl content of 25.02 percent.

** Unoxidized material from fraction B solids of oxidations 1 through 4.

poor yield of compounds that would move from the starting line when chromatographed, making it seem that the oxidation products were of an intermediate molecular weight. The oxidations run at 180°C seemed to give a slightly better yield of aldehydes than 160°C. This was desirable since it was difficult to find a spray reagent for the detection of the methylated acids.

While the above investigation gives only an indication of the effect of variables for this oxidation, conditions prevailing in oxidation number 5 were chosen for the oxidation of 100 grams of the methylated phenolic acid. Five runs were made by dispersing 20 grams of the methylated phenolic acid in a solution of 80 grams of sodium hydroxide in 800 milliliters of water, adding 80 grams of nitrobenzene and sealing the mixture in a bomb. The apparatus was the same as that used for the copper oxide oxidation. The temperature was raised to 180°C and maintained as close to this temperature as possible for three hours.

The cooled mixture was extracted with ether to remove nitrobenzene reduction products and neutral oxidation products. Steam distillation, the method frequently used for this step, was tried in one case. After collection of 20 liters, the distillate still contained nitrobenzene reduction products and this method was discarded in favor of ether extraction.

When the reduction products had been removed, the solution was adjusted to pH 6.5-7.5 and extracted continuously for 10-15 hours with benzene. The pH was then adjusted to 1.0 and extraction was continued for 10-20 hours with a fresh batch of benzene. This was followed by a continuous ether extraction of 10-15 hours. The methylation procedure, methoxyl content and yields for the five runs are shown in Table XXIII.

TABLE XXIII

YIELD OF NITROBENZENE OXIDATION PRODUCTS

Methylation	Methoxyl Percent	Benzene Soluble pH 7	Benzene Soluble pH 1.0	Ether Soluble pH 1.0	Insoluble Precipitate pH 1.0
Acetone-K_2CO_3	25.02	11.2	20.8	25.7	36.5
Acetone-K_2CO_3	26.69	6.0	10.0	21.0	46.0
Acetone-K_2CO_3	25.02	11.0	20.0	39.5	--
Aqueous NaOH	22.24	16.8	16.7	23.7	22.5
Aqueous NaOH	22.24	9.7	29.9	9.8	20.5

Apparently the high methoxyl content of the sample in run 2 above stabilized the material toward oxidation. This is indicated by the low yield of ether and benzene soluble compounds and the high recovery of insoluble material. Qualitatively, chromatograms of all the corresponding fractions were identical, regardless of the methylation procedure. The only effect of the methylation procedure was the decreased yield noted above. The yields noted in Table XXIII for the soluble fractions isolated at pH 1.0

seem to vary considerably from run to run. This was probably caused by variations in the time of extraction with the benzene and the ether. The total yield is much more constant at this pH than the yield obtained from either of these extractions. In general, the benzene extraction was continued for at least ten hours. The solvent was changed at any time thereafter which induced a large variation in the extraction time. If the extraction time had been more constant, the yield of benzene soluble and ether soluble materials would probably have followed a more constant pattern.

Bisulfite Soluble Fraction From the Benzene Extraction at pH 7.0

The main reason for this oxidation was to determine if the phenolic acid after methylation resembled wood lignin. Therefore, only the bisulfite soluble fractions from this oxidation were analyzed.

Each of the fractions listed in Table XXIII were extracted to isolate the aldehydes. Acidification of the bisulfite solution was followed by heating and the passage of carbon dioxide through the solution to remove the sulfur dioxide. When cool, the solution was extracted with ether. The ether was dried over sodium sulfate and distilled to leave a residue of nearly pure vanillin. Chromatographic examination of the residue showed two very faint spots in addition to the spot for vanillin. Attempts to isolate any

aldehyde other than vanillin were unsuccessful. Vanillin was identified as described previously under the discussion of the copper oxide oxidation. The yields of vanillin from the various runs were 2.01 percent, 0.50 percent, 1.35 percent 2.15 percent and 2.05 percent respectively.

