# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS OF ANALYSIS</td>
<td>18</td>
</tr>
<tr>
<td>EXPERIMENTAL PART</td>
<td></td>
</tr>
<tr>
<td>(a) Preliminary Treatment of the Seed</td>
<td>21</td>
</tr>
<tr>
<td>(b) Preparation and Properties of the Mixed Mucilage</td>
<td>22</td>
</tr>
<tr>
<td>(c) Fractionation of the Mucilage</td>
<td>24</td>
</tr>
<tr>
<td>(d) Preparation of the Mucilage Acids</td>
<td>27</td>
</tr>
<tr>
<td>(e) Hydrolysis of the Mucilage</td>
<td>31</td>
</tr>
<tr>
<td>(f) Identification of the Sugars</td>
<td>33</td>
</tr>
<tr>
<td>(g) The Barium Salts of the Uronic Acid-Sugar Compounds</td>
<td>34</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>37</td>
</tr>
</tbody>
</table>
The author wishes to express his appreciation and gratitude to Dr. Ernest Anderson for the advice, assistance, and encouragement given during the course of this investigation.
THE COMPOSITION AND STRUCTURE OF THE MUCILAGE FROM WILD INDIAN WHEAT, PLANTAGO FASTIGIATA, T.

INTRODUCTION

Desert Indian Wheat (Plantago fastigiata, T.) grows in certain arid portions of the Southwestern United States. It is high in palatability and is so abundant on some ranges that it is classed as an important spring forage. Thornber ranks the native plantains, or Indian Wheats, next in importance to the alfileria among the winter annuals of Southern and Western Arizona. The Papago and Pima Indians sometimes grind the seed for meal which is used in cooking.

The seed used in this investigation was obtained from Prof. J.J. Thornber of the University of Arizona. It was harvested in the Fall of 1937.

Since other varieties of plantago have some commercial uses, it would be interesting to see if the Indian Wheats could also be used commercially. The purpose of the present investigation was to determine the composition and structure of the mucilage from Indian Wheat and to compare its composition with that of the mucilage from Psylla seed (Plantago psyllium, L).

In 1892, Schultze isolated some carbohydrate fractions from different plants by extraction with dilute alkali and precipitation with acid. He called these substances hemi-celluloses. He defines them as follows:
1. They are insoluble in water in the natural state.

2. They are hydrolyzed by weak acids into sugars.

3. These sugars are usually mixtures of pentoses and hexoses.

4. The hemicelluloses are extracted from the plant by a 4% solution of sodium hydroxide.

5. They are precipitated from the alkaline solution by neutralizing with acid, especially if alcohol is added.

Until recently the hemicelluloses of Schultze were regarded as true hexosans or pentosans, or more commonly hexo- (5) pentosans containing both units. In 1923, O'Dwyer established (6) the presence of a sugar acid in hemicellulose.

Norris and Schryver reported that they obtained small amounts of hemicelluloses by treating various preparations of pectin with alkali. From this fact they assumed that hemicelluloses were associated with pectin.

O'Dwyer extracted a hemicellulose from the American white oak using dilute alkali, and precipitating with acetic acid and alcohol. Analysis gave 51.5% xylose, 18.5% arabinose, and 30% mannann and galactan. Later she isolated two hemicelluloses, A and B, from beech wood. Hemicellulose A was precipitated directly by acidification and gave 11% glucuronic acid; hemicellulose B was precipitated by the addition of alcohol and gave carbon dioxide corresponding to 63% galacturonic acid.
Norman states that the sugar units usually found in the plant hemicelluloses belong to distinct configurational groups and that in purified preparations the appearance of units from both groups is unusual. The two groups are (1) Glucose series: d-glucose, d-glucuronic acid, and d-xylose; Galactose series: d-galactose, d-galacturonic acid, and l-arabinose. Gandlin and Schryver suggested the word polyuronide to describe a group of substances which consist of sugars linked to a uronic acid. Among these are the hemicelluloses, pectins, gums, and mucilages. Gortner groups these in his classification of the carbohydrates:

Polysaccharides or non-sugars
   a. Pentosans
   b. Methyl pentosans
   c. Hexosans
   d. Mixed pentosans
   e. Mixed hexosans
   Gums
   Mucilages
   Hemicelluloses
   Pectins
   Celluloses

Since natural hemicelluloses are formed by the union of hexoses, pentoses, and a uronic acid with the loss of one molecule of water between each unit, it is evident that they are typical polyuronides and have much in common with the other members of this group. In fact, hemicelluloses are more
closely related to pectin than to cellulose as the name would imply.

The pectic substances have been more extensively investigated than either hemicelluloses or gums. Fruits of all types contain large quantities of pectic substances, the presence of which gives jams and preserves their jellying property. The study of pectic substances has been carried on for over one hundred years. In 1825 Braconnot discovered a gelatinous substance in fruit juices, which he called pectin.

Candlin and Schryver give the following general characteristics of substances belonging to the pectin group:

1. They cannot be extracted from tissues by cold water or alkalies, but can be extracted with hot water or with dilute ammonium oxalate (0.5%) solution or solutions of other salts whose anion forms an insoluble calcium salt.

2. They form gels under various conditions.

3. They contain the uronic acid group and therefore can be classed as "polyuronides".

4. They undergo partial decarboxylation on heating with boiling 0.5% sodium hydroxide solution.

The pectic substances are classified as: (1) pectic acid which is synonymous with cyto-pectic acid, (2) pectin which was formerly known as pectinogen, (3) protopectin which is identical with insoluble pectin and pecto-cellulose. These names were approved by the American Chemical Society, which in 1925 held a Pectin Symposium to choose the best fitted
Pectic acid is the basis of all pectic substances. The formula suggested by Nanji, Paton, and Ling, but based largely on results of previous workers, has been widely accepted.

