Structure Determination of Polysaccharides in *Aloe saponaria* (Hill.) Haw. (Liliaceae)

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Abstract □ Neutral polysaccharides that inhibit carrageenin-induced edema in rats were isolated from the nondialysate of the pulp of *Aloe saponaria* by gel filtration. These were shown to be a linear polymer of a 1,4-linked β-D-mannopyranose (mol. wt. 15,000) containing 18% acetyl groups (As mannan 1), and a 1,4-linked α-D-mannopyranose polymer containing a single branch on the principal chain consisting of D-glucose residues linked at C-2 and C-4 (mol. wt. 66,000), with 10% acetyl groups (As mannan 2). As mannan 1 inhibited carrageenin-induced hind paw edema at 50 mg/kg ip in rats; As mannan 2 was not tested for pharmacological activity. A crude preparation of both As mannans was effective when given intraperitoneally, but was ineffective when given orally.

Keyphrases □ *Aloe saponaria*—isolation of neutral polysaccharides, As mannan 1, As mannan 2, structural determinations □ As mannans—isolation from *Aloe saponaria*, structural determinations, inhibition of carrageenin-induced edema in rats □ Edema, carrageenin-induced—inhibition in rats by neutral polysaccharides isolated from *Aloe saponica*, As mannan 1, As mannan 2

Pharmacological evidence for an anti-inflammatory effect was provided by the isolation from a nondialysate fraction of *Aloe saponaria*[1] of a glycoprotein with kininase activity (1). Further studies on the nondialysate led to the isolation of neutral polysaccharides that inhibit carrageenin-induced edema in rats. This paper describes the structural studies of the polysaccharides, As mannans 1 and 2, and the pharmacological evaluation of As mannan 1.

[1] This plant is also called spotted aloe or soap aloe.

EXPERIMENTAL[2]

Materials—The following materials were obtained commercially: agarose gel[3], diethylaminoethyl cellulose[4], dialysis membrane[5], bromelain[6], protease[7], anti-inflammatory drug[8], and a series of dextrans[9].

Methods of Analysis—The carbohydrate content of the sample was determined colorimetrically by the phenol–sulfuric acid method (2). Elution of material through gel[3] and diethylaminoethyl cellulose[4] filtration was monitored by absorbance of the effluent at 490 nm. Acid hydrolysis was carried out with 1M H_2SO_4 at 90°C for 2 h. Excess sulfuric acid was removed by precipitation as barium sulfate. The filtrate was passed through an ion-exchange column[10] to remove the remaining salts, and then the filtrate was evaporated to dryness. Partial hydrolysis was performed with 0.5M H_2SO_4 at 70°C for 4 h. Analysis of the carbohydrates by methylation was performed according to the method of Hakomori (3). This procedure was performed twice, to give permethylate showing no hydroxyl absorption in the IR spectrum. Paper partition chromatography[11] of the sugar moiety was performed using butanol–pyridine–water (6:4:3) and aniline hydrogen phthalate detection. Ultracentrifugation[12] was performed on an analytical ultracentrifuge with

[2] The IR spectra were obtained with a KOKEN DS-301 spectrometer. The ^1H- and ^{13}C-NMR spectra were recorded with a JEOL PS-100 (100 MHz) and a JEOL FX-100 (25 MHz) spectrometer, respectively. Chemical shifts were recorded in δ units relative to the internal standards 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt or tetramethylsilane. Optical rotations were obtained on a JASCO DIP-4.

[3] Sepharose 6B; Pharmacia Fine Chemicals, Uppsala, Sweden.

[4] DE 32; Whatman Ltd., England.

[5] Visking tube; Union Carbide, Co.

[6] Nakarai Chemical Co., Ltd., Kyoto, Japan.

[7] Type III from papaya; Sigma Chemical Co.

[8] Indomethacin.

[9] Pharmacia Fine Chemicals, Uppsala, Sweden.

[10] Amberlite MR-3; Rohm and Hass Co., Ltd.

[11] Toyo Filter Paper No 50; Toyo Roshi, Co., Tokyo.

[12] Spinco Model E; Beckman Instrument Inc.

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a schlieren optical system in a single sector at 20°C and 56,000 rpm.

