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Plant Mucilages. XXXII.[1] A Representative Mucilage, "Althaea-Mucilage R," from the Roots of *Althaea rosea*

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A representative mucilage, named Althaea-mucilage R, was isolated from the roots of *Althaea rosea* CAVAILLES (hollyhock). The final preparation was homogeneous as determined by ultracentrifugal analysis, cellulose acetate and glass-fiber electrophoresis, and gel chromatography. Its water solution gave an intrinsic viscosity value of 33.4. It was composed of partially acetylated acidic polysaccharide and protein in a ratio of approximately 10.3 : 1.0, and its molecular weight was estimated to be 41700. The polysaccharide moiety was composed of D-galactose, D-glucose, L-rhamnose, D-galacturonic acid, and D-glucuronic acid in the molar ratio of 2 : 1 : 6 : 4 : 6. Reduction of carboxyl groups followed by methylation analysis, and partial acid hydrolysis studies made it possible to deduce the structural features of the polysaccharide moiety in the mucilage (Chart 4).

Keywords——Althaea-mucilage R; *Althaea rosea* (hollyhock); mucilage; intrinsic viscosity; molecular weight; sugar and *O*-acetyl composition; amino acid composition; methylation analysis; partial hydrolysis; structure of polysaccharide

The roots of *Althaea rosea* CAVAILLES (hollyhock) have been used as a crude drug with emollient and demulcent properties. It is well known that the roots contain relatively large amounts of mucilages. Recently, Salikhov *et al.*[2] reported that the crude polysaccharides of the roots were made up of arabinose, xylose, galactose, glucose, rhamnose, galacturonic acid, and glucuronic acid. However, the homogeneity and the mucosity of the mucilages obtained by them were uncertain, and no structural study on the mucilages in the roots has yet been reported. We have now obtained a representative mucilage from the roots of *Althaea rosea*. Its properties and main structural features are described in the present paper.

The fresh roots were sliced, homogenized and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol, then dissolved in dilute sodium sulfate solution. The solution was treated with cetyltrimethylammonium bromide, and the precipitate obtained was dissolved in sodium chloride solution. The resulting solution was poured into ethanol, then the precipitate was dissolved in water, reprecipitated with ethanol, and dialyzed against distilled water. A pure mucilage was obtained by lyophilization of the dialysate.

The mucilage was homogeneous as determined by ultracentrifugal analysis (Fig. 1), and gave a single spot on zone electrophoresis in both cellulose acetate membrane with a pyridine–acetic acid buffer and glass-fiber paper with an alkaline borate buffer. Furthermore, it gave a single peak on gel chromatography with Toyopearl HW 65 (Fig. 2).

The mucilage showed a positive specific rotation ($[\alpha]_D^{20}+51.7°$ in H_2O, $c=0.1$), and its solution in water gave the high intrinsic viscosity value of 33.4 at 30 °C. The relative viscosity of the solution of the pure mucilage was about 1.6 times that of the crude mucilage. In view of this result and the yield, it is reasonable to assume that the pure mucilage is the representative mucous substance in the water extract from the roots. The name "Althaea-mucilage R" is proposed for this substance.

As component sugars of the mucilage, D-galactose, D-glucose, L-rhamnose, D-

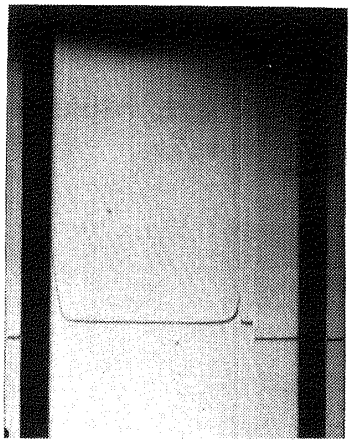

Fig. 1. Ultracentrifugal Pattern of Althaea-Mucilage R

0.2% in H_2O, 20 °C, 48 min, 60000 rpm, Hitachi UCA-1A ultracentrifuge.

