*The Isolation of Oligosaccharides from Gums and Mucilages. Part II.**

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Hydrolysis of lemon gum yields a mixture of monosaccharides, and acidic and neutral oligosaccharides, including one to which the structure 4-*O*-(4-*O*-methyl-α-D-glucuronosyl)-L-arabinose (I) has been assigned.

LEMON GUM has been shown by Connell, Hainsworth, Hirst, and Jones (*J.*, 1950, 1696) to contain D-galactose, L-arabinose, and D-glucuronic acid. They reported a 4% methoxy-content, and this fact, together with new paper-chromatographic evidence, indicates that the glucuronic acid exists as a monomethyl derivative, probably the 4-methyl ether. These authors also isolated an aldobiuronic acid, which on methylation and hydrolysis yielded 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid and 2 : 3 : 6-tri-*O*-methyl-D-galactose, from the mixture of sugars produced when the gum was hydrolysed with boiling dilute sulphuric acid. The quantity of aldobiuronic acid obtained accounted for about a third of the total uronic acid in the gum, and it was suggested that the gum contains other aldobiuronic acid units. It is now shown that this is, in fact, the case.

Lemon gum, dissolved in N-sulphuric acid and kept at room temperature, underwent a slow hydrolysis which after some weeks had virtually ceased. The degraded gum so produced was composed of galactose and uronic acid residues only. The aldobiuronic acid described by Connell *et al.* (*loc. cit.*) was not detected amongst the sugars liberated during this hydrolysis, and therefore is undoubtedly a unit in the structure of the degraded gum. The chief monosaccharide liberated by this hydrolysis was L-arabinose, but galactose and rhamnose were also detected in trace amounts. Several oligosaccharides were produced during the degradation, two of them, both acidic, being in quantities sufficient for further examination.

The sugar acids of low molecular weight were isolated by use of an anion-exchange resin and then fractionated on a cellulose column, an acidic eluent being used. The first major component to be eluted was an *O*-methylaldobiuronic acid, to which the structure 4-*O*-(4-*O*-methyl-α-D-glucuronosyl)-L-arabinose (I) has been assigned. It was so resistant to hydrolysis that insufficient of its two component sugars could be obtained for their

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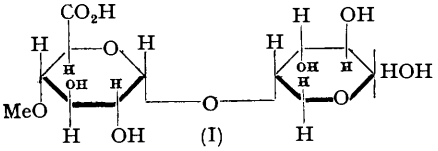
complete characterisation, but their rates of movement on the paper chromatogram and their colour reactions with *p*-anisidine hydrochloride corresponded with those of arabinose and 4-*O*-methylglucuronic acid. In order to elucidate further the structure of (I), it was



methylated with methyl sulphate and sodium hydroxide, and the non-reducing methylated acid was isolated, converted into its methyl ester by treatment with Purdie's reagents, and reduced to the corresponding glucosylarabinose derivative with lithium aluminium hydride. Finally, the newly-formed primary alcoholic group was methylated (Purdie's reagents), and the resultant methylated neutral disaccharide was hydrolysed with dilute acid. The resultant mixture consisted of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 2 : 3-di-*O*-methyl-L-arabinose, which sugars were separated on a sheet-paper chromatogram and identified by their physical properties and by conversion into well-characterised crystalline *N*-phenylglycosylamine derivatives.

The isolation of these two sugars proves that in lemon gum some of the *O*-methyl-D-glucuronic acid units are united through either $C_{(4)}$ or $C_{(5)}$ of a similar number of L-arabinose residues. The high positive optical rotation ($[\alpha]_D$ +134°) of (I) indicates both that the arabinose unit is in the pyranose form, and that the 1-4′-type linkage has the α-configuration.

In addition to (I), another methoxy-containing acidic oligosaccharide was isolated from the mixture of sugar acids of low molecular weight. This one appears to be a trisaccharide, for paper-chromatographic examination indicated that on hydrolysis it yielded equimolecular amounts of (I) and arabinose. A comparison of its optical rotation ($[\alpha]_D$ *ca.* +47°) with that of (I) suggests that the additional arabinose unit is in the furanose form, but as yet there is no conclusive evidence for its structure.

Experimental

Chromatographic separations were on Whatman No. 1 paper, with the following solvent mixtures: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (*b*) *n*-butanol–pyridine–water (10 : 3 : 3); and (*c*) *n*-butanol–ethanol–water (40 : 11 : 19) (all v/v). *p*-Anisidine hydrochloride was used as spray reagent to detect sugars. The rates of movement on the paper chromatogram of oligosaccharides are quoted relative to that of galactose, *i.e.*, they are R_{gal} values.

Optical rotations were determined at 20° ± 2° in water. Solutions were evaporated under reduced pressure. Microanalyses are by Mr. B. S. Noyes of Bristol.

Hydrolysis of the Gum.—Lemon gum (15 g.) was dissolved in cold N-sulphuric acid (150 c.c.) and the solution set aside at 20° ± 5°. Samples of the solution were withdrawn at intervals and examined on the paper chromatogram.

From 12 to 24 weeks after the start the chromatographic pattern appeared to be constant. Separation in solvent (*b*), in which uronic acids remain on the starting line, indicated the presence of much arabinose, a trace of galactose, and two neutral pentose-containing oligosaccharides, the one with R_{gal} 0·68 being present in considerable amount and the other with R_{gal} *ca.* 1·2 being present in traces only. In solvent (*a*) the picture was more complex as the uronic acids also moved on the chromatogram: the major acidic components that were present had R_{gal} 1·1 and 0·65.

