672. Anogeissus schimperi *Gum.*

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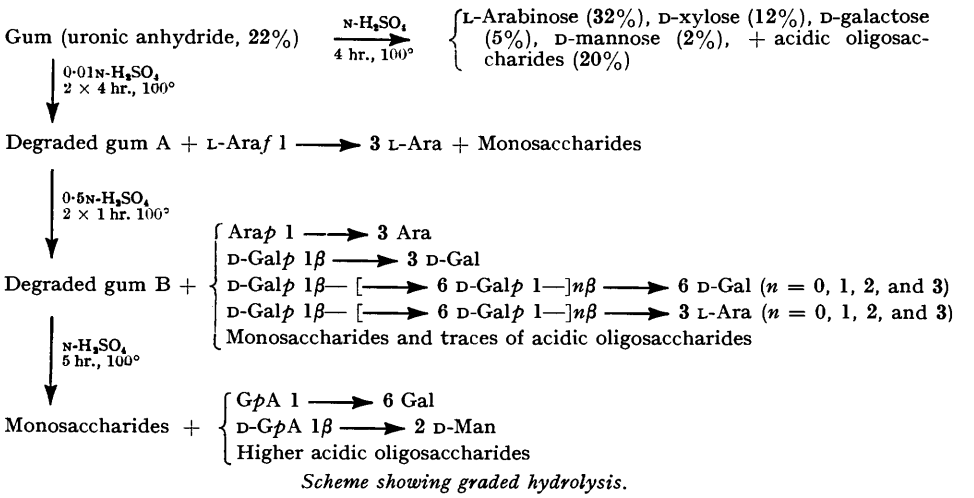
Anogeissus schimperi gum contains residues of L-arabinose, D-xylose, D-galactose, D-mannose, and D-glucuronic acid. Controlled acid hydrolysis of the gum removes some of the arabinose residues preferentially, and 3-*O*-L-arabinofuranosyl-L-arabinose has been isolated during the early stages of the hydrolysis. Further graded hydrolysis afforded complex mixtures of neutral and acidic oligosaccharides amongst which the following have been characterised: 3-*O*-β-D-galactopyranosyl-D-gaclatose, the first four members of the series *O*-β-D-galactopyranosyl-[(1 → 6)-*O*-β-D-galactopyranosyl]$_n$-(1 → 3)-L-arabinose (n = 0—3), and 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose. The recognised structural features of the gum are compared with those of gum ghatti.

The gum exudate from *Anogeissus schimperi*, or marike gum, is isolated from a tree found in Northern Nigeria and the eastern parts of the Sudan. McIlroy [1] showed the presence in it of arabinose, galactose, and glucuronic acid residues. A quantity of the gum was kindly placed at our disposal by Professor R. J. McIlroy and some of the structural features of the gum have been investigated by an examination of its partial acid hydrolysis.

A preliminary investigation (with Dr. Barbara J. Auret and Mr. H. Wilkie) showed the gum to contain ~22% of uronic anhydride, and to give on hydrolysis, D-xylose (12%), L-arabinose (32%), D-galactose (5%), D-mannose (2%), traces of rhamnose, ribose, and fucose, and a mixture of acidic oligosaccharides (20%), which was shown by paper chromatography to contain a main component indistinguishable from 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose. In view of the low recovery of sugars in this experiment, the quoted proportions of sugars are minimum values, and it is probable that those of acidic oligosaccharides and hexose sugars are relatively lower than those of pentose sugars.

In preliminary experiments on the partial acid hydrolysis of the gum under varying conditions many different oligosaccharides, both neutral and acidic, were detected as products. Accordingly, graded acid hydrolysis was carried out in three stages (see flow sheet), degraded gums A and B being separated from the soluble sugars after the first and



Scheme showing graded hydrolysis.

[1] McIlroy, *J.*, 1952, 1918.

the second stage. At each stage the various oligosaccharides were fractionated by adsorption and/or partition chromatography.

