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Plant Mucilages. VII.[1] Six Oligosaccharides obtained from Odoratan and Falcatan by Partial Acid Hydrolysis

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Controlled acid hydrolysis of odoratan, the mucilage from *Polygonatum odoratum* Druce var. *japonicum* Hara rhizome, and of falcatan, the mucilage from *Polygonatum falcatum* A. Gray rhizome, has led to the isolations of six oligosaccharides. Analysis of components, periodate oxidation, methylation and partial degradation studies provided the evidences that they are O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-D-glucopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(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.

The mucilage from the rhizome of *Polygonatum odoratum* Druce var. *japonicum* Hara named odoratan[3] and the mucilage from the rhizome of *Polygonatum falcatum* A. Gray named falcatan[4] were extracted and their properties investigated in this laboratory. Both substances are composed of D-fructose, D-mannose, D-glucose and D-galacturonic acid, although their molar ratios are different. Both polysaccharides show similar values of the specific rotation and of the viscosity, and it is interesting to note that they have similarly high molecular weight, while they contain many fructose residues as components. In this paper, the isolation and identification of six oligosaccharides as partial acid hydrolysates of both odoratan

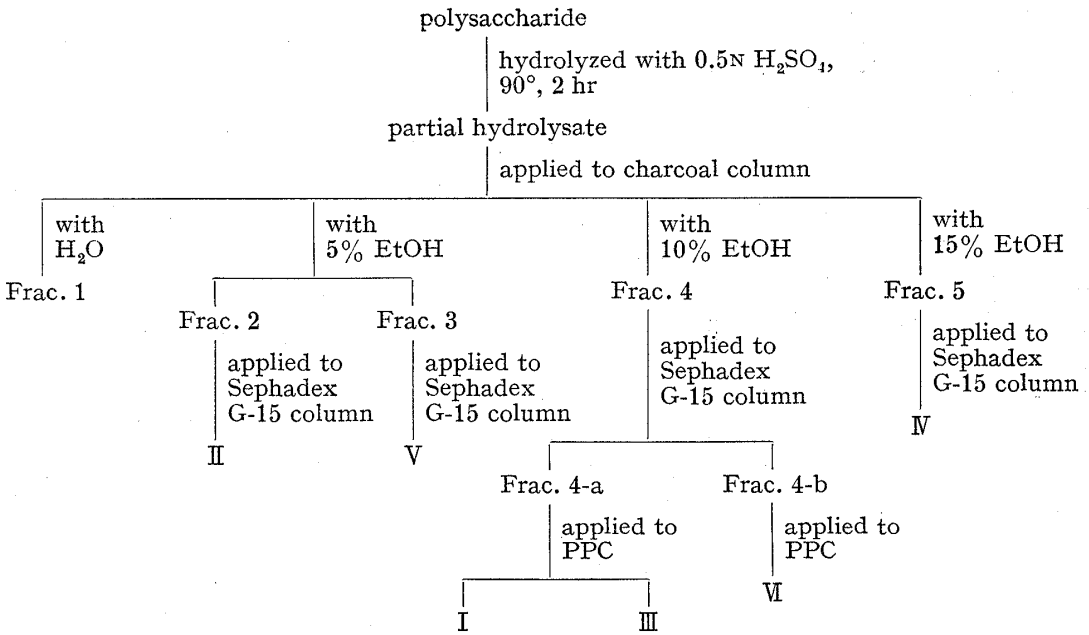

Chart 1. Isolation of Oligosaccharides

1) Part VI: M. Tomoda and M. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 989 (1973).
2) Location: *1-5-30, Shibakoen, Minato-ku, Tokyo, 105, Japan.*
3) M. Tomoda, Y. Yoshida, H. Tanaka and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 2173 (1971).
4) M. Tomoda and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **20**, 2491 (1972).

and falcatan are described, and data on the aldohexose chains in both polysaccharides are discussed.

