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Plant Mucilages. X.[1] Isolation and Characterization of a Mucous Polysaccharide, "Lilium-A-glucomannan," from the Bulbs of *Lilium auratum*

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A mucous polysaccharide, named Lilium-A-glucomannan, has been isolated from the bulbs of *Lilium auratum* Lindl. It was homogeneous on glass-fiber paper electrophoresis and by ultracentrifugal analysis. It was composed of D-mannose and D-glucose in the molar ratio of 8: 3, and its molecular weight was estimated at 35700. The O-acetyl groups in it were identified and the content was 5.1%. The O-acetyl groups were located in both positions 3 and 6 of a part of D-mannose units. Methylation, periodate oxidation and partial acid hydrolysis studies suggested that the polysaccharide is mainly composed of β-1→4 linked aldohexopyranose residues having a small degree of branching at position 2 of D-mannose residues and the non-reducing ends were terminated by D-mannose units.

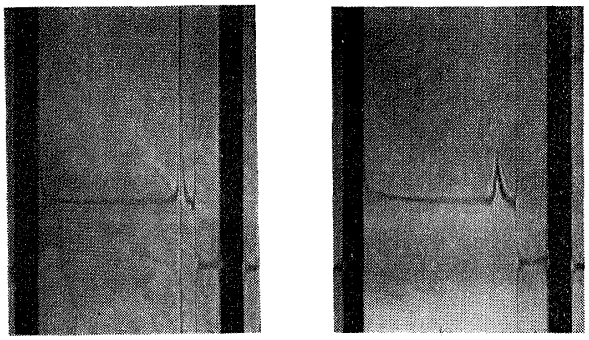
The bulbs of *Lilium auratum* Lindl. have been used as a crude drug for the purpose of analeptic and cough medicine. Lily bulbs contain, in addition to starch, a water-soluble mucous reserve polysaccharide. And it has been reported that the mucilage was composed of mannose and glucose in the approximate molar ratio of 2: 1.[3] Structural studies on glucomannans isolated from the bulbs of *Lilium candidum*, *L. henryii* and *L. umbellatum* suggested that the majority of hexose units were linked together by β-1→4 glycosidic bonds to form long chains.[4] In this paper, the isolation and the structural feature of a new pure mucous polysaccharide from the fresh bulbs of *Lilium auratum* are described.

The material bulbs were crushed and extracted with hot methanol, then the residue was extracted with cold water. The crude mucilages were precipitated from the water extract by addition of methanol. The solution of the precipitate was applied to a column of DEAE-cellulose (carbonate form), and a mucous polysaccharide was obtained from the eluate with water.

The polysaccharide gave one spot on glass-fiber paper electrophoresis in alkaline borate buffer, and it was found to be homogeneous by the ultracentrifugal analysis (Fig. 1). Characteristic sharp sedimentation pattern (Fig. 1-a) of high molecular weight viscous substances was shown as described in the former report.[5]

It showed a negative specific rotation ($[\alpha]_D^{23}$ −37.9° in H_2O, c=1.0). Its solution in water gave the intrinsic viscosity value of 2.4 at 27°. As the component sugars of it, mannose and glucose were identified by means of cellulose thin-layer chromatography (TLC) of the hydrolysate and gas–liquid chromatography (GLC) of trimethylsilyl derivative of the methanolysate. Quantitative determination of them showed that the molar ratio of mannose: glucose is 8: 3. The measurement of osmotic pressure gave the value of 35700 as the molecular weight of the polysaccharide and this value was also supported by the result of gel chromatography on Sephadex G-200.

1) Part IX: M. Tomoda, S. Nakatsuka and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **22**, 2710 (1974).
2) Location: *1-5-30, Shibakoen, Minato-ku, Tokyo, 105, Japan.*
3) T. Takahashi, *Nippon Nogeikagaku Kaishi*, **6**, 791, 861 (1930); *idem, ibid.*, **7**, 219 (1931).
4) P. Andrews, L. Hough and J.K.N. Jones, *J. Chem. Soc.*, **1956**, 181.
5) M. Tomoda, S. Nakatsuka, M. Tamai and M. Nagata, *Chem. Pharm. Bull.* (Tokyo), **21**, 2667 (1973).



