367. Virgilia oroboides *Gum. Part IV.*[1] *Methylation Study of the Partly Degraded Gum.*

By A. M. Stephen.

Methylation and hydrolysis of *Virgilia oroboides* gum that had been submitted to prolonged, mild acid treatment showed that mainly unbranched acidic fragments of short chain length had been produced. The linkages shown to be present in these fragments are consistent with the pattern of molecular structure suggested by earlier studies of the gum.

Studies of the molecular structure of *Virgilia oroboides* gum by the techniques of fragmentation analysis in which acid hydrolysis [2] and investigation of the fully methylated gum were used led to broad conclusions which were summarised in Part III.[1] This paper describes the structure of the degraded acidic material obtained from the polysaccharide by relatively mild acid treatment. That the degree of breakdown was in fact rather high has been reported briefly elsewhere.[3]

Gum that had been stored for some years in the acid form (easily hydrolysed groups may become lost during this process) [4] was heated with 0·01N-sulphuric acid until the bulk of arabinoside linkages had been severed, and the material precipitated by ethanol was collected; this comprised just over one-third of the weight of original gum. Galactose and hexose-containing oligosaccharides were liberated during the hydrolysis. The equivalent weight of the precipitated, partly degraded gum showed nearly four hexose residues for each hexuronic acid; the molecular-size distribution and the relative proportions of acidic and neutral molecules have not been determined.

Methylation and hydrolysis of the acid-degraded gum revealed a high proportion of non-reducing end-groups, consisting of D-galactopyranose, D-glucopyranuronic acid, and some D-mannopyranose, but only negligible amounts of sugars representing branch-points. The bulk of the sugar residues are 1 ⟶ 6-linked D-galactopyranose and 1 ⟶ 2-linked D-mannopyranose with a much smaller proportion of 1 ⟶ 3-linked D-galactopyranose. The absence of di-*O*-methylgalactoses shows that the sugar residues at positions 3 and 4 of the 1 ⟶ 6-linked galactose chains, which make an important contribution to the extensive branching in the polysaccharide, are almost completely removed by mild acid treatment. Similarly, the sugar residues linked 1 ⟶ 4 to glucuronic acid are liberated. On the other hand, more than one-fifth of the sugars linked to position 3 of D-mannopyranose are retained (cf. damson gum).[5] The presence in acid-degraded *Virgilia oroboides* gum of mannose non-reducing end-groups suggests that glucuronic acid is not directly bonded to these mannose units in the gum itself.

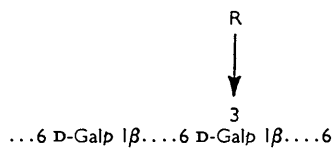
In this experiment the extent of acid degradation is such that it is not possible to

[1] Part III, Stephen, *J.*, 1962, 2030.
[2] Smith and Stephen, *J.*, 1961, 4892.
[3] Stephen, *S. Afr. J. Lab. Clinical Med.*, 1962, 76.
[4] Stephen, *S. Afr. Industrial Chemist*, 1963, in the press.
[5] Hirst and Jones, *J.*, 1946, 506, and references there cited.

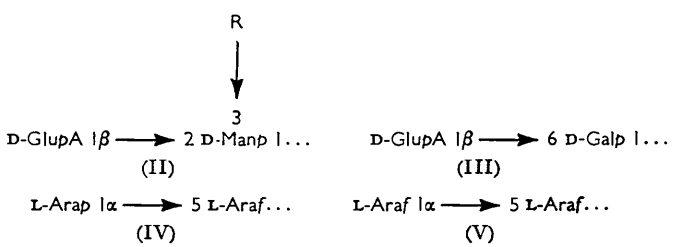
differentiate between the positions of attachment of arabinoside and galactoside linkages to the main galactose chains. Further, it becomes necessary to postulate acid-labile linkages at all branch-points and at short intervals in the main chains themselves. Oligosaccharides with hexoses linked glycosidically to arabinose have, however, not been isolated from the products of graded hydrolysis (though there is evidence that such might be present), and this is not easy to explain if the arabinofuranoside linkages are the cause of acid-lability. It may be that the carboxyl groups present in the glucuronic acid residues participate in the severing of glycosidic bonds at positions two or more sugars removed from the hexuronic acid, though even neutral arabinogalactans are known [6, 7] to be more extensively decomposed by acid than would be expected from a knowledge of the relative stability of furanoside and pyranoside linkages in simple compounds. Such deep-seated decomposition of gums by mild acid treatment has been observed, *e.g.*, with *Acacia cyanophylla* gum,[8] whereas, by contrast, the hydrolysis of a neutral arabinogalactan from larch wood [9] leaves a residual polymer of comparatively high molecular weight. A study of the action of mild acid on diborane-reduced gum is accordingly being undertaken to see whether in solution kept at some fixed pH (*e.g.*, 2) there is in fact more extensive degradation of the acidic than of the neutral polysaccharide.

