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A COMPLEX POLYSACCHARIDE FROM THE SEEDS OF *Anthocephalus indicus* A. RICH

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ABSTRACT

The seeds of *Anthocephalus indicus* contain a water-soluble polysaccharide composed of D-xylose, D-mannose, and D-glucose in the molar ratios 1:3:5. Methylation analysis afforded 2,3,4-tri-*O*-methyl-D-xylose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose in the molar ratios 7:21:12:15:8. Periodate oxidation and methylation data indicated 22.5% and 21.9% of end groups, respectively. The above findings, together with the results of partial hydrolysis with acid, indicate the polysaccharide to consist of a linear chain of (1→4)-linked β-D-mannosyl and β-D-glucosyl residues to which α-D-xylosyl and β-D-glucosyl groups are attached by (1→6)-linkages.

INTRODUCTION

Anthocephalus indicus A. Rich belongs to the Rubiaceae family, and we now report on a polysaccharide isolated from the seeds.

RESULTS AND DISCUSSION

The pure polysaccharide, $[\alpha]_D^{25}$ +45° (water), was isolated from dried, crushed, and defatted seeds by extraction with 1% acetic acid and by repeated precipitation from its solution therein with ethanol. The homogeneity of the polysaccharide was verified by fractional precipitation, *via* acetylation–deacetylation, and by zone electrophoresis. The polysaccharide was water-soluble, had an ash content of 0.14%, and negligible contents of methoxyl and acetyl groups and uronic acid.

Acid hydrolysis of the polysaccharide gave D-xylose, D-mannose, and D-glucose in the molar ratios 1:3:5. Graded, acid hydrolysis of the polysaccharide released first xylose, followed by mannose and glucose. This finding indicates that D-mannose and D-glucose are present in the main chain, and that D-xylose is located on the periphery as non-reducing end-groups.

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Hydrolysis of the methylated polysaccharide, $[\alpha]_D^{25}$ +28° (chloroform), gave 2,3,4-tri-*O*-methyl-D-xylose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose in the molar ratios 7:21:12:15:8. The identity of the methylated monosaccharides was established on the basis of crystalline derivatives. The percentage of terminal groups calculated from methylation studies was 21.9. The conversion of all the D-xylose (7 mol.) in the polysaccharide into 2,3,4-tri-*O*-methyl-D-xylose (which also confirmed the results of graded hydrolysis) indicated its exclusive presence as non-reducing end-groups. Similarly, the formation of 2,3,6-tri-*O*-methyl-D-mannose (21 mol.) and 2,3,6-tri-*O*-methyl-D-glucose (12 mol.) indicate the presence of (1→4)-linked D-mannose and D-glucose residues. Those D-glucose residues that yield 2,3-di-*O*-methyl-D-glucose (15 mol.) are linked through positions 1, 4, and 6. The remaining 8 units of D-glucose, which gave rise to 2,3,4,6-tetra-*O*-methyl-D-glucose, must be non-reducing end-groups.

