J. Biochem., 76, 861–869 (1974)

Structural Studies on Anti-inflammatory Polysaccharides from *Streptomyces fradiae*

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Received for publication, March 9, 1974

Two kinds of L-arabinans have been isolated from a broth of *Streptomyces fradiae* 3739 strain. Both of these arabinans, named P-1 and P-2, possess potential anti-carrageenin abscess activity. Chromatographic differences on TEAE-cellulose between P-1 and P-2 can be attributed to differences in the contents of D-galacturonic acid in these polysaccharides. From the results of various experiments, it is concluded that these L-arabinans have almost linear structures made up of 1→5 linked α-L-arabinofuranose units. D-Galacturonic acid was found to be linked to L-arabinose by an α-glycosidic linkage. The molecular weights of P-1 and P-2 were determined to be 9,800 and 4,700, respectively, by ultracentrifugal analyses. Periodate oxidation of these polysaccharides resulted in loss of biological activity.

During studies on biologically active components produced by bacteria, we found that *Streptomyces fradiae* 3739 strain produces certain substances having potential anti-carrageenin abscess activity (*1*). We characterized these substances as polysaccharides, and were able to purify them by ion exchange cellulose chromatography. The fractions which were eluted by 0.05 M and 0.08 M sodium chloride on TEAE-cellulose retained remarkable biological activity. These fractions, termed P-1 and P-2, respectively, were finally purified on TEAE-cellulose by continuous NaCl gradient elution. They were both composed of over 90% of L-arabinose, with small amounts of D-galacturonic acid.

In the present paper, we describe the structural determination of P-1 and P-2 and some of their biological properties.

Abbreviations used: Ara, arabinose; GalUA, galacturonic acid; Gal, galactose; GLC, gas-liquid chromatography; *St.*, *Streptomyces*; EDTA, ethylenediaminetetraacetic acid disodium salt.

MATERIALS AND METHODS

α-Galactosidase (α-D-galactoside galactohydrolase [EC 3.2.1.22]) from ficin (*2*) was a gift from Dr. Yu-Teh Li, Tulane University, Louisiana, U.S.A. Zizyphus arabinan (*3*) was donated by Dr. M. Tomoda of Kyoritsu College of Pharmacy, Tokyo. TEAE-cellulose, DEAE-cellulose, and CM-cellulose were purchased from Serva. L-Arabinitol was prepared by reduction of L-arabinose with sodium borohydride. Methyl 2,3,5-tri-O-methyl-L-arabinofuranoside was prepared by methylation of

methyl L-arabinofuranoside (*4*). Methyl 2-O-methyl-L-arabinoside was prepared by methylation of methyl 3,4-O-isopropylidene-β-L-arabinopyranoside (*5*) followed by hydrolysis. Methyl 3-O-methyl-L-arabinoside and methyl 2,3-di-O-methyl-L-arabinoside were prepared by methylation of Zizyphus arabinan (*3*) followed by methanolysis.

Vegetable medium was used for the cultivation of *St. fradiae*, which was composed of 8.0% soluble starch, 1.0% soybean meal, 2.5% peanut meal, 0.3% sodium chloride, 0.5% calcium carbonate, 0.05% lard oil, 0.025% P-2000 and water.

Assay Method for Anti-carrageenin Abscess Activity (*1*) — Six-week-old, male Sprague-Dawley rats (Clea) weighing from 160 to 180 g were used. They were given subcutaneous injections of carrageenin (2%) mixed with the test samples, then after 24 hr they were killed and the abscesses were removed from the carcasses. Their weights were compared with those of control abscesses.

Gas Liquid Chromatography—A Perkin-Elmer gas chromatograph model 881 equipped with a glass column (6 feet in length) of 0.7% OV-1 on gaschrome Q (100–120 mesh) was employed. For analysis of the polysaccharide components, the column was operated at 150° under 1.5 atm of carrier nitrogen. Samples were methanolyzed with 1 N HCl in methanol at 80°C for 12 hr. The methanolysates were dried *in vacuo*, dissolved in pyridine, and then trimethylsilylated (*6*). For analysis of O-methylated-L-arabinose, the same column was operated at 100°C under 1.5 atm of carrier nitrogen. For analysis of Smith degradation products, the column temperature was programmed from 100 to 150°C at 4°C per minute after keeping it for 6 min at 100°C.

