THE ISOLATION AND ANALYSIS OF THE HEMICELLULOSES
OBTAINED FROM LEMON WOOD

by

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1938

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THE ISOLATION AND ANALYSIS OF THE HEMICELLULOSES OBTAINED FROM LEMON WOOD

INTRODUCTION

(1) Schulze (1890) applied the name "hemicellulose" to that class of carbohydrates soluble in alkali occurring in the plant cell distinct from cellulose. The identifying characteristics of the class are: solubility in dilute alkali, precipitation in alcohol or acid solution, and ease of hydrolysis in the presence of hot dilute acids. This classification has been generally adopted, but many substances that are not true hemicelluloses show characteristics indicative of hemicelluloses. Mechanical treatment may so alter pure cellulose that its behavior becomes similar to that of a true hemicellulose as defined by Schulze.

Until the last decade the hemicelluloses of Schulze were believed to be true pentosans or hexosans, but the presence of sugar acids of the uronic type in hemicelluloses has now been found to be the rule rather than the exception. It is significant that the sugar units commonly found in hemicelluloses from plants are of two distinct groups: (1) The glucose series consisting of d-glucose, d-glucuronic acid, and d-xylose; (2) The galactose series consisting of d-galactose, d-galacturonic acid, and l-arabinose. The combinations usually found, therefore, are gluco-xylans containing glucuronic acid, and (2) galacto-arabans containing galacturonic acid. Hawley and
Norman in 1932 classified hemicelluloses as (a) those substances very intimately associated with the cellulose and never containing a uronic acid, and (b) those incrusting substances not closely associated with the cellulose, and containing in practically all cases a uronic acid. The term "cellulosan" has been applied to hemicelluloses which are strictly pentosans, hexosans, or mixtures of the two. Gandlin and Schryver have applied the term "polyuronide" to those hemicelluloses whose molecule consists of a uronic acid conjugated with sugars. This latter term must not be applied to hemicelluloses alone, as pectins and plant gums also contain uronic acids. Both classes of hemicelluloses are characteristic in their solubility in alkali and their insolubility in alcohol and acid solutions.

Norman (1932), in an attempt to summarize the chemistry and nomenclature of hemicelluloses, has set a criterion for a true hemicellulose. He defines a hemicellulose as that cell-wall polysaccharide which may be extracted from plant tissues with hot or cold dilute alkalies, but not with water and which may be hydrolyzed to constituent sugars and sugar acids by boiling with hot dilute mineral acids. This definition would exclude those water soluble substances that bear great similarity to hemicelluloses. He does not, however, exclude hemicellulose preparations originally extracted with alkali and which may later be partially water soluble. Apparently all that is demanded is initial water insolubility.
The fact should be born in mind that the process of solution may render a change in the chemical nature of a hemicellulose and hence lead to water solubility of the polysaccharide. An alkali extract as ordinarily employed for the preparation of hemicelluloses is likely to contain compounds from two sources—the cell wall incrusting material, and the material intimately associated with the cellulose itself (the Cross and Bevan cellulose fraction). The fact that a mixture is obtained is not particularly serious, because hemicellulose as isolated from wood or straw has never been considered a single chemical compound. O'Dwyer brought about a separation of a crude hemicellulose preparation into two fractions, which differed slightly in proportions of sugars and uronic acids, by precipitating first with acid and then with alcohol. Norman points out that the dual source of hemicellulose has been insufficiently appreciated by most workers. The cellulosan fraction is composed of one or not more than two sugar units and contains carboxyl groups in only small amounts. The incrusting hemicelluloses are found to contain uronic acid in conjugation with other sugars. Emphasis is placed on the fact that all polyuronides are not hemicelluloses, because pectins, gums, and mucilages also contain uronic acids and may be similarly classified. Norman applies the term "polyuronide hemicelluloses" where distinction is necessary.

The following table presents his method of differentiation.
THE DIFFERENTIATION OF HEMICELLULOSES

HEMICELLULOSES

Extracted by dilute alkalis
Hydrolysed by hot dilute acids
Giving hexoses and pentoses and often uronic acids

not associated with cellulosic fraction

associated with natural cellulose

containing uronic acid

not containing uronic acid

not containing uronic acid

associated with lignin?

POLYoses

POLYURONIDES

CELLULOSANS

HEXOSANS
PENTOSANS
HEXO-PENTOSANS

RESERVES?

