379. Virgilia oroboides *Gum. Part III.*[1] *Products of Hydrolysis of the Methylated Gum.*

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Acid hydrolysis of methylated *Virgilia oroboides* gum yields the following neutral sugars: 2,3,5- and 2,3,4-tri- and 2,3-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,4-, 2,4,6-, and 2,3,6-tri-, 2,4-, 2,3-, and 2,6-di-, and 2- and 4-mono-*O*-methyl-D-galactose, and 4,6-di-*O*-methyl-D-mannose, together with traces of other sugars none of which constitutes more than 1% of the total. The hydrolysis products which are acidic, after reduction to neutral fragments and further acid hydrolysis, yield 2,3,4-tri- and 2,3-di-*O*-methyl-D-glucose, 2,3,4-tri- and 2,3-di-*O*-methyl-D-galactose, and 4,6-di-*O*-methyl-D-mannose, together with traces of other sugars. The structural significance of these results is discussed in relation to earlier work on products of partial hydrolysis from the gum, and a comparison is made with the similarly constituted gum ghatti.

Earlier investigation of *Virgilia oroboides* gum [1] showed that the gum acid (equiv., 2100) is composed principally of residues of D-galactose and L-arabinose, with D-glucuronic acid and D-mannose in smaller amount. Traces of D-xylose and 4-*O*-methyl-D-glucuronic acid have also been identified, and there is chromatographic evidence for the presence of some rhamnose. The chief structural features of the gum, as shown by the identification of fragments after successive partial acid hydrolyses, are as follows: long D-galactopyranose chains linked $\beta 1\rightarrow 6$, a smaller proportion of D-galactopyranose linked $\beta 1\rightarrow 3$, L-arabinopyranose (partially replaced by D-xylopyranose) residues $\alpha 1\rightarrow 5$ linked to L-arabinose, L-arabinofuranose $\alpha 1\rightarrow 5$ linked to L-arabinose, D-glucopyranuronic acid linked $\beta 1\rightarrow 2$ to D-mannose and $\beta 1\rightarrow 6$ to D-galactose, and the 4-*O*-methyl-D-glucuronic acid linked to D-galactose. Practically all of the arabinose (*ca.* 40% of the gum) is removed by mild acid treatment and is therefore probably linked through arabinofuranoside to the main fragment of the gum. The acidic residues are obtained, as is usual, in the form of aldobio- and aldotrio-uronic acids after hydrolysis of the partially-degraded gum under more severe conditions. An indication was obtained that galactose might be joined to arabinose in the gum.

The gum was converted into its fully methylated derivative, which was hydrolysed with N-sulphuric acid. By using ion-exchange resins the neutralised hydrolysate was separated into a syrupy mixture (A) of neutral sugars and an acidic mixture; the latter was then reduced with lithium aluminium hydride, and re-hydrolysed to give a second neutral sugar mixture (B). The mixtures A and B were each separated into fractions by partition chromatography on cellulose columns, and from these the following methylated sugars were identified by the usual preliminary examination followed by crystallisation of the sugars and/or by the formation of crystalline derivatives: from A, 2,3,4,6-tetra-, 2,3,4- and 2,4,6-tri-, 2,4-, 2,3-, and 2,6-di-, and 2- and 4-mono-*O*-methyl-D-galactose, 2,3,4- and 2,3,5-tri- and 2,3-di-*O*-methyl-L-arabinose; from B, 2,3-di-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-galactose. In addition the following sugars, present in minor amounts and sometimes in the form of mixtures difficult to separate, were identified on the basis of optical rotation, by paper chromatography and paper ionophoresis of the sugars and of the products obtained from them by demethylation or by complete methylation, and by chromatography of the products of periodate oxidation of the sugars and of their derived glycitols: from A, 2,3,6-tri-*O*-methyl-D-galactose and another tri-*O*-methyl-galactose (presumably 3,4,6-), 3,4-di-*O*-methyl-D-galactose, 3- and 6-*O*-methyl-D-galactose, 4,6-di-*O*-methyl-D-mannose and (?)6-*O*-methylmannose; from B, 2,3,4-tri-*O*-methyl-D-glucose, 4,6-di-*O*-methyl-D-mannose, and 2,3-di-*O*-methylgalactose.

[1] Part II, Smith and Stephen, *J.*, 1961, 4892.

