A COMPARISON OF THE COMPOSITION OF THE HEMICELLULOSES FROM VARIOUS HARDWOODS

by

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A COMPARISON OF THE COMPOSITION OF THE HEMICELLULOSES FROM VARIOUS HARDWOODS

INTRODUCTION

Since 1931 when Schulze first gave the name "hemicellulose" to a newly discovered class of carbohydrates, it has been the aim of many workers in this field to establish similarities or dissimilarities in the physical and chemical properties of the hemicelluloses isolated from various sources and from these properties, if possible, to arrive at a definite formula for their composition. Although the same general method of isolation has been used since the first hemicellulose was obtained, numerous methods of preliminary treatment, precipitation, fractionation, and purification have been used with the result that the data in the literature are based on materials prepared in different ways. It is generally believed that the hemicelluloses are really mixtures which are fractionated by making use of solubility relationships. If this is true, it is obvious that any change in these procedures would change the nature of the fraction obtained. During the past two years hemicelluloses from four hardwoods have been prepared and analyzed in this laboratory. The purpose of this investigation is to compare the composition of these hemicelluloses with the various hemicelluloses previously described in the literature.
Review of the Literature

A comparison of the data in the literature indicates that the hemicelluloses are very complex mixtures whose composition varies even for those isolated from the same substance at different times; however, when approximate qualitative allowances are made for the presence of impurities, especially pectin, the evidence as shown in the following review of the literature seems to point to a general structure for at least all hemicelluloses from similar sources.

In early investigations the hemicelluloses were isolated by extraction from the untreated cell membranes with dilute alkali and precipitated from the extract with dilute acids. This simple procedure could be easily and accurately duplicated. Schulze and Tollens followed this scheme in isolating the hemicelluloses from wheat straw and maize stocks. They reported xylose as the sole product of hydrolysis in both cases. Schulze obtained both xylose and arabinose from wheat and rye brans and in collaboration with Pfenniger found the pods of Pisum sativum and Phaseolus vulgaris to be rich in hemicelluloses whose hydrolytic products were galactose and arabinose. Xylose was found to be the only sugar in the hemicelluloses from beech wood, analyzed by Tollens and Allen, and cherry wood, analyzed by Wheeler and Tollens.

In 1926 O'Dwyer separated the hemicellulose from beech wood into two fractions whose chemical and physical proper-
ties were distinctly different. The first fraction was precipitated by acidifying the alkaline extract with acetic acid; this water insoluble portion was called fraction A. When an equal volume of 95% ethanol was added to the filtrate, water soluble fraction B was precipitated. O'Dwyer also showed that these hemicelluloses evolved carbon dioxide under conditions described by Nanji, Paton, and Ling, which is characteristic of the uronic acid. These uronic acids were known to form fufural on treatment with dilute hydrochloric acid. This fact explained why some hemicelluloses yielded fufural and gave no traces of pentoses.

Following the work of O'Dwyer, Butler and Cretcher showed that the substance from gum arabic that Salkowski had reported to be pure arabinose contained xylose and galactose and also evolved carbon dioxide when treated with dilute hydrochloric acid. In view of this work, Anderson pointed out the need for reinvestigating some of the hemicelluloses which are reported to yield only sugars on hydrolysis, especially since very few have actually been proven to be pure polysaccharides.

Buston summed up these observations in the statement that the water insoluble fraction A generally consisted of pentosans and hexosans; whereas the water soluble fraction contained high percentages of uronic acid. It has been found since, that polyuronide hemicelluloses are separated into fractions each of which contains uronic acids in addition to
pentoses and hexoses. The high uronic acid referred to by Buton in his summary is undoubtedly due to the presence of pectin as an impurity. Pectin precipitates more completely as the alcohol concentration of the solution increases, and since the higher the pectin concentration of the hemicellulose becomes the greater will be the uronic acid content, it would be expected that the fractions isolated from solutions of higher alcohol concentration should contain higher percentages of uronic acid. Thus, the uronic acid attributed to the hemicelluloses would depend first on the amount of pectin originally present in the cell with the hemicellulose, second on the completeness of removal of pectic material before the isolation of the hemicellulose, third on the amount of alcohol used in the precipitation of the fraction, and fourth on the physical and chemical nature of the pectin.

This pectic material is not readily soluble in the concentration of sodium hydroxide used to extract the hemicelluloses so the amount brought into solution during the alkaline extraction would depend on two factors, the length of time the sodium hydroxide extraction is carried out and the amount of pectin present in situ with the hemicellulose. When the alkaline extractions are acidified for the precipitation of fraction A, the acid concentration is probably too low to cause any appreciable precipitation of the pectin present, although the higher the acid concentration becomes
the more the pectin will tend to precipitate. However, fraction B is precipitated under conditions suitable for the precipitation of pectin. Since the greater the alcohol concentration used for each fraction the greater the amount of pectin precipitated, then the properties of the hemicellulloses from fraction A through C should take on characteristics more and more similar to pectin, such as higher uronic anhydride, higher hexose content, higher percentage of arabinose, and to a less definite extent, a lower rotation. This latter property would depend mostly on the sugars present in each fraction and would therefore not vary in any definite manner from one fraction to the next. Water soluble fractions of a hemicellulose obtained from sources low in pectin content should have a smaller uronic acid content than the identical fractions of a hemicellulose isolated from sources higher in pectin content. So in comparing these water soluble fractions for possible similarity, it is evidently very necessary to make sure that the pectic material be reduced to as nearly the same amount as possible or allowances be made for the relative amounts of pectic material present. There is no reason for assuming that identical pectin extraction processes for hemicelluloses from two different sources or the same source at two different times will accomplish this removal to the same degree of completeness. This might explain why identical fractions isolated at different times from the same source frequently analyze differently.
Until 1923 the hemicelluloses were all precipitated from the alkaline extract by the addition of glacial acetic acid. The sodium acetate and acetic acid buffer mixture thus formed prevented the pH from becoming such that any appreciable pectin would be precipitated. These hemicelluloses were probably quite free from pectic material. In 1923 the first precipitation by alcohol was made by O'Dwyer, who found that after acidification, if an equal volume of 95% ethanol were added, complete precipitation took place. This hemicellulose had been extracted from untreated American white oak wood and all the pectic material dissolved during extraction would be completely precipitated by the above procedure. As a consequence, O'Dwyer reported traces of arabinose and galactose in addition to xylose. If it is assumed that the arabinose and galactose be present as hydrolytic products of pectin then the composition of the American white oak hemicellulose would be identically the same as those isolated from wheat straw and maize stock, maize cobs, beech wood, and cherry wood. Each one of these hemicelluloses mentioned is isolated from the structural portion of the plant and serves the same purpose in every case, which makes their marked similarity expected rather than unusual.

