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STRUCTURAL ANALYSIS OF SUISEN GLUCOMANNAN*

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ABSTRACT

The main component of the mucilage in the bulbs of Suisen (*Narcissus tazetta* L., var. chinensis Roem) has been shown to be a glucomannan composed of D-glucose and D-mannose in the ratio of 2:3 and having a relatively low degree of branching. Acetolysis of the polysaccharide led to the isolation of β-(1→4)- and β-(1→3)-linked oligosaccharides composed of D-mannose and/or D-glucose residues. The average chain length (c.l.) of the polysaccharide was determined by methylation analysis to be about 22.

INTRODUCTION

Narcissus tazetta L., var. chinensis Roem, named Suisen in Japanese, is a perennial that grows wild or is cultivated for use as on ornamental plant throughout Japan. In 1931, Y. Kihara[1] suggested that the bulbs of this plant contained, in addition to starch, a polysaccharide that exists as a main constituent of the cold-water-soluble mucilage in the parenchymatous cells of the bulb scale. As this polymer gave D-glucose and D-mannose in the ratio of 1:2 on acid hydrolysis, it was termed "Suisen-glucomannan" by Kihara. However, its physical homogeneity and chemical structure have not as yet been established, and this paper is concerned with the structural features of the glucomannan deduced from fragmentation and methylation studies.

RESULTS AND DISCUSSION

Extraction of triturated bulbs of *N. tazetta* L. var. chinensis Roem with cold water, followed by purification as a copper complex, gave a white, powdery polysaccharide whose acetate was ultracentrifugally homogeneous and which yielded three parts of D-mannose and two parts of D-glucose on complete acid hydrolysis. The purified polysaccharide was insoluble in water, but dissolved in aqueous sodium hydroxide to give a viscous solution; $[\alpha]_D$ −44.3° (*c* 1.1, 10% sodium hydroxide).

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Neutralization of the alkaline solution with acid gave a gelatinous precipitate. The i.r. spectrum of the polysaccharide was similar to that of a glucomannan obtained from *Amorphophallus konjac*[2a].

Hydrolysis of the methylated polymer yielded mainly 2,3,6-tri-*O*-methyl-D-mannose and -D-glucose, isolated by carbon-column chromatography and identified respectively as the 1,4-di-(*p*-nitrobenzoate) and the crystalline ether.

2,3,4,6-Tetra-*O*-methyl-D-glucose and -D-mannose were identified as their methyl glycosides by gas chromatography. A small proportion of di-*O*-methylhexose was also detected, but no attempt was made to characterize this fraction. The latter is believed to be 2,6-di-*O*-methyl-D-mannose, which may represent a branching point of the polysaccharide, as the β-D-(1→3)-linked mannobiose was detected in an acetolyzate of the polymer. These results indicate that the polysaccharide is a glucomannan containing chains of β-(1→4)-linked D-glucose and D-mannose residues terminated at the non-reducing end by both hexose residues, with chains being (1→3)-joined by a β-D-mannose residue.

Di-*O*-methyl-D-glucose and a (1→3)-linked disaccharide containing D-glucose residues were not detected by methylation analysis and fragmentation analysis, respectively. This result may be a consequence of lack of branching at the D-glucose residues, or else because of the lower incidence of branching (if any) at the D-glucose residues than at the D-mannose residues.

The molar ratio of the tetra-, tri-, and di-*O*-methyl glycosides indicates an average of about 22–23 hexose residues per non-reducing end-group (Table I).

TABLE I

GAS CHROMATOGRAPHY OF METHANOLYSIS PRODUCT OF THE METHYLATED GLUCOMANNAN

Methyl glycoside of	*Relative retention time*	*Molar ratio*
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00 (β)	1.00
	1.38 (α)	
2,3,4,6-Tetra-*O*-methyl-D-mannose	1.22	
2,3,6-Tri-*O*-methyl-D-glucose	1.84 (β)	20.6
	2.25 (α)	
2,3,6-Tri-*O*-methyl-D-mannose	2.31	
2,6-Di-*O*-methyl-D-mannose	3.50	0.7

Further structural details on the polymer were obtained from fragmentation analysis by acetolysis. Acetolysis yielded a mixture of D-mannose, D-glucose, and the oligosaccharides composed of D-mannose and/or D-glucose residues.

