894. *Gum Ghatti (Indian Gum). Part III.*[1] *Neutral Oligosaccharides formed on Partial Acid Hydrolysis of the Gum.*

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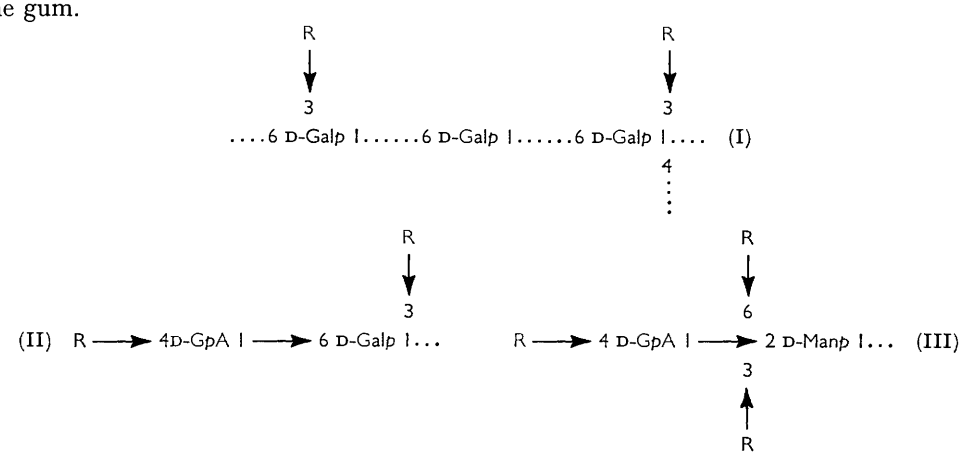
Partial acid hydrolysis of the gum affords two homologous series of oligosaccharides together with small amounts of 3-*O*-β-D-galactopyranosyl-D-galactose and other disaccharides. The first three members of the series *O*-β-D-galactopyranosyl-[(1 → 6)-*O*-β-D-galactopyranosyl]$_n$-(1 → 6)-D-galactose (n = 0, 1, and 2) and the first four members of the series *O*-β-D-galactopyranosyl-[(1 → 6)-*O*-β-D-galactopyranosyl]$_n$-(1 → 3)-L-arabinose (n = 0, 1, 2, and 3) have been characterised. The significance of these results is discussed.

The results of previous structural studies on gum ghatti[1] can be summarised in the partial structures (I), (II), and (III). The gum contains chains of 1 : 6-linked β-D-galactopyranose residues (I) to which are attached either directly or through one or more 1 : 6-linked galactose residues the aldobiouronic acid units (II and III). The majority of the substituents R are single non-reducing L-arabofuranose end-groups. We now report an examination of the neutral oligosaccharides formed on partial acid hydrolysis of the gum.



The gum was partially hydrolysed by hot dilute sulphuric acid, and the mixture of neutral sugars formed was fractionated by chromatography on charcoal, followed where necessary by partition chromatography on cellulose, and the oligosaccharide fractions were examined. The main products were members of an homologous series of galactose-containing oligosaccharides. The first member was the disaccharide, 6-*O*-β-D-galactopyranosyl-D-galactose (IV; n = 0), whose structure was proved by the isolation of equimolecular proportions of 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-galactose on hydrolysis of the methylated derivative. The presence of a β-glycosidic linkage was indicated by the low positive optical rotation ([α]$_D$ +31°) of the disaccharide, which was similar to that recorded for the 1 : 6-linked galactobiose isolated from the partial acid hydrolysis of golden-apple gum [2] and markedly different from that reported for 6-*O*-α-D-galactopyranosyl-D-galactose, the major product of the acid-catalysed reversion of D-galactose.[3] The structure of the second member of the series, *O*-β-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 6)-D-galactose (IV; n = 1), follows from the