Mannan,
Araban,
Xylan, &c.

'CYTO-URONIDES' ENCRUSTING SUBSTANCE

Pentose + uronic acid
Hexitose + uronic acid
Pentose + hexose + uronic acid

CELLULOSIC FRAMEWORK SUBSTANCE

Xylan,
Mannan.
Glucosan?
The lack of definition of hemicelluloses, together with difficulty of isolation and fractionation, renders a rigorous systematization almost impossible.

In addition to carboxyl and sugar groups, ether linked methoxyl groups have been found in a number of hemicelluloses. Anderson and co-workers have found methoxylated hexuronic acids present in every hemicellulose isolated from the wood of a number of trees. The source of the methoxyl group has been considered by O'Dwyer. Her conclusions excluded pectic materials as a source of methoxyl, since the sodium hydroxide used in the extraction of hemicelluloses would bring about a de-esterification of methoxyl linked to those substances.

In addition, procedures are taken to remove the greater portion of pectic material by use of ammonia and N/20 hydrochloric acid, previous to extraction of hemicelluloses. The possibility of lignin as a source of methoxyl groups is not to be overlooked since a soluble form of lignin, known as "metalignin", would be extracted with the sodium hydroxide. The metalignin, however, is soluble in alcohol and would be removed from any hemicellulose preparation on thorough washing with that reagent. In view of these facts, O'Dwyer assumes that the hemicellulose itself is the source of the methoxyl groups.

A number of explanations have been offered as to the mode of formation and purpose of the hemicellulose portion of a plant. Schulze considered hemicellulose to be an in-
intermediate in the formation of cellulose. Von Fehlenburg and Ehrlich suggest the possibility of a transition in the plant from pectic substances to lignin. O'Dwyer indicates that hemicelluloses may be more akin to pectin than to cellulose, and that they might even bear some relation to lignin. This she supports with a study of the relationship of pectin and hemicellulose content of plants as lignification proceeds. She showed that pectin was present in greater amounts than hemicellulose in unli gnified tissues, and was present only in traces in lignified material. Norman admits the theory is attractive but adds that it is difficult to see from a chemical standpoint how the transformation could take place. He advances a suggestion in ramification of the theory of Tollens and de Cnalmont that primary alcohol groups in terminal positions undergo oxidation to form a series of conjugated uronic acids which on de-carboxylation yield pentoses. This theory offers an explanation of a mechanism whereby cellulose is converted to a hemicellulose. It is evident that such a transformation would result in the association of d-glucose, d-glucuronic acid, and d-xylose and likewise in the association of d-galactose, d-galacturonic acid, and l-arabinose. Significantly, that is found to be the case. Anderson is of the opinion that starch or dextrin may undergo oxidation and result in the formation of hemicellulose on loss of carbon dioxide.

The hemicelluloses were formerly known as reserve
celluloses, the theory being that they were stored against periods of great metabolic activity. Certain investigators believe that pentosans do not serve as reserve materials, while others postulate that they may serve as food after complete consumption of the more readily utilized carbohydrates.

The author has desired to present only the salient topics in this discussion on the history and nature of hemicellulose. A more complete review will be found in the theses of Nutter, Seigle, and Kinsman.
PRELIMINARY TREATMENT OF WOOD

The lemon wood used in this study was obtained from the vicinity of Santa Paula, California. The preparation and analysis of the sawdust was carried out by J. C. Fruin in 1932. The results of analytical methods described by Schorger are given in Table I.
TABLE I *

Analysis of lemon wood sawdust

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>4.95%</td>
</tr>
<tr>
<td>Ash</td>
<td>1.46%</td>
</tr>
<tr>
<td>Cold water solubility</td>
<td>6.69%</td>
</tr>
<tr>
<td>Hot water solubility</td>
<td>9.55%</td>
</tr>
<tr>
<td>Hot water solubility corrected for cold water solubility</td>
<td>2.86%</td>
</tr>
<tr>
<td>1 percent NaOH solubility</td>
<td>21.99%</td>
</tr>
<tr>
<td>10 percent KOH solubility corrected for NaOH solubility</td>
<td>12.44%</td>
</tr>
<tr>
<td>10 percent KOH solubility</td>
<td>31.86%</td>
</tr>
<tr>
<td>Ether solubility</td>
<td>1.05%</td>
</tr>
<tr>
<td>Total solubility in the above solvents</td>
<td>32.73%</td>
</tr>
</tbody>
</table>