(a) (b)

Fig. 1. Ultracentrifugal Pattern of Lilium-A-glucomannan

a: 0.5% in H_2O, 20°, 75 min, 60000 rpm
b: 0.1% in H_2O, 20°, 48 min, 60000 rpm
Hitachi model UCA-1A ultracentrifuge

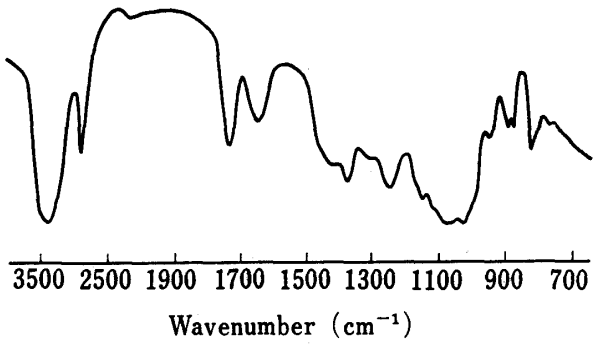
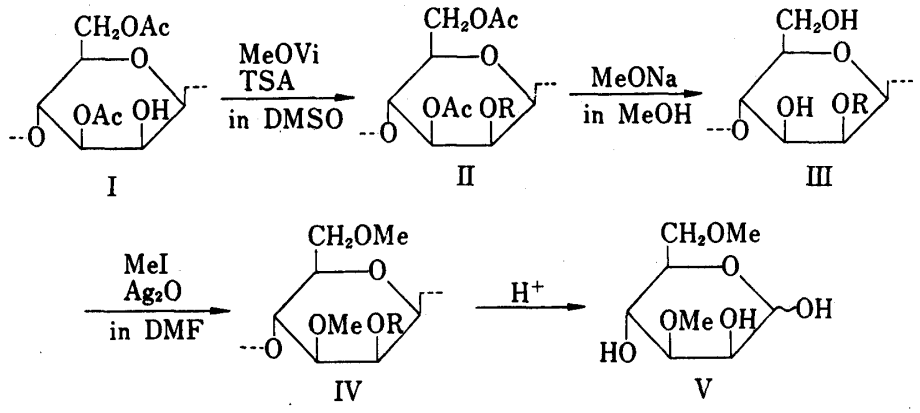


Fig. 2. IR Spectrum of Lilium-A-glucomannan

As shown in Fig. 2, the infrared (IR) spectrum of it has the absorption bands of 1735 and 1250 cm^{-1} suggesting the presence of ester linkages in addition to the absorption of 890 cm^{-1} being due to β-glycosidic linkages. The acid hydrolysate of the polysaccharide was analyzed by GLC,[1] and it gave one peak, whose retention time was precisely equal to that of authentic sample of acetic acid. The acetyl content of the polysaccharide was determined to be 5.1% by GLC. Thus the pure mucilage obtained by us has different properties from those described in the former reports,[3,4] and the name "Lilium-A-glucomannan" is proposed for the polysaccharide.

The location of O-acetyl groups in Lilium-A-glucomannan was established by the application of the method[6] using methyl vinyl ether as a protective reagent for the free hydroxyl groups. The sequence of reactions is illustrated in Chart 1. The polysaccharide (I) was dissolved in dimethylsulfoxide and treated with methyl vinyl ether in the presence of *p*-toluenesulfonic acid for conversion of the free hydroxyl groups to 1-methoxyethyl ethers (II). Deacetylation of the derivative (II) was accomplished by refluxing with methanolic sodium methoxide and gave the partially-O-(1-methoxyethyl)-glucomannan (III), then it was methylated with methyl iodide and silver oxide in dimethylformamide.[7] Each product was purified by gel chromatography using a column of Sephadex LH-20. The resulting par-



