486. *Gum Ghatti (Indian Gum). Part V.*[1] *Degradation of the Periodate-oxidised Gum*

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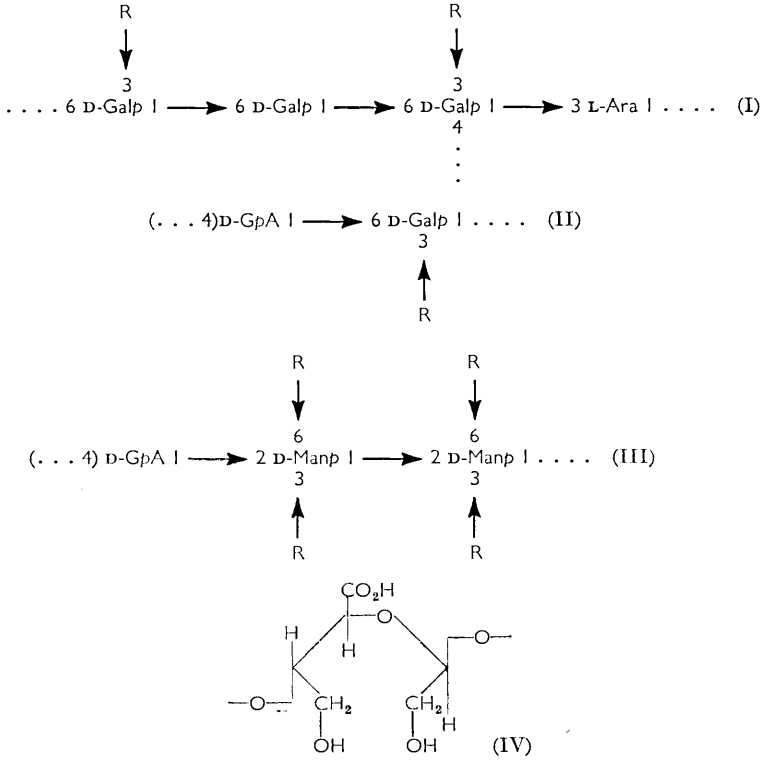
Reduction of periodate-oxidised gum ghatti, followed by controlled acid hydrolysis, affords degraded gum B. The degraded polysaccharide has been examined by methylation, partial acid hydrolysis, and periodate oxidation, and the structure of the gum is discussed in the light of the results obtained.

The known structural features of gum ghatti [1] may be summarised in the partial structures (I), (II), and (III), which carry, at the indicated sites, various acid-labile side-chains (R), which are most frequently single L-arabinofuranose residues, but may also be multiple units of L-arabinose residues or single L-rhamnopyranose residues. In order to obtain further structural information the gum has been degraded by Smith's procedure [2] of periodate oxidation, reduction, and mild acid hydrolysis, to give a degraded polysaccharide (hereinafter referred to as degraded gum B, in contradistinction to degraded gum A which is formed on autohydrolysis of the gum acid [1b]). Degraded gum B has been examined by methylation, partial hydrolysis, and periodate oxidation procedures.



Periodate-oxidised gum ghatti was reduced with potassium borohydride, and the resulting polyalcohol was hydrolysed with cold dilute sulphuric acid under conditions which resulted in minimum cleavage of glycosidic linkages, and degraded gum B was isolated by

[1] (*a*) Part I, *J.*, 1955, 1160; (*b*) Part II, *J.*, 1958, 221; (*c*) Part III, *J.*, 1958, 4408; (*d*) Part IV, preceding Paper.

[2] I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.

precipitation with ethanol. The supernatant liquid consisted largely of glycerol (from cleaved sugar residues), but included some glycosides of low molecular weight which were not examined in detail. Although previous studies [15] showed that all the D-glucuronic acid residues in the gum would be expected to be cleaved by periodate, degraded gum B still contained acidic groups (Equiv., 1100). These acidic groups were not those of unmodified D-glucuronic acid residues since neither this sugar nor acidic oligosaccharides could be detected on partial hydrolysis of the degraded gum. Since the subsequent hydrolysis of methylated degraded gum B furnished *inter alia* methoxyacetaldehyde, it is probable that the acidic groups were present in cleaved sugar units (*e.g.*, IV) whose acetal linkages were not hydrolysed by cold dilute acid. Further experiments showed that the conditions required to cleave these acetal linkages caused appreciable hydrolysis of normal glycosidic linkages.

