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

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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, 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 \rightarrow 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 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. Furthermore, it gave a single peak on gel chromatography with Sephacryl S-200.

The substance showed a negative specific rotation ($[\alpha]_D^{25} -40.7^\circ$ 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 TLC of the hydrolysate and 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.

The 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 hydrolysate of it by GLC and

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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. 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. The resulting product was hydrolyzed and analyzed by 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-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. The fully methylated product was hydrolyzed and analyzed by GLC-MS after conversion into 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, 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 showed the presence of *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannopyranose, *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-glucopyranose, *O*- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannopyranose, *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannopyranose, *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannopyranose, *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-glucopyranose, *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannopyranose, and *O*- β -D-glucopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannopyranose in addition to the component monosaccharides.

These results indicated that the glucomannan is mainly composed of β -1 \rightarrow 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. 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 and the presence of 1.7% of *O*-acetyl groups was determined by GLC of its hydrolysate. Based on these results, it can be deduced that about one third of the *O*-acetyl groups in the original glucomannan block periodate cleavage.

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 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 be attributed to partial overoxidation.

The Liliu-glucomannans obtained by us until now may be divided into three types regarding the location of *O*-acetyl groups: the first, Liliu-A-glucomannan and Liliu-S-glucomannan; the second, Liliu-La-glucomannan and Liliu-J-glucomannan; and the third, Liliu-Lo-glucomannan and Liliu-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. 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.

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