TABLE II

Solvent	Water			50% Alcohol			Pyridine		
Hours	3	24	48	3	24	48	3	24	48
D-Galactose	20.5	64.5	73.0	18.5	56.5	67.0	28.2	60.5	71.0
D-Glucose	0.0	8.4	0.0	7.9	14.3	18.0	3.4	38.2	64.0
D-Xylose	30.4	38.5	51.3	23.9	46.4	48.7	29.8	50.5	54.5
L-Arabinose	38.8	41.5	51.1	46.0	36.4	43.5	48.0	41.3	41.3
L-Rhamnose	17.5	42.9	13.7	25.5	22.0	12.7	32.4	45.0	24.0

a solution of 7.5 g. of sodium nitrite in 15 ml. of water. This was diluted to 93 ml.

Preparation of Formazans.—A mixture of 0.45 g. of aldose (corresponding to 0.0025 mole of hexose, or 0.0030 mole of pentose or 0.00275 mole of 6-deoxyhexose) and 0.45 g. of phenylhydrazine (0.00418 mole) was dissolved (i) in 2.5 ml. of water, (ii) in 2.5 ml. of 50% alcohol, (iii) in 2.5 ml. of pyridine[6] and allowed to stand at room temperature (20 ± 2°).

After the desired period of time, 2.5 ml. of pyridine was added to the aqueous and 50% alcohol–water solution, respectively, and 2.5 ml. of absolute alcohol to the pyridine solution. They were refrigerated and then coupled by dropwise addition of the diazonium solution prepared from 0.24 g. of aniline (1 mole + 7.5%) and allowed to stand for 10 minutes. After this time, the solutions were poured into ice-water five times their volume, and kept in the ice-box overnight. The precipitated, bright-red, mostly crystalline formazans were then collected, dried in air at room temperature, and weighed. In the case of mannose, from all three solvents the precipitated phenylhydrazone was collected, washed with alcohol, water and ether, then dissolved in 68 ml. of pyridine. On adding 7 ml. of ethanol, the solution was finally coupled with diazo solution prepared from 0.24 g. of aniline; yields 0.63, 0.43, 0.48 g. of formazan (67.5, 45.7, 51.4%).

No formazan could be observed from L-sorbose or D-fructose in any of the media; merely a little of a yellowish-pinky, oleaginous, sticky substance precipitated.

Yields, in per cent., obtained in aqueous solution, in 50% alcohol, and in pyridine are given in Table II.

For identification, the crude products were crystallized to constant m.p. from hot butanol, with the exception of the formazan obtained from D-xylose, which was crystallized by washing the crude product with abs. ether, dissolving it in hot alcohol, and adding hot water to it (alcohol to water 3:2).

Anal. Calcd. for $C_6H_{20}O_4N_4$: N, 16.27. Found for D-xylose: N, 16.12, 16.06; for L-arabinose: N, 16.12, 16.03.[7]

Solubility of the resulting formazans was determined with various methods. In distilled water at 20° it was found to be 5–10 mg./100 ml.

(6) With galactose 90% *aqueous* pyridine was used.

(7) For the analyses of the other sugar formazans *cf.* our previous papers.[1,2]

BUDAPEST, HUNGARY

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Acetolysis of the Glucomannan of Iles Mannan[1]

BY F. SMITH AND H. C. SRIVASTAVA

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The glucomannan of Iles mannan, the polysaccharide extracted from the tubers of *Amorphophallus* plants gives on acetolysis followed by deacetylation a mixture of oligosaccharides in addition to D-glucose and D-mannose. Three of the oligosaccharides have been obtained in a crystalline form and have been shown to be 4-*O*-β-D-glucopyranosyl-α-D-mannopyranose, 4-*O*-β-D-glucopyranosyl-β-D-glucopyranose (cellobiose) and 4-*O*-β-D-mannopyranosyl-α-D-glucopyranose. The structure of the last-named hitherto unknown disaccharide has been proved by methylation studies. The structural pattern of the glucomannan polysaccharide is discussed.

Iles mannan obtained from the tubers of the *Amorphophallus oncophyllus* and *A. variabilis* has been shown[2] to be a mixture of two polysaccharides, a glucomannan and an amylose-like polyglucosan, in the ratio of approximately 6:1. Methylation studies[2] showed that the glucomannan component was composed of D-glucopyranose and D-mannopyranose units linked together by 1,4′-β-linkages, the ratio of mannose to glucose being approximately 2:1.

