THE CHEMICAL NATURE OF THE "LIGNIN" OF
THE BARK OF DOUGLAS FIR,
PSEUDOTSUGA MENZIESII (MIRB.) FRANCO
by
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THE CHEMICAL NATURE OF THE "LIGNIN" OF
THE BARK OF DOUGLAS FIR,
PSEUDOTSUGA MENZIESII (MIRB.) FRANCO

INTRODUCTION

This study was undertaken with the hope that, by investigating the lignin components of Douglas-fir bark, further elucidation of the general nature of bark lignins might be possible. Furthermore, such information would be a valuable aid in the future utilization of the 1.5 million tons of Douglas-fir bark annually produced as a byproduct of the lumber industry.

An anatomical investigation of Douglas-fir bark showed that it is composed of several constituents. The bark between the wood cambium and the cork cambium is designated the inner bark and is composed of bast fibers, sieve cells, and phloem parenchyma. The outer bark is made up of yellow layers of cork which are separated by reddish-brown areas of phloem tissue that contain bast fibers and crushed parenchyma. Any problem concerned with the study of Douglas-fir bark lignin must take into consideration the distinctly different nature of these lignin-containing bark elements. Failure to do so will only serve to increase the complexity of such a problem. Physical methods for the isolation of these Douglas-fir bark components in a relatively pure form have been previously described (12, pp.59-60 and 20, p.14).
An earlier study of Douglas-fir bark bast fiber lignin showed that it can be divided into two principal fractions, the usual dioxane-hydrochloric acid lignin and an alkali-soluble lignin which contained 4.3 per cent methoxyl (20, pp.16-18). The low-methoxyl lignin, found as a thin encrusting layer around the needle-shaped bast fibers, possessed a building unit of molecular weight 850-918. It contained one carboxyl, one methoxyl, two alcoholic hydroxyl and four phenolic hydroxyl groups. Differences between this building unit and that of native lignin, as proposed by Brauns, are quite apparent (29, p.299). Because of its chemical nature, this low-methoxyl lignin was termed a phenolic acid.

An investigation of Douglas-fir bark cork demonstrated that approximately 40 per cent of the cork could be isolated as an alkali-soluble phenolic acid whose methoxyl content was identical to that of the Douglas-fir bark bast fiber phenolic acid (12, p.65). As it occurs naturally in the cork, the phenolic acid is esterified in the form of a phenolic acid-hydroxy acid complex.

Several lignin fractions have been isolated from the bark of redwood, *Sequoia sempervirens* Endl., including a low-methoxyl alkali-soluble lignin which was reported to contain a carboxyl group (16, p.119).
Further investigations concerning the chemical nature of bark lignins have been quite limited. Attempts to point out differences or similarities between bark lignin and wood lignin, in relation to their chemical nature and properties, have been hindered by the more complex constitution of the bark. It is hoped that this work may serve as a means of correlating relationships between the lignins from these two sources.
EXPERIMENTAL PROCEDURE

I. COLLECTION AND PREPARATION OF SAMPLES.

The bark used in this work was collected November 4, 1951 on the holdings of Caffall Brothers near Estacada, Oregon. Fresh moist samples were selected from mature newly-felled trees.

Following collection, the large bark slabs were sawed lengthwise into strips approximately two inches wide and four feet long. The inner bark was then removed from these strips by sawing through the region of the cork cambium. Small sections of outer bark not removed from the inner bark by this procedure were eliminated by hand. Hence, the bark samples were divided into two fractions, the inner bark and the outer bark; the yield of the former was 11.1 per cent of the oven-dry weight of the whole bark with the remainder being outer bark. The two fractions, containing approximately 18 per cent moisture, were then ground separately on a Greundler hammer mill, air dried, and screened by means of a Rotap shaker to segregate the components according to particle size. Results are shown in Table I.
TABLE I

PARTICLE SIZE DISTRIBUTION OF GROUND INNER AND OUTER BARK
(percentage based on oven-dry weight of bark component)

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>Inner Bark (%)</th>
<th>Outer Bark (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larger Than 20 Mesh</td>
<td>24.5</td>
<td>51.0</td>
</tr>
<tr>
<td>20-35 Mesh</td>
<td>19.0</td>
<td>15.6</td>
</tr>
<tr>
<td>35-60 Mesh</td>
<td>27.6</td>
<td>12.8</td>
</tr>
<tr>
<td>60-100 Mesh</td>
<td>21.2</td>
<td>9.3</td>
</tr>
<tr>
<td>Smaller Than 100 Mesh (by difference)</td>
<td>7.7</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The fraction of the inner bark which failed to pass a 20 mesh screen was composed of particles containing bast fibers, sieve tubes and phloem parenchyma that had not been broken down into their individual components. Fractions 20-35, 35-60, and 60-100 mesh were made up primarily of individual bast fibers. Those particles which passed through a 100 mesh screen were a mixture of small bast fibers, crushed sieve cells, and phloem parenchyma and were designated fines.

The fraction of the outer bark which was larger than 20 mesh consisted of large cork particles with a small amount of phloem tissue. It was used later as a source.
of cork phenolic acid. The 20-35 mesh fraction contained large bast fibers and small cork particles. The 35-60 mesh fraction was made up of bast fibers in admixture with phloem tissue. Small bast fibers and a considerable amount of phloem tissue were found in the 60-100 mesh fraction. The outer bark fines, particles smaller than 100 mesh, contained finely divided phloem tissue together with a moderate amount of broken bast fibers.

Following the screening operation, the individual fractions from the outer and inner barks were placed in sealed glass jars for storage.

To obtain a large quantity of bast fibers from the outer bark it was necessary to carry out additional work since the grinding and screening procedure did not provide a sample of sufficient purity for conclusive chemical examination. The 35-60 mesh outer bark fraction was made up primarily of bast fibers. Since it provided particles of a size which would promote efficient solvent extraction, an attempt was made to remove the phloem impurities by the procedure of Kiefer and Kurth (20, p.14).

The outer bark 35-60 mesh material was placed in distilled water. The lighter phloem particles floated on top of the water while the bast fibers sank to the bottom of the container. The phloem impurities were scraped from the surface and the bast fibers removed by filtration.
However, microscopic examination of this purified bast fiber fraction showed considerable impurities still present. To increase the efficiency of the purification treatment, the density of the suspending medium was increased to approximately 1.2 by saturating a water solution with sodium chloride at 25°C. The 35-60 mesh fraction was placed in approximately six times its volume of the sodium chloride solution. After stirring vigorously and then allowing to stand for fifteen minutes, the lighter impurities were skimmed from the surface, washed thoroughly with distilled water on a Büchner funnel, air dried and stored for future use in a sealed glass container. Microscopic examination of this material demonstrated that it consisted primarily of crushed sieve tubes and phloem parenchyma. In order to simplify further discussions concerning this fraction and to distinguish it from the outer bark bast fibers and cork, it was designated outer bark phloem. The outer bark bast fibers were filtered from the salt solution, washed with distilled water, air dried and stored in a similar manner. By this procedure, the 35-60 mesh outer bark fraction was found to contain 76 per cent pure bast fibers and 24 per cent phloem.

In all, six physically separable bark fractions were prepared. These consisted of inner bark bast fibers, inner bark fines, outer bark bast fibers, outer bark phloem, outer bark fines, and cork. To obtain conclusive
information concerning the nature of Douglas-fir bark lignin, it was decided that examination of each of these six fractions was necessary.

Extractive-free samples of the 35-60 mesh inner bark bast fibers, outer bark bast fibers and outer bark phloem were prepared in accordance with T.A.P.P.I. method T 12 m-45 which consisted of making successive extractions with alcohol-benzene, alcohol, and hot water (28). Similar samples of the cork, outer bark particles larger than 20 mesh, and the outer and inner bark fines were obtained by this procedure.

The yields and nature of the extractives from Douglas-fir bark cork and bast fibers have been previously determined. The total extractive content of the bast fibers was 13.5 per cent (20, p.15); that of the cork was 37.6 per cent (12, p.60). The yields of extractives from the inner and outer bark fines were found to be 13.4 and 18.9 per cent, respectively, as shown in Table 2. The outer bark phloem contained 28.9 per cent extractives as given in Table 3.
### TABLE 2

**EXTRACTIVE CONTENT OF INNER AND OUTER BARK FINES**

(Percentages based on oven-dry weight of unextracted fines)

<table>
<thead>
<tr>
<th>Component</th>
<th>Inner Bark Fines (%)</th>
<th>Outer Bark Fines (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol-Benzene Solubles</td>
<td>6.7</td>
<td>13.6</td>
</tr>
<tr>
<td>Alcohol Solubles</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Hot-Water Solubles</td>
<td>5.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Total Extractives</td>
<td>13.4</td>
<td>18.9</td>
</tr>
</tbody>
</table>

### TABLE 3

**EXTRACTIVE CONTENT OF OUTER BARK PHLOEM**

(Percentages based on oven-dry weight of unextracted phloem)

<table>
<thead>
<tr>
<th>Component</th>
<th>Outer Bark Phloem (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol-Benzene Solubles</td>
<td>18.1</td>
</tr>
<tr>
<td>Alcohol Solubles</td>
<td>3.5</td>
</tr>
<tr>
<td>Hot-Water Solubles</td>
<td>7.3</td>
</tr>
<tr>
<td>Total Extractives</td>
<td>28.9</td>
</tr>
</tbody>
</table>
The fact that a higher yield of extractives was obtained from the outer bark fines than from the inner bark fines may be ascribed to the variation in the physical components of the two fractions. The inner bark fines were composed primarily of small bast fibers which contain relatively low yields of extractives. The outer bark fines contained bast fibers and, in addition, a considerable amount of extractive-rich phloem tissue. The presence of this phloem tissue would result in the increased yield from the outer bark fines.
II. EXTRACTION AND DISTRIBUTION OF BARK PHENOLIC ACID

Extraction Of Phenolic Acid.

In previous work, Douglas-fir bark bast fiber phenolic acid was obtained by leaching the fibers with a one per cent sodium hydroxide solution on a steam bath for one hour (20, pp.16-17). To determine whether or not the yield of phenolic acid might be increased, the solubility of the inner bark bast fibers in various alkaline solutions was determined by a modification of T.A.P.F.I. method T 4 m-44(28). The phenolic acid was then precipitated from these alkaline extracts and isolated by the procedure of Kiefer and Kurth (20, pp.16-17).

Two gram samples of the fibers were treated for one hour with the following solutions at 90\degree C.: one per cent sodium hydroxide, five per cent potassium carbonate, 0.1 normal alcoholic potassium hydroxide, and two per cent sodium sulfite. The insolubles were removed from each extract by filtration on sintered glass crucibles of c-porosity. The residue was washed with 100 milliliters of hot water, 50 milliliters of ten per cent acetic acid, and with 200 milliliters of hot water, respectively. It was then dried for two hours in an oven at 105\degree C. and weighed. Similar solubility determinations were made by extracting the fibers for 24 hours with one per cent
sodium hydroxide and five per cent potassium carbonate at 25°C. Results of these analyses are listed in Table 4.

The filtered alkaline aqueous extracts from these determinations were acidified to pH 4 with concentrated hydrochloric acid. In each case, this resulted in the formation of a reddish-brown flocculent precipitate which was removed by filtration on a sintered glass crucible and washed with 25 milliliters of distilled water. The moist precipitate was dissolved in 150 milliliters of dioxane and the resultant solution dried over anhydrous sodium sulfate overnight. After filtering, the dried dioxane solution was evaporated to 25 milliliters and poured into 150 milliliters of diethyl ether with vigorous stirring to precipitate the phenolic acid. The phenolic acid was removed by filtration on a sintered glass crucible, washed with 100 milliliters of ether, and dried in an oven at 105°C; the yields are shown in Table 4.

