THE CONSTITUTION OF THE HEMICELLULOSE OF APPLE WOOD

by

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B.Sc., University of British Columbia, 1958

A THESIS SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER
OF SCIENCE

in the Department
of
CHEMISTRY

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1960
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Date Sept. 27/60
ABSTRACT

The hemicellulose isolated from apple wood (var. Golden Transparent) by alkaline extraction has been shown to contain a 4-O-methylglucuronoxylan. Hydrolysis of the hemicellulose yielded neutral sugars and uronic acids. Paper chromatographic examination of the neutral sugars showed D-xylose to be the major component with small amounts of other sugars corresponding to rhamnose, arabinose and galactose also being present. Two other sugars with $R_f$ values greater than that of xylose were found but the identity of these components has not been established. An aldobiouronic acid was isolated and characterized as the crystalline acetate of 2-0-($\xi$-0-methyl-\(\xi\)-D-glucopyranosyluronic acid)-D-xylose.

In order to determine the mode of union of the component sugars the polysaccharide was methylated and then hydrolyzed to give 2,3,4-tri-O-methyl-D-xylose, 2,3 di-O-methyl-D-xylose, 2-0-(2,3-di-O-methyl-D-xylopyranosyl)-2,3-di-O-methyl-D-xylose, a dimethylated lyxose, 2-0- and 3-0-methyl-D-xylose, 2-0-(2,3,4-tri-O-methyl-D-glucopyranosyluronic acid)-D-xylose, and probably a trimethylated rhamnose. (The dimethylated lyxose isolated is not an integral constituent of the native polysaccharide since no lyxose was obtained in the acid hydrolyzate. It is thought to arise by epimerization of 2,3-di-O-methyl-D-xylose.) Quantitative analysis of the methylated hemicellulose has shown the tri-, di- and monomethyl pentose and 2,3,4-tri-O-methyl-D-\(\xi\)-
glucuronic acid to be present in the mole ratios of 1:97:21:19, respectively.

The methyl ester of the methylated aldobiouronic acid was reduced with lithium aluminum hydride and the resulting neutral disaccharide hydrolyzed. The cleavage products were identified as 3-0-methyl-D-xylose and 2,3,4-tri-0-methyl-D-glucose indicating the uronic acid portion of the molecule to be linked through its reducing end to position 2 of a xylose moiety.

Results obtained in this work show that the carbohydrate polymer isolated from apple wood consists of a backbone of approximately 119 anhydro-D-xylopyranose linked by 1,4-β-glycosidic bonds. The side chains are composed of single units of 4-0-methyl-D-glucuronic acid which occur at every sixth xylose residue. A rhamnose unit may perhaps be present as a side chain too; although it is not known whether this sugar is an integral constituent of the glucuronoxylan.

The general features of the hemicellulose are very similar to glucuronoxylns isolated from other hardwoods and especially resemble white elm and cherry wood in its structure and high uronic acid content.

We are grateful to Dr. C. T. Bishop who presented this work at the 43rd conference of The Chemical Institute of Canada, Ottawa, June 1960.
ACKNOWLEDGEMENTS

To Dr. G. G. S. Dutton I wish to express my deep appreciation for his excellent guidance and encouragement throughout the course of this work.

I also wish to thank Dr. T. E. Timell for samples of 2-O-methyl-D-xylose and of the crystalline acetate of 2-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylose.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. HISTORICAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. APPLE WOOD HEMICELLULOSE</td>
<td>23</td>
</tr>
<tr>
<td>III. EXPERIMENTATION</td>
<td>34</td>
</tr>
<tr>
<td>A. ISOLATION OF APPLE WOOD HEMICELLULOSE</td>
<td>34</td>
</tr>
<tr>
<td>B. HYDROLYSIS OF APPLE WOOD HEMICELLULOSE</td>
<td>35</td>
</tr>
<tr>
<td>C. SEPARATION OF THE ACIDIC COMPONENT OF APPLE WOOD HEMICELLULOSE</td>
<td>36</td>
</tr>
<tr>
<td>D. PREPARATION OF THE CRYSTALLINE DERIVATIVE OF 2-O-(4-O-METHYL-(\alpha)-D-GLUCOPYRANOSYLURONIC ACID)-D-XYLOPYRANOSE</td>
<td>37</td>
</tr>
<tr>
<td>1. The Methyl Ester Methyl Glycoside</td>
<td>37</td>
</tr>
<tr>
<td>2. Methyl 2-O- Methyl (2,3-di-O-acetyl-4-O-methyl-(\alpha)-D-glucopyranosyl) uronate - 3,4-Di-O-acetyl-D-xylopyranoside</td>
<td>38</td>
</tr>
<tr>
<td>E. IDENTIFICATION OF D-XYLOSE</td>
<td>38</td>
</tr>
<tr>
<td>F. ACETYLATION OF APPLE WOOD HEMICELLULOSE</td>
<td>39</td>
</tr>
<tr>
<td>G. METHYLATION OF APPLE WOOD HEMICELLULOSE</td>
<td>40</td>
</tr>
<tr>
<td>1. Methylation I</td>
<td>40</td>
</tr>
<tr>
<td>2. Methylation II</td>
<td>41</td>
</tr>
<tr>
<td>3. Methylation III</td>
<td>42</td>
</tr>
<tr>
<td>H. FRACTIONATION OF THE METHYLATED HEMICELLULOSE</td>
<td>42</td>
</tr>
<tr>
<td>I. METHANOLYSIS OF METHYLATED APPLE WOOD HEMICELLULOSE</td>
<td>46</td>
</tr>
<tr>
<td>J. SAPONIFICATION OF METHYL ESTER OF THE ALDOBIOURONIC ACID</td>
<td>46</td>
</tr>
</tbody>
</table>
K. SEPARATION OF THE ACIDIC COMPONENT OF METHYLATED APPLE WOOD HEMICELLULOSE ........ 47

L. PREPARATION OF METHYL 2-0-(2,3,4-TRI-0-METHYL-D-GLUCURONOSYL)-3-0-METHYL-D-XYLOSIDE METHYL ESTER ............... 47
   1. Preparation of Diazomethane .......... 47
   2. Preparation of Methyl Ester of Methyl Glycoside of the Aldobiouronic Acid ........ 47

M. REDUCTION OF THE METHYL ESTER OF METHYL 2-0-(2,3,4-TRI-0-METHYL-D-GLUCURONOSYL)-3-0-METHYL-D-XYLOSE ........... 48

N. HYDROLYSIS OF METHYL 2-0-(2,3,4-TRI-0-METHYL-D-GLUCOPYRANOSYL)-3-0-METHYL-D-XYLOSE ................. 49
   1. Identification of 2,3,4-Tri-0-Methyl-D-Glucose ......................... 50
   2. Identification of 3-0-Methyl-D-Xylose 50

O. SEPARATION OF THE NEUTRAL COMPONENTS OF METHYLATED APPLE WOOD HEMICELLULOSE ........ 51

P. IDENTIFICATION OF THE COMPONENTS ............... 53
   1. Component 1, Tri-0-Methyl-Rhamnose 53
   2. Component 2, 2,3,4-Tri-0-Methyl-D-Xylose ......................... 53
   3. Component 3, 4-0-(2,3-di-0-methyl-D-xylopyranosyl)-2,3-di-0-methyl-xylose 53
   4. Component 4, 2,3-Di-0-Methyl-D-xylene 54
   5. Component 5, Di-0-methyl-Lyxose 54
      a. Demethylation of Di-0-methyl-Pentose ......................... 54
p. IDENTIFICATION OF THE COMPONENTS (continued)

6. Component 6, 2-O- and 3-O-Methyl-
   D-xylose ................. 55

7. Component 7, D-Xylose ....... 55

Q. QUANTITATIVE COMPOSITION OF APPLE WOOD

   HEMICELLULOSE ............. 55

BIBLIOGRAPHY .................. 58
**LIST OF TABLES**

**TABLE IA**

FRACTIONAL PRECIPITATION OF METHYLATED
APPLE WOOD HEMICELLULOSE II .......................... 44

**TABLE IB**

FRACTIONAL PRECIPITATION OF METHYLATED
APPLE WOOD HEMICELLULOSE III ....................... 45

**TABLE II**

SEPARATION OF METHYLATED SUGARS of
APPLE WOOD HEMICELLULOSE ............................ 52

**TABLE III**

QUANTITATIVE ANALYSIS of METHYLATED
SUGARS of APPLE WOOD HEMICELLULOSE
by PHENOL-SULPHURIC ACID METHOD .................... 57
HISTORICAL INTRODUCTION

Of universal occurrence, hemicelluloses constitute a large group of polysaccharides whose abundance, as far as organic material is concerned, is surpassed only by cellulose. Hemicelluloses, and in particular xylans, are commercially valuable as a source of furfural and furan derivatives. They are said to be of value when present in small quantities in paper pulps. Accordingly, where once these carbohydrate polymers were regarded as a nuisance in the pulping of wood, they now represent a rich, almost untapped source of raw material.

As early as 1891, Schulze (1) obtained, by alkaline extraction of plant material, a group of carbohydrate polymers which were more readily hydrolyzed than cellulose. Because of their chemical and physical similarities to cellulose, he designated the group hemicellulose. In the classic work by O'Dwyer (2), in 1926, polysaccharides which were precipitated from alkaline solutions by simple acidification and by addition of ethanol to the filtrate were designated as hemicellulose A and hemicellulose B, respectively. Subsequent investigations by other workers show inconsistent terminology. For example, hemicellulose A has also been described as the fraction extracted with a% potassium hydroxide and hemicellulose B as the fraction
extracted from the residue with 24% sodium hydroxide (3).

With the advent of modern techniques such as paper chromatography and electrophoresis, the field of polysaccharide chemistry has been vastly augmented. No standard nomenclature for these macromolecules is, as yet, available and classification of polysaccharides varies with the investigator. Inconsistencies in terminology appear in the literature mainly due to this lack of standard nomenclature and classification.