Fractionation of the mixture by carbon-column chromatography followed by preparative paper-chromatography allowed the isolation of each oligosaccharide (Fig. 1). Isolation of the oligosaccharides 4-*O*-β-mannosylcellobiose (Man→Glc→Glc) and 4-*O*-β-mannosylepicellobiose (Man→Glc→Man) shows that the glucomannan

contains both isolated and contiguous D-glucose residues. This result contrasts with that found for a glucomannan obtained from loblolly pine[3], which contains only isolated D-glucose residues, although some glucomannans are known that have isolated and contiguous D-glucose residues.

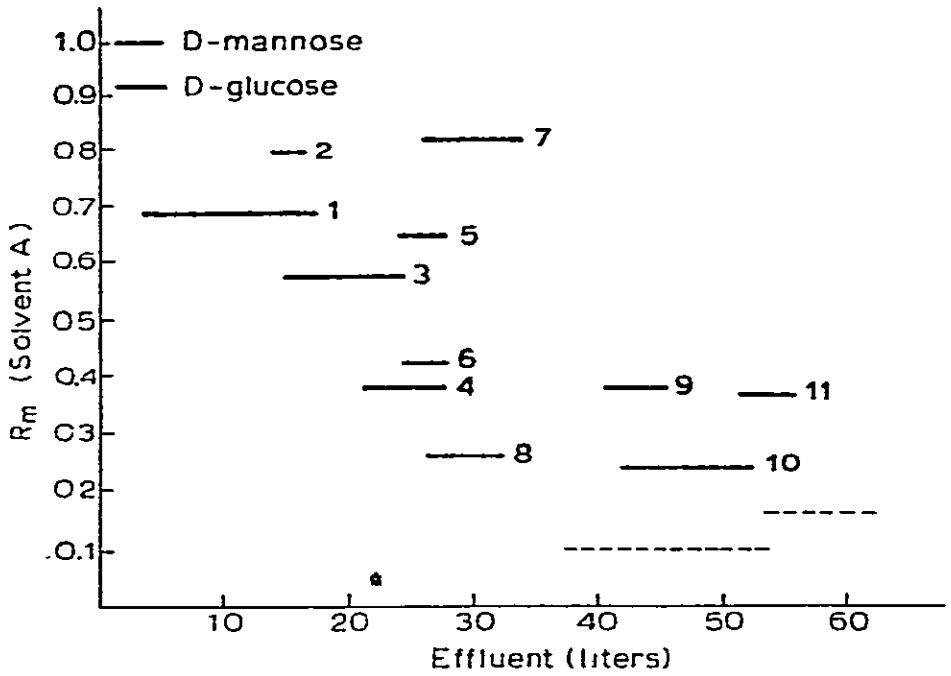


Fig. 1. Carbon-column and paper-chromatographic behavior of *O*-de-acetylated products of acetolyzate of the glucomannan: β-D-Man-(1→4)-D-Man (**1**), β-D-Man-(1→3)-D-Man (**2**), β-D-Man-(1→4)-D-Glc (**3**), β-D-Man-(1→4)-β-D-Man-(1→4)-D-Man (**4**), β-D-Glc-(1→4)-D-Glc (**5**), Mannotriose having β-(1→4) and β-(1→3) linkages (**6**), β-D-Glc-(1→4)-D-Man (**7**), β-D-Man-(1→4)-β-D-Man-(1→4)-D-Man (**8**), β-D-Glc-(1→4)-β-D-Man-(1→4)-D-Man (**9**), β-D-Man-(1→4)-β-D-Glc-(1→4)-D-Glc (**10**), β-D-Man-(1→4)-β-D-Glc-(1→4)-β-D-Man (**11**).