Information concerning the sequence of the sugar residues in the glucomannan may be obtained by stepwise degradation and characterization of oligosaccharides of varying degree of polymerization. This paper is concerned with the partial degradation of the glucomannan component of Iles mannan by acetolysis and the isolation and characterization of three oligosaccharides, namely, 4-*O*-β-D-glucopyranosyl-β-D-glucopyranose (cellobiose), 4-*O*-β-D-glucopyranosyl-α-D-mannopyranose and 4-*O*-β-D-mannopyranosyl-α-D-glucopyranose.

The glucomannan was separated from Iles mannan meal by precipitation as a copper complex and subsequent decomposition of the latter with dilute acid as previously described.[2] Preliminary experiments were carried out in order to establish the conditions under which acetolysis produced the largest amounts of oligosaccharides. The maximum yield of the oligosaccharide acetates was obtained when the acetolysis[3] was conducted initially at 0° and after 24 hours completed by heating. The nature and relative amounts of the products formed by acetolysis was determined by deacetylation of the mixture of acetates followed by qualitative paper chromatography. Acetolysis was found to give several oligosaccharides as well as D-glucose and D-mannose. The mixture of mono- and oligosac-

(1) Paper No. 3459, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper will form part of a thesis to be submitted by H. C. Srivastava to the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D.

(2) P. A. Rebers and F. Smith, THIS JOURNAL, **76**, 6097 (1954).

(3) *Cf.*, K. S. Barclay, E. J. Bourne, M. Stacey and M. Webb, *J. Chem. Soc.*, 1501 (1954).

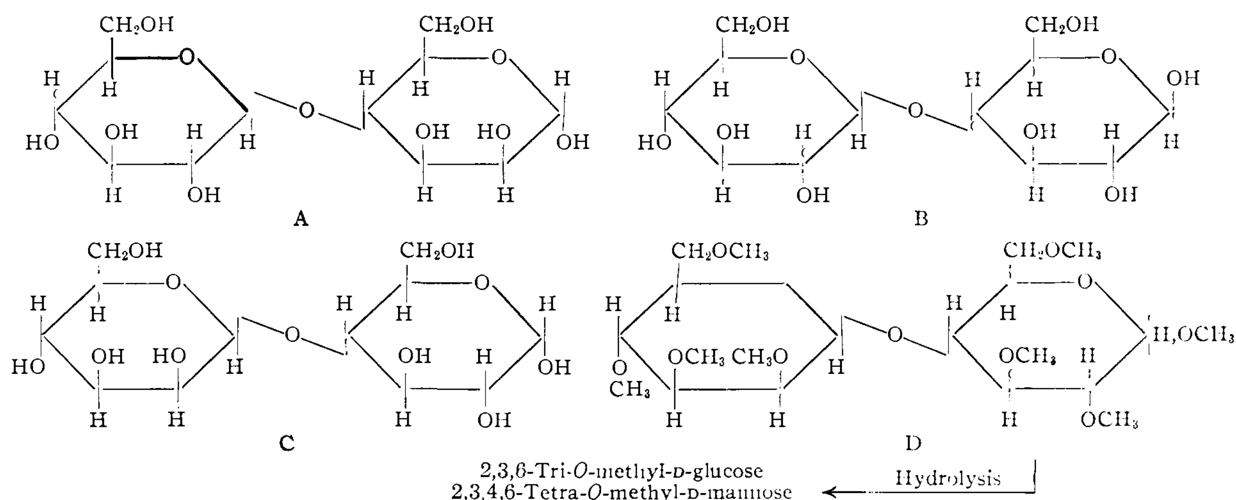


Fig. 1.

charides was resolved into its components by chromatography first on a charcoal-celite column[4] and then on a cellulose column.[5] In certain cases sheet paper chromatography proved to be effective for final purification of the oligosaccharides.

That the oligosaccharides were not reversion products was shown by the fact that when a mixture of glucose and mannose (in the ratio of 1:2) was treated with acetic anhydride and sulfuric acid under conditions similar to those in the acetolysis of the glucomannan and the reaction product deacetylated, chromatographic analysis on paper showed no evidence for the formation of oligosaccharides.

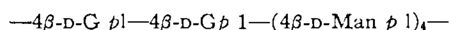
Three of the oligosaccharides produced by acetolysis and subsequent deacetylation have been crystallized and identified as 4-*O*-β-D-glucopyranosyl-α-D-mannopyranose (A), 4-*O*-β-D-glucopyranosyl-β-D-glucopyranose (cellobiose, β) and 4-*O*-β-D-mannopyranosyl-α-D-glucopyranose (C).

