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Plant Mucilages. XIII.[1] Isolation and Characterization of a Mucous Polysaccharide, "Lilium-S-glucomannan," from the Bulbs of *Lilium speciosum*

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A mucous polysaccharide, named Lilium-S-glucomannan, has been isolated from the bulbs of *Lilium speciosum* Thunb. It was homogeneous on glass-fiber paper electrophoresis and by ultracentrifugal analysis. The component sugars of it were D-mannose and D-glucose in the molar ratio of 2: 1, and its molecular weight was estimated at 388000. Methylation, periodate oxidation and partial acetolysis studies suggested that the polysaccharide is mainly composed of β-1→4 linked aldohexopyranose residues and it contains about six aldohexose units per one end group on the average. Mannose units occupy nonreducing terminal positions and branch points at position 3. The O-acetyl groups in the polysaccharide were identified and determined as the content of 3.3%. They were located in both positions 3 and 6 of a part of D-mannose units.

In a previous paper[3] of this series, the isolation and the structural feature of a glucomannan from the bulbs of *Lilium auratum* have been reported by us and the necessity of reinvestigation of the presence of O-acetyl groups in the native Lilium glucomannans was pointed out. Now we obtained a new pure mucous polysaccharide from the fresh bulbs of *Lilium speciosum* Thunb., and its properties are described in the present paper.

After treatment with hot methanol, the crushed bulbs were extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol. 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 a single spot on glass-fiber paper electrophoresis in alkaline borate buffer, and was homogeneous when analyzed 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 cases of Bletilla-glucomannan[4] and Lilium-A-glucomannan.[3]

The polysaccharide showed a negative specific rotation ($[\alpha]_D^{23}$ $-29.5°$ in H_2O, $c=0.2$). Its solution in water gave the intrinsic viscosity value of 6.6 at 25°. Cellulose thin-layer chromatography (TLC) of the hydrolysate and gas-liquid chromatography (GLC) of trimethylsilyl derivative of the methanolysate of the polysaccharide revealed that its component sugars are mannose and glucose. Quantitative determination of them showed that the molar ratio of mannose: glucose is 2: 1. The measurement of osmotic pressure gave the value of 388000 as the molecular weight of the polysaccharide.

As shown in Fig. 2, its infrared (IR) spectrum has the absorption bands of 1730 and 1250 cm^{-1} suggesting the presence of ester linkages. When the acid hydrolysate of the polysaccharide was analyzed by GLC,[5] it gave one peak, whose retention time was the same as that of authentic sample of acetic acid. The acetyl content of the polysaccharide was deter-

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[1] Part XII: M. Tomoda and S. Kimura, *Chem. Pharm. Bull.* (Tokyo), 24, 1807 (1976).
[2] Location: *1-5-30, Shibakôen, Minato-ku, Tokyo, 105, Japan.*
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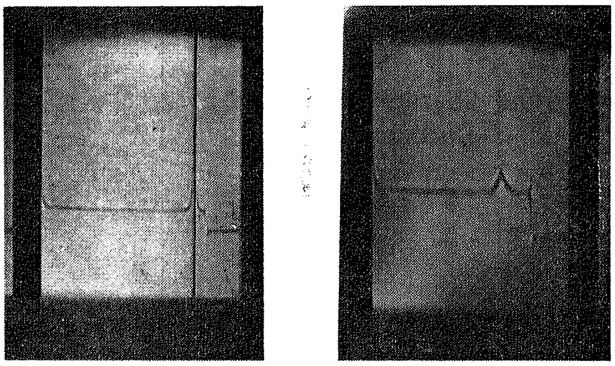

(a) (b)

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

a: 0.5% in H_2O, 20°, 60 min, 60000 rpm
b: 0.1% in H_2O, 20°, 75 min, 60000 rpm
Hitachi model UCA-1A ultracentrifuge

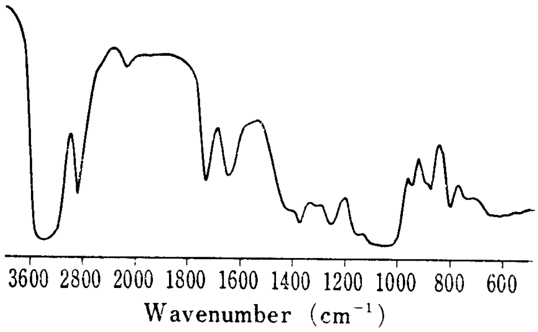

Fig. 2. IR Spectrum of Lilium-S-glucomannan

mined to be 2.0% by GLC. The name "Lilium-S-glucomannan" is proposed for the polysaccharide.