Gas chromatography (GC)[13] of the trimethylsilylated sugar from the hydrolysate was performed on a column of silicone SE 52 (3 mm × 2 m) on Chromosorb W AWDMCS (60–80 mesh) at 160°C with a nitrogen flow rate of 45 mL/min. Quantitative analysis of the acetyl content was determined by GC using a column of styrene divinylbenzene polymer (3 mm × 2 m)[14] at 158°C with a nitrogen flow rate of 55 mL/min, using propionic acid as an internal standard (4). GC analysis of the methanolysate was performed on a column of 1% neopentyl glycol succinate polyester (3 mm × 2 m) on Chromosorb W AWDMCS (60–80 mesh) at 147°C, with a nitrogen flow rate of 40 mL/min, and on a column of 5% 1,4-butanediol succinate (3 mm × 2 m) on Chromosorb W[15] (60–80 mesh) at 180°C, with a nitrogen flow rate of 60 mL/min.

The molecular weight estimation was determined on an agarose gel column (1.5 × 51.5 cm) using 0.1 M NaCl at a flow rate of 26 mL/h. A series of dextrans (10, 20, 40, 70, and T-500[9]) were used as a standard. The molecular weight was estimated from a linear correlation between the logarithm of the molecular weight of the polysaccharides and the ratio of their elution volume to the void volume from the column. The viscosities of As mannans 1 and 2 in an aqueous solution were determined by a capillary viscometer[16] at 26°C. The intrinsic viscosity (η) was obtained by the extrapolation to zero concentration of the straight line obtained by plotting η_{sp}/C *versus* concentration of each sample, where C is the concentration. η_{sp} is calculated by $\eta_{sp} = (t_1\rho_1/t_0\rho_0) - 1$, where t_1 is the time of efflux of the solution, ρ_1 is its density, t_0 is the time of efflux of the solvent, and ρ_0 is its density.

Assay Method for Carrageenin-Induced Edema—Male Wistar albino rats weighing 120–150 g were used. Edema was induced by the injection of 0.1 mL of 1% (w/v) carrageenin dissolved in saline beneath the plantar aponeuroses of the hind paws. One to twelve hours before the injection of carrageenin, drugs were administered intraperitoneally or per os. The swelling (in percent) of the rat hind paws was measured by a volume differential meter[17].

Preparation of Crude Material—The fresh leaves of *A. saponaria*[18] were harvested in September 1980 from the greenhouse of the herbal garden of Kyushu University. Fresh leaves were cut in half in a parallel manner, and colorless gelatinous pulp was separated carefully by scraping from the green cortical layer containing yellow phenolics. The homogenized pulp (3 kg) was centrifuged at 10,000 rpm for 30 min. The supernatant was membrane[5] dialyzed against distilled water for 48 h, and nondialysate was lyophilized to afford a colorless soft material (6 g).

Isolation and Purification of As Mannans 1 and 2—Crude material (yield, 0.012% from the fresh leaves) isolated from the pulp harvested in September 1980 was dissolved in 0.3 M NaCl and was subjected to column fractionation (4.8 × 20.5 cm, agarose gel) using the same solution as a solvent at a flow rate of 28 mL/h. The fraction (elution volume, 110–250 mL) containing carbohydrate was pooled, dialyzed, and lyophilized to give a colorless soft material (yield, 50.0%). This material (0.1 g) dissolved in 0.02 M NH_4HCO_3 (5 mL) was subjected to column fractionation (1.5 × 44 cm, DEAE-cellulose[4]) using the same solution as a solvent at a flow rate of 25 mL/h, and the fraction (elution volume, 40–75 mL) was pooled, dialyzed, and lyophilized to give As mannan 1 (yield, 35% from the crude material). In a similar manner, the crude material (yield, 0.05% from the fresh leaves) isolated from the pulp harvested in December 1980 was treated to give As mannan 2 (yield, 16% from the crude material).

Chemical and Physical Properties of As Mannans 1 and 2—Since As mannan 1 in 1% solution and As mannan 2 in 0.5% solution in 0.1 M NaCl gave single peaks having $S_{20,w} = 1.25$ *S* and 1.17 *S*, respectively, each appears to be homogeneous.

As Mannan 1—$[\alpha]_D^{20}$ −40.5° (c = 0.7, H_2O); ^{1}H-NMR [3-(trimethylsilyl)-1-propanesulfonic acid sodium salt, D_2O]: δ 2.13 (m, CH_3COO), 4.76 (br s, anomeric proton of the nonreducing carbon atom), 4.17, and 5.50 ppm (CH_3COOCH—); ^{13}C-NMR [3-(trimethylsilyl)-1-propanesulfonic acid sodium salt, 0.1 M NaCl]: δ 176.4 (CH_3COO) and 22.9 ppm (CH_3COO), with other signals listed in Table I. IR (KBr) ν_{max}: 1730 and 1250 cm^{-1}. This compound is very slightly soluble in water.

