THE ISOLATION AND ANALYSIS OF HEMICELLULOSES AND PECTIC MATERIALS FROM LEAVES OF CORN, ZEA MAYS

by

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A Thesis submitted to the faculty of the Department of Chemistry in partial fulfillment of the requirements for the degree of Master of Science in the Graduate College University of Arizona 1941

Approved: Ernest Anderson
Director of Thesis

Date: May 20, 1941
To the faculty of the University of Arizona

I hereby certify that this paper is the product of my own research and that I have not used the work of others without proper acknowledgment.

Signature:

Date:

[Stamp: University of Arizona Library]
The author wishes to express his sincere appreciation and gratitude to Dr. Ernest Anderson for his constant advice, assistance, and encouragement throughout this investigation.
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THE ISOLATION AND ANALYSIS OF HEMICELLULOSES AND PECTIC MATERIALS FROM LEAVES OF CORN, ZEA MAYS

INTRODUCTION

The term hemicelluloses has been given to a large class of polysaccharides which fill to a certain extent the wide gap between cellulose on the one hand and starch on the other. They are closely related to the gums, plant mucilages, and pectic materials.

Pringsheim (1) points out that the literature on the subject is a mass of inaccurate and partly self-contradictory statements. The nomenclature is unsatisfactory and the classifications are often obscure. Norman (2) says that the chemistry of the hemicelluloses remains to be written. It may be seen that the structural complexities and physical properties of the substances included in this group make their examination, separation, and purification difficult and uncertain.

Norman (3), Pringsheim (4), and Shorger (5) agree that the term hemicellulose is an unfortunate one. However, until more is known, it cannot be easily dispensed with. The term was introduced by E. Schultze in 1892. His definition has been modified by more modern writers. We now think of hemicelluloses as those materials which
may be extracted from plant tissues by treatment with dilute alkalies, either hot or cold, but not with water, and which may be hydrolysed to the constituent simple sugars and sugar acids by heating with dilute mineral acids (6).

Hemicelluloses have been divided into the polyuronide and non-polyuronide types depending upon whether the molecule contains uronic acid groupings. It is now believed that hemicelluloses of the latter type are rare, except in the form of cellulosans. Norman (7) and Anderson (8) point out that many hemicelluloses which have been reported as true hexosans or pentosans have revealed, upon re-examination, uronic acid groupings in the molecule. Norman (9) points out further that all polyuronides are not hemicelluloses since pectin, gums, and mucilages contain uronic acid units.

In considering the polysaccharides from the aspect of function, Onslow (10) lists the following types: First, those which form the structural cell-wall, i.e. cellulosans, hemicellulosans, and pectic substances. Second, those which form reserves of carbohydrates, i.e. starch and inulin. Third, there are 'degradation products' of the cell-wall, the gums and mucilages.

The pectic materials have been more extensively studied than the other polyuronides. Branfoot (11) lists an extensive bibliography of this work in her
monograph. Onslow (12) points out that the literature on the subject is confusing owing to the different methods employed in extraction, the impure state of the products obtained, and the changes brought about by the treatment during extraction.

Branfoot (13) considers the following points concerning the pectic materials to be supported by satisfactory evidence:

"1. An insoluble pectic compound occurs in unripe fruits and other plant tissues, and is generally described as pectose or protopectin. 2. Pectin is a neutral methyl ester of pectic acid. Between pectin and pectic acid are intermediate forms which exhibit increasing acidic properties with a decrease in their content of methyl alcohol. These intermediate forms are classed together as pectinic acids to distinguish them from true pectin. 3. Pectic Acid may be regarded as the basal molecule of pectin, and is a complex galacturonic acid combined with arabinose and galactose. In pectin, the carboxyl groups of the galacturonic acid are replaced by methyl alcohol, and both methyl alcohol groups and carboxyl groups are present in pectinic acids. 4. Calcium pectate is a definite chemical compound with an ash content found by experiment to be 7.62 per cent of calcium."

Protopectin is the mother substance of the group, and gives rise, by hydrolysis, to the other substances.

