carried out with isopropyl ether–ethanol–water (16:4.5:1, v./v.) and 20-ml. fractions of eluate collected. Fractions 190 to 272 contained adenine. Fractions 360 to 475 were pooled and evaporated to dryness. The residue was extracted with hot methanol (3 × 100 ml.) and the combined extracts were freed of solvent *in vacuo*. The product (150 mg.) was dissolved in *ca.* 5 ml. of water, treated with a small amount of decolorizing carbon, filtered and concentrated to a volume of 2 ml. When seeded and kept at 5° overnight the solution deposited 42.4 mg. (6.3%) of 2'-deoxyadenosine as colorless crystals melting partially *ca.* 165° and finally at 190–192° (cor.).[15] Admixture with authentic natural nucleoside gave no depression. In water (*c* 0.40) it rotated $[\alpha]^{20}D$ −27.0°. Like the natural nucleoside it showed an absorption minimum at 225 mμ and a maximum at 260 mμ, the A_M at this latter wave length being 15,220.

(15) This behavior on melting is highly characteristic of the substance; see refs. 1 and 4.

Fractions 500 to 630 contained a component with the chromatographic characteristics of 9-(2-deoxy-α-D-ribofuranosyl)-adenine. They were pooled and evaporated to dryness. The residue was dissolved in 40 ml. of hot absolute alcohol and, after filtration, the solution concentrated to a volume of *ca.* 10 ml. After cooling at −5° overnight there was obtained 55.0 mg. (8.1%) of 9-(2-deoxy-α-D-ribofuranosyl)-adenine, melting at 201–205° (cor.). Two recrystallizations from absolute alcohol gave 35.5 mg. of pure nucleoside melting at 208–210° (cor.) and rotating $[\alpha]^{20}D$ +69° in water (*c* 0.32). At 260 mμ it showed an absorption maximum: A_M 16,290. A minimum was shown at 228 mμ. Ness and Fletcher[1] reported for 9-(2-deoxy-α-D-ribofuranosyl)-adenine m.p. 209–211° (cor.), $[\alpha]^{20}D$ +71° and A_M 15,900 at 260 mμ. A mixed melting point of the samples from the two sources was undepressed. No evidence was found for the presence of a 7-(2-deoxy-D-ribofuranosyl)-adenine in any of the fractions of this chromatography.

[CONTRIBUTION FROM THE PULP AND PAPER RESEARCH INSTITUTE OF CANADA AND THE DEPARTMENT OF CHEMISTRY, MCGILL UNIVERSITY, MONTREAL, CANADA]

Isolation and Properties of an *O*-Acetyl-4-*O*-methylglucurono-xyloglycan from the Wood of White Birch (*Betula papyrifera*)[1]

BY T. E. TIMELL

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Extraction with dimethyl sulfoxide of a chlorine holocellulose from the wood of white birch has yielded a water-soluble *O*-acetyl-4-*O*-methylglucurono-xyloglycan containing one α-(1 → 2)-linked 4-*O*-methyl-D-glucuronic acid residue and 3.6 *O*-acetyl groups per 10 xylose residues. Hydrolysis of the methylated and reduced polysaccharide gave D-yxlose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose in a mole ratio of 1:4.8:3.0:15.6:2.7. The hemicellulose consumed 6.6 moles of periodate per average repeating unit, giving D-xylose as the only reducing sugar after reduction and hydrolysis. It is tentatively concluded that the *O*-acetyl groups were located in the xylan framework and probably mostly attached to C_3, and that the carboxyl groups were neither lactonized nor ionized. The number- and weight-average degrees of polymerization of the polysaccharide as determined by osmometry and light scattering were 180 and 470, respectively.

In an earlier investigation[2] it was shown that untreated wood contains few, if any, formyl ester groups, and that the formyl groups previously found probably had been formed during the analysis by alkaline or acid decomposition of wood polysaccharides. It was concluded that all acyl groups in wood are acetyl groups. Ritter and co-workers[3–6] were the first to show that the acyl groups in wood are associated with the holocellulose portion. This observation was confirmed by later investigators,[7,8] and it was found[9] that the acyl groups are probably attached to the acidic xylans present in both hardwoods and softwoods.

