Plant Gums of the Genus *Sterculia*. Part IV.[1] Acidic Oligosaccharides from *Sterculia urens* Gum

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The acidic oligosaccharides formed on partial depolymerisation of *Sterculia urens* gum have been further examined. Previously uncharacterised oligosaccharides include 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose, *O*-(galactopyranosyluronic acid)-(1 → 2)-*O*-rhamnopyranosyl-(1 → 4)-galactose, *O*-(galactopyranosyluronic acid)-(1 → 2)-*O*-rhamnopyranosyl-(1 → 4)-galacturonic acid, and 3-*O*-(glucopyranosyluronic acid)-galacturonic acid from partial acid hydrolysis, and *O*-galactopyranosyl-(1 → 2)-*O*-(galactopyranosyluronic acid)-(1 → 4)-galactose from partial acetolysis. The configuration of the glycosidic linkage in 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose has been confirmed by degradation to 2-*O*-α-D-galactopyranosylglycerol.

PREVIOUS studies on the acidic polysaccharide obtained on deacetylation of *Sterculia urens* gum established some of its main structural features. We have now re-examined the complex mixture of acidic oligosaccharides formed on partial depolymerisation of the gum.

Partial acid hydrolysis of the deacetylated gum gave acidic oligosaccharides (I)—(VI). The identities of the previously characterised[2] aldobiouronic acid (II) and the trisaccharide (V) were confirmed by methylation. Likewise the structure of (I), which was assigned previously on indirect evidence,[2] and that of (VI), which may be derived from the trisaccharide (V) but was not isolated before, were confirmed by methylation. The structures of the new trisaccharides (III) and (IV) are assigned on the basis of paper chromatography of the products of partial hydrolysis of the sugars and the derived glycitols, of alkaline degradation with aqueous calcium hydroxide, and of gas chromatography of the products of cleavage of the methylated oligosaccharides and/or the methylated reduction products. The enantiomeric configurations in the oligosaccharides are assigned by analogy with the known sugar constituents of the gum.[2]

α-D-Gal*p*A 1 → 4 D-Gal*p* (I)

α-D Gal*p*A 1 → 2 L-Rha (II)

D-Gal*p*A 1 → 2 L-Rha*p* 1 → 4 D-Gal*p* (III)

D-Gal*p*A 1 → 2 L-Rha*p* 1 → 4 D-Gal*p*A (IV)

β-D-G*p*A 1 → 3 D-Gal*p*A 1 → 2 L-Rha (V)

β-D-G*p*A 1 → 3 D-GalA (VI)

D-Gal*p* 1 → 2 D-Gal*p*A 1 → 4 D-Gal*p* (VII)

The configuration of the glycosidic linkage in the aldobiouronic acid (II), which is formed on partial hydrolysis of several gums and mucilages,[3] has been assigned previously on the basis of its high positive specific rotation ($[\alpha]_D$ +90°). The aldobiouronic acid was converted into the methyl ester of the derived glycitol, which was treated with dilute sodium periodate solution under conditions expected to result in preferential oxidation of acyclic diols,[4] and then reduced with sodium borohydride. The sequence of reactions yielded 2-*O*-α-D-galactopyranosylglycerol; thus the assigned configuration was confirmed. The n.m.r. spectrum of 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnitol showed a doublet at τ 4·70 (*J* 2·8 Hz), characteristic of the anomeric protons of α-D-glycosides.[5]

The β-D-configuration was tentatively assigned to the glycosidic linkage in the aldobiouronic acid (I), formed on partial hydrolysis of the structurally similar *S. caudata*,[6] *S. setigera*,[1] and *Cochlospernum gossypium*[1] gums. This assignment, however, must be revised since the n.m.r. spectrum of the glycitol derived from the aldobiouronic acid (I) from *S. urens* gum showed a doublet at 4·80 (*J* 3·4 Hz), characteristic of the anomeric protons of α-D-glycosides.[5]

Partial acetolysis of polysaccharides frequently leads to markedly different fragmentation patterns from partial hydrolysis with aqueous mineral acid.[3] *S. urens* gum was partially degraded by acetolysis, the products were deacetylated, and paper chromatography then indicated the presence of one oligosaccharide which had not been detected in the products of partial hydrolysis of the gum. The oligosaccharide was separated by chromatography on diethylaminoethyl-Sephadex, followed by filter sheet chromatography, and the structure, *O*-D-galactopyranosyl-(1 → 2)-*O*-(D-galactopyranosyluronic acid)-(1 → 4)-D-galactose (VII), has been assigned on the basis of the following experiments. Partial hydrolysis of the oligosaccharide gave the aldobiouronic acid (I), but this disaccharide was not detected in the products of partial hydrolysis of the oligosaccharide glycitol. Gas chromatography of the methanolysis products from the methylated oligosaccharide gave methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose, and 3,4-di-*O*-methylgalacturonic acid. The structural significance of the various oligosaccharides formed from *S. urens* gum is assessed in the following paper in the light of further studies on the carboxy-reduced polysaccharide.

