The Gum of Acacia pycnantha.

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The gum exuded by *Acacia pycnantha* has been compared with other types of *Acacia* gum. It gives on hydrolysis D-galactose (65%), L-arabinose (27%), L-rhamnose (1—2%), and D-glucuronic acid (5%). Partial hydrolysis affords the aldobiuronic acid 6-*O*-β-D-glucuronosyl-D-galactose and a disaccharide, probably 3-*O*-β-D-galactopyranosyl-D-galactose. Evidence has been obtained that the molecular structure is complex and probably highly branched and it appears that gums from various species of *Acacia* must differ markedly in structural details.

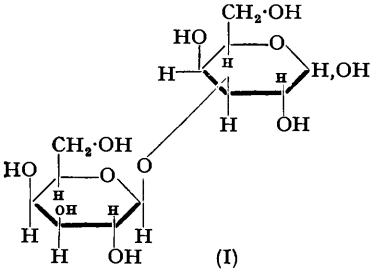
Gums have been isolated from many species of *Acacia* and, of these, gum arabic has been studied most extensively (O'Sullivan, *J.*, 1884, **41**; Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1519; Challinor, Haworth, and Hirst, *J.*, 1931, 258; Smith, *J.*, 1939, 744, 1724; Jackson and Smith, *J.*, 1940, 74, 79; Dillon, O'Ceallachain, and O'Colla, *Proc. Roy. Irish Acad.*, 1953, **55**, *B*, 331). In this gum L-rhamnose (1 mol.), L-arabinose (3 mols.), D-galactose (3 mols.), and D-glucuronic acid (1 mol.) are glycosidically linked in a highly branched, complex pattern. The gum of *Acacia mollissima* Willd. (black-wattle gum) is of similar type but differs in containing a smaller proportion (approximately half) of glucuronic acid. Like gum arabic it gives rise on partial hydrolysis to the aldobiuronic acid 6-glucuronosido-galactose (Stephen, *J.*, 1951, 646). The gum of *Acacia pycnantha*, which is the

subject of the present paper, does not appear to have been examined and it was of interest therefore to determine what relation it bears to the other gums of the *Acacia* family.

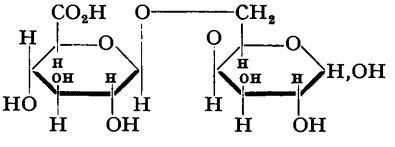
The gum was made available through the kindness of Mr. J. E. Cummins of the Australian Scientific Liaison Office, London, and Mr. B. H. Bednall, Conservator of Forests, Adelaide. It was in the form of nodules exuded by *A. pycnantha* growing in South Australia. Most of the crude gum was readily soluble in water but approximately 3% formed a dense gel. The soluble portion was isolated by precipitation with alcohol as colourless, acidic, slightly reducing powder. Hydrolysis and examination of the products on the paper chromatogram indicated the presence of L-rhamnose (1—2%), L-arabinose (27%), and D-galactose (65%). The uronic acid residues (D-glucuronic acid) amounted to approximately 5% of the total. It is possible that the rhamnose is present in an adherent impurity and is not a main structural feature of *A. pycnantha* gum. The insoluble gel was not hydrolysed completely by acid, but the mixture of sugars liberated had essentially the same composition as for the soluble gum, the uronide carbon dioxide figure being somewhat higher and the nitrogen content considerably higher. The gel was evidently a mixture and was not examined further.

The arabinose residues were removed by hydrolysis under mild conditions. The rhamnose residues were less labile but were liberated more rapidly than galactose, permitting the use of graded hydrolysis to effect a partial separation of rhamnose and arabinose from the greatly predominating galactose. The mixture of free sugars was then separated by partition chromatography on a cellulose column. This gave L-rhamnose, identified as the crystalline hydrate, and larger quantities of L-arabinose and D-galactose, identified as characteristic hydrazones. After removal of the sugars the column was washed with water, giving a mixture of oligosaccharides, which were partially separated on a charcoal–Celite column (Whistler and Durso, *J. Amer. Chem. Soc.*, 1951, **13**, 4189; Peat and Whelan, personal communication). Development of the charcoal column with 7·5% alcohol afforded a fraction from which a crystalline disaccharide was isolated (m. p. 159—160°, $[\alpha]_D$ +62·1° in H_2O) which on hydrolysis yielded only galactose. Evidence obtained by oxidation by lead tetra-acetate (Perlin, forthcoming publication) indicated that this was 3-*O*-β-galactopyranosyl-D-galactose (I). This disaccharide was isolated by Smith (*loc. cit.*) after partial hydrolysis of gum arabic.