The data given in Table 4 showed that, of the alkaline solutions tested, not one per cent sodium hydroxide produced the highest yield of phenolic acid. However, both the potassium carbonate and the sodium sulfite extractions gave comparable values when carried out at 90°C. Reduction in the temperature at which the extraction was carried out gave a decided decrease in the yield of phenolic acid.
TABLE 4

SOLUBILITY OF INNER BARK BAST FIBERS IN ALKALINE SOLUTIONS
AND RESULTANT YIELDS OF PHENOLIC ACID
(percentages based on oven-dry weight
of extractive-free fibers)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Temperature (°C.)</th>
<th>Total Extractives (%)</th>
<th>Phenolic Acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Sodium Hydroxide</td>
<td>90</td>
<td>23.7</td>
<td>8.14</td>
</tr>
<tr>
<td>1% Sodium Hydroxide</td>
<td>25</td>
<td>8.5</td>
<td>2.92</td>
</tr>
<tr>
<td>5% Potassium Carbonate</td>
<td>90</td>
<td>20.1</td>
<td>7.37</td>
</tr>
<tr>
<td>5% Potassium Carbonate</td>
<td>25</td>
<td>7.7</td>
<td>2.80</td>
</tr>
<tr>
<td>0.1 N. Alcoholic Potassium Hydroxide</td>
<td>90</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>2% Sodium Sulfite</td>
<td>90</td>
<td>20.0</td>
<td>6.53</td>
</tr>
</tbody>
</table>

Distribution Of Phenolic Acid.

Because of the small amount of phenolic acid which was found in the inner bark bast fibers, the yields of this material from other physical components of the bark were ascertained. The inner bark fines and outer bark fines, phloem, bast fibers and cork were extracted with one percent sodium hydroxide at 90° C. and at 25° C. as previously described and their phenolic acid contents determined.
Extraction of each bark fraction with hot alkali gave a significantly greater yield of phenolic acid than did cold alkali. Almost 60 per cent of the outer bark fines was isolated as phenolic acid. Notable amounts were also obtained from the cork, outer bark phloem, and the inner bark fines. Results of these determinations are summarized in Table 5.

**TABLE 5**

**YIELDS OF PHENOLIC ACID OBTAINED BY ONE PER CENT SODIUM HYDROXIDE EXTRACTION OF BARK COMPONENTS AT 25° AND 90° C.**

(Percentages based on oven-dry weight of extractive-free bark component)

<table>
<thead>
<tr>
<th>Bark Component</th>
<th>25° C. (%)</th>
<th>90° C. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner Bark Bast Fibers</td>
<td>2.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Outer Bark Bast Fibers</td>
<td>3.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Inner Bark Fines</td>
<td>0.1</td>
<td>21.6</td>
</tr>
<tr>
<td>Outer Bark Fines</td>
<td>12.0</td>
<td>58.8</td>
</tr>
<tr>
<td>Outer Bark Phloem</td>
<td>5.1</td>
<td>23.9</td>
</tr>
<tr>
<td>Cork</td>
<td>21.6</td>
<td>39.2</td>
</tr>
</tbody>
</table>
The methoxyl content of each of the phenolic acid fractions isolated from the various bark components by extraction with one per cent sodium hydroxide at 90° C., were determined by T.A.P.P.I. method T 2 m-43 (28). Results are listed in Table 6. It was found that the methoxyl contents of the phenolic acid materials from the inner bark components were considerably higher than those from the outer bark. Values ranged from 8.19 per cent for the inner bark bast fiber phenolic acid to 4.21 per cent for that from cork. An investigation of Eucalyptus bark lignin by Stuart and Harvey also showed the outer bark lignin to have a lower methoxyl content than that from the inner bark; 2.37 per cent compared to 3.23 per cent (26, p.17).
## TABLE 6

**METHOXYL CONTENTS OF PHENOLIC ACID FRACTIONS OBTAINED BY ONE PER CENT SODIUM HYDROXIDE EXTRACTION OF BARK COMPONENTS AT 90° C.**

(Percentages based on oven-dry weight of phenolic acid)

<table>
<thead>
<tr>
<th>Phenolic Acid Source</th>
<th>Methoxyl Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner Bark Bast Fibers</td>
<td>8.19</td>
</tr>
<tr>
<td>Outer Bark Bast Fibers</td>
<td>4.95</td>
</tr>
<tr>
<td>Inner Bark Fines</td>
<td>6.78</td>
</tr>
<tr>
<td>Outer Bark Fines</td>
<td>4.35</td>
</tr>
<tr>
<td>Outer Bark Phloem</td>
<td>4.53</td>
</tr>
<tr>
<td>Cork</td>
<td>4.21</td>
</tr>
</tbody>
</table>
III. ISOLATION AND PROPERTIES OF BARK PHENOLIC ACID

Isolation Of Phenolic Acid.

The next phase of this work dealt with the isolation of large quantities of Douglas-fir bark phenolic acid for use in further investigations. The inner bark bast fibers were obtained as a fraction which was relatively free from other bark components. Because of its high degree of purity, this fraction was selected as a source of phenolic acid for these investigations. The cork was chosen as an additional source due to the large amounts of cork available and because of its high phenolic acid content.

**Inner bark bast fiber phenolic acid.** During a series of orientation experiments, fifty grams of extractive-free inner bark bast fibers were placed in a one-liter beaker and 700 milliliters of one per cent sodium hydroxide added. The mixture was then heated for two hours on a steam bath with constant agitation being provided by an air-driven stirrer. The hot solution was filtered on a Büchner funnel to remove undissolved fibers and this residue washed with five 50 milliliter portions of distilled water. These washings were added to the filtrate which was then cooled to room temperature.
The clear reddish-brown extract was acidified to pH 3 with concentrated hydrochloric acid. This resulted in the formation of a brown flocculent precipitate. After allowing the precipitate to settle overnight, most of the supernatent liquor could be decanted. The remaining liquor and precipitate were transferred to centrifuge tubes and centrifuged for one hour. Following this treatment, the standing liquor was easily poured off. In order to obtain acid-free material, 200 milliliters of distilled water were added to the precipitate and the mixture thoroughly stirred. After centrifuging for one hour, the light-red wash liquor was decanted and the washing procedure repeated twice using fresh portions of distilled water. This attempt to obtain an acid-free precipitate was unsuccessful due to the tendency of the material to dissolve in the wash water. As the pH of the washings increased, the solubility of the precipitate increased. Almost all of the precipitate had dissolved or was suspended in the water after the third washing. A similar phenomenon was observed when a phenolic acid-like material was isolated from redwood bark (16, p119).

A small amount of the combined washings was made distinctly acid to litmus and, after a few minutes, a brown flocculent precipitate was observed. Further work showed that the dissolved material could be reprecipitated
from solution by the addition of sodium chloride. This suggested that the reaction between the precipitate and water was due, at least in part, to a colloidal phenomenon.

The ability of sodium chloride to prevent the phenolic acid precipitate from dissolving in distilled water was utilized in the isolation of 100 grams of inner bark bast fiber phenolic acid. The bast fibers were extracted with one per cent sodium hydroxide at 25°C for 24 hours. Although the yield of phenolic acid produced by this extraction is less than that obtained by treatment at 90°C., as shown in Table 5, it was felt that the cold extraction was justified in that it reduced the possibility of degradative action of the alkali on the phenolic acid. The alkaline extract was filtered and acidified as described earlier. The precipitate which resulted was washed in a centrifuge with five per cent sodium chloride until the washings were acid-free. The moist precipitate was then stirred into a large volume of dioxane in which the phenolic acid is soluble. This solution was dried over anhydrous sodium sulfate overnight, filtered and evaporated to a thin syrup on a steam bath. The concentrated dioxane solution was poured into ten volumes of diethyl ether with vigorous stirring. The precipitated phenolic acid was removed by filtration, washed thoroughly with ether, air dried, and placed in sealed glass jars for storage.
Cork phenolic acid. Three-hundred grams of air-dry extractive-free Douglas-fir bark cork were treated with three liters of one per cent sodium hydroxide at 25°C for 24 hours. Efforts to remove the undissolved cork particles by filtration were unsuccessful due to the syrupy nature of the extract. Therefore, the entire extraction mixture was acidified with concentrated hydrochloric acid to pH 3. Following acidification, the solid material which consisted of undissolved cork particles and precipitated phenolic acid and hydroxy acids was easily removed by filtration on a Büchner funnel through No. 410 S & S filter paper. Attempts to wash the residue with distilled water proved unsuccessful because of the tendency of the phenolic acid to go into solution upon removal of the mineral acid. Therefore, the unwashed reddish-brown residue was air dried on the Büchner funnel.

The air-dried residue was then placed in a large cylinder made from a thick sheet of dissolving pulp and extracted with two liters of diethyl ether in a large Soxhlet-type apparatus for 24 hours to remove the hydroxy acids. A 200 milliliter aliquot portion of this extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The yield of ether soluble material was 21.5 per cent of the oven-dry weight of the extractive-free cork.
After the ether extraction, the cylinder and its contents were air dried. This was followed by a 24 hour extraction with two liters of 95 per cent ethanol to remove the cork phenolic acid. The alcohol extract was filtered, evaporated to 200 milliliters on a steam bath, and poured into one liter of distilled water with vigorous stirring to precipitate the phenolic acid. The precipitate was removed by filtration on a Buchner funnel, washed with one liter of distilled water and air dried. Approximately 55 grams of cork phenolic acid, an 18.3 per cent yield, were obtained by this procedure.

Tannin-Like Nature Of Phenolic Acid.

The dried phenolic acid samples from each bark component exhibited similar properties. They were soluble in dilute alkali and slightly soluble in numerous organic solvents. During isolation, the moist precipitates were found to be soluble in acetone, dioxane, ethyl acetate, and water.

Hydrolysis of the phenolic acid with boiling two per cent sulfuric acid for eight hours failed to show the presence of any sugars. This indicated the absence of carbohydrate material in the phenolic acid. Unlike wood lignin, the phenolic acid failed to give a positive-color
test with phloroglucinol and hydrochloric acid. The material decomposed on heating above 310°C.

Fifty grams of extractive-free inner bark bast fibers were extracted with 700 milliliters of one per cent sodium hydroxide for two hours on a steam bath. The insoluble residue was removed by filtration. The alkaline extract was acidified to pH 3, centrifuged and the supernatant liquor decanted from the precipitate which resulted, as previously described. The moist precipitate was then stirred vigorously with one liter of distilled water so as to obtain an aqueous solution of the phenolic acid. This solution was used in various tests to determine properties of the phenolic acid.

The addition of lead acetate to a small portion of the aqueous solution resulted in the formation of a brown flocculent precipitate. After the precipitate had settled, the supernatent liquid was colorless.

A green color was produced upon the addition of a few drops of freshly prepared ferric chloride reagent to the solution. This indicated the presence of phenolic hydroxyl groups and a green color with ferric chloride is indicative of the presence of a catechol nucleus in the phenolic acid.

Treatment of the solution with gelatin caused the immediate formation of a reddish-brown precipitate.
After allowing the precipitate to settle, the standing liquor was colorless. A similar precipitate was formed when a few drops of bromine were added to a portion of the phenolic acid solution.

Three drops of dilute sulfuric acid were added to five milliliters of the solution. Boiling this mixture resulted in the formation of a reddish-brown flocculent precipitate. This precipitate could not be redissolved in distilled water which indicated that dehydration had occurred.

According to Freudenberg, these reactions are characteristic of materials which belong to the non-hydrolyzable class of tannins called phlobatannins (29, p.421).

The tannin content of the aqueous phenolic acid solution was determined by the hide powder method of the American Leather Chemists (1). Results of this determination are shown in Table 7. It was found that 7.5 per cent of the extractive-free inner bark bast fibers demonstrated a tannin-like nature since it was readily adsorbed by hide powder. This yield is approximately equal to that of the phenolic acid obtained from the inner bark bast fibers shown in Table 5. These results clearly indicated the tannin-like nature of the phenolic acid.

A sheepskin skivver was tanned with an aqueous
solution of the phenolic acid similar to that used in the determination of tannin by the hide powder method. The tanned skivver was dark-brown in color and lacked uniformity. Although inferior in quality, it showed no evidence of decomposition after 18 months.

Similar tannin-like properties were shown by the phenolic acid preparations obtained from the other bark components. Analyses of aqueous solutions of these materials for their tannin content by the hide powder method were not carried out.

TABLE 7

TANNIN ANALYSIS OF ALKALI EXTRACTIVES FROM INNER BARK BAST FIBERS

(percentages based on oven-dry weight of extractive-free inner bark bast fibers)

<table>
<thead>
<tr>
<th>Component</th>
<th>Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>18.5</td>
</tr>
<tr>
<td>Soluble Solids</td>
<td>15.7</td>
</tr>
<tr>
<td>Insolubles</td>
<td>2.8</td>
</tr>
<tr>
<td>Tannin</td>
<td>7.5</td>
</tr>
<tr>
<td>Non-Tannin</td>
<td>8.2</td>
</tr>
</tbody>
</table>
IV. INNER BARK BAST FIBER DIOXANE LIGNIN

Douglas-fir inner bark bast fiber dioxane lignin was isolated so that it could be used as a comparison to the bark phenolic acid preparations in later investigations.

