THE HEMICELLULOSES OF ASPEN WOOD

(POPULUS TREMULOIDES)

A Thesis

by

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GENERAL INTRODUCTION

The solvent effect of liquid ammonia on woody materials, particularly sugar maple wood, was studied in this laboratory by Yan in 1947. It was found that nearly 6% by weight of maple wood was soluble and the extract consisted of a mixture of lignin, polysaccharides and acetamide, the latter substance being derived from the ammonolysis of acetyl groups combined in the wood.

Since the wood residue appeared to be chemically modified, Neubauer extracted with hot water and found that 2% of polysaccharide material, not originally water-soluble, could be removed. Evidence was presented to show that in addition to the acetyl ester linkage other esters were also cleaved by the ammonia.

As a result of the previous studies, Milford initially delignified black spruce wood in order to facilitate the extraction of hemicelluloses, after which a route similar to that employed by Neubauer was used. In contrast to maple wood, on the wood basis liquid ammonia removed 4.95% by weight from delignified spruce, and subsequent cold and hot water extractions removed an additional 12.7%, most of which was polysaccharide material.

In the present investigation a similar line of approach was followed to isolate the hemicelluloses from aspen wood. Holocellulose, the wood residue after delignification, was soluble in liquid ammonia to the extent of 12.6%, and when

the residue was extracted with cold and hot water, an additional 11.4% was removed, 80% of which consisted of hemicellulose contaminated with pectin.

A study showed that the hemicellulose was a mixture of polyuronides similar in composition. The greater part of the work was concerned with the separation of these polysaccharides from extraneous material and with a study of the structure of one of them.

HISTORICAL INTRODUCTION

The cell walls of mature plants are principally composed of cellulose with lesser amounts of lignin and of polysaccharides that are described as hemicelluloses. Numerous x-ray studies have shown that the cellulose macromolecules are for the most part crystallized against each other to form submicroscopic crystallites, but submicroscopic interconnecting interstices result where the cellulose macromolecules are in random arrangement. It is into these interstices that amorphous lignin and hemicellulose are deposited in and around the cell wall. This arrangement of the constituents permits only occasional contact between lignin and the enormous surface of the cellulose but intimate contact between lignin and hemicellulose (1).

The generic term 'hemicellulose' was introduced by Schulze (2) to describe a group of polysaccharides in plant tissues which were extracted by dilute alkali but not by water, and which were more easily hydrolyzed with dilute acid than the cellulose proper. According to Norman (3,4,5) non-cellulosic cell-wall polysaccharides which are extracted by cold or hot alkali should be classed in either of two groups (i) the polyuronide hemicelluloses and (ii) the cellulosans. The polyuronide hemicelluloses, composed in part of methylhexuronic acid, are closely associated with lignin, and complete extraction of either species cannot be attained without a little concomitant removal of the other. Similarly, no solvent system has been found that will completely separate the cellulosans

from cellulose. Some of the cellulosans, xylan in straws and hardwoods, and mannan in softwood, are alkali-insoluble and remain in alpha-cellulose. Their association with the cellulose is supposed to correspond to the relationship between the components of a solid solution or a mixed crystal (6). However, the recent discovery of a disaccharide of glucose and mannose in the acetolysis products of slash pine alpha-cellulose would suggest an actual linkage (7). Polyuronide hemicelluloses and cellulosans may be separated by preparing Cross and Bevan cellulose (8).

Although hemicelluloses are not water soluble, they frequently form opalescent solutions in water after extraction with alkali and precipitation with acid. This behaviour suggests either a physical protection of the hemicelluloses by the other components of the plant, or a chemical linkage with the cell-wall constituents which is cleaved by alkali. This linkage is thought to be with lignin. A large amount of evidence has been amassed in favour of this postulate but unfortunately most of it is indirect, and until a lignin-hemicellulose complex has been isolated it cannot be said unequivocally that the linkage exists. One approach to the relationship between lignin and carbohydrates in maple wood was made by Harris, Sherrard and Mitchell (9) through chlorination, and methylation with dimethyl sulphate and alkali. Under conditions which were applicable to isolated lignin, lignin <u>in situ</u> was not methylated, but methylation did proceed after preliminary hydrolysis of the wood with dilute acid. Chlorination

of lignin in wood proceeded 50% further than chlorination of isolated lignin. These observations were explained by assuming that combination with other constituents of maplewood masked all lignin hydroxyl groups, and that enolic hydroxyl groups reverted to the ketonic form when these combinations were cleaved, thereby reducing chlorination.

The extraction of wood with dilute alkali removes only part of the hemicelluloses and extraction with progressively increasing concentrations of aqueous alkali dissolves hemicelluloses in a stepwise manner. However, Anderson and coworkers (10) and Sands and Nutter (11) found that the solubility of hemicelluloses in alkali increased when chlorination preceded extraction. Norman (8, 12) reported that in the preparation of Cross and Bevan cellulose, chlorination of the wood prior to extraction with sodium sulphite solution was as necessary to remove lignin as it was to remove hemicellulose. Moreover, after chlorination an aqueous extraction was nearly as effective as one with sulphite in removing hemicellulose. These facts lend support to the presence of a lignin-carbohydrate combination in wood.

Recently, Brauns and Seiler (13) showed that sprucewood when beaten in aqueous suspension was partly homogenized and could be separated as a colloidal solution. Although native lignin was extracted from the original wood, no additional amounts were recovered from the homogenized wood, and even when homogenized wood was methylated with diazomethane, and with dimethyl sulphate and alkali, lignin was not extracted

with indifferent solvents. These results indicated that lignin was present in the wood in either a highly condensed state or in chemical combination with other wood constituents, since a mere physical occlusion would probably have been overcome by the far-reaching mechanical disintegration the wood underwent during the homogenization.

As already mentioned, the difficulty of removing all lignin from mature plants without simultaneous removal of polysaccharides has been cited as additional evidence for lignin-hemicellulose union. Ritter and Kurth (14) selectively removed Klason lignin from maplewood by chlorination and extraction of the chlorolignin with a solution of pyridine in alcohol. The residue, termed 'holocellulose', had the general appearance of the plant material and retained nearly all the polysaccharides and associated groups. This method for preparing holocellulose was subsequently improved and shortened by the use of ethanolamine as solvent for chlorolignin (15). In subsequent studies on the preparation of holocellulose from various woods (16, 17), and in studies on the isolation of holocellulose by the use of sodium chlorite (18, 19, 20), it was demonstrated that when the lignin content of a holocellulose sample dropped below 2-3% an appreciable portion of the hemicellulose was removed. March (21) found that 30% of the hemicelluloses was lost in the wash waters when the lignin of chlorited aspenwood was reduced to 0.2 to 0.3%.

Finally, recent work on the action of liquid ammonia

on wood meal or holocellulose furnished evidence which was not inconsistent with the facts pertaining to a lignin-hemicellulose union. By extraction of sugar maplewood with liquid ammonia, Yan (22) was able to isolate a lignin fraction which was partly soluble in methanol and dioxane but which was free from nitrogen. Removal of lignin in this manner could result from either a purely physical process of solution, or a chemical process involving ammonolysis of ammonia-sensitive links. These links might be ester links between hydroxyl groups of the lignin and carboxyl groups of the holocellulose residue. In this event any amide units formed would be attached to the carbohydrate and not to the lignin constituent. The wood residue did retain nitrogen, and as a result of chemical analysis Neubauer (23) assumed the increase to be due principally to formation of acid amide. Similarly, nitrogen was retained in the residue of liquid ammonia extracted spruce chlorite holocellulose, but Milford (24) had to class approximately 60% of the nitrogen as of an undetermined type. Part was present as an ammonium salt, but little if any of the nitrogen could be shown to originate from amides. However, polyuronides not water-soluble before treatment with liquid ammonia and amounting to 13% of the wood were rendered water-soluble as a result of the treatment. Neubauer had previously reported the isolation of a water-soluble polyuronide hemicellulose from sugar maple under similar conditions, though not through the holocellulose route. The polyuronide, including pectic material, amounted to 2% of the ammonia extracted wood. Since ammonolysis of acetyl ester

links had been repeatedly demonstrated through the isolation of acetamide, it was not unreasonable to assume from the above evidence that uronic acid esters had been cleaved by the ammonia which resulted in the solubilization of the hemicelluloses with water. Foster, Schwerin and Cohen (25) recently presented evidence that the carboxyl groups of uronic acids in wood were esterified with lignin, but the possibility of a carbohydrate hydroxyl-uronic acid ester was not excluded. A more detailed and extensive account of the possible association of lignin and hemicelluloses in mature plants may be found in a number of reviews (1,4,26).

Recently, an excellent review of the chemistry of the hemicelluloses themselves (26) covered the work up to 1952 and supplemented other reviews (1,4,5,27). In consequence, the following account of the hemicelluloses was restricted to items of immediate importance for this Thesis. The researches started by Miss M.H. O'Dwyer in 1923 and continued until 1940 (28-33) produced a lasting contribution to the chemistry of hemicellulose, and also furnished a general line of approach for other workers. Most of her work was done on the heartwood and sapwood of oak. 'Hemicellulose A' was isolated from a 4% alkaline extract of the wood by treatment with acetic acid, and 'Hemicellulose B' by precipitation of the filtered mother liquor from 'A' by excess alcohol. When hemicellulose A from sapwood was given a prolonged treatment with takadiastase, a blue colouration given by iodine and characteristic of starch disappeared,

leaving a polysaccharide, with a specific rotation in 2% caustic soda of $[\alpha]_D$ - 97.5° and averaging 11 xylose units to 1 methylglucuronic acid unit. When further treated with takadiastase, the polysaccharide became water soluble. The addition of alcohol to the aqueous solution precipitated a polysaccharide with $[\alpha]_D$ - 51°, and an analysis indicated 1 uronic acid group, 6 xylose groups and 1 methoxyl group. This analysis was strikingly similar to that of hemicellulose B, $[\alpha]_D$ - 52° to - 53°, isolated from heartwood. Glucose was found in the sapwood but not in the heartwood hemicelluloses. A hemicellulose B of the same composition was obtained by Preece from a cold alkaline extract of boxwood (34).

Anderson (10, 35) followed the same general technique as O'Dwyer but chlorinated the wood residue after extraction with alkali to render further amounts of the hemicellulose accessible to aqueous alkali. Much of Anderson's work was on the hemicelluloses of the hardwoods, black locust, lemon wood, white birch and cottonwood. In the largest hemicellulose fragments, the ratio of xylan and methyluronic acid was almost 19:1, whereas in the smallest fragments it was about 8:1. Working with mesquite wood but not using chlorination, Sands and Gary (36) also isolated fractions containing xylose and methyluronic acid units in the same ratios as O'Dwyer's hemicellulose A and B. When Sands and Nutter (11) attempted to get a more quantitative hemicellulose separation from mesquite by using an intermediate chlorination, the clean-cut ratios previously obtained in the

case of more soluble hemicellulose fractions, disappeared.

Much less research has been done on the softwoods. Anderson (37) reported that white pine yielded hemicelluloses which contained 36% to 46% of mannan, 44% to 50% of xylan and 10% to 15% of methyluronic acid together with a qualitative test for glucose. Recently Milford (24) isolated from spruce chlorite holocellulose a "pure" hemicellulose which contained uronic acid residues, and the sugars xylose, arabinose and galactose, the molar ratio of which was 7.19:1:0.32.

As an alternative route to the isolation of hemicellulose, most workers have turned to extracting the wood residue after removal of all but a small percentage of the lignin. Ritter and Kurth (14, 39) modified the Cross and Bevan (38) preparation of wood pulp by extracting the chlorolignin with pyridine in alcohol and losses of hemicelluloses encountered in the earlier method were eliminated. Later, the use of alcoholic ethanolamine by Van Beckum and Ritter (15), to extract the chlorolignin, permitted the preparation of holocellulose in quantity. Schmidt and co-workers (40, 41) had used chlorine dioxide rather than chlorine for the removal of lignin in the preparation of Cross and Bevan cellulose, but by using chlorine dioxide and pyridine together he reported that the resulting wood residue contained all of the carbohydrate substances, or "skelettsubstanzen".

Schmidt's work did not attract particular attention for some years, since the method required 26 days, but never-

theless it did furnish a new route to holocellulose. Jayme (18) treated thin sections of wood in aqueous suspension with sodium chlorite and acetic acid at pH 4 and 60°C, and in this way brought about delignification by generating chlorine dioxide in situ. The time of delignification with acidulated sodium chlorite was shortened by Wise, Murphy and D'Addieco, and the method has subsequently become a standard procedure (19).

Delignification of wood by either Van Beckum and Ritter's method or by Wise's method, however, did not result in a true holocellulose, i.e. a wood residue free from lignin and containing all of the original carbohydrate material. Earlier results showed that analyses of Klason lignin plus holocellulose totalled very nearly 100% by weight for a number of woods (14, 15, 42-47) but subsequent work showed that a loss of carbohydrate was compensated by a retention of lignin, undetectable by the Klason method (46, 48, 49). A similar situation arose in chlorite holocellulose and Campbell and MacDonald (50, 51) neatly demonstrated that "acid soluble" lignin, i.e. non-Klason lignin, remained in the holocellulose and amounted to as much as 5 to 7% in beech and spruce. Some attempts have been made to eliminate losses of carbohydrate by either method, notably by chlorination in carbon tetrachloride at 0°C according to Thomas (49), or in water at 0°C according to Sitch (20, 52), to prevent localized overheating and by allowing 2-3% of the lignin to remain in the residue (18, 19). Timell and Jahn (20) showed that chlorination in aqueous suspension at 6°C, the original Van

Beckum and Ritter procedure, and the chlorite method of Wise, effectively removed all but 0.4, 2.3 and 4.9% respectively of the lignin in birch wood meal before any pentosan was lost.

While both the chlorine-ethanolamine procedure as modified by Thomas and Sitch, and Wise's chlorite procedure, have provided a starting material from which hemicelluloses may be easily removed, the weight of evidence from studies on the molecular dimensions of cellulose and hemicellulose in the respective holocellulses would indicate that less degradation occurred during chlorination than through the action of chlorine dioxide or a buffered solution of sodium chlorite.

Many references may be found in the literature to the average D.P. (degree of polymerization) of cellulose in wood or holocellulose, but because of factors then unknown the numerical values have little importance, and the D.P's quoted for a particular species of wood vary widely. However, more recently some attempts have been made to compare systematically the methods of preparing holocellulose. Atchison (46) applied the method of Van Beckum and Ritter to wood meal from black spruce and found that degradation of the holocellulose did not occur until the lignin content reached 1%, when a slow depolymerization started. On the other hand, application of sodium chlorite or chlorine water resulted in considerable degradation. The procedures investigated were able to bring about the isolation of holocellulose with increasing degradation in the order as follows: chlorination according to Van Beckum and Ritter, sod-

ium chlorite in water buffered with acetic acid, chlorination in water, unbuffered aqueous solution of sodium chlorite. Jorgensen (53, 54) found that cellulose nitrate from aspenwood had a D.P. of 2,900 but after applying Wise's procedure the D.P. was reduced to 2,070. These values were recalculated on the assumption that Km in Staudinger's relationship was 10.10^{-4} for solutions of cellulose nitrate in acetone. Correspondingly, the D.P's of cellulose nitrate from spruce was 2,250 and 1,940 respectively. A more systematic investigation was carried out by Timell and Jahn (20). The D.P. of cellulose nitrate from paper birch was 2,975 but this value was reduced to 2,500 when Wise's procedure was used, and the lignin content was still 4.9%. By comparison when the chlorine-ethanolamine method of Sitch was applied, the D.P. was 2,800 and the lignin content was reduced to 1.2%.

Wethern (55) prepared black spruce holocellulose by: (a) the chlorine dioxide-pyridine procedure of Schmidt; (b) the chlorite procedure of Wise; and (c) the chlorine-ethanolamine procedure (with Thomas' modification). Hemicelluloses were extracted with 5 and 16% aqueous potassium hydroxide from each holocellulose sample and the viscosities of the extracts were determined. In each case the magnitude of the viscosity of the 16% extract was higher than that of the 5% extract, and the viscosity of the hemicelluloses, obtained from each type of holocellulose, increased in the order of the methods listed above. Wise and Thompson (56) found higher intrinsic viscosities for fractions isolated directly from big tooth aspen than

from holocellulose, and suggested that chain degradation occurred during the preparation of the holocellulose, using the chlorine-ethanolamine method of Thomas.

Although the extent of cellulose degradation by the various procedures decreased in the same order as with the hemicellulose, these findings are somewhat surprising in the light of the results obtained with cotton linters. When Staudinger and Jurisch (57) treated cotton linters with chlorine water, the D.P. decreased to a value which was only 38% of the original after 1 hour at 20°C, and only 6% after 25 hours, whereas the corresponding values for chlorine dioxide were 73% in both cases and 60% after 144 hours. Atchison (46) found that the T.A.P.P.I. viscosity of cotton linters dropped from 19.2 to 5.1 after treatment with buffered sodium chlorite, but Lovell (58) reported that essentially no change occurred. These conflicting results seem to have been resolved by Timell and Jahn (20) who showed that cotton linters were degraded both by the Van Beckum and Ritter method and by Wise's chlorite method, although much more severely by the former. Degrees of polymerization, determined viscometrically on the nitrate esters, dropped from 1,950 to 900 after 7 chlorinations but only to 1,700 after 3 treatments with sodium chlorite. Depolymerization also resulted from chlorination in water at 0°C.

It would thus appear that present knowledge of cellulose, hemicellulose and lignin is insufficient to explain why an apparent degradation of the carbohydrates occurred during

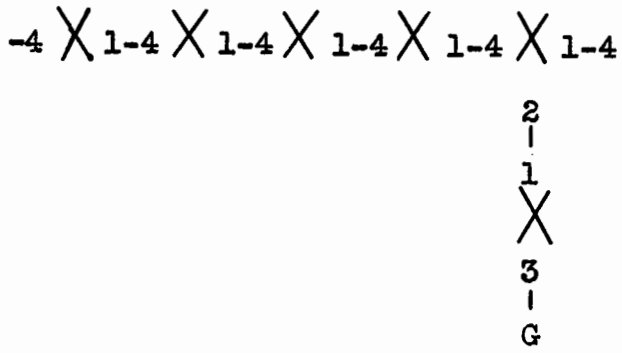
isolation from the plant when degradation was less for cellulose itself, or in the absence of lignin.

Considerable attention has been given to the chemical constitution of polysaccharides from gums, mucilages, seeds and straws, but little is known of the chemical constitution of the hemicelluloses of wood. Reference to the structure of polysaccharides from various sources may be found in a review of polysaccharide chemistry by Whistler and Smart (59).

After hydrolysis of wood with dilute acid, a mixture of carbohydrates results, the constituents of which can be identified, as for example, rhamnose, arabinose, xylose, mannose (?), glucose, galactose, oligosaccharides and various acidic materials from aspen (60). Earlier reference was made to the fact that a part of the hemicelluloses of hardwoods appears to be composed of varying amounts of xylose and methyluronic acids (10, 35, 36, 28-33) but little is known of the nature of the linkages which unite the units in these polysaccharides. Neubauer (23) isolated a polyuronide from sugar maple wood. Acetylation, acid hydrolysis, methylation and oxidation with aqueous sodium periodate suggested that the material had as its repeating structure a main chain of xylan units linked 1-4, and on the average every fifth unit was substituted by a side chain of one xylose unit linked 1-2 and terminated by a 1-3 linkage to a 3-methylglucuronic acid unit.

FIGURE 1

Structure of Polyuronide Hemicellulose



X represents anhydro-D-xylose and G a 3-methylglucuronic acid residue.

This was the first structure proposed for a hemicellulose from wood. From aspen, however, Jones and Wise (60,61) showed that an aldobiuronic acid, isolated from the hydrolysis products of the wood, was composed of 4-methylglucuronic acid linked to the 2-position of a xylose residue. Similarly, White (62) and Smith (63) found that mesquite gum contained 4-methylglucuronic acid. Among the hydrolysis products of aspen wood, Jones and Wise (60) also found a disaccharide, and a trisaccharide of D-xylose. These two materials had earlier been isolated by Whistler and Tu (64,65) from the hydrolysis products of corn cob holocellulose and shown to have 1,4-β linkages. The structure of xylan from esparto grass, now fully elucidated (66,67) consists of a singly-branched chain molecule containing 75 ($\pm$5) D-xylopyranose units, the single

branching point being formed by a 1-3 union. The high negative rotations of the polysaccharide and its derivatives indicated that the linkages were β .

Adams (68) isolated a hemicellulose from wheat straw holocellulose, and proposed a structure consisting of approximately thirty-two D-xylose units linked 1,4- in a chain to which five L-arabinose units and three D-glucuronic acid units were attached as side groups by 1,3- glycosidic bonds. From the structure of the methylated aldobiuronic acid, isolated by acid hydrolysis of the methylated polysaccharide, D-glucuronic acid units were considered to be linked to the xylose units in the 3-position. Bishop (69), by isolating an aldobiuronic acid from wheat straw holocellulose and by the synthesis of the β - anomer, definitely proved that D-glucuronic acid was linked in the α configuration to the 3-position of a xylose unit, which supported Adam's opinion.

Studies on the chain length of hemicelluloses by viscometric and osmometric determinations indicated an average D.P. of approximately 150-160, for beech, straw and spruce (70), and also black spruce (55) and big tooth aspen (56). Wethern (55) has shown that at least for black spruce the hemicelluloses are composed of fractions with D.P's ranging from 50 to 300. The latest constant K^1 reported, in the relation $[\eta] = K^1$ (D.P.) where $[\eta]$ is the intrinsic viscosity, was 4.4×10^{-3} (56).

DISCUSSION OF RESULTS

Throughout the present investigation every effort was made to isolate the hemicelluloses from aspen wood in a state as unchanged from their original one as possible.

A study was first made of the methods of preparing holocellulose from wood to provide a basis for the selection of a starting material which would yield hemicelluloses with minimum degradation. After being extracted exhaustively with alcohol-benzene, the wood meal was converted into holocellulose by two procedures. The first one was the chlorite method of Wise, Murphy and D'Addieco (19), giving holocellulose I. The second one, a modification of the method of Ritter and Kurth (14), giving holocellulose II, was similar to the method used by Sitch (52) and by Timell and Jahn (20) who chlorinated paper birch wood meal in ice water. Although ethanolamine is more commonly used as a solvent for chlorolignin (15), pyridine was employed in an attempt to maintain the loss of carbohydrate material at a minimum during extractions. As a criterion for evaluating these procedures, the change in the degree of polymerization (D.P.) of the cellulose in the wood by the action of the delignifying agents was used.

