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**Plant Mucilages. XXVIII. Isolation and Characterization  
of a Mucilage, "Althaea-mucilage OL," from the  
Leaves of *Althaea officinalis*\***

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In the previous papers of this series, the isolation and structural features of a representative mucilage, named Althaea-mucilage O, from the roots of *Althaea officinalis* L. have been reported from this laboratory. The roots of this plant have been used in a well-known crude drug "Althaeae Radix" as an emollient, demulcent, and cough medicine. In addition, the leaves of this plant have been used as a crude drug "Althaeae Folium" for the same purposes. The extract with water from the leaves of this plant contains many mucilages, but no structural study on the mucilages has been reported so far. In the present study we obtained a new representative mucilage from the leaves of *Althaea officinalis*. Its properties and main structural features are described in the present paper.

The fresh leaves were homogenized and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol. The solution of the crude mucilage was applied to a column of DEAE-Sephadex A-25 (carbonate form). After elution with 0.2 M ammonium carbonate, a mucilage was obtained from the eluate with 0.5 M ammonium carbonate solution. The mucilage was homogeneous as determined by ultracentrifugal analysis, 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.

The mucilage showed a positive specific rotation ( $[\alpha]_D^{25} +61.6^\circ$  in 0.1%  $\text{NH}_4\text{OH}$ ,  $c=0.1$ ), and its solution in water gave the high intrinsic viscosity value of 49.0 at  $30^\circ$ . The relative viscosity of the solution of the pure mucilage was about 9.6 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 leaves. Gel chromatography gave a value of approximately 1800000 for the molecular weight. The name "Althaea-mucilage OL" is proposed for this substance.

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.

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

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sugar units. Quantitative determination showed that the mucilage contained 34.9% rhamnose, 30.6% galacturonic acid, and 27.7% glucuronic acid, and that their molar ratio was 1.5:1.1:1.0. The infrared (IR) spectrum has absorption bands at 1250 and 1720  $\text{cm}^{-1}$ , suggesting the presence of ester linkages. When the acid hydrolysate was analyzed by gas-liquid chromatography (GLC), 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 1.0%. The determination of protein content was carried out by the method of Lowry *et al.*, and a value of 3.3% was obtained. No compound other than carbohydrates and amino acids was detected in the hydrolysate of the mucilage.

The methylation of the carboxyl-reduced mucilage was performed with the methylsulfinyl anion and methyl iodide in dimethyl sulfoxide. The fully methylated product was 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; 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 were identified in a molar ratio of 1.5:1.0:0.1:1.0.

The mucilage was hydrolyzed with 1 *N* sulfuric acid for 2 hr, then neutralized and applied to a column of Dowex 50W ( $\text{H}^+$ ). The eluate with water was applied to a column of DEAE-Sephadex A-25 (formate form). Five oligosaccharides (I to V) were obtained by stepwise elution with dilute formic acid, then purified by rechromatography. Based on the results of component sugar analysis, and by comparing its chromatographic and electrophoretic properties and the values of specific rotation with those of authentic samples, I to V were identified as the following five oligosaccharides (Chart 1).

The combined yields of I, II, III, IV, and V accounted for over 72% of the total mono- and oligosaccharide fractions obtained from the partial hydrolysate of the mucilage. Consequently, it can be concluded that these oligosaccharides do represent the structural features of the bulk of *Althaea*-mucilage OL.

Based on these results, it can be concluded that the polysaccharide moiety of the mucilage is mainly composed of (1 $\rightarrow$ 4)-[*O*- $\beta$ -(D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)]-*O*- $\alpha$ -(D-galactopyranosyluronic acid)-(1 $\rightarrow$ 2)-*O*- $\alpha$ -L-rhamnopyranosyl units. In view of the results of methylation analysis, however, we concluded that one-eleventh of the D-galacturonic acid residues has no branch, and that eleven-fifteenths of the L-rhamnose residues link to position 4 of D-galacturonic acid units but four-fifteenths of the L-rhamnose residues link to each other by 1 $\rightarrow$ 2 glycosidic linkages.

