INVESTIGATION OF THE SEED MUCILAGE FROM

PLANTAGO ARENARIA.

-by-

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INTRODUCTION.

The plant mucilages are polysaccharides of high molecular weight which possess chemical structures of an extremely complex and highly-branched nature.

Mucilages occur widely in nature, existing as secondary membrane thickening or as intracellular material in a large variety of plants. They are obviously of considerable physiological importance, providing a food reserve for the plant,[1,2] and on account of their hydrophilic nature acting as a water reservoir for the use of the plant during dry conditions.[1,3] They occur in large quantities in the coating of certain seeds, such as linseed, flax, quince and plantain seeds, and it is reasonable to suppose that here their function is partly to provide a moist environment for the seed during germination.

The plant gums are very similar to the mucilages, notably in their chemical structure and their property of forming viscous solutions in water, although their biological function is very different. Whereas mucilages are normal products of plant metabolism, gums are produced by certain trees in response to some mechanical injury or invasion by bacteria or fungi. They are produced as an exudation from the tree and dry to form a protective layer over the injured area.

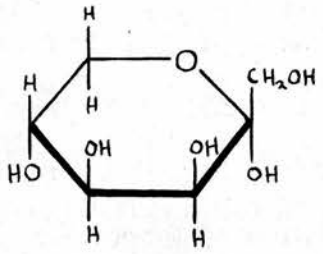
The origin of the gums is uncertain but a disappearance of starch granules has been observed during their formation, suggesting a transformation of starch into

gum. This would involve an alteration in the stereochemical configuration of the glucose to form other sugar residues which are present in the gum, and the mechanism of such a change is obscure. An interesting point is the resemblance which has been observed between gums and certain pneumococcus polysaccharides. Furthermore, degraded gums have been found to give a precipitin reaction with certain types of pneumococcus antisera;[4] and it has been suggested that the gum is actually synthesised by the invading organism. However, the constitution of gums from different species varies considerably but is remarkably consistent for a given type of tree;[5] and this seems to suggest that the gum is synthesised by the tree from materials within the cell in order to protect the damaged portion from further bacterial attack.

The plant gums and mucilages are of considerable commercial importance, their main application being in the preparation of adhesives and thickening agents, due to their property of forming viscous solutions in water. Thus they are widely used in the paper-making, textile and confectionery industries, and they also form the basis of a large number of pharmaceutical preparations.

With regard to their chemical structure, the plant gums and mucilages are all complex, highly-branched polysaccharides of high molecular weight, consisting of several different sugars linked together in the most

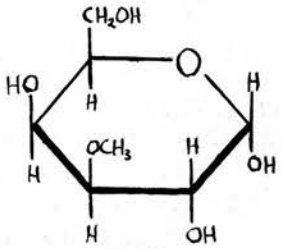
diverse ways within the same molecule. The sugars which most commonly constitute the building units of this type of polysaccharide are D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, L-rhamnose and L-fucose, although other sugars have been found occasionally. For example, the rare ketose D-tagatose(I) has been found in the gum from the tree Sterculia setigera.



I

The plant gums and most of the mucilages contain also uronic acid residues which exist as neutral salts with metals. This enables them to be classified under the heading of acid polysaccharides or polyuronides, which are defined as polysaccharides containing one or more uronic acid residue(s) in the repeating unit. This group also includes such substances as pectins, hemicelluloses, alginic acid from algae and bacterial- and muco-polysaccharides.

As well as unsubstituted sugars and uronic acids, methylated or acetylated derivatives are occasionally encountered in the gums and mucilages. The mucilage obtained from the bark of the Slippery Elm tree contains 3-methyl-D-galactose(II),[6] and the glucuronic acid present in mesquite gum was found to have a methoxyl group on the 4 position (III)[7].



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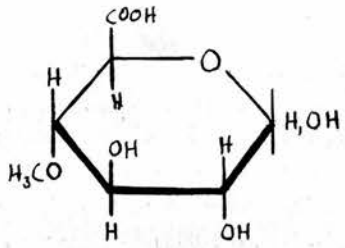


III

It is interesting to note that D-glucuronic acid is usually characteristic of the gums, whereas D-galacturonic acid is obtained from the mucilages, but this classification is not invariable. For example, galacturonic acid, and not glucuronic acid is found in gum tragacanth.[8] The rule is generally true however, and this may account for some of the inherent differences in properties of the two groups.

Until comparatively recently little work was carried out on these complex polysaccharides and this concerned only the nature of the component sugars. However, during the last fifteen years great strides have been made in the elaboration of their molecular structure and size. Early work was amazingly accurate, but it was not until the technique of methylation was developed by W.N.Haworth that structural studies could be undertaken with any degree of success. By this method the free hydroxyl groups in the polysaccharide are methylated, and hydrolysis of the fully methylated product yields a mixture of partially methylated monoses which can be separated and identified. The free hydroxyl groups in these sugars indicate the points of linkage of the various sugars in the polysaccharide.

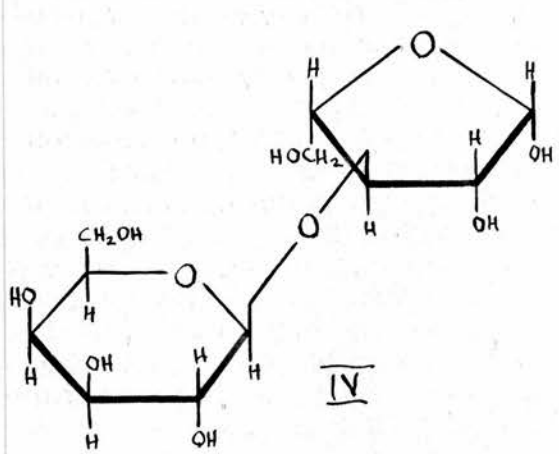
One of the greatest obstacles in this technique lies in the separation of the complex mixture of closely related sugar derivatives, and the recently developed chromatographic methods are proving to be of immense value in the identification[9], quantitative separation[10] and micro-determination[11] of unsubstituted and methylated sugars.

Initial work on these polysaccharides involves the preparation of a pure and homogeneous sample of the material in its free acid form. It is, however, difficult to be certain that the material comprises a single homogeneous substance, e.g. cholla gum[4] can be fractionated to give a soluble and insoluble portion, and gum tragacanth[8,13] has been shown to be a mixture of a complex acid polysaccharide, tragacanthic acid, and a neutral polysaccharide composed principally of L-arabinose residues. However, many of the gums seem to have an invariable and characteristic composition[5] and it has even been suggested that a quantitative knowledge of the constituent sugars of a gum may provide an identification of the botanical species or variety of the plant from which the gum is obtained. The equivalent weight, specific rotation, uronic acid and pentosan content etc. of the polyuronide are determined by the usual methods. Acid hydrolysis yields the component sugars which are determined by conversion into crystalline derivatives such as anilides and phenylhydrazones, or

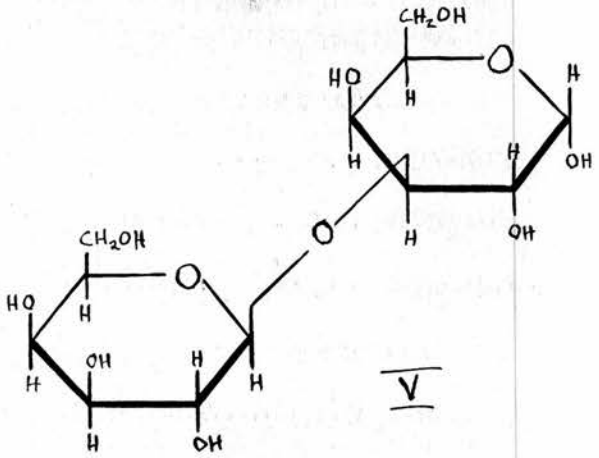
more recently, by paper chromatographic techniques.

Autohydrolysis often removes labile arabofuranose residues[5,14] together with other neighbouring sugars which form a kind of outer layer, leaving a degraded skeleton containing a stable nucleus of hexose and uronic acid residues. A comparison of the hydrolysis products obtained from the fully methylated natural and degraded gums is of great value in ascertaining the structure of the whole molecule.

Mild hydrolysis at different rates often removes disaccharides whose structures may be determined by the usual methods of methylation and hydrolysis. This indicates the mutual linkage of two of the sugars, thus supplying evidence concerning the order of arrangement of the residues in the molecule. An example of this is the isolation of 3-D-galactosido-L-arabinose(IV) and 3-D-galactosido-D-galactose(V) from gum arabic.[15]



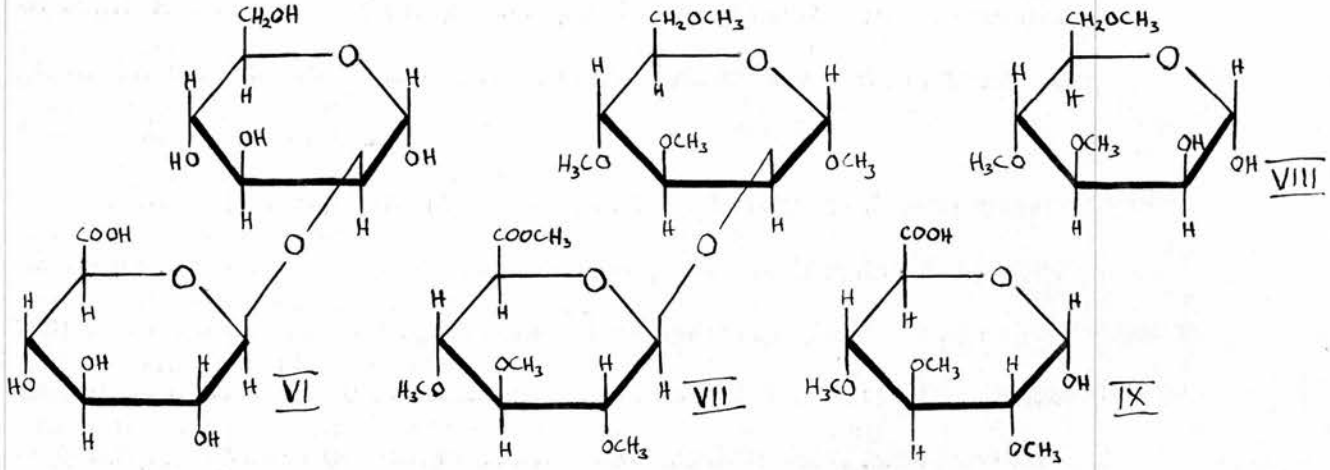
IV



V

Most gums and mucilages also contain an aldobiuronic acid grouping, comprising a uronic acid combined with a sugar residue, which is very resistant to hydrolysis and

is therefore easily isolated and identified. For example, the aldobiuronic acid(VI) obtained by hydrolysis of damson and cherry gums[16,5] gives on methylation a heptamethyl methyl aldobiuronoside(VII), which on acid hydrolysis yields 3:4:6-trimethyl-D-mannose(VIII) and 2:3:4: trimethyl-D-glucuronic acid(IX).



The aldobiuronic acid is thus characterised as 2-D-glucuronosido-D-mannose.

A new method of approach is afforded by oxidation with periodic acid which attacks the linkage between consecutive carbon atoms possessing free hydroxyl groups.[17]

Oxidation of the polysaccharide with this reagent yields valuable evidence concerning the mode of linkage of the sugar residues and branching of the chains,[18] in which respect it has been used in the study of egg-plum gum.[19] It is also of great value in the identification of methylated derivatives.

Physical methods have been used also in investigations

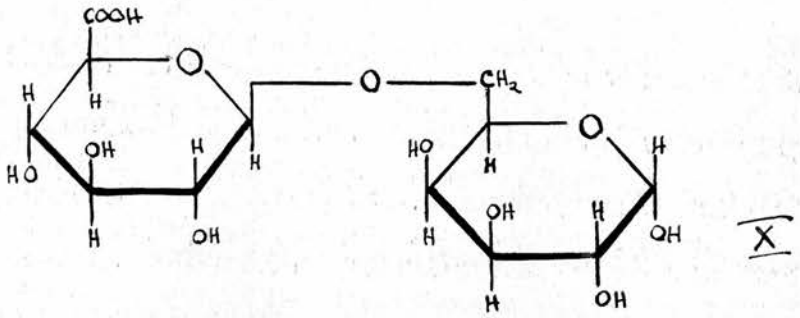
in this field, e.g. molecular weight determinations by sedimentation in the ultracentrifuge and viscosity and osmotic pressure measurements. X-ray investigations, particularly by Astbury,[20] indicating the geometrical arrangement within the molecule, are also of value.

Although no unique structure can as yet be assigned to any of these complex polysaccharides, their molecular constitution has been extensively studied along the lines discussed and a rough picture of the structure of many of them has been obtained.

Gum arabic is the most important and thoroughly studied of the gums and a detailed review of its investigation provides a good example of the complexity of the problems involved and the general method of attack in studies of this type.

Acid hydrolysis of gum arabic by Butler and Cretcher[21] yielded the component sugar units which were identified as D-galactose (2 units), L-arabinose (3 units), L-rhamnose (1 unit), and an aldobiuronic acid, consisting of D-galactose and D-glucuronic acid (1 unit).

The aldobiuronic acid was identified as 6-β-D-glucuronosido-α-D-galactose(X) by methanolysis of the methyl ether to give 2:3:4-trimethyl methyl-D-galactosides and the methyl ester of 2:3:4-trimethyl methyl-D-glucuronoside[22]



X

A detailed series of investigations were carried out by F. Smith[15,22,23] involving the hydrolysis of gum arabic and its methyl ether under varying conditions, and the examination of the cleavage fragments thus obtained.

Autohydrolysis afforded L-arabinose, L-rhamnose and a disaccharide, identified as 3-D-galactosido-L-arabinose (IV). Separation of these labile residues left a degraded arabic acid which on further prolonged autohydrolysis yielded a small quantity of a disaccharide shown to be 3-D-galactosido-D-galactose(V).

The isolation of these disaccharides indicates the attachment of galactosido residues to the 3-position of both arabinose and galactose in the gum arabic molecule.

The degraded gum arabic was then methylated and hydrolysed to give the following products:-

2:3:4:6-tetramethyl-D-galactose (1 mole)

2:3:4-trimethyl-D-galactose (5 moles)

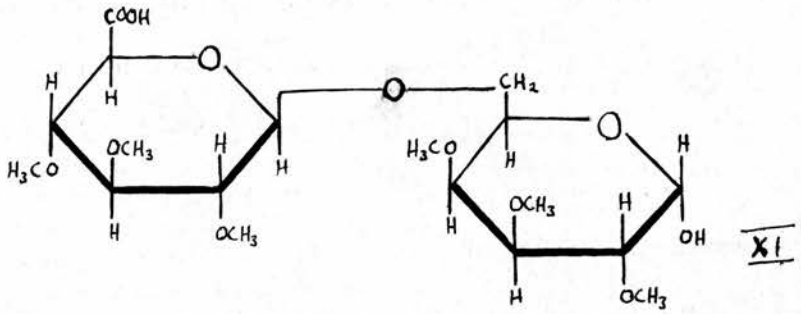
2:4-dimethyl-D-galactose (3 moles)

2:3:4-trimethyl-D-glucuronic acid (3 moles).

From this it is evident that one molecule of tetramethyl galactose and 3 molecules of trimethyl glucuronic acid must constitute the end-groups, and the existence of four of these in a molecular complex

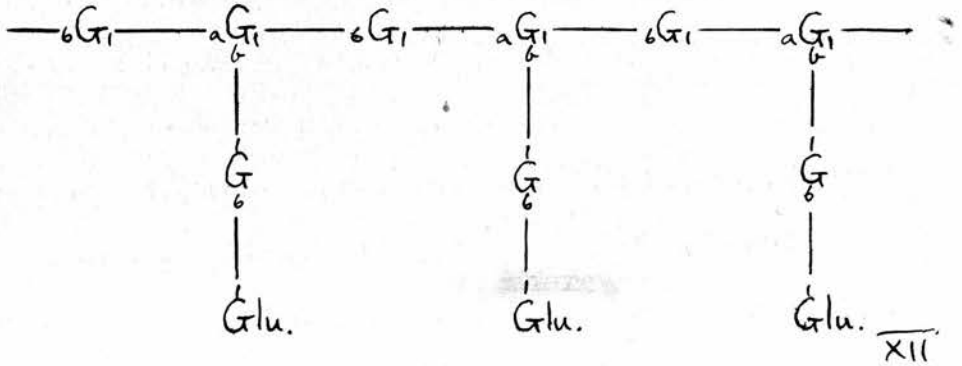
consisting of only twelve residues indicates the branched chain character of the product. The isolation of the 2:3:4-trimethyl galactose indicates that galactose is linked with other residues in the complex through positions 1 and 6. Similarly, the galactose residues which give rise to 2:4-dimethyl galactose must be linked through positions 1, 3 and 6.

In later work Smith was also able to isolate by controlled hydrolysis of the methylated degraded gum 6-(2:3:4-trimethyl-D-glucuronosido)-2:3:4-trimethyl-D-galactose(XI), which is evidence that the side-chains consist of aldobiuronic acid units.



XI

From a consideration of all these results, Smith put forward the following structure(XII) for the repeating unit of 12 residues in degraded arabic acid.



XII

where

G = D-galactopyranose

Glu = D-glucuronic acid

a = 6, b = 3 or vice versa.

A study of the hydrolysis products of methylated arabic acid itself furnished further evidence of the positions of the arabinose, rhamnose and galactose units. These were identified as follows:-

2:3:4:6-tetramethyl-D-galactose.

2:3:4-trimethyl-L-rhamnose.

2:3:5-trimethyl-L-arabinose.

2:5-dimethyl-L-arabinose.

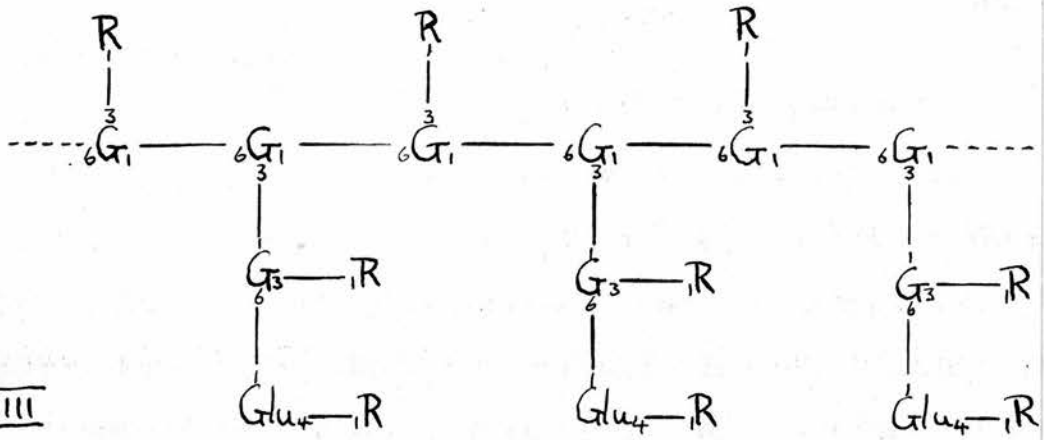
2:4-dimethyl-D-galactose.

2:3-dimethyl-D-glucuronic acid.

2:3:4-trimethyl-D-glucuronic acid.

From these results it is obvious that the labile rhamnopyranose, arabofuranose and 3-galactosido-L-arabofuranose residues are attached to the degraded nucleus by glycosidic linkages. Evidence is also obtained as to the positions at which further branching occurs. As no 2:3:4-trimethyl galactose was obtained, and only 2:4-dimethyl galactose, the 3 position of galactose and, similarly, the 4 position of glucuronic acid, must be engaged in the linkage of side-chains consisting of the labile residues.

On this evidence Smith suggested the following structure(XIII) for the repeating unit in gum arabic as one of a few very similar possibilities.



XIII

where A = L-arabofuranose

R = L-rhamnopyranose or L-arabofuranose or 3-β-D-galactosido-L-arabofuranose

G = D-galactopyranose

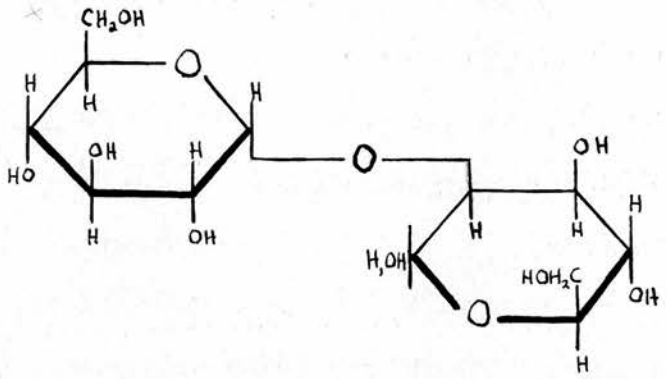
Glu = D-glucopyruronic acid.

Thus, although the possibilities have been greatly narrowed down, further work is still necessary before a unique configuration can be ascribed to the gum arabic molecule.

Damson Gum has been studied by Hirst & Jones[5,24] and seems to be related to gum arabic in its general structure and size of the repeating unit, but it differs from it in that it contains D-mannose and D-xylose but no L-rhamnose. Again labile pentose residues are present in the outer shell of the branched molecule, and are easily removed by autohydrolysis. However, its structure is much more complicated than that of gum arabic, as is shown by the hydrolysis products of the methylated whole and degraded gum:-

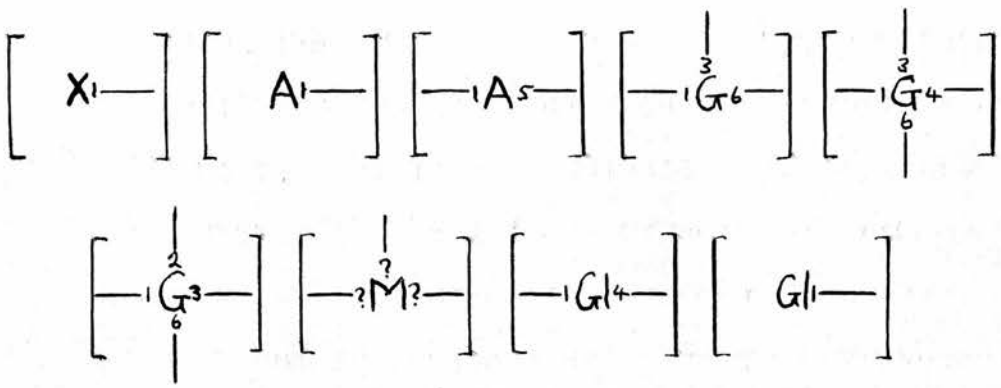
Degraded damson gum	Damson gum
2:3:4-trimethyl-D-xylose	2:3:4-trimethyl-D-xylose
2:3:4:6-tetramethyl-D-galactose	2:3:5-trimethyl-L-arabinose
2:3:4-trimethyl-D-galactose	2:3-dimethyl-L-arabinose
2:4:6-trimethyl-D-galactose	2:4:6-trimethyl-D-galactose
2:4-dimethyl-D-galactose	2:4-dimethyl-D-galactose
? dimethyl-D-mannose	2-methyl-D-galactose
2:3:4-trimethyl-D-glucuronic acid	4-methyl-D-galactose
2:3-dimethyl-D-glucuronic acid	? dimethyl-D-mannose
	2:3:4-trimethyl-D-glucuronic acid
	2:3-dimethyl-D-glucuronic acid.

The aldobiuronic acid is 2-D-glucuronosido-D-mannose (XIV), the same as that present in Cherry Gum.



XIV

It is probable that the main backbone of the molecule of damson gum comprises a chain of D-galactose residues with 1:3- and 1:6-linkages, but very little can be deduced regarding the structure except that it is a highly branched molecule containing residues with the following linkages(XV), the order of arrangement being undefined as yet.



Where A = L-arabofuranose

X = D-xylopyranose

G = D-galactopyranose

M = D-mannopyranose

Gl = D-glucuronic acid.

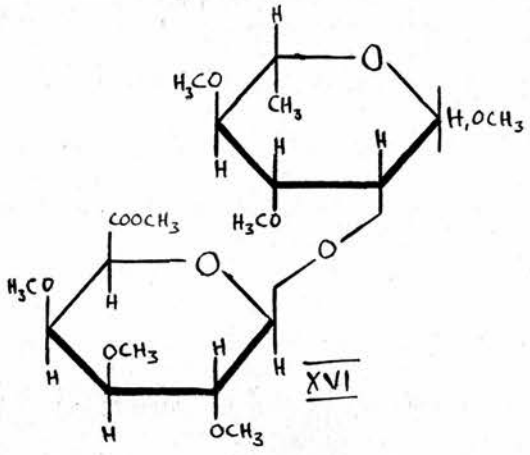
Other gums have been investigated, notably cherry gum;[25] Mesquite gum;[7,26] egg-plum gum[27] and gum tragacanth;[8,13] and have been found to possess complex structures. It is obvious, however, that until newer methods of attack are developed, no detailed formulae can be put forward for these complex substances.

The plant mucilages are very similar to the gums and can be roughly divided into 3 classes:- (1) neutral mucilages; (2) mucilages, obtained from algae, which contain sulphate ester groupings; (3) acidic polysaccarides usually containing D-galacturonic acid as the acid residue.

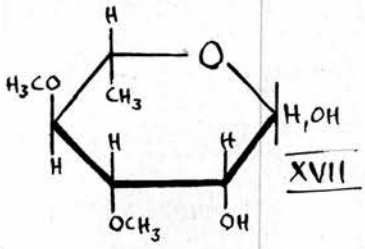
The acidic mucilages resemble the gums very closely in their general molecular structure, although they have not been subjected to such intensive study as the latter. Little was known about them prior to 1930 when the subject was first investigated by Anderson, whose work, however, concerned only the component sugars obtained

as the result of hydrolysis.

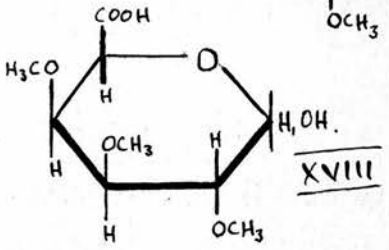
The mucilage from the bark of the Slippery elm tree (Ulmus Fulva) was investigated by Anderson in 1934[28] and found to contain D-galactose and an aldobiuronic acid consisting of L-rhamnose and D-galacturonic acid. No further work was carried out until Gill, Hirst & Jones undertook a study of the mucilage in 1939[29] when they identified the aldobiuronic acid as 2-D-galacturonosido-L-rhamnose using the methylation technique. Thus they identified as the hydrolysis products of the methylated aldobiuronic acid(XVI) 3:4-dimethyl-L-rhamnose(XVII) and 2:3:4-trimethyl-D-galacturonic acid(XVIII).



XVI



XVII



XVIII

This aldobiuronic acid is also found in linseed mucilage[30] and the mucilage from Plantago ~~arenaria~~ ovata[32] and is interesting from the point of view that it contains the unusual 1:2 linkage which is also found in the aldobiuronic acid obtained from damson and cherry gums.

Later, hydrolysis of the fully methylated mucilage[31] yielded the following methylated sugars:-

2:3-dimethyl-D-galacturonic acid (4 parts)

2:3:4-trimethyl-D-galacturonic acid (trace)

2:3:4:6-tetramethyl-D-galactose (2 parts)

2:4:6-trimethyl-D-galactose (1 part)

2:3:6-trimethyl-D-galactose (1 part)

3:4-dimethyl-L-rhamnose (2 parts)

4-methyl-L-rhamnose (2 parts).

Further work[6] resulted in the isolation of 3-methyl-D-galactose from the products of hydrolysis, the various monosaccharide residues being shown to be present in the following proportions:-

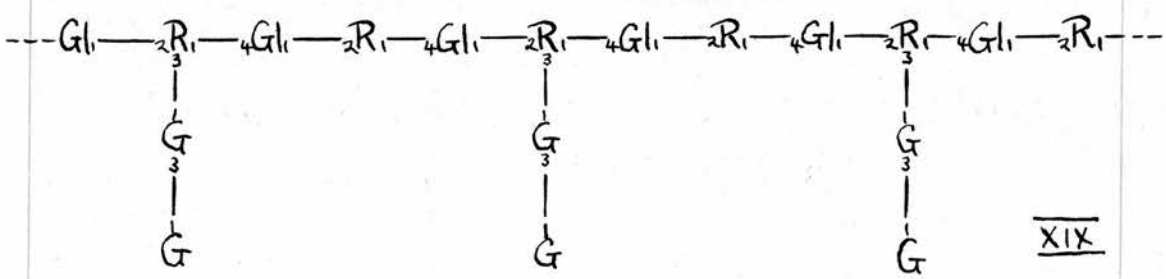
D-galactose (1 part)

3-methyl-D-galactose (1 part)

L-rhamnose (2 parts)

D-galacturonic acid (2 parts).

