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SHORT COMMUNICATION

THE STRUCTURE OF A GALACTOMANNAN FROM THE SEEDS OF *DESMODIUM PULCHELLUM*

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Abstract—The galactomannan from the seeds of *Desmodium pulchellum* contains D-galactose and D-mannose in the ratio of 1:2. Hydrolysis of the methylated galactomannan gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose. Galactose has been found to constitute the end-group of the polysaccharide. The galactomannan contains α-linked D-galactopyranose combined with 1,4,6- and 1,4-β-linked D-mannopyranose units.

INTRODUCTION

Desmodium pulchellum was earlier examined by Ghosal and Mukherjee[1] who reported the presence of seven alkaloids. Tiwari, Srivastava and Shukla[2] examined the seed fat of the plant.

The seed polysaccharide has now been studied.

RESULTS

The purified polysaccharide $[\alpha]_D^{20°} + 60$ in 0·5 M NaOH was obtained as an amorphous white powder which analysed negatively for acetyl, methoxyl, pentosan and uronide. Hydrolysis gave galactose and mannose. On complete hydrolysis with 1 M H_2SO_4, the hydrolysate had $[\alpha]_D^{20°} + 36°$, indicating a 1:2 ratio for these two sugars,[3] which was confirmed by quantitative analysis. The first sugar to appear on partial hydrolysis with 0·2 M H_2SO_4 was galactose, indicating that this hexose may have occupied terminal positions in the polymer. The methylated polysaccharide[4,5] was hydrolysed to give 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose, identified by comparing R_g values and by preparing suitable derivatives. The galactomannan thus appears to be highly branched with galactose units occupying the terminal positions; the branch-points of the mannose units were at positions 1, 4 and 6.

On periodate oxidation, the polysaccharide consumed 3·2 moles of periodate for every three hexose residues, with the liberation of 1 mole of formic acid. These results showed that the galactomannan should contain 1 → 3 linkages between mannose units. Had this been the case, one of the methylated mannoses would have been 2,4,6-tri-*O*-methyl-D-mannose in place of 2,3,6-tri-*O*-methyl-D-mannose which, however, was not the case. This

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[3] C. M. RAFIQUE and F. SMITH, *J. Am. Chem. Soc.* 72, 4635 (1950).
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[5] T. PURDIE and J. C. IRVINE, *J. Chem. Soc.* 83, 1021 (1903).

discrepancy could possibly be accounted for by the presence of some mannose, which had survived oxidation and was found in the solution containing the oxidized products. The periodate oxidation was repeated using excess of sodium metaperiodate and the titration was done at longer intervals. This time, the same amount of polysaccharide reduced 4 moles of periodate for every three hexose residues and the solution containing the oxidized products was free of mannose. The resistance of combined mannose to periodate oxidation has been reported in several other galactomannans.[6-8]

From the above observations and from i.r. studies[9] of the galactomannan (which showed peaks at 815 and 876 cm^{-1}) it appears that the galactomannan of *Desmodium pulchellum* consists of a linear chain (1 → 4) of D-mannopyranose units with position 6 of one out of two mannose units being attached to galactose by an α (1 → 6) linkage. This is in agreement with the specific rotation of the polysaccharide, $[\alpha]_D^{20°} + 60°$ (0·5 M NaOH) and of its methylated derivative $[\alpha]_D^{20°} + 42{\cdot}2°$ ($CHCl_3$), when compared with values for related galactomannans of known structure.[7,8]

EXPERIMENTAL

All specific rotations are equilibrium values and all m.ps are uncorrected. Paper chromatography was carried out at room temperature by descent, using (1) *n*-BuOH–EtOH–H_2O–NH_4OH (45:5:49:1), (2) *n*-BuOH–HOAc–H_2O (4:1:5) and (3) *n*-BuOH–EtOH–H_2O (5:1:4).

Isolation and Purification

2 kg of crushed defatted seeds of *Desmodium pulchellum* were suspended in water acidified with 1% HOAc and the extract was concentrated in a rotary film evaporator, the polysaccharide being precipitated by adding 90% ethanol. The polysaccharide was repeatedly dissolved in water and reprecipitated with ethanol, until the ash content was reduced to a constant value (1·23 per cent). The polysaccharide was washed with ethanol and ether and dried (20 g). The purified polysaccharide (3 g) dissolved in water (100 ml); by fractional precipitation, two samples of polysaccharide were prepared which were hydrolysed with 2 N H_2SO_4 separately. The sugars released were quantitatively the same as those from a large-scale preparation, confirming its homogeneity. The purified polysaccharide was an amorphous white powder. Its aqueous solution gave an insoluble complex with Fehling's solution but did not reduce it, even on prolonged boiling, thus indicating the absence of free sugars.