Odoratan and falcatan were respectively hydrolyzed with 0.5N sulfuric acid at 90° for 2 hr, and the products were fractionated by active charcoal column chromatagraphy. Then the fractions were applied to a column of Sephadex G-15, and when necessary, the fractions obtained by gel chromatography were further purified by paper partition chromatography (PPC). Three disaccharides (I, II and III), two trisaccharides (IV and V) and a tetrasaccharide (VI) were obtained from odoratan, and five oligosaccharides but III were obtained from falcatan. The outline of the preparation and isolation of the partial hydrolysates is shown on Chart 1.

The homogeneity of each oligosaccharide was checked by the cellulose thin-layer chromatography (TLC) and by the gas-liquid chromatography (GLC) of its trimethylsilyl derivative. Most of the trimethylsilyl derivatives of the oligosaccharides gave two anomeric peaks on GLC, but the trimethylsilyl derivatives of their reduction products showed single sharp peaks. Table I gives the *Rf* values on TLC and the retention times on GLC in various conditions.

Table I. *Rf* Values of Oligosaccharides and Retention Times of their Trimethylsilyl Derivatives

	Cellulose TLC (*Rf*)		GLC (t_R)	
	Solvent A	Solvent B	Condition A	Condition B
Oligosaccharide I	0.38	0.53	39.9, 42.1	35.0, 36.9
Oligosaccharide II	0.30	0.42	41.8, 43.7	36.6, 38.5
Oligosaccharide III	0.23	0.33	42.2, 43.6	37.9, 39.1
Oligosaccharide IV	0.19	0.27	60.5, 64.9	52.9, 54.5
Oligosaccharide V	0.16	0.22	62.1	53.4, 55.6
Oligosaccharide VI	0.05	0.09	—	—
Reduction product of I			43.9	37.7
Reduction product of II			44.9	39.0
Reduction product of III			45.1	39.2
Reduction product of IV			66.2	54.6
Reduction product of V			73.1	56.0

See "Experimental" on the solvents and conditions.

After recrystallization, I was obtained as hygroscopic colorless rods (monohydrate), mp 134—136°, $[\alpha]_D^{23}$ +3.4° (H_2O, c=1.3); II was obtained as colorless rods, mp 204—205°, $[\alpha]_D^{27}$ −6.7° (H_2O, c=3.9); IV was obtained as colorless prisms, mp 161—163°, $[\alpha]_D^{23}$ −5.8° (H_2O, c=2.2); V was obtained as colorless prisms, mp 164—166°, $[\alpha]_D^{27}$ −20.9° (H_2O, c=2.3). Specific rotations of the other oligosaccharides were as follows: III, $[\alpha]_D^{23}$ +6.0° (H_2O, c=0.3); VI, $[\alpha]_D^{27}$ −25.9° (H_2O, c=1.6).

The TLC of the hydrolysates and the GLC of the trimethylsilyl derivatives of the methanolysates of the oligosaccharides showed that I, III and IV are composed of mannose and glucose, and that II, V and VI are composed of mannose. After reduction of the oligosaccharides with sodium borohydride, the products were methanolyzed, then the identification of the methanolysates was carried out by GLC after trimethylsilylation. The results revealed that I is D-glucosyl-D-mannose, II is D-mannosyl D-mannose, III is D-mannosyl-D-glucose, IV is D-mannosyl-D-glucosyl-D-mannose, V is D-mannosyl-D-mannosyl-D-mannose, and VI is D-mannosyl-D-mannosyl-D-mannosyl-D-mannose.