These results indicate the general structure of the slippery elm mucilage, although no unique formula can be put forward at present. The following structure(XIX) for the mucilage molecule has been suggested as one of several possibilities, but which of the galactose residues are substituted on C_3 by methoxyl groups is as yet obscure.



XIX

where Gl = D-galacturonic acid

G = D-galactose

R = L-rhamnose.

Also contained in the acidic mucilage class are the majority of the seed mucilages. Of these, only the Plantago mucilages have been investigated by the methylation technique, and these will now be considered in detail.

It should be mentioned here that some confusion has existed in the past regarding the identity of some of the specimens investigated, due to the difficulty in obtaining reliable homogeneous samples of the different seeds from the Plantago species. Samples sold under the trade name of "Ispaghula" have been found to be uniform, and have been identified as Plantago ovata Forsk, but commercial samples of "Psyllium" seeds did not always consist of seeds of P. psyllium. These were classed as "light psyllium" and "dark psyllium", the light variety consisting of seeds of P. ovata Forsk, and the dark variety often consisting of seeds of either or both P. arenaria or P. lanceolata.[32]

Early work by Anderson on Plantago mucilages was concerned mainly with the identification of the component sugars. He found that the mucilages from both Indian Wheat (Plantago fastigiata)[33] and light psyllium seeds (Plantago ovata Forsk)[34] contained D-xylose, L-arabinose and D-galacturonic acid. Extraction of the mucilage

from Plantago fastigiata under different conditions produced fractions with varying compositions. The solubility of the fractions was found to decrease with increase of the pentosan content, and it was suggested that each mucilage molecule contained only one uronic acid residue, and that its size and solubility were determined by the number of pentosan units in the molecule.

The first detailed investigations in this field were carried out on the seed mucilage from Plantago lanceolata by Mullan & Percival in 1940.[35] It was found to be composed of 72% pentosan (thought to be mainly D-xylose), 11% methyl pentosan (unidentified) and 11% uronic anhydride, which yielded mucic acid on oxidation and was therefore considered to be D-galacturonic acid.

The aldobiuronic acid obtained on hydrolysis was not identified, but analysis of its calcium salt indicated that it was mainly the salt of an aldobiuronic acid containing the methyl pentose residues.

Methanolysis of the methylated mucilage yielded 2:3:4-trimethyl methyl-xylosides(30%), dimethyl methyl-xylosides(28%), 2:4:6-trimethyl methyl-galactosides, glycosides of lower methoxyl content(22%) and a partly methylated uronic acid. The dimethyl pentose fraction, which was identified as 3:4-dimethyl xylose, formed a crystalline lactone, mp.67° whose rate of hydrolysis was that of a δ-lactone, indicating substitution of position

4, and the corresponding amide readily gave hydrazodicarbonamide when tested by the Weerman reaction,[36] showing the presence of a free hydroxyl group on position 1.

In further work on this mucilage by Percival and Willox,[37] the presence of 3:4-dimethyl xylose could not be substantiated, the dimethyl glycosides being found to consist almost entirely of 2:3- and 2:4-dimethyl methyl-xylosides. However, a syrupy fraction was obtained after removal of the crystalline 2:4-dimethyl xylose, the amide of which gave a positive Weerman test. This was attributed to the probable presence of small amounts of 3:4-dimethyl xylose or 3-methyl xylose. At this stage the dimethyl pentose fraction obtained by Mullan & Percival was shown to be a complex mixture, and it was found that the lactone mp.67° which was then thought to be 3:4-dimethyl-D-xylonolactone did not depress the mp. of 2-methyl-D-xylonolactone, mp.66-68°.

Among the methylated hydrolysis products, Percival & Willox identified, as well as those found previously, 2:3:4:6-tetramethyl methyl-D-galactosides (2.4%), 2-methyl methyl-D-xylosides (8%), 3-methyl methyl-D-xylosides(ca.5%) and methyl-D-xylosides (7%).

The aldobiuronic acid, which was thought to be a mixture, was not identified, but the acidic moiety was identified as D-galacturonic acid. These results indicate the highly-branched nature and complexity of the mucilage, but as yet no speculation regarding its

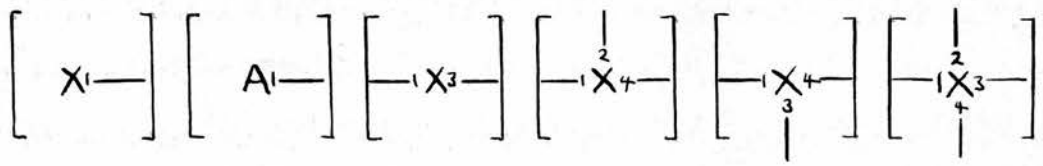
structure can be made.

Anderson and Fireman studied the mucilage from light psyllium seeds (Plantago ovata Forsk[34]) and suggested that it might be composed of a chain of xylose residues with an attached aldobiuronic acid composed of D-galacturonic acid and L-arabinose. They also found that the composition of the mucilage varied with the conditions of extraction. This was also experienced by Laidlaw & Percival in later work on this mucilage.[38] Extraction of the seeds with cold water gave a polysaccharide PI which had an equivalent weight of 700 and contained pentosan (52%), methyl pentosan(18%) and uronic anhydride(20%). Another polysaccharide PII was extracted from the residual seeds by water at 90-95° and this had an equivalent weight of 4,000 and contained pentosan(90%) and uronic anhydride(3%). This indicates that the mucilages examined were probably mixtures of at least two different polysaccharides, one containing the uronic acid residues and the other neutral.

Hydrolysis of PI gave an insoluble residue(2%) which was probably due to associated cellulose or lignin, D-xylose(46%), L-arabinose(7%) and an aldobiuronic acid which on methylation and hydrolysis yielded 3:4-dimethyl -L-rhamnose and 2:3:4-trimethyl-D-galacturonic acid, identified as the crystalline methyl trimethyl mucate. It was thus shown to be 2-D-galacturonosido-L-rhamnose which is identical with the aldobiuronic acid obtained

from slippery elm mucilage and flax seed mucilage. Acetylation and methylation of PI, followed by methanolysis gave the following derivatives:-
2:3:4-trimethyl methyl-D-xylosides (6 parts),
2:3:5-trimethyl methyl-L-arabinosides (1 part),
2:4-dimethyl methyl-xylopyranosides (1 part),
3-methyl methyl-D-xylopyranosides (4 parts),
2-methyl methyl-D-xylopyranosides (1 part), and
methyl-D-xylopyranosides (2 parts).

These results indicate that the sugar residues are linked as follows in the polysaccharide:-



X = D-xylopyranose A = L-arabofuranose

It was considered that the unsubstituted xylose residues were present in too great a quantity to have arisen simply as the result of either incomplete methylation or demethylation during hydrolysis. Furthermore, it is obvious that the highly-branched nature of the molecule necessitates the presence of a relatively high proportion of free xylose residues in the methylated product.

No methylated uronic acid or rhamnose derivatives were found in the hydrolysis products of the methylated mucilage, and the acetylated mucilage was also found to

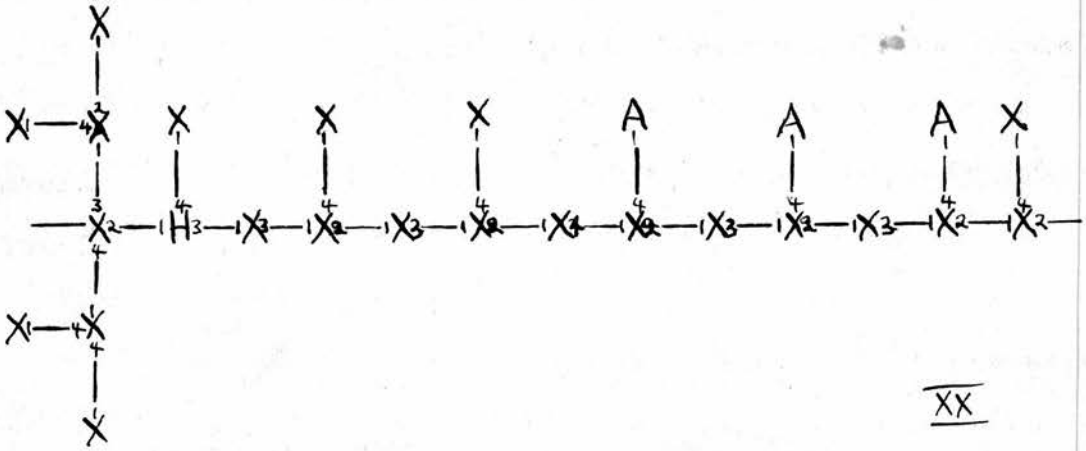
be devoid of uronic acid. A direct methylation of the polysaccharide showed that it could be separated into a soluble partly methylated product containing uronic acid residues, and an insoluble fraction containing no uronic acid. This supports the previous evidence that the mucilage does not comprise a single acidic polysaccharide but is probably a mixture of a neutral mucilage and an associated polyuronide.

Investigation of the polysaccharide PII[39] extracted from Plantago ovata seeds with hot water subsequent to the cold water extraction showed it to consist of an insoluble residue(3%), D-xylose(80%), L-arabinose(14%), D-galactose(trace) and an aldobiuronic acid(0.3%). Methylation and hydrolysis yielded the following:-

trimethyl-D-xylopyranose (8 parts),
trimethyl-L-arabofuranose (3 parts),
tetramethyl-D-galactopyranose (ca.0.3%),
2:4-dimethyl-D-xylopyranose (4 parts),
3-methyl-D-xylopyranose (8 parts),
D-xylopyranose (1 part),
and a dimethyl fraction (1 part).

The dimethyl fraction was not completely identified, but was thought to be a 2:6-dimethyl aldohexose. No evidence was obtained to indicate the order in which the sugars are linked in the molecule, and therefore no definite structure could be assigned to PII, but the

following type of structure(XX) was suggested which would explain the experimental evidence.



XX

where X = D-xylopyranose

A = L-arabofuranose

H = hexose.

It was suggested that 13-14 such repeating units are linked together and terminated by a D-galactopyranose residue to form the mucilage molecule.

Periodate oxidation of PII gave products which coincided reasonably quantitatively with those which would be obtained theoretically from such a molecule.

The mucilage from the seeds of Plantago arenaria (the so-called "dark psyllium" seeds) which is the subject of the present research, was studied by Nelson & Percival in 1942.[40] It was shown to have an equivalent weight of ca.2000 and on hydrolysis it yielded D-xylose (70%), L-arabinose(9.5%), D-galactose(3.3%) and an

aldobiuronic acid(13%) which was shown to be composed of D-galacturonic acid and D-xylose.

Hydrolysis of the methylated mucilage gave trimethyl xylopyranose (ca.30%), 2-methyl xylose (ca.23%) and a mixture which was thought to be composed chiefly of 3:4-dimethyl xylose. Oxidation of the fraction identified as 3:4-dimethyl xylose with bromine in water yielded a δ-lactone which was not obtained crystalline (cf. 3:4-dimethyl xylonolactone[35]), but the corresponding amide gave a positive Weerman test and oxidation yielded an unsymmetrical hydroxydimethoxyglutaric acid. A great deal of confusion has existed about the isolation of 3:4-dimethyl xylose from this mucilage and also Plantago lanceolata, and later workers were of the opinion that the positive Weerman reaction may have been caused by the presence of small amounts of 3-methyl xylose which could not be completely separated from the dimethyl fraction by the method of fractional distillation. Furthermore the dimethyl fraction obtained from Plantago arenaria also yielded an anilide which had m.p.170°, $[\alpha]_D^{11}$,-74° in acetone. This anilide was not identified, but was found by analysis to be a dimethyl pentose anilide and it was thought probable that it was a dimethyl-L-arabinose anilide. In the light of further work on the Plantago mucilages, it is almost certain that this was 2:4-dimethyl xylose anilide, which is known to have m.p.170° and $[\alpha]_D^{20}$,-82° in dioxan.[41]

No methylated galacturonic acid or arabinose derivatives were identified in the hydrolysis products, but analogy with the work done on Plantago ovata suggests that the latter may be present as end group along with the trimethyl xylose.

It was with these problems in mind that the following investigations on the Plantago arenaria mucilage were undertaken, in the hope that, with the aid of the more delicate methods of separation and newer tools now at the disposal of the carbohydrate chemist, some further insight might be gained into the structure of the molecule.

N O T E.

All distillations described in the experimental section were carried out at 40°C/18 m.m. pressure unless otherwise stated.

All melting points quoted were uncorrected and were performed by the method described in Campbell's "Qualitative Organic Chemistry" p.7.

Methoxyl determinations were carried out by the Micro-Zeisel method as described in Pregl-Roth's "Quantitative Organic Microanalysis, p.171 (3rd edition.)

Carbon, hydrogen and nitrogen analyses were carried out at Drs. Weiler and Strauss' laboratories, Oxford. All other estimations were performed by the author.

R_G values quoted in paper chromatographic studies are those found in butanol/ethanol/water/ammonia (40/10/49/1) solvent, unless otherwise stated.

EXPERIMENTAL

The *Plantago arenaria* seeds from which the mucilage was extracted were thin and elliptical in shape, about 2mm. long, and dark reddish brown in colour. They appeared to be quite homogeneous.

Preparation of the mucilage.

The seeds (1000gm.) were soaked in water (16 litres) at room temperature for 48 hours, with occasional stirring. An extremely viscous solution was obtained, which was separated from the seeds, with difficulty, initially by filtration through muslin, and later by the use of a filter press. A further quantity of water (4-5 litres) was added to the residual seeds, and the extraction repeated. The mucilage was dehydrated by the addition of three times its volume of methanol with vigorous stirring, and washed with methanol until crisp. It was then filtered and allowed to stand overnight in ether, and finally dried over P_2O_5 in a vacuum dessicator. A 3% yield of the brownish-white fibrous material was obtained.

No further material was extracted on treating the seeds with hot water after cold extraction. It was found that if the seeds were soaked for longer than 48 hours, a bluish-purple coloration developed, and although this coloured material was soluble in alcohol, it could not be completely separated from the mucilage.

Ash determination of mucilage.

About 0.5gm. mucilage was incinerated in a platinum

crucible until combustion was complete, and the residue weighed. The ash was also determined as sulphate by treatment of the residue with sulphuric acid.

Found:- Ash = 5.3%

as sulphate, 6.0%

Preparation of the "free-acid" polysaccharide.

To the mucilaginous solution prepared above was added three times its volume of acidified alcohol(40c.c. concentrated hydrochloric acid per litre). The "free-acid" polysaccharide thus obtained was triturated with methanol in a mortar until free from chloride ions, and dried in ether and in a vacuum dessicator as before. The product was white and fibrous and again obtained in ca. 3% yield:-

Found:- ash - 1.15%

Unless otherwise stated, this "free-acid" mucilage was used in all subsequent investigations and dried at 40°C/13mm. over P_2O_5 before all quantitative determinations.

Equivalent weight determination.

The mucilage (ca.0.2gm.) was dissolved in standard decinormal sodium hydroxide(25c.c.) and allowed to stand overnight. The excess alkali was then estimated by titration with standard decinormal hydrochloric acid, using phenolphthalein as indicator. A blank experiment was also carried out.

Found:- equivalent weight = ca.1700 (average figure)

In a typical estimation, 0.1613gm. mucilage was

equivalent to 0.94c.c. 0.1020N HCl, corresponding to an equivalent weight of 1681.

Estimation of uronic acid.

The uronic anhydride content was determined by heating the mucilage(0.2gm.) with 19% hydrochloric acid at 145°C and absorbing the carbon dioxide evolved in a known volume of standard sodium hydroxide, according to the method of Swenson, McCready and MaClay.[42] Blank experiments were also carried out.

Found:- Average uronic anhydride = 7.2%

In a typical estimation, 0.2230gm. mucilage gave carbon dioxide equivalent to 1.66c.c. 0.1021N hydrochloric acid, indicating a uronic anhydride content of 7.0%.

Pentose estimations.

The mucilage(ca.0.15gm.) was heated with 13.13% hydrochloric acid containing sodium chloride according to the method of Marshall and Norris.[43] The furfural obtained was distilled off, converted to the insoluble thiobarbiturate and weighed. A correction was applied for the furfural derived from the uronic acid[44] and the weight of furfural from the pentose calculated. The pentose in the mucilage was then calculated as anhydroxylose using Marshall and Norris' factors, viz.

Anhydroxylose = 0.6293T + 0.0013 ± 0.00044.

where T = weight of thiobarbiturate.

A corrected equation was derived, viz.

Anhydroxylose = 0.6293T - 0.3304U - 0.00267

where U = wt. uronic anhydride in sample

(based on the fact that U/P = 2.37 for galacturonic acid and anhydroxylose = 0.783P + 0.00397 ± 0.00103,

where P = weight of phloroglucide complex)

Found:- mean pentose = ca. 80%

Control experiments carried out on pure dry xylose showed that it gave only 92% of the theoretical yield of furfural. Thus in one experiment 0.0952gm. xylose gave 0.1370gm. furfural thiobarbiturate, corresponding to 91.9% anhydroxylose. A correction was made for this in the analysis of the mucilage.

In a typical estimation 0.1566gm. mucilage which contains 7.2% or 0.0128gm. uronic anhydride, yielded 0.1937gm. furfural thiobarbiturate. Hence the pentosan content, as anhydroxylose, is 81.4%.

Methylpentose determination[45]

The mucilage was hydrolysed with 3% oxalic acid for 6 hours and the resulting solution oxidised for 2.5 hours with periodic acid in a current of nitrogen. Any acetaldehyde formed was collected as a complex with sodium bisulphite and estimated by decomposition of the complex with sodium bicarbonate and titration of the bisulphite liberated with N/100 iodine solution.

The following results were obtained:-

(1) 0.2290gm. mucilage was hydrolysed with 3% oxalic acid (7c.c.) for 6 hrs. The acetaldehyde produced was

equivalent to 0.87c.c. 0.00895N iodine, indicating a methyl pentose content of 0.3%.

(2) 0.3472gm. mucilage was hydrolysed with 3% oxalic acid(7c.c.) for 24hrs. The acetaldehyde produced was equivalent to 0.88c.c. 0.00946N iodine, indicating a methyl pentose content of 0.2 %.

(3) 0.2806gm. mucilage was hydrolysed with 3% oxalic acid (7c.c.) for 48hrs. The acetaldehyde produced was equivalent to 1.360c.c. 0.00946N iodine indicating a methyl pentose content of 0.5 %.

From these results it appears that there is present in the mucilage some methyl pentose, but that it exists in some relatively stable combination, as only 0.6% can be detected after prolonged hydrolysis.

Autohydrolysis of the mucilage.

The mucilage (ca.0.3gm.) was heated with water(25c.c.) for 24hrs. on a boiling water bath. An insoluble residue remained which was filtered off and the solution was neutralised with barium carbonate and filtered. The filtrate was evaporated to ca.5c.c. and poured into methanol(400c.c.). A white precipitate of the degraded mucilage was obtained which was removed by centrifugation and washed with methanol. The alcoholic solution and washings were evaporated to dryness and the syrup obtained dissolved in a few c.c. water, the solution deionised by shaking with Amberlite resins IR100 and IR4B, and examined on a paper chromatogram. A small amount of

arabinose only was detected.

Acid Hydrolysis of the mucilage.

The mucilage(2gm.) was hydrolysed with 3% oxalic acid (100c.c.) on a boiling water bath. The course of the hydrolysis was followed by polarimetric measurements and also by hypoiodite titrations which were carried out as follows:- 5c.c. hydrolysate were removed from time to time and 0.1N sodium hydroxide(50c.c.) and 0.1N iodine (10c.c.) added. These were allowed to react for 45 minutes at room temperature, and then after acidification with N sulphuric acid(10c.c.) the residual iodine was estimated by titration with 0.05N sodium thiosulphate.

Results:-

Time(hours)	$[\alpha]_D^{17}$	Iodine absorbed
0.5	?	2.55c.c.
1.0	?	4.68
1.5	?	5.86
2.0	20°	6.60
3.0	34°	7.29
4.0	39°	7.47
5.0	46°	7.93
6.0	46°	7.82
6.5	46°	7.70

For the first 1.5 hours the solution was too cloudy for polarimetric observations, even after filtration.

Both polarimetric measurements and hypoiodite titrations indicate that the hydrolysis is complete after

5 hours. The slight decrease in the volume of iodine absorbed after 5 hours is probably due to partial breakdown of the sugars after hydrolysis is complete.

After hydrolysis with oxalic acid a dark brown insoluble residue remained in the solution. This was removed by filtration and the solution neutralised with barium carbonate, the mixture being heated at 100°C for 30 minutes to decompose any barium bicarbonate formed. After filtration the solution was evaporated to dryness under reduced pressure at 40°C. The remaining syrup was dissolved in a few c.c. water and a little inorganic material removed by filtration. The solution was then poured into alcohol(250c.c.) from which the barium salt of the aldobiuronic acid separated as a fine white precipitate. This was removed and the filtrate evaporated under reduced pressure to yield a yellow syrup which was strongly reducing and showed $[\alpha]_D^{16}$, +40°C(c, 0.5 in water).

On examination of this syrup on a paper chromatogram, three sugars separated which were shown to be xylose, arabinose and galactose by direct comparison with controls.

Partial hydrolysis of the mucilage was effected by heating with oxalic acid for short periods.

The mucilage(5gm.) was heated at 100°C with 3% oxalic acid(50c.c.) for 30 minutes and the mixture cooled, neutralised with barium carbonate and filtered. The filtrate was evaporated to dryness and the residue exhaustively extracted with alcohol. The remaining degraded mucilage was then hydrolysed as before for

further periods of 45 minutes, +1 hour , +1½ hours.

Evaporation of the alcohol extracts to small volume and examination by paper chromatography in each case showed the presence of xylose, arabinose and galactose as well as various oligosaccharides, one with R_G value 0.10 and several with R_G values of less than 0.06.

Hydrolysis of the autohydrolysed mucilage with 3% oxalic acid at 100°C for 6 hours gave after the usual treatment a syrup which was shown by paper chromatography to contain xylose, arabinose and galactose.

Estimation of the products of hydrolysis.

Many determinations were carried out and the following are some typical results.

Insoluble Residue.

(1) The mucilage(2.0480gm.) was hydrolysed with 3% oxalic acid(100c.c.) at 100°C for 6 hours. The dark brown residue was removed from the solution by filtration through a sintered glass crucible (porosity 3) and dried in an oven at 100°C until constant weight(0.0405gm.). This corresponds to 1.95% insoluble residue in the mucilage.

(2) The mucilage(0.7859gm.) was hydrolysed with 3% oxalic acid(50c.c.) at 100°C for 6 hours and yielded 0.0165gm. insoluble residue. This corresponds to 2.1%.

Thus the mucilage contains ca.2% insoluble residue.

Free sugars and aldobiuronic acid.

(1) The mucilage(10.00gm.) was hydrolysed with 3% oxalic

acid(250c.c.) for 6 hours at 100°C. The mixture was neutralised with barium carbonate, filtered and evaporated to dryness. The residue was dissolved in a small volume of water, filtered free from barium salts and again evaporated to dryness. The residue was exhaustively extracted with boiling alcohol and the combined extracts evaporated to dryness to give a syrup comprising the free sugars(3.686gm.).

The residual barium salt, which had a brown coloration, was dissolved in water and the solution heated with charcoal at 100°C for ten minutes. On filtration a clear solution was obtained which was evaporated to dryness to give the pure barium aldobiuronate (0.649gm.). This corresponds to aldobiuronic acid(0.542gm.) (calculated on the assumption that this is a combination of a methyl pentose and a hexuronic acid).

Thus, assuming equal loss of free sugars and barium aldobiuronate during the experimental procedure, and allowing for 2% insoluble residue, these figures indicate 12.6% aldobiuronic acid and 85.4% free sugars in the mucilage.
(2) The mucilage(1.05gm.) was heated at 100°C with 3% oxalic acid(50c.c.) for 6 hours. The solution was neutralised with barium carbonate, filtered, and the solution evaporated to dryness. The residue was dissolved in a small volume of water (ca.10c.c.),filtered, and the solution evaporated to dryness. The residue was dissolved in 2-3c.c. water and the solutions poured into methanol to precipitate the barium salt of the aldobiuronic

acid which was centrifuged off and washed with methanol. The methanolic solution was still opalescent and was therefore evaporated to dryness and the residue extracted several times with methanol. The two precipitates were dissolved in water and the solutions combined and evaporated to dryness to give the barium salt(0.0988gm.). The methanolic solution containing the free sugars was evaporated to dryness and dried over P_2O_5 *in vacuo* to give a syrup (0.5380gm.).

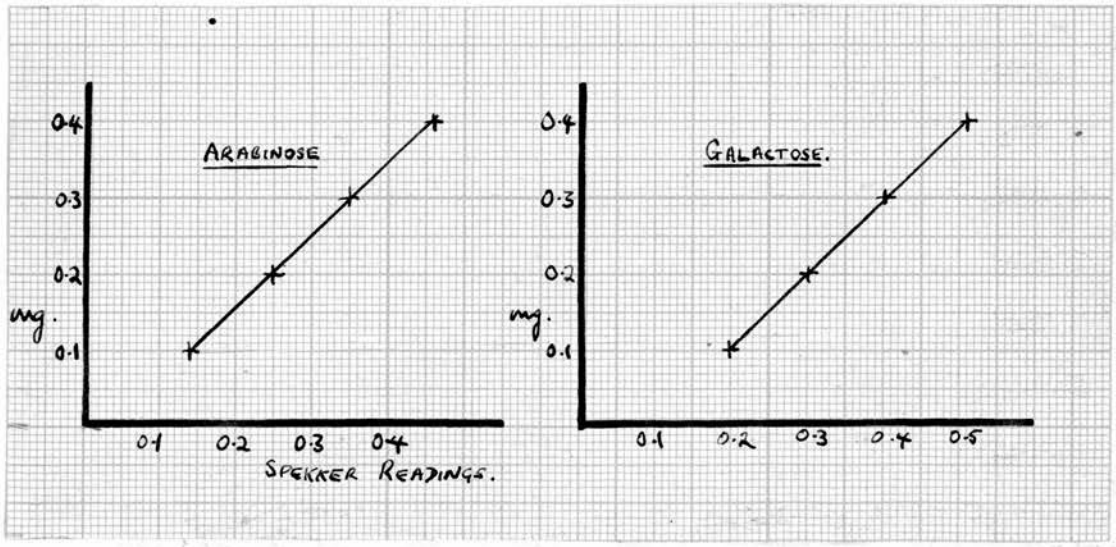
0.0988gm. barium aldobiuronate corresponds to 0.0823gm. free acid, and therefore the mucilage comprises 13.0% aldobiuronic acid and 85.0% free sugars.

Estimation of the individual sugars.

The mucilage(0.7859gm.) was hydrolysed with 3% oxalic acid at 100°C for 6 hours and the solution neutralised with barium carbonate and treated as before to give the syrup containing the free sugars. This was deionised by dissolving in water and passing through two columns containing Amberlite IR100 and IR4B resins respectively, and the solution evaporated to dryness to give a syrup(0.595gm.)

The relative proportions of the sugars in this syrup were estimated on a paper chromatogram as follows.

The syrup(0.26gm.) was dissolved in water (5c.c.) and an equal volume of this solution spotted onto six paper chromatograms by means of a micro-pipette (volume 0.027c.c.).[46] The sugars were separated by running the papers in ethyl acetate/acetic acid/water (3/1/3) for


ARABINOSE
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mg.
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SPEKKER READINGS.
GALACTOSE.
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0·3
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mg.
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0·1
0·2
0·3
0·4
0·5

45 hours. The acetic acid was removed by hanging the paper strips over a dish of water in a vacuum dessicator and evacuating for 12 hours. The sugars were then extracted from the paper strips by elution with cold water[47] and estimated as follows.