In a previous study[10] it was shown that the polysaccharide obtained on alkaline extraction of wood or holocellulose from white birch contains a linear framework of about 200 (1 → 4)-linked β-D-xylopyranose residues,[11] every tenth of which, on the average, carries a 4-*O*-methyl-D-glucuronic acid residue as a terminal side chain, attached through C_2. In this hemicellulose all acyl groups originally present had, of course, been removed by the alkali. A polysaccharide still containing *O*-acetyl groups has now been isolated from the wood of the same species. This paper is concerned with the isolation and molecular properties of this *O*-acetyl-4-*O*-methylglucurono-xyloglycan and also attempts to establish tentatively the location of its *O*-acetyl groups and the nature of the carboxyl groups.

The extractive-free wood meal contained 5.07% *O*-acetyl groups, identified through two crystalline acetates. Treatment of the wood with chlorine and alcoholic ethanolamine[12] gave a holocellulose which contained almost all of the original *O*-acetyl groups. The holocellulose was exhaustively extracted with dimethyl sulfoxide[9] to give an *O*-acetyl-4-*O*-methylglucurono-xyloglycan containing 9.33% *O*-acetyl groups and representing 50% of the total amount of this polysaccharide present in the wood. *O*-Acetylated sugars, when treated similarly, were recovered unchanged, and variation in experimental conditions did not alter the yield or *O*-acetyl content of the hemicellulose. The partly acetylated polysaccharide was a fluffy powder, easily soluble in water, dimethyl sulfoxide, dimethylformamide, formamide and aqueous alkali.

(1) Paper presented at the Symposium on Wood Hemicelluloses before the Division of Cellulose Chemistry at the 136th Meeting of the American Chemical Society, Atlantic City, N. J., September, 1959.

(2) T. E. Timell, *Svensk Papperstidn.*, **60**, 762 (1957).

(3) G. J. Ritter and E. F. Kurth, *Ind. Eng. Chem.*, **25**, 1250 (1933).

(4) E. F. Kurth and G. J. Ritter, THIS JOURNAL, **56**, 2720 (1934).

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(6) R. L. Mitchell, S. C. Rogers and G. J. Ritter, *Ind. Eng. Chem.*, **40**, 1528 (1948).

(7) F. W. Klingstedt, *Paperi ja Puu*, **19**, 613, 648 (1937); **20**, 104 (1938).

(8) W. Klauditz, *Holzforsch.*, **11**, 47 (1957).

(9) E. Hägglund, B. Lindberg and J. McPherson, *Acta Chem. Scand.*, **10**, 1160 (1956).

(10) C. P. J. Glaudemans and T. E. Timell, THIS JOURNAL, **80**, 941, 1209 (1958).

(11) C. P. J. Glaudemans and T. E. Timell, *Svensk Papperstidn.*, **61**, 1 (1958).

(12) T. E. Timell and E. C. Jahn, *ibid.*, **54**, 831 (1951).

Its uronic anhydride, methoxyl and *O*-acetyl contents suggested the presence of one 4-*O*-methyl-D-glucuronic acid residue and 3.6 *O*-acetyl groups per 10 xylose residues.

When the hemicellulose was subjected to one methylation according to Kuhn and co-workers,[13,14] a product was obtained which contained 90% of the expected *O*-acetyl and methoxyl groups. A second methylation did not increase the methoxyl content and lowered the *O*-acetyl content considerably. The mixture of reducing sugars obtained on methanolysis, reduction[15] and hydrolysis of the methylated hemicellulose recovered after one methylation was resolved[16] on a column of Darco G-60[17] charcoal and Celite[18] by gradient elution[19,20] to yield D-xylose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose in a molar ratio of 1:4.8:3.0:15.6:2.7.

In considering these results, it has to be remembered that the polysaccharide could not be methylated to completion, that some *O*-acetyl groups were lost and that acetyl migration might have occurred during the methylation.[14,21] The following conclusions are therefore only tentative.

The ratio between xylose and glucose residues in the mixture of methylated sugars was 9:1, in approximate agreement with the presence of one 4-*O*-methyl-D-glucuronic acid residue per 10 xylose residues in the original polysaccharide. This, in conjunction with the fact that no other *O*-methylglucoses could be detected, suggested that few of the *O*-acetyl groups were attached to the acid side chains. This conclusion was further corroborated by the fact that on complete oxidation of the *O*-acetylated polysaccharide with periodate, followed by reduction with sodium borohydride and hydrolysis,[22] the only reducing sugar obtained was D-xylose. No traces could be detected of the mono- or biouronic acids to be expected if the side chains had been protected from attack by the periodate by substitution with *O*-acetyl groups at C_2 or C_3.