After periodate oxidation, the values of periodate consumption, formic acid and formaldehyde liberations per one mole were established for each oligosaccharide and are shown in Table II. These data suggest the presence of 1→2 or 1→4 glycosidic linkages for the two trisaccharides and the tetrasaccharide, while the possibility of 1→3 glycosidic linkage in

Table II. Mole Values of Periodate Consumption, Formic Acid and Formaldehyde Liberations per One Mole of Oligosaccharides

	Periodate consumption	Formic acid liberation	Formaldehyde liberation
Oligosaccharide I	5.2	3.1	1.0
Oligosaccharide II	5.1	3.1	1.0
Oligosaccharide III	4.7	2.8	1.0
Oligosaccharide IV	6.0	2.7	1.0
Oligosaccharide V	6.0	2.9	1.0
Oligosaccharide VI	7.2	3.1	1.0

addition to 1→2 and 1→4 linkages also should be taken into consideration for the three disaccharides.

On the other hand, the oligosaccharides were subjected to oxidation with bromine followed by mild periodate oxidation and Smith degradation.[5] Trimethylsilyl derivatives of the products were analyzed by GLC, and the results showed that all the samples yielded glycolic acid, glycerol and erythritol. These results support the presumption that all the disaccharides and trisaccharides have only a 1→4 glycosidic linkage. According to these results, it is also conceivable that the tetrasaccharide is composed of 1→4 linked D-mannopyranose residues and in this case the coexistence of a 1→2 linkage is not inconsistent.

Finally, methylation provided evidences which confirm the presence of 1→4 glycosidic likages in all the oligosaccharides. The samples were methylated with sodium hydride and methyl iodide in dimethyl sulfoxide.[6] The fully methylated products were methanolyzed and the methanolysates were analyzed by GLC. The methyl glycosides of 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-mannose were identified from I. The methyl glycosides of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-mannose were identified from II, V and VI. And the methylglycosides of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose were identified from III. In the case of IV, methyl-2,3,4,6-tetra-O-methyl-D-mannoside was identified, but it was observed that the methylglycosides of 2,3,6-tri-O-methyl D-glucose and of 2,3,6-tri-O-methyl-D-mannose interfere with each other on GLC. The structure of IV was demonstrated by the results of its partial hydrolysis.

IV, V and VI were hydrolyzed with 0.5N sulfuric acid at 90° for 1 hr and the resulting products were identified by the TLC and GLC of their trimethylsilyl derivatives. As the results of this treatment, the partial hydrolysates obtained were I and III from IV, II from V, and II and V from VI, in addition to monosaccharides.

The values of the specific rotation and of the melting point of I, II and V coincide with those given in the literature for O-β-D-glucopyranosyl-(1→4)-D-mannopyranose,[7,8] O-β-D-mannopyranosyl-(1→4)-D-mannopyranose[9,10] and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose.[11–13] These results gave the evidence that all oligosaccharides have β-glycosidic linkages of D-mannopyranose and D-glucopyranose.

The present study has made clear that the configuration of the glycosidic linkages of all the D-mannopyranose and D-glucopyranose residues in odoratan and falcatan is of the

5) H. Yamaguchi and M. Kuriyama, *J. Biochem.*, **71**, 239 (1972).
6) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
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13) O. Perila and C.T. Bishop, *Can. J. Chem.*, **39**, 815 (1961).

β-type, and elucidated the fact that most of the D-mannopyranose and D-glucopyranose are connected one another by β-(1→4) glycosidic linkages. At least, both polysaccharides have two kinds of aldohexose chain unit, which are, 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.

As the result of the Smith degradation of falcatan,[4] mannose was detected as one of the resulting products in addition to glycerol and erythritol; a similar result was also obtained by the Smith degradation of odoratan. From the results stated above, it can be put forward that in the present investigation no other oligosaccharides than those showing a 1→4 glycosidic linkage have been found, which does not exclude the possibility of a part of D-mannopyranose residues of both polysaccharides occupying branching position or part of D-mannopyranose residues having 1→3 glycosidic linkages. Therefore, it will be necessary to apply different conditions for partial hydrolysis in order to elucidate further the structure of these compounds.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Melting points were determined on a micro melting point apparatus (an air-bath type) and are uncorrected. Specific 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 ion detector.

