319. *Gum Tragacanth. Part II.*[1] *The Arabinogalactan.*

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Hydrolysis of the methylated derivative of the arabinogalactan from gum tragacanth furnishes 2,3,5-tri-, 2,3-, 2,5-, and 3,5-di-, 2- and 3-*O*-methyl-L-arabinose, L-arabinose, 2,4,6-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-galactose, D-galactose, 4-*O*-methyl-L-rhamnose, 2,3,4-tri- and 2,3-di-*O*-methyl-D-galacturonic acid, and traces of other sugars. Degraded polysaccharides have been prepared by mild acid hydrolysis and by degradation of the periodate-oxidised arabinogalactan, and the cleavage products from their methylated derivatives have been examined by chromatographic techniques.

In Part I [1] it was shown that the arabinogalactan component of gum tragacanth may be isolated conveniently by extraction of the gum with ethanol–water (7 : 3). This polysaccharide preparation was essentially homogeneous when chromatographed on diethylaminoethylcellulose and hydrolysis afforded small proportions of galacturonic acid and rhamnose as constituent sugars in addition to arabinose and galactose. In earlier studies on the polysaccharide James and Smith [2] showed that when gum tragacanth is methylated

[1] Part I, preceding paper.
[2] James and Smith, *J.*, 1945, 749.

the derivative of the arabinogalactan may be separated from that of the major polysaccharide component, tragacanthic acid. The following sugars were recognised as cleavage products from the methylated arabinogalactan, 2,3,5-tri- and 2,3-di-*O*-methyl-L-arabinose, L-arabinose, and an unidentified di-*O*-methyl-D-galactose. These results indicated that the polysaccharide was highly branched, and, although strict evidence of homogeneity was not available, suggested that the structure was based on a core of D-galactose residues to which were attached highly ramified chains of L-arabinofuranose residues. This conclusion is supported by the results of the present investigation in which further structural details have been established.

Methylated arabinogalactan was prepared by etherification of the separated polysaccharide and by methylation of the water-soluble portion of the gum followed by separation of the methylated derivative. The methylated arabinogalactan was hydrolysed and the following sugars were characterised by the formation of crystalline derivatives: 2,3,5-tri-, 2,3-, 2,5- and 3,5-di-, 2- and 3-*O*-methyl-L-arabinose, L-arabinose, 2,4,6-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-galactose, D-galactose, 4-*O*-methyl-L-rhamnose, and 2,3,4-tri- and 2,3-di-*O*-methyl-D-galacturonic acid. In addition, evidence was obtained for the presence in the hydrolysate of an unknown di-*O*-methylgalactose and 3,4-di-*O*-methylrhamnose. These results show that the L-arabinose residues, presumably present in the furanose form only, are involved in almost all the possible combinations of 1,2-, 1,3- and 1,5-linkages, and that the D-galactose residues are present mainly as single or double branching points.

Under controlled conditions partial acid hydrolysis of the arabinogalactan resulted in selective cleavage of arabinose residues, and a degraded galactan was isolated showing that the polysaccharide contains a core of contiguous D-galactose residues. A preliminary examination of this material showed that the degraded polysaccharide was still branched but that 1,6-linkages predominated. Paper chromatography of the products of partial acid hydrolysis indicated that 6-*O*-galactosylgalactose was the disaccharide formed in greatest amount, but that the 1,3-linked isomer was also produced. Gas–liquid chromatography [3,4] of the mixture of methyl glycosides of methylated sugars formed on methanolysis of the methylated degraded galactan indicated the presence of methyl glycosides of 2,3,4-tri- (major component), 2,3,4,6-tetra-, 2,3,6- and 2,4,6-tri-, and 2,4-di-*O*-methyl-galactose.