The two D-glucose ethers, which appear in B but not in A, clearly arise from the reduction of the corresponding D-glucuronic acid residues in the hydrolysate of methylated *Virgilia oroboides* gum. The molar ratio of the 2,3,4-tri- to 2,3-di-*O*-methylglucose recovered indicates that of the (approximately) 8·5 acidic residues present in every 100 sugar residues of the gum an average of 6 are there as end groups, the remainder carrying a substituent sugar at position 4; of the six end groups, one appears to be *O*-methylated at position 4. More than half the acidic residues are β1→2 linked to D-mannopyranose which in turn carries a substituent at position 3, the rest of the acidic residues being β1→6 linked to D-galactopyranose. The presence of more 2,3- than 2,4-di-*O*-methylgalactose in B suggests that some of the galactose to which glucuronic acid is linked may be 4-*O*-substituted.

The D-galactose ethers isolated show that, of the galactose residues present in the gum, *ca.* 4% are end groups, over 40% are unsubstituted units in 1→6 linked chains, 5% are 1→3 linked, 20% occur as branch-points linked through positions 3 and 6, and 15% as branch-points linked through positions 4 and 6. The remaining galactose (10% of the total galactose, and representing *ca.* 5% of the whole gum) is recovered as methyl ethers of such variety that it is impossible to assess which indicate other modes of linking and which arise as a result of incomplete methylation of (presumably) hindered hydroxyl groups in the highly-branched gum molecules or even by demethylation during the acid hydrolysis.

In contrast to this diversity of galactose derivatives only three methyl ethers of L-arabinose have been identified in hydrolysate A, and there is possibly one in hydrolysate B. The bulk of the arabinose accounted for appears as pyranose and as furanose end groups, the rest as 1→5 linked arabinofuranose units. The yield of arabinose ethers is, however, low compared with that of the arabinose obtained from the gum by partial acid hydrolysis, and this is probably due to the relatively high volatility of 2,3,5-tri-*O*-methylarabinose. The proportion of end groups revealed by the methylation and hydrolysis procedure is somewhat less (by *ca.* 10%) than the proportion of branch-points, and this may partly be accounted for by arabinofuranose residues lost by evaporation of the fully methylated compound. Acid-labile arabinofuranose residues may also have been lost during the process of methylating the gum. The position of attachment of the arabinose residues to the rest of the gum structure should be revealed for certain by examination of the hydrolysis products from methylated partially-hydrolysed (*i.e.*, arabinose-free) gum, but the results so far obtained indicate that they are joined predominantly to positions 3 and 4 on D-galactopyranose and to position 3 on D-mannopyranose residues. The failure to isolate any *O*-methylated derivatives of rhamnose or of xylose makes it impossible to suggest what part these sugars may play in the constitution of the gum.

These results show that there is a general similarity in the molecular structure of *Virgilia oroboides* gum to those of a number of others, particularly damson [2] and cherry [3] gums and the exudates of *Anogeissus latifolia*, Wall (gum ghatti),[4] and *A. schimperi*.[5] The resemblance to gum ghatti is especially close. Each contains similar component sugars, the D-glucopyranuronic acid residues (some substituted on position 4) are linked in the same manner to D-mannose and to D-galactose, the D-galactopyranose residues are predominantly β1→6 linked, and there is a surrounding sheath of L-arabinofuranose residues. *Virgilia oroboides* gum, however, contains a large proportion of L-arabinopyranose as well as -furanose end groups, and α1→5 linkages between arabinose residues, whereas in gum ghatti the arabinose occurs principally as L-arabinofuranose end groups, the pyranose residues are parts of chains, and 1→5 linkages play only a minor role. There is a qualitative difference in the proportion of 1→3 linkages between D-galactopyranose

[2] Hirst and Jones, *J.*, 1946, 506.
[3] Jones, *J.*, 1947, 1055.
[4] Aspinall, Auret, and Hirst, *J.*, 1958, 4408.
[5] Aspinall and Christensen, *J.*, 1961, 3461.

units (more in *Virgilia oroboides* gum), and the D-mannopyranose units in this gum, although they are branch-points, do not carry a sugar attached to position 6. A lower proportion of the D-glucuronic acid residues in *Virgilia oroboides* gum is substituted by sugars in position 4, but to offset this there is evidence of 4-*O*-methylation.

