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Plant Mucilages. XII.[1] Fourteen Oligosaccharides obtained from Bletilla-glucomannan by Partial Acetolysis

Masashi Tomoda and Satomi Kimura (née Nakatsuka)

Kyoritsu College of Pharmacy[2]

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Partial acetolysis of Bletilla-glucomannan, the mucilage from the tubers of *Bletilla striata* Reichenbach fil., has led to the isolations of fourteen oligosaccharides. Analysis of components, methylation and partial degradation studies provided the evidences that they are O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-α-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-D-glucopyranose, O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-(1→4)-D-glucopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-glucopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-glucopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, and O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose. Among them, it is conceivable that mannobiose having α-glycosidic linkage is the artifact produced during the reaction.

The properties of Bletilla-glucomannan, the mucous polysaccharide isolated from the tubers of *Bletilla striata* Reichenbach fil., were investigated in this laboratory.[3,4] Periodate oxidation and methylation studies revealed that the substance is mainly composed of 1→4 linked aldohexopyranose residues having a branched structure with 1→2 branch point at a part of mannose unit. Furthermore, it was identified that about 80% of glucose residues in the polysaccharide possess 3-O-acetyl groups. Partial acid hydrolysis of it gave five main oligosaccharides and the confirmation of them also supported the conclusion on β-1→4 linked structure of the main chain.

The present work was undertaken to isolate and identify the oligosaccharides as the products of partial acetolysis of Bletilla-glucomannan. Data on the aldohexose chains in the polysaccharide are discussed in this paper.

Bletilla-glucomannan was dissolved in formamide and acetylated with acetic anhydride and pyridine. The acetate obtained was partially degraded with sulfuric acid in acetic anhydride. After deacetylation, the products were fractionated by active charcoal column chromatography. Then the fractions were further purified by paper partition chromatography (PPC). Five disaccharides (I, II, III, IV, and V), six trisaccharides (VI, VII, VIII, IX, X, and XI) and three tetrasaccharides (XII, XIII, and XIV) were obtained. The outline of the preparation and isolation of the partial acetolysis products is shown in Chart 1.

The homogeneity of each oligosaccharide was checked by the cellulose thin-layer chromatography (TLC). For the disaccharides and the trisaccharides, the gas–liquid chromatography (GLC) of their trimethylsilyl derivatives was also carried out. Most of the trimethylsilyl

1) Part XI: M. Tomoda and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **24**, 230 (1976).
2) Location: *1-5-30, Shibakôen, Minato-ku, Tokyo, 105, Japan.*
3) M. Tomoda, S. Nakatsuka, M. Tamai, and M. Nagata, *Chem. Pharm. Bull.* (Tokyo), **21**, 2667 (1973).
4) M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **22**, 2710 (1974).

