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Plant Mucilages. X. Isolation and Characterization of a Mucous Polysaccharide, "Lilium-A-glucomannan", from the Bulbs of *Lilium auratum**

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The bulbs of *Lilium auratum* LINDL. have been used as a crude drug for the purpose of analeptic and cough medicine. Lily bulbs contain, in addition to starch, a water-soluble mucous reserve polysaccharide. And it has been reported that the mucilage was composed of mannose and glucose in the approximate molar ratio of 2 : 1.[1] Structural studies on glucomannans isolated from the bulbs of *Lilium candidum*, *L. henryii* and *L. umbellatum* suggested that the majority of hexose units were linked together by β-1→4 glycosidic bonds to form long chains.[2] In this paper, the isolation and the structural feature of a new pure mucous polysaccharide from the fresh bulbs of *Lilium auratum* are described.

The material bulbs were crushed and extracted with hot methanol, then the residue was extracted with cold water. The crude mucilages were precipitated from the water extract by addition of methanol. The solution of the precipitate was applied to a column of DEAE-cellulose (carbonate form), and a mucous polysaccharide was obtaind from the eluate with water.

The polysaccharide gave one spot on glass-fiber paper electrophoresis in alkaline borate buffer, and it was found to be homogeneous by the ultracentrifugal analysis. It showed a negative specific rotation ($[\alpha]_D^{23}$ −37.9° in H_2O, c=1.0). Its solution in water gave the intrinsic viscosity value of 2.4 at 27°. As the component sugars of it, mannose and glucose were identified by means of cellulose thin-layer chromatography (TLC) of the hydrolysate and gas-liquid chromatography (GLC) of trimethylsilyl derivative of the methanolysate. Quantitative determination of them showed that the molar ratio of mannose : glucose is 8 : 3. The measurement of osmotic pressure gave the value of 35700 as the molecular weight of the polysaccharide and this value was also supported by the result of gel chromatography on Sephadex G-200.

The infrared (IR) spectrum of it has the absorption bands of 1735 and 1250 cm^{-1} suggesting the presence of ester linkages in addition to the absorption of 890

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1) T. Takahashi, *Nippon Nogeikagaku Kaishi* **6**, 791, 861 (1930) ; *idem*, *ibid.*, **7**, 219 (1931).

2) P. Andrews, L. Houghand J. K. N. Jones, *J. Chem. Soc.*, **1956**, 181.

cm^{-1} being due to β-glycosidic linkages. The acid hydrolysate of the polysaccharide was analyzed by GLC, and it gave one peak, whose retention time was precisely equal to that of authentic sample of acetic acid. The acetyl content of the polysaccharide was determined to be 5.1 % by GLC. Thus the pure mucilage obtained by us has different properties from those described in the former reports,[1,2] and the name "Lilium-A-glucomannan" is proposed for the polysaccharide.

The location of O-acetyl groups in Lilium-A-glucomannan was established by the application of the method using methyl vinyl ether as a protective reagent for the free hydroxyl groups. The sequence of reactions is illustrated in Chart 1. The polysaccharide (I) 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 (II). Deacetylation of the derivative (II) was accomplished by refluxing with methanolic sodium methoxide and gave the partially-O-(1-methoxyethyl)-glucomannan (III), then it was methylated with methyl iodide and silver oxide in dimethylformamide. Each product was purified by gel chromatography using a column of Sephadex LH-20. The resulting partially-O-methyl-O-(1-methoxyethyl)-glucomannan (IV) was finally subjected to acid hydrolysis, and the products were analyzed by paper partition chromatography (PPC) and by GLC of the alditol acetate after reduction and acetylation of the hydrolysate. Besides mannose and glucose, a hexose methyl ether was detected and identified as 3,6-di-O-methyl-D-mannopyranose (V) by comparison with the synthetic specimen.