Experimental

Paper chromatography was carried out on Whatman Nos. 1 and 3 MM filter-papers, the following solvent systems (v/v) being used: (*a*) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer), (*b*) benzene–ethanol–water (169 : 47 : 15, upper layer), and (*c*) butan-1-ol–pyridine–water (9 : 2 : 2); unless otherwise stated, solvent (*a*) was used for methylated sugars, and R_G values are rates relative to 2,3,4,6-tetra-*O*-methyl-D-glucose. Paper ionophoresis was in 0·1M-borate buffer at 400v for 2—4 hr., migration rates (M_g) being expressed relative to glucose.[6] Optical rotations were measured in water at *ca.* 25° unless otherwise stated. Demethylations of methylated sugars [7] were carried out by heating samples with 48% hydrobromic acid (E. Merck A.G., Darmstadt) in sealed tubes for 15—25 min., and the products (almost invariably the parent sugar together with a mixture of partially methylated sugars) were examined by paper chromatography and paper ionophoresis, with the products obtained by a similar treatment of authentic methylated sugars as reference compounds. Glycitols derived from the methylated sugars were prepared by reduction with sodium borohydride in aqueous solution at room temperature for 18 hr.[8] followed by treatment with Amberlite IR-120(H) resin, extraction with methanol, and evaporation of the extract; excess of boric acid was removed by repeated addition of methanol and evaporation.[9] Periodate oxidations of the methylated sugars and glycitols were carried out for 2—3 hr. at 0°, otherwise following the procedure of Lemieux and Bauer.[10] Sugars were normally revealed on chromatograms by spraying with *p*-anisidine hydrochloride in moist butan-1-ol, a little glacial acetic acid being added before spraying of ionophoretograms. Aqueous solutions were evaporated *in vacuo* below 50°. M. p.s were taken on a hot-stage apparatus calibrated with substances of known m. p.

Methylation of Virgilia oroboides *Gum.*—The polysaccharide (30 g.), purified as described in Part II,[1] was allowed to swell in water (100 ml.) and was then stirred vigorously with methyl sulphate (100 ml.) and 40% w/v sodium hydroxide (200 ml.) at 0° under nitrogen. Three further additions of the same quantities of reagents were made on successive days at room temperature, and on the fifth day the mixture was warmed to 60° for several hours. The cooled product was neutralised with sulphuric acid to pH 6, filtered, and the filtrate dialysed until free from sulphate ions. The dialysed solution was evaporated to dryness and the residue was extracted with chloroform. The dried extract was heated under reflux in a mixture of methanol (50 ml.), acetone (50 ml.), and methyl iodide (100 ml.), and silver oxide (25 g.) was added portionwise during 3 days. The partially-methylated gum was recovered by chloroform extraction and remethylated by using further quantities of methyl iodide (150 ml.) and silver oxide (50 g.) during 12 days. The product (31 g.) was dissolved in chloroform (130 ml.), light petroleum (b. p. 60—80°; 130 ml.) was added, and the mixture was centrifuged. The solid obtained was re-fractionated, and the insoluble material (4·5 g.) discarded. The solutions were combined, evaporated to dryness, and again methylated with methyl iodide and silver oxide. This process was repeated three times, fractions insoluble in chloroform–light petroleum (1 : 2) being re-methylated before being combined with the more soluble fractions, there being obtained the methylated gum as a glass (*A*) (15·4 g.) and a more soluble gelatinous fraction (*B*) (4·6 g.). Because there was little or no difference in methoxyl content (42·6%) and specific rotation (−22° in $CHCl_3$) between fractions *A* and *B*, a portion of the major fraction (*A*) was methylated twice more to give the fully methylated product (*C*).

Hydrolysis of Methylated Polysaccharide C and Identification of the Neutral Sugars.—Methylated polysaccharide *C* (6 g.) was heated at 96° for 11 hr. in N-sulphuric acid (200 ml.), and the solution was cooled and neutralised with barium carbonate. The mixture was filtered,