Bisulfite Soluble Fraction at pH 1.0

Yields of the bisulfite soluble components isolated from the extractions carried out at pH 1.0 are shown in Table XXIV.

TABLE XXIV

YIELD OF BISULFITE SOLUBLE PRODUCTS FROM BENZENE AND ETHER EXTRACTION OF THE NITROBENZENE OXIDATION MIXTURE AT pH 1.0

Run	Bisulfite Soluble From Benzene Extract	Bisulfite Soluble From Ether Extract	Total
1	8.8%	10.1	18.9
2	1.0%	9.1	10.1
3	8.3%	6.0	14.3
4	6.2%	12.3	18.5
5	15.1%	3.6	18.7

The total bisulfite soluble fraction at pH 1.0 was about 18 percent regardless of the methylation, with the exception already noted. The differences in yield of bisulfite soluble compounds between the benzene and ether soluble appeared to be due to incomplete extraction with benzene. This is demonstrated in run number 5 where the benzene extraction was continued for 20 hours.

Chromatographic comparison of these aldehyde fractions showed that they were composed of the same compounds. There were more "non aldehyde" components on the chromatogram of the bisulfite soluble fraction isolated from the ether solution than from the benzene solution. These "non aldehydes" were compounds showing up on the chromatograms under ultra violet light but not reacting with the 2,4 dinitrophenylhydrazine spray reagent. These compounds were probably found in the bisulfite fraction because of partion coefficients between water and the organic solvent which favored water solubility. A factor favoring the extraction of water soluble components with the bisulfite solution was the large excess of this solution used in the above separations. The p-hydroxyazobenzene which was incompletely extracted at pH 7 was also present in the extracts obtained at pH 1.0. This compound gave a positive test with 2,4 dinitrophenylhydrazine. This compound made it necessary to use an excess of bisulfite to insure complete extraction of the aldehydes.

Because these bisulfite soluble mixtures contained the same aldehydes, they were combined and treated as a single fraction. First attempts to separate the individual compounds by selective solvent action failed. Therefore, the streak method of chromatographic separation described earlier was employed for the separation of these compounds.

To handle the large number of bands cut from the chromatograms, extraction with ethanol in Soxhlet apparatus was employed for the elution. The extracts were allowed to accumulate from one oxidation to the next, with one change of solvent after the third oxidation. Nine fractions were collected, and after all of the bisulfite material had been chromatographed, the ethanol solutions were concentrated to 20 milliliters and filtered to remove paper fibers that had been carried into the boiling flasks from the extraction thimbles. The solutions were then evaporated to dryness to determine the yields. Table XXV shows the yields of these fractions.

TABLE XXV

YIELD OF ACIDIC ALDEHYDES FROM NITROBENZENE OXIDATION OF METHYLATED PHENOLIC ACID

Band	Yield In Grams
Original Streak	2.5
1	2.4
2	4.6
3	1.8
3a	1.9
4	0.8
5	0.6
6	0.6
7	0.2
Losses	0.7

Bisulfite Soluble Bands 1 and 2. The residue from bands 1 and 2 was very difficult to redissolve in ethanol. Solutions of these materials were a red brown color, the

solution of band 1 material being darker than that from band 2. Both of these solutions were decolorized with activated carbon. The carbon was thoroughly washed with hot alcohol and the washings were added to the main solution. This treatment reduced the yields to 1.1 and 2.3 grams respectively. Acetone added to these solutions caused a precipitate to form. After standing for two hours the solutions were filtered. Recrystallized from alcohol, the precipitate weighed 0.3 gram and melted with evolution of gas at 160-165°C. When the melt was tested with 2,4 dinitrophenylhydrazine, it no longer gave a positive reaction. This compound gave a red-brown color with five percent ferric chloride solution, which is characteristic of veratroylformic acid. The ultra violet spectrum measured in 95 percent ethanol was quite similar to that of vanillin.

