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Plant Mucilages. XXXV.[1] Isolation and Characterization of a Mucous Polysaccharide, Hippeastrum-H-glucomannan, from the Bulbs of *Hippeastrum hybridum*

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A mucous polysaccharide, named Hippeastrum-H-glucomannan, was isolated from the bulbs of *Hippeastrum hybridum* HORT. The final preparation was homogeneous as determined by ultracentrifugal analysis, glass-fiber electrophoresis, and gel chromatography. It was composed of D-mannose and D-glucose in the molar ratio of 5:2, and its molecular weight was estimated to be 331000. *O*-Acetyl groups were identified and their content amounted to 13.2%. They were located at positions 2, 6 of some of the D-mannose units. Methylation and partial acid hydrolysis studies showed that the glucomannan is mainly composed of β-1→4-linked aldohexopyranose residues, and that it contains about eighty-three aldohexose units per six non-reducing groups on average. Both D-mannose and D-glucose units occupy non-reducing terminal positions and branching points linked through position 3.

Keywords——*Hippeastrum hybridum*; bulb; Hippeastrum-H-glucomannan; acetyl-rich polysaccharide; intrinsic viscosity; molecular weight; structure; 2,6-di-*O*-acetyl group

Acetyl-rich mucous glucomannans have been isolated from the bulbs of several plants in the Amaryllidaceae family.[2-4] These polysaccharides possess β-1→4 glycosidic main chains in common, but differ in types of branching and acetyl group location. In addition, different values of component sugar ratio and molecular weight were observed. We have now obtained a new acetyl-rich mucous polysaccharide from the bulbs of *Hippeastrum hybridum* HORT. This plant provides a famous ornamental flower. The properties and the structural features of the polysaccharide are described in the present paper.

The bulbs were sliced and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol, then dissolved in water and applied to a column of diethylaminoethyl (DEAE)-cellulose (acetate form). A mucous polysaccharide was obtained from the eluate with water. The polysaccharide gave a single spot on glass-fiber paper electrophoresis, and was homogeneous as determined by ultracentrifugal analysis (Fig. 1).

The polysaccharide was readily soluble in water and it showed a negative specific rotation ($[\alpha]_D^{22}$ −35.1° in H_2O, $c=0.8$). Its aqueous solution gave an intrinsic viscosity value of 4.0 at 30 °C. It gave a single peak on gel chromatography with Sephacryl S-400, and a value of 331000 was obtained for the molecular weight from a calibration curve based on the elution volumes of standard dextrans (Fig. 2).

Mannose and glucose were identified as the component sugars, and quantitative determination showed that the molar ratio of mannose : glucose is about 5 : 2. Total hexose content was estimated to be 86.3%. The polysaccharide contained no nitrogen. The name Hippeastrum-H-glucomannan is proposed for this compound.

The infrared (IR) spectrum of the glucomannan has absorption bands at 1245 and 1730 cm^{-1}, suggesting the presence of ester linkages. The proton magnetic resonance (^1H-NMR) spectrum showed acetyl signals at δ 1.90 and δ 2.18 ppm. Analysis of the acid

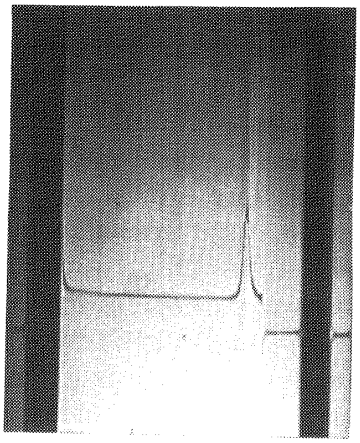

Fig. 1. Ultracentrifugal Pattern of Hippeastrum-H-glucomannan

0.5% in H_2O, 20°C, 63 min, 60000 rpm, Hitachi UCA-1A ultracentrifuge.

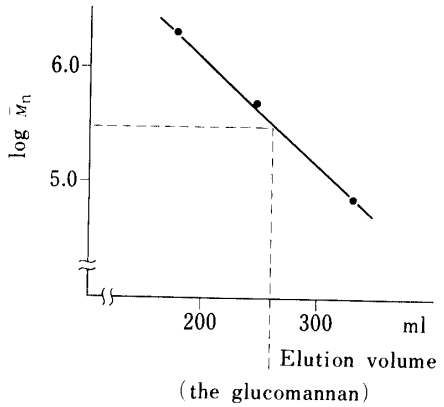

Fig. 2. Plot of Elution Volume against log $\bar{M}_n$ for Dextran Fractions on Sephacryl S-400

hydrolysate of the glucomannan by gas-liquid chromatography (GLC)[5] showed the occurrence of acetic acid, and the content of *O*-acetyl groups was determined to be 13.2%.