The methylation of the polysaccharide was performed with sodium methylsulfinyl carbanion and methyl iodide in dimethylsulfoxide.[6] The fully methylated product was formolyzed with formic acid and hydrolyzed with dilute sulfuric acid, then analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) after conversion to alditol acetates.[7,8] 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 2,6-di-O-methyl-D-mannose were identified and obtained in a molar ratio of 1.0: 2.2: 1.9: 0.3. The tetramethyl ether of mannose was also confirmed as its methyl glycoside by GLC.

As the result of periodate oxidation at room temperature, 1.07 mole of periodate per one mole of component anhydro sugar unit of the polysaccharide was consumed with 0.27 mole of formic acid liberation. The periodate-oxidized polysaccharide was treated with sodium borohydride,[9] and the reduction product was obtained after purification with gel chromatography on Sephadex G-15. Analysis of trimethylsilyl derivative of the methanolysate of it by GLC revealed the presences of erythritol and mannose as the main products and the yields of them were 31.6% and 14.9%.

These results indicated that the polysaccharide is mainly composed of 1→4 linked aldohexopyranose units and has some mannopyranose residues as terminals and branching points linking through position 3. From the result of methylation study and the value of quantitative analysis of mannose in Smith degradation product, it is able to conclude that the polysaccharide contains about six aldohexose units per one non-reducing end group. The yield of the dimethyl ether of mannose was too low in comparison with that of the tetramethyl ether of it. But none of the other di- and mono-methyl ethers and free aldose was detected in the hydrolysate of the methylated product by GLC or TLC. One of the causes for such a discrepancy may be attributed to unavoidable degradation and losses of methylated sugars.[10] The value of formic acid liberation, on the contrary, was too high in comparison with that estimated from the branching degree. When periodate oxidation was carried out at 5°, the maximum

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values of periodate consumption and formic acid liberation were 0.79 mole and 0.11 mole per one mole of component anhydro sugar unit. This result suggested that the excessive value was probably due to partial overoxidation.

Partial acetolysis of Lilium-S-glucomannan also gave the evidence that the straight chain parts in the polysaccharide are composed of β-1→4 linked aldohexopyranose residues. The polysaccharide 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 gel chromatography using Sephadex G-15. The comparison by TLC and by GLC of trimethylsilyl derivative with authentic samples[1,11] showed that the oligosaccharides obtained 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-glucopyranosyl-(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, on the whole, the manner of sequence of the main chain is similar to those of Bletilla-glucomannan[4] and Lilium-A-glucomannan.[3]

The method of de Belder and Norrman[12] was applied to the determination of the location of O-acetyl groups in Lilium-S-glucomannan. Because the polysaccharide is hardly soluble in dimethylsulfoxide, the sample was digested with a hemicellulase preparation. After gel chromatography using a column of Sephadex G-15, the partially degraded polysaccharide having all ester linkages was isolated. It 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. The derivative was deacetylated by refluxing with methanolic sodium methoxide, then methylated with methyl iodide and silver oxide in dimethylformamide.[13] The resulting product was subjected to acid hydrolysis, and the final products were analyzed by GLC–MS of the alditol acetate after reduction and acetylation of the hydrolysate and by paper partition chromatography (PPC) of it. Besides mannose and glucose, a hexose methyl ether was detected and identified as 3,6-di-O-methyl-D-mannopyranose. None of hexose monomethyl ether was detected as the product of these reactions.

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 the polysaccharide. The value of quantitative analysis indicated that one mannose residue in about twenty-six component hexose units has 3,6-di-O-acetyl groups.