Approximately 20 grams of extractive-free inner bark bast fibers were treated with 500 milliliters of one per cent sodium hydroxide for two hours at 90° C. to remove the phenolic acid. These fibers were filtered from the alkaline extract and washed with 500 milliliters of distilled water, 100 milliliters of five per cent acetic acid, and an additional 500 milliliters of water. They were then air dried, transferred to an Alundum crucible, and extracted in a Soxhlet extractor for 24 hours with a dioxane solution which contained 0.4 per cent by weight of dry hydrochloric acid. At the end of this time, the extract was a light-red color which indicated that only a small amount of dioxane lignin had been removed from the fibers. Therefore, the extract was replaced with a fresh portion of dioxane solution and the fibers were extracted for an additional 24 hours. Before the color of the solvent returning to the receiving flask indicated complete removal of the dioxane lignin, the fibers had to be treated with two additional portions of fresh solvent for 24 hours each. This gave a total extraction time of 96
hours. Fresh solvent was used for each 24 hour extraction period so that four lignin fractions would be available for determining the homogeneity of the dioxane soluble materials.

The extract obtained from the first 24 hour extraction was evaporated on a steam bath to a thin syrup and stirred into one liter of distilled water. The tan precipitate which resulted was washed with distilled water in a centrifuge and dissolved in 100 milliliters of dioxane. The dioxane solution was dried over anhydrous sodium sulfate and filtered. It was poured into one liter of diethyl ether with vigorous agitation to precipitate the lignin which was then removed by filtration, washed with ether and hexane, and dried in a vacuum oven for three hours at 55°C. The lignin from this first 24 hour extraction was termed inner bark bast fiber dioxane lignin I. The yield was 2.80 per cent based on the oven-dry weight of the extractive-free fibers. The lignins from the second, third, and fourth 24 hour extractions were isolated in a similar manner and were termed inner bark bast fiber dioxane lignin II, III, and IV, respectively. The yield of fraction II was 5.44 per cent, that of fraction III was 2.39 per cent while fraction IV comprised 1.29 per cent of the oven-dry weight of the extractive-free inner bark bast fibers. The methoxyl content of each lignin
preparation was determined by T.A.P.P.I. method T 12 m-43 (28). Table 8 contains a summary of these results.

The methoxyl contents of the dioxane lignin fractions varied from 15.0 per cent for that from the first 24 hour extraction to 11.8 per cent for dioxane lignin IV. A significant difference observed between the dioxane lignins from the inner bark bast fibers and wood was that the bark lignin failed to give a positive test with phloroglucinol and hydrochloric acid.

**TABLE 8**

**DIOXANE LIGNIN FROM INNER BARK BAST FIBERS**

(yields based on oven-dry weight of extractive-free fibers)

<table>
<thead>
<tr>
<th>Extraction Sequence</th>
<th>Dioxane Lignin Fraction</th>
<th>Yield (%)</th>
<th>Methoxyl In Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 24 Hours</td>
<td>I</td>
<td>2.80</td>
<td>15.0</td>
</tr>
<tr>
<td>Second 24 Hours</td>
<td>II</td>
<td>5.44</td>
<td>15.0</td>
</tr>
<tr>
<td>Third 24 Hours</td>
<td>III</td>
<td>2.39</td>
<td>13.5</td>
</tr>
<tr>
<td>Fourth 24 Hours</td>
<td>IV</td>
<td>1.29</td>
<td>11.8</td>
</tr>
<tr>
<td>Total Yield</td>
<td></td>
<td>11.92</td>
<td></td>
</tr>
</tbody>
</table>
In a similar determination, the dioxane lignin content of the outer bark bast fibers was evaluated. After extracting for eight hours with dioxane-hydrochloric acid solution, the solvent returning to the receiving flask was colorless which indicated that the major portion of the lignin had been removed. The yield was 12.1 per cent based on the oven-dry weight of extractive-free outer bark bast fibers. This dioxane lignin had a methoxyl content of 13.2 per cent. Like the inner bark bast fiber dioxane lignins, it gave a negative test with phloroglucinol and hydrochloric acid.

The ease with which the dioxane lignin can be removed from the outer bark bast fibers as contrasted to the difficulties encountered in removing that lignin from the inner bark bast fibers indicated a significant difference in the fibers from these two fractions. It appears likely that the lignin in the inner bark fibers is combined in some form of stable chemical union with the carbohydrate components of the fibers. As the bast fibers from the inner bark move into the outer bark during the growth process, there is a partial disruption of the uniting forces. This may be brought about by weathering, or natural oxidation, of a lignin-cellulose linkage.
V. COMPARISON OF BARK LIGNINS AND RELATED MATERIALS BY PAPER CHROMATOGRAPHY

The resolution of various lignin preparations by paper chromatography has been achieved by Bailey who used such developing solvents as dioxane, 95 per cent ethanol, acetone, butyl acetate, and aniline (2, pp. 205-209). In later work, butanol lignin from numerous woods was resolved using butyl acetate as the developing solvent (3, pp. 395-398). This work demonstrated the heterogeneous nature of the lignins and the chromatographic pattern appeared to be a specific function of the botanical source of lignin.

Bland and Gatley have recently reported an extensive investigation of the methanol lignin from eucalyptus wood (5, pp. 1-12). The use of a wide variety of developing solvents indicated that this material contained at least two different lignin fractions together with impurities. The lignin fractions were identified as such by their ultraviolet absorption spectra.

In order to more fully ascertain the nature of the phenolic acid fractions isolated from the physically separable components of Douglas-fir bark and to compare these acids with related phenolic materials, extensive paper chromatographic studies were carried out.
The inner bark bast fibers and outer bark bast fibers, phloem, and cork were leached with one per cent sodium hydroxide for 24 hours at 25° C. and the phenolic acid isolated from each extract as previously described. An additional sample of cork phenolic acid was prepared by extraction at 90° C. The inner bark bast fiber dioxane lignin fractions I, II, III and IV, described in the preceding section, were also used in this study. The wood from western hemlock, Tsuga heterophylla Sarg., was used as a source of native lignin (6, pp.213-214). White fir, Abies concolor Lindl. and Gord., wood was extracted with dioxane and hydrochloric acid and the dioxane lignin isolated by conventional methods. Other samples used were Douglas-fir cork phlobaphene (12, p.61), ethyl acetate soluble Douglas-fir bark tannin (15, p.606), and white fir tannin (13, p.144).

A Fisher strip-paper chromatography apparatus was used throughout this investigation. The spotting solutions, prepared by dissolving three grams of phenolic material in 100 milliliters of two normal sodium hydroxide, were applied to strips of No. 1 Whatman filter paper. The spots were acidified by passing the paper rapidly over a porcelain dish which contained boiling glacial acetic acid. After air drying, these strips were placed in the developing apparatus, equilibrated with the solvent.
system for three hours, and developed by the descending method at a constant temperature of 21° C.

Duplicate chromatograms of each sample were prepared. One of the developed strips was examined under ultraviolet light and the spots observed were outlined with a soft lead pencil and labeled as to color. The second was sprayed with an indicator prepared by mixing equal amounts of freshly prepared one per cent solutions of ferric chloride and potassium ferricyanide (4, p. 249) which gives a deep-blue color with phenolic materials. A permanent record was obtained by washing the sprayed strip in dilute hydrochloric acid and then with water.

The solvent systems used in this work are listed in Table 9 together with a brief description of the results obtained for the inner bark bast fiber phenolic acid. It is evident, upon close examination of the developing solvents, that the presence of a large percentage of water had a tendency to increase the resolution of the phenolic acid materials. In cases where the constituents of the developer formed two immiscible layers, it was found that better resolution was obtained with the aqueous phase than with the organic phase. Some resolution was achieved using distilled water alone as the developer.

Of the systems investigated, the most significant information was obtained with methyl ethyl ketone:
water (1:5), acetone:methyl isopropyl ketone:water (140:75:40), and acetone:methyl ethyl ketone:water (25:75:100). The latter was found to give excellent resolution of methanol lignin from eucalyptus wood (5, p.1). These three solvent systems were then used to prepare chromatograms of other phenolic materials. Results are shown in Tables 10, 11 and 12.

Photographs which compare several of the chromatograms after they had been sprayed with ferric chloride-potassium ferricyanide indicator and as they appeared under ultraviolet light are shown in Figures 1 through 14.

Examination of the chromatograms showed that a significant portion of each fraction investigated gave a blue color with the ferric chloride-potassium ferricyanide indicator which demonstrated its phenolic nature. However, certain spots were highly colored only at their extreme edges, the center areas being a faint-blue. This was found to be due to the inability of the indicator to wet areas in which the phenolic acid was highly concentrated. If the indicator was applied by means of a piece of absorbent cotton with a gentle rubbing action, the entire spot became deep-blue. This inability of the indicator to wet spots which contained concentrated amounts of phenolic material, however, resulted in the formation of chromatograms which were extremely distinct when the
solvent system containing acetone:methyl isopropyl ketone: water (140:75:40) was used. In this case, the indicator reacted with the paper to produce a deep-blue color while the areas which contained adsorbed materials were only slightly colored. Figures 1, 2, 9 and 14 are illustrative of this phenomenon.

Analysis of the data contained in Table 9 showed conclusively that the inner bark bast fiber phenolic acid was heterogeneous. In every chromatogram developed, there was a certain amount of material which remained at the base line. The remainder was further divided into as many as three additional zones when observed under ultraviolet light. However, when such resolution was achieved, there always appeared to be one significant spot or zone which stood out because of the strong intensity of its fluorescence and because of the intense blue spot which resulted after application of the indicator. These two factors suggested that most of the phenolic acid was located in one spot, the other zones containing a much smaller percentage of the original material. It was concluded, therefore, that the inner bark bast fiber phenolic acid was primarily a definite chemical entity together with smaller amounts of impurities.

The outer bark bast fiber and phloem phenolic acids show strong similarities as shown in Figure 1. This fact
was in agreement with their methoxyl contents which were 4.95 and 4.53 per cent, respectively. Figure 2 indicated a slight difference between these two fractions and the inner bark bast fiber phenolic acid whose methoxyl content was 8.19 per cent.

No significant difference was noted between the cork phenolic acids obtained by extraction with alkali at 90° C. and at 25° C. Figure 3 showed them to give very similar chromatograms. The cork phenolic acid obtained by hot alkali extraction was found to be distinctly contrasting in nature to the cork phlobaphene as evidenced in Figures 4 and 5. Figures 6 and 7 indicate a slight difference between the cork phenolic acid isolated at 25° C. and the inner bark bast fiber phenolic acid. These two figures, in addition to Figures 8 and 9, indicated a high degree of homogeneity in these materials.

The methoxyl contents of the inner bark bast fiber dioxane lignin fractions I, II, III and IV were 15.0, 15.0, 13.5 and 11.8, respectively. The chromatograms of these materials, as pictured in Figures 10 and 11, indicated them to be relatively homogeneous. Fractions I, II and III gave identical chromatograms while IV showed slight differences from the other three.
A comparison of chromatograms of white fir dioxane lignin and western hemlock native lignin is provided in Figure 12. Prominent dissimilarities in these two lignins were found using methyl ethyl ketone:water (1:5) as the developing solvent.

