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Plant Mucilages. XXIX.[1] Isolation and Characterization of a Mucous Polysaccharide, "Plantago-mucilage A," from the Seeds of *Plantago major* var. *asiatica*

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A representative mucous polysaccharide, named Plantago-mucilage A, was isolated from the seeds of *Plantago major* L. var. *asiatica* Decaisne (=*Plantago asiatica* L.). The final preparation was homogeneous as determined by ultracentrifugal analysis, glass-fiber electrophoresis, and gel chromatography. It was readily soluble in water and its solution gave an intrinsic viscosity value of 39.5. It was composed of L-arabinose, D-xylose, D-glucuronic acid, and D-galacturonic acid in the molar ratio of 4.0: 10.8: 3.3: 0.7, and its molecular weight was estimated to be about 1500000. *O*-Acetyl groups were identified in it and their content amounted to be 4.8%. Reduction of carboxyl groups, methylation analysis, controlled Smith degradation, and partial acid hydrolysis studies showed that the mucilage possesses a main chain composed of β-1→4 linked D-xylopyranose residues having other D-xylopyranose side chains at position 3 and branches composed of *O*-α-(D-glucopyranosyluronic acid)-(1→3)-α-L-arabinofuranose and of *O*-α-(D-galactopyranosyluronic acid)-(1→3)-α-L-arabinofuranose at position 2 of the residual D-xylopyranose units.

Keywords——Plantago-mucilage A; *Plantago major* var. *asiatica* (=*Plantago asiatica*); representative mucous polysaccharide; intrinsic viscosity; molecular weight; analysis of components: reduction and methylation; controlled Smith degradation; partial hydrolysis; nuclear magnetic resonance

Plantaginis Semen (Japanese name, Shazenshi), the seeds of *Plantago major* L. var. *asiatica* Decaisne (=*Plantago asiatica* L.), is a well-known crude drug used as an antiphlogistic, diuretic, antidiarrheic, and cough medicine. In 1971, Tomoda *et al.*[2] reported the isolation of a mucous polysaccharide, called plantasan, from the seeds of this plant. Structural studies on plantasan were performed by Smith degradation[3] and by partial acid hydrolysis[4] followed by methylation analysis, and the results indicated the existence of β-1,4-linked D-xylopyranose backbone chains having branches at position 2 and of β-D-glucopyranosyluronic acid-(1→5)-L-arabinofuranose side chains. We have now isolated a new mucous polysaccharide which has a much higher water-solubility and a higher intrinsic viscosity value in aqueous solution than plantasan. The properties and the structural features of this new polysaccharide are described in the present paper.

The seeds were extracted with 0.2% sodium carbonate at room temperature, and after centrifugation, the polysaccharide was prepared from the supernatant by repeated precipitation with ethanol, followed by dialysis and lyophilization. The polysaccharide was homogeneous as determined by ultracentrifugal analysis (Fig. 1) and gave a single spot on glass-fiber paper electrophoresis. Furthermore, it gave a single peak on gel chromatography with Sepharose 4B (Fig. 2).

The polysaccharide was readily soluble in water and showed a negative specific rotation ($[\alpha]_D^{24}$ −38.1° in H_2O, c=0.2). Its solution in water gave the high intrinsic viscosity value of 39.5 at 30°C. Gel chromatography gave a value of approximately 1500000 for the molecular weight. The name "Plantago-mucilage A" is proposed for this polysaccharide.

As component sugars of the mucilage, D-xylose, L-arabinose, D-glucuronic acid, and D-galacturonic acid were identified by cellulose thin-layer chromatography (TLC) of the hydrolysate. These sugars were isolated by preparative paper partition chromatography (PPC) and proved to have the configurations given above.

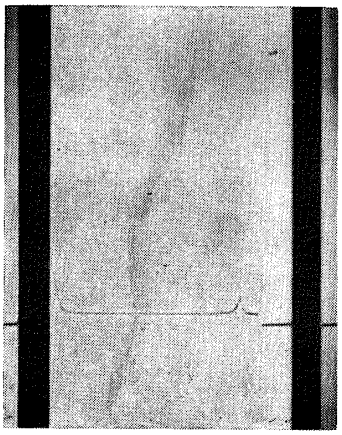

Fig. 1. Ultracentrifugal Pattern of Plantago-mucilage A

0.2% in H_2O, 20°C 36 min, 51200 rpm, Hitachi UCA-1A ultracentrifuge.

