THE POLYSACCHARIDE CONSTITUENTS OF THE GRAMINEAE

BY

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ABSTRACT OF THESIS

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Title of Thesis THE POLYSACCHARIDE CONSTITUENTS OF THE GRAMINEAE.

1. The structures of xylans isolated from barley husks from two different harvests, from the roots and stems of perennial rye grass, and from a Norway spruce wood were investigated using methylation techniques. The various methyl glycoside methyl ethers of xylose and arabinose obtained from the different xylans were identified by gas-liquid chromatography. The xylans were found to correspond to the pattern of the typical xylan with regard to their main structural features, but to differ in some cases with regard to their fine structure.

2. A partial acid hydrolysis carried out on a perennial rye grass root xylan yielded the characteristic xylo-oligosaccharides, together with two arabinose containing oligosaccharides. These were shown to be 2-O- -D-xylopyranosyl-L-arabinose and D-galactopyranosyl(1--4) -D-xylopyranosyl(1--2)-L-arabinose and it appears probable that these units are attached as side-chains to the xylan backbone through the arabinose residues.

3. The Smith degradation was applied to a barley husk xylan and a rye flour xylan. That the xylopyranosylarabinose units in the husk xylan are attached through position 3 of D-xylose residues in the basal chain follows from the isolation and characterisation of the degradation product L-arabinofuranosyl (1--3) -D-xylopyranosyl-glycerol. In both xylans a random

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distribution of branch points along the xylan backbone is indicated from the isolation and characterisation of the xylo-glycerol oligosaccharides formed.

4. The work on the alkaline degradation of arabinoxylans started by Aspinall et al, was continued by the synthesis and alkaline degradation of the following model compounds : 3,4-di-O-methyl-L-arabinose, 3,4-di-O-methyl-D-xylose, and 3-O-methyl(4-O- -D-xylopyranosyl)-D-xylose. The disaccharide, 4-O- -D-xylopyranosyl-D-xylose(xylobiose) was also synthesis.

5. In the alkaline degradation of 3-O-methyl-(4-O- -D-xylopyranosyl)-D-xylose, a double elimination from C_3 and C_4 was shown to have taken place, thus providing clear evidence for the by-passing of a branch point during the alkaline degradation of arabinoxylans.

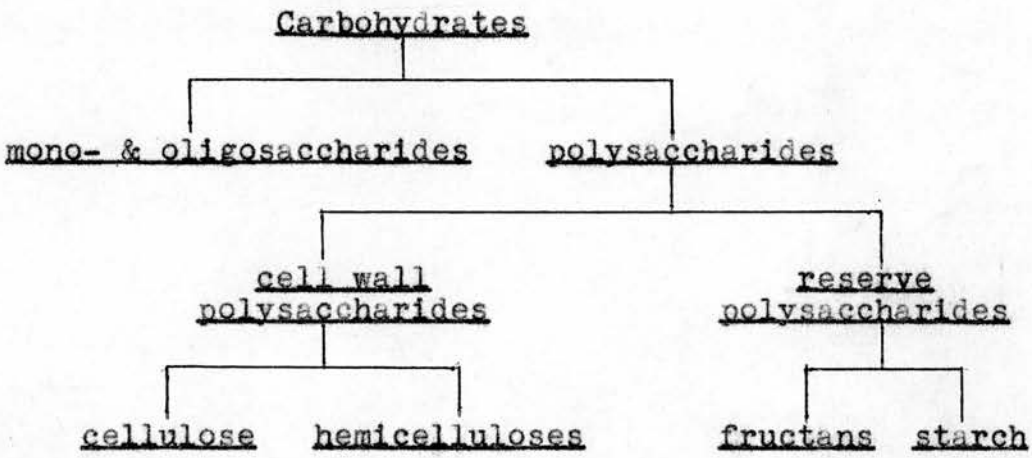
6. In similar degradations of the other model compounds, complex mixtures of compounds were obtained, suggesting that the degradation mechanism is not simple, and that various competing reactions are probably taking place simultaneously.

Table of Contents.

INTRODUCTION.

The Gramineae, which include the commonly known grains and grasses, are flowering plants consisting of approximately 4,500 species found in many parts of the world. In the temperate zone they are characterised in wheat, barley, rye, oats, and in the many grasses used for grazing. The economically more important species in the tropics are maize, rice, and millet.

The carbohydrates of the Gramineae can be classified as follows:



Glucose and fructose are the most commonly found monosaccharides (1) in the Gramineae, and sucrose the most abundant disaccharide (2).

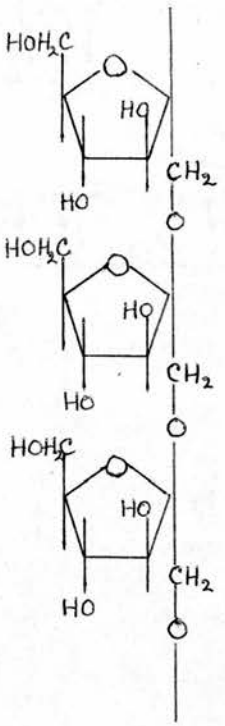
The bulk of the plant however, as in all plants, consists of polysaccharides, existing either as reserve or cell wall polysaccharides.

Reserve Polysaccharides.

Fructans

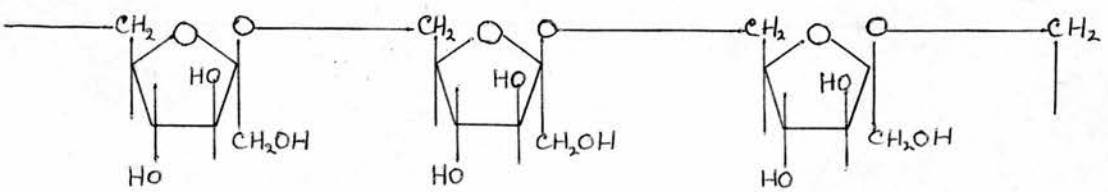
These polymers of fructose residues are water soluble and exist in three distinct forms:

a) Polymers consisting of 2',1-linked-β-D-fructofuranose residues. Inulin is a typical example of this class.



(I)

b) Polymers consisting of 2',6-β-D-fructofuranose residues, of the levan and phlein type.



(II)

Fructans isolated from the grasses are almost invariable of this type, and in most cases are unbranched.

c) Polymers containing both 2',1 and 2',6-β-D- fructofuranose residues.

Since the order of occurrence of the different types of linkage in many cases is not known, assignation of a specific formula is not yet possible.

Fructans from the cereals are usually branched, and can possess either the inulin or levan type of structure, or both.

It is now generally accepted that fructans from all three classes have sucrose residues in terminal positions(3). It appears possible that fructans are built up in plants by the additions of fructose residues to a sucrose residue, and indeed, oligosaccharides containing up to ten fructose residues have been isolated from barley (4).

Starch.

Starch is the main reserve polysaccharide in cereals and occurs in the form of discrete granules. It is commonly believed to consist of two polymers of glucose, differing markedly in properties and structure, called amylose and amylopectin. In both polymers, the α-D-glucopyranose residues are linked through positions 1 and 4.

The principle differences in properties are summerised in Table 1.

Table 1.

	Amylose	Amylopectin
molecular configuration	essentially linear	branched
molecular weight	ca 10^6	ca 10^8
x-ray diffraction	crystalline patterns	amorphous or weakly crystalline patterns
complex formation	readily forms complexes with iodine and polar substances	very limited complex with iodine and polar substances
stability in aqueous solutions	unstable. Tends to "retrograde" in concentrated solutions	stable at any concentration
β-amylolysis	ca 70-80% conversion into maltose	ca 55% conversion into maltose

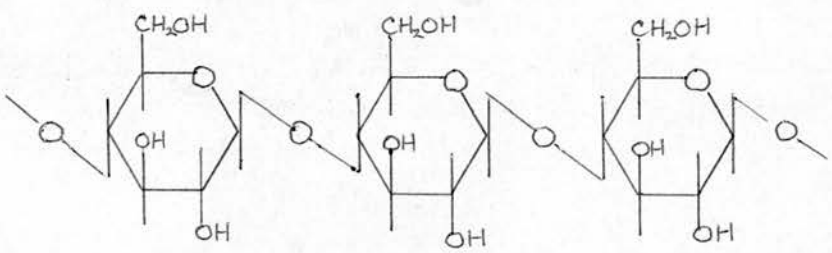
Despite the considerable progress in the field of starch during the last twenty years, as yet few starches have been completely characterised.

Cell Wall Polysaccharides

Cellulose

Of all naturally occurring organic compounds cellulose is the most abundant, and makes up about one-third of all vegetable matter. It is the basic constituent of the cell wall of land plants.

Most celluloses studied have been obtained from wood or cotton due to their economic importance, but it appears probable that all celluloses have essentially the same chemical structure, consisting of unbranched chains of 1,4-β-D-glucopyranose residues.



(III)

Within the cell wall, the cellulose is believed to acquire an organised structure by the long thread-like macromolecules being aligned in bundles.

Hemicelluloses

Cellulose almost invariably occurs in association with other polysaccharides. This is particularly true in

lignified tissues where the cellulose bundles are embedded in an amorphous mass of lignin, and other polysaccharides called the hemicelluloses.

The exact nature of the association between cellulose, lignin, and the other non-cellulosic polysaccharides is not yet known, although some evidence in favour of a chemical link between lignin and a part of the hemicellulose is produced by Lindgrens electrophoretic studies (6). This is contrary to the views of Nelson and Scheurch (7) who believe the association to be purely physical

The term "hemicellulose" is usually taken to cover only cell wall polysaccharides extracted by alkali, but this is unsatisfactory since it excludes polysaccharides similar in chemical structure and biological function. It is less confusing simply to classify these polymers (non-cellulosic cell wall) by their main constituent sugar; thus a polymer of mainly xylose residues is called a xylan, that of mainly galactose residues a galactan etc... When any one of the three sugars, xylose, galactose, or mannose is present in the polymer it is usually present as the main constituent, but other sugars, namely L-arabinose, D-glucose, L-rhammose, D-glucuronic acid and its 4-O-methyl ether, can compose part of the molecule.

The biological function of these polysaccharides is still not clear. Whistler and Young (8) have recently shown

that in oats, they do not act as reserve polysaccharides but exist purely in the cell wall.

Xylans

Xylans are by far the most common group of the non-cellulosic cell wall polysaccharides found in the Gramineae, and it is with their fine structure and behaviour in alkali that this work is mainly concerned.

Extraction and Purification

Before isolation of xylans, grasses and cereals are given a preliminary extraction with an organic solvent, often followed by some delignification procedure.

Of the methods devised for removal of lignin, in essence only two have been adopted. In one the plant tissue is treated with chlorine and the chlorolignin extracted with some alkaline solvent (9); in the other the lignin is directly transformed into a water soluble compound by treatment with either chlorine dioxide or sodium chlorite (10). By neither of these methods can the lignin be completely removed without appreciable loss of carbohydrate. Timell and John (11) have studied methods of delignification and concluded that the chlorination method is superior, producing a holocellulose containing about 1% lignin without appreciable loss of carbohydrate.

Treatment of the holocellulose with alkali, usually about 4%, extracts the xylans, which can be recovered from solution by neutralisation and precipitation with acetone. Oxygen free conditions must be used to minimize the degradative effect of the alkali, a topic discussed on page 28.

Direct extraction of cereal grains with warm water yields a mixture of polysaccharides, often called the cereal gums. These normally contain xylans with a high proportion of arabinose, which, although similar in chemical structure to the water insoluble xylans, appear to differ in biological function.

On extraction xylans are invariably contaminated by other polysaccharides and some form of purification is always necessary. Purification in this sense simply means the isolation of polymers containing the same constituent sugars. These polymers have a molecular weight range, and although possessing the same constituent sugars they can differ from each other in the proportions of these sugars present.

A suitable method of purification for any particular case can only be found by trial and error, but generally belongs to one of the four following classes:

a) Fractional precipitation by the addition of a non-solvent, or fractional dissolution in mixtures of solvent and non-solvent.

b) Precipitation as specific complexes.

Both complexing with copper ions (12), and with cetavlon (cetyltrimethyl ammonium bromide)(13), has been used successfully for certain xylans.

c) Salting out.

Ammonium sulphate has been used for graded precipitation of xylans from aqueous solutions (14).

d) Chromatography.

The recent introduction of diethylaminoethyl (DEAE) cellulose columns (15) has produced good results with certain polysaccharides.

Electrophoresis (16) and ultracentrifugation (17) have been used in small scale separations of polysaccharide species and are valuable as analytical tools.

Despite the use of these various methods, efficient purification is still a major problem in polysaccharide chemistry.

Methods of Structural Investigation

Initial examination of any polysaccharide involves the determination of optical rotation, percentage ash, methoxyl, acetyl and uronic acid content, and most important, the constituent sugars. These sugars are easily determined by acid hydrolysis of the polysaccharide, followed by separation identification and estimation (18) of the products.

Methylation

Methylation followed by hydrolysis has for long been the most important and valuable technique in structural polysaccharide chemistry. It is the main method of determining the modes of linkage between monsaccharides.

The method involves the exhaustive methylation and subsequent hydrolysis of the polysaccharide to give partially methylated sugars, the free hydroxyl groups of which indicate the positions of linkage in the molecule.

The most widely used method of methylation involves treatment with sodium hydroxide and dimethyl sulphate (19), the procedure being repeated several times. The use of concentrated sodium hydroxide can lead to some degradation and depolymerisation, particularly in the presence of oxygen. For this reason oxygen free conditions are favoured.

Since this method of Haworth's is seldom completely successful, it is usually followed by treatment with silver oxide and methyl iodide, according to Purdie and Irvine (20). Silver oxide causes degradation by oxidation but this effect is minimised when most of the hydroxyl groups are already methylated. The procedure is repeated until constant methoxyl determination is achieved.

The Purdie technique has recently been improved by Kuhn et al (21), by using dimethyl formamide as a solvent.

In this way oligosaccharides can be methylated directly without any preliminary treatment with dimethyl sulphate and sodium hydroxide. A further modification has been the introduction of barium oxide for silver oxide (22).

Some success in the methylation of polysaccharides has been achieved by workers using a method developed by Fear and Menzies (23) which involves reaction of thallous complexes of polysaccharides with methyl iodide. Another method favoured by many makes use of liquid ammonia, by dissolving the potassium derivative of the polysaccharide in it, then adding methyl iodide (24).

Complete methylation, though sometimes difficult, is very important, since undermethylation can give rise to, on hydrolysis, methyl ethers which might incorrectly be assumed to have structural significance. The same danger follows from any partial demethylation during hydrolysis.

Hydrolysis of the methylated polysaccharide can be carried out by any of the following methods; methanolysis followed by hydrolysis of the methyl glycosides; formolysis followed by hydrolysis of the formate esters; or prehydrolysis in fairly concentrated sulphuric acid followed by dilution and warming.

The methylated sugars resulting from hydrolysis are usually separated on cellulose columns (25), but charcoal columns (26) have also been used.

In 1958 Bishop and Cooper (27), and more recently Aspinall et al, (28) successfully separated the methyl glycosides of methylated sugars by gas-liquid chromatography. Using argon as the vapour phase, and various liquid phases, the resolution of many mixtures of methylated sugar glycosides has been achieved.

Partial Hydrolysis

Acid hydrolysis under various conditions is used to isolate fragments of the molecule whose structures may be completely determined, thus providing information, both about the main structural features and about minor details of the parent polysaccharide.

The various types of linkage present in polysaccharides are hydrolysed at different rates. Furanoside linkages are the first to break, being destroyed by .01N-sulphuric acid at 100° for a few hours. Pyranoside linkages tend to split using 0.1N-acid, while uronic acid glycosidic linkages are very resistant, and in the harsh conditions required for their hydrolysis the sugars may decompose. By utilising these facts it is possible to a limited extent to preferentially hydrolyse the different linkages present.

Acid hydrolysis is a reversible reaction (29) and if concentrated solutions are used there may be formed oligosaccharides which are not characteristic of the

polysaccharide hydrolysed. However in solutions of less than 1% this is unimportant. It has also been observed that on more prolonged heating true fragments hydrolyse to monosaccharides whereas reversion products do not.

Products of partial acid hydrolysis are generally separated on charcoal columns using either step-wise or gradient elution, but Wolfrom et al (25) have developed the use of calcium acid silicate, while Jones and Wall (32,31) have shown how ion-exchange resin columns can be used to separate some sugars.

Aldobiouronic acids are easily isolated from the hydrolysates of acidic polysaccharides by ion-exchange chromatography, and their structure can then be determined by reducing the methyl ester methyl glycoside and applying standard techniques.

When pure enzyme preparations are available, hydrolysis with them sometimes yields information that acid hydrolysis cannot. Enzymic methods have mainly been confined to the field of starch, but are becoming increasingly important in other polysaccharide fields.

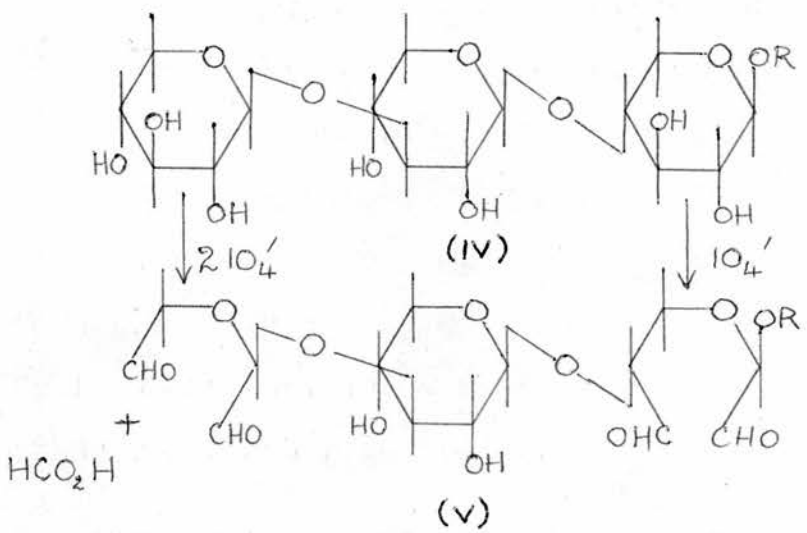
Periodate Oxidation (33)

When hydroxyl groupings are present on adjacent carbon atoms, the carbon-carbon bond is attacked by periodic acid or its salts.

The most readily oxidised compounds are open chain glycols, followed by cyclic cis-glycols, then cyclic trans-glycols, although when trans-glycols are fixed in unfavourable conformations they are unattacked. The reactivity may also be affected by steric effects of neighbouring groups.

In structural investigations on polysaccharides measurement of periodate consumed, together with the determination of the reaction products and possibly isolation of unattacked residues, can go far towards elucidation of some of the structural features present in the molecule.

Reference to the following hypothetical trisaccharide illustrates the general principles:

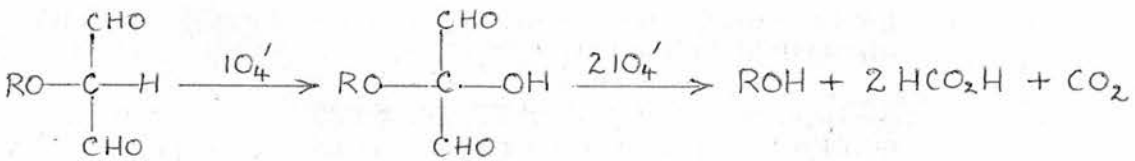


(V)

The residue containing three adjacent hydroxyl groupings reacts with two moles of periodate, liberating one mole of formic acid, whereas the residue containing two adjacent

hydroxyl groupings reacts with only one mole of periodate. The central residue, containing no adjacent hydroxyl groupings, remains unattacked.

Residues containing carbon linked hydrogen atoms activated by two adjacent carbonyl groupings (VI) are sometimes formed as intermediates and can undergo further oxidation thus ;



(VI) (VII)

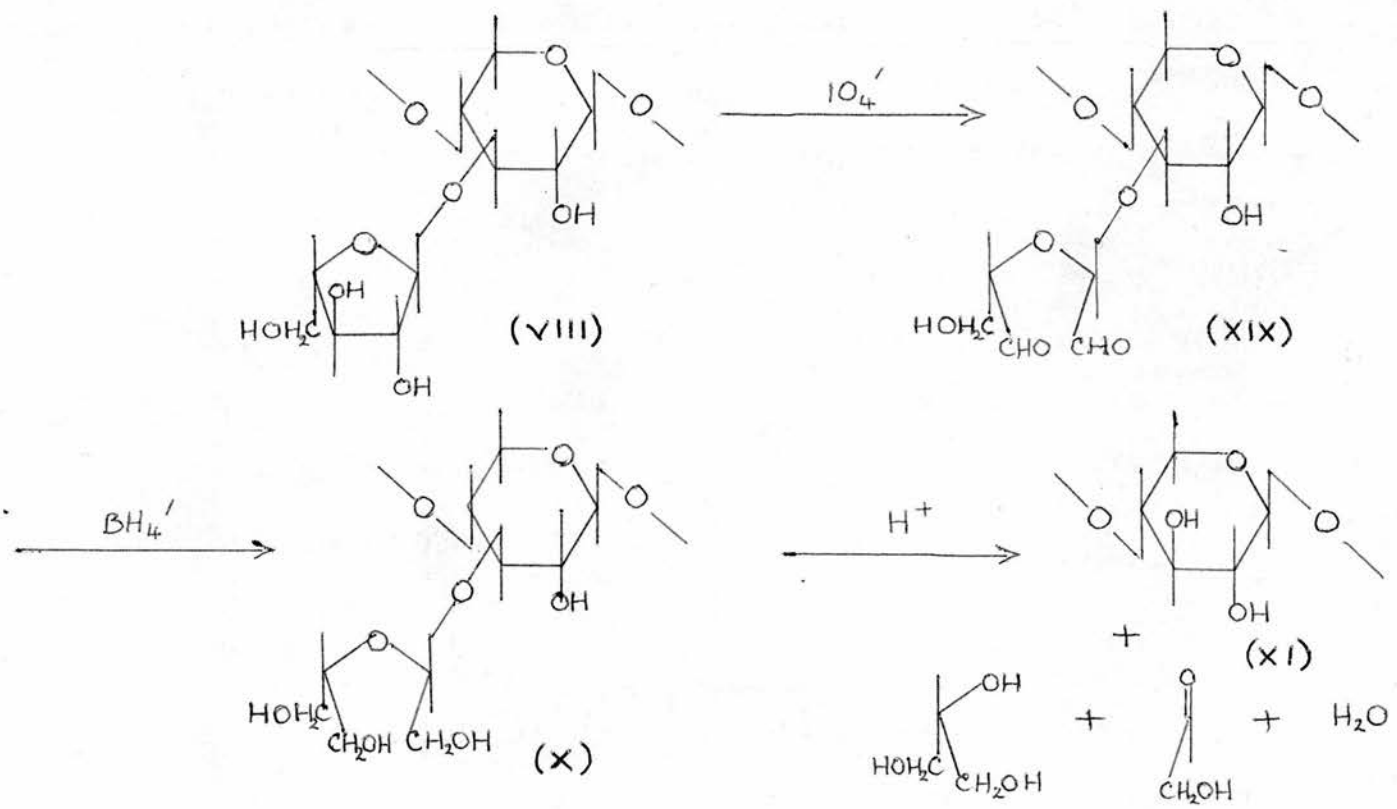
In order to study the arrangement of sugar residues in a polysaccharide the degradation devised by Barry (34) has often been used. It involves oxidation by periodate, followed by treatment with phenylhydrazine in dilute acetic acid, to give a compound containing one phenylhydrazine residue for each pair of aldehydic groupings formed. When this is treated with acetic acid and excess phenylhydrazine, the oxidised residues split off as phenylosazones. Identification of these, together with an investigation of the unoxidised residues remaining, yields information about the original structure.

Smith Degradation (35)

This is in many ways an improvement on the Barry

degradation, and may ultimately replace it as a means of degrading a polysaccharide to one of simpler structure, which can then be more easily investigated.

Residues in the polysaccharide (Vlll) containing glycols are attacked by periodate producing a polyaldehyde (lX), which is fairly resistant to acid hydrolysis. However, reduction of the aldehydic groups with borohydride gives a polyalcohol (X), the acetal linkages of which are very readily split by cold dilute acid, leaving the glycosidic linkages intact (Xl).

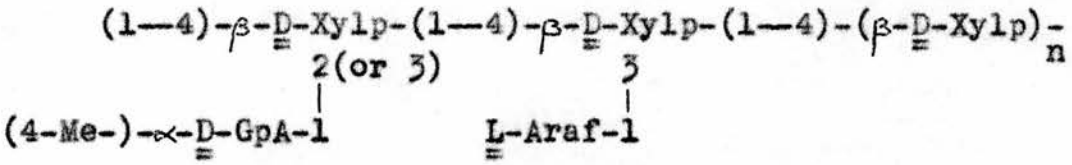


When the degradation is applied to a polysaccharide containing both periodate resistant and periodate labile residues in the main chain, the polymer is brocken up with production of relatively low molecular weight units.

A newer reagent for glycol cleavage is lead tetra-acetate (36) but this has not been extensively used as yet.

Molecular Structure of Xylans. (37)

All xylans from land plants belong to the same general type (XII) but differ from one another mainly in the proportions and types of side chains they possess:

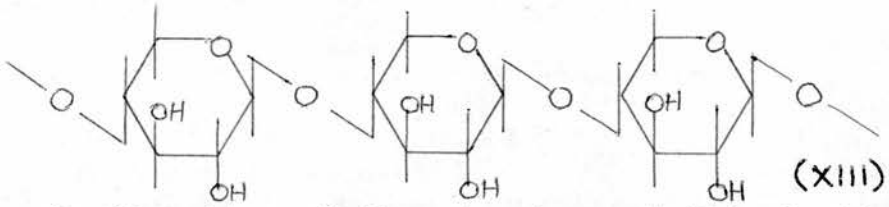


(XII)

Xylans from the Gramineae generally possess L-arabinofuranose residues linked to the backbone as single unit side chains, usually to position 3 of D-xylose, as their main characteristic, but in many cases a smaller proportion of D-glucuronic acid or its 4-O-methyl ether is also present, usually linked to position 2 of D-xylose.

That the backbone in xylans consists of 1,4 linked -D-xylopyranose residues has been shown by the isolation of 2,3-di-O-methyl-D-xylose as a major product of hydrolysis of

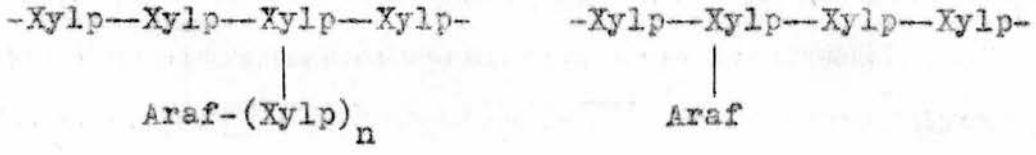
methylated xylans from many sources. This sugar could also arise from 1,5 linked residues, but Haworth and Percival (38) as long ago as 1931 proved that in esparto grass xylan at least, the xylose residues were in the pyranoid form. From a comparison of optical rotation and rate of hydrolysis of other xylans with this xylan, it appears probable that they all possess xylopyranose residues (XIII);



(XIII)

Confirmation of the structure of this backbone chain was obtained from the partial hydrolysis work of Whistler and Tu (39) who isolated from a maize cob xylan a series of oligosaccharides containing 1,4-linked β-D-xylopyranose residues ranging from xylobiose up to xyloheptaose. The lower members of this range have since been isolated from various other xylans.

In 1934 Haworth, Hirst, and Oliver (40) isolated from the hydrolysate of methylated esparto grass xylan 2,3,5-tri-O-methyl-L-arabinose and concluded that the arabinose was in the furanose form and terminated side chains (XIV), or was directly attached to the xylan backbone (XV):

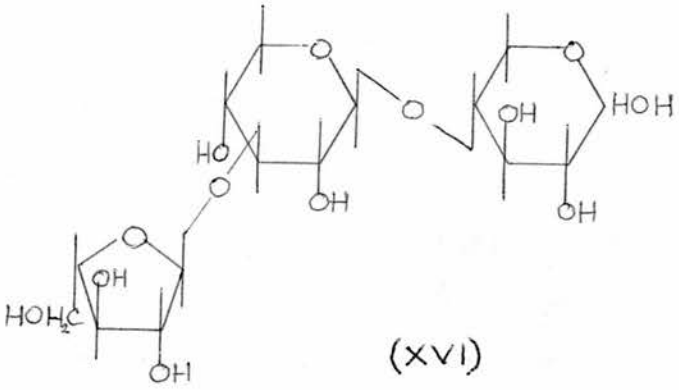


(XIV) (XV)

Using periodate oxidation techniques on a xylan from wheat flour, Perlin (41) was able to show that structure (XV) was probably correct, and Aspinall et al (42,43,44) were able to agree with this view as a result of their investigations on xylans from barley and rye.

It was long suspected from methylation and hydrolysis studies that the non-reducing arabinose side chains were attached through position 3 of the xylose residues, since 2-O-methyl-D-xylose was generally, although not always, the only monomethyl ether of xylose found. Direct proof of this has recently been obtained in certain cases by enzymic hydrolysis (45) and by catalytic oxidation (46) results.

Partial acid hydrolysis splits the arabinofuranose linkages, but enzyme preparations have been found which attack xylans giving arabinose containing oligosaccharides. Bishop and Whitaker (45) have isolated from wheat straw xylan a series of oligosaccharides containing arabinose and xylose residues as partial hydrolysis products, using an enzyme preparation from the mould Myrothecium verrucaria, and one of the components was shown to be the trisaccharide O-L-arabino-furanosyl-(1—3)-O-β-D-xylopyranosyl-(1—4)-D-xylose (XVI). This trisaccharide has since been isolated in a similar way by Aspinall and coworkers (47) from rye flour and cocksfoot grass xylans.

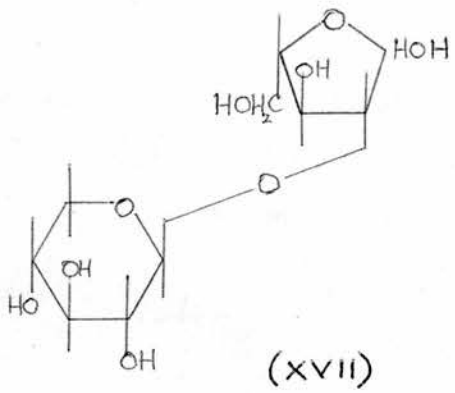


(XVI)

Aspinall and Cairncross (46), using rye flour xylan, have shown that catalytic oxidation results in selective oxidation of some of the primary alcoholic groupings, with the formation of carboxylic acid groupings. Graded hydrolysis of the oxidised polysaccharide afforded the aldobiouronic acid, (L-arabinofuranosyluronic acid)-(1→3)-D-xylose.

