Structure Determination of Polysaccharides in *Aloe arborescens* var. *natalensis*

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Abstract: Neutral polysaccharides and a glycoprotein were isolated by gel filtration from a nondialyzable fraction of *Aloe arborescens* var. *natalensis* Berger. These were shown to contain the following: a linear polymer of a (1, 6)-*O*-linked α-D-glucopyranose (mol. wt. 15,000); a branched polymer of an arabinogalactan (mol. wt. 30,000, molar ratio of galactose to arabinose 1 : 1.5) with two principle linear chains of (1-2)-*O*-L-arabinopyranose and (1-2)-*O*-D-galactopyranose at O-2 and O-6 of the D-galactopyranose residue; a linear polymer of a (1, 4)-O-linked β-D-mannopyranose (mol. wt. 40,000) with 10 % acetyl group; a glycoprotein (mol. wt. 40,000; protein, 57 %; hexosamine, 4 %; and carbohydrate, 34 %) consisting of glucose, mannose, galactose, glucosamine, galactosamine, *N*-acetylglucosamine (2 : 2 : 1 : 1 : 4 : 1) and amino acids (Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Phe, Orn, Lys, Arg, and Pro). The linear chain of (1, 4)-*O*-linked β-D-mannopyranose with 10 % acetyl groups and the glycoprotein enhanced phagocytosis in adult bronchial asthmatic.

Introduction

Polysaccharides consisting of D-mannose (1), D-galactose and D-mannose (2), and equal amounts of D-mannose and D-glucose together with a small amount of uronic acid (3) were found in the pulp of *Aloe* leaves. As for glycoprotein, aloctin A and B were isolated and their biological activities reported (4). In an earlier experiment it was shown that oral administration of *Aloe* extract showed efficacy for chronic bronchial asthmatics of various ages as well as intrinsic types (5). In the screening of *Aloe* extracts for phagocytosis and nitroblue tetrazolium chloride (NBT) reduction, glycoprotein and polysaccharide fractions showed phagocytosis-enhancing activity (6). The present work was undertaken to isolate the active component of the nondialyzable fraction. This paper deals with the determination of structure and evaluation of polysaccharides and glycoproteins as active phagocytosis promoters.

Materials and Methods

General

Infrared (IR) spectra were obtained with a KOKEN DS-301 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL PS-100 (100 M Hz) and JEOL FX-100 (25 MHz) spectrometers, respectively. Chemical shifts were recorded in δ units relative to the internal standard, 3-(trimethylsilyl)-propanesulfonic acid sodium salt; s = singlet, d = doublet, t = triplet, and m = multiplet; *J* values are in Hz. Optical rotations were obtained on a JASCO DIP-4.

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Plant material

The fresh leaves of the plant were harvested in June, 1981, at the green house of the herbal garden of Kyushu University where a specimen is available for inspection.

Methods of Analysis

Elution of the polysaccharide fraction from a Sepharose 6B (Pharmacia Fine Chemicals) column, 2.8 × 46 cm and diethylaminoethyl cellulose (DEAE-cellulofine, Chisso Corp.) column, 4.5 × 28 cm, and of the glycoprotein fraction from a Sepharose 6B column, 4.5 × 28 cm, was monitored by absorbance of effluents at 490 nm using the phenolsulfuric acid method (7) and at 260 nm.

Acid hydrolyses of polysaccharide A (3 mg) and C (4 mg) were carried out in 2N H_2SO_4 at 90° C for 3 h. Each hydrolyzate was analyzed by PC and GC to give D-glucose and D-mannose, respectively. Acid hydrolysis of polysaccharide B (3 mg) was carried out in the same manner as in polysaccharide A, and arabinose and galactose were detected on PC and GC analyses. The spots corresponding to arabinose and galactose were extracted from the paper with water. The extracts were evaporated to a syrup and determined to be arabinose (0.6 mg), $[\alpha]_D^{22}$ +107° (c = 0.06, H_2O) and galactose (0.4 mg), $[\alpha]_D^{22}$ +75° (c = 0.04, H_2O). By comparing each sugar weight, the molar ratio of arabinose to galactose was estimated to be 1.5 : 1.0.

