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Plant Mucilages. XXVII.[1] Isolation and Characterization of a Mucous Polysaccharide, "Narcissus-T-glucomannan," from the Bulbs of *Narcissus tazetta* var. *chinensis*

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A mucous polysaccharide, named Narcissus-T-glucomannan, was isolated from the bulbs of *Narcissus tazetta* L. var. *chinensis* Roemer. 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: 1, and its molecular weight was estimated to be 119000. The *O*-acetyl groups in the glucomannan was identified and the content amounted to 22.7%. They were located at positions 6 and 2, 6 of most of the D-mannose units. Methylation, periodate oxidation, partial acetolysis, and enzymatic degradation studies showed that the glucomannan is mainly composed of β-1→4 linked aldohexopyranose residues, and that it contains about 42 aldohexose units per non-reducing group on average. D-Mannose units occupy non-reducing terminal positions and branching points linked through position 3.

Keywords——Narcissus-T-glucomannan; *Narcissus tazetta* var. *chinensis*; native polysaccharide; intrinsic viscosity; molecular weight; analysis of components; location of *O*-acetyl groups; structure of main chain; branching points

Narcissus tazetta L. var. *chinensis* Roemer provides an ornamental flower. The bulb of this plant has been used as a crude drug to treat tumors of the breast. In 1931, Kihara[3] reported the isolation of a water-soluble polysaccharide, called Suisen-glucomannan, from the bulbs. He reported the approximate molar ratio of mannose and glucose in the polysaccharide as 2: 1, but the homogeneity of the polymer was not established. More recently, Kato *et al.*[4] reported that the polysaccharide purified by a copper complex method was composed of mannose and glucose in the molar ratio of 3: 2, and suggested that the majority of hexose units were linked together by β-1→4 glycosidic bonds to form long chains. They stated that the polysaccharide became insoluble in water after isolation by the copper complex method. Treatment with an alkaline solution readily causes *O*-deacetylation, and it is unsuitable for the isolation of native polysaccharides having *O*-acetyl groups, as has already been pointed out by several investigators.[5-7] We have now obtained a native pure mucous polysaccharide from the fresh bulbs of this plant, and its properties and structural features are described in the present paper.

The bulbs were crushed and extracted with cold water after treatment with hot methanol. 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 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

1) Part XXVI: M. Tomoda, K. Shimada, Y. Saito, and M. Sugi, *Chem. Pharm. Bull.*, **28**, 2933 (1980).
2) Location: *1-5-30, Shibakōen, Minato-ku, Tokyo, 105, Japan.*
3) Y. Kihara, *Nippon Nogeikagaku Kaishi*, **7**, 1061 (1931).
4) K. Kato, Y. Kawaguchi, and T. Mizuno, *Carbohydr. Res.*, **29**, 469 (1973).
5) A. Misaki, T. Ito, and T. Harada, *Agric. Biol. Chem.*, **36**, 761 (1972).
6) T. Matsuo and T. Mizuno, *Agric. Biol. Chem.*, **38**, 465 (1974).
7) M. Tomoda, S. Kaneko, and S. Nakatsuka, *Chem. Pharm. Bull.*, **23**, 430 (1975).

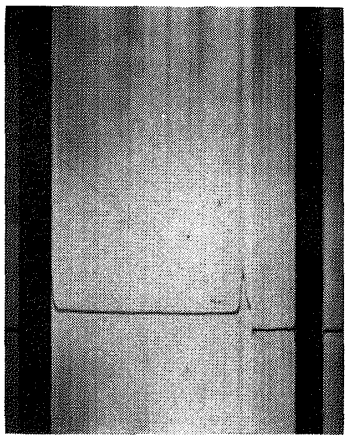

Fig. 1. Ultracentrifugal Pattern of Narcissus-T-glucomannan

0.5% in H_2O, 20°, 24 min, 60000 rpm, Hitachi UCA-1A ultracentrifuge.

