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Note

Structural studies of the glucomannan from *Aloe vahombe**

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For centuries, the fresh mucilaginous jelly from the leaves of plants belonging to the genus *Aloe (Liliaceae)* has been used in the treatment of burns and skin diseases[1]. Recently, the jelly extracted from various members of this genus has been investigated and its composition shown to vary widely with the species. The jelly from *A. vera* contains four glucomannans[2], that from *A. plicatilis* a single, linear glucomannan[3], and that from *A. barbadensis* a branched glucomannan[4]. The jelly from *A. arborescens* contains an acetylated β-D-mannan which has antitumour activity in mice[5]. β-D-Mannans were also isolated from *A. saponaria* and *A. valebalensi*[6]. The immunoadjuvant activity of a crude extract from leaves of *A. vahombe* has been described[7] and we now report on the structure of the polysaccharide extracted from this species.

A. vahombe leaves used in this work were collected in Madagascar, and their first, crude, aqueous extract was a gift from Service d'Extraction et de Fermentation, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette. The extract was a fibrous, coloured powder which was fractionated by graded precipitation with ethanol from aqueous solution. The polysaccharide fraction (precipitate II, see Experimental) was subjected to gel chromatography on Sephadex G 100, to give fractions *A* and *B* (Fig. 1).

The major fraction *A* had $[\alpha]_D$ −36° (*c* 0.5, water), contained 90% of carbohydrate estimated as mannose, and, on acid hydrolysis, gave D-glucose and D-mannose in the molar ratio ~1:3 (determined by g.l.c.–m.s. of the alditol acetates). Determination of sugars by Wilson's method[8] gave the molar ratio 1:2.6. Fraction *A* did not contain nitrogen, phosphorus, or uronic acid. I.r. bands at 1250 and 1730 cm^{-1} and an n.m.r. signal at δ 2.09 indicated the presence of acetyl groups. The *O*-acetyl content (0.33, determined by saponification) was lower than those of polymers from *A. plicatilis*[3] (0.67) and *A. vera*[2] (0.78), but similar to that

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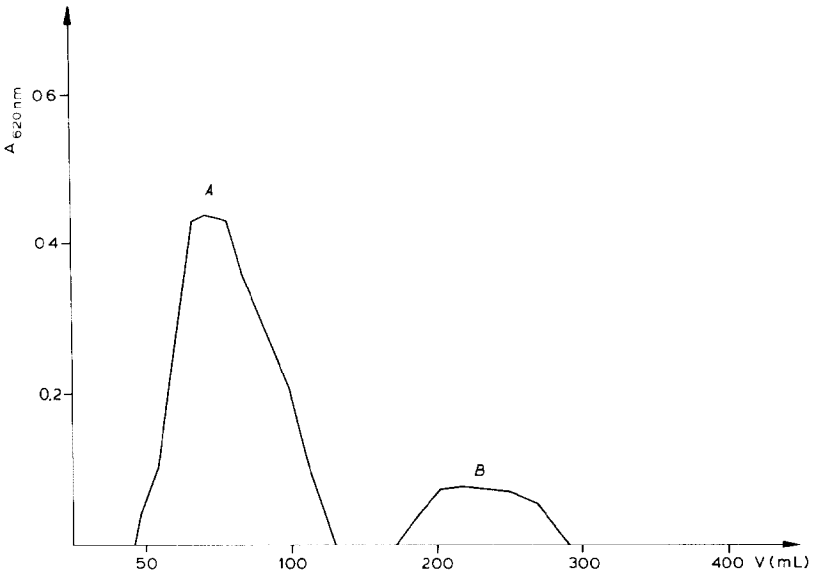

Fig. 1. Elution profile of precipitate II from a column (30 × 2.8 cm) of Sephadex G-100 (V_o 45 mL, V_o + V_i 400 mL). Fractions (1 mL) were analysed for total carbohydrate. Fraction *B* contained 20% of reducing sugars, 10% of Ca, and 0.1–1% of Cl, K, S, Si, Zn, and Sr.

of the acetylated glucomannan isolated from *Pinus silvestris*[3]. Thus, fraction *A* contained approximately one acetyl group per three sugar units. The ^{1}H-n.m.r. spectrum of the partially depolymerised glucomannan (d.p. ~15) contained two well-separated doublets in the region for anomeric protons, namely, δ 4.72 with a $J_{1,2}$ value close to that (0.9 Hz) observed for mannose residues in legume-seed galactomannans[9], and δ 4.52 with a $J_{1,2}$ value (7.5 Hz) comparable to that for cellobiose[10].

Fraction *A* was methylated once by the Haworth method[11] and twice by the Hakomori method[12], and then hydrolysed. P.c. (solvent *C*) and t.l.c. (solvents *E* and *F*) of the hydrolysate revealed only 2,3,6-tri-*O*-methyl-glucose and -mannose; a specific detection agent[13] was used. Analysis by g.l.c. of the alditol acetates gave the same result. No alditol derivative corresponding to a tetramethylhexose arising from end groups was detected, which is consistent with the very high molecular weight of the polysaccharide as shown by gel filtration. Smith degradation (*i.e.*, periodate oxidation, borohydride reduction, acid hydrolysis) gave erythritol, glucose, and traces of glycerol.