Systematic classification and nomenclature, based on normal carbohydrate nomenclature, has been attempted although it has not been generally accepted. Hemicellulose have been divided into three fractions: (1) pentosans, (2) polyuronides, including molecules composed of hexose and/or pentose sugars and uronic acids (the term "polyuronide", is a misnomer since it implies that a substance is composed of uronic acid residues when, in fact, the polymer consists of chains of hexoses or pentoses with a few uronic acid units joined through its hemiacetal hydroxyl to the other hemicellulosic constituents), and (3) cellulosans consisting of xylan, mannan, and perhaps a "glucan" (4). The term non-cellulosic cell-wall polysaccharide has been suggested (5), but it is admitted that the term fails because the dividing line between true cellulose and hemicellulose is arbitrary. Other workers consider this group of polymers to be a mixture of unmodified glycans composed of either pentose or hexose sugar units and of modified glycans containing one or more glucuronic acids joined to the main chain of the molecule (6). Thus, for example, in a classification such as the latter, the hemicellulose group of polysaccharides
can be classified into (1) true xylans, (2) araboyxylans, (3) glucuronoxyylans, (4) araboglucuronoxyylans, and (5) complex xylans.

A wide range of molecular sizes and shapes exist among these carbohydrates from different plants and from different parts of the plant. Due to these differences and differences in their acidic properties, hemicellulose vary widely among themselves in solubility. Consequently, it is difficult to prepare a pure molecular type by the fractionation procedures used to date. It apparently seems, however, that many tissues contain not more than three or four polysaccharide types which differ primarily in molecular weight.

Pretreatment of plant materials is necessary for the isolation of hemicelluloses. This includes solvent extraction and delignification. There are certain plant constituents which occur in association with the polymeric carbohydrate material which are generally extractable with alcohol-benzene azeotrope and consist of compounds such as lipids, waxes, resins, organic acids, free sugars etc. (7).

Pectin and pectic substances are sometimes removed prior to hemicellulose extraction especially when dealing with plant materials containing a large amount of these substances, such as the cambium layer of wood or the leaves and stems of plants. Extraction with 0.5% solutions of ammonium oxalate removes water insoluble pectic substances if they are not trapped or chemically bound to plant materials (8).
Hemicelluloses and lignin occur in close association with cellulose. Their apparent function appears to be that of cementing together cellulose fibers thus imparting strength to the plant tissue. The presence of lignin in plant tissue presents a problem since it retards the isolation of hemicellulose. This difficulty arises presumably because of mechanical obstruction or because of, as yet, unknown covalent bonding between the lignin, thought to be polymeric phenylpropane units, and the hemicellulose. The former is soluble in alkali and thus hampers the purification of hemicelluloses by extraction with aqueous alkali.

Many procedures have been proposed for the removal of lignin from plants. In one of the earlier methods of delignification, plant material was extracted with 50% ethanol containing 1% sodium hydroxide (9). This method, however, is not recommended since at boiling temperatures, the uronic acid containing polyuronides are degraded (10). Removal of the lignin is retarded at lower temperatures. Several other techniques have been developed for the selective removal of lignin but the method developed by Jayme (11) and improved by Wise and co-workers (12) is most widely used. Selective removal of the lignin is achieved by treating the wood sample with acidified sodium chlorite followed by subsequent extraction of the hemicellulose with alkali.

For deciduous plants, a simpler method of delignification has been developed by McDonald (13) in which 0.1N sodium hydroxide was found to remove a major portion of the alkali.
soluble impurities. The loss of lignin was found to be approximately constant above an alkali strength of 0.1N so that pentosan removal with 1N sodium hydroxide was facilitated.

Since the time of Schulze (1), alkaline extractions have been the principal method used for isolating the hemicellulose from plant materials. Sodium and potassium hydroxides of varying strengths have been employed. An alkali concentration of 1N is the most commonly used but the ideality of the concentration has been found to differ among different plants. For example, Wise and co-workers (14) showed that the solubility of hemicellulose of slash pine holocellulose increases as the potassium hydroxide concentration of the extracting solution increases with optimum solubilization obtained at 16%. In most cases, concentrations of alkali higher than 16% result in small additional solubilization of hemicellulose.

Initial subdivision of polysaccharide mixtures have been obtained by fractional precipitation. Acidification of alkali extract was found to precipitate, in some cases, a large portion of hemicellulose, termed hemicellulose A (2). This procedure takes advantage of the fact that high molecular weight polysaccharides precipitate first while lower molecular weight polymers and uronic acid containing ones are precipitated by the addition of excess alcohol.

Further purifications have been achieved by complexing the hemicellulose in aqueous solutions with copper compounds (15). Sometimes more efficient separation of polysaccharides
are attained by the fractional precipitation of their acetyl or methyl derivatives (16-18). Other workers have used organic solvents for obtaining fractionation (19), while still others have employed inorganic salts, for example ammonium sulphate (18, 20), selective extraction with aqueous ethanol (21) and precipitation with Getavalon (cetyltrimethyl ammonium bromide) (22).

The above described methods for obtaining hemicelluloses have proved very useful but it must be borne in mind that degradation occurs when strong oxidizing agents such as chlorine and chlorite are employed. Degradation is also known to occur in the presence of strong alkali. Hence, polysaccharides obtained by chemical means may not necessarily be that found in the native polysaccharide material. Also, no method is as yet available for determining, unequivocally, the homogeneity of polymeric fractions although steps have been taken in this direction by the use of electrophoresis (23-27).

Acid hydrolysis, followed by qualitative and quantitative analysis, give an insight into the nature of the sugar residues present in the macromolecule and the proportions in which they are present. Modern chromatographic techniques have opened new horizons in carbohydrate chemistry and chromatographic identification of monosaccharides, after hydrolysis, are identified without the tedious task of synthesizing derivatives, as least for the first analysis. Quantitative determination of monosaccharides is also facilitated by chromatography
and colorimetry (28).

In determining the architecture of the macromolecules, applications of various analytical techniques are involved including methylation, graded hydrolysis, enzymatic degradation and periodate oxidation—all require the use of chromatography.

Methylation, a procedure developed approximately sixty years ago, still proves to be the most powerful weapon for determining the mode of linkage in carbohydrate polymers. Methyl ether derivatives of the polysaccharides are obtained when subjected to Haworth methylations (29). This involves the treatment of material with dimethyl sulphate and alkali. Following this, reaction with methyl iodide and silver oxide (Purdie methylation) generally produces a completely methylated polysaccharide (30). Modifications to the above procedures have been made to expedite this step of the analysis (31-33). Hydrolysis of the methylated polysaccharide and identification of the cleavage products reveal the nature of the linkage in the polymers. Information with regard to the number of end groups, linearity, or branched nature is also obtained. This procedure does not, however, reveal the sequence of the sugar residues in the molecule.

Partial hydrolysis and graded hydrolysis are rapidly becoming useful techniques for determining the order of linkage. Both chemical and enzymatic depolymerization have been employed (26, 34-40).

Much useful information can be derived from the study of periodate on the macromolecules. End group determinations,
the nature and the number of residues so linked that they are not attacked by periodate, and the proportions of residues giving rise to dialdehydes can all be dealt with in this way (42).

It is practically impossible to completely methylate all the free hydroxyl groups present in a large polysaccharide. Similarly, it is difficult to determine the extent of over- or under-oxidation in the periodic oxidation of polysaccharides (42). In addition to chemical and enzymatic degradation, data as to the kinds of monosaccharides present are required, this being obtained by complete hydrolysis of the molecule. It is possible to offset some of the problems involved in correctly interpreting the results obtained if the analytical methods described above are used in conjunction with each other.

In this present work a hemicellulose consisting primarily of D-xylose units was investigated. Therefore, works on xylose-containing polysaccharides will be reviewed.

Xylans occur in practically all land plants and are said to be present in some marine algae (43,44). They are the most abundant of the group of hemicelluloses which include xylans, glucomannans, mannans, arabogalactans etc. and are particularly abundant in agricultural residues such as corn cobs, corn stalks, grain hulls and stems, and these materials have been extensively investigated. Amounts ranging from 15-30% of xylan have been obtained. Wood xylans are found in smaller amounts in comparison to annual crops. Hardwoods are
found to contain 20-25% xylan while softwoods contain 7-12% (45).

Xylans have the general properties of insolubility in water, solubility in alkaline solutions, ease of acid hydrolysis, high negative optical rotation and non-reducing actions toward Fehling's solution. They fall into either of three general classes: (1) pentosan (2) glycan or (3) hemicellulose because they are composed almost entirely of pentose units, are largely a polymer of unmodified sugars and are extractable by hemicellulose extraction procedures (6).

Upon acid hydrolysis, they release a variety of sugars and hexuronic acids, namely, D-xylose, 2-O- and 3-O-methyl-D-xylose, L-arabinose, L-rhamnose, 3-O-methyl-L-rhamnose, D- and L-galactose, D-mannose, D-glucose, L-fucose, D-galacturonic acid, D-glucuronic acid and 4-O-methyl-D-glucuronic acid. On the basis of their chemical constitution, therefore, further subdivision of this large class of polysaccharides may be made. Thus, the hemicellulose group of carbohydrate polymers can be classified as (i) true xylans, (ii) araboxylans, (iii) glucuronoxylans, (iv) arabo-glucuronoxylans and (v) complex xylans (46).

True xylans are polysaccharides composed solely of anhydroxylopyranose units. This type of hemicellulose from land plants is not too common and only two such examples are known, to date, to be completely devoid of other sugar residues, those from esparto grass (47) and tamarind seed (48).
Esparto grass, which has been studied extensively, was originally thought to be composed of D-xylose and L-arabinose residues (49). After hydrolysis of the fully methylated derivative of the xylan, the principal products obtained, in this early work, were 2,3 di-O-methyl-D-xylose and 2,3,5-tri-O-methyl-L-arabinose roughly in the proportions of 20:1. The polysaccharide was, therefore assumed to consist of 1,4-linked D-xylopyranose residues as evidenced by the isolation of the dimethyl pentose. L-Arabinose units were considered to exist as terminal non-reducing end groups joined to the main chain of D-xylopyranose units.

Further investigations have revealed that L-arabofuranose units are, in fact, not an integral part of the molecule and that rather, one of the hemicelluloses from this plant is actually a true xylan. Chanda and co-workers (47) have, by vigorous fractionation of the esparto grass pentosan, isolated a xylan free of other sugar residues. The ready formation of an insoluble copper complex (15) by the hemicellulose was taken advantage of. (This method of fractionation is gentle enough chemically not to disturb the labile arabofuranosyl linkages should they be present in the molecule.) Re-examination of the pure polysaccharide was shown to contain no detectable amount of L-arabinose. Investigation of the arabinose-free material by standard methylation procedures and hydrolysis clearly showed it to be a true xylan composed of 1,4-β linked D-xylose units with a terminal non-reducing xylose residue every 40-repeating units. Isolation of
2-O-methyl-D-xylose as a cleavage product of the methylated xylan indicated that perhaps the side chain xylose was linked 1,3-β to the main chain.

