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CHARACTERIZATION OF ANTI-COMPLEMENTARY ACIDIC HETEROGLYCANS FROM THE SEED OF *COIX LACRYMA-JOBI* VAR. *MA-YUEN*

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Key Word Index—*Coix lacryma-jobi* var. *ma-yuen*; Gramineae; polysaccharide; acidic arabinogalactan; anti-complementary activity.

Abstract—The anti-complementary polysaccharides, CA-1 and CA-2, were purified from the seed of *Coix lacryma-jobi* L. var. *ma-yuen*. CA-1 consists of rhamnose, arabinose, xylose, galactose, galacturonic acid and glucuronic acid in molar ratios of 1.8:43.8:10.8:33.2:3.2:7.2, and CA-2 consists of rhamnose, arabinose, xylose, mannose, galactose, glucose, galacturonic acid and glucuronic acid in molar ratios of 2.4:37.0:11.8:1.7:35.6:2.9:2.6:6.0. CA-1 and CA-2 contained 8 ~ 11% protein. Their M_rs were estimated to be 160 000 in CA-1 and 70 000 in CA-2 by gel filtration. CA-2 showed more potent anti-complementary activity than CA-1 in low dose.

Methylation analysis of CA-1, its carboxyl-reduced products (reduced CA-1a and CA-1b) and CA-2 were carried out by the use of GC/MS and the results suggested that CA-1 has a very complicated and highly branched structure, and CA-2 is also composed of the same glycosidic linkages as CA-1 in different molar proportions. The results of exo α-L-arabinofuranosidase treatment and partial acid hydrolysis suggested that CA-1 and CA-2 contained arabino 3,6-galactan moiety and most of the arabinose was present as an α-L-furanosyl residues in the non-reducing terminals and (1 → 5)-linked side chains which mostly attached to the O-3 of (1 → 6)-linked galactopyranosyl residues. The results also suggested that CA-1 and CA-2 contained rhamnogalacturonan moiety which has a main chain consisting of (1 → 4)-linked galacturonic acid and (1 → 2)-linked rhamnose, and arabino 3,6- and 4-galactan might be attached to the rhamnosyl residue at the O-4. All glucuronic acid residues were present at the non-reducing terminals.

INTRODUCTION

The seed of *Coix lacryma-jobi* L. var. *ma-yuen* STAPF without the husks, has been used in a Chinese herbal medicine as a diuretic, stomachic, nourishment, anodyne and antispasmodic. Recently, we have been reported the isolation and the structure analysis of a major polysaccharide from the hot water extract of the seed of *C. lacryma-jobi*, and this polysaccharide was characterized to be an amylopectin-like glucan mixtures [1]. Various polysaccharides and polysaccharide containing materials are known to have anti-complementary activity which regulate the complement system [2–15]. Anti-tumour polysaccharides also have an anti-complementary activity [16, 17]. We have now isolated anti-complementary polysaccharide from the same extract of *C. lacryma-jobi* and report on its purification and chemical characterization.

RESULTS AND DISCUSSION

The crude polysaccharide fraction (CS-1) was separated on the column of DEAE-Sepharose (Cl^-) into an unabsorbed fraction (CS-I) and the absorbed fractions (CS-IIa and IIb) which were eluted stepwise with 0.2 and 0.5 M NaCl solution, respectively. CS-I was characterized as amylopectin-type glucan mixture as reported previously [1]. CS-IIa was divided into three fractions IIa-1, IIa-2 and IIa-3 and most potent anti-complementary activity was observed in CS-IIa-2 (data not shown). The order of the activities of these fractions was IIa-2 > IIa-3 > IIb > IIa-1 > I. The yield of CS-IIa-2 was 0.65% from the crude polysaccharide fraction. When CS-IIa-2 was subjected to gel filtration on Sephadex G-100 by the elution with 0.2 M NaCl, the active fraction (CS-IIa-2A) was eluted in the void vol (data not shown). CS-IIa-2A yielded arabinose, galactose, xylose, rhamnose, galacturonic acid and glucuronic acid and traces of mannose and glucose on hydrolysis. CS-IIa-2A was further purified by affinity chromatography on Con-A Sepharose (data not shown). However the amount of the Con-A bound fraction obtained by this affinity chromatography was insignificant, and all activity was recovered in the Con-A unbound fraction. The Con-A unbound fraction was further subjected to Sepharose CL-6B chromatography in 0.2 M NaCl solution (Fig. 1). In these conditions, trace amount of polysaccharide voided the column but the major polysaccharide was included as a single carbohydrate peak in the gel, and uronic acid was also coeluted with this peak. However, predominant anti-complementary activity was observed in the later eluted fractions in the carbohydrate peak (Fig. 1).