When the first hemicellulose fractionations were made, the greatest difference in the composition of the two fractions was the amount of uronic acid present in each. O'Dwyer's beech wood, which was previously reported by Wheeler and
(6). Tollens as containing only xylose, fractionated into a water insoluble and water soluble fraction. The water insoluble fraction should contain very little pectic material when first precipitated, and after purification by resolution in sodium hydroxide and reprecipitation with glacial acetic acid the pectin content should be practically zero. This fraction gave 2.75% carbon dioxide and on hydrolysis xylose alone was identified. If this entire carbon dioxide content is attributed to pectin, the amount of galactose and arabinose present would still be so small as to make identification highly improbable, especially since the concentration of xylose present is in great excess of the galactose and arabinose, which reduces the sensitivity of their tests. The fraction precipitated by alcohol is purified by resolution in sodium hydroxide and reprecipitated with alcohol causing any pectin present after the sodium hydroxide treatment to be completely precipitated. This fraction evolved 16% carbon dioxide and when oxidized with nitric acid yielded 4.4% mucic acid corresponding to 4.3% galactose. This value is probably much too low due to the interference of the other sugars. The pentoses were not identified, but the high percentage of uronic anhydride together with a positive proof of the presence of galactose not previously reported by Wheeler and Tollens indicates strongly the presence of pectic materials. Her preliminary treatment of
the alkaline extract with lime water in an attempt to remove
the pectin was evidently not successful.

(15)

Norman obtained water insoluble and water soluble frac­tions from oat and rye straws not previously treated for the
removal of pectin. Fraction A from oat straw gave 2.7% carbon
dioxide and arabinose, xylose, and galactose on hydro­
lysis. Fraction B with 7.95% carbon dioxide hydrolyzed to
give sugars identified as only arabinose with traces of un­
identified hexose. The uronic acid contents of fractions A
and B from rye straw are very nearly the same as those from
oat straw; however, since the products of hydrolysis were
not determined and were assumed to be the same, it is impos­
sible to draw any conclusions as to their similarity. The
fact that both water soluble fractions contain high uronic
acids indicates pectin, which would be present in such small
quantities that its identification could easily have been
overlooked. The preponderance of galactose and arabinose
in fractions A as compared to the composition of wheat straw
seems to indicate that here we have a hemicellulose of the
type galactose-arabinose-galacturonic acid rather than a
glucose-xylose-glucuronic acid. A complete and careful com­
parison of these two hemicelluloses of practically the same
type origin should prove interesting.

(14)

Norris and Freece realizing the necessity of a pre­
liminary removal of extraneous cell wall material preceded
the sodium hydroxide extraction of the wheat bran by ammonium
oxalate and 1% sodium hydroxide-alcohol treatments for the specific purposes of removing pectin and lignin. Norman criticizes the use of the alkaline alcohol extraction, claiming that some of the hemicelluloses will be removed with lignin; however, Angell and Norris later showed that this treatment leaves all the hemicellulose substance intact.

Fractionations were carried out as directed by O'Dwyer with the exception that a half volume of acetone was used wherever she had used one volume of alcohol. Another fraction C was obtained when one half volume of acetone was added to the filtrate from B. Fractions B and C were further separated by the addition of Fehling's solution (Baker and Pope). The less soluble parts of the fractions were precipitated and centrifuged off. These were called hemicelluloses B_1 and C_1. To the centrifuged was added one half volume of acetone, which resulted in the precipitation of the more soluble portions B_2 and C_2. Four distinct hemicelluloses were thus obtained, A, B_1, B_2, and C_2. Fractions B_1 and C_2 consisted chiefly of xylose with 1.85% carbon dioxide and 1.93% carbon dioxide respectively. B_2 was unique in that it was found to be a glucosan with a trace of pentosan, a substance not generally considered a constituent of hemicelluloses. Fraction A was found to be a polysaccharide hemicellulose yielding arabinose and xylose, which confirms the finding of Schulze whose "wheat bran hemicellulose" corresponds to this. The success of the pectin removal is confirmed by the complete absence of
any indications of pectin; however, the presence of the polysaccharide substances is questionable.

Following exactly the procedures used by Norris and Preece, Preece isolated four separate hemicelluloses from maize cobs, which also had been previously reported as pure xylan. All four fractions contained uronic anhydride in amounts varying from 4.8% to 7.6%. In no single fraction was a trace of hexose sugars reported. This is a good indication of the absence of pectin.

