102. *The Structure of Peach Gum. Part I. The Sugars produced on Hydrolysis of the Gum.*

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Peach gum on hydrolysis yields a mixture of D-galactose (5 parts), L-arabinose (6 parts), D-xylose (2 parts), rhamnose (*ca.* 2%), and D-glucuronic acid (1 part). The rate of hydrolysis indicates that the arabinose residues are in the furanose form; the other sugars are probably present in the pyranose form. Graded hydrolysis of the gum gives an aldobiuronic acid identified as 6-D-glucuronosido-D-galactose. Oxidation of the gum with periodic acid yields approximately two mols. of formic acid per repeating unit of equivalent weight 2040. Eight sugar residues are oxidised, of which four are arabinose, two are xylose and two are galactose.

Samples of peach gum (*Prunus persica* L.) were kindly supplied from Piedmont, Italy, by Professor A. Nasini of the University of Turin, and from Bristol by Mr. R. Stitch. Examination of the physical constants of fractions of the gum indicated that it was essentially homogeneous, but it may be a mixture not separable by the methods in general use. Analysis of the sugars produced on hydrolysis of the fractions, by the use of partition chromatography (Flood, Hirst, and Jones, *J.*, 1948, 1679; Hirst and Jones, *J.*, 1949, 1659), showed that the ratio of sugars was D-galactose (5 parts), L-arabinose (6 parts), D-xylose (2 parts), and D-glucuronic acid (1 part; by difference) per equivalent weight of 2040. L-Rhamnose (*ca.* 2%) was also present. The identity

of the sugars was confirmed by graded hydrolysis of the gum. The rate of hydrolysis of the sugars, estimated by chromatographic analysis on paper, showed that L-arabinose was hydrolysed very rapidly, indicating that it is combined in the furanose form in the gum (cf. damson gum; Hirst and Jones, *J.*, **1946**, 506). The D-xylose was hydrolysed rather more slowly than the arabinose, but faster than the D-galactose, which is very probably in the pyranose form. From the products of hydrolysis of the gum, pure crystalline specimens of L-arabinose, D-galactose, D-xylose, and L-rhamnose were isolated. Oxidation of the gum with potassium periodate solution led to the consumption of ten moles of periodate and the formation of approximately two moles of formic acid per equivalent of gum (2040). During this oxidation all the xylose and approximately two-thirds (4 moles) of the arabinose and two-fifths (2 moles) of the galactose are destroyed. These results follow from a quantitative analysis of the sugars present in the oxidised gum by paper partition chromatography. Some of the L-arabofuranose residues must therefore be linked through $C_{(2)}$ and/or $C_{(3)}$ because only thus will the α-glycol group [·CH(OH)·CH(OH)·] be absent in the molecule. The formic acid may have arisen from the oxidation of a D-xylopyranose, a D-galactopyranose, or a uronic acid end group. If the D-xylose was an end group, then galactopyranose residues containing 1 : 2- or 1 : 4-glycosidic linkages were present in the gum. If the galactose residues occupy terminal positions, then xylose residues substituted on $C_{(2)}$ or $C_{(4)}$ only must be present in the gum molecule.

Methylation of the barium salt of the aldobiuronic acid fraction, obtained by graded hydrolysis of the polysaccharide, yielded a non-reducing methylated aldobiuronic acid which was built up of D-glucuronic acid linked through $C_{(6)}$ of a D-galactose residue, since on hydrolysis the methylated acid gave equimolecular proportions of 2 : 3 : 4-trimethyl D-glucuronic acid (I) and 2 : 3 : 4-trimethyl D-galactose (II). The former was identified as the crystalline methyl ester of 2 : 3 : 4-trimethyl D-glucosaccharolactone, and the identity of (II) was confirmed from its rate of movement on the paper chromatogram and by the formation of its characteristic anilide. The aldobiuronic acid is therefore composed of the same sugar residues as are the aldobiuronic acids present in arabic acid and egg plum gum.

EXPERIMENTAL.

