986. *The Seed Mucilage of* Lepidium sativum (*Cress*). *Part I. Identification of the Components and Some Neutral Oligosaccharides Derived from the Mucilage, Together with the Examination of a Pentosan Fraction*

By Jean M. Tyler

L-Arabinose, D-xylose, D-galactose, L-rhamnose, D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid have been identified as the main components of cress-seed mucilage, and D-glucose and mannose as components of the dispersed fibrous material (cellulose) associated with the polysaccharide(s). The oligosaccharides 3-*O*-α-D-xylopyranosyl-L-arabinose, *O*-α-D-xylopyranosyl-(1 ⟶ 3)-*O*-α-L-arabinofuranosyl-(1 ⟶ 3)-L-arabinopyranose, and 4-*O*-α-D-xylopyranosyl-D-galactose were identified among the products of autohydrolysis of the mucilage.

Methylation of the whole mucilage led to the separation of a fraction, composed mainly of pentose units, which yielded 2,3,4-tri-*O*-methyl-D-xylose, and 2,5-di- and 2-mono-*O*-methyl-L-arabinose in approximately equimolar proportions, together with less 2,3-di-*O*-methyl-L-arabinose, on hydrolysis. The structures of the first two oligosaccharides above complement the methylation data, and indicate the presence of a xylo-araban, consisting of a backbone of 1,5-linked arabinofuranose residues probably bearing side-chains of 3-*O*-α-D-xylopyranosyl-L-arabinofuranose units at the 3-position, as a fraction of the mucilage.

A second fraction obtained after methylation was later found to give methyl ethers of galactose, rhamnose, and uronic acids as the main components.

Cress-seed mucilage is thus heterogeneous, consisting of a cellulose component, a xylo-araban, and a complex acidic polysaccharide containing D-galacturonic acid, and 4-*O*-methyl-D-glucuronic acid.

A RE-EXAMINATION of the mucilage of cress seeds (*Lepidium sativum*) was of interest because of the soluble, or dispersed, cellulose reported [1,2] to be present, and because the earlier investigation was conducted without the use of chromatography.

In the previous chemical investigation [1] L-arabinose, D-galactose, L-rhamnose, and D-galacturonic acid were all identified as major components of the mucilage. Fibrous material, obtained on hydrolysis, was subjected to a Monier-Williams degradation; [3] because crystalline D-glucose was recovered, the material was considered to be cellulose, even though a pentose other than arabinose was also detected in the hydrolysate. Acid hydrolysis of the mucilage readily released arabinose and some galactose, whereas galacturonic acid, rhamnose, and the remaining galactose residues were more difficult to liberate. Subsequent experiments on the mucilage showed that alcohol precipitation, or treatment with baryta followed by alcohol precipitation, effected some fractionation.

In the present investigation three successive cold-water extractions of the seeds were made to give fractions 1, 2, and 3. The capsular gel (fraction 4) was difficult to dislodge; a little material dissolved thereafter in hot water (fraction 5). Most of the polysaccharide was present in the first two fractions. Fractions were precipitated with ethanol, or acidic ethanol, as the polysaccharide salt or acid. In addition, the first three fractions were combined and precipitated with ethanol at two concentrations; ethanol (71%) precipitated material containing a high percentage of fibrous matter, referred to as the cellulose-rich mucilage R, whereas ethanol (83%) gave material M, virtually free from cellulose and suitable for a methylation study (cf. later, and Part II [3a]).

[1] K. Bailey, *Biochem. J.*, 1935, **29**, 2477.
[2] K. Mühlethaler in "Experimental Cell Research," Academic Press, New York, 1950, vol. I, p. 341.
[3] G. W. Monier-Williams, *J.*, 1921, **119**, 803.
[3a] Part II, following Paper.

Chromatographic examination of dilute acid hydrolysates of fractions R and M confirmed Bailey's results, for both fractions contained arabinose, galactose, rhamnose, and galacturonic acid as major components; only traces of glucose were present. Xylose and 4-*O*-methylglucuronic acid, neither detected previously, were observed to be major components of R and M. (The small but significant methoxyl content of R and M had indicated that an *O*-methyl derivative such as the above acid might be present.) Glucurone and glucuronic acid were absent from all hydrolysates of R and M. Similar equivalent-weight values were obtained for R and M, of 620 (corrected for the cellulose content) and 570, respectively, which corresponded to hexuronic acid anhydride contents of 28·4 and 30·9%. The proportions, as anhydro-sugars, of galactose : arabinose : xylose : rhamnose were 10 : 13 : 7 : 7 in R, and 10 : 12 : 8 : 8 in M. Thus, except for the cellulose present in R, the two fractions of mucilage are similar.

D-Galacturonic acid and 4-*O*-methyl-D-glucuronic acid were characterised after being isolated from an acid hydrolysate of R. Further evidence for the latter acid was available when a sample of mucilage degraded by autohydrolysis was esterified, reduced, and hydrolysed; a new component, behaving as a 4-*O*-methylhexose on periodate oxidation,[4] was identified as 4-*O*-methyl-D-glucose. Methylation results on the whole mucilage [3a] suggest that the galacturonic acid and 4-*O*-methylglucuronic acid are present in approximately equimolar proportions.

Attempts to discover the amount of fibrous material present in the various batches of mucilage were, of necessity, crude, but indicated that it was mainly associated with the capsular gel.

Preliminary experiments on acid and autohydrolysis of R and M revealed (as partly shown by Bailey) [1] that under both conditions arabinose (major component), galactose, and xylose were liberated rapidly and in appreciable amount; rhamnose was more resistant to acid hydrolysis, and a negligible amount was released on autohydrolysis. This suggested that arabinose occurs in the furanose form in the mucilage, and that rhamnose may form part of an aldobiouronic acid unit. Pentose-containing oligosaccharides, detectable over a prolonged period of autohydrolysis, are not reversion products, for the same oligosaccharides were present in the early stages of mild acid hydrolysis, but disappeared later.

For convenience, cellulose-rich mucilage R was used in a large-scale autohydrolysis. Insoluble cellulose U, a mixture of neutral and acidic sugars S, and a degraded mucilage T were isolated in yields of 9, 49, and 33%, respectively. Similar results were obtained in a second experiment. The neutral anhydro-sugars in the degraded mucilage samples were present in the proportions of galactose : arabinose : xylose : rhamnose, 10 : 3 : 3 : 10 and 10 : 4 : 3 : 9.

Further autohydrolysis was used to procure a fraction of mucilage, albeit degraded, which it was hoped would be free from pentose residues. The resulting samples of twice degraded mucilage were almost free from arabinose, and contained galactose, xylose, and rhamnose in the proportions (as anhydro-sugars) of 10 : 2 : 16 and 10 : 2 : 15. The persistent xylose residues were accounted for later. Galacturonic acid, but considerably less 4-*O*-methylglucuronic acid, was present in the degraded mucilage. The chromatographic patterns of the released neutral and acidic sugars resembled those given by the first autohydrolysis syrup S, although pentoses and pentose-containing moieties were less evident.[3a]

The fibrous material U, obtained after autohydrolysis, was heated repeatedly with acid, and the sugars present in each hydrolysate were identified. The final residue was digested by the Monier-Williams method,[3] and D-glucose identified. Some mannose was present. The monosaccharides in the sequential hydrolysates indicated that the mucilage was closely associated with the cellulose (xylose, particularly, persisted after several hydrolyses) but was gradually hydrolysed away leaving a glucomannan and/or a true cellulose. Whether the association is other than a physical one was not determined.

[4] R. U. Lemieux and H. F. Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

The sugars contained in the autohydrolysis syrup S1 were separated on charcoal–Celite,[5] and acidic material was removed from the fractions with a resin. After partition of the aqueous eluate on cellulose, L-arabinose (major component), D-xylose, D-galactose, and L-rhamnose were identified as crystalline derivatives.

Two disaccharides, A and B, mainly eluted with 2 and 5% ethanol, and a trisaccharide C, eluted with 10% ethanol, were further purified. Higher oligosaccharide fractions were not investigated. (The syrup S2 gave similar yields of mono- and oligosaccharides.)