Mild acid hydrolysis resulted in cleavage of most of the arabinose and xylose residues, leaving degraded gum A with a relatively resistant core, composed largely of galactose, mannose, and glucuronic acid residues. The main disaccharide, isolated at this stage, appeared to be chromatographically and ionophoretically homogeneous and gave only arabinose on hydrolysis. Methylation and then hydrolysis of this disaccharide gave 2,3,5-tri- and 2,4-di-*O*-methyl-L-arabinose as the principal products, with smaller amounts of 2,3,4-tri-, and 2,5- and 3,4-di-*O*-methylarabinose. It follows that the fraction was composed largely of 3-*O*-L-arabinofuranosyl-L-arabinose, which was contaminated with other arabinose-containing oligosaccharides. The optical rotation ($[\alpha]_D$ +107°) of the fraction was rather higher than those reported for 3-*O*-L-arabinofuranosyl-L-arabinose isolated from sugar-beet araban [2] and *Acacia pycnantha* gum,[3] again suggesting contamination with other sugars. Other fractions were shown by chromatography of the sugars and their hydrolysis products to contain three oligosaccharides composed solely of arabinose residues (one of them indistinguishable from 3-*O*-β-L-arabinopyranosyl-L-arabinose), 3-*O*-β-galactopyranosylarabinose, and a disaccharide containing mannose and xylose residues.

The second stage of the partial hydrolysis gave mainly neutral oligosaccharides, including the following crystalline sugars which were identified by comparison with authentic specimens: 3-*O*-β-D-galactopyranosyl-D-galactose; 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—3), which have been previously characterised as partial hydrolysis products of gum ghatti.[4] The four members of this polymer-homologous series were only identified by the chromatographic mobilities of the sugars and their partial hydrolysis products, but there can be little doubt as to their identity in view of the isolation of the crystalline polygalactosylarabinose oligosaccharides. An arabinobiose was chromatographically and ionophoretically indistinguishable from 3-*O*-β-L-arabinopyranosyl-L-arabinose, and hydrolysis of the methylated derivative gave 2,3,4-tri- and 2,4-di-*O*-methylarabinose. In addition, small amounts of three acidic oligosaccharides were found at this stage, two of them being chromatographically indistinguishable from 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose; the third acidic sugar was a trisaccharide, *O*-(glucosyluronic acid)-(1 → 6)-*O*-galactosylarabinose, since hydrolysis of the glycitol (obtained by borohydride reduction) gave 6-*O*-(glucosyluronic acid)galactose, galactose, and arabitol, and reduction of the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave glucose, galactose, and arabinose.

The third stage of the graded hydrolysis of the gum gave a mixture of acidic oligosaccharides. The main component was the aldobiouronic acid, 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, whose structure was proved from the following experiments. Hydrolysis of the sugar gave glucuronic acid, glucurone, and mannose. Periodate oxidation gave formaldehyde in low yield (0·3 mol.), presumably owing to incomplete oxidation, but since complete oxidation of the derived glycitol [5] gave only 1 mol. of formaldehyde, the presence of a 2-*O*-substituted mannose residue was suspected. Reduction of the derived methyl ester methyl glycosides with potassium borohydride afforded two neutral components, which were presumably anomeric glycosides since both gave glucose and mannose on hydrolysis and reacted with periodate in a similar manner. The glycoside formed in largest amount was methylated and on subsequent hydrolysis furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose. The β-configuration of the glycosidic

[2] Andrews, Hough, and Powell, *Chem. and Ind.*, 1956, 658.
[3] Aspinall, Hirst, and Nicolson, *J.*, 1959, 1697.
[4] Aspinall, Hirst, and Wickstrøm, *J.*, 1955, 1160; Aspinall, Auret, and Hirst, *J.*, 1958, 221, 4408.
[5] Hough, Woods, and Perry, *Chem. and Ind.*, 1957, 1100.

linkage in the aldobiouronic acid may be assigned since the optical rotation ($[\alpha]_D$ −35°) was similar to that ($[\alpha]_D$ −32°) of the aldobiouronic acid from gum ghatti,[4] which has been degraded by Perlin's procedure [6] to 2-*O*-β-D-glucopyranosylglyceritol.[7]

