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Plant Mucilages. XXXI.[1] An Acetyl-rich Mucous Polysaccharide, "Lycoris-R-glucomannan," from the Bulbs of *Lycoris radiata*

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A mucous polysaccharide, named Lycoris-R-glucomannan, was isolated from the bulbs of *Lycoris radiata* Herbert. 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 12: 1, and its molecular weight was estimated to be 468000. *O*-Acetyl groups were identified in the glucomannan and their content amounted to 15.5%. They were located at positions 2,6 of some of the D-mannose units. Methylation, periodate oxidation, and partial acid hydrolysis studies showed that the glucomannan is mainly composed of β-1→4 linked aldohexopyranose residues, and that it contains about thirteen aldohexose units per three non-reducing groups on average. D-Mannose units occupy non-reducing terminal positions and branching points linked through both positions 3 and 6.

Keywords——acetyl-rich polysaccharide; Lycoris-R-glucomannan; *Lycoris radiata*; intrinsic viscosity; molecular weight; analysis of components; 2,6-di-*O*-acetyl groups; structure of main chain; branching points

The bulbs of *Lycoris radiata* Herbert (Amaryllidaceae) have been used as a crude drug with expectorant and emetic properties. In addition to various alkaloids,[2] starch, and fructans, the isolation of a glucomannan was reported by Hayashi *et al.*[3] in 1953. They reported that the glucomannan was purified by an alkaline copper complex method and it became insoluble in water, and in addition, the homogeneity of the polysaccharide was not established. The deacetylation of native water-soluble glucomannans having *O*-acetyl groups is known to result in insolubility of the products in water.[4–6] Thus it is necessary to reexamine the glucomannans obtained by treatment with an alkaline solution. We have now obtained a native, pure, highly acetylated polysaccharide from the fresh bulbs of this plant, and its properties and structural features are described in the present paper.

The bulbs were sliced and extracted with cold water. The crude mucilage obtained was applied to a column of diethylaminoethyl (DEAE)-cellulose (acetate form), and a mucous

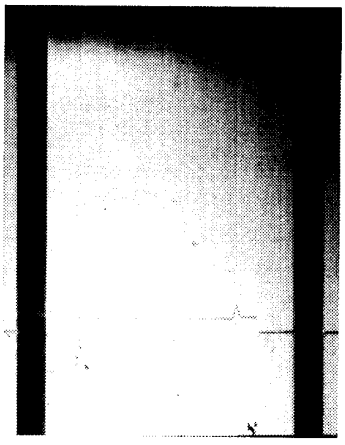

Fig. 1. Ultracentrifugal Pattern of Lycoris-R-glucomannan

0.25% in H_2O, 20°C, 48 min, 55400 rpm, Hitachi UCA-1A ultracentrifuge.

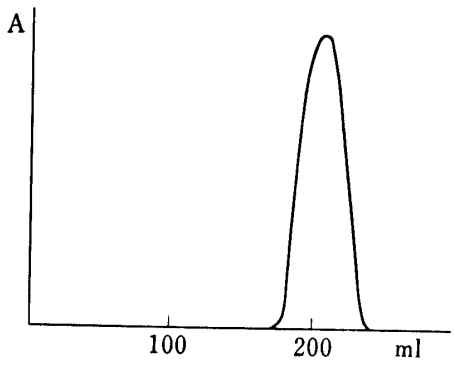

Fig. 2. Chromatogram of Lycoris-R-glucomannan on Sepharose CL-4B

polysaccharide was obtained from the eluate with water. The polysaccharide was homogeneous as determined by ultracentrifugal analysis (Fig. 1), and gave a single spot on glass-fiber paper electrophoresis in both a pyridine–acetic acid buffer and an alkaline borate buffer. Furthermore, it gave a single peak on gel chromatography with Sepharose CL-4B (Fig. 2).