The component unit having the repeating structure (1 $\rightarrow$ 4)-[*O*- $\beta$ -(D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)]-*O*- $\alpha$ -(D-galactopyranosyluronic acid)-(1 $\rightarrow$ 2)-*O*- $\alpha$ -L-rhamnopyranose is common in the mucilages from the roots and the leaves of *Althaea officinalis*. On the other hand, the lack of galactosyl galactose branches at position 4 of the L-rhamnose residues and the partial lack of glucuronic acid branches at position 3 of the D-galacturonic acid residues

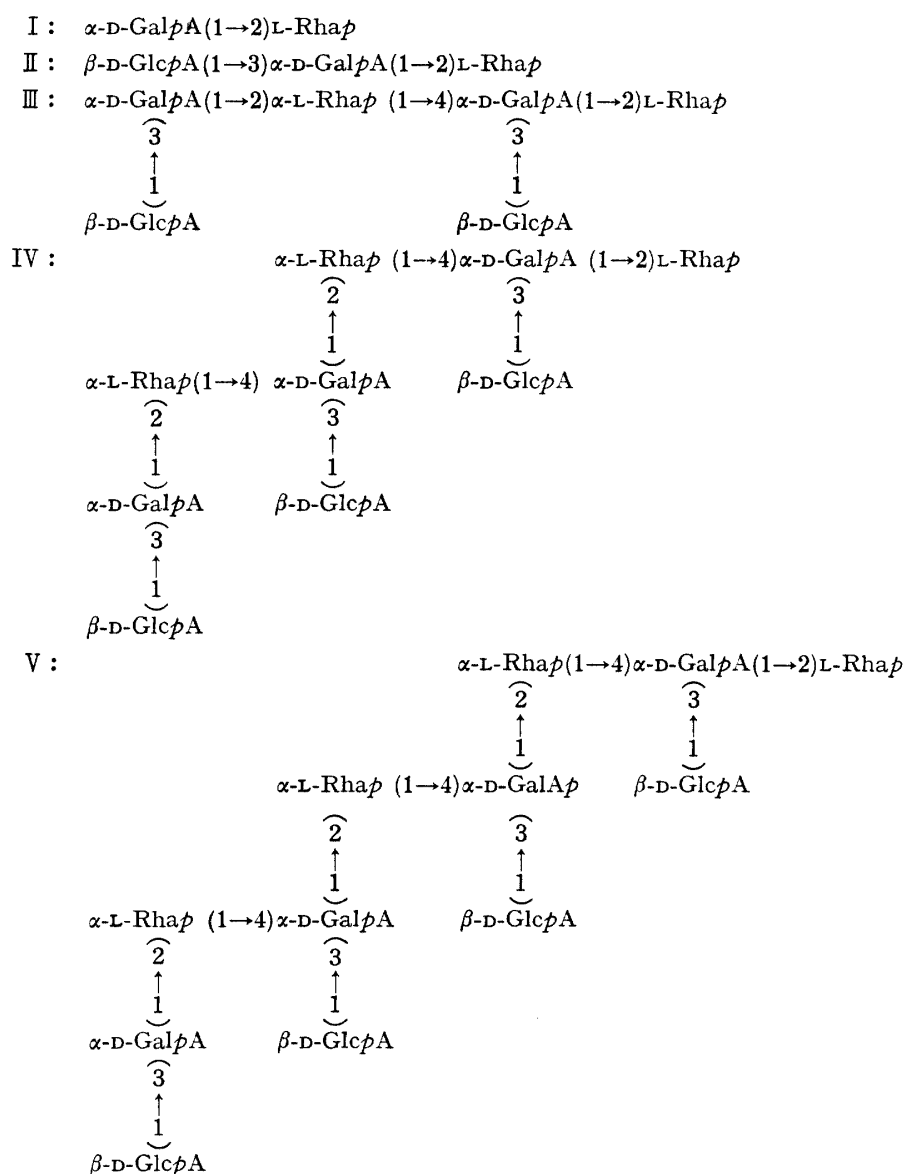


Chart 1. Structural Features of the Oligosaccharides

in the main chain were not found in *Althaea*-mucilage O. These are common characteristics of structures in both *Althaea*-mucilage OL and *Abelmoschus*-mucilage G. In addition, the presence of rhamnosyl rhamnose units in the main chain in *Althaea*-mucilage OL is unique, compared with the other four mucilages which have been isolated from Malvaceae plants and reported in the previous papers in this series. The results of aetailed analysis of the structure will be reported in subsequent papers.