Smaller amounts of other acidic oligosaccharides were partially identified by chromatography of the sugars and their derivatives. One was chromatographically and ionophoretically indistinguishable from 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and another was probably a second *O*-(glucosyluronic acid)mannose. Three higher acidic oligosaccharides were composed of glucuronic acid and mannose residues, and may be members of a polymer-homologous series since all gave staining reactions with aniline oxalate similar to that given by 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose, and the absence of a colour reaction with triphenyltetrazolium hydroxide [8] indicated that in each case the reducing residue was substituted at position 2. One of the oligosaccharides was chromatographically indistinguishable from the aldotriouronic acid, *O*-(D-glucopyranosyluronic acid)-(1 ⟶ 2)-*O*-D-mannopyranosyl-(1 ⟶ 2)-D-mannose, which has been isolated as a partial hydrolysis product from gum ghatti.[7]

The results of this investigation of *Anogeissus schimperi* gum may be compared with those from studies of gum ghatti,[4,7] which is derived from the botanically related species, *Anogeissus latifolia*. The two gums contain L-arabinose, D-galactose, D-mannose, and D-glucuronic acid as the main constituent sugars and, although an exact quantitative comparison of compositions is not possible, it is clear that *A. schimperi* gum contains a substantially higher proportion of D-glucuronic acid and probably also of D-mannose residues. Whereas xylose is only a minor constituent in gum ghatti it is present in substantial proportions in *A. schimperi* gum. In gum ghatti [4] it is probable that this sugar arises from a minor contaminating polysaccharide, and it is possible that in *A. schimperi* gum the xylose is derived from such a second polysaccharide component present in larger amount. Similarly the traces of rhamnose, ribose, and fucose may also be formed from minor contaminants. Even in the absence of proof of homogeneity, it is clear that the two gums contain structural features in common, and the present results may be assessed by considering, in turn, the acid-labile arabinose residues, the framework of D-galactose residues, and the acidic oligosaccharide units.

As in the case of gum ghatti, mild acid hydrolysis of *A. schimperi* gum removes the majority of the L-arabinose residues, and it is probable that these are in the outer parts of the molecular structure and that many are present in the furanose form. The characterisation of 3-*O*-L-arabinofuranosyl-L-arabinose as a partial acid hydrolysis product establishes the presence of adjacent L-arabinose residues in the gum, and chromatographic evidence for the formation of other arabinobioses as partial hydrolysis products indicates that arabinose residues in the gum are mutually linked in a variety of ways. Gum ghatti contains a high proportion of L-arbinose residues as end-groups in the furanose form, but other arbinose residues are present in non-terminal positions linked in a variety of ways.[4] Although no arabinobioses have yet been fully characterised as partial hydrolysis products of gum ghatti, it is probable that the outer chains contain some adjacent arabinose residues since mild acid hydrolysis of the gum removes some of the non-terminal in addition to the terminal L-arabinofuranose units.[4] Furthermore, reduction of the periodate-oxidised gum followed by mild acid hydrolysis affords a degraded gum still containing arabinofuranose end-groups; it is probable that these units have originated from non-terminal arabinose residues in the outer chains, which are also resistant to attack by periodate, since the degradation procedure would remove the outer shell of arabinofuranose end-groups.[7] *A. schimperi* gum, like gum ghatti, contains some arabinose residues in the interior chains

[6] Charlson, Gorin, and Perlin, *Canad. J. Chem.*, 1956, **34**, 1811; Gorin and Perlin, *ibid.*, 1958, **36**, 999.

[7] Christensen, Ph.D. Thesis, Edinburgh, 1960.

[8] Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351; Bailey, Barker, Bourne, Grant, and Stacey, *J.*, 1958, 1895.

of the polysaccharide since partial hydrolysis affords the same homologous series of oligosaccharides, *O*-β-D-galactopyranosyl-[(1 ⟶ 6)-*O*-β-D-galactopyranosyl]$_n$-(1 ⟶ 3)-L-arabinose.

