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A NEW WATER-SOLUBLE POLYSACCHARIDE FROM THE SEEDS OF *Cassia multijuga*

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ABSTRACT

A polysaccharide consisting of D-galactose, D-mannose, and D-xylose in the molecular ratios 5:1:2 has been isolated from the defatted seeds of *Cassia multijuga*. Methylation analysis yielded 2,3-di-*O*-methyl-D-galactose (2 mol), 2,3,6-tri-*O*-methyl-D-galactose (4 mol), 2,3,4,6-tetra-*O*-methyl-D-galactose (4 mol), 2,3-di-*O*-methyl-D-mannose (2 mol), 2-*O*-methyl-D-xylose (1 mol), 2,3-di-*O*-methyl-D-xylose (2 mol), and 2,3,4-tri-*O*-methyl-D-xylose (1 mol). Periodate oxidation indicated 32.4% of end-groups and methylation indicated 31.2%. Partial hydrolysis with acid gave 6-*O*-α-D-galactosyl-D-galactose, 6-*O*-α-D-galactosyl-D-mannose, 4-*O*-β-D-galactosyl-D-xylose, and 3-*O*-β-D-xylosyl-D-xylose, together with monosaccharides. The polysaccharide is highly branched, consisting of galactosyl, mannosyl, and xylosyl residues in the main chain, with (1→4)-β linkages, and galactosyl and xylosyl end-groups.

INTRODUCTION

Species of *Cassia* (Leguminoseae) are rich sources of polysaccharide[1], and the plant possesses important medicinal properties. We now report on a new water-soluble polysaccharide isolated from the seeds.

RESULTS AND DISCUSSION

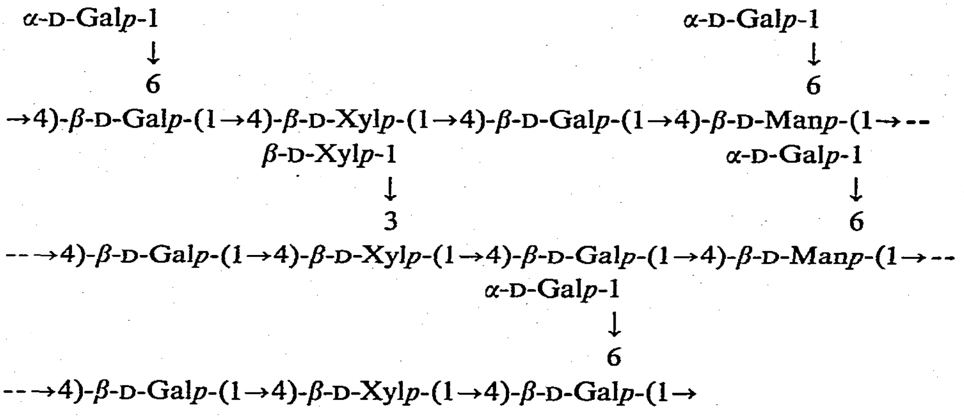
The polysaccharide was conveniently extracted from the defatted seeds with 1% acetic acid and precipitated with alcohol. Repetition of this process eventually gave a product having an ash content of 0.3%. Homogeneity of the polysaccharide was tested by fractional precipitation and by zone electrophoresis. The dry polysaccharide had $[\alpha]_D^{20}$ +61° (water), was soluble in water at room temperature, and had a negligible content of methoxyl and acetyl groups and uronic acid. Complete hydrolysis with acid yielded D-galactose, D-mannose, and D-xylose in the molar ratios 5:1:2. When graded hydrolysis with 25mM sulphuric acid was monitored by chromatography, galactose was found to be released first, followed by mannose and xylose simultaneously. Thus, the end-groups mainly consist of galactosyl groups.

Treatment of the polysaccharide by the Haworth method gave only a partly methylated polysaccharide, $[\alpha]_D^{24}$ +58° (chloroform). Subsequent treatment with the Purdie reagents gave a completely methylated product, $[\alpha]_D^{30}$ +60° (chloroform). Hydrolysis of the methylated polysaccharide gave 2,3-di-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-galactose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3-di-*O*-methyl-D-mannose, 2-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-D-xylose in the ratios 2:4:4:2:1:2:1. Thus, it is clear that the non-reducing terminal groups are composed mainly of D-galactosyl groups and a few D-xylosyl groups. Also, the ratio of 2,3-di-*O*-methyl-D-galactose or 2,3-di-*O*-methyl-D-mannose to the total of other methylated sugars was 2:14. Thus, two residues each of galactose and mannose are linked at positions 1, 4, and 6 in the main chain. Similarly, the formation of 2-*O*-methyl-D-xylose indicates that one xylose residue is linked through positions 1, 3, and 4 in the main chain.

