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# Plant Mucilages. XXIV.[1] The Structural Features of Althaea-mucilage O, a Representative Mucous Polysaccharide from the Roots of *Althaea officinalis*

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Partial acid hydrolysis of Althaea-mucilage O, a representative mucous polysaccharide isolated from the roots of *Althaea officinalis* L., led to the isolation of five oligosaccharides. Analysis of their components, as well as reduction and methylation, and partial degradation studies showed that these oligosaccharides are *O*-α-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose, *O*-β-(D-glucopyranosyluronic acid)-(1→3)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose, and the hexasaccharide, the nonasaccharide, and the dodecasaccharide composed of a repeating unit having the structure of the trisaccharide through position 4 of the D-galacturonic acid residue. The polysaccharide was also subjected to chromium trioxide oxidation, and to controlled Smith degradation. The configuration and the mode of branching were determined. The structural features of the mucilage are discussed.

**Keywords**——Althaea-mucilage O; *Althaea officinalis*; partial hydrolysis; isolation of five oligosaccharides; reduction and methylation analysis; proton magnetic resonance; structures of five oligosaccharides; chromium trioxide oxidation; controlled Smith degradation; structural features of polysaccharide

The isolation and properties of Althaea-mucilage O, the representative mucous substance obtained from the root of *Althaea officinalis* L., were reported in a previous paper of this series.[3] The substance is an acidic polysaccharide composed of L-rhamnose, D-galactose, D-galacturonic acid, and D-glucuronic acid in a molar ratio of approximately 3 : 2 : 3 : 3. Methylation studies of the original and the carboxyl-reduced polysaccharides showed that the substance was composed of 1→2 linked L-rhamnopyranose units, 1→2 linked L-rhamnopyranose units having branches at position 4, D-galactopyranosyl-(1→4)-D-galactopyranose side chains, 1→4 linked D-galactopyranosyluronic acid units having branches at position 3, and terminal D-glucopyranosyluronic acid units in a ratio of approximately 2 : 1 : 1 : 3 : 3.

The purpose of this study was to examine the sequence of the component sugars and the configurations of the glycosidic linkages. It was found that the main chain of the mucilage is similar in structural type to that of the polysaccharide moiety of Abelmoschus-mucilage M,[1,4] although the latter possesses no galactosyl galactose side chain.

The mucilage was hydrolyzed with 1 N sulfuric acid at 100° for 2 hr, then neutralized and applied to a column of Dowex 50W-X8 ($H^+$). The eluate with water was applied to a column of diethylaminoethyl (DEAE)-Sephadex A-25 (formate form), and five oligosaccharides (I to V) were obtained from the fractions by stepwise elution with dilute formic acid. Each of them was further purified by paper partition chromatography (PPC). The preparation of the partial hydrolysates is summarized in Chart 1.

The homogeneity of each oligosaccharide was checked by PPC and by paper electrophoresis. Table I gives the $R_{\mathrm{Rha}}$ values on PPC and the mobilities on paper electrophoresis.

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[1] Part XXIII: M. Tomoda, Y. Suzuki, and N. Satoh, *Chem. Pharm. Bull.*, **27**, 1651 (1979).