Buston and Chambers investigated the lichen commonly known as "Iceland Moss" (Cetraria islandica) and reported the absence of pectic material and all hemicelluloses except fraction B. The lichen was extracted exhaustively with hot 0.5% ammonium oxalate solution and the extracts precipitated with alcohol. After purification by solution in water, reprecipitation with alcohol followed by resolution in water, the pectic material was precipitated as calcium pectate. The small amount of material obtained gave 11.3% carbon dioxide and 16% calcium, whereas pure calcium pectate gives 17.6% carbon dioxide and 7.45% calcium. This was not reported as pectin but was assumed to be the salt of one of the lichen acids. The possibility that this substance was impure pectin was discarded despite the fact that small quantities of furfural were obtained that could not be accounted for by the amount of uronic acids etc. present. The lichen was therefore given no preliminary treatment for the removal of pectin.
before the hemicellulose was isolated. When the alkaline extract was made slightly acid with acetic acid, slight turbidity resulted; when one half volume of acetone was added, a copious precipitate was produced. It was concluded that the water insoluble fraction was absent. However, Angell and Norris report that the adjustment of the pH for the complete precipitation of fraction A is as important as obtaining the iso electric point for the complete precipitation of the proteins. It is therefore very possible that fraction A was precipitated along with the water soluble fraction. From previous work it has been noted that the water insoluble fraction almost invariably is present in quantities many times greater than the water soluble fractions; so it would be expected that the one hemicellulose obtained by Buston and Chambers has properties very similar to water insoluble fraction A. The fact that the carbon dioxide content is a little too high may easily be explained by assuming pectin, the presence of which was indicated and the absence of which not proven. After fractionation with Fehling’s solution, B1 gave 2.4% carbon dioxide and B2 gave 2.01% carbon dioxide, which are approximately the same values obtained by Norris and Prooce for their water insoluble fractions which were mixed with traces of pectin. These interesting results have two very important interpretations. First, if hemicellulose A is actually absent, then this is another evidence for the theory that all hemicellulososes contain relatively the same
amount of uronic acid which would simplify the general structure of these complex molecules; and second, if A and B are actually different and if A is present in this mixed fraction in an amount greater than that of B, then A must have a carbon dioxide content of about 1.3%, which is close to values obtained for fraction A of the pectin free hemicelluloses from maize cobs, wheat bran, and Iceland Moss. This is additional evidence for the general nature of the water insoluble fractions. Another hemicellulose extracted with 17% alkali from the residue left after the 4% treatment analyzed almost the same as those just mentioned, which may substantiate the existence of "free" and "combined" hemicellulose. There is a possibility, however, that this hemicellulose is the result of inefficient 4% extraction.

Sands and Gary obtained four separate fractions A₁, A₂, C₁, and C₂ from mesquite wood, which had previously been treated for the removal of pectic substances by two two-hour extractions with ammonium oxalate. Fraction A was precipitated in definitely acid solution and redissolved in two and one half percent sodium hydroxide. Alcohol was added to the alkaline solution until it was 35% with respect to alcohol giving fraction A₁. The solution was then made 35% with respect to the ethanol which caused the precipitation of A₂. Any pectin present would tend to come down more completely with A₂ which is precipitated by higher alcohol concentration. C₁ and C₂ were precipitated in exactly the same
manner, with the exception that the alkaline extract is made definitely acid before the addition of the alcohol. The last three fractions were precipitated under conditions most favorable for the precipitation of pectin and should therefore have higher carbon dioxide than the first fraction. From the following results, this is shown to be true. A₁ gave 2.54% carbon dioxide, A₂ gave 4.38% carbon dioxide, and C₂ gave 4.46% carbon dioxide. Again the uronic acid content of the most insoluble hemicellulose is found to be strikingly similar to those reported by previous workers. The fact that no mucic acid or arabinose was found does not prove the absence of pectin, which would be present in very small quantities as evidenced by the low carbon dioxide.

(22) Anderson and Krznarich separated a hemicellulose extracted from oat hulls which had been previously treated exhaustively with boiling ammonium oxalate. The fractions obtained by a number of different modifications of the acid-alcohol separation analyzed very nearly 1.5% carbon dioxide. A uronic acid was identified in quantities great enough to account for this carbon dioxide which seems to exclude the possibility of the presence of pectin. Attention is again called to the relatively consistent carbon dioxide content of the water insoluble fractions.

The first workers to realize that the yield of fraction A was dependent on the pH at which it was precipitated were (20) Angell and Norris, who by a series of trials found the acid
concentration at which this fraction was most rapidly and completely thrown out of solution. This acid concentration was so high that hydrochloric acid had to be used rather than acetic acid, which has a tendency to buffer the solution at a pH near 7. Since the protein molecule is also held in solution by charge and hydration factors and is most completely precipitated at its isoelectric point, it is natural that the hemicellulose which in this way resembles a protein solution be precipitated at a certain acid concentration. Angell and Norris suggested that each hemicellulose fraction had a characteristic pH at which it was precipitated and working with the hemicellulose from the hop demonstrated this fact by showing the effect of pH adjustment before each separation on the yields of these fractions. These fractions were further separated by a glycerol-copper sulfate mixture rather than the strongly alkaline Fehling's solution which had heretofore been used. The uronic anhydride contents of these eight fractions, $A_1$, $B_1$, $C_1$, and $C_2$ from maize cobs and $A_1$, $B_1$, $B_2$, and $C_2$ from hops ranged from 7.45% to 9.7%. Fractions B in all cases gave values just slightly higher than the carbon dioxide content of A.

In every case cited above the carbon dioxide content of the water insoluble fraction varied from 1.3% to 2.7%. The hemicelluloses yielding the highest carbon dioxide were either extracted from the plant before the pectin was removed or precipitated from the sodium hydroxide extract by alcohol.
Both conditions would favor the precipitation of pectic material in fraction A, which would account for the high carbon dioxide found. The hemicelluloses isolated from oat hulls, which are naturally low in pectin content and which had been extracted exhaustively with ammonium oxalate, contained a lower percent of uronic acid than any yet isolated, and may represent the most complete pectin removal that has ever been obtained. It is possible that even a more complete removal of pectin could be obtained yielding a hemicellulose of very low uronic acid content. No such purification has yet been reported. The water soluble fractions show variations in carbon dioxide from slightly greater than 2% to 16%. In every case those yielding approximately 2% carbon dioxide were isolated from pectin free or near pectin free sources and resembled the insoluble fraction A; whereas, those containing the higher percentage of carbon dioxide were obtained by alkaline extraction of untreated sources and resembled A in almost every respect except the uronic acid content, which can be explained by assuming the presence of pectin. These are all strong evidences for the possibility that if these hemicelluloses are not pure compounds by mixtures, they are mixtures of substances very similar in composition.