Partial acid hydrolysis of degraded gum B furnished a complex mixture of neutral oligosaccharides, which was fractionated by chromatography on charcoal–Celite, followed where necessary by partition chromatography on cellulose. The main oligosaccharides were characterised as members of the two polymer-homologous series (V; $n = 0$, 1, 2, 3, and 4) and (VI; $n = 0$, 1, 2, and 3). In addition, 3-*O*-β-D-galactopyranosyl-D-galactose was isolated as the crystalline sugar, a trace amount of an unknown galactosylmannose was detected, and three other galactose-containing fragments were isolated in small amount but not fully characterised.

D-Gal*p* I — [⟶ 6 D-Gal*p* I —]$_n$ ⟶ 6 D-Gal (V)

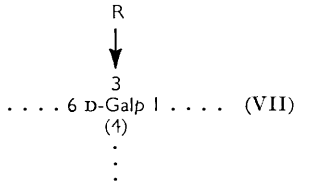
D-Gal*p* I — [⟶ 6 D-Gal*p* I —]$_n$ ⟶ 3 L-Ara (VI)

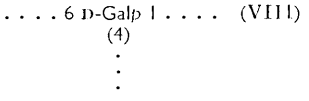
Hydrolysis of methylated degraded gum B furnished methoxyacetaldehyde and the following methylated sugars which were characterised as crystalline derivatives: 2,3,5-tri- and 2,4-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3- and 2,4-di-*O*-methyl-D-galactose, and 3,4,6-tri-*O*-methyl-D-mannose. In addition, smaller amounts of the following sugars were characterised by optical rotation, paper chromatography of the sugars and their derivatives, and gas chromatography of their methyl glycosides: 2,3,4-tri-, 2,5-di-, and 2,-*O*-methyl-L-arabinose, 2,3,6- and 2,4,6-, and 2-*O*-methyl-D-galactose, and 2,3,4,6-tetra-, (?) 4,6-di-, and 4-*O*-methyl-D-mannose.

Degraded gum B still contained a substantial proportion of periodate-resistant sugar residues, and a second Smith degradation afforded polymeric material, degraded gum C, which was precipitated from aqueous solution on addition of ethanol. Gas chromatography of the cleavage products from methylated degraded gum C indicated the presence of methyl glycosides of 2,3,4- and 2,3,5-tri-, and 2,4- and 2,5-di-*O*-methylarabinose, 2,3,4,6-tetra-, and 2,3,4- and 2,4,6-tri-*O*-methylgalactose, and 2,3,4,6-tetra-, 2,4,6- and 3,4,6-tri-, and possibly 4,6-di-*O*-methylmannose. Partial acid hydrolysis of degraded gum C afforded four disaccharides. 3-*O*-, and 6-*O*-β-D-Galactopyranosyl-D-galactose were characterised as crystalline derivatives. 3-*O*-β-D-Galactopyranosyl-L-arabinose was identified by paper chromatography of the disaccharide and its derivatives, and by gas chromatography of the cleavage products of the methylated derivative. The fourth disaccharide was assigned the structure 3-*O*-L-arabinopyranosyl-D-mannose on the basis of the following experiments. Hydrolysis gave arabinose and mannose, whereas hydrolysis of the derived glycitol gave arabinose and mannitol. Methanolysis of the methylated derivative gave a mixture of methyl glycosides, whose major components were identified by gas chromatography as methyl glycosides of 2,3,4-tri-*O*-methylarabinose and 2,4,6-tri-*O*-methylmannose.

The results of these experiments, although not open to unambiguous interpretation, may be assessed by considering, in turn, the chains of 1,6-linked D-galactopyranose residues (I), the L-arabinose residues which terminate these chains, the L-arabinose residues in the outer chains, and the D-mannose residues in the inner chains (III). Previous methylation

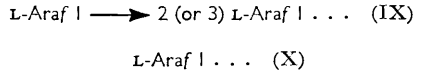
experiments on gum ghatti [1b] showed that approximately one in three of the 1,6-linked D-galactopyranose residues were unbranched, but the isolation of oligosaccharides up to and including the hexasaccharide indicates that there are parts of the molecular structure in which at least six contiguous galactose residues are each present as branching points, and suggests that attachment of side-chains to these units is not statistically regular. The extent of methylation and site of substitution of the methyl ethers of D-galactose, which were isolated from hydrolysis of methylated degraded gum B, indicates that the Smith degradation has resulted in removal of substituents from C-3 of D-galactopyranose residues, which in the original gum were also substituted at C-6 or at C-4 and C-6 (cf. VII ⟶ VIII). The present results do not provide any further indication as to the location of the small proportion of the 3-*O*-β-D-galactopyranosyl-D-galactose units in relation to other structural units, although the isolation of the 1,3-linked galactobiose on partial hydrolysis of both degraded sums B and C indicates that this unit arises from interior chains of the molecule.