(i) by periodate oxidation[48]

To the sugar solutions and paper blanks was added 0.25M sodium periodate(1c.c.). The solutions were heated in tubes provided with cooling coils in a boiling water bath for half an hour and cooled. Ethylene glycol(0.3c.c.) was added to each and the solutions titrated with standard N/100 sodium hydroxide using as indicator methyl-red-methylene blue.

Xylose (extracted from 1 paper)

The formic acid liberated was equivalent to 1.586c.c. NaOH (0.0127N). This corresponds to 0.76mg. xylose.

Arabinose (extracted from 2 papers)

The formic acid was equivalent to 0.840c.c. NaOH(0.0127N). This corresponds to 0.20mg. arabinose, on one paper.

(ii) by the Arsenomolybdate-Somogyi method[46,49]

The arabinose and galactose were estimated colorimetrically by use of Arsenomolybdate-Somogyi reagent. The sugars were eluted from the paper strips with cold water as before and the solutions obtained heated at 100°C for 15 minutes, in order to equalise the amount of oxygen in each solution. Two sets of standard solutions containing 0.1mg., 0.2mg., 0.3mg. and 0.4mg. of galactose and arabinose respectively and also water and paper blank

solutions were treated simultaneously under the same conditions. After cooling, copper reagent(2c.c.) was added and the solutions heated at 100°C for 20 minutes and cooled. Arsenomolybdate reagent(2c.c.) was then added when a blue-green colour developed, and the solutions were made up to 25c.c. with water. The standard solutions were examined against the water blank in a Spekker Electrophotometer using "green 604" filters, and the readings plotted against the sugar concentration. A straight line was obtained from which could be read the weight of sugar in the unknown solutions after comparison with the paper blank in the instrument.

Two standard solutions containing 0.1mg. galactose and arabinose respectively per c.c. were prepared. 1c.c., 2c.c., 3c.c. and 4c.c. of these solutions were used and gave the following results when compared with the water blank.

Concn. of sugar/25c.c.	Spekker Reading.
0.1mg. arabinose	0.14
0.2mg. "	0.25
0.3mg. "	0.35
0.4mg. "	0.46
0.1mg. galactose	0.20
0.2mg. "	0.30
0.3mg. "	0.40
0.4mg. "	0.51

Two solutions were obtained by eluting the arabinose from one paper chromatogram and two by combining the galactose

eluates from three and two papers respectively.

(a) Arabinose (1 paper) gave reading 0.28 } Average value
(b) " " " " 0.26 } 0.27

This corresponds to 0.22mg. arabinose.

(c) Galactose (3 papers) gave reading 0.32.

This corresponds to 0.22mg., i.e. 0.073mg. on one paper.

(d) Galactose (2 papers) gave reading 0.25, corresponding to 0.15mg., i.e. 0.075mg. on one paper.

Average value = 0.074mg. galactose.

A combination of the above results gave the following figures for the composition of the syrup:-

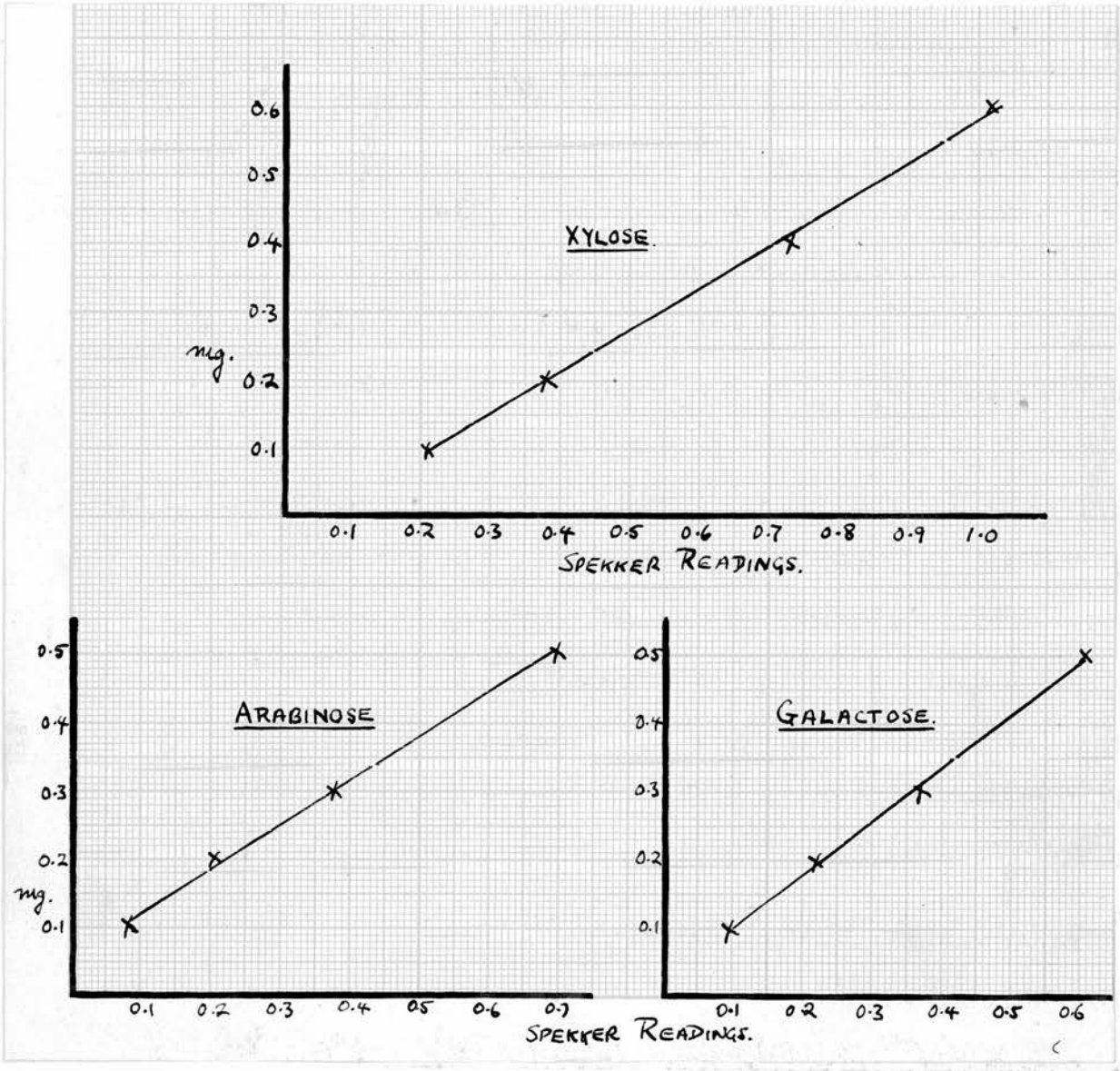
		Calculated as anhydro sugars
Xylose 0.756 mg.	72.6%	72.5%
Arabinose 0.21 mg.	20.2%	20.2%
Galactose 0.074 mg.	7.1%	7.3%

Thus the composition of the mucilage is as follows:-

	% by weight	relative no. of molecules (approx).
Insoluble residue	2%	
Aldobiuronic acid	13%	1
Xylose	62%	12
Arabinose	17%	3
Galactose	6%	1
	100%	

Examination of the Insoluble Residue.

(1) The dark brown insoluble residue, which was removed from the autohydrolysed solution by filtration, was heated at 100°C with 3% oxalic acid overnight, the mixture


XYLOSE.
mg.
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SPEKKER READINGS.
ARABINOSE
mg.
0.5
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0.1 0.2 0.3 0.4 0.5 0.6 0.7
GALACTOSE.
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0.3
0.2
0.1
0.1 0.2 0.3 0.4 0.5 0.6
SPEKKER READINGS.

neutralised with barium carbonate and filtered. On pouring the evaporated solution into alcohol no precipitate was obtained. The methanolic solution was evaporated to small volume, and examination on a paper chromatogram showed the presence of only a trace of glucose. This would seem to indicate that the insoluble residue is of a cellulosic nature.

(2) The insoluble residue(0.2gm.) obtained after hydrolysis of the mucilage with 3% oxalic acid was suspended in 72% sulphuric acid(2c.c.) and allowed to stand at room temperature for ten days, according to the method of Monier-Williams.[50] The mixture was diluted to 200c.c. with water and heated under reflux at 100°C for 15 hours. A dark brown residue still remained which was probably of a ligneous nature. The solution was neutralised with barium carbonate, filtered and evaporated to dryness. The residue was extracted with methanol and the solution examined on a paper chromatogram which showed the presence of glucose.

The Neutral Mucilage.

Hydrolysis and Estimation of the free sugars.

The mucilage(1gm.), precipitated in non-acidified alcohol as the natural salt, was hydrolysed with 3% oxalic acid(50c.c.) at 100°C for 6 hours and treated as usual to give the syrup(0.50gm.) containing the free sugars.

The syrup was dissolved in 30c.c. water and 0.030c.c. of this solution spotted on each of five paper chromatograms

by means of a micro-pipette. The xylose, arabinose and galactose were separated and the sugar from several papers combined to give the necessary concentration for estimation by the Arsenomolybdate-Somogyi method.

Straight line relationships between sugar concentration and Spekker reading were obtained with standard solutions of xylose, arabinose and galactose. The sugars eluted from the papers gave the following results.

Sugar	Spekker reading	Wt. sugar on 1 paper
Xylose (1 paper)	0.48	0.26 mg.
Xylose (1 paper)	0.48	0.26
Arabinose (2 papers)	0.15	0.075
Arabinose (3 papers)	0.25	0.073
Galactose (5 papers)	0.14	0.026

Thus the composition of the syrup is:-

Xylose	.26 mg.	72.3%
Arabinose	.074 mg.	20.5%
Galactose	.026 mg.	7.2%

Periodate oxidation of the mucilage.

Periodate uptake.

The mucilage(ca.0.4gm.) was shaken with water(40c.c.) until it had dissolved and then molar sodium metaperiodate (10c.c.) added, making the solution approx. 0.2 molar. At intervals 5c.c. solution were withdrawn and the periodate present estimated. This solution was diluted, excess solid sodium bicarbonate added, followed by 0.1N sodium arsenite solution(25c.c.) and excess solid potassium

iodide. The solution was allowed to stand at room temperature for 15 minutes and then titrated with iodine solution (0.09924N).

A similar estimation was carried out on a solution of the reagent to give a blank value.

Results. (1) Wt. mucilage used = 0.4215 gm.

Time(days)	Titre/50c.c.	Periodate uptake (mols./equivalent)
1	46.2	13.32
3	48.1	13.88
4	47.8	13.79

(2) Wt. mucilage used = 0.4052 gm.

Time(days)	Titre/50c.c.	Periodate uptake (mols./equivalent)
1	44.9	13.47
3	46.6	13.98
4	46.2	13.87

The titre was constant thereafter.

No. of anhydroxylose residues/equivalent = 2450/132 = 18.57

13.9 molecules periodate are absorbed per 18.57 residues

i.e. 0.75 molecules periodate per residue.

Formic Acid Liberated.[48]

The mucilage(ca.0.4gm.) was shaken with water(100c.c.) until dissolved. To the solution were added sodium metaperiodate(0.23M;25c.c.) and potassium chloride(5gm.) and the mixture was shaken gently in the dark at room temperature. At intervals 10c.c. portions of the supernatant liquid were withdrawn, ethylene glycol(1c.c.) added and the solution titrated with sodium hydroxide

(0.00968N), using methyl red-methylene blue indicator.

A solution of the reagents alone was neutral to methyl red-methylene blue. A solution of 0.3950gm. mucilage in 100c.c. water required 11.6c.c. NaOH.

(1) 0.4226gm. mucilage used.

Time(days)	Titre/125c.c.	Mols. formic acid liberated/equivalent
1	50.21c.c.	2.82
3	75.71	4.25
5	85.47	4.80
7	90.8	5.10
10	97.9	5.50
12	99.5	5.60
14	101.3	5.70
16	101.3	5.70

(2) 0.4637gm. mucilage used.

Time(days)	Titre/125c.c.	Mols. formic acid liberated/equivalent
1	44.13c.c.	2.26
3	64.13	3.28
5	73.64	3.77
7	81.24	4.16
10	95.68	4.90
12	102.1	5.22
14	106.6	5.45
16	108.6	5.55
18	111.3	5.69
20	111.2	5.69

The fact that (2) was slower than (1) was attributed to incomplete dissolution of the mucilage in experiment (2).

Hydrolysis of the periodate-oxidised mucilage.

The mucilage(0.2836gm.) was oxidised with sodium metaperiodate(0.2M;35c.c.) for 5 days, the solution neutralised with barium carbonate and filtered. Pure re-crystallised D-ribose(0.0964gm.) was then added to the solution as a standard sugar for comparison and the iodic acid removed by bubbling sulphur dioxide through the solution for 30 minutes.[51] The solution was then made 3% with respect to oxalic acid and hydrolysed for 5 hours at 100°C. After neutralisation with barium carbonate the inorganic ions were removed by electrodialysis[46] and the solution evaporated to dryness to give a syrup(0.151gm.)

The syrup was dissolved in water(5c.c.) and 0.030c.c. of this solution spotted onto 2 paper chromatograms. The sugars present in the mixture were xylose, arabinose and ribose, with no galactose, and these were separated and estimated by means of the arsenomolybdate reagent.

Found: Wt. ribose on each paper = 0.35mg.

Wt. xylose " " " = 0.18mg.

Wt. arabinose " " " = 0.03mg.

$$\text{Wt. unattacked anhydroxylose} = \frac{0.18}{0.35} \times 0.0964 \times \frac{132}{150}$$

$$= 0.04363\text{gm.}$$

Sim. wt. unattacked anhydroarabinose = 0.007586gm.

Now 0.2836gm. mucilage contain 62% or 0.1758gm. anhydroxylose (12 residues)

and 17% or 0.04820gm. anhydroarabinose(3 residues)

∴ 25% of the xylose and 16% of the arabinose present in the mucilage is unattacked by periodate.

i.e. 1 in every 4 xylose residues and 1 in every 6 arabinose residues are unattacked.

INVESTIGATION OF THE ALDOBIURONIC ACID.

Preparation of the barium salt.

The mucilage(150gm.) was hydrolysed with 3% oxalic acid at 100°C for 12 hours. The mixture was cooled, neutralised with barium carbonate and filtered to give a brown solution which was digested with charcoal for 20 minutes, filtered and evaporated to ca. 250c.c. This solution was poured slowly into vigorously stirred methanol(4 l.) to give a pale brown precipitate of the barium aldobiuronate. This was filtered, washed thoroughly with methanol and hydrolysed with 2% sulphuric acid at 100°C for 7 hours. The barium salt was obtained as before and the methanol washings were shown by paper chromatography to contain some xylose, arabinose and galactose. Therefore the barium salt was further purified by hydrolysis with 2% sulphuric acid for 5 hours and after precipitation of the salt the washings contained only a trace of xylose with some rhamnose. This, as well as its analysis figures, showed that the barium salt was fairly pure and it was dried to give a brownish-white powder(20 gm.).

The pure barium salt had $[\alpha]_D^{16}$, +65°(c,0.3 in water)

Ba, 16.2% ; CO_2, 10.6%

Calc. for $C_{12}H_{19}O_{11}Ba/2$: Ba, 16.8% ; CO_2, 10.8%

Hydrolysis of the barium salt.

The pure barium salt(0.5gm.) was heated at 100°C

with 9% sulphuric acid(20c.c.) for 20 hours. The resulting brown solution was diluted with water, neutralised with barium carbonate, the mixture digested with charcoal and filtered. Evaporation of the filtrate yielded a white residue which was extracted with hot methanol. The methanol extracts were evaporated to give a syrup which when examined on a paper chromatogram was found to consist of rhamnose.

The residue was dissolved in water, shaken with Amberlite resin IR100 and the solution examined by paper chromatography which showed the presence of galacturonic acid.

Oxidation of the barium salt.

The barium salt(1gm.) was oxidised by the method of Heidelberger and Goebel[52] by heating at 100°C with 6% hydrobromic acid(10c.c.) containing bromine(1c.c.) for 12 hours. The brown solution was left at room temperature for 2 days, heated with charcoal for 10 minutes and filtered to give a clear solution. After one week at 0°C mucic acid had separated, m.p.215°(d) and mixed m.p. 214°(d).

After removal of the mucic acid, the mother liquor was neutralised with silver carbonate and the Ag precipitated as sulphide. The solution was filtered, aerated and evaporated to give a syrup which partially crystallised.

This mixture was heated with phenylhydrazine in alcohol at 80°C for 2 hours. On cooling, crystals of

rhamnonic acid phenylhydrazide separated. These were recrystallised from absolute alcohol and had m.p. 193°C, $[\alpha]_D^{17}$, +17°C(c,1.1 in water).

Methylation of the barium salt.

The barium salt(12gm.) was dissolved in water(100c.c.) and 30% sodium hydroxide(200c.c.) and dimethyl sulphate (100c.c.) added over a period of 4 hours. After standing overnight the solution was found to be non-reducing, and solid sodium hydroxide(60gm.)was added and dimethyl sulphate(100c.c.) run in over a period of 6 hours. The solution was again left overnight, heated at 100°C for 30 minutes, cooled and neutralised with concentrated sulphuric acid. The slightly acid solution was exhaustively extracted with chloroform and after washing with water the extracts were evaporated to give a sludge which was remethylated as before. The product was extracted with chloroform, the extracts dried (sodium sulphate) and evaporated to give a syrup which was methylated twice by the Purdie method. It was then methylated with diazomethane in ethereal solution for 14 hours at 0°C. The diazomethane was allowed to evaporate slowly at room temperature and the ether evaporated to yield a syrup which was distilled over barium carbonate, B.P.180-200°C(bath temperature)/0.05mm. A pale yellow syrup(0.63gm.) was obtained. n_D^{16} ,1.4684 OMe,45.6% (Calculated for $C_{19}H_{34}O_{11}$: OMe, 49.5%).

Hydrolysis of the methylated product.

The syrup(0.569gm.) was heated at 100°C with 2N hydrochloric acid(25c.c.) for 12 hours, the course of the hydrolysis being followed by polarimetric observations.

$[\alpha]_D^{16}$ +85°(15mins.); +77°(2 hrs.); +76°(4 hrs); +72°(10 hrs., constant).

The solution was diluted, neutralised with silver carbonate, filtered and Ag^+ removed as sulphide. After filtration the solution was aerated, warmed with barium carbonate, filtered and evaporated to dryness.

The residue was exhaustively extracted with dry ether, leaving a white solid R(0.21gm.). On evaporation of the ether extracts a syrup S(0.195gm.) was obtained.

Identification of the syrup S.

The syrup did not crystallise. It had $[\alpha]_D^{16}$,+19° (c,2.6 in water) n_D^{16},1.4728. OMe,31.8%(Calculated for $C_6H_{12}O_5$: OMe, 32.3%)

When examined on a paper chromatogram it gave a yellow-brown colour with aniline oxalate and had R_G value 0.85, which is that quoted for 3:4 dimethyl rhamnose.

The syrup(9mg.) was oxidised with periodic acid[45] and gave no acetaldehyde.

The lactone was prepared by oxidation of the syrup (0.26gm.) with bromine in water until non-reducing(8 days). Distillation gave a poor yield(0.07gm.) of a brownish syrup B.P.140°-160°(bath temperature)/0.01mm. This had $[\alpha]_D^{16}$, -148°(10mins.); -142°(7hrs.); -126°(31hrs.);-120°(50hrs.)

-117°(72hrs.,constant) (c,0.92 in water).

The amide was obtained by treatment of the lactone with methanolic ammonia at 0°C for 2 days. Removal of the solvent in a vacuum dessicator gave a syrup which partially crystallised. On subjecting this to a Weerman test[36] a precipitate of hydrazodicarbonamide was obtained.

Identification of the residue R.

The residue R(0.2gm.) was dissolved in water(10c.c.) and oxidised with bromine(2c.c.) till non-reducing(3 days). The solution was aerated to remove the bromine, neutralised with barium carbonate, filtered and evaporated to yield a syrup. This was esterified by heating at 80°C with 3% methanolic hydrogen chloride(40c.c.) for 14 hours. The solution was neutralised with silver carbonate, filtered and evaporated to give a syrup which was distilled at 140°C(bath temperature)/0.05mm. The distillate, n_D^{17}, 1.4545, crystallised on leaving overnight at 0°C. The mixture was triturated with 1:3 ether-light petroleum and the crystalline material recrystallised from 1:5 ether-light petroleum, m.p.100-101°C, not depressed on admixture with an authentic specimen of dimethyl 2:3:4 trimethyl mucate, $[\alpha]_D^{17}$, $+28^{\circ}$ (c,0.86 in water).

INVESTIGATION OF THE ACETYLATED AND METHYLATED MUCILAGE.

Acetylation of the mucilage.

The mucilage(60gm.) was moistened with alcohol and thoroughly dispersed in pyridine(900c.c.) with shaking. To the mixture acetic anhydride(750c.c.) was added slowly in small amounts. Heat developed and a jelly-like mass was obtained which was heated on the boiling water bath for 3 hours. The resulting extremely viscous mixture was then allowed to stand for 48 hours at room temperature with frequent shaking. It was then poured into a large volume of vigorously stirred iced water and the semi-solid gelatinous mass hardened by grinding with ice and water in a mortar. The solid was filtered off and washed in running water for 24 hours and after a quick washing with alcohol was dried in air to give a greyish-white powder(75gm.)

Acetyl content of the acetylated mucilage.

To the acetate(ca. 0.1gm.) were added acetone(25c.c.) and standard N/10 sodium hydroxide(25c.c.) and the mixture allowed to stand overnight. The excess sodium hydroxide was estimated by titration with standard N/10 hydrochloric acid using phenolphthalein as indicator. Blank experiments were carried out concurrently.

Found: CH_3CO, 34.6% (average value).

Re-acetylation of a small portion of acetate.

The acetate(10gm.) was re-acetylated as above with pyridine(150c.c.) and acetic anhydride(100c.c.) and the

product was found to have CH_3CO, 35.4%.

It was evident that little increase in acetyl content had been obtained, and therefore the main bulk of the acetylated mucilage was not re-acetylated.

Fractionation of the acetylated mucilage.

The acetate was found to be partially soluble in acetone and chloroform and a fractionation was therefore attempted as follows:-

The acetate(3gm.) was extracted for 24 hours with a mixture of equal volumes of chloroform and acetone in a Haanen Badum extractor. 80% of the acetate formed a brown jelly-like mass while 20% went into solution.

The pale yellow viscous solution was evaporated to give an almost colourless glass, which was dissolved in a little chloroform and precipitated by pouring into light petroleum (B.P.60°-80°). It was obtained as a white fibrous solid(0.494gm.) and showed $[\alpha]_D^n$, -95° (c, 0.5 in chloroform).

The insoluble portion was obtained as a greyish-white amorphous powder(2.123gm.) by stirring in light petroleum (B.P.60°-80°) until crisp.

Acetyl contents of soluble and insoluble acetates.

Soluble fraction: CH_3CO, 37.4%

Insoluble fraction: CH_3CO, 30.2%

Fractionation of acetylated mucilage on a larger scale.

The acetate(20gm.) was extracted for 24 hours on a boiling water bath with a mixture of equal volumes of

chloroform and acetone. The soluble and insoluble fractions were separated by centrifugation and filtration through a sintered glass filter(17-X-3). The insoluble portion was extracted twice again and the filtrates combined. The acetates were precipitated in light petroleum as before to yield:-

6gm. soluble fraction (30%), $[\alpha]_D^{14}$, -88°(c,0.3 in $CHCl_3$)

14gm. insoluble fraction (70%).

Following this a further 31gm. were fractionated to give:- 80% insoluble fraction (25gm.),

20% soluble fraction (6gm.) $[\alpha]_D^{16}$,-99°(c,0.2 in $CHCl_3$).

By this method a very good separation was attained, the soluble acetate having a high negative rotation. In general it was found that removal of the insoluble acetate by centrifugation alone gave a slightly turbid solution which yielded an acetate with $[\alpha]_D^{13}$, -65°, whereas centrifugation followed by filtration through a fine sinter gave a clear solution which yielded an acetate with $[\alpha]_D^{14}$, -95° in $CHCl_3$.

The two fractions of the acetylated mucilage obtained were subsequently methylated and studied separately.

Uronic acid content of the acetates.

Unfractionated acetate: uronic anhydride, 3.6%

Soluble acetate: " " , 2.3%

Insoluble acetate: " " , 3.9%

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Methylation of the insoluble acetate.

The acetate was methylated by the method used by

Mullan and Percival.[35]

To the insoluble acetate(12gm.) in acetone(480c.c.) were added dimethyl sulphate(240c.c.) and 30% sodium hydroxide(600c.c.) in ten portions at ten minute intervals. The solution was stirred vigorously throughout the additions and the temperature of the water bath was allowed to rise slowly from 40° to 60°C so that towards the end the acetone was gently refluxing. After all the additions had been made the acetone was removed in a partial vacuum, the temperature being allowed to rise to 70°-80°C. After 1 hour at the latter temperature the product was filtered hot and washed quickly with boiling water to remove sodium sulphate.

The product was subjected to three further methylations, small samples being withdrawn after the third and fourth methylations. These were thoroughly washed with boiling water and refluxed with chloroform and anhydrous sodium sulphate. The dry chloroform solutions were evaporated to small volume and precipitated as a white fibrous material by pouring into light petroleum (B.P.60°-80°).

	$[\alpha]_D^{14}$ (c,0.4,$CHCl_3$)	OMe
3 methylations:	-102°	35.9%
4 methylations:	-103°	36.5%

Fractionation of the methylated material obtained from insoluble acetate.

The dry methylated compound(45gm.;OMe,36.5%) was

dissolved in chloroform(500c.c.) and light petroleum(B.P. 60°-80°) added in 50c.c. portions with continuous stirring. When a stable precipitate formed it was removed by filtration and further portions of light petroleum added to the filtrate until all the methylated material had been precipitated.

	Vol.petroleum added	Weight	OMe	$[\alpha]_D^{14}$(c,0.2,$CHCl_3$)
Fraction A	2200c.c.	3.75gm.	36.2%	-102°
Fraction B	3100c.c.	0.28gm.	36.6%	-103.5°
		4.03gm.		

Methylation on a larger scale.

In order to obtain sufficient material for a more complete investigation of fraction A, a further 25gm. insoluble acetate were methylated four times as before to give a product, OMe, 36.5%. Fractionation was again effected by precipitation from a chloroform solution with light petroleum to give fractions A and B in approximately the same proportions as before.

Methylation at room temperature in an atmosphere of nitrogen.

The insoluble acetate(25gm.) was dispersed in acetone (350c.c.) and dimethyl sulphate(400c.c.) and 30% sodium hydroxide(1000c.c.) added slowly in an atmosphere of nitrogen with vigorous stirring. Considerable heat was evolved and it was necessary to cool the flask occasionally. When all the reagents had been added, the mixture was heated to 40°C and the acetone removed in a partial vacuum. It was then heated to 90°C for half an hour,

filtered hot and the semi-solid mass washed once with hot water.

The solid was remethylated by dispersing in acetone (250c.c.) and adding dimethyl sulphate(240c.c.) and sodium hydroxide(600c.c.) as before. Ten methylations were carried out thus, samples being removed after the fourth and tenth methylations and dried and precipitated in light petroleum as before.

4 methylations: OMe, 34.7%

10 methylations: OMe, 35.5%; $[\alpha]_D^{16} = -104^\circ$(c,0.2,$CHCl_3$).

After ten methylations, hydrolysis of a small sample and examination on a paper chromatogram indicated approximately the same degree of methylation as before, i.e. approximately the same proportion of tri-/di-/mono-methyl pentoses as was obtained after four methylations at 40-60°C.

Followingthis a further attempt was made to raise the methoxyl content of this material.

Methylation in dioxan with heating.

The above material was dissolved in about 150c.c. dioxan (diethylene dioxide) and the mixture heated to 40°C. Dimethyl sulphate(250c.c.) and 30% sodium hydroxide(700c.c.) were added over a period of 2 hours with vigorous stirring, the temperature being raised gradually from 40° to 80°C. The mixture was then heated at 90°C for 30 minutes, and the semi-solid material filtered hot. The filtrate was neutralised with glacial acetic acid and evaporated to a sludge to which was added

500c.c. hot water. The mixture was heated at 100°C for 30 minutes, whereby the methylated material was precipitated as a semi-solid mass which was removed by filtration and added to the main bulk of the product and the whole remethylated as follows.

