[CONTRIBUTION FROM THE OLYMPIC RESEARCH DIVISION, RAYONIER, INC.]

The Hemicelluloses of Western Red Cedar: The Constitution of a Glucomannan[1]

BY J. KELVIN HAMILTON AND E. VERNON PARTLOW

RECEIVED MARCH 29, 1958

Extraction with 18% sodium hydroxide of a western red cedar (*Thuja plicata* Donn) chlorite holocellulose, which had been previously extracted with 0.1 *N* and 4% sodium hydroxide, led to the isolation of a glucomannan in which the ratio of glucose-to-mannose was approximately 1 to 2.5. The hydrolyzate of the fully methylated polysaccharide yielded 2,3,6-tri-*O*-methyl-D-mannose as the predominant methylated sugar, a small amount of 2,3,6-tri-*O*-methyl-D-glucose, a smaller amount of 2,3,4,6-tetra-*O*-methyl-D-glucose, and trace amounts of di-*O*-methylgalactose and 2,3,4,6-tetra-*O*-methylgalactose, presumably from a contaminating galactoglucomannan. Graded acid hydrolysis of the glucomannan, followed by qualitative paper chromatographic analysis of the hydrolyzate, showed the presence of substances corresponding to glucosidomannose, mannobiose, mannosidoglucose, cellobiose and a mannotriose. The above methylation and graded hydrolysis results, in conjunction with certain physical and chemical properties, indicate that this glucomannan is a short, predominantly straight chain polymer composed of glucose and mannose in a ratio of 1 to 2.5 and joined mainly by 1—4-β-glycosidic bonds. It is similar to glucomannans isolated from other woods.

The hemicelluloses of conifers constitute approximately one-fifth of the weight of the wood, and the mannose-containing polymers account for about one-half of this hemicellulosic material. The mannose has been found to occur predominantly, if not exclusively, as the major component of heteropolymers such as glucomannans,[3-6] galactoglucomannans[7-9] or perhaps as galactomannans. A very small percentage may be due to the presence of a true mannan, although no concrete evidence for the existence of such a polymer from a conifer has been presented thus far. Since the original disclosure of the presence of glucomannans in western hemlock,[3] similar polymers have been isolated from a number of wood cellulose systems.[10-16]

This paper is specifically concerned with the isolation and structure of the glucomannans present in western red cedar (*Thuja plicata* Donn) holocellulose. A second objective was to gain more information concerning the nature of the polysaccharides extracted from the holocellulose by sodium hydroxide solutions of varying concentrations. A third objective was to obtain information with respect to the sugars and polysaccharides extracted from cedar wood shavings with acetone, water, and dilute sodium hydroxide. A number of excellent papers have been published on the extractives[17,18] and phenolic[19] materials present in this wood.

Cedar holocellulose,[20] rather than wood itself, was used for the study on the glucomannan in order to increase the yield of alkali-soluble hemicellulose. The fibrous holocellulose was extracted successively with aqueous 0.1 *N*, 4.0% and 18.0% sodium hydroxide with the purpose of subdividing the cedar hemicelluloses into less complex mixtures.[21,22] The polysaccharides were isolated by conventional procedures.

Following hydrolysis, each of these three polysaccharide fractions was quantitatively analyzed for neutral sugars by means of paper partition chromatographic techniques.[23] Uronic acids were determined by qualitative paper chromatography. The results (see Table I) showed that a partial separation of the xylose- and mannose-containing polysaccharides had been achieved. This partial separation of polysaccharide material on the basis of solubility in alkaline solutions of varying concentration also has been shown to occur when western hemlock cellulose is extracted with alkali.[3] It is of general interest that all three of the polysaccharide fractions obtained from the cedar holocellulose were water soluble immediately upon isolation but, after storage for several months, could only be partially dissolved in water.

In order to ascertain the mode of union of the building units, one of the polysaccharide fractions (from the 18.0% sodium hydroxide extraction of cedar holocellulose) was chosen for methylation studies. The polysaccharide was acetylated,[24] but could not be fractionated into its components. The mixture of polysaccharide acetates was methylated, first with sodium hydroxide and methyl sulfate[25] and then with silver oxide and methyl iodide.[26] Fractional precipitation of the methylated polysaccharide from a chloroform solution with petroleum ether indicated the presence of two components, as did chromatographic examination of the hydrolyzates of each fraction. The less soluble methylated polysaccharide, upon hydrolysis, was found chromatographically to consist principally of 2,3-di-O-methyl-D-xylose and was not

(1) Contribution No. 36.

(2) Presented at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April, 1958.

(3) J. K. Hamilton, H. W. Kircher and N. S. Thompson, THIS JOURNAL, **78**, 2508 (1956).

(4) J. K. N. Jones, E. Merler and L. E. Wise, *Can. J. Chem.*, **35**, 634 (1957).

(5) H. F. Lewis, L. E. Wise and B. L. Browning, *Tappi*, **33**, 92 (1950).

(6) F. E. Brauns and H. F. Lewis, *Paper Trade J.*, **105**, No. 10, 35 (Sept. 2, 1957).

(7) J. K. Hamilton, E. V. Partlow and N. S. Thompson, *Tappi*, in press.

(8) G. A. Adams, *Tappi*, **40**, 721 (1957).

(9) G. G. S. Dutton and F. Smith, private communication.

(10) J. K. N. Jones and T. J. Painter, *J. Chem. Soc.*, 669 (1957).

(11) T. E. Timell and A. Tyminski, *Tappi*, **40**, 519 (1957).

(12) B. Immergut and B. Råuby, *Svensk Papperstidn.*, **60**, 573 (1957).