Selected fractions from Sepharose CL-6B column (Fig. 1) were analysed for neutal and acidic glycosyl residues by the alditol acetate method. The result showed that the early eluted fractions (CA-1) are rich in arabinosyl

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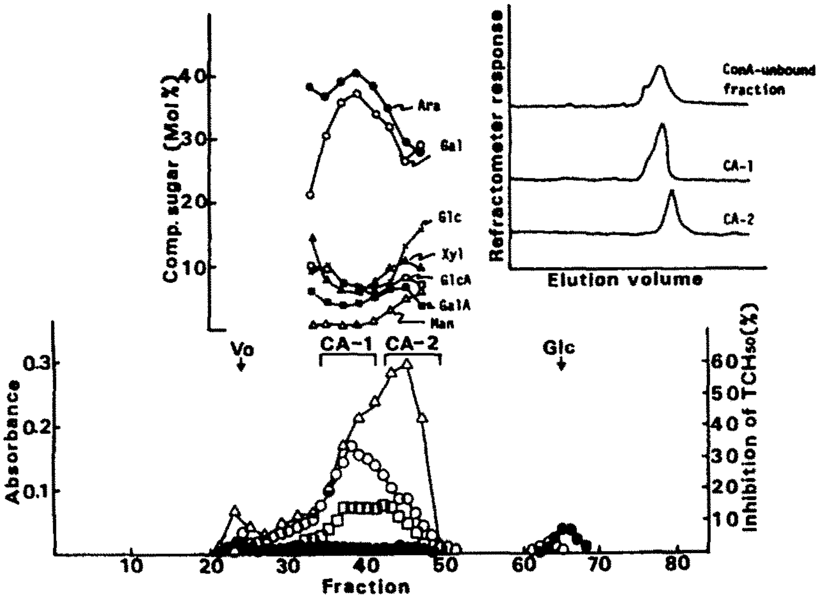

Fig. 1. Gel filtration of CS-IIa-2A Con-A unbound fraction on Sepharose CL-6B. Vo, void volume; Glc, glucose.-O-O-, carbohydrate, 490 nm; -●-, 280 nm; -□-, uronic acid, 520 nm; -Δ-, anti-complementary activity (%). Inset, HPLC of Con-A unbound fraction, CA-1 and CA-2.

and galactosyl residues, whereas the later eluted fractions (CA-2) are rich in xylosyl, glucosyl, glucuronosyl and galacturonosyl residues. This result indicates that the Con-A unbound fraction is not a structurally homogeneous polymer. When CA-1 and CA-2 were applied to HPLC on Asahipak GS-510 + GS-320, each fraction was eluted as a single peak with different elution volume (Fig. 1 inset).

The apparent M_rs of CA-1 and CA-2, as determined by gel filtration on Sepharose CL-6B column, were *ca* 1.6×10^5 and 7.0×10^4 (compared to standard dextrans), respectively. The total hexose contents of CA-1 and CA-2 were 92% and 89%, respectively. CA-1 and CA-2 contained 8 and 11% protein, respectively. Sugar composition analysis showed that CA-1 consisted of rhamnose, arabinose, xylose, galactose, glucose, galacturonic acid and glucuronic acid in the molar ratios of 1.8:43.8:10.8:33.2:3.2:7.2 and CA-2 consisted of rhamnose, arabinose, xylose, mannose, glactose, galacturonic acid and glucuronic acid in the molar ratios of 2.4:37.0:11.8:1.7:35.6:2.9:2.6:6.0 (Table 1). ^{1}H NMR of CA-1 and CA-2 mixture in D_2O showed the signals of C-methyl proton at δ1.42, *O*-acetyl proton at δ2.25 and anomeric protons due to α-configuration at δ5.15, 5.30 and 5.40 and due to β-configuration at δ4.5 and 4.8. ^{13}C NMR of CA-1 and CA-2 mixture showed a signal at δ109.8 in the region of an anomeric carbon due to α-L-arabinofuranosyl residue.

The carboxyl groups of uronic acid residues in the major polysaccharide, CA-1, were reacted with the carbodiimide reagent, then reduced with $NaBD_4$ to the corresponding neutral sugar units. After this reaction, the carboxyl-reduced reaction product was purified by gel filtration on Sephadex G-10, and the carbohydrate fractions were eluted in both void vol (reduced CA-1a) and included vol (reduced CA-1b) (data not shown). Reduced CA-1a and CA-1b were composed of rhamnose, arabinose, xylose, galactose and glucose in the molar ratios of 2.3:45.2:2.5:42.1:4.9 and trace:43.0:25.4:28.0:3.6, respectively (Table 1). Small amounts of glucuronic acid and galacturonic acid still remained in reduced CA-1a. The presence of galacturonic acid and glucuronic acid in CA-1 was also confirmed by the increase of galactose and glucose content in reduced CA-1a and CA-1b. When reduced CA-1b was applied to the column of Bio-gel P-2, all of the carbohydrate fraction was recovered in the void vol.