The isolation of the same two series of oligosaccharides containing 1,6-linked β-D-galactopyranose residues from both gums shows that the interior chains are similarly constituted. In contrast, *A. schimperi* gum affords a relatively larger amount of 3-*O*-β-D-galactopyranosyl-D-galactose on partial hydrolysis. There is no evidence yet for the structural environment of these 1,3-linked galactose residues, although it may be noted that no oligosaccharides containing contiguous 1,3-linkages could be detected on partial hydrolysis of the gum.

The two gums furnish different proportions of acidic oligosaccharides, and it is possible that these may reflect not only differences in the proportions of similar structural units, but differences in the types of linkage present. Whereas gum ghatti affords approximately equal proportions of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose on partial hydrolysis, *A. schimperi* gum affords only the mannose-containing aldobiouronic acid in substantial amount. *A. schimperi* gum also gives rise to 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, but this aldobiouronic acid is formed in small amount and hitherto has only been identified by paper chromatography of the sugar and its derivatives. A similar identification of an aldotriouronic acid, *O*-(glucosyluronic acid)-*O*-galactosylarabinose, however, suggests that the galactose glucosiduronic acid units in the gum may be attached to arabinose residues in the outer chains rather than being present as part of the acid-resistant core of the molecular structure. The structural environment of the 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose units in the gum remains to be fully established, although the isolation of higher acidic oligosaccharides composed of glucuronic acid and mannose residues only indicates that the main aldobiouronic acid are not simply attached to basal chains of galactose residues. The isolation from gum ghatti of similar higher acidic oligosaccharides, including the trisaccharide, *O*-(D-glucopyranosyluronic acid)-(1 ⟶ 2)-*O*-D-mannopyranosyl-(1 ⟶ 2)-D-mannose,[7] shows that in this respect the two gums have common structural features, and that the molecular structure of gum ghatti is yet more complex than hitherto supposed.[4]

Experimental

Paper chromatography was carried out on Whatman Nos. 1, 3MM, and 31 papers with the following solvent systems (v/v): (A) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (B) ethyl acetate–pyridine–water (10 : 4 : 3); (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (D) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (E) benzene–ethanol–water (11 : 3 : 1, upper layer); (F) butan-1-ol–ethanol–water (1 : 1 : 1); (G) ethyl acetate–acetic acid–formic acid–water (18 : 8 : 3 : 9); (H) butan-2-one, saturated with water. Paper ionophoresis was in borate buffer at pH 10. Optical rotations were observed for aqueous solutions at 18° ± 2°.

The gum was received from Professor R. J. McIlroy as a light grey powder which had been reprecipitated with ethanol from aqueous solution; it had uronic anhydride (by decarboxylation), *ca.* 22%.

Hydrolysis of the Gum and Identification of Neutral Sugars (with Dr. Barbara J. Auret and Mr. H. Wilkie).—The gum (5 g.) was heated in N-sulphuric acid (300 ml.) on the boiling-water bath for 4 hr. Insoluble material, which separated during the hydrolysis, was filtered off, and the cooled filtrate was neutralised with barium carbonate and filtered. The filtrate was concentrated to a syrup which was poured into methanol (1 l.), precipitated barium salts (1·0 g.) were separated, and the supernatant liquid was concentrated to a syrupy mixture (2·55 g.) of sugars. The sugars were separated on cellulose, with butan-1-ol, half saturated with water, to give six fractions.

Fraction 1 (0·053 g.) contained rhamnose and ribose. Fraction 2 (0·501 g.) had $[\alpha]_D$ +18·7° (equil.), and after recrystallisation from ethanol m. p. and mixed m. p. (with D-xylose) 144°; it was characterised as the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 210°. Fraction 3 (0·651 g.) had $[\alpha]_D$ +103° (equil.) and, after recrystallisation from methanol, m. p.