The isola tion of this acid, taken together with the evidence of enzymic degradation, shows that at least some of the terminal L-arabinofuranose residues in xylans are attached through position 3 of D-xylose.

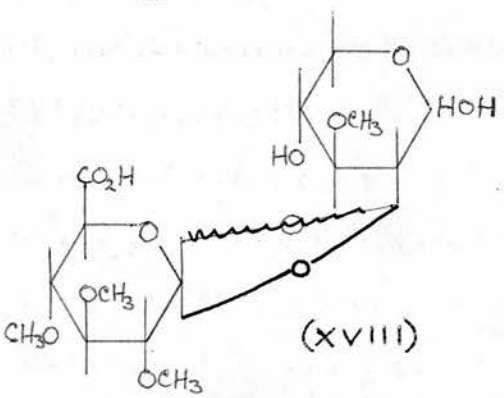
Most of the arabinose residues found in xylans are in the form of terminal groupings, but some have been shown to occupy non-terminal positions in certain cases, by the presence of partially methylated arabinose residues in the hydrolysates of the methylated xylans, and by the isolation of the disaccharide 2-O-β-D-xylopyranosyl-L-arabinose (XVII) on partial hydrolysis (48,43,49).



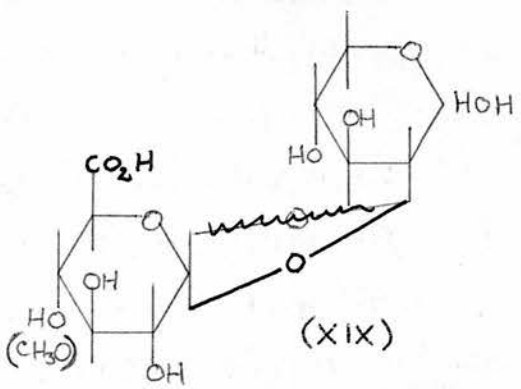
(XVII)

Many xylans from the Graminèae contain residues of D-glucuronic acid, or 4-O-methyl-D-glucuronic acid, as well as L-arabinose residues, although these are almost invariably present in smaller proportion.

Methylation studies usually yield 2-O-(2,3,4-tri-O-methyl-α-D-glucosyluronic acid)-3-O-methyl-D-xylose (XVIII) (50), the structure of which is indicated by conversion to the methyl ester methyl glycoside followed by reduction and hydrolysis to 3-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-glucose.



(XVIII)

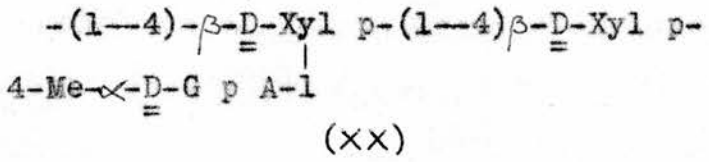


(XIX)

Similarly partial acid hydrolysis of acidic xylans generally yield aldobiouronic acids (XIX) which on reduction,

methylation, and hydrolysis of the methyl ester methyl glycoside give rise to 3,4-di-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-glucose.

From these results it is seen that glucuronic acid or its 4-O-methyl ether is most commonly found linked through position 2 of D-xylose. In all xylans so far examined, hexauronic acid residues have been found to be linked directly to the basal chain (XX):



(XX)

It is not yet known whether the backbone chain in xylans is strictly linear or whether there is some degree of branching, but the failure of anyone to isolate by partial hydrolysis a purely xylose containing di-or trisaccharide with (1—2) or (1—3) linkages points to a widespread lack of branching.

Grass and Straw type Xylans.

Chanda et al (12) have obtained an arabinose free xylan from esparto grass by repeated fractionation by means of the insoluble copper complex. Since an arabinose rich fraction has been isolated from the same source (51), it appears

that there exists in the plant a range of closely related polysaccharides. The presence of some non-terminal arabinose in the arabinose rich xylan is indicated by the isolation from it of the disaccharide (XVll) on partial hydrolysis (48).

In studies on perennial rye grass roots xylan using methylation techniques, the isolation of tetramethyl-D-galactose (52) suggests the presence of some galactose in terminal positions.

Xylans from wheat straw are among the most studied and some interesting variations in fine structure have been observed. By repeated fractionation, one xylan has been obtained free of arabinose but still containing a small amount of uronic acid (53). Another has (1—3) linked uronic acid (54), while another has yielded a small proportion of 2,6-di-O-methyl-D-glucose on methylation and hydrolysis (55). The trisaccharide (XVl) has been obtained by enzymic degradation of a wheat straw xylan (45).

Xylans from cocksfoot grass (50), wheat leaf (56), and oat straw (57) have been found to conform to the general pattern of the typical xylan.

Husk type xylans

Xylans extracted from husks show a somewhat greater complexity than those from the endosperm, barley husks

probably being the simplest (43) although even here 1,2-linked arabinose is found.

Partial hydrolysis of the complex impure xylan from maize hulls (or fibre) has produced some surprising oligosaccharides. Whistler and Corbett (58) have isolated and characterised 3-O-α-D-xylopyranosyl-L-arabinose (XXI) and O-L-galactopyranosyl-(1—4)-O-D-xylopyranosyl-(1—2)-L-arabinose (XXII), while Smith et al (59,60) have characterised 5-O-β-D-galactopyranosyl-L-arabinose (XXIII) and 4-O-β-D-galactopyranosyl-D-xylose (XXIV).

α-D-Xylp-(1—3)-L-Ara (XXI)

L-Galp-(1—4)-D-Xylp-(1—2)-L-Ara (XXII)

β-D-Galp-(1—5)-L-Ara (XXIII)

β-D-Galp-(1—4)-D-Xyl (XXIV)

The three oligosaccharides containing reducing arabinose residues are probably attached as side chains to the xylan backbone through arabinofuranosyl linkages.

Corn cob hemicellulose can be separated into two fractions, A and B, differing in their water solubility (61). On partial hydrolysis of the more complex B fraction an aldobiouronic acid (XXV)(62) and an aldotriouronic acid (XXVI)(63) were isolated in addition to the normal type of aldobiouronic acid (XIX):

$$\alpha\text{-}\underline{D}\text{-GpA-}(1\text{--}4)\text{-}\underline{D}\text{-Xyl}$$

(XXV)

$$\alpha\text{-}\underline{D}\text{-GpA-}(1\text{--}4)\text{-}\beta\text{-}\underline{D}\text{-Xylp }(1\text{--}4)\text{-}\underline{D}\text{-Xyl}$$

(XXVI)

This suggests the termination of some xylan chains by uronic acid groupings. Non-terminal arabinose is also present in this xylan (49).

From wheat bran Adams (64) isolated a unique polysaccharide in that it contained a higher proportion of L-arabinose than D-xylose. The L-arabinose was present as non-reducing end-groupings and in non-terminal positions linked through positions 1 and 3, positions 1,2 and 3, and positions 1,2, and 5(or 4).

Flour type xylans.

Xylans containing a high proportion of arabinose can be extracted by water from the flour of cereals, particularly wheat (41,65,66) and rye (44,46,47). Although their biological function is not clear, they do not appear to be structural polysaccharides and, together with -glucans with which they are associated, are often called cereal gums. They can usually be separated from -glucans by ammonium sulphate fractionation (14).

In all the examples studied, no uronic acid has been found, and the L-arabinose, which is all present as single

unit side-chains, can sometimes be attached through positions 2 and 3 of the same D-xylose residue in the basal chain.

Biosynthesis of Xylans

Tracer studies on the biosynthesis of xylans (67,68,69) using labelled sugars have shown that the xylose units involved in xylan synthesis are probably derived from glucose by oxidation at C_6 followed by decarboxylation, The conversion is assumed to take place at the monosaccharide level (70), in view of the differences in xylan fine structure and the absence of naturally occurring poly-D-glucosiduronic acids.

Neish and Altermatt (68) have proposed a hypothetical scheme for polysaccharide synthesis, suggesting that the polysaccharides are built up by transglycosylation reactions involving uridine disphosphate (U.D.P.) glycoside intermediates, which are interconvertible.

Since, however, arabinoxylans contain D-xylose and L-arabinose residues in pyranose and furanose forms respectively, simple conversion of a derivative of the one pentose to a derivative of the other seems doubtful.

It is possible that all xylans are not built up by the same process, and as yet their biosynthesis is not well understood.

Molecular size of xylans.

Few measurements of molecular weights have been carried out in the xylan field, partly due to the experimental difficulty encountered. Most determinations have been made by the isothermal distillation method on methylated or acetylated derivatives in benzene. The degree of polymerisation of various xylans has been found to be between 50 and 100 by this method.

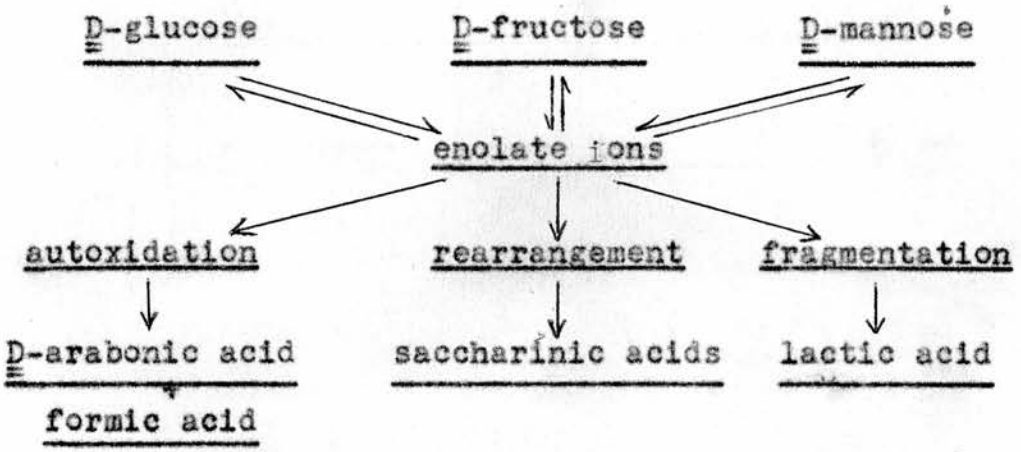
Methods of determining the size and shape of polysaccharides has been reviewed by Greenwood (71) and by Glandemans and Timell (72).

Alkaline Degradation of Carbohydrates.

The effect of alkali on carbohydrates has been subjected to study by chemists for well over a hundred years. It is only recently however, that the degradative effect of alkali on polysaccharides has been vigorously investigated. In the particular case of xylans, the importance of this lies in the fact that most xylans are extracted by alkali, thus raising the possibility of structural modification before investigation of their structure begins.

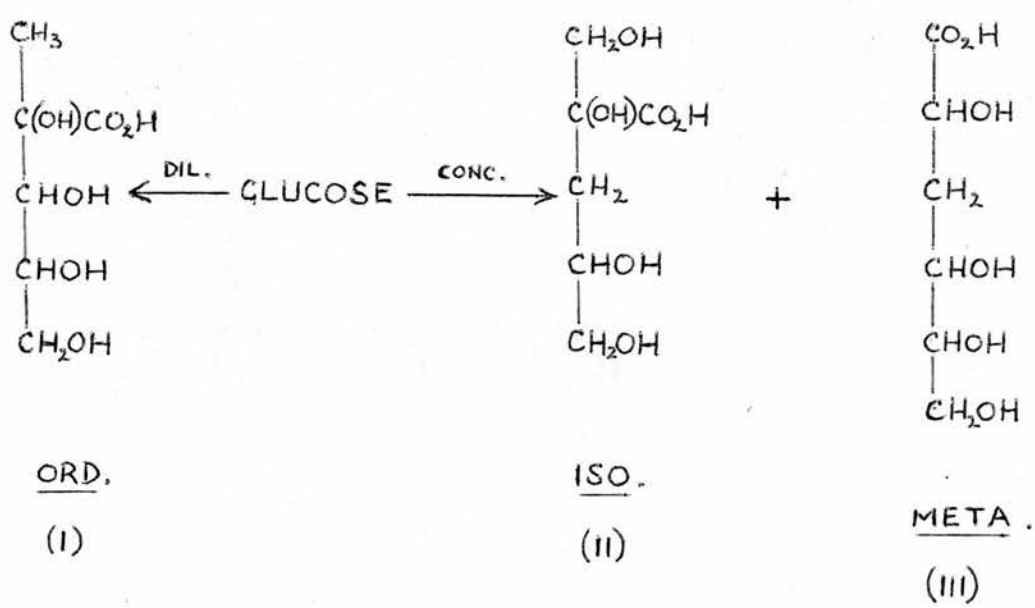
With regard to polysaccharides in general it has been suggested that their reaction with alkali may find some use as a tool in structural investigations by identification of the acids produced. Indeed the structure of many carbohydrates ultimately established by methylation analysis could have been determined much earlier by the use of alkali had its effect been understood.

Oxygen free conditions are generally observed in all studies of alkaline solutions on carbohydrates as the presence of oxygen introduces many more degradative pathways (73), some indication of which is obtained from the Lobry de Bruyn van Ekenstein interconversion and sugar degradations (74):



In the absence of oxygen, rearrangement of the sugars to saccharinic acids is the predominant reaction, but even so, formation of these acids is normally accompanied by some production of carboxylic acids with fewer carbon atoms, by some process involving fragmentation of the sugar molecule.

Thus when glucose is degraded under oxygen free conditions, mainly ordinary saccharinic acid (I) is formed with dilute alkali, and a mixture of the iso (II) and metasaccharinic (III) acids with strong alkali (75):



The three pairs of stercoisomeric acids are also isomeric with the parent monosaccharide, and readily lactonise to form saccharins.

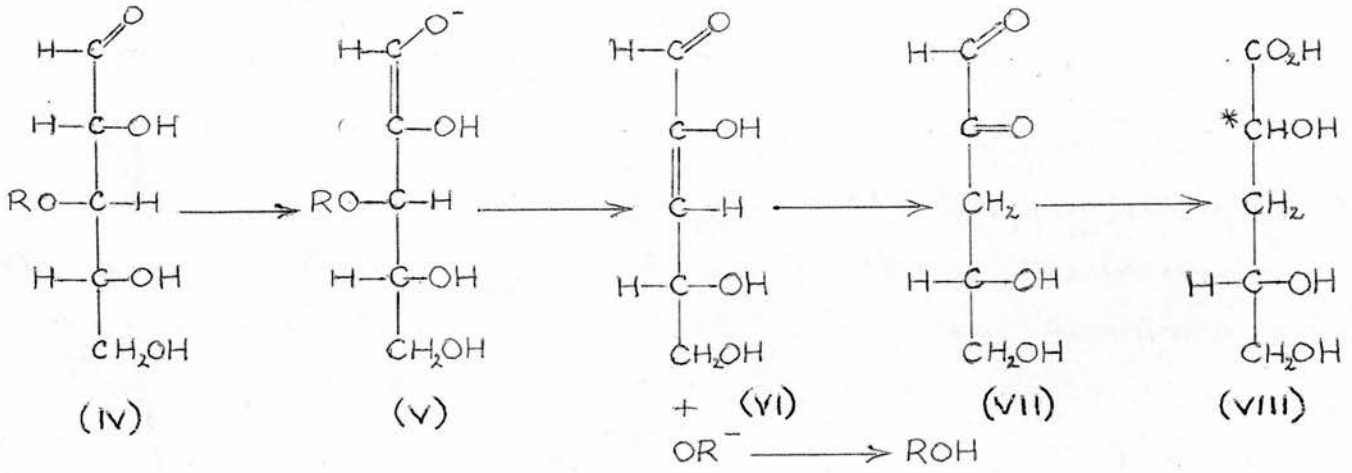
The mechanism of alkaline degradation of polysaccharides has become much clearer now that the effect of alkali on certain monosaccharides and substituted monosaccharides has been studied.

Kenner and co-workers have examined the action of lime-water on a whole range of reducing disaccharides and substituted monosaccharides. They found that D-glucometasaccharinic acid was formed as the principle product from the action of lime-water on the following 3-substituted hexoses: 3-O-methyl glucose, 3-O-methyl fructose (76), laminaribiose, turanose (77), and 3,6-anhydro-D-glucose (78). The corresponding D-glucoisosaccharinic acid was obtained on the degradation of lactose (79), cellobiose, cellotetraose, cellobiulose (80), lactulose, maltose, maltulose (81), 4-O-methyl glucose, and 4-O-methyl fructose (82).

Thus in the degradation of substituted monosaccharides, the formation of one type of saccharinic acid predominates, the nature of which is dependent upon the point of attachment of the substituent grouping. Kenner explained the mechanism of degradation on the basis of the elimination of an alkoxide ion from a β-alkoxyketone (83), a type of mechanism first

suggested by Isbell (84), and which has now received general acceptance. The mechanisms in the pentose series can be represented as follows:

Degradation of 3-substituted pentoses. FIGURE 2



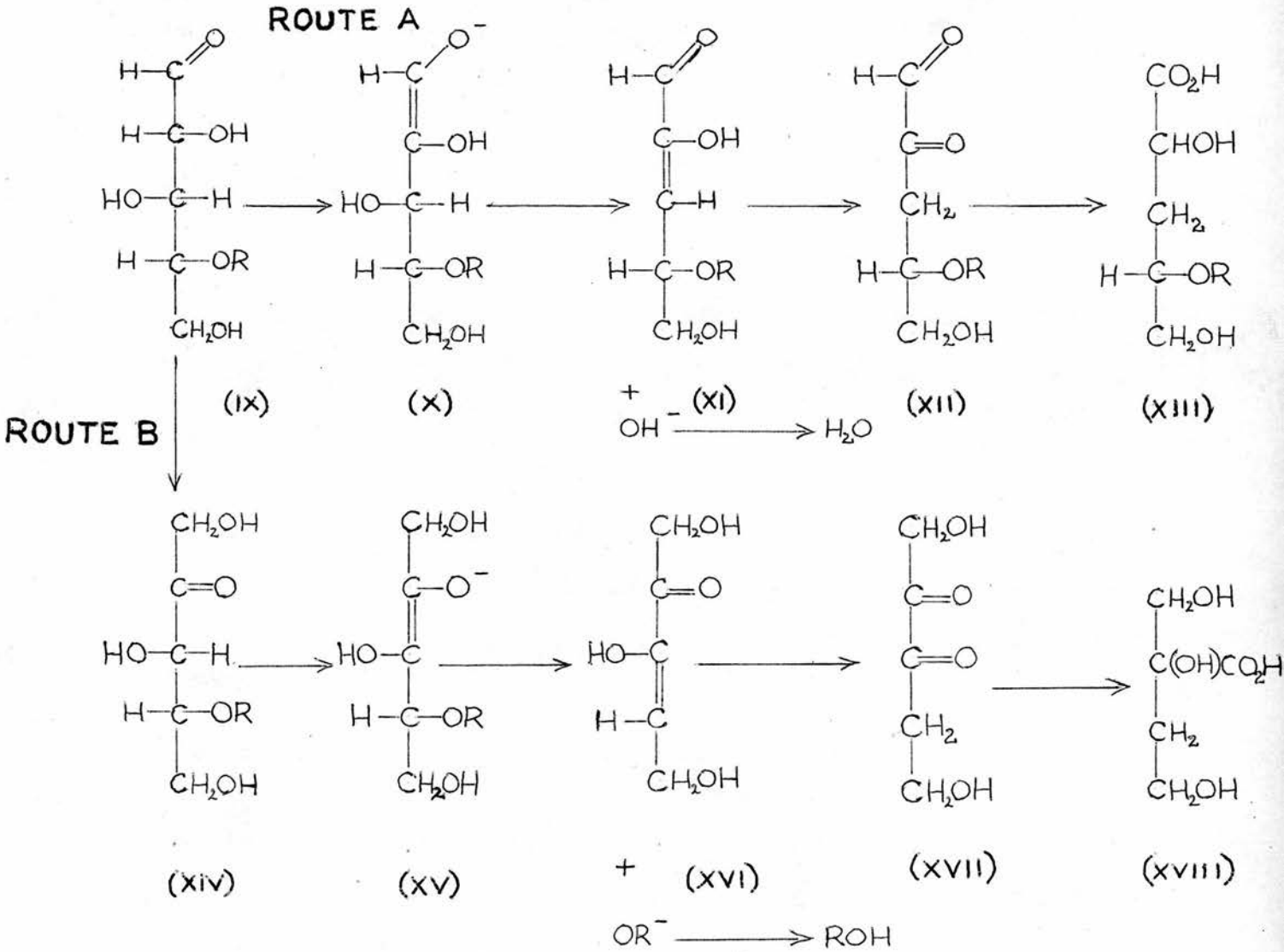
eg. 3-O-methyl xylose ⟶ α-and β-xylometasaccharinic acid + methanol.

3-substituted monosaccharides (IV) are particularly labile to alkali. The eliminated alkoxide ion picks up a proton to give the alcohol ROH. After elimination the α-diketone (VII) is assumed to undergo a benzilic acid type rearrangement with the formation of a pair of stereoisomeric acids (VIII).

In the case of 4-substituted sugars, two modes of degradation resulting in the formation of saccharinic acids

are possible, with one being preferred to the other:

Degradation of 4-substituted pentoses. FIGURE 3



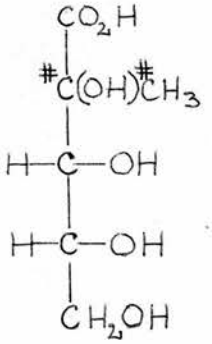
eg. 4-O-methyl xylose → α-and β-isosaccharinic acids + methanol

Kenner et al believe that pathway B is preffered to pathway A because of the greater receptivity of the aqueous alkali for an alkoxide ion than for a hydroxyl ion. They found that when methanol was used instead of water for the reaction medium, the rate of reaction was greatly retarded (85). That pathway A can be followed however, has been demonstrated by Richards et al (86) by their degradation of a 1,4-linked glucose polymer to an alkali stable polysaccharide.

Acetal linkages of 1-substituted monosaccharides have been found to be stable to dilute alkali under oxygen free conditions. Similarly 2-substituted sugars are also stable, since they are unable to form the necessary carbonyl group in the position to the substituted hydroxyl grouping. Whistler and Corbett (87) demonstrated this with 2-O-β-D-xylopyrannsyl-L-arabinose, which they found to be stable in cold dilute alkali.

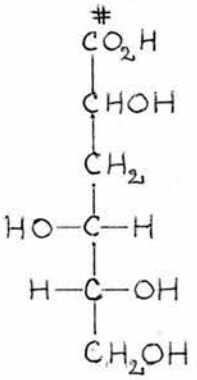
In the case of unsubstituted sugars, saccharinic acid formation involves the expulsion of a hydroxyl group. Accordingly the formation of different acids under different conditions is due to the expulsion of different hydroxyl groups from the several charged ionic species. Generally ordinary saccharinic acid is formed in dilute alkali, and a mixture of the iso-and meta-acids in more concentrated alkali.

That the degradative pathways can be more complicated than indicated by the β-alkoxyketone elimination mechanism has been shown by radioactive tracer studies on some sugars. Sowden and Keunne (88) found that on degrading D-mannose-C_1^{14}, the D-glucosaccharinic acid (XIX) produced contained its radioactivity both in C_2 and in the carbon of the methyl group attached to C_2. This is contrary to the elimination mechanism described, which predicts the appearance of the radioactivity entirely at the methyl carbon atom.



(XIX)

$C^{\#} = C^{14}$



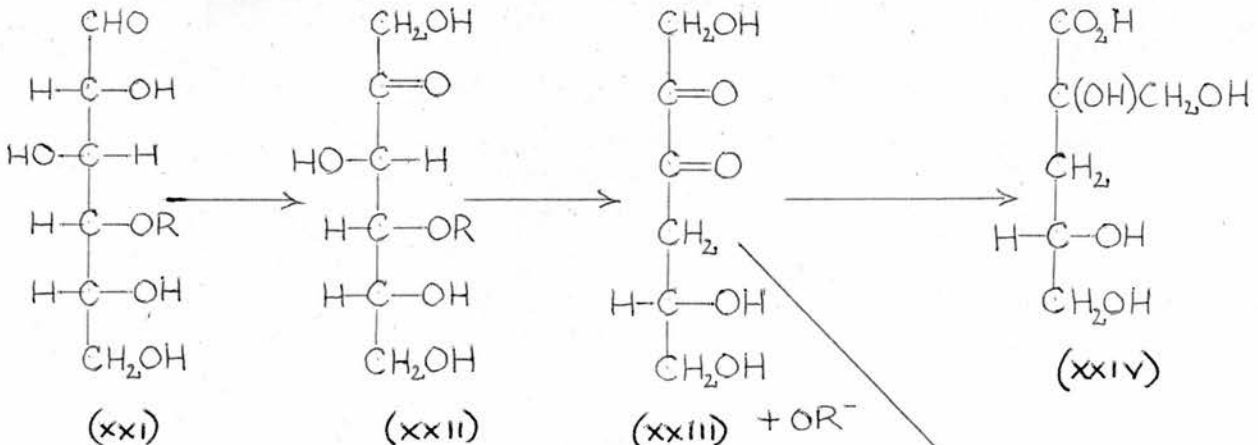
(XX)

On degrading D-galactose-C_1^{14} however, the metasaccharinic acid (XX) produced contained 95% of the radioactivity originally present, in the carboxyl group which is consistant with the mechanism outlined. To explain the unexpected result using D-mannose-C_1^{14} Sowden and Keunne raised the possibility of intermolecular recombination of fission fragments.

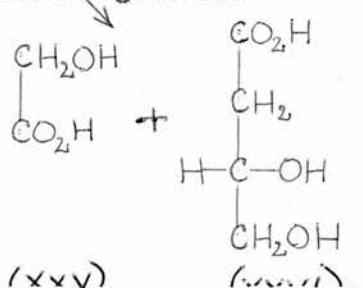
The first evidence that different bases may produce

different mechanisms came from further tracer studies by Sowden and co-workers (89). The isomerisation of D-glycerose-C_1^{14} by dilute soduim hydroxide led to lactic acid labelled almost equally and only in C_1 and C_3. In contrast, saturated lime water converted D-glycerose-C_1^{14} to lactic acid with significant amounts of label in all three carbon atoms. The authors believe the mechanism can be interpreted in terms of an α-dicarbonyl intermediate followed by two different types of rearrangement to saccharinic acids.

A much fuller investigation of the effect of different bases in the degradation of sugars was carried out by Machell and Richards (90). They found that in the degradation of the 4-substituted glucose derivatives, 4-O-methyl-D-glucose, maltose, amylose, and cellulose, calcium hydroxide favoured the formation of D-glucosaccharinic acid whereas in sodium hydroxide solution fragmentation predominated. They believe the reactions can be represented by the following scheme:



R=methyl group, glucosyl residue, or 1,4 linked glucan.



(xxv)

The dicarbonyl compound (XXlll) was isolated in small yield from the degradation products of maltose and shown to be 4-deoxy-3-oxo-D-fructose. Upon treatment with lime-water this dicarbonyl intermediate rearranged to D-glucosaccharinic acid in 90% yield, but on treatment with sodium hydroxide considerable fragmentation to glycollic, βγ-dihydroxybutyric, and formic acids occurred.

Acids (yields as percentage of total acid) formed in alkaline degradation of maltose and cellulose are shown in Table 11 below:

Conditions	Substrate	Formic (%)	D-glucoiso-saccharinic (%)	Glycollic (%)	β-hydroxy-γ-butyro-lactone ≠
.04N-lime water at25°	maltose	2	89		
	cellulose	5	88		
.05N-NaOH at 25°	maltose	11	13	23	33
	cellulose	7	20	22	25

≠ Corresponding free acid not determined.

Polysaccharides.

Studies on the alkaline degradation of polysaccharides has been mainly carried out using glucose polymers due to the economic importance of cellulose and starch. The general method of investigation has been to study the action of alkali on suitably chosen model compounds. As already

pointed out 4-substituted sugars give rise to largely the isosaccharinic acid while 3-substituted sugars give rise to largely the metasaccharinic acid.

Kenner and his co-workers have degraded 1,4-linked compounds such as cellobuilose, cellobiose, and cellotetraose using lime-water (80). Complete degradation to the isosaccharinic acid was found to take place, and the following scheme represents the step-wise degradation of cellotetraose:

$$G—G—G—G \longrightarrow G—G—G—Fru \longrightarrow G—G—G + S \longrightarrow$$

$$G—G—Fru + S \longrightarrow G—G + 2S \longrightarrow G—Fru + 2S \longrightarrow G + 3S \longrightarrow A + 3S$$

G=glucose; Fru=fructose; S=isosaccharinic acid;
A=acids from the degradation of glucose.

Glucose, fructose, cellobiose and cellobiulose were detected in the degradation products; thus starting from the reducing end of the molecule, degradation is seen to proceed along the chain by a "peeling" mechanism which splits each glycosidic link in turn exposing a new reducing end-group to attack. This lends support to the original suggestion by Davidson (91) that attack by alkali occurs only at the reducing end of the cellulose molecule.

It has since been shown that when the reducing groups of cellulose are blocked (92), or modified by reduction (93)

or oxidation (94), no degradation takes place in dilute alkaline solutions. Lindberg (95) however has shown that under drastic conditions, glycosides can degrade.

The degradation of cotton hydrocellulose by hot dilute sodium hydroxide has been studied in some detail by Machell, Richards and Sephton (96,97). They found that D-glucoisosaccharinic acid was a major degradation product, as would be expected from the work of Kenner et al, but that a complex mixture of other acids was also formed. These workers were also able to show that degradation of the polymer chain by no means goes to completion, and that ultimately an alkali stable polysaccharide is formed. Isolation of the alkali stable polysaccharide, followed by partial hydrolysis, yielded D-glucometasaccharinic acids, and it was concluded that these acids originated by alkaline rearrangement of the reducing end-group of the cellulose molecule, thus imparting stability. When alkali stable hydrocellulose, from which most of the alkali-stable groups had been removed by hydrolysis, was again treated with alkali at 100°, it suffered the same extent of degradation as the original hydrocellulose.