Alkaline hydrolysis of the polysaccharide C (4 mg) was performed with 1N NaOH (2 ml) at 90° C for 10 min, and the reaction mixture was dialyzed against distilled water followed by lyophilization to give deacetylpolysaccharide C (2 mg), $[\alpha]_D^{15}$ −40° (c = 0.1, 1N NaOH), with no absorption band in the IR spectrum due to an acetyl group. Deacetylpolysaccharide C is barely soluble in water.

PC of the sugar moiety was performed on a paper chromatogram (Toyo Roshi No. 50) and a developing solution of BuOH/pyridine/H_2O (6 : 4 : 3); detection was by spraying with aniline hydrogen phthalate followed by heating. TLC was done on a cellulose F_{254} (Merck) with EtOAc/BuOH/AcOH/H_2O (6 : 8 : 5 : 8) and BuOH/pyridine/H_2O (6 : 4 : 3) as solvent systems.

GC of the trimethylsilylated sugar which was obtained from the reaction of a sugar moiety with *N*-trimethylsilylimidazole was performed on a column of 1.5 % silicone SE 52 (3 mm × 2 m) on chromosorb W (AWDMCS, 60–80 mesh) at 200° C with a nitrogen flow rate of 45 ml/min. The following retention times (min) were obtained: arabinose 5.6, 6.8; mannose 9.2, 14.6; glucose 13.6, 21.4; galactose 11.4, and the hydrolysate of polysaccharide A, B or C: 13.6, 21.6; 5.6, 6.8, 11.4; 9.2, 14.6 min; glucosamine 8.0, 9.2; galactosamine 6.8, 9.6; *N*-acetylglucosamine 13.2, and the hydrolysate of glycoprotein A: 9.2, 11.6, 13.8, 14.6, 21.4; 6.8, 8.0, 9.2, 9.6 and 13.2.

Methylation analysis of carbohydrate was performed according to the method of Hakomori (8). This procedure was carried out twice to give permethylates showing no absorption band in the IR spectrum due to a hydroxyl group. Each sample of the permethylate of polysaccharide A, B or C (5 mg) was subjected to methanolysis with 4 % hydrogen chloride in MeOH (1 ml) in a sealed tube at 90° C for 2 h. After the removal of the solvent, each methanolysate (dissolved in EtOAc) was chromatographed on a silica gel column using EtOAc and EtOAc/MeOH as solvents to give material which showed one spot on TLC (EtOAc/MeOH, 25 : 1). GC of each methanolysate was performed under conditions (a) and (b) as shown in Table I.

Methylation analysis of polysaccharide A and C gave methyl 2, 3, 4-trimethyl-*O*-glucopyranoside and methyl 2, 3, 6-trimethyl-*O*-man-

Table I. Rentention times (min) of methylated sugars and the methanolysates of polysaccharide A, B, and C

