317. *The Location of* L-*Rhamnopyranose Residues in Gum Arabic.*

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D-Glucuronic acid residues in gum arabic are reduced to D-glucose residues by treatment of the acetylated gum with diborane. Acetolysis of the reduced polysaccharide, followed by deacetylation, furnishes a mixture of oligosaccharides amongst which the disaccharide, 4-*O*-α-L-rhamnopyranosyl-D-glucose, has been characterised. These experiments establish the location of some of the L-rhamnopyranose residues in the gum.

L-RHAMNOPYRANOSE residues in *Acacia senegal* gum (gum arabic) are known to occur mainly, if not exclusively, as non-reducing end groups.[1] Since L-rhamnose and D-glucuronic acid residues are present in the approximate ratio of 1 : 1 in gum arabic, and also in *Acacia mollissima*[2] and *Acacia cyanophylla*[3] gums, Charlson, Nunn, and Stephen[3] suggested that this might indicate that L-rhamnose is glycosidically linked to D-glucuronic acid residues in these three gums. A similar suggestion has been made by Smith and

[1] Smith, *J.*, 1940, 1035.
[2] Stephen, *J.*, 1951, 646.
[3] Charlson, Nunn, and Stephen, *J.*, 1955, 269.

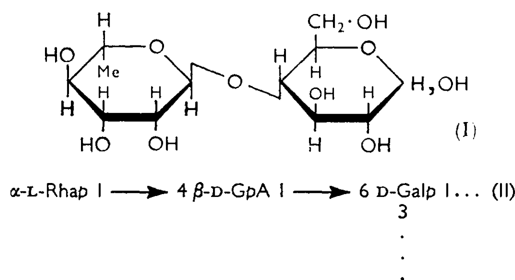
Montgomery.[4] Hamilton and Thompson [5] have shown that alkaline hydrolysis of gum arabic at 160° leads to the loss of both glucuronic acid and rhamnose residues with the formation of an arabinogalactan. Since this type of reaction appears to result in the selective cleavage of glycosiduronic acid linkages, sugar units attached to glucuronic acid residues would also be lost. Although other interpretations are possible, this observation seems to support the contention that L-rhamnose is attached to D-glucuronic acid residues in gum arabic. Very recently, O'Colla and his collaborators [6] have shown that degradation of gum arabic with Fenton's reagent yields 10% of dialysable material which includes rhamnose, arabinose, galactose, and a disaccharide. The mechanism of this degradation is not yet understood, but since glycosiduronic acid linkages are apparently cleaved an association of rhamnose and other sugar units with glucuronic acid residues is indicated. We now report definite evidence for such a rhamnose–glucuronic acid linkage.

In this study *Acacia senegal* gum, obtained through the courtesy of Mr. Videl-Hall of the Forest Department of the Sudan Government, has been used. In order to show that this sample of gum arabic contained similar structural features to those of the sample used by Smith in his investigations,[1] the gum was methylated and the methanolysis products from the methylated gum were examined by gas–liquid partition chromatography.[7,8] This highly selective analytical technique showed the presence therein of components having the same retention times as those of the methyl glycosides of all the major cleavage products which had been characterised by Smith,[1] namely, 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,5-tri- and 2,5-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra- and 2,4-di-*O*-methyl-D-galactose, and 2,3,4-tri- and 2,3-di-*O*-methyl-D-glucuronic acid; smaller relative amounts of methyl glycosides of 2,3,4-tri-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose were also indicated. Further indications that the D-glucuronic acid residues in the gum were 4-*O*-substituted, and, to a smaller extent, present as end groups, were obtained by gas-chromatographic examination of the methanolysis products of the methylated gum after reduction of the glucuronic acid to glucose residues with lithium aluminium hydride, and also after subsequent remethylation. In these methanolysis products the components having the retention times of methyl glycosides of 2,3-di- and 2,3,4-tri-*O*-methyl-D-glucuronic acid were replaced by components having the retention times of methyl glycosides of 2,3-di- and 2,3,4-tri-*O*-methyl-D-glucose and of 2,3,6-tri- and 2,3,4,6-tetra-*O*-methyl-D-glucose, respectively.