[1] (*a*) Part I, *J.*, 1955, 1160; (*b*) Part II, *J.*, 1958, 221.
[2] Lindgren, *Acta Chem. Scand.*, 1957, **11**, 1365.
[3] Turton, Bebbington, Dixon, and Pacsu, *J. Amer. Chem. Soc.*, 1955, **77**, 2565.

isolation of 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-galactose in the proportion of 1 : 2 on hydrolysis of the methylated trisaccharide. The optical rotation ($[\alpha]_D$ +20°) of the trisaccharide was consistent with the presence of two β-D-galactopyranosyl linkages. A third galactose-containing oligosaccharide was probably galactotetraose (IV; $n = 2$, as its optical rotation ($[\alpha]_D$ +14°) and its chromatographic mobility were consistent with the addition of another β-D-galactopyranosyl residue and since partial acid hydrolysis gave only one disaccharide, the 1 : 6-linked galactobiose.

(IV) D-Gal*p* $\overset{\beta}{1}$—[—6 D-Gal*p* $\overset{\beta}{1}$—]$_n$—6 D-Gal*p* D-Gal*p* $\overset{\beta}{1}$—[—6 D-Gal*p* $\overset{\beta}{1}$—]$_n$—3 L-Ara (V)

A second series of oligosaccharides was isolated in smaller amount. These new oligosaccharides, which were all obtained crystalline, contained galactose residues and one arabinose residue per molecule. In each case the arabinose was at the reducing end of the molecule since reduction with potassium borohydride followed by hydrolysis of the derived glycitol gave only galactose. The first member of the series was 3-*O*-D-galactopyranosyl-L-arabinose (V; $n = 0$) since hydrolysis of the methylated disaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose and 2 : 4-di-*O*-methyl-L-arabinose, together with traces of 2 : 5-di-*O*-methyl-L-arabinose. If it is assumed that the reducing arabinose residue in the disaccharide is present in the pyranose form, the observed optical rotation {$[\alpha]_D$ +62° (equil.)} is only consistent with the presence of a β-D-galactopyranosyl linkage and may be compared with that observed for 3-*O*-α-D-galactopyranosyl-L-arabinose ($[\alpha]_D$ +152°), which has been isolated from the partial acid hydrolysis of *Acacia cyanophylla* gum [4] and as its methylated derivative from gum arabic.[5] The presence of an α-glycosidic linkage in the latter has recently been proved by its degradation to 2-*O*-α-D-galactopyranosylglycerol.[6] Preliminary evidence for the structure of the second member of the series was the detection of 3-*O*-β-galactopyranosylarabinose and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis, and the mode of linkage of the sugars was proved by the isolation of 2 : 3 : 4 : 6-tetra-*O*-methyl- and 2 : 3 : 4-tri-*O*-methyl-D-galactose, and 2 : 4- and in small amount 2 : 5-di-*O*-methyl-L-arabinose from hydrolysis of the methylated trisaccharide. Since arabinose is present as the reducing residue it follows that the trisaccharide is *O*-β-D-galactopyranosyl-(1 ⟶ 6)-*O*-β-D-galactopyranosyl-(1 ⟶ 3)-L-arabinose (V; $n = 1$). The tetrasaccharide (V; $n = 2$) and pentasaccharide (V; $n = 3$) were established as members of the same homologous series by the following observations: (i) a plot of the R_M values [7] of the series, galactosylarabinose to tetragalactosylarabinose, against degree of polymerisation was a straight line; (ii) hydrolysis gave galactose and arabinose in the proportions of 2·8 : 1 and 3·7 : 1; (iii) chromatography of the products of partial acid hydrolysis showed only two disaccharides, 3-*O*-galactosylarabinose and 6-*O*-galactosylgalactose, together with arabinose, galactose, and higher oligosaccharides.