Prolonged hydrolysis of the gum followed by neutralisation with barium carbonate and removal of the free sugars afforded a mixture of barium salts. On the paper chromatogram the main component appeared as a slow-moving spot, but there were present some glucuronic acid (as glucurone) and traces of galactose and arabinose. The slow-moving component, obtained as a syrup by elution from the chromatogram, had equivalent weight 347, and $[\alpha]_D$ +16° in water. On further hydrolysis this gave galactose and glucurone. Methano-



(I)



(II)

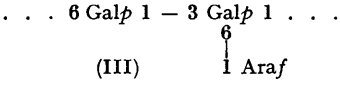
lysis, followed by reduction of the ester with sodium borohydride (Wolfrom and Anno, *J. Amer. Chem. Soc.*, 1952, **74**, 5583) and hydrolysis of the resulting glycosides, gave a mixture of galactose and glucose. These results indicated that the main component of the acidic hydrolysis product was an aldobiuronic acid composed of galactose and glucuronic acid.

By methylation a fully methylated aldobiuronic acid was prepared and this on hydrolysis yielded 2 : 3 : 4-tri-*O*-methyl-D-galactose, identified as the crystalline anilide, and 2 : 3 : 4-

tri-*O*-methyl-D-glucuronic acid, identified as the amide and by conversion into the methyl ester of 2 : 3 : 4-tri-*O*-methyl-D-saccharolactone. Reduction of this methyl ester with sodium borohydride (Wolfrom and Anno, *loc. cit.*) gave only 2 : 3 : 4-tri-*O*-methylglucose, indicating the absence of galacturonic acid. In addition to these two components of the aldobiuronic acid a small quantity of another methylated sugar (R_G, 0·81 ; OMe, 35%) was found in the products of hydrolysis. The principal aldobiuronic acid is, therefore, 6-β-D-glucuronosyl-D-galactose (II), but in view of the presence of the unidentified sugar the possibility remains that a small proportion of the glucuronic acid residues in the polysaccharide may be linked in some other way.

Periodate oxidation of the gum resulted in consumption of 0·86 mole of periodate per residue with the formation of 0·32 mole of formic acid per residue. Many of the sugar residues in the gum were not attacked by periodate and it follows that the polysaccharide may be highly branched or may contain a proportion of 1 : 3-glycosidic linkages, or both. Since the periodate-oxidised polysaccharide yielded on hydrolysis galactose accompanied by only traces of arabinose it appears that the arabinose residues in the polysaccharide are more open to attack. Since over 90% of the gum is composed of D-galactose and L-arabinose residues in the proportion 2 : 1 one possible type of structural unit would therefore be (III), but it is already clear, as the result of preliminary methylation studies, that the structure must in fact be much more complex.

(III)



The fully methylated polysaccharide was prepared and a preliminary examination of the products of hydrolysis on the paper chromatogram indicated the presence of at least six methylated sugars. From their positions on the paper these appeared to be (in order) 2 : 3 : 5-tri-*O*-methylarabinose (faint), 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (moderate), an unknown sugar (trace), 2 : 3 : 4-tri-*O*-methylgalactose (moderate), a di-*O*-methylgalactose (strong), an unknown sugar (faint), and a methylated uronic acid (trace). This mixture will be further investigated but these observations are sufficient to support the indication given by periodate oxidation that the polysaccharide has a highly branched structure.

The polysaccharide of *A. pycnantha* therefore resembles gum arabic and black-wattle gum in that it contains the same constituent sugars and yields the same aldobiuronic acid. Nevertheless, the proportions of the sugar residues present in the various *Acacia* gums are markedly different (see Table), and the detailed structures must therefore differ considerably.

	Rhamnose	Arabinose	Galactose	Glucuronic	Equiv. wt.
Arabic [a]	14	34	42	15	1000
Black wattle [b]	8	46	38	8	1880
A. pycnantha	2	27	65	4	3700

[a] Pigman and Goepp, " Chemistry of the Carbohydrates," Academic Press, New York, 1948, p. 633.
[b] Stephen, *loc. cit.*

Experimental

The gum was composed of amber to dark brown nodules admixed with small particles of bark. The nodules were taken up in water, and the solution was strained through muslin, and then centrifuged, depositing a small quantity of dense gel. The clear straw-coloured supernatant liquor was acidified (pH 2) with dilute hydrochloric acid, and alcohol was added to a concentration of 80%. The precipitate was removed on the centrifuge, washed with alcohol and ether, and dried *in vacuo*. Addition of alcohol to the gel caused solidification and the solid was washed with alcohol and ether and dried *in vacuo*. In a typical preparation, 100 g. of crude gum yielded 78·2 g. of the water-soluble fraction and 2·85 g. of gel.