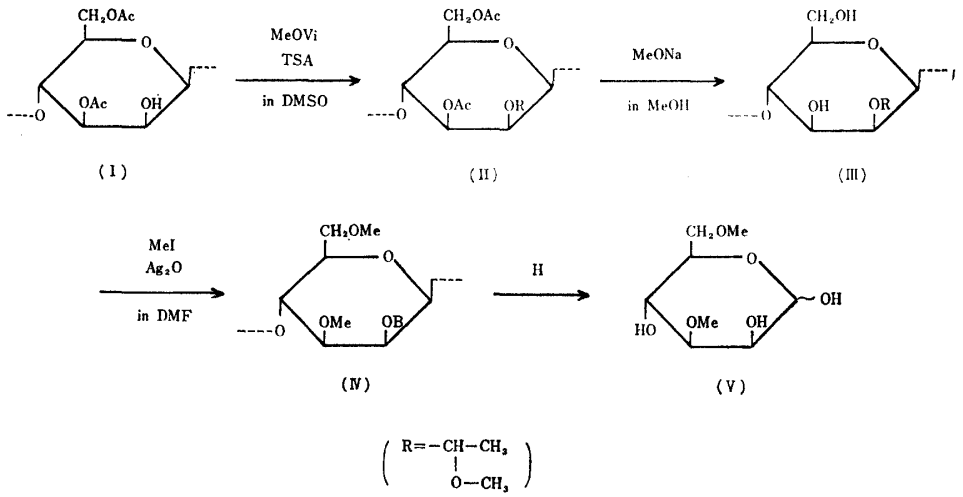

Chart 1

Owing to this result, it is able to conclude that the O-acetyl groups are attached to positions 3 and 6 of a part of D-mannopyranose units in Lilium-A-glucomannan. The value of quantitative analysis indicated that one mannose residue in

about eight component hexose units has 3,6-di-O-acetyl groups.

The methylation of the polysaccharide was performed with sodium methylsulfinylcarbanion and methyl iodide in dimethylsulfoxide. The fully methylated product was hydrolyzed with formic acid and dilute sulfuric acid. The products were separated by PPC, then analyzed by GLC 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 and 3,6-di-O-methyl-D-mannose were obtained in a molar ratio of 1.0 : 6.3 : 2.9 : 0.7. These methyl ethers of component sugars were also identified as their methyl glycosides by GLC.

These results suggested that the polysaccharide is mainly composed of 1→4 linked aldohexopyranose units and has some mannopyranose residues as branching points linked through position 2 with an average of about eleven component sugar units per non-reducing end group.

As the result of periodate oxidation, 1.01 mole of periodate per one mole of component anhydro sugar unit of the polysaccharide was consumed with 0.09 mole of formic acid liberation. The periodate-oxidized polysaccharide was treated with sodium borohydride, and the reduction product was methanolyzed. Analysis of trimethylsilyl derivative of the methanolysate by GLC revealed the presences of erythritol and mannose as the main products and showed that the yields of erythritol and mannose were 26.0% and 8.0%. These results, especially the value of formic acid liberation after periodate oxidation and the yield of mannose by Smith degradation, supported the conclusion of branching structure obtained by methylation studies.

Partial acid hydrolysis of Lilium-A-glucomannan also gave the evidence that the straight chain parts in the polysaccharide are composed of β-1→4 linked aldohexopyranose residues. The mucilage was hydrolyzed with 0.5 N sulfuric acid at 90° for 2.5 hr, and 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 showed that they are 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-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose. The results elucidated the fact that most of D-mannopyranose and D-glucopyranose residues are connected one another by β-1→4 glycosidic linkages, and at least, the mucilage has two kinds of aldohexose chain unit, which are O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyran-

osyl-(1→4)-D-mannopyranose and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose.

Andrews, *et al.*[2] reported that the glucomannans isolated from the bulbs of *Lilium umbellatum* and *L. henryii* had D-glucopyranosyl units as their non-reducing ends and *L. umbellatum* glucomannan had a small number of D-glucopyranosyl units as branching points linked through positions 3 and 6. Thus Lilium-A-glucomannan has different properties from those of the other Lilium glucomannans obtained until now in points of not only molar ratio of component sugars but also branching structure. And this is the first report describing the presence of partially 3,6-di-O-acetylated D-mannopyranosyl units in natural glucomannan.

The former investigators[1,2] used copper complex method for the purification of Lilium glucomannans, and they described that the polysaccharides became insoluble in water after such a treatment. The treatment with alkaline solution causes easily O-deacetylation, and it is unsuitable for the isolation of native polysaccharides having O-acetyl groups. So it will be necessary to reexamine the presence of O-acetyl groups in the other native Lilium glucomannans.