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Note

Isolation and structure analysis of a glucomannan from the leaves of *Aloe arborescens* var. Miller

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The leaves of *Aloe arborescens* var. Miller (Asphodelaceae) contain a mucilage (aloegel), which is typical for other members of the genus Aloe. The mucilage possesses various pharmacological activities, such as antiphlogistic and antiedematic effects as well as a treatment for burns including those caused by X-rays[1,2], and is used mainly in cosmetics as a humectant and against sunburn[3]. The most important component of the aloegel is a polysaccharide that consists of a neutral glucomannan together with smaller proportions of acidic polysaccharides[4,5].

There are contradictory data on the structure of the water-soluble polysaccharides of *A. arborescens*. Yagi *et al.*[6] found a partially acetylated mannan (mol. wt. 15 000) and reported later[7] an additional mannan (mol. wt. 40 000) with an acetyl content of 10%, a (1→6)-α-glucan, and a branched arabinogalactan. Kodym *et al.*[8] reported mannose, glucose, arabinose, and galactose to be the constituents of a neutral polysaccharide, whereas other authors isolated mainly acidic, pectin-like polysaccharides[9,10].

We now report on the isolation and structures of a neutral glucomannan and an acidic arabinogalactan from the fresh leaf material of *A. arborescens*.

Powdered dry leaves of *A. arborescens* were extracted with hot ethanol, dried, and then extracted with phosphate buffer (pH 7.0) to give the crude water-soluble polysaccharides (0.10%). This polysaccharide fraction contained protein (0.8%) and uronic acid (2%), and had i.r. bands for ester groups at 1740 and 1250 cm^{-1}.

Hydrolysis of the polysaccharide gave mannose, glucose, arabinose, and galactose in the molar ratios 60:17:11:11 (g.l.c. of the alditol acetates), and glucuronic acid (identified by t.l.c.). The total hexose content of the crude polysaccharide fraction was 40% and it was resistant to alpha-amylase, thus proving the absence of starch.

Fractionation of the crude polysaccharide on DEAE-Sephacel gave neutral and acidic fractions. The acidic fraction (10.6%), eluted with 0.4M phosphate buffer, was composed of arabinose, galactose, rhamnose, and glucose (molar ratios

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43:43:7:7), contained glucuronic acid (6%) and protein (2%), and had no i.r. absorption for ester. The molecular weight was estimated to be 50 000 by gel-permeation chromatography on Superose™ 12.

The neutral fraction (50.2%) was composed of mannose, glucose, arabinose, and rhamnose (molar ratios 87:4:4:5), and contained protein (0.2%) and uronic acid (0.6%). The i.r. spectrum showed absorption for ester groups. After gel-permeation chromatography on Superose™ 12 or Superose™ 6, most of the polysaccharide was present in the void volume and there was a minor fraction with a mol. wt. of 12 000. The molecular weight of the fraction in the void volume, determined using Sephacryl S-500, was 1×10^6.

The lyophilized high-molecular-weight fraction, obtained after gel-permeation chromatography, was water-soluble, had i.r. absorption for ester, and was free of protein and uronic acids. Total hydrolysis yielded only mannose and glucose in the molar ratio of 95:5. The total hexose content of this fraction was 73% (excluding the acetyl content).

The results of methylation analysis, shown in Table I, accorded with a (1→4)-linked glucomannan; only traces of branched sugar residues were detected. The ^{1}H-n.m.r. spectrum contained signals at δ 1.99, 2.01, and 2.05 for OAc groups, and the signals at δ 4.16 and 5.39 indicated the acetyl groups to be located at C-6 and C-2,3, respectively. The anomeric proton signal at δ 4.70 confirmed[17] that the sugar residues were linked β-glycosidically, which accords with the i.r. band at 870 cm^{-1}.

The ^{13}C-n.m.r. spectrum (Table II) showed downfield shifts for the resonances of C-1 and C-4 similar to those of other (1→4)-linked glucomannans[18]. There were distinct signals for OAc groups at δ 20.2–20.7 and 173.5–174.1, and the acetyl content was calculated to be 25%. A chemical method[19] for the determination of acetyl groups gave a value of 25.2% for the acetyl content.

The location of the OAc groups was determined[20] by reaction with methyl vinyl ether, followed by *O*-deacetylation, methylation[16], hydrolysis, reduction, and acetylation. The methyl groups marked the positions originally occupied by acetyl groups. The results in Table III indicated an acetyl content of 26%, corresponding to a d.s. of 1.3, and the acetyl groups to be attached to the mannose residues at positions 2, 3, and 6. Similar proportions of mono-, di-, and tri-substituted mannose residues were detected together with 20% of unsubstituted mannose.

