[Chem. Pharm. Bull. 32(6)2182—2186(1984)]

Plant Mucilages. XXXIV.[1] The Location of *O*-Acetyl Groups and the Structural Features of Plantago-Mucilage A, the Mucous Polysaccharide from the Seeds of *Plantago major* var. *asiatica*

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(Received September 28, 1983)

The *O*-acetyl groups in Plantago-mucilage A, the representative mucous polysaccharide isolated from the seeds of *Plantago major* L. var. *asiatica* DECAISNE (= *Plantago asiatica* L.), were located at position 2 of about one-fourth of L-arabinofuranosyl residues, about two-fifths of the terminal D-xylopyranosyl residues, and about one-ninth of the non-terminal D-xylopyranosyl residues. Reinvestigation of earlier methylation analysis results, showed that the mucilage possesses a main chain composed of β-1→4-linked D-xylopyranose residues having other β-D-xylopyranose units and branches composed of *O*-α-(D-glucopyranosyluronic acid)-(1→3)-α-L-arabinofuranose and of *O*-α-(D-galactopyranosyluronic acid)-(1→3)-α-L-arabinofuranose at position 3 as side chains.

Keywords——Plantago-mucilage A; *Plantago major* var. *asiatica* (= *Plantago asiatica*); seed mucilage; methylation analysis; structural feature; acetyl location; deacetylation influence

In a previous paper,[2] Tomoda *et al.* have reported the isolation and the characterization of Plantago-mucilage A, a representative mucous polysaccharide from the seeds of *Plantago major* L. var. *asiatica* DECAISNE (= *Plantago asiatica* L.). The mucilage has the main chain composed of highly branched β-1→4-linked D-xylopyranose residues, β-D-xylopyranosyl side chains, and two kinds of aldobiouronic acid side chains. In addition, the mucilage contains 4.8% *O*-acetyl groups. The present work was undertaken to elucidate the location of the *O*-acetyl groups. This paper also reports the revision of some previously proposed structural features of Plantago-mucilage A.

The analytical data showed that the molar ratio of L-arabinose : D-xylose : D-glucuronic acid : D-galacturonic acid : *O*-acetyl group was 4.0 : 14.5 : 3.3 : 0.7 : 3.7 in Plantago-mucilage A. As reported previously,[2] the controlled Smith degradation product was obtained from the original mucilage by periodate oxidation and reduction followed by mild hydrolysis.[3] The product was composed of L-arabinose, D-xylose, and *O*-acetyl group in a molar ratio of 4.0 : 9.0 : 2.0. Thus, some of the analytical values given in a previous report should be corrected; the cause of the error in the previous analysis may be attributed to incomplete acetylation of the alditols.

Both the mucilage and the controlled Smith degradation product were exhaustively treated with methyl vinyl ether, as a protective reagent for the free hydroxyl groups, in the presence of *p*-toluenesulfonic acid as a catalyst in dimethyl sulfoxide.[4] After conversion of the free hydroxyl groups to 1-methoxyethyl ethers, the derivatives were deacetylated, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide.[5] The resultant products were subjected to acid hydrolysis, and the final products were analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates.[6] 2-*O*-

TABLE I. Molar Ratios of Methylated Products

	Products after acetalation followed by deacetylation		Carboxyl-reduced mucilage	Smith degradation product
	Mucilage	Smith degradation product		
2,3,5-Me-L-Arabinose	—	—	—	4.3
2,5-Me-L-Arabinose	—	—	4.0	—
2-Me-L-Arabinose	1.0	1.0	—	—
2,3,4-Me-D-Xylose	—	—	5.3	—
2,3-Me-D-Xylose	—	—	—	5.0
2-Me-D-Xylose	3.0	1.0	9.2	4.1
2,3,4,6-Me-D-Glucose	—	—	3.4	—
2,3,4,6-Me-D-Galactose	—	—	0.6	—

Abbreviations: Me=methyl (*e.g.*, 2,3,5-Me-=2,3,5-tri-*O*-methyl-).

Methyl-L-arabinose and 2-*O*-methyl-D-xylose were detected and identified in a molar ratio of 1.0 : 3.0 in the product derived from Plantago-mucilage A. On the other hand, both 2-*O*-methyl-L-arabinose and 2-*O*-methyl-D-xylose were also identified in the product derived from the controlled Smith degradation product, but their molar ratio was 1.0 : 1.0 (Table I).

