661. *Cherry Gum. Part III. An Examination of the Products of Hydrolysis of Methylated Degraded Cherry Gum, using the Method of Paper Partition Chromatography.*

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Methylated degraded cherry gum yields on hydrolysis a mixture of methylated sugars which have been separated by means of partition chromatography on a column of cellulose. After separation, 2 : 3 : 4-trimethyl and 2 : 4-dimethyl D-xylose, 2 : 3 : 4 : 6-tetramethyl, 2 : 4 : 6-trimethyl, 2 : 4-dimethyl, and 2 : 6-dimethyl D-galactose, 2 : 3 : 4-trimethyl and 2 : 3-dimethyl D-glucuronic acid were identified. Two unidentified sugars, probably derivatives of mannose, were also separated. Oxidation of cherry gum with periodate results in destruction of the xylose and of about half of the arabinose; the galactose is unaffected. The significance of these results is discussed.

Earlier work (Jones, *J.*, 1939, 558) has shown that cherry gum yields on hydrolysis a mixture of L-arabinose (52% as $C_5H_8O_4$), D-xylose (*ca.* 1% as $C_5H_8O_4$), D-galactose (26% as $C_6H_{10}O_5$), D-mannose (10% as $C_6H_{10}O_5$), and D-glucuronic acid (11% as $C_6H_8O_6$). These analytical figures have been confirmed by the separation and estimation of the products of hydrolysis of the gum by the method of Flood, Hirst, and Jones (*J.*, 1948, 1679; see Experimental section). Graded hydrolysis of the ash-free acidic polysaccharides yields L-arabinose and a degraded polysaccharide gum composed of D-xylose, D-galactose, D-mannose, and D-glucuronic acid residues. This arabinose-free polysaccharide has now been methylated by the thallium hydroxide–methyl iodide method (Menzies, *J.*, 1926, 937; Hirst and Jones, *J.*, 1938, 502). Examination, on the paper chromatogram (Brown, Hirst, Hough, Jones, and Wadman, *Nature*,

1948, **161**, 720), of the sugars produced on hydrolysis of the methylated degraded gum showed that they were a complex mixture. Partial separation of these sugar derivatives was achieved by partition chromatography on a column of cellulose using the procedure of Hough, Jones, and Wadman (*Nature*, 1949, **163**, 177; this vol., p. 2511). This method of analysis led to the isolation of pure fractions of sugars, but owing to the small quantity of material available no attempt was made at this stage to determine quantitatively the amounts of sugars present.

The following sugars were separated by this procedure and subsequently identified:

(1) 2 : 3 : 4 : 6-Tetramethyl D-galactose (cf. I). This, as well as (2), (3), (4), and (6), were identified as their crystalline anilides.

(2) 2 : 3 : 4-Trimethyl D-xylose (cf. II), isolated as the crystalline sugar.

(3) 2 : 4 : 6-Trimethyl D-galactose (cf. III), identified as its crystalline anilide.

(4) 2 : 4-Dimethyl D-xylose (cf. IV), isolated as its crystalline anilide.

(5) 2 : 6-Dimethyl D-galactose (cf. V), the structure being indicated by its non-identity with 2 : 4-, 3 : 4-, and 4 : 6-dimethyl D-galactose and by its conversion on methylation into 2 : 3 : 4 : 6-tetramethyl D-galactose; this proves it to be a derivative of D-galactose and to possess a hydroxyl group on $C_{(5)}$. Its identity with 2 : 6-dimethyl galactose was confirmed by its rate of movement on the paper chromatogram.

(6) 2 : 4-Dimethyl D-galactose (cf. VI), identified as its crystalline anilide.

(7) A trimethyl mannose (?) and a dimethyl mannose (?) derivative were also detected on the paper chromatogram. They were considered to be derivatives of mannose from their rate of movement on the chromatogram. It is known that at least one derivative of D-mannose must be present since the degraded gum on further hydrolysis yields an aldobionic acid consisting of D-glucuronic acid linked through $C_{(2)}$ of a D-mannose residue. It follows that either 3 : 4 : 6-trimethyl D-mannose or other derivatives such as 4 : 6-, 3 : 6-, or 3 : 4-dimethyl or a monomethyl derivative of mannose must be present in the products of hydrolysis of the methylated degraded gum.