Quantitative Analyses of Components—Neutral sugar was determined by the anthrone reaction (*7*) and by the phenol-sulfuric acid method (*8*). Uronic acid was determined by the carbazole-sulfuric acid method (*9*). Total protein was determined by Lowry's method (*10*).

Determination of Molecular Weight — Molecular weights of polysaccharides were determined on a Hitachi UCA Analytical ultracentrifuge. Samples were dissolved in water.

Paper Chromatography and Paper Electrophoresis—Toyo No. 51A was used for paper chromatography with a solvent system of *n*-butanol : pyridine : water (6 : 4 : 3), developing with silver nitrate reagent (*11*). Electrophoresis was carried out in phosphate buffer, pH 7.6, at 1,000 V per 40 cm for 1 hr. Spots were stained with silver nitrate reagent (*11*).

RESULTS

Preparation of Active Material—St. fradiae 3739 strain was grown in a culture medium for three days. The broth was centrifuged to remove mycelia, and to the supernatant (20 L) was added ammonium sulfate to 80% saturation. The precipitate was collected and dialyzed against water for three days. The dialyzate was passed through a column of DEAE-cellulose previously equilibrated with 0.05 M sodium acetate, pH 7.5, then passed through CM-cellulose previously equilibrated at pH 6.0 with 0.06 M phosphate buffer. The effluent was dialyzed for two days and lyophilized. The yellow powder (19 g) obtained showed 57% anti-carrageenin abscess activity at 0.2 mg per site.

Fraction of Active Material on TEAE-Cellulose—The biologically active material (19 g) described above was dissolved in 5 ml Tris-maleate buffer, pH 9.0, and applied to a column of TEAE-cellulose (5.2×30 cm) previously equilibrated at pH 9.0 with the same buffer. Three fractions were eluted stepwise with the same buffer, 0.1 M sodium chloride, and 2.0 M sodium chloride, with yields of 35, 24, and 13%, respectively. The 0.1 M NaCl fraction showed strong biological activity and was further chromatographed on TEAE-cellulose.

Rechromatography of the 0.1 M NaCl Fraction on TEAE-cellulose—The 0.1 M NaCl fraction was dialyzed for two days and lyophilized. This material was dissolved in 5 mM Tris-maleate buffer, pH 9.0 (50 ml), and applied to a column of TEAE-cellulose (3.4×55 cm) equilibrated with the same buffer. The column was eluted stepwise with the same buffer 0.02 M NaCl, 0.05 M NaCl, 0.08 M NaCl, 0.15 M NaCl,

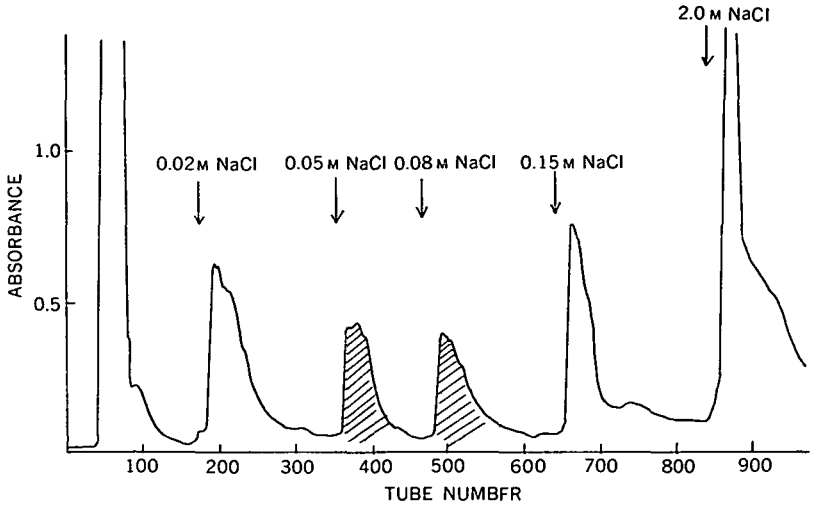

Fig. 1. Chromatography of 0.1 M NaCl fraction on TEAE-cellulose. Fractionation was carried out on a column (3.4×55 cm) at pH 9.0. The column was eluted stepwise with 0.005 M Tris-maleate buffer of pH 9.0, aqueous 0.02 M NaCl, 0.05 M NaCl, 0.08 M NaCl, 0.15 M NaCl, and 2.0 M NaCl. Ten milliliters fractions were collected and the absorbancy at 280 nm was followed. The two fractions indicated by hatching were collected and designated as 0.05 M fraction and 0.08 M fraction, respectively.

and 2.0 M NaCl. The elution pattern is shown in Fig. 1. Each fraction was dialyzed and lyophilized. The biological activity was concentrated in the fractions eluted with 0.05 M and 0.08 M NaCl. These fractions, indicated in Fig. 1 by hatching and designated as the 0.05 M and 0.08 M fractions, respectively, were collected and further purified on TEAE-cellulose by a linear gradient method.