The oligosaccharide A which had m.p. 134–139° and showed $[\alpha]^{24}\text{D}$ +4.7° (final) in water gave equal parts of glucose and mannose upon acid hydrolysis as ascertained by paper chromatographic analysis. Bromine oxidation followed by hydrolysis afforded glucose and mannonic acid. This showed that the oligosaccharide was a glucosyl-mannose. Since the glucomannan had already been shown to possess only 1,4′-linkages, it appeared likely that this disaccharide was a 4-glucosyl-mannose. This proved to be correct for the disaccharide was found to be identical with the 4-*O*-β-D-glucopyranosyl-α-D-mannopyranose previously synthesized.[6,7] Moreover, it gave rise to octa-*O*-acetyl-4-*O*-β-D-glucopyranosyl-D-mannose[8] upon acetylation with sodium acetate and acetic anhydride.

The oligosaccharide B which had m.p. 227° and showed $[\alpha]^{26}\text{D}$ +28° → +34° in water and gave only glucose upon treatment with β-glucosidase proved to be identical with 4-*O*-β-D-glucopyranosyl-β-D-glucopyranose (cellobiose). Its identity was confirmed by converting it into the corresponding known β-octaacetate.

The third oligosaccharide C having m.p. 203–204° and $[\alpha]^{20}\text{D}$ +30° → +19° in water gave upon hydrolysis glucose and mannose in equal amounts. Its molecular weight determined by oxidation with alkaline hypoiodite[9] and by bromine water corresponded to that of a disaccharide. Hydrolysis of the bromine oxidation product yielded mannose and gluconic acid. Moreover, β-glucosidase had no hydrolytic action on this oligosaccharide. It was apparent therefore that C was a mannosylglucose and since the original glucomannan was known to be composed of glucose and mannose units joined by 1,4′-glycosidic bonds, the oligosaccharide C must also contain the 1,4′-linkage. Proof of this followed from the observation that methylation of C to give D followed by hydrolysis of the latter gave rise to 2,3,4,6-tetra-*O*-methyl-D-mannose, identified as the anilide,[10] and 2,3,6-tri-*O*-methyl-D-glucose identified as the 1,4-bis-*p*-nitrobenzoate.[2] From the fact that the oligosaccharide has a low initial rotation (+30°, water) it is believed that the biose linkage is of the β-type and since it displays a downward mutarotation, the configuration at C_1 is probably α. The disaccharide is therefore designated 4-*O*-β-D-mannopyranosyl-α-D-glucopyranose.

Since it has already been established that the glucomannan has a linear structure[2] and is composed of two parts of mannose and one part of glucose, and if it may be assumed that the structural pattern is repeated throughout the polymer molecule, then the isolation of cellobiose only, and none of its related homologs such as cellotriose or cellotetraose requires that the structural pattern of the repeating unit in the polysaccharide be tentatively formulated as



By joining two of these together the formation of the two other disaccharides, 4-*O*-β-D-glucopyranosyl-D-mannose and 4-*O*-β-D-mannopyranosyl-D-glu-

(4) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(5) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(6) M. Bergmann and H. Schotte, *Ber.*, **54**, 1570 (1921).

(7) W. N. Haworth, E. L. Hirst, H. R. L. Streight, H. A. Thomas and J. I. Webb, *J. Chem. Soc.*, 2636 (1930).

(8) D. H. Brauns, THIS JOURNAL, **48**, 2776 (1926).

(9) J. L. Baker and H. F. E. Hulton, *Biochem. J.*, **14**, 754 (1920).

(10) J. C. Irvine and D. McNicoll, *J. Chem. Soc.*, **97**, 1449 (1910).

cose during degradation of the glucomannan becomes apparent. Since no mannobiose or mannotriose was isolated, it would appear that the linkage between the mannose units is cleaved more readily than the others during the process of acetolysis. Further support for the above formulation for the glucomannan is now being sought in the identification of the other oligosaccharides produced by acetolysis.

