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STRUCTURE OF THE GLUCOMANNAN ISOLATED FROM THE LEAVES OF *Aloe barbadensis* MILLER*

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ABSTRACT

The polysaccharide mixture obtained by hot-water extraction of *A. barbadensis* pulp was fractionated by stepwise treatment with calcium chloride solution and Fehling solution. This yielded a pure glucomannan fraction containing glucose and mannose in the molar ratio of 1:22. Methylation analysis of the glucomannan furnished 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylmannose, and 2,3,6-tri-*O*-methylglucose in the molar ratios of 1.3:18.3:1.2:1.0. The glucomannan reduced one molar equivalent of periodate per hexosyl residue, and none of the monosaccharides survived the oxidation. Smith degradation furnished mainly erythritol and a trace of glycerol. From these results, a structure has been assigned to the repeating unit of the glucomannan. The number-average, molecular weight ($\overline{M}n$) of the permethylated glucomannan was found to be 1.5×10^4.

INTRODUCTION

Previously, we have reported[1] that the polysaccharide composition of the leaves of *A. barbadensis* changes with the season of the year. This change seems to have helped in lowering the uronic acid content to ~10% by $CaCl_2$ treatment of the crude, polysaccharide mixture. The resulting polysaccharide mixture enriched in hexose (especially mannose) could be readily separated into (*i*) a pure glucomannan fraction (as its copper complex), and (*ii*) a mixture of a galactan, an arabinan, and pectic acid. We now report the structure of the glucomannan.

RESULTS AND DISCUSSION

A. barbadensis leaves used in this investigation were collected in October, 1977. The crude polysaccharide (A) was isolated from the pulp by extraction with hot water,

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*Characterization of the Polysaccharides of *Aloe barbadensis* Miller, Part II. For Part I, see ref. 1.
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TABLE I

COMPOSITION OF POLYSACCHARIDES A, A_1, A_2, A_3, AND A_4

Method of isolation		*Ps fractions*[a]	$[\alpha]_{589.5}$ *(degrees)*[b]	*GalA (%)*	*Total hexose (%)*	*Mole ratio*[c]					
						Glc	*Man*	*Gal*	*Ara*	*Xyl*	*Rha*
Hot-water extraction		A	+180.0 (0.45)	70.50	9.95	+	+	+	+	+	+
$CaCl_2$ treatment on	Ps-A solution (0.15%)	A_1	+65.3 (0.47)	35.20	50.30	trace	3.16	2.28	1.00	trace	1.17
	Ps-A_1 solution (0.22%)	A_2	+47.5 (0.50)	9.96	65.70	trace	4.78	2.67	1.00		trace
Fehling solution treatment on Ps-A_2	Residue	A_3	—		90.00	1.00	21.90				
	Supernatant liquor	A_4	+10.2 (1.50)	10.20	64.12	trace		4.56	1.00		trace

[a]Ps = polysaccharide. [b]In water; concentration in parentheses. [c]From g.l.c. of the alditol acetates; —, could not be determined; +, present; blank spaces indicate absence.

as already described[1], and found to contain GalA, 70.5%, and total hexose, 9.95%. Fractionation of A by treatment with $CaCl_2$ solution furnished polysaccharide A_1 containing GalA, 35.2%, and a repetition of the purification process furnished polysaccharide A_2 containing GalA, 9.96%, and total hexose, 65.7%. The major, neutral sugars of A_2 were Man, Gal, and Ara in the molar ratios of 4.78 : 2.67 : 1.0, along with traces of Glc and Rha. It is significant that the uronic acid content could not be lessened to such a low value during the isolation of the D-galactan (Fraction C_4, see[1] Part I). Also, Man, which was present as a trace in polysaccharide C_4, was now the major component of A_2. Polysaccharide A_2 showed no ester bands[2] (*viz.*, at 1730 and 1260 cm^{-1}) in its i.r. spectrum. Hence, considering the method of isolation, it would be highly improbable that all of the *O*-acetyl groups originally present had been hydrolyzed.

As mannans are amenable to complexing with copper solution[3], further fractionation was achieved by treating the aqueous solution of A_2 with Fehling solution at 4°; this resulted in the isolation of polysaccharide fractions A_3 (from the precipitated, copper complex) and A_4 (from the supernatant liquor). An interesting observation was that, although A_2 itself was quite soluble in water, its sub-fraction A_3 (containing the glucomannan) was very insoluble, and sub-fraction A_4 (containing the galactan, arabinan, and galacturonan components) was fairly soluble.