* The preliminary treatment of the wood and the analysis of the sawdust were carried out by J. C. Fruin in 1950. The results are not corrected for ash in solubility determinations.
A total of 2000 grams of lemon wood were weighed out and divided equally among four six-liter flasks. In order to free the wood of terpenes and lipoids, it was extracted repeatedly with boiling acetone under reflux, until, on filtering, the acetone was free of any color. The same treatment was carried out using eighty-five percent alcohol. The wood was then covered with water and held at ninety-seven degrees centigrade for a period of two hours, filtered, and subjected to the same treatment a second and third time. Seigle found that boiling water removed a great portion of starch present in the wood. When preliminary treatment was completed, the sawdust was a very light yellow in color. It was washed thoroughly on a Buchner funnel and placed in four six-liter flasks for further treatment.
EXTRACTION OF HEMICELLULOSE BEFORE CHLORINATION

The wood was next treated for a period of four days with five-percent sodium hydroxide in the cold in order to extract the hemicelluloses. The wood was filtered and again subject­ed to extraction with sodium hydroxide for the same length of time. The two extracts were united and reserved for further treatment.
REMOVAL OF PECTIC MATERIAL

The wood, after the sodium hydroxide extraction, was washed with dilute hydrochloric acid and then with water, in order to insure removal of all sodium hydroxide. The wood was covered with N/20 hydrochloric acid and placed in a boiling water bath for a period of three hours. The liquid was filtered off, and the wood was washed thoroughly with water. Pectic materials were removed by extraction with cold five-percent ammonium hydroxide. According to (27) Anderson, the treatment with N/20 hydrochloric acid removes the calcium from calcium pectate and leaves the pectic acid to be dissolved later by the ammonium hydroxide. The pectin was precipitated by use of alcohol and acid. A discussion of the pectic materials in lemon wood will be found in the theses of Seigle and Marteny.
CHLORINATION OF THE WOOD

The wood was washed thoroughly with distilled water and placed in four six-liter flasks. Sufficient water was added to cover the pulp and the mixture acidified with hydrochloric acid. The flasks were then placed on a shaking device and chlorine gas passed into the pulp suspension. Due to danger of oxidation of cellulose, chlorination of the wood continued for only four hours. The wood was filtered and placed under reflux with alcohol. After refluxing for an hour, the mixture was again filtered and the sawdust thoroughly washed with alcohol to effect removal of chlorination products.

Norman and Shilkrande offer evidence for a linkage (30) between polyuronide hemicelluloses and lignin. The process of chlorination renders lignin soluble, due to substitution (31) and oxidation. Fremy and Terrell developed a quantitative determination for cellulose on the observation that chlorination rendered the incrusting substances soluble in dilute alkali and alcohol. The presence of moisture is absolutely essential since dry lignocellulose does not react with (32) chlorine even when heated to eighty degrees. The furfural-producing carbohydrates are found to be unchanged by chlorination, as has been shown by the furfural yields from
a fiber before and after chlorination. Heuser and Sieber, in a study of the action of chlorine on spruce wood, found that up to the time of complete removal of lignin, 31.1 percent of chlorine was obtained as hydrochloric acid, while only 9.47 percent had combined with the lignin. From these facts they concluded that the action of chlorine on lignin is one of oxidation. A formation of oxy-cel lulose was found on prolonging the chlorination period for twenty-two hours, but as long as any lignin remained, oxy-cel lulose was not produced. The lignon chloride formed during chlorination is soluble in alcohol, and, hence, was removed by alcohol washings.
REMOVAL OF PECTIC MATERIALS AFTER CHLORINATION

The sawdust was returned to the six-liter flasks and extracted two times with cold five-percent ammonium hydrox­ide for periods of two days. This extraction removed pectic materials and any lignon chloride left undissolved by the alcohol treatment. The removal of any lignon chloride at this point was decidedly advantageous, for it enabled a purer subsequent extraction of the hemicellulose. Any lignon chloride left in the pulp after the pectin extract­ion would dissolve in the five-percent sodium hydroxide solution and result in contamination of the hemicellulose. Following the above treatment, the wood was filtered on a Buchner funnel and washed thoroughly with water.
EXTRACTION OF THE HEMICELLULOBSES AFTER CHLORINATION