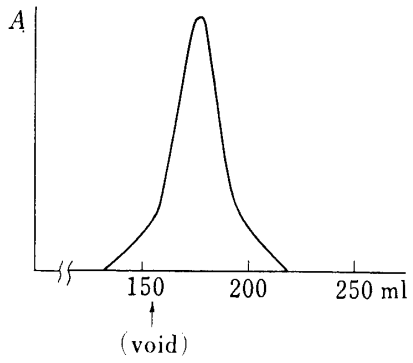

Fig. 2. Chromatogram of Plantago-mucilage A on Sepharose 4B

The carboxyl groups of hexuronic acid residues in the mucilage were reacted with a carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units.[5] Quantitative determination of component sugars of the product was carried out by gas–liquid chromatography (GLC) of alditol acetates derived from the hydrolysate. The result showed that the molar ratio of xylose: arabinose: glucuronic acid: galacturonic acid was 10.8: 4.0: 3.3: 0.7 in the original mucilage. In addition, the presence of *O*-acetyl groups in the original mucilage was found by GLC of its hydrolysate,[6] and the acetyl content was determined to be 4.8%.

Methylation of the carboxyl-reduced mucilage was performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[7] The fully methylated product was hydro-

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

	Molar ratios		Relative retention times[a]	Main fragments (*m/e*)
	Carboxyl-reduced mucilage	Smith degradation product		
Peak 1	—	4.3	0.46	43, 45, 71, 87, 101, 117, 129, 161
Peak 2	2.0	—	0.60	43, 101, 117, 161
Peak 3	3.9	—	0.89	43, 45, 113, 117, 233
Peak 4	3.4	—	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
Peak 5	—	—	1.05	43, 117, 233
Peak 6	—	2.2	1.17	43, 87, 101, 117, 129, 189
Peak 7	0.6	—	1.21	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
Peak 8	9.2	7.0	2.04	43, 87, 117, 129, 189, 261
1,4-Ac-2,3,5-Me-L-Arabinitol			0.46	43, 45, 71, 87, 101, 117, 129, 161
1,5-Ac-2,3,4-Me-D-Xylitol			0.60	43, 101, 117, 161
1,3,4-Ac-2,5-Me-L-Arabinitol			0.89	43, 45, 113, 117, 233
1,5-Ac-2,3,4,6-Me-D-Glucitol			1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,3,5-Ac-2,4-Me-D-Xylitol			1.05	43, 117, 233
1,4,5-Ac-2,3-Me-D-Xylitol			1.17	43, 87, 101, 117, 129, 189
1,5-Ac-2,3,4,6-Me-D-Galactitol			1.21	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,3,4,5-Ac-2-Me-D-Xylitol			2.04	43, 117, 261
1,2,4,5-Ac-3-Me-D-Xylitol			2.04	43, 87, 129, 189

a) Relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol.
Abbreviations: Ac=acetyl; Me=methyl (*e.g.*, 1,4-Ac-2,3,5-Me-=1,4-di-O-acetyl-2,3,5-tri-O-methyl-).

lyzed and the products were analyzed by gas-liquid chromatography-mass spectrometry (GLC–MS) after conversion into alditol acetates[8]; 2,5-di-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2- and 3-*O*-methyl-D-xyloses, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-galactose were identified in a molar ratio of 3.9: 2.0: 9.2: 3.4: 0.6. The alditol acetates of the two mono-*O*-methyl xyloses were not separated from each other under the conditions used, but the coexistence of both 2-*O*-methyl and 3-*O*-methyl D-xyloses was proved by the mass fragmentation pattern of the superimposed peak (Table I).