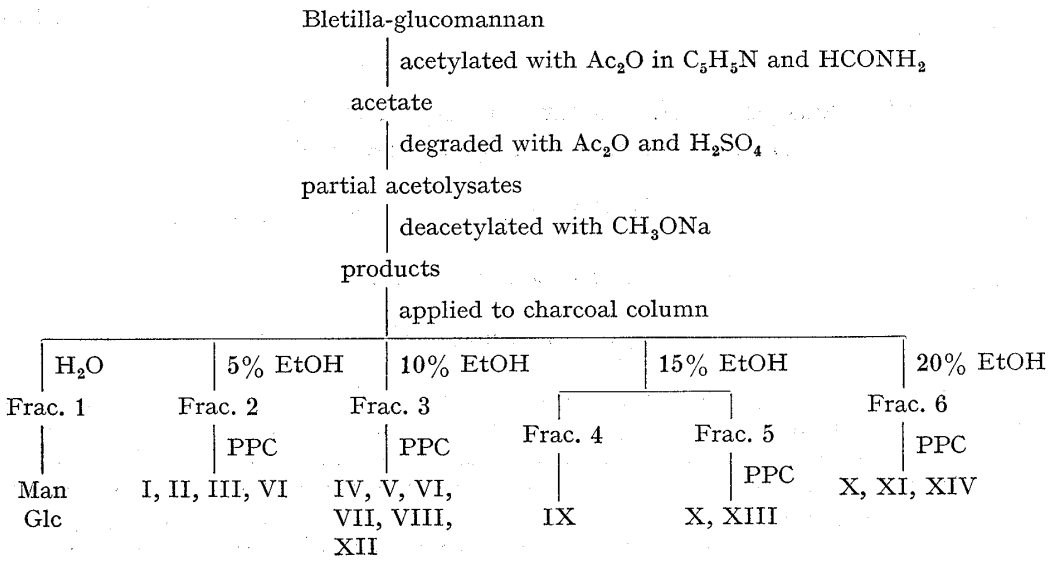

Chart 1. Isolation of Oligosaccharides

TABLE I. *Rf* Values of Oligosaccharides and Retention Times (min) of Trimethylsilyl Derivatives of Oligosaccharides and their Reduction Products

Oligosaccharides	Cellulose TLC (*Rf*)			GLC (t_R)			
				Condition A		Condition B	
	Solvent A	Solvent B	Solvent C	Original	Reduced	Original	Reduced
I	0.55	0.36	0.54	36.6,[a] 38.5	39.0	41.6,[a] 43.6	44.9
II	0.59	0.41	0.60	34.3,[a] 36.9	36.8	39.0,[a] 41.8	42.9
III	0.47	0.27	0.48	37.9, 39.1[a]	39.2	42.2, 43.6[a]	45.1
IV	0.65	0.44	0.63	35.0,[a] 36.9	37.6	39.9,[a] 42.1	43.9
V	0.57	0.35	0.55	39.4,[a] 40.6	37.9	43.8,[a] 41.7	43.8
VI	0.28	0.18	0.41	53.6,[a] 55.8	56.0	62.1,[a] 66.0	72.9
VII	0.39	0.22	0.46	52.5,[a] 54.7	56.1	60.2	68.9
VIII	0.19	0.13	0.37	56.7	57.9	65.6,[a] 68.4	72.3
IX	0.22	0.15	0.39	51.9,[a] 53.6	55.9	59.0	62.9
X	0.35	0.20	0.44	52.9,[a] 54.5	56.3	59.9,[a] 62.8	66.2
XI	0.51	0.26	0.50	53.0,[a] 55.0	56.6	60.4,[a] 64.3	69.8
XII	0.09	0.05	0.27	—	—	—	—
XIII	0.16	0.09	0.35	—	—	—	—
XIV	0.15	0.10	0.33	—	—	—	—

For the solvents and the conditions, see "Experimental."
a) main peaks

derivatives of the oligosaccharides gave two anomeric peaks on GLC, but those of the reduced oligosaccharides showed their own single peak. Table I shows the *Rf* values on TLC and the retention times on GLC under several conditions.

The TLC of the hydrolysates and the GLC of the trimethylsilyl derivatives of the methanolysates of the oligosaccharides showed their component sugars. After reduction of the oligosaccharides with sodium borohydride, the products were methanolyzed, then analyzed by GLC after trimethylsilylation. The results revealed their constitutions and reducing terminals. These results and specific rotations of the oligosaccharides in water are shown in Table II.

The methylation of each oligosaccharide was performed with sodium methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[5] The fully methylated products were

5) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

Table II. Specific Rotations and Component Sugars of the Oligosaccharides and Molar Ratios of Components of Reduced Oligosaccharides