(8) 2 : 3 : 4-Trimethyl D-glucuronic acid (cf. VII) was identified, after oxidation and esterification, as the methyl ester of 2 : 3 : 4-trimethyl D-glucosaccharolactone

(9) 2 : 3-Dimethyl D-glucuronic acid (cf. VIII) was converted into 2 : 3-dimethyl D-glucosaccharic acid and identified as the methyl ester of 2 : 3-dimethyl D-glucosaccharofuranolactone.

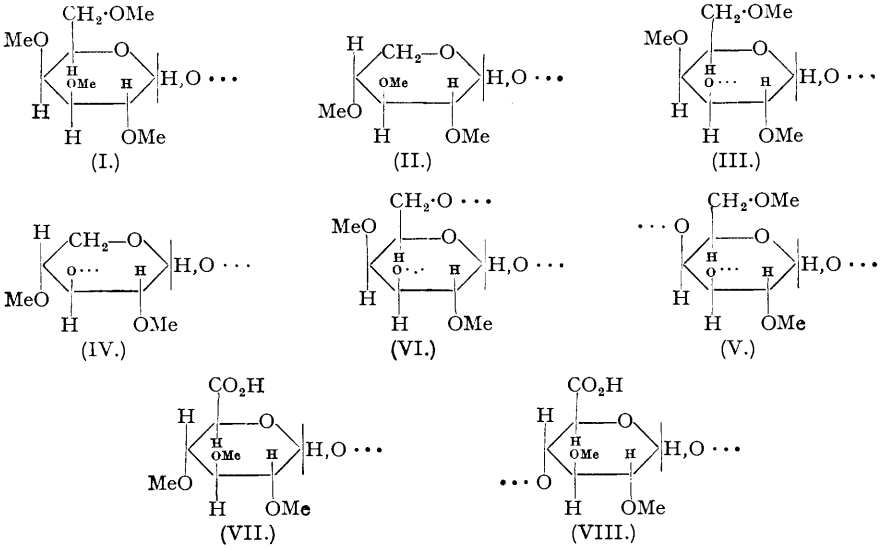
The isolation of these methylated sugars shows that degraded cherry gum (arabinose-free) is a branched-chain polysaccharide containing terminal residues of D-galactopyranose and D-xylopyranose. The isolation of 2 : 4 : 6-trimethyl and 2 : 4-dimethyl D-galactose shows that the 1 : 3-type of linkage present in damson gum (Hirst and Jones, *J.*, 1946, 506) is also present in cherry gum. The isolation of 2 : 6-dimethyl D-galactose (cf. Bell, *J.*, 1945, 682; Dewar and Percival, *Nature*, 1945, **156**, 633) shows that a 1 : 4-type linkage is also present. Methylated cherry gum on hydrolysis yields a complex mixture of sugars, not all of which were identified (Jones, *J.*, 1947, 1055). Evidence was given for the presence of a dimethyl pentose fraction which was not affected by sodium periodate; this therefore contained no adjacent hydroxyl groups, and gave on oxidation a pyranolactone, showing that $C_{(4)}$ was substituted with a methoxyl group. The isolation of 2 : 4-dimethyl D-xylose from methylated degraded cherry gum is strong indication that this sugar was probably present also in the products of hydrolysis of methylated cherry gum. It is of interest that in this case the D-xylopyranose residue is substituted through $C_{(3)}$, not through $C_{(2)}$, as in some of the plant mucilages (Mullan and Percival, *J.*, 1940, 1501) and tragacanthic acid (James and Smith, *J.*, 1945, 741), or through $C_{(4)}$ as in xylan (Bywater, Haworth, Hirst, and Peat, *J.*, 1937, 1983).

