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Plant Mucilages. III.[1] Smith Degradation Products of Plantasan

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Plantasan, the seed mucilage of *Plantago major* L. var. *asiatica* DECAISNE, has been subjected to Smith degradation. The polysaccharide obtained as the degradation product was an arabinoxylan, and the molar ratio of D-xylose: L-arabinose was 9: 1. After methanolysis, the O-methyl derivatives characterised from the methylated polysaccharide were methyl glycosides of 2,3,5-trimethyl L-arabinose, 2,3-dimethyl D-xylose and 3-methyl D-xylose. As the result of these investigations and periodate oxidation, a possible structure of the arabinoxylan is proposed. In addition to this polysaccharide, glycerol, glycolaldehyde, ethylene glycol, D-xylose, L-arabinose and their glycosides were determined as Smith degradation products obtained from plantasan.

The seed mucilage of *Plantago major* L. var. *asiatica* DECAISNE named plantasan has been isolated and recognized as an acidic polysaccharide composed of D-xylose, L-arabinose, L-rhamnose, D-galactose and D-galacturonic acid.[3] The previous investigation showed that the molar ratio of them was 15:3:2:0.4:4. This result suggests a high branched structure for the polysaccharide like the other many plant gums and mucilages,[4] and the evidences of it produced by Smith degradation[5] and following methylation and periodate oxidation studies of a product are described in the present paper.

Plantasan was subjected to periodate oxidation, and after stropping of the reaction by addition of ethylene glycol, the product was reduced with sodium borohydride. The controlled hydrolysis of acetal linkages was achieved with 1N sulfuric acid at room temperature for two days. After neutralization, the solution was concentrated and dialyzed against water. The dialyzable fraction was converted into trimethylsilyl or trifluoroacetyl derivatives and analyzed by gas liquid chromatography (GLC) and also by thin-layer chromatography (TLC). The non-dialyzable fraction was applied to a column of Sephadex G-25, and a polysaccharide was obtained. The outline of the process is shown in Chart 1.

As the result of periodate oxidation, 1.35 mole of periodate per one mole of component anhydro sugar unit of plantasan was consumed with 0.81 mole of formic acid liberation. Smith degradation of plantasan produced glycerol (11.6% of the dialysate), glycolaldehyde (5.6% of the dialysate), ethylene glycol (3.2% of the dialysate), D-xylose glycoside (42.8% of the dialysate), L-arabinose glycoside (23.3% of the dialysate), D-xylose (4.2% of the dialysate) and L-arabinose (12.5% of the dialysate), and a new polysaccharide (14.4% yield from plantasan). The glycosides are estimated to be compounds which pentose combines with C-2-substituted glycerol, and some of glycosidic bonds may be cleaved during hydrolysis of acetal linkages with cold dilute acid.

1) Part II: M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 2173 (1971).
2) Location: *1-5-30, Shibakôen, Minato-ku, Tokyo.*
3) M. Tomoda and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 1214 (1971).
4) *a*) F. Smith and R. Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold, New York, 1959; *b*) G.O. Aspinall, "The Carbohydrates, Chemistry and Biochemistry" Vol. IIB ed. by W. Pigman and D. Horton, Academic Press, New York and London, 1970, p. 515.
5) I.J. Goldstein, G.W. Hay, B.A. Lewis and F. Smith, "Methods in Carbohydrate Chemistry" Vol. V ed. by R.L. Whistler, Academic Press, New York and London, 1965, p. 361.

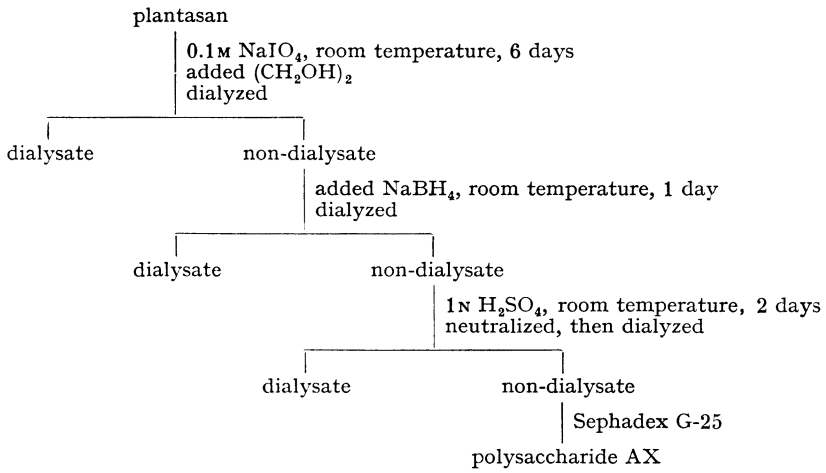

Chart 1. Smith Degradation of Plantasan

The polysaccharide showed a negative specific rotation ($[\alpha]_D^{30}$ −107.2°, c=0.9, H_2O), and it is homogeneous on a glass-fiber paper electrophoresis and gel filtration. We named provisionally it polysaccharide AX. Its number-average molecular weight was estimated from the calibration curve given by gel filtration,[3] and the value of 9200 was obtained. Acid hydrolysis produced D-xylose and L-arabinose, and determination of them showed that the molar ratio was 9:1.

