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Plant Mucilages. XXVI.[1] Isolation and Structural Features of a Mucilage, "Okra-mucilage F," from the Immature Fruits of *Abelmoschus esculentus*

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A representative mucilage, named Okra-mucilage F, has been isolated from the immature fruits of *Abelmoschus esculentus* MOENCH (=*Hibiscus esculentus* L.; Okra). The final preparation was homogeneous as determined by ultracentrifugal analysis, cellulose acetate membrane electrophoresis, and gel chromatography. Its water solution gave an intrinsic viscosity value of 30.1. It was composed of partially acetylated acidic polysaccharide and protein in a ratio of approximately 8.1: 1.0, and its molecular weight was estimated to be about 1700000. Analysis of component sugars, together with reduction and methylation, and partial degradation studies showed that the polysaccharide moiety in the mucilage possesses a main chain having the repeating structure (1→4)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranose, and that half the L-rhamnose residues in the main chain have branches composed of 4-*O*-β-D-galactopyranosyl D-galactopyranose at position 4.

Keywords——*Abelmoschus esculentus*; Okra-mucilage F; ultracentrifugal analysis; electrophoresis and gel chromatography; intrinsic viscosity; analysis of components; reduction and methylation analysis; partial hydrolysis; proton magnetic resonance; structural features of polysaccharide moiety

The immature fruits of *Abelmoschus esculentus* MOENCH(=*Hibiscus esculentus* L.; Okra) are widely used as a food. It is well known that the immature fruits contain relatively large amounts of mucilage. The mucilage has been used as a plasma expander[3] and as an intravenous circulation agent.[4]

Relatively many studies on the mucilage of this material have been published. Whistler and Conrad[5] reported that the mucilage was a polysaccharide composed of D-galactose, L-rhamnose, and D-galacturonic acid units, and they obtained 1→4 linked galactobiose and 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnose from the mucilage by partial acid hydrolysis. Amin[6] isolated a polysaccharide composed of 80% galactose, 10% rhamnose, 3% arabinose, and 6% galacturonic acid. He suggested that the main components were 1→4 linked galactose and 1→2 linked rhamnose residues. On the other hand, Kelkar *et al.*[7] reported that hydrolysis of the mucilage produced glucose, glucosamine, and six amino acids. Kishida *et al.*[8] found that the mucilage was composed of glucose, fructose, and fourteen amino acids, while Woolfe *et al.*[9] concluded that the mucilage was composed of galacturonic acid, galactose, rhamnose, and glucose in the ratio of 1.3: 1.0: 0.1: 0.1. However, the homogeneity and the mucosity of the mucilages obtained by the former investigators were uncertain, and differences

1) Part XXV: M. Tomoda, M. Arai, Y. Suzuki, M. Ohmura, and H. Takayama, *Chem. Pharm. Bull.*, **28**, 1546 (1980).
2) Location: *1-5-30, Shibakōen, Minato-ku, Tokyo, 105, Japan.*
3) H.B. Benjamin, H.K. Ihrig, and D.A. Roth, *Rev. Canad. Biol.*, **10**, 215 (1951).
4) P.I. Pilimeni, J. Al-Sadir, and A.F. Cutilletta, *Ger. Offen.*, 2734849 [*C.A.*, **89**, P 17232*n* (1978)].
5) R.L. Whistler and H.E. Conrad, *J. Am. Chem. Soc.*, **76**, 1673, 3544 (1954).
6) E.S. Amin, *J. Chem. Soc.*, **1956**, 828.
7) G.M. Kelkar, T.R. Ingle, and B.V. Bhide, *J. Indian Chem. Soc.*, **39**, 557 (1962) [*C.A.*, **58**, 1623*g* (1963)].
8) T. Kishida and H. Fukui, *Kaseigaku Zasshi*, **18**, 71 (1967).
9) M.L. Woolfe, M.F. Chaplin, and G. Otchere, *J. Sci. Food Agric.*, **28**, 519 (1977).

in these factors presumably account for the disagreements in their conclusions. No further structural study on the mucilage has yet been reported. However, we have now isolated a pure mucilage from the immature fruits of this plant. On the basis of the relative viscosity of the solution of the mucilage and its yield, we consider it is probable that the pure mucilage obtained by us is the major component accounting for the mucosity of the water extract from the material. Its properties and structural features are described in the present paper.

