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A Study of the Carbohydrate Fraction of Spruce Chlorite Liquors

by Walter J. Bublitz, Jr.

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A STUDY OF THE CARBOHYDRATE FRACTION
OF SPRUCE CHLORINE LIQUORS

A thesis submitted by

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INTRODUCTION AND HISTORICAL

For many years attempts have been made to separate quantitatively the entire carbohydrate fraction of wood from the lignin fraction. Such a method, if possible, would be a great contribution to the field of wood chemistry, for it would undoubtedly help to establish the relationship between lignin and polysaccharides in wood.

The early attempts have followed in the footsteps of the classical Gross and Dovn method. Schmidt, et al. (1-10) devised a method for the quantitative removal of plant incrustants by treating the material alternately with chlorine dioxide and sodium sulfite solution. Later work (11, 12) proved that Schmidt's method removed carbohydrate material and, thus, could not be considered a quantitative method for the separation of lignin and carbohydrates.

In 1933, Bitter and Kurth (13) suggested a new method for the separation of the lignin from the entire polysaccharide fraction of wood (which they termed "holocellulose"); this method consisted of treating the wood alternately with chlorine and alcohol-pyridine to remove the lignin. VanDeEem and Bitter (14, 15) later modified this procedure by using an ethanolic solution of monothanolamine as the extracting agent. This method is somewhat lengthy and arduous, however.

In recent years, sodium chlorite has become available commercially, both in a technical grade and in a reagent grade for laboratory use. Its availability has encouraged its use as a delignification agent and much research has been directed toward this end. Jayne, et al. (16-19) have conducted considerable research work on the use of sodium
chlorite to delignify wood and concluded (16) that it was possible to effect a sharp quantitative separation of lignin and phenolic compounds by his method.

Vise, et al. (20, 21) have developed a single-stage isolation of holocellulose. The advantages of this method lay in its relative simplicity and in the shorter time required, as compared with Jayne's method (12).

Considerable work has been done on the holocellulose fractions isolated by these different methods, yet little attention has been directed toward the soluble products from these procedures. The first to investigate the chlorite liquors were Sahn and Reiff (22), who acidified the liquors and obtained a white precipitate which they assumed to be hemicelluloses. Jayne, et al. (16-19) have extended Sahn and Reiff's work.

**Work of Jayne and Co-workers**

Jayne and his associates (12) treated sprucewood with aqueous solutions of sodium chlorite and acetic acid stepwise, acidified the resultant liquor, and obtained a precipitate which was subsequently dialyzed. In other cases, the liquor was dialyzed or electrodialyzed without previous acidification. In all cases, the purified liquor was evaporated to dryness and the weights of the residue determined. The yield thereof varied from \(2\%\) to \(13\%\) of the weight of the wood, or up to \(50\%\) of the weight of the material (presumably "lignin") removed from the wood during the chlorite digestion. The remainder of the material removed from the wood could not be recovered.
These residues were subsequently analyzed in toto; the investigators evidently did not realize that they could be physically fractionated by means of alcohol, dioxane, or some other inert organic solvent. The analyses showed 8 to 10% methoxyl, 46 to 53% carbon, 4 to 6% hydrogen, and 1.5 to 4.5% chlorine. The methoxyl and carbon contents fall between the respective average values for these constituents in carbohydrates and lignin. Hydrolysis of these materials showed the presence of reducing sugars; these were tentatively and rather nebulously identified as glucose. In addition, the presence of an aldonic acid was established. The percentage of glucose was calculated as 20 to 28% of the weight of the original liquor residues, and aldonic acids as 5 to 10%; the total identifiable carbohydrates thus constituted about 30 to 40% of the weight of the residues.

Joyce and Henke (19) made a balance of the carbohydrate materials isolated from the chlorite procedure and found that there was an excess of such material; this they termed "überrechene Polysaccharid." Thus the yield of "lignin-free" holocellulose (yield of holocellulose minus the analytically determined false lignin therein) plus the yield of carbohydrate materials isolated from the chlorite liquor was more than the theoretical yield of holocellulose (weight of original wood minus the analytically determined false lignin therein).

On the basis of this isolation of carbohydrate materials and a theoretical carbon balance of wood and lignin, Joyce and Henke (19) have constructed a theory of a lignin-carbohydrate complex which exists in wood. Their conclusions may be traced back to three major assumptions:
1. That the true holocellulose fraction of extractive-free wood may be calculated by subtracting the analytically determined false (or Klassen) lignin value from 100; 

2. That the lignin-free yield of a holocellulose fraction may be calculated by subtracting the amount of analytically determined false (or Klassen) lignin in said fraction from the total amount of holocellulose; and 

3. That the carbon content of isolated spruce lignin is 65 to 65%, as indicated by Freundenberg (23).

In a later paper, Jayme and Finck (24) have indicated that the second assumption is probably not true, and that a false lignin determination on holocellulose may give low results. They likewise abandoned the earlier concept of "dberschusses Polysaccharid" (19); hydrolytic work had failed to prove the existence of the excess polysaccharide material which had been previously hypothesized. Rather, it was indicated that the existence of this material could be traced to the second assumption listed above which, the authors have admitted, is incorrect.

Mueller (25) likewise indicated that excess polysaccharide material was present in beechwood, when calculated by the conventional method. He showed that there was lignin present in the holocellulose of beechwood and that some polysaccharides were present in cuprammonium hydroxide lignin.

INVESTIGATION OF BARTON

Barton (26) made a rather thorough investigation of the liquor from the chlorite oxidation of slash pine. By acidification of the liquor,
he was able to obtain a precipitate which could subsequently be separated into a lignin and a carbohydrate fraction. The former he termed "Chlorite Lignin A." By concentration of the mother liquor and extraction with ether, he obtained another fraction which he termed "Chlorite Lignin B." In the course of the purification of the latter, a dioxane-insoluble fraction was obtained which proved to be partially carbohydrate in nature. Other fractions were separated and compounds were isolated which are not pertinent to this discussion.

Of prime interest is the fact that Barton was able to resolve the components of the chlorite liquor into lignin and predominantly non-lignin fractions by physical means. It was conclusively proved that the purified chlorite lignins contained no polycoccharidic material and that the dioxane-insoluble fractions were at least partly carbohydrate in nature, containing mannose and galactose. Barton characterized and indicated a possible formula for the chlorite lignin.

Whereas Jayne and Henke concluded that the lignin and carbohydrate materials were chemically bound to each other because they were present in a solution which presumably contained only lignin degradation products, Barton showed that these components were simply a physical mixture. Whether they were chemically combined in situ is a point which was and is not settled.

The action of sodium chlorite and chlorine dioxide is incompletely understood. Taylor, et al. (27) have stated that, at a pH of 2.5 or greater, a sodium chlorite solution liberates primarily chlorine dioxide. James and Isbell (28) made an intensive investigation of the
effect of sodium chlorite solutions on simple sugars. With neutral or weakly alkaline solutions, there was little effect on the sugars at room temperature. With acid solutions (as low as pH 2) the reaction was much more rapid. They concluded that the primary action of sodium chlorite solutions on sugars is the oxidation of the free aldehyde group to carboxyl, according to the following equations:

\[ \text{RCCHO} + \text{HClO}_2 \rightarrow \text{RCOCH} + \text{HCl} \]

or

\[ \text{RCCHO} + 3\text{HClO}_2 \rightarrow \text{RCO} + \text{Cl}_2 + \text{HCl} + \text{H}_2\text{O}. \]

Boist (29) studied the oxidation potentials of aqueous solutions of chlorine dioxide and sodium chlorite, and found that chlorine dioxide was a stronger and more effective oxidizing agent than sodium chlorite.
This thesis was originally intended to be an extension of Barton’s work (26) on chlorite lignins. He isolated and partially characterized these products and established a tentative unit weight for them. It was planned to isolate enough chlorite lignin to allow extensive research work thereon. Such properties as the molecular weight and the ultimate structural formula were the goal.

Black spruce wood (Picea marina D.C.F.) was used for this investigation instead of slash pine; a different wood was used to determine whether similar results could be obtained. In addition, a sufficient supply of spruce was available and, inasmuch as considerable research work has been conducted on this wood, it was felt that a correlation of the results with other published data would be simpler.

For reasons to be described later, Barton’s procedure for the isolation of the chlorite lignin was modified and, in the course of the new procedures, a large fraction, presumably carbohydrate in nature, was isolated. Such a fraction has not been reported previously and, in view of the fact that chlorite liquor should contain at most only small amounts of carbohydrate materials (according to the previously accepted theory), it was decided to devote the main effort toward the analysis of this fraction. It was hoped that its origin could be determined as well.
EXPERIMENTAL SECTION

CHEMICAL

ANALYTICAL PROCEDURES

All Klason lignin determinations in this work were carried out according to Institute Method 13. The methoxyl determinations were performed on a semimicro scale according to Institute Method 18. Incre-
ash determinations followed Institute Method 4, whereas micro-ash and sulfated ash determinations are described in Institute Micro Method 712.

The Carius tube method for microchlorine analysis in organic compounds is described in Institute Micro Method 709a. Carbon and hydrogen analyses were carried out according to Institute Micro Method 706, and Institute Method 3 was used for the determination of moisture in wood and holocellulose. Moisture samples of isolated lignin and carbohydrate materials were dried in vacuo at 60° C., either in a vacuum oven or in an Aderhelden pistol.

Pentosans were gravimetrically determined by Institute Method 23 and Krober's tables (30). Uronic acids were determined by Institute Method 25, also described by Browning (31).

SOLUTION PREPARATION

Absolute ethanol was used for washing the carbohydrate frac-
tions after their precipitation with U.C.P. 95% ethanol. Absolute ether (c.p.) was used for the precipitations and washings of various products. The used ether was recovered by distillation over sodium metal. Reagent-
grade petroleum ether was used for the final washings of the lignin
fractions. Two fractions were employed: low-boiling petroleum ether, boiling range 30 to 60°C, and high-boiling petroleum ether, boiling range 65 to 110°C. These solvents were recovered by distillation over sodium metal.

Anhydrous 1,4-dioxane was prepared by refluxing technical grade dioxane over sodium metal until the surfaces of the sodium spheres were bright and shiny, indicating that all reaction with glycols, alcohols, and water was complete. The dioxane was then distilled and the fraction boiling from 100 to 101.5°C was retained for use. Used dioxane was purified in the same manner.

WOOD PREPARATION

The sample of black spruce employed was a batch of 13 black spruce logs cut from a swamp near Debo, Wisconsin, in May, 1947, and was kept for about five months before use. The maximum number of growth rings visible was 63, with an average of about 40 rings. The largest stick was 13 inches in diameter, and no heartwood was evident in any stick. The sticks had been previously peeled for an investigation of the bark at the Institute of Paper Chemistry in the summer of 1947.

These sticks were reduced to two-inch chips in a commercial chipper, and were hand sorted to remove knots, splinters, etc. The chips were further processed in a hammer mill and finally were reduced in a Wiley mill, using an eight-mesh screen. The meal was then screened with a 48-mesh screen, only that part which was retained being saved. Effectively, then, the particle size of the wood meal used was 8 to 48 mesh.
The wood meal was extracted batchwise with a 2:1 mixture of absolute ethanol-benzene in a large percolating extractor described by Horton. About 30 pounds of wood meal could be extracted at a time; the operation required 16 days, after which the extraction seemed complete.

The unextracted wood was analyzed for alcohol-benzene solubility according to Institute Method II; it contained 4.45 soluble material. Analysis of the air-dried extracted wood gave the data recorded in Table I.

### Table I

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxyl, $%$</td>
<td>5.07</td>
</tr>
<tr>
<td>Ash, $%$</td>
<td>0.10</td>
</tr>
<tr>
<td>Klossor lignin, $%$</td>
<td>27.9</td>
</tr>
<tr>
<td>Uronic anhydride, $%$</td>
<td>4.00</td>
</tr>
<tr>
<td>Corrected pentoses, $%$</td>
<td>10.7</td>
</tr>
</tbody>
</table>

*All results are on an oven-dry basis.

**Orientation Work**

All digestions or extractions with aqueous solutions of sodium chlorite and acetic acid will be referred to as "cooks."

**Cook 1**

The first three cooks were orientative in nature. In Cook 1, 30 grams (oven-dry basis) of wood were digested according to the conditions listed in Table II which is the method Horton devised for his cooks. This method was subsequently used in this work, unless otherwise noted. Acidification of the filtrate produced a milky white precipitate, and no further work was done.
Cooking Conditions

| Consistency of solution, ° | 6 |
| Temperature, °C | 75-80 |
| Length of cook, hrs. | 4.5 |
| Additions of NaSO₂ | 4 |
| Amount NaSO₂ per addition (based on wood), ° | 27.5 |
| Total NaSO₂ (based on wood), ° | 110 |
| Additions of acetic acid | 4 |
| Volume of acid per addition, ml. | 4.5 |

*Applies to this cook only; varying amounts of acetic acid added to other cooks.