Workers in the chemistry laboratories at the University of Arizona have isolated, fractionated, and analyzed in exactly the same manner the structural hemicelluloses from four different hardwoods for the purpose of making a comparison
of the composition of these substances. The results of these analyses and conclusions drawn from them constitute the experimental portion of this paper.
PRECIPITATION, FRACTIONATION, AND PURIFICATION OF THE HEMICELLULOSES

In the Fall of 1937 the extraction of the hemicelluloses from the four hardwoods, Lemon wood, White Birch, Black Locust Heartwood, and Black Locust Sapwood was begun for the purpose of comparing their compositions. The work on the first three woods mentioned was completed the following Spring and the results published in the theses of W. T. Stewart, J. R. Redd, and D. G. Westerbeke; however a comparison of these data was withheld pending the completion of the work on the hemicelluloses of the Sapwood of Black Locust. The results of the analysis of the hemicelluloses from all four woods follow.

The methods used in the preliminary treatment of the wood and the methods of analysis are identical with those employed for the other three woods and are given in detail in the above mentioned theses. The methods of precipitation, fractionation, and purification of these hemicelluloses, however, were complicated by the presence of large quantities of starch which necessitated modifications in the procedures.

Four lots of 450 grams each of the sapwood extracted by organic solvents as indicated above was extracted in the cold for 48 hours with approximately 4% solution of sodium hydroxide and then filtered through muslin. The sapwood was again extracted with fresh 4% solution of sodium hydroxide for the
same length of time and again filtered. This indicated the necessity of a further extraction. The third filtrate obtained was only slightly yellow and gave a barely perceptible precipitate when acidified. No more extractions were carried out. The filtrates were combined and filtered several times through four thicknesses of muslin to remove the last traces of the sawdust. The resulting solution was amber colored and water clear. Angell and Norris emphasized the necessity of obtaining the optimum pH for complete precipitation of the fractions; hence twenty-four five cc portions of the solution were exactly neutralized, and the pH of each adjusted to vary from 6.9 to 1.0. The solution at a pH of about one showed the most rapid and complete precipitation. The entire sodium hydroxide filtrate was neutralized with dilute hydrochloric acid, but before the neutral point was reached, a large dirty-brown precipitate formed. At the neutral point settling had begun. The precipitate was allowed to settle for a period of 24 hours, at which time considerable sediment had collected on the bottom of the container. In view of the fact that this fraction might be different from the ordinary water insoluble fraction A the precipitate was centrifuged free of the neutral solution and washed from the cups with recovered alcohol. This fraction was called "N" and was analyzed separately. Since the filtrate from hemicellulose "N" could not be obtained water clear even after numerous filtrations and centrifugations, it was acidified to a pH of one. A heavy, brown precipitate was
formed which settled within approximately five minutes. This was called fraction A. After standing 24 hours the clear supernatant liquid was tested for complete precipitation by varying its acid concentration. No turbidity resulted indicating that all the water insoluble fraction had been removed. The supernatant liquid was siphoned off and the precipitate was centrifuged free of the solution and washed into a beaker with recovered alcohol. To the clear filtrate was added one volume of 95% alcohol. Immediate precipitation resulted and after standing for 24 hours, it had settled out completely leaving a clear, light yellow supernatant liquid. This fraction B was white in color; whereas the two previous fractions had been muddy in appearance. Hemicellulose B was removed from the solution as previously indicated and washed into recovered alcohol where it was left until the other fractions had been isolated.

Upon the addition of a second volume of alcohol to the filtrate from B, the solution became cloudy within five minutes and at the end of three hours time the precipitate had begun to settle. Precipitation and settling in this case were also complete in 24 hours, leaving an almost colorless supernatant liquid. This fraction C was removed as described above. The filtrate was tested with alcohol for further hemicellulose content, but the amount of precipitate formed when two additional volumes of alcohol were added was insignificant. The filtrate was therefore discarded. No fraction C was ob-
tained from the other three hardwoods and this one may be due to incomplete precipitation of B. The analyses described later show that hemicelluloses B and C are identical. Each fraction obtained above was centrifuged from the alcohol and redissolved in 4% sodium hydroxide. The solutions varied from a brown color for fraction N to a yellow color for fraction C. The solutions were filtered until they were water clear. A few drops of each solution were made slightly acid and a drop of iodine solution added. Hemicellulose N developed a deep blue color characteristic of starch. A and B gave royal purple and reddish purple respectively which is characteristic of the higher dextrans. C gave a negative test.

