Plant Mucilages. XXI.¹⁾ Isolation and Characterization of a Mucous Polysaccharide, "Lilium-Ma-glucomannan," from the Bulbs of *Lilium maculatum*

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A mucous polysaccharide, named Lilium-Ma-glucomannan, has been isolated from the bulbs of *Lilium maculatum* THUNB. It was homogeneous on glass-fiber paper electrophoresis and by ultracentrifugal analysis. The component sugars of the glucomannan were D-mannose and D-glucose in the molar ratio of 7:4, and its molecular weight was measured at 184000. Methylation, periodate oxidation, and partial acetolysis studies suggested that the polysaccharide is mainly composed of β -1 $\rightarrow$ 4 linked aldohexopyranose residues and it contains about seven aldohexose units per one end group on the average. D-Mannose units occupy non-reducing terminal positions and branching points linked through positions 2 or 3. The O-acetyl groups in the glucomannan were identified and determined as the content of 4.7%. They were located in positions 2, 3, 6, 3,6, and 2,3,6 of a part of D-mannose units, and 6 and 2,3,6 of a part of D-glucose units.

Keywords—mucous polysaccharide; Lilium-Ma-glucomannan; *Lilium maculatum*; molar ratio of component sugars; intrinsic viscosity; molecular weight; structure of main chain; branching points; location of acetyl groups

In the previous papers of this series,³⁻⁶⁾ the structural features of O-acetylated glucomannans from the bulbs of *Lilium auratum*, *Lilium speciosum*, *Lilium lancifolium*, and *Lilium longiflorum* have been reported from this laboratory. Now we obtained a new mucous polysaccharide from the fresh bulbs of *Lilium maculatum* THUNB. This paper is concerned with its properties and structure.

After treatment with hot methanol, the material was extracted with cold water. The crude mucilage obtained was applied to a column of diethylaminoethyl (DEAE)-cellulose

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

a: 0.1% in H₂O, 23°, 48 min, 60000 rpm.
b: 0.1% in H₂O, 23°, 60 min, 60000 rpm.
Hitachi model UCA-1A ultracentrifuge.

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

- 1) Part XX: M. Tomoda, N. Satoh, and G. Matsumura, *Chem. Pharm. Bull.* (Tokyo), 26, 3215 (1978).
- 2) Location: 1-5-30, Shibakôen, 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).

(carbonate form), and a mucous polysaccharide was isolated from the eluate with water. It 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 substance showed a negative specific rotation ($[\alpha]_D^{25} -32.4^\circ$ in H_2O , $c=0.3$). Its solution in water gave the intrinsic viscosity value of 5.6 at 28° . 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 7: 4. The measurement of osmotic pressure gave the value of 184000 as the molecular weight of the polysaccharide. The name "Lilium-Ma-glucomannan" is proposed for it.

The glucomannan was methylated with methylsulfinylmethyl sodium in dimethyl sulfoxide and methyl iodide.⁷⁾ The fully methylated product was hydrolyzed and analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates.⁸⁾ As the hydrolysis products of the methylated polysaccharide, 2,3,4,6-tetra-*O*-methyl- β -D-mannose, 2,3,6-tri-*O*-methyl- β -D-mannose, 2,3,6-tri-*O*-methyl- β -D-glucose, 2,6-di-*O*-methyl- β -D-mannose, and 3,6-di-*O*-methyl- β -D-mannose were identified and obtained in a molar ratio of 1.0: 19.0: 11.5: 0.4: 0.3. The tetramethyl ether of mannose was also confirmed as its methyl glycoside by GLC.

As the result of periodate oxidation, 0.97 mol of periodate per one mol of component anhydro sugar unit of the glucomannan was consumed with 0.15 mol of formic acid liberation. The periodate-oxidized polysaccharide was reduced⁹⁾ and hydrolyzed. Analysis of the products showed that the yields of erythritol and mannose were 61.5% and 15.1%.

On the other hand, partial acetolysis of the glucomannan was carried out. The sample was acetylated, then partially degraded with sulfuric acid in acetic anhydride. After deacetylation, the products were applied to high performance liquid chromatography (HPLC), and the fractions obtained were analyzed by TLC and by GLC of trimethylsilyl derivatives. The comparison with authentic samples¹⁰⁾ showed the presence of *O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)- β -D-mannopyranose, *O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-mannopyranose, *O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranose, *O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranose, *O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-*O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)- β -D-mannopyranose, *O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-mannopyranose, and *O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-*O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)- β -D-mannopyranose in addition to the component monosaccharides.