Experimental

All evaporations were done under reduced pressure at 35–45°. The melting points are uncorrected. The following solvents were used for partition chromatography: (1) pyridine–ethyl acetate–water (1:2.5:3.5—upper layer),[11] (2) 1-propanol:water azeotrope, (3) 2-butanone-water azeotrope,[12] (4) 1-butanol–ethanol–water (5:1:4—upper layer), (5) benzene-ethanol–water (200:47:15—upper layer)[13] and (6) 1-butanol–acetic acid–water (4:1:5—upper layer). The following spray reagents were used for the detection of sugars and their derivatives: (a) acetonic silver nitrate and alcoholic caustic soda,[14] (b) *p*-anisidine trichloroacetate,[15] (c) periodate–benzidine[16] and (d) hydroxylamine–ferric chloride.[17]

Isolation of the Glucomannan from Iles Mannan.—To Iles mannan flour (20 g.) was added sodium xylene sulfonate (40 ml., 50% solution) and the mannan allowed to swell.[2] Sodium hydroxide (200 ml., 30% by weight) was then added and the mixture allowed to stand for 0.5 hour when the material swelled still further. The mixture was heated at 60° and water (1800 ml.) in portions of 100 ml. was added with stirring to effect solution. The solution was centrifuged to remove the undissolved residue and to the supernatant liquid Fehling solution B (400 ml.) and Fehling solution A (300 ml.) were added with stirring. The copper complex, which precipitated as a jelly, was filtered, washed with dilute Fehling solution and decomposed by suspending it in water at 0° and adding *N* hydrochloric acid dropwise. The portion (A) of the complex which did not dissolve was removed (centrifuge). The supernatant was poured into 95% ethanol when there separated a white jelly-like precipitate with a greenish-blue tinge. The color due to copper was removed by trituration with acetone–acetic acid.

The portion A of the copper complex, which had not dissolved in *N* hydrochloric acid, was dissolved in ammonium hydroxide (sp. gr., 0.9) and the deep-blue solution was dialyzed against distilled water until no more color could be removed. The glucomannan was precipitated by pouring the dialyzed solution into 95% ethanol. The light blue-green color of the polysaccharide was removed by trituration with acetone–acetic acid as described previously. The two fractions of the glucomannan polysaccharide thus obtained from the decomposition of the copper complex were combined and reprecipitated by dissolving in sodium hydroxide (30%) and adding Fehling solution, the copper complex of the polysaccharide being decomposed with 2 *N* hydrochloric acid as in the previous manner. The polysaccharide was precipitated by pouring the solution into alcohol and the copper ions removed, with acetone–acetic acid. The glucomannan thus obtained was a white amorphous granular powder (16.3 g.) which had $[\alpha]^{25}D$ −32° (*c* 1) in 30% sodium hydroxide.

Acetolysis of the Glucomannan. Experiment (a).—The glucomannan (0.5 g.) was shaken with a mixture of acetic anhydride (2.5 ml.), acetic acid (2.8 ml.) and concentrated sulfuric acid (0.14 ml.) for 6.5 days at room temperature. When the reaction mixture was poured with stirring into water no precipitate was formed. Extraction of the mixture with chloroform gave a sirupy acetate (0.4 g.). Deacetylation of the acetate mixture in methyl alcoholic solution with a catalytic amount of sodium[18] gave a mixture of reducing sugars and oligosaccharides which were examined by paper chromatography using solvent 1 and spray reagent a. The results are given in Table I.

Experiment (b).—In a second experiment the glucomannan (0.5 g.) was shaken with acetic anhydride (10 ml.) and sulfuric acid (0.3 ml.) for 38 hours. The yield of acetolysis product which separated on pouring the reaction mixture into water was 0.4 g. The components produced upon deacetylation are given in Table I.

TABLE I

CHROMATOGRAPHIC ANALYSIS OF THE COMPONENTS PRODUCED BY ACETOLYSIS OF GLUCOMANNAN

$R_{mannose}$ (R_m) values (solvent 1, spray reagent a)

Experiment (a) Component	R_m	Experiment (b) Component	R_m
1	0.17	a	0.10
2	.37	b	.32
3	.49	c	.45
4	.70		
5 (glucose)	.86	5 (glucose)	.85
6 (mannose)	1.0	6 (mannose)	1.00
7 (maltose standard)	0.55	7 (maltose standard)	0.55

It is believed that components 2 and 3 of experiment (a) are similar if not identical with components b and c of experiment (b). Component a of experiment (b) having R_m 0.1, however, is believed to be a higher oligosaccharide and is not identical with component 1 of experiment (a).

