

Structure of *Chloroxylon Swietenia* Gum.* Part I. Nature of Sugars Present and the Structure of Aldobiouronic Acid

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Chloroxylon swietenia gum on hydrolysis yields a mixture of D-galactose, L-arabinose, D-galacturonic acid, 4-O-methyl-D-glucuronic acid, and three other minor sugars. Graded hydrolysis of the gum provides an aldobiouronic acid, which has been shown to be 6-O-(α -D-galactopyranosyluronic acid)-D-galactose from methylation studies.

Different parts of *Chloroxylon swietenia* DC^{1,2} (Fam. Rutaceae), namely, its timber³, sun-dried bark⁴, and seeds⁵, have been chemically examined.

A preliminary investigation⁶ of the gum as to its chemical nature and purification has already appeared. In the present communication, a detailed study of the composition of the gum and the structure of an aldobiouronic acid, obtained from it on graded hydrolysis, has been described.

Complete hydrolysis of the gum⁶ has shown that it is mainly composed of D-galactose, L-arabinose, D-galacturonic acid, and 4-O-methyl-D-glucuronic acid. The occurrence of two different uronic acids in a gum is rare and has been reported so far in two other gums, namely, Kotha⁷ (*Feronia elephantum*) and *Khaya grandifolia*⁸ gum.

Aqueous solutions of the gum are sufficiently acidic (pH 2.4) to undergo slow autohydrolysis when the solution is heated on a water bath. L-Arabinose was liberated within 4 hours, whereas the presence of D-galactose in the hydrolysate was detected after 30 hours. The autohydrolysate was resolved into two fractions: (A) the neutral sugar mixture and (B) the barium salt of the degraded gum. Fraction (A), when subjected to paper chromatography, revealed the presence of D-galactose, L-arabinose, and three other minor sugars⁶, having R_f 0.26, 0.27, and 0.30 respectively; one of them is rhamnose and the other two (R_f 0.26, 0.27) appear to be methylated in nature. Isolation of the three minor sugars in an homogeneous state could not be made due to closeness of their rate of migration on paper and also due to paucity of the material. Fraction (B), the barium salt of the degraded gum, was found to be associated with D-galacturonic as well as 4-O-methyl-D-

*Communication No. 20 on the Structure of plant polysaccharides from National Sugar Institute, Kanpur.

1. "The Wealth of India. Raw Materials", Vol. II, C. S. I. R., New Delhi, 1950, p. 131.

2. Howes, "Vegetable Gums and Resins", Chronica Botanica Company, Waltham, Mass., U.S.A., 1949, p. 57.

3. Auld, *J. Chem. Soc.*, 1900, 95, 965; *Bull. Imp. Inst.*, 1907, 7, 93.

4. Mookerjee and Bose, this *Journal*, 1946, 23, 1.

5. Rau and Simonsen, *Indian Forest Rec.*, 1922, 7, 97.

6. Bose *et al.*, *Indian J. Chem.*, 1963, 1, 324.

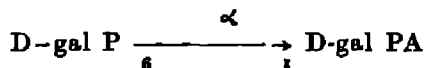
7. Heidelberger *et al.*, *Immunology*, 1962, 5, 686.

8. Aspinall *et al.*, *J. Chem. Soc.*, 1956, 989.

glucuronic acid. Partial hydrolysis of fraction (B) with 0.1N-H₂SO₄ provided a mixture of neutral sugars (Fraction C) and the barium salt of the aldobiouronic acid (Fraction D). Resolution of fraction (C) on cellulose column furnished L-arabinose and D-galactose, which were identified in the usual way⁶.