The 3.0 moles of 3-*O*-methyl-D-xylose corresponded approximately to the 2.7 moles of 2,3,4-tri-*O*-methyl-D-glucose, thus suggesting that few of the *O*-acetyl groups were located at C_2 in the xylose residues. The presence of 4.8 moles of 2-*O*-methyl-D-xylose indicated that the majority of the *O*-acetyl groups were attached to C_3 while the presence of D-xylose, albeit partly attributable to the incomplete methylation, suggested that a minor number of these groups might also be located at both C_2 and C_3 in the same xylose residue. These conclusions were supported by results obtained on oxidation of the polysaccharide with periodate. It can be calculated[23] that if the present polysaccharide were randomly acetylated, approximately 6.9 moles of periodate should be consumed for each repeating unit of ten xylose and one 4-*O*-methyl-D-glucuronic acid residues. The minimum and maximum consumption are 6.4 and 8.2 moles, corresponding to only single or only double substitution at each xylose residue. The actual consumption was 6.6 moles, thus suggesting that only one *O*-acetyl group was attached to each xylose residue.

While the present investigation was in progress, a preliminary report appeared,[14] dealing with an *O*-acetylated 4-*O*-methylglucuronoxylan from a mixture of *Betula verrucosa* and *B. pubescens* wood. The composition of the sugar mixture obtained from the methylated and reduced polysaccharide was similar to that found in the present case and the conclusions drawn by the authors as to the location of the *O*-acetyl groups in this hemicellulose were the same as those arrived at here.

The exact nature of the carboxyl groups present in the *O*-acetyl-4-*O*-methylglucurono-xyloglycans as they occur in the wood is a problem still open to debate. One suggestion[24] involves the presence of an ester linkage between these groups and other wood constituents. The present polysaccharide was salt-free and could thus be expected to contain few ionized carboxyl groups. The correctness of this assumption was borne out by a comparison between the infrared spectra of different xylans (all in the solid state) in the 1500 to 1900 cm.$^{-1}$ region (Fig. 1). A neutral esparto xylan exhibited a slight absorption at 1640 cm.$^{-1}$ due to traces of adsorbed water. The potassium salt of a 4-*O*-methylglucuronoxyloglycan from white birch wood had a characteristic band at 1600 cm.$^{-1}$, indicative of the presence of ionized carboxyl groups. The corresponding free acid of the same polysaccharide exhibited a band at 1725 cm.$^{-1}$ due to C=O stretching of an un-ionized carboxyl group. The pronounced band in the 1725 to 1765 cm.$^{-1}$ region for the *O*-acetylated xylan was interpreted as being due to the presence of *O*-acetyl groups, together with either un-ionized or lactonized carboxyl groups.

When an attempt was made to reduce the *O*-acetyl-4-*O*-methylglucurono-xyloglycan with sodium borohydride in an aqueous solution containing borate buffer,[25,26] almost all the acid groups were recovered after hydrolysis as 4-*O*-methyl-D-glucuronic acid or 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose. Only trace amounts of 4-*O*-methyl-D-glucose could be detected in the neutral portion of the hydrolyzate. D-Glucurono-(6 → 3)-lactone, under identical conditions, gave D-glucitol. It thus appears probable that hardly any of the carboxyl groups in the polysaccharide were lactonized. This conclusion was further corroborated by the rate at which the polysaccharide released iodine from an aqueous iodide–

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(14) H. O. Bouveng, P. J. Garegg and B. Lindberg, *Chemistry & Industry*, 1727 (1958).

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(18) A product of Johns–Manville Co., New York, N. Y.

(19) R. S. Alm, *Acta Chem. Scand.*, **6**, 1186 (1952).

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(24) L. G. Neubauer and C. B. Purves, *Can. J. Chem.*, **35**, 388 (1957).

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(26) R. H. Marchessault and B. G. Rånby, *Svensk Papperstidn.*, **62**, 230 (1959).

iodate solution. In this respect, the hemicellulose behaved more like D-galacturonic acid than like D-glucurono-(6 → 3)-lactone.