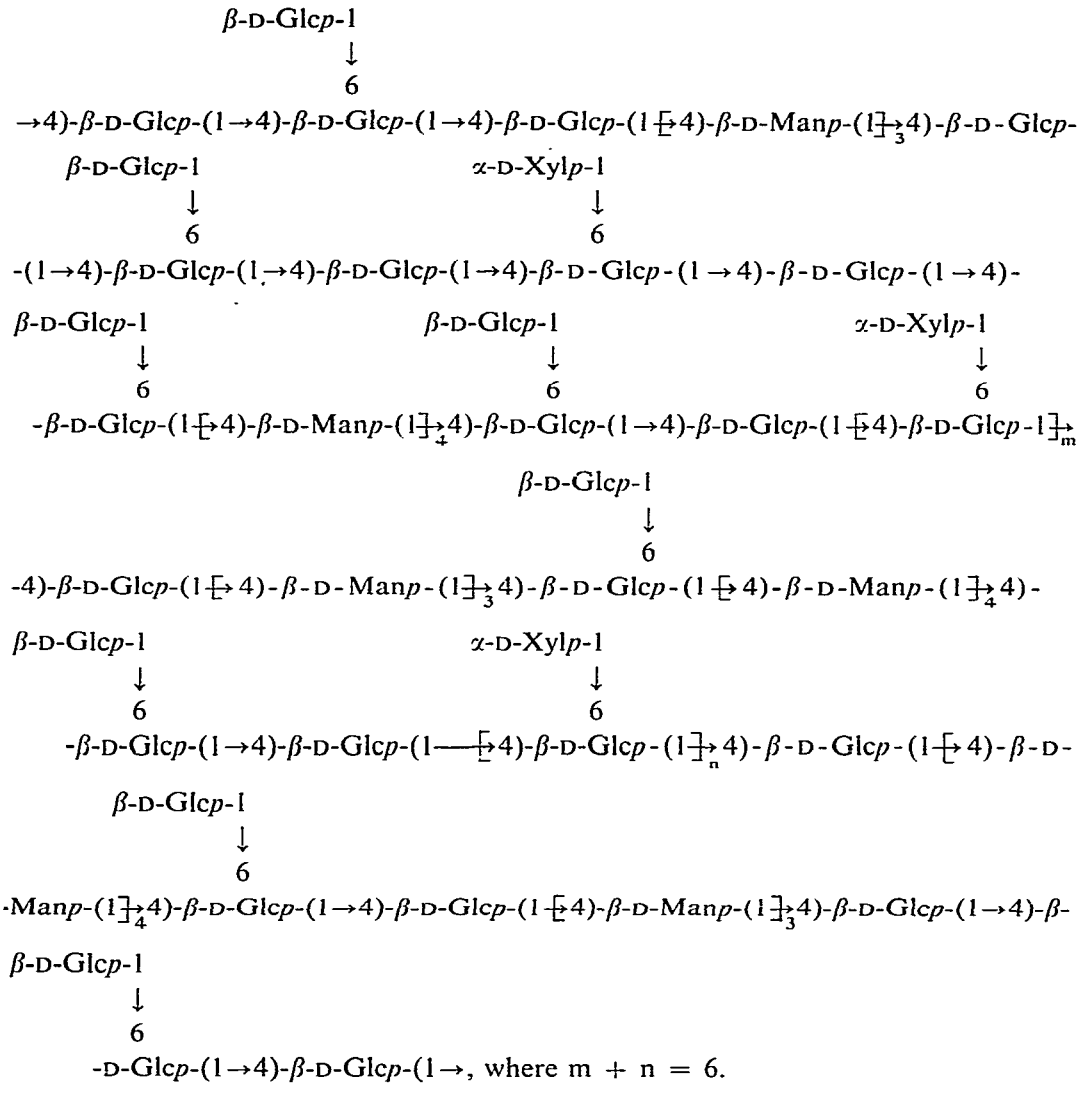
The formic acid liberated by periodate oxidation indicated 22.5% of end groups (*cf.* 21.9% from the methylation data). After oxidation of the polysaccharide for 60 h, small amounts of D-mannose and D-glucose survived, but no xylose. After 96 h, all of the hexose had been destroyed.

The above results indicate that the polysaccharide consists of a linear chain of (1→4)-linked β-D-mannopyranose (21) and β-D-glucopyranose (27) residues, to which α-D-xylosyl (7) and β-D-glucosyl (8) groups are attached by (1→6)-linkages. The polysaccharide showed i.r. absorption at 822 and 880 cm^{-1}, indicating[1] the presence of α and β linkages.

Thus, the repeating unit of the polysaccharide involves a main chain of 48 →4)-β-D-Glc*p*-(1→4)-β-D-Man-(1→ units, 7 side-chain α-D-Xyl*p* residues attached to position 6 of D-glucosyl residues, and 8 side-chain β-D-Glc*p* residues attached to position 6 of D-glucosyl residues.