159° and mixed m. p. (with L-arabinose) 158°, and was characterised as the benzoylhydrazone, m. p. 187° and mixed m. p. 186°. Fraction 4 (0·681 g.) contained arabinose and traces of mannose. Fraction 5 (0·071 g.) contained D-mannose and L-arabinose in smaller amount; D-mannose was characterised as the phenylhydrazone, m. p. and mixed m. p. 198°. Fraction 6 (0·200 g.) had $[\alpha]_D$ +82° (equil.) and, after recrystallisation from methanol, m. p. and mixed m. p. 168°, and was characterised as the 1-methyl-1-phenylhydrazone, m. p. and mixed m. p. 185°. A sample of the barium salts was dissolved in water, barium ions were removed with Amberlite resin IR-120(H), and chromatographic examination of the resulting solution showed a main component with the mobility of 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannose.

Partial Hydrolysis of the Gum (1).—The gum (23 g.) was heated in 0·01N-sulphuric acid (1600 ml.) on the boiling-water bath for 4 hr., and the cooled solution was neutralised with Amberlite resin IR-4B(OH). The solution was concentrated and poured into ethanol (4 vol.), the precipitated degraded polysaccharide (17 g.) was separated, and the supernatant liquid was concentrated to a syrup (3 g.). The degraded polysaccharide (17 g.) was rehydrolysed under the same conditions and yielded degraded gum A (13 g.) and syrup (2 g.). The combined syrups (5 g.) were separated on cellulose (50 × 3·5 cm.) with butan-1-ol, half saturated with water, as eluant to give four fractions. Fraction 1 (50 mg.) contained rhamnose, ribose, fucose, xylose, and arabinose. Fraction 2 (2·8 g.) contained xylose and arabinose. Fraction 3 (0·41 g.) contained arabinose, oligosaccharides *a* and *b*, and galactose. Fraction 4 (80 mg.) contained oligosaccharides *c*, *d*, *e*, and *f*. Monosaccharides were identified only by paper chromatography; oligosaccharides were further fractionated by chromatography on filter sheets with solvent C.

Oligosaccharide *a* (130 mg.) had $R_{galactose}$ 1·25 in solvent C and $[\alpha]_D$ +107° (*c* 1·0), and gave arabinose only on hydrolysis. Methyl sulphate (1 ml.) and 30% aqueous sodium hydroxide (1 ml.) were added to the sugar (102 mg.) in water (3 ml.) at 0°. Further additions of methyl sulphate (2 × 6 ml.) and 30% sodium hydroxide (2 × 10 ml.) were made at room temperature and the reaction was completed by heating the solution on the boiling-water bath for 1 hr. The methylated oligosaccharide (90 mg.) was isolated by extraction with chloroform. Hydrolysis of a sample (3 mg.) gave tri- and di-*O*-methylarabinoses with only traces of products of incomplete methylation. Hydrolysis of the methylated oligosaccharide (87 mg.) with N-hydrochloric acid at 100° for 5 hr., followed by neutralisation with Amberlite resin IR-45(OH), furnished a syrup (60 mg.) which was separated on cellulose (40 × 1·5 c.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, as eluant to give five fractions. Fraction (i) (20 mg.), R_G 0·95 in solvent D, was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, identified by m. p. and mixed m. p. 129—131°, and by an *X*-ray powder photograph. Fraction (ii) (5 mg.), R_G 0·83, was chromatographically and ionophoretically indistinguishable from 2,5-di-*O*-methyl-L-arabinose. Fraction (iii) (2 mg.), R_G 0·82 in solvent D, was indistinguishable from 2,3,4-tri-*O*-methyl-L-arabinose in solvent E. Fraction (iv) (14 mg.), R_G 0·60 in solvent D, was chromatographically and ionophoretically indistinguishable from 2,4-di-*O*-methyl-L-arabinose and was characterised by conversion into the aniline derivative, m. p. 148—149° and mixed m. p. (with sample, m. p. 144—146°) 144—145°. Fraction (v) (3 mg.), R_G 0·51 in solvent D, was chromatographically and ionophoretically indistinguishable from 3,4-di-*O*-methyl-L-arabinose. Oligosaccharide *b* (20 mg.), $R_{galactose}$ 1·1 in solvent C, gave arabinose only on hydrolysis. Oligosaccharide *c* (15 mg.), $R_{galactose}$ 0·7 in solvent C, gave arabinose only on hydrolysis and was chromatographically indistinguishable from 3-*O*-β-L-arabinopyranosyl-L-arabinose in solvents, A, B, and C. Oligosaccharide *d* (6 mg.), $R_{galactose}$ 0·55 in solvent C, gave arabinose only on hydrolysis. Oligosaccharide *e* (3 mg.), $R_{galactose}$ 0·5 in solvent C, was stained brown with aniline oxalate (characteristic of substituted hexoses) and yielded mannose and xylose on hydrolysis. Oligosaccharide *f* (10 mg.), $R_{galactose}$ 0·4 in solvent C, was chromatographically indistinguishable from 3-*O*-β-D-galactopyranosyl-L-arabinose.