The acetylated gum was then reduced by using the diborane procedure of Smith and Stephen; [9] examination of the reduced polysaccharide, which was isolated in high yield after deacetylation of the reaction product, showed that *ca.* 82% of the carboxyl groups had been reduced. Partial hydrolysis of the reduced gum was effected by acetolysis with acetic anhydride–sulphuric acid [10] for 24 hours at room temperature. Paper-chromatography of the sugars formed on deacetylation of the acetolysis products showed the presence of monosaccharides and at least eight oligosaccharides. Column chromatography on cellulose and charcoal–Celite led to the isolation of a syrupy disaccharide which was characterised as its crystalline phenylosazone. The structure of the disaccharide as 4-*O*-α-L-rhamnopyranosyl-D-glucopyranose (I) follows from the following considerations. Glucose and rhamnose were detected on paper chromatograms after hydrolysis of the disaccharide, but only rhamnose was detected on hydrolysis of the derived phenylosazone. The nature of the reducing portion of the disaccharide was established by the isolation of D-glucitol on hydrolysis of the glycitol formed on reduction of the disaccharide with sodium

[4] Smith and Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold Publ. Corp., New York, 1959.
[5] Hamilton and Thompson, *Pulp and Paper Mag. Canada*, 1960, **61**, No. 4, 263.
[6] O'Colla, O'Donnell, and Feeley, *Proc. Chem. Soc.*, 1962, 68.
[7] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[8] Aspinall, *J.*, 1963, 1676.
[9] Smith and Stephen, *Tetrahedron Letters*, 1960, No. 7, 17.
[10] Smith and Srivastava, *J. Amer. Chem. Soc.*, 1956, **78**, 1406.

borohydride. Oxidation of the disaccharide with an excess of lead tetra-acetate in acetic acid containing 2% of water for 2 hours at room temperature [11] followed by hydrolysis of the product gave rhamnose and erythrose. These experiments show that the disaccharide is a rhamnosylglucoside and that 1 ⟶ 2 and 1 ⟶ 3 linkages are absent. Oxidation of the disaccharide with periodate gave 0·55 mol. of formaldehyde, which could only have arisen from the glucose portion, thus indicating the absence of 1 ⟶ 5 and 1 ⟶ 6 linkages. Positive evidence in favour of a 1 ⟶ 4 linkage was obtained by examining the cleavage products from the methylated disaccharide. The methanolysis products from the methylated disaccharide were examined by gas–liquid chromatography,[7,8] and the presence was indicated of methyl glycosides of 2,3,4-tri-*O*-methylrhamnose and 2,3,6-tri-*O*-methylglucose. Hydrolysis of the methylated disaccharide gave 2,3,4-tri-*O*-methyl-L-rhamnose and 2,3,6-tri-*O*-methyl-D-glucose, both sugars being characterised by the formation of crystalline derivatives. The configuration of the glycosidic linkage is indicated by the similarity of the optical rotation ($[\alpha]_D$ −6° in water) to that ($[\alpha]_D$ −0·1°) of the isomeric disaccharide, 6-*O*-α-L-rhamnopyranosyl-D-glucose, for whose configuration independent evidence has been obtained by Gorin and Perlin.[12]



The characterisation of 4-*O*-α-L-rhamnopyranosyl-D-glucopyranose (I) as a partial hydrolysis product of reduced gum arabic provides clear evidence that some of the L-rhamnopyranose residues in the gum are glycosidically linked to C-4 of D-glucuronic acid residues. Since earlier work [1] on gum arabic showed that L-rhamnopyranose residues were present as terminal groups, that the majority of D-glucuronic acid residues carried substituents at position 4, and that the majority of D-galactopyranose residues were 3,6-di-*O*-substituted, and since the aldobiouronic acid, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, is formed on graded hydrolysis of the gum, it may be concluded that the structural unit (II) is an important fragment of the gum molecule.

