Investigation of Product VII.—The chromatographic properties of product VII were compared with those of product VIII, following the procedure of Wolfrom and Arsenault.[21] With the exception of the absence of the equivalent of zones 1 and 4 in the chromatogram of product VII, the chromatographic properties of the two products were the same.

An amount (1 g.) of product VII was recrystallized twice from nitrobenzene to afford 222 mg. of glyoxal bis-(2,4-dinitrophenylhydrazone), m.p. 329–332° dec. undepressed on admixture with authentic material. The recrystallized material was further shown to be glyoxal bis-(2,4-dinitrophenylhydrazone) by infrared spectrum. The mother liquor from the first recrystallization was black, indicating that a large amount of tars was present in product VII.

Gas-Liquid Partition Chromatography of the Condensate.—The columns employed were packed with 40% polyethyleneglycol-400 on 30–60 mesh Firebrick C-22. The smaller column (5 mm. diam. by 1.2 m.) was operated at 30° using a helium flow of 50 ml. per min. while the larger column (13 mm. diam. by 4.9 m.) was operated at 80° with a nitrogen flow of 450 ml. per min. Both columns gave similar results. A typical chromatogram obtained on the small column is shown by Fig. 2; the larger column gave better resolution of the minor components. Freshly prepared red oil[4] found in the first spiral trap (Fig. 1[4]) was injected into the column with a hypodermic syringe inserted through a heated inlet. Considerable carbonization of the oil was noted.

As the components emerged from the columns, they were collected in cold traps containing carbon disulfide. Acetaldehyde, ethyl acetate, acrolein and hyrogen cyanide were each identified by elution times and infrared absorption spectra in comparison with known samples. Acetone was identified only by comparison of elution times. Although the carrier gas flow was continued for 8 times the elution time of the last zone (Fig. 2), no further zones appeared.

Comparison of the areas of the zones ascribed to acetaldehyde, ethyl acetate and acrolein to the areas produced by known amounts of these substances indicated that 0.02, 0.01 and less than 0.001 mmole, respectively, had been obtained per mmole[7] of cellulose nitrate ignited.

The well-defined zone (unknown, Fig. 2) produced a strong single peak at 10.5 μ in the infrared absorption spectrum. The infrared spectrum of the hydrogen cyanide zone contained an absorption maximum at 5.8 μ which has not been explained.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

The Constitution of a Glucomannan from White Spruce (Picea glauca)

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A glucomannan has been isolated from the wood of white spruce (*Picea glauca* (Moench) Voss) with its mannose component representing 82% of the mannose residues present in this species. The electrophoretically homogeneous polysaccharide contained only mannose and glucose residues in a ratio of 3:1. Partial acid hydrolysis yielded crystalline 4-*O*-β-D-mannopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose, 4-*O*-β-D-glucopyranosyl-D-mannose, *O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose and *O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose. Hydrolysis of the fully methylated polysaccharide gave 2,3,6-tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-glucose, in addition to traces of tetra-*O*-methylhexoses. The number-average degree of polymerization of the nitrated glucomannan was 107 and its weight-average value was 182. It is suggested that the glucomannan present in white spruce contains, on the average, slightly more than 100 D-mannose and D-glucose residues, linked together by (1 → 4)-β-glycosidic bonds. The composition of the partial hydrolyzate indicates the presence of very few contiguous glucose residues. The degree of branching of the polysaccharide has yet to be determined.

In the last few years, hemicelluloses composed of glucose and mannose residues have been isolated from several species of coniferous woods. These glucomannans have been shown to contain most, if not all, of the mannose residues occurring in these woods. The present study is concerned with the isolation and constitution of a glucomannan from the wood of white spruce, a species unusually rich in mannose residues.[1,2] The structures of a water-soluble arabogalactan[3] and two acidic oligosaccharides[4] isolated from the same wood have recently been reported.