These results indicate that 2-*O*-acetyl-L-arabinose and 2-*O*-acetyl-D-xylose units are present in the mucilage in a molar ratio of 1 : 3. Two-thirds of the 2-*O*-acetyl-D-xylose units must be located at the terminals in th mucilage because of their disappearance after periodate oxidation. On the basis of the molar ratio of *O*-acetyl groups to component sugars, it can be concluded that hexuronic acid residues do not possess *O*-acetyl groups.

Reinvestigations of the methylation analysis of the mucilage and the controlled Smith degradation product were carried out. Methylation was performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[7] The fully methylated products were hydrolyzed, reduced, and acetylated. The products were analyzed by GLC-MS as described above; 2,5-di-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, 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 4.0 : 5.3 : 9.2 : 3.4 : 0.6 in the product derived from the carboxyl-reduced mucilage. In the previous work,[2] the alditol acetates of 2-*O*-methyl and 3-*O*-methyl D-xyloses were not separated from each other under the conditions used. In the present study, we used different conditions with a 0.3% OV 275+0.4% XF 1150 column[8] for GLC and GLC-MS, and the mass fragmentation pattern of the corresponding peak indicated that 2-*O*-methyl D-xylose was the sole monomethyl xylose in the methylation products. In the case of methylation analysis of the controlled Smith degradation product, 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, and 2-*O*-methyl-D-xylose were identified as the partially methylated alditol acetates in a molar ratio of 4.3 : 5.0 : 4.1 by GLC-MS. These results are also listed in Table I.

As already reported in a previous paper,[2] *O*-β-D-xylopyranosyl-(1→3)-D-xylopyranose, *O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose, *O*-α-(D-glucopyranosyluronic acid)-(1→3)-L-arabinofuranose, and *O*-α-(D-galactopyranosyluronic acid)-(1→3)-L-arabinofuranose were obtained and identified as partial hydrolysates from the mucilage. In addition, the presence of α-glycosidic linkages of L-arabinofuranose units in the mucilage was already proved on the basis of the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of the controlled Smith degradation product.[2]

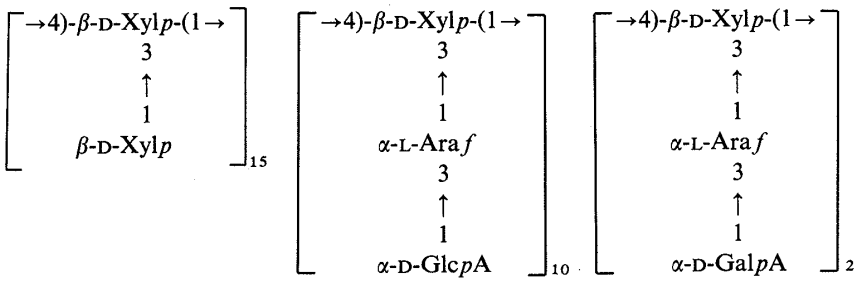

Chart 1. Minimal Component Units in the Structure of Plantago-Mucilage A

The terminal xylose units consist of D-Xyl*p*:2-Ac-D-Xyl*p* (3:2), and the intermediate xylose units consist of D-Xyl*p*:2-Ac-D-Xyl*p* (8:1).
The intermediate arabinose units consist of L-Ara*f*:2-Ac-L-Ara*f* (3:1).
Abbreviations: Xyl*p*, xylopyranose; Ara*f*, arabinofuranose; Glc*p*A, glucopyranosyluronic acid; Gal*p*A, galactopyranosyluronic acid; Ac, acetyl.

TABLE II. Properties of the Mucilage and the Deacetylated Product

	Plantago-mucilage A	Deacetylated product
$[\alpha]_D^{24}$ (in H_2O)	−38.1°	−59.8°
Solubility (in H_2O at 25 °C)[a]	>2.7%	>7.5%
Molecular weight (by gel chromatography)	1500000	1400000
Intrinsic viscosity (in H_2O at 30 °C)	39.5	24.2

a) These values show the measurement limits because of the high viscosity of concentrated solutions.

Based on the accumulated evidence described above, it can be concluded that Plantago-mucilage A contains the units shown in Chart 1.

On the basis of these results, we concluded that the molar ratios of D-xylopyranose and 2-*O*-acetyl-D-xylopyranose are approximately 3:2 in the terminal units and approximately 8:1 in the intermediate units, and that the molar ratio of L-arabinofuranose and 2-*O*-acetyl-L-arabinofuranose is approximately 3:1.