$(R = -\underset{\underset{O-CH_3}{|}}{CH}-CH_3)$

Chart 1

6) A.N. de Belder and B. Norrman, *Carbohyd. Res.*, **8**, 1 (1968).
7) R. Kuhn, H. Trischmann and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

tially-O-methyl-O-(1-methoxyethyl)-glucomannan (IV) was finally subjected to acid hydrolysis, and the products were analyzed by paper partition chromatography (PPC) and by GLC of the alditol acetate after reduction and acetylation[8] of the hydrolysate. Besides mannose and glucose, a hexose methyl ether was detected and identified as 3,6-di-O-methyl-D-mannopyranose (V) by comparison with the synthetic specimen.[9]

Owing to this result, it is able to conclude that the O-acetyl groups are attached to positions 3 and 6 of a part of D-mannopyranose units in Lilium-A-glucomannan. The value of quantitative analysis indicated that one mannose residue in about eight component hexose units has 3,6-di-O-acetyl groups.

The methylation of the polysaccharide was performed with sodium methylsulfinylcarbanion and methyl iodide in dimethylsulfoxide.[10] The fully methylated product was hydrolyzed with formic acid and dilute sulfuric acid. The products were separated by PPC, then analyzed by GLC after conversion to alditol acetates.[8] As the hydrolysis products of the methylated polysaccharide, 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose and 3,6-di-O-methyl-D-mannose were obtained in a molar ratio of 1.0: 6.3: 2.9: 0.7. These methyl ethers of component sugars were also identified as their methyl glycosides by GLC.

These results suggested that the polysaccharide is mainly composed of 1→4 linked aldohexopyranose units and has some mannopyranose residues as branching points linked through position 2 with an average of about eleven component sugar units per non-reducing end group.

As the result of periodate oxidation, 1.01 mole of periodate per one mole of component anhydro sugar unit of the polysaccharide was consumed with 0.09 mole of formic acid liberation. The periodate-oxidized polysaccharide was treated with sodium borohydride,[11] and the reduction product was methanolyzed. Analysis of trimethylsilyl derivative of the methanolysate by GLC revealed the presences of erythritol and mannose as the main products and showed that the yields of erythritol and mannose were 26.0% and 8.0%. These results, especially the value of formic acid liberation after periodate oxidation and the yield of mannose by Smith degradation, supported the conclusion of branching structure obtained by methylation studies.

Partial acid hydrolysis of Lilium-A-glucomannan also gave the evidence that the straight chain parts in the polysaccharide are composed of β-1→4 linked aldohexopyranose residues. The mucilage was hydrolyzed with 0.5N sulfuric acid at 90° for 2.5 hr, and the products were fractionated by active charcoal column chromatography. Most of the fractions were applied to PPC, and several oligosaccharides were obtained. The comparison by TLC and by GLC of trimethylsilyl derivatives with authentic samples[12] showed that they are O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, O-β-D-glucopyranosyl-(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 results elucidated the fact that most of D-mannopyranose and D-glucopyranose residues are connected one another by β-1→4 glycosidic linkages, and at least, the mucilage has 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.

8) H. Björndal, B. Lindberg and S. Svensson, *Acta Chem. Scand.*, **21**, 1801 (1967).
9) N. Handa and R. Montgomery, *Carbohyd. Res.*, **11**, 467 (1969).
10) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
11) M. Abdel-Akher, J.K. Hamilton, R. Montgomery and F. Smith, *J. Am. Chem. Soc.*, **74**, 4970 (1952).
12) M. Tomoda, S. Nakatsuka and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **21**, 2511 (1973).

Andrews, *et al.*[4] reported that the glucomannans isolated from the bulbs of *Lilium umbellatum* and *L. henryii* had D-glucopyranosyl units as their non-reducing ends and *L. umbellatum* glucomannan had a small number of D-glucopyranosyl units as branching points linked through positions 3 and 6. Thus Lilium-A-glucomannan has different properties from those of the other Lilium glucomannans obtained until now in points of not only molar ratio of component sugars but also branching structure. And this is the first report describing the presence of partially 3,6-di-O-acetylated D-mannopyranosyl units in natural glucomannan.