Compound A, $[\alpha]_D$ +185°, which crystallised, was characterised as 3-*O*-α-D-xylopyranosyl-L-arabinose (an authentic sample was kindly supplied by Dr. L. Hough, University of Bristol), a disaccharide previously obtained on autohydrolysis of golden apple gum [6] and by mild acid hydrolysis of corn fibre [7] and corn hull [8] hemicelluloses.

General procedures established that B was a xylosylgalactose, with a linkage other than 1 → 2, 1 → 6, or 1 → 5. The structure was established as 4-*O*-D-xylopyranosyl-D-galactose, a previously unknown disaccharide, from the products obtained on hydrolysis of the methylated disaccharide (crystalline 2,3,4-tri-*O*-methyl-D-xylose, and 2,3,6-tri-*O*-methyl-D-galactose were identified). Application of Hudson's rules [9] to determine the anomeric configuration indicates that the glycosidic linkage is α rather than β. The $[\alpha]_D$ values, +169°, +112°, +53°, −4°, were calculated for the appropriate α-α, α-β, β-α and β-D-xylopyranosyl-β-D-galactose respectively, using A and B values quoted by Klyne.[10] Though the purest sample of disaccharide B had $[\alpha]_D$ +96°, the actual rotation is probably slightly lower, for the trace of 2,4-di-*O*-methylarabinose detected on hydrolysis of the methylated disaccharide indicated the presence of the highly dextrorotary 3-*O*-α-D-xylopyranosyl-L-arabinose as a contaminant.

The third oligosaccharide C, $[\alpha]_D$ +95°, eventually crystallised, but was extremely hygroscopic, and attempts to prepare an *X*-ray powder photograph failed. Hydrolysis, partial hydrolysis, hydrolysis of the derived aldonic acid, examination of the products of lime-water degradation [11] (unchanged C, 3-*O*-α-xylosylarabinose, and xylose), and determination of the molecular weight by hypoiodite oxidation [12] indicated that C was a pentose trisaccharide. Formaldehyde production [13] (1 mole/mole of trisaccharide) on periodate oxidation indicated that the reducing unit was not linked through positions 5 or 4. Position 2 was excluded by the results of lime-water degradation. The observations implied that trisaccharide C had the constitution *O*-α-D-xylopyranosyl-(1 → 3)-*O*-L-arabinofuranosyl-(1 → 3)-*O*-L-arabinopyranose (the central arabinofuranose residue becoming pyranose when xylosylarabinose was liberated on mild hydrolysis), which was confirmed by methylation. The three components, produced in approximately equimolar amounts and identified as crystalline derivatives, were 2,3,4-tri-*O*-methyl-D-xylose, 2,5-di-*O*-methyl-L-arabinose, and 2,4-di-*O*-methyl-L-arabinose.

A decrease in optical rotation is observed between pairs of related compounds where an L-arabinopyranose residue is replaced by an L-arabinofuranose residue (*e.g.*, 5-*O*-α-L-arabinopyranosyl-L-arabinofuranose,[14] $[\alpha]_D$ −18° and 5-*O*-α-L-arabinofuranosyl-L-arabinofuranose,[15] $[\alpha]_D$ −94°; also, 3-*O*-β-L-arabinopyranosyl-L-arabinopyranose,[7] $[\alpha]_D$ +214° and 3-*O*-β-L-arabinofuranosyl-L-arabinopyranose,[5] $[\alpha]_D$ +94°). Thus, the contribution of rotation due to a 3-*O*-α-D-xylopyranosyl-L-arabinofuranose moiety is expected to be about

[5] P. Andrews, L. Hough, and D. B. Powell, *Chem. and Ind.*, 1956, 658.
[6] P. Andrews and J. K. N. Jones, *J.*, 1954, 4134.
[7] R. L. Whistler and W. M. Corbett, *J. Amer. Chem. Soc.*, 1955, **77**, 6328.
[8] R. Montgomery, F. Smith, and H. C. Srivastava, *J. Amer. Chem. Soc.*, 1957, **79**, 698.
[9] C. S. Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 66; 1916, **38**, 1566.
[10] W. Klyne, in "Determination of Organic Structures by Physical Methods," Braude and Nachod, Academic Press, New York, 1955, p. 96.
[11] W. M. Corbett and J. Kenner, *J.*, 1953, 2245.
[12] E. L. Hirst, L. Hough, and J. K. N. Jones, *J.*, 1949, 928.
[13] R. E. Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.
[14] F. Smith and A. M. Stephen, *J.*, 1961, 4892.
[15] P. A. J. Gorin, *Canad. J. Chem.*, 1962, **40**, 275.

100° less than the rotation observed for 3-*O*-α-D-xylopyranosyl-L-arabinose ($[\alpha]_D$ +185°). In addition, the rotation of 3-*O*-α-L-arabinofuranosyl-L-arabinopyranose has recently been recorded [14] as *ca.* 0°, whereas the β-anomer mentioned above has $[\alpha]_D$ +94°. The data indicate that the glycosidic linkage between the arabinose units in the trisaccharide is probably also of the α-configuration (cf. later discussion on the methylated polysaccharide).

Whole cellulose-free mucilage, M, could be separated, after methylation and neutralisation, into chloroform- and water-soluble fractions. The former was converted, after fractional precipitation, into fully methylated polysaccharide A (17% yield), $[\alpha]_D$ −28·5° (OMe, 39·3%); and a syrup B, $[\alpha]_D$ −5·7° (OMe, 40·7%) (5% yield), was recovered from the fractionating liquors. The hydrolysate of B was examined only by chromatography. The methylated fraction of M insoluble in chloroform, but soluble in water, had a high uronic acid content and was subsequently [3a] converted into the methylated methyl ester C (48% yield), having $[\alpha]_D$ +66° (OMe, 38·3%) which yielded mainly methyl ethers of galactose, rhamnose, galacturonic acid, and glucuronic acid on hydrolysis.

Methylated polysaccharide A gave mainly a mixture of methylated pentoses after hydrolysis, with some methylated barium uronates. After fractionation on cellulose,[16] the first major fraction, chromatographically identical with 2,3,4-tri-*O*-methylxylose, was unfortunately lost. 2,5-Di- and 2,3-di-*O*-methyl-L-arabinose, major components, were identified as crystalline derivatives. The fourth major component, a monomethylpentose, appeared to be 2-*O*-methyl-L-arabinose, and co-chromatographed with an authentic sample which was synthesised [17,18] later. The molar proportions of the 2,5-di-, 2-mono-, and 2,3-di-*O*-methyl ethers were 1 : 1 : 0·5. In order to identify the first major component, the methylation of M was repeated, but only the first two major components of the hydrolysate of the chloroform soluble material collected. Crystalline 2,3,4-tri-*O*-methyl-D-xylose and 2,5-di-*O*-methyl-L-arabinose were obtained in the molar ratio of 0·8 : 1.

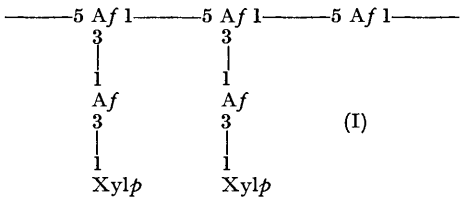
3-*O*-Methyl-L-rhamnose was a minor component. Traces of 2,3,5-tri-*O*-methylarabinose, tetra-*O*-methylgalactose, 2,3,6-tri-*O*-methylgalactose, and a di-*O*-methylhexose (probably 2,6-di-*O*-methylgalactose) were identified by chromatography, as were arabinose, xylose, rhamnose, and 2,3,4-tri-*O*-methylglucuronic acid.

The methylated barium uronates (about 10% of the hydrolysate) were investigated only chromatographically. 2,3,4-Tri-*O*-methylglucuronic acid and possibly di-*O*-methylgalacturonic acid were identified among the free acids. Prolonged hydrolysis afforded 3-*O*-methylrhamnose and a dimethylhexose. From subsequent work [3a] it is apparent that the above methyl ethers, here present in small amounts, are due to contamination of the pentosan by the uronic acid–galactose–rhamnose fraction of the mucilage.

