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Isolation and Characterization of Biopolymers Extracted from the Bark of *Acanthopanax sessiliflorus* and Their Anticomplement Activity

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Abstract The crude biopolymer (AS-S1) and endo-biopolymer (AS-S2) were isolated from the dry stem bark of *Acanthopanax sessiliflorus* and tested for anti complement activity. The two potent anticomplement biopolymers, AS-1 and AS-2-Fr.I, were isolated by the combination of ion-exchange chromatography and gel filtration methods from the endo-biopolymers (AS-S2). The anticomplement activity of AS-1 (MW 12 kDa) and AS-2-Fr.I (MW 180 kDa) were found to be 84.4% and 100.0%, respectively, at the concentration of 25 μg/ml. Activated pathway of the complement system occurred in both classical and alternative pathways, as evidenced by crossed immunoelectrophoresis (CIEP), where a major pathway was detected to be the classical one. It was found that the anticomplement activities of the periodate oxidized were decreased significantly, but those of pronase digested biopolymers of AS-1 and AS-2-Fr.I were decreased very little. The AS-1 contained 2,4,6-tri-*O*-methyl-D-glucitol, 2,3,6-tri-*O*-methyl-D-glucitol, and 2,3,6-tri-*O*-methyl-D-galacitol, which indicated that AS-1 contained a (1→3), (1→4)-linked glucopyranosyl residue and a (1→4)-linked galactosyl residue. AS-2-Fr.I contained mainly 2,4-di-*O*-methyl-D-mannitol and 2,3,4-tri-*O*-methyl-D-galacitol, which contained (1→3), (1→6) linked mannosyl and (1→6) linked galactosyl residues.

Key words: Anticomplement activity, biopolymer, *Acanthopanax sessiliflorus*

Acanthopanax species belong to Araliaceae, which is a shrub present mainly in Korea, Japan, and China. The stems and roots of these plants have potential medicinal value and are used in folk medicine for many ailments including diabetes, neuralgia, palsy [10], gastric ulcer [7], learning-behavior difficulties, and cancer [38]. The biologically active components of this plant comprise phenylpropanoids, lignins, sterols, coumarins, and mono- and polysaccharides [14]. *Acanthopanax sessiloflorus* is known to be one of the most abundant species, and had reported antitumor and immunostimulating activities in its of fruits [25]. In recent years, clinical studies have been focused on natural polysaccharides that were proven to have a group of potentially immunostimulating [13, 17], complement-activating [24], anti-inflammatory [40], and phagocytosis-stimulating [6] activities.

In the present investigation, we report the isolation and characterization of biopolymers from the dry stem bark of *A. sessiliflorus* Seem. (Araliaceae) as well as their anticomplement activity, for the first time.

Materials and Methods

Plant Material

The authentic dry stem bark of *Acanthopanax sessiliflorus* Seem (Araliaceae) was obtained from a local Korean herbal market in South Korea.

Preparation and Purification of Biopolymer

The dry stem bark of *Acanthopanax sessiliflorus* was cut into small pieces and autoclaved for 2 h with water. The preparation and isolation of crude and endo-biopolymers from the plants were as described previously [14].

The endo-biopolymer (AS-S2) solution was applied to a DEAE-Sepharose CL-6B column (6.6×50 cm) equilibrated with H_2O and then eluted with 0.1–1 M NaCl in a stepwise gradient until no sugar was detected. The polysaccharide fractions (AS1-AS7) were dialyzed with a dialysis membrane

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and freeze-dried. The AS-1 and AS-2 fractions were further fractionated and purified by gel filtration on Sephadex G-100 and the activity tested.

Determination of Total Anticomplement Activity
The anticomplement activity was measured by the complement fixation test based on complement consumption and degree of red blood cell lysis by the residual complement [16]. The anticomplement activity was assayed as described previously [11] and the activity expressed as the percentage inhibition of the TCH_{50} of control.

