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Plant Mucilages. XXII.[1] Isolation and Characterization of a Mucous Polysaccharide, "Lilium-J-glucomannan," from the Bulbs of *Lilium japonicum*

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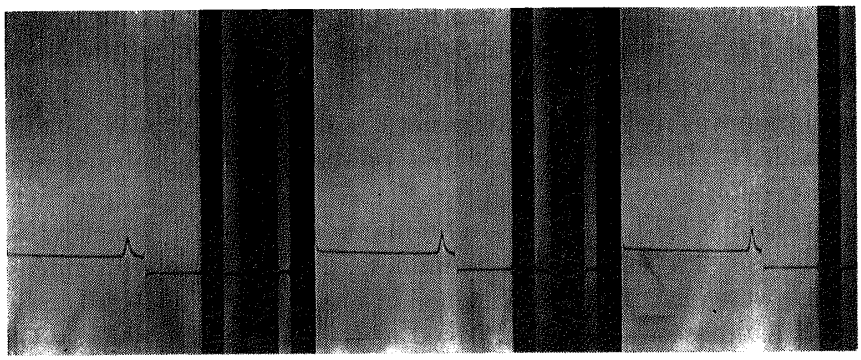
A mucous polysaccharide, named Lilium-J-glucomannan, has been isolated from the bulbs of *Lilium japonicum* Thunb. It was homogeneous on glass-fiber paper electrophoresis, by ultracentrifugal analysis, and on gel chromatography. The component sugars of it were D-mannose and D-glucose in the molar ratio of 5 : 2, and its molecular weight was measured to be 318000. The *O*-acetyl groups in the glucomannan were identified and determined as the content of 5.0%. They were located in positions 2, 3, and 6 of a part of D-mannose units. Methylation, partial acetolysis, and periodate oxidation studies suggested that the glucomannan is mainly composed of β-1→4 linked aldohexopyranose residues and it contains about seventeen aldohexose units per one non-reducing group on the average. D-Mannose units occupy non-reducing terminal positions and branching points linked through positions 2 or 3. A few D-glucose residues are also present as branching points linked through position 3. The comparison of the structure and the property of the glucomannan with those of the other five Lilium-glucomannans was described.

Keywords——mucous polysaccharide; *Lilium japonicum*; Lilium-J-glucomannan; molar ratio of components; intrinsic viscosity; molecular weight; location of *O*-acetyl groups; structure of main chain; branching points; comparison of Lilium-glucomannans

The bulbs of *Lilium japonicum* Thunb. have been used as a crude drug for the purpose of analeptic and cough medicine. In the previous papers of this series,[1,3–6] the isolations and the structural features of mucous glucomannans from the bulbs of *Lilium auratum*, *Lilium speciosum*, *Lilium lancifolium*, *Lilium longiflorum*, and *Lilium maculatum* have been reported from this laboratory. The lily glucomannans all belong to a group of branched polysaccharides and possess the main chain having β-1→4 glycosidic linkages. However, there are various values of molecular weight, molar ratio of components, and *O*-acetyl content in them. Moreover, they have their own types of branching and locations of *O*-acetyl groups. The present paper is concerned with the isolation and the structure of a pure mucous polysaccharide from the fresh bulbs of *Lilium japonicum*. The comparison of the structure and the property of the polysaccharide with those of the other Lilium-glucomannans was also described in this report.

The material bulbs were crushed and extracted with cold water after treatment with hot methanol. The crude mucilage obtained was applied to a column of diethylaminoethyl (DEAE)-cellulose (carbonate form), and a mucous polysaccharide was isolated 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). Furthermore, it gave a single peak on gel chromatography with Sephacryl S-200 (Fig. 2).