Table 1. Chemical properties of CA-1, CA-2, and reduced CA-1a and CA-1b

			Reduced	
	CA-1	CA-2	CA-1a	CA-1b
	wt (%)			
Protein (as BSA)	8.0	11.0	n.d.*	n.d.*
Carbohydrate (as Ara:Xyl:Gal = 5:1:4)	92.0	89.0	n.d.*	n.d.*
Component sugars	Mol (%)			
Rha	1.8	2.4	2.3	trace
Ara	43.8	37.0	45.2	43.0
Xyl	10.8	11.8	2.5	25.4
Man	—	1.7	—	—
Gal	33.2	35.6	42.1	28.0
Glc	trace	2.9	4.9	3.6
GalA	3.2	2.6	1.7	—
GlcA	7.2	6.0	1.3	—

*Not detected.

CA-2 showed more potent activity than CA-1 in low dose such as 100 μg/ml (Table 2). However, the activity of reduced CA-1a decreased in comparison with CA-1 or CA-2.

CA-1, CA-2 and carboxyl-reduced CA-1a and CA-1b were methylated by the method of ref. [18]. The fully methylated product was hydrolyzed with acid, and the products were converted into the alditol acetates. The partially methylated alditol acetates were identified by their retention time in GC and their fragmentation pattern in MS. CA-1 and CA-2 (Table 3) contained arabinofuranosyl, galactopyranosyl, xylopyranosyl and glucopyranosyl residues as non-reducing end-groups, 3-*O*-substituted arabinofuranosyl and 4- or 5-*O*-substituted arabinosyl groups, 4-*O*-, 3-*O*-, 6-*O*-, 3,4-di-*O*-, 4,6-di-*O*-, 3,6-di-*O*- and 3,4,6-tri-*O*-substituted galactopyranosyl residue, 4-*O*- and 3,4-di-*O*-substituted xylopyranosyl residues, 2,4-di-*O*-substituted rhamnopyranosyl residues and 4-*O*-substituted glucopyranosyl residues in the glycosyl chain. The relative abundance of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylgalactitol in the methylated CA-1 and CA-2 suggested that both polysaccharides contained arabino 3,6-galactan moiety. Total ion and selected ion chromatograms of partially methylated alditol acetates derived from fully methylated CA-1 and reduced CA-1a are shown in Fig. 2. In reduced CA-1a, *m/z* 207, 163 and 47 were abundant in peak A due to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol-6,6-*d,d*, and *m/z* 235 and 47 were in peaks B and C due to 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylgalactitol-6,6-*d,d* and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol-6,6-*d,d*. However, these fragment ions were not abundant in CA-1. Reduced CA-1b also gave same alditol acetate derivatives (Table 4). These results indicated that CA-1 contained non-reducing terminal and 4-*O*-linked glucuronic acid and 4-*O*-linked galacturonic acid residues.

In the present results, CA-1 and CA-2 were suggested to be structurally related polysaccharides, therefore CA-1 and CA-2 mixture (CA, major neutral component sugar; rhamnose, arabinose, xylose, and galactose in the molar ratios of 0.2:3.5:1.0:3.0) was used for the following structural analysis.

CA-1 and CA-2 mixture was digested with *exo* α-L-arabinofuranosidase from *R. flava*. About 40% of this polysaccharide was hydrolysed by the enzyme. This liberated sugar content corresponded to 66% of the total arabinose in CA-1 and CA-2 mixture. The reaction products were fractionated on a column of Bio-gel P-2 (data not shown), and two carbohydrate fractions that were eluted in the void and included vols were obtained. Arabinose was the only sugar detected in the included vol by TLC and GC. The material (AF-CA) recovered in the void vol consisted of rhamnose, arabinose, xylose, galactose and uronic acids in the molar ratios of 0.2:1.2:1.0:3.7 as major neutral sugars. These results lead to the conclusion that *ca* 70% of the arabinosyl residues in CA-1 and CA-2 were linked in an α-L-configuration in the furanose form. The results of methylation analysis of AF-CA showed that the molar ratios of the terminal, (1 → 5)-linked arabinofuranosyl residues and (1 → 3,6)-linked galactopyranosyl residues had decreased significantly, and that the molar ratio of the terminal and (1 → 6)-linked galactopyranosyl residues had increased compared with CA-1 and CA-2 mixture (Table 5). These results suggested that CA-1 and CA-2 contained (1 → 5)-linked arabinofuranosyl side chains attached at O-3 of some of the (1 → 6)-linked galactosyl residues in the main chain or the interior chains of galactose residues mutually joined by (1 → 3)- and (1 → 6)-linkages. This moiety was very similar to the anti-complementary arabino 3,6-galactans from the roots of *Angelica acutiloba* Kitagawa (AR-arabinoglactan IIa and IIb-1) [7–8, 14–15], anti-complementary polysaccharides from the leaves of *Artemisia princeps* PAMP (AAFIIb-2 and IIb-3) [10] and the arabinogalactans from *Zea mays* shoots [19].

