CEREAL POLYSACCHARIDES
with Special Reference to
THE HEMICELLULOSES OF RYE

By

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TO MY MOTHER

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The Hemicelluloses

Many polysaccharides, closely associated with cellulose, but differing from it due to their solubility in alkali, were originally termed hemicelluloses by Schultze.[1] He found the material, which occurs in all land plants, to be much more readily hydrolysed than cellulose, and considered these cell wall components to be chemically and structurally related to cellulose and to serve as a reserve supply as well. The most common constituent sugar is D-xylose, but hemicelluloses containing L-arabofuranose, D-galactose, D-glucose, D-mannose, and L-rhannose are known. The presence of 2-methyl ethers in the form of 2-O-methyl-L-fucose[2] and 2-O-methyl-D-xylose[3] has been detected in hemicellulose sources, although it is possible that the original source may have been pectic material.

Classification of the hemicellulose group is permissible by sub-division into pentosans and hexosans, with further divisions into groups of polysaccharides all having the same constituent sugars, such as arabinose and xylose, arabinose and galactose, glucose and mannose, etc., although the bulk of the hemicellulose mixture consists of a single polysaccharide xylan consisting of xylose units together with smaller amounts of arabinose and glucuronic acid units.

The wide range of molecular sizes and molecular shapes, existing among the polysaccharides of the hemicellulose group, cause wide variations in solubility, thus creating difficulties in the separation of a pure molecular type by fractionation

procedures.

The function of the hemicelluloses in the plant is not clearly understood. Suggestions have been made that they act as plasticisers rendering flexibility to the tissue. Although an intimate association between cellulose and hemicellulose is indicated, X-ray evidence shows that xylan is not a constituent of the crystalline micellar regions of cellulose. The chemical relationships which the hemicelluloses bear to cellulose and lignin are not clearly understood. Difficulties are encountered in trying to separate xylan and cellulose due to the similarity of the two species, while the relationship between hemicellulose and lignin may be a physical one, as studies by Nelson and Scheurch[4] have shown.

Extraction and Purification of Hemicelluloses

Fats, waxes, resins, lipids and other soluble materials are generally removed from the tissue with a preliminary treatment with an organic solvent, such as benzene-ethanol azeotrope, and if pectic substances are present in any quantity they are usually removed by extraction with 0.5% aqueous ammonium oxalate.[11]

The extraction of the plant tissue with alkali gives rise to polysaccharide mixtures highly contaminated with lignin. Many methods have been devised for its removal, few being acceptable due to their degradative effect on the polysaccharide. Early workers used chlorine gas and sodium sulphite solution,[5] boiling 50% aqueous ethanol and 1% NaOH,[6] and chlorine dioxide in pyridine and water.[7] None of these methods achieves complete

removal of lignin without degradation of the carbohydrate. The most widely used method in recent years is the acid-chlorite method[8] in which complete removal of lignin is claimed with a minimum removal and of physical and chemical change of the polysaccharide. Experiments by Staudinger and Jurich[9] have shown that chlorine dioxide has a very slow depolymerisation action on cellulose, but Timell et al[141] have shown that some degradation of hemicellulosic material can take place. They found that polysaccharides directly extracted from wood and from chlorine holocellulose had approximately the same D.P., but material extracted after chlorite treatment had a much lower D.P. The action of chlorites on simple sugars has been studied by Jeanes and Isbell[10] who found no evidence for the scission of glycosidic linkages, but solutions of the sugars were converted to the corresponding aldonic acids.

Hemicellulosic materials are then extracted by means of subsequent treatment with water and then alkali at increasing concentrations.

Cereal grains normally give pentosan rich fractions on aqueous extraction at 40°. It has been shown that there is not a complete removal of water soluble hemicellulose at this temperature, pentosans are obtained together with large quantities of starch at higher temperatures, the polysaccharides being recovered on enzymic degradation of the starch.[12]

Hot water extraction of wood tissues mainly yield water soluble polysaccharides which mainly consist of units of galactose and arabinose.