[6] Cf. Smith and Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold Publishing Corp., New York, 1959, pp. 85—95.
[7] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[8] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.
[9] Zill, Khym, and Cheniae, *J. Amer. Chem. Soc.*, 1953, **75**, 1339.
[10] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

concentrated to a small bulk, and passed in succession through columns of Amberlite resins IR-120(H) (until barium-free) and IR-4B(OH). The eluate on evaporation yielded a syrupy mixture (*D*) (5 g.) of neutral sugars. From the anion-exchange resin there was obtained by elution with N-sodium hydroxide, followed by removal of sodium with cation-exchange resin, an acidic solution which was neutralised with barium carbonate, filtered, and evaporated to give a mixture of barium salts (0·8 g.); this product (*E*) was shown by paper chromatography (solvent *c*) to be free from neutral sugars. The mixture *D* was placed on a cellulose column (90 × 4·2 cm.) which was kept at 30° by means of a water-jacket. The column was eluted with mixtures of light petroleum (b. p. 100—120°) and water-saturated butan-1-ol (7 : 3 changed in nine steps to 7 : 50), followed by butan-1-ol half-saturated with water, to give 21 fractions containing neutral methylated sugars.

Fraction 1. The chromatographically pure mobile syrup (280 mg.), R_G 0·95, $[\alpha]_D$ −33° (*c* 3·9), was identified as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into the arabonolactone and corresponding arabonamide, m. p. 133—135° undepressed on admixture with an authentic sample.

Fraction 2. The syrup (126 mg.) had R_G 0·88, $[\alpha]_D$ + 111° (*c* 2·1), and on demethylation gave products identical with those obtained from 2,3,4,6-tetra-*O*-methyl-D-galactose. The aniline derivative had m. p. and mixed m. p. (with 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactosylamine) 194°.

Fraction 3. A portion of the syrup (170 mg.), R_G 0·76, $[\alpha]_D$ +130° (*c* 2·0), gave products identical with those expected from 2,3,4-tri-*O*-methyl-L-arabinose on demethylation. The derived lactone was converted into 2,3,4-tri-*O*-methyl-L-arabonophenylhydrazide, m. p. and mixed m. p. 158°.

Fraction 4. The mixture of sugars was separated by paper chromatography with solvent *b* into four fractions, one of which was 2,3,4-tri-*O*-methyl-L-arabinose (identified with the material in fraction 3). The other components were: 4a (5 mg.), M_g 0·00; 4b (15 mg.), consisting of three substances, M_g 0·05, 0·45, and 0·72 (the central spot corresponded chromatographically and ionophoretically to 3,4,6-tri-*O*-methylmannose); 4c (9 mg.), M_g 0·05.

Fraction 5. The chromatographically pure syrup (40 mg.) had R_G 0·74, $[\alpha]_D$ +94° (*c* 0·75), and on demethylation gave products identical with those from 2,3,6-tri-*O*-methyl-D-galactose. Reduction of a portion of the syrup and treatment with periodate gave a product (2,3-di-*O*-methyl-L-threose) chromatographically identical with a specimen prepared from 2,3-di-*O*-methyl-D-galactose.

Fraction 6. The crystalline sugar (137 mg.) had R_G 0·73, m. p. (after recrystallisation from ether–acetone) 103—103·5°, $[\alpha]_D$ +112° ⟶ +82° (*c* 4·4). The aniline derivative had m. p. 174° and mixed m. p. (with authentic 2,4,6-tri-*O*-methyl-*N*-phenyl-D-galactosylamine) 173°.

Fraction 7. The syrup (940 mg.), $[\alpha]_D$ +101° (*c* 5·1), was a mixture of two components, R_G 0·70 and R_G 0·65, chromatographically identical with 2,3,4-tri-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose. The mixture was not resolved by paper chromatography in solvent *b* or by paper ionophoresis. Demethylation gave the products expected of these two methylated sugars. Borohydride reduction of a portion of the mixture afforded 2,3,4-tri-*O*-methyl-D-galactitol, needles from acetone, m. p. and mixed m. p. 120°,[11] which on periodate oxidation gave a sugar of R_G 0·89 and 0·81 (solvents *a* and *b*) whose red stain corresponded to that of 2,3,4-tri-*O*-methylarabinose and which is presumably 2,3,4-tri-*O*-methyl-L-lyxose. The mother liquor after separation of the galactitol gave this product together with a second (yellow stain), slightly faster-moving on paper chromatography, which was identical with the sugar formed by similar oxidation of 2,3-di-*O*-methyl-L-arabitol. The syrupy mixture comprising fraction 7 (200 mg.) was converted into a mixture of aldonamides from which 2,3-di-*O*-methyl-L-arabonamide was obtained (from methanol–acetone), m. p. 161° and mixed m. p. (with specimen of m. p. 156—157°) 158°—160° (Found: OMe, 31·0. Calc. for $C_7H_{15}NO_5$: OMe, 32·1%).