After removal of the seeds, the fresh immature fruits 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 cetyltrimethyl ammonium 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 cellulose acetate membrane electrophoresis in both a pyridine–acetic acid buffer and an alkaline borate buffer. Furthermore, it gave a single peak on gel chromatography with Sepharose 4B. It showed a positive specific rotation ($[\alpha]_D^{20}$ +51.8° in 1 N NaOH, $c=0.1$), and its solution in water gave the high intrinsic viscosity value of 30.1 at 30°. The relative viscosity of the solution of the pure mucilage was about 1.6 times that of the crude mucilage. Gel chromatography gave a value of approximately 1700000 for the molecular weight. The name "Okra-mucilage F" is proposed for this substance.

As component sugars of the mucilage, D-galactose, L-rhamnose, 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 shown to have the configurations given above. Quantitative determination showed that the mucilage contained 25.2% galactose, 21.8% rhamnose, and 27.3% galacturonic acid. As shown in Fig. 2, 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),[10] 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 5.5%.

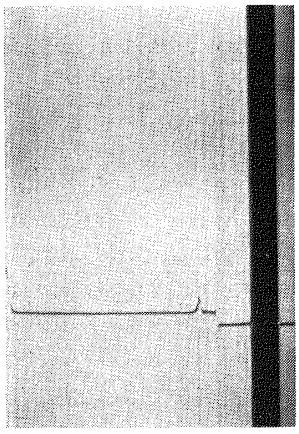


Fig. 1. Ultracentrifugal Pattern of Okra-mucilage F (0.1% in 0.1% NH_4OH, 20°, 30 min, 55430 rpm, Hitachi UCA-1A ultracentrifuge)

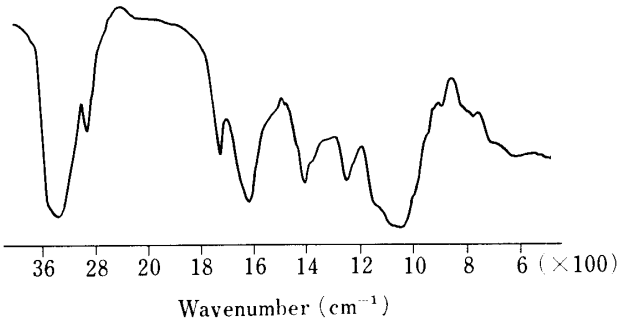


Fig. 2. IR Spectrum of Okra-mucilage F

10) M. Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.*, **26**, 2768 (1978).

The mucilage contained 1.66% nitrogen. Determination of protein content was carried out by the method of Lowry *et al.*,[11] and a value of 10.8% was obtained. The amino acid composition after hydrolysis with 6 N hydrochloric acid is listed in Table I, together with that of Abelmoschus-mucilage M.[12] No nitogen-containing compound other than amino acids was detected in the hydrolysate. There is no significant difference in amino acid composition between Okra-mucilage F and Abelmoschus-mucilage M, except for the values of aspartic acid and glutamic acid.

Table I. Amino Acid Compositions (Molar Percent)

	Okra-mucilage F	Abelmoschus-mucilage M
Lysine	5.56	7.05
Histidine	2.49	2.42
Arginine	5.15	4.46
Aspartic acid	9.84	6.75
Threonine	5.77	5.28
Serine	6.37	5.44
Glutamic acid	9.56	12.58
Proline	4.60	5.97
Glycine	10.35	10.34
Alanine	8.20	8.93
Valine	7.37	6.62
Methionine	2.08	2.03
Isoleucine	5.75	6.49
Leucine	9.01	7.71
Tyrosine	3.06	2.94
Phenylalanine	4.84	4.97

The carboxyl groups of galacturonic acid residues in the mucilage were reacted with a carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units.[13] Quantitative determination showed that the carboxyl-reduced mucilage was composed of 69.3% galactose and 30.7% rhamnose.