Cook 2

Cook 2 was a series of four small-scale cooks, each identical with the conditions of Cook 1, except that varying amounts of acetic acid were added to each to produce different acivities in the liquors. The pH of the liquors varied from 4.3 to 5.4 at the end of the cooks; it was observed that the liquor with pH 4.6 gave the clearest filtrate and also the clearest precipitate upon acidification of the liquor with sulfuric acid. Consequently, with later cooks, the pH was maintained as near 4.5 to 4.6 as possible by periodic sampling of the liquor, determination of the pH by meter reading, and addition of the proper quantity of acetic acid.

Cook 3

Cook 3 was performed on a scale six times as large as Cooks 1 and 2. The pH of the solution was adjusted by judicious additions of acetic acid, as described above. The resultant liquor were divided into aliquot parts to which varying amounts of sulfuric acid were added. The pH for optimum precipitation of the lignin was 1.8 to 2.0 and, in future
cooks, the chlorite liquor was acidified to this pH to produce the best precipitation.

Cook 4

Cook 4 was the first full-scale cook to be carried out. The cooking conditions were essentially the same as those described for Cook 1, but the technique was slightly different.

Twenty-eight liters of distilled water were heated in a 305 x 610-mm. Pyrex battery jar (capacity 35 liters) over a steam bath in a ventilated hood. A temperature of 60° C. was the maximum obtainable by this method, so that the water had to be heated to 75° C. by direct steam heating. One thousand eight hundred grams of wood (sawdust basis—1930 grams on air-dry basis) were added to the water and the first addition of 482 grams of reagent-grade sodium chlorite was made. The solution was stirred constantly with a 'Lightnin' mixer (with a glass stirring rod) to prevent foaming. Usually 10 to 20 ml. of glacial acetic acid were added at the beginning of the cook to accelerate the formation of chlorous dioxide and thus initiate the reaction. After five to ten minutes, a small sample of the solution was withdrawn, cooled to 20° C., and the pH was determined with a Beckman pH meter. Acetic acid was then added to the solution in the amount necessary to keep the pH about 4.5; readings were taken every half hour during the cook to maintain a close check on the solution. For the first two to three hours, the pH would remain low with the addition of very little acetic acid. After this time, the acidity would fell rapidly and larger quantities of acetic acid
were required to keep the pH at 4.5. Generally, 110 to 130 ml. of acid were required per cook.

Sodium chlorite was added at the end of 1, 2, and 3 hours, and the mixture was allowed to react for 1.5 hours after the last addition of chlorite—a total cooking time of 4.5 hours. The hemicellulose was filtered immediately on a 30-ml. Buchner funnel fitted with a coarse filter paper. The liquor was collected in a four-liter filter flask and subsequently stored in five-gallon jugs. The filtered hemicellulose was washed immediately with distilled water until no further odor of chlorine dioxide could be detected. The liquor and washings were combined.

A more complete description of the treatment accorded the liquor of Cook 4 will be found in the Appendix. Briefly, the filtered chlorite liquor was acidified with concentrated sulfuric acid to a pH of 2.9, the supernatant liquor was siphoned off, and the resultant precipitate was centrifuged. The precipitate was triturated with anhydrous dioxane, filtered, and the dioxane solution dehydrated by vacuum distillation. The distillate was periodically tested for water, first by the use of anhydrous copper sulfate powder and, finally, by the freezing point method. When the freezing point of the distillate was identical with that of the stock anhydrous dioxane (11.5° C.), the dehydration was terminated. The insoluble materials were filtered or centrifuged, and a 10-ml. batch of the dioxane solution of lignin (approximately 10° concentration) was precipitated dropwise into a 250-ml. centrifuge jar filled with anhydrous ether and stirred vigorously. The resulting mixture was centrifuged, and the precipitate was washed successively with
other, high-boiling petroleum ether, and twice with low-boiling petroleum ether. It was then dried in a vacuum desiccator over pereflin, concentrated sulfuric acid, and phosphorus pentoxide. This method has been described in detail by Brame (32) and will hereafter be referred to as "the usual method of lignin purification" for the sake of brevity.

The yield of Chlorite lignin A was 15 grams, for less than the 15 to 50 grams that Borton had isolated in a similar manner. The methanol content was 12.2%. Because of the low yield of material, a different treatment was tried in Cook 5 in the hope of obtaining larger yields of lignin.

Cook 5

In Cook 5, the conditions were the same but the liquor was concentrated in a large vacuum evaporator (33) to 2.5 liters prior to acidification with sulfuric acid. A violent reaction caused some difficulty when the concentrated acid was added to the concentrated liquor; consequently, the remaining acid was diluted before addition.

The precipitate was centrifuged and various methods were employed to precipitate as much remaining lignin as possible from the mother liquor; salting out with sodium sulfate and chilling the solution were tried. For a more complete description of this process, see the Appendix. Some insoluble material was recovered which was combined with the original precipitate and purified in the usual manner. The yield

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*Caution: The solution being concentrated must be kept in circulation and not allowed to evaporate to dryness. Any mixtures of sodium chlorite (and related decomposition products) and organic materials are extremely dangerous and explosive. An explosion completely crooked the large evaporator used in this work.
of crude lignin having a methoxyl content of 10.5% was 35 grams; after two further purifications, the yield was 11.5 grams with a methoxyl content of 11.2%.

Infrared Absorption Curves

This purified lignin was subjected to an infrared absorption analysis by Edward Jones of The Institute of Paper Chemistry (34). The absorption curves for this chlorite lignin and a sample of Brauns' native lignin (32) are plotted for comparison in Figure 1. The abscissa of the curve is the frequency plotted in reciprocal centimeters (cm.⁻¹), or the wave number; the ordinate is the percentage of transmission, so that absorption maxima appear as minima on this graph.

The first minimum of interest occurs near a wave number of 3350 cm.⁻¹; this corresponds to the absorption by the hydroxyl group. The absorption at this point by native lignin is much stronger than that for chlorite lignin, suggesting that some hydroxyl groups have been destroyed during the chlorite oxidation. The next minima, at 2900 and 2830 cm.⁻¹, correspond to aliphatic carbon-hydrogen single-bond linkages; there is no great difference between the two lignins at these points. The minimum at 1727 cm.⁻¹ is rather significant. This corresponds to the carbonyl group, C=O, which may be aldehydic, ketonic, or carboxylic. Chlorite lignin has much stronger carbonyl activity than native lignin, presumably because of the presence of more carboxyl groups. Barton has indicated the presence of two carboxyl groups in Chlorite Lignin A with a unit weight of approximately 600; these curves tend to support his findings.
**Figure 1**

Infrared Absorption Spectrum

- Spruce native lignin
- Chlorite lignin (5-5)

*Frequency, cm$^{-1}$*
The minima at 1533 and 1538 cm.\(^{-1}\) are considered representative of the phenyl nucleus; both are apparent for chlorite lignin as well as for native lignin, though they are somewhat weaker for the former. This indicates that the aromatic nucleus of lignin is not completely destroyed by the cooking conditions employed. This finding is in contrast with the work of Schmidt (35, 9), Rehm and Konig (36), and Vincent, et al. (37), who claimed that sodium chlorite in acid solutions will, in general, oxidize and destroy phenols. However, it agrees with that of Barker (38), who stated that substituted phenols are less readily oxidized than phenols with free hydroxyl groups.

The minima below 1400 cm.\(^{-1}\) are not well characterized as yet, and offer no definite conclusions. In general, the infrared absorption curves for the two lignin products show that:

1. Chlorite lignin retains the aromatic nucleus, although less markedly than native lignin.

2. Chlorite lignin has stronger carbonyl activity than native lignin.

3. Chlorite lignin has weaker hydroxyl activity than native lignin.

4. Both lignins retain an aliphatic carbon-hydrogen activity.

Cook 6

Inasmuch as poor yields of chlorite lignin had been obtained in both Cooks 4 and 5, the former using Barton's technique and the latter using a modification thereof, it was decided to try a third method. Noting that Jayne and Burke (19) had obtained rather good yields by
dialyzing their solutions, it was decided to dialyze the chlorite liquor, evaporate the liquor to dryness, and resolve the materials into lignin and carbohydrate fractions by conventional methods. This would eliminate much of the inorganic material which was contaminating the constituents of the liquor.

The conditions were the same as those for Cooks 4 and 5, and the liquor was concentrated to three liters. It was dialyzed for three weeks in a cellulose sausage-skin casing; tap water, and then distilled water, were used as the dialyzing medium. The dialyzed liquor was evaporated to dryness and triturated with dioxane. Surprisingly, only eight mg. of lignin could be recovered, and the remainder of the material was ethanol insoluble, indicating carbohydrate material. This unexpected result proved that chlorite lignin could be lost entirely by dialysis because of its low molecular weight. The yield of the alcohol-insoluble fraction was 52 grams.

Cook 7

Since dialysis had resulted in the loss of dioxane-soluble lignin from the chlorite liquor, a return to the older method of lignin isolation by acid precipitation was indicated. As a side experiment, an aliquot of the chlorite liquor was electrodialyzed to determine whether this too would cause the loss of all lignin; it was thought that such would be the case and that perhaps Joyce and Lanks had isolated only a carbohydrate fraction instead of a carbohydrate-lignin mixture in their work.

The electrodialysis was carried out in a small apparatus with a capacity of 100 ml. The time of treatment was 45 hours; at the end
of that time, a thick, creamy precipitate had formed on the anode membrane and the color of solution had changed from an initial, fairly clear, green to a turbid, milky white. The diozone treatment of the anodic precipitate and the solution resulted in the isolation of 0.3 gram of lignin with a methoxyl content of 13.6%, the highest methoxyl of any lignin isolated thus far.

The main portion of the chlorite liquor was treated by a modification of the treatment accored Cook 5 (see Appendix); 43 grams of lignin were isolated. This was the best yield obtained and was comparable with Barton's yields.

FINAL EXPERIMENTAL PROCEDURE

Cook 6

The small scale electrodialysis of Cook 7 indicated that this treatment might be a good method for purifying the chlorite lignin without undue loss. The liquor from Cook 6 was concentrated to four liters and electrodialyzed in a large apparatus designed for this purpose (Figure 2). Because of the size and the necessity for having a high rate of water flow through the apparatus, it was impractical to use distilled water, and tap water had to be substituted. The liquor was stirred vigorously during the process to improve the efficiency of electrodialysis. The current was maintained at approximately six amperes throughout the process. The voltage at the beginning was 49 volts, whereas at the end, the maximum voltage obtainable (150 volts) produced a current of only three amperes through the cell. This was due to a fall in conductance of the liquor caused by the removal of the ionic inorganic impurities. At the end of 48 hours, the liquor gave a negative test for chlorite.
FIGURE 2
Large Electrodialysis Apparatus
The dialyzed liquor, with a volume of approximately seven liters, was concentrated to about one to two liters. This solution was then precipitated into eight volumes of 95% ethanol. The heavy white voluminous precipitate settled readily. This was filtered on a 15-cm. Schlenk funnel, washed with ethanol and ether, and dried in a vacuum desiccator. The yield was 182 grams.

The strongly colored alcohol used for precipitation was evaporated to dryness and triturated with diethylether; the lignin was isolated in the usual manner. The yield of this lignin was good (58 grams) and it had a high methoxyl content (15.2%). In view of the high yields of material and relative ease of manipulation, the procedure described above (see flowchart, Figure 3) was used for all subsequent cooks, with only slight modifications which will be noted. It is recommended as a method of isolation of relatively pure carbohydrate and lignin fractions from chlorite liquor.

Change of Thesis Goal

Preliminary analyses indicated that the alcohol-insoluble fraction (coded 8-1) was composed of about 50% of reducing sugars. So such large carbohydrate fraction had been reported as being isolated from chlorite liquor, although Barton had obtained diethylether-insoluble fractions (predominantly carbohydrate in nature) which totaled 25 grams in weight. Joyce and Fenke, of course, had not separated their lignin and carbohydrate materials. It was felt that this large carbohydrate fraction would yield information on the mechanism of the chlorite process and might shed some light on the relationship of lignin to carbohydrate in
Alcohol-benzene extracted wood

- Digested with NaOCl and acetic acid
- 4.5 hours, filtered, and washed

Filtrate

- Concentrated to 3-4 liters and
electrolyzed

Holocellulose

Purified liquor

- Concentrated to 1-2 liters and
precipitated into EtOH, filtered,
 washed with EtOH, ether, and
petroleum ether, dried

Precipitate

(8-1, 9-1, etc.)