[2] Location: *1-5-30, Shibakōen, Minato-ku, Tokyo, 105, Japan.*

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

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

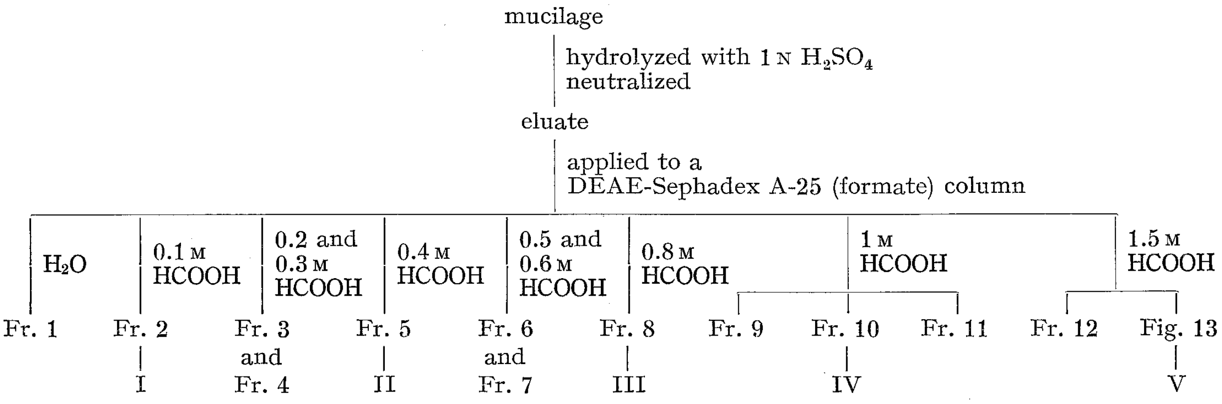

Chart 1. Isolation of the Oligosaccharides

Table I. $R_{Rha}$ and $M_{GalA}$ Values of the Oligosaccharides

| Oligosaccharides | PPC ($R_{Rha}$) | Paper electrophoresis ($M_{GalA}$)[a] |
|---|---|---|
| I | 0.79 | 0.73 |
| II | 0.66 | 1.10 |
| III | 0.39 | 1.32 |
| IV | 0.22 | 1.38 |
| V | 0.13 | 1.42 |

For the solvent and the buffer, see "Experimental."
*a*) Electrophoretic mobility relative to that of D-galacturonic acid.

Cellulose thin–layer chromatography (TLC) of the hydrolysates of the oligosaccharides was carried out to identify their component sugars. Quantitative determination of the component sugars was carried out by gas–liquid chromatography (GLC) of alditol acetates derived from the hydrolysates of the carboxyl-reduced oligosaccharides as described in the previous paper,[1] and by colorimetric methods. All five oligosaccharides bear a L-rhamnose residue as a common reducing terminal. The results and specific rotations of the oligosaccharides in water are listed in Table II.

Table II. Specific Rotations and Component Sugars of the Oligosaccharides

| Oligosaccharides | Specific rotations in water (final values) | Components | Molar ratios | Rhamnitol in reduced samples |
|---|---|---|---|---|
| I | $[\alpha]_D^{20}$ +93.2° ($c$=0.3) | GalA: Rha | 1.0:1.0 | 45.4% |
| II | $[\alpha]_D^{20}$ +84.1° ($c$=1.7) | GlcA: GalA: Rha | 1.0:1.1:1.0 | 31.8% |
| III | $[\alpha]_D^{20}$ +81.4° ($c$=1.4) | GlcA: GalA: Rha | 1.1:1.0:1.0 | 15.9% |
| IV | $[\alpha]_D^{20}$ +78.0° ($c$=2.2) | GlcA: GalA: Rha | 1.0:1.0:1.0 | 10.5% |
| V | $[\alpha]_D^{20}$ +73.1° ($c$=2.1) | GlcA: GalA: Rha | 1.0:1.0:1.0 | 8.3% |

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

Each carboxyl-reduced oligosaccharide was methylated with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide.[5] The fully methylated products were hydrolyzed and analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) after conversion into alditol acetates.[6] The products obtained by hydrolysis of the methylated carboxyl-reduced oligosaccharides are listed in Table III.

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5) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
6) H. Björndall, B. Lindberg, and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).

Table III. Products isolated from the Hydrolysates of Methylated Carboxyl-reduced Oligosaccharides and Their Molar Ratios

| Oligosaccharides | Products | Molar ratios |
|---|---|---|
| Carboxyl-reduced I | 2,3,4,6-Me-Gal: 3,4-Me-Rha | 1.1: 1.0 |
| Carboxyl-reduced II | 2,3,4,6-Me-Glc: 2,4,6-Me-Gal: 3,4-Me-Rha | 1.0: 1.1: 1.0 |
| Carboxyl-reduced III | 2,3,4,6-Me-Glc: 2,4,6-Me-Gal: 2,6-Me-Gal: 3,4-Me-Rha | 2.0: 1.0: 0.9: 1.8 |
| Carboxyl-reduced IV | 2,3,4,6-Me-Glc: 2,4,6-Me-Gal: 2,6-Me-Gal: 3,4-Me-Rha | 2.9: 1.1: 1.7: 3.0 |
| Carboxyl-reduced V | 2,3,4,6-Me-Glc: 2,4,6-Me-Gal: 2,6-Me-Gal: 3,4-Me-Rha | 4.0: 1.2: 2.7: 4.4 |

Abbreviations: Me=methyl; Gal=D-galactose; Rha=L-rhamnose; Glc=D-glucose (*e.g.*, 2,3,4,6-Me-Gal=2,3,4,6-tetra-*O*-methyl-D-galactose).