Cellulose in the wood and holocellulose samples were isolated as the nitrate esters by direct nitration at 24°C. for 5 hours with a nitration acid composed of 64% nitric acid, 26% phosphoric acid and 10% phosphorus pentoxide, according to Alexander and Mitchell (71). Intrinsic viscosities of the nitrates

were determined from the viscosity of acetone solutions (Fig. 2) and degrees of polymerization were calculated using 10.10^{-4} for the constant Km in Standinger's equation (72). When not based on this value the degrees of polymerization reported in the literature were recalculated here. The D.P. found for holocellulose I was 1,530, whereas the D.P. of holocellulose II was 1,900 and that of aspen wood was 2,110.

The value for the D.P. of the aspen wood cellulose was considerably lower than that reported by Jörgensen (53,54) for the same material (2,900) and by Timell and Jahn (20) for paper birch cellulose (2,975). The other results, however, were not inconsistent with the findings of Timell and Jahn. Birch holocellulose prepared by a slightly modified Van Beckum and Ritter procedure was somewhat degraded, the D.P. of the cellulose portion being 2,800, and a considerable degradation apparently occurred during chloriting operations since the D.P. was lowered to 2,500. In comparison with birch chlorite holocellulose, Jörgensen found a value of 2,070 for the corresponding holocellulose from aspen. Both values however were higher than the value reported here.

Although degradation of the cellulose was apparently more extensive by the action of chlorite than chlorine, chlorite holocellulose was chosen as a starting material to isolate the hemicellulose portion of the wood. Atchison (46) and Thomas (49) have shown previously that ethanolamine was retained by holocellulose during extractions of chlorolignin, and in

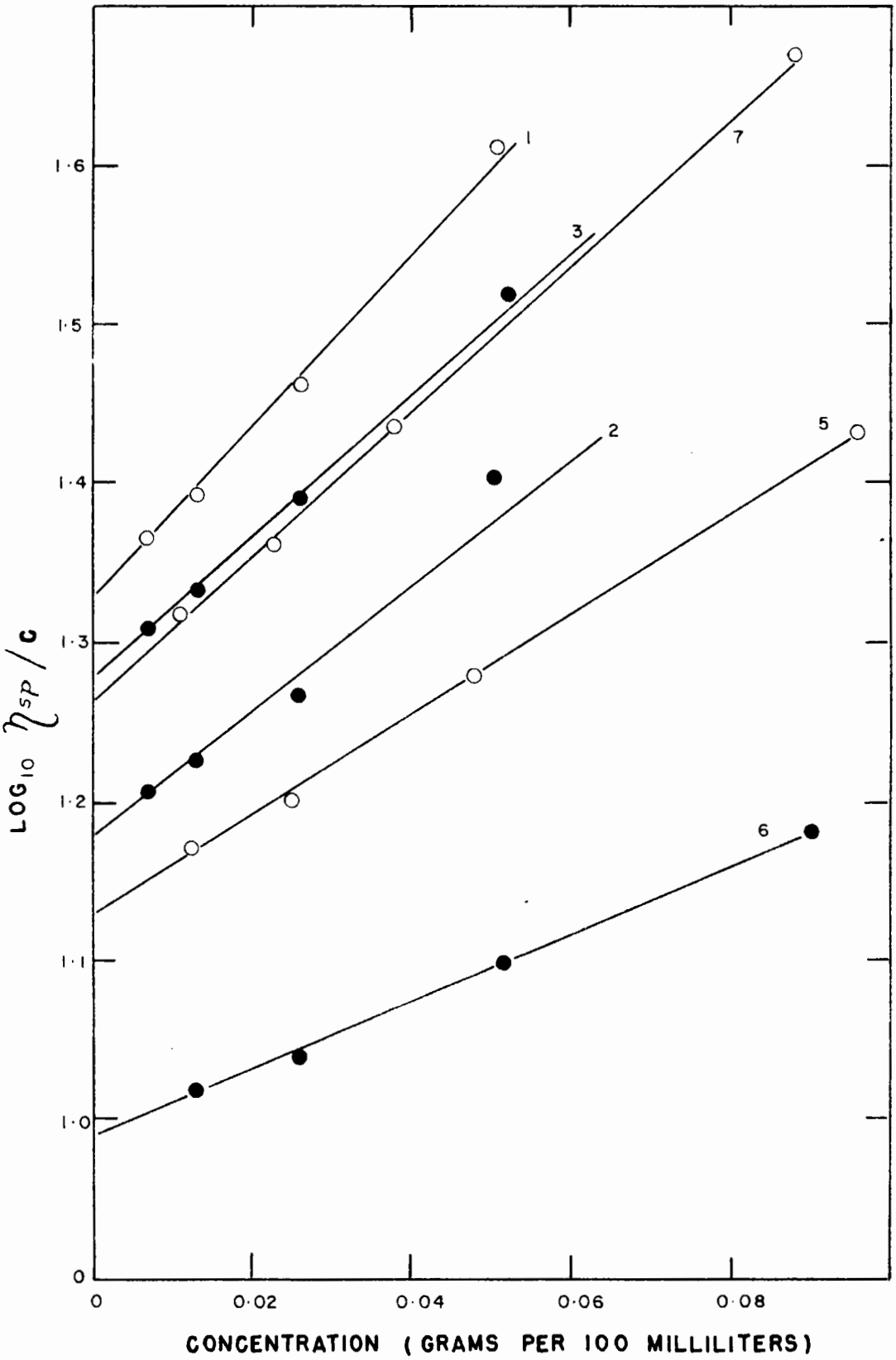


FIG. 2. Plot of Viscosity against Concentration for Samples of Cellulose Nitrate in Acetone referred to in Table XVII.

the present case a similar retention was also found with pyridine. This was seen by the increase in nitrogen content from 0.095% in the wood to 0.27% in the holocellulose. In consequence, since liquid ammonia was subsequently employed in an intermediary stage during the extraction of hemicellulosic material, any combined nitrogen in the holocellulose originating from ammonia would be masked by the nitrogen of pyridine. As a result, any information concerning lignin-carbohydrate linkages derived from an investigation of the combined nitrogen would not be attained.

Since products prepared from spruce wood by Milford (24) were comparable to those prepared from aspen in the present study, it was considered of interest to determine the changes in D.P. of the spruce cellulose during the isolation of the hemicelluloses. The nitrating medium was changed to a composition of 22% phosphorus pentoxide and 78% nitric acid in the hope that better yields of acetone-soluble cellulose nitrate would be attained. This acid was used previously to nitrate cotton linters and found to give good results (73,74). Spruce wood, however, did not yield a soluble nitrate when nitrated at either 6°C. for 24 hours or at 26°C. for 6 hours, although aspen wood could be nitrated. Jörgensen (53,54) also found that spruce behaved quite differently from aspen when nitrated and that isolation of cellulose from spruce wood could not be achieved without considerable degradation. This was reflected in the observed D.P. of 2,730 for the cellulose in a bisulphite pulp but only 2,250 for cellulose isolated by direct nitration.

The D.P. of a cellulose nitrate prepared at 6°C. from Milford's chlorite holocellulose was only 1,330 and holocellulose extracted first with liquid ammonia at approximately 20°C. and then with water yielded a nitrate with a D.P. of 980. Cellulose nitrate prepared in the same manner from aspen wood had a D.P. of 1,860, a value slightly lower than that obtained with Mitchell's acid. These degrees of polymerization were calculated as described previously for the other cellulose nitrates, the intrinsic viscosities being calculated from the viscosity-concentration plots shown in Fig. 2.

It would thus appear that liquid ammonia had some degradative action on holocellulose although Björkqvist and Jörgensen (75) reported no marked drop in the D.P. of cellulose in birch and spruce holocellulose during liquid ammonia treatment and subsequent extractions. In this connection, Bishop (76) found that an isolated polyuronide from wheat straw holocellulose was degraded by 8.4% in liquid ammonia. These conflicting results and the low yields of acetone-soluble nitrate from holocellulose and extracted holocellulose, namely 45.5% and 81.7%, respectively, made it uncertain whether the drop in D.P. was due to the presence of ammonia-sensitive linkages or whether the observed decrease in D.P. occurred during the nitration. The former alternative, however, would seem more probable.

A quantity of chlorite holocellulose, the analysis of which is shown in Table I, was next prepared from 1 kg. of sol-

vent-extracted wood meal in 84.4% yield and with a lignin content of 2.9%. Timell and Jahn (20) showed that loss of carbohydrate material in birch chlorite holocellulose did not occur until the lignin content was reduced to approximately this level, while March (21) found that chlorite holocellulose from aspen was severely degraded when the lignin content was reduced to 0.2 to 0.3%, as demonstrated by the loss of 30% of the hemicelluloses. In contrast to these results, an aspen holocellulose prepared by a modified Van Beckum and Ritter method was found by Thomas (48) to have suffered only a slight loss in carbohydrates at as low a lignin content as 0.4%.

After delignification the holocellulose was extracted twice with water at 65° to 70°C. for 3 hours at pH 5 to 6 to remove residual soluble material which amounted to 3.6%.

Soluble materials in chlorite liquors have been investigated by various workers. Jayme and Hanke (77) dialyzed and electrodialyzed chlorite liquors from spruce wood and after evaporating the solution to dryness analyzed the residue in toto for methoxyl, carbon and hydrogen. The data obtained fell somewhere between average values for lignin and carbohydrate materials. Sugars were produced on acid hydrolysis and tentatively identified as glucose, together with gluconic acid. Barton (78) found that carbohydrate material and lignin in chlorite liquors from slash pine were precipitated together when the liquor was acidified with concentrated sulphuric acid. However, the lignin could be removed by dissolving in dioxane and the

TABLE I

Comparative Analyses[a] of Holocellulose and Wood

Component	On Basis of Wood %	On Basis of Holocellulose %	Recovery of Component On Basis of Content in Wood %
Moisture	6.37,6.37	- - -	- - -
Ash	0.41,0.41	0.46,0.43	- - -
Lignin	19.3,19.5	2.8,3.0	12.7
Acetyl	4.25,4.30	5.02,5.02	99.0
Pentosan	20.9,20.9	23.3,23.0	93.7
Xylan[b]	19.3,19.3	21.2,20.7	91.4
Uronic Anhydride	4.78,4.60	6.08,6.20	110.0
Yield	100	84.4	- - -
Yield (Lignin-free)	80.6	81.5	

(a) Based on moisture and ash-free material.

(b) Corrected for uronic anhydride.

conclusion was reached that carbohydrate material and lignin in the liquors were physically occluded. In an investigation of the components of spruce chlorite liquors, Bublitz (79) found that alcohol precipitated a large carbohydrate fraction which, on acid hydrolysis, yielded considerable amounts of mannose, galactose and uronic acids with smaller quantities of xylose and arabinose. Lignin was also present in the fraction and was obtained as an insoluble residue after hydrolysis. This residue, when isolated from various sub-fractions of the alcohol-insoluble material, had a methoxyl content of 9.5% and an ultraviolet absorption spectrum which resembled that of lignin derivatives; the yields amounted to 2.0 to 18.8%. As a result of this evidence, Bublitz concluded that part of the alcohol-insoluble, water-soluble fraction may have been a carbohydrate-lignin complex.

Water-soluble material remaining in the holocellulose presumably should be comparable to material dissolved in the chlorite liquors and a study was made to determine whether or not evidence could be obtained which would indicate the presence of a carbohydrate-lignin complex. The material was divided into Fraction I, insoluble in 70% aqueous alcohol, Fraction II, precipitated after the addition of excess alcohol, and Fraction III, soluble in the mother liquor from Fraction II and recovered on evaporation of the solution to dryness.

Fraction I with a methoxyl content of 3.6% constituted the combined material isolated in the above manner from the first

hot water extraction (Fraction AI with $[\alpha]_D^{22}$ +9.7° (in water)) and from the second hot water extraction (Fraction BI with $[\alpha]_D^{22}$ +35.6° (in water)) and yielded xylose, arabinose, galactose, and uronic acid derivatives on acid hydrolysis as indicated by a paper chromatogram using ethyl acetate-water-pyridine (2:2:1) as the solvent. After oxidative hydrolysis with bromine and hydrobromic acid by the method of Heidelberger and Goebel (80), 0.11 g. of mucic acid was produced from a 1.75 g. sample. This yield of mucic acid indicated that the fraction was in part composed of pectic material.

Fraction II had a methoxyl content of 6.8% and was further fractionated in an attempt to isolate fractions with constant properties.

As seen from Table II, no such fractionation was accomplished as indicated by the methoxyl values of the sub-fractions. However, sub-fractions 3,4,5, 6 and 7 (Fraction IIa) had an ultraviolet absorption spectrum (Fig. 3) similar to lignin and on acid hydrolysis yielded xylose, arabinose(?) mannose, galactose, glucose and uronic derivatives as indicated by paper chromatography; an insoluble residue in the hydrolysate amounted to 6.5%. It would thus appear that Fraction II was probably an intimate mixture of polysaccharides and "modified" lignin, which could not be separated by the fractional precipitation technique, rather than a carbohydrate-lignin complex.

The methoxyl content of Fraction III, 12.2%, was higher than that of Fraction II and only traces of xylose and arabinose

TABLE II

Sub-fractions of Fraction II, an Aqueous Extract of Holocellulose

Sub-fraction	Yield g.	Methoxyl %	Ash %	Ethanol Concentration[a]
1	0.1675	1.50	52.0	50
2	0.1735	4.57	8.9	62
3	0.4414	4.05	7.3	64
4	2.4325	5.24	7.7	69
5	1.0505	5.68	7.8	72
6	0.6471	6.92	6.2	74
7	0.9382	7.56	6.8	77
8	0.5146	9.00	7.7	81
9	1.4877	11.20	10.2	-
Recovery	7.853, 96.5%			

(a) Required to precipitate the fraction from aqueous solution.

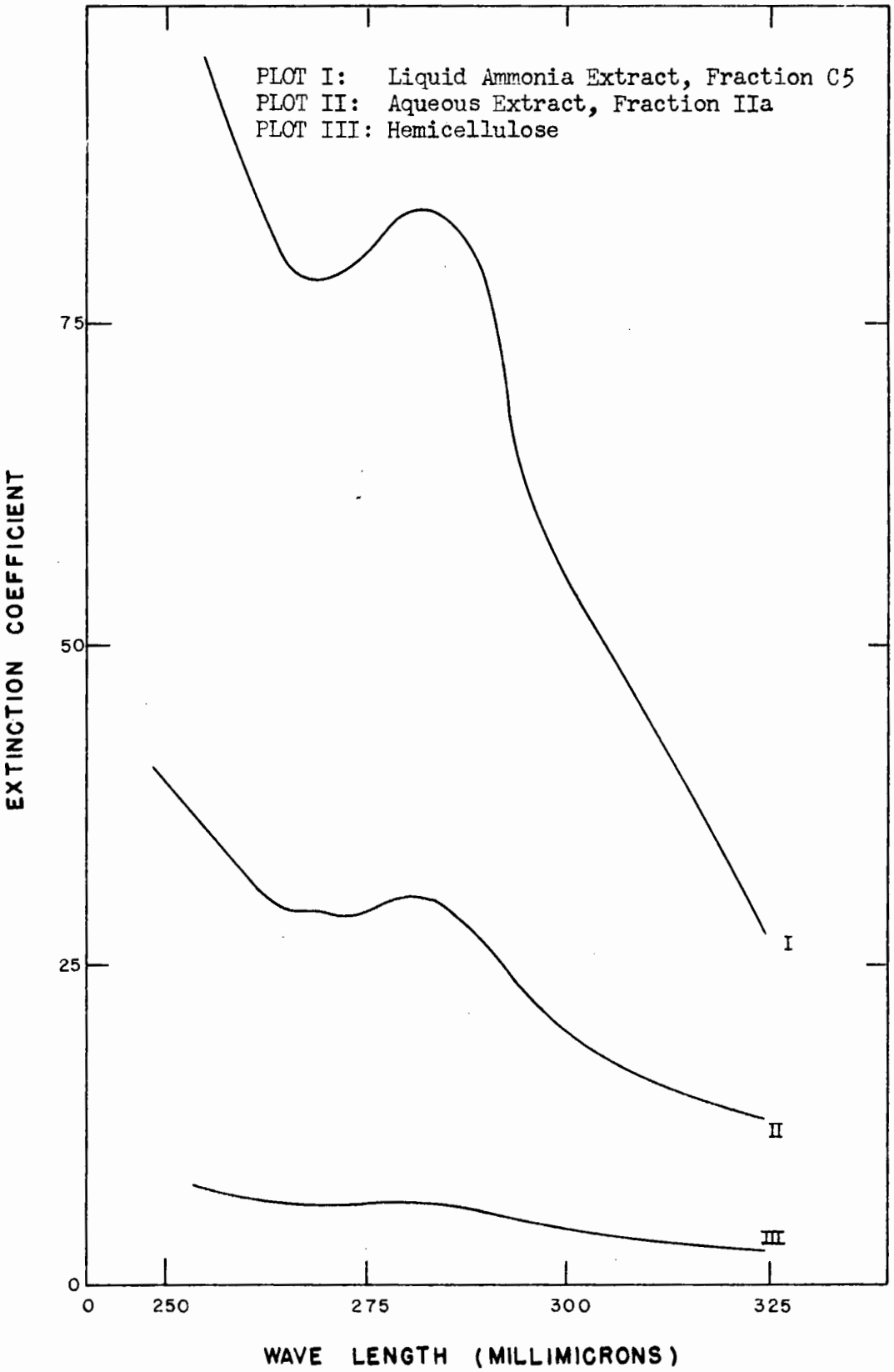


FIG. 3. Ultraviolet Absorption Spectra for Various Materials in N Sodium Hydroxide Solution.

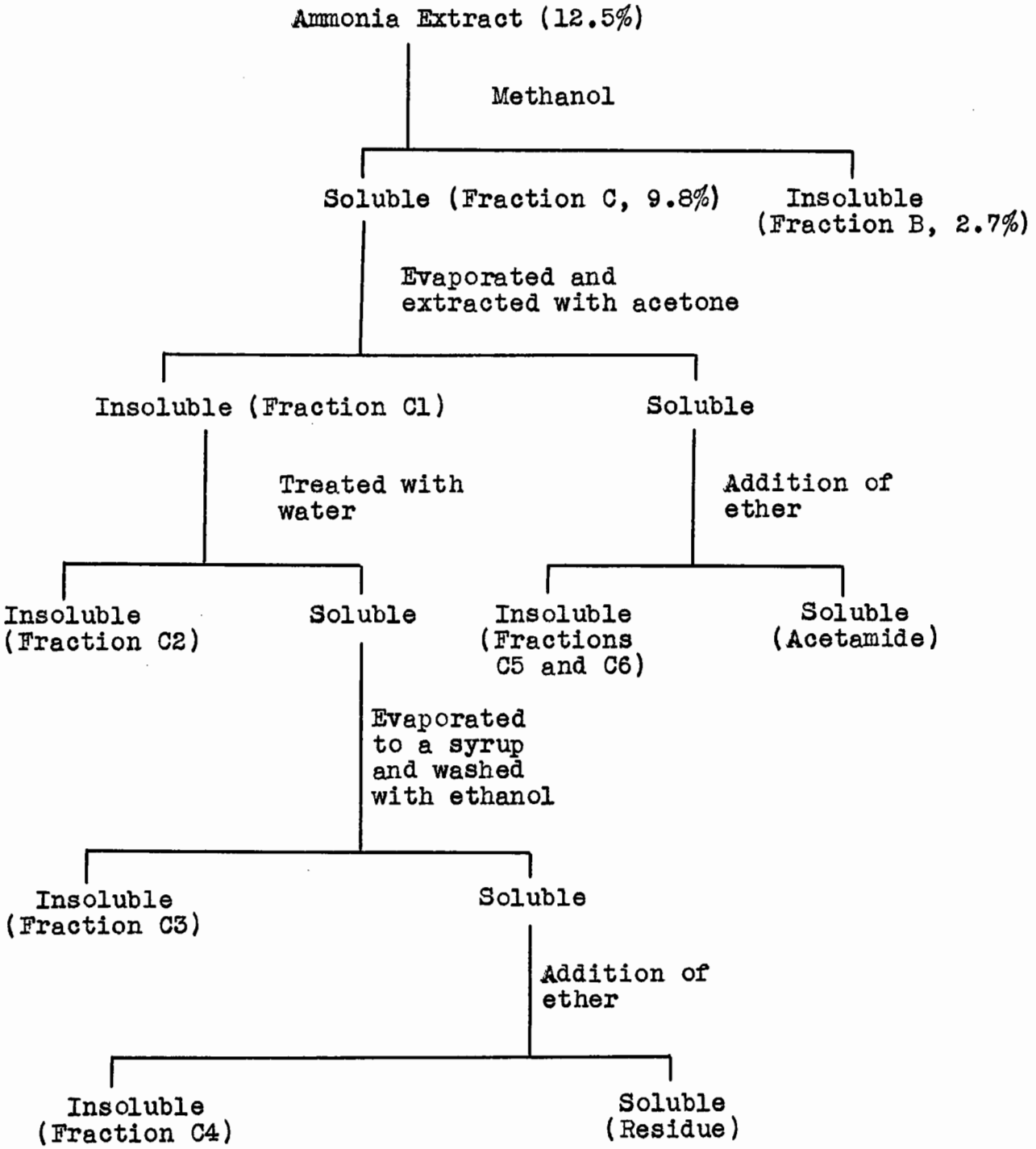
in the acid hydrolysate of the fraction were detected on the paper chromatogram, a large insoluble residue (32%) being also present. This fraction, closely resembling sub-fraction 9 (Table II), consisted mainly of "modified" lignin.

Fraction III was also analyzed for uronic anhydride, the apparent value being approximately 11% as calculated from the amount of carbon dioxide evolved according to the method of McCready, Swenson and Maclay (81). However, since no uronic acids could be detected on the paper chromatogram, carboxyl groups originating from some other source must have been present. This anomalous result will be referred to later.

The holocellulose, now free from water-solubles, was dried over phosphorus pentoxide in vacuo to a moisture content of less than 1%. Dr. Jablonski and Mr. Sanderson had noted previously that this step was essential for the subsequent extraction with liquid ammonia in order to avoid an initial rapid evolution of heat. The Experimental Section describes in detail the apparatus and technique of conducting the extractions at room temperature and about 150 p.s.i. pressure.