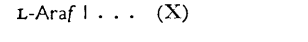


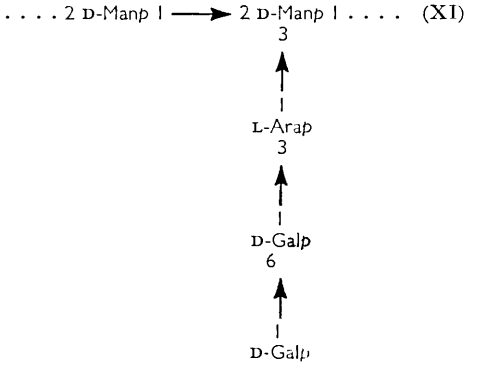


Gum ghatti contains 3-*O*-substituted L-arabinose residues in both furanose and pyranose forms, but previous studies provided no evidence for the ring size of the L-arabinose residues which give rise to the reducing groups of the galactosylarabinose oligosaccharides (VI). Partial acid hydrolysis of degraded gum B also furnished these oligosaccharides, and since hydrolysis of the methylated derivative furnished 2,4-di-*O*-methyl-L-arabinose in larger amount than the 2,5-dimethyl ether (approximate ratio, 3 : 1), it is probable that the majority of the interior 3-*O*-substituted L-arabinose residues are in the pyranose form. Degraded gum C, which was formed after a second Smith degradation, also afforded 3-*O*-β-D-galactopyranosyl-L-arabinose on partial hydrolysis, but since both 2,4- and 2,5-di-*O*-methyl-arabinose (the latter in smaller but significant amount) were recognised as cleavage products of the methylated derivative, the nature of the ring size of these interior L-arabinose residues is not unequivocally settled.

The majority of L-arabinose residues in gum ghatti, as in many other exudate gums, are present in the furanose form in the outer chains. In gum ghatti most of these residues are present as end-groups, but a small proportion are in non-terminal positions in a variety of types of substitution. The markedly reduced proportion of L-arabinofuranose residues in







degraded gum B indicated that the majority of these residues were indeed removed during the Smith degradation. The L-arabinofuranose end-groups (X), which are present in the

degraded gum, must have originated from 2- and/or 3-*O*-substituted residues in gum ghatti which probably carried L-arabinofuranose end-groups in arabinobiose units (IX).

D-Mannopyranose residues in gum ghatti are 2-*O*-substituted (as in III), but the majority of these residues carry other substituents at C-3 and C-6. Since hydrolysis of methylated degraded gum B gave 3,4,6-tri- and (?) 4,6-di-*O*-methyl-D-mannose together with a little of the 4-methyl ether, it is clear that most of the substituents at C-6 and some of those at C-3 were removed during the Smith degradation. Further information on the structural relationship of D-mannopyranose residues (in III) and other units in the polysaccharide emerges from the results of studies on degraded gum C. Degraded gum C, although of sufficient molecular size to be precipitated from aqueous solution with ethanol, is probably of relatively low molecular weight and is likely to be a mixture of related fragments rather than a discrete molecular species. The recognition of 2,3,4-tri- and 2,4-di-*O*-methylarabinose, tetra- and 2,3,4-tri-*O*-methylgalactose, and tetra-, 2,4,6- and 3,4,6-tri-, and possibly 4,6-di-*O*-methylmannose amongst the cleavage products of the methylated derivative, and the characterisation of 6-*O*-β-D-galactopyranosyl-D-galactose, 3-*O*-β-D-galactopyranosyl-L-arabinose, and 3-*O*-L-arabinopyranosyl-D-mannose amongst the partial hydrolysis products of degraded gum C may be accommodated in the partial structure (XI) if the major part of the degraded polysaccharide is considered as a mixture of fragments, each of which is composed in part of various of the possible sequences of sugar residues in this structure. The formulation of this new partial structure (XI), which provides a key to the relationship of the partial structures (I and III), is based on the reasonable, but not uniquely proven, assumption that the L-arabinose units in (I) are in the pyranose form.