EXPERIMENTAL

Paper chromatography was carried out on Whatman nos. 1, 3MM, and 4 papers with the following systems (v/v): (A)

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[3] G. O. Aspinall, *Adv. Carbohydrate Chem.*, 1969, **24**, 333.

[4] M. J. Clancy and W. J. Whelan, *Chem. and Ind.*, 1959, 673.

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ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) ethyl acetate–acetic acid–formic acid–water (18 : 8 : 3 : 9); (D) ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3); (E) butan-1-ol–pyridine-water (3 : 2 : 2); (F) butanone–acetic acid–water (9 : 1 : 1, saturated with boric acid). Ionophoresis was performed in borate buffer at pH 10. Unless otherwise stated, optical rotations were measured for aqueous solutions at *ca.* 18 °C.

Gas chromatography was performed on columns of acid-washed Celite coated with (*a*) 5% (w/w) of neopentyl glycol adipate polyester (operating temperature 150°), (*b*) 10% of butane-1,4-diol succinate polyester (175°), (*c*) 15% of ethylene glycol adipate polyester (175°), (*d*) 10% of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] (200°), (*e*) 3% of silicone gum XE-60 (150°), and (*f*) 10% of polyester-silicone gum ECNSS-M. Retention times (R_t) of methylated sugar derivatives are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Partial Hydrolysis of Gum Acid.—Deacetylated gum acid [1] (12 g) was heated in N-sulphuric acid (500 ml) on a boiling-water bath for 6 h. The solution was neutralised with barium hydroxide and barium carbonate, and insoluble barium salts were removed by centrifugation and washed with water. The combined supernatant liquid and washings were concentrated, treated with Amberlite resin IR-120(H^+) to remove barium ions, and further concentrated to a syrup (9 g). The syrup (9 g) in water (5 ml) was adsorbed on a column of diethylaminoethyl-Sephadex A-25 (formate form; 30 g), and the column was eluted with water to ensure complete removal of neutral sugars. Elution of the column successively with 0·05M-, 0·4M-, and 0·5M-formic acid gave a series of fractions (total 3·08 g) containing acidic sugars which were further separated by filter sheet chromatography in solvent (B) to give oligosaccharides (I)—(VI).

Examination of Acidic Oligosaccharides.—Oligosaccharide (I). The sugar (230 mg), $R_{\text{galacturonic acid}}$ 0·23 in solvent (B), $[\alpha]_D$ +73° (*c* 0·83), gave galacturonic acid and galactose on hydrolysis. Hydrolysis of the derived glycitol gave galacturonic acid as the sole reducing sugar. Gas chromatography of the methanolysis products from the methylated [7] disaccharide on column (*a*) showed the presence of compounds with the retention times of methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid and 2,3,6-tri-*O*-methylgalactose. The n.m.r. spectrum of the disaccharide glycitol in deuterium oxide showed a doublet attributable [5] to the anomeric proton [τ 4·8 (*J* 3·4 Hz)].

Oligosaccharide (II). The sugar (360 mg), $R_{\text{galacturonic acid}}$ 0·75 in solvent (B), $[\alpha]_D$ +88° (*c* 0·73), gave galacturonic acid and rhamnose on hydrolysis. Hydrolysis of the derived glycitol gave galacturonic acid as the sole reducing sugar. Gas chromatography of the methanolysis products from the methylated [7] disaccharide on column (*a*) showed the presence of compounds with the retention times of methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid and 3,4-di-*O*-methylrhamnose. The n.m.r. spectrum of the disaccharide glycitol in deuterium oxide showed a doublet attributable to the anomeric proton [τ 4·7 (*J* 2·8 Hz)].