When the results of arabinofuranosidase digestion were calculated from only xylosyl residues, it was suggested that some α-L-arabinofuranosyl residues might attach at O-3 of some of (1 → 4)-linked xylopyranosyl residues.

To obtain more detailed information about the structure of CA-1 and CA-2, the arabinofuranosidase digested CA-1 and CA-2 mixture (AF-CA) was hydrolysed partially with 0.1 M HCl at 100° for 3 hr. After neutralization, the reaction products were fractionated on a column of Bio-gel P-2 (data not shown), and five carbohydrate fractions that were eluted in the void vol, tetrasaccharide, trisaccharide, disaccharide regions and the retained vol were obtained. Arabinose, xylose and galactose

Table 2. Anti-complementary activity of CA-1, CA-2 and reduced CA-1a

	Concentration (μg/ml) 1000	500	100
	Anti-complementary activity (%)		
CA-1	80	75	53
CA-2	75	73	69
Reduced CA-1a	72	59	32

Table 3. Methylation analysis of CA-1 and CA-2

Peak No.	Methylated sugars	Mol. % CA-1	CA-2	Linkages
1	2,3,5-tri-*O*-Me Ara	29.0	28.2	$\mathrm{Ara}f^{1}\rightarrow$
2	2,3,4-tri-*O*-Me Xyl	0.6	0.9	$\mathrm{Xyl}^{1}\rightarrow$
3	2,5-di-*O*-Me Ara	2.6	2.7	$\rightarrow{}^{3}\mathrm{Ara}f^{1}\rightarrow$
4	2,3,-di-*O*-Me Ara	12.7	9.7	$\rightarrow{}^{4}\mathrm{Ara}^{1}\rightarrow$ or $\rightarrow{}^{5}\mathrm{Ara}f^{1}\rightarrow$
5	2,3-di-*O*-Me Xyl	2.8	3.5	$\rightarrow{}^{4}\mathrm{Xyl}^{1}\rightarrow$
6	2,3,4,6-tetra-*O*-Me Glc	0.4	0.9	$\mathrm{Glc}^{1}\rightarrow$
7	2,3,4,6-tetra-*O*-Me Gal	2.2	3.6	$\mathrm{Gal}^{1}\rightarrow$
8	2-mono-*O*-Me Xyl	8.7	10.5	$\begin{smallmatrix}\rightarrow 4\\ \rightarrow 3\end{smallmatrix}\mathrm{Xyl}^{1}\rightarrow$
9	3-mono-*O*-Me Rha	1.5	1.4	$\begin{smallmatrix}\rightarrow 4\\ \rightarrow 2\end{smallmatrix}\mathrm{Rha}^{1}\rightarrow$
10	2,3,6-tri-*O*-Me Gal	2.9	2.4	$\rightarrow{}^{4}\mathrm{Gal}^{1}\rightarrow$
11	2,3,6-tri-*O*-Me Glc	0.5	1.3	$\rightarrow{}^{4}\mathrm{Glc}^{1}\rightarrow$
12	2,4,6-tri-*O*-Me Gal	7.1	7.6	$\rightarrow{}^{3}\mathrm{Gal}^{1}\rightarrow$
13	2,3,4-tri-*O*-Me Gal	1.0	2.7	$\rightarrow{}^{6}\mathrm{Gal}^{1}\rightarrow$
14	2,6-di-*O*-Me Gal	1.8	1.2	$\begin{smallmatrix}\rightarrow 4\\ \rightarrow 3\end{smallmatrix}\mathrm{Gal}^{1}\rightarrow$
15	2,3-di-*O*-Me Gal	0.6	0.9	$\begin{smallmatrix}\rightarrow 6\\ \rightarrow 4\end{smallmatrix}\mathrm{Gal}^{1}\rightarrow$
16	2,4-di-*O*-Me Gal	12.6	12.5	$\begin{smallmatrix}\rightarrow 6\\ \rightarrow 3\end{smallmatrix}\mathrm{Gal}^{1}\rightarrow$
17	2-mono-*O*-Me Gal	2.5	1.6	$\begin{smallmatrix}\rightarrow 6\\ \rightarrow 4\end{smallmatrix}\underset{\uparrow 3}{\mathrm{Gal}}^{1}\rightarrow$