The former investigators[3,4] used copper complex method for the purification of Lilium glucomannans, and they described that the polysaccharides became insoluble in water after such a treatment. The treatment with alkaline solution causes easily O-deacetylation, and it is unsuitable for the isolation of native polysaccharides having O-acetyl groups. So it will be necessary to reexamine the presence of O-acetyl groups in the other native Lilium glucomannans.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Viscosity was measured with an Ubbelohde-type viscosimeter. Optical rotation was determined with JASCO model DIP-SL automatic polarimeter. IR spectra were measured with Hitachi model EPI-G3 infrared spectrophotometer. GLC was carried out by the use of Hitachi model 063 gas chromatograph equipped with hydrogen flame ion detector.

Isolation of Polysaccharide——The material was obtained in September of 1973 from the plants cultivated in Saitama prefecture. The fresh bulbs (235 g), which contain 72.9% of water, were crushed, then extracted with hot methanol (940 ml) for 30 min. After suction filtration, the extraction was similarly repeated. The extracts were combined, concentrated and lyophilized. Light brown powder (8.80 g) was obtained. After extraction with methanol, the residue was extracted with water (1880 ml) under stirring at room temperature for 1 hr twice. After suction filtration, the extracts were combined and poured into two volumes of methanol, then filtered. The precipitate was treated with absolute methanol, then dried *in vacuo*. Gray powder (8.34 g) was obtained. A part of the crude mucilage (0.8 g) was dissolved in water and applied to a column (4.5×60 cm) of DEAE-cellulose (Brown Co.). DEAE-Cellulose was used as carbonate form by previous successive treatments with 0.5N sodium hydroxide, water, 1M ammonium carbonate and water. The column was eluted with water, and fractions of 100 ml were collected and analyzed by phenol-sulfuric acid method.[13] The eluates obtained from tubes 5 to 14 were combined, concentrated and lyophilized. Lilium-A-glucomannan (0.66 g) was obtained as white powder.

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF 83 glass-fiber and alkaline borate buffer of pH 9.2 (0.025M borax: 0.1N NaOH, 10: 1) in the same manner as a preceding report[14] of this series. The condition of 380 volt for 2 hr was used. The sample gave one spot at a distance of 10.4 cm from the origin toward the cathod. Distance moved by standard glucose was 14.0 cm.

Qualitative Analyses of Component Sugars——The sample was hydrolyzed with 2N sulfuric acid in a sealed tube at 100° for 6 hr followed by neutralization with barium carbonate. The hydrolysate was applied to TLC using Avicel SF cellulose and the following three solvent systems: A, AcOEt: pyridine: AcOH:

Table I. *Rf* Values of Component Sugars and Retention Times of Trimethylsilyl Derivatives

	Cellulose TLC (*Rf*)			GLC (t_R) Condition A
	Solvent A	Solvent B	Solvent C	
Hydrolysate	0.58, 0.54	0.44, 0.37	0.41, 0.35	
Methanolysate				15.6, 19.6
Mannose	0.58	0.44	0.41	
Glucose	0.53	0.37	0.35	
Methylmannoside				15.6
Methylglucoside				19.6

13) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
14) M. Tomoda, Y. Yoshida, H. Tanaka and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 2173 (1971).

H_2O (5:5:1:3); B, BuOH: pyridine: H_2O (6:4:3); C, C_6H_5OH: 1% NH_4OH (2:1). Component sugars were revealed with silver nitrate reagent[15] and naphthoresorcinol-phosphoric acid reagent.[16]

On the other hand, the sample was methanolyzed with 4% methanolic HCl in a sealed tube at 75° for 16 hr. After removal of HCl by the repeated addition and evaporation of methanol, the methanolysate was trimethylsilylated[18] and applied to GLC. GLC was carried out under condition A, a column (0.3 cm × 2 m long spiral stainless steel) packed with 2% OV 17 on Chromosorb W (80 to 100 mesh) and with a flow of 20 ml per min of N_2. The programmed temperature was increased 3° per min from 120° to 200°. Table I shows *Rf* values in TLC and retention times in GLC of components.

