138. Acacia mollissima *Willd. Part I. The Component Sugars and Aldobiuronic Acid of Black Wattle Gum.*

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Black wattle gum differs from gum arabic in the proportions of sugars and uronic acid produced on acid hydrolysis. L-Arabinose (6), L-rhamnose (1), D-galactose (5), and D-glucuronic acid (1) have been shown to be combined in the approximate molecular ratios indicated. The equivalent weight (*ca.* 1880) is nearly double that of gum arabic. The aldobiuronic acid constituent, 6-(D-glucuronosido)-D-galactose, is the same as that present in gum arabic, egg-plum gum, almond-tree gum, and peach gum.

Black wattle gum is an exudate from the bark of *Acacia mollissima* Willd., produced on injury, or pathologically as a result of " gummosis." Unlike gum arabic, a product of various *Acacia* species, this complex acidic polysaccharide has not hitherto been investigated in detail or put to commercial use. The approximate molar proportions of the monosaccharide residues in gum arabic are L-arabinose (3), D-galactose (2), L-rhamnose (1), and D-glucuronosido-D-galactose (1), giving an equivalent weight of 1000—1200 (Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1519; Mukherji and Ghosh, *J. Indian Chem. Soc.*, 1949, **26**, 277). This composition may vary, because in certain samples no rhamnose has been detected (see Pigman and Goepp, " Carbohydrate Chemistry," 1948, p. 633). Removal of arabinose, rhamnose, and 3-L-arabinose-D-galactoside by mild hydrolysis leaves a degraded polysaccharide containing a basic repeating chain of six galactose residues, to numbers 1, 3, and 5 of which are linked terminal aldobiuronic acid groups. The structure of undegraded gum arabic is exceedingly complex; sugars other than galactose may constitute the main chains, and the glucuronic acid residues may not necessarily be present exclusively as end-groups (Smith, *J.*, 1939, 744, 1724; 1940, 1035; Jackson and Smith, *J.*, 1940, 74, 79). It is of interest that commercial sources of gum arabic are invariably trees located in hot dry climates (Pigman and Goepp, *op. cit.*).

Three samples of purified gum, prepared from fresh exudate by dissolution in weak alkali, acidification, and precipitation with ethanol, were similar in physical properties, thus indicating the raw material to be essentially homogeneous. The equivalent weight (*ca.* 1900) is considerably higher than that recorded for gum arabic. The number-average molecular weight of black wattle gum, determined by Mr. F. J. Joubert of this laboratory, is of the order of 92,000, decreasing slowly on account of hydrolysis. During the determination fragments are split off which are small enough to pass through a membrane capable of retaining particles of molecular weight *ca.* 30,000. Acid hydrolysis readily removes L-arabinose and smaller amounts of L-rhamnose and D-galactose (detected on one- and two-dimensional chromatograms), leaving a polysaccharide consisting predominantly of galactose and glucuronic acid residues in the proportion 5 : 1. The course of hydrolysis resembles that of the other plant gums, indicating that pentose units are present in the furanose form and hexose units in the pyranose form. More concentrated acid splits off four galactose residues per equivalent of polysaccharide, an aldobiuronic acid remaining which is resistant to dilute sulphuric acid at the ordinary pressure. Methylation of this acid, followed by hydrolysis, yields 2 : 3 : 4-trimethyl galactose and 2 : 3 : 4-trimethyl D-glucuronic acid, proving the aldobiuronic acid to be 6-(D-glucuronosido)-D-galactose. It is thus established that the components of black wattle gum are similar to those of gum arabic but are combined in different proportions. The polysaccharide remaining after removal of pentose and methylpentose, for example, can be represented in the same way as that obtained from gum arabic (Jackson and Smith, *locc. cit.*) except that in black wattle gum every fourth and not every second galactose residue in the repeating chain is linked to a 6-(D-glucuronosido)-D-galactose unit. In other words, the composition of degraded black wattle gum is the same as that of degraded gum arabic with every second aldobiuronic acid unit omitted.