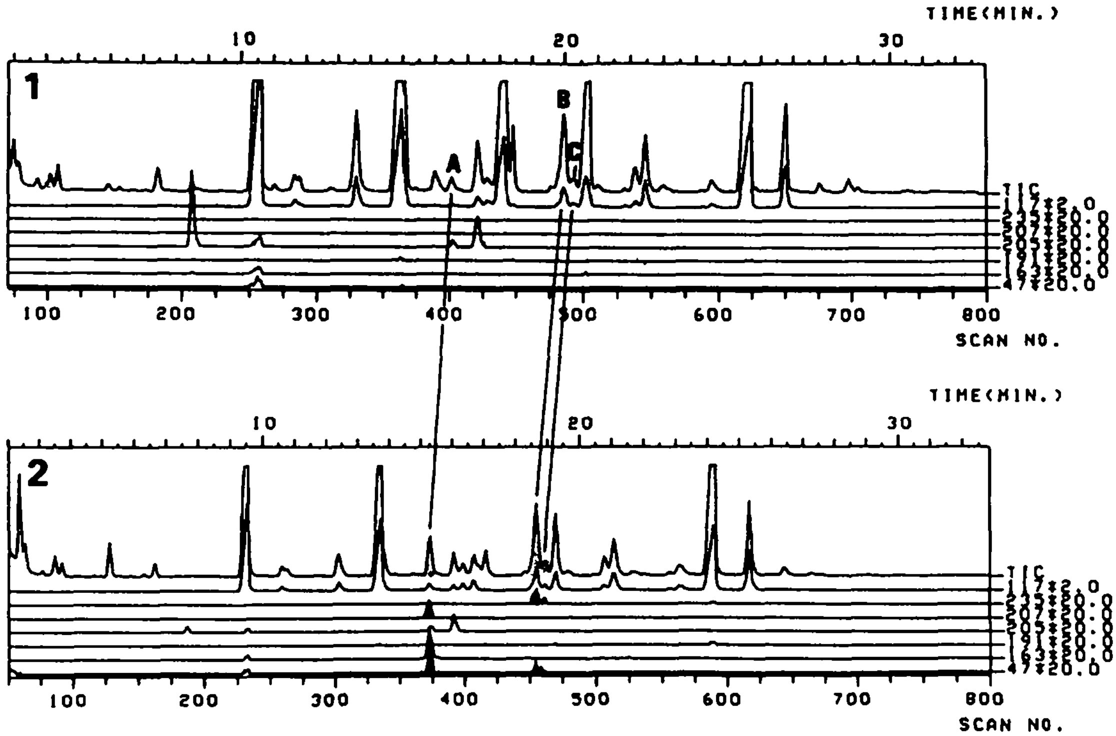

Fig. 2. Mass chromatogram of CA-1 (1) and reduced CA-1a (2). TIC, total ion chromatogram; m/z 47, 117, 163, 191, 205, 207 and 235 were monitored. A; 1,5-di-OAc-2,3,4,6-tetra-OMe-glucitol; B, 1,4,5-tri-OAc-2,3,6-tri-OMe-galactitiol; C, 1,4,5-tri-OAc-2,3,6-tri-OMe-glucitol

Table 4. Diagnostic ions of partially methylated alditol acetates from reduced CA-1b

Alditol acetates derivatives	Fragments (m/z)	Linkages
1,5-di-OAc-2,3,4,6-tetra-OMe-glucitol-6,6-*d,d*	43, 47, 71, 89, 102, 117, 131, 147, 163, 207	GlcA^1 →
1,4,5-tri-OAc-2,3,6-tri-OMe-galactitol-6,6-*d,d*	43, 47, 87, 99, 101, 113, 117, 235	→ ^{4}GalA^1 →
1,4,5-tri-OAc-2,3,6-tri-OMe-glucitol-6,6,*d,d*,	43, 47, 87, 99, 101, 113, 117, 235	→ ^{4}GlcA^1 →

(1.4:1.0:2.7 by mol.) were detected in the retained vol by TLC and GC. The material (PD-CA) recovered in the void vol consisted of rhamnose, arabinose, xylose and galactose in the molar ratios of 0.5:0.2:1.0:3.1, and significant amounts of galacturonic acid and glucuronic acid (1.0:5.5 by mol). The result of methylation analysis of PD-CA showed that the molar ratios of all of the arabinosyl, 3,4-di-*O*-substituted xylopyranosyl and (1 → 4)-linked galactopyranosyl residues had decreased significantly, and that the molar ratios of the (1 → 4)-linked xylopyranosyl and (1 → 6)-linked and 4,6-di-*O*-substituted galactopyranosyl residues had increased compared to those of AF-CA (Table 5). However, the molar ratios of 2,4-di-*O*-substituted rhamnose and (1 → 4)-linked galacturonic acid did not change significantly. These results suggested that the inner core of CA-1 and CA-2 consisted of rhamnogalacturonan moiety which was substituted (1 → 4)- and/or (1 → 6)-linked galactan, and (1 → 4)-linked xylosyl residues attached at O-4 of rhamnosyl residues. Some (1 → 6)-linked galactan may be attached to 4-linked galactan moiety at O-6, and some arabinofuranosyl side chains also may be attached to (1 → 6)-linked galactan moiety at O-3. These partial structure moieties of CA-1 and CA-2 were similar to the pectic polysaccharides such as polysaccharides isolated from cotyledon meals and hulls of *Glycine max* [20, 21], leaves of *Medicago sativa* [22], suspension cultured sycamore cell walls [23, 24], rice endosperm cell walls [25], the fruits of *Zizyphus jujuba* Millar var. *inermis* Reahd [26], suspension cultured cells and leaves of *Nicotiana tabacum* [27, 28], the root of *A. acutiloba* [14, 15], and the leaves of *A. princeps* [10, unpublished]. Because pectic polysaccharide from the fruits of *Z. jujuba* did not show potent anti-complementary activity [12], it was suggested that the side chain structure of rhamnogalacturonan moiety may be important to the expression of the anti-complementary activity.