Oligosaccharide (II) (360 mg) in water (10 ml) was neutralised with aqueous 1% potassium hydroxide and treated with potassium borohydride (300 mg) for 20 h. Potassium ions were removed with Amberlite resin IR-120(H^+) and boric acid was removed as methyl borate by repeated distillation with methanol. Chromatography of the resulting syrup showed that reduction was complete, and a sample was methylated.[7] Gas chromatography of the methanolysis products from the methylated derivative on column (*a*) showed the presence of compounds with the retention times of 1,3,4,5-tetra-*O*-methylrhamnitol and methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid. The syrupy glycitol (205 mg) was treated with methanolic 1% hydrogen chloride (30 ml) at room temperature for 18 h and then heated under reflux for 1 h. The cooled solution was neutralised with silver carbonate, filtered, and concentrated. The resulting syrup (166 mg, 465 μmol) was treated with sodium periodate solution (300 μM, 5 l) and the consumption of oxidant was followed spectrophotometrically.[8] After 1·25 h the consumption of periodate corresponded to 2·25 mol per mol of sugar, and excess of periodate was destroyed with ethylene glycol. Inorganic ions were removed with Amberlite resins IR-120(H^+) and IR-45(OH^-), the solution was concentrated (to 10 ml), potassium borohydride (300 mg) was added, and the solution was kept for 18 h. Potassium ions and boric acid were removed as before and the solution was concentrated to a syrup (92 mg). Chromatography in solvent (B) showed a main component with the mobility of 2-*O*-α-D-galactopyranosylglycerol, with traces of other substances. The syrup was heated under reflux with acetic anhydride (5 ml) and anhydrous sodium acetate (40 mg), and the product was separated by t.l.c. on Kieselgel in benzene–methanol (4 : 1) to give 2-*O*-α-D-galactopyranosylglycerol hexa-acetate, m.p. and mixed m.p. 99°.

Oligosaccharide (III). The sugar (35 mg) $R_{\text{galacturonic acid}}$ 0·03 and 0·30 in solvents (B) and (C), $M_{\text{galactose}}$ 0·73 gave galacturonic acid, galactose, and rhamnose on hydrolysis. Hydrolysis of the derived glycitol and examination of the hydrolysate in solvents (A), (B), and (F) showed the presence of galacturonic acid, rhamnose, and galactitol. Colorimetric estimations of galacturonic acid (carbazole reagent [9]), rhamnose (cysteine reagent [10]), and total sugar (phenol–sulphuric acid reagent [11]) in the oligosaccharide and the oligosaccharide glycitol indicated that residues of galacturonic acid, rhamnose, and galactose were present in the proportions of 1 : 1 : 1. Treatment of the oligosaccharide with saturated calcium hydroxide solution furnished 2-*O*-(galactopyranosyluronic acid)rhamnose. Samples of the oligosaccharide and the derived glycitol were methylated [7] and the methanolysis products from the methylated derivatives were examined by gas chromatography on column (*c*). Both afforded methyl glycosides of 2,3,4-tri-*O*-methylgalacturonic acid and 3,4-di-*O*-methylrhamnose, together with methyl glycosides of 2,3,6-tri-*O*-methylgalactose from the former and 1,2,3,5,6-penta-*O*-methylgalactitol from the latter.

Oligosaccharide (IV). The sugar (25 mg), $R_{\text{galacturonic acid}}$ 0·13 and 0·53 in solvents (B) and (C), $M_{\text{galactose}}$ 0·83, gave galacturonic acid and rhamnose on hydrolysis. Hydrolysis of the derived glycitol gave galacturonic acid, rhamnose, and

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galactonic acid. Colorimetric estimations of galacturonic acid, rhamnose, and total sugar in the oligosaccharide and the oligosaccharide glycitol indicated that residues were present in the proportions of 2 : 1. Partial acid hydrolysis of the oligosaccharide and the oligosaccharide glycitol gave 2-*O*-(galactopyranosyluronic acid)rhamnose from both compounds, and galacturonic acid from the former only. Treatment of the oligosaccharide with saturated calcium hydroxide solution gave 2-*O*-(galactopyranosyluronic acid)-rhamnose. Samples of the oligosaccharide and the oligosaccharide glycitol were each treated with methanolic 1% hydrogen chloride at room temperature for 18 h and then heated under reflux for 1 h. The solutions were neutralised with silver carbonate, and concentrated. The resulting syrups were reduced with aqueous sodium borohydride, and the products were methylated with methyl sulphate and sodium hydroxide. The methanolysis products from the methylated derivatives were examined by gas chromatography on columns (*a*) and (*c*). Both compounds afforded methyl glycosides of 2,3,4,6-tetra-*O*-methylgalactose and 3,4-di-*O*-methylrhamnose, together with methyl glycosides of 2,3,6-tri-*O*-methylgalactose from the former and 1,2,3,5,6-penta-*O*-methylgalactitol from the latter.