The crude samples of gum consisted of a mixture of large and small nodules admixed with bark and organic debris. The gum was essentially homogeneous since nodules gave, after purification, substantially identical constants. The gum was fractionated by extracting separate nodules successively with (*a*) cold water, yielding a cold-water fraction, and (*b*) hot N-sodium hydroxide to give an alkali-soluble fraction. The insoluble residue consisted of bark and inorganic material. The fractions were purified by precipitation from aqueous solution with glacial acetic acid (twice) (Bell and Young, *Biochem. J.*, 1934, **28**, 882). This procedure removed practically all the ash and colouring matter. The gum was finally precipitated from aqueous solution by addition of methanol in order to remove acetic acid. It was thus obtained as a white powder, initially freely soluble in water, but gradually becoming insoluble. Drying in a partial vacuum rapidly caused the gum to become insoluble in water. The cold-water-soluble fraction had $[\alpha]_D^{20}$ $-46°$ (*c*, 0·23 as the sodium salt in water) (Found : equiv., 2300; sulphated ash, 0·4%). The gum soluble in hot sodium hydroxide had $[\alpha]_D^{20}$ $-47°$ (*c*, 0·22 as the sodium salt in water) (Found : equiv., 2200; sulphated ash, 0·3%). Samples of gum from Turin and from Bristol were not distinguishable by physical properties or chemical reactions.

A sample of each of these fractions was hydrolysed on the boiling water-bath with N-sulphuric acid for 5 hours. The cooled solution was neutralised with barium carbonate and filtered, and the filtrate examined on the paper chromatogram; galactose, arabinose, and xylose were detected in each case. Rhamnose was not detected until a later stage (see below).

Quantitative Analysis of the Sugars produced on Hydrolysis of the Gum.—Peach gum (cold-water-soluble; 100·4 mg.) was hydrolysed with N-sulphuric acid (5 c.c.) in a sealed tube in a boiling water-bath for 12 hours. The cooled tube was opened and its contents were added to a solution of ribose (41·6 mg.); acids (including uronic acids) were then removed with Amberlite Resin IR4B, and the filtered solution was concentrated to about 2 c.c. Portions (0·001 ml.) of the solution were then placed on the starting line of a sheet paper chromatogram, an Agla micrometer syringe being used, and the sugars were separated and isolated by the procedure of Flood, Hirst, and Jones (*J.*, 1948, 1679) as modified by Hirst and Jones (*J.*, 1949, 1659) (Found : galactose, 0·93; arabinose, 0·93; xylose, 0·34; ribose, 0·90 mg.; whence the percentage of galactose is 38·6 calc. as $C_6H_{10}O_5$, and that of arabinose 37·8 and of xylose 13·8 calc. as $C_5H_8O_4$). A polysaccharide of equiv. 2040 and containing five galactose residues, six arabinose residues, two xylose residues, and one uronic acid residue per average repeating unit requires: galactose, 39·6 (calc. as $C_6H_{10}O_5$); arabinose, 38·8; xylose, 12·9% (calc. as $C_5H_8O_4$). Peach gum (soluble in sodium

hydroxide) on hydrolysis and analysis, by the method detailed above, gave a mixture of galactose, arabinose, and xylose [Found: galactose, 40·2 (calc. as $C_6H_{10}O_5$); arabinose, 38·4; xylose 13·6% (calc. as $C_5H_8O_4$)]. Owing to the small amount present, rhamnose was not detected in these analyses.

Oxidation of Peach Gum with Potassium Periodate Solution.—The gum (547 mg. of neutral sodium salt) was dissolved in water (50 c.c.) containing potassium chloride (5 g.), sodium metaperiodate solution (50 c.c.; 0·25M.) added, and the whole shaken in the dark and in an air-tight vessel. After various time intervals, portions (25 c.c.) of the solution were withdrawn, ethylene glycol (excess) was added, and the formic acid titrated with 0·01N-sodium hydroxide, methyl-red being used as indicator [Found: 12·7 c.c. (190 hours); 12·5 c.c. (220 and 260 hours)]. This corresponds to the production of one mol. of formic acid per 1100 g. of gum, or approx. two mols. per equivalent of 2040.