Partial Hydrolysis of the Gum (2).—The gum (30 g.) was heated in 0·5N-sulphuric acid (1 l.) on the boiling-water bath for 1 hr., and the cooled solution was neutralised to pH 5 with barium hydroxide and subsequently with barium carbonate. Inorganic material was removed at the centrifuge, the solution was concentrated and poured into ethanol (4 vol.), the precipitated degraded polysaccharide was separated, and the supernatant liquid was concentrated to a syrup (10·6 g.). The degraded polysaccharide was rehydrolysed under the same conditions and furnished degraded gum B (8 g.) and syrup (1 g.). The combined syrups (11·6 g.) dissolved in water were adsorbed on charcoal–Celite (1 : 1; 40 × 7 cm.). Elution with water gave

fraction 1 (9·7 g.) containing xylose, arabinose, galactose, and traces of mannose. Elution with water containing 2·5% of ethanol gave fraction 2 (30 mg.) containing oligosaccharide 1, fraction 3 (30 mg.) containing oligosaccharide 2, and fraction 4 (300 mg.) containing oligosaccharide 3. Elution with water containing 5% of ethanol gave fraction 5 (20 mg.) containing acidic oligosaccharides *a* and *b*, fraction 6 (140 mg.) containing neutral oligosaccharide 4 and acidic oligosaccharides *a* and *b*, and fraction 7 (20 mg.) containing oligosaccharide 5. Elution with water containing 7·5% of ethanol gave fraction 8 (60 mg.) containing oligosaccharide 6, and fraction 9 (50 mg.) containing oligosaccharides 6 and 7, and acidic oligosaccharide *c*. Elution with water containing 10% of ethanol gave fraction 10 (20 mg.) containing oligosaccharide 8, and fraction 11 (10 mg.) containing oligosaccharides 9 and 10. The weights of the fractions are only approximate but give some indication of the relative amounts of pure oligosaccharides, which, in most cases, were isolated after chromatographic separation on filter sheets. Further quantities of several oligosaccharides were isolated in a similar manner after hydrolysis of degraded gum A with 0·5N-sulphuric acid at 100° for four periods of 0·5 hr. (degraded polysaccharide was separated from soluble sugars at the end of each period).

Oligosaccharide 1 had $R_{galactose}$ 0·8 in solvent B and gave arabinose only on hydrolysis. The sugar was indistinguishable from 3-*O*-β-L-arabinopyranosyl-L-arabinose and from oligosaccharide *c* when examined by paper ionophoresis and by paper chromatography in solvents A, B, and C. Hydrolysis of the derived methylated oligosaccharide afforded 2,3,4-tri- and 2,4-di-*O*-methylarabinose.

Oligosaccharide 2, $[\alpha]_D$ +17° (*c* 1·2) and $R_{galactose}$ 0·3 in solvent B, was chromatographically and ionophoretically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose.

Oligosaccharide 3, $R_{galactose}$ 0·6 in solvent B, crystallised and, after recrystallisation from ethanol–water, had $[\alpha]_D$ +97° (2 min.) ⟶ +67° (2 hr., equil.) (*c* 0·45). Hydrolysis of the sugar gave galactose and arabinose, and of the derived glycitol only galactose. The sugar was identified as 3-*O*-β-D-galactopyranosyl-L-arabinose by its m. p. and mixed m. p. 204—205°, and by an *X*-ray powder photograph.