Partial Hydrolysis and Isolations of Six Oligosaccharides——In each case of odoratan and falcatan, the polysaccharide (3 g) was dissolved in 0.5N H_2SO_4 (300 ml) and heated under reflux at 90° for 2 hr. After neutralization with barium carbonate, the filtrate and washings were combined and concentrated, then applied to a column (2×28 cm) of active charcoal (for chromatographic use, Wako-Junyaku Co.). The charcoal was treated before use with hot 15% AcOH followed by washing with hot water. In the case of the hydrolysate of odoratan, the column was eluted successively with water (550 ml), 5% ethanol (900 ml), 10% ethanol (1000 ml), 15% ethanol (1050 ml) and 20% ethanol (700 ml). Fractions were collected at 50 ml and carbohydrates in eluates were measured by phenol–sulfuric acid method.[14] The eluates obtained from the column were divided into seven groups: Frac. 1, tubes 3 to 8; Frac. 2, tubes 9 to 13; Frac. 3, tubes 14 to 18; Frac. 4, tubes 19 to 29; Frac. 5, tubes 30 to 51; Frac. 6, tubes 52 to 70; Frac. 7, tubes 71 to 84. The yields were 947 mg in Frac. 1, 146 mg in Frac. 2, 221 mg in Frac. 3, 233 mg in Frac. 4, 316 mg in Frac. 5, 294 mg in Frac. 6 and 191 mg in Frac. 7. On the other hand, in the case of the hydrolysate of falcatan, the column was eluted successively with water (500 ml), 5% ethanol (750 ml), 10% ethanol (750 ml), 15% ethanol (500 ml) and 20% ethanol (400 ml), and the eluates obtained from the column were divided into six groups: Frac. 1, tubes 3 to 8; Frac. 2, tubes 9 to 16; Frac. 3, tubes 17 to 25; Frac. 4, tubes 26 to 35; Frac. 5, tubes 36 to 45; Frac. 6, tubes 46 to 58. The yields were 2294 mg in Frac. 1, 339 mg in Frac. 2, 195 mg in Frac. 3, 223 mg in Frac. 4, 94 mg in Frac. 5 and 79 mg in Frac. 6. In the each case, Fractions 2, 3, 4, and 5 were respectively applied to a column (5×78 cm) of Sephadex G-15 (fine, Pharmacia Co.). The column was eluted with water by ascending method, and fractions were collected at 20 ml and analyzed by phenol–sulfuric acid method. II was obtained from the eluates in tubes 42 to 52 from Frac. 2. IV was obtained from the eluates in tubes 40 to 44 from Frac. 5. And V was obtained from the eluates in tubes 39 to 43 from Frac. 3. From Frac. 4, the eluates in tubes 32 to 38 and the eluates 45 to 51 were separately combined, concentrated and applied to PPC. PPC was carried out by ascending method using Toyo-Roshi No. 50. Solvent C, BuOH: pyridine: H_2O (1: 1: 1), was used for the former, and VI was obtained from a part showing *Rf* value of 0.23 by means of the extraction with water. Solvent A in Table I, BuOH: pyridine H_2O (6: 4: 3), was used for the latter, and I was obtained from a part showing *Rf* value of 0.36 and III was obtained from a part showing *Rf* value of 0.20. The yields were 83.8 mg in I, 263 mg in II, 8.8 mg in III, 70 mg in IV, 168 mg in V and 58 mg in VI from odoratan, and 27.5 mg in I, 267 mg in II, 19.7 mg in IV, 102 mg in V and 47.4 mg in VI from falcatan.

TLC and GLC of Oligosaccharides——TLC was carried out using Avicel SF cellulose and solvent B, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3) in addition to solvent A. Samples were revealed with benzidine[15] and silver nitrate[16] reagents.