The gum (516 mg.), under conditions of oxidation similar to those described above, consumed sodium periodate (4·9 c.c.; N.), whence 1 l. of M-periodate is equivalent to 211 g. of gum. It follows that approx. ten mols. of periodate are consumed per 2040 g. of gum.

Determination of the Sugars produced on Hydrolysis of the Oxidised Gum.—The gum (0·50 g.) was oxidised with potassium periodate as described above. Ethylene glycol was added to the solution after 200 hours, and the solution dialysed against distilled water until iodate and chloride ions could no longer be detected in the solution. The oxidised product (0·3 g.) was isolated by concentrating the dialysed solution to *ca.* 10 c.c. and precipitating it with methanol. The product, a pale yellow powder, was dried in a partial vacuum at 100°.

The oxidised polysaccharide (104·9 mg.) was hydrolysed by heating it with sulphuric acid (2N.; 5 c.c.) in a sealed tube in a boiling water-bath for 7 hours. The tube was cooled and the contents washed into a solution of ribose (89·3 mg.) in water. The solution was neutralised with Amberlite Resin IR4B and filtered. The filtrate was concentrated to *ca.* 1 c.c., and portions of the solution (0·001 ml.) were placed on the starting line of a sheet paper chromatogram. After separation and isolation the sugars were determined as before [Found: galactose, (*a*) 1·51, (*b*) 1·50; arabinose, (*a*) 0·57, (*b*) 0·61; ribose, (*a*) 4·35, (*b*) 4·45 mg.]. These figures correspond to the presence of the following percentage of sugars, calculated as $C_6H_{10}O_5$ and $C_5H_8O_4$, respectively: galactose, (*a*) 26·6, (*b*) 25·8; arabinose, (*a*) 10·0, (*b*) 10·4. No xylose could be detected.

Hydrolysis of Ash-free Peach Gum.—The gum (1·0 g.) was dissolved in 0·5N-sulphuric acid (25 c.c.) containing ribose (0·198 g.). The solution was heated in a closed vessel on a boiling water-bath, and at intervals samples were withdrawn and analysed [Found (reducing sugar calculated as hexose): 874 mg. (1 hour); 969 mg. (2 hours); 1·269 mg. (5 hours and 9 hours)].

Portions of the solution were withdrawn at intervals, cooled, neutralised with barium carbonate, and filtered, and the filtrate concentrated. The residual solution of sugars were analysed by the method of sheet paper chromatography [Found (percentage of sugars calc. as $C_6H_{10}O_5$ and $C_5H_8O_4$, respectively): galactose, 6·8; arabinose, 35; xylose, 8 (1 hour); galactose, 27·0; arabinose, 37·0; xylose, 13·3 (4 hours); galactose, 38·8; arabinose, 37·0; xylose, 12·8 (9 hours)]. Rhamnose was not detected in this experiment.

Autohydrolysis of the Gum.—The ash-free gum (10·52 g.) was dissolved in water (100 c.c.), and the thick brown solution heated on the steam-bath for 66 hours. At intervals, samples were withdrawn and analysed. It was not possible to follow changes in optical rotation owing to the darkness of the solution. Analysis on the paper chromatogram showed that arabinose was the first reducing sugar to appear in solution and that, after 41 hours, arabinose, xylose, and three other sugars, possibly disaccharides (their rate of movement on the paper chromatogram was about that of maltose), were present in the solution. After 52 hours, galactose, arabinose, and xylose could be detected. At this stage, the solution contained 5·4 g. of reducing sugars (calc. as hexose). This figure remained unaltered on heating of the solution for a further 14 hours.

The cooled solution was neutralised with barium hydroxide solution and filtered. The filtrate was concentrated to *ca.* 100 c.c. and poured into methanol (1 l.). The precipitated barium salts were redissolved in water and reprecipitated in methanol to remove reducing sugars, yielding a product (*A*, 2·75 g.) containing no free galactose, arabinose, or xylose (paper chromatogram). The methanolic solution on concentration gave a syrup which was exhaustively extracted with boiling methanol, yielding an alcohol-insoluble residue (*B*; 1·7 g.) and soluble sugars (*C*; 7·5 g.) obtained on removal of the solvent.