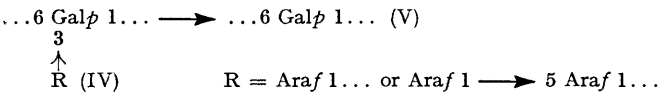
Further information concerning the highly ramified outer chains of L-arabinofuranose residues in the polysaccharide and an indication of the way in which some of these chains are attached to the inner chains of D-galactopyranose residues was obtained by degrading the arabinogalactan in a stepwise manner by removing those sugar residues which were attacked by periodate in the procedure of Smith and his co-workers.[5] The periodate-oxidised arabinogalactan was reduced with potassium borohydride, and controlled hydrolysis with cold dilute acid then afforded degraded arabinogalactan A. Two further degradations furnished successively degraded arabinogalactans B and C. After each degradation the degraded polysaccharide was recovered by precipitation from aqueous solution and products of low molecular weight were isolated from the mother-liquors. Paper-chromatographic examination of the low-molecular-weight products, before and after acid hydrolysis, showed that glycerol was the main component of each of the mixtures and that only small amounts of glycosidically linked components were present. It follows that the degradations resulted mainly in gradual erosion of the outer layers of sugar residues in the polysaccharide. At each stage of the degradation the relative proportions of arabinose to galactose residues in the degraded polysaccharides decreased, showing that arabinose residues were being removed preferentially.

Degraded arabinogalactans A and B were methylated and the relative proportions of

[3] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[4] Aspinall, *J.*, 1963, 1676.
[5] Goldstein, Hay, Lewis, and Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.

the cleavage products from the methylated derivatives were assessed semiquantitatively, together with those from the methylated undegraded polysaccharide; this was done by comparing relative intensities of spots on paper chromatograms given by the sugars formed on hydrolysis and relative peak heights from the gas–liquid chromatography of the methyl glycosides formed on methanolysis. By using these two techniques it was possible to detect all the methyl ethers of arabinose and galactose which had been characterised as cleavage products from the original methylated arabinogalactan. Methylated degraded arabinogalactans A and B furnished qualitatively similar mixtures of sugars and in each case 2,3,5-tri-*O*-methylarabinose was a major cleavage product. These residues, which appeared as end groups in the degraded polysaccharides, arose from interior chains in the parent polysaccharide. Since the first two degradations resulted in the exposure of new arabinofuranose end groups in the degraded polysaccharides it is certain that the majority and probably that all the L-arabinose residues in the polysaccharide are present in the furanose form. New arabinofuranose end groups (III) would be exposed by the degradation of sequences of sugar residues as in (I) or (II) in which the sequence is attached to another sugar residue which is resistant to glycol cleavage.

Changes in the mode of linkage of most of the galactose residues on degradation of the arabinogalactan were not readily apparent, but the results of the above experiments showed that 2,3,4-tri-*O*-methylgalactose could be detected as a cleavage product from each of the methylated degraded polysaccharides but not of the methylated undegraded arabinogalactan. It follows that such 1,6-linked galactopyranose residues (V) arose from residues which carried side-chains necessarily composed of arabinofuranose residues which were cleaved by periodate, *e.g.*, (IV). Since the arabinogalactan contains arabinofuranose residues in the outer chains and galactopyranose residues in the inner chains only, such a degradative sequence (IV → V) indicates the way in which some of the units of the two sugars are joined.



The present results establish the general structural character of the arabinogalactan from gum tragacanth. The polysaccharide is composed of interior chains of D-galactopyranose residues, in which the majority of units are mutually joined by 1,6-linkages, and a smaller proportion by 1,3-linkages. These basal chains carry highly ramified exterior chains of L-arabinofuranose residues which are mutually joined by 1,2-, 1,3-, and 1,5-linkages. The polysaccharide also contains small proportions of D-galacturonic acid and L-rhamnose residues, whose modes of linkage were indicated by methylation. In view of the chromatographic homogeneity of the polysaccharide on DEAE-cellulose it is probable that these sugars are integral, albeit minor, constituents of the arabinogalactan, but nothing is yet known of the way in which units of these sugars are incorporated into the general molecular structure. The polysaccharide is notable in containing a high ratio of arabinose to galactose residues, but these results show that the polysaccharide has many structural features in common with those from other plant gums.[6]

Experimental

The general experimental procedures were as described in Part I.

