987. *The Seed Mucilage of* Lepidium sativum (*Cress*). *Part II.*[1] *Products of Hydrolysis of the Methylated Mucilage and the Methylated Degraded Mucilage*

By Jean M. Tyler

The fraction of whole cress-seed mucilage of high uronic acid content, when methylated and hydrolysed, afforded the neutral sugars 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, and L-rhamnose, together with traces of other galactose and rhamnose methyl ethers. The acidic portion, after reduction and hydrolysis, yielded 2,3,4-tri-*O*-methyl-D-glucose and 2,3,6-tri-, 2,6-di-, and 2,3-di-*O*-methyl-D-galactose. The above sugars were produced, though in different proportions, from the methylated degraded mucilage. 3,4-Di- and 4-mono-*O*-methyl-L-rhamnose were also identified among the neutral sugars, and 2,3,4-tri-*O*-methyl-D-galactose from the reduced acidic fraction. The general structural features which emerged closely paralleled those of certain plant gums, and were dissimilar to other seed mucilages so far investigated.

In the preceding Paper [1] it was shown that the mucilage extracted from cress seeds (*Lepidium sativum*) consisted of units of L-arabinose, D-xylose, D-galactose, L-rhamnose,

[1] Part I, preceding Paper.

D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid, together with D-glucose and traces of mannose, which arose from the " cellulose " present mainly in the tenacious gelatinous capsule surrounding the water-soaked seed. Virtually all the arabinose, but only part of the xylose, was found to constitute a xylo-araban, that was separable from a fraction of high uronic-acid content during the methylation of the whole cellulose-free mucilage M.

The degraded mucilage V, which was methylated, was not derived from the whole mucilage M, but by autohydrolysis, in two stages, of the cellulose-rich mucilage R. As previously pointed out, the properties of M and R are similar, when allowance is made for the cellulose content of R. After the first autohydrolysis cellulose was easily removed, and the degraded mucilage T recovered at this stage was further degraded to V.

After removal of the methylated pentosan from the methylated whole mucilage, the remaining uronic-acid-containing fraction was fully methylated and yielded C ($[\alpha]_D$ +67°; OMe, 38·3%), which showed little hydroxyl absorption in the infrared. C was subjected to methanolysis and hydrolysis. The products were separated on cellulose into mixtures of neutral methylated sugars and of methylated barium uronates. Fractionation on cellulose [2] yielded 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-*O*-methyl-D-galactose, 3-*O*-methyl-L-rhamnose, and L-rhamnose, which were all identified as the crystalline sugar or a derivative and, except for the tetramethylgalactose, were all significant components; rhamnose, its 3-methyl ether, and tri-*O*-methylgalactose were the most abundant. 2,3,4-Tri- and 3,4-di-*O*-methyl-L-rhamnose (both identified by rotation, chromatography, and the latter by demethylation [3] and ionophoresis [4]), and 6-*O*-methylgalactose (identified by chromatography, ionophoresis, periodate oxidation,[5] and chromatography of the osazone [6]) were minor components, the last being contaminated with traces of the 2-isomer, and with glucose.

Small, though approximately equivalent, amounts of 2,5-di- and 2-mono-*O*-methylarabinose, and a trace of the 2,3-dimethyl ether, were identified by chromatography. Although the amounts of the arabinose derivatives render them insignificant structurally, it is obvious that they, and a small portion of the 2,3,4-tri-*O*-methylxylose, are derived from contaminating xylo-araban.[1] Methylated barium uronates were converted into the free acids, which were separated into three fractions (A, major, fast-moving; B, minor; C, trace) by paper chromatography. Portions of each fraction were examined chromatographically after prolonged hydrolysis, and the remainders converted into the methyl ester methyl glycosides which were reduced with lithium aluminium hydride. The product was hydrolysed and the methylated neutral sugars examined.

Free 2,3,4-tri-*O*-methylglucuronic acid was present in the mixture of acids comprising fraction A. Prolonged acid hydrolysis gave 2,3,6-tri-*O*-methylgalactose, 2,3,4-tri-*O*-methylglucuronic acid, and a di-*O*-methylhexuronic acid as the major components, and 2,6-di-*O*-methylgalactose, and 3-*O*-methylrhamnose as minor ones; a trace of di-*O*-methylglucuronic acid was thought to be present. When the neutral sugars from the reduction were resolved on cellulose, 2,3,4-tri-*O*-methyl-D-glucose, and 2,3,6-tri-, 2,3-di-, and 2,6-di-*O*-methyl-D-galactose were identified as crystalline derivatives. A little 3-*O*-methylrhamnose, and traces of 2,3-di-*O*-methylglucose and 6-*O*-methylgalactose were identified by chromatography.

2,3,4-Tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-galactose had not occurred previously among the methylated neutral sugars, and must have arisen from the related methylated uronic acids, 2,3,4-tri-*O*-methyl-D-glucuronic acid and 2,3-di-*O*-methyl-D-galacturonic acid. The proportions of the neutral sugars, and the high mobility of the original fraction

[2] L. Hough, J. K. N. Jones, and W. H. Wadman, *J.*, 1949, 2511.
[3] L. Hough, J. K. N. Jones, and W. H. Wadman, *J.*, 1950, 1702.
[4] A. B. Foster, *Chem. and Ind.*, 1952, 828.
[5] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.
[6] V. C. Barry and P. W. D. Mitchell, *J.*, 1954, 4020.

A, indicated that, in addition to much free 2,3,4-tri-*O*-methyl-D-glucuronic acid, the aldobiouronic acid 2,3,6-tri-*O*-methyl-4-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl-uronic acid)-D-galactose was a major constituent of the mixture. Lack of information concerning aldobiouronic acids precludes the assignment of aldobiouronic acid structures to the minor components.

3-*O*-Methyl-L-rhamnose and a di-*O*-methylhexuronic acid, identified as 2,3-di-*O*-methyl-D-galacturonic acid by the isolation of 2,3-di-*O*-methyl-D-galactose after reduction, were the major components of fraction B. As the galactose and rhamnose methyl ethers were isolated in the approximate ratio of 2 : 1, it seemed likely that fraction B consisted mainly of an aldotriouronic acid *O*-2,3-di-*O*-methyl-D-galactopyranuronosyl-(1 ⟶ 4)-*O*-2,3-di-*O*-methyl-D-galactopyranuronosyl-[1 ⟶ 2 (4)]-3-*O*-methyl-L-rhamnose.