Fraction (*C*) crystallised on trituration with methanol. The crystals (3·66 g.) were filtered off and identified as L-arabinose, $[\alpha]_D^{20}$ 105° (in water), m. p. 160°. These crystals contained traces of xylose only as impurity (paper chromatogram). The non-crystalline residue was analysed on the paper chromatogram [Found: galactose, 1·27; arabinose, 0·75; xylose, 0·66 mg.; whence the weights of galactose, arabinose, and xylose present in the syrup (3·84 g.) were 1·81, 1·07, and 0·95 g., respectively]. The presence of these sugars was confirmed by the isolation of L-arabinose benzoylhydrazone, m. p. 186°, D-galactose phenylmethylhydrazone, m. p. and mixed m. p. 186°, and dibenzylidene D-xylose dimethyl acetal, m. p. 210°, not depressed on admixture with an authentic specimen. L-Rhamnose was not detected until later (see below).

The syrupy sugars (3·0 g.) from fraction (*C*) were separated on a column of cellulose by the method of Hough, Jones, and Wadman (*J.*, 1949, 2511), using *n*-butanol saturated with water as eluent. Examination of the eluate showed that the first tubes contained pure L-rhamnose, which was isolated as the crystalline hydrate (52 mg.) after recrystallisation from acetone–methanol, m. p. and mixed m. p. 100°, $[\alpha]_D$ +9° (*c*, 1·1 in water). The next few tubes contained D-xylose, which was obtained crystalline on concentration of the solvent. After recrystallisation, the product had m. p. 146° and $[\alpha]_D$ +18° (in water). Thereafter arabinose and galactose were eluted from the column but were not further examined. The residual sugars on the column were then eluted with water, the eluate was concentrated, and the residual syrupy sugars (0·52 g.) separated by the filter-paper strip method of Flood, Hirst, and Jones (*loc. cit.*), yielding the following fractions: (*a*) arabinose, (*b*) galactose, (*c*), (*d*), and (*e*) sugars moving at a rate corresponding closely to that of a disaccharide. Fractions (*a*) and (*b*) were not further examined. Fraction (*e*), which had moved very slowly indeed, was a barium salt and was discarded. Fractions (*c*) and (*d*) on hydrolysis yielded galactose, identified by its rate of movement on the chromatogram.

Fraction (*B*) (1·7 g.) was hydrolysed with 0·5N-sulphuric acid (50 c.c.) on the boiling water-bath: $[\alpha]_D^{20}$ rose to +47° (*c*, 3·4 in 0·5N-sulphuric acid; constant value, 6 hours). The cooled solution was neutralised with barium carbonate and filtered. The filtrate was concentrated, and the syrupy residue extracted exhaustively with methanol, leaving an insoluble barium salt (0·8 g.). The methanol extract contained galactose (0·82 g.), arabinose (0·07 g.), and xylose (0·04 g.), determined by analysis on the paper chromatogram. D-Galactose was further identified by the isolation of the crystalline sugar (0·6 g.), m. p. 165°, $[\alpha]_D^{20}$ +82° (in water). The insoluble residue of barium salt (0·8 g.) was added to the barium salt obtained from fraction (*A*) (see below).

Fraction (*A*) (2·03 g.) was hydrolysed with boiling 0·1N-sulphuric acid (75 c.c.) for 12 hours: $[\alpha]_D$ rose from +29° to +47°. The cooled solution was neutralised with barium hydroxide solution and filtered, the filtrate concentrated to dryness, and the residue exhaustively extracted with methanol, leaving a barium salt (1·58 g.). Concentration of the methanolic extract gave crystalline D-galactose (0·46 g.), $[\alpha]_D^{20}$ +80°, m. p. 165°. This sample of sugar contained traces of arabinose and xylose (paper chromatogram).

