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# Plant Mucilages. XIV.[1] Isolation and Characterization of a Mucous Polysaccharide, "Lilium-La-glucomannan" from the Bulbs of *Lilium lancifolium*

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A mucous polysaccharide, named Lilium-La-glucomannan, has been isolated from the bulbs of *Lilium lancifolium* Thunb. It was homogeneous on glass-fiber paper electrophoresis and by ultra centrifugal analysis. The component sugars of it were D-mannose and D-glucose in the molar ratio of 5: 2, and its molecular weight was measured at 417000. The O-acetyl groups in the polysaccharide were identified and determined as the content of 1.2%. They are located in positions 2, 3, and 6 of a part of D-mannose units. Methylation, periodate oxidation and partial acetolysis studies suggested that the polysaccharide is mainly composed of $\beta$-1→4 linked aldohexopyranose residues and it contains about thirty aldohexose units per one end group on the average. D-Mannose units occupy non-redncing terminal positions and branch points linked through positions 2 and 3.

Water-soluble mucous glucomannans have been found in several lily bulbs.[1,3–6] The polysaccharides possess $\beta$-1→4 glycosidic bonds to form main chains of component sugars, but different types of branching and different values of molecular weight are present in them. The presence and the location of O-acetyl groups in the native glucomannans are also important problems in connection with their properties.[1,5,6] In this paper, the isolation and the structural feature of a new pure mucous polysaccharide from the fresh bulbs of *Lilium lancifolium* Thunb. are described. The bulbs have been used as a crude drug for the purpose of analeptic and cough medicine.

The material was crushed and extracted with hot methanol, then the residue was extracted with cold water. The crude mucilage was precipitated from the water extract by addition of ethanol. The solution of the precipitate was applied to a column of diethylaminoethyl (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 found to be homogeneous when analyzed by the ultracentrifugal analysis (Fig. 1).

The polysaccharide showed a negative specific rotation ($[\alpha]_D^{22}$ −33.7° in $H_2O$, $c$=0.2). Its solution in water gave the intrinsic viscosity value of 5.1 at 31°. Monnose and glucose were identified as the component sugars by means of cellulose thin-layer chromatography (TLC) of the hydrolysate and gas-liquid chromatography (GLC) of trimethylsilyl derivative of the hydrolysate in the other condition. Quantitative determination of them showed that the molar ratio of mannose: glucose is 5: 2. The measurement of osmotic pressure gave the value of 417000 as the molecular weight of the polysaccharide. The name "Lilium-La-glucomannan" is proposed for it.

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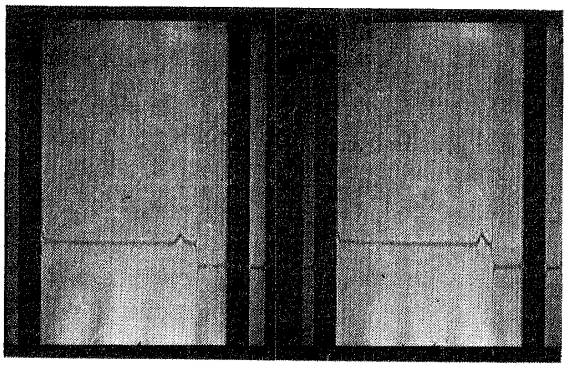

Fig. 1. Ultracentrifugal Pattern of Lilium-La-glucomannan

a: 0.1% in $H_2O$, 20°, 30 min, 60000 rpm
b: 0.1% in $H_2O$, 20°, 45 min, 60000 rpm
Hitachi model UCA-1A ultracentrifuge