Preparation and Hydrolysis of Methylated Arabinogalactan.—Arabinogalactan (fraction A14,[1] 5 g.) was methylated with methyl sulphate and sodium hydroxide and partly methylated

[6] Smith and Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold Publ. Corp., New York, 1959.

polysaccharide (4 g.) was isolated by extraction with chloroform (Found: OMe, 34·0%). This product was combined with similar material isolated during the preparation of methylated tragacanthic acid [1] (combined wt., 6 g.), and further methylation with methyl iodide and silver oxide furnished methylated arabinogalactan (4·2 g.), $[\alpha]_D$ −97° (*c* 1·7 in $CHCl_3$) (Found: OMe, 39·5%).

Methylated arabinogalactan (3·1 g.) was suspended in 2N-hydrochloric acid (100 ml.) for two days at room temperature. After addition of methanol (100 ml.) solution was complete and the mixture was heated slowly to 100°, care being taken that no material was precipitated as methanol was removed by distillation. The mixture was diluted with water so that the resulting solution was made N with respect to acid, and the hydrolysis was continued on the boiling-water bath for a further 15 hr. (constant rotation). The cooled solution was neutralised with silver carbonate and filtered, precipitation of silver was completed with hydrogen sulphide, and the hydrolysate was shaken with Amberlite resin IR-45(OH), to remove acidic sugars, and concentrated to a syrup (2·96 g.), which was separated on cellulose (74 × 4 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1), saturated with water, (ii) butan-1-ol, half saturated with water, and (iii) water being used as eluants to give twelve fractions. A further fraction containing acidic sugars was eluted from the ion-exchange resin with N-formic acid.

Fraction 1. The chromatographically pure syrup (961 mg.), R_G 0·95 and $[\alpha]_D$ −40° (*c* 1·2), gave arabinose only on demethylation and was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. 135—136° and mixed m. p. (with sample, m. p. 134—136°) 134—136°.

Fraction 2. Chromatography of the syrup (181 mg.), $[\alpha]_D$ −20° (*c* 0·51), in solvents A, B, and C showed a complex mixture of sugars including 2,3,5-tri-, 2,5/3,5-, and 2,3-di-*O*-methylarabinose, di-*O*-methylrhamnose, and tri-*O*-methylgalactose, and demethylation of the syrup gave arabinose, galactose, and rhamnose. The syrup (170 mg.) was refractionated on cellulose (45 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (4 : 1), saturated with water, as eluant, to give fractions 2*a* (28 mg.), 2*b* (18 mg.), 2*c* (85 mg.), and 2*d* (24 mg.). Fraction 2*a*, R_G 0·95 and $[\alpha]_D$ −39° (*c* 1·4), was chromatographically pure 2,3,5-tri-*O*-methyl-L-arabinose. Chromatography of fraction 2*b*, $[\alpha]_D$ +34° (*c* 0·9) in solvents A, B, and C, and ionophoresis indicated 3,4-di-*O*-methylrhamnose (major component) and a mixture of 2,5- and 3,5-di-*O*-methylarabinose. Gas chromatography of the derived methyl glycosides on column *a* showed components having the retention times of methyl glycosides of 3,4-di-*O*-methylrhamnose (*T* 0·97) and 2,5-di-*O*-methylarabinose (*T* 2·02). Demethylation of fraction 2*b* gave rhamnose and arabinose. Chromatography and ionophoresis of fraction 2*c*, $[\alpha]_D$ −18°, showed 2,5- and 3,5-di-*O*-methylarabinose as major components with traces of 2,3-di-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose. Chromatographically and ionophoretically pure samples of the two main components were obtained after ionophoresis on filter sheets. 2,5-Di-*O*-methyl-L-arabinose (45 mg.) was characterised by conversion into 2,5-di-*O*-methyl-L-arabonolactone, m. p. 59–60° (depressed on admixture with 3,5-di-*O*-methyl-L-arabonolactone) (Smith [7] gives m. p. 60° for 2,5-di-*O*-methyl-L-arabonolactone). 3,5-Di-*O*-methyl-L-arabinose (30 mg.) was characterised by conversion into 3,5-di-*O*-methyl-L-arabonolactone, m. p. and mixed m. p. 76—77°. Chromatography of fraction 2*d*, $[\alpha]_D$ +94° (*c* 1·2), in solvents A, B, and C showed 2,3-di-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose, and demethylation gave arabinose and galactose. Gas chromatography of the derived methyl glycosides on column *c* showed main components having the retention times of methyl glycosides of 2,3-di-*O*-methylarabinose (*T* 0·62, 0·82, 0·93) and 2,4,6-tri-*O*-methylgalactose (*T* 2·09, 2·36).