In addition to isolating the rhamnose-containing disaccharide (I), we have obtained evidence that higher oligosaccharides containing rhamnose residues are produced in the acetolysis. One of these appeared to be an acidic trisaccharide, resulting from incomplete reduction of hexuronic acid units, which gave, on graded hydrolysis, sugars with the chromatographic mobilities of L-rhamnose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. It was shown subsequently (unpublished results) that this substance is more readily formed from the unmodified gum. It is noteworthy that several oligosaccharides containing terminal 6-deoxyhexose residues have been isolated by using acetolysis for the cleavage of glycosidic linkages. Kuhn and his collaborators [13] have isolated 2-*O*-α-L-rhamnopyranosyl-D-galactose and 2-*O*-α-L-rhamnopyranosyl-(3-*O*-β-D-galactopyranosyl-D-glucose) on acetolysis of the steroidal glycoside solanin. In this laboratory 2-*O*-α-L-fucopyranosyl-D-xylose has been recently isolated from gum tragacanth

[11] Charlson and Perlin, *Canad. J. Chem.*, 1956, **34**, 1200.
[12] Gorin and Perlin, *Canad. J. Chem.*, 1959, **37**, 1930.
[13] Kuhn, Löw, and Trischmann, *Chem. Ber.*, 1955, **88**, 1492.

by a similar procedure.[14] It is clear, therefore, that acetolysis provides a valuable alternative method for the linkage analysis of polysaccharides containing 6-deoxyhexose residues which are relatively labile towards hydrolysis with aqueous mineral acid.

EXPERIMENTAL

Paper chromatography was carried out on Whatman Nos. 1 and 3MM papers with the following solvent system (v/v): (A) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (B) butan-1-ol–acetic acid–water (4 : 1 : 5, upper layer); (C) benzene–ethanol–water (169 : 47 : 15, upper layer); (D) butan-2-one, half saturated with water; (E) ethyl acetate–pyridine–water (10 : 4 : 3); ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4).

Gas–liquid partition chromatography of the methyl glycosides of methylated sugars was carried out in a Pye argon chromatograph according to the procedure of Bishop and Cooper [7] (see also accompanying paper [8]). Separations were carried out on the following columns (120 × 0·5 cm.) at gas flow rates of 80—100 ml./min.: (*a*) 15% by weight of butan-1,4-diol succinate polyester [7] on acid-washed Celite at 175°; (*b*) 10% by weight of polyphenyl ether [*m*-di-(*m*-phenoxyphenoxy)benzene] on acid-washed Celite at 200°; (*c*) 20% by weight of Apiezon M on acid-washed Celite at 150°. Retention times (T) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Optical rotations were observed at *ca.* 18°.

Methylated Gum Arabic and Derivatives.—Gum arabic (20 g.) was methylated successively with methyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide, to give methylated gum arabic (11·2 g.), $[\alpha]_D$ −48° (*c* 1·0 in $CHCl_3$) (Found: OMe, 41·5%). Lithium aluminium hydride (150 mg.) in tetrahydrofuran (5 ml.) was added to methylated gum arabic (150 mg.) in tetrahydrofuran (5 ml.), and after 0·5 hr. at room temperature the mixture was refluxed for 3 hr. The excess of hydride was destroyed with ethyl acetate and water, and the resulting mixture was shaken with dilute sulphuric acid and extracted with chloroform. The chloroform extract afforded reduced methylated gum arabic (105 mg.), $[\alpha]_D$ −43·5° (*c* 1·06 in $CHCl_3$) (Found: OMe, 39·6%). Hydrolysis of a sample of the reduced methylated gum followed by chromatography of the products in solvents A and B showed that reduction of hexuronic acid residues was complete. Reduced methylated gum (90 mg.) was methylated in *NN*-dimethylformamide with methyl iodide and silver oxide, to give methylated reduced gum arabic (78 mg.), $[\alpha]_D$ −47·5° (*c* 1·02 in $CHCl_3$) (Found: OMe, 41·3%).