Gradient Chromatography of the 0.05 M Fraction on TEAE-cellulose—The sample (1.65 g) was dissolved in 16 ml of Tris-maleate buffer, pH 9.0, and applied to a column of TEAE-cellulose (2.4×53 cm) equilibrated with the same buffer. After the column had been eluted with 540 ml of the same buffer, linear NaCl gradient elution from 0.02 M to 0.1 M was carried out. The chromatogram is shown in Fig. 2. Fractions No. 180 to 340 were collected and lyophilized to give 1,040 mg of a material which was biologically active (*1*), designated as P-1.

Gradient Chromatography of the 0.08 M Fraction of TEAE-cellulose—The sample (950 mg) was dissolved in 16 ml of 5 mM Tris-maleate buffer, pH 9.0, and applid to a column of TEAE-cellulose (3.5×47 cm). The column was eluted with the same buffer (1,350 ml), followed by an NaCl gradient from 0.05 M to 0.15 M. Fractions No. 150 to 350 (see Fig. 3) were collected and lyophilized to give 750 mg of a material which was biologically active (*1*), designated as P-2.

Gel Filtration of P-1 and P-2 on Biogel P-60—Each sample (20 mg) was applied to a column of Biogel P-60 (1.2×100 cm). The column was eluted with 0.1 M NaCl, and neutral sugar and protein were followed by the phenol-sulfuric acid method and by absorbancy at 280 nm. Both of the samples showed almost homogeneous elution patterns. The chromatogram of P-1 is shown in Fig. 4.

Chemical Compositions and Some Properties of P-1 and P-2—Quantitative analysis results for components and assays for anti-carrageenin abscess activity are summarized in Table I.

Identification of Neutral Sugars of P-1 and P-2—P-1 and P-2 were methanolysed with 1 N HCl in methanol at 80°C overnight. Thin-layer chromatograms of each methanolysate

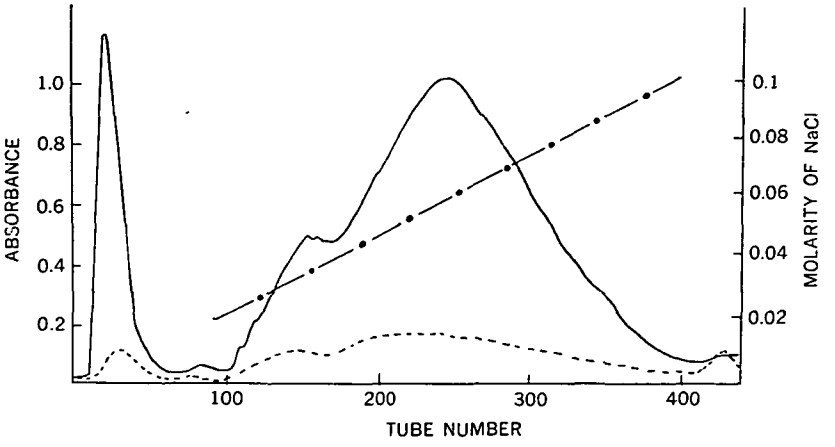

Fig. 2. Gradient chromatography of the 0.05 M fraction on TEAE-cellulose. Fractionation was carried out on a column (2.4×53 cm) at pH 9.0. The column was eluted with 0.005 M Tris-maleate buffer (540 ml), followed by a linear NaCl gradient from 0.02 M to 0.1 M, —•—. Aliquots of the fractions were used for component analyses, ——, anthrone reaction (620 nm); ----, absorbancy at 280 nm. Fractions No. 180 to 340 were collected and designated as P-1.

