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## Note

# Structural studies of a neutral polysaccharide from the root bulb of *Mirabilis jalapa*

BIMALENDU RAY, PRADYOT K. GHOSAL, SWAPNADIP THAKUR, AND SUBHENDU G. MAJUMDAR
*Department of Chemistry, Natural Products Laboratory, The University of Burdwan, Burdwan 713 104 (India)*

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The medicinal plant[1] *Mirabilis jalapa* Linn. (Nyctaginaceae) grows abundantly in India, and its root and seed are widely used for treating syphilitic sores, reducing inflammation, and as a purgative. The structure of the polysaccharide obtained from the seed cotyledon has been investigated[2] and we now report on a D-glucan isolated from the root bulb.

Extraction of the shredded root bulb with water gave a polysaccharide which, on chromatography on DEAE-cellulose, gave fractions MJ-1/2 (eluted with water) and MJ-3/4 (eluted with 0.05M sodium hydroxide). The major fraction MJ-1 has $[\alpha]_{589.6}^{26}$ +33° (*c* 0.1, water), constituted 43% of the polysaccharide mixture, gave a single peak on gel filtration through Sephadex G-100, and was homogeneous in high-voltage paper electrophoresis.

Complete acid hydrolysis of the polysaccharide gave D-glucose only, and Hakomori methylation[3] followed by conventional methylation analysis gave 2,3,4,6-tetra-*O*-methyl- (0.9 mol), 2,3,6-tri-*O*-methyl- (8 mol), and 2,4,6-tri-*O*-methyl-D-glucose (26 mol), analysed by g.l.c. of the alditol acetates[4]. The results indicated the D-glucan to be linear and to contain (1→3) and (1→4) linkages.

The glucan consumed[5,6] 0.3 mol of periodate per glucosyl residue during 12 h, which accorded with the value expected on the basis of the results of methylation analysis. On Smith degradation of the glucan, 75% of the glucosyl residues survived. A second Smith-degradation gave a product that could be precipitated with ethanol from aqueous solution. These results indicated the presence of an extended sequence of (1→3)-linked glucosyl residues.

Graded acid hydrolysis of the glucan gave five fractions which were characterised conventionally (see Experimental), and I–V were shown to have the structures in Table I.

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TABLE I

STRUCTURE OF THE PRODUCTS OF PARTIAL ACID HYDROLYSIS OF THE GLUCAN FROM *Mirabilis jalapa*

| *Compound* | $[\alpha]_D^{26}$ *(water) (degrees)* | $R_{GLC}$ | $M_G$ | *Products of methylation analysis (mol)*[a] | | | *Proposed structure* |
|---|---|---|---|---|---|---|---|
| | | | | *2,3,4,6-Glc* | *2,3,6-Glc* | *2,4,6-Glc* | |
| I | +53 | 1 | 1 | 1 | | | D-Glucose |
| II | +22 | 0.72 | 0.67 | 0.95 | | 1 | Glc-(1→3)-Glc |
| III | −13 | 0.53 | 0.65 | 0.9 | 1 | 1 | Glc-(1→4)-Glc-(1→3)-Glc |
| IV | +2 | 0.22 | 0.61 | 0.9 | | 4 | Glc-[(1→3)-Glc]$_2$-(1→3)-Glc |
| V | +5 | 0.1 | 0.25 | 0.9 | 1 | 5 | Glc-[(1→3)-Glc]$_5$-(1→4)-Glc |

[a] 2,3,4,6-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, etc.

EXPERIMENTAL

*General.*—Solvents were removed under diminished pressure at < 50°. Optical rotations were determined with a Perkin–Elmer Model 241 MC spectropolarimeter. Ascending p.c. was performed on Whatman No. 1 and 3 MM papers, using *A*, the upper layer of 4:1:5 1-butanol–acetic acid–water; *B*, 4:1:5 1-butanol–ethanol–water; *C*, 8:3:1 ethyl acetate–pyridine–water; and *D*, 6:4:3 1-butanol–pyridine–water; and detection with alkaline silver nitrate and aniline oxalate. T.l.c was performed on Silica Gel G (Merck), using solvents *B* and *C*, and detection with anisaldehyde–sulphuric acid and aqueous 2% sulphosalicylic acid[7]. I.r. spectra were recorded with an Acculab 10 Beckman instrument for KBr pellets. High-voltage paper electrophoresis at 2° was conducted with a Labor Model No. OE 201 instrument, on Whatman No. 1 and 3MM papers using borate buffer (pH 9.5). Periodate oxidation was monitored at 225 nm with a Beckman Model 26 spectrophometer. G.l.c. was conducted on Packard Model 419 and Hewlett-Packard Model 5713 A gas chromatographs each equipped with a flame-ionisation detector, and columns of *1*, 3% of ECNSS-M on Gas Chrom Q (100–200 mesh); and *2*, 3% of OV-225 on Gas Chrom Q (100–200 mesh). G.l.c.–m.s. was conducted with a Hewlett-Packard 5895 instrument, using *3*, a fused-silica capillary column (30 m × 0.25 mm) coated with a 0.2 μm film of OV-1. The ionisation potential was 70 eV and the temperature of the ion source was 200°.