Determination of Anticomplement Activity Through the Alternative Pathway
The alternative complement pathway was determined in 10 mM EGTA containing 2 mM $MgCl_2$ in GVB^{--} (Mg^{++}-EGTA-GVB^{--}) by a modified method of Platt-Mills and Ishizaka [26]. The sample was incubated with Mg^{++}-EGTA-GVB^{--} and NHS at 37°C for 30 min, and the residual complement mixtures were measured by hemolysis of sheep erythrocytes (5×10^7 cells/ml) incubated with Mg^{++}-EGTA-GVB^{--}.

Detection of C3 and C3b by Crossed Immunoelectrophoresis
The specific activation of C3 complement component by biopolymer in NHS was assessed by comparative measurements of C3 cleavage. The ratio between the heights of the C3 and C3b peaks was calculated [3]. The experimental procedures are fully described elsewhere [12].

Pronase Digestion of Biopolymer
The biopolymer (40 mg) was dissolved in 40 ml of 50 mM Tris-HCl buffer (pH 7.9, containing 10 mM $CaCl_2$) and 10 mg pronase then added. The reaction mixture was incubated at 37°C for 48 h with a small amount of toluene. The reaction was terminated by boiling for 5 min and then dialyzed against DIW for 2 days. The nondialyzable portion was lyophilized [37].

Periodate Oxidation of Biopolymer
The biopolymer (40 mg) was dissolved in 40 ml of 50 mM acetate buffer (pH 4.5) and 10 ml of 50 mM $NaIO_4$ added. The reaction mixture was incubated at 4°C in the dark room for 3 days. Ethylene glycol was added to destroy the excess periodate, and the mixture was dialyzed against DIW for 2 days. The nondialyzable solution was concentrated to 10 ml and 20 mg of sodium borohydride added. The mixture was continuously stirred for 12 h at room temperature. The reaction mixture was neutralized with acetic acid. The boric acid contained in the reaction mixture sample was removed by the repeated addition and evaporation of methanol. The resulting oxidized sample was lyophilized after the dialysis [37].

Determination of Molecular Weight
Molecular weight of the biopolymer was determined by HPLC using the Shodex GS520+GS320+GS220 packed column. Standard pullulans (P1600, 800, 400, 200, 100, 50, 20, 10, and 5 from Sigma, U.S.A.) were used for the determination of molecular weight as the standard.

Analysis of Protein and Sugar Compositions of Biopolymer
Total protein content of the biopolymer was determined by the Lowry method [26]. The protein was hydrolyzed and the amino acid composition was analyzed by a Biochrom 20 (Pharmacia Biotech. Ltd., U.S.A.) amino acid auto analyzer with a Na-form column. The total sugar content was determined by the phenol sulfuric acid method [4] using a galactose and glucose mixture (1:1) as a standard. The sugar composition was analyzed by a Varian GC3600 gas chromatography equipped with a flame-ionization detector (FID) on an SP-2380 capillary column (15 m, 0.25 mm i.d., 0.2-μm film: SUPELCO) based on the hydrolysis and acetylation method [15].

Infrared (IR) Spectroscopy
The IR spectra (4,000–400 cm^{-1}) were recorded with a Mattson Instrument Fourier transform infrared spectrophotometer (FTIR) Genesis II. The obtained spectra were interpreted by comparison with published data [34].

Methylation of Biopolymer
Each biopolymer was methylated by using Hakomori's method [10]. The biopolymer (2 mg) was dissolved in dimethyl sulfoxide (0.1 ml) by ultrasonication in a nitrogen atmosphere. The solution was treated with methylsulfinyl carbanion (0.1 ml) for 4 h at room temperature, and then with methyl iodide (0.1 ml) for 12 h at room temperature. Each methylated biopolymer was purified by using a Sep-pak C_{18} cartridge (Waters Assoc.). The permethylated biopolymer was hydrolyzed with 2 M trifluoroacetic acid (1.5 ml) for 1 h at 121°C, reduced with sodium borohydride, and acetylated. The resulting methylated alditol acetates were analyzed by gas-liquid chromatography (GC) and gas-liquid chromatography-mass spectrometry (GC-MS). GC was performed on a Varian model STAR 3600CX gas chromatography equipped with a FID on an SP-2380 capillary column (30 m, 0.25 mm i.d., 0.2-μm film: SUPELCO). GC-MS (70 eV) was performed on a Shimadzu QP5050 instrument equipped with the same capillary column. Peaks were identified on the basis of relative retention time and fragmentation patterns. The mol% for each sugar was calibrated by using the peak areas.