The mucilage was subjected to periodate oxidation followed by reduction with sodium borohydride.[9] The maximal values of periodate consumption and formic acid liberation were 0.84 mol and 0.45 mol per mol of anhydrosugar unit. The reduction product was treated with 0.5 N sulfuric acid overnight at room temperature. After neutralization and dialysis, the controlled Smith degradation product was isolated by gel chromatography on Sephadex G-25. It gave a single spot on glass-fiber paper electrophoresis. It showed a negative specific rotation ($[\alpha]_D^{27}$ −113.2° in H_2O, c=0.2). Gel chromatography gave a value of 29200 for the molecular weight. It contained 28.1% arabinose, 67.2% xylose, and 4.7% *O*-acetyl groups; their molar ratio was thus 3.8: 9.0: 2.0.

Methylation analysis of the controlled Smith degradation product was carried out as described above, and the products were identified as 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, and 2-*O*- and 3-*O*-methyl-D-xyloses (Table I). They were obtained in a molar ratio of 4.3: 2.2: 7.0.

As shown in Table II, the chemical shift of 108.1 ppm for the C-1 signal of the arabinofuranosyl residue in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of the controlled Smith degradation product indicates that L-arabinofuranose units are α-linked.[10,11] The chemical shifts of 102.8 and 103.5 ppm for the C-1 signals of the xylopyranosyl residues also suggest that D-xylose residues are β-linked.[12] The presence of the *O*-acetyl groups was confirmed by the presence of a signal at 24.5 ppm in the ^{13}C-NMR spectrum.

TABLE II. Chemical Shifts[a] in the ^{13}C-NMR Spectrum of the Smith Degradation Product

	C-1	C-2	C-3	C-4	C-5	CH_3-
Arabinofuranose	108.1	79.3	83.3	81.6	63.2	
Xylopyranose	103.5	81.2	83.9	76.5	61.3	
	102.8	73.4	75.5			
Acetyl						24.5

a) Chemical shifts are expressed in terms of ppm.

On the other hand, the mucilage was hydrolyzed with 0.1 N sulfuric acid for 2 h, then neutralized and applied to a column of Sephadex G-25. The low molecular weight fraction obtained was applied to a column of diethylaminoethyl (DEAE)–Sephadex A-25 (formate form). The eluate with water was rechromatographed on a column of Sephadex G-25, and the fraction containing disaccharides was obtained. Two disaccharides (I and II) were isolated from this fraction by preparative PPC. The eluate with 0.1 M formic acid from the DEAE–Sephadex A-25 column gave a third disaccharide (III). The fraction containing high molecular weight substances obtained by the first Sephadex G-25 column chromatography was mixed with the insoluble material in the hydrolysate and the mixture was hydrolyzed with 0.5 N sulfuric acid for 2 h. After neutralization and application to a column of Sephadex G-25, a fourth disaccharide (IV) was obtained. The preparation of the partial hydrolysates is summarized in Chart 1.

The homogeneity of each disaccharide was checked by cellulose TLC and by GLC of trimethylsilylated derivatives. Table III gives the *Rf* values on TLC, retention times on GLC, and specific rotations of the disaccharides in water.