(13) Rep. For. Prod. Res. Board, 1953, p. 43.

(14) G. O. Aspinall and R. A. Laidlaw and R. B. Rashbrook, *J. Chem. Soc.*, 4444 (1957).

(15) B. Lindberg, H. Meier, *Svensk Papperstidn.*, **60**, 785 (1957).

(16) J. K. Hamilton and H. W. Kircher, THIS JOURNAL, **80**, 4703 (1958).

(17) G. M. Barton and J. A. F. Gardner, *Pulp Paper Mag. Can.*, **55**, 132 (1954).

(18) H. Erdtman and J. Gripenberg, *Acta Chem. Scand.*, **2**, 625 (1948).

(19) A. B. Anderson and J. Gripenberg, *ibid.*, **2**, 644 (1948).

(20) E. L. Lovell, *Ind. Eng. Chem.*, **37**, 1034 (1945).

(21) J. K. Hamilton and G. R. Quimby, *Tappi*, **40**, 781 (1957).

(22) J. K. Hamilton and N. S. Thompson, THIS JOURNAL, **79**, 6464 (1957).

(23) R. M. McCready and E. A. McComb, *Anal. Chem.*, **26**, 1645 (1954).

(24) G. G. S. Dutton and F. Smith, THIS JOURNAL, **78**, 3744 (1956).

(25) W. N. Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

(26) T. Purdie and J. C. Irvine, *ibid.*, **83**, 1021 (1903).

further examined. The more soluble methylated polysaccharide was hydrolyzed and subjected to chromatographic separation on paper.

The 2,3,6-tri-O-methyl-D-mannose was identified, following oxidation with bromine, as the crystalline phenylhydrazide of 2,3,6-tri-O-methyl-D-mannono-γ-lactone. The 2,3,6-tri-O-methyl-D-glucose crystallized and was identified both chromatographically and by its optical rotation. The 2,3,4,6-tetra-O-methyl-D-glucose was identified by conversion into its characteristic N-phenylglycosylamine, and the very small amount of 2,3,4,6-tetra-O-methylgalactose also was identified by its characteristic N-phenylglycosylamine. A trace of a probable di-*O*-methyl component (less than 0.7%) was observed and upon demethylation was shown to be derived from galactose.

From the preceding experimental data, some structural features of the glucomannan polymer can be deduced. It is evident that the 2,3,4,6-tetra-*O*-methyl-D-glucose is representative of terminal D-glucopyranose units in the glucomannan polymer. From the large amounts of 2,3,6-tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-glucose, it is concluded that the main body of the polymer consists of D-glucose and D-mannose pyranose units linked through positions 1 and 4. The optical rotations of both the polymer and its methylated derivative indicate the β-configuration.

The conclusion that the glucomannan is essentially a straight chain polymer is supported by the products obtained on partial acid hydrolysis. If glucose and mannose are randomly distributed along a 1→4-linked chain and if it is assumed that the resistance of the 1→4-glycosidic bonds toward cleavage by acid hydrolysis are roughly equal, it would follow that 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose and 4-*O*-β-D-glucopyranosyl-D-glucose and a mannotriose should be found in the partial acid hydrolyzates.[3,16,27,28] The fact that substances corresponding to these compounds were qualitatively identified by paper partition chromatography in the glucomannan partial acid hydrolyzates appears to be in agreement with the conclusion reached from the methylation study; *i. e.*, the glucomannan is a linear (1→4)-β-D-linked polymer.

Although the small amounts of methylated galactose isolated from the hydrolyzates of the methylated polysaccharide cannot be entirely ignored, it is believed that this galactose is not structurally significant but rather is representative of contamination with a small amount of galactoglucomannan. Such polymers are known to occur in conifers,[7-9] and work on the structure of a typical galactoglucomannan (pine) is now in progress and will be reported at a later date.[7] Results from a subsequent similar extraction of cedar holocellulose with aqueous 0.1 *N*, 4.0% and 18.0% sodium hydroxide solutions showed the 18.0% sodium hydroxide-extracted polysaccharide, following hydrolysis, to be chromatographically free of galactose. This supports the view that galactose is not structurally significant in the present cedar glucomannan but arises from the presence of a small amount of galactoglucomannan which was incompletely separated from the glucomannan.

From these facts a simplified structure for a cedar hemicellulose glucomannan may be proposed

$$G_1-{}_4M_1-{}_4M_1-{}_4M_1-{}_4G_1-{}_4G_1-{}_4M_1-{}_4M_1-{}_4M_1-{}_4M_1-{}_4M_1-{}_4G_1-{}_4M_1-{}_4M_1-{}_4M$$

This information concerning the glucomannan of western red cedar hemicellulose in conjunction with that already known about the glucomannans of other coniferous woods[3-16] emphasizes the view that all of these polysaccharides are essentially linear polymers composed of a D-glucopyranose and D-mannopyranose framework. This linear type of structure, resembling that of cellulose, suggests that glucomannans also should resemble cellulose in other characteristics. The fact that both a mechanical disruption and a mild acid hydrolysis prior to alkaline extraction were required to essentially free holocellulose of mannose-containing polymers[21] implies that glucomannans must be intimately associated with cellulose and may also be dependent on their degree of polymerization for solubility in alkaline solutions. This behavior of glucomannans is in sharp contrast to that of the xylose-containing polymers[3,22] which have single side chains of 4-*O*-methyl-D-glucuronic acid and/or L-arabinose[7] or the still more highly branched galactoglucomannans[7] both of which are more readily extracted with dilute sodium hydroxide solutions.