Conditions (a)		Conditions (b)	
Methyl glucopyranosides of		Methyl glucopyranosides of	
2, 3, 4, 6-tetramethyl-*O*-	0.5	2, 3, 4, 6-tetramethyl-*O*-	0.3
3, 4, 6-trimethyl-*O*-	1.1	3, 4, 6-trimethyl-*O*-	6.5
2, 4, 6-trimethyl-*O*-	1.7	2, 4, 6-trimethyl-*O*-	3.5
2, 3, 6-trimethyl-*O*-	1.6	2, 3, 6-trimethyl-*O*-	5.8
2, 3, 4-trimethyl-*O*-	1.3	2, 3, 4-trimethyl-*O*-	2.0
the methanolysate of polysaccharide A	0.5; 1.3	the methanolysate of polysaccharide A	0.3; 2.0
Methyl mannopyranosides of		Methyl mannopyranoside of	
2, 3, 4, 6-tetramethyl-*O*-	1.8	2, 3, 4, 6-tetramethyl-*O*-	4.1
3, 4, 6-trimethyl-*O*-	2.8	3, 4, 6-trimethyl-*O*-	8.6
2, 4, 6-trimethyl-*O*-	3.3	2, 4, 6-trimethyl-*O*-	10.1
2, 3, 6-trimethyl-*O*-	4.3	2, 3, 6-trimethyl-*O*-	12.3
2, 3, 4-trimethyl-*O*-	2.7	2, 3, 4-trimethyl-*O*-	8.5
the methanolysate of polysaccharide C	1.8; 4.3	the methanolysate of polysaccharide C	4.1; 12.3
Methyl arabinopyranosides of		Methyl arabinopyranosides of	
3, 4-dimethyl-*O*-	1.2	3, 4-dimethyl-*O*-	5.8
2, 4-dimethyl-*O*-	1.6	2, 4-dimethyl-*O*-	5.2
2, 3-dimethyl-*O*-	1.0	2, 3-dimethyl-*O*-	5.2
4-monomethyl-*O*-	2.4	4-monomethyl-*O*-	6.0
3-monomethyl-*O*-	2.5	3-monomethyl-*O*-	6.0
2-monomethyl-*O*-	3.6	2-monomethyl-*O*-	6.2
Methyl galactopyranosides of		Methyl galactopyranosides of	
3, 4, 6-trimethyl-*O*-	3.0	3, 4, 6-trimethyl-*O*-	11.8
2, 4, 6-trimethyl-*O*-	3.4	2, 4, 6-trimethyl-*O*-	12.4
2, 3, 6-trimethyl-*O*-	4.0	2, 3, 6-trimethyl-*O*-	12.2
2, 3, 4-trimethyl-*O*-	7.6	2, 3, 4-trimethyl-*O*-	12.4
2, 6-dimethyl-*O*-	9.2	2, 6-dimethyl-*O*-	–
3, 6-dimethyl-*O*-	7.4	3, 6-dimethyl-*O*-	29.0
4, 6-dimethyl-*O*-	5.4	4, 6-dimethyl-*O*-	12.8
2, 3-dimethyl-*O*-	11.8	2, 3-dimethyl-*O*-	–
2, 4-dimethyl-*O*-	14.4	2, 4-dimethyl-*O*-	–
3, 4-dimethyl-*O*-	13.4	3, 4-dimethyl-*O*-	31.0
the methanolysate of polysaccharide B	1.2; 3.0; 13.6	methanolysate of polysaccharide B	5.8; 11.8; 31.4

Condition (a): a column of 1 % neopentylglycol succinate polyester (3 mm × 2 m) on chromosorb W (AWDMCS, 60 – 80 mesh) at 147° C with a nitrogen flow rate of 40 ml/min.
Condition (b): a column of 5 % 1, 4-butandiol succinate (3 mm × 2 m) on chromsorb W (AWDMCS, 60 – 80 mesh) at 180° C with a nitrogen flow rate of 60 ml/min.

nopyranoside, respectively. Methylation analysis of polysaccharide B gave methyl 3, 4-dimethyl-*O*-arabinopyranoside, methyl 3, 4, 6-trimethyl-*O*-galactopyranoside and methyl 3, 4-dimethyl-*O*-galactopyranoside. By comparing the area of each methylated sugar, the molar ratio was estimated to be 1.56 : 1.00.

Quantitative analysis of the acetyl content was determined by GC with a column of chromosorb 102 (Johns-Manville, 3 mm × 2 m) at 158° C, a nitrogen flow rate of 55 ml/min, and propionic acid as an internal standrad (9). The acetyl content of polysaccharide C was estimated to be 10.0 %.

Ultracentrifugation was performed on an analytical ultracentrifuge with a Schlieren optical system in a single sector at 20° and 2×10^5 g (9).

Molecular weights were estimated from gel filtration on a Sepharose 6B column (1.5 × 51.5 cm) eluted with 0.3 M NaCl at a flow rate of 26 ml/h. A series of dextrans, 10, 20, 40, 70, and T-500 (Pharmacia Fine Chemicals) was used as standards and molecular weights were estimated from the linear correlation between the logarithm of the molecular weights of the standards and the ratios of their elution volumes to the void volume of the column (9). Molecular weight of polysaccharide A, B, and C and glycoprotein A were determined to be 15,000, 30,000, and 40,000 and 40,000, respectively.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed accoding to Laemmli (10) on a 10 % gel at pH 7.2. Samples were prepared in buffer containing 1 % (w/v) SDS and 5 % (v/v) 2-mercaptoethanol and left for 3 min at 100° C before application. Electrophoresis was carried out with a current of 3 mA per gel until the bromphenol blue marker reached the bottom of the gel. Gels were stained with Coomassie brilliant blue R250 and periodate Schiff reagents (11).