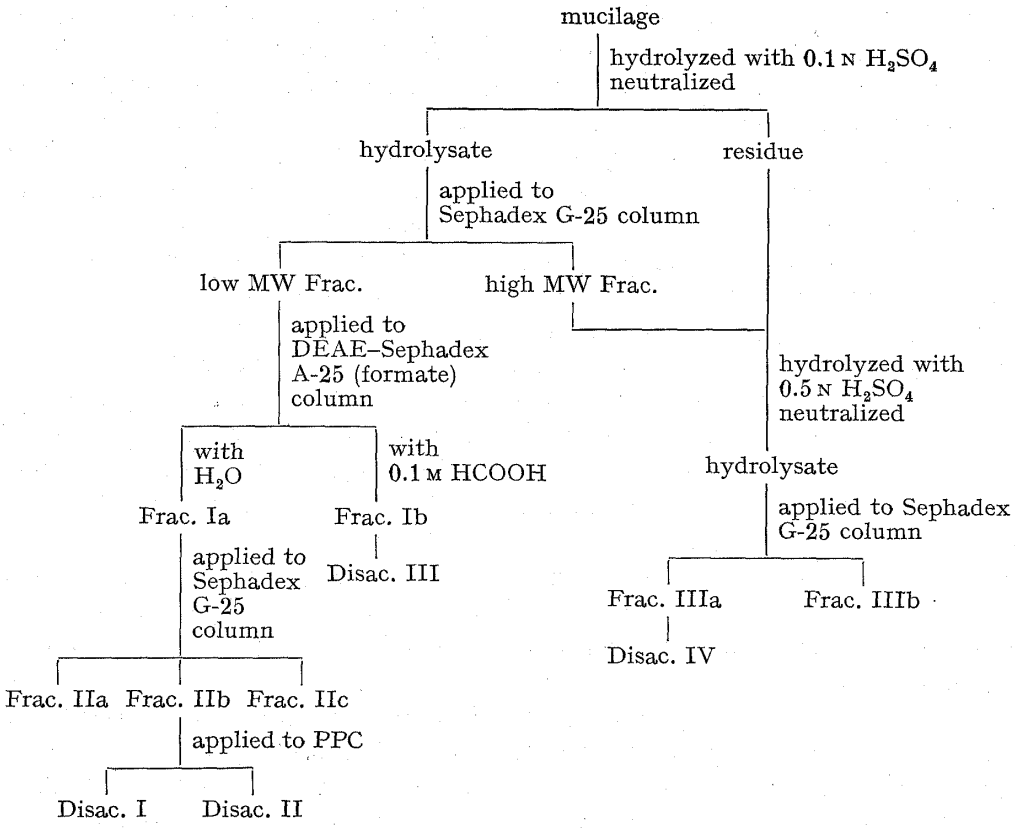

Chart 1. Isolation of the Disaccharides

TABLE III. Specific Rotations, *Rf* Values on TLC, and Retention Times on GLC of the Disaccharides

Disaccharides	Specific rotations in water (final values)	Cellulose TLC (*Rf*)		GLC (t_R)[a] Under condition B
		Solvent A	Solvent B	
I	$[\alpha]_D^{25}$ − 22.6° (c=0.2)	0.74	0.47	26.2, 27.1
II	$[\alpha]_D^{25}$ − 17.8° (c=0.4)	0.62	0.32	29.3, 29.8
III	$[\alpha]_D^{16}$ +152.6° (c=1.0)	0.23	0.20	28.7, 30.5
IV	$[\alpha]_D^{16}$ +132.6° (c=0.5)	0.23	0.19	28.5, 30.2

a) Retention times (min) of trimethylsilylated derivatives.
Solvent A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3) at 23°C.
Solvent B, BuOH: pyridine: H_2O (6: 4: 3) at 23°C.
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°C per min from 150°C to 300°C at a nitrogen flow rate of 30 ml per min.

The hydrolysates of I and II gave D-xylose, and the component sugars of III were L-arabinose and D-glucuronic acid. However, the hydrolysis of IV gave L-arabinose, D-glucuronic acid, and D-galacturonic acid in the molar ratio of 1.0: 0.9: 0.15. Based on the result of component sugar analysis, and by comparing its chromatographic properties and the value of specific rotation with those of an authentic sample,[4] II was identified as *O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose.

Disaccharides III and IV were converted into the corresponding carboxyl-reduced derivatives of the methyl ester methyl glycosides with sodium borohydride. Methylation analyses of disaccharide I, and the carboxyl-reduced III and IV indicated the production of 2,4-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-xylose in a molar ratio of 1.1: 1.0

from I, and 2,5-di-*O*-methyl-L-arabinose and 2,3,4,6-tetra-*O*-methyl-D-glucose in a molar ratio of 1.0: 1.1 from carboxyl-reduced III, whereas the carboxyl-reduced IV gave 2,5-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-galactose in a molar ratio of 1.0: 1.0: 0.17.