A portion of the molecule may be represented as

\[
\text{Xyl}p_1 - 4-\beta - \text{Xyp}_1 - \text{Xyl}_p 1 - 4-\beta - \text{D-Xyl}_p 1
\]

\[
\text{Xyl}_p 1 - 4-\beta - \text{Xyp}_1
\]

Another pure xylan of the type in esparto grass was isolated from tamarind seeds (48). It is quite similar to the former in that a non-reducing end group is present for every 35 ± 5 D-xylose residues with branching through carbon 3 of the xylose of a linear chain.

An interesting xylan of the above type was found to exist in marine red alga, Rhodymenia palmatta (44). It is a true xylan since on acid hydrolysis only D-xylose is obtained. However, structural determination indicated a novel feature of this polysaccharide. In addition to the common linear backbone of 1,4-β linked D-xylose residues, isolation of 2,4 di-O-methyl-D-xylopyranose suggested the presence of 1,3-β linked D-xylose chain, these being present in the proportions of 4:1, respectively. The main chain, containing these two types of backbone, is terminated by a xylose unit, the whole chain consisting of 17 xylose units. Nothing is yet known concerning the order in which the 1,4- and 1,3-β links are arranged in the linear molecule.
Araboxylans, as the name suggests, are polymers composed of arabinose and xylose residues only. Such a polymer was isolated from wheat flour and the L-arabinose was shown conclusively, by Perlin (40), to be an integral constituent of the xylan. Furthermore, he showed that all the L-arabinose units in wheat flour pentosan were present as non-reducing end-groups in the furanose form. Graded hydrolysis of the soluble pentosan of wheat flour readily liberated three-quarters or more of the arabinose units leaving a water-insoluble araboxylan. Methylation and periodate oxidation of the pentosan showed that it consisted of D-xylopyranose units linked 1,4-\(\beta\). From this chain, radiated L-arabofuranose residues attached at the 2 or 3- positions of the xylose framework.

The hemicellulose of the endosperm of wheat (50), gave on hydrolysis of the methylated polysaccharide, 2,3,5-tri-0-methyl-L-arabinose, 2,3-di-0-methyl-D-xylose, 2-0-methyl-D-xylose and D-xylose in the molar ratio of 3:19:6:4. The "squeegee" fraction of wheat flour (39) on similar treatment gave the sugars in the ratio of 14:24:7:4. Again, a backbone of 1,4-\(\beta\) linked anhydroxylose units is suggested. In both cases, the hemicelluloses are of a highly branched nature since comparatively large amounts of D-xylose and 2-0-methyl-D-xylose are obtained. Side chains of L-arabofuranose residues emanate from the framework and are joined to positions 2 and/or 3 of the latter. A high proportion of arabinose in the araboxylan complex appears to be a general feature of hemicelluloses of wheat grains.

The water soluble polysaccharide from rye-flour (51)
was found to be similar to those from wheat with the exception that branching occurred primarily through carbon 3 of the xylose moiety whereas in the wheat pentosans branching was observed through carbons 2 and 3.

A study of the arabinose-rich fraction of esparto grass hemicellulose also yielded araboxylan. In addition to D-xylose and L-arabinose, however, Aspinall and co-workers (52) found that D-glucose and D-galactose were also present in the hydrolyzate of the hemicellulose. Isolation of 2,3,5 tri-O-methyl-L-arabinose, 2,3,4-di-O-methyl D-xylose and 2-O-methyl-D-xylose (1:3:1) together with small traces of dimethylated arabinose, tetra-, tri- and di-methyl galactose suggest that perhaps mixtures of polysaccharides exist. This information, together with the previous isolation of a true xylan (47) indicate that esparto grass hemicellulose is a mixture of at least two and perhaps three different polysaccharide entities.

Many polysaccharides of the xylan group contain D-glucuronic acid or 4-O-methyl-D-glucuronic acid. Aldobiouronic acids, consisting of a glycosiduronic acid and a sugar are particularly resistant to acid hydrolysis. Consequently, they can be isolated by graded hydrolysis of xylans and other acidic polysaccharides. The mode of linkage between the two moieties can be established by identification of the hydrolysis products of the methylated derivatives. Thus, Jones and Wise (53) isolated the aldobiouronic acid from aspenwood. Identification of the acid proceeded as follows: the acid resistant acidic portion of the hemicellulose was subjected to prolonged acid hydrolysis. Chromatographic examination revealed the presence
of D-xylose and 4-O-methyl-D-glucuronic acid. Methylation of the aldobiouronic acid, followed by hydrolysis, yielded 2,3,4-
tri-O-methyl-D-glucuronic acid and 3,4-di-O-methyl-D-xylose.
Reduction of the aldobiouronic acid with lithium aluminum
hydride (54,55) and methylation afforded 2-0-(2,3,4,6-tetra-
O-methyl-β-D-glucopyranosyl)-3,4-di-O-methyl-D-xylopyranose.
A high positive optical rotation of the disaccharide indicated
the glycosidic linkage between the pentose and the hexuronic
acid moieties to be α and the acid present in aspenwood hemi­
cellulose was therefore 2-0-(4-O-methyl-β-D-glucopyranosyl-
uronic acid)-D-xylopyranose.

That the configuration of the glycosidic linkage in
4-O-methyl-D-glucopyranuronosyl-aldobiouronic acids was, in
fact, α was proved conclusively by Gorin and Perlin (56). The
2-0-(4-O-methyl-D-glucopyranosyl uronic acid)-D-xylose was con­
verted to 2-0-(4-O-methyl-D-glucopyranosyl)-glycerol and then
methylated to yield the hexamethyl ether derivative. A compari­
son of its infrared spectrum and specific rotation with those
of the synthesized hexamethyl ethers of 2-0-α and 2-0-β-D-
glucopyranosyl-glycerol showed the configuration of the oxidized
product to be α.

A crystalline acetate derivative of this acid has
been prepared by Timell (57) and the neutral methylated
disaccharide has been prepared by Dutton and Smith (16). The
β anomer of the glycosiduronic acid, has been synthesized and
characterized by Bowering and Timell (58). The hexuronic acid,
4-O-methyl glucuronic acid, has been obtained by Gorin (59) by the oxidation of the aldobiouronic acid with lead tetraacetate.

The unmethylated aldobiouronic acid, 2-0(α-D-glucopyranosyl uronic acid)-D-xylose, has been isolated by graded hydrolysis. Some less common acids have also been found in plants. Wheat straw (60) and sunflower heads (61) studied by Bishop, and pear cell wall (62) studied by Chanda et al were found to contain 3-0(α-D-glucopyranosyluronic acid)-D-xylose, while New Zealand pine (63) contained the 4-methyl ether derivative, 3-0-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose. An even more rare glycosidic linkage, 1,4-, was observed in hemicellulose B of corn cob (64) as 4-O-(α-D-glucopyranosyluronic acid)-D-xylose.

In all polysaccharides examined so far, the hexuronic acids have been shown to be linked to the xylan backbone as single unit side chains. If the glycosiduronic acid were present as terminal end groups as illustrated below then the 2,3-di-O-methyl-4-0- or 3,4-di-O-methyl-2-0-(2,3,4-tri-O-methyl-D-glucuronic acid)-D-xylose would be obtained. In all cases this was not observed.

D-GpA 1—4-D-Xylp1 or D-GpA 1—2-D-Xylp

Glucuronoxylans are most commonly found in wood and occasionally in graminae. The constitutional analysis of the hemicellulose of wheat straw, which had previously been obtained free from arabinose by extraction with hot 70% aqueous alcohol was shown to consist of approximately 40-45 anhydro-D-xylose units linked 1,4-β in a chain to which a D-glucuronic acid was
attached at carbon 2 of a xylose moiety (21). Araboglucuronoxylans have also been isolated from wheat straw as will be seen later.

Kapok (65) and milkweed floss (66), examined by Timell and co-workers were found to be quite similar except that kapok contained twice as many aldobiouronic acid units as milkweed floss. Pear cell-wall (62) glucuronoxylan also showed structural similarities with the exception that the acid fragment was linked to carbon 3 of a xylose unit instead of carbon 2 as in the previous two examples.

Hemicelluloses from wood constitute a large proportion of polysaccharides which can be classed as glucuronoxylan. Thus, hemicellulose A of European beechwood (*Fagus sylvatica*) (67) gave on hydrolysis D-xylose and 4-O-methyl-D-glucuronic acid. Methylation and periodate oxidation studies showed that the molecule consisted of approximately 70 1,4-β linked D-xylopyranose units with every tenth unit carrying a uronic acid residue at carbon 2 of a xylose unit. Norway spruce (68) was found to consist of 80 ± 5 xylopyranose residues linked in a similar fashion but with every 5th xylose residue carrying a side chain of 4-O-methyl-D-glucuronic acid linked through carbon 2.

The water soluble fraction of American beechwood (*Fagus grandifolia*) (69) is a smaller molecule containing 45 pentose units glycosidically linked 1,4-β with branching at carbon 2 and hence the molecule has 2 non-reducing end groups and one reducing end group. Five units of 4-O-methyl-D-glucuronic acid are joined as single terminal side chains to the xylose unit of the main structure by 1,2 bonds.
The pentosan fraction from Loblolly pine, studied by Jones and co-workers (70-72) was found to be essentially the same as European beechwood. Seven xylose residues were present for one 4-0-methyl-D-glucuronic acid with ten repeating units per molecule. Branching may occur through position 2 of xylose.

The xylan fraction of hemicellulose of white elm (73,74) consists of a backbone of 1,4β linked anhydroxylopyranose units with a 4-0-methyl glucuronic acid glycosidically linked 1,2- to the backbone. It is essentially a linear chain composed of 185 pentose residues, every 7th one containing an acid residue. White birch (75,76) (Betula papyrifera) has a similar structural pattern with the exception that every eleventh xylose residue carries a 4-0-methyl-D-glucuronic acid residue. Sugar maple (77), again shows similar architecture but it is slightly larger, the molecule containing 200 xylose units with every tenth unit carrying an acid side chain.