For amino acid analysis, the sample was hydrolyzed in 6N HCl at 110° C for 20 h in an evacuated sealed tube. The hydrolyzate was then evaporated to dryness, dissolved in 0.2N HCl, and analyzed with a Hitachi 835 amino acid analyzer.

Hydrolysis of glycoprotein A (20 mg) was carried out with pronase E (Kaken Kagaku) at pH 7.8 in phosphate buffer (2 ml) for 24 h at 37° C and lyophilized before gel-filtration (Sephadex G-25). The isolated carbohydrate moiety was hydrolyzed with 2N H_2SO_4. GC analysis revealed glucose, mannose and galactose. A sample of the glycoprotein A (20 mg) was hydrolyzed with 2N H_2SO_4 (2 ml) at 90° C for 3 h. After removal of excess $BaSO_4$, the solution was subjected to cation exchange resin chromatography (Dowex 50 W). The neutral fraction which was eluted from the column with water was examined by GC and showed glucose, mannose and galactose. Elution of the column with 2N NH_4OH yielded glucosamine, galactosamine and *N*-acetylglucosamine, as identified by GC analysis. The composition of the sugar moiety in glycoprotein A was determined to be glucose: mannose: galactose: glucosamine: galactosamine: *N*-acetylglucosamine (2 : 2 : 1 : 1 : 4 : 1). A part of the alkaline eluted fraction from the cation

exchange resin was evaporated to dryness, and the residue was gel-filtrated on Sephadex G-25 with monitoring at 260 nm. The composition of the peptide fraction was determined to be Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Phe, Orn, Lys, Arg, and Pro.

Quantitative analysis of the hexosamine and protein moieties in glycoprotein A was performed by the methods of Elson-Morgan (12) after acid hydrolysis with 6N HCl for 6 h, and Lowry (13), respectively.

Enzymatic hydrolysis of glycoprotein A (5 mg), suspended in 0.01M phosphate buffer (2 ml) at pH 5.3, was carried out with β-D-glucosidase (2 mg, almond emulsin, Sigma). The hydrolysate was examined as a trimethylsilyl ether on GC and showed glucose. A similar hydrolysis with mannosidase (5 mg, Seikagaku kogyo) followed by GC analysis revealed mannose.

Methylation analysis of glycoprotein A (40 mg) was carried out in a similar way as in polysaccharide A. From the GC analysis of the methanolysate, methyl 2, 3, 4, 6-tetra-*O*-methyl glucopyranoside, methyl 2, 6-di-*O*-methyl glucopyranoside, methyl 2, 3, 4, 6-tetra-*O*-methyl mannopyranoside and methyl 2, 6-di-*O*-methyl galactopyranoside were identified.

Procedure for biological assay

Preparation of leukocytes from venous blood of adult bronchial asthmatics and the procedures for the assay of NBT reduction and phagocytosis were according to the methods in a preceding paper (6). Aseptic techniques were employed throughout. Leukocytes from peripheral blood of patients were prepared for both phagocytosis and NBT reduction.

Preparation of leukocytes

To 20 ml of venous blood from adult bronchial asthmatic collected in a plastic syringe, 4 ml of 4.5 % dextran B (mol. wt. 1.7×10^5) in saline and 500 units of heparin were added. This was throughly mixed by gentle shaking and allowed to stand for 50 min at room temperature to sediment the erythrocytes. The supernate, rich in leukocytes, was removed by pressing and washed twice with Hank's balanced salt solution (Nakarai Chemicals Ltd.). Neutrophils were counted in an aliquot after Giemsa staining. The cells were suspended in a volume of Hank's balanced salt solution at a concentration of 2×10^6 neutrophils per milliliter for the NBT reduction test and 2×10^7 neutrophils per milliliter for the phagocytosis experiment.