The sawdust was allowed to stand for three days with cold five-percent sodium hydroxide. At the end of this period, the mixture was filtered and the wood again extracted for three days with sodium hydroxide. For ease of manipulation, only three liters of alkali solution were used in the treatment of five hundred grams of wood. Both extracts were a deep brown in color, due probably to the presence of lignin and other coloring matter. The sawdust was washed free of alkali, dried, and set aside.
The sodium hydroxide extracts made before and after chlorination of the wood were subjected to a number of purification measures. In both cases the extracts were made slightly acid with hydrochloric acid and warmed to approximately forty degrees. On testing the acidified solution with iodine, a distinct blue color, indicative of starch, was observed. One gram of Taka-diastase was added to each three-liter portion of acidified extract and the mixture maintained at forty degrees for twenty-four hours. This same treatment was continued for another twenty-four hours, as the extracts made before chlorination showed a slight starch test. Further treatment for the removal of starch was thought inadvisable due to danger of hydrolysis of the hemicellulose.

At this time it was thought best to carry out a precipitation of the hemicellulose, disregarding any attempt at fractionation. A number of ten-cubic centimeter portions of the hemicellulose solutions were acidified with varying amounts of hydrochloric acid. The acidified mixtures were allowed to stand over night and observed the next day. Varying amounts of precipitate were observed in all cases. In determining the optimum precipitation, consideration was
made to the amount and nature of precipitated material. In some cases the precipitated material was too colloidal to be effectively filtered, while in other cases an easily filterable precipitate was formed. Estimation of the yields was made in all cases, and one precipitation seemed to be ideal. A p.H. of the solution was determined with a quinhydrone electrode and found to be 1.7. All subsequent precipitations were carried out in solutions of this acidity.

The precipitation and fractionation of hemicelluloses has been the subject of considerable discussion. Buston raised a question as to the system of classification of hemicelluloses. Angel and Norris emphasize the fact that systematic designations indicate, not definite classes of hemicellulose, but particular methods of preparation. They point out that any one fraction does not represent a particular hemicellulose of definite chemical entity. The fractionation is very largely physical, and it is probable that the fractions obtained are more or less mixtures. Angel and Norris found hemicellulose from maize cobs to precipitate best at a p.H. of 4, but in their work on hop flowers they observed that ideal precipitation occurred at different p.H. values depending on the particular fraction being precipitated. This would seem to indicate that the optimum p.H. of precipitation was characteristic of each fraction. The hemicelluloses in this study seemed to show ideal precipitation at the same p.H. throughout the
fractionation.

The solutions of hemicellulose before and after chlorination were acidified to a pH of 1.7 with hydrochloric acid, using thymol sulphone pthalein as an indicator. Angel and Norris used acetic acid in this acidification, but the use of that reagent results in the formation of a buffer system. The sodium acetate and acetic acid present so control the pH of the solution that the addition of a large quantity of acetic acid is necessary. In light of this fact, the use of dilute hydrochloric was thought to be preferable. After acidification, three volumes of eighty-five percent ethanol were added and the hemicellulose allowed to settle out for twenty-four hours. The supernatant liquid was siphoned off and the precipitated hemicelluloses centrifuged out, filtered, and dried. The hemicelluloses before and after chlorination were redissolved separately in five percent sodium hydroxide solution and the solutions filtered until clear. Both solutions were slightly acidified with hydrochloric acid in preparation for bromination. Liquid bromine was added in sufficient quantities to color the solutions a light brown. Successive additions of this reagent were required as it was rapidly used in the purification. Bromine behaves toward lignin in the same way that chlorine does but less energetically. Any lignin that dissolved with the five percent alkali was thus combined
with bromine to form bromolignin, imparting a dark red color to the solution. The bromination was carried out for a period of twenty-four hours. At the end of this time the acidity was increased to a pH of 1.7 and the hemicellulose allowed to precipitate over night. The fraction precipitating at this point was termed 'A' in case of the hemicellulose extracted before chlorination of the wood, and 'C' in case of the hemicellulose extracted after chlorination of the wood. The hemicellulose in each case was centrifuged out, washed thoroughly with ninety-five percent alcohol, filtered, and dessicated over calcium chloride. The resulting preparations were fine white powders. Two volumes of alcohol were then added to the solution containing hemicellulose that did not precipitate on acidification. The solution was allowed to stand over night and the supernatant liquid siphoned off. The fractions on being washed, filtered, and dried were termed 'B' and 'D'. 'B' indicating the hemicellulose extracted before chlorination of the wood, and 'D' the fraction after chlorination of the wood. Another volume of alcohol was added to the solutions from which these fractions were obtained; and no further precipitation was evident, indicating that all of the hemicelluloses had been recovered.
ALANYSIS OF HEMICELULOSES

The complete analysis of the hemicelluloses required a number of different determinations. Moisture and ash determinations were made in all cases since it was necessary to know the amount of pure hemicellulose present in any sample.