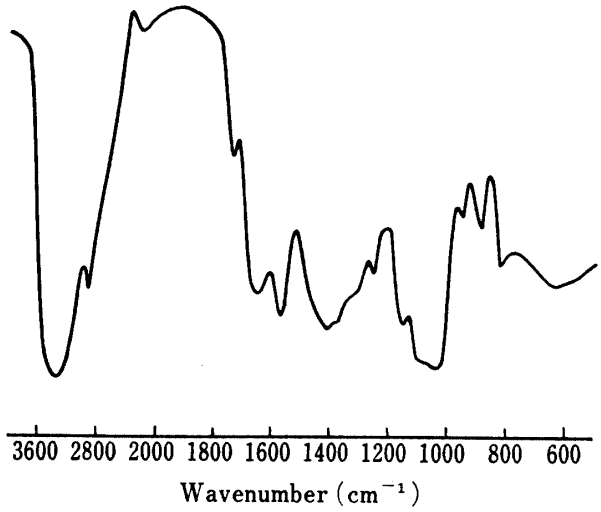


Fig. 2. IR Spectrum of Lilium-La-glucomannan

As shown in Fig. 2, the infrared (IR) spectrum of it has the absorption bands of 1730 and 1250 $cm^{-1}$ suggesting the presence of ester linkages in addition to the absorption of 890 $cm^{-1}$ being due to $\beta$-glycosidic linkages. When acid hydrolysate of the polysaccharide was analyzed by GLC,[7] it gave one peak, whose retention time was the same as that of authentic sample of acetic acid. The acetyl content of the glucomannan was determined to be 1.2% by GLC.

The location of O-acetyl groups in the glucomannan was established by the application of the method of de Belder and Norrman.[8] Because the polysaccharide is hardly soluble in dimethylsulfoxide, it was first digested with a hemicellulase preparation. The partially degraded polysaccharide having all ester linkages was isolated by the gel chromatography using Sephadex G-25. Then 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 with methanolic sodium methoxide, then methylated with methyl iodide and silver oxide in dimethylformamide.[9] The resulting product was subjected to acid hydrolysis, and the final products were analyzed by paper partition chromatography (PPC) and by gas-liquid chromatography-mass spectrometry (GLC–MS) after conversion to alditol acetates.[10,11] Besides mannose and glucose, three hexose methyl ethers were detected and identified as 6-mono-O-methyl-D-mannopyranose, 2-mono-O-methyl-D-mannopyranose and 3-mono-O-methyl-D-mannopyranose. The result of GLC showed that the molar ratio of them is 1.0: 0.9: 1.5. Owing to this result, it is able to conclude that the residues of 6-mono-O-acetyl-D-mannopyranose, 2-mono-O-acetyl-D-mannopyranose and 3-mono-O-acetyl-D-mannopyranose are partially present in the molecule of the glucomannan.

The methylation of the glucomannan was performed with sodium methylsulfinyl carbanion and methyl iodide in dimethylsulfoxide.[12] The fully methylated product was successively hydrolyzed with formic acid and dilute sulfuric acid. The products were separated by PPC, then analyzed by GLC–MS after conversion to alditol acetates. 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, 2,6-di-O-methyl-D-mannose and 3,6-di-O-methyl-D-

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mannose were identified and obtained in a molar ratio of 1.0: 53.3: 24.5: 0.6: 1.1. The tetramethyl ether of mannose was also confirmed as its methyl glycoside by GLC.

As the result of periodate oxidation, 1.04 mole of periodate per one mole of component anhydro sugar unit of the glucomannan was consumed with 0.033 mole of formic acid liberation. The periodate-oxidized polysaccharide was treated with sodium borohydride,[13] and the reduction product was isolated by the gel chromatography using Sephadex G-15. Analysis of alditol acetates derived from its hydrolysate by GLC showed that the yields of erythritol and mannose were 61.4% and 3.6%.

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 positions 2 and 3 in part. From the value of formic acid liberation and the yield of mannose as the hydrolysis product after periodate oxidation, it is able to conclude that the glucomannan has about thirty aldohexose units per one non-reducing group on the average. The yield of tetramethyl ether of mannose was low in comparison with these values. The cause for such a discrepancy may be attributed to unavoidable losses of sugar methyl ethers.

Partial acetolysis of the glucomannan also gave the evidence that the straight chain parts in the polysaccharide are composed of β-1→4 linked aldohexopyranose residues. The sample was dissolved in formamide and acetylated with acetic anhydride and pyridine. The product was partially degraded with sulfuric acid in acetic anhydride. After deacetylation, 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[14,15] showed that they 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, 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-mannopyranose, O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, 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)-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.

