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STRUCTURAL STUDIES OF POLYSACCHARIDES FROM *Aloe vera*

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ABSTRACT

The mucilaginous jelly from the leaves of *Aloe vera* is composed of at least four different partially acetylated glucomannans that differ in their glucose-to-mannose ratios and acetyl contents. Methylation and periodate-oxidation studies indicate that the glucomannans are linear polymers containing (1→4)-glycosidic linkages.

INTRODUCTION

Recently, it has been reported[1] that the mucilaginous jelly from *Aloe plicatilis* Miller is a linear (1→4)-linked glucomannan having a glucose-to-mannose ratio of 1:2.8. The mucilaginous jelly present in the leaves of *Aloe vera* is reported[2] to be a polysaccharide composed of equal amounts of D-glucose and D-mannose together with 2.4% of uronic acid. However, the structure of the polysaccharide has not been investigated. In view of the medicinal importance[3,4] of the mucilaginous jelly of *Aloe vera*, a detailed study of its chemical nature has been undertaken.

RESULTS AND DISCUSSION

Extraction of the mucilaginous jelly from the leaves of locally available *Aloe vera* with 50% aqueous ethanol, followed by precipitation with ethanol, gave a polysaccharide mixture which, after dissolution in 0.02M HCl, dialysis, and precipitation with ethanol, was obtained as a coarse, white, fibrous powder in 0.7% yield based on the weight of the wet jelly. The material was composed of glucose and mannose in the molar ratio 1:6, together with traces of galacturonic acid, arabinose, xylose, and galactose.

Fractionation of the polysaccharide mixture by graded precipitation with ethanol, from aqueous solution, gave three polysaccharide fractions: A_1, A_2, and B. Further fractionation of A_1 with 0.05M sodium tetraborate gave polysaccharides A_{1a} and A_{1b}. The polysaccharides A_{1b}, A_2, and B were further purified by repeated precipitation from aqueous solution with ethanol, until there was no change in the glucose-to-mannose ratio. Polysaccharide A_{1a} could not be purified, as it was insoluble in water. The yield, specific rotation, *O*-acetyl content, and sugar composition of these polysaccharides are given in Table I.

TABLE I

COMPOSITION OF THE *Aloe vera* POLYSACCHARIDES

Polysaccharide fraction	*Physical appearance*	*Molecular weight*[a]	$[\alpha]_D$[b] *(degrees)*	*Phosphate*[c] *(%)*	*O-Acetyl*[d] *(%)*	*Glc/Man ratio*[e]
A_{1a}	Grey powder	—	—	—	1.1	1.5:1 (traces of GalA, Xyl, Ara, and Gal are also present)
A_{1b}	White powder	$>2 \times 10^5$	−21.5	—	9.25	1:4.5
A_2	White powder	$>2 \times 10^5$	−32	0.15	10.3	1:13.5
B	Coarse, white, fibrous powder	$>2 \times 10^5$	−40	0.17	17.2	1:19

[a]By gel filtration. [b]In M sodium hydroxide (*c* 0.25); values uncorrected for possible deacetylation. [c]Determined by spectrophotometry as the molybdovanadophosphoric acid complex. [d]Determined by saponification. [e]Determined by g.l.c. as the alditol acetates.

From their compositions, it is evident that the four polysaccharides are partially acetylated glucomannans that are similar to the glucomannan of *Aloe plicatilis*[1], which has a high degree of *O*-acetyl substitution (d.s. 0.67). Polysaccharide B has the highest *O*-acetyl content (d.s. ~0.78) and, with water, only this polysaccharide gave a mucilaginous jelly resembling that of the native jelly; polysaccharides A_{1b} and A_2 formed solutions that were only opalescent. Further, alkali-treated polysaccharide B lost its ability to form a mucilaginous jelly, indicating that the mucilaginous behaviour may be due to the high *O*-acetyl content.

Hakomori methylation[5] of the polysaccharides, followed by g.l.c. analysis of the derived, partially methylated sugars as their alditol acetates[6], indicated the presence of only 2,3,6-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylmannose. These results (Table II) indicate that both glucose and mannose are (1→4)-linked in each of these polysaccharides and that the polymers are linear. Since these polysaccharides have high molecular weights ($>2 \times 10^5$), no alditol acetate derivative corresponding to a glucose or mannose end-group could be detected. The results of periodate oxidation on alkali-treated polysaccharides A_2 and B indicated that all of the sugar residues

TABLE II

G.L.C. DATA FOR THE PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM THE POLYSACCHARIDES A_{1a}, A_{1b}, A_2, AND B

Alditol acetates of	T[a]	*Molar ratio*			
		A_{1a}	*A_{1b}*	*A_2*	*B*
2,3,6-Tri-*O*-methylglucose	2.48	1.25	1	1	1
2,3,6-Tri-*O*-methylmannose	2.19	1	4.8	14	20

[a]Retention times relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.

are attacked, thus supporting the results of methylation analysis. Further, the negative specific rotations of the polysaccharides suggest the presence of β-D-glycosidic linkages.