Holocellulose, prepared from the extractive-free wood by the chlorite method,[5] was exhaustively extracted with 17.5% sodium hydroxide[6] containing 4% boric acid.[7] Fehling solution[8] was added to the extract and the precipitated copper complex was thoroughly washed with water and subsequently decomposed with acid. Addition of alcohol gave a crude polysaccharide in a yield of 12.7% of the original wood. After two more precipitations as the copper complex, the final product corresponded to 6.7% of the wood. The sugar composition of the polysaccharides[9] is presented in Table I. The crude polysaccharides apparently still contained minor quantities of galactose, arabinose and xylose residues in addition to small amounts of uronic acids which were not determined. In the final product all these sugars had disappeared except for a faint trace of xylose. The ratio of mannose to glucose remained essentially unchanged throughout and approximated 3:1, thus indicating the homogeneous nature of the product. When subjected to electrophoresis in a borate buffer,[10] the polysaccharide traveled as one compact spot on the glass paper, thus corroborating the above

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evidence. The specific rotation of the pure polysaccharide, $-34°$ in alkali, and the intrinsic viscosity in cupriethylenediamine, 0.24 dl./g., were in close agreement with the corresponding values reported for a similar glucomannan from western hemlock.[11]

TABLE I

PERCENTAGE COMPOSITION OF GLUCOMANNANS

Component sugar	A[a]	B[a]	C[b]
Galactose	1.1	1.0	Nil
Glucose	22.8	21.6	25.0
Mannose	72.6	73.7	75.0
Arabinose	1.7	Nil	Nil
Xylose	1.8	3.7	Trace
Mannose/glucose	3.2	3.4	3.0

[a] Crude products from duplicate experiments. [b] Final product.

The wood contained 11.6% mannose and of this 82% was present in the crude glucomannan whereas the purified glucomannan contained 43% of all the combined mannose in the wood. Several conifers have been reported to contain galactoglucomannans in addition to glucomannans.[12-15]

Fractional extractions of the chlorite holocellulose with alkali failed to produce any evidence of the presence of a galactoglucomannan in white spruce. Since true mannans have never been isolated from any wood species, it appears probable that most, if not all, of the mannose residues in the present species occurred as the major component of a glucomannan.

Partial hydrolysis of the glucomannan with aqueous formic acid yielded a sugar mixture which was resolved on a charcoal column by elution with aqueous ethanol, the alcohol concentration of which was increased stepwise. Nine chromatographically pure oligosaccharides were obtained, five of which crystallized and were fully identified. These included three disaccharides, namely, 4-*O*-β-D-mannopyranosyl-D-mannose (mannobiose), 4-*O*-β-D-mannopyranosyl-D-glucose and 4-*O*-β-D-glucopyranosyl-D-mannose, one mannotriose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannose, and a mannotetraose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannose. The first three compounds have previously been isolated from several coniferous woods,[13,14,16-20] and the mannotriose has been obtained crystalline from loblolly pine wood[13] and was probably present also in glucomannan hydrolyzates from western hemlock,[16] red cedar[14] and spruce sulfite pulp.[18] The mannotetraose appears to have been isolated previously only from vegetable ivory.[21]

The remaining four oligosaccharides could not be fully identified. One of them was chromatographically identical with cellobiose and gave only glucose on hydrolysis. A presumed trisaccharide contained glucose and mannose in a ratio of 1:2 and yielded glucosylmannose and mannosylglucose on partial hydrolysis, thus suggesting the presence of an *O*-mannosyl-*O*-glucosylmannose. The paucity of material prevented further characterization. A similar oligosaccharide has previously been obtained from another spruce glucomannan.[18]

Hydrolysis of the fully methylated polysaccharide yielded a mixture of methylated hexoses which was resolved on a charcoal column by elution with aqueous ethanol. A di-*O*-methylhexose, constituting 0.5% of the total hydrolyzate, might be indicative of a branched structure but could as well have originated from incomplete methylation or demethylation during hydrolysis. Elution with 2% ethanol gave pure, sirupy 2,3,6-tri-*O*-methyl-D-mannose, identified through its crystalline di-*p*-nitrobenzoate. Further elution with 5–7% ethanol yielded crystalline 2,3,6-tri-*O*-methyl-D-glucose. Prolonged elution with more concentrated alcohol solutions gave only small quantities (0.1%) of a tetra-*O*-methylhexose (or hexoses).