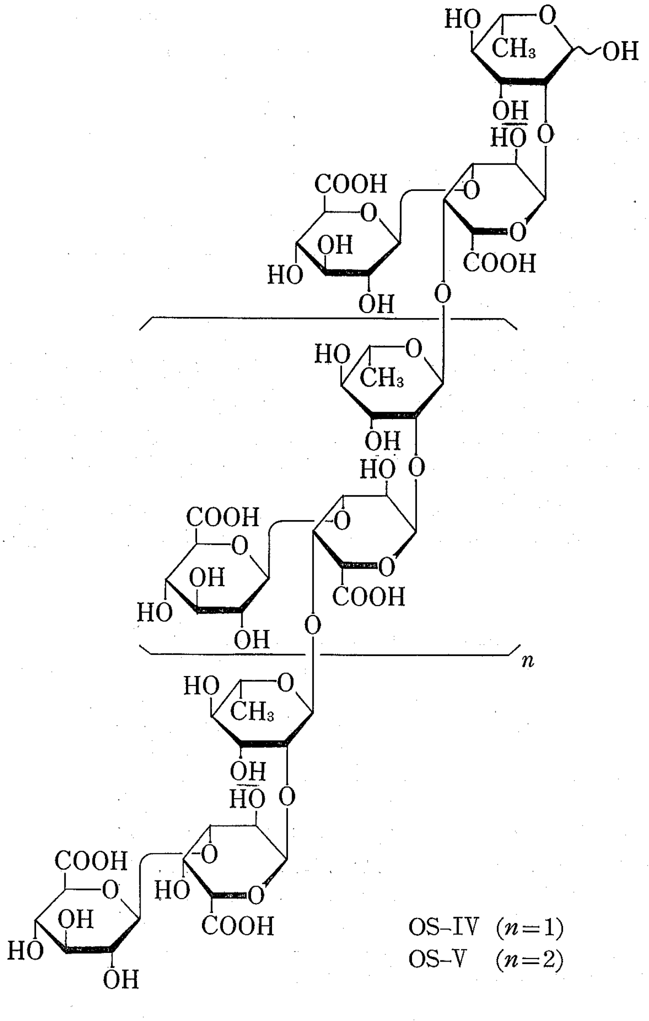

Chart 2. Structures of Oligosaccharides IV and V (OS=oligosaccharide)

Based on the results of the methylation analysis, and by comparing their chromatographic properties, the proton magnetic resonance (PMR) spectra, and the values of specific rotation with those of authentic samples,[1] I, II, and III were identified as *O*-α-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose, *O*-β-(D-glucopyranosyluronic acid)-(1→3)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose, and *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→3)]-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-L-rhamnopyranose, respectively.

Marked production of II was observed on partial hydrolysis of IV and V with 1 N sulfuric acid at 100° for 1 hr. The hydrolysate was analyzed by PPC and determined by GLC after conversion of the carboxyl-reduced derivatives into alditol acetates. The ratios of II, disaccharides, and monosaccharides were 7.5: 1.0: 2.3 in the hydrolysate from IV, and 7.8: 1.0: 2.2 in the hydrolysate from V. In addition, the formation of III was detected in the hydrolysates of IV and V by PPC and by paper electrophoresis.

Oligosaccharides IV and V were reduced with sodium borohydride. The PMR spectra of the resulting alditols derived from IV and V showed four anomeric proton signals at δ 4.80 (3H, d, $J$=7 Hz), δ 5.20 (2H, d, $J$=2 Hz), δ 5.32 (1H, d, $J$=3 Hz), and δ 5.48 (2H, d, $J$=3 Hz), and a methyl signal at δ 1.30 (9H, d, $J$=6 Hz) in the non-reducing derivative from IV, and four anomeric proton signals at δ 4.80 (4H, d, $J$=7 Hz), δ 5.20 (3H, d, $J$=2 Hz), δ

5.31 (1H, d, $J=3$ Hz), and $\delta$ 5.47 (3H, d, $J=3$ Hz), and a methyl signal at $\delta$ 1.28 (12 H, d, $J=6$ Hz) ins the non-reducing derivative from V. These data suggest that the D-glucuronic acid residues in IV and V are $\beta$-linked, and that the L-rhamnose and D-galacturonic acid residues in IV and V are $\alpha$-linked.[1]

Based on the accumulated evidence described above, IV and V were identified as a nonasaccharide and a dodecasaccharide composed of a repeating unit having the structure II (Chart 2).