*Isolation and fractionation of the polysaccharide.* — Finely shredded root bulb (50 g) of *Mirabilis jalapa* was stirred vigorously with cold water (1.5 L) for 3 h, and then centrifuged, and the supernatant solution was diluted with cold ethanol (5 L). The precipitate was collected by centrifugation, washed four times with acetone, and dried. A solution of the polysaccharide in water (200 mL) was diluted with ethanol (600 mL), and the precipitate was collected by centrifugation. This process was repeated thrice. A portion (100 mg) of the resulting polysaccharide (5 g) was then eluted from a column (75 × 2.5 cm) of DEAE-cellulose with water (500 mL) and then 0.05M sodium hydroxide (500 mL). Fractions (5 mL) were assayed for carbohydrate by the phenol–sulphuric acid method[8]. Four fractions (MJ-1/4) were obtained, of which MJ-1, $[\alpha]_{589.6}^{26}$ +33° (*c* 0.1, water), constituted 43% of the polysaccharide and was used in the subsequent studies.

Gel filtration in borate buffer (pH 9.5), through a column (79 × 2.5 cm) of Sephadex G-100, and high-voltage paper electrophoresis at 2° in the same borate buffer showed MJ-1 to be homogeneous.

*Acid hydrolysis.*— The polymer (10 mg) was hydrolysed with 0.5M sulphuric acid (5 mL) for 16 h at 100° in a sealed tube, and 15 mg of *myo*-inositol was added as internal standard. The hydrolysate was neutralised with barium carbonate, deionised with Amberlite IR-120 ($H^+$) resin, and concentrated. T.l.c. (solvents *B* and *C*) and p.c. (solvents *A*, *C*, and *D*) of the hydrolysate revealed glucose, the identity of which was confirmed by g.l.c. (columns *1* and *2*) of the derived alditol acetate. The D configuration was indicated by the $[\alpha]_{589.6}^{26}$ value [+53° (*c* 0.2,

water)] of the sugar isolated by preparative p.c. (solvent *D*).

*Methylation analysis.*— The glucan (10 mg) was methylated by the method of Hakomori[3] and the product showed no i.r. absorption for hydroxyl. The methylated polymer was treated with aqueous 90% formic acid (5 mL) for 2 h at 100°, and the product was isolated and hydrolysed with 0.5M sulphuric acid (5 mL) for 12 h at 100°. The partially methylated sugars were converted into their alditol acetates and analysed by g.l.c. (columns *1* and *2*).

*Periodate oxidation.* — (*a*) The glucan was treated with 0.04M sodium metaperiodate in the dark at 5° and the consumption of oxidant, monitored spectrophotometrically[5,6], became constant (0.3 mol per glucosyl residue) after 12 h.

(*b*) The glucan (80 mg) was oxidised with 0.04M sodium metaperiodate (200 mL) in the dark for 13 h at 5°, and the product was isolated conventionally and reduced with sodium borohydride (800 mg). A part (6 mg) of the product (67 mg) was hydrolysed (together with an internal standard) with 0.5M sulphuric acid (5 mL) for 16 h at 100°. G.l.c. (column *2*) of the derived alditol acetate indicated that 75% of the glucose was resistant to periodate. The remaining part (61 mg) was treated with 0.5M sulphuric acid for 24 h at room temperature. The hydrolysate was neutralised with $BaCO_3$ and centrifuged, and the supernatant solution was decationised with Amberlite IR-120 ($H^+$) resin and concentrated. The residue was subjected to a second Smith-degradation and the final solution was added to 500 mL of cold ethanol. The precipitate was collected by centrifugation and dried (yield, 1 mg).

*Graded acid hydrolysis.* — A solution of the glucan (200 mg) in aqueous 40% formic acid (50 mL) was heated for 4 h at 100°. The formic acid was removed under diminished pressure by co-distillation with water. P.c. (Whatman No.3MM paper, solvent *D*) of the hydrolysate gave homogeneous fractions I–V (Table I). To establish the sequence of linkages in the oligomers, they were subjected to paper electrophoresis in borate buffer[9,10]. The results are given in Table I. The oligomers were then subjected to conventional methylation analysis. The resulting partially methylated alditol acetates were analysed[4,11,12] by g.l.c. (columns *1* and *2*) and g.l.c.-m.s. (column *3*). The results are presented in Table I.

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