The presence of unlactonized carboxyl groups in the polysaccharide was also evident from the viscosity behavior of the latter in various solvents. While linear relationships between concentration and reduced viscosities were observed in cupriethylenediamine and dimethyl sulfoxide, an upward curvature with decreasing concentration was noticeable in water, a phenomenon which became less pronounced in aqueous sodium chloride. This is a behavior typical of polyelectrolytes.[27] The intrinsic viscosities in cupriethylenediamine and dimethyl sulfoxide were 0.87 and 0.98 dl./g., respectively, compared to 0.89 and 0.68 dl./g. after removal of the acetyl groups with alkali. The higher intrinsic viscosity in dimethyl sulfoxide of the *O*-acetylated polysaccharide was probably due to the tendency of the O-acetyl groups to become solvated in this solvent, thus causing an increase in the effective hydrodynamic volume of the polymer. The intrinsic viscosity in water was approximately 1.20 dl./g., compared to only 0.90 dl./g. in aqueous sodium chloride, the sodium ions presumably causing the macromolecules to assume a more coiled configuration and thus a smaller volume.

A portion of the *O*-acetyl-4-*O*-methylglucuronoxyloglycan was converted to the fully substituted acetate derivative.[11,28] Osmotic pressure measurements[11] suggested a number-average degree of polymerization ($\bar{P}_n$) of 180. Light-scattering measurements[29] gave a weight-average molecular weight of 78,200, corresponding to a degree of polymerization ($\bar{P}_w$) of 470. The ratio between $\bar{P}_w$ and $\bar{P}_n$ indicated that the polysaccharide was polymolecular.[11] The values obtained were somewhat lower than those observed when alkali was used to extract a larger portion (76%) of the 4-*O*-methylglucuronoxyloglycan from the same wood, 215 and 500, respectively.[29] The difference was probably due to the fact that shorter molecules were preferentially removed on extraction of the wood with dimethyl sulfoxide.

Experimental

All specific rotations are equilibrium values and were determined with water as the solvent and at 20° unless otherwise specified. Melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°.

Paper Chromatography.—Solvents (v./v.) used for separating sugars were (A) ethyl acetate–acetic acid–water (9:2:2), (B) butan-1-ol–pyridine–water (10:3:3), (C) butan-1-ol–ethanol–water (40:11:19) and (D) butanone–water (89:11). Separations were carried out on Whatman No. 1 filter paper by the descending technique. *o*-Aminobiphenyl was used as a spray reagent for reducing sugars[30] and periodate–permanganate[31] for sugar alcohols. Paper electrophoresis was carried out with Whatman No. 3 MM filter paper and with 0.05 *M* borate buffer.[32]

Isolation of the Hemicellulose.—Extractive-free wood meal (40–60 mesh) was prepared from a sound, 60-year-old specimen of white birch, cut in 1958.[33]

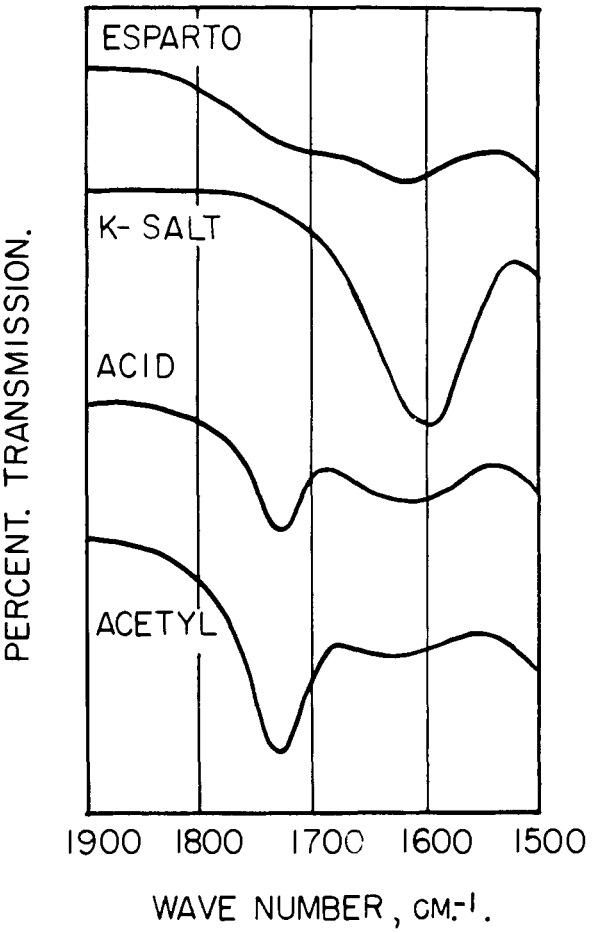

Fig. 1.—Infrared spectra of four xyloglycans in the 1500–1900 cm.$^{-1}$ region.