Solution

- Evaporated to dryness and triturated
with dioxane, centrifuged

Solution

- Dehydrated and
centrifuged

Moist dioxane insoluble fractions (A)

- A and B combined in all
cooks to form one fraction,
8-2, 9-2, etc.

Dry dioxane insoluble fractions (A)

Precipitated into ether, washed with
ether, petroleum ether, and dried

Lignin

(8-3, 9-3, etc.)
the wood itself. At this point, all research work on the lignin was terminated and, subsequently, the investigation of the carbohydrate fraction comprised the main effort.

Cook 9 and 10

These two cooks were performed primarily for the purpose of isolating sufficient carbohydrate material for analysis. The cooks were conducted in the same manner as Cooks 4 to 6, and the liquors were treated by the procedure outlined in Figure 3, with the following exception: after precipitation of the carbohydrate fractions (9-1, 10-1) into alcohol, the alcohol was concentrated to about 100 ml., and reprecipitated into eight volumes of fresh alcohol. The insoluble material was filtered off, the alcohol concentrated to a small volume, and again reprecipitated into eight volumes of fresh alcohol. The precipitate was filtered off, and the filtrate was evaporated to dryness, taken up in dioxane, and the lignin isolated in the usual manner. This method is considered superior to the treatment outlined in the flow sheet, inasmuch as difficulty is experienced in dehydrating the dioxane solution when appreciable amounts of dioxane-insoluble materials are present. The solution then has a tendency to emulsify, and the capillary tube will frequently become clogged with solid material. With the three-stage precipitation suggested above, the bulk of the dioxane-insoluble materials will be removed prior to the dioxane treatment, and the dehydration operation will proceed more smoothly. The yield of 9-1 was 105 grams; of 10-1, 126 grams.
Hot Water Extraction of Wood

In an attempt to establish the origin of the alcohol-insoluble fractions, an investigation was made of the hot water extract of spruce-wood. It had been suggested that part or all of the alcohol-insoluble fractions (2-1, 9-1, and 10-1) consisted of materials which could be extracted from wood by hot water. To test this, a small-scale orientation experiment was conducted on the alcohol-benzene extracted wood. One hundred grams of air-dry wood were extracted with hot water under the conditions of an actual chlorite cook—i.e., 60 consistency, 75-80° C. temperature, and 4.5 hours extraction time. No acetic acid or sodium chlorite was added. The pH of the distilled water used was 5.9; after extraction, the pH of the aqueous solution was 4.9, showing the removal of acidic constituents. The wood was filtered and dried; the solution was concentrated and precipitated into ethanol. A white precipitate formed which was filtered, washed, and dried.

Two 2-kilogram batches of wood (oven-dry basis) were similarly extracted. The results of the three experiments are listed in Table III.

**TABLE III**

RESULTS OF HOT WATER EXTRACTIONS

<table>
<thead>
<tr>
<th></th>
<th>Small scale,</th>
<th>Large scale,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Recovery of extracted wood</td>
<td>97.9</td>
<td>97.2</td>
</tr>
<tr>
<td>Yield alcohol-insoluble material</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>Yield alcohol-soluble material</td>
<td>0.38</td>
<td>0.36</td>
</tr>
<tr>
<td>Total recovery</td>
<td>99.22</td>
<td>98.52</td>
</tr>
</tbody>
</table>

Alcohol-soluble materials lost
The results of the different extractions are in good agreement with each other; these data indicate that slightly less than 1% of the water-soluble material of the unextracted wood is removed and recovered in the alcohol-insoluble portion of the chlorite liquors—about 16 grams in a full-scale cook. This must be considered a maximum value of the materials extracted under the conditions, since these materials are subjected to oxidation and hydrolysis during the cook and to losses during the electrodialysis treatment. During the isolation of native lignin, Brown has reported the presence of a water-soluble polyaccharide in sprucewood (29). He obtained a yield of 0.1-0.2% of the wood, whereas nearly 1% has been isolated in this case. However, Brown's material was isolated under different conditions and underwent considerably more treatment than the water-soluble materials in this work; consequently, the two fractions are not necessarily identical.

Cook 18

This cook was performed on 1000 grams (oven-dry basis) of hot water-extracted wood; by mistake, acetic acid was not added to the cook until 15 minutes after the first addition of sodium chlorite. Otherwise, the cook and the treatment of the liquor were identical up to the electrodialysis stage. During this process, one of the membranes sprung a leak and part of the liquor was lost. The remaining liquor was then dialyzed for one week against distilled water and subsequently treated in the usual manner—i.e., precipitation into alcohol, etc. An alcohol-insoluble fraction of 100 grams was isolated by the first precipitation and, subsequently, an additional 36 grams were obtained by the second and third precipitations.
This experiment showed that the major portion of the alcohol-insoluble fractions previously isolated could not be from the hot water-soluble fraction of ©.recessed. This cook turned out to be less than quantitative (because of chemical loss during electrodialysis) and yet the yield of the alcohol-insoluble materials was comparable with those of previous cooks, where the parent wood had not been water extracted. Obviously, the alcohol-insoluble fractions must have arisen from sources other than the hot water-soluble materials of ©.recessed.

There are at least two major possibilities for the origin of this material: (1) that the chlorite cooking conditions as employed in this work had been somewhat more drastic than necessary—such that, in the course of the lignin removal, some carbohydrate material normally conceived to be part of the holocellulosic fraction had been simultaneously removed; or (2) that lignin in situ consists in part of carbohydrate material, termed "Überschuss Polysaccharide" by Joyce and Lieske (19), which is liberated by the chlorite process. There are at least two possibilities for this latter theory: (a) that the polysaccharides are polymerized with lignin in a lignin-carbohydrate complex actually present in wood as postulated by London (40); or (b) that these polysaccharides are not present as such in the lignin but rather arise from some parent material during chlorite oxidation. Theory (c) presupposes that these polysaccharides will be analytically determined as lignin in the Klenze or Ileson lignin determination.

The first possibility was investigated by altering the cooking conditions. It was conceivable that these were too drastic. The Klenze
lignin residues, for example, in the holocelluloses of Cooks 8, 9, and 10 are 1.6, 1.5, and 1.2%, respectively. Wise (21) has stated that the Klason lignin content of the holocellulose should not be allowed to fall below 2-4% (the exact figure being dependent on the type of wood); otherwise, it is well known that considerable degradation of the holocellulose takes place. Using Wise's criterion, it was possible that the large alcohol-insoluble fractions were isolated from chlorite liquors simply because cooking conditions were too drastic and the true holocellulose was partially degraded and dissolved.

Cook 14

In this cook, the time was shortened to three hours, and only three additions of 482 grams of sodium chlorite were made, at zero, one, and two hours. The liquor was concentrated and electrodialysis was started but, unfortunately, a membrane broke again and this time the entire batch of liquor was lost. The holocellulose was analyzed for Klason lignin (5.9%).

Cook 15

The cooking conditions were more drastic than in Cook 14, in the hope of obtaining a holocellulose with about 4% Klason lignin, this being the upper limit of Wise's criterion. Water-extracted wood was used as the source material. Three hourly additions of 560 grams of sodium chlorite were made and the cooking time was three hours. The liquor was treated in the usual manner, and the yield of the alcohol-insoluble fraction was 100 grams. The Klason lignin content of the holocellulose was 3.1%, somewhat less than the value which had been anticipated.
Cook 16

Another cook was performed to see if stillilder cooking conditions would produce an appreciable alcohol-insoluble fraction. This time the goal was to produce a holocellulose with a bleach lignin content well above the 4% already mentioned as a criterion for holo-
cellulose degradation. Unbleached wood was used, and again the cooking time was three hours. Three additions of 450 grams of sodium chlorite were made; otherwise the cooking conditions and treatment of liquor were identical with those already described. The yield of the alcohol-insoluble fraction was 102 grams; the bleach lignin content of the holocellulose was 5.9%.

Cook 17

At this point, it was noted that, inadvertently, the order of addition of acetic acid and sodium chlorite had been reversed, such that the salt was added first. The prescribed method of Wise, et al. (21) calls for the acidification of the solution before addition of the salt. To establish the effect of the deviation from the method, Cook 17 was carried out.

Two hundred grams (oven dry basis) of hot water-extracted wood were digested with sodium chlorite under the usual conditions of consistency and temperature. Additions of three, two, and two ml. of acetic acid preceded the hourly additions of 50 grams of sodium chlorite, and the total cooking time was three hours. These conditions paralleled those of Cook 16 but on a smaller scale. The holocellulose was filtered and washed, and the liquor electrodialyzed for 14 hours without being
previously concentrated. At the end of this time, the current in the apparatus had fallen to a steady value, and it was felt that the purification was complete. The liquor was concentrated, precipitated into alcohol, etc.

The yield of holocellulose from this cook was 87.8%, and the Klason lignin content thereof was 8.4%. Both values are somewhat higher than those of Cook 16 and indicate that the treatment had been less drastic than in the latter case.

The yield of the alcohol-insoluble fraction was 25 grams; the ash content was 22.5% (indicating incomplete electrodialysis); the reducing value after 24 hours' hydrolysis with 2% sulfuric acid was 11.0% (calculated as glucose). The latter value, calculated on the basis of the wood, was 1.37%, and compares with a similar yield of reducing sugars from 16-1 of 1.38% (based on the wood). In view of the good agreement between the two procedures, it seems probable that the order of addition of sodium chlorite and acetic acid at the beginning of the cook is immaterial as long as the two are added within a few seconds of each other. No difference is evident in the amount of reducing sugars that can be isolated from the liquor.

IDENTIFICATION OF ALCOHOL-INSOLUBLE FRACTIONS

For the sake of brevity, the alcohol-insoluble fractions will subsequently be identified by a code number; this designation corresponds to the nonconcretion of the flowchart for Cook 8 in Figure 3. The first figure is the cook number; the number following the hyphen is the fraction isolated. Thus, "16-1" refers to the initial alcohol-insoluble fraction (suffix -1) from Cook 16 (prefix 16-).
Purification of Fractions

Considerable work was done on the purification of the original alcohol-insoluble fraction, S-1. The methoxyl content of the original precipitate was 3.04%, which seemed somewhat high for a carbohydrate type of material. Subsequent work indicated that the best means of purification was by precipitation of an aqueous solution of this material into ethanol. Not all of S-1 was water soluble; a finely dispersed crystalline substance present was water insoluble. A qualitative analysis, using the procedure of Boyes (11), showed that the material consisted mainly of sodium oxalate. Smaller amounts of calcium, potassium, and chloride ions were also present.

Prior to the second precipitation, the aqueous solution of S-1 was filtered to remove this insoluble material, and the clear filtrate was precipitated into ethanol. The methoxyl content was 2.95%. Subsequent small-scale purifications failed to change the methoxyl content, and further purification of the main body of the fraction was not attempted.

Soxhlet Extraction of Material

Separate samples of S-1 were exhaustively extracted in Soxhlet extractors with anhydrous dioxane and absolute ethanol, respectively. Neither removed more than traces of material, and the methoxyl contents of the extracted substances were nearly the same as formerly—namely, 2.92 and 2.75% for the alcohol and dioxane-extracted material, respectively.

*All samples of carbohydrate materials that had once in contact with ethanol during their isolation still retained traces of ethanol that were difficult to remove by solvent washing. The samples for methoxyl determination were dissolved in water and the aqueous solution evaporated to dryness. The residue was then tested for methoxyl.
Subsequently, a somewhat larger sample of 8-1 was similarly extracted with absolute ethanol and the methoxyl content of the dried extract determined. It was 9.50%, showing that some material with high methoxyl content (presumably lignin) was removed during this process.

**Analyses for Carbohydrate Materials**

**Time-Hydrolysis Curves**

Preliminary investigation had revealed that 8-1 consisted of approximately 50% reducing sugars (calculated as glucose). This was determined by the Leman-Walker method (12). To establish the maximum amount of reducing sugars that could be determined, rate of hydrolysis curves were determined for the various alcohol-insoluble fractions.

The method used was as follows: 2 to 2.5 grams of the alcohol-insoluble fraction were dissolved in exactly 150 ml. of 25 sulfuric acid, and the mixture (normally cloudy at the beginning) was slowly heated to boiling in a glycerin bath. A reflux condenser was provided to prevent loss of acid. The temperature of the glycerin was about 120° C. Zero time was calculated from the commencement of boiling, usually about 30 minutes after heating had started.