Statistical Analysis
All the experiments were performed in triplicate for statistical analysis. Results were expressed as the mean± standard deviation (SD).

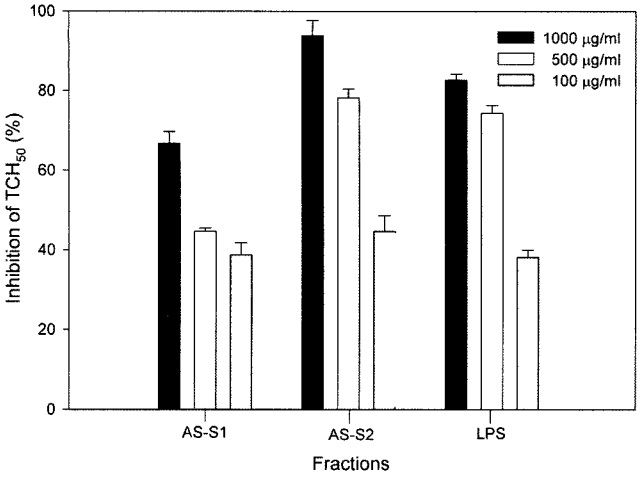

Fig. 1. Anticomplement activities of crude and endo-biopolymers obtained from *Acanthopanax sessiliflorus*.
AS-S1, Crude biopolymer; AS-S2, Endo-biopolymer; LPS, Positive control (lipopolysaccharide from *Escherichia coli* 0127: B8).

RESULTS

Anticomplement Activities of Biopolymers

The yields of crude (AS-S1) and endo-biopolymers (AS-S2) of dry stem bark of *A. sessiliflorus* were 31.7 g/kg and 6.0 g/kg, respectively. Their anticomplement activities were tested at the concentrations of 100, 500, and 1,000 μg/ml (Fig. 1). It was found that the anticomplement activities of both crude and endo-biopolymers were increased with increasing concentration. However, the endo-biopolymer was shown to have higher activity than crude biopolymer at all concentrations. At 1,000 μg/ml, the anticomplement activities of crude and endo-biopolymers were 67.1% and 95.1%, respectively.

The endo-biopolymers (AS-S2) were further fractionated into seven fractions by using ion-exchange chromatography.

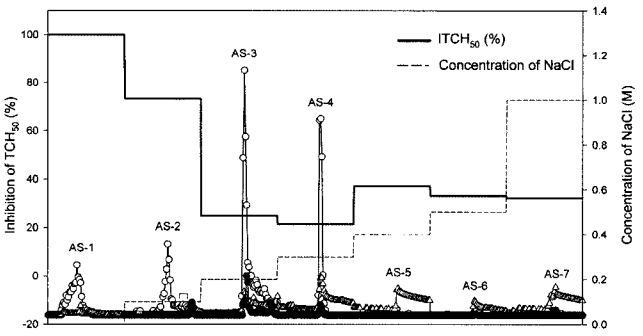

Fig. 2. Fractionation and anticomplement activities of endo-biopolymers extracted from *Acanthopanax sessiliflorus* on the DEAE Sepharose CL-6B column.
All the fractions were tested at the concentration of 1,000 μg/ml. LPS: Positive control (lipopolysaccharide from *Escherichia coli* 0127: B8). Symbols: (○) sugar (absorbance at 490 nm), (△) protein (absorbance at 280 nm), (●) uronic acid (absorbance at 520 nm).

The percentage contents of biopolymers of AS-1, AS-2, AS-3, AS-4, AS-5, AS-6, and AS-7 were 8.7, 8.6, 42.8, 28.7, 1.8, 4.1, and 5.5, respectively. The anticomplement activities of these seven fractions are shown in Fig. 2. The highest activity appeared in AS-1, followed by AS-2, AS-5, AS-6, AS-7, AS-3, and AS-4. The AS-1 and AS-2 fractions showed higher activity at lower concentrations (25 μg/ml) than total endo-biopolymers (AS-S2) at high concentrations (1,000 μg/ml). The fraction AS-1 was composed of 91.10% neutral sugar and 8.90% protein. The AS-2 Fr.I and Fr.II fractions contained 79.26 and 54.36% of neutral sugar, 8.57 and 24.65% of uronic acid, and 12.17 and 20.99% of protein, respectively (Table 2).