In separate experiments, polysaccharide material was extracted from western red cedar wood shavings with acetone, water and aqueous 5.0% sodium hydroxide. The acetone-extracted material, which has been shown to be essentially phenolic in nature,[16-18] was found on chromatographic analysis, both before and after acid hydrolysis, to contain arabinose[29] as the principal constituent sugar. On the other hand, the water extract, though primarily carbohydrate in nature, chromatographically was found to contain principally galactose in the acid hydrolyzates, along with small amounts of glucose, mannose, arabinose, xylose, accompanied by traces of rhamnose and glucuronic acid. The high galactose content of this polysaccharide material suggests that the principal polysaccharide present in this water extract may well be an arabogalactan or galactan similar to those found in water extracts of larches.[30-32] Polysaccharide materials extracted from cedar wood with aqueous sodium hydroxide, however, appear to be more complex than either of the previously mentioned acetone- or water-extracted polysaccharides. Upon hydrolysis of this sodium hydroxide-extracted polysaccharide material and qualitative chromatographic analysis, appreciable quantities of galactose, glucose, mannose, arabinose, xylose, a small amount of 4-*O*-methyl-D-glucuronic acid and a trace of rhamnose were observed. These findings would appear to be indicative of the presence of a mini-

(27) J. G. Leach, *Tappi*, **35**, 249 (1952).
(28) A. Anthis, *ibid.*, **39**, 401 (1956).
(29) A. B. Anderson and H. Erdtman, THIS JOURNAL, **71**, 2927 (1949).
(30) A. W. Schorger and D. F. Smith, *Ind. Eng. Chem.*, **8**, 494 (1916).
(31) L. E. Wise and F. C. Peterson, *ibid.*, **22**, 362 (1930).
(32) E. V. White, THIS JOURNAL, **63**, 2971 (1941).

mum of two polymer systems in this hemicellulose material.

Experimental

Paper Chromatography.—Chromatographic solvents employed for the separation of uronic acids and sugars were (A) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), (B) ethyl acetate–acetic acid–water (9:2:2), (C) ethyl acetate–pyridine–water (8:2:1), (D) ethyl acetate–acetic acid–water (6:3:2), (E) butanol–pyridine–water (10:3:3), (F) 2-butanone–formic acid–water (26:1:4). All solvents were made up on a volume-to-volume basis. The solvent used for separation of methylated sugars was (G) 2-butanone–water (10:1).

The indicators employed were ammoniacal silver nitrate, brom cresol blue adjusted to *p*H 6 for carboxylic acids, and *p*-anisidine trichloroacetic acid. A modification of the last spray reagent to which 4% by volume of pyridine had been added was used to detect pentoses and pentose oligosaccharides.[22] This reagent gives pink to reddish-brown spots (intensified under ultraviolet light) with all four pentoses and with oligouronides, while hexoses give yellow-brown spots.

Quantitative paper chromatographic analysis was by a method similar to that used by McCready, *et al.*[23] The indicator employed was aniline phthalate and relative spot densities were determined with a Beckman spectrophotometer. Chromatographic papers employed were Whatman Nos. 1, 3MM and 50.

Starting Material.—The cedar shavings used in this study were cut from green western red cedar (*Thuja plicata* Donn). The shavings were air classified to remove fines and averaged 0.014 inch in thickness.

Acetone Extraction.—Cedar shavings were extracted with acetone for 24 hours in a soxhlet-type apparatus. The acetone extract was filtered to remove wood debris, evaporated, and the reddish-brown sirup dried to constant weight in a yield of 3.1% (based on oven-dry wood).

Paper partition chromatographic studies on the acetone extract (solvent A and *p*-anisidine spray containing 4% pyridine) showed the presence of a substance corresponding to arabinose. A portion of the acetone extract was hydrolyzed for 12 hours with 1.0 *N* sulfuric acid, neutralized with barium carbonate and filtered to remove insoluble barium salts. The filtrate was deionized with a cation exchange resin (Amberlite IR-120) and evaporated under reduced pressure to a sirup. Chromatographic study (solvent C and ammoniacal silver nitrate spray) of this sirup showed spots corresponding to those of authentic specimens of arabinose, glucose, xylose and rhamnose. Spot size and intensity indicate that the major portion of the sugar in this sirup was arabinose with glucose being present in secondary quantities, while only trace quantities of xylose and rhamnose appeared to be present.

Cold Water Extraction.—Cedar shavings (previously extracted with acetone) were soaked for 24 hours in water at 5°. Following decantation, the shavings were further soaked in water at 5° for an additional 60 hours. The combined water extracts were evaporated at diminished pressure to 200 ml. Methanol (4 vol.) was added and, after standing for 24 hours, the precipitate was removed by centrifugation. This precipitate was dissolved in water containing a small quantity of chlorine dioxide (sufficient to persist for 30 minutes at room temperature) and then was reprecipitated with methanol (4 vol.).[33] This precipitate was successively washed with methanol (twice), acetone and finally with diethyl ether (twice). On drying to constant weight in a vacuum desiccator, a yield of 0.014% (based on oven dry wood) was obtained.

A portion of this water-extracted polysaccharide material (10 mg.) was dissolved in 1 ml. of 72% sulfuric acid (25°) followed by cautious dilution (to avoid reprecipitation) to 25 ml. with water and immediate heating of the solution on a boiling water-bath for 12 hours. The cooled solution was neutralized with barium carbonate and deionized with a cation exchange resin (Amberlite IR-120), filtered, and the filtrate treated with an anion exchange resin (Duolite A-4). After shaking overnight, the mixture was filtered and the Duolite A-4 ion exchange resin washed twice with water. The filtrate and washing contained neutral sugars.