A portion of the wood meal was partially delignified by a minimum of chlorite treatments in an attempt to avoid at least some of the concomitant depolymerization.[22-24] The glucomannan portion of the holocellulose was converted to its nitrate derivative without any apparent degradation.[25] Osmotic pressure measurements indicated a number-average molecular weight of 30,500, corresponding to a degree of polymerization of 107. The corresponding weight-average values were 51,700 and 182, respectively, as determined by light scattering.

From the above evidence a tentative structure can now be suggested for the glucomannan present in the wood of white spruce. The isolation of 2,3,6-tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-glucose from the methylated hemicellulose shows that the polysaccharide is composed of D-mannose and D-glucose residues linked together by (1→4)-glycosidic bonds. This conclusion is further corroborated by the consumption of 0.97 mole oxidant per hexose residue on periodate oxidation of the glucomannan. The negative rotation of the latter indicates that the hexose residues are present in the β-configuration. The isolation of the oligosaccharides referred to above corroborates these conclusions. The nature of these oligosaccharides, and notably the presence of the mannobiose, mannotriose and mannotetraose in conjunction with the paucity of any cellobiose or higher glucose oligosaccharides, clearly suggests that few of the glucose residues in the glucomannan are contiguous. Low yields of cellobiose have been reported on several occasions on partial hydrolysis of softwood glucomannans.[12,16,18]

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Whether the glucomannan is linear or branched cannot be decided on the basis of the present evidence. The relatively low intrinsic viscosity of the glucomannan, as compared to its weight-average degree of polymerization, would tend to make a somewhat branched structure appear rather plausible.

The ratio of 3:1 between mannose and glucose residues found here is of the same order of magnitude as those reported for other softwood glucomannans, such as red cedar (2.5),[14] western hemlock (3),[11,13] loblolly pine (2.7),[16] Scots pine (3.1 to 3.7),[26] white pine (3),[27] Norway spruce (3.5 to 4)[28,29] and Sitka spruce (2.5).[17] Similarly, the number-average degree of polymerization (107) is within the same range as the $\bar{P}_n$ values reported for glucomannans from western hemlock (130),[12] Scots pine (70–115)[26] and Norway spruce (68–100).[29] Whether these values represent the molecular weights of the native polysaccharides is, however, extremely doubtful in view of the depolymerizing effect of the acid chlorite used for delignification of the wood in these cases.

The ratio between the weight- and number-average molecular weights is 1.70, a value not very far from that expected for a polymer with a Flory distribution ($\bar{M}_w/\bar{M}_n = 2$). The polysaccharide is accordingly quite polymolecular. This ratio does not agree with those recently reported for a glucomannan from Norway spruce.[28] In this case, however, the weight-average values were derived from viscosity measurements.

The relative ease with which the present, as well as other spruce glucomannans,[28,29] can be isolated and purified is in pronounced contrast to the difficulties encountered in isolating glucomannans from various species of pine.[16,26,27] In the case of white pine,[27] for example, the copper complex method is far less useful and precipitation with barium hydroxide[30] has to be carried out repeatedly for a complete removal of the acidic xylan. Elimination of galactose residues requires additional delignification at this stage,[26] followed by further purifications *via* the copper complex. This difference between the *Picea* and the *Pinus* genera might be due partly to the absence of any larger quantities of galactoglucomannans in the former, but it might also be caused by morphological differences between the two genera.

Experimental

All specific rotations are equilibrium values and were determined at 20°. Melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°.

Paper Chromatography.—Chromatographic separation of sugars was carried out on Whatman No. 1 or (for preparative purposes) No. 3MM filter papers by the descending technique. The solvents (v./v.) used were (X) ethyl acetate–acetic acid–water (9:2:2), (Y) butan-1-ol–pyridine–water (10:3:3) and (Z) butanone–water (89:11). *o*-Aminobiphenyl[9] was used as a spray reagent.

Electrophoretic separations were carried out on glass-fiber paper in a borate buffer at 750 volts for 3–5 hr.[10] α-Naphthol (1 g.) in butan-1-ol (100 ml.) containing concentrated sulfuric acid (5 ml.) was used for detecting polysaccharides.