The tissues are then subjected to a series of alkaline extraction treatments with increasing concentrations of alkali, normally from 4% up to 24%, giving materials which are probably mixtures of polysaccharide species. Different polysaccharide species in the plant are closely associated, and the similarity in the chemical and physical properties of these species make the isolation of a pure hemicellulose a very difficult problem, the solution of which is undoubtedly of the greatest importance. Several fractionation techniques have been developed recently but there is no set procedure which can be applied to any particular type of polysaccharide, each case having to be carefully studied on its own. The selective extraction with increasing concentrations of alkali has not been completely successful in separating molecular species, but this method is still used in the isolation of wood polysaccharides into xylan, and mannan rich fractions. O'Dwyer[13] took advantage of the fact that neutralisation of alkaline hemicellulose solutions caused precipitation of higher molecular weight glycans which she called Hemicellulose 'A', the more soluble fraction, Hemicellulose 'B', being precipitated by two volumes of ethanol.

Fractional precipitation of the derived acetates with light petroleum from chloroform solution has been found to give effective separations of hexosans from pentosans.[14] Salkowski[15] found that xylans form insoluble complexes with copper ions, while the common contaminating polysaccharides do not. Thus Chanda et al[16] have obtained an arabinose free xylan from esparto grass by repeated use of this procedure.

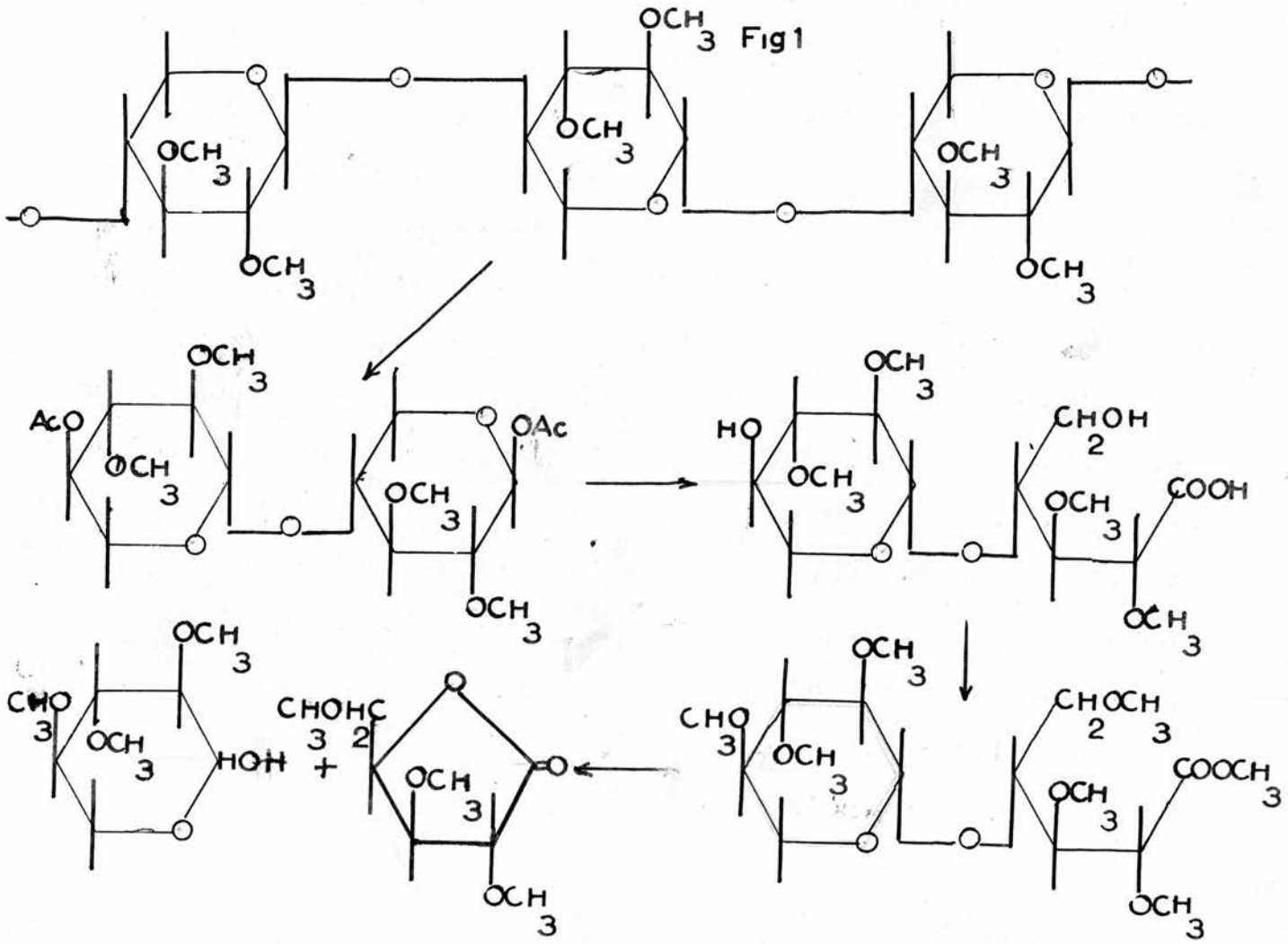
From the same source, Aspinall et al[17] have obtained a polysaccharide containing appreciable amounts of arabinose by extraction with aqueous 70% alcohol. A more recent method, which is finding increasing use, is the graded precipitation of the polysaccharide from aqueous solution with ammonium sulphate,[18] while the use of concentrated urea solutions has been claimed to be suitable in the fractionation of barley polysaccharides.[19]

