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STRUCTURAL STUDIES OF THE POLYSACCHARIDE FROM *Aloe plicatilis* MILLER

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ABSTRACT

The interior part of the leaves of the succulent *Aloe plicatilis* Miller *(Liliaceae)* consists of a transparent jelly containing mainly an acetylated glucomannan. Structural studies show that glucose and mannose, which are present in the ratio 1:2.8, are (1 → 4)-linked and that there is no branching. The hexose residues are randomly and variously substituted at positions 2,3,6, 2,3, 2, and 3 with acetyl groups; a small proportion of the sugar units is unsubstituted.

INTRODUCTION

Most of the members of the genus *Aloe (Liliaceae)* have fleshy leaves, the interior of which is composed of a white, mucilaginous jelly. The jelly from *A. vera* is a polysaccharide composed[1] of equal amounts of glucose and mannose in addition to 2.4% of uronic acid. For centuries, the fresh mucilaginous jelly has been used in the treatment of burns and skin irritation, and is incorporated in ointments used for the treatment of burns and certain skin diseases[2].

We now report on the chemical structure of the mucilaginous jelly from *Aloe plicatilis* Miller.

RESULTS AND DISCUSSION

Leaves from the succulent *Aloe plicatilis* Miller *(Liliaceae)* were collected in the Botanical Garden, Funchal, Madeira, at Christmas 1973 and Easter 1975. The chlorophyll-containing outer layer of the leaves was removed, exposing the inner part as a transparent, colourless jelly. The jelly was either suspended in water or dissolved in methyl sulphoxide. Exhaustive dialysis against distilled water precipitated the bulk of the material inside the dialysis bag as an insoluble jelly, which was recovered by freeze-drying. The yield was 0.3% based on the wet weight of the jelly present in the plant.

The freeze-dried polymer was only partly soluble in water, methyl sulphoxide, M sulphuric acid, or 90% formic acid. After vigorous stirring in 20% sodium hydroxide,

complete dissolution could be achieved, but these conditions would hydrolyse ester bonds.

Preliminary investigations of the polymer indicated the presence of glucose and mannose. Acetylated glucomannans have been found in various pines[3-7], spruce[8], orchids[9-11], and various lilies[12,13], and they show[4,10,12,13] i.r. bands at 1260 and 1730 cm^{-1} characteristic of ester groups. The polymer from *A. plicatilis* showed i.r. bands at 1250 and 1735 cm^{-1}, which were absent from the i.r. spectrum of the alkali-treated material. Ester groups are therefore present in the native polymer. The acid component was found to be acetic acid by g.l.c. after weak acid hydrolysis of the polymer[10].

α-D-Galactose, (1 → 6)-linked to a glucomannan, occurs in various plants[14]. When native or alkali-treated polymer from *A. plicatilis* was incubated with α-D-galactosidase, no galactose was liberated (p.c.). The molecular weight, carbohydrate composition, and acetyl content of the polymer are given in Table I; the $[\alpha]_D$ value was not measured, because of the very limited solubility of the material. The polymer gave a negative protein test, but the measured content of carbohydrate and acetyl groups does not account for 100% of the material; as the polymer is extremely hygroscopic, this may reflect the presence of water.

The results in Table I show that the polymer contains ~4 acetyl groups/6 sugar units, corresponding to a degree of substitution (d.s.) of 0.67. The highest d.s. previously reported[5] was 0.36 for an acetylated glucomannan from *Pinus silvestris*.

Treatment with M methanolic hydrogen chloride will normally completely

TABLE I

COMPOSITION OF THE POLYSACCHARIDE ISOLATED FROM *A. plicatilis*

M_w[a]	*Carbohydrate composition*[b]	*Carbohydrate content*[c]	*Acetyl content*	
			d	*e*
1.2×10^6	Glucose 1 Mannose 2.8	60%	16.5%	13.7%

[a]Determined by gel filtration. [b]Determined by g.l.c. of *O*-trimethylsilyl derivatives of the methyl glycosides. [c]Determined by the phenol–sulphuric acid method. [d]Determined by the hydroxamate test. [e]Determined by g.l.c.