In addition to these major products of partial acid hydrolysis of the gum, very small quantities of other disaccharides were isolated. 3-*O*-β-D-Galactopyranosyl-D-galactose, previously characterised as a partial hydrolysis product of *Acacia pyanantha* gum,[8] golden-apple gum,[2] and of larch ε-galactan,[9] was identified as the crystalline sugar. A second minor component is tentatively believed to be an α-linked galactosylglucose. The structural significance of this disaccharide is not yet clear as glucose has not been detected previously as a constituent of the gum. Four arabinose-containing disaccharides were detected chromatographically, but in insufficient quantity for further study. Since the two disaccharides present in greatest amount in the mixture were chromatographically

[4] Charlson, Nunn, and Stephen, *J.*, 1955, 269.
[5] Smith, *J.*, 1939, 744.
[6] Charlson, Gorin, and Perlin, *Canad. J. Chem.*, 1957, **35**, 365.
[7] Bate-Smith and Westhall, *Biochem. Biophys. Acta*, 1950, **4**, 427.
[8] (*a*) Hirst and Perlin, *J.*, 1954, 2622; (*b*) Perlin, *Analyt. Chem.*, 1955, **27**, 396.
[9] Aspinall, Hirst, and Ramstad, *J.*, 1958, 593.

indistinguishable from the two reducing disaccharides, 3- and 4-*O*-β-L-arabopyranosyl-L-arabinose, formed by acid-catalysed reversion from L-arabinose,[10] it is not certain that these substances are true fragments of the original polysaccharide.

The isolation of the first homologous series (IV) of oligosaccharides from partial acid hydrolysis of gum ghatti was to be expected since the formation of 2 : 3 : 4-tri-*O*-methyl-D-galactose as the major product of hydrolysis of the methylated degraded gum [1b] indicated that chains of 1 : 6-linked D-galactopyranose residues (I) formed the backbone of the molecular structure of the gum. It is probable that 3-*O*-β-D-galactopyranosyl-D-galactose, isolated only in small amount, has structural significance and is not an artefact, since it is known that the major product of the acid-catalysed reversion of D-galactose is 6-*O*-α-D-galactopyranosyl-D-galactose [3] and no trace of such a disaccharide was found amongst the hydrolysis products of the gum. As a small amount of 2 : 4-di-*O*-methyl-D-galactose was isolated on hydrolysis of the methylated degraded gum,[1b] it is possible that the 1 : 3-linked galactobiose arises from a small degree of branching in the galactan backbone of the gum.

The isolation of the second homologous series (V) of oligosaccharides from gum ghatti points to a previously unsuspected structural feature, namely, the presence of L-arabinose residues in the backbone of the gum structure. Hitherto, structural investigations on plant gums have only shown arabinose residues, usually in the furanose form, to be present in the outer chains of the polysaccharides, and indeed, in gum ghatti the majority of arabinose residues are thus located. Here, the structural significance of 3-*O*-β-D-galactopyranosyl-L-arabinose as a fragment of gum ghatti may be contrasted with that of 3-*O*-α-D-galactopyranosyl-L-arabinose in *Acacia cyanophylla* gum [4] and gum arabic [5] where the disaccharide unit is removed under very mild conditions of hydrolysis and probably originates from the periphery of the gum molecule. Indications of the presence of arabinose residues in the more acid-resistant part of the gum molecule were obtained in earlier experiments.[1] Autohydrolysis of the gum resulted in cleavage of the more acid-labile linkages and a degraded gum was isolated which still contained a small proportion of arabinose residues,[1a] but such arabinose residues were not found in the methylated degraded gum.[1b] Further experiments now suggest that the majority of arabinose residues in the degraded gum are reducing groups terminating chains of 1 : 6-linked galactose residues.