Methylations of the original and the carboxyl-reduced mucilages were performed with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide.[14] 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,[15] and identified as 3,4-di-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 3-*O*-methyl-L-rhamnopyranose, and 2,3,6-tri-*O*-methyl-D-galactopyranose. They were obtained in a molar ratio of 1.00: 0.93: 1.05: 1.10 from the original mucilage, and in a molar ratio of 1.00: 1.01: 0.94: 3.16 from the carboxyl-reduced product (Figs. 3, 4). These results show that two-thirds of the trimethyl galactose units in the methylation product of the carboxyl-reduced mucilage are derived from galacturonic acid residues in the original material.

The mucilage was hydrolyzed with 1 N sulfuric acid for 2 hr, then neutralized and applied to a column of Dowex 50W (H^+). The eluate with water was applied to a column of diethylaminoethyl (DEAE)-Sephadex A-25 (formate form). Only one oligosaccharide was obtained by stepwise elution with dilute formic acid, in addition to component sugars. Based on the results of component sugar analysis, and by comparing its chromatographic and electrophoretic

11) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
12) M. Tomoda and Y. Suzuki, *Chem. Pharm. Bull.*, **25**, 3061 (1977).
13) R.L. Taylor and H.E. Conrad, *Biochemistry*, **11**, 1383 (1972).
14) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
15) H. Björndal, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).

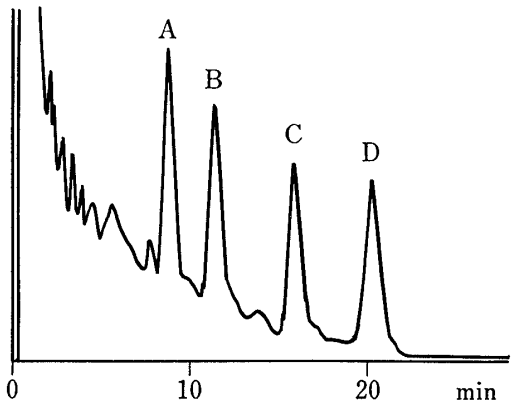


Fig. 3. Gas–Liquid Chromatogram of *O*-Methylated Alditol Acetates obtained from the Methylated Original Mucilage

Peak A:
1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol.
Peak B:
1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol.
Peak: C:
1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl-L-rhamnitol.
Peak D:
1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-galactitol.

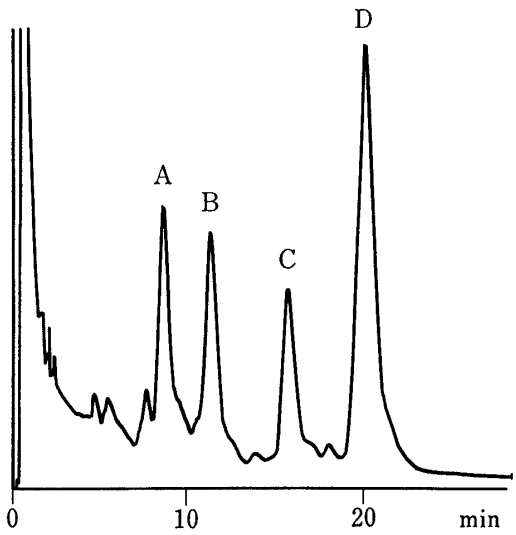


Fig. 4. Gas–Liquid Chromatogram of *O*-Methylated Alditol Acetates obtained from the Methylated Carboxyl-reduced Mucilage

Peaks A to D: the same as in Fig. 3.