The percentage of moisture was determined by drying weighed samples in an oven at 100 degrees for twenty-four hours.

Ash percentages were determined by ignition at a low red heat. The ash was undoubtedly sodium chloride formed when the hemicelluloses were precipitated.

The determination of hexuronic acid was one of greatest importance. The estimation of uronic acid is dependent upon the fact that a molecule of uronic acid gives quantitatively one molecule of carbon dioxide upon being heated in boiling twelve percent hydrochloric acid. The percent of carbon dioxide was determined by the method of Lefevre and Toliens. Essentially the determination is as follows: A weighed sample of material is heated in boiling twelve percent hydrochloric acid. The apparatus is so arranged that a stream of carbon dioxide-free air sweeps the evolved carbon dioxide through a vertical column containing a known volume of standard baryta water. The columns are filled with glass
beads in order to bring about intimate contact between the carbon dioxide and baryta water. Anderson has shown that uronic acid does not start to decompose until the hydrochloric acid has boiled for five minutes or longer. Furthermore, he has shown that only carbonates and similar matter will evolve carbon dioxide in the cold. The determination is continued for four hours. At the close of this period, the baryta solution is titrated with standard hydrochloric acid, using phenythalein as an indicator. The percent of carbon dioxide is given by the following formula:

\[
\%CO_2 = 100 \times \frac{(\text{Blank} - \text{Titration}) \times .022 \times \text{Normality of Acid}}{\text{Weight of pure sample}}
\]

Where, \text{Titration} = Number of cubic centimeters of standard hydrochloric acid required to neutralize excess barium hydroxide.

\text{Blank} = Number of cubic centimeters of standard hydrochloric acid required to neutralize the barium hydroxide when determination is carried out without sample present.

The difference between blank and titration gives the amount of hydrochloric acid equivalent to carbon dioxide evolved.

A more complete and thorough description of the apparatus and technique involved has been given by Krznarich.

Pentosan material was determined using the phloroglucinol method which is as follows: A weighed sample of material is placed in a distilling flask and covered with one hundred cubic centimeters of twelve percent hydrochloric acid. The flask is connected to a condenser and the acid
brought to a boil. The rate of boiling is so adjusted that thirty cubic centimeters of distillate are collected every ten minutes. Thirty cubic centimeters of twelve percent hydrochloric acid are added to the distilling flask every ten minutes in order to keep the volume in the flask constant. Distillation is allowed to continue until four hundred and twenty cubic centimeters of distillate have been collected. At this time fifty cubic centimeters of phloroglucinol reagent are added to the distillate and the solution thoroughly stirred. The phloroglucinol reagent is made up by dissolving ten grams of phloroglucinol in 300 cubic centimeters of twelve percent hydrochloric acid and adding sufficient water to make a total volume of fifteen hundred cubic centimeters. The distillate, with phloroglucinol added, is allowed to stand with occasional stirring for approximately thirty-six hours. Standing seems to bring about a coagulation of the furfural-phloroglucinol precipitate, and leads to greater ease in filtering. The precipitates are filtered on previously prepared Gooch crucibles, dried, and weighed.

The action of boiling twelve percent hydrochloric acid on pentoses results in a nearly quantitative production of furfural. A molecule of pentose loses three molecules of water and is converted to furfural. This fact is the basis of the pentosan determination. Furfural distills over and is collected and precipitated with the phloroglucinol reagent.
The relation of the weight of furfural-phenol to weight of pentose material can be found in Krober's tables. Since the yield of furfural is dependent upon the particular pentose present, it is required to know what pentose is present. Hydrolysis of the hemicellulose showed xylose to be the sugar present and the results were calculated on that basis. No methyl pentosan was found in any of the fractions.

