The Composition of Acacia cyanophylla *Gum.*

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Acacia cyanophylla gum on hydrolysis gives L-rhamnose (5 mols.), L-arabinose (2 mols.), D-galactose (11 mols.), and D-glucuronic acid (5 mols.), and its equivalent weight is therefore unusually low in comparison with those of other gums from *Acacia* species. The acid residues are recovered as 6-*O*-β-D-glucuronosyl-D-galactose on autohydrolysis of the gum acid and on partial hydrolysis of the resulting degraded polysaccharide. The disaccharide 3-*O*-α-D-galactopyranosyl-L-arabinose occurs in the products of autohydrolysis. The structure of the gum must therefore differ considerably from that exuded by *A. pycnantha* (Hirst and Perlin, *J.*, 1954, 2622), a species very closely related botanically to *A. cyanophylla*.

DESPITE the widespread use of a general term " gum arabic " to denote the polysaccharide gums from such diverse sources as *A. senegal*, *A. verek*, and others (Pigman and Goepp, " Chemistry of the Carbohydrates," Academic Press, New York, 1948, p. 631; Whistler and Smart, " Polysaccharide Chemistry," Academic Press, New York, 1953, p. 304), it has become apparent that considerable variation is to be expected in the structure of gums isolated from different *Acacia* species. *A. mollissima* gum, for example, contains the same monosaccharide and uronic acid components but has an equivalent weight greater than the average value found for gums hitherto classed as gum arabic (Stephen,

J., 1951, 646), while a very recent paper by Hirst and Perlin (*J.*, 1954, 2622) has shown the equivalent weight of *A. pycnantha* gum to be even higher. As part of a programme to elaborate further the variation of gum composition with species of *Acacia*, the gums of *A. cyanophylla* and *A. karroo* have been examined by us during the past two years, and the first part of this work is now reported. A fair-sized, leafy tree, *A. cyanophylla* Lindl. (Port Jackson willow; golden willow) was introduced from Australia into the winter-rainfall region of the Cape Province in the middle of the 19th century, and has flourished in sandy coastal areas; it has proved to be a comparatively poor source of gum. *A. pycnantha* Benth. is closely related botanically to *A. cyanophylla* but is rare in the Western Cape; we intend to collect gum from this species for comparison with the material of South Australian origin described by Hirst and Perlin (*loc. cit.*).

Purified *A. cyanophylla* gum acid has a negative specific rotation, in common with those of the other *Acacia* gums hitherto examined, but the equivalent weight (740) is considerably lower. No significant differences have been detected between samples obtained from trees grown within a radius of several miles, and a preparation investigated electrophoretically showed single boundaries on both the ascending and the descending side. Although the descending boundaries exhibited considerable spreading, the ascending boundaries were very sharp. No further proof of homogeneity can be put forward. Commercial "gum arabic," procured from Eimer and Amend Co., New York, moves at a very different rate from cyanophylla gum acid (Joubert, *J. S. African Chem. Inst.*, in the press).

Paper-chromatographic examination of the hydrolysis of the gum acid in water at 95° showed the liberation within 4 hours of rhamnose, arabinose, and a disaccharide, followed, after 23 hours, by galactose and an aldobiuronic acid. The sugars were identified as L-rhamnose, L-arabinose, and D-galactose by chromatographic separation (Hough, Jones, and Wadman, *J.*, 1949, 2511), and the preparation of crystalline derivatives. The disaccharide formed an osazone whose m. p. corresponded with that obtained for 3-*O*-α-D-galactopyranosyl-L-arabinose phenylosazone by Jones (*J.*, 1953, 1672), and on methylation gave a crystalline heptamethyl derivative whose physical constants agree with those given by Smith (*J.*, 1939, 744) for the methylated disaccharide; it has not been isolated from *A. pycnantha* gum. These workers obtained their material from "gum arabic, Turc. variety" and "gum kordofan" respectively. The aldobiuronic acid liberated on autohydrolysis was 6-*O*-β-D-glucuronosyl-D-galactose, the same product as has been isolated from *A. mollissima* and *A. pycnantha* gums. It was homogeneous according to prolonged paper chromatography in an acid–ethyl acetate mixture, and formed a crystalline hepta-*O*-methyl methyl ester (cf. Challinor, Haworth, and Hirst, *J.*, 1931, 258).