The combined yields of II, III, IV and V went up to over 60% of the total mono- and oligosaccharides obtained from the partial hydrolysate of the mucilage. Consequently, it can be concluded that these oligosaccharides do represent the structural features of the main chain in Althaea-mucilage O.

The glycosidic linkage of the D-galactose residue is much more easily cleaved than those of the other component sugars in the mucilage.[3] Therefore, no oligosaccharide having D-galactose as a component was obtained by partial acid hydrolysis. The results of partial hydrolysis of the mucilage during the carboxyl-reducing reaction and the methylation studies,[3] however, indicate that branches composed of 4-*O*-D-galactopyranosyl D-galactopyranose are attached to position 4 of about one-third of the L-rhamnose moieties in the main chain.

Oxidation of the acetylated mucilage with chromium trioxide in acetic acid resulted in zero recovery of D-galactose after 1 hr. This result indicates that the D-galactose residues in the mucilage are $\beta$-linked.[7]

The mucilage was subjected to periodate oxidation followed by reduction with sodium borohydride.[8] As a result of the oxidation, 1.18 mol of periodate was consumed per mol of component anhydro sugar units with the liberation of 0.16 mol of formic acid. The reduction product was isolated and selective cleavage of the acetal linkages was achieved by mild hydrolysis with dilute sulfuric acid. After reduction, the controlled Smith degradation products were separated by gel chromatography on Sephadex G-15. In addition to galacturonic acid-(1→2)-glycerol (=product d), three products (a to c) containing galacturonic acid, rhamnose, and glycerol were obtained. Analysis revealed that the molar ratios of galacturonic acid : rhamnose : glycerol in the products were 4: 3: 1 in product a, 3: 2: 1 in product b, and 2: 1: 1 in product c. The molecular weights were 1200 for a, 910 for b, 590 for c, and 260 for d. The ratio of the yields of the products a, b, c, and galacturonic acid-(1→2)-glycerol was 1.5: 1.0: 1.2: 1.7.

Based on the results of controlled Smith degradation, we concluded that there are three types of the positions of the galactosyl galactose side chains linking to rhamnose residues

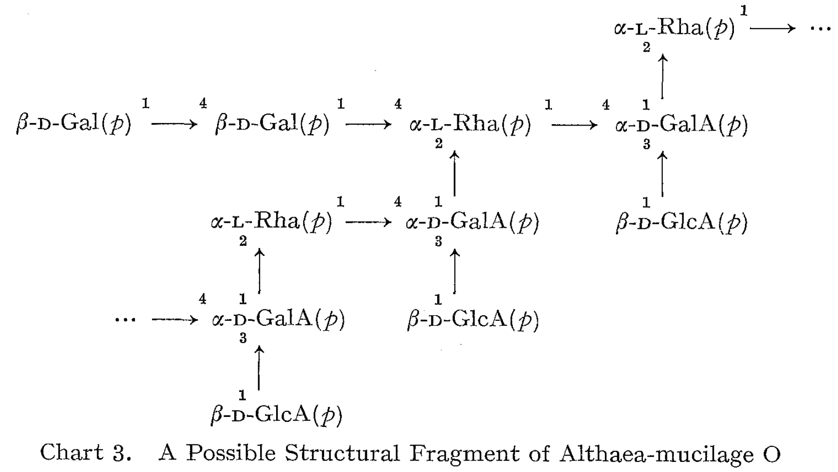

Chart 3. A Possible Structural Fragment of Althaea-mucilage O

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7) S.J. Angyal and K. James, *Aust. J. Chem.*, 23, 1209 (1970).

8) M. Abder-Akher, J.K. Hamilton, R. Montgomery, and F. Smith, *J. Am. Chem. Soc.*, 74, 4970 (1952).

in the main chain. The branching seems to be rather random. In the two types which produce the controlled Smith degradation products a and b, the side chains are linked to neighboring rhamnose residues. In the simplest part, Althaea-mucilage O contains the following undecasaccharide repeating unit (Chart 3).

