863. *The Composition of* Hakea acicularis *Gum.*

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The polysaccharide gum from *Hakea acicularis* has an acid equivalent weight of *ca.* 2000, and on hydrolysis yields L-arabinose (19%), D-xylose (8%), D-galactose (58%), D-mannose (7%), and D-glucuronic acid (8%). Its complexity resembles that of wild cherry gum and gum ghatti, and the partial hydrolysis products have not hitherto yielded a pure neutral disaccharide. Isolation of 2-*O*-β-D-glucuronosyl-D-mannose indicates that the mode of union of the mannose and acid residues is the same as in gum ghatti and in damson and cultivated cherry gums; mannose is almost certainly linked to galactose in the polysaccharide. The gum has been submitted to periodate oxidation both before and after mild acid hydrolysis.

Hitherto the majority of plant gums studied have been collected from trees of the families *Leguminosae* and *Rosaceae*, and there is no record of work on *Proteaceae* gums. In view of the growing tendency [1-3] to relate gum composition to source of origin, it is desirable that the field of investigated types should be extended as widely as possible. The present work is a survey of the composition of the gum exudate from the bark of *Hakea acicularis*, a species introduced into South Africa from Australia during the past century. The tree grows and increases rapidly, and has spiny leaves and characteristic almond-like fruit. Gum occurs infrequently but, when it does, it may be found in very large quantity on one tree. The family (*Proteaceae*) to which *Hakea* belongs is widely distributed in the western regions of the Cape Province.

[1] Challinor, Haworth, and Hirst, *J.*, 1931, 258; Smith, *J.*, 1939, 744, 1724; Jackson and Smith, *J.*, 1940, 74, 79.
[2] Stephen, *J.*, 1951, 646; Hirst and Perlin, *J.*, 1954, 2622; Charlson, Nunn, and Stephen, *J.*, 1955, 269, 1428.
[3] Aspinall, Hirst, and Matheson, *J.*, 1956, 989.

Purified by precipitation in acidified ethanol in the usual way,[4] the gum has an equivalent weight as acid of *ca.* 2000, a value difficult to obtain with certainty owing to the low solubility of the material in water. The homogeneity of the gum is open to some doubt on account of this insolubility (which is not due, as in *Khaya grandifolia* gum,[3] to the presence of a small proportion of acetate groups). Acid hydrolysis of the gum reveals the presence of sugars corresponding in chromatographic behaviour to arabinose, xylose, mannose, and galactose, together with glucuronic acid (glucurone). Hydrolysis with 0·01N-sulphuric acid splits off a relatively low proportion (*ca.* 12% by weight) of the sugar residues present in the gum, most of the arabinose and some xylose and galactose being removed by this treatment. The residual polysaccharide, on being heated with N-sulphuric acid, liberates the remaining arabinose, together with xylose and much galactose, without the production of neutral oligosaccharides in quantity sufficient for structural examination. Mannose and glucuronic acid are split off about the same time, there remaining at this point an aldobiouronic acid (2-*O*-β-D-glucuronosyl-D-mannose), together with oligosaccharides in small amount containing this acid linked to D-galactose. The yield of aldobiouronic acid available is low on account of the low acid content of the gum and the apparent firmness of bonding of mannose to galactose. The sugars and glucuronic acid (all in their customary configurations) were identified and assayed by their isolation and the formation of suitable derivatives. Some unidentified monosaccharide material (0·3% by weight) was obtained on dilute acid hydrolysis of the gum, but this is of questionable constitutional importance.

Mannose and glucuronic acid are present in approximately equimolecular amounts in the gum, and it appears that all of the mannose is bound through $C_{(2)}$ to glucuronic acid; this is not the case in gum ghatti,[5] which *Hakea acicularis* gum resembles in the complexity of sugar residues present. Hydrolysis under comparable conditions gives no evidence for the liberation from *Hakea acicularis* gum of a glucuronosyl galactose, which is produced from gum ghatti.

Preliminary oxidation of the gum with periodate under the usual conditions resulted in uptake of the oxidant and liberation of acid per equivalent similar to that found for gum ghatti. The gum after mild acid hydrolysis consumed rather less periodate per equivalent but gave a relatively higher yield of acid, suggesting the presence of a high proportion of 1 : 6-linked galactose units in the resistant backbone of the gum molecule.

Experimental

The paper (Whatman No. 1) chromatograms were run at *ca.* 20° in butanol–ethanol–water (5 : 1 : 4, upper layer), in ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), or in butanol–pyridine–water (9 : 2 : 2). Specific rotations were measured in water unless otherwise stated. The trees were identified through the courtesy of the Bolus herbarium.

