519. *The Hemicelluloses Present in Aspen Wood* (Populus tremuloides). *Part I.**

By J. K. N. Jones and Louis E. Wise.

Hydrolysis of extractive-free aspen sawdust yields L-rhamnose, L-arabinose, D-xylose, D-galactose, xylobiose, xylotriose, 4-methyl D-glucuronic acid, and several oligosaccharides containing uronic acids. The action of periodate on the sawdust and on isolated hemicellulose has been examined. The results are discussed.

Wood cell wall material is composed, in the main, of carbohydrates, by far the greater portion of which is cellulose. The remainder consists of "hemicelluloses," their percentage being dependent on the type and age of wood examined. Hemicelluloses have been described by Norman ("The Biochemistry of Cellulose, Hemicelluloses, Polyuronides and Lignins," Oxford Univ. Press, 1937, p. 37) as "Those polysaccharides extractable from plant tissues by treatment with dilute alkalis, either hot or cold, but not with water, and which may be hydrolysed to constituent sugars and sugar acids by dilute mineral acids." However, celluloses and hemicelluloses cannot be very sharply differentiated (cf. Wise and Ratcliff, *Ind. Eng. Chem., Anal. Ed.*, 1947, **19**, 459). As Norman has pointed out, these hemicelluloses will have their origin either in the structural cell wall or in the encrusting materials.

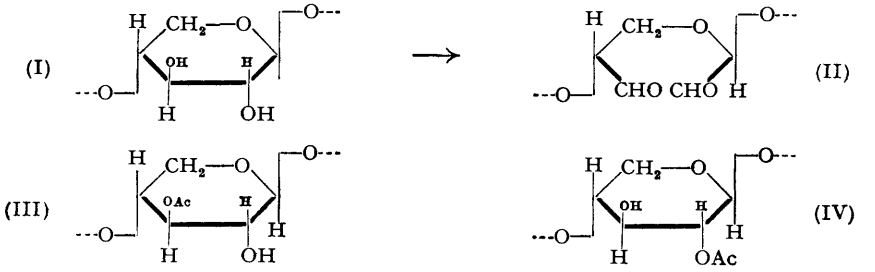
The chemical constitution of the hemicelluloses of wood has been little investigated and little is known of the nature of the linkages which unite the sugar units in these polysaccharides. For example, the question whether these carbohydrates are united in the form of one giant molecule or whether the sugar units are combined in the form of a mixture of polysaccharides, each of which is built up of one or more types of sugar only or whether the hemicelluloses are joined to cellulose or lignins is still largely undecided. The evidence of Hirst (*J.*, 1949, 522), O'Dwyer (*Biochem. J.*, 1940, **34**, 149), Anderson (*J. Biol. Chem.*, 1938, **126**, 175; 1941, **140**, 563), McIlroy (*J.*, 1949, 121), and others indicates that some plant hemicelluloses consist of mixtures of sugars and uronic acid units. Isherwood, who separated certain hemicelluloses by electrophoresis (1st Intern. Congr. Biochemistry, 1949, Abstracts, p. 515), and others have shown that such substances give, on hydrolysis, one sugar only. The problem is further complicated by the fact that the composition of the polysaccharides may alter with the age of the tree and vary in different parts of the wood as, for example, in the sapwood and heartwood of the oak (cf. O'Dwyer, *loc. cit.*), and further that the hemicelluloses may be modified during isolation. Schoettler, of The Institute of Paper Chemistry, showed that when aspen sawdust is heated with hot potassium hydroxide

* The substance of this paper was presented at the 75th Anniversary Meeting of The American Chemical Society, New York, September, 1951.