A small sample of the filtrate from the separation of the above material was evaporated to dryness to remove the acetone. The residue was taken up in water and treated with an excess of 2,4 dinitrophenylhydrazine dissolved in 6N hydrochloric acid. The precipitate weighed 0.25 gram. Recrystallized from water-alcohol, the hydrazone darkened when heated to 190°C, softened with evolution of a gas at 206-207°C and became completely liquid at 230°C. This corresponds to the description of this derivative of vanilloylformic acid.

The ammonium salt was formed from an authentic sample of vanilloylformic acid. The salt decomposed at 157-165°C, corresponding fairly well with the compound isolated above. Therefore, a small sample of this material was dispersed in alcohol and a few drops of concentrated hydrochloric acid were added. The sample immediately dissolved in the cold alcohol.

To isolate the free acid, the filtrate from the separation of the ammonium salt was concentrated to a small volume and treated with an excess of sodium bicarbonate. The bicarbonate solution was extracted with ether to remove any non acidic impurities. The solution was then acidified and re-extracted with ether. The ether was dried and distilled leaving a residue of 2.1 grams. This residue was dissolved in boiling benzene which had been dried by distillation over sodium. As the benzene solution cooled, an oily deposit collected and was removed by decantation. Evaporation of the benzene caused more of the oily deposit to form. This deposit was removed as it formed, by decantation. After removal of 1.1 grams, the precipitate became crystalline in nature. The benzene was allowed to evaporate to dryness leaving 1.0 gram of orange crystals. After recrystallization five times in dry benzene, the crystals melted at 125-129°C. A sample of 0.1 gram was sublimed at five millimeters mercury and 100°C. The sublimate melted at 129-131°C and the melting point was

not depressed when the sublimate was mixed with an authentic sample of vanilloylformic acid.

A sample of 0.1 gram of the vanilloylformic acid was converted to methylveratroylformate by treatment with diazomethane. The melting point of this compound after crystallization from dilute alcohol was 59-60°C. The literature value for the melting point of this compound is 60°C (10, p.2411).

The remaining 1.1 grams of impure vanilloylformic acid, separated as an oily residue, was not further purified. Crystallization from benzene is not satisfactory unless the compound is nearly pure. It was noted that each time the vanilloylformic acid was dissolved in benzene during the purification of the crystalline fraction there was some decomposition. This was probably due to lengthy boiling required to achieve solution. Several solvents and solvent mixtures were investigated, however none of them showed any improvement over benzene.

Chromatographic examination of the impure fraction, the purified material and an authentic sample of vanilloylformic acid with three solvent systems was carried out. In every instance, only one spot was observed for each sample, and that corresponded with the authentic vanilloylformic acid.

Although the isolated vanilloylformic acid was only 2.4 percent of the methylated phenolic acid, the author

feels that the yield is actually closer to the value of 7.0 percent, the yield of the crude fraction. The difficulty in dissolving the ammonium salt in alcohol after determination of the yield indicated the low solubility in this solvent. Subsequent decolorization with activated carbon reduced the yield of this fraction by about half, although the carbon was washed carefully with hot alcohol. The yield should have been redetermined by the oxidation of a new sample, however the supply of methylated phenolic acid had been exhausted.

Bisulfite Soluble Compounds From Bands 3 and 3a. From the experience with the ammonium salt of vanilloyl-formic acid, these fractions were taken up in bicarbonate, extracted with ether, acidified and re-extracted with ether. The ether solutions were dried and distilled leaving dark brown residues. Extraction with hot benzene dissolved 1.1 and 0.9 grams respectively from these residues. The material insoluble in benzene was a tan colored powder which gradually softened, turned red and then carbonized on the melting point block. This tan powder was discarded.

