Chem. Pharm. Bull.
28(5)1546—1551(1980)

Plant Mucilages. XXV.[1] Isolation and Structural Features of a Mucilage, "Abelmoschus-mucilage G," from the Roots of *Abelmoschus glutinotextilis*

MASASHI TOMODA, MAEMI ARAI, YŌKO SUZUKI, MASAKO OHMURA,
and HIROMI TAKAYAMA

Kyoritsu College of Pharmacy[2]

(Received November 17, 1979)

A representative mucilage, named Abelmoschus-mucilage G, has been isolated from the roots of *Abelmoschus glutinotextilis* KAGAWA. The final preparation was homogeneous as determined by ultracentrifugal analysis, cellulose acetate membrane electrophoresis, and gel chromatography. Its water solution gave the high intrinsic viscosity value of 52.8. It was composed of acidic polysaccharide and protein in a ratio of approximately 3.9: 1.0, and its molecular weight was estimated to be 67900. Analysis of component sugars, together with reduction and methylation, and partial degradation studies revealed the structural features of the polysaccharide moiety in the mucilage.

Keywords——*Abelmoschus glutinotextilis*; Abelmoschus-mucilage G; ultracentrifugal analysis; electrophoresis; gel chromatography; intrinsic viscosity; analysis of components; reduction and methylation analysis; partial acid hydrolysis; structural features of polysaccharide moiety

In the previous papers of this series,[3,4] the isolation and structural features of a representative mucilage, named Abelmoschus-mucilage M, from the roots of *Abelmoschus manihot* have been reported from this laboratory. In the present study we obtained a new representative mucilage from the roots of *Abelmoschus glutinotextilis* KAGAWA. Its properties and main structural features are described in the present paper. The extract with water from the root of this plant contains many mucilages. It has been used as a size similar to that obtained from the root of *Abelmoschus manihot*, and its fruit is used as a food.

The fresh roots were crushed 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 acetone, then the precipitate was dissolved in water, reprecipitated with acetone, 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 Sephadex G-200. It showed a positive specific rotation ($[\alpha]_D^{20}$ $+53.3°$ in H_2O, $c=0.1$), and its solution in water gave the high intrinsic viscosity value of 52.8 at 30°. The relative viscosity of the solution of the pure mucilage was about 2.2 times that of the crude mucilage. In view of this result and the yield, it is conceivable that the pure mucilage is the representative mucous substance in the water extract from the roots. Gel chromatography gave a value of 67900 for the molecular weight. The name "Abelmoschus-mucilage G" is proposed for this substance.

---

1) Part XXIV: M. Tomoda, N. Satoh, and K. Shimada, *Chem. Pharm. Bull.*, **28**, 824 (1980).

2) Location: *1-5-30, Shibakôen, Minato-ku, Tokyo, 105, Japan.*

3) M. Tomoda and Y. Suzuki, *Chem. Pharm. Bull.*, **25**, 3061 (1977).

4) M. Tomoda, Y. Suzuki, and N. Satoh, *Chem. Pharm. Bull.*, **27**, 1651 (1979).

As component sugars of the mucilage, L-rhamnose, D-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.

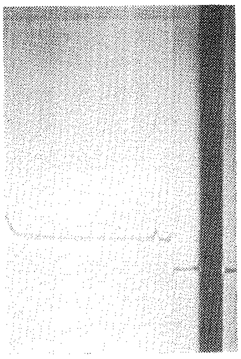
Fig. 1. Ultracentrifugal Pattern of Abelmoschus-mucilage G (0.1% in H_2O, 26°, 18 min, 60000 rpm, Hitachi UCA-1A ultracentrifuge)

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 determinations of the neutral component sugars of the original and the carboxyl-reduced mucilages were carried out by gas-liquid chromatography (GLC) of alditol acetates derived from the hydrolysates, and hexuronic acids in the original mucilage were estimated by a colorimetric method. The results showed that the mucilage contained 24.5% rhamnose and 51.1% hexuronic acids, and that the molar ratio of rhamnose: galactose: glucose was 10.0: 9.8: 7.6 in the carboxyl-reduced mucilage.