An araboglucuronoxylan isolated from corn cobs has been extensively studied. By partial degradation of the pentosan, Whistler and co-workers have isolated a homologous series of oligosaccharides from xylose to xyloheptaose (78,79) all with the normal 1,4β links; 2-0-α-D-xylopyranosyl-L-arabinose (80); three aldobiouronic acid fragments, namely, 2-0-(4-0-methyl-α-D-glucopyranosyluronic acid)-D-xylose (81), 4-0-(α-D-glucopyranosyluronic acid)-D-xylose (64), and 2-0-(α-D-glucopyranosyluronic acid)-D-xylose (64); and an aldotriouronic acid, 0-α-D-glucopyranosyluronic acid (1,4)-0β-
D-xylopyranosyl-(1,4)-D-xylose (82). This and other data (83), show that corn cob xylan has a backbone of 1,4-α linked xylose residues to which are attached side chains of D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and L-arabinose residues. Ten side chains of L-arabofuranose are proposed to exist for every 4/4 sugar units and one mono-methyl D-glucuronic acid for each 9-11 sugar units.

Aspinall and co-workers (21), in 1954, obtained a glucuronoxylan by fractionation with hot 70% alcohol which contained only a trace of arabinoxylan. Examination of the arabinoxylan-rich fraction of wheat straw hemicellulose (84), by the same group in 1956, revealed a structure in which this sugar was an integral part of the polysaccharide and that this same sugar occurred exclusively as end-groups in the furanose form. These end-groups are postulated to occur as side chains linked glycosidically through carbon 3 of a xylose residue with the glucuronic acid linked through carbon 2. The structural pattern of this araboglucuronoxylan has been depicted as:

\[
\text{D-Xyl}_p^{3} \xrightarrow{\text{4D-Xyl}} \text{Xyl}_p^{1} \xrightarrow{\text{L-Arab}} \text{D-GpA}
\]

The polyuronide of wheat straw examined by Adams (36) was proposed to consist of approximately 32 anhydro-D-xylose units for every 5 anhydro-L-arabofuranose and 3 D-glucuronic acid units. This hemicellulose has the distinction in that the acid fragment is glycosidically linked 1,3- to the backbone (60). In order to
prove that L-arabinose was indeed an integral part of the molecule and does not originate from an araban, Bishop and Whitaker (86) carried out degradative studies to isolate an oligosaccharide containing arabinose and xylose. A cellulolytic enzyme from mold, *Myrothecium verrucaria*, was used for depolymerization. The enzyme preparation was found to hydrolyze linear chains of 1,4-β linked xylose chains and a series of oligosaccharides containing D-xylose and L-arabinose (di- to heptasaccharides) were isolated. A trisaccharide was characterized as O-(α or β)-L-arabofuranosyl(1,3)-O-β-D-(xylopyranosyl(1,4)-D-xylopyranose (38). These results demonstrate that an araboxylan is present and that isolation of 2-O-methyl-D-xylose arose from branch points in the molecule. Wheat leaf polyuronide is structurally similar to wheat straw polyuronide studied by Adams (87). Hemicelluloses of barley husk (88) and oat-straw xylan (89) also show similarity.

Wheat bran, which was fractionated to remove L-arabinose present as araban (34,90), was shown electrophoretically to consist of D-glucuronic acid, D-xylose and L-arabinose (35). Enzymatic degradation showed the molecules to be linear with 7-8 xylose residues per hexuronic acid units. Isolation of mono-, di-, and tri methylated arabinose indicated the arabo-glucuronoxylan to be highly branched although the xylan portion of the molecule was essentially linear and similar to those found in other wheat plants.

The xylan fraction of western hemlock (*Tsuga heterophylla*) was studied in detail by Dutton and Smith. Hydrolysis
of the hemicellulose gave L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose and an aldobiouronic acid 4-O-(4-O-methyl-D-glucopyranosyluronic acid)-D-xylose (91). The constitution of the xylan polysaccharide, based on methylation studies (16), reveals a branched structure:

\[
\begin{align*}
\beta\text{-D-Xyl}_{1} & \quad \left[ 4\beta\text{-D-Xyl}_{1} \right] & \quad 4\beta\text{-D-Xyl}_{1} & \quad \left[ 4\beta\text{-D-Xyl}_{1} \right] & \quad 4\beta\text{-D-Xyl}_{1} \\
2 & \quad 3 & \quad 3 & \quad 7 \\
\overset{1}{4-O-Me-\alpha-DG} & \quad \text{Arab} & \quad \text{f}
\end{align*}
\]

From mole ratios obtained by quantitative analysis, the polysaccharide was shown to contain three aldobiouronic acid residues associated with about 13 xylose units. This high proportion of uronic acid residues is a distinguishing feature of the hemicellulose of western hemlock. The pentosan fraction of European larch (Larix decidua) (92) contains one hundred 1,4-linked D-xylose, every fifth or sixth xylose residue carrying a terminal 4-O-methyl-D-glucuronic acid linked through position 2 and L-arabinose attached to position 3 of xylose.

In addition to D-xylose, L-arabinose and hexuronic acids, other neutral sugars are occasionally present in some hemicelluloses. These polysaccharides are characterized by the complexity of their chemical architecture. These hemicelluloses are generally highly branched.

Corn hull hemicellulose, which shows promise as an adhesive, thickener or stabilizer is a by-product of the corn milling industry. It has been a subject of intensive investigation in respect to its chemical constitution and structure.
by Smith and co-workers and Whistler and co-workers. On acid hydrolysis D- and L-galactose has been obtained besides the usual sugar residues. Cleavage of the methylated polysaccharide has yielded the following products, 2,3,5-tri-O-methyl-L-arabinose, 3,5-di-O-methyl-L-arabinose, 3-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D and L-galactose, 2,3,4-tri-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, 3-O- and 2-O-methyl-D-xylose, 2-O-2,3,4, tri-O-methyl-D-glucuronic acid)3-O-methyl-D-xylose (93). Graded hydrolysis gives a number of neutral and acidic oligosaccharides including 2-O-(α-D-glucopyranosyluronic acid)-D-xylose (37), 3-O-α-D-xylopyranosyl-L-arabofuranose, 0-L-galactopyranosyl (1,4)-0-xylopyranosyl-(1,2)-L-arabinose, 4-O-(β-D-galactopyranosyl)-β-D-xylose (93), 4-O-β-D-xylopyranosyl-D-xylose and 5-O-(D-galactopyranosyl)-L-arabofuranose (94).

These data show a complex architecture which is unique. Isolation of the tetramethyl galactose indicates that all the galactose residues constitute the non-reducing ends, a fact which is supported by the isolation of oligosaccharides containing galactose. Unlike other hemicelluloses where L-arabinose is invariably found as non-reducing end groups, a few such units occupy a non-terminal position evidenced by the isolation of mon- and di- methylated arabinose. The oligosaccharides provide evidence that the hemicellulose from corn hulls has a highly branched structure and that a variety of branches emanate from the main xylan chain. Further, that some of the xylose residue have multiple branching is indicated by the identification of xylose in the hydrolysis product of the methylated polymer.
The hemicellulose of flax straw (*Linum Usitatissimum* Sp.) (95,96) was shown to contain L-rhamnose. Hydrolysis of the methylated polysaccharide produced a dimethylated L-rhamnose so that either a rhamnoaraboglucuranoxylan is present or an arabo-glucuronoxylan with impurities of rhamnose. The latter possibility is illustrated as

\[
\beta-D-Xyl_1\rightarrow [4-\beta-D-Xyl_1\rightarrow 4-\beta-D-Xyl_2\rightarrow 4-\beta-D-Xyl_1\rightarrow 4-O-Me-\alpha-D-G A_1 \rightarrow \]

Should the former be the case, then rhamnose is joined 1, 3 by glycosidic bonds to the main chain in which case this portion of the molecule may be represented as

\[
4-D-Xyl_1\rightarrow 3 L-Rha_1\rightarrow 4-D-Xyl_1\rightarrow
\]

Wheat straw xylan, studied by Ehrenthal and co-workers (85), gives as a hydrolysis product of the methylated polysaccharide 2,6-di-O-methyl-glucose besides the mono-, di- and tri- methyl xyloses and L-arabinose. The dimethylated glucose is either a 1, 3 and 5 linked glucofuranose or a 1, 3 and 4 linked glucopyranose. Periodate oxidation followed by reduction of the resulting polyaldehyde with Raney nickel yielded a polyalcohol which on hydrolysis gave no glucose. This evidence pointed to a 3,4- linked glucopyranose unit, the reducing end being free.
It is thus seen that the xylans from land plants belong to a general group in possessing an essentially linear molecular chain of repeating xylose units linked glycosidically by 1,4-$\beta$ bonds. Differences in the structures are reflected by the proportions and type of side chains present and the mode of attachment of these side chains. Closely related molecular species exist in which variations in detailed structure are manifested by variations in molecular size. Properties of hemicelluloses vary considerably probably due to the nature of the side chains attached to the main structural backbone and to the extent of regularity or irregularity of branching.
II

APPLE WOOD HEMICELLULOSE

The structures of many hemicelluloses from land plants have been extensively investigated in the last ten years. Most of the work has, however, been confined to examination of cereal crops and hardwoods and softwoods of commercial importance so that in this work the hemicellulose of a fruit tree, apple (var. Transparent Golden), has been studied. Striking similarities have been noticed between the polysaccharides of apple wood and cherry wood (97).

Finely ground apple wood was rendered free of extractives by continuous extraction with hot alcohol-benzene (1:2) azeotrope (7). Delignification of the sawdust was accomplished by shaking the extractive-free material in 0.1N sodium hydroxide at room temperature. The holocellulose, containing the total water-insoluble carbohydrate portion of the plant, was extracted with 1N sodium hydroxide according to the method of McDonald (13). Precipitation of the hemicellulose was effected by pouring the alkali extract into an excess of acidified alcohol. The resulting amorphous solid was dried by solvent exchange. The hemicellulose content of apple wood was found to be 8-12% of the original material.

When the hemicellulose was hydrolyzed with acid there was a marked increase in optical rotation \( ([\alpha]_p -62^\circ \rightarrow [\alpha]_p +41^\circ ) \).
This is generally attributed to the cleavage of \( \beta \) -glycosidic bonds which are characteristic of xylans.