Physical methods such as electrophoresis and ultracentrifugation can be used in the small scale separation of polysaccharide species, and are valuable as an analytical tool in determining the degree of homogeneity of a specimen, but so far have not been found to be of general application to the problem of isolating a pure polymer in quantity. A combined electrophoresis and column chromatography technique has been used by Northcote[22] in the separation of a mixture of polysaccharides on a 1 g. scale.

Molecular Structure of Xylans[23,24,25]

D-Xylose was shown by Wheeler and Tollens[26] in 1889 to be the hydrolysis product of material from alkaline extraction of wood. Johnson[27] proposed the empirical formula of xylan to be $(C_5H_8O_4)_n$ and this was confirmed in 1929 by Hampton, Haworth and Hirst[28] from their work on esparto grass. These workers obtained 90% of 2:3 di-O-methyl-D-xylose methyl glycoside from the methanolysis of the methylated polysaccharide thus proving the greater part of the polymer to consist of D-xylose units linked through 1:4 or 1:5 positions. The presence of pyranose

rings was suspected due to the relative stability of the polysaccharide to acid hydrolysis and this was confirmed by Haworth and Percival[29] by acetolysis of the methylated xylan with acetic anhydride and sulphuric acid. A xylobiose derivative was obtained which on deacetylation, oxidation, remethylation and hydrolysis, gave 80% 2:3:4-tri-O-methyl-D-xylose and 73% 2:3:5-tri-O-methyl-D-xylonolactone, see Fig. (I). The presence of the lactone proves that the C_4 of the reducing xylose unit of the disaccharide was involved in the glycosidic link, thus confirming the existence of the sugars in the pyranose form. Xylan had therefore been shown to be basically a polymer of 1:4 linked β-D-xylopyranose units.



2:3:5-Tri-O-methyl-L-arabinose and monomethylxylose were found in approximately equimolar proportions in the hydrolysate of methylated esparto xylan by Haworth, Hirst and Oliver.(30) This was taken as an indication that L-arabinose units were attached directly to the xylan chain (II) or that they terminated side chains (III).

(II) (III)

-x- 1:4 linked β-D-xylopyranose.

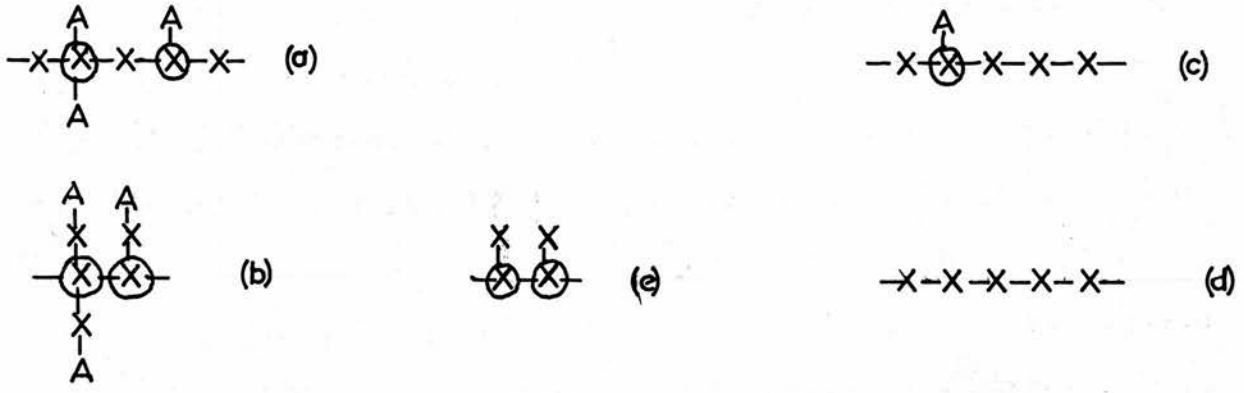
A- 1-linked L-arabofuranose.