Hakea acicularis *Gum.*—Initially soft, the exudate from the bark of *Hakea acicularis* dried to an intensely hard, horny mass, colourless unless contaminated by reddish extractives from the bark. Dispersion of the aged gum in water was almost impossible, whereas fresh soft gum dissolved completely. On storage of the hardened gum in 0·5N-hydrochloric acid for several months, partial hydrolysis with the liberation of sugars occurred, and a solution of high viscosity was obtained. Crushed nodules of gum, extracted with hot ethanol, contained no *O*-acetyl group. When purified by precipitation of an aqueous solution with ethanol in the usual way, fresh gum was converted into a colourless acidic powder, $[\alpha]_D$ −13° (*c* 0·8 in dilute alkali) (Found: equiv., *ca.* 2000; ash, <1%; I val. by Baker and Hulton's method,[6] 1·48 c.c. of 0·1N-iodine per g. of gum). Oxidation of the gum with periodate [7] for 3 days caused the liberation of 4 equivs. of acid with uptake of *ca.* 11 mols. of reagent per equiv. of gum.

Hydrolysis of the Gum.—When the gum (183 g.) was heated with 0·01N-sulphuric acid at 96° for 68 hr., there was an upward change in rotation and an increase in iodine value which fell off to a constant level; the product was then separated, by precipitation of the concentrated neutralised (barium carbonate) solution with methanol, into a mixture of sugars (19·5 g.), a degraded polysaccharide (155 g., as barium salt), and a partially soluble oligosaccharide (1·6 g., as barium salt) of $[\alpha]_D$ *ca.* +10°.

[4] Brown, Hirst, and Jones, *J.*, 1948, 1677.
[5] Aspinall, Hirst, and Wickstrøm, *J.*, 1955, 1160.
[6] Baker and Hulton, *Biochem. J.*, 1920, **14**, 754.
[7] Hirst and Jones, *J.*, 1947, 1064.

The sugar mixture was composed of L-arabinose {(15·9 g.; a portion recrystallised from methanol had m. p. and mixed m. p. 160°, $[\alpha]_D$ +102°, and yielded a phenylosazone, m. p. 164° (decomp.) (lit., m. p. 164—166°)}, D-galactose (2·1 g.; this yielded mucic acid, m. p. and mixed m. p. 219°, on oxidation), D-xylose (1·0 g.), and unidentified monosaccharides (0·5 g.) which included material similar in chromatographic behaviour and appearance on spraying to ribose and rhamnose. The weights of the individual sugars were obtained from the quantities separated from an aliquot part on a cellulose column by using aqueous butanol (water–butanol ratio, 1 : 10).

The degraded polysaccharide barium salt (Found: Ba, 3·7%), after reprecipitation through the free acid, had $[\alpha]_D$ +8°. Periodate oxidation caused liberation of 4 equivs. of acid per equivalent (1800) of gum, while 8 mols. of periodate were consumed. Further hydrolysis of the polysaccharide (81 g.) in boiling N-sulphuric acid for 10 hr. gave a mixture which, after conversion into barium salts and precipitation in ethanol in the usual way, could be divided into a sugar fraction A (61·2 g.), and a barium salt fraction B (10·4 g.) which had $[\alpha]_D^{20}$ −30° (*c* 0·34) (Found: Ba, 17·1%). Separation of the sugar fraction into its components by standard techniques yielded D-galactose {41·8 g.; m. p. 161—164° and $[\alpha]_D^{18}$ +80° (*c* 1·4) after recrystallisation from methanol–acetone; oxidation afforded mucic acid}, L-arabinose {6·5 g.; m. p. and mixed m. p. 157°, $[\alpha]_D^{20}$ +100° (*c* 0·5); derived benzoylhydrazone, m. p. and mixed m. p. 205°, and $[\alpha]_D^{20}$ +46° (*c* 0·45 in pyridine; one week in storage)}, D-xylose {5·6 g.; m. p. and mixed m. p. 150—151°, $[\alpha]_D^{20}$ +24° (*c* 1·1); derived mono-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 212—213° and $[\alpha]_D^{20}$ −3° (*c* 1·5 in $CHCl_3$)}, and D-mannose {2·4 g.; derived phenylhydrazone, m. p. and mixed m. p. 197° (decomp.) and $[\alpha]_D^{22}$ +29° ⟶ +12° in 6 hr. (*c* 0·48 in pyridine)}.[8] In addition, elution with water after the monosaccharides had been taken off the cellulose column with water–butanol (3 : 40) afforded a mixture of oligosaccharides (5·0 g.), R_{gal} 0·63 and 0·45 in the acid solvent but with much streaking; further hydrolysis of this gave mainly galactose, with some arabinose, mannose, and a uronic acid component (paper-chromatographic identification). The weights of sugars recorded above were computed from the recovery of each sugar on elution of a portion (18·4 g.) of the original mixture from a cellulose column. Hydrolysis of the polysaccharide for 5 hr., instead of 10 hr. as described above, gave galactose, arabinose, and xylose with only a trace of mannose, together with glucuronosylmannose and two slower-moving (paper chromatogram) fractions which each yielded complicated mixtures of sugars and sugar acids on hydrolysis.