Samples of 12 to 13 ml. were intermittently removed by a pipette, cooled to 20° C, immediately, neutralized with a small amount of sodium bicarbonate, and filtered on a Whatman No. 50 filter paper if the hydrolysate was not clear. Exactly ten ml. of the filtered hydrolysate were removed by pipette and analyzed by the Leman-Walker method for reducing sugars. The curves for six alcohol-insoluble materials are given in Figures 4 and 5.
The alcohol-insoluble fractions give basically similar hydrolysis curves, although the absolute amounts of sugars vary widely. It seems likely that there are two types of materials present—one that is rapidly hydrolyzed within the first three to four hours, and another type that is more difficultly hydrolyzed. In any case, a maximum degree of hydrolysis is reached in about 24 hours, and the curve remains essentially flat for the next 24 hours, beginning to fall off after 48 hours. For all subsequent hydrolytic work, the reaction time was 24 hours.

Qualitative Sugar Tests

Mannose. Burton (26) reported the presence of mannose in the hydrolysate of one of his dioxene-insoluble fractions. Accordingly, a simultaneous qualitative and quantitative test for mannose was performed on 8-1 according to the Egglund-Bratt method (43) as modified by Wise (44). Essentially, this consists of treating the hydrolysate of the material with phenylhydrazine to obtain mannose phenylhydrazone which is gravimetrically determined; in the quantitative test, a known amount of mannose is added to the hydrolysate prior to the addition of the phenylhydrazine (this “booster charge” ensures quantitative precipitation of the phenylhydrazone); in the qualitative portion of the test, no mannose is added. In the latter test, a precipitate of mannose phenylhydrazone was obtained with a melting point of 195º C. after two recrystallizations from 95º ethanol. This melting point was determined on a Fisher-Johns block. The reported melting point in the literature is 199-203º C. Quantitatively, 8-1 analyzed 18.3 (estimated accuracy of determination, ± 1.5).
Gelactose. Gelactose in S-1 was determined according to Institute Method 27. This consists in the oxidation of gelactose to sulfuric acid by nitric acid and the subsequent gravimetric determination of the sulfuric acid. However, the recoveries are less than quantitative, especially with only small amounts of gelactose present. Qualitatively, a good crop of crystals of sulfuric acid was obtained without seeding. The first melting point was 214–215°C.; after recrystallization from water, the acid melted at 211–212°C. The reported melting point is 212–215°C. This confirms the finding of Barton, who also reported the presence of gelactose in his dioxane-insoluble fraction.

Gelacturonic acid. A naphthoresorcinol test established qualitatively the presence of uronic acids in S-1. This consisted of boiling a few milligrams of S-1 in a test tube with five ml. of water, 0.5 ml. of 1° naphthoresorcinol in ethanol, and 0.5 ml. concentrated hydrochloric acid. The boiling was continued for five to ten minutes. The solution was allowed to stand for five minutes, cooled, and shaken with an equal volume of benzene. A bluish color was obtained in the benzene phase, which indicated the presence of uronic acids. Anderson, et al. (45, 46) have reported the presence of pectic materials containing D-galacturonic acid from the inner bark and cambial zone of black spruce, and it was thought that the uronic acids in S-1 might similarly contain D-galacturonic acid. A Heidelberger-Goebel's test (47) for this substance was performed but gave negative results. This is not in conflict with Anderson's findings, inasmuch as the wood used in this work had been thoroughly worked and peeled before reduction to wood meal. Actually, Anderson's material was isolated from the logs which were subsequently used in this work.
Anderson (49) has likewise reported the isolation of pectic material (containing galacturonic acid) from the holocellulose of black spruce by alkaline extraction. The data presented do not indicate that this material will be removed from the holocellulose under normal chlorite cooking conditions, although, under very drastic conditions, some is removed and would be expected to be isolated from the chlorite liquor. The cooks in this work are possibly mild enough so that pectic material is not removed from the holocellulose.

**Paper chromatography.** In recent years, a new tool for the identification of sugars has been developed—paper chromatography (49). Briefly, the method is as follows: an aqueous solution of the unknown sugars is spotted on a strip of Whatman's No. 50 filter paper (about 10 cm. by 40 cm.) on a horizontal line 5 cm. from the top. Spots of known sugar solutions are placed on this line alongside the unknown sugar spots. The top of this strip of paper is placed in a trough containing an alcohol solution (four parts of butanol and one part of ethanolic saturated with water) and the strip is allowed to hang vertically in a large, sealed container saturated with water vapor. Best results are obtained if the temperature is constant throughout the adsorption process.

The process is carried out for 16 to 20 hours, at which time the paper strip is removed, the solvent front marked, and the strip oven dried at 100°C. for about 30 minutes. The length of travel of the solvent is measured and the paper is cut into three longitudinal strips about 25 cm. long. The strips are brushed with an ammonical solution of silver nitrate and immediately developed in an oven at 105°C.
Dark spots of reduced silver will appear where the sugar spots have advanced along the paper; different sugars will travel different distances depending on their relative solubilities in the alcohol solution and water. By comparing the positions of the unknown sugar spots with those of the known sugars, it is possible to determine qualitatively the sugars in a mixture. A given sugar will travel a definite fraction of the distance (referred to the horizontal line on which the sugar solutions were spotted) which the solvent front has traveled; this will be of aid in identifying the sugars. Quantitative methods for the sugars have yet to be developed.

Preparation of the sugar solution for this chromatographic work is important. It is essential to remove as much as possible of the uralonic acids. A sample of 1-1 was hydrolyzed for 24 hours with 23 sulfuric acid, neutralized with barium carbonate, concentrated, precipitated into alcohol, filtered, and the alcohol-soluble fractions were concentrated and precipitated into alcohol twice more. This removed the uronic acids as insoluble barium salts. The final alcohol solution was evaporated to dryness and dissolved in a small amount of water. This aqueous solution was used to spot the paper.

The results confirmed the presence of large quantities of mannose and galactose, and very small amounts of xylose. No glucose could be identified. This is in contrast to the findings of Jahn and Lenke (19) who reported that the sugars in the hydrolysate of materials in their chlorite liquor were predominantly glucose.
Arabinose, if present in 8-I, would not be identifiable in this chromatographic test, since the arabinose and xylene occupy nearly identical positions. In the presence of large amounts of xylene, a large and rather diffuse spot is obtained. This obliterates a spot due to arabinose and makes the visual separation impossible.

A confirmatory test for xylene was attempted with negative results. This is the method of Brooks and Jones (50) as modified by Wise (51), and consists in the formation of an insoluble dibenzylidene dimethyl acetal of xylene. However, if only small quantities of xylene are present, this test is not conclusive.

Quantitative Sugar Tests

**Differential fermentation.** A neat and rapid method for the quantitative determination of various sugars has been developed by Wise and Appling (52–54) and Auernheimer, Wickham, and Schiopp (55). This consists of the differential fermentation of the various sugars by different strains of yeast organisms. These organisms have been carefully selected and tested by the Northern Regional Research Laboratory at Peoria, Illinois. For brevity, the organisms will be designated by the number assigned to them at this laboratory—e.g., N.R.R.L. No. 379.

The three sugars which may thus be determined are D-galactose, D-xylene, and L-arabinose. The action of the different organisms is shown in Table IV.

The method may be summarized as follows: the unknown poly-saccharide material is hydrolysed the requisite time with 7% sulfuric
### TABLE IV

**Action of Yeast Organisms on Different Sugars**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sugars Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.B.R.L. No. 379 (Saccharomyces carlsbergensis)</td>
<td>Mannose, D-glucose, fructose, and D-galactose</td>
</tr>
<tr>
<td>U.B.R.L. No. 566 (Saccharomyces bayanus)</td>
<td>Mannose, D-glucose, and fructose</td>
</tr>
<tr>
<td>U.B.R.L. No. 488 (Candida Guilliermondii)</td>
<td>D-Xylose and L-arabinose</td>
</tr>
<tr>
<td>U.B.R.L. No. 238 (Knezema canescens)</td>
<td>D-Xylose</td>
</tr>
</tbody>
</table>

Hexoses must be fermented with U.B.R.L. No. 379 prior to fermentation with these organisms.

Acid, neutralized with sodium bicarbonate (neutralization with barium carbonate will introduce barium ions which may poison the organisms), and 35-ml. aliquots of the hydrolysate are mixed with 15 ml. of yeast extract. These are sterilized, inoculated with the appropriate organisms, capped, and shaken for 24 hours at 30° C. The solutions are then filtered and tested for reducing sugar content by the Munson-Walker method. Knowing the reducing sugar content of the original uninoculated hydrolysate, the amount of sugars fermented by the various organisms can be calculated. A special table of Munson-Walker reducing values for various sugars has been prepared by Wise and McCormack (50) and is necessary for these calculations.

The pentose sugars require a total of two days for fermentation; for the first 24 hours, the hydrolysates are fermented with U.B.R.L. No. 379 (to remove the hexoses), after which they are steriley inoculated.
separately with the pentose fermenters, N.R.E.L. Nos. 838 and 938. The fermentation proceeds for another 24 hours, at which time the samples are analyzed for reducing sugars.

Controls on known pure sugars were run simultaneously to determine whether mutations of the organisms had developed which would behave abnormally. In all this work, the yeasts fermented properly.

Controls on the sterile uninoculated hydrolyzate were run to determine the stability of the solution; results indicated that there was no loss of reducing sugars over the 48-hour period.

The calculated results for two alcohol-insoluble fractions which were thus hydrolyzed and fermented are given in Table V.

### Table V

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Alcohol insoluble fraction</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-1 (A)</td>
<td>16-1 (B)</td>
</tr>
<tr>
<td>Hunson-Dolker reducing value of original hydrolyzate (as glucose)</td>
<td>23.3</td>
<td>22.9</td>
</tr>
<tr>
<td>Inositol</td>
<td>17.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Galactose</td>
<td>16.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Xylose</td>
<td>2.0</td>
<td>0.3b</td>
</tr>
<tr>
<td>Arabinose</td>
<td>3.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Tunic acid hydridec</td>
<td>2.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Total</td>
<td>53.3</td>
<td>20.3</td>
</tr>
</tbody>
</table>

*Calculated by assuming that the hydrolyzates were composed entirely of mannose and galactose. Then N.R.E.L. No. 966 ferments only mannose.

*The xylose value of 16-1 is questionable, because of the small amount of xylose present.

*The tunic acid hydride values are calculated by assuming that all the residual reducing value after fermentation with N.R.E.L. No. 468 results from tunic acid. So far as is known, these are not significantly fermented by any of the organisms.
In both 8-1 and 16-1, mannose and galactose are present in about a 1:1 ratio and, together, these two sugars account for the largest portion of the alcohol-insoluble fractions. The mannose value of 17.7, determined by differential fermentation, agrees well with the mannose value of 18.5 determined gravimetrically. This is further proof that glucose is absent from the hydrolyzate, as already mentioned and justifies the assumption that the fermentation by U.B.R.I. No. 966 consists of mannose utilization alone.

The presence of arabinose in both 8-1 and 16-1 is established by this method. That it should be present in 16-1 is somewhat surprising in view of the fact that the hot water extraction of the wood prior to chlorite oxidation had removed material which was known to contain arabinose. However, no attempt was made at exhaustive extraction under which conditions it is conceivable that all the arabin could have been removed from the wood. The ratio of arabin in 8-1 to arabin in 16-1 is about the same as the corresponding ratio of mannose and galactose.

This work likewise established the presence of xylose in 8-1. This sugar had been tentatively identified by chromatographic analysis, as already mentioned, but the fermentation results are confirmation of this. For 16-1, the presence of xylose is doubtful; the calculated values of xylose are far below the range of accuracy of the Hanson-Walker tables. If xylose is removed from spruce wood only as a result of degradation of the holocellulose by drastic cooking conditions, it would be expected that proportionately less xylose would be found in 16-1, which is a product of considerably milder cooking conditions. Column 8 tends to confirm this.
A confirmation of the presence of pentose sugars was made by the chromatogram method. The excess hydrolyzate fermented by E. coli No. 379 but not used for the Monon-Fischer determination was chromatographed. The fermentation had quantitatively removed the hexoses, mannose, and galactose, leaving the pentoses. Results of the chromatogram confirmed the presence of both arabinose and xylose. Previously, arabinose had not been identified chromatographically because of the presence of mannose; the spots of the two sugars are nearly inseparable. But with the removal of the mannose by fermentation, the arabinose was clearly evident.

A control was simultaneously conducted to determine whether the fermentation of hexoses by E. coli No. 379 could produce pentoses or materials that would give reducing tests. A sample of pure galactose solution that had been quantitatively fermented with E. coli No. 379 was chromatographed. No reducing materials of any kind were evident on the paper; this proved that the apparent arabinose and xylose spots were actually caused by these sugars and not by waste products of fermentation. This test was final evidence for the presence of the pentoses in the hydrolyzate.