The results indicated the fact that the manner of sequence of component sugars in the main chain is similar to those of Lilium-A-glucomannan[6] and Lilium-S-glucomannan.[1] These two glucomannans and Lilium-La-glucomannan all possess D-mannopyranose residues as the branching points and as the terminals in the molecules. However, they have their own types of branching, and also their own values of molecular weight and of the molar ratio of component sugars. Moreover, there are various values of O-acetyl content in them, and Lilium-La-glucomannan has different O-acetylated D-mannopyranose units from those of the other two lily glucomannans previously obtained by us. Further studies on the mucilages from 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 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.

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**Isolation of Polysaccharide**——The material was obtained in September of 1975 from the plants grew in Saitama prefecture. The fresh bulbs (252 g), which contain 53.2% of water, were crushed, then extracted with hot methanol (1000 ml) for 30 min twice. After suction filtration, the residue was extracted with water (2000 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 (9.49 g) was obtained. A part of the crude mucilage (1.3 g) was dissolved in water and applied to a column (5×60 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.[16] The eluates obtained from tubes 13 to 27 were combined, concentrated and lyophilized. Lilium-La-glucomannan (0.80 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[17] of this series. The condition of 380 volts for 1.5 hr was used. The sample gave one spot at a distance of 10.3 cm from the origin toward the cathod. Distance moved by standard glucose was 11.5 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 hydrochloride reagent[18] and naphthoresorcinol-phosphoric acid reagent.[19] In addition, the sample was hydrolized with 2 M trifluoroacetic acid in a sealed tube at 120° for 2 hr. After removal of the acid by the repeated addition and evaporation of methanol, the hydrolysate was trimethylsilylated in pyridine with hexamethyldisilazane and trimethylchlorosilane[20] and applied to GLC. GLC was carried out under following two conditions; 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 130 to 280°; B, a column (0.3 cm×2 m long spiral stainless steel) packed with 3% SE 52 on Chromosorb W (80 to 100 mesh); carrier gas and programmed temperature are the same as condition A. *Rf* values in TLC and retention times in GLC are shown in Table I.

Table I. *Rf* Values of Hydrolysates and Retention Times (min) of Trimethylsilyl Derivatives

| | Cellulose TLC (*Rf*) | | GLC ($t_R$) | |
|---|---|---|---|---|
| | Solvent A | Solvent B | Condition A | Condition B |
| Hydrolysate | 0.56, 0.49 | 0.50, 0.42 | 12.2, 15.2, 17.9 | 16.9, 19.5, 22.6 |
| Mannose | 0.56 | 0.50 | 12.2 | 16.9 |
| Glucose | 0.49 | 0.42 | 15.2, 17.9 | 19.5, 22.6 |

**Determination of Component Sugars**——The sample (10 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 2 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, 4 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 C, 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 74.5% of mannose and 29.7% 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[21] of this series.

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

The sample (8 mg) was hydrolyzed with 1 N hydrochloric acid (0.1 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 D, 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.

**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 under the addition of toluene, then concentrated and applied to a column (5 × 73 cm) of Sephadex G-25. The column was eluted with water and fractions were collected at 50 ml. The eluates obtained from tubes 16 to 20 were combined, concentrated and lyophilized, and this was the only fraction having the IR absorption bands suggesting the presence of ester linkages.

**Treatment with Methyl Vinyl Ether**——The sample (100 mg) was dissolved in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (28 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 (4 × 13 cm) of Sephadex LH-20. The column was eluted with acetone, and fractions were collected at 5 ml. The eluates obtained from tubes 13 to 18 were combined and concentrated. The product was treated again under the same condition. The IR spectrum of the final residue had no absorption near 3400 $cm^{-1}$.

**Deacetylation of the O-Acetyl-O-(1-methoxyethyl)-derivative**——The O-acetyl-O-(1-methoxyethyl)-derivative (254 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 and applied to a column (4 × 19 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 20 ml, and the eluates obtained from tubes 5 to 11 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 (232 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 (5 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 (4 × 19 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 5 ml, and the eluates obtained from tubes 17 to 35 were combined and concentrated.

**Hydrolysis and Analysis of the O-Methyl-derivative**——O-Methyl-O-(1-methoxyethyl)-derivative (82 mg) was hydrolyzed with 2 N sulfuric acid (10 ml) at 100° for 5.5 hr, then neutralized with Dowex 2 ($OH^-$). The filtrate and washings with water and methanol were combined and evaporated and the hydrolysate (57 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 (0.75, 0.61, 0.56, 0.40, and 0.34; 2,3,4,6-tetra-O-methyl-D-glucose = 1.00) of the five spots detected with *p*-anisidine hydrochloride reagent were identical with those of authentic 6-mono-O-methyl-D-mannose, 2-mono-O-methyl-D-mannose, 3-mono-O-methyl-D-mannose, D-mannose and D-glucose.