The substance showed a negative specific rotation ($[\alpha]_D^{24}$ $-28.5°$ in H_2O, $c=0.4$), and its solution in water gave an intrinsic viscosity value of 4.7 at 30°C. The measurement of osmotic pressure gave a value of 468000 for the molecular weight of the polysaccharide. Mannose and glucose were identified as the component sugars by means of cellulose thin-layer chromatography (TLC) of the sulfuric acid hydrolysate and by gas–liquid chromatography (GLC) of the trimethylsilyl derivatives. Quantitative determination showed that the molar ratio of mannose; glucose was 12: 1. The name "Lycoris-R-glucomannan" is proposed for this compound.

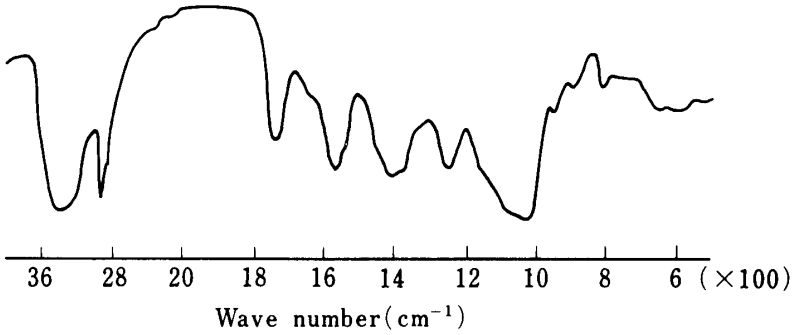

Fig. 3. IR Spectrum of Lycoris-R-glucomannan

As shown in Fig. 3, the infrared (IR) spectrum of the glucomannan has absorption bands at 1240 cm^{-1} and 1735 cm^{-1}, suggesting the presence of ester linkages, while the absorption at 890 cm^{-1} is due to β-glycosidic linkages. The proton magnetic resonance (^{1}H-NMR) spectrum showed acetyl signals at δ 1.88 and δ 2.16, and the acetyl content of the glucomannan was determined to be 15.5%.

In order to elucidate 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.[7] After conversion of the free hydroxyl groups into 1-methoxyethyl ethers, the derivative was deacetylated, then methylated with methyl iodide and siliver oxide in *N*,*N*-dimethylformamide.[8] The resulting product was hydrolyzed and analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) after conversion into alditol acetates.[9] A hexose methyl ether was detected and identified as 2,6-di-*O*-methyl-D-mannose. 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.[10] The fully methylated product was hydrolyzed and analyzed by GLC–MS after conversion into alditol acetates; 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 2-*O*-methyl-D-mannose were identified in a molar ratio of 6.0: 14.8: 2.2: 3.2. The identity of the tetra-*O*-methyl mannose was also confirmed by GLC of the methyl glycoside.

In order to remove the *O*-acetyl groups, the glucomannan was treated with dilute alkali solution, and after neutralization, the product was oxidized with periodate. In this periodate oxidation, 0.9 mol of periodate per mol of component anhydro sugar unit was consumed. The periodate-oxidized product was reduced,[11] hydrolyzed, and analyzed. The yields of mannose and erythritol were 13.0% and 35.3%, respectively.

On the other hand, the glucomannan was partially hydrolyzed with dilute sulfuric acid. The products were analyzed by TLC and by GLC of the trimethylsilylated derivatives. Com-

parison with authentic samples[12] showed the presence of D-mannose, D-glucose, *O*-β-D-mannopyranosyl-(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 relative yield ratios of these mono-, di-, and trisaccharides were 100: 7.2: 13.7: 0.7: 0.9: 7.9.

Based on these results, it can be concluded that the glucomannan is mainly composed of β-1→4 linked aldohexopyranose units and has some mannopyranose residues as terminals and branching points, linked in part through positions 3 and 6. The terminal mannopyranose units, the intermediate β-1→4 linked mannopyranose and glucopyranose units, and mannopyranose units at the branching points must be present in a molar ratio of 6: 15: 2: 3. From the value of acetyl content, it can be presumed that about nine out of every twenty-one terminal and intermediate mannose residues carry 2,6-di-*O*-acetyl groups.