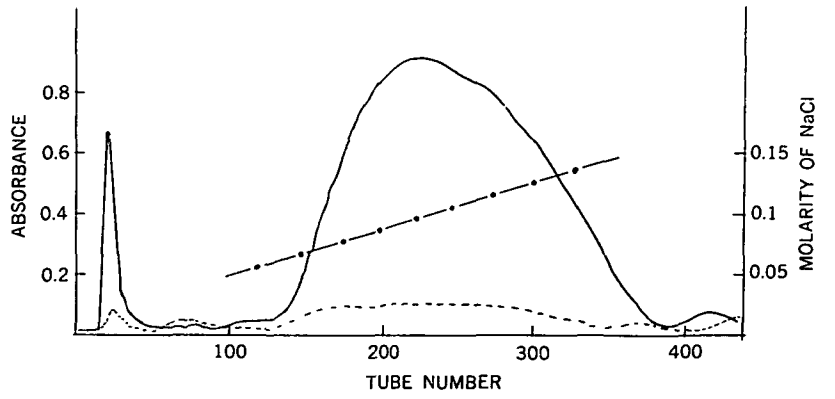

Fig. 3. Gradient chromatography of 0.08 M fraction on TEAE-cellulose. Fractionation was carried out on a column (3.5×47 cm) at pH 9.0. The column was eluted with 0.005 M Tris-maleate buffer (1,350 ml), followed by a linear NaCl gradient from 0.05 M to 0.15 M, —•—. Aliquots of the fractions were used for component analyses, ——, anthrone reaction (620 nm); ----, absorbancy at 280 nm. Fractions No. 150 to 350 were collected and designated as P-2.

TABLE I. Chemical compositions and some properties of P-1 and P-2. The anti-carrageenin abscess activity was assayed using 0.2 mg per site.

	Activity (%)	Protein (%)	Neutral sugar (%)	Amino sugar (%)	Uronic acid (%)	C	H	N	P	$[\alpha]_D^{26}$
						(%)				
P-1	60.0	2.0	92.0	none	5.0	44.52	6.91	trace	0.79	−100±3.0
P-2	65.0	1.0	90.0	none	9.0	44.32	6.38	trace	none	−76.3±2.4

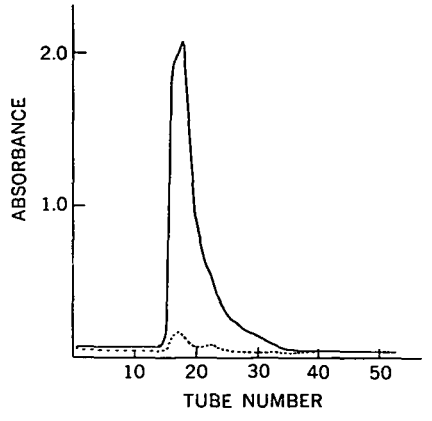

Fig. 4. Gel filtration of P-1 on Biogel P-60. Chromatography was carried out on a Biogel P-60 column (1.2×100 cm). The column was eluted with 0.1 M aqueous sodium chloride at a flow rate of ten milliliters per hr. Aliquots of the fractions were used for component analyses. ——, phenol-sulfuric acid (480 nm); ----, absorbancy at 280 nm.

were identical with that of the methanolysate of authentic L-arabinose. The methanolysates of P-1 and P-2 were applied to a column of Dowex 1×4 (OH^-) and eluted with water according to the method of Matsushima and Miyazaki (*12*). Methyl β-L-arabinopyranoside (m.p. 171°C, $[\alpha]_D^{22.5}=+234\pm13.0$) and methyl α-L-arabinopyranoside (m.p. 131°C, $[\alpha]_D^{23.5}=+12.3\pm5.4$) were isolated in a pure state. This shows arabinose to be in the L-form. The gas-liquid chromatograms of methanolysates of P-1 and P-2 were almost identical with that of the methanolysate of L-arabinose. Neither hexose nor methylpentose were detected.

Identification of Uronic Acid—The sample was hydrolyzed with 1 ml of 1 N HCl at 100°C for 2 hr. The hydrolysate was neutralyzed with 1 N NaOH, concentrated to 1 ml, and applied to a Norit column (1×4 cm). The column was washed with water (30 ml) to remove salt, then eluted with 10% aqueous ethanol (30 ml). The ethanol solution was evaporated *in vacuo* and the residue was subjected to paper chromatography with the solvent system described previously. Two spots were detected. The main spot was arabinose and there was another spot appearing near the starting line. The latter spot was eluted with water and analyzed by paper electrophoresis and GLC after trimethylsilylation. This sample was identified as galacturonic acid.