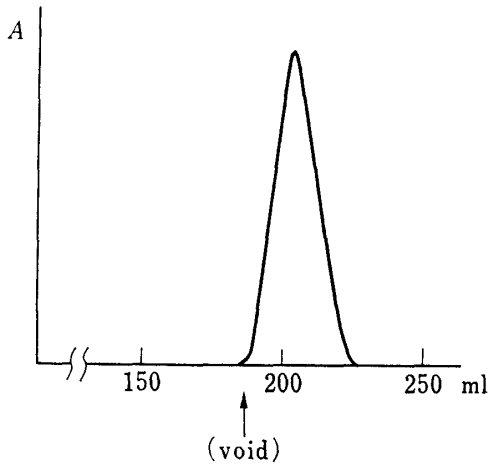

Fig. 2. Chromatogram of Narcissus-T-glucomannan on Sephacryl S-200

and an alkaline borate buffer. Furthermore, it gave a single peak on gel chromatography with Sephacryl S-200 (Fig. 2).

The substance was readily soluble in water and it showed a negative specific rotation ($[\alpha]_D^{20}$ −24.3° in H_2O, $c=1.0$). Its solution in water gave an intrinsic viscosity value of 2.6 at 30°. Gel chromatography gave a value of approximately 119000 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 5: 1. The name "Narcissus-T-glucomannan" is proposed for this compound.

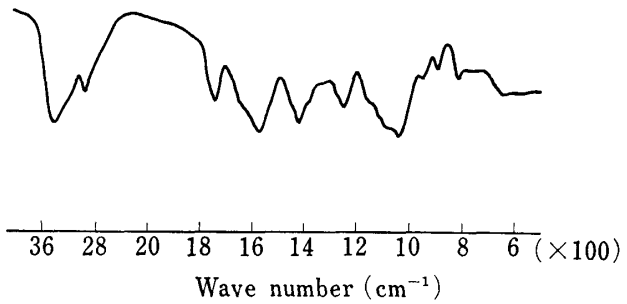

Fig. 3. IR Spectrum of Narcissus-T-glucomannan

As shown in Fig. 3, the infrared (IR) spectrum of the glucomannan has absorption bands at 1250 cm^{-1} and 1740 cm^{-1}, suggesting the presence of ester linkages in addition to the absorption of 890 cm^{-1}, which is due to β-glycosidic linkages. The proton magnetic resonance (^{1}H-NMR) spectrum showed an acetyl signal at δ 1.92, and the acetyl content of the glucomannan was determined to be 22.7%.

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.[8] After conversion of the free hydroxyl groups into 1-methoxyethyl ethers, the derivative was deacetylated, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide.[9] The resulting product was hydrolyzed and analyzed by GLC and gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates.[10] Two hexose methyl ethers were detected and identified as 6-*O*-methyl-D-mannose and 2,6-di-*O*-methyl-D-mannose in a molar ratio of 1.0: 2.0. In addition, free D-mannose and D-glucose were detected

8) A.N. DeBelder and B. Norrman, *Carbohydr. Res.*, **8**, 1 (1968).
9) R. Kuhn, H. Trischman, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
10) H. Björndall, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).

in the hydrolysate in a molar ratio of 1.0: 1.1. These results indicate that many residues of 6-*O*-acetyl-D-mannose and 2,6-di-*O*-acetyl-D-mannose are present in the glucomannan.

The glucomannan was methylated with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide.[11] 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 2,6-di-*O*-methyl-D-mannose were identified in a molar ratio of 1.3: 30.7: 6.7: 1.0. The identity of the tetra-*O*-methyl mannose was also confirmed by GLC of methyl glycoside.

In order to avoid the blocking effect of *O*-acetyl groups, the original glucomannan was treated with dilute alkali solution, and the water-insoluble deacetylated polysaccharide thus obtained was oxidized with periodate under stirring. In this periodate oxidation, 1.06 mol of periodate per mol of component anhydro sugar unit was consumed with liberation of 0.05 mol of formic acid. The periodate-oxidized product was reduced,[12] hydrolyzed, and analyzed. The yields of mannose and erythritol were 2.4% and 68.7%.