Partial hydrolysis of the polysaccharide with acid yielded D-xylose, D-mannose, and D-glucose; together with five disaccharides, namely, β-D-Man*p*-(1→4)-D-Man*p* (5.2%), β-D-Glc*p*-(1→4)-D-Glc*p* (1.7%), β-D-Glc*p*-(1→6)-D-Glc*p* (6.4%), β-D-Glc*p*-(1→4)-D-Man*p* (2.2%), and α-D-Xyl*p*-(1→6)-D-Glc*p* (4.6%); and five trisaccharides, namely, α-D-Xyl*p*-(1→6)-β-D-Glc*p*-(1→4)-D-Glc*p* (4.4%), β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p* (6.3%), β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→4)-D-Glc*p* (3.4%), β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-D-Man*p* (2.0%), and β-D-Glc*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p* (1.9%); and the tetrasaccharide β-D-Man*p*-(1$\frac{}{}$4)-β-D-Man*p*-(1$\frac{}{2}$4)-D-Man*p*) (6.2%); all of which (see Experimental section) were characterised.

The above results accord with the following structure.



Such a polysaccharide should consume 1.2 mol (78 mol per 63 units) of periodate per repeating unit. The experimental value was 1.27 mol.

EXPERIMENTAL

Solutions were concentrated at diminished pressure and low temperature unless stated otherwise. All residues were dried *in vacuo* over anhydrous calcium chloride. Melting points are uncorrected and $[\alpha]_D$ values are for equilibria. P.c. was carried out at room temperature with *A*, 1-butanol–ethanol–water (5:1:4); *B*, 1-butanol–ethanol–water (4:1:5); *C*, 1-butanol–acetic acid–water (4:1:5); *D*, 1-butanol–2-propanol–water (11:6:3); *E*, 1-butanol–ethanol–water–ammonia (40:10:49:1);

F, butanone–water–ammonia (100:50:3); *G*, ethyl acetate–pyridine–water (10:4:3); *H*, ethyl acetate–pyridine–water (2:1:2); *I*, benzene–acetic acid–methanol (1:1:3); *J*, 1-butanol–acetic acid–water (5:1:4); and *K*, benzene–ethanol–water (69:47:15); and detection with aniline hydrogen phthalate.

Polysaccharide from Anthocephalus indicus. — (*a*) *Isolation.* Dried, crushed, and defatted seeds of *Anthocephalus indicus* (1.5 kg) were extracted successively with light petroleum (60–80°) and ethanol, and then stirred with 1% acetic acid at room temperature for 20 h. The acid extract was added slowly, with stirring, to ethanol (2.5 vol.). The crude polysaccharide (15 g) was collected, washed with ethanol, and dried. The dry product was re-precipitated from solution in 1% acetic acid by ethanol, to yield non-reducing, white, amorphous material (12 g; ash content, 0.14%), $[\alpha]_D^{25}$ +45° (water).

(*b*) *Homogeneity.* The polysaccharide (1.5 g) was fractionally[2] precipitated from the aqueous solution (300 ml) by the addition of ethanol (400 and 800 ml, respectively). The fractions (*a* and *b*) were collected by centrifugation, washed, and dried by washing with ethanol and then ether. Hydrolysis of fraction *a*, $[\alpha]_D^{25}$ +45° (water), and fraction *b*, $[\alpha]_D^{25}$ +44° (water), gave D-xylose, D-mannose, and D-glucose, in the molar ratios 1:3:5.

The polysaccharide (800 mg) was treated[3] with sodium acetate–acetic anhydride, and the resulting acetate (650 mg) had $[\alpha]_D^{25}$ +22° (chloroform) (Found: OAc, 42.8%). Deacetylation[3] generated material with $[\alpha]_D^{25}$ +45° (water).

The polysaccharide (60 mg) was subjected to conventional zone-electrophoresis on Whatman No. 1MM paper in borate buffer (pH 9.2). The intensity of the characteristic, yellow-orange colour developed in the aqueous eluate of each segment of the paper, using the phenol–sulphuric acid reagent, was measured in a Klett–Summerson Photoelectric Colorimeter (filter No. 50). A plot of absorbance against segment no. showed only a single, sharp peak.