The polysaccharide remaining after hydrolysis in water for 75 hours had almost the same equivalent weight (700) as the original gum acid, the splitting off of sugar residues being compensated by the liberation of aldobiuronic acid. Hydrolysis of this polysaccharide with dilute sulphuric acid gave D-galactose and an aldobiuronic acid identical with that produced during autohydrolysis.

Assay by the method of Hirst and Jones (*J.*, 1949, 1659) of the sugars produced on prolonged acid hydrolysis of the gum showed that L-rhamnose (5), L-arabinose (2), D-galactose (11), and D-glucuronic acid (5 mols.) must be linked together in the gum in the proportions indicated. The low proportion of arabinose necessitates the postulation of a repeating unit containing not 1 but at least 5 acid residues, in much the same way as the low rhamnose content of *A. pycnantha* gum (Hirst and Perlin, *loc. cit.*) requires 4 acid residues per repeating unit.

The composition of the gum from *A. cyanophylla* is therefore markedly different from that of *A. pycnantha* despite a close botanical connection and the identity of the ultimate hydrolysis products of the two gums. The rhamnose and uronic acid contents of *A. cyanophylla* gum are unusually high, but the ratio of these two residues is very nearly unity, as for the gums from *A. senegal* (Butler and Cretcher, *J. Amer. Chem. Soc.*, 1929, **51**, 1519) and *A. mollissima*. This may mean that rhamnose is linked glycosidically to the acid residues in these gums (cf. Charlson, Ph.D. thesis, Cape Town, 1954).

Experimental

Unless otherwise stated, concentration of solutions was carried out at 40°/20 mm., and specific rotations were measured in aqueous solution. Paper chromatograms were run at 27° in butanol–ethanol–water (20 : 1 : 3), ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4) (Jones, *loc. cit.*), or butanol–pyridine–water (9 : 2 : 2).

Purification of A. cyanophylla *Gum.*—The bulk of the gum was collected during autumn from a small plantation near Retreat in the Western Province of the Cape; the trees were identified by courtesy of the Bolus Herbarium. No attempt was made to separate the clear transparent jelly-like material from dried-out resinous lumps.

Crude gum (100 g.) was purified in the standard manner (Smith, *J.*, 1939, 744) by precipitation in ethanol from dilute hydrochloric acid and then aqueous solution (four times in all) and after drying at 45° in partial vacuum for 30 hr. was a white amorphous powder (65 g.), $[\alpha]_D^{16}$ −20° (*c*, 0·68) [Found: Loss at 100°/20 mm., 10·4; sulphated ash, 0·73; N, 0·17%; equiv., 740 (on dry basis); iodine absorption, 2·1 c.c. of 0·1N-iodine per g. after 20 minutes' oxidation]. Samples from different trees and different plantations had almost identical specific rotations and equivalent weights.

Hydrolyses of the Gum.—(a) *Total hydrolysis.* The purified gum acid was completely hydrolysed by 2N-sulphuric acid at 100° for 14 hr., as shown by filter paper chromatography of the neutralised hydrolysate (Partridge, *Nature*, 1946, **158**, 270; Flood, Hirst, and Jones, *J.*, 1948, 1679). Quantitative analysis (Hirst and Jones, *loc. cit.*) indicated that the products were galactose, arabinose, and rhamnose in the approximate molar ratio of 11·1 : 2 : 5 (mean of six determinations).

(b) *Partial hydrolysis.* The progress of hydrolysis of the gum acid (54 g.) in water (1 l.) at 90—95° was followed by polarimetric and iodometric observations: $[\alpha]_D$ −20° (initial), +18° (14 hr.), +23° (20 hr.), +25° (24 hr.), +27° (32 hr.), +28° (38 hr.), *ca.* +30° (50 hr.). Iodine values expressed as c.c. of 0·1N-iodine per g.: 2·3 (initial), 15·7 (4 hr.), 24·0 (8 hr.), 29·4 (12 hr.), 38·6 (20 hr.), 47·0 (32 hr.), 50·1 (38 hr.), 55·6 (50 hr.), 62·5 (66 hr.), 63·2 (75 hr.). Examination on a paper chromatogram after 4 hr. showed rhamnose, arabinose, and a disaccharide, which later increased slowly in amount; galactose and an aldobiuronic acid were found after 23 hr. The cooled, filtered solution was concentrated to 400 c.c. and poured into ethanol (1·5 l.), whereupon a degraded acid (C), $[\alpha]_D$ *ca.* 0° (*c*, 0·58), was precipitated (27·3 g., dried at 45° *in vacuo*) [Found: Loss at 100°/20 mm., 6·7%; equiv., 700 (on dry basis)]. Concentration of the neutralised (barium carbonate) filtrate (to 80 c.c.) followed by pouring into methanol (1 l.) afforded a slimy precipitate of barium salts (B; 8·3 g.), and, from the filtrate, a syrupy mixture of reducing sugars (A; 17·5 g.)

