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CHARACTERIZATION OF ANTI-COMPLEMENTARY NEUTRAL POLYSACCHARIDES FROM THE ROOTS OF *BUPLEURUM FALCATUM*

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Key Word Index—*Bupleurum falcatum*; Umbelliferae; polysaccharide; arabinan; glucan; arabinoglucan; anti-complementary activity.

Abstract—The anti-complementary neutral polysaccharide, BR-5-I, was purified from the roots of *Bupleurum falcatum*. It seemed to be a homogeneous polymer from the results of gel filtration, HPLC and electrophoresis. BR-5-I mainly consists of L-arabinose and D-glucose in a molar ratio of 9:10. Its M_r was estimated to be 18 500 by gel filtration. From the results of methylation analysis, Smith degradation and NMR studies, it appeared that BR-5-I contained a three-branched (1→5)linked α-L-arabinan moiety and a (1→4)linked α-D-glucan moiety. When BR-5-I was treated with exo-α-L-arabinofuranosidase, a glucan-rich polymer was obtained, but when it was treated with β- and α-amylase, an arabinan-rich polymer was obtained. Both polymers showed reduced anti-complementary activities and had the same M_r as BR-5-I. BR-5-I was, however, separated into Con-A unbound arabinan and Con-A bound glucan containing arabinose and glucan by affinity chromatography on Con-A Sepharose. These results indicated that BR-5-I was a mixture of highly 3-branched (1→5)linked α-L-arabinan, (1→4)linked α-D-glucan containing arabinose, and (1→4)linked α-D-glucan. Surprisingly, these polymers have almost the same M_r and same electrophoretic mobility. All the polysaccharides showed potent anti-complementary activity but the α-L-arabinan showed the highest activity.

INTRODUCTION

The roots of *Bupleurum falcatum* L. have been used in Chinese and Japanese herbal medicine (Kampo medicine) for the treatments of chronic hepatitis, nephrosis syndrome and auto-immune diseases. The hot water extract from the roots of *B. falcatum* has been reported to have several immunomodulating activities such as the enhancement of antibody response and an inhibitory effect on mitogen-induced lymphocyte transformation [1].

It is known that *B. falcatum* contains several saikosaponins [2], α-spinasterol [3], stigmasterol [4], fatty acids such as palmitic acid, oleic acid, linoleic acid and linoceric acid [5], adnitol and (−)-anomalin [6]. A large variety of pharmacological activities of saikosaponins have been reported [7–9], however studies on polysaccharides from *B. falcatum* have not been undertaken although some polysaccharide molecules in other herbs have immunomodulating activities [10].

In a study of the polysaccharide components in Chinese herbs, those from *B. falcatum* were fractionated and water-soluble neutral polysaccharides which contain arabinan, arabinoglucan and glucan, were obtained as the potent anti-complementary polysaccharides. The present paper deals with the purification and chemical properties of these polysaccharides.

RESULTS AND DISCUSSION

The crude hot water soluble polysaccharide fraction (BR-I) from the roots of *B. falcatum* was fractionated into four polysaccharide-containing fractions (BR-2–BR-5).The neutral polysaccharide fraction (BR-5) showed marked anti-complementary activity and was further fractionated to give BR-5-I, IIa, IIb and IIc. The major neutral polysaccharide fraction (BR-5-I) showed marked anti-complementary activity, and gave mainly arabinose and glucose after acid hydrolysis. When subjected to gel filtration on Sepharose CL-6B (Fig. 1) or to HPLC on

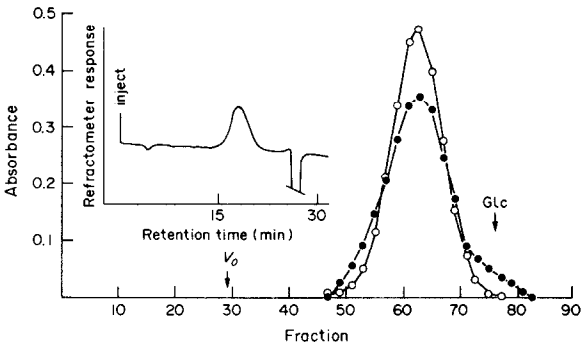

Fig. 1. Gel filtration of BR-5-I on Sepharose CL-6B. V_0, void volume; Glc, glucose; –●–, carbohydrate, 490 mm; –○–, pentose, 552–510 nm. Inset, HPLC of BR-5-I. BR-5-I was eluted with 0.2 M NaCl in gel filtration and HPLC.