Fraction 8. The syrup (750 mg.) was chromatographically homogeneous and identical with 2,3,4-tri-*O*-methyl-D-galactose, present in fraction 7. Nucleation induced complete crystallisation as needles, m. p. 70° (from ether containing a few drops of acetone), $[\alpha]_D$ +137° ⟶ +108° (const.) (*c* 2·1) (Found: OMe, 39·0. Calc. for $C_9H_{18}O_6,H_2O$: OMe, 38·8%). The sugar was characterised as its aniline derivative, m. p. and mixed m. p. 166—168°, and as the derived glycitol, m. p. 121—122°.

Fraction 9. The syrup (90 mg.) contained two components R_G 0·67 and R_G 0·65, the former

[11] Bishop, *Canad. J. Chem.*, 1960, **38**, 1636.

predominating. The sugars had M_g 0·48 and 0·07, respectively. Demethylation gave mostly mannose and monomethyl ethers of mannose. The major component was identical with that of fraction 10.

Fraction 10. The syrup (35 mg.) had M_g 0·48, $[\alpha]_D$ +50° (*c* 0·5), and gave mannose on demethylation. The compound was identical with fraction (v) (below), and was examined with it.

Fraction 11. The syrup (30 mg.) had R_G 0·70, M_g 0·42, and gave galactose and galactose ethers on demethylation, the R_G and M_g of which support the view that the sugar may be 3,4,6-tri-*O*-methyl-D-galactose.

Fraction 12. The syrup (15 mg.), R_G 0·60, M_g 0·39, gave on demethylation a number of products which suggest that the sugar is a di-*O*-methylarabinose. This is by no means certain as paper ionophoresis of the demethylation products did not confirm arabinose.

Fraction 13. The crystalline sugar (70 mg.), $[\alpha]_D$ +82° (*c* 0·7), R_G 0·58, gave galactose and two monomethyl ethers on demethylation. Recrystallisation from ethyl acetate afforded 2,6-di-*O*-methyl-D-galactose, m. p. and mixed m. p. 118—120°. Periodate oxidation gave products identical with those obtained from authentic 2,6-di-*O*-methylgalactose.

Fraction 14. The syrup (330 mg.) had R_G 0·56, M_g 0·32, $[\alpha]_D$ +79° ⟶ +95° (20 hr.; *c* 3·3); the sugar and its demethylation products corresponded to 2,3-di-*O*-methyl-D-galactose and its derivatives. The derived glycitol on periodate oxidation gave 2,3-di-*O*-methyl-L-threose, identical with that from authentic 2,3-di-*O*-methyl-D-galactitol. Periodate oxidation of the sugar (Reeves's method) [12] gave formaldehyde (dimedone derivative, m. p. and mixed m. p. 184—186°, in good yield). The derived aldonolactone from fraction 14 had $[\alpha]_D$ −45° ⟶ −36° (3 days; *c* 6·9), and from this was prepared the aldonamide, m. p. 138°, undepressed by authentic 2,3-di-*O*-methyl-D-galactonamide.

Fraction 15. The syrup (10 mg.) had R_G 0·50, M_g 0·42.

Fraction 16. The crystalline sugar (600 mg.), R_G 0·54, M_g 0·25, was recrystallised from acetone–ether and had m. p. and mixed m. p. (with 2,4-di-*O*-methyl-α-D-galactose monohydrate) 98—100°, $[\alpha]_D$ +117° ⟶ +84° (*c* 2·2) (Found: OMe, 27·6. Calc. for $C_8H_{16}O_6,H_2O$: OMe, 27·4%). The aniline derivative had m. p. and mixed m. p. (with 2,4-di-*O*-methyl-*N*-phenyl-D-galactosylamine) 214—215°. On periodate oxidation the sugar was unchanged, but the derived glycitol (m. p. 127—128° without recrystallisation) gave a single sugar whose chromatographic behaviour (R_G 0·70 in *a*, 0·30 in *b*) was consistent with that of the expected 2,4-di-*O*-methyl-L-lyxose.