Figure 13 demonstrated that it was possible to show considerable differences between Douglas-fir cork phlobaphene and bark tannin by paper chromatography. Another tannin, that of white fir bark, is shown in Figure 14.
<table>
<thead>
<tr>
<th>Developing Solvent</th>
<th>Developing Time (Hrs.)</th>
<th>Ultraviolet Light</th>
<th>FeCl₃-K₃Fe(CN)₆ Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether saturated with water</td>
<td>48</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Blue spot at Rf = 0.00.</td>
</tr>
<tr>
<td>Ethanol (95%)</td>
<td>5</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Blue spot at Rf = 0.00.</td>
</tr>
<tr>
<td>Acetone</td>
<td>6</td>
<td>Lavender fl. spot at Rf = 0.00 and at Rf = 0.09 - 0.45.</td>
<td>Blue spot at Rf = 0.00.</td>
</tr>
<tr>
<td>Acetone:water (1:1)</td>
<td>2</td>
<td>Brown fl. spot at Rf = 0.78 - 0.89. Tan fl. at Rf = 0.90 - 1.00.</td>
<td>Blue streak at Rf = 0.00 - 0.74 and colorless spot at Rf = 0.78 - 0.85 surrounded by bright-blue border. Faint streak at Rf = 0.89 - 1.00.</td>
</tr>
</tbody>
</table>

1. fl. = fluorescent or fluorescence.
<table>
<thead>
<tr>
<th>Developing Solvent</th>
<th>Developing Time (Hrs.)</th>
<th>Ultraviolet Light</th>
<th>FeCl₃-K₃Fe(CN)₆ Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone:water (1:3)</td>
<td>2</td>
<td>Reddish-brown fl. spot at Rf = 0.00. Light-blue fl. at Rf = 0.00 - 0.10. Brown fl. spot at Rf = 0.71 - 0.86.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.69. Bright-blue spot at Rf = 0.69 - 0.90.</td>
</tr>
<tr>
<td>Acetone:water (1:5)</td>
<td>1.5</td>
<td>Brown fl. spot at Rf = 0.00. Yellow fl. spot at Rf = 0.70 - 0.77. Tan fl. spot at Rf = 0.77 - 0.83.</td>
<td>Blue spot at Rf = 0.00. Blue spot at Rf = 0.75 - 0.90. Diffuse faint-blue streak at Rf = 0.90 - 1.00.</td>
</tr>
<tr>
<td>Acetone:water (1:6)</td>
<td>2</td>
<td>Brown fl. spot at Rf = 0.00. Brown fl. spot at Rf = 0.81 - 0.90. Tan fl. streak at Rf = 0.90 - 1.00.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.05. Bright-blue spot at Rf = 0.79 - 0.90. Blue streak at Rf = 0.90 - 1.00.</td>
</tr>
<tr>
<td>Acetone:water (3:1)</td>
<td>2</td>
<td>Brown fl. spot at Rf = 0.00. Faint-brown fl. streak at Rf = 0.00 - 0.64. Brown fl. spot at Rf = 0.77 - 0.83.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.64. Blue spot at Rf = 0.64 - 0.75. Colorless spot at Rf = 0.77 - 0.97 with narrow bright-blue border.</td>
</tr>
<tr>
<td>Developing Solvent</td>
<td>Developing Time (Hrs.)</td>
<td>Ultraviolet Light:</td>
<td>FeCl₃-K₃Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------------------------</td>
<td>--------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Acetone:water (6:1)</td>
<td>2</td>
<td>Dark-brown fl. spot at Rf = 0.00. Brown fl. streak at Rf = 0.00 - 0.23. Yellow fl. at Rf = 0.23 - 0.30. Bright-yellow fl. at Rf = 0.98 - 1.00.</td>
<td>Dark-blue spot at Rf = 0.00 - 0.23. Faint-blue streak at Rf = 0.23 - 0.46. Dark-blue at Rf = 0.98 - 1.00.</td>
</tr>
<tr>
<td>Water</td>
<td>1.5</td>
<td>Reddish-brown fl. spot at Rf = 0.00. Tan fl. at Rf = 0.00 - 0.03. Brown fl. spot at Rf = 0.85 - 0.94.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.82. Well-defined blue spot at Rf = 0.82 - 0.97.</td>
</tr>
<tr>
<td>Methyl ethyl ketone: water (1:5)</td>
<td>2</td>
<td>Light-brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.74 - 0.82.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.74. Faint-blue spot at Rf = 0.74 - 0.90.</td>
</tr>
<tr>
<td>Acetone:methyl ethyl ketone:water (1:3:4)</td>
<td>1.5</td>
<td>Brown fl. at Rf = 0.00 - 0.31. Brown fl. spot at Rf = 0.73 - 0.85.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.65. Bright-blue well-defined spot at Rf = 0.65 - 0.86. Faint-blue streak at Rf = 0.86 - 1.00.</td>
</tr>
<tr>
<td>Developing Solvent</td>
<td>Developing Time (Hrs.)</td>
<td>Ultraviolet Light</td>
<td>FeCl₃-K₃Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Acetone:methyl isopropyl ketone:water (1:3:4)</td>
<td>2</td>
<td>Brown fl. spot at Rf = 0.00. Faint-brown fl. at Rf = 0.00 - 0.48. Yellow fl. at Rf = 0.63 - 0.88.</td>
<td>Blue spot at Rf = 0.00. Blue streak at Rf = 0.00 - 0.81 surrounded by blue border.</td>
</tr>
<tr>
<td>Acetone:methyl isopropyl ketone:water (140:75:40)</td>
<td>1.5</td>
<td>Brown fl. spot at Rf = 0.00. Blue fl. spot at Rf = 0.25 - 0.50. Tan fl. = 0.00 - 0.18. Faint band at Rf = 0.83 - 0.88. Blue spot at Rf = 0.22 - 0.45. Blue band at Rf = 0.82 - 0.90.</td>
<td>Blue spot at Rf = 0.00. Diffuse blue streak at Rf.</td>
</tr>
<tr>
<td>Acetone:methyl isopropyl ketone:water (115:75:80)</td>
<td>2</td>
<td>Brown fl. spot at Rf = 0.00. Bright-blue fl. spot at Rf = 0.59 - 0.82.</td>
<td>Deep-blue spot at Rf = 0.00. Diffuse blue streak at Rf.</td>
</tr>
<tr>
<td>N-butanol:water (1:1) (aqueous layer)</td>
<td>2</td>
<td>Brown fl. spot at Rf = 0.00. Brown fl. spot at Rf = 0.75 - 0.85. White fl. streak at Rf = 0.35 - 1.00. Small brown fl. spot at Rf = 1.00.</td>
<td>Blue spot at Rf = 0.00. Bright-blue well-defined spot at Rf = 0.71 - 0.86. Faint-blue streak at Rf = 0.86 - 1.00. Small blue spot at Rf = 1.00.</td>
</tr>
<tr>
<td>Developing Solvent</td>
<td>Developing Time (Hrs.)</td>
<td>Ultraviolet Light</td>
<td>( \text{FeCl}_3 \cdot \text{K}_3\text{Fe(CN)}_6 ) Indicator</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------------------------</td>
<td>-------------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>N-butanol:water (1:1). (organic layer)</td>
<td>2</td>
<td>Brown fl. spot at ( R_f = 0.00 ). Well-defined yellow fl. spot at ( R_f = 0.00 - 0.07 ) with a thin border of reddish-brown fl. material.</td>
<td>Intense-blue spot at ( R_f = 0.00 - 0.07 ). Faint-blue streak at ( R_f = 0.07 - 0.14 ).</td>
</tr>
<tr>
<td>N-butanol:benzene:water (1:1:2). (aqueous layer)</td>
<td>2</td>
<td>Brown fl. spot at ( R_f = 0.00 ). Brown fl. spot at ( R_f = 0.75 - 0.90 ) with a light-yellow fl. border.</td>
<td>Blue spot at ( R_f = 0.00 ). Blue oblong spot at ( R_f = 0.80 - 0.91 ). Light-blue streak at ( R_f = 0.89 - 1.00 ).</td>
</tr>
<tr>
<td>N-butanol:benzene:water (1:1:2). (organic layer)</td>
<td>4</td>
<td>Brown fl. spot at ( R_f = 0.00 ). Well-defined bright-yellow fl. spot at ( R_f = 0.00 - 0.04 ) with a narrow border of reddish-brown fl. material.</td>
<td>Blue spot at ( R_f = 0.00 ). Blue oblong spot at ( R_f = 0.80 - 0.91 ). Light-blue streak at ( R_f = 0.89 - 1.00 ).</td>
</tr>
<tr>
<td>Dioxane:water (3:1)</td>
<td>7</td>
<td>Brown fl. spot at ( R_f = 0.00 - 0.04 ). Brown fl. streak at ( R_f = 0.45 - 0.94 ).</td>
<td>Faint-blue spot at ( R_f = 0.00 - 0.04 ). Dark-blue streak at ( R_f = 0.50 - 0.86 ). Faint-blue streak at ( R_f = 0.95 - 1.00 ).</td>
</tr>
<tr>
<td>Developing Solvent</td>
<td>Developing Time (Hrs.)</td>
<td>Ultraviolet Light</td>
<td>FeCl₃-K₃Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------------------</td>
<td>-------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Butyl acetate</td>
<td>8</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Bright-blue spot at Rf = 0.00.</td>
</tr>
<tr>
<td>Carbonic acid (pH 4.2)</td>
<td>1.5</td>
<td>Brown fl. spot at Rf = 0.00. Brown fl. oblong spot at Rf = 0.74 - 0.98 surrounded by a light-yellow diffuse border.</td>
<td>Blue spot at Rf = 0.00. Colorless spot at Rf = 0.74 - 0.97 with a diffuse blue border.</td>
</tr>
</tbody>
</table>
### TABLE 10

**CHROMATOGRAPHY OF BARK LIGNINS USING ACETONE:METHYL ETHYL KETONE:WATER (25:75:100)**

(Development time: two hours)