solution (16%), some 70% of the total (*ca.* 23%) hemicelluloses in the sawdust is extracted, the remainder being possibly combined with the cellulose or lignin, inaccessible to the alkaline reagent or perhaps of different molecular weight or constitution. If the sawdust is first delignified with chlorine dioxide [Wise, Murphy, and D'Addieco, *Paper Trade J.*, **125**, No. 11, 57 (September 1947)], practically all the hemicellulosic material can be extracted with sodium or potassium hydroxide from the resultant "holocellulose." This comparative ease of extraction may result because of the rupture of a lignin–hemicellulose bond with removal of the lignin or the hemicellulose may be more accessible to the reagent (Lange, Symposium, September 1951, Appleton, Wisconsin), or it may be that the hemicelluloses have been modified (*i.e.*, oxidised or degraded) by the reagents and thus rendered more soluble in the alkaline solution. That a degradation, at least in molecular magnitude, *may* occur on treatment with chlorite, has been demonstrated by Wethern (Thesis, Institute of Paper Chemistry, 1951) for sprucewood hemicelluloses, and by Timell and Jahn (*Svensk Papperstidnung*, 1951, **54**, 831) for birchwood hemicellulose. In order to eliminate the latter possibility, the aspen hemicellulose which was used in this work was prepared by direct extraction of the aspen sawdust, suitably freed from non-cellular extraneous materials by use of organic solvents, with dilute alkalis in an atmosphere of nitrogen. The extract may not, therefore, represent a true picture of the properties of the *total* hemicelluloses of the aspen as, under these conditions, some were not extracted and remained in the residue. Furthermore, it is possible that the extracted hemicellulose may have been modified. Certainly the acetyl groups, over 3% of which occur combined in the wood (Sherrard and Blanco, *Ind. Eng. Chem.*, 1923, **15**, 611; Scholler, *Chim. ind. agr. Biol.*, 1939, **15**, 195; Mitchell and Ritter, *J. Amer. Chem. Soc.*, 1940, **62**, 1958; Bertrand and Brooks, *Compt. rend.*, 1941, **212**, 739), very probably to the xylan, will have been eliminated (see below).

In an attempt to determine the position of these acetyl groups and also to decide whether any considerable amount of end groups were present in aspen wood, the action of sodium metaperiodate solution at 2°, both on aspen wood and on an isolated aspen hemicellulose, was studied. Metaperiodates will normally react with carbohydrate residues containing glycol groups [–CH(OH)·CH(OH)–] (cf., however, Alexander, Dimler, and Miltretter, *J. Amer. Chem. Soc.*, 1951, **73**, 4658) and will liberate one mole of formic acid per pyranose sugar residue which contains hydroxyl groups on each of three contiguous carbon atoms (Halsall, Hirst, and Jones, *J.*, 1947, 1427). The rate of oxidation has been shown to depend in part on the configuration of the hydroxyl groups in the molecule (cf. "Organic Reactions," Adams, Wiley, Vol. 2, p. 343), and unpublished work (Andrews, Hough, and Jones) has indicated that steric effects may slow down the rate of oxidation of some of the sugars (cf. Hirst, Jones, and Roudier, *J.*, 1948, 1779; Gibbons and Boissonnas, *Helv. Chim. Acta*, 1950, **33**, 1477). Potter and Hassid (*J. Amer. Chem. Soc.*, 1948, **70**, 3488) have shown that amylose and amylopectin were rapidly oxidised in heterogeneous solution by sodium metaperiodate at 2° and yield the requisite amounts of formic acid in about 25 hours. Aspen wood and aspen hemicellulose were oxidised much more slowly by the reagent, but eventually gave practically constant yields of formic acid. Isolated xylan is rapidly oxidised by metaperiodate and eventually passes into solution (Jayme and Sätre, *Ber.*, 1942, **75**, 1840; 1944, **77**, 242, 248). No such effect was noticed during the oxidation of aspen sawdust. The oxidation of both aspen sawdust and isolated aspen xylan was extended to ensure that, so far as possible, all available carbohydrate residues were oxidised. If no glycol groups are present in the sugar residues, they will remain substantially unaffected by the metaperiodate, and the unoxidised sugar units can then be detected chromatographically after acidic hydrolysis of the residual oxidised polysaccharide. Aspen sawdust, on oxidation with sodium metaperiodate solution at 2° in the dark (Potter and Hassid, *loc. cit.*) yielded formic acid, the amount corresponding to one mole per *ca.* 5700 g. of sawdust (containing *ca.* 84% of carbohydrate on the extractive-free basis). This means either that the amount of sugar residues containing the –CH(OH)·CH(OH)·CH(OH)– grouping, which per mole will yield one mole of formic acid on oxidation with periodate, was very small, or that the polysaccharides present in the wood were oxidised incompletely (because of greater crystallinity?). It was found that, on hydrolysis, 14·3% of xylose (as $C_5H_8O_4$) was liberated together with 2·8% of glucose (as $C_6H_{10}O_5$). Most of the xylan