Fraction (D), consisting of aldobiouronic acid with traces of D-galacturonic and 4-O-methyl-D-glucuronic acid, was hydrolysed completely with 2N-H₂SO₄ to obtain the uronic acid moieties (Fraction E). Paper chromatographic examination of fraction (E) revealed a strong spot for D-galacturonic acid (*R_f* 0.15) and a very weak spot (*R_f* 0.27) for 4-O-methyl-D-glucuronic acid. Fraction (E) after reduction with sodium borohydride⁹ and hydrolysis afforded only D-galactose, thus confirming the presence of D-galacturonic acid. The nature of the second uronic acid in *Chloroxylon swietenia* gum was established in two ways⁶. Reduction of the gum and the degraded gum with sodium borohydride furnished a mixture of neutral sugars consisting of D-galactose, L-arabinose, and an unknown sugar⁶, identified as 4-O-methyl-D-glucose from its specific rotation [α]_D³⁰ +47.6° (water) and rate of migration in different solvent systems. The methoxyl values of the products during graded hydrolysis (gum, 4.4; fraction B, 3.6; fraction D, 3.01; fraction E, 1.95%) show a gradual decrease, contrary to the expectations. This shows that 4-O-methyl-D-glucuronic acid is being gradually decomposed during graded hydrolysis, thus making its isolation as such not possible. Such decomposition of 4-O-methyl-D-glucuronic acid was also observed earlier¹⁰.

The uronic acid impurities in fraction (D) was removed by repeated extraction with hot methanol. Pure aldobiouronic acid (*R_f* 0.053) on further hydrolysis yielded D-galactose and D-galacturonic acid. Reduction of the aldobiouronic acid with sodium borohydride afforded D-galactose only. Finally, the structure of the aldobiouronic acid was established from methylation studies. The fully methylated derivative of the aldobiouronic acid is *dextro*-rotatory and on hydrolysis furnishes equal amounts of 2,3,4-tri-O-methyl-D-galactose (I) and 2,3,4-tri-O-methyl-D-galacturonic acid (II) (vide Experimental). 2,3,4-Tri-O-methyl-D-galacturonic acid (II) was identified from its specific rotation, rate of migration on paper chromatogram, by preparing crystalline methyl 2,3,4-tri-O-methyl-mucate (m.p. 101°), and also by reducing it to 2,3,4-tri-O-methyl-D-galactose. Isolation of compounds (I) and (II) indicates that D-galacturonic acid is linked through C₆ of D-galactose residue in the aldobiouronic acid. Hence the aldobiouronic acid is 6-O-(α -D-galacto-pyranosyluronic acid)-D-galactose (III).



(III)

D-gal P = D-galactopyranose.

D-gal PA = D-galactopyranosyluronic acid.

Among the various gums studied so far, *jeol*¹¹⁻¹³, *Semul*¹³, and *Chloroxylon swietenia* gums produce only one aldobiouronic acid, composed of D-galactose and D-galacturonic

9. Hirst and Perlin, *J. Chem. Soc.*, 1954, 2622.

10. Whistler et al., *J. Amer. Chem. Soc.*, 1954, 76, 1668.

11. Mukherjee and Chakravarti, *this Journal*, 1945, 25, 113.

12. Dhar and Mukherjee, *J. Sci. Ind. Res.*, 1959, 18B, 219.

13. Bose and Dutta, *this Journal*, 1963, 40, 259.

acid. It is rather interesting to observe that the aldobiouronic acids, occurring in *Semul* and *Chloroxylon swietenia* gums, carry the similar linkage (6 $\rightarrow$ 1), but they differ in anomeric configuration, the former being β and the latter, α .

EXPERIMENTAL

Paper chromatographic analyses were carried out by the descending method on Whatman No. 1 filter papers, using non-aqueous phase of any one of the following solvent systems: (A) *n*-butanol-ethanol-water (5:1:4); (B) *n*-butanol-acetic acid-water (4:1:5); (C) ethyl acetate-acetic acid-pyridine-water (5:1:5:3); (D) ethyl acetate-acetic acid-formic acid-water (18:3:1:4); (E) ethyl acetate-pyridine-water (2:1:2); (F) ethyl-acetate-acetic acid-formic acid-water (20:3:1:4). All specific rotations are equilibrium values and all melting points are uncorrected. Unless otherwise stated, all evaporations were carried out at 40-50° under reduced pressure.