White Spruce Wood.—A 40-year old, healthy specimen of white spruce[31] was converted to wood meal. The 40–60 mesh fraction was exhaustively extracted with ethanol–benzene (1:2, v./v.) and with cold water. Analysis[1,2] showed that the wood contained α-cellulose (44.8), pentosan (9.8), lignin (27.1), acetyl (1.3), ash (0.3), uronic anhydride (3.6), galactose (1.2), glucose (45.6), mannose (11.6), arabinose (1.6) and xylose (6.8%).

Isolation of the Glucomannan.—Holocellulose was prepared in 78.6% yield by seven treatments with acid chlorite[5]: pentosan content: calcd. 12.5, found 11.8%.

Holocellulose (89.7 g.) was extracted in a nitrogen atmosphere at +30° with 1.5 liters of a solution containing (w./w.) 17.5% sodium hydroxide and 4% boric acid.[7] The mixture was cooled to +5° and the undissolved material (69%) was removed by filtration and washed with water. Fehling solution[8] was added to the combined filtrates and the blue precipitate formed was stirred with distilled water which was removed by centrifuging. This treatment was repeated twice and the combined washings were added to ethanol, when a second precipitate was formed. The main portion was suspended in ice-water and the copper complex was destroyed by addition of aqueous *N* hydrochloric acid. After removal of a small amount of insoluble material by centrifuging, the clear solution was added to ethanol when a precipitate was formed (12.7 g., 11.2% of the original wood). The second, minor precipitate of the copper complex was treated similarly to yield 1.73 g. (1.5%) of material.

The crude glucomannan was dissolved in the same alkaline solution, Fehling solution was added as before and the precipitate was washed seven times with distilled water. The copper complex was decomposed and the glucomannan was precipitated by addition of ethanol. This series of treatments was repeated once. The product was washed with diethyl ether and dried *in vacuo* at 50°. The pure glucomannan (7.6 g., 6.7% of the wood) was a white powder, easy soluble in 10% alkali.

Acid hydrolysis of the crude glucomannan which showed $[\alpha]_D$ −85° (*c* 1.0 in 17.5% aqueous sodium hydroxide containing 4% borate) and −34° (*c* 1.0 in 17.5% aqueous sodium hydroxide), gave a mixture of sugars containing glucose and mannose as revealed by paper chromatography (solvents X and Y). Mannose was identified as its phenylosazone, m.p. and mixed m.p. 198°.

Partial Hydrolysis of the Glucomannan.—The glucomannan (12 g.) was dissolved in 90% formic acid (150 ml.) and the solution was diluted with water to 300 ml. and subsequently heated at 96–98° for 4 hr.[16] After cooling, the insoluble portion was discarded. Most of the formic acid was removed by repeated evaporations from water and methanol. The portion insoluble in 80% aqueous methanol was dissolved in formic acid and again subjected to partial hydrolysis as above. This treatment was repeated five times to give a mixture of partially hydrolyzed sugars (9.5 g.)

Resolution of the Hydrolyzate.—The hydrolyzate (9.5 g.) was added to the top of a column (4.5 × 46 cm.) containing Nuchar[32] activated charcoal. Elution was effected with aqueous ethanol at a rate of 2.5 liters per day, the concentration of the alcohol being increased in steps of 0.25%. Monosaccharides were removed by eluting with 0.5% aqueous ethanol (25 l.). Elution with 0.5–1.0% alcohol (30 l.) gave a pure oligosaccharide (A) which was also obtained in admixture with another oligosaccharide (B) on further elution with 1.75% ethanol (50 l.). This mixture was resolved by paper chromatography (solvent Y). A third oligosaccharide (C) was removed with 2.0% alcohol (60 l.) and the same compound was obtained together with another oligosaccharide (D) on elution with 2.25% alcohol (65 l.). The sugars were separated on large sheets of filter paper (solvent B). Elution with 2.5–3.5% ethanol yielded three more oligosaccharides which could not be identified due to the small quantities obtained. The first yielded only glucose on hydrolysis, the second both glucose and mannose, and the third only mannose. Further elution with 3.5–5.5% ethanol (45 l.) gave a pure oligosaccharide (E) which was purified by paper chromatography (solvent B).