Experimental

Paper chromatography was carried out with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer); (E) butan-2-one, half saturated with water; (F) butan-2-one–acetic acid–water (9 : 1 : 1, saturated with boric acid); (G) butan-1-ol–ethanol–water (1 : 1 : 1). R_G Values of methylated sugars refer to rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent C. Demethylations of methylated sugars were performed with boron trichloride.[3] Chromatography of the periodate oxidation products of methylated sugars was carried out by Lemieux and Bauer's method.[4] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas chromatography of methylated and partially methylated methyl glycosides was carried out on columns of (a) 15% by weight of butane-1,4-diol succinate polyester on Celite at 175°; (b) 10% by weight of polyphenyl ether [*m*-bis-(*m*-phenoxyphenoxy)benzene] on Celite at 200°.[5] Retention times (T) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Preparation of Degraded Gum B.—Gum ghatti (80 g.) was oxidised by sodium metaperiodate (225 g.) in water (5 l.) at room temperature for 3 days (consumption of reagent was constant). Excess of periodate was destroyed by the addition of ethylene glycol, the solution was dialysed to remove iodate ions and concentrated (to 1·6 l.), and potassium borohydride (15 g.) was added during 2 days. Excess of hydride was destroyed, and potassium ions were removed by treatment of the solution with Amberlite resin IR-120(H), and addition of ethanol containing 5% of light petroleum (4 vol.) precipitated the polyalcohol (44 g.), $[\alpha]_D$ −4° (*c* 3·0). The polyalcohol (40 g.) was hydrolysed with N-sulphuric acid (520 ml.) at room temperature for 12 hr., the solution was neutralised with barium hydroxide and barium carbonate, and the filtrate was passed through Amberlite resin IR-120(H) to remove barium ions and poured into ethanol (4 vol.) to give degraded gum B (20 g.), $[\alpha]_D$ −3·5° (*c* 4·0) and equiv. wt., 1100. Concentration of the superantant liquid furnished a syrup (9 g.). Chromatography of the syrup showed glycerol to be the dominant component together with traces of arabinose, threitol, and a number of non-reducing glycosides which gave galactose and glycerol on hydrolysis. A sample of

[3] T. G. Bonner, E. J. Bourne, and S. McNally, *J.*, 1960, 2929.
[4] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[5] G. O. Aspinall, *J.*, 1963, 1676.

degraded gum B was heated in 0·01N-sulphuric acid on the boiling-water bath and aliquot portions were withdrawn periodically. Examination of the polysaccharide, which was precipitated with ethanol, and of the soluble cleavage products indicated that the proportion of acidic groups in the polysaccharide decreased but that cleavage of glycosidic linkages took place with the release of arabinose.

Partial Hydrolysis of Degraded Gum B.—Degraded gum B (20 g.) was hydrolysed with 0·5N-sulphuric acid on a boiling-water bath for three periods of 0·5 hr. (degraded polysaccharide was separated from soluble sugars at the end of each period by precipitation with ethanol and rehydrolysed), to give a syrupy mixture (16 g.) of sugars which was adsorbed on charcoal–Celite (1 : 1; 50 × 7 cm.). Elution with water gave arabinose, galactose, and mannose (11 g.), and subsequent elution with water containing 2·5, 5·0, and 10·0% of ethanol gave fourteen oligosaccharide fractions, which were further separated, as required, by chromatography on filter sheets.

Oligosaccharide 1 (0·5 g.), $[\alpha]_D$ +23° (*c* 1·1) and $R_{galactose}$ 0·3 in solvent A, gave galactose only on hydrolysis and was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose.

Oligosaccharide 2 (90 mg.), $R_{galactose}$ 0·6 in solvent A, gave galactose and arabinose on hydrolysis, and was recrystallised from ethanol–water to give 3-*O*-β-D-galactopyranosyl-L-arabinose, m. p. and mixed m. p. 204—205°.

Oligosaccharide 3 (60 mg.), $R_{galactose}$ 0·4 in solvent A, gave galactose only on hydrolysis and was recrystallised from ethanol–water to give 3-*O*-β-D-galactopyranosyl-D-galactose monohydrate, which was identified by m. p. and mixed m. p. 154—156°, and by *X*-ray powder photograph.