Since the effect of starch on subsequent procedures was not known, attempts to remove it were made. The solutions were made so slightly acid that they turned blue litmus red but did not affect congo red. 0.5 grams of take-diastase was made into a thin paste with distilled water and added to each solution. After three days the solutions still gave positive tests for starch. The color obtained was quite definitely less but did not further disappear even after fresh portions of take-diastase were added and the solutions incubated at 35 degrees C (26) for several hours. According to Thatcher the completeness of enzyme action in some cases depends on the dilution of the substrate. As a last attempt to remove starch the solutions were diluted with two volumes of water and allowed to stand one week. They still gave tests for starch and even after hydrolyzing for
90 minutes with 2% hydrochloric acid, which is a very drastic treatment, a blue-green color developed on addition of iodine solution. It has been suggested that the hemicelluloses themselves give color reactions with the iodine solution. Since the hemicelluloses are in a general way similar to starch in composition, this fact seems probable, especially since the above tests show that the hemicelluloses of larger equivalent weight give colors similar to the dextrins and starch. However, a fraction isolated from this same wood after chlorination has a greater equivalent weight than hemicellulose N and does not give a positive test with the iodine solution. Likewise, every fraction isolated from the heartwood of Black Locust, which is a starch-free wood, gave negative color tests with iodine. This seems to indicate that the color is due to substances other than the hemicelluloses. If the starch were physically entrapped by the hemicellulose molecule, it should be easily hydrolyzed by treatment with hydrochloric acid or taka-diastase. The fact that it is not destroyed by these treatments makes the possibility of occlusion or adsorption improbable. The only possibility left is that of a chemical union between the hemicellulose and starch. Evidence for this union is given by the following experiment:

The least soluble of the fractions, N, was partially dissolved in boiling water and filtered. The filtrate was acidified and one drop of iodine solution was added. The solution became blue immediately; however, after the solution had
cooled, the hemicellulose began settling, carrying all of the blue color down with it. After two days the supernatant liquid was perfectly colorless, and the sediment a definite blue. A very dilute starch solution was treated in exactly the same manner, and although some of the starch settled out, the deep blue color was still evident in the solution. From these data it is reasonable to assume that a combination between the hemicelluloses and starch does exist. Attempts to prepare starch-free hemicelluloses therefore were abandoned and purification and fractionation was carried out as though starch were absent. The solutions were all made slightly acid with hydrochloric acid and a slight excess of liquid bromine added. The solution was allowed to stand 24 hours. From time to time it was necessary to add more bromine to replace that which had been used in the process of purification. Hemicellulose B and C were perfectly white when first isolated, but as a precaution they were also brominated. After 24 hours, all the solutions except fraction N were acidified to the optimum pH and alcohol added to remove the excess bromine. This treatment removes the greater part of the lignin by forming the alcohol soluble lignin chloride. The above process precipitated the hemicelluloses completely from solution. Hemicellulose N and A were still slightly colored; hence, they were centrifuged from the solution and the treatment with bromine repeated a second and a third time. The resulting hemicelluloses were white. All four fractions were then centrifuged from the solution and washed onto a suction
filter with 35% alcohol. After washing with increasing strengths of alcohol, the hemicelluloses were dried on porous plates to a fine white powder. The yields were as follows: N, 26 grams; A, 43 grams; B, 16.5 grams; and G, 14.5 grams.

Fractionation of the Hemicelluloses

In order to study any possible effect of strong mineral acids on the hemicelluloses, fraction N was kept in alkaline or very slightly acid solutions up to this point. In fractionating it, the same precaution was taken. The hemicellulose was dissolved in 4% sodium hydroxide and made exactly neutral. The precipitate formed was centrifuged off. This was called hemicellulose N1. To the filtrate was added one volume of alcohol. This fraction was called hemicellulose N2. Both were removed from solution and dried as described above.

Hemicellulose A was dissolved in 4% sodium hydroxide and the pH adjusted to the optimum. The resulting precipitate, called hemicellulose A1, could not be centrifuged off and filtration was unsuccessful. Following a method employed by Sands, a hollow clay candle attached to suction was used. The candle was lowered into the solution in which the precipitate was suspended and the suction turned on. The clear filtrate was sucked into a trap that was connected between the clay candle and the suction pump, leaving the precipitate...
clinging to the candle. After the filtration was complete, the hemicellulose layer was removed from the candle with a spatula. No scraping was necessary and the mechanical loss of the yield was negligible. Hemicellulose $A_1$ was dried by alcohol as described above. To the clear filtrate was added one volume of alcohol. The resulting hemicellulose $A_2$ was also separated by use of the clay candle. It was dried as previously described. When the alkaline solution of hemicellulose $B$ and $C$ were acidified to the optimum pH no turbidity resulted. This proved the absence of hemicellulose $B_1$. When one volume of alcohol was added, hemicellulose $B_2$ was precipitated, but the solution of hemicellulose $C$ remained perfectly clear. This indicated the absence of hemicellulose $C_1$. Hemicellulose $C_2$ precipitated after a second volume of alcohol was added. Fractions $B_2$ and $C_2$ were removed from solution by centrifuging and were washed with increasing strengths of alcohol. They were dried on a clay plate to a fine, white powder. The yields of these hemicelluloses were: $M_1$, 14 grams; $N_2$, 12 grams; $A_1$, 20 grams; $A_2$, 3.5 grams; $B_2$, 11 grams; $C_2$, 6.7 grams.

**Extraction of the Hemicelluloses**
**After Chlorination of the Wood**

The sapwood from the extractions with sodium hydroxide was washed thoroughly with water and dried by suction. Removal of pectin material and chlorination of the wood was carried out according to the description given by Stewart.
In order to isolate the hemicelluloses after chlorination, the pectin and lignin-free wood was extracted twice with a 4% solution of sodium hydroxide in the cold. Precipitation, purification, and fractionation of the hemicelluloses from this extract was carried out exactly as described for the hemicellulloses before chlorination. In these latter hemicelluloses, however, starch was not present which simplified their purification. Fractions D, E, F corresponding to A, B, and C were obtained, but an acid concentration twice that used in precipitating A was required for precipitating D. Fraction D separated into D₁ and D₂, but E and F were not separated. The yields of the hemicelluloses were: D₁, 17 grams; D₂, 1 gram; E, 3 grams; F, 1 gram. Table I gives the results of the analyses of these fractions.
ANALYSES OF THE HEMICELLULOSES