For analysis by GLC, samples were trimethylsilylated in the same way as described in the previous report[1] and following two conditions were used: A, column 3% SE 52 on Chromosorb W (80 to 100 mesh) (0.3 cm×2 m long stainless steel); programmed column temperature, increase in 3° per min from 130 to

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15) J.S.D. Bacon and J. Edelman, *Biochem. J.*, **48**, 114 (1951).
16) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).

280°; carrier gas, N_2 (20 ml per min); B, column, 2% OV 17 on Chromosorb W (80 to 100 mesh) (0.3 cm × 2 m long stainless steel); programmed column temperature and carrier gas are the same as Condition A.

Reduction of Oligosaccharides——Each sample (10 mg) was dissolved in water (1 ml) and added sodium borohydride (5 mg). After standing for 1 hr at room temperature, the pH of the reaction mixture was adjusted to 5 by addition of Dowex 50W × X8 (H^+). The resins were filtered off, and after washing with water and methanol, the filtrate and washings were combined and evaporated. Methanol was added to the residue and evaporated three times, then the final residue was dissolved in small amount of water and lyophilized.

Analyses of Components——Oligosaccharides were hydrolyzed with 2N H_2SO_4 in a sealed tube at 100° for 6 hr, then neutralized with barium carbonate. The hydrolysates were analyzed by cellulose TLC as described above.

On the other hand, oligosaccharides and their reduction products were methanolyzed 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 in the same conditions as described above. *Rf* values on TLC and retention times on GLC are shown in Table III. Determinations of the components of reduced oligosaccharides were carried out by GLC (Condition B) of the methanolysates. The results revealed that approximate molar ratios of mannitol: mannose are 1: 1 in reduced II, 1: 2 in reduced V and 1: 3 in reduced VI; mannitol: glucose is 1: 1 in reduced I; sorbitol: mannose is 1: 1 in reduced III; mannitol : mannose: glucose is 1: 1: 1 in reduced IV.

Table III. *Rf* Values of Hydrolysates and Retention Times of Trimethylsilyl Derivatives of Methanolysates

	Cellulose TLC (*Rf*)		GLC (t_R)	
	Solvent A	Solvent B	Condition A	Condition B
Hydrolysate of I	0.49, 0.42	0.60, 0.54		
Hydrolysate of II	0.49	0.60		
Hydrolysate of III	0.49, 0.42	0.60, 0.54		
Hydrolysate of IV	0.49, 0.42	0.60, 0.54		
Hydrolysate of V	0.49	0.60		
Hydrolysate of VI	0.49	0.60		
Mannose	0.49	0.60		
Glucose	0.42	0.54		
Methanolysate of I			16.0, 19.1	12.7, 16.9
Methanolysate of II			16.0	12.7
Methanolysate of III			16.0, 19.1	12.7, 16.9
Methanolysate of IV			16.0, 19.1	12.7, 16.9
Methanolysate of V			16.0	12.7
Methanolysate of VI			16.0	12.7
Methanolysate of reduced I			19.1, 20.2	15.1, 16.9
Methanolysate of reduced II			16.0, 20.2	12.7, 15.1
Methanolysate of reduced III			16.0, 20.4	12.7, 15.4
Methanolysate of reduced IV			16.0, 19.1, 20.2	12.7, 15.1, 16.9
Methanolysate of reduced V			16.0, 20.2	12.7, 15.1
Methanolysate of reduced VI			16.0, 20.2	12.7, 15.1
Methyl mannoside			16.0	12.7
Methyl glucoside			19.1	16.9
Mannitol			20.2	15.1
Sorbitol			20.4	15.4

Periodate Oxidation——Each oligosaccharide (3 mg) was oxidized with 0.05M sodium metaperiodate in 0.2M acetate buffer (pH 4.4) (3 ml) at 5° in a dark place. The periodate consumption was measured by a spectrophotometric method.[17] For the determination of formic acid liberation, the oxidations were performed with water solution of sodium metaperiodate. The oxidations were completed after 13 days for I, 10 days for II, 27 days for V and VI, 20 days for III, and 16 days for IV. Formic acid liberation was measured by a titration with 0.01N NaOH, and formaldehyde was estimated by chromotropic acid method.[18]

17) *a*) J.S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954); *b*) G.O. Aspinall and R.J. Ferrier, *Chem. Ind.*, **1957**, 1216.
18) M. Lambert and A.C. Neish, *Can. J. Research*, **28B**, 83 (1950).

