[Chem. Pharm. Bull. 31(11)3878—3882(1983)]

Plant Mucilages. XXXIII.[1] An Acetyl-Rich Mucilage, "Lycoris-S-glucomannan," from the Bulbs of *Lycoris squamigera*

MASASHI TOMODA,* NORIKO SHIMIZU, KAZUYO SHIMADA, TAKAE ISHII, and MOTOKO OGAWA

Kyoritsu College of Pharmacy, Shibakōen, Minato-ku, Tokyo 105, Japan

(Received March 4, 1983)

A mucilage, named Lycoris-S-glucomannan, was isolated from the bulbs of *Lycoris squamigera* MAXIM. The final preparation was homogeneous as determined by ultracentrifugal analysis, glass-fiber electrophoresis, and gel chromatography. It was mainly composed of D-mannose and D-glucose in the molar ratio of 7:2, and its molecular weight was estimated to be about 1800000. *O*-Acetyl groups were identified in the glucomannan and their content amounted to 16.7%. They were located at positions 2, 6 of about half 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 twenty aldohexose units per three non-reducing groups on average. D-Mannose units occupy all branching points linked through position 3, while both D-mannose and D-glucose units occupy non-reducing terminal positions.

Keywords——mucilage; Lycoris-S-glucomannan; *Lycoris squamigera*; intrinsic viscosity; molecular weight; analysis of component; 2,6-di-*O*-acetyl group; structure

The bulbs of *Lycoris squamigera* MAXIM. were used as a source of galanthamine.[2] In addition, the isolation of various alkaloids has been reported.[3-5] The bulbs of this plant also contain many mucous polysaccharides, but no structural study on the mucilages has been reported so far. The present paper is concerned with the isolation and the structural analysis of a pure mucilage from the fresh bulbs of this plant.

The bulbs were sliced and treated with hot methanol, then the residue was extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol. The crude mucilage obtained was applied to a column of diethylaminoethyl (DEAE)-cellulose (acetate form), and a mucous polysaccharide was obtained from the eluate with water. The polysaccharide gave a single spot on glass-fiber paper electrophoresis in both a pyridine–acetic acid buffer and an alkaline borate buffer, and was homogeneous as determined by ultracentrifugal analysis (Fig. 1). Furthermore, it gave a single peak on gel chromatography with Sephacryl S-400 (Fig. 2).

The substance was readily soluble in water and it showed a negative specific rotation ($[\alpha]_D^{22}$ −23.1° in H_2O, $c=1.0$). Its aqueous solution gave an intrinsic viscosity value of 3.9 at 30 °C. Gel chromatography gave a value of approximately 1800000 for the molecular weight. Mannose and glucose were identified as the component sugars by cellulose thin-layer chromatography (TLC) of the hydrolysate and by gas-liquid chromatography (GLC) of the trimethylsilyl derivatives. Quantitative determination showed that the molar ratio of mannose:glucose was 7:2. The name "Lycoris-S-glucomannan" is proposed for this compound.

The glucomannan was methylated with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[6] The fully methylated product was hydrolyzed and analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates;[7]