After methylation of polysaccharide AX with sodium hydride and methyl iodide in dimethyl sulfoxide,[6] methylated product was methanolyzed and the methanolysate was analyzed by GLC and TLC. Methyl glycosides of 2,3-di-O-methyl D-xylose, 3-O-methyl D-xylose and 2,3,5-tri-O-methyl L-arabinose were identified.

By periodate oxidation of polysaccharide AX, 0.73 mole of periodate per one mole of component anhydro sugar unit was consumed and no formic acid liberation was observed. Smith degradation of polysaccharide AX produced very small amounts of a xylan in addition to glycerol, glycolaldehyde, ethylene glycol, D-xylose glycoside, L-arabinose glycoside, D-xylose and L-arabinose. Their yields in the dialysate were 35.0%, 0.7%, 0.2%, 25.4%, 0.6%, 1.2% and 0.4%.

Thus, Smith degradation of plantasan removed all the D-galacturonic acid, L-rhamnose and D-galactose residues, together with some of D-xylose and L-arabinose. The methylation study provided the evidence that polysaccharide AX has a main chain of 1→4 linked D-xylo-

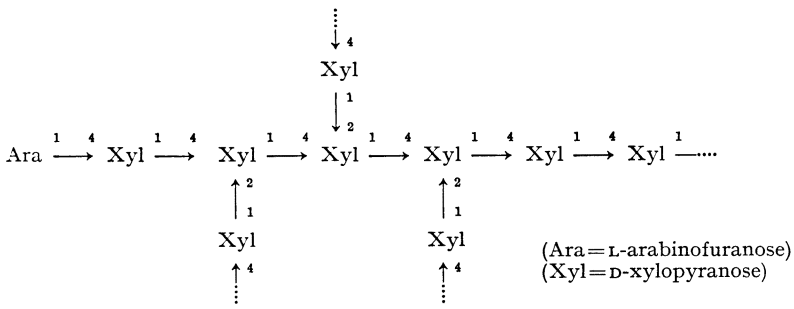

Chart 2. Possible Structural Unit of Polysaccharide AX

6) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

pyranose units having a highly branched structure with 1→2 branch point. From the result of periodate oxidation, it is concluded that the molar ratio of the methyl glycosides of 2,3-di-O-methyl D-xylose, 3-O-methyl D-xylose and 2,3,5-tri-O-methyl L-arabinose was approximately 6:3:1.

A possible structural unit shown in Chart 2 could be proposed to polysaccharide AX, although further detailed study is still necessary for the confirmation of branching positions. Of course, in the molecule of plantasan, all the D-xylose residues of polysaccharide AX must combine with short or long side-chains.

The results of present investigation indicated that D-galacturonic acid, L-rhamnose and D-galactose residues exist as linear chains or terminal residues in plantasan, on the other hand, D-xylose forms high branched backbone structure and a part of L-arabinose residue also occupy branching points. Further evidence obtained from partial hydrolysis for the proposed structure will be described in following papers.

Experimental

Solutions were evaporated at 40° or below with rotary evaporators under reduced pressure. Specific rotation was measured by the use of JASCO model DIP-SL automatic polarimeter. Gas chromatography was carried out by the use of Hitachi Model F6D gas chromatograph equipped with hydrogen flame ion detector.