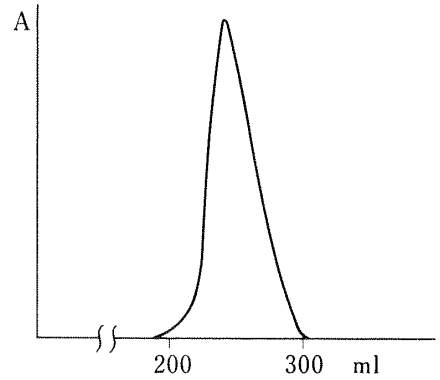

Fig. 2. Chromatogram of Althaea-Mucilage R on Toyopearl HW 65

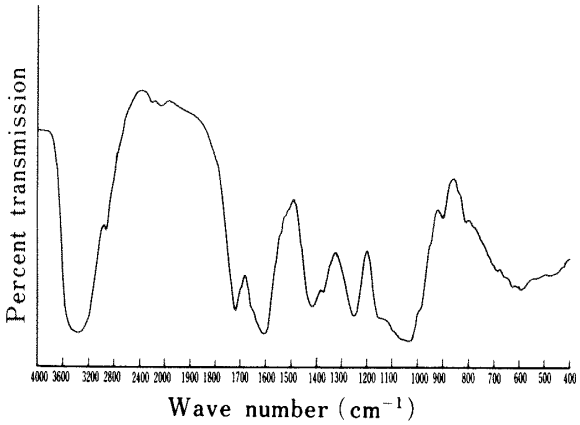

Fig. 3. IR Spectrum of Althaea-Mucilage R

galacturonic acid, and D-glucuronic 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.

The carboxyl groups of hexuronic acid residues in the mucilage were reacted with a carbodiimide reagent, then reduced with sodium borohydride to give the corresponding neutral sugar units.[3] Quantitative determination showed that the mucilage contained 8.4% galactose, 4.1% glucose, 22.6% rhamnose, 18.2% galacturonic acid, and 27.6% glucuronic acid, and that their molar ratio was 2.0 : 1.0 : 6.1 : 4.1 : 6.2. As shown in Fig. 3, the infrared (IR) spectrum has absorption bands at 1250 and 1730 cm^{-1}, suggesting the presence of ester linkages. When the acid hydrolysate was analyzed by gas-liquid chromatography (GLC),[4] it gave a single peak, with a retention time equal to that of acetic acid. The acetyl content of the mucilage was determined to be 9.8%. The measurement of osmotic pressure gave the value of 41700 as the molecular weight of the mucilage.

The mucilage contained 1.33% nitrogen. Determination of protein content was carried out by the method of Lowry *et al.*,[5] and a value of 8.8% was obtained. The amino acid composition after hydrolysis with 6 N hydrochloric acid is listed in Table I, together with those of Abelmoschus-mucilage M,[6] Abelmoschus-mucilage G,[7] and Okra-mucilage F.[8] No nitrogen-containing compound other than amino acids was detected in the hydrolysate. There is no significant difference in amino acid composition among them, except for the values of aspartic acid, methionine, and tyrosine.

TABLE I. Amino Acid Compositions (Molar Percent)

	Althaea-mucilage R	Abelmoschus-mucilage M	Abelmoschus-mucilage G	Okra-mucilage F
Lysine	6.16	7.05	6.28	5.56
Histidine	1.86	2.42	2.17	2.49
Arginine	4.18	4.46	3.49	5.15
Aspartic acid	10.26	6.75	11.26	9.84
Threonine	7.52	5.28	5.50	5.77
Serine	7.21	5.44	6.12	6.37
Glutamic acid	10.24	12.58	11.39	9.56
Proline	5.46	5.97	5.64	4.60
Glycine	9.92	10.34	9.59	10.35
Alanine	10.00	8.93	8.39	8.20
Valine	6.63	6.62	8.17	7.37
Methionine	1.70	2.03	1.01	2.08
Isoleucine	4.55	6.49	6.10	5.75
Leucine	8.20	7.71	8.73	9.01
Tyrosine	2.02	2.94	1.76	3.06
Phenylalanine	4.08	4.97	4.41	4.84