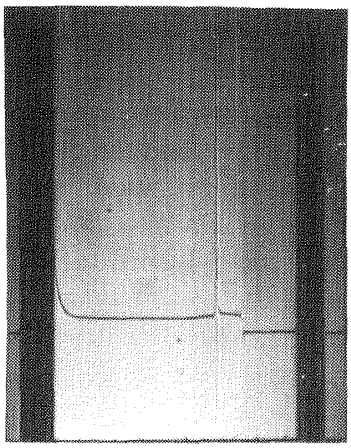

Fig. 1. Ultracentrifugal Pattern of Lycoris-S-glucomannan

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

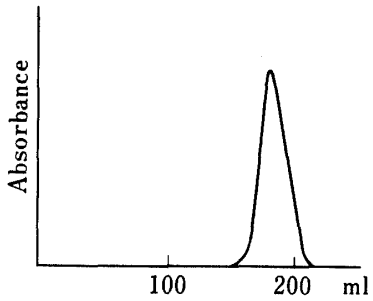

Fig. 2. Chromatogram of Lycoris-S-glucomannan on Sephacryl S-400

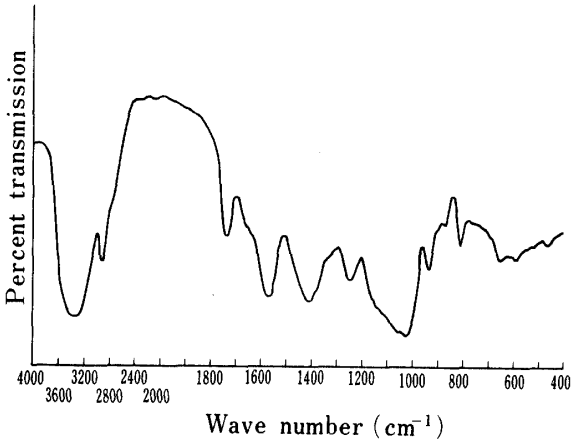

Fig. 3. IR Spectrum of Lycoris-S-glucomannan

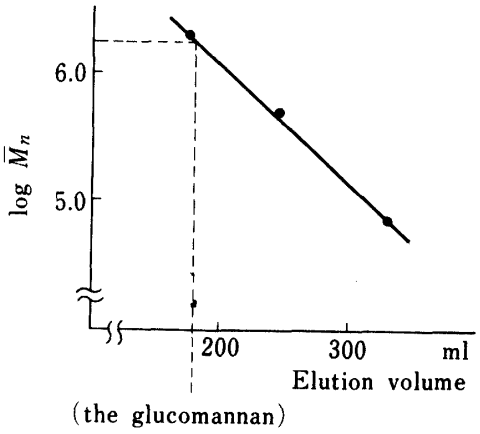

Fig. 4. Plot of Elution Volume against log $\bar{M}_n$ for Dextran Fractions on Sephacryl S-400

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, and 2,6-di-*O*-methyl-D-mannose were identified in a molar ratio of 2.0 : 1.0 : 10.5 : 3.5 : 2.9. 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.

Furthermore, the glucomannan was partially hydrolyzed with dilute sulfuric acid. The products were analyzed by TLC and by GLC of the trimethylsilylated derivatives. Comparison with authentic samples[8] showed the presence of D-mannose, D-glucose, *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-mannopyranose, and *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose.

As shown in Fig. 3, the infrared (IR) spectrum of the glucomannan has absorption bands at 1240 and 1735 cm^{-1}, suggesting the presence of ester linkages, while the absorption at 870 cm^{-1} is due to β-glycosidic linkages. The proton magnetic resonance (^{1}H-NMR) spectrum showed acetyl signals at δ 1.89 and δ 2.17, and the acetyl content of the glucomannan was determined to be 16.7%. In addition, the glucomannan contains a small quantity of protein. The determination of protein content was carried out by the method of Lowry *et al.*,[9]

and a value of 2.5% was obtained.

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.[10] After conversion of the free hydroxyl groups into 1-methoxyethyl ethers, the derivative was de-*O*-acetylated, then methylated as described above, and the methylation was completed with methyl iodide and silver oxide in *N,N*-dimethylformamide.[11] The resulting product was hydrolyzed and analyzed by GLC-MS after conversion into alditol acetates. 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 treated with dilute alkali solution, and after neutralization, the resulting de-*O*-acetylated product was oxidized with periodate. As a result of the reaction, 0.94 mol of periodate per mol of component anhydrohexose unit was consumed with liberation of 0.12 mol of formic acid. The periodate-oxidized product was reduced,[12] hydrolyzed, and analyzed. The yields of mannose and erythritol were 12.4 and 36.4%, respectively.

Based on these results, it can be concluded that the glucomannan is mainly composed of β-1→4 linked aldohexopyranose units and has both mannopyranose and glucopyranose residues as terminals, and that it has branches at position 3 of some mannopyranose units. The terminal mannopyranose and glucopyranose 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 approximately 4 : 2 : 21 : 7 : 6. Upon partial hydrolysis, β-1→4 linked mannobiose and mannotriose were major oligosaccharides in the products, while no cellobiose was detected as a product. Therefore it is highly probable that the presence of D-glucose residues is discontinuous in the polysaccharide moiety. From the value of acetyl content, it can be presumed that about half of the mannose residues carry 2,6-di-*O*-acetyl groups.