The barium salt fraction B, when chromatographed on paper with the acid solvent, was found to contain a uronic acid, an aldobiouronic acid (R_{gal} 0·5), and a trace of galactose. The de-ionised (Amberlite IR-120 H) mixture was chromatographed on cellulose, elution being with butanol–water–formic acid (45 : 4 : 1); there were obtained the following fractions: (1) Traces of sugars (xylose, arabinose, galactose), followed by D-glucuronic acid, reprecipitated as barium salt (2·3 g.), $[\alpha]_D^{22}$ +15° (*c* 1·02) (Found: Ba, 27·5%); removal of barium ion [Amberlite IR-120(H) resin] and concentration of the aqueous solution gave gluctronic acid and glucurone (paper-chromatographic identification). (2) Aldobiouronic acid, isolated as barium salt (3·7 g.), $[\alpha]_D^{23}$ −30° (*c* 0·96), R_{gal} 0·56 (acid solvent) (Found: Ba, 16·5%). (3) Residues, washed from the column with water and recovered as barium salt (3·0 g.), R_{gal} 0·6 with streaking from origin (there being indications of material concentrated at R_{gal} 0·6, 0·4, and 0·25) in acid solvent; no movement in basic solvent.

Identification of Aldobiouronic Acid.—Hydrolysis of the above-mentioned aldobiouronic acid, by 2N-sulphuric acid at 100° for 16 hr., yielded mannose, glucuronic acid, and glucurone (paper chromatograms). Methylation of the aldobiouronic acid (2·5 g.) with dimethyl sulphate–sodium hydroxide and then methyl iodide–silver oxide gave, after extraction with chloroform and removal of solvent, a syrup (1·25 g.), b. p. 180—190° (bath)/0·02 mm., $[\alpha]_D^{18}$ −25° (*c* 0·52). This was heated for 12 hr. with 1·5N-sulphuric acid at 100°, and the mixture was then neutralised with barium carbonate, centrifuged, and evaporated to near dryness under reduced pressure. Exhaustion of the concentrate with chloroform yielded a pale yellow syrup (0·18 g.), R_{MG} 0·94 (acid solvent), 0·95 (basic solvent), and 0·96 (neutral solvent), together with a faster-moving impurity, R_{MG} 1·06, 1·13, and 1·13 (R_{MG} 1·00 for tetra-*O*-methylgalactose). When sprayed with the periodate–benzidine reagent of Cifonelli and Smith [9] only the slower-moving material was detectable. The two components were separated on filter-paper sheets, yielding the slower-moving material as a syrup which crystallised after long storage in a desiccator. Recrystallisation from ether gave elongated colourless prisms, m. p. 104°, proved identical (*X*-ray powder

[8] Butler and Cretcher, *J. Amer. Chem. Soc.*, 1931, **53**, 4358.
[9] Cifonelli and Smith, *Analyt. Chem.*, 1954, **26**, 1132.

diagram) with authentic 3 : 4 : 6-tri-*O*-methyl-D-mannose. The minor component, $[\alpha]_D$ +49° (*c*, 3·65 in $CHCl_3$), yielded 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid on mild alkaline hydrolysis followed by removal of cation with Amberlite IR-120(H) resin (see below). It was furthermore indistinguishable in its behaviour on paper chromatograms from the methyl ester of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, and in the orange colour given on spraying with *p*-anisidine hydrochloride and aniline hydrogen oxalate.

The aqueous solution of the methylated barium salt obtained as above after hydrolysis of the methylated aldobiouronic acid was treated in the usual way, to yield a syrup (0·31 g.) whose rate of movement on paper, in all three chromatographic solvents used, was the same as that of a specimen of 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid (prepared by methylation of D-glucurono-lactone). The acid was converted into its barium salt and washed with ether to remove traces of neutral material, a chromatographically pure white solid (0·23 g.), $[\alpha]_D$ +33° (*c* 2·3), being obtained. Identification of the acid was completed by conversion of this barium salt into the amide of methyl 2 : 3 : 4-tri-*O*-methyl-α-D-glucuronoside, m. p. and mixed m. p. 182° after recrystallisation from ether.

Direct paper-chromatographic comparison of the aldobiouronic acid with 2-*O*-β-D-glucuronosyl-D-mannose obtained from gum ghatti [5] showed identity of the two specimens. The homogeneity of the acid was further demonstrated by paper ionophoresis [10] in alkaline borate buffer, a single discrete spot being obtained.

Hydrolysis of Residues.—A portion of the residues (3, above) (0·5 g.) was heated on a water-bath with N-sulphuric acid (10 c.c.), samples being taken at 2, 4, and 6 hr. and chromatographed on paper. There were liberated arabinose, xylose (traces), mannose, galactose, and glucuronic acid, while the three main oligosaccharide components remained (they must obviously have diminished in quantity during hydrolysis). The total oligosaccharide fraction was separated from the sugars by precipitation as barium salt, then placed on filter-paper sheets in order to separate three fractions, R_{gal} 0·64, 0·43, and 0·28 (acid solvent) (yields: 35, 10, and 11 mg. respectively). The fastest-moving of these was chromatographically identical with the above aldobiouronic acid, and gave mannose, glucuronic acid, and glucurone on further hydrolysis. Each of the two other fractions furnished galactose as well as mannose and glucuronic acid on hydrolysis and so are not aldobiouronic acids (though they may contain traces of aldobiouronic acid).

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[10] Consden and Stanier, *Nature*, 1952, **169**, 783.