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Note

Structural studies of a hemicellulose from the floss of *Calotropis gigantea*

D Channe Gowda and Yernool V Anjaneyalu
Department of Post-Graduate Studies and Research in Chemistry, University of Mysore, Manasa Gangothri, Mysore-570 006 (India)

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The hemicelluloses of kapok[1] (*Ceiba pentandra*) and milkweed[2] (*Asclepias syriaca*) floss are 4-*O*-methyl-D-glucuronoxylans that contain a linear chain of (1→4)-linked β-D-xylopyranosyl residues having single 4-*O*-methyl-D-glucopyranosyluronic acid groups attached to O-2 Similar hemicelluloses have been reported from other sources[3–5] We now report on the structural features of a hemicellulose isolated from the floss (seed hairs) of *Calotropis gigantea*, which have an important role in the wind-dispersal of seeds

The seed hairs of *Calotropis gigantea* were treated with benzene–ethanol and then with water, and the fibres were extracted with aqueous potassium hydroxide in a nitrogen atmosphere The hemicellulose was precipitated from the alkaline extract by acidification to pH 4 5–5 0 with acetic acid The product was dissolved in aqueous sodium hydroxide and, after centrifugation, reprecipitated with acetone The polysaccharide was further purified by precipitation with Fehling's solution Two more precipitations with Fehling's solution did not change the specific rotation, $[\alpha]_D$ −106°, and sugar composition (D-xylose 88%, and 4-*O*-methyl-D-glucuronic acid 12%) The $[\alpha]_D$ value indicated a preponderance of β-D-glycosidic linkages in the polysaccharide

Partial hydrolysis of the polysaccharide and preparative paper chromatography gave an aldobiouronic acid that was identified as 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylose

The hemicellulose was methylated in sequence by the procedures of Haworth[6], Falconer and Adams[7], and Purdie[8], to give a permethylated product that showed no i r absorption for hydroxyl The methylated polysaccharide was hydrolysed first with formic acid and then with aqueous sulphuric acid The hydrolysate was separated into neutral and acidic fractions The neutral, partially methylated sugars were converted into the alditol acetates, and g l c [9,10] revealed the presence of alditol acetates of 2,3,4-tri-*O*-methylxylose (1 5%), 2,3-di-*O*-methylxylose (90%), and 3-*O*-methylxylose (8 5%) The partially methylated, acidic sugar was converted into its methyl ester methyl glycoside, which was then reduced with sodium borohydride The

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resulting neutral sugar was identified by conversion into the alditol acetate and by g l c , which showed the presence of mainly 2,3,4-tri-*O*-methylglucose derived from 2,3,4-tri-*O*-methylglucuronic acid

On the basis of the methylation analysis, it is concluded that the hemicellulose contains a linear chain of (1→4)-linked *β*-D-xylopyranosyl residues, and that one in every ten D-xylosyl residues has a 4-*O*-methyl-D-glucosyluronic acid group attached to O-2 The results of periodate-oxidation studies[11] accord with such a structure The polysaccharide consumed 1 03 mol of periodate per mol of pentosyl residue Acid hydrolysis of the periodate-oxidised polysaccharide gave (paper chromatography) mainly glycerol together with small proportions of xylose and unidentified components

EXPERIMENTAL

General methods — Descending paper chromatography (p c) was performed on Whatman No 1 and 3MM papers with *A*, 1-butanol–benzene–pyridine–water (5 1 3 3, upper layer), *B*, ethyl acetate–pyridine–water (8 2 1), *C*, 1-butanol–acetic acid–water (4 1 5, upper layer), and detection with *p*-anisidine hydrochloride[12] and alkaline silver nitrate[13] Total carbohydrate was determined by the phenol–sulphuric acid method[14], and uronic acid by the carbazole method[15]

Acid hydrolysis was performed with 0 5M sulphuric acid for 8–10 h at 100° The hydrolysates were neutralised with barium carbonate, filtered, deionised with Amberlite IR-120 (H^+) and IRA-400 (CO_3^{2-}) resins, and concentrated under diminished pressure The residues were examined by p c , and also analysed by g l c after conversion into the alditol acetates The Amberlite IRA-400 (CO_3^{2-}) column was eluted with 2M formic acid, the eluate evaporated to dryness, and the residue examined by p.c The enantiomeric identity of the sugars was determined by isolating them by preparative p c and measuring their optical rotations