Chromatographic examination of the hydrolyzate revealed the polysaccharide to be composed of D-xylose, traces of other sugars and uronic acids. Separation of the neutral sugars and acidic fragments was effected by the use of ion-exchange columns. D-Xylose was found to be the major component of the neutral fraction other sugars, namely, L-arabinose, D- or L-galactose, L-rhamnose and two other components with \( R_f = 0.168 \) and \( R_f = 0.297 \) being present in trace amounts. D-Xylose was characterized by its melting point and optical rotation.

It is of interest to note that young apple wood, investigated by Gerhard (95) in 1929, was shown to contain D-xylose and L-arabinose in the ratio of 7:1. The intensity of the spots obtained on paper chromatograms indicated that in the hemicellulose investigated in this present work such a high ratio of L-arabinose was not present.

L-Rhamnose has been found in hydrolyzates of wood hemicelluloses but no structural significance has been placed on this desoxy sugar. Hemicellulose from flax straw (95), however, has been suggested to be a rhamnoaraboglucuronoxylan, this suggestion being based on the isolation of a dimethylated rhamnose, 2-4-di-0-methyl-L-rhamnose, as a cleavage product of the methylated polysaccharide. A trimethylated rhamnose was isolated from methylated apple wood hemicellulose but whether L-rhamnose is an integral constituent of the glucuronoxylan is not known. The
identity of the sugars with $R_f = 0.168$ and 0.297 have not been established.

On paper chromatograms, the uronic acid fragments showed three spots corresponding to uronic acid, aldobiouronic acid, and probably aldotriouronic acid. The aldobiouronic acid was isolated by streaking the acidic mixture on paper, irrigating with ethylacetate: acetic acid; formic acid: water solvent system with subsequent elution of areas of the paper containing the desired acid. An acetylated derivative was prepared from the methyl ester methyl glycoside of the aldobiouronic acid according to the method of Timell (57) and the constituent acid characterized as methyl-2-O-\((2,3\text{ di-O}-\text{acetyl-L-D-glucopyranosyl})\) uronate -3,4 di-O-acetyl-D-xylopyranoside (I).
This acetate shows that the aldobiouronic acid from apple wood hemicellulose is 2-O(4-O-methyl-\(\alpha\)-D glucopyranosyluronic acid)-D-xylopyranose (II).

The acetylated polysaccharide was prepared with intention of fractionating it and also methylating the fully acetylated derivative. Unfortunately the product was insoluble in organic solvents. No further attempt was made to fractionate this product.

The hemicellulose from apple wood was methylated six times by Haworth's procedure and three times by Purdie's method. The resulting product showed the absence of a hydroxyl band when examined by infrared. The methylated polysaccharide was fractionally precipitated with petroleum ether. Amalgamation of six fractions yielded enough material for a qualitative examination of the pentosan. (Further portions of hemicellulose were methylated and fractionated. One fraction from this was used for
quantitative analysis of the methylated sugars obtained after hydrolysis of the fully methylated polysaccharide.) The methylated polysaccharide was cleaved with methanolic hydrogen chloride and after the methyl ester of the acidic component had been saponified with barium hydroxide the acidic and neutral components were separated by ion exchange.

In order to further confirm the identity of the acidic portion it was esterified with diazomethane and reduced with lithium aluminum hydride (54,55). The neutral disaccharide glycoside failed to crystallize even on seeding with an authentic sample. Hydrolysis of the syrup and subsequent separation of the mixture on a cellulose-hydrocellulose column yielded 3-0-methyl-D-xylose and 2,3,4-tri-0-methyl-D-glucose which were characterized as their crystalline anilides. The methylated aldobiouronic acid is therefore, 2-0-(2,3,4-tri-0-methyl-\(\alpha\)-D-glucopyranosyluronic acid)-3-0-methyl-D-xylose (III). This further substantiates the aldobiouronic acid as being (II).

![Diagram](III)
The presence of a free hydroxyl group at position 4 of the xylose unit establishes the fact that the xylose moiety is joined to other units through this position. Since this particular xylose residue is probably linked to other residues through its reducing end, the former constitutes a branch point in the molecular complex. Clearly, the 4-0-methyl-D-glucuronic acid is attached as a single unit side chain to the 2 position of the xylose moiety by a glycosidic bond because only the 3-0-methyl-D-xylose is obtained from the methylated aldobiouronic acid of the methylated polysaccharide.

The mixture of neutral sugar glycosides obtained from the methanolysis of the methylated apple wood hemicellulose was hydrolyzed and resolved on a cellulose-hydrocellulose column. The mixture was found to contain tri-0-methyl-rhamnose, 2,3,4-tri-0-methyl-D-xylose, 2,3-di-0-methyl-D-xylose, 2-0 and 3-0-methyl xylose, 4-0-(2,3-di-0-methyl-D-xylopyranosyl)-2,3-di-0-methyl-D-xylose, and small amounts of D-xylose and di-0-methyllyxose.

The fastest running component from the separation was tentatively identified as a trimethylated rhamnose with Rf 0.856. The 2,3,4-tri-0-methyl-D-xylose was identified by qualitative chromatography. The trimethylated xylose gave a spot on a paper chromatogram which corresponded to the Rf of an authentic sample of 2,3,4-tri-0-methyl-D-xylose. Preparation of a crystalline anilide derivative of the sugar failed. Optical rotation indicated that the syrup was only 60% pure this probably being the reason for the anilide not crystallizing. The 2,3-di-0-methyl-
D-xylose was identified by its transformation to the characteristic crystalline anilide. The monomethyl xyloses were obtained as a mixture of the 2-O- and 3-O-methyl-D-xylose. That they were in fact a mixture was established by the comparison of chromatographic and electrophoretic mobilities of the sample and of authentic samples of 2-O- and 3-O-methyl-D-xylose.

The component with $R_f$ 0.71 gave on hydrolysis 2,3-di-O-methyl-D-xylose. Geedes and Smith (95) also obtained a component with the same $R_f$ value, and which on hydrolysis gave the same dimethylated xylose. They established the sugar to be 4-O-(2,3-di-O-methyl-D-xylopyranosyl)-2,3-di-O-methyl-D-xylose. In this work, this constituent was obtained crystalline and had optical rotation $[\alpha]_D^\circ +112^\circ$, while Geedes and Smith obtained their xylobiose as a syrup, $[\alpha]_D^\circ 100^\circ$. It has been stated previously that high negative optical rotations are indicative of $\beta$ glycosidic linkages. Since this xylobiose has a high positive rotation, it can be deduced that the bond between the two xyloses is of the $\alpha$-type. Jones and co-workers (72), have shown that on treatment of D-xylose with acid condensation polymerization occurs with the formation of oligosaccharides. Thus, when the polysaccharide is hydrolyzed by acid, the monosaccharides undergo condensation with the formation of oligosaccharides which contain glycosidic linkages differently placed, and of a type differing from those present in the original polymer. In this present work, whether some glycosidic bonds of the $\alpha$-type do exist in the natural polymer or whether some reversion of the 2,3-di-O-methyl-D-xylose has occurred to produce an artefact is
not known. (Oligosaccharides of xylose isolated from corncobs have $\beta$ glycosidic linkages as evidenced by their negative rotations (78).)

The component leaving the column between 2,3 di-0-methyl-D-xylose and the mono-methyl xyloses had an $R_f$ of 0.396 and an optical rotation of $[\kappa]_b = -26^\circ$ (ca) in methanol. Larger amounts of a similar unknown component, which had an optical rotation of $[\kappa]_b = -36^\circ$ in water and which appeared to be chromatographically and electrophoretically similar to the unknown constituent in methylated apple wood hemicellulose, were also isolated from cherry wood, (97) studied by Dutton and McKelvey. In the case of cherry wood the sugar was found to have a methoxyl content of 34.5% which is indicative of a dimethyl pentose. It was further shown by these workers that the unknown component was not 2,4-di-0-methyl-D-xylose since on chromatography the $R_f$ values of the unknown pentose and 2,4-di-0-methyl-D-xylose were not identical. The possibility that this sugar was 3,4- or 3,5-di-0-methyl-D-xylose was also discarded since the latter sugars will show electrophoretic migration while the unknown pentose showed no migration. Demethylation of the unknown sugar with boron trichloride (98) showed the parent sugar to be lyxose. Chromatographic examination of the dimethylated lyxose from cherry wood with an authentic sample of 2,3-di-0-methyl-D-lyxose showed these two sugars to be identical. A mixed melting point also indicated that they were identical. (The dimethylated lyxose was not obtained crystalline from methylated apple wood.)
It is of interest to note that Montgomery and Smith (39) also obtained an unknown dimethyl pentose from wheat flour which crystallized spontaneously but failed to give a crystalline anilide.

No lyxose was found by acid hydrolysis of the hemicellulose from either cherry wood or apple wood. Hence, this pentose could conceivably arise from epimerization of 2,3-di-O-methyl-D-xylose to 2,3-di-O-methyl-D-lyxose. The mechanism may be proposed

![Chemical structure](image)

Prentice and co-workers (99), have taken advantage of this principle to prepare 2,4,6-tri-O-methyl-mannose from 2,4,6-tri-O-methyl-glucose, a reaction which involves the epimerization of carbon 2.

From the above experimental evidence certain structural features of the hemicellulose can be deduced. It is clear that the 2,3,4-tri-O-methyl-D-xylose is derived from terminal xylopyranose units in the polysaccharide and if the trimethylated rhamnose is an integral part of the polysaccharide then it would also occur as non-reducing end groups attached to the linear backbone. From the large amount of 2,3-di-O-methyl-D-xylose it can be deduced that the backbone consists primarily of 1,4-linked xylose units. Some of the 3-O-methyl-D-xylose probably
arises due to the hydrolysis of the aldobiouronic acid. The presence of a mono-uronic acid supports this assumption. The remaining amounts of 3-0-methyl-D-xylose and the 2-0-methyl-D-xylose may very well arise from branching in the xylan although in view of the evidence presented by Croon and Timell (100), the mono-methyl xyloses may be due to incomplete methylation. It has previously been stated that the 4-0-methyl-D-glucuronic acid is linked by \( \alpha \) glycosidic bonds to carbon 2 of the xylose moiety.