Fraction 3. Chromatography of the syrup (282 mg.) {$[\alpha]_D$ +92° (*c* 1·0) in solvents A, B, and C} showed 2,3-di-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose, and demethylation gave arabinose and galactose. The syrup (260 mg.) was fractionated on charcoal–Celite by gradient elution with water containing 5—25% of ethanol to give pure samples of both sugars and a small amount of a mixture (24 mg.). 2,3-Di-*O*-methyl-L-arabinose (98 mg.), $[\alpha]_D$ +101° (*c* 0·5), was characterised by conversion into 2,3-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 160—161°. 2,4,6-Tri-*O*-methyl-D-galactose (121 mg.), $[\alpha]_D$ +90° (*c* 1·2), was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 172—173°.

Fraction 4. The syrup (64 mg.), R_G 0·57, 0·54, 0·42 and $[\alpha]_D$ +77° (*c* 0·8), was separated on

[7] Smith, *J.*, 1939, 744.

filter sheets with solvent C into fractions 4*a* (10 mg.; 2,4-di-*O*-methylgalactose), 4*b* (7 mg.; 2-*O*-methylarabinose), and 4*c* (40 mg.). Fraction 4*c*, $[\alpha]_D$ +20° (*c* 0·8), contained two components, R_G 0·54, 0·57, which were resolved by ionophoresis on filter sheets. Both sugars gave positive colour reactions with triphenyltetrazolium hydroxide (unsubstituted at C-2). The first sugar (19 mg.), R_G 0·54 and $[\alpha]_D$ +14° (*c* 0·4), crystallised from acetone–water, had m. p. 130—135°, and gave galactose on demethylation (Found: OMe, 26·0. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). The second sugar, R_G 0·57 and $[\alpha]_D$ +10° (*c* 0·5), crystallised from acetone–water, gave rhamnose on demethylation, and had m. p. 113—115° and mixed m. p. with 4-*O*-methyl-L-rhamnose (m. p. 121—122°) 115—118°.

Fraction 5. Chromatography of the syrup (66 mg.), $[\alpha]_D$ +105° (*c* 1·4), showed 2,3-di-*O*-methylgalactose (R_G 0·46) as the main component with traces of 2,4-di-*O*-methylgalactose (R_G 0·42) and 2-*O*-methylarabinose (R_G 0·38). The main component (40 mg.) was separated from the two minor components (9 mg.) by chromatography on filter sheets in solvent C. The sugar, R_G 0·47 and $[\alpha]_D$ +81° (*c* 0·8), was converted into 2,3-di-*O*-methyl-*N*-phenyl-D-galactosylamine, which was identified by m. p. 137—139° and by *X*-ray powder photograph.

Fraction 6. Chromatography of the syrup (47 mg.), $[\alpha]_D$ +104° (*c* 1·1), showed 2-*O*-methylarabinose (R_G 0·38) with some 2,4-di-*O*-methylgalactose (R_G 0·42). Demethylation gave arabinose and a trace of galactose. The main portion (40 mg.) of the syrup was fractionated on a filter sheet in solvent B, to give 2-*O*-methyl-L-arabinose (34 mg.) which was characterised by conversion into the toluene-*p*-sulphonylhydrazone, m. p. 144—146° and mixed m. p. (with a sample of m. p. 147—148°) 145—148°.

Fraction 7. Chromatography of the syrup (81 mg.), $[\alpha]_D$ +91° (*c* 1·8), showed approximately equal amounts of 2-*O*-methylarabinose and 2,4-di-*O*-methylgalactose. Demethylation gave arabinose and galactose. Chromatography of the periodate oxidation products showed methoxymalondialdehyde (from 2-*O*-methylaldose) and unchanged 2,4-di-*O*-methylgalactose. 2,4-Di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 98—99°, gradually separated from the syrup.