The foregoing data are consistent with the simplest repeating-unit of the polysaccharide containing 16 sugar units, of which 1 xylosyl and 4 galactosyl units (31.2% of the total repeating-unit) are non-reducing terminal groups. Periodate oxidation of the polysaccharide liberated 0.2 mol of formic acid per 100 g of polysaccharide, indicating 32.4% of terminal groups; the galactose and mannose residues were completely oxidised in 48 h, whereas the xylose required 72 h. These data may be explained on the basis of xylose residues linked through position 3, a conclusion that is also confirmed by the methylation studies.

Partial hydrolysis of the polysaccharide with 0.05M sulphuric acid at 100° for 14 h gave 6-*O*-α-D-galactosyl-D-galactose, 6-*O*-α-D-galactosyl-D-mannose (epimelibiose), 4-*O*-β-D-galactosyl-D-xylose, and 3-*O*-β-D-xylosyl-D-xylose, in addition to galactose, mannose, and xylose.

Thus, it may be concluded that the main chain of the polysaccharide consists of (1→4)-linked residues of β-D-galactose, β-D-mannose, and β-D-xylose, and that α-D-galactosyl groups are (1→6)-linked to mannose residues. The following repeating-unit, which accords with the foregoing data and is tentatively assigned, should consume 20 mol of periodate with the liberation of 5 mol of formic acid per repeating-unit. The observed values are 19.8 mol of periodate and 4.95 mol of formic acid.



$$\begin{array}{ccccccccc}
 & \alpha\text{-D-Gal}p\text{-}1 & & & & & & \alpha\text{-D-Gal}p\text{-}1 & \\
 & \downarrow & & & & & & \downarrow & \\
 & 6 & & & & & & 6 & \\
\rightarrow 4)\text{-} & \beta\text{-D-Gal}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Xyl}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Gal}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Man}p & \text{-}(1\rightarrow \text{--} \\
 & & & \beta\text{-D-Xyl}p\text{-}1 & & & & \alpha\text{-D-Gal}p\text{-}1 & \\
 & & & \downarrow & & & & \downarrow & \\
 & & & 3 & & & & 6 & \\
\text{--}\rightarrow 4)\text{-} & \beta\text{-D-Gal}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Xyl}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Gal}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Man}p & \text{-}(1\rightarrow \text{--} \\
 & & & & & \alpha\text{-D-Gal}p\text{-}1 & & & \\
 & & & & & \downarrow & & & \\
 & & & & & 6 & & & \\
\text{--}\rightarrow 4)\text{-} & \beta\text{-D-Gal}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Xyl}p & \text{-}(1\rightarrow 4)\text{-} & \beta\text{-D-Gal}p & \text{-}(1\rightarrow & &
\end{array}$$

EXPERIMENTAL

All concentrations were carried out under diminished pressure at low temperature. Melting points are uncorrected and all optical rotations are equilibrium values. P.c. was performed at room temperature by the descending technique on Whatman No. 1 paper with *A*, 1-butanol–ethanol–water[2] (5:1:4); *B*, 1-butanol–2-propanol–water[3] (11:6:3); *C*, ethyl acetate–pyridine–water[4] (10:4:3); *D*, ethyl acetate–pyridine–water[5] (2:1:2); and *E*, ethyl acetate–pyridine–water[6] (5:2:7); and detection with aniline hydrogen phthalate.

Isolation of the polysaccharide. — The defatted seeds (500 g) of *Cassia multijuga* were extracted with light petroleum and ethanol, and then suspended in 1% acetic acid overnight. The mixture was filtered, and the product was precipitated from the filtrate by the addition of 3 vol. of ethanol. Dissolution and reprecipitation were repeated six times, to give a product (18 g) having minimum ash content (0.3%) and $[\alpha]_D^{20}$ +61° (water).

The homogeneity of the polysaccharide was checked by the following methods.

(*a*) *Fractional precipitation*[7]. A solution of the polysaccharide (2 g) in distilled water (200 ml) at 60° was treated first with 100 ml of ethanol, to give fraction A, and then with 1 litre of ethanol, to give fraction B. Fractions A and B had similar $[\alpha]_D$ values, and p.c. of their hydrolysates revealed identical compositions.