Smith Degradation of Plantasan——Plantasan (0.5 g) was dissolved in water (60 ml) as its sodium salt and 0.25M sodium metaperiodate solution (40 ml) was added. The oxidation was performed in darkness at room temperature, and the periodate consumption was measured by a spectrophotometric method.[7] The oxidation was completed after five days (Fig. 1), then formic acid liberation was measured by a titration with 0.01N NaOH, and the reaction was stopped by addition of ethylene glycol (1 ml). The solution was dialyzed against running tap water for two days, then concentrated to 50 ml and sodium borohydride (0.5 g) was added. The mixture was kept at room temperature for 24 hr, then dialyzed for further two days. Equal volume of 2N sulfuric acid was added to the solution and the polyalcohol was hydrolyzed at room temperature for two days, after which the solution was neutralized with barium carbonate and the filtrate was deionized by treatment with a small column of Amberlite IR-120 (H^+). After concentration to 10 ml, the solution was dialyzed against water (500 ml). The dialysate was concentrated and lyophilized. Yellow syrup (0.143 g) was obtained. The non-dialyzable fraction was further dialyzed against running tap water for two days, then applied to a column (4×50 cm) of Sephadex G-25 (Pharmacia Co.). Fractions of 20 ml were collected and analyzed by phenol-sulfuric acid method.[8] Polysaccharide AX (0.072 g) was obtained as white powder from tubes 14 to 19 by lyophilization.

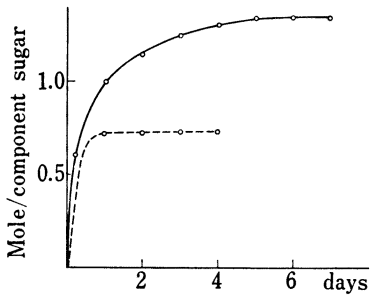

Fig. 1. Periodate Oxidation

—— plantasan
------ polysaccharide AX

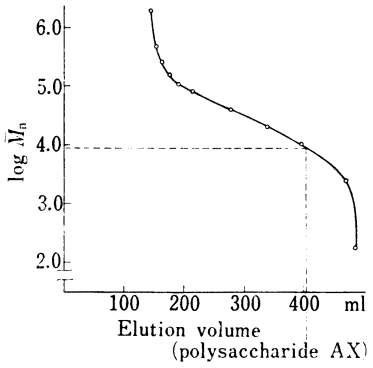

Fig. 2. Plot of Elution Volume against log $\bar{M}_n$ for Dextran Fractions on Sephadex G-200 with 0.1M Ammonium Formate

7) *a*) J.S. Dixon and D. Lipkin, *Anal. Chem.* **26**, 1092 (1954); *b*) G.O. Aspinall and R.J. Ferrier, *Chem. Ind.*, **1957**, 1216.
8) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

Analysis of Dialyzable Fraction——The dialyzable fraction (2 mg) was dissolved in water (0.4 ml) containing hydroxylamine hydrochloride (10 mg) and heated at 80° for 30 min in a sealed tube.[9] The reaction mixture was dried *in vacuo*, and the residue was dissolved in pyridine (0.2 ml) containing trimethylolpropane (1 mg) as an internal standard, then subjected to trimethylsilylation by addition of hexamethyldisilazane (0.1 ml) and trimethylchlorosilane (0.06 ml). The product was applied to a gas chromatograph and determined.

GLC: column, 5% SE 30 on Chromosorb G (80 to 100 mesh) (0.3 cm × 1 m long stainless steel); programmed column temperature, increase in 5° per min from 60° to 180°; carrier gas, N_2 (30 ml per min); t_R, ethylene glycol 6.3; glycolaldehyde 9.1; glycerol 15.1; trimethylolpropane 18.7; L-arabinose 24.3; D-xylose 29.0.

Determinations of monosaccharides and their glycosides were carried out in the same way as described in the article of analysis of component sugars.

Gel Filtration on Sephadex G-200 Column——A column (2.6 × 96 cm) of Sephadex G-200 (Pharmacia Co.) was prepared and the elution was carried out as described in the first report of this series.[3] Fractions of 5 ml were collected and analyzed by phenol-sulfuric acid method.[8] The calibration curve is shown in Fig. 2.

Glass-fiber Paper Electrophoresis——Electrophoresis was done with Toyo-Roshi GB 60 glass-fiber paper (12 × 24 cm long) and alkaline borate buffer of pH 9.2 (0.025M borax: 0.1N NaOH, 10: 1) at the condition of 220 volt for 90 min. Samples were applied in line at 8 cm from the anode, and moved toward the cathode. The inside of the apparatus was cooled with dry ice. *p*-Anisidine-sulfuric acid reagent[10] was used for detection. Polysaccharide AX gave one spot at a distance of 4.6 cm from the origin. Distance moved by D-glucose was 5.1 cm.