Fraction C gave approximately equal amounts of rhamnose and 3-*O*-methylrhamnose, and a larger proportion of 2,3-di-*O*-methylgalacturonic acid (identified by reduction to 2,3-di-*O*-methylgalactose); the low mobility of the fraction suggests it is an oligouronide containing adjacent 1,4-linked galacturonic acid residues.

Fully methylated degraded mucilage D ($[\alpha]_D$ +82°; OMe, 39·1%; no hydroxyl absorption in the infrared) was prepared from V, subjected to methanolysis and hydrolysis, and the products were subjected to the same fractionation procedures as those from the methylated whole mucilage. Of the resulting methylated neutral sugars, 2,3,4,6-tetra-, 2,3,6-tri-, and 2,6-di-*O*-methyl-D-galactose, 3,4-di-, 4-mono-, and 3-mono-*O*-methyl-L-rhamnose and L-rhamnose were obtained as crystalline derivatives. 2,3,4-Tri-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-L-rhamnose (minor components) were identified by chromatographic means and from the rotation.

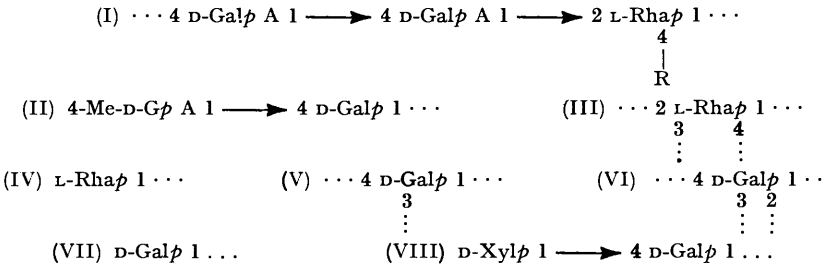
The mixture of methylated uronic acids obtained from the degraded mucilage was separated into fractions A′, B′, and C′ (as for the methylated whole mucilage). Fractions B′ and C′ behaved almost identically to fractions B and C, and provided further evidence for the presence of contiguous 1,4-linked galacturonic acid residues in the mucilage.

Of the methylated neutral sugars obtained from reduced fraction A′, 2,3,4-tri-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-L-rhamnose, 2,3,4-tri-, 2,3-di-, and 2-mono-*O*-methyl-D-galactose were isolated as crystalline derivatives. 3-*O*-Methylrhamnose and 2,3,6-tri- and 2,6-di-*O*-methyl-D-galactose were also identified. The 2,3,4-trimethylglucose and 2,3,4-tri-and 2,3-di-methylgalactose were derived from the related uronic acid residues.

The major differences from the sugars obtained from the methylated whole mucilage lay in the isolation of 4-*O*-methyl-L-rhamnose, the production of increased proportions of 3,4-di-*O*-methyl-L-rhamnose and 2,3,4,6-tetra-*O*-methyl-D-galactose, and the decreased proportions of 2,3,4-tri-*O*-methyl-D-xylose, rhamnose and its 3-methyl ether, 2,3,6-tri- and 2,6-di-*O*-methyl-D-galactose, and 2,3,4-tri-*O*-methyl-D-glucuronic acid. 2,3,4-Tri-*O*-methyl-D-galacturonic acid residues were present only in the methylated degraded mucilage.

The two most important features of the whole mucilage which emerge from the above results are represented in formulæ (I) (R = side-chain) and (II). (I) constitutes, in part, the backbone or resistant core of the mucilage, for the main constituents of the degraded mucilage as deduced from the methylation data, are galacturonic acid and rhamnose; and (II) represents the major side-chain. The absence of 2,3,4-tri-*O*-methylgalacturonic acid in the products from the methylated whole mucilage shows that all the galacturonic acid therein is 1,4-linked. (Terminal galacturonic acid residues in the degraded mucilage must arise through scission of the main galacturonic acid–rhamnose chain). Fractions B and B′ indicated that the uronic acid residues are linked to the 2- or 4-position of rhamnose. Assignment of the linkage to the 2- rather than to the 4-position is based on the observation that although 1,2,4-linked rhamnose residues account for well over half the rhamnose units in the whole mucilage, 1,2-linked residues are predominant in the degraded mucilage. About one in every three rhamnose residues in the whole mucilage is fully substituted, as represented by (III). Such residues appear to be genuine, for they persist in the degraded

mucilage structure; they presumably give rise, after loss of a side-chain from position 4, to the 4-methylrhamnose which is isolated from the degraded mucilage. (Fully substituted rhamnose residues have been reported recently [7] as a feature of one of the acidic fractions of linseed mucilage). Occasional non-reducing terminal rhamnose residues (IV) are present in the whole cress-seed mucilage.



(D-Gal*p* A = D-galacturonic acid; 4-Me-D-Gp A = 4-*O*-methyl-D-glucuronic acid: D-Gal*p* = D-galactopyranose; L-Rha*p* = L-rhamnopyranose; D-Xyl*p* = D-xylopyranose.)

4-*O*-Methyl-D-glucuronic acid, occurring only as a terminal residue in the whole and degraded mucilages, constitutes about half the acidic residues of the whole polysaccharide. It is possibly attached to the 4-position of a galactose residue, as an aldobiouronic acid unit (II), in the whole mucilage, for the total amount of the major galactose methyl ether isolated (the 2,3,6-isomer) was almost equivalent to that of 2,3,4-tri-*O*-methyl-D-glucose (formed from the related acid). On degradation, the proportions of galactose and 4-*O*-methylglucuronic acid residues are decreased relative to the galacturonic acid and rhamnose residues, thus emphasising the close association of the first two residues and their possible situation in the outer chains of the polysaccharide. The unit (II), representing the major side-chain in the whole mucilage structure, is possibly attached directly to position 4 of the rhamnose residues, as shown in (I).