Both methylated cherry gum and methylated degraded cherry gum yield, on hydrolysis, mixtures of 2 : 3 : 4-trimethyl and 2 : 3-dimethyl D-glucuronic acid, indicating that these residues are not substituted by L-arabofuranose residues.

The sugar residues (I)—(VIII) are present in methylated degraded cherry gum and are united through the carbon atoms indicated.

Oxidation of cherry gum with potassium periodate gave approximately 2 molecules of formic acid per equivalent of the gum. As glucuronic acid residues are present as end groups in the polysaccharide, it is doubtful whether this yield of formic acid has any structural significance (see Halsall, Hirst, and Jones, *J.*, 1947, 1427). During this oxidation some seven moles of periodate were consumed. The production of a mole of formic acid requires the reduction of two moles of periodate; approximately three sugar residues per equivalent of the gum were therefore oxidised by the periodate, in addition to the uronic acid residue. Analysis of the oxidised gum showed that the galactose was unaffected (25·6% changing to 24·5%), but that the xylose was oxidised and that the arabinose content fell from 52·2 to 20·2% (hexose

and pentose calculated as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively). The fate of the mannose and uronic acid is unsettled.



These results confirm the view that the galactose is not present as a derivative containing α-glycol groups and that arabinose is present in part (*ca.* 50%) in a form which is not oxidised by periodate. Methylated cherry gum on hydrolysis yields an amount of 2 : 5-dimethyl L-arabinose corresponding to *ca.* 50% of the total arabinose content of the gum; the sugar residue present in the gum which leads to this derivative contains no free α-glycol group when in combination in the polysaccharide and would therefore resist oxidation by the periodate ion.

Experimental.

Hydrolysis of Cherry Gum.—Cherry gum (ash free; 143·5 mg.) was dissolved in N-sulphuric acid (5 c.c.) and heated in a sealed glass tube on the boiling water-bath for 5 hours. The contents were then washed into a beaker containing ribose (41·6 mg.). The solution was neutralised with Amberlite resin 1R4B and filtered, and the filtrate concentrated to about 1 c.c. Spots of the resulting sugar solution were placed on the starting line of a sheet-paper chromatogram by means of an Agla micrometer syringe. The sugars were separated in the chromatography apparatus, with a mixture of *n*-butanol (400 parts), ethanol (100 parts), and water (190 parts) for development. The positions of the sugars were detected as described previously (Flood, Hirst, and Jones, *loc. cit.*) and the sugars extracted and determined by the method of Hirst and Jones (this vol., p. 1659) (Found: galactose, 1·06 mg.; arabinose, 2·21 mg.; xylose, 0·12 mg.; and ribose 1·08 mg.; whence the percentages of sugars, calculated as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively, were: galactose, 25·6; arabinose, 52·2; and xylose, 2·8%).

In a second experiment, the gum (63·2 mg.) from an ornamental double white cherry tree was hydrolysed and added to ribose (41·6 mg.), and the following figures were obtained on analysis: galactose, 0·26 mg.; arabinose, 0·54 mg.; xylose, 0·04 mg.; ribose, 0·60 mg.; whence the percentages of sugars, calculated as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively, were: galactose, 25·5, arabinose, 52·0, and xylose, 3·9%.

Oxidation of Cherry Gum with Periodate.—(*a*) Cherry gum (690 mg.; neutral sodium salt) was dissolved in water (50 c.c.) and oxidised with potassium periodate in the presence of potassium chloride (5 g.). After 8 days, ethylene glycol was added to the solution, and the acid produced determined by titration with 0·1N-sodium hydroxide (Found: titre, 8·4 c.c., equivalent to a yield of one g.-mol. of formic acid per 820 g. of polysaccharide).

(*b*) Cherry gum (577 mg.; neutral sodium salt) was oxidised as above, but for 9 days (Found: titre, 7·3 c.c., equivalent to a yield of one g.-mol. of formic acid per 792 g. of polysaccharide).