Preliminary Examination.—The main fraction was a white powder, $[\alpha]_D$ −8·1° (*c*, 2·7 in H_2O) [ash, 0·33% ; N, 0·5% ; reducing power, 10 g. required 4·4 c.c. of 0·1N-iodine ; CO_2H (by titration), 0·8% ; uronide CO_2 liberated by boiling 12 or 19% hydrochloric acid, 1·0—1·2% (corrected for non-uronide CO_2)]. When the substance was hydrolysed at 2% concentration with 0·5N-sulphuric acid in a sealed tube at 100° the specific rotation ($[\alpha]_D$) rose to the constant value +83°. Examination of the neutralised hydrolysate on the paper chromatogram indicated the presence

of rhamnose, galactose, and arabinose. Quantitative estimation of the sugars separated on the chromatogram by periodate oxidation (Hirst and Jones, *J.*, 1949, 1659) and by the Somogyi reagent (Somogyi, *J. Biol. Chem.*, 1945, **160**, 61) gave rhamnose 1—2%, arabinose 27%, and galactose 65%. The rotation of an aqueous solution of L-arabinose and D-galactose mixed in these proportions is $[\alpha]_D$ +87°. These figures together with 5% of uronic anhydride indicate the approximate composition of the polysaccharide. In another experiment a single nodule of the gum, purified as described above, had the same composition as that of the large-scale preparation, indicating the gum to be essentially homogeneous.

The gel (see above), when dried, gave a coarse grey powder [N, 1·3%; uronide CO_2, 1·7% (corr.)]. Under conditions of hydrolysis as for the soluble gum it did not all dissolve {$[\alpha]_D$ (final) +68°}. On the paper chromatogram of the products only a trace of rhamnose was present but arabinose and galactose were present in the molar ratio 1 : 2·4. The gel was not further examined.

Qualitative experiments in which the gum was boiled (*a*) in aqueous solution and (*b*) with 0·05N-sulphuric acid, indicated that much of the arabinose was liberated rapidly and rhamnose somewhat more slowly, whilst the galactose residues were more resistant to hydrolysis.

Graded Hydrolysis.—The gum (30 g.) in 0·1N-sulphuric acid (1 l.) was heated for 8 hr. at 95°. The solution was then concentrated at 30—35° to 150 c.c. and poured into alcohol (1 l.), giving a precipitate which was separated and dried (12·9 g.) (This precipitate was found on hydrolysis to contain galactose and uronic acid residues.) The supernatant liquor was neutralised with barium carbonate and concentrated to a syrup of which 9 g. were placed on a cellulose column and eluted with *iso*propyl alcohol–water (9 : 1) (Hough, Jones, and Wadman, *J.*, 1949, 2511). The eluted fractions were examined on the paper chromatogram. By combination of appropriate fractions and removal of the solvent the following were obtained: fraction 1, α-L-rhamnose hydrate (120 mg.), $[\alpha]_D$ +9° (*c*, 1·35 in H_2O, final), m. p. 91°, not depressed by admixture with authentic α-L-rhamnose hydrate, m. p. 92°; the *X*-ray powder diagram was identical with that of α-L-rhamnose hydrate. Fraction 2, L-arabinose (2·5 g.), $[\alpha]_D$ +100° (*c*, 1·1 in H_2O, equil.) [benzoylhydrazone, m. p. and mixed m. p. 185—187° (decomp.)]. Fraction 3, D-galactose (2·4 g.), $[\alpha]_D$ +81·5° (*c*, 1·0 in H_2O, final); recrystallised from aqueous methanol this gave α-D-galactose hydrate, m. p. 119° (methylphenylhydrazone, m. p. and mixed m. p. 182°).

The cellulose column was washed with water, and the eluate was concentrated to a syrup (3·2 g.), which showed several components on a paper chromatogram, the fastest of which corresponded to di- and tri-saccharides. The syrup was transferred to a charcoal–Celite (1 : 1) column (Whistler and Durso, *loc. cit.*) which was developed with 7·5% alcohol, 100-c.c. fractions being collected and examined chromatographically. The early fractions contained at least three components; fractions 6—10 appeared to consist mainly of one component, which appeared to be a disaccharide. Fractions 6—10 were combined and concentrated to a syrup (0·45 g.) a portion of which, on hydrolysis, gave on a paper chromatogram galactose (strong) and arabinose (weak). This syrup was then boiled under reflux with 85% methanol. After several weeks in the cold the extract deposited crystals (0·11 g.), m. p. 159—160·5°, $[\alpha]_D$ +62° (equil.; *c*, 0·58 in H_2O), reducing equivalent 366 (a disaccharide, containing two hexose residues, requires 342; for a monohydrate, 360). After hydrolysis with 0·5N-sulphuric acid $[\alpha]_D$ was +76·5° and the hydrolysate, examined on a paper chromatogram, contained only galactose.