These results indicated that the glucomannan is mainly composed of β -1 $\rightarrow$ 4 linked aldohexose units and has some mannopyranose residues as terminals and branching points linking through positions 2 or 3 in part. From the value of formic acid liberation after periodate oxidation and the yield of mannose as the Smith degradation product, it is able to conclude that the glucomannan has about seven aldohexose units per one non-reducing group on the average. The yields of tetramethyl and dimethyl ethers of mannose were low in comparison with these values. The cause for such a discrepancy may be attributed to unavoidable loss of the methyl ethers.

As shown in Fig. 2, the infrared (IR) spectrum of the glucomannan has the absorption bands of 1250 cm^{-1} and 1740 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 showed the occurrence of acetic acid. The acetyl content of the glucomannan was determined to be 4.7% by GLC.

7) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

8) H. Björndal, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).

9) M. Abdel-Akher, J.K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, **74**, 4970 (1952).

10) M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull. (Tokyo)*, **21**, 2511 (1973).

The location of *O*-acetyl groups in the glucomannan was established by the application of the method of DeBelder and Norrman.¹¹⁾ After conversion of the free hydroxyl groups into 1-methoxyethyl ethers by the exhaustive treatment with methyl vinyl ether in the presence of *p*-toluenesulfonic acid in dimethyl sulfoxide, the derivative was deacetylated, then methylated by Kuhn method.¹²⁾ The resulting product was hydrolyzed and analyzed by GLC-MS after conversion into alditol acetates. Seven hexose methyl ethers were detected and identified as 2-mono-*O*-methyl-D-mannose, 3-mono-*O*-methyl-D-mannose, 6-mono-*O*-methyl-D-mannose, 6-mono-*O*-methyl-D-glucose, 3,6-di-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-glucose in a molar ratio of 2.3: 1.4: 6.1: 1.3: 1.0: 2.9: 2.3.

Based on this result, it is able to conclude that the residues of 2-mono-*O*-acetyl-D-mannose, 3-mono-*O*-acetyl-D-mannose, 6-mono-*O*-acetyl-D-mannose, 6-mono-*O*-acetyl-D-glucose, 3,6-di-*O*-acetyl-D-mannose, 2,3,6-tri-*O*-acetyl-D-mannose, and 2,3,6-tri-*O*-acetyl-D-glucose are partially present in *Lilium-Ma*-glucomannan. We have already reported the presence of partially 2,3,6-tri-*O*-acetylated D-manno- and D-gluco-pyranosyl units in addition to several di- and mono-*O*-acetylated hexose residues in the glucomannan isolated from the bulbs of *Lilium longiflorum*.⁶⁾ Thus this is the second example showing the presence of partially tri-*O*-acetylated hexose units in lily glucomannans. 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 measured with JASCO model DIP-SL automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. IR spectra were recorded on Hitachi model EPI-G3 infrared spectrophotometer. 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.

Isolation of Polysaccharide—The material was obtained in October of 1976 from the plants cultivated in Saitama prefecture. The fresh bulbs (100 g), which contain 41.2% of water, were crushed, then extracted with hot methanol (400 ml) for 30 min twice. After suction filtration, the residue was extracted with water (800 ml) twice under stirring at room temperature for 1 hr each. The extracts were combined and poured into two volumes of ethanol, then filtered. The precipitate was treated with ethanol again, then dried *in vacuo* (yield, 3.1 g). A part of this crude mucilage (0.38 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.¹³⁾ The eluates obtained from tubes 11 to 20 were combined, concentrated and lyophilized. *Lilium-Ma*-glucomannan (0.19 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 preceding report¹⁴⁾ of this series. The condition of 380 volts for 2 hr was used. The sample gave one spot at a distance of 15.1 cm from the origin toward the cathod. Standard glucose moved to a distance of 17.8 cm.

Qualitative and Quantitative Analyses of Component Sugars—These were carried out in the same manners as in a former report⁶⁾ of this series. The results revealed that the sample was composed of 62.8% of mannose and 36.2% 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 former report¹⁵⁾ of this series.

Methylation of Polysaccharide—Sodium hydride (250 mg) was mixed with dimethyl sulfoxide (5 ml) and the mixture was stirred at 70° for 1 hr. The sample (210 mg) was dissolved in dimethyl sulfoxide (20 ml) under stirring at 60° and the solution of methylsulfinylmethyl sodium was added into this mixture. After

11) A.N. DeBelder and B. Norrman, *Carbohydr. Res.*, **8**, 1 (1968).