The concentrated benzene solution of band 3 deposited 81 milligrams of pale yellow crystals. The crystals gave a negative reaction with five percent ferric chloride and 2,4 dinitrophenylhydrazine reagents. The compound melted

at 247-248°C, subliming rapidly as the melting point was approached. The qualitative ultra violet absorption spectrum was determined and was similar to that of vanillic acid. Chromatography of this material with butanol saturated with two percent ammonium hydroxide revealed a single spot at R_f 0.09-0.13, which reacted to produce a red color with bis-diazotized benzidine. A neutralization equivalent determined on a 15 milligram sample gave the value of 168 for the equivalent weight. This value is between that of a hydroxy-methoxy benzoic acid and a dimethoxy benzoic acid. A survey of this type of compounds listed in Beilstein showed that the melting point corresponded quite closely to that of isovanillic acid, the melting point being listed at 250°C. All other compounds of the classes listed above had melting point values no higher than 206-207°C, although all possible compounds of this type were not characterized.

Further concentration of the benzene produced a semi-crystalline mixture. Dissolving the material in benzene and adding ligroin until the solution was cloudy, followed by evaporation caused an oily deposit to form. Further attempts to crystallize the mixture from organic solvents failed. The solution was evaporated to dryness and taken up in hot water. Cooling for 48 hours at 10°C produced a precipitate weighing 0.2 grams. Chromatograms of this

material showed two spots, one due to the compound described above and the other due to vanillic acid, as shown by a comparison spot of vanillic acid. These two spots overlapped considerable. This is a further indication of the similarity of these compounds. This was recrystallized from chloroform and from chloroform-hexane. In each case the recrystallized product was still a mixture.

Chromatography of the mother liquor from the separation of this mixture revealed the presence of more of these two compounds along with a faint spot which reacted with 2,4 dinitrophenylhydrazine and corresponded with the spot of an authentic sample of veratroylformic acid. The mother liquor was extracted with ether which in turn was extracted with sodium bisulfite. The bisulfite solution was carefully back extracted with ether and acidified. When the sulfur dioxide had been removed, the solution was extracted with ether. The ether solution was dried and distilled leaving a sticky orange residue of 15 milligrams. The residue reacted to produce a red-orange color with five percent ferric chloride, characteristic of veratroylformic acid. Attempts to obtain a crystalline product from this residue failed.

Evaporation of the ether solution remaining after the above bisulfite extraction left a residue of 0.15 gram. This was a mixture of the two compounds indicated earlier. Attempts to separate these compounds by selective solvent

action failed. Some success was achieved by fractional sublimation, but a pure product was never obtained.

Evaporation of the benzene solution of the band 3a fraction left a mixture of crystals and oily residue. The residue was sublimed at 130°C and 5-7 millimeters mercury. The sublimate was a pale yellow and weighed 0.4 gram. Recrystallization from water, resublimation and recrystallization from chloroform-ligroin produced a white powder with a sharp melting point. The melting point was 172-173°C and was not depressed when mixed with an authentic sample of veratric acid, (melting point 176-177°C). The compound gave only a weak color reaction with bis-diazotized benzidine. This is characteristic for fully methylated compounds since the phenolic activation of the benzene ring is required for color formation with this reagent.

Bisulfite Soluble Fraction From Bands 4 and 5. Bicarbonate solution of these fractions was incomplete. Ether extraction of the bicarbonate removed 0.4 and 0.2 gram respectively from these fractions. Either these compounds were not soluble in benzene and therefore had not been removed from the original oxidation mixture at pH 7, or there had been decarboxylation during the isolation procedure.

The bicarbonate solutions were acidified and extracted with ether. The ether solutions were distilled leaving

dark brown residues. The residues were taken up in absolute alcohol and ligroin was added until the solutions were cloudy. After standing for three hours the solutions were filtered. The precipitates were brown powders which weighed 0.11 and 0.13 gram respectively. The samples carbonized without melting and the material was discarded. Evaporation of the filtrates to dryness left red tacky residues. These were taken up in hot water, decolorized with activated carbon and allowed to stand at 10°C for 24 hours. At the end of this time, a total of 0.13 gram of light tan precipitate was removed from these fractions. The product isolated from both of these solutions was identified as 5-carboxy vanillin by melting point, mixed melting point and comparison chromatography as described in the discussion of the copper oxide oxidation products.