On the other hand, the glucomannan was peracetylated with acetic anhydride and pyridine in formamide, then partially degraded with sulfuric acid in acetic anhydride. After deacetylation, the products were analyzed by TLC and by GLC of trimethylsilylated derivatives. Comparison with authentic samples[13] 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-mannopyranosyl-(1→4)-D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(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 16.6: 7.8: 45.8: 5.1: 20.3: 4.4.

In addition, the glucomannan was treated with a β-D-mannanase obtained from Driselase (Kyowa Hakko Kogyo Co.) prepared from culture solutions of *Irpex lacteus*. The products were analyzed as described above, and the results showed the presence of D-mannose, D-glucose, β-D-1→4-linked mannobiose, β-D-1→4-linked glucosyl mannose, β-D-1→4-linked mannotriose, and β-D-1→4-linked mannosyl glucosyl mannose in relative yield ratios of 4.9: 6.0: 59.1: 6.6: 22.4: 1.0. The appearance of free glucose is probably due to the coexistence of a β-D-glucanase activity in the enzyme preparation.

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 position 3. The average chain length of the polysaccharide was determined by methylation analysis and Smith degradation to be about 42.

In the results of both acetolysis and enzymatic degradation, β-D-1→4-linked mannobiose and mannotriose were major products, while no cellobiose was detected as a product in these treatments. Therefore it is possible that the presence of D-glucose residues is discontinuous in the polysaccharide.

An important characteristic of Narcissus-T-glucomannan is its fairly high acetyl content. On the basis of the content and the location of *O*-acetyl groups, we concluded that the molar ratio of D-mannose, 6-*O*-acetyl-D-mannose, and 2,6-di-*O*-acetyl-D-mannose residues was 2.0: 3.0: 6.0 in the glucomannan. This conclusion was also supported by the fact that free D-mannose and D-glucose were detected in a molar ratio of 1.0: 1.1 in the hydrolysate of the product obtained after treatment of the glucomannan by the method of DeBelder and Norrman.[8]

The nature of the relationship between the presence of many *O*-acetyl groups and the high water-solubility is still unclear. Further studies are in progress.

11) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
12) M. Abdel-Akher, J.K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, **74**, 4970 (1952).
13) M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull.*, **21**, 2511 (1973).

Experimental

Solutions were concentrated at or below 40° in rotary evaporators under reduced pressure. Optical rotation was measured with a JASCO DIP-SL automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. Infrared (IR) spectra were recorded on a Hitachi EPI-G3 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. Nuclear magnetic resonance (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°.

Isolation of Polysaccharide——The material was obtained in September 1979 from plants cultivated in Saitama prefecture. The fresh bulbs (429 g), which contained 72.0% water, were crushed, then extracted twice with hot methanol (1300 ml) for 30 min each time. After suction filtration, the residue was extracted twice with water (1300 ml) under stirring at room temperature for 1 hr each time. The extracts were combined and poured into two volumes of ethanol, then filtered. The precipitate was treated with ethanol again, then dried *in vacuo* (yield, 7.72 g). A part of this crude mucilage (1 g) was dissolved in water and applied to a column (4.2 × 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 7 to 22 were combined, concentrated and lyophilized. Narcissus-T-glucomannan (0.79 g) was obtained as a white powder. The glucomannan was readily soluble, and its solubility in water at 20° was more than 14%. This value represents the limit of measurement because of the high viscosity of the resulting solution. The IR spectrum of the glucomannan showed ester absorption bands. IR ν_{max}^{KBr} cm^{-1}: 1740, 1250 (ester), 890 (β-glycosidic linkage).

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF 83 glass-fiber 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 volts for 90 min; B, 0.025 M borax: 0.1 N sodium hydroxide (10: 1, pH 9.3) at 570 volts for 60 min. The sample gave a single spot at distances of 7.1 cm (A) and 9.3 cm (B) from the center toward the cathode. Standard glucose moved to distances of 8.2 cm (A) and 10.7 cm (B).

Gel Chromatography——This was carried out in the manner described in a previous report[16] of this series using a column (2.6 × 95 cm) of Sephacryl S-200 superfine. Standard dextrans having known molecular weights were run on the column and gave the calibration curve shown in Fig. 4.