Oligosaccharide 4 (80 mg.), $[\alpha]_D$ +11·4° (*c* 0·7) and $R_{galactose}$ 0·4 and 0·25 in solvents A and B, gave galactose only on hydrolysis but was chromatographically distinct from the 1,3-, 1,4-, and 1,6-linked galactobioses. The sugar gave a strong positive colour reaction with triphenyltetrazolium hydroxide,[6] indicating the absence of a substituent at C-2.

Oligosaccharide 5 (*ca.* 5 mg.), $R_{galactose}$ 0·7 in solvent A, gave galactose and mannose on hydrolysis, whereas the derived glycitol furnished galactose as the sole reducing sugar.

Oligosaccharide 6 (230 mg.), R_F 0·20 in solvent G, gave galactose and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis. The sugar was crystallised from ethanol–water and was characterised as *O*-β-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 6)-D-galactose by m. p. and mixed m. p. (with sample obtained by direct partial hydrolysis of the gum [1c]) 135—140° (decomp.), $[\alpha]_D$ +17° (3 min.) → +21° (4 hr., equil.) (*c* 0·8), and by *X*-ray powder photograph.

Oligosaccharide 7 (110 mg.), R_F 0·25 in solvent G, gave galactose, arabinose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis. Crystallisation from ethanol–water furnished *O*-β-D-galactopyranosyl-(1 → 6)-*O*-β-D-galactopyranosyl-(1 → 3)-L-arabinose, which was identified by m. p. and mixed m. p. 184—186°, and by *X*-ray powder photograph.

Oligosaccharide 8 (120 mg.), R_F 0·12 in solvent G and $[\alpha]_D$ +14° (*c* 1·3), was chromatographically indistinguishable from the 1,6-linked galactotetraose,[1c] and gave galactose only on hydrolysis, and 6-*O*-galactopyranosylgalactose was the only disaccharide which could be detected on partial hydrolysis.

Oligosaccharide 9 (20 mg.), R_F 0·16 in solvent G, gave galactose, arabinose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis. The sugar was crystallised from ethanol–water and was characterised as *O*-β-D-galactopyranosyl-[(1 → 6)-*O*-β-D-galactopyranosyl]$_2$-(1 → 3)-L-arabinose by m. p. and mixed m. p. 170—173°, and by *X*-ray powder photograph.

Oligosaccharide 10 (30 mg.), R_F 0·07 in solvent G and $[\alpha]_D$ +9° (*c* 0·9), had an R_M value [7] consistent with that of a 1,6-linked galactopentaose, and gave galactose as the only monosaccharide and 6-*O*-β-galactopyranosylgalactose as the only disaccharide on partial hydrolysis.

Oligosaccharide 11 (30 mg.), R_F 0·17 in solvent G, and $[\alpha]_D$ 0° (*c* 0·5), and gave galactose and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis, but the R_M value did not correspond to that of a member of the 1,6-linked series of galactose-containing oligosaccharides.

[6] D. S. Feingold, G. Avigad, and S. Hestrin, *Biochem. J.*, 1956, **64**, 351; R. W. Bailey, S. A. Barker, E. J. Bourne, P. M. Grant, and M. Stacey, *J.*, 1958, 1895.

[7] E. C. Bate-Smith and R. G. Westhall, *Biochem. Biophys. Acta*, 1950, **4**, 427.

Oligosaccharide 12 (10 mg.), R_F 0·10 in solvent G, gave galactose, arabinose, 3-*O*-β-galactopyranosylarabinose, and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis. The sugar was crystallised from ethanol–water and was characterised as *O*-β-D-galactopyranosyl-[(1 → 6)-*O*-β-D-galactopyranosyl]$_3$-(1 → 3)-L-arabinose by m. p. and mixed m. p. 170—174°, and by *X*-ray powder photograph.

Oligosaccharide 13 (10 mg.), R_F 0·04 in solvent G, had an R_M value [7] consistent with that of a 1,6-linked galactohexaose, and gave galactose as the only monosaccharide and 6-*O*-β-galactopyranosylgalactose as the only disaccharide on partial hydrolysis.

Oligosaccharide 14 (*ca.* 3 mg.), R_F 0·10 in solvent G, gave galactose and 6-*O*-β-galactopyranosylgalactose on partial hydrolysis, but the R_M value did not correspond to that of a member of the 1,6-linked series of galactose-containing oligosaccharides.