Fraction 8. The sugar (121 mg.), R_G 0·41 and $[\alpha]_D$ +84° (*c* 1·2), which contained a trace of contaminating 2-*O*-methylarabinose (R_G 0·38), was recrystallised from acetone containing 1% of water to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 101—102°, $[\alpha]_D$ +120° ⟶ +85° (*c* 0·7) (aniline derivative, m. p. and mixed m. p. 210°).

Fraction 9. Chromatography of the syrup (40 mg.), $[\alpha]_D$ +83° (*c* 0·8), showed 2,4-di-*O*-methylgalactose (R_G 0·41) and 3-*O*-methylarabinose (R_G 0·32). Demethylation gave galactose and arabinose. Chromatography of the periodate oxidation products showed a main component (R_F 0·72, grey stain with aniline oxalate) and a trace of methoxymalondialdehyde (R_F 0·24) characteristic of the products from a 3-*O*-methylpentose, and unchanged 2,4-di-*O*-methyl galactose.

Fraction 10. The chromatographically pure sugar (288 mg.), R_G 0·32 and $[\alpha]_D$ +113° (*c* 2·2), gave arabinose on demethylation and was characterised as 3-*O*-methyl-L-arabinose by conversion into 3-*O*-methyl-L-arabonolactone, m. p. 76—77° and mixed m. p. (with sample, m. p. 75—77°) 75—76°.

Fraction 11. The sugar (256 mg.), R_G 0·29 and $[\alpha]_D$ +82° (*c* 2·0), which contained a trace of contaminating arabinose (R_G 0·21), recrystallised from ethanol–water to give 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 145—146°, $[\alpha]_D$ +55° ⟶ +84° (*c* 1·9).

Fraction 12. Chromatography of the syrup (506 mg.), $[\alpha]_D$ +82° (*c* 2·1), showed arabinose (R_G 0·21), galactose (R_G 0·14), and methylated uronic acids (R_G 0·31 and 0·07). The syrup was separated on filter sheets in solvent E into fractions 12*a* (240 mg.), 12*b* (25 mg.), 12*c* (17 mg.), and 12*d* (52 mg.). Fraction 12*a* was L-arabinose, m. p. and mixed m. p. 158—159°, $[\alpha]_D$ +160° ⟶ +104° (*c* 1·4) (toluene-*p*-sulphonylhydrazone, m. p. and mixed m. p. 154—155°). Fraction 12*b* was D-galactose, m. p. and mixed m. p. 166—167°, $[\alpha]_D$ +140° ⟶ +80° (*c* 1·2) (mucic acid, m. p. and mixed m. p. 210—211°). Fractions 12*c* and 12*d* were combined with fractions 13*a* and 13*b* for further examination.

Fraction 13. The syrup (101 mg.; eluted from Amberlite resin IR-45) contained methylated uronic acids (R_G 0·31 and 0·07) and neutral sugars which had been retained by the resin. Neutral sugars were separated from the barium salts of the acids by extraction with boiling acetone. The acids were released after removal of barium ions on Amberlite resin IR-120(H), and fractionation on filter sheets in solvent E gave fractions 13*a* (13 mg.) and 13*b* (28 mg.). The combined fractions 12*c* and 13*a* were converted into methyl ester methyl glycosides,

reduced with lithium aluminium hydride in tetrahydrofuran, and hydrolysed to furnish a syrup (10 mg.), $[\alpha]_D$ +115° (*c* 1·0), R_G 0·72, which crystallised from ethanol to give 2,3,4-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 80—81°, $[\alpha]_D$ +147 ⟶ +120° (*c* 1·1). The combined fractions 12*d* and 13*b* similarly afforded 2,3-di-*O*-methyl-D-galactose (41 mg.), R_G 0·49, $[\alpha]_D$ +85° (*c* 0·9), which was characterised as the aniline derivative, identified by m. p. 133—135° and *X*-ray powder photograph.