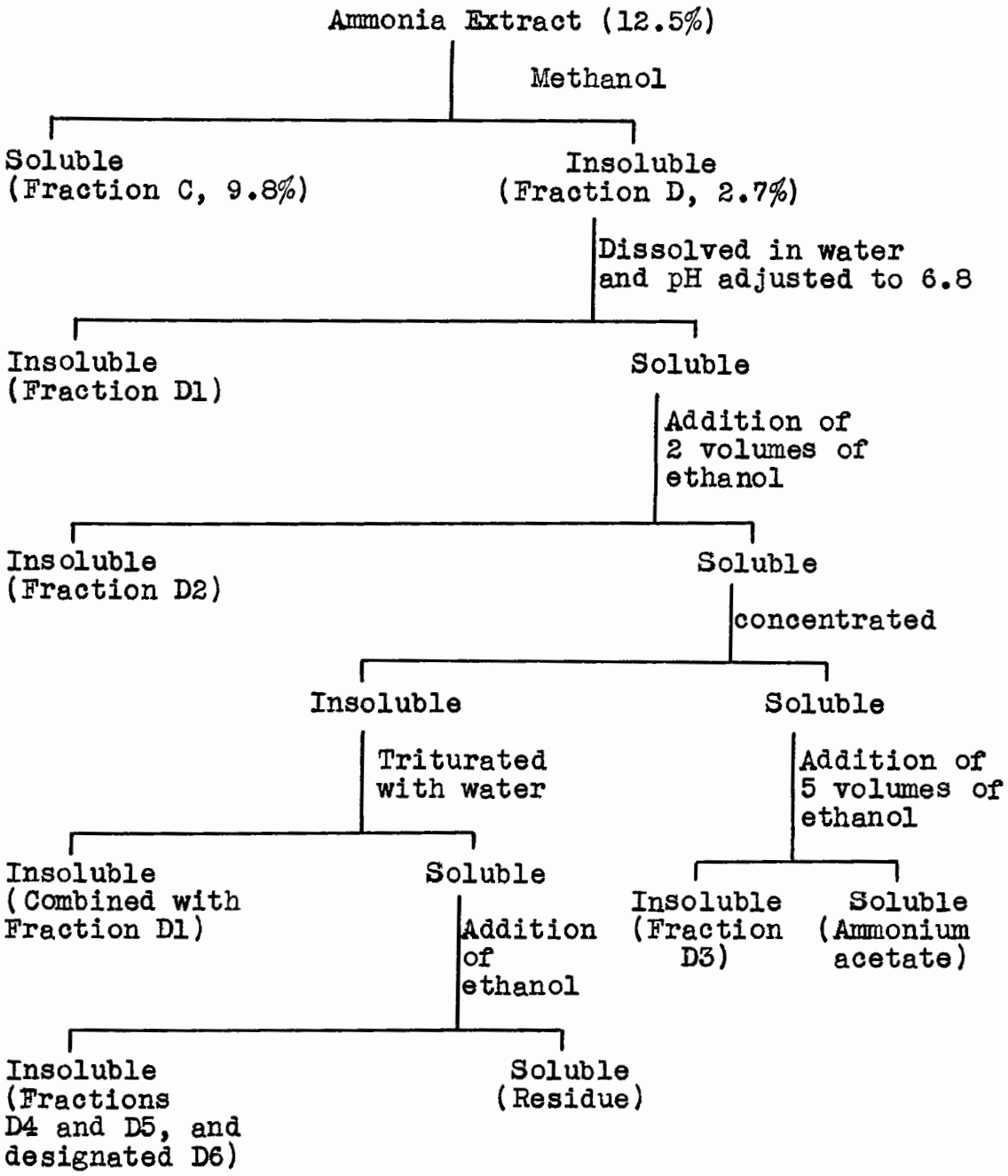
The liquid ammonia extract constituted 12.5% of the holocellulose and was fractionated extensively in order to discover evidence of "modified" lignin and polysaccharides in addition to the amount previously identified in the aqueous extract. Fig. 4 and Fig. 5 are flowsheets giving the essential details of the fractionations of the methanol-soluble and methanol-insoluble portions, respectively.

FIG. 4. Fractionation of Liquid Ammonia Extract[a] (Methanol-Soluble)



(a) Percentages based on moisture-free holocellulose.

FIG. 5. Fractionation of Liquid Ammonia Extract (Methanol-Insoluble)



(a) Percentages based on moisture-free holocellulose.

The methanol-soluble portion, amounting to 9.8% of the holocellulose was first fractionated with acetone for the purpose of removing soluble acetamide. This compound had been repeatedly identified in liquid ammonia extracts (22,23,24,76) and its presence demonstrated that liquid ammonia was not a neutral solvent. Acetamide comprised 56% of the methanol-solubles or 45% of the total extract, and accounted for 82% of the acetyl groups in the holocellulose. Some of the acetyl groups (8.5%), however, were protected in an unknown manner and remained in the ammonia-extracted holocellulose residue.

Fractions C2, C3, C5 and C6, amounting to 34% of the methanol-soluble portion and 3.4% of the holocellulose, were dark brown solids with methoxyl contents of the order of 16%. A Molisch test on each fraction was negative. Milford (24) recently isolated lignin derivatives from spruce chlorite holocellulose in a similar manner and characterized them as "acid-soluble" lignin of the type isolated by Campbell and McDonald (50, 51). As shown in Fig. 3 the ultraviolet absorption plot of Fraction C5 was typical of lignin and since the methoxyl content of lignin in hardwoods is approximately 20%, part of these fractions probably were also of the "acid-soluble" type. Mr. Thompson in this laboratory discovered recently that periodate lignin (82) was appreciably demethoxylated by the action of chlorine dioxide, a finding which was not inconsistent with the methoxyl content of the various "modified" lignins extracted from the holocellulose.

Fraction D, the methanol-insoluble portion of the ammonia extract, amounted to 2.7% of the holocellulose and consisted of material very similar to the aqueous extract of the holocellulose. An investigation of Fractions D2 and D3 by hydrolyzing a sample of each and chromatographing the hydrolysates showed that part of each fraction was composed of polysaccharides and that the sugars in the hydrolysates, equivalent to glucose, amounted to 42.5% and 6.7%, respectively. Glucose, galactose, xylose and uronic acid derivatives were identified in the hydrolysates of both D2 and D3 but while mannose was also found in the former, arabinose was found in the latter. In accordance with the carbohydrate content, the methoxyl content of Fraction D2 was lower than that of Fraction D3, the values being 5.64% and 12.40%, respectively.

Fraction D6 was similar to Fraction D3 both in appearance and methoxyl content (14.06%) and these two fractions were combined, dialyzed and fractionated from an aqueous solution with ethanol, with the result shown in Table III.

Except for the first and last sub-fractions the methoxyl contents were fairly constant, but after combining sub-fractions 4 to 11 and hydrolyzing, uronic acid derivatives and the same sugars found in Fraction D3 were identified on the paper chromatogram. Evidence thus obtained from the ammonia extract again indicated that "modified" lignin and polysaccharides existed as a mixture in the holocellulose, and the presence of a carbohydrate-lignin complex appeared unlikely.

TABLE III

Fractionation of Fractions D3 and D6 by precipitation from Water with Ethanol(a)

Sub-fraction	Yield g.	Methoxyl %	Ash %
1 (water insoluble)	1.3325	20.40	- - -
2	0.0568	- - -	- - -
3	0.1652	- - -	- - -
4	0.1642	11.13	- - -
5	0.6012	11.54	5.2
6	0.8945	10.67	- - -
7	0.6165	10.13	5.3
8	0.3963	10.85	- - -
9	0.4467	10.32	4.4
10	0.4177	10.42	- - -
11	0.5167	11.25	- - -
12	3.1416	15.85	- - -
Recovery	9.75, 81.7%		

(a) Original weight 11.9 g. before dialysis and centrifuging.

Before proceeding to an account of the hemicelluloses, whose study was the principal object of this research, it will be convenient first to present an explanation for the anomalous uronic anhydride content of Fraction III (an aqueous extract of holocellulose).

As previously mentioned, an examination of the acid hydrolysate of Fraction III by paper chromatography showed that uronic acid derivatives were absent although the apparent uronic anhydride content of the fraction was approximately 11%. Furthermore, holocellulose itself was found to contain an uronic anhydride content which was 10% in excess of that present in the wood (Table I). These results implied that "modified" lignin remaining in the holocellulose contained carboxyl groups which were presumably formed through oxidation. Any carboxyl groups thus formed would contribute to the calculated uronic anhydride content.

In order to test the oxidizing action of chlorine dioxide (formed from sodium chlorite during delignification) a sample of periodate lignin (82) which had been oxidized with chlorine dioxide by Mr. Thompson was analyzed for uronic acids. The carbon dioxide content was 5.32% which was equivalent to 21.3% uronic anhydride. Periodate lignin itself analyzed for 8.44% uronic anhydride, although no furfural-yielding components were present. These results showed that both the uronic anhydride and corrected pentosan content of chlorite holocellulose would be in error unless a suitable correction were made for the

presence of "modified" lignin.

Although exhaustive extraction with water at 65° to 70°C. removed 3.6% of the holocellulose, after liquid ammonia treatment two cold water extractions and two hot water extractions (70°C.) removed an additional 11.4%, or 9.6% based on the original wood. As mentioned previously, this increase in solubility may have resulted from the swelling action of liquid ammonia and ammonolysis of esters. The individual aqueous extracts were carefully concentrated under reduced pressure at pH 6, and impure hemicelluloses were precipitated by adding alcohol to a concentration of 70%. Soluble material in the mother liquors was isolated by evaporating the solutions to dryness under reduced pressure but was not investigated further. The yields of material isolated from each operation are shown in Table IV.

TABLE IV

Yield of Material Removed from Ammonia-Treated Holocellulose by Aqueous Extraction

Material	Yield On Basis of Holocellulose %	Yield On Basis of Wood %
Cold Water Extract		
Insol. in 70% Alcohol	5.8	4.9
Sol. in 70% Alcohol	1.9	1.6
Hot Water Extract		
Insol. in 70% Alcohol	3.3	2.8
Sol. in 70% Alcohol	0.4	0.3

Throughout all previous successive stages in the isolation of the hemicelluloses, the appearance of the original wood meal remained unchanged except for colour. However, during the hot water extractions of the ammonia-treated holocellulose, marked swelling of the fibres occurred and the holocellulose residue resembled a typical commercial pulp. This material was quite different from the holocellulose residue from spruce at this stage of extraction, as found by Milford (24), and it was of interest to determine some of its properties. The physical measurements recorded in Table V were kindly determined by Mr. W.C. Lodge of the Pulp and Paper Research Institute, Montreal.

An examination of Table V shows that all properties of the pulp were extremely poor although this was not unexpected since the original wood meal was 40 to 80 mesh. Information of more value on the pulp qualities could be obtained by using wood chips as a starting material. However, the fact that the final holocellulose residue was highly swollen with water corroborates the assumption made earlier that liquid ammonia disrupted some chemical linkages present in the wood.

In a comparison of methods for purifying hemicelluloses, a sample of the hot water-extracted hemicellulose was redissolved in water, the solution centrifuged to remove finely-divided insoluble material, and the hemicelluloses reprecipitated by the addition of alcohol. Part of the hemicellulose was then precipitated three times from N sodium hydroxide solution in an atmosphere of nitrogen, a process which McDonald (83)

TABLE V

Effect on the "Pulp" of Beating in a Waring Blender[a]

Stirring Time (min.)	Can. Stand. Freeness	Bulk	Burst Factor	Tear Factor	Breaking Length (metres)	% Stretch
0	522	2.73	5.3	0.21	1105	2.0
5	394	2.04	14.6	0.24	3320	2.0
15	339	2.17	13.3	0.30	3430	1.9
30	284	2.06	16.1	0.34	3580	2.2

(a) Aspen chlorite holocellulose after extraction with liquid ammonia and water.

found would remove acid-soluble lignin from a xylan isolated directly from beech wood, and the other part was bleached at room temperature for 5 hours with a buffered solution of sodium chlorite. The resulting products were only partly soluble in water, approximately 14% being insoluble. The water-soluble fractions, however, had similar analyses (Table VI) and no apparent degradation of the hemicelluloses resulted from either treatment. As seen from Fig. 3 and Fig. 6, a small amount of "modified" lignin still remained in the untreated hemicellulose, and both treatments, while removing equal amounts of the lignin, did not remove it entirely (Fig. 6).

On the basis of Table VI and Fig. 6, hemicelluloses from the cold water extract and the hot water extract were bleached separately and the products isolated as shown in Fig. 7. The insolubility of Fractions L1 and L2, although originally soluble, must have resulted from a change in colloidal properties, presumably from a change in the zeta-potential, through the action of sodium chlorite or sodium hydroxide.

The fractions were hydrolyzed with 2½% sulphuric acid for 5 hours at 100°C. and the hydrolysates were examined on a paper chromatogram. In each case xylose was present predominately with traces of glucose, arabinose and rhamnose; uronic acid derivatives were also present. Jones and Schoettler (84) and Björkqvist and Jörgensen (85) proved recently that L-rhamnose occurred as a constituent sugar in aspen wood through its isolation and formation of derivatives. Fraction L1 (insol-

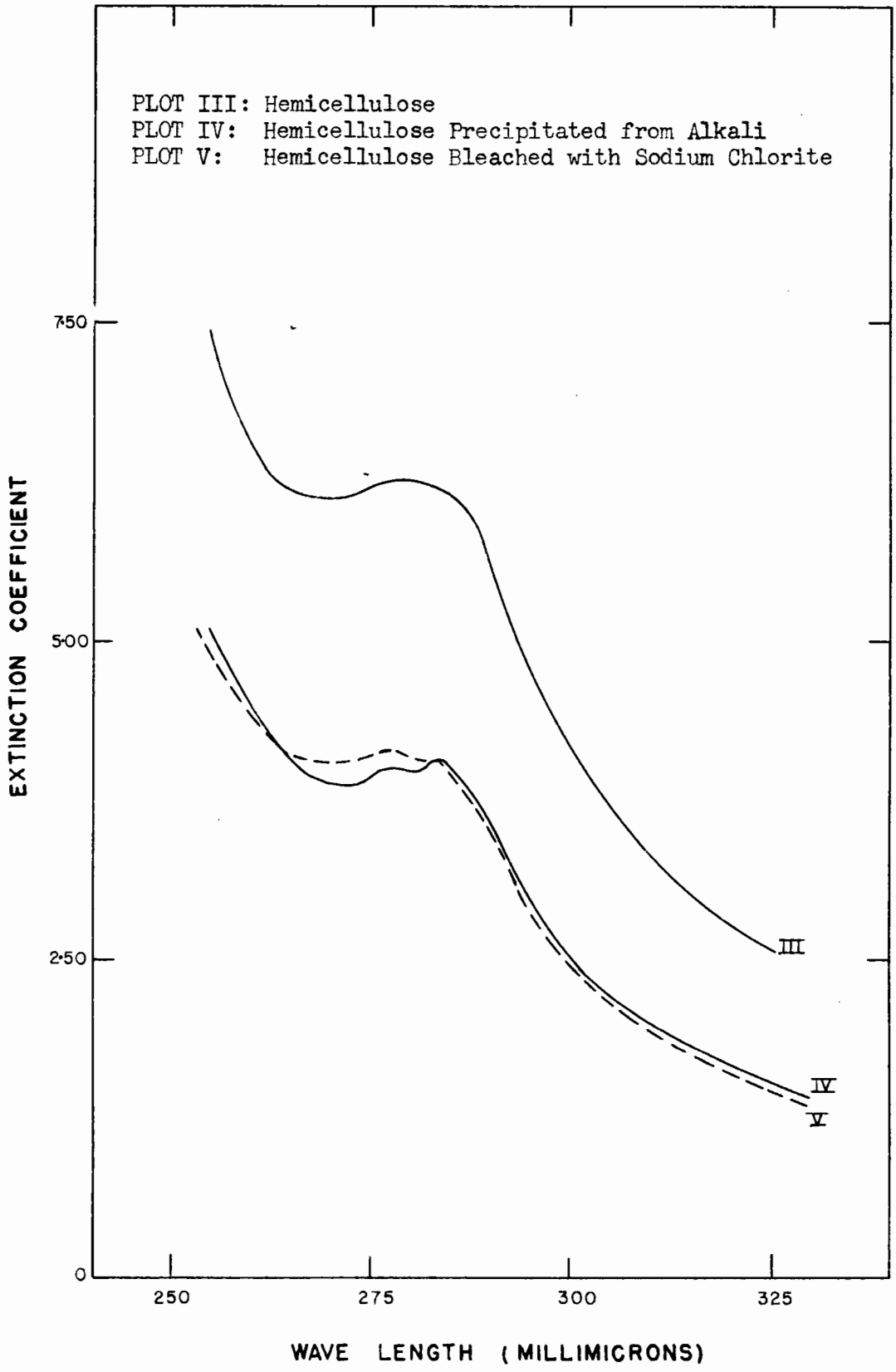


FIG. 6. Ultraviolet Absorption Spectra for Various Hemicelluloses in N Sodium Hydroxide Solution.

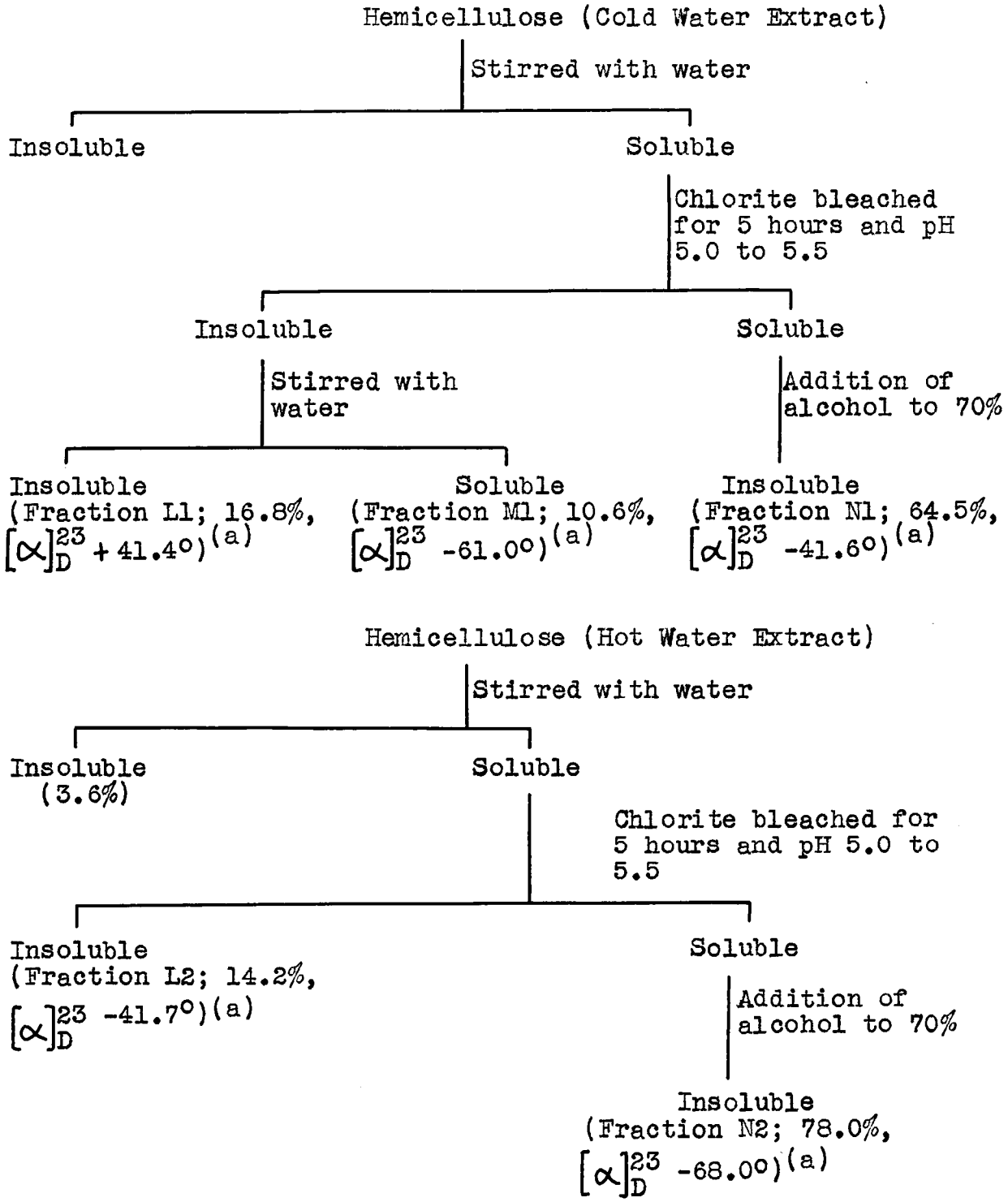
TABLE VI

Comparative Analyses of Bleached Hemicellulose and Hemicellulose Precipitated from Alkaline Solution

	Ash %	Glucose Equivalent %	$[\alpha]_D^{23}$ in Water	$\eta_{sp/c}$ in 10% KOH(a)	Extinction Coefficient at 280 mμ
Hemicellulose	2.5	76.5	-63.0°	0.459	6.3
Alkali-Precipitated Hemicellulose (after dialysis)	3.4	75.3	-74.5°	0.465	4.0
Bleached Hemicellulose	2.9	75.0	-64.8°	0.519	4.1

(a) Material present in 0.5% concentration.

FIG. 7. Effect of Bleaching on Water-Soluble Hemicellulose



(a) Optical rotations were determined on N NaOH solutions.

uble in water) with a specific rotation of $+41.4^{\circ}$ (in N sodium hydroxide) differed from the other fractions by its resistance to acid hydrolysis, a large, insoluble residue remaining after 5 hours. The fact that this resistance and the high positive rotation were probably due to the occurrence of pectin as a contaminant was substantiated by the characterization of pectin through the isolation of 0.49 g. of impure mucic acid from a 1.51 g. sample.

As a second step in the purification of the hemicelluloses, the material was acetylated and the acetates fractionated by fractional precipitation from chloroform solution with petroleum ether. Neubauer (23) found that pectic material contaminating maple hemicelluloses could be effectively removed by acetylation since acetylated pectin was water-soluble while the acetylated hemicellulose was not. In addition, Perlin (86) showed that fractional precipitation of the acetates of crude pentosans from wheat flour removed contaminating galactans and glucans; a similar result was reported by Adams and Castagne (87) for wheat straw hemicelluloses.

Preliminary acetylations of Fraction N1 ($[\alpha]_D^{23}$ -41.6°), according to the method of Carson and Maclay (88), and deacetylation, according to Peterson and others (89), showed that an acetate isolated from the acetylation mixture by precipitating with ice-water yielded a hemicellulose with a higher negative rotation than one derived from an acetate precipitated from cold alcohol. All hemicellulose fractions soluble in water

(Fraction M1, N1 and N2), accordingly, were acetylated to a constant acetyl content, 36%, in 124% yield with recovery of the product after each acetylation by precipitation from ice-water.