present in the wood (*ca.* 19%) had, therefore, escaped oxidation, very probably because it was in part acetylated (see below). The substitution of any hydroxyl by acetyl in a xylose unit in a xylan will prevent it from being oxidised by periodate (see I—IV).

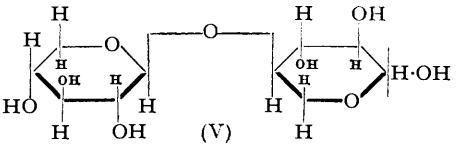


A sample of the hemicellulose, which had been extracted from aspen wood with 16% potassium hydroxide solution, was oxidised by an excess of sodium metaperiodate solution. The hemicellulose behaved quite differently from the sawdust sample, in that oxidation caused it to dissolve, in part, in water. After dialysis, the material was hydrolysed and the products of hydrolysis were determined quantitatively. In this instance, only 1·7% of xylose (calc. as $C_5H_8O_4$) had escaped oxidation by the metaperiodate. This indicates that either the hemicellulose is more easily oxidised when it has been separated from the wood, or that it has been modified in some way (deacetylated?) (cf. Bell and Wright, *J. Amer. Chem. Soc.*, 1950, **72**, 1485) and thus rendered vulnerable to oxidation by metaperiodate. The original hemicellulose was not a pure xylan and contained small quantities of materials producing, on hydrolysis, sugars which moved at the same rate on the chromatogram as did glucose, galactose, arabinose, and rhamnose. All these sugar residues were destroyed during the periodate oxidation and were not subsequently detected in the products of hydrolysis of the oxidised material.

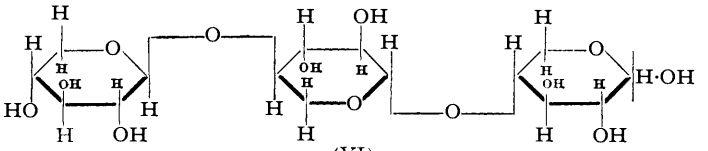
Preliminary work by Schoettler (Thesis, Institute of Paper Chemistry, Appleton, Wisconsin, 1951) had shown that the aspen hemicelluloses on hydrolysis yield several uronic acid-containing materials. Methods which were developed by the authors to isolate and identify these materials are described in the present paper. In order to avoid ambiguity, isolation of the hemicelluloses as such was not attempted; instead, the products formed on hydrolysis of benzene–alcohol extracted aspen sawdust were examined. This procedure avoids the possibility of formation of acidic material, *e.g.*, 'onic acid residues during the chlorite procedure, and the possible loss of any carbohydrate during this stage, and ensures that no sugars or uronic acids which may be attached to the cellulose or lignin are discarded. Preliminary experiments had shown that the great majority of the uronic acid-containing materials were liberated in the early stages of the hydrolysis and, to avoid the formation of overwhelming amounts of xylose, the hydrolysis of the aspen sawdust was not taken to completion. Norman (*loc. cit.*) has noted that some xylose units in wood are hydrolysed with more difficulty than are others. This may, however, be a physical rather than a chemical difference (cf. Jorgensen, Internat. Symposium on The Fundamental Chemistry of Cellulose and Lignin, Montreal, September, 1951).