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Asahipak GS-510+GS-320 (Fig. 1 inset), the carbohydrate was eluted as a single peak. The pentose was also co-eluted with the carbohydrate peak on gel filtration (Fig. 1). BR-5-I had $[\alpha]_D + 26.2°$ (water; *c* 1.0), and gave a single spot on glass-fibre paper electrophoresis. These results suggested that BR-5-I was homogeneous and pure enough for structural studies. BR-5-I contained 95.2% of total carbohydrate, and was composed of L-arabinose and D-glucose, in a molar ratio of 9:10, with traces of galactose and mannose. Nitrogen and sulphate were not detected by elementary analysis. The M_r of BR-5-I was estimated to be 18 500 by gel filtration on Sepharose CL-6B.

The ^1H NMR spectrum of BR-5-I in D_2O contained signals of anomeric protons in the α-configuration at δ 5.08, 5.11 and 5.15 (due to L-arabinofuranosyl residues) and δ 5.36 (due to D-glucopyranosyl residues). The ^{13}C NMR spectra of BR-5-I gave signals in the anomeric carbon region at δ 102.6 due to C-1 of the α-D-glucopyranosyl residue, and at δ 110.1 and 110.3 due to C-1 of the α-L-arabinofuranosyl residue.

BR-5-I was methylated by the method of ref. [11] and the fully methylated product was hydrolysed with acid and converted into alditol acetates. The partially methylated alditol acetates were analysed by GC and GC-MS, and 1,4-di-*O*-acetyl-2,3,5-tri-methylarabinitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylarabinitol, 1,3,4,5-tetra-*O*-acetyl-2-mono-*O*-methylarabinitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol were identified (Table 1). The results of methylation analysis indicated that BR-5-I contained mainly (1→5)linked arabinofuranosyl residues, (1→4)linked glucopyranosyl residues and 3,5-substituted arabinofuranosyl residues.

BR-5-I consumed 0.83 mol of periodate per anhydrosugar residue. Oxidised BR-5-I was treated with sodium borohydride, and the resulting polyalcohol on complete hydrolysis with acid gave glycerol, erythritol and L-arabinose in the molar ratios of 10:59:18. The presence of unchanged L-arabinose and the formation of erythritol were indicative of branched arabinofuranosyl residues and (1→4)linked glucosyl residues, respectively. These results accorded with those of methylation analysis. A comparison of the molar ratios between the methylated sugars and the Smith-degradation products indicated that half of the expected arabinose in the acid hydrolysate of the polyalcohol was lost, probably because of the instability of arabinosyl residue.

The polyalcohol was hydrolysed with a weak acid (0.05 M H_2SO_4) at room temp. for 12 hr (controlled Smith degradation). Fractionation of the neutralised reaction product on Bio-gel P-2 gave di-, tri-, tetra-, and pentasaccharides (B1-4, respectively) and higher oligo-saccharides (B5-7, respectively) (Fig. 2). On hydrolysis B1, B2, B3 and B4 gave mainly arabinose. B5 and B6 yielded arabinose and erythritol. While B7 yielded erythritol, arabinose and glucose (49:10:6 by mol). These results indicated that BR-5-I contained branches made up of two to five or more adjoining arabinosyl residues.