Thus, unlike the jelly of *Aloe plicatilis*, which is reported to be a single, linear glucomannan, the mucilaginous jelly of *Aloe vera* contains a mixture of at least four linear glucomannans that differ in their *O*-acetyl content and glucose-to-mannose ratio. Hence, the early report[2] that the mucilaginous jelly of *Aloe vera* is a single glucomannan is not correct.

EXPERIMENTAL

Materials and methods. — Descending paper chromatography (p.c.) was performed on Whatman No. 1 paper with *A*, 1-butanol–benzene–pyridine–water (5:1:3:3, upper layer); *B*, 1-butanol–acetic acid–water (4:1:5, upper layer); *C*, ethyl acetate–pyridine–water (8:2:1); and detection with *p*-anisidine hydrochloride[7]. G.l.c. was performed on a Willy Giede GCHF 18.3 gas chromatograph fitted with a flame-ionization detector and a stainless-steel column (3 m × 4 mm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh), with nitrogen as carrier gas. Retention times (*T*) are given relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. Gel filtration[8] was performed with Sephadex G-200 by using 0.1M sodium chloride as eluant; the elution of polysaccharides was monitored by the phenol–sulphuric acid method[9].

The polysaccharide precipitates were collected by centrifugation, and dried by solvent exchange with ethanol, acetone, and dry ether, and finally by storage over phosphorus pentaoxide. The *O*-acetyl and phosphate contents of the polysaccharides were determined by the saponification[10] and spectrophotometric[11] methods, respectively.

The polysaccharide fractions were hydrolysed with 0.25M sulphuric acid for 10–12 h on a boiling water-bath. The hydrolysates were made neutral with barium carbonate, filtered, and deionised with Amberlite IR-120(H^+) and Amberlite IRA-400(CO_3^{2-}) resins. The neutral hydrolysates were concentrated under diminished pressure below 45° and examined by p.c. The neutral sugars were also analysed by g.l.c. as their alditol acetates[12]. The Amberlite IRA-400 (CO_3^{2-}) resin was eluted with 2M formic acid, the eluate was evaporated to dryness, and the residue was examined by p.c. for acidic sugars.

Isolation of the polysaccharides. — From the leaves of *Aloe vera*, the mucilaginous jelly (2.5 kg) was scraped out, and extracted with 50% aqueous ethanol. The insoluble, fibrous material was removed by centrifugation. To the clear solution was added ethanol (4 vol.) with vigorous stirring. The precipitate was collected, and dissolved in 0.02M hydrochloric acid (500 ml), and the solution was dialysed for 48 h against distilled water and then poured into ethanol (4 vol.). The precipitated polysaccharide mixture was collected and dried; yield, 17.5 g. After acid hydrolysis of the polysaccharide, p.c. indicated the presence of glucose and mannose in the molar ratio ~1:6, together with traces of galacturonic acid, xylose, arabinose, and galactose.

Fractionation of the polysaccharides. — To a solution of the polysaccharide mixture (12 g) in water (2 litres) was added ethanol (4 litres) and the precipitate (fraction A, 8.7 g) was removed. To the clear centrifugate was added ethanol (4 litres), and the precipitated polysaccharide (B, 3.0 g) was collected. To a solution of fraction A (8.7 g) in water (1 litre) was added ethanol (1.5 litres), and the precipitated polysaccharide (A_1, 5.4 g) was removed. To the clear solution was added ethanol (1.5 litres), and the polysaccharide precipitate (A_2, 3.1 g) was collected. Fraction A_1 (5.4 g) was dissolved in water (500 ml) and mixed with 0.1M sodium tetraborate (500 ml). The precipitated polysaccharide (A_{1a}) was suspended in 5% aqueous acetic acid (100 ml) and dialysed, and the polysaccharide (2.65 g) was recovered by the addition of ethanol (200 ml). The clear solution was acidified with 50% aqueous acetic acid and dialysed, and ethanol (2 vol.) was added to give the polysaccharide A_{1b} (2.8 g). The polysaccharides A_{1b}, A_2, and B were further purified by two fractional precipitations involving the gradual addition of ethanol to a well-stirred aqueous solution (1%) of each fraction and collecting the bulk of the material that was sharply precipitated. The sugar compositions of the polysaccharides (Table I) remained unaltered. These polysaccharides were excluded from Sephadex G-200, indicating the molecular weights to be higher than 2×10^5.