The structure of III was regarded as the correct one when Haworth et al(31) found that the arabinose units could be preferentially removed by dilute acid and that remethylation and hydrolysis of the degraded xylan produced an equivalent number of 2:3:4-tri-O-methyl-D-xylose units.

The position of attachment of the terminal arabinose units to the rest of the xylan molecule appears to be generally, but not exclusively through position 3. Perlin established a structure of this kind for an araboxylan from wheat flour.(14) In an attempt to settle the issue as to whether the terminal arabinose units comprised single unit side chains or not, he investigated the effect of the periodate ion on the pentosan. From methylation studies, the polysaccharide appeared to have a type of structure corresponding to either (a) or (b) in Fig.(IV).

The periodate ion will attack all sugar units except these marked by circles. Both (a) and (b) would liberate very small quantities of formic acid, but on preferential removal of the terminal arabofuranose units, (a) would expose a xylose unit to oxidation giving a corresponding increase in uptake, but no increase in acid liberated, as in (c), whereas a molecule (e) derived from structure (b) would have no appreciable increase in uptake, but a marked increase in formic acid would be found. Perlin found that on removal of all but 7% of the arabinose, the polysaccharide consumed 0.94 moles of periodate/mole, with little or no increase in formic acid liberated which is consistent with (d) being derived from (a).

Fig. IV



An extension of this method has been used[32] in which the xylose residues unattacked by periodate were measured after oxidation and hydrolysis of both the polysaccharide and degraded polysaccharide. The decrease in xylose residues unattacked after degradation and oxidation indicated that most of the terminal arabinose units were attached directly to the backbone.

A series of arabinose containing oligosaccharides was

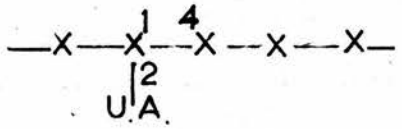
obtained by Bishop and Whittaker[33] from wheat straw xylan by means of an enzyme preparation from the mould Myrothecium Verrucaria, thus proving beyond all doubt that the arabinose is an integral part of the xylan molecule. In the isolation of the trisaccharide, O-L-arabofuranosyl (1→3)-O-D-xylopyranosyl (1→4)-D-xylopyranose, Bishop[34] was able to prove at least some of the arabinose to be in the form of single side chains.

Several years previous to this, an attempt was made by Hirst and co-workers[17] to establish whether the arabinose found in esparto xylan was due to an araboxylan or to a contaminating araban. Extraction of esparto holocellulose with 70% aqueous alcohol yielded an araboxylan containing 26% arabinose,[17] and it was shown that most of the arabinose units were terminal and linked on to the xylan backbone. A hemicellulose, containing only xylose units, was also obtained from esparto by repeated fractionation of the copper complex. Methylation studies and molecular weight determinations indicated a molecule of 80 D-xylopyranose units linked β 1:4 in a chain containing one branch point through position 3. Non-terminal arabinose units have now been found to occur in several sources. In the polysaccharide obtained by extracting esparto holocellulose with 70% aqueous ethanol, most of the arabinose units were found to be in the end position, but small quantities of 2:3-di-O-methyl-L-arabinose and another unidentified dimethyl arabinose were obtained. Since then 2-O-D-xylopyranosyl-L-arabinose has been isolated by partial hydrolysis from the original polysaccharide,[37] maize cobs[35] and barley husks.[32] That the arabinose is present in the

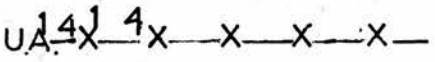
furanose form was suspected from studies of the rates of hydrolysis and confirmed by methylation studies.(75)