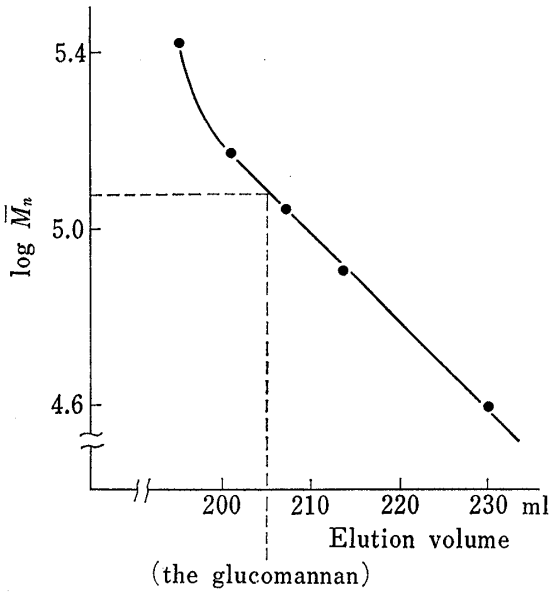

Fig. 4. Plot of Elution Volume against log $\bar{M}_n$ for Dextran Fractions on Sephacryl S-200

Qualitative and Quantitative Analyses of Component Sugars——These were carried out by the methods described in a previous report[16] of this series. The results revealed that the sample was composed of 63.9% mannose and 12.6% glucose in addition to acetyl groups.

Determination of Acetyl Groups——The sample was hydrolyzed with 1 N hydrochloric acid in a sealed tube at 100° for 2 hr, then neutralized with sodium hydroxide. Acetic acid in the hydrolysate was determined by a colorimetric method.[17]

Treatment with Methyl Vinyl Ether——The sample (93 mg) was suspended in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (20 mg) was added. The mixture was stirred at 15°, then methyl vinyl ether (5 ml), condensed at −10°, was added in portions under stirring. The reaction mixture was stirred at 15° for 4 hr, then dialyzed against running water overnight. The non-dialyzable fraction was concentrated to dryness, and the reaction procedure was repeated three times. The final solution was applied to a column (3.1 × 25 cm) of Sephadex LH-20. The column was eluted with acetone, and fractions of 10 ml were

14) 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.
15) M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. Pharm. Bull.*, **19**, 2173 (1971).
16) M. Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.*, **26**, 2768 (1978).
17) Y. Kasai, T. Tanimura, and Z. Tamura, *Anal. Chem.*, **47**, 34 (1975).

collected. The eluates in tubes 10 to 17 were combined and concentrated. The IR spectrum of the final product had no absorption near 3400 cm^{-1}.

Deacetylation of the *O*-Acetyl-*O*-(1-methoxyethyl) Derivative——The product was dissolved in methanol (15 ml), then 0.2 M methanolic sodium methoxide (15 ml) was added under stirring. The solution was refluxed at 75° for 4 hr, then concentrated and applied to a column (4.2 × 30 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions of 10 ml were collected, and the eluates obtained from tubes 14 to 24 were combined and concentrated. The absence of ester absorption bands in the IR spectrum of the residue confirmed that deacetylation was complete.

Methylation of the *O*-(1-Methoxyethyl) Derivative——The product was dissolved in *N*,*N*-dimethylformamide (5 ml), then methyl iodide (2 ml) and silver oxide (0.4 g) were added successively under stirring. The reaction mixture was stirred at room temperature for 20 hr in the dark. After filtration and washing with chloroform, the filtrate and washing were combined and concentrated. Methyl iodide (2 ml) and silver oxide (0.4 g) were added again to the residual solution, and the reaction procedure was repeated six times. The final reaction mixture was filtered and washed with chloroform (4 ml). The filtrate and washing were combined, then water (20 ml) and 10% potassium cyanide (4 ml) were added to the solution. The mixture was extracted with chloroform (10 ml each) five times. The extracts were combined and washed with water (50 ml each) five times, then dried over sodium sulfate and the filtrate was concentrated. The final solution was applied to a column (2 × 20 cm) of Sephadex LH-20. The column was eluted with chloroform: methanol (2: 1) mixture, and fractions of 3 ml were collected. The eluates in tubes 9 to 15 were combined and concentrated to dryness. The final residue (98 mg) was a reddish-yellow syrup. Its IR spectrum showed no absorption near 3400 cm^{-1}.

