[Chem. Pharm. Bull. 26(6)1729—1736(1978)]

UDC 547.458.02.05 : 581.192

Polysaccharides of Azuki Beans. I. Neutral Homopolysaccharides "Arabinans" from Alkaline Extract of the Seeds of *Phaseolus radiatus* L. var. *aurea* PRAIN[1]

SHIGEO UKAI, CHIHIRO HARA, TADASHI KIHO, and KAZUO HIROSE

Gifu College of Pharmacy[2]

(Received November 4, 1977)

Two kinds of arabinans AL-I and AL-II, as neutral homoglycan, have been isolated by alkaline extraction from azuki beans, which are the seeds of *Phaseolus radiatus* L. var. *aurea* PRAIN. Both arabinans were homogeneous as judged by gel filtration (Sephadex G-200), and electrophoresis.

Structural analysis indicated that these arabinans had a chain of 1→5 linked L-arabinofuranose units bearing a highly branched structure with branching points either at each of position 2 and 3 or at both these positions, to which non-reducing terminal furanosyl residues were attached. At the branching points, the proportion of 1→2 linkage was larger than that of 1→3 linkage.

Both arabinans were similar, but some significant differences between AL-I and AL-II were observed in physical properties such as molecular weight and solubility in 80% aqueous ethanol solution. The structural differences between two were found in such respects that the amount of non-reducing terminal residues in AL-I was slightly larger than that in AL-II, and the proportion of 1→5 linkage bearing branch point at position 2 or 3 in AL-I was lower than that in AL-II.

Keywords——*Phaseolus radiatus* L. var. *aurea* PRAIN; azuki bean; polysaccharide; neutral homoglycan; arabinan; rate of acid hydrolysis; specific rotatory change; methylation analysis; Smith degradation

Azuki beans have long been used in Japan as food and antidote. The constituents of the beans have so far been studied on many substances such as free sugars,[3a] oligosaccharides,[3b] starch,[3c] free amino acids,[4a] proteins,[4b] fat[5] and saponins.[6] However, no study on polysaccharides except for starch has been reported.

For the purpose of examining the nutritive value and biological activities of polysaccharides, we have now isolated two kinds of arabinans AL–I and AL–II as a neutral homoglycan from the alkaline extract of the seeds of *Phaseolus radiatus* L. var. *aurea* PRAIN. The present paper deals with the purification, characterization and structural analysis of both AL–I and AL–II.

The flour of the dehulled beans was first treated with hot and cold aqueous ethanol solution to inactivate any enzyme and to remove low molecular materials.[7,8] The ethanol-free residue was repeatedly extracted with hot water to remove starch and other polysaccha-

1) Presented partly at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1975.
2) Location: *6–1, Higashi-5-chome, Mitahora, Gifu, 502, Japan.*
3) *a*) A. Matsushita, *Nippon Nogeikagaku Kaishi*, **41**, 646 (1967); *b*) T. Narasaki and S. Kawamura, *Kagawa Daigaku Nogakubu Gakujutsu Hokoku*, **9**, 157 (1958); *c*) S. Kawamura, *Nippon Nogeikagaku Kaishi*, **33**, 300 (1959).
4) *a*) A. Matsushita, *Nippon Nogeikagaku Kaishi*, **32**, 833 (1958); *b*) S. Akune and S. Takagi, *Agric. Biol. Chem.* (Tokyo), **26**, 63 (1962).
5) N. Ito, *Nippon Nogeikagaku Kaishi*, **17**, 1005 (1941).
6) N. Toya and S. Iseda, *Nippon Nogeikagaku Kaishi*, **38**, 273 (1964).
7) I.A. Preece and K.G. Mackenzie, *J. Inst. Brewing*, **58**, 353 (1952).
8) O. Igarashi and Y. Sakurai, *Agric. Biol. Chem.* (Tokyo), **29**, 678 (1965).

rides. Then, the swollen residue was directly treated with 5% aqueous sodium hydroxide solution overnight at room temperature. After neutralization and dialysis, large amounts of starch and concomitant proteins in the extract were removed by enzymatic hydrolysis with α-amylase and then with Pronase, followed by the Sevag method[9] and dialysis. To the non-dialysable fraction was added ethanol up to 70% ethanol solution to separate a polysaccharide fraction ALP as precipitate, whose structural analysis will be reported in a near future. The polysaccharide fraction AL prepared from the ethanolic supernatant was subjected to glucoamylase digestion to remove remaining dextrin, followed by dialysis. The non-dialysable fraction thus obtained was chromatographed on DEAE-Sephadex A-25 column with 0.02 N sodium acetate buffer (pH 6.1). The neutral fraction eluted was divided into two fractions AL-I (supernatant) and AL-II (precipitate) by treatment with 80% aqueous ethanol solution. The fraction AL-I and AL-II were respectively lyophilized to afford polysaccharides as hygroscopic and slightly yellowish flakes in a low yield (less than 0.2% of whole beans).