From the quantitative analysis the tri-, di- and mono-methylated pentoses and uronic acid were found to be present in the mole ratio of (1:97:21:19). The results obtained indicate that the hemicellulose has a chain length of approximately 119 units with a uronic acid residue attached to about every sixth residue as single unit side chain. From the small amounts of monomethylated xyloses present one can only conclude that the molecule is only slightly branched or that it is essentially linear. The structure of the glucuronoxylan may be represented as IV.

\[
\begin{align*}
\text{D-Xyl}_{1} & \left[ 4-\beta-\text{D-Xyl}_{1} \right]^{97} \left[ 4-\beta-\text{D-Xyl}_{1} \right]^{2} \left[ 4-\beta-\text{D-Xyl}_{1} \right]^{1} \left[ 4-0-\text{Me-}\alpha-\text{D-G}_{pA} \right]^{19} \\
\end{align*}
\]

(IV)
The constitution of the hemicellulose of apple wood is similar, although not identical, to that of other methyl glucuronoxylans isolated from deciduous woods. These polysaccharides are all essentially linear with the exception of possibly American beechwood (69), and contain side chains of 1,2-\(\alpha\) linked 4-O-methyl-D-glucuronic acid. Of the hardwoods studied all contain hemicellulose with 10-11 xylose units per acid side group except white elm (73,74), and cherry (97), which contain 7 xylose residues per acid side chain. Thus, an unusual feature of apple hemicellulose is apparent in that it has a high uronic acid content (6:1), a general characteristic feature of softwoods.
EXPERIMENTAL

The following solvent systems (v/v) were used to separate sugars on paper chromatograms; (A) ethyl acetate: acetic acid: formic acid: water, 18:3:1:4; (B) n-butanol: ethanol: water: ammonia, 40:11:19:1; (c) methyl ethyl ketone-water azeotrope; (D) n-butanol: acetone: water, 5:4:1. Whatman No. 1 paper was used for all qualitative separations and Whatman No. 3MM for quantitative separations. With solvent system (D) phosphate impregnated papers, which were prepared by dipping papers into a buffer solution of disodium hydrogen phosphate and potassium dihydrogen phosphate (pH = 5) were used (101). Electrophoresis was carried out on Whatman No. 1 paper in 0.05M sodium borate (pH = 9.2) (102). Sugars were detected with p-anisidine trichloroacetic acid and aniline phosphate sprays. (103)

A. ISOLATION OF APPLE WOOD HEMICELLULOSE

A log of apple wood was reduced to sawdust in a Wiley mill. The sawdust (200 gm. portions) was exhaustively extracted with hot alcohol-benzene (1:2) for four hours, and air-dried. Delignification of the sawdust was effected by suspending the extracted sawdust in 0.1N sodium hydroxide (1.8 l.) for 24 hours, at room temperature after which the mixture was filtered and the
colored liquor discarded. The residue was washed free of alkali and then extracted with N sodium hydroxide (1.8 l.) at room temperature for 48 hours, according to the method of McDonald (13). The alkaline extract was collected and the residue washed with water to make up a volume of 2 l.

The combined alkali extract (500 ml. portions) was acidified with acetic acid (25 ml.) and precipitated by the addition of 3 volumes of ethanol. The light brown colored hemicellulose thus obtained was centrifuged and washed successively with ethanol, diethyl ether, petroleum ether and dried. A typical yield from 100 gm. sawdust was 8 gm. of hemicellulose which had $[\alpha]_D^{20} -62^\circ$ (c,1.2 in 8% NaOH), neutralization equivalent of 741, and ash content of 4.66%.

A second batch of apple wood sawdust was extracted as above except that the mixture of holocellulose and N sodium hydroxide was agitated for 48 hours. This procedure increased the yield of hemicellulose to 12%.

B. HYDROLYSIS OF APPLE WOOD HEMICELLULOSE

A solution of hemicellulose (214 mg.) in 2N sulphuric acid (20 ml.) was heated for 9 hours on a steam bath at which time the optical rotation had changed from $[\alpha]_D^{20} -62^\circ$ to $[\alpha]_D^{20} +41^\circ$. The dark colored solution was neutralized with saturated barium hydroxide. Barium sulphate was removed by centrifugation and the yellow solution passed through Amberlite IR 120 resin. The eluate from the cation resin was passed through Duolite A-4 resin which
selectively absorbed the acidic components. The columns, in each case, were washed until the eluates gave a negative Molisch test. The acidic components were liberated from the anion resin with 2N sodium hydroxide (5 ml.) and passed through a fresh column of Amberlite IR-120. The eluate containing the acidic components was concentrated (24.5 mg.) and examined chromatographically using solvent system A as the irrigating solvent (7 hours). Three spots appeared on spraying with p-anisidine trichloroacetic acid corresponding to uronic acid, aldobiouronic acid, and probably triouronic acid, \( R_x = 1.2, 1.0, 0.69 \), respectively.

The eluate containing the non-acidic sugars was evaporated to a syrup (144 mg.). Chromatographic examination of the neutral sugars in various solvent systems (A - D) showed a large spot corresponding to xylose. In solvent system (D) faint spots corresponding to arabinose, rhamnose and galactose were obtained while in solvent system (C) two spots with \( R_f \) values of 0.168 and 0.297 also appeared.

C. SEPARATION OF THE ACIDIC COMPONENT OF APPLE WOOD HEMICELLULOSE

The wet hemicellulose was hydrolyzed in larger quantity (ca 30 gm.). It was dissolved in 2N sulphuric acid (1000 ml.), heated for 11 hours, neutralized with barium carbonate and passed through the resin columns as above. The eluate containing the acidic components was concentrated in vacuo at 35-40°C, yielding a dark brown syrup (5.844 gm.). The eluate containing the neutral sugars was also concentrated in vacuo (22.14 gm.).
A portion of the syrupy acidic constituent was streaked on Whatman No. 3MM (146 X 57 cms.) filter paper which had been previously washed with the irrigating solvent (A). The sheets were irrigated for 16 hours. Strips, 2.5 cm. in width, were cut off the sheets and developed with p-anisidine trichloroacetic acid. Only areas containing the desired aldobiouronic acid as shown by the spray reagent were cut out. These portions containing the product were cut into 1 mm. squares, packed in a column (3.5 X 14 cms.) and eluted with methanol until a negative Molisch test was obtained. The solution was evaporated to dryness in vacuo at 35-40°C. Yield of the aldobiouronic acid was 146 mg.

D. PREPARATION OF THE CRYSTALLINE DERIVATIVE OF 2-O-((4-O-METHYL-\(\alpha\)-D-GLUCOPYRANOSYLURONIC ACID)-D-XYLOPYRANOSE

1. The Methyl Ester Methyl Glycoside

The methyl ester of the aldobiouronic acid was prepared by dissolving the acid (146 mg.) in methanol (15 ml.). Methanolic hydrogen chloride was added (15 ml. methanol, 1.6 ml. acetyl chloride) and the solution boiled under reflux for nine hours. After neutralization with silver carbonate the excess Ag was removed by passage of hydrogen sulphide gas through the solution. Filtration followed by evaporation yielded a syrup (119.4 mg.).
2. **Methyl 2-0- Methyl (2,3-di-O-Acetyl-4-0-methyl-α-D-glucopyranosyl) uronate -3,4-Di-O-acetyl-D-xylopyranoside**

The methyl ester methyl glycoside of the aldobiouronic acid (119.4 mg.) was dissolved in dry pyridine (15 ml.) and redistilled acetic anhydride (5 ml.) was added. After standing 21 hours at room temperature, the solution was poured into ice water (150 ml.) and the aqueous solution extracted with chloroform (ca 130 ml.). The chloroform layer was washed with ice-cold 10% HCl (10 X 10 ml.), followed by saturated sodium bicarbonate (3 X 20 ml.) and subsequently with water (1 X 50 ml.). After drying over magnesium sulphate, the chloroform layer was evaporated to a pale yellow syrup (89.2 mg.) which was dissolved in boiling diethyl ether. On cooling the solution, crystals immediately formed. The white crystals (11 mg.) were washed with cold diethyl ether. M.p. 191-193°C., mixed m.p. 194-196°C. (57). Insufficient material was available for measurement of optical rotation.

E. **IDENTIFICATION OF D-XYLOSE**

Concentration of the eluate from the Duolite A-4 column afforded D-xylose as the major component. Recrystallization from methanol-water gave crystals of D-xylose, m.p. 143-145°C., mixed m.p. 143.5-145.5°C., \([\alpha]_D^{25} + 17.7^\circ\) (c, 1.69, water).

The trace sugar, presumed to be rhamnose, was shown to be, in fact, chromatographically identical to rhamnose when run in solvent system (D). It was also identical electrophoretically to the same sugar. In both cases, the spots gave a yellow color, on
spraying, which was characteristic rhamnose. Arabinose and galactose were also identified chromatographically in solvent (D).

The mother liquor, after the majority of xylose had been removed, showed two other components with $R_f = 0.168$ and 0.297 when examined chromatographically in solvent (C).

**F. ACETYLATION OF APPLE WOOD HEMICELLMULOSE**

Apple wood hemicellulose (1.02 gm.) was dissolved, with heating, in dimethyl sulfoxide (110 ml.). The undissolved hemicellulose was centrifuged off. Pyridine (30 ml.) was added, followed by dropwise addition of acetic anhydride (30 ml.). During the reaction the mixture was maintained at a low temperature (0°C.) and then allowed to stand 3 days at room temperature. The solution was precipitated into hydrochloric acid and ice-water. The acetylated polysaccharide oiled out.

The yellow oil and water mixture was extracted with chloroform, evaporated and reacetylated with pyridine and acetic anhydride. This was allowed to stand 24 hours. The solution was then poured into ethereal methanolic hydrochloric acid but no precipitate resulted. Further isolation of a solid derivative was not attempted. Failure to obtain the acetylated polysaccharide was probably due to its high solubility in dimethyl sulfoxide.

A further portion of hemicellulose (10 gm.) was treated in a similar manner as described above except that dimethylformamide was used as the solvent instead of dimethylsulfoxide. Precipitation of the solution, containing the acetylated hemicellu-
lose, into methanolic hydrochloric acid resulted in the precipitation of brownish-yellow flocculent solid (13.4 gm.).

Fractionation of the acetylated derivative was not attempted since the dried product was not soluble in organic solvents such as chloroform, acetone and dimethylformamide.