1) Part XXI: M. Tomoda and C. Odaka, *Chem. Pharm. Bull.* (Tokyo), **26**, 3373 (1978).
2) Location: *1–5–30, Shibakōen, Minato-ku, Tokyo, 105, Japan.*
3) M. Tomoda, S. Kaneko, and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **23**, 430 (1975).
4) M. Tomoda and S. Kaneko, *Chem. Pharm. Bull.* (Tokyo), **24**, 2157 (1976).
5) M. Tomoda, S. Kaneko, C. Ohmori, and T. Shiozaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 2744 (1976).
6) M. Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.* (Tokyo), **26**, 2768 (1978).



(c) 45 min (b) 36 min (a) 27 min

Fig. 1. Ultracentrifugal Pattern of Lilium-J-glucomannan
Hitachi model UCA-1A ultracentrifuge, 0.1% in H_2O, 20°, 60000 rpm

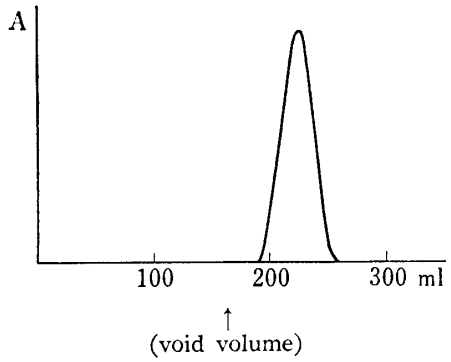


Fig. 2. Chromatogram of Lilium-J-glucomannan on Sephacryl S-200

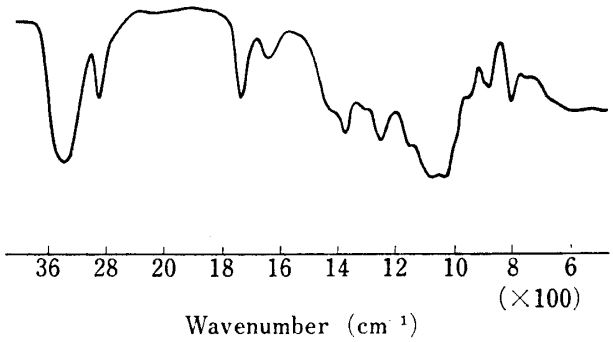


Fig. 3. IR Spectrum of Lilium-J-glucomnnan

The substance showed a negative specific rotation ($[\alpha]_D^{23}$ −40.7° in H_2O, c=0.6). Its solution in water gave the intrinsic viscosity value of 7.2 at 25°. Mannose and glucose were identified as the component sugars by means of cellulose thin-layer chromatography (TLC) of the hydrolyzate and gas-liquid chromatography (GLC) of its trimethylsilyl derivative. Quantitative determination of them showed that the molar ratio of mannose: glucose is 5: 2. The measurement of osmotic pressure gave the value of 318000 as the molecular weight of the polysaccharide. The name "Lilium-J-glucomannan" is proposed for it.

As shown in Fig. 3, the infrared (IR) spectrum of the glucomannan has the absorption bands of 1250 cm^{-1} and 1735 cm^{-1} suggesting the presence of ester linkages in addition to the absorption of 890 cm^{-1} being due to β-glycosidic linkages. Analysis of the acid hydrolyzate of it by GLC and high performance liquid chromatography (HPLC) showed the occurrence of acetic acid, and the acetyl content of the glucomannan was determined to be 5.0%.

For the elucidation of the location of *O*-acetyl groups, the glucomannan was exhaustively treated with methyl vinyl ether in the presence of *p*-toluenesulfonic acid in dimethyl sulfoxide.[7] After conversion of the free hydroxyl groups into 1-methoxyethyl ethers, the derivative was deacetylated, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide.[8] The resulting product was hydrolyzed and analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates. Three hexose methyl ethers were detected and identified as 2-*O*-methyl-D-mannose, 3-*O*-methyl-D-

7) A.N. DeBelder and B. Norrman, *Carbohydr. Res.*, **8**, 1 (1968).
8) R. Kuhn, H. Trischman, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

mannose, and 6-*O*-methyl-D-mannose in a molar ratio of 1.3: 1.0: 1.5. These results indicate that the residues of 2-*O*-acetyl-D-mannose, 3-*O*-acetyl-D-mannose, and 6-*O*-acetyl-D-mannose are partially present in the glucomannan.