The mucilage contained 2.88% nitrogen. The determination of protein content was carried out by the method of Lowry, *et al.*,[6] and a value of 19.4% was obtained. The amino acid composition of the mucilage after hydrolysis with 6 N hydrochloric acid is listed in Table I, together with that of Abelmoschus-mucilage M. No nitrogen-containing compound, other than amino acids, was detected in the hydrolysate. There is no significant difference in amino acid composition between Abelmoschus-mucilage G and Abelmoschus-mucilage M except for the values of aspartic acid, methionine and tyrosine.

TABLE I. Amino Acid Compositions (Molar Percent)

	Abelmoschus-mucilage G	Abelmoschus-mucilage M
Lysine	6.28	7.05
Histidine	2.17	2.42
Arginine	3.49	4.46
Aspartic acid	11.26	6.75
Threonine	5.50	5.28
Serine	6.12	5.44
Glutamic acid	11.39	12.58
Proline	5.64	5.97
Glycine	9.59	10.34
Alanine	8.39	8.93
Valine	8.17	6.62
Methionine	1.01	2.03
Isoleucine	6.10	6.49
Leucine	8.73	7.71
Tyrosine	1.76	2.94
Phenylalanine	4.41	4.97

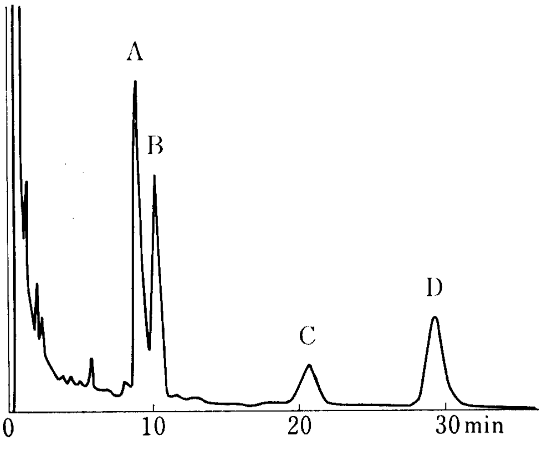

Fig. 2. Gas-Liquid Chromatogram of O-Methylated Alditol Acetates obtained from the Methylated Carboxyl-reduced Mucilage

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

---

5) R.L. Taylor and H.E. Conrad, *Biochemistry*, **11**, 1383 (1972).

6) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

The methylation of the carboxyl-reduced mucilage was performed with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide.(7) The fully methylated product was hydrolyzed successively with formic acid and dilute sulfuric acid. The products were analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) after conversion into alditol acetates,(8) and identified as 3,4-di-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, 2,3,6-tri-*O*-methyl-D-galactopyranose, and 2,6-di-*O*-methyl-D-galactopyranose. They were obtained in a molar ratio of 4.0: 3.0: 1.0: 3.0 (Fig. 2).

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). Four oligosaccharides (I to IV) were obtained by stepwise elution with dilute formic acid and were purified by rechromatography.