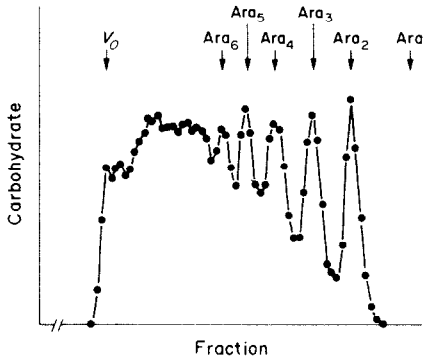

Fig. 2. Gel filtration of BR-5-I controlled Smith degradation product on Bio-gel P-2. Arrows indicate the eluting position of arabino-oligosaccharides. Ara, arabinose; Ara_2, arabinobiose; Ara_3, arabinotriose; Ara_4, arabinotetraose; Ara_5, arabinopentaose; Ara_6, arabinohexaose. The sample was eluted with water.

BR-5-I was treated with exo-α-L-arabinofuranosidase. The reaction products were applied to a column of Bio-gel P-2, and the void volume and the inner volume collected as a series of fractions (Fig. 3a, inset). Arabinose was only detected (TLC and GC) in the fractions of the inner volume. The void volume fractions were applied to a column of Sepharose CL-6B but the elution volume of this arbinofuranosidase resistant fraction did not change in comparison with that of BR-5-I although this fraction consisted of arabinose, glucose and galactose in molar ratios of 21:313:10 (Fig. 3a).

The arabinofuranosidase resistant fraction was subjected to methylation analysis and 2,3,4,6-tetra-*O*-methylglucose, 2,3,6-tri-*O*-methylglucose, 2,3,4-tri-*O*-methylglucose, 2,3-di-*O*-methylarabinose and 2,3,5-tri-O-methylarabinose were detected in molar ratios of 94:863:8:35: trace by GC (Table 2). To decompose the α-glucan moiety of BR-5-I, it was treated with β-amylase and the reaction products were fractionated on a column of Sepharose CL-6B (Fig. 3b), and fraction I and the fraction of the inner volume (fraction II) were obtained. Glucose and maltose were detected in fraction II by TLC. This glucose might be due to the contaminant in the commercial β-amylase [12]. Fraction I was eluted in the same elution volume as BR-5-I. Fraction I was further treated with α-amylase and the products were fractionated into the

Table 1. Methylation analysis of BR-5-I

Methylated alditol acetate derivatives	Mol%	Major mass fragments (*m/z*)	Linkages
2,3,5-tri-*O*-Me Ara	11.7	43,45,101,117,129,161	Ara*f*^{1}→
2,3-di-*O*-Me Ara	19.0	43,101,117129,189	→^{5}Ara*f*^{1}→
2-mono-*O*-Me Ara	32.4	43,117,127,201,261	→$^{3}_{5}$Ara*f*^{1}→
2,3,4,6-tetra-*O*-Me Glc	3.2	43,45,101,117,129,233	Glc^1→
2,3,6-tri-*O*-Me Glc	33.7	43,45,101,117,233	→^{4}Glc^1→