The present report is the first to describe the presence of *O*-acetyl groups and the location of them in mucilages obtained from plants of the Plantago genus. The native water-soluble glucomannans obtained from the bulbs of plants in the Liliaceae and Amaryllidaceae families possess *O*-acetyl groups, and deacetylation causes insolubility of the products in water.[9-12] In contrast to these neutral polysaccharides, the deacetylation of paniculatan, the acidic polysaccharide composed of 4-*O*-methyl-D-glucuronic acid, D-glucuronic acid, D-galacturonic acid, D-galactose, L-rhamnose, and 3-*O*-acetyl-L-rhamnose, isolated from the inner barks of *Hydrangea paniculata* (Saxifragaceae), increases the solubility in water.[13] In the case of Plantago-mucilage A, increase of water solubility and lowering of the molecular weight and viscosity in aqueous solution were observed upon deacetylation, as in the case of paniculatan (Table II). However, the effects were less marked than in the case of paniculatan.

Several studies on the properties and structures of the mucilages obtained from the seeds of plants in the *Plantago* genus have been reported. As components of the seed mucilages, D-xylose, D-galactose, and D-galacturonic acid in *Plantago lanceolata*,[14] D-xylose, L-arabinose, L-rhamnose, and D-galacturonic acid in *Plantago ovata*,[15,16] and D-xylose, L-arabinose, D-galactose, L-rhamnose, and D-galacturonic acid in *Plantago arenaria*[17] were reported. In the cases of mucilages from the seeds of *Plantago ovata* and *P. arenaria*, the presence of *O*-α-(D-galacturonic acid)-(1→2)-L-rhamnose units as side chains was reported. In Plantago-mucilage

A, however, neither D-galactose nor L-rhamnose was found as a component, and there were two kinds of aldobiouronic acid side chains, namely *O*-α-(D-glucopyranosyluronic acid)-(1→3)-L-arabinofuranose as the major one and *O*-α-(D-galactopyranosyluronic acid)-(1→3)-L-arabinofuranose as the minor one, in addition to β-D-xylose side chains. Thus, Plantago-mucilage A has characteristic side chains and location of *O*-acetyl groups, although the main chain, composed of β-1→4-linked D-xylose residues, is similar to those of the seed mucilages from other plants in the *Plantago* genus.

Experimental

Solutions were concentrated at or below 40 °C with rotary evaporators under reduced pressure. Infrared (IR) spectra were recorded on a JASCO IRA-2 infrared spectrophotometer. GLC was carried out on a Shimadzu GC-7AG 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. Optical rotation was measured with a JASCO DIP-140 automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter.

Preparation of the Mucilage and the Controlled Smith Degradation Product——These were carried out as described in a previous report[2] of this series.

Determination of Components——This was carried out by the methods described in a previous report[2] of this series, but GLC of alditol acetates was carried out under condition A, using a column (0.3 cm × 2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 200 °C with a helium flow of 50 ml per min. Quantitative determination showed that Plantago-mucilage A contained 57.8% xylose, 16.0% arabinose, 17.5% glucuronic acid, 3.7% galacturonic acid, 4.8% acetyl group, and 0.15% sodium, and that the controlled Smith degradation product contained 66.0% xylose, 29.3% arabinose, and 4.7% acetyl group.

Treatment with Methyl Vinyl Ether——The dried sample (100 mg) was suspended in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (20 mg) was added. The mixture was stirred at 15 °C, then methyl vinyl ether (5 ml), condensed at −10 °C, was added in portions under stirring. The reaction mixture was stirred at 15 °C for 6 h, then dialyzed against running water overnight. The non-dialyzable fraction was concentrated to dryness, and the reaction procedure was repeated twice. The final solution was applied to a column (4 × 33 cm) of Sephadex LH-20. The column was eluted with acetone, and fractions of 10 ml were collected. The eluates obtained from tubes 15 to 18 were combined and concentrated. The reaction procedure of acetalation followed by purification on a Sephadex LH-20 column was similarly repeated once more. The final product showed no hydroxyl absorption in its IR spectrum.

Deacetylation of the *O*-Acetyl-*O*-(1-methoxyethyl) Derivative——Half of the product was dissolved in methanol (10 ml), then 0.2 M methanolic sodium methoxide (10 ml) was added under stirring. The solution was refluxed at 75 °C for 4 h, then concentrated and applied to a column (4 × 42 cm) of Sephadex LH-20, and the column was eluted with methanol. Fractions of 10 ml were collected, and the eluates obtained from tubes 19 to 23 were combined and concentrated. The absence of ester absorption bands in the IR spectrum of the residue confirmed that deacetylation was complete.