Examination of the hydrolysis products on the paper chromatogram showed the presence of rhamnose, arabinose, xylose, mannose (?), glucose, galactose, oligosaccharides, and various spots due to the presence of acidic material (cf. Gustafsson *et al.*, *Paper and Timber, B*, 1951, no. 10). The acidic sugars were removed on Amberlite resin IR-4B and the neutral sugars separated by fractional elution from charcoal (Darco G.60) by the method of Whistler and Durso (*J. Amer. Chem. Soc.*, 1950, **72**, 677) and then by partition chromatography on sheets of filter paper. In this manner, L-rhamnose, L-arabinose, D-xylose, D-galactose, a disaccharide (V), and a trisaccharide (VI), composed of D-xylose units, were separated and identified. The last two materials had earlier been prepared by Whistler and Durso (*loc. cit.*) by graded hydrolysis of xylan. The crystalline sugars were compared with samples kindly provided by Professor Whistler and were found to be identical with his materials. The isolation of these products (albeit in small yield) indicates that there is

present in aspen wood a polysaccharide which contains D-xylopyranose residues united through positions 1 and 4 and joined by β-linkages (cf. Whistler and Durso, *loc. cit.*). A polysaccharide containing D-xylose residues only, termed xylan, is present in esparto grass (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289) and it may be that the xylose formed on hydrolysis of aspen wood is present as a xylan of similar constitution (cf. esparto xylan, which after oxidation with periodate gives a product containing 1·3% of xylose). The



(V)



(VI)

xylan in pear cell wall also contains D-glucuronic acid as part of the molecule (Chanda, Hirst, and Percival, *J.*, 1951, 1240; O'Dwyer, *loc. cit.*) (see below).

The sugar acids present in the hydrolysate from aspen sawdust were displaced from the Amberlite IR-4B resin with sulphuric acid, the latter then removed as barium sulphate, and inorganic material removed with Amberlite IR-120 resin. This procedure gave a product which, on examination on the paper chromatogram, was found to be a complex mixture of acids containing a little free xylose. This sugar was detected by examining the mixture chromatographically with a butanol–pyridine–water mixture in which only the neutral sugar moved, the uronic acids remaining on the starting line. The uronic acid fraction was separated first on a charcoal (Darco G.60)–celite column (Whistler and Durso, *loc. cit.*) and then on a column of cellulose, using butanol–formic acid–water as eluant; thus, five fractions containing uronic acid were separated. The fastest-moving fraction, which gave a bright red colour with the *p*-anisidine hydrochloride spray (Hough, Jones, and Wadman, *J.*, 1950, 1702), was identified as 4-methyl D-glucuronic acid. The ester glycoside of this material, on reaction with alcoholic ammonia, gave the amide of 4-methyl α-methyl-D-glucuronoside, characterised by Smith (*J.*, 1948, 1146; 1951, 2646) and by White (*J. Amer. Chem. Soc.*, 1947, **69**, 715), and was oxidised by sodium periodate with inversion of sign of optical rotation (cf. Smith, *loc. cit.*). Reduction of the methyl ester of 4-methyl αβ-methyl-D-glucuronoside with lithium aluminium hydride (cf. Lythgoe and Trippet, *J.*, 1950, 1983) gave 4-methyl αβ-methyl-D-glucoside, converted by acidic hydrolysis into 4-methyl D-glucose. This was characterised by its rate of movement on the chromatogram and by its conversion into 4-methyl D-glucosazone, identical with a synthetic specimen.