TABLE II

G.L.C.–M.S. DATA FOR THE METHYLATED ALDITOL ACETATES DERIVED FROM THE POLYMER OF *A. plicatilis*

	T[a]	*Primary fragments* (m/e)
2,3,6-Tri-*O*-methylmannose	1.81	45, 117, (161), 233
2,3,6-Tri-*O*-methylglucose	2.03	45, 117, (161), 233

[a]Retention times relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucitol diacetate on column (*b*).

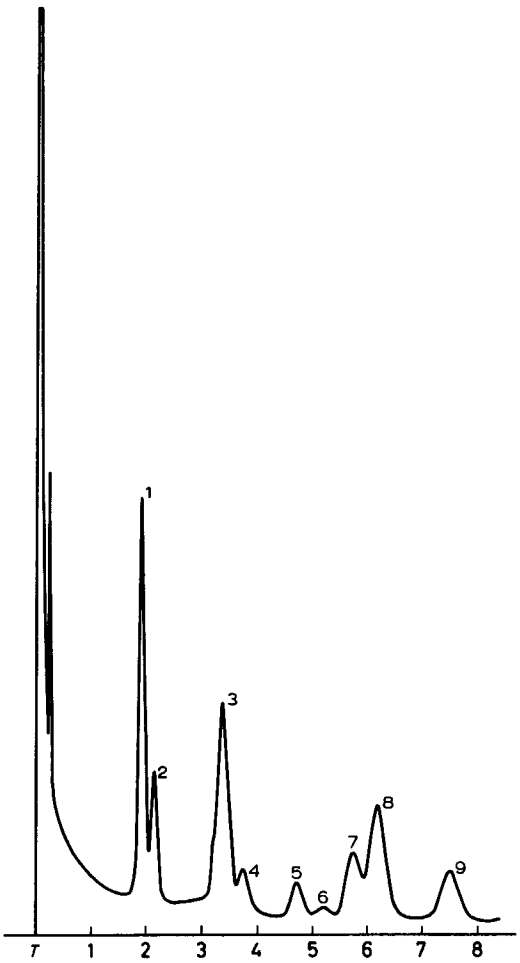

Fig. 1. Gas chromatogram of partly methylated alditol acetates derived from *B* and *C*: **1**, 2,3,6-tri-*O*-methylmannitol triacetate; **2**, 2,3,6-tri-*O*-methylglucitol triacetate; **3**, 2,3-di-*O*-methylmannitol tetra-acetate; **4**, 2,3-di-*O*-methylglucitol tetra-acetate; **5**, 2-*O*-methylmannitol penta-acetate; **6**, 2-*O*-methylglucitol penta-acetate; **7**, 3-*O*-methylmannitol penta-acetate; **8**, mannitol hexa-acetate + 3-*O*-methylglucitol penta-acetate(?); **9**, glucitol hexa-acetate. *T* values relative to that of 2,3,4,6-tetra-*O*-methylglucitol diacetate.

degrade a polysaccharide[15], but 6M acid was required for the *A. plicatilis* polymer; oligomers were obtained when M acid was used.

Two Hakomori treatments were necessary to effect complete methylation of the polymer. The product was hydrolysed, and the derived, partly methylated monosaccharides were converted into the corresponding alditol acetates and analysed by g.l.c.–m.s.[16]. The results (Table II) show that both glucose and mannose are (1 → 4)-linked and that the polymer is linear. No alditol derivative corresponding to a glucose or mannose end-group was detected, which is consistent with the polysaccharide's having a very high molecular weight.

Various methods have been used for determination of the position of *O*-acetyl groups in polysaccharides; blocking the free hydroxyl groups by reaction with methyl vinyl ether, followed by methylation of the product, has been shown to give reliable results[19].