Identification of Uronic Acid Fraction.—The barium salts (1 g.) from fraction (*A*) (above) were heated with sulphuric acid (2N.; 25 c.c.) for 15 hours on the boiling water-bath to hydrolyse any remaining oligosaccharide. The cooled solution was then neutralised (a small quantity of the syrup was examined on the paper chromatogram and spots corresponding to the presence of glucurone and glucuronic acid were detected), and the sugars were converted into their methyl ethers by reaction first with methyl sulphate and sodium hydroxide until the solution was non-reducing to Fehling's reagents, and then by the addition of 30% sodium hydroxide and methyl sulphate with stirring. After completion of the addition of the reagents, the solution was boiled to destroy sodium methyl sulphate, cooled, extracted with chloroform, acidified, and again extracted with chloroform. Concentration of the latter chloroform extracts yielded trimethyl methyl-D-glucuronoside (0·42 g.) as a syrup (Found: OMe, 49·0%; equiv., 254. Calc. for $C_{10}H_{18}O_7$: OMe, 49·6%; equiv., 250). The D-glucuronoside (0·4 g.) was hydrolysed with N-hydrochloric acid on the boiling water-bath for 5 hours, the solution cooled, and bromine water added. When the solution no longer reduced Fehling's solution (5 days) it was aerated to remove bromine, neutralised with silver carbonate, filtered before and after the passage of hydrogen sulphide, and then concentrated to the syrupy 2 : 3 : 4-trimethyl D-glucosaccharic acid. This product was esterified by boiling it with methanolic hydrogen chloride, and the solution was then neutralised with silver carbonate and filtered. Concentration of the filtrate yielded the methyl ester of 2 : 3 : 4-trimethyl D-glucosaccharolactone, m. p. and mixed m. p. 111°.

Isolation of a crystalline acid derivative. The residual barium salts (1·15 g.) from fractions (*A*) and (*B*) were methylated with methyl sulphate and sodium hydroxide by the procedure described above. After completion of the methylation, the solution was cooled, acidified with dilute sulphuric acid, and extracted with chloroform. Concentration of the extracts left a syrup (0·8 g.) which was methylated with Purdie's reagents and then distilled. Two fractions and a still residue were obtained: Fraction (1) (0·11 g.), b. p. 130°/0·05 mm., n_D^{18} 1·4484 (Found: OMe, 50·0%); this fraction on hydrolysis gave 2 : 3 : 4-trimethyl D-glucuronic acid, identified by its rate of movement on the chromatogram and by its conversion into the methyl ester of 2 : 3 : 4-trimethyl D-glucosaccharolactone, m. p. 111°. Fraction (2) (0·23 g.), b. p. 200—230°/0·05 mm., n_D^{20} 1·4662 (Found: OMe, 51%; equiv., 450). Still residue (0·42 g.) (Found: OMe, 40·3%). Fraction (2) (0·20 g.) was hydrolysed with 2N-hydrochloric acid (25 c.c.) for 15 hours. It was not possible to follow any change in optical rotation owing to darkening of the solution. The solution was neutralised with silver carbonate and filtered, and the filtrate treated with hydrogen sulphide and again filtered. The filtrate was concentrated to 10 c.c. and then neutralised with barium carbonate and filtered. Concentration of the filtrate left a glass which was exhaustively extracted with ether, leaving the barium salt of 2 : 3 : 4-trimethyl D-glucuronic acid, identified as the methyl ester of 2 : 3 : 4-trimethyl D-glucosaccharolactone, m. p. and mixed m. p. 111°. The ethereal extract contained 2 : 3 : 4-trimethyl D-galactose, since a small quantity of the syrup remaining after removal of the solvent moved at the same rate as this compound on the paper chromatogram (Brown, Hirst, Hough, Jones, and Wadman, *Nature*, 1948, **161**, 720). This was confirmed by converting the syrup {0·08 g.; $[\alpha]_D^{21}$ +87° (*c*, 0·8 in water)} into the characteristic anilide, m. p. 169°, not depressed on admixture with an authentic sample.

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