Methylations of the original and the carboxyl-reduced mucilages were performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[9] The fully methylated products were hydrolyzed with dilute sulfuric acid in acetic acid. The products were analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates.[10] Methyl ethers of the hexuronic acids were removed from the hydrolysis products of the methylated original mucilage by treatment with an anion-exchange resin, and the residual products were identified as 3,4-di-*O*-methyl-L-rhamnopyranose, 3-*O*-methyl-L-rhamnopyranose, 2,3,6-tri-*O*-methyl-D-glucopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, and 2,3,6-tri-*O*-methyl-D-galactopyranose in a molar ratio of 5.1 : 1.1 : 1.3 : 1.0 : 1.2, while 3,4-di-*O*-methyl-L-rhamnopyranose, 3-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, 2,3,6-tri-*O*-methyl-D-glucopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 2,3,6-tri-*O*-methyl-D-galactopyranose, and 2,6-di-*O*-methyl-D-galactopyranose were identified in a molar ratio of 4.8 : 1.1 : 3.8 : 3.2 : 1.0 : 1.2 : 4.6 from the carboxyl-reduced product.

These results suggested that the minimal repeating unit of Althaea-mucilage R is composed of eight kinds of component sugar units as shown in Chart 1.

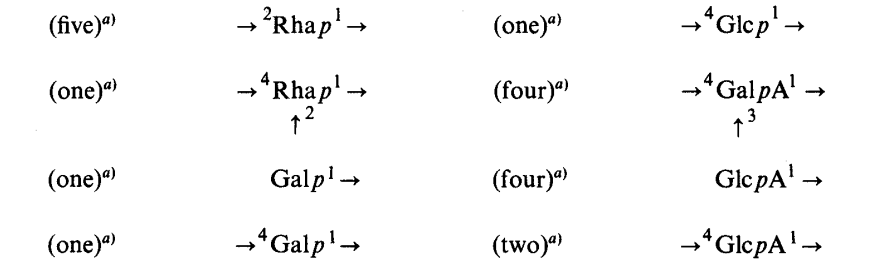

Chart 1. Component Sugar Residues in the Minimal Repeating Unit in the Structure of Althaea-Mucilage R

a) number of residues.

Rha*p*, rhamnopyranose; Gal*p*, galactopyranose; Glc*p*, glucopyranose; Gal*p*A, galactopyranosyluronic acid; Glc*p*A, glucopyranosyluronic acid.

The mucilage was hydrolyzed with 1 N sulfuric acid for 2 h, then neutralized and applied to a column of Dowex 50 W (H^+). The eluate with water was applied to a column of

diethylaminoethyl (DEAE)-Sephadex A-25 (formate form). Four oligosaccharides (I to IV) were obtained by stepwise elution with dilute formic acid, then purified by rechromatography with Sephadex G-25. Based on the results of component sugar analysis, and by comparing their chromatographic properties, the proton magnetic resonance (^{1}H-NMR) spectra, and the values of specific rotation with those of authentic samples,[11] I, II, and III were identified as the following three oligosaccharides (Chart 2).

I: α-D-Gal*p*A $^{1}\rightarrow{}^{2}$L-Rha*p*

II: β-D-Glc*p*A $^{1}\rightarrow{}^{3}$ α-D-Gal*p*A $^{1}\rightarrow{}^{2}$ L-Rha*p*

III: α-D-Gal*p*A $^{1}\rightarrow{}^{2}$ α-L-Rha*p* $^{1}\rightarrow{}^{4}$ α-D-Gal*p*A $^{1}\rightarrow{}^{2}$ L-Rha*p*
↑ $^{3}_{1}$ ↑ $^{3}_{1}$
β-D-Glc*p*A β-D-Glc*p*A