Hydrolysis of As Mannan 1—A sample of As mannan 1 (10 mg) was hydrolyzed to give D-mannose as the only sugar moiety by paper and gas (as a trimethylsilyl ether) chromatographic analyses. R_f values: glucose 0.31; galactose 0.25; mannose 0.34; and hydrolysate 0.34. GC retention times (in minutes): mannose 10.1 and 15.1; glucose 14.4 and 22.1; galactose 10.7, 12.6, and 15.7; the hydrolysate 10.1 and 15.1.

As Mannan 2—$[\alpha]_D^{20}$ +70.0° (c = 0.8, H_2O); ^{1}H-NMR [3-(trimethylsilyl)-1-propanesulfonic acid sodium salt, D_2O]: δ 2.13 (m, CH_3COO) and 5.00 ppm (br s, anomeric proton of the nonreducing carbon atom). ^{13}C-NMR [3-(trimethylsilyl)-1-propanesulfonic acid sodium salt, 0.1 M NaCl): δ 176.1 (CH_3COO) and 22.8 ppm (CH_3COO), with other signals listed in Table I. IR (KBr) ν_{max}: 1730 and 1245 cm^{-1}. This compound is soluble in water.

Hydrolysis of As Mannan 2—A sample of As mannan 2 (15 mg) was hydrolyzed to give D-mannose as the major sugar component and a minor amount of D-glucose by paper and gas (as a trimethylsilyl ether) chromatographic analyses. R_f values: mannose 0.37; glucose 0.31; galactose 0.26; the hydrolysate 0.31 and 0.36. GC retention times (in minutes): mannose 10.1 and 15.1; glucose 14.4 and 22.1; galactose 10.7, 12.6, and 15.7; the hydrolysate 10.1, 14.8, and 22.4. Mannose and glucose were detected using GC in a ratio of 95:5.

Analysis of As Mannan 1 by Methylation—A sample of As mannan 1 (20 mg) was methylated according to Hakomori's method (3). The product was chromatographed on a silica gel column using chloroform–methanol (20:1) to give material which showed no hydroxyl absorption in the IR spectrum; proton signals due to a methoxyl group (δ 3.3–3.8 ppm) and anomeric proton signals (δ 4.61 ppm) were observed in the ^{1}H-NMR spectrum. The permethylate (5 mg) was subjected to methanolysis with 4% hydrogen chloride in methanol (1 mL) in a sealed tube at 90°C for 2 h. After the removal of the solvent, the methanolysate (dissolved in ethyl acetate) was chromatographed on a silica gel column using ethyl acetate and ethyl acetate–methanol as solvents to give material which showed one spot on TLC [ethyl acetate–methanol (25:1)]. The methanolysate was subjected to GC analysis on columns of 1% neopentyl glycol succinate (*a*) and 5% 1,4-butanediol succinate (*b*). The following retention times were obtained: (*a*) methyl mannopyranosides of 2,3,4,6-tetramethyl-*O*-, 3,4,6-trimethyl-*O*-, 2,4,6-trimethyl-*O*-, 2,3,6-trimethyl-*O*-, and 2,3,4-trimethyl-*O*- derivatives: 1.8, 2.8, 3.3, 4.3, and 2.7 min and the methanolysate: 1.8 and 4.3 min. (*b*) methyl mannopyranosides of 2,3,4,6-tetramethyl-*O*-, 3,4,6-trimethyl-*O*-, 2,4,6-trimethyl-*O*-, 2,3,6-trimethyl-*O*-, and 2,3,4-trimethyl-*O*- derivatives: 4.1, 8.6, 10.1, 12.3, and 8.5 min and the methanolysate: 4.1 and 12.3 min.