The AS-1 and AS-2 fractions were purified by gel filtration on Sephadex G-100 and the AS-1 showed a single symmetrical peak. The AS-2 was further fractionated into AS-2-Fr.I and AS-2-Fr.II (Figs. 3A and 3B). As shown in Fig. 3, the anticomplement activity of AS-2-Fr.I was higher than that of AS-1. The AS-2-Fr.II had no significant anticomplement activities, as shown in Fig. 3, nor lowering of the overall activity of AS-2.

The AS-2-Fr.I exhibited high anticomplement activity at all concentrations compared with LPS, which was used as a positive control. The anticomplement activity was 4-times higher than LPS at 25 μg/ml. Moreover, the AS-2-Fr.I showed a higher $ITCH_{50}$ value (100.0%) than the AS-1 fraction (84.4%) at 25 μg/ml (Fig. 3). The relative $ITCH_{50}$ values were obtained. The results suggest that AS-1 and AS-2-Fr.I of two distinct biopolymers showed higher activity in the lower concentration range of 5–10 μg/ml. These two biopolymer fractions (AS-1 and AS-2-Fr.I) may play an important role for the overall anticomplement activity of the polysaccharides in *A. sessiliflorus*.

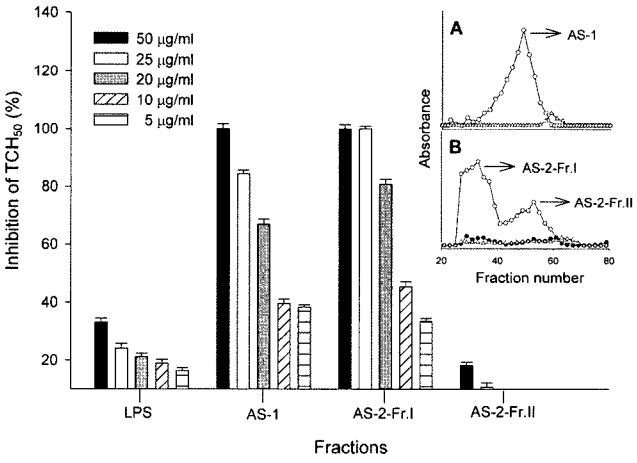

Fig. 3. Anticomplement activities of biopolymers extracted from *Acanthopanax sessiliflorus* on the Sepharose CL-6B column.
Gel chromatography profiles of AS-1 (**A**) and AS-2 (**B**) on the Sephadex G-100 column. LPS: Positive control (lipopolysaccharide from *Escherichia coli* 0127: B8).

Table 1. Anticomplement activities of biopolymers extracted from *Acanthopanax sessiliflorus* in the presence or absence of Ca^{++} and Mg^{++}.

	Inhibition of TCH_{50} (%) ±SD[a]	
	AS-1	AS-2-Fr.I
GVB^{++}[b]	66.7±1.3	79.9±1.4
Mg^{++}-EGTA-GVB^{--}[c]	23.3±1.2	23.7±1.3
EDTA-GVB^{--}[d]	7.5±1.1	6.4±1.2

[a]Each value is the mean±SD for triple determinations.
[b]Gelatin veronal buffer with two divalent metal ions; activated both pathways.
[c]EGTA-gelatin veronal buffer with Mg^{++} ion; activated the alternative pathway.
[d]EDTA-gelatin veronal buffer without two divalent metal ions; blocked both pathways.

Activation Mode of Complement System by Biopolymers

In order to evaluate the complement-activated pathway, AS-2-Fr.I was studied in different buffer systems. The anticomplement activity was found to be 79.9% in GVB^{++} condition, which is the result of both classical and alternative pathways leading to the cellular lysis. However, the anticomplement activity of AS-2-Fr.I showed only 23.7% in Ca^{++}-depleted experimental conditions, and 6.0% anticomplement activity was detected in the EDTA-GVB^{--} system (Table 1). These results suggest that the complement system was activated *via* both the classical (56.0%) and alternative (17%) pathways by AS-2-Fr.I.