On hydrolysis pectic acid breaks down to give l-arabinose, d-galactose, and d-galacturonic acid. Ehrlich reports three molecules of acetic acid when investigating the pectic acid from beet residues. Fellenberg isolated a pectic acid from beet residues, consisting of eight molecules of galacturonic acid, two of arabinose, one of galactose and one of a methyl pentose: \( (C_5H_9O_4)_2(C_6H_{10}O_5)(C_6H_7O_4\cdot COOH)_8 \cdot 2H_2O \). Fellenberg reported the presence of a methyl pentose because some of the furfural phloroglucide obtained from the pectic acid was soluble in alcohol. However since the Rosenthaler test for a methyl pentose is negative, this alcohol-soluble compound may be due to some decomposition product originating from the pectic acid and not from a methyl pentose. Analyses of the salts of pectic acid which were purified by repeated solution in water and precipitation with alcohol show that four molecules of galacturonic acid occur in the pectic acid molecule. The union is similar to that in polysaccharides, water splitting off between an aldehyde and a hydroxyl group so that a glucosidic union occurs. The reduction of Fehling's solution is so slight that no free aldehyde group can exist. Also it is known that there are four carboxyl groups, and as further hydrolysis of the tetragalacturonic acid does not
increase the acidity of the substance, the four carboxyl groups must be free. Summarizing all the facts that are known about pectic acid, the analysis of the acid shows that besides containing four molecules of galacturonic acid, one molecule of d-galactose, and one molecule of l-arabinose, there are three molecules of acetic acid. All these substances are combined with the loss of eight molecules of water. The acetic acid is present as acetyl groups joined to the molecule through hydroxide groups, but whether these are hydroxyl groups of the carbohydrate or the galacturonic acid has not yet been definitely proven. The four carboxyl groups are free, and there is no free aldehyde group. Nanji, Paton, and Ling, noting these facts suggested the following structural formula for pectic acid.

\[
\begin{align*}
\text{GA} & : \text{galacturonic acid} \\
\text{G} & : \text{galactose} \\
\text{A} & : \text{arabinose}
\end{align*}
\]

The purest preparations of calcium pectate obtained had a calcium content of 7.34% and contained 70.56% galacturonic acid anhydride yielding about 20.0% furfuraldehyde. Assuming their formula for pectic acid to be correct, the theoretical composition of the calcium salt would be as follows: 7.36% calcium, 69.7% galacturonic acid anhydride, and 19.5%
furfuraldehyde. These figures are in fair agreement with many preparations from various sources, and the formula (5) proposed by Nanji, Paton, and Ling has been widely accepted.

The major contradictions to this theory have been made by Baur and Link and Norris and Rosch. Bonner reviewed the evidence that disproves the formula of Nanji, Paton, and Ling. Various workers have obtained various percentages of galacturonic acid in pectic acid. It may vary from 65% to 95% of the total and the cyclic formula does not allow for this great variation.

The units of pectic acid which are dispersed in colloidal solution are greatly elongated. The colloidal micelles are one hundred times or more longer than they are wide. This elongated structure is shown by the fact that when pectic acid is dried under tension in the form of threads, the micelles show a good orientation in the direction of tension. The chain structure of pectic acid can be verified by X-ray analysis, which was so successful in the application to cellulose. Bonner concludes that pectic acid is made up of long chains of galacturonic acid with an occasional galactose and arabinose scattered along the chain.

Pectin includes derivatives of pectic acid in which some or all of the carboxyl groups are esterified by methyl alcohol. Pellenberg, who first discovered the presence of methoxyl groups in pectic compounds, gives the name pectin to the completely esterified form of his pectic acid, namely
the octomethyxy ester. The pectin of Ehrlich from beet residues contains also calcium and magnesium. Norris and Schryver, adopting the formula given by Nanji, Paton, and Ling, determined the methyl alcohol in pectin from turnips, onions, and pea-pods. Their values for methyl alcohol content were variable, but the value was never greater than the value corresponding to three esterfied carboxyl groups. They therefore concluded that three carboxyl groups were esterfied while the fourth was left free to form salts. A similar result was found by Norris for the pectin from both the juice and pulp of the orange, showing the esterification of three carboxyl groups. Onslow concludes that there is possibly some hydrolysis of the esterfied groups thereby giving variable methoxy contents. Norman, after purifying the pectin from lemons by precipitation with iron and later removing the iron, found that the results were in accordance with fully esterfied pectic acid. Pectin therefore should vary in composition according to method of preparation and source, having various ratios between methyl groups and inorganic cations.

The third type of pectic substance known as protopectin is insoluble in water and occurs in the cell wall. Fellenberg considers that the insolubility of protopectin is due to the combination of one or more of the carboxyl groups of pectin, others being methylated, with cellulose residues by condensation with elimination of water. Carre' has put for-
ward a rather similar theory that protopectin is a pectin-cellulose complex in which a variable number of pectin methoxyl groups are replaced by linkage with cellulose. Tutin has questioned the existence of protopectin on the grounds that the retention of pectin in a form apparently insoluble in water is solely due to mechanical difficulties of penetration.

Ehrlich states that the pectic substances stand in some generic relationship to hemicelluloses, different types of gums and plants mucilages.