(*c*) *Hydrolysis.* The polysaccharide (1.5 g) was hydrolysed at 100° with M H_2SO_4 (50 ml) for 20 h. P.c. (solvents *A* and *C*) of the hydrolysate revealed xylose, mannose, and glucose, as did t.l.c. (solvents *I* and *J*, Silica Gel G plates impregnated with 33mM boric acid and 20mM borate buffer).

The syrupy hydrolysis-product was eluted with solvent *B* from a column of cellulose to give, first, D-xylose, m.p. 151–153° (from aqueous methanol), mixture m.p. 150–152°, $[\alpha]_D^{25}$ +22° (water). The derived benzylphenylhydrazone had m.p. 97–99°. Eluted second was D-mannose, m.p. 128–130° (from aqueous methanol), mixture m.p. 127–129°, $[\alpha]_D^{25}$ +13° (water). The derived phenylhydrazone had m.p. 194–196°. Eluted third was D-glucose, m.p. 145–147° (from ethanol), mixture m.p. 143–145°, $[\alpha]_D^{25}$ +53° (water). The derived phenylosazone had m.p. 206–208°.

The components in the hydrolysate were separated by p.c. (solvent *D*) and quantified[2] by periodate oxidation; the molar ratios of D-xylose, D-mannose, and D-glucose were 1:3:5.

(*d*) *Graded hydrolysis*[4]. The polysaccharide (60 mg) was hydrolysed with

0.25M H_2SO_4 (50 ml), and the hydrolysis was monitored by p.c. (solvent *D*). Xylose was detected first (15 min), followed by mannose and glucose (25 min).

(*e*) *Periodate oxidation*[5]. To a solution of polysaccharide (300 mg) in water (30 ml) were added KCl (2.5 g) and 0.25M sodium metaperiodate (25 ml). The volume was made up to 100 ml with water, and the mixture was stored in the dark at room temperature. Aliquots (5 ml) were withdrawn at intervals and titrated with 0.07M sodium hydroxide after reducing the excess of periodate with ethylene glycol. The amount of formic acid liberated (60 h) corresponded to 22.5% of end groups.

After 60 h, excess (30 ml) of ethylene glycol was added, the solution was concentrated, and the residue was hydrolysed. P.c. (solvent *D*) of the hydrolysate revealed mannose and glucose, but no xylose.

To a solution of polysaccharide (300 mg) in water (30 ml) was added 0.25M sodium metaperiodate (25 ml). The volume was made up to 100 ml with water. Aliquots (5 ml) were withdrawn at intervals and titrated against 25mM sodium thiosulphate[6]. The consumption of periodate after 96 h was 1.27 mol per "anhydro unit". After 96 h, the oxidised polysaccharide was hydrolysed as described above; neither mannose nor glucose was detected.

(*f*) *Methylation*. The polysaccharide (3 g) was methylated by the method of Andrew *et al.*[7], to give a product (1.7 g), $[\alpha]_D^{25}$ +28° (chloroform) (Found: OMe, 40.9%). The methylated polysaccharide (1.5 g) was hydrolysed[5] with 90% formic acid and then with 0.5M H_2SO_4, and the products were fractionated on Whatman No. 3 paper with solvent *A*, to give the following products.

2,3,4-Tri-*O*-methyl-D-xylose (90 mg), m.p. 90–92° (from light petroleum), $[\alpha]_D^{25}$ +19° (water), R_{TMG} (mobility relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose) 0.94 and 0.92 (solvents *E* and *F*); lit.[8] m.p. 91–92°, $[\alpha]_D$ +64→+18° (water). The derived anilide had m.p. 96–97°, $[\alpha]_D^{25}$ +45° (ethanol); lit.[9] m.p. 98–100°, $[\alpha]_D^{25}$ +47° (ethanol).