NBT reduction test

To siliconized 15-cm conical centrifuge tube the following were added: 0.1 ml of 0.01M potassium cyanide, 0.05 ml of 0.81 μ latex spherules (Difco Lab.), 0.05 ml of aloe sample, and 0.5 ml of the cell suspension of neutrophils (2×10^6) in Hank's balanced salt solution. This mixture was preincubated in a water bath at 37° C for 7 min. Then, 0.5 ml of 0.1 % NBT (Nakarai Chemicals, Ltd.) in 0.15M phosphate buffer, pH 7.2, was added. The reaction was allowed to proceed for 60 min, then stopped by addition of 10 ml of 1N HCl. The reaction mixture was centrifuged at 700 g for 10 min, and the supernatant was discarded. To the precipitate was added 3 ml of dimethyl sulfoxide, and the reaction mixture was heated at 100° C for 20 min. The optical density of the visible purple color of the reduced NBT was determined at 515 nm with a Hitachi spectrophotometer against the control which consisted of the described reagents minus the neutrophil suspension.

Phagocytosis

The reaction mixture consisted of 0.2 ml of neutrophils (2×10^7) in 1 ml of Hank's balanced salt solution containing 5 % fetal calf serum and 0.05 ml of aloe sample. This mixture was preincubated in a water bath at 37° C for 7 min. A suspension of yeast particles was diluted with saline solution to 25×10^7 particles per milliliter and heated at 100° C for 30 min. To the preincubated reaction mixture, 0.1 ml of the suspension of yeast particles was added and incubation continued at 37° C for 60 min. The number of yeast particles phagocytosed by neutrophils was counted after staining with 5 % Fuchsin in phenol solution.

$$\text{Phagocytic index} = \frac{\text{number of yeast phagocytosed by 100 neutrophils}}{\text{control}}$$

Control: The aloe sample was replaced by 0.05 ml of 0.15M phosphate buffer, pH 7.2.

Results and Discussion

As shown in Fig. 1, 2, and 3, polysaccharide A, B, and C and glycoprotein A were isolated from the fresh leaves in a yield of 3×10^{-4}, 1×10^{-4}, 1×10^{-4} and 3×10^{-3} %, respectively.

Properties of polysaccharide A: $[\alpha]_D^{22}$ +95.0° (c = 2.0 %, 0.15M NaCl), Anal. Calcd for $(C_6H_{10}O_5)_n$ C, 40.44; H, 6.71. Found. C, 40.89; H, 6.65, N(-), Ash (-). $S_{20,w}$ = 1.49 S (c = 0.5 % in 0.1M NaCl), ^{1}H-NMR (D_2O) δ: 5.04 (br. s., anomeric H of a non-reducing sugar residue).