G. METHYLATION OF APPLE WOOD HEMICELLULOSE

1. Methylation I

Hemicellulose (ca 20 gm.) was dissolved, by mechanical stirring, in 8% sodium hydroxide (92 ml.). After dissolution of the hemicellulose sodium hydroxide pellets (31.3 gm.) were added to bring the concentration of the alkaline solution to 30%. The polysaccharide was methylated at 50°C. by the dropwise addition of dimethyl sulphate (300 ml.) and 30% sodium hydroxide (900 ml.) over a period of 2 hours. Acetone was added to control the frothing and to reduce the viscosity. During the methylation procedure, the hemicellulose began to precipitate out. After addition of the reagents the mixture was heated in a boiling water bath for 1 hour to decompose the excess dimethyl sulphate and to distill off the acetone. The partially methylated polysaccharide precipitated on the sides of the reaction flask so that the liquor was able to be decanted off. The solid was washed with boiling water. No attempt was made at this time to reduce the content of the inorganic salts occluded onto the solid.

The above Haworth methylation procedure was repeated
5 more times. After the sixth methylation a portion of the solid was dialyzed overnight against hot water and then cold water. The aqueous solution was acidified with sulphuric acid and extracted with chloroform. The chloroform was washed with water until neutral to litmus paper and evaporated to a dark brown syrup. (Yield, 3.745 gm.)

2. Methylation II

Since the yield of partially methylated polysaccharide was too little for subsequent methylation and fractionation the procedure was repeated with further quantity of hemicellulose (26 gm.).

After five Haworth methylations, one-half of the product was suspended in ice-cold water and acidified with sulphuric acid to precipitate the polysaccharide. The mixture was then made alkaline (pH 8), centrifuged and the precipitate extracted with acetone. On refluxing the solid, a saturated salt layer separated. This was exhaustively extracted with acetone.

The acetone extract, evaporated to dryness, was redissolved in acetone (200 ml.), methanol (60 ml.) and methyl iodide (100 ml.). Silver oxide (30 gm.) was added over a period of 3 hours to the refluxing solution. The mixture was stirred mechanically for 23.5 hours to effect the methylation according to Purdie. The excess of methyl iodide was recovered by distillation and the insoluble residue repeatedly extracted with boiling methanol. Evaporation of the combined methanolic extracts
gave a syrup which was shown to be incompletely methylated when examined by infra-red spectroscopy.

After two more Purdie methylations no apparent hydroxyl peak appeared on the infra-red spectrum. A small sample of the syrup, dissolved in chloroform, was poured into an excess of petroleum ether to yield a white powder, OCH$_3$ 38.7%.

3. Methylation III

The remaining one-half of the partially methylated polysaccharide from (2) was dialyzed to remove the occluded inorganic salts. A small portion of the solid was wetted with a little water and dialyzed against running hot water for 4 days. The aqueous suspension (700 ml.) was evaporated to a small bulk (250 ml.), acidified and extracted with chloroform as before. The chloroform layer was evaporated in vacuo to a syrup (3.241 gm.). The remainder of the salt enriched material was similarly treated. Total yield, 11.15 gm.

The extracted product (11.15 gm.) was dissolved in acetone and methylated three times by Purdie method, OCH$_3$ 38.7%.

H. FRACTIONATION OF THE METHYLATED HEMICELLULOSE

The syrup obtained from the third Purdie methylation II was dissolved in chloroform (100 ml.), diluted with diethyl ether to precipitate any inorganic impurities and allowed to stand over-night. The solution was centrifuged and excess petroleum ether was added to the mechanically stirred solution until a cloudiness was observed. The mixture was centrifuged and the oil obtained
was redissolved in chloroform (5 ml.) and again precipitated into petroleum ether (200 ml.). The white precipitate obtained was dried by solvent exchange. The centrifugate, after precipitation of the first fraction, was treated in a similar manner until no more polysaccharide precipitated upon further additions of petroleum ether. The results of this fractionation are shown in Table IA. Table IB shows a similar set of results obtained from the fractionation of Methylation III.

Fractions 2-7 were amalgamated and further structural analysis was conducted. Since, however, these fractions were not completely methylated, as shown by methoxyl determinations, fraction 3 from Methylation III was used for quantitative analysis.
# TABLE IA

**FRACTIONAL PRECIPITATION OF METHYLATED APPLE WOOD HEMICELLULOSE II**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total petroleum ether added, ml.</th>
<th>Weight, gm.</th>
<th>$[\alpha]_p$ in CHCl$_3$</th>
<th>$%$OCH$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>215</td>
<td>0.2357</td>
<td>-</td>
<td>37.4</td>
</tr>
<tr>
<td>2</td>
<td>240</td>
<td>0.6180</td>
<td>-52.6</td>
<td>39.9</td>
</tr>
<tr>
<td>3</td>
<td>290</td>
<td>0.7354</td>
<td>-54.1</td>
<td>38.6</td>
</tr>
<tr>
<td>4</td>
<td>310</td>
<td>0.1586</td>
<td>-51.3</td>
<td>38.6</td>
</tr>
<tr>
<td>5</td>
<td>390</td>
<td>0.2954</td>
<td>-50.0</td>
<td>38.4</td>
</tr>
<tr>
<td>6</td>
<td>430</td>
<td>0.1672</td>
<td>-50.6</td>
<td>41.7</td>
</tr>
<tr>
<td>7</td>
<td>530</td>
<td>0.4544</td>
<td>-44.8</td>
<td>38.9</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1.2282</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fraction</td>
<td>Total petroleum ether added, ml.</td>
<td>Weight, gm.</td>
<td>[α]_D in CHCl₃</td>
<td>%OCH₃</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------</td>
</tr>
<tr>
<td>1</td>
<td>400</td>
<td>0.4720</td>
<td>-39.0</td>
<td>35.2</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>0.9351</td>
<td>-55.5</td>
<td>38.3</td>
</tr>
<tr>
<td>3</td>
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<td>1.2851</td>
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<tr>
<td>4</td>
<td>780</td>
<td>0.3657</td>
<td>-52.6</td>
<td>38.5</td>
</tr>
<tr>
<td>5</td>
<td>980</td>
<td>1.0930</td>
<td>-51.6</td>
<td>39.8</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>2.6314</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
I. METHANOLYSIS OF APPLE WOOD HEMICELLULOSE

The methylated hemicellulose from fraction 2-7 (Methylation II) (2.0186 gm.) was suspended in methanol (25 ml.). Methanolic hydrogen chloride (2 ml. acetyl chloride in 25 ml. methanol) was added and the mixture refluxed on a steam bath. The methanolysis could not be readily followed polarimetrically but it was allowed to proceed for 7 hours when the optical rotation seemed apparently constant. The solution was neutralized with silver carbonate, filtered and the methyl glycosides concentrated to a dark syrup (2.1306 gm.).

J. SAPONIFICATION OF METHYL ESTER OF THE ALDOBIOURONIC ACID

The syrup (2.1306 gm.) was dissolved in barium hydroxide (25 ml., saturated at room temperature) and heated for 2 hours at 60°C. to saponify the methyl ester of the acidic component. The aqueous solution was then continuously extracted with petroleum ether (200 ml.) for 20 hours. It was filtered from traces of solid and passed through a column of Amberlite IR 120 resin to remove the barium ions. The column was washed until the washings gave a negative Molisch test.

The eluate from above was passed through a column of Duolite A-4 resin which selectively absorbed the acidic component. The effluent from this column was concentrated to a syrup. The petroleum ether extract was added to this syrup and the whole reevaporated to constant weight (1.5172 gm.). This syrup
was subsequently separated and the neutral sugars identified.

K. **SEPARATION OF THE ACIDIC COMPONENT OF METHYLATED APPLE WOOD HEMICELLULOSE**

The acidic component was displaced from the Duolite A-4 column with 2N sodium hydroxide (25 ml.) and passed through a fresh column of Amberlite IR 120. The eluate and washings were evaporated to a dark syrup (475.5 mg.).

L. **PREPARATION OF METHYL 2-O-(2,3,4-TRI-O-METHYL-D-GLUCURONOSYL)-3-O-METHYL-D-XYLOSE METHYL ESTER**

1. **Preparation of Diazomethane**

   An ethereal solution of p-tolysulfonylmethyl nitrosonamide (4 gm. in 23 ml. of diethyl ether) was added dropwise to a solution containing potassium hydroxide (1.1 gm. in 2 ml. water), carbitol (6.5 ml.) and diethyl ether (2 ml.) which had previously been heated to 70-75°C. in a water bath. The diazomethane produced by this reaction was distilled into diethyl ether (38 ml.) which was cooled to -5°C.

2. **Preparation of Methyl Ester of Methyl Glycoside of Aldobiouronic Acid**

   To the dissolved partially methylated aldobiouronic
acid (475.5 mg in 25 ml. methanol), was added one-half of ethereal diazomethane prepared above. The solution was allowed to stand overnight after which the excess diazomethane and solvent were removed by distillation. The 2-O(2,3,4-tri-O-methyl-D-glucuronosyl)-2-O-methyl-D-xyloside methyl ester (464 mg.) gave a positive hydroxamic acid test for esters.

M. REDUCTION OF THE METHYL ESTER OF METHYL 2-O-(2,3,4-TRI-O-METHYL-D-GLUCURONOSYL)-3-O-METHYL-D-XYLOSIDE

The ester (464 mg.) was dissolved in dried tetrahydrofuran (75 ml. dried by distillation from lithium aluminium hydride). A solution of lithium aluminium hydride was prepared by refluxing for 1 hour finely ground hydride (500 mg.) and dry tetrahydrofuran (20 ml.). The solution containing the ester was added slowly and at room temperature to the reducing agent and then the whole refluxed for 1 hour after the addition was complete. Excess hydride present was decomposed by the addition of ethereal ethyl acetate followed by dilute acetic acid. The mixture was evaporated to dryness and acetylated without attempting to separate the reduced material.

Anhydrous sodium acetate (500 mg.) and redistilled acetic anhydride (15 ml.) were added to acetylate the reaction mixture. Simultaneous cleavage of the inorganic addition complex and acetylation of the disaccharide was achieved by heating the mixture for 3 hours on a steam bath. The excess acetic anhydride was removed by distillation. Dilute hydrochloric acid
(20 ml.) was added to dissolve the inorganic salts and the acidic solution extracted with chloroform (125 ml.). The chloroform layer was washed with water until free of chloride ion and then evaporated to dryness, to yield a thick clear syrup (363 mg.).