<table>
<thead>
<tr>
<th>Material</th>
<th>Ultraviolet Light</th>
<th>FeCl₃-K₃Fe(CN)₆ Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner bark bast fiber dioxane lignin I.</td>
<td>Brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.70 - 0.77.</td>
<td>Blue spot at Rf = 0.00. Diffuse light-blue streak at Rf = 0.00 - 0.70. Faint-blue spot at Rf = 0.70 - 0.82. Blue streak at Rf = 0.82 - 1.00.</td>
</tr>
<tr>
<td>Inner bark bast fiber dioxane lignin II.</td>
<td>Brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.70 - 0.77.</td>
<td>Dark-blue spot at Rf = 0.00. Diffuse light-blue streak at Rf = 0.00 - 0.70. Faint-blue spot at Rf = 0.70 - 0.82. Blue streak at Rf = 0.82 - 1.00.</td>
</tr>
<tr>
<td>Inner bark bast fiber dioxane lignin III.</td>
<td>Brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.65 - 0.77.</td>
<td>Dark-blue spot at Rf = 0.00. Diffuse light-blue streak at Rf = 0.00 - 0.65. Faint-blue well-defined spot at Rf = 0.65 - 0.82. Blue streak at Rf = 0.82 - 1.00.</td>
</tr>
<tr>
<td>Inner bark bast fiber dioxane lignin IV.</td>
<td>Brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.73 - 0.81.</td>
<td>Dark-blue spot at Rf = 0.00. Very faint-blue diffuse streak at Rf = 0.00 - 0.62. Faint-blue spot at Rf = 0.62 - 0.86.</td>
</tr>
<tr>
<td>Material</td>
<td>Ultraviolet Light</td>
<td>FeCl₃-K₂Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Outer bark bast fiber phenolic acid.</td>
<td>Brown fl. spot at Rf = 0.00.  Faint-brown fl. streak at Rf = 0.00 - 0.45. Dark brown fl. streak at Rf = 0.45 - 0.79 with yellow fl. border.</td>
<td>Dark-blue spot at Rf = 0.00.  Diffuse faint-blue streak at Rf = 0.00 - 0.40. Dark-blue spot at Rf = 0.40 - 0.78.  Faint-blue diffuse streak at Rf = 0.78 - 1.00.</td>
</tr>
<tr>
<td>Outer bark phloem phenolic acid.</td>
<td>Dark-brown fl. spot at Rf = 0.00.  Faint-tan fl. at Rf = 0.00 - 0.58. Light-yellow fl. spot at Rf = 0.58 - 0.74.  Brown fl. streak at Rf = 0.71 - 0.81.</td>
<td>Dark-blue spot at Rf = 0.00.  Diffuse faint-blue streak at Rf = 0.00 - 0.60. Blue spot at Rf = 0.60 - 0.80.  Diffuse faint-blue streak at Rf = 0.80 - 1.00.</td>
</tr>
<tr>
<td>Inner bark bast fiber phenolic acid.</td>
<td>Faint-brown fl. streak at Rf = 0.00 - 0.31. Brown fl. spot at Rf = 0.73 - 0.85 surrounded by a diffuse yellow fl. border.</td>
<td>Blue spot at Rf = 0.00.  Faint-blue streak from Rf = 0.00 - 0.65. Bright-blue well-defined spot at Rf = 0.65 - 0.86.  Faint-blue streak at Rf = 0.86 - 1.00.</td>
</tr>
<tr>
<td>Material</td>
<td>Ultraviolet Light</td>
<td>FeCl₃-K₂Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Inner bark bast fiber phenolic acid.</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Blue spot at Rf = 0.00. Diffuse blue streak at Rf = 0.00 - 0.20</td>
</tr>
<tr>
<td></td>
<td>Brown fl. streak at Rf = 0.00 - 0.12. Blue fl. spot at Rf = 0.31 - 0.62. Tan fl. band at Rf = 0.82 - 0.88.</td>
<td>Faint-blue spot at Rf = 0.28 - 0.50. Blue band at Rf = 0.83 - 0.89.</td>
</tr>
<tr>
<td>Outer bark phloem phenolic acid.</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Dark-blue spot at Rf = 0.00.</td>
</tr>
<tr>
<td></td>
<td>Light-brown fl. streak at Rf = 0.00 - 0.23. Diffuse blue fl. spot at Rf = 0.23 - 0.49. Tan fl. band at Rf = 0.82 - 0.88.</td>
<td>Faint-blue streak at Rf = 0.00 - 0.23. Faint blue spot at Rf = 0.24 - 0.48. Blue band at Rf = 0.85 - 0.89.</td>
</tr>
<tr>
<td>Cork phenolic acid. (25° C. alkali treatment)</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Blue spot at Rf = 0.00. Blue streak at Rf = 0.00 - 0.08.</td>
</tr>
<tr>
<td></td>
<td>Brown fl. streak at Rf = 0.00 - 0.08. Yellow fl. spot at Rf = 0.08 - 0.11. Dark-brown fl. streak at Rf = 0.07 - 0.16. Yellow fl. spot at Rf = 0.16 - 0.20. White fl. spot at Rf = 0.20 - 0.26. Diffuse yellow fl. streak at Rf = 0.26 - 0.40.</td>
<td>Dark-blue spot at Rf = 0.08 - 0.16. Blue streak at Rf = 0.16 - 0.26. Dark-blue spot at Rf = 0.26 - 0.35. Blue streak at Rf = 0.35 - 0.46.</td>
</tr>
<tr>
<td>Material</td>
<td>Ultraviolet Light</td>
<td>FeCl₃-K₂Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>White fir bark tannin</td>
<td>Brown fl. spot at Rf = 0.00.</td>
<td>Blue spot at Rf = 0.00.</td>
</tr>
<tr>
<td></td>
<td>Brown fl. streak at Rf = 0.00 - 0.30. Tan fl. spot at Rf =</td>
<td>Diffuse faint-blue streak at Rf = 0.00 - 0.35.</td>
</tr>
<tr>
<td></td>
<td>0.30 - 0.39. Blue fl. spot at Rf = 0.39 - 0.69. Brown fl.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>streak at Rf = 0.69 - 0.92. Tan fl. band at Rf = 0.92 - 0.93.</td>
<td>Light-blue spot at Rf = 0.35 - 0.63. Diffuse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blue streak at Rf = 0.63 - 0.90. Dark-blue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>band at Rf = 0.90 - 0.92.</td>
</tr>
</tbody>
</table>
### TABLE 12

CHROMATOGRAPHY OF PHENOLIC MATERIALS USING METHYL ETHYL KETONE:WATER (1:5)

(Development time: two hours)

<table>
<thead>
<tr>
<th>Material</th>
<th>Ultraviolet Light</th>
<th>( \text{FeCl}_3-\text{K}_3\text{Fe(CN)}_6 ) Indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cork phenolic acid (25° C. alkali treatment)</td>
<td>Brown fl. spot at ( \text{Rf} = 0.00 ). Tan fl. spot at ( \text{Rf} = 0.71 - 0.83 ).</td>
<td>Blue spot at ( \text{Rf} = 0.00 ). Diffuse faint-blue streak at ( \text{Rf} = 0.00 - 0.72 ). Light-blue spot at ( \text{Rf} = 0.72 - 0.88 ). Blue streak at ( \text{Rf} = 0.88 - 0.97 ).</td>
</tr>
<tr>
<td>Cork phenolic acid (90° C. alkali treatment)</td>
<td>Brown fl. spot at ( \text{Rf} = 0.00 ). Blue fl. spot at ( \text{Rf} = 0.69 - 0.77 ). Brown fl. spot at ( \text{Rf} = 0.77 - 0.89 ).</td>
<td>Blue spot at ( \text{Rf} = 0.00 ). Diffuse faint-blue streak at ( \text{Rf} = 0.00 - 0.68 ). Blue spot at ( \text{Rf} = 0.68 - 0.78 ). Dark-blue spot at ( \text{Rf} = 0.78 - 0.92 ). Diffuse blue streak at ( \text{Rf} = 0.92 - 1.00 ).</td>
</tr>
<tr>
<td>Cork phlobaphene</td>
<td>Brown fl. spot at ( \text{Rf} = 0.00 ). Tan fl. spot at ( \text{Rf} = 0.73 - 0.77 ). Brown fl. spot at ( \text{Rf} = 0.75 - 0.87 ).</td>
<td>Blue spot at ( \text{Rf} = 0.00 ). Faint-blue streak at ( \text{Rf} = 0.00 - 0.70 ). Bright-blue spot at ( \text{Rf} = 0.70 - 0.87 ). Blue streak at ( \text{Rf} = 0.87 - 1.00 ).</td>
</tr>
<tr>
<td>Western hemlock wood native lignin</td>
<td>Blue fl. spot at ( \text{Rf} = 0.75 - 0.81 ).</td>
<td>Blue spot at ( \text{Rf} = 0.00 ). Diffuse blue streak at ( \text{Rf} = 0.00 - 0.73 ). Blue oblong spot at ( \text{Rf} = 0.73 - 0.85 ).</td>
</tr>
<tr>
<td>Material</td>
<td>Ultraviolet Light</td>
<td>FeCl₃-K₃Fe(CN)₆ Indicator</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------</td>
</tr>
<tr>
<td>White fir bark tannin.</td>
<td>Brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.63 - 0.77. Yellow fl. spot at Rf = 0.77 - 0.90.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.58. Intense-blue spot at Rf = 0.58 - 0.78. Blue spot at Rf = 0.78 - 0.94.</td>
</tr>
<tr>
<td>Douglas-fir bark tannin.</td>
<td>Brown fl. spot at Rf = 0.00. Blue fl. spot at Rf = 0.11 - 0.20. Tan fl. spot at Rf = 0.72 - 0.76. Blue fl. spot at Rf = 0.76 - 0.94.</td>
<td>Blue spot at Rf = 0.00. Diffuse faint-blue streak at Rf = 0.00 - 0.70. Deep-blue spot at Rf = 0.70 - 0.87. Faint-blue streak at Rf = 0.87 - 1.00.</td>
</tr>
<tr>
<td>Inner bark bast fiber</td>
<td>Light-brown fl. spot at Rf = 0.00. Tan fl. spot at Rf = 0.74 - 0.82.</td>
<td>Blue spot at Rf = 0.00. Faint-blue streak at Rf = 0.00 - 0.74. Faint-blue spot at Rf = 0.74 - 0.90.</td>
</tr>
</tbody>
</table>
FIGURE 1

PAPER CHROMATOGRAM OF OUTER BARK PHLOEM AND BAST FIBER PHENOLIC ACIDS


1. Outer bark bast fiber phenolic acid.
2. Outer bark phloem phenolic acid.
FIGURE 2

PAPER CHROMATOGRAM OF INNER BARK BAST FIBER AND OUTER BARK PHLOEM PHENOLIC ACIDS

Time: 2.5 hours.

1. Inner bark bast fiber phenolic acid.
2. Outer bark phloem phenolic acid.
FIGURE 3

PAPER CHROMATOGRAM OF CORK PHENOLIC ACIDS ISOLATED BY ALKALI EXTRATION AT 25° AND 90° C.

Time: Two hours.

1. Cork phenolic acid (90° C. alkali treatment).
2. Cork phenolic acid (25° C. alkali treatment).
PAPER CHROMATOGRAM OF CORK PHLOBAPHENE AND CORK PHENOLIC ACID (90° C. ALKALI TREATMENT)

**FIGURE 4**

Solvent: Acetone:water (1:5).
Time: 1.5 hours.

1. Cork phlobaphene.
2. Cork phenolic acid (90° C. alkali treatment).
FIGURE 5

PAPER CHROMATOGRAM OF CORK PHLOBAPHENE AND CORK PHENOLIC ACID (90° C. ALKALI TREATMENT)

1. Cork phlobaphene.
2. Cork phenolic acid (90° C. alkali treatment).
FIGURE 6

PAPER CHROMATOGRAM OF CORK PHENOLIC ACID (25° C. ALKALI TREATMENT)
AND INNER BARK BAST FIBER PHENOLIC ACID

Solvent: Methyl ethyl ketone:water (1:5).
Time: Two hours.

2. Inner bark bast fiber phenolic acid.
FIGURE 7

PAPER CHROMATOGRAM OF CORK PHENOLIC ACID (25° C. ALKALI TREATMENT)
AND INNER BARK BAST FIBER PHENOLIC ACID

FeCl₃ - K₃Fe(CN)₆ Indicator

1 2

Ultraceanet Light

1 2

brown

brown

0.00

0.25

0.50

yellow

yellow

tan
tan

0.75

1.00

Solvent: Acetone:water (1:5).
Time: 1.5 hours.

2. Inner bark bast fiber phenolic acid.
FIGURE 8

PAPER CHROMATOGRAM OF CORK PHENOLIC ACID (25° C. ALKALI TREATMENT) AND INNER BARK BAST FIBER PHENOLIC ACID

FeCl₃ - K₃Fe(CN)₆
Indicator

Ultrasound
Light

1. Inner bark bast fiber phenolic acid.
2. Cork phenolic acid (25° C. alkali treatment).

Time: Two hours.
FIGURE 9

PAPER CHROMATOGRAM OF CORK PHENOLIC ACID (25° C. ALKALI TREATMENT)
AND INNER BARK BAST FIBER PHENOLIC ACID

FeCl₃ - K₂Fe(CN)₆ Indicator

<table>
<thead>
<tr>
<th></th>
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<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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Ultraviolet Light

<table>
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<tr>
<td>1</td>
<td>brown</td>
<td>brown</td>
</tr>
<tr>
<td>2</td>
<td>blue</td>
<td>blue</td>
</tr>
</tbody>
</table>

Time: Two hours.

1. Inner bark bast fiber phenolic acid.
2. Cork phenolic acid (25° C. alkali treatment).
FIGURE 10

PAPER CHROMATOGRAM OF INNER BARK BAST FIBER DIOXANE LIGNINS I AND II

Time: Two hours.

1. Inner bark bast fiber dioxane lignin I.
2. Inner bark bast fiber dioxane lignin II.
FIGURE 11

PAPER CHROMATOGRAM OF INNER BARK BAST FIBER DIOXANE LIGNINS III AND IV

Time: Two hours.

1. Inner bark bast fiber dioxane lignin III.
2. Inner bark bast fiber dioxane lignin IV.
PAPER CHROMATOGRAM OF WHITE FIR WOOD DIOXANE LIGNIN AND WESTERN HEMLOCK NATIVE LIGNIN

FeCl₃ - K₃Fe(CN)₆

Indicator

1 2

Ultraviolet

Light

1 2

Rf

Solvent: Methyl ethyl ketone:water (1:5).
Time: Two hours.

1. White fir dioxane lignin.
2. Western hemlock native lignin.
FIGURE 13

PAPER CHROMATOGRAM OF DOUGLAS-FIR BARK TANNIN AND CORK PHLOBAPHENE

Solvent: Methyl ethyl ketone:water (1:5).
Time: Two hours.

1. Cork phlobaphene.
2. Douglas-fir bark tannin.
FIGURE 14

PAPER CHROMATOGRAM OF WHITE FIR BARK TANNIN

Time: 2.5 hours.

1. White fir tannin.
VI. ABSORPTION SPECTRA OF BARK LIGNINS

Infrared.