Methylation Study of P-1 and P-2—P-1 and P-2 (20 mg each) were methylated according to the method of Hakomori (*13*). The reaction mixture was dialyzed against water and the product was extracted with chloroform. This procedure was repeated twice until no hydroxyl group absorption was detected on infrared analysis. The methylated samples (1 mg) were methanolyzed in a sealed tube with 0.5 N HCl in methanol (1 mg) at 80°C overnight. The methanolysates were then passed through a column of CG-4B (OH^-) to remove HCl, and evaporated to dryness *in vacuo*. These samples were dissolved in methanol and applied to a 0.7% OV-1 GLC column. The gas chromatograms of P-1 and P-2 showed almost the same patterns. Methyl glycosides of 2, 3-di-O-methyl-arabinose formed the main peak, and small amounts of 2, 3, 5-tri-O-methyl-arabinofuranose and 2-O-methyl-arabinose were also detected. Accordingly, P-1 and P-2 appear to have almost linear structures.

Smith Degradation of P-1 and P-2—Samples of P-1 and P-2 (5 mg each) were oxidized with 0.05 M sodium metaperiodate (3 ml) at 4°C for 96 hr (*14*). One drop of ethylene glycol was then added to the reaction solution to destroy excess sodium metaperiodate. The formic acid liberated was determined by titration with 0.01 M NaOH (*15*). The solution was dialyzed overnight and lyophilized. The product was reduced with sodium borohydride (30 mg) at 4°C overnight, 1 N HCl was added until the pH reached 5.0, then the solution was passed through columns of Dowex 50×8 (H^+) and CG-4B (OH^-). The eluate was evaporated to dryness *in vacuo*, then further treated with methanol to remove boric acid. The residue was hydrolyzed with 0.2 N HCl at 100°C for 5 hr, then lyophilized. The hydrolyzates were dissolved in pyridine and trimethylsilylated (*6*). The conditions for GLC were as described in "MATERIALS AND METHODS." The chromatograms of the degradation products from P-1 and P-2 showed that glycerol was the main product, accompanied by a trace of arabinose, indicating the absence of brached

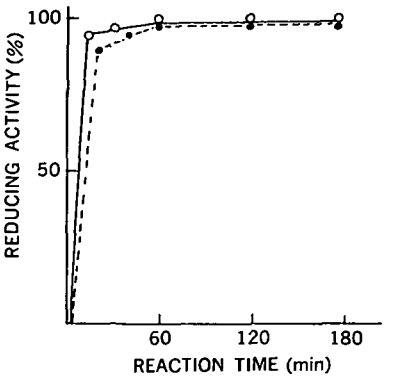

Fig. 5. Increase of reducing power on acid hydrolysis of P-1. The sample was hydrolyzed with 1 N aqueous sulfuric acid at 100°C. Hydrolysis rates were followed by measuring reducing power. ——, P-1; ----, Ziziphus arabinan.

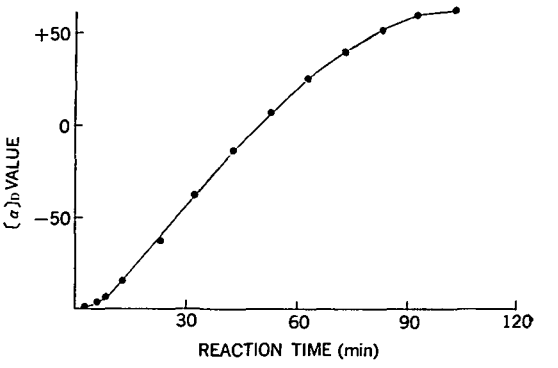

Fig. 6. Optical rotatory changes during acid hydrolysis. The sample (P-1) was diluted to 1.12% with 1 N aqueous sulfuric acid. Optical rotation was measured at 70°C.

structure in the polysaccharides.

Time Course of Acid Hydrolysis—A sample of P-1 (7 mg) was hydrolyzed with 1 N H_2SO_4 (5.5 ml) at 100°C. After various periods of time, 1 ml of the hydrolysate was taken, neutralized with 1 N NaOH, and diluted to 10 ml with water. 0.5 ml of the diluted solution was taken and analyzed for reducing power by the method of Park and Johnson (*16*). The increase in reducing power is shown in Fig. 5. In comparison with the case of Zizyphus arabinan (*3*), this rapid rate of acid hydrolysis strongly suggests that the arabinose units are of furanose type.

Optical Rotatory Changes during Acid Hydrolysis—The sample was diluted to 1.12% with 1 N H_2SO_4 and heated at 70°C. Optical rotation was measured after various periods of time. The value changed from levo- to dextrorotatory as shown in Fig. 6. This shows that the arabinose units are connected by α-glycosidic linkages.