The Duolite A-4 ion exchange resin was shaken for 24 hours with *N* sulfuric acid, filtered, and washed with water until the washings were neutral. The filtrate and washings were neutralized with barium carbonate, filtered, freed of barium ion with Amberlite IR-120 ion exchange resin and evaporated to a sirup. This sirup contained the uronic acids.

Qualitative Chromatographic Analysis of Cold Water Extracts.—The sirups obtained from the cold water extracts were diluted with a small volume of water and were analyzed for their constituent neutral sugars (Solvent C) and uronic acids (Solvent F) by paper partition chromatographic techniques. The studies showed the presence of substances, the R_x values (distance traveled relative to that of xylose) of which corresponded to galactose (large), glucose (small), mannose (small), arabinose (small), xylose (small), rhamnose (trace) and glucuronic acid (trace).

Two parallel water extractions of cedar shavings at 20 and 50° also were made with similar results except that, additionally, 4-*O*-methyl-D-glucuronic acid was found in trace quantities. Solvents employed were B, D, E and F.

Caustic Extraction of Cedar Shavings.—Cedar shavings were extracted with sodium hydroxide (5% solution concentration) for 30 minutes at 20°, filtered, and the filtrate made slightly acidic with acetic acid. After standing two days at room temperature, the solution was centrifuged and the precipitate so obtained was suspended in water and dialyzed against running tap water for seven days. This suspension was evaporated under diminished pressure to 200 ml. and then bleached by adding small portions of chlorine dioxide until a slight excess (on standing one hour at room temperature) was obtained. Methanol was added (4 vol.) to precipitate polysaccharide material. The precipitate was washed successively with methanol, acetone and petroleum ether (30–60°). On drying this polysaccharide material to constant weight, a yield of 0.13% (based on oven dry wood) was obtained.

Following removal of this water-insoluble polysaccharide material by centrifugation, the previously mentioned supernatant was dialyzed against running tap water for nine days and then was concentrated at diminished pressure to a small volume (200 ml.). Methanol (4 vol.) was added and the precipitate so obtained was dissolved in 100 ml. of water and bleached with chlorine dioxide as previously described.[33] After the bleaching (1 hr.), methanol was added (4 vol.) and the precipitate was washed successively with methanol (3 times), acetone (twice) and finally with diethyl ether (twice). On drying this water-soluble polysaccharide material to constant weight at reduced pressure, a yield of 1.28% (based on oven-dry weight of wood) was obtained.

Portions of both the water-soluble and water-insoluble polysaccharide fractions recovered from the sodium hydroxide extraction of cedar shavings were hydrolyzed, neutralized, deionized, and evaporated to thick sirups by the procedure previously described (see Cold Water Extraction).

Qualitative Chromatographic Analysis of 5% Sodium Hydroxide Extracts.—The sirups from the various hydrolyzates were dissolved in a small volume of water and analyzed for their constituent sugars by paper partition chromatographic techniques with solvents B, D and E for neutral sugars and A, B and D for acidic monomers and oligouronides. These studies showed the presence of substances the R_x values (distance traveled relative to that of xylose) of which corresponded to galactose (large), glucose (medium), mannose (large), arabinose (large), xylose (very large), rhamnose (trace), 4-*O*-methyl-D-glucuronic acid (small) and 2-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-xylose (small).

Preparation of Cedar Holocellulose.—Holocellulose was prepared from cedar shavings by the method of Lovell.[20] Shavings (154 g.), previously extracted with acetone, were placed in an erlenmeyer flask containing 3 liters of 6% sodium chlorite solution (adjusted to *p*H 4.7 with acetic acid). The solution was held thermostatically at 50 ± 3° for 12 hours at which time the spent solution was drained from the shavings and replaced with a fresh charge of sodium chlorite solution (same concentration and *p*H as initial charge) and held at 50 ± 3° for 24 hours. After a third similar sodium chlorite treatment for 8 hours, the soft, white shavings were drained of liquor and covered several times with distilled water. Further washing and soaking in changes of distilled

(33) Control experiments on bleaching hemicelluloses with aqueous chlorine dioxide showed that the colored material associated with the hemicellulose was largely solubilized by this treatment and remained in the methanolic layer of the precipitating solution. The cupriethylenediamine hydroxide (cuene) intrinsic viscosity of the hemicellulose was not lowered by this bleaching treatment.

water over a period of several days was required to free the gelatinous fibers of the last traces of reagent. Disintegration of the shavings into a pulp, which was nearly complete at this point, was accomplished by use of a laboratory stirrer. The gelatinous pulp so obtained was filtered, dehydrated with methanol, and dried at room temperature.

The cedar holocellulose contained α-cellulose[33a] (69.5%), β-cellulose[a] (1.4%), γ-cellulose[a] (29.1%), ash (1.1%), silica (0.03%), galactose (1.0%), glucose (large amount but not quantitatively determined), mannose (12.3%), arabinose (trace), xylose (5.6%), rhamnose (trace), galacturonic acid (trace), 4-*O*-methyl-D-glucuronic acid (0.6%, gravimetrically), soluble lignin[34] (3.6%), insoluble lignin[34] (0.1%). The yield (O.D.) was 61% and the intrinsic viscosity in *M* cupriethylenediamine hydroxide was 8.3 (expressed as dl./g.).