The proportions of the components of black wattle gum have been determined (i) by complete hydrolysis of the gum with sulphuric acid and estimation of the resultant sugars by chromatographic separation and oxidation with periodate (Jones, *J.*, 1950, 534; Hirst and Jones, *J.*, 1949, 1659), and (ii) by partial hydrolysis of the gum to equal weights of the degraded acid polysaccharide (as its barium salt) and a mixture of sugars, followed by independent examination of the two fractions. The sugar mixture was shown to contain L-arabinose and L-rhamnose (cf. Hough, Jones, and Wadman, *J.*, 1949, 2511) by isolation of the pure sugars,

the ratios being determined by paper chromatography. In addition to employing the periodate oxidation method, roughly quantitative estimations were carried out by using the linear relation of spot area to the logarithm of sugar content of the spot (Fisher, Parson, and Morrison, *Nature*, 1948, **161**, 764). Instead of spraying ammoniacal silver nitrate the β-naphthylamine reagent recently evolved in this laboratory (Novellie, *ibid.*, in the press) was employed, the coloured spots being developed at 150° in order to obtain the clearest boundaries. The degraded polysaccharide mentioned above was further hydrolysed to an aldobiuronic acid and a mixture of sugars from which pure D-galactose was separated; the proportions of galactose, arabinose, and rhamnose in the residual syrup were then estimated by the means of spot areas.

Galactose, arabinose, and rhamnose have thus been shown to be in the molar ratio of 5·15 : 6 : 1 in the total hydrolysate of wattle gum, and this together with the known equivalent weight of the gum indicates a composition of D-galactose (5 parts), L-arabinose (6 parts), L-rhamnose (1 part), and D-glucuronic acid (1 part) with a calculated equivalent weight of 1940. As expected from a gum of this composition, the combined weights of arabinose and rhamnose are equal to that of the barium salt of degraded polysaccharide, and analysis of the mixed sugars has confirmed the arabinose : rhamnose ratio as 6 : 1. Both the barium content of the degraded polysaccharide salt and the weight of barium aldobiuronate recovered after removal of galactose are in agreement with a galactose : glucuronic acid ratio of 5 : 1.

Oxidation of the neutral gum with potassium periodate for two weeks (Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785) caused the production of very nearly two mols. of formic acid per equivalent of gum. If the acid is produced from $-[CH{\cdot}OH]_3-$ units only, this is consistent with the aldobiuronic acid residues being terminal as suggested above; each would be expected to liberate 2 mols. of formic acid on gentle oxidation. The network of galactose, arabinose, and rhamnose residues forming the main structure of the gum cannot possess this structural feature, indicating that either the D-galactopyranose residues are not linked in the 1 : 6 positions or, if they are so linked, they are engaged in glycosidic unions with pentose and methylpentose units.

Experimental.

The gum was collected from small trees in a native plantation in the Wartburg district of Natal (by courtesy of Mr. J. Hunt Holley). The trees were of varying ages and no attempt was made to discriminate between "wound" and "gummosis" gum. Collection took place during a two-week period in February towards the close of the heavy-rain season, when the gum was found as soft transparent jelly-like lumps which fermented on storage and dried to a horny mass during several months.

The crude gum was received as clear jelly-like lumps discoloured by reddish extracts from the bark. The bulk of it dissolved on gentle warming in sodium hydroxide solution, and purification was effected by repeated precipitation of the filtered, acidified solution in ethanol (see, *e.g.*, Brown, Hirst, and Jones, *J.*, 1948, 1677). The gum did not appreciably reduce Fehling's solution or form an insoluble copper derivative (see Deuel and Neukom, *Makromol. Chem.*, 1949, **4**, 97). It had $[\alpha]_D$ −49° in water. The iodine value, determined by Baker and Hulton's method (*Biochem. J.*, 1920, **14**, 754) was high and variable (5·6—11·5 c.c. of 0·1N-iodine per g. of gum after 20 minutes' oxidation) [Found: loss on vacuum desiccation at room temperature, 6·0; OMe, 0·35; ash, 1·2; pentosan, 48·5% (from Kröber's tables); equiv., 1880 (by titration with 0·01N-sodium hydroxide using methyl-red)]. The barium salt of the gum was prepared by addition of barium carbonate to an aqueous solution, filtration, and precipitation in ethanol (Found, on material dried at 100° *in vacuo*: Ba, 3·2. A polysaccharide of equiv. wt. 1880 requires Ba, 3·5%).