The use of infrared spectroscopy has been an important tool in recent studies of the structural nature of lignin. However, difficulties in the interpretation of the spectra have prevented a more complete answer to the lignin problem. The primary purpose behind the use of infrared absorption in this work was to provide a further means of comparing the various bark lignin fractions obtained.

A model 12c Perkin-Elmer spectrometer adapted to automatic double-beam operation was used in this investigation. A sodium chloride prism was utilized in surveying the region between 700 and 3900 wave numbers. The samples were mulled in Nujol and run against a sodium chloride plate blank.

All samples were prepared from Douglas-fir bark by methods described earlier. They consisted of inner bark bast fiber phenolic acid, dioxane lignin fractions I and IV, outer bark bast fiber phenolic acid, and cork phlobaphene. Cork phenolic acid samples, obtained by extraction with one per cent sodium hydroxide at both 25° and 90° C., were also investigated. Close examination of Figures 15 through 21 showed that the infrared spectra
of these materials possess close similarities to each other. The principal absorption peaks of each fraction are listed in Tables 13 and 14 together with the functional group to which the absorption has been tentatively attributed (18, pp.19-51).

Besides demonstrating an overall similarity between the samples investigated, the carbonyl absorption of these fractions was of significant interest. A study of spruce native lignin has shown that carbonyl absorption occurs at 1663 centimeters\(^{-1}\) (19, p.168). This band was attributed to an aldehyde or ketone grouping.

More recently, black spruce native lignin has been fractionated and the infrared spectra of these fractions determined (14, pp.318-319). The high-molecular weight fractions showed a weak broad inflection in the region from 1700 to 1760 centimeters\(^{-1}\) together with a strong band at 1665 centimeters\(^{-1}\). As the molecular weight of the fractions decreased, a band developed at 1710 to 1722 centimeters\(^{-1}\) and, finally, the band at 1665 centimeters\(^{-1}\) was reduced in magnitude until it was only an inflection. Hence, it was demonstrated that black spruce native lignin exhibited carbonyl absorption at two regions of the infrared spectrum. It was indicated that the intensity of this absorption was related to the molecular weight of the lignin fraction.
### Table 13

**Infrared Absorption Bands of Douglas Fir Bark Lignins**

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Cork Phenolic Acid (25°C NaOH)</th>
<th>Cork Phenolic Acid (90°C NaOH)</th>
<th>Outer Bark Bast Fiber Phenolic Acid</th>
<th>Inner Bark Bast Fiber Phenolic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl</td>
<td>3260</td>
<td>3240</td>
<td>3220</td>
<td>3220</td>
</tr>
<tr>
<td>CH₂ or CH₃</td>
<td>2880</td>
<td>2860</td>
<td>2860</td>
<td>2860</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>1703</td>
<td>1696</td>
<td>1692</td>
<td>1692</td>
</tr>
<tr>
<td>Phenyl Rings</td>
<td>1589</td>
<td>1597</td>
<td>1594</td>
<td>1594</td>
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<tr>
<td></td>
<td>1509</td>
<td>1503</td>
<td>1506</td>
<td>1500</td>
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<tr>
<td></td>
<td>1451</td>
<td>1455</td>
<td>1454</td>
<td>1449</td>
</tr>
<tr>
<td>-C-H- (Nujol)</td>
<td>1375</td>
<td>1377</td>
<td>1371</td>
<td>1367</td>
</tr>
<tr>
<td></td>
<td>1260</td>
<td>1257</td>
<td>1260</td>
<td>1260</td>
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<td>877</td>
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<td></td>
<td>819</td>
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<td>813</td>
<td>819</td>
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<td></td>
<td>724</td>
<td>722</td>
<td>720</td>
<td>722</td>
</tr>
</tbody>
</table>
### TABLE 14

INFRARED ABSORPTION BANDS OF DOUGLAS-FIR BARK DIOXANE LIGNINS AND CORK PHLOBAPHENE

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Cork Phlobaphene</th>
<th>Dioxane Lignin I</th>
<th>Dioxane Lignin IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl</td>
<td>3260</td>
<td>3305</td>
<td>3305</td>
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<tr>
<td>CH₂ or CH₃</td>
<td>2845</td>
<td>2845</td>
<td>2845</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>1700</td>
<td>1704</td>
<td>1692</td>
</tr>
<tr>
<td>Phenyl Rings</td>
<td>1618</td>
<td>1581</td>
<td>1584</td>
</tr>
<tr>
<td>-C-H- (Nujol)</td>
<td>1506</td>
<td>1500</td>
<td>1500</td>
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<tr>
<td>Unassigned</td>
<td>1447</td>
<td>1454</td>
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<td>Unassigned</td>
<td>724</td>
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</tbody>
</table>
The Douglas-fir bark lignin fractions studied gave no evidence of absorption at 1663-1665 centimeters\(^{-1}\). The carbonyl absorption of these samples ranged from 1704 centimeters\(^{-1}\) for the inner bark bast fiber dioxane lignin I to 1688 centimeters\(^{-1}\) in the case of the inner bark bast fiber phenolic acid. In this respect, the carbonyl absorption of Douglas-fir bark lignin and phlobaphene preparations appeared closely related to that of low-molecular weight black spruce native lignin. The essential difference between these preparations and native lignin was the lack of carbonyl absorption in the region of 1663-1665 centimeters\(^{-1}\). Similar results were obtained in a study of white fir bark phenolic acid and phlobaphene (13, p.142).

**Ultraviolet.**

The ultraviolet absorption spectra were obtained of the inner and outer bark bast fiber phenolic acids, cork phlobaphene, inner bark bast fiber dioxane lignins I and IV, and cork phenolic acids isolated with alkali at 25° and 90° C. Solutions which contained ten milligrams of sample per liter of 95 per cent ethanol were examined in the region between 220 and 340 millimicrons with a Beckman model DU quartz spectrophotometer.
Once again, an overall similarity between the materials was exhibited as shown by the curves in Figures 22 and 23 where per cent absorption, 100 minus the per cent transmission, is plotted versus the wavelength. Each fraction had an absorption band in the region of 280 millimicrons. Bands at 260 millimicrons were exhibited by the cork phenolic acid (90° C. alkali treatment), inner bark bast fiber dioxane lignins I and IV, outer bark phloem phenolic acid, and the inner bark bast fiber phenolic acid. Similar absorptions are exhibited by native lignin preparations (14, p.317).
FIGURE 15.
INNER BARK BAST FIBER PHENOLIC ACID INFRARED ABSORPTION SPECTRUM
FIGURE 16.
OUTER BARK BAST FIBER PHENOLIC ACID
INFRARED ABSORPTION SPECTRUM

WAVE NUMBER (CM⁻¹)

PER CENT TRANSMISSION

WAVE NUMBER (CM⁻¹)
FIGURE 17
CORK PHENOLIC ACID (25 DEG. C. ALKALI TREATMENT)
INFRARED ABSORPTION SPECTRUM

PER CENT TRANSMISSION

100 80 60 40 20

3900 3600 3300 3000 2700 2400 2100 1800 1500
WAVE NUMBER (CM$^{-1}$)

WAVE NUMBER (CM$^{-1}$)

1500 1400 1300 1200 1100 1000 900 800 700

100 80 60 40 20

0 20 40 60 80 100
FIGURE 18.
CORK PHENOLIC ACID (90 DEG. C. ALKALI TREATMENT)
INFRARED ABSORPTION SPECTRUM

WAVE NUMBER (CM$^{-1}$)

PER CENT TRANSMISSION
FIGURE 19.
CORK PHLOBAPHENE
INFRARED ABSORPTION SPECTRUM

PER CENT TRANSMISSION

WAVE NUMBER (CM⁻¹)

100 80 60 40 20 0
3900 3600 3300 3000 2700 2400 2100 1800 1500

20 10 0
1500 1400 1300 1200 1100 1000 900 800 700

WAVE NUMBER (CM⁻¹)
FIGURE 20.
INNER BARK BAST FIBER DIOXANE LIGNIN I
INFRARED ABSORPTION SPECTRUM
**Figure 21**

Dioxane Lignin IV

Infrared Absorption Spectrum

![Graph depicting infrared absorption spectrum of dioxane lignin IV.](image)
FIGURE 22.
BARK LIGNINS
ULTRAVIOLET ABSORPTION
SPECTRA

1. OUTER BARK BAST FIBER PHENOLIC ACID
2. INNER BARK BAST FIBER DIOXANE LIGNIN IV
3. INNER BARK BAST FIBER PHENOLIC ACID
4. OUTER BARK PHLOEM ACID
FIGURE 23.
BARK LIGNINS AND CORK PHLOBAPHENE
ULTRAVIOLET ABSORPTION SPECTRA

1. CORK PHLOBAPHENE
2. CORK PHENOLIC ACID (90 DEG. C. TREATMENT)
3. CORK PHENOLIC ACID (25 DEG. C. TREATMENT)
4. INNER BARK BAST FIBER DIOXANE LIGNIN I
VII. ALKALINE NITROBENZENE OXIDATION OF BARK PHENOLIC ACIDS

The alkaline nitrobenzene oxidation of lignin produces aromatic aldehydes. Softwood lignins yield vanillin while vanillin and syringaldehyde are derived from the lignin of hardwoods (7, pp. 32-37). In recent work, certain lignified materials such as corn stalks, and sphagnum peat have been shown to produce significant amounts of p-hydroxybenzaldehyde (8, pp. 37-38 and 22, pp. 311-312).

The alkaline nitrobenzene oxidation of Douglas-fir bark bast fiber phenolic acid was previously found to produce 1.63 per cent vanillin (20, p. 17). No further attempt was made to identify additional aldehydes possibly present. This small yield of vanillin should be expected due to the relatively low methoxyl content of bark phenolic acid. However, the possibility that other aromatic aldehydes, containing no methoxyl groups, might also have been formed should not be overlooked. Therefore, the products from the alkaline nitrobenzene oxidation of both the cork and inner bark bast fiber phenolic acids were investigated.

A hydrogenation apparatus, American Instrument Company model 406-801 DA, equipped with an automatic shaking mechanism, was used to carry out the oxidations. The
electrically-heated stainless steel bomb had a total capacity of approximately four liters. The temperature was controlled by observation of a calibrated potentiometer connected to an iron/constantan thermocouple which had been inserted in the temperature well of the reaction vessel.

Thirty grams of air-dry phenolic acid, 20 milliliters of nitrobenzene, and 600 milliliters of two normal sodium hydroxide were sealed into the bomb which was then heated for two hours at 160° C. with continuous shaking. At the end of this time, the shaking was discontinued, the bomb cooled to room temperature, and the reaction mixture washed into a two-liter distilling flask where it was steam distilled to remove aniline and azobenzene.

Treatment of a small portion of the distillate with 2,4 dinitrophenylhydrazine reagent indicated that no volatile aldehydes were present. The steam-distilled alkaline oxidation mixture was cooled to room temperature and filtered on a Büchner funnel. The residue from the filtration was found to consist entirely of azobenzene which is insoluble in aqueous solutions and was not completely removed by the distillation. The filtrate was acidified to pH 3 with concentrated hydrochloric acid, transferred to a liquid-liquid extractor and continuously extracted with two liters of diethyl ether for 48 hours.
SEPARATION OF COMPONENTS FROM NITROBENZENE OXIDATION
OF CORK PHENOLIC ACID

Oxidation Mixture

Steam distillation

Non-volatiles

Volatile

Concentrated HCl
Ether extraction

Ether insolubles
Ether solubles

Ether extraction

20% NaHSO₃

NaHSO₃ solubles

Ether solubles

Concentrated HCl
Ether extraction

8% NaHCO₃

Ether soluble aldehydes
(9.11%)

NaHCO₃ solubles
Ether solubles

Concentrated HCl
Ether extraction

5% NaOH

Ether soluble acids
(10.0%)

NaOH solubles
Neutrals

Concentrated HCl
Ether extraction

Ether soluble phenols
(1.50%)
This ether extract was then treated as outlined in Figure 24 to remove aldehydes, acids and phenols.

**Cork Phenolic Acid.**

**Aldehydes.**

The ether extract of the cork phenolic acid nitrobenzene oxidation mixture was shaken with eight 100 milliliter portions of 20 per cent sodium bisulfite to remove aldehydes. The bisulfite extract was acidified with concentrated hydrochloric acid and the resultant sulfur dioxide removed by passing a stream of air through the cold solution under reduced pressure. It was then shaken with eight 150 milliliter portions of diethyl ether to remove ether soluble aldehydes. This ether extract was dried over anhydrous sodium sulfate overnight, filtered, and evaporated to dryness at room temperature. The yield of aldehydes was 9.11 per cent, based on the oven-dry weight of the cork phenolic acid. This value was considerably less than those obtained from other lignin-containing materials where 27-52 per cent of the lignin was isolated as aldehydes (9, p.3049).