Preparation and Hydrolysis of Methylated Degraded Gum B.—Degraded gum B (2·6 g.) was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, to give methylated degraded gum B (1·96 g.) (Found: OMe, 42·1%). The methylated polysaccharide (1·76 g.) was hydrolysed with N-hydrochloric acid on a boiling-water bath for 4 hr., and the hydrolysate was neutralised with Amberlite resin IR-45(OH). The solution was concentrated to a syrupy mixture (1·50 g.) of methylated sugars, and methoxyacetaldehyde in the distillate was characterised by conversion into the *p*-nitrophenylhydrazone, m. p. and mixed m. p. 116°. The mixture of sugars was separated on cellulose, (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1), saturated with water, and (ii) butan-1-ol, half saturated with water, being used as eluants to give 13 fractions. Table 1 summarises the results of preliminary examination of the various fractions.

TABLE 1

Analysis of hydrolysate of methylated degraded gum B

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography * R_G	Sugar	Sugars given on demethylation	Other evidence †
1	122	−22°	1·00	Me_4mannose		g.l.c.
			0·98	2,3,5-Me_3arabinose		
2	149	+112°	1·00	Me_4mannose (t)		g.l.c.
			0·91	Me_4galactose		
3	64	+42°	0·90	Me_4galactose		D, I, g.l.c.
			0·84	2,3,4-Me_3arabinose		
				2,5-Me_2arabinose		
			0·77	3,4,6-Me_3mannose		
4	80	+64°	0·84	2,3,4-Me_3arabinose		D, g.l.c.
			0·77	3,4,6-Me_3mannose		
5	276	+96°	0·70	2,3,4-Me_3galactose		g.l.c.
				2,3,6-Me_3galactose (t)		
				2,4,6-Me_3galactose (t)		
6	163	+58°	0·70	2,3,4-Me_3galactose		D, E, g.l.c.
			0·66	Me_2mannose		
			0·62	2,4-Me_2arabinose		
7	19		0·70	2,3,4-Me_3galactose (t)		D
			0·66	Me_2mannose	galactose	
			0·56	Me_2galactose	mannose	
			0·54	(?) Me arabinose	arabinose	
8	121	+79°	0·55	2,3-Me_2galactose		P
				2-Me arabinose (t)		
9	19	+88°	0·50	2,4-Me_2galactose		
10	8		0·50	2,4-Me_2galactose		
			0·40	4-Me mannose		
11	27		0·40	4-Me mannose		A, B
12	14		0·40	4-Me mannose	mannose	A, B, D
			0·35	Unknown	arabinose	
					galactose	
13	9		0·30	2-Me galactose		

* t = trace. † A, B, D, and E = paper chromatography in solvents A, B, D, and E. P = paper chromatography of the periodate-oxidised sugar. g.l.c. = gas chromatography of the methyl glycosides.

Characterisation of Sugars from Hydrolysis of Methylated Degraded Gum B.—Fraction 1. The syrup (105 mg.) was fractionated on charcoal–Celite (1 : 1; 10 × 1·5 cm.) by gradient elution with water containing 5·0—7·5% of butan-2-one. The first fraction (88 mg.), $[\alpha]_D$ −31° (*c* 1·5),

was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 143—144°. The second fraction (11 mg.), $[\alpha]_D$ +5° (*c* 0·78), was chromatographically indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-mannose.

Fraction 2. The main component was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 207°.

Fraction 3. The syrup (60 mg.), was fractionated on charcoal–Celite (1 : 1; 10 × 1·5 cm.) by gradient elution with water containing 2·5—7·5% of butan-2-one, and the fractions were examined by chromatography in solvent D. The first fraction (10 mg.) contained 2,3,4-tri- and 2,5-di-*O*-methylarabinose in the approximate proportion of 2 : 1. The second fraction (38 mg.) contained 3,4,6-tri-*O*-methylmannose and tetra-*O*-methylgalactose. This fraction was separated on filter sheets using solvent D, to give 3,4,6-tri-*O*-methyl-D-mannose (22 mg.), $[\alpha]_D$ +11° (*c* 0·72), m. p. and mixed m. p. 98—100°, and tetra-*O*-methylgalactose (13 mg.).

Fraction 4. The syrup (80 mg.) was partially separated by chromatography on filter sheets using solvent D to give (*a*) 3,4,6-tri-*O*-methylmannose (24 mg.) containing a trace of 2,3,4-tri-*O*-methylarabinose, (*b*) a mixture (14 mg.) of the two sugars, and (*c*) chromatographically pure 2,3,4-tri-*O*-methyl-L-arabinose (34 mg.), $[\alpha]_D$ +109° (*c* 1·1).