12) R. Kuhn, H. Trischman, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

13) 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.

14) M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 2173 (1971).

15) M. Tomoda and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **20**, 2491 (1972).

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 six more times under the same conditions. 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 85% formic acid at 90° for 16 hr and 0.5 N sulfuric acid at 100° for 2 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 and GLC-MS were carried out under the same conditions as in a former report.⁶⁾

In addition, a part of the product was methanolized with 4% hydrogen chloride in methanol in a sealed tube at 75° for 16 hr. After removal of hydrogen chloride by evaporation, 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, Smith Degradation, and Analysis of Products—These were carried out in the same manners as in a former report⁶⁾ of this series.

Partial Acetolysis—The acetylation and the partial degradation of the polysaccharide were carried out in the same manners as in a former report.⁶⁾

Analysis of Partial Acetolysis Products—The products were dissolved in methanol and deacetylated with 0.2 N sodium hydroxide at 5° overnight. After neutralization with acetic acid, the solvent was evaporated off. The residue was dissolved in water and passed successively through the columns ($1 \times 3\text{ cm}$) of Dowex 50W (H^+) and Dowex 2 (OH^-). The eluate and washing were combined and evaporated to dryness. The residue was dissolved in water, and a part (80 μl) of the 10% solution was applied to HPLC. HPLC was carried out using a column ($0.8 \times 50\text{ cm}$) packed with Shodex Ionpak S-801. The column was eluted with water under a flow of 1 ml per min at 50° . Three fractions were obtained from peaks showing retention times (min) of 11.8, 14.0, and 15.9. 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⁾ Fraction 1 contained higher oligomers and polymers. $\text{Man} \rightarrow \text{Man} \rightarrow \text{Man}$, $\text{Man} \rightarrow \text{Glc} \rightarrow \text{Man}$, and $\text{Man} \rightarrow \text{Man} \rightarrow \text{Man} \rightarrow \text{Man}$ were found in fraction 2. $\text{Man} \rightarrow \text{Man}$, $\text{Glc} \rightarrow \text{Man}$, $\text{Man} \rightarrow \text{Glc}$, and $\text{Glc} \rightarrow \text{Glc}$ were detected in fraction 3.

Determination of O-Acetyl Groups—The IR spectrum of the glucomannan showed the absorption bands of ester. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1740, 1250 (ester), 890 (β -glycosidic linkage).

The determination of O-acetyl groups was performed in the same manner as in a former report.⁶⁾

Treatment with Methyl Vinyl Ether—The sample (100 mg) was suspended in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (25 mg) was added. The mixture was stirred at 15° , then methyl vinyl ether (6 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 ($4 \times 23\text{ cm}$) of Sephadex LH-20. The column was eluted with acetone, and fractions were collected at 10 ml each. The eluates obtained from tubes 11 to 14 were combined and concentrated. The reaction was repeated once again under the same conditions. The IR spectrum of the final residue had no absorption near 3400 cm^{-1} .

Deacetylation of the O-Acetyl-O-(1-methoxyethyl)-derivative—The product was dissolved in methanol (6 ml), then 0.2 M methanolic sodium methoxide (6 ml) was added under stirring. The solution was refluxed at 80° for 4 hr, then concentrated and applied to a column ($4 \times 30\text{ cm}$) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 10 ml, and the eluates obtained from tubes 16 to 20 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 (180 mg) was dissolved in *N,N*-dimethylformamide (10 ml), then methyl iodide (4 ml) and silver oxide (0.8 g) were added successively under stirring. The reaction mixture was stirred at room temperature for 24 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 five times. The final reaction mixture was filtered and washed with chloroform (4 ml). The filtrate and washing were combined, then water (20 ml) was added into the solution. The mixture was extracted with chloroform (30 ml each) five times. The extracts were combined and washed with water (150 ml each) five times, then filtered and evaporated. After addition of chloroform and water (6 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—O-Methyl-O-(1-methoxyethyl)-derivative was successively treated with 85% formic acid and 0.5 N sulfuric acid, then the hydrolyzate was reduced and acetylated as

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 I.

TABLE I. Relative Retention Times on GLC and Fragments in MS of Partially Methylated Alditol Acetates

	Relative retention times ^{a)}	Main fragments (<i>m/e</i>)
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
1,2,4,5-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
1,2,3,4,5-Ac-6-Me-D-Glucitol	4.45	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., 1,4,5-Ac-2,3,6-Me=1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl)

described above. GLC and GLC-MS were carried out under the same conditions as in a former report.⁶⁾

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