Uronic Acids and Pentoses

These substances were determined quantitatively by Institute Methods 25 and 23, respectively. The alcohol-insoluble fractions were thus analyzed and the results are given in Table VI.

The apparent pentose figure is corrected for the presence of uronic acids by the following formula:
Let \( \Delta = \) weight of sample for pentose determination,
\( \Delta = \) Percentage of carbon dioxide in sample (from uronic acid determination), and
\( \Delta = \) weight of furfural phloroglucinol precipitate from pentose sample.

Then \( \Delta \times D \times 1.47 = \Delta = \) weight of furfural phloroglucinol arising from uronic acid and
\( \Delta - \Delta = \) weight of furfural phloroglucinol arising from true pentose material.

**Table VI**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>S-1</th>
<th>9-1</th>
<th>10-1</th>
<th>12-1</th>
<th>15-1</th>
<th>16-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uronic acid hydride</td>
<td>17.6</td>
<td>12.0</td>
<td>17.1</td>
<td>17.4</td>
<td>19.4</td>
<td>17.6</td>
</tr>
<tr>
<td>Agarose pentose</td>
<td>6.5</td>
<td>6.9</td>
<td>6.8</td>
<td>9.7</td>
<td>6.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Corrected pentose</td>
<td>5.2</td>
<td>5.8</td>
<td>5.8</td>
<td>5.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pentose by fermentation</td>
<td>5.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.7</td>
</tr>
</tbody>
</table>

It must be emphasized that this is an empirical correction and that the corrected pentose values are not accurate. For example, the known pentose contents of S-1 and 16-1 are 5.2 and 1.7, respectively, as determined by fermentation. Yet the corrected pentose figures are 5.2 and 0.7, respectively, errors of 20 to 100%. With low percentages of pentose, this correction is obviously only approximate.

In the case of both Cook 8 and Cook 16, more pentose may be accounted for by fermentation than by direct analysis. This also illustrates the inaccuracy of the pentose correction.

Second, the uronic acids are only partially hydrolyzed in a similar hydrolysis. It is known that the uronic acid hydride contents of S-1 and 16-1 are over 17% and yet only 9 and 5% uronic acid hydride can be
calculated by fermentation results. This is in harmony with the findings of other investigators (57-60) who have reported the presence of uronic acid residues that are quite stable to acid hydrolysis.

Isolation of Barium Salt

The presence of uronic acid material that apparently could not be hydrolyzed led to the adoption of a technique of analysis that had been used by Anderson, et al. (61), Sands and Butts (59), and O'Dayor (57). This method consists of the isolation and analysis of a barium salt of an aldonic acid. The technique is as follows.

A sample of the uronic acid-containing material is hydrolyzed with sulfuric acid, neutralized with barium carbonate, filtered, concentrated in vacuo, and precipitated into ethanol. The precipitate is filtered and purified by precipitation from an aqueous solution into alcohol to a constant barium content (or some other criterion of purity). Analyses are normally made for barium, methoxyl, pentoses, uronic acids, and optical rotation.

In this work, 30 grams of 2-1 were hydrolyzed for 24 hours with 2250 ml. of 2% sulfuric acid, neutralized with barium carbonate, filtered, concentrated in vacuo to 300 ml., and precipitated into ethanol. The crude salt was filtered, dried, and analyzed for methoxyl. It was dissolved in a small amount of water, decolorized with carbon, and precipitated into ethanol. This process was repeated without the decolorization step. The methoxyl contents after the first, second, and third precipitations were 1.20, 0.40, and 0.37%, respectively. In view of the constant methoxyl contents, the purifications were discontinued.
The yield of the purified salt was 1.40 grams. The analyses of this material are given in Table VII.

**TABLE VII**

**ANALYSES OF BARIUM SALT**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>$%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxyl</td>
<td>0.37</td>
</tr>
<tr>
<td>Uronic anhydride</td>
<td>24.08</td>
</tr>
<tr>
<td>Apparent pentoses</td>
<td>4.9</td>
</tr>
<tr>
<td>Corrected pentoses</td>
<td>0.0</td>
</tr>
<tr>
<td>Sulfated ash</td>
<td>54.7</td>
</tr>
<tr>
<td>Barium</td>
<td>31.0</td>
</tr>
<tr>
<td>Carbon</td>
<td>23.7</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>3.54</td>
</tr>
<tr>
<td>Specific rotation $[\alpha]_D$</td>
<td>$6.0^\circ$</td>
</tr>
</tbody>
</table>

The ultraviolet absorption curve for this material is plotted in Figure 6. The material was titrated conductimetrically by adding an excess of standard alkali and back titrating with standard acid. The curve for this experiment is given in Figure 7.

From these data, two facts are evident: (1) The material isolated is not a barium salt of a xylomethylaldehydeic acid, and (2) the material is not aromatic in nature.

The first point is proven by comparison with the theoretical analysis of such a barium salt. O'Dwyer (67) lists the theoretical analysis of a barium salt of a xylomethylaldehydeic acid as 16.86% barium, 7.61% methoxyl, and 43.19% uronic anhydride. The barium salt isolated in this work has nearly twice as much barium, practically no methoxyl, and a little more than half the uronic anhydride content. In addition, there is no pentosean material evident. However, in one aspect,
FIGURE 6

Ultraviolet Absorption Spectrum of Barium Salt

Specific extinction

Wavelength, mmu
FIGURE 7

Conductimetric Titration of Barium Salt

0.1815 g. salt
5.00 ml. 0.1132N NaOH added
Back-titrated with 0.0499N HCl
it agrees with a similar barium salt isolated by Joyce and Hinke (12); the only reported analysis of this salt is the barium content (39.1%), which agrees reasonably well with the value of 31.0% found in this work. It must be emphasized that these barium salts have been isolated from material which has undergone rather drastic oxidative treatment; in contrast, the salts isolated by O'Dwyer, Anderson, and others originated from material which had undergone no such treatment. Therefore, close agreement between the two materials is not necessarily to be anticipated.

That the salt is not aromatic in nature is shown by the complete absence of absorption in the ultraviolet spectrum and is substantiated by the low carbon content.

The titration of the salt indicates the presence of 17.25 total, 14.05 combined, and 3.25 free acid material (calculated as carbon dioxide). The carbon dioxide resulting from the acetic acids is 5.05, leaving 11.25 carbon dioxide from other sources. Some of this may be from alicyclic acids; it suggests that this may be the salt of an oxidized carbohydrate material. If so, this would explain the high barium content and the low carbon content.

**Klason Lignin and Hydrolysis Residues**

A Klason lignin determination on 3-1 gave a residue of 1.92%. The methoxyl content of this residue was 9.5%. In the hydrolysis of 16-1 preliminary to the differential fermentation, an acid-insoluble residue of 18.8% was obtained with a methoxyl content of 9.4%. The graph for the ultraviolet absorption curve of the former fraction is given in Figure 8.
FIGURE 8

Ultraviolet Absorption Spectrum of Hydrolysis Residue
Those experiments leave little doubt that the acid-insoluble residues are primarily lignin in nature. In view of the fact that these residues are derived from materials which have been extracted with alcohol and dioxane but which are water soluble and considering that a drastic acid hydrolysis is necessary for the formation of the residues, there can be little doubt that the lignin-like materials are chemically bound to the carbohydrates. Evidence to support this belief is given by Larson (40, 53), who states that it is generally believed that lignin is chemically combined with the polyuronide hemicelluloses, and that removal of the latter from wood is difficult without simultaneous removal of the former.

Analysis of Water-Soluble Extract

The hot water-soluble portion of the wood (removed prior to Courses 12, 15, and 16) which was alcohol insoluble was analyzed. A sample of this material was hydrolyzed with 7.5 sulfaric acid for 24 hours, giving a Munson-Bulker reducing value of 92.3 (calculated as galactose). In the following table, the analyses of this material are compared with those of Dreuna's material (32):

<table>
<thead>
<tr>
<th></th>
<th>Dublita</th>
<th>Dreuna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>8.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Uronic anhydride</td>
<td>12.1</td>
<td>13.2</td>
</tr>
<tr>
<td>Apparent pentoses</td>
<td>19.6</td>
<td>--</td>
</tr>
<tr>
<td>Corrected pentoses</td>
<td>10.7</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Note: All values except ash content are calculated on an ash-free basis.
The agreement is good, indicating that the same type of material has been isolated in both cases.

A sample of this material was chromatographed according to the method already described. The results showed the presence of large amounts of galactose and considerably smaller amounts of arabinose. This likewise confirms Drewes' findings. No other sugars could be identified by the chromatographic method.

Balance of Alcohol-Insoluble Fractions

The analytically determined constituents of the alcohol-insoluble fractions are listed in Table II. Although there was little doubt that these fractions were predominantly carbohydrate, only part of them could be determined as sugars; the less drastic the cook, the less reducing sugars were present. From the solubility and other characteristics, the material appeared to be mainly polysaccharidic in nature, yet the possibility could not be dismissed that the nonreducing portions were aromatic in nature or lignin degradation products. However, there are several pieces of evidence that rule against this theory:

1. The carbon content of 8-1 is 40.0\% (ash-free basis). The carbon contents of the various chlorite lignins isolated in this work vary between 50 and 55\%, whereas the theoretical carbon contents of pentosans and hexosans are 45.5 and 44.4\%, respectively. It is hardly conceivable that the 35\% of 8-1 not analytically determined as ash, reducing sugars, or acid-insoluble residue (primarily lignin in nature) could itself be lignin in nature. Rather, with a carbon content lower than that of pentosans or hexosans, it appears possible that this undetermined material may consist of oxidation products of polysaccharides.
### TABLE IX

#### A—ANALYSIS OF ASCOIDE-INDICATED MATERIALS

<table>
<thead>
<tr>
<th></th>
<th>6-1</th>
<th>9-1</th>
<th>10-1</th>
<th>12-1</th>
<th>15-1</th>
<th>16-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (on wood)</td>
<td>7.9</td>
<td>5.8</td>
<td>10.3</td>
<td>5.5</td>
<td>5.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Acid-insoluble residue</td>
<td>2.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>18.8</td>
</tr>
<tr>
<td>Ash</td>
<td>6.8</td>
<td>13.7</td>
<td>12.6</td>
<td>9.3</td>
<td>7.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Mexican reducing power (as glucose)</td>
<td>56.2</td>
<td>55.0</td>
<td>43.7</td>
<td>50.0</td>
<td>34.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Mannan</td>
<td>17.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>7.1</td>
</tr>
<tr>
<td>Celulosen</td>
<td>16.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>6.4</td>
</tr>
<tr>
<td>Xylan</td>
<td>2.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.3</td>
</tr>
<tr>
<td>Araban</td>
<td>3.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.4</td>
</tr>
<tr>
<td>Uronic anhydride</td>
<td>9.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>5.1</td>
</tr>
<tr>
<td>Pentosan, gravimetric</td>
<td>8.6</td>
<td>8.9</td>
<td>8.5</td>
<td>9.7</td>
<td>6.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Uronic anhydride</td>
<td>17.6</td>
<td>12.0</td>
<td>17.1</td>
<td>17.4</td>
<td>19.4</td>
<td>17.6</td>
</tr>
<tr>
<td>Corrected pentosan</td>
<td>4.2</td>
<td>4.8</td>
<td>3.8</td>
<td>4.0</td>
<td>0.0</td>
<td>1.7</td>
</tr>
</tbody>
</table>

aThis is the residue from the acid hydrolysis
bCalculated from differential fermentation results
cCalculated from carbon dioxide evolution
dCalculated from differential fermentation results; corrected pentosan yield is zero

### B—SOLUTION OF MAJOR COMPOUNDS OF 6-1 AND 16-1

<table>
<thead>
<tr>
<th></th>
<th>6-1</th>
<th>16-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing polysaccharides</td>
<td>48.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Net uronic anhydrides</td>
<td>8.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Acid-insoluble residue</td>
<td>2.0</td>
<td>18.8</td>
</tr>
<tr>
<td>Ash</td>
<td>6.8</td>
<td>12.3</td>
</tr>
<tr>
<td>Total</td>
<td>65.7</td>
<td>63.9</td>
</tr>
<tr>
<td>Deficiency</td>
<td>34.3</td>
<td>36.1</td>
</tr>
</tbody>
</table>

aThis is the difference between the gravimetrically determined uronic anhydride content (by carbon dioxide evolution) and the uronic anhydride content from differential fermentation

*All results are on an oven dry basis*
2. The methoxyl contents of 8-1 and 15-1, corrected for the methoxyl of the known lignin contents (acid-insoluble residues), are 2.7 and 3.2\textsuperscript{1}, respectively. This is again well below the range of methoxyl values for known lignin materials.