All the fractions isolated in sufficient quantities were analyzed for (a) moisture, (b) ash, (c) hexuronic acid, (d) xylan, (e) methoxyl, (f) specific rotation. The six determinations run on each fraction are described in detail in the theses of Stewart, Redd, and Westerbeke. The number of xylose units per molecule of uronic acid and the equivalent weight of each fraction were calculated from the results of the analyses. However, since an interpretation of these results is to follow, it is necessary to point out the accuracy of each determination which is involved in the following discussion. The CO₂ determination is perhaps the most accurate. From this value is calculated the equivalent weight of the hemicellulose and the number of xylose units per uronic acid. However, a variation of only 0.10% in the CO₂ causes a difference of 143 in the equivalent weight and one xylose unit in the length. Experimental error in the CO₂ determination amounting to 0.15% often occurs. In making comparisons of equivalent weights and lengths of molecules based on CO₂ determinations these facts must be born in mind. For example; a hemicellulose giving 1.95% CO₂ will have an equivalent weight of 2378 plus or minus 143 and 16 plus or minus 1 molecules of xylose per uronic acid. Thus it is only possible to distinguish satisfactorily hemicellulose fractions which vary by 3 or more
It is well known that the pentose determinations are not very accurate when applied to materials such as the hemicelluloses. However, the pentosan determinations give results that are good approximations, and are useful in predicting the general size of the molecule. The fact should be kept in mind that these values may easily vary two to five percent from one determination to the next.

The quantitative determination of methoxyl is run primarily to determine the number of methoxyl groups present per molecule of uronic acid. This type of interpretation does not require a high degree of accuracy; hence approximate values are satisfactory.

No generalization can be made relative to the specific rotations of the hemicelluloses except that they all rotate strongly laevo. It is quite possible that when a sufficient amount of data are available on the rotations of these substances, definite conclusions can be drawn as to the methods of linking. This knowledge would make the chemical and physical properties of the hemicellulose more understandable.

In the following discussion of the data in Table I, the results are interpreted as being quantities variable within the limits mentioned above.
<table>
<thead>
<tr>
<th>Fractions</th>
<th>N₁</th>
<th>N₂</th>
<th>A₁</th>
<th>A₂</th>
<th>B</th>
<th>B₂</th>
<th>C</th>
<th>C₂</th>
<th>D₁</th>
<th>D₂</th>
<th>E</th>
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<tbody>
<tr>
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<td>1.86</td>
<td>1.69</td>
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<td>2.34</td>
<td>2.56</td>
<td>2.83</td>
<td>2.53</td>
<td>1.77</td>
<td>2.45</td>
<td>2.25</td>
</tr>
<tr>
<td>Methylated Uronic Acid</td>
<td>8.15</td>
<td>8.16</td>
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<td>10.31</td>
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<td>89.00</td>
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<td>89.00</td>
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<td>-53.82</td>
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<td>1.39</td>
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<td>1.65</td>
<td>1.83</td>
<td>1.99</td>
<td>1.79</td>
<td>1.33</td>
<td>1.90</td>
<td>1.95</td>
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<tr>
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<td>2.30</td>
<td>2.26</td>
<td>2.63</td>
<td>1.29</td>
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<td>1556</td>
<td>2435</td>
<td>1796</td>
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<tr>
<td>No. of Xylose Molecules</td>
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<td>16.1</td>
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<td>14.0</td>
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<td>10.99</td>
<td>9.96</td>
<td>11.3</td>
<td>17.0</td>
<td>11.0</td>
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<td>98.21</td>
<td>99.72</td>
<td>101.07</td>
<td>89.49</td>
<td>88.99</td>
<td>89.72</td>
<td>87.55</td>
<td>100.32</td>
<td>99.65</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE I**
DISCUSSION OF THE ANALYSES OF THE HEMICELLULOSES

From CO₂ and xylan determinations, hemicelluloses N₁, N₂, A₁, and A₂ are practically identical in size and composition, with the exception of A₂ which appears to be shorter by one or two xylose units. The order of precipitation would lead to the same conclusion, because the shorter molecules appear to be the more soluble and would precipitate last. No definite borderline of solubility was observed in precipitating hemicelluloses N₁, N₂, and A₁; from the analyses it appears that the same substance was precipitating throughout the entire range of pH covered during the isolation of hemicellulose N and A. Quantitative determinations of methoxyl indicate that in all four cases there is one methoxyl group for every molecule of uronic acid. After hydrolysis the methoxyl group always remains with the acid portion of the molecule and appears to be attached to the uronic acid. The nucleus of the molecule then is a mono methoxy hexuronic acid. All these hemicelluloses are combined with starch as shown by the tests with iodine solution, but from the total percentage accounted for, it is evident that very little starch material is present. Evidently the presence of starch in these fractions has no effect on the results other than the production of color with iodine solution. No explanation can be offered for the variations in the specific rotations.
The results of analyses indicate these four fractions are the same in composition and are made up of one methoxy uronic acid with 16 plus or minus one xylose units. The minimum solubility is at a pH of approximately one, but precipitation begins at a pH greater than seven and continues until this optimum pH is reached.

The insoluble hemicelluloses B, B₂, C, and C₂ are very similar in composition and size, but the fact that B and C were separated by alcohol would indicate that B was more insoluble than C. It is more probable that fraction C is the result of incomplete precipitation of fraction B, especially since the analytical results including the rotations are identical for both substances. One volume of alcohol was not sufficient to cause complete precipitation of fraction B and when a second volume was added, the remainder of this fraction precipitated. This was mistaken for a new fraction which was obviously incorrect. The separation of the water insoluble and water soluble fractions cannot be explained in this way. It is evident that the two fractions vary in some way which makes their reactions differ toward certain reagents.