Only 57.7% of the hemicellulose acetate, however, was soluble in chloroform. The soluble portion was fractionally precipitated by the addition of light petroleum ether (b.p. 65° to 110°C.) with the results shown in Table VII and Fig. 8. Of the soluble acetates, the specific rotations increased slowly from -74.1° with increasing solubility of the fractions to a maximum of -84.8° when the rotation suddenly decreased to -58.5°C. This last fraction, recovered by evaporating the solution to dryness, presumably contained the more soluble acetates of hexosans. Refractionation of Fractions 2b,3,4, 9 and 10 did not change the physical properties appreciably (Table VIII). The slow downward drift in the intrinsic viscosities of successive fractions suggested that little if any degradation had occurred during the isolation of the hemicelluloses from wood, and indicated a narrow-size distribution for the water-soluble portion of the hemicelluloses.

Since part of the original hemicellulose acetates were insoluble in chloroform, separate deacetylation of the soluble and insoluble fractions was warranted. Of the soluble acetates, Fractions 5,6, 7 and 8 (Table VII) and subfractions IV,V,VI and VII (Table VIII) had specific rotations and intrinsic viscosities which were not too widely distributed, and this group was chosen for further study.

TABLE VII

Fractionation of Hemicellulose Acetate[a]

Fraction	Yield g.	Yield %	$[\alpha]_D^{22}$ in Chloroform	$[\eta]$ in Chloroform	Solution Composition, % Chloroform
1	12.19	26.7	Insoluble	- - -	- - -
2a	7.12	15.6	Insoluble	- - -	- - -
2b	2.31	5.1	-74.1°	0.44	- - -
3	1.46	3.2	-76.2°	0.47	87
4	3.97	8.7	-76.4°	0.44	85
5	2.97	6.5	-79.0°	0.44	83
6	2.69	5.9	-79.8°	0.42	81
7	3.04	6.6	-81.8°	0.40	79
8	1.56	3.3	-82.5°	0.38	77
9	4.04	8.8	-84.8°	0.33	72
10	1.50	3.3	-82.8°	0.24	68
11	1.14	2.5	-58.5°	- - -	- - -
Total	43.99	or 96.6%			

(a) A 45.7 g. sample in 1 litre of chloroform.
Non-solvent: petroleum ether.

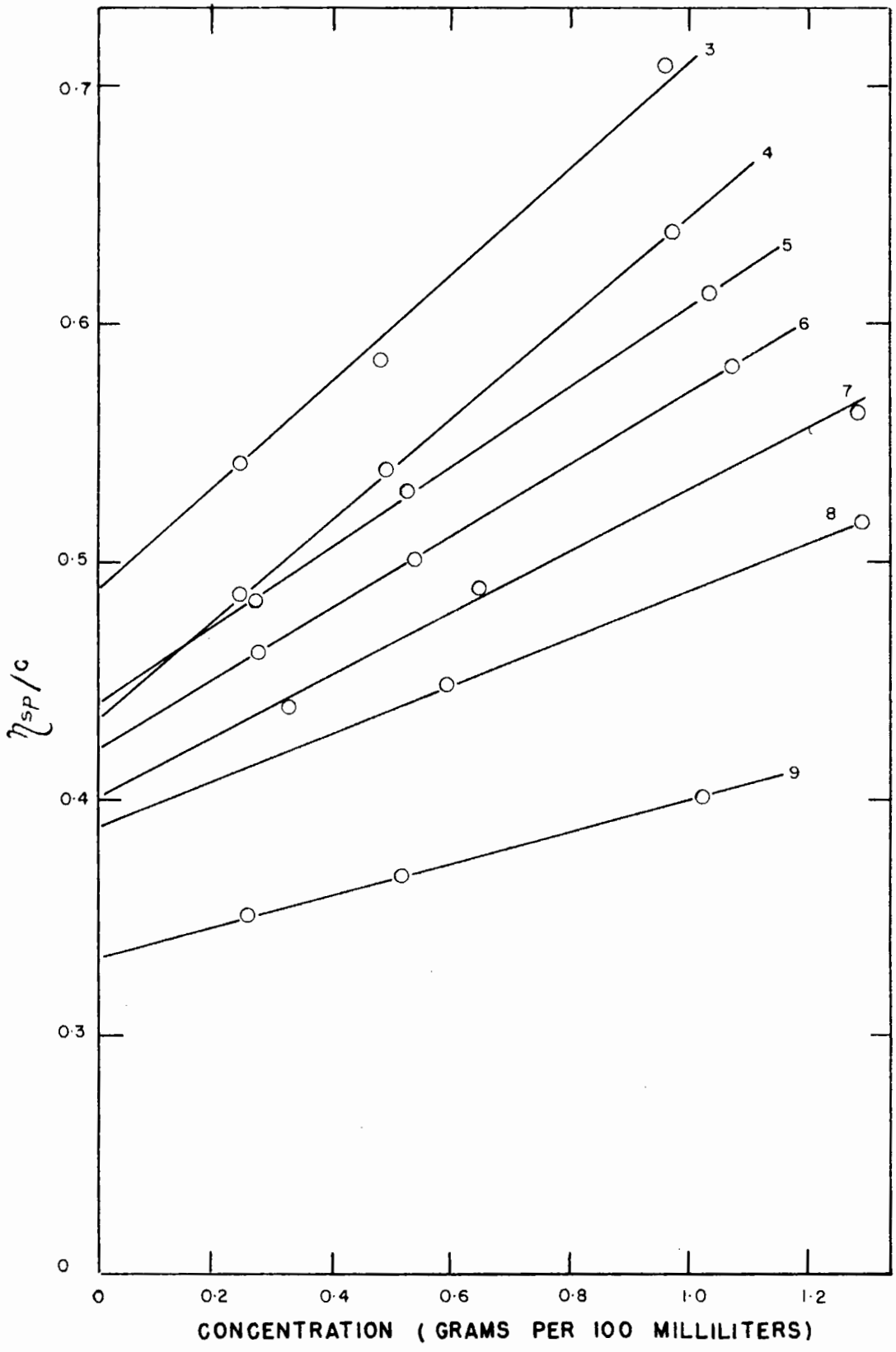


FIG. 8. Plot of Viscosity against Concentration for Hemicellulose Acetate Fractions in Chloroform referred to in Table VII.

TABLE VIII

Refractionation[a] of Hemicellulose Acetate Fractions 2b,3,4,9 and 10

Fraction	Sub-fraction	Yield g.	$[\alpha]_D^{22}$ in Chloroform	$[\eta]$ in Chloroform	Solution Composition, % Chloroform
2b,3,4	I	3.75	Insoluble	- - -	- - -
	II	1.06	-70.2°	- - -	89
	III	0.73	- - -	- - -	87
	IV	1.36	-81.3°	0.42	82
9,10	V	0.80	-83.3°	0.35	79
	VI	1.08	-85.5°	0.31	77
	VII	1.61	-84.0°	0.33	- - -
	VIII	1.62	-81.5°	- - -	- - -

(a) By successive addition of petroleum ether to a chloroform solution.

After deactylation, and fractionation of the products from 2N sodium hydroxide solution by the addition of alcohol, hemicelluloses derived from either the chloroform-insoluble acetates or the chloroform-soluble acetates were not uniform with respect to either specific rotation or intrinsic viscosity, and fractions were obtained which were common to both groups (Table IX and Fig. 9). The degrees of polymerization (D.P.) listed were calculated from the relation $[\eta] = K^1$ (D.P.) using a value of 4.4×10^{-3} for K^1. This value was obtained by Thompson and Wise (56) from a correlation of the observed osmotic pressures and intrinsic viscosities of hemicelluloses from big tooth aspen in 10% potassium hydroxide solution. The analyses of the various hemicellulose fractions, when combined into four main groups according to their physical properties, are shown in Table X.

As a result of the fractionation studies of both the hemicellulose acetates and the hemicelluloses it can be seen that polyuronide hemicelluloses from aspen wood varied in chain length and composition. From a consideration of the specific rotations of the hemicellulose fractions, the absence of any marked change in the rotation of successive fractions of the acetates showed that these fractions must have consisted of approximately the same mixture of acetylated polyuronides. However, as Ritter (90) has pointed out, a long chain hemicellulose containing uronic acid groups may have the same solubility as a short chain material with no acid groups. In accordance with these results, the effect of composition on the

TABLE IX

Fractionation of Deacetylated Hemicellulose Acetates from 2N Sodium Hydroxide with Alcohol

Fraction	Yield %	$[\alpha]_D^{22}$ in 10% KOH	$[\eta]$ in 10% KOH	D.P.	Solubility in Water
	From Acetate Fractions 1 and 2a, insoluble in Chloroform				
H1	5.02	-83.5°	0.44	100	Insoluble
H2	1.32	-81.5°	0.42	96	do
H3	0.63	-79.5°	0.43	98	do
H4	2.02	-71.3°	0.39	89	Soluble
H5	1.07	-71.3°	0.24	55	do
H6	0.35	-71.3°	- - -	- - -	do
	From Acetate Fractions 5,6,7,8 and Sub-fractions IV,V,VI,VII, soluble in Chloroform				
H7	3.54	-77.0°	0.48	111	Soluble
H8	3.02	-63.0°	0.33	75	do
H9	1.40	-59.0°	0.22	50	do
H10	0.88	-50.8°	- - -	- - -	do
H11	0.12	- - -	- - -	- - -	do

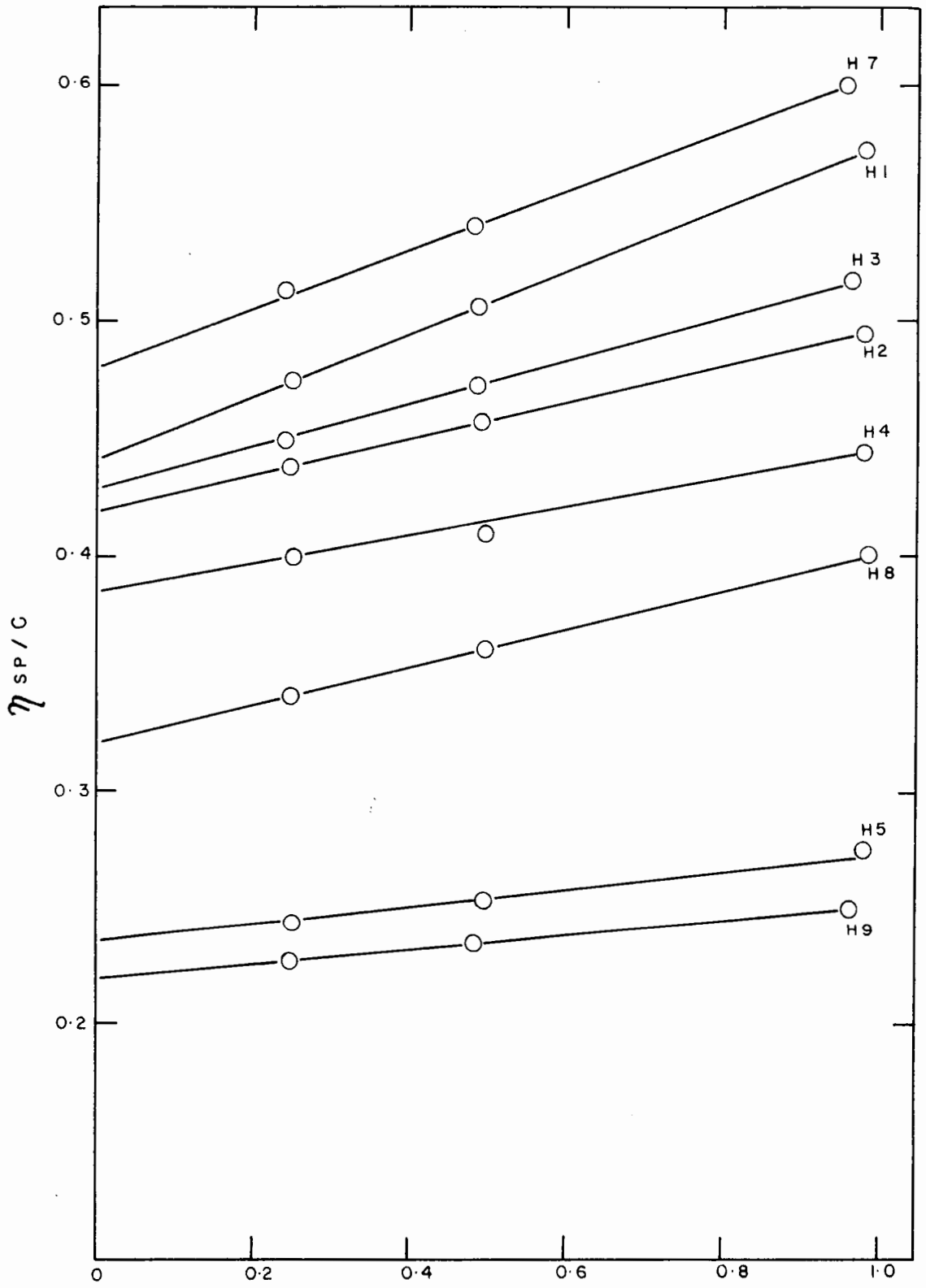


FIG. 9. Plot of Viscosity against Concentration for Hemicellulose Fractions in 10% KOH referred to in Table IX.

TABLE X

Analyses[b] of Hemicellulose Fractions R,X,Y and Z

Fraction[a]	Xylan %	Uronic Acid Anhydride %	Methoxyl %	Ash %	Molar Ratio, methoxyl:uronic anhydride	Molar Ratio, anhydroxylose: methoxyl:uronic anhydride
R	84.7	15.04	1.77	3.5	2:2.94	22:2:3
	87.4	15.24	1.86	3.7		
X	76.8	17.84	2.26	3.9	2:2.88	16:2:3
	76.7	18.48	2.19	3.8		
Y	75.4	24.64	2.74	4.4	2:3.2	13:2:3
	75.3	23.52	2.59	4.2		
Z	68.3	23.44	2.66	3.0	2:3	12:2:3
			2.72	2.7		

(a) Fraction R was formed from H1,H2,H3 and H7; Fraction X from H4,H5 and H6; Fraction Y from H8; Fraction Z from H9.

(b) Analyses were calculated on a moisture and ash-free basis.

solubilities of the polyuronide acetates must have been compensated by the effect of chain length.

The degrees of polymerization (D.P.) of the hemicelluloses fractions ranging from 50 to 110 were of a much lower order of magnitude than the average degree of polymerization reported by Thompson and Wise (56) for the hemicelluloses of big tooth aspen, namely, 150. Wethern (55) found from osmotic pressure measurements that the average D.P. for the hemicelluloses of black spruce was also 150, although fractionation of the butyrate esters from acetone solutions yielded fractions which had degrees of polymerization ranging from 50 to 300. The above hemicelluloses, however, all originated from holocelluloses prepared by the chlorination procedure and could thus be expected to be less degraded than those isolated here from the chlorite holocellulose. Some of the longer chain hemicelluloses might also have escaped extraction and thus remained in the residual holocellulose. Milford (24) estimated that the hemicelluloses, isolated by him from black spruce in the same manner as those studied here, had an average D.P. of 80 to 90.

As mentioned previously, pectic material was found in the aqueous extract of the holocellulose, and as a contaminant of the polyuronide hemicelluloses in the aqueous extract of the ammonia-treated holocellulose. In addition, this material was also found in the aqueous liquors from the precipitations of the hemicellulose acetates, a result which confirmed Neubauer's finding (23) that pectic material could be removed through acet-

ylation. The widespread occurrence of pectin showed that this high molecular weight material was only separated with difficulty, while its presence would have greatly affected the determinations of the intrinsic viscosities of the hemicelluloses. Anderson and Wise (91) also found pectic material in the alcohol-insoluble fraction isolated by Thomas (48) from the wash waters of a holocellulose preparation.

All hemicellulose fractions (R,X,Y and Z) differed only in their ratios of anhydroxylose to uronic acid residues, the latter component being present as two methyluronic acid units to one uronic acid unit. That some of the uronic acid units in the hemicelluloses from aspen did not contain a methyl group was noted previously by Thomas (49) from a study of the composition of the hemicelluloses extracted with alkali from a holocellulose preparation. Sitch (52) also found that methyluronic acids and uronic acids were present as constituents in the hemicelluloses of paper birch, the ratio of the two being 2:1.

Hemicellulose Fraction R was used for a study of the points of linkage of the various constituents, while Fractions X, Y and Z were hydrolyzed with acid for a study of the uronic acid fraction and for establishing the identity of xylose. After hydrolysis of X, Y and Z, the uronic acids were separated from the neutralized hydrolysate by precipitation as the barium salts with alcohol, and then converted to the free acids by percolation in aqueous solution through a column of Amberlite

IR-120. The hydrolysate mother liquor was passed successively through columns of Amberlite IR-120 and Amberlite IR-4B. After concentrating the final eluate to a syrup, crystals were deposited after one week over phosphorus pentoxide in vacuo. These crystals were identified as D-xylose, when recrystallized from glacial acetic acid, by the melting point, 144° to 145°C., and optical rotation, $[\alpha]_D^{23}$ +19.0° (in water). The melting point was not depressed on admixture with an authentic sample of D-xylose.

The free uronic acids, 1.0 g., were chromatographed on large sheets of Whatman No. 1 filter paper with an acid solvent composed of ethyl acetate-acetic acid-formic acid-water (18:3:1:4), according to Jones and Wise (60), and the various uronic acid derivatives extracted from the sheets with water. Of the eight components in the original mixture only two were present in sufficient quantity for further study. Fraction 2, R_f 0.07, giving a red spot on the chromatogram was crystalline and amounted to 0.31 g.; Fraction 5, R_f 0.16, giving an orange spot amounted to 0.53 g. and could not be crystallized. Both fractions were analyzed for molecular weight by oxidation with alkaline hypoiodite, according to Hirst, Hough and Jones (92) and for alkali equivalents; specific rotations were also determined. The results are shown in Table XI.

The analyses of Fraction 2 suggested that this material was an aldotriuronic acid containing one reducing group, one acid group, and one methoxyl group. The analytical data

TABLE XI

Analyses of Uronic Acid Fractions 2 and 5, and D-Glucurone

Material	Methoxyl %	Alkali Equivalent	Determined Molecular Weight		$[\alpha]_D^{22}$ in Water
			Hypoiodite	Methoxyl	
D-Glucurone	- - -	169 173	160 170	- - -	- - -
Fraction 2	5.90 5.96	435 450	476 476	520	+46.9°
Fraction 5	6.65 6.65	204 209	428 434	465	+81.2°
Calculated for:					
Fraction 2					
2 anhydro-D-xylose units + 1 methylhexuronic acid unit					
	6.55	472	472	472	
Fraction 5					
1 anhydro-D-xylose unit + 1 methylhexuronic acid unit + 1 hexuronic acid unit					
	6.00	258	516	516	

obtained were very similar to those valid for a compound consisting of two anhydro-D-xylose units and one methylhexuronic acid unit. On the other hand Fraction 5, while being of approximately the same molecular weight, had one reducing group, one methyl group and two acid groups. A structure involving one anhydro-D-xylose unit, one methylhexuronic acid unit, and one hexuronic acid unit was not inconsistent with this analysis. In accordance with these structures, Fraction 5 with two acid groups had a higher mobility than Fraction 2 which contained only one acid group. Variance of the two fractions was also indicated by the difference in specific rotations, the values for Fractions 2 and 5 being $+46.9^{\circ}$ and $+81.2^{\circ}$, respectively. In order to account for this difference, both glycosidic linkages in Fraction 5 must have been α-, whereas an α- and a β-linkage probably existed in Fraction 2.

In order to establish the nature of the constituents in both fractions, a sample of each was reduced with sodium borohydride according to the method of Wolfrom and Anno (93) and the products, after hydrolysis with 2% sulphuric acid, examined for constituent sugars by paper chromatography using methyl ethyl ketone saturated with water-ethanol (4:1) as solvent. A comparison with 2-, 3-, and 4-methyl-D-glucose which had distinctly different R_f values of 0.34, 0.36, 0.32, respectively, showed that 4-methyl-D-glucose was present as a constituent sugar in both of the reduced aldotriuronic acids. Jones and Wise (61) previously identified 4-methyl-D-glucuronic and

2-α-(4-methyl-D-glucuronosyl)D-xylose in the acid fraction of the hydrolysis products from aspen wood. This finding was substantiated through the identification of 4-methyl-D-glucose in the above reduction products. In the same manner D-xylose was identified but D-glucose was not detected. These results were unexpected since D-glucose should have been formed from Fraction 5, and D-glucurone subjected to the same treatment yielded D-glucose. As will be shown in a later part of the Thesis, a sugar or sugar derivative was present in hemicellulose R with three adjacent hydroxyl groups, the amount of which was equivalent to the content of uronic acid not containing a methyl group. This latter result would exclude the existence of a xyluronic acid residue in Fraction 5. As yet, no explanation has been obtained to account for this anomaly.

Hemicellulose Fraction R was oxidized with 0.1671 M aqueous sodium metaperiodate, and the results were referred to a basic molecular weight of 3,460, calculated from the composition of the material (22 anhydro-D-xylose units:2 methyluronic acid units: 1 uronic acid unit) listed in Table X. Extrapolation of the plots in Fig. 10 to zero time showed that 17 moles of periodate were consumed and 1.1 moles of formic acid produced from the oxidation of 1 base mole of polyuronide.