Some 1,3,4-linked galactose residues (V) occur in the whole mucilage, but whether these are present as additional units in the side-chain or in the core of the polysaccharide is uncertain. 1,2,3,4-Linked galactose residues (VI) occur infrequently, and only a small proportion of the terminal residues of the whole mucilage are galactose units (VII).

D-Xylose, present as a terminal group in the degraded and the whole mucilage, is a minor though integral part of the mucilage, as substantiated by the previous isolation [1] of the disaccharide 4-*O*-α-D-xylopyranosyl-D-galactose from the autohydrolysis products of the whole mucilage. The unit (VIII) therefore constitutes a minor side-chain of the mucilage.

Two other seed mucilages with a high cellulose content are those from quince (*Cydonia vulgaris*) [8] and white mustard (*Brassica alba*) [9]; both mucilages have a high uronic acid content (20—30%), and appreciable methoxyl content (3 and 2%, respectively). Some evidence suggested that white-mustard-seed mucilage contained galacturonic acid and glucuronic acid (presumably as a methyl ether), and the occurrence of a galacturonosyl-rhamnose unit was indicated; Bailey [10] has indicated the heterogeneity of the mucilage.

Other seed mucilages which have been investigated, though having a negligible cellulose content, include those from members of the *Plantago* species (*P. arenaria*,[11,12] *P. fastigiata*,[13]

[7] K. Hunt and J. K. N. Jones, *Canad. J. Chem.*, 1962, **40**, 1266.
[8] A. G. Renfrew and L. H. Cretcher, *J. Biol. Chem.*, 1932, **97**, 503.
[9] K. Bailey and F. W. Norris, *Biochem. J.*, 1932, **26**, 1609.
[10] K. Bailey, *Biochem. J.*, 1935, **29**, 2477.
[11] W. A. G. Nelson and E. G. V. Percival, *J.*, 1942, 58.
[12] E. L. Hirst, E. G. V. Percival, and C. B. Wylam, *J.*, 1954, 189.
[13] E. Anderson, L. A. Gillette, and M. G. Seeley, *J. Biol. Chem.*, 1941, **140**, 569.

P. lanceolata,[14,15] and *P. ovata* Forsk [16,17]). Each consists principally of a highly branched xylan (cf. Discussion, Part I [1]). 2-*O*-α-(D-Galactopyranosyluronic acid)-L-rhamnose was isolated from two of the mucilages,[12,16] from which Erskine and Jones [18] also isolated small fractions rich in rhamnose and galacturonic acid.

Linseed mucilage (from *Linum usitatissimum*), unusual in containing L-galactose,[19] is the most extensively investigated seed mucilage. A highly branched xylan (containing some arabinose), and two fractions containing 1,4-linked galacturonic acid residues, and 1,2- and 1,2,3-linked rhamnose residues have been isolated;[18,20] some fully substituted rhamnose residues occur in one fraction, which also contains L-fucose.

The only polysaccharides possessing overall structural features which are paralleled by those of the uronic acid-rich fraction of cress-seed mucilage, are the gum from *Khaya grandifolia* [21] and the major fraction of the gum from *Khaya senegalensis*.[22] 2-*O*-(D-Galactopyranosyluronic acid)-L-rhamnose and 4-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-galactose were isolated from both gums, and it was established that both contain a backbone of 1,4-linked galacturonic acid residues, and 1,2-linked rhamnose residues which are substituted at position 4, almost certainly by the side-chain (II). 1,4-Linked galactose residues also occur in the backbone—a feature not established for cress-seed mucilage.

Although various other gums contain a high percentage of either D-galacturonic acid or 4-*O*-methyl-D-glucuronic acid, only Ketha gum [23] (no adequate structural work is available), the two *Khaya* gums, and this fraction of cress-seed mucilage have been shown to contain both acids.

Experimental

Paper chromatography was performed on Whatman No. 1 paper with the upper phase of, or, the following solvent systems (v/v): *A*, ethyl acetate–pyridine–water (10 : 4 : 3); *B*, benzene–ethanol–water (169 : 47 : 15); *C*, ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); *D*, butanol–acetic acid–water (4 : 1 : 5); *E*, butanol–ethanol–water (4 : 1 : 5). Sugars were revealed with aqueous aniline hydrogen oxalate and the colours of the stains are indicated occasionally. R_G values refer to the rate of movement of methylated sugars relative to 2,3,4,6-tetra-*O*-methylglucose in solvent *E*, except where stated otherwise. The products of periodate oxidation [5] were chromatographed in solvent *E*. Other operations were carried out as in Part I.[1]

Preparation of Fully Methylated Whole Mucilage C.—The partially methylated polysaccharide C (cf. Part I [1]) (6·67 g., $[\alpha]_D$ +88·7°; ash, 14·4; OMe, 28·5%), dissolved in water, was deionised with IR-120 (H) resin and converted into its silver salt by treatment with excess of silver carbonate. The amorphous silver salt (6·12 g.), obtained after filtration and evaporation, was suspended in methanol (20 ml.) and methyl iodide (50 ml.), and the mixture heated under reflux for 0·5 hr., and left at room temperature for 5 days. The isolated ester (5·24 g.), $[\alpha]_D$ +69·9° (*c* 0·29 in $CHCl_3$) (Found: OMe, 33·7%), was methylated six times with Purdie reagents, giving methylated polysaccharide C (4·83 g.) with $[\alpha]_D$ +66·6° (*c* 1·44 in $CHCl_3$) (Found: OMe, 38·3%), which showed no significant hydroxyl absorption in the infrared.