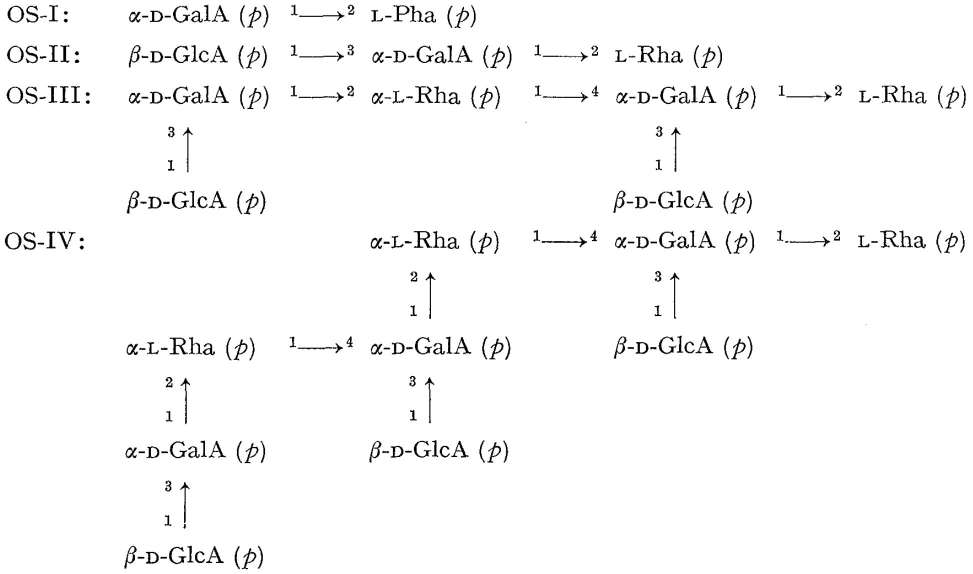

Chart 1. Structural Features of the Oligosaccharides (OS=Oligosaccharide)

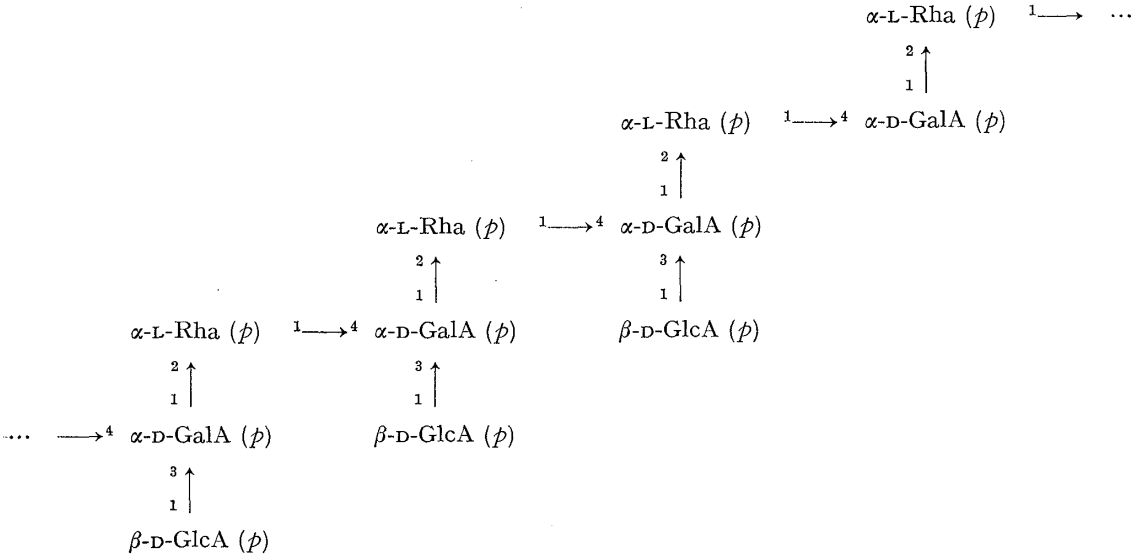

Chart 2. A Possible Structural Fragment of the Polysaccharide Moiety of Abelmoschus-mucilage G

---

7) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

8) H. Björndal, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).

Based on the results of component sugar analysis, and by comparing their chromatographic and electrophoretic properties and the values of specific rotation with those of authentic samples,[1,4] I to IV were identified as the following four oligosaccharides (Chart 1).

Based on the accumulated evidence described above, it can be concluded that the polysaccharide moiety of the mucilage has a main chain composed of (1→4)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranosyl repeating units. Three-quarters of the D-galacturonic acid residues in the main chain possess β-D-glucuronic acid residues at position 3, but a quarter of the D-galacturonic acid residues has no branch. A possible structure of the polysaccharide moiety of Abelmoschus-mucilage G is shown in Chart 2.

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 of *Abelmoschus glutinotextilis*, *Abelmoschus manihot*,[3,4] and *Althaea officinalis*,[1,9] and from the inner bark of *Hydrangea paniculata*.[10,11] Abelmoschus-mucilage M appears to possess the simplest repeating structure in its polysaccharide moiety. On the other hand, the partial lack of branches at position 3 of the D-galacturonic acid residues in the main chain is characteristic of Abelmoschus-mucilage G. Further studies on the mucilages from other plants in the Malvaceae family are in progress.