Examination of the Sugars (*A*).—Paper chromatography disclosed rhamnose, arabinose, galactose, a disaccharide, and a small amount of mixed barium salts at the origin. The syrup (0·2 g.) was then heated with 2N-sulphuric acid (5 c.c.) in a sealed tube at 100° for 14 hr. and analysed (Hirst and Jones, *loc. cit.*): rhamnose, arabinose, and galactose were found in the molar ratio 5·0 : 1·9 : 1·2.

Trituration of syrup (A) (16·5 g.) with cold methanol and storage in the ice-chest gave crystals (5 g.) which after separation on a porous tile and recrystallisation from ethanol proved to be α-L-rhamnose hydrate, m. p. and mixed m. p. 93—94°, $[\alpha]_D^{16}$ +9° (*c*, 2·1) [phenylosazone m. p. and mixed m. p. 175° (decomp.)]. Half of the residual syrup (5·1 g.) was separated by elution from a cellulose column (Hough, Jones, and Wadman, *loc. cit.*) with half-saturated aqueous butanol, α-L-rhamnose (1·9 g.), L-arabinose (1·2 g.), and D-galactose (0·6 g.) being obtained crystalline. The arabinose was identified through its m. p. and mixed m. p., 156°, $[\alpha]_D^{20}$ +105° (*c*, 1·0), and formation of its benzoylhydrazone, m. p. and mixed m. p. 204° (from ethanol); the galactose had m. p. and mixed m. p. 164°, $[\alpha]_D^{18}$ +79° (*c*, 1·2), and gave mucic acid on oxidation. Further elution, with 96% aqueous ethanol, afforded a chromatographically pure reducing disaccharide (0·6 g.), $[\alpha]_D^{16}$ +152° (*c*, 2·3), R_{gal} (relative to galactose) 0·50 in the basic solvent mixture; it consisted of galactose and arabinose which were detected on a paper chromatogram after hydrolysis of a small portion with 0·5N-sulphuric acid. The rate of movement of this disaccharide was identical with that of the chief disaccharide component of autohydrolysed commercial "gum arabic" acid. A mixture of barium salts (0·5 g.) was finally washed from the column with water.

Examination of the Barium Salts (*B*).—The mixed salts (B) constituted a white amorphous powder which reduced Fehling's solution strongly and had $[\alpha]_D^{17}$ +4° (*c*, 0·46) (Found: Ba,

13·8; iodine absorption, 38 c.c of 0·1N-iodine per g. Calc. for $C_{12}H_{19}O_{12}Ba_{0·5}$: Ba, 16·2%; iodine absorption 47 c.c.). On a descending paper chromatogram run in the acid solvent the main component had R_{gal} 0·27, but there were present also a uronic acid spot, R_{gal} 1·05, and two faint spots between R_{gal} 0·10 and the origin. After hydrolysis of the salts (B) (0·1 g.) with 2N-sulphuric acid (5 c.c.) in a sealed tube at 100° for 14 hr., the neutralised (barium carbonate) product was examined on a paper chromatogram; galactose and a uronic acid were present. Galactose was estimated by adding ribose (53·7 mg.) to a hydrolysate (258 mg.) prepared in this way, and determining the galactose–ribose ratio (Found: galactose, 40·0. Calc. for $C_{12}H_{19}O_{12}Ba_{0·5}$; galactose, 42·5%).