Because of its insolubility in water or alkali, further purification of A_3 was possible only by dispersion in water, and centrifugation. For component identification, A_3 was dissolved in 70% H_2SO_4, the solution diluted, and the polysaccharide hydrolyzed. The component monosaccharides were found to be Glc and Man in the molar ratio of 1 : 21.9. The hexose content of A_3 (as estimated, after hydrolysis, by g.l.c., using *myo*-inositol as the internal standard) was found to be 90%, and this probably reflects the very hygroscopic nature of the material. The compositions of fractions A, A_1, A_2, A_3, and A_4 are given in Table I.

Methylated A_3 was obtained by one Hakomori[4] and two Purdie[5] treatments: the product contained OMe, 43.9%, and had $[\alpha]_{589.5}$ −10.2°. Attempted fractionation of the permethylated product by adding petroleum ether to its chloroform solution furnished essentially one fraction, which was indistinguishable from the parent compound in respect of methoxyl content and value of specific rotation; this indicated that the permethylated product was homogeneous. Methylation analysis of the product furnished (*a*) 2,3,4,6-tetra-, (*b*) 2,3,6-tri-, and (*c*) 2,3-di-*O*-methylmannose, and (*d*) 2,3,6-tri-*O*-methylglucose, in the molar ratios of 1.3 : 18.3 : 1.2 : 1.0 (see Table II).

The molar ratio $(d) : (a + b + c) = 1.0 : 20.8$, being also of the same order as that of Glc and Man in A_3, corroborated the homogeneity of the glucomannan. As g.l.c. was not conclusive enough to identify the tetra-*O*-methyl sugar, the latter was identified by demethylating it, and characterizing the resulting mannose in the usual way.

From these results, it is possible to deduce some conclusions regarding the structure of the repeating unit of the glucomannan. Characterization of the tri-*O*-

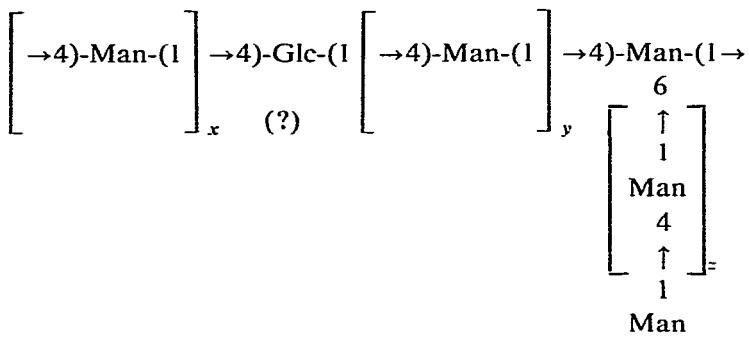
TABLE II

IDENTIFICATION OF THE METHYLATED SUGARS OBTAINED FROM THE GLUCOMANNAN OF *A. barbadensis*

Components (as alditol acetates of)	*RRT*[a]		*Mole ratio*
	Col. I	*Col. II*	
2,3,4,6-Tetra-*O*-methylmannose	**1.00** (1.00)	**1.00** (0.99)	1.3:1
2,3,6-Tri-*O*-methylmannose	2.24 (2.20)	2.06 (2.03)	18.3:1
2,3,6-Tri-*O*-methylglucose	2.52 (2.50)	2.35 (2.32)	1.0:1
2,3-Di-*O*-methylmannose	5.00 (4.83)	3.79 (3.69)	1.2:1

[a]Relative retention-times with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity; col. = column. Literature values[18] are given in parentheses.

methyl sugars indicated that the hexosyl residues (glucose and mannose) are linked through O-1 and O-4 in the main chain, and, possibly, also in the side chains in the repeating unit. The occurrence of 2,3-di-*O*-methylmannose indicated that this polymer is branched, and that the branching took place only from the mannosyl residues, probably through O-6. The isolation of only tetra-*O*-methylmannose indicates that the nonreducing ends of the repeating unit are composed of mannosyl residues. In view of these results and the molar ratios of the methylated sugars, structure **1** may be suggested as being the average repeating-unit of the glucomannan.



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where $(x + y + z) = 20$ mannosyl residues.