Disaccharides I, III, and IV were reduced with sodium borohydride. The proton magnetic resonance (^{1}H-NMR) spectra showed an anomeric proton signal at δ 4.72 (d, J=7 Hz) in the derivative from I, an anomeric proton signal at δ 5.33 (d, J=3 Hz) in the derivative from III, and two anomeric proton signals at δ 5.28 (d, J=3 Hz) and δ 5.33 (d, J=3 Hz) in the derivative from IV. These data suggest that the non-reducing terminal D-xylose is β-linked,[13] and that both the D-glucuronic acid residues in III and IV are α-linked.[13,14]

Based on the results described above, I and III were identified as *O*-β-D-xylopyranosyl-(1→3)-D-xylopyranose and *O*-α-(D-glucopyranosyluronic acid)-(1→3)-L-arabinofuranose, but IV must be a mixture of III and *O*-α-(D-galactopyranosyluronic acid)-(1→3)-L-arabinofuranose. Further purification of IV was unsuccessful because of the similar chromatographic behavior of the two disaccharides as shown in the values for IV in Table III.

The combined yield of these four disaccharides and monosaccharides was over 94% of total sugars obtained from the partial hydrolysate of the mucilage. Consequently, it can be deduced that these disaccharides do represent the structural features of the mucilage.

Based on the accumulated evidence described above, it can be concluded that the minimal unit of Plantago-mucilage A was composed of the five kinds of sugar units shown in Chart 2.

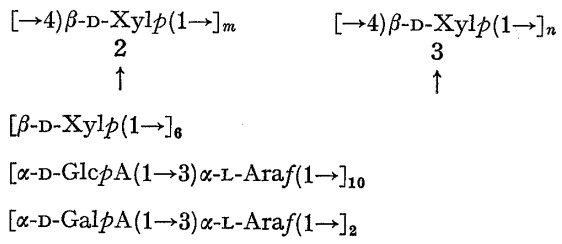

Chart 2. Minimal Component Units in the Structure of Plantago-mucilage A ($m+n=27$)

Abbreviations: Xyl*p*, xylopyranose; Ara*f*, arabinofuranose; Glc*p*A, glucopyranosyluronic acid; Gal*p*A, galactopyranosyluronic acid.

The glycosidic linkages of arabinofuranose residues in the mucilage are much more easily cleaved than those of the other component sugars. Therefore no direct evidence showing the mode of linkage of arabinosyl xylose was obtained. On the other hand, only two xylobioses were found as products of partial hydrolysis; those were β-1→3 and β-1→4 linked xylobioses. This result shows the presence of a β-1→4 linked D-xylopyranose backbone chain having the other xylose side chains at position 3 of D-xylopyranose units. Consequently, it can be presumed that *O*-α-(D-glucopyranosyluronic acid)-(1→3)-α-L-arabinofuranose and *O*-α-(D-galactopyranosyluronic acid)-(1→3)-α-L-arabinofuranose units link to position 2 of D-xylopyranose units in the backbone chain.

The location of the acetyl groups remains to be determined. The results of detailed analysis of the structure will be reported in subsequent papers.

Experimental

Solutions were concentrated at or below 40°C with rotary evaporators under reduced pressure. Optical rotations were measured with a JASCO DIP-SL automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. 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. Infrared (IR) spectra were recorded on a JASCO IRA-2 infrared

spectrophotometer. ^{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. The ^{13}C-NMR spectrum was recorded on a JEOL JNM-FX 100 NMR spectrometer in heavy water containing benzene as an internal standard at 25°C.

Isolation of the Mucilage——The seeds (100 g) were extracted twice with 0.2% sodium carbonate (1000 ml each) under stirring at room temperature for 1 h each time. After centrifugation (12000 rpm, 20 min), the extracts were combined and poured into five volumes of ethanol, then centrifuged. The precipitate was dissolved in water and treated with ethanol again. The treatment with ethanol was repeated three times. The final precipitate was dissolved in water, followed by dialysis against running distilled water. Plantago-mucilage A (3.5 g) was obtained as a grayish-white powder after lyophilization.

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 a buffer of 0.025 M borax: 0.1 N sodium hydroxide (10: 1, pH 9.3) at 570 V for 1 h. Plantago-mucilage A, the carboxyl-reduced mucilage, and the Smith degradation product each gave a single spot at distances of 6.3, 9.8, and 8.0 cm from the center toward the cathode, respectively. Standard glucose moved to a distance of 11.6 cm.