The Zeisel method was used in determination of the methoxyl content. Weighed samples of hemicellulose are heated at 150-140 degrees with hydriodic acid in a stream of carbon dioxide. The methyl iodide formed is swept through the apparatus and absorbed in an alcoholic solution of silver nitrate. The alcoholic solution is evaporated on a water bath and the precipitated silver iodide filtered on a previously prepared Gooch crucible. The percent methoxyl is calculated using the following relationship:

\[
\text{percent methoxyl} = 100 \times \frac{31 \times \text{wt. of silver iodide}}{254.8 \times \text{wt. of pure hemicellulose}}
\]

The determination is very accurate, but great care must be taken in its performance. The samples must be thoroughly dried in vacuo as the presence of ethanol will put the results in error. Seigle noted a methoxyl content of 1.38 percent in starch from lemon wood, when he used the method of Zeisel; yet the qualitative test of von Fehlenberg and Deniges was negative. The presence of ethanol used in precipitation was concluded to have
caused the contradiction. The starch had been dried over boiling toluene in an Abderhalden drier for one hundred hours. This gives some indication of the difficulty involved in removing interfering substances. The hydriodic acid used must be of high quality. "Merck's Blue Label" reagent is found to give satisfactory results. The acid should be prepared for use in the following manner: One hundred cubic centimeters of acid are added to a distilling flask and distilled from a glycerine bath. The first and last twenty-five cubic centimeter portions of distillate being discarded, the remaining portion is retained for use in the analysis.
TABLE I

<table>
<thead>
<tr>
<th>Hemicellulose</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>C₁</th>
<th>C₂</th>
<th>D</th>
<th>D₁</th>
<th>D₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Dioxide</td>
<td>2.01%</td>
<td>3.37%</td>
<td>2.03%</td>
<td>1.89%</td>
<td>2.17%</td>
<td>2.96%</td>
<td>2.54%</td>
<td>3.11%</td>
</tr>
<tr>
<td>Methoxylated Uronic Acid</td>
<td>9.52%</td>
<td>15.93%</td>
<td>9.56%</td>
<td>8.95%</td>
<td>10.22%</td>
<td>13.98%</td>
<td>11.98%</td>
<td>14.70%</td>
</tr>
<tr>
<td>Xylan</td>
<td>90.39%</td>
<td>84.02%</td>
<td>90.79%</td>
<td>90.56%</td>
<td>89.36%</td>
<td>86.45%</td>
<td>90.00%</td>
<td>85.46%</td>
</tr>
<tr>
<td>Total</td>
<td>99.91%</td>
<td>99.95%</td>
<td>100.37%</td>
<td>99.51%</td>
<td>99.58%</td>
<td>100.43%</td>
<td>101.98%</td>
<td>100.16%</td>
</tr>
<tr>
<td>Methoxal</td>
<td>1.43%</td>
<td>2.38%</td>
<td>1.57%</td>
<td>___</td>
<td>___</td>
<td>1.39%</td>
<td>1.66%</td>
<td>1.66%</td>
</tr>
<tr>
<td>[α]₀</td>
<td>-86.1°</td>
<td>-61.08°</td>
<td>-85.43°</td>
<td>-75.92°</td>
<td>-85.99°</td>
<td>-77.70°</td>
<td>-82.26°</td>
<td>-75.91°</td>
</tr>
</tbody>
</table>

Results of Hemicellulose Analyses Corrected for Moisture and Ash
TABLE II

<table>
<thead>
<tr>
<th>Hemicellulose</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>C₁</th>
<th>C₂</th>
<th>D</th>
<th>D₁</th>
<th>D₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivalent Weight from Uronic Acid</td>
<td>2189</td>
<td>1306</td>
<td>2171</td>
<td>2322</td>
<td>2031</td>
<td>1489</td>
<td>1736</td>
<td>1415</td>
</tr>
<tr>
<td>Mols of Xylose Per Mol of Uronic Acid</td>
<td>15</td>
<td>8.31</td>
<td>14.78</td>
<td>16.02</td>
<td>13.8</td>
<td>9.70</td>
<td>11.57</td>
<td>9.14</td>
</tr>
<tr>
<td>Theoretical Mol Wt.</td>
<td>2188</td>
<td>1264</td>
<td>2188</td>
<td>2420</td>
<td>2056</td>
<td>1528</td>
<td>1792</td>
<td>1396</td>
</tr>
</tbody>
</table>