properties and the value of specific rotation with those of an authentic sample,[1,16,17] the oligosaccharide was identified as 2-*O*-α-(D-galactopyranosyluronic acid)-L-rhamnopyranose. The ratio of the yields of the disaccharide, galactose, rhamnose, galacturonic acid, and the residual water-insoluble fraction composed of rhamnose and galacturonic acid together with a small amount of amino acids was 2.2: 11.0: 1.0: 0.8: 19.0. On the other hand, a part of the solution of the partial hydrolysate was applied to a column of Sephadex G-25, and it was confirmed that the solution contained no polysaccharide fraction. Thus, no galactose was found in any oligosaccharide or polysaccharide fraction after partial hydrolysis. On the other hand, about 90% of the rhamnose and galacturonic acid residues were present as components in the disaccharide and the water-insoluble polysaccharide fraction. These results suggest that branches in the polysaccharide moiety of the mucilage are composed of galactose residues.

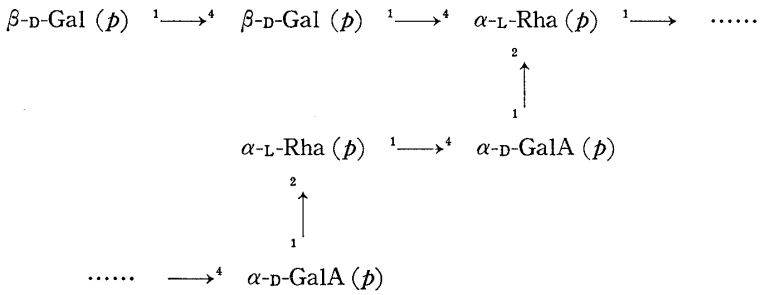
The proton magnetic resonance (^{1}H-NMR) spectrum of the carboxyl-reduced mucilage showed three anomeric proton signals at δ 4.76 (d, J=8 Hz), δ 5.06 (d, J=2 Hz), and δ 5.32 (d, J=2 Hz), an acetyl signal at δ 2.22 (s), and a methyl signal at δ 1.37 (d, J=6 Hz). The ratio of their integrals was 1.0: 1.0: 1.0: 1.5: 3.0. Half of the D-galactose units must be β-linked, because of the high value observed for the coupling constant of the signal at δ 4.76 in the ^{1}H-NMR spectrum. This result also suggests that the remaining D-galactose units are α-linked (the signal at δ 5.32 in the ^{1}H-NMR spectrum), and that L-rhamnose units are α-linked (the signal at δ 5.06 in the ^{1}H-NMR spectrum) in the substance.[16,18,19] Based on the results of the partial hydrolysis, it is evident that D-galacturonic acid residues are α-linked in the original mucilage, so D-galactose residues in the mucilage must be β-linked.

The mucilage was subjected to periodate oxidation followed by reduction with sodium brohydride. The maximal periodate consumption was 0.74 mol per mol of anhydrosugar unit. The reduction product was isolated by gel chromatography on Sephadex G-15. It contained 16.7% rhamnose, 9.6% galacturonic acid, and 5.3% *O*-acetyl groups; their molar ratio was

16) M. Tomoda, Y. Suzuki, and N. Satoh, *Chem. Pharm. Bull.*, **27**, 1651 (1979).
17) M. Tomoda, N. Satoh, and K. Shimada, *Chem. Pharm. Bull.*, **28**, 824 (1980).
18) B. Lindberg and W. Nimmich, *Carbohydr. Res.*, **48**, 81 (1976).
19) G.G.S. Dutton and K.L. Mackie, *Carbohydr. Res.*, **62**, 321 (1978).

thus 2.1: 1.0: 2.2. In addition, a samll proportion (3.8%) of protein still remained, but no galactose was found in it. These results indicate that about two-thirds of L-rhamnose and about one-third of D-galacturonic acid in the mucilage survived after periodate oxidation. It can be deduced that the *O*-acetyl groups are attached in susch a manner that they block periodate cleavage of the surviving sugar units at positions 3 or 4 of about one-third of straight chain rhamnose residues, at position 3 of about one-third of branched rhamnose residues, and at positions 2 or 3 of about one-third of galacturonic acid residues.