Gel Chromatography——This was carried out in the manner described in a previous report[16] of this series using a column (2.6 × 84 cm) of Sepharose 4B.

Hydrolysis, Isolation, and TLC of Component Sugars——These were carried out in the manner described in a previous report[17] of this series. The *Rf* values of component sugars on TLC using Avicel SF cellulose and with the solvent systems A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3), and B, BuOH: pyridine: H_2O (6: 4: 3), are listed in Table IV.

Table IV. *Rf* Values on TLC, and Retention Times on GLC of Component Sugars

Components and derivatives	Cellulose TLC (*Rf*)		GLC (t_R)
	Solvent A	Solvent B	Under condition A
Xylose	0.72	0.49	
Arabinose	0.60	0.41	
Glucuronic acid	0.26	0.18	
Galacturonic acid	0.21	0.15	
Arabinitol pentaacetate			7.0
Xylitol pentaacetate			8.8
Galactitol hexaacetate			19.0
Glucitol hexaacetate			21.1

Solvents A and B are the same as those in Table III.
Condition A: a column (0.3 cm × 2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 210°C with a nitrogen flow of 30 ml per min.

Reduction of the Mucilage——The mucilage (100 mg) was dissolved in water (30 ml), then 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (1 g) was added. The pH of the r action mixture was maintained at 4.75 by titration with 0.1 N hydrochloric acid under stirring for 2 h, then 2 M sodium borohydride (10 ml) was added gradually to the reaction mixture during 4 h while the pH was maintained at 7.0 by titration with 4 M hydrochloric acid under stirring at room temperature. The solution was dialyzed against running water overnight, then the non-dialyzable fraction was concentrated to 30 ml. The product was reduced three times more under the same conditions. The final non-dialyzable fraction was applied to a column (5 × 80 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 28 to 33 were combined and lyophilized. Yield, 58 mg.

Determination of Components——After acid hydrolysis, component aldoses were converted into alditol acetates and analyzed by GLC as described in a previous report[14] of this series. 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 210°C with a nitrogen flow of 30 ml per min. *Myo*-inositol was used as an internal standard. The retention times (min) of alditol acetates are also listed in Table IV. Hexuronic acids were estimated by a modification of the carbazole method.[18] Quantitative determination showed that the original mucilage contained 50.9% xylose, 18.9% arabinose, 20.8% glucuronic acid, and 4.4% galacturonic acid. Determination of *O*-acetyl groups was carried out in the manner described in a previous report[6] of this series.

Methylation of the Carboxyl-reduced Mucilage——The sample (18.1 mg) was dissolved in dimethyl sulfoxide (5 ml). Sodium hydride (40 mg) was mixed with dimethyl sulfoxide (8 ml) in an ultrasonic bath for 30 min; the mixture was stirred at 70°C for 1 h, then added to the sample solution. The reaction mixture

was stirred at room temperature for 4 h, then methyl iodide (8 ml) was added and the whole was stirred overnight at room temperature. All procedures were carried out under nitrogen. After addition of water (20 ml), the reaction mixture was extracted five times with chloroform (20 ml each). The combined extract was washed five times with water (100 ml each), then dried over sodium sulfate, and the filtrate was concentrated to dryness. The residue was methylated four times 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 13 were combined and concentrated to dryness. The final product (20.6 mg) was a yellow powder. Its IR spectrum showed no absorption of hydroxyl groups.

Analysis of the Methylated Product——A part of the product was hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated in the manner described in a previous report[16] of this series. GLC–MS was carried out under the same conditions as in a previous report.[16] The relative retention times of the products (peaks 2, 3, 4, 7, and 8) with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GLC and their main fragments in the mass spectra are listed in Table I.

Periodate Oxidation——The mucilage (1.0 g) was oxidized with 0.05 M sodium metaperiodate (400 ml) at 5°C in the dark. The periodate consumption was measured by a spectrophotometric method.[19] The oxidation was completed after four days. The formic acid liberation was measured by titration with 0.01 N sodium hydroxide after addition of ethylene glycol.