Recent developments in the paper chromatographic analysis of the aldehydes produced by the nitrobenzene oxidation of lignin provided a highly-sensitive means
for further investigation of this fraction. Stone and Blundell have shown that a satisfactory separation of vanillin, p-hydroxybenzaldehyde, and syringaldehyde can be achieved utilizing a solvent system which contained six parts of ligroine (boiling range 100° to 120° C.) and one part of n-butyl ether saturated with water (27, p.772). Furthermore, they have utilized paper chromatography in conjunction with spectrophotometric analysis for the quantitative determination of these aldehydes. This procedure was used in further examination of the aldehydes derived from the alkaline nitrobenzene oxidation of cork phenolic acid.

A Fisher strip-paper chromatography apparatus was used in the preparation of the chromatograms. A strip of No. 1 Whatman filter paper 22 inches long was used as the supporting medium. An alcoholic solution of the aldehyde fraction which resulted from the oxidation was applied to a spot four inches from one end of the strip by means of a capillary pipet. Alcoholic solutions of vanillin, p-hydroxybenzaldehyde, and syringaldehyde were spotted alongside the unknown mixture to serve as reference materials. The chromatogram was then developed for 13 hours using ligroine:n-butyl ether:water (6:1:1) as the solvent system. After development, the strip was air-dried for one hour and sprayed with a 2,4 dinitro-
phenylhydrazine reagent prepared by dissolving one gram of 2,4 dinitrophenylhydrazine in 300 milliliters of concentrated hydrochloric acid and diluting to one liter with distilled water.

**Vanillin.** The only aldehyde from the oxidation mixture which could be identified by comparison with the three reference compounds was vanillin. No evidence for the presence of syringaldehyde or p-hydroxybenzaldehyde was found. A bright orange-red spot at the base line of the chromatogram indicated that a considerable part of the aldehyde fraction was not affected by the solvent system.

The yield of vanillin was determined by the method of Stone and Blundell (27, pp.772-773). The supporting medium was a strip of Whatman No. 1 filter paper six inches wide and 22 inches long. The strip was ruled lengthwise so as to give a five inch lane and a one inch lane. The aldehyde fraction was dissolved in 40 milliliters of absolute ethanol. A 0.06 milliliter aliquot portion of this solution was spotted along the base line of the five inch lane. An authentic sample of vanillin was spotted on the base line of the one inch lane to serve as a reference material. The chromatogram was then developed for 13 hours with ligroine:n-butyl ether:water (6:1:1). The one inch lane which contained the reference
material was cut from the five inch lane and sprayed to reveal the location of the vanillin. Next, a three-inch-wide strip was cut from the five inch lane to include all of the adsorbed vanillin. This strip was placed in a small Soxhlet extractor and extracted with 50 milliliters of absolute ethanol to remove the vanillin. Eight milliliters of 0.2 per cent alcoholic potassium hydroxide were added to the extract which was then diluted to 100 milliliters with absolute ethanol. The per cent transmission of this solution was measured at 353 millimicrons by means of a Beckman model DU spectrophotometer and the amount of vanillin present was obtained directly from a standard curve. The yield of vanillin was only 0.60 per cent of the oven-dry weight of the cork phenolic acid.

*Protocatechualdehyde*. Although no syringaldehyde or p-hydroxybenzaldehyde could be detected on the chromatogram of the aldehydes obtained from the nitrobenzene oxidation of the cork phenolic acid, an intense orange-red spot was observed at a point one centimeter from the base line. On spraying a similar chromatogram with a one per cent ferric chloride solution, a bright-green spot developed at this same position. The formation of a green color when treated with ferric chloride is characteristic of materials containing a catechol nucleus.
These tests were indicative of an ortho-dihydroxy aromatic aldehyde. A comparison of this spot with an authentic sample of protocatechualdehyde when developed with ligroine:n-butyl ether:water (6:1:1) for 13 hours and sprayed with 2,4 dinitrophenylhydrazine is shown in Figure 25. Results indicated that the material was protocatechualdehyde.

In an attempt to isolate protocatechualdehyde from the resinous ether-soluble aldehyde fraction, two grams of the mixture were extracted with five 20 milliliter portions of boiling water in which vanillin and protocatechualdehyde are soluble. The aqueous extract was filtered to remove suspended particles, reheated to boiling and decolorized with charcoal. After filtering, the clear extract was cooled and shaken with three 100 milliliter portions of diethyl ether in a separatory funnel. The ether extract was dried over anhydrous sodium sulfate overnight, filtered and evaporated to dryness at room temperature. Approximately 150 milligrams of a brown solid residue were obtained.

This residue was stirred into two milliliters of cold water, in which vanillin is relatively insoluble, and filtered. The aqueous solution was allowed to evaporate to approximately one milliliter in a refrigerator at 4°C. The colorless crystals which formed were
FIGURE 25

PAPER CHROMATOGRAPHIC RESOLUTION OF ALDEHYDES FROM THE
NITROBENZENE OXIDATION OF CORK PHENOLIC ACID

Time: 13 hours.
Indicator: 2,4 dinitrophenylhydrazine.
removed by filtration and air dried. They melted at 145-148° C. The melting point of protocatechualdehyde is 152-153° C. This indicated the presence of vanillin impurities. However, further purification was impossible due to the small amount of material isolated. A chromatogram was prepared by spotting No. 1 Whatman paper with an alcoholic solution of the remaining crystals. It was developed as previously described and sprayed with an aqueous one per cent ferric chloride solution. A bright-green spot corresponded to that of an authentic sample of protocatechualdehyde while a trace of vanillin was also indicated.

In alkaline ethanol solutions, the long wave length bands of p-hydroxy aldehydes and p-hydroxy ketones are displaced into the high ultraviolet (328-370 millimicrons) and their absorption intensities are considerably increased (21, p.846). This phenomenon was used by Stone and Blundell, in conjunction with paper chromatography, for the quantitative determination of vanillin, syringaldehyde and p-hydroxybenzaldehyde as previously described. Because of its chemical structure, a similar method was devised for the quantitative determination of protocatechualdehyde.

To obtain the yield of protocatechualdehyde, a chromatogram of the aldehyde fraction from the nitro-
benzene oxidation of the cork phenolic acid was spotted and developed in the same manner as that described in the determination of vanillin. The portion of the paper strip which contained protocatechualdehyde was removed and extracted with 50 milliliters of absolute ethanol in a Soxhlet extractor to dissolve the adsorbed aldehyde. Eight milliliters of 0.2 per cent alcoholic potassium hydroxide were added to this extract which was then diluted to 100 milliliters with absolute ethanol. The ultraviolet spectrum of protocatechualdehyde showed maximum absorption to occur at 352 millimicrons as shown in Figure 26. The per cent transmission of the alkaline solution was measured at this wavelength and the amount of protocatechualdehyde obtained directly from the standard curve shown in Figure 27; duplicate determinations showed the yield to be 0.66 per cent of the oven-dry weight of the cork phenolic acid.

A search of the literature revealed that the formation of protocatechualdehyde by the alkaline nitro-benzene oxidation of lignin had never been reported. A recent investigation of the reaction products from a similar oxidation of vanillin resulted in the isolation of protocatechualdehyde (17, p.575). Its presence was obviously due to demethylation of the vanillin. The protocatechualdehyde was separated by paper chromatography
FIGURE 26
ULTRAVIOLET ABSORPTION SPECTRUM
OF PROTOCATECHUALDEHYDE
FIGURE 27.
RELATIONSHIP BETWEEN OPTICAL DENSITY AND CONCENTRATION OF PROTocatechualdehyde IN ALKALINE SOLUTION AT 352 MILLIMICRONS

OPTICAL DENSITY

CONCENTRATION, MG IN 100 ML.
but its absolute yield was not ascertained. However, it was less than two per cent based on the original weight of vanillin (24). In the study of aldehydes from the cork phenolic acid, the yield of protocatechualdehyde was actually greater than that of vanillin. It was evident from this data that the major part of the protocatechualdehyde was formed directly from the phenolic acid since treatment of vanillin under similar conditions resulted in less than two per cent conversion to the demethylated product.

The instability of protocatechualdehyde is well-known. The ortho-dihydroxy grouping lends itself to oxidation (23, p.506). The colorless needle-like crystals became reddish-brown after short exposure to air. On melting, the crystals formed a dark-red liquid which indicated partial conversion of the catechol nucleus to the ortho-quinone configuration (10, p.724).

An authentic sample of protocatechualdehyde was subjected to an alkaline nitrobenzene oxidation at 160° C. as previously described. Only 31 per cent of the original material could be recovered as protocatechualdehyde. Therefore, the conditions to which the phenolic acid was subjected during the nitrobenzene oxidation decomposed a significant part of the protocatechualdehyde formed. This indicated that the chemical nature of the phenolic
acid was such that it contained a greater amount of the
nucleus necessary for the production of protocatechualdehyde than was implied by the 0.66 per cent yield of
this aldehyde.

Less than 14 per cent of the aldehyde fraction
obtained from the oxidation of the cork phenolic acid
was identified; vanillin comprised 6.58 per cent of
this fraction while protocatechualdehyde accounted for
7.24 per cent. The remainder of the material could not
be resolved by the chromatographic technique discussed
previously but remained on the base line where it formed
a bright orange-red spot after spraying with 2,4 dinitro-
phenylhydrazine reagent. This indicated that the spot
contained complex aldehyde materials which may have
resulted from polymerization or decomposition of resultant
products or incomplete oxidation.

The formation of aromatic aldehydes by alkaline
nitrobenzene oxidation was found to be characteristic
of both wood lignins and cork phenolic acid. However,
the relatively low aldehyde yield and the formation of
protocatechualdehyde from the latter indicated a sig-
nificant difference between the two materials.

This study showed that further examination of the
products from the oxidation of cork phenolic acid is
necessary. Masking of the phenolic groups through
methylation would prevent oxidation of the material at the ortho-dihydroxy position and could result in the formation of the more stable veratric aldehyde. Variations in the oxidizing agent, temperature, concentrations, and duration of the cooking time should serve to give a more complete insight into the mechanism of the formation of protocatechualdehyde from this lignin-like material.

Acids.

Following removal of the aldehydes from the ether extract of the oxidation mixture, the ether solution was shaken with six 100 milliliter portions of eight per cent sodium bicarbonate to obtain the acids. The dark-red bicarbonate extract was acidified with concentrated hydrochloric acid and shaken with six 150 milliliter portions of diethyl ether. This ether extract was dried over anhydrous sodium sulfate overnight, filtered and evaporated to dryness on a steam bath. The yield of ether-soluble acids was 10.0 per cent of the oven-dry weight of the cork phenolic acid. A similar study of numerous lignin preparations gave yields of less than two per cent acids (9, p. 3049).

The dried ether-soluble acids were a dark-brown amorphous solid. After dissolving the material in hot hexane and allowing the solution to stand in a refrigerator
at 4° C. for 24 hours, tan crystals separated from the solution and were removed by filtration. Recrystallization from hexane and acetone resulted in a white microcrystalline product in a yield of approximately one percent of the oven-dry weight of the cork phenolic acid. The crystals melted at 72-73° C. and had a neutral equivalent of 233.1. These properties indicated that the crystalline acid fraction was a mixture of the hydroxy acids that have previously been described by Hergert and Kurth who isolated a phenolic acid-hydroxy acid complex from Douglas-fir cork (12, pp.65-66).

The isolation of cork phenolic acid by treatment of the cork with dilute caustic at room temperature evidently resulted in only a partial saponification of the hydroxy acid-phenolic acid complex. However, this treatment was sufficient to render the complex soluble. Subjecting this material to the conditions under which the alkaline nitrobenzene oxidation was carried out completed the saponification. Therefore, the acid fraction obtained from the oxidation mixture of the cork phenolic acid was a combination of hydroxy acid and phenolic acid degradation products which resulted in a comparatively high yield of acids.

Further attempts to obtain additional acid precipitates from a variety of solvents were unsuccessful.
Phenols.