Why is fraction A precipitated by acid whereas B is not? Both are practically identical in composition, and vary only slightly in size. The hemicellulose molecule involves so many units and has so many possible linking combinations, it may have many structural patterns. Is it possible that variations in the structure of the molecule could account for the
solubilities and rotations? Some of the molecules may be straight chained and like cellulose hold very small amounts of water of hydration. These would be the water insoluble fractions, and would precipitate readily; whereas, molecules that are coiled up and heavily hydrated would remain soluble until a high enough concentration of alcohol is added to dehydrate them. The water soluble fractions would resemble this type. From equivalent weight and length of fractions A and B it is evident that they are about the same size and so it is possible that the only dissimilarity between them is the structural pattern of their molecules, which results in a different degree of hydration and therefore of solubility. All these possibilities should be considered as probable explanations of the chemical and physical behavior of the hemicelluloses.

The low xylan content of all four water soluble fractions, together with the low total percentage accounted for, indicates that some component has been omitted. Anderson isolated a fermentable sugar of rotation plus fifty-two from the hydrolytic products of a hemicellulose from the sapwood of this tree in the Spring of 1954. He concluded that it was glucose from the starch. Owing to the small quantities of these four fractions left after all other analyses were complete, a hydrolysis could not be carried out. It was assumed on the basis of Anderson's work that the fractions B and C contained about nine percent glucose, which may come from the dextrin material shown to be present by the iodide tests. It is pos-
sible that this glucose is a part of the hemicellulose molecule and does not come from the hydrolysis of the dextrin. A comparison of the actual results obtained with a theoretical molecule, which contains one unit of glucose as a part of its composition, indicates that these fractions contain glucose in their molecules. A theoretical molecule made up of one methoxy hexuronic acid, 10 plus or minus one xylose units, and one glucose unit would have an equivalent weight of about 1354 and yield 2.39% CO₂ corresponding to about 13% methoxy hexuronic acid, 78.31% xylan, and 8.6% glucose. This is practically the analysis obtained for the four water insoluble fractions.

Fractions D₁ and D₂ after chlorination corresponding to A₁ and A₂ before chlorination compare very closely even to rotation and solubility. It is therefore evident that the first fractions isolated from the sodium hydroxide extract are identical. Fraction E, however, which is of the same general size, contains no glucose as evidenced by the xylan and total accounted for values. It differs from the water insoluble fractions isolated before chlorination in this one respect. Particular note should be paid to the specific rotation. In the case of the fraction B and C, which contain the positive rotating glucose, the specific rotation was rather low; fraction E, which is of the same size but differs from B and C in that it does not contain glucose, rotates somewhat more laevo. Whether this is the result of the glucose or some other factor
can not be determined by present methods and at present must be left unexplained.

The results of the Fehling's reduction and starch tests have no significance except to indicate the presence of reducing material and starch or dextrins. Whether the hemicellulose is the reducing agent or some extraneous material is responsible for the reduction is not known.

From the results in Table I it is evident that two different fractions of hemicelluloses were obtained from the sodium hydroxide extract of the hardwood. Those extracted before chlorination were the same as those extracted after chlorination. Water insoluble fractions which were separated into sub fractions all of which were practically identical, closely resemble the water insoluble fractions isolated by all the workers previous to this time. Water soluble fractions, which were also separated into identical fractions by alcohol, vary slightly in composition from the water insoluble fractions and may owe their greatest dissimilarity to a difference in structure.
HYDROLYSIS OF THE HEMICELLULOSES

In January, 1934, Anderson hydrolyzed separately hemicellulose A and hemicellulose B from the sapwood of Black Locust and isolated d-xylose, a calcium salt of an aldonic acid, and d-glucose. The following method of procedure was used:

100 grams of the hemicellulose were mixed with one liter of 4% sulfuric acid and heated in a bath of boiling water for 14 hours. The solution was filtered and decolorized by Norit. Excess calcium carbonate was added and the calcium sulfate was filtered off. After cooling, the filtrate was treated with just enough barium sulfate to remove excess sulfate ion and the barium sulfate was filtered off. The syrup was poured into 95% alcohol and the calcium salt filtered from the solution. It was dissolved in water, decolorized again, filtered, concentrated in vacuo, and reprecipitated by alcohol. The white calcium salt was dried on clay plates and analyzed. The results are given in Table II. The alcoholic sugar solution was concentrated, dissolved in glacial acetic acid, and seeded with d-xylose. A large crop of sugar crystallized out. Sugar A from hemicellulose A and sugar B from hemicellulose B were combined at this point and identified as d-xylose by its rotation and by the Bertrand's tests. The combined mother liquor from sugars A and B were again concentrated, dissolved
in glacial acetic acid, and from this syrup crystalline d-glucose was obtained. This was identified by its specific rotation and by conversion to potassium acid saccharate. An inspection of Table II indicates that the calcium salt isolated above approximates closely the theoretical composition of the salt of a methoxy uronic acid combined with one molecule of d-xylose.

ANALYSES OF THE CALCIUM SALT

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>%CO₂</td>
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<tr>
<td>Calcium Salt Determined</td>
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<tr>
<td>Theory</td>
</tr>
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CARBON DIOXIDE DETERMINATIONS ON THE HEMICELLULOSES

BEFORE CHLORINATION

<table>
<thead>
<tr>
<th>Fractions</th>
<th>A</th>
<th>A₁</th>
<th>A₂</th>
<th>B</th>
<th>B₁</th>
<th>B₂</th>
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<tbody>
<tr>
<td>Black Locust Sapwood</td>
<td></td>
<td>1.69</td>
<td>2.20</td>
<td>2.34</td>
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<td>2.56</td>
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<td>1.68</td>
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<td>1.89</td>
<td>3.37</td>
<td>1.60</td>
<td>1.95</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>3.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Birch Wood</td>
<td>1.83</td>
<td>2.05</td>
<td></td>
<td>2.35</td>
<td>3.16</td>
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</table>

AFTER CHLORINATION

<table>
<thead>
<tr>
<th>Fractions</th>
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<th>C₂</th>
<th>D</th>
<th>D₁</th>
<th>D₂</th>
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<tbody>
<tr>
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<td>1.68</td>
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</table>