To confirm the above result, The CIEP experiment was carried out after incubation of NHS with AS-2-Fr.I in both GVB^{++} and Mg^{++}-EGTA-GVB^{--}. The activated C3 complement component was separated into subunits C3a and C3b in both pathways, and C3b was a major fragment deposited on the microbial surface where the complement is activated [1]. As shown in Fig. 4, the C3b precipitin line was obtained in the serum treated with AS-2-Fr.I in both GVB^{++} and Mg^{++}-EGTA-GVB^{--} buffer systems, and the height of the C3b precipitin line in GVB^{++} (Fig. 4A) was higher than that in Mg^{++}-EGTA-GVB^{--} (Fig. 4B), whereas

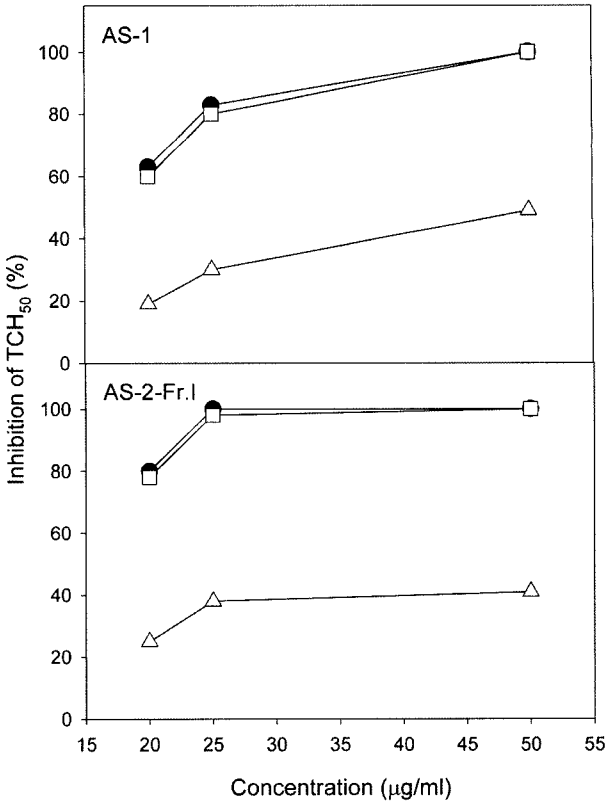

Fig. 5. Effects of pronase digestion and periodate oxidation on the anticomplement activities of biopolymers of *Acanthopanax sessiliflorus*.
Symbols: (●) native biopolymer, (□) pronase-digested biopolymer, (△) periodate-oxidized biopolymer.