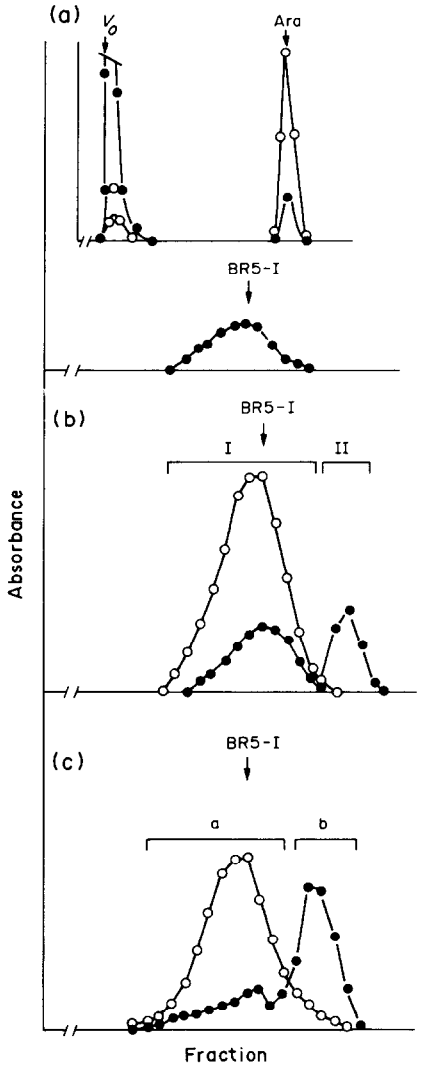

Fig. 3. Gel filtration of enzymatic digestion products of BR-5-I. a, elution of exo-α-L-arabinofuranosidase resistant fraction from BR-5-I on Sepharose CL-6B with 0.2 M NaCl; arrow indicates the eluting position of BR-5-I. Inset, elution of exo-α-L-arabinofuranosidase digested BR-5-I on Bio-gel P-2 with water. b, elution of β-amylase digested BR-5-I on Sepharose CL-6B with 0.2 M NaCl. c, elution of α-amylase digested fraction I (Fig. 3b) on Sepharose CL-6B with 0.2 M NaCl. All symbols are the same as in Fig. 1.

pentose-rich fraction-a and hexose-rich fraction-b on a column of Sepharose CL-6B (Fig. 3c). Although the pentose-rich fraction consisted of arabinose, glucose and galactose in molar ratios of 215:13:10, it still had the same elution volume as BR-5-I. Fraction-a was subjected to methylation analysis and 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylarabinose, 2-mono-*O*-methylarabinose, 2,3,4,6-tetra-*O*-methylglucose and 2,3,4-tri-*O*-methylglucose were detected in molar ratios of 148:323:506:21:1 by GC (Table 2). α-L-Arabinofuranosidase resistant BR-5-I and β- and α-amylase resistant BR-5-I (Fraction-a) both had lower anti-complementary activities than BR-5-I, and the activity of β- and α-amylase resistant

Table 2. Methylation analysis of enzymic digestion product of BR5-I

Methylated alditol acetate derivatives	α-L-Arafase* resistant fraction	β- and α-Amylase resistant fraction
	(Mol%)	
2,3,5-tri-*O*-Me Ara	trace	14.8
2,3-di-*O*-Me Ara	3.5	32.3
2-mono-*O*-Me Ara	—	50.6
2,3,4,6-tetra-*O*-Me Glc	9.4	2.1
2,3,4-tri-*O*-Me Glc	0.8	0.1
2,3,6-tri-*O*-Me Glc	86.3	—

*exo-α-L-Arabinofuranosidase.

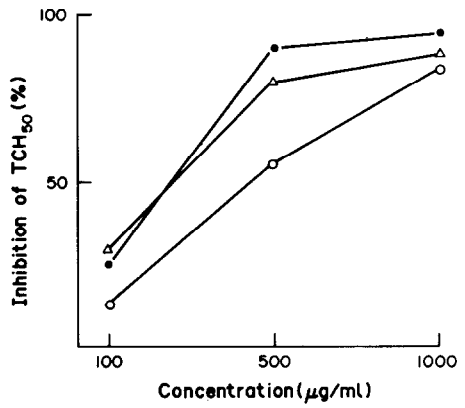

Fig. 4. Effects of enzymatic treatment of BR-5-I on anti-complementary activity. –●–, BR-5-I; –△–, exo-α-L-arabinofuranosidase digested BR-5-I (the enzymatic resistant fraction); –○–, β- and α-amylase digested BR-5-I (fraction-a from Fig. 3c).

BR-5-I was much lower than that of arabinofuranosidase resistant BR-5-I (Fig. 4). These results suggested that a majority of BR-5-I is a mixture of α-arabinan and α-glucan.

To confirm this possibility, BR-5-I was subjected to affinity chromatography on Con-A Sepharose, and the Con-A unbound fraction and the Con-A bound fraction eluted (Fig. 5a). The unbound fraction consisted of arabinose and glucose in a molar ratio of 6:5 whereas the bound fraction consisted of glucose and arabinose in a molar ratio of 189:2. The Con-A bound fraction showed strong iodine binding suggesting the presence of α-(1→4)linked glucan. The Con-A unbound fraction was further applied to a Con-A Sepharose column, and a second Con-A unbound fraction (A-1), a retarded fraction (A-2) and a Con-A bound fraction (B) were obtained (Fig. 5b). A-1 consisted of only arabinose, A-2 arabinose and glucose in a molar ratio of 1:10, and B only glucose. These results indicated that BR-5-I was a mixture of α-arabinan, α-arabinoglucan and α-glucan. The analytical results suggested that the α-arabinan was a (1→5)-α-L-arabinan possessing numerous arabinofuranosyl side-chains at position 3 as shown in Fig. 6. The first Con-A bound glucan-rich fraction, the second Con-A bound glucan (B) and the second Con-A retarded arabinoglucan (A-2)