Chart 2. Structural Features of Oligosaccharides I, II, and III

Oligosaccharide IV showed a positive specific rotation ($[\alpha]_D^{23}+69.5°$ in H_2O, $c=0.1$), and was composed of L-rhamnose, D-galacturonic acid, and D-glucuronic acid in a molar ratio of 2:2:3. It has an L-rhamnose residue as a reducing terminal. The oligosaccharide was converted into the corresponding carboxyl-reduced oligosaccharide by reduction of the methyl ester methyl glycoside with sodium borohydride.[11] Methylation of the carboxyl-reduced derivative of IV was performed as described above. The fully methylated product was hydrolyzed and the hydrolysate was analyzed by GLC-MS after conversion into alditol acetates; 3,4-di-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, 2,3,6-tri-*O*-methyl-D-glucopyranose, 2,4,6-tri-*O*-methyl-D-galactopyranose, 2,6-di-*O*-methyl-D-galactopyranose were identified in a molar ratio of 1.9:1.8:1.0:0.8:1.1.

The reducing terminal rhamnose unit of IV was converted into the corresponding alditol by reduction with sodium borohydride. The ^{1}H-NMR spectrum of the resulting alditol derived from IV showed five anomeric proton signals at δ 4.69 (2H, d, $J=7$ Hz) [due to terminal glucuronic acid residue], δ 4.77 (1H, d, $J=7$ Hz) [due to a chain glucuronic acid residue], δ 5.12 (1H, d, $J=2$ Hz), [due to a rhamnose residue], δ 5.28 (1H, d, $J=3$ Hz) [due to a chain galacturonic acid residue], and δ 5.35 (1H, d, $J=3$ Hz) [due to branching point], [11] and two methyl signals at δ 1.23 (3H, d, $J=6$ Hz) and δ 1.26 (3H, d, $J=6$ Hz). These data suggest that the D-glucuronic acid residues in IV are β-linked and that L-rhamnose and D-galacturonic acid residues in IV are α-linked.[11]

On the other hand, the alditol derivative of IV described above was hydrolyzed with 1 N sulfuric acid for 1 h and the hydrolysate was determined by GLC after conversion into trimethylsilyl derivatives. The results showed that the ratio of reduced II, II, disaccharides, and monosaccharides in the hydrolysate was 1.0:1.7:1.1:1.8. Thus marked productions of II and reduced II were observed on the partial hydrolysis of IV, and the yield of II was much greater than that of reduced II. The glycosidic linkage of the L-rhamnose residue is more easily cleaved than those of hexuronic acids in acidic oligosaccharides.[11,12] Consequently, the results of the partial hydrolysis described above suggested the presence of the unit of triose II in the alditol derivative of IV. It is conceivable that the reduced II is a secondary product derived from the residual tetrasaccharide unit in the alditol derivative of IV in the process of partial hydrolysis.

Based on the accumulated evidence described above, IV was identified as *O*-β-(D-glucopyranosyluronic acid)-(1→3)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranosyl-(1→4)-[*O*-β-(D-glucopyranosyluronic acid)-(1→4)-*O*-β-(D-glucopyranosyluronic acid)-(1→3)]-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose (Chart 3).

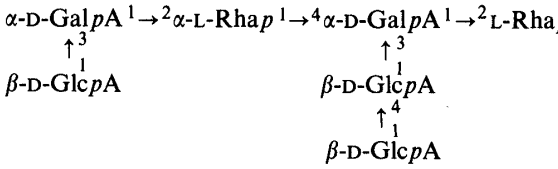

Chart 3. Structural Feature of Oligosaccharide IV

The ratio of yields of rhamnose, galactose, glucose, glucuronic acid, I, II, III, and IV obtained from the partial hydrolysate of Althaea-mucilage R was 3.3 : 12.0 : 1.0 : 1.4 : 3.8 : 11.7 : 11.1 : 11.7. The large difference in yields between galactose and glucose, in addition to the results of methylation analysis of the mucilage, suggests the presence of a galactosyl-(1→4)-galactosyl-(1→4)-glucose side chain linking to position 4 of one-sixth of the rhamnose residues in the backbone chain. Thus, Althaea-mucilage R contains the following unit (Chart 4).