Hydrolysis of the Fully Methylated Polysaccharide C.—Methylated polysaccharide (3·0 g.) was heated at 90—95° for 18 hr. with methanolic 3% hydrogen chloride (70 ml.). Methanol was removed, the residue was hydrolysed (100°; 6 hr.) with N-hydrochloric acid (150 ml.), the hydrolysate neutralised (silver carbonate), and filtered. The silver ions were removed by hydrogen sulphide, the filtrate concentrated, and the resulting syrup taken up in methanol to free it from silver salts. After removal of methanol, the residue was dissolved in water (20

[14] J. Mullan and E. G. V. Percival, *J.*, 1940, 1501.
[15] E. G. V. Percival and I. C. Willox, *J.*, 1949, 1608.
[16] R. A. Laidlaw and E. G. V. Percival, *J.*, 1949, 1600.
[17] R. A. Laidlaw and E. G. V. Percival, *J.*, 1950, 528.
[18] A. J. Erskine and J. K. N. Jones, *Canad. J. Chem.*, 1956, **34**, 821.
[19] E. Anderson, *J. Biol. Chem.*, 1933, **100**, 249.
[20] A. J. Erskine and J. K. N. Jones, *Canad. J. Chem.*, 1957, **35**, 1174.
[21] G. O. Aspinall, E. L. Hirst, and N. K. Matheson, *J.*, 1956, 989.
[22] G. O. Aspinall, M. J. Johnston, and A. M. Stephen, *J.*, 1960, 4918.
[23] M. Heidelberger, J. M. Tyler, and S. Mukherjee, *Immunol.*, 1962, **5**, 666.

ml.), and acidic material converted into the barium salts with barium carbonate. Evaporation of the filtrate gave a syrup (3·29 g.) containing methylated neutral sugars and barium salts. Separation on cellulose (4·5 × 45 cm.) with butan-1-ol half-saturated with water gave neutral sugars (0·89 g.), followed by some acidic material (73 mg.) which was combined with the water eluate containing the barium salts (2·05 g.).

Identification of the Neutral Methylated Sugars.—The sugars were separated on cellulose (3·2 × 60 cm.) with water-saturated light petroleum (b. p. 100—120°)–butanol-1-ol (7 : 3; later 6 : 4 and 1 : 1), followed by butan-1-ol saturated with water, and finally with water, to give thirteen fractions. The results of the preliminary examinations are given in Table 1.

TABLE 1

Analysis of methylated neutral sugars from the whole mucilage

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography R_G	Paper chromatography sugar	Sugars given on demethyln.	Other evidence †
1	78	+17°	0·95	2,3,4-$(Me)_3$-xylose		
2	10		1·01	2,3,4-$(Me)_3$-rhamnose (*t*)	Rhamnose	*A*
			0·92	2,3,4-$(Me)_3$-xylose	Xylose	
			0·85	$(Me)_2$-rhamnose (*t*)	Galactose (*t*), Glucose (*t*)	
3	15	+31	0·86	$(Me)_2$-rhamnose	Rhamnose	I
			0·91	2,3,4,6-$(Me)_4$-galactose (*t*)	Galactose (*t*)	
4	26	+86	0·91	2,3,4,6-$(Me)_4$-galactose	Galactose	
5	10		0·83	2,5-(Me_2)-arabinose, 3,5-$(Me)_2$-arabinose (*t*)	Arabinose	I
6	22	+38	0·85	3,4-$(Me)_2$-rhamnose, 2,3,6-$(Me)_3$-galactose	Rhamnose, Galactose, Xylose (*t*), Glucose (*t*), Arabinose (*t*)	*A*, *B*, I
7	105	+101	0·78	2,3,6-$(Me)_3$-galactose	Galactose, Glucose (*t*)	*A*, P (-ve)
8	66	+90	0·78	2,3,6-$(Me)_3$-galactose	Galactose	*A*, P (-ve)
			0·69	2,3-$(Me)_2$-arabinose (*t*)		
			0·60	Me-rhamnose (*t*)		
9	168	+36	0·61	3-Me-rhamnose	Rhamnose	*A*, I, P
10	101	+80	0·50	2,6-$(Me)_2$-galactose, Me-pentose (*t*)	Galactose	I, P
11	9	+58	0·41	2-Me-arabinose, 2,6-$(Me)_2$-galactose (*t*)	Arabinose, Galactose, Xylose (*t*), Glucose (*t*)	*A*, P
12	148	+13	0·32	Rhamnose		I
13	25		0·19	6-Me-galactose, 2-Me-galactose (*t*)	Galactose	*A*, I, P
			0·09	Glucose	(Glucose)	P, I

t = Trace. † *A* and *B* = paper chromatography in solvents *A* and *B* respectively; I = paper ionophoresis; P = paper chromatography of the periodate oxidized sugar in solvent *E*, (-ve) where component unchanged.

Fraction 1 crystallised, had $[\alpha]_D$ +64·0° ⟶ +17·4° (*c* 0·34), and was identified as 2,3,4-tri-*O*-methyl-D-xylose by m. p. 86—87° and mixed m. p. 83—84°, with an authentic sample, m. p. 82—83°; and by *X*-ray powder photography.

Fraction 3. The main component was identified from the low rotation, $[\alpha]_D$ +31° (*c* 0·29), and ionophoresis (M_G 0·31) as 3,4-di-*O*-methyl-L-rhamnose.

Fraction 4 was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 189—190°.

Fraction 6. The rotation of the syrup was consistent with 3,4-di-*O*-methyl-L-rhamnose being the main component.

Fraction 7. The syrup, $[\alpha]_D$ +72·3° ⟶ +100·5° (*c* 2·06), of 2,3,6-tri-*O*-methyl-D-galactose, was characterised by conversion into the aldono-lactone, m. p. 96—97° and mixed m. p. 96°. The aldono-lactone was revealed as a single component by the hydroxylamine–ferric chloride spray [24] after chromatography in solvents *C* and *D*.

[24] M. Abdel-Akher and F. Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

Fraction 8. The syrup, $[\alpha]_D$ +63·9° ⟶ +89·5° (*c* 1·33), consisted almost entirely of 2,3,6-tri-*O*-methyl-D-galactose; the derived 2,3,6-tri-*O*-methyl-D-galactonolactone had m. p. and mixed m. p. 96—97°.