Fraction 17. The syrup (35 mg.), R_G 0·44, M_g 0·26, gave three compounds on demethylation, corresponding chromatographically and ionophoretically to galactose and its 3- and 4-*O*-methyl derivatives. Periodate oxidation of the sugar and of its glycitol (elongated, blunt-ended prisms, m. p. 195° from methanol) gave a chromatographically homogeneous product, R_G 0·70 in *a*, 0·20 in *b* (reddish-black), as is to be expected of 3,4-di-*O*-methylgalactose (⟶ 2,3-di-*O*-methyl-D- and -L-lyxoses).

Fraction 18. The mixture (55 mg.) consisted of material judged by paper chromatography and ionophoresis to contain the sugars present in fraction 17 (major component) and fraction 19 (minor component), with traces of two other sugars.

Fraction 19. The syrup (30 mg.), R_G 0·43, M_g 0·58, gave mannose on demethylation. The stain (vermilion) produced by spraying the sugar with *p*-anisidine hydrochloride was clearly different from those produced from 2- and 3-*O*-methylmannose. Periodate oxidation gave a major product identical with that obtained from 6-*O*-methyl-, not from 4-*O*-methyl-D-galactose.

Fraction 20. The syrup (95 mg.), R_G 0·38, $[\alpha]_D$ +86° (*c* 1·4), deposited crystalline nodules (68 mg.) (from acetone), m. p. and mixed m. p. (with 2-*O*-methyl-D-galactose) 150—152°. The syrupy mother liquor contained more 2-*O*-methyl-D-galactose (M_g 0·35) and a smaller quantity of 6-*O*-methyl-D-galactose (M_g 0·85). Periodate oxidation of the crystalline 2-*O*-methyl derivative gave the expected methoxymalondialdehyde (canary-yellow spot at R_F 0·20 in solvent *a*), whereas periodate oxidation of the derived glycitol gave none of this compound on paper chromatography but one which gave a diffuse dull-yellow streak of R_F *ca.* 0·70 (presumably 2-*O*-methyl-L-glyceraldehyde). Periodate oxidation of the mother liquor confirmed the presence in it of some 6-*O*-methylgalactose.

Fraction 21. When warmed with methanol–acetone the syrup (55 mg.), R_G 0·34, deposited 4-*O*-methyl-β-D-galactose as elongated prisms, m. p. 211° (lit., m. p. of β-form 207°, 218—221°),

[12] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.

$[\alpha]_D$ +76° ⟶ +86° (*c* 0·8); demethylation of the sugar (which was ionophoretically identical, M_g 0·19, with 4-*O*-methylgalactose) gave galactose, and periodate oxidation of the sugar gave as major product a sugar R_F 0·58 (presumably 2-*O*-methyl-D-threose). The mother liquor after crystallisation of the sugar was concentrated to a syrup (*ca.* 12 mg.) which contained two components, M_g 0·19 and 0·65. The first of these corresponds to 4-*O*-methyl- and the second to 3-*O*-methyl-galactose, and the presence in the mixture of the latter was further indicated by (i) reduction and paper ionophoresis whereupon a single spot corresponding in rate to 4-*O*-methyl-D-galactitol (=3-*O*-methyl-L-galactitol) was obtained, and (ii) periodate oxidation of the mixture of sugars which gave two sugars, R_F 0·58 and R_F 0·40 (presumably 2-*O*-methyl-D-lyxose).

Elution of the column with aqueous ethanol gave a residue (70 mg.) containing (paper chromatography) at least ten components, including traces of mannose, galactose, and an immobile residue, which was not examined further.

Examination of the Acidic Components E.—A portion (40 mg.) of the acidic mixture *E*, obtained by hydrolysis of the methylated gum, was heated in 2N-sulphuric acid for 12 hr. at 100°, and the neutralised (barium carbonate) and filtered solution was examined by paper chromatography. Two main cherry-red spots (R_G 0·29 and 0·22) appeared with traces only of sugars, of higher R_G. The bulk of the mixture *E* was converted into methyl ester methyl glycosides by de-ionising it with Amberlite resin IR-120(H) and by heating the dried syrupy residue in 3% methanolic hydrogen chloride under reflux for 8 hr. The mixture was neutralised (silver carbonate), filtered, and evaporated, giving a product (530 mg.) which was then heated with lithium aluminium hydride (450 mg.) under reflux in tetrahydrofuran (20 ml.) for 2 hr. The reduced product (400 mg.) was isolated by addition of ethyl acetate and 2N-sulphuric acid, followed by chloroform extraction. The bulk of this material was heated in N-sulphuric acid (20 ml.) under reflux for 7 hr. to give a methanol-soluble mixture of neutral sugars (360 mg.) which was separated into its components by cellulose column chromatography (55 × 4 cm.) as described above. In this manner 11 fractions were collected.