The ether solution which remained after extractions with sodium bisulfite and sodium bicarbonate was next shaken with six 100 milliliter portions of five per cent sodium hydroxide to remove phenols. The alkaline extract was acidified with concentrated hydrochloric acid and shaken with six 150 milliliter portions of ether. This ether extract was dried over anhydrous sodium sulfate overnight, filtered and evaporated to dryness on a steam bath. The resultant solid amorphous residue of phenolic materials comprised 1.50 per cent of the oven-dry weight of the cork phenolic acid. The acidified aqueous solution from which the ether soluble phenols were isolated was tested for the presence of additional phenols by means of ferric chloride. Negative results were obtained which demonstrated that all the phenolic material was removed by the ether extraction.

Considerable work has been carried out by other investigators concerning the separation and identification of phenols by paper chromatography. The phenols from the cork phenolic acid oxidation were subjected to paper chromatographic analysis in accordance with the procedure of Barton, Evans and Gardner which utilizes carbonic acid, pH 4.2, as the developing solvent (4, pp.249-250). The
indicator used was prepared by mixing equal amounts of one per cent aqueous solutions of ferric chloride and potassium ferricyanide as described in the section on chromatography of bark lignins.

An ether solution of the phenol fraction was used to spot No. 1 Whatman paper strips which were then developed for three hours. After spraying, four distinct spots were found on the paper and resolution was excellent. However, further work with this procedure showed that definite Rf values could not be assigned to individual phenols because the distance which they moved down the paper was affected by several variables. Essentially, it was found that a phenol when developed in admixture with other phenols would not give the same Rf value as obtained when the pure phenol was developed under identical conditions. In addition, the width of the paper on which the chromatogram was developed affected the Rf values. This made it impossible to identify any of the phenols from the oxidation either by Rf values or by comparison of the spots obtained to those of known phenols.

The phenols from the oxidation were developed next with n-amyl alcohol and water, mutually saturated, according to the method outlined by Riley (25, pp. 5782-5783). In this procedure, separation of mono-, di-, and tri-hydroxy phenols may be achieved although resolution
of individual members within a group is not very satisfactory. Results indicated that dihydroxy phenols comprised the major portion of the phenols from the nitrobenzene oxidation of the cork phenolic acid. No mono- or tri-hydroxy phenols were present.

Riley used an additional solvent system composed of n-butanol:benzene:water (1:19:20) to resolve individual members of the simple dihydroxy phenols. Application of this procedure to the phenols from the cork phenolic acid failed to demonstrate the presence of either catechol, resorcinol or hydroquinone.

Therefore, it was concluded that no simple phenols were produced by the alkaline nitrobenzene oxidation of the cork phenolic acid. The phenols formed were complex in nature. In addition, the use of paper chromatography demonstrated that at least four different chemical entities comprised this fraction.

**Inner Bark Bast Fiber Phenolic Acid.**

**Aldehydes.**

The aldehydes produced by the alkaline nitrobenzene oxidation of the inner bark bast fiber phenolic acid were isolated in the same manner as that described in the earlier section on the aldehydes from the cork phenolic
acid. The 8.35 per cent yield obtained compared closely with that from the cork phenolic acid. The qualitative and quantitative determinations of the aldehydes present in this fraction were carried out using the paper chromatographic techniques previously described.

**Vanillin.** The methoxyl content of the inner bark bast fiber phenolic acid, 8.19 per cent, suggested that this fraction should give a higher yield of vanillin than was obtained from the cork phenolic acid whose methoxyl content was only 4.0 per cent. The yield of vanillin was 2.6 per cent of the oven-dry weight of the inner bark bast fiber phenolic acid which served to substantiate this conclusion. However, the yield is still relatively low when compared to those obtained from other lignin materials.

A small amount of vanillin was isolated from the aldehyde fraction by use of the sublimation technique of Hawkins, Wright and Hibbert (11, p.2447). The colorless crystals obtained melted at 81°C and formed a 2,4 dinitrophenylhydrazone derivative which melted at 268-269°C with decomposition.

**Protocatechualdehyde.** The presence of protocatechualdehyde in the aldehyde fraction produced by the alkaline nitrobenzene oxidation of inner bark bast fiber
phenolic acid was indicated by paper chromatography. The yield was 0.79 per cent of the oven-dry weight of the phenolic acid and was quite similar to that which resulted from the cork phenolic acid.

Syringaldehyde and p-hydroxybenzaldehyde. Faint spots which corresponded to syringaldehyde and p-hydroxybenzaldehyde were found upon examination of the chromatogram of the aldehyde fraction from the inner bark bast fiber phenolic acid. It was clearly evident that only traces of these two materials were present in the mixture. Therefore, quantitative determinations were not carried out.

Acids.

After treatment with sodium bisulfite to remove the aldehydes, the ether extract of the oxidation mixture was shaken with six 100 milliliter portions of eight per cent sodium bicarbonate to remove acids. The isolation of this fraction was then carried out as described in the earlier section concerning the acids from the cork phenolic acid. The yield of acids was 2.72 per cent of the oven-dry weight of the inner bark bast fiber phenolic acid.
This value is considerably less than that obtained for the cork phenolic acid, 10.0 per cent, and corresponds more closely with yields of acids from other lignin-containing materials (9, p3049). No crystalline products could be isolated from a variety of solvents.

**Phenols.**

The phenols which resulted from the alkaline nitrobenzene oxidation of the inner bark bast fiber phenolic acid were isolated as described earlier in the section on the phenols from the cork phenolic acid. The yield of phenols was 0.79 per cent based on the oven-dry weight of the phenolic acid. Because of this relatively low value, the fraction was not investigated further.
DISCUSSION

Because of the complex nature of its components, Douglas-fir bark was separated into six fractions before examination of the bark lignin materials was begun. This was necessary before any definite conclusions could be drawn concerning the chemical nature of bark lignin since earlier investigators have shown that Douglas-fir cork and bast fibers are dissimilar (12, pp. 59-66 and 20, pp. 14-19).

Extraction of the extractive-free bark components with hot alkaline solutions removed almost four times the amount of phenolic acid as was obtained when similar extractions were carried out at room temperature. This suggested that the removal of phenolic acids from the bark was a saponification process. Indeed, the phenolic acid of Douglas-fir cork has been previously shown to exist naturally in the form of a phenolic acid-hydroxy acid ester (12, pp. 65-66). Whether or not the phenolic acid is actually bound to the other bark components by a similar mechanism remains to be determined.

The outer bark bast fibers contained a slightly greater amount of phenolic acid than the fibers from the inner bark. This was most likely due to the presence of a small quantity of phenolic acid-rich phloem impurities
in the outer bark bast fiber fraction. All the phenolic acid fractions isolated from the outer bark components had methoxyl contents between 4.95 and 4.21 per cent while the inner bark phenolic acids contained 6.78-8.19 per cent methoxyl. The inner bark bast fiber phenolic acid had a methoxyl content of 8.19 per cent whereas that of the outer bark bast fiber phenolic acid was 4.95 per cent. This indicated that demethylation of the bast fiber phenolic acid occurred as the fibers aged during the process of tree growth.

Freshly isolated bark phenolic acids showed strong tendencies to dissolve in water. Chemical tests indicated that the phenolic acid possessed phlobatannin properties. An aqueous solution of the phenolic acid was used to tan a sheepskin skivver. Although the leather produced was inferior in quality, the use of bark phenolic acid for such things as an additive for oil-drilling muds would bear further investigation. Thousands of tons of this material are available from the 1.5 million tons of Douglas-fir bark produced each year.

Considerable difficulties were experienced during the isolation of dioxane lignin from inner bark bast fibers. It was necessary to extract the fibers with dioxane-hydrochloric acid for 96 hours before it could be concluded that the major part of the dioxane lignin
had been removed. On the other hand, the dioxane lignin could be isolated from outer bark bast fibers by extraction with dioxane-hydrochloric acid for a period of eight hours. This suggested that as the fibers move outward during bark growth, there is a weakening of the forces which bind the lignin to the fibers. The methoxyl contents of the bast fiber dioxane lignins were similar to those of wood dioxane lignins. However, bast fiber dioxane lignins failed to give the color test with phloroglucinol and hydrochloric acid that is characteristic of wood lignins. Further differences between the dioxane lignins from these two sources were demonstrated by paper chromatography and infrared and ultraviolet spectral analysis.

The solvent systems previously shown to give resolution of various wood lignin preparations did not give satisfactory results with bark lignins. The highest degree of resolution of bark lignins was obtained with developers which contained a high percentage of water. It was even possible to obtain limited resolution by using pure distilled water as the developing agent. Non-aqueous systems caused considerable streaking of the chromatograms. It was clearly demonstrated that paper chromatography can be used as a readily-available tool for comparison of bark lignin preparations and results are highly reproducible.
This investigation also indicated that further purification of bark lignins could be carried out by column chromatography. It seems likely that powdered cellulose would provide a satisfactory absorbant for such a study and numerous aqueous developing solvents were found which gave satisfactory resolution of the lignins on paper strips. The fluorescence of the bark lignins under ultraviolet light could be used to the progress of the development.

The infrared spectra of bark lignins showed carbonyl absorption to occur at 1688-1704 centimeters⁻¹. Designation of absorption in this region to a specific carbonyl structural configuration is impossible at this time. Carboxylic acid and ester absorption occur in the region of 1725 centimeters⁻¹. Aldehyde and ketone carbonyl absorption are found at 1663-1665 centimeters⁻¹ for numerous lignin preparations. An earlier methylation study of Douglas-fir bast fiber phenolic acid demonstrated the presence of a carboxylic acid group in this material (20, pp. 17-18). Hence, it appears likely that absorption in the region of 1688-1704 centimeters⁻¹ is associated with the carboxylic acid group. However, this group is modified in such a manner that it produces the absorption band observed. Absorption in the region of 1700 centimeters⁻¹ is characteristic of other lignin preparations.
in addition to the samples used in this work. Among these are white fir bark phenolic acid and low-molecular weight black spruce native lignin (13, p.142 and 14, pp.318-319). Further examination of model compounds will be necessary before a definite assignment of this absorbance band can be made.

Wood lignin contains guaiacyl and syringyl nuclei as part of its structural makeup. The production of protocatechualdehyde by alkaline nitrobenzene oxidation of bark phenolic acids indicated that a catechol nucleus may be an important building stone in the structure of these materials. Other low-methoxyl phenolic materials, such as phlobatannin and phlobaphene, could also result in the formation of protocatechualdehyde. Therefore it is evident that further investigations concerned with the production of protocatechualdehyde by nitrobenzene oxidation of low-methoxyl lignins and related materials are necessary.
SUMMARY

In this investigation, Douglas-fir bark was divided into six separate fractions; inner bark bast fibers, outer bark bast fibers, inner bark fines, outer bark fines, outer bark phloem parenchyma and sieve tubes, and cork. The solubilities of the inner bark bast fibers in various alkaline solutions and the resultant yields of phenolic acid were ascertained. The yields of phenolic acid from the six bark components were determined by extraction with one per cent sodium hydroxide at 90°C. The inner and outer bark fines and the outer bark phloem and cork were found to be relatively rich in this low-methoxyl lignin-like material. It was demonstrated that bark phenolic acids possess tannin-like properties. Inner and outer bark bast fiber dioxane lignins were prepared and their nature determined. A comparison of the bark lignins and related materials was made by paper chromatography. An extensive search was made for solvent systems which would promote sufficient resolution for this comparison. It was clearly demonstrated that the phenolic acid preparations were not homogeneous but contained one principal common component in admixture with small amounts of other dissimilar materials.
Further comparisons of bark phenolic acid, dioxane lignin, and cork phlobaphene were made by preparation and examination of their infrared and ultraviolet spectra. The carbonyl absorption exhibited in the infrared region by the bark phenolic acid differed from that of conventional wood lignin preparations. The cork and inner bark bast fiber phenolic acids were subjected to alkaline nitrobenzene oxidation. Vanillin and protocatechualdehyde were obtained from the cork phenolic acid oxidation products. Oxidation of the inner bark bast fiber phenolic acid produced vanillin, protocatechualdehyde, and traces of syringaldehyde and p-hydroxybenzaldehyde.
BIBLIOGRAPHY


13. _____. The chemical nature of the extractives from white fir bark. TAPPI 35:137-144. 1953.