One or more of the uronic acid-containing fractions (which moved more slowly than did 4-methyl D-glucuronic acid on the chromatogram) were combined with D-xylose, since this sugar was produced on hydrolysing them. The source, whether from polysaccharide or lignin, of the 4-methyl D-glucuronic acid-containing fractions is not certain. It may be that they are combined with xylan as in pear cell wall xylan (Chanda, Hirst, Isherwood, and Percival, *loc. cit.*) and in the hemicellulose of *Phormium tenax* (McIlroy, *J.*, 1949, 121).

4-Methyl D-glucuronic acid has also been isolated from mesquite gum (White, *loc. cit.*; Smith, *loc. cit.*) and from gum myrrh (Hough, Jones, and Wadman, *J.*, 1952, 796; cf. Bertrand and Brooks, *Ann. Fermentations*, 1940, **5**, 537).

Experimental

Examination of the Products of Hydrolysis of Aspen Sawdust.—Aspen (*Populus tremuloides*) sawdust (pentosan content, *ca.* 20·5%, uncorrected for uronic anhydride) (270 g., air-dry) was extracted under reflux for 12 hours with 2 : 1 (v/v) benzene–alcohol (95%) (750 c.c.). The

residue was dried in air and then extracted several times (for a total of 2 hours) by shaking it with cold water, until the extracts were colourless and gave a negative Molisch test. The sawdust was filtered off, dried by suction, and then suspended in N-sulphuric acid (1500 c.c.). The mixture, after 60 hours at 25°, was heated at 90° for 8 hours. The slurry was cooled and filtered, and the residual sawdust, which had become reddish-brown, was washed with water (2 l.) until the washings gave only a faint positive Molisch test. The combined filtrates were brought to pH 3 by the addition of barium hydroxide solution and refiltered. The filtrate was concentrated to about 700 c.c. and barium and other inorganic anions removed with Amberlite resin IR-120. Acids were then removed by passing the solution through a column of Amberlite resin, IR-4B, yielding an effluent (*A*) which contained neutral sugars (30 g.) and traces only of uronic acid derivatives. The Amberlite resin IR-4B, which had been well washed with water, was stirred with an excess of N-sulphuric acid, and the solution filtered. The resin was washed several times with water until the washings were free from carbohydrates. To the combined filtrate and washings, barium hydroxide solution was added to pH 3 and the precipitated barium sulphate was filtered off. Barium ions were removed from the filtrate with Amberlite resin IR-120 (or IR-100), and the filtrate evaporated under reduced pressure at 40° to a syrupy mixture (3·8 g.) of uronic acid derivative (*B*). A pronounced odour of acetic acid was present in the distillate.

Separation of Sugars in (A).—A sample of the sugars (5·4 g.) was dissolved in water (15 c.c.) and poured on to a column (6 × 1″) of 1 : 1 (w/w) charcoal (Darco G.60)–celite. Monosaccharides were washed through the column with water. When sugars were no longer detected (Molisch test) in the effluent (*A*I), water was replaced by water containing 5% of ethanol and the solution of oligosaccharides was collected (*A*II). Chromatographic examination of the syrup produced on concentration of the effluents, *A*I and *A*II, showed that xylose was the main component in *A*I, together with traces of rhamnose, arabinose, glucose, mannose (?), and galactose. *A*II contained xylose and at least three oligosaccharides.

*A*I (5·0 g.) slowly crystallised. Trituration with alcohol yielded D-xylose (3·1 g.), m. p. and mixed m. p. 144° after recrystallisation from methanol, $[\alpha]_D^{22}$ +19° (equilibrium) (*c*, 4·1 in water). A portion of the syrupy residue was separated on sheets of filter paper (9″ wide), with ethyl acetate–acetic acid–water (9 : 2 : 2) as solvent. The location of the sugars was determined by spraying guide strips cut from the edge of the filter papers (cf. Flood, Hirst, and Jones, *loc. cit.*). Extraction of the appropriate strips yielded crystalline L-rhamnose (22 mg.), m. p. and mixed m. p. 102°, $[\alpha]_D^{22}$ +9° (equilibrium) (*c*, 1·4 in water), which furnished the characteristic benzoylphenylhydrazone, m. p. 187° (Schoettler and Jones, *TAPPI*, Feb., 1952), L-arabinose ($[\alpha]_D^{22}$ +95° in water), isolated as its diphenylhydrazone, m. p. 207° and mixed m. p. 204°, and D-galactose ($[\alpha]_D^{22}$ +80°; *c*, 0·12 in water), identified as its *N*-methyl-*N*-phenylhydrazone, m. p. 186°.