Samples of the methylated gum, reduced methylated gum, and methylated reduced gum were heated with methanolic 2·5% hydrogen chloride in sealed tubes at 100° for 16 hr., the cooled solutions were neutralised with silver carbonate, filtered, and concentrated, and the resulting syrups were examined by gas-chromatography, with results shown in the Tables.

Carboxyl-reduced Arabic Acid.—Arabic acid (equivalent weight, 1380) was prepared from *Acacia senegal* gum by precipitation from aqueous solution which had been acidified with hydrochloric acid by the addition of ethanol (4—5 vol.), and the gum acid was acetylated in formamide solution with acetic anhydride and pyridine by Carson and Maclay's method [15] to give acetylated arabic acid, $[\alpha]_D$ −21° (*c* 0·94 in $CHCl_3$). Acetylated arabic acid (30 g.) was dissolved in 1,2-dimethoxyethane (400 ml.), lithium borohydride (6·5 g.) was dissolved in the solution, and diborane was generated *in situ* by the addition of portions (*ca.* 5 ml.) of a solution of boron trifluoride–ether complex (10 g.) in 1,2-dimethoxyethane (40 ml.) during 1·5 hr. After each addition the stoppered flask was shaken gently and then vigorously to break up the gel which separated, and further solvent (200 ml.) was added during the additions when the mixture became too thick. The mixture was set aside overnight and was then poured into ice–water (1·5 l.). The mixture was made just alkaline and was concentrated under reduced pressure to a thick paste. The paste was dissolved in 0·1N-sodium hydroxide, and the solution was adjusted to pH 9 and heated for 2 hr. at 55°. The resulting solution was dialysed against tap water for 48 hr. and against distilled water for 24 hr., filtered, concentrated, and poured into a mixture of ethanol (750 ml.) and ether (250 ml.). The gummy precipitate was triturated with ethanol and dried to give a residue (20 g.). The crude polysaccharide was dissolved in water (200 ml.), and the solution was deionised by passage through columns of Amberlite resins

[14] Aspinall and Baillie, *J.*, 1963, 1702.
[15] Carson and Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.

TABLE 1.

Relative retention times (T) of methyl glycosides on column (*a*).

Sugar	Methylated gum arabic	Reduced methylated gum arabic	Methylated reduced gum arabic
2,3,4-Tri-*O*-methylrhamnose	0·44	0·44	0·45
2,3,5-Tri-*O*-methylarabinose	0·55, 0·72	0·54, 0·71	0·55, 0·71
2,3,4-Tri-*O*-methylarabinose	1·04	1·04	(1·02)
2,5-Di-*O*-methylarabinose	(1·84), 3·47	(1·86), 3·41	(1·87) (3·50)
2,3,4,6-Tetra-*O*-methylgalactose	(1·84)	(1·86)	(1·87)
2,4,6-Tri-*O*-methylgalactose	4·19, 4·80	4·15, 4·70	4·17, (4·82)
2,3,4-Tri-*O*-methylglucuronic acid *	2·52, 3·28		
2,3-Di-*O*-methylglucuronic acid *	8·4, 9·3		
2,3,4-Tri-*O*-methylglucose		2·54, 3·70	
2,3,4,6-Tetra-*O*-methylglucose			(1·02) (1·49)
2,3,6-Tri-*O*-methylglucose			3·50, 4·82
Unknown sugar	1·51	1·49	(1·49)

* Methyl glycosides present as methyl esters.

Figures in parentheses indicate T values of components which were incompletely resolved.

TABLE 2.

Relative retention times (T) of methyl glycosides on column (*b*).