Fruits of all types contain large quantities of pectic materials. Schorger (14) states that only small amounts of pectin exist in wood. Anderson (15) found that the sap-wood of black locust contains less than three per cent and the cambium layer less than thirteen
per cent pectic materials. He also suggests that the water-insoluble pectic material in wood seems to be present largely as a calcium salt.

Gums and mucilages belong to an ill-defined group which has long been known to be predominately carbohydrate. No clear line of demarcation exists between the two. Since from the uronic acids present they contain free carboxyl groups, Norman (16) suggests that they may be classed as 'acidic polyuronides', along with the pectic materials, and thus distinguished in this way from the polyuronide hemicelluloses which are usually considered to be 'neutral polyuronides.' Onslow (17) considers both gums and mucilages as abnormal products of the cell-wall, the former being associated with injury. Other workers class the mucilages as normal constituents of plants.

The chemistry of lignin seems to be even more obscure and controversial than that of the other members of this group lying between cellulose and starch. Since drastic measures are necessary in its extraction, the lignin examined is probably quite different from that in the plant cell-wall. Armstrong and Armstrong (18) point out that lignified tissues contain relatively large amounts of hemicelluloses and lignins, but only traces of pectin. However, non-lignified tissues contain large amounts of pectic materials, small amounts of
hemicelluloses, and no lignin.

The polysaccharides that have been defined are of great importance since they make up part of the cell-wall of all plants. The constituents of the cell-wall vary with the age and function of the tissue. Of these constituents, we are most interested in the condensation products of the sugars. Onslow (19) says that the cell-wall may be composed of a least six sugars of the pentose and hexose type, together with other derivatives, and these units may be condensed by different linkages in varying proportions. While cellulose is the chief representative, other constituents include modified cellulose, hemicelluloses, and various lignified and un lignified materials.

Purified cotton cellulose is glucose polysaccharide. If this material is treated with seventeen and one half per cent sodium hydroxide in the cold (20), alpha-cellulose remains undissolved. If the strong alkaline extract is acidified, the resulting precipitate is termed beta-cellulose. The fractional part not recovered is called gamma-cellulose. Hydrocellulose is probably a natural stage in the hydrolysis of cellulose. The action of strong oxidizing agents on pure cellulose give rise to an oxidized product of indefinite nature known as oxycellulose.

The natural cellulosic aggregate of most plants
consist only partially of true cellulose. The other constituents are described as cellulosans for convenience. Xylan is the most commonly occurring cellulosan, mannan is well known, araban and others have been found. The hemicelluloses are probably joined to cellulose by an ester linking, the other end of the hemicellulose molecule being joined to lignin, starch, or other material. Anderson (21) points out that both ester and glucosidic unions are probably present in plant materials.

Cellulose, starch, and pectic materials exist, more or less, as the same definite chemical compounds, no matter where they are found. The question then arises as to whether or not the hemicelluloses are alike in different materials, or even in the same material. The evidence is to the contrary. Mutter (22) found at least three distinct hemicelluloses represented in eight fractions of mesquite wood. Anderson, Seeley, Stewart, Redd and Westerbeke (23) found a mixture of hemicelluloses present in the hardwoods investigated. Similarly, Kesselman (24) and Bennett (25) found more than one hemicellulose in white pine. Kaster (26) found mixtures in cottonwood. This also applies to hemicelluloses from sources other than wood. Thus, Anderson, Hechtman, and Seeley (27) found a mixture in cottonseed hulls; and Anderson and Krznarich (28) found that oat hulls give a mixture of hemicelluloses.
It is quite possible that the hemicelluloses are changed somewhat during extraction and purification. However, this cannot be accepted as an explanation for the mixtures found, since mucilages, which are water-soluble and not changed during extraction, exist in plants as mixtures.