Fraction *A*II (0·3 g.) was separated into its components as described above, and yielded xylobiose (76 mg.), $[\alpha]_D^{22}$ −26° (*c*. 1·1 in water), m. p. and mixed m. p. 189° (decomp.), and xylotriose (77 mg.), $[\alpha]_D^{22}$ +48° (*c*, 1·0 in water), m. p. and mixed m. p. 214° (decomp.). These specimens were chromatographically pure and were not distinguishable chromatographically from authentic specimens when butanol–pyridine–water (10 : 3 : 3), ethyl acetate–acetic acid–water (9 : 2 : 2), or butanol–ethanol–water (40 : 11 : 19) were used as solvents. The rates of movement relative to rhamnose (R_F 0·3 assumed) were 0·098, 0·036; 0·109, 0·034; 0·111 and 0·039 respectively in these solvent mixtures. Both fractions, on hydrolysis with N-sulphuric acid, gave D-xylose only; the trisaccharide on hydrolysis with hot 0·1N-sulphuric acid gave both D-xylose and xylobiose, which were detected chromatographically. The sugars produced on hydrolysis of the oligosaccharides were separated on the chromatogram with the butanol–pyridine–water as solvent, thus avoiding the necessity of neutralising the hydrolysis mixture before the separation.

Separation of the Acids in (B).—A sample of the sugar acids was separated on filter paper with ethyl acetate–acetic acid–water (9 : 2 : 2) as solvent. At least eight components were detected on the chromatogram, by using the *p*-anisidine hydrochloride spray. When butanol–pyridine–water was used as solvent, the uronic acids stayed on the starting line and the neutral sugar, xylose, present in small amount, was the only component which moved.

Attempted separation on a charcoal column. The sugar acids (14 g., from several preparations) were dissolved in water (25 c.c.) and poured on a celite–charcoal (Darco G.60) (1 : 1) column previously wetted with water. Water was passed down the column until the effluent was neutral. Concentration of the effluent yielded a syrup, *B*I (9·1 g.), which contained four components. When water containing 5% ethanol was substituted as eluant, an acidic fraction

*B*II (1·6 g.), containing a uronic acid derivative which moved at the rate of glucose was obtained. Elution with 50% acetic acid yielded a mixture of uronic acid derivatives, *B*III (0·2 g.), which moved very slowly on the chromatogram. A portion (6·3 g.) of fraction *B*I was fractionated further on a column of cellulose, with equilibrated butanol–formic acid–water (50 : 1 : 5) as eluent. The effluent was collected fractionally (cf. Hough, Jones, and Wadman, *loc. cit.*) and by selection of the appropriate portions, followed by refractionation, five different fractions (*B*I*a*, 1·1 g.; *B*I*b*, 0·3 g.; *B*I*c*, 2·8 g.; *B*I*d*, 1·3 g.; *B*I*e*, 1·08 g.) containing uronic acids were obtained. The first fraction (*B*I*a*) moved at the same rate as rhamnose in the ethyl acetate–acetic acid–formic acid–water solvent (18 : 3 : 1 : 4) and was identified as 4-methyl D-glucuronic acid (see below). The third fraction moved at about the same rate as the acid in fraction *B*II and these two samples were combined, $[\alpha]_D^{20}$ +90° (*c*, 6·96 in water) (Found : OMe, 7·8%; equiv., 284. Calc. for $C_{12}H_{20}O_{11}$: OMe, 9·0%; equiv. 340). The remaining fractions were combined with fraction *B*III and refractionated. On hydrolysis with N-sulphuric acid of a portion of these fractions, D-xylose was liberated, having m. p. 143°, $[\alpha]_D$ +19° (in water), which was separated on sheets of paper with the butanol–pyridine–water as solvent (see above). Identification of the other components of these fractions will form the substance of a later communication.