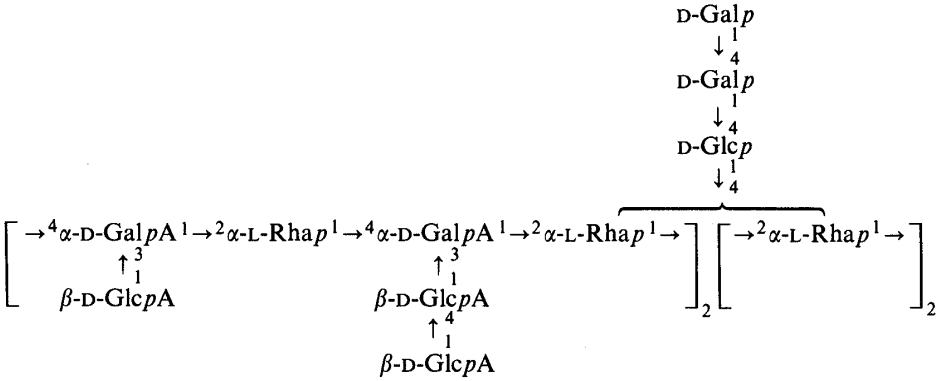

Chart 4. A Possible Structural Fragment of the Polysaccharide Moiety of Althaea-Mucilage R

As already reported in previous papers[7,8,11-13] of this series, the component unit having the repeating structure (1→4)-[*O*-β-(D-glucopyranosyluronic acid)-(1→3)]-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranose is common in the mucilages from the roots[12] and the leaves[13] of *Althaea officinalis*, the roots[11] of *Abelmoschus manihot*, and the roots[7] of *Abelmoschus glutinotextilis*. However, they do not possess a repeating unit having the structure IV. The heptasaccharide IV is the characteristic structural unit in Althaea-mucilage R. The mucilages from the roots[12] of *Althaea officinalis* and the immature fruits[8] of *Abelmoschus esculentus* have branches composed of 4-*O*-β-D-galactopyranosyl D-galactopyranose at position 4 of a part of the L-rhamnose units in the main chain. In contrast, the presence of the side chains composed of both D-galactose and D-glucose is another characteristic of the structure of Althaea-mucilage R. 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 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. Amino acids were determined with a Hitachi KLA-5 amino acid analyzer. ^{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 the Mucilage——The material was obtained at the beginning of September 1981 from plants

cultivated in Saitama prefecture. The fresh roots (480 g), which contained 69.1% water, were sliced and homogenized, then extracted with water (2500 ml) under stirring for 3 h at room temperature. After centrifugation, the supernatant was poured into two volumes of ethanol, then filtered. The precipitate was lyophilized, and a light brown powder (4.4 g) was obtained. A part of the crude mucilage (1.1 g) was dissolved in water (400 ml) and 0.02% sodium sulfate (400 ml) was added, followed by 10% cetyltrimethyl ammonium bromide (20 ml). The precipitate was separated by centrifugation, and dissolved in 2 M sodium chloride (450 ml). The solution was centrifuged to remove small amounts of impurities. The supernatant thus obtained was poured into two volumes of ethanol. The resulting precipitate was dissolved in water, and treated again with ethanol, followed by dialysis against running distilled water. Althaea-mucilage R (0.39 g) was obtained as a grayish-white powder after lyophilization.

Cellulose Acetate Membrane Electrophoresis——This was carried out as described in a previous report[8] of this series with 0.08 M pyridine–0.04 M acetic acid (pH 5.4) at 420 V for 1 h. The sample gave a single spot at a distance of 4.5 cm from the center toward the anode.

Glass-Fiber Paper Electrophoresis——This was carried out as described in a previous report[14] of this series with 0.025 M borax–0.1 N sodium hydroxide (10 : 1, pH 9.3). The sample gave a single spot at a distance of 2.0 cm from the center toward the cathode.

Gel Chromatography——The sample (3 mg) was dissolved in 0.1 M potassium dihydrogen phosphate and applied to a column (2.6 × 94 cm) of Toyopearl HW 65. Elution was carried out by the ascending method with the same solution. Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method.[15]

Hydrolysis, Isolation and TLC of Component Sugars——These were carried out as described in a previous report[16] of this series.