Alkaline Extraction of Holocellulose.—A sample of holocellulose was extracted successively with alkaline solutions (0.1 *N*, 4.0% and 18.0% sodium hydroxide) so that the extractable hemicelluloses could be subdivided on an alkaline solubility basis. Conditions used for alkaline extraction were as follows: A portion of holocellulose (120 g.) was slurried with 0.1 *N* sodium hydroxide for 20 minutes and filtered on a Buchner funnel, and the pulp pad was washed by displacement with fresh 0.1 *N* sodium hydroxide until 4 liters of filtrate was collected. The holocellulose sample previously extracted with 0.1 *N* sodium hydroxide was subjected to a second similar extraction made with 4.0% sodium hydroxide. A third similar extraction with 18.0% sodium hydroxide was also employed.

Each of these three filtrates was similarly treated to recover polysaccharide material from the alkaline solutions. Each filtrate was refiltered through medium porosity, sintered glass filters (to remove traces of fiber), acidified with acetic acid and dialyzed against running water for ten days. The dialyzed solutions were concentrated to one-twentieth of their original volume under diminished pressure. (It is of interest to note that no precipitation occurred either following neutralization, dialysis or concentration.) These polysaccharide fractions were precipitated with methanol (4 vol.), and after standing overnight the precipitates were removed by centrifugation. The precipitates were dissolved in water (100 ml.), bleached with chlorine dioxide at room temperature[33] (sufficient chlorine dioxide to give a slight excess after one hour) and then were reprecipitated with methanol (4 vol.). The precipitates were removed by centrifugation and were solvent exchanged with methanol (twice), acetone, and finally with diethyl ether. Following drying to constant weights in a vacuum desiccator, yields of 4.16% (0.1 *N* sodium hydroxide extract), 6.32% (4.0% sodium hydroxide extract) and 6.99% (18.0% sodium hydroxide extract) (all values based on oven dry holocellulose) were obtained. Table I indicates some of the physical properties of these polysaccharides.

Graded Acid Hydrolysis of 18.0% Sodium Hydroxide Extract from Holocellulose.—About 200 mg. of the 18.0% sodium hydroxide-extracted polysaccharide was swollen in water (50 ml.) overnight. Sufficient sulfuric acid was added to this slurry to make a solution 0.2 *N* in respect to sulfuric acid and was heated at boiling bath temperature for two hours. After cooling, the supernatant solution was removed by centrifugation. The solid residue was hydrolyzed twice more in the same manner. The three hydrolyzates were neutralized with barium carbonate, combined and filtered, and the filtrate was ion exchanged with Amberlite IR-120 and evaporated to a sirup.

The sirup was streaked on a sheet of Whatman No. 3 MM paper and chromatographed in solvent D. The area containing oligosaccharides was cut from the paper, eluted, ion exchanged with Amberlite IR-120, evaporated to a sirup, and examined chromatographically on solvents B and D. In addition to xylose oligosaccharides present as impurities, spots corresponding to those of authentic specimens of 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose, and 4-*O*-β-D-glucopyranosyl-D-glucose (trace quantity) and a mannotriose were found.

Acetylation of 18.0% NaOH Extract from Holocellulose.—The polysaccharide recovered from 18.0% sodium hydroxide extraction of holocellulose (5.2 g.) was wet with water (25 ml.). After standing three days, the water was displaced with methanol on a filter. To the methanol-wet precipitate was added 150 ml. of pyridine and 120 ml. of acetic anhydride in portions (40 ml. of acetic anhydride at hourly intervals) with shaking. The acetylation reaction was carried out for 22 hours at room temperature, followed by 16 hours at boiling bath temperature. The cooled acetylation mixture was poured into acidified water (1% HCl) with stirring. The precipitate was removed by filtration and washed with water, methanol, and diethyl ether. The weight of recovered dry acetylated material was 6.2 g. Separation of the acetylated polysaccharide on the basis of acetone solubility into an acetone-soluble and an acetone-insoluble fraction proved to be of limited value in that, on hydrolysis, glucose, mannose and xylose were detected in both fractions with only small variations in apparent relative concentrations.

TABLE I

PROPERTIES AND COMPOSITION OF HEMICELLULOSE FRACTIONS

Sample	0.1 *N* NaOH ext.	4.0% NaOH ext.	18.0% NaOH ext.
$[\alpha]^{23}D$	−1.76°[a]	−44.6°[b]	−37.2°[c]
Intrinsic viscosity[d]	0.38	0.42	0.44
Ash, %	3.0	3.1	1.6
Galactose[e]	3.5	2.9	1.0
Glucose[e]	2.2	8.8	18.3
Mannose[e]	21.5	22.4	46.6
Arabinose[e]	1.0	1.0	0.0
Xylose[e]	16.2	24.1	8.1
Rhamnose[f]	Trace	None	None
4-*O*-Methyl-D-glucuronic acid[f]	M	M	M
2-*O*-(4-*O*-Methyl-glucuronopyranosyl)-D-xylose[f]	M	M	M
Galacturonic acid[f]	S	None	None
Yield (based on oven-dry holocellulose), %	4.16	6.32	6.99

[a] *c* 3.2% in water. [b] *c* 2.3% in water. [c] *c* 0.9% in water. [d] One *M* cupriethylenediamine hydroxide. [e] Calculated from quantitative paper chromatography data expressed as molar ratios. [f] Qualitative paper chromatography: M = medium, S = small (solvents A and B).