Fraction 9. The crystalline sugar had $[\alpha]_D$ +21·7° ⟶ +35·7° (*c* 1·15) (Found: OMe, 17·1. Calc. for $C_7H_{14}O_5$: OMe, 17·4%), and was distinct in solvent *A* from the 4-methyl ether; on chromatography it reacted with triphenyltetrazolium hydroxide [25] spray (*i.e.*, C-2 unsubstituted), and was identical with 3-mono-*O*-methylrhamnose on ionophoresis. By m. p. 115—116°, mixed m. p. 113—114° (authentic sample m. p. 111—113°), and *X*-ray powder photography the sugar was characterised as 3-*O*-methyl-L-rhamnose.

Fraction 10. The crystalline sugar had $[\alpha]_D$ +56·5° ⟶ +80·0° (*c* 0·94) and was identified as 2,6-di-*O*-methyl-β-D-galactose hydrate by m. p. and mixed m. p. 106—108° (Found: OMe, 29·5. Calc. for $C_8H_{16}O_6$: OMe, 29·8%); derived anilide, m. p. and mixed m. p. 109—111°.

Fraction 12. The syrup later crystallised, and had m. p. and mixed m. p. 89—90° with authentic L-rhamnose hydrate; benzoylhydrazone derivative, m. p. 185°, and mixed m. p. 187—188° (authentic sample, m. p. 190°; *X*-ray powder photograph identical with that of L-rhamnose hydrate.

Fraction 13. Residual material was converted into the osazone (a tar), which was examined by circular paper chromatography [6] using toluene–ethanol–water (270 : 30 : 1) as solvent. Components, which darkened on spraying with ammoniacal silver nitrate, corresponding to the osazones of glucose and 6-*O*-methylgalactose, were present.

Examination of the Methylated Acidic Fraction.—Chromatography of a sample in solvent *E* showed free neutral methylated sugars to be absent. A portion (1·20 g.) of the methylated barium uronates was treated with IR-120 (H) resin, and the free methylated acids (895 mg.) were separated on thick paper with (fresh) solvent *D* into three fractions (R_G values given for *D*): A, R_G 0·69—1·01 (522 mg.)—this fraction contained several overlapping regions, and a major component, R_G 0·88 (approx.) was noted; B, R_G 0·40 (191 mg.); C, R_G 0·28 (74 mg.).

Samples (10—15 mg.) of each fraction were hydrolysed with N-hydrochloric acid for 18 hr. at 100°, and, after neutralisation (Ag_2CO_3), were examined in solvent *E* for neutral sugars, and, after passage of hydrogen sulphide, in solvent *D* for methylated acids.

Fraction A contained a trace of 3-*O*-methylrhamnose, 2,3,6-tri-*O*-methylgalactose, R_G 0·76, as the major component, and 2,6-di-*O*-methylgalactose, R_G 0·50, as a minor component. In solvent *D*, additional components with R_G values 0·46 (major, di-*O*-methylgalacturonic acid), *ca.* 0·57 (minor, di-*O*-methylglucuronic acid), and 0·84—0·88 (major, tri-*O*-methylglucuronic acid) were observed.

Fraction B gave 3-*O*-methylrhamnose, R_G 0·62, as the major component, and rhamnose and a di-*O*-methylrhamnose in trace amounts (R_G values 0·38 and 0·88); di-*O*-methylgalacturonic acid, R_G *ca.* 0·50, was the predominant component in solvent *D*.

Fraction C. The hydrolysate contained rhamnose, R_G 0·38, and 3-*O*-methylrhamnose, R_G 0·62, in approximately equal amounts, and a di-*O*-methylhexuronic acid, R_G *ca.* 0·50, in slightly greater proportion.

Reduction, Followed by Hydrolysis, of the Methylated Acidic Fractions.—Fraction A (450 mg.) was converted into the methyl ester methyl glycosides by heating under reflux with 1·5% methanolic hydrogen chloride (30 ml.). The glycosides were dissolved in tetrahydrofuran (25 ml.), and reduced with lithium aluminium hydride (*ca.* 0·4 g.). The mixture was heated under reflux for 2 hr. Excess of hydride was destroyed with water, ethyl acetate added, and the organic layer removed. The suspension of lithium hydroxide was evaporated to dryness, and the residue extracted with dry acetone. The combined extracts, together with the ethyl acetate layer, were evaporated to dryness. Hydrolysis of the reduction product (370 mg.) with N-hydrochloric acid (25 ml.; 6 hr.; 100°), followed by neutralisation (Ag_2CO_3), gave a mixture of neutral sugars (280 mg.), which was separated on cellulose (2·2 × 45 cm.) as described previously, into six fractions (see Table 2).

Fraction 1 was identified as 2,3,4-tri-*O*-methyl-D-glucose (aniline derivative m. p. 135—136°, and mixed m. p. 139—140°, with a sample of m. p. 144°).

Fraction 2 was characterised as 2,3,6-tri-*O*-methyl-D-galactose by conversion into 2,3,6-tri-*O*-methyl-D-galactonolactone, m. p. and mixed m. p. 96—97°.

Fraction 4. The syrup, having $[\alpha]_D$ +80·4° (*c* 0·53), later crystallised; m. p. 113—115°, and mixed m. p. 110—112°, with authentic 2,6-di-*O*-methyl-D-galactose hydrate of m. p. 109—110°.

[25] K. Wallenfels, *Naturwiss.*, 1950, **37**, 491.

Fraction 5. The principal sugar was identified as 2,3-di-*O*-methyl-D-galactose by conversion into 2,3-di-*O*-methyl-D-galactonamide, m. p. 137°, and mixed m. p. 138—139°, (authentic sample, m. p. 139—140°).