Isolation of an Aldobiuronic Acid.—The gum (40 g.) in N-sulphuric acid (800 c.c.) was heated at 95° for 7·5 hr. The solution was neutralised ($BaCO_3$), concentrated to 150 c.c., and added slowly with stirring to ethanol (1 l.). The precipitate was collected on the centrifuge, extracted 4 times with boiling methanol, washed with ether, and dried *in vacuo* (1·57 g.; Ba, 15·5%; OMe, 1·4%). On a paper chromatogram (developed with ethyl acetate–acetic acid–formic acid–water, 9 : 1·5 : 0·5 : 2) (Jones and Wise, *J.*, 1951, 2750), the crude barium salt gave a main component close to the origin, traces of two slightly faster moving substances, and traces of arabinose, galactose, and uronic acid; the last two travelled at similar rates but were differentiated by development with *p*-anisidine hydrochloride (Hough, Jones, and Wadman, *J.*, 1950, 1702) and examination in ultra-violet light. Galactose gave a yellow, and the uronic acid a deep red, fluorescence. The main component, isolated by chromatography on sheets of Whatman 3MM paper, had an equivalent 347 (an aldobiuronic acid requires 356) and $[\alpha]_D$ +16° (*c*, 0·2 in H_2O). On hydrolysis in a sealed tube at 100° for 3·5 hr. with 3N-sulphuric acid it yielded galactose and glucuronic acid, the latter appearing on the chromatogram as glucurone when the partly neutralised hydrolysate was examined. A portion (200 mg.) of the aldobiuronic acid was boiled with 8% methanolic hydrogen chloride for 7 hr.; the solution was neutralised and the alcohol removed. A solution of the product in water (2 c.c.) was added dropwise with stirring during

5 min. to a solution of sodium borohydride (0·1 g.) in water (1·5 c.c.) (Wolfrom and Anno, *loc. cit.*). After 15 min., the excess of borohydride was destroyed with dilute acetic acid, the solution was de-ionised with resins, and the glycosides were hydrolysed with sulphuric acid. On a paper chromatogram the hydrolysate was then found to contain galactose and glucose. Treatment of the mixture of sugars with a sample of glucose oxidase (notatin), known to be specific for glucose, removed the material which had previously corresponded to glucose on the chromatogram and left only galactose.

Structure of the Methylated Aldobiuronic Acid.—The crude barium salt (3·1 g.) prepared by hydrolysis of the gum (74 g.) under the conditions previously described was methylated twice with methyl sulphate (20 c.c.) and sodium hydroxide (40 c.c.; 40%). The alkaline solution was extracted with chloroform to remove non-acidic methylated sugars, and after acidification (sulphuric acid) again extracted with chloroform. The second extract was concentrated to a syrup which was methylated twice with methyl iodide and silver oxide giving a syrup (1·2 g.; OMe, 45%). Further treatment with methyl iodide and silver oxide failed to raise the methoxyl value. By repeatedly extracting the syrup with boiling light petroleum (b. p. 40—60°) a clear pale yellow oil (0·93 g.; n_D^{20} 1·4680; OMe, 49·5%) was removed leaving some dark insoluble wax. The oil, purified by distillation, had b. p. (bath-temp.) 160—190°/0·02 mm., n_D^{17} 1·4640, $[\alpha]_D$ −5° (*c*, 3·0 in H_2O) (OMe, 50·6. Calc. for a fully methylated aldobiuronic acid, 53·0%).