The glucomannan was exhaustively treated with methyl vinyl ether, as a protective reagent for the free hydroxyl groups, in the presence of *p*-toluenesulfonic acid in dimethyl sulfoxide.[6] After conversion of the free hydroxyl groups to 1-methoxyethyl ethers, the derivative was deacetylated, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide.[7] The product was hydrolyzed, reduced, and acetylated. The final products were analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS).[8] A partially methylated alditol acetate was detected and identified as 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-mannitol. This result indicates that 2,6-di-*O*-acetyl-D-mannose units are present in the glucomannan.

The glucomannan was methylated with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[9] The fully methylated product was hydrolyzed, reduced, and acetylated, and the products were analyzed by GLC-MS. As the hydrolysis products of the methylated polysaccharide, 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-mannose, and 2,6-di-*O*-methyl-D-glucose were identified and obtained in a molar ratio of 5.0 : 1.0 : 49.8 : 21.0 : 4.8 : 0.9. The identity and the ratio of the two tetra-*O*-methyl hexoses were confirmed by GLC of the methyl glycosides obtained by methanolysis of the methylated product.

Further, partial hydrolysis of the glucomannan was carried out with dilute trifluoroacetic acid. The products were analyzed by thin-layer chromatography (TLC) and by GLC of the trimethylsilylated derivatives. Comparison with authentic samples[10] showed the presence of D-mannose, D-glucose, *O*-β-D-glucopyranosyl-(1→4)-D-mannose, *O*-β-D-mannopyranosyl-(1→4)-D-mannose, *O*-β-D-mannopyranosyl-(1→4)-D-glucose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannose, and *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannose. The relative yield ratio of these mono-, di-, and tri-saccharides was 100 : 40.4 : 27.7 : 48.9 : 14. 9 : 42.6 : 42.6.

These results indicated that the glucomannan is mainly composed of β-1→4 linked D-aldohexopyranose units and has both mannopyranose and glucopyranose residues as terminals. It has branches at both position 3 of some mannopyranose units and position 3 of some glucopyranose units. The molar ratio of the terminal mannose and glucose units, the intermediate β-1→4 linked mannose and glucose units, and the branched mannose and

glucose units must be approximately 5 : 1 : 49 : 22 : 5 : 1. The presence of branched glucose units is a unique feature of Hippeastrum-H-glucomannan among the Amaryllidaceae glucomannans obtained by us. Upon partial hydrolysis, there is no great difference in the yields of three β-1→4 linked disaccharides and two β-1→4 linked trisaccharides, in contrast to Narcissus-T-glucomannan,[2] Lycoris-R-glucomannan,[3] and Lycoris-S-glucomannan.[4] No cellobiose was detected as a product, and it can be concluded that the presence of D-glucose residues is discontinuous in the polysaccharide, as in the other three Amaryllidaceae glucomannans.

Hippeastrum-H-glucomannan is the fourth example of acetyl-rich polysaccharides from the bulbs of plants in the Amaryllidaceae family. On the basis of the content and the location of *O*-acetyl groups, we concluded that the molar ratio of D-mannose and 2,6-di-*O*-acetyl-D-mannose residues was approximately 7 : 5 in the glucomannan. It is interesting that 2,6-di-*O*-acetyl-D-mannose is a common unit in the four acetyl-rich glucomannans from the Amaryllidaceae plants.

Experimental

Solutions were concentrated at or below 40 °C with rotary evaporators under reduced pressure. Optical rotation was measured with a JASCO DIP-140 automatic polarimeter. IR spectra were recorded on a JASCO IRA-2 infrared spectrophotometer. ^{1}H-NMR spectrum was recorded on a JEOL JNM-GX270 FT NMR spectrophotometer in heavy water containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard at 27 °C. GLC was carried out on a Shimadzu GC-7AG gas chromatograph equipped with a hydrogen flame ionization detector. GLC-MS was performed with a JEOL JGC-20K gas chromatograph and a JEOL JMS-D100 mass spectrophotometer.

Isolation of Mucilage——The material was obtained in September 1981 from plants cultivated in Chiba prefecture. The fresh bulbs (650 g), which contained 89.4% water, were sliced and extracted with water (3250 ml) under stirring at room temperature for 1 h. After centrifugation, the supernatant was poured into two volumes of ethanol. The precipitate was obtained by centrifugation, then dried *in vacuo* (yield, 5.2 g). A part of this crude mucilage (0.5 g) was dissolved in water (75 ml), and after centrifugation, the supernatant was applied to a column (4 × 37 cm) of DEAE-cellulose (acetate form). The column was eluted with water, and fractions of 20 ml were collected and analyzed by the phenol–sulfuric acid method.[11] The eluates obtained from tubes 6 to 15 were combined, concentrated, and lyophilized. Hippeastrum-H-glucomannan (0.21 g) was obtained as a white powder.