Quantitative Hydrolysis.—Dry purified black wattle gum (0·259 g.) was heated with 1·8N-sulphuric acid (10 c.c.) in a sealed tube in a boiling water-bath for 16 hours, and the cooled, partly neutralised (barium carbonate) hydrolysate poured through a column of Amberlite IR-4B anion-exchange resin. The neutral filtrate was then evaporated to small bulk (7·0 c.c.) under diminished pressure and analysed on an ascending paper partition chromatogram (cf. Partridge, *Nature*, 1946, **158**, 270; Flood, Hirst, and Jones, *J.*, 1948, 1679), with *n*-butanol–ethanol–water (4 : 1 : 5, upper layer). If the chromatogram was kept longer than 16—18 hours at 30° merging of the component sugars towards the solvent front, 18—23 cm. above the starting line, occurred. The positions of the different sugars on strips cut from the sheet were revealed by spraying the strips with Novellie's β-naphthylamine reagent and development at 140—150°. R_F values varied from one chromatogram to the next but the relative separation of the sugars was practically constant: rhamnose 0·63 (yellow), arabinose 0·43 (rose-pink), and galactose 0·33 (ochre). The individual sugars were extracted from the main sheet by cutting out appropriate strips, subdividing these into pieces no longer than 20 × 5 mm., and eluting them with three changes of warm water. Use of a Soxhlet device was unnecessary. Estimation of the sugars according to Hirst and Jones (*loc. cit.*) indicated the molar proportions of galactose, arabinose, and rhamnose in black wattle gum hydrolysate to be 5·15 : 6 : 1 (average of four determinations).

Graded Hydrolyses.—(a) *Autohydrolysis.* The changes in iodine value (expressed as c.c of 0·1N-iodine per g. of gum) on heating of black wattle gum (purified material, 12 g.) in water (100 c.c.) were as follows: 5·5 (initial value), 49 (17 hours), 61 (41 hours), 71 (65 hours). The polysaccharide recovered

by pouring the cooled, concentrated solution into ethanol had an increased iodine value (22) and retained moisture after vacuum-desiccation at room temperature (loss on drying at 100° *in vacuo*, 5·9%). During the hydrolysis samples were withdrawn and diluted to 2% concentration, and known volumes (0·01—0·02 ml.) placed on the starting line of an ascending chromatogram together with solutions of arabinose, rhamnose, and galactose of known strength. From the areas of the spots produced after the chromatogram (aqueous phenol at 31°) had run for 40 hours and then been sprayed with the β-naphthylamine reagent, the weights (in g.) of sugars produced from 1 g. of gum were calculated as follows: arabinose 0·25, rhamnose hydrate 0·03 (17 hours); arabinose 0·31, rhamnose hydrate 0·05 (41 hours); arabinose 0·335, rhamnose hydrate 0·06, galactose 0·025 (65 hours).

(b) *Hydrolysis with* 0·01N-*sulphuric acid.* The purified gum (7·5 g.) in 0·01N-sulphuric acid (250 c.c.) was heated on the boiling water-bath and the course of the reaction followed polarimetrically: $[\alpha]_D$ −47° (initial value); −10·7° (4 hours); +34·7° (21 hours); +36·7° (27 hours); +42·7° (43 hours). Previous experiment had shown the rate of change to be very slow during the last 10 hours. Iodine titration values were 69 (21 hours); 74 (27 and 43 hours). The neutralised (barium carbonate) reaction mixture was then filtered, evaporated to dryness at *ca.* 20 mm., and extracted with boiling methanol, giving a residue consisting of the barium salt of a degraded polysaccharide (3·33 g.; fraction A) and a syrupy mixture of sugars (3·765 g.; fraction B; containing *ca.* 0·20 g. of a barium salt) which crystallised partly when rubbed with cold methanol–acetone. Treatment of the mixed sugars (fraction B) with N-sulphuric acid (100 c.c.) on the water-bath for an hour caused no appreciable change in optical rotation ($[\alpha]_D$ +72°), indicating the absence of oligosaccharide. The recovered sugar mixture (3·56 g.) was then divided into a white solid powder (1·91 g., dried at 100°) and a viscous golden-brown syrup by trituration with methanol. The white solid contained a small proportion of barium (1·17%, corresponding to 7·2% of barium aldobiuronate) and had m. p. 143—145°, $[\alpha]_D^{18}$ +95·3° (*c*, 1·91 in water); examination on a paper chromatogram showed this to be mainly arabinose with a very faint galactose spot and a trace of barium salt at the origin. From this the arabinose content of the solid was estimated as 1·76 g. (92%). Recrystallisation of a portion of the solid from methanol and acetone yielded pure L-arabinose, m. p. and mixed m. p. 156°, $[\alpha]_D^{26}$ +105° (*c*, 0·785 in water). The benzoylhydrazone (see Hirst, Jones, and Woods, *J.*, 1947, 1048) had m. p. 195—199° (decomp.), raised to 206° by recrystallisation from ethanol; no depression when mixed with authentic L-arabinose benzoylhydrazone.