Methylation of 18.0% Sodium Hydroxide Extract from Holocellulose.—The acetylated polysaccharide (5.1 g.) was dispersed in acetone (50 ml.) by shaking. To the partially solubilized suspension, 30% sodium hydroxide (190 ml.) and methyl sulfate (60 ml.) were added in aliquot tenths at 10-minute intervals with stirring.[35] The temperature schedule followed was a 5° rise per 10 minutes from the initial temperature (25°) to the maximum temperature used (55°). Small amounts of acetone were added periodically to control foaming. After the addition of the reagents, the solution was held an additional two hours at 55°. Methyl sulfate (13 ml.) and 30% sodium hydroxide (31 ml.) were added to the solution, and the flask heated slowly to boiling bath temperature and held at this temperature for 30 minutes in order to destroy the excess of methyl sulfate and distil off the acetone. The cooled solution was made slightly acid with sulfuric acid (5 *N*) and was dialyzed 3 days against tap water, and the residual solution evaporated to a small volume.

A second and third similar methylation was performed at 55° using 30% sodium hydroxide (211 ml.) and methyl sulfate (73 ml.), together with acetone as needed. The product was recovered in each case by dialysis. After the third methylation, the dialyzed and concentrated aqueous solution was chloroform extracted and the chloroform layer washed three times with water. The chloroform solution was then evaporated to a viscous brown sirup.

The partially methylated sirup was dissolved in a mixture of acetone (25 ml.) and methyl iodide (25 ml.); silver oxide[26] (5 g.) and anhydrous calcium sulfate (3 g.) were added and the resulting mixture was refluxed for 8 hours. After the dilution with chloroform, the insoluble residue was removed by centrifugation, and the residue was extracted twice with fresh portions of chloroform. Evaporation of the combined

(33a) Method similar to Tappi Standard Method No. T203m-55.

(34) B. L. Browning and L. O. Bublitz, *Tappi*, **36**, 452 (1953).

TABLE II

FRACTIONAL PRECIPITATION OF METHYLATED CEDAR HEMICELLULOSE

Fraction	Total petroleum ether added, ml.	Wt., g.	OMe, %	$[\alpha]_D^{23}$ (c 1.0% in chloroform)
1	300	0.19	40.4	−25.9°
2[a]	400	.55	43.3	....
3	800	.95	44.7	−18.6
4[a]	[b]	.40	44.8	....

[a] Fractions 2 and 4 were precipitated as oils. They were refractionated as shown in Table III. [b] Recovered by evaporation of mother liquor from fraction 3.

extracts gave a sirup which was soluble in methyl iodide, and was remethylated six more times by Purdie's method[2] without the addition of acetone. A small sample of the final sirup which was dissolved in chloroform and poured into an excess of petroleum ether, gave a near white powder (OMe, 45.0%).

Fractionation of the Methylated Hemicellulose.—The sirup obtained following the seventh methyl iodide methylation was dissolved in chloroform (60 ml.), diluted with diethyl ether (60 ml.), and fractionated by addition of petroleum ether (30–60°) to a mechanically stirred solution. Results are shown in Tables II and III.

From both the fractionation data and chromatographic study of the hydrolyzates of 20-mg. samples of the various fractions, it was concluded that at least two polysaccharides were present. Polysaccharide A was composed of fractions 2A, 2B, 3 and 4A, while polysaccharide B was composed of fraction 1 (not further used). The ensuing experimental data refer only to polysaccharide A ($[\alpha]^{22}D$ −19.4°, chloroform, 44.2% OMe).

Hydrolysis of Polysaccharide A.—Polysaccharide A was dissolved in methanol (50 ml.) containing 2% hydrochloric acid and was refluxed for 12 hours. The solution was evaporated to a thick sirup and then was dissolved in normal sulfuric acid (100 ml.), and the solution was heated at boiling bath temperature for 13 hours. Following neutralization with barium carbonate, filtration and deionization with Amberlite IR-120 and Duolite A-4 ion exchange resins, this solution containing neutral methylated sugars was evaporated under diminished pressure to a thick sirup. On treatment of the Duolite A-4 anion exchange resin in the usual manner, uronic acids were found to be absent.

Separation of Methylated Sugars.—Portions of the hydrolyzate (80 mg.) were streaked on Whatman 3MM papers ($18^1/_4'' \times 11^1/_4''$) and separated using solvent G for 4.5 hours in a chromatographic cabinet. Although the di-*O*-methyl, tri-*O*-methyl and tetra-*O*-methyl sugars were separated from each other by this method, the 2,3,6-tri-*O*-methyl-D-glucose was poorly separated from 2,3,6-tri-*O*-methyl-D-mannose. It was possible, however, to resolve the two 2,3,6-tri-*O*-methylhexoses quite readily by the use of a Whatman No. 50 wick which was sewn onto the Whatman 3MM papers. This increased the time for the irrigating solvent to reach the bottom of the paper from 5–6 to 20 hours or more. Through the use of this technique, the 2,3,6-tri-*O*-methyl-D-glucose was separated readily from the 2,3,6-tri-*O*-methyl-D-mannose. The di-*O*-methyl and tetra-*O*-methyl fractions also were chromatographed using this refinement in technique and these various sugars were obtained: 2,3,6-tri-*O*-methyl-D-mannose (sirup, 394 mg.), 2,3,6-tri-*O*-methyl-D-glucose (crystallized spontaneously, 102 mg.), 2,3,4,6-tetra-*O*-methyl-D-glucose (56 mg., based on equilibrium rotation of chromatographically pure fraction), 2,3,4,6-tetra-*O*-methylgalactose (sirup, 16 mg.) and a di-*O*-methyl galactose (sirup, 4 mg.). Based on the above, the molar ratio of these sugars is 7.7:2:1:0.3:0.07 which, when the trace galactose residues are ignored, corresponds to a linear polymer of approximately 10–12 anhydrohexose units.