Partial Acid Hydrolysis of Arabinogalactan (with R. N. FRASER and R. STIRLING).—Arabinogalactan (0·25 g.) was heated in 0·1N-hydrochloric acid (25 ml.) on the boiling-water bath. Samples were withdrawn at intervals, degraded polysaccharide was precipitated by the addition of ethanol (4 vol.), and the supernatant liquid was neutralised with Amberlite resin IR-45(OH) and concentrated. The soluble sugars and the hydrolysate from the degraded polysaccharide were each examined chromatographically. The results showed that arabinose was rapidly released together with small amounts of galactose and traces of rhamnose. After 22 hr. the degraded polysaccharide gave mainly galactose and only traces of arabinose on hydrolysis. Under the same conditions arabinogalactan (7 g.) furnished degraded galactan (1·1 g.). Degraded galactan (1·0 g.) was methylated with methyl sulphate and sodium hydroxide, to give partially methylated galactan (0·55 g.), which was further methylated with methyl iodide and silver oxide to give methylated galactan (*ca.* 200 mg.), $[\alpha]_D$ +26° (*c* 0·47 in $CHCl_3$) (Found: OMe, 45·5%). Chromatography of the hydrolysate from the methylated galactan in solvent A indicated tetra- (medium), tri- (strong), and di-*O*-methylgalactose (weak). Methylated galactan was heated with methanolic 4% hydrogen chloride in a sealed tube for 18 hr., the cooled solution was neutralised with silver carbonate, filtered, and concentrated, and the resulting syrup was examined by gas chromatography on columns *b* and *c*. Table 1 shows the relative retention times (*T*) of methyl glycosides of methylated sugars which were indicated.

TABLE 1.

Examination of methanolysis products from methylated degraded galactan by gas chromatography.

Sugar	Approx. relative propns.	Relative retention times (*T*) of methyl glycosides: Column *b*	Column *c*
2,3,4,6-Tetra-*O*-methylgalactose	++	1·80	1·53, (1·61)
2,3,4-Tri-*O*-methylgalactose	+++	7·5	2·62, 2·89
2,3,6-Tri-*O*-methylgalactose	+	3·25, 3·93 (4·30) (4·75)	(1·60) (2·11) 2·22, (2·48)
2,4,6-Tri-*O*-methylgalactose	++	4·19, (4·75)	(2·11), 2·38
2,4-Di-*O*-methylgalactose	+		3·74, 4·44

Arabinogalactan (0·1 g.) was heated in 0·5N-sulphuric acid (10 ml.) on the boiling-water bath for 1 hr. The cooled solution was neutralised with barium carbonate, filtered, and concentrated. The resulting syrup was eluted from charcoal–Celite (1 : 1; 10 g.) with water (500 ml.) and with water containing 10% of ethanol (250 ml.). Chromatography of the latter eluant showed disaccharides with the mobilities of 6- (major product) and 3-*O*-β-D-galactopyranosyl-D-galactose, and a third disaccharide (possibly a 1,4-linked galactobiose in trace amounts).

Degraded Arabinogalactans A, B, and C.—Arabinogalactan (17 g.) was oxidised with 0·1M-sodium metaperiodate (2·445 l.) for 30 hr. (uptake of reagent was constant and corresponded to the consumption of 0·58 mole of reagent with the release of 0·04 mole of titratible acid per sugar residue), and the excess of reagent was destroyed by addition of ethylene glycol (17 g.). The solution was passed through Amberlite resin IR-120(H) to remove sodium ions, and iodic acid was neutralised with barium hydroxide. The filtered solution was treated with potassium borohydride (14 g.) for 24 hr., the excess of which was then destroyed. Potassium ions were removed by Amberlite resin IR-120(H), and the solution was concentrated, methanol being added to facilitate removal of boric acid as methyl borate. The concentrated solution was made N with respect to sulphuric acid and left at room temperature for 3 hr. The resulting solution was neutralised with barium hydroxide, barium sulphate was removed at the centrifuge, and degraded arabinogalactan A (7 g.), $[\alpha]_D$ −21° (*c* 1·3), was precipitated by the addition of ethanol (4 vol.). Hydrolysis of degraded arabinogalactan A gave arabinose and galactose in the approximate proportions of 2·5 : 1, and traces of rhamnose. Concentration of the mother-liquor from the precipitation gave a syrup which was shown by chromatography to contain

glycerol as the main component together with two non-reducing substances, $R_{arabinose}$ 1·10 and 0·95 (traces only). These minor components were probably glycerol glycosides of arabinose and galactose since hydrolysis of the syrup gave arabinose and a trace of galactose.