TABLE I

METHYLATION ANALYSIS OF THE HIGH-D.P. NEUTRAL POLYSACCHARIDE FRACTION

Derivative[a]	T *(min)*	*Mol%*	*Type of linkage*
1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol	16.1	1.3	1-Glucose
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol	23.5	95.0	1,4-Mannose
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol	24.2	2.7	1,4-Glucose
1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol	30.8	1.0	1,4,6-Glucose

[a]Partially methylated alditol acetates analysed by g.l.c.–m.s.

TABLE II

^{13}C-N.M.R. DATA FOR THE HIGH-D.P. NEUTRAL POLYSACCHARIDE FRACTION

Assignment	*Signals*[a]	
Acetyl groups (C=O, CH_3)	174.11	20.72
	174.02	20.45
	173.54	20.22
Sugar residue		
C-1	100.3	
C-2	70.2	
C-3	71.6	
C-4	77.0	
C-5	75.0	
C-6	60.6	

[a]P.p.m. downfield from the signal for external Me_4Si.

TABLE III

LOCATION OF THE ACETYL GROUPS IN THE *Aloe* GLUCOMANNAN

Derivative[a]	T *(min)*	*Mol%*	*Location of OAc*
1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol	21.4	20.0	2,3,6
1,2,4,5-Tetra-*O*-acetyl-3,6-di-*O*-methyl-D-mannitol	28.0	6.0	3,6
1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol	29.3	25.0	2,3
1,2,3,4,5-Penta-*O*-acetyl-6-*O*-methyl-D-mannitol	29.9	23.0	6
Hexa-*O*-acetyl-D-mannitol	38.9	21.0	—
Hexa-*O*-acetyl-D-glucitol	41.3	5.1	—

[a]Partially methylated alditol acetates analysed by g.l.c.–m.s.

The neutral fraction with mol. wt. 12 000 had structural features similar to those of the high-molecular-weight glucomannan, *i.e.*, a mannose–glucose ratio of 95:5, a total hexose content of 70%, and an acetyl content of 18%. Methylation analysis again demonstrated the presence of 4-linked mannose, 4-linked glucose, and terminal glucose in the molar ratios 93:2:4.5. Thus, the glucomannans of low and high molecular weight are produced by the same route of biosynthesis.

From the viewpoint of chemotaxonomy, glucomannans are typical of the family Liliaceae. Differences are mainly in the mannose–glucose ratio, in molecular weight, and in patterns of branching and acetyl substitution. Highly acetylated glucomannans have been isolated from *A. plicatilis*[4] (d.s. 0.67) and *A. vera*[5] (d.s. 0.78). The glucomannan of *A. arborescens* has a significantly higher d.s. of 1.3. The glucomannans of *Narzissus tazetta*[21] and *Lycoris radiata*[22], which belong to the family Amaryllidaceae, are also highly acetylated. The characteristics of *A. arborescens* glucomannan are comparable to those of glucomannans from other *Aloe* species[4,5], which confirms the importance of glucomannans for chemotaxonomic classification.

EXPERIMENTAL

General. — Solutions were concentrated at 40° under reduced pressure. I.r. spectra were recorded with a Beckman Acculab 3 spectrophotometer. The ^1H- and ^{13}C-n.m.r. spectra were recorded with a Bruker WM spectrometer (250 MHz) at 25° for solutions in D_2O (external Me_4Si). Dialysis was carried out in Spectrapor 3 tubes (mol. wt. of cut-off, 3500) for 72 h with repeated changes of water. Protein was determined according to Sedmark and Grossberg[11] with Coomassie Brilliant Blue and bovine serum albumin as the standard. Total sugar contents were determined by the anthrone method[15], and uronic acid according to Blumenkrantz *et al.*[12] as modified by Kram *et al.*[13]. Starch contamination was estimated by treatment[14] with pancreatic alpha-amylase. Acetyl groups were determined by the method of Paulsen *et al.*[4] Acetyl groups were located following the method of De-Belder and Norman[20], using 30 mg of *Aloe* glucomannan.

Plant material. — *Aloe arborecens* var. Miller was grown in a greenhouse. The fresh leaves were harvested during January–July 1988.

Isolation of the mucilage polysaccharides. — Fresh leaves were crushed in a Sorvall Omnimixer with cooling in ice, and then lyophilized. The residue was pulverised in a mortar, then extracted with hot aqueous 96% ethanol in a Soxhlet apparatus for 3 h. After centrifugation (10 000*g*, 10 min), the residue was dried, then extracted with 0.1M phosphate buffer (pH 7.0) for 3 h at 52°. After centrifugation (10 000*g*, 10 min) and re-extraction of the pellet, the phosphate buffer extracts were combined, concentrated, dialyzed, and lyophilized. The yield of the crude polysaccharide fraction was 0.10% of the fresh weight.

The crude polysaccharide fraction was eluted from a column (2.8 × 25 cm) of DEAE-Sephacel (PO_4-form) (Pharmacia), at 50 mL.h^{-1} with (*1*) water (400 mL), (*2*) a phosphate buffer (pH 6.0) gradient (0→M, 600 mL), and (*3*) 0.2M NaOH (400 mL). Anthrone-positive fractions were combined, concentrated, dialysed, and lyophilized.