The syrup (1·65 g.; from fraction B) after removal of arabinose was examined on paper chromatograms in two ways. (i) 2% Solutions of the syrup in water (0·01 ml.) were run on an ascending chromatogram with known solutions of arabinose, galactose, and rhamnose hydrate, with aqueous phenol as solvent, and from the areas of the resulting spots the weights of the three sugars present were estimated as 0·92, 0·24, and 0·49 g., respectively. (ii) The syrup (3% solution; 0·1 ml.) was separated into its constituents and analysed by the periodate oxidation method of Hirst and Jones (*loc. cit.*); arabinose (0·83 g.), galactose (0·295 g.), and rhamnose hydrate (0·53 g.) were found and in addition a fourth component (faint pink spot with the β-naphthylamine reagent; R_F slightly lower than that of rhamnose) which was, however, insufficient in amount to estimate or identify. From the above results the weights of arabinose and rhamnose hydrate produced on mild hydrolysis of black wattle gum are in the ratio of 2·59 : 0·53 (molar ratio of 5·9 : 1).

Hydrolysed as before, the gum (90 g.) yielded a syrupy mixture of sugars from which crystalline L-arabinose (13 g.) was separated by trituration with methanol. A further crop crystallised after some weeks, and the residual syrup was then chromatographed on cellulose using water-saturated *n*-butanol (Hough, Jones, and Wadman, *loc. cit.*). Rhamnose hydrate, crystallising as long rectangular blades from the first eluate, had m. p. 98° and $[\alpha]_D^{20}$ +11° (*c*, 0·76 in water) after crystallisation from methanol–acetone. There was no m. p. depression on admixture with an authentic specimen of L-rhamnose hydrate, and the osazone had m. p. and mixed m. p. 180—181° (decomp.).

(c) *Examination of the degraded polysaccharide* (*fraction* A *above*). The polysaccharide salt remaining after removal of pentose and methylpentose by mild acid hydrolysis (fraction A) was dissolved in water, acidified with dilute sulphuric acid, and centrifuged to remove barium sulphate. The aqueous solution was then concentrated and poured into excess of ethanol kept at 5°, yielding a colourless flocculent polysaccharide which was dried in a vacuum (2·73 g.; loss on heating at 100° *in vacuo*, 9·0%). On evaporation of the bulk of the aqueous ethanol, treatment with barium carbonate and concentration of the filtrate a barium salt was recovered (0·40 g.) (Found: Ba, 9·0%) consisting of further hydrolysed material. The bulk of the degraded polysaccharide (2·68 g.) was made up to 100 c.c. with dilute sulphuric acid (2N.), and a portion (10 c.c.) of the solution reprecipitated in ethanol (100 c.c.) (Found, on material dried at 100° *in vacuo*: equiv., 930, by titration with 0·015N-sodium hydroxide using methyl-red; I absorption, 21·4 c.c. of 0·1N-iodine per g.).

The sulphuric acid treatment to remove barium had caused some hydrolysis, as results from a second experiment showed: in this case the barium salt of the polysaccharide, after thorough extraction of monosaccharides in boiling methanol, was dissolved in water and reprecipitated in ethanol [Found, on material dried at 100° *in vacuo*: Ba, 6·5; I absorption, 16 c.c. (as above). Calc. for polysaccharide salt containing 1 barium glucuronate and 5 galactose residues: Ba, 6·4%; equiv., 1004]. Hydrolysis of this substance in 0·1N-sulphuric acid at 96° for 9 hours caused a change in $[\alpha]_D$ from −13° to 0° (*c*, 1·84), and from the product a small quantity of galactose, arabinose, and rhamnose (in the approximate ratio of 3 : 3 : 1 from a paper chromatogram) and the barium salt of a polysaccharide (Found: Ba, 7·0%) were recovered.

The changes on hydrolysis of the polysaccharide (*c*, 2·68) in 2N-sulphuric acid on the boiling water-bath were as follows: $[\alpha]_D^{25}$ −12° (initial value), +6° (1 hour), +21° (4·5 hours), *ca.* +45° (6·5 hours); at this stage the solution was too dark to permit accurate measurement. I val. (as above): 53 c.c. (1 hour), 71 c.c. (2·5 hours), 79 c.c. (4·5 hours), 87 c.c. (6·5 hours), 89 c.c. (9 hours); the final titre corresponds to 0·80 g. of hexose in solution per g. of polysaccharide hydrolysed. Barium carbonate was added

in excess to the cooled solution, and after filtration the clear yellow liquid was evaporated under reduced pressure. The residue was extracted with boiling methanol (200 c.c.), leaving a yellow powder (0·78 g.; $[\alpha]_D$ 0°) which was strongly reducing (Found : Ba, 16·0; I absorption, 53 c.c. Calc. for $C_{12}H_{19}O_{12}Ba_{0\cdot5}$: Ba, 16·2%; I absorption, 47 c.c., calculated from the known value for galactose). The yield on the weight of dry polysaccharide taken (43·8%) was in agreement with that (42·2%) expected from a polysaccharide containing one uronic acid and five hexose residues. No sugars were present, a paper chromatogram showing only a distinct spot at the origin.