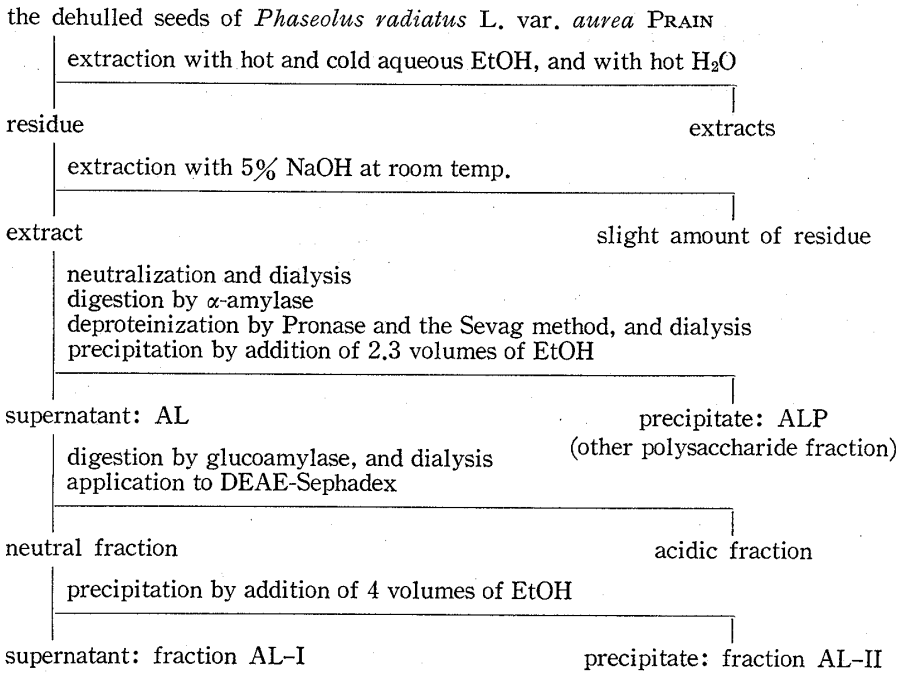

Chart 1. Fractionation of Alkali-Soluble Constituents

These polysaccharides were homogeneous on gel chromatography with Sephadex G-200 as shown in Fig. 1 and gave one spot on glass fiber paper electrophoresis in 0.05 M sodium tetraborate buffer of pH 9.3. The approximate molecular weight ($\bar{M}w$) of each polysaccharide was 13000 for AL-I and 22000 for AL-II respectively, which was estimated from the calibration curve given by gel chromatography with Sephadex G-200 of standard dextran fractions of known molecular weights.

These polysaccharides were found to contain a trace of nitrogen by elemental analysis as shown in Table I. The sugar content of each polysaccharide was 98.3% for AL-I and 99.3% for AL-II (by phenol-sulfuric acid method).[18] Their infrared (IR) spectra were essentially identical each other and had characteristic absorptions at 855 cm^{-1} and 800 cm^{-1}.

Both polysaccharides were readily hydrolyzed with 1 N sulfuric acid at 100° and the reducing activity reached a maximal value in 90 min (Fig. 2).

Paper partition and thin-layer chromatography (PPC and TLC) of the hydrolysate prapared from each polysaccharide and gas-liquid chromatography (GLC) of the trifluoro-

9) M.G. Sevag, *Biochem. Z.*, **273**, 419 (1934).

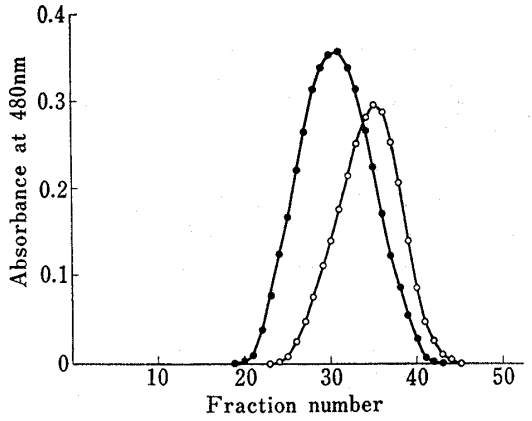

Fig. 1. Gel Chromatography of the Arabinans with Sephadex G-200
—○—: AL-I, —●—: AL-II.