It is now clear that in 1,4-linked polysaccharide degradation there are two types of competing reaction occurring, which may be referred to as "peeling" and "stopping" reactions.

The "stopping" reaction (compare pathway A figure 3.) occurs at a much slower overall rate than the "peeling" reaction, (compare pathway B figure 3.) and so there is considerable degradation of any given molecule before the "stopping" reaction renders it stable.

Xylans

It would be expected from the work of Kenner et al that 1,4-linked xylo-oligosaccharides would be degraded by alkali with the formation of xyloisosaccharinic acid. That this is in fact the case has been shown by Aspinall et al (98) and Whistler and Corbett (99) by their isolation of xyloisosaccharinolactone as the main product from the degradation of xylobiose and xylotriose. The structure of this lactone was proved by synthesis. Small amounts of three other lactones were also detected and probably arose from the degradation of the liberated xylose. Sodium hydroxide was used to simulate the conditions used in xylan extraction.

$$Xly\text{–}Xly\text{–}Xly \longrightarrow Xly\text{–}Xly + S \longrightarrow Xly + 2S \longrightarrow A + 2S$$

Xly=xylopyranosyl residues.

S =xyloisosaccharinic acids

A =acids from degradation of xylose.

These results indicate a "peeling" type reaction as already demonstrated in the hexose series by Kenner et al (80). Each successive reducing sugar residue gives rise to acidic products with the simultaneous exposure of a new reducing group. Since appreciable amounts of non-lactonisable acids were also formed it was clear that other reactions were also taking place (98).

The sodium hydroxide degradation of 4-O-methyl-D-xylose has since been investigated (100), and again D-xyloisosaccharinolactone was produced as the main product, with formic acid as the only non-lactonisable acid found. It would therefore appear that in the degradation of these model compounds with sodium hydroxide the fragmentation reaction is not so prominant as Machell and Richards found in the hexose series using maltose (see Table 11)(90).

Aspinall et al (100) have recently studied the effect of sodium hydroxide on some xylans, particularly a water extracted rye flour xylan, by analysis of the acids formed, and by number average molecular weight determinations on the xylans and alkali degraded xylans. They have succeeded in confirming the following points:

a) Alkaline degradation proceeds only from the reducing end of the molecule.

When borohydride reduced rye flour xylan was treated

with sodium hydroxide no free acids were produced. Number average molecular weight determinations on the polysaccharide, before and after treatment with alkali, were almost identical showing that no random cleavages of glycosidic linkages had taken place. (See Table 111).

b) Alkaline degradation of xylans causes about 20% reduction in molecular weight.

Table 111

Xylan	Derivative	Molecular weight (±500)	Degree of Polymerisation (±3)
Rye flour A	acetate	13,200	64
Rye flour A	methylate	9,100	57
Rye flour B	acetate	15,700	72
Rye flour B	methylate (1)	10,800	67
Rye flour B	methylate (11)	10,400	65
Alkali degraded rye flour B	acetate	12,500	58
Reduced rye flour B	acetate	15,100	70
Alkali degraded reduced rye flour B	acetate	15,100	70

It is clear from the earlier work, both on cellulose and amylose (96,97,101), and on xylo-oligosaccharides (98,99) that degradation proceeds in a step-wise manner with elimination of one xylose residue at a time, and exposure of a new reducing end group (Fig.3,pathway B, where R=1,4-linked xylan). At some point however the "stopping" type reaction takes place with elimination from C_3 instead of C_4 (Fig.3,pathway A, where R=1,4-linked xylan). Using a water extracted rye flour xylan, Aspinall et al found that this appeared to occur after a loss in molecular weight of about 20%. Repeating Machell and Richards work, using the alkali-stable xylan instead of the alkali-stable cellulose, they showed that degradation was probably halted by rearrangement of a reducing group to a metasaccharinic acid residue.

The authors point out that degradation takes place so slowly under the conditions used (1N sodium hydroxide under oxygen free conditions at room temperature) that it is probable that relatively little degradation occurs during the extraction of xylans.

c) Analysis of low molecular weight acids formed during alkaline degradation of xylans show the presence of glycollic, lactic, lactyllactic, and other acids. (See Table 1V).

Table IV.

xylan degraded	isoS	metaS	glycollic acid	lactic acid	lactyl-lactic acid	other acids
Rye flour B	++	tr.	++	+++	++	++
Reduced rye flour B	-	-	-	-	-	-
Barley Husks	++	tr.	++	+++	+	++
Oat straw A	++	tr.	++	+++	+	++
Oat straw B	++	tr.	++	++	+	++

isoS.=xylo*iso*saccharinolactone.

metaS.=xylo*meta*saccharinolactones.

Although the *iso*saccharinolactone was present in significant amounts from all the xylans treated, the presence of these other acids in fair yield indicated that other reactions were taking place, possibly involving some form of fragmentation of an intermediate similar to that described for maltose (Fig.4).

Whistler and BeMiller (102) have put forward the theory that in the degradation of a 1,4-linked polymer, branches on C_3 would be liberated from the main chain and the main chain would be terminated by a stable *meta*saccharinate end-unit derived from that glycose unit in the main chain to which the branch is attached. The main chain would then be stable

to alkali but branches would degrade further, depending on the type of linkages present.

This means that in a typical xylan with its single unit arabinose side chains attached to C_3, alkaline erosion would terminate at the first branch point reached. In view of the results obtained by Aspinall et al (100)(see Tables 111 and 1V) with rye flour xylan, this appears to be unlikely, since this xylan has approximately one arabinose residue to every two xylose residues. The exception would be if all the arabinose side chains were remote from the reducing end of the molecule, but this again is unlikely.

Further indication that it may be possible for degradation to bypass a branch point at C_3 is obtained from a comparison of the amounts of lactic acid produced from different sources. This acid was undetected in the degradation of the model compounds 4-O-methyl-D-xylose, xylobiose, and xylotriose, and an inference is that its production is associated with the liberation of arabinose side chains, particularly since more of it was produced from degradation of the highly branched rye flour xylan then from the xylans containing less arabinose. Nef (75) as long ago as 1910 found that lactic acid could be formed from arabinose on treatment with alkali. It is conceivable that lactic acid could arise both from degradation of any liberated arabinose, and from fragmentation of some

intermediate.

Introduction to the Present Work.

Although the main structural features of xylans from the Gramineae are now well established, some aspects of their fine structure still require investigation. Section I of this work is concerned with an investigation of a variety of xylans using methylation techniques, and the fine structure of a perennial rye grass roots xylan is further investigated by partial acid hydrolysis.

Some aspects of branching in the xylan molecule have never been satisfactorily elucidated, partially due to a lack of sufficiently accurate experimental techniques. In Section II the Smith degradation is applied to barley husk and rye flour xylans in an attempt to solve some of these problems.

The work on the alkaline degradation of xylans started by Aspinall et al (100) is continued in Section III of this work, by the synthesis and degradation of model compounds.

A part of this work is published in the Journal of the Chemical Society, September, 1961.

General Procedures.

Paper Chromatography

Qualitative and quantitative chromatograms were run on Whatman No.1 paper. For quantitative sugar estimations two side strips were drawn on the chromatogram. After irrigation and drying of the paper, these strips were cut off and developed with a suitable spray to show the positions of the sugar bands on the main chromatogram. The bands were then cut out, the sugars eluted with water and estimated by some method of microdetermination.

Large scale separations were carried out on Whatman No.3MM filter sheets which had previously been extracted with methanol in a soxhlet apparatus.

Chromatography solvent systems (V/V)

(A) Ethyl acetate : pyridine : water (10:4:3).
(B) Ethyl acetate : acetic acid : water (3:1:3, upper layer).
(C) Ethyl acetate : acetic acid : formic acid : water (18:3:1:4).
(D) Butan-1-ol : ethanol : Water (4:1:5, upper layer).
(E) Butan-1-ol : acetic acid : water (4:1:5, upper layer).
(F) Benzene : butanol : pyridine : water (1:5:3:3, upper layer).

The R_F value of a sugar refers to its rate of movement relative to the solvent front and the R_{xylose} value refers to

the rate of movement of the sugar relative to xylose.

Chromatographic spray reagents.

(A) Aniline oxalate (reducing sugars)

Unless otherwise stated chromatograms were sprayed with a saturated aqueous solution of aniline oxalate and developed at 120-140° for 2-3 minutes.

(B) Silver nitrate (reducing and non-reducing sugars)

This reagent was used to reveal non-reducing sugars, sugar glycosides, and sugar alcohols. The dried chromatograms were dipped in the silver nitrate reagent (saturated aqueous silver nitrate solution (1ml.) added to acetone (20ml.)), dried, and then dipped into ethanolic sodium hydroxide solution (1 pellet of sodium hydroxide dissolved in water (0.5ml.) and diluted with ethanol (25ml.)). The chromatograms were finally washed with aqueous sodium thiosulphate solution (10%), then with water, and dried.

Sugars showed up as grey to black spots, with reducing sugars appearing immediately.

(C) Hydroxylamine-ferric chloride (103) (esters and lactones)

Chromatograms were sprayed with a methanolic solution of hydroxylamine, followed after 10 minutes by a ferric chloride solution (2%) in hydrochloric acid (1%). Lactones and esters gave mauve spots.

(D) Bromothymol blue indicator (acids).

Acidic compounds appeared as yellow spots on a blue-green background on spraying the dried chromatograms with a solution of bromothymol blue (0.1%) in ethanol (80%) adjusted to pH8.

(E) Potassium iodate-potassium iodide-starch. (acids).

Chromatograms were sprayed with an aqueous solution containing potassium iodate (1%), potassium iodide (1%) and starch indicator. Acidic compounds appeared as brown spots.

(F) Potassium permanganate (unsaturated compounds).

Chromatograms were sprayed with an aqueous solution of potassium permanganate (0.5%). Unsaturated compounds showed up immediately as green spots on a purple background.

(G) 2,4-Dinitrophenylhydrazine (carbonyl compounds).

Carbonyl compounds were located as brown spots on a yellow background by spraying the chromatogram with a saturated alcoholic solution of 2,4-dinitrophenylhydrazine.

(H) Triphenyltetrazolium salt (104) (2-O-substituted reducing sugars).

Chromatograms were sprayed with a chloroform solution of the salt (0.5%), dried, and then sprayed with ethanolic sodium hydroxide solution and heated for a few seconds at 100°. Reducing sugars gave red spots but those in which a substituent was adjacent to the reducing group gave little or no colour.

(J) Periodate-permanganate reagent (111)(sugar alcohols)

The chromatograms were sprayed with a mixture of 4 parts 2% aqueous sodium periodate with 1 part 1% potassium permanganate in 2% aqueous sodium carbonate. On standing at room temperature for about 15 minutes, sugars gave yellow spots on a pink background.

Solvents.

Organic solvents were purified and dried as described by Vogel. (105).

Charcoal Columns.

Equal weights of acid washed B.D.H. charcoal and acid washed celite (grade 545) were mixed into a slurry with water and poured into a column plugged at one end with cotton wool. After thoroughly washing with water, the sugar mixture was applied to the top of the column, and separation of the sugars effected by water elution followed by elution with aqueous ethanol containing increasing proportions of alcohol. If the eluate was found to be acidic on concentrating to a small volume, neutralisation was achieved by means of amberlite resin 1R-4B(OH) before final removal of solvent.

Alumina Columns.

Activated alumina (Type H,100/200 S mesh, supplied by Peter Spence and Sons Ltd.) was shaken overnight with N-acetic

acid, washed free of acid by decantation, and dried at 260° for 6 hours. A slurry was prepared by adding light petroleum (60-80°) to the alumina with stirring and this was then poured into a column containing a glass wool plug at the bottom. The sugar mixture was dissolved in the minimum amount of benzene and applied to the top of the column. Displacement of the sugars was carried out by irrigating the column with light petroleum, benzene, ether, ethanol, and various mixtures of these solvents.

Resin Column.

Dowex 50WX3(200-400 mesh, Ba ++.) was used to separate neutral oligosaccharides.

The column was sealed at the bottom with a plug of polyurethane foam held in place by a rubber stopper with a short piece of glass capillary tubing through it. The capillary outlet was attached via a 4-foot length of rubber tubing to a syringe needle. This allowed regulation of the rate of flow by altering the hydraulic head. The resin was washed with N-sulphuric acid, then with a saturated solution of barium chloride, and finally with water until free from chloride ions.

A slurry of the resin in water was poured into the column and backwashed for several hours at a rate sufficient to expand the bed to twice its final dimensions, after which the resin was allowed to settle by gravity. The sugar mixture was

applied to the top of the column and eluted with water.

Paper ionophoresis.(106)

Ionophoretograms were run for 4-5 hours in borate buffer at pH 10 using a potential of 500 volts, dried, and sprayed with aniline oxalate containing glacial acetic acid (5%).

Methoxyl determinations were carried out by the Ziesel semi-micro procedure. (107).

Evaporations were carried out under reduced pressure at 40°.

Optical rotations were observed at 18° ± 2°.

Periodate oxidation of methylated sugars (108)

The methylated sugar (2-3mg.) was treated with sodium metaperiodate (0.5N ; 0.2ml.) at 0° for 1 hour. Ethylene glycol (1 drop) was added, and the solution allowed to come to room temperature. Sufficient sodium hydroxide solution was added to make the solution alkaline to phenolphthalein. The solution was then examined chromatographically using solvent D.

Kuhn methylations. (21)

The oligosaccharide (2-5mg.) was dissolved in dimethyl formamide (0.2-0.5ml.), then shaken with silver oxide (0.2-0.5g.) and methyl iodide (0.2-0.5ml.) at room temperature in

the dark for 18 hours. The mixture was filtered and the residue washed with chloroform. The combined filtrate and washings were dried over anhydrous calcium sulphate and evaporated to dryness.

Methanolyses.

The methylated compound (2-10mg.) was heated in a sealed tube with methanolic hydrogen chloride (3.5%) for 18 hours, the solution neutralised with silver carbonate and filtered, and the solvent removed in vacuo.

Hydrolyses.

Compounds were hydrolysed with N-sulphuric acid, unless otherwise stated. The sample (5mg.) was heated for 6 hours with acid (2ml.), the solution cooled, neutralised with barium carbonate, and filtered. Ions were removed with 1R-120(H) and 1R-4B(OH) resins and the solution evaporated to dryness.

Borohydride reduction of sugars.

The compound was dissolved in water and treated with an excess of potassium borohydride. After standing at room temperature overnight, the solution was deionised with 1R-120(H) resin. Evaporations with methyl alcohol removed borate ions as the volatile methyl borate.

Periodate consumption by sugars.

The compound was dissolved in a two fold excess of aqueous sodium metaperiodate (0.15M.). The concentration of periodate was measured at intervals spectrophotometrically and the values plotted against time. By extrapolation of the straight line portion of the graph, it was possible to measure the number of moles of periodate consumed per mole of compound.

Determination of sugars by phenol-sulphuric acid (109)

To the sample (10-70ug.) in water (2ml.) was added 5% aqueous phenol solution (1ml.) and concentrated sulphuric acid (5ml.). The solution was thoroughly mixed and after 10 minutes placed in a water bath at 25-30° for a further 10-20 minutes. The absorbance of the solution was measured spectrophotometrically and the sugar concentration obtained from a standard graph.

Determination of formaldehyde by chromotropic acid (110)

To the sample (up to 100ug.) in water (0.5-1.0ml.) was added chromotropic acid reagent (10% aqueous solution)(0.5ml.) and concentrated sulphuric acid (5ml.). The solution was heated on a boiling-water bath for 30 minutes, then cooled and made up to 50ml. with water. The transmittance was measured using a spectrometer and the amount of formaldehyde determined from a standard graph.

SECTION 1.

A Comparison of Some Xylans using Methylation and Partial Hydrolysis Techniques.

Discussion.

The xylans studied were obtained from the husks of barley from two different harvests, from the stems and roots of perennial rye grass, and from a sample of Norway spruce wood.

The polysaccharides were subjected to methylation followed by methanolysis, to give methyl glycoside mixtures of partially methylated sugars which were investigated by gas-liquid chromatography (27,28), using argon as the vapour phase and liquid phases of apiezon M, butanediol succinate polyester, and m- bis-(m-phenoxy-phenoxy)-benzene supported on celite. The polyester column was found to give satisfactory resolution of the tri-and dimethyl derivatives of xylose and arabinose methyl glycosides, while the column of apiezon M was useful in detecting compounds with higher retention times, such as methyl glycosides of monomethyl derivatives and methyl ester methyl glycosides of methylated aldobiouronic acids. Unfortunately peaks due to these derivatives of aldobiouronic acids were not characterised due to a lack of the necessary standards. Use of the polyphenol column helped to resolve tetramethyl galactose glycosides from the glycosides of other sugars.

Husks obtained from a 1959 crop of Carlsberg barley were given a preliminary extraction with an organic solvent to remove fats, monosaccharides, colouring matter etc., and then delignified using the acid chlorite method. The holocellulose was extracted with N-alkali in an atmosphere of nitrogen for two periods of 48 hours. Acidification of the extract with acetic acid and the addition of acetone yielded a polysaccharide which was purified by reprecipitation.

The polysaccharide was methylated using standard procedures to give a methylated product having OMe,38.5%. Treatment with methanolic hydrogen chloride yielded a sugar mixture which was shown to contain, by gas-liquid chromatography, the methyl glycosides of the following methylated monosaccharides : 2,3,4 tri-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, 2,3,5-tri-O-methyl-L-arabinose, 3,5-di-O-methyl-L-arabinose and 2-O-methyl-D-xylose. The glycosides of 2,3-di-O-methyl-D-xylose predominated.

A xylan was isolated from the husks of a 1953 crop of Carlsberg barley by Aspinall and Ferrier (43). This xylan had been extracted by alkali without any preliminary delignification of the husks. Methylation of this polysaccharide and investigation of the methanolysis products of the methylated polymer by gas-liquid chromatography showed the presence of the same methyl glycosides as above in apparently the same

ratio. These results are in agreement with the methylation results of Aspinall and Ferrier obtained using the same polysaccharide (43). These workers found on methylation and hydrolysis of the xylan, and separation of the products on a cellulose column, that the same methyl derivatives of xylose and arabinose were present in the following ratios : 2,3,5-tri-O-methyl-L-arabinose (1 part), 2,3,4-tri-O-methyl-D-xylose (2), 3,5-di-O-methyl-L-arabinose (1), 2,3-di-O-methyl-D-xylose (14), 2-O-methyl-D-xylose (3), and 3-O-methyl-2-O-(2,3,4-tri-O-methylglucuronosyl)-D-xylose (1).

Thus these two xylans isolated from barley husks grown in different seasons appear to have essentially the same structure, both containing non-terminal arabinose residues.

Xylans from the roots and stems of perennial rye grass were investigated using the same techniques. The xylan isolated from the stems corresponded to the pattern of the typical xylan, methylation studies revealing 2,3-di-O-methyl-D-xylose as the major derivative with small amounts of 2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose. Since no 3,5-di-O-methyl-L-arabinose methyl glycoside was detected it must be assumed that all the arabinose residues were present as non-reducing terminal units in the furanose form.

In contrast, the xylan isolated from the roots of the plant gave on methylation and methanolysis the glycosides of

3,5-di-O-methyl-L-arabinose, indicating the presence of non-terminal arabinose as in the husk xylans. In addition, peaks characteristic of the methyl glycosides of 2,3,4,6-tetra-O-methyl-D-galactose were obtained, which suggested the termination of some polysaccharide chains by non-reducing galactose residues. Further evidence for the presence of this sugar was obtained by Aspinall and Cairncross, who isolated crystalline 2,3,4,6-tetra-O-methyl-D-galactose during methylation studies on the same xylan (52).

The glycosides of 2,3,4-tri-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, and 2,3,5-tri-O-methyl-L-arabinose were present in the methanolysis products in amounts similar to that of the other xylans studied. A trace of 3,4-di-O-methyl-D-xylose methyl glycoside was also present but its structural significance, if any, is not clear.

A xylan from Norway spruce wood was similarly examined (112).

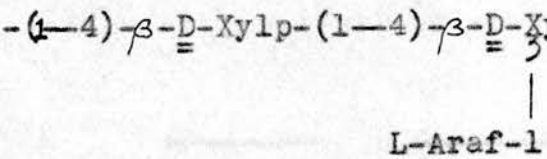
Although not isolated from the Graminae family of plants, it possessed essentially the same features as the other xylans with regard to the glycosides of the tri-and dimethyl sugars found. No 3,5-di-O-methyl-L-arabinose methyl glycoside was detected, and unidentified peaks were obtained which probably arose from derivatives of the relatively large amounts of aldobiouronic acids which characterise xylans from woods.

The following table gives the approximate relative amounts of the methyl glycoside methyl ethers of the sugars obtained from the various methylated xylans.

Table V.

methyl glycoside	barley husks 1959	barley husks 1953	P.R.G. stems	P.R.G. roots	Norway spruce wood
2,3,4-Me Xyl 3	*	+	+	+	+
2,3-Me Xyl 2	+++	+++	+++	+++	+++
2,3,5-Me Ara 3	++	++	++	++	+
3,5-Me Ara 2	+	+		+	
2,3,4,6-Me Gal 4				+	

These xylans therefore conform to the general xylan pattern with regard to their main structural features. They appear to contain the usual backbone of 1,4-linked-β-D-xylose residues, with single unit arabofuranose side-chains (1) :



(I)

It is only when their fine structure is considered that some differences in structure become apparent. That the 3,5-di-O-methyl-L-arabinose derived from barley husk xylan is not in fact an artifact has been proved by Aspinall and Ferrier, by their isolation and characterisation of the disaccharide 2-O-β-D-xylopyranosyl-L-arabinose on partial hydrolysis of their 1953 barley husk xylan (43).

Perennial rye grass roots xylan was examined by similar techniques to those used by Aspinall and Ferrier, and the same disaccharide, 2-O-β-D-xylopyranosyl-L-arabinose, obtained, together with xylo-oligosaccharides and a galactose containing trisaccharide.

The xylan had been extracted from the roots of the plant by Aspinall and Cairncross, using the same procedure as that described for barley husk xylan. Analysis of the polysaccharide showed the presence of the following sugars ; xylose (46.0%), arabinose (13.2%), glucose (8.0%) and galactose (6.4%). The polysaccharide also gave uronic anhydride (9.7%) and ash (11.0%). It proved to be almost insoluble in hot water, but a colloidal solution was obtained by dissolving in sodium hydroxide solution and dialysing.

Treatment of the xylan with 0.5N-sulphuric acid for 30 minutes gave a mixture of mono- and oligosaccharides which were fractionated on a charcoal-celite column. Elution of the column with water and 2% ethanol removed all the xylose, arabinose, glucose, and galactose. Elution with 5% ethanol gave a mixture of two oligosaccharides having R_{xylose} values 0.70, and 0.64 in solvent A. Fractionation on thick filter sheets gave, using solvent A, chromatographically pure samples of each.

The faster moving sugar was obtained crystalline and hydrolysed to xylose and arabinose on treatment with acid. It was chromatographically indistinguishable from an authentic sample of 2-O-β-D-xylopyranosyl-L-arabinose and both samples gave the same X-ray powder photograph. The melting point of the crystals was undepressed on admixture with the authentic sample.

The other sugar obtained from this fraction hydrolysed to xylose alone, and gave a crystalline phenylosazone on treatment with phenylhydrazine whose melting point was identical to that of xylobiose phenylosazone. Its melting point was undepressed on admixture with an authentic sample and the sugar itself was chromatographically indistinguishable from xylobiose, 4-O-β-D-xylopyranosyl-D-xylose.

Elution of the column with 7% ethanol and fractiona-

tion of the sugars obtained on thick filter sheets gave an oligosaccharide, R_{xylose} 0.31 in solvent A, which hydrolysed to xylose alone and appeared to be xylotriose.

From the 10% ethanol eluate a sugar was obtained in trace amount which hydrolysed to galactose, xylose, and arabinose. In an attempt to increase the yield of this trisaccharide the hydrolysis of the xylan was repeated using 0.02N-acid and heating for 5 hours. The products were fractionated on a charcoal-celite column as before and a sugar having the same R_{xylose} value to the above sugar isolated from the 10% ethanol eluate. Hydrolysis gave galactose, xylose, and arabinose, in approximately equal amounts.

Reduction with borohydride followed by hydrolysis and chromatographic examination of the products showed arabinose to be the reducing end of the molecule. The absence of reaction to spray H suggested a substituent in position 2 of the reducing sugar residue. Methylation by Kuhn's (21) method and examination of the methanolysis products by gas-liquid chromatography showed the presence of 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-D-xylose, 3,4-di-O-methyl-L-arabinose, and a possible trace of 3,5-di-O-methyl-L-arabinose, indicating the trisaccharide to have the following structure(11) :

D-Gal-(1—4)-D-Xyl-(1—2)-L-Ara.

(11)

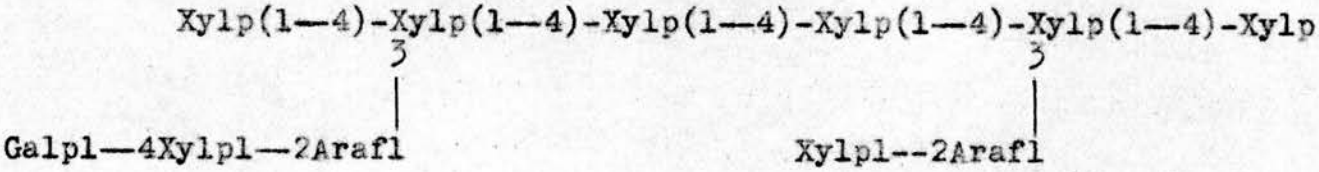
Periodate oxidation studies showed an uptake of 4.6 moles of periodate per mole of sugar, with the formation of 0.8 of a mole of formaldehyde. On the basis of structure (11) being correct, the expected periodate consumption would be 5 moles per mole of sugar, with a corresponding release of one mole of formaldehyde. No periodate resistant residues could be detected on chromatographic examination of the hydrolysis products of the oxidised sugar.

Chromatographic examination of the products of partial hydrolysis showed the presence of the expected monosaccharides together with 2-O-β-D-xylopyranosyl-L-arabinose. Since no spot corresponding to a galactose-xylose containing disaccharide was obtained it seemed likely that the galactose-xylose link was preferentially attacked.

These results are all in agreement with the trisaccharide being D-galactopyranosyl-(1—4)-D-xylopyranosyl-(1—2)-L-arabinose. A similar trisaccharide, but containing L-instead of D-galactose, has been isolated by Whistler and Corbett (58) from maize hulls polysaccharide. 4-O-β-D-Galactopyranosyl--D-xylose and other galactose and arabinose containing disaccharides have also been obtained from the same source (59,60).

It seems probable from previous work on xylans that the xylosylarabinose and galactosylxylosylarabinose units are

attached as side chains, through the arabinose residues in the furanose form, to position 3 of xylose residues in the xylan backbone (111). This view is supported by the absence on partial hydrolysis of higher arabinose or galactose containing oligosaccharides.



(111)

It is not possible to say whether the xylosylarabinose units actually exist as such in the polysaccharide, or whether they are liberated from the galactosylxylosylarabinose units during hydrolysis.

Experimental.

Xylan from Barley Husks (1959).

Carlsberg barley (1959 harvest)(7Kg.) was treated in batches (30g.) for 30 seconds in an automatic polishing machine to remove the husks, which were then separated from powdered barley by sieving (360g.).

Preliminary extraction of the husks.

In order to deactivate enzymes, and remove colouring matter etc., the husks were continuously extracted in a Soxhlet extractor for 24 hours with benzene-ethanol(2:1), and then air dried.

Delignification

The husks (100g.) in water (3.5l.) were heated to 70° and maintained at that temperature for 4 hours. At hourly intervals additions of sodium chlorite (30g.) and glacial acetic acid (10ml.) were made. The delignified husks were washed free of inorganic ions with cold water, and then with water at 70-80° until the washings gave no colouration with iodine. The husks were finally washed with acetone and air dried.

The remainder of the husks were treated in the same way.

Extraction of Xylan.

The holocellulose (200g.batches) was treated for 48 hours with 4% sodium hydroxide (2.5l.) in an atmosphere of nitrogen in a ball-mill, the resultant sludge was centrifuged, the supernatant stored at 0° and the sediment treated with a further quantity of sodium hydroxide (2.5l.) for 48 hours. The second extract was centrifuged, and the combined supernatants acidified to pH5 with glacial acetic acid before precipitation of the polysaccharide by the addition of acetone (1.5 volumes).

Reprecipitation of Xylan.

The xylan (46g.) was shaken with water (2l.), then heated to 60°, cooled and centrifuged. The supernatant was acidified to pH5, the xylan precipitated with acetone (2 volumes), removed at the centrifuged and dried, 41g.

Analysis of Xylan.

$[\alpha]_D$,-92°(C,0.5 in 0.5 N sodium hydroxide).

Xylan (50mg.) was hydrolysed, cooled, a known weight of rhamnose added, and the solution neutralised. Quantitative chromatography in solvent F gave the following results :

Sugar	%
Xylose	76.3
Arabinose	11.0
Glucose	9.4
Galactose	2.0

Methylation.

The polysaccharide was methylated initially by the standard method of Haworth using sodium hydroxide and dimethyl sulphate, and then by Purdie's method using silver oxide and methyl iodide.

The xylan (0.50g.) in water (15ml.) was shaken overnight to effect solution. To the continually stirred xylan solution was added dropwise 30% sodium hydroxide solution (13.5ml.) and dimethyl sulphate (4.5ml.) over a period of several hours. Methylation was carried out in an atmosphere of nitrogen to minimize degradation due to alkali.

Six daily additions of the above quantities were made. The partially methylated polysaccharide started to come out of solution after the fourth treatment and acetone was added to keep it in solution as far as possible. After the sixth methylation the solution was allowed to stir overnight, and on removal of the acetone in vacuo the methylated polysaccharide

appeared as insoluble lumps. The lumps were shaken for 2 hours with chloroform, the solution then centrifuged, deionised with IR-120(H) resin, washed with water, and dried over anhydrous sodium sulphate. Removal of the solvent left a syrup, OMe, 35.8%.

Purdie methylation.