Based on the accumulated evidence described above, it can be concluded that the polysaccharide moiety of the mucilage has the following hexasaccharide repeating unit (Chart 1).



Gal=galactose; Rha=rhamnose; GalA=galacturonic acid

Chart 1. A Possible Structural Fragment of The Polysaccharide Moiety of Okra-mucilage F

The presence of a main chain having the repeating structure (1→4)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranose is common in the mucilages from the roots of *Althaea officinalis*,[17] *Abelmoschus manihot*,[16] and *Abelmoschus glutinotextilis*,[1] from the fruit of *Abelmoschus esculentus*, and from the inner bark of *Hydrangea paniculata*.[20] In the structures of Althaea-mucilage O,[17] Abelmoschus-mucilage M,[16] and paniculatan,[20] all the D-galacturonic acid residues possess β-D-glucuronic acid branches at position 3. In Abelmoschus-mucilage G,[1] three-quarters of the D-galacturonic acid residues possess β-D-glucuronic acid branches at position 3. In contrast to these mucilages, Okra-mucilage F has no branch at the D-galacturonic acid residues. It has branches composed of 4-*O*-β-D-galactopyranosyl D-galactopyranose at position 4 of half the L-rhamnose units in the main chain. Among the mucilages described above, Althaea-mucilage O possesses similar galactosyl galactose branches at position 4 of about one-third of the L-rhamnose units.[17]

The precise locations of acetyl groups and the mode of polysaccharide-protein linkage in Okra-mucilage F remain 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° 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. GLC was carried out on a Hitachi 063 gas chromatograph equipped with a hydrogen 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 JEOL JLC-6AH amino acid analyzer. 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°.

Isolation of the Mucilage——The material was obtained in the beginning of September 1979 from plants cultivated in Saitama prefecture. After removal of seeds, the fresh fruits (410 g), which contained 88.3%

20) M. Tomoda and N. Satoh, *Chem. Pharm. Bull.*, **25**, 2910 (1977).

water, were sliced and homogenized, then extracted with water (2000 ml) under stirring for 1 hr at room temperature. After centrifugation, the extraction was repeated with water (2000 ml). The extracts were combined and poured into two volumes of ethanol, then centrifuged. The precipitate was treated with ethanol, then dried *in vacuo*. A light brown powder (1.45 g) was obtained. A part of the crude mucilage (290 mg) was dissolved in water (150 ml), and after centrifugation, 0.02% sodium sulfate (150 ml) was added to the supernatant, followed by 10% cetyltrimethyl ammonium bromide (20 ml). The precipitate was separated by centrifugation, and dissolved in 2 M sodium chloride (120 ml). The solution was centrifuged to remove small amounts of impurities. The supernatant 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. Okra-mucilage F (150.4 mg) was obtained as a grayish-white powder after lyophilization.

Cellulose Acetate Membrane Electrophoresis——Electrophoresis was carried out with Separax (Fuji Film Co., 6×21 cm long) using the following two buffers: A, 0.08 M pyridine–0.046 M acetic acid (pH 5.3); B, 0.025 M borax: 0.1 N sodium hydroxide (10: 1, pH 9.3), at 420 volts for 1 hr. The inside of the apparatus was cooled with dry ice. The sample was applied in a line at the center, and was visualized with 0.5% toluidine blue in 3% acetic acid. The sample gave a single spot at distances of 5.0 cm in buffer A and 4.3 cm in buffer B from the origin towards the anode.