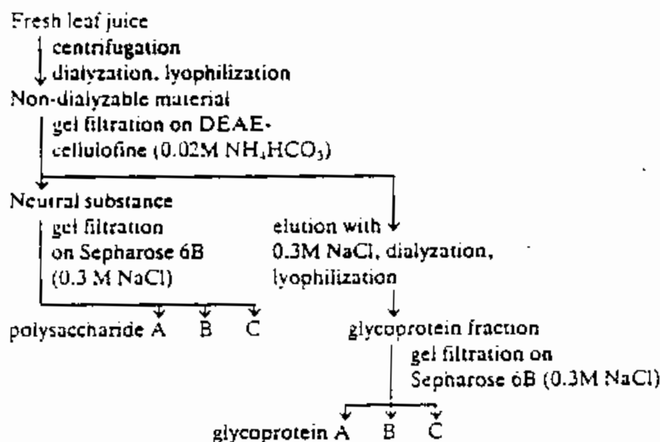

Fig. 1. Fractionation scheme of polysaccharides and glycoproteins

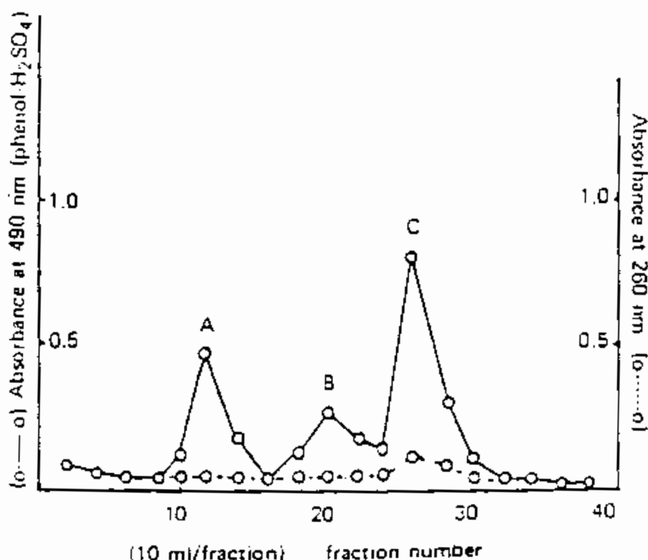

Fig. 2. Chromatography of polysaccharide fraction on Sepharose 6B

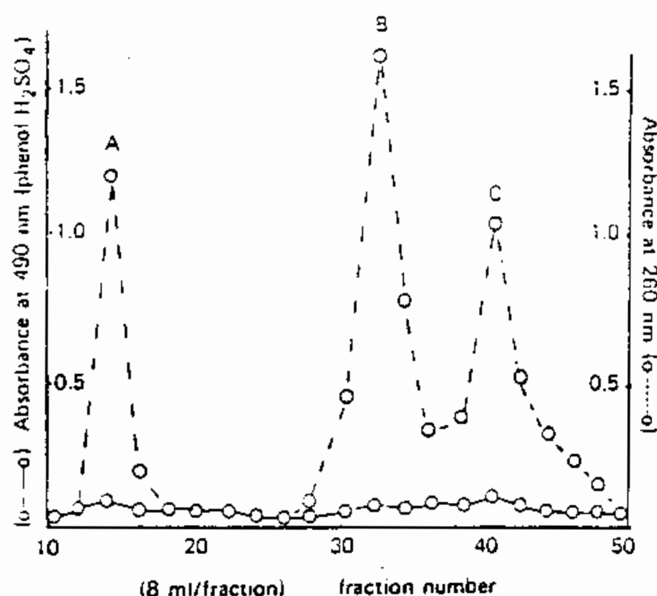

Fig. 3. Chromatography of glycoprotein fraction on Sepharose 6B

Polysaccharide B: $[\alpha]_D^{16}$ −66.7° (c = 0.25 %, H_2O), $S_{20,w}$ = 3.67 (c = 0.5 % in H_2O). ^{1}H-NMR (D_2O) δ: 4.46 (br. s., anomeric H of a non-reducing sugar residue).

Polysaccharide C: $[\alpha]_D^{20}$ −190° (c = 0.5 %, 0.1M NaCl), $S_{20,w}$ = 1.08 (c = 0.5 % in 0.1M NaCl). ^{1}H-NMR (D_2O) δ: 2.13 (br. s., CH_3COO), 4.10 (br. s., CH_3COOCH_2), 4.74 (br. s., anomeric H of a non-reducing sugar residue), 5.50 (CH_3COOCH). ^{13}C-NMR (0.1M NaCl) δ: 176.5 (CH_3COO), 22.9 (CH_3COO), and other signals are listed in Table II.

Glycoprotein A: $[\alpha]_D^{20}$ −10.0° (88 % HCOOH, c = 0.6 %), $S_{20,w}$ = 3.34 S (c = 1.2 % in 0.02M NH_4HCO_3), IR ν_{max}^{KBr} cm^{-1}: 3300, 1650, 1540, 1070. ^{1}H-NMR (DCOOD) δ: 2.07–2.28 (CH_3CONH), 3.9–4.42 (CHOH), 4.5–4.7 (anomeric H). Carbohydrate, hexosamine and protein contents were estimated to be 34,4, and 57 %, respectively.

Structure elucidation

^{13}C-NMR spectrometry has povided a very useful method for the determination of the structure of polysaccharide (14, 15). Table II summarizes the ^{13}C-NMR spectral data of polysaccharide A, B and C, together with As mannan 1[(1-4)-*O*-β-D-mannan with 18 % acetyl group, mol. wt. 15,000] which was isolated from *Aloe saponaria* (9). Comparison of the chemical shifts of polysaccharide A with those of isomaltodextrin [(1-6)-*O*-α-D-glucan (16, 17)] reveals a significant similarity. The C6 resonance of polysaccharide A is deshielded by 6 ppm relative to that of α-D-glucose due to the formation of 1-6-linkages. Accordingly, a linear formation of (1-6)-*O*-α-D-glucan in polysaccharide A was established.