The sticky material was dissolved in dioxan(200c.c.) and dimethyl sulphate(200c.c.) and 30% NaOH(560c.c.) added over 2 hours at 40-80°C as before. As the temperature was raised the methylated material started to separate out and further quantities of dioxan were added to keep it in solution. After addition of all the reagents, a further 150c.c. dioxan were added and the mixture stirred vigorously at 90°C for 30 minutes. The mixture now comprised two immiscible layers, most of the methylated material being dissolved in the upper dioxan layer which was separated off.

The lower aqueous layer was extracted twice by shaking with hot dioxan and the extracts added to the main dioxan solution. In order to ensure complete extraction of the methylated material, the aqueous solution was then diluted to twice its volume with water and heated at 90°C for 30 minutes. A small amount of methylated material was precipitated which was filtered off and added to the dioxan extracts.

The combined extracts were evaporated to ca. 200c.c. and the methylation procedure repeated as before.

After four methylations, the dioxan solution was evaporated to dryness, anhydrous sodium sulphate and

chloroform added and the mixture allowed to stand overnight. The chloroform solution was evaporated to small volume and precipitated in light petroleum (B.P.60-80°C) to give a white fibrous solid (8gm.).

OMe, 36.8%

A trial hydrolysis and examination on a paper chromatogram showed little change in the ratio of the methylated sugars.

Methylation of the soluble acetate.

The acetate(10gm.) in acetone(400c.c.) was methylated as above by addition of dimethyl sulphate(200c.c.) and 30% sodium hydroxide(500c.c.) to yield a white fibrous compound(5gm.)

	$[\alpha]_D^{16}$ (c,0.2,$CHCl_3$)	OMe
3 methylations:	-104°	36.0%
4 methylations:	-104°	36.3%

Fractionation of the methylated material obtained from the soluble acetate.

The methylated compound(5gm.) was dried and dissolved in chloroform(250c.c.) and fractionally precipitated by addition of light petroleum (B.P.60-80°C) as before.

	Vol.petroleum added	Weight	OMe	$[\alpha]_D^{14}$ (c,0.2,$CHCl_3$)
Fraction C	1200 c.c.	4.21gm.	36.9%	-105°
Fraction D	1900 c.c.	0.29gm.	36.2%	-104°
		4.50gm.		

Methylation of fraction C by the Purdie method.

A small amount of the methylated polysaccharide fraction C (OMe,36.9%) was hydrolysed overnight with 3% methanolic hydrogen chloride in a sealed tube, and then overnight with 2% nitric acid in a sealed tube, and the hydrolysate examined on a paper chromatogram. This appeared to comprise the same mixture of methylated sugars as fraction A, but the proportion of end group appeared to be small, and therefore, in order to ensure that the polysaccharide was fully methylated, it was methylated a further twice by the Purdie method as follows. Fraction C (8.5gm.) was dissolved in methyl iodide(65c.c.). The resulting solution was extremely viscous and therefore it was diluted by the addition of chloroform(25c.c.). The solution still fairly viscous, was heated at 45°C and silver oxide(50gm.) added over a period of six hours and the solution kept at this temperature for a further 20 hours. The resulting viscous mixture was diluted with chloroform, centrifuged and filtered, and the silver oxide extracted four times by shaking with chloroform for an hour and filtering. The combined extracts were evaporated to give a yellowish sticky mass, which was subjected to another methylation as before.

The final product was a yellow syrup which was dissolved in chloroform and precipitated with light petroleum (B.P.60-80°). An impure greyish-white fibrous solid was obtained(OMe,30.0%). This almost certainly

still contained some silver iodide, as the chloroform solution had an opaque yellow colour and turned pink on standing in sunlight, and was therefore fractionally precipitated in order to obtain a pure sample for analysis. Two fractions were obtained:-

1) slightly discoloured,

2) white OMe, 36.6%; ($[\alpha]_D^{16}$ = -104°(c,0.2 in $CHCl_3$).

Methanolysis, hydrolysis and examination on a paper chromatogram showed a mixture of sugars very similar to that obtained from fraction A, and as the methoxyl content and specific rotation had not been altered by two Purdie methylations, it was concluded that fraction C was fully methylated.

Direct methylation of the mucilage.

The mucilage(12gm.) was methylated with dimethyl sulphate and 30% sodium hydroxide as before. A viscous solution was obtained which was neutralised with glacial acetic acid and freed from inorganic ions by dialysis in a cellophane bag. The solution was then evaporated to ca. 500c.c. and the methylation procedure repeated. After the second methylation some solid material separated out; this was removed and combined with the solution after dialysis and concentration. After three and four methylations samples of the solid material were dissolved in chloroform, dried (sodium sulphate) and precipitated with light petroleum(B.P.60-80°). After four methylations no soluble material was obtained on evaporation of the dialysate.

3 methylations: OMe, 30.6%

4 methylations: OMe, 31.0%

Viscosity measurements.

Viscosity measurements were carried out on 7c.c. of solutions of the acetylated and methylated polysaccharide in m-cresol in order to obtain some indication of the amount of degradation which had occurred during the acetylation and methylation processes.

η_{sp} = specific viscosity

c = concentration in gm. substance per 100c.c. solution.

c^1 = concentration in gram molecules of acetylated or methylated anhydroxylose residues per litre, assuming the repeating unit to be a dihydroxy anhydroxylose $C_5H_8O_4$.

Substance	c	c^1	Time of flow (seconds)	η_{sp}	η_{sp}/c	η_{sp}/c'
m-cresol			412			
Soluble acetate.	0.326	0.01509	1586	2.76	8.47	183
Compound obtained after 3 methylations of soluble acetate.	0.334	0.02085	2171	4.14	12.40	199
Compound obtained after 4 methylations of soluble acetate.	0.327	0.02023	1920	3.66	11.20	181
Methylated compound from insoluble acetate(4 methylations at 40-60°)	0.252	0.01575	3063	6.44	25.55	408

Substance	c	c^1	Time of flow (seconds)	η_{sp}	η_{sp}/c	η_{sp}/c'
Compound obtained by direct methylation.	0.306	0.01913	1290	2.14	7.00	112
Fraction A	0.346	0.02224	4820	10.48	30.3	482
Fraction B	0.343	0.02122	1665	3.04	8.87	146
Fraction C	0.336	0.02100	2205	5.36	15.93	254
Fraction D	0.342	0.02136	750	0.82	2.4	39
Compound from insoluble acetate after 4 methylations at room temperature.	0.106	0.006624	1361	2.305	21.7	348
Compound from insoluble acetate after 10 methylations at room temperature.	0.159	0.00994	1563	2.793	17.6	281
Compound from insoluble acetate after 10 methylations at room temperature and 4 in dioxan.	0.219	0.009714	595	0.443	2.02	46
Fraction C after 2 Purdie methylations.	0.333	0.0208	981	1.381	4.15	66

Methanolysis of fraction A.

The methylated polysaccharide A(8gm.; OMe,37.4%) was hydrolysed at 100°C with methanolic hydrogen chloride(3%; 160c.c.) until the rotation became constant. $[\alpha]_D^{16}$, +54° (20 hours). The solution was neutralised with silver carbonate, filtered and evaporated to dryness. The residue was extracted with hot methanol, the solution filtered and evaporated to give a mobile non-reducing syrup (9.12gm.).

A small portion of this syrup was hydrolysed with 2% nitric acid in a sealed tube at 100°C for 7 hours. The solution was neutralised with barium carbonate and deionised by shaking with Amberlite resins IR100 and IR4B. Paper chromatography indicated the presence of the following sugars, by direct comparison with controls:-

Trimethyl xylose, R_G value 0.94
Tetramethyl galactose, R_G value 0.88
A sugar of R_G 0.81
2:3-dimethyl xylose, R_G 0.74
2:4-dimethyl xylose, R_G 0.66
monomethyl xylose, R_G 0.38
xylose, R_G 0.15.

Fractionation of the methyl glycosides.

An attempt to achieve a separation of the fully methylated, partially methylated and free sugars was made by the method of Solvent Extraction, based on the original procedure of Brown and Jones.(53) Two continuous extractors and a condenser are fitted one above the other and

connected to a reservoir flask containing boiling light petroleum. The vapour is condensed and bubbles through an aqueous solution of the methyl glycosides in the top extractor. The fully methylated glycosides should be extracted in this way, and the extracts are washed by passing through water in the lower extractor before returning to the flask. The extraction is interrupted at intervals and the solvent evaporated and the syrup weighed.

The syrup(9.05gm.) was dissolved in 50c.c. water and extracted with 200c.c. purified light petroleum (B.P.40-45°) to give the following fractions:-

	Time of extraction	Weight	Temp.(°C)	n_D
(1)	2 hours	1.818gm.	18	1.4400
(2)	+ 2 "	0.582	18	1.4440
(3)	+ 2 "	0.215	18	1.4483
(4)	+ 4 "	0.318	18	1.4489
(5)	+ 6 "	0.226	15	1.4535
(6)	+ 6 "	0.170	15	1.4542
(7)	+16 "	0.397	16	1.4517
		3.726		

The residual aqueous solution was evaporated to dryness to give a mobile syrup (4.178gm.)
Recovery 88%.

Hydrolysis of small portions of fractions (1)-(7) with 2% nitric acid as before and examination on a paper chromatogram showed that no effective separation had been achieved. All the fractions consisted of mixtures

of fully methylated, dimethyl and small amounts of monomethyl methyl-glycosides. After the seventh extraction, however, only monomethyl methyl-xylosides and methyl-xylosides remained in the aqueous solution.

It was thought that the light petroleum used may have had too high a boiling point to effect an efficient separation and therefore the extraction was repeated, using lower boiling-point light petroleum.

Fractions (1)-(7) were combined with the syrup from the aqueous solution and the syrup(7.35gm.)was extracted as before using light petroleum (B.P.38-40°), with the addition of a little barium carbonate to the aqueous solution to prevent acidity. The following fractions were obtained:-

	Time of extraction	Weight	Temp.(°C)	n_D
(1)	16 hours	2.406gm.	16	1.4448
(2)	+ 6 "	0.167	18	1.4520
(3)	+16 "	0.459	15	1.4552
(4)	+45 "	0.356	15	1.4560
		3.388		

The aqueous solution was evaporated to dryness to give a syrup (3.579gm.).

Recovery 95%.

Fractions (1)-(4) were shown by hydrolysis and paper chromatography to contain fully methylated and dimethyl methyl-glycosides with only a slight trace of monomethyl methyl-glycosides, and were combined to give fraction I.

All of the fully methylated and dimethyl methyl-glycosides had been removed from the aqueous extract and the syrup(3.58gm.) was dissolved in water(30c.c.) and extracted in the same way as before with chloroform using a Soxhlet-type extractor. After 18 hours the chloroform and aqueous extracts were evaporated to give the following fractions:-

Fraction II (chloroform extract)3.325gm., containing monomethyl methyl-xylosides and traces of dimethyl methyl-xylosides and other glycosides.

Fraction III (water extract)0.250gm., containing monomethyl methyl-xylosides and methyl-xylosides.

Recovery 100%.

Hydrolysis of fraction I and separation of the sugars on a column of cellulose.

Fraction I(3.39gm.) was hydrolysed at 100°C with 2% nitric acid(150c.c.) until the rotation became constant. $[\alpha]_D^{15}$, +49°(3½hrs.); +43°(8hrs.); +36°(9½hrs.); +31°(12hrs., constant).

The solution was neutralised with barium carbonate, evaporated to dryness and the residue extracted with hot methanol to yield a reducing syrup (3.054gm.).

The syrup(3.019gm.) was fractionated on a column of cellulose (27" x 1.2") according to the method of Hough, Jones & Wadman.[10] The eluent used was light petroleum (B.P.100-120°)/butanol (7/3) saturated with water. Every tenth tube of solution collected was evaporated and

tested on a paper chromatogram for presence of sugars, and all the tubes containing the same sugars combined and the solvent evaporated. In order to purify them, all the syrups thus obtained were dissolved in water, digested with charcoal and filtered hot; after evaporation of the water, the residue was exhaustively extracted with boiling acetone (unless otherwise stated), filtered hot, evaporated, dried in vacuo over P_2O_5 and weighed.

The following fractions were obtained and identified as stated by direct comparison with controls on a paper chromatogram. The papers were sprayed with aniline oxalate.

Fraction	Weight	Rg value	Colour of spot	Possible identity of sugar.
(a)	1.013gm.	0.94	pink	trimethyl-xylose (and -arabinose?)
(b)	0.457gm.	0.90	brown	tetramethyl galactose
(c)	0.214gm.	0.81	grey-black	?
(d)	0.128gm.	0.81	grey-black	?
		0.64	pink	2:3-dimethyl xylose
(e)	0.416gm.	0.64	pink	2:3-dimethyl xylose
(f)	0.230gm.	0.66	pink	2:4-dimethyl xylose
(g)	0.159gm.	0.66	pink	2:4-dimethyl xylose
		0.52	brown	?
		0.38	pink	monomethyl xylose
		0.15	"	xylose.
	2.617gm.			

Fraction (g) was washed out of the column with water. Recovery 88%.

Hydrolysis of fraction II and separation of the sugars on a column of cellulose.

Fraction II(3.325gm.) was hydrolysed with 2% nitric

acid(150c.c.) at 100°C for 6 hours. $[\alpha]_D^{16}$,+60°(zero time); +49°(1hr.); +36°(3hrs.); +29°(6hrs.,constant). The solution was neutralised and treated as usual to give a reducing syrup (3.171gm.)

The syrup(2.80gm.) was fractionated on a column of cellulose (27" x 1.2") in the same way as the sugars from Fraction I, the eluent again being light petroleum (B.P. 100-120°)/butanol (7/3) saturated with water. The following fractions were obtained:-

Fraction	Weight	R_G	Colour of spot (aniline oxalate)	Possible identity of sugar
(h)	0.085gm.	0.80	grey-black	?
(i)	0.158	0.80	grey-black	?
		0.74	pink	2:3-dimethyl xylose
(j)	0.183	0.74	pink	2:3-dimethyl xylose
		0.66	pink	2:4- " "
(k)	0.205	0.66	pink	2:4-dimethyl xylose
		0.58	brown	?
		0.50	brown	?
(l)	0.077	0.50	brown	?
		0.38	pink	monomethyl xylose
(m)	1.822	0.38	pink	monomethyl xylose
(n)	0.063	0.38	pink	monomethyl xylose
		0.24	pink	?
		0.15	pink	xylose
	2.595gm.			

Fraction (n) was washed out of the column with water. For purification it was extracted with 80% ethanol.

Fraction (m) was extracted with ethanol.

Recovery 93%.

Hydrolysis of fraction III.

The syrup(0.250gm.) was hydrolysed with 2% nitric

acid(30c.c.) until the rotation became constant. $[\alpha]_D^{17}$, +47°(1hr.); +25°(4hrs.); +24°(5hrs., constant).

The solution was neutralised with barium carbonate and treated in the usual way to yield a reducing syrup (0.215gm.).

When examined on a paper chromatogram and compared with standards this was seen to consist of the following sugars:-

2-methyl xylose	ca. 0.1gm.
xylose	ca. 0.1gm.
arabinose	(trace).
sugar R_G 0.24	(trace).

Methanolysis of fraction C.

Fraction C after two Purdie methylations(4.5gm.) was heated at 100°C with 3% methanolic hydrogen chloride(90c.c.) until the rotation became constant $[\alpha]_D^{16}$, +57°(3hrs.); +64° (22hrs., constant). The solution was neutralised with silver carbonate, filtered and evaporated to dryness to give a syrup (ca.5gm.).

Hydrolysis of the methyl glycosides.

The syrup(5gm.) was heated at 100°C with 2% nitric acid(80c.c.) until the rotation became constant $[\alpha]_D^{16}$, +29°(4hrs.); +27°(5hrs., constant). The solution was neutralised with barium carbonate and treated as usual to give a syrup (3.71gm.).

Separation of the free sugars on a column of cellulose.

A partial separation of the sugars in the syrup(3.71gm.) was obtained on a column of cellulose (27" x 1.2"). The eluent was 70/30 light petroleum (B.P.100-120°)/butanol saturated with water, which was gradually changed to a 50/50 mixture as the sugars were eluted.

No effective separation of the fully methylated sugars was achieved, and therefore the first 200 tubes containing these were combined and re-columned.

Fraction	Weight	Rg value	Colour of spot (aniline oxalate)	Possible identity
(o)	1.657gm.			
(p)	0.280gm.	0.74	pink	2:3-dimethyl xylose
(q)	0.285gm.	0.66	pink	2:4-dimethyl xylose
(r)	0.141gm.	0.58	brown	?
(s)	0.020gm.	0.58 0.50	brown brown	?
(t)	0.052gm.	0.50 0.38	brown pink	?
(u)	0.153gm.	0.38	pink	3-or 2-methyl xylose
(v)	0.438gm.	0.38	pink	2-methyl xylose
(w)	0.015gm.	0.24	pink	?
(x)	0.064gm.	0.15	pink	xylose
	3.105gm.			

Recovery 85%.

The syrup (1.657gm.) containing the fully methylated sugars was separated on another column (27" x 1.2") using a 70/30 mixture of the same solvent. The following fractions were obtained:-

Fraction	Weight	R_G value	Colour of spot (aniline oxalate)	Possible identity
(O_1)	0.805gm.	0.94	pink	trimethyl xylose
(O_2)	0.136	0.94 0.88	pink brown	trimethyl xylose tetramethyl galactose
(O_3)	0.098	0.88	brown	tetramethyl galactose
(O_4)	0.233	0.80 0.74	grey-black pink	? 2:3-dimethyl xylose
(O_5)	0.114 1.386gm.	0.74	pink	2:3-dimethyl xylose

Recovery 84%.

Separation of the sugar with R_G 0.80 and 2:3-dimethyl xylose on a column of cellulose.

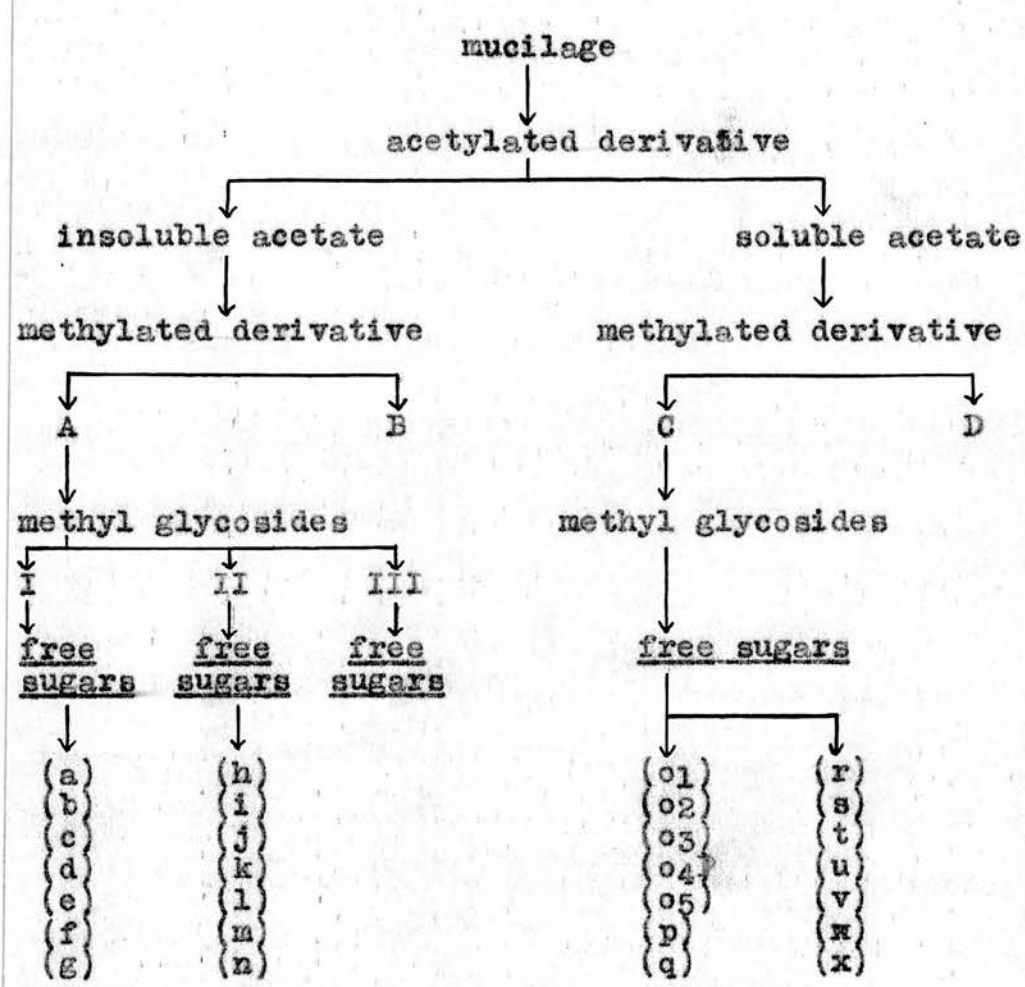
Fractions (d), (i) and (O_4) were combined to give a syrup(0.50gm.) which was separated on a column of cellulose (19" x 1") using a 70/30 mixture of the usual solvent to give the following fractions:-

Fraction	Weight	R_G value	Colour of spot (aniline oxalate)	Possible identity
(i)	0.014gm.			trace of each.
(ii)	0.244gm.	0.80	grey-black	?
(iii)	0.087	0.80 0.74	grey-black pink	? 2:3-dimethyl xylose
(iv)	0.094 0.439gm.	0.74	pink	2:3-dimethyl xylose

Recovery 88%.

Fractions (c), (h) and (ii) were compared on a chromatogram and found to comprise the same sugar, all giving a characteristic grey-black spot with aniline oxalate. Therefore, in order to obtain sufficient material for identification, these were combined to give fraction H.

To summarise, the following fractions have been obtained:-



$(d) + (i) + (o_4) \longrightarrow$ (i), (ii), (iii), (iv)

$(c) + (h) + (ii) \longrightarrow$ H.

EXAMINATION OF THE METHYLATED SUGAR FRACTIONS.

Sugars obtained from fraction A.

Identification of fraction (a) as a mixture of trimethyl xylopyranose and trimethyl arabofuranose.

The syrup(1.013gm.) partially crystallised and the crystals (0.537gm.) were separated by tiling. These crystallised as long needles from dry ether and had m.p.90-92°C and mixed m.p. with an authentic specimen of 2:3:4-trimethyl-α-D-xylopyranose 89-91°C.
$[\alpha]_D^{16}$, +66.7°(zero time); +51°(15mins.); +29°(1½hrs.); +19°(3hrs., constant). (c, 0.6 in water).

Found: C, 50.16%; H, 8.5%; OMe, 47.7%.

Calc.for $C_8H_{16}O_5$: C,50.0%; H, 8.4%; OMe, 48.4%.

The syrup(0.432gm.) was extracted from the tile with boiling acetone and had $[\alpha]_D^{19}$, +9.6°.

Lactone formation.

The syrup was dissolved in water(15c.c.) and oxidised with liquid bromine until it was found to be non-reducing after removal of the bromine by aeration. The solution was neutralised with silver carbonate, filtered and the free acid obtained by passing hydrogen sulphide through the solution for 15 minutes. The silver sulphide was removed by filtration and the solution evaporated to dryness. The syrup was dissolved in acetone, filtered free from any silver salts, the acetone removed and the resulting syrup heated at 100°/18mm. for 2 hours and then fractionally distilled to yield the following syrupy

distillates:-

(1a) 0.104gm. B.P.90°-110°(bath temp.)/0.15mm.

(2a) 0.080gm. B.P.110°-120°(bath temp.)/0.10mm.

Study of the lactone fraction (1a).

This showed $[\alpha]_D^{16}$, +6.7°(5mins.); +1.9°(22hrs.); 0° (45hrs.); +12.5°(100hrs.,constant). (c, 1.04 in water).

Titration. 2c.c. of the polarimetric solution were titrated immediately on solution with 0.0322N NaOH, using phenolphthalein as indicator. 3.39c.c. were required.

Calculated for $C_8H_{14}O_5$, 3.40c.c.

Neutralisation was slow.

Amide formation. The polarimetric solution was evaporated to dryness and the syrup redistilled to give the lactone which was treated with methanolic ammonia at 0°C for four days. The solvent was removed in a vacuum dessicator to give the syrupy amide which was inoculated with a crystal of 2:3:5-trimethyl arabonamide and left at 0°C. After 10 months it had partially crystallised and the crystals were separated by tiling and recrystallised as needles from ethyl acetate. They had m.p.136° alone or in admixture with an authentic specimen of 2:3:5-trimethyl arabonamide (m.p. 139°).

Study of the lactone fraction (2a).

This showed $[\alpha]_D^{16}$, -1°(½hr.); +6°(1hr.); +13°(4hrs.); +15°(72hrs.); +18°(200hrs.,constant). (c, 2.0 in water.)

Titration. 0.0163gm. required 2.54c.c. 0.0322N NaOH for neutralisation.

Calculated for $C_8H_{14}O_5$, 2.66c.c.

Neutralisation was slow.

Identification of fraction (b) as 2:3:4:6-tetramethyl-D-galactose.

The syrup crystallised almost entirely on keeping for a few weeks. The crystals were separated from the syrup by tiling and recrystallised from light petroleum (B.P.40-60°) as long needles, m.p. and mixed m.p. with 2:3:4:6-tetramethyl-D-galactose 70-72°.
The crystals had $[\alpha]_D^{16}$, +142°(5mins.); +129°(30mins.); +119°(1hr.); +116.6°(3hrs.,constant). (c, 1.09 in water).
They gave the following figures on analysis:-
Found: C, 50.9%; H, 8.7%; OMe, 52.0%.
Calc. for $C_{10}H_{20}O_6$: C, 50.8%; H, 8.5%; OMe, 52.5%.
The residual syrup had $[\alpha]_D^{18}$, +114°, OMe, 51.7%.
Anilide formation. To the syrup was added freshly distilled aniline(0.13gm.) and absolute alcohol(2.c.c.) and the solution was heated under reflux at 80°C for 1.5hours. Crystals separated immediately on cooling the solution and were recrystallised twice from alcohol. They had m.p. 192-194° alone and m.p. 193-194° on admixture with an authentic specimen of 2:3:4:6-tetramethyl-D-galactose anilide.
Found: C,61.8%; H,8.0%;N,4.3%; OMe,39.4%.
Calc. for $C_{16}H_{25}O_5N$: C,61.7%; H,8.1%;N,4.5%; OMe,39.9%.
The anilide had $[\alpha]_D^{17}$,+41.5° (equilibrium rotation)
(c, 0.60 in acetone.)

Examination of fraction (d).

This constituted a mixture of fractions (c) and (e).

The relative proportion of these was determined by separating the sugars on a paper chromatogram, extracting them from the paper strips with boiling water for 45 minutes and estimating them by oxidation with alkaline hypoiodite.[54] A phosphate buffer pH 11.4[55] was used instead of bicarbonate, in order to avoid loss of solution by effervescence during the subsequent neutralisation.

To the cooled solutions were added 0.1N iodine solution(1c.c.) and phosphate buffer pH 11.4(2c.c.). The tubes containing the solutions were closed with ground glass stoppers moistened with 10% potassium iodide and left at room temperature for 3 hours. The solutions were then diluted to ca. 25c.c., acidified by the addition of 2N sulphuric acid(2c.c.) and titrated with 0.00982N sodium thiosulphate. Paper blanks were also run.

The following results were obtained:-

(1) fraction (c) ≡ 0.568c.c. $Na_2S_2O_3$

fraction (e) ≡ 0.737c.c. $Na_2S_2O_3$

Assuming (c) to be a sugar of molecular weight 194 (see p. 88) and (e) to be a dimethyl pentose, molecular weight 178, then

$$\frac{\text{Wt. fraction (c)}}{\text{Wt. fraction (e)}} = \frac{0.568 \times 194}{0.737 \times 178} = \frac{45.7\%}{54.3\%}$$

(2) fraction (c) ≡ 0.519c.c. $Na_2S_2O_3$

fraction (e) ≡ 0.652c.c. $Na_2S_2O_3$

$$\frac{\text{Wt. fraction (c)}}{\text{Wt. fraction (e)}} = \frac{46.4\%}{53.5\%}$$

∴ fraction (d) (0.128gm.) comprises a mixture of the sugars in fractions (c) and (e) in the proportion 46%

(c) (0.059gm.) and 54% (e) (0.069gm.)