Xylans in general have been found to confirm to the same general pattern as esparto xylan, except that D-glucuronic acid or its 4-methyl ether are present in a large number of the xylans obtained from the lignified tissues of land plants. Wood xylans are only now being submitted to a full chemical examination, although many have been studied by partial hydrolysis methods. The structures of the aldobiouronic acids isolated by this means indicate that the acidic residues usually form single unit side chains attached to the backbone of the molecule. In the woods 4-O-methyl-D-glucuronic acid is found almost exclusively, and in most cases being linked to the xylan through position 2, although a 3 linked aldobiouronic acid has recently been isolated and characterised.(36) The aldobiouronic acid 2-O-(4-O-methyl-α-D-glucuronopyranosyl)-D-xylose has been found in American beechwood,(38) European beechwood,(39) European larch,(40) aspen wood,(41) birch wood,(42) Norway spruce,(43) black spruce,(44) Scots pine,(44) white birch,(45) white elm,(46) oat straw,(47) oat hulls,(48) wheat straw,(49) wheat bran(50) and maize cobs.(51) The unmethylated glucoronic acid appears to be commonly attached through position 2 but this is variable. It has been found in wheat straw,(49) maize cobs,(52) maize hulls,(53) oat hulls,(48) barley husks(32) and wheat bran,(50) while in wheat straw,(39,54,55) and wheat leaf(56) a 3 linked acid is thought to occur. Bishop(57) isolated and characterised 3-O-(D-glucuronopyranosyl)-D-xylose from wheat straw thus confirming this linkage. These acidic

residues have been shown by methylation studies to constitute single side chains attached to the xylan backbone (V) although glucuronic acid has also been reported linked to C_4 of xylose, but it is not known if this is occupying a position at the end of the xylan chain (VI).



(V)



(VI)

U.A. = uronic acid

The biouronic[52] and triouronic acids[35] forming the end two and three units respectively of this type of chain have been isolated from maize cobs and characterised.

It is of particular interest that the araboxylans of cereal grains form a different group of substances having a higher proportion of arabinose but no uronic acid residues, while the straw type has low uronic acid and significant arabinose contents, and the wood hemicelluloses have little or no arabinose but high uronic acid content.

Among the more significant contributions to cereal carbohydrate chemistry in recent years has been the accumulation of information concerning the water soluble hemicelluloses, known as the cereal gums. They have been referred to as "gums" despite important differences in chemical composition from the more typical plant gums, which occur as exudates on the fruit and bark of trees, frequently as the result of injuries.

O'Sullivan initiated the study of cereal gums giving the

name "amylan" to the water soluble polysaccharides extracted from a number of cereals. Later studies were carried out by Piratzky and Wiecha[58] on the "amylan" of germinating barley, whilst Morris[59] isolated a polysaccharide from oats which closely resembled lichenin.

Modern fractionation techniques have been applied to the hemicellulose components of cereal grains, the water soluble components of which seem to contain large amounts of araban, xylan and glucan, with smaller amounts of galactan and mannan.

Thus Preece and Mackenzie[18] using the ammonium sulphate fractionation procedure were able to give approximate figures for the amount of glucan and pentosan in grains (Table I). Both α and β glucans are present although the β type predominates in barley and oats, and is only present in trace amounts in maize and wheat.

Table I

Water soluble Hemicelluloses of some cereals

Cereal	-glucan %	Araban %	Xylan %
Barley	0.80	0.18	0.11
Maize	0.04	0.03	0.01
Oats	0.35	0.16	0.05
Wheat	0.06	0.17	0.18
Rye	0.18	0.25	0.40

The authors point out that the figures in the table are illustrative only, as the percentage levels for each constituent

vary widely in one particular type of cereal but similar patterns were found in all cereals of each type examined.

By means of this fractionation procedure, a pure β-linked glucose polymer has been obtained from barley[18] and oats[60]. The barley β-glucan has been examined structurally by Aspinall and Telfer[61] who found it to consist of unbranched chains of β-linked glucopyranose units, approximately equal numbers of β1:3 and β1:4 linkages being present. The distribution of these linkages is still a matter of doubt. Aitken et al[62] suggested at least the greater proportion of the linkages occur in separate homologous groups, but Preece and Hoggan[63] concluded that both types of linkage are distributed generally throughout the molecule. Both groups of workers reject the idea of simple alternation. This β-glucan appears to have a structure similar to that of lichenin,[147] which was found to

Table II

Structural Features of Cereal Pentosans

Molecular Proportions

Xylan	Xl-	Al-	-4X-1-	-4X-1- (3)	-4X-1- (2,3)	Other Features	Ref.
a) Flour							
Barley	1	12	4	2			105
Barley	3	3	1	1		-4 Xl- (2)	65
Wheat	13	19	6	4		-4 Xl- (2)	106
Wheat (squeegee)	14	24	7	4			107
Wheat	3	3	1	1			14
Wheat	6	6	1	1		4-Gal.1 (3)	66