The occurrence of almost equimolar proportions of 2,3,4-tri-*O*-methyl-D-xylose, 2,5-di-, and 2-mono-*O*-methyl-L-arabinose (0·8 : 1·0 : 1·0), together with less 2,3-di-*O*-methyl-L-arabinose (0·5 molar proportion) suggests a structure such as (I), a xylo-araban, for the pentose-rich fraction of cress-seed mucilage. (Araboxylans, frequently isolated from cereals, refer to structures containing a xylan backbone and possessing arabinose-containing side-chains). Terminal arabinofuranose units are not a feature of the polysaccharide, and the proportion of 2,5-dimethylarabinose tends to preclude the occurrence of more than one 1,3-linked arabinofuranose unit in the side-chain. Alternatively, the backbone may contain 1,4-linked arabinopyranose units.

An alternative structure which could accommodate the methylation results would contain single-unit side-chains of xylose, and a backbone of 1,5- and 1,3-linked arabinofuranose residues. The identification of 3-*O*-α-D-xylopyranosyl-L-arabinose, in reasonable yield, and of the trisaccharide *O*-α-D-xylopyranosyl- (1 ⟶ 3)-*O*-α-L-arabinofuranosyl-(1 ⟶ 3)-L-arabinopyranose among the autohydrolysis products of the whole mucilage supports structure (I).

[16] L. Hough, J. K. N. Jones, and W. H. Wadman, *J.*, 1949, 2511.
[17] J. Honeyman, *J.*, 1946, 990.
[18] G. G. S. Dutton and Y. Tanaka, *Canad. J. Chem.*, 1961, **39**, 1797.



(Xylp = D-xylopyranose, *Af* = L-arabinofuranose.)

The pectic arabans so far investigated (apple,[19] citrous,[20] peanut,[21,22] and sugar beet [23]) are recognised as highly branched structures. The ease of hydrolysis, the highly negative rotation of the polysaccharides and of the methylated derivatives (*e.g.*, peanut araban,[21] $[\alpha]_D$ −160°; methylated derivative [22] $[\alpha]_D$ −180°), and the isolation of two disaccharides, 3-*O*-α- and 5-*O*-α-L-arabinofuranosyl-L-arabinose, from sugar-beet araban, suggest that the arabans are composed of α-linked arabinofuranose units.

The methylated pentosan fraction isolated from cress-seed mucilage had $[\alpha]_D$ −29°, a far more positive value than the arabans display, which must be attributed to the dextrorotary contribution of the α-linked D-xylopyranosyl residues, and only to a slight extent to the contaminating acidic fraction.

The pentosan, thus crudely separated from the uronic acid–galactose–rhamnose fraction of whole cress-seed mucilage during methylation, differs from the mucilages extracted from seeds of *Plantago arenaria*,[24] *P. lanceolata*,[25] and *P. ovata* Forsk,[26] which are essentially highly branched xylans containing some terminal arabinofuranose units. The heterogeneity of the mucilages from *P. arenaria* and *P. ovata* has recently been demonstrated.[27] Linseed mucilage (from *Linum usitatissimum*) which has been rigorously fractionated,[27,28] yields a neutral pentosan similar to those present in the mucilages of the *Plantago* species. Like cress-seed mucilage, quince-seed [29] and white-mustard-seed [30] mucilages contain high percentages of cellulose and uronic acid. Some components have been identified, but no structural features of either mucilage have been determined. The heterogeneity of white-mustard-seed mucilage has been indicated.[1]

Although this investigation was initiated before the more elegant fractionation techniques, such as ion-exchange cellulose chromatography,[31] were regularly applied to carbohydrates, it is evident that the mucilage is heterogeneous, and consists of (at least) three polysaccharides, namely, cellulose, a xylo-araban, and a complex acidic polysaccharide containing D-galacturonic and 4-*O*-methyl-D-glucuronic acids, besides D-galactose, L-rhamnose, and D-xylose.

Experimental

Paper partition chromatography was performed on Whatman No. 1 paper with the upper layers of, or, the following solvent systems (v/v): *A*, ethyl acetate–pyridine–water (10 : 4 : 3); *B*, ethyl acetate–acetic acid–water (3 : 1 : 3); *C*, ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); *D*, butan-1-ol–pyridine–water–benzene (5 : 3 : 3 : 1); *E*, butan-1-ol–ethanol–water (4 : 1 : 5); *F*, butan-1-ol–acetic acid–water (4 : 1 : 5); *G*, pentan-2-one, 75% water

[19] E. L. Hirst and J. K. N. Jones, *J.*, 1939, 454.
[20] G. H. Beaven, E. L. Hirst, and J. K. N. Jones, *J.*, 1939, 1865.
[21] E. L. Hirst and J. K. N. Jones, *J.*, 1939, 452.
[22] E. L. Hirst and J. K. N. Jones, *J.*, 1947, 1221.
[23] E. L. Hirst and J. K. N. Jones, *J.*, 1948, 2311.
[24] W. A. G. Nelson and E. G. V. Percival, *J.*, 1942, 58; E. L. Hirst, E. G. V. Percival, and C. B. Wylam, *J.*, 1954, 189.
[25] J. Mullan and E. G. V. Percival, *J.*, 1940, 1501; E. G. V. Percival and I. C. Willox, *J.*, 1949, 1608.
[26] R. A. Laidlaw and E. G. V. Percival, *J.*, 1949, 1600; 1950, 528.
[27] A. J. Erskine and J. K. V. Jones, *Canad. J. Chem.*, 1956, **34**, 821.
[28] A. J. Erskine and J. K. N. Jones, *Canad. J. Chem.*, 1957, **35**, 1174; K. Hunt and J. K. N. Jones, *ibid.*, 1962, **40**, 1266.
[29] A. G. Renfrew and L. H. Cretcher, *J. Biol. Chem.*, 1932, **97**, 503.
[30] K. Bailey and F. W. Norris, *Biochem. J.*, 1932, **26**, 1609.
[31] H. Neukom, H. Deuel, W. J. Heri, and W. Kündig, *Helv. Chim. Acta*, 1960, **43**, 67.

saturated; *H*, benzene–ethanol–water (169 : 47 : 15); *I*, ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3).[32] R_{Gal}, R_{Rh}, R_{GluA}, R_{GalA}, R_G refer to the R_F values with respect to galactose, rhamnose, glucuronic acid, galacturonic acid, and 2,3,4,6-tetra-O-methylglucose respectively, the latter in solvent *E*. Sulphuric acid hydrolysates were neutralised with barium carbonate or barium hydroxide; barium sulphate was removed, the residues washed with water, and the combined solutions evaporated to dryness and examined in a basic solvent (*A* or *D*) for neutral sugars. When hydrolysates were examined for acidic fragments, barium ions were removed with IR-120 (H) resin, and the concentrated solutions examined in solvents *C*, *F*, or *I*. Hydrochloric acid hydrolysates were neutralised with silver carbonate, and the silver ions removed with hydrogen sulphide when free acids were to be examined. Paper ionophoresis [33] was in borate buffer at pH 10. Sugars were detected with aniline hydrogen oxalate,[34] which was acidified (acetic acid) when used on ionophoretograms. Specific rotations were measured in water, except when stated otherwise, and at 20° ± 2°. Equivalent weights were determined by titration with carbonate-free 0·01N-sodium hydroxide solution, and uronic acid anhydride content by the decarboxylation method [35] (by, or through the courtesy of, Dr. D. W. A. Anderson). Periodate oxidation products [4] of methylated sugars were examined on chromatograms in solvent *E*. Demethylations were effected with hydrobromic acid [36] and the products examined chromatographically using solvent *A*. Aldonic acids were prepared by oxidation of the reducing sugar with bromine, the excess being removed by aeration; hydrobromic acid was neutralised with silver carbonate, and hydrogen sulphide passed to give the free aldonic acid. Evaporations were carried out under reduced pressure at 40°.