Examination of fraction (e).

This did not crystallise and had n_D^{17}, 1.4727. OMe, 34.1%. Calc. for $C_7H_{14}O_5$, OMe, 34.8%. $[\alpha]_D^{16}$, was +24°(15mins.); +25.6°(6hrs.,constant).

Examination of the syrup by paper chromatography showed it to be a mixture. After running the paper for 40 hours in benzene(167)/ethanol(47)/water(16) solvent an "8-shaped" spot was obtained which consisted of mostly 2:3-dimethyl xylose and a sugar which travelled slightly faster than the latter. Both gave a pink colour with aniline oxalate.

Lactone formation. The syrup was oxidised with bromine until non-reducing (7 days) and treated as usual to give a syrup which was distilled to give the lactone(0.117gm.). B.P. 125-135°(bath temp.)/0.03mm.
This had $[\alpha]_D^{16}$, +28°(initial reading).

Amide formation. The lactone was treated with methanolic ammonia at 0°C to give a brown glass which did not crystallise. A Weerman test[36] was carried out on this and a precipitate of hydrazodicarbonamide was obtained, m.p. and mixed m.p. with an authentic specimen 257°C. A control test on gluconamide gave hydrazodicarbonamide and one on 2:3-dimethyl xylonamide was negative.

As 2:3-dimethyl xylonamide does not yield hydrazodicarbonamide this result indicates that the second component in the mixture must possess a free hydroxyl group on the second carbon atom.

Attempted separation of the lactone.

Following this, a fresh portion of the free sugar was oxidised with bromine and the lactone fractionally distilled as follows:-

(1b) 0.025gm. B.P.120-125°(bath temp.)/0.04mm. mobile syrup.

(2b) 0.009gm. B.P. 150°(bath temp.)/0.03mm. viscous yellow syrup.

Fraction (1b) $[\alpha]_D^{16}$, +47°(4hrs.); +38°(25hrs.); +35°(68hrs.); +31°(120hrs.); +29°(215hrs.); +27.5°(270hrs., constant). (c, 1.27 in water).

Fraction (2b) n_D^{17} = 1.4669

$[\alpha]_D^{16}$, +25.6°(½hrs.); +19°(5hrs.); +12.8°(18hrs.); 0°(47hrs.); +10.6°(121hrs.); +21°(143hrs.); +17°(166hrs.); +15° (196hrs.); +12.8°(212hrs.); +8.5°(289hrs., constant). (c, 0.47 in water.)

Anilide formation.

The syrup(0.035gm.) was heated with freshly distilled aniline(0.023gm.) in absolute alcohol(1c.c.) at 80°C for 1.5 hours. The alcohol was removed in a vacuum dessicator to give a brown syrup which failed to crystallise.

Demethylation.

Fraction (e) was demethylated by the method of Hough Jones and Wadman.(56) The syrup(10mg.) was heated at 100°C with hydrobromic acid(48%;1c.c.) in a sealed tube for 7 minutes. The solution was cooled and diluted to 10c.c. immediately, neutralised with silver carbonate and filtered. Ag^+ was removed as silver sulphide and the solution evaporated to give a syrup which was examined on a paper chromatogram. The following spots were

obtained on spraying the paper with aniline oxalate:-

	R_G value	Colour of spot	Possible Identity
(1)	0.64	pink	Unchanged material
(2)	0.46	pink	? arabinose derivative
(3)	0.38	pink	2- and/or 3-methyl xylose
(4)	0.24	pink	? arabinose derivative
(5)	0.15	pink	xylose
(6)	0.12	pink	arabinose.

These results indicate that fraction (e) must contain a dimethyl arabinose derivative which gives arabinose on demethylation.

It also seems possible that the unidentified sugar, R_G value 0.24, which occurs in fractions (n), III and (w) is the same as that obtained above (3) and is therefore a monomethyl arabinose derivative.

Identification of fraction (f) as 2:4-dimethyl xylose.

This crystallised almost entirely. Trituration with acetone: light petroleum(B.P.40-60°) (1:3) and recrystallisation from acetone: light petroleum(B.P.40-60°) (1:10) gave 2:4-dimethyl-β-D-xylose as long needles, m.p.111°, not depressed on admixture with an authentic specimen.

$[\alpha]_D^{17}$, -27.6°(5mins.); +10.6°(50mins.); +21.5°(2hrs., constant). (c, 0.47 in water).

Found: C, 47.1%; H, 7.6%; OMe, 34.3%.

Calc.for $C_7H_{14}O_5$: C, 47.2%; H, 7.9%; OMe, 34.8%.

Lactone formation. The sugar(0.10gm.) was oxidised with

bromine till non-reducing and treated as usual to give the lactone, which was distilled to give a syrup which did not crystallise. B.P.135-140°(bath temp.)/0.01mm.

$[\alpha]_D^{20}$, -14°(8mins.); -7°(2hrs.); 0°(4½hrs.); +14°(21hrs.); +18°(28hrs.); +23°(48hrs.); +27°(118hrs., constant); (c, 0.3 in water.)

Amide formation. The lactone was treated with methanolic ammonia at 0°C and the solvent removed in a vacuum dessicator to give a syrupy amide. This gave a negative Weerman test, indicating substitution of position 2 by a methoxyl group.

Fraction (g)

Paper chromatography indicated that this comprised mainly equal proportions of monomethyl xylose and a sugar of R_G 0.52, with a trace of 2:4-dimethyl xylose and xylose.

Examination of fraction (i).

This comprised a mixture of fraction (h) and the first sugar in fraction (j), i.e. 2:3-dimethyl xylose. The relative proportions of these were estimated by oxidation with alkaline hypoiodite after separation on a paper chromatogram in the same way as fraction (d).

(1) fraction (h) ≡ 0.15c.c. $Na_2S_2O_3$

2:3-dimethyl xylose ≡ 0.38c.c. $Na_2S_2O_3$.

$$\therefore \frac{\text{fraction (h)}}{\text{2:3-dimethyl xylose}} = \frac{30.1\%}{69.9\%}$$

(2) fraction (h) ≡ 0.30c.c. $Na_2S_2O_3$

dimethyl xylose ≡ 0.86c.c. $Na_2S_2O_3$.

$$\therefore \frac{\text{fraction (h)}}{\text{2:3-dimethyl xylose}} = \frac{27.6\%}{72.4\%}$$

Therefore the mixture(0.158gm.) consists of 29% fraction (h) (0.046gm.) and 71% 2:3-dimethyl xylose(0.112gm.)

Examination of fraction (j).

Paper chromatography showed this to be a mixture of 2:3- and 2:4-dimethyl xyloses. The proportion of 2:3-dimethyl xylose in the mixture was determined by oxidation with periodic acid and estimation of the formaldehyde produced as the dimedon compound, according to the method of Reeves.[57]

Dr. S.K. Chanda found that maximum oxidation of 2:3-dimethyl xylose was achieved after four days. A control experiment carried out with pure crystalline 2:3-dimethyl xylose as follows showed that it was 70% oxidised after this time.

2:3-dimethyl xylose(16.12mg.) was dissolved in 2c.c. water in a test tube, 1.0N sodium bicarbonate(2c.c.) and 0.3M periodic acid(2c.c.) added and the solution left in the dark at room temperature for 96 hours. Then 1.0N hydrochloric acid(3c.c.) and 1.2N sodium arsenite(2c.c.) were added and the solution mixed. When the precipitate and yellow colour had disappeared 1.0M sodium acetate (2c.c.) and dimedon solution(85mg./c.c. 95% alcohol;1c.c.) were added and the mixture heated at 100°C for 5 minutes. After standing overnight the precipitate was filtered, dried at 85°-95° for 20 minutes and weighed. 18.52mg. of the compound were obtained, m.p.189-190°. This is

equivalent to 1.902mg. formaldehyde, indicating 70% oxidation of the 2:3-dimethyl xylose.

Under similar conditions fraction (j)(16.95mg.)gave 7.60mg. dimedon-formaldehyde compound or 0.7806mg. formaldehyde. This corresponds to the presence of 39% 2:3-dimethyl xylose in the mixture.

i.e. fraction (j) (0.183gm.) comprises 2:3-dimethyl xylose (0.112gm.) and 2:4-dimethyl xylose(0.112gm.).

Fractions (k) and (l).

These were mixtures of 2:4-dimethyl xylose, two sugars of R_G values 0.58 and 0.50, and a trace of monomethyl xylose. Both the unknown sugars gave brown spots on spraying with aniline oxalate.

Demethylation of fraction (k) (10mg.) with hydrobromic acid(48%;1c.c.) at 100°C for 7 minutes and treatment as usual yielded a syrup which was examined by paper chromatography and gave the following spots when sprayed with aniline oxalate:-

	R_G value	Colour of spot	Possible identity.
(1)	0.66 0.58 0.50	pink) brown) brown)	Unchanged material
(2)	0.37	pink	2 methyl xylose
(3)	0.24	pink	? monomethyl arabinose
(4)	0.15	pink	xylose (trace)
(5)	0.12	pink	arabinose

The two unidentified sugars were originally suspected to be dimethyl galactose derivatives, since

they gave a brown colour with aniline oxalate, but no galactose was obtained on demethylation and the results seem to indicate that they are arabinose derivatives.

These results also lend support to the theory (see p.77) that the sugar, R_G 0.24, is an arabinose derivative.

Fractions (k) and (l) were both too small for satisfactory separation and identification.

Characterisation of fraction (m) as 2-methyl xylose.

Paper chromatography indicated that this consisted of 2-methyl xylose, by direct comparison with a control. Purity of the crude material. The crude sugar(0.0506gm.) was dissolved in water(50c.c.). 1c.c. portions of this solution were oxidised with alkaline hypoiodite as usual and were equivalent to 1.225c.c. 0.00853N sodium thiosulphate. This indicates 94% aldose in the material.

The sugar crystallised completely and was recrystallised from n-butanol to give crystals m.p.155-7°, not depressed on admixture with a specimen of 2-methyl xylose. $[\alpha]_D^{17}$, -17°(3mins.); +12.5°(15mins.); +33.6°(75mins.); +35°(100mins.,constant). (c, 0.7 in water.)

Found: C, 43.7%; H, 7.6%; OMe, 18.6%.

Calc.for $C_6H_{12}O_5$: C, 43.9%; H, 7.4%; OMe, 18.9%.

The syrup reclaimed from the mother liquor after crystallisation of the 2-methyl xylose had $[\alpha]_D^{16}$, +34.9° (c, 0.6 in water).

Anilide formation. The crystals(0.12gm.) were heated with aniline(0.072gm.) and absolute alcohol(2c.c.) for

1.5hrs. at 80°C. The alcohol was removed in a vacuum dessicator to leave a whitish-brown mass of crystals. These were washed with cold dry ether to remove excess aniline and recrystallised from absolute alcohol to give 2-methyl xylose anilide, m.p.124-5° alone or in admixture with an authentic specimen.

$[\alpha]_D^{16}$, +213°. (c, 0.7 in ethyl acetate).

Found: C, 60.0%; H, 7.04%; OMe, 13.2%.

Calc.for $C_{12}H_{17}O_4N$: C, 60.3%; H, 7.1%; OMe, 12.9%.

Fraction (n).

Paper chromatography showed this to be a mixture of 2-methyl xylose, free xylose and a sugar of R_G value 0.24. The latter gave a pink spot on spraying with aniline oxalate and is probably a monomethyl arabinose derivative. (see pp.77,81).

The syrup was too small for satisfactory investigation.

Sugars obtained from fraction C.

Identification of fraction (o_1) as a mixture of trimethyl xylopyranose and trimethyl arabofuranose.

The syrup(0.805gm.) partially crystallised and was tiled to give a white solid(0.527gm.) which was recrystallised from dry ether to give needles, m.p. and mixed m.p. with 2:3:4-trimethyl-D-xylose, 89-91°C.

The tile was extracted with acetone and the extracts evaporated to give a syrup(0.256gm.).

This syrup was oxidised with bromine water and treated as usual to give the lactone which was fractionally distilled to give:-

fraction (1c) B.P.80-100°C(bath temp.)/0.07mm.
0.0626gm. n_D'', 1.4750.

fraction (2c) B.P.100-125°C(bath temp.)/0.07mm.
0.0677gm. n_D'', 1.4583.

Fraction (1c) was dissolved in 4.0c.c. water. 1c.c. of this solution was titrated with 0.01964 N sodium hydroxide.

Neutralisation was slow.

Volume required: 4.29c.c.

Calc. for $C_8H_{14}O_5$: 4.42c.c.

$[\alpha]_D^{16}$, -5.7°(1hr.); -5.1°(18hrs.); -0.64°(94hrs.); +1.9°(118hrs.); +3.2°(140hrs.); +4.5°(284hrs., constant). (c, 1.57 in water).

The polarimetric solution was evaporated to dryness and lactonised by heating at 100°/18mm./3 hours and distilling as before.

The lactone thus obtained was treated with methanolic ammonia at 0°C to give a syrupy amide which was inoculated with a crystal of 2:3:5-trimethyl arabonamide. After 6 months at 0°C the syrup had failed to crystallise.

Fraction (2c) was dissolved in 4c.c. water. 1c.c. of this solution was titrated with 0.01964 N sodium hydroxide. Neutralisation was slow.

Volume required: 4.69c.c.

Calc. for $C_8H_{14}O_5$: 4.72c.c.

$[\alpha]_D^{17}$, -4.2°(10mins.); -1.2°(25hrs.); +1.8°(43hrs.); +9° (93hrs.); +10°(167hrs.); +12°(200hrs., constant)(c,1.7 in water).

Investigation of fraction (α_2).

Paper chromatography showed this to be a mixture of 2:3:4-trimethyl xylose and 2:3:4:6-tetramethyl galactose. The relative proportions of these were estimated by oxidation with alkaline hypoiodite after separation on a paper chromatogram.

The syrup(0.136gm.) was dissolved in water(5c.c.)and 0.027c.c. of this solution spotted on two paper chromatograms which were run in butanol(40)/ethanol(10)/water(49)/ ammonia(1) solvent for 42 hours to effect separation of the sugars. After extraction and oxidation of the sugars the excess iodine was titrated with 0.01002N sodium thiosulphate with the following results:-

Trimethyl xylose ≡ 0.331c.c. $Na_2S_2O_3$.

Tetramethyl galactose ≡ 0.238c.c. $Na_2S_2O_3$.

$\therefore \frac{\text{Weight trimethyl xylose}}{\text{Weight tetramethyl galactose}} = \frac{0.331 \times 192}{0.238 \times 236} = \frac{53.1\%}{46.9\%}$

$\therefore$ fraction (o_2) (0.136gm.) consists of trimethyl xylose (0.072gm.) and tetramethyl galactose(0.064gm.).

Identification of fraction (o_3) as tetramethyl-D-galactopyranose.

The syrup had $[\alpha]_D^{17}$, +113.5°(c, 0.13 in water).

Anilide formation. The syrup(0.051gm.) was heated at 80°C with freshly distilled aniline(0.02gm.) and absolute alcohol(5c.c.) for 1.5 hours. The solution was cooled and evaporated to smaller volume, when needles separated out. These were recrystallised from absolute alcohol and had m.p.193-195°C, not depressed on admixture with an authentic specimen of 2:3:4:6-tetramethyl-D-galactose anilide.

Investigation of fraction (p).

Paper chromatography showed this to consist of a mixture of the same two sugars as were present in fraction (e) (p.75). 2:3-dimethyl xylose was present in major proportion and the other component, which travelled slightly faster than 2:3-dimethyl xylose in benzene(167)/ethanol(47)/water(16) solvent and gave a pink colour with aniline oxalate, was probably a dimethyl arabinose derivative, by analogy with fraction (e).

The syrup(0.280gm.) was inoculated with a crystal of 2:3-dimethyl xylose obtained from Dr. S.K. Chanda[69] and after 7 months at 0°C had partially crystallised. The cubic crystals were removed, tiled and quickly washed

with light petroleum to give a white powder(0.024gm.)m.p. 80-82°C, and mixed m.p. with Dr. Chanda's specimen 79-80°C.

The crystals had $[\alpha]_D^{16}$, +61°(7mins.); +43.5°(15mins.); +31°(50mins.); +26°(70mins.); +24°(3hrs.); +22.6°(18hrs., constant). (c, 0.6 in water).

Identification of fraction (q) as 2:4-dimethyl xylose.

This crystallised almost completely. It was triturated with acetone/light petroleum (B.P.60-80°)(1/3) and recrystallised from a 1/10 mixture of the same solvents to give long needles m.p.111°, not depressed on admixture with an authentic specimen of 2:4-dimethyl-β-D-xylose.

The crystals had $[\alpha]_D^{17}$, -26°(10mins.); +11°(50mins.); +22°(2hrs., constant).

Examination of fraction (r).

The syrup when examined by paper chromatography was found to have an R_G value of ca. 0.58 and gave a brown colour with aniline oxalate.

It had $[\alpha]_D^{18}$, +78°(c, 0.4 in water).

Fractions (s) and (t).

These were examined by paper chromatography. Fraction (s) was found to consist of two sugars, with R_G values of 0.58 and 0.50, which both gave a brown colour with aniline oxalate. Fraction (t) comprised a mixture of the sugar with R_G value 0.50 and a small amount of monomethyl xylose.

The two unidentified sugars were obviously the same as those which were found in fraction (k) (p.80) and by

analogy with this fraction, which yielded arabinose on demethylation, they are probably arabinose derivatives.

The fractions were too small for satisfactory investigation.

Examination of fraction (u).

This comprised the first 100 tubes of the monomethyl xylose fraction separated on the cellulose column and was examined separately in order to ascertain whether any 3-methyl xylose was present in the methylated mucilage, the latter sugar travelling slightly faster than 2-methyl xylose.

The syrup had $[\alpha]_D^{16}$, +34°(c,1.2 in water). Therefore it seems obvious that this fraction is 2-methyl xylose, whose equilibrium rotation is quoted as +36°(58)

Identification of fraction (v) as 2-methyl xylose.

The syrup crystallised almost completely. The crude syrupy material had $[\alpha]_D^{18}$,+9°(5mins.); +28°(3hrs.); +31° (24hrs.,constant).

The material was recrystallised from n-butanol to give crystals,m.p. and mixed m.p. with 2-methyl xylose 136°. $[\alpha]_D^{17}$,-18°(2mins.);+14°(16mins.);+29°(1hr.);+36°(2hrs.,constant). (c, 1.4 in water).

Fraction (w), comprised a sugar which when examined by paper chromatography was found to have an R_G value of 0.24 and gave a pink colour when sprayed with aniline oxalate.

Since fractions (e)(p.75) and (k)(p.80) on demethylation

both furnished a sugar of R_G value 0.24 as well as arabinose, it seems very possible that this is a monomethyl arabinose derivative.

Fraction (x) was shown to consist of unsubstituted xylose, by direct comparison with controls on a paper chromatogram.

Investigation of the dimethyl fraction H.

This was a combination of fractions (c), (h) and (ii). It has so far defied identification, but the following data have been obtained.

The syrup had OMe, 31.6%

$[\alpha]_D^{16}$, - 18° (c, 1.10 in water)

Paper chromatographic studies indicated that it probably consisted of only one sugar, as one distinct spot was obtained after running it for 30 inches in butanol/ethanol/water/ammonia (40/10/49/1)solvent. It had an R_G value of ca. 0.80 and gave a grey-black colour when sprayed with aniline oxalate.

Oxidation with alkaline hypoiodite.

1c.c. of a solution containing 0.0240gm. syrup/5c.c. was oxidised with alkaline hypoiodite as usual(p.74)and

was found to absorb iodine equivalent to 4.69c.c. 0.01055 N sodium thiosulphate. This indicates that the sguar is an aldose of molecular weight ca. 194.

Anilide formation.

The syrup(11mg.) was heated at 80°C with aniline (10mg.) and absolute alcohol(1c.c.) for 1.5 hours. Removal of the alcohol left a brown syrup which failed to crystallise.

Methyl furanoside and pyranoside formation.

The syrup(0.1279gm.) was dissolved in methanolic hydrogen chloride(1%;15c.c.). The solution was divided into two portions and polarimetric observations made as follows.

(1) The solution was allowed to stand at room temperature. $[\alpha]_D^{16}$, -15°(5mins.); -9°(15mins.); 0°(30mins.); +21°(80mins.); +28°(150mins.); +28°(4hrs.); +18°(5hrs.); +11°(6hrs.); -59° (26hrs.)

(2) The solution wasmaintained at 80°C (bath temperature). $[\alpha]_D^{16}$, -67°(50mins.); -71°(100mins.); -74°(220mins., constant).

These observations seem to indicate that formation of the methyl furanoside, $[\alpha]_D^{16}$, +28°, was effected at room temperature. This then changed to the more stable pyranoside, $[\alpha]_D^{16}$ -74°, the reaction being much more rapid at 80°C.

Methylation.

The syrup(30mg.) was heated at 80°C with methanolic hydrogen chloride(2%;5c.c.) for 12 hours. The solution was neutralised with silver carbonate, filtered and evaporated to yield a non-reducing syrup which was methylated twice with the Purdie reagents. On

evaporation of the solvent the methyl glycoside was obtained as a mobile syrup(28mg.).

The methyl glycoside was hydrolysed with 2% nitric acid(10c.c.) at 100°C for 6 hours. The solution was neutralised with barium carbonate, filtered and evaporated to give a white residue which was extracted with boiling acetone. Evaporation of the acetone extracts yielded the free sugar as a syrup which failed to crystallise. On examination on a paper chromatogram this gave only one spot of R_G value 0.96 which gave a brown-black colour with aniline oxalate. No crystalline anilide could be obtained.

Demethylation.

The syrup(7mg.) was demethylated by heating at 100°C with hydrobromic acid(48%;1c.c.) in a sealed tube for 7 minutes and treated as usual(p.76) to yield a syrup which was investigated by paper chromatography. The following spots were obtained on spraying the paper with aniline oxalate.

	R_G value	Colour of spot	Identity
(1)	0.80	grey-black	unchanged material
(2)	ca. 0.45	faint brown	?
(3)	0.34	pink	monomethyl arabinose?
(4)	0.12	pink	arabinose

The arabinose was identified by direct comparison with a control.

Lactone formation.

The syrup(0.1gm.) was oxidised with bromine in water

until non-reducing (6 days). The usual treatment yielded the lactone which distilled at 120-130°/0.06mm. The product(85mg.) did not crystallise and had OMe, 28.6%. $[\alpha]_D^{17}$, -51°(zero time); -50°(24hrs.); -44°(192hrs.); -39°(336hrs.); -36°(384hrs.); -33°(456hrs.); -32°(480hrs., constant). (c, 0.8 in water).

Amide formation.

The above lactone was reclaimed from the polarimetric solution by evaporation and redistillation. It was treated with methanolic ammonia at 0°C for two days. On evaporation of the solvent in a vacuum dessicator a white solid(25mg.) was obtained which after recrystallisation from absolute alcohol had m.p.131-132°C.

A Weerman test carried out on the pure amide(10mg.) was negative; gluconamide treated under the same conditions yielded hydrazodicarbonamide.

Found: C, 42.95% ; H, 7.7% ; N, 6.9%.

Calc.for $C_7H_{15}O_5N$: C, 43.5% ; H, 7.7% ; N, 7.25%.

Calc.for $C_8H_{17}O_6N$: C, 43.0% ; H, 7.7% ; N, 6.3%.

Calc.for $C_8H_{17}O_5N$: C, 46.4% ; H, 8.2% ; N, 6.8%.

Periodate Oxidations.

Periodate uptake.

The syrup H(0.0169gm.) was dissolved in water(2c.c.), sodium metaperiodate(0.3M;2c.c.) added and the solution allowed to stand at room temperature for 50 hours in the dark. Sodium arsenite(0.1N;25c.c.) and excess solid potassium iodide were then added and the solution left for 15 minutes at room temperature before titrating with

0.1N iodine solution. Blank estimations were carried out concurrently.

The solution required 4.97c.c. 0.09924N iodine solution, corresponding to an uptake of 2.8 molecules periodate per molecule of syrup.

Glucose(0.0217gm.) was oxidised under the same conditions and required 9.53c.c. iodine solution, corresponding to an uptake of 3.9 molecules periodate per molecule of glucose. Theoretically 5 molecules should be absorbed and therefore the glucose was only 78% oxidised.

4-methyl rhamnose(0.0189gm.) required 3.17c.c. iodine solution, indicating an uptake of 1.5 molecules periodate per molecule of sugar. Theoretically 2 molecules should be absorbed and therefore the 4-methyl rhamnose was 74% oxidised.

Analysis for formaldehyde produced.

The syrup(17.8mg.) was dissolved in water(2c.c.)and oxidised with periodic acid by Reeves' method, as described on p.79. Two hours after the addition of dimedon reagent, a white precipitate(2.9mg.) appeared. Although this charred slightly at 186°C it had failed to melt at 230°C and was therefore considered to consist largely of inorganic salt. Any formaldehyde-dimedon compound present was negligible.

Glucose(17.8mg.) was oxidised under the same conditions and yielded a precipitate of fine white needles ten minutes after the addition of the dimedon solution. The dimedon-formaldehyde compound(15mg.) had

m.p.189°. A solution of the reagents alone yielded no precipitate.

Thus fraction H was found to yield no formaldehyde on periodic acid oxidation, indicating the non-existence of a free primary alcoholic grouping in the molecule.

Analysis for acetaldehyde produced.

Fraction H was oxidised with periodic acid and the products analysed for the presence of acetaldehyde by the method of Nicolet and Shinn(loc.cit.) with the following results:-

(1) The syrup(9.6mg.) on oxidation gave a product which formed a complex with sodium bisulphite. The complex was decomposed by the addition of saturated sodium bicarbonate(10c.c.) and the bisulphite liberated, was found to require 0.875c.c. 0.009458N iodine solution. This corresponds to the presence of 8.5% dimethyl methyl pentose in the syrup.

(2) After oxidation of the syrup(10.8mg.) the bisulphite liberated required 0.583c.c. 0.009412N iodine, indicating the presence of 5.1% dimethyl methyl pentose in the syrup.

Oxidation of glucose(9.9mg.) and similar treatment gave a titre of 0.374c.c. 0.009458N iodine.

With 2:3-dimethyl mannose(18.5mg.) the titre was 0.540c.c. 0.009412N iodine.

10c.c. saturated sodium bicarbonate required no iodine.

These figures indicate a "blank" value of ca. 3.2% methyl pentose. The cause of this is probably incomplete

retention of the formaldehyde by the alanine(0.2gm.) in the reaction mixture. If this is the case, and if fraction H were composed entirely of dimethyl methyl pentose, then this "blank" figure would be inapplicable since no formaldehyde would be produced.

Subtraction of the blank value from the above results gives values of,

(1) 5.3% dimethyl methyl pentose

(2) 1.9% " " "

Oxidation of the amide of fraction H and titration of the bisulphite from the complex gave a titre of 0.969c.c. 0.009412N iodine. This corresponds to 4.7% dimethyl methyl pentonamide, or 1.6% allowing for the blank value.

Thus it seems possible that there may be some methyl pentose present in fraction H since methylated sugars are often very poorly oxidised with periodic acid, but the results are inconclusive.

Investigation of the effect of acid hydrolysis on the yield of end group.

The yield of the trimethyl xylose fraction from fraction A was lower than would be expected, and although this may have been due to incomplete methylation, it was thought that some loss may have been suffered as the result of demethylation during hydrolysis or as the result of volatilisation of the trimethyl methyl-xylosides during the subsequent evaporation of the solvent. Therefore the following experiments were carried out.

Trimethyl xylose was treated with methanolic hydrogen chloride under exactly the same conditions as were used for the methanolysis of fraction A. Thus, pure crystalline trimethyl-α-D-xylopyranose(0.371gm.) was heated at 100°C with methanolic hydrogen chloride(3%;60c.c.) for 21 hours. The solution was then neutralised with silver carbonate, and filtered and evaporated at 45°C to give a syrup and a distillate D. The syrup still contained some silver salts and was therefore extracted with boiling acetone, the solution filtered and the acetone removed at 30°C to give a pale yellow mobile syrup(0.301gm.).