CA-1 and CA-2 contained large amount of arabinofuranosyl groups as non-reducing terminal, significant amount of protein and traces of glucose. In order to determine whether these molecules contribute to the expression of the anti-complementary activity, CA-1 and

Table 5. Methylation analysis of exo α-L-arabinofuranosidase digested CA and partial acid degraded CA

	Mol.%							
	CA		AF–CA		PD-CA			
Methylated sugars	Neutral*	Galactan†	Neutral*	Galactan†	Total‡	Galactan†	Neutral*	Linkages
2,3,5-tri-*O*-Me Ara	10.9	—	1.8	—	0.4	—	0.5	$\mathrm{Ara}f^{1}\rightarrow$
2,5-di-*O*-Me Ara	2.3	—	1.9	—	0.2	—	0.3	$\rightarrow{}^{3}\mathrm{Ara}f^{1}\rightarrow$
2,3-di-*O*-Me Ara	7.2	—	1.2	—	0.5	—	0.7	$\rightarrow{}^{4}\mathrm{Ara}^{1}\rightarrow$ or $\rightarrow{}^{5}\mathrm{Ara}f^{1}\rightarrow$
2,3,4-tri-*O*-Me Xyl	0.7	—	n.d.§	—	0.5	—	0.7	$\mathrm{Xyl}^{1}\rightarrow$
2,3-di-*O*-Me Xyl	6.4	—	6.1	—	7.6	—	10.5	$\rightarrow{}^{4}\mathrm{Xyl}^{1}\rightarrow$
2-mono-*O*-Me Xyl	18.7	—	10.1	—	2.1	—	3.0	${}^{\rightarrow 4}_{\rightarrow 3}\mathrm{Xyl}^{1}\rightarrow$
3-mono-*O*-Me Rha	0.9	—	0.8	—	0.5	—	0.7	${}^{\rightarrow 4}_{\rightarrow 2}\mathrm{Rha}^{1}\rightarrow$
2,3,4,6-tetra-*O*-Me Gal	3.1	6.5	9.9	12.7	7.1	11.7	9.8	$\mathrm{Gal}^{1}\rightarrow$
2,3,6-tri-*O*-Me Gal	12.9	30.0	15.2	19.5	1.9	3.1	2.6	$\rightarrow{}^{4}\mathrm{Gal}^{1}\rightarrow$
2,4,6-tri-*O*-Me Gal	10.9	22.8	9.1	11.7	7.8	12.9	10.8	$\rightarrow{}^{3}\mathrm{Gal}^{1}\rightarrow$
2,3,4-tri-*O*-Me Gal	3.4	7.1	24.9	31.9	28.1	46.5	38.9	$\rightarrow{}^{6}\mathrm{Gal}^{1}\rightarrow$
2,6-di-*O*-Me Gal	1.9	3.9	0.9	1.2	n.d.§	n.d.§	n.d.§	${}^{\rightarrow 4}_{\rightarrow 3}\mathrm{Gal}^{1}\rightarrow$
2,3-di-*O*-Me Gal	0.9	1.9	1.2	1.5	4.3	6.0	7.2	${}^{\rightarrow 4}_{\rightarrow 6}\mathrm{Gal}^{1}\rightarrow$
2,4-di-*O*-Me Gal	14.7	37.5	16.9	21.6	11.3	18.6	15.6	${}^{\rightarrow 3}_{\rightarrow 6}\mathrm{Gal}^{1}\rightarrow$
6,6-*d*,*d*-2,3,4-tri-*O*-Me Glc	—	—	—	—	25.7‖	—	—	$\mathrm{GlcA}^{1}\rightarrow$
6,6-*d*,*d*-2,3-di-*O*-Me Gal	—	—	—	—	2.1¶	—	—	$\rightarrow{}^{4}\mathrm{GalA}^{1}\rightarrow$

*Calculated from neutral glycosyl residues.

†Calculated from only galactosyl residues (galactan).

‡Calculated from all glycosyl residues.

§Not detected.

‖Calculated from the ratio of the intensities of the fragment ions at *m/z* 233 and 235.

¶Calculated from the ratio of the intensities of the fragment ions at *m/z* 261 and 263.