Methylation analysis. — Each polysaccharide (10 mg) was methylated according to the Hakomori method. A single methylation of each gave a completely methylated product, as two further methylations by the Hakomori method, and also methylation of the fully acetylated polysaccharide[13] by the Hakomori method, did not alter the proportions of the methylated sugars. Each fully methylated polysaccharide was hydrolysed first with 90% formic acid (1 ml) at 100° for 2 h in a sealed tube and then, after evaporation of the formic acid, with 0.25M sulphuric acid for 12 h at 100°. The resulting, partially methylated sugars were converted into their alditol acetates and analysed by g.l.c. on 3% ECNSS-M, which indicated the presence of only 2,3,6-tri-*O*-methylglucose and 2,3,6-tri-*O*-methylmannose from polysaccharides A_{1b}, A_2, and B. For polysaccharide A_{1a}, in addition to the foregoing methylated sugars, an appreciable amount of 2,3-di-*O*-methylxylose and traces of three sugar derivatives that could not be identified were also observed.

Periodate oxidation of the polysaccharides A_2 and B. — As the majority of the sugar residues in the untreated polysaccharides resisted periodate oxidation due to the high *O*-acetyl content, the alkali-treated polysaccharides were subjected to periodate oxidation.

Polysaccharides A_2 and B (150 mg of each) were separately taken up in M sodium hydroxide (10 ml), warmed at 60° for 4 h, and then kept overnight at room temperature. The resulting solutions were dialysed, and the polysaccharides were recovered by the addition of ethanol (4 vol.); yields, 90 and 85 mg, respectively, for A_2 and B.

The alkali-treated polysaccharides A_2 and B (50 mg of each) were oxidised with 45mM sodium metaperiodate at 5° in the dark. The periodate consumption[14] was ~1 mol/mol of hexose residue for both A_2 and B. The periodate-oxidised material

was treated with ethylene glycol, reduced with sodium borohydride, and then hydrolysed with 0.5M sulphuric acid for 8 h at 100°; p.c. of the hydrolysate indicated that all of the sugar residues in the deacetylated polysaccharides had been attacked by periodate.

REFERENCES

1 B. S. PAULSEN, E. FAGERHEIM, AND F. ØVERBYE, *Carbohydr. Res.*, 60 (1978) 345–351.
2 E. ROBOZ AND A. J. HAAGEN-SMIT, *J. Am. Chem. Soc.*, 70 (1948) 3248–3249.
3 V. E. TYLER, L. R. BRADY, AND J. E. ROBERRS, *Pharmacognosy*, Lea and Febiger, Philadelphia, 7th edition, 1976, pp. 81–83.
4 *The Wealth of India–Raw Materials*, C.S.I.R. Publication, New Delhi, India, 1 (1948) 60–62.
5 S. I. HAKOMORI, *J. Biochem (Tokyo)*, 55 (1964) 205–208.
6 P. JANSSON, L. KENNE, H. LIEDGREN, B. LINDERG, AND J. LÖNNGREN, *Chem. Commun., Univ. Stockholm*, 8 (1976) 1–76.
7 L. HOUGH, J. K. N. JONES, AND W. H. WADMAN, *J. Chem. Soc.*, (1950) 1702–1706.
8 S. C. CHURMS, *Adv. Carbohydr. Chem. Biochem.*, 25 (1970) 13–51.
9 J. E. HODGE AND B. T. HOFREITER, *Methods Carbohydr. Chem.*, 1 (1962) 388–389.
10 T. H. SCHULTZ, *Methods Carbohydr. Chem.*, 5 (1965) 187–189.
11 R. J. SMITH AND J.-L. CARVSO, *Methods Carbohydr. Chem.*, 4 (1964) 42–46.
12 J. H. SLONEKER, *Methods Carbohydr. Chem.*, 6 (1972) 20–24.
13 J. F. CARSON AND W. D. MACLAY, *J. Am. Chem. Soc.*, 68 (1946) 1015–1017.
14 G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 357–361.