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF83 glass-fiber papers in the manner described in a previous report[12] of this series, with the following buffers and conditions: A, 0.08 M pyridine–0.04 M acetic acid (pH 5.4) at 570 V for 90 min; B, 0.025 M borax: 0.1 N sodium hydroxide (10 : 1, pH 9.3) at 570 V for 45 min. The sample gave a single spot at distances of 6.3 cm (A) and 7.5 cm (B) from the center toward the cathode.

Gel Chromatography——The sample was dissolved in water and applied to a column (2.6 × 94 cm) of Sephacryl S-400. Elution was carried out by the descending method with 0.1 M Tris-HCl buffer (pH 7.0) as an eluant. Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method. Standard dextrans having known molecular weights were run on the column to obtain the calibration curve shown in Fig. 2.

Qualitative and Quantitative Analyses of Component Sugars——These were carried out by the methods described in a previous report[5] of this series. Sugars were also determined by the chromotropic acid method.[13] The results revealed that the sample was composed of 61.3% mannose and 25.0% glucose in addition to acetyl groups.

Determination of *O*-Acetyl Groups——The IR spectrum of the glucomannan showed ester absorption bands. IR ν_{max}^{KBr} cm^{-1}: 1730, 1245 (ester), 890 (β-glycosidic linkage). The determination was carried out by the method described in a previous report.[14]

1-Methoxyethylation of Glucomannan Followed by De-*O*-acetylation——These procedures were carried out by the methods described in a previous report[2] of this series.

Methylation of the *O*-(1-Methoxyethyl) Derivative——This was also carried out in the manner described in a previous report[2] of this series.

Analysis of the *O*-Methyl Derivative——The product was hydrolyzed with 88% formic acid and 0.5 N sulfuric acid, then the hydrolysate was reduced and acetylated by the methods described in a previous report[4] of this series. The partially methylated alditol acetate obtained was analyzed by GLC and GLC-MS. GLC and GLC-MS were carried out under condition A, using a column (0.3 × 200 cm long spiral glass) packed with 3% OV 225 on Gas-chrom Q (100 to 120 mesh) at 200 °C with a helium flow of 60 ml per min. The relative retention time of the product with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and its main fragments in the mass spectrum are listed in Table I.

Methylation of Glucomannan and Analysis of the Products——Methylation was carried out in the manner

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

	Relative retention times[a]	Main fragments (*m/z*)
1,5-Ac-2,3,4,6-Me-D-Mannitol	0.98	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,5-Ac-2,3,4,6-Me-D-Glucitol	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,4,5-Ac-2,3,6-Me-D-Mannitol	1.84	43, 45, 87, 99, 101, 113, 117, 233
1,4,5-Ac-2,3,6-Me-D-Glucitol	2.07	43, 45, 87, 99, 101, 113, 117, 233
1,3,4,5-Ac-2,6-Me-D-Mannitol	2.65	43, 45, 87, 117, 129
1,3,4,5-Ac-2,6-Me-D-Glucitol	2.94	43, 45, 87, 117, 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-).

described in a previous report[2] of this series. The methylation reaction was repeated four times. The product was hydrolyzed with dilute sulfuric acid in the manner described in a previous report[15] of this series. After neutralization with Dowex 2 (OH^-), the hydrolysate was reduced, acetylated, and analyzed by GLC and GLC-MS as described above. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and their main fragments in mass spectra, are also listed in Table I. A part of the methylation product was methanolyzed and the product was analyzed by GLC in the manner described in a previous report[4] of this series; both methyl 2,3,4,6-tetra-*O*-methyl-D-glucoside and methyl 2,3,4,6-tetra-*O*-methyl-D-mannoside were identified.

Partial Acid Hydrolysis and Analysis of Degradation Products——The sample (9 mg) was hydrolyzed with 0.5 M trifluoroacetic acid (3 ml) at 90 °C for 3 h. After removal of acid by evaporation, the products were subjected to cellulose TLC and identified by comparison with subjected to cellulose TLC and identified by comparison with authentic samples in the manner described in a previous report[16] of this series. In addition, the products were trimethylsilylated and subjected to GLC under the same conditions as in a previous report.[15] The relative yields of the products were evaluated by GLC.

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