The polysaccharide was methylated with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide.[9] The fully methylated product was hydrolyzed and analyzed by GLC–MS after conversion into alditol acetates.[10] 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, 2,6-di-*O*-methyl-D-glucose, and 3,6-di-*O*-methyl-D-mannose were identified and obtained in a molar ratio of 1.0: 10.8: 4.6: 0.2: 0.2: 0.5. The tetra-*O*-methyl mannose was also confirmed as its methyl glycoside by GLC.

On the other hand, the polysaccharide was acetylated with acetic anhydride and pyridine in formamide, then partially degraded with sulfuric acid in acetic anhydride. After deacetylation, the products were applied to a column of active charcoal. The fractions obtained were analyzed by TLC and by GLC of trimethylsilyl derivatives. The comparison with authentic samples[11] showed the presence of *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-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, and *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose in addition to the component monosaccharides.

These results indicated that the glucomannan is mainly composed of β-1→4 linked aldohexopyranose units and has some mannopyranose residues as terminals and branching points linking through positions 2 or 3 in part. In addition, only a few glucopyranose residues are also present as branching points linking through position 3. Based on the result of methylation analysis, it is able to conclude that the glucomannan has about seventeen aldohexose units per one non-reducing group on the average.

The glucomannan was subjected to periodate oxidation followed by reduction with sodium borohydride.[12] The maximum values of periodate consumption and formic acid liberation were 0.65 mol and 0.02 mol per one mol of anhydrohexose unit. The reduction product was isolated by the gel chromatography using a column of Sephadex G-15. The IR spectrum of the product has the absorption bands suggesting the presence of ester linkages (Fig. 4), and the presence of 1.7% of *O*-acetyl groups was determined by GLC of its hydrolyzate. Based on these results, it can be deduced that about one third of the *O*-acetyl groups in the original glucomannan block periodate cleavage.

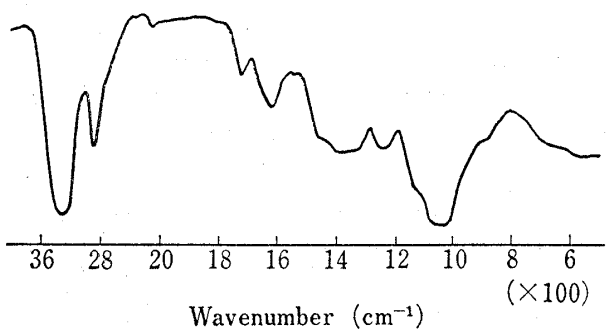


Fig. 4. IR Spectrum of Periodate Oxidation-Reduction Product

The water-soluble original polysaccharide was treated with dilute alkali solution, and the water-insoluble deacetylated glucomannan obtained was oxidized with periodate under stirring. As the result of this case, 0.99 mol of periodate per one mol of component anhydro sugar

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10) H. Björndall, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).
11) M. Tomoda and S. Kimura, *Chem. Pharm. Bull.* (Tokyo), **24**, 1807 (1976).
12) M. Abdel-Akher, J.K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, **74**, 4970 (1952).

unit was consumed with 0.20 mol of formic acid liberation. The periodate-oxidized product was reduced, hydrolyzed, and analyzed. The yields of mannose, glucose, and erythritol were 4.2%, 1.8%, and 42.8%. Thus the conclusion obtained by methylation analysis was also supported by the results of periodate oxidation followed by Smith degradation of the deacetylated glucomannan. A few excessive value of formic acid liberation may attributed to partial overoxidation.