Further Hydrolysis of the Acid (*C*).—The polysaccharide (C) (13 g.) was heated with 0·5N-sulphuric acid (200 c.c.) on a boiling-water bath for 15 hr., during which the iodine absorption rose from 11 c.c. to 86 c.c. and $[\alpha]_D$ from +3° to +35°, with the customary levelling off of these values with time. The neutralised (barium carbonate) hydrolysate was then concentrated (to 50 c.c.) and poured into excess of methanol, whereupon a white amorphous precipitate of barium salts (D) (9 g.), $[\alpha]_D^{17}$ +13° (*c*, 0·7), was formed (Found: Ba, 16·2%; iodine absorption, 57 c.c. of 0·1N-iodine per g.). A slow-moving aldobiuronic acid (R_{gal} 0·27) together with traces of galactose and an uronic acid were present according to a paper chromatogram. (Complete hydrolysis gave a uronic acid and galactose only.) Concentration of the aqueous-methanolic mother-liquors yielded crude D-galactose (5·9 g.), which was purified by one recrystallisation from absolute ethanol and identified in the usual way.

Identification of the Disaccharide Isolated from the Sugars (*A*).—A portion of this disaccharide (0·1 g.) was oxidised with bromine water and barium benzoate (Hudson and Isbell, *Bur. Stand. J. Res.*, 1929, **3**, 58), and after hydrolysis of the product with dilute sulphuric acid the solution was neutralised using Amberlite IR-4B resin. The solution contained galactose (paper chromatogram); arabinose was therefore the aglycone in the disaccharide. On treatment with aqueous phenylhydrazine acetate at 100° for 30 min. the disaccharide formed a pale-yellow phenylosazone which, crystallised from ethanol, had m. p. 235° (decomp.) (Found: C, 55·9; H, 6·3; N, 12·0. Calc. for $C_{23}H_{30}O_8N_4$: C, 56·4; H, 6·1; N, 11·4%); Jones (*loc. cit.*) gives m. p. 240°.

Methylation of the disaccharide (1 g.) in the usual way afforded its crystalline heptamethyl derivative, m. p. 87—88° [from light petroleum (b. p. 40—60°)], $[\alpha]_D^{16}$ +168° (*c*, 1·2) (Found: C, 52·5; H, 8·3; OMe, 52·8. Calc. for $C_{18}H_{34}O_{10}$: C, 52·7; H, 8·4; 7OMe, 52·9%). Smith (*loc. cit.*) reported m. p. 82° and $[\alpha]_D^{18}$ +162° for hepta-*O*-methyl-3-*O*-α-D-galactopyranosyl-L-arabinose. On hydrolysis with dilute sulphuric acid the methylated substance (0·52 g.) gave a syrup (0·48 g.) which showed two large spots R_{MG} 1·0 (relative to tetramethylgalactose) and R_{MG} 0·78, and a much smaller spot R_{MG} 0·60 on a paper chromatogram (basic solvent). By use of butanol containing light petroleum (b. p. 100—120°) as mobile phase the mixture was separated on a cellulose column into (i) a pale yellow syrup (0·21 g.), R_{MG} 1·0, which afforded *N*-phenyl-D-galactosylamine 2 : 3 : 4 : 6-tetramethyl ether, m. p. and mixed m. p. 192°, on treatment with aniline in ethanol, (ii) a mixture (0·13 g.) of the methylated sugars, and (iii) a di-*O*-methylarabinose (0·09 g.), R_{MG} 0·78. Fraction (iii) had $[\alpha]_D^{15}$ +135° (*c*, 1·1) and did not evolve formaldehyde on periodate oxidation, but formed a crystalline derivative, m. p. 137°, on treatment with aniline in ethanol (Found: OMe, 24·6. Calc. for $C_{13}H_{19}O_4N$: 2OMe, 24·6%). These constants are consistent with this derivative's being the aniline compound of 2 : 4-di-*O*-methyl-L-arabinose (lit., m. p. 126°, 142°, 145—146°; aniline derivative of the 2 : 3-dimethyl ether, m. p. 139°). 2 : 3-Di-*O*-methyl-L-arabinose has $[\alpha]_D$ +107°, but should evolve 1 mol. of formaldehyde on periodate oxidation. The recorded $[\alpha]_D$ of the 2 : 4-isomer is +129°, and it should not evolve formaldehyde on periodate oxidation.