Several suggestions have been offered as to the origin of hemicelluloses, pectic materials, and lignin in plants. Anderson (29) suggests that these substances may be formed from carbohydrates by a process of intermolecular oxidation and reduction with a liberation of energy for use by the growing plant. Schorger (30) points out that pentoses are formed from hexoses by oxidation of the terminal primary alcohol group and the splitting out of carbon dioxide from the molecule. Pentosans could result similarly from hexosans with uronic acids as possible intermediate products. Norman (31) notes the significant fact that sugar units found in plant hemicelluloses and pectic materials belong to either the glucose series of d-glucose, d-glucuronic acid and d-xylose, or to the galactose series of d-galactose, d-galacturonic acid and l-arabinose. Haworth (32) also believes that the oxidation-reduction mechanism connects the starches and cellulose with the pentosans. An excellent summary of the various theories concerning the origin of these materials may be found
in chapter seven of Norman.

Doree (33) lists the following steps as being typical in the study of hemicelluloses:

"(a) isolation of a crude product by extraction with alkali.... (b) its purification.... (c) its fractionation.... (d) determination of rotation in alkaline solution. (e) furfural yield, and carbon dioxide yield and calculations of the distribution of the pectin, pentose, and uronic acid constituents. (f) the hydrolysis to carbohydrates, usually with sulfuric acid.... (g) the separation and identification of the individual sugars obtained".

This general procedure was followed in the present investigation.
EXPERIMENTAL

Preparation of Material

Source

The material consisted of leaves of mature corn, *Zea mays*, grown on the University of Arizona Farms near Tucson. The leaves were collected in October, 1939, air-dried, and ground to a fine powder in a Wiley mill.

Analysis

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.32 %</td>
<td>Moisture</td>
</tr>
<tr>
<td>14.96</td>
<td>Ash</td>
</tr>
<tr>
<td>8.00</td>
<td>Protein Nitrogen</td>
</tr>
<tr>
<td>0.32</td>
<td>Fat</td>
</tr>
<tr>
<td>28.58</td>
<td>Crude Fiber</td>
</tr>
<tr>
<td>41.82</td>
<td>Carbohydrate (By difference)</td>
</tr>
<tr>
<td>100.00 %</td>
<td></td>
</tr>
</tbody>
</table>

Preliminary Treatment

Five hundred grams of powdered leaves were placed in each of six six-liter flasks and extracted with acetone four times. The liquid assumed a distinct green color during the first extractions. This color was due to chlorophyll and other plant pigments which are insoluble in water, but soluble in acetone and other organic solvents. (34)

Four extractions were then made in the same way using 85 per cent ethanol. The liquid became dark brown during the first extraction, but gradually lightened to a light straw-color during the subsequent extractions.
Isolation of the Hemicelluloses and Pectic Materials

**Extraction and Precipitation of Water-Soluble Material**

The leaf pulp from three of the flasks was filtered through muslin following the preliminary extractions. The material was washed with distilled water and then refluxed in boiling water for eight hours. The water which had become dark brown was filtered off. This procedure was repeated four times. The extract was tested for starch with negative results. The water-soluble extract was precipitated with three volumes of ethanol.

The precipitate was allowed to settle, and the supernatant liquid was siphoned off. The residue was centrifuged, washed with 95 per cent ethanol, and filtered on a Buchner. When first precipitated, the residue was a light cream color. Upon standing and upon exposure to air, it gradually changed to a dark brown. The precipitate was dried, first on porous clay plates, then in a desiccator. It was finally bottled and weighed.

**Extraction and Precipitation of the Hemicelluloses before Chlorination**

After the water extractions the pulp was placed in 4 per cent sodium hydroxide solution. The liquid having become very dark brown at the end of a week, the pulp was filtered through muslin. The extract was then fil-
tered on a Buchner to remove all foreign matter. The extraction was repeated with fresh sodium hydroxide solution.

The sodium hydroxide extracts were made acidic by the addition of hydrochloric acid. Previous tests had been made upon small portions, and a pH of four was found to be optimum for precipitation. At this acidity a dark brown suspension was formed which could not be removed by centrifuging. Therefore, three volumes of ethanol were added to precipitate completely the hemicelluloses.

The precipitate was treated in precisely the same manner as described under water-soluble material. The light brown powder that resulted was bottled and weighed.