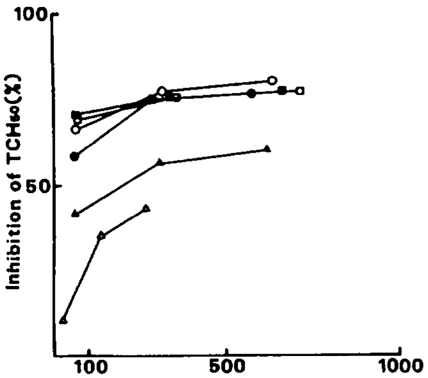

Fig. 3. Effects of the enzymic and chemical treatments of CA-1 and CA-2 mixture on anti-complementary activity. –●–, CA-1 and CA-2 mixture; –○–, α-L-arabinofuranosidase digest; –■–, β-amylase digest; –□–, pronase digest; –▲–, mild acid hydrolysate; –△–, IO_4^- oxidation product.

CA-2 mixture was digested with exo α-L-arabinofuranosidase, pronase and β-amylase, respectively. This did not affect the anti-complementary activity of CA-1 and CA-2 mixture (Fig. 3). When CA-1 and CA-2 mixture was treated with mild acid (0.01 M HCl, 100°, 1 hr) or oxidized with $NaIO_4$ followed by reduction with $NaBH_4$, the resulting products were shown to decrease the activity than CA-1 and CA-2 mixture (Fig. 3). These data suggested that at least the side chain structure and/or tertiary structure of the polysaccharide molecule are required for the expression of anti-complementary activity.

The another anti-complementary pectic arabinogalactan (AGIIb-1) from *A. acutiloba* also decreased the activity by the treatment of mild acid hydrolysis [14]. Overall structures of AGIIb-1 was suggested to consist of one neutral and two acidic arabinogalactans and one neutral arabinan which were linked to each other by acid-labile linkages [15]. However, the presence of the unit structure is not yet known in CA-1 and 2. More recently, polysaccharides having similar compositions to CA-1 and 2 have been isolated from *C. lacryma-jobi* seed as active principles of hypoglycemic activity [29], but their structures are not yet known.

EXPERIMENTAL

Materials. The seed of *Coix lacryma-jobi* var. *ma-yuen* without husks (Yoku-inin), from China, was purchased from Tochimoto Tenkaido Co. Ltd. (Osaka, Japan). DEAE-Sepharose CL-6B, Sepharose CL-6B, concanavalin-A (Con-A) Sepharose and Sephadex G-100 were purchased from Pharmacia and Bio-gel P-2 (200–400 mesh) from Bio-Rad. α-L-Arabinofuranosidase from *Rhodotorula flava* was a gift from Dr Naoto Shibuya (National

Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Japan). Sep-pak C_{18} cartridge was purchased from Waters Associates Inc. β-Amylase from sweet potato was purchased from Sigma, and Pronase from Kaken-kagaku Co. Ltd. (Tokyo, Japan).

General methods. Total carbohydrate and uronic acid contents were determined by the PhOH–H_2SO_4 method [30] and *m*-hydroxydiphenyl method [31] respectively, using a mixture of arabinose, xylose, and galactose in molar ratios of 5.0:1.0:4.0 and galacturonic acid as the respective standards. Protein was assayed by the method of ref. [32] with bovine serum albumin as the standard. TLC was performed on cellulose-coated plastic sheets (Merck) with EtOAc–pyridine–HOAc–H_2O (5:5:1:3) and sugars were detected by using alkaline $AgNO_3$ [33] for reducing sugars and *p*-anisidine HCl [34] for uronic acid. GC (Shimadzu GC-6A) was equipped with FID and a glass column (0.3 × 200 cm) of 3% ECNSS-M or 1% Silicone OV-225 on Uniport HP at 180 or 200°, respectively. N_2 was used as a carrier gas at flow rate of 40 ml/min. HPLC was conducted by using Waters Model ALC/GPC 244 equipped with columns (0.76 × 50 cm) of Asahi-Pak GS-510 + GS-320 (Asahi Chemical Industry Co. Ltd., Japan) and developed with H_2O as reported previously [1]. Polysaccharide (0.1–1 mg) was hydrolyzed with 2 M TFA for 1.5 hr at 121° [10]. The hydrolysate containing neutral sugar and uronic acid was converted to alditol acetates by the method of ref. [35]. The resulting alditol acetates were analysed to determine component sugars by GLC [10].

Preparation of crude anti-complementary polysaccharides. Crude anti-complementary polysaccharide (CS-1) was prepared by hot H_2O extraction and EtOH pptn from *C. lacryma-jobi* as reported previously [1]. CS-1 was further purified by a column (3.0 × 33.0 cm) of DEAE-Sepharose CL-6B (Cl^- form) [1]. Anti-complementary polysaccharide fraction, CS-IIa-2 from the absorbed fraction was recovered by elution of 0.2 M NaCl.