Bisulfite Soluble Bands 6 and 7. The components isolated by elution of bands 6 and 7 were treated with bicarbonate solution. A solid residue, insoluble in bicarbonate and ether was filtered from these solutions. This substance weighed 0.31 and 0.15 gram respectively from fractions 6 and 7. Heating this material on the melting point block caused it to carbonize. From the lack of solubility in ether and bicarbonate and the carbonization on heating, it seemed that this might be a product isolated from degradation of a small amount of the filter paper.

The ammonia in the solvent might have produced a soluble component from the paper that collected at high R_f values and was removed by the long extraction with alcohol.

The bicarbonate solutions were acidified and extracted with ether. The ether solutions were dried and distilled leaving dark residues of 0.21 and 0.02 grams. Attempts to purify these fractions were continued until there was insufficient material for further work.

Table XXVI provides a summary of the analysis of the bisulfite soluble products obtained by nitrobenzene oxidation.

TABLE XXVI

YIELD OF BISULFITE SOLUBLE PRODUCTS FROM NITROBENZENE OXIDATION OF METHYLATED PHENOLIC ACID

Oxidation Product	Percent Yield Based on Phenolic Acid
Vanillin	0.50-2.15
Vanilloylformic Acid	2.5-7.0
Vanillic Acid Isovanillic Acid?	0.28
Veratroylformic Acid?	Trace
Veratric Acid	0.34
5-carboxyvanillin	0.13
Unknown Aldehyde (Band 6)	Trace
Unknown Aldehyde (Band 7)	Trace

DISCUSSION

Extensive search for a chromatographic solvent system which would resolve the phenolic acid was only partially successful. The evidence obtained from this investigation favored the conclusion that the material was homogeneous, or contained very small amounts of impurities. The conclusion was tentative since none of the solvents investigated were entirely satisfactory. A better criterion for the purity of this material is needed. For this type of material, electrophoretic methods of separation might provide the solution and should be investigated in any further study of the phenolic acid.

Exploratory oxidations with nitric acid and permanganate were unsuccessful from the standpoint of degradation products. They did confirm that the phenolic acid was a highly condensed and highly active phenolic compound. The amorphous nitro-products isolated from the nitric acid oxidation mixtures indicate a similarity to lignin since "amorphous nitro-lignin" products and oxalic acid are the usual products from the action of nitric acid and lignin.

The results from attempts to hydrolyze the phenolic acid in concentrated potassium hydroxide ruled out a structure of the chromone type, unless chromone groups were further linked by carbon to carbon bonds. Even if this were the case, a small yield should have been isolated

from irregularities of the polymer and from end groups.

Alkaline fusion products obtained from both white fir and Douglas fir were of the same composition, however the yield of crude products was higher for the white fir. This provides evidence both for similarity and differences between these phenolic acids. The presence of phloroglucinol among the fusion products was the first evidence obtained for a basic structural difference between phenolic acid and wood lignin. As far as the author knows, no phloroglucinol nucleus has been isolated from lignin degradation.

Further comparison between Douglas fir and white fir cork phenolic acids was obtained from alkaline nitrobenzene oxidation. Qualitative analysis of the oxidation products from white fir revealed the presence of protocatechualdehyde, vanillin and p-hydroxybenzaldehyde. Only the first two of these aldehydes were isolated from the Douglas fir oxidation mixture. This, along with the difference in yield from the alkaline fusions, shows some difference in these phenolic acids.

Oxides of copper and mercury were somewhat more successful in providing degradation products. Both of these reagents produced the same mixture of oxidation products. The low yield of identified products was qualitatively similar to the products isolated from oxidation of lignin. There were a number of unidentified compounds which were

of an unstable nature. Further work should be carried out since these compounds would be of great interest in the elucidation of the structure of the phenolic acid. From very little evidence, it is the author's opinion that much of the amorphous material isolated in the copper oxide oxidation arises from decomposition of the unknown compound labeled acid 1 in Table XIV.

