[Chem. Pharm. Bull. 26(9)2768—2773(1978)]

UDC 547.458.02 : 581.192

Plant Mucilages. XIX.[1] Isolation and Characterization of a Mucous Polysaccharide, "Lilium-Lo-glucomannan," from the Bulbs of *Lilium longiflorum*

Masashi Tomoda, Noriko Satoh, and Chieko Ohmori

Kyoritsu College of Pharmacy[2]

(Received March 13, 1978)

A mucous polysaccharide, named Lilium-Lo-glucomannan, has been isolated from the bulbs of *Lilium longiflorum* Thunb. It was homogeneous on glass-fiber paper electrophoresis and in ultracentrifugal analysis. The component sugars of it were D-mannose and D-glucose in the molar ratio of 5 : 2, and its molecular weight was estimated at 263000. 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 ten aldohexose units per non-reducing terminal group on the average. D-Mannose units occupy non-reducing terminal positions and branch points linked through positions 2 or 3. The *O*-acetyl groups in the polysaccharide were identified and determined as the content of 3.2%. They were located in positions 6, 2,6, 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.

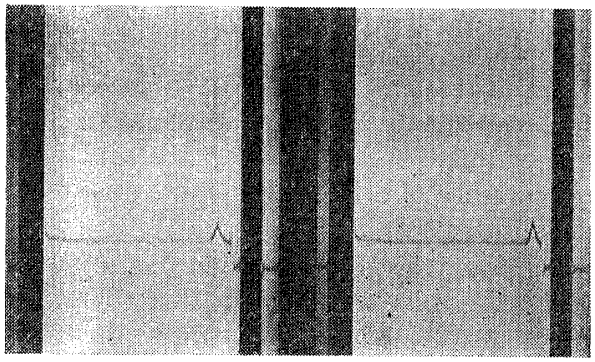
Keywords——mucous polysaccharide; Lilium-Lo-glucomannan; *Lilium longiflorum*; molar ratio of component sugars; intrinsic viscosity; molecular weight; methylation analysis; Smith degradation; partial acetolysis; location of acetyl groups

The isolations and the structural features of several *O*-acetylated glucomannans from lily bulbs have been reported by us.[3–5] In 1974, Matsuo and Mizuno[6] reported the isolation of a water-soluble glucomannan from commercial bulbs in the dormant stage of *Lilium longiflorum* Thunb. var. "*Georgia*," and observed the presence of *O*-acetyl groups in the polysaccharide. However, the structure of the glucomannan and the location of *O*-acetyl groups were not described in their report. The present paper is concerned with the isolation and the structure of a pure mucous polysaccharide from the fresh bulbs of *Lilium longiflorum* Thunb. This plant provides a famous ornamental flower.

The material bulbs were crushed and treated with hot methanol, then the residue was 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 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 in the ultracentrifugal analysis (Fig. 1). Further, it gave a single peak on gel chromatography with Sephacryl S-200.

The polysaccharide showed a negative specific rotation ($[\alpha]_D^{23}$ −33.2° in H_2O, c=0.3). Its solution in water gave the intrinsic viscosity value of 4.1 at 28°. Cellulose thin-layer chromatography (TLC) of the hydrolyzate and gas–liquid chromatography (GLC) of trimethylsilyl derivative of the hydrolyzate revealed that the component sugars are mannose and glucose. Quantitative determination of them showed that the molar ratio of mannose: glucose is about 5 : 2. The measurement of osmotic pressure gave the value of 263000 as

1) Part XVIII: M. Tomoda and Y. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **25**, 3061 (1977).
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) T. Matsuo and T. Mizuno, *Agr. Biol. Chem.*, **38**, 465 (1974).



(b) (a)

36 min 24 min

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

Hitachi model UCA-1A ultracentrifuge, 0.1% in H_2O, 23°, 60000 rpm.

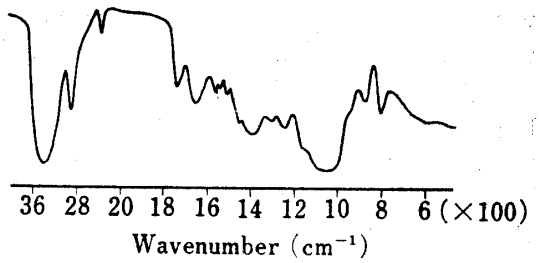


Fig. 2. IR Spectrum of Lilium-Lo-glucomannan

the molecular weight of this polysaccharide. The name "Lilium-Lo-glucomannan" is proposed for it.