The presence of O-acetyl groups in native glucomannans from lily bulbs and the fact that deacetylation of them causes the insolubility of the products in water have been already pointed out by us[3] and the other investigators,[14] so the location of O-acetyl groups in native polysaccharides is an interesting problem in connection with their properties. Lilium-S-glucomannan has 3,6-di-O-acetylated D-amnnopyranosyl units and β-1→4 linked aldohexose main chains just as Lilium-A-glucomannan,[3] although the two glucomannans possess respectively different types of branching and widely different values of molecular weight. The structural investigations of glucomannans from the other lily bulbs are now in progress.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Optical rotation was determined with JASCO model DIP-SL automatic polarimeter. Viscosity was measured with

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[14] T. Matsuo and T. Mizuno, *Agr. Biol. Chem.*, **38**, 465 (1974).

an Ubbelohde-type viscosimeter. 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 ionization detector. GLC–MS was performed by the use of JEOL model JGC-20K gas chromatograph and JEOL model JMS-D100 mass spectrometer.

Isolation of Polysaccharide——The material was obtained in September of 1974 from the plants grew in Saitama prefecture. The fresh bulbs (366 g), which contain 63.3% of water, were crushed, then extracted with hot methanol (740 ml) for 10 min. After suction filtration, the residue was extracted with water (1830 ml) under stirring at room temperature for 1 hr twice. The extracts were combined and poured into two volumes of ethanol, then filtered. The precipitate was treated with absolute ethanol, then dried *in vacuo*. Gray powder (14.05 g) was obtained. A part of the crude mucilage (1 g) was dissolved in water and applied to a column (4×37 cm) of DEAE-cellulose (carbonate form). The column was eluted with water, and fractions of 50 ml were collected and analyzed by phenol–sulfuric acid method.[15] The eluates obtained from tubes 8 to 26 were combined, concentrated and lyophilized. Lilium-S-glucomannan (0.81 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 (0.025 M borax: 0.1 N sodium hydroxide, 10: 1) in the same manner as a preceding report[16] of this series. The condition of 380 volt for 2 hr was used. The sample gave one spot at a distance of 12.4 cm from the origin toward the cathod. Standard glucose showed a spot at a distance of 14.4 cm.

Qualitative Analyses of Component Sugars——The sample was hydrolyzed with 2 N 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 two solvent systems: A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3); B, BuOH: pyridine: H_2O (6: 4: 3). Component sugars were revealed with *p*-anisidine reagent[17] and alkaline silver nitrate reagent.[18] In addition, the sample was methanolyzed with 4% hydrogen chloride in methanol in a sealed tube at 75° for 16 hr. After removal of hydrogen chloride by the repeated addition and evaporation of methanol, the methanolysate was trimethylsilylated 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 in the programmed temperature increasing 3° per min from 120 to 280°. *Rf* values in TLC and retention times in GLC were the same as described in a previous report.[3]

Determination of Component Sugars——The sample (5 mg) was hydrolyzed with 2 N sulfuric acid in a sealed tube at 100° for 6 hr, then neutralized with barium carbonate. The hydrolysate was reduced in water with sodium borohydride (10 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 glass) packed with 3% ECNSS-M on Gaschrom Q (100 to 120 mesh) at 195° with a flow of 30 ml per min of N_2. Xylose was used as an internal standard. Retention times of acetates of xylitol, mannitol and sorbitol were 11.5, 19.7, and 26.8 min, respectively. The results revealed that the sample was composed of 65.1% of mannose and 31.9% 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[19] of this series.

Determination of O-Acetyl Groups——The IR spectrum of the polysaccharide showed the absorption bands of ester. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1730, 1250 (ester), 890 (β-glycosidic linkage).

The sample (3 mg) was hydrolyzed with 1 N hydrochloric acid (0.05 ml) containing propionic acid (0.1 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 (min), acetic acid 7.2; propionic acid (internal standard) 11.3.

Methylation of Polysaccharide[20]——Sodium hydride (750 mg) was mixed with dimethyl sulfoxide (15 ml) and the mixture was stirred at 50° for 45 min. The polysaccharide (1 g) was suspended in dimethyl sulfoxide (50 ml) and stirred at 60° for 1 hr and, after cooling to room temperature, the solution of methylsulfinylcarbanion was added into this mixture. After stirring at room temperature for 4 hr, methyl iodide (3 ml) was added and the mixture was stirred overnight at room temperature. All procedures were carried out

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20) H.E. Conrad, *Meth. Carbohyd. Chem.*, **6**, 361 (1972).

in nitrogen atmosphere. Then the reaction mixture was dialyzed against running water overnight. The non-dialyzable fraction was concentrated and lyophilized. The product was methylated six more times under the same condition. The final non-dialyzable fraction was extracted with chloroform (200 ml) eight times. The extracts were combined and dried over sodium sulfate and the filtrate was evaporated to dryness. The IR spectrum of the final product (1.15 g) had no absorption near 3400 cm^{-1}.