Oligosaccharide 4, $R_{galactose}$ 0·4 in solvent B, crystallised and, after recrystallisation from ethanol–water, had $[\alpha]_D$ +84° (3 min.) ⟶ +61° (3 hr., equil.), m. p. and mixed m. p. 169—170°, and gave an *X*-ray powder photograph identical with that of 3-*O*-β-D-galactopyranosyl-D-galactose.

Oligosaccharide 5, R_F 0·20 in solvent F, was chromatographically indistinguishable from *O*-β-D-galactopyranosyl-(1 ⟶ 6)-*O*-β-D-galactopyranosyl-(1 ⟶ 6)-D-galactose. Partial acid hydrolysis gave galactose and 6-*O*-β-galactopyranosylgalactose.

Oligosaccharide 6, R_F 0·25 in solvent F, gave galactose, arabinose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose on partial acid hydrolysis. After recrystallisation from ethanol–water the sugar was identified as *O*-β-D-galactopyranosyl-(1 ⟶ 6)-*O*-β-D-galactopyranosyl-(1 ⟶ 3)-L-arabinose by its m. p. and mixed m. p. 190—191°, and by an *X*-ray powder photograph.

Oligosaccharide 7, R_F 0·12 in solvent F, was chromatographically indistinguishable from the 1,6-linked galactotetraose isolated from gum ghatti.[4] Hydrolysis gave only galactose, and 6-*O*-β-galactopyranosylgalactose was the only disaccharide detected on partial acid hydrolysis.

Oligosaccharide 8, R_F 0·16 in solvent F, gave galactose, arabinose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose on partial acid hydrolysis. After recrystallisation from ethanol–water the sugar was identified as *O*-β-D-galactopyranosyl-[(1 ⟶ 6)-*O*-β-D-galactopyranosyl]$_2$-(1 ⟶ 3)-L-arabinose by its m. p. and mixed m. p. 167—172°, and by an *X*-ray powder photograph.

Oligosaccharide 9, R_G 0·075 in solvent F, had an R_M value [9] consistent with that of a 1,6-linked galactopentaose.

Oligosaccharide 10, R_F 0·10 in solvent F, gave galactose, arabinose, 3-*O*-galactopyranosylarabinose, and 6-*O*-galactopyranosylgalactose on partial acid hydrolysis. After recrystallisation from ethanol–water the sugar was identified as *O*-β-D-galactopyranosyl-[(1 ⟶ 6)-*O*-β-D-galactopyranosyl]$_3$-(1⟶ 3)-L-arabinose by its m. p. and mixed m. p. 178—182°, and by an *X*-ray powder photograph.

Acidic oligosaccharides a and *b* were chromatographically identified as 2-*O*-(glucosyluronic acid)mannose and 6-*O*-(glucosyluronic acid)galactose.

[9] Bate-Smith and Westhall, *Biochem. Biophys. Acta*, 1950, **4**, 427.

Acidic oligosaccharide c had $[\alpha]_D$ +6° (*c* 0·3) and R_F 0·23 in solvent F and R_F 0·16 in solvent G, and gave a pink stain with aniline oxalate characteristic of reducing pentose derivatives. Hydrolysis gave arabinose, galactose, glucuronic acid, and 6-*O*-(glucosyluronic acid)galactose, and reduction of the derived methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave arabinose, galactose, and glucose in approximately equal proportions. Hydrolysis of the derived glycitol (borohydride reduction of the acid) gave 6-*O*-(glucosyluronic acid)galactose, galactose (trace), and arabitol.