Identification of 2,3,6-Tri-*O*-methyl-D-mannose.—This sugar was isolated as a sirup, $[\alpha]^{22}D$ −12.3° (*c* 3.9% in water). The 2,3,6-tri-*O*-methyl-D-mannose gave the corresponding N-phenylglycosylamine which proved to be very difficult to purify and was discarded.

The 2,3,6-tri-*O*-methyl-D-mannose (100 mg.) was dissolved in water (5 ml.), bromine (0.25 ml.) was added, and the resulting mixture stored in the dark for seven days. The reaction mixture was worked up in the usual manner, and the corresponding γ-lactone was obtained. This latter compound was dissolved in methanol (4 ml.); phenylhydrazine (0.05 ml.) was added and the solution was refluxed for 40 minutes. Crystallization and recrystallization from absolute ethanol afforded the anhydrous phenylhydrazide derivative, m.p. 142–143°, $[\alpha]^{22}D$ −16.0° (*c* 1.0% in water). Literature values[35–37] are m.p. 144°. $[\alpha]^{20}D$ −16.5° (*c* 0.7% in water).

TABLE III

REFRACTIONATION OF FRACTIONS 2 AND 4

Fraction	Total petroleum ether added, ml.	Wt., g.[e]	OMe, %	$[\alpha]_D^{23}$ (c 1.0% in chloroform)
2A[a]	460	0.21	43.7	−20.3°
2B	[b]	.27	44.1	−20.3
4A[c]	600	.20	44.8	−17.6
4B	[d]	.20	..	....

[a] Fraction 2 was dissolved in 60 ml. of chloroform and 60 ml. of diethyl ether, and petroleum ether was added slowly with stirring. [b] Recovered by evaporation of mother liquor from fraction 2A. [c] Fraction 4 was dissolved in 10 ml. of chloroform and petroleum ether was added with stirring. [d] Recovered by evaporation of mother liquor from fraction 4A. (This black sirup was not further used.) [e] Fractions 2A, 2B and 4A precipitated as oils. They were dissolved in chloroform (10 ml.), poured into petroleum ether (500 ml.) and precipitated as flocculent near white solids.

Identification of 2,3,6-Tri-*O*-methyl-D-glucose.—This chromatographically pure sugar crystallized spontaneously from a sirup. The crystals were placed on a porous tile for 24 hours and then were washed with a few drops of diethyl ether (m.p. 112–114° obtained at this stage). Recrystallization from ether–petroleum ether mixture gave m.p. and mixed m.p. 121–122°, $[\alpha]^{22}D$ +67.9° (*c* 0.9% in water containing a trace of ammonia). The literature[38] quotes m.p. 121–123°, $[\alpha]D$ +70° (H_2O).

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—Preliminary paper chromatography experiments using authentic samples of tetra-*O*-methyl-D-glucose and tetra-*O*-methyl-D-mannose and a large number of solvent systems showed that none of these solvent systems satisfactorily resolved these two components. The most promising results were obtained with a petroleum ether (60–110°)–2-butanone–methanol (10:1:1) system, but results could not be readily duplicated presumably because of the volatility of the solvent system.

The tetra-*O*-methyl component (88 mg.) obtained from the paper eluates had $[\alpha]^{22}D$ +53.5° (*c* 0.9% in water) which indicated a purity of about 64%. The aniline derivative was prepared from the crude crystals separated from the adhering sirup by use of a porous tile. These crystals were recrystallized from ether–petroleum ether mixture into three fractions and the melting point of each fraction determined: first fraction, m.p. 135–136°; second fraction, m.p. 135–136°; and third fraction, m.p. 120°. The literature m.p. for the N-phenyl-D-glucopyranosylamine-2,3,4,6-tetramethyl ether is 137–138°,[39] while the m.p. of the corresponding aniline derivative of 2,3,4,6-tetra-*O*-methyl-D-mannose is 144–145°.[40]

Previous work[15] has shown that a mixture of the aniline derivatives of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose may be separated by fractional crystallization. It therefore was concluded from the fractional crystallization m.p. data that only 2,3,4,6-tetra-*O*-methyl-D-glucose was present in this component.

Identification of 2,3,4,6-Tetra-*O*-methylgalactose.—This sirup (16 mg.) which chromatographically corresponded to authentic 2,3,4,6-tetra-*O*-methyl-D-galactose was treated with aniline to give 2,3,4,6-tetra-*O*-methylgalactose N-phenylglycosylamine, m.p. and mixed m.p. 189–190° after sublimation and recrystallization from ether–petroleum ether; lit. m.p. for aniline derivative 192°.[35]

(35) C. M. Rafique and F. Smith, THIS JOURNAL, **72**, 4634 (1950).
(36) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).
(37) F. Smith, THIS JOURNAL, **70**, 3249 (1948).
(38) H. C. Carrington, W. N. Haworth and E. L. Hirst, *ibid.*, **55**, 1084 (1933).
(39) M. L. Wolfrom and W. L. Lewis, *ibid.*, **50**, 837 (1928).
(40) W. N. Haworth, R. L. Heath and S. Peat, *J. Chem. Soc.*, 833 (1941).

Identification of a Di-*O*-methylgalactose.—This sirup (4 mg.) constituted less than 0.7% of the hydrolyzed methylated sugars and its mobility on solvent G placed it in the di-*O*-methylhexose class. On demethylating the sirup in a sealed tube (1 ml. of 48% hydrobromic acid on boiling water-bath for 20 minutes), this compound was shown chromatographically (solvent C) to correspond to galactose.

Acknowledgment.—The authors wish to express their thanks to Drs. H. W. Kircher and N. S. Thompson for many most valuable suggestions and discussions.