When a sample of the degraded gum was partially hydrolysed with acid 3-*O*-β-D-galactopyranosylarabinose was easily detected by chromatography as one of the hydrolysis products. However, if the degraded gum was either oxidised with bromine water or treated with lime-water, and the product partially hydrolysed, only traces of 3-*O*-β-galactopyranosylarabinose were detectable. The arabinose residues in the degraded gum were therefore, either oxidised to arabonic acid residues or degraded with alkali, probably with the formation of saccharinic acids.[11] Since 1 : 3-linked carbohydrates are very susceptible to degradation by alkali, methylation of the degraded gum under alkaline conditions would easily result in loss of the reducing arabinose moiety. It is probable that the exposure of these reducing arabinose residues during the autohydrolysis results from the cleavage of acid-labile glycosidic linkages in the inner chains of the gum structure, and that in the original gum chains of 1 : 6-linked β-D-galactopyranose residues are joined through 1 : 3-linked L-arabinose residues as in (VI). Our earlier methylation studies [1b] showed that both 1 : 3-linked L-arabofuranose and L-arabopyranose residues were present in the gum, but it is probable that the formation of the degraded gum involves cleavage of arabofuranosyl linkages.

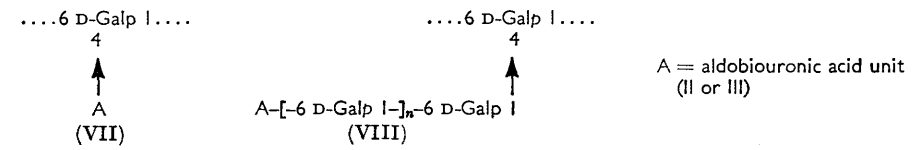
....6 D-Galp 1 ⟶ 6 D-Galp 1 ⟶ 3 L-Araf (or L-Arap) 1..... 6 D-Galp 1.... (VI)

The aldobiouronic acid units (II and III) in gum ghatti are present as side-chains and are probably linked to galactose through position 4, but in our earlier experiments it was

[10] Jones and Nicholson, *J.*, 1958, 27.
[11] Kenner, *Chem. and Ind.*, 1955, 727.

not possible to distinguish between direct attachment of these units to the backbone (VII) and attachment through one or more 1 : 6-linked galactose residues (VIII). Since the glycopyranosyluronic acid linkages in aldobiouronic acids are extremely resistant to acid hydrolysis, the isolation on partial hydrolysis of the gum of a neutral disaccharide containing a 1 : 4-linked galactose residue would only be expected if the aldobiouronic acid units were not directly linked to the backbone (VIII). The failure to detect such a disaccharide provides negative evidence in favour of direct attachment (VII). Experiments to obtain positive evidence for the mode of linkage of aldobiouronic acid units (II and III) to the galactan backbone are in progress.

D-Mannose residues in the gum are known to be present in the aldobiouronic acid unit (III). Since glucopyranosyluronic acids are resistant to acid hydrolysis it would only be expected that neutral mannose-containing disaccharides would be formed as products



of partial acid hydrolysis of the gum if some mannose residues were linked to neutral sugars only. The apparent absence of such neutral disaccharides in the partial hydrolysis products provides negative evidence for the occurrence of mannose residues linked only in aldobiouronic acid units (III). Since glucuronic acid is also linked to galactose in the gum there should be present a higher proportion of glucuronic acid than of mannose residues. Whereas previous results [1a] indicated the presence of glucuronic acid and mannose residues in the proportion of 1 : 1 in the original and also in the degraded gum, re-analysis of the degraded gum shows the proportion to be greater than 2 : 1.

Recent experiments on the ionophoresis of polysaccharides on glass-fibre paper [12] have indicated that gum ghatti is heterogeneous. Professor F. Smith (personal communication) informs us that the glass-fibre ionophoreses show gum ghatti to contain a major component and two minor components, one of which might be a pentosan. The results of methylation experiments [1b] suggested that the gum might contain one minor component since xylose residues present in the original gum [1a] were not found in the methylated polysaccharide, but we have not yet found evidence for a second minor component. Further experiments will be necessary before the nature of the heterogeneity of gum ghatti can be ascertained.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5; upper layer); (C) butan-1-ol–ethanol–water (1 : 1 : 1); (D) ethyl acetate–acetic acid—formic acid–water (18 : 3 : 1 : 4). Optical rotations were observed at 18° ± 2°.