Table II (contd.)

b) **Husk**							
Barley	2	1	14	3	1	$\overset{2}{\text{Al-}}$, G.A.1-	32
Wheat Bran	5	6	4	4	3	$\overset{2}{\text{Al-}}$, $\overset{2}{\text{-5Al-}}$, G.A.1-, M.G.A.1-	78
Maize Cob	1	2	28	5		$\overset{4}{\text{-3G.}}$	76
Maize Fibre		8	10	8	2	Gal.1-, $\overset{3}{\text{Al-}}$, $\overset{2}{\text{-5Al-}}$, G.A.1-	68
Maize Fibre (de-graded)	14		47	19	2	G.A.1- or M.G.A.1-	70
c) **Straw**							
Wheat	2.4		93	3.4		G.A.1-	80
Wheat	1	3	41	4		G.A.1-, M.G.A.1-,	49
Wheat	1	5	50	10		$\overset{4}{\text{-3G.}}$	76
Wheat	0.5	3	27	6		$\overset{4}{\text{-3G.}}$	77
Wheat	1	5	25	5		G.A.1-, M.G.A.1-	54
Oat	1	1	41	1		M.G.A.1-	47

Xl- = D-Xyl. 1-

Al- = L-Ara. 1-

G.A. = D-Glu. A.

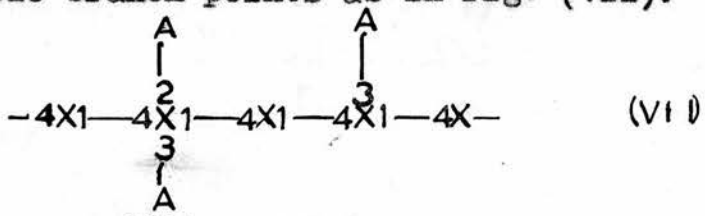
M.G.A. = 4-O-Methyl-D-Glu. A.

Gal. 1- = Galactose

G. = D-glucose

contain β 1:3 and β 1:4 linkages in the approximate ratio of 30:70. In the case of a similar glucan isolated from oats, named oat lichenin,[59] periodate oxidation studies have shown that the proportion of β 1:3 to β 1:4 linkages to be about 1:3. In partial hydrolysis studies, Peat et al have isolated cellobiose, laminaribiose, and the trisaccharides, 4-O-β -D-laminaribiosylglucose and 3-O-β -D-cellobiosylglucose.[20]

On removal of the bulk of the β -glucan by ammonium sulphate precipitation, considerable amounts of pentosans are obtained, usually containing arabinose and xylose residues in the ratio of 2:3. All evidence put forward so far indicates that these pentosans are araboxylans comprising of a backbone of β 1:4 linked xylosyl residues with side chains of single arabofuranosyl residues linked mainly 1:3 and sometimes 1:2 to the xylosyl backbone (Table II). The structure of the wheat flour pentosan was proposed by Perlin (see page 7), to be a highly branched araboxylan with a certain number of the xylosyl backbone units containing double branch points as in Fig. (VII).

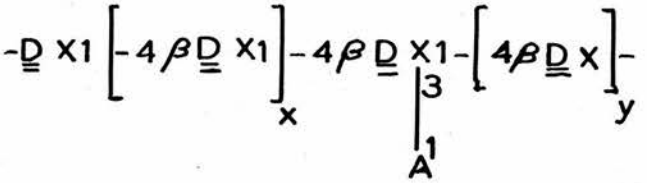


(VII)

Recently, Howard[64] obtained a series of xylose oligosaccharides, from xylobiose to xylopentose by means of enzymic hydrolysis of the pentosan with ruminal micro-organisms, thus establishing the β 1:4 linked backbone of the molecule, but he was unable to obtain any evidence of arabinose containing oligosaccharides, to give definite proof of the presence of

single unit arabofuranosyl side chains. The araboxylan isolated from barley flour by Aspinall and Ferrier[65] was shown to have similar features as the wheat flour xylan. Several years previously, Ford and Peat[66] isolated a polysaccharide associated with β-amylase in wheat flour, and obtained 2:4-di-O-methyl-D-galactose in the products derived from methylation, but the significance of this finding is not understood.