Analysis of the Methylated Product——One tenth of the product was successively treated with 90% formic acid at 90° for 16 hr and 0.5 N sulfuric acid at 100° for 2.5 hr. After neutralization with barium carbonate, the hydrolysate was reduced with sodium borohydride, then acetylated with acetic anhydride–pyridine mixture as described above. GLC of partially methylated alditol acetates was carried out under condition B' using the same column as condition B but at 180° with a flow of 30 ml per min of N_2. Relative retention times of the products to 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-glucitol and fragments of the products in the mass spectra were as follows: 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-mannitol, 0.98, *m/e* 43, 45, 71, 87, 101, 117, 129, 145, 161, 205; 2,3,6-tri-O-methyl-1,4,5-tri-O-acetyl-D-mannitol, 2.13, *m/e* 43, 45, 87, 99, 101, 113, 117, 233; 2,3,6-tri-O-methyl-1,4,5-tri-O-acetyl-D-glucitol, 2.44, *m/e* 43, 45, 87, 99, 101, 113, 117, 233; 2,6-di-O-methyl-1,3,4,5-tetra-O-acetyl-D-mannitol, 3.13, *m/e* 43, 45, 87, 117, 129.

On the other hand, a part of the product 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 the same two conditions as described in a previous report.3)

Periodate Oxidation——The sample (200 mg) was oxidized with 0.05 M sodium metaperiodate (100 ml) at 25° in a dark place. The periodate consumption was measured by a spectrophotometric method.21) The oxidation was completed after twenty days, then 1 ml of the solution was used for the measurement of formic acid liberation by a titration with 0.01 N sodium hydroxide after addition of one drop of ethylene glycol. On the other hand, the reaction was carried out at 5° in the similar manner with 0.05 M sodium metaperiodate in water or in 0.2 M acetate buffer (pH 4.4) for three months. Two kinds of solvent for the reagent gave the same results.

Smith Degradation and Analysis of Products——The residue of the reaction mixture was neutralized with 0.1 N sodium hydroxide and treated with ethylene glycol (0.5 ml). After evaporation, the solution was poured into two volumes of ethanol. The precipitate was separated by centrifugation, then dissolved in water. The solution was treated with sodium borohydride (900 mg) at 5° for 16 hr, then adjusted to pH 5 by addition of acetic acid. The solution was concentrated and applied to a column (5.5 × 82 cm) of Sephadex G-15. Fractions were collected at 50 ml, and the eluates obtained from tubes 11 to 14 were combined, concentrated and lyophilized. The product was methanolyzed with 4% methanolic hydrogen chloride at 70° for 16 hr, and the determinations of mannose and erythritol were carried out by GLC as described in a previous paper.5)

Partial Acetolysis——The polysaccharide (0.5 g) was suspended in formamide (12 ml), then pyridine (6 ml) and acetic anhydride (4 ml) were added under stirring. The mixture was stirred for three days at room temperature, then poured into three volumes of methanol. The precipitate was filtered off, washed with methanol and dried. The product was dissolved in acetic anhydride (8 ml) and, after the addition of a cold mixture of acetic anhydride: sulfuric acid (5: 1, 4 ml) under cooling, the solution was kept at 5° for one day. The reaction mixture was poured into ice water (100 ml), then the products were extracted with chloroform (100 ml) three times. The extracts were combined and washed successively with 10% sodium bicarbonate and water, then evaporated to dryness. The products were dissolved in methanol (10 ml) and deacetylated with 0.2 N sodium hydroxide (10 ml) overnight at 5° and neutralized with acetic acid. The solvent was evaporated off.