Fraction B (150 mg.) was similarly converted into the methyl ester methyl glycoside, which

TABLE 2

Analysis of methylated neutral sugars from the major reduced methylated acidic fraction of the methylated whole mucilage *

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography R_G	Sugar	Sugars given on demethyln.	Other evidence
1	114	+78°	0·86	2,3,4-(Me)$_3$-glucose	Glucose	*B*, I
2	59	+85	0·78	2,3,6-(Me)$_3$-galactose	Galactose	*A*, I, P (-ve)
3	11		0·61	3-Me-rhamnose	Rhamnose	*A*, I
				2,3-(Me)$_2$-glucose	Glucose	
4	19	+80	0·52	2,6-(Me)$_2$-galactose	Galactose	I, P
5	38	+95	0·47	2,3-(Me)$_2$-galactose	Galactose	*A*, I, P
				2,6-(Me)$_2$-galactose (*t*)		P
6	5		0·47	2,3-(Me)$_2$-galactose (*t*)		*A*, I, P
			0·40	2-Me-arabinose		
				6-Me-galactose (*t*)		

* The footnotes to Table 1 apply.

was reduced, and the product (124 mg.) hydrolysed. Chromatography of the hydrolysate indicated the presence of 2,3-di-*O*-methylgalactose, R_G 0·52, and 3-*O*-methylrhamnose, R_G 0·62, as major components; a trace of 2,3,4-tri-*O*-methylglucose, R_G 0·86, was observed. A portion (36 mg.) of the hydrolysate was separated on thick paper with solvent *E* and the two main fractions, (i) 12 mg. and (ii) 12 mg., were isolated.

Fraction (i), $[\alpha]_D$ +90° (*c* 0·60), was identified as 2,3-di-*O*-methyl-D-galactose by chromatography, demethylation, and chromatography of the periodate oxidation products (components with R_F values 0·65, 0·72, and 0·83 present.)

Fraction (ii), $[\alpha]_D$ +55° (*c* 0·60), was shown by chromatography in *A* and *E*, by ionophoresis (one component, M_G 0·25, was identical with 3-*O*-methylrhamnose), and by periodate oxidation, to consist of 2,3-di-*O*-methyl-D-galactose and 3-*O*-methyl-L-rhamnose in the approximate proportions of 1 : 2. Only galactose was detected on demethylation.

Fraction C. Hydrolysis of the reduced acidic fraction gave 3-*O*-methylrhamnose, 2,3-di-*O*-methylgalactose (predominant) and rhamnose, and a minor component which was further resolved by solvent *A* into 2- and 6-*O*-methyl-galactose.

Preparation of Methylated Degraded Mucilage.—The two samples (each of 1·70 g.) of degraded mucilage, V (cf. Part I [1]) which were combined for the methylation, had similar properties: $[\alpha]_D$ +92°, 71°; Equiv., 481, 549; methoxyl, 1·6, 1·2%; proportions of galactose : xylose : rhamnose, 10 : 2 : 15 and 10 : 2 : 16; both samples contained small proportions of glucose, mannose, and arabinose.

The mucilage as methylated five times with methyl sulphate and 30% w/v sodium hydroxide solution. Excess of methyl sulphate was destroyed, the mixture neutralised to pH 7, and extracted with chloroform, giving, after concentration, methylated Dl(204 mg.), $[\alpha]_D$ +29·6° (*c* 1·82 in $CHCl_3$). The aqueous solution was dialysed (3 days) and then evaporated to dryness. The product (3·41 g.) was converted into the silver salt by treatment with silver carbonate. The dry silver salt was triturated with methyl iodide (60 ml.) and methanol (30 ml.), refluxed for 1 hr., and the ester (3·45 g.) isolated after filtration and evaporation. (Found: OMe, 33·3%). Methylation (six times) with Purdie reagents gave methylated polysaccharide D (3·11 g.), a well-defined solid with $[\alpha]_D$ +82·2° (*c* 0·88 in $CHCl_3$) (Found: OMe, 39·1%); the i.r. spectrum showed no hydroxyl absorption.

Hydrolysis of the Methylated Degraded Polysaccharide Dl.—After appropriate treatment, the hydrochloric acid hydrolysate (18 hr.) was examined in solvent *E* for neutral sugars, and in solvent *D* for free acids. The chromatographic patterns were similar (*i.e.*, acidic material inappreciable), and the following sugars were identified, those marked * predominating (R_G values given): 2,3,4,6-tetra-*O*-methylglucose, 1·0; 2,3,4-tri-*O*-methylxylose,* 0·94; 2,3,4,6-tetra-*O*-methylgalactose, or 2,3,4-tri-*O*-methylglucose, 0·89; di-*O*-methylrhamnose,* 0·82; di-*O*-methylhexose,* 0·52; and trace products with R_G 0·70, 0·60. The product was not investigated further.

Hydrolysis of the Methylated Degraded Polysaccharide D.—Methylated polysaccharide (2·00 g.) was methanolysed and hydrolysed, and worked up in the same way as the methylated whole mucilage C. Neutral sugars (0·753 g.) were separated, on a cellulose column (4·5 × 45 cm.), by elution with butan-1-ol half-saturated with water, from the methylated barium uronates (1·23 g.), which were eluted with water.

Identification of the Neutral Methylated Sugars.—Partition of the sugars on cellulose (3·2 × 60 cm.), as described previously, afforded twelve fractions (Table 3).

TABLE 3

Analysis of methylated neutral sugars from the methylated degraded mucilage *

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography R_G	Sugar	Sugars given on demethyln.	Other evidence
1	14	+35°	1·05	Unknown		
2	21	+41	1·02	2,3,4-(Me)$_3$-rhamnose		*A*
				Unknown (*t*)		*B*
3	14		0·97	2,3,5-(Me)$_3$-arabinose	Arabinose	*B*
				2,3,4-(Me)$_3$-xylose (*t*)	Xylose	
				Unknown (*t*)	Galactose	*B*
4	25		0·96	2,3,4-(Me)$_3$-xylose	Xylose	*B*
				Me$_2$ rhamnose (*t*)	Galactose	
				Unknown (*t*)		
5	74	+36	0·87	3,4-(Me)$_2$-rhamnose	Rhamnose	*B*, I, P
			0·92	2,3,4,6-(Me)$_4$-galactose		
6	128	+59	0·87	3,4-(Me)$_2$-rhamnose	Galactose	*B*, I, P
			0·92	2,3,4,6-(Me)$_4$-galactose		
7	65	+76	0·87	3,4-(Me)$_2$-rhamnose	Galactose	*B*, I, P
			0·92	2,3,4,6-(Me)$_4$-galactose		
8	104		0·78	2,3,6-(Me)$_3$-galactose	Galactose	*A*, I, P
			0·60	4-Me-rhamnose		
9	64	+33	0·60	3-Me-rhamnose		*A*, I, P
10	22	+59	0·53	2,6-(Me)$_2$-galactose	Galactose	I, P
11	67	+16	0·35	Rhamnose		*A*, I
12	19		0·19	6-Me-galactose		*A*
			0·10	Glucose		

* The footnotes to Table 1 apply.