Anal. α-Cellulose,[34] 46.9; pentosan,[34] 28.7; lignin,[34] 20.6; acetyl,[2,35] 5.07; uronic anhydride,[36] 4.2; estd. content of *O*-acetyl-4-*O*-methylglucurono-xyloglycan, 33%.

Wood meal (185 g.) was subjected to three treatments with chlorine in ice-water for 5 minutes,[12] followed by exhaustive extraction of the chlorinated lignin with alcoholic ethanolamine (3%, v./v.). After the final treatment the product was washed with water, ethanol and petroleum ether (30–60° b.p.), and dried *in vacuo* to yield a holocellulose (157 g., 85%).

Anal. *O*-acetyl, 5.65; Klason lignin, 1.90; ash, nil.

The process was repeated five times to yield 920 g. of holocellulose.

Holocellulose (900 g.) was treated with technical dimethyl sulfoxide (6 l.) at room temperature on a shaker for 3 days. The extract was removed by filtration on sintered glass and the solid residue was washed with dimethyl sulfoxide (4 l.). The brown-colored extract and washings were poured into ethanol (25 l.) to give a colloidal solution (*p*H 7) from which a precipitate soon formed. After standing overnight, the precipitated hemicellulose could be removed as a semi-solid cake, which was broken up, washed on filter paper with ethanol and petroleum ether, and dried *in vacuo* at room temperature over potassium hydroxide for 3 days to give a powder (175 g., 50% of the xylan content of the wood). On hydrolysis the polysaccharide yielded xylose in addition to minor quantities of an aldobiouronic acid and 4-*O*-methylglucuronic acid.

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(28) J. F. Carson and W. D. Maclay, THIS JOURNAL, **68**, 1015 (1946).

(29) D. A. I. Goring and T. E. Timell, unpublished results.

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(34) "Testing Methods of the Technical Association of the Pulp and Paper Industry," New York, N. Y.

(35) R. L. Whistler and A. Jeanes, *Ind. Eng. Chem., Anal. Ed.*, **15**, 317 (1943).

(36) B. L. Browning, *Tappi*, **37**, 336 (1954).

Anal. acetyl, 9.33; methoxyl, 2.09; uronic anhydride, 9.8; ash, nil., $[\alpha]_D - 86°$ (*c* 1.3 in water).

The unextracted portion was stirred with cold water for several days and filtered. Evaporation of the filtrate gave a brown, brittle product (2.9 g.). The residual material was extracted with hot water (80–90°) to give a black material (14 g.). A second extraction with dimethyl sulfoxide of the remaining holocellulose (675 g.) did not remove any further hemicellulose. Similar yields were obtained when anhydrous or 90% aqueous dimethyl sulfoxide was used.

Identification of O-Acetyl Groups.—Wood meal (5 g.) was heated to boiling with 0.1 *N* sodium methoxide in methanol[2,35] (100 ml.). Distillation was allowed to take place immediately and the methyl acetate collected was identified through the *p*-nitrobenzyl acetate, m.p. and mixed m.p. 78°, and the *p*-bromophenacyl acetate, m.p. and mixed m.p. 84.5–85°.

Acetylation of the Hemicellulose.—The hemicellulose (2.0 g.) was dissolved in dry formamide[28] (40 ml.) and treated with pyridine (80 ml.) and acetic anhydride (60 ml.) at room temperature for 2 days. The acetylated product was isolated as described previously[11] to yield a grayish powder (2.6 g.)

Anal. Calcd. for the fully acetylated hemicellulose: acetyl, 37.8. Found: acetyl, 37.9.