**Extraction and Precipitation of the Pectic Materials before Chlorination**

The leaf pulp was washed nearly free of sodium hydroxide solution and set aside to dry in large evaporating dishes. After drying, it was washed with distilled water and then with .05 normal hydrochloric acid. The pulp was then extracted for two hours with .05 normal hydrochloric acid. At the end of that time it was filtered and washed, first with .05 normal hydrochloric acid and then with distilled water.

The filtrate was precipitated with four volumes of ethanol. The dried precipitate, after treating as above,
was nearly white.

The leaf pulp was then extracted with 4 per cent ammonium hydroxide solution. After two such extractions, the pulp was washed and dried in evaporating dishes prior to chlorination.

The ammonium hydroxide extracts were made acid and precipitated with four volumes of ethanol. After treating the precipitate as above, a dark brown material was obtained.

**Chlorination of the Leaf Pulp**

The leaf pulp that had been drying was moistened with distilled water, neutralized, and then made very slightly acid with dilute hydrochloric acid. The material was filtered through muslin, washed free of salts, and sucked dry on a Buchner funnel.

The pulp was placed in flasks, covered with distilled water, placed in a mechanical shaker, and chlorine gas was passed into the solution for one and a quarter hours. At first the pulp became slightly pink. This color faded in a very short time and no other color change except bleaching was noticed. In about forty-five minutes the material began to foam. At the end of another thirty minutes the degree of foaming indicated that the reaction had proceeded far enough.

The pulp was filtered, washed with distilled water several times, and then with dilute ethanol. It was
then extracted with ethanol for three hours under reflux. The pulp was filtered, washed with ethanol, and then with water.

**Extraction and Precipitation of the Pectic Materials after Chlorination**

The pulp was extracted again with 4 per cent ammonium hydroxide solution. The pectic material was precipitated and dried in exactly the same way as before chlorination. The dry material obtained was light tan in color.

**Extraction and Precipitation of the Hemicelluloses after Chlorination**

The leaf pulp was again extracted twice with four per cent sodium hydroxide solution. The extracts were precipitated and dried as before chlorination.

**Purification of the Hemicelluloses and Pectic Materials**

All of the material obtained by the different extractions was purified in the same way. The hemicelluloses were redissolved separately in fifty times their weight of 4 per cent sodium hydroxide solution. The solution was filtered until perfectly clear and then acidified slightly with hydrochloric acid. Liquid bromine was added in slight excess. The mixture was shaken from time to time, and bromine was added as long as it was used up. After twenty-four hours the excess bromine was destroyed by addition of ethanol. The mixture was acidified to the correct pH, and the purified hemicellu-
loses were precipitated. The hemicelluloses extracted after chlorination were snow white and required no further purification. The hemicelluloses obtained before chlorination were cream colored and it was necessary to brominate them a second time before they became white.

The pectic materials were purified in exactly the same way. An attempt was made to dissolve the material in sodium hydroxide solution. Part of the water-soluble extract and the ammonium hydroxide extract before chlorination dissolved in the sodium hydroxide solution. The pectic material which did not dissolve in sodium hydroxide solution was centrifuged out, made acid, and centrifuged again. It was then dissolved in fifty times its weight of 4 per cent ammonium hydroxide solution.

During the above operation the loss in material was so great that it did not seem wise to brominate it a second time, though the product was still somewhat colored.

**Fractionation of the Hemicelluloses and Pectic Materials**

In order to separate the hemicelluloses into more soluble and less soluble fractions, they were dissolved in fifty times their weight of 3 per cent sodium hydroxide solution, filtered until clear, and made very slightly acid. Three fractions were obtained from the hemicelluloses extracted before chlorination. At a pH of four, a water-insoluble fraction formed and was centri-
fuged out, filtered and dried. It was called Hemicellulose "A". A second fraction ("B₁") was separated by adding three quarters of a volume of ethanol. The remainder of the hemicelluloses obtained before chlorination were then precipitated by addition of five volumes of ethanol. This fraction was known as "B₂".

The more insoluble portion of the hemicelluloses obtained after chlorination was precipitated with one-half of a volume of ethanol. It was called "C₁". The remainder of the hemicelluloses was precipitated as "C₂" by addition of four volumes of ethanol.