A β-D-(1→4)-linked mannotetraose was not isolated, but it is reasonable to consider, from the ratio of yields of mannobiose and cellobiose, that such a component occurs in the main chain of the polymer. If this were not so, the ratio of yields of mannobiose and cellobiose would be expected to be smaller than the observed value[2c] (mannobiose/cellobiose = 1200 mg/40 mg) because the linkage between mannose residues is cleaved more readily during acetolysis[4] than other linkages.

Although it is not possible to conclude total structure from the present results, if it may be assumed that the structural pattern is repeated throughout the polymer molecule, the repeating unit of the main chain can be formulated as:

→Glc→Glc→Man→Man→Man→Man→Glc→Man→; the branching point occurs at C-3 of a β-(1→4)-linked D-mannose residue.

The foregoing results is very similar to that observed for a glucomannan obtained from *A. konjac*[2a,b]. However, a smaller sedimentation coefficient of the acetyl derivative of this polymer (4.85×10^{-13}) than konjac glucomannan acetate[2d] (6.13×10^{-13}) shows that suisen-glucomannan has lower molecular-weight than konjac glucomannan.

EXPERIMENTAL

General methods — All concentrations were conducted under diminished pressure at 40–45°. Specific rotations were determined at 20 ±5°. The melting points are uncorrected. Paper-chromatographic separation of sugars was performed by the double-ascending method on Toyo No. 50 filter paper, with the solvents: (*A*) 4:6:3 pyridine–1-butanol–water[5], and (*B*) butanone–water azeotrope[6]. Unless otherwise stated, the first of these solvent systems was used. Paper electrophoresis was conducted on Toyo No. 51 filter paper at about 15 V/cm for 3 h in borate buffer[7] (pH 9.8) and at about 25 V/cm for one h in molybdate buffer[8] (pH 5.0). The position of the compounds on the paper chromatograms and electrophoretograms was located by spraying with aniline hydrogen phthalate[9] for reducing sugars and silver nitrate–sodium hydroxide[10] for alditols. M_G and M_S refer to the mobility of a compound on paper electrophoretogram relative to D-glucose and D-glucitol, respectively.

Reduction of the oligosaccharides to the corresponding alditols was performed with sodium borohydride by the usual method. Complete acid hydrolysis of the oligosaccharides and alditols was performed by heating the compounds (*ca.* 5 mg) in a sealed tube with 0.5M sulfuric acid (0.5 ml) for 3 h at 100°. Partial acid hydrolysis of the oligosaccharides and alditols was achieved by heating the compounds (*ca.* 5 mg), under reflux, with 100 parts of 50mM sulfuric acid for 3 h in a boiling-water bath.

Gas-chromatographic analysis[11] was conducted at 200° on a 1.5 m column (3 mm i.d.) containing 10% of polyphenyl ether (5 ring) on Celite 545 (acid-washed, 80–100 mesh) with a Hitachi gas chromatograph, Model 063, fitted with a flame-ionization detector and employing nitrogen as the carrier gas (25 ml/min).