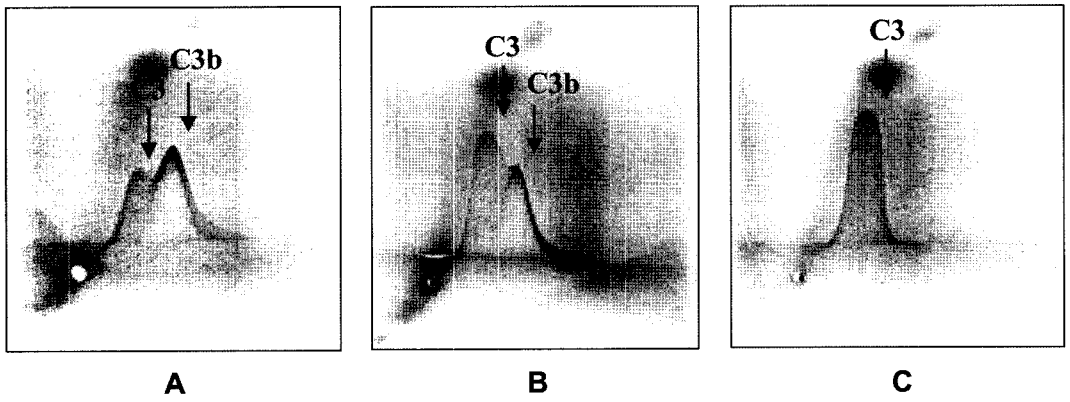

Fig. 4. Crossed immunoelectrophoretic patterns of C3 converted by AS-2-Fr.I from *Acanthopanax sessiliflorus* in the presence or absence of divalent metal ions.
Normal human serum was incubated with GVB^{++}, Mg^{++}-EGTA-GVB^{--}, or EDTA-GVB^{--} at 37°C for 30 min. The sera were then subjected to immunoelectrophoresis using anti-human C3 sera to locate C3 cleavage products. Anode is to the right. **A**: GVB^{++}, in the presence of Ca^{++} and Mg^{++}. **B**: Mg^{++}-EGTA-GVB^{--}, in the presence of Mg^{++} and absence of Ca^{++}. **C**: EGTA-GVB^{--}, in the absence of Ca^{++} and Mg^{++}.

Table 2. Chemical composition of the biopolymer fractions obtained from *Acanthopanax sessiliflorus* by Sepharose CL-6B gel chromatography.

	AS-1	AS-2	
		Fr-I	Fr.II
Neutral sugar (%)	91.1	79.3	54.4
Uronic acid (%)	0	8.6	24.7
Protein (%)	8.9	12.2	21.0
	Neutral sugar (molar ratio)		
Fucose	Trace	Trace	0.13
Arabinose	Trace	0.60	0.69
Xylose	Trace	0.22	0.72
Mannose	0.28	0.23	0.14
Galactose	1.00	1.00	1.00
Glucose	0.98	0.32	0.26
		Amino acid (%)	
Asp	3.79	5.56	6.86
Thr	4.18	6.38	7.61
Ser	8.82	18.85	16.08
Glu	4.57	9.93	9.48
Gly	8.13	13.84	12.34
Ala	3.95	9.74	8.48
Cys	7.51	2.64	2.87
Val	10.22	7.74	6.86
Met	4.88	2.19	2.99
Iso	7.04	5.37	3.62
Leu	7.89	6.74	4.61
Phe	trace	trace	2.37
Lys	7.28	3.10	4.86
Arg	21.75	7.92	10.97

[a]Molar ratios were calculated on the basis of total sugar and molar mass.

the C3b precipitin line was not observed in the EDTA-GVB^{--} buffer system (Fig. 4C). This result also explained that the AS-2-Fr.I participated in the complement activation system as an activator.

Determination of the Major Anticomplement Active Component of Biopolymers

As shown in Fig. 5, the anticomplement activities of enzyme degradation biopolymers were compared with native fraction at the concentrations of 20 to 50 μg/ml. It was found that the anticomplement activities of the periodate-oxidized AS-1 and AS-2-Fr.I were decreased significantly (53.0 and 61.0%, respectively). However, those of the pronase-digested biopolymers (AS-1 and AS-2-Fr.I) were decreased by 3.0% at 25 μg/ml.

Chemical Compositions of the Biopolymers

Total sugar contents of AS-1 and AS-2-Fr.I were 91.10% and 87.83%, respectively. The sugar compositions of these fractions are summarized in Table 2. AS-1 was composed of neutral sugar, which contained mainly galactose and glucose, whereas AS-2-Fr.I consisted of neutral sugar (79.26%) and uronic acid (8.57%). The sugar components of AS-2-Fr.I were arabinose, xylose, mannose, galactose, and glucose in the molar ratios of 0.60:0.22:0.23:1.00:0.32 by GC analysis.

Total protein contents of AS-1 and AS-2-Fr.I were 8.90% and 12.17%, respectively. The amino acid composition of biopolymers of *A. sessiliflorus* is summarized in Table 2.

The molecular weights of *A. sessiliflorus* biopolymers were determined by HPLC. The molecular weight of AS-1 and AS-2-Fr.I were found to be about 12 and 180 kDa, respectively. These results were matched with the results of gel filtration on Sephadex G-100 chromatography.