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 the carrier gas

Extraction of the hemicellulose — The seed hairs of *Calotropis gigantea* were exhaustively treated with benzene–ethanol (2 1) and then with cold water The defatted fibers (25 g) were extracted with 6% aqueous potassium hydroxide (500 ml), in an atmosphere of nitrogen, according to the method of Whistler and Feather[16] The alkaline slurry was filtered through a cloth, and the residue was washed with water The combined extract and washings were cooled in an ice-bath, and the hemicellulose was precipitated by acidification to pH 4 5–5 with 50% aqueous acetic acid The precipitate was collected by centrifugation, washed with water (at pH 4 5), ethanol, and acetone, and then dried, to yield the crude polysaccharide (5 5 g) A solution of the crude hemicellulose in M sodium hydroxide (100 ml) was centrifuged, and the supernatant solution was dialysed against running tap-water for 24 h and then against distilled water for 24 h The polysaccharide was precipitated by the addition

of acetone (4 vol), recovered by centrifugation, washed with acetone, and then dried over phosphorus pentaoxide *in vacuo*, yield, 4 8 g

Purification of the hemicellulose — To a solution of the hemicellulose (3 0 g) in 0 1M sodium hydroxide (100 ml) was added Fehling's solution until precipitation was complete The polysaccharide was collected by centrifugation, dispersed in 0 05M hydrochloric acid, and dialysed for 48 h against distilled water, and then ethanol (4 vol) was added The polysaccharide, which was collected by centrifugation, washed with ethanol and acetone, and then dried over phosphorus pentaoxide *in vacuo*, was a white, amorphous powder (2 4 g), $[\alpha]_D$ −106° (*c* 0 4, M sodium hydroxide) Two more precipitations of the polysaccharide with Fehling's solution, as described above, did not change its specific rotation or sugar composition (D-xylose 88%, and 4-*O*-methyl-D-glucuronic acid 12%)

Characterisation of the aldobiouronic acid obtained by partial hydrolysis of the hemicellulose. — A suspension of the hemicellulose (500 mg) in 0 25M sulphuric acid was kept for 6 h at 100° After the usual work-up, p c (solvent *C*) of the acidic sugars indicated the presence of an aldobiouronic acid (R_{GlcA} 0 72) in addition to 4-*O*-methyl-D-glucuronic acid Preparative p c gave the pure aldobiouronic acid (6 8 mg), acid hydrolysis of which gave xylose and 4-*O*-methylglucuronic acid in equal amounts After reduction of the aldobiouronic acid with sodium borohydride, acid hydrolysis gave 4-*O*-methylglucuronic acid, but no xylose The aldobiouronic acid (3 mg) was treated with 3% methanolic hydrogen chloride and heated under reflux for 5 h A portion of the resulting methyl ester methyl glycoside was reduced with sodium borohydride, and hydrolysis with acid then gave (p c) xylose and 4-*O*-methylglucose Another portion was subjected, in sequence, to periodate oxidation, borohydride reduction, and acid hydrolysis, and p c then indicated the absence of intact sugars, but the presence of glyceraldehyde in addition to other periodate-degradation products Hence, the aldobiouronic acid was 2-*O*-(4-*O*-methyl-D-gluco-pyranosyluronic acid)-D-xylose

Methylation analysis — The polysaccharide (400 mg) was methylated twice by the Haworth procedure[6] and then by the method of Falconer and Adams[7] with dimethyl sulphate and solid sodium hydroxide in dry tetrahydrofuran, and finally four times by the Purdie[8] method The resulting product (200 mg) showed no i r absorption for hydroxyl

The methylated product (20 mg) was hydrolysed with 90% formic acid in a sealed tube for 2 h, and then, after evaporation of the formic acid, with 0 5M sulphuric acid for 10 h at 100°. After the usual work-up, the neutral sugars were converted into the alditol acetates, g l c on 3% of ECNSS-M then revealed the alditol acetates of 2,3,4-tri-*O*-methylxylose (1 5%), 2,3-di-*O*-methylxylose (90%), and 3-*O*-methyl-xylose (8 5%) The acidic sugar was converted into its methyl ester methyl glycoside, reduced with sodium borohydride, and hydrolysed The resulting neutral sugar was converted into its alditol acetate, and g l c showed the presence mainly of the derivative of 2,3,4-tri-*O*-methylglucose

Periodate oxidation — The polysaccharide (100 mg) was oxidised with 0 05M

sodium metaperiodate (100 ml) in the dark at room temperature The periodate uptake became constant after 120 h, when 1 03 mol of periodate had been consumed per mol of pentosyl residue The reaction was then stopped by the addition of ethylene glycol (0 5 ml), the solution was dialysed against running tap-water for 24 h, and the product was reduced with sodium borohydride The resulting polyalcohol was hydrolysed with 0 5M sulphuric acid for 8 h at 100° P c of the hydrolysate revealed mainly glycerol together with small proportions of xylose and unidentified components

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