Extraction of the Mucilage.—*Lepidium sativum* seeds (1 kg.) were soaked in water (10 l.) for 20 hr. Centrifugation gave a viscous supernatant (fraction 1) and forced some gelatinous capsular material away from the seeds. Further extraction (for 20 hr. with 10 l. and 6 l. of water, respectively) gave less-viscous solutions (fractions 2 and 3). The seeds were squeezed in muslin to remove some capsular gel, fraction 4. Extraction with water at 80° gave another fraction, 5. Each fraction was evaporated down to *ca.* 1 l. and precipitated into ethanol (4 volumes) or acidified ethanol (containing 20 ml. concentrated hydrochloric acid/l.) as the mucilage salt (series I) or acid (series II). The samples were dried by solvent exchange (ethanol, followed by ether), and finally over phosphorus pentoxide *in vacuo*.

Fraction	1	2	3	4	5
Series I (g.)	62	28	11	12	
Series II (g.)	21	9	4	4	11
Ash (%) in II	0·4	0·3	0·7	1·0	10·0

In a second series of experiments, 2 kg. of seeds were used to obtain three fractions corresponding to 1—3 above; these were bulked, reduced to 3 l., and precipitated into 9 l. of acidified ethanol, when a fibrous material R (43 g.) separated; addition of an equal volume of ethanol to the supernatant gave a white powdery precipitate M (27 g.). R and M were used in the principal experiments (autohydrolysis and methylation). [Found, for R and M respectively (the presence in R of 9% cellulose, a value obtained from the large-scale autohydrolysis experiment, is allowed for in the calculations) (ash-free): Equiv., 620, 570; uronic acid anhydride (by calculation), 28·4, 30·9%; $[\alpha]_D$ +54° (?), +66°; N, 0·5, 0·2%; OMe, *ca.* 2, 2—3%].

Acid Hydrolysis of Cellulose-rich Whole Mucilage, R, *and Cellulose-free Whole Mucilage*, M.—Separation, isolation,[37] and determination [38] of the neutral sugars liberated after 16 hr. hydrolysis (N-H_2SO_4; 100°) showed the presence of galactose, arabinose, xylose, and rhamnose in the proportions, as anhydro-sugars, of 10 : 13 : 7 : 7 in R, and 10 : 12 : 8 : 8 in M. Galacturonic acid and 4-*O*-methylglucuronic acid were major components in R and M, but glucuronic acid and glucurone were absent, on chromatograms run in solvent *I*, from both samples. Traces of glucose were detected. A component, R_{Gal} 1·13, was present in the half-hour hydrolysates of R and M, and another, R_{Gal} 0·72, was detected in the hydrolysate of M; only traces of rhamnose

[32] F. G. Fischer and H. Dörfel, *Z. physiol. Chem.*, 1955, **301**, 224.
[33] A. B. Foster, *Chem. and Ind.*, 1952, 828.
[34] S. M. Partridge, in "Partition Chromatography," Biochemical Society Symposia, No. 3, Cambridge University Press, 1951, p. 5.
[35] D. W. A. Anderson, *Talanta*, 1959, **2**, 73.
[36] L. Hough, J. K. N. Jones, and W. H. Wadman, *J.*, 1950, 1702.
[37] A. E. Flood, E. L. Hirst, and J. K. N. Jones, *J.*, 1948, 1679.
[38] M. Somogyi, *J. Biol. Chem.*, 1952, **195**, 19.

were liberated under these conditions, although arabinose, galactose, and xylose were major components.

Isolation and Identification of the Uronic Acids from Whole Cellulose-rich Mucilage, R.—The mucilage (2·50 g.) in N-sulphuric acid (125 ml.) was heated at 98° (6 hr.). The cold hydrolysate was neutralised (barium carbonate, etc.), concentrated, and barium ions removed. The eluate and washings were concentrated and dried (2·07 g.). After dissolution in water, acidic material was absorbed on Duolite A4 (OH) resin, and the neutral sugars were washed through (1·34 g. recovered, 54% yield). Acids were eluted with 0·5N-ammonium hydroxide, the column washed, the eluate deionised with IR-120 (H) resin, and free acids recovered (0·64 g., 26% yield). Chromatography in solvent *I*, against authentic samples, revealed the presence of galacturonic acid and 4-*O*-methylglucuronic acid (R_{GluA} 1·30, R_{GalA} 1·45), together with a complex mixture of components of lower R_F. Separation on 3 MM paper, with solvent *I*, afforded galacturonic acid (63 mg.), $[\alpha]_D$ +67° (*c* 0·61) (lit., +51°), contaminated with a component which was probably an aldobiouronic acid containing 4-*O*-methyl-glucuronic acid. Glucuronic acid was absent. Formation of the *p*-bromophenylhydrazine *p*-bromophenylhydrazone galacturonate [39] (m. p. 133—135°, mixed m. p. 139—141° with an authentic specimen, m. p. 145—146°), and oxidation to mucic acid, m. p. 210°, and mixed m. p. 213—215° with an authentic sample of m. p. 218—219°, confirmed the presence of D-galacturonic acid. The second fraction (11 mg.), $[\alpha]_D$ +25° (*c* 0·32) (lit.,[40] for 4-*O*-methyl-D-glucuronic acid, +35°), gave 4-*O*-methyl-glucose (identical with an authentic sample) after esterification, borohydride reduction, and hydrolysis; the sugar gave glucose on demethylation.

Dissociation of " Cellulose " from Whole Cellulose-rich Mucilage, R.—Samples (2·00 g.) of series (II/1—II/4) were heated with 2N-sulphuric acid (200 ml.) for 2·5 hr. on a boiling-water bath. Suspended material was filtered off, washed, and dried. The fibrous residues constituted 6, 3, 8, and 18% of the original samples.

Samples (1·00 g.) of mucilage II/1 were heated with 0·05, 0·1, 0·2, 0·5, and 1·0N-sulphuric acid (100 ml.) for 2 hr. as above. The insoluble material constituted 9, 8, 7, and 7% of the original sample. The hydrolysates were neutralised (barium carbonate, etc.), reduced in volume to 5 ml., and degraded mucilage recovered after precipitation with ethanol (100 ml.) in 56, 53, 35, 21, and 6% yields, respectively. Supernatants were concentrated and examined in solvent *A*, when xylose and arabinose were found to predominate; the amount of galactose increased with increasing acid strength. A trace of rhamnose was present in the 1N-acid hydrolysate. Components with R_{Gal} 1·13 and 0·90 were evident in the hydrolysates produced at lower acid concentration, but less so, in proportion to the monosaccharides, in the 0·5N- and 1N-acid hydrolysates.

Autohydrolysis of the Cellulose-free Whole Mucilage, M.—The mucilage (10 g.) in water (2 l.) was heated at 98° for 96 hr. Only 0·4% of fibrous material was recovered. Degraded mucilage N [recovered in 38% yield by precipitating the evaporated solution (100 ml.) into ethanol (750 ml.)] had $[\alpha]_D$ +90° (*c* 0·50), Equiv., 463, and proportions of galactose : arabinose : xylose : rhamnose of 10 : 1 : 1 : 14 (16 hr., 1N-sulphuric acid, 100°). Arabinose and galactose, some xylose, a trace of rhamnose, and oligosaccharides with R_{Gal} 1·10 (which partly obscured the galactose), and 0·87 were distinct in the autohydrolysis syrup. Oligosaccharides of lower R_F were present. The pattern resembled that given by syrup S, from the 72-hr. autohydrolysate of mucilage R, (cf. later).

Preliminary Experiments on the Autohydrolysis of Cellulose-rich Whole Mucilage, R, *and Acid Hydrolysis of the Recovered Degraded Mucilage*.—Autohydrolysis of 0·5% or 1% aqueous suspensions of mucilage, R, heated at 98°, were followed (after removal of fibrous material by centrifugation) by reducing power, optical rotation (which did not alter appreciably), and chromatography. The reducing power continued to increase at 95 hr. In 6 hr., small amounts of arabinose and xylose, less galactose, and a pentose-containing oligosaccharide, R_{Gal} 1·11 in solvent *A*, were produced, and all were present in considerable quantity after 23 hr. Two other oligosaccharides, R_{Gal} 0·87 and 0·73 in *A*, were obvious after 30 hr. After 72 or 92 hr., arabinose was the main component; xylose and galactose (partly obscured by the component, R_{Gal} 1·1) were present; rhamnose was scarcely detectable, but the three oligosaccharides were evident, together with oligosaccharides of lower R_F.