Fraction (*i*). The mobile syrup (30 mg.) gave no colour when a chromatogram was sprayed with *p*-anisidine reagent, but after being hydrolysed for 4 hr. at 100° with 2N-sulphuric acid it was converted into a mixture of sugars indistinguishable from those in fraction (ii) (major portion) and fractions (v), (vi), and (vii) (minor portion).

Fraction (*ii*). The syrupy sugar (120 mg.), R_G 0·88, M_g 0·00, $[\alpha]_D$ +57° (*c* 0·95), was chromatographically indistinguishable (solvents *a* and *b*) from 2,3,4-tri-*O*-methyl-D-glucose. Demethylation gave glucose, and methylation gave 2,3,4,6-tetra-*O*-methylglucose. The derived tri-*O*-methylglucitol was oxidised with periodate, giving a sugar (R_G 0·95 in *a*, 0·85 in *b*) identical with that produced similarly from authentic 2,3,4-tri-*O*-methyl-D-glucitol.

Fraction (*iii*). The syrup (15 mg.) had R_G 0·75 (solvent *a*), but was separated by solvent *b* into two components, similar in chromatographic rate to 2,3,4-tri-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose.

Fraction (*iv*). The syrup (30 mg.), R_G 0·70, was chromatographically identical with 2,3,4-tri-*O*-methyl-D-galactose. Its identity was confirmed by reduction to the corresponding galactitol, m. p. and mixed m. p. 121—122°. Periodate oxidation of the mother liquors from the crystallisation of the galactitol indicated that there was a trace of 2,3-di-*O*-methylarabinose mixed with the 2,3,4-tri-*O*-methyl-D-galactose.

Fraction (*v*). The syrup (40 mg.), R_G 0·67, was chromatographically and ionophoretically identical with the material in fraction 10 (above). Demethylation gave mannose and two monomethyl ethers. Periodate oxidation of the sugar and of the derived glycitol gave the same two products, R_F 0·78 (brown, possibly 2,4-di-*O*-methyl-D-erythrose) and 0·69 (reddish-black, possibly 3,5-di-*O*-methyl-D-arabinose); relatively more of the faster-moving product was obtained from the sugar than from the glycitol. The methyl glycoside mixture prepared from the sugar consumed 1 mol. of periodate in 20 hr. at pH 8. These results are consistent with the sugar in fractions (v) and 10 being 4,6-di-*O*-methyl-D-mannose.

Fraction (*vi*). The mixture (*ca.* 7 mg.) contained two components, readily distinguishable by paper ionophoresis, corresponding to the substances in fractions (v) and (vii).

Fraction (*vii*). The syrup (40 mg.), R_G 0·66 (yellow fluorescent stain), M_g 0·16, $[\alpha]_D$ +60° (*c* 5·4), gave glucose on demethylation. The sugar was inert towards periodate but the derived glycitol reacted to give a single product (2,3-di-*O*-methyl-L-threose) identical with that obtained above from 2,3-di-*O*-methyl-D-galactitol and from 2,3-di-*O*-methyl-L-arabitol. The sugar is

thus distinguished from 2,4- and from 3,4-di-*O*-methyl-D-glucose (whose behaviour on similar oxidation and reduction/oxidation treatment was as predicted); it was characterised as 2,3-di-*O*-methyl-D-gluconophenylhydrazide, m. p. and mixed m. p. 173—175°.

Fraction (*viii*). The mixture (10 mg.) consisted of at least four components and was not further investigated.

Fraction (*ix*). The syrup (15 mg.), R_G 0·56, was identical with 2,3-di-*O*-methyl-D-galactose on paper chromatography and ionophoresis, and the derived glycitol gave the same product on periodate oxidation as was given by 2,3-di-*O*-methyl-D-galactitol.

Fraction (*x*). The syrup (5 mg.), R_G 0·54, was shown to contain two components, M_g 0·61 and 0·23, by paper ionophoresis (the slower moving corresponds to 2,4-di-*O*-methyl-galactose).

Fraction (*xi*). The residues (12 mg.) washed from the column contained a major component, R_G 0·41, and a number of minor components.

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