Determination of Molecular Weight—Ultracentrifugal analyses of P-1 and P-2 gave homogeneous peaks at concentrations of 6.54 and 6.38 mg/ml water, with sedimentation constants of 1.52 and 1.29S, respectively. The diffusion constants of P-1 and P-2 were calculated to be 9.90×10^{-7} and 17.7×10^{-7} (*17*). Molecular weights were calculated from the following equation.

$$\text{M.W.} = \frac{\text{sRT}}{\text{D}(1-\bar{v}\rho_0)}$$

The value 0.62 was used as the partial specific volume ($\bar{v}$) for pentose (*18*). The calculated molecular weights were 9.8×10^3 for P-1 and 4.7×10^3 for P-2.

Partial Acid Hydrolysis of P-2—P-2 (100 mg) was hydrolyzed with 0.25 N HCl (6 ml) at 100°C for 1 hr (*19*). The solution was neutralized with 1 N NaOH and applied to a column of Norit (2.5×2 cm). The column was washed with water (20 ml) and eluted with 10% aqueous ethanol (20 ml) and 30% ethanol (20 ml), respectively. The fraction which was eluted with 10% ethanol contained arabinose, and the fraction eluted with 30% ethanol was shown to contain uronic acid by the carbazole sulfuric acid test (*9*). The 30% ethanol fraction was evaporated *in vacuo* to 1 ml, applied to a Biogel P-2 column (1.2×100 cm), and eluted with water. The pattern of the chromatogram is shown in Fig. 7. Fraction A, which was positive in the carbazole-sulfuric acid reaction, was collected and aliquots were subjected to paper chromatography and paper electrophoresis at pH 7.6. Two spots, corresponding to galacturonic acid and a disaccharide, were observed.

Identification of the Disaccharide—Fraction A was collected and the concentrated solution was subjected to preparative paper chromatography as shown in Fig. 8. The band PC-1,

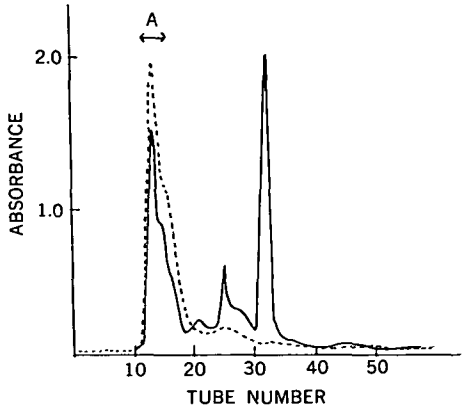

Fig. 7. Fractionation of the 30% ethanol fraction on Biogel P-2. Chromatography was carried out on a Biogel P-2 column (1.2×100 cm). The column was eluted with water at a flow rate of 22 ml per hr. Four-milliliter fractions were collected. Aliquots of the fractions were used for component analyses. ——, phenol-sulfuric acid (480 nm); ----, carbazole-sulfuric acid (535 nm). Fractions No. 12 to 18 were collected and designated as fraction A.

corresponding to a disaccharide, was eluted with water. The eluate was concentrated and an aliquot was chromatographed on paper with the solvent system described previously. The chromatogram showed one spot, with no contamination by galacturonic acid. This sample was subjected to GLC analysis after methanolysis, and methyl glycosides of galacturonic acid and arabinose were found in a ratio of 1.2 : 1.0. The disaccharide (*ca.* 50 μg) was dissolved in a mixture of methanol and water (1 : 1) and its galacturonic acid moiety was esterified with diazomethane in ether (*20*). The solution was evaporated *in vauco*, 0.2 ml of 1 M aqueous sodium borohydride was added and the pH adjusted to 9.0 with aqueous boric acid, then the solution was kept at 4°C with stirring. This procedure was repeated three times at two-hr intervals, then the solution was left to stand at room temperature overnight. The reaction mixture was passed through a column of Dowex 50×8 (H^+), and the eluate was evaporated to dryness. The residue was treated three times with methanol to remove boric acid. The reduced disaccharide was dissolved in 0.5 ml of 0.05 M citrate buffer, pH 4.5, and incubated with fig α-galactosidase

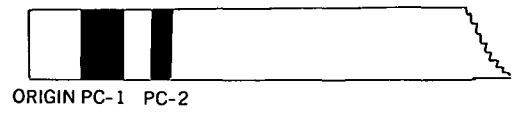

Fig. 8. Preparative paper chromatography of fraction A isolated by gel chromatography (Fig. 7). The paper was impregnated with *n*-butanol : pyridine : water (6 : 4 : 3). Band PC-2 corresponded to D-galacturonic acid, and PC-1 to D-galacturonyl L-arabinose. The bands were developed with silver nitrate reagent.