Partial Acid Hydrolysis of Gum Ghatti and Fractionation of Neutral Oligosaccharides.—Gum ghatti (100 g.) in water (1·95 l.) was heated to 100°, 4N-sulphuric acid (50 ml.) was added, and the solution was boiled for 90 min. The cooled solution was neutralised with barium hydroxide (to pH 4) and barium carbonate, filtered, concentrated to 400 ml., and poured into ethanol (2 l.). The precipitated degraded polysaccharide A (*ca.* 50 g.) was separated, and the supernatant liquor was concentrated to 200 ml., passed through Amberlite resin IR-120(H) to remove barium ions and Amberlite resin IR-4B(OH) to remove acidic sugars, and concentrated to a syrup B(i) (*ca.* 35 g.). The degraded gum A (50 g.) in water (1 l.) was heated to 100°, 4N-sulphuric acid (140 ml.) was added, and the solution was boiled for 30 min. The hydrolysis products were worked up in the same way to give another syrupy mixture of sugars B(ii) (*ca.* 15 g.). The combined syrups B(i) and B(ii) (*ca.* 50 g.) in water (200 ml.) were adsorbed on charcoal–Celite (800 g.; 1 : 1). Elution with water afforded monosaccharides (36 g.) [arabinose, galactose, xylose, and rhamnose (trace)] and a small fraction (0·8 g.) in which

[12] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.

L-rhamnose was identified as the crystalline hydrate, m. p. 90—91° and mixed m. p. 89—90°, $[\alpha]_D$ −2·0° (5 min.) ⟶ +8·6° (1 hr., equil.) (*c* 1·63). Oligosaccharides were eluted with ethanol–water containing increasing proportions of ethanol. Ten fractions contained either pure oligosaccharides or mixtures from which pure compounds were isolated on refractionation. Other fractions which contained mixtures of oligosaccharides were not examined further.

Examination of Oligosaccharide-containing Fractions.—Fraction 1. Chromatography of the syrup (0·23 g.; eluted with water containing 2·5% of ethanol) showed 6-*O*-β-galactopyranosylgalactose, 3-*O*-β-arabopyranosylarabinose, and a pentose-containing disaccharide (R_{gal} 0·30, 0·83, and 0·67). Hydrolysis of the mixture gave galactose and arabinose. The two arabinose-containing disaccharides (R_{gal} 0·83 and 0·67) were chromatographically indistinguishable from the two reducing disaccharides formed by acid-catalysed reversion of L-arabinose.[10]

Fraction 2. The syrup (1·1 g.; eluted with water containing 2·5% of ethanol) contained a main component, R_{gal} 0·29 in solvent A, R_F 0·31 in solvent 3, together with traces of galactose, arabinose, and arabinose-containing disaccharides. The syrup (1·0 g.) was fractionated on cellulose (45 × 2 cm.) with solvent A to give fractions 2*a* (0·15 g.) and 2*b* (0·56 g.). Fraction 2*a* contained arabinose, galactose, and a mixture of arabinose-containing disaccharides in which 3-*O*-β-arabopyranosylarabinose predominated. Fraction 2*b*, $[\alpha]_D$ +31° (*c* 0·99 in water), was chromatographically pure and gave galactose only on hydrolysis. Methyl sulphate (1 ml.) and sodium hydroxide (1 ml.; 30%) were added dropwise during 1 hr. to the sugar (200 mg.) in water (5 ml.). Three more additions of methyl sulphate (12 ml.) and sodium hydroxide (17 ml.; 30%) were made, each during a period of 3 hr. and followed by stirring overnight. The reaction was completed by heating the solution on the boiling-water bath for 30 min., and the methylated sugar (202 mg.) was isolated by extraction with chloroform for 16 hr. Hydrolysis of the methylated disaccharide (150 mg.) with N-hydrochloric acid (7 ml.) at 100° for 4 hr., followed by neutralisation with silver carbonate yielded a mixture of sugars (140 mg.) which were separated into three fractions on cellulose (35 × 1·6 cm.) with light petroleum–butan-1-ol (7 : 3) saturated with water. Fraction (i) (40 mg.), $[\alpha]_D$ +105° (*c* 0·78 in water), R_G 0·90 in solvent B, was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose [aniline derivative, m. p. 198° and mixed m. p. (with sample of m. p. 189—190°) 190—192°]. Fraction (ii) (35 mg.) contained a mixture of tetra- and 2 : 3 : 4-tri-*O*-methylgalactose and a trace of a sugar (possibly 2 : 3 : 5-tri-*O*-methylgalactose), R_G 0·82 in solvent B. Fraction (iii) (39 mg.), $[\alpha]_D$ +106° (*c* 0·77 in water), R_G 0·70 in solvent B, was identified as 2 : 3 : 4-tri-*O*-methyl-D-galactose [aniline derivative, m. p. 159—160° and mixed m. p. (with sample of m. p. 164—165°) 161—162°].