(*c*) Cherry gum (472 mg.; neutral sodium salt) was oxidised as described above. After 8 days, the consumption of sodium periodate was determined (Found: 20·5 c.c. of 0·1M-sodium periodate consumed, *i.e.*, one mol.-equiv. of α-glycol group per 230 g. of gum).

Determination of the Sugars produced on Hydrolysis of Periodate-oxidised Cherry Gum.—The periodate-oxidised gum (182 mg.) was hydrolysed in a sealed tube with N-sulphuric acid (3 c.c.) for 6 hours at 100°. The solution was then washed into a beaker containing ribose (62·2 mg.) in water (5 c.c.). Acidic materials were removed with Amberlite resin 1R4B, and the neutral solution was filtered and evaporated to a thin syrup (containing about 10% solids). The solution was then analysed as described above (Found, in duplicate experiments: galactose, 1·05, 1·08; arabinose, 0·875, 0·92; ribose, 1·3, 1·38 mg.; whence the percentages of sugars present as $C_6H_{10}O_5$ and $C_5H_8O_4$ respectively were: galactose, 24·8, 24·1; arabinose, 20·2, 20·1%).

Methylation of Degraded Cherry Gum.—Degraded cherry gum (arabinose-free) (Jones, *J.*, 1939, 558) (30 g.) was methylated first by the thallium hydroxide–methyl iodide method, then with thallium ethoxide–methyl iodide, and finally with silver oxide–methyl iodide (for details see Hirst and Jones, *loc. cit.*). The product, a pale yellow solid (26·7 g.) (Found: OMe, 41·5%), was fractionated from chloroform solution by the gradual addition of light petroleum (b. p. 40—60°), giving (i) (11·8 g.), $[\alpha]_D^{20}$ +5° (*c*, 0·51 in methanol) (Found: OMe, 40·9%), (ii) (3·1 g.), $[\alpha]_D^{20}$ +6° (*c*, 0·68 in methanol) (Found: OMe, 41·0%), and (iii) (7·6 g.), $[\alpha]_D^{20}$ +5° (*c*, 0·57 in methanol) (Found: OMe, 39·5%). Fraction (iv) (4·2 g.) was obtained by concentrating the chloroform–light petroleum mother-liquor. It was a sticky solid which was not further examined. Fractions (i), (ii), and (iii) were pale yellow crisp solids with very similar properties; they were therefore combined. The methylated product was soluble in methanol, acetone, chloroform, benzene, and cold water. It was soluble, with hydrolysis, in cold 14N-sulphuric acid (Jackson and Smith, *J.*, 1940, 74), $[\alpha]_D^{20}$ +24° (*c*, 1·4), rising to +78° (constant value) in 20 hours. (Owing to enemy action, the majority of the methylated derivative was lost and only a small portion could be used for analysis.)

Hydrolysis of the Methylated Derivative.—A portion (2·90 g.) was heated with methanolic hydrogen chloride (4%; 50 c.c.) in a sealed tube at 100° for 12 hours. The solution was then neutralised with silver carbonate and filtered. Concentration of the filtrate gave a syrup (3·31 g.) which was hydrolysed with N-hydrochloric acid (50 c.c.) at 95° for 8 hours. The cooled solution was neutralised with silver carbonate and filtered, and the filtrate treated with hydrogen sulphide and again filtered. After aëration to remove hydrogen sulphide, barium carbonate was added to neutralise the uronic acids, and the solution filtered and concentrated to a syrup which was exhaustively extracted with ether, leaving a residue of insoluble barium salts (1·42 g.). Concentration of the ethereal extract left a syrup (1·78 g.) which was separated, in part, into its constituents by partition chromatography on a column of cellulose, using *n*-butanol, half saturated with water, as the mobile phase and auramine as the indicator (Hough, Jones, and Wadman, *loc. cit.*). Auramine has a R_G value of *ca.* 1·0 and it was found that, by use of a column of dimensions 40 × 4 cm., auramine was detected in the eluent after about 400 c.c., and sugars after 430 c.c., of solvent had passed through the column. The eluent, collected in *ca.* 6-c.c. portions, was examined on the paper chromatogram by the method described by Hough, Jones, and Wadman, and was thus separated into the following 6 fractions.