Experiment (c).—The glucomannan (4.6 g.) in small portions was added with stirring to a mixture of acetic anhydride (17.5 ml.) and sulfuric acid (1.8 ml.) cooled to 0°. After all the polysaccharide was added, the mixture was stirred (at 0°) for another 2 hours, and then at room temperature for 12 hours. More acetic anhydride (4.6 ml.) was added and the mixture stirred for a further period of 33 hours after which time the reaction was completed by heating at 80–90° for 15 minutes (rapid darkening of the solution took place during heating). The reaction mixture was poured with stirring into cold water when a grey-white precipitate was obtained. It was filtered, washed with water and dried (7.8 g.). The mixture of acetates gave upon deacetylation[18] four oligosaccharides in addition to glucose and mannose.

An attempt to resolve the mixture of the acetates of the mono- and oligosaccharides using a calcium carbonate column according to the procedure of Bredereck, *et al.*,[19] failed.

Examination of Possible Reversion during Acetolysis.—To a mixture of glucose (0.5 g.) and mannose (1.0 g.) was added a cold (0°) mixture of acetic anhydride (6.6 ml.) and sulfuric acid (0.67 ml.) and the reaction mixture stored in the cold (5°) with occasional shaking for 24 hours. The reaction mixture was further allowed to stand at room temperature (20–25°) for another 24 hours, and then heated at 80-90° for 15 minutes. The reaction mixture was poured into water with stirring and the acetates, after extraction with chloroform, isolated in the usual manner. Upon deacetylation of the sirupy mixture of acetates and chromatographic analysis, only glucose and mannose were found to be present.

Separation of the Mono- and Oligosaccharides on a Charcoal Column.—A portion (1.0 g.) of the mixture of sugars obtained in (b) was fractionated on a charcoal-celite column[4] in the usual way. The column was eluted successively with water (2 l.),[20] 5% aqueous ethanol (1.5 l.), 15% aqueous ethanol (1 l.) and 50% aqueous ethanol.

The product eluted with 50% ethanol was a degraded

(11) E. F. McFarren, K. Brand and H. R. Rutkowski, *Anal. Chem.*, **23**, 1146 (1951).

(12) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

(13) G. A. Adams, *Can. J. Chem.*, **33**, 56 (1955).

(14) W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

(15) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(16) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954).

(17) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 5859 (1951).

(18) G. Zemplén, *Ber.*, **59**, 1258 (1926).

(19) H. Bredereck, H. Dürr and K. Ruck, *Ber.*, **87**, 526 (1954).

(20) In other experiments, it was found better to pack the charcoal-Celite mixture as a slurry in 2.5% aqueous ethanol, and to begin the separation of the mixture of sugars with the same solvent rather than with water. This procedure results in a more effective separation of the monosaccharides from the oligosaccharides than is the case with water (Allene Jeanes, C. A. Wilham, R. W. Jones, H. M. Tsuchiya and C. E. Rist, THIS JOURNAL, **75**, 5911 (1953)).

TABLE II

FRACTIONATION OF SUGARS OBTAINED FROM GLUCOMANNAN ON THE CHARCOAL COLUMN

Solvent, 5% ethanol			Solvent, 15% ethanol		
Fraction	Wt. (mg.)	Component[a]	Fraction	Wt. (mg.)	Component[a]
1, 2, 3	27	Glucose, Mannose, Oligosaccharides A and B	1, 2	200	Oligosaccharides A, B and C
4, 5	126	Oligosaccharide C plus small amount of B	3, 4, 5	116	Oligosaccharides A, B, C and a fourth higher oligosaccharide
6, 7	48	Oligosaccharide C			

[a] The components in each fraction were ascertained by paper chromatography using solvent I.

polysaccharide (300 mg.) which did not move on a paper chromatogram using solvent 1.

Separation of the Oligosaccharides on Cellulose Column.—The combined mixture of sugars (314 mg.) from fractions 1, 2, 3, 4 and 5 of the 15% alcohol eluate from the charcoal column (Table II) was dissolved in 1-propanol:water azeotrope and put on a cellulose column previously equilibrated with the same solvent. The column was developed with solvent 2 and the fractions (10 ml.) collected at intervals of 30 minutes. The fractions containing pure oligosaccharides as checked by paper chromatography were combined. On evaporation *in vacuo* and crystallization from aqueous ethanol, oligosaccharides A, B and C were obtained in a pure state.