Fraction 5. The sugar was characterised as 2,3,4-tri-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 174—175°.

Fraction 6. The syrup (152 mg.) was fractionated on charcoal–Celite (1 : 1; 21 × 1·5 cm.) by gradient elution with water containing 0·0—2·5% and 2·5—5·0% of butan-2-one. The first fraction (19 mg.), $[\alpha]_D$ +130° (*c* 0·6), was chromatographically indistinguishable from 2,4-di-*O*-methyl-L-arabinose and was characterised by conversion into the aniline derivative, m. p. 126—128° and mixed m. p. 121—123°. The second fraction (11 mg.) contained a mixture of sugars found in the previous and subsequent fractions. The third fraction (100 mg.) contained a mixture of 2,3,4-tri-*O*-methylgalactose (40 mg.) and a di-*O*-methylmannose (58 mg.) which were separated by chromatography on filter sheets using solvent D. The latter sugar, R_G 0·64 and $[\alpha]_D$ +29° (0·35), gave a positive colour reaction with triphenyltetrazolium hydroxide,[7] indicating the presence of a free hydroxyl group at C-2, and afforded mannose on demethylation. Gas chromatography of the methyl glycoside(s) showed a single component (*T* 11·1 and 2·9 on columns *a* and *b*) which was distinct from that derived from 3,4-di-*O*-methyl-D-mannose (*T* 7·3 and 2·2). Chromatography of the periodate oxidation products of the sugar showed three components (R_G 0·76, 0·86, and 0·99). In a parallel experiment periodate oxidation of 4,6-di-*O*-methyl-D-galactose furnished three components (R_G 0·73, 0·85, 0·99), whereas 3,6-di-*O*-methyl-D-glucose gave a single component (R_G 0·87).

Fraction 8. The syrup (120 mg.) was fractionated on charcoal–Celite (1 : 1; 20 × 1·5 cm.) by gradient elution with water containing 0·0—2·0% of butan-2-one, to give (*a*) 2-*O*-methylarabinose (5 mg.), and (*b*) 2,3-di-*O*-methyl-D-galactose (79 mg.), $[\alpha]_D$ +57° (*c* 0·8). The latter sugar was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 130°.

Fraction 9. The sugar was chromatographically indistinguishable from 2,4-di-*O*-methyl-D-galactose, and crystallisation from acetone–water afforded the monohydrate, m. p. and mixed m. p. 88—90°.

Degradation of Periodate-oxidised Degraded Gum B.—Degraded gum B (8 g.) was oxidised with sodium metaperiodate (34 g.) in water (800 ml.) at room temperature for 48 hr. (consumption of reagent was constant). Excess of periodate was destroyed by the addition of ethylene glycol, sodium ions were removed with Amberlite resin IR-120(H), and iodate was precipitated by the addition of barium hydroxide followed by barium carbonate. The filtered solution was concentrated (100 ml.) and potassium borohydride (4 g.) was added during 2 days. Excess of hydride was destroyed and potassium ions were removed by treatment of the solution with Amberlite resin IR-120(H), boric acid was removed as methyl borate by repeated distillation with methanol, and the reduction product was hydrolysed with N-sulphuric acid (80 ml.) at room temperature for 12 hr. The solution was neutralised with barium carbonate, concentrated to a syrup, and poured into ethanol (8 vol.) to give degraded gum C (2 g.), $[\alpha]_D$ +22° (*c* 2·0). Chromatography of degraded gum C in solvent G indicated the presence of tri- and higher oligosaccharides. Concentration of the supernatant liquid gave a syrup (4 g.) which consisted entirely of chromatographically mobile substances and was not examined further.

Partial Hydrolysis of Degraded Gum C.—Degraded gum C (1·4 g.) was hydrolysed with 0·5N-sulphuric acid on a boiling-water bath for 0·5 hr., the solution was neutralised with barium carbonate, and concentration of the filtrate afforded a syrupy mixture (0·85) of sugars which was

adsorbed on charcoal–Celite (1 : 1; 20 × 3 cm.). Elution of the column with water gave monosaccharides (0·78 g.), and gradient elution with water containing 0—30% of ethanol gave three main oligosaccharide-containing fractions.