Methylation of the Hemicellulose.—The hemicellulose (20 g.) was dissolved in dry dimethylformamide (1 l.). Freshly prepared silver oxide (50 g.) and methyl iodide (50 ml.) were added and the reaction mixture was shaken at room temperature for 24 hr.[13] Silver oxide (50 g.) and methyl iodide (50 ml.) were again added. After an additional 24 hr., the polysaccharide was extracted with chloroform (3 l.) on the centrifuge and the extract was washed with 5% aqueous potassium cyanide (1 l.), followed by three washings with water (1 l. each). The chloroform solution was dried over anhydrous sodium sulfate, filtered and evaporated to a volume of 300 ml. The solution was poured with stirring into petroleum ether (3 l.) to yield a precipitate which was recovered by filtration, washed with petroleum ether and dried *in vacuo* at room temperature to give a white, fluffy powder (15 g.). The infrared diagram of the product indicated the presence of hydroxyl and *O*-acetyl groups. A second methylation of another preparation gave a product containing 4.2% acetyl and 27.6% methoxyl.

Anal. Calcd. for the fully methylated polysaccharide: acetyl, 8.1; OMe, 29.9. Found: acetyl, 7.2; OMe, 28.1.

Methanolysis, Reduction and Hydrolysis of the Methylated Hemicellulose.—The methylated hemicellulose (14.0 g.) was boiled under reflux with 2% methanolic hydrogen chloride (300 ml.) for 10 hr. After removal of the acid with silver carbonate, filtration through Celite and evaporation to dryness, the sirup obtained (14.5 g.) was dissolved in anhydrous ether (300 ml.) and reduced with lithium aluminum hydride[15] (3 g.). The recovered product was boiled under reflux with *N* sulfuric acid (300 ml.) for 8 hr. Neutralization (barium carbonate), filtration through Celite, treatment with Amberlite IR-120 exchange resin,[37] filtration and evaporation to dryness yielded a pale, yellow sirup (13.0 g.). Examination by paper chromatography (solvents C and D) suggested the presence of three partly methylated xyloses and a tri-*O*-methylglucose.

Resolution of the Hydrolyzate.—A portion of the hydrolyzate (800 mg.) was added to the top of a column (3.5 × 55 cm.) containing a 1:1 (w./w.) mixture of Darco G-60 charcoal[17] and Celite.[18] Gradient elution[19,20] was effected with 3 liters each of the following solvents: water→8% aqueous ethanol, 8% ethanol→20% ethanol, 20% ethanol→40% ethanol. Fractions, 25-ml. each, were collected with an automatic collector and every third fraction was examined by paper chromatography (solvents C and D). The following main fractions were obtained in the order of their appearance in the eluate: xylose, 3-*O*-methylxylose, a mixture of 3-*O*- and 2-*O*-methylxylose, 2-*O*-methylxylose, 2,3-di-*O*-methylxylose and 2,3,4-tri-*O*-methylglucose. The mixture of mono-*O*-methylxyloses was resolved by paper electrophoresis.

Identification of the Methylated Sugars.—All sugars were chromatographically or electrophoretically identical to authentic specimens.

(37) A product of Rohm and Haas Co., Philadelphia, Pa.

D-Xylose.—The crystalline sugar (20 mg.) had m.p. and mixed m.p. 144–145°, $[\alpha]_D + 18°$.

3-*O*-Methyl-D-xylose.—The sirupy sugar (65 mg.), $[\alpha]_D + 17°$,[38] was converted to a sirupy aniline derivative, the infrared spectrum of which was identical with that of an authentic sample. The rate of movement of the sugar on the paper electrophoretogram was identical to that of an authentic specimen.

2-*O*-Methyl-D-xylose.—This fraction (107 mg.) crystallized spontaneously, m.p. and mixed m.p. 133–134°, $[\alpha]_D + 35°$.[39]

Anal. Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 18.4.

2,3-Di-*O*-methyl-D-xylose.—The crystalline sugar (370 mg.) had m.p. 94–96°,[40,41] $[\alpha]_D + 32°$. The 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine had m.p. and mixed m.p. 125–127°.[42] Its infrared spectrum was identical with that of an authentic specimen.

2,3,4-Tri-*O*-methyl-D-glucose.—The sirupy sugar (80 mg.), $[\alpha]_D + 70°$, was converted to its aniline derivative, m.p. and mixed m.p. 144°.[43]

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 42.4.