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 Hitachi KLA-5 amino acid analyzer. Infrared (IR) spectra were recorded on a JASCO IRA-2 infrared spectrophotometer.

Isolation of the Mucilage——The material was obtained in October 1978 from plants cultivated in Saitama prefecture. The fresh roots (1025 g), which contain 61.2% water, were crushed, then extracted with water (5000 ml) under stirring for 3 hr at room temperature. After centrifugation, the extraction was repeated with water (5000 ml). The extracts were combined and poured into two volumes of ethanol, then filtered. The precipitate was treated with ethanol, then dried *in vacuo*. A light brown powder (7.62 g) was obtained. A part of the crude mucilage (3.57 g) was dissolved in water (700 ml) and 0.02% sodium sulfate (700 ml) was added, followed by 10% cetyltrimethyl ammonium bromide (200 ml). The precipitate was separated by centrifugation, and dissolved in 2M sodium chloride (500 ml). The solution was centrifuged to remove small amounts of impurities. Acetone (750 ml) was added to the supernatant thus obtained. The resulting precipitate was dissolved in water, and treated again with acetone, followed by dialysis against running distilled water. Abelmoschus-mucilage G (2.21 g) was obtained as a grayish-white powder after lyophilization.

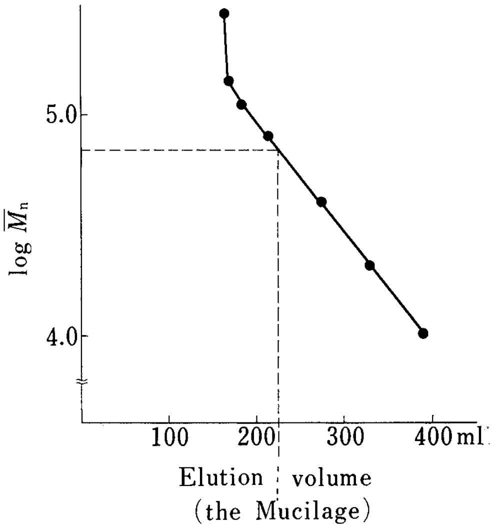

Fig. 3. Plot of Elution Volume against log $\bar{M}_n$ for Dextran Fractions on Sephadex G-200

Cellulose Acetate Membrane Electrophoresis——Electrophoresis was carried out with Separax (Fuji Film Co., 6×21 cm long) using the following two buffers: A, 0.04M acetic acid: 0.08M pyridine (1:1, pH 5.4); B, 0.025M borax: 0.1N 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 5.2 cm in buffer A and 4.9 cm in buffer B from the origin towards anode.

---

[9] M. Tomoda, S. Kaneko, M. Ebashi, and T. Nagakura, *Chem. Pharm. Bull.*, **25**, 1357 (1977).

[10] M. Tomoda and N. Satoh, *Chem. Pharm. Bull.*, **24**, 230 (1976).

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

Hydrolysis, Isolation and TLC of Component Sugars——These were carried out as described in a previous report[3] of this series.

Gel Chromatography——The sample (3 mg) was dissolved in 0.1% ammonium hydroxide and applied to a column (2.6 × 90 cm) of Sephadex G-200. 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.[12] Standard dextrans having known molecular weights were run on the column and gave the calibration curve shown in Fig. 3.

Determination of Components——Hexuronic acids were determined by a modification of the carbazole method.[13] Based on the result of analyses of the carboxyl-reduced product, the values of hexuronic acids were calculated on the assumption that the molar ratio of galacturonic acid and glucuronic acid is 4: 3. Neutral sugars in the original and the carboxyl-reduced mucilages were analyzed by GLC as described in a previous report[4] of this series. The amino acid composition was estimated after hydrolysis with 6 N hydrochloric acid at 108° for 22 hr.