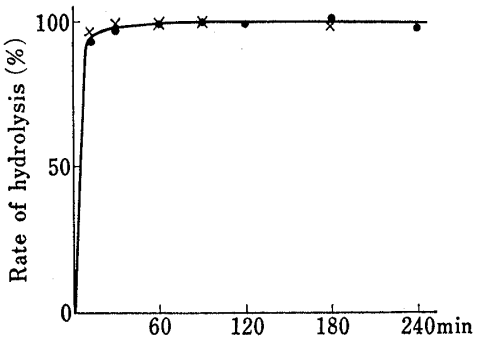

Fig. 2. Increase of Reducing Activity during Acid Hydrolysis
×: AL-I, ●: AL-II.

acetate of the reduction product from the hydrolysate indicated that the component sugar of each polysaccharide was only arabinose.

The hydrolysate of AL–I was treated with 95% aqueous ethanol solution to give colorless crystals, mp 152—154°, $[\alpha]_D^{13}$ +105.1° equil., in water, c=0.039. The crystalline substance was identified as L-arabinose by mixed melting point test and by its IR spectral comparison with an authentic sample.

These polysaccharides showed highly negative specific rotations as follows: AL–I ($[\alpha]_D^{20}$ −178° in water), AL–II ($[\alpha]_D^{20}$ −173° in water).

Some properties of both polysaccharides are summarized in Table I.

Table I. Some Properties of the Arabinans from Azuki Bean

		AL-I	AL-II
Specific rotation $[\alpha]_D^{20}$ in H_2O		−178° (c =0.138)	−173° (c =0.242)
Elemental analysis	C	45.32	43.38
	H	6.13	6.06
	N	0.44	0.41
IR spectra (cm^{-1}) by KBr tablet		800, 855	800, 855
Periodate consumption (mol per anhydroarabinose unit)		0.70	0.66
Molecular weight ($\overline{M}w$)[a]		13000	22000

a) estimated by gel chromatography (Sephadex G–200)

During acid hydrolysis, the initial value ($[\alpha]_D^{15}$ −178.5° in 1 N sulfuric acid, c=0.028) of the specific rotation of AL–I has gradually increased to reach +100° in 200 min as shown in Fig. 3. On the other hand, authentic L-arabinose showed $[\alpha]_D^{24}$ +105.5° equil., in water, c=0.091. As is evident from above results, the rapid rate of acid hydrolysis and the change of the specific rotation from levo- to dextrorotatory suggest[10] that the arabinose units in each polysaccharide are of furanose type and the majority of the sugar residues are of the α-L type. The characteristic absorptions at 855 cm^{-1} and 800 cm^{-1} in IR spectra might be attributed to those of furanose ring.[11]

10) C.S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909); S. Karacsonyi, R. Toman, F. Janecek, and M. Kubackova, *Carbohyd. Res.*, **44**, 285 (1975); H. Arita, H. Tsuzuki, K. Morihara, and J. Kawanami, *J. Biochem.* (Tokyo), **76**, 861 (1974); M. Tomoda, M. Takahashi, and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **21**, 707 (1973).

11) S.A. Barker and R. Stephens, *J. Chem. Soc.*, **1954**, 4550.

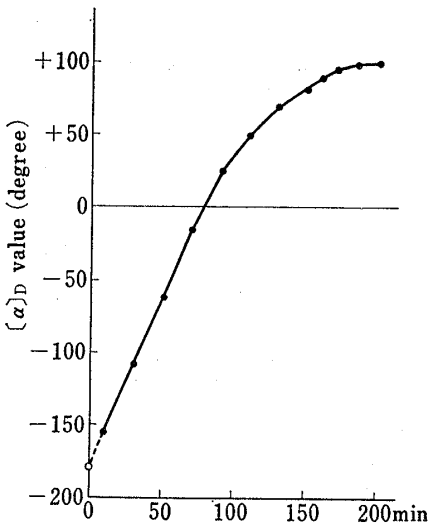

Fig. 3. Specific Rotatory Changes during Acid Hydrolysis
AL-I; ○: $[\alpha]_D$ at 15°, —●—: $[\alpha]_D$ at 69°

These polysaccharides were methylated by the methods of Hakomori[12] and Purdie.[13] After methanolysis and hydrolysis of these fully methylated polysaccharides, the resulting O-methylated arabinoses were converted into the corresponding alditol acetates, followed by analyzing those by GLC. The O-methylated arabinitol acetates were identified by comparison with the relative retention times of authentic samples and gas-liquid chromatography-mass spectrometry (GLC–MS). The mass spectra were compatible with those in the literature.[14] The results of the methylation analysis are given in Table II. Approximate molar ratio of 2,3,5-tri-, 2,3-di-, 2- and 3-mono-O-methylarabinitol acetates and penta-O-acetylarabinitol obtained from AL–I and AL–II was 1.00: 0.49: 0.10: 0.43: 0.21 for AL–I and 1.00: 0.59: 0.15: 0.47: 0.23 for AL–II.