Sugar	Methylated gum arabic	Reduced methylated gum arabic	Methylated reduced gum arabic
2,3,4-Tri-*O*-methylrhamnose	(0·46)	(0·46)	(0·46)
2,3,5-Tri-*O*-methylarabinose	(0·46), 0·59	(0·46), 0·59	(0·46), 0·59
2,3,4-Tri-*O*-methylarabinose	0·84	0·83	0·82
2,5-Di-*O*-methylarabinose	0·69, 1·06	0·70, 1·05	0·69, (1·06)
2,3,4,6-Tetra-*O*-methylgalactose	1·52, 1·61	1·52, 1·61	1·52, 1·61
2,4,6-Tri-*O*-methylgalactose	2·09, (2·41)	2·10, (2·46)	(2·21), 2·39
2,4-Di-*O*-methylgalactose	3·71, 4·40	3·68, 4·40	3·69, 4·40
2,3,4-Tri-*O*-methylglucuronic acid *	1·78, 2·24		
2,3-Di-*O*-methylglucuronic acid *	(2·41), 3·11		
2,3,4-Tri-*O*-methylglucose		1·36, 1·86	
2,3-Di-*O*-methylglucose		(2·46), 3·28	
2,3,4,6-Tetra-*O*-methylglucose			(1·02), 1·34
2,3,6-Tri-*O*-methylglucose			1·72, 2·21

* Methyl glycosides present as methyl esters.

Figures in parentheses indicate T values of components which were incompletely resolved.

IR-120(H) and IR-45(OH), concentrated to 100 ml., and freeze-dried to give the reduced polysaccharide (15 g.), which had $[\alpha]_D$ −16° (*c* 0·94 in H_2O) and equivalent weight 7800. Hydrolysis of the reduced polysaccharide followed by paper chromatography of the products showed rhamnose, arabinose, galactose, and glucose with only traces of aldobiouronic acid (6-*O*-β-D-glucuronosyl-D-galactose).

Acetolysis of Carboxyl-reduced Arabic Acid.—The reduced polysaccharide (14 g.) was added with stirring to acetic anhydride (250 ml.) and concentrated sulphuric acid (7·5 ml.) at 3°, stirring was continued for 0·5 hr. at 3° and at room temperature for 6 hr., and the mixture was kept overnight. The resulting dark solution was poured into ice–water (500 ml.), and after being stirred for 15 min. the mixture was extracted with chloroform (4 × 225 ml.). The chloroform extract was washed with water and sodium hydrogen carbonate solution, dried, and concentrated to a syrup (23·9 g.). The syrup was treated with methanolic 5% sodium methoxide (50 ml.) for 18 hr. at 3°, and the resulting mixture was neutralised with acetic acid and concentrated to a syrup, which was dissolved in water, deionised by passage through cation- and anion-exchangers and concentrated to a syrup (10 g.). A further quantity (9 g.) of the mixture of sugars was similarly formed from the reduced polysaccharide (10 g.).

The combined syrups (19 g.) were fractionated on cellulose (35 × 6·8 cm.) by elution with benzene–ethanol (5 : 1 in steps to 1 : 5) containing small amounts of water (0·5 to 5%), and later with ethanol–water (4 : 1) and with water. The earlier fractions contained monosaccharides (rhamnose, arabinose, galactose, and glucose) and substances of higher chromatographic mobility (probably partially acetylated sugars). Later, four fractions containing oligosaccharides were collected.

Fractionation of Oligosaccharides.—Fraction 1. Chromatography of the syrup (0·7 g.; eluted with 1 : 1 benzene–ethanol containing 1% and 2% of water) indicated galactose, oligosaccharide 1 ($R_{galactose}$ 0·69 and 0·75 in solvents A and F), and traces of arabinose and galactosylarabinose. Galactose (70 mg.) separated from the syrup and the remaining syrup was fractionated on charcoal–Celite (1 : 1; 29 × 3·8 cm.). Elution with water afforded galactose (150 mg.), elution with water containing 2% of ethanol (3 × 500 ml.) gave fractions 1*a* to 1*c*, and elution with water containing 4% of ethanol gave fraction 1*d*. Although no sugars could be detected in fractions 1*a* and 1*d*, these fractions were concentrated and combined with fraction 2 for re-fractionation. Fraction 1*b* was concentrated, dissolved in methanol, filtered, and taken to dryness to give oligosaccharide 1 (110 mg.), $[\alpha]_D$ −6° (*c* 0·97 in H_2O), which was substantially pure except for a trace of galactosylarabinose. Concentration of fraction 1*c* gave further oligosaccharide 1 (150 mg.) probably contaminated with Celite.