The partially methylated xylan (0,38g.) was refluxed in the dark with methyl iodide (10ml.) for 6 hours, silver oxide (0.2g.) being added at hourly intervals over the first 4 hours. The silver residues were filtered off and washed with hot chloroform. The methyl iodide and chloroform extracts were combined and the solvents removed in vacuo. Treatment of the resultant syrup with silver oxide and methyl iodide was repeated until constant methoxyl determinations on the syrup were obtained. The methylated xylan was precipitated from chloroform solution by the addition of light petroleum (60-80°), 0.32g. OMe, 38.5%

Methanolysis.

The methylated xylan (5mg.) was heated at 100° in a sealed tube with 3% methanolic hydrogen chloride (2ml.) for 12 hours. The mixture of methyl glycosides obtained was investigated by gas-liquid chromatography (Fig. 1).

Other Xylans.

The other xylans investigated were extracted and methylated in a similar manner to that described above for barley husks xylan. The products of methanolysis of the methylated xylans were investigated by gas-liquid chromatography (Fig 2-5).

Fig. 1. Gas-liquid partition chromatogram of methanolysis products of methylated barley husks xylan (1959 harvest).

Conditions : Butanediol succinate polyester-20% on Celite.
Temperature 150°.
90ml. argon/min.
Methyl glycoside of :

Peak 1	2,3,4-tri-O-methyl-D-xylose.
Peak 11	2,3,5-tri-O-methyl-L-arabinose.
Peak 111	2,3,4-tri-O-methyl-D-xylose.
Peak 1V	2,3,5-tri-O-methyl-L-arabinose.
Peak V	3,5-di-O-methyl-L-arabinose.
Peak V1	2,3-di-O-methyl-D-xylose.
Peak V11	2,3-di-O-methyl-D-xylose.
Peak V111	2,3-di-O-methyl-D-xylose.
Peak 1X	3,5-di-O-methyl-L-arabinose.

Fig. 2. Gas-liquid partition chromatogram of methanolysis products of methylated barley husks xylan (1953 harvest).

Conditions : Butanediol succinate polyester - 20% on Celite.
Temperature 150°
100 ml. argon/min.
Methyl glycoside of :

Peak	I	2,3,4-tri-O-methyl-D-xylose.
Peak	II	2,3,5-tri-O-methyl-L-arabinose.
Peak	III	2,3,4-tri-O-methyl-D-xylose.
Peak	IV	2,3,5-tri-O-methyl-L-arabinose.
Peak	V	3,5-di-O-methyl-L-arabinose.
Peak	VI	2,3-di-O-methyl-D-xylose.
Peak	VII	2,3-di-O-methyl-D-xylose.
Peak	VIII	2,3-di-O-methyl-D-xylose.
Peak	IX	3,5-di-O-methyl-L-arabinose.

Fig. 3. Gas-liquid partition chromatogram of methanolysis products of methylated perennial rye grass stems xylan.

Conditions : Butanediol succinate polyester - 20% on Celite.
Temperature 150°
90ml. argon/min.
Methyl glycoside of :

Peak	1	2,3,4-tri-O-methyl-D-xylose.
Peak	11	2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose.
Peak	111	2,3,5-tri-O-methyl-L-arabinose.
Peak	1V	2,3-di-O-methyl-D-xylose.
Peak	V	2,3-di-O-methyl-D-xylose.
Peak	V1	2,3-di-O-methyl-D-xylose.

Fig. 4. Gas-liquid chromatogram of methanolysis products of methylated perennial rye grass roots xylan.

Conditions : Butanediol succinate polyester - 20% on Celite.
Temperature 150°
90ml. argon/min.
Methyl glycoside of :

Peak	I	2,3,4-tri-O-methyl-D-xylose.
Peak	II	2,3,5-tri-O-methyl-L-arabinose.
Peak	III	2,3,4-tri-O-methyl-D-xylose.
Peak	IV	2,3,5-tri-O-methyl-L-arabinose.
Peak	V	3,5-di-O-methyl-L-arabinose.
Peak	VI	3,4-di-O-methyl-D-xylose.
Peak	VII	2,3-di-O-methyl-D-xylose.
Peak	VIII	2,3-di-O-methyl-D-xylose and 3,4-di-O-methyl-D-xylose.
Peak	IX	2,3-di-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-galactose.
Peak	X	2,3,4,6-tetra-O-methyl-D-galactose.
Peak	XI	3,5-di-O-methyl-L-arabinose.

Fig. 5. Gas-liquid chromatogram of methanolysis products of methylated Norway spruce wood xylan.

Conditions : Butanediol succinate polyester - 20% on Celite.
Temperature 150°.
90ml. argon/min.
Methyl glycoside of :

Peak	1	2,3,4-tri-O-methyl-D-xylose.
Peak	11	2,3,5-tri-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-D-xylose.
Peak	111	2,3,5-tri-O-methyl-L-arabinose.
Peak	1V	2,3-di-O-methyl-D-xylose.
Peak	V	2,3-di-O-methyl-D-xylose.
Peak	V1	2,3-di-O-methyl-D-xylose.

FIGURE 1

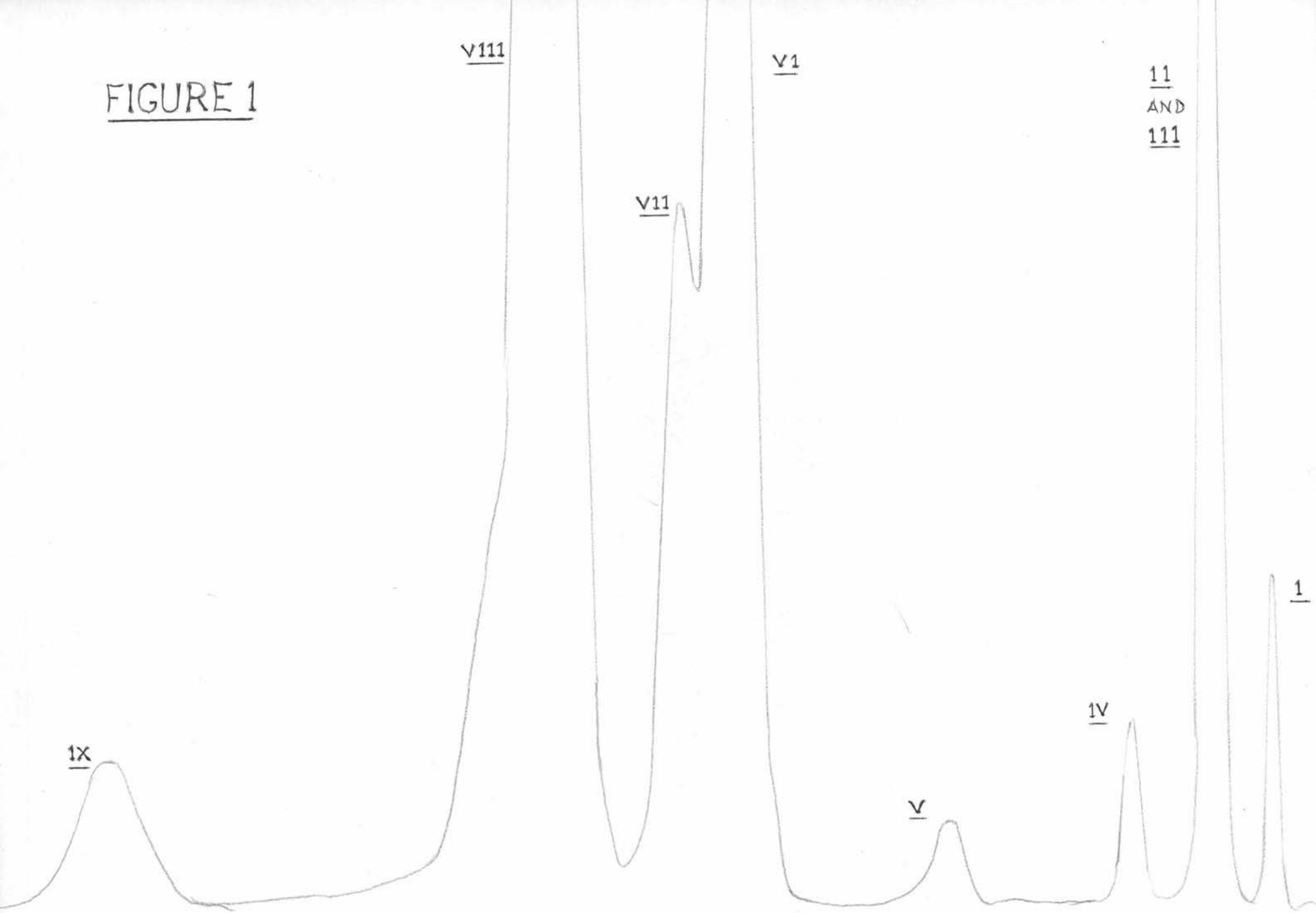


FIGURE 2

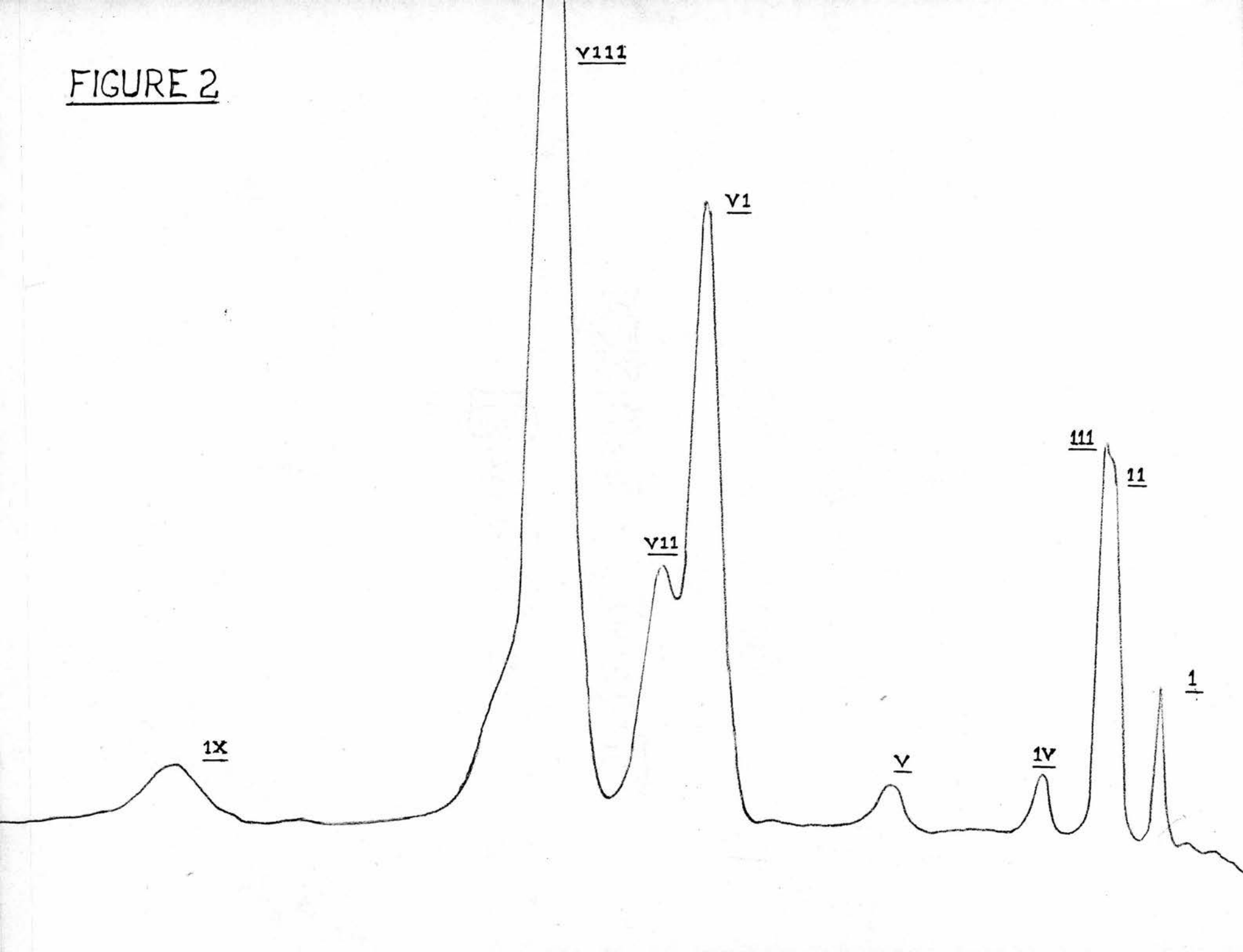


FIGURE 3

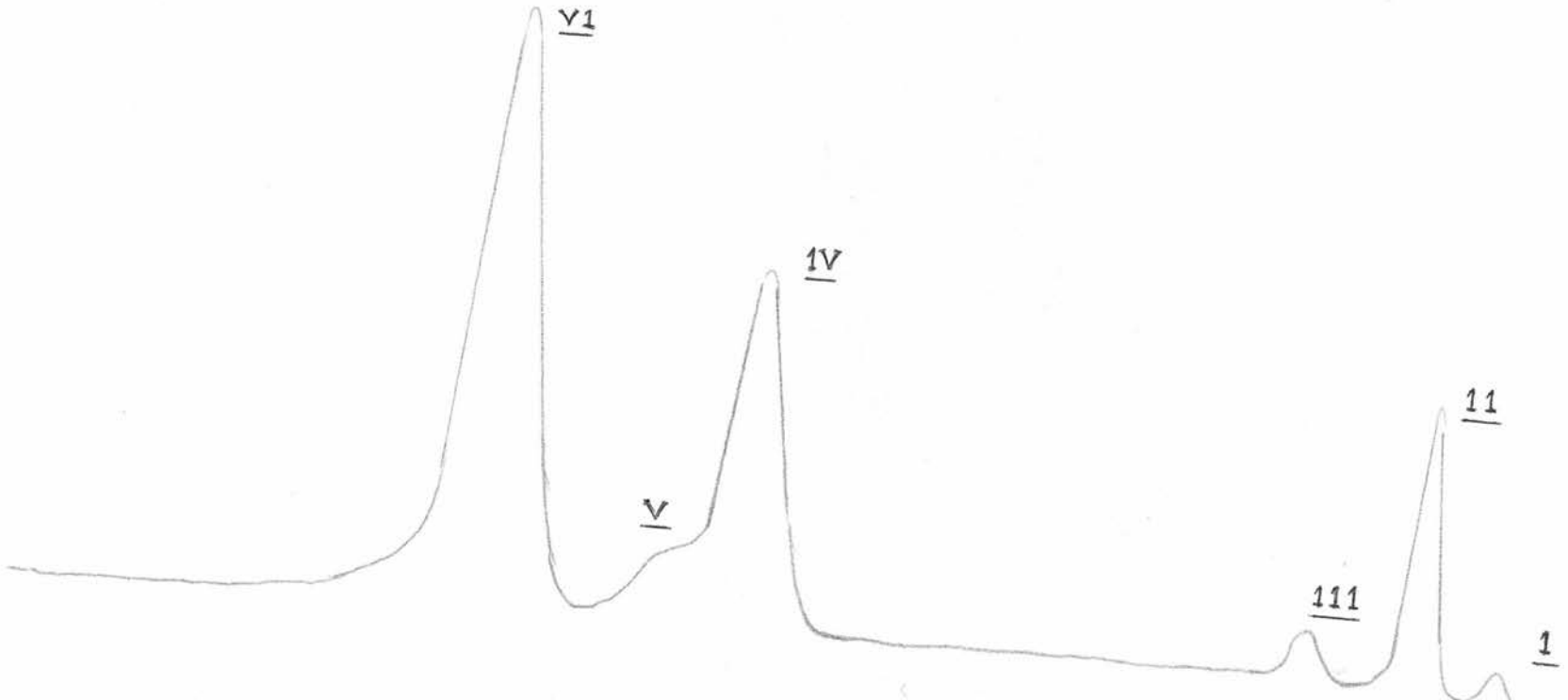


FIGURE 4

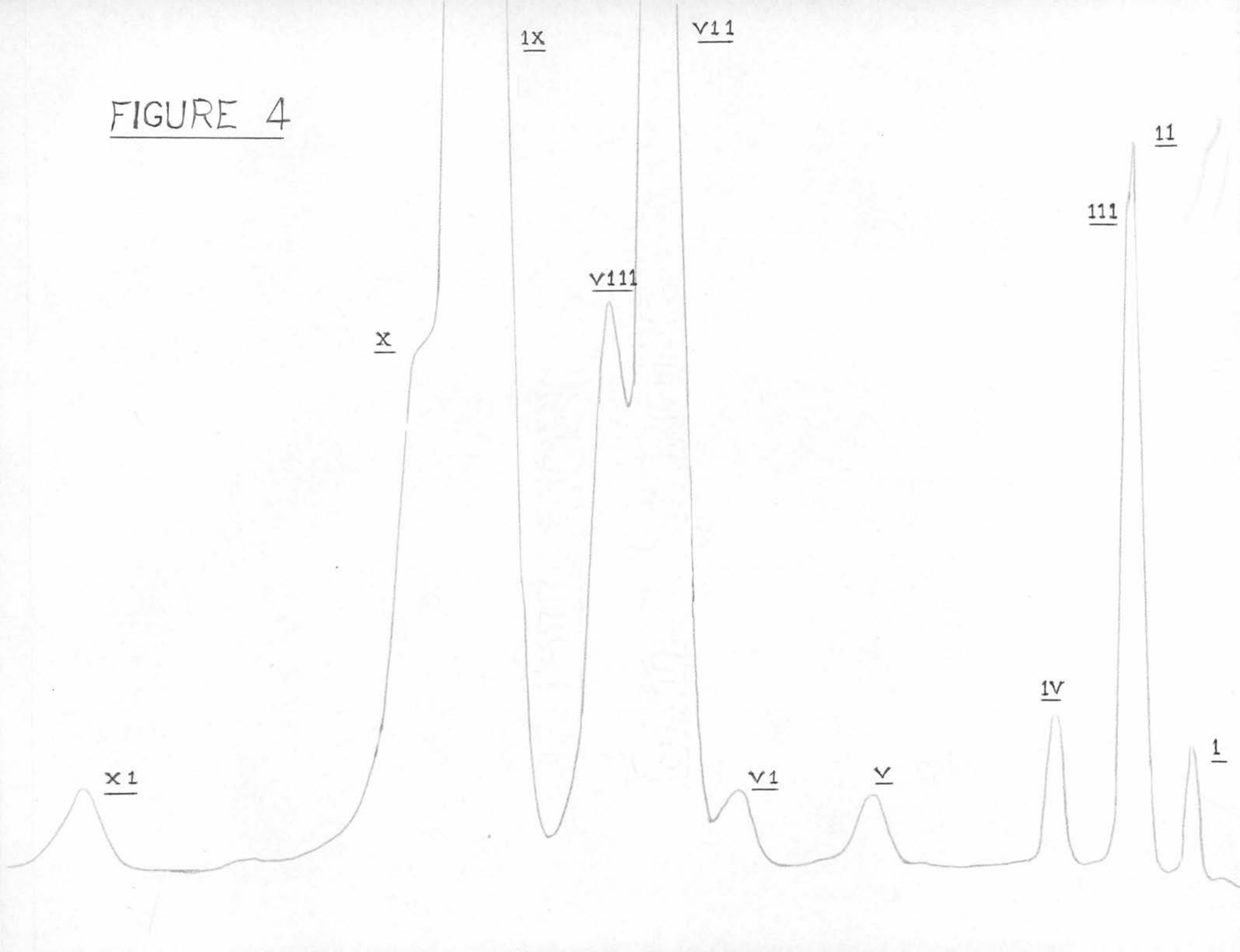
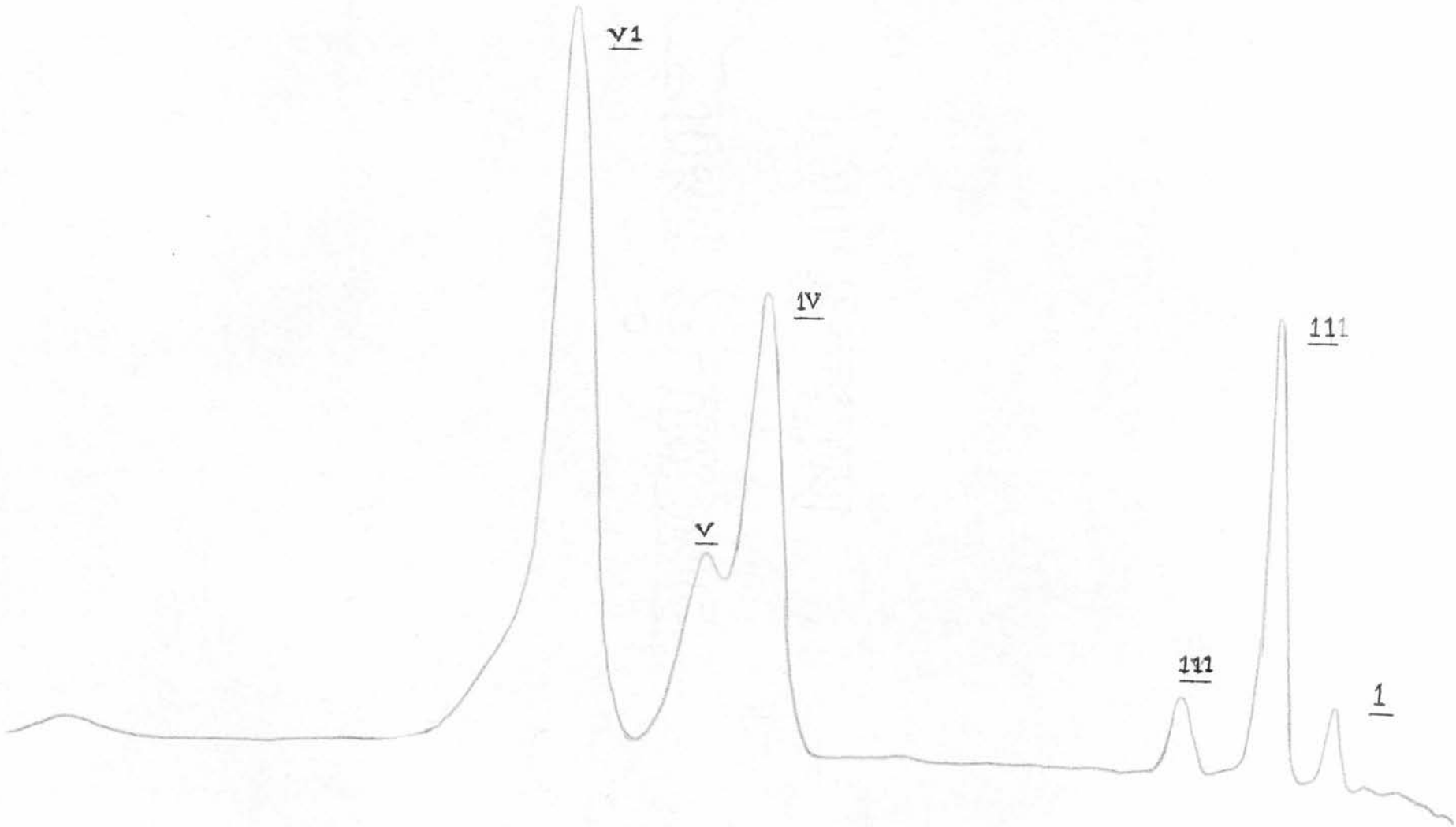


FIGURE 5



Partial Acid Hydrolysis of Perennial Rye Grass Roots Xylan.

Extraction and Purification.

(For full details of extraction and purification procedure, see I.M. Cairncross, Ph.D. thesis, Edinburgh 1959).

The roots were cut into small pieces, extracted with boiling 80% ethanol, and then with cold and hot water. Delignification was carried out by the sodium chlorite/acetic acid method as already described for barley husks.

Treatment of the holocellulose with sodium hydroxide (4%) for 48 hours in a ball-mill extracted the xylan which was precipitated by the addition of acetone (1.5 volumes) to the solution adjusted to pH5 with glacial acetic acid.

Analysis of Xylan.

Quantitative estimation of the constituent sugars gave xylose (46.0%), arabinose (13.2%), glucose (8.0%), galactose (6.4%), uronic anhydride (9.7%), ash (11.0%).

Rotation

$[\alpha]_D$, -100° (C,1.0 in 0.5N sodium hydroxide).

Solubility.

The xylan proved to be almost insoluble in boiling water

and cold 72% sulphuric acid. Partial solution was effected by shaking the polysaccharide (10g.) with N-alkali (2l.) overnight in an atmosphere of nitrogen, neutralising by addition of glacial acetic acid, then dialysing for 3 days to remove inorganic ions. In this way a colloidal solution of the xylan was obtained (2l.)

Trial hydrolysis using 0.5N sulphuric acid.

The polysaccharide colloid (15ml.) was made acid by the addition of sulphuric acid (5ml.,2N) and heated on a boiling-water bath. Samples were removed at 15 minute intervals, neutralised with barium carbonate, filtered, and concentrated to syrups which were examined chromatographically in solvents A and B. The first four samples all contained an oligosaccharide (O_2) which travelled on chromatograms at the same rate as xylobiose. The concentration of this sugar appeared to reach a maximum after about 30 minutes and thereafter quickly decreased. After 1.5 hours the hydrolysate consisted almost entirely of the monosaccharides xylose, arabinose, galactose and glucose, with traces of oligosaccharides.

Hydrolysis using 0.5N sulphuric acid.

The colloidal solution of the xylan (1.6l.), made 0.5N with respect to sulphuric acid by addition of this acid (200ml.,

4.5N), was heated on a boiling-water bath for 30 minutes then quickly cooled. Polysaccharide which had precipitated out during hydrolysis was centrifuged off and the solution then neutralised with barium carbonate. After removal of inorganic salts the solution was concentrated to a small volume and degraded polysaccharide precipitated by the addition of alcohol (2 volumes). The precipitates of polysaccharide were united and given a further treatment with sulphuric acid (0.5N) for 30 minutes. In all the hydrolysis was repeated five times, each time using the degraded polysaccharide recovered from the previous hydrolysis. The combined hydrolysates were deionised with resins and concentrated to a syrup, 3.60g.

Chromatographic examination in solvents A and C showed the presence of xylose, arabinose, glucose, galactose, and oligosaccharides (O_1 — O_5) whose R_{xylose} values in solvent A were 0.70, 0.64, 0.40, 0.34, 0.28. Small amounts of higher xylo-oligosaccharides were also present. The fastest moving oligosaccharide (O_1) gave an orange colour on chromatograms with spray A and was readily distinguishable under ultraviolet light by its bright orange fluorescence. All other oligosaccharides gave brown to pink spots. O_2 travelled a t the same rate as xylobiose.

When the syrup was examined in solvent C without previous

treatment with 1R-~~120(H)~~ 4B(OH) resin, an additional spot to the above was obtained, R_{xylose} 0.62, orange in colour and fluorescing under ultraviolet light. This was in all probability an aldobiouronic acid.

The syrup was fractionated on a charcoalcelite column (2.5cmX45cm.). The column was initially eluted with water, and then with increasing concentrations of aqueous alcohol.

Fraction	Vol. of eluant	eluant	weight	Sugars present
1	1,500ml.	water	2.31g.	Ara,Xyl,Gal,Glu.
2	1,000ml.	2%ethanol	0.230g.	Ara,Xyl,Glu,Gal, Traces of O_1 and O_2.
3	500ml.	5%ethanol	0.084g.	O_1, some O_2 .
4	1,000ml.	5%ethanol	0.312g.	O_1 and O_2.
5	1,000ml.	5%ethanol	0.094g.	O_1,O_2 and trace O_3.
6	1,000ml.	7%ethanol	0.056g.	O_2, O_3, and O_4.
7	2,000ml.	10%ethanol	0.062g.	O_3, O_4, and higher oligosaccharides.

Fraction 3.

0.084g. The sugars were separated on Whatman 3MM sheets in solvent A and the components eluted with water. A chromatographically pure sample of O_1 was obtained which crystallised on standing. $[\alpha]_D$ +28°(C,0.5 in water). Slow recrystallis-

ation from water yielded prismatic crystals.

Hydrolysis of the sugar with N-sulphuric acid at 100° for 6 hours and examination of the products chromatographically showed approximately equal amounts of xylose and arabinose.

Reduction of the sugar (5mg.) with potassium borohydride (20mg.) in water (2ml.) overnight at room temperature, followed by removal of ions by resin and evaporations with methyl alcohol gave a syrup which on hydrolysis with N-sulphuric acid at 100° for 6 hours yielded xylose as the only reducing sugar on chromatographic examination using spray A. Examination using spray B showed in addition the presence of arabitol.

Chromatographic examination of the oligosaccharide using spray H gave no coloured spot, indicating a substituent in 2 in the reducing sugar residue.

The sugar travelled identically with an authentic sample of 2-O-β-D-xylopyranosyl-L-arabinose in solvents A,B, and D. In none of these solvents was a mixture of O_1 and the characterised dissacharide resolvable. Both sugars gave a characteristic orange colour and bright orange fluorescence under ultraviolet light when treated on chromatograms with spray A.

A sharp melting point of the crystals was not obtained, the crystals melting over the range 84-92°, undepressed on admixture with an authentic sample, m.p. 90-94°.

The x-ray powder photographs of O_1 and an authentic sample of 2-O-β-D-xylopyranosyl-L-arabinose were identical.

Fraction 4.

0.312g. This fraction contained a mixture of O_1 and O_2. Fractionation of the syrup on Whatman 3MM filter sheets using solvent A followed by elution of the components with water yielded a chromatographically pure sample of O_2, R_{xylose} 0.64 in solvent A $[\alpha]_D$, -22(C,0.5 in water).

Hydrolysis of a sample with N-sulphuric acid at 100° for 6 hours and chromatographic examination of the product showed the presence of xylose alone.

The sugar was chromatographically indistinguishable from an authentic sample of 4-O-β-D-xylopyranosyl-D-xylose (xylobiose) in solvents A,B and D. The syrup refused to crystallise in the desicator or from methanol-water.

Phenylozasone derivative.

To the syrup (30mg.) in water was added phenylhydrazine (0.1ml.), 20% acetic acid (0.5ml.) and sodium bisulphite (5mg.). The solution was heated for 2 hours at 100°. Crystals of the hemihydrate which separated were washed with water and recrystallised from 60% ethanol, melting point and mixed melting point 203-5° (dec.).

Fraction 6

0.056g. This fraction consisted of a mixture of O_2, O_3 and O_4, with O_4 predominating. Fractionation of the mixture on Whatman 3MM filter sheets using solvent B gave a sample of O_4 still contaminated by a trace of O_3.