Methylation with dimethy sulfate resulted in the separation of the phenolic acid into two fractions. This separation did not produce homogeneous fractions, as further methylation caused a further separation. Analysis of the methoxyl content showed that the difference between these fractions was more than a difference in the degree of polymerization. The larger fraction, making up 80-90 percent of the phenolic acid had a functional group content quite similar to that of other phenolic acid preparations. The minor fraction had a much higher carboxyl content and a lower hydroxyl content. The differences were such that the minor fraction could not have been derived from a carboxylation of the phenolic acid during the isolation of the phenolic acid from the cork.

For a further comparison with wood lignin, methylated phenolic acid was oxidized with alkaline nitrobenzene. Results indicate that phenolic acid is not a low methoxyl wood lignin. The yield of vanillin did not increase to a

very great extent, reaching a maximum of 2.15 percent. While the yield of vanillin is not conclusive, production of vanilloylformic acid as a major product is strong evidence for a basic difference in the structures of phenolic acid and wood lignin. This compound has never been reported among the products isolated from the nitrobenzene oxidation of wood lignin and has only recently been reported in mixtures from copper oxide oxidation (10, pp.2409-2412). In this instance it was obtained as a minor product, the yield being 0.4 percent based on the lignin.

This same report indicates that mercuric oxide also produces vanilloylformic acid in yields up to two percent when employed as the oxidant in place of copper oxide.

Evidence for the presence of veratroylformic acid in the mixture of products obtained from alkaline fusion of methylated wood lignin has been reported (3, p.570), and small amounts of this compound have been isolated from lignin by treatment with alkali at 270°C followed by methylation and permanganate oxidation (3, p.403 and p.405).

The low yield of this structural type compound and the fact that it has never been reported among the products from alkaline nitrobenzene oxidation of lignin is a strong indication for a difference in the basic structure of the side chains linking the benzene nuclei in phenolic acid and in lignin.

Before the implications of vanilloylformic acid can be understood, oxidation of model compound should be performed. In the extensive study of lignin model compounds by nitrobenzene oxidation, there has been only one indication for the presence of vanilloylformic acid and in this case isolation and identification were not performed (39, p.893). The model compound under investigation was propioguaiacone.

Another factor which should be investigated is the stability of vanilloylformic acid under conditions of nitrobenzene oxidation. It seems likely that an alpha-keto acid would undergo decomposition in alkaline solutions at 180°C. If this were true, a recovery experiment would give an indication of the actual amount of vanilloylformic acid produced by this oxidation.

Other research suggested by the results of this work include: further investigation of methods for the isolation of the phenolic acid, preferably by reagents and procedures that can not introduce carboxyl groups; research on separation and purification of the fractions of the phenolic acid; completion of the analysis of the products obtained from the nitrobenzene oxidation of the methylated phenolic acid; methylation and nitrobenzene oxidation of Douglas fir phenolic acids; methylation and nitrobenzene oxidation of the chlorophene from white fir; and the investigation of the degradation of phenolic acid by hydrogenation.

SUMMARY

Exploratory oxidation of white fir cork phenolic acid with nitric acid and potassium permanganate failed to provide identifiable degradation products other than oxalic acid. Alkaline hydrolysis was equally unsuccessful, while alkaline fusion produced low yields of the same products from both white fir and Douglas fir cork phenolic acids.

Alkaline nitrobenzene oxidation supplied further evidence for the similarity of these materials. Qualitative examination of the oxidation mixture showed the presence of the same compounds reported earlier from Douglas fir.

Mercuric and cupric oxides in alkaline solution cleaved the phenolic acid providing low yields of compounds similar to those isolated from degradation of lignin with these reagents.

A method for the separation of the phenolic acid into two non homogeneous fractions resulted when the material was methylated with dimethyl sulfate. Analysis of the major of these two fractions showed that the functional group content was similar to phenolic acids isolated from the bark of other trees.

Analysis of the bisulfite soluble products from nitrobenzene oxidation of a methylated sample of the phenolic acid showed that vanilloylformic acid was the major

component. This provides definite evidence for a basic difference in structure between the phenolic acid and wood lignin and indicates a path for future investigation in the analysis of the structure of the phenolic acid.

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