Purification of the Gum.—The finely powdered gum (100 g., sulphated ash 7.36%) was stirred with water (150 ml) to form a gel. It was mixed with 3*N*-NaOH solution (150 ml) and heated at 60° for 2 hr. The gum solution after filtration was cooled, acidified with acetic acid, and poured into acetone (2 litres) with continuous stirring. The precipitated gum was dissolved in water (200 ml), acidified with HCl (Congo-red paper), and reprecipitated with acetone (2 litres). This procedure was repeated three times to obtain the gum acid in the form of a slightly brown powder (sulphated ash 1.8%). Final purification of the gum sample was effected by passing its aqueous solution (400 ml) through a column of freshly regenerated Duolite C-25 cation-exchange resin, washing the column with water (100 ml), and pouring the effluent in acetone (4 litres). The white precipitate, thus obtained, was washed with ether and dried *in vacuo* (P_2O_5). The pure gum acid (yield 50 g.), $[\alpha]_D^{20}$ -20.96° (c, 1 in 0.01*N*-NaOH) has an equivalent (by titration) 1200; sulphated ash, 0.43; furfural, 17.95; pentosan, 30.38; pentoses, 34.53; methoxyl, 4.4%. It did not reduce Fehling's solution and nitrogen, sulphur, and halogens were found absent.

Complete Hydrolysis of the Gum.—The purified gum (10 g.) was dissolved in 2*N*- H_2SO_4 (200 ml) and hydrolysed completely (30 hr.) over a boiling water bath. The hydrolysate was neutralised (barium carbonate) and filtered. The filtrate was concentrated to a syrup. To obtain the neutral sugars, the syrup was exhaustively extracted with boiling methanol and the methanolic extract was again concentrated to a syrup. The amorphous residue, insoluble in methanol, consisting of the barium salts of the uronic acids, was washed thoroughly with methanol and dried *in vacuo*. Paper chromatography of the syrup, using solvent A and silver nitrate¹⁴ spray reagent, revealed a strong spot of arabinose and a fairly strong spot of galactose.

Resolution of the sugar syrup on cellulose column using *n*-butanol, half saturated with water, as eluent yielded (a) L-arabinose, m.p. and mixed m.p. 159°, $[\alpha]_D^{20}$ +108.1° (c, 1 in water); phenylosazone, m.p. and mixed m.p. 162°; *p*-nitro-*N*-phenyl-L-arabinosylamine, m.p. and mixed m.p. 201°; (b) D-galactose, m.p. and mixed m.p. 166-167°, $[\alpha]_D^{20}$ +81.56°.

14. Trevelyan *et al.*, *Nature*, 1950, 166, 444.

(c, 0.6 in water); methylphenylhydrazone, m.p. and mixed m.p. 186°; *p*-nitro-*N* phenyl-D galactosylamine, m.p. and mixed m.p. 205°.

A small portion of the barium salt of uronic acid was acidified with H_2SO_4 (dil.) and the free uronic acids on paper chromatographic examination (solvent B and *p*-anisidine phosphate spray reagent) produced two pink spots: a strong spot due to D-galacturonic acid (R_f 0.15) and a faster moving weak spot (R_f 0.27) due to 4-*O*-methyl-D-glucuronic acid.

Autohydrolysis.—A solution of the gum (50 g.) in water (1 litre) was heated over a boiling water bath till the hydrolysis was complete (100 hr.), as determined iodometrically¹⁵ since the solution was too dark for polarimetric observation. Paper chromatographic examination of the hydrolysate was carried out every hour. L-Arabinose was liberated within 4 hr. and galactose was detected after 30 hr. The autohydrolysate was worked up in the usual way to obtain the neutral sugars (Fraction A) and barium salts of the degraded gum (Fraction B; yield 24 g.). Paper chromatographic examination of fraction A (solvent A; silver nitrate spray reagent) revealed two very weak spots (R_g 0.26 and 0.30 respectively) in addition to galactose and arabinose. Resolution of the syrup on the cellulose column yielded four fractions. The first two fractions, obtained in a very small amount, when examined paper chromatographically using solvents A and B, were found to be mixtures of three sugars having R_g 0.26, 0.27, and 0.30 respectively. When sprayed with *p*-anisidine hydrochloride, the sugar having R_g 0.30 developed a colour indicative of rhamnose, whereas sugars with R_g 0.26 and 0.27 exhibited a colour, reminiscent of methylated sugars. The third and fourth fractions, consisting of L-arabinose and D-galactose, were identified in the manner, described earlier⁶.