Oligosaccharides	Specific rotations in water (final values)	Component sugars	Molar ratios of components of reduced oligosaccharides
I	$[\alpha]_D^{27}$ − 6.7° (c=3.9)	mannose	mannose: mannitol =1.0:1.0
II	$[\alpha]_D^{23}$ +42.6° (c=0.4)	mannose	mannose: mannitol =1.0:1.0
III	$[\alpha]_D^{23}$ + 6.7° (c=0.3)	mannose, glucose	mannose: glucitol =1.0:1.0
IV	$[\alpha]_D^{23}$ + 3.4° (c=1.3)	mannose, glucose	glucose: mannitol =1.0:1.0
V	$[\alpha]_D^{23}$ +33.0° (c=0.8)	glucose	glucose: glucitol =1.0:1.0
VI	$[\alpha]_D^{27}$ −20.9° (c=2.3)	mannose	mannose: mannitol =2.0:1.0
VII	$[\alpha]_D^{25}$ −13.2° (c=0.6)	mannose, glucose	mannose: glucose: mannitol =1.0:0.9:1.0
VIII	$[\alpha]_D^{25}$ − 7.2° (c=0.4)	mannose, glucose	mannose: glucitol =2.1:1.0
IX	$[\alpha]_D^{20}$ + 5.7° (c=0.5)	mannose, glucose	mannose: glucose: glucitol =1.1:1.1:1.0
X	$[\alpha]_D^{23}$ − 5.8° (c=2.2)	mannose, glucose	mannose: glucose: mannitol =1.1:1.0:1.0
XI	$[\alpha]_D^{25}$ + 5.8° (c=0.3)	mannose, glucose	glucose: mannitol =1.9:1.0
XII	$[\alpha]_D^{27}$ −25.9° (c=1.6)	mannose	mannose: mannitol =2.9:1.0
XIII	$[\alpha]_D^{25}$ −20.8° (c=0.3)	mannose, glucose	mannose: glucose: mannitol =1.9:0.9:1.0
XIV	$[\alpha]_D^{25}$ −16.1° (c=0.5)	mannose, glucose	mannose: glucose: mannitol =2.1:1.0:1.0

Table III. Products in Hydrolysates obtained from Methylated Oligosaccharides and Molar Ratios of Them

Oligosaccharides	Products	Molar ratios
I	2,3,4,6-me-Man: 2,3,6-me-Man	1.0:1.0
II	2,3,4,6-me-Man: 2,3,6-me-Man	1.0:0.9
III	2,3,4,6-me-Man: 2,3,6-me-Glc	1.0:1.0
IV	2,3,4,6-me-Glc: 2,3,6-me-Man	1.0:0.9
V	2,3,4,6-me-Glc: 2,3,6-me-Glc	1.0:1.1
VI	2,3,4,6-me-Man: 2,3,6-me-Man	1.0:2.0
VII	2,3,4,6-me-Glc: 2,3,6-me-Man	1.0:1.9
VIII	2,3,4,6-me-Man: 2,3,6-me-Man: 2,3,6-me-Glc	1.0:1.2:1.1
IX	2,3,4,6-me-Man: 2,3,6-me-Glc	1.0:2.1
X	2,3,4,6-me-Man: 2,3,6-me-Man: 2,3,6-me-Glc	1.0:0.9:1.0
XI	2,3,4,6-me-Glc: 2,3,6-me-Man: 2,3,6-me-Glc	1.0:0.9:1.2
XII	2,3,4,6-me-Man: 2,3,6-me-Man	1.0:3.1
XIII	2,3,4,6-me-Glc: 2,3,6-me-Man	1.0:2.8
XIV	2,3,4,6-me-Man: 2,3,6-me-Man: 2,3,6-me-Glc	1.0:2.1:1.1

abbreviations: me=methyl; Man=D-mannose; Glc=D-glucose.
(*e.g.*, 2,3,4,6-me-Man=2,3,4,6-tetra-O-methyl-D-mannose)

hydrolyzed with formic acid and dilute sulfuric acid. The hydrolysates were derived to corresponding alditol acetates,[6] then analyzed by GLC. The products obtained by hydrolysis of the methylated oligosaccharides are shown in Table III.

The comparison by TLC and by GLC of trimethylsilyl derivatives with authentic samples[7] and their values of the specific rotation showed that I, III, IV, V, VI, X and XII are O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-D-glucopyranose, O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-(1→4)-D-glucopyranose

6) H. Björndal, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, **21**, 1801 (1967).
7) M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **21**, 2511 (1973).

(=cellobiose), O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, respectively.

Finally, the structures of the trisaccharides and the tetrasaccharides were determined from the results of controlled acid hydrolysis of them. The samples were hydrolyzed with 0.5N sulfuric acid at 90° for 1 hr, and the products were separated by PPC and characterized by TLC and by GLC of trimethylsilyl derivatives. The results are shown in Table IV.