Analysis of the *O*-Methyl Derivative——The product was hydrolyzed with dilute sulfuric acid in acetic acid in the manner described in the preceding report[1] of this series. After neutralization with Dowex 2 (OH^-), the hydrolysate was reduced with sodium borohydride and then acetylated with acetic anhydride–pyridine mixture. GLC and GLC–MS of the partially methylated alditol acetates obtained were carried out under the same conditions as in the preceding report.[1] 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. GLC of the alditol acetates of free component sugars was carried out under the same conditions as in a previous report.[16]

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

	Relative retention times[a]	Main fragments (*m/e*)
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
1,2,3,4,5-Ac-6-Me-D-Mannitol	3.59	43, 45, 87, 115, 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-).

Methylation of Polysaccharide——The sample (20 mg) was dissolved in dimethyl sulfoxide (5 ml). Sodium hydride (35 mg) was mixed with dimethyl sulfoxide (7 ml) in an ultrasonic bath for 30 min, followed by stirring at 70° for 1 hr, then the mixture was added to the sample solution. The reaction mixture was stirred at room temperature for 4 hr, then methyl iodide (8 ml) was added and the whole was stirred overnight at room temperature. All procedures were carried out under nitrogen. The reaction mixture was then dialyzed against running water for two days. The non-dialyzable fraction was concentrated to dryness. The product was methylated once more under the same conditions. After addition of water (45 ml), the reaction mixture was extracted five times with chloroform (45 ml each). The combined extract was washed five times with water (225 ml each), then dried over sodium sulfate, and the filtrate was concentrated to dryness. The residue was methylated once more under the same conditions. The final residue was dissolved in chloroform–methanol mixture (2: 1), then applied to a column (2 × 20 cm) of Sephadex LH-20. The column was eluted with the same solvent, and fractions of 3 ml were collected. The eluates obtained from tubes 7 to 14 were combined and concentrated to dryness. The final product (22 mg) was a yellow powder. Its IR spectrum showed no absorption near 3400 cm^{-1}.

Analysis of the Methylated Product——A part of the product was hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated as described above. GLC–MS was carried out under the same conditions as in the preceding report.[1] 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.

In addition, a part of the product was methanolyzed and GLC of the methyl glycosides of the partially methylated hexoses was carried out under the same conditions as in a previous report.[16]

Deacetylation of Polysaccharide followed by Periodate Oxidation——The sample (50 mg) was dissolved in water (5 ml), then 0.2 N sodium hydroxide was added. After standing at room temperature for 10 min, the solution was poured into 0.1 N ethanolic hydrochloric acid. The precipitate was separated, washed with ethanol, and lyophilized. The deacetylated polysaccharide obtained was insoluble in water. The absence of ester absorption bands in its IR spectrum confirmed that deacetylation was complete. The deacetylated polysaccharide (26 mg) was oxidized with 0.05 M sodium metaperiodate (13 ml) at room temperature under stirring in the dark. The periodate consumption was measured by a spectrophotometric method.[18] The oxidation was completed after seven days. The formic acid liberation was measured by titration with 0.01 N sodium hydroxide after addition of ethylene glycol.

Smith Degradation and Analysis of Products——The residue of the reaction mixture (11 ml) was successively treated with ethylene glycol (0.1 ml) and sodium borohydride (100 mg) at 5° for 16 hr, then adjusted to pH 5 by addition of acetic acid. The solution was dialyzed against running water for two days. The non-dialyzable fraction was concentrated and applied to a column (5 × 79 cm) of Sephadex G-15. Fractions of 20 ml were collected, and the eluates obtained from tubes 27 to 30 were combined and lyophilyzed. The product was hydrolyzed and analyzed in the manner described in a previous report.[16]

Partial Acetolysis——The polysaccharide (26 mg) was dissolved in formamide (0.6 ml), then pyridine