The term gum is a generic name given to a group of non-crystalline carbohydrates which occur in plants and to a limited extent in animals. Less is known about gums than either of the two foregoing polyuronides. This is largely due to the heterogeneity of the samples. The gums are divided into two groups, water-insoluble and water-soluble. This classification overlaps that of mucilages.

Gum arabic is the most important and best known water-soluble gum. It is used extensively as an adhesive. It occurs naturally as a salt from which the free acid may be obtained by solution in warm water, acidification with hydrochloric acid and precipitation with alcohol. Butler and Gretchuer found that on hydrolysis the arabic acid yielded the following constituents: 28.3% galacto-glucuronic acid, 29.5% galactose, 34.4% arabinose, and 14.2% rhamnose. The ease with which it is hydrolyzed off indicates that the
Arabinose is linked by glucosidic unions to the rest of the molecule. The above figures agree very well with the theory for a molecule consisting of an acid complex of one galactose and one glucuronic acid, to which are attached two galactoses, three arabinoses, and one methyl pentose, with the loss of seven molecules of water. Butler and Cretcher have suggested the following structure for the aldobionic acid in the nucleus.

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{OH} \\
\text{H} & \quad \text{OH} \\
\text{COOH} & \\
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{COH} \\
\text{H} & \quad \text{OH} \\
\text{H} & \quad \text{OH} \\
\text{HO} & \quad \text{CHO} \\
\end{align*}
\]

Although L-arabinose and D-galactose have long been known to be constituents of gum arabic, the presence of rhamnose has not previously been reported, furthermore, it has not been found in some other samples of the gum.

Anderson and Sands, and Anderson and Otis reported that mesquite gum consists of d-glucuronic acid containing one methoxyl group and combined with three molecules of D-galactose and four molecules of l-arabinose. They state that the arabinose must be attached by a loose union since it is completely hydrolyzed off by heating to 80 degrees C for six hours with 3% sulfuric acid. Since the free acid
did not reduce Fehling's solution, there must be either a dicarboxyl union involving at least one molecule of arabinose or a glucosidic union between the aldehyde of the end arabinose and some hydroxyl in the molecule. They suggested that the dicarboxyl union is between the first two molecules of arabinose. The galactose units are more firmly attached in the molecule than the arabinose. Since the different degradation products contained only one free aldehyde, it appears that the three molecules of d-galactose are united in a chain by glucosidic unions. It was proved conclusively that the uronic acid is attached by a glucosidic union involving the aldehyde group of the uronic acid and a hydroxyl group of the galactose. Anderson and Otis suggested the following structure for mesquite gum:

\[
\begin{align*}
&\text{CH}_2\text{O} - \text{H} - \text{OH} - \text{H} - \text{OH} - \text{COOH} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O} \\
&\text{H} \quad \text{H} \quad \text{H} \\
&\text{HC} \quad \text{OH} \quad \text{O}
\end{align*}
\]

Cholla gum contains both soluble and insoluble fractions. Sands and Klass found that the insoluble portion consisted of 11.5% galacturonic acid, 53.2% arabinose, 5.5% rhamnose, and 8.4% galactose.

Butler and Gargether, working on the gum from wild cherry trees identified arabinose, xylose, galactose, mannose, and
(52) glucuronic acid.

Mucilages are complex carbohydrate substances that form slimy liquids with water. The mucilages are normal constituents of certain plants. They can occur in the plant either as membrane mucilage or as cell mucilage. They are present as a semi-solid mass dispersed throughout the cell and may occur in any organ of the plant, in the root (Althea rosea), in the bark (Cinnamomum), in the stem (Malvaceen), in the leaves (Buccu), in the flowers (Malvaceen), in the seed husk (cacao).

The function of mucilages on epidermal surfaces apparently is to reduce transpiration, as in epiphytic plants such as orchids. The plant mucilages favor water absorption by the aerial parts of the plant. Also, they may reduce diffusion through the walls as in aquatic plants and in some cases prevent the penetration of ions of heavy metals. The presence of mucilages on the surface of leaves favors the under-cooling so that they will not freeze so easily. Mucilages covering the surface of seeds, as in Flax, enable the seed to imbibe water and thus hold a moisture supply for the germinating embryo. In certain water plants the mucilages on the surface of seeds prevent too great desiccation of the embryo. In some seed coats, such as mustard seed (Brassica alba), the special mucilage cells are of importance in the imbibition of moisture requisite for germination.
It has been suggested that mucilages are produced by the partial oxidation of the cell wall constituents either as a normal process or under the action of bacteria. Since the mucilages are usable carbohydrates in many plants, they must be regarded as possible reserve forms, although this function is probably minor.

The classifications of mucilages are varied and overlapping. Tschirch classifies them according to their behavior toward a solution of iodine in zinc chloride as cellulose mucilages and true mucilages. The cellulose mucilages (celluloseschleime) give the color reactions of cellulose and on oxidation with nitric acid give only oxalic acid. The true mucilages are colored yellow to brown by a solution of iodine in zinc chloride, and on oxidation with nitric acid give oxalic and mucic acids.