(1) (0·43 g.) (Found: OMe, 46%) contained 2 : 3 : 4-trimethyl D-xylose (R_G 0·96), 2 : 3 : 4 : 6-tetramethyl D-galactose (R_G 0·88), and traces of an unknown sugar (R_G 0·83).

(2) (0·22 g.) (Found: OMe, 39·8%) contained 2 : 4 : 6-trimethyl D-galactose (R_G 0·69) and traces of 2 : 3 : 4-trimethyl xylose, 2 : 3 : 4 : 6-tetramethyl D-galactose and an unknown sugar (R_G 0·83).

(3) (0·41 g.) (Found: OMe, 39·7%) contained mainly 2 : 4 : 6-trimethyl D-galactose (R_G 0·69) with traces of an unknown sugar (R_G 0·56).

(4) (0·20 g.) (Found: OMe, 35·8%) contained 2 : 4-dimethyl D-xylose (R_G 0·64) and an unidentified D-mannose (?) derivative (R_G 0·56).

(5) (0·12 g.) (Found: OMe, 29·9%) consisted of a mixture of 2 : 6-dimethyl D-galactose (R_G 0·44) and 2 : 4-dimethyl D-galactose (R_G 0·39).

(6) (0·09 g.) (Found: OMe, 29·0%) contained 2 : 4-dimethyl D-galactose.

Examination of the fractions. Fractions (1) and (2) were combined and refractionated, with a mixture of *n*-butanol (1 vol.) and light petroleum (b. p. 80—100°) (9 vols.). Six fractions were isolated. The first, (*a*), on concentration yielded crystalline 2 : 3 : 4-trimethyl D-xylose (0·05 g.), m. p. and mixed m. p. 91°. Fraction (*b*) (107 mg.) contained a mixture of 2 : 3 : 4-trimethyl D-xylose and 2 : 3 : 4 : 6-tetramethyl D-galactose, identified as its anilide (m. p. and mixed m. p. with an authentic specimen, 194°). Fraction (*c*) (80 mg.) contained only 2 : 3 : 4 : 6-tetramethyl D-galactose (R_G 0·86), identified as its anilide, m. p. 195°. Fraction (*d*) (40 mg.) also contained only 2 : 3 : 4 : 6-tetramethyl D-galactose (anilide m. p. 194°). Fraction (*e*) (90 mg.) contained a little 2 : 3 : 4 : 6-tetramethyl galactose (anilide, m. p. 192°) and an unidentified sugar (trimethyl mannose?) of R_G 0·83. The last fraction (250 mg.) contained mainly 2 : 4 : 6-trimethyl D-galactose (anilide, m. p. 179°, not depressed on admixture with an authentic specimen). Traces of unidentified sugars with R_G values of 0·82 and 0·79 were also present.

Fraction (3) was boiled with alcoholic aniline (10%; 5 c.c.). On cooling, 2 : 4 : 6-trimethyl D-galactose anilide separated, having m. p. 174° (176° on admixture with an authentic specimen of m. p. 178°). A specimen of the anilide (*ca.* 0·1 mg.) was dissolved, with gentle warming, in dilute hydrochloric acid, and the solution examined on the paper chromatogram. Only 2 : 4 : 6-trimethyl galactose could be detected, characterised by its rate of movement compared with that of an authentic specimen of 2 : 4 : 6-trimethyl galactose anilide treated in a like manner.