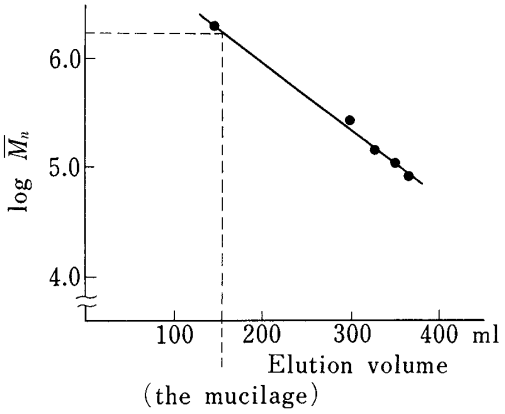


Fig. 5. Plot of Elution Volume against log $\bar{M}_n$ for Dextran Fractions on Sepharose 4B

Gel Chromatography——The sample (3 mg) was dissolved in water and applied to a column (2.6×86 cm) of Sepharose 4B. Elution was carried out by the ascending method with 0.1 M Tris-HCl buffer (pH 7.0). Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method.[21] Standard dextrans having known molecular weights were run on the column and gave the calibration curve shown in Fig. 5.

Hydrolysis, Isolation and TLC of Component Sugars——These were carried out as described in a previous report[22] of this series.

Determination of Components——Aldoses were analyzed by GLC as described in a previous report[16] of this series. Galacturonic acid was determined by a modification of the carbazole method.[23] The amino acid composition was estimated after hydrolysis with 6 N hydrochloric acid at 108° for 22 hr.

Determination of O-Acetyl Groups——The IR spectrum of the mucilage showed ester absorption bands. IR ν_{max}^{KBr} cm^{-1}: 1250, 1730 (ester).

The sample was hydrolyzed with 1 N hydrochloric acid containing propionic acid as an internal standard in a sealed tube at 100° for 2 hr. The hydrolysate was directly subjected to GLC under the same conditions as in a previous report[10] of this series.

Reduction of the Mucilage——The mucilage (153 mg) was suspended in water (50 ml), then 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (1.53 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 hr, then 2 M sodium borohydride (15 ml) was added gradually to the reaction mixture during 4 hr 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 50 ml. The product was reduced twice more under the same conditions. The final non-dialyzable fraction was applied to a column (5×78 cm) of Sephadex G-15. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 29 to 35 were combined and lyophilized. Yield, 84 mg.

Methylation——The carboxyl-reduced mucilage (8 mg) was dissolved in dimethyl sulfoxide (3 ml). Sodium hydride (20 mg) was mixed with dimethyl sulfoxide (4 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 (3 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 hr. The non-dialyzable fraction was concentrated to dryness. The product was methylated twice more under the same conditions. The non-dialzable fraction was extracted five times with chloroform (20 ml each). The combined extract was washed five times with water (100 ml

21) J.E. Hodge and B.T. Hoffreiter, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York and London, 1962, p. 388.
22) M. Tomoda, S. Kaneko, M. Ebashi, and T. Nagakura, *Chem. Pharm. Bull.*, **25**, 1357 (1977).
23) T. Bitter and H. Muir, *Anal. Biochem.*, **4**, 330 (1962).

each), then dried over sodium sulfate, and the filtrate was concentrated to dryness. The residue was dissolved in chloroform, and applied to a column (1.5×30 cm) of Sephadex LH-20. The column was eluted with chloroform, and fractions of 3 ml were collected. The eluates obtained from tubes 9 to 13 were combined and concentrated. The IR spectrum of the final residue showed no absorption near 3400 cm^{-1}. Yield, 9 mg. In the case of the original mucilage, the sample was methylated five times under the same conditions.

Analysis of the Methylated Products——The product (4 mg) was dissolved in 0.5 N sulfuric acid in 95% acetic acid (10 N sulfuric acid: acetic acid=5: 95; 0.8 ml) and heated at 80° for 16 hr, then 0.5 N sulfuric acid (0.8 ml) was added to the solution. The solution was treated at 100° for 3 hr. After neutralization with Dowex 2 (OH^-), the hydrolysate was dissolved in water (10 ml) in an ultrasonic bath for 20 sec, followed by reduction with sodium borohydride (20 mg) at 5° overnight. After treatment with Dowex 50W-X8 (H^+) up to pH 5, the filtrate was concentrated and boric acid was removed by repeated addition and evaporation of methanol. The product was acetylated with acetic anhydride–pyridine mixture (1: 1) at 100° for 1 hr. After concentration of the solution, the residue was dissolved in chloroform and subjected to GLC. GLC was carried out under condition A, using a column (0.3 cm×2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 180° with a nitrogen flow of 40 ml per min. GLC-MS was carried out under the same conditions, but using 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 II.