Margin distinguishes between cellulose mucilages, pecto-mucilages, and scar tissue mucilages besides mixed mucilages and undetermined mucilages, in which the pecto-mucilages correspond to the true mucilages of Tschirch. The cellulose mucilages coagulate in a mixture of alcohol and hydrochloric acid; the fibres dissociate in ammonium oxalate solution, but do not swell or dissolve. These show the properties of cellulose and are colored by the same dyes as cellulose. The pecto-mucilages swell rapidly in water and are dissolved almost completely to a ropy solution. They are coagulated by lead acetate, alum, ferrous
sulphate, and other salts. There are numerous color tests for this group. The scar tissue mucilages swell but little in water at first and then suddenly dissolve. After swelling they dissolve in phosphoric acid or in calcium chloride solution. They swell without dissolving in ammonium and sodium carbonate solutions. These are colored acid and neutral dyes. The mixed mucilages consist of a mixture of cellulose and pecto-mucilages. To this group belong the mucilages from the quince, linseed, plantago, and others. The undetermined mucilages are not stained by dyes. They are insoluble in alcohol, ether, and other organic solvents. They are sometimes soluble and sometimes insoluble in Schweitzer's reagent. The mucilage from St. Johns Bread belongs in this class.

The mucilage from linseed has long been known to give sugars on hydrolysis. Hilger found that hydrolysis of this mucilage with 1% sulfuric acid gave dextrose, galactose, arabinose, xylose, and a residue which was acidic in nature and formed a barium salt. Neville repeated this work and confirmed the findings of Hilger. The mucilage prepared by soaking the seeds with water and precipitate by alcohol is almost neutral. Neville soaked the seeds in dilute acid and precipitated the mucilage acid which could be titrated with sodium hydroxide. In addition Neville found that the mucilage was dextro rotary in alkaline solution. After hydrolysis with 4% sulfuric acid Anderson and Crowder isolated the
calcium salt of an aldobionic acid which was identified as galacturonic acid linked to L-rhamnose. The galacturonic acid was later identified as d-galacturonic acid. Among the hydrolytic products L-galactose and d-xylose were found to be present, but no L-arabinose or dextrose as had previously been reported.

Bailey and Norris studied the mucilage from white mustard seed (Brassica alba). They reported the presence of rhamnose, arabinose, galactose, galacturonic acid, and cellulose. By the addition of saturated baryta to the water solution, a heavy gel was obtained leaving a filtrate which was precipitated by alcohol and called fraction I. The gel was treated with 4% sodium hydroxide solution for four hours to precipitate the cellulose. The extract was acidified and precipitated with alcohol; this was designated as fraction III. On analysis it gave the following results:

<table>
<thead>
<tr>
<th></th>
<th>Fr. I</th>
<th>Fr. III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium</td>
<td>9.45</td>
<td></td>
</tr>
<tr>
<td>Pentosan</td>
<td>6.80</td>
<td>9.4</td>
</tr>
<tr>
<td>Methyl Pentosan</td>
<td>10.00</td>
<td>nil</td>
</tr>
<tr>
<td>Uronic Anhydride</td>
<td>24.20</td>
<td>14.8</td>
</tr>
<tr>
<td>Methoxyl</td>
<td>4.90</td>
<td>3.4</td>
</tr>
<tr>
<td>Hexosan (by diff)</td>
<td>44.65</td>
<td>72.4</td>
</tr>
</tbody>
</table>

Arabinose and galactose were identified in the products of hydrolysis of each fraction. Rhamnose was found in fraction I but not in fraction III. Glucuronic acid was identified in fraction III. Fraction II consisted of cellulose.

Quince seed mucilage has been studied by Renfrew and
Cretcher. The mucilage after hydrolysis with 0.5 N sulfuric acid yielded a cellulose fraction along with a mixture of arabinose, xylose, and methylated uronic acids. Quince seed mucilage is probably an acid polysaccharide containing arabinose, and xylose-methoxy-hexuronic acid linked to cellulose.

Bailey investigated the mucilage from cress seed (Lepidium sativum). This mucilage, like white mustard and quince seed mucilages, contains a dispersable cellulose component and gives rise on acid hydrolysis to l-arabinose, d-galactose, l-rhamnose, d-glucose, the cellulose component, and d-galacturonic acid. The cellulose isolated from the cress seed mucilage has an exceptionally high xylan content. In this respect it differs from the cellulose from mustard seed mucilage, which has a low xylan content.

The mucilage from psyllium seed (Plantago psyllium) has repeatedly been investigated. Kirchner and Tollens in 1875 extracted a dextro-rotary gum-like material, which on hydrolysis gave fermentable sugars. Schmidt and Bauer found xylose in the hydrolytic products of this mucilage. They reported 8.9% pentosan and 5.7% galactan. Giraud found xylose and arabinose in the hydrolytic products together with small amounts of dextrose and galactose. Anderson and Fireman reported that this mucilage is a non-homogeneous mixture, since its composition varies with the procedure followed in its isolation. The uronic acid con-
tent varied from 7.8% to 13.6%, and the pentosan content from 78% to 90%. These analyses correspond to 9-36 pentose units per uronic acid, indicating equivalent weights from 1300 to 4800. On hydrolysis the mucilage gave xylose, arabinose, and galacturonic acid, but no galactose or dextrose as reported by Giraud.
The ash was determined by igniting a sample slowly in a weighed porcelain crucible and heating to constant weight.

Samples that did not darken at 100 degrees C were heated for 10 hours in the oven at this temperature and considered moisture free. Other samples were dried in an Abderhalden vacuum, dried with phosphorus pentoxide at 100 degrees and the moisture calculated from the loss in weight.

The uronic acid anhydride was determined by the modified method of Lefevre and Tollens. This procedure is very accurate and the results from it are reliable.

The pentosan content was determined according to the Methods of Analysis A.O.A.C. (1925). The calculations were made using the Krober tables in van der Haar. The results obtained are questionable since the tables are made using pure samples and not mixtures as are present in the mucilage.