Degraded mucilage recovered from a 72-hour hydrolysate had $[\alpha]_D$ +76° (*c* 1·00), Equiv.,

[39] C. Neimann, E. Schoeffel, and K. P. Link, *J. Biol. Chem.*, 1933, **101**, 337.
[40] W. D. S. Bowering and T. G. Timell, *Canad. J. Chem.*, 1960, **38**, 311.

515, and ratios of anhydrogalactose : arabinose : xylose : rhamnose of 10 : 4 : 4 : 10; galacturonic acid and 4-*O*-methylglucuronic acid were also detected.

Large-scale Autohydrolysis of Whole Cellulose-rich Mucilage, R.—A solution of the mucilage (20·0 g.) in water (2 l.) had pH 2·9, and was heated at 98° for 72 hr. Suspended gelatinous material was centrifuged down, washed (with water, ethanol, and ether) and the brown fibrous residue, U1, dried (1·75 g., 9%). The supernatant and washings were reduced to 120 ml., and the solution was poured into ethanol (2 l.) to precipitate the degraded mucilage, T1, which was finally obtained as a heavy white powder (6·68 g., 33%). Evaporation of the supernatant and washings gave a syrup S1 (9·73 g., 49%) which was fractionated on charcoal–Celite, etc.

Repetition of the experiment gave insoluble residue, U2 (1·72 g.), degraded mucilage, T2 (8·54 g.), and syrup, S2 (8·08 g.).

The degraded mucilages, T1 and T2, had, respectively, $[\alpha]_D$ +79° (*c* 0·89), +92° (*c* 0·53); Equiv., 492, 470; proportions of anhydrogalactose: arabinose : xylose : rhamnose (16 hr. hydrolysis, N-sulphuric acid, 100°) 10 : 3 : 3 : 10 and 10 : 4 : 3 : 9. Traces of glucose and mannose were distinct in both hydrolysates. In solvent *I*, galacturonic acid (major component) and 4-*O*-methylglucuronic acid (minor component) were present, but glucurone and glucuronic acid were absent. Several components of low R_F were present.

Further Autohydrolysis of the Degraded Mucilages, T1 *and* T2, *Derived from Cellulose-rich Whole Mucilage*.—1% Solutions of the mucilages (6·0 and 8·0 g. of T1 and T2, respectively) were autohydrolysed for 72 hr. then evaporated to small volume (40 and 60 ml., respectively) and precipitated into ethanol (2 l.). Degraded mucilages, V1 and V2, (2·29 and 2·47 g.) were recovered. Syrups (2·89 and 3·69 g.), recovered from the supernatants were examined in solvent *A*, when galactose (main component), arabinose, and xylose were detected. Rhamnose was a distinct but minor component, and the oligosaccharides with R_{Gal} 1·13 and 0·73 were evident. No glucurone was present on chromatograms run in solvent *F*. Galacturonic acid was present, and several oligosaccharides of low R_F. The chromatographic patterns resembled those of syrups from the first degradation, but the pentoses and pentose-containing oligosaccharides were less prominent.

The mucilage samples had, respectively, $[\alpha]_D$ +71° (*c* 0·77), +92° (*c* 0·48); Equiv., 549, 481; OMe, 1·2, 1·6, and contained galactose, xylose, and rhamnose in the ratios 10 : 2 : 16 and 10 : 2 : 15. Both hydrolysates showed traces of glucose, mannose and arabinose (the last two sugars were separated in solvent *B*). The hydrolysate of V2 was examined in solvents *F* and *I*, after Ba^{2+} ions had been removed. Galacturonic acid and 4-*O*-methylglucuronic acid were present, the former predominating, but no traces of glucurone or glucuronic acid were detected.

Esterification, Reduction, and Hydrolysis of the Twice-Degraded Mucilage, V2.—The mucilage (500 mg.), $[\alpha]_D$ +92°, was esterified by refluxing for 6 hr. with 1% methanolic hydrogen chloride. The isolated esterified polysaccharide (490 mg.), in water (10 ml.) was reduced with potassium borohydride solution (500 mg. in 5 ml. water) and the mixture left at room temperature for 5 hr. K^+ ions were removed with IR-120 (H) resin, and borate by repeated distillation with methanol. Portions of the final product, having $[\alpha]_D$ +87° (*c* 1·24), were hydrolysed with N-sulphuric acid at 100° for 4 and 16 hr. Traces of glucose, mannose, and xylose were present. Galactose and rhamnose were the main components, and a new component, R_{Rh} 0·87, was identical with authentic 4-*O*-methylglucose.

The remainder of the reduced polysaccharide (275 mg.) was hydrolysed (20 ml.; N-H_2SO_4; 4 hr.; 100°). The sugars isolated after paper separation were galactose (95 mg., crystalline, and chromatographically pure), xylose (8 mg.), 4-*O*-methylglucose (13 mg.) and rhamnose (73 mg.). The 4-*O*-methylglucose, $[\alpha]_D$ +48° (*c* 0·34), contained a trace of rhamnose. Periodate oxidation [4] gave the component (citrine), R_F 0·60 in solvent *E*, characteristic of a 4-monomethylhexose. Attempts to prepare the osazone were unsuccessful.

Examination of the Fibrous Residue, U.—A sample (500 mg.) was heated with 2N-sulphuric acid (25 ml.) for 4 hr. on a boiling-water bath. Gelatinous material was centrifuged down, dried, and similarly treated with a proportionately smaller amount of 2N-acid. The process was repeated six times. Each time the centrifugate and aqueous washings were neutralised ($BaCO_3$, etc.) and examined by chromatography in solvents *A*, *B*, and *I*. Galactose, glucose, mannose, arabinose, xylose (main component), rhamnose, and galacturonic acid were present in the first hydrolysate, but only glucose (in increasing intensity), mannose, and xylose (in decreasing intensity) were present thereafter.

The final fibrous residue (126 mg.) was suspended in 72% (w/w) sulphuric acid (5 ml.) at room temperature for 7 days. The acid was diluted to 2N, the suspension heated (98°; 4 hr.), and insoluble material (7 mg.) centrifuged down. The supernatant was neutralised ($BaCO_3$, etc.) and examined in solvents *A* and *I*, when a trace of xylose, some mannose, and much glucose were found.

A portion of the syrup was fractionated on 3 MM paper and gave: (*a*) mannose (3 mg.; containing a trace of glucose) identified as its phenylhydrazone,[41] m. p. and mixed m. p. 189—190° with an authentic sample of m. p. 190—191°: (*b*) a sample (11 mg.), $[\alpha]_D$ +57° ⟶ +50° (*c* 0·72), of chromatographically pure D-glucose. The derived 2,4-dinitrophenylhydrazone had m. p. 108—110° and mixed m. p. 117—120°, with an authentic specimen of m. p. 122—124°.

Separation and Identification of the Monosaccharides and Neutral Oligosaccharides obtained on Autohydrolysis of Cellulose-rich Mucilage, R.—The syrup, S1, (9·7 g.) in water (10 ml.) was applied to a charcoal–Celite column (1 : 1; 6·8 × 15 cm.). Water, followed by dilute ethanol, was used as eluant. Each fraction (0·5 l.) was treated with IR-4B (OH) resin to remove acidic sugars. The products were examined by chromatography in *A*, and appropriate fractions combined.

Fraction 1, (0·215 g., eluted with water), crystalline, was arabinose.