TABLE IV. Oligomers in the Partial Acid Hydrolysates of Trisaccharides and Tetrasaccharides

Materials	Products
VI	Man$^{\beta\text{-}4}$Man (I)
VII	Glc$^{\beta\text{-}4}$Man (IV), Man$^{\beta\text{-}4}$Man (I)
VIII	Man$^{\beta\text{-}4}$Man (I), Man$^{\beta\text{-}4}$Glc (III)
IX	Man$^{\beta\text{-}4}$Glc (III), Glc$^{\beta\text{-}4}$Glc (V)
X	Man$^{\beta\text{-}4}$Glc (III), Glc$^{\beta\text{-}4}$Man (IV)
XI	Glc$^{\beta\text{-}4}$Glc (V), Glc$^{\beta\text{-}4}$Man (IV)
XII	Man$^{\beta\text{-}4}$Man (I), Man$^{\beta\text{-}4}$Man$^{\beta\text{-}4}$Man (VI)
XIII	Glc$^{\beta\text{-}4}$Man (IV), Man$^{\beta\text{-}4}$Man (I), Glc$^{\beta\text{-}4}$Man$^{\beta\text{-}4}$Man (VII), Man$^{\beta\text{-}4}$Man$^{\beta\text{-}4}$Man (VI)
XIV	Man$^{\beta\text{-}4}$Glc (III), Glc$^{\beta\text{-}4}$Man (IV), Man$^{\beta\text{-}4}$Man (I), Man$^{\beta\text{-}4}$Glc$^{\beta\text{-}4}$Man (X), Glc$^{\beta\text{-}4}$Man$^{\beta\text{-}4}$Man (VII)

Man=D-mannopyranose unit, Glc=D-glucopyranose unit

Thus VII, VIII, IX, XI, XIII and XIV are O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-glucopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-glucopyranose, O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose and O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, respectively.

As described in a former report,[3] we have already obtained five oligosaccharides, *i.e.*, I, III, IV, VI and XII, from the partial acid hydrolysate of Bletilla-glucomannan. In addition to these oligosaccharides, the isolations and the identifications of V, VII, VIII, IX, X, XI, XIII and XIV as the partial acetolysis products of the polysaccharide provided new informations on the structures of Bletilla-glucomannan. Thus the polysaccharide possesses these diverse component aldohexose units having β-1→4 glycosidic linkages. On the other hand, neither partial acid hydrolysis nor partial acetolysis gave pure oligosaccharides having 1→2 glycosidic linkage. From these results, it is able to presume that 1→2 glycosidic linkages in the glucomannan are cleft much more easily than 1→4 glycosidic linkages by the action of acid. In the present work, we have obtained di-, tri-, and tetra-saccharides, so there is a possibility of presences of some higher oligosaccharides having 1→2 glycosidic linkages in the residual part adsorbed in the active charcoal column. Studies on them and the elucidation of the sequence of component oligosaccharide units are remaining problems.

The results of component sugar determination and methylation analysis and the value of the specific rotation indicated that oligosaccharide II is O-α-D-mannopyranosyl-(1→4)-D-mannopyranose. To make sure whether α-glycosidic linkage is essentially present in the polysaccharide or not, authentic mannotetraose (XII) having only β-1→4 glycosidic linkage was undergone the similar partial acetolysis, and after deacetylation, the products were analyzed by TLC and GLC. The results showed the formation of a slight amount of II in addition to abundant mannose, I and VI. In partial acid hydrolysates of the glucomannan or manno-

tetraose (XII), II was entirely absent. From these facts and the low yield (less than 0.2%) of II, it is conceivable that II is not the substance showing an essential component unit in Bletilla-glucomannan, but the artifact produced during the reaction of acetolysis. Further investigation of this problem is now under progress.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Optical rotations were measured with JASCO model DIP-SL automatic polarimeter. GLC was carried out by the use of Hitachi model 063 gas chromatograph equipped with hydrogen flame ionization detector.

