Title	Plant mucilages. XXIX. isolation and characterization of a mucous polysaccharide, "plantago-mucilage A," from the seeds of plantago major var. asiatica
Sub Title	
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Publisher	共立薬科大学
Publication year	1981
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.26 (1981.) ,p.70- 73
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000026-0070

Plant Mucilages. XXIX. Isolation and Characterization of a Mucous Polysaccharide, "Plantago-mucilage A," from the Seeds of *Plantago major* var. *asiatica**

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Plantaginis Semen (Japanese name, Shazenshi), the seeds of *Plantago major* L. var. *asiatica* Decaisne (=*Plantago asiatica* L.), is a well-known crude drug used as an antiphlogistic, diuretic, antidiarrheic, and cough medicine. In 1971, Tomoda *et al.* reported the isolation of a mucous polysaccharide, called plantasan, from the seeds of this plant. Structural studies on plantasan were performed by Smith degradation and by partial acid hydrolysis followed by methylation analysis, and the results indicated the existence of β-1,4-linked D-xylopyranose backbone chains having branches at position 2 and of β-D-glucopyranosyluronic acid-(1→5)-L-arabinofuranose side chains. We have now isolated a new mucous polysaccharide which has a much higher water-solubility and a higher intrinsic viscosity value in aqueous solution than plantasan. The properties and the structural features of this new polysaccharide are described in the present paper.

The seeds were extracted with 0.2% sodium carbonate at room temperature, and after centrifugation, the polysaccharide was prepared from the supernatant by repeated precipitation with ethanol, followed by dialysis and lyophilization. The polysaccharide was homogeneous as determined by ultracentrifugal analysis and gave a single spot on glass-fiber paper electrophoresis. Furthermore, it gave a single peak on gel chromatography with Sepharose 4B.

The polysaccharide was readily soluble in water and showed a negative specific rotation ($[\alpha]_D^{24}$ −38.1° in H_2O, c=0.2). Its solution in water gave the high intrinsic viscosity value of 39.5 at 30°. Gel chromatography gave a value of approximately 1500000 for the molecular weight. The name "Plantago-mucilage A" is proposed for this polysaccharide.

As component sugars of the mucilage, D-xylose, L-arabinose, D-glucuronic acid, and D-galacturonic 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 configuration 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 sugar units. Quantitative determination of component sugars of the product was carried out by gas-liquid chromatography (GLC) of alditol acetates derived from the hydrolysate. The result showed that the molar ratio of xylose: arabinose: glucuronic dcid: galacturonic

* 本報告は *Chem. Pharm. Bull.*, **29**, 2877—2884 (1981) に発表。

acid was 10.8: 4.0: 3.3: 0.7 in the original mucilage. In addition, the presence of *O*-acetyl groups in the original mucilage was found by GLC of its hydrolysate, and the acetyl content was determined to be 4.8%.

Methylation of the carboxyl-reduced mucilage was performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide. The fully methylated product was hydrolyzed and the products were analyzed by gas-liquid chromatography-mass spectrometry (GLC–MS) after conversion into alditol acetates; 2.5-di-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2-*O*- and 3-*O*-methyl-D-xyloses, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-galactose were identified in a molar ratio of 3.9: 2.0: 9.2: 3.4: 0.6. The alditol acetates of the two mono-*O*-methyl xyloses were not separated from each other under the conditions used, but the coexistence of both 2-*O*-methyl and 3-*O*-methyl D-xyloses was proved by the mass fragmentation pattern of the superimposed peak.

The mucilage was subjected to periodate oxidation followed by reduction with sodium borohydride. The maximal values of periodate consumption and formic acid liberation were 0.84 mol and 0.45 mol per mol of anhydrosugar unit. The reduction product was treated with 0.5 N sulfuric acid overnight at room temperature. After neutralization and dialysis, the controlled Smith degradation product was isolated by gel chromatography on Sephadex G-25. It gave a single spot on glass-fiber paper electrophoresis. It showed a negative specific rotation ($[\alpha]_D^{27}$ $-113.2°$ in H_2O, $c=0.2$). Gel chromatography gave a value of 29200 for the molecular weight. It contained 28.1% arabinose, 67.2% xylose, and 4.7% *O*-acetyl groups; their molar ratio was thus 3.8: 9.0: 2.0.

Methylation analysis of the controlled Smith degradation product was carried out as described above, and the products were identified as 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, and 2-*O*- and 3-*O*-methyl-D-xyloses. They were obtained in a molar ratio of 4.3: 2.2: 7.0.

The chemical shift of 108.1 ppm for the *C*-1 signal of the arabinofuranosyl residue in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of the controlled Smith degradation product indicates that L-arabinofuranose units are α-linked. The chemical shifts of 102.8 and 103.5 ppm for the *C*-1 signals of the xylopyranosyl residues also suggest that D-xylose residues are β-linked. The presence of the *O*-acetyl groups was confirmed by the presence of a signal at 24.5 ppm in the ^{13}C-NMR spectrum.