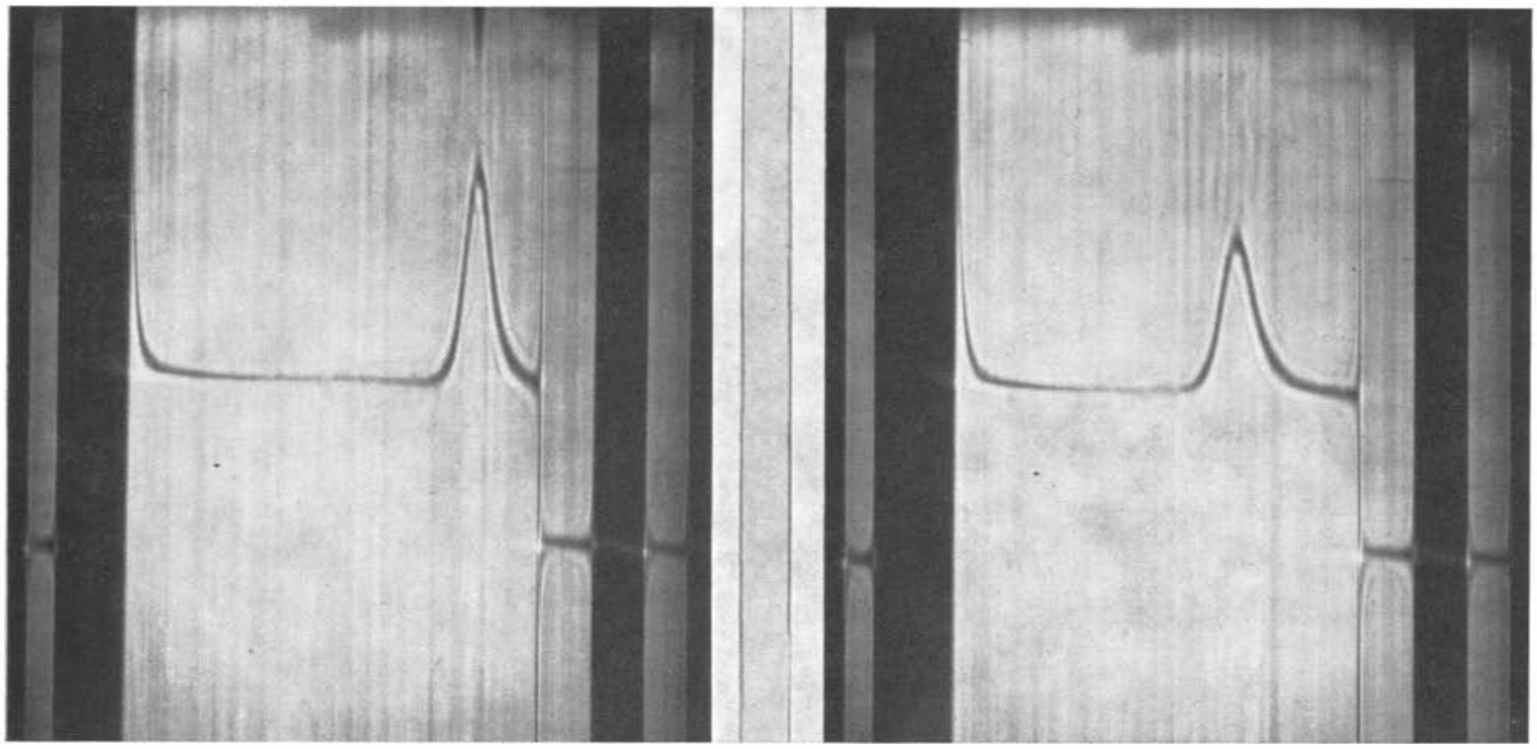
Isolation of the glucomannan. — The bulbs (6.7 kg) of *N. tazetta* L. var. chinensis Roem were collected at the beginning of their dormant period. They were triturated in a mixer with a 10-fold volume of water. The mixture was centrifuged at 13,000 r.p.m. to remove insoluble materials, including starch. To the supernatant solution, which gave no color with iodine, Fehling's solution was added with vigorous stirring, and the resulting copper-complex was collected by centrifugation at 13,000 r.p.m. and converted into the dry glucomannan by the usual method; yield 128 g. The purified glucomannan contained N, 0.15–0.20% and ash, 0.05–0.06%.

Complete acid hydrolysis of the glucomannan. — The glucomannan (50 mg) was heated in a sealed tube with 0.5M sulfuric acid (5 ml) for 48 h at 100°, and the solution was neutralized with barium carbonate. Paper-chromatographic and electrophoretic analyses revealed two spots, corresponding to D-glucose and D-mannose. D-Glucose and D-mannose were determined, after separation by paper chromatography, by the phenol–sulfuric acid method[12], and their ratio was found to be 2:3.