Sodium metaperiodate will oxidize glycols to dialdehydes or diketones, one mole of periodate being consumed per mole of glycol oxidized. If three adjacent carbon atoms in a chemical compound each carry an hydroxyl group, then two

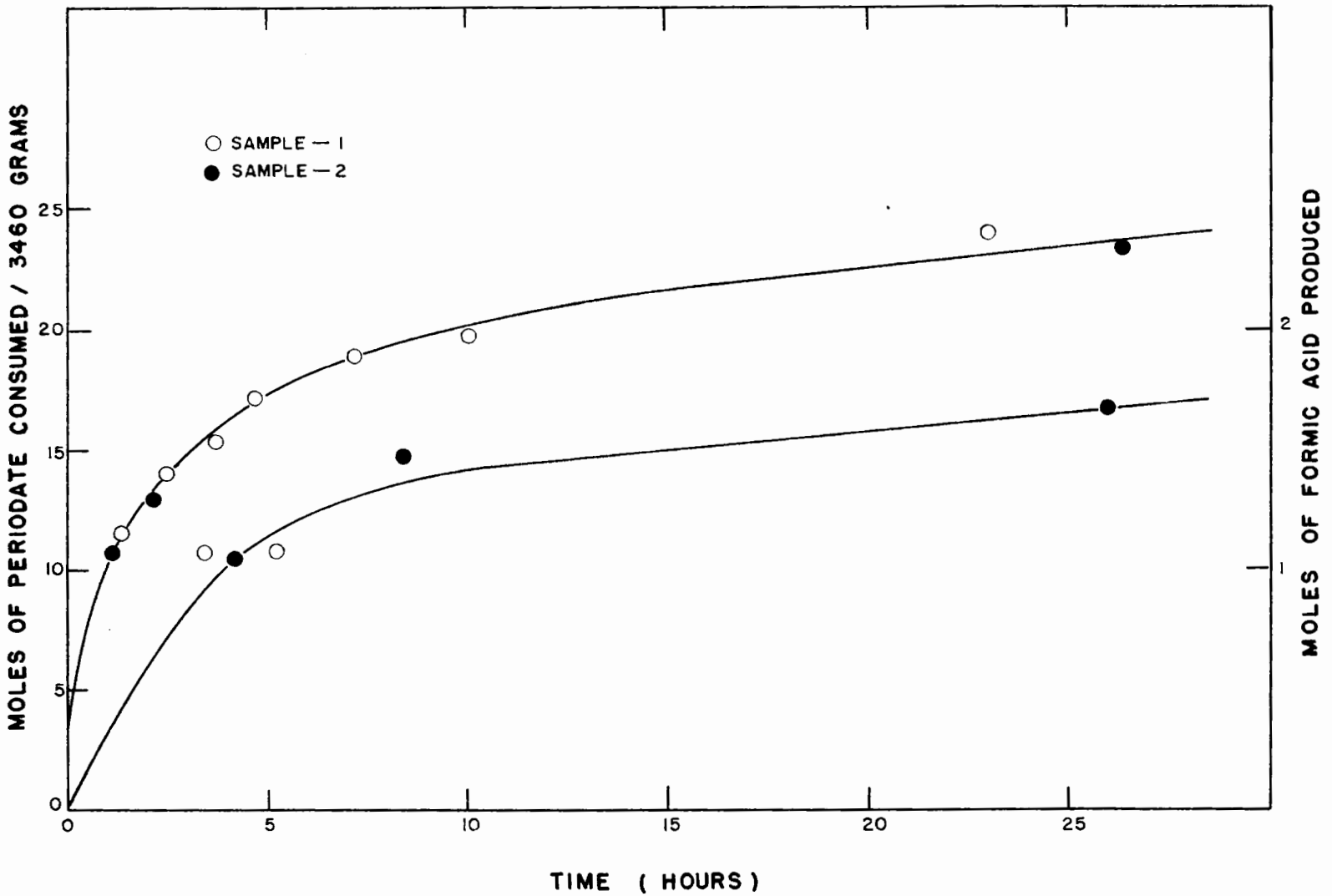


FIG. 10. Oxidation of Fraction R with Sodium Metaperiodate.

sample-1, 0.1404 g.; sample-2, 0.1421 g.

Periodate consumption (upper curve); Formic acid production (lower curve).

moles of periodate are consumed in the oxidation, one mole of formic acid is produced from the central carbon atom, and the two terminal hydroxyl groups are oxidized to carbonyl groups.

The amount of formic acid produced from the oxidation of hemicellulose Fraction R, namely, 1.1 moles, was only slightly in excess of that expected if the non-methylated uronic acid were an hexuronic acid, and consequently the number of xylose end groups appeared to be negligible. However, the total amount of periodate consumed, 17 moles, did not correspond to the amount which would be consumed by a chain of 20 xylose units linked at carbon atoms 1 and 4 with one aldobiuronic acid unit and one aldotriuronic acid unit (containing an hexuronic acid) as branches. This structure should consume 23 moles of periodate and consequently the consumption of periodate indicated that the amount of branching should be greater than that shown by the postulated structure. Although accumulated data strongly suggested the presence of a hexuronic acid, presumably glucuronic acid, evidence for this acid as glucose was not obtained on reduction, as previously explained.

Methylation of hemicellulose Fraction R by the thallous hydroxide, thallous ethylate, methyl iodide route yielded a chloroform soluble portion with a methoxyl content of 34.8%. Two methylations with methyl iodide and silver oxide raised the methoxyl content to 36.3% and an additional methylation with thallous ethylate and methyl iodide brought the content to 36.8%.

Fractionation of the fully-methylated material from chloroform solution by the addition of petroleum ether gave fractions whose analyses are listed in Table XII.

TABLE XII

Fractionation of Methylated Hemicellulose[(a)]

Fraction	Yield g.	Methoxyl %	$[\alpha]_D^{22}$ in Chloroform	$[\eta]$ in Chloroform
1	0.28	37.0, 37.0	-54.2°	0.68
2	0.20	37.0, 37.0	-55.8°	0.67
3	2.43	36.5, 36.6	-56.0°	0.59
4	3.39	36.5, 36.6	-51.4°	0.42
5	1.60	36.8, 36.8	-54.8°	0.30

(a) Sample, 8.5 g. in 200 ml of chloroform.
Non-solvent: petroleum ether.

By extrapolating the specific viscosity-concentration plots in Fig. 11 to zero concentration, intrinsic viscosities of the fractions were obtained. These fractions, differing only in their apparent chain length, were combined for further study.

The polysaccharide was hydrolyzed for 4 hours with 2% methanolic-hydrogen chloride and then for 6 hours after the addition of an equal volume of water. After fractionation of the hydrolysis products on a cellulose column with methyl ethyl ketone saturated with water as solvent four fractions

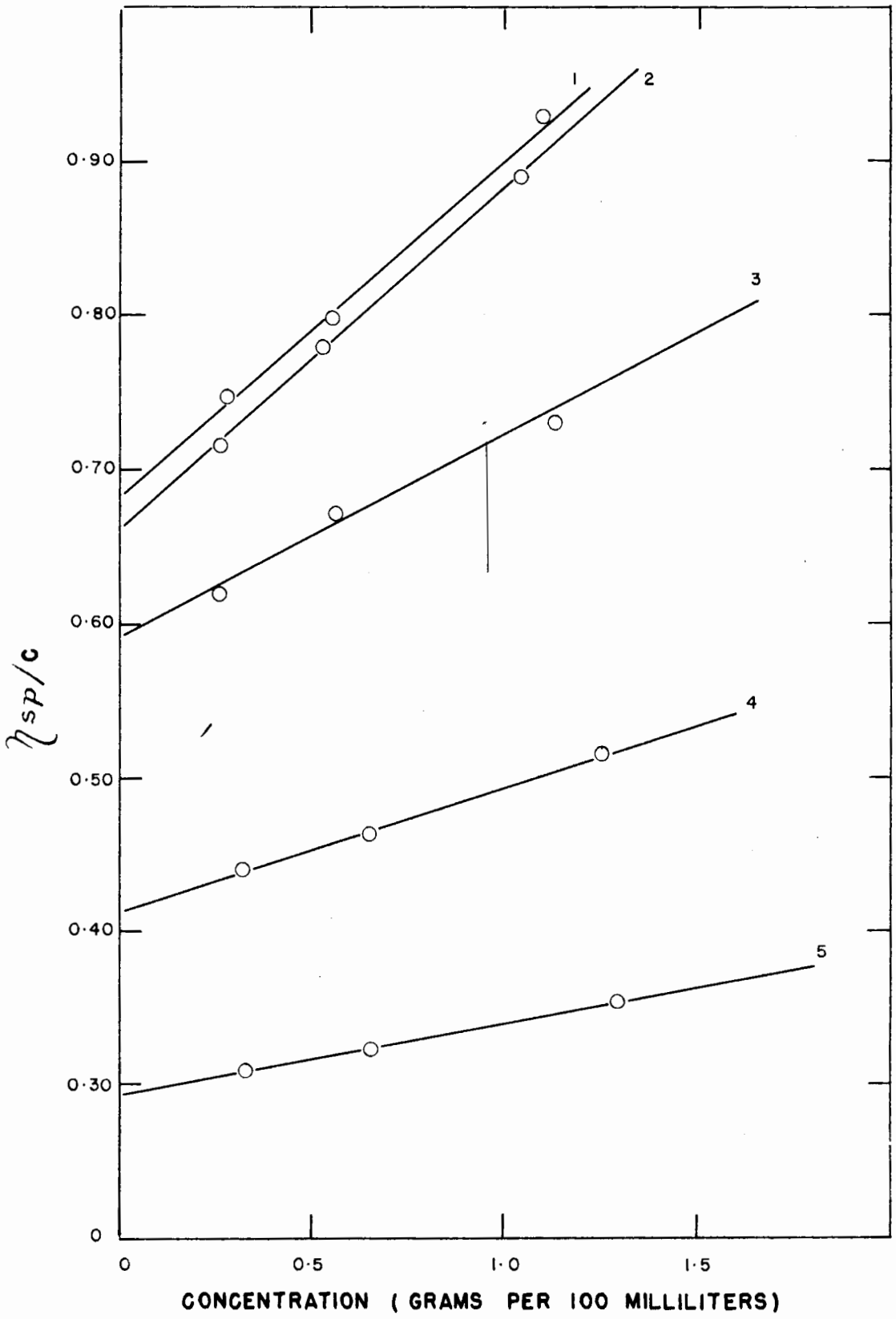


FIG. 11. Plot of Viscosity against Concentration for Fractions of Methylated Hemicellulose R in Chloroform referred to in Table XII.

were obtained, the first two consisting of the larger part of the original mixture. The first fraction did not give a spot on the paper chromatogram while the second one, although giving a spot which corresponded to dimethyl xylose, had a high specific rotation, $[\alpha]_D^{22}$ +75.5° (in water); the third and fourth fractions consisted of monomethyl xylose and methylated uronic acid derivatives, respectively. From a consideration of the preliminary analyses of the first two fractions it was apparent that these materials consisted mainly of methyl glycosides. Accordingly, these fractions were hydrolyzed with 2% hydrochloric acid to constant rotation; the solutions were combined and neutralized, and the components were again fractionated on the cellulose column with methyl ethyl ketone saturated with water.

During both fractionations, however, methylated uronic acid derivatives were found to be present in the dimethyl- and monomethyl xylose fractions, while, in contrast, these acid derivatives moved only slightly on a paper chromatogram when the above solvent was used. No explanation could be presented for this difference in mobility. As a result, it was necessary to remove the uronic acids from the methylated sugar fractions with a mixed resin of Amberlite IR-120 and Amberlite IR-4B, and a study of the methylated uronic acid fraction, accordingly, was not possible.

2,3-Dimethyl-D-xylose, 2.78 g., was identified by its anilide, m.p. 124° to 125°C., its optical rotation, $[\alpha]_D^{22}$ +23.8° (in water), and its methoxyl content, 34.5% (calc'd 34.8%).

This sugar could only arise from xylose units in the original polysaccharide which had linkages at the 1 and 4 or 1 and 5 carbon atoms. Since in all cases D-xylose units in polysaccharides hitherto examined were present in the pyranose form it was doubtful that linkages at the 1 and 5 carbon atoms existed. The high negative rotations of the original polyuronides (Table IX), the acetate derivatives (Table VII), and the methyl derivative of Fraction R (Table XII) suggested that the xylose units were joined by β-linkages predominately. Recently, Jones and Wise (60) isolated two oligosaccharides from aspen wood which were identical to xylobiose and xylotriose, previously isolated from corn cob xylan by Whistler and Tu (65) who showed that the D-xylose units were joined by 1,4-β linkages. This evidence corroborated the present findings.

The monomethyl fraction, 0.20 g., consisted of two sugars, namely, 2-methyl-D-xylose and 3-methyl-D-xylose. The identification of these sugars was established from the following evidence. Two spots identical in position to 2-methyl- and 3-methyl-D-xylose were nearly separated on the paper chromatogram with methyl ethyl ketone saturated with water-ethanol (4:1) after 12.5 hours; the optical rotation, $[\alpha]_D^{22}$ + 23.0° (in water) was between that of 2-methyl-D-xylose with $[\alpha]_D$ + 35.9° (94) and 3-methyl-D-xylose with $[\alpha]_D$ +14.8° (95); after oxidizing with lead tetraacetate in glacial acetic acid, 0.52 molecular equivalents of oxidant were consumed for each mole of monomethyl xylose. Mahoney and Purves (96) found that with a mixture of ethylated glucose derivatives, those derivatives containing an

hydroxyl group on carbon atoms 1 and 2 were oxidized at this glycol within 15 minutes. Since one molecular equivalent of lead tetraacetate would be consumed by one mole of 3-methyl-D-xylose after 15 minutes, approximately half of the mixture was composed of this xylose derivative. However, a small amount of 2,3-dimethyl-D-xylose was also present as shown by a chromatogram and by the methoxyl content of the mixture, 19.9%. Neither this sugar nor 2-methyl-D-xylose would consume a noticeable amount of lead tetraacetate within 15 minutes. The amount of dimethyl xylose, calculated from the methoxyl content of the mixture was approximately 9%, and consequently the percentage of 2-methyl-D-xylose was about 40%.

While insufficient evidence was obtained for the exact structure of the polyuronide hemicellulose, the isolation of 2-methyl- and 3-methyl-D-xylose suggested that points of branching in the molecule occurred at both carbon atoms 2 and 3 of the xylose units joined by 1,4β-glycosidic linkages. Studies on the structure of the unmethylated polysaccharide indicated that for every base molecular weight of the polyuronide, two 4-methyl-D-glucuronic acid residues and one unidentified acid residue were present in the molecule. Some indication of the points of attachment of these acid residues was found previously by Jones and Wise (61) through the isolation of 2-α-(4-methyl-D-glucuronosyl)D-xylose from aspen wood and the occurrence of this material accounted for at least part of the origin of 3-methyl-D-xylose. While uronic acid units may also have been linked to the 3 carbon atom of a xylose unit, it

appeared more likely that at this point of branching another xylose unit was attached from which a xylose chain may have commenced. In addition, it was shown previously that D-xylose, 4-methyl-D-glucuronic acid, and the unidentified acid were linked together as an aldotriuronic acid. Reduction of the methylated aldotriuronic acid fraction should give information concerning the mode of attachment of these units and the precursor of the unidentified acid. Through periodate oxidation it was shown that if only two branches were present in the basic unit, which together contained the acid residues, 23 moles of periodate should be consumed, whereas actually only 17 moles were consumed. These findings, together with the methylation studies, indicated that the polyuronide hemicellulose probably consisted of a highly branched complex structure.

EXPERIMENTAL

Analytical Methods

Moisture

One gram to 2 g. samples were dried in an oven at 105°C. for 16 hours, and the loss in weight noted.

Ash

The residue from the determination of moisture was ignited at 600° to 650°C. in an electric muffle furnace. On a micro scale 0.01 to 0.03 g. was ashed by the procedure of Niederl and Niederl (97).

Klason Lignin

The standard Tappi method (98), which specified a digestion period of 2 hours at 18° to 20°C. with 72% sulphuric acid, was used. Loss of finely-divided lignin during filtration was avoided by using an asbestos mat in the filtering crucible.

Pentosan

Furfural was determined using an apparatus similar to that described by Bray (99). A blank run was made using all of the reagents prepared according to the Tappi method (100), but the amount of bromate-bromide was increased from 20 to 25 ml.

When the pentosan content was calculated without a correction for the presence of uronic acids the following form-

ula was used:

$$\text{Pentosan, \%} = \frac{7.5 \times N \times (v_2 - v_1)}{w}$$

where v_2 = ml. of thiosulphate in the blank determination,

v_1 = ml. of thiosulphate in the test determination,

N = normality of thiosulphate

and w = weight of sample in grams.

The factor 7.5 was the product of $\frac{100 \times 0.048}{0.727 \times 0.88}$, where 0.048 was the weight of furfural in grams corresponding to 1 ml. of N thiosulphate solution, 0.727, the theoretical conversion factor of pentosan to furfural, and 0.88 a factor to compensate for the incomplete conversion of pentosan to furfural.

Most of the results were calculated as xylan, since this material was the principal or only furfural-yielding component present, excluding uronic acid residues. Furfural was calculated by substitution in the expression:

$$\text{Furfural, \%} = \frac{(v_2 - v_1) \times N \times 0.048 \times 100}{w}$$

and in the final calculation of xylan, a correction factor was made for a 21.5% yield of furfural from glucuronic acid (101). The expression for xylan was thus:

$$\text{Xylan, \%} = \frac{F - 0.0215U}{0.73 \times 0.88}$$

where F = % furfural

and U = % uronic acid

Uronic Acid

The method of McCready, Swenson and Maclay was used (81), and the determination was made on 0.08 g. to 0.50 g. of material. The sample was heated with 19% hydrochloric acid at 145°C. for 1.5 hours, a stream of carbon dioxide-free air being drawn through the apparatus as a carrier gas for the carbon dioxide generated. The contents of the absorption tower, containing 0.25N sodium hydroxide, was then drained into the accompanying receiver; carbon dioxide was precipitated as barium carbonate and the mixture was titrated with 0.1N hydrochloric acid. The determination was repeated without a sample and the uronic anhydride carbon dioxide was calculated by substitution in the expression:

$$CO_2, \% = \frac{(v_2 - v_1) \times N \times 0.022 \times 100}{w}$$

where v_2 = ml. of hydrochloric acid used for blank determination,

v_1 = ml. of hydrochloric acid used for test determination,

N = normality of hydrochloric acid

and w = weight of sample in grams.

$$\% \text{ Uronic anhydride } = \% \text{ Uronic anhydride carbon dioxide} \times \frac{4.0}{\text{(mol. wt. 176)}} \quad \text{(mol. wt. 44)}$$

Methoxyl

The method of Vieböck and Schwappach (102) and Vieböck and Brecher (103), with the modification of Peniston and Hibbert (104) was used. The solution in the scrubber was a 1:1 mixture

of 5% aqueous sodium thiosulphate and 5% aqueous cadmium sulphate, as recommended by Friedrich (105).

Nitrogen

Nitrogen was determined on a semi-micro scale by the Kjeldahl method described by Gunning (106) but methyl green was a more suitable indicator for titration of ammonia, as the end point was readily determined by a colour change from green through grey to purple. The nitrogen content was calculated from the formula:

$$N, \% = \frac{(v_2 - v_1) \times N \times 14 \times 100}{w \times 1000}$$

where v_2 = ml. of hydrochloric acid used for test determination,

v_1 = ml. of hydrochloric acid used for blank determination,

N = normality of hydrochloric acid

and w = weight of sample in grams.

Acetyl

The method of Genung and Mallatt (107) as modified to a semi-micro scale by Lemieux (108) was used for the determination of acetyl in wood.

Acetyl in acetylated polysaccharides was determined on 0.01 to 0.02 g. samples by the semi-micro method of Clark (109). The volume of distillate was increased to 100 ml. and no correction factor was applied. A small blank, 0.4 to 0.5 ml. of base, was obtained from cigarette paper, the carrier for the sample.

Copper Reducing Power

The Shaffer and Somogyi copper solution described by Brown and Zerban (110) was standardized against solutions of glucose and xylose of various strengths. From the calibration curves, milligrams of sugar equivalent to millilitres of thiosulphate was determined. Excellent reproducibility was obtained when the alkalinity of the copper reagent was increased by using sodium carbonate in place of sodium bicarbonate as recommended.

The copper solution prepared according to Heidt and co-workers (111) was found to be too unstable, with copper precipitating as a complex after a few days.

Paper Chromatography

The chromatographic apparatus received from the Fisher Scientific Co. was similar to those described previously (112, 113). An aliquot of the solution to be examined, 0.01 ml., containing about 1 mg. of solids, was spotted on a sheet of Whatman No. 1 filter paper, 4½ x 22 inches, on the starting line 3 inches from the top. The chromatogram was hung from the trough and developed with one or other of the following solvents: (A) ethyl acetate-water-pyridine (2:2:1) (114); (B) ethyl acetate-acetic acid-formic acid-water (18:3:1:4) (60); (C) methyl ethyl ketone saturated with water; (D) n-butanol-ethanol-water-ammonia (40:10:49:1) (115); (E) methyl ethyl ketone saturated with water-ethanol (4:1). The paper was removed from the tank, air-dried and sprayed with a solution of

aniline phthalate in water-saturated n-butanol (116). After heating the paper in an oven at 100°C. for 3 to 5 minutes, coloured spots were produced which were tentatively identified by comparison with the spots produced from known sugars simultaneously chromatographed. Solvent E was prepared in this laboratory by Mr. Falconer in order to separate mixtures of methyl glucoses.

Ultraviolet Absorption Spectra

Ultraviolet absorption measurements were carried out by means of a Beckman Quartz Spectrophotometer, using solutions of 0.004% concentration in N sodium hydroxide. The extinction coefficient, $E^{1\%}_{1\ cm}$, was calculated from the expression

$$E^{1\%}_{1\ cm} = \frac{D}{CL}$$

where D = optical density

C = concentration in g./100 ml.

and L = width of the cell (1 cm.)

Preliminary Preparations and Nitrations

A peeled bolt of aspen wood was kindly sent from the Howard Smith Paper Mills by Dr. George Tomlinson II in October 1951. The bolt was approximately four feet long, was free from noticeable imperfections, and had been cut the previous year and allowed to dry in a rack. The age of the log was estimated to be approximately 26 years from the annular rings. In order to prepare the wood for subsequent work, it was chip-

ped into pieces 1 inch square, shredded and ground in a Wiley mill to pass a 40 mesh but not an 80 mesh screen. The wood meal was extracted continuously for 8 hours in a Soxhlet extractor with ethanol-benzene (1:2) and then allowed to air-dry until free from the solvent.

The first sample of holocellulose, holocellulose I, was prepared according to the method of Wise, Murphy and D'Addieco (19). A suspension of 19.2 g. of air-dried wood meal in 640 ml. of distilled water was warmed in a 1-litre Erlenmeyer flask to 75°C. Six grams of sodium chlorite and 2 ml. of glacial acetic acid were added to the suspension with stirring. The flask was loosely stoppered with an inverted 150 ml. Erlenmeyer flask, and the temperature was maintained at approximately 75°C. for 1 hour, with occasional shaking of the mixture. At the end of this time, 2 ml. of glacial acetic acid was added together with 6.0 g. of sodium chlorite. The flask was shaken, and heating was continued for another hour. After another similar treatment, the wood residue was recovered on a filter and washed well with cold water until the effluent was neutral to litmus and free from chlorite ion as shown by its failure to turn acidified potassium iodide-starch solution blue. The water was partially removed with acetone and the nearly white residue was dried at 37°C. under reduced pressure. All operations involving chlorite were performed in a well-ventilated fume hood in order to remove the chlorine dioxide evolved during the delignification.