Periodate oxidation of these polysaccharides was carried out with micro scale-analysis used by Ikekawa.[15] The amounts of periodate consumption per anhydroarabinose unit were 0.70 mol for AL–I and 0.66 mol for AL–II, respectively.

Table II. Relative Retention Times of O-Methylated L-arabinitol Acetates from the Methylated Azuki Arabinans

O-methylated L-arabinitol acetate	Relative retention times[b]		
	Condition A[c]	Condition B[d]	Condition C[e]
1,4-Ac_2-2,3,5-Me_3-Ara[a]	0.74	0.77	0.76
1,4,5-Ac_3-2,3-Me_2-Ara	2.10	1.90	1.91
1,3,4,5-Ac_4-2-Me-Ara	4.06	3.23	3.53
1,2,4,5-Ac_4-3-Me-Ara	4.34	3.52	3.77
1,2,3,4,5-Ac_5-Ara	6.06	4.58	5.27

a) Abbreviations: Ac=acetyl; Me=methyl; Ara=arabinitol.
b) Relative to 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-arabinitol.
c) 3% ECNSS-M, 170°.
d) 3% Silicone OV-225, 176°.
e) 15% LAC-4R 886 grease, 198°.
Gas chromatographic conditions are described in the experimental section.

The experimentally determined consumptions were in good agreement with those of 0.67 mol for AL–I and 0.65 mol for AL–II per pentose residue, evaluated from the methylation analysis. Smith degradations of AL–I and AL–II produced glycolaldehyde, large amounts of glycerol and arabinose. The detection of glycerol and none of ethylene glycol support the idea that non-reducing terminal arabinose is furanose type.

From the above results, it is concluded that both arabinans have a chain of 1→5 linked L-arabinofuranose units bearing a highly branched structure with branching points either at each of position 2 and 3 or at both these positions, to which non-reducing terminal furanosyl residues are attached. At the branching points, the proportion of 1→2 linkage is larger than

12) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
13) T. Purdie and J.C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).
14) H. Björndal, B. Lindberg, and S. Svensson, *Carbohyd. Res.*, **5**, 433 (1967).
15) T. Ikekawa, *J. Biochem.* (Tokyo), **54**, 328 (1963).

that of 1→3 linkage. These arabinans were similar to each other on the structure on the basis of the experimental results described above. However, some significant differences between AL–I and AL–II were observed in physical properties such as molecular weight and solubility in 80% aqueous ethanol solution. In addition, the structural analysis indicated the following differences: the amount of non-reducing terminal residues in AL–I was slightly larger than that in AL–II; the proportion of 1→5 linkage bearing 1→2 or 1→3 branching point in AL–I was slightly lower than that in AL–II.

Table III. Molar Composition of the Methylated Arabinans from Different Sources

O-methylated L-arabinitol acetate	Soybean[16a] arabinan	Mustard seed[16b] arabinan	Rapeseed[16c] arabinan	Azuki bean arabinan	
				AL-I	AL-II
1,4-Ac_2-2,3,5-Me_3-Ara[a]	1.00	1.00	1.00	1.00	1.00
1,4,5-Ac_3-2,3-Me_2-Ara	0.77	0.64	0.76	0.49	0.59
1,3,4,5-Ac_4-2-Me-Ara	0.36	0.72	0.93	0.10	0.15
1,2,4,5-Ac_4-3-Me-Ara	0.15	trace	trace	0.43	0.47
1,2,3,4,5-Ac_5-Ara	0.27	0.16	0.26	0.21	0.23

a) abbreviations are the same as described in Table II.

A comparison is made in Table III between results obtained on methylation analysis of the present and other arabinans from different sources. The proportions of AL–I and AL–II were similar to ordinary arabinans of plant origin[16a—d] on the highly branched structure composed of L-furanoid units and on the relatively low molecular weight. However, both azuki arabinans differ from ordinary arabinans in the fact that AL–I and AL–II possess larger proportion of 1→2 linkage than that of 1→3 linkage at the branching points of 1→5 linked chain. Furthermore, as is distinct from ordinary arabinans except for the case of mustard seed,[16b] AL–I and AL–II are composed of only arabinose and are not contaminated with any other sugar residue.