Structure Analyses of the Biopolymers

Each of the purified biopolymers was recorded as KBr tablets on an FT-IR model. The spectra of all fractions shows absorbances at wavenumbers characteristic of natural polysaccharide: O-H stretching (3,300–3,500 cm^{-1}), C-H stretching (3,000–2,850 cm^{-1}), carbonyl (-CHO, -CO) stretching (–1,700 cm^{-1}) [24, 28].

Table 3. Identification of partially methylated alditol acetates of major active biopolymers from *Acanthopanax sessiliflorus*.

Methylated sugar	Major mass spectral fragments (m/e)[c]	Mol%[a]		linkages
		AS-1	AS-2-Fr.I	
2,3,4,6-Tetra-*O*-Me-D-Glc[b]	43,45,71,87,101,117,129,145,161,205	-	7.1	$Glc^{1}\rightarrow$
2,4,6-Tri-*O*-Me-D-Glc	43,45,87,101,117,129,161,233	15.1	4.5	$\rightarrow^{3}Glc^{1}\rightarrow$
2,3,6-Tri-*O*-Me-D-Glc	43,87,99,101,117,129,161,189,233	14.1	7.2	$\rightarrow^{4}Glc^{1}\rightarrow$
2,3,4-Tri-*O*-Me-D-Glc	43,45,87,99,101,113,117,233	1.1	4.7	$\rightarrow^{6}Glc^{1}\rightarrow$
2,3,6-Tri-*O*-Me-D-Gal	43,87,99,101,117,129,161,189,233	31.3	9.5	$\rightarrow^{4}Gal^{1}\rightarrow$
2,3,4-Tri-*O*-Me-D-Gal	145,161,189,233	-	22.1	$\rightarrow^{6}Gal^{1}\rightarrow$
2,3-Di-*O*-Me-D-Ara	43,101,117,129,189	-	9.2	$\rightarrow^{5}Ara^{1}\rightarrow$
2,4-Di-*O*-Me-D-Gal	43,87,117,129,189	-	3.7	$\rightarrow^{3,6}Gal^{1}\rightarrow$
2,4-Di-*O*-Me-D-Man	87,101,129,159,189	7.1	16.9	$\rightarrow^{3,6}Man^{1}\rightarrow$

[a]Calculated from peak areas and response factors of hydrogen flame ionization detector on GLC (Sweet, Shapiro, & Albersheim, 1975).
[b]2,3,4,6-Tetra-*O*-Me-D-Glc=2,3,4,6-tetra-*O*-methyl-D-glucitol, etc.
[c]The fragments were obtained from GC-MS spectra of the methylated alditol acetates recorded on a Shimazu QP5050 MS spectrometer. The excluded unidentified peaks may represent multibranched sugars or the consequences of slight undermethylation.

All fractions show major characteristic absorption around 1,590 cm^{-1} owing to carbohydrate [34], and the small band at 890 to 900 cm^{-1} is characteristic of β-glycosidic linkage [2]. The absorption around 1,200 cm^{-1} reflects the presence of an ester (COOR and C-O-C) group [36].

Table 3 shows the characterized predominant peaks of AS-1 and AS-2-Fr.I. The AS-1 contains 2,4,6-tri-*O*-methyl-D-glucitol, 2,3,6-tri-*O*-methyl-D-glucitol, and 2,3,6-tri-*O*-methyl-D-galacitol, which indicated that AS-1 contains (1→3) and (1→4)-linked glucopyranosyl residue, and (1→4)-linked galactosyl residue. The AS-2-Fr.I contains mainly 2,4-di-*O*-methyl-D-mannitol, and 2,3,4-tri-*O*-methyl-D-galacitol, which contain (1→3) and (1→6)-linked mannosyl residue and (1→6)-linked galactosyl residue.

DISCUSSION

The *Acanthopanax* species have been reported to have various biological efficacies such as antitumor, anti-inflammation, antihypoglycemic, and hypocholesterolemic effects [5], but no reports are available on the anticomplement activity and chemical characterization of its biopolymer.