On the other hand, the mucilage was hydrolyzed with 0.1 N sulfuric acid for 2 hr, then neutralized and applied to a column of Sephadex G-25. The low molecular weight fraction obtained was applied to a column of DEAE–Sephadex A-25 (formate form). The eluate with water was rechromatographed on a column of Sephadex G-25, and the fraction containing disaccharides was obtained. Two disaccharides (I and II) were isolated from this fraction by preparative PPC. The eluate with 0.1 M formic acid from the DEAE–Sephadex A-25 column gave a third disaccharide (III). The fraction containing high molecular weight

substances obtained by the first Sephadex G-25 column chromatography was mixed with the insoluble material in the hydrolysate and the mixture was hydrolyzed with 0.5 N sulfuric acid for 2 hr. After neutralization and application to a column of Sephadex G-25, a fourth disaccharide (IV) was obtained.

The hydrolysate of I and II gave D-xylose, and the component sugars of III were L-arabinose and D-glucuronic acid. However, the hydrolysis of IV gave L-arabinose, D-glucuronic acid, and D-galacturonic acid in the molar ratio of 1.0: 0.9: 0.15. Based on the result of component sugar analysis, and by comparing its chromatographic properites and the value of specific rotation with those of an authentic sample, II was identified as *O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose.

Disaccharides III and IV were converted into the corresponding carboxyl-reduced derivatives by reduction of the methyl ester methyl glycosides with sodium borohydride. Methylation analyses of disaccharide I, and the carboxyl-reduced III and IV indicated the production of 2,4-di-*O*-methyl-D-xylose and 2,3,4- tri-*O*-methyl-D-xylose in a molar ratio of 1.1 : 1.0 from I, and 2,5-di-*O*-methyl-L-arabinose and 2,3,4,6-tetra -*O*-methyl-D-glucose in a molar ratio of 1.0: 1.1 from carboxyl-reduced III, whereas the carboxyl-reduced IV gave 2,5-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-galactose in a molar ratio of 1.0: 1.0: 0.17.

Disaccharides I, III, and IV were reduced with sodium borohydride. The proton magnetic resonance (^{1}H-NMR) spectra showed an anomeric proton signal at δ 4.72 (d, J=7 Hz) in the derivative from I, an anomeric proton signal at δ 5.33 (d, J=3 Hz) in the derivative from III, and two anomeric proton signals at δ 5.28 (d, J=3 Hz) and δ 5.33 (d, J=3 Hz) in the derivative from IV. These data suggest that the non-reducing terminal D-xylose is β-linked, and that both the D-glucuronic acid residues in III and IV are α-linked.

Based on the results described above, I and III were identified as *O*-β-D-xylopyranosyl-(1→3)-D-xylopyranose and *O*-α-(D-glucopyranosyluronic acid)-(1→3)-L-arabinofuranose, but IV must be a mixture of III and *O*-α-(D-galactopyranosyluronic acid)-(1→3)-L-arabinofuranose. Further purification of IV was unsuccessful because of the similar chromatographic behavior of the two disaccharides.

The combined yield of these four disaccharides and monosaccharides was over 94% of total sugars obtained from the partial hydrolysate of the mucilage. Consequently, it can be deduced that these disaccharides do represent the structural features of the mucilage.

Based on the accumulated evidence described above, it can be concluded that the minimal unit of Plantago-mucilage A was composed of the five kinds of sugar units shown in Chart 1.

The glycosidic linkages of arabinofuranose residues in the mucilage are much more easily cleaved than those of the other component sugars. Therefore no direct evidence showing the mode of linkage of arabinosyl xylose was obtained. On the other hand, only two xylobioses were found as products of partial hydrolysis; those were β-1→3 and β-1→4

$$[\rightarrow 4)\beta\text{-D-Xyl}p(1\rightarrow]_m \qquad [\rightarrow 4)\beta\text{-D-Xyl}p(1\rightarrow]_n$$
$$\begin{matrix} 2 \\ \uparrow \end{matrix} \qquad\qquad \begin{matrix} 3 \\ \uparrow \end{matrix}$$

$$[\beta\text{-D-Xyl}p(1\rightarrow]_6$$

$$[\alpha\text{-D-Glc}p\text{A}(1\rightarrow 3)\alpha\text{-L-Ara}f(1\rightarrow]_{10}$$

$$[\alpha\text{-D-Gal}p\text{A}(1\rightarrow 3)\alpha\text{-L-Ara}f(1\rightarrow]_2$$

Chart 1. Minimal Component Units in the Structure of Plantagomucilage A
(m+n=27)

linked xylobioses. This result shows the presence of a β-1→4 linked D-xylopyranose backbone chain having the other xylose side chains at position 3 of D-xylopyranose units. Consequently, it can be presumed that *O*-α-(D-glucopyranosyluronic acid)-(1→3)-α-L-arabinofuranose and *O*-α-(D-galactopyranosyluronic acid)-(1→3)-α-L-arabinofuranose units link to position 2 of D-xylopyranose units in the backbone chain.

The location of the acetyl groups remains to be determined. The results of detailed analysis of the structure will be reported in subsequent papers.