The water and alkaline extracts of azuki beans contain some other neutral and pectin-like acidic polysaccharides. Structural studies of these polysaccharides are now in progress.

Experimental

Specific rotations were measured by the use of a JASCO Model DIP-4 automatic polarimeter. IR spectra were recorded on a Japan Spectroscopic Co., Model IRA-I spectrometer. GLC was carried out with a glass column (2 m long × 0.3 cm inner diameter) by the use of a JEOL Model JGC-1100 gas chromatograph equipped with a hydrogen flame ionization detector. GLC-MS was carried out by the use of a Shimadzu Model LKB-9000 gas chromatograph and mass spectrometer.

Materials——Commercial azuki beans (seeds of *Phaseolus radiatus* L. var. *aurea* Prain, cultivated type: dainagon) harvested in Hokkaido (Japan) were used as a material source. Partially methylated L-arabinitol acetates used as reference compounds for GLC and GLC-MS were newly prepared. Enzymes, namely, α-amylase (Type III, 50—100 units/mg), glucoamylase (pure grade, 20 units/mg) and Pronase (45000 PUK/g) were purchased from Sigma Chemicals, Pharmacia Fine Chemicals and Kaken Chemical Ind., Ltd., respectively. DEAE-Sephadex A-25, Sephadex G-200 and standard dextrans (Dextran T-10, T-20, T-40 and T-70) were purchased from Pharmacia Fine Chemicals.

Extraction and Isolation of Polysaccharides——Azuki beans were pulverized and sieved (200 mesh) to remove the hulls. The dehulled flour (250 g), thus obtained, was refluxed in 80% EtOH for 1 hr. After dilution with H_2O up to 70% concentration, the mixture was allowed to stand overnight at room temperature. The extraction with 70% EtOH was repeated 2 times. The solid residue obtained was extracted with hot

16) *a*) G.O. Aspinall and I.W. Cottrell, *Can. J. Chem.*, **49**, 1019 (1971).; *b*) E.L. Hirst, D.A. Rees and N.G. Richardson, *Biochem. J.*, **95**, 453 (1965).; *c*) I.R. Siddiqui and P.J. Wood, *Carbohyd. Res.*, **36**, 35 (1974).; *d*) W. Pigman and D. Horton, "The Carbohydrates, Chemistry and Biochemistry," Vol IIB, 2nd ed., Academic Press, 1970, p. 517.

H_2O (93—98°) for 4 hr (3 times). After centrifugation, the swollen residue was directly treated with 5% NaOH overnight at room temperature (3 times), and centrifuged at 5000 rpm for 30 min. The supernatant solution was immediately neutralized with dil. HCl. Then, the combined mixture was dialyzed against running water for 3 days. The internal solution separated from a small amount of insoluble materials by centrifugation was concentrated to about 1 liter under reduced pressure, to which was added equal volume of 0.1 N phosphate buffer (pH 7.0). The mixture was treated with α-amylase (2 g) at 37° until iodine reaction was negative (3 days), and dialyzed against running water for 3 days. After removal of a small amount of insoluble materials by centrifugation, the internal solution was concentrated to about 1 liter under reduced pressure and adjusted to pH 7.8 with 1 N NaOH. To the mixture was added Pronase at 0 hr (600 mg), 48 hr (300 mg) and 96 hr (150 mg), respectively. The reaction mixture was incubated at 37° for 146 hr, while pH value was sometimes adjusted to 7.8. After dialyzing against running water for 3 days, the internal solution was further deproteinized by the Sevag method.[9] The supernatant separated by centrifugation was concentrated to 80 ml under reduced pressure, to which was added EtOH (187 ml) with stirring up to 70% concentration. The mixture was allowed to stand in a refrigerator for 2 days to liberate a crudy precipitate. The precipitate was collected by centrifugation at 8000 rpm for 20 min and dissolved in a small amount of H_2O, and lyophilized to give fraction ALP as brown flakes (yield: 2.2 g). The supernatant was concentrated to a small volume under reduced pressure, and lyophilized to afford fraction AL as pale brown flakes (yield: 2.6 g).