These results support the finding obtained from the methylation analysis of polysaccharide A. By comparison of spectra with those of so called ivory nut mannan (18) [(1-4)-*O*-β-D-mannan, mol. wt. 13,000], a complete assignment of ^{13}C-NMR signals in polysaccharide C can be made. With respect to linkage formation, it is evident that the C4 resonance in polysaccharide C is deshielded by 11 ppm relative to the C4 resonance in β-D-mannose. Thus, the D-mannose in polysaccharide C was confirmed to have (1-4)-*O*-linkages. The ^{13}C-NMR spectral data are in accord with the results obtained from the ^{13}C-NMR study on As mannan 1 (9), the structure of which was established to be (1-4)-*O*-β-D-mannan with 18 % acetyl group. These results are consistent with those from the methylation analysis on polysaccharide C.

Methylation analysis of polysaccharide B, composed of arabinose and galactose in molar ratios of 1.5 : 1.0, indicated that the major chain consists of both (1-2)-*O*-linked arabinose and galactose and the branching point is O-2 and O-6 of galactopyranose. These findings explain the ^{13}C-NMR data of C2 resonances at 83.9 and 88.2 ppm which are deshielded by 14 and 18 ppm relative to those of methyl β-L-arabinopyranoside and methyl β-D-galactopyranoside. Therefore, the main chain in polysaccharide B consists of (1-2)-*O*-L-arabinose and (1-2)-*O*-D-galactose linkages with a branching point located at O-2 and O-6 of D-galactose (19). The direct bond C-H coupling constant of C1 ($J_{C1\text{-}H}$) of hexopyranose is characteristic of the anomeric configuration (20), however, it is difficult to assign α or β in polysaccharides having a broad signal due to anomeric protons of the non-reducing carbon atom. The chemical shifts due to equatorial anomeric hydrogen of non-reducing carbon atoms in polysaccharides always appear at a lower field (δ 5.02–4.92), although deviation was observed depending on substituents, the aglycone, and the measurement conditions (20–25). Based on the ^{1}H-NMR spectral study together with a comparison of specific rotation values, the configuration of the sugar linkages in polysaccharide A, B, and C were determined to be α, β, and β, respectively. A molar ratio of 2.3 : 1.0 of D-mannose to the acetyl group in polysaccharide C was observed in the ^{1}H-NMR spectra.

Table II. ^{13}C-NMR Spectral Assignments of Polysaccharides A, B, and C[a]

	Polysaccharides			As mannan 1[e]	Isomalto-dextrin[f]	Ivory nut mannan[g]
	A[b]	B[c]	C[d]			
C1	100.4	100.6	102.7	102.7	99.3	101.7
C2	74.2	83.9; 88.2	72.6	72.6	73.0	72.2
C3	76.2	73.5	74.1	74.1	74.8	73.5
C4	72.3	71.3	79.0	79.1	71.1	78.8
C5	72.9	75.4	77.6	77.6	71.1	78.8
C6	68.2	63.7	63.2	63.3	67.1	62.1

[a]Chemical shifts (δ) was recorded at 25.5 MHz with 3-(trimethylsilyl)-propanesulfonic acid sodium salt as an internal standard at 28° C.
[b]30 mg/ml in 0.3M NaCl.
[c]50 mg/ml in D_2O.
[d]20 mg/ml in 0.1M NaCl.
[e]10 mg/ml in 0.1M NaCl.
[f]See Ref. 15.
[g]See Ref. 17.

Table III. Effect of Polysaccharides A, B, C and Glycoprotein A on Phagocytosis and NBT reduction[a]

Samples	Dilution	Phagocytosis	NBT reduction
Polysaccharide A	1 : 1	1.029 ± 0.031	0.954 ± 0.115
	1 : 5	1.024 ± 0.057	0.924 ± 0.073
	1 : 10	1.040 ± 0.085	1.003 ± 0.067
Polysaccharide B	1 : 1	0.997 ± 0.121	0.887 ± 0.077
	1 : 5	1.150 ± 0.087	1.095 ± 0.154
	1 : 10	1.225 ± 0.196	1.010 ± 0.178
Polysaccharide C	1 : 1	1.025 ± 0.034	1.065 ± 0.059
	1 : 5	0.914 ± 0.062	1.166 ± 0.022*
	1 : 10	1.182 ± 0.054**	1.045 ± 0.047
Glycoprotein A	1 : 1	1.248 ± 0.079**	1.584 ± 0.476
	1 : 5	1.182 ± 0.086***	1.119 ± 0.078***
	1 : 10	1.189 ± 0.051*	1.078 ± 0.081