Consumption of Periodate.—Samples of hemicellulose (150–200 mg.) were dissolved in 0.05 *M* sodium periodate (50 ml.) and the reaction was allowed to proceed at room temperature in the dark for various lengths of time. The consumption of periodate was determined by the excess arsenite method.[44] The following moles of periodate (average of duplicate or triplicate determinations) were consumed per repeating unit of the polysaccharide (hr.): 20 (6.6), 30 (7.1), 50 (7.5), 55 (7.6), 72 (7.9), 96 (8.3), 100 (8.5), 120 (8.7), 135 (9.1), 165 (9.4), 210 (10.3). Extrapolation of the almost linear conversion-time curve to zero time gave a value of 6.6 glycol groups per repeating unit.

Periodate Oxidation, Reduction and Hydrolysis of the Hemicellulose.—The hemicellulose (1 g.) was oxidized for 4 weeks in the dark at +4° with 0.4 *M* aqueous sodium periodate (50 ml.).[22,45,46] Iodate was removed with barium acetate and the solution was concentrated to 100 ml. Sodium borohydride (1 g.) was added to a portion of the solution (50 ml.) and the reduction was allowed to proceed with stirring overnight. After dialysis against distilled water for one day, the remaining solution was freeze-dried to give a brittle powder (0.4 g.). A portion of the material was hydrolyzed to yield a mixture of sugars which was resolved by paper chromatography (solvents A and B) into two nonreducing and one reducing compound. The latter crystallized and proved to be D-xylose, m.p. and mixed m.p. 145–146°, $[\alpha]_D + 18°$. No trace of any aldobiouronic acid or 4-*O*-methylglucuronic acid could be detected. The remaining portion of the solution with the oxidized polysaccharide was hydrolyzed with *N* sulfuric acid to give a mixture of sugars (0.4 g.) containing xylose but no traces of any uronic acids.

Attempted Reduction of the Hemicellulose.—*O*-Acetyl-4-*O*-methylglucurono-xyloglycan (5.0 g.) was dissolved in water (100 ml.) containing sodium borate (5 g.), and sodium borohydride (5 g.) was added in small portions over a period of 2 hr. After another hour, sodium borohydride (5 g.) was added in the same way and this treatment was repeated twice more. The solution was allowed to stand overnight. Excess borohydride was destroyed with glacial acetic acid, and the solution was poured into a mixture (1:1 v./v.) of ethanol and acetone (500 ml.). The precipitate was collected on a Büchner funnel, washed successively with 70% aqueous ethanol, 100% ethanol and petroleum ether,

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(39) G. J. Robertson and T. H. Speedie, *J. Chem. Soc.*, 824 (1934).

(40) J. K. Hamilton, E. V. Partlow and N. S. Thompson, *Tappi*, **41**, 811 (1958).

(41) A. L. Currie and T. E. Timell, *Can. J. Chem.*, **37**, 922 (1959).

(42) I. Ehrenthal, M. C. Rafique and F. Smith, THIS JOURNAL, **74**, 1341 (1952).

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(44) P. F. Fleury and J. Lange, *J. pharm. chim.*, [8] **17**, 107, 196 (1933).

(45) J. K. Hamilton and F. Smith, THIS JOURNAL, **78**, 5907, 5910 (1956).

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and finally dried *in vacuo* to give a white powder (5.0 g.). A 4-*O*-methylglucurono-xyloglycan (potassium salt, 5.0 g.), when treated similarly, yielded a fluffy product (5.0 g.).

Both hemicelluloses were hydrolyzed to give a mixture of reducing sugars, each of which was resolved into a neutral and an acidic portion by passage through a column of Dowex 1-X4 exchange resin (acetate form).[47] The acidic fractions of the hemicelluloses (0.5 and 0.7 g., respectively) contained the expected aldobiouronic acid and 4-*O*-methylglucuronic acid as indicated by paper chromatography (solvent A). The neutral fraction from the 4-*O*-methylglucurono-xyloglycan contained only D-xylose, m.p. and mixed m.p. 144–145°, $[\alpha]_D$ +18°. The neutral fraction from the *O*-acetylated hemicellulose (4.0 g.) was added to the top of a Darco G-60 charcoal–Celite column (3.5 × 55 cm.). Xylose was removed by washing with water. Gradient elution with 3 l. each of water and 20% aqueous ethanol yielded only 4-*O*-methylglucose (20 mg.) which was identified chromatographically (solvents A, B, C and D).