Reduction of the Mucilage——The mucilage (66 mg) was dissolved in water (30 ml), then 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (0.66 g) was added. The pH of the reaction mixture was maintained at 4.75 by titration with 0.1 N hydrochloric acid under stirring for 2 h, then 2 M sodium borohydride (7 ml) was added gradually to the reaction mixture during 4 h while the pH was maintained at 7.0 by titration with 4 N 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 twice more under the same conditions. The final non-dialyzable fraction was centrifuged, then the supernatant was concentrated and applied to a column (5 × 86 cm) of Sephadex G-15. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 30 to 35 were combined and lyophilized. Yield, 18.7 mg.

Determination of Components——Neutral sugars in the original and the carboxyl-reduced mucilages were analyzed by GLC after conversion into alditol acetates as described in a previous report[11] of this series. Rhamnose was also determined by the thioglycolic acid method,[17] and hexuronic acids in the original mucilage were estimated by a modification of the carbazole method.[18]

Determination of *O*-Acetyl Groups——The IR spectrum of the mucilage showed ester absorption bands. IR ν_{max}^{KBr} cm^{-1}: 1250, 1730 (ester). A sample was hydrolyzed with 1 N hydrochloric acid at 100°C for 2 h. Propionic acid was used as an internal standard, and the hydrolysate was directly subjected to GLC under the same conditions as in a previous report[4] of this series.

Determination of Molecular Weight——The measurement of osmotic pressure was carried out by the use of a Knauer electronic membrane osmometer at 61°C. The sample was dissolved in water, and 0.39, 0.31, 0.19, and 0.11% solutions were used.

Methylation——The sample (5 mg) was dissolved in dimethyl sulfoxide (2 ml). Sodium hydride (10 mg) was mixed with dimethyl sulfoxide (2 ml) in an ultrasonic bath for 30 min, followed by stirring at 70°C for 1 h, then the mixture was added to the sample solution. The reaction mixture was stirred at room temperature for 4 h, then methyl iodide (2 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 48 h. The non-dialyzable fraction was concentrated to dryness. The product was methylated three times more under the same conditions. The non-dialyzable fraction was extracted five times with chloroform (15 ml each). The combined extract was washed five times with water (75 ml each), then dried over sodium sulfate, and the filtrate was concentrated to dryness. The residue was dissolved in chloroform–methanol mixture (2 : 1), and applied to a column (1 × 18 cm) of Sephadex LH-20. The column was eluted with the same solvent, and fractions of 1 ml were collected. The eluates obtained from tubes 5 to 7 were combined and concentrated. The IR spectrum of the final residue showed no absorption of hydroxyl groups. Yield, 5.7 mg.

Analysis of the Methylated Products——Each product (3 mg) was hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated in the manner described in a previous report[8] of this series. GLC-MS was carried out under the same conditions as in a previous report.[8] The relative retention times of the products 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 II.

Partial Hydrolysis and Isolation of Oligosaccharides——The mucilage (500 mg) was suspended in 1 N sulfuric acid (50 ml) and heated under reflux at 100 °C for 2 h. The resulting water-insoluble fraction (46 mg) was separated by centrifugation, then the supernatant was neutralized with barium carbonate, and after filtration, the filtrate was

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

	Relative retention times[a]	Main fragments (m/z)
1,2,5-Ac-3,4-Me-L-Rhamnitol	0.88	43, 89, 129, 131, 189
1,2,4,5-Ac-3-Me-L-Rhamnitol	1.60	43, 87, 101, 129, 143, 189, 203
1,5-Ac-2,3,4,6-Me-D-Glucitol	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,5-Ac-2,3,4,6-Me-D-Galactitol	1.16	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,4,5-Ac-2,3,6-Me-D-Galactitol	2.04	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-Galactitol	2.90	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.*, 1,2,5-Ac-3,4-Me = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-).