Partial Hydrolysis of the Gum (3).—Degraded gum B (7·5 g.) was heated in N-sulphuric acid (300 ml.) on the boiling-water bath for 5 hr., the cooled solution was neutralised with barium hydroxide and barium carbonate, and the filtered solution was concentrated to a syrup (5·3 g.). The syrup (5·3 g.) in water was treated with Amberlite resin IR-120(H) to remove barium ions, and the acidic solution was passed through a column of Amberlite resin IR-45(OH) to absorb acids. Elution of the resin with water gave neutral sugars (0·9 g.) and elution with water containing 10% of formic acid afforded a mixture (4·0 g.) of acidic oligosaccharides. Chromatographically pure acidic oligosaccharides I—IV were obtained after separation on filter sheets by using solvent C. Acidic oligosaccharides V and VI, $R_{galactose}$ 0·12 and 0·05 in solvent G, which were not isolated individually, when stained with aniline oxalate showed the same characteristic orange-brown fluorescence in ultraviolet light as acidic oligosaccharides I and IV.

Acidic oligosaccharide I (1·1 g.), $[\alpha]_D$ −35° (*c* 1·85) and $R_{galactose}$ 0·35 and 0·74 in solvents C and G, was chromatographically and ionophoretically indistinguishable from 2-*O*-(glucosyluronic acid)mannose. Hydrolysis gave glucuronic acid, glucurone, and mannose. Oxidation of the sugar with sodium metaperiodate at pH 7·5 gave 0·3 mol. of formaldehyde (estimation by the method of O'Dea and Gibbons [10]). Reduction of the sugar with potassium borohydride, followed by oxidation with periodate, gave 1·0 mol. (constant value) of formaldehyde.

The sugar (0·5 g.) was converted into the methyl ester methyl glycosides by boiling it with methanolic hydrogen chloride for 6 hr. The ester glycosides were treated with potassium borohydride (50 mg.) in methanol (10 ml.) for 24 hr., and two neutral glycoside fractions *a* (150 mg.), $R_{galactose}$ 1·2 in solvent B, and *b* (25 mg.), $R_{galactose}$ 0·8, were isolated after chromatographic separation on filter sheets. Fractions *a* and *b* were probably anomeric disaccharide methyl glycosides since both gave glucose and mannose on hydrolysis and both consumed 2·8 mol. of periodate, and they furnished chromatographically similar products on reduction of the periodate-oxidised glycosides followed by hydrolysis. Fraction *a* (130 mg.) was methylated in the usual way and furnished methylated disaccharide (130 mg.), hydrolysis of which afforded tetra-*O*-methylglucose (R_G 1·0 in solvent D) and tri-*O*-methylmanose (R_G 0·85 in solvent D) with only traces of products of incomplete methylation. The methylated sugars were identified as (i) 2,3,4,6-tetra-*O*-methyl-D-glucose, $[\alpha]_D$ +88·5° (3 min.) ⟶ +77° (3 hr., equil.) (*c* 0·7), by its m. p. and mixed m. p. 81—82°, and by an *X*-ray powder photograph, and (ii) 3,4,6-tri-*O*-methyl-D-mannose, by its m. p. and mixed m. p. 101—103°, and by an *X*-ray powder photograph.

Acidic oligosaccharide II (30 mg.), $R_{galactose}$ 0·25 and 0·67 in solvents C and G, gave glucuronic acid, glucurone, and mannose on hydrolysis. Reduction of the methyl ester methyl glycosides followed by hydrolysis gave glucose and mannose.

Acidic oligosaccharide III (35 mg.), $R_{galactose}$ 0·15 and 0·51 in solvents C and G, was chromatographically and ionophoretically indistinguishable from 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. Hydrolysis gave glucuronic acid, glucurone, and galactose, and reduction of the methyl ester methyl glycosides followed by hydrolysis gave glucose and galactose.

Acidic oligosaccharide IV (40 mg.), $R_{galactose}$ 0·07 and 0·32 in solvents C and G, was chromatographically indistinguishable from *O*-(D-glucopyranosyluronic acid)-(1 ⟶ 2)-*O*-D-mannopyranosyl-(1 ⟶ 2)-D-mannose from gum ghatti, and when stained with aniline oxalate gave the same characteristic orange-brown fluorescence in ultraviolet light as acidic oligsaccharide I. Hydrolysis gave glucuronic acid, glucurone, and mannose, and reduction of the methyl ester methyl glycosides followed by hydrolysis gave glucose and mannose.

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[10] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.