The samples of pectic materials were too small to be further fractionated. They were designated as follows:

Pectic Material A — Hydrochloric acid extract.

Pectic Material B₁ — Sodium hydroxide soluble fraction of the ammonium hydroxide extract before chlorination.

Pectic Material B₂ — Ammonium hydroxide soluble fraction of the ammonium hydroxide extract before chlorination.

Pectic Material C — All the pectic material obtained after chlorination of the leaves.

Pectic Material W₁ — Sodium hydroxide fraction of the water extract.

Pectic Material W₂ — Ammonium hydroxide fraction of the water extract.
Analysis of the Hemicelluloses and Pectic Materials

Moisture

Weighed samples of the hemicellulose fractions were dried to constant weight in an oven at 100-105°C. The percent moisture was calculated from the loss in weight.

The entire fractions of pectic materials were dried in the oven, and the moisture-free materials were used in the analyses.

Ash

Weighed samples of the hemicelluloses and pectic materials were ashed. They were heated at a low temperature for fifteen minutes, then at the highest temperature of the Bunsen flame to constant weight. The ashes of all the hemicellulose fractions were white, but those of most of the pectic materials were colored.

In all of the remaining determinations, the actual weights were corrected to a moisture-free and ash-free basis.

Hexose Uronic Acid

The per cent uronic acid in the hemicelluloses was determined by the method of Lefevre and Tollens (35). The details of the determination are given in the thesis of Krznarich (36).
Blanks were first run to determine the amount of carbon dioxide drawn through the apparatus from the atmosphere. Weighed samples were then determined, and the per cent of carbon dioxide calculated from the following formula (37):

$$\% \text{CO}_2 = \frac{100(\text{Blank} - \text{Titration})(0.032)(\text{Normality of acid})}{\text{Weight of pure sample}}$$

where, Blank = Number of cubic centimeters of standard hydrochloric acid required to neutralize the barium hydroxide when the sample is omitted.

and, Titration = Number of cubic centimeters of standard acid required to neutralize the excess barium hydroxide when a weighed sample is used.

The per cent of uronic acid anhydride is obtained by multiplying the per cent of carbon dioxide by four.

**Pentosan**

Methods for determining pentoses and pentosans were first developed by Tollens and his workers (38). They all depend upon the conversion of the pentose sugars into furfural by distilling with strong hydrochloric acid. The furfural was precipitated in this case as the phloroglucide as described in "Methods of Analysis of the Association of Official Agricultural Chemists" (39). The amount of pentosan was determined from Krober's tables (40) from the weight of furfural phloroglucide.
Methoxyl

The uronic acid unit of hemicelluloses often has a methoxyl group substituted in it. In determining the per cent of methoxyl, a semi-micro modification of the Zeisel-Viebock-Schwappach method as described by Bennett (41) was used.

Optical Activity

Weighed samples of hemicelluloses and pectic materials were dissolved in 3 per cent sodium hydroxide and ammonium hydroxide solutions respectively. The rotations given in sodium vapor light at 250°C. were taken. The specific rotations, \([\alpha]_D^{15}\), were calculated from these data.
TABLE I.

YIELD OF PURIFIED HEMICELLULOSES AND PECTIC MATERIALS FROM 1500 g. OF POWDERED LEAF PULP

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield in Grams</th>
<th>Per cent Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemicellulose A</td>
<td>4.8 *</td>
<td>0.320 %</td>
</tr>
<tr>
<td>Hemicellulose B₁</td>
<td>44.6 *</td>
<td>2.973</td>
</tr>
<tr>
<td>Hemicellulose B₂</td>
<td>46.8 *</td>
<td>3.120</td>
</tr>
<tr>
<td>Hemicellulose C₁</td>
<td>3.6</td>
<td>0.240</td>
</tr>
<tr>
<td>Hemicellulose C₂</td>
<td>12.5</td>
<td>0.833</td>
</tr>
<tr>
<td>Pectic Material A</td>
<td>1.4</td>
<td>0.093</td>
</tr>
<tr>
<td>Pectic Material B₁</td>
<td>1.7</td>
<td>0.127</td>
</tr>
<tr>
<td>Pectic Material B₂</td>
<td>3.8</td>
<td>0.254</td>
</tr>
<tr>
<td>Pectic Material C</td>
<td>3.8</td>
<td>0.254</td>
</tr>
<tr>
<td>Pectic Material W₁</td>
<td>2.0</td>
<td>0.133</td>
</tr>
<tr>
<td>Pectic Material W₂</td>
<td>1.5</td>
<td>0.100</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>126.5 g.</strong></td>
<td><strong>8.447 %</strong></td>
</tr>
</tbody>
</table>