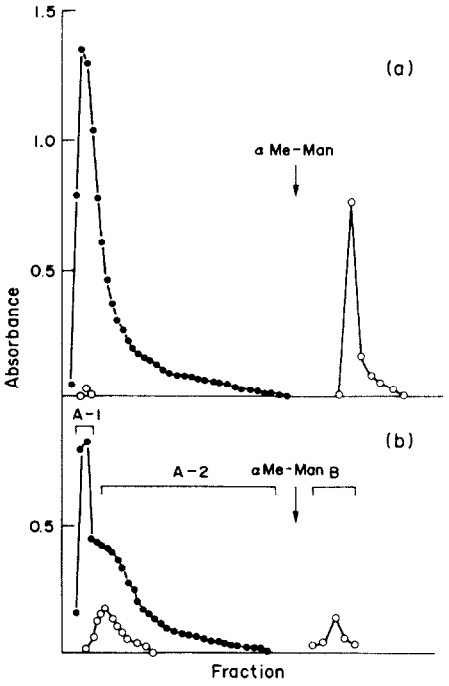

Fig. 5. Affinity chromatography of BR-5-I on Con-A Sepharose. a, first affinity chromatography of BR-5-I. –●–, carbohydrate, –○–, iodine binding, 465 nm; αMe-Man, 0.1 M α-methyl mannoside. b, second affinity chromatography of Con-A unbound fraction from Fig. 5a. Con-A bound fraction was only detected by iodine binding method.

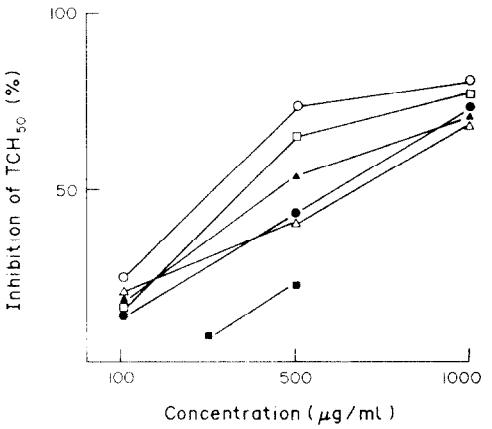

Fig. 7. Anti-complementary activities of fractions obtained by Con-A Sepharose affinity chromatography of BR-5-I. □, BR-5-I; ●, first Con-A bound glucan-rich fraction (Fig. 5a); ○, second Con-A unbound fraction (A-1) (Fig. 5b); ▲, second Con-A retard fraction (A-2); Δ, second Con-A bound fraction (B); ■, commercial amylose.

showed lower anti-complementary activity than BR-5-I, but the second Con-A unbound arabinan (A-1) showed higher activity (Fig. 7).

Pure arabinan has been obtained from *Pinus pinaster* Wood [13], mustard seeds [14] and the inner bark of *Rosa glauca* [15]. Other arabinans contain small proportions of other sugar residues such as galactose [16, 17]. BR-5-I contained pure arabinan in addition to α-glucan and glucose-rich arabinoglucan, but it is not known whether the arabinose in arabinoglucan is due to contaminantion by some other α-arabinan. Arabinans are generally represented as an α-L-(1→5)linked arabinofuranan backbone which is branched through C-3 and/or C-2 to side branches of α-L-arabinofuranosyl chains (α-L-3,5-arabinan and/or α-L-2,5-arabinan), however the length of the arabinofuranosyl side chains is not been known. α-L-3,5-Arabinan in BR-5-I showed a potent anti-complementary activity. α-L-2,5-Arabinan isolated from the dried fruits of *Zizyphus jujuba* var. *inermis* also showed a potent activity [18]. Pectic arabinogalactan (AGIIb-I), obtained from the roots of *Angelica acutiloba* Kitagawa, consists of four kinds of carbohydrate unit which are joined through an acid labile linkage [19, 20]. One of the carbohydrate units is an α-L-3,5-arabinan unit (N-II) [20], and is known to have potent anti-complementary activity [unpublished]. Beet arabinan also mainly consisted of terminal, 5-linked and 3,5-substituted arabinofuranosyl residues in addition to small amounts of 2-, 3-linked and 2,3-, 2,5- and 2,3,5-substituted arabinofuranosyl residues [21], but no anti-complementary activity was observed [unpublished]. These observations suggest that a (1→5)linked α-L-arabinan which is branched through C-3 or C-2, is essential for the expression of potent anti-complementary activity by pure arabinan, but other arabinosyl linkages may suppress the expression of this activity.

