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Plant Mucilages. VIII[1]. Isolation and Characterization of a Mucous Polysaccharide, "Bletilla-glucomannan," from *Bletilla striata* Tubers*

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The tuber of *Bletilla striata* Reichenbach fil. (Orchidaceae) has been used as a crude drug for the purpose of hemostatic and anti-swelling. On the constituent of this tuber, presence of a mucilage was reported by Ohtsuki.[2] He has concluded that the mucilage is composed of D-mannose and D-glucose in the molar ratio of 4: 1, but its homogeneity was uncertain and the structure has been unknown until now. We have now isolated a pure mucous polysaccharide from the tuber of this plant, and its properties are described in the present paper.

The fresh tubers were extracted with hot methanol, then the residue was extracted with hot 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 obtained from the eluate with water. For the purpose of comparison, the material was also extracted with water at room temperature as described in Ohtsuki's report,[2] then the crude mucilage was purified by the use of DEAE-cellulose column chromatography. And it was observed that the mucilage obtained by hot water extraction gives much higher values than that obtained by cold water extraction on both yield and viscosity. So we decided the application of hot water extraction method for the isolation of the mucilage.

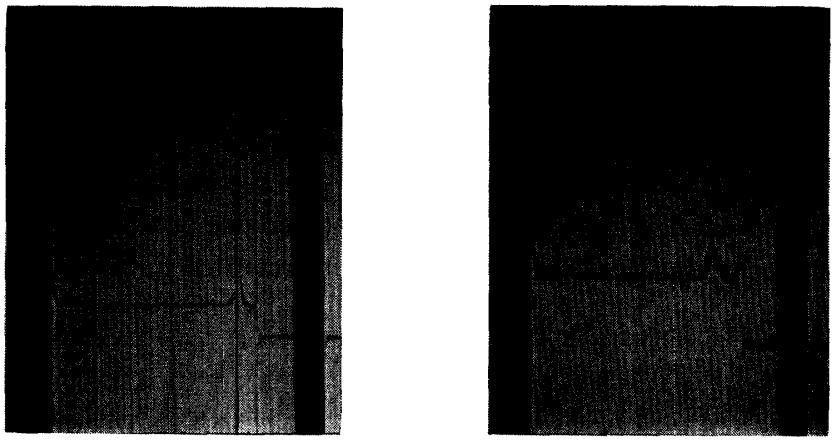
The polysaccharide gave one spot on glass-fiber paper electrophoresis in alkaline borate buffer, and it was found that the polysaccharide is homogeneous by the ultracentrifugal analysis (Fig. 1) and shows a characteristic sharp sedimentation pattern (Fig. 1-a) of high molecular weight viscous substances.

The polysaccharide showed a negative specific rotation ($[\alpha]_D^{23}$ $-31.6°$ in H_2O, $c=0.9$). Its solution in water gave the intrinsic viscosity value of 8.10 at 31°, while the mucilage obtained by cold water extraction gave the value of 4.75. 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 3: 1. Thus the pure mucous polysaccharide obtained by us has different properties from those described in the former report,[2] and the name "Bletilla-glucomannan" is proposed for the polysaccharide. The measurement of osmotic pressure

* 本報告は *Chem. Pharm. Bull.* (Tokyo), **21**, 2667 (1973) に発表.

1) Part VII: M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **21**, 2511 (1973).

2) T. Ohtsuki, *Acta Phytochim.*, **10**, 29 (1937).



(a) (b)

Fig. 1. Ultracentrifugal Pattern of Bletilla-glucomannan
(a), 0.5% in H_2O, 25°, 69 min, 60000 rpm
(b), 0.1% in H_2O, 25°, 45 min, 60000 rpm
Hitachi model UCA-1A ultracentrifuge

gave the value of 182000 as the molecular weight of Bletilla-glucomannan.

As the result of periodate oxidation, 0.98 mole of periodate per one mole of component anhydro sugar unit of the polysaccharide was consumed with 0.17 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 39.0% and 15.1%.

From the value of formic acid liberation after periodate oxidation, it is conceivable that Bletilla-glucomannan contains six aldohexose units per one end group on the average. And on the basis of the yield of mannose by Smith degradation, it is probable that a part of mannose residues occupies branching positions. The formation of a large quantity of erythritol by Smith degradation and the value of periodate consumption suggest that the straight chain parts in Bletilla-glucomannan are composed of 1→4 linked aldohexopyranose residues. These presumptions were supported by the results of partial acid hydrolysis, and the presence of any chain composed of aldohexoses having 1→3 or 1→6 glycosidic linkages was not found.

Bletilla-glucomannan was hydrolyzed with 0.5N sulfuric acid at 90° for 2.5 hr, and the product was fractionated by active charcoal column chromatography. The fractions were applied to paper partition chromatography (PPC), and several oligisaccharides were obtained. From among them, three disaccharides (**I**, **II**, and **III**), a trisaccharide (**IV**) and a tetrasaccharide (**V**) were identified.

Analysis of trimethylsilyl derivatives of their methanolysates by GLC, methylation studies and the comparison TLC and GLC with authentic samples showed that **I** is O-β-D-mannpoyranosyl-(1→4)-D-mannopyranose, **II** is O-β-D-mannopyranosyl-(1→4)-D-gluco-

pyranose, **III** is O-β-D-glucopyranosyl-(1→4)-D-mannopyranose, **IV** is O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose, and **V** is O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannopyranose.

These results gave the evidence that most of the D-mannopyranose and D-glucopypyranose residues in Bletilla-glucomannan are combined one another by β-1→4 glycosidic linkage. Detailed elucidation of the structure will be reported in following papers.