Fraction 3. The syrup (205 mg.; eluted with water containing 5% of ethanol) contained a mixture of sugars and was fractionated on cellulose (45 × 2 cm.) with solvent A to give four fractions. Fraction 3*a* (65 mg.) contained arabinose, galactose, and three arabinose-containing disaccharides (R_{gal} 1·9, 1·33, and 1·22 in solvent A). Fraction 3*b* (10 mg.) contained 3-*O*-β-arabopyranosylarabinose (R_{gal} 0·83 in solvent A). Fraction 3*c* (30 mg.), $[\alpha]_D$ +125° (*c* 1·1 in water) and R_{gal} 0·35 in solvent A, contained a single component which gave galactose and glucose on hydrolysis. Hydrolysis of the derived glycitol gave only galactose. Fraction 3*d* (50 mg.) contained only 6-*O*-β-galactopyranosylgalactose.

Fraction 4. The sugar (155 mg.; eluted with water containing 5% of ethanol), after recrystallisation from ethanol–water, had m. p. 202—203°, $[\alpha]_D$ +80° (5 min.) ⟶ +62° (90 min., equil.) (*c* 0·77 in water), R_{gal} 0·60 in solvent A and R_F 0·38 in solvent C. Hydrolysis of the sugar gave galactose and arabinose, and of the derived glycitol only galactose. The sugar (100 mg.) was methylated as described above, giving the methylated disaccharide (100 mg.). Hydrolysis of this with N-hydrochloric acid at 100° for 3 hr. followed by neutralisation with silver carbonate, gave a syrup (85 mg.) which was separated into four fractions on cellulose (40 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water. Fraction *a* (28 mg.), $[\alpha]_D$ +76° (*c* 0·52 in water), contained 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (aniline derivative, m. p. and mixed m. p. 192—193°) and a trace of 2 : 5-di-*O*-methylarabinose. Fraction *b* (5 mg.), $[\alpha]_D$ *ca.* −20° (*c* 0·1 in water), R_G 0·85 in solvent B, was chromatographically indistinguishable from 2 : 5-di-*O*-methyl-L-arabinose. Fraction *c* (10 mg.) was a mixture of tri-*O*-methylgalactose (probably from incomplete methylation) and 2 : 4-di-*O*-methylarabinose. Fraction *d* (20 mg.), $[\alpha]_D$ +120° (*c* 0·3 in water), was chromatographically indistinguishable from 2 : 4-di-*O*-methyl-L-arabinose (R_G 0·60 in solvent B) and clearly distinct from the 2 : 3- and 3 : 4-dimethyl ethers in solvents B and D. An impure specimen of the

aniline derivative, m. p. 114—115° and mixed m. p. 114—117°, gave an *X*-ray powder photograph identical with that of the authentic specimen, m. p. 139—140°.