Table I. Properties and Structural Features of Lilium-glucomannans

	L-J-GM	L-A-GM[3]	L-S-GM[4]	L-La-GM[5]	L-Lo-GM[6]	L-Ma-GM[1]
$[\alpha]_D^{23}$ (in H_2O)	−40.7°	−37.9°	−29.5°	−33.7°	−33.2°	−32.4°
Intrinsic viscosity (in H_2O at 25°)	7.2	2.4	6.6	5.1	4.1	5.6
Solubility (in H_2O at 4°)	>14%[a]	>30%[a]	1.26%	0.95%	1.78%	2.24%
Molar ratio (Man: Glc)	5 : 2	8 : 3	2 : 1	5 : 2	5 : 2	7 : 4
Molecular weight	318000	35700	388000	417000	263000	184000
Main linkage	β-1→4	β-1→4	β-1→4	β-1→4	β-1→4	β-1→4
Branching positions	2 and 3 of Man 3 of Glc	2 of Man	3 of Man	2 and 3 of Man	2 and 3 of Man	2 and 3 of Man
Average number of component units per one end group	17	11	6	30	10	7
Acetyl content	5.0%	5.1%	3.3%	1.2%	3.2%	4.7%
Location of acetyl groups	2, 3, and 6 of Man	3,6 of Man	3,6 of Man	2, 3, and 6 of Man	2,3,6, 2,6, 3,6, and 6 of Man 2,3,6, and 6 of Glc	2,3,6, 3,6, 2, 3, and 6 of Man 2,3,6, and 6 of Glc

a) These values show measurement limits because of the high viscosity of concentrated solutions.
Abbreviations: L=Lilium; GM=glucomannan; Man=D-mannose, Glc=D-glucose.

As shown in Table I, the Lilium-glucomannans obtained by us until now may be divided into three types regarding the location of *O*-acetyl groups: the first, Lilium-A-glucomannan and Lilium-S-glucomannan; the second, Lilium-La-glucomannan and Lilium-J-glucomannan; and the third, Lilium-Lo-glucomannan and Lilium-Ma-glucomannan. The native water-soluble glucomannans from lily bulbs possess *O*-acetyl groups, and the deacetylation of them causes the insolubility of the products in water.[3,13] Therefore, the content and the location of *O*-acetyl groups in native polysaccharide are interesting problems in connection with their properties. However, the solubility of the glucomannans in water is not necessarily proportional to the acetyl content. The relationship between the solubility and the location of *O*-acetyl groups is also not clear. Of course, branching structures and molecular weights of the polysaccharides are certainly the other dominant factors concerning their solubilities and viscosities.

Lilium-A-glucomannan and Lilium-J-glucomannan have particularly high solubility in water. Although it is interesting that these possess the highest acetyl content among the six Lilium-glucomannans, the fundamental elucidation of the factors governing the solubility is still undissolved problem. Further investigation is now under progress.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Optical rotation was measured with JASCO model DIP-SL automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. IR spectra were recorded on JASCO model IRA-2 infrared spectrophotometer.

13) T. Matsuo and T. Mizuno, *Agr. Biol. Chem.*, **38**, 465 (1974).

GLC was carried out by the use of Hitachi model 063 gas chromatograph equipped with a 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. HPLC was carried out by the use of JASCO model FLC-A700 automatic high pressure liquid chromatograph equipped with Mitsumi model SF-1107 RI monitor.

Isolation of Polysaccharide——The material was obtained in June of 1977 from the plants grew in Ishikawa prefecture. The fresh bulbs (94.0 g), which contain 71.4% of water, were crushed, then extracted twice with hot methanol (380 ml) for 30 min. After suction filtration, the residue was extracted with water (760 ml) twice under stirring at room temperature for 1 hr each. Each extract was poured into two volumes of ethanol, then filtered. The precipitates were combined and treated with ethanol again, then dried *in vacuo* (yield, 4.25 g). A part of this crude mucilage (1.31 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.[14] The eluates obtained from tubes 11 to 42 were combined, concentrated and lyophilized. Lilium-J-glucomannan (0.93 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 in a previous report[15] of this series. The condition of 380 volts for 90 min was used. The sample gave one spot at a distance of 11.2 cm from the origin toward the cathod. Standard glucose moved to a distance of 12.4 cm.