The second sample of holocellulose, holocellulose II,

was prepared by modifying the method of Ritter and Kurth (14). Air-dried wood meal (10.0 g.), suspended in 100 ml. of ice-water contained in a beaker, was chlorinated for 5 minutes by introducing chlorine gas through a filter stick (20,52). The contents were transferred to a coarse sintered-glass funnel, filtered, and the meal was washed twice with ice-water, and three times with ethanol. After the final ethanol wash, the meal was covered with a 15% solution of technical pyridine in ethanol to remove any remaining acids, and then extracted four times with the hot solvent to remove chlorinated lignin. Although all of the chlorolignin had not been extracted, as was evident from the amber colour of the solvent, the meal was prepared for the next chlorination by washing twice with ethanol and twice with cold water. In all, the meal was chlorinated three times, the last two chlorinations being carried out in a filter funnel surrounded by an ice-water jacket. Extraction of the chlorolignin after each chlorination was according to the manner described.

Since a substantial amount of the chlorolignin still remained in the holocellulose, the concentration of technical pyridine was increased to 30%, and extractions with the hot solvent were continued until the filtrate was light yellow. This extraction required about 6 days and the solvent was changed during the days after every half-hour. Holocellulose prepared in this manner was darker than the original wood meal.

Nitration of Aspen Wood and Holocellulose

One-half gram samples of solvent-extracted wood meal

TABLE XIII

Analytical Data for Samples of Aspen Holocellulose[a]

	Wood	Holocellulose I	Holocellulose II
Moisture, %	4.24,4.31	5.83,6.00	5.40,5.49
Ash, %	0.39,0.40	0.74,0.73	0.50,0.50
Pentosan[b], %	21.2,21.3	23.4,23.8	23.8,23.8
Lignin, %	19.1,19.1	3.73,3.61	7.00,7.13
Nitrogen, %	0.094,0.096	0.038,0.050	0.28,0.26
Yield, %	100	85.21	87.88
Yield (lignin-free), %	80.9	81.5	80.8

(a) analyses were calculated on a moisture and ash-free basis;

(b) pentosan uncorrected for uronic acid.

and of both types of holocellulose were separately nitrated with 30 ml. of a solution consisting of 64% nitric acid, 26% phosphoric acid and 10% phosphorus pentoxide (71), (nitrating acid I), at 6°C. for 24 hours in glass-stoppered bottles. Acid was sucked from the crude cellulose nitrates with caution in order to exclude air, and the remaining acid was removed with large amounts of ice-water, and then with water at room temperature. Nitrolignin was partly removed by shaking the crude nitrates with 60 ml. of methanol (53,54) and finally as insoluble material when the cellulose nitrates were dissolved in acetone. Cellulose nitrate was recovered in a nearly white fibrous state by pouring the acetone solutions into water. However, samples of cellulose nitrate prepared in this manner could not be redissolved completely in acetone, and consequently they were unsuitable for determinations of the degree of polymerization (D.P.).

Nitrations were repeated on samples of the wood and holocellulose, but at room temperature (24°C.) for 5 hours, and the products were recovered by filtration as before. The remaining acid was removed with 100 ml. of 50% ethanol at -17°C. (73,74) and boiled three times for 15 minutes with 100 ml. volumes of 96% ethanol. Treatment with methanol failed to remove any nitrolignin. The products were dried at 50°C. for 3 hours under reduced pressure and then purified by dissolution in acetone and precipitation by the addition of water (54). The results are given in Table XIV.

TABLE XIV

Nitration[a] of Aspen Wood, Holocellulose I, and Holocellulose II

Sample	wt. g.	Yield g.	Yield %	Ppted. from Acetone %	Acetone Insol. %	Nitrogen[b] %
Wood	0.4949	0.4642	94.0	83.8	9.1	13.26 13.24
Holocellulose I	0.5046	0.5473	108.5	87.6	3.9	13.26 13.21
Holocellulose II	0.4842	0.5073	105.0	66.0	24.2	13.38 13.42

(a) Nitration at 24°C. Analyses calculated on a moisture and ash-free basis.

(b) On acetone-soluble portion (column 5).

Nitration of Spruce Wood and Spruce Holocellulose

Samples of spruce wood, spruce chlorite holocellulose, and spruce holocellulose which had been extracted with liquid ammonia and water were nitrated at 6°C. for 24 hours. These samples had been prepared in this laboratory by Dr. Milford. The nitration mixture was prepared according to Brown and Purves (74), (nitrating acid II), and consisted of 22% phosphoric anhydride and 78% nitric acid. No acetone-soluble cellulose nitrate could be obtained from spruce wood when the wood was nitrated at either 6°C. for 24 hours or 6 hours at 26°C. The results from the nitration of the other two samples are given in Table XV. Equivalent data are reproduced for aspen wood nitrated under the same conditions.

Degree of polymerization of Cellulose Nitrates by Viscometry

Acetone solutions of the cellulose nitrates were prepared for viscosity determinations by adding 50 mg. samples (weighed accurately to ± 0.1 mg.) to 50 ml. of acetone contained in 100 ml. volumetric flasks, and shaking the mixture for 24 hours in a mechanical shaker. The solutions were made up to volume and then diluted to prepare concentrations in the range of 0.05, 0.025, 0.0125 and 0.006%. Viscosity measurements were carried out at 25 ± 0.05°C. in a Cannon-Fenske viscometer, No. 50, A.S.T.M.; the time of flow for pure acetone was 105.0 seconds and no kinetic energy correction was made. The flow times were repeated until triplicate times agreed to 0.1 seconds.

TABLE XV

Nitration of Spruce Chlorite Holocellulose

Sample	wt. g.	Yield g.	Yield %	Ppted. from Acetone %	Acetone Insol. %	Nitrogen %
Holocellulose	0.6907	1.0309	149.3	45.5	34.0	13.33 13.42
Holocellulose[a] (extracted)	0.6693	1.1253	168.2	81.7	0	13.43 13.51
Aspen Wood	0.7215	0.7846	108.5	52.0	34.0	13.36 13.31

(a) Holocellulose extracted first with liquid ammonia near 20°C., and then with water.

For calculating the "viscosity average" degrees of polymerization (D.P.) the relationship

$$D.P. = K.[\eta]$$

was used. The intrinsic viscosity, $[\eta]$, was calculated by plotting $\log_{10}\eta_{sp/c}$ of the solutions against the concentration, c, and extrapolating to zero concentration. This extrapolation gave the relationship $\log_{10}[\eta] = \lim_{c\to 0} \log_{10}\eta_{sp/c}$, where $\eta_{sp/c} = \eta - \eta_o/\eta_o c$, and from which $[\eta]$ was obtained. The specific viscosity was η_{sp}, η, the viscosity of the solutions in seconds, η_o, the viscosity of the solvents in seconds, c, the concentration of the solute in grams/ 100 ml., and K, a constant.

A value of 100 for the constant K in the above relationship was used, corresponding to a value of 10.10^{-4} for Km in Staudinger's equation (72), $\eta_{sp/c_{c\to 0}} = Km.Mw$, where Km was a constant, Mw, the molecular weight of the solute, and c, the concentration of the solute expressed as a fraction of the base molar concentration. An example is shown in Table XVI.

TABLE XVI

Viscosity Data for Cellulose Nitrate from Aspen Wood in Acetone

Conc. (g./100 ml.)	0.0510	0.0255	0.0127	0.0064
Flow Time (sec.)[a]	322.2	182.8	138.2	120.4
$\eta_{sp/c}$	40.58	29.08	24.82	23.08
$\log_{10}\eta_{sp/c}$	1.608	1.464	1.395	1.363

(a) Flow time for acetone, 105 sec.

The degree of polymerization (D.P.) and intrinsic viscosity of cellulose nitrates prepared by nitrating the various samples of wood and holocellulose are listed in Table XVII, and the plots from which the intrinsic viscosities were determined are shown in Fig. 2.

TABLE XVII

Degree of Polymerization of Samples of Cellulose Nitrate

Sample	$[\eta]$	D.P.
Nitrating Acid I[(a)]		
1. Aspen Wood	21.1	2,110
2. Holocellulose I	15.3	1,530
3. Holocellulose II	19.0	1,900
Nitrating Acid II[(b)]		
4. Spruce Wood	- - -	- - -
5. Holocellulose	13.3	1,330
6. Holocellulose (extracted)	9.8	980
7. Aspen Wood	18.6	1,860

(a) HNO_3:H_3PO_4: P_2O_5 = 64:26:10. Nitration at 6°C.

(b) HNO_3:P_2O_5 = 78:22. Nitration at 6°C.

Large-Scale Preparation of Holocellulose

In 100 g. batches, 1 kg. of solvent-extracted wood meal was treated with acidulated sodium chlorite solution, as previously described, according to the method of Wise and co-workers (19). Complete analyses of wood and holocellulose are shown in Table I, on a moisture and ash-free basis.

Aqueous Extraction of Holocellulose and Examination of the Extracts

Air-dried holocellulose (740 g., moisture and ash-free) was extracted in 200 g. batches with 5-litre volumes of distilled water at 65° to 75°C. for 3 hours at pH 5 to 6. The residue was recovered on a filter and re-extracted with water. The first and second extracts were separately evaporated to dryness under reduced pressure to yield Fraction A, 17.5 g. (2.4%) and Fraction B, 9.3 g., (1.25%), respectively. Both fractions were brown friable solids, and neither fraction was found to contain free sugars as indicated by paper chromatography, using solvent A (p. 68) as the developer.

Fractionation of Fractions A and B

Fraction A (16.9 g.) was stirred with 100 ml. of water until dissolution had ceased, when an insoluble residue (0.47 g., 3%) was removed by centrifugation. Two volumes of alcohol were slowly added to the clear solution with vigorous stirring, to precipitate Fraction AI (5.20 g., 31%) which, after reprecipitation and solvent-exchange with ether, had $[\alpha]_D^{22}$ +9.7° (c, 0.930 in water). The alcoholic mother liquors were

concentrated to about 50 ml. and then flooded with alcohol. The precipitate, Fraction AII, was reprecipitated, solvent-exchanged and dried *in vacuo* to a light-brown solid. The yield was 4.05 g. (24%) but no measurement of optical rotation was possible because the colour of even a very dilute solution was intense. Evaporation of the alcoholic mother liquors of Fraction AII left Fraction AIII, a light-brown glass which amounted to 5.65 g. (33%).

Fraction B was refractionated into sub-fractions in a similar manner to give: insoluble in water (0.2 g., 2%); Fraction BI (1.8 g., 20%) with $[\alpha]_D^{22} + 35.6^\circ$ (c, 0.925 in water); Fraction BII (4.1 g., 45%); Fraction BIII (3.3 g., 35%).

Sub-fractions of Fractions A and B, since they had similar appearance and physical properties, were combined into three main fractions, Fractions I, II and III. The Molisch test for carbohydrates on Fractions I and II was positive, but negative on III.

Analysis: Fraction I methoxyl 3.60, 3.64%, ash 26.0, 28.0%; Fraction II methoxyl 6.79, 6.92%, ash 6.9, 7.1%; Fraction III methoxyl 12.22, 12.16%, ash 13.0, 12.9%, uronic anhydride 9.68, 12.40%.

Simultaneous Oxidation and Hydrolysis of Fraction I

A sample of Fraction I, 1.75 g., was oxidized by the method of Heidelberger and Goebel (80). The material was heat-

ed under reflux for 24 hours with 50 ml. of N hydrobromic acid containing 0.5 ml. of bromine. The bromine was replaced from time to time as it was removed by vaporization.

The solution was then evaporated under reduced pressure to about 3 ml. and placed in a cold room at 6°C. for 24 hours. Crystals (0.11 g.) were removed by filtration and washed well with water. The melting point of the crystals was 208°C. and was undepressed by an authentic sample of mucic acid.

Fractionation of Fraction II

Fraction II was further fractionated to obtain, if possible, a homogeneous substance which might be a lignin-carbohydrate complex. A solution of Fraction II, 8.126 g. in 20 ml. of water, was vigorously stirred in a 250 ml. centrifuge tube. Alcohol was added dropwise from a burette until a permanent turbidity resulted, when the precipitate was separated in the centrifuge, washed first with an alcohol-water solution of the same concentration as the mother liquor and then with ether. After a final extraction with petroleum ether the sub-fraction was dried *in vacuo*. The alcoholic wash waters were combined with the mother liquor, and fractionation was continued. The last fraction was recovered by evaporating the last aqueous alcoholic mother liquor to dryness under reduced pressure. Table II gives the results.

Examination of the Constituent Sugars in Fractions I, IIa and III

Sub-fractions 3,4,5,6 and 7 from the fractionation were combined and designated as Fraction IIa, and this fraction, like

Fraction III, was insoluble in N sulphuric acid. Approximately 20 mg. each of Fractions I,IIa and III were hydrolyzed with 0.6 ml. of N sulphuric acid at 100°C. for 15 hours in sealed tubes. An insoluble residue which remained in each tube was separated and the hydrolysates were neutralized with barium carbonate. These residues amounted to 4.1%, 6.5% and 32% of the fractions, respectively. The hydrolysates together with reference sugars were chromatographed on sheets of filter paper using solvent A (p. 68) as the developer. Constituent sugars in the fractions are listed in Table XVIII.

Liquid Ammonia Extraction of Water-Extracted Holocellulose

Small-Scale Extraction

Since liquid ammonia at room temperature had a pressure near 150 p.s.i., a stainless steel bomb having a capacity of about 250 ml. was used. The bomb had a thick steel lid with a steel tube and needle valve, and the inside opening of the tube (which did not protrude past the bottom of the lid) was covered with a fine wire gauze. This arrangement permitted a convenient removal of liquid ammonia from the holocellulose when the bomb was inverted and the needle valve opened.

Ten grams of water-extracted holocellulose which had been exhaustively dried in vacuo over phosphorus pentoxide was placed in the bomb and was covered by liquid ammonia, the lid, fitted with a gasket, was quickly replaced and securely fastened by a bolt threaded through a heavy steel yoke which encompassed the whole bomb. The assembly was tested at room temp-

TABLE XVIII

Examination of Hydrolysates from Fractions I, IIa and III

	Reduction as Glucose[a] %	Chromatogram					
		Glucose	Mannose	Galactose	Xylose	Arabinose	Uronic Acid
I	20	-	-	+	+	+	+
IIa	43	+	+	+	+	?	+
III	14	-	-	-	trace	trace	-

(a) By Schaffer-Somogyi method.

erature for leaks and was shaken overnight. The liquid ammonia was removed by filtration and collected in a filter flask protected from moisture by a potassium hydroxide guard tube. Fresh ammonia was then added to the contents of the bomb twice more, after which no more material was removed from the holocellulose.

Large-Scale Extraction

The bomb used consisted of a stainless steel pipe 48" long and 6.5" in diameter, sealed at one end and threaded on the outside of the open end. A heavy top was fitted to the bomb by means of threads on the inside of the top. The top was pierced by 8 bolts which, when screwed down, pressed a thick, Teflon-gasketted lid against the top of the bomb to produce a tight closure. This lid was pierced and fitted with two 1/4" steel tubes which passed through openings in the top, and were connected to steel needle valves serving as inlet and outlet tubes for the liquid ammonia. The ends of these tubes which opened into the bomb were covered with a disk of 60 mesh steel screen, to prevent the loss of holocellulose during the filling and emptying of the bomb.

The holocellulose (668.4 g. moisture and ash-free) to be extracted was dried over phosphorous pentoxide to a moisture content of less than 1%, placed in the bomb and the bomb and its contents were cooled to 6°C. in a cold room. The bomb was then placed on a heavy-capacity platform scale, the inlet tube was connected to a cylinder of ammonia, inverted to deliver

the liquid, and a tube from the outlet was extended into a fume cupboard. After weighing the bomb to the nearest 1/8 kg., both valves were opened and liquid ammonia was run in slowly. The evaporation of the first portion of liquid ammonia soon cooled the bomb and its contents to the point where the rate of addition of liquid ammonia could be increased. Five kg. of ammonia was charged in less than one-half hour, and the cylinder valve, the inlet valve and outlet valve were then closed in that order. The inlet line contained an extra valve, vented in the fume cupboard, to bleed off ammonia from the line before disconnecting it from the bomb.

The bomb was then transferred to a shaking apparatus, designed by the author's colleagues, Dr. Jablonski and Mr. Sanderson, which consisted of a stand on which the bomb was mounted in a horizontal position at the centre of balance fastened at one end through a shaft to an eccentric wheel, driven by a motor, to produce a rocking motion. After the bomb had been rocked overnight at room temperature, it was inverted on the stand and the outlet tube was connected to a length of copper tubing which passed through a rubber stopper into a 6-litre Pyrex flask. This inlet tube extended about 3" further through the stopper than an outlet tube which was vented to the hood by means of rubber tubing.

The outlet valve on the bomb was slowly opened and the liquid ammonia was forced into the flask, which contained 500 ml. of anhydrous methanol, by the pressure of gaseous amm-

onia in the bomb. When the flow of ammonia had ceased, the bomb was disconnected and charged with a further 5 kg. of the liquid and rocked as before. The Pyrex flask was transferred to the fume hood, and after attaching potassium hydroxide guard tubes to the inlet and outlet lines, the ammonia was allowed to evaporate. This process was assisted by circulating cold water around the flask. A total of three extractions of the holocellulose sufficed to remove the substances soluble in liquid ammonia, as indicated by the small-scale extractions, and all extracts were collected in the same flask.

Prior to removing the holocellulose residue from the bomb, most of the free ammonia gas was sucked out by the vacuum of a water-pump. The residue was free from the remaining ammonia after having been kept for 48 hours under vacuum over phosphorus pentoxide, and amounted to 582.5 g. or 87.5% of the holocellulose when corrected for moisture and ash. Continuous extraction for 12 hours with ethanol removed only 1.9 g. from this residue. The analysis of the residue, and the percentage of the wood constituents in the wood and original holocellulose which were retained by the residue are given in Table XIX.

Examination of the Liquid Ammonia Extract

Methanol-Soluble Fraction

The presence of methanol in the round-bottom flask, into which the liquid ammonia extract was discharged, provided a preliminary fractionation of the extracted material, and prevented the deposition of the residue as a physically unmanage-

TABLE XIX

Analysis and Recovery of Wood Constituents in Liquid Ammonia-Extracted Holocellulose (a)

	Holocellulose Residue after Liquid Ammonia Extractions %	Recovery of Wood Constituents	
		On Basis of Content in Holocellulose %	On Basis of Content in Wood %
Moisture	10.4,10.3	- - -	- - -
Ash	0.28,0.31	- - -	- - -
Lignin	0.77,0.80	23	2.9
Pentosan	26.5,26.7	96.5	90.5
Xylan (b)	24.9	100	91.6
Uronic Anhydride	5.12,5.28	71.3	78.7
Acetyl	0.50,0.51	8.5	8.4

(a) Based on moisture and ash-free material

(b) Corrected for uronic anhydride.

able lacquer, according to the experience of Dr. Jablonski and Mr. Sanderson.

After evaporation of the ammonia, that part of the extract which remained undissolved in the methanol was washed twice with 100 ml. volumes of methanol. Evaporation of the combined methanol solutions to dryness left a hard brown residue, designated as Fraction C, which amounted to 68.5 g. or 9.8% of the holocellulose.

Fraction C was extracted three times with 200 ml. volumes of hot acetone to dissolve acetamide. The insoluble residue, Fraction C1, was then vigorously stirred with three 100 ml. volumes of water, and insoluble material, Fraction C2, was removed by centrifugation. After evaporation of the combined aqueous solutions nearly to dryness, the residual syrup was washed with ethanol and an excess of ether was added to the ethanol solution. These operations provided a rough fractionation, and yielded Fraction C3, which did not dissolve in the ethanol wash, and Fraction C4, which was precipitated by ether. Unfortunately, the ether-alcohol solution was discarded before it was realized that 5.71 g. of Fraction C remained unrecovered.

The acetone solution, containing dissolved acetamide, was treated with ether until precipitation was complete. The mother liquors were decanted, and the syrupy precipitate was exhaustively extracted with fresh acetone. Insoluble granular material constituted Fraction C5. The acetone solution was

again treated with ether until precipitation was complete, and the precipitate was again extracted with acetone. These operations were repeated until an acetone solution had been prepared from which nothing further was precipitated by the addition of ether, with a residue, Fraction C6, which was extractive-free.

The acetone mother liquors of Fraction C6 were concentrated under reduced pressure at 40° to 45°C. until crystallization had commenced. The crystals were collected on a filter, triturated with ether, and dried; yield, 15.44 g. The filtrate and ether wash were then concentrated to a syrup, ether was added, and a second crop of crystals was obtained which amounted to 17.83 g. After recrystallization from ether the crystals melted at 80° to 81°C., and this melting point was undepressed by admixture with an authentic sample of acetamide.

Analysis: Calculated for acetamide,
C_2H_5ON; N, 23.7%
Found: N, 24.4%.

A partly crystalline residue amounting to 5.30 g. was recovered from the mother liquors of the second concentrate and this weight of material was used in calculating the yield of acetamide.

The flowsheet for this fractionation is shown in Fig. 4, the yields and methoxyl content of the fractions are given in Table XX, and an ultraviolet absorption plot for Fraction C5 is shown in Fig. 3.

TABLE XX

Yield and Methoxyl Content of Fractions from the Methanol-Soluble Liquid Ammonia Extract

Fraction	Yield g.	Isolated from Holocellulose %	Methoxyl %
C2	6.50	0.93	17.25,16.90
C3	6.71	0.96	15.10,14.90
C4	0.58	0.1	- - -
C5	9.13	1.48	15.25,15.55
C6	1.14		
Acetamide	38.57	5.60	- - -

Fractions C2, C3, C5 and C6 did not give a positive Molisch test for carbohydrates.

Methanol-Insoluble Fraction

The residue which remained after removing the methanol solubles from the liquid ammonia extract was dissolved in 290 ml. of water, the pH was adjusted to 6.8 with acetic acid, and 680 ml. of ethanol was added. After standing overnight at 6°C., a precipitate was removed in the centrifuge, stirred with 100 ml. of water, and collected again in the centrifuge as Fraction D1. Fraction D2 precipitated from the mother liquors when alcohol was added to a concentration of

70%, but when the alcoholic solution was concentrated to about 40 ml., an additional amount of material was rendered insoluble. This material was removed in the centrifuge and the aqueous solution was diluted with 5 volumes of alcohol to precipitate Fraction D3. Trituration of the aforementioned insoluble material with water left a solid which was combined with Fraction D1, and Fraction D4 was precipitated from the wash waters with 5 volumes of alcohol. When the mother liquors of Fraction D4 were again concentrated, a small fraction, Fraction D5, was removed by flooding with alcohol. For convenience, Fractions D4 and D5 were combined and designated Fraction D6.