Fraction 2 (1·88 g., eluted with water) was fractionated[6] on a cellulose column (4·5 × 45 cm.) with butan-1-ol, saturated with water. The following fractions were obtained: (*a*) L-rhamnose (29 mg.), $[\alpha]_D$ +17·5° ⟶ +10·5° (equil., *c* 0·57), (2,5-dichlorophenylhydrazone,[42] m. p. 167—168°, and mixed m. p. 169—170°, with an authentic specimen of m. p. 172°); (*b*) a mixture (15 mg.) of rhamnose, ribose, and xylose; ribose predominated and was identified chromatographically in solvents *A*, *D*, and *E*; (*c*) a syrup (35 mg.) consisting of xylose and ribose (trace); (*d*) crystalline D-xylose (138 mg.), $[\alpha]_D$ +30·2° ⟶ +18·9° (*c* 0·53), m. p. and mixed m. p. 144—145° (dibenzylidene dimethyl acetal derivative,[43] m. p. 208—209°, and mixed m. p. 210—211°); (*e*) a syrup (238 mg.) consisting of D-xylose and L-arabinose with $[\alpha]_D$ +42° (equil., *c* 1·29) (*i.e.*, 73% D-xylose present); (*f*) crystalline L-arabinose (592 mg.), $[\alpha]_D$ +150·6° ⟶ +107·0° (*c* 0·60), m. p. 160—161°, and mixed m. p. 158—159°, with an authentic specimen of m. p. 155° (benzoylhydrazone,[44] m. p. and mixed m. p. 195—196°); (*g*) crystalline D-galactose (156 mg.), $[\alpha]_D$ +133° ⟶ +73° (*c* 0·17), of m. p. 159—160° and mixed m. p. 163—164° with an authentic specimen of m. p. 163—164° (methylphenylhydrazone,[44] m. p. 181—182° and mixed m. p. 184—185° with an authentic specimen of m. p. 186—187°; 2,5-dichlorophenylhydrazone,[42] m. p. and mixed m. p. 197—198°); (*h*) a syrup (66 mg.) consisting of galactose and disaccharide A.

Fraction 3, (2·70 g., eluted with water, 2% ethanol, and 5% ethanol), a syrup, contained traces of monosaccharides, the oligosaccharide, R_{Gal} 1·11, as the main component, a second with R_{Gal} 0·72, and traces of higher oligosaccharides. Fractionation of a part (1·68 g.) on cellulose (3·2 × 15 cm.) with butan-1-ol saturated with water, followed by separations on thick paper with solvent *A*, gave as the main fractions (*a*) disaccharide A (694 mg., crystalline, m. p. 100—105°); (*b*) crude disaccharide B (138 mg.); (*c*) a mixture of oligosaccharides (108 mg.) containing some trisaccharide C.

Identification of Disaccharide A.—Crystallisation from aqueous ethanol gave small prisms of m. p. 115—116°, with $[\alpha]_D$ +185° (*c* 0·20); no mutarotation was observed. No change in the optical rotation occurred on heating a solution of the material (11·7 mg.) in 0·01N-hydrochloric acid (5 ml.) at 85° for 6 hr. Hydrolysis (N-H_2SO_4; 2 hr.; 100°) gave arabinose and xylose in equal amounts (visual estimate). Hydrolysis of the derived aldonic acid gave xylose. Lime-water degradation,[12] the product being examined in solvent *A*, gave only xylose. The osazone, chromatographically pure on circular paper chromatography,[45] using toluene–ethanol–water (270 : 30 : 1), had m. p. 228° (lit.,[7] 226°), and from its absorption[46] at 394 mμ had *M* 444 (Calc. for a pentose disaccharide osazone, 460). After periodate oxidation,[14] the formaldehyde dimedone derivative (m. p. and mixed m. p. 188—189°) was isolated in 61% yield (*i.e.*, for 1 mole formaldehyde/mole disaccharide). Chromatography in solvents *A*, *C*, and *E* (R_{Gal} 1·11, 0·94, 0·78, respectively) identified disaccharide A as 3-*O*-α-D-xylopyranosyl-L-arabinose, confirmed by mixed m. p. and *X*-ray powder photography.

[41] E. Glaser and N. Zuckerman, *Z. physiol. Chem.*, 1927, **167**, 37.
[42] I. Mandl and C. Neuberg, *Arch. Biochem. Biophys.*, 1952, **35**, 326.
[43] L. J. Breddy and J. K. N. Jones, *J.*, 1945, 738.
[44] E. L. Hirst, J. K. N. Jones, and E. A. Woods, *J.*, 1947, 1048.
[45] V. C. Barry and P. W. D. Mitchell, *J.*, 1954, 4020.
[46] V. C. Barry, J. E. McCormick, and P. W. D. Mitchell, *J.*, 1955, 222.

Purification and Identification of Disaccharide B.—After three fractionations on paper with solvent *A*, the recovered material (53 mg.), which could not be crystallised, had $[\alpha]_D$ +118·2° ⟶ +116·3° (*c* 1·48); chromatographic examination in solvent *C* revealed traces of disaccharide A and trisaccharide C. The material was treated as follows. Hydrolysis gave xylose and galactose in equal amounts (visual estimate), and a trace of arabinose; hydrolysis of the bromine oxidation product gave xylose; degradation by lime-water gave xylose. After periodate oxidation,[14] the formaldehyde dimedone derivative (m. p. and mixed m. p. 187—188°) was isolated in 81% yield (for 1 mole formaldehyde/mole disaccharide). Alkaline hypoiodite oxidation [13] (6 hr.), gave *M* 305 (Calc. for $C_{10}H_{18}O_9$, 312). The remaining material was fractionated once, prior to methylation, on paper with solvent *C*. The product, $[\alpha]_D$ +96° (*c* 2·61) retained a trace of material with a lower R_F value.

Methylation of Disaccharide B (36 mg.) by Haworth's reagents followed Srivastava and Smith's method,[47] and the isolated partially methylated product (38 mg.) was twice methylated with methyl iodide (10 ml.) and silver oxide (1 g.). The methylated disaccharide (33 mg.) had $[\alpha]_D$ +104·9° (*c* 0·61), and was hydrolysed (10 ml. N-HCl; 3 hr.; 100°). After neutralisation (Ag_2CO_3), the recovered sugars (29 mg.), were fractionated on No. 1 paper with solvent *E*. *Fraction* (*a*) (5·1 mg., a loss was incurred) crystallised, and had $[\alpha]_D$ +25·0° (*c* 0·44). The sugar was chromatographically pure and identical in solvents *A* and *E*, R_G 0·97, with 2,3,4-tri-*O*-methylxylose, and was identified, after recrystallisation, as 2,3,4-tri-*O*-methyl-D-xylose by m. p. and mixed m. p. 83—84°, and from its rotation. *Fraction* (*b*) (11·5 mg.), a syrup with $[\alpha]_D$ +80·7° ⟶ +95·0° (equil., *c* 0·50), was chromatographically pure, R_G 0·77, and identical in solvent *A* with 2,3,6-tri-*O*-methylgalactose (R_G 0·80), and distinct from the 2,4,6-isomer (R_G 0·73). The derived aldonolactone had m. p. and mixed m. p. 95—96° with an authentic sample of 2,3,6-tri-*O*-methyl-D-galactonolactone. *Fraction* (*c*) (1 mg.) was chromatographically pure and identical in solvent *E* with 2,4-di-*O*-methylarabinose, R_G 0·63.

Fraction 4 (443 mg.; eluted with 10% ethanol) consisted mainly of trisaccharide C, R_{Gal} 0·87 in solvent *A*, together with some mono- and di-saccharides. After fractionation on cellulose (3·0 × 37 cm.) with butan-1-ol saturated with water, purified trisaccharide C (212 mg.) was recovered as a glass.

Identification of Trisaccharide C.—After rigorous drying, a crystalline mass of m. p. 99—100°, with $[\alpha]_D$ +98·2° ⟶ +95·2° (*c* 0·34), was obtained. The *trisaccharide* was extremely hygroscopic and efforts to recrystallise it failed, as did attempts to prepare a specimen for an *X*-ray powder photograph. The sugar appeared to be chromatographically pure in solvents *A* (R_{Gal} 0·87—0·90), *C*, and *D* (R_{Gal} 0·84). Hydrolysis (2 hr.; N-acid; 100°) gave xylose and arabinose in the ratio of 1 : 1·9. Hydrolysis of the derived aldonic acid gave xylose and arabinose in equal proportions (visual estimate). Alkaline hypoiodite oxidation gave *M* 428 (Calc. for $C_{15}H_{26}O_{13}$: *M*, 414). The product fram lime-water degradation showed the presence of unchanged trisaccharide C, 3-*O*-α-D-xylopyranosyl-L-arabinose, and xylose, in solvent *A*.