Characterization of 4-O-β-D-Glucopyranosyl-α-D-mannopyranose.—Oligosaccharide A from the cellulose column obtained as a sirup had $[\alpha]^{25}D$ +4.7° (equilibrium value) in water (*c* 2), and the same R_f in solvents 1, 2 and 4 as 4-*O*-β-D-glucopyranosyl-D-mannose. When a solution of A in ethanol was allowed to evaporate slowly in the air, large crystals of 4-*O*-β-D-glucopyranosyl-α-D-mannopyranose monohydrate were obtained, m.p. and mixed m.p. 134–139°. When the oligosaccharide A was hydrolyzed with *N* sulfuric acid and the resulting mixture of glucose and mannose resolved and the sugars determined quantitatively according to the procedure described below in the identification of 4-*O*-β-D-mannosyl-D-glucose, the oligosaccharide was found to be composed of equal parts of glucose and mannose.

Acetylation of the crystals (25 mg.) by treatment with acetic anhydride (3 ml.) and anhydrous sodium acetate (0.1 g.) for 2.5 hours on a boiling water-bath gave octa-*O*-acetyl-4-*O*-β-D-glucopyranosyl-α-D-mannopyranose (needles from methanol) m.p. and mixed m.p. 203°, $[\alpha]^{20}D$ +34° in chloroform (*c* 0.6). Brauns' reports m.p. 202-203° and $[\alpha]D$ +36° in chloroform for this compound.

Oxidation of 4-O-β-D-Glucopyranosyl-D-mannose with Bromine.—To a solution of A (7 mg. in 2 ml. H_2O), barium carbonate (10 mg.) and bromine (2 drops) were added. The reaction mixture was shaken and kept at room temperature (25°) in the dark for 2.5 days. Excess bromine was removed by aeration, the mixture filtered and the filtrate evaporated. The residual sirup was hydrolyzed in a sealed tube with *N* sulfuric acid on a boiling water-bath for 11 hours. After neutralization ($BaCO_3$) and filtration, the filtrate was evaporated and the residue extracted with hot absolute ethanol. The ethanol extract, after filtration and evaporation and chromatographic analysis, was found to contain glucose only. The residue left after ethanol extraction was dissolved in water and passed through a column of cation-exchange resin. The effluent after evaporation was chromatographed using solvent 6 and spray reagent d. The results indicated the presence of D-mannono-γ-lactone.

Identification of 4-O-β-D-Glucopyranosyl-β-D-glucopyranose (β-Cellobiose).—Oligosaccharide B, obtained from the cellulose column in a crystalline form, had m.p. 227° (undepressed on admixture with β-cellobiose) and showed $[\alpha]^{25}D$ +28 → +34° (equilibrium value) in water (*c* 1). Upon incubation of a solution of B in sodium acetate buffer (*p*H 5) with β-glucosidase in a melting point tube at 38° for 24 hours according to the procedure of Porter, *et al.*,[21] the oligosaccharide was found, by paper chromatography, to be completely hydrolyzed to glucose.

(21) W. L. Porter and N. Hoban, *Anal. Chem.*, **26**, 1846 (1954).

Octa-O-acetyl-β-cellobiose.—The disaccharide B (60 mg.), anhydrous sodium acetate (0.5 g.) and acetic anhydride (5 ml.) were shaken overnight and then heated on a boiling water-bath for 6 hours. The reaction mixture was poured into water when a granular precipitate was obtained. It was extracted with chloroform and the chloroform extract, after washing successively with a solution of sodium bicarbonate and water and drying, was evaporated to give a crystalline acetate (65 mg.). It was recrystallized from 95% ethanol; m.p. 188° and $[\alpha]^{25}D$ −5.4° in chloroform (*c* 1.5) (m.p. and $[\alpha]D$ in chloroform of octa-*O*-acetyl-β-cellobiose reported by Hudson[22] 202°, −14.7° and that by Maquenne[23] 191-192° and −7.4°, respectively); mixed m.p. with an authentic specimen of octa-*O*-acetyl-β-cellobiose (m.p. 195-196°) 191-195°.

Crystallization of 4-O-β-D-Mannopyranosyl-D-glucose.—The white amorphous solid (48 mg.) obtained on evaporation of the 5% aqueous ethanol eluate (fractions 6 and 7) from the charcoal column was refluxed with absolute ethanol (10 ml.) and a drop of water, until all the oligosaccharide was dissolved. On cooling the solution, crystals in the form of needles separated. After keeping in the cold (5°) for 3 days, the crystals were filtered, washed with ethanol and dried m.p. 185-188° (yield 16 mg.). After recrystallization from ethanol and a few drops of water the 4-*O*-β-D-mannopyranosyl-α-D-glucopyranose had m.p. 202-203° and $[\alpha]^{25}D$ −30° changing in 4.5 hours to +19° in water (*c* 1).