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 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 (10 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 30 ml. The product was reduced five more times under the same conditions. The final non-dialyzable fraction was applied to a column (5 × 82 cm) of Sephadex G-15. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 26 to 32 were combined and lyophilized. Yield, 24.5 mg.

Methylation of the Reduction Product——A sample (12 mg) was dissolved in dimethyl sulfoxide (2 ml). Sodium hydride (20 mg) was mixed with dimethyl sulfoxide 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 (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 overnight. The non-dialyzable fraction was concentrated to dryness. The product was methylated once 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 methylated three more times under the same conditions. The IR spectrum of the final residue showed no absorption near 3400 cm^{-1}.

Analysis of the Methylated Products——The product was treated successively with 88% formic acid at 90° for 16 hr and 0.5 N sulfuric acid at 100° for 2.5 hr. After neutralization with Dowex 2 (OH^-), the hydrolysate was derivatized into partially methylated alditol acetates and analyzed by GLC and GLC-MS as described in a previous report[4] of this series. 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-Rhamnitol	0.88	43, 89, 129, 131, 189
1,5-Ac-2,3,4,6-Me-D-Glucitol	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,4,5-Ac-2,3,6-Me-D-Galactitol	2.04	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-).

Partial Hydrolysis and Isolation of Oligosaccharides——The mucilage (0.5 g) was suspended in 1 N sulfuric acid (50 ml) and heated under reflux at 100° for 2 hr. The solution was neutralized with barium carbonate, and after filtration, the filtrate was passed through a column (1 × 12 cm) of Dowex 50W-X8 (H^+). The

---

12) 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.

13) T. Bitter and H. Muir, *Anal. Biochem.*, 4, 330 (1962).

Table III. Specific Rotations, Sugar Compositions, R_{Rha} and M_{GalA} Values of Oligosaccharides, and Retention Times of Alditol Acetates of the Carboxyl-reduced Oligosaccharides

Oligosaccharides	$[\alpha]_D^{20}$ in H_2O	Sugar compositions	PPC (R_{Rha})	Paper electrophoresis (M_{GalA})	GLC (t_R) (min)
I	+93.7°	GalA: Rha=1: 1	0.79	0.73	18.3
II	+83.9°	GlcA: GalA: Rha=1: 1: 1	0.66	1.10	29.3
III	+80.5°	GlcA: GalA: Rha=1: 1: 1	0.39	1.32	—
IV	+77.8°	GlcA: GalA: Rha=1: 1: 1	0.22	1.38	—

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

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 (125 ml), 0.1 M formic acid (60 ml), 0.2 M formic acid (225 ml), 0.3 M formic acid (135 ml), 0.4 M formic acid (200 ml), 0.5 M formic acid (200 ml), 0.6 M formic acid (100 ml), 0.8 M formic acid (100 ml), 1 M formic acid (250 ml), and 1.5 M formic acid (300 ml). Fractions of 5 ml were collected and analyzed by the phenol-sulfuric acid method. The eluate obtained from the column were divided into seven groups: Frac. 1, tubes 8 to 13; Frac. 2, tubes 50 to 59; Frac. 3, tubes 95 to 101; Frac. 4, tubes 131 to 139; Frac. 5, tubes 158 to 176; Frac. 6, tubes 250 to 268; Frac. 7, tubes 280 to 324. The yields were 37.6 mg in Frac. 1, 7.6 mg in Frac. 2, 3.5 mg in Frac. 3, 2.9 mg in Frac. 4, 24.3 mg in Frac. 5, 26.0 mg in Frac. 6, and 19.6 mg in Frac. 7. I, II, III, and IV were obtained from Fracs. 2, 5, 6, and 7, respectively, after rechromatography under the same conditions.

Analysis of the Oligosaccharides——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 the preceding report[1] of this series. GLC of the alditol acetates of the carboxyl-reduced derivatives was carried out as described in a previous report.[4] The results are listed in Table III.

Acknowledgement The authors wish to thank Prof. G. Matsumura, School of Pharmaceutical Sciences, Showa University, for ultracentrifugal analysis, and Dr. S. Kobayashi, Division of Physiology, this College, for the determination of amino acids.