+ Correspond to D₁, D₂, and E respectively

TABLE III
PENTOSAN DETERMINATIONS OF THE HEMICELLULOSES

BEFORE CHLORINATION

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<tr>
<th>Fractions</th>
<th>A</th>
<th>A₁</th>
<th>A₂</th>
<th>B</th>
<th>D₁</th>
<th>D₂</th>
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</thead>
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AFTER CHLORINATION

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<th>Fraction</th>
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<th>C₁</th>
<th>C₂</th>
<th>D</th>
<th>D₁</th>
<th>D₂</th>
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<td></td>
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+ Correspond to D₁ and E respectively

TABLE IV
EQUIVALENT HEIGHT AND NUMBER OF XYLOSE UNITS PER MOLECULE OF METHOXY URONIC ACID

BEFORE CHLORINATION

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<td>(14)</td>
<td>(12)</td>
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<td>Black Locust Heartwood</td>
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<td>(19)</td>
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<td>(15)</td>
<td>(12)</td>
<td>(9)</td>
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</table>

AFTER CHLORINATION

<table>
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<tr>
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<th>$C_2$</th>
<th>$D$</th>
<th>$D_1$</th>
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<td>(16)</td>
<td>(14)</td>
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<td>(11.5)</td>
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<td>(18)</td>
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</tr>
</tbody>
</table>

+ Number of Xylose Units  TABLE V
A COMPARISON OF THE COMPOSITIONS OF THE HEMICELLULOSES FROM THE FOUR HARDWOODS

An inspection of tables III, IV, and V indicates that fractions A and C are very similar if not identical. The averages of the analyses for these fractions of the hemicelluloses from the four woods are: hemicellulose A, 1.91% CO₂, 90.37% xylan, equivalent weight 2255, and 16.0 xylose units. In only one case does the CO₂ vary from this mean by more than 0.15% CO₂. Hemicellulose A₁ of the Heartwood of Black Locust gives 1.68% CO₂ which is about 0.25% CO₂ lower than the average values. All xylan determinations are within 1.5% of the average. Equivalent weights and lengths of the molecules in xylose units are all close to the average values obtained.

The average values for hemicellulose C are almost the same as those for A. The percent CO₂ was 2.03%, xylan was 90.69%, equivalent weight was 2255, and the length in xylose units was 15.5. The agreement of each individual fraction with the average value of all fractions is even closer than in the case of fractions A. It should be noticed in every case that the less soluble sub fractions of A and C are higher in uronic acid content while the xylan content remains the same. These sub fractions are precipitated with alcohol which also precipitates any pectin present in the sodium hydroxide extract. Pectin would cause an increase in carbon dioxide but would not affect the xylan. Since all four woods contain relatively large...
amounts of pectin and received no preliminary treatment for its removal before the hemicelluloses were removed, the precipitation of pectin with the hemicelluloses would be expected.

The slight increase in carbon dioxide and decrease in xylan contents in fractions B and D for all woods except the Heartwood of Black Locust indicates a shortening of the molecule. Fraction B from the Heartwood of Black Locust seems to be identical with fraction A. There is a possibility that fraction A was not completely removed and later precipitated with B, because this fraction is the only one which does not compare with those isolated from the other three woods. The xylan content of fractions B and C from Sepwood of Black Locust are low due to the presence of glucose as already described. Glucose was not identified by any of the other three workers. Evidently fraction B of this wood differs from fraction B of the other woods only by one glucose molecule per methoxy uronic acid, since the carbon dioxide and equivalent weights are similar.

The average values of the results for all B and C fractions, with the exception of the xylan determination in the Sepwood and the carbon dioxide determination in the Heartwood of Black Locust, compare favorably with the individual analyses. However, it is evident that fraction B and D are contaminated with the water insoluble fractions in some cases. B₁ and B₂ of Heartwood of Black Locust and D of White Birch are probably mixtures of the water soluble and water insoluble fractions due
to the incomplete precipitation of fractions A and C respectively. The average values of the analyses are: B; 2.56% CO₂, 33.63% xylan, 1628 equivalent weight, and 10.4 xylose units. C: 2.50% CO₂, 37.30% xylan, 1711 equivalent weight, and 11.0 xylose units. The agreement in the analytical results of the two fractions is strong evidence of the identity of the water soluble hemicelluloses.

It thus appears that the hemicelluloses from the four hardwoods are identical and consist of two distinct types, water insoluble and water soluble.

Fractions A and C, the water insoluble fractions, are identical and consist of one methoxyl uronic acid with 16 plus 1 xylose units. Fractions B and D, the water soluble fractions are likewise identical and consist of one methoxyl uronic acid with 11 plus 1 xylose units. Fraction B isolated from the Sapwood of Black Locust is an exception and contains one molecule of glucose in place of one of the xyloses. Their minimum solubility is at a pH of about one, and they all rotate laevo.

The sugar obtained by the hydrolysis of the hemicellulose was d-xylose with the single exception previously mentioned. The salts of the aldobionic acids, Table VI show that hydrolysis proceeds to about the same degree in each hemicellulose to give a product of approximately the same composition. This indicates their similarity in chemical properties.
SUMMARY

1. The hemicelluloses have been isolated from the Sapwood of Black Locust and compared with those from various hardwoods.

2. The hemicelluloses are of two distinct types.

3. The solubility of the hemicelluloses may not depend on the size of the molecule but on their structure.

4. Each hemicellulose fraction contains one methoxy uronic acid.

5. Xylose is the only sugar present in the hemicelluloses with the exception of one fraction.

6. The hemicelluloscs are polyuronides of the glucose, glucuronic acid, xylose type.

7. Similar fractions isolated from the four hardwoods are identical in composition.

8. The hydrolytic products are the same for all fractions.
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