Acetylation of Polysaccharide——The polysaccharide (5 g) was suspended in formamide (120 ml) and stirred for 5 hr at room temperature. After three times addition of pyridine (20 ml each), acetic anhydride (40 ml) was added during 40 min at 20 to 25° under continuous stirring. The mixture was stirred for three days at room temperature, then poured into methanol (1000 ml) to precipitate the acetate. The precipitate was filtered off, washed with methanol and ether, and dried *in vacuo*; yield, 7.9 g.

Partial Acetolysis and Isolation of Oligosaccharides——The acetate (4 g) was dissolved in acetic anhydride (56 ml) and kept overnight at 5°. A cold mixture (5: 1, 30 ml) of acetic anhydride and sulfuric acid was added into the solution and the whole mixture was kept for 30 min at 5°. The reaction mixture was left for four days at room temperature, then poured into ice water (800 ml). The products were extracted with chloroform followed by washing with water, 10% sodium bicarbonate and water. After evaporation and dryness, the products were de-acetylated with 0.1 M sodium methoxide (150 ml) overnight at 5° and neutralized with acetic acid. The solvent was evaporated off and the residue was dissolved in water, then applied to a column (2 × 30 cm) of active charcoal (for chromatographic use, Wako-junyaku Co.). The charcoal was treated before use with hot 15% acetic acid followed by washing with hot water. The column was eluted successively with water (400 ml), 5% ethanol (900 ml), 10% ethanol (850 ml), 15% ethanol (900 ml) and 20% ethanol (500 ml). Fractions were collected at 50 ml and carbohydrates in eluates were measured by phenol-sulfuric acid method.[8] The eluates obtained from the column were divided into six groups: Frac. 1, tubes 3 to 8; Frac. 2, tubes 9 to 26; Frac. 3, tubes 27 to 43; Frac. 4, tubes 44 to 50; Frac. 5, tubes 51 to 61; Frac. 6, tubes 62 to 71. The yields were 2155 mg in Frac. 1, 679 mg in Frac. 2, 197 mg in Frac. 3, 57 mg in Frac. 4, 141 mg in Frac. 5 and 85 mg in Frac. 6. Fractions 2,3,5, and 6 were respectively applied to PPC. PPC was carried out by ascending method using Tôyô-Roshi No. 50 and with solvent A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3). Table V shows the charcoal column fractions from which oligosaccharides were obtained, solvents and *Rf* value regions of

Table V. Fractions for the Isolation of Oligosaccharides and Yields of Oligosaccharides

Oligosaccharides	Charcoal column fractions	Preparative PPC		Yields (mg) from the acetate (4 g)
		Solvents	*Rf* value regions of extraction parts	
I	Frac. 2	(first) A (second) B	0.43 to 0.49 0.25 to 0.27	166.7
II	Frac. 2	(first) A (second) B	0.43 to 0.49 0.28 to 0.34	5.0
III	Frac. 2	A	0.33 to 0.41	187.7
IV	Frac. 3	A	0.53 to 0.62	70.8
V	Frac. 3	A	0.44 to 0.50	12.6
VI	Frac. 2	A	0.26 to 0.31	52.0
	Frac. 3	A	0.26 to 0.31	
VII	Frac. 3	A	0.33 to 0.38	22.4
VIII	Frac. 3	A	0.19 to 0.24	21.0
IX	Frac. 4	—	—	57.0
X	Frac. 5	A	0.29 to 0.37	122.6
	Frac. 6	A	0.29 to 0.37	
XI	Frac. 6	A	0.37 to 0.42	38.8
XII	Frac. 3	A	0.07 to 0.12	17.0
XIII	Frac. 5	A	0.09 to 0.14	17.5
XIV	Frac. 6	A	0.15 to 0.20	22.8

8) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

extraction parts in preparative PPC and yields of oligosaccharides. The mixture of I and II was obtained from Frac. 2 by PPC with solvent A, and it was again applied to PPC with solvent B, BuOH: pyridine: H_2O (6: 4: 3). IX was directly obtained from Frac. 4.

TLC and GLC of Oligosaccharides——TLC was carried out using Avicel SF cellulose and solvent C, BuOH: pyridine: H_2O (1: 1: 1) at 23° in addition to solvent A at 23° and solvent B at 28°. Samples were revealed with *p*-anisidine hydrochloride[9] and silver nitrate[10] reagents.