Fraction 5. The mixture of sugars (80 mg.) was separated on cellulose (45 × 2 cm.) with solvent A to give four fractions. Fraction 5*a* (25 mg.) contained a mixture of arabinose containing disaccharides (R_{gal} 1·18, 1·40, and 2·0). Fraction 5*b* (5 mg.) contained 3-*O*-β-galactopyranosylarabinose. Fraction 5*c* (25 mg.) crystallised from acetone–water, had m. p. 151—152°, $[\alpha]_D$ +69° ⟶ +55° (equil.) (*c* 1·4 in water), R_{gal} 0·35 in solvent A, and gave galactose only on hydrolysis. Oxidation of the disaccharide and of 3-*O*-β-D-galactopyranosyl-D-galactose with lead tetra-acetate followed by hydrolysis [9b] gave the same products including galactose and lyxose. The *X*-ray powder photograph of the sugar was identical with that of 3-*O*-β-D-galactopyranosyl-D-galactose. Fraction 5*d* (3 mg.) contained 6-*O*-β-galactopyranosylgalactose.

Fraction 6. The syrup (0·83 g.; eluted with water containing 7·5% of ethanol) contained a main component together with traces of 6-*O*-β-galactopyranosylgalactose and monosaccharides. A portion (0·58 g.) was separated on cellulose (45 × 2 cm.) with solvent A and a chromatographically pure sample (0·45 g.) of the oligosaccharide, $[\alpha]_D$ +20° (*c* 0·99 in water), R_F 0·20 in solvent C, was isolated. Hydrolysis of the sugar gave only galactose, and 6-*O*-β-galactopyranosylgalactose was the only disaccharide formed on partial hydrolysis. The sugar (200 mg.) was methylated as described previously to give the methylated trisaccharide (220 mg.), hydrolysis of which with N-sulphuric acid at 100° for 4 hr. afforded a mixture of methylated sugars which were separated on filter sheets with solvent B to give two fractions. Fraction (i) (36 mg.), $[\alpha]_D$ +100° (*c* 0·53 in water), contained 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, R_G 0·90 (aniline derivative, m. p. and mixed m. p. 189—190°), and a trace of a second sugar, R_G 0·82 (possibly 2 : 3 : 5-tri-*O*-methylgalactose). Fraction (ii) (80 mg.), $[\alpha]_D$ +104° (*c* 0·77 in water), contained 2 : 3 : 4-tri-*O*-methyl-D-galactose, R_G 0·70 (aniline derivative, m. p. and mixed m. p. 159—160°), together with traces of the sugar with R_G 0·82.

Fraction 7. The sugar (265 mg.; eluted with water containing 7·5% of ethanol) after recrystallisation from ethanol–water had m. p. 191°, $[\alpha]_D$ +39° (equil.) (*c* 0·91 in water), R_{gal} 0·18 in solvent A, R_F 0·25 in solvent C. Partial acid hydrolysis of the trisaccharide afforded arabinose, galactose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose, and of the derived glycitol only galactose and 6-*O*-β-galactopyranosylgalactose. The trisaccharide (200 mg.) was converted into its methylated derivative (202 mg.), hydrolysis of which with N-hydrochloric acid at 100° for 4 hr. gave a mixture of methylated sugars (180 mg.) which was separated into four fractions on cellulose (50 × 1·6 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water. Fraction *a* (47 mg.), $[\alpha]_D$ +97° (*c* 2·1 in water), R_G 0·90 in solvent B, was 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose (aniline derivative, m. p. and mixed m. p. 190—191°). Fraction *b* (5 mg.), R_G 0·85 in solvent B, was identified as 2 : 5-di-*O*-methyl-L-arabinose by conversion into 2 : 5-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 122°. Fraction *c* (40 mg.), R_G 0·70, was 2 : 3 : 4-tri-*O*-methyl-D-galactose [aniline derivative, m. p. 167—168° and mixed m. p. (with sample m. p. 164—165°) 164—165°]. Fraction *d* (32 mg.), $[\alpha]_D$ +107° (*c* 0·51 in water), R_G 0·60 in solvent B, was characterised by conversion into the aniline derivative of 2 : 4-di-*O*-methyl-L-arabinose, identified by m. p. and mixed m. p. 122° and by *X*-ray powder photograph.