3. The barium salt previously discussed is representative of the nonreducing material but shows no evidence of lignin substance therein. The extremely low methoxyl and carbon content, as well as the flat ultraviolet absorption curve, rule out the possibility that this material is aromatic in nature.

In view of this evidence, there is little possibility that this nonreducing material can be lignin in nature. The work of Isbell (23) has indicated that reducing sugars are oxidized by sodium chlorite solutions to aldehydic acids, and Joyce (19) has reported the identification of gluconic acid from the chlorite liquor of sprucewoods. Based on this information, various attempts were made to identify the sugar acids.

First, attempts were made to prepare the alkaloidal salts which are useful in identification. The method was essentially that described by Joyce (19): an aqueous solution of the barium salt (whose preparation has been described) was freed of barium by addition of the calculated amount of sulfuric acid, filtered, and boiled under reflux for several hours with an alcoholic solution of the alkaloid. The excess alkaloid was extracted with chloroform, and the aqueous solution was concentrated to a small volume. Two alkaloids were used (brucine and cinchonine) but no insoluble salts could be isolated or identified.
Two alcohol-insoluble fractions were titrated conductimetrically to determine the amount of carboxyl groups. Orientation experiments indicated that the best results were obtained when an excess of standard alkali was added and the solution was back titrated with standard acid. The curves for 8-1 and 16-1 are given in Figure 9. Table I summarizes the calculations from these graphs.

**Table I**

**CONDUCTIMETRIC TITRATION CALCULATIONS**

<table>
<thead>
<tr>
<th></th>
<th>8-1</th>
<th>16-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Free carboxyl (as CO₂)</td>
<td>9.22</td>
<td>8.35</td>
</tr>
<tr>
<td>2. Combined carboxyl (as CO₂)</td>
<td>0.46</td>
<td>6.90</td>
</tr>
<tr>
<td>3. Total carboxyl (as CO₂)</td>
<td>9.68</td>
<td>15.25</td>
</tr>
<tr>
<td>4. ( \frac{3}{2} ) corrected for lignin( ^a )</td>
<td>9.04</td>
<td>10.20</td>
</tr>
<tr>
<td>5. Uronic anhydride (as CO₂)</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>6. ( 4 - 5 ) (as CO₂)</td>
<td>4.6</td>
<td>5.5</td>
</tr>
<tr>
<td>7. ( 6, ) calculated as aldonic anhydride</td>
<td>16.4</td>
<td>23.2</td>
</tr>
</tbody>
</table>

\( ^a \)This correction was made by assuming that chlorite lignin has four titratable acidic groups. Correction is made for the theoretical amount of chlorite lignin (10.3 \( \% \) methoxyl) in the acid-insoluble fractions (9.5 \( \% \) methoxyl) from the respective carbohydrate materials.

The uronic acid content had previously been determined gravimetrically. These values indicate that considerably more carboxyl groups are present than exist as uronic acid groups and support the hypothesis that aldonic acids are present in these carbohydrate fractions. Line 7 expresses the excess carbon dioxide as aldonic acids (calculated as the polyosaccharide). This figure is purely hypothetical; some of the carboxyl may exist as dicarboxylic acids, such as succinic acid.
or as lower molecular weight carbohydrate acids, with only three or four carbon atoms per chain. This is unlikely, inasmuch as a saccharic acid could not exist as a polymer; the glycosidic linkage would have to be broken before these could be formed, as for example, by oxidation of polyuronide material. Thus, it would be improbable that they would be isolated as alcohol-insoluble monomers.

The presence of such large amounts of aldonic acids presupposes short chain length poly saccharide materials. Poly saccharides are mainly nonreducing, because of the glycosidic linkage covering carbon atom 1 of the sugar molecule; the only reducing sugar group in a polymer would be an end group not associated with a glycosidic linkage. Consequently, this would be the only group capable of oxidation by sodium chlorite, and a high ratio of end-group sugars (aldonic acids) to reducing sugars necessitates short chain length material. The extreme water solubility of these alcohol-insoluble materials lends credence to this hypothesis.

Summary of Cooks

In Table XI, the analysis of sprucewood is summarized and, in Table XII, the cooking conditions and summary of the principal products of the cooks are given.

| TABLE XI |
| ANALYSIS OF CRUDEWOOD |
| Methoxyl | 6 |
| Ash | 4.19 |
| Klason lignin | 27.9 |
| Uronic anhydride | 4.80 |
| Pentosans | 12.8 |
| Corrected pentosans | 10.7 |
| Theoretical yield of holocellulose | 72.1 |

*All results are on an oven-dry basis
### TABLE XII

#### A-DISTRIBUTION OF CARBOHYDRATES AND PROTEINS

<table>
<thead>
<tr>
<th>Cook</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>12</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of wood, g.</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
<tr>
<td>Length of cook, hr.</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Biocel (on wood), %</td>
<td>10.7</td>
<td>10.7</td>
<td>10.7</td>
<td>10.7</td>
<td>9.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Yield holocellulose (on wood), %</td>
<td>73.5</td>
<td>73.5</td>
<td>73.6</td>
<td>75.7</td>
<td>81.6</td>
<td>84.7</td>
</tr>
<tr>
<td>Glucosyl lignin in holocellulose, %</td>
<td>1.00</td>
<td>1.05</td>
<td>1.26</td>
<td>1.84</td>
<td>3.10</td>
<td>5.30</td>
</tr>
<tr>
<td>Yield lignin-free holocellulose (on wood), %</td>
<td>72.4</td>
<td>73.4</td>
<td>72.7</td>
<td>71.3</td>
<td>73.1</td>
<td>79.7</td>
</tr>
<tr>
<td>Trionic anhydride, holocellulose, %</td>
<td>6.2</td>
<td>6.5</td>
<td>6.6</td>
<td>--</td>
<td>6.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Corrected pentosans in holocellulose, %</td>
<td>10.6</td>
<td>11.9</td>
<td>12.1</td>
<td>--</td>
<td>11.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Yield alcohol-insoluble fraction (on wood), %</td>
<td>7.9</td>
<td>8.8</td>
<td>10.3</td>
<td>5.9</td>
<td>9.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Yield reducing sugars (on alcohol-insoluble fraction), %</td>
<td>56.2</td>
<td>55.0</td>
<td>47.7</td>
<td>50.0</td>
<td>34.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Yield reducing sugars (on wood), %</td>
<td>4.4</td>
<td>3.2</td>
<td>4.5</td>
<td>2.8</td>
<td>1.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Trionic anhydride (alcohol-insoluble fraction), %</td>
<td>17.6</td>
<td>12.0</td>
<td>17.1</td>
<td>17.3</td>
<td>19.4</td>
<td>17.6</td>
</tr>
<tr>
<td>Pentosans (alcohol-insoluble fraction), %</td>
<td>3.6</td>
<td>3.9</td>
<td>3.4</td>
<td>9.7</td>
<td>6.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Corrected pentosans (alcohol-insoluble fraction), %</td>
<td>4.2</td>
<td>4.8</td>
<td>3.6</td>
<td>4.0</td>
<td>0</td>
<td>1.7</td>
</tr>
<tr>
<td>Theoretical aldehyde acids (alcohol-insoluble fraction), %</td>
<td>12.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>23.2</td>
</tr>
</tbody>
</table>

*All figures are on an oven-dry basis

*b) Wet water-extracted wood

b) Differential fermentation results

### B-DISTRIBUTION OF CARBOHYDRATE FRACTIONS

<table>
<thead>
<tr>
<th></th>
<th>8-1</th>
<th>16-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction sugars</td>
<td>48.3</td>
<td>20.3</td>
</tr>
<tr>
<td>Total pentosans</td>
<td>18.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Acid-insoluble resins</td>
<td>46.2</td>
<td>16.8</td>
</tr>
<tr>
<td>Calculated aldehyde acids</td>
<td>13.4</td>
<td>33.2</td>
</tr>
<tr>
<td>Ash</td>
<td>6.8</td>
<td>12.3</td>
</tr>
<tr>
<td>Total</td>
<td>66.7</td>
<td>87.1</td>
</tr>
</tbody>
</table>

*All carbohydrate materials are calculated as polysaccharides, rather than as simple sugars*
As indicated in Table XI, the theoretical yield of lignin-free holocellulose is 72.1%. This value was obtained in Cokes 8 and 10 and only slightly more than this value in Cokes 9 and 12. In all four cokes, the conditions were identical. With milder conditions (Cokes 15 and 16), the yields of lignin-free holocellulose were progressively larger. Thus, it is apparent that the term, "lignin-free" holocellulose should not be used; the present work indicates that the true holocellulose residue of an impure holocellulose fraction cannot be determined by subtracting the Elnson lignin content from the latter value. It is quite possible that, after wood has been treated with sodium chlorite, it is no longer amenable to the Elnson lignin determination and that results obtained by this method will be low. It is probable that chlorite treatment renders the residual lignin in the impure holocellulose soluble in the Elnson lignin treatment. This view has been expounded by Joyce and Finck (24).

It is interesting to note that, in each coke, there is an apparent increase in the amount of uronic anhydride during the cooking process. In view of the results of James and Isbell (28), it is unlikely that such material is actually produced by oxidation during the process. The uronic anhydride content of spruce wood is 4.0%, whereas the uronic anhydride contents of the holocelluloses vary from 4.6 to 5.9% (based on the wood) from Coke 8 to Coke 16. In addition, the uronic anhydride of the alcohol-insoluble fractions must be considered. Paradoxically, the holocellulose produced under the mildest cooking conditions gave the highest yield of uronic anhydride.
It is known that organic carboxyl groups other than uronic acid carboxyl groups will evolve carbon dioxide (though not quantitatively) under the conditions of the uronic acid determination. For example, oxidized lignin remaining in the holocellulose may yield carbon dioxide, giving an erroneously high uronic anhydride result. This may explain why the holocellulose of Cook 16 has an apparent uronic anhydride content much higher than that of Cook 3.

The presence of carboxyl groups other than uronic acids in the alcohol-insoluble fractions of the cooks may also explain the discrepancy between the corrected pentosan contents and the pentosan content determined by fermentation. If the uronic anhydride content of these fractions is erroneously high, the pentosan correction will be too large and the resulting pentosan value will be too low. This is most evident in 8-1, where the corrected pentosan value is less than that found by fermentation, and in 15-1 and 16-1, where the corrected pentosan values are zero, even though it is known that some pentosan material is present.

The yields of these holocelluloses are not strictly comparable with those of Jayne and Benke (19), inasmuch as the methods of cooking are different. All their cooks were made in either three or four stages and at a lower temperature (60° C.). Their conditions are listed briefly in Table XIII.

The advantage of a single-stage, higher temperature chlorite oxidation is that the yield of holocellulose with a given lignin content is higher. In the case of single-stage cooks as performed in this
TABLE XIII

SUMMARY OF JAYE AND BARKER'S COOKS

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stages</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Cooking time, hr.</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Total NaClO₂ (on wood), g</td>
<td>120</td>
<td>90</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>False lignin in holocellulose, g</td>
<td>2.0</td>
<td>3.1</td>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Yield of lignin-free holocellulose (on wood), g</td>
<td>69.2</td>
<td>71.8</td>
<td>71.6</td>
<td>69.3</td>
</tr>
<tr>
<td>Theoretical yield of holocellulose (on wood), g</td>
<td>72.1</td>
<td>72.1</td>
<td>72.1</td>
<td>72.8</td>
</tr>
</tbody>
</table>

work with 107.5 sodium chlorite (based on wood), "lignin-free" holocelluloses are obtained with yields of 72.4 to 74.3. The Elason lignin contents of the crude holocellulose fractions vary from 1.25 to 1.843. For Jaye and Barker's cooks with 120.5 sodium chlorite, crude holocellulose fractions with Elason lignin contents of 1.1 to 2.03 are obtained, with corresponding "lignin-free" holocellulose yields of 69.23.

A comparison of chlorite cooks on sprucewood with those on slash pine in Barton's work (25) indicates that there will be a greater loss of material from pine when cooking to a given lignin content in the holocellulose. Barton's cooking conditions are presumably identical with those of Cooks 8, 9, and 10 in this work, so that the comparisons are valid. The condensed data are given in Table XIV.