BR-5-I also contained a linear α-glucan which consisted mainly of α-(1→4)linked glucosyl residue with a very low proportion of α-(1→6)linked glucosyl residues, and it showed a considerable anti-complementary activity. Nevertheless amylose showed negligible activity (Fig. 7). The fine structure of the α-glucan in BR-5-I needed for the expression of anti-complementary activity must await further studies.

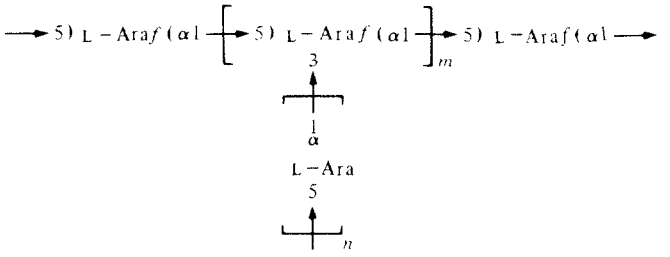

Fig. 6. A possible structural unit of α-arabinan from *B. falcatum*.

EXPERIMENTAL

Materials. The roots of *Bupleurum falcatum* L. (Japanese name = Saiko) were purchased from Uchida Wakanyaku Co. Ltd, Tokyo, Japan. Sweet potato β-amylase and potato amylose (type III) were purchased from Sigma, and *Bacillus subtilis* α-amylase (Liquefying type) from Seikagaku Kogyo Co. Ltd, Tokyo, Japan. *Rhodotorula flava* exo-α-L-arabinofuranosidase was a gift from Dr Naoto Shibuya (National Food Research Institute, Ministry of Agliculture, Forestry and Fisheries, Japan).

General methods. The total carbohydrate and uronic acid contents were determined by the phenol–H_2SO_4 [22] and *m*-hydroxybiphenyl [23] methods, respectively, using arabinose and galacturonic acid as the respective standards. Protein was determined by the method of ref. [24], with bovine serum albumin as standard. Polysaccharides were hydrolysed with 2 M TFA at 121° for 1.0 hr. The hydrolysates were analysed by TLC on cellulose-coated plastic sheets (Merck, 5577) with EtOAc pyridine–HOAc–H_2O (5:5:1:3) as the solvent system, and by GC as their alditol acetates [19]. Reducing sugars were detected with alkaline $AgNO_3$ [25], and uronic acid with *p*-anisidine hydrochloride [26]. FID/GC (Shimadzu GC-6A) analysis was performed on a glass column (0.3 × 200 cm) packed with 1% silicon OV-225 on Uniport HP at 190°. N_2 was used as carrier gas at a flow rate of 60 ml/min. Mol ratios of sugars were calculated from peak areas and M_rs of the corresponding alditol acetates. HPLC (Waters Model ALC/GPC 244) was performed on column (0.76 × 50 cm) of Asahipak GS-510 + GS-320 (Asahi Chemical Industry Co., Ltd, Japan) developed with 0.2 M NaCl. Gel filtration was carried out on Sepharose CL-6B (2.6 × 87 cm) and Bio-Gel P-2 (2.0 × 52 cm) eluted with 0.2 M NaCl and H_2O, respectively. Con-A Sepharose chromatography was carried out by the method of Kennedy and Rosevear [27]. Con-A binding glucan was detected by the iodine-binding method of Yamada *et al* [28]. Electrophoresis on glass fibre paper (Toyo-Roshi GA-100) was carried out in 26 mM borate buffer (pH 9.2) at a constant current of 40 V/cm for 1 hr. Carbohydrate was detected with the 1-naphthol–H_2SO_4 reagent [29].