Treatment of the *A. plicatilis* polymer with methyl vinyl ether gave a product (*B*), which was deacetylated with methanolic sodium methoxide to give product *C*. *B* and *C* were subjected to methylation analysis as described above. The results, which were similar for both products, are shown in Fig. 1. Since *O*-methyl groups in the products are present at positions originally occupied by *O*-acetyl groups, the results in Fig. 1 show that the native polymer is composed of 2,3,6-tri-*O*-acetyl, 2,3-di-*O*-acetyl, 2-*O*-acetyl, and 3-*O*-acetyl derivatives of both glucose and mannose. The alditol acetate from 3-*O*-acetylglucose would be masked by that from mannose under the conditions used. The polymer also contains unsubstituted glucose and mannose residues.

O-Acetyl groups in acetylated glucomannans previously studied[3-5,7,10] are mainly located at positions 2 and 3. Only one example of an *O*-acetyl group at position 6 has been reported[13], namely, for the Lilium-A-glucomannan. This polymer contained 3,6-di-*O*-acetylmannose residues. There has been no previous report of the presence of 2,3,6-tri-*O*-acetyl-glucose or -mannose residues in a naturally occurring, acetylated polysaccharide.

Material *C* was subjected in sequence to chlorine oxidation, treatment with alkali, and mild hydrolysis with acid[7]. This procedure degraded all sugar units carrying *O*-acetyl groups. Isolated, unsubstituted sugar units, or sequences of such sugar units, survive. The material only partly dissolved at the various reaction stages, and the insoluble material was removed before proceeding to the next stage. Thus, the results cannot be regarded as quantitative.

Gel filtration on BioGel P2 of the degraded material (*E*) gave, after hydrolysis, glucose as the only sugar present in the compound eluted with the void volume, and a mixture of glucose and mannose, in the ratio 7.8:1, as monosaccharides in the last peak. These results indicate that the distribution of acetylated sugar units in the original polymer is random. Certain areas consist of contiguous, unsubstituted sugar units, whereas others have a single unsubstituted sugar unit next to acetylated units.

EXPERIMENTAL

Concentrations were performed under diminished pressure at 40°. Dialysis was carried out against distilled water. Carbohydrate contents were determined by the phenol–sulphuric acid method[20]. I.r. spectra were recorded on a Beckman IR-20 spectrophotometer using potassium bromide discs. Paper chromatography (p.c.) was carried out on Whatman Nos. 1 and 3MM papers with *A*, 1-butanol–ethanol–water (40:11:19); *B*, ethyl acetate–pyridine–water (8:2:1); *C*, 1-butanol–pyridine–water (6:4:3); and detection with aniline oxalate.

Methanolysis was performed by heating the sample (2 mg) with M or 6M

hydrogen chloride in methanol (0.5 ml) at 80° for 24 h. The product was then prepared for analysis by g.l.c. as the *O*-trimethylsilyl derivatives, as described by Reinhold[15], with mannitol (0.17 mg) as the internal standard.

Methylation was performed by the modified Hakomori method[21]. The methylated polymer was hydrolysed, and the partly methylated monosaccharides were converted[16] into the corresponding alditol acetates for analysis by g.l.c. and g.l.c.–m.s.

G.l.c. was performed on a Varian 1400 gas chromatograph fitted with a flame-ionization detector and glass columns (400 × 0.2mm) of (*a*) 3% of SE 52 on Varaport 30, (*b*) 3% of OV-225 on Varaport 30, or (*c*) 10% of SP-1200/1% H_3PO_4 on Chromosorb W (80/100 mesh).

G.l.c.–m.s. was performed with column (*b*) fitted in a Varian 1400 gas chromatograph coupled to a Varian CH-7 low-resolution mass spectrometer. The instrumental details are published elsewhere[22].

Isolation of the polymer. — Leaves from *Aloe plicatilis* Miller consist of two distinct parts; the chlorophyll-containing layer, which is 2–3 mm thick, and an interior part consisting of a white transparent jelly. The inner part was used for the present investigation.

The first portion was suspended in water, dialysed, and freeze-dried to give A_1. The other portion was dissolved in methyl sulphoxide, and the polymer was recovered by freeze-drying after dialysis. During dialysis, the polymer precipitated as a jelly (A_2) inside the dialysis bag. A_1 and A_2 were incompletely soluble in water and were similar; 1244 g of jelly gave 4.2 g of freeze-dried polymer.