Deactylation was effected by the addition of methanolic sodium hydroxide (6 ml. of 1N sodium hydroxide in 10 ml. methanol) and heating under reflux for 1 hour on a steam bath. Deionization and purification of the glycoside disaccharide was effected by passage of the solution through columns of Amberlite IR 120 and Duolite A-4 respectively.

The clear neutral syrup (344 mg.) failed to crystallize even after seeding with an authentic sample of methyl 2-O-(2,3,4-tri-O-methyl-D-glucopyranosyl)-3-O-methyl-D-xyloside.

N. HYDROLYSIS OF METHYL 2-O-(2,3,4-TRI-O-METHYL-D-GLUCOPYRANOSYL)-3-O-METHYL-D-XYLOSIDE

The syrupy disaccharide (186 mg.) from above was dissolved in methanol (10 ml.) containing acetyl chloride (0.5 ml.). After refluxing for 5 hours on a steam bath the solution was neutralized with silver carbonate. Extraction of the mixture with ethyl acetate followed by filtration and evaporation of the extracted liquor yielded the methyl glycosides as a syrup (202.5 mg.).

The methyl glycosides were hydrolyzed in N sulphuric acid (5 ml.) for 7 hours on a steam bath. Sulphuric acid was removed by deionization with Duolite A-4 resin. Concentration of the eluate yielded the hydrolyzed products as a syrup
(126 mg.). Paper chromatograms and electrophorograms revealed spots identical to 2,3,4-tri-O-methyl-D-glucose and 3-O-methyl-D-xylose as the major components and a trace of what was assumed to be incompletely hydrolyzed material. The remainder of the syrup was added to a cellulose-hydrocellulose column by dissolution of the syrup with a little irrigating solvent (C). The eluate was collected (12 ml. per fraction) at 20-minute intervals.

1. **Identification of 2,3,4 Tri-O-Methyl-D-Glucose**

Concentration of the solutions of tubes 11-17 gave a syrup (37.2 mg.) which was chromatographically identical to 2,3,4-tri-O-methyl-D-glucose, \([\chi]_D^{+} 73.1^\circ (c, 0.7, \text{ethyl acetate}).\) The anilide was prepared by dissolving the syrup (37.2 mg.) in ethanol (1 ml.), adding redistilled aniline (0.2 ml.) and refluxing for 3 hours. The excess solvent and aniline were evaporated in vacuo and the residue left to crystallize. Recrystallization from ethyl acetate-petroleum ether yielded white needle-like crystals, m.p. 141-143°C. Literature value, 145-146°C. (104).

2. **Identification of 3-O-Methyl-D-Xylose**

Amalgamation of the contents of tubes 60-80 afforded a syrup (23.6 mg.) which failed to crystallize. The anilide of 3-O-methyl-D-xylose which was prepared in a similar manner as above, had m.p. 133-134°C, after recrystallization from ethyl acetate-petroleum ether.
0. SEPARATION OF THE NEUTRAL COMPONENTS OF METHYLATED APPLE WOOD HEMICELLULOSE

The methyl glycosides (1.357 gm.) were hydrolyzed in N sulphuric acid (60 ml.) under reflux for 8 hours. The solution was neutralized with barium carbonate, centrifuged and the residue extracted with methanol. Concentration of the centrifugate and washing yielded a brown syrup (1.156 gm.).

The hydrolyzed monosaccharides (1.042) were dissolved in methyl ethyl ketone-water azeotrope (1.5 ml.) and placed on a cellulose-hydrocellulose column and irrigated with the same solvent. One hundred fractions were collected at 10 minute intervals. Further 100 fractions were collected at one-half hour intervals. The positions of the sugars were determined by placing 5 spots of solution from each tube on paper and spraying with p-anisidine trichloroacetic acid. Where approximate breaks occurred the respective tubes were concentrated and investigated for purity by paper chromatography and electrophoresis. Fractions containing chromatographically pure samples were amalgamated. The results of the separation are shown on Table II.
## TABLE II

SEPARATION OF METHYLATED SUGARS  

of  

APPLE WOOD HEMICELLULOSE

<table>
<thead>
<tr>
<th>Component</th>
<th>Tube Number</th>
<th>Weight, mg.</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6-9</td>
<td>17.0</td>
<td>Tri-O-methyl-rhamnose</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>1 + 2</td>
</tr>
<tr>
<td>2</td>
<td>11-13</td>
<td>21.9</td>
<td>2,3,4-Tri-O-methyl-D-xylose</td>
</tr>
<tr>
<td>14-15</td>
<td></td>
<td>14.7</td>
<td>2 + 3</td>
</tr>
<tr>
<td>3</td>
<td>16-22</td>
<td>56.0</td>
<td>Methylated xylobiose</td>
</tr>
<tr>
<td>23-31</td>
<td></td>
<td>32.6</td>
<td>3 + 4</td>
</tr>
<tr>
<td>4</td>
<td>32-53</td>
<td>637.6</td>
<td>2,3-Di-O-methyl-D-xylose</td>
</tr>
<tr>
<td>54-59</td>
<td></td>
<td>22.0</td>
<td>4 + 5</td>
</tr>
<tr>
<td>5</td>
<td>60-75</td>
<td>23.0</td>
<td>Di-O-methyl-lyxose</td>
</tr>
<tr>
<td>76-108</td>
<td></td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>109-113</td>
<td>15.6</td>
<td>2- and 3-Monomethyl-D-xylose</td>
</tr>
<tr>
<td>7</td>
<td>160-185</td>
<td>8.6</td>
<td>D-Xylose</td>
</tr>
</tbody>
</table>

Recovery 83%
P. IDENTIFICATION OF THE COMPONENTS

1. Component 1, Tri-0-Methyl Rhamnose

This fraction obtained in very small quantity gave a spot, on paper chromatography, with a large Rf value of 0.856. This was assumed to be a tri-0-methyl rhamnose. Further investigation into the exact identity of this sugar was not conducted.

2. Component 2, 2,3,4 Tri-0-Methyl-D-Xylose

This fraction, also obtained in small quantity, was found to be chromatographically identical to 2,3,4-tri-0-methyl-D-xylose. The optical rotation of the syrup [α]D²⁰ ca 5.68°(c, 0.4 in methanol) indicated that it was impure. The anilide failed to crystallize. The neutral sugar was regenerated by heating the anilide in hot water and adding a little Amberlite IR 120 resin (105). Optical rotation of the recovered syrup (8.5 mg.) was found to be [α]D°+11.2°(c, 0.5 in methanol), indicating that it was still only 60% pure. Theory [α]D°+18.5° (Methanol). No further attempt was made to identify the sugar as a crystalline derivative.

3. Component 3, 4-0-(2,3-Di-O-methyl-D-xylopyranosyl)-2,3-Di-O-methyl-D-xylose

The syrup obtained from this fraction had an Rf of 0.71 and an optical rotation of 112°(c, 3.1, methanol). It crystallized readily, on evaporation of the solvent, to long feathery needles. However, all attempts to recrystallize the
substance from various solvents failed. (M.p. 83.0-83.5°C.). Hydrolysis of a small portion of this material and subsequent chromatography showed it to yield only 2,3-di-O-methyl-D-xylose.

4. **Component 4, 2,3 Di-O-methyl-D-xylose**

This component (R_f 0.495) was found to be the major constituent of the methylated hemicellulose. It failed to recrystallize from ethyl acetate-petroleum ether and was therefore identified as the crystalline anilide, m.p. 122-123°C.

5. **Component 5, Di-O-methyl Lyxose**

This fraction (R_f 0.396) which was present in only a small amount has not been completely elucidated but appears to be a di-methylated pentose. A similar fraction was obtained from cherry wood hemicellulose (97). It has [α]_D -26° (ca) in methanol.

a. **Demethylation of Di-O-methyl Pentose**

Boron trichloride (redistilled) was distilled into a receiver containing a solution of dimethyl pentose (9.6 mg.) in dried dichloromethane (3 ml.). The solution was kept at -78°C. for 30 minutes and then allowed to come to room temperature. It was then allowed to evaporate overnight under anhydrous conditions. The remaining residue was dissolved in aqueous methanol. Electrophoresis of the demethylated sugar gave a spot identical to that of D-lyxose. Paper chromatography of the demethylated component in solvent (D) also indicated the presence of D-lyxose.
6. Component 6, 2-O and 3-O-Methyl-D-xylose

This fraction appeared to be homogeneous on paper chromatograms but was readily shown by electrophoresis to be a mixture of 2- and 3-O-methyl-D-xylose. No further attempt was made to separate the two mono-methyl xyloses.

7. Component 7, D-Xylose

Concentration of this fraction yielded a very small amount of syrup which did not crystallize. Chromatographic identification indicates it may be D-xylose due to incomplete methylation.

Q. QUANTITATIVE COMPOSITION OF APPLE WOOD HEMICELLULOSE

For the quantitative analysis of methylated polysaccharide, fraction 3 from Methylation III was used, \([\alpha]_D^\circ -57.8^\circ (\text{CHCl}_3), \text{OCH}_3 39.1\%\).

Fraction 3 was hydrolyzed as before and the neutral sugars separated on Whatman No. 1 paper using solvent system (C). Guide strips were cut out and sprayed with aniline phosphate to locate the sugars. Appropriate zones, containing the tri-, di- and monomethyl pentoses were cut out and eluted with water. The concentrations of the sugars were determined using the phenol-sulphuric acid method (28). Standard curves (Figure 1) prepared by McKelvey (97) were used. The results obtained are shown in Table III.
STANDARD COLORIMETRIC CURVES

2,3,4-TRI-O-METHYL-D-XYLOSE

2-O-METHYL-D-XYLOSE

2,3-DI-O-METHYL-D-XYLOSE

OPTICAL DENSITY

CONCENTRATION IN MICROGM. PER 2 ML.
TABLE III

QUANTITATIVE ANALYSIS
of
METHYLATED SUGARS of APPLE WOOD HEMICELLULOSE
by
PHENOL-SULPHURIC ACID METHOD

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Mole Ratio</th>
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</thead>
<tbody>
<tr>
<td>Trimethyl xylose</td>
<td>1</td>
</tr>
<tr>
<td>Dimethyl pentose</td>
<td>97</td>
</tr>
<tr>
<td>Monomethyl xyloses</td>
<td>2</td>
</tr>
<tr>
<td>2-O-(2,3,4-Tri-O-methyl-D-glucopyranosyluronic acid)-3-O-methyl-D-xylose (by weight)</td>
<td>19</td>
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</table>
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