Degraded arabinogalactan A (3·5 g.) was similarly degraded by oxidation with sodium metaperiodate (0·52 mole of reagent was consumed with the release of 0·06 mole of titratable acid per sugar residue), reduction with potassium borohydride, and hydrolysis with cold N-sulphuric acid, to furnish degraded arabinogalactan B (1·5 g.), $[\alpha]_D$ +15° (*c* 0·9), hydrolysis of which gave arabinose and galactose in the approximate proportions of 1·5 : 1, and a trace of rhamnose. In a further degradation degraded arabinogalactan B (0·75 g.) (0·61 mole of periodate were consumed per sugar residue) afforded degraded arabinogalactan C (0·35 g.) $[\alpha]_D$ +48° (*c* 2·1), hydrolysis of which gave arabinose and galactose in approximately equimolecular proportions.

Analysis of Cleavage Products from Methylated Arabinogalactan and Methylated Arabinogalactans A and B.—Methylated arabinogalactan was heated with methanolic 4% hydrogen chloride in a sealed tube for 18 hr., the cooled solution was neutralised with silver carbonate, filtered, and concentrated, and the resulting syrup was examined by gas chromatography on columns *b* and *c*. Table 2 shows the relative retention times (*T*) of methyl glycosides of methylated sugars which had been characterised previously by the formation of crystalline derivatives.

TABLE 2.

Examination of methanolysis products from methylated arabinogalactan by gas chromatography.

Sugar	Relative retention times (*T*) of methyl glycosides: Column *b*	Column *c*
2,3,5-Tri-*O*-methylarabinose	0·56, 0·72	0·46, (0·59)
2,3-Di-*O*-methylarabinose	1·56, 1·78 (1·89)	0·64, (0·84) 0·96
2,5-Di-*O*-methylarabinose	1·89, 3·48	0·69, 1·02
3,5-Di-*O*-methylarabinose	1·06, 2·54	(0·59) (0·84)
2-*O*-Methylarabinose	6·10	(1·04) (1·47)
3-*O*-Methylarabinose	4·34, 6·96	1·27, (1·47), 1·59
2,4,6-Tri-*O*-methylgalactose	4·15, 4·74	2·08, 2·35
2,3-Di-*O*-methylgalactose		2·47, 3·15 (3·71) (4·20)
2,4-Di-*O*-methylgalactose		(3·71) 4·41
2,3-Di-*O*-methylgalacturonic acid *	5·30	2·19

* Present as methyl ester. Figures in parentheses indicate *T* values of components which were incompletely resolved.

TABLE 3.

Examination of cleavage products from methylated arabinogalactans.

Sugar	Methylated arabinogalactan	Methylated degraded arabinogalactan A	Methylated degraded arabinogalactan B
2,3,5-Tri-*O*-methylarabinose	++++	+++	+++
2,3-Di-*O*-methylarabinose	++	++	++
2,5-Di-*O*-methylarabinose	+	+	+
3,5-Di-*O*-methylarabinose	+	+	+
2-*O*-Methylarabinose	++	+	tr.
3-*O*-Methylarabinose	+++	+	+
arabinose	+++	+	tr.
2,4,6-Tri-*O*-methylgalactose	++	++	++
2,3,4-Tri-*O*-methylgalactose		++	++
2,4-Di-*O*-methylgalactose	++	++	++
2-*O*-Methylgalactose	++	++	++
galactose	Trace	Trace	Trace

Methylated degraded arabinogalactans A (Found: OMe, 40·3%) and B (Found: OMe, 41·0%) were prepared by treatment of the parent polysaccharides (*ca.* 0·5 g.) with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide.

The cleavage products from methylated degraded arabinogalactans were similarly examined by (*a*) paper chromatography of the hydrolysates in solvents A, B, C, and D, and (*b*) gas

chromatography of the methyl glycosides formed on methanolysis on columns *b* and *c*. Table 3 indicates the relative proportions of the cleavage products from the various methylated arabinogalactans.

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