Thus, Lycoris-R-glucomannan has a unique mode of branching, and also possesses an extraordinarily high ratio of mannose to glucose. Recently, we reported a highly acetylated glucomannan from the bulbs of *Narcissus tazetta* L. var. *chinensis* ROEMER.[13] This substance, named Narcissus-T-glucomannan, was composed of mannose and glucose in the molar ratio of 5: 1, and the *O*-acetyl groups in it were located at positions 2 and 2,6 of most of the mannose units. Lycoris-R-glucomannan is the second example of acetyl-rich glucomannans from the bulbs of plants in the Amaryllidaceae family.

Experimental

Solutions were concentrated at or below 40°C with rotary evaporators under reduced pressure. Optical rotation was measured with a JASCO DIP-SL automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. IR spectra were recorded on a JASCO IRA-2 infrared spectrophotometer. GLC was carried out on a Hitachi 063 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 spectrometer. ^{1}H-NMR spectra were recorded on a JEOL MH-100 NMR spectrometer in heavy water containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard at 70°C.

Isolation of Polysaccharide——The material was obtained in July 1981 from plants cultivated in Saitama prefecture. The fresh bulbs (330 g), which contained 71.9% water, were sliced, then extracted twice with water (1650 ml) under stirring at room temperature for 1 h each time. The extracts were combined and poured into two volumes of ethanol, then the mixture was filtered. The precipitate was treated with ethanol again, then dried *in vacuo* (yield, 1.68 g). A part of this crude mucilage (0.22 g) was dissolved in water and applied to a column (4×24 cm) of DEAE-cellulose (acetate form). The column was eluted with water, and fractions of 50 ml were collected and analyzed by the phenol–sulfuric acid method.[14] The eluates obtained from tubes 8 to 17 were combined, concentrated and lyophilized. Lycoris-R-glucomannan (0.13 g) was obtained as a white powder. It contains no nitrogen.

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF 83 glass-fiber papers in the manner described in a previous report[15] of this series, with the following buffers and conditions: A, 0.08 M pyridine–0.046 M acetic acid (pH 5.3) 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 40 min. The sample gave a single spot at distances of 7.0 cm (A) and 4.4 cm (B) from the center toward the cathode. Standard glucose moved to distances of 7.6 cm (A) and 5.3 cm (B).

Gel Chromatography——The sample (3 mg) was dissolved in water and applied to a column (2.6×94.5 cm) of Sepharose CL-4B. Elution was carried out by the ascending method with 0.02 M phosphate buffer (pH 7.8) as an eluant. Fractions were collected at 5 ml and analyzed by the phenol–sulfuric acid method.

Determination of Molecular Weight——The measurement of osmotic pressure was carried out with a Knauer electronic membrane osmometer in the same manner as in a previous report[16] of this series.

Qualitative and Quantitative Analyses of Component Sugars——These were carried out by the methods described in a previous report[17] of this series. The results revealed that the sample was composed of 79.2% mannose and 6.6% 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}: 1735, 1240 (ester), 890 (β-glycosidic linkage).

The sample (4 mg) was hydrolyzed with 1 N hydrochloric acid (0.2 ml) in a sealed tube at 100°C for 2 h, then neutralized with sodium hydroxide. Acetic acid in the hydrolysate was determined by a colorimetric method.[18]

1-Methoxyethylation of Polysaccharide followed by Deacetylation——These procedures were carried out by the methods described in a previous report[13] of this series.

Methylation of the *O*-(1-Methoxyethyl) Derivative——This was also carried out in the manner described in a previous report[13] of this series.

Analysis of the *O*-Methyl Derivative——The product (20 mg) was hydrolyzed with 88% formic acid (4 ml) in a sealed tube at 90°C for 16 h. After removal of the acid by evaporation, the residue was dissolved in 1 M trifluoroacetic acid (4 ml) and the solution was heated in a sealed tube at 100°C for 5 h. After removal of the acid by evaporation, the hydrolysate was dissolved in water (50 ml) and reduced with sodium borohydride (100 mg) at 5°C overnight. After treatment with Dowex 50W-X8 (H^+) up to pH 5, the filtrate was concentrated and boric acid was removed by repeated addition and evaporation of methanol. The product was acetylated with acetic anhydride–pyridine mixture (1: 1) at 100°C for 1 h. After concentration of the solution, the residue was dissolved in chloroform–methanol mixture (1: 1) and subjected to GLC. GLC was carried out under condition A, using a column (0.3 cm × 2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 180°C with a nitrogen flow of 30 ml per min. GLC–MS was carried out under the same conditions, but with helium as a carrier gas. The relative retention time of the product