After 24 weeks the gum solution was brought to pH 5 with barium hydroxide and filtered. The filtrate was concentrated to *ca.* 100 c.c., passed down a column of Amberlite resin IR120, and then down a column of Amberlite resin IR4B to remove uronic acids of low molecular weight. The effluent was neutralised with barium hydroxide, concentrated to 40 c.c., and poured into alcohol. The precipitated barium salt of the degraded gum was collected, dried, and extracted exhaustively with methanol. The yield of degraded gum was 6 g.; on hydrolysis it gave galactose and uronic acid derivatives, including a trace of an *O*-methyluronic acid, but no pentose. Concentration of the filtrate from the barium salt gave a syrupy mixture of sugars (6·5 g.) which partly crystallised. After trituration with methanol, crystalline L-arabinose

(0·4 g.), m. p. and mixed m. p. 158°, was filtered off. The filtrate was concentrated and the resulting syrup examined chromatographically [solvent (*b*)]. It consisted of arabinose (mainly), the pentose-containing disaccharide with R_{gal} 0·68, and traces of galactose, rhamnose, and two other pentose-containing saccharides (R_{gal} 1·2 and 0·35).

The Acids of Low Molecular Weight.—These were displaced from the column of Amberlite resin IR4B by elution with N-sulphuric acid. The effluent was neutralised with barium hydroxide solution and filtered, and the filtrate passed down a column of Amberlite resin IR120. The effluent contained a mixture of the ash-free uronic acids and was concentrated to dryness at 25° (yield, 1·5 g.). Chromatographic examination of this mixture [solvent (*a*)] indicated 4-*O*-methylglucuronic acid, an acidic disaccharide (I) with R_{gal} 1·1 and an acidic tri(?)saccharide with R_{gal} 0·65.

The syrup (1·5 g.) was placed on a cellulose column (14″ × 1″) and fractionally eluted with ethyl acetate–acetic acid–water (9 : 2 : 1 v/v). 4-*O*-Methyl-D-glucuronic acid (?) and traces of arabinose were first eluted followed by the *aldobiuronic acid* (I) (0·43 g.), which had R_{gal} 1·1 in solvent (*a*) and $[\alpha]_D$ +134° (*c*, 4·3) (Found: OMe, 10·4. $C_{12}H_{20}O_{11}$ requires OMe, 9·1%). The solvent was changed to ethyl acetate–acetic acid–water (9 : 2 : 2 v/v), and a slower moving component (yield, 0·9 g.) having R_{gal} 0·65 [in solvent (*a*)] was then eluted, admixed with a little of (I). The mixture had $[\alpha]_D$ +47° (*c*, 8·0) (Found: OMe, 6·0. Calc. for $C_{17}H_{28}O_{15}$: OMe, 6·6%). Hydrolysis of this fraction with dilute acid gave arabinose and a roughly equivalent amount of the aldobiuronic acid (I), detected chromatographically. The uronic acids remaining on the cellulose column were eluted with water (yield, 0·2 g.). Chromatographic examination indicated the presence in this material of three slow-moving uronic acid-containing fragments [R_{gal} 0·65, 0·42, and 0·25 in solvent (*a*)].

The aldobiuronic acid (I) was hydrolysed with 2N-sulphuric acid at 100° for 24 hr. Paper-chromatographic examination of the very dark hydrolysate indicated arabinose and 4-*O*-methylglucuronic acid but mainly unchanged (I).

Methylation of (I). The aldobiuronic acid (I) (0·42 g.) was methylated with 30% sodium hydroxide and methyl sulphate in the usual manner. The acidic product (0·47 g.), isolated by continuous chloroform extraction of the reaction mixture after acidification with sulphuric acid, was converted by treatment with Purdie's reagents into its methyl ester (yield, 0·46 g.) which was then reduced with lithium aluminium hydride by adding it dropwise in ethereal solution to a solution of this reagent in ether (yield of glucosylarabinose derivative, 0·28 g.). Unchanged methylated aldobiuronic acid (0·12 g.) was recovered by acidifying the reaction mixture with sulphuric acid and extracting it continuously with chloroform. The *glucosylarabinose derivative* was further methylated with Purdie's reagents, and the product distilled [yield, 0·25 g.; b. p. 170° (bath temp.)/1 mm.] (Found: OMe, 48·8. $C_{18}H_{34}O_{10}$ requires OMe, 52·9%).

The methylated disaccharide (0·24 g.) was hydrolysed in boiling N-hydrochloric acid: $[\alpha]_D$ +108° ⟶ + 74° (constant value, 7½ hr.). The cooled solution was neutralised with silver carbonate and filtered. The filtrate was concentrated to a syrup (0·19 g.) consisting of two sugars, which were separated on a sheet of filter paper by using solvent (*c*), and were isolated in the usual manner. The faster-moving component (0·116 g.) was identical with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose. After recrystallisation from ether–light petroleum (b. p. 60—80°) it had m. p. and mixed m. p. 90°, and the derived *N*-phenylglucosylamine had m. p. and mixed m. p. 137° after recrystallisation from ethanol. The slower-moving fraction (0·07 g.) (Found: OMe, 34·2. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), having R_G 0·82 in solvent (*a*), was identical with 2 : 3-di-*O*-methyl-L-arabinose. It had $[\alpha]_D$ +98° (*c*, 2·7), and when it was heated with alcoholic aniline *N*-phenyl-L-arabinosylamine 2 : 3-dimethyl ether was produced, m. p. and mixed m. p. 138° (Found: N, 5·5. Calc. for $C_{13}H_{19}O_4N$: N, 5·5%).

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