SHELTON, WASH.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Synthesis of Amino Sugars by Reduction of Hydrazine Derivatives[1]

BY M. L. WOLFROM, F. SHAFIZADEH AND R. K. ARMSTRONG

RECEIVED APRIL 28, 1958

Reduction of the hydrazino compounds formed by the replacement of sulfonyloxy group in the sugar series provides a convenient approach to the synthesis of amino sugars. 3-Amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and a methyl 2-amino-2-deoxy-3,4-*O*-isopropylidene-β-L-pentopyranoside have been prepared by this method. The former product was further characterized as the picrate and salicylaldehyde Schiff base. The latter product has been isolated as the salicylaldehyde Schiff base and converted to the corresponding methyl 2-amino-2-deoxy-β-L-pentopyranoside hydrochloride and 2-amino-2-deoxy-α-L-pentose hydrochloride. Some of the properties of this compound have been compared with those of other 2-amino-2-deoxyaldoses.

In a previous paper[2] a convenient method has been described for the synthesis of a variety of amino-deoxy-alditols and 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose, through the reduction of the phenylhydrazone derivative of the corresponding aldose or carbonyl compound. Application of this method for the synthesis of other amino sugars is naturally limited by the availability of the corresponding phenylhydrazone derivatives. The present work provides an extension of this work to the reduction of hydrazino compounds which may be obtained through the replacement of sulfonyloxy groups. Direct replacement of the secondary sulfonyloxy groups in general is very difficult[3] and the results obtained by the drastic treatment of methyl 3,4,6-tri-*O*-methyl-2-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside[4] and the corresponding galactopyranoside derivative[5] with alcoholic ammonia have been discouraging as a method of synthesis. The direct replacement of the secondary sulfonyloxy groups with hydrazine, however, appears to be more practical. Thus, Freudenberg and Brauns[6] obtained 3-deoxy-3-hydrazino-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (I) in 60% yield. Reduction of this compound with Raney nickel catalyst in the Parr hydrogenation apparatus readily provides 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (II), which has been converted to the salicylaldehyde Schiff base and the picrate salt. The above compound and some of its derivatives have been obtained previously by Freudenberg and associates,[7] less conveniently and in a somewhat smaller yield, through the replacement of a *p*-tolylsulfonyloxy group by ammonia (under pressure). The fact that this reaction proceeds without Walden inversion has been established by the synthesis of 3-amino-3-deoxy-D-glucose derivatives through the treatment of methyl 3,4-anhydro-β-D-allopyranoside and methyl 2,3-anhydro-4,6-benzylidene-α-D-allopyranoside with ammonia.[8]

Application of the above process to methyl 3,4-*O*-isopropylidene-2-*O*-*p*-tolylsulfonyl-β-L-arabinopyranoside[9] (III) provided a methyl 2-amino-2-deoxy-3,4-*O*-isopropylidene-β-L-pentopyranoside (IV), which could have either the L-*arabino* or the L-*ribo* configuration. This product was isolated as the salicylaldehyde Schiff base. Hydrolysis of the isopropylidene group with hydrochloric acid gave the corresponding methyl 2-amino-2-deoxy-β-L-pentopyranoside hydrochloride (V) in crystalline form. As may be expected from the well established properties of 2-amino-2-deoxy-D-glucose derivatives,[10-12] the glycosidic group of the above compound was quite resistant toward acid hydrolysis. Consequently the free sugar, a 2-amino-2-deoxy-α-L-pentose hydrochloride (VI), was obtained from the acetylation of the methyl glycoside and subsequent hydrolysis of the sirupy acetate with hydrochloric acid. The free sugar displayed the rapid mutarotation shown in Fig. 1, and on acetylation with silver acetate and acetic anhydride furnished a 2-acetamido-2-deoxy-L-pentose.

Previously the only known 2-amino-2-deoxypentose, was the D-xylose derivative, synthesized by Wolfrom and Anno[13] from the configurationally related 2-amino-2-deoxy-D-glucose. The above compound is the second member of this series. The L-arabinose configuration may be assigned to the new 2-amino-2-deoxypentose on the assumption that the direct replacement of sulfonyloxy group with

(1) Supported by Grant No. CY-3232 from the Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda 14, Md.

(2) M. L. Wolfrom, F. Shafizadeh, J. O. Wehrmüller and R. K. Armstrong, *J. Org. Chem.*, **23**, 571 (1958).

(3) R. S. Tipson, *Advances in Carbohydrate Chem.*, **8**, 107 (1953).

(4) W. O. Cutler and S. Peat, *J. Chem. Soc.*, 782 (1939); W. N. Haworth, E. L. Hirst and L. Panizzon, *ibid.*, 154 (1934).

(5) L. F. Wiggins, *ibid.*, 522 (1944).

(6) K. Freudenberg and F. Brauns, *Ber.*, **55**, 3233 (1922).

(7) K. Freudenberg, O. Burkhart and E. Braun, *ibid.*, **59**, 714 (1926).

(8) S. Peat and L. F. Wiggins, *J. Chem. Soc.*, 1810 (1938).

(9) J. Honeyman, *ibid.*, 990 (1946).

(10) J. C. Irvine and A. Hynd, *ibid.*, **101**, 1128 (1912); **105**, 698 (1914).

(11) R. C. G. Moggridge and A. Neuberger, *ibid.*, 745 (1938).

(12) A. B. Foster, D. Horton and M. Stacey, *ibid.*, 81 (1957).

(13) M. L. Wolfrom and K. Anno, THIS JOURNAL, **75**, 1038 (1953).