The barium in the salts was precipitated as the sulphate by the addition of dilute sulfuric acid in excess and digested for one hour. The precipitate was filtered through an ashless filter and washed free from sulfate. The precipitate and filter were ignited carefully in a weighed platinum crucible and heated to constant weight. The results always tend to be high in the mono basic acids. The free aldehyde (CHO) was determined by the method of Cajori. The results obtained
from this determination are dependable. In the case of pure glucose this method gives quantitative results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured Value</th>
<th>Expected Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.50</td>
<td>4.50</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.75</td>
<td>3.75</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.60</td>
<td>2.60</td>
</tr>
<tr>
<td>Sample 4</td>
<td>1.87</td>
<td>1.87</td>
</tr>
<tr>
<td>Sample 5</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Sample 6</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Sample 7</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Sample 8</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Sample 9</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>
TABLE I

COMPOSITION OF INDIAN WHEAT SEED COMPARED WITH PSYLLA SEED

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Indian Wheat</th>
<th>Psylla Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.53%</td>
<td>-----%</td>
</tr>
<tr>
<td>Ash</td>
<td>6.87%</td>
<td>3.45%</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3.25%</td>
<td>7.00%</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>19.52%</td>
<td>12.57%</td>
</tr>
<tr>
<td>Protein</td>
<td>16.19%</td>
<td>19.49%</td>
</tr>
<tr>
<td>Nitrogen free extractives</td>
<td>48.59%</td>
<td>57.49%</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>68.11%</td>
<td>70.06%</td>
</tr>
</tbody>
</table>
EXPERIMENTAL PART

Preliminary Treatment of the Seed

The seed as it was received was mixed with chaff, stems, and other foreign material. These substances were removed by a seed cleaning machine. The seed was next treated with boiling acetone for two periods of six hours each. The extract was dark brown in color. The seed was then extracted with boiling 85% ethanol for two periods of six hours each. The solvents were removed by distillation and the combined residue was concentrated to a small volume in vacuo. This residue had the odor of caramel and contained reducing sugars. It was not investigated thoroughly. When poured into absolute ethanol, a black gum precipitated. When the residue was treated with glacial acetic acid, a dark red-brown color was produced. From these simple tests, it is evident that a free aldehyde group is present, possibly as a free sugar. The portion that is soluble in absolute ethanol is non-fermenting.

After the above treatment the seed was much lighter in color. It was dried and stored for use.
Preparation and Properties of the Mixed Mucilage

100 grams of the extracted seed were mixed with twenty times their weight of water. After 24 hours, the thick mucilaginous mixture was pressed through a double thickness of cloth. Since the composition of the mucilage varies with the force used in pressing it from the seed, each portion was squeezed until all the liquid was forced out. The seed cannot be extracted mechanically with much force because the inner part ruptures, giving a protein impurity to the mucilage. Some mucilage was prepared from the unextracted seed for comparison.

The mucilage was precipitated from the water extract by addition of four times its volume of 85% ethanol. This mixture was allowed to stand for six or more hours and the supernatent liquid was siphoned off. One volume of 95% ethanol was added to the flocculated mucilage and it was allowed to stand for one hour. The mucilage was filtered off and washed with ethanol and ether and dried at room temperature. Additional extractions of the seed yield very little mucilage. The dried mucilage weighed 19 grams or 19% of the weight of the seed used.

The mucilage has a light gray color and is neutral to litmus. It has very little taste and no odor. It gives no test for starch nor does it reduce Fehling's solution even after boiling for five minutes.
Analyses of the Mixed Mucilages and Comparison with the Results of Fireman

<table>
<thead>
<tr>
<th>Component</th>
<th>Untreated seed</th>
<th>Treated seed</th>
<th>White Psylla seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>3.37%</td>
<td>5.85%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.53%</td>
<td>5.84%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.50%</td>
<td>5.85%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.47%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td></td>
<td>5.85%</td>
<td></td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>1.56%</td>
<td>2.02%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.55%</td>
<td>2.25%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.56%</td>
<td>2.14%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.54%</td>
<td></td>
<td>1.85%</td>
</tr>
<tr>
<td>Avg. Theory</td>
<td></td>
<td>2.02%</td>
<td></td>
</tr>
<tr>
<td>Pentosan</td>
<td>92.98%</td>
<td>83.86%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90.50%</td>
<td>82.20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90.88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>91.19%</td>
<td>83.03%</td>
<td>87.07%</td>
</tr>
<tr>
<td>Theory</td>
<td>93.84%</td>
<td>91.11%</td>
<td></td>
</tr>
<tr>
<td>Total Percent</td>
<td>97.43%</td>
<td>91.59%</td>
<td>94.57%</td>
</tr>
<tr>
<td>Pentose Units Per Molecule Theory</td>
<td>20.02</td>
<td>14.84</td>
<td>16.51</td>
</tr>
<tr>
<td>Equivalent Weight Theory</td>
<td>2820</td>
<td>2154</td>
<td>2373</td>
</tr>
</tbody>
</table>
Discussion of Table II

A study of table II shows that the extraction of the seed removes some pentose material. This is further evidence that the extracted material is a free sugar or possibly a glycoside.

Another interesting relationship is that the average of the values for the mucilages of treated and untreated seed approximate very closely the results for psylla seed mucilage. The average for carbon dioxide is 1.85% as compared with 1.85% for the psylla seed mucilage. The average for pentosan is 87.11% as compared with 87.07% for the psylla seed mucilage. This is evidence that the mucilages from the two seeds are closely related and indicates that the wild seed may have the same commercial uses as the cultivated seed.