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(31) Kindly supplied by Mr. Sigmund Wang, President, Industrial Cellulose Research Ltd., Haukesbury, Ontario.

(32) A product of Fisher Scientific Co., Fair Lawn, N. J.

Identification of the Oligosaccharides. (A) **4-*O*-β-D-Mannopyranosyl-D-mannose.**—This compound (1,070 mg.) moved on the paper chromatogram (solvents X and Y) at the same rate as an authentic sample of 4-*O*-β-D-mannopyranosyl-D-mannose.[33] Chromatographic examination of the hydrolyzate indicated the presence of mannose only. The disaccharide crystallized from methanol, m.p. and mixed m.p. 203–204°, $[\alpha]_D$ −7° (*c* 1.0 in water).[34] The X-ray diffraction pattern and infrared absorption diagram were identical with those of the authentic specimen.

(B) **4-*O*-β-D-Mannopyranosyl-D-glucose.**—This disaccharide (82 mg.) was chromatographically identical with a sample of authentic 4-*O*-β-D-mannopyranosyl-D-glucose.[35] Paper chromatographic analysis indicated equal amounts of mannose and glucose in the hydrolyzate. Crystals were obtained from ethanol containing a little water, m.p. and mixed m.p. 201–202°, $[\alpha]_D$ +18° (*c* 1.0 in water).[36]

(C) ***O*-β-D-Mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose.**—This compound (481 mg.) was chromatographically identical to an authentic specimen of *O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose.[35] On complete hydrolysis, only mannose was obtained while partial hydrolysis yielded both mannose and mannobiose. After seeding with an authentic crystal, the product crystallized from ethanol, m.p. and mixed m.p. 165–169°, $[\alpha]_D$ −25° (*c* 2.5 in water).[16]

(D) **4-*O*-β-D-Glucopyranosyl-D-mannose.**—The disaccharide (383 mg.) was chromatographically identical with an authentic specimen of 4-*O*-β-D-glucopyranosyl-D-mannose.[35] Equal amounts of mannose and glucose were formed on hydrolysis. The compound crystallized from ethanol, m.p. and mixed m.p. 135–137°, $[\alpha]_D$ +16° (*c* 0.8 in water). The derived octaacetate, prepared with acetic anhydride and zinc chloride, had m.p. 204–205°, unchanged on admixture with an authentic specimen,[37] $[\alpha]_D$ +36° (*c* 1.0 in chloroform).[36]

(E) ***O*-β-D-Mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose.**—Complete hydrolysis of this compound (100 mg.) indicated the presence of mannose only in the hydrolyzate, while partial hydrolysis gave rise to mannose, mannobiose and mannotriose on the paper chromatogram. Application of the method of Peat, Whelan and Roberts[38] suggested the presence of a tetrasaccharide, while oxidation with hypoiodite indicated an equivalent weight of 700 (calcd. 666).[39] The product crystallized spontaneously from methanol–water, m.p. 212–214°, raised to 232–234° after recrystallization from methanol, $[\alpha]_D$ −31° (*c* 1.6 in water).[21]

Oligosaccharide F.—When hydrolyzed, this oligosaccharide (50 mg.) gave rise to glucose and mannose in a ratio of 1:2. The presumed trisaccharide moved faster on the paper chromatogram (solvents X and Y) than mannotriose. Partial hydrolysis with formic acid yielded mannose, glucosylmannose, glucose and mannosylglucose, with the first two sugars predominating.

Methylation of the Glucomannan.—Glucomannan (20 g.) was dissolved in 12% sodium hydroxide (500 ml.) in an atmosphere of nitrogen at 0°. Dimethyl sulfate (100 ml.) was added dropwise with stirring to the cooled solution over a period of 5 hr. Solid sodium hydroxide (100 g.) was then added, followed by dimethyl sulfate (200 ml.). This treatment was repeated eight times, water being added whenever necessary for efficient stirring. The cooled reaction mixture was neutralized with acetic acid and evaporated to 2.5 liters. The resulting solution was extracted exhaustively together with precipitated salts at 40° with chloroform and the combined extracts were evaporated to dryness. The residue was dissolved in tetrahydrofuran (500 ml.) and the resulting solution was added dropwise to petroleum ether (3 l., b.p. 30–60°), producing a grayish precipitate (16.8 g.).