Hydrolysis of a sample with N-sulphuric acid at 100° for 6 hours gave xylose as the only product on chromatographic examination.

$$[\alpha]_D - 44°(C,0.2 \text{ in water})$$

The sugar had R_{xylose} 0.34 in solvent A and in all probability was xylotriose.

Fraction 7

0.062g. This fraction consisted of O_3, O_4, and higher xylo-oligosaccharides. Fractionation of the syrup on Wha tman 3MM filter sheets in solvent C yielded a chromatographically pure sample of O_3.

Hydrolysis with N-sulphuric acid followed by chromatographic examination of the products in solvents A and B showed the presence of xylose, arabinose, and galactose in approximately equal amounts.

In an attempt to obtain a larger quantity of this oligosaccharide the partial acid hydrolysis was repeated on a further quantity of the xylan, using milder conditions.

Trial hydrolysis using 0.02N sulphuric acid.

To the colloidal solution of the polysaccharide (9ml.) was added sulphuric acid (1ml. 0.2N) and the solution heated on a boiling-water bath. Samples were withdrawn at 30 minute intervals and examined as previously described. Arabinose production increased with time and reached a maximum after 4-5 hours. Similarly production of xylosylarabinose and oligosaccharide O_3 increased with time in the early stages. From 4 hours onwards the amount of xylose liberated appreciably increased, thus the optimum conditions for production of the sugar O_3 were taken as being 0.02N acid for 5 hours.

Hydrolysis with 0.02N sulphuric acid.

The xylan solution (5g. 1 .) was converted to a 0.02N sulphuric acid solution by the addition of this acid (10ml. 2N) and then heated on a boiling-water bath for 5 hours. The solution was rapidly cooled and treated as previously described for the 0,5N acid hydrolysis. The degraded polysaccharide was isolated and subjected to a further treatment with 0.02N acid for 5 hours.

After neutralisation and centrifugation, the hydrolysates were combined, concentrated, deionised with 1R120(H) and 1R4B (OH) resins, and taken to a syrup, 0.60g.

The syrup was fractionated on a charcoal-celite column (1.5cmx20cm.) as before. Water and ethanol (2%) removed from the column all the arabinose plus the traces of xylose and glucose 0.490g. Ethanol (5%) removed the xylosylarabinose and xylobiose. 7% and 10% ethanol gave a syrup containing O_3 together with O_4 and traces of higher oligosaccharides 0.084g. Fractionation on Whatman 3MM filter sheets using solvent C gave a pure sample of O_3, chromatographically indistinguishable from the sample isolated using 0.05N acid, R_{xylose} 0.40 in solvent A. The fractions were combined, 25mg.

Characterisation of the Trisaccharide.

$[\alpha]_D$, + 20° (C, 0.2 in water).

Hydrolysis of the sugar with N-sulphuric acid at 100° for 6 hours followed by chromatographic examination of the products showed the presence of xylose, arabinose, and galactose in approximately equal amounts.

A sample (4mg.) was reduced with potassium borohydride (10mg.) in water (2ml.) overnight at room temperature. Deionisation with resin and methyl alcohol, followed by hydrolysis with N-sulphuric acid and chromatographic examination of the products with spray A showed galactose and xylose as the only reducing sugars present. Spray B revealed in addition the

presence of arabitol.

Chromatographing the sugar in solvents A and C and treating the chromatograms with spray H gave no coloured spots, indicating the presence of a substituent in position 2 of the sugar unit constituting the reducing end of the molecule.

Methylation.

The trisaccharide (10mg.) was shaken with methyl iodide (1.0ml.),dimethyl formamide (1.0ml.) and silver oxide (2g.) at room temperature in the dark for 18 hours. The methylated sugar was isolated, refluxed with methanolic hydrogen chloride (2%) for 12 hours, and the products investigated by gas-liquid chromatography using an apiezon M column and a polyester column as liquid phases , and argon as the gas phase.

The following results were obtained using the polyester column at 150° (see Fig. 6)

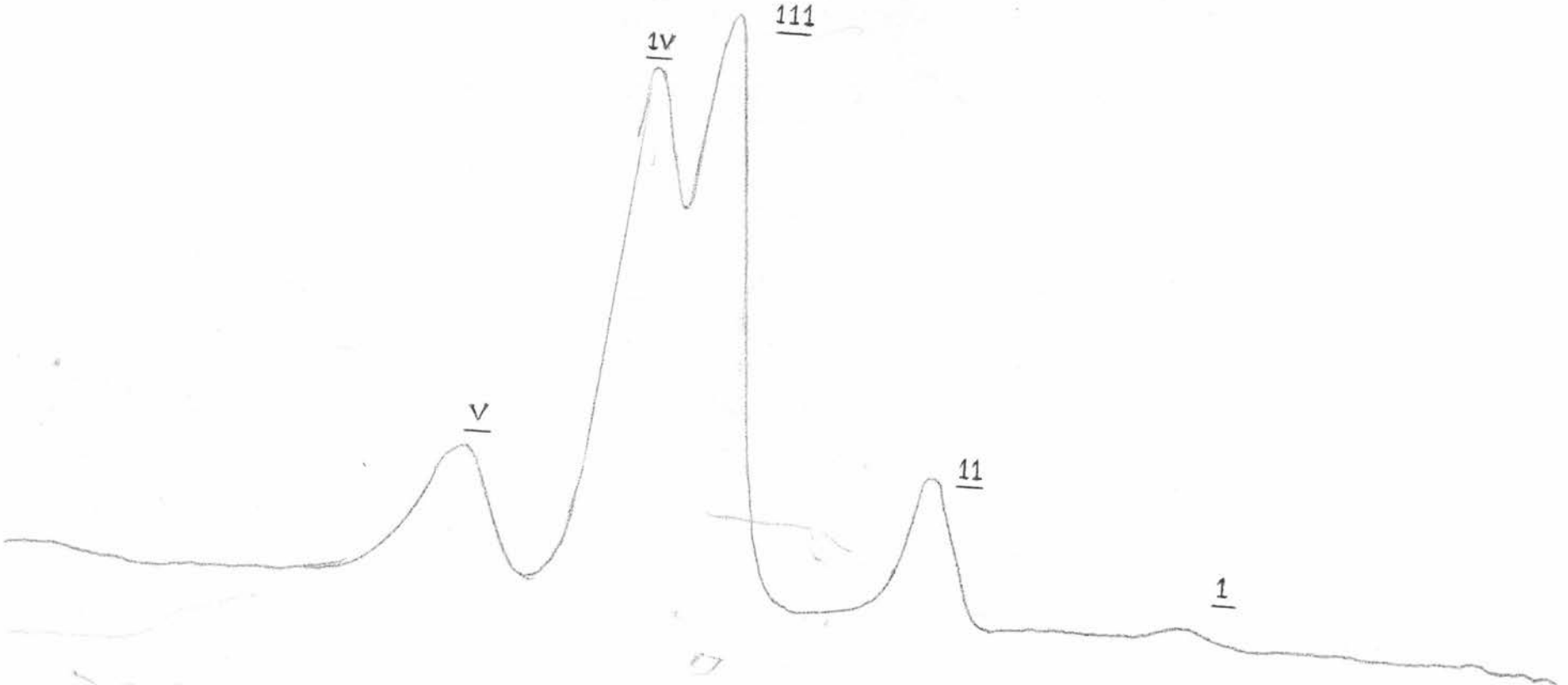
Fig. 6. Gas-liquid partition chromatogram of methanolysis products of methylated trisaccharide.

Conditions : Butanediol succinate polyester - 20% on Celite.
Temperature 150°.
90 ml. argon/min.
Methyl glycoside of :

Peak 1 3,5-di-O-methyl-L-arabinose (trace).
Peak 11 2,3-di-O-methyl-D-xylose.
Peak 111 2,3-di-O-methyl-D-xylose and 2,3,4,6-tetra-O-methyl-D-galactose.
Peak 1V 2,3,4,6-tetra-O-methyl-D-galactose.
Peak V 3,4-di-O-methyl-L-arabinose.

A gas-liquid chromatogram obtained using apiezon M as liquid phase helped to confirm the presence of the above sugars.

FIGURE 6



Periodate oxidation studies.

The trisaccharide (8mg.) was reduced with potassium borohydride (15mg.) in water (2ml.) at room temperature and after 18 hours the ions were removed with 1R-120(H) resin and evaporations with methyl alcohol. The solution was taken to dryness and the syrup obtained dissolved in water (10ml.). The amount of sugar present was estimated by the phenol-sulphuric acid method, 6.5mg.

Periodate uptake.

The periodate uptake of the sugar (6.5mg.) in aqueous sodium metaperiodate (1.0ml.:0.15M) was measured spectrophotometrically and was found to be 4.6 moles per mole of sugar.

Formaldehyde release.

The formaldehyde present on completion of the oxidation was estimated using chromotropic acid. The formaldehyde present corresponded to 0.8 of a mole per mole of sugar oxidised.

Examination for unattacked residues.

The excess periodate in the solution containing the oxidised sugar was destroyed by addition of ethylene glycol (1 drop) and the sodium ions removed using 1R-120(H) resin. The iodate was precipitated by addition of an excess of barium carbonate

and removed by filtration. Hydrolysis of the oxidised sugar with N-sulphuric acid and examination of the products chromatographically using spray A showed the absence of any unattacked sugar residues.

Partial Hydrolysis.

The sugar (2mg.) was hydrolysed with sulphuric acid (0.1N;2ml.) at 100° for 15 minutes. Chromatographic examination of the products showed the presence of xylose, arabinose, galactose and 2-O-β-D-xylopyranosyl-L-arabinose.

SECTION 11

Smith Degradation Applied to Xylans.

Discussion

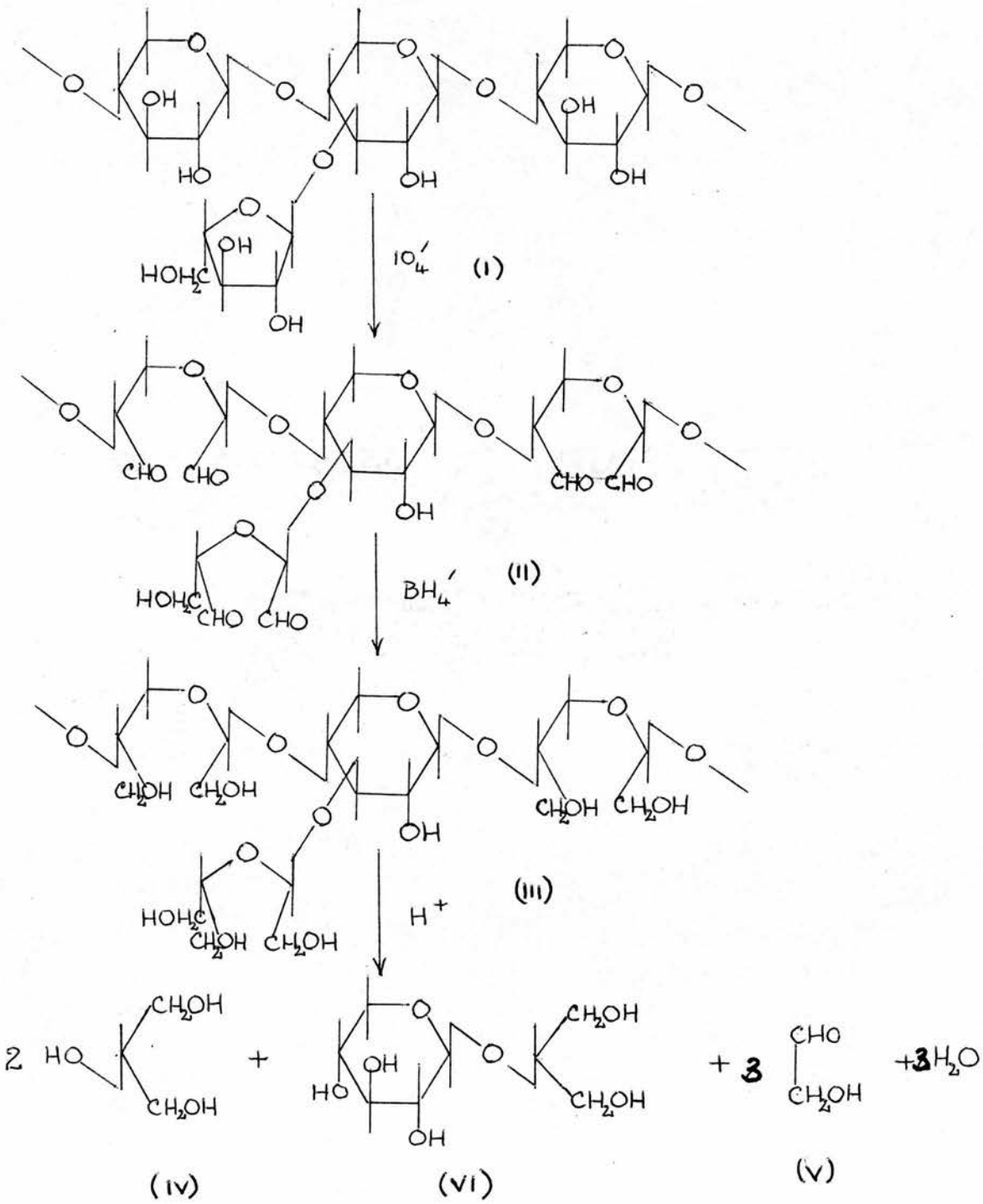
The xylans studied were the xylan isolated from 1959 barley husks already discussed in Section 1, and a xylan extracted from rye flour by Aspinall and Sturgeon (44).

Smith degradation (35) involves periodate oxidation of the polysaccharide, followed by reduction of the aldehydic groups formed to primary alcoholic groups. When the polyalcohol is hydrolysed with cold acid, the acetal links are destroyed leaving any glycosidic links intact.

This means in the case of xylans, where side-chains are attached to position 3(or2) of 1,4-linked β-D-xylose residues (1), that xylosyl-glycerol(VI) would be expected as a degradation product if the side-chains are evenly distributed along the basal xylan chain. If, however, the side-chains are attached to two, three, or four etc., adjacent xylose residues then xylobiosyl-glycerol, xylotriosyl-glycerol, or xylotetra - osyl-glycerol etc., would result.

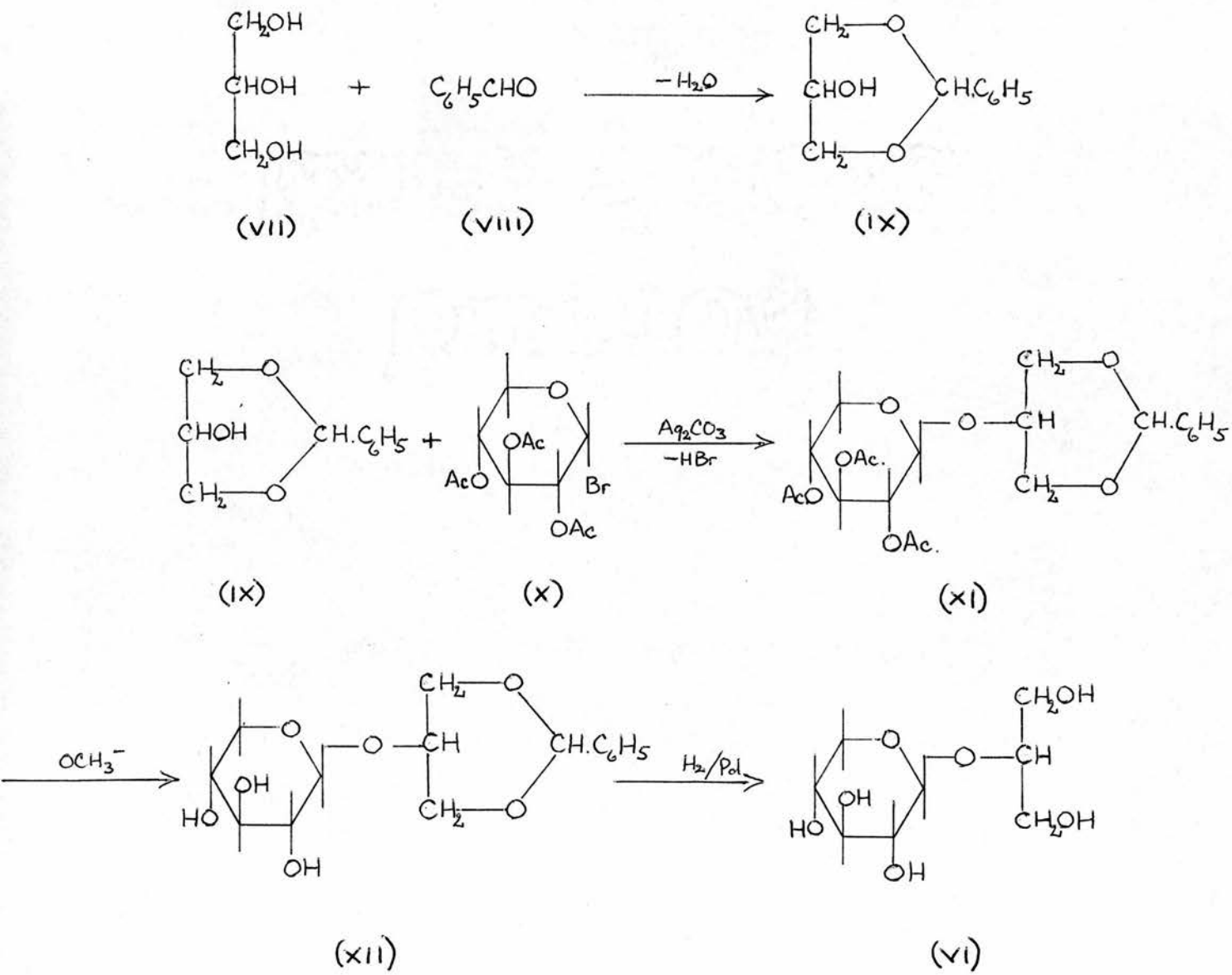
In addition large amounts of glycerol (IV) and glycollic aldehyde (V) would be expected to arise from the periodate attacked residues.

Figure 1. Smith degradation of xylan.



The synthesis of 2-O-β-D-xylopyranosyl-glycerol was carried out (Figure 2.) in order to study its chromatographic behaviour before embarking on these degradations, thus facilitating its isolation from the degradation products. Synthesis also supplies conclusive proof of structure.

Figure 2. Synthesis of 2-O-β-D-xylopyranosyl-glycerol.



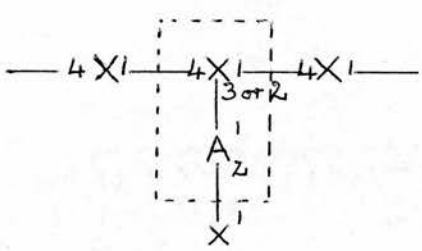
Condensation of glycerol (VII) with benzaldehyde (VIII)(113) at 170° gave the condensation product 1,3-O-benzylidene-glycerol (cis isomer)(IX) on vacuum distillation of the reaction mixture. Treatment with hydrogen chloride caused crystallisation to occur. A further condensation, this time of the Koenigs-Knorr type, between the 1,3-O-benzylidene-glycerol and 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide (X) in the presence of silver carbonate gave a product in low yield, separated from the reaction mixture by alumina chromatography, which gave on analysis C,57.7%; H,6.04%. This was in agreement with the compound being 1,3-O-benzylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-glycerol (XI). Treatment of this compound with sodium methoxide removed the acetyl groupings to give the crystalline 1,3-O-benzylidene-2-O-β-D-xylopyranosyl-glycerol (XII), which lost the protecting benzylidene grouping on hydrogenation over a palladium catalyst to yield the syrupy 2-O-β-D-xylopyranosyl-glycerol (VI).

2-O-β-D-xylopyranosyl-glycerol was found to be absorbed on charcoal, and to be eluted from it by water containing a very small quantity of ethanol.

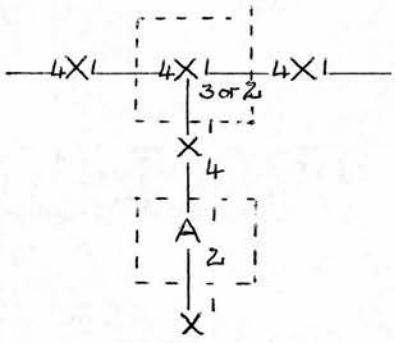
Barley husk xylan.

A Smith degradation was carried out on this xylan with a view to obtaining further information about branching in the

molecule. In their studies on barley husk xylan Aspinall and Ferrier have shown this type of xylan molecule to contain side-chains of terminal L-arabinofuranose residues, single unit D-glucuronic acid (or its 4-O-methyl ether) residues, and 2-O-β-D-xylopyranosyl-L-arabinose residues. In the case of the xylosylarabinose side-chains, two types of structure are possible : the residues can be attached directly to the xylan backbone (XIII) or they can terminate longer side-chains (XIV):



(XIII)



(XIV)

X=D-xylopyranosyl residues

A=L-arabinofuranosyl residues.

Residues enclosed by the dotted line are unattacked by periodate ions.

In structure (XIV), the arabinose residue cannot be linked through position 3 of xylose, since this would lead to the

formation on methylation and hydrolysis, of 2,4-di-O-methyl-D-xylose, a sugar undetected by Aspinall and Ferrier. Thus isolation and characterisation of any arabinose containing oligosaccharides from a Smith degradation must differentiate between the two possible structures.

In a trial degradation of the xylan, inorganic ions were removed at the various stages by dialysis. This appeared to result in serious loss of material and so oxidation was carried out using periodic acid, since the iodate ions formed could be precipitated as barium iodate.

The polysaccharide was dissolved in periodic acid at 5° and the periodate uptake corresponded to 0.94 of a mole per mole of anhydropentose residue, measured titrimetrically by the method of Fleury and Lange (114). Reduction of the polyaldehyde was achieved using potassium borohydride, inorganic ions being removed with resins and methyl alcohol. Cold sulphuric acid was used in the partial hydrolysis, which was terminated after 5 hours since acetal linkages are quickly destroyed in acid solution. Anattempt to follow the hydrolysis by change in specific rotation proved unsuccessful, only a very small change in rotation being observed.

Chromatographic examination of the syrup obtained from the hydrolysate showed it to contain 70-80% glycerol, together

with small amounts of both reducing and non-reducing sugars. The syrup was fractionated on a charcoal-celite column by employing a gradient elution, ranging from water alone up to water containing 30% ethanol. A poor fractionation of the constituent sugars was achieved, and in fact no sugar was obtained chromatographically pure by this means alone. Ten fractions were collected, some containing up to four constituents. From R_{xylose} values and examination of hydrolysis products the fractions appeared to consist of mixtures of two or more of the following sugars (listed in order of their chromatographic mobilities in solvent A) :

ethylene glycol	Xylosyl-glycerol
Glycollic aldehyde	arabinose
glycerol	arabinosylxylosyl-glycerol
	(or xylosylarabinosyl-glycerol)
erythritol	xylobiosyl-glycerol
xylose	xylobiose

Traces of unidentified higher xylo-oligosaccharides and a xylose-arabinose containing oligosaccharide were also detected.

Glycollic aldehyde and glycerol are to be expected in the Smith degradation of a xylan (Figure 1), while the erythritol

probably arose from a contaminating glucan or galactan. Xylose, xylobiose, and the trace of arabinose could only have arisen from the splitting of glycosidic linkages at some stage, probably during the partial hydrolysis.

The fractions were further investigated by carrying out separations on thick paper and chromatographically pure samples of the major sugar components were obtained.

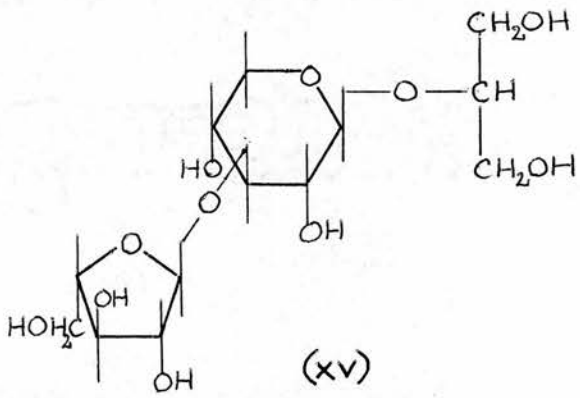
In the following discussion of low molecular weight compounds obtained by degradation of this xylan (and of the rye flour xylan which follows), xylose and arabinose residues are assumed to have the D and L configurations respectively, while the xylose residues are assumed to be β-linked, an assumption bourne out by the negative rotations of the compounds.

From fractions 3 and 4 a chromatographically pure sample was obtained of a non-reducing sugar which hydrolysed to xylose and glycerol alone in almost equimolecular amounts. Methylation and periodate oxidation studies showed the compound to be 2-O- -D-xylopyranosyl-glycerol (VI). It travelled on chromatograms in solvents A,C and D at the same rate as an authentic sample of this sugar and in no solvent was a mixture of the two resolvable.

From fraction 5 a pure sample was obtained of a non-reducing sugar which hydrolysed to xylose, arabinose, and

glycerol in almost equimolecular amounts. Methylation studies showed the arabinose residue to be linked through position 1 only, while the xylose residue was shown to be linked through positions 1 and 3. The sugar had a periodate consumption corresponding to 0.8 of a mole of periodate per mole of sugar, with no formic acid or formaldehyde formed as by products, indicating that the arabinose was in the furanose form and that the glycerol was linked through position 2. The fact that the xylose residue remained unattacked by periodate confirmed its position as the central residue.

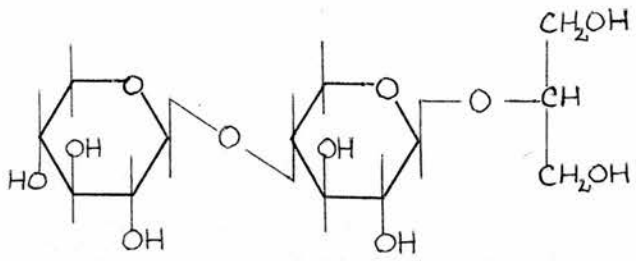
From these results the structure of the sugar must be L-arabinofuranosyl (1—3)-β-D-xylopyranosyl (1—2)-glycerol(XV):



(xv)

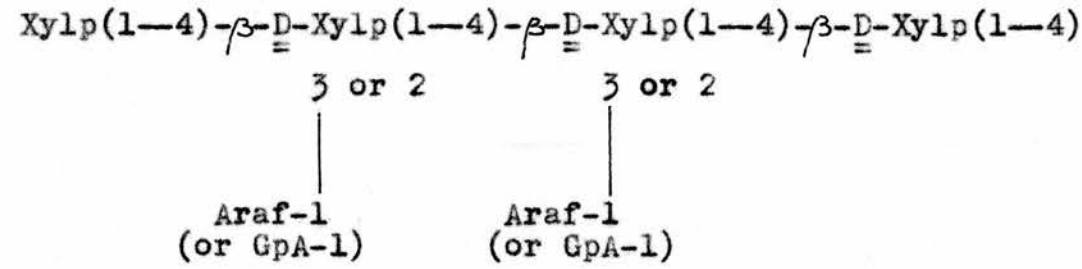
From fraction 8 a pure sample of a non-reducing sugar was obtained which hydrolysed to give 1.8 moles of xylose and 0.9 of a mole of glycerol per mole of sugar hydrolysed, on quantitative estimation. Methylation studies revealed 1,4-

linked xylose and 1-linked xylose to be present in the ratio of 1 to 1, and periodate oxidation studies showed that 1 mole of sugar consumed 2.8 moles of periodate, liberating 1.0 mole of acid, no formaldehyde, and leaving no unattacked xylose residues. The sugar was therefore β-D-xylopyranosyl (1—4)-β-D-xylopyranosyl-glycerol(xylobiosyl-glycerol)(XVl) :



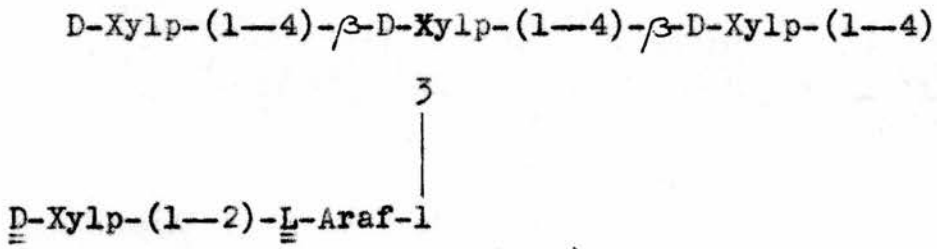
(xvi)

The total amount of xylosyl-glycerol, xylobiosyl-glycerol and arabinosylxylosyl-glycerol obtained on degradation of the xylan is directly proportional to the degree of branching in the molecule. Xylosyl-glycerol arises from any 1,4-linked xylose residue carrying a branch point through positions 2 or 3, and flanked by xylose residues susceptible to periodate attack, while xylobiosyl-glycerol results from two adjacent xylose residues carrying branch points in positions 2 or 3 (XVll):



(XVII)

The isolation of arabinosylxylosyl-glycerol(XVI) shows that the xylosylarabinose side-chains in the molecule must be attached directly to the xylan backbone, as in structure (XIII) Since the arabinose was linked to position 3 of xylose in this oligosaccharide, this establishes the mode of attachment of these side-chains (XVIII) :



(XVIII)

The complexity of the sugar mixture obtained from the xylan degradation prevented an accurate quantitative determination of the above oligosaccharides. However xylosyl-glycerol was present in much greater amount than xylobiosyl-glycerol,

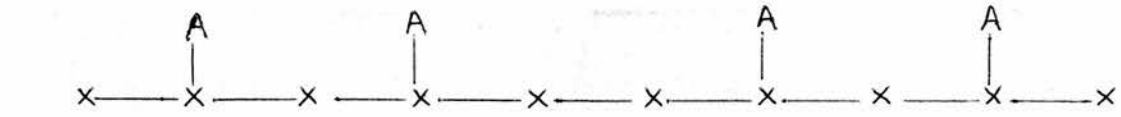
and higher xylo- and arabinoxylo--ligosaccharides (other than the sugar represented by structure (XV)) were present in trace amounts only. This indicates a random distribution of branch points along the xylan backbone.

Rye flour xylan.