Hydrolysis of the Barium Salt (B) and Isolation of Aldobiouronic Acid.—The fraction (B) (24 g., Ba, 11.68; -OMe, 3.7%) containing traces of D-galacturonic acid and 4-*O*-methyl-D-glucuronic acid, as detected paper chromatographically, was dissolved in H_2SO_4 (480 ml) of such strength that the resulting solution was 0.1*N*. It was heated over a boiling water bath till complete hydrolysis (50 hr.). The solution was neutralised (barium carbonate) and worked up to obtain the neutral sugar fraction (C) and the barium salt of the aldobiouronic acid (Fraction D; yield 11.0 g.). Partition chromatography of fraction (C) on filter paper and also on cellulose column showed it to be composed of L-arabinose and D-galactose.

Fraction (D), barium salt of the aldobiouronic acid [Ba, 15.61; OMe, 3.01; monomethyl aldobiouronate ($\text{C}_{13}\text{H}_{21}\text{O}_{12}$)₂ Ba requires Ba, 15.70; OMe, 7.08%] was treated with freshly regenerated Duolite C-25 cation-exchange resin and chromatographed on filter paper, using solvent B and *p*-anisidine phosphate spray reagent. Three pink spots were observed: (a) strong spot of aldobiouronic acid (R_f 0.053); (b) weak spot of D-galacturonic acid (R_f 0.15); (c) a faint spot of 4-*O*-methyl-D-glucuronic acid (R_f 0.27).

Hydrolysis of Aldobiouronic Acid to Uronic Acids.—Fraction D (5.0 g.) on complete hydrolysis with 2*N*- H_2SO_4 for 25 hr. yielded a neutral sugar fraction, identified as D-galactose and the barium salts of the uronic acid fraction (E) [yield 2.6 g.; Ba, 24.43; OMe, 1.95; monomethyl hexuronate ($\text{C}_7\text{H}_{11}\text{O}_7$)₂ Ba requires Ba, 24.92; OMe, 11.24%]. Paper chromatography of the free uronic acids, using solvents B, C, D, and F and *p*-anisidine phosphate spray reagent, revealed two pink spots, the stronger one corresponding to D-galacturonic

acid and the weaker one agreeing with 4-*O*-methyl-D-glucuronic acid. A portion of fraction (E) (0.2g.) was refluxed with 4% methanolic hydrogen chloride for 8 hr., neutralised (silver carbonate), filtered, and the filtrate evaporated to dryness. A solution of this product in water (2ml) was added dropwise to a solution of sodium borohydride (0.1 g.) in water (2 ml) during 5 min. After keeping for 1 hr., the excess borohydride was removed (acetic acid). The solution was deionised by passing successively through the columns of freshly regenerated Duolite C-25 and Duolite A-7 ion-exchange resins. The deionised solution was concentrated and hydrolysed on a boiling water bath with *N*-H₂SO₄ for 20 hr. The solution on being worked up provided a single spot for D-galactose, when examined paper chromatographically (solvents A and B).

Demethylation of the Gum.—Purified gum (2g.) and freshly distilled hydroiodic acid (20 ml) were heated in a sealed tube on a boiling water bath for 1 hr. The solution was neutralised with 2*N*-H₂SO₄ for 25 hr. and on working up furnished a syrup, which on paper chromatography (solvent B and *p*-anisidine phosphate spray reagent) provided in addition to the yellow spots for galactose and arabinose, two pink spots: a combined spot (*R*_f 0.15) for D-galacturonic acid and D-glucuronic acid and a spot for D-glucurone (*R*_f 0.33).