Determination of Component Sugars——The sample (4 mg) was hydrolyzed with 2N sulfuric acid in a sealed tube at 100° for 6 hr, then neutralized with barium carbonate. The hydrolysate was reduced in water (5 ml) with sodium borohydride (5 mg) for 1 hr. After neutralization with Dowex 50W (H^+), the filtrate was evaporated and boric acid was removed by the repeated addition and evaporation of methanol. Then the products were acetylated with acetic anhydride–pyridine mixture (1:1, 2 ml) at 100° for 20 min. After evaporation of the solution, the residue was dissolved in chloroform–methanol mixture (1:1) and applied to GLC. GLC was carried out under condition B, a column (0.3 cm × 2 m long spiral stainless steel) packed with 3% ECNSS–M on Gaschrom Q (100 to 120 mesh) at 200° with a flow of 40 ml per min of N_2. Xylose was used as an internal standard. Retention times of acetates of xylitol, mannitol and sorbitol were 9.2, 16.0 and 21.1. The result revealed that the sample was composed of 69.1% of mannose and 25.9% of glucose in addition to acetyl group.

Determination of Molecular Weight——The measurement of osmotic pressure was carried out by the use of Knauer Electronic Membrane Osmometer in the same manner as a former report[17] of this series.

For the gel chromatography, a column (2.6 × 96 cm) of Sephadex G-200 (Pharmacia Co.) was prepared and the elution was carried out as described in the first report[18] of this series. Fractions of 5 ml were collected and analyzed by phenol-sulfuric acid method.

Determination of O-Acetyl Groups——The IR spectrum of the polysaccharide showed the absorption bands of ester. IR ν_{max}^{KBr} cm^{-1}: 1735, 1250 (ester), 890 (β-glycosidic linkage).

The sample (3 mg) was hydrolyzed with 1N hydrochloric acid (0.05 ml) containing propionic acid (0.2 mg) as an internal standard in a sealed tube at 100° for 2 hr. The hydrolysate was directly applied to GLC. GLC was carried out under condition C, a column (0.3 cm × 2 m long spiral stainless steel) packed with 20% tetramethyl cyclobutanediol adipate-4% phosphoric acid on Chromosorb W (80 to 100 mesh) at 120° with a flow of 20 ml per min of N_2; t_R, acetic acid 6.0; propionic acid (internal standard) 9.4.

Treatment with Methyl Vinyl Ether——The sample (100 mg) was dissolved in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (20.8 mg) was added. The solution was stirred at 15°, then methyl vinyl ether (5 ml), condensed at −10°, was added in portions under stirring. The reaction mixture was stirred at 15° for 3.5 hr. The clear, deep orange solution was then applied to a column (4 × 20 cm) of Sephadex LH-20 (Pharmacia Co.). The column was eluted with anhydrous acetone, and fractions were collected at 20 ml. The eluates obtained from tubes 6 to 11 were combined and concentrated. The IR spectrum of the residue had no absorption near 3400 cm^{-1}.

Deacetylation of the O-Acetyl-O-(1-methoxyethyl)-polysaccharide——The O-acetyl-O-(1-methoxyethyl)-polysaccharide (262 mg) was dissolved in methanol (5 ml), then 0.2M methanolic sodium methoxide (5 ml) was added under stirring. The solution was refluxed at 80° for 4 hr, then concentrated to 5 ml at room temperature. The solution was applied to a column (4 × 30 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 50 ml, and the eluates obtained from tubes 3 to 6 were combined and concentrated. The absence of carbonyl absorption in the IR spectrum of the residue proved the complete deacetylation.

Methylation of the O-(1-Methoxyethyl)-derivative——The O-(1-methoxyethyl)-derivative (200 mg) was dissolved in dimethylformamide (5 ml), then methyl iodide (1 ml) and silver oxide (0.4 g) were added successively under stirring. The reaction mixture was stirred at room temperature for 20 hr in a dark. After filtration, methyl iodide (1 ml) and silver oxide (0.4 g) were added again into the filtrate, then the reaction was similarly repeated. The reaction mixture was filtered, and the silver salts were washed with dimethylformamide (4 ml). The filtrate and washing were combined, then benzene (20 ml) was carefully added into the mixture. The precipitate was filtered off, and benzene was evaporated. The residual solution was applied to a column (4 × 30 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 50 ml, and the eluates obtained from tubes 3 to 5 were combined and concentrated.