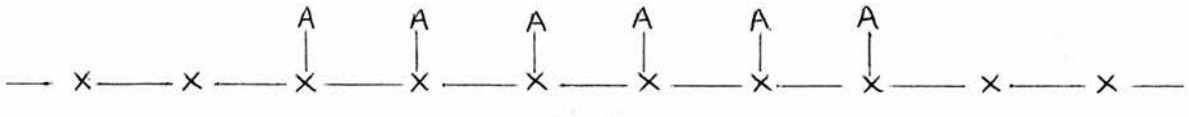
A similar type of degradation was carried out on a xylan isolated from rye flour. This xylan had been extracted by Aspinall and Sturgeon (44) using water at 40°, and has been used by them in alkaline degradation studies (100). In structural studies on this non-acidic xylan these workers showed that all the arabinose was in the form of non-reducing terminal residues, and was present with xylose in the ratio of 1 to 2.(44).

The available data do not define how the branches are arranged in this, and other, polysaccharides and a variety of possibilities must be considered (structures (XlV),(XX),(XXl)). On applying a Smith degradation, structure (XlX) would afford xylosyl-glycerol (Vl), structure (XX) a polymeric xylan linked to a glycerol residue, and structure (XXl) a mixture of xylo-oligosaccharides all terminated by a glycerol residue. Thus isolation and characterisation of the unoxidised fragments from a Smith degradation must indicate the type of structure

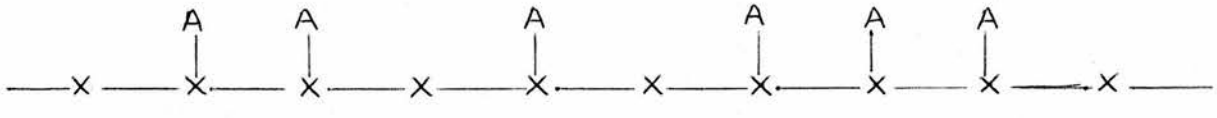
existing in the molecule.



(xIx)



(xx)



(xxI)

A contaminating glucan was partially removed by enzymic hydrolysis, and the purified xylan then oxidised with sodium metaperiodate. The periodate uptake, measured titrimetrically, corresponded to 0.67 of a mole per mole of anhydropentose residue. The reduction of the polyaldehyde and hydrolysis of the polyalcohol formed were carried out as described for the husks xylan, with the exception that the hydrolysis time was cut from 5 to 3 hours to minimize the danger of destroying glycosidic linkages.

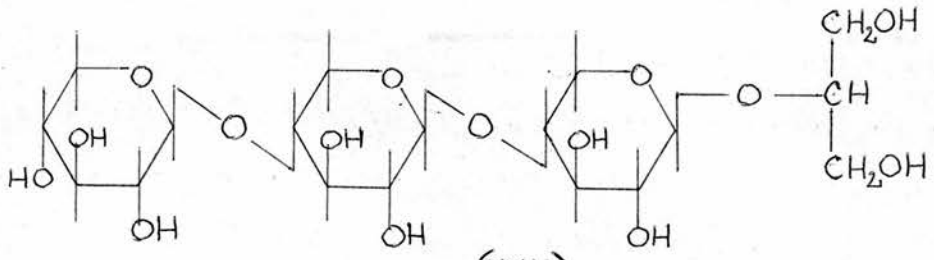
On chromatographic examination of the syrup obtained from the hydrolysate, the presence of glycerol and glycollic aldehyde was indicated, together with smaller amounts of three non-reducing compounds having R_{xylose} values of 1.00, 0.62, and 0.35 in solvent A. The sugars were separated on a resin column in the manner described by Jones and Wall (31,32). Slow elution of the column with water supplied almost pure samples of the two slower moving oligosaccharides, the sugar having the lower R_{xylose} value being eluted first. Chromatographically pure samples of each sugar was obtained by the usual procedure of thick paper separation.

The first compound to be eluted hydrolysed to xylose and glycerol alone and quantitative estimation of these showed that 2.8 moles of xylose and 1.1 moles of glycerol were liberated from 1 mole of sugar. Methylation studies showed the molecule to contain 1-linked and 1,4-linked xylose in the ratio of 1 to 2. When the sugar was treated with sodium metaperiodate, 3.8 moles of periodate were consumed and 1.1 moles of formic acid liberated per mole of sugar attacked. The absence of formaldehyde in the reaction mixture indicated the presence of 2-linked glycerol.

The results are all in agreement with the sugar being

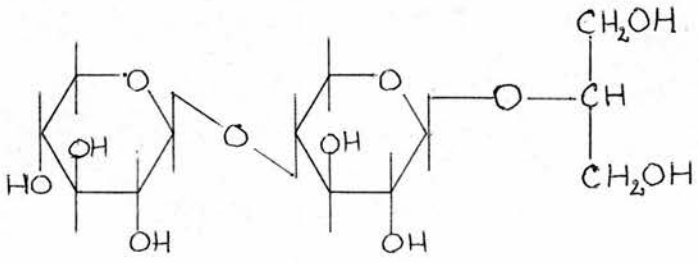
β-D-xylopyranosyl-(1—4)-β-D-xylopyranosyl-(1—4)-β-D-

xylopyranosyl-glycerol (xylotriosyl-glycerol)(XXII).



(XXII)

During elution of the column this compound was followed by another having a higher R_{xylose} value and appearing to belong to the same homologous series. It hydrolysed to xylose and glycerol alone, 2.1 moles of xylose and 1.1 mole of glycerol being liberated from 1 mole of sugar. Methylation studies showed the presence of 1-linked and 1,4-linked xylose in approximately equimolecular amounts, and the sugar consumed the equivalent of 2.8 moles of periodate per mole of sugar, liberating 1 mole of formic acid but no formaldehyde. The sugar was therefore β-D-xylopyranosyl(1—4)-β-D-xylopyranosyl(1—2)-glycerol, (xylobiosyl-glycerol)(XXIII) :



(XXIII)

Further elution of the column yielded the lowest member of the series. The fraction was highly contaminated with erythritol but a thick paper separation yielded a chromatographically pure sample. The sugar hydrolysed to yield 0.9 of a mole of xylose and 1.1 moles of glycerol per mole of sugar, and methylation and periodate oxidation studies showed it to be 2-O-β-D-xylopyranosyl-glycerol(VI). It ran on chromatograms at the same rate as an authentic sample of this sugar and in no solvent was a mixture of the two resolvable.

Aspinall and Sturgeon (44) deduced from their experiments with this xylan that the degree of branching in the xylan backbone is very slight, and that most of the arabinose at least, is attached as single-unit terminal residues. This means that the xylo-oligosaccharides discussed above are derived from xylose residues in the backbone carrying arabinose side-chains. When arabinose residues are attached to three adjacent xylose residues xylotriosyl-glycerol results, when attached to two adjacent xylose residues xylobiosyl-glycerol results, and when attached to non-adjacent xylose residues xylosyl-glycerol results (see structure (XXI)). Quantitative estimations carried out on the degradation reaction mixture showed the amounts of xylosyl-glycerol, xylobiosyl-glycerol, and xylotriosyl-glycerol to be present in the ratio of 3.2 :

1.5 : 1 by weight, which is a molar ratio of 7.5 : 2.2 : 1, thus indicating a random distribution of arabinose side-chains along the xylan backbone.

The absence of any arabinose containing oligosaccharide points to an absence of any non-terminal arabinose in the polysaccharide, unless it possesses 1,4-linkages. No such arabinose has been detected in the previous investigations on this xylan (44).

The branching in this xylan therefore is essentially similar to that in a wheat flour xylan examined by Ewald and Perlin (115) using the Barry degradation. These workers found in this xylan that the arabinose sometimes occurred on isolated xylose residues, sometimes on two adjacent xylose residues, and sometimes on three, but never on four, five, etc.. In this xylan, however, the amount of arabinose linked to two adjacent xylose residues was higher, while the amount linked to three adjacent xylose residues was present in trace amounts only.

Experimental.

Synthesis of 2-O-β-D-xylopyranosyl-glycerol.

1,3-O-Benzylidene-glycerol (1X).

Glycerol (1)(92g.) was heated with an excess of freshly distilled benzyaldehyde (11)(127g.) at 145-150° for 1½ hours, and then at 165-170° for a further 2 hours. A stream of carbon dioxide was passed through the reaction mixture during heating to remove water. The mixture was distilled in vacuo and the fraction boiling at 140-150° at 4mm. pressure collected as a clear syrup. Hydrogen chloride was bubbled through the syrup at 0° for 5 minutes, and after standing for 2 days in a stoppered flask at 5°, the syrup crystallised to a white solid mass which was then shaken with aqueous ammonia solution (1%) for 1 hour. Benzene (900ml.) was added and solution of the solid effected by warming. The benzene layer was separated, washed several times with aqueous ammonia solution (1%), and concentrated to a small volume (300ml.). Addition of light petroleum (60-80°)(450ml.) caused precipitation of the 1,3-O-benzylidene-glycerol(1X), and recrystallisation from benzene-light petroleum (60-80°)(3:4) afforded the pure compound, 34.4g.

m.p.81-82°, undepressed on admixture with an authentic sample of the cis isomer.

2,3,4-Tri-O-acetyl-α-D-xylopyranosyl bromide (X).

This compound was prepared by the standard reaction of 1,2,3,4-tetra-O-acetyl-β-D-xylose with hydrogen bromide in glacial acetic acid.

1,3-O-Benzylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-glycerol(XI).

1,3-O-Benzylidene-glycerol(IX)(25g.), freshly prepared silver carbonate (50g.), and anhydrous calcium sulphate (100g.) were shaken in dry benzene (300ml.) overnight in the dark. 2,3,4-Tri-O-acetyl-α-D-xylopyranosyl bromide (X)(37.4g.) in dry benzene (300ml.) was added gradually and the mixture shaken for 3 days in the dark, with occasional release of carbon dioxide. The mixture was finally refluxed for 15 minutes then filtered. Evaporation of the filtrate gave a white solid.

The solid was dissolved in benzene and chromatographed on alumina. Elution with light petroleum (60-80°)-benzene (3:2) furnished 1,3-O-benzylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-glycerol(XI) as a white solid, 1.60g.

The compound was recrystallised from light petroleum (40-60°)-ethanol(2:1).

m.p. 183-4°. $[\alpha]_D$,-33.8°.

Found : C,57.6% : H,6.04%($C_{21}H_{26}O_{10}$ requires C,57.5% ; H,5.94%).

1,3-O-Benzylidene-2-O-β-D-xylopyranosyl-glycerol(XII).

1,3-O-Benzylidene-2-O-(2,3,4-tri-O-acetyl-β-D- xylopyranosyl)-glycerol(XI)(1.30g.) was treated with sodium methoxide (0.8g.) in methanol (40ml.) for 18 hours at room temperature. The solution was deionised with 1R-120(H) and 1R-4B (OH) resins, filtered, and the solution evaporated to a syrup which crystallised on standing. The crystalline 1,3-O-benzylidene-2-O-β-D-xylopyranosyl-glycerol(XII) was recrystallised from methanol-ether (2:1), 0.86g.

m.p. 132-134°. $[\alpha]_D$,-41.4°.(C,1 in water).

2-O-β-D-Xylopyranosyl-glycerol(XIII).

1,3-O-Benzylidene -2-O-β-D-xylopyranosyl-glycerol(XII) (0.80g.) was dissolved in ethanol (50ml.) and the solution shaken in hydrogen at atmospheric pressure over 10% palladium -charcoal(0.80g.) for 48 hours. The product, 2-O-β-D-

xylopyranosyl-glycerol(XIII) was obtained as a syrup by filtration and evaporation of the solution, 524mg.

$[\alpha]_D$, -36° (C,2 in water).

Oxidation by periodate. The syrup (10mg.) was found to consume the equivalent of 1.9 moles of periodate per mole of sugar attacked when dissolved in aqueous sodium metaperiodate (1.0ml. : 0.10M.), with the liberation of 1.02 moles of formic acid. No formaldehyde was detected.

Benzylidene derivative. The compound (20mg.) was shaken with freshly distilled benzaldehyde (1ml.) and powdered fused zinc chloride (0.1g.) for 24 hours. The solution was added to a mixture of light petroleum (40-60°)(5ml.) and water (5ml.) which was then shaken for 5 minutes. The aqueous layer was separated, filtered, and washed with light petroleum (40-60°). Removal of the solvent gave the 1,3-O-benzylidene glycerol derivative as a syrup which crystallised from methanol-ether (2:1).

m.p.130-132°, undepressed on admixture with an authentic sample.

$[\alpha]_D$,-40.8°. (C 0.5 in water).

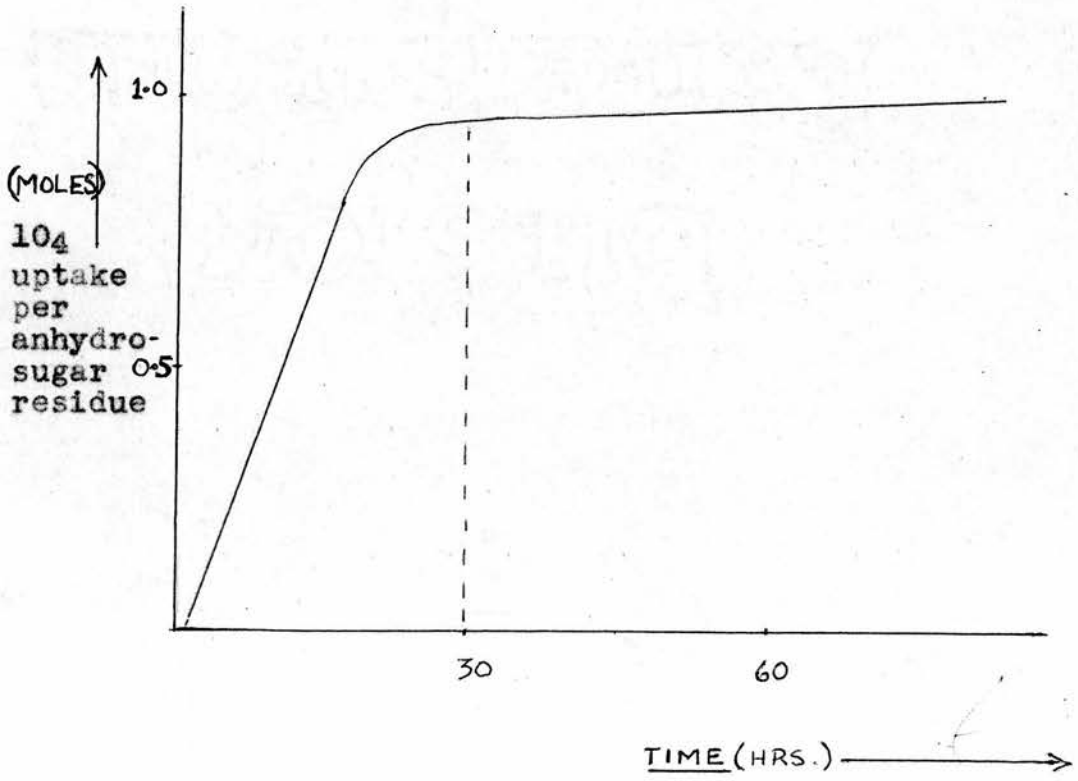
Smith Degradation of Barley Husk Xylan.

Extraction.

The extraction and analysis of this xylan from the husks of 1959 Carlesberg barley is described in Section 1 of thiswork.

Oxidation.

The xylan (1g.) was dissolved in periodic acid (100ml. ; 0.15M.) and the solution kept in the dark at 5°. The concentration of periodate ions was measured periodically using the titrimetric method of Fleury and Lange. By plotting periodate consumption against time, it was clear that normal glycol cleavage was complete within 30 hours.



The xylan (20g.) was treated with periodic acid (2l. ; 0. 5M.) at 5° in the dark for 30 hours, when the periodate uptake was found to be 0.94 mole per anhydro pentose residue.

The remaining periodate ions were destroyed by addition of ethylene glycol and the iodate ions precipitated as barium iodate, by addition of barium hydroxide and barium carbonate. The solution was centrifuged and filtered, and finally deionised with resins.

Borohydride reduction.

The oxypolysaccharide in water (1l.) was reduced by treatment with potassium borohydride (10g.) overnight at room temperature. Ions were removed by shaking alternatively with 1R-120(H) and 1R-4B(OH) resins, filtering, and finally evaporating several times with methyl alcohol.

Partial hydrolysis.

The syrup was dissolved in sulphuric acid (500ml. ;1N.) and the solution allowed to remain at room temperature. Its specific rotation rose slightly during the first hour.

After 5 hours the solution was neutralised with barium hydroxide and barium carbonate, then centrifuged, filtered, and concentrated to a small volume. Treatment with resins removed ions and removal of the solvent left a syrup, 18.8g.

Chromatographic examination

Chromatographic examination of the syrup in solvents A and C using sprays A and B and J showed the syrup to contain a very high proportion of glycerol and glycollic aldehyde with traces of slower moving compounds, both reducing and non-reducing.

Fractionation on charcoal column.

The syrup was placed on top of a charcoal-celite column (4cm.x28cm.) and a gradient elution of the column carried out ranging from water alone up to water containing 30% ethanol. The eluant (6l.) was drawn from equal reservoirs of water (3l.) maintained at the same height. 40 minute fractions were collected, each containing 40-45ml.. A sample (3ml.) from every third fraction was evaporated to dryness and examined chromatographically in solvent A using sprays B and J, in order to establish the elution pattern.

Batches of tubes were bulked to give 10 fractions which were evaporated to syrups and examined chromatographically in solvents A and C using sprays A and B. Each fraction (4mg.) was hydrolysed with N-sulphuric acid at 100° for 6 hours and the products examined in solvents A and C using sprays A and B. The results are given in table (V11).

Table (V11).

Fraction	Tube Nos.	Weight (mg.)	R_{xylose} values in solvent A.	Hydrolysis products.
1.	0-20 (water eluant)	17.4g.	1.70;1.31;1.18;	Xylose,arabinose (trace)
			1.0;0.84.	glycerol,erythritol,etc.
2.	0-39	206	1.31;1.0;0.84.	xylose,arabinose (trace) glycerol.
3.	40-44	94	1.31;1.0	xylose, glycerol.
4.	45-48	70	1.31;1.0	xylose, glycerol.
5.	49-56	96	1.0;0.75	xylose,arabinose, glycerol.
6.	57-62	130	1.0;0.75;0.34	xylose, arabinose, glycerol.
7.	62-65	14	0.62;0.34	xylose, glycerol.
8.	66-75	80	0.62;0.34	xylose, glycerol.
9.	76-88	74	0.62;0.64;0.55	xylose,arabinose, glycerol.
10.	89-105	58	0.55;0.19	xylose,arabinose, glycerol.
	105-144	Nil.		

From the information in table (V11) and from the approximate relative amounts of hydrolysis products, the fractions appeared to have the following probable composition :

Fraction 1. Ethylene glycol, glycerol, erythritol, xylose, arabinose (trace).

Fraction 2. Glycerol, xylose(trace), xylosyl-glycerol, arabinose (trace).

Fraction 3. Glycerol, xylosyl-glycerol.

Fraction 4. Glycerol(trace), xylosyl-glycerol.

Fraction 5. Xylosyl-glycerol, arabinosylxylosyl-glycerol, (or xylosylarabinosyl-glycerol,)

Fraction 6. Xylosyl-glycerol(trace), arabinosylxylosyl-glycerol, unidentified xylo-oligosaccharide.

Fraction 7. Xylo-oligosaccharide, xylosylxylosyl-glycerol,

Fraction 8. Xylo-oligosaccharide, xylosylxylosyl-glycerol.

Fraction 9. Xylosylxylosyl-glycerol, xylosylxylose (xylobiose), arabinose-xylose containing oligosaccharide.

Fraction 10. Arabinose-xylose containing oligosaccharide, higher xylo-oligosaccharide.

Fractions 3,4,5,and 8 were further investigated. By fractionation of each syrup on Whatman 3MM filter sheets using either solv-

ent A or C a chromatographically pure sample of the major component of each syrup was obtained.

Fractions 3 and 4.

From each of these fractions a non-reducing sugar was obtained having R_{xylose} 1.00 in solvent A.

$[\alpha]_D$, -32.1°(C, 2 in water).

Hydrolysis of a sample with sulphuric acid at 100° gave xylose and glycerol alone.

The sugar ran on chromatograms at the same rate as an authentic sample of 2-O-β-D-xylopyranosyl-glycerol in solvents A,B, and D and in no solvent was a mixture of the two resolvable.

Fraction 5.

From this fraction a chromatographically pure sample of a non-reducing sugar was obtained having an R_{xylose} value of 0.75 in solvent A.

$[\alpha]_D$, -22.4° (C, 1 in water).

Hydrolysis of a sample with sulphuric acid at 100° gave arabinose, xylose, and glycerol alone and in approximately

equimolecular amounts.

Fraction 8.

A non-reducing sugar was obtained from this fraction having an R_{xylose} value of 0.62 in solvent A.

$[\alpha]_D$, -50° (C,0.5 in water)

Hydrolysis of a sample with sulphuric acid at 100° yielded xylose and glycerol alone.

The structures of the above three sugars were determined using the following techniques :

Quantitative estimation of components.

The sugar (30mg. or less estimated by the phenol-sulphuric acid method) was hydrolysed with N-sulphuric acid at 100° for 6 hours and the products separated chromatographically using solvents F and C for the arabinose containing sugar and solvent C alone for the others. Rhamnose was added as a standard for the xylose and arabinose and the percentage glycerol loss found by chromatographing a known weight of glycerol on a separate paper.

The sugars were eluted, made up to a known volume with water, and samples used to determine the sugar content.

Xylose, arabinose, and rhamnose were estimated by the phenol-sulphuric acid method and glycerol estimated by oxidation with an excess of sodium metaperiodate (0.1M.) and determination of the formaldehyde formed by chromotropic acid reagent.

Methylation.

Samples of each sugar (3-4mg.) were dissolved in dimethyl formamide and methylated by Kuhn's method using silver oxide and methyl iodide. The methylated sugar was isolated, methanolysed with methanolic hydrogen chloride (3%) and the methyl glycosides formed identified by gas-liquid chromatography, using a column of butanediol succinate polyester on celite as liquid phase, and argon as vapour phase. The chromatograms were run at a temperature of 150°. Besides the peaks due to the glycosides of xylose and arabinose methyl ethers, a peak was obtained in all cases with an extremely low retention time which was probably due to 1,3-di-O-methyl-glycerol.

Periodate Oxidation.

The sugars (10-20mg.) were each dissolved in aqueous sodium metaperiodate solution (0.1M.) at room temperature and the periodate uptake measured spectrophotometrically. Any formic acid formed was determined by titration with standard sodium hydroxide (0.01N) using methyl red as indicator.

Samples of the solutions were used to test for formaldehyde by chromotropic acid reagent.

The solutions were deionised by shaking with 1R-120(H) and 1R-4B(OH) resins and then evaporated to syrups which were hydrolysed with N-sulphuric acid at 100° for 6 hours. Chromatographic examination of the products showed the presence or absence of unattacked sugar residues (other than glycerol).

The results of these investigations are shown in table (V111).

The two different methyl ethers of xylose from the Fraction 8 sugar occurred in approximately equimolecular amounts, as did the xylose and arabinose methyl ethers from Fraction 5 sugar.

Table (VIII).

Experiment		Sugar isolated from :		
		Fractions 3&4	Fraction 5	Fraction 8.
Quantitative estimation (mole/sugar mole)		xylose = 1.0 glycerol = 1.1	xylose = 0.9 arabinose=0.9 glycerol =1.1	xylose = 1.8 glycerol =0.9
Methylation studies (methyl glycosides)		2,3,4Me_3Xyl. 1,3Me_2Gly.(?)	2,4Me_2Xyl. 2,3Me_3Ara 1,3Me_2Gly(?)	2;3;4Me_3Xyl. 2,3Me_2Xyl. 1,3Me_2Gly(?)
PERIODATE STUDIES	Uptake mole/sugar mole	1.9	0.8	2.8
	acid formed (mole/sugar mole	0.9	Nil.	1.0
	Formaldehyde formed	Nil.	Nil.	Nil.
	Unattacked residues	Nil.	Xylose	Nil.

Xyl=D-xylose; Ara=L-arabinose; Gly=glycerol; Me=methyl.

Smith Degradation of a Rye Flour Xylan.

Extraction.

(For full details of extraction and fractionation, see R. Sturgeon, Ph.D. Thesis, Edinburgh, 1958).

The raw grain was ground to a fine powder and extracted with 80% ethanol. Extraction of the residue with water at 40° and centrifugation of the extract gave a clear solution, to which, after concentrating, was added acetone (2 volumes) to precipitate polysaccharide. The dried precipitate represented 0.85% of the rye flour.

Purification.

The polysaccharide (4.9g.) was dissolved in water (200ml) and added to a solution of salivary α-amylase (200ml.) in sodium acetate buffer (pH5). After incubation at 35° for 3 days the polysaccharide was precipitated with acetone (2 volumes) and separated off. Examination of the filtrate showed the presence of glucose.

The polysaccharide was reprecipitated twice from water and dried.

Analysis of the Xylan.

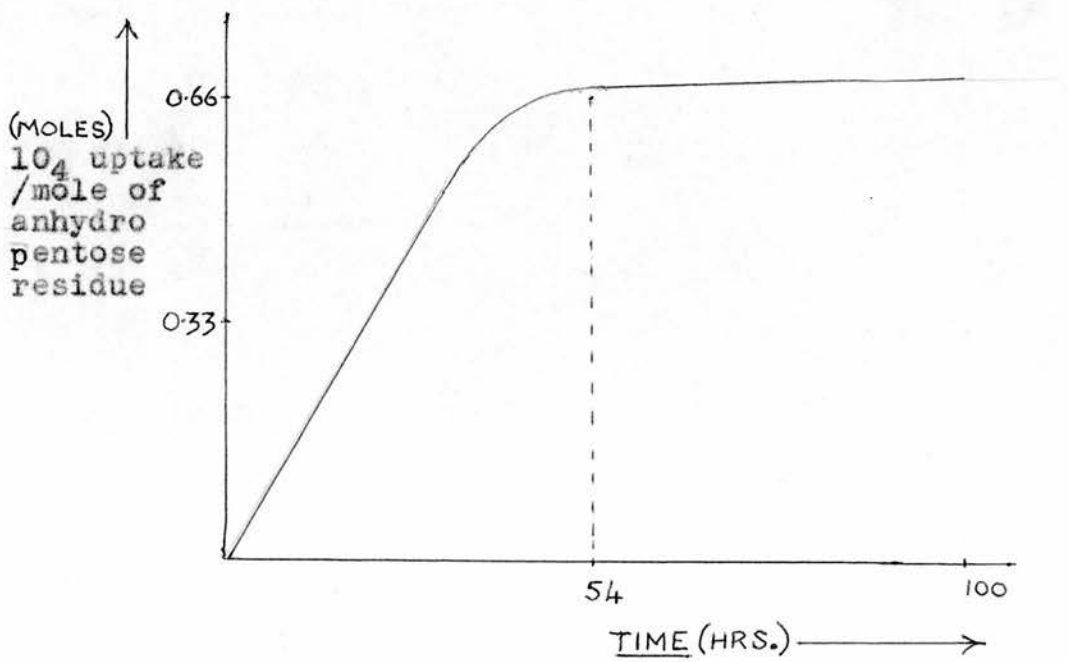
$[\alpha]_D$ -97° (C,1 in 0.5N-sodium hydroxide).

Hydrolysis of the polysaccharide with N-sulphuric acid at 100° for 6 hours and estimation of the sugars formed by quantitative paper chromatography using solvent F gave the following results :

Sugar	%
xylose	57.6
arabinose	25.2
glucose	8.5

Periodate oxidation.

The polysaccharide (0.5g.) was dissolved in water (23ml.) by warming and aqueous sodium metaperiodate solution (23ml. ; 0.3M) added to the cooled solution. The solution was kept in the dark and the periodate uptake measured periodically by the method of Fleury and Lange. The uptake was plotted against time to obtain the time required for normal glycol cleavage to go to completion.



The xylan (4.4g.) was treated as before with aqueous sodium metaperiodate (400ml. ;0.15M.) and the periodate uptake after 54 hours found to be 0.67 of a mole per mole of anhydro pentose residue, measured titrimetrically.

The excess of periodate ions was destroyed by addition of ethylene glycol and the solution shaken with 1R-120(H) resin. Iodate ions were precipitated by addition of barium hydroxide and barium carbonate, the solution centrifuged, and finally filtered.

Borohydride reduction.

The solution was treated with potassium borohydride (3.0g.) at room temperature for 20 hours. Excess borohydride was destroyed by shaking with 1R-120(H) resin and several evaporations with methyl alcohol removed borate ions.

Partial hydrolysis.

Sulphuric acid (100ml. ; 2N) was added to a solution of the polyalcohol in water (100ml.). After 3 hours at room temperature the acid was neutralised by addition of barium hydroxide solution and barium carbonate. The solution was centrifuged and filtered, concentrated to a small volume, and ethanol (5 volumes) added. A slight precipitate was obtained which was removed and subjected to a further treat-

ment with N-sulphuric acid for 2 hours. The solution was treated as before but on addition of alcohol to the neutral concentrated solution no precipitate was obtained.

The combined ethanolic solutions were evaporated to a syrup, 4.1g.

Examination of syrup.

Chromatographic examination of the syrup in solvents A and B using spray B showed the presence of compounds with identical R_{xylose} values to glycerol and xylosyl-glycerol. Two slower moving compounds were also detected having R_{xylose} values of 0.62 and 0.35 in solvent A, together with faster moving compounds having R_{xylose} values of 1.21 and 1.30. No reducing spots were detected except one corresponding to glycollic aldehyde.

Separation of oligosaccharides.

The syrup (3.6g.) was fractionated on a column of Dowex 50 WX3 resin of 200-400mesh, in the form of its barium salt (2.8cm.x 100cm.). The column was eluted with water and 15 minute fractions, each of 1.75ml. volume, collected. Every second fraction was concentrated and examined chromatographically in solvent A using sprays B and J.

Table (1X) shows the elution pattern.

Table (IX).

Fraction	Weight	Tube Nos.	Total eluant	R_{xylose} values (solvent A)
1	-	1-104	181ml.	no sugar present
2	87mg.	105-114	15.8ml.	0.618(trace);0.352
3	170mg.	115-130	26.2ml.	0.618;0.352(trace)
4	-	131-133	3.5ml.	no sugar present.
5	764mg.	134-145	19.2ml.	1.00(major);1.21(minor)
6	0.8g.	146-152	10.5ml.	1.00;1.21;1.3(major)
7	1.8g.	153-174	36.8ml.	1.00(trace);1.21 (trace) 1.3(major).