The new evidence confirms some of the structural features already put forward for *Virgilia oroboides* gum, as isolated, and these are accommodated in the following partial formulæ. In addition to uncertainty as to which sugar residues are attached to positions 3 and 4 of the galactose chains there are a number of features, mentioned in earlier papers, whose significance cannot yet be evaluated.

To the chains of 1 ⟶ 6-linked D-galactopyranose units (I) are joined groups of sugars R as shown, the approximate proportions of unbranched, 3-linked, and 4-linked galactose units being 3 : 2 : 1. A small quantity of galactose is trisubstituted at positions 3, 4, and 6. Of every three residues R, two are neutral and one is acidic, which suggests the acidic side-chains may be attached at position 4. These acidic fragments consist of units (II) and (III) (the former predominating) which cannot be separated by more than a few sugar



units from the main galactose chains. Some of the glucuronic acid (which is present to an extent of *ca.* 8%, on a molar basis, in the gum) carries an easily hydrolysed sugar residue at position 4 and is therefore not terminal. Because of the acid-lability of the sugar at position 4 the molar proportion (3%) of this feature indicated by earlier work on methylated gum is a minimum figure. Some of the units (III) carry a 4-methoxyl substituent on the acid residue.



[6] Aspinall, Hirst, and Ramstad, *J.*, 1958, 593.
[7] Bouveng, *Acta Chem. Scand.*, 1961, **15**, 78.
[8] Charlson, Nunn, and Stephen, *J.*, 1955, 269.
[9] Campbell, Hirst, and Jones, *J.*, 1948, 774.

L-Arabinose constitutes nearly two-fifths of the gum, and about one-half of the total arabinose in the isolated gum appears as non-reducing end-groups, some pyranose but mostly furanose. In addition to the units (IV) and (V), the presence of single L-arabinofuranose end-groups, attached to other sugars, is indicated by the occurrence of arabinose chains at least three units long.

Experimental

General techniques for separating and identifying the sugars produced on hydrolysis of methylated polymers were as described in Part III.[1] Solvent systems (v/v) used in paper chromatography were the upper layers of (*a*) butan-1-ol–ethanol–water (4 : 1 : 5) and (*b*) benzene–ethanol–water (169 : 47 : 15). Unless otherwise stated, R_G values are relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent (*a*).

Preparation of Acid-degraded Gum.—*Virgilia oroboides* gum (part of the bulk preparation used previously in methylation studies; 30 g.) was heated in 0·01N-sulphuric acid (1 l.) for 40 hr. at *ca.* 95°. The hydrolysate was neutralised with barium carbonate and concentrated to 400 c.c. Addition of ethanol precipitated the partly degraded gum as barium salts; reprecipitation in ethanol from aqueous solution gave the product (A) as a white powder (10·9 g.), $[\alpha]_D$ +24° (*c* 1·7) (equiv., 810). The derived acetate had $[\alpha]_D$ 0° (*c* 1·6 in $CHCl_3$). Acid hydrolysis of a portion of material A showed it to be free from pentose. The mother-liquors from precipitation of the partly degraded gum were concentrated to a syrup (18 g.) which contained arabinose, considerably less galactose, and traces of oligosaccharides.

Methylation and Hydrolysis of Material A.—Methylation of this material A (4 g.) by Haworth's procedure and then by successive treatments with methyl iodide and silver oxide until the methoxyl content was not increased by re-methylation gave methylated partly-degraded *Virgilia oroboides* gum (2·6 g.), $[\alpha]_D$ +56° (*c* 10·4 in $CHCl_3$) (Found: OMe, 48·2%). Hydrolysis under conditions essentially similar to those used for hydrolysis of the methylated gum itself then gave a neutral sugar mixture (B) (1·5 g.) and an acidic fraction recovered as mixed barium salts (C) (0·9 g.). Judged by paper chromatography the separation into neutral and acid components was complete. Reduction of the methyl ester methyl glycosides of products C in the usual way gave a mobile syrup (0·8 g.) which on hydrolysis afforded a second mixture of neutral sugars (D).

Partion Chromatography of Sugar Mixtures B and D.—Fractionation on a cellulose column of the mixture of sugars B gave the following compounds:

(i) A syrupy sugar (15 mg.) chromatographically [solvents (*a*) and (*b*)] identical with 2,3-5-tri-*O*-methyl-L-arabinose.

(ii) A syrup (70 mg.), R_G 0·95 in solvent (*a*) and 1·01 in (*b*), $[\alpha]_D$ +6° (*c* 2), which afforded mannose on demethylation. The aniline derivative had m. p. 145°, $[\alpha]_D$ −10° (final value; *c* 2·6 in MeOH), and gave an identical *X*-ray powder diagram (comparison kindly undertaken by Dr. D. Feil) with that of authentic 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine.