Analysis of As Mannan 2 by Methylation—A sample of As mannan 2 (8 mg) was methylated according to Hakomori's method (3). In the same manner as for As mannan 1, the product was treated to give the methanolysate which showed a single spot on TLC [ethyl acetate–methanol (25:1)]. The methanolysate was subjected to GC analysis to give the following retention times on columns of 1% neopentyl glycol succinate (*a*) and 5% 1,4-butanediol succinate (*b*): (*a*) methyl mannopyranosides of 3,4,6-trimethyl-*O*-, 2,4,6-trimethyl-*O*-, 2,3,6-trimethyl-*O*-, and 2,3,4-trimethyl-*O*- derivatives: 3.4, 4.3, 5.6, and 3.4 min, methyl glucopyranosides of 3,4,6-trimethyl-*O*-, 2,4,6-trimethyl-*O*-, 2,3,6-trimethyl-*O*-, and 2,3,4-trimethyl-*O*- derivatives: 3.4, 5.4, 5.2, and 4.1 min, and the methanolysate 5.6 min. (*b*) methyl mannopyranosides of 2,3,4,6-tetramethyl-*O*-, 3,4,6-trimethyl-*O*-, 2,4,6-trimethyl-*O*-, 2,3,6-trimethyl-*O*-, 2,3,4-trimethyl-*O*-, 4,6-dimethyl-*O*-, 3,6-dimethyl-*O*-, and 3,4-dimethyl-*O*- derivatives: 4.9, 10.3, 12.0, 15.3, 12.0, 22.4, 20.5, and 20.2 min, methyl glycopyranosides of 4,6-dimethyl-*O*-, 3,6-dimethyl-*O*-, and 3,4-dimethyl-*O*-derivatives: 28.5, 29.3, and 25.7 min, and the methanolysate: 15.3 and 29.1 min.

Determination of the Molecular Weights of As Mannans 1 and 2—The molecular weights were estimated on a column of agarose gel using a series of dextrans as a standard. The molecular weights of As mannans 1 and 2 were determined to be 15,000 and 66,000, respectively.

Determination of the Viscosities of As Mannans 1 and 2—The viscosities were obtained with a capillary viscometer[16]. The intrinsic viscosities of As mannans 1 and 2 was determined to be 15.6 and 2.5 dL/g, respectively.

Alkaline Hydrolysis of As Mannans 1 and 2—Each sample of As mannan 1 (5 mg) or 2 (5 mg) was hydrolyzed with 4% NaOH for 30 min, and the mixture was dialyzed against distilled water followed by lyophilization to give material which showed no absorption in the IR spectrum due to acetyl groups. Deacetyl As mannan 1: ^{1}H-NMR [3-(trimethylsilyl)-1-propanesulfonic acid sodium salt, 1 M NaOD]: δ 4.55 ppm (br s); deacetyl As mannan 2: ^{1}H-NMR [3-(trimethylsilyl)-1-propanesulfonic acid sodium salt, 1 M NaOD]: δ 4.81 ppm (br s).

Partial Hydrolysis of As Mannan 1—On acid hydrolysis with 0.5M H_2SO_4 at 70°C for 4 h, As mannan 1 (38 mg) yielded the oligosaccharides. The products were fractionated on a cellulose column using acetone–water. On elution with acetone–water (10:1), (6:1), and (3:1), mannose

[13] Gas chromatograph GC-4BM; Shimadzu Seisakusho Ltd., Japan.
[14] Chromosorb 102; Johns-Manville.
[15] Shimalite W; Shimadzu Seisakusho Ltd., Japan.
[16] Ubbelohde instrument.
[17] Ugo Basile Co., Ltd., Italy.
[18] A voucher specimen is available for inspection at the Herbal Garden of Kyushu University, Fukuoka, Japan.

Table I—^{13}C-NMR Spectral Assignments

	As Mannan 1		As Mannan 2		Ivory Nut Mannan[c]	Native D_1[f]
	1,4-β-Man[a]	Deacetyl[b]	1,4-α-Man[c]	Deacetyl[d]		
C-1	102.7[g]	103.0	100.1	101.3	101.7	102.1; 101.9
C-2	72.6	73.5	72.1	73.0	72.7	70.9; 71.1
C-3	74.1	74.7	72.7	73.4	73.5	70.2
C-4	79.1	78.6	76.1	77.5	78.8	74.8; 74.5
C-5	77.6	78.6	74.0	74.9	78.8	71.8; 69.3
C-6	63.3	63.4	63.2	63.6	62.1	64.1; 61.3
			67.3[h]	68.6[h]		

[a] Concentration: 30 mg/mL of 0.1 M NaCl at 28°C. [b] Concentration: 30 mg/mL of 0.1 M NaOD at 28°C. [c] Concentration: 50 mg/mL of 0.1 M NaCl at 28°C. [d] Concentration: 10 mg/mL of 0.1 M NaOD at 28°C. [e] From Ref. 5. [f] From Ref. 7; see text for chemical name. [g] Chemical shift (δ) was recorded at 25.5 MHz using tetramethylsilane as the internal standard. [h] Not assigned.