The partially methylated glucomannan (16.5 g.) was dissolved in anhydrous dimethylformamide.[38] Methyl iodide (60 g.) and freshly prepared silver oxide (60 g.) were added and the reaction mixture was shaken for 24 hr. at room temperature. The methylated glucomannan was recovered in the usual way.[40] The final product was redissolved in tetrahydrofuran (100 ml.) and added slowly to petroleum ether (500 ml.) to yield a precipitate (13.7 g.) which was dried *in vacuo*, $[\alpha]_D$ −20° (*c* 2.0 in chloroform), OMe 44.0%. An infrared diagram of the product indicated the absence of any hydroxyl groups.

Hydrolysis of the Metylated Glucomannan and Resolution of the Mixture of Methylated Hexoses.—The methylated glucomannan (5 g.) was dissolved in 90% formic acid (100 ml.), diluted with water (100 ml.) and heated at 97° for 4 hr. Formic acid was removed by repeated evaporation and the water-soluble hydrolyzate (4.6 g.) was added to the top of a column (4 × 82 cm.) containing Nuchar[32] activated charcoal. Elution with 1.75% aqueous ethanol gave a disubstituted hexose (23 mg.). Desorption with 2% alcohol (72 liters) yielded 2,3,6-tri-*O*-methyl-D-mannose. 2,3,6-Tri-*O*-methyl-D-glucose was obtained by elution with 5–7% ethanol (50 l.). When the alcohol concentration was increased to 13–15%, small amounts of a tetra-*O*-methylhexose were obtained.

Identification of 2,3,6-Tri-*O*-methyl-D-mannose.—This compound (1,870 mg.) was chromatographically identical (solvent Z) with an authentic specimen of 2,3,6-tri-*O*-methyl-D-mannose. Demethylation[41] yielded mannose only. The slightly colored sirup had $[\alpha]_D$ −12° (*c* 1.0 in water). The derived di-*p*-nitrobenzoate derivative[42] had m.p. 187–188°.

Identification of 2,3,6-Tri-*O*-methyl-D-glucose.—This sugar moved on the paper chromatogram (solvent Z) at a rate identical to that of an authentic specimen of 2,3,6-tri-*O*-methyl-D-glucose. Demethylation[41] gave glucose only. The product, after recrystallization from ethyl acetate–ethyl ether, had m.p. and mixed m.p. 120–121°, $[\alpha]_D$ +70° (*c* 1.0 in water).

Periodate Oxidation of the Glucomannan.—Samples, 150 mg. each, were oxidized for various lengths of time in the dark at 30° with 50 ml. of 0.05 *M* sodium metaperiodate, the consumption of which was measured by the usual excess arsenite method. The polysaccharide consumed 0.97 mole of oxidant within the first 72 hr.

Preparation of the Nitrate Derivative of the Glucomannan.—Glucomannan (1 g.) was treated with a mixture of nitric acid, phosphoric acid and phosphorus pentoxide[43] at 17° for 1 hr. The reaction mixture was poured into a mixture of acetic acid and water (1:1), cooled to −16°. The nitrate was recovered by filtration and washed with ice-water until the washings were neutral; yield 1.6 g., nitrogen content[44] 13.82%. A duplicate experiment gave the same yield of product containing 13.38% nitrogen.

Determination of the Number-average Molecular Weight of the Nitrated Glucomannan.—The osmometers used were of the Zimm–Myerson[45] type as improved by Stabin and Immergut.[46] Gel cellophane membranes which had never been allowed to dry were used,[47] the solvent was freshly distilled *n*-butyl acetate and the temperature was kept at 30°. The osmotic pressure was determined by the static method and the following values were obtained for h/w (h = osmotic height in cm. solvent and w = concentration in g./kg. solution) at different values of w: 1.074(6.544), 1.037(5.444), 1.009(4.658), 0.982(3.829), 0.994(3.345), 0.964 (2.944), 0.962(1.873). Extrapolation to zero concentration gave a value of 0.845 for $(h/w)_0$, corresponding to a number-average molecular weight of 30,500 and a $\bar{P}_n$ value of 107 (base mol. wt. 285).