Recently, Tomoda *et al.* reported two highly *O*-acetylated glucomannans from plants in the Amaryllidaceae family, namely, the bulbs of *Narcissus tazetta* L. var. *chinensis* ROEMER[13] and *Lycoris radiata* HERBERT.[14] Lycoris-S-glucomannan is the third example of acetyl-rich glucomannans from the bulbs of plants in the Amaryllidaceae family. This substance has both mannopyranose units and glucopyranose units as terminals, and contains a small amount of protein. In addition, the glucose content in it was relatively high, while the molar ratios of mannose and glucose were 5 : 1 in Narcissus-T-glucomannan[13] and 12 : 1 in Lycoris-R-glucomannan.[14] However, the latter two glucomannans contain no protein, and they have no glucose residue terminals. It is interesting that these three acetyl-rich glucomannans possess 2,6-di-*O*-acetyl-D-mannose residues in common, although Narcissus-T-glucomannan[13] has about half 6-*O*-acetyl-D-mannose units in addition. Further studies on the mucilages from other plants in the Amaryllidaceae family are in progress.

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 Mucilage——The material was obtained in September 1982 from plants cultivated in Saitama prefecture. The fresh bulbs (175 g), which contained 61.6% water, were sliced, then extracted with hot methanol (525 ml) for 30 min. After suction filtration, the residue was homogenized and extracted twice with water (525 ml each) under stirring at room temperature for 1 h each time. The extracts were combined and poured into two volumes

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	1.91	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

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-).

of ethanol, then the mixture was filtered. The precipitate was treated with ethanol again, then dried *in vacuo* (yield, 6.35 g). A part of this crude mucilage (1.0 g) was dissolved in water and applied to a column (4.8 × 43 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.[15] The eluates obtained from tubes 36 to 68 were combined, concentrated and lyophilized. Lycoris-S-glucomannan (0.23 g) was obtained as a white powder.

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF 83 glass-fiber papers in the manner described in a previous report[16] 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 2.4 cm (A) and 7.2 cm (B) from the center toward the cathode. Standard glucose moved to distances of 2.6 cm (A) and 8.3 cm (B).

Gel Chromatography——The sample (3 mg) 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 and gave the calibration curve shown in Fig. 4.

Qualitative and Quantitative Analyses of Component Sugars——These were carried out by the methods described in a previous report[17] of this series. Sugars were also determined by the chromotropic acid method.[18] The results revealed that the sample was composed of 62.3% mannose and 17.8% glucose in addition to acetyl groups and protein.

Determination of *O*-Acetyl Groups——The IR spectrum of the glucomannan showed ester absorption bands. IR ν_{max}^{KBr} cm^{-1}: 1735, 1240 (ester), 870 (β-glycosidic linkage).

The sample (3 mg) was hydrolyzed with 1 N hydrochloric acid (0.05 ml) in a sealed tube at 100 °C for 2 h. The hydrolysate was directly applied to GLC, and propionic acid was used as an internal standard. GLC was carried out under condition A, using a column (0.3 × 200 cm long spiral glass) packed with 5% Thermon-1000–0.5% phosphoric acid on Chromosorb W (80 to 100 mesh) at 100 °C with a flow of 46 ml per min of nitrogen; t_R (min), acetic acid 4.0; propionic acid (internal standard) 5.8.

Methylation of Glucomannan 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 by GLC and GLC-MS. GLC was carried out under condition B, using a column (0.3 × 200 cm 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 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 listed in Table I.

A part of the methylation product (6 mg) was methanolyzed with 4% methanolic hydrogen chloride (3 ml) in a sealed tube at 80 °C for 16 h. After removal of hydrogen chloride by evaporation, the product was subjected to GLC under condition C, using a column (0.3 × 200 cm stainless steel) packed with 15% poly-butane 1,4-diol succinate on Chromosorb W (80 to 100 mesh) at 175 °C with a nitrogen flow of 20 ml per min. The relative retention times of the resulting methyl glycosides with respect to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside were 1.00, 1.37 for methyl 2,3,4,6-tetra-*O*-methyl-D-glucoside and 1.32, 1.86 for methyl 2,3,4,6-tetra-*O*-methyl-D-mannoside. The separation of methyl glycosides of tri-*O*-methyl and di-*O*-methyl hexoses in the products was not clear.

