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## Note

# Structural studies of polysaccharides from *Aloe saponaria* and *Aloe vanbalenii*

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The mucilaginous jelly from *Aloe plicatilis* Miller[1] is a linear, (1→4)-linked glucomannan having a glucose-to-mannose ratio of 1 2 8, and that of *Aloe vera*[2] is a mixture of partially acetylated glucomannans that differ in their glucose-to-mannose ratios The mucilaginous jelly from the leaves of *Aloe arborescens* var *natalensis*[3] is a partially acetylated, (1→4)-linked β-D-mannan and is reported to have antitumour activity in mice In view of the medicinal importance[4,5] of the plants belonging to the genus *Aloe*, other species have been investigated, and this report deals with the chemical nature of the mucilaginous jellies from the leaves of *Aloe saponaria* and *Aloe vanbalenii*

Extraction of the mucilaginous jelly from the leaves of *Aloe saponaria* with 50% aqueous ethanol, followed by precipitation with ethanol, gave a polysaccharide mixture in 0 45% yield based on the total weight of the fresh leaves This was composed of glucose and mannose in the ratio ~1 10 together with traces of galactose, arabinose, and xylose

Fractionation of the polysaccharide mixture by graded precipitation from aqueous solution with ethanol gave fractions AS1 and AS2 in 15 9 and 77 3% yields, respectively, based on the total polysaccharide The polysaccharide AS2, which consisted of mannose together with traces of glucose, was purified by two more precipitations from aqueous solution with ethanol to give a pure mannan Further fractionation of AS1 with 0 05M sodium tetraborate gave polysaccharides AS1a and AS1b The nature and compositions of these fractions are given in Table I Since fraction AS1a was insoluble in water, and both AS1a and AS1b were obtained in low yields, they were not further studied

Extraction of the mucilaginous jelly from the leaves of *Aloe vanbalenii*, as described above, gave a polysaccharide mixture in 0 25% yield based on the total weight of the fresh leaves This was composed mainly of mannose together with small proportions of glucose and galactose Fractionation of this polysaccharide mixture, as in the case of *Aloe saponaria* polysaccharides, gave two minor fractions (AV1a and AV1b) and a major fraction (AV2) The polysaccharide AV2, which consisted of

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TABLE I

COMPOSITION OF THE POLYSACCHARIDES FROM *Aloe saponaria* AND *Aloe vanbalenii*

| *Polysaccharide fraction* | *Physical appearance* | *Yield (%)*[a] | *Solubility in water* | *O-acetyl content*[b] | *Sugar composition* |
|---|---|---|---|---|---|
| *Aloe saponaria* | | | | | |
| AS1a | Coarse, grey, fibrous powder | 7 1 | Insoluble | 0 | Glc, traces of Gal, Ara, and Xyl |
| AS1b | Greyish powder | 6 8 | Soluble | 0 | Man Glc Gal = 1 25 1 0 0 25 |
| AS2 | White powder | 77 3 | Soluble | 20 7 | Man |
| *Aloe vanbalenii* | | | | | |
| AV1a | Greyish powder | 1 7 | Insoluble | 0 | Glc, traces of Gal |
| AV1b | Greyish powder | 7 5 | Soluble | 0 | Man Glc Gal = 1 0 1 0 0 5 |
| AV2 | White powder | 82 0 | Soluble | 19 5 | Man |

[a]Based on the total polysaccharide [b]Determined by saponification

mannose and traces of glucose and galactose, gave a pure mannan on two more precipitations from aqueous solution with ethanol The nature and compositions of these fractions are given in Table I

Thus, it is evident that the mucilaginous jelly from each of these *Aloe* species consists mainly of a mannan It is interesting to note that these mannans have a high content of *O*-acetyl (d s 0 87 and 0 81, respectively, for AS2 and AV2) The highest d s previously reported[2] was 0 78 for an acetylated mannan from *Aloe vera*

Hakomori methylation[6] of AS2 and AV2, followed by g l c analysis of the derived, partially methylated sugars as their alditol acetates[7], indicated the presence of only 2,3,6-tri-*O*-methylmannose in each case These results indicate that the *Aloe saponaria* and *Aloe vanbalenii* mannans are linear and contain (1→4)-glycosidic linkages After periodate oxidation[8] of each mannan and acid hydrolysis of the product, no intact sugars remained, indicating the absence of branch points and supporting the results of methylation analysis