Investigation of the polymer. — (*a*) *Alkali-treatment.* Polymer (0.5 g) was added to 20% sodium hydroxide (20 ml) at 20°, and nitrogen was bubbled through the stirred solution. After ~1 h, the polymer had dissolved. The solution was dialysed and freeze-dried to give the deacetylated product (0.4 g).

(*b*) *Incubation with* α-D-*galactosidase.* Samples (2 mg) of the native and deacetylated polymer were separately incubated with a suspension (0.1 ml) of α-D-galactosidase (α-D-galactoside galactohydrolase, EC 32.1.22, from green coffee beans, Boehringer) in 0.1M acetate buffer (1 ml, pH 5.1) at 37° for 15 h, 1 drop of toluene being added to prevent microbial growth. The enzyme activity was destroyed by keeping the mixture in boiling water for 2 min. The cooled mixture was deionised with Zerolit DMF (CO_3^{2-}) resin and concentrated, and the residues were examined by p.c. (solvents *A*–*C*) for the presence of free galactose.

(*c*) *Determination of the molecular weight.* The molecular weight was determined by gel filtration on a column (3.0 × 40 cm) of Sepharose 4B by elution with 25mM Tris–HCl buffer (pH 7.2). Fractions of 3 ml were collected. The column was calibrated against dextrans of known molecular weight (Dextran T-series from Pharmacia).

(*d*) *Determination of the component sugars.* After drying *in vacuo* over phosphorus pentaoxide, the polymer was treated with 6M methanolic hydrogen chloride, and the resulting methyl glycosides were trimethylsilylated, and analysed by g.l.c.

on column (*a*) using a temperature programme starting at 140° and increasing at 2°/min for 10 min, and then at 4°/min.

(*e*) *Determination of* O-*acetyl groups.* The native material had i.r. bands at 1250 and 1735 cm^{-1} which were absent from the spectrum of the alkali-treated material. The *O*-acetyl content was determined by (*a*) the hydroxamate test[23] modified as follows: after the addition of the alkaline hydroxylamine reagent, the test solutions were kept at 100° for 15 min in order to obtain a quantitative reaction; (*b*) by g.l.c.[10]: the polymer (2.4 mg) was hydrolysed for 2 h with M hydrochloric acid (0.1 ml) at 100° in a sealed tube. Propionic acid was added as the internal standard. The hydrolysate was analysed by g.l.c. on column (*c*).

(*f*) *Methylation.* Complete methylation proved to be difficult. When the modified Hakomori methylation procedure was applied twice to the polymer, complete methylation was achieved. The fully methylated polymer was hydrolysed at 100° with 90% formic acid (1 ml) for 6 h in a sealed tube. After the addition of water (3 ml), heating at 100° was continued for 2 h. Formic acid was removed by evaporation, and the partly methylated monosaccharides were analysed by g.l.c.–m.s. after conversion into the corresponding alditol acetates.

(*g*) *Acetalation and deacetylation.* A solution of the native polymer (100 mg) and toluene-*p*-sulphonic acid (20 mg) in methyl sulphoxide (20 ml), in a serum flask with a rubber cap, was cooled to 15° and kept at this temperature. Methyl vinyl ether (10 ml) at −50° was added and the mixture was stirred for 4 h at 15° to give a clear, orange solution. Excess of methyl vinyl ether was removed in a stream of nitrogen, and the mixture was dialysed against distilled water, and then concentrated to dryness. The i.r. spectrum of the product (*B*) showed no absorption for hydroxyl groups.

A suspension of *B* (50 mg) in methanol (5 ml) was filtered, and treated with 0.25M methanolic sodium methoxide (5 ml) with stirring. The mixture was kept at 80° for 4 h, and then cooled, dialysed, and concentrated to dryness to give *C*, the i.r. spectrum of which indicated the presence of small amounts of hydroxyl groups; 5 mg of *C* was subjected to methylation analysis.