Partial Acid Hydrolysis and Analysis of Degradation Products——The sample (5 mg) was hydrolyzed with 0.5 N sulfuric acid (1 ml) at 90 °C for 3 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.[20] In addition, the products were trimethylsilylated in the usual way,[21] then subjected to GLC under the same conditions as in a previous report[19] of this series. The relative yields of mannose, glucose, mannosyl mannose, mannosyl glucose, mannosyl glucosyl mannose, and mannosyl mannosyl mannose were 63.3 : 16.1 : 11.4 : 1.5 : 1.0 : 6.6. *Rf* values and retention times of the products were the same as listed in a previous report[14] of this series.

1-Methoxyethylation of Glucomannan Followed by De-*O*-acetylation——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 (4.5 ml) in a sealed tube at 90 °C for 16 h. After removal of the acid by evaporation, the residue was dissolved in 0.5 N sulfuric acid (4.5 ml) and the solution was heated in a sealed tube at 100 °C for 3 h. After neutralization with Dowex 2 (OH^-), the hydrolysate was reduced, acetylated, and analyzed as described above. 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 MS are also listed in Table I.

De-*O*-acetylation of Glucomannan Followed by Periodate Oxidation——The sample (30.5 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 M acetic acid and the total volume was adjusted to 6 ml with water. After addition of 0.1 M sodium metaperiodate (6 ml), the reaction mixture was kept at 3 °C in the dark. The periodate consumption was measured by a spectrophotometric method.[22] The oxidation was completed after six 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.

Acknowledgement We are grateful to Prof. G. Matsumura, School of Pharmaceutical Sciences, Showa University, for ultracentrifugal analysis.

References and Notes

1) Part XXXII: M. Tomoda, K. Shimada, and N. Shimizu, *Chem. Pharm. Bull.*, **31**, 2677 (1983).
2) Shionogi & Co., Ltd. (by K. Kimura, S. Uyeo, and Y. Yamamoto), Japan. Patent 485 (1963) [*Chem. Abstr.*, **59**, P3094*e* (1963)].
3) K. Yamaguchi, H. Ogawa, and S. Natori, *Eisei Shikenjo Hokoku*, No. 80, 17 (1962).
4) S.-H. Hung and K.-E. Ma, *Yao Hsueh Hsueh Pao*, **11**, 1 (1964) [*Chem. Abstr.*, **61**, 3154*d* (1964)].
5) C.-C. Yuan, S.-L Liu, K. Yao, and K.-M. Hu, *Chung Ts'ao Yao*, **12**, 39 (1981) [*Chem. Abstr.*, **95**, 156434*s* (1981)].
6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
7) H. Björndal, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).
8) M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull.*, **21**, 2511 (1973).
9) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
10) A. N. DeBelder and B. Norrman, *Carbohydr. Res.*, **8**, 1 (1968).
11) R. Kuhn, H. Trischman, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
12) M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, **74**, 4970 (1952).
13) M. Tomoda, M. Yokoi, A. Torigoe, and K. Maru, *Chem. Pharm. Bull.*, **28**, 3251 (1980).
14) M. Tomoda and N. Shimizu, *Chem. Pharm. Bull.*, **30**, 3965 (1982).
15) J. E. Hodge and B. T. Hofreiter, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York and London, 1962, pp. 388—389.
16) M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. Pharm. Bull.*, **19**, 2173 (1971).
17) M. Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.*, **26**, 2768 (1978).
18) B. Klein and M. Weissman, *Anal. Chem.*, **25**, 771 (1953).
19) M. Tomoda, K. Shimada, Y. Saito, and M. Sugi, *Chem. Pharm. Bull.*, **28**, 2933 (1980).
20) M. Tomoda and S. Kimura, *Chem. Pharm. Bull.*, **24**, 1807 (1976).
21) C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
22) *a*) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954); *b*) G. O. Aspinall and R. J. Ferrier, *Chem. Ind.* (London), **1957**, 1216.