Hydrolysis and Analyses of the O-Methyl-derivative——O-Methyl-O-(1-methoxyethyl)-derivative (140 mg) was hydrolyzed with 2N sulfuric acid at 100° for 5.5 hr, then neutralized with barium carbonate. The filtrate was evaporated and the hydrolysate (80 mg) was obtained.

15) W.E. Trevelyan, D.P. Procter and J.S. Harrison, *Nature*, **166**, 444 (1950).
16) V. Prey, H. Berbalk and M. Kausz, *Mikrochim. Acta*, **1961**, 968.
17) M. Tomoda and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **20**, 2491 (1972).
18) M. Tomoda and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 1214 (1971).

The hydrolysate was applied to PPC with Tôyô-Roshi No. 51 and solvent system D, AcOEt: HCOOH: H_2O (3:1:1). The R_G values (0.72, 0.40 and 0.34; 2,3,4,6-tetra-O-methyl-D-glucose=1.00) of the three spots detected with benzidine reagent[19] were identical with those of authentic 3,6-dimethyl-D-mannose, D-mannose and D-glucose.

On the one hand, the hydrolysate was reduced with sodium borohydride and then acetylated with acetic anhydride–pyridine mixture as described above. The alditol acetates were applied to GLC under condition B′, a column (0.3 cm×2 m) packed with 3% ECNSS–M on Gaschrom Q at 180° with a flow of 40 ml per min of N_2. The result revealed that the product has 3,6-di-O-methyl-1,2,4,5-tetra-O-acetyl-D-mannitol as the sole partially methylated alditol acetate, whose relative retention time to 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-glucitol was 4.38.

Methylation of Polysaccharide——Sodium hydride (200 mg) was mixed with dimethyl sulfoxide (7 ml) and the mixture was stirred at 70° for 1 hr. The sample (100 mg) was dissolved in dimethyl sulfoxide (20 ml) and the solution was added into this mixture. After stirring at 70° for 30 min, the reaction mixture was cooled to room temperature, then methyl iodide (10 ml) was added and the mixture was stirred overnight at room temperature. All procedures were carried out in nitrogen atmosphere. After dilution with water (100 ml), the mixture was extracted with chloroform (100 ml) three times. The combined extract was washed with water (400 ml) three times, then dried over sodium sulfate and the filtrate was evaporated. The residue was methylated two more times under the same condition. The IR spectra of the final product had no absorption near 3400 cm^{-1}.

Hydrolysis and Analysis of the Methylated Product——The half of the fully methylated polysaccharide was successively hydrolyzed with 90% formic acid at 90° for 16 hr and with 0.5N sulfuric acid at 100° for 2.5 hr, then the hydrolysate was applied to PPC with the solvent system E, BuOH: EtOH: H_2O (4:1:5, upper layer), as described in the preceding report.[1] The each of tetramethyl, trimethyl and dimethyl hexose-fractions was reduced with sodium borohydride, then acetylated with acetic anhydride–pyridine mixture as described above. GLC of partially methylated alditol acetates were carried out under condition B′. Table II shows relative retention times of the products to 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-glucitol.

Methanolysis and Analysis of the Methylated Product——A part of the fully methylated polysaccharide was methanolyzed with 4% methanolic HCl in a sealed tube at 75° for 16 hr. After removal of HCl by the repeated addition and evaporation of methanol, GLC of methyl glycosides of partially methylated hexoses were carried out under following two conditions: D, a column (0.3 cm×2 m long spiral 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; E, a column (0.3 cm×2 m long spiral 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. Relative retention times of the products to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside are also shown in Table II.