2,3,6-Tri-*O*-methyl-D-mannose (410 mg), syrup, $[\alpha]_D^{25}$ −11° (water), R_{TMG} 0.80 and 0.54 (solvents *E* and *F*); lit.[10] $[\alpha]_D^{25}$ −10° (water) (Found: OMe, 42.5%. Calc. for tri-*O*-methylhexose: OMe, 41.8%). The derived hydrazide had m.p. 121–131°; lit.[11] m.p. 131°.

2,3,6-Tri-*O*-methyl-D-glucose (250 mg), m.p. 121–123°, $[\alpha]_D^{25}$ +69° (water), R_{TMG} 0.81 and 0.66 (solvents *E* and *F*); lit.[12] $[\alpha]_D$ +70.5° (water) (Found: OMe, 40.6%. Calc. for tri-*O*-methylhexose: OMe, 41.8%). The derived 1,4-bis(*p*-nitrobenzoate) had m.p. 186–188°, $[\alpha]_D^{25}$ −32° (chloroform); lit.[12] m.p. 189–190°, $[\alpha]_D$ −33° (chloroform).

2,3-Di-*O*-methyl-D-glucose (290 mg), m.p. 115–117° (from ethyl acetate), $[\alpha]_D^{25}$ +51° (acetone), R_{TMG} 0.57 and 0.25 (solvents *E* and *F*); lit.[13] m.p. 117–119°, $[\alpha]_D$ +50.9° (acetone). The derived phenylazobenzoate had m.p. 184–186°; lit.[14] m.p. 187–190°.

2,3,4,6-Tetra-*O*-methyl-D-glucose (98 mg), m.p. 91–93° (from light petroleum), $[\alpha]_D^{25}$ +86° (water); lit.[15,16] m.p. 84–97°, $[\alpha]_D$ +92°→+84° (water) (Found: OMe, 51.5%. Calc. for tetra-*O*-methylhexose: OMe, 52.5%). The derived anilide

had m.p. 134–136°, $[\alpha]_D^{25}$ +225° (acetone); lit.[17] m.p. 137–138°, $[\alpha]_D$ +229.5° (acetone).

The methylated polysaccharide (55.2 mg), together with D-glucose (15 mg), was treated with 2% methanolic hydrogen chloride (20 ml) at 100° for 15 h. The product was then hydrolysed with M HCl (15 ml) at 100° for 10 h. The neutralised mixture was concentrated, and the methylated sugars were separated on Whatman No. 1 paper (solvent *K*) and quantified by alkaline hypoiodite[17]. Found (results expressed as ml of 5mM iodine consumed); tri-*O*-methylhexose, 1.05, 1.12, 0.96; tri-*O*-methylmannose, 3.15, 3.36, 2.93; tri-*O*-methylglucose, 1.79, 1.93, 1.68; di-*O*-methylglucose, 2.25, 2.40, 2.10; tetra-*O*-methylglucose, 1.20, 1.27, 1.13 (glucose, 1.38, 1.65, 1.10). These figures correspond to average molar ratios of 7:21:12:15:8.

(*g*) *Partial, acid hydrolysis.* A suspension of the powdered polysaccharide (3.5 g) in water (500 ml) was stirred for ~15 h. Conc. HCl (20 ml) was added, and the mixture was kept for 4 h at 80 ±2° and then neutralised, filtered, and concentrated. P.c. of the resulting syrup revealed fourteen products, including glucose, mannose, and xylose, which were isolated by preparative p.c.

Mannobiose [β-D-Man*p*-(1→4)-D-Man*p*] (1.85 mg, 5.2%), R_{Man} 0.52 (solvent *G*), R_{Glc} 0.64 (solvent *H*), m.p. 203–205° (from ethanol), $[\alpha]_D^{25}$ −9° (water); lit.[18,19] m.p. 193–210°, $[\alpha]_D^{25}$ −5→−9° (water). The derived phenylosazone had m.p. 202–204°; lit.[19] m.p. 203–206°. Acid hydrolysis gave (p.c.) mannose, and almond emulsin hydrolysed the disaccharide, indicating a β linkage. During 48 h, the disaccharide consumed 5.2 mol of periodate with the liberation of 2.8 mol of formic acid. Methylation (methyl sulphate and sodium hydroxide) followed by acid hydrolysis gave (p.c.) 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose.