The methylation of the polysaccharide was performed with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide.[7] The fully methylated product was successively hydrolyzed with formic acid and dilute sulfuric acid, then the products were analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) after conversion into the corresponding alditol acetates.[8] As the hydrolysis products of the methylated polysaccharide, 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-mannose, and 3,6-di-*O*-methyl-D-mannose were identified and obtained in a molar ratio of 1.0: 30.4: 14.0: 0.3: 0.6. The identification of the tetra-*O*-methyl mannose was also carried out by GLC as its methyl glycoside.

The polysaccharide consumed 0.99 mol of periodate per anhydrohexose unit with 0.12 mole of formic acid liberation. The periodate-oxidized polysaccharide was treated with sodium borohydride,[9] and the reduction product was isolated by the gel chromatography using a column of Sephadex G-15. The product was hydrolyzed and then converted into alditol acetates. Analyses of them by GLC showed that the yields of erythritol and mannose were 64.0% and 9.6%.

Further, partial acetolysis of the glucomannan was carried out. The sample was suspended 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 trimethylsilylated and analyzed by GLC. The comparison with authentic samples[10] showed the presences of *O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, *O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose, *O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose, and *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose in addition to the component monosaccharides.

These results indicated that the polysaccharide is mainly composed of β-1→4 linked aldohexose units and has some mannopyranosyl residues as non-reducing 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 ten aldohexose units per one non-reducing group on the average. The yields of tetra-*O*-methyl and di-*O*-methyl mannoses

7) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
8) H. Björndal, B. Lindberg, and S. Svensson, *Carbohyd. 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).

were low in comparison with these values. The cause for such a discrepancy may be attributed to unavoidable losses of a part of the methyl ethers.

As shown in Fig. 2, the infrared (IR) spectrum of this 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 the glucomannan by GLC showed the occurrence of acetic acid, and the content of *O*-acetyl groups in the glucomannan was determined to be 3.2%.

For the elucidation of the location of *O*-acetyl groups, the polysaccharide was repeatedly treated with methyl vinyl ether in the presence of *p*-toluenesulfonic acid in dimethyl sulfoxide.[11] After conversion of the free hydroxyl groups into 1-methoxyethyl ethers, the derivative was deacetylated with methanolic sodium methoxide, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide.[12] The resultant product was subjected to acid hydrolysis, and the final products were analyzed by GLC–MS after conversion into alditol acetates. Six hexose methyl ethers were detected and identified as 6-mono-*O*-methyl-D-mannopyranose, 6-mono-*O*-methyl-D-glucopyranose, 2,6-di-*O*-methyl-D-mannopyranose, 3,6-di-*O*-methyl-D-mannopyranose, 2,3,6-tri-*O*-methyl-D-mannopyranose, and 2,3,6-tri-*O*-methyl-D-glucopyranose in a molar ratio of 5.0: 1.4: 1.0: 1.0: 2.4: 1.8.

These results indicate that the residues of 6-mono-*O*-acetyl-D-mannopyranose, 6-mono-*O*-acetyl-D-glucopyranose, 2,6-di-*O*-acetyl-D-mannopyranose, 3,6-di-*O*-acetyl-D-mannopyranose 2,3,6-tri-*O*-acetyl-D-mannopyranose, and 2,3,6-tri-*O*-acetyl-D-glucopyranose are partially present in the glucomannan. All the results indicated that the manner of sequence of component sugars in the main chain and the type of branching were similar to those of Lilium-La-glucomannan.[5] However, Lilium-Lo-glucomannan has different *O*-acetylated hexopyranose units from Lilium-A-glucomannan,[3] Lilium-S-glucomannan,[4] and Lilium-La-glucomannan.[5] Very recently, Paulsen, *et al.*[13] reported the presences of the hexose residues substituted at positions 2,3,6 with acetyl groups in the glucomannan obtained from the leaves of *Aloe plicatilis* MILLER. The present report is the second example describing the presences of partially 2,3,6-tri-*O*-acetylated D-mannopyranosyl and D-glucopyranosyl units in natural glucomannans.