A portion (350 mg) of the polysaccharide fraction AL was dissolved in 0.02 N sodium acetate buffer (pH 4.7, 40 ml) and further treated with glucoamylase (6 mg). The reaction mixture was incubated at 28° for 77 hr in cellulose tubing under dialyzing against the same buffer and then successively dialyzed against deionized water for 3 days. The internal solution was heated for 5 min in boiling water bath and the insolubilized enzyme was removed by filtration. Then, the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in 0.02 N sodium acetate buffer (pH 6.1, 8 ml) and applied to a column (2.6 × 26.5 cm) of DEAE-Sephadex A-25, which was previously prepared with 0.02 N sodium acetate buffer at pH 6.1. The neutral fraction eluted with the buffer was dialyzed against deionized water for 3 days. The dialysate was concentrated to 20 ml under reduced pressure, to which was added EtOH (80 ml) with stirring up to 80% concentration. After being allowed to stand in a refrigerator for 2 days, the mixture was divided into fraction AL-I (supernatant) and AL-II (precipitate) by centrifugation at 8000 rpm for 30 min. Two fractions thus obtained was respectively lyophilized to afford polysaccharides AL-I (53 mg) and AL-II (36 mg) as slightly yellowish flakes. These polysaccharides were rather hygroscopic.

Zone Electrophoresis——Zone electrophoresis was carried out on Whatman GF-81 glass fiber paper (4 × 40 cm) with 0.05 M sodium tetraborate buffer (pH 9.3) at the condition of 240 volt for 2 hr. Each polysaccharide was applied in line at 13 cm from the anode. The spot was detected with α-naphthol–H_2SO_4 reagent.[17] Each polysaccharide gave one spot at a distance of 11 cm from the origin.

Gel Chromatography with Sephadex G-200——Each polysaccharide (1.5—2.0 mg) was dissolved in 0.1 M NaCl (0.5 ml) and applied to a column (1.5 × 96.5 cm) of Sephadex G-200 previously prepared. The elution was carried out by descending method with 0.1 M NaCl as an eluent at a flow rate of 9.5 ml per hr. Fractions were collected at 4 ml and an aliquot of each was analyzed for carbohydrate by phenol–H_2SO_4 method.[18]

Estimation of Molecular Weight ($\bar{M}w$)——The following commercial dextrans were used as standards for estimation of molecular weight ($\bar{M}w$): Dextran T-10 ($\bar{M}w$, 10400), Dextran T-20 ($\bar{M}w$, 22300), Dextran T-40 ($\bar{M}w$, 39500) and Dextran T-70 ($\bar{M}w$, 70000). Gel chromatography of the standard dextrans was carried out under similar conditions as used for our polysaccharides. Elution volume (Ve) was determined by calculation from each elution diagram. Then the Ve were plotted against logarithms of $\bar{M}w$ to give a linear relationship. Ve: Dextran T-10 134.8 ml, Dextran T-20 113.4 ml, Dextran T-40 97.0 ml, Dextran T-70 80.2 ml, AL-I 129.5 ml, AL-II 113.6 ml. The approximate molecular weight ($\bar{M}w$) of each polysaccharide was 13000 for AL-I and 22000 for AL-II, respectively.

Rate of Acid Hydrolysis——Each polysaccharide (1.6 mg) was hydrolyzed with 1 N H_2SO_4 (16 ml) at 100° in a sealed glass tube. After various periods of time, 1 ml of the hydrolysate was taken up, neutralized with 1 N NaOH (1 ml) and analyzed for reducing activity by the method of Somogyi–Nelson.[19] The increase of reducing activity is shown in Fig. 2.

Qualitative Analyses of Component Sugar——Each polysaccharide (1.5—3.0 mg) was hydrolyzed with 1 N H_2SO_4 (2 ml) in a sealed glass tube at 100° for 2—4 hr. The hydrolysate was neutralized with $BaCO_3$ and filtered. The filtrate was passed through a column of Amberlite CG-120 (H^+) and the eluate was concentrated to a small volume under reduced pressure.

PPC was carried out on Toyo Roshi No. 51A filter paper by ascending method with the following solvent systems (v/v): (A) AcOEt: pyridine: H_2O (10: 4: 3), (B) *n*-BuOH: pyridine: H_2O (6: 4: 3), (C) *n*-

17) H. Jacin and A.R. Mishkin, *J. Chromatogr.*, **18**, 170 (1965).
18) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
19) M. Somogyi, *J. Biol. Chem.*, **160**, 69 (1945); **195**, 19 (1952).

BuOH: EtOH: H_2O (4: 1: 5, upper layer). Sugar spots were detected with *p*-anisidine–HCl[20] and alkaline $AgNO_3$[21] reagents.

TLC was carried out on Kieselguhl G (Merck) containing 0.1 M AcONa with the following solvent system, *n*-BuOH: pyridine: H_2O (70: 15: 15), and naphthoresorcinol–H_2SO_4 reagent[22] was used for detection.