Table II. Relative Retention Times of Methylated Products

	Condition B′[a] (3% ECNSS-M)	Condition D[b] (15% BDS)	Condition E[b] (5%NPGS)
2,3,4,6-Tetra-O-methyl-1,5-di-O-acetyl-D-mannitol	0.99		
2,3,6-Tri-O-methyl-1,4,5-tri-O-acetyl-D-mannitol	2.10		
2,3,6-Tri-O-methyl-1,4,5-tri-O-acetyl-D-glucitol	2.43		
3,6-di-O-Methyl-1,2,4,5-tetra-O-acetyl-D-mannitol	4.38		
Methyl 2,3,4,6-tetra-O-methyl-D-mannoside		1.34, 1.86	1.42, 2.23
Methyl 2,3,6-tri-O-methyl-D-mannoside		3.62, 4.24	3.39, 4.53
Methyl 2,3,6-tri-O-methyl-D-glucoside		3.68, 4.07	3.36, 4.28
Methyl 3,6-di-O-methyl-D-mannoside		8.90	7.80, 9.14

a) for partially methylated alditol acetates
b) for methyl glycosides of partially methylated aldoses

Periodate Oxidation, Smith Degradation and Analysis of the Products——These were carried out as described in a previous report[5] of this series.

19) J.S.D. Bacon and J. Edelman, *Biochem. J.*, **48**, 114 (1951).

Partial Acid Hydrolysis——The polysaccharide (1 g) was dissolved in 0.5N sulfuric acid (200 ml) and heated under reflux at 90° for 2.5 hr. After neutralization with barium carbonate, the filtrate and washing were combined and concentrated, then applied to a column (2×15 cm) of active charcoal (for chromatographic use, Wakô-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 (650 ml), 5% ethanol (650 ml), 10% ethanol (400 ml), 15% ethanol (500 ml) and 20% ethanol (350 ml). Fractions were collected at 50 ml and carbohydrates in eluates were measured by phenol-sulfuric acid method. The eluates obtained from the column were divided into six groups: Frac. 1, tubes 1 to 5; Frac. 2, tubes 6 to 13; Frac. 3, tubes 14 to 26; Frac. 4, tubes 27 to 34; Frac. 5, tubes 35 to 44; Frac. 6, tubes 45 to 51. Disaccharide I was obtained from Frac. 2, and Fracs. 3 to 6 were respectively applied to PPC by ascending method using Tôyô-Roshi No. 50 and solvent system A. In the case of Frac. 3, disaccharides I, II, III and trisaccharide IV were obtained from parts showing *Rf* values of 0.31, 0.41, 0.24 and 0.17. In the case of Frac. 4, disaccharides II, III and tetrasaccharide VI were obtained from parts showing *Rf* values of 0.41, 0.24 and 0.07. In the case of Frac. 5, trisaccharide V and tetrasaccharide VI were obtained from parts showing *Rf* values of 0.20 and 0.08. And in the case of Frac. 6, trisaccharide IV and tetrasaccharide VI were obtained from parts showing *Rf* values of 0.17 and 0.07. Yields, 116.6 mg in I; 98.8 mg in II; 14.6 mg in III; 49.0 mg in IV; 83.1 mg in V; 45.3 mg in VI.

TLC and GLC of Partial Acid Hydrolysates——These were carried out as described in a previous report[12] of this series. Table III gives *Rf* values of partial acid hydrolysates on TLC and retention times of their trimethylsilyl derivatives on GLC in several conditions.

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

	Cellulose TLC (*Rf*)		GLC (t_R)			
	Solvent A	Solvent B	Condition A′[a]		Condition F[b]	
I (Man→Man)	0.42	0.30	36.6	38.5	41.7	43.7
II (Glc→Man)	0.53	0.38	35.0	36.9	39.9	42.1
III (Man→Glc)	0.33	0.23	37.9	39.1	42.2	43.6
IV (Man→Man→Man)	0.22	0.16	53.6	55.8	62.1	
V (Man→Glc→Man)	0.27	0.19	52.9	54.5	59.9	62.8
VI (Man→Man→Man→Man)	0.09	0.05	—		—	

a) condition A′, 2% OV 17 on Chromosorb W, from 130 to 280° (3°/min)
b) condition F, 3% SE 52 on Chromosorb W, from 130 to 280° (3°/min)

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