

Title	Plant mucilages. XXXII. a representative mucilage, "althaeamucilage R," from the roots of althaea rosea
Sub Title	
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Publisher	共立薬科大学
Publication year	1983
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.28 (1983.) ,p.65- 69
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000028-0065

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

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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.* 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, 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.

The mucilage showed a positive specific rotation ($[\alpha]_D^{20} + 51.7^\circ$ in H_2O , $c=0.1$), and its solution in water gave the high intrinsic viscosity value of 33.4 at $30^\circ C$. 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-galacturonic acid, and D-glucuronic acid were identified by cellulose TLC of the hydrolysate. These sugars were isolated by preparative PPC and proved to have the configurations given above.

The carboxyl groups of hexuronic acid residues in the mucilage were reacted with

* 本報告は *Chem. Pharm. Bull.*, 31, 2677—2684 (1983) に発表

a carbodiimide reagent, then reduced with sodium borohydride to give the corresponding neutral sugar units. 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. The infrared spectrum has absorption bands at 1250 and 1730 cm^{-1} , suggesting the presence of ester linkages. When the acid hydrolysate was analyzed by 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 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.*, and a value of 8.8% was obtained. No nitrogen-containing compound other than amino acids was detected in the hydrolysate.

Methylations of the original and the carboxyl-reduced mucilages were performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide. The fully methylated products were hydrolyzed with dilute sulfuric acid in acetic acid. The products were analyzed by GLC-MS after conversion into alditol acetates. 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

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 DEAE-Sephadex A-25 (formate form). Four oligosaccharides (I to IV) were obtained by stepwise elution with dilute formic acid, then purified by rechromato-

graphy with Sephadex G-25. Based on the results of component sugar analysis, and by comparing their chromatographic properties, the ^1H -NMR spectra, and the values of specific rotation with those of authentic samples, I, II, and III were identified as the following three oligosaccharides (Chart 2).

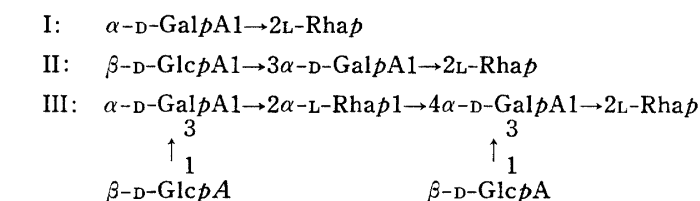


Chart 2. Structural Features of Oligosaccharides I, II, and III

Oligosaccharide IV showed a positive specific rotation ($[\alpha]_D^{25} + 69.5^\circ$ 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. 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 ^1H -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 residues], 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 a branching point], 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.

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. 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 $\rightarrow$ 3)-*O*- α -(D-galactopyranosyluronic acid)-(1 $\rightarrow$ 2)-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-[*O*- β -(D-glucopyranosyluronic acid)-(1 $\rightarrow$ 4)-*O*- β -(D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)]-*O*- α -(D-galactopyranosyluronic acid)-(1 $\rightarrow$ 2)-L-rhamnopyranose (Chart 3).

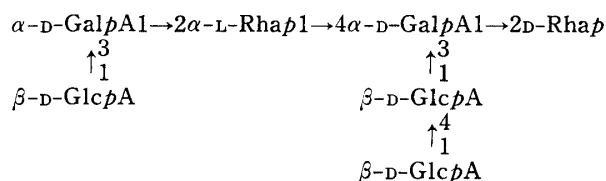
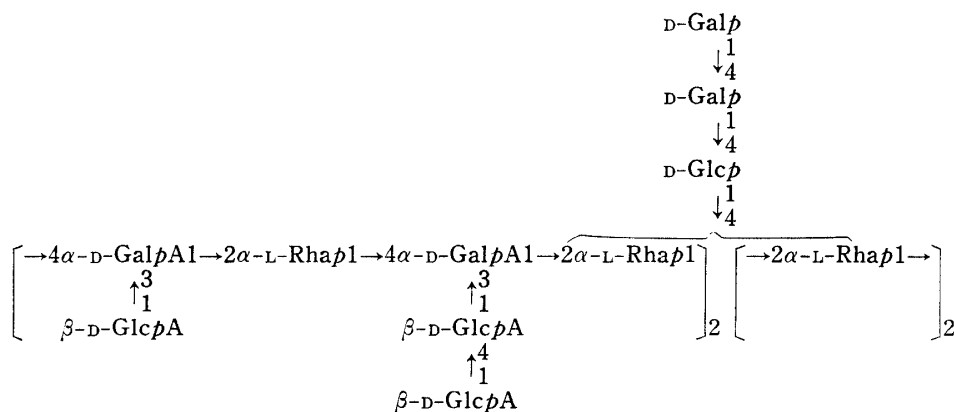


Chart 3. Structural Feature of Oligosaccharide IV

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 $\rightarrow$ 4)-galactosyl-(1 $\rightarrow$ 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 of this series, the component unit having the repeating structure (1 $\rightarrow$ 4)-[*O*- β -(D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)]-*O*- α -(D-galactopyranosyluronic acid)-(1 $\rightarrow$ 2)-*O*- α -L-rhamnopyranose is common in the mucilages from the roots and the leaves of *Althaea officinalis*, the roots of *Abelmoschus manihot*, and the roots 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 of *Althaea officinalis* and the immature

fruits 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.