Then each hydrolysate was reduced with $NaBH_4$ (6 mg) for 4 hr at room temperature to convert into corresponding alditol. The excess borohydride was decomposed by addition of Amberlite CG-120 (H^+) resin. After removal of the resin by filtration, the filtrate was concentrated to dryness and freed from boric acid by repeated evaporations with MeOH. The well-dried residue was treated with trifluoroacetic anhydride in AcOEt for GLC.

GLC of the trifluoroacetate derivative prepared from each hydrolysate was carried out under the following condition, a glass column packed with 2% XF-1105 on Chromosorb P (80 to 100 mesh) at 137° with a flow rate of 37 ml per min of N_2.

Recovery of Crystalline Arabinose from the Acid Hydrolysate of AL-I——The hydrolysate of AL-I was concentrated to dryness and crystallized from 95% EtOH (0.5 ml) by allowing to stand in a freezer for 2 weeks. The crystals (mp 152—154°, $[\alpha]_D^{13}$ +105.1° equil., in H_2O, c=0.039) thus obtained were identified as L-arabinose by mixed melting point test and by its IR spectral comparison with an authentic sample.

Specific Rotatory Changes during Acid Hydrolysis——Polysaccharide AL-I (2.27 mg) was dissolved in 1 N H_2SO_4 (8 ml) at 15° and then hydrolyzed at 69°. Specific rotations were measured at various periods of time. The initial value of highly negative specific rotation, $[\alpha]_D^{15}$ −178.5° (c=0.028, 1 N H_2SO_4), changed gradually from levo- to dextrorotatory to reach a constant value, $[\alpha]_D^{69}$ +100° in 200 min. Authentic L-arabinose showed as follow, $[\alpha]_D^{24}$ +105.5° equil., in H_2O, c=0.091.

Methylation——NaH (250 mg, 50% mineral oil) was mixed with Me_2SO (5 ml) and the mixture was stirred at 60—70° for 1 hr. The mixture (3 ml) was added into Me_2SO (2 ml) solution containing polysaccharide (10 mg). After stirring for 5 hr at room temperature, MeI (2 ml) was added dropwise with cooling and the reaction mixture was stirred overnight at room temperature. Above all procedures were carried out in nitrogen atmosphere. After dilution with a small amount of H_2O, the solution was dialyzed against running water. Then the internal solution was concentrated to dryness under reduced pressure.

To the above residue dissolved in MeI (25 ml) was added Ag_2O (4 g) in small portions. The mixture was stirred under reflux for 8 hr, and stirred overnight at room temperature and then filtered. The filtrate was evaporated to dryness under reduced pressure. Above methylation procedure was repeated 3 times. The final methylated products showed no hydroxyl absorption band in the IR spectra.

Methanolysis, Hydrolysis and Analyses of the Methylated Sugars——Each fully methylated polysaccharide was methanolyzed with 4% methanolic hydrogen chloride (4 ml) in a sealed glass tube at 100° for 15 hr. After neutralization with Ag_2CO_3 followed by filtration, the filtrate was carefully evaporated to dryness under a mild condition.

Then each methanolysate was hydrolyzed with 1 N H_2SO_4 (4 ml) in a sealed glass tube at 100° for 14 hr. The hydrolysate was neutralized with $BaCO_3$, filtered and concentrated to a small volume under reduced pressure. The resulting methylated sugars were reduced with $NaBH_4$ (20 mg) for 24 hr at room temperature to convert into corresponding methylated alditols. After neutralization with Amberlite CG-120 (H^+), the mixture was evaporated with repeated additions of MeOH. The products were acetylated with Ac_2O and dry pyridine (1: 1, 1 ml) at 95° for 1.5 hr. The reaction mixture was directly injected into the GLC column. GLC was carried out under the following conditions; condition A: a glass column packed with 3% ECNSS-M on AW-Gaschrom Q (100 to 120 mesh) at 170° with a flow rate of 43 ml per min of N_2. condition B: a glass column packed with 3% Silicone OV-225 on AW-DMCS-Chromosorb W (80 to 100 mesh) at 176° with a flow rate of 43 ml per min of N_2. condition C: a glass column packed with 15% LAC-4R 886 grease[23] on Chromosorb W (80 to 100 mesh) at 198° with a flow rate of 38 ml per min of N_2. Table II shows relative retention times of the O-methylated arabinitol acetates to authentic 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-arabinitol under above three conditions.