Chromatographically pure samples of the major constituents of fractions 2,3 and 5 were isolated by separation of the syrups on Whatman 3MM filter sheets using solvent A for fractions 2 and 3, and solvent C for fraction 5.

Fraction 2.

The sugar isolated from this fraction had R_{xylose} 0.35 in solvent A and gave only a very faint spot with spray A.

$[\alpha]_D$, -59°(C, 1 in water).

Hydrolysis with sulphuric acid at 100° gave xylose and glycerol alone.

Fraction 3.

The sugar isolated from this fraction had R_{xylose} 0.62 in solvent A and gave only a very slight spot with spray A.

$[\alpha]_D$, -49.1°(C, 1 in water).

Hydrolysis with sulphuric acid at 100° gave xylose and glycerol alone.

Fraction 5

The sugar isolated from this fraction had R_{xylose} 1.00 in solvent A.

$[\alpha]_D$, -34.4°(C, 2 in water).

Hydrolysis with sulphuric acid at 100° gave xylose and glycerol.

The chromatographic mobility of the sugar in solvents A, C and D was identical to that of an authentic sample of 2-O-β-D-xylopyranosyl-glycerol, and in no solvent was a mixture of the two resolvable.

The structures of the compounds were determined using the same techniques of quantitative estimation of sugar resid-

ues, methylation, and periodate oxidation described for the structural work on the oligosaccharides from barley husk xylan. The results are shown in table (X).

Table (X).

Experiment.		Sugar isolated from :		
		Fraction 2	Fraction 3	Fraction 5
Quantitative estimation (Moles/sugar mole)		xylose = 2.8 glycerol=1.1	xylose = 2.1 glycerol=1.1	xylose = 0.9 glycerol=1.0
Methylation studies. (methyl glycosides)		2,3,4Me_3Xyl. 2,3Me_2Xyl. 1,3Me_2Gly(?)	2,3,4Me_3Xyl. 2,3Me_2Xyl. 1,3Me_2Gly(?)	2,3,4Me_3Xyl. 1,3Me_2Gly.(?)
PERIODATE STUDIES	Uptake (Mole/sugar mole)	3.8	2.8	1.9
	Acid formed (mole/sugar mole)	1.1	1.0	1.0
	Formaldehyde formed	Nil.	Nil.	Nil.
	Unattacked residues	Nil.	Nil.	Nil.

Xyl = xylose ; Ara = arabinose ; Gly = glycerol. ; Me = methyl

The compound from fraction 2 gave in methylation studies, the trimethyl and dimethyl derivatives of xylose in the approximate ratio of 1 to 2, whereas the same sugars derived from the compound from fraction 3 were in the approximate ratio of 1 to 1.

A quantitative estimation carried out on the degradation mixture showed the foregoing three oligosaccharides to be present in the molar ratio of 7.5:2.2:1. The sugars were estimated by the phenol-sulphuric acid method.

SECTION 111.

Synthesis and Alkaline Degradation of Model Compounds.

Discussion.

The understanding of alkaline degradation of polysaccharides greatly increased after the work of Kenner, Richards, and Corbett (76-83) on the degradation of model compounds. From their studies on monosubstituted monosaccharides, highly significant results were obtained which led to an interpretation of the mechanism of polysaccharide degradation on the basis of a β-elimination from a dicarbonyl intermediate, formed from the reducing moiety. In this way degradation can proceed in a step-wise manner along a linear chain of sugar residues starting from the reducing end.

When the more complicated problem of branched polysaccharides is considered however, the degradative mechanism in some cases is not clear, and indeed it has been open to doubt whether, in fact, alkaline degradation is capable of passing certain types of branch points in the polymer chain or not. Whistler and BeMiller (102) have postulated that in a 1,4-linked polysaccharide containing branching through position 3 (for example arabinoxylans), alkaline degradation must cease on reaching a branch point. This appears to be contrary to

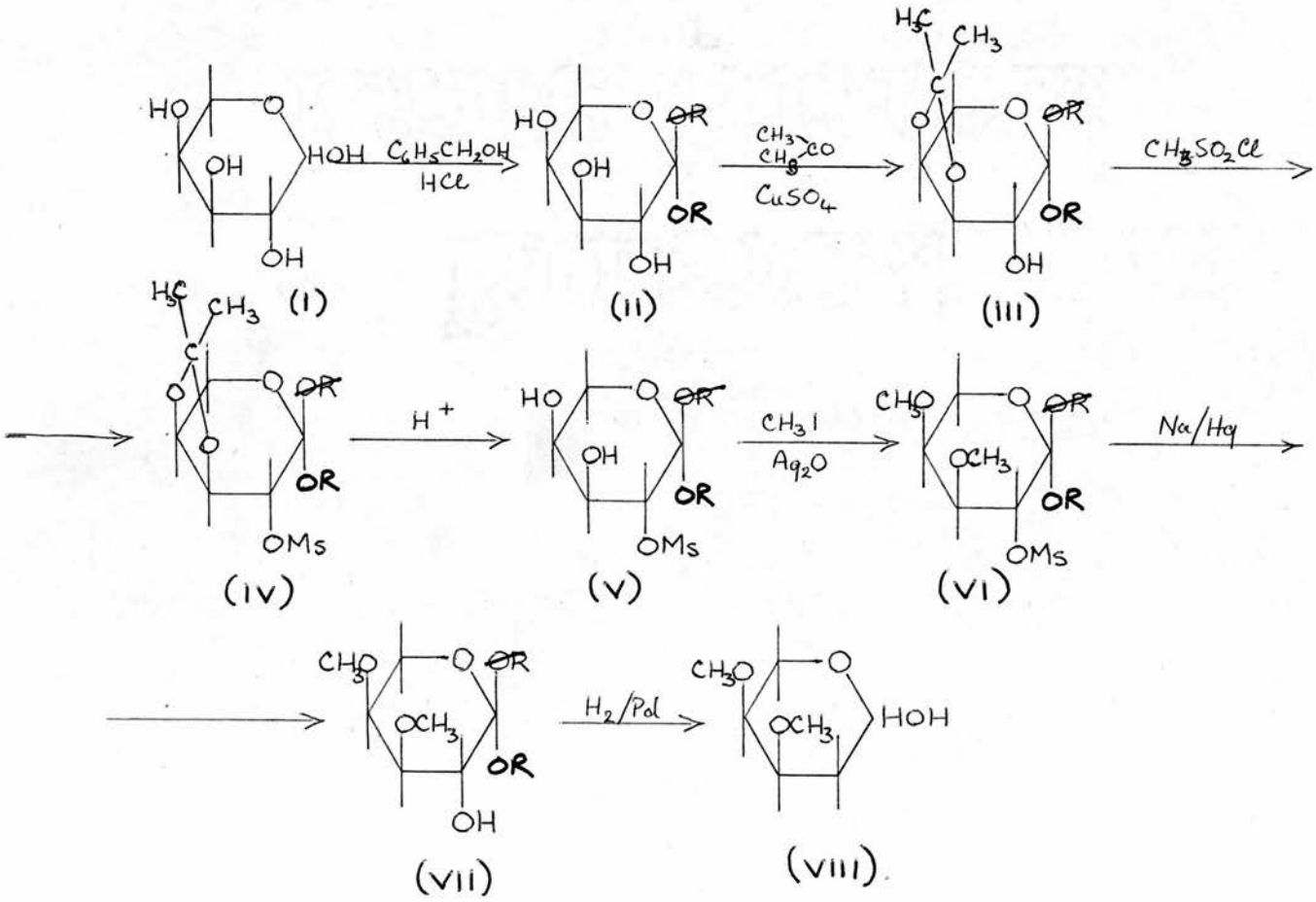
the recent findings of Aspinall et al, (100), who detected a decrease in molecular weight of about 20% on degrading a rye flour xylan containing a high proportion of arabinose side-chains. This particular highly branched xylan is the one examined in Section 11 of this work, and the conclusion reached was that the branch points are randomly distributed along the basal xylan chain. Thus in order to achieve a molecular weight decrease of 20% it is inevitable that at least one branch point, and probably many more, has been successfully by-passed in the step-wise degradation. Further support for this view comes from the suggestion of Aspinall et al(100) that the high proportion of lactic acid formed during their degradation experiments originated from liberated arabinose side-chains.

In an attempt to solve this problem, and to investigate the degradative mechanism involved, the following model compounds were synthesised and subjected to treatment with alkali: 3,4-di-O-methyl-L-arabinose, 3,4-di-O-methyl-D-xylose, and 3-O-methyl(4-O-β-D-xylopyranosyl)-D-xylose. In the course of the synthesis of the last named sugar, the parent disaccharide was synthesised by a parallel series of reactions. This disaccharide, 4-O-β-D-xylopyranosyl-D-xylose (xylobiose), has been detected, and in some cases isolated, by partial hydrolysis

of many xylans (37), and its synthesis supplies absolute proof of its structure.

3,4-Di-O-methyl-L-arabinose was synthesised by the series of reactions outlined in Figure 1. After introducing blocking groups into positions 1,2,3, and 4 of L-arabinose, positions 3 and 4 were freed, leaving them susceptible to methylation (116,122). Removal of the substituents in positions 2 and 1 left the required dimethyl ether of L-arabinose.

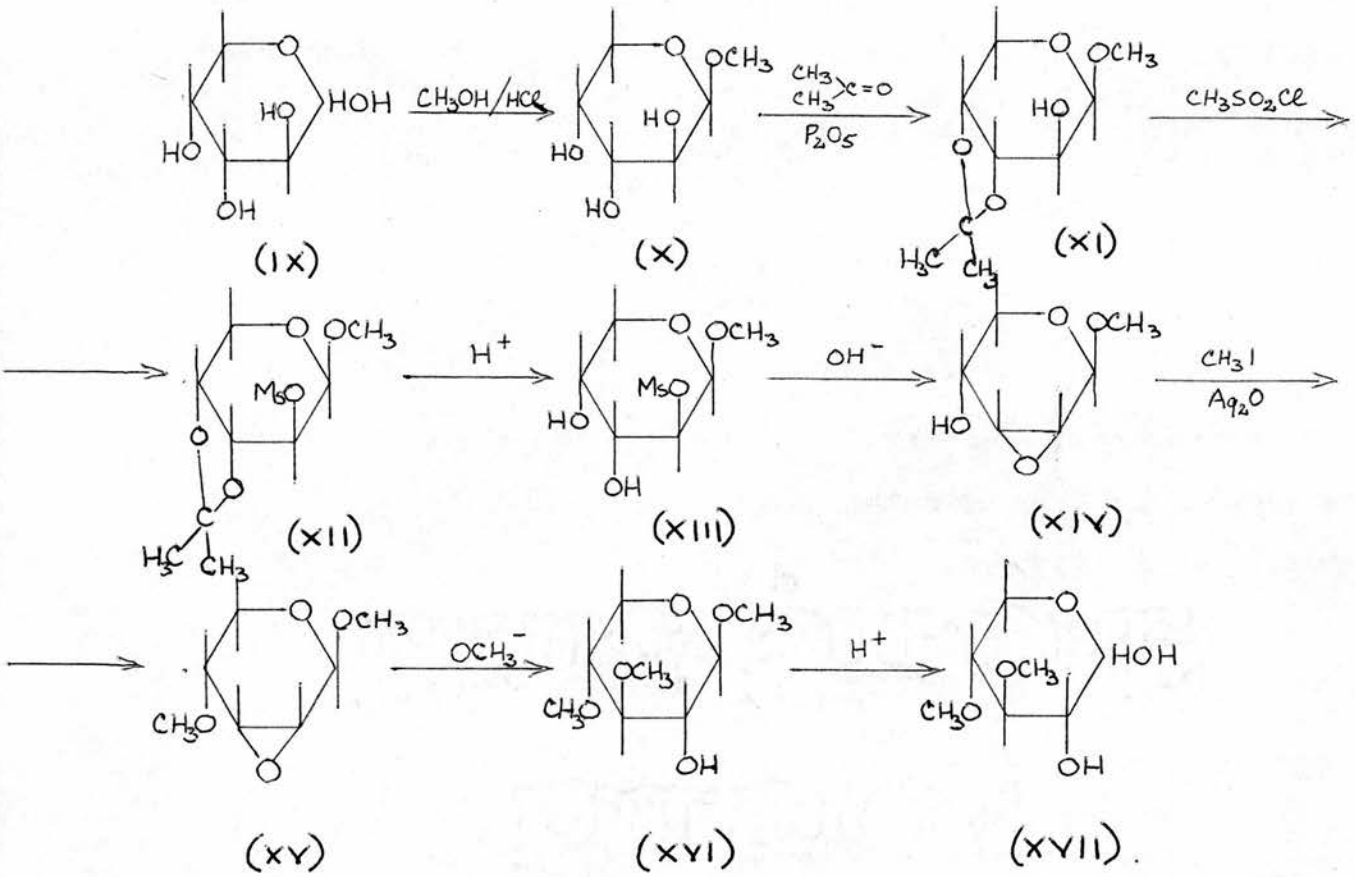
Figure 1. Synthesis of 3,4-di-O-methyl-L-arabinose.



L-Arabinose (1) was converted to the corresponding β-benzyl glycoside (11), which on condensation with acetone gave benzyl 3,4-O-isopropylidene-β-L-arabinopyranoside (111). Treatment of this compound with methane sulphonyl chloride in pyridine furnished the 2-methanesulphonyl derivative (1V). The isopropylidene grouping was removed by hydrolysing with N-sulphuric acid and the product obtained (V) was methylated by Purdie's method to give the crystalline benzyl 3,4-di-O-methyl-2-O-methanesulphonyl β-L-arabinopyranoside. Demesylation of this compound was carried out using sodium amalgam, and removal of the benzyl grouping by hydrogenation over a palladium catalyst yielded 3,4-di-O-methyl-L-arabinose as a syrup.

3,4-Di-O-methyl-D-xylose was synthesised by the method of Hough and Jones (117) with the exception that the 2-O-methanesulphonyl derivative of D-arabinose ~~used~~ instead of the 2-O-toluenesulphonyl derivative was used, since the 2-O-mesyl derivatives are crystalline. The method involves the synthesis of methyl 2,3-anhydro-β-D-ribopyranoside, which can easily be methylated in position 4. On opening the epoxide ring with sodium methoxide the dimethyl ether of methyl β-D-xylopyranoside only is obtained and not the D-arabinose derivative. The series of reactions is outlined in Figure 2.

Figure 2. Synthesis of 3,4-di-O-methyl-D-xylose.



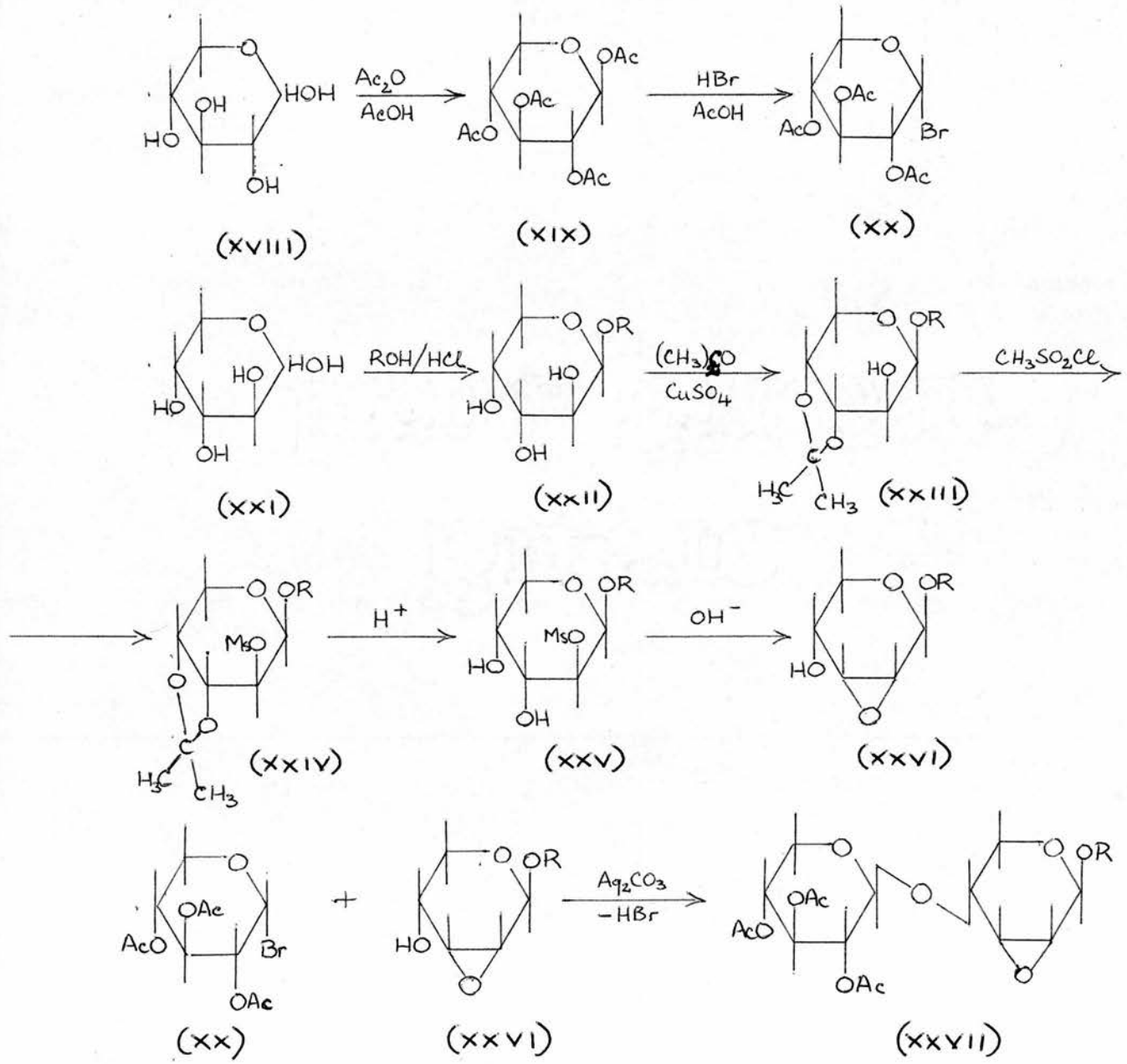
Ms = methanesulphonyl.

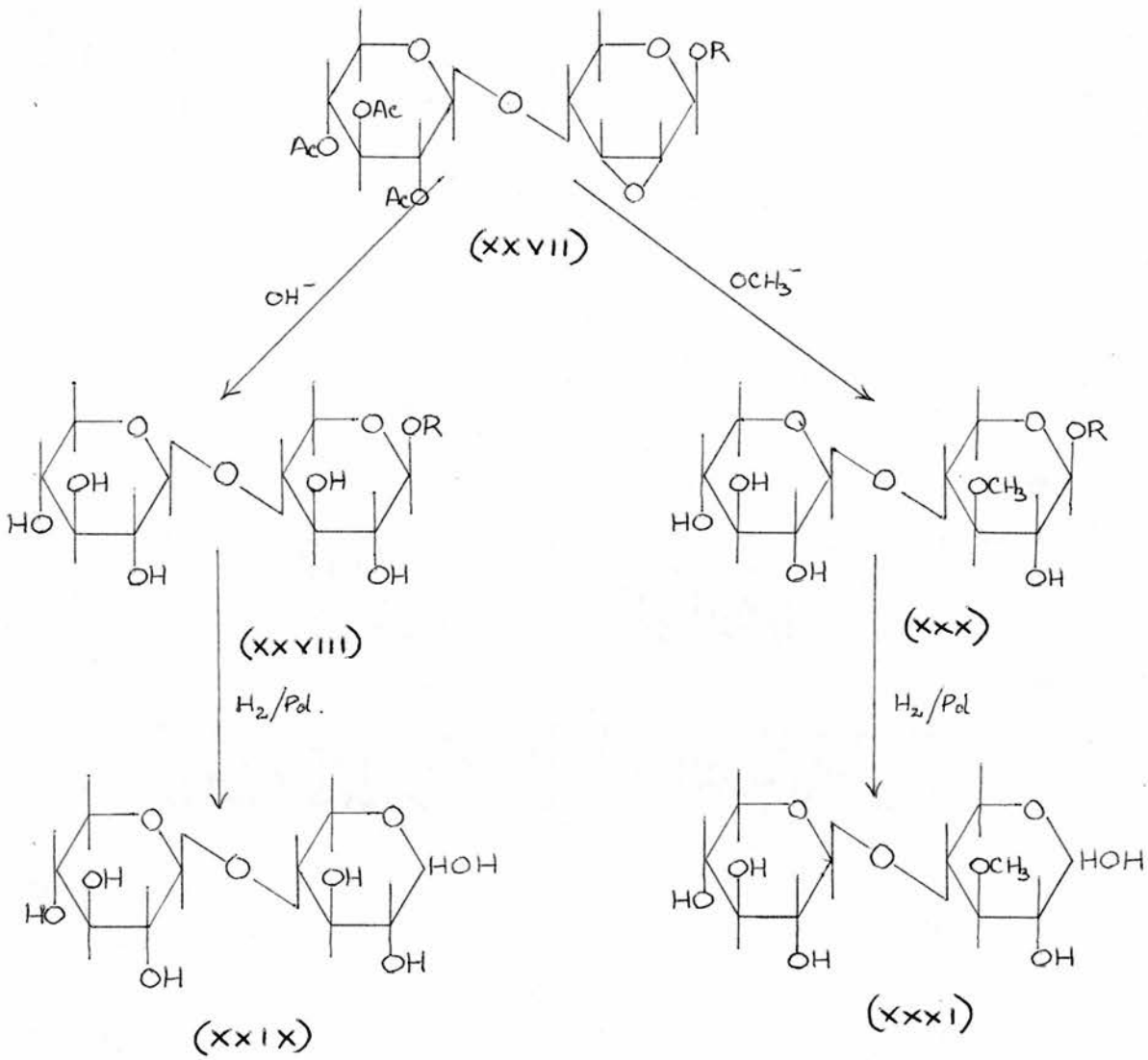
Methyl-β-D-arabinopyranoside (X), prepared from D-arabinose (IX) by treatment with methanolic hydrogen chloride, was condensed with acetone in the presence of phosphorus pentoxide to yield methyl 3,4-O-isopropylidene-β-D-arabinopyranoside

(XI). Treatment of this compound with methanesulphonyl chloride in pyridine afforded the crystalline 2-O-methanesulphonyl derivative (XII). The isopropylidene grouping was removed by hydrolysing with N-sulphuric acid and treatment of the compound produced (XIII) with dilute sodium hydroxide at 75° furnished methyl 2,3-anhydro-β-D-ribopyranoside (XIV). A Purdie methylation using silver oxide and methyl iodide converted this compound (XIV) to its 4-O-methyl ether (XV), which gave methyl 3,4-di-O-methyl-β-D-xylopyranoside (XVI) on refluxing with sodium methoxide. The glycosidic group was removed by acid hydrolysis and 3,4-di-O-methyl-D-xylose (XVII) was obtained as a syrup.

The disaccharides 3-O-methyl-4-O-(β-D-xylopyranosyl)-D-xylose (XXXI) and 4-O-β-D-xylopyranosyl-D-xylose (XXIX) were synthesised via a Koenigs-Knorr condensation of 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide (XX) with benzyl 2,3-anhydro-β-D-ribopyranoside (XXVI). The series of reactions involved are outlined in Figure 3.

Figure 3. Synthesis of 4-O-(β-D-xylopyranosyl-D-xylose and 3-O-methyl-4-O-(β-D-xylopyranosyl)-D-xylose.





Ms = CH_3SO_2 R = $CH_2C_6H_5$

Initially benzyl 2-O-methanesulphonyl-β-D-arabinopyranoside (XXV) was prepared, using the method of Wood and Fletcher (116), already described for the L-series of arabinose derivatives in the synthesis of 3,4-di-O-methyl-L-arabinose. The action of dilute sodium hydroxide on this compound afforded the crystalline epoxide, benzyl 2,3-anhydro-β-D-ribopyranoside (XXVI).

A Koenigs-Knorr condensation of this epoxide with 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide (XX) furnished benzyl 2,3-anhydro-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-ribopyranoside (XXVII) in much better yield than is often obtained in disaccharide synthesis involving condensations of "acetohalogen sugars" with secondary hydroxyl groups of sugar derivatives. The hydroxyl group in the epoxide (XXVI) is much less sterically hindered than most secondary hydroxyl groups in otherwise fully substituted sugar derivatives. It is noteworthy that Jones and Curtis (118) have made a similar observation with regard to the condensation of "acetohalogen sugars" with secondary hydroxyl groups in acyclic derivatives of sugars.

In most applications of the Koenigs-Knorr reaction it has been found that a Walden inversion accompanies replacement of the halide group, and the glycosidic linkage formed thus

possesses a configuration opposite to that of the parent glycosyl halide. Nevertheless several reactions have been reported in which the anomeric configuration of the product is the same as in the glycosyl halide used. In general, when a glucosyl or xylosyl halide is used, inversion of configuration takes place if the substituent on C_2 is cis with respect to the halogen atom, but if this substituent is in the transposition, inversion of configuration does not necessarily follow.

Treatment of the condensation product (XXVII) with sodium hydroxide resulted in de-acetylation and opening of the epoxide ring to give benzyl 4-O-β-D-xylopyranosyl-β-D-xylopyranoside (XXVIII) and similar reaction of the disaccharide epoxide with sodium methoxide gave benzyl 3-O-methyl-4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXX). In both cases opening of the epoxide ring proceeded with formation of the D-xylose derivative, and no trace of the D-arabinose derivative could be detected. The relationship between the two benzyl glycosides was confirmed since both afforded the same crystalline pentamethyl ether on methylation with methyl sulphate and sodium hydroxide, and since hydrolysis of benzyl 3-O-methyl-4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXX) gave D-xylose and 3-O-methyl-D-xylose.

Both benzyl glycosides, on hydrogenation over palladium-charcoal, furnished the corresponding disaccharides (XXlX, and XXXl). Although neither disaccharide was obtained crystalline, the identity of 4-O-β-D-xylopyranosyl-D-xylose with the xylobiose formed on partial acid hydrolysis of oak heartwood xylan (119) and other xylans was established by paper chromatography of the sugar and by conversion into the crystalline phenylosazone.

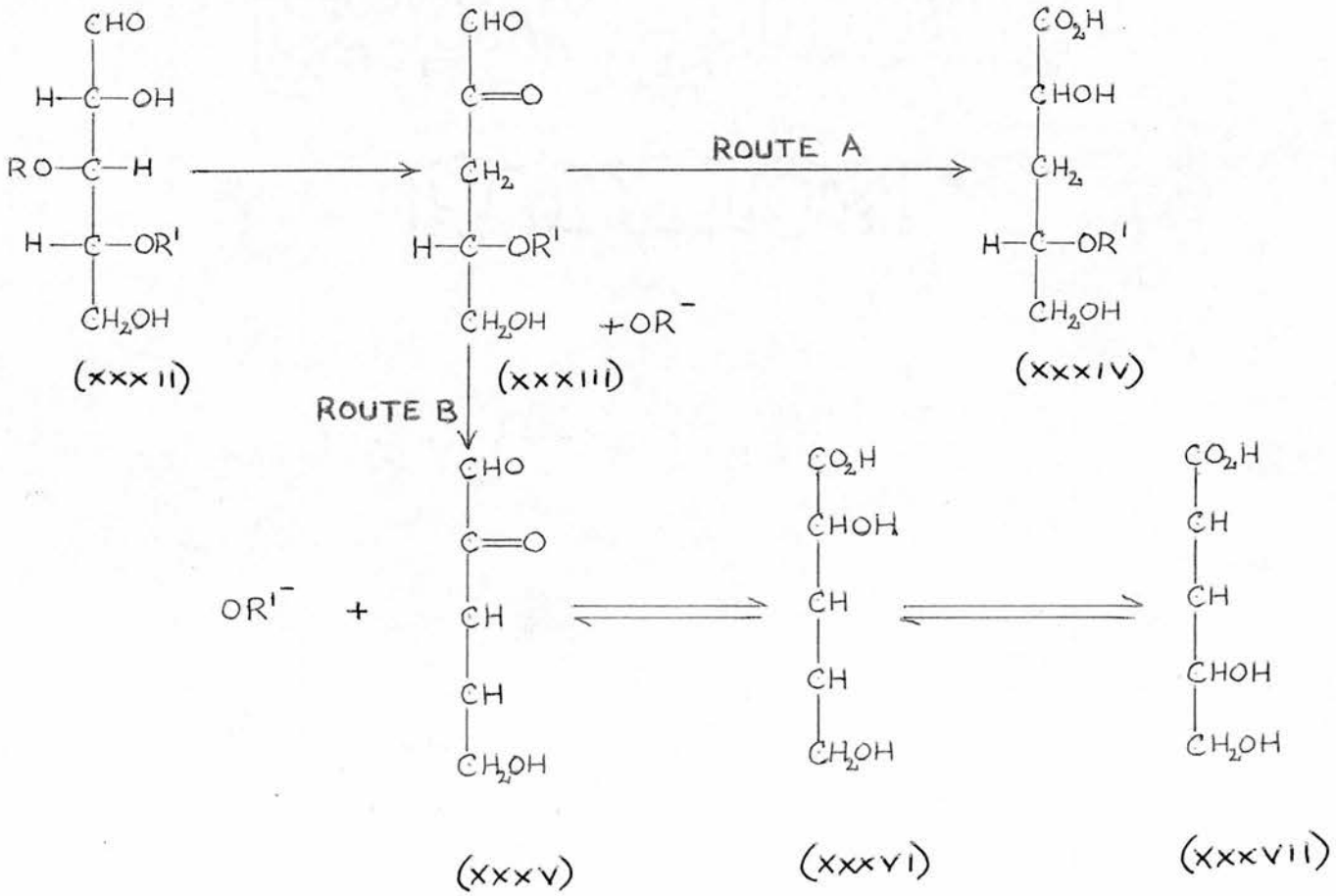
Alkaline degradation of model compounds.

When 3-O-methyl-4-O-(β-D-xylopyranosyl)-D-xylose was treated with cold dilute sodium hydroxide, the disaccharide was almost completely destroyed after 6 hours with the elimination of the substituents at C_3 and C_4. Under the conditions employed no further degradation of xylose could be detected. Methanol was identified by conversion into methyl iodide and by oxidation to formaldehyde, and D-xylose was identified as the di-O-benzylidene dimethylacetal.

