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Plant Mucilages. XVI. Isolation and Characterization of a Mucous Polysaccharide, "Althaea-mucilage O", from the Roots of *Althaea officinalis**

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The roots of *Althaea officinalis* L. have been used as a crude drug for the purpose of emollient, demulcent, and cough medicine. It is well known that the roots contain relatively large amounts of mucilage. But no structural study on the whole molecule of the genuine mucilage has been reported until present time. We have now isolated a new mucous polysaccharide having glucuronic acid in addition to galacturonic acid, rhamnose, and galactose as its component sugars. The relative viscosity of the solution of the new mucous polysaccharide was about 2.3 times as high as the value of the crude mucilage. From this result and the yield, it is conceivable that the polysaccharide is the representative substance in the mucosity of water extract from the material. The properties and the structural features of it are described in the present paper.

The fresh roots were crushed and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol. The solution of the precipitate was applied to a column of DEAE-Sephadex A-25 (carbonate form). None of the substances adsorbed was eluted with water and 0.1 M ammonium carbonate solution, and a mucous polysaccharide was obtained from the eluate with 0.5 M ammonium carbonate solution.

The polysaccharide was homogeneous by the ultracentrifugal analysis, and gave a single spot on glass-fiber paper electrophoresis in alkaline borate buffer. It contained no nitrogen, and showed a positive specific rotation ($[\alpha]_D^{26} + 50.5^\circ$ in H_2O , $c=0.1$). Its solution in water gave the high intrinsic viscosity value of 50.0 at 23° .

As the component sugars of it, L-rhamnose, D-galactose, D-galacturonic acid, and D-glucuronic acid were identified by means of cellulose thin-layer chromatography (TCL) of the hydrolyzate. These sugars were isolated by preparative paper partition chromatography (PPC) and proved to have the configurations given above. The infrared (IR) spectrum has the weak absorption bands of 1235 and 1730 cm^{-1} suggesting the presence of ester linkages. When the acid hydrolyzate of the polysaccharide was analyzed by gas-liquid chromatography (GLC), it gave one peak, whose retention time was equal to that of authentic sample of acetic acid. The acetyl content of the polysaccharide was determined to be 0.7% by GLC. The measurement of osmotic pressure gave the value of 34000 as the molecular weight of the ammonium salt of the polysaccharide. The name "Althaea-mucilage O" is proposed for the polysaccharide.

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

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a carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units. Quantitative determinations of the neutral component sugars of the original and the carboxyl-reduced polysaccharides were carried out by GLC of alditol acetates derived from the hydrolyzates, and hexuronic acids in the original polysaccharide were estimated by a colorimetric method. The results showed that the original polysaccharide contained rhamnose, galactose, and hexuronic acids in the molar ratio of 3.3 : 2.0 : 6.0, and showed that the carboxyl-reduced polysaccharide was composed of rhamnose, galactose, and glucose in the molar ratio of 2.3 : 3.1 : 2.0.

The methylations of the original and carboxyl-reduced polysaccharides were performed with methylsulfinymethyl sodium and methyl iodide in dimethyl sulfoxide. The fully methylated products were successively hydrolyzed with formic acid and dilute sulfuric acid. The products were analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion to alditol acetates. Methyl ethers of the hexuronic acids were removed from the hydrolysis products of the methylated original polysaccharide by treatment with an anion-exchange resin, and the residual products were identified as 3,4-di-*O*-methyl-L-rhamnopyranose, 3-mono-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, and 2,3,6-tri-*O*-methyl-D-galactopyranose. They were obtained in the molar ratio of 2.0 : 1.0 : 1.1 : 1.0. In the case of the hydrolysis products of the methylated carboxyl-reduced polysaccharide, 3,4-di-*O*-methyl-L-rhamnopyranose, 3-mono-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*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 and obtained in the molar ratio of 4.1 : 2.0 : 5.9 : 1.8 : 1.0 : 6.0.

These results suggested that the minimal repeating unit of *Althaea*-mucilage O was composed of six kinds of the component sugar units. Owing to these results, it can be concluded that the backbone chain in the mucilage is composed of rhamnose and galacturonic acid in the approximate molar ratio of 1 : 1. The value of molar ratio of galacturonic acid corresponded to that of glucuronic acid, and the value of molar ratio of the terminal galactose was approximately equal to that of the rhamnose unit being situated in a branching point. Consequently, it can be presumed that each galacturonic acid residue forms a branch having a terminal glucuronic acid, and about one third of rhamnose residues form branching points having the side chains which are composed of two 1→4 linked galactose units.

As described above, the results of the component sugar determination and of the methylation analysis revealed that the approximate molar ratio of rhamnose: galactose: hexuronic acids in the original mucilage was 6 : 4 : 12 and that of rhamnose: original galactose: the hexoses derived from hexuronic acids in the carboxyl-reduced polysaccharide was 6 : 3 : 12. So it is conceivable that the carboxyl-reduced polysaccharide has lost about one fourth of galactose units in the original polysaccharide in the process of reduction. It seems likely that about a half of galactose-galactose linkages in the mucilage were hydrolyzed during the reaction.