The methanol extract, after evaporation to dryness and washing with a little cold methanol–acetone, yielded crystalline D-galactose (0·68 g.), m. p. 160—165° (foaming), $[\alpha]_D^{20}$ +98° ⟶ +77° in 4 hours, final value +74°, and a syrup (0·22 g.). A portion of the solid (0·228 g.) with methylphenylhydrazine in aqueous ethanol (Hirst, Jones, and Woods, *loc. cit.*) yielded a derivative (0·318 g.), m. p. and mixed m. p. 187—188° (decomp.), raised to 190° (decomp.) on recrystallisation from aqueous ethanol. A specimen of pure D-galactose gave a similar weight of the methylphenylhydrazone on treatment in the same way. A second sample of the solid yielded mucic acid, m. p. and mixed m. p. 220° (decomp.), on oxidation with nitric acid. The syrup, oxidised similarly, gave approx. 70% as much mucic acid, indicating that it contained 0·15 g. of galactose; a paper chromatogram revealed the presence of galactose with traces only of arabinose and rhamnose.

Identification of the Aldobiuronic Acid.—Black wattle gum (dry weight 25·0 g.; equiv. 1880) in 2N-sulphuric acid (75 c.c.) was hydrolysed for 6 hours and worked up in the usual way, to give a methanol-insoluble barium salt (4·35 g.; calc. yield of barium aldobiuronate, 5·6 g. Found : Ba, 15·9%). A portion (0·1 g.), after 3·5 hours' heating with 3N-sulphuric acid (1·5 c.c.) in a sealed tube immersed in a boiling water-bath, did not contain galacturonic acid residues according to Ehrlich's basic lead acetate test (*Ber.*, 1932, **65**, 352). The barium aldobiuronate (1·65 g.) was methylated and the resulting methyl heptamethylaldobiuronate {0·52 g.; b. p. *ca.* 220° (bath-temp.)/0·15 mm.; n_D^{15} 1·4664, $[\alpha]_D^{16}$ +28° (*c*, 1·32 in water)} characterised by hydrolysis with 1·5N-hydrochloric acid to give (i) 2 : 3 : 4-trimethyl galactose, n_D^{18} 1·4650, $[\alpha]_D$ +94° (*c*, 1·62 in water) (Found : OMe, 43·3. Calc. for $C_9H_{18}O_6$: 3OMe, 41·9%) [anilide, colourless needles, m. p. 163—165° after being washed with ethanol (lit., 168°, 169°)], and (ii) the ether-insoluble barium salt of an acid which on oxidation with bromine and esterification with methanolic hydrogen chloride afforded the methyl ester, m. p. 108—109° (distilled at 0·1 mm. and recrystallised from ethanol–*iso*hexane) (lit., m. p. 107°, 110°, 111°), of 2 : 3 : 4-trimethyl D-glucosaccharolactone. The aldobiuronic acid component of black wattle gum is thus identical with that of gum arabic (Smith, *J.*, 1939, 1724), egg-plum gum (Hirst and Jones, *ibid.*, 1947, 1064), almond-tree gum (Brown, Hirst, and Jones, *loc. cit.*) and peach gum (Jones, *loc. cit.*). Confirmation of the structure of the galactose derivative was obtained by oxidizing it with periodate, whereupon formaldehyde (dimedone compound, m. p. and mixed m. p. 188°) was evolved.

Periodate Oxidation of the Gum.—The purified gum (0·293 g.) was neutralised with 0·01N-sodium hydroxide, and after addition of sodium metaperiodate solution (20 c.c.; 0·2M.) and potassium chloride (2 g.), was made up to 50 c.c. in a standard flask. The stoppered flask was kept in the dark and shaken occasionally. At intervals portions (5 c.c.) were withdrawn and titrated with 0·01N-sodium hydroxide after addition of ethylene glycol (5 drops, excess) [Found : 2·85 c.c. (190 hours); 3·03 c.c. (330 hours); 3·68 c.c. (1 month). Methyl-red used as indicator]. These results correspond to the production of 1 g.-mol. of formic acid from 1030, 970, 800 g. of gum respectively, *i.e.*, 1·83, 1·93, and 2·35 mols. of acid from one equivalent of gum (1880).

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