(*b*) *Zone electrophoresis*[8,9]. Electrophoresis of the polysaccharide was performed in 0.05M sodium tetraborate (pH 9.2) for 6 h at 320 volts and 3.7 mA. A plot of the absorbance against segment number showed a sharp peak, confirming the homogeneous nature of the polysaccharide.

Investigation of the polysaccharide. — (*a*) *Hydrolysis.* The purified polysaccharide (1 g) was hydrolysed with M H_2SO_4 at 100° for ~24 h. P.c. (solvent *C*) of the hydrolysate revealed galactose (R_F 0.15), mannose (R_F 0.21), and xylose (R_F 0.29). Identities and absolute configurations were confirmed by co-chromatography with authentic samples and preparation of derivatives: D-galactose phenylosazone, m.p. 164°, $[\alpha]_D^{30}$ +80° (water); D-galactose phenylhydrazone, m.p. 153°; D-mannose, m.p. 131°, $[\alpha]_D^{30}$ +14° (water); D-mannose phenylhydrazone, m.p. 196°; D-xylose, m.p. 151°, $[\alpha]_D^{30}$ +23° (water); D-xylose phenylhydrazone, m.p. 98°.

The polysaccharide (0.3 g), together with ribose (30 mg) as reference, was treated with M H_2SO_4 for 24 h at 100°. A portion (1 ml) of the hydrolysate was subjected to p.c. (solvent *C*), and the individual monosaccharides were determined by periodate oxidation. The results (expressed as mol) were as follows: galactose, 2.92, 3.05, 2.80; mannose, 0.58, 0.61, 0.56; xylose, 1.16, 1.22, 1.12; and ribose, 0.46, 0.54, 0.49. Assuming 100% recovery of ribose, the molar ratios of galactose, mannose, and xylose were 5:1:2.

The polysaccharide (0.2 g) was hydrolysed with 25mM H_2SO_4 (40 ml) at 100° for 7 h. The hydrolysate was subjected to p.c. (solvent *B*) after 15, 30, 45, 60, 120, 150, 180, 240, 300, 330, 360, 390, and 420 min. Galactose was found to be liberated first, followed by xylose and mannose simultaneously.

(*b*) *Periodate oxidation.* Periodate oxidation, when carried out by the method of Andrews *et al.*[10], liberated 0.20 mol of formic acid with the consumption of 0.77 mol of periodate per 100 g of the polysaccharide; an aliquot (5 ml) of the reaction mixture after 72 h required 3.0 ml of M/110.5 NaOH. After 72 h, p.c. of the solution revealed only xylose.

(*c*) *Methylation.* The polysaccharide was subjected to four Haworth methylations[11] followed by four Purdie methylations[12]. The completely methylated polysaccharide had $[\alpha]_D^{28}$ +61° (chloroform).

The methylated derivative (20 mg) was hydrolysed with 85% formic acid for 6 h at 100° and then with 0.75M H_2SO_4 for 14 h at 100°. The hydrolysate was subjected to preparative p.c. (solvent *A*) with 2,3,4,6-tetra-*O*-methyl-D-glucose (TMG) as the reference. The following products were isolated.

(*1*) 2,3-Di-*O*-methyl-D-galactose, syrup, R_{TMG} 0.37, $[\alpha]_D^{18}$ +105° (water); lit.[13] $[\alpha]_D^{18}$ +106°. The anilide had $[\alpha]_D$ +57° (ethanol); lit.[13] $[\alpha]_D$ +58°. Oxidation with bromine water for 43 h gave 2,3-di-*O*-methyl-D-galactono-1,4-lactone, $[\alpha]_D$ −49° (water) (lit.[13] $[\alpha]_D$ −49°), from which was prepared 2,3-di-*O*-methyl-D-galactonamide, m.p. 141°, $[\alpha]_D^{20}$ +13° (water); lit.[13] m.p. 142°, $[\alpha]_D^{20}$ +14°.

(*2*) 2,3,6-Tri-*O*-methyl-D-galactose, R_{TMG} 0.72, m.p. 121°, $[\alpha]_D$ +73° (water); lit.[14] m.p. 122°, $[\alpha]_D$ +74°. The di-*p*-nitrobenzoate had m.p. 188°, $[\alpha]_D$ −33° (chloroform); lit.[14] m.p. 190°, $[\alpha]_D$ −35°.