The observation that both substituents are rapidly eliminated when this 3,4-di-O-substituted D-xylose is treated with alkali provides clear support for the existance of some "by-passing" mechanism to account for the results of alkaline degradation of branched arabinoxylans.

A possible pathway for this type of degradation (Figure 6) involves the initial formation of an intermediate (XXXlll). If this intermediate (XXXlll) undergoes a benzilic acid type of rearrangement the product (routeA) would be a 4-O-substituted metasaccharinic acid (XXXlV). The foregoing results suggest however, that this intermediate (XXXlll), which is also a β-alkoxy-carbonyl compound, undergoes β-elimination of alkoxide ion OR'^-(route B) with the formation of a compound (XXXV) or some similar compound.

Figure 6. By-passing mechanism.



A compound with no alkyl substituent at C_4 but otherwise identical to structure (XXXlll) has been isolated and characterised by Kato (120), from the reaction mixture of D-xylose and n-butylamine in acid medium.

An attempt was made to characterise the alkali stable compounds formed by the degradation of the above model compounds. Paper chromatography using solvent B showed that the degradation mixtures from each sugar consisted of a variety of compounds.

3,4-Di-O-methyl-L-arabinose gave rise to three acids, the faster moving, R_F 0.49, predominating. The reaction with permanganate of the first and last suggested the presence of unsaturation in the molecules. An appreciable amount of a lactone having R_F 0.54 was also formed, and was followed by a small amount of a slower moving one.

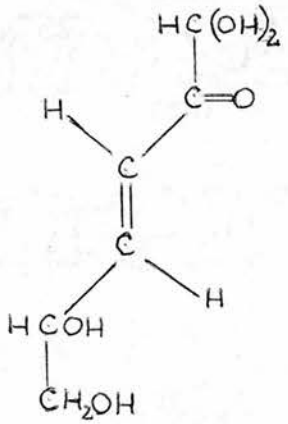
Degradation of 3,4-di-O-methyl-D-xylose gave the same lactones as the arabinose derivative but in smaller amounts. The main acid was the one having R_F 0.43, while the total amount of acid formed appeared to be less than that from dimethyl-arabinose.

Both the arabinose and xylose methyl ethers gave a carbonyl compound, since a precipitate was obtained with 2,4-dimitrophenylhydrazine. This compound contained no methoxyl

group, indicating a double elimination to have taken place.

Degradation of the disaccharide gave the same lactones and acids as the other sugars but in smaller amounts, the main acid being the one which predominated among the degradation products of dimethylxylose.

The ultra-violet spectra of the degradation products suggested the presence of a compound (or compounds) possessing a keto-enol system, which was stable in acid medium. Anet (121) has isolated and characterised unsaturated hexosones (XXXVlll) as by-products in the preparation of 3-deoxy-D-erythro-hexosone from difructoseglycine.



(xxxVlll)

Both the cis and trans isomers were unstable in acid medium, dehydrating to hydroxymethylfurfural. Nevertheless it is possible that compounds similar to (XXXVlll) but contain-

ing five carbon atoms, are formed in the degradation of these sugars.

In conclusion, it is obvious that the degradation mechanism is not simple and that several competing reactions must be taking place simultaneously. However, the evidence obtained clearly shows the existence of some mechanism whereby alkaline erosion of arabinoxylans is not necessarily arrested at branching points through position 3 of D-xylose residues.

Experimental

Synthesis of 3,4-di-O-methyl-L-arabinose.

Benzyl β-L-arabinopyranoside (11)

L-Arabinose (1)(20g.) in benzyl alcohol (100ml.) was cooled in an ice-salt bath and saturated with hydrogen chloride (bubbled for 20 minutes). On shaking overnight at room temperature, crystallisation occurred. Ether (200ml.) was added slowly and the flask and contents left at 5° for 4 hours. The glycoside was filtered off, washed with ether, and recrystallised from alcohol.

Yield, 18.8g. m.p.,171°.

Benzyl 3,4-O-isopropylidene-β-L-arabinopyranoside (111)

Benzyl-β-L-arabinopyranoside (11)(5g.) was suspended in dry acetone (250ml.) and shaken vigorously with phosphorus pentoxide (4 g.) for 5 minutes. The acetone was decanted, the residue washed with acetone (3x10ml.), and the combined extracts shaken with solid potassium carbonate. After filtration and removal of the solvent a pale yellow syrup

was obtained which contained acetone condensation products. The syrup was dissolved in ether and chromatographed on alumina. Elution with ether containing ethanol (5%) yielded the crystalline isopropylidene derivative (111).

Yield, 3.1g. m.p., 57°.

Benzyl-β-L-arabinopyranoside (5g.) in dry acetone (250ml.) was shaken with anhydrous cupric sulphate (15g.) and concentrated sulphuric acid (0.25ml.) for 24 hours. The acetone solution was separated, the acid neutralised by passing in dry ammonia, salts removed by filtration, and the solution concentrated to a syrup. The syrup was purified as before by chromatographing on alumina.

Yield, 4.2g. m.p., 57-58°.

Since this method afforded the better yield it was used in a larger scale preparation of the isopropylidene compound (111).

Benzyl 3,4-di-O-isopropylidene-2-O-methylsulphonyl-β-L-arabinopyranoside (1V).

Benzyl 3,4-di-O-isopropylidene-β-L-arabinopyranoside (111)(10.0g.) was added with stirring to a mixture which had been made from dry pyridine (30ml.) and methane sulphonyl chloride (3.65ml.) precooled to 10°. After 2 hours at room temperature the excess of methane sulphonyl chloride was de-

composed by the addition of a little water. An excess of aqueous sodium bicarbonate solution (10%) was then added and the solution evaporated to dryness, several additions of water being made to remove the final traces of pyridene. The residue was extracted with chloroform and removal of the solvent yielded the mesyl compound (IV) as a syrup.

Yield, 10.8g.

Benzyl 2-O-methylsulphonyl-β-L-arabinopyranoside (V).

The syrupy benzyl 3,4-di-O-isopropylidene-2-O-methane sulphonyl-β-L-arabinopyranoside (IV)(10.8g.) was diluted with acetone (15ml.) and sulphuric acid (150ml.,1N.) and the solution refluxed for 3 hours, most of the acetone escaping in the process. The product, benzyl 2-O-methylsulphonyl-β-L-arabinopyranoside (V) crystallised on cooling and was removed by filtration. Recrystallisation from methanol-water (3:1), then from water alone, yielded the pure compound.

Yield, 7.2g. m.p., 128-129°

$[\alpha]_D$, +170(C2 in chloroform-ethanol(5:1)).

Benzyl 3,4-di-O-methyl-2-O-methanesulphonyl β-L-arabinopyranoside (VI)

Benzyl 2-O-methanesulphonyl-β-L-arabinopyranoside (V) (7.0g.) was dissolved in dry acetone (30ml.) and methyl iodide added (60ml.). The solution was refluxed for 6 hours, silver oxide (12g.) being added in four additions over the first 4 hours. On cooling the silver salts were removed by filtration and washed with chloroform (3 x 30ml.). The filtrated were combined and removal of the solvent yielded a yellow syrup, OMe, 6.2%. The methylation was repeated six times and the syrup obtained chromatographed on alumina. Elution with light petroleum (60-80°) - benzene (1:4) yielded a syrup which crystallised in the vacuum desiccator. Recrystallisation from water-ethanol(2:1) afforded colourless crystals, 6.8g.

m.p. 55-57° $[\alpha]_D$, +188° (C,2 in methanol.).

OMe, 19.8%. $C_{15}H_{22}O_7$ requires OMe, 19.7%.

Benzyl 3,4-di-O-methyl-β-L-arabinopyranoside (VII).

Benzyl 3,4-di-O-methyl-2-O-methanesulphonyl-β-L-arabinopyranoside (VI),(6.7g.) in methanol-water (4:1) was stirred for 48 hours with 4% sodium amalgam (100g.). The

solution was decanted from the mercury and neutralised by passage of carbon dioxide gas. Inorganic salts were removed by filtration, and the solution concentrated. Extraction of the solution with chloroform and removal of the dried solvent left a syrup, 5.1g.

$[\alpha]_D$, +221°(C,0.6 in chloroform).

OMe, 23.1%. $C_{14}H_{20}O_5$ requires OMe, 23.1%.

3,4-Di-O-methyl-L-arabinose (VIII).

Benzyl 3,4-di-O-methyl-β-L-arabinopyranoside (VII)(5.1g.) was dissolved in ethanol (100ml.) and shaken in hydrogen at atmospheric pressure over 10% palladium-charcoal (5g.) for 48 hours. The catalyst was filtered off and concentration of the filtrate afforded syrupy 3,4-di-O-methyl-L-arabinose (VIII), 3.1g.

$[\alpha]_D$, +110° (C,2 in water).

OMe,34.7% $C_7H_{14}O_5$ requires OMe, 34.8%.

Arabonamide. The sugar (30mg.) was oxidised with bromine water for 2 days at room temperature. The lactone was recovered in the usual way and dissolved in methanol (2ml.)

saturated with ammonia. After standing at 0° overnight the solvent was removed to give the crystalline arabonamide, which was recrystallised from acetone.

m.p., 133° undepressed on admixture with an authentic sample.

Synthesis of 3,4-di-O-methyl-D-xylose.

Methyl-β-D-arabinopyranoside (X)

D-Arabinose (30g.)(1X) was refluxed for 7 hours with methanolic hydrogen chloride (1%). The methyl glycoside crystallised out during removal of the solvent, was filtered off and washed with methanol (3 x 50ml.). The filtrates were combined and recycled to yield more crystalline glycoside. Recrystallisation from ethanol afforded pure methyl-β-D-arabinopyranoside (X), 24.3g.

m.p., 168-170°. $[\alpha]_D$, -233° (C,0.77 in water).

Methyl 3,4-O-isopropylidene-β-D-arabinopyranoside (X1).

Methyl-β-D-arabinopyranoside (X)(24.2g.) was suspended in acetone (1l.) and shaken with phosphorus pentoxide (16g.) for 10 minutes. The acetone was decanted, the residue washed with acetone (3 x 100ml.), and the combined extracts shaken with anhydrous potassium carbonate to remove any acid. Filtration and removal of the solvent gave a pale yellow syrup which was dissolved in ether and chromatographed on alumina. Elution with ether containing ethanol (5%) yielded the isopropylidene derivative as a colourless syrup, 20.5g.

$[\alpha]_D$, -200° (C,0.66 in chloroform.)

OMe, 15.3%. $C_9H_{16}O_5$ requires OCH_3, 15.3%.

Methyl 3,4-O-isopropylidene-2-O-methanesulphonyl-β-D-arabinopyranoside (XII).

Methyl 3,4-O-isopropylidene-β-D-arabinopyranoside (XI), (20g.) in dry pyridine (100ml.) was cooled and treated with methanesulphonyl chloride (10ml.). After standing overnight at 40°, the solution was cooled, diluted with water, and the product crystallised out spontaneously. Recrystallisation from alcohol gave clear needle-like crystals, 21.5g.

m.p. 138-139° $[\alpha]_D$, -184° (C,1.1 in chloroform)

OMe_3,10.8%. $C_{10}H_{18}O_7S$ requires OMe, 10.9%.

Methyl-2-O-methanesulphonyl-β-D-arabinopyranoside (XIII).

Methyl 3,4-O-isopropylidene-2-O-methanesulphonyl-β-D-arabinopyranoside (XII)(21.4g.) was dissolved in sulphuric acid (500ml.,1N.) and acetone (100ml.) and refluxed for 4 hours. The cooled solution was neutralised with barium hydroxide and barium carbonate, filtered, and concentrated to a syrup which crystallised from alcohol-water (5:1) at

-5°, 13.1g.

m.p. 67°, $[\alpha]_D$, -160° (C,2 in chloroform-ethanol (5:1)).

OCH_3, 12.8%. $C_7H_{14}O_7S$ requires OMe, 12.8%.

Methyl 2,3-anhydro-β-D-ribopyranoside (XIV).

2N-sodium hydroxide was added with stirring to methyl-2-O-methylsulphonyl-β-D-arabinopyranoside (V)(8.5g.) in ethanol (25ml.) at 75° until the solution was permanently alkaline, Sodium methanesulphonate was filtered off and the filtrate taken to dryness. Extraction of the residue with ethyl acetate (5 x 10ml.), and removal of the solvent gave a colourless syrup which was chromatographed on alumina. Elution with ether-ethanol (1:1) afforded crystalline methyl 2,3 anhydro-β-D-ribopyranoside, 3.0g.

m.p. 48-50°. $[\alpha]_D$, -50.5° (C,0.66 in $CHCl_3$).

~~Hough and Jones~~ HONEYMAN report $[\alpha]_D$ +36.8° for the L isomer.

OMe, 21.4% $C_6H_8O_4$ requires OMe, 21.5%.

Methyl 4-O-methyl 2,3-anhydro-β-D-ribopyranoside (XV).

Methyl 2,3-anhydro-β-D-ribopyranoside (VI)(3.0g.) was

dissolved in methyl iodide (40ml.) and refluxed for 6 hours, silver oxide (5g.) being added gradually over the first 4 hours. Silver salts were removed from the cooled solution by filtration and washed with chloroform (3 x 15ml.). The filtrates were combined and evaporation of the solvent gave a colourless syrup which partially crystallised on standing in the desiccator. The methylation was repeated and recrystallisation of the product from light petroleum (40-60°) afforded crystalline methyl 4-O-methyl-2,3-anhydro-β-D-ribopyranoside (XV), 2.8g.

m.p. 72-73°. $[\alpha]_D$, -7° (C, 2 in water).
OMe, 38.6% $C_7H_{10}O_4$ requires OMe, 38.8%.

Methyl 3,4-di-O-methyl-β-D-xylopyranoside (XVI).

Methyl 4-O-methyl-2,3-anhydro-β-D-ribopyranoside (XV) (2.8g.) was heated in methanol (100ml.) containing sodium (6g.) for 24 hours. The solution was concentrated, diluted with water, and continuously extracted with hot chloroform for 24 hours. The extract was dried over anhydrous sodium sulphate, filtered, and evaporation of the solvent gave methyl 3,4-di-O-methyl-β-D-xylopyranoside (XVI) which recrystallised from ether-light petroleum (40-60)(1:1), 2.6g.

m.p. 85-87°. $[\alpha]_D$, -68° (C, 2 in water).

OMe, 48.5%, $C_8H_{16}O_5$ requires OMe, 48.4%.

3,4-Di-O-methyl-D-xylose (XVII).

Methyl 3,4-di-O-methyl- -D-xylopyranoside (XVI)(2.5g.) was hydrolysed with hydrochloric acid (50ml.,0.5N) on a boiling-water bath for 6 hours. Neutralisation of the acid with silver carbonate, followed by filtration, and evaporation of the solvent gave syrupy 3,4-di-O-methyl-D-xylose (XVII), 2.0g.

$[\alpha]_D$, +12° (C, 1. 5 in methanol).

OMe, 34.8%. $C_7H_{14}O_5$ requires OMe, 34.8%.

Lactone The sugar (20mg.) was oxidised with bromine water at 30° for 48 hours. The lactone formed was isolated and recrystallised from ether.

m.p. 68°, undepressed on admixture with an authentic sample.

Synthesis of 4-O-β-D-xylopyranosyl-D-xylose and 3-O-methyl-4-O-(β-D-xylopyranosyl)-D-xylose

2,3,4-Tri-O-acetyl-α-D-xylopyranosyl bromide (XX)

This compound was prepared from 1,2,3,4-tetra-O-acetyl-β-D-xylose (XIX) by the standard reaction using hydrobromic acid in glacial acetic acid.

Benzyl-β-D-arabopyranoside (XXII)

D-Arabinose (XXI)(50g.) in benzyl alcohol (300ml.) was cooled in an ice-salt bath and saturated with hydrogen chloride (bubbled for 20 minutes). On shaking overnight at room temperature crystallisation occurred. Ether (500ml.) was added slowly and the flask and contents left at 5° for four hours. The glycoside was filtered off, washed with ether, and recrystallised from absolute alcohol, 54g., m.p.171°.

Benzyl-3,4-O-isopropylidene-β-D-arabinopyranoside (XXIII)

Benzyl-β-D-arabinopyranoside (XXII)(53.9g.) in dry acetone (2.5l.) was shaken with anhydrous copper sulphate (150g.) and concentrated sulphuric acid (2.5ml.) for twenty four hours. The acetone solution was separated, the acid

neutralised by passing in dry ammonia gas, the salts removed by filtration, and the solution concentrated to a syrup. The syrup was purified by chromatographing on activated alumina. Elution with ether containing 5% ethanol yielded the crystalline isopropylidene derivative, 41.2g.

m.p. 57°, $[\alpha]_D$ -209° (C2 in ethanol).

Benzyl 3,4-O-isopropylidene-2-O-methanesulphonyl-β-D-arabinopyranoside (XXIV)

Benzyl 3,4-O-isopropylidene-β-D-arabinopyranoside (XXIII) (41g.) was added with stirring to a mixture which had been made from pyridine (125ml.) and methanesulphonyl chloride (11.5ml.) precooled to 10°. After two hours at room temperature the excess of methanesulphonyl chloride was decomposed by the addition of a little water. An excess of aqueous sodium bicarbonate solution (10%) was then added and the solution evaporated to dryness, several additions of water being made to remove the last traces of pyridene. The residue was extracted with chloroform and removal of the solvent yielded the mesyl derivative as a syrup, 52g.

Benzyl 2-O-methanesulphonyl-β-D-arabinopyranoside (XXV).

The syrupy benzyl 3,4-O-isopropylidene-2-O-

methanesulphonyl-β-D-arabinopyranoside (52g.) was diluted with acetone (60ml.) and sulphuric acid (750ml.,1N.), and the solution refluxed for three hours, most of the acetone escaping in the process. The product, benzyl 2-O-methanesulphonyl-β-D-arabinopyranoside, (XXV), crystallised on cooling and was removed by filtration. Recrystallisation from methanol-water (3:1), then from water alone, yielded the pure compound, 30.6g.

m.p. 128-129°, $[\alpha]_D$, -186°, (C,3.2 in chloroform-ethanol,5:1).

Benzyl 2,3-anhydro-β-D-ribopyranoside (XXVI)

2N-Sodium hydroxide was added with stirring to benzyl 2-O-methanesulphonyl-β-D-arabinopyranoside (XXV)(30.5g.) in ethanol (80ml.) at 75° until the solution was permanently alkaline. Sodium methanesulphonate was filtered off and the filtrate was taken to dryness. The residue was extracted several times with warm ethyl acetate, and after removal of solvent the resulting solid was recrystallised from light petroleum (60-80°) to give benzyl 2,3- anhydro-β-D-ribopyranoside (XXVI) as long colourless needles, (18.4g.).

m.p. 75-76°, $[\alpha]_D$,-58°(C,0.66 in chloroform).

Found : C,64.7%,H,6.3%. $C_{12}H_{14}O_4$ requires C,64.8%,H,6.3%.

Benzyl 2,3-anhydro-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-ribopyranoside (XXVII)

Benzyl 2,3-anhydro-β-D-ribopyranoside (18.2g.), freshly prepared silver carbonate (32.5g.) and "drierite" (100g.-dried for three hours at 240°) were shaken overnight in dry benzene (250ml.). After addition of iodine (6g.), 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide (XX)(27.6g.) in benzene (250ml.) was added slowly with stirring during one hour. The mixture was shaken in the dark for three days (with occasional release of carbon dioxide); the benzene solution then gave no opalescence with ethanolic silver nitrate. The filtered solution was concentrated to a syrup which was chromatographed in benzene on alumina, and elution with light petroleum-benzene (1:1) furnished benzyl 2,3-anhydro-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-ribopyranoside (XXVII),(9.1g.) The compound was recrystallised from light petroleum-ethanol (2:1).

m.p. 131-132°, $[\alpha]_D$,-48°(C,0.66 in chloroform).

Found : C,56.7%; H,5.7%. $C_{23}H_{28}O_{11}$ requires C,57.6%; H,5.8%.

Benzyl 3-O-methyl-4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXX)

The disaccharide epoxide (XXVll)(6g.) was refluxed in methanol (180ml.) containing sodium methoxide (13.9g.) for 24 hours, and the cooled solution was diluted with water, neutralised with N-sulphuric acid, and evaporated to dryness. The residue was extracted with hot acetone giving syrupy benzyl 3-O-methyl-4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXX) (3.8g.)

$[\alpha]_D$, -115° (C,2.0 in water).

Found : OMe,8.1%, $C_{18}H_{26}O_9$ requires OMe,8.0%.

Acetylation of the benzyl glycoside gave benzyl 2-O-acetyl-3-O-methyl-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside,

m.p. 154-156°, $[\alpha]_D$, -101° (C,0.66 in Chloroform).

Found : C,56.3% ; H,6.0% ; OMe,5.6%.

$C_{26}H_{34}O_{13}$ requires C,56.4% ; H,6.1% ; OMe, 5.6%.

Methylation of the benzyl glycoside gave benzyl 2,3-di-O-methyl-4-O-(2,3,4-tri-O-methyl-β-D-xylopyranosyl)-β-D-xylopyranoside.

m.p. 88-90°, $[\alpha]_D$, -80° (C,0.4 in chloroform).

Found : C,59.8% ; H,7.5% ; OMe,35.0%.

$C_{22}H_{34}O_9$ requires C,59.7% ; H,7.7% ; OMe,35.1%.

Hydrolysis of the benzyl glycoside (XXX)(75mg.) with N-sulphuric acid at 100° for 4 hours, followed by neutralisatiom with barium carbonate gave two sugar components which were separated chromatographically using solvent A. The sugars were characterised as :

a) 3-O-methyl-D-xylose by formation of the phenylosazone, m.p. and mixed m.p. 170°.

b) D-xylose by conversion into the di-O-benzylidene dimethylacetal, m.p. and mixed m.p. 211°, $[\alpha]_D$, -9° (C,0.66 in chloroform.)

Chromatography of the periodate oxidation products of the 3-O-methylxylose fraction showed no methoxymalondialdehyde which would be formed from 2-O-methylarabinose.

3-O-methyl-4-O-(β-D-xylopyranosyl)-D-xylose (XXXl).

Benzyl 3-O-methyl-4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXX)(3.1g.) was dissolved in ethanol-water (120ml.) and shaken in hydrogen at atmospheric pressure over 10% palladium-charcoal (3g.) for 48 hours. The catalyst was filtered off and concentration of the filtrate afforded chromatographically pure syrupy 3-O-methyl-4-O-(β-D-xylopyranosyl)-D-xylose (XXXl), 1.9g.

$[\alpha]_D$, -18° (C,2.0 in water).

Found : OMe, 10.4%. $C_{11}H_{20}O_9$ requires OMe, 10.5%.

R_{xylose}, 0.61 in solvent C.

Benzyl 4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXVll).

The compound (XXVll), benzyl 2,3-anhydro-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-ribopyranoside (1g.) was heated in 2N-sulphuric acid (50ml.) on a boiling-water bath for 16 hours. The cooled solution was deionised with Amberlite resins 1R-120(H) and 1R-45(OH) and concentrated to give syrupy benzyl 4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXVlll), 0.81g.

$[\alpha]_D$, -120° (C, 1.6 in water).

The benzyl glycoside was chromatographically homogeneous (R_{xylose} 1.58 in solvent C), and hydrolysis gave xylose alone. Methylation with methyl sulphate and sodium hydroxide afforded the pentamethyl ether, m.p. and mixed m.p. 88-90°.

4-O-β-D-xylopyranosyl-D-xylose (XXlX).

Benzyl 4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (XXVlll) (0.6g.) was dissolved in ethanol-water (25ml.) and shaken in hydrogen at atmospheric pressure over 10% palladium-charcoal (0.6g.) for 48 hours. The catalyst was filtered off and

concentration of the filtrate gave a syrup (0·4g.) which contained xylobiose (R_{xylose} 0.31 in solvent C) and a trace of xylose. Chromatographically pure 4-O-β-D-xylopyranosyl-D-xylose (0.22g.) was isolated after separation on filter sheets using solvent C.

$[\alpha]_D$, -22° (C, 2.0 in water).

The disaccharide was characterised by conversion into the **phenylosazone**, identified by m.p. and mixed m.p. 207° (dec.), $[\alpha]_D$, -6° (5 min.) → -50° (24 hours, const.) (C,0.7 in pyridene-ethanol (7:3)), and by x-ray powder photograph.

Alkaline Degradation of Model Compounds.

Degradation of 3-O-methyl xylobiose.

The sugar (10mg.) was dissolved in oxygen-free sodium hydroxide (5ml. ; 1N.) under an atmosphere of nitrogen. Samples were withdrawn at 2 hour intervals, deionised with 1R-120(H) resin and concentrated to syrups, which were chromatographed in solvent B. The chromatograms were sprayed with spray A and the presence of xylose was detected in every sample. Xylose production increased with time while the amount of disaccharide present decreased. After 6 hours approximately two-thirds of the disaccaride originally present had been destroyed and xylose production appeared near its maximum.

The disaccharide had R_{xylose} 0.61 in solvent C but ran at almost the same rate as xylose in solvents A,D and F. A good resolution of the two was obtained by electrophoresis using a borate buffer.

The disaccharide (100mg.) was treated with oxygen-free N-sodium hydroxide solution (20ml.) at room temperature for 6 hours and the solution then deionised with 1R-120(H) resin. The solution was evaporated to a syrup under reduced pressure, the receiver being cooled with ice to trap volatile products.

Methanol was characterised in the first few drops of distillate by :

a) oxidation to formaldehyde, identified by formation of the dimedone derivative, m.p. 189°, undepressed on admixture with an authentic sample.

b) conversion to methyl iodide, identified by its infrared spectrum.

The residual syrup was chromatographed on Whatman 3MM filter sheets using solvent B and a pure sample of xylose obtained.

Di-O-benzylidene dimethylacetal. The sugar (30mg.) was dissolved in methanolic hydrogen chloride containing benzaldehyde (1ml.) After 7 days the derivative crystallised. m.p. 210°, undepressed on admixture with an authentic sample.

Acid Production.

3,4-di-O-methyl D-xylose (100mg.) and 3,4-di-O-methyl L-arabinose (100mg.) were degraded by N-sodium hydroxide in the usual manner, a blank alkali solution being run simultaneously. At intervals the alkali remaining was measured by titration with standard 0,5 N-hydrochloric acid, and the acid formed during the degradations calculated.

Me$_2$Ara - 1.3 moles of acid formed per mole of sugar

Me$_2$Xyl - 1.1" " " " " " " " "

Heating of the reaction mixtures to 100° failed to increase acid production.

Ultra-violet spetra.

The degradation products of all three model compounds were examined spectrophotometrically. Dimethyl-xylose and dimethyl-arabinose gave the same spectrum (Figure 4) which differed slightly from that of monomethyl-xylobiose (Figure 5).

FIGURE 4

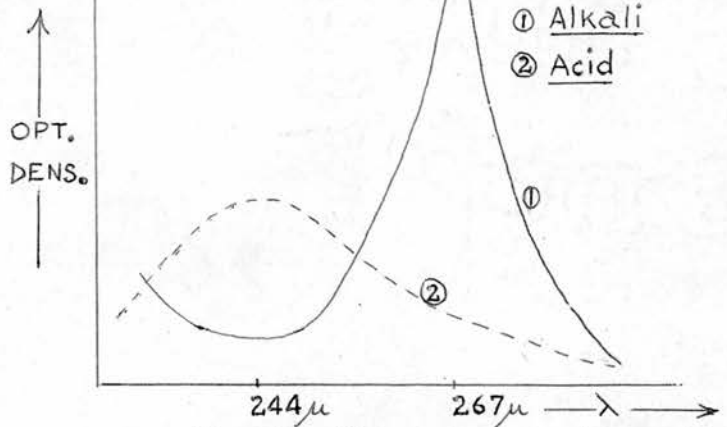
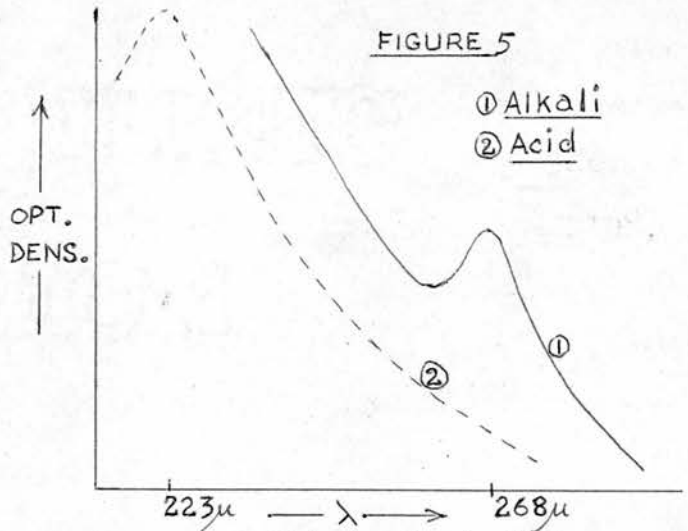


FIGURE 5



In all three cases absorption maxima were obtained at 268μ in alkaline solution. On acidifying the solutions these peaks were immediately replaced by lower peaks at 244μ in the case of the two dimethyl sugars, and by a higher peak at 223μ in the case of disaccharide. When the solutions were made alkaline again the original peaks reappeared. In both media the peaks were stable over 21 days.

Chromatographic examination of degradation products.

Samples of all three model compounds were degraded with N-sodium hydroxide in the manner already described, and the products obtained were investigated by paper chromatography using solvent B. Various sprays were used in an attempt to characterise as far as possible the products formed. The R_F values of the various products from each sugar are shown in TableXl .

Table Xl.

Spray	Me Arabinose 2	Me xylose 2	3Me xylobiose.
C (lactones)	0.54;0.37	0.54;0.37	0.54;0.37.
D (acids)	0.49;0.43;0.28	0.49;0.43;0.37	0.49;0.45;0.43; 0.28.
E (acids)	0.49;0.43;0.28	0.49;0.43;0.37	0.49;0.45;0.43; 0.28.
F (unsaturated) compounds	0.49;0.28	0.49	0.49;0.28

Addition of an alcoholic solution of 2,4-dinitrophenylhydrazine to solutions of the degradation products of the dimethyl sugars gave orange precipitates on standing, which had zero methoxyl contents.

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