Oligosaccharide (V). The sugar (283 mg), $R_{\text{galacturonic acid}}$ 0·20 in solvent (B), $[\alpha]_D$ +81° (*c* 0·90), gave glucuronic acid, galacturonic acid, and rhamnose on hydrolysis. Hydrolysis of the derived glycitol gave glucuronic and galacturonic acids as the sole reducing sugars. The oligosaccharide was treated as described for oligosaccharide (IV) and gas chromatography of the methanolysis products from the methylated carboxy-reduced derivative on column (*a*) showed the presence of compounds with the retention times of methyl glycosides of 2,3,4,6-tetra-*O*-methylglucose, 2,4,6-tri-*O*-methylgalactose, and 3,4-di-*O*-methylrhamnose.

Oligosaccharide (VI). The sugar (31 mg), $R_{\text{galacturonic acid}}$ 0·25 in solvent (B), gave glucuronic acid and galacturonic acid on hydrolysis, together with a trace of rhamnose. Chromatography in solvent (C) and ionophoresis showed the presence of the major component with traces of oligosaccharide (V). A sample of the oligosaccharide was reduced as described for oligosaccharide (V) and the reduction product was methylated.[7] Gas chromatography of the methanolysis products from the methylated derivative on column (*a*) showed the presence of components with the retention times of methyl glycosides of 2,3,4,6-tetra-*O*-methylglucose, and 2,4,6-tri-*O*-methylgalactose, and (in trace amount) of 3,4-di-*O*-methylrhamnose.

Acetolysis of Gum Acid.—Gum acid (10 g) was dispersed in formamide (500 ml) and pyridine (250 ml), and acetic anhydride (180 ml) was added with stirring at 30° during 4 h. The mixture was stirred for a further 5 h at 30° and for 18 h at room temperature, and then poured into 2N-hydrochloric acid (2 l). The precipitated polysaccharide acetate was reprecipitated from chloroform with light petroleum (b.p. 40—60°). Polysaccharide acetate (11 g) was added slowly to acetic anhydride (240 ml), acetic acid (240 ml), and concentrated sulphuric acid (24 ml) at 0°. The mixture was shaken for 8 h until complete dissolution was achieved, and kept at room temperature for 112 h. The solution was poured into ice–water (1·5 l) and the precipitate which separated was filtered off and extracted with chloroform (4 × 250 ml). The filtrate was neutralised to pH 5 with sodium hydrogen carbonate, and extracted with chloroform (4 × 500 ml). The combined extracts were dried and concentrated to a syrup. The syrup was dissolved in methanol (25 ml) and 0·5N-barium methoxide was added until the solution was alkaline. The solution was kept at 0° for 24 h and then poured into water (1 l). The solution was then treated with Amberlite resin IR-120(H^+), filtered, and concentrated to a syrup (4·5 g).

The syrup, in water (5 ml), was adsorbed on a column of diethylaminoethyl-Sephadex A-25 (formate form; 30 g), and the column was eluted with water to ensure complete removal of neutral sugars. Elution of the column successively with 0·05M-, 0·4M-, and 0·5M-formic acid gave a series of fractions containing acidic sugars. Chromatography of the fractions in solvent (B) indicated the presence of one oligosaccharide which had not been detected on partial hydrolysis of the gum. Filter sheet chromatography of the appropriate fraction afforded oligosaccharide (VII).

Oligosaccharide (VII) (18 mg), $R_{\text{galacturonic acid}}$ 0·09 and 0·43 in solvents (B) and (C), gave galactose and galacturonic acid on hydrolysis. Hydrolysis of the derived glycitol gave galactose, galacturonic acid, and galactitol. Oligosaccharide (I) was detected in the products of partial acid hydrolysis of oligosaccharide (VII) but not of the derived glycitol. A sample of the oligosaccharide was methylated [7] and gas chromatography of the methanolysis products from the methylated derivative on column (*c*) showed the presence of compounds with the retention times of methyl glycosides of 2,3,4,6-tetra- and 2,3,6-tri-*O*-methylgalactose, and 3,4-di-*O*-methylgalacturonic acid.

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