Gel Chromatography——This was carried out in the same manner as in a former report[6] of this series.

Qualitative and Quantitative Analyses of Component Sugars——These were carried out in the same manners as in a former report[6] of this series. The results revealed that the sample was composed of 68.8% of mannose and 27.0% 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 in a previous report[16] of this series.

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

The determination of acetyl groups by GLC was performed in the same manner as in a former report.[6]

On the other hand, the sample (5 mg) was hydrolyzed with 1% phosphoric acid (0.25 ml) containing 0.2% succinic acid as an internal standard in a sealed tube at 100° for 16 hr. A part (100 μl) of the hydrolyzate was applied to HPLC. HPLC was carried out using a column (0.8×50 cm) packed with Shodex Ionpak C-811. The column was eluted with 0.1% phosphoric acid under a flow of 1 ml per min at 60°. The result was shown in Fig. 5. GLC and HPLC gave the same value of acetyl group.

Treatment with Methyl Vinyl Ether——The sample (106 mg) was suspended in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (20 mg) was added. The mixture was stirred at 15°, then methyl vinyl

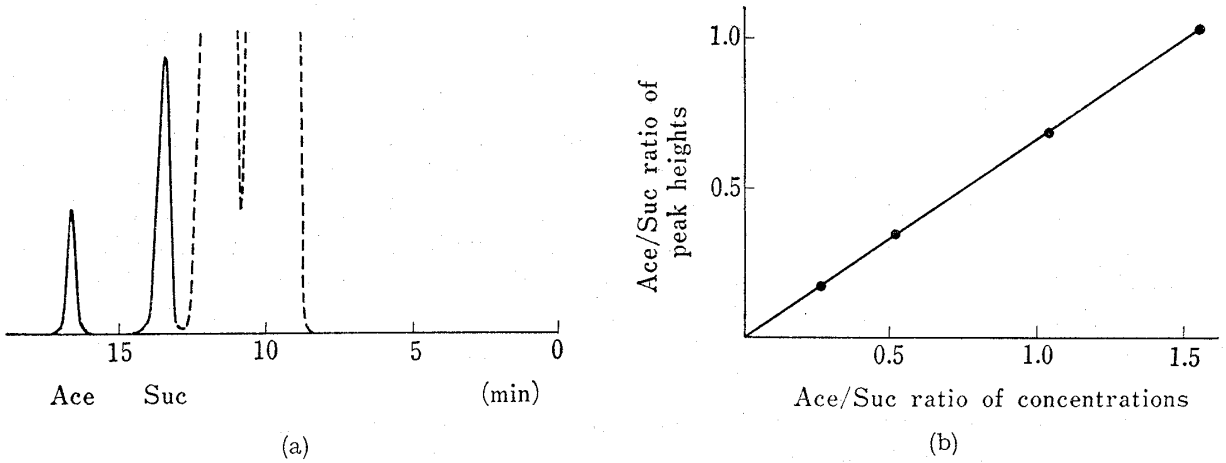

Fig. 5. HPLC of the Hydrolyzate of Lilium–J–glucomannan for the Determination of Acetyl Content

(a) Chromatogram of the Hydrolyzate
abbreviations: Ace=acetic acid; Suc=succinic acid (internal standard).
------: degradation products (sugars) from the polysaccharide.
(b) Calibration Curve

14) J.E. Hodge and B.T. Hofreiter, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York and London, 1962, p. 388.
15) M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 2173 (1971).
16) M. Tomoda and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **20**, 2491 (1972).

ether (5 ml), condensed at −10°, was added in portions under stirring. The reaction mixture was stirred at 15° for 4 hr, then dialyzed against running water overnight. The non-dialyzable fraction was concentrated to dryness, then the reaction was similarly repeated. The final solution was applied to a column (4×20 cm) of Sephadex LH-20. The column was eluted with acetone, and fractions were collected at 10 ml each. The eluates obtained from tubes 10 to 16 were combined and concentrated. The reaction was repeated eight times under the same condition. The IR spectrum of the final product had no absorption near 3400 cm^{-1}.