Isolation of water-soluble polysaccharides. Roots of *B. falcatum* (1 kg) were boiled with 10 l H_2O to half vol. and the residual material extracted (x3) by the same procedure. The extracts were combined and centrifuged (7500 rpm, 20 min) to remove insoluble material. The supernatant was lyophilized to give the water-soluble extract (BR-0, yield 136.3g). BR-0 was refluxed with 3 l MeOH for 1 hr (× 5) and centrifuged to give a MeOH-insoluble ppt. The ppt. was dissolved in H_2O and then 5 vols of EtOH were added. The resulting ppt. was redissolved in H_2O and dialysed against running H_2O for 3 days. The non-dialysable portion was centrifuged to remove H_2O-insoluble material and the supernatant (crude polysaccharide) was lyophilized (BR-1, yield 35.3 g).

Fractionation of crude polysaccharide. Crude polysaccharide (BR-1) was fractionated into four fractions as follows. BR-1 (15.8 g) was dissolved in H_2O (790 ml) and treated with an equal vol. of an 8% soln of cetyltrimethylammonium bromide (cetavlon) by the method of Yamada *et al.* [30, 31]. After standing at 20° for 20 hr, the resulting ppt. was collected by centrifugation and redissolved in 10% NaCl. KOAc and 5 vols of EtOH were added to the soln, and the resulting ppt. was dissolved in H_2O, followed by dialysis against running H_2O. The acidic polysaccharide fraction (BR-2) was then obtained as the lyophilisate of the non-dialysable fraction (yield, 22% from BR-2).

The supernatant was added to an equal vol. (790 ml) of 1% H_3BO_3, and the pH was adjusted 8.8 by the addition of 2 M NaOH followed by stirring for 24 hr. The resulting ppt. was washed with 0.5% Na borate buffer (pH 8.8), and dissolved in 2% AcOH (300 ml). Five vols of EtOH were added to the soln. together with KOAc, and the resulting ppt. was dissolved in H_2O and then dialysed. The non-dialysable fraction (BR-3) was obtained as the lyophilisate (yield, 25% from BR-1). The pH of the supernatant was readjusted to 9.5 by the addition of 2 M NaOH. The resulting ppt. was collected as the non-dialysable lyophilisate (BR-4) by the same procedure as that used to obtain BR-3. The final supernatant of the cetavlon fractionation was acidified with HOAc and 5 vols of EtOH were added together with KOAc, and then the ppt. was obtained by centrifugation. This ppt. was washed with 2% AcOH–EtOH and EtOH, and was dissolved in H_2O, followed by dialysis. The non-dialysable portion was lyophilysed to obtain BR-5 (yield 37% from BR-1).

Ion-exchange chromatography of BR-5. BR-5 (500 mg) was applied to a column (3.2 × 40 cm) of DEAE-Sepharose CL-6B (Cl^- from) equilibrated with H_2O. The column was eluted first with H_2O (500 ml) until sugar was no longer detected, and then the polysaccharide fractions were eluted by elution (stepwise) with 0.1 M–2 M NaCl. The unabsorbed fraction (BR-5-I) and three absorbed fractions (BR-5-IIa, IIb and IIc) were obtained as lyophilysates after dialysis.

NMR studies. ^1H NMR: 90 MHz, D_2O at 80°; ^{13}C NMR: at 25MHz and room temp. using a Fourier transform spectrometer with complete proton decoupling. Chemical shifts were expressed as δ values (ppm) relative to that of the internal standard Na 3-(trimethysilyl)propane-1-sulphonate (TSP).