Fraction 2. Chromatography of the syrup [1·62 g.; eluted with benzene–ethanol (1 : 2, later 1 : 5, containing 3% and 5% of water, respectively)] indicated galactose, arabinose, and oligosaccharides 1 and 2. Elution of the syrup from charcoal–Celite (1 : 1; 33 × 3·8 cm.) with water (1 l.) afforded monosaccharides, but further elution with water (6·5 l.), and with water (2 l.) containing 3% of ethanol, gave an unresolved mixture (1·15 g.) of oligosaccharides 1 and 2. The oligosaccharides were fractionated on cellulose (38 × 2·3 cm.), elution with butan-1-ol, half saturated with water, furnishing oligosaccharide 1 (fraction 2*a*; 0·31 g.) containing only a trace of galactosylarabinose, and elution with methanol–water (9 : 1) furnished oligosaccharide 2 (fraction 2*b*; 0·57 g.), which was chromatographically indistinguishable from 3-*O*-α-D-galactopyranosyl-L-arabinose, $R_{galactose}$ 0·50 and 0·72 in solvents A and E.

Fraction 3. Chromatography of the syrup [(1·16 g.; eluted with benzene–ethanol–water (1 : 10 : 1) and ethanol–water (4 : 1)] indicated oligosaccharides 2 and 3 ($R_{galactose}$ 0·2, 0·50, and 0·29 in solvents A, E, and F) and traces of galactose and arabinose. Oligosaccharide 3 was probably a galactobiose since a sub-fraction from chromatography on charcoal–Celite, which was rich in this component, gave mainly galactose on hydrolysis.

Fraction 4. Chromatography of the syrup (6·63 g.; eluted with water) indicated a complex mixture of higher oligosaccharides. The syrup was chromatographed on charcoal–Celite (35 × 6·8 cm.), elution with water removing traces of monosaccharides and elution with water containing 2, 5, 10, and 20% of ethanol furnishing fractions 4*a* (0·2 g.), 4*b* (0·7 g.), 4*c* (0·62 g.), and 4*d* (0·91 g.). Fraction 4*a*, $R_{galactose}$ 0·17 and 0·0 in solvents F and E, was probably an acidic trisaccharide which gave rhamnose, an aldobiouronic acid (probably 6-*O*-β-D-glucuronosyl-D-galactose), and a trace of galactose on hydrolysis. Fractions 4*c* and 4*d* contained higher rhamnose-containing oligosaccharides which gave rhamnose, glucose, and galactose amongst the hydrolysis products.

Examination of oligosaccharide 1. Oligosaccharide 1 had $R_{galactose}$ 0·69 and 0·75 in solvents A and F, but cochromatographed with glucose in solvent E and afforded rhamnose and glucose on hydrolysis. The sugar (fraction 2*a*; 0·2 g.) was reduced with sodium borohydride (0·1 g.) in water (10 ml.) for 18 hr. The solution was acidified with acetic acid, shaken with Amberlite resin IR-120(H), filtered, concentrated, and taken to dryness with methanol to remove boric acid. The product was chromatographically homogeneous, but treatment of a portion with acetic anhydride and pyridine failed to yield a crystalline acetyl derivative. The remainder of the glycitol was hydrolysed with 0·5N-sulphuric acid at 100° for 5 hr., and chromatography of the hydrolysate indicated rhamnose and glucitol. The hydrolysate was heated with aqueous barium hydroxide at 100° for 3 hr. to destroy reducing sugar, and the solution was acidified with dilute sulphuric acid, filtered, neutralised with Amberlite resin IR-45 (OH), and taken to dryness. The residue was treated with acetic anhydride and pyridine, and the product after recrystallisation from ethanol furnished D-glucitol hexa-acetate, $[\alpha]_D$ +9° (*c* 2·4 in Me_2CO), which was identified by m. p. and mixed m. p. 100° and by its infrared spectrum.