Partial hydrolysis of the trisaccharide (10 mg.) by 0·1N-sulphuric acid (2 ml.) at 100° was followed chromatographically in solvents *A* and *C* from 10 min. to 2 hr. (3-*O*-α-D-Xylopyranosyl-L-arabinose, 3-*O*-β-L-arabinopyranosyl-L-arabinose, and trisaccharide C had R_{Gal} values of 0·90, 0·77, and 0·62, respectively, in solvent *C*). The intensity of the trisaccharide spot gradually decreased; a trace of xylose was detected in 10 min., but did not increase appreciably thereafter. Arabinose and 3-*O*-α-D-xylopyranosyl-L-arabinose, both present in 10 min., increased in intensity during the hydrolysis. No 3-*O*-β-L-arabinopyranosyl-L-arabinose was detected.

Formaldehyde, as the dimedone derivative (m. p. and mixed m. p. 190—191°) was obtained in quantitative yield (1 mole/mole of trisaccharide) after periodate oxidation.[13]

Methylation of Trisaccharide C.—The trisaccharide (240 mg.) was twice methylated with Haworth reagents and the product isolated, after neutralisation, by continuous (16 hr.) chloroform extraction. After two methylations with Purdie reagents, the methylated trisaccharide (225 mg.) was hydrolysed with N-hydrochloric acid (20 ml.). The syrup (208 mg.), recovered after neutralisation with silver carbonate, was fractionated on cellulose (1·4 × 43 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, as eluant, and yielded the following fractions. *Fraction* (*a*), (52 mg.) identical in solvents *E* (R_G 0·95) and *H* with 2,3,4-tri-*O*-methylxylose, was crystalline and had $[\alpha]_D$ +59·5° ⟶ +23·8° (*c* 0·42). The sample, after recrystallisation, had m. p. 83—84° and mixed m. p. 84—85°, with an authentic sample

[47] H. C. Srivastava and F. Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 982.

of 2,3,4-tri-*O*-methyl-D-xylose of m. p. 84°. *Fraction* (*b*), a syrup (64 mg.), was shown by chromatography in *E* (R_G 0·83) and *H* to be 2,5-di-*O*-methylarabinose contaminated with a trace of 2,3,4-tri-*O*-methylxylose. The optical rotation $[\alpha]_D$ −21·9° (*c* 1·28), indicated that 97% of the fraction was 2,5-di-*O*-methyl-L-arabinose (lit.,[48] value for synthetic D-form is +23°) and the sugar was characterised by conversion into 2,5-di-*O*-methyl-L-arabonamide of m. p. and mixed m. p. 128—129°. *Fraction* (*c*), a syrup (45 mg.) with $[\alpha]_D$ +135·4° (*c* 0·84), gave only arabinose on demethylation. The syrup gave no colour with triphenyltetrazolium hydroxide spray,[49] indicating the presence of a methoxyl group at C-2, and was chromatographically pure in solvents *H* and *E* (R_G 0·63) and distinct from 2,3-di-*O*-methylarabinose (R_G 0·68). The sugar was identified by conversion into the aniline derivative having m. p. and mixed m. p. 124—126° with authentic 2,4-di-*O*-methyl-L-arabinose anilide; and by *X*-ray powder photography. *Fraction* (*d*) (4 mg.) contained 2,4-di-*O*-methylarabinose and a component with R_G 0·55. Arabinose was produced on demethylation. *Fraction* (*e*) (7 mg.), R_G 0·44, gave arabinose on demethylation, and was chromatographically identical with 2-*O*-methylarabinose.

Methylation of Whole Cellulose-free Mucilage.—The mucilage M (10 g.) was methylated five times by Haworth's procedure and, after careful neutralisation (pH 7) with acetic acid, the mixture was thoroughly extracted with chloroform. The resulting syrup (4·29 g.) (Found: OMe, 32·7%) was fractionally precipitated from chloroform (20 ml.) by light petroleum (160 ml., b. p. 60—80°) and gave, after trituration with light petroleum, a pale yellow powder A (2·07 g.) having $[\alpha]_D$ −23·6° (*c* 0·42 in chloroform) (Found: OMe, 35·0%). Methylation (seven times) with Purdie reagents gave a glass (1·70 g.) with $[\alpha]_D$ −28·5° (*c* 0·98 in $CHCl_3$) (Found: OMe, 39·3. Calc. for a dimethyl pentosan: OMe, 38·8%).

Evaporation of the chloroform–light petroleum liquors gave a syrup B (0·51 g.), $[\alpha]_D$ −5·7° (*c*, 0·35 in chloroform) (Found: OMe, 27·1%), which after six Purdie methylations had a methoxyl content of 40·7%.

The aqueous fraction from the Haworth methylations was dialysed for five days. It was then evaporated, giving an amorphous solid which was dissolved in water (40 ml.). Polysaccharide C, recovered by precipitation into ethanol (1 l.) had $[\alpha]_D$ +88·7° (*c* 0·45; ash free) (Found: ash, 14·4; OMe, 28·5%), yield 6·67 g. It was examined separately.[3a]

Hydrolysis of the Chloroform-soluble Methylated Polysaccharide A *and Identification of the Resulting Methylated Sugars.*—The polysaccharide (1·63 g.) was hydrolysed with 2N-sulphuric acid (200 ml.) at 37° for 16 days, and for four 6-hour periods at 97° during this time. The hydrolysate was neutralised [$Ba(OH)_2$, etc.]. The residue of methylated sugars and barium uronates was extracted with chloroform–methanol and yielded a syrup (1·185 g.) which was partitioned in cellulose (2·8 × 50 cm.) in the usual way.

Fraction 1, R_G 0·97, was chromatographically identical with 2,3,4-tri-*O*-methylxylose, but was lost.

Fraction 2. The syrup, (216 mg.) R_G 0·86, gave arabinose, and traces of glucose and galactose on demethylation. After fractionation on thick paper the main component, recovered as a syrup, $[\alpha]_D$ −21·1° (*c* 0·76) (Found: OMe, 34·8. Calc. for $C_7H_{14}O_5$: OMe 34·8%), was identified as 2,5-di-*O*-methyl-L-arabinose; paper ionophoresis showed no 3,5-isomer. The sugar was unchanged by periodate oxidation. The derived aldonamide had m. p. and mixed m. p. 128—129° with authentic 2,5-di-*O*-methyl-L-arabonamide. Accompanying trace components were identified by chromatography in solvents *A*, *E*, and *H* as 2,3,5-tri-*O*-methylarabinose, R_G 0·97, 2,3,6-tri-*O*-methylgalactose, R_G 0·75, and probably tetra-*O*-methylgalactose, R_G 0·92.

Fraction 3 (40 mg.) contained 2,3-di-*O*-methylarabinose (R_G 0·71 in *A*, 0·69 in *E*) as the main component, with traces of 2,3,5-tri-*O*- and 2,5-di-*O*-methylarabinose and some tri-*O*-methylhexose. Arabinose, and traces of glucose and galactose, were produced on demethylation.

Fraction 4. The syrup (92 mg.) contained a major component, R_G 0·69, and minor components, R_G 0·86, 0·79, and 0·59. Fractionation on thick paper with solvent *E* gave two fractions.

Fraction (*a*), a syrup (66 mg.), chromatographically pure after a second fractionation on paper, R_G 0·70, $[\alpha]_D$ +83·1° (*c* 0·60), and M_G 0, gave no colour with triphenyltetrazolium hydroxide spray (*i.e.*, C-2 substituted). Demethylation gave arabinose. The sugar was identical in solvent *A* with 2,3-di-*O*-methylarabinose, and distinct from the 2,4-isomer; the

[48] J. Fried and D. E. Walz, *J. Amer. Chem. Soc.*, 1952, **74**, 5468.
[49] K. Wallenfels, *Naturwiss.*, 1950, **37**, 491.

derived aldonamide had m. p. 147° and mixed m. p. 149—150° with an authentic sample of 2,3-di-*O*-methyl-L-arabonamide of m. p. 152°.