Methylation analysis. Methylation was performed using the method of ref. [11]. The methylated polysaccharides were hydrolysed with 2 M TFA at 121° for 1 hr, and the partially methylated sugars reduced with $NaBH_4$ to the corresponding alditols. These were then acetylated with Ac_2O in the presence of AcONa at 121° for 3 hr [32]. The resulting alditol acetates were subjected to GC and GC-MS. Solns of partially methylated alditol acetates in Me_2CO were injected into a DB-1 capillary column (0.25 μm film thickness, 30 m × 0.25 mm i.d., J and W Scientific Inc. U.S.A.) with splitless injection. The flow rate of the carrier gas, He was 0.9 ml/min. The oven temp. was programmed at 60° for 1 min, 18°/min to 150° and then 2°/min to 210°. GC-MS was performed on a JEOL DX-300 instrument equipped with SPB-1 fused silica capillary column (25 μm film thickness, 25 m × 0.25 mm i.d., SPELCO) at 120 to 210° (4°/min) with splitless injection and operated at an ionisation voltage of 70 eV with an ionisation current of 300 μA.

Enzymic hydrolysis. (i) Exo-α-L-arabinofuranosidase digestion. BR-5-I (10.4 mg) was dissolved in 3 ml 0.1 M citrate Pi buffer (pH 3.0) and incubated at 50° with 0.7 units of α-L-arabinofuranosidase. After 48 hr, the incubation mixture was adjusted to pH 6.0 with 0.5% NaOH to inactivate the enzyme and lyophilized. The lyophilisate was applied to a column (2.0 × 52cm) of Bio-gel P-2 kept at 55° and eluted with H_2O. Fractions (1 ml) were collected and assayed for total carbohydrate and pentose with phenol–H_2SO_4 [22] and phloroglucinol-AcOH reagents [33], respectively. The material recovered in the void vol. was applied to a column of Sepharose CL-6B with 0.2M NaCl.

(ii) β-Amylase and α-amylase digestions. BR-5-I (20 mg) was dissolved in 10 ml of 0.1 M Ac buffer (pH 4.0) and β-amylase (1140 units) added, and then incubated at 25° for 68 hr in the presence of one drop of toluene. The incubation mixture was neutralized and heated at 100° for 5 min and centrifuged to remove the insoluble residue. The supernatant was lyophilized and applied to a column of Sepharose CL-6B. Fractions (6.2 ml) eluted with 0.2 M NaCl were collected and analysed. The fraction eluted in the same elution vol. with BR-5-I was further treated with α-amylase (3060 unit) in 8.5 ml of 50 mM Ac buffer (pH 6.0) at 40° for 3 days. The reaction mixture was

neutralized, heated and centrifuged as above. The α-amylase digest was also subjected to CC on Sepharose CL-6B (same conditions).

Periodate oxidation and Smith degradation. BR-5-I (20 mg) was dissolved in 10 ml of 50 mM Ac buffer (pH 5.2) and oxidized with 0.369 mmol $NaIO_4$ at 4° in the dark. The IO_4^- consumption was determined at various time-intervals by the method of ref. [34]. After 123 hr, ethylene glycol was added to decompose the excess of IO_4^- and the reaction mixture was dialysed. The oxidised polymer was reduced with $NaBH_4$ (27.9 mg) for 24 hr at room temp., then the mixture was dialysed against H_2O for 2 days after decomposition of excess $NaBH_4$ by the addition of AcOH. The BR-5-I polyalcohol was isolated by lyophilization. The polyalcohol was hydrolysed with 2M TFA at 121° for 1 hr and the hydrolysate converted into alditol acetates and analysed by GC (130→210° at 10°/min).

Controlled Smith degradation. BR-5-I polyalcohol (6.6 mg) was hydrolysed with 0.05 M H_2SO_4 (2 ml) for 12 hr at room temp., then neutralized with $BaCO_3$, and desalted with AG50WX8 (H^+ form) resin. The controlled Smith-degradation product gave seven oligosaccharides and oligosaccharide alcohols on a column of Bio-gel P-2 at 55°.

Anti-complementary activity. The anti-complementary activity was measured according to the previously described procedure [31, 35], except H_2O was used for dilutions.

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