Controlled Smith Degradation——The reaction mixture was successively treated with ethylene glycol (2 ml) and sodium borohydride (2 g) at 5°C for 16 h, 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 to 50 ml, then the same volume of 1 N sulfuric acid was added to the solution. After standing at room temperature for 16 h, the solution was neutralized with barium carbonate and filtered. The filtrate was concentrated and passed through a column (0.7 × 4 cm) of Dowex 50W-X8 (H^+). The eluate with water was concentrated and dialyzed against water, then the non-dialyzable fraction was applied to a column (5 × 80 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 28 to 33 were combined and lyophilized. Yield, 0.34 g.

Methylation Analysis of the Smith Degradation Product——This was carried out as described above. The relative retention times of the products (peaks 1, 6, and 8) with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GLC and their main fragments in the mass spectra are also listed in Table I.

Partial Hydrolysis and Isolation of Disaccharides——The mucilage (908 mg) was suspended in 0.1 N sulfuric acid (182 ml) and heated under reflux at 100°C for 2 h. After centrifugation, the supernatant was neutralized with barium carbonate. After filtration, the filtrate was passed through a column (1 × 5 cm) of Dowex 50W-X8 (H^+). The eluate with water was concentrated and applied to a column (5 × 80 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 29 to 56 and the precipitate of the centrifugation described above were combined and subjected to secondary partial hydrolysis. The eluates obtained from tubes 57 to 72 were combined, concentrated and applied to a column (1 × 10 cm) of DEAE-Sephadex A-25 (formate form, Pharmacia Co.). The column was eluted successively with water (100 ml), 0.1 M formic acid (55 ml), 0.2 M formic acid (25 ml), and 0.5 M formic acid (25 ml). Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method.[20] The eluates obtained from the column were divided into two groups: Frac. Ia, tubes 1 to 7; Frac. Ib, tubes 24 to 29. No sugar was found in the eluates with 0.2 M and 0.5 M formic acid. Frac. Ia was applied to a column (5 × 80 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from the column were divided into three groups: Frac. IIa, tubes 52 to 59; Frac. IIb, tubes 60 to 63; Frac. IIc, tubes 64 to 69. The yields were 30 mg in Frac. IIa, 191.5 mg in Frac. IIb, and 166 mg in Frac. IIc. Fracs. IIa and IIc were mixtures of trisaccharides and of monosaccharides, respectively. Disaccharides I and II were isolated from Frac. IIb by preparative PPC with Tōyō-Roshi No. 50 paper and solvent A. The yields were 45 mg for disaccharide I and 139.5 mg for disaccharide II. Disaccharide III was directly obtained from Frac. Ib.

The secondary partial hydrolysis was performed with 0.5 N sulfuric acid (200 ml) at 100°C for 2 h. After neutralization with barium carbonate followed by treatment with Dowex 50W-X8 (H^+) as described above, the hydrolysate was applied to a column (5 × 80 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from the column were divided into two groups: Frac. IIIa, tubes 51 to 63; Frac. IIIb, tubes 64 to 69. The yields were 49.5 mg for Frac. IIIa and 137.6 mg for Frac. IIIb. Disaccharide IV was directly obtained from Frac. IIIa, and Frac. IIIb gave xylose.

GLC of Disaccharides——Samples were trimethylsilylated in the usual way,[21] then subjected to GLC. GLC was carried out under condition B, using 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°C per min from 150°C to 300°C at a nitrogen flow rate of 30 ml per min. Retention times (min) of the products are also listed in Table III.

Determination of Component Sugars of Disaccharides——The carboxyl groups in the acidic disaccharides were reduced, hydrolyzed and derivatized into alditol acetates, then analyzed by GLC as described in a previous report[14] of this series.

Methylation Analysis of I and Carboxyl-reduced Disaccharides——This was carried out as described above, but the amount of the sample used was 4 mg and the reaction was repeated three times. The relative retention times of the products (peaks 2, 3, 4, 5, and 7) with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GLC and their main fragments in the mass spectra are also listed in Table I.

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References and Notes

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