Deacetylation of the *O*-Acetyl-*O*-(1-methoxyethyl)-derivative——The product was dissolved in methanol (9 ml), then 0.2 M methanolic sodium methoxide (9 ml) was added under stirring. The solution was refluxed at 80° for 4 hr, then concentrated and applied to a column (4×27 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 10 ml each, and the eluates obtained from tubes 27 to 36 were combined and concentrated. The absence of acetyl absorption in the IR spectrum of the residue proved the complete deacetylation.

Methylation of the *O*-(1-Methoxyethyl)-derivative——The product was dissolved in *N*,*N*-dimethylformamide (5 ml), then methyl iodide (2 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 chloroform, the filtrate and washing were combined and evaporated. Methyl iodide (2 ml) and silver oxide (0.4 g) were added again into the residual solution, then the reaction was similarly repeated six times. The final reaction mixture was filtered and washed with chloroform (4 ml). The filtrate and washing were combined, then water (15 ml) was added into the solution. The mixture was extracted with chloroform (15 ml each) four times. The extracts were combined and washed with water (60 ml each) four times, then filtered and evaporated. After addition of chloroform and water (9 ml each) into the residue, the mixture was centrifuged. The chloroform layer separated was concentrated to dryness. The IR spectrum of the final residue had no absorption near 3400 cm^{-1}.

Analysis of the *O*-Methyl-derivative——A part of *O*-methyl-*O*-(1-methoxyethyl)-derivative (50 mg) was successively treated with 90% formic acid (2.5 ml) at 90° for 16 hr and 0.5 N sulfuric acid (2.5 ml) at 100° for 2.5 hr. After neutralization with Dowex 2 (OH^-), the hydrolyzate was reduced with sodium borohydride, then acetylated with acetic anhydride-pyridine mixture as described in a former report.[6] GLC-MS was carried out under the same condition as in a former report.[6] Relative retention times of the products to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol and main fragments of them in the mass spectra are shown in Table II.

Table II. Relative Retention Times on GLC and Fragments in MS of Partially Methylated Alditol Acetates

	Relative retention times[a)]	Main fragments (*m/e*)
1,5-Ac-2,3,4,6-Me-D-Mannitol	0.98	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,4,5-Ac-2,3,6-Me-D-Mannitol	1.91	43, 45, 87, 99, 101, 113, 117, 233
1,4,5-Ac-2,3,6-Me-D-Glucitol	2.15	43, 45, 87, 99, 101, 113, 117, 233
1,3,4,5-Ac-2,6-Me-D-Mannitol	2.76	43, 45, 87, 117, 129
1,3,4,5-Ac-2,6-Me-D-Glucitol	3.06	43, 45, 87, 117, 129
1,2,4,5-Ac-3,6-Me-D-Mannitol	3.30	43, 45, 87, 99, 113, 129, 189, 233
1,2,3,4,5-Ac-6-Me-D-Mannitol	3.59	43, 45, 87, 115, 129
1,3,4,5,6-Ac-2-Me-D-Mannitol	5.12	43, 117, 139
1,2,4,5,6-Ac-3-Me-D-mannitol	6.44	43, 85, 87, 99, 127, 129, 189, 261

a) Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol under the condition with 3% OV 225 on Gaschrom Q at 180°.
abbreviations: Ac=acetyl; Me=methyl.
(*e.g.*, 1,5-Ac-2,3,4,6-Me=1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl)