The distillate D was evaporated to dryness at 30°C to yield a syrup(0.010gm.). This syrup was hydrolysed with nitric acid(2%;5c.c.) at 100°C for 7 hours. The solution was neutralised with barium carbonate, evaporated to dryness and the residue extracted with acetone; the acetone extracts were filtered and the solution evaporated to small volume(ca. 2c.c.) and examined on a paper

chromatogram. Direct comparison with a control showed the presence of trimethyl xylose only.

No sugar was detected in distillates obtained when the evaporations were carried out at 30°C.

These results show that the loss of trimethyl-methyl xylosides suffered by their volatilisation during evaporation of the solvent at 40-45°C/18mm. was $^{0.01}/_{0.311}$ or 3.2%.

In order to determine whether any demethylation had occurred during this treatment with methanolic hydrogen chloride, or during the subsequent hydrolysis with nitric acid, the above syrup of the trimethyl methyl-xylosides (0.301gm.) was hydrolysed with 2% nitric acid(15c.c.) at 100°C for 7 hours. The solution was neutralised with barium carbonate, filtered and the solution evaporated to dryness; the residue was extracted with boiling acetone and the solution filtered and evaporated to give a syrup which crystallised almost immediately. This was dissolved in water and examined on a paper chromatogram which showed a large spot corresponding to trimethyl xylose and a very faint elongated spot corresponding to 2:3- and 2:4-dimethyl xyloses, which could not amount to more than 1%.

Thus it would seem that the yield of trimethyl xylose was affected to only a very small extent by losses due to either of the above causes.

In order to verify further the percentage of trimethyl xylose in fraction A, the following experiment was carried out.

Quantitative estimation of the trimethyl xylose in fraction A.

Fraction A, to which had been added a known weight of a standard sugar, was hydrolysed on a small scale under carefully controlled conditions, the sugars separated by paper chromatography and the trimethyl xylose and control sugar estimated by oxidation in buffered hypoiodite solution.

Fraction A(0.1034gm.) and pure galactose(0.0201gm.) were heated with methanolic hydrogen chloride(3%;3c.c.)at 75°C for 20 hours. The solution was neutralised with silver carbonate, filtered and evaporated at 30°C to give a syrup. This syrup was heated at 100°C with nitric acid (2%;3c.c.)for 5½ hours. The solution was neutralised with barium carbonate, filtered and evaporated to dryness at 30°C. The residue was dissolved in water and the solution deionised by shaking with Amberlite resins IR100 and IR4B and evaporated to give a syrup(0.0905gm.). The sugars in the syrup were separated on a paper chromatogram, and the trimethyl xylose and galactose estimated by oxidation with alkaline hypoiodite as usual. Three estimations were carried out as follows:-

(1) Trimethyl xylose ≡ 1.146c.c. 0.01153N $Na_2S_2O_3$.

i.e. 0.001269gm. trimethyl xylose.

galactose ≡ 1.072c.c. 0.01153N $Na_2S_2O_3$.

i.e. 0.001113gm. galactose.

This corresponds to 20.1% anhydro-trimethyl xylose in fraction A.

(2) Trimethyl xylose ≡ 0.861c.c. 0.01153N $Na_2S_2O_3$.

i.e. 0.0009530gm.

galactose ≡ 0.766c.c. 0.01153N $Na_2S_2O_3$.

i.e. 0.0007947gm.

i.e. 21.1% anhydro-trimethyl xylose in fraction A.

(3) Tetramethyl galactose which had separated on the paper chromatogram was also estimated as a check on the method.

Trimethyl xylose ≡ 0.878c.c. 0.01055N $Na_2S_2O_3$

i.e. 0.0008894gm.

galactose ≡ 0.890c.c. 0.01055N $Na_2S_2O_3$

i.e. 0.0008450gm.

Tetramethyl galactose ≡ 0.273c.c. 0.01055N $Na_2S_2O_3$

i.e. 0.0003399gm.

i.e. 18.5% anhydro-trimethyl xylose in fraction A.

7.2% anhydro-tetramethyl galactose

∴ Fraction A contains ca.20% anhydro-trimethyl xylose.

EXPERIMENTS ON THE ALKALI SENSITIVITY OF THE MUCILAGE.

Deacetylation of the acetylated mucilage.

The unfractionated acetate(1gm.) was dispersed in chloroform(15c.c.). The mixture was cooled in a freezing mixture and a solution of 0.05gm. sodium in 5c.c. absolute methanol added.[59] The mixture was shaken for 4 hours at room temperature, ice water(5c.c.) added, followed by acetic acid(10%;1c.c.) and finally water(10c.c.). The resulting mixture was poured into methanol(200c.c.) when a white solid was precipitated. On examination, this appeared to consist of two distinct substances: I, a greyish-white fibrous material, presumably the deacetylated mucilage and II, a fine gelatinous white precipitate which was very similar in appearance to the barium aldobiuronate. This was separated from the heavier coarse precipitate I by decantation after the latter had settled, and was centrifuged off.

Examination of I.

This was hydrolysed with 3% oxalic acid in the usual way and gave a syrup which when examined on a paper chromatogram was found to consist of xylose, arabinose and galactose in approximately the same proportions as before. No barium salt was precipitated on pouring the neutral hydrolysed solution into methanol.

Examination of II.

This was expected to be the sodium salt of the aldobiuronic acid or a polyuronide. It gave a strong

positive reaction when tested with naphthoresorcinol as follows:-

To a solution of a small amount of the solid in 5c.c. water were added naphthoresorcinol in methanol(1%;1c.c.) and concentrated hydrochloric acid(6c.c.). The solution was boiled for 1 minute and set aside for 4 minutes. It was then cooled, and an equal volume of ether added; on shaking, the ether layer acquired a deep violet-blue coloration. A solution of galacturonic acid was treated in the same way and gave a similar result, whereas a blank solution gave only a very faint pink coloration.

The remainder of the precipitate II was dissolved in water, filtered and shaken for 3 hours with Amberlite resin IR100 to yield the free acid. The solution was filtered, evaporated to dryness and dried on the hyvac pump at 40°C to give a transparent film of the acid (0.0177gm.). This was dissolved in water and titrated with 0.005135N sodium hydroxide. It required 12.23c.c. This corresponds to an equivalent weight of 282.

An aldobiuronic acid consisting of a hexuronic acid and a methyl pentose has the formula $C_{12}H_{20}O_{11}$, equivalent weight, 340.

As the above specimen must have been slightly impure, it seems possible that it may be a molecule of this type.

Treatment of the mucilage with alkali.

Following the result of deacetylation of the acetylated

mucilage with sodium methoxide, it was decided to study the effect of the alkali on the mucilage itself and investigate any change in the uronic acid content which may have occurred.

The mucilage(ca. 1-2gm.) was heated with 2N sodium hydroxide(120c.c.) for 15 minutes. After this time a slight yellow coloration was observed and therefore the heating was discontinued and the mixture allowed to stand overnight at room temperature. The mobile solution was then neutralised with glacial acetic acid, when the viscous jelly-like character of the mucilage returned. The solution was diluted, dialysed in a cellophane bag against running water for 5 days and evaporated to small volume. The mucilage was then precipitated in methanol, allowed to stand overnight in ether, dried on the hyvac pump at 40°C and the uronic acid content determined as usual.

Average uronic anhydride content, 4.3%.

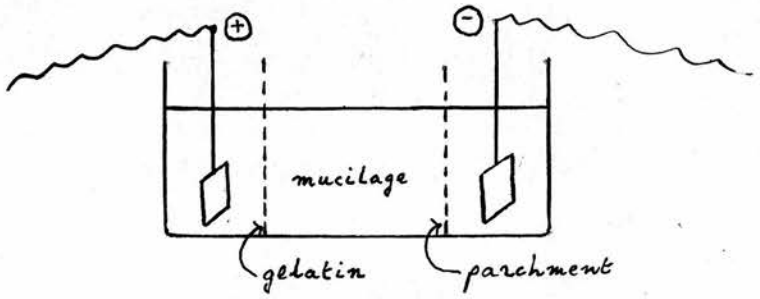
In a typical estimation 0.2261gm. mucilage liberated CO_2 equivalent to 1.10c.c. 0.0986N HCl, corresponding to uronic anhydride 4.2%.

Before the alkaline treatment the uronic anhydride content of the mucilage was 7.2%. Thus there seems to be a considerable decrease in the uronic acid content, although it has not completely disappeared. This may be due to incomplete removal by dialysis. For this reason the following experiment was carried out.

Electrodialysis of the alkali-treated mucilage.

The mucilage was allowed to stand in 2N sodium

hydroxide at room temperature for a week. The solution was neutralised with dilute sulphuric acid and an attempt was made to separate the uronic acid component by electrodialysis as follows:-



The membranes used were parchment and a piece of muslin coated with gelatin. A potential difference of 130 volts D.C. was applied between the electrodes and a current of 900 milliamps. developed, which dropped slowly after the water in the electrode compartments had been changed several times. It reached a minimum value of 30 milliamps after 1.5 hours.

The electrodialysed mucilage was then dialysed in a cellophane bag for a week after which the solution was evaporated to small volume, and the mucilage precipitated in methanol and dried as usual to give a crisp brownish solid.

Uronic anhydride, 2.5%.

The solution in the anode compartment was evaporated to small volume and was found to give a positive naphthoresorcinal test.

Attempted electrodialysis of mucilage.

In view of the possibility that the uronic acid component may not be an integral part of the mucilage molecule, an attempt was made to separate it from the latter by electrodialysis, without success.

A dilute solution of the mucilage was treated as above. A current was passed for 2 hours and remained constant throughout, the only effect being that a thick layer of the mucilage collected on the inside of the gelatin membrane.

The solution in the anode compartment on evaporation to small volume gave no colour when tested with naphthoresorcinol in hydrochloric acid.

DISCUSSION.

The present research on the mucilage obtained from *Plantago arenaria* seeds was undertaken in order to gain a more detailed knowledge of the structure of the molecule than could be obtained by previous workers[40] by making use of the more delicate techniques which have been developed during the last ten years.

On account of the confusion which has existed concerning the identity of *Plantago* seeds investigated in the past, the present investigations were carried out on the mucilage obtained from seeds which were undoubtedly *P. arenaria*. The seeds, which were quite homogeneous, were purchased as genuine *P. arenaria* seeds and fitted the description of this variety given by Skryme[32] in two papers discussing the identification of seeds of the Plantago species.

When the seeds were soaked in cold water a solution of the mucilage was obtained which was precipitated in alcohol as a fibrous solid in 3% yield. The "free acid" polysaccharide was also prepared by precipitation of the mucilaginous solution in acidified alcohol and this material was used in all structural investigations.

Extraction of the seeds with hot water subsequent to the cold water extraction yielded no further quantity of material, in contrast with the behaviour of *P. ovata* from which at least two different polysaccharides were obtained on cold and hot water extraction.[38,39] Anderson also found that variation of the conditions of extraction

of both *P. ovata*[34] and *P. fastigiata*[35] seeds gave fractions with different compositions. Thus in the present case there exists the question as to whether the starting material is a distinct chemical entity or whether it is a mixture of two or more components. This problem is still undecided and is discussed again later.

The polysaccharide contained 80% anhydropentose (calculated as xylose), 7.2% uronic anhydride and had an equivalent weight, as determined by titration, of *ca.* 1700.

The uronic anhydride corresponds to an equivalent weight of 2450, which differs considerably from the value obtained by titration(1700). The former value seems more likely to be accurate since the uronic anhydride content was determined by estimation of the carbon dioxide evolved whereas the titration method might be considerably affected by any impurities in the mucilage. It is possible that there may have been traces of acid still adhering to the fibres after precipitation of the mucilage in alcoholic hydrogen chloride, although the material was triturated with alcohol until all chloride ions appeared to have been removed. If this were the case a high alkali titre would be obtained which would give a low value for the equivalent weight. For this reason an equivalent weight of 2450 was assumed in subsequent investigations.

Periodate oxidations carried out on the hydrolysed mucilage liberated small amounts of acetaldehyde. The amount obtained increased with the time of hydrolysis but even after hydrolysis for 48 hours with 3% oxalic

acid, acetaldehyde equivalent to only 0.5% methyl pentose was obtained. This seemed to indicate that some methyl pentose was present but that it must exist in some relatively stable combination, and it suggested the possibility that a methyl pentose residue might be linked with the uronic acid in the form of an aldobiuronic acid which would be very resistant to hydrolysis.

Hydrolysis of the mucilage with 3% oxalic acid was complete after heating at 100°C for 6 hours. A dark brown insoluble residue remained which amounted to 2% of the mucilage. This so-called "x-body" is commonly found on hydrolysis of gums and mucilages and Anderson and Fireman (Loc.cit.) were of the opinion that it might be an integral part of the molecule. However, treatment with 72% sulphuric acid by the Monier-Williams method yielded glucose but did not completely hydrolyse the material, a large proportion of it remaining apparently unchanged. This unchanged material was probably a lignin and the glucose was in all probability derived from an associated cellulose.

The hydrolysate, after removal of this insoluble residue, yielded a barium salt(15.6%) insoluble in alcohol and an alcohol-soluble fraction(85%) which was shown by paper chromatography to comprise xylose, arabinose and galactose. These sugars, after separation on a paper chromatogram, were estimated to be present in the proportions, xylose 72.6%, arabinose 20.2% and galactose 7.1%.

Drastic hydrolysis of the barium salt and identification of the products by paper chromatography showed it to be the

salt of an aldobiuronic acid whose components were galacturonic acid and rhamnose.

Thus the constituent units of the mucilage have been identified and the molecule has been shown to be composed as follows:-

	% by weight	Relative no. of molecules
Insoluble residue	2	
Anhydroaldobiuronic acid	13	1
Anhydroxylose	62	12
Anhydroarabinose	17	3
Anhydrogalactose	6	1
	100	

A molecule comprising these residues would have an equivalent weight of 2482 which is in fairly good agreement with the value of 2450 obtained by direct experiment.

Partial hydrolysis of the mucilage was effected by heating with 3% oxalic acid for short periods. Xylose, arabinose and galactose were liberated, as well as several oligosaccharides, but these studies yielded no information regarding the structure of the molecule.

The mucilage was autohydrolysed by heating at 100°C with water for 24 hours. An alcohol-soluble syrup was obtained which was shown by paper chromatography to consist of arabinose only. The removal of some of the arabinose residues indicates that they are probably of the labile arabofuranose type and that they must form part of the "outer shell" of the molecule.

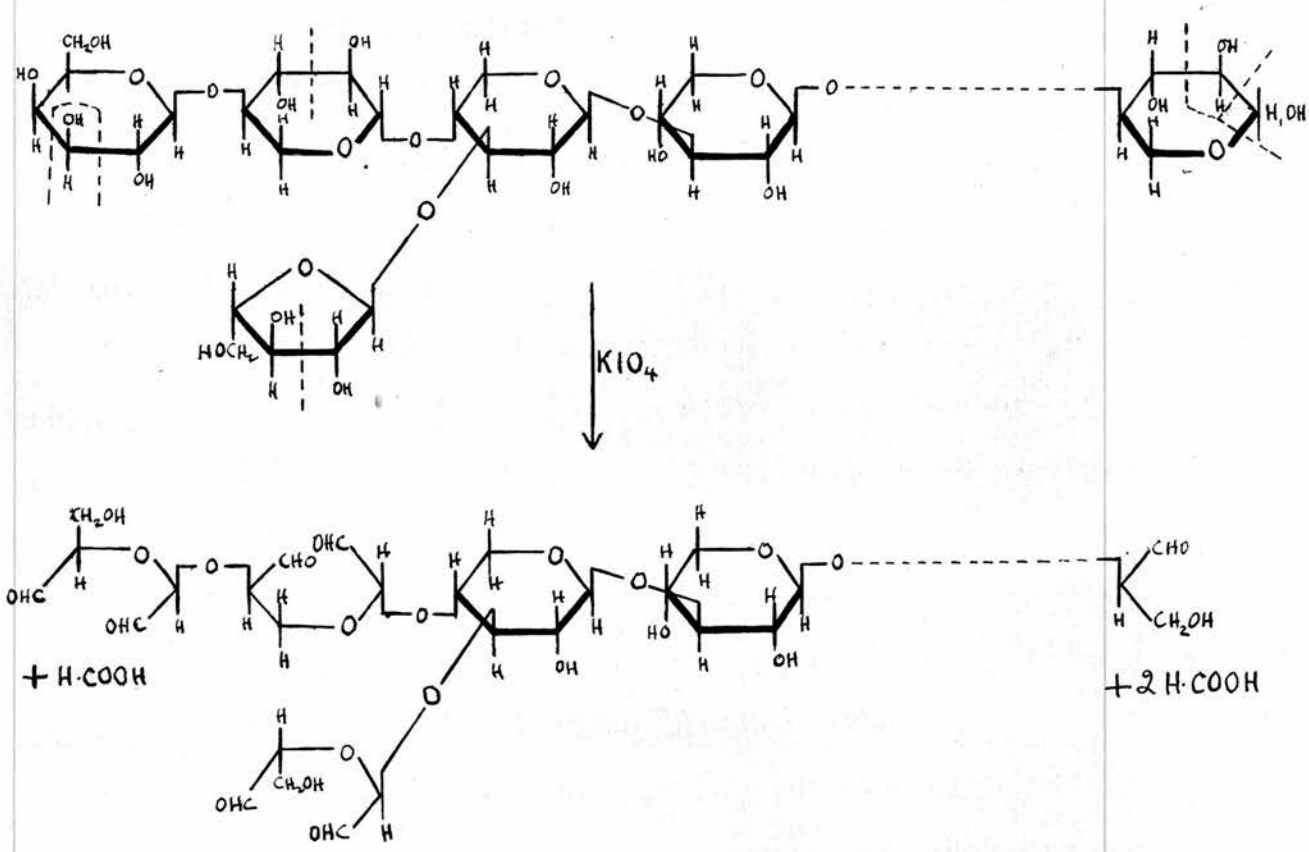
The possibility arose here that, since the arabofuranose residues are so labile that they are split

off simply by autohydrolysis, the molecule may have suffered some degradation during the precipitation of the "free acid" mucilage in acidified alcohol. This has been found in the case of arabic acid whose equivalent weight decreased on dialysis, due to the splitting off of the labile residues.

In order to investigate this possibility, the mucilage was precipitated as the natural salt in non-acidified alcohol and hydrolysed with 3% oxalic acid. The relative proportions of the free sugars obtained were estimated by paper chromatographic techniques to be xylose 72.3%, arabinose 20.5% and galactose 7.2%. These results are identical with those obtained for the free sugars in the "free acid" mucilage, thus proving that no loss of arabofuranose residues had occurred during the acid precipitation of the mucilage.

Evidence regarding the architecture of the molecule was obtained by oxidation of the mucilage with periodate. Since this reagent is specific for α-glycol links, the determination of its uptake by the mucilage gives an idea of the configuration of the molecule and as formic acid can arise only from the terminal groups these can be estimated by titration of the formic acid produced. Thus a molecule built up of 1:3- and 1:4- linked D-xylose units, terminated by D-xylopyranose, L-arabofuranose and

D-galactopyranose residues would react as follows:-



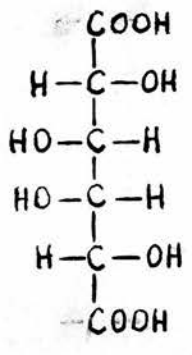
The mucilage was found to absorb 13.9 molecules of sodium metaperiodate per equivalent. Oxidation with potassium periodate yielded 5.7 molecules formic acid per equivalent, showing that the mucilage possesses 5.7 end groups per equivalent.

Hydrolysis of the periodate-oxidised mucilage showed that all the galactose had been broken down. The remaining xylose and arabinose were estimated after separation by paper chromatography and it was found that 25% of the xylose and 16% of the arabinose originally present in the mucilage had been unattacked. i.e. One in every four xylose residues and one in every six arabinose residues are so linked in the molecule that they do not possess

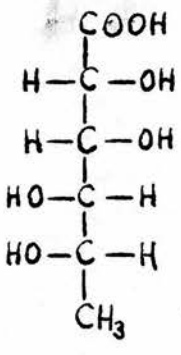
two adjacent free hydroxyl groups. These residues must either constitute branching points in the molecule or, if they are present in the pyranose form, must be linked through the 3-position in a chain, or if in the furanose form, through the 2- or 3-position.

Identification of the aldobiuronic acid (p.44)

Preliminary investigation of the barium salt obtained after oxalic acid hydrolysis of the mucilage showed it to be the salt of an aldobiuronic acid composed of galacturonic acid and rhamnose, as described earlier. This was confirmed by oxidation of the salt with bromine and hydrobromic acid which yielded crystalline mucic acid (I) and L-rhamnonic acid (II), identified as the crystalline phenylhydrazide.(60)



I



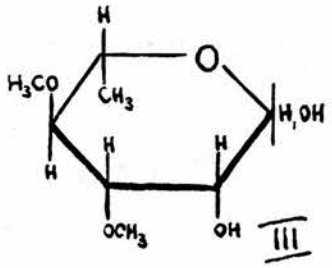
II

Methylation was effected by the Haworth and Purdie methods and finally by esterification with diazomethane. A syrup was obtained, OMe, 45.6% (Calc. for $C_{19}H_{34}O_{11}$, OMe, 49.5%), which was hydrolysed with 2N hydrochloric acid, the course of the hydrolysis being followed by polarimetric observations. The solution finally showed $[\alpha]_D^{16}$, +72°(the rotation of an equimolecular mixture of 2:3:4-trimethyl-D-galacturonic acid and 3:4-dimethyl-L-

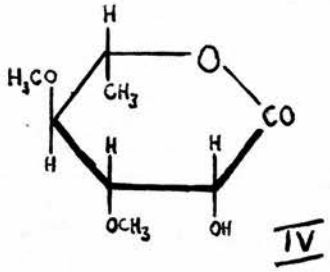
rhamnose is calculated to be $+70^{\circ}$.). The mixture was neutralised and separated by extraction with ether to give a syrup S and a residue R.

The syrup S had the methoxyl content of a dimethyl methyl pentose and had $[\alpha]_D^{16}$, $+19^{\circ}$, which is the value quoted for 3:4-dimethyl-L-rhamnose (III).[30,61] It had the same R_G value as the latter and gave no acetaldehyde on oxidation with periodic acid, indicating that one of the methoxyl groups must be on either $C_{(4)}$ or $C_{(5)}$.

The lactone was obtained in poor yield. It did not crystallise but was characterised as 3:4-dimethyl-L-rhamnonolactone (IV)[29] by its mutarotation in water.

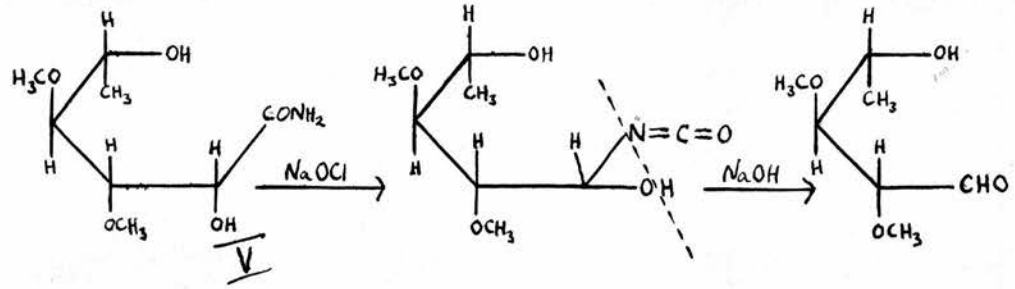


III



IV

The amide (V) partially crystallised and when subjected to a Weerman test (<u>loc.cit.</u>) gave a precipitate of hydrazodicarbonamide. In this reaction, the amide of an α-hydroxy carboxylic acid is degraded when treated with alkaline sodium hypochlorite. Sodium isocyanate is split off and this can be condensed with semicarbazide hydrochloride and sodium acetate to yield the insoluble hydrazodicarbonamide.



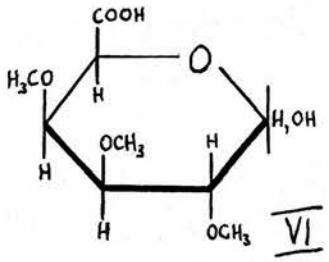
V

$NH_2.CO.NHNH_2$ + OCNH ⟶ $NH_2.CO.NH.NH.CONH_2$.

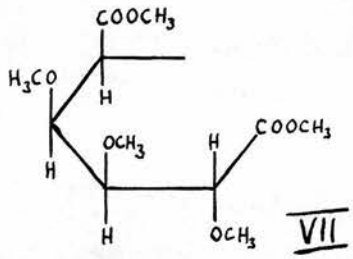
semicarbazide isocyanic acid hydrazodicarbonamide

Thus the syrup S was shown to possess a free hydroxyl group on C(2) which in conjunction with the above evidence identifies it as 3:4-dimethyl-L-rhamnose.

The residue R was shown to be the barium salt of 2:3:4-trimethyl-D-galacturonic acid(VI) which on oxidation and esterification yielded the dimethyl ester of 2:3:4-trimethyl mucic acid(VII) which was identified by its m.p., mixed m.p. and specific rotation.[22,62]



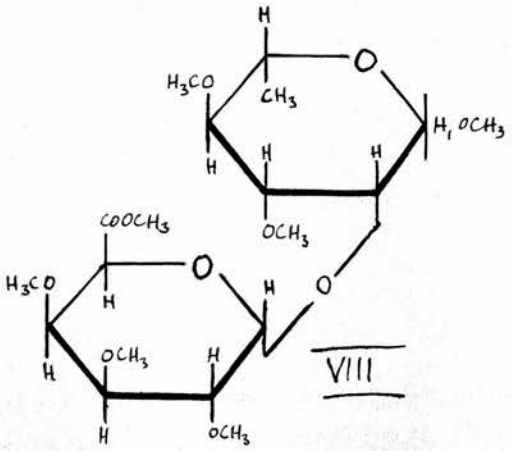
VI



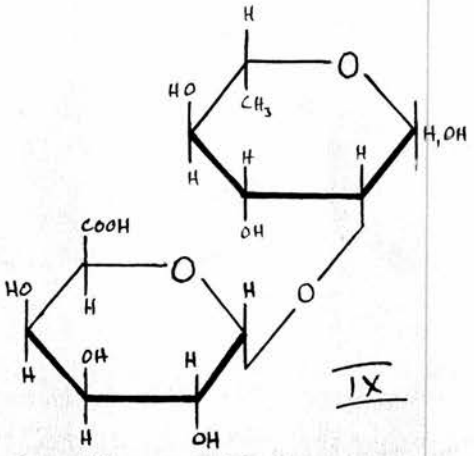
VII

There is at present no definite proof as to whether the glycosidic link between the two residues in the aldobiuronic acid is of the α- or β- form, but it seems very probable that it is an α-linkage, on account of the high positive rotations of both the barium salt and the methylated acid.

The fully methylated compound is therefore the methyl ester of 2-(2:3:4-trimethyl-α-D-galacturonosido)-3:4-dimethyl methyl L-rhamnopyranoside(VIII) and the aldobiuronic acid is 2-α-D-galacturonosido-L-rhamnose(IX), which is the same as that isolated from flax-seed,[30] *Ulmus fulva*[29] and *Plantago ovata*.[38]



VIII



IX

Acetylation and methylation studies on the mucilage.

Acetylation of the mucilage gave a greyish-white powder(CH_3CO,34.6%) in good yield. This was found to be partially soluble in a mixture of acetone and chloroform and it was fractionated to give a soluble portion(20%; $[\alpha]_D^{17}$, -95° in chloroform; CH_3CO,37.4%; uronic anhydride 2.3%) and an insoluble portion(80%:CH_3CO,30.2%; uronic anhydride 3.9%). Since the unsubstituted mucilage contained 7.2% uronic anhydride it can be calculated that the soluble and insoluble acetates should have uronic anhydride contents of 4.5% and 5.0% respectively. From the above results it is obvious that a loss of uronic acid has occurred during the acetylation process.