Acetylation of the glucomannan[13]. — The glucomannan (60 g) was stirred for 5 h at room temperature with formamide (250 ml). After the addition of pyridine (380 ml), acetic anhydride (250 ml) was added dropwise with stirring during 40 min. The temperature was kept at 20–25° with cooling until all of the anhydride had been added. Thereafter, the mixture was stirred for 3 days at room temperature and then

poured into methanol to precipitate the glucomannan acetate. The precipitate was filtered off, washed with methanol and ether, and dried *in vacuo*; yield 60 g. The acetate thus obtained had $[\alpha]_D$ −23.5° (*c* 1.1, chloroform).

Sedimentation analysis. — The sedimentation pattern (Fig. 2) was obtained in a Hitachi type UCA-1A ultracentrifuge by using a 1% solution of the acetate in acetone, at a rotor speed of 55,430 r.p.m; photographs were taken at every 9 min after attaining full speed. The sedimentation coefficient, calculated in the usual way, was 4.85×10^{-13}.



(A) 9 min. (B) 36 min.

Fig. 2. Sedimentation pattern of the glucomannan acetate (sedimented from right to left).

Methylation analysis. — A sample of the glucomannan acetate (3 g) was methylated first by the modified Haworth method[13] and then twice by the method of Hakomori[14]. The methylated polymer was extracted with chloroform and the extract was evaporated to dryness. The final product was dissolved in tetrahydrofuran and added slowly to three volumes of petroleum ether to yield a precipitate, which was dried in vacuo; yield 2 g. The i.r. spectrum of this material in KBr was compared with that of a disk of KBr alone. No difference could be detected in the area of hydroxyl absorption.

The methylated glucomannan (630 mg) was dissolved in 70% sulfuric acid (30 ml) at 5° and the mixture was kept for 11 h at room temperature. The solution was then diluted with water to 300 ml and refluxed for 7 h. Acid was removed by barium carbonate and the filtrate was concentrated to dryness; yield 600 mg.

A part of the hydrolyzate (550 mg) was dissolved in the minimum amount of water, poured onto a carbon column (3 × 19 cm) and fractionated by stepwise elution with water and aqueous ethanol (1.75–50%). The effluent was collected in 300-ml

fractions and each fraction was examined after concentration by paper chromatography (solvent *B*). Chromatographically identical fractions were combined and evaporated to dryness.

2,3,6-Tri-*O*-methyl-D-mannose (300 mg, syrup) was eluted by 1.75% ethanol was characterized as the 1,4-di-(*p*-nitrobenzoate)[4], m.p. and mixed m.p. 187–189°, $[\alpha]_D$ +29° (*c* 0.8, chloroform); yield 75 mg from 120 mg of syrup. The i.r.-spectrum was identical with that of an authentic specimen.

2,3,6-Tri-*O*-methyl-D-glucose (200 mg, syrup) was eluted by 1.75–5% ethanol and was obtained as the crystalline solid from a 1:4 mixture of ethyl acetate and ether. Recrystallization from ether gave product having m.p. and mixed m.p. of 122–123°. The i.r. spectrum was identical with that of an authentic specimen.

2,3,4,6-Tetra-*O*-methyl-D-mannose and -D-glucose were detected in the fraction eluted by 20–50% ethanol, and they were identified as methyl glycosides by gas chromatography.

Di-*O*-methylhexoses were detected in the fraction eluted with water, but were not examined further.