As already reported in previous papers,[1,9] of this series, the component unit having *O*-β-(D-glucopyranosyluronic acid)-(1→3)-*O*-α-(D-galactopyranosyluronic acid)-(1→2)-*O*-α-L-rhamonopyranose is common in the mucilages from the roots of *Althaea officinalis* and *Abelmoschus manihot* (Malvaceae), and from the inner barks of *Hydrangea paniculata* (Saxifragaceae). Among them, however, the last mucilage, paniculatan,[9] does not possess a repeating unit having the structure IV. The nonasaccharide IV is the common repeating structural unit in both Althaea-mucilage O and Abelmoschus-mucilage M, but the presence of 4-*O*-β-D-galactopyranosyl-D-galactopyranose side chains is characteristic of the former mucilage. Further studies on the mucilages from other plants in the Malvaceae family are now 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. Infrared (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. PMR spectra were recorded with a JEOL MH-100 NMR spectrometer in heavy water containing acetone as an internal standard at 70°.

**Partial Hydrolysis and Isolation of Oligosaccharides**——The mucilage (1.0 g) was suspended in 1 N sulfuric acid (100 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 (1×15 cm) of Dowex 50W-X8 ($H^+$). The eluate with water was concentrated and applied to a column (1.5×28 cm) of DEAE-Sephadex A-25 (formate form, Pharmacia Co.). The column was eluted successively with water (100 ml), 0.05 M formic acid (100 ml), 0.1 M formic acid (230 ml), 0.2 M formic acid (200 ml), 0.3 M formic acid (200 ml), 0.4 M formic acid (270 ml), 0.5 M formic acid (250 ml), 0.6 M formic acid (220 ml), 0.8 M formic acid (300 ml), 1 M formic acid (500 ml), and 1.5 M formic acid (260 ml). Fractions of 10 ml were collected and analyzed by the phenol–sulfuric acid method.[10] The eluates obtained from the column were divided into thirteen groups: Frac. 1, tubes 4 to 7; Frac. 2, tubes 32 to 40; Frac. 3, tubes 53 to 58; Frac. 4, tubes 67 to 78; Frac. 5, tubes 96 to 107; Frac. 6, tubes 111 to 124; Frac. 7, tubes 145 to 150; Frac. 8, tubes 167 to 180; Frac. 9, tubes 188 to 215; Frac. 10, tubes 216 to 229; Frac. 11, tubes 230 to 235; Frac. 12, tubes 243 to 252; Frac. 13, tubes 253 to 263. The yields were 98.9 mg in Frac. 1, 12.9 mg in Frac. 2, 6.0 mg in Frac. 3, 2.3 mg in Frac. 4, 50.5 mg in Frac. 5, 7.4 mg in Frac. 6, 0.8 mg in Frac. 7, 68.2 mg in Frac. 8, 16.9 mg in Frac. 9, 64.5 mg in Frac. 10, 4.2 mg in Frac. 11, 8.0 mg in Frac. 12, and 61.4 mg in Frac. 13. I, II, III, IV, and V were obtained from Fracs. 2, 5, 8, 10, and 13, respectively, after purification by PPC with solvent A. IV was obtained as a water-soluble colorless powder by lyophilization, and decomposed at 177—180°. *Anal.* Calcd for $C_{54}H_{80}O_{49}$: C, 42.86; H, 5.33. Found: C, 42.17; H, 5.01. V was obtained as a water-soluble colorless powder by lyophilization, and decomposed at 181—184°. *Anal.* Calcd for $C_{72}H_{106}O_{65}$: C, 42.99; H, 5.31. Found: C, 42.73; H, 5.26.

**PPC and Paper Electrophoresis**——PPC was carried out with Tōyō-Roshi No. 51 paper by the descending method at 30°. The following solvent system was used; A, AcOEt: pyridine: AcOH: $H_2O$ (5: 5: 1: 3). Electrophoresis was carried out with Tōyō-Roshi No. 51 paper (12×38 cm long) and 0.025 M borax: 0.1 N sodium hydroxide buffer (10: 1, pH 9.4) at 570 volts for 2 hr. Samples were applied in a line at the center. The inside of the apparatus was cooled with dry ice. The oligosaccharides were visualized with silver nitrate reagent.[11]

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

**Determination of Component Sugars**——The carboxyl groups in the samples were reduced, hydrolyzed and derivatized into alditol acetates, then analyzed by GLC as described in the preceding report[1] of this series. The determination of the reducing terminal of each sample was also carried out under the same

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

[10] 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.