Separation and Analysis of Partial Acetolysis Products——The residue was dissolved in water and applied to a column (4.8 × 78 cm) of Sephadex G-15. The column was eluted with water and fractions were collected at 50 ml. Carbohydrates in eluates were measured by phenol–sulfuric acid method and oligosaccharides were obtained from tubes 17 to 20. Each fraction was applied to PPC by ascending method using Tôyô-Roshi No. 50 and solvent system B. In the fraction from tube 20, disaccharides I and II were obtained from parts showing *Rf* values of 0.37 and 0.30. In the fraction from tube 19, disaccharides III and IV were obtained from parts showing *Rf* values of 0.25 and 0.17. In the fraction from tube 18, trisaccharide V was obtained from a part showing *Rf* value of 0.14. And in the fraction from tube 17, trisaccharides V, VI, and tetrasaccharide VII were obtained from parts showing *Rf* values of 0.14, 0.10, and 0.06. Yields, 4.1 mg in I; 3.7 mg in II; 8.7 mg in III; 9.2 mg in IV; 18.5 mg in V; 5.4 mg in VI; 2.8 mg in VII. Each oligosaccharide was identified by comparing with authentic samples as described in a previous report.1) Table I gives *Rf* values of oligosaccharides on TLC and retention times of their trimethylsilyl derivatives on GLC in several conditions.

Enzymic Degradation——The polysaccharide (200 mg) was dissolved in water (32 ml) and, after addition of 0.07 M phosphate buffer (pH 5.3, 8 ml), hemicellulase (10 mg, Tokyo Kasei Co.) was added. The solution was incubated at 37° for 48 hr, then concentrated and applied to a column (5 × 83.5 cm) of Sephadex G-15.

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21) *a)* J.S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954); *b)* G.O. Aspinall and R.J. Ferrier, *Chem. Ind.*, **1957**, 1216.

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

	Cellulose TLC (*Rf*)		GLC (t_R)	
	Solvent A	Solvent B	Condition A'[a]	Condition D[b]
I (Glc→Man)	0.65	0.44	35.0, 36.9	39.9, 42.1
II (Glc→Glc)	0.58	0.36	39.4, 40.6	43.8, 41.7
III (Man→Man)	0.54	0.34	36.6, 38.5	41.6, 43.7
IV (Man→Glc)	0.47	0.27	37.9, 39.1	42.2, 43.6
V (Man→Glc→Man)	0.35	0.21	52.9, 54.5	59.9, 62.8
VI (Man→Man→Man)	0.28	0.17	53.6, 55.8	62.1, 66.0
VII (Man→Man→Man→Man)	0.09	0.05	—	—

a) condition A', 2% OV 17 on Chromosorb W, from 130 to 280° (3°/min), N_2 20 ml/min
b) condition D, 3% SE 52 on Chromosorb W, from 130 to 280° (3°/min), N_2 20 ml/min

The column was eluted with water and fractions were collected at 50 ml. The eluates obtained from tubes 13 to 15 were combined, concentrated and lyophilized, and this fraction has exclusively the IR absorption bands of 1730 and 1250 cm^{-1} suggesting the presence of ester linkages.

Treatment with Methyl Vinyl Ether——The sample (80 mg) was dissolved in dimethyl sulfoxide (9 ml) and then *p*-toluenesulfonic acid (20 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 solution was then applied to a column (2.6×46 cm) of Sephadex LH-20. The column was eluted with acetone, and fractions were collected at 6 ml. The eluates obtained from tubes 13 to 17 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)-derivative——The O-acetyl-O-(1-methoxyethyl)-derivative (240 mg) was dissolved in methanol (5 ml), then 0.2 M methanolic sodium methoxide (5 ml) was added under stirring. The solution was refluxed at 80° for 4 hr, then concentrated and applied to a column (2.6×48 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 30 ml, and the eluates obtained from tubes 3 to 5 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 (145 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 and washing with dimethylformamide (4 ml), 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 (2.6×48 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 35 ml, and the eluates obtained from tubes 3 to 5 were combined and concentrated.

Hydrolysis and Analysis of the O-Methyl-derivative——O-Methyl-O-(1-methoxyethyl)-derivative (70 mg) was hydrolyzed with 2 N sulfuric acid at 100° for 5.5 hr, then neutralized with Dowex 2 (OH^-). The filtrate was evaporated and the hydrolysate (30 mg) was obtained.

The hydrolysate was applied to PPC with Tôyô-Roshi No. 51 and solvent system C, 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 *p*-anisidine reagent were identical with those of authentic 3,6-dimethyl-D-mannose, D-mannose and D-glucose.

On the other 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'. 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 and fragments in the mass spectra were 3.96, *m/e* 43, 45, 87, 99, 113, 129, 189, 233.

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