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 use of Hitachi model 063 gas chromatograph equipped with hydrogen flame ionization detector. GLC–MS was performed by use of JEOL model JGC-20K gas chromatograph and JMS-D100 mass spectrometer.

Isolation of Polysaccharide——The material was obtained in October of 1976 from the plants cultivated in Saitama prefecture. The fresh bulbs (152 g), which contain 63.6% of water, were crushed, then extracted with hot methanol (600 ml) for 30 min twice. After suction filtration, the residue was extracted with water (1200 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 ethanol again, then dried *in vacuo* (yield, 4.2 g). A part of this crude mucilage (0.62 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 20 were combined, concentrated and lyophilized. Lilium-Lo-glucomannan (0.46 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

11) A.N. DeBelder and B. Norrman, *Carbohyd. Res.*, **8**, 1 (1968).
12) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
13) B.S. Paulsen, E. Fagerheim, and E. Øverbye, *Carbohyd. Res.*, **60**, 345 (1978).
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.

preceding report[15] of this series. The condition of 380 volts for 2 hr was used. The sample gave one spot at a distance of 15.5 cm from the origin toward the cathod. Standard glucose moved to a distance of 17.8 cm.

Gel Chromatography——The sample (3 mg) was dissolved in water and applied to a column (2.6×97.5 cm) of Sephacryl S-200 superfine. The elution was carried out by ascending method with 0.04 M pyridine–0.02 M acetic acid mixture (1: 1) as an eluant. Fractions were collected at 5 ml and analyzed by phenol-sulfuric acid method. The sample peak was detected in fraction No. 45. The void volume was 200 ml (fraction No. 40).

Qualitative Analysis of Component Sugars——The sample was hydrolyzed with 2 N sulfuric acid in a sealed tube at 100° for 6 hr, then neutralized with barium carbonate. The hydrolyzate was applied to TLC using Avicel SF cellulose. In addition, the sample was hydrolyzed with 2 M trifluoroacetic acid in a sealed tube at 120° for 2 hr. After removal of the acid by evaporation, the hydrolyzate was trimethylsilylated in the usual way and applied to GLC. TLC and GLC were carried out under the same conditions as in a former report[5] of this series.

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 hydrolyzate was reduced in water with sodium borohydride (15 mg) for 2 hr. After neutralization with Dowex 50W-X8 (H^+), the filtrate was evaporated and boric acid was removed by repeated addition and evaporation of methanol. Then the products were acetylated with acetic anhydride-pyridine mixture (1: 1) at 100° for 40 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 A, a column (0.3×200 cm long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 210° with a flow of 60 ml per min of nitrogen. Xylose was used as an internal standard. Retention times of the corresponding peracetates of xylitol, mannitol and glucitol were 8.9, 17.7, and 21.2 min, respectively. The result revealed that the sample was composed of 68.6% of mannose and 28.4% of glucose in addition to *O*-acetyl group.

Determination of Molecular Weight——The measurement of osmotic pressure was carried out by use of Knauer Electronic Membrane Osmometer in the same manner as in a former report[16] of this series.

Methylation of Polysaccharide——Sodium hydride (100 mg) was mixed with dimethyl sulfoxide (5 ml) and the mixture was stirred at 70° for 1 hr. The sample (100 mg) was dissolved in dimethyl sulfoxide (5 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 (50 ml each) five times. The extracts were combined and washed with water (250 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——The product (5 mg) was successively treated with 90% formic acid (0.5 ml) at 90° for 16 hr and 0.5 N sulfuric acid (0.5 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 above. GLC was carried out under condition B, using the same column as condition A at 180° with a flow of 60 ml per min of nitrogen. GLC–MS was also carried out under condition

Table I. 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	1.92	43, 45, 87, 99, 101, 113, 117, 233
1,4,5-Ac-2,3,6-Me-D-glucitol	2.18	43, 45, 87, 99, 101, 113, 117, 233
1,3,4,5-Ac-2,6-Me-D-mannitol	2.83	43, 45, 87, 117, 129
1,2,4,5-Ac-3,6-Me-D-mannitol	3.39	43, 45, 87, 99, 113, 129, 189, 233
1,2,3,4,5-Ac-6-Me-D-mannitol	3.68	43, 45, 87, 115, 129
1,2,3,4,5-Ac-6-Me-D-glucitol	4.57	43, 45, 87, 115, 129

a) Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.
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).