(*3*) 2,3,4,6-Tetra-*O*-methyl-D-galactose, R_{TMG} 0.87, m.p. 72–73°, $[\alpha]_D^{32}$ +120° (water); lit.[15] m.p. 74°, $[\alpha]_D^{32}$ +121°. The anilide had m.p. 192–193°, $[\alpha]_D^{32}$ +43° (acetone); lit.[15] m.p. 194°, $[\alpha]_D^{32}$ +45°.

(*4*) 2,3-Di-*O*-methyl-D-mannose, R_{TMG} 0.53, m.p. 107–108°, $[\alpha]_D^{25}$ −16° (water); lit.[16] m.p. 108°, $[\alpha]_D$ −15.8°. The anilide had m.p. 136°; lit.[16] m.p. 138°.

(*5*) 2-*O*-Methyl-D-xylose[17], R_{TMG} 0.36, m.p. 133–135°, $[\alpha]_D^{17}$ −34° (water); lit.[17] m.p. 136°, $[\alpha]_D^{17}$ −35°. The anilide had m.p. 126–128°, $[\alpha]_D^{16}$ +212° (water); lit.[17] m.p. 127°, $[\alpha]_D^{16}$ +210°.

(*6*) 2,3-Di-*O*-methyl-D-xylose, R_{TMG} 0.75, m.p. 79–80°, $[\alpha]_D$ +23° (water); lit.[18] m.p. 81°, $[\alpha]_D$ +24°. The anilide had m.p. 115–118°, $[\alpha]_D^{12}$ +116° (water); lit.[18] m.p. 116°, $[\alpha]_D^{10}$ +118°.

(*7*) 2,3,6-Tri-*O*-methyl-D-xylose, R_{TMG} 0.93, m.p. 87–90°, $[\alpha]_D$ +19° (water); lit.[19] m.p. 88°, $[\alpha]_D$ +19°. The anilide had m.p. 95–96°, $[\alpha]_D^{30}$ +42° (ethanol); lit.[19] m.p. 97°, $[\alpha]_D^{30}$ +41°.

The methylated polysaccharide (2 g), together with D-glucose as reference, was treated with 0.75M H_2SO_4 for 18 h at 100°. The resulting methylated sugars were separated by p.c. (solvent *A*) and determined by alkaline hypoiodite. The molar ratios of fractions 1–7 were 2:4:4:2:1:2:1.

(*d*) *Partial, acid hydrolysis.* Polysaccharide (4 g) was hydrolysed with 0.05M H_2SO_4 at 100° for 12 h. The hydrolysate, when subjected to preparative p.c. (solvent D) and elution of fractions with distilled water, gave D-galactose, D-mannose, and D-xylose, and the following disaccharides.

(*1*) 6-*O*-α-D-Galactosyl-D-galactose[20], m.p. 128°, $[\alpha]_D$ +144° (water); lit.[20]

m.p. 130°, $[\alpha]_D$ +130.9°. It reduced Fehling's solution and the Tollens reagent, and afforded only D-galactose on hydrolysis. Methylation and hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-galactose. It was resistant to emulsin, indicating an α linkage. The octa-acetate had m.p. 222–227°, $[\alpha]_D$ +180° (chloroform); lit.[20] m.p. 224°, $[\alpha]_D$ +180°.

(*2*) 6-*O*-α-D-Galactosyl-D-mannose[21] (epimelibiose), m.p. 199°, $[\alpha]_D^{32}$ +120.5° (water); lit.[21] m.p. 200°, $[\alpha]_D^{32}$ +121°. Acid hydrolysis gave galactose and mannose, and methylation followed by hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-mannose.

The $[\alpha]_D^{32}$ value and resistance to emulsin are consistent with an α linkage.

(*3*) 4-*O*-β-D-Galactosyl-D-xylose[22], m.p. 190–191°, $[\alpha]_D$ −17.5° (water); lit.[22] m.p. 192°, $[\alpha]_D$ −18°. Acid hydrolysis gave galactose and xylose, and methylation followed by hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-D-xylose. The disaccharide was cleaved by emulsin, indicating a β linkage.

(*4*) 3-*O*-β-D-Xylosyl-D-xylose[23], m.p. 190–191°, $[\alpha]_D$ −17.5° (water); lit.[23] m.p. 192°, $[\alpha]_D$ −18°. Acid hydrolysis gave D-xylose only, and methylation followed by hydrolysis afforded 2,3,4-tri-*O*-methyl-D-xylose and 2,4-di-*O*-methyl-D-xylose. The disaccharide was cleaved by emulsin, indicating a β linkage.

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