Gel-permeation chromatogaphy was carried out on a column (1 × 30 cm) of Superose™ 12 and Superose™ 6 by elution with 0.1M NaCl. Fractions were monitored by the anthrone method. The column was calibrated with standard pullulans (Macherey–Nagel). A column (1.6 × 50 cm) of Superose™ 12 was used for larger scale separations. The molecular weight of the high-d.p. polysaccharide was determined on a column (1.6 × 90 cm) of Sephacryl S 500 (Pharmacia) by elution with 0.1M NaCl. The column was calibrated as above.

Determination of the sugar composition and linkage analysis. — Polysaccharides were hydrolysed with 2M trifluoroacetic acid for 60 min at 120°. The hydrolysate was repeatedly co-concentrated with water. The residue was analysed by t.l.c. on Silica Gel 60 (Merck), using acetone–water (87:13, v/v) or ethyl acetate–methanol–boric acid–acetic acid (55:20:15:10, v/v), and detection with aniline–diphenylamine–phosphoric acid[23].

The monosaccharides in the hydrolysate were converted[24] into the alditol

acetates, then analysed by g.l.c. at 220° for 30 min with a Varian 3500 instrument, fitted with a fused-silica column DB 225 (0.25 mm × 30 m) and a flame-ionization detector, with N_2 as the carrier gas at 0.8 mL.min^{-1}, a split ratio of 1:50, and *myo*-inositol as the internal standard.

Methylation analysis was performed according to Harris *et al.*[16]. Partially methylated alditol acetates were analysed with a Hewlett–Packard GC 5890 A, using a mass-selective detector 5970 B, a Durabond fused-silica column (DB 225, 0.25 mm × 30 m), and a temperature program of 170°→210° at 10°.min^{-1} followed by an isothermic phase.

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REFERENCES

1 P. Hoffenberg, *Seifen, Öle, Fette, Wachse,* 105 (1979) 499–502.
2 B. Rovatti and R. J. Brennan, *Ind. Med. Surg.,* 28 (1959) 364–368.
3 R. Henry, *Cosmet. Toiletr.,* 94 (1979) 42–48.
4 B. S. Paulsen, E. Fagerheim, and E. Overbyl, *Carbohydr. Res.,* 60 (1978) 345–351.
5 D. C. Gowda, B. Neelisiddaiah, and Y. V. Anjaneyalu, *Carbohydr. Res.,* 72 (1979) 201–205.
6 A. Yagi, K. Makino, I. Nishioka, and Y. Kuchino, *Planta Med.,* 31 (1977) 17–20.
7 A. Yagi, H. Nishimura, T. Shida, and I. Nishioka, *Planta Med.,* 3 (1986) 213–218.
8 A. Kodym, H. Broda, and I. Dragumowicz, *Herba Polonica,* 3 (1979) 193–199.
9 R. Ovodova and V. F. Lapschik, *Khim. Prir. Soedin.,* 11 (1975) 3–5.
10 M. Hranisavljevic-Jakovljevic, *Glas. Hem. Drus. Beograd,* 46 (1981) 269–273.
11 J. J. Sedmark and S. E. Grossberg, *Anal. Biochem.,* 79 (1977) 544–549.
12 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.,* 54 (1973) 484–489.
13 G. Kram, Dissertation, Regensburg, 1984.
14 T. Asamizu and A. Nishi, *Planta,* 146 (1979) 49–54.
15 D. L. Morris, *Science,* 107 (1948) 254–255.
16 P. J. Harris, R. J. Henry, A. B. Blakeney, and B. A. Stone, *Carbohydr. Res.,* 127 (1984) 59–73.
17 A. Yagi, K. Hamada, K. Mihashi, N. Harada, and I. Nishioka, *J. Pharm. Sci.,* 73 (1984) 62–65.
18 T. Usui, T. Mizumo, K. Kato, M. Tomoda, and G. Miyajima, *Agric. Biol. Chem.,* 43 (1979) 863–865.
19 B. S. Paulsen, *Carbohydr. Res.,* 60 (1978) 345–351.
20 A. N. DeBelder and B. Norman, *Carbohydr. Res.,* 8 (1969) 1–8.
21 M. Tomoda, M. Yokoi, A. Torigoe, and K. Maru, *Chem. Pharm. Bull.,* 28 (1980) 3251–3256.
22 M. Tomoda and N. Shimizu, *Chem. Pharm. Bull.,* 30 (1982) 3965–3970.
23 R. W. Bailey and E. J. Bourne, *J. Chromatogr.,* 4 (1960) 206–210.
24 A. B. Blakeney, P. J. Harris, R. J. Henry, and B. A. Stone, *Carbohydr. Res.,* 113 (1983) 291–299.