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).

B. However, helium was used as carrier gas. 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.

In addition, a part of the product was methanolyzed with 4% methanolic hydrogen chloride 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] Methyl glycosides of hexose trimethyl ethers were not separated from each other and those 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 room temperature in a dark place. The periodate consumption was measured by a spectrophotometric method.[17] The oxidation was completed after nine days, then 2 ml of the solution was used for the measurement of formic acid liberation by titrating 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 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 × 72 cm) of Sephadex G-15. Fractions were collected at 50 ml, and the eluates obtained from tubes 10 to 14 were combined, evaporated and lyophilized. The product was hydrolyzed with 1 N sulfuric acid at 100° for 6 hr, and the hydrolyzates were derived to alditol acetates as described above and determined by GLC under condition A. Retention time of erythritol tetraacetate was 2.5 min.

Partial Acetolysis——The polysaccharide (50 mg) was suspended in formamide (1.2 ml), then pyridine (0.6 ml) and acetic anhydride (0.4 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 ether, then dried *in vacuo*. The product was dissolved in acetic anhydride (0.8 ml), then a cold mixture of acetic anhydride: sulfuric acid (5: 1, 0.4 ml) was added under cooling, and the solution was kept at 5° for 24 hr. The reaction mixture was poured into ice-water (10 ml), then the products were extracted with chloroform (10 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 and deacetylated with 0.2 N sodium hydroxide (1 ml) at 5° overnight. After neutralization with acetic acid, the solvent was evaporated off.

Analysis of Partial Acetolysis Products——The residue was dissolved in water and passed through a column (1 × 3 cm) of a mixture of Dowex 50W-X8 (H^+) and Dowex 2 (OH^-). The eluate and washing were combined and evaporated to dryness. The residue was trimethylsilylated in the usual way and applied to GLC. GLC was carried out under the same conditions as a former report[5] of this series.

Determination of *O*-Acetyl Groups——The IR spectrum of the polysaccharide showed the absorption bands of ester. IR ν_{max}^{KBr} cm^{-1}: 1740, 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 hydrolyzate was directly applied to GLC. GLC was carried out under condition C, a column (0.3 × 200 cm long spiral glass) packed with 5% Thermon-1000-0.5% phosphoric acid on Chromosorb W (80 to 100 mesh) at 110° with a flow of 20 ml per min of nitrogen; t_R (min), acetic acid 3.5; propionic acid (internal standard) 5.1.

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 three times. 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. The eluates obtained from tubes 9 to 13 were combined and concentrated. The product was further treated twice 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 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 75° for 4 hr, then concentrated and applied to a column (5 × 20 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions were collected at 10 ml, and the eluates obtained from tubes 12 to 20 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 product (200 mg) was dissolved in *N*,*N*-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 chloroform, the filtrate and washing were combined and evaporated. Methyl iodide (1 ml) and silver oxide (0.4 g) were added again into the residual solution, then the reaction was similarly repeated ten

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

times. The final reaction mixture was filtered and washed with chloroform (4 ml). The filtrate and washing were combined, then water (10 ml) was added into the solution. The mixture was extracted with chloroform (15 ml each) five times. The extracts were combined and washed with water (75 ml each) five times, then filtered and evaporated. After addition of chloroform and water (3 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 (5 mg) was successively treated with 90% formic acid (0.5 ml) and 0.5 N sulfuric acid (0.5 ml), then the hydrolyzate was reduced and acetylated as described above. GLC and GLC–MS were carried out under condition B. 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 I.

Acknowledgement The authors are grateful to Prof. G. Matsumura, School of Pharmaceutical Sciences, Showa University, for ultracentrifugal analysis. They also thank Misses K. Ohshima and Y. Ohshima for their technical assistance in a part of this work.