Identification of 4-methyl D-*glucuronic acid.* The acid (*B*I*a*; 1·1 g.) $[\alpha]_D^{19}$ +83° (*c*, 1·1 in water) (Found : OMe, 11·3. Calc. for $C_7H_{12}O_7$: OMe, 14·6%), was boiled under reflux with methanolic hydrogen chloride (50 c.c.; 1%) for 6 hours. The cooled solution was neutralised with silver carbonate and filtered and the filtrate evaporated to a syrup (1·0 g.) (Found : OMe, 37·1. Calc. for $C_9H_{16}O_7$: OMe, 39·8%).

A portion of the syrup (82 mg.) in water (1 c.c.) showed the following changes in optical rotation on oxidation at 0° with sodium metaperiodate solution (2 c.c.; 0·5M) : +75° (initial); +12° (10 mins.); −11° (16 mins.); −22° (22 mins.); −36° (34 mins.); −46° (55 mins.); −51° (80 mins.); −54° (148 mins.); −56° (constant; 20 hours). No formic acid was produced during the reaction.

A portion of the syrup (0·1 g.), $[\alpha]_D^{19}$ +75° (*c*, 1·1 in water), with alcoholic ammonia yielded a mixture of amides from which the less soluble amide of 4-methyl α-methyl-D-glucuronoside was isolated by recrystallisation from ethanol. This material had m. p. 236°, not depressed on admixture with an authentic specimen supplied by Professor F. Smith of the University of Minnesota (Found : N, 6·3; OMe, 26·0. Calc. for $C_8H_{15}O_6N$: N, 6·3; OMe, 28·1%).

The methyl ester methylglucuronoside (0·5 g.) in dioxan (5 c.c.) was added to a cooled, stirred suspension of lithium aluminium hydride (1 g.) in ether (50 c.c.). After 30 minutes, the mixture was diluted with ice-water and filtered. The filtrate was passed through a column of Amberlite mixed resins IR-120 and IR-400, and the effluent evaporated to a syrup (0·32 g.; n_D^{17} 1·4840) (Found : OMe, 29·0. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). The glucoside was hydrolysed with boiling N-sulphuric acid (25 c.c.) for 5 hours and the cooled solution neutralised with barium hydroxide and filtered. Concentration of the filtrate yielded 4-methyl D-glucose (0·18 g.) as a pale yellow syrup, soluble in acetone and sparingly soluble in ether, $[\alpha]_D^{18}$ +65° (*c*, 1·1 in water) (Found : OMe, 15·7. Calc. for $C_7H_{14}O_6$: OMe, 16·0%). This material gave the same colour reactions and moved at the same rate on the chromatogram as did 4-methyl glucose in a variety of solvents (cf. Hough, Jones, and Magson, *J.*, 1952, 1525). A sample of the syrupy glucose derivative was heated at 70° for 4 hours with phenylhydrazine acetate. On cooling, 4-methyl D-glucosazone separated, m. p. and mixed m. p. 158° (after recrystallisation from benzene).