In structure **1**, the length of the side chain (z) and the location of the glucosyl residue (in the main or side chain) remain uncertain. The negative specific rotation (−10.2°) of the permethylated glucomannan indicates a preponderance of β-linkages.

On periodate oxidation, A_3 reduced one molar equivalent of the oxidant per hexosyl residue within 48 h (constant thereafter). During oxidation, the solution

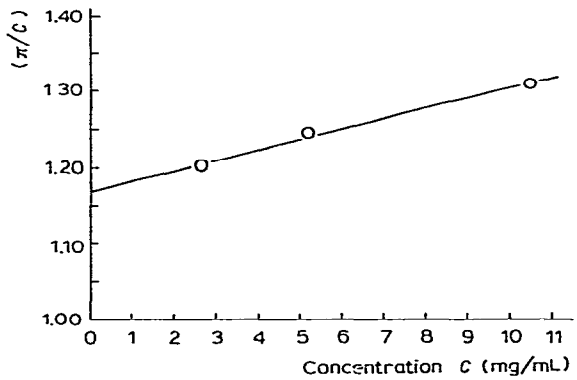

Fig. 1. Plot of π/C against C for permethylated A_3 (glucomannan fraction). (From the intercept, the value of $(\pi/C)_{C\to 0}$ was found to be 1.17.)

became acidic, but the results of the formic acid estimation were not reproducible, probably due to the initial, heterogeneous state of the reaction mixture. Smith degradation[6] of the oxidized product furnished erythritol as the major component, and a trace of glycerol, and none of the monosaccharides survived. These findings are also consistent with the structure proposed.

The number-average, molecular weight ($\overline{M}n$) of permethylated A_3 was determined by osmometry as described earlier[1], using the same solvent system ($CHCl_3$–EtOH). From the plot of π/C against C (see Fig. 1), the intercept corresponding to $(\pi/C)_{C\to 0}$ was found to be 1.17, and, from this, the $\overline{M}_n$ value was calculated to be 1.5×10^4.

It is evident, therefore, that this glucomannan is significantly different from those previously described[7,8] in being neither linear nor acetylated, and it has a much lower molecular weight.

EXPERIMENTAL

Materials and methods. — Paper partition-chromatography (p.c.) was conducted on Whatman Nos. 1 and 3 MM papers, using the following solvent systems (v/v): (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 4:1:5 1-butanol–ethanol–water (upper layer), and (*C*) 40:11:19 1-butanol–ethanol–water[9]. The staining reagents used were (*a*) alkaline silver nitrate[10], (*b*) benzidine periodate[11], and (*c*) aniline hydrogen-oxalate[12].

Unless otherwise stated, all evaporations were conducted at 35–40° under diminished pressure. Analytical methods and instruments were the same as reported earlier[1]. Specific rotations were recorded at equilibrium, at ~20°. For g.l.c., glass columns (1.83 m × 6 mm) containing (*I*) 3% of ECNSS-M on Gas Chrom Q (100–200 mesh) at 190° (for alditol acetates of neutral sugars) and at 170° (for partially methylated alditol acetates), and (*II*) 5% of OV-225 on SIL. RUB. (80–100 mesh) at 170° (for partially methylated alditol acetates) were used.

Isolation of the glucomannan. — Crude polysaccharide A was isolated in ~13.5% yield from the pulp of *A. barbadensis* leaves by extraction with hot water as described[1] in Part I. This material contained GalA, 70.5%, and hexose, 9.95%. The solution of crude A (1.5 g) in H_2O (1 L) was fractionally precipitated with 5% $CaCl_2$ solution (60 mL), and the hexose-enriched polysaccharide A_1 (325 mg) was isolated from the supernatant liquor. Polysaccharide A_1 (260 mg) was refractionated from a 0.22% aqueous solution by treatment with the same $CaCl_2$ solution. The resulting polysaccharide, A_2 (110 mg), contained GalA, 9.96%, and hexose, 65.7%. The compositions of A_1 and A_2 are given in Table I.