(4.3 mg), mannobiose (4.5 mg), and mannotriose (2.5 mg) were obtained. Mannobiose: $[\alpha]_D^{20}$ −6.4° (c = 0.66, H_2O); ^1H-NMR(tetramethylsilane, D_2O): δ 3.4–4.2 (m, 8, CHOH), 4.70 (s, 1, C_1 α-H), 4.88 (d, J = 0.7 Hz, C_1 α-H), and 5.15 ppm (d, J = 1.0 Hz, C_1 β-H); ^{13}C-NMR(tetramethylsilane, D_2O): δ 63.7, 63.1 (C_6, $C_{6'}$), 69.3 ($C_{4'}$), 71.6, 72.8, 73.2, 73.6, 77.4, 79.0 (C_2, C_3, C_5, $C_{2'}$, $C_{3'}$, $C_{5'}$), 79.5 (C_4), 96.4 (C_1), and 102.8 ppm ($C_{1'}$). The C—H coupling constants of the nonreducing anomeric carbon ($J_{C(1)-H}$) and of the reducing anomeric carbon ($J_{C(1)-H}$) were 163 and 172 Hz, respectively (nuclear Overhauser effect).

RESULTS AND DISCUSSION

To characterize the active component(s) in the crude material, 25 mg/kg ip of the crude material, treated by the following procedures, was administered to rats prior to the induction of edema by carrageenin. On heating at 100°C for 10 min or 1 h, crude material (150 mg) dissolved in distilled water (50 mL) did not show any change in the inhibitory activity, and on treatment of crude material (100 mg) with artificial gastric juice (40 mL, pH 1.2) at 37°C for 13 h, the same result was obtained. By allowing crude material (100 mg) to stand in 1% NH_4OH (20 mL) at room temperature for 13 h, followed by dialysis and lyophilization, the inhibitory activity of the material recovered (11 mg) appeared to be markedly elevated. On enzymatic hydrolysis of the crude material (160 mg) with protease[7] (5 mg) in 10 mM phosphate buffer (40 mL, pH 6.2) at 37°C for 57 h, followed by the precipitation of protease with trichloroacetic acid, filtration, dialysis, and lyophilization, the inhibitory activity of the material recovered (51 mg) appeared to be enhanced. From the above experiments it is concluded that the active substance(s) in the crude material is not an enzyme or protein. Furthermore, these results suggest that the inhibitory activity in the crude material may be due to a neutral polymer stable to heat, acid, and alkali. Neutral polysaccharides, As mannans 1 and 2, were isolated from the leaves harvested in September and December 1980, respectively, by gel filtration on agarose and DEAE–cellulose columns.