Hydrolysis of the Galactomannan

The galactomannan (1 g) was hydrolysed with 100 ml of 2 N H_2SO_4 for 18 hr. Chromatographic examination of the hydrolysate in solvents A and B revealed the presence of D-mannose and D-galactose. The syrup obtained after the neutralization of the hydrolysate was eluted from the cellulose column with solvent A and two sugars were obtained: D-mannose, R_f 0·20 in solvent B, recrystallized from aq. methanol. It had $[\alpha]_D^{25°} + 12°$ (water) and melted at 126–127°; and D-galactose, R_f 0·15 in solvent B, recrystallized from 4% aq. methanol, m.p. 164–165°, and $[\alpha]_D^{25°} + 81{\cdot}7°$.

The molecular proportion of the sugars determined by periodate oxidation,[10] after separation in solvent A, was 1·00 (galactose):2·00 (mannose).

Methylation of Galactomannan

The polysaccharide (3 g) was methylated by the method of Parikh *et al.*[4] followed by Purdie's[5] method using MeI and AgO, when a yellowish thick syrup (3·2 g $[\alpha]_D^{20°} + 42{\cdot}2°$ ($CHCl_3$), methoxyl, 41·5%) was obtained.

[6] R. L. WHISTLER and J. Z. STENI, *J. Am. Chem. Soc.* 73, 4187 (1951).
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[8] P. ANDREWS, L. HOUGH and J. K. N. JONES, *J. Am. Chem. Soc.* 74, 4029 (1952).
[9] S. A. BARKER, E. J. BOURNE and D. H. WHIFFEN, in *Method of Biochemical Analysis* (edited by D. GLICK), Vol. 3, p. 213, Interscience (1956).
[10] E. L. HIRST and J. K. N. JONES, *J. Chem. Soc.* 1659 (1949).

Hydrolysis of Methylated Galactomannan

2·0 g of methylated galactomannan was hydrolysed with 30 ml of methanolic HCl (6%) and heated on a water-bath for 18 hr. The methylated sugars were separated on Whatman No. 1 paper using solvent A.

Fraction I. (200 mg). R_g in solvent A, 0·92 (found OCH_3, 51·8%), calculated for $C_{10}H_{20}O_6$, OCH_3, 52·5%). $[\alpha]_D^{25°} + 160°$ (C, 0·5 in water, lit.[3] $[\alpha]_D^{24°} + 162°$ (water). It gave a red colour with *p*-anisidine. The syrup (100 mg) recovered from the mother liquor afforded with boiling alcohol and aniline (40 mg of aniline) 2,3,4,6-tetra-*O*-methyl-D-galactose anilide, m.p. 185–186°, lit.[3] m.p. 186–188°.

Fraction II. (150 mg). R_g in solvent A, 0·83 (found OCH_3, 42·0%, calculated for $C_9H_{18}O_6$, OCH_3 41·9%) $[\alpha]_D^{25°} + 120°$ (C, 0·05 in water), lit.[3] for 2,3,6-tri-*O*-methyl-D-mannose $[\alpha]_D^{25°} + 15°$ in water (C, 1·0). On oxidation, 2,3,6-tri-*O*-methyl-D-mannose with Br_2 afforded 2,3,6-tri-*O*-methyl-D-mannose lactone, m.p. 82–83°, lit.[3] m.p. 79°.

Fraction III. (150 mg). R_g in solvent A, 0·62 (found OCH_3, 29·7%, calculated for $C_8H_{16}O_6$, OCH_3, 29·8%) $[\alpha]_D^{25°} + 20°$ in water (C_1 0·8), lit.[3] $[\alpha]_D^{25°} + 22°$ in water (C_1 0·7) for 2,3-di-*O*-methyl-D-mannose. This dimethyl-D-mannose (60 mg) afforded an anilide which failed to crystallize.

Periodate Oxidation

Periodate oxidation was performed according to the method of Andrews *et al.*[7] The results are summarized in the table below.

Time (hr)	0·5	5	10	15	20	48
Periodate reduced (ml of 0·01 N thiosulphate)	4·18	6·12	8·14	10·00	10·4	10·4
Acid liberated (ml of 0·01 alkali)	1·47	1·50	1·53	1·56	1·6	1·6

The periodate uptake after 20 hr assumes a constant value which corresponds to reduction of 1 mole of periodate per 192 g of polysaccharide. Similarly the titre value 1·6 ml of 0·01 N alkali indicated that, after 20 hr, 1 mole of formic acid was liberated per 625 g of polysaccharide.

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