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

Standard and products	Cellulose TLC (*Rf*)		GLC (t_R) under condition B
	Solvent A	Solvent B	
Man	0.69	0.57	3.5
Glc	0.62	0.50	4.8
β-1,4-Glc→Man	0.65	0.44	20.4,[a] 22.6
β-1,4-Man→Man	0.55	0.36	22.2,[a] 24.2
β-1,4-Man→Glc	0.47	0.27	23.0, 24.5[a]
β-1,4-Man→Glc→Man	0.35	0.20	37.8, 38.3[a]
β-1,4-Man→Man→Man	0.28	0.17	39.1,[a] 40.7
β-1,4-Man→Man→Man→Man	0.09	0.05	—
Partial acetolysis products	0.69	0.57	3.5
	0.62	0.50	4.8
	0.55	0.36	22.2
	0.47	0.27	23.0
	0.35	0.20	24.3
	0.28	0.17	37.8
	0.09	0.05	38.3
			39.1
			40.7
Enzymatic degradation products	0.69	0.57	3.5
	0.65	0.50	4.8
	0.62	0.44	20.4
	0.55	0.36	22.2
	0.35	0.20	22.6
	0.28	0.17	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°.
Solvent B, BuOH: pyridine: H_2O (6: 4: 3) at 28°.
Condition B: a column (0.3 cm × 2 m long spiral glass) packed with 2% OV 101 on Uniport HP (80 to 100 mesh) and with a programmed temperature increase of 3° per min from 180° to 300° at a nitrogen flow rate of 30 ml per min.
a) Main peaks.

18) *a*) J.S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954); *b*) G.O. Aspinall and R.J. Ferrier, *Chem. Ind.*, **1957**, 1216.

(0.3 ml) and acetic anhydride (0.2 ml) were added under stirring. The mixture was stirred for three days at room temperature, then poured into four volumes of methanol. The precipitate was filtered off, washed with methanol and ether, then dried *in vacuo*. The product was acetylated once more under the same conditions. The final product was dissolved in acetic anhydride (0.6 ml) and, after addition of a cold mixture of acetic anhydride: sulfuric acid (5: 1, 0.3 ml) under cooling, the solution was kept at 5° for 24 hr. The reaction mixture was poured into ice-water (10 ml), then the products were extracted three times with chloroform (10 ml each). The extracts were combined and washed successively with 10% sodium bicarbonate and water, dried over sodium sulfate, then evaporated to dryness. The residue was dissolved in acetone (0.5 ml), deacetylated by treatment with 0.2 N sodium hydroxide (0.5 ml) overnight at 5°, then neutralized with acetic acid. The solvent was evaporated off. Yield, 11.8 mg.

Enzymatic Degradation——Driselase (1 g) was suspended in 0.1 M Tris-HCl buffer (pH 8, 20 ml) under cooling. The suspension was centrifuged (15000 rpm, 15 min) and the supernatant was dialyzed against 0.01 M Tris-HCl buffer (pH 8, 500 ml) overnight at 2°. The non-dialyzable fraction was applied to a column (1 × 8 cm) of DEAE-cellulose at 2° and eluted with a gradient of 0 to 0.2 M potassium chloride in 0.01 M Tris-HCl buffer (pH 8, 200 ml). Fractions of 5 ml were collected. The eluates in tubes 14 and 15 were combined, and adjusted to pH 6 by addition of 5% acetic acid. The polysaccharide (5 mg) was dissolved in this enzyme solution (1 ml). The solution was incubated at 37° with a drop of toluene for three days. The resulting solution was successively passed through columns (1 × 2 cm each) of Dowex 50W-X8 (H^+) and Dowex 2 (OH^-), and the eluate was concentrated to dryness. Yield, 3.5 mg.

Analysis of Degradation Products——The products were subjected to cellulose TLC and identified by comparison with authentic samples in the manner described in a previous report.[19] In addition, samples were trimethylsilylated in the usual way,[20] then subjected to GLC under the same conditions as in the preceding report.[1] The relative yields of the products were evaluated by GLC. *Rf* values and retention times of the products are shown in Table II.

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

19) M. Tomoda and S. Kimura, *Chem. Pharm. Bull.*, **24**, 1807 (1976).
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