Fraction (*b*) (14 mg.), further separated with solvent *E*, gave 2,3-di-*O*-methylarabinose, R_G 0·69, and a component, R_G 0·59, identical in solvent *A* with 3-*O*-methylrhamnose and distinct from the 4-methyl ether. Periodate oxidation [4] gave a component, with R_F 0·75, identical with that from 3-*O*-methylrhamnose. Trace components were identified as 2,5-di-*O*-methylarabinose, tri-*O*-methylgalactose, and a di-*O*-methylhexose.

Fraction 5. The syrup (101 mg.) contained two main components, R_G 0·59 and 0·44, and a trace of 2,3-di-*O*-methylarabinose, R_G 0·69. Separation on paper with solvent *C* gave fractions (*a*) (45 mg.) and (*b*) (50 mg.). *Fraction* (*a*) on refractionation gave: (i) a syrup (21 mg.), $[\alpha]_D$ +20·0° (*c* 0·90), contained traces of 2,3-di-*O*-methylarabinose and a di-*O*-methylhexose (R_G 0·52), but the main component (R_G 0·59) was identified by ionophoresis, and from the rotation, as 3-*O*-methyl-L-rhamnose. Periodate oxidation [4] gave a component with R_F 0·16 (yellow) characteristic of a 2-methylaldose (*e.g.*, from a 2,6-di-*O*-methylhexose), and a second with R_F 0·73, characteristic of 3-*O*-methylrhamnose. *Fraction* (ii) contained 2,3-di-*O*-methylarabinose, 3-*O*-methylrhamnose (confirmed by periodate oxidation), and di-*O*-methylgalactose. *Fraction* (*b*), R_G 0·44, a syrup with $[\alpha]_D$ +88·2° (*c* 0·41) (Found: OMe, 17·0. Calc. for $C_6H_{12}O_5$: OMe, 18·9%), migrated as a single spot on ionophoresis. Demethylation gave arabinose. No red colour was produced with triphenyltetrazolium hydroxide spray (*i.e.*, C-2 substituted), and periodate oxidation yielded the yellow methoxymalondialdehyde characteristic of a 2-methylaldose. Attempts to prepare the crystalline aldonamide failed. The fraction was considered to be 2-*O*-methyl-L-arabinose, and this was subsequently confirmed by chromatography.

Fraction 6. The syrup (136 mg.), R_G 0·46, after purification on thick paper, had $[\alpha]_D$ +71·3° (*c* 0·88). The main component was concluded to be 2-*O*-methyl-L-arabinose from the rotation, behaviour on periodate oxidation, and demethylation, and it was chromatographically identical with a synthetic sample available later. The anilide and phenylhydrazone were prepared, but failed to crystallise. A trace of 2,3,4-tri-*O*-methylglucuronic acid was present in the crude fraction.

Fraction 7. (22 mg.) was a mixture of 2-*O*-methylarabinose, rhamnose, arabinose, xylose and several unknowns of lower R_F values.

Examination of the Acidic Fraction.—The water-wash (115 mg.) containing methylated barium uronates was examined in solvent *E*. Neutral sugars were absent. A sample (3 mg.) was converted into the free acids with IR-120 (H) resin, and the product examined in solvent *F*. Two main components, R_G 0·83 (identical with 2,3,4-tri-*O*-methylglucuronic acid) and 0·50 (possibly di-*O*-methylgalacturonic acid), were present, together with traces of components with lower R_G values (0·32, 0·25, 0·19).

Samples (5 mg.) were hydrolysed with N-hydrochloric acid (0·5 ml.) for 1, 8, and 18 hr., and examined, after neutralisation, for neutral sugars in solvent *E*, and in solvent *F* for free acids. A component with R_G 0·60, corresponding to 3-*O*-methylrhamnose, was produced in increasing amount during the hydrolysis, and was accompanied (18-hr. sample) by components with R_G 0·47 (a di-*O*-methylhexose) and 0·18. 2,3,4-Tri-*O*-methylglucuronic acid (in increasing amount) and 3-*O*-methylrhamnose, R_G 0·62, were identified in the acidic solvent *F*. Acidic material of low R_F and a streak (R_G 0·42 approx., probably di-*O*-methylgalacturonic acid) were also present.

Examination of the Chloroform-soluble Methylated Polysaccharide, Fraction B.—A sample (10 mg.) was heated at 100° for 12 hr. with methanolic 2% hydrogen chloride (1 ml.). Methanol was removed, the residue hydrolysed with N-hydrochloric acid (1 ml.) at 100° for 6 hr., and the hydrolysate examined in solvents *E* and *F*.

The pattern of sugars was similar in solvent *E* to that obtained from methylated polysaccharide A, but rhamnose and galactose derivatives were more prominent. The principal components were 2,3,4-tri-*O*-methylxylose, 2,5-di- and 2-mono-*O*-methylarabinose. 2,3-Di-*O*-methylarabinose, 3-*O*-methylrhamnose, and a di-*O*-methylgalactose were present in appreciable amounts, also traces of 2,3,4,6-tetra-*O*-methylglucose, 2,3,4,6-tetra-*O*-methylgalactose, tri-*O*-methylgalactose, and (?) arabinose. The pattern in *F* resembled that in *E*. Some 2,3,4-tri-*O*-methylglucuronic acid was probably present, but was partially obscured by the 2,5-di-*O*-methylarabinose.

Repetition of the Methylation of Whole Cellulose-free Mucilage, M, *Followed by the Isolation*

of the Chloroform-soluble Fraction and Its Examination.—A sample (5·0 g.) of mucilage was methylated and the chloroform-soluble material extracted as described previously, and concentrated. After chloroform extraction of the residue the resulting syrup (1·51 g.) was fully methylated (Found: OMe, 38·5. Calc. for dimethylpentosan: OMe, 38·8%), but the product (0·76 g.), having $[\alpha]_D$ −12·7° (*c* 2·02 in $CHCl_3$), had not been fractionated with chloroform–light petroleum. A portion (400 mg.) was refluxed with 3% methanolic hydrogen chloride (20 ml.) for 12 hr., methanol removed, and the residue hydrolysed with N-hydrochloric acid (20 ml.; 6 hr.; 100°). The hydrolysate was neutralised (Ag_2CO_3, etc.). The resulting syrup (360 mg.) was examined by chromatography, and showed the same components as the previous mixture. The sugars were partitioned on cellulose (2·2 × 45 cm.) as before. The first two components were collected.

Fraction 1 (80 mg.) which crystallised, had R_G 0·97, $[\alpha]_D$ +45·5° ⟶ +22·0° (*c* 0·68), gave xylose on demethylation, and was identified as 2,3,4-tri-*O*-methyl-D-xylose by m. p. 84—86°, and mixed m. p. 83—85° (authentic sample, m. p. 78—80°).

Fraction 2. The syrup (89 mg.), R_G 0·83, $[\alpha]_D$ −16·1° (*c* 0·81), M_G 0 (traces of components with M_G 0·3 and 0·65—? a trace of the 3,5-dimethyl isomer) gave arabinose and a trace of glucose on demethylation. The derived aldonamide had m. p. and mixed m. p. 128—129° with authentic 2,5-di-*O*-methyl-L-arabonamide. Chromatography in *A*, *E*, and *H* indicated that traces of a tri-*O*-methylglucose, R_G 0·72, and 2,3-di-*O*-methylarabinose were present.

2-*O*-*Methyl*-L-*arabinose*.—This was synthesised from methyl 3,4-isopropylidene-β-L-arabinopyranoside essentially by the method used by Dutton and Tanaka.[18] The product had $[\alpha]_D$ +97·2° (*c* 0·99), R_G 0·44. The aldonolactone, m. p. 85—86° from methanol–ethyl acetate (lit.,[18] 86°) and aldonamide, m. p. 126—127° from methanol–acetone (lit.,[22] 130°) were prepared.

The author is most grateful to Professor Sir Edmund Hirst, F.R.S., and Dr. G. O. Aspinall for their interest and advice, and thanks the University of Edinburgh for the award of an Imperial Chemical Industries Limited Fellowship.

Department of Chemistry, The University of Edinburgh.

[Present address: Department of Medicine, Royal Free Hospital, Gray's Inn Road, London W.C.1.] [*Received, January 19th,* 1965.]