Both the soluble and insoluble acetates were methylated separately four times by the Haworth method and each product was fractionally precipitated to give two fractions of presumably different molecular sizes. The insoluble acetate yielded fractions A and B and the soluble acetate yielded fractions C and D. These fractions had the following properties:-

Fraction	% yield	OMe	$[\alpha]_D^{14}$ ($CHCl_3$)	$\eta_{sp/c}$ (m-cresol)
A	74	36.2%	-102°	30.0
B	6	36.6%	-103.5°	8.9
C	19	36.9%	-105°	15.9
D	1	36.2%	-104°	2.4

c is the concentration in gm. per 100c.c.

Fraction C was subjected to two further methylations by the Purdie method to give a material which had OMe, 36.6%; $\eta_{sp/c}$ in m-cresol, 4.2. Another sample of the insoluble acetate after 14 methylations by the Haworth method had OMe, 36.8%; $\eta_{sp/c}$ in m-cresol, 2.0. It was therefore concluded that the material was as fully methylated as possible after four methylations.

Direct methylation of the mucilage was unsuccessful, giving a material with OMe, 31.0%; $\eta_{sp/c}$ in m-cresol, 7.0.

An attempt was made to determine the amount of degradation which had occurred during the acetylation and methylation procedures by carrying out viscosity measurements in m-cresol. However, owing to the highly branched nature of the mucilage and since the limiting viscosity could not be ascertained accurately, these measurements could give only a very general indication of the size of the molecule. A decrease in the specific viscosity of the material may be caused either by degradation of the molecule or by removal of the effects of hydrogen bonding of the hydroxyl groups as the result of further substitution of methoxyl groups in the molecule.

For example, in the case of the methylation of

fraction C cited above, the fall in the value of η_{sp}/c from 15.9 to 4.2 may have been caused by either of the above effects and it is probable that relatively little degradation had actually occurred, since a value of 4.2 still represents a very large molecule.

Since the methylated products from both the acetate fractions were very similar in their appearance and constants, it seems very probable that the differences in the properties of the acetates were due only to the differences in the degree of acetylation and not to any essential difference in their molecular structures. The only constant in which there was any appreciable difference was the specific viscosity, that of fraction A being approximately twice that of fraction C. However, this may quite well be due to a difference in the degree of methylation, as explained above; this was in fact substantiated by investigation of the hydrolysis products of the two fractions, fraction A seeming to be less fully methylated than fraction C. From this it would appear that the mucilage was essentially homogeneous.

From a consideration of the fact that the acetylated and methylated derivatives of the mucilage had in every case a highly negative specific rotation and since the proportion of L-arabinose residues present is relatively small, it can be concluded that there is probably a predominance of β-linkages in the molecule. This was also found to be the case in the mucilages from P. ovata and P. lanceolata.

Fraction A was hydrolysed with methanolic hydrogen

chloride and an attempt was made to fractionate the resulting complex mixture of methyl glycosides by the method of solvent extraction. This was based on the original procedure of Brown and Jones (loc.cit.) and depends on the difference in the distribution coefficients of fully and partially methylated methyl glycosides between low-boiling light petroleum and water. After extraction with light petroleum, the aqueous solution containing the partially methylated methyl glycosides was extracted with chloroform to remove unsubstituted sugars. No clear-cut fractionation was achieved, but three fractions were obtained: fraction I contained mainly the fully methylated and dimethyl methyl glycosides; fraction II contained monomethyl and traces of dimethyl methyl glycosides; fraction III contained monomethyl and unsubstituted methyl glycosides.

These fractions were hydrolysed with 2% nitric acid to give mixtures of the free methylated sugars which were separated on columns of cellulose to give the following fractions:-

A ⟶ methyl glycosides ⟶ I ⟶ (a),(b),(c),(d),(e),(f),(g).
methyl glycosides ⟶ II ⟶ (h),(i),(j),(k),(l),(m),(n).
methyl glycosides ⟶ III

Fraction C was hydrolysed with methanolic hydrogen chloride to give a mixture of methyl glycosides which on hydrolysis with 2% nitric acid yielded a mixture offree methylated sugars which was fractionated on columns of cellulose as follows:-

C ⟶ methyl glycosides ⟶ $(o_1),(o_2),(o_3),(o_4),(o_5),(p),(q),(r),$ $(s),(t),(u),(v),(w),(x).$

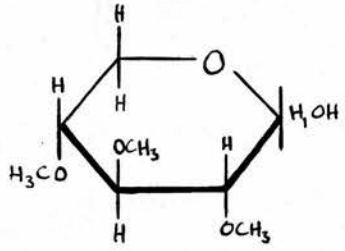
Fractions (d),(i) and (o_4) were combined and the mixture separated on a column of cellulose to give fractions (i),(ii),(iii) and (iv).

Fractions (c), (h) and (ii) were combined to give fraction H.

The fractions containing the main sugars will be considered first.

Investigation of the sugar fractions from fraction A.

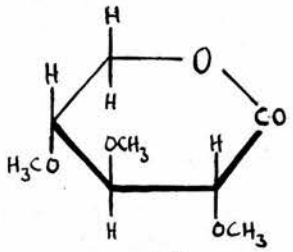
Fraction (a) (p.71) yielded crystalline 2:3:4-trimethyl-D-xylose(X)[38,63] which was identified by its m.p., mixed m.p., analysis and mutarotation in water.



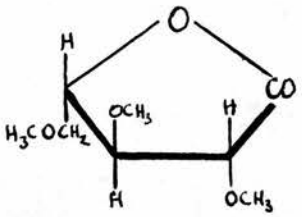
X

It was evident however that there was another component in this fraction. After removal of the crystalline trimethyl xylose a syrup remained whose specific rotation was lower than that of trimethyl xylose; this was oxidised with bromine in water to give a mixture of 2:3:4-trimethyl-D-xylonolactone(XI) and 2:3:5-trimethyl-L-arabonolactone(XII). The mixture was fractionated by high-vacuum distillation to give fractions (1a) and (2a); fraction (1a) yielded crystalline 2:3:5-trimethyl-L-

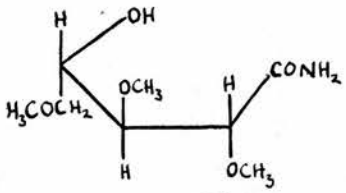
arabonamide(XIII);[15,23,26,64] characterised by its m.p. and mixed m.p. with an authentic specimen.



XI



XII



XIII

The relative proportion of the two lactones in the mixture was calculated from the equilibrium rotations of fractions (1a) and (2a) which were, respectively,

$[\alpha]_D^{16}$, $+12.5^{\circ}$(water)

and $[\alpha]_D^{16}$, $+18^{\circ}$ (water)

Now at equilibrium, 2:3:4-trimethyl-D-xylonolactone has

$[\alpha]_D^{20}$, $+21.4^{\circ}$[8,65]

and 2:3:5-trimethyl-L-arabonolactone has

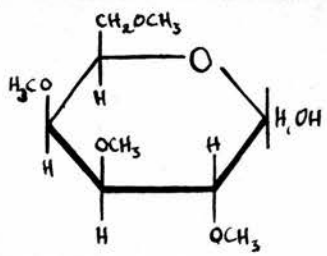
$[\alpha]_D^{20}$, -25.2°[66]

The following composition was calculated from these figures:-

	Trimethyl-D-xylonolactone	Trimethyl-L-arabonolactone	Total
Fraction (1a)	0.084gm.	0.020gm.	0.104gm.
Fraction (2a)	0.074gm.	0.006gm.	0.080gm.
Total.	0.158gm.	0.026gm.	0.184gm.

From all the above data it was calculated that fraction (a) contained 93.7% trimethyl-D-xylopyranose and 6.3% trimethyl-L-arabofuranose.

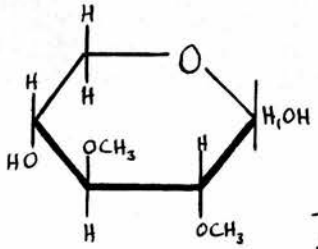
Fraction (b) yielded crystalline 2:3:4:6-tetramethyl-D-galactose(XIV)[23,67] which was identified by its m.p., mixed m.p., analysis and mutarotation in water.



XIV

It yielded crystalline 2:3:4:6-tetramethyl-D-galactose anilide, identified by its m.p., mixed m.p. and analysis. Its equilibrium rotation in water was +41.5°, which is the value quoted for tetramethyl-D-galactopyranose anilide.[23]

Fraction (e) (p.75) was a syrup which had the methoxyl content of a dimethyl pentose. Direct comparison with a control on a paper chromatogram showed that it consisted of a mixture of 2:3-dimethyl xylose(XV) and a sugar which travelled slightly faster than the latter; this was suspected to be a dimethyl pentose since it gave a pink colour with aniline oxalate.

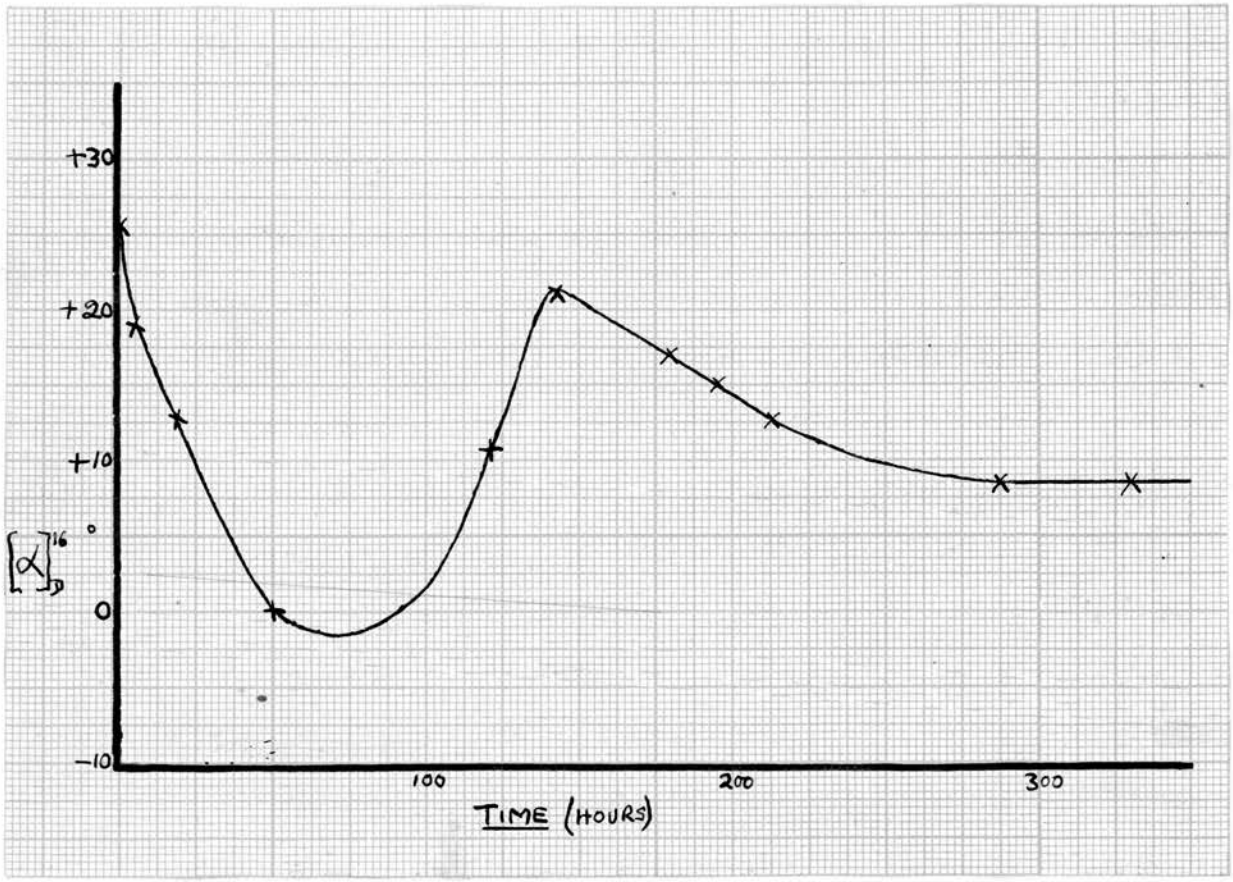


XV

The syrup had $[\alpha]_D^{16}$, +25.6°, which is the value quoted for 2:3-dimethyl-D-xylose.[63,68] It did not yield a crystalline anilide.

The syrup was oxidised with bromine to yield a mixture of lactones which had $[\alpha]_D^{16}$, +28°(initial reading); this is much lower than the value quoted for pure 2:3-dimethyl-D-xylonolactone($+97^\circ \longrightarrow +62.5^\circ$[63]), and therefore the unknown component must have a lower rotation than the latter.

The lactone mixture yielded the corresponding amides which when subjected to a Weerman test gave hydrazodicarbonamide, indicating that one of the amides


+30
+20
+10
0
−10
$[\alpha]_D^{16}$ °
100
200
300
TIME (HOURS)

must possess a free hydroxyl group on $C_{(2)}$.

A fresh portion of fraction (e) was oxidised and an attempt was made to separate the lactones by fractional distillation. Two fractions, (1b) and (2b), were obtained.

Fraction (1b) was a mobile syrup which had $[\alpha]_D^{16}$, $+47^\circ \longrightarrow +27.5^\circ$ (270hrs.); this is not characteristic of 2:3-dimethyl-D-xylonolactone and therefore this fraction must still have contained a fair amount of the other component. Nelson and Percival (loc.cit.) obtained among the methylated products of P. arenaria a dimethyl fraction, thought to contain 3:4-dimethyl-D-xylose, which on oxidation gave a lactone with $[\alpha]_D$, $+46^\circ \longrightarrow +29^\circ$ and yielded an amide which gave a positive Weerman test. It is very probable that this comprised the same mixture of lactones as is present in fraction (1b).

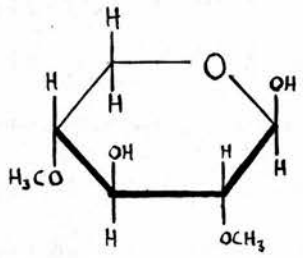
Fraction (2b) was a very viscous syrup and the course of its hydrolysis in water to the carboxylic acids was followed by polarimetric measurements which were recorded graphically. From this it was obvious that fraction (2b) contained a mixture of lactones, one of which probably had a fairly negative rotation.

Demethylation of fraction (e) with 48% hydrobromic acid yielded 2-methyl xylose, xylose and arabinose, as well as other unidentified sugars which were probably arabinose derivatives; this indicates that the second component in the mixture may be a dimethyl arabinose derivative. This sugar was probably present in the complex mixture of dimethyl sugars obtained by Nelson and

Percival and its amide may have been responsible for the positive Weerman test which they obtained with the dimethyl fraction.

Thus fraction (e) has been found to be a mixture of 2:3-dimethyl-D-xylose and an α-hydroxy dimethyl arabinose derivative.

Fraction (f)(p.77) crystallised to give 2:4-dimethyl-β-D-xylopyranose(XVI) which was identified by its analysis, mutarotation, m.p. and mixed m.p. with an authentic specimen.[38,41]



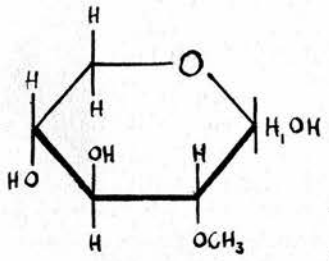
XVI

Oxidation with bromine gave a syrupy lactone. The constants observed for this lactone indicated that it was 2:4-dimethyl-D-xylonolactone. On hydrolysis in water it showed $[\alpha]_D^{20}$, $-14^\circ \longrightarrow +27^\circ$(118 hours). The figures quoted for authentic 2:4-dimethyl-D-xylonolactone are $[\alpha]_D^{20}$, $-15^\circ \longrightarrow +30^\circ$,[41] the rate of hydrolysis being approximately the same.

The lactone yielded the syrupy 2:4-dimethyl-D-xylonamide which gave no hydrazodicarbonamide when subjected to a Weerman test, indicating substitution of $C_{(2)}$ by a methoxyl group.

Paper chromatography showed 2:4-dimethyl xylose to be the only sugar in fraction (f).

Fraction (m)(p.81) yielded crystalline 2-methyl-D-xylopyranose(XVII), identified by its analysis, mutarotation[58] m.p.[37] and mixed m.p. with an authentic specimen.



XVII

Treatment with alcoholic aniline yielded a crystalline anilide which was identified by its specific rotation, analysis, m.p. and mixed m.p. with an authentic specimen of 2-methyl-D-xylose anilide.[63]

Paper chromatography indicated that 2-methyl xylose was the only sugar present in fraction (m).

Fraction H(p.88) was a combination of fractions (c), (h) and (ii) which were shown by paper chromatography to comprise the same sugar.

The syrup had a methoxyl content of 31.6% which is approximately that of a dimethyl methyl pentose(32.3%). Its specific rotation in water was -18°.

Paper chromatographic investigations seemed to indicate that it consisted of only one sugar; one spot was obtained which on spraying with aniline oxalate gave a grey-black colour which is characteristic of no known pentose, hexose or methyl pentose derivatives. It travelled at approximately the rate of a trimethyl hexose or dimethyl methyl pentose, having an R_G value of ca. 0.80.

It did not yield a crystalline anilide.

The syrup was oxidised with alkaline hypoiodite[54] and was therefore an aldose.

$$I_2 + 2NaOH \longrightarrow NaOI + NaI + H_2O$$

$$R.CHO + NaOI \longrightarrow R.COOH + NaI.$$

After acidification the excess iodine was estimated by titration with sodium thiosulphate.

$$NaOI + NaI + H_2SO_4 \longrightarrow Na_2SO_4 + I_2 + H_2O$$

The result gave a value of 194 for the molecular weight of the syrup, which approximates to that of a dimethyl methyl pentose(192); a dimethyl pentose has M.W. 178 and a dimethyl hexose, M.W.208.

Oxidation with bromine in water yielded a syrupy lactone which had OMe, 28.6%; $[\alpha]_D^{17}$, $-51^\circ \longrightarrow -32^\circ$(20days); the slow rate of hydrolysis is characteristic of a γ-lactone, showing the hydroxyl group on $C_{(4)}$ to be free.

The lactone gave a crystalline amide in good yield. This had m.p.131-2° and on analysis was found to have C, 42.95%; H,7.7%; N,6.9%. The C and H contents approximate to those of either a dimethyl pentonamide or a dimethyl hexonamide, while the N is nearer the figure for a dimethyl methyl pentonamide.

	C%	H%	N%
dimethyl pentonamide:	43.5	7.7	7.25
dimethyl hexonamide:	43.0	7.7	6.3
dimethyl methyl pentonamide:	46.4	8.2	6.8

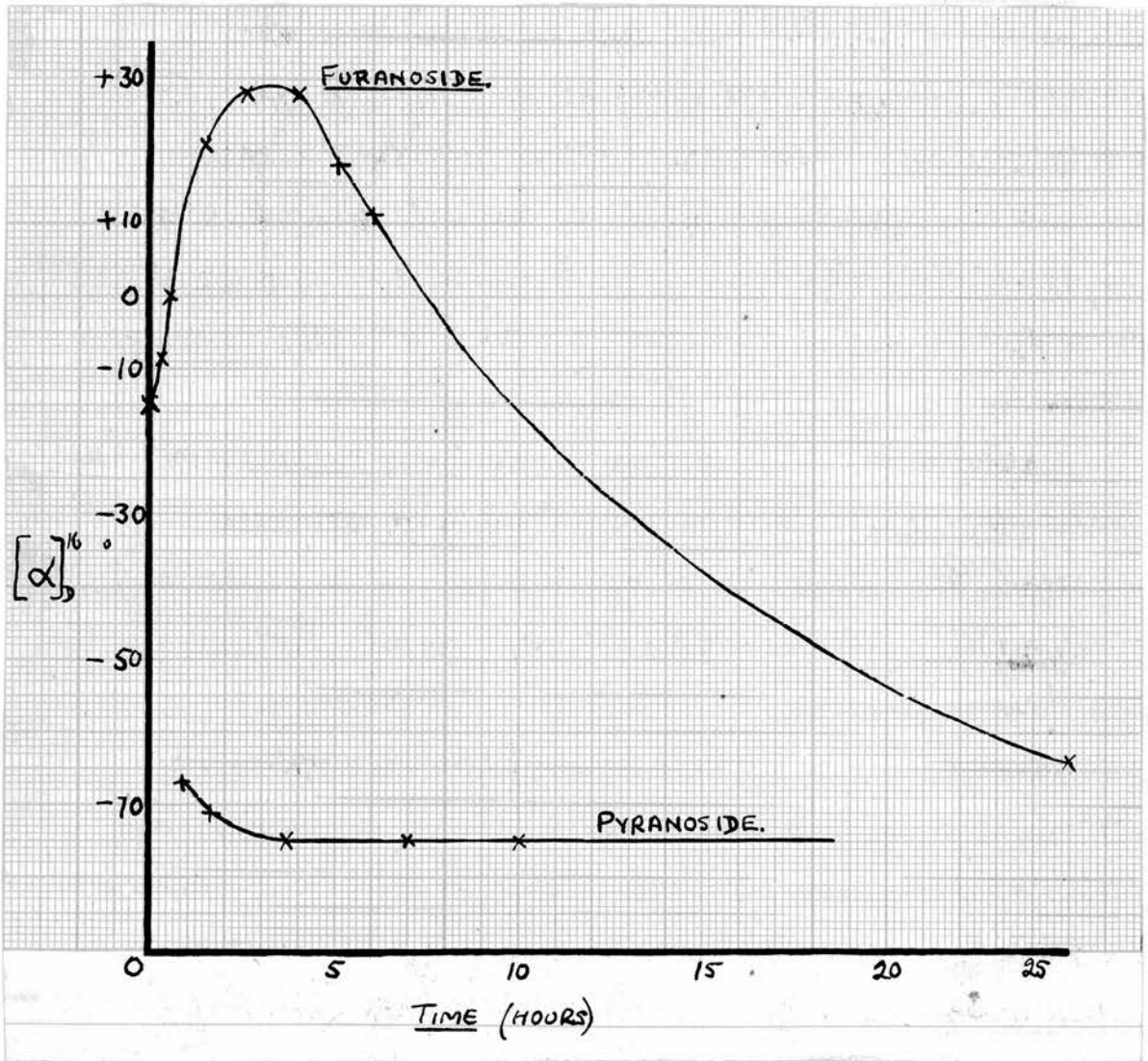
The amide gave a negative Weerman test indicating that $C_{(2)}$ must be substituted by a methoxyl group.

Methylation of fraction H and hydrolysis yielded a product which gave only one spot on a paper chromatogram. It had R_G value 0.96 and gave a brown-black colour when sprayed with aniline oxalate.

From a consideration of all the above data, this fraction seems to be very similar to a fraction obtained from P. ovata by Laidlaw and Percival.[39] The latter had OMe, 33.6%; $[\alpha]_D$,-19° in water; R_G value 0.80. Oxidation with alkaline hypoiodite indicated a M.W. of 180 and it yielded a lactone which had OMe, 32.8% and $[\alpha]_D$ -50° ⟶ -33°(18 days). It yielded a crystalline amide with the same m.p. and analysis figures (C,42.9%;H,7.7%;N,6.8%; OMe,30.0%) as the amide of fraction H, and which gave a negative Weerman test. Methylation also gave only one sugar, R_G value 0.96, the methyl glycoside of which had OMe, 58.1%.

Since these two fractions and their derivatives have almost identical constants and as they are both obtained from mucilages of the same species, it seems almost certain that they comprise the same substance.

Demethylation of fraction H by treatment with 48% hydrobromic acid gave a mixture of sugars which were identified by paper chromatography. Arabinose or a sugar indistinguishable from arabinose was obtained in good yield, as well as a sugar, R_G value 0.34, which gave a pink colour with aniline oxalate and might have been a monomethyl arabinose derivative. Thus it would seem


+30
+10
0
-10
-30
-50
-70
$[\alpha]_D^{16}$ °
FURANOSIDE.
PYRANOSIDE.
0
5
10
15
20
25
TIME (HOURS)

that fraction H might either be an arabinose derivative or have a structure which is closely related to that of arabinose, but the nature of this relationship is obscure.

It is also possible that fraction H may be a mixture of an arabinose derivative and another component which is inseparable from the latter on a paper chromatogram but this seems unlikely since methylation gives also only one spot on the chromatogram. Furthermore, the close similarity between fraction H and the fraction obtained from P. ovata, and particularly the identical results obtained for the mutarotation of the lactones makes this seem improbable, since it is unlikely that a mixture of two sugars should be isolated from two different sources in exactly the same proportions.

Polarimetric observations during methyl glycoside formation of fraction H furnished valuable evidence concerning the structure of the molecule. A solution of the syrup in methanolic hydrogen chloride kept at room temperature had $[\alpha]_D^{16}$, $-15^\circ \longrightarrow +28^\circ$(150mins.) $\longrightarrow$ -59°(24hrs.), whereas the same solution maintained at 80°C had a final rotation of -73°(200mins.). From this it was evident that the methyl furanoside ($[\alpha]_D^{16}$, $+28^\circ$) was being formed first and then reverting to the more stable pyranoside ($[\alpha]_D^{16}$, -73°). This shows that $C_{(4)}$ and $C_{(5)}$ are both free, and since the signs of the specific rotations of the furanoside and pyranoside are opposite, it would seem to indicate that the hydroxyl groups on $C_{(4)}$ and $C_{(5)}$ are on different sides of the ring.

When oxidised with sodium metaperiodate, one mole of syrup H was found to absorb 2.8 moles of periodate; glucose and 4-methyl rhamnose oxidised under the same conditions were only 75% oxidised. Unless the syrup had been over-oxidised, this abnormally high periodate uptake is quite inexplicable, since a dimethyl-methyl pentose or -hexose should absorb only one mole of periodate.

No formaldehyde was released on periodate oxidation (Reeves, loc.cit.). Since $C_{(4)}$ and $C_{(5)}$ are free this must indicate that the sugar is either a hexose derivative with a methoxyl group on $C_{(6)}$ or a 6-deoxy-hexose derivative.

Estimation of the acetaldehyde produced on oxidation with periodic acid (Nicolet and Shinn, loc.cit.) indicated a dimethyl methyl pentose content of 2.8%. It is possible that this low figure may represent a considerable amount of dimethyl methyl pentose since the latter have often been found to give a very low yield of acetaldehyde, probably due to some steric effect. For this reason an open chain derivative might be expected to be more completely oxidised and therefore the amide was oxidised and found to yield acetaldehyde equivalent to 2.5% dimethyl methyl pentonamide. Thus these results are very inconclusive.

To summarise, the following facts are known about fraction H:-

1) It must be a derivative of xylose, arabinose or

galactose, or of a sugar which is indistinguishable from one of the latter on a paper chromatogram.

2) It is a dimethyl aldose.

3) It forms a furanolactome. $\therefore C_{(4)}$ is free.

4) The analytical figures for the amide only partially coincide with those of a dimethyl-hexose, -pentose or -methyl pentose.

5) The amide gives a negative Weerman test. $\therefore C_{(2)}$ is not free.

6) It gives on demethylation arabinose or a sugar which is indistinguishable from the latter on a paper chromatogram.

7) It forms a furanoside and a pyranoside. $\therefore C_{(4)}$ and $C_{(5)}$ are free.

8) The hydroxyls on $C_{(4)}$ and $C_{(5)}$ are probably on opposite sides of the ring.

9) It yields no formaldehyde on periodate oxidation. $\therefore C_{(6)}$ is not free.

10) It yields 2-8% acetaldehyde on periodate oxidation.

Laidlaw and Percival thought it might possibly be a dimethyl gulose derivative, gulose having the same R_G value as arabinose, but this seems rather unlikely since its R_G value is not characteristic of a dimethyl hexose, and the analytical figures do not exactly coincide with those of a dimethyl hexose.