Methylation analysis of polysaccharides. Polysaccharides were methylated by the method of ref. [18] and completeness of methylation was checked using triphenylmethane [36]. The methylated polysaccharides were purified by Sep-pak C_{18} cartridge using CH_3CN and EtOH as eluent [37]. The fully methylated polysaccharides were heated with aq 88% HCOOH at 100° for 5 hr, and then with 1 M TFA at 100° for 5 hr [7], and the hydrolysate was converted into alditol acetates [7]. The methylated alditol acetates were analysed by GC (Shimadzu GC9A) with OV-1 fused silica capillary column (25 m × 0.25 mm i.d., Gaskuro Kogyo Inc., Tokyo) at 145 to 205° (1°/min) with a split ratio of 80:1. He carrier flow was 1 ml/min. GC/MS of partially methylated alditol acetates was performed on a Hitachi M-80 mass spectrometer equipped with the same column and condition as GC analysis operated at an ionization voltage of 20 eV and an ion source at 180°. Fully methylated PD-CA was reduced with $NaBD_4$ in 95% EtOH–THF [27:73 (v/v)] [38]. Methyl esterified hexosyluronic aid residues were converted into the corresponding 6,6-di-deuterated hexosyl residue by this reducing procedure [37].

*Carboxyl reduction of CA-*1. Carboxyl reduction of CA-1 was carried out by the method of ref. [39]. CA-1 (5 mg) was dissolved in 0.1 M NaOH (1 mg) and kept at 12° for 10 min. The soln was neutralized with 0.1 M HCl and diluted up to 3 ml with H_2O, then 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulphonate (100 mg) was added. The pH of the reaction mixture was maintained at 4.75 by titration with 0.07 M HCl under stirring for 2 hr, then 2 M $NaBD_4$ (1 ml) was added gradually to the reaction mixture during 4 hr while the pH was maintained at 7 by titration with 4 M HCl while stirring at room temp. After the soln was lyophilized, the carboxyl reduction product was purified by gel filtration on Sephadex G-10 using H_2O as eluant [14]. The carbohydrate fractions were combined and lyophilized. The fraction eluted in the included vol was further purified by gel filtration on Bio-gel P-2 at 55°.

NMR studies. ^1H NMR spectrum was obtained for 5% soln in D_2O as 90 MHz and 80° using Varian EM-390. Chemical shifts were expressed relative to that of sodium 3-(trimethylsilyl) propane-1-sulphonate-d_4 (TSP). ^{13}C NMR spectrum was obtained at 25 MHz and room temp. using JEOL PS-100/EC-100 Fourier transform spectrometer with complete proton decoupling. Chemical shifts were expressed as δ values (ppm) from the signal of TSP.

Exo *α-L-arabinofuranosidase digestion of polysaccharides.* CA-1 and CA-2 mixture (CA, 14 mg) was dissolved in 3 ml of 0.1 M acetate buffer (pH 4) and α-L-arabinofuranosidase (0.7 unit) was added. The reaction mixture was incubated at 50° for 20 hr in the presence of one drop of toluene. Following this the pH of the mixture was adjusted to 6 with 0.5% NaOH to inactivate the enzyme, and then the mixture was lyophilized. The lyophilizate was separated by gel filtration on a column (2.0 × 52 cm) of Bio-gel P-2 at 55°. The fraction eluted in the included vol was desalted with cation exchange resin, AG 50 W × 8 (H^+ form), and analysed by TLC and GC. The material (AF-CA) recovered in the void vol was lyophilized, and the component sugar and the glycosidic linkage of this enzyme resistant polysaccharide were analysed.

Partial acid hydrolysis of exo *α-L-arabinofuranosidase digested polysaccharide. Exo* α-L-arabinofuranosidase digested polysaccharide (AF-CA, 6 mg) was hydrolysed with 2 ml of 0.1 M HCl at 100° for 3 hr. After hydrolysis, the soln was neutralised with 0.1 M NaOH. The neutralized soln was applied to a column of Bio-gel P-2, followed by gel filtration with H_2O at 55°, and the fraction (PD-CA) eluted in the void vol, oligo- and monosaccharide fractions were obtained.

Anti-complementary activity. The anti-complementary activity was measured as described previously [6], except H_2O was used for dilutions.

Enzymatic degradation procedure. CA-1 and CA-2 mixture was digested with β-amylase, *exo* α-L-arabinofuranosidase and pronase, separately as reported previously [7, 9]. Each reaction mixture was heat denatured and the resulting supernatant was dialysed against H_2O, and the non-dialysable portions were lyophilized. The anti-complementary activity of these lyophilizates was then assayed.

Chemical degradations. CA-1 and CA-2 mixture was treated with 0.01 M HCl, 100° for 1 hr. Then the reation mixture was neutralised with 1 M NaOH, and dialysed against H_2O, and the non-dialysable portion was lyophilized. Periodate oxidation of CA-1 and CA-2 was carried out as previously [9]. The oxidation product was reduced with $NaBH_4$ followed by dialysis and lyophilization. These lyophilizates were used for the assay of the anti-complementary actvity.

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