* These fractions were brominated twice.
### TABLE II.

**MOISTURE AND ASH DETERMINATIONS**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Moisture</th>
<th>Ash</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemicellulose A</td>
<td>6.12 %</td>
<td>50.86 %</td>
<td>56.98 %</td>
</tr>
<tr>
<td>Hemicellulose B1</td>
<td>9.39</td>
<td>14.00</td>
<td>23.39</td>
</tr>
<tr>
<td>Hemicellulose B2</td>
<td>4.39</td>
<td>4.43</td>
<td>8.82</td>
</tr>
<tr>
<td>Hemicellulose C1</td>
<td>12.55</td>
<td>2.42</td>
<td>14.75</td>
</tr>
<tr>
<td>Hemicellulose C2</td>
<td>8.48</td>
<td>1.45</td>
<td>9.93</td>
</tr>
<tr>
<td>Pectic Material A</td>
<td>----</td>
<td>2.62</td>
<td>2.62</td>
</tr>
<tr>
<td>Pectic Material B1</td>
<td>----</td>
<td>6.57</td>
<td>6.57</td>
</tr>
<tr>
<td>Pectic Material B2</td>
<td>----</td>
<td>2.41</td>
<td>2.41</td>
</tr>
<tr>
<td>Pectic Material C</td>
<td>----</td>
<td>6.19</td>
<td>6.19</td>
</tr>
<tr>
<td>Pectic Material W1</td>
<td>----</td>
<td>3.18</td>
<td>3.18</td>
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<tr>
<td>Pectic Material W2</td>
<td>----</td>
<td>28.03</td>
<td>28.03</td>
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</table>
TABLE III.

SUMMARY OF HEMICELLULOSE ANALYSIS CORRECTED FOR ASH AND MOISTURE

<table>
<thead>
<tr>
<th>Fraction</th>
<th>A</th>
<th>B₁</th>
<th>B₂</th>
<th>C₁</th>
<th>C₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentosan, %</td>
<td>69.70</td>
<td>83.92</td>
<td>79.37</td>
<td>97.37</td>
<td>67.96</td>
</tr>
<tr>
<td>Uronic Anhydride, %</td>
<td>5.19</td>
<td>2.66</td>
<td>6.06</td>
<td>5.55</td>
<td>5.32</td>
</tr>
<tr>
<td>Methoxyl, %</td>
<td>0.98</td>
<td>0.46</td>
<td>0.98</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Hexose, % (By difference)</td>
<td>24.26</td>
<td>13.21</td>
<td>14.12</td>
<td>0.00</td>
<td>26.24</td>
</tr>
<tr>
<td>Rotation, [α]_D</td>
<td>----</td>
<td>-76.86</td>
<td>-51.01</td>
<td>-58.54</td>
<td>-51.09</td>
</tr>
<tr>
<td>Calculated Equivalent Weight</td>
<td>3392</td>
<td>6614</td>
<td>2902</td>
<td>3172</td>
<td>3308</td>
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<tr>
<td>Theoretical Equivalent Weight</td>
<td>3376</td>
<td>6562</td>
<td>2908</td>
<td>3226</td>
<td>3262</td>
</tr>
<tr>
<td>Pentose Units per Hexuronic Acid</td>
<td>17.91</td>
<td>42.05</td>
<td>17.45</td>
<td>23.40</td>
<td>17.03</td>
</tr>
<tr>
<td>Methoxyl per Hexuronic Acid</td>
<td>1.08</td>
<td>0.97</td>
<td>0.92</td>
<td>1.05</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Hemicellulose Molecule Probably Consists of:
(a) Monomethylated
   Hexuronic Acid 1 1 1 1 1
(b) Pentose units 18 42 18 23 17
(c) Hexose units 5 5 2 0 5
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pentosan</th>
<th>Uronic Acid</th>
<th>Methoxyl</th>
<th>Rotation, $[\alpha]_D^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>64.46</td>
<td>26.28</td>
<td>0.60</td>
<td>27.33</td>
</tr>
<tr>
<td>B₁</td>
<td>75.64</td>
<td>12.25</td>
<td>1.14</td>
<td>7.13</td>
</tr>
<tr>
<td>B₂</td>
<td>42.66</td>
<td>38.42</td>
<td>1.82</td>
<td>6.82</td>
</tr>
<tr>
<td>C</td>
<td>71.19</td>
<td>17.84</td>
<td>.86</td>
<td>5.21</td>
</tr>
<tr>
<td>W₁</td>
<td>37.70</td>
<td>15.23</td>
<td>---</td>
<td>40.38</td>
</tr>
<tr>
<td>W₂</td>
<td>18.82</td>
<td>21.37</td>
<td>---</td>
<td>55.91</td>
</tr>
</tbody>
</table>
Discussion of Analysis