On the other hand, the hydrolysate was applied to preparative PPC with Tôyô-Roshi No. 50 and solvent system D, BuOH: EtOH: $H_2O$ (4: 1: 5, upper layer). The mannose monomethyl ethers were obtained from parts showing *Rf* values of 0.56, 0.41, and 0.38. They were respectively reduced with sodium borohydride, then acetylated with acetic anhydride-pyridine mixture as described above. The alditol acetates were applied to GLC under condition C′ using the same column as condition C but at 180° with a flow of 30 ml per min of $N_2$. Relative retention times of the derivatives to 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-glucitol and fragments of them in the mass spectra were as follows: 6-mono-O-methyl-1,2,3,4,5-penta-O-acetyl-D-mannitol, 4.31, *m/e* 43, 45, 87, 115, 129; 2-mono-O-methyl-1,3,4,5,6-penta-O-acetyl-D-mannitol, 6.89, *m/e* 43, 117, 139; 3-mono-O-methyl-1,2,4,5,6-penta-O-acetyl-D-mannitol, 9.23, *m/e* 43, 85, 87, 99, 127, 129, 189, 261.

**Methylation of Polysaccharide**——Sodium hydride (250 mg) was mixed with dimethyl sulfoxide (5 ml) and the mixture was stirred at 50° for 45 min. The polysaccharide (200 mg) was suspended in dimethyl sulfoxide (20 ml) and stirred at 60° for 1 hr and, after cooling to room temperature, the solution of methylsulfinyl carbanion was added into this mixture. After stirring at room temperature for 5 hr, methyl iodide (2 ml) was added and the mixture was stirred overnight at room temperature. All procedures were carried out in nitrogen atmosphere. Then the reaction mixture was dialyzed against running water overnight. The nondialyzable fraction was concentrated and lyophilized. The product was methylated five more times under the same condition. The final nondialyzable fraction was extracted with chloroform (100 ml each) five times. The extracts were combined, washed with water (100 ml each) five times and dried over sodium sulfate and

the filtrate was evaporated to dryness. The IR spectrum of the final product had no absorption near 3400 $cm^{-1}$.

**Analysis of the Methylated Product**——A part 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 applied to preparative PPC with Tôyô-Roshi No. 51 and solvent system D. The hexose tetramethyl ether, trimethyl ethers and dimethyl ethers were obtained from parts showing *Rf* values of 0.91, 0.80, and 0.62. They were respectively 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 C′. Relative retention times of the products to 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-glucitol and fragments of them 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, 2.16, *m/e* 43, 45, 87, 99, 101, 113, 117, 233; 2,3,6-tri-O-methyl-1,4,5-tri-O-acetyl-D-glucitol, 2.47, *m/e* 43, 45, 87, 99, 101, 113, 117, 233; 2,6-di-O-methyl-1,3,4,5-tetra-O-acetyl-D-mannitol, 3.23, *m/e* 43, 45, 87, 117, 129; 3,6-di-O-methyl-1,2,4,5-tetra-O-acetyl-D-mannitol, 4.00, *m/e*, 43, 45, 87, 99, 113, 129, 189, 233.

In addition, a part of the product of methylation 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, GLC of methyl glycosides of partially methylated hexoses were carried out under the same two conditions as described in a previous report.[6] Methyl glycosides of mannose dimethyl ethers were not detected in these conditions.

**Periodate Oxidation**——The sample (50 mg) was oxidized with 0.05 M sodium metaperiodate (25 ml) at 5° in a dark place. The periodate consumption was measured by a spectrophotometric method.[22] The oxidation was completed after forty days, then 2 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.

**Smith Degradation and Analysis of Products**——The residue of the reaction mixture (16 ml) was successively treated with ethylene glycol (0.3 ml) and sodium borohydride (120 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 × 78 cm) of Sephadex G-15. Fractions were collected at 50 ml, and the eluates obtained from tubes 12 and 13 were combined, evaporated and lyophilized. The product was hydrolyzed with 1 N sulfuric acid at 100° for 6 hr, and the hydrolysates were derived to alditol acetates and determined by GLC under condition C as described above. Retention time of erythritol tetraacetate was 2.6 min.