Reduction of the Parent Gum and Degraded Gum (Fraction B).—The parent gum (5g.) was converted into the methyl ester methylglycoside by treating with 4% methanolic hydrogen chloride (80 ml). It was then reduced with sodium borohydride (3g.) in the same manner, described earlier⁶. The reduction product was hydrolysed with 2*N*-H₂SO₄ for 25 hr. Subsequent working up furnished a syrup (yield 3.7g.), which on paper chromatography (solvent A) revealed in addition to the spots for galactose and arabinose, a faster moving (*R*_f 0.265) spot. The faster moving sugar was isolated by paper chromatographic resolution of the syrup on Whatman No. 3 MM paper and identified as 4-*O*-methyl-D-glucose (OMe, 15.2; monomethylhexose C₆H₁₄O₆ requires OMe, 15.9%); [α]_D³⁰ +47.6° (c, 0.3 in water); *R*_{xylose} values¹⁰ in solvents B, D and E were 1.29, 1.17, and 1.12 respectively. Degraded gum (Fraction B) was also reduced and hydrolysed in the same manner and the presence of 4-*O*-methyl-D-glucose in the hydrolysate was confirmed.

Reduction of the Aldobiouronic Acid (Fraction D).—Fraction (D) was freed of the associated uronic acid impurities by extraction with hot methanol (90%) for 50 hr. in a Soxhlet. The residue left furnished a single pink spot (*R*_f 0.053) on a paper chromatogram. Pure aldobiouronic acid (0.5g.) was converted into methyl ester methylglycoside and reduced with sodium borohydride (0.4g.). The reduction product after hydrolysis in the usual way provided a single spot for galactose on a paper chromatogram (solvents A and B).

Methylation of the Aldobiouronic Acid and its Hydrolysis.—The pure aldobiouronic acid (Ba salt, 9g.) was dissolved in water (100 ml) and methylated three times with dimethyl sulphate and NaOH according to the procedure of Aspinal *et al.*¹⁶. The reaction product was cooled, acidified (pH 4.0), and extracted with chloroform in a liquid-liquid extractor. The chloroform extract was concentrated to leave a residue (7.2 g.). This was methylated twice by Purdie's method (yield 6.9g., OMe 43.7%). The product was further methylated thrice with Purdie's reagent; yield 6.2g., [α]_D²³ +51.0° (c, 2 in CHCl₃); OMe, 48.1% Treatment of this product with Purdie's reagent once more did not increase its methoxyl content.

The fully methylated aldobiouronic acid (2 g.) was hydrolysed with 4% MeOH-HCl (100ml) for 50 hr. Methanol was distilled under reduced pressure and the concentrate was further hydrolysed with aqueous *N*-HCl (100ml) for 20 hr. The solution was neutralised (silver carbonate) and filtered. Silver ions were removed from the filtrate as silver sulphide and the filtrate was concentrated to a small volume. This was made alkaline (*pH* 9.5) and extracted with chloroform in a liquid-liquid extractor. The chloroform extract consisting of methylated galactose only was evaporated to a syrup (yield 0.5 g.) and identified as 2,3,4-tri-*O*-methyl-D-galactose from its migration rate (R_f 0.63 in solvent A) on a paper chromatogram and also by preparing its crystalline anilide; m.p. and mixed m.p. 164°. (Found: OMe, 40.9. Calc. for tri-*O*-methyl galactose $C_9H_{18}O_6$: OMe, 41.9%). The identity of the sugar was further confirmed by treating a portion (0.04 g.) with sodium metaperiodate¹⁷ when formaldehyde was liberated, furnishing the characteristic dimedone derivative, m.p. 186°.

The aqueous solution left after chloroform extraction was acidified (*pH* 2.0) with H_2SO_4 (dil.) and again extracted with chloroform. The chloroform extract consisting of methylated uronic acid was concentrated to a syrup; yield 0.55 g., $[\alpha]_D^{25} +97.7^\circ$ (*c*, 0.7 in water). This was identified as 2,3,4-tri-*O*-methyl-D-galacturonic acid from its R_f value (0.63) in solvent B and by preparing methyl tri-*O*-methylmucate, m.p. and mixed m.p. (with a sample obtained earlier¹³) 101°.

A small portion (0.1 g.) of the methylated uronic acid was esterified by 4% MeOH-HCl and reduced with sodium borohydride. The reduction product after hydrolysis developed a single spot (R_f 0.63 in solvent A) on paper chromatography, corresponding to 2,3,4-tri-*O*-methyl-D-galactose.

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17. Reeves, *J. Amer. Soc.*, 1941, 63, 1476.