This methyl hepta-*O*-methylaldobiuronate (0·3 g.) was heated at 100° for 7 hr. The solution was neutralised with silver carbonate, treated with hydrogen sulphide, and neutralised with barium carbonate. The filtrate was concentrated to a syrup from which traces of water were removed by repeated addition and distillation of alcohol. Exhaustive extraction of the syrup with boiling ether (A) left a barium salt {0·16 g., $[\alpha]_D$ +66° (*c*, 1·0 in H_2O)}. This was boiled with 3% methanolic hydrogen chloride, and the product with methanolic ammonia gave the amide of methyl 2 : 3 : 4-tri-*O*-methyl-α-D-glucuronide, m. p. 184—185°, after recrystallisation from acetone–ether. The amide and the material in the mother-liquors were transformed into the corresponding methyl ester by reaction with 3% methanolic hydrogen chloride. The ester was reduced with sodium borohydride as described previously. After hydrolysis the product gave only one spot on a paper chromatogram, its position being identical with that of 2 : 3 : 4-tri-*O*-methylglucose and well separated from that of 2 : 3 : 4-tri-*O*-methylgalactose. Another sample (0·24 g.) of the barium salt of the methylated uronic acid prepared by hydrolysis of the fully methylated aldobiuronic acid (0·4 g.) was oxidised with bromine. The product was esterified (3% methanolic hydrogen chloride) and distilled; it had b. p. (bath-temp.) 134—145°/0·1 mm., n_D^{18} 1·4530, and crystallised after 6 weeks at 0°; recrystallisation from ether gave the methyl ester, m. p. 106—107°, of 2 : 3 : 4-tri-*O*-methyl-D-saccharolactone (Found : OMe, 49·5. Calc. for $C_{10}H_{16}O_7$: OMe, 50·0%).

The ether extract (A, above) was concentrated to a syrup (0·15 g. from 0·4 g. of methylated aldobiuronic acid), $[\alpha]_D$ +63·5° (*c*, 0·77 in H_2O) (OMe, 36·1%). On a chromatogram it gave a strong spot with R_G that of 2 : 3 : 4-tri-*O*-methylgalactose, and a faint spot (R_G 0·81) which travelled more rapidly. These components were separated by chromatography on sheets of 3MM paper. The major component {OMe, 39·2%; $[\alpha]_D$ +80° (*c*, 1·0 in H_2O)} on treatment with aniline in boiling alcohol gave 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-galactopyranosylamine, m. p. and mixed m. p. 164—165° after recrystallisation from alcohol–ether. The minor component {MeO, 35·0%; $[\alpha]_D$ +4·5° (*c*, 0·5 in H_2O)} was oxidised by sodium metaperiodate with formation of formaldehyde as indicated by the Rimini ferricyanide–phenylhydrazine test (*Bull. Soc. chim.*, 1898, **20**, 896).

Oxidation of the Gum with Sodium Metaperiodate.—The gum (100 mg.) was dissolved in water (58 c.c.) containing sodium metaperiodate (0·4 g.), and the solution was kept at 16° in the dark. Portions of 5 c.c. were withdrawn at intervals and titrated with 0·005N-sodium hydroxide after destruction of excess of periodate with ethylene glycol: 3·21 c.c. (24 hr.), 3·46 c.c. (72 hr.), 3·65 c.c. (120 hr.), the last corresponding to *ca.* 0·32 mole of formic acid per residue (consumption of periodate at this stage was 0·86 mole per residue). Potassium chloride was added to the remaining solution of oxidised gum, and the salts were removed by dialysis. The product, after hydrolysis with boiling 0·5N-sulphuric acid, gave on a paper chromatogram indications of galactose (strong) and arabinose (trace).

Methylation of the Gum.—The gum (24 g.) in water (300 c.c.) was methylated in the usual way by sodium hydroxide (310 c.c.; 40%) and methyl sulphate (230 c.c.) under nitrogen. The methylation was repeated twice and the product was then methylated five times with silver oxide and methyl iodide. The product was fractionally precipitated by light petroleum (b. p. 60—80°) from its solution in chloroform. Almost the whole of it came down as material having

$[\alpha]_D$ −48° in $CHCl_3$ (*c*, 1·0) and OMe, 40·5%. No change was effected by further methylation and the material could not be separated by fractional dissolution into fractions differing appreciably in proportions. This methylated polysaccharide (50 mg.) was dissolved in dry methanol (5 c.c.), dry IR-120 resin (150 mg.) was added, and the mixture heated in a sealed tube for 18 hr. at 100°. The product was heated with 0·6N-hydrochloric acid (5 c.c.) in a sealed tube at 100° for 8 hr. The product, isolated in the usual way, was examined on a paper chromatogram, at least six components being observed. By comparison with authentic materials these components corresponded, in decreasing order of rate of movement, to 2 : 3 : 5-tri-*O*-methylarabinose (faint), 2 : 3 : 4 : 6-tetra-*O*-methylgalactose (moderate), an unidentified substance (trace), 2 : 3 : 4-tri-*O*-methylgalactose (moderate), a di-*O*-methylgalactose (strong), an unidentified substance (faint), and a methylated uronic acid (faint).

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