[11] W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).

conditions by GLC of the alditol acetates. Rhamnose and hexuronic acids were estimated by the thioglycolic acid method[12] and by a modified carbazole method,[13] respectively.

**Methylation of Carboxyl-reduced Oligosaccharides**——Each carboxyl-reduced oligosaccharide (1 mg) was dissolved in dimethyl sulfoxide (1 ml). Sodium hydride (5 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 (1 ml) was added and the mixture was stirred overnight at room temperature. All procedures were carried out under nitrogen. After dilution with water (15 ml), the mixture was extracted four times with chloroform (15 ml each). The combined extract was washed five times with water (60 ml each), then dried over sodium sulfate, and the filtrate was evaporated to dryness. The residue was methylated twice more under the same conditions. The IR spectrum of the final residue showed no absorption near 3400 $cm^{-1}$.

**Analysis of Methylated Products**——The product was successively treated with 85% formic acid (0.2 ml) at 100° for 1.5 hr and 0.5 N sulfuric acid (0.2 ml) at 100° for 6 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 the preceding report[1] of this series.

**Partial Hydrolysis of IV and V and Analysis of the Products**——These procedures were carried out as described in the preceding report[1] of this series.

**Chromium Trioxide Oxidation**——The mucilage (8.8 mg) was dissolved in formamide (0.8 ml), then treated with acetic anhydride (0.8 ml) and pyridine (0.6 ml) at room temperature for three days with stirring. After concentration, the same reaction was repeated five times. The acetylated polysaccharide with *myo*-inositol hexaacetate (1.2 mg) as an internal standard was dissolved in glacial acetic acid (1 ml), and chromium trioxide (10 mg) was added to this solution. The reaction mixture was held in an ultrasonic bath at room temperature for 1 hr. The resulting solution was poured into water (5 ml), which was then extracted three times with chloroform (5 ml each). The extracts were combined and washed sequentially with water (15 ml each), saturated aqueous sodium hydrogen carbonate (15 ml each), and water (15 ml each) three times. The chloroform solution was dried over sodium sulfate and the filtrate was concentrated. The residue was successively treated with 85% formic acid (1 ml) at 100° for 3 hr and 0.5 N sulfuric acid in 90% acetic acid (1 ml) at 100° for 6 hr. After neutralization with Dowex 2 ($OH^-$), the hydrolysate was derivatized into alditol acetates and analyzed by GLC as described above. The retention time (min) of *myo*-inositol hexaacetate was 22.0. It was found that 100% of the galactose and 35% of the rhamnose residues were consumed.

**Controlled Smith Degradation**——The mucilage (230 mg) was oxidized with 0.05 M sodium metaperiodate (120 ml) at 3° for seven days in the dark. The periodate consumption was measured by a spectrophotometric method.[14] The sample consumed 1.02 mol of periodate per anhydro component sugar unit. The reaction mixture was treated with ethylene glycol (0.6 ml) for 1 hr, then reduced with sodium borohydride at 5° for 16 hr. After the addition of acetic acid up to pH 5, the solution was dialyzed against running water overnight, then concentrated and applied to a column (5 × 70 cm) of Sephadex G-15. The column was eluted with water, and fractions of 50 ml were collected and analyzed by the phenol–sulfuric acid method. The eluates obtained from tubes 12 to 20 were combined, concentrated and lyophilized. Yield, 163 mg. The product was oxidized again with 0.025 M sodium metaperiodate (80 ml) at 3° for five days. The sample consumed 0.16 mol of periodate per component unit and no further oxidation was observed after seven days. After treatment with ethylene glycol, followed by reduction with sodium borohydride, the resulting solution was dialyzed, concentrated and applied to a column (5 × 82 cm) of Sephadex G-15 as described above. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 26 to 37 were combined, concentrated and lyophilized. Yield, 160 mg. The product was hydrolyzed with 1 N sulfuric acid at 20° for five days. After neutralization with barium carbonate, followed by reduction with sodium borohydride, the resulting solution was passed through a column (1 × 3.5 cm) of Dowex 50W-X8 ($H^+$). A quarter of the eluate was applied to a column (5 × 82 cm) of Sephadex G-15. The column was eluted with water, and fractions of 10 ml were collected and analyzed by a modified carbazole method and by the thioglycolic acid method (Fig. 1). The eluates obtained from the column were divided into four groups: Frac. a, tubes 52 to 54; Frac. b, tubes 55 to 57; Frac. c, tubes 58 to 61; Frac. d, tubes 67 to 70. The products a, b, c, and d were obtained from Fracs. a, b, c, and d after rechromatography on the same column. The yields of the products were 5.4 mg for a, 3.6 mg for b, 4.3 mg for c, and 6.1 mg for d. Each product gave a single spot on glass-fiber paper electrophoresis.