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

	Relative retention times[a)]	Main fragments (*m/e*)
1,2,5-Ac-3,4-Me-L-Rhamnitcl	0.88	43, 89, 129, 131, 189
1,5-Ac-2,3,4,6-Me-D-Galactitol	1.17	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,2,4,5-Ac-3-Me-L-Rhamnitol	1.62	43, 87, 101, 129, 143, 189, 203
1,4,5-Ac-2,3,6-Me-D-Galactitol	2.06	43, 45, 87, 99, 101, 113, 117, 233

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-).

Partial Hydrolysis and Isolation of an Oligosaccharide——The mucilage (200 mg) was suspended in 1 N sulfuric acid (200 ml) and heated under reflux at 100° for 2 hr. The resulting water-insoluble fraction (80 mg) was separated by centrifugation, then the supernatant was neutralized with barium carbonate, and 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 (2×18 cm) of DEAE-Sephadex A-25 (formate form). The column was eluted successively with water (75 ml), 0.1 M formic acid (90 ml), 0.2 M formic acid (220 ml), 0.5 M formic acid (140 ml), 1 M formic acid (140 ml), 1.5 M formic acid (140 ml), and 2 M formic acid (140 ml). Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method. The eluates obtained from the column were divided into three groups: Frac. 1, tubes 6 to 12; Frac. 2, tubes 44 to 54; Frac. 3, tubes 58 to 61. The yields were 50.6 mg in Frac. 1, 9.2 mg in Frac. 2, and 3.2 mg in Frac. 3. Frac. 1 contained galactose and rhamnose in a ratio of 11: 1. 2-*O*-α-(D-Galacturonic acid)-L-rhamnose and D-galacturonic acid were obtained directly from Fracs. 2 and 3, respectively.

A part of the partial hydrolysate (10 mg) was dissolved in water and applied to a column (2.6×89 cm) of Sephadex G-25. The column was eluted with water, and fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method. The disaccharide and monosaccharides were recovered from the eluates of tubes 44 to 49 and of tubes 82 to 91, respectively. Oligosaccharide V (MW=2010) obtained from Althaea-mucilage O[17)] was used as a standard sugar, and its elution volume was 190 ml.

Analysis of Fractions——Analysis of component sugars was carried out as described above. PPC by the descending method and paper electrophoresis with an alkaline borate buffer were carried out as described in a previous report[17)] of this series. In addition, samples were trimethylsilylated in the usual way, 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° per min from 180° to 300° at a nitrogen flow rate of 30 ml per min. The retention times (min) of the trimethylsilyl derivatives were 2.1 and 2.7 in L-rhamnose, 3.9, 4.3 and 5.0 in D-galactose, 4.3, 5.2 and 6.6 in D-galacturonic acid, and 20.8 and 21.3 in 2-*O*-α-(D-galacturonic acid)-L-rhamnose.

Periodate Oxidation——The sample (120 mg) was oxidized with 0.05 M sodium metaperiodate (60 ml) at 3° in the dark. The periodate consumption was measured by a spectrophotometric method.[24)] The

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

oxidation was completed after five days. The formic acid liberation was measured by titration with 0.01 N sodium hydroxide after addition of ethylene glycol, and a value of 0.16 mol per mol of anhydrosugar unit was obtained.

Reduction and Isolation of the Product——The residue of the reaction mixture was treated with ethylene glycol (0.3 ml) for 1 hr, then sodium borohydride (300 mg) was added. After standing at 5° for 16 hr, the solution was adjusted to pH 5 by addition of acetic acid, followed by dialysis 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 26 to 41 were combined and lyophilized. The product was analyzed as described above.

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