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.1; H, 6.5. Found: C, 42.3; H, 6.8.

Identification of 4-O-β-D-Mannopyranosyl-α-D-glucopyranose: Determination of Molecular Weight (a).—To a solution of C (10.7 mg. in 1 ml. water) was added a solution of iodine (5 ml., 0.05 *N*) followed by sodium hydroxide (7.5 ml., 0.05 *N*). After 10 minutes the solution was acidified and titrated with 0.01 *N* sodium thiosulfate (6.2 ml.). The equivalent weight by this method was 346 (calcd. for a disaccharide 342). **(b) Bromine Oxidation.**—A solution of C (20 mg.) in water (1 ml.) was treated with bromine (0.3 ml.) and barium carbonate (50 mg.). After keeping the reaction mixture at room temperature for 72 hours and removing the excess of bromine, chromatographic analysis showed that oxidation was incomplete. Oxidation was completed by adding more bromine (0.3 ml.) and heating the mixture at 55-60° for 6 hours in presence of barium carbonate (50 mg.). The reaction mixture was freed from bromine, centrifuged and the supernatant passed through a cation-exchange resin (Amberlite IR 120). The effluent was neutralized (Ag_2O), filtered and the filtrate evaporated. The residue was extracted with water and the extract filtered and the filtrate evaporated. The residue was extracted with water and the extract filtered after treatment with hydrogen sulfide. The filtrate was passed through a cation-exchange resin (Amberlite IR 120) and the effluent evaporated to a sirup which was dried *in vacuo* over sulfuric acid (yield 18.8 mg.). The equivalent weight of the acid derived from the oligosaccharide was determined by titration with 0.01 *N* sodium hydroxide. Found: equiv. wt., 310 (calcd. for a disaccharide acid, 358).

The sugar acid was hydrolyzed with *N* sulfuric acid in a sealed tube on a boiling water-bath for 22 hours. The hydrolysate was neutralized ($BaCO_3$), filtered and the filtrate passed first through a cation-exchange resin and then through an anion-exchange resin. The effluent was evaporated and the resulting sirup was found by paper chromatography to contain only mannose. The acid adsorbed by the anion-exchange resin was displaced by *N* sodium hydroxide and the effluent passed through a column of cation-exchange resin (Amberlite IR 120). Evaporation of the effluent and chromatographic analysis of the resulting sirup using solvent 6 and spray reagent d demonstrated the presence of D-glucono-γ-lactone.

Determination of the Glucose:Mannose Ratio in C.—A solution of oligosaccharide C (10 mg.) in sulfuric acid (2 ml., *N*) was heated for 24 hours in a sealed tube in a boiling water-bath. The solution, after neutralization ($BaCO_3$) and filtration, was evaporated and the resulting sirup chro-

(22) C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 1276 (1915).

(23) L. Maquenne and W. Goodwin, *Bull. soc. chim.*, [3] **31**, 856 (1904).

matographed on a sheet of paper using solvent 1. The zones containing glucose and mannose were cut out by the help of marginal guide strips and the sugars, after extraction from the paper with water, determined colorimetrically by the method of Dubois, *et al.*,[24] using phenol and sulfuric acid. The oligosaccharide was found to be composed of equal parts of glucose and mannose.

When a solution of the oligosaccharide was incubated with β-glucosidase in the manner described above, no hydrolysis occurred.