For determination of the average chain-length, a portion of the hydrolyzate (10 mg) was subjected to gas-chromatographic analysis after conversion into the corresponding methyl glycosides by 0.5M methanolic hydrogen chloride (0.5 ml). The molar ratio of the tetra-, tri-, and di-*O*-methyl sugars was determined by the areas under the peaks

Fragmentation analysis by acetolysis. — The glucomannan acetate (30 g) was dissolved in acetic anhydride (425 ml) and kept overnight at 5°. To this solution was added 195 ml of a cold mixture of acetic anhydride (5 parts) and sulfuric acid (1 part), and the whole mixture was kept for 30 min at 5°. Thereafter, the reaction mixture was kept for 5 days at room temperature, and then poured into ice–water. The derived acetates were isolated by extraction with chloroform in the usual way; the degree of hydrolysis as determined for the *O*-de-acetylated product was 46.1%. The products were *O*-de-acetylated with 0.02M sodium methoxide at 5°, and fractionated first on a carbon column (8 × 25 cm) by stepwise elution with water and aqueous ethanol (2.5–15%) and then on Toyo No. 525 filter paper. The results are shown in Fig. 1. The oligosaccharides were characterized as follows.

β-D-*Man*-(*1→4*)-D-*Man* (**1**). This compound (1.2 g, syrup), eluted by water and 2.5% ethanol, had $[\alpha]_D$ −10.2° (*c* 1.1, water) and migrated with the same characteristics as an authentic sample of 4-*O*-*β*-D-mannopyranosyl-D-mannose on paper chromatograms and electrophoretograms (M_G 0.62, M_S of the corresponding alditol <0.1). The i.r. spectrum was similar to that of a crystalline, authentic sample of *β*-(1→4)-linked mannobiose: 980, 960, 940, 900 (type 2b), 870 (type 2c), 810 (type 3), and 750 cm^{-1}. Acid hydrolysis indicated the presence of D-mannose only.

β-D-*Man*-(*1→3*)-D-*Man* (**2**). This compound (10 mg, syrup), eluted by 2.5% ethanol, gave only D-mannose on acid hydrolysis and co-migrated with 3-*O*-*β*-D-mannopyranosyl-D-mannose prepared from konjac glucomannan[2d] on paper chromatograms and electrophoretograms (M_G 0.69, M_S<0.1). Furthermore, this

oligosaccharide was not hydrolyzed by almond emulsin, which is known to have α-mannosidase activity[15].

β-D-*Man*(*1→4*)-D-*Glc* (**3**). This compound (630 mg, syrup), eluted by 2.5% ethanol, had $[\alpha]_D$ +16° (*c* 1.4, water) and co-migrated with an authentic sample of 4-*O*-*β*-D-mannopyranosyl-D-glucose on paper chromatograms and electrophoretograms (M_G 0.44, M_S 0.30). This oligosaccharide, on acid hydrolysis, yielded D-glucose and D-mannose in the ratio of 1:1. Reduction, followed by hydrolysis, gave D-mannose as the only reducing sugar. Crystallization from methanol gave a m.p. and mixed m.p. of 198–201°. The i.r. spectrum was identical with that of an authentic specimen.

β-D-*Man*-(*1→4*)-*β*-D-*Man*-(*1→4*)-D-*Man* (**4**). This compound (90 mg, syrup), eluted by 5% ethanol had $[\alpha]_D$ −20.5° (*c* 1.3, water) and co-migrated with an authentic sample of the *β*-D-(1→4)-linked mannotriose on paper chromatograms and electrophoretograms (M_G 0.61, $M_S<0.1$). Complete acid hydrolysis gave only D-mannose. Graded acid hydrolysis gave D-mannose, *β*-D-(1→4)-linked mannobiose and the unhydrolyzed original sugar. Log $(R_F/1-R_F)$ values[16] of these sugars, when plotted against degree of polymerization, gave a straight-line graph.

β-D-*Glc*-(*1→4*)-D-*Glc* (**5**). This compound (40 mg, syrup), eluted by 5% ethanol, gave only D-glucose on acid hydrolysis and co-migrated with an authentic specimen of cellobiose on paper chromatograms and electrophoretograms (M_G 0.32, M_S 0.31). The octaacetate had m.p. 195° unchanged on admixture with cellobiose octaacetate. The i.r. spectrum was identical with that of an authentic specimen.