Table of Equivalent Weights
DISCUSSION OF THE ANALYSES

The uronic acid content indicates that all of the hemicellulose fractions are of the polyuronide type. Analysis indicates that one uronic acid is linked to a chain of from eight to sixteen xylose molecules. The equivalent weights, calculated from the percent of methoxylated uronic acid, vary from 1306 to 1322. There seems to be two hemicelluloses present in the fractions obtained before and after chlorination, one of high equivalent weight and one of low equivalent weight. Fraction 'B' and 'C' show an equivalent weight of 1306 and 1322, respectively, and all other fractions seem to be a mixture of these two hemicelluloses. Surprising agreement is found in the equivalent weights of the more insoluble fractions isolated before and after chlorination. Uronic acid determinations show that hemicellulose 'A' has an equivalent weight of 2189 and that 'C' has an equivalent weight of 2171. The equivalent weights of the more soluble hemicelluloses isolated before and after chlorination are not found to agree so closely. Fractions 'B' and 'D' show equivalent weights of 1306 and 1489 respectively. From methoxyl determinations it was concluded that each uronic acid contains an ether linked methoxyl group. In all cases the percent of methoxylated uronic acid and pentosan approximate very closely
100 percent, indicating that the hemicelluloses are quite pure. Reda and Westerbeke, in studies on the hemicelluloses found in Black locust heartwood and White Birch, have isolated fractions that seem to be of the same nature as those found in this investigation.
HYDROLYSIS AND SEPARATION OF HYDROLYTIC PRODUCTS

Seventy grams of starch-free hemicellulose were mixed with a liter of four percent sulfuric acid and boiled under reflux for sixteen hours. The solution was neutralized with barium hydroxide and barium carbonate. Bone charcoal was added and the solution filtered. A thick syrup resulted on concentrating the solution in vacuo. This syrup was diluted with a small amount of water and poured into absolute alcohol. Barium salt 'A' precipitated at this point and was filtered out. The filtrate was concentrated in vacuo and the sugar crystallized out in alcohol and filtered. This filtrate was concentrated in vacuo and a further precipitation of barium salt occurred on the addition of absolute alcohol. This barium salt was termed 'B'. It was redissolved in water, filtered, and barium salt 'B₁' precipitated by the addition of alcohol. Barium salt 'B₂' was obtained from the filtrate on concentrating in vacuo and adding alcohol.
ANALYSIS OF THE BARIUM SALTS

The barium salts 'A', 'B_1', and 'B_2' were dried over phosphorous pentoxide for several days in preparation for analysis.

The determination of uronic acid content was made by the method of Leevre and Tollens as used previously. The percent carbon dioxide shows that the hydrolysis resulted in a mixture of methylated uronic acid sugar complexes, consisting of one methylated uronic acid and from one to two xylose molecules. According to theory, a molecule of complex containing one molecule of methylated uronic acid and two molecules of xylose should yield 8.15 percent carbon dioxide, and a molecule of complex containing one methylated uronic acid and one molecule of xylose should yield 10.79 percent carbon dioxide. Barium salt 'A' gave a yield of 8.21 percent carbon dioxide, barium salt 'B_1' a yield of 9.56 percent carbon dioxide, and barium salt 'B_2' a yield of 10.82 percent carbon dioxide. From these data, it is evident that fraction 'A' consists of two molecules of sugar and one molecule of methylated uronic acid; fraction 'B_2' consists of one molecule of sugar and one molecule of methylated uronic acid, and fraction 'B_1' represents a mixture of salts 'A' and 'B_2'.
IDENTIFICATION OF THE SUGAR FROM THE HYDROLYSIS

The sugar obtained from the hydrolysis of the hemi-cellulose was washed with acetic acid and a little absolute alcohol and dried over calcium chloride for several days. Its specific rotation was determined as -19.3 degrees, indicating that the sugar was xylose, which in the pure form has a specific rotation of -18.5 degrees.
SUMMARY

The results of this study may be summarized as follows:

1. A number of hemicellulose fractions have been isolated from lemon wood.

2. The fractions seem to be a mixture of two distinct hemicelluloses.

3. Additional amounts of hemicellulose can be extracted after treatment of the wood with chlorine gas.

4. Analysis shows that the hemicelluloses extracted before and after chlorination of the wood are of about the same composition.

5. Removal of lignin from hemicellulose can be effected by treatment with bromine.

6. The hemicelluloses treated in this study bear great similarity to those isolated from other woods.
BIBLIOGRAPHY

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46. Van der Haar, *opus citatum*, p. 82.