Fraction (4) (0·20 g.) was refractionated, with *n*-butanol–water as solvent. Three fractions were isolated. Fraction (*a*) had R_G 0·64, and chromatographic analysis showed it to contain one main component and traces of another sugar. When boiled with alcoholic aniline, it gave an anilide, m. p. 174° after recrystallisation from ether. This was 2 : 4-dimethyl D-xylose anilide; the m. p. was depressed to 158° on admixture with 2 : 3 : 4-trimethyl D-galactose anilide, but not depressed on admixture with an authentic sample of 2 : 4-dimethyl D-xylose anilide (cf. Wintersteiner and Klingsberg, *J. Amer. Chem. Soc.*, 1949, **71**, 939). Fraction (*b*) consisted of a mixture of 2 : 4-dimethyl xylose (R_G 0·64) and an unknown sugar, also contained in fraction (*c*), with R_G 0·54, $[\alpha]_D^{20}$ +30° (*c*, 0·38 in water). This material (dimethyl mannose?) did not crystallise and did not give a crystalline anilide. The lactone, prepared in the usual manner also failed to crystallise.

Fraction (5) crystallised on storage. It was recrystallised from acetone–ether. The crystals of 2 : 6-dimethyl galactose had m. p. 134° and by comparison of their rate of movement in the chromatographic apparatus with that of authentic specimens, they were shown to differ from 2 : 4-, 4 : 6-, and 3 : 4-dimethyl galactose. When the crystals were kept, their melting point fell to that of the corresponding hydrated derivative, m. p. 98—100°, not depressed on admixture with an authentic specimen of 2 : 6-dimethyl D-galactose monohydrate. Methylation, with Purdie's reagent, of a small portion of the crystals gave 2 : 3 : 4 : 6-tetramethyl αβ-methyl-D-galactoside, identified after hydrolysis as the anilide of 2 : 3 : 4 : 6-tetramethyl D-galactose.

Fraction (6), when boiled with alcoholic aniline, yielded 2 : 4-dimethyl D-galactose anilide, m. p. 204°, not depressed on admixture with an authentic specimen.

Examination of the barium salts. The barium salts (1·42 g.) were dissolved in water, and barium ions removed with Amberlite resin I.R.100. The filtered solution was concentrated under reduced pressure to a syrup which was dissolved in butanol and separated on a column of cellulose, by means of the top layer of a mixture of *n*-butanol (50 parts), *n*-butyl acetate (6 parts), acetic acid (4 parts), and water (40 parts).

Four fractions were isolated, each appearing to be homogeneous since only one sugar could be detected on the paper chromatogram. Portions of these syrups gave bright red colours on filter paper after spraying it with aniline trichloroacetate solution and warming the paper (optical rotations were not observable) : (*A*) (0·3 g.) (Found : OMe, 32·3%; equiv., 240); (*B*) (0·4 g.) (Found : OMe, 26·0%; equiv., 230); (*C*) (0·1 g.) (Found : OMe, 19·6%; equiv., 260); and (D) (0·4 g.) (Found : OMe, 14·1%; equiv., 320) (retained at the top of the column and recovered after washing the column with methanol).

Fraction (*A*) (0·2 g.) was oxidised with bromine water until non-reducing (3 days at 20°), and the acid isolated in the usual manner after removal of bromine ions from the solution. Esterification of the acid with methanolic hydrogen chloride, followed by distillation under reduced pressure, yielded the methyl ester (0·18 g.), m. p. 110° (Found : OMe, 49·8. Calc. for $C_{10}H_{16}O_7$: OMe, 50·0%), of 2 : 3 : 4-trimethyl D-glucosaccharolactone.

Fraction (*B*) (0·3 g.), after oxidation with bromine water followed by esterification with methanolic hydrogen chloride, yielded, on distillation, 2 : 3-dimethyl D-glucosaccharofuranolactone methyl ester (0·25 g.), m. p. 101°, not depressed on admixture with an authentic specimen (Found : OMe, 39·0. Calc. for $C_9H_{14}O_7$: OMe, 39·7%).

Fractions (*C*) and (*D*) were oligosaccharides, since on further hydrolysis they yielded mixtures of sugars and uronic acids, and they were not further examined.

The author is indebted to Professor E. L. Hirst, F.R.S., for his interest and advice.

THE UNIVERSITY, BRISTOL. [*Received, August 29th,* 1949.]