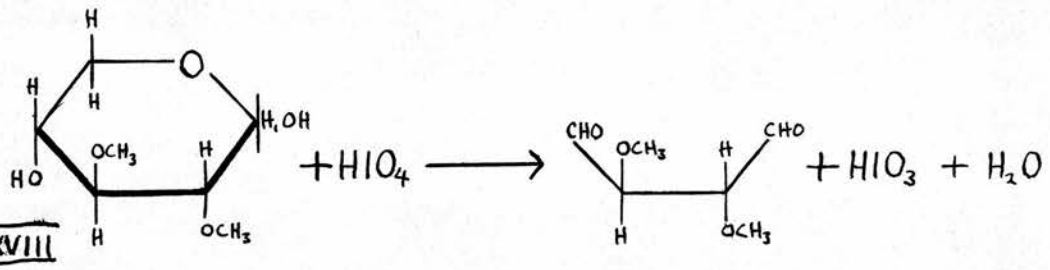
It has been suggested later in this discussion that this residue may contain an unsaturated grouping but no experimental evidence has been obtained to support this.

At present no definite conclusions can be drawn regarding its structure.

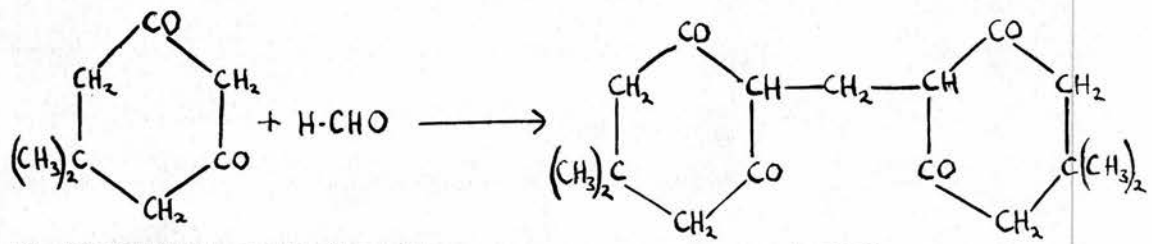
Fractions (d) and (i) (pp.73,78) consisted of mixtures of 2:3-dimethyl-D-xylose and the sugar constituting fraction H. The relative proportions of these were estimated by oxidation with alkaline hypoiodite after separation of the sugars by paper chromatography.

Fraction (d) was found to comprise 52% 2:3-dimethyl-D-xylose and 48% H and fraction (i) 71% 2:3-dimethyl-D-xylose and 29% H.

Fraction (j) (p.79) was a mixture of 2:3- and 2:4-dimethyl-D-xyloses. Since 2:4-dimethyl xylose possesses no α-glycol links, the amount of 2:3-dimethyl xylose(XVIII) could be determined by oxidation of the mixture with periodic acid and estimation of the formaldehyde produced as the insoluble dimedon compound(XIX) (Reeves, loc.cit.).



XVIII



5:5-dimethyl-cyclohexane-1:3-dione (dimedon)

XIX

Oxidation of the mixture gave formaldehyde-dimedon compound equivalent to 39% 2:3-dimethyl xylose.

Fractions (k) and (l) (p.80) were mixtures of mono- and di-methyl-D-xyloses as well as two sugars of R_G 0.50 and 0.58 which were at first suspected to be galactose derivatives since they gave a brown colour with aniline oxalate. However demethylation of fraction (k) with hydrobromic acid yielded arabinose as well as xylose and therefore these are probably methylated arabinose derivatives, unless, of course, they are monomethyl derivatives of the dimethyl sugar present in fraction H. A sugar was also obtained on demethylation which had R_G 0.24 and gave a pink colour with aniline oxalate and is very probably a monomethyl arabinose derivative. This is probably the same sugar as one which appears in fractions (n),III and (w). It was also obtained on demethylation of fraction (e) in conjunction with arabinose.

Fraction III (p.67) contained equal amounts of 2-methyl xylose and xylose which were identified by paper chromatography, as well as a trace of arabinose and a sugar with R_G value 0.24 which was probably a monomethyl arabinose derivative (see above).

The quantitative results for the building units of fraction A were as follows:-

	Anhydrosugar %	Relative no. of molecules (approx.)
Trimethyl-D-xylopyranose	20.0	11
trimethyl-L-arabofuranose	2.1	1
tetramethyl-D-galactopyranose	9.8	5
Fraction H	8.5	5
2:3-dimethyl-D-xylose	13.7	9

(Cont'd)

	Anhydrosugar %	Relative no. of molecules(approx.)
2:4-dimethyl-D-xylose	7.2	5
2-methyl-D-xylose	40.0	29
D-xylose	1.9	1.5
? dimethyl arabinose?	4.2	2
? dimethyl arabinose?	1.2	0.5

If the material were fully methylated, one molecule of end-group would be obtained for every molecule of monomethyl xylose, since the latter constitutes a branching point in the molecule. A study of the above figures indicates that such is not the case, only 17 molecules of end-group being obtained with 30 molecules of monomethyl xylose and xylose. The conclusion was drawn from this that either the mucilage had been incompletely methylated or that some of the end-group had not been accounted for.

It was thought possible that some trimethyl xylose may have been lost as the result of either volatilisation of the methyl glycosides or demethylation during the course of acid hydrolysis. In order to investigate the extent of either of these losses, some control experiments were carried out (p.95) by treating pure trimethyl xylose under exactly the same conditions as were used during the hydrolysis of fraction A. It was shown that not more than 3% of the trimethyl methyl xylosides could have been lost by volatilisation and also that very little loss was suffered by demethylation. Dr. S.K. Chanda (loc.cit.) has also proved that at the most 1.5% dimethyl xylose and 0.4% monomethyl xylose is obtained from

trimethyl xylose under the same conditions of acid hydrolysis.

In order to verify the yield of end-group obtained above, a small amount of fraction A was hydrolysed under carefully controlled conditions and the trimethyl-xylose and -arabinose estimated by oxidation with alkaline hypoiodite after separation from the other components by paper chromatography. An average result of 20% was obtained, which is in reasonable agreement with the yield of trimethyl pentoses obtained above.

It is evident therefore that fraction A was incompletely methylated and it is impossible to formulate a structure for the molecule on the basis of the above figures, since the proportion of monomethyl xylose residues to end-groups is too high. The results do, however, give an idea of the highly branched nature of the molecule and the diversity of linkages involved, although they do not give a quantitative determination of the degree of branching, nor any idea of the order in which the various residues are combined.

It is difficult to say whether the unsubstituted xylose residues arise solely as the result of incomplete methylation and partial demethylation or whether they are an integral part of the molecule. It seems probable however, considering the extremely highly branched nature of the molecule, that a certain proportion of free xylose units might be present in the fully methylated molecule.

Investigation of the sugar fractions from fraction C.

Paper chromatography indicated that this fraction

yielded a mixture of the same sugars as were obtained from fraction A.

Fraction (α_1)(P.83) was identified as a mixture of trimethyl-D-xylopyranose(91%) and trimethyl-L-arabofuranose (9%) in the same way as fraction (a).

After removal of the crystalline trimethyl xylose from the mixture the residual syrup was oxidised with bromine to give two lactone fractions (1c) and (2c). At equilibrium, fraction (1c) had $[\alpha]_D^{16}$, +4.5° and fraction (2c) had $[\alpha]_D^{17}$, +12°. From a consideration of the equilibrium rotations of pure 2:3:4-trimethyl-D-xylonolactone and 2:3:5-trimethyl-L-arabonolactone, the lactone fractions were calculated to have the following compositions:-

	Trimethyl-D-xylonolactone	Trimethyl-L-arabonolactone	Total.
fraction (1c)	0.0401gm.	0.0225gm.	0.0626gm.
fraction (2c)	0.0542gm.	0.0135gm.	0.0677gm.
	0.0943gm.	0.0360gm.	0.1303gm.
	72%	28%	

From these figures, the composition of the original mixture was calculated.

Fraction (α_2) (p.84) was a mixture of 2:3:4-trimethyl-D-xylose and 2:3:4:6-tetramethyl-D-galactose. The relative proportions of these were found to be 53% and 47% respectively by separation on a paper chromatogram and oxidation with alkaline hypoiodite.

Fraction (α_3) (p.85) had $[\alpha]_D^{17}$, +114°, the value quoted for authentic tetramethyl-D-galactopyranose being

+117°. It yielded crystalline 2:3:4:6-tetramethyl-D-galactose anilide, identified by its m.p., mixed m.p. with an authentic specimen and equilibrium rotation.

Fraction (p) (p.85) was shown by paper chromatography to consist of a mixture of the same two sugars as were found in fraction (e), i.e. 2:3-dimethyl xylose and a sugar which was suspected to be a dimethyl arabinose derivative.

The syrup was inoculated with a crystal of pure authentic 2:3-dimethyl-D-xylose and it had partially crystallised after 7 months at 0°C. The crystals had m.p. 80-82°C and mixed m.p. 79-80°C.

They had $[\alpha]_D^{16}$, +61° ⟶ +23° (18hrs.) in water, the value quoted for 2:3-dimethyl-D-xylose being $[\alpha]_D^{15}$ +70° ⟶ +23° (14hrs.).[69]

Fraction (q) (p.86) crystallised completely and was identified as 2:4-dimethyl-β-D-xylopyranose by its m.p., mixed m.p. with an authentic specimen and mutarotation in water.

Fraction (r) (p.86) was a syrup which had R_G value 0.58 and gave a brown colour with aniline oxalate when examined on a paper chromatogram. By analogy with fraction (k) it was suspected to be a dimethyl arabinose derivative. It had $[\alpha]_D^{18}$, +78° in water and it is possible that it may be 2:3-dimethyl-L-arabinose,[70] quoted as having $[\alpha]_D^{18}$, +86° in water and R_G value 0.64.

Fraction (u) (p.87) comprised the first 100 tubes

of monomethyl xylose solution which came out of the column. It was investigated separately in order to ascertain whether any 3-methyl xylose were present. This would be expected to be eluted first as it travels slightly faster than 2-methyl xylose. However it had $[\alpha]_D^{16}$, $+34^\circ$ in water, the value quoted for 2-methyl-D-xylose being $+36^\circ$(58) and that for 3-methyl-D-xylose $+17^\circ$;(38) proving fairly conclusively that it must be 2-methyl-D-xylose.

Fraction (v) (p.87) was identified as 2-methyl-D-xylopyranose by its m.p., mixed m.p. with an authentic specimen and mutarotation in water.

Fraction (x) was identified as xylose by paper chromatography.

All other fractions were investigated by paper chromatography with the results shown on pages 68 and 69. They were mostly mixtures which were too small for satisfactory investigation.

Thus the above fractions were shown to comprise the same sugar derivatives as were obtained from fraction A, in the following proportions:-

	Anhydro sugar %	Relative no. of molecules (approx)
Trimethyl-D-xylopyranose	30.6	18
trimethyl-L-arabofuranose	3.3	2
tetramethyl-D-galactose	5.6	3
fraction H	9.0	5
2:3-dimethyl-D-xylose	13.4	8
2:4-dimethyl-D-xylose	9.2	6
2-methyl-D-xylose	18.8	13

	Anhydro sugar %	Relative no. of molecules (approx.)
D-xylose	2.0	2
unidentified sugars.	7.2	ca. 3

The unidentified sugars were probably di- and mono-methyl arabinose derivatives.

These figures show that fraction C consists of 19 dimethyl residues to which are attached two unsubstituted xylose residues from which two branches must emerge, and 13 residues from which one branch emerges; these require 1, 4 and 13 end-groups respectively, making a total of 18, whereas 23 end-groups were actually found. If the three unidentified residues constituted branching points, the structural requirements would be more or less satisfied; otherwise, this excess of end-groups is inexplicable.

Hence the polysaccharide from which fraction C was derived, and possibly the whole mucilage, since fraction C was probably a more fully methylated form of fraction A, as discussed earlier, is built up of the following units:-

18 D-xylopyranose residues(XX) linked through $C_{(1)}$ only

2 L-arabofuranose residues(XXI) linked " " "

3 D-galactopyranose residues(XXII) linked " " "

5 unidentified residues (fraction H)

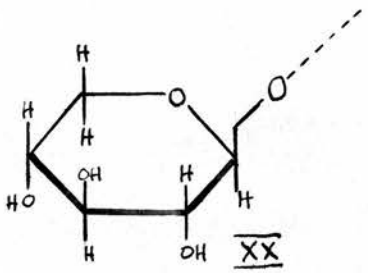
8 D-xylopyranose residues(XXIII) linked through $C_{(1)}$ and $C_{(4)}$

6 D-xylopyranose residues(XXIV) linked through $C_{(1)}$ and $C_{(3)}$

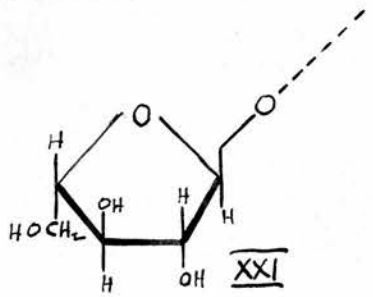
13 D-xylopyranose residues(XXV) linked through $C_{(1)}C_{(3)}$ and $C_{(4)}$

2 D-xylopyranose residues(XXVI) linked through $C_{(1)}$, $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$.

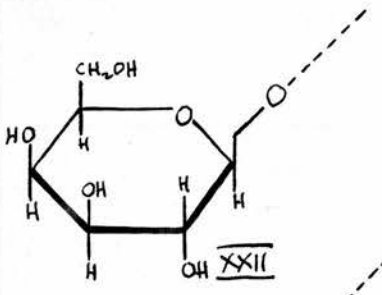
3 residues consisting of unidentified sugars.



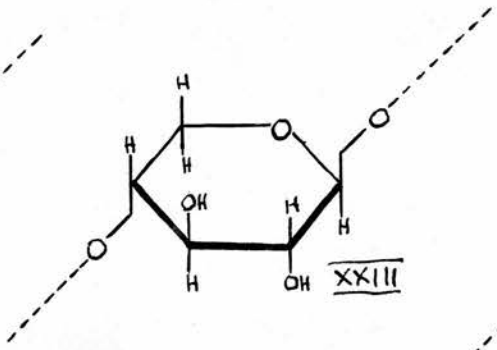
XX



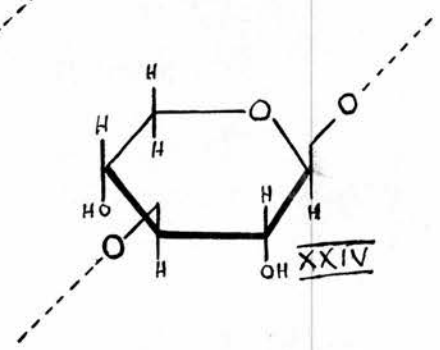
XXI



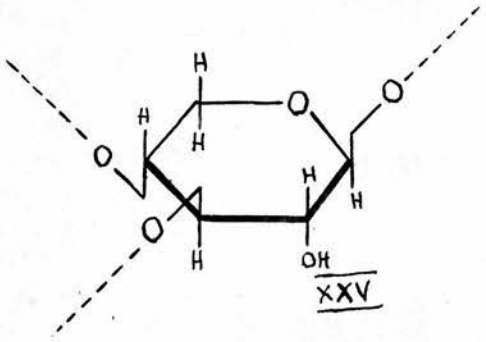
XXII



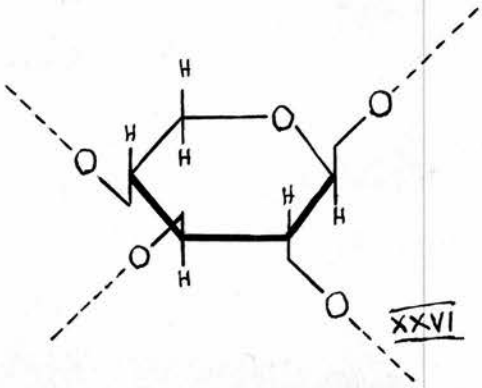
XXIII



XXIV



XXV



XXVI

Such a molecule would absorb 63 molecules periodate, corresponding to an uptake of 14.4 molecules per equivalent, which agrees with the figure obtained(13.9). The end groups in this molecule would liberate 5.3 molecules formic acid per equivalent which also agrees with the result obtained(5.7).

However these results did not lend support to those

obtained on hydrolysis of the mucilage after periodate oxidation, which indicated that 1 in 4 xylose residues and 1 in 6 arabinose residues constituted branching points. Here we have 15 xylose residues in 59 which would be unattacked, i.e. 1 in 2.8; no arabinose residues which would be unattacked have been identified.

On examination of the products of the mucilage obtained after methylation and hydrolysis it is at once obvious that some of the original components have not been recovered as their methylated derivatives.

Analysis of the mucilage showed that it contained 17% arabinose, or one residue in five. Now after methylation and hydrolysis methylated arabinose derivatives have not been found to this extent; in fact, only 2 residues of trimethyl arabinose in a total of 60 residues have been identified, although the 3 unidentified residues are very probably arabinose derivatives. It must also be remembered that fraction H may be an arabinose derivative or a derivative of a sugar which is indistinguishable from arabinose on a paper chromatogram and was considered to be arabinose in the original analysis of the mucilage. If this is the case, then 10 methylated arabinose or "pseudo-arabinose" derivatives have been recovered in a total of 60 residues, i.e. 1 in 6, which is approximately the number found to be present in the mucilage (3 in 16).

If, however, fraction H is not an arabinose or "pseudo-arabinose" derivative, then 50% of the arabinose

has not survived the acetylation and methylation processes. A loss of arabinose has been experienced in previous studies on the Plantago mucilages,[35,37] and it may be due to the extreme lability of the arabofuranose residues which may be split off during any slightly acid conditions which arise during the experimental procedure. The possibility also exists that there may be associated with the mucilage another polysaccharide containing some of the arabinose which is lost during the acetylation or methylation on account of solubility differences. This may also be the case with some of the uronic acid which was shown to be lost during acetylation. This question of the macromolecular composition of the starting material is as yet unsolved and constitutes one of the biggest problems in work of this kind.

Another very curious fact which emerges from this work is that no methylated galacturonic acid or rhamnose derivatives were found among the methylated hydrolysis products. This phenomenon was also encountered with the mucilages from P. ovata and P. lanceolata, no trace of any methylated products of the aldobiuronic acid being obtained from either of them. This is not found to be the case with other gums and mucilages and must therefore be due to some molecular peculiarity in the mucilages of the Plantago species.

However some light was thrown on this phenomenon by some results which arose during the investigation of the

acetylated mucilage. This was deacetylated with sodium methoxide and gave two fractions on precipitation; one was the deacetylated mucilage which on hydrolysis yielded xylose, arabinose and galactose in approximately the same proportions as before. The other fraction was a gelatinous precipitate which appeared to be the sodium salt of the aldobiuronic acid; it gave a positive naphthoresorcinol test which is characteristic of uronic acids, and the free acid was found by titration to have an equivalent weight of the same order as the aldobiuronic acid. This seemed to indicate that the aldobiuronic acid had actually been split off from the rest of the molecule and so, in order to investigate this effect further, the mucilage itself was treated with alkali and its uronic anhydride content examined.

The mucilage was allowed to stand overnight in 2N sodium hydroxide and after neutralisation and dialysis the uronic anhydride content was found to have fallen from 7.2% to 4.3%. Another sample was treated with 2N sodium hydroxide for a week and a more effective removal of the uronic acid component was attempted by electrodialysis of the solution. The resulting material had uronic anhydride, 2.5%, and after electrodialysis, the solution in the anode compartment gave a positive naphthoresorcinol test. Electrodialysis of a dilute solution of the natural mucilage did not remove any uronic acid. These results seem to indicate that the aldobiuronic acid is an integral part of the molecule

which is split off by the action of alkali.

Since dilute alkali produces scission of the aldobiuronic acid, it is obvious that this must occur during methylation of the mucilage in 30% sodium hydroxide solution, thus explaining why no methylated derivatives of the aldobiuronic acid were isolated.

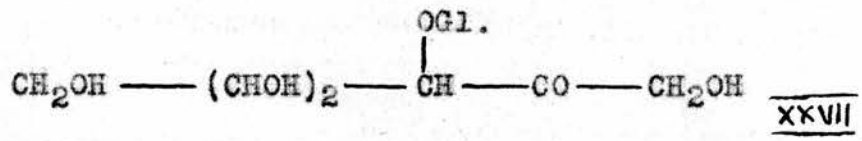
The reason for this alkali lability is not clear but it may be due to one of the following possibilities:-

1) Sugar residues in polysaccharides of this type have always been found to be joined by glycosidic linkages, which are normally quite stable to alkali. It is conceivable that in this case the aldobiuronic acid is attached to the rest of the molecule by means of some other type of linkage, such as an ester linkage through the carboxyl group of the uronic acid. Such a link would be attacked by either acid or alkali.

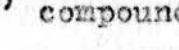
2) The alkali sensitivity may be due to some peculiarity in the structure of the residue to which the aldobiuronic acid is attached. If this is true, this residue must be a sugar which is common only to the Plantago mucilages and has not been encountered before in the alkali-stable gums and mucilages. The only sugar which might fit such a description is the so far unidentified fraction H which has been found in P. ovata as well as P. arenaria.

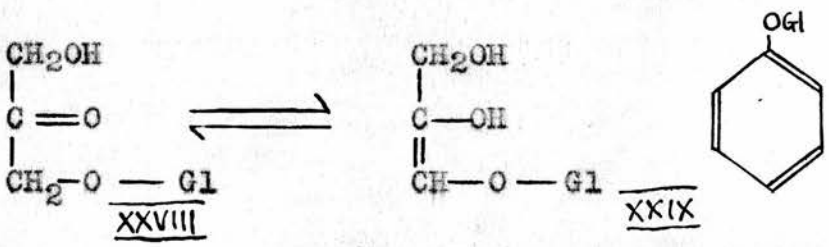
Mention might be made here of the alkali-sensitivity of the oligosaccharide turanose (3-glucosyl-fructose) (XXVII)[71] which is considered to be due to the 1:3 link (although 1:3 links have been found in alkali-stable

polysaccharides) or to the proximity of the unsaturated carbonyl group of the fructose.



XXVII

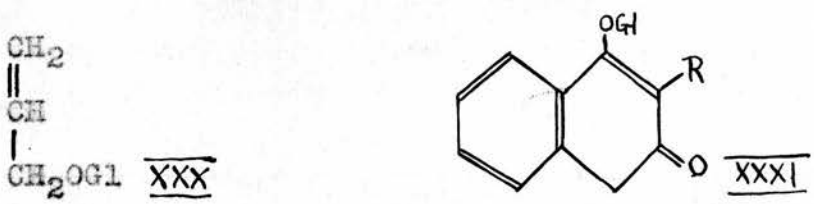
It is interesting to note that, as observed by Evans and his associates,(72) compounds possessing the common structural unit  , which in the case of turanose is the enol isomer, have been found to be alkali-sensitive. Examples of this are dihydroxy acetone glucoside(XXVIII) and the phenyl glucosides(XXIX).



XXVIII XXIX

When the double bond is removed further from the glycosidic link, as in the case of allyl glucoside(XXX) the alkali sensitivity is lost.

Link, in his study of alkali sensitive derivatives of 4-hydroxy coumarin glycosides(XXXI), also found that the above basic structure was necessary.(73)



XXX XXXI

It is possible that in the present case the alkali-sensitivity may be caused by the presence of an unsaturated structure of this type in the residue to which the

aldobiuronic acid is attached and it is therefore feasible that fraction H may contain an unsaturated grouping.

To summarise, our present state of knowledge of the structure of the mucilage from P. arenaria is as follows. The nucleus is a highly branched chain polysaccharide, as is evidenced by methylation followed by hydrolysis. The uronic acid, L-rhamnose and a large proportion of the L-arabinose residues must be situated on the periphery of the molecule, since they are so easily removed without loss of the macromolecular properties of the mucilage. The L-rhamnose and D-galacturonic acid are combined in the form of an aldobiuronic acid which is 2-α-D-galacturonosido-L-rhamnose. The complex structure contains D-xylopyranose, L-arabofuranose and D-galactopyranose residues linked through $C_{(1)}$ only, xylose units linked through $C_{(1)}$ and $C_{(3)}$ and also through $C_{(1)}$ and $C_{(4)}$, a large proportion of xylose units linked through $C_{(1)}$, $C_{(3)}$ and $C_{(4)}$ and a small proportion linked through $C_{(1)}$, $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$. It also contains a small proportion of a residue which has not yet been identified. No information is available concerning the order in which these are linked together in the molecule.

Valuable evidence would be obtained regarding the arrangement of the residues in the molecule by the isolation and identification of the oligosaccharides obtained by partial hydrolysis of the mucilage under different conditions.

Further suggestions for future research would

include an attempt to discover whether the mucilage is a single chemical entity or not; this subject might be approached by investigation of any solubility differences in the copper complex.

It would also be of interest to separate the arabinose obtained on hydrolysis of the mucilage on a large scale by partition chromatography and determine whether any other component were present. If such were the case, its structure could be examined in relation to the alkali sensitivity of the molecule. The latter problem could also be approached by a study of the action of alkali on synthetic oligosaccharides containing unsaturated sugar residues combined by different types of linkages.

Thus this subject opens up quite a large field of research which will have to be carried out before any definite conclusions can be reached with regard to the detailed structure of the molecule.

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SUMMARY.

1. The mucilage was extracted from Plantago arenaria seeds and precipitated in acidified alcohol as the "free-acid" polysaccharide.
2. The mucilage had an equivalent weight ca. 2450, uronic anhydride 7.2%, anhydropentose 80%. Periodic acid oxidation indicated that some methyl pentose was present and formed part of a relatively stable nucleus.
3. Autohydrolysis of the mucilage yielded L-arabinose.
4. Hydrolysis with 3% oxalic acid gave an insoluble residue(2%), D-xylose(62%), L-arabinose(17%),D-galactose (6%) and an aldobiuronic acid(13%).
5. Drastic hydrolysis of the aldobiuronic acid yielded D-galacturonic acid and L-rhamnose and oxidation yielded mucic acid and L-rhamnonic acid. On methylation and hydrolysis, 2:3:4-trimethyl-D-galacturonic acid and 3:4-dimethyl-L-rhamnose were obtained. Thus the aldobiuronic acid was identified as 2-α-D-galacturonosido-L-rhamnose.
6. Treatment of the insoluble residue with 72% sulphuric acid indicated that it was composed of cellulose and lignin.
7. On oxidation with periodate, the mucilage absorbed 13.9 molecules periodate per equivalent and liberated 5.7 molecules formic acid per equivalent. Hydrolysis of the periodate-oxidised mucilage indicated that ¼ of the xylose residues and 1/6th of the arabinose residues had been unattacked.

8. Acetylation of the mucilage gave two fractions which were found to have lost ca. 20% of their uronic anhydride content during the process.

9. Methylation of the two acetate fractions separately yielded two main fractions, A and C.

10. Hydrolysis of fractions A and C and separation and identification of the methylated sugar derivatives showed them to be built up of the following units:-

	fraction A	fraction C
2:3:4-trimethyl-D-xylose	11 mols.	18 mols.
2:3:5-trimethyl-L-arabinose	1 mol.	2 mols.
2:3:4:6-tetramethyl-D-galactose	5 mols.	3 mols.
Unidentified fraction (H).	5 mols.	5 mols.
2:3-dimethyl-D-xylose.	9 mols.	8 mols.
2:4-dimethyl-D-xylose.	5 mols.	6 mols.
2-methyl-D-xylose.	29 mols.	13 mols.
D-xylose.	1.5 mols.	2 mols.
Unidentified residues.	2.5 mols.	ca. 3 mols.

11. Treatment of the mucilage with 2N sodium hydroxide at room temperature effected scission of the aldobiuronic acid from the rest of the molecule.

12. The results obtained have shown that the mucilage has a highly branched structure, consisting of D-xylopyranose, L-arabofuranose and D-galactopyranose residues linked through $C_{(1)}$ only; D-xylose residues linked through $C_{(1)}$, and $C_{(4)}$ and also through $C_{(1)}$ and $C_{(3)}$; D-xylose residues linked through $C_{(1)}$, $C_{(3)}$ and $C_{(4)}$; a small proportion of D-xylose residues linked through $C_{(1)}$, $C_{(2)}$, $C_{(3)}$ and $C_{(4)}$; a small proportion of an unidentified residue and aldobiuronic acid residues, identified as

2-α-D-galacturonosido-L-rhamnose, linked through $C_{(1)}$ of the rhamnose moiety. No evidence was obtained concerning the order in which these residues were arranged within the molecule.

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