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
1,4,5-Ac-2,3,6-Me-D-Mannitol	1.91	43, 45, 87, 99, 101, 113, 117, 233
1,4,5-Ac-2,3,6-Me-D-Glucitol	2.15	43, 45, 87, 99, 101, 113, 117, 233
1,3,4,5-Ac-2,6-Me-D-Mannitol	2.76	43, 45, 87, 117, 129
1,3,4,5,6-Ac-2-Me-D-Mannitol	5.12	43, 117, 139

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-).

Table II. *Rf* Values and Retention Times (min) of Trimethylsilyl Derivatives of Standard Sugars and Partial Hydrolysis Products

Standard and products	Cellulose TLC (*Rf*) Solvent A	Cellulose TLC (*Rf*) Solvent B	GLC (t_R) under condition B
Man	0.69	0.57	3.6,[a] 4.6
Glc	0.62	0.50	4.9,[a] 6.1
β-Man-(1→4)-Man	0.55	0.36	22.3,[a] 24.2
β-Man-(1→4)-Glc	0.47	0.27	23.0, 24.5[a]
β-Man-(1→4)-β-Glc-(1→4)-Man	0.35	0.20	37.8, 38.3[a]
β-Man-(1→4)-β-Man-(1→4)-Man	0.28	0.17	39.1,[a] 40.7
β-Man-(1→4)-β-Man-(1→4)-β-Man-(1→4)-Man	0.09	0.05	—
Partial hydrolysis products	0.69	0.57	3.6
	0.62	0.50	4.6
	0.55	0.36	4.9
	0.47	0.27	6.1
	0.35	0.17	22.3
	0.28	0.05	23.0
	0.09		24.2
			37.8
			38.3
			39.1
			40.7

Man=D-mannopyranose; Glc=D-glucopyranose.
Solvent A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3) at 23°C.
Solvent B, BuOH: pyridine: H_2O (6: 4: 3) at 28°C.
Condition B: a column (0.3 cm × 2 m spiral glass) packed with 2% OV 101 on Uniport HP (80 to 100 mesh) and with a programmed temperature increase of 3°C per min from 180°C to 300°C at a nitrogen flow rate of 30 ml per min.
a) Main peaks.

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 Polysaccharide and Analysis of the Products——Methylation was carried out in the manner described in a previous report[13] of this series. The methylation reaction was repeated four times. The product was hydrolyzed with dilute sulfuric acid in acetic acid and neutralized with Dowex 2 (OH^-) in the manner described in a previous report[19] of this series. The hydrolysate was reduced, acetylated, and analyzed 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 the mass spectra are also listed in Table I.

Deacetylation of Polysaccharide followed by Periodate Oxidation——The sample (25 mg) was dissolved in water (2 ml), then 0.02 N sodium hydroxide (2 ml) was added. After standing at room temperature for 10 min, the solution was neutralized with 0.1 N acetic acid and the total volume was adjusted to 5 ml with water. After addition of 0.1 M sodium metaperiodate (5 ml), the reaction mixture was kept at 5°C in the dark. The periodate consumption was measured by a spectrophotometric method.[20] The oxidation was completed after four days.

Smith Degradation and Analysis of Products——These procedures were carried out in the manner and under the conditions described in a previous report[17] of this series.

Partial Acid Hydrolysis and Analysis of Degradation Products——The sample (10 mg) was hydrolyzed with 0.5 N sulfuric acid (1 ml) at 90°C for 2 h. After neutralization of the mixture with barium carbonate, the products were subjected to cellulose TLC and identified by comparison with authentic samples in the manner described in a previous report.[21] In addition, the products were trimethylsilylated in the usual way,[22] then subjected to GLC under the same conditions as in a previous report[19] of this series. The relative yields of the products were evaluated by GLC. *Rf* values and retention times of the products are shown in Table II.

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