As Mannan 1—On acid hydrolysis D-mannose was the only sugar moiety detected by paper and gas chromatographic analyses. However, the IR spectrum gave a peak indicative of an *O*-acetyl substituent, and the ^1H- and ^{13}C-NMR spectral signals supported the presence of *O*-acetyl groups. The acetyl content was determined to be 18% by GC, and a molar ratio (1.2:1) of D-mannose to *O*-acetyl groups was confirmed by ^1H-NMR spectroscopy. On analysis by methylation the permethylate provided only 1,4-linked D-mannose residues on the GC spectrum. This indicated a linear structure made up of 1,4-linked D-mannopyranose. Table I indicated the downfield shifted signal due to 1,4-linkages at 102.7 and 79.1 ppm assigned to C-1 and C-4, respectively. On comparison of the ^{13}C-NMR spectral data with those of ivory nut mannan [1,4-linked β-D-mannan (5)], the assignment of the carbon resonances of As mannan 1 was reasonably accomplished. Accordingly, a linear chain structure having a repeating unit of 1,4-linked D-mannopyranose was proposed as As mannan 1. This conclusion was further confirmed by the partial hydrolysis. On treatment with 0.5M H_2SO_4 for 4 h at 70°C, As mannan 1 provided a mannobiose whose configuration was determined to be 4-*O*-β-D-mannopyranosyl-D-mannose based on the optical rotation value and the $J_{C(1)-H}$ coupling constants of the nonreducing anomeric carbon (163 Hz) and the reducing anomeric carbon (172 Hz) (6). As mannan 1 gave a $[\alpha]_D$ −40.5°, suggesting that all mannopyranose residues were β,D-linked. The direct bonded C—H coupling constant of the anomeric carbon ($J_{C(1)-H}$) may be useful for determining the configuration of the anomeric carbons in mannose-containing polysaccharides. However, it is evident that the wide diversity of ^{13}C-NMR spectra from mannose-containing polysaccharides is caused by differences in chain structure of the polysaccharides, and each peak width in the coupling between C-1 and H in the anomeric carbons in As mannan 1 are too broad. ^1H-NMR spectra have been used extensively for the characterization of anomeric protons of nonreducing carbon atoms in polysaccharides. It has been found that chemical shifts due to anomeric protons with an equatorial configuration appear further downfield (δ 5.02–5.92 ppm) than those with an axial configuration (δ 4.55–4.93 ppm) although deviation was observed depending on substituents, the aglycone, or the measurement condition. The anomeric proton signal at δ 4.76 was assigned as an axial proton, and the configuration of As mannan 1 was determined to be β-linked. Further evidence about the configuration of the glycosidation bonds was obtained from ^1H-NMR spectral data of the permethylate, indicating that the anomeric proton of the nonreducing carbon atom appeared at δ 4.61 ppm, and from the $J_{C(1)-H}$ coupling constant (163 Hz) of the nonreducing carbon atom in the mannobiose, indicating a repeating unit. The location of the *O*-acetyl substituents was suggested to be at C-6, by the ^{13}C-NMR spectral data, when comparing the chemical shift of C-6 with those of deacetyl As mannan 1 or ivory nut mannan. The signals at δ 4.17 ppm are assigned to the C-6 proton attached to the acetyl group, using comparison with signals of deacetyl As mannan 1 or D-mannopyranose pentaacetate on the ^1H-NMR spectra.

As Mannan 2—On acid hydrolysis, As mannan 2 gave D-mannose (major) and D-glucose (minor) components by paper and gas chromatographic analyses. The IR spectrum showed absorption indicative of an *O*-acetyl substituent, and the ^1H- and ^{13}C-NMR spectral signals supported the presence of *O*-acetyl groups. The acetyl content was determined to be 10% by GC, and a molar ratio of (2.4:1) of D-mannose to acetyl groups was confirmed by ^1H-NMR spectroscopy. On analysis by methylation the permethylate provided a 1,4-linked D-mannose residue (major) and a 1,4 and 1,2-linked glucose residue (minor) on GC. Further evidence for a 1,4-linkage of D-mannopyranose was shown by ^{13}C-NMR. Table I shows that the downfield shifted signal due to the 1,4-linkage was found at δ 100.1 and 76.1 ppm, assignable to C-1 and C-4, respectively.

Table II—Effect of Intraperitoneally Administered Crude Material, As mannan 1, and Indomethacin on Carrageenin-Induced Edema

Drug (Dose)	Postadministration Swelling, %[a]				
	1 h	2 h	3 h	4 h	5 h
Crude material (50 mg/kg)	16.2 ± 4.6	23.0 ± 5.3	17.6 ± 5.3	30.0 ± 13.1	43.4 ± 8.2
Indomethacin (2.5 mg/kg)	18.3 ± 6.8	20.8 ± 1.0	32.6 ± 5.5	37.7 ± 2.2	53.4 ± 6.6
As mannan 1 (50 mg/kg)	22.3 ± 6.7	19.1 ± 6.7	20.3 ± 9.4	37.1 ± 6.8	48.9 ± 12.8
Control (Saline)	24.8 ± 7.0	43.5 ± 10.1	63.1 ± 14.0	62.9 ± 16.6	72.4 ± 11.6

[a] The values are means ± *SD* from five rats.