It is obvious that lower yields of lignin-free holocellulose are obtained with higher residual lignin contents in the holocellulose from slash pine. In cooking to a given lignin content in the holocellulose, less holocellulose will be recovered from pine than from spruce.
Table XIV

Summary of Holocellulose Fractions of Cocos by Brand*  

<table>
<thead>
<tr>
<th>Cook</th>
<th>7</th>
<th>8</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of holocellulose (on wood), $%$</td>
<td>72.9</td>
<td>73.2</td>
<td>72.7</td>
</tr>
<tr>
<td>Lignin in holocellulose, $%$</td>
<td>2.2</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Yield of lignin-free holocellulose (on wood), $%$</td>
<td>71.3</td>
<td>71.4</td>
<td>70.8</td>
</tr>
<tr>
<td>Theoretical yield of holocellulose (on wood), $%$</td>
<td>72.4</td>
<td>72.4</td>
<td>72.4</td>
</tr>
</tbody>
</table>

*All figures are on an oven-dry basis

The major portions of the two carbohydrate fractions, 8-1 and 16-1, have been identified and summarized in Table XIII. In each case, about 67% of the total material may tentatively be accounted for. The fact that less than 100% may be accounted for is probably due in part to the destruction of some reducing sugars on acid hydrolysis. The values for aldehydic acids must not be considered more accurate than possibly approximately 2 to 3; several assumptions and corrections have entered into their calculations.

General Consideration of Results

The different theories for the origin of the carbohydrate materials isolated from the chlorite liquors have been discussed previously. The work on Cooks 15 and 16 with mild cooking conditions has indicated that, even under conditions yielding a "lignin-free" holocellulose far in excess of theoretical, there is still an appreciable carbohydrate fraction that may be isolated. This disproves the idea that drastic cooking conditions alone were responsible for the carbohydrates in the liquor.
Another possibility is that lignin may be oxidized by sodium chlorite to a carbohydrate-like material. If lignin is formed in the living tree by reduction of carbohydrate materials, as has been frequently suggested (68), the process may conceivably be reversed and carbohydrate materials may be formed from lignin by oxidation. Barton (25) has shown that, under proper conditions, vanillin may be oxidized with sodium chlorite to 2,4-furandicarboxylic acid. This process has opened the benzene ring without complete destruction of the molecule. It is conceivable that, during the chlorite cooking of wood, lignin is partially oxidized to carbohydrates or generically related materials.

To test this hypothesis, a known lignin product, Indulin "A" (hereafter referred to as "Indulin"), an isolated kraft lignin of the West Virginia Pulp andPaper Company, was subjected to a chlorite oxida-
tion. Barton had performed a similar oxidation to see whether Indulin would produce oxidation products similar to those from wood. In general, this was so; fractions were separated from the Indulin chlorite liquor that were quite similar to those from slash pine chlorite liquor.

Experimental Work

Barton’s general method of cooking was followed with a few modifications. Five grams of Indulin and 15 grams of sodium chlorite were added to two liters of water at 75-80° C.; usually a few drops of acetic acid were necessary to start the reaction. Once started, the reaction proceeded spontaneously and the pH dropped rapidly, so that it was necessary to add about five grams of sodium acetate to act as a buffer; under these conditions, the pH at the end of the cook was about
and the liquor remained clear. If the pH dropped much lower, there was a precipitation of organic materials from the solution.

Three such additions of Indulin and sodium chlorite were made per cock—a total of 15 grams of Indulin and 45 grams of sodium chlorite. Usually 10 to 15 minutes were required for each of the three stages of the cook; the criterion of finality was the disappearance of all solid Indulin in each stage. Seven such cooks were made, and the resultant liquors were combined and treated in a manner analogous to that for spruce chlorite liquor. This method is given in the flowsheet in Figure 10.

The analytical results of the Indulin work are summarized in Table IV.

<table>
<thead>
<tr>
<th>Indulin</th>
<th>Methoxyl, %</th>
<th>Sulfur, %</th>
<th>Ash, %</th>
<th>Klason Lignin, %</th>
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</thead>
<tbody>
<tr>
<td>Yield, g.</td>
<td>Yield (based on Indulin), %</td>
<td>Methoxyl, %</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>12.0</td>
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<td>3.25</td>
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<td>2</td>
<td>5.5</td>
<td>5.2</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17.5</td>
<td>16.7</td>
<td>10.60</td>
<td></td>
</tr>
</tbody>
</table>

*Data by Barlow.*

All data are on an oven-dry basis.

105 grams of Indulin were oxidized.

Hydrolysis of Indulin-1

One gram of Indulin-1, the alcohol-insoluble fraction, was hydrolyzed 24 hours with 50 ml. of 25% sulfuric acid, giving an insoluble residue of 25.0%. The dark solution was neutralized with barium
Indulin-A

Digested with NaClO₂

Chlorite liquor

Concentrated to 3 liters and electrodialyzed

Purified liquor

Concentrated to 2 liters and precipitated into ethanol, washed with ethanol, ether, and dried

Alcohol soluble

Evaporated to dryness, triturated with dioxane, centrifuged

Dioxane-insoluble (Indulin-2)

Solution

Dehydrated, centrifuged, and precipitated into ether, washed with ether, petroleum ether, dried

Lignin (Indulin-3)

Alcohol insoluble (Indulin-1)

FIGURE 10

TREATMENT

Treatment of Indulin Chlorite Liquor
carbonate, filtered, and clarified by the following procedure: 85 ml. of the filtered hydrolysate were treated with 10 ml. of saturated neutral lead acetate solution and the mixture was allowed to stand for 10 minutes. The precipitate was filtered on a Whatman No. 1 filter paper, washed with small amounts of distilled water, and solid sodium acetate was added to the filtrate to precipitate the excess lead. The precipitate was filtered on a Whatman No. 50 filter paper, and the filtrate diluted to 50 ml. Previous tests had indicated that this method would remove the dark colored constituents of the hydrolysate without a corresponding removal of reducing sugars. A clarification was essential in this Indulin-1 hydrolysate to remove any lignin degradation products which might have reducing power in the Hansen-Julicher procedure.

The clarified hydrolysate, analyzed for reducing sugars by the Hansen-Julicher method, gave a reducing value of 3.23 (calculated as glucose) based on Indulin-1, or 0.43 based on the original Indulin. This is a very small amount, and could conceivably be caused by contamination of the Indulin by polysaccharide materials that ultimately were isolated in the Indulin-1 fraction. To test this, 10 grams of Indulin were hydrolyzed for five hours with 500 ml. of 23 sulfuric acid, and the hydrolysate was neutralized with barium carbonate, filtered, clarified with neutral lead acetate, and tested for reducing power by the Hansen-Julicher method. The reducing value of this hydrolysate was 0.853 (calculated as glucose) based on the Indulin. Thus, twice as much reducing material was present in the Indulin before chlorite oxidation as could be isolated from Indulin-1 after the oxidation.
Although it was obvious that the oxidation of Indulin did not produce reducing sugars, nevertheless the possibility remained that nonreducing carbohydrate materials were produced by such an oxidation. Indulin-1 was primarily nonreducing, yet its other characteristics were remarkably similar to those of the alcohol-insoluble fractions from spruce chlorite liquors. The solubility characteristics and methoxyl contents were similar. The lignin-free yield of Indulin-1 was 9.0% of the Klason lignin of the original Indulin. The yields of the nonreducing fractions of C-1 and C-16 were 5.7 and 7.1%, respectively, of the Klason lignin in the original wood. These yields, although not identical, were of the same order of magnitude and suggest that there is a similarity between Indulin-1 and the nonreducing portions (tentatively identified as chloric acids) of C-1 and C-16.

Indulin-1 was obviously composed of more than one material, so that direct analysis seemed inadvisable. Consequently, an attempt was made to prepare a barium salt to determine whether such a salt would have any relationship to the barium salt isolated from C-1. The remaining 11 grams of Indulin-1 were hydrolyzed 24 hours with 500 ml. of 95% sulfuric acid, filtered, neutralized with barium carbonate, filtered, concentrated in vacuo, decolorized with activated carbon, and precipitated into alcohol. Only 60 mg. of a white substance could be isolated; analysis showed the absence of barium, indicating that this was not a salt; further analyses were not attempted. Unfortunately, no Indulin-1 was available for further work.

It is thought that the decolorisation step with carbon removed the main body of material which would have been precipitated by the
alcohol. Although this material would have been colored, in contrast to the twice-purified berisium salt from spruce which was white, the analytical relationship between the two might have been fairly close. This point must remain unsettled for the present; it is to be hoped that future work will establish the identity of Indulin-1 more closely.

It is evident that no reducing sugars are formed by the oxidation of Indulin, although the question of the production of nonreducing carbohydrates is unsettled. The results of this work must not be considered as final or conclusive for the following reasons:

1. Indulin is a condensed lignin that has undergone a drastic treatment during the Kraft pulping process which might render it highly resistant to further oxidation. Native lignin might be more amenable to oxidation.

2. The conditions employed for oxidizing Indulin are somewhat less drastic than the cooking conditions employed for spruce wood. The total cooking time for Indulin never exceeded 45 minutes, whereas a minimum of three hours was employed for the spruce cooks. It is conceivable that considerable oxidation might take place after all the Indulin has gone into solution so that, after three to four hours, carbohydrate-type materials (reducing or nonreducing) could be formed. Most probably the latter type would be formed; even after the possible formation of reducing sugars, the aldehyde group would likely be oxidized to carboxyl.

On the basis of this work, it must be concluded that reducing carbohydrate materials are not produced from Indulin by chlorite oxidation under the conditions described.
In general, the salient features of Barton's work have been reproduced and confirmed. Chlorite lignin fractions have been isolated from sprucewood that are quite similar in physical and chemical properties to those isolated from slash pine. In both cases, it has been shown that these fractions contain no materials that give reducing tests. By contrast, Joyce and Hanke, not having attempted a physical separation of these materials, indicated that the reducing sugars were chemically combined with the lignin in the chlorite liquor.

The application of Barton's treatment of pine chlorite liquor to spruce chlorite liquor gives a lower yield of chlorite lignin. Different methods had to be devised to obtain yields of lignin that were comparable with Barton's. The yields of Chlorite Lignins A and B vary from 40 to 50 grams in his work; by using a different technique, these two materials are isolated as a single fraction with yields from 32 to 37 grams. It is probable that Barton did not extract all the lignin from the liquor.

Overall, it would seem that the method developed in this study for the treatment of the chlorite liquor is somewhat more direct. The carbohydrates and lignins may be separated into two large fractions which may be subsequently fractionated further. In this treatment, with the exception of the materials lost by electrodialysis, all
the materials present in the liquor may be accounted for. This lost material is of low-molecular weight, and the evidence indicates that only small amounts are lost. The electrodialysis serves the purpose of removing most of the inorganic constituents, thus yielding purer carbohydrate materials than could be obtained otherwise.

SIGNIFICANCE OF ALCOHOL-INSOLUBLE FRACTION

This work has revealed the presence of hitherto unsuspected quantities of carbohydrate materials in the liquor from a sodium chlorite oxidation of wood. The yield of such material is a function of cooking conditions and, the more drastic the cook, the more material isolated. This is indicated by Table XII-A; the lower the lignin content of the holocellulose, the higher the yield of the alcohol-insoluble fraction. Thus, 76% of 8-1 is conceived to be carbohydrate (both reducing and nonreducing), or 6.0% based on the original wood; 56% of 16-1 is carbohydrate, or 3.4% of the wood. In either case, it is surprising to find such a large amount of material; Cook 8 gave a holocellulose in approximately theoretical yield, whereas the yield of the holocellulose from Cook 16 was about 8% above the theoretical.

Regardless of the exact nature of this material, it is evident that appreciable amounts of carbohydrate materials are removed from wood by chlorite oxidation well before the theoretical holocellulose stage is reached. This finding questions the validity of the sodium chlorite method for producing quantitative yields of holocellulose; it can no longer be considered a means of sharp differentiation between lignin and carbohydrates.
The origin of the carbohydrates in the alcohol-insoluble fractions is undetermined and questionable at present. There are at least three major possibilities that may be considered:

1. Carbohydrates are an oxidation product of lignin.

2. They are a part of the cell-wall carbohydrates, such as the celluloses and true cellulose.

3. They are part of the incrusted polyuronides associated with lignin.

The first possibility has been investigated and discussed in connection with the oxidation of Indulin; on the basis of the data presented, the possibility of the production of reducing carbohydrates by this method may be discarded.

The second possibility must remain incompletely answered. There is no evidence, for example, that true cellulose itself has been degraded enough to go into solution in the chlorite liquor; no glucose could be identified in this work and all evidence indicates that there is none. Small amounts of celluloses, however, appear to be present in the liquors. Norman (65) states that, in softwoods, xylan and arabinan are the principal celluloses. The yields of arabinan in Cooke 8 and 16 are 1.4 and 0.45 of the original wood, respectively. Uise (66) has indicated that there is a loss of arabinan during the isolation of holocellulose by the chlorite method. In the case of black spruce, this loss amounts to 1.9% of the wood; as indicated above, 1.4% may be recovered from the chlorite liquor. The rest may be lost in the
electrodialysis. The yields of xylan are even smaller, 0.16% of the wood from Cook 3 and probably zero for Cook 16. Regardless of the presence of xylose, the identification of xarose indicates the presence of cellulose in both 3-1 and 16-1.