Methylation of Polysaccharide——Sodium hydride (75 mg) was mixed with dimethyl sulfoxide (3 ml) and the mixture was stirred at 70° for 1 hr. The sample (100 mg) was dissolved in dimethylsulfoxide (3 ml) under stirring at 60° and the solution of methylsulfinylmethyl sodium was added into this mixture. After stirring at room temperature for 5 hr, methyl iodide (5 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 non-dialyzable fraction was concentrated and lyophilized. The product was methylated four more times under the same condition. The final non-dialyzable fraction was extracted with chloroform (100 ml each) five times. The extracts were combined and washed with water (500 ml each) five times, then 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 and 0.5 N sulfuric acid, then the hydrolyzate was reduced and acetylated as described above. GLC–MS was carried out under the same condition as in a former report.[6] Relative retention times of the products to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol and main fragments of them in the mass spectra are also shown in Table II.

In addition, a part of the product was methanolyzed and GLC of methyl glycosides of partially methylated hexoses was carried out as described in a previous report[3] of this series.

Partial Acetolysis——The acetylation and the partial degradation of the polysaccharide were carried out in the same manners as in a previous report.[5]

Separation and Analysis of Partial Acetolysis Products——The products (50 mg) were dissolved in water and applied to a column (1 × 7.5 cm) of active charcoal (for chromatographic use, Wakō-Junyaku Co.). The column was eluted with water (60 ml), 5% ethanol (100 ml), 10% ethanol (70 ml), and 15% ethanol (90 ml). Fractions were collected at 10 ml each and measured by phenol-sulfuric acid method. The eluates obtained from the column were divided into four groups: Frac. 1, tubes 1 to 6; Frac. 2, tubes 7 to 16; Frac. 3, tubes 17 to 23; Frac. 4, tubes 24 to 32. Yields, 7.2 mg in Frac. 1; 10.6 mg in Frac. 2; 3.9 mg in Frac. 3; 7.6 mg in Frac. 4. Each fraction was applied to TLC using Avicel SF cellulose and to GLC after conversion into their trimethylsilyl derivatives. TLC and GLC were carried out under the same conditions as in a previous report.[5] Frac. 1 contained component monosaccharides. Man→Man and Man→Glc were found in Frac. 2, Man→Man→Man in Frac. 2 and 3, Glc→Man and Man→Man→Glc in Frac. 3, Man→Glc→Man in Frac. 3 and 4, and Man→Man→Man→Man and Glc→Man→Man→Man in Frac. 4.

Periodate Oxidation——The sample (50 mg) was oxidized with 0.05 M sodium metaperiodate (25 ml) at 7° in a dark place. The periodate consumption was measured by a spectrophotometric method.[17] The maximum value of consumption was obtained after ten days, and the value was unaltered on further standing during thirty days. The formic acid liberation was measured by titrating with 0.01 N sodium hydroxide after addition of ethylene glycol.

Deacetylation of Polysaccharide followed by Periodate Oxidation——The sample (135 mg) was dissolved in water (25 ml), then 0.2 N sodium hydroxide (25 ml) was added. After standing at room temperature for 10 min, the solution was poured into 0.1 N ethanolic hydrochloric acid. The precipitate was separated, washed with ethanol, and lyophilized. The deacetylated polysaccharide obtained (41 mg) was oxidized with 0.05 M sodium metaperiodate (20 ml) at room temperature under stirring in a dark place. The oxidation was completed after four days.

Smith Degradation and Analysis of Products——The residue of the reaction mixture (18 ml) was successively treated with ethylene glycol (0.6 ml) and sodium borohydride (190 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 (2.6 × 87 cm) of Sephadex G-15. Fractions were collected at 20 ml, and the eluates obtained from tubes 8 to 10 were combined, concentrated and lyophilized. The product was hydrolyzed and analyzed in the same manners as in a former report.[6]

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