TABLE III. Specific Rotations, Sugar Compositions, R_{Rha} Values of Oligosaccharides, and Retention Times of the Trimethylsilylated Derivatives

Oligosaccharides	$[\alpha]_D^{23}$ in H_2O	Sugar compositions	Rhamnitol in reduced samples	PPC (R_{Rha})	GLC (t_R) (min)
I	+93.2°	GalA : Rha = 1 : 1	45.4%	0.79	20.7, 21.2
II	+85.4°	GlcA : GalA : Rha = 1 : 1 : 1	31.8%	0.66	35.8, 37.7
III	+82.1°	GlcA : GalA : Rha = 1 : 1 : 1	15.9%	0.40	
IV	+69.5°	GlcA : GalA : Rha = 3 : 2 : 2	12.6%	0.34	

Abbreviations: GalA = galacturonic acid; GlcA = glucuronic acid; Rha = rhamnose.

passed through a column (1 × 15 cm) of Dowex 50W-X8 (H^+). The eluate with water was concentrated and applied to a column (1.5 × 20 cm) of DEAE-Sephadex A-25 (formate form). The column was eluted successively with water (120 ml), 0.2 M formic acid (240 ml), 0.4 M formic acid (400 ml), 0.6 M formic acid (200 ml), 0.8 M formic acid (480 ml), and 1 M formic acid (460 ml). Fractions of 10 ml were collected and analyzed by the phenol–sulfuric acid method. The eluates obtained from the column were divided into six groups: Frac. 1, tubes 5 to 9; Frac. 2, tubes 15 to 25; Frac. 3, tubes 43 to 80; Frac. 4, tubes 81 to 101; Frac. 5, tubes 102 to 136; Frac. 6, tubes 145 to 190. The yields were 42.5 mg for Frac. 1, 14.1 mg for Frac. 2, 30.5 mg for Frac. 3, 11.8 mg for Frac. 4, 34.0 mg for Frac. 5, and 26.8 mg for Frac. 6. Frac. 1 contained rhamnose, galactose, and glucose, and the ratio of them was determined by GLC as described above. Frac. 2 was dissolved in water and applied to a column (2.6 × 93.5 cm) of Sephadex G-15. The column was eluted with water and fractions of 5 ml were collected. The eluates obtained from the column were divided into two groups: Frac. a, tubes 48 to 58; Frac. b, tubes 61 to 65. The yields were 10.0 mg for Frac. a and 3.5 mg for Frac. b. Oligosaccharide I and glucuronic acid were obtained from Frac. a and Frac. b, respectively. Oligosaccharide II was obtained in quantitative yield from Frac. 3. Oligosaccharide III was obtained from Frac. 4, and, in addition, was present in Frac. 5. Fracs. 5 and 6 were each dissolved in water, and applied to a column (2.6 × 95 cm) of Sephadex G-25. The column was eluted with 0.08 M pyridine–0.04 M acetic acid buffer (pH 5.4) and fractions of 5 ml were collected. The eluates obtained from the column were divided into two groups: Frac. c, tubes 53 to 58 from Frac. 5; Frac. d, tubes 47 to 50 from both Frac. 5 and 6. Oligosaccharide III was obtained from Frac. c in addition to Frac. 4. Oligosaccharide IV was obtained from Frac. d. The yields were 28.8 mg for III and 30.4 mg for IV.

Analysis of the Oligosaccharides——Analysis of component sugars was carried out as described above. PPC by the descending method was carried out as described in a previous report[12] of this series. I and II were trimethylsilylated in the usual way, then subjected to GLC under the same conditions as in a previous report.[8] The results are listed in Table III.

Partial Hydrolysis of the Alditol Derived from IV and Analysis of the Products——IV was reduced with sodium borohydride in the usual way, and the resulting alditol was hydrolyzed with 1 N sulfuric acid at 100°C for 1 h. After

neutralization, the products were trimethylsilylated and analyzed by GLC as described above. The retention time (min) of the trimethylsilylated alditol derivative from II was 38.5.

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References and Notes

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