The AS-2-Fr.I fraction of *A. sessiliflorus* showed potential anticomplement activity. Many investigators have reported that higher anticomplement activity appeared in the acidic polysaccharide fraction than in the neutral fractions [20, 25]. However, the neutral fraction obtained from *Bupleurum falcatum* showed a high anticomplement activity [37]. The main active fractions (AS-2-Fr.I and AS-1) were mainly composed of a neutral sugar (72% and 91%, respectively), whereas the inert fraction (AS-2-Fr.II) consisted of 24.65% acidic sugar and 54.36% neutral sugar. The molecular weights of AS-2-Fr.I and AS-1 were approximately 180 and 12 kDa. AS-2-Fr.I was mainly composed of galactose, arabinose, and glucose followed by xylose and mannose, whereas AS-1 was mainly composed of galactose and glucose. Therefore, it can be estimated that the anticomplement activity of the biopolymers extracted from *A. sessiliflorus* originated from neutral sugar content, as for *Bupleurum falcatum* [40].

It is known that both Mg^{++} and Ca^{++} ions are essential for the activation of the classical pathway, but only Mg^{++} ion is needed for the activation of the alternative pathway [23]. Our results indicated that the activation of the complement system by AS-2-Fr.I was not only via the classical pathway but also via the alternative pathway. This pattern was similar to that of AR-arabinogalactan [38] and LPS [28], which appear to activate both pathways [30].

It was reported that the anticomplement activity includes both activation and inhibition of the complement system [12]. As shown in Fig. 4, an activated C3b molecular precipitin line was found when a serum treated with the AS-2-Fr.I was subjected to electrophoresis and agar diffusion against anti-human C3 (see Fig. 4A). AS-1 also showed two activated complement molecular precipitin lines (data not shown). Therefore, this result can be explained in that the AS-2-Fr.I and AS-1 that participated in the complement activation system were activators of the complement system. From these results, it suggests that the AS-2-Fr.I and AS-1 might play an important role in humoral immunity.

The molecular weights of AS-1 and AS-2-Fr.I were found to be about 12 and 180 kDa, respectively. It was reported that an immunomodulating biopolymer isolated from the roots of *A. senticosus* was found to be a low molecular weight component (7 kDa), which consisted of glucose, galactose, and arabinose [35]. Another investigator isolated 30 kDa and 150 kDa biopolymers from *A. senticosus* with high immunomodulatory activities [6]. Therefore, it can be estimated that the anticomplement activities of *A. sessiliflorus* biopolymers originated from its chemical structure but not from molecular weight.

As shown in Fig. 5, the anticomplement activities of the two biopolymers were drastically decreased by periodate oxidation and slightly decreased by pronase digestion. It indicates that the anticomplement activities of these fractions were mainly attributed to the carbohydrate moiety. These results are consistent with the findings of Yamada *et al.* [37] and Shin *et al.* [32], who reported the involvement of the carbohydrate moiety in executing anticomplement activity.

It was found that the most active biopolymer fraction (AS-2-Fr.I) isolated from dry stem bark of *A. sessiliflorus* was a complex form of polysaccharides, where galactose and arabinose constituted two of the major components, indicating their arabinogalactan nature. It has been reported that arabinogalactan has potent anticomplement activity [38]. The second active fraction of AS-1 was mainly composed of galactose and glucose, with traces of arabinose. Therefore, it may be postulated that anticomplement activities of biopolymers are derived by the presence of galatose, arabinose, and glucose in their chemically complex structures.

Since the activation of the complement system requires steric recognition, the geometry of the molecules should play an important role. For example, it has been observed that the presence of an arabinosyl and galactosyl side chain is an important factor for the expression of the activity of different petic polysaccharides [8, 19, 21, 37, 39, 42]. The existence of branched structures has also been pointed out as a determinant for the anticomplement activity of the neutral polysaccharides [20]. Also, it was generally accepted that the steric factor in various polysaccharide molecules seems to play a key role in anticomplement activity [33]. From our results, AS-1 and AS-2-Fr.I were consisted of glucopyranosyl residue, galactosyl residue, and mannosyl residue, and have various branched structures. Therefore, the potent anticomplement activities of these biopolymers

(AS-1 and AS-2-Fr.I) may be influenced by its chemical structure.

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