Cellobiose (60 mg, 1.7%), m.p. 230° (from ethanol), $[\alpha]_D^{25}$ +33° (water); lit.[20] m.p. 234–240°, $[\alpha]_D$ +33.3° (water). The derived octa-acetate had m.p. 226–228°; lit.[21] m.p. 224–229°.

Gentiobiose (225 mg, 6.4%), m.p. 192° (from ethanol), $[\alpha]_D^{25}$ +9° (water); lit.[22] m.p. 190–195°, $[\alpha]_D$ +9.8° (water).

Epicellobiose [β-D-Glc*p*-(1→4)-D-Man*p*] (80 mg, 2.2%), m.p. 134–135° (from ethanol), $[\alpha]_D^{25}$ +6° (water); lit.[18,23] m.p. 133–139°, $[\alpha]_D$ +5° (water). The derived octa-acetate had m.p. 198–200°; lit.[23] m.p. 202–204°.

Isoprimeverose [α-D-Xyl*p*-(1→6)-D-Glc*p*] (160 mg, 4.6%), m.p. 200–202° (from 95% methanol), $[\alpha]_D^{25}$ +123° (water); lit.[24] m.p. 205°, $[\alpha]_D$ 121–127° (water). The derived methyl glycoside hepta-acetate had m.p. 120–122°, $[\alpha]_D^{25}$ +65° (chloroform); lit.[24] m.p. 123–124°, $[\alpha]_D$ +66°.

6^2-α-D-Xylosylcellobiose [α-D-Xyl*p*-(1→6)-β-D-Glc*p*-(1→4)-D-Glc*p*] (155 mg, 4.4%), m.p. 144–145° (from 80% ethanol), $[\alpha]_D^{25}$ +158° (water); lit.[25] m.p. 147–150°, $[\alpha]_D$ +150° (water).

Mannotriose [β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*] (220 mg, 6.3%), m.p. 211–213° (from ethanol), $[\alpha]_D^{25}$ −18° (water); lit.[18,19] m.p. 214–215°, $[\alpha]_D$ −15→−26° (water).

4-β-Gentiobiosyl-D-glucose [β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→4)-D-Glc*p*] (120

mg, 3.4%), $[\alpha]_D^{25}$ +10° (water); lit.[26] $[\alpha]_D$ +9.2° (water). The derived acetate had m.p. 200–203°, $[\alpha]_D^{25}$ −12.5° (chloroform); lit.[26] m.p. 205°, $[\alpha]_D$ −13° (chloroform).

4-β-Cellobiosyl-D-mannose [β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-D-Man*p*] (72 mg, 2.07%), m.p. 157–159° (from ethanol; monohydrate), $[\alpha]_D^{25}$ −3° (water); lit.[27,28] m.p. 158–160° (monohydrate), $[\alpha]_D$ −3.4° (water).

4^2-β-D-Glucosylmannobiose [β-D-Glc*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*] (68 mg, 1.9%), m.p. 147–150° (from ethanol; monohydrate), $[\alpha]_D^{25}$ −10° (water); lit.[27] m.p. 148–152°, $[\alpha]_D$ −10.8° (water).

Mannotetraose $\{\beta\text{-D-Man}p\text{-}(1\frac{}{}4)\text{-}\beta\text{-D-Man}p\text{-}(1\frac{}{}_2^{}4)\text{-D-Man}p\}$ (270 mg, 6.2%), m.p. 231–233° (from ethanol), $[\alpha]_D^{25}$ −30° (water): lit.[8,29] m.p. 232–234°, $[\alpha]_D$ −31° (water).

The identity of the foregoing saccharides was also confirmed by data from hydrolysis and, where appropriate, partial hydrolysis, response to almond β-emulsin, periodate oxidation, and methylation analysis.

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