Methylation of the *O*-(1-Methoxyethyl) Derivative——The product was dissolved in *N*,*N*-dimethylformamide (5 ml), then methyl iodide (4 ml) and silver oxide (0.4 g) were added successively under stirring. The reaction mixture was stirred at room temperature for 20 h in the dark, then filtered. The filtrate was washed with chloroform, then the filtrate and washing were combined and concentrated. Methyl iodide (4 ml) and silver oxide (0.4 g) were added again to the residual solution, and the reaction procedure was repeated four times. The final reaction mixture was filtered and washed with chloroform (4 ml). The filtrate and washing were combined, then water (15 ml) and 10% potassium cyanide (3.5 ml) were added to the solution. The mixture was extracted with chloroform (30 ml each) five times. The extracts were combined and washed with water (150 ml each) five times, then dried over sodium sulfate and the filtrate was concentrated. The final solution was applied to a column (4 × 42 cm) of Sephadex LH-20. The column was eluted with chloroform–methanol (2 : 1) mixture, and fractions of 10 ml were collected. The eluates in tubes 19 to 22 were combined and concentrated to dryness. The final residue (100 mg) was a reddish-yellow syrup, and showed no hydroxyl absorption in its IR spectrum.

Analysis of the *O*-Methyl Derivative——The product (8 mg) was dissolved in 90% formic acid (2 ml) and heated at 100 °C for 16 h. After removal of the acid by evaporation, the residue was treated with 0.5 N sulfuric acid (2 ml) at 100 °C for 3 h. After neutralization with Dowex 2 (OH^-), the hydrolysate was reduced with sodium borohydride and then acetylated with acetic anhydride-pyridine mixture.[18] GLC and GLC-MS were carried out under the following two conditions; B, using a column (0.3 cm × 2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 200 °C with a helium flow of 60 ml per min; C, using a column (0.3 cm × 2 m long spiral glass) packed with 0.3% OV 275 + 0.4% XF 1150 on Gaschrom Q (100 to 120 mesh) and with a programmed temperature increase of 1 °C per min from 130 to 180 °C at a helium flow rate of 60 ml per min. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and their main fragments in the mass spectra, are

Table III. Relative Retention Times on GLC and Main Fragments in MS of Partially Methylated Alditol Acetates

	Relative retention times[a]		Main fragments (m/z)
	Condition B	Condition C	
1,4-Ac-2,3,5-Me-L-Arabinitol	0.49	0.56	43, 45, 71, 87, 101, 117, 129, 161
1,3,4-Ac-2,5-Me-L-Arabinitol	0.91	1.06	43, 45, 113, 117, 233
1,3,4,5-Ac-2-Me-L-Arabinitol	1.73	1.58	43, 117, 261
1,5-Ac-2,3,4-Me-D-Xylitol	0.61	0.73	43, 101, 117, 161
1,4,5-Ac-2,3-Me-D-Xylitol	1.18	1.33	43, 87, 101, 117, 129, 189
1,3,4,5-Ac-2-Me-D-Xylitol	1.98	1.88	43, 117, 261
1,5-Ac-2,3,4,6-Me-D-Glucitol	1.00	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,5-Ac-2,3,4,6-Me-D-Galactitol	1.16	1.17	43, 45, 71, 87, 101, 117, 129, 145, 161, 205

a) Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.
Abbreviations: Ac = acetyl; Me = methyl (*e.g.*, 1,4-Ac-2,3,5-Me- = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-).

listed in Table III.

Methylation of Polysaccharides and Analysis of the Products——Methylation of the carboxyl-reduced mucilage and the controlled Smith degradation product followed by acid hydrolysis, reduction, and acetylation were carried out by the methods described in a previous report[2] of this series. GLC and GLC-MS of the resultant partially methylated alditol acetates were carried out under the conditions B and C as described above. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and their main fragments in the mass spectra, are also listed in Table III.

Deacetylation of the Mucilage——The mucilage (50 mg) was dissolved in 0.1 N sodium hydroxide (20 ml), and the solution was left at 30 °C for 10 min. After neutralization with 1 N hydrochloric acid, the solution was dialyzed against distilled water overnight. The non-dialyzable fraction was concentrated and applied to a column (5 × 83.5 cm) of Sephadex G-15. The column was eluted with water, and fractions of 50 ml were collected. The eluates obtained from tubes 11 to 28 were combined, concentrated, and lyophilized. The absence of ester absorption bands in the IR spectrum of the product (25 mg) confirmed that deacetylation was complete.

References and Notes

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