(iii) 2,3,4,6-Tetra-*O*-methyl-D-galactose (450 mg.), $[\alpha]_D$ +104° (*c* 4·2), characterised as the aniline derivative, m. p. and mixed m. p. 194°.

(iv) A syrup (100 mg.) consisting mainly of a sugar identical on paper chromatography and ionophoresis with 3,4,6-tri-*O*-methyl-D-mannose; nucleation with a crystal of this sugar afforded colourless prisms, m. p. and mixed m. p. 102°, $[\alpha]_D$ +28 ⟶ +17° (*c* 1·8). The periodate-oxidation product resembled (paper chromatography) that from 3,4,6-tri-*O*-methyl-D-mannose.

(v) A discoloured syrup (25 mg.), $[\alpha]_D$ −17° (*c* 1·2), which was homogeneous by criteria of paper chromatography (R_G 0·89) and ionophoresis (M_G zero). The results of periodate oxidation of the sugar and of the derived glycitol, taken in conjunction with these data, suggest that the sugar is 2,5-di-*O*-methyl-L-arabinose.

(vi) 2,4,6-Tri-*O*-methyl-D-galactose (40 mg.), R_G 0·24 [solvent (b)], $[\alpha]_D$ +90°, obtained as elongated prisms, m. p. 103—104°, on nucleation of the syrupy product dissolved in ether–acetone. The derived *N*-phenylgalactosylamine had m. p. and mixed m. p. 172—173°.

(vii) 2,3,4-Tri-*O*-methyl-D-galactose (550 mg.) which crystallised spontaneously and had $[\alpha]_D$ +140° ⟶ +109°(*c* 1·6) (Found: OMe, 39·0. Calc. for $C_9H_{18}O_6,H_2O$: OMe, 38·8%). The aniline derivative had m. p. and mixed m. p. 167°.

(viii) A syrup (25 mg.), $[\alpha]_D$ +29° (*c* 1·15), identical on paper chromatography and ionophoresis with the sugar tentatively identified as 4,6-di-*O*-methyl-D-mannose in Part III. Demethylation indicated mannose, and periodate oxidation of the sugar and its glycitol gave products corresponding to those from fraction (v) of Part III.

(ix) A syrup (15 mg.), $[\alpha]_D$ *ca.* +80°, which resembled 2,3-di-*O*-methyl-D-galactose on paper chromatography and ionophoresis and was similarly resistant to periodate. The derived glycitol gave the expected single product on periodate treatment.

(x) A crystalline sugar (10 mg.), m. p. and mixed m. p. (with 2,4-di-*O*-methyl-α-D-galactose hydrate) 96—98°. The derived 2,4-di-*O*-methyl-*N*-phenyl-D-galactosylamine had m. p. and mixed m. p. 214—215°.

(xi) A syrup (6 mg.) chromatographically similar to 3,4-di-*O*-methylgalactose.

In a similar manner, partition chromatography on a cellulose column of the mixture of neutral sugars D, derived from the acid hydrolysis products by reduction, gave a number of fractions.

(*a*) The mobile syrup (130 mg.), which was eluted ahead of the first sugar detectable by spraying paper chromatograms with *p*-anisidine hydrochloride in wet butan-1-ol, gave on acid hydrolysis sugars indistinguishable by paper chromatography and ionophoresis from those contained in fraction (*b*).

(*b*) The syrup (420 mg.), $[\alpha]_D$ +14°, showed a single spot on paper chromatography [solvents (*a*) and (*b*)], but two spots (M_G zero and 0·42) on paper ionophoresis. Acid hydrolysis of the syrup yielded no sugars other than the two indicated. Ionophoretic separation of the mixture on thick filter-paper sheets yielded a syrup (in minor amount) identical with 3,4,6-tri-*O*-methylmannose on paper chromatography, ionophoresis and reaction towards periodate, together with 2,3,4-tri-*O*-methyl-D-glucose. This sugar was unaffected by periodate, and the derived glycitol gave a product identical with that from 2,3,4-tri-*O*-methyl-D-glucitol on oxidation by periodate. Demethylation of this periodate-oxidation product (presumably 2,3,4-tri-*O*-methyl-L-xylose) by the method of Bonner *et al.*[10] gave a sugar chromatographically indistinguishable from xylose.

(*c*) This fraction consisted of a syrup (45 mg.), shown by paper chromatography to contain at least two components. The bulk of the material was identical with that in fraction (*d*).

(*d*) The syrup (50 mg.), $[\alpha]_D$ +80°, M_G zero, was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 167°.

(*e*) The syrup (20 mg.) corresponded in chromatographic and ionophoretic mobility with the (?)4,6-di-*O*-methylmannose in fraction (viii).

Traces of three slower-moving sugars (<10 mg. each) were finally eluted from the column.

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S. AFRICA. [*Received, October 4th,* 1962.]

[10] Bonner, Bourne, and McNally, *J.*, 1960, 2929.