Methylation of 4-*O*-β-D-Mannopyranosyl-D-glucopyranose.—To a solution of C (137 mg.) in water (5 ml.) cooled in ice, potassium hydroxide (5 ml., 50%) and methyl sulfate (1 ml.) were added dropwise with vigorous stirring. In this manner 6 ml. of methyl sulfate and 30 ml. of potassium hydroxide solution were added over a period of 1.5 hours. During the addition of the reagents the temperature was maintained below 10°. The solution was stirred for another 6 hours and then more methyl sulfate (6 ml.) and potassium hydroxide (30 ml., 50%) were added at room temperature (25°). The reaction mixture which at this stage was nonreducing, was then heated to 50–60° and the methylation continued by the dropwise addition of methyl sulfate (5 ml.) and potassium hydroxide (25 ml., 40%) in the previous manner. The reaction mixture was heated at 90–100° for 1 hour and the product extracted from the cooled reaction mixture with chloroform. The extract on drying and evaporation gave a sirup (24 mg.). Since the yield of the methylated material was poor, the aqueous solution left after chloroform extraction was neutralized (sulfuric acid) and then diluted with an equal amount of methanol to precipitate the sodium sulfate. The precipitate was filtered and the filtrate evaporated. The material thus obtained was combined with the chloroform soluble product and, after adding acetone (20 ml.), methylated with methyl sulfate (10 ml.) and potassium hydroxide (50 ml., 40%) at 50–60° in the manner described above. The reaction mixture was heated at 90–100° for 1.5 hours, and, after cooling, extracted with chloroform. Evaporation of the dried ($MgSO_4$) chloroform extract afforded a sirup (75.5 mg.). The aqueous solution left after chloroform extraction was worked up in the previous manner and the material thus obtained was methylated separately with methyl sulfate and alkali. In this way 19 mg. more of chloroform soluble methylated material was obtained. It was combined with the product obtained previously, dissolved in ether, filtered and the filtrate evaporated to give a sirup (94 mg.). The latter was methylated twice with methyl iodide and silver oxide and the sirupy methylated product (82 mg.) isolated in the usual manner.

Hydrolysis of Octa-*O*-methyl-4-*O*-β-D-mannopyranosyl-D-glucopyranose.—The methylated oligosaccharide D (75 mg.) was refluxed with *N* sulfuric acid (5 ml.) for 18 hours, and the resulting solution, after neutralization ($BaCO_3$) and filtration evaporated to a sirup. The sirup was chromatographed on paper using solvents 3 and 5 and the methylated sugars detected by spray reagent b. The presence of 2,3,6-tri-*O*-methylglucose (component I) and 2,3,4,6-tetra-*O*-methylmannose (component II) was recognized by comparison with authentic samples of these two sugars (see Table III).

TABLE III

Methylated sugars	Solvent 3 R_G[a]	Solvent 5 R_M[b]
Component I	0.68	0.30
Component II	.98	0.97
2,3,4,6-Tetra-*O*-methyl-D-mannose	.98	1.00
2,3,6-Tri-*O*-methyl-D-mannose	.60	0.39
2,3,6-Tri-*O*-methyl-D-glucose	.68	.31
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.0	.91

[a] R_g = R (2,3,4,6-tetra-*O*-methyl-D-glucose). [b] R_m = R (2,3,4,6-tetra-*O*-methyl-D-mannose).

The mixture of methylated sugars, obtained on hydrolysis of the methylated oligosaccharide, was resolved into pure components I and II by chromatography on sheets of paper using solvent 3, when 33 mg. of 2,3,6-tri-*O*-methyl-D-glucose (I) and 39 mg. of 2,3,4,6-tetra-*O*-methyl-D-mannose (II), both in the form of sirup, were obtained.

Identification of 2,3,6-Tri-*O*-methyl-D-glucose.—Component I (22 mg.) which had $[\alpha]^{26}D$ +43° in methanol (*c* 0.7) was converted into a bis-*p*-nitrobenzoate by treatment of it in pyridine solution with *p*-nitrobenzoyl chloride.[2] After recrystallization from methanol the derivative (10 mg.) had m.p. and mixed m.p. with an authentic sample of 2,3,6-tri-*O*-methyl-D-glucose 1,4-bis-*p*-nitrobenzoate 192° and showed $[\alpha]^{28}D$ −34° in chloroform (*c* 2).[2]

Identification of 2,3,4,6-Tetra-*O*-methyl-D-mannose.—Component II (20 mg.), which had $[\alpha]^{25}D$ +24° in methanol (*c* 0.7), was converted into the corresponding anilide by boiling for 7 hours with ethanol (3 ml.) containing aniline (100 mg., freshly distilled). After keeping the reaction mixture overnight, the solvent and excess of aniline were removed *in vacuo* when the anilide crystallized. Recrystallization from ethanol–petroleum ether gave needles of 2,3,4,6-tetra-*O*-methyl-D-mannose anilide,[10] m.p. and mixed m.p. 144°, $[\alpha]^{25}D$ −7° (equil. value) in methanol (*c* 0.8). When mixed with 2,3,4,6-tetra-*O*-methyl-D-glucose anilide the m.p. was 118–127°.

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ST. PAUL, MINNESOTA

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