**Glass-Fiber Paper Electrophoresis**——Electrophoresis was carried out with Whatman GF 83 glass-fiber paper, using the same alkaline borate buffer as in the paper electrophoresis. The conditions of electrophoresis were 380 volts for 1 hr. The *p*-anisidine–sulfuric acid reagent[15] was used for detection. The products

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12) M.N. Gibbons, *Analyst* (London), **80**, 268 (1955).

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

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

15) K.W. Fuller and D.H. Northcote, *Biochem. J.*, **64**, 657 (1956).

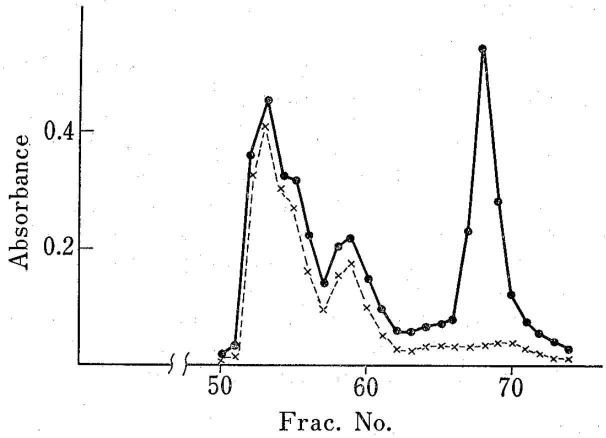

Fig. 1. Chromatogram of the Controlled Smith Degradation Products

—●—: a modified carbazole method,
---×---: the thioglycolic acid method.

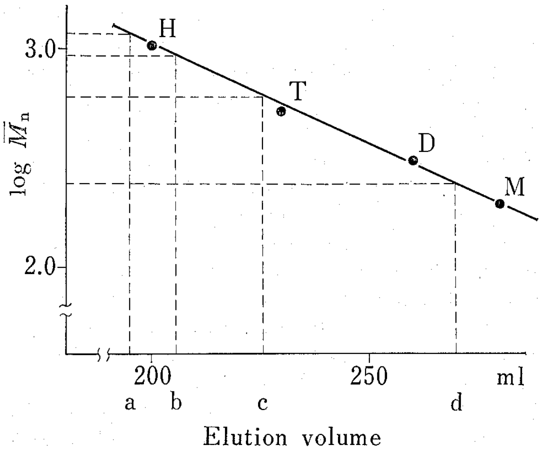

Fig. 2. Plot of Elution Volume against log $\bar{M}_n$ for Standard Acidic Sugars on Sephadex G-15

Standards: H, oligosaccharide III; T, oligosaccharide II; D, D-glucuronic acid-(1→5)-L-arabinose; M, D-galacturonic acid

and the standard glucose moved toward the cathode. The values of electrophoretic mobility of the products relative to that of glucose were 0.57 for a, 0.61 for b, 0.66 for c, and 0.71 for d.

**Determination of Molecular Weight**——Sephadex G-15 was treated with 0.1 M Tris-HCl buffer (pH 7.0) and a column (2.6×92 cm) was prepared. Elution was carried out by a descending method with the same buffer as an eluant. Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method. The results are shown in Fig. 2.

**Determination of Components**——The sample was hydrolyzed with 1 N sulfuric acid at 100° for 6 hr. The hydrolysate was derivatized into alditol acetates as described above, then subjected to GLC. GLC was carried out using a column (0.3 cm×2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) and with a programmed temperature increase of 10° per min from 100° to 210° at a nitrogen flow of 60 ml per min. The retention times (min) of the acetates of glycerol and rhamnitol were 8.5 and 16.7. Rhamnose and galacturonic acid were also estimated by colorimetric methods as described above.

**D-Galacturonic Acid-(1→2)-glycerol**——This was obtained by the Smith degradation of oligosaccharide II. The product d was identified as this substance by component analysis, by molecular weight determination, and by comparing its chromatographic behavior, electrophoretic mobility, and IR spectrum with those of an authentic sample.

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