Fraction 1 was chromatographically homogeneous (orange-yellow stain) but not identified; demethylation gave no monosaccharides.

Fraction 2. Mainly 2,3,4-tri-*O*-methyl-L-rhamnose, from specific rotation; a trace of unidentified material had R_G 1·17 in solvent *B* (orange-pink stain).

Fraction 3. Unidentified, R_G 1·17 in solvent *B*.

Fraction 4. Unidentified, (orange stain), R_G 1·03 in solvent *B*.

Fraction 5. The crystalline sugar had $[\alpha]_D$ +27·0° ⟶ +36·3° (*c* 1·70); a sublimed portion had m. p. 92° and gave an *X*-ray powder photograph distinct from those of 2,3- and 2,4-di-*O*-methyl-L-rhamnose. The red colour characteristic of a 2-unsubstituted sugar was produced with triphenyltetrazolium hydroxide spray,[25] and the main component, M_G 0·37, was identical with authentic 3,4-di-*O*-methylrhamnose; the minor component had M_G 0. Thus, the fraction consists of 3,4-di-*O*-methyl-L-rhamnose (61 mg.) and 2,3,4,6-tetra-*O*-methyl-D-galactose (13 mg.).

Fraction 6. The syrup crystallised, and had $[\alpha]_D$ +50·1° ⟶ +59·1° (*c* 1·98). Chromatography and ionophoresis showed approximately equal amounts of 3,4-di-*O*-methylrhamnose and 2,3,4,6-tetra-*O*-methylgalactose, and the presence of the latter was confirmed by formation of the aniline derivative, m. p. and mixed m. p. 190—191°. From the observed rotation, the fraction consisted of 2,3,4,6-tetra-*O*-methyl-D-galactose (54 mg.) and 3,4-di-*O*-methyl-L-rhamnose (74 mg.).

Fraction 7. 2,3,4,6-Tetra-*O*-methyl-D-galactose in the mixture was identified by conversion into its aniline derivative. From the observed optical rotation the mixture consisted of 2,3,4,6-tetra-*O*-methyl-D-galactose (37 mg.) and 3,4-di-*O*-methyl-L-rhamnose (28 mg.).

Fraction 8. The two components were separated on thick paper with solvent *E*. *Fraction* (*a*) (31 mg.) a syrup, $[\alpha]_D$ +59·6° ⟶ +81·1° (*c* 0·60), was chromatographically pure. Demethylation gave galactose. The derived aldonolactone had m. p. and mixed m. p. 94—95° with an authentic specimen of 2,3,6-tri-*O*-methyl-D-galactonolactone, and was chromatographically homogeneous in solvent *D*. *Fraction* (*b*) (27 mg.) a syrup, $[\alpha]_D$ +9·4° (*c* 0·53), was identical with 4-mono-*O*-methylrhamnose in solvents *A* and *E*, and triphenyltetrazolium hydroxide spray

gave a red colour confirming the unsubstituted C-2 hydroxyl group. The derived aldonolactone had m. p. 84—91°, and mixed m. p. 81—89° with authentic 4-*O*-methyl-L-rhamnonolactone of m. p. 84—89°. The recoveries on paper separation showed that the original mixture contained 56 mg. of 2,3,6-tri-*O*-methyl-D-galactose and 48 mg. of 4-*O*-methyl-L-rhamnose.

Fraction 9. The sugar, which later crystallised and had $[\alpha]_D$ +25·4° ⟶ +32·9° (*c* 1·06), gave, after chromatography, a red colour with triphenyltetrazolium hydroxide spray, indicating an unsubstituted C-2 position. The crystalline product was identified as 3-*O*-methyl-L-rhamnose by m. p. 113—114°, and mixed m. p. 114—115° with an authentic specimen of m. p. 114—115°, and by *X*-ray powder photography.

Fraction 10. The syrup, which later crystallised, was not revealed on chromatograms with triphenyltetrazolium hydroxide, confirming the presence of a C-2 methyl group which had been indicated by periodate oxidation. The aniline derivative could not be prepared. From its rotation, the above evidence, and the *X*-ray powder photograph, the component was identified as 2,6-di-*O*-methyl-D-galactose.

Fraction 11. The *X*-ray powder photograph together with the specific rotation, $[\alpha]_D$ +16·0° (*c* 1·19), of the crystalline sugar confirmed its identity as L-rhamnose.

Fraction 12 (19 mg.) was obtained on washing the column with water. Chromatograms run in solvents *A* and *E* were streaked, but regions corresponding to glucose and (?) 6-*O*-methylgalactose were visible, Paper ionophoresis and periodate oxidation were inconclusive.

Examination of the Methylated Acidic Components.—No free methylated neutral sugars were observed on chromatography in solvent *E*. Barium uronates (1·10 g.) were converted into the free acids with IR-120 (H) resin, and recovered as a syrup (830 mg.). The complex mixture of acids similar to that given by the methylated uronates from the whole mucilage was resolved by chromatography on thick paper with solvent *D*, yielding: fraction A′, R_G 0·63—0·88 (530 mg.), which contained a rhamnose-containing major component of R_G 0·69 and another in the region 0·81—0·88; fraction B′, R_G 0·49 (176 mg.); fraction C′, R_G 0·28 (44 mg.).

Samples of the three fractions were hydrolysed as before, and examined for neutral sugars and acidic components.

Fraction A′. The hydrolysate contained di-*O*-methylrhamnose and 2,3,6-tri-*O*-methylgalactose as major components, together with traces of 3-*O*-methylrhamnose, and 2,6-di-*O*-methylgalactose. In solvent *D*, 2,3,4-tri-*O*-methylglucuronic acid, R_G 0·84, and tri-*O*-methylgalacturonic acid, R_G 0·67, were present as minor components, and di-*O*-methylgalacturonic acid, R_G 0·46, as the major component.