[a]Each sample (100 mg) was dissolved in 5 ml of 0.15M phosphate buffer at pH 7.2 and tested for phagocytosis and NBT reduction according to the method described in the experimental section. The values for phagocytosis are presented as phagocytic index. The phagocytic index of the control was calculated to be 1.000. The optical density of reduced NBT was determined at 515 nm. The optical density of the control was calculated to be 1.000.
Data were analyzed using Student's t-test to determine the significant difference. The values in the assay are means ± s. e. of 6 samples.
*, ** and ***: statistically significant from the control at $p < 0.02, 0.05$, and 0.1, respectively.

The position of the acetyl group in polysaccharide C is speculated to be at O-6 and O-2 (and/or O-3) because the signals appeared at δ 4.10 and 5.50 ppm in a ratio of peak height of 5 : 1 are assigned to C6 and C2 (and/or C3) protons attached to the acetyl group, respectively, when compared with those of D-mannopyranose pentaacetate. This speculation was ascertained from the ^{13}C-NMR spectral data that the chemical shifts due to C6 in polysaccharide C appear down-field relative to those of ivory nut mannan (17). Glycoprotein A was not sufficiently soluble in water, alkali or 88 % HCOOH, for ^{13}C-NMR spectral measurement.

Glycoprotein A appeared to be a single band on disc polyacrylamide electrophoresis on a 7 % gel at pH 9.5 and SDS polyacrylamide electrophoresis on a 10 % gel at pH 7.2, and showed a single symmetrical peak with a sedimentation coefficient of $S_{20,w} = 3.34$ S. The IR spectrum of glycoprotein A indicated an absorption band due to an amide bond and a sharp peak due to an *N*-acetyl group, in addition to the broad signals of anomeric proton noted in the ^{1}H-NMR spectrum. Glycoprotein A was composed of glucose, mannose, galactose, glucosamine, galactosamine and *N*-acetylglucosamine (2 : 2 : 1 : 1 : 4 : 1) and the following amino acids: Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Phe, Orn, Lys, Arg, and Pro. Enzymatic hydrolysis and methylation analysis of glycoprotein A showed that the sugar moiety is composed of terminal glucose and mannose residues, and glucose and galactose residues having branching points at O-3 and O-4. However, the linkages in the hexosamine and *N*-acetylglucosamine residues and the mode of covalent connection between the carbohydrate and amino acid could not be determined because of the structure complexity.

In vitro assay

Recently, many kinds of biologically active polysaccharides have been isolated from various sources, notably compounds having antitumor activity. It is possible that this activity is due to a host-mediated action of the polysaccharides; they may activate the depressed functions of phagocytes in tumor-bearing hosts. In an assay for phagocytosis it is preferable to use neutrophils from patients similar with regard to sex, age, type of asthma (chronic or acute), and personal clinical history (endogeneous or exogeneous). In addition, use of neutrophils from patients who have not been treated with corticosteroid is important (6).

Among the polysaccharides studied here, polysaccharide C showed some phagocytosis-enhancing effect (Table III). This may be closely related to the host-mediated action of the antitumor aloemannan [mol. wt. 15,000, $S_{20,w} = 1.55$ S (1)] which has a structural profile similar to that of polysaccharide C.

As shown in Table III, glycoprotein A demonstrated phagocytic activity in a dose-dependent fashion. Heat treatment destroyed the phagocytic activity, which suggests that the native structure of the protein moiety in glycoprotein A is essential for activity.

The phagocytic activity of polysaccharide C and glycoprotein A seen here may provide biomedical evidence for the anti-inflammation activity of *Aloe arborescens* var. *natalensis*. In a recent study (26), glycoprotein A was shown to stimulate deoxyribonucleic acid synthesis in baby hamster kidney cells and to have the properties of a lectin which reacts with sheep blood cells.

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