**Partial Acetolysis**——The polysaccharide (1 g) was suspended in formamide (20 ml), then pyridine (12 ml) and acetic anhydride (8 ml) were added under stirring. The mixture was stirred for three days at room temperature, then poured into four volumes of methanol. The precipitate was filtered off, washed with methanol and ether, then dried *in vacuo*. The product was dissolved in acetic anhydride (20 ml) and, after addition of a cold mixture of acetic anhydride: sulfuric acid (5: 1, 10 ml) under cooling, the solution was kept at 5° for three days. The reaction mixture was poured into ice water (100 ml), then the products were extracted with chloroform (130 ml each) three times. The extracts were combined and washed successively with 10% sodium bicarbonate and water, and dried over sodium sulfate, then evaporated to dryness. The products were dissolved in methanol (20 ml) and de-acetylated with 0.2 N sodium hydroxide (20 ml) overnight at 5°, then neutralized with acetic acid. The solvent was evaporated off.

Table II. *Rf* Values of Oligosaccharides and Retention Times (min) of Trimethylsilyl Derivatives

| | Cellulose TLC (*Rf*) | | GLC ($t_R$) | |
|---|---|---|---|---|
| | Solvent A | Solvent B | Condition A | Condition B |
| I (Man→Man) | 0.55 | 0.36 | 36.6, 38.5 | 41.6, 43.7 |
| II (Man→Glc) | 0.47 | 0.27 | 37.9, 39.1 | 42.2, 43.6 |
| III (Glc→Man) | 0.65 | 0.45 | 35.0, 36.9 | 39.9, 42.1 |
| IV (Glc→Glc) | 0.57 | 0.34 | 39.4, 40.6 | 43.8, 41.7 |
| V (Glc→Man→Man) | 0.39 | 0.22 | 52.5, 54.7 | 60.2 |
| VI (Man→Man→Man) | 0.28 | 0.18 | 53.6, 55.8 | 62.1, 66.0 |
| VII (Glc→Glc→Man) | 0.51 | 0.26 | 53.0, 55.0 | 60.4, 64.3 |
| VIII (Man→Glc→Man) | 0.35 | 0.20 | 52.9, 54.5 | 59.9, 62.8 |
| IX (Man→Man→Man→Man) | 0.09 | 0.07 | — | — |
| X (Man→Glc→Man→Man) | 0.15 | 0.10 | — | — |

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

**Separation and Analysis of Partial Acetolysis Products**——The residue was dissolved in water and 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 (250 ml), 5% ethanol (350 ml), 10% ethanol (500 ml), 15% ethanol (450 ml) and 20% ethanol (450 ml). Fractions were collected at 50 ml and measured by phenol-sulfuric acid method. The eluates obtained from the column were divided into five groups: Frac. 1, tubes 1 to 5; Frac. 2, tubes 6 to 12; Frac. 3, tubes 13 to 22; Frac. 4, tubes 23 to 31; Frac. 5, tubes 32 to 40. Yields, 449 mg in Frac. 1; 97 mg in Frac. 2; 47 mg in Frac. 3; 38 mg in Frac. 4; 11 mg in Frac. 5. Fractions 2 to 4 were respectively applied to PPC with Tôyô-Roshi No. 51 and solvent system A. In Frac. 2, disaccharides I and II were obtained from parts showing *Rf* values of 0.37 and 0.26. In Frac. 3, disaccharides III and IV were obtained from parts showing *Rf* values of 0.54 and 0.39, and trisaccharides V and VI were obtained from parts showing *Rf* values of 0.25 and 0.14. In Frac. 4, trisaccharides VII and VIII were obtained from parts showing *Rf* values of 0.32 and 0.22, and tetrasaccharide IX was obtained from a part showing *Rf* value of 0.11. Tetrasaccharide X was detected in Frac. 5. Each oligosaccharide was identified by comparing with authentic samples as described in a previous report.[15] Table II gives *Rf* values of oligosaccharides in TLC and retention times of their trimethylsilyl derivatives in GLC under several conditions.

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