The analysis of the hemicelluloses indicates that they are of the polyuronide type. The carbon dioxide determination proves the existence of the hexuronic acid group. The methoxyl determination indicates one methoxyl group per hexuronic acid.

The equivalent weights of the fractions were calculated from the carbon dioxide determination. They varied from 2902 to 6614. These values agreed fairly well with the theoretical equivalent weights. The differences are probably due to experimental error and to the inexactness of the carbon dioxide determination.

The lengths of the hemicellulose molecules vary and are inversely proportional to the solubility, with the exception of fraction A. The abnormally low solubility of fraction A is probably due to the fact that it contains 50.86 per cent ash of which 58.01 per cent is silica. Probably the silicic acid is present as a part of the hemicellulose molecule.

The hemicellulose molecules vary in constitution from 17 to 42 pentoses. In addition Fractions A, B1, B2, and C2 contain from 2 to 5 hexose units.

Since the material was not hydrolysed, the sugars present were not accurately determined. However, all qualitative tests for methyl pentoses (42) were negative while the results pointed toward the existence of xylose.
as the only pentose present. Since the starch tests were negative, the hexoses, which were calculated by difference, may be assumed to be an integral part of the molecule.

The hemicelluloses appear to exist as a mixture. They differ from those found in other sources, but are quite similar to those found in the rind of corn stalks by Scott (43).

The high carbon dioxide content, positive rotation, and relatively low pentosan content prove the existence of pectic materials. However, in view of the criterion set forth by Anderson, Siegle, Krznarich, Richards, and Marteny (44), to the effect that pectic materials containing less than 16.5 per cent carbon dioxide and having specific rotations below plus 200 degrees are contaminated with hemicelluloses, all the fractions of pectic materials contained large amounts of hemicelluloses.
SUMMARY

Hemicelluloses of the polyuronide type were isolated from leaves of corn, *Zea mays*. They are mixtures whose equivalent weights vary from 2902 to 6614. All contain one molecule of a monomethylated hexuronic acid combined with a series of pentose units. Fractions A, B₁, B₂, and C₂ contain in addition from 2 to 5 hexose units. Their solubility, with the exception of fraction A, decreases as the size of the molecule increases. The abnormally low solubility of fraction A is probably due to the fact that it contains 50.86 per cent ash of which 58.01 per cent is silica. Probably the silicic acid is present as a part of the hemicellulose molecule. The hemicelluloses isolated after chlorination of the pulp were similar to those obtained before chlorination except that they contain no silica.

In addition to the hemicelluloses, small quantities of pectic materials were isolated. Analysis indicated that these were contaminated with hemicelluloses.


43. Scott, Donald, Master's Thesis, Univ. of Ariz., (1941)