(*h*) *Oxidation and degradation of the acetalated, deacetylated polymer.* Methyl sulphoxide (10 ml) was added to a M solution (25 ml) of chlorine in dichloromethane at −45° with vigorous stirring. A white precipitate formed. A suspension of *C* in dichloromethane (10 ml) was added and the mixture was stirred at −45° for 5 h. Triethylamine (8 ml) was added, and the mixture was left for 10 min, allowed to attain room temperature, dialysed overnight against 1% pyridine in water, and concentrated to dryness to give *D*.

To a solution of *D* in dichloromethane (6 ml), M ethanolic sodium ethoxide (3 ml) was added. The mixture was stirred at room temperature for 1 h, neutralised with acetic acid, and concentrated to dryness. The residue was dissolved in methanol and treated with Dowex 50 (H^+) resin to pH 4, and the solution was then filtered and concentrated to dryness. 50% Acetic acid (10 ml) was added to the residue, and the mixture was stored overnight at 100° in a sealed vessel, cooled, filtered, and

concentrated to dryness. The residue was partitioned between chloroform and water, and the aqueous phase was freeze-dried to yield *E* (25 mg).

A portion (0.1 ml) of a solution of *E* in water (0.2 ml) was applied to a column (45 × 0.5 cm) of Biogel P2 and eluted with water. Fractions (10 drops) were collected and tested for carbohydrate (phenol–sulphuric acid method). The first peak came with the void volume and was followed by a second monosaccharide peak. Each fraction was subjected to methanolysis, and the products were converted into the *O*-trimethylsilyl derivatives prior to analysis by g.l.c. on column (*a*).

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REFERENCES

1 E. Roboz and A. J. Haagen-Smit, *J. Am. Chem. Soc.*, 70 (1948) 3248–3249.
2 V. E. Tyler, L. R. Brady, and J. E. Robbers, *Pharmacognosy*, Lea and Febiger, Philadelphia, 7th edition, 1976, pp. 81–83.
3 H. Meier, *Acta Chem. Scand.*, 15 (1961) 1381–1385.
4 G. Katz, *Tappi*, 48 (1965) 34–41.
5 B. Lindberg, K. G. Rosell, and S. Svensson, *Sven. Papperstidn.*, 10 (1973) 383–384.
6 M. Kolera and C. Achtardjieff, *Pharmazie*, 30 (1975) 111–113.
7 L. Kenne, K. G. Rosell, and S. Svensson, *Carbohydr. Res.*, 44 (1975) 69–76.
8 E. Hägglund, B. Lindberg, and J. McPherson, *Acta Chem. Scand.*, 10 (1956) 1160–1164.
9 D. H. Juers, H. A. Swenson, and S. F. Kurath, *J. Polym. Sci.*, 5 (1967) 361–375.
10 M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. Pharm. Bull.*, 22 (1974) 2710–2713.
11 A. J. Buchala, G. Franz, and H. Meier, *Phytochemistry*, 13 (1974) 163–166.
12 T. Matsuo and T. Mizuno, *Agric. Biol. Chem.*, 38 (1974) 465–466.
13 M. Tomada, S. Kaneko, and S. Nakatsuka, *Chem. Pharm. Bull.*, 23 (1975) 430–436.
14 R. L. Whistler and E. L. Richards, in W. Pigman and D. Horton (Eds.), *The Carbohydrates*, 2nd edition, Academic Press, New York, Vol. IIA, 1972, pp. 461–462.
15 V. N. Reinhold, *Methods Enzymol.*, 25 (1972) 244–249.
16 B. Smestad, A. Haug, and S. Myklestad, *Acta Chem. Scand. Ser. B*, 28 (1974) 662–666.
17 H. O. Bouveng, *Acta Chem. Scand.*, 15 (1961) 96–100.
18 T. Koshijima, *Nippon Mokuzai Gakkaishi*, 6 (1960) 194–198.
19 A. N. de Belder and B. Norrman, *Carbohydr. Res.*, 8 (1968) 1–6.
20 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
21 S. I. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
22 E. Jellum, D. Stokke, and L. Eldjarn, *Clin. Chem.*, 18 (1972) 800–809.
23 S. Hestrin, *J. Biol. Chem.*, 180 (1949) 249–261.