Fractionation of the Mucilage

Many unsuccessful attempts were made to fractionate the mucilage by adding different volumes of ethanol to the water extract of the seed. The mucilage solution has an alcohol number of about 2.5. Below this value, the ethanol does not precipitate the mucilage, while above this value the precipitation is almost complete. The fractionation was accomplished as follows:

100 grams of the seed were mixed with 2 liters of 25% ethanol and allowed to stand for 24 hours. The dissolved
mucilage was pressed through cloth and precipitated by the addition of 4 volumes of 85% ethanol. The seed residue from the above treatment was then mixed with 2 liters of water and allowed to stand for 24 hours. The mucilage was pressed out and precipitated as above. This gave two fractions of mucilage. The portion containing the smaller molecule was more soluble in the 25% ethanol. This was established by analysis of the mucilage. The mucilage obtained from the extraction with ethanol is called mucilage A and has an equivalent weight of 1290. Mucilage B, the less soluble portion obtained from the water extraction, has an equivalent weight of 2445 and hence is a larger molecule. 

Fireman investigated the mucilage from psylla seed. His methods of preparation were as follows:

Crop 5A was prepared by soaking the seed in 12 volumes of water for 23 hours. The mucilage was pressed out gently and precipitated with ethanol. Crop 5B was prepared by soaking the seed residue from 5A in 12 volumes of water and heating for an hour on a boiling water bath. The mixture was thoroughly pressed from the seed and precipitated with ethanol.
Analyses of Mucilages A and B from Indian Wheat and
Comparison with the Analyses from Psylla Seed

<table>
<thead>
<tr>
<th>Component</th>
<th>Mucilage A</th>
<th>Mucilage B</th>
<th>Mucilage 5A</th>
<th>Mucilage 5B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>4.88%</td>
<td>2.93%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>4.64%</td>
<td>2.89%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.75%</td>
<td>2.91%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>3.39%</td>
<td>1.81%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>3.38%</td>
<td>1.80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theory</td>
<td>3.39%</td>
<td>1.81%</td>
<td>3.70%</td>
<td>1.23%</td>
</tr>
<tr>
<td>Pentosan</td>
<td>86.31%</td>
<td>90.13%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>87.50%</td>
<td>95.16%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theory</td>
<td>86.90%</td>
<td>92.64%</td>
<td>77.21%</td>
<td>85.86%</td>
</tr>
<tr>
<td>Total Percent</td>
<td>100.50%</td>
<td>99.84%</td>
<td>92.01%</td>
<td>90.78%</td>
</tr>
<tr>
<td>Pentose Units per Molecule</td>
<td>8.34</td>
<td>17.06</td>
<td>7.56</td>
<td>25.60</td>
</tr>
<tr>
<td>Theory</td>
<td>8.00</td>
<td>17.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equivalent Weight</td>
<td>1290</td>
<td>2445</td>
<td>1191</td>
<td>3570</td>
</tr>
<tr>
<td>Theory</td>
<td>1249</td>
<td>2437</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion of Table III

A study of the analyses given in table III proves conclusively that the mucilage is a mixture. By allowing the seed to soak in ethanol-water mixtures of varying concentrations, a fractionation of the mucilage results. Fireman accomplished a fractionation by successive treatments with water. In his process the smaller molecules first dissolve. This fact suggests that the mucilage may exist in the seed in the form of layers, with the smaller molecules on the outside and the larger molecules on the inside.

In this connection Wiesner states that the seed coat of Plantago psyllium is composed of two layers. The outer layer swells in water more readily than does the inner layer. This fact probably has a definite function in the plant. The outer or the more absorptive layer tends to take up moisture while the inner layer prevents the absorption of an excess amount of water. Too much water might result in the bursting of the seed or the cytosis of the cells of the embryo.

Preparation of the Mucilage Acids

100 grams of the seed were allowed to stand for 24 hours with water. The mucilage was pressed out and precipitated by the addition of 4 volumes of 85% ethanol which had been made 0.1 N with hydrochloric acid. The precipitated mucilage was freed from chloride ion by repeated washings with ethanol. This mucilage acid is slightly soluble in 2% sodium hydroxide.
solution. The entire crop was dissolved in 2% sodium hydroxide solution. The solution was made neutral with hydrochloric acid and divided into three portions.

The first portion was reprecipitated by the addition of 4 volumes of 85% ethanol which had been made 0.1 N with hydrochloric acid. The precipitated mucilage was washed free of chloride ion with ethanol and finally dried by washing with ether. The yield was 8% of the weight of the seed used. This mucilage was called mucilage acid I. It reduced Fehling's solution on boiling, indicating that the treatment had caused a slight hydrolysis of the molecule.

The second portion of the mucilage solution was treated in the same way except that it was reprecipitated by the addition of ethanol which had been made 0.5 N with hydrochloric acid. It was purified exactly as described for mucilage acid I. This mucilage was called mucilage acid II. It also reduces Fehling's solution on boiling.