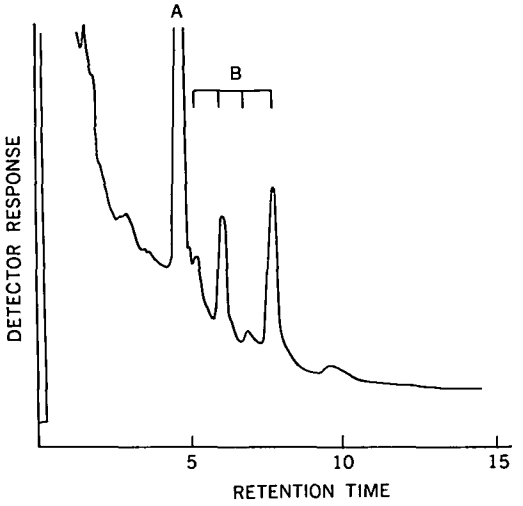

Fig. 9. Analysis of enzymic reaction products by gas liquid chromatography. Enzyme-digested sample was trimethylsilylated and analyzed at 165°C under 1.5 atm pressure of carrier nitrogen. Peak A corresponded to authentic arabinitol and peak B to galactose.

(*2*) at 37°C overnight. The solution was lyophilized and subjected to GLC analysis after trimethylsilylation (*6*). The trimethylsilylated sample was subjected to GLC on a 0.7% OV-1 column (6 feet in length) operated at 165°C. As shown in Fig. 9, arabinitol and galactose were detected. From these data, it is obvious that galacturonic acid was connected to arabinose by an α-glycosidic linkage.

Partial Periodate Oxidation of P-1 and P-2—Samples (6 mg) were dissolved in aqueous 10^{-2} M sodium metaperiodate (2 ml) and kept at 0°C overnight. One drop of ethylene glycol was added, then the solutions were dialyzed and lyophilized. The biological activity was found to be completely lost.

Treatment of P-1 and P-2 with EDTA—Samples (4 mg) were dissolved in 2 ml aqueous 10^{-2} M EDTA and incubated at 37°C for two hr. The solutions were dialyzed and lyophilized. The original biological activities were found to be retained in both P-1 and P-2.

DISCUSSION

Gel chromatography and ultracentrifugal analysis showed that the L-arabinans (P-1 and P-2) from *St. fradiae* were of high homogeneity. From the methylation and Smith degradation studies, it is obvious that P-1 and P-2 have almost linear structures consisting of 1→5 linked L-arabinose units. The rapid rate of hydrolysis and optical transition from levo- to dextro-rotation during acid hydrolysis suggest that the constituent arabinose residues are furanose type and are connected by α-glycosidic linkages. Liberation of formic acid in amounts approximately equivalent to the uronic acid content by periodate oxidation of P-1 and P-2 shows that galacturonic acid is present at a non-reducing end, which may be connected at the C-3 position of an arabinose unit, as indicated by the methylation study. Partial acid hydrolysis and subsequent reduction of P-2 afforded a reduced disaccharide, galactosyl-arabinitol, and liberation of galactose from this reduced disaccharide by fig α-galactosidase (*2*) revealed that galacturonic acid units are connected to arabinose units by α-glycosidic linkages. On the basis of the component analyses and molecular weights, P-1 may be composed of about sixty L-arabinose and two D-galacturonic acid residues, whereas P-2 may contain about thirty L-arabinose and two D-galacturonic acid residues. L-Arabinans from peanut meal consists of 1→5 linked arabinose units in a highly branched chain structure with 1→3 branch points (*21–25*). Thus, P-1 and P-2 are very different from peanut arabinans, having a linear structure. The highest activity against carrageenin abscess was obtained after three days culture, and further cultivation yielded biologically active L-arabinans containing, arabinose as the major component, and rhamnose and galacturonic acid as minor components. Considering that L-arabinan is not thought to be produced by bacteria, it may be assumed that P-1 and P-2 are derived from peanut L-arabinans by enzymatic modifications. Small amounts of protein were found to exist in P-1 and P-2 (Table I), and various kinds of amino acids, lysine, histidine, arginine, aspartic acid, threonine, serine, proline, and glycine, were detected by amino acid analysis of hydrolysates of P-1 and P-2. These proteins could not be removed from P-1 and P-2 by chromatographic methods, so it is possible that these proteins participate in the anti-carrageenin abscess activity. However, complete loss of anti-carrageenin abscess activity on partial periodate oxidation indicates the requirement for an intact polysaccharide structure for biological activity. EDTA treatment of P-1 and P-2 had no effect on their activities. The pH range for stable activity was 5.8–9.1; the arabinans were stable on heating at 50°C, but lost activity on heating at 60°C for 10 min. The minimum effective dose of P-2 was only 25 μg per site.