Identification of the Uronic Acid.—Barium aldobiuronate (11·5 g.), obtained by the hydrolysis of *A. cyanophylla* gum acid (30 g.) with 0·5N-sulphuric acid for 18 hr., was heated with 2N-sulphuric acid (120 c.c.) in a sealed bottle at 100° for 16 hr. The neutralised (barium carbonate) solution was poured into methanol (1 l.), and the precipitated barium salt was purified by elution from a cellulose column with water, after washing through traces of galactose with propan-2-ol (yield, 3·6 g.) (Found: Ba, 26·0. Calc. for $C_6H_9O_7Ba_{0·5}$: Ba, 26·3%). Barium was removed from an aqueous solution with Amberlite IR-100H resin; the resulting acid solution was then concentrated to a syrup, placed on a cellulose column, and fractionated with half-saturated aqueous butanol as eluant. D-Glucurone, m. p. 179° (lit., m. p. 175—176°), and D-glucuronic acid, m. p. 154°, $[\alpha]_D^{17}$ +33° (*c*, 2·0) (lit., m. p. 156°, $[\alpha]_D^{23}$ +35°), were thereby isolated. The uronic acid was characterised by oxidation with dinitrogen tetroxide in chloroform and condensation of the product with *o*-phenylenediamine to give the dibenziminazole

derivative of D-glucosaccharic acid, m. p. and mixed m. p. 234° (decomp.) (Lohmar, Dimber, Moore, and Link, *J. Biol. Chem.*, 1942, **143**, 551).

Identification of the Aldobiuronic Acid in Fraction (D).—Aldobiuronic acid was obtained from barium salts (D) (4 g.) by de-ionising them with Amberlite IR-100H resin and chromatography on cellulose, wet butanol being used to elute galactose and uronic acid, and 90% aqueous ethanol to elute the aldobiuronic acid (2 g.); this was a stable white solid, $[\alpha]_D^{16}$ −3° (*c*, 2·0), R_{gal} 0·27 (acid solvent) (Found: equiv., 380. Calc. for $C_{12}H_{20}O_{12}$: equiv., 356). The acid (1·3 g.) was methylated in the usual way to give a viscid syrup (1·0 g.), n_D^{20} 1·464, which slowly crystallised. Recrystallisation from light petroleum gave needles, m. p. 86—87°, $[\alpha]_D^{18}$ −35° (*c*, 1·2 in $CHCl_3$) (Found: C, 50·8; H, 7·7; OMe, 53·0. Calc. for $C_{20}H_{36}O_{12}$: C, 51·3; H, 7·8; 8OMe, 53·0%), constants which agree with those reported by Challinor, Haworth, and Hirst (*loc. cit.*) for the methyl ester of methyl hexa-*O*-methyl-6-*O*-β-D-glucuronosyl-β-D-galactoside.

Hydrolysis of this methyl ester (cf. Brown, Hirst, and Jones, *J.*, 1948, 1677) gave an ether-soluble syrup, from which a tri-*O*-methylgalactose (0·14 g.) was isolated on a large paper chromatogram. This methylated sugar had $[\alpha]_D^{20}$ +100° (*c*, 0·9) and R_{MG} 0·87, and gave a crystalline *N*-phenyl-D-galactosylamine trimethyl ether, m. p. 161—162° (lit. m. p. of the 2 : 3 : 4-ether 164—165° to 170°) after recrystallisation from ethanol (Found: OMe, 31·3. Calc. for $C_{15}H_{23}O_5N$: 3OMe, 31·3%); this depressed the m. p. of *N*-phenyl-D-galactosylamine 2 : 4 : 6-trimethyl ether from 169° to 157°. The sugar ether was proved to be 2 : 3 : 4-tri-*O*-methyl-D-galactose by oxidation of a portion (15 mg.) with sodium periodate, whereupon formaldehyde (dimedone compound, m. p. and mixed m. p. 188°; yield 12 mg.) was evolved. The original aldobiuronic acid was therefore 6-*O*-β-D-glucuronosyl-D-galactose.

Identification of Aldobiuronic Acid in Fraction (B).—Barium was removed from the salts (B) by treatment with Amberlite IR-100H, and the acidic syrup obtained therefrom was fractionated on cellulose (cf. previous section) to yield an aldobiuronic acid (2 g.), R_{gal} 0·27 in the acid solvent. Complete methylation of this afforded a crystalline derivative, m. p. 86°, $[\alpha]_D^{17}$ −30° (*c*, 1·2 in $CHCl_3$); there was no depression of m. p. on admixture with the hepta-*O*-methyl methyl ester of 6-*O*-β-glucuronosyl-D-galactose described above.

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