For GLC-MS, a portion of the acetylation mixture was evaporated with a small amount of toluene and the residue was dissolved in few drops of $CHCl_3$. GLC-MS was carried out under the following condition, a glass column (0.4×100 cm) packed with 3% ECNSS-M on AW-Gaschrom Q (100 to 120 mesh) at 188° with a flow rate of 30 ml per min of helium. The mass spectra were recorded at an ionizing potential of 70 eV, an ionizing current of 60 μA and a temperature of the ion surce of 290°. The identities of the O-methylated arabinitol acetates prepared from these methylated polysaccharides were confirmed by comparison with ion peaks in authentic samples and by the appearance of base peaks as reported by H. Björndal.[14]

Periodate Oxidation——Periodate oxidation procedure was carried out with the method used by Ikekawa.[15] Polysaccharides AL-I (5.8 mg), AL-II (4.9 mg) together with methyl α-D-glucoside (3.0 mg) as

20) L. Hough, J.K.N. Jones, and W.H. Wadman, *J. Chem. Soc.*, **1950**, 1702.
21) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature* (London), **166**, 444 (1950).
22) G. Pastuska, *Z. Analyt. Chem.*, **179**, 69 (1961).
23) S.C. Williams and J.K.N. Jones, *Can. J. Chem.*, **45**, 275 (1967).

a standard were oxidized with 2.5 mM $NaIO_4$ in 0.025 N sodium acetate buffer (pH 4.6, 40 ml) at room temperature (5—12°) in a dark place, respectively. After various periods of time, the periodate consumption was measured by a spectrophotometric method (290 nm) and calculated from the calibration curve of $NaIO_4$ (0 to 3 mM).

The oxidation of these polysaccharides was completed in 842 hr and the amounts of periodate consumption per component anhydroarabinose unit was 0.70 mol for AL-I and 0.66 mol for AL-II, respectively. In this experiment, 1.98 mol of periodate consumption per methyl α-D-glucoside was extremely theoretical.

Smith Degradations and Analyses of the Products——Each oxidized polysaccharide solution, after addition of ethylene glycol (0.2 ml), was dialyzed against deionized water for 2 days and the internal solution was concentrated to a small volume under reduced pressure. The oxidized product was reduced with $NaBH_4$ (15 mg) for 48 hr at room temperature. After neutralization with Amberlite CG-120 (H^+), the mixture was evaporated with repeated additions of MeOH. The residue containing polyalcohol was obtained. A portion of each polyalcohol was mildly hydrolyzed with 0.3 N HCl (1 ml) for 48 hr at room temperature in a sealed glass tube. Then the hydrolysate was treated with hydroxylamine hydrochloride (8 mg) at 80° for 30 min in a sealed tube, and the reaction mixture was concentrated under reduced pressure. The residue was dissolved in pyridine (0.1 ml) containing trimethylolpropane as an internal standard and trimethylsilylated with hexamethyldisilazane (0.05 ml) and trimethylchlorosilane (0.03 ml)[24] for GLC. GLC was carried out under the following condition, dual columns packed with 5% SE-30 on AW-Chromosorb W (60 to 80 mesh); programmed column temperature increasing in 4° per min from 90° to 180°; carrier gas, N_2 (43 ml per min). Retention times of trimethylsilyl derivatives of the low molecular Smith degradation products were as follows; glycolaldehyde (oximes) 6.2, 6.5 min, glycerol 11.0 min, trimethylolpropane (internal standard) 15.9 min.

A residual portion of each polyalcohol was completely hydrolyzed with 0.5 N H_2SO_4 (4 ml) at 100° for 4 hr. After neutralization with $BaCO_3$ followed by filtration, the filtrate was passed through a small column of Amberlite CG-120 (H^+) and the eluate was concentrated to a small volume under reduced pressure. By PPC, glycerol and arabinose were detected in each sample, *Rf*: glycerol 0.37, arabinose 0.21. Solvent system: (A), Spray reagent: alkaline $AgNO_3$.[21] Then, each hydrolysate was reduced with $NaBH_4$ and acetylated by the method as described above. GLC was carried out under the following condition, dual columns packed with 3% ECNSS-M on AW-Gaschrom Q (100 to 120 mesh); programmed column temperature increasing in 6° per min from 60° to 180°; carrier gas, N_2 (43 ml per min). Retention times of acetate derivatives of sugar alcohols were as follows; small amount of ethylene glycol (derived from glycolaldehyde by reduction) 7.9 min, glycerol 18.1 min, arabinitol 40.8 min, erythritol (internal standard) 24.4 min.

Acknowledgement We wish to thank Dr. I. Himuro, Research Laboratories, Chugai Pharmaceutical Co., Ltd., for measurement of GLC-MS spectra. Thanks are also due to the members of the Central Analysis Room of this college for elemental analyses.

24) C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).