The mechanism of the inflammatory process is not yet well understood, and it is, therefore, rather difficult to clarify the mechanism by which these simple polysaccharides have such biological activity. One model for anti-inflammatory action involves stabilization of the lysosomal membrane (*26*). These polysaccharides showed no activity for stabilization of murine hepatic lysosomal membrane, but actually caused labilization of the lysosomal membrane.

Recently, many kinds of biologically active polysaccharides have been isolated from various natural sources, notably compounds having anti-tumor activity (*27*) or interferon-inducing activity (*28*). The relationship between structure and biological activity in these compounds is not yet clarified, and poses an interesting problem for future work.

The authors wish to thank Dr. Y.-T. Li, Tulane University, Convigton, La., U.S.A., for the gift of α-galactosidase from ficin and Dr. M. Tomoda, Kyoritsu College of Pharmacy, Tokyo, for the gift of Ziziphus arabinan. The authors are also pleased to acknowledge Dr. K. Hirose and Dr. H. Inoue of this laboratory for the assay of anti-carrageenin abscess activity and for determination of the molecular weights of the polysaccharides.

REFERENCES

1. K.F. Benitz and L.M. Hall, *Arch. Int. Pharmacodyn. Ther.*, **144**, 185 (1963).
2. Y.-T. Li and S.C. Li, *J. Biol. Chem.*, **246**, 3739 (1971).
3. M. Tomoda, M. Takahashi, and S. Nakatzuka, *Chem. Pharm. Bull.* (Tokyo), **21**, 707 (1973).
4. I. Augestad and E. Berner, *Acta Chem. Scand.*, **8**, 251 (1954).
5. J.K.N. Jones, P.W. Kent, and M. Stacey, *J. Chem. Soc.*, 1341 (1947).
6. C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
7. J.H. Roe, *J. Biol. Chem.*, **212**, 335 (1955).
8. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
9. Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).
10. O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
11. W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).
12. Y. Matsushima and T. Miyazaki, *J. Biochem.*, **55**, 464 (1964).
13. S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
14. H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem.*, **63**, 553 (1968).
15. T.G. Halsall, E.L. Hirst, and J.K.N. Jones, *J. Chem. Soc.*, 1427 (1947).
16. J.T. Park and M.J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).
17. H.K. Schachman, "Methods in Enzymology," ed. by S.P. Colowick and N.O. Kaplan, Academic Press Inc., New York, Vol. IV, p. 32 (1957).
18. R.V. Webber, *J. Am. Chem. Soc.*, **78**, 536 (1956).
19. S.M. Rosen, M.J. Osborn, and B.L. Horecker, *J. Biol. Chem.*, **239**, 3196 (1964).
20. S. Hase, Y. Tsuji, and Y. Matsushima, *J. Biochem.*, **72**, 1549 (1972).
21. E.L. Hirst, J.K.N. Jones, and E. Williams, *J. Chem. Soc.*, 1062 (1947).
22. E.L. Hirst and J.K.N. Jones, *J. Chem. Soc.*, 1321 (1947).
23. E.L. Hirst and J.K.N. Jones, *J. Chem. Soc.*, 2311 (1948).
24. L. Hough and D.B. Powell, *J. Chem. Soc.*, 16 (1960).
25. G.O. Aspinall, "The Carbohydrates, Chemistry, and Biochemistry," ed. by W. Pigman and D. Horton, Academic Press, New York and London, Vol. IIB, p. 517 (1970).
26. G. Weissmann and L. Thomas, *Rec. Progr. Hormone Res.*, **20**, 215 (1964).
27. Y. Yoshika, T. Sano, and T. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 1772 (1973).
28. S. Suzuki, M. Suzuki, M. Imaya, and F. Chaki, *Japan. J. Microbiol.*, **16**, 1 (1972).