The third portion of the mucilage solution was made slightly acid with hydrochloric acid and allowed to stand for 24 hours in the presence of excess bromine. The excess bromine was removed by the addition of ethanol and the mucilage was precipitated by the addition of 4 volumes of ethanol which had been made 0.1 N with hydrochloric acid. This was called mucilage acid III. It differed from the other mucilage acids, both in appearance and composition. Mucilage acids I and II were white and soft, while mucilage acid III was grey and
Attempts to determine the specific rotation of the mucilage acids were unsuccessful. The solutions were opaque in all cases.
### Analyses of the Mucilage Acids

<table>
<thead>
<tr>
<th>Component</th>
<th>Acid I</th>
<th>Acid II</th>
<th>Acid III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>1.67%</td>
<td>1.79%</td>
<td>1.19%</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.52%</td>
<td>1.79%</td>
<td>1.19%</td>
</tr>
<tr>
<td>Ash</td>
<td>1.59%</td>
<td>1.79%</td>
<td>1.19%</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.59%</td>
<td>1.79%</td>
<td>1.19%</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>1.68%</td>
<td>1.65%</td>
<td>2.26%</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.80%</td>
<td>1.81%</td>
<td>2.15%</td>
</tr>
<tr>
<td>Theory</td>
<td>1.70%</td>
<td>1.73%</td>
<td>2.21%</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>1.71%</td>
<td>1.71%</td>
<td>2.33%</td>
</tr>
<tr>
<td>Avg.</td>
<td>1.70%</td>
<td>1.73%</td>
<td>2.21%</td>
</tr>
<tr>
<td>Theory</td>
<td>1.71%</td>
<td>1.71%</td>
<td>2.33%</td>
</tr>
<tr>
<td>Pentosan</td>
<td>89.28%</td>
<td>88.75%</td>
<td>92.00%</td>
</tr>
<tr>
<td>Avg.</td>
<td>89.29%</td>
<td>90.38%</td>
<td>92.30%</td>
</tr>
<tr>
<td>Theory</td>
<td>89.24%</td>
<td>89.57%</td>
<td>92.15%</td>
</tr>
<tr>
<td>Pentosan</td>
<td>92.50%</td>
<td>92.50%</td>
<td>90.48%</td>
</tr>
<tr>
<td>Avg.</td>
<td>92.50%</td>
<td>92.50%</td>
<td>90.48%</td>
</tr>
<tr>
<td>Theory</td>
<td>92.50%</td>
<td>92.50%</td>
<td>90.48%</td>
</tr>
<tr>
<td>Total Percent</td>
<td>96.04%</td>
<td>96.49%</td>
<td>100.93%</td>
</tr>
<tr>
<td>Pentose Units Per Molecule</td>
<td>18.15</td>
<td>17.81</td>
<td>13.63</td>
</tr>
<tr>
<td>Theory</td>
<td>18.00</td>
<td>18.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Equivalent Weight</td>
<td>2590</td>
<td>2544</td>
<td>1798</td>
</tr>
<tr>
<td>Theory</td>
<td>2567</td>
<td>2567</td>
<td>1348</td>
</tr>
</tbody>
</table>
Discussion of Table IV

It is evident from a study of table IV that variation in the acidity of the ethanol within the limits used, does not affect the composition of the precipitated mucilage acids. The process has hydrolyzed off some of the pentose molecules. As already pointed out, the mucilage precipitated from neutral solution did not reduce Fehlings solution.

The treatment with bromine in acid solution led to a greater change in the molecule than the treatment with the acid alone.

The mucilage acids were titrated with standard base, using phenolphthalein as indicator. The results obtained from back titration gave 1.19% carbon dioxide, while the results from the method of Tollens and Lefevre showed 1.70% carbon dioxide. In this connection, Norman states that the hemicelluloses cannot be titrated with base. The hemicelluloses are acidic molecules of the same general structure as mucilages.

Hydrolysis of the Mucilage

Numerous hydrolyses of the mucilage were carried out using 50 gram portions. In all these the mucilage was mixed with 60 times its weight of 4% sulfuric acid solution, shaken until it dissolved, and then heated in a bath of boiling water for periods varying from 15 to 20 hours. During the
process a dark flocculent precipitate always formed. This was filtered off at the close of the hydrolysis, dried and found to be 3% of the mucilage used. It probably consists of polymerized furfural. Barium hydroxide solution was carefully added to the filtrate until it was just neutral to litmus. The barium sulfate was filtered off and the solution decolorized by Darco. This filtrate was concentrated in vacuo to a small volume. The barium salt of the uronic acid-sugar compound was precipitated from the sugar solution by pouring the syrup slowly into a large volume of 95% ethanol. The salt was triturated until granular. It was then filtered from the sugar solution, washed with ethanol, dried on a porous plate and in vacuo. The salts were usually purified by redissolving them in water, filtering the solution and reprecipitating them by the addition of ethanol. After drying on a porous plate and in vacuo the yield varied from 10 to 16% depending on the length of heating. The further treatment of these salts is described below.

The combined ethanol solutions of the sugars from which the barium salts had been precipitated were concentrated in vacuo to a small volume. The syrup was filtered from any remaining barium salts. The concentrated syrup was used as described below in the identification of the sugars.

x The best concentration for precipitation of the barium salts is reached when the refractive index of the syrup varies from 1.4 to 1.5.
Identification of the Sugars

The syrup was mixed with a small volume of glacial acetic acid and seeded with crystals of d-xylose. The mixture was placed in a refrigerator and allowed to stand for 24 hours. It became solid with crystals which were filtered off and washed with glacial acetic acid and absolute ethanol. The sugar was identified by its melting point, 150°C, and by its \([\alpha]_o\) plus 18.5. It also gave the characteristic crystals of the cadmium bromide-cadmium xylonate double salt. Two more crops of the sugar were obtained but in each case the rotation was higher indicating the presence of other higher rotating sugars.