Determination of the Weight-average Molecular Weight of the Nitrated Glucomannan.—Light-scattering intensities were measured with a Brice–Phoenix photometer as modified

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by Huque, Jaworzyn and Goring.[48] The solvent was *n*-butyl acetate and the liquid surrounding the cell was a mixture of 2 parts of ethylene glycol and 1 part of water. The light-scattering cells were designed to achieve efficient removal of micellar debris, which, if present, caused vertical striations, making accurate measurements impossible. All solutions were clarified twice by ultracentrifugation, first, at 40,000 r.pm. and subsequently in the light-scattering cells at 20,000 r.p.m. The wave length of the light used was 5460 Å. The refractive index increment was measured with a Brice–Phoenix differential refractometer at a wave length of 4358 Å. The value obtained was 0.085 ml./g. No correction was applied for dissymetries which averaged 1.1 to 1.2 and which did not vary with the concentration. The following values were obtained for the ratio $C/I_{90°}$ at the respective concentrations C (mg./ml.): 2.084(1.98), 2.933(3.99), 3.423(6.06), 3.912(8.16) and 4.420(9.90). Extrapolation to zero concentration gave a value of 1.75 for $(C/I_{90°})_{c=0}$. The molecular weight was calculated from the relationship $1/M_w = H(C/\tau)_0$ where the symbols have their usual significance.

Determination of Intrinsic Viscosity.—The viscometer used was a Craig–Henderson instrument.[49] Reduced viscosities were estimated at 25° at seven different concentrations and extrapolated to zero concentration in the usual way. The solvent was *M* cupriethylenediamine.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, McGILL UNIVERSITY, AND THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

Synthesis and Characterization of 2-O-(β-D-Glucopyranosyluronic Acid)-D-xylopyranose[1]

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2-O-(β-D-Glucopyranosyluronic acid)-D-xylopyranose has been synthesized by condensation of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucuronate with methyl 3,5-O-isopropylidene-D-xyloside in a Koenigs–Knorr reaction. The aldobiouronic acid was characterized by reduction, methylation and hydrolysis when 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-D-xylose were obtained.

In connection with an attempted synthesis of the β-anomer of the ubiquitous 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose[2] the unmethylated aldobiouronic acid 2-O-(β-D-glucopyranosyluronic acid)-D-xylopyranose was also synthesized. The α-anomer of this compound was first obtained by partial hydrolysis of hemicellulose B of corn cob[3,4] and has since been isolated from hemicelluloses in wheat bran,[5] oat hulls,[6] chagual gum,[7] corn hull,[8,9] groundnut shell,[10] wheat straw[11] and the wood of maritime pine.[12] It has been fully characterized through the crystalline methyl ester-pentaacetate of its methyl α- and β-D-glycosides.[9]

The 2-O-(β-D-glucopyranosyluronic acid)-D-xylopyranose was synthesized from D-glucurono-(3→6)-lactone and D-xylose. D-Glucurone was converted by three different methods[13–15] to sirupy methyl (D - glucopyranosyl) - uronate. Acetylation[15,16] yielded the α- and β-anomers of methyl 1,2,3,4 - tetra - O - acetyl - D - glucuronate which both crystallized. The compound was treated with titanium tetrachloride in chloroform[18] when the crystalline methyl 2,3,4-tri-O-acetyl-1-chloro-1-deoxy-α-D-glucuronate was obtained. Treatment with hydrogen bromide in acetic acid converted the tetraacetate to the corresponding bromo compound which was also crystalline, albeit rather unstable.[13,17,18]

D-Xylose was converted to methyl α,β-D-xylofuranoside, either directly by treatment with methanolic hydrogen chloride[19,20] or *via* the 3,5-O-isopropylidene derivative.[21,22] The α- and β-anomers of 3,5-O-isopropylidene D-xyloside were obtained by subsequent condensation with acetone under carefully controlled conditions.[21]

Condensation of methyl 2,3,4-tri-O-acetyl-1-chloro-1-deoxy-α-D-glucuronate with methyl 3,5-

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