Further fractionation of A_2 by treatment with alkaline copper solution[3]. — To an ice-cold solution of A_2 (60 mg) in water (20 mL) was added, dropwise, freshly prepared Fehling solution (2 mL), with stirring. A thick, pale-blue, gelatinous precipitate appeared instantly; this was allowed to settle for 2 h at 4°, and then collected by centrifuging. The residue was washed with ice-cold water (3 × 10 mL) by centrifuging, and the washings were pooled with the supernatant liquor. The precipitated copper complex was then decomposed by being macerated with ice-cold, ethanolic hydrogen chloride [5% (v/v); 5 mL], and the suspension centrifuged at 0°. The supernatant liquor was discarded, and the residue was washed by centrifugation with cold ethanol (3 × 10 mL), in order to remove cupric chloride, until the washing failed to give any yellow color with acetone. The resulting, polysaccharide A_3 (21 mg) was extremely insoluble in water or alkali, and further purification was effected only by dispersion in water (50 mL) and centrifugation. Subsequent lots were isolated in the same way.

Identification of the component monosaccharides of A_3. — Polysaccharide A_3 (0.4 mg) was dissolved by rubbing with ice-cold H_2SO_4 (70%, 0.2 mL). To this solution was added ice-cold water (2.37 mL) and the polysaccharide was hydrolyzed in a boiling-water bath for 12 h. After the usual processing, the resulting monosaccharides, identified by p.c. (solvent *A*, staining agent *a*), and by g.l.c. of their alditol acetates, were found to be glucose and mannose in the molar ratio of 1:21.9; the total hexose was estimated[13] to be 90%.

Methylation analysis. — Polysaccharide A_3 (50 mg) was dried over P_2O_5, and methylated[4] as described earlier[1]. After the usual processing, the resulting product (partially methylated, as evidenced by its i.r. spectrum) was subjected to two Purdie[5] treatments, to yield the permethylated glucomannan (36.4 mg) having $[\alpha]_{589.5}$ −10.0° (*c* 1.0, chloroform), and OMe, 43.8%. This compound was dissolved in chloroform (1 mL) and fractionally precipitated with increasing volumes of petroleum ether (b.p. 60–80°). The first turbidity appeared on adding 5 vol. of petroleum ether; this fraction was separated by centrifugation, to yield the permethylated polysaccharide (33.0 mg) having $[\alpha]_{589.5}$ −10.2° (*c* 1.0, chloroform) and OMe, 43.9%. Evaporation of the supernatant liquor furnished a second fraction (~3 mg). Permethylated A_3 (2 mg) was hydrolyzed[7], and the resulting, partially methylated monosaccharides were characterized by p.c. (solvents *D* and *E*, staining agent *c*), and the results confirmed by g.l.c. of their alditol acetates (see Table II).

Identification of tetra-O-methylmannose. — Permethylated A_3 (~6 mg) was

hydrolyzed[7], and the tetra-*O*-methyl sugar was isolated in the pure state by preparative p.c. (solvent *C*) on 3 MM paper. This compound was demethylated[14], and the resulting product was found to contain mannose (p.c.: solvent *A*, stain *a*), along with some partially methylated sugars.

Periodate oxidation. — Polysaccharide A_3 (4 mg, in duplicate) was dispersed in water, and subjected to periodate oxidation[15] at 10°. The initial turbidity disappeared within 18 h, when the rate of uptake could be determined spectrophotometrically. The uptake became constant in 48 h, with reduction of 0.93 mol of the oxidant per hexosyl residue. In another experiment, A_3 (16 mg) in H_2O (8 mL), in duplicate, was oxidized with periodate under the same conditions. Aliquots were titrated for formic acid with 0.01M NaOH at 20, 30, 40, and 48 h in the usual way[16], but the results were not satisfactorily reproducible. Remaining portions of the duplicate reaction-mixture were pooled, and decomposed with ethylene glycol (1 mL). This mixture was dialyzed, and the solution concentrated by lyophilization. The resulting polysaccharide was reduced with KBH_4 (100 mg) for 24 h at 4°, and the reduction product (~9 mg) isolated in the usual way[17]. Hydrolysis of the reduced polysaccharide revealed the presence of erythritol (major, R_{Gal} 1.85; solvent *A*, stain *b*) and a trace of glycerol (R_{Gal} 2.31; solvent *A*, stain *b*). No hexose survived the oxidation.

Determination of molecular weight. — The number-average, molecular weight ($\overline{M}_n$) of permethylated A_3 was determined by osmometry (see Part I). Measurements were made by using duplicate solutions, of concentrations (mg/mL) 10.49, 5.245, and 2.6225, in 24:1 (v/v) $CHCl_3$–EtOH at 30°.

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