Mannotriose having *β*-D-(*1→3*) *and* *β*-D-(*1→4*) *linkages* (**6**). This compound (20 mg, syrup), eluted by 5% ethanol, gave only D-mannose on acid hydrolysis. Partial acid hydrolysis gave *β*-D-(1→4)-linked mannobiose and *β*-D-(1→3)-linked mannobiose as reducing disaccharides, indicating that this sugar is one of the three possible trisaccharides having both *β*-D-(1→4) and *β*-D-(1→3) linkages. Although further studies to characterize the sugar were not attempted, the larger M_G value (0.69) of this sugar than that (0.61) of *β*-D-(1→4)-linked mannotriose suggests that the linkage at the reducing end is 1→3.

β-D-*Glc*-(*1→4*)-D-*Man* (**7**). This compound (150 mg, syrup), eluted by 5% ethanol, had $[\alpha]_D$ +5.0° (*c* 1.1, water) and co-migrated with an authentic sample of epicellobiose on paper chromatograms and electrophoretograms (M_G 0.51, $M_S<0.1$). When hydrolyzed, this oligosaccharide gave D-mannose and D-glucose in the ratio of 1:1.

The reduction product of this sugar, on hydrolysis, gave D-glucose as the reducing sugar. The octaacetate had $[\alpha]_D$ +34° (*c* 1.1, chloroform) and m.p. 202° unchanged on admixture with epicellobiose octaacetate. The i.r. spectrum was identical with that of an authentic specimen.

β-D-*Man*-(*1→4*)-*β*-D-*Man*-(*1→4*)-D-*Glc* (**8**). This compound (120 mg, syrup), eluted by 5% ethanol, had $[\alpha]_D$ −7.0° (*c* 1.0, water) and M_G 0.41, on acid hydrolysis it gave D-glucose and D-mannose in the ratio of 1:2. Acid hydrolysis of the reduction product of this sugar gave D-mannose as the only reducing sugar, indicating that the reducing end of this oligosaccharide is D-glucose. On partial acid hydrolysis, it gave

D-glucose, D-mannose, β-D-(1→4)-linked mannobiose, a mannosylglucose and the unhydrolyzed original sugar. Log $R_F/1-R_F$ values[16] of these sugars except D-mannose, when plotted against degree of polymerization, gave a straight-line graph.

β-D-Glc-(1→4)-β-D-Man-(1→4)-D-Man (**9**). This compound (110 mg, syrup), eluted by 10% ethanol, had $[\alpha]_D$ −11° (*c* 1.1, water) and M_G 0.50 and on acid hydrolysis gave D-glucose and D-mannose in the ratio of 1:2. Partial hydrolysis of the sugar gave epicellobiose and β-D-(1→4)-linked mannobiose as disaccharides, whereas epicellobiose was the sole reducing disaccharide in the partial acid hydrolyzate of the corresponding alditol.

β-D-Man-(1→4)-β-D-Glc-(1→4)-D-Glc (**10**), This compound (100 mg, syrup), eluted by 10–15% ethanol, had M_G 0.37 and on acid hydrolysis gave D-glucose and D-mannose in the ratio of 2:1. Partial acid hydrolysis gave cellobiose and β-D-(1→4)-linked mannosylglucose as disaccharide components, with the former preponderating. However, β-D-(1→4)-linked mannosylglucose was detected as the only reducing disaccharide in the partial acid hydrolyzate of the corresponding alditol.

β-D-Man-(1→4)-β-D-Glc-(1→4)-D-Man (**11**). This compound (120 mg, syrup), elutad by 15% ethanol, had M_G 0.59 and on acid hydrolysis gave D-glucose and D-mannose in the ratio of 1:2. Partial acid hydrolysis gave epicellobiose and β-D-(1→4)-linked mannosylglucose as disaccharides, with the former preponderating. However, no epicellobiose was detected in the partial acid hydrolyzate of the corresponding alditol.

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