Fraction B′ gave 3-*O*-methylrhamnose (major), a di-*O*-methylrhamnose (minor), and rhamnose (trace). Solvent *D* showed di-*O*-methylgalacturonic acid, R_G 0·47, to be a major component, and tri-*O*-methylgalacturonic acid, R_G 0·67, a minor one.

Fraction C′. Rhamnose, 3-*O*-methylrhamnose, di-*O*-methylgalacturonic acid, and a mono-*O*-methylhexuronic acid, R_G 0·25, were present.

Reduction, followed by Hydrolysis, of the Methylated Acidic Fractions.—*Fraction* A′. The syrup of methylated acids (465 mg.) was converted into the methyl ester methyl glycosides, which were reduced, and the product (423 mg.) was isolated as before. A portion (360 mg.) was hydrolysed. The methylated sugars (300 mg.) were fractionated on a cellulose column (2·2 × 45 cm.) as described previously (Table 4).

Fraction 1. The syrup, $[\alpha]_D$ +33·2° ⟶ +42·2° (*c* 0·66), contained two components with M_G 0·29, and 0, corresponding to 3,4-di-*O*-methylrhamnose and 2,3,4-tri-*O*-methylglucose. The syrup crystallised when seeded with authentic 3,4-di-*O*-methyl-L-rhamnose and after recrystallisation, a sample having m. p. and mixed m. p. 89—91° (authentic sample, m. p. 90—91°) was obtained; from the original rotation, 3,4-di-*O*-methyl-L-rhamnose was concluded to form 60% of the mixture.

Fraction 2. The syrup consisted of 2,3,4-tri-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-L-rhamnose in the approximate proportion 7 : 1. The identity of the glucose derivative was confirmed by formation of the aniline derivative, m. p. 135—137° and mixed m. p. 139—141°, with a sample of m. p. 144°.

Fraction 3 appeared to be chromatographically pure and identical with 2,3,6-tri-*O*-methylgalactose, although periodate oxidation revealed a trace of the component, R_F 0·82 produced by 3,4-di-*O*-methylrhamnose. On chromatography of the derived aldonolactone, two components, one identical with 2,3,6-tri-*O*-methylgalactonolactone, were revealed by the hydroxylamine–ferric chloride spray.

Fraction 4. The sugar, $[\alpha]_D$ +102·5° (*c* 0·41), was identified as 2,3,4-tri-*O*-methyl-D-galactose by conversion into its aniline derivative, m. p. 157—158°, and mixed m. p. 158—160° (authentic sample, m. p. 166—167°).

Fraction 5 contained 2,3,4-tri-*O*-methylgalactose as the major component and 3-*O*-methyl-

TABLE 4

Analysis of methylated sugars from the major reduced methylated acidic fraction from the methylated degraded mucilage *

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography R_G	Sugar	Sugars given on demethyln.	Other evidence
1	23	+42°	0·90	2,3,4-(Me)$_3$-glucose 3,4-(Me)$_2$-rhamnose	Glucose Rhamnose	*B*, I, P
2	46	+71	0·90	2,3,4-(Me)$_3$-glucose 3,4-(Me)$_2$-rhamnose	Glucose Rhamnose (*t*)	*B*, I
3	18	+69	0·75	2,3,6-(Me)$_3$-galactose	Galactose	*A*
4	50	+103	0·71	2,3,4-(Me)$_3$-galactose	Galactose	*A*, P (-ve)
5	29	+97	0·71 0·60	2,3,4-(Me)$_3$-galactose 3-Me-rhamnose	Galactose Rhamnose	*A*, I, P
6	36	+90	0·56	2,6-(Me)$_2$-galactose 2,3-(Me)$_2$-galactose	Galactose	I, P
7	45	+117	0·53	2,3-(Me)$_2$-galactose	Galactose	P
8	14	+39	0·53 0·41	2,3-(Me)$_2$-galactose Rhamnose	Galactose	*A*, P *A*
9	7		0·35	2-Me-galactose	Galactose	P

* The footnotes to Table 1 apply.

rhamnose as the minor one; the presence of 4-*O*-methylrhamnose was excluded by paper ionophoresis and periodate oxidation.

Fraction 6. The syrup $[\alpha]_D$ +89·6° (*c* 0·56) was a mixture of 2,6-di-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-D-galactose.

Fraction 7. Formation of 2,3-di-*O*-methyl-D-galactonamide of m. p. and mixed m. p. 139—140°, showed the original sugar to be 2,3-di-*O*-methyl-D-galactose.

Fraction 8 contained two components which were identified as 2,3-di-*O*-methyl-D-galactose and L-rhamnose from the rotation, and chromatographic examination.

Fraction 9 crystallised and was identified as 2-*O*-methyl-D-galactose by m. p. 144—146°, and mixed m. p. 147—150°, with an authentic sample of m. p. 148—151°.

Fraction B′ (135 mg.) was similarly reduced, and the product (95 mg.) hydrolysed. Chromatography of the hydrolysate indicated 2,3-di-*O*-methylgalactose (predominant) and 3-*O*-methylrhamnose, together with traces of 2,3,4-tri-*O*-methylgalactose, and a di-*O*-methylrhamnose. Separation of a portion (26 mg.) of the hydrolysate on thick paper with solvent *E*, and recovery of the two main fractions, gave: *fraction* (i) a syrup (10 mg.), $[\alpha]_D$ +104° (*c* 0·50), which gave galactose on demethylation: from the rotation, and the chromatographic pattern of the periodate oxidation products, the sugar was identified as 2,3-di-*O*-methyl-D-galactose; *fraction* (ii), (6 mg.), $[\alpha]_D$ +24° (*c* 0·25), was identical with 3-*O*-methylrhamnose, M_G 0·24, and gave the characteristic product, R_F 0·67, on periodate oxidation.

Fraction C′ (24 mg.) gave rhamnose, 3-*O*-methylrhamnose, 2,3-di-*O*-methylgalactose (main component), and some mono-*O*-methylhexoses (2-*O*-methylgalactose probably present), after hydrolysis of its reduced methyl ester methyl glycoside.

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