Oxidation of Aspen Sawdust with Periodate.—Extractive-free aspen sawdust (pentosan content 17%; 2·1 g.) was oxidised with sodium metaperiodate (5·18 g.) in water (80 c.c.) at 2° in the dark by Potter and Hassid's method (*loc. cit.*). The mixture was shaken from time to time and at intervals samples of the clear supernatant solution were withdrawn, ethylene glycol was added to destroy excess of sodium periodate, and the formic acid titrated (methyl red) [Found (c.c. of 0·01N-formic acid produced per 2·1 g. of sawdust) : 18·6 (48 hours); 29·8 (144 hours); 35·7 (216 hours); 36·9 (380 hours)]. This last figure corresponds to the formation of 3 moles of formic acid per *ca.* 17,000 g. of sawdust. If it is assumed that the lignin present (about 16%) yields no formic acid on oxidation with periodate and that the acid is produced solely from the polysaccharides (3 moles per mole of polysaccharide), this figure corresponds to the presence of one end group in approx. every 21,300 g. of the polysaccharide components of sawdust.

Quantitative Hydrolysis of Oxidised Sawdust.—The oxidised sawdust produced in the experiment described above was filtered off, washed well with water, and air-dried at 25° to constant

weight (2·1 g.). The material was hydrolysed by boiling it with N-sulphuric acid (100 c.c.) for 20 hours. To the cooled hydrolysate, ribose (45 mg.) was added and the solution filtered. The residue, which also contained lignin, weighed 0·717 g. (33%). The filtrate was neutralised with Amberlite resin IR-4B and concentrated to a syrup which was separated by using (*a*) butanol–pyridine–water and (*b*) ethyl acetate–acetic acid–water as solvents. Quantitative determination of the sugars by the method of Flood, Hirst, and Jones (*loc. cit.*) gave, with solvent (*a*) ribose, 1·25 mg., xylose, 4·97 mg. and glucose, 1·84 mg., and with solvent (*b*), ribose, 1·35 mg., xylose, 4·81 mg. and glucose, 1·86 mg. These figures correspond to an average recovery of xylose, 14·3% and glucose, 2·8% from the wood (calc. as $C_5H_8O_4$ and $C_6H_{10}O_5$ respectively). Traces of sugars corresponding to arabinose and galactose also appeared to be present.

Oxidation of a Hemicellulose Sample obtained from Aspen Sawdust.—A sample of hemicellulose supplied by Dr. J. Schoettler (1·42 g.; xylan content, 60·3%) which had been isolated from the same aspen sawdust by direct extraction with sodium hydroxide solution (16%) was oxidised with sodium metaperiodate solution (0·25M; 100 c.c.) in darkness at 2° for 96 hours. Much of the material dissolved during the oxidation (cf. Jayme and Sätre, *loc. cit.*). At the end of this time, the metaperiodate uptake (0·95 mole per $C_5H_8O_4$) was almost constant. Ethylene glycol was added to destroy excess of periodate, and the solution was dialysed against distilled water until free from inorganic impurities. The solution and suspension were evaporated to a syrup and hydrolysed with N-sulphuric acid (50 c.c.) for 12 hours at 90°. The reaction mixture was cooled, ribose (33·5 mg.) added, and the solution neutralised with Amberlite resin IR-4B and filtered. The filtrate was concentrated to a dark brown syrup and analysed by the method of Flood, Hirst, and Jones (*loc. cit.*) (Found: ribose, 1·17 mg.; xylose, 0·965 mg.). These figures correspond to a recovery of approx. 1·7% of xylose (calculated as $C_5H_8O_4$). No other sugar could be detected on the chromatogram.

We thank Professor R. L. Whistler for gifts of xylobiose and xylotriose, and Professor Fred Smith for the gift of derivatives of 4-methyl D-glucuronic acid. One of us (J. K. N. J.) is indebted to the Institute of Paper Chemistry, Appleton, Wisconsin, U.S.A., for a Fellowship and for the facilities placed at his disposal. A gift of charcoal, Darco G.60, from The Darco Department, Atlas Powder Company, New York, U.S.A., is gratefully acknowledged.

THE INSTITUTE OF PAPER CHEMISTRY,
APPLETON, WISCONSIN, U.S.A.

[*Received, March 10th*, 1952.]