Reduction of D-Glucurono-(6→3)-lactone.—D-Glucurono-(6→3)-lactone, (1 g.) when reduced with sodium borohydride (5.0 g.) in an aqueous borate buffer yielded D-glucitol, identified through its hexaacetate, m.p. 98.5–99°, $[\alpha]_D$ +10° (*c* 3.0 in chloroform).[48]

Infrared Analysis.—The esparto xylan[49] was isolated as described previously[50] from *Stipa tenacissima*. The birch wood xylan was obtained by extracting wood from *Betula papyrifera* with 24% (w./w.) potassium hydroxide.[51] The potassium salt of the hemicellulose was obtained by using acetic acid for neutralizing the alkali. Neutralization of another portion to *p*H 2.5 with dilute hydrochloric acid gave the free acid form of the same polysaccharide. All samples used were dried over phosphorus pentoxide at 100° *in vacuo* for 4 days. Infrared spectra were obtained with a Perkin–Elmer model 21 spectrophotometer. The potassium bromide pellet technique was used. No bands for water (3500 and 1640 cm.$^{-1}$) were observed with potassium bromide alone.

Treatment with Iodide–Iodate.—D-Glucurono-(6→3)-lactone (176 mg.), D-galacturonic acid (194 mg.) and *O*-acetyl-4-*O*-methylglucurono-xyloglycan (1660 mg.) were dissolved in water (50 ml.). The solutions were diluted to 100 ml. with a solution containing 60 g. of potassium iodide and 12 g. of potassium iodate per liter. Aliquots (10 ml.) were withdrawn after various intervals of time and the iodine formed was determined by titration with 0.01 *N* sodium thiosulfate. Iodine released in percentage of theory was for the three compounds: 2.1, 82, 33 (5 min.); 2.3, 83, 35 (75 min.); 70, 96, 84 (3 days); and 90, 103, 96 (5 days).

Viscosity Measurements.—Viscosity measurements were carried out with a Craig–Henderson viscometer[52] at 30° in *M*-cupriethylenediamine, redistilled dimethyl sulfoxide containing 2% (v./v.) water, *M* sodium chloride in water and water. Reduced viscosities were determined at 6–7 different concentrations (0.1–1.2 g./dl.) and were extrapolated to zero concentration according to Huggins,[53] thus yielding the intrinsic viscosity.

Osmotic Pressure Measurements.—The osmometers used were of the Zimm–Myerson type,[54] later improved by Stabin and Immergut.[55] Gel cellophane membranes which had never been allowed to dry were used,[56] the temperature was 30° and the osmotic height was measured by the static method. The solvent was a mixture of chloroform and ethanol (9:1, v./v.). The following values were obtained for h/w (h = osmotic height in cm. solution and (w = concentration in g./kg. solution)) at different values of w: 0.696 (3.680) 0.676 (3.107), 0.676 (2.425), 0.681 (1.872), 0.641 (1.470), 0.630 (0.800). Extrapolation to zero concentration gave a value of 0.600 for $(h/w)_{w=0}$, corresponding to a number-average molecular weight of 42,800 and a $\bar{P}_n$ value of 180.

Light-Scattering Measurements.—Light-scattering intensities were measured with a Brice-Phoenix photometer as modified by Huque, Goring and Mason.[57] The hemicellulose was dissolved in freshly redistilled dimethyl sulfoxide containing 2% (v./v.) of water and the solution was clarified by ultracentrifugation,[58] first at 140,000 g. and subsequently at 35,000 g., the latter in light-scattering cells specifically designed for elimination of micellar debris.[58,59] The wave length of the light was 5460 Å. The solution was strongly fluorescent and a filter had to be used to obviate this difficulty. Correction was applied for dissymmetries which averaged 1.08. The following values were obtained for $C/I_{90°}$ at the respective concentrations C (mg./ml.): 2.212 (1.88), 2.298 (3.54), 2.566 (5.44), 2.667 (7.44), 2.768 (8.86). Extrapolation to zero concentration gave a value of 2.08 for $(C/I_{90°})_{c=0}$. The refractive index increment was determined with a Brice–Phoenix differential refractometer at a wave length of 4358 Å. The average value used was 0.0641 ml./g. The molecular weight (78,200) was obtained from the relationship $1/\bar{M}_w = H(C/\tau)_{c=0}$, where the symbols have their usual significance.

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