## EXPERIMENTAL

*General methods* — The leaves of locally available *Aloe saponaria* and *Aloe vanbalenii* were used 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), and *C*, ethyl acetate–pyridine–water (6 4 3) Detection was effected with *p*-anisidine hydrochloride[9] and alkaline silver nitrate[10] G l c was performed on a Willy Giede GCHF 18 3 gas chromatograph fitted with a flame-ionisation 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

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 contents were determined by the saponification method[11]

Unless otherwise stated, all acid hydrolyses were performed with 0 5M sulphuric acid 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 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* — The mucilaginous jelly was scraped out from the leaves of *Aloe saponaria* (1 kg) and extracted with 50% aqueous ethanol (1 litre) The extract was centrifuged to remove suspended impurities, and ethanol (6 vol ) was added to the supernatant solution The precipitate was collected, and dissolved in 20mM hydrochloric acid (20 ml), and the solution was dialysed for 48 h against distilled water and then poured into excess of ethanol The precipitated polysaccharide mixture was collected and dried, yield, 445 mg Acid hydrolysis of this polysaccharide followed by p c indicated the presence of glucose and mannose in the ratio ~1 10, together with traces of galactose, arabinose, and xylose No acidic sugars could be detected on paper chromatograms

Similar extraction of mucilaginous jelly from *Aloe vanbalenii* leaves (1 kg) gave a polysaccharide mixture (250 mg), acid hydrolysis of which gave (p c ) mainly mannose together with small proportions of galactose and glucose

*Fractionation of the polysaccharides* — To a solution of *Aloe saponaria* polysaccharide (440 mg) in water (100 ml) was added ethanol (200 ml), and the precipitate (AS1, 70 mg) was collected More ethanol (400 ml) was added to the solution, and the precipitate (AS2, 340 mg) was collected Acid hydrolysis of AS2 gave (p c ) mainly mannose together with traces of glucose The polysaccharide AS2 was dissolved in water (100 ml), ethanol (2 vol ) was added, and the small amount of precipitate was removed The polysaccharide was precipitated by the further addition of ethanol (4 vol ) Repetition of this procedure afforded a pure mannan, acid hydrolysis of which gave (p c ) only mannose

A solution of AS1 (70 mg) in water (10 ml) was mixed with 0 1M sodium tetraborate (10 ml) The precipitated polysaccharide (AS1a) was collected and then suspended in 5% aqueous acetic acid (5 ml) and dialysed The polysaccharide (32 mg) was recovered by centrifugation after the addition of ethanol (10 ml) The supernatant solution was acidified with 50% aqueous acetic acid and dialysed, and ethanol (2 vol ) was added to give polysaccharide AS1b (30 mg)

The nature and compositions of these polysaccharides are given in Table I

Similar fractionation of *Aloe vanbalenii* polysaccharide (240 mg) gave the fractions AV1a (4 mg), AV1b (18 mg), and AV2 (205 mg) The polysaccharide AV2 (consisting mainly of mannose, together with traces of galactose and glucose) was

purified, as in the case of AS2, to give a pure mannan The nature and compositions of these polysaccharides are given in Table I

*Methylation analysis of the mannans* — The polysaccharides AS2 and AV2 (10 mg of each) were methylated by the Hakomori method[6] Each 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 5M sulphuric acid for 10 h on a boiling-water bath The resulting, partially methylated sugar(s) were converted into the alditol acetate(s) and analysed by g l c , which indicated the presence of only 2,3,6-tri-*O*-methylmannose in each polysaccharide

*Periodate oxidation of the mannans* — Separate solutions of the polysaccharides AS2 and AV2 (20 mg of each) in M sodium hydroxide (2 ml) were kept at 60° for 3 h and then overnight at room temperature Each solution was dialysed and then mixed with an equal volume of 0 1M sodium metaperiodate The oxidation was allowed to proceed at room temperature in the dark for 148 h Each solution was then treated with ethylene glycol (0 2 ml), dialysed, and treated with sodium borohydride Acid hydrolysis of the periodate-oxidised materials followed by p c indicated the absence of intact sugars

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