Table III—Effect of Orally Administered Crude Material and Indomethacin on Carrageenin-Induced Edema

Drug (Dose)	Postadministration Swelling, %[a]				
	1 h	2 h	3 h	4 h	5 h
Crude material (400 mg/kg)	10.3 ± 3.8	32.3 ± 12.4	51.9 ± 19.1	53.7 ± 18.1	42.6 ± 19.3
Indomethacin (20 mg/kg)	1.7 ± 2.4[b]	5.2 ± 5.0[b]	9.5 ± 12.8[c]	17.3 ± 16.3	7.9 ± 9.3[c]
Control (saline)	14.1 ± 10.0	34.1 ± 7.1	41.4 ± 9.8	46.9 ± 4.8	33.4 ± 9.5

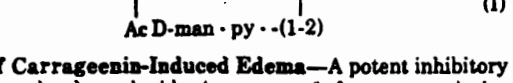
[a] The values are means ± *SD* from five rats; the drugs were administered per os. [b] Statistically significantly different from the control at $p < 0.05$. [c] Statistically significantly different from the control at $p < 0.01$.

Table IV—Effect of Crude Material and Bromelain Administered Orally 2, 4, 6, and 12 h Before Induction of Edema by Carrageenin Injection

Drug (Dose)	Time of Administration	Postinjection Swelling, %[a]				
		1 h	2 h	3 h	4 h	5 h
Crude material (100 mg/kg)	2 h	11.5 ± 1.9	39.3 ± 12.0	40.5 ± 15.1	55.7 ± 16.5	51.5 ± 8.0
Bromelain (100 mg/kg)		13.3 ± 1.3	29.9 ± 10.7	33.2 ± 14.1	46.5 ± 15.5	41.8 ± 13.5
Control (saline)		18.8 ± 8.3	40.4 ± 10.7	37.6 ± 6.7	47.0 ± 3.9	49.7 ± 5.6
Crude material (100 mg/kg)	4 h	14.1 ± 5.4	56.8 ± 6.8	61.7 ± 7.0	62.6 ± 6.7	60.4 ± 5.4
Bromelain (100 mg/kg)		13.1 ± 6.1	45.8 ± 14.7	51.9 ± 11.4	57.3 ± 7.1	56.9 ± 6.7
Control (saline)		12.8 ± 6.1	54.6 ± 13.4	58.6 ± 12.7	60.6 ± 11.7	61.7 ± 8.1
Crude material (100 mg/kg)	6 h	25.5 ± 3.7	42.4 ± 8.8	43.6 ± 9.6	58.4 ± 8.9	59.1 ± 7.3
Bromelain (100 mg/kg)		17.9 ± 9.9	34.1 ± 15.7	37.4 ± 14.5	63.9 ± 4.6	61.5 ± 5.3
Control (saline)		25.8 ± 9.0	48.5 ± 11.7	49.8 ± 12.3	64.7 ± 12.5	64.6 ± 8.9
Crude material (100 mg/kg)	12 h	17.9 ± 9.1	23.4 ± 7.3	35.8 ± 15.6	48.4 ± 17.1	49.9 ± 17.3
Bromelain (100 mg/kg)		22.7 ± 6.4	29.7 ± 9.1	38.6 ± 12.1	47.2 ± 9.6	41.0 ± 7.5
Control (saline)		16.9 ± 5.5	28.0 ± 5.6	36.3 ± 7.8	48.1 ± 6.7	48.2 ± 5.9

[a] The values are means ± *SD* from five rats.

On comparison of the ^{13}C-NMR spectral data with those of 1,4- and 1,2-linked α-D-mannan having an acetyl group at C-6 [native D_1 (7)], carbon resonances of As mannan 2 were assigned. As mannan 2 gave a $[\alpha]_D$ + 70°, suggesting mannopyranose residues were α,D-linked. On comparison of the ^1H-NMR spectra with those of As mannan 1, the anomeric proton signal of the nonreducing carbon atom at δ 5.00 ppm was assigned to be an equatorial proton, and the configuration of As mannan 2 was determined to be α-linked. The location of the acetyl group was ambiguous, because of a low content of acetyl groups in As mannan 2. Thus, a branched structure (I) was proposed for As mannan 2.



(I)

Inhibition of Carrageenin-Induced Edema—A potent inhibitory effect of the neutral polysaccharide, As mannan 1, for carrageenin-induced edema by the intraperitoneal route was found (Table II). With oral administration, however, both crude material and bromelain failed to induce any response in test animals at the dose used, while indomethacin gave apparent inhibition (Table III). No inhibitory activity of crude material or bromelain was found, even at a dose of 100 mg/kg, when given per os 2, 4, 6, or 12 h before induction of edema (Table IV). This may be due to poor absorption of As mannan when given orally.

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