In order to recover the original methanol-insoluble material completely, the mother liquors from Fraction D3 were concentrated to a thick syrup and were stored over phosphorus pentoxide. After an extended period of time, large transparent hygroscopic crystals formed which were not investigated but which were assumed to be ammonium acetate, originating from the neutralization of the ammonia extract. The flowsheet for this fractionation is given in Fig. 5.

Table XXI shows that the methoxyl contents of Fractions D3 and D6, while similar, were quite different to that of D2. In consequence, D3 and D6 were combined for further analysis.

A semi-quantitative determination of the carbohydrate portion of the fractions and tentative identification of the constituent sugars was made in the following manner. Accurately weighed samples (0.1 g.) were heated in sealed tubes at 100°C.

TABLE XXI

Yield and Analyses of Fractions from the Methanol-Insoluble Liquid Ammonia Extract

Fraction	Yield %	Methoxyl %	Ash %	Glucose Equivalent	Carbohydrate Constituents
D1	1.76 + 1.46	- - -	- - -	- - -	- - -
D2	2.34	5.64,5.64	2.0,1.7	42.5	glucose, galactose, mannose, arabinose (?), xylose, uronic acid derivatives
D3	8.90	12.30,12.50	2.9,2.4	6.75	glucose, galactose, mannose (?), arabinose, xylose, uronic acid derivatives
D6 (D4+D5)	3.58	14.05,14.09	2.7,3.4	- - -	- - -

with 2 ml. of 2½% sulphuric acid for 5 hours; the tubes were opened cautiously, and the contents were quantitatively transferred to 100 ml. volumetric flasks and diluted to the mark with water. Two 1 ml. aliquots were removed and glucose equivalents were determined by the Schaffer-Somogyi copper reducing power; 10 ml. aliquots were neutralized with barium carbonate, filtered, and the filtrate evaporated to a thin syrup and examined by paper chromatography, using solvent A (p. 68). No free sugars were detected before hydrolysis when the fractions were examined in a similar manner.

Fractions D3 and D6 together (11.92 g.) were dissolved in 150 ml. of water and were subjected to further fractionation. The clear solution was first dialyzed against running tap water for 24 hours to remove any accompanying salts, as for example ammonium acetate, and as a result material which had been apparently stabilized in the colloidal state by the presence of the salts became insoluble in water. The resulting fine suspension was separated in the centrifuge only after coagulation through shaking with a few drops of n-butanol. The mother liquor was then evaporated to dryness, the residue was dissolved in 40 ml. of water and sub-fractions were isolated by the addition of ethanol in the manner previously described. The results are given in Table III.

Samples of sub-fractions 6 to 10, when combined, hydrolyzed and chromatographed, were shown to contain the same mixture of sugars found in Fraction D3 (Table XXI).

Aqueous Extraction of Residual Holocellulose

The residual 615.7 g. from successive extractions of the holocellulose with water, liquid ammonia, and alcohol was stirred with 5 l. of water at room temperature and pH 5 to 6 for 5 hours. The aqueous extract was filtered, and the wet residue was again extracted under the same conditions. After the second extraction, since marked swelling of the wood fibres was apparent, a sample of the swollen material, 66.4 g. when dried at 100°C., was set aside for examination, with the results shown in Table V.

The removal of soluble hemicellulose was completed by two hot water extractions using 5 l. volumes of water at 70°C.

Dissolved hemicelluloses were then recovered separately from the cold and hot water extracts by concentrating the solutions to about 1 litre under reduced pressure and adding alcohol to a concentration of 70%.

The products, collected in the centrifuge, were solvent-exchanged with alcohol and ether. Non-precipitated material was recovered by evaporating the mother liquors to dryness.

Based on the original holocellulose, crude hemicelluloses precipitated from the cold and hot water extracts amounted to 5.8% (39.0 g.), and 3.3% (17.0 g.), respectively; the yields of non-precipitated material were 1.9% (13.0 g.) and 0.35% (2.0 g.) (Table IV). Appropriate corrections were made

to account for the sample removed when the percentage yields were calculated.

Attempted Purification of Hemicelluloses

A sample of the hemicellulose extracted with hot water, 1.00 g., was stirred in water until dissolution was almost complete, the insoluble material (1%) was removed on the centrifuge, and 2.5 volumes of alcohol were added to the solution to precipitate 95% of the sample which was recovered in the usual manner.

Analysis: ash 2.45, 2.48%; glucose equivalent 76.5%; $[\alpha]_D^{23}$ -63.0° (c, 0.483 in water); $\eta_{sp/c}$ 0.459 (c, 0.489 in 10% potassium hydroxide solution); extinction coefficient, 6.3 (at 280 mμ) (Fig. 6).

An alkaline solution of the crude hemicellulose was prepared by dissolving 1.20 g. in 8 ml. of N sodium hydroxide; 16 ml. of alcohol was added with vigorous stirring, and the resulting precipitate was recovered in the centrifuge. The product was reprecipitated twice by diluting an aqueous solution with an equal volume of 2N sodium hydroxide, followed by the addition of alcohol to a concentration of 70%. Air was excluded during these operations by bubbling nitrogen through the mixture. The final alcoholic mother liquor was colourless, and the yield was 1.34 g. or 111%. After dialyzing an aqueous solution containing 1.30 g. against running tap water for 43 hours, 4% of the product was lost to the dialysate, 13% was insoluble

in water, and 1.08 g. (83%) was precipitated by 2.5 volumes of alcohol. This fraction was analyzed.

Analysis: ash 3.32, 3.60%; glucose equivalent 75.3%; $[\alpha]_D^{23}$ -74.5° (c, 0.483 in water); $\eta_{sp/c}$ 0.465 (c, 0.483 in 10% potassium hydroxide solution); extinction coefficient 4.0 (at 280 mμ) (Fig. 6).

One gram of hemicellulose in 30 ml. of water was bleached for 5.5 hours with 1.0 g. of sodium chlorite, glacial acetic acid being added at various intervals to maintain the pH at 5.0 to 5.5. Insoluble material (15%) which resulted from the treatment was then removed in the centrifuge, and the clear mother liquor was diluted with 2.5 volumes of alcohol to precipitate 0.82 g. (82%) of hemicellulose.

Analysis: ash 2.68, 3.08%; glucose equivalent 75.0%; $[\alpha]_D^{23}$ -64.8°(c, 0.973 in water); $\eta_{sp/c}$ 0.519 (c, 0.486 in 10% potassium hydroxide solution); extinction coefficient 4.1 (at 280 mμ) (Fig. 6).

Initial Purification of Hemicellulose Fractions

The crude hemicellulose fraction (38.50 g.) from the cold water extract was dissolved in water and, after centrifuging the solution to remove insoluble material, was bleached with sodium chlorite in the manner described above. As before, some of the material separated from solution during the bleach,

and this portion was removed by centrifuging, mixed with water, and the viscous mixture was dialyzed against running tap water for 5 days. Since a positive test for chlorite ion was still obtained by the acid-potassium iodide-starch method, after this time the mixture was concentrated at room temperature under reduced pressure to a thick paste. Two volumes of alcohol were added, and the resulting precipitate was washed with 70% alcohol until the test for chlorite ion was negative. The alcohol-swollen sample was then extracted twice with 100 ml. volumes of water, and after concentrating the extract to about 50 ml., dissolved material was precipitated by adding alcohol to a concentration of 70%. The water-insoluble portion was designated as Fraction L1, and the precipitate as M1.

The bulk of the hemicelluloses remaining in the chlorite liquor were recovered by precipitation from 70% alcohol. This precipitate, Fraction N1 was freed of chlorite ions in the manner previously mentioned.

Hemicellulose from the hot water extract, 13.78 g., was bleached and worked up in the same manner, with the exception that material which precipitated during the bleach was easily washed with water and the wash waters were combined with the chlorite mother liquors. The corresponding fractions consequently were Fractions L2 and N2. The various operations for these fractionations are represented by a flowsheet in Fig. 7, and the analyses of the fractions are listed in Table XXII.

For the analysis of the constituent sugars, samples

TABLE XXII

Analyses of Hemicellulose Fractions after Bleaching with Buffered Aqueous Sodium Chlorite

Fraction	Yield g.	Yield %	$[\alpha]_D^{23}$ in N NaOH	Constituent Sugars
Cold Water Extract				
Initially water insoluble	1.48	3.84	- - -	- - -
L1	6.47	16.8	+41.4°	xylose≫ arabinose, glucose, uronic acid derivatives,> rhamnose
M1	4.09	10.6	-61.0°	do
N1	24.84	64.5	-41.6°	do
Hot Water Extract				
Initially water insoluble	0.05	3.6	- - -	- - -
L2(a)	1.96	14.2	-41.7°	do
N2	10.72	78.0	-68.0°	do

(a) Only partly dissolved after hydrolyzing for 5 hours.

of the fractions were hydrolyzed with 2½% sulphuric acid for 5 hours at 100°C., the hydrolysates were neutralized with barium carbonate, and the intensity of the spots produced by aniline phthalate, after chromatographing with solvent A, (p. 68), were compared.

Acetylation of Various Hemicellulose Fractions

(a) One gram of hemicellulose Fraction N1 was dispersed in 20 ml. of formamide by stirring at 65°C. for 1 hour. Twenty ml. of freshly-distilled pyridine was added and the dispersion cooled to room temperature. Acetic anhydride (16.5 ml.) was added dropwise over a period of 3 hours and the solution was allowed to stand overnight. This method was similar to the procedure of Carson and Maclay (88).

From this acetylation mixture equal volumes (20 ml.) were removed and added separately to 160 ml. of cold ethanol (6°C.), from which acetate A precipitated, and to 120 ml. of water containing 40 g. of ice, from which acetate B precipitated. After collecting the precipitated materials in the centrifuge, the products were solvent-exchanged into alcohol and ether and were dried *in vacuo*. The yields of acetate A and B were 0.58 g. and 0.57 g., respectively.

Partial dissolution of the acetates in 18 ml. of dioxane separated the materials into soluble and insoluble fractions. Both fractions were deacetylated with 0.4N potassium hydroxide in alcohol by the method of Peterson and others (89),

and the products were washed successively with 1% acetic acid in alcohol, with alcohol, and finally with ether. From acetate A, the yield of deacetylated material insoluble in dioxane was 0.03 g. with $[\alpha]_D^{22}$ -39.4° (c, 0.813) and the soluble fraction was 0.20 g., with $[\alpha]_D^{22}$ -51.5° (c, 0.718). Similarly, from acetate B, the fractions were 0.10 g. with $[\alpha]_D^{22}$ -52.0° (c, 0.712) and 0.12 g. with $[\alpha]_D^{22}$ -64.8° (c, 0.710), respectively. All optical rotations were measured in N sodium hydroxide solution.

(b) Large-scale acetylations were carried out on 23.06 g. of Fraction N1 and 14.48 g. of Fractions M1 and N2 in the manner just described. The acetates were isolated by pouring the solutions into 3-litre volumes of water containing 500 g. of ice, the products were removed in the centrifuge and solvent-exchanged with alcohol, and benzene. To make sure that acetylation was complete, the product from Fraction N1 (20.44 g.), for example, was dissolved in 250 ml. of pyridine at 55°C., 70 ml. of acetic anhydride was added and the solution was allowed to stand for 19 hours at room temperature. The product was recovered as before. Three acetylations sufficed to give constant acetyl values, the values for the acetates from Fraction N1 and from M1 and N2, being 35.8 and 36.1%, respectively, with corresponding yields of 26.90 (117%) and 19.17 g. (132%).

The aqueous mother liquors obtained during the isolation of the hemicellulose acetates were combined and evaporated to dryness. The residue amounted to 4.04 g. or 11% of Fractions N1, M1 and N2. This residue was subjected to oxidative

hydrolysis with bromine and hydrobromic acid, as previously described, with a subsequent recovery of 0.10 g. of mucic acid, m.p. 118°C., from a 1.40 g. sample. The melting point was not depressed by an authentic sample of mucic acid.

Fractionation of Hemicellulose Acetate

Before being fractionally precipitated, the above hemicellulose acetates were combined (45.70 g.) and were stirred with 1000 ml. of chloroform. Insoluble material, Fraction 1, was separated from the chloroform solution in the centrifuge, and the turbid, more dense solution was removed by siphoning. Fraction 1 was then stirred with acetone, the resulting solution was concentrated to 50 ml., and the material precipitated with petroleum ether from this extract was redissolved in the chloroform solution. The addition of 150 ml. of petroleum ether reduced the density of the latter solution sufficiently to permit the recovery of more suspended material (Fraction 2) by centrifuging. This fraction was washed with chloroform: acetone (50:50 v/v) to separate insoluble material (Fraction 2a) and soluble material (Fraction 2b), which was recovered by precipitation. Soluble fractions were then precipitated from the clear chloroform solution by the addition of varying increments of petroleum ether. Each fraction was washed with a solution of the same composition as its mother liquor, solvent-exchanged with petroleum ether, and dried *in vacuo*. The last fraction was recovered by removal of the solvent. The results of the fractionation are shown in Table VII and Fig. 8.

For further fractionation, Fractions 2b, 3, and 4, with almost identical specific rotations and intrinsic viscosities, and Fractions 9, and 10, also similar, were combined into two groups each of which was redissolved in chloroform and sub-fractions precipitated with petroleum ether. In the first group some material insoluble in chloroform which was not removed in the previous fractionation was recovered as sub-fraction I. Like Fractions 1 and 2a of the previous fractionation, the optical rotation and intrinsic viscosity of this sub-fraction could not be determined. The results of the fractionation are shown in Table VIII.

Deacetylation of Hemicellulose Acetate and Fractionation of the Product

Combined hemicellulose acetate Fractions 1 and 2a, 19.0 g. (Table VII) were immersed overnight in 450 ml. of peroxide-free dioxane at room temperature, and the mixture was then warmed at 45°C. for 3 hours to complete swelling. The procedure for deacetylation with 0.4N potassium hydroxide in alcohol was previously described, but in this case precaution was taken to exclude air by continuously bubbling nitrogen through the mixture. In order to ensure complete removal of acetate groups, the product was dissolved in 125 ml. of 3N caustic soda under nitrogen and then precipitated by the addition of excess alcohol. Acetic acid (50% v/v) was mixed with the precipitate to neutralize the base and salts were removed by washing with 70% alcohol.

At this stage, the alcohol-wet material was dissolved in 320 ml. of 2N caustic soda under nitrogen and fractionated by the addition of alcohol in the usual manner, with the results shown in Table IX. Although some of the precipitates were insoluble in water, each fraction in aqueous suspension or solution was treated with 50% acetic acid to neutralize the base and was then solvent-exchanged with alcohol and benzene and dried in vacuo. In a similar manner, material consisting of Fractions 5,6,7 and 8 (Table VII) and sub-fractions IV,V,VI and VII (Table VIII) was deacetylated and fractionated as one unit (Table IX), since their specific rotations and intrinsic viscosities were rather similar.

Hemicellulose fractions of similar physical properties were combined into four main groups, namely, Fraction R from H1,H2,H3 and H7; Fraction X from H4,H5 and H6; Fraction Y from H8, and Fraction Z from H9. After hydrolysis of 10 mg. of each fraction with 0.5 ml. of 2.5% sulphuric acid for 5 hours, and examination of the neutral hydrolysates on the chromatogram with solvent A (p. 68), each fraction was found to give a predominant spot which was identified as xylose by comparison with an authentic sample. Spots corresponding to uronic acid derivatives were present in all cases, but in Fractions Y and Z traces of glucose were also present. The analyses of the fractions are in Table X .

To determine quantitatively the amount of glucose in the fractions, samples were hydrolyzed and the hydrolysates

were prepared, according to Purves and Hudson (117), for fermentation with yeast.

Accurately weighed samples (40 mg.) were hydrolyzed with 3 ml. of 2.5% sulphuric acid for 4 hours, and after the addition of a crystal of ammonium acetate and sodium dihydrogen phosphate, the pH was adjusted to 4.5 with 2N sodium hydroxide and the volume of the solution was brought to 10 ml. A 2 ml. sample was removed and diluted to 25 ml., and from this solution two 5 ml. volumes were taken to determine the glucose equivalent by copper reduction. The remaining 8 ml. of the hydrolysate was treated with 3 mg. of yeast and the mixture was held at 32°C. for 2 days. After this time a 2 ml. sample was removed and the copper reducing power was determined as before. Glucose (a few mg.) was then added to the fermented hydrolysate which was analyzed again after 2 days at 32°C. The results of the fermentation are shown in Table XXIII. Although fermentation of sugars other than xylose was obvious, no additional attempts were made to determine quantitatively the traces of glucose originating from Fractions Y and Z.

Acid Hydrolysis of Fractions X, Y and Z

(a) A sample of the combined fractions (0.2215 g., ash-free) was suspended in 25 ml. of 1% sulphuric acid and heated in a boiling water bath. At intervals 0.20 ml. aliquots were withdrawn and the copper reducing power, calculated as xylose, was determined. The reducing power reached a maximum after 235 minutes' hydrolysis (Fig. 12), the maximum value being equival-

TABLE XXIII

Fermentation of Hydrolyzed Hemicellulose Fractions R,X,Y and Z

Fraction	Milligrams of Sugar Present as Glucose[a]			Percent of Original Sugar Fermented	
	Initially	After First Fermentation	After Second Fermentation	Before Addition of Glucose	After Addition of Glucose
R	35.75	30.25	17.00	15	52
X	36.50	32.50	23.25	11	36
Y	31.50	22.25	13.75	29	56
Z	27.25	23.50	14.75	14	46

(a) By the Shaffer-Somogyi copper reduction method.

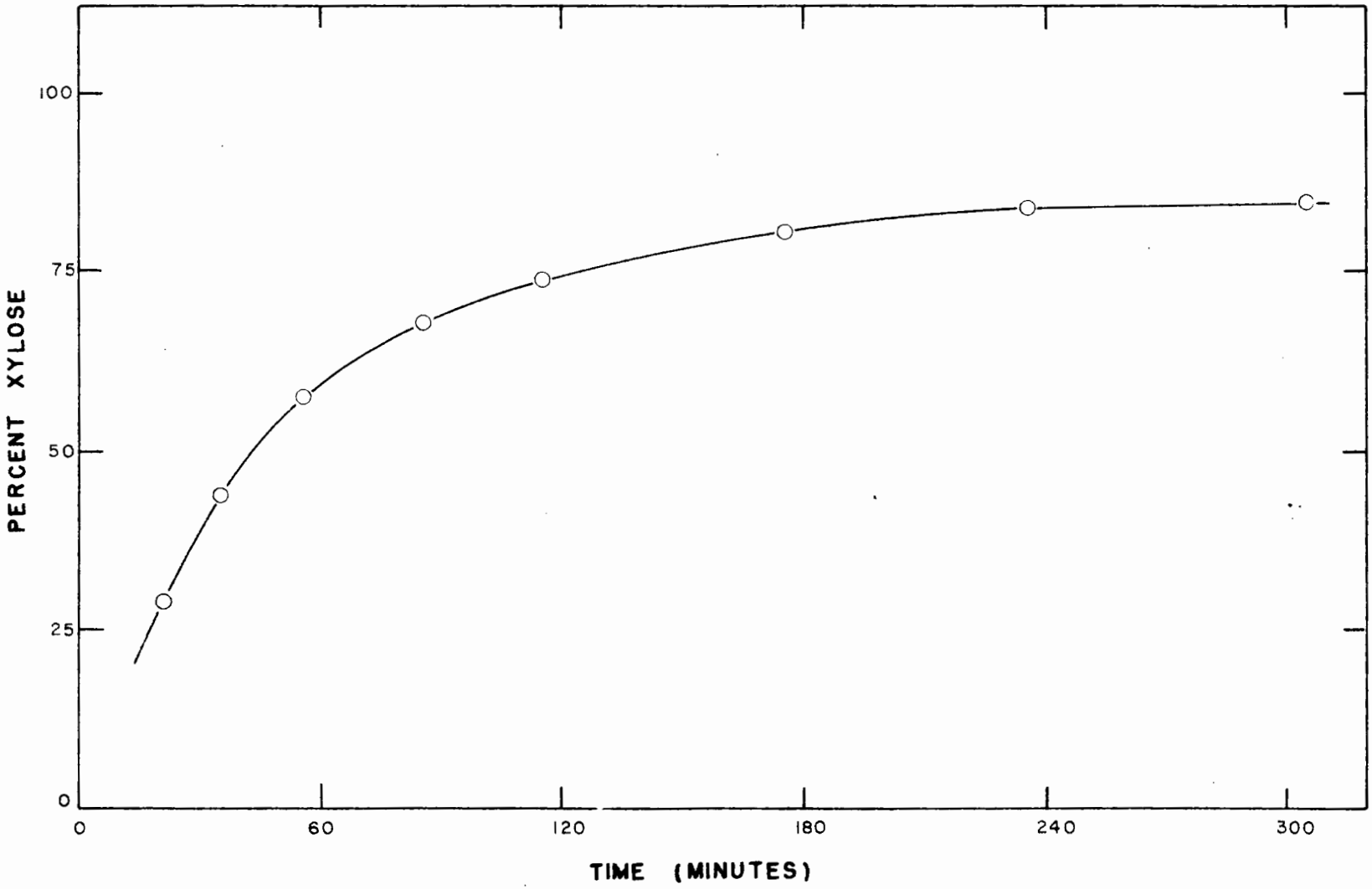


FIG. 12. Hydrolysis of Polyuronide Fractions X,Y and Z with 1% Sulphuric Acid at 100°C.

ent to 0.189 g. or 85% xylose.

(b) The rest of the material (4.47 g.) was hydrolyzed in a similar manner with 446 ml. of 1% sulphuric acid for 4½ hours. Sulphate ions were then removed by the addition of barium carbonate, and the precipitate was collected on the filter and washed well with hot water; the combined neutral filtrate and wash waters were carefully concentrated under vacuum to approximately 50 ml.

To facilitate the examination of the constituent units in the polyuronides, the concentrate was treated with 500 ml. of alcohol to precipitate barium uronates. This material was collected in the centrifuge, taken up in 20 ml. of water, and reprecipitated by the addition of alcohol. The product was dried, again dissolved in water, and the solution was allowed to percolate through a cation-exchange column of Amberlite IR-120. The column was washed with water until the eluate failed to give a naphthoresorcinol test for uronic acids. Evaporation of the eluate to dryness left a syrup, 1.00 g.