Fraction 1. The sugar (24 mg.) was chromatographically indistinguishable from 6-*O*-β-D-galactopyranosyl-D-galactose, $R_{galactose}$ 0·3 in solvent A, and was characterised by conversion into the phenylosazone, m. p. and mixed m. p. 178—180°.

Fraction 2. The syrup (15 mg.) contained two disaccharides which were separated by chromatography on a filter sheet using solvent A. The first component (4 mg.) was chromatographically indistinguishable from 3-*O*-β-D-galactopyranosyl-L-arabinose ($R_{galactose}$ 0·61 and 0·45 in solvents A and B). Hydrolysis of the disaccharide gave galactose and arabinose, whereas hydrolysis of the derived glycitol (reduction with potassium borohydride) furnished galactose and arabitol. Methylation of the disaccharide (2 mg.) with methyl sulphate and sodium hydroxide, followed by methanolysis of the methylated derivative, gave a mixture of methyl glycosides which were shown by gas chromatography on columns *a* and *b* to have retention times of methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,4- and, as a minor component, 2,5-di-*O*-methyl-L-arabinose. The second component (4 mg.), $R_{galactose}$ 0·86 and 0·60 in solvents A and B, gave arabinose and mannose on hydrolysis, whereas the derived glycitol (reduction with potassium borohydride) furnished arabinose and mannitol (detected by chromatography in solvent F). The disaccharide (*ca.* 2 mg.) was methylated with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, and methanolysis of the methylated derivative gave, as major components, substances which were shown by gas chromatography on columns *a* and *b* to have the retention times of methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinose and 2,4,6-tri-*O*-methyl-D-mannose.

Fraction 3. The syrup (38 mg.) contained a mixture of sugars, but the sugar of highest mobility was isolated by chromatography on a filter sheet using solvent B and was characterised as 3-*O*-β-D-galactopyranosyl-D-galactose (5 mg.) by crystallisation of the monohydrate, m. p. and mixed m. p. 168°, from ethanol–water.

Methylation of Degraded Gum C.—Degraded gum B (250 mg.) was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, to give methylated degraded gum C (126 mg.). The hydrolysate from a sample of the methylated derivative was examined by chromatography in solvents C, D, and E, and 2,3,4- and 2,3,5-tri-*O*-methylarabinose, tetra- and 2,3,4-tri-*O*-methylgalactose, and tetra-, 3,4,6-tri-, and (?) 4,6-di-*O*-methylmannose were recognised. A further sample of the methylated derivative was methanolysed and the resulting methyl glycosides were examined by gas chromatography on columns *a* and *b*, as shown in Table 2.

TABLE 2

Sugar	Relative proportions	Column *a*		Column *b*	
		S	*M*	*S*	*M*
2,3,4-Me_3arabinose ...	+++	1·04	1·04	0·83	0·82
2,3,5-Me_3arabinose ...	++	0·56, 0·72	0·55, 0·72	0·47, 0·59	0·47, 0·60
2,4-Me_3arabinose	+	2·24, 2·32	2·26, 2·34	1·08, 1·13	1·07, 1·12
2,5-Me_2arabinose	Trace	1·89, 3·47	(1·83) n.d.	0·70, 1·03	0·71, 1·02
Me_4galactose	+++	1·80	1·83	1·52, 1·60	1·52, 1·60
2,3,4-Me_3galactose	++	7·5	7·5	2·62, 2·89	2·62 (2·90)
2,4,6-Me_3galactose	+ +	4·15, 4·70	4·17, 4·76	2·08, 2·38	2·08, 2·38
Me_4mannose	+ + +	1·42	1·43	1·29	1·32
2,4,6-Me_3mannose	+ +	3·86	3·91	2·01	2·01
3,4,6-Me_3mannose	+ + +	3·08	3·14	1·71	1·70
? 4,6-Me_2mannose	++	11·1	11·1	2·90	(2·90)

S = Methyl glycosides from authentic sugars, *M* = methyl glycosides from methanolysis products from methylated degraded gum C. n.d. = not detected. Relative retention times shown in parentheses are those of components which were not resolved.

The authors thank Professor Sir Edmund Hirst, C.B.E., F.R.S., for his interest and advice, the University of Edinburgh for the award of a post-graduate studentship (to V. P. B.), and the Rockefeller Foundation, Norges Teknisk-Naturvitenskapelige Forsknongsråd, and Papirindustriens Forskningsinstitutt (Oslo) for grants.

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