Bromine Oxidation followed by Smith Degradation——This was carried out in the same manner as described in the previous report.[1] The products were analyzed by GLC.

GLC: column, 5% SE 30 on Chromosorb G (80 to 100 mesh) (0.3 cm × 2 m long stainless steel); programmed column temperature, increase in 5° per min from 60° to 200°; carrier gas, N_2 (30 ml per min); t_R, glycolic acid 12.3; glycerol 19.4; erythritol 26.0; trimethylolpropane (internal standard) 23.0.

Methylation and Methanolysis——Sodium hydride (5 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 20 min stirring 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 (20 ml), the mixture was extracted with chloroform (20 ml) three times. The combined extract was washed with water (60 ml) three times, then dried over Na_2SO_4 and the filtrate was evaporated. The residue was methylated again under the same condition. The infrared spectra of the final product had no absorption near 3400 cm^{-1}. The fully methylated product was heated with 3% methanolic HCl in a sealed tube at 70° for 16 hr. After cooling, the solution was evaporated and HCl was removed by the repeated addition and evaporation of methanol.

Analysis of Methanolysate——Chloroform solution of the methanolysate was applied to a gas chromatograph. The following two conditions were used: C, a column (0.3 cm × 2 m long stainless steel) packed with 15% Poly-butane 1,4-diol succinate on Chromosorb W (80 to 100 mesh) at 175° with a flow of 20 ml per min of N_2; D, a column (0.3 cm × 2 m long stainless steel) packed with 5% Neopentylglycol succinate on Chromosorb G (60 to 80 mesh) at 150° with a flow of 20 ml per min of N_2. Table IV shows relative retention times of the products obtained by methanolysis to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside in the two conditions.

Table IV. Relative Retention Times[a] of Methylation Products

	Condition C (15% BDS)	Condition D (5% NPGS)
Methanolysate of methylated I	1.00, 1.37, 3.62, 4.24	1.00, 1.43, 3.38, 4.52
Methanolysate of methylated II	1.33, 1.86, 3.62, 4.24	1.42, 2.23, 3.39, 4.53
Methanolysate of methylated III	1.33, 1.86, 3.67, 4.07	1.42, 2.23, 3.36, 4.28
Methanolysate of methylated IV	1.33, 1.87, 3.68, 4.17	1.42, 2.23, 3.36, 4.44
Methanolysate of methylated V	1.33, 1.86, 3.62, 4.24	1.42, 2.23, 3.39, 4.53
Methanolysate of methylated VI	1.33, 1.86, 3.62, 4.24	1.42, 2.23, 3.39, 4.53
Methyl 2,3,4,6-tetra-O-methyl-D-glucoside	1.00, 1.37	1.00, 1.43
Methyl 2,3,6-tri-O-methyl-D-glucoside	3.67, 4.07	3.36, 4.28
Methyl 2,3,4,6-tetra-O-methyl-D-mannoside	1.33, 1.86	1.42, 2.23
Methyl 2,3,6-tri-O-methyl-D-mannoside	3.62, 4.24	3.39, 4.53

a) methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside=1.00

Partial Hydrolysis of Oligosaccharides——The sample was dissolved in 0.5N H_2SO_4 and heated under reflux at 90° for 1 hr. After neutralization with barium carbonate, the hydrolysate was analyzed by TLC and its trimethylsilyl derivative was analyzed by GLC as described above.