Arabinose and galactose may be present in these fractions as a part of the material that could be removed by exhaustive extraction of sprucewood with hot water. No such extraction has been made in this work, so that, in the case of Cook 16, where the wood had been extracted for 4.5 hours with hot water previous to the chlorite cook, both arabinose and galactose have been identified in appreciable quantities. It is possible that these sugars could have been removed by more exhaustive extraction. At best, though, it seems that only a small part of the total alcohol-insoluble material can be attributed to such materials.

Apparently the incrusting polyuronides are the source of the major portion of these fractions. Norman (129, 63) has given abundant evidence that lignin removal is difficult without the corresponding removal of some polyuronide hemicellulose material and vice versa. It is commonly accepted that the two are, at least in part, chemically bound to one another. In view of the high uronic acid content of the carbohydrate fractions, it is plausible to assume that the major source of these fractions is the incrusting polyuronides.

PROJECT STATUS OF DTH CHLORITE UNIDO

In view of the results of this work and the findings of Joyce and Perks, some of the previous concepts of the chlorite process must
be discarded or revised. In the first place, the quantitative concept of the cooking with sodium chlorite is erroneous, and the summations previously calculated have been fortuitous in adding up to 100%. The idea that sodium chlorite solution removes only lignin in the earlier stages of the process is likewise erroneous, as can be seen from the results of Cook 16. Here a "lignin-free" holocellulose is obtained in a yield of nearly 80%—nearly 60% above the theoretical—yet a fraction containing about 3% carbohydrate material (based on wood) is obtained from the chlorite liquor.

It seems obvious that some carbohydrate material must be removed simultaneously with the lignin. Whether this takes place immediately at the start of the cook or whether it begins at a certain stage of lignin removal cannot be determined at present. By the time the residual lignin in the holocellulose is decreased to 6%, some carbohydrate is removed. The amount of such material removed does not seem to increase proportionately with further cooking.

There is little doubt that the alcohol-insoluble fractions isolated must consist in part of a lignin-carbohydrate complex. That lignin-like material is present is proven by the acid-insoluble residue produced on hydrolysis, with a methoxyl content of 9 to 10%, and a characteristic lignin ultraviolet absorption curve. The extraction of the fractions with alcohol and acetic fails to remove this lignin, indicating a definite chemical bond.

With milder cooking conditions, the lignin content of the alcohol-insoluble fractions increases. Evidently, the longer the cook,
the greater is the degree of hydrolysis of the alcohol-insoluble fraction, and subsequently the lower the lignin content of the material. This suggests that, in a chlorite cook, the main body of the lignin carbohydrate complex is removed nearly intact and that subsequent hydrolysis may take place in solution.
SUMMARY AND CONCLUSIONS

1. A new method has been developed for the treatment of chlorite liquors. In this method, the lignin and carbohydrate materials are isolated essentially in two main fractions.

2. An alcohol-insoluble fraction has been isolated from the chlorite liquor that yields large amounts of mannose, galactose, and uronic acids, with smaller amounts of arabinose and xylose. An appreciable part of these fractions consists of nonreducing, carbohydrate-like materials, presumably aldonic acids.

3. Within the range of the experimental cooking conditions employed in this work, the carbohydrate fractions may be isolated in varying amounts from all chlorite liquors, regardless of the cooking conditions.

4. In view of the fact that carbohydrate materials may be isolated from chlorite liquors produced under widely varying cooking conditions, the sodium chlorite method for the isolation of holocellulose may no longer be considered a method for the quantitative separation of lignin and true holocellulose. Sodium chlorite holocellulose may be considered an approximation of the true holocellulose content of wood.
### APPENDIX

#### ANALYTICAL DATA FOR CODE 4

<table>
<thead>
<tr>
<th>Code</th>
<th>Fraction</th>
<th>Yield, g.</th>
<th>Ash, %</th>
<th>NfO, %</th>
<th>C, %</th>
<th>H, %</th>
<th>N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-1</td>
<td>Dioxane insolubles</td>
<td>10.7</td>
<td>70.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4-2</td>
<td>Dioxane insolubles</td>
<td>8.0</td>
<td>--</td>
<td>4.52</td>
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<tr>
<td>4-3</td>
<td>Lignin</td>
<td>15.5</td>
<td>--</td>
<td>10.82</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
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<td>Dioxane insolubles</td>
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<td>--</td>
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<tr>
<td>4-5</td>
<td>Lignin</td>
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<td>0.63</td>
<td>12.34</td>
<td>54.4</td>
<td>4.63</td>
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</table>

Eelson lignin content of holocellulose, % 2.05

#### ANALYTICAL DATA FOR CODE 5

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<th>Ash, %</th>
<th>NfO, %</th>
<th>C, %</th>
<th>H, %</th>
<th>N, %</th>
<th>Cl, %</th>
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<td>5-1</td>
<td>Carbohydrate</td>
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<td>Lignin</td>
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<td>Dioxane insolubles</td>
<td>5.1</td>
<td>6.3</td>
<td>9.68</td>
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<td>Lignin</td>
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<td>0.69</td>
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<td>Lignin</td>
<td>11.5</td>
<td>0.96</td>
<td>11.18</td>
<td>51.8</td>
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<td>8.27</td>
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<tr>
<td>5-7</td>
<td>Carbohydrate</td>
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<td>8.1</td>
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<td>--</td>
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Eelson lignin content of Holocellulose, % 1.85

#### ANALYTICAL DATA FOR CODE 6

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<th>Ash, %</th>
<th>NfO, %</th>
<th>C, %</th>
<th>H, %</th>
<th>N, %</th>
<th>Cl, %</th>
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Eelson lignin content of holocellulose, % 1.31

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<td>--</td>
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<td>0.93</td>
<td>12.66</td>
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<td>5.70</td>
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<td>Yield, %</td>
<td>Acid, %</td>
<td>Lignin, %</td>
<td>Uronic Acid, %</td>
<td>Corrected Pentosan, %</td>
<td>A.A.</td>
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<td>Cook 10</td>
<td>Holocellulose</td>
<td>1325</td>
<td>--</td>
<td>1.24</td>
<td>6.63</td>
<td>12.1</td>
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<tr>
<td>10-1</td>
<td>Alcohol Insoluble</td>
<td>126</td>
<td>12.6</td>
<td>2.98</td>
<td>--</td>
<td>17.1</td>
<td>3.8</td>
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<td>10-2</td>
<td>Dioxane Insoluble</td>
<td>12.3</td>
<td>6.40</td>
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<tr>
<td>10-3</td>
<td>Lignin</td>
<td>32</td>
<td>11.3</td>
<td>--</td>
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<tr>
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<td>1.84</td>
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<tr>
<td>12-1</td>
<td>Alcohol Insoluble</td>
<td>100</td>
<td>5.3</td>
<td>--</td>
<td>--</td>
<td>17.4</td>
<td>4.0</td>
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<tr>
<td>Cook 13</td>
<td>Holocellulose</td>
<td>1360</td>
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<tr>
<td>Cook 14</td>
<td>Holocellulose</td>
<td>1482</td>
<td>--</td>
<td>6.00</td>
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<td>Cook 15</td>
<td>Holocellulose</td>
<td>1470</td>
<td>--</td>
<td>3.03</td>
<td>6.50</td>
<td>11.0</td>
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<tr>
<td>15-1</td>
<td>Alcohol Insoluble</td>
<td>100</td>
<td>7.5</td>
<td>4.62</td>
<td>--</td>
<td>19.4</td>
<td>0</td>
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<td>Cook 16</td>
<td>Holocellulose</td>
<td>1525</td>
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<td>5.90</td>
<td>7.04</td>
<td>10.6</td>
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<td>16-1</td>
<td>Alcohol Insoluble</td>
<td>106</td>
<td>12.3</td>
<td>5.00</td>
<td>--</td>
<td>17.6</td>
<td>1.7</td>
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</tr>
</tbody>
</table>
Alcohol-benzene extracted wood

Chlorited and filtered, washed

Chlorite liquor  Holocellulose

Acidized and acidified, centrifuged

Solution  Precipitate

E triturated with dioxane, centrifuged

Precipitate  Solution

Concentrated and dehydrated, centrifuged

Precipitate  Solution

Dissolved in HCl, precipitated in DICH, washed with DICH, petroleum ether, dried

Precipitate (4-2)  Precipitate (4-3)

Precipitated into ether, washed with petroleum ether, dried

E triturated with dioxane

Precipitate  Solution

Precipitated into ether, washed with petroleum ether, dried

Precipitate (4-5)
Alcohol-benzene extracted wood

Chlorite and filtered, washed

Filtrate → Polysaccharide

Concentrated in vacuo

Solution [Insoluble inorganic matter]

Acidified and centrifuged

Solution [Insoluble]

Neutralized to pH 5.5, cooled, acidified to pH 2.7

Solution [Insoluble] → Eriturate with dioxane

Soluble (Solution I) → Insoluble

Precipitate [Cellulose solution]

Dissolved in DMSO

Soluble (Solution II)

Clear, insoluble, washed with EtOH, petroleum ether, dried

Precipitate (5-1)

Solution I (Lignin in dioxane)

Concentrated and dehydrated

Solution [Insoluble] → Dissolved in DMSO

Soluble (Solution II)

Precipitated in ether, etc.

Precipitate (5-2)

Repurified by dioxane treatment

Soluble → Insoluble (5-3)

Precipitated in ether, washed, etc.

Precipitate (5-4)

Repurified by dioxane treatment

Insoluble [EtOH, ether, etc.] → Precipitate (5-6)

Soluble [EtOH, ether, etc.]

Precipitated in ether, washed, etc.

Precipitate (5-5)
Alcohol-benzene extracted wood

Chlorited and filtered, washed

Filtrate Holocellulose

Concentrated to 2 liters

Solution Insoluble inorganic matter

Dialyzed

Purified solution

Evaporated to dryness, dissolved in LML, precipitated into EtOH

Solution Precipitate

Evaporated to dryness, dissolved in LML, precipitated into EtOH, washed with EtOH, ether, etc.

Washed with EtOH, ether, etc., dried

Precipitate (6-2)

Precipitate (6-1)

Evaporated to dryness, dissolved in LML, precipitated into EtOH, washed with EtOH, ether, etc.

Precipitate (6-3)
Alcohol-benzene extracted wood

Chlorited and filtered, washed

Filtrate

Dilucrose

Concentrated to 4 liters

Solution Insoluble inorganic materials

\[ \text{Aliquot portion} \rightarrow \text{Electrodialyzed} \rightarrow \text{Purified solution} \]

Acidified and centrifuged

Precipitate Solution

Insoluble Solution

Etriturated with dioxene, centrifuged

Soluble Insoluble

Rehydrated and centrifuged

Solution Insoluble

Precipitated in ether, washed, etc.

Precipitate (7-3)

Precipitate (7-2)

Dissolved in DCH, precipitated in DCH, washed, etc.

Precipitate (7-1)

Precipitated in DCH, washed, etc.

Solution

Solution

Precipitate (7-4)

Dissolved to dryness, dissolved in DCH, precipitated in DCH, washed, etc.

Precipitate (7-5)

Alcohol solubles

Precipitate
APPENDIX

Ultraviolet Absorption Spectrum
Chlorite Lignin (4-5)
APPENDIX

Conductimetric Titration
Chlorite Lignin (7-3)

0.2243 g.

15.00 ml. 0.1132N NaOH Added

0.0499N HCl added, ml.
CALCULATIONS FROM CONDUCTIMETRIC TITRATION OF
CHLORITE LIGNIN

This experiment attempted to determine the amount of titratable acidic groups on chlorite lignin. Barton (26) indicated the presence of two hydroxyl and two carboxyl groups per unit weight of 600 for chlorite lignin.

Assuming that two phenolic hydroxyl groups are present that may be titrated with alkali, then the carboxyl content (calculated as carbon dioxide) of this chlorite lignin is 1.28%. If these hydroxyl groups are not titratable, then the carboxyl content is 15.9%. The theoretical carboxyl content of chlorite lignin (calculated as carbon dioxide) with two carboxyl groups per unit weight of 600 is 14.7%.
2. Schmidt, Erich, and Duyse, Franz, Ber. 54B:3241-3244(1921).


35. Schmidt, Erich, and Atterer, Matthies, Ber. 60B:1671-1679(1927).


43a. Hägglund, Erik, and Brett, L. G., Degier-Tabr. 34:100-103 (1936).