The sugar (fraction 1*b*; 95 mg.) was heated with phenylhydrazine hydrochloride (0·2 g.) and sodium acetate (0·3 g.) in water (2 ml.) at 100° for 2·5 hr. and furnished a phenylosazone which had m. p. 165—167° after recrystallisation from ethanol–water (Found: C, 55·3; H, 6·4; N, 10·8; and after drying at 100°: C, 57·1; H, 6·9. $C_{24}H_{32}N_4O_8,H_2O$ requires C, 55·2; H, 6·6; N, 10·7. $C_{24}H_{32}N_4O_8$ requires C, 57·2; H, 6·4%). Hydrolysis of the phenylosazone gave rhamnose as the sole reducing sugar.

Lead tetra-acetate (15 mg.) in acetic acid (1 ml.) was added to the sugar (fraction 1*b*; 5 mg.) in acetic acid (3 ml.) and water (0·08 ml.), and the solution was kept at room temperature for

2·5 hr. The excess of lead tetra-acetate was destroyed and lead was precipitated by the drop-wise addition of 10% oxalic acid in acetic acid. The filtered solution was concentrated, the residue was hydrolysed and chromatography of the hydrolysate showed rhamnose and erythrose. Periodate oxidation of the sugar (fraction 1*b*; 10 mg.) in sodium hydrogen carbonate buffer [16] afforded formaldehyde, identified as the dimedone derivative, m. p. 190°.

The sugar (fraction 1*c*; 130 mg.) was methylated successively with methyl sulphate and sodium hydroxide, methyl iodide and silver oxide, and methyl iodide and silver oxide in *NN*-dimethylformamide, to give methylated disaccharide (128 mg.), $[\alpha]_D$ −23° (*c* 1·33 in $CHCl_3$). Essentially complete etherification was indicated by (*a*) paper chromatography of the hydrolysate, which showed two main components, and (*b*) gas-chromatography of the methanolysate on columns *a* and *c*, which showed the presence of major components having the retention times of methyl glycosides of 2,3,4-tri-*O*-methyl-L-rhamnose (*T* 0·46 and 0·46 on columns *a* and *c*) and 2,3,6-tri-*O*-methyl-D-glucose (*T* 3·50 and 4·78, and 1·31 and 1·61 on columns *a* and *c*) and only traces of other components. The methylated disaccharide (95 mg.) was hydrolysed in 0·5N-sulphuric acid at 90° for 4 hr., and after neutralisation with barium carbonate furnished a mixture of sugars (85 mg.) which was chromatographed on cellulose with benzene–ethanol (20 : 1; later, 10 : 1) containing a trace of water, to give three fractions. Chromatography in solvent A indicated that fraction 1 (44 mg.) contained 2,3,4-tri-*O*-methylrhamnose, fraction 2 (14 mg.) contained a mixture of sugars, and fraction 3 (12 mg.) contained 2,3,6-tri-*O*-methyl-glucose. Fraction 1 had $[\alpha]_D$ +18° (*c* 1·1 in H_2O) and the sugar was characterised as 2,3,4-tri-*O*-methyl-L-rhamnose by conversion into the aniline derivative, which was identified by m. p. and mixed m. p. 112—114° and by its infrared spectrum. The relative yield of tri-*O*-methyl-glucose (fraction 3) was low and it is possible that the methylated disaccharide was incompletely hydrolysed. Attempts to characterise the sugar by the formation of a crystalline derivative failed, but in a separate experiment methylated disaccharide (from fraction 2*a*) was hydrolysed and the corresponding methylated sugar was characterised as 2,3,6-tri-*O*-methyl-D-glucose by conversion into the 1,4-di-*p*-nitrobenzoate, m. p. and mixed m. p. 191°.

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[16] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1477.