On the other hand, samples were trimethylsilylated in the usual way[11] and following two conditions were used for GLC: A, a column (0.3 cm × 2 m long spiral stainless steel) packed with 2% OV 17 on Chromosorb W (80 to 100 mesh) in the programmed temperature increasing 3° per min from 130 to 280° with a flow of 20 ml per min of N_2; B, a column (0.3 cm × 2 m long spiral stainless steel) packed with 3% SE 52 on Chromosorb W (80 to 100 mesh) using the same programmed temperature and carrier gas as used in condition A.

Reduction of Oligosaccharides——Each sample (2 mg) was dissolved in water (1 ml) and added sodium borohydride (2 mg). After standing for 1 hr at room temperature, Dowex 50 W (H^+) was added up to pH 5. The resin was filtered off, and after washing with water and methanol, the filtrate and washing were combined and evaporated. Methanol was added to the residue and evaporated five times, then the final residue was dissolved in small amount of water and lyophilized.

Analysis of Sugar Components——Samples were hydrolyzed with 2 N sulfuric acid in a sealed tube at 100° for 6 hr, then neutralized with barium carbonate. The hydrolysates were analyzed by cellulose TLC as described above. *Rf* values of mannose and glucose were 0.69 and 0.62 in solvent A, 0.57 and 0.50 in solvent B.

Oligosaccharides and their reduction products were methanolized with 4% methanolic HCl in a sealed tube at 75° for 16 hr, then HCl was removed by the repeated addition and evaporation of methanol. The methanolysates were trimethylsilylated and applied to GLC under the same conditions as described above. Determinations of components of reduced oligosaccharides were carried out by GLC of the methanolysates in condition A. Retention times of trimethylsilyl derivatives of methyl mannoside, methyl glucoside, mannitol and sorbitol were 12.7, 16.9, 14.9 and 15.2 in condition A, and 16.4, 19.1, 20.2, and 20.4 in condition B.

Methylation of Oligosaccharides——Sodium hydride (2 mg) was mixed with dimethyl sulfoxide (1 ml) and the mixture was stirred at 70° for 1 hr. The sample (1 mg) was dissolved in dimethyl sulfoxide (1 ml) and the solution was added into this mixture. After stirring for 1 hr at room temperature, methyl iodide (1 ml) was added and the reaction mixture was stirred overnight at room temperature. All procedures were carried out in nitrogen atmosphere. After dilution with water (10 ml), the mixture was extracted with chloroform (10 ml) three times. The combined extract was washed with water (30 ml) three times, then dried over sodium sulfate and the filtrate was evaporated. The residue was methylated again under the same condition.

Analysis of Methylated Products——Each of the fully methylated oligosaccharides was hydrolyzed with 90% formic acid at 90° for 16 hr. After removal of formic acid by repeated evaporation, the residue was successively hydrolyzed with 0.5 N sulfuric acid at 100° for 2 hr followed by neutralization with Dowex 2 (OH^-). The product was reduced with sodium borohydride as described above, then acetylated with the mixture (1: 1) of acetic anhydride and pyridine at 100° for 20 min. GLC of partially methylated alditol acetates were carried out under condition C: a column (0.3 cm × 2 m long spiral glass) packed with 3% ECNSS-M on Gaschrom Q (100 to 120 mesh) at 180° with a flow of 30 ml per min of N_2. Relative retention times of the products to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol were as follows: 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-mannitol, 0.99; 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-mannitol, 2.16; 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-glucitol, 2.47.

Partial Acid Hydrolysis——Each of trisaccharides and tetrasaccharides was dissolved in 0.5 N sulfuric acid and heated in a sealed tube at 90° for 1 hr. After neutralization with barium carbonate, the oligosaccharides in the hydrolysate were separated by PPC with solvent A or B. TLC and GLC of trimethylsilyl derivatives were carried out for analysis of them as described above.

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10) W.E. Trevelyan, D.P. Procter and J.S. Harrison, *Nature*, **166**, 444 (1950).
11) C.C. Sweeley, R. Bentley, M. Makita and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).