After most of the d-xylose had been removed, the ethanol-acetic acid solution was concentrated in vacuo to a syrup and seeded with L-arabinose. After standing for several days in the refrigerator the crystalline sugar was filtered off and washed with absolute ethanol. This sugar was identified as L-arabinose by its \([\alpha]_o\) plus 92.05 and by converting it to the \(\alpha\)-benzyl phenyl hydrazone which had a melting point of 168°C and a \([\alpha]_o\) -12.2. Tests for galactose and methyl pentoses were negative. The absence of fermentable sugars was established by the lack of fermentation with ordinary yeast. D-Xylose was the only sugar obtained from the 15 hour hydrolysis of the mucilage proving that the xylose units are attached to the outer end of the molecule. L-Arabinose was the only sugar identified, obtained by hydrolysis of the barium salt in the autoclave. This proves that the arabinose
is attached to the uronic acid.

Inspection of table II indicates the presence of 19 pentose units attached to one uronic acid. Hydrolysis of this mucilage gave a brown salt containing three arabinose units. In the sugars obtained from this hydrolysis, the ratio of the d-xylose to the l-arabinose was approximately 3 to 1. Since there were 19 pentose units on the original mucilage and 3 remained, there must have been 16 pentose units hydrolyzed off. This would correspond to 12 units of d-xylose and four units of free arabinose. Hence in the original mucilage there were approximately 7 units of l-arabinose and 12 units of d-xylose.

The Barium Salts of the Uronic Acid-Sugar Compounds

These barium salts were obtained by hydrolysis of the mucilage as already described. The presence of a uronic acid in the salts was established by the naphtho-resorcinol test and by the yield of carbon dioxide by the method of Lefevre and Tollens. The uronic acid was identified as galacturonic acid by the method of Heidelberger and Goebel. The mucic acid obtained melted at 217 C.

To identify the sugar in the barium salt, 10 grams of the salt were dissolved in 300 cc of 4% sulfuric acid solution and heated for 10 hours in an autoclave at 17 pounds gauge pressure. The solution was treated with barium hydroxide
solution until neutral to litmus. The barium salt and the sugar were separated from each other as already described. 1-Arabinose was identified in the sugar solution by conversion to the α-benzyl phenylhydrazone with melting point 169°C.

Four lots of barium salts were prepared as already described, and one of these was oxidized to the dibasic salt in the following manner:

13.5 grams of the barium salt, which had been obtained from the twenty hour hydrolysis of the mucilage, were dissolved in 50 cc of water and to this was added 575 cc of 0.3 N iodine in barium iodide. This mixture was made basic with 900 cc of saturated barium hydroxide solution. The mixture was allowed to stand for 2 days. The solution was then made acid with sulfuric acid and the barium sulfate filtered off. An excess of lead carbonate was added to the filtrate and the mixture filtered. The filtrate was concentrated in vacuo to a small volume. A saturated solution of silver sulfate was added to precipitate the iodide ions. The solution was filtered and hydrogen sulfide passed into the filtrate to remove any lead and silver. This solution was neutralized with barium hydroxide, filtered, and the filtrate concentrated in vacuo to a small volume. The barium salt was precipitated in the usual way and is called Ba₆.

Barium salt, Ba₁, was prepared as already described. Barium salt, Ba₂, was precipitated in absolute ethanol after the removal of Ba₁. Barium salt, Ba₃, was prepared in the reg-
ular way except that it was hydrolyzed for 15 hours. The re-
sults obtained on analyses of their salts are given in table V.

**TABLE V**

**SUMMARY OF THE ANALYSES OF THE BARIUM SALTS**

<table>
<thead>
<tr>
<th></th>
<th>Ba₁ 20 hour hydrolysis</th>
<th>Ba₂ 20 hour hydrolysis</th>
<th>Ba₃ 15 hour hydrolysis</th>
<th>Ba₅ 20 hour hydrolysis (dibasic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium</td>
<td>20.69%</td>
<td>20.57%</td>
<td>15.02%</td>
<td>23.09%</td>
</tr>
<tr>
<td>Average Theory</td>
<td>20.77%</td>
<td>20.62%</td>
<td>14.87%</td>
<td>23.08%</td>
</tr>
<tr>
<td>Theory</td>
<td>10.45%</td>
<td>10.45%</td>
<td>8.70%</td>
<td>22.50%</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>6.55%</td>
<td>7.28%</td>
<td>5.40%</td>
<td>3.97%</td>
</tr>
<tr>
<td>Average Theory</td>
<td>6.60%</td>
<td>7.05%</td>
<td>5.22%</td>
<td>3.92%</td>
</tr>
<tr>
<td>Theory</td>
<td>6.60%</td>
<td>6.69%</td>
<td>5.57%</td>
<td>7.21%</td>
</tr>
</tbody>
</table>

Specific rotation +55.96 +55.96 +35.96

**Discussion of Table V**

An inspection of table V shows that after hydrolysis for 20 hours three pentose units remain attached to the uronic acid. The results of the barium analyses indicate that the free aldehyde group of the molecule holds some barium in com-
bination with it.
S U M M A R Y

The results of this investigation may be summarized as follows:

1. The seed from Wild Indian Wheat has been found to yield a mucilage which amounts to approximately 20% of the weight of the seed used.

2. The mucilage is a mixture which has been separated into two fractions with the equivalent weights, 1290 and 2445.

3. The mucilage is the salt of a complex carbohydrate from which the free acid has been prepared.

4. The constituents of the mucilage have been identified as d-galacturonic acid, l-arabinose, and d-xylose.

5. Barium salts of the uronic acid-sugar complex have been isolated and analyzed.

6. The mucilage obtained from Wild Indian Wheat resembles closely the mucilage obtained from imported Psylla seed.

7. On the basis of the experimental results, a structure has been suggested for the mucilage.

\[ \text{Chemical Structure Diagram} \]
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