Analysis of Component Sugars——Polysaccharide AX was hydrolyzed with 2N sulfuric acid at 100° for 2 hr, then neutralized with barium carbonate. The filtrate was passed through a small column of Dowex 50W-X8 (H^+) for the removal of barium ion. TLC using Avicel SF cellulose was carried out as described in the first report of this series.[3] Three solvent systems were used; A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3, by vol.); B, AcOEt: pyridine: AcOH: H_2O (30: 10: 1: 6, by vol.); C, C_6H_5OH: 1% NH_4OH (2: 1, by vol.). The component sugars were revealed with silver nitrate reagent[11] and *p*-Anisidine reagent.[12] On the other hand, a part of the hydrolysate was trimethylsilylated and applied to a gas chromatograph as described in the previous paper.[1]

GLC: column, 3% SE 52 on Chromosorb W (80 to 100 mesh) (0.3 cm × 1 m long stainless steel); programmed column temperature, increase in 2.5° per min from 130° to 180°; carrier gas, N_2 (20 ml per min).

Table I shows *Rf* values of component sugars in TLC and retention times of their trimethylsilyl derivatives in GLC.

TABLE I. *Rf* Values of Component Sugars and Retention Times of their Trimethylsilyl Derivatives

	TLC (*Rf*)			GLC (t_R)
	Solvent A	Solvent B	Solvent C	
D-Xylose	0.61	0.28	0.43	8.0, 9.5
L-Arabinose	0.54	0.20	0.52	5.9

For the determination of component sugars, GLC[13] of the trifluoroacetate of reduction product of hydrolysate was carried out using a column (0.4 cm × 2 m long spiral glass) packed with 2% XF 1105 on Gas-Chrom P (80 to 100 mesh) at 140° with a flow of 30 ml per min of nitrogen. D-Mannose was used as an internal standard. The value of pentose was also supported by the result of orcinol method.[14] The investigation showed that polysaccharide AX contains 90.9% of D-xylose and 10.1% of L-arabinose.

Methylation and Methanolysis——Sodium hydride (100 mg) was mixed with $(CH_3)_2SO$ (2 ml) and the mixture was stirred at 70° for 1 hr. Polysaccharide AX (10 mg) in $(CH_3)_2SO$ (2 ml) was added into this mixture. After 10 min stirring at 70°, CH_3I (4 ml) was added and the reaction mixture was stirred overnight at room temperature. The procedures were carried out in nitrogen atmosphere. After dilution with water, the mixture was extracted with $CHCl_3$ thrice. The extract was dried and the solvent was evapo-

9) H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem.*, **68**, 253 (1970).
10) K.W. Fuller and D.H. Northcote, *Biochem. J.*, **64**, 657 (1956).
11) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).
12) L. Hough, J.K.N. Jones, and W.H. Wadman, *J. Chem. Soc.*, **1950**, 1702.
13) T. Imanari, Y. Arakawa, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1967 (1969).
14) M. Tomoda, *Chem. Pharm. Bull.* (Tokyo), **11**, 809 (1963).

rated *in vacuo*. The residue was methylated again under the same condition. The infrared spectra of the final product had no absorption near 3400 cm^{-1}. The fully methylated product was heated with 0.5N methanolic HCl (1 ml) in a sealed tube at 70° for 8 hr. After cooling, the solution was treated with Amberlite IR4B (OH^-) to remove HCl, then evaporated *in vacuo*.

Analysis of Methanolysate——Chloroform solution of the methanolysate was applied to a gas chromatograph. The following two conditions were used; A, a column (0.3 cm × 1 m long stainless steel) packed with 15% Poly-butane 1,4-diol succinate on Chromosorb W (80 to 100 mesh) at 175° with a flow of 20 ml per min of N_2; B, a column (0.3 cm × 1 m long stainless steel) packed with 5% Neopentylglycol succinate on Chromosorb G (60 to 80 mesh) at 150° with a flow of 20 ml per min of N_2. Table II shows relative retention times of the products obtained by methanolysis to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside in the two gas chromatographic conditions. On the other hand, TLC using Wako-gel B5 was carried out with the solvent system of benzene: acetone (5: 1, by vol.) and 10% sulfuric acid was used for detection at 150°. R_G values of the products to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside are also shown in Table II.

TABLE II. Relative Retention Times and R_G Values of Methylation Products

	GLC		TLC
	Condition A (15% BDS)	Condition B (5% NPGS)	
Methyl 2,3,5-tri-O-methyl-L-arabinofuranoside	0.61	0.54	1.05
Methyl 2,3-di-O-methyl-D-xylopyranoside	1.80	3.33	0.57
Methyl 3-O-methyl-D-xylopyranoside	3.54	7.72	1.19

Smith Degradation of Polysaccharide AX——The sample (150 mg) was oxidized with 150 ml of 0.05M sodium metaperiodate in 0.2M acetate buffer (pH 4.4) at 5° to 10° in a dark place. The oxidation was completed after two days (Fig. 1). The remaining procedures of the degradation were carried out in the same way as the case of plantasan.