Fraction 8. The chromatographically pure sugar (0·47 g.; eluted with water containing 10% of ethanol) had $[\alpha]_D$ +14° (*c* 1·95 in water), R_F 0·12 in solvent C. Hydrolysis gave only galactose, and 6-*O*-β-galactopyranosylgalactose was the only disaccharide detected on partial acid hydrolysis.

Fraction 9. The crystalline sugar (100 mg.; eluted with water containing 12·5% of ethanol), after recrystallisation from ethanol–water had m. p. 171°, $[\alpha]_D$ +26° (equil.) (*c* 0·90 in water), R_F 0·16 in solvent C. Hydrolysis of the tetrasaccharide gave galactose and arabinose in the proportion of 2·8 : 1. Arabinose, galactose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose were formed on partial acid hydrolysis of the sugar, whereas similar treatment of the derived glycitol gave only galactose and 6-*O*-β-galactopyranosylgalactose.

Fraction 10. The sugar (115 mg.; eluted with water containing 15% of ethanol), after recrystallisation from ethanol–water, had m. p. 177—179° (decomp.), $[\alpha]_D$ +19° (equil.) (*c* 1·05 in water), R_F 0·10 in solvent C. Hydrolysis gave galactose and arabinose in the proportion of 3·7 : 1. Partial acid hydrolysis of the sugar gave arabinose, galactose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose, and partial hydrolysis of the derived glycitol gave galactose and 6-*O*-β-galactopyranosylgalactose.

Experiments on Degraded Gum Ghatti.—A sample (158 mg.) of degraded gum ghatti [2] (uronic anhydride, 22%) was hydrolysed with 2N-sulphuric acid (5 ml.) at 100° for 24 hr. Ribose (23·3 mg.) was added to the cooled hydrolysate, and the mixture was neutralised with Amberlite resin IR-4B(OH), filtered, and concentrated. Samples (*ca.* 20 mg.) were separated on filter sheets with ethyl acetate–acetic acid–water (3 : 1 : 3; upper layer) and the quantities of mannose and ribose were determined by Flood, Hirst, and Jones's [13] method. The quantities of mannose corresponded to the presence of 6·3% of anhydromannose in the degraded gum (mean of three determinations). This is a minimum value since the hydrolysis conditions would not cause complete cleavage of aldobiouronic acids.

Bromine (0·5 ml.) was added to a solution of degraded gum (100 mg.) in water (100 ml.) over calcium carbonate (2 g.) and the mixture kept for 1 week at room temperature in the dark. Excess of bromine was removed by aeration, and the solution treated with silver carbonate, filtered, treated with hydrogen sulphide, again filtered, and concentrated. A second sample of degraded gum (100 mg.) was kept in oxygen-free saturated lime water (20 ml.) for 1 week at room temperature. The solution was neutralised with carbon dioxide, filtered, and concentrated to a syrup.

Samples (*ca.* 50 mg.) of degraded gum, degraded gum oxidised with bromine, and degraded gum treated with lime water were each hydrolysed with 0·5N-sulphuric acid at 100° for 30 min. The hydrolysates were absorbed on columns of charcoal–Celite (1 : 1; 25 × 1·8 cm.), monosaccharides were eluted with water (150 ml.), and water containing 20% of ethanol eluted mixtures of oligosaccharides.

Chromatography in solvent A showed that the degraded gum gave 3-*O*-β-galactosylarabinose and galactose-containing oligosaccharides as products of partial hydrolysis, but that the gums oxidised with bromine and treated with lime water gave galactose-containing oligosaccharides and only traces of 3-*O*-galactosylarabinose.

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[13] Flood, Hirst, and Jones, *J.*, 1948, 1679.