Identification of D-Xylose

The aqueous alcohol solution from which the barium uronates had been precipitated was concentrated to about one-quarter of its volume and passed first through a cation-exchange column of Amberlite IR-120 and then through an anion-exchange column of Amberlite IR-4B in order to remove any uronic acid derivatives not previously precipitated. In each case the columns were washed well with water until an aliquot of the

eluates failed to give a positive Molisch test for carbohydrates. The eluate from the second column was concentrated to a thick syrup, and after standing for one week under vacuum over phorphorus pentoxide the syrup crystallized completely in a yield of 3.46 g. The crystals were taken up in a minimum of boiling glacial acetic acid and allowed to crystallize slowly at room temperature. The crystals were collected on the filter and dried under reduced pressure in the presence of sodium hydroxide pellets. This material had a melting point of 144° to 145°C. which was not depressed on admixture with an authentic sample of D-xylose. In accordance with D-xylose, the specific rotation of a 2% aqueous solution at 23°C. was + 19.0°.

Examination of Uronic Acid Fraction

A chromatogram of the uronic acid fraction using solvent B (p. 68) showed that 8 components were present in the mixture with the following R_f values (1) 0.03, (2) 0.07, (3) 0.10, (4) 0.12, (5) 0.16, (6) 0.21, (7) 0.29, and (8) 0.37, obtained by comparison with L-rhamnose, R_f 0.30. The spot with R_f 0.21 had the same mobility and colour as D-xylose.

In order to fractionate the mixture, a thin syrup containing 1.0 g. of solids was applied along the starting lines of 10 sheets of Whatman No. 1 filter paper each 18¼" long x 22½" wide, the application covering approximately 22" in a band ¼" deep. Triangular sections were cut from the top of the sheet to form tabs such that the sheet could be evenly hung from a circular glass trough, while anchored with a circular glass rod

in the bottom of the trough. The trough was supported by glass tubing which fitted into posts attached both to the bottom of the trough and to a circular glass rod in the bottom of the tank. Small pieces of cork were wedged between the side of the trough and the tank for added stability. A thin glass rod which acted as a lip was attached to the rim of the trough in order to hold the paper from contact with the glass surface.

The chromatogram was developed in the usual manner by downward displacement with solvent B (p. 68) for 17 hours and then air-dried in a fume hood. Three strips 1/6" wide were cut lengthwise from the paper and sprayed with aniline phthalate to locate the uronic acid containing zones, and the unsprayed zones were then cut out. The appropriate papers were combined from the 10 large sheets and broken up in a Waring blender with a minimum of water; the pulp was removed on the filter and washed well with water. The uronic acid fractions were recovered on evaporation of the extract to dryness.

Of the extracted materials, Fraction 2 (R_f 0.07, weight 0.31 g.) crystallized from a thick syrup and gave a red spot with aniline phthalate. Fraction 5 (R_f 0.16, weight 0.53 g.) gave an orange spot and could not be crystallized. Both fractions were analyzed for molecular weight by oxidation with alkaline hypoiodite according to Hirst, Hough and Jones (92) and for alkali equivalence, methoxyl content and specific rotations in the following manner. Approximately 0.15 g. was dissolved in water in a 10 ml. volumetric flask, the solution

was brought to volume, and the optical rotation was measured. A 2 ml. aliquot was removed and mixed with 10 ml. of standard 0.1N iodine solution and 50 ml. of a solution containing 0.2M sodium hydrogen carbonate and 0.2M sodium carbonate (pH 10.6). After being kept in a glass-stoppered flask for 2½ hours in the dark, the solution was acidified and the excess iodine was titrated with standard N/20 sodium thiosulphate solution using starch indicator. From this titration the weight of material which would consume 2 equivalents of iodine was calculated. The alkali equivalents were determined by adding an excess of standard alkali (0.00705N) to 1 ml. of the stock solutions and after 4 hours back-titrating to pH 8.2 to 8.3 with 0.1N hydrochloric acid and the aid of the pH meter. The analytical results, including those for pure D-glucurone, are given in Table XI.

Identification of the Uronic Acid Units

Fractions 2 and 5, and also D-glucurone were reduced with sodium borohydride to yield sugar units according to the method of Wolfrom and Anno (93), after first forming their ester glycosides.

Samples, 0.12 to 0.29 g., were heated under reflux in a concentration of 2% with 1.8% methanolic-hydrogen chloride for 8 hours, and the solutions were neutralized with silver carbonate. Silver chloride was removed on the filter, washed with dry methanol, and hydrogen sulphide was passed through the filtrate. A colloidal solution of silver sulphide resulted, but

after concentration in a water bath (50 C.) with a jet of air, the precipitate coagulated sufficiently to be removed on the filter. The filtrates were then evaporated to dryness to leave the methyl ester methyl glycosides as syrups.

In the reduction, the product from Fraction 5, 0.24 g., for example, was dissolved, in 3 ml. of water and the solution was continuously stirred for 25 minutes with 0.10 g. of sodium borohydride in 2 ml. of water. The reaction mixture was then diluted with 10 ml. of water and hydrolyzed under reflux with 12 ml. of 4% sulphuric acid for 5 hours. After being cooled and neutralized with barium carbonate, the solution was filtered and the filtrate was passed through a column containing equal amounts by weight of Amberlite IR-120 and Amberlite IR-4B (cation and anion exchange resins). The effluent was concentrated to a syrup.

Examination of a portion of this syrup on a chromatogram with solvent A (p. 68) showed that only two constituents were present, one of which was xylose, while the other had a mobility greater than xylose. The final product from Fraction 2 was similar, but xylose appeared to be present in a relatively greater amount. On comparison with authentic samples of 2-, 3-, and 4-methyl-D-glucose, which had the distinctly different mobilities of R_f 0.34, 0.36, and 0.32, respectively, using solvent E (p. 68), the constituent other than xylose in both products was seen to have a mobility and colour identical with that of 4-methyl-D-glucose. The reaction product from D-gluc-

urone gave a spot identical with that of D-glucose on the chromatogram.

Examination of Fraction R

Oxidation with Sodium Metaperiodate

The procedure outlined by Hayward (118) was used. This procedure had been adapted from the work of several authors (119,120,121,122) and revealed the polarimetric change, the final amount of periodate consumed, and the amount of formic acid and formaldehyde produced, from a single 100 to 200 mg. sample. Since Fraction R was not water soluble, the polarimetric change could not be determined, and the oxidation was carried out in the dark with duplicate samples of approximately 0.15 g.

The sample was suspended in 10 ml. of water contained in a 25 ml. volumetric flask, and 15 ml. of 0.1671M sodium metaperiodate was added from a pipette. The mixture was shaken vigorously and the pH was immediately determined on a 5 ml. sample with a calibrated Beckman pH-meter. The sample was then returned to the flask. At various intervals, an aliquot, 2 ml., was removed, diluted with 10 ml. of water and buffered with 1.5 g. of sodium bicarbonate. Ten ml. of 0.1N sodium arsenite solution and 0.2 g. of potassium iodide were then added, and after standing 10 to 15 minutes at room temperature, the excess arsenite was titrated with standard 0.1N iodine. From the iodine titre and that of a suitable blank the amount of periodate consumed was calculated. This estimation is described in

detail by Jackson (119).

To determine the amount of formic acid product, a 5 ml. aliquot was titrated with standard 0.01N potassium hydroxide solution to the original pH with the aid of the pH meter. The results are in Table XXIV and Fig. 10.

No formaldehyde was detected by the dimedon reagent (122) in the combined last three oxidized solutions.

Methylation of Fraction R

Fraction R, 8.73 g., was methylated by the thallous hydroxide-thallous ethylate-methyl iodide technique of Hirst and Jones (123). The ethylate was prepared by shaking 10 to 15 g. of clean thallium shavings in 250 ml. of dry ethanol for 10 hours in an atmosphere of oxygen. The supply of oxygen was maintained by attaching a balloon containing the gas to a tube fitted into the top of the flask. After nearly or all of the thallium had reacted, the solution was decanted into a bottle and stored in the dark at -5°C. until crystallization of the ethylate was complete. A fresh supply of metal was added to the mother liquors and the process continued. This procedure was a modification of the method of Assaf, Haas and Purves (124) and was described in detail by Manchester (125). Thallous hydroxide was prepared by adding a solution of the ethylate in benzene to water and removing the benzene by distillation under vacuum.

The addition of the polysaccharide to 140 ml. of N

TABLE XXIV

Oxidation of Hemicellulose Fraction R with Sodium Metaperiodate Solution

Sample 1, 0.1404 g. (ash-free) in 25 ml.

Time hrs:mins.	Iodine Titre ml.	pH	KOH Titre ml.
0	5.87	4.89	- - -
1:25	6.56	- - -	- - -
2:25	3.73	- - -	- - -
3:25	- - -	4.28	0.85
3:40	6.83	- - -	- - -
4:40	6.93	- - -	- - -
5:15	- - -	4.10	0.85
7:10	7.05	- - -	- - -
10:10	7.10	- - -	- - -
23:00	7.37	- - -	- - -

Sample 2, 0.1421 g. (ash-free) in 25 ml.

1:10	6.53	- - -	- - -
2:10	6.69	- - -	- - -
4:10	- - -	4.25	0.85
8:25	- - -	3.80	1.18
26:00	- - -	3.70	1.35
26:25	7.38	- - -	- - -

thallous hydroxide caused the formation of a heavy yellow precipitate which was stirred under nitrogen for 2 hours. The precipitate was recovered on the filter, and was washed with cold water and a small amount of ethanol. After drying for 1 week in vacuo over phosphorus pentoxide, the pale yellow solid, 27.15 g., was ground to pass a 120-mesh screen and heated under reflux with 90 ml. of freshly distilled methyl iodide for 12 hours. Excess methyl iodide was removed by distillation, 150 ml. of 0.88 N thallous ethylate in benzene was added, and the whole was evaporated to dryness under reduced pressure. The mixture was again methylated for 12 hours with 90 ml. of methyl iodide.

Prior to any further methylation, the dried mixture of thallous iodide and partly methylated polysaccharide was extracted with methanol, chloroform, acetone and pyridine, which together removed 4.61 g. of material (OCH_3, 31.3%). The extracted residue was then re-extracted with hot water, and 5.39 g. (OCH_3, 3.61%) was recovered on evaporation of the dispersion to dryness. Since it was apparent that a protective coating had been formed around a large portion of the original polysaccharide during the reaction with thallous hydroxide, this portion was redispersed in water and shaken with 150 ml. of 0.88 N thallous ethylate in benzene. Both benzene and water were removed by distillation under reduced pressure in an atmosphere of nitrogen and the residue was dried for 16 hours at 35°C. under vacuum. The grey solid was powdered to pass a 120-mesh screen, heated under reflux with methyl iodide for 36 hours and

recovered by extraction with chloroform in a yield of 4.70 g. with a methoxyl content of 30.9%.

The two portions, now of approximately equal methoxyl content, were combined and dissolved in purified dioxane. Another methylation with thallous ethylate in benzene increased the methoxyl content to 34.8%.

Further methylation was accomplished by heating a solution of the material in 90 ml. of methyl iodide with 5 g. of freshly prepared silver oxide for 6 hours under reflux. Silver oxide was removed on the filter and methyl iodide by distillation. The methoxyl content of the product, 36.3%, was not increased by a further treatment with methyl iodide and silver oxide but was increased to 36.8% on treatment with thallous ethylate and methyl iodide (calc'd for $C_7H_{12}O_4$:OCH_3, 38.8%). The final yield was 8.50 g.

Examination of Methylated Fraction R

The methylated product, 8.50 g., was dissolved in 200 ml. of chloroform, and light petroleum ether, b.p. 30° to 60°C., was added in successive portions as in the fractionation of the hemicellulose acetate. The results are given in Table XII and Fig. 11.

The fractions, uniform in methoxyl content and similar in specific rotations, were combined in spite of their divergence in intrinsic viscosity. A sample of the combined material, 0.1 g., was heated in a sealed tube at 100°C. with 5 ml. of 1.8%

methanolic-hydrogen chloride for 6 hours; the tube was cautiously opened, the contents diluted with 5 ml. of water and the hydrolysis to reducing sugars was continued for another 6 hours. The solution was then neutralized by the silver carbonate-hydrogen sulphide method and chromatographed with solvents C and D (p. 68). Only two spots, corresponding to a mono- and dimethyl xylose, could be detected on the chromatogram in the area occupied by methylated xylose derivatives. Spots whose smaller mobility suggested uronic acids were also present.

In an attempt to separate the methylated sugars from the methylated uronic acid derivatives, a 0.55 g. sample was heated under reflux with 2% methanolic-hydrogen chloride. The following changes in optical rotation occurred: $+73^{\circ}$ (2 hours); $+73^{\circ}$ (3 hours); $+70.5^{\circ}$ (5 hours). The solution was neutralized with silver carbonate and the silver removed with hydrogen sulphide. According to the method of Adams (68), the neutral solution was concentrated to a light-yellow syrup, 0.49 g., and warmed on a steam bath at 60°C. for 3 hours with 7 ml. of saturated barium hydroxide solution. Excess barium hydroxide was removed by carbon dioxide, the solution was heated for 15 minutes at 85°C. to decompose any barium hydrogen carbonate, filtered, and the precipitate thoroughly washed with hot water. The aqueous solution was concentrated to about 12 ml. under reduced pressure, and was continuously extracted for 18 hours with chloroform. Unlike Adam's experience, the methyl dimethyl- and monomethyl xylosides were not completely removed from the

methyl uronosides. This conclusion was reached by hydrolyzing the fractions to reducing sugars, which when chromatographed with solvent E (p. 68) revealed partly methylated xylose and uronic acids both in the water-soluble and chloroform-soluble portions.

Since methyl ethyl ketone saturated with water (solvent C; p. 68) was found to separate all three constituents of the hydrolyzed polysaccharide effectively on the paper chromatogram, the operation was repeated on a larger scale. The methylated polysaccharide, 6.39 g., was hydrolyzed, first with 330 ml. of 2% methanolic-hydrogen chloride under reflux for 4 hours ($[\alpha]_D^{22}$ +72°), and then after the addition of 300 ml. of water for 6 hours. This hydrolysate was neutralized by the silver carbonate-hydrogen sulphide method and the solvents were removed under reduced pressure. The resulting mixture, 6.70 g., a light-yellow syrup, was dissolved in 7 ml. of methyl ethyl ketone saturated with water and applied to a powdered cellulose column, 3.7 x 71 cm., previously washed with the solvent. The solvent was allowed to percolate through the column at a rate of 6 ml. in 5 minutes, and the eluate was collected in test tubes, just prior to the elution of carbohydrate material, by a mechanical automatic receiver changer, constructed by Mr. Sanderson in this laboratory. Samples of the eluate were spotted in chronological order on large sheets of filter paper, chromatographed with solvent E (p. 68) and sprayed with aniline phthalate. Appropriate fractions were then combined, evaporated to dryness, and examined again on the chromatogram using

solvent E.

The eluates from 1 to 45 yielded 2.76 g. of syrup but did not give a spot on the chromatogram; eluates 46 to 108 yielded 3.28 g. of syrup which, although giving a spot for dimethyl xylose had $[\alpha]_D^{22}$ +75.5° (c, 1.50 in water); eluates 138 to 164 yielded 0.06 g. of syrup which gave a spot corresponding to monomethyl xylose. The column was washed with water to remove 0.07 g. of uronic acid derivatives. Uronic acid derivatives were also present in all fractions as contaminants.

Syrups from eluates 1 to 45, 2.70 g., and from 46 to 108, 2.67 g., were refluxed separately in 130 ml. of 2% hydrochloric acid for 6 hours, after which the optical rotation had changed from +58.8° to +26.4° for the former solution and from +74.0° to +25.5° for the latter solution. The solutions were combined, neutralized by the silver carbonate-hydrogen sulphide method and evaporated to a thin syrup. This syrup, contaminated with colloidal silver sulphide, was chromatographed on a cellulose column and the eluate was analyzed in the manner previously described.

The eluate from tubes 16 to 56 yielded a syrup which analyzed for both uronic acid derivatives and dimethyl xylose on the chromatogram; the eluate in tubes 59 to 110 when evaporated to a thin syrup contained uronic acid derivatives, monomethyl xylose and a trace of dimethyl xylose. No uronic acid derivatives were present in the aqueous washings of the column. Both syrups were dissolved separately in 50ml. of water and the

solutions were shaken continuously with 10 g. of Amberlite resin IR-120 (cation-exchange) and 10 g. of Amberlite IR-4B (anion-exchange) for 25 minutes. The resins were then removed on the filter, washed well with water, and the filtrates were evaporated to a syrup under reduced pressure. The syrups were dried in vacuo over phosphorus pentoxide.

Monomethyl D-Xylose

The syrup, 0.20 g. with $[\alpha]_D^{22}$ +23.0° (c, 1.00 in water) gave a principal spot, R_f 0.43, and a light spot, R_f 0.65, when chromatographed with methyl ethyl ketone saturated with water-ethanol (4:1), solvent E for 2.5 hours, and had a methoxyl content of 19.9, 19.8% (calc'd 18.9%). After 12.5 hours the principal spot had nearly divided into two spots the centres of which having moved 36.3 cm. and 38.3 cm., respectively, were identical in position to 2-methyl- and 3-methyl-D-xylose. A solution of 87.0 mg. in 100 ml. of glacial acetic acid was prepared, 10 ml. aliquots were removed and the solutions were treated with 10 ml. of 0.1N lead tetraacetate in glacial acetic acid. After 1,2,5,15 and 30 minute intervals, 20 ml. of an aqueous solution containing 20 g. of sodium iodide and 250 g. of sodium acetate per litre was added and the liberated iodine was titrated with standard sodium thiosulphate solution in the presence of starch indicator, according to Hockett and McClenahan (126). Sodium thiosulphate, 0.1N, however, was inadvertently used rather than 0.02N. A titre of 0.55 ml. of 0.1000N sodium thiosulphate was constant after 2 minutes oxidation, equivalent to 0.52 molecular equi-

valents of lead tetraacetate consumed per mole of monomethyl xylose. When another 10 ml. of the sugar solution was oxidized for 15 minutes, a titre of 2.73 ml. of 0.0200N sodium thiosulphate was obtained.

2,3-Dimethyl-D-xylose

The syrup, 2.87 g., having a methoxyl content of 34.4, 34.6% (calc'd 34.8%) and $[\alpha]_D^{22}$ + 23.8° (c, 2.00 in water), consisted only of 2,3-dimethyl-D-xylose with R_f 0.65 (solvent E, p. 68). An anilide was prepared by refluxing 0.25 g. in 5 ml. of methanol containing 1 ml. of freshly-distilled aniline for 2.5 hours, the methanol was removed and the reaction mixture was allowed to stand for 1 week. Excess aniline was dissolved in cold ether and the crystals were collected on the filter. After recrystallization from ethyl acetate and petroleum ether the melting point of these crystals and a mixed melting point with an authentic sample of 2,3-dimethyl-D-xylose anilide, kindly supplied by Dr. G.A. Adams, was 124° to 125°C.

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. The degree of polymerization of cellulose isolated by direct nitration of aspen wood was found to be 2110, whereas the cellulose component of an aspen holocellulose, prepared by a chlorination procedure, and an aspen chlorite holocellulose averaged only 1,900 and 1,530, respectively. These results corroborated the earlier view that, although both procedures cause a degradation of the carbohydrates in wood, the chlorination method brings about much less degradation than the chlorite procedure.

2. The degree of polymerization of the cellulose component of black spruce chlorite holocellulose before and after extraction with liquid ammonia and water was 1,330 and 980, respectively. The low yield of cellulose nitrate in the latter case, however, made it uncertain whether or not degradation had occurred during the treatment with liquid ammonia.

3. Hot water extraction of the aspen chlorite holocellulose yielded 3.6% of a material containing lignin and carbohydrates, which was subdivided into three fractions with aqueous alcohol. The first fraction consisted of polysaccharides and pectic material. The components of the second portion, comprising polysaccharides and "modified" lignin, could be partly separated by fractional precipitation. The third fraction consisted mainly of "modified" lignin but had an apparent uronic anhydride content of 11%. This fact, together with an ob-

served increase in uronic anhydride on conversion of wood to holocellulose, and the apparent uronic anhydride content of 21.3% in periodate oxy-lignin, suggested that chlorine dioxide was capable of producing carboxyl groups in lignin. The uronic anhydride and corrected pentosan content of the chlorite holocellulose would thus be in error.

4. Treatment of the water-extracted holocellulose with liquid ammonia removed 12.5% of material, the methanol-soluble portion of which amounted to 9.8% and consisted of "modified" lignin and acetamide, the amount of the latter corresponding to 82% of the acetyl groups in the holocellulose. This material was further subdivided by fractional precipitation. Four of the fractions which apparently contained no carbohydrates had methoxyl contents of the order of 16% and one had an ultraviolet absorption spectrum typical of lignin derivatives. The methanol-insoluble portion consisted of a mixture of "modified" lignin and polysaccharides. Neither these results nor those in (3) indicated the presence of a lignin-carbohydrate complex.

5. Extraction of the remaining holocellulose with cold and hot water removed a further 11.4% of material, the residue being highly swollen, suggesting that liquid ammonia caused chemical changes in the wood other than removal of acetyl groups. The portion of the extracts that was insoluble in aqueous alcohol was bleached with sodium chlorite, and, after removal of pectin rendered insoluble by the treatment, the remaining hemi-

celluloses were acetylated to yield a product, 42% of which was soluble in chloroform. This portion was further subdivided by fractional precipitation but the resulting fractions varied little in either specific rotation or intrinsic viscosity, the solubility behaviour of the acetates evidently being influenced both by chemical and physical properties.

6. Deacetylation of the chloroform-soluble and -insoluble acetates and fractional precipitation of the resulting hemicelluloses yielded fractions whose degrees of polymerization approximated values of 50 to 110 and whose optical rotations were widely divergent. Analyses of four of the fractions indicated that their elementary compositions were similar but that the ratios of the building units were different. The constituent units were D-xylose, methyluronic acids and uronic acids, the last two being present in the ratio of 2:1.

7. Hydrolysis of three of the polyuronide fractions yielded D-xylose and two aldotriuronic acids, the analysis of which suggested that one of them consisted of two D-xylose units and one 4-methyl-D-glucuronic acid unit, joined by α- and β-glycosidic linkages, whereas the other one consisted of one D-xylose unit, one 4-methyl-D-glucuronic acid unit and one unknown uronic acid unit, joined by α-glycosidic linkages only. Periodate and lead tetraacetate oxidation and methylation studies indicated that the fourth polyuronide fraction had a highly branched structure with D-xylose units joined by 1,4-β-linkages with branches originating at the 2 and 3 carbon atoms terminat-

ed by the uronic acid units.

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