These findings indicated fraction *A* to be an acetylated, linear, (1→4)-linked β-D-glucomannan containing glucose and mannose in the ratio ~1:3. The presence of glucose after Smith degradation suggested that the acetyl groups were located mainly on the glucose residues. Thus, fraction *A* is comparable to the glucomannan derived from *A. plicatilis* Miller[3].

EXPERIMENTAL

I.r. spectra were recorded with a Perkin–Elmer spectrophotometer, using potassium bromide discs. Specific rotations were measured with a Roussel-Jouan polarimeter. The n.m.r. spectrum was recorded with a Cameca 250 instrument for a solution in D_2O at 40°. G.l.c. of partially methylated alditol acetates was performed with a Carlo Erba 2400 T apparatus, with a flame-ionisation detector, a stainless-steel column (2 m × 4 mm) containing 3% of ECNSS M on Chromosorb Q, and nitrogen as carrier gas. G.l.c.–m.s. of alditol acetates involved a NERMAG R 1010 instrument with a capillary column CPSil 5 (25 m) and a temperature programme from 120→250° at 3°/min.

P.c. was carried out on Whatman No. 1 (analytical) or No. 3 (preparative)paper with *A*, 1-butanol–acetic acid–water (4:1:5); *B*, 1-butanol–pyridine–water (6:4:3); *C*, 1-butanol–ethanol–water–ammonia (40:10:49:1), ascending method; and *D*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4). T.l.c. was performed on cellulose (Merck) and on silica gel 60 F 254 (Merck) with *E*, acetone–water (9:1); and *F*, acetone–water–conc. ammonia (250:3:1). Reducing sugars and polyols were detected with alkaline $AgNO_3$, and reducing sugars with aniline hydrogen phthalate and with *N*,*N*-dimethylaniline trichloroacetate (for partially methylated sugars[13]). The absence of uronic acids was checked by p.c. of the acid hydrolysate and detection with a specific spray[14].

Carbohydrate content was determined by the anthrone method[15]. The ratio of glucose and mannose was determined by Wilson's method[8] after their chromatographic separation, and by g.l.c. as their alditol acetates.

Investigation of the polysaccharide. — *(a) Isolation.* The dried, crude, aqueous extract was dissolved with stirring in warm water (60°), and the insoluble material was removed by filtration. To the clear, aqueous solution (1 vol.) was added ethanol (0.5 vol.), and the precipitate (I) was collected by centrifugation (yield, 30%; low sugar content). To the supernatant solution (1 vol.) was added more ethanol (1 vol.), and the precipitate (II) was collected as described above, washed with ethanol, and dried *in vacuo* (yield, 35%).

(b) Hydrolysis. The polysaccharide was hydrolysed with 1.5M HCl at 100° for 8 h in a sealed tube. The hydrolysate contained (solvents *B* and *D*) mannose and glucose, which were isolated by preparative p.c.: their absolute configurations were assigned on the basis of the $[\alpha]_D$ values.

For g.l.c.–m.s., the hydrolysis products were reduced for 18 h with $NaBH_4$ and then acetylated with acetic anhydride in pyridine.

Sugar	*Ratio*	*T*	*Primary fragments*, m/z (%)
Mannose	3	19.57	361 (1.3), 289 (1.8), 259 (2.0), 217 (3.3), 187 (2.6), 157 (2.6), 145 (4.1), 115 (4.2), 43 (100)
Glucose	1	20.11	289 (3.0), 259 (2.2), 217 (5.2), 187 (4.5), 157 (3.7), 103 (4.8), 43 (100)

(c) Smith degradation. To a solution of the polysaccharide (10 mg) in water (3 mL) was added freshly prepared 0.1M sodium metaperiodate (3 mL). The solution was stored in the dark at 15° for 120 h and then treated with ethylene glycol to reduce the excess of periodate. The sodium iodate was precipitated with ethanol at 0° and removed, and the filtrate was concentrated. The product was reduced with $NaBH_4$ overnight, the excess of $NaBH_4$ was destroyed with acetic acid, and the mixture was treated with Dowex 50 (H^+) resin. Boric acid was removed conventionally as methyl borate. The residue was hydrolysed with 2M HCl at 100° for 4 h, and subjected to p.c. (solvents *A* and *B*) and cellulose t.l.c. (solvent *E*).

(d) Methylation. The polysaccharide was methylated first by the Haworth method[11] and then twice by the Hakomori method[12]. The product, which showed no i.r. absorption for hydroxyl groups, was hydrolysed with 2M HCl at 100° for 6 h, and the methylated hexoses were subjected to p.c. (solvent *C*, ascending technique) and t.l.c. (solvent *F*). The products of hydrolysis were also conventionally reduced with $NaBH_4$, to give the corresponding alditols, and these were acetylated with 1:1 acetic anhydride–pyridine and then subjected to g.l.c.–m.s.

Sugar	T[a]
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylmannitol	1.88
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol	2.12

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

(e) Partial degradation. The glucomannan (0.25 g) was ground with syrupy 85% orthophosphoric acid (30 mL) at 20° for 2 h and the mixture was shaken at 4° for 4 weeks[8]. Ice-cold ether (150 mL) was then added to the solution with vigorous stirring. The resulting precipitate was collected by decantation, extracted by repeated grinding with fresh ether, and then dissolved in water. The solution was neutralised with barium carbonate, filtered, centrifuged, and concentrated to dryness, to give partially depolymerised polysaccharide (0.05 g).

The $d.p._n$ of the product was determined by g.l.c. after reduction, hydrolysis, and acetylation.

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