The repeating unit for an average araboxylan molecule can be written thus:



Husk Type Hemicelluloses

The outer coating or pericarp of cereal grains contains a high proportion of pentosan material which appears to be more complex than those found in the endosperm. After removal of starch from the tissues, typical hemicelluloses can be extracted by treatment with 4% aqueous sodium hydroxide solution. Fractionation at this stage is usually possible by means of ammonium sulphate or Fehling's solution.

The polysaccharides investigated from this source are barley husks,[32] maize hulls,[53,67] maize cobs[76,77] and wheat bran.[78] Apart from the usual methylation studies, valuable information regarding the nature of side chains has been obtained by partial hydrolysis studies (Table III).

Table III

Source	Fragment	Structure	Author	Ref.
Barley Husks	2-O-β-D-xylopyranosyl-L-arabinose	Xl→2A	Aspinall	32
Maize Hulls	3-O-α-D-xylopyranosyl-L-arabinose	Xl→3A	Whistler Smith	69 71
	O-L-galactopyranosyl(1→4)-O-D-xylopyranosyl(1→2)-L-arabinose	L-Gal. 1→4 X 1→2 A	Whistler	69
	4-O-D-galactopyranosyl-D-xylose	D-Gal. 1→4 X	Smith	71
	5-O-β-D-galactopyranosyl-L-arabinose	D-Gal. 1→5 A	Smith	72
Maize Cobs	2-O-β-D-xylopyranosyl-L-arabinose	Xl→2A	Whistler	75

In the barley husk hemicellulose[32] about 4% glucuronic acid was found, together with a xylose:arabinose ratio of 6:1. An interesting feature of the polysaccharide is the presence of some non-terminal L-arabofuranose residues. Mild hydrolysis liberated 2-O-β-D-xylopyranosyl-L-arabinose, which together with the methylation results indicated side chains of single unit L-arabofuranose and 2-O-β-D-xylopyranosyl-L-arabofuranose through position 3, and glucopyranuronic acid residues through position 2.

Uronic acid residues have also been reported in maize hulls,[53,57] wheat bran,[78] and oat hulls.[48] In a maize hull hemicellulose, Wolf et al[67] found it to consist of xylose, arabinose, galactose and glucuronic acid residues. Methylation results obtained by Whistler et al[68] showed the presence of 2:5-di-O-methyl-L-arabinose and 3-O-methyl-L-arabinose, indicating the presence of non-terminal

arabinose units. It was also shown that galactose was present only in terminal positions. Partial hydrolysis[69] afforded 3-O-α-D-xylosyl-L-arabinose, and O-L-galactopyranosyl (1→4)-O-D-xylopyranosyl- (1→2)-L-arabofuranose. Investigation of the degraded polysaccharide[70] indicated it to be devoid of arabinose and galactose units.

The acidic fraction was investigated by Smith[53] in which he found D-glucuronic acid linked (1→2) to the xylan backbone. He also isolated the 3-linked xylosylarabinose[71] and 4-O-D-galactopyranosyl-D-xylose, which is significant since the trisaccharide isolated by Whistler et al would furnish 4-O-L-galactopyranosyl-D-xylose on further hydrolysis. Later Smith et al[72] isolated 5-O-β-D-galactopyranosyl-L-arabofuranose, which they found identical to the synthesised material.[73]

The presence of a complex polysaccharide from maize cobs has been reported[35,51,52] in which three aldobiouronic acids and a triouronic acid have been characterised from the hydrolysis products of the "B" fraction of the hemicellulose. As was found in the maize hull fraction, D-glucuronic acid was found to be linked to xylose (1→2), as well as to the 4 position. 4-O-Methyl-D-glucuronic acid was also present in attachments to position 2 on the backbone.

By partial hydrolysis of the "A" fraction, Whistler and Tu[74] obtained a series of β 1:4 linked xylo-oligosaccharides ranging from xylobiose to xyloheptaose. As in the barley husk and maize fibre materials, non-terminal arabinose was shown by isolation of 2-O-β-D-xylosyl-L-arabinose.[75]

A minor neutral fraction from maize cobs was obtained by Smith and co-workers.[76,77] From such a fraction they obtained a small amount of 2:6-di-O-methyl-D-glucose from the hydrolysate of the methylated material. They postulate a structure in which two xylan units are attached through positions 3 and 4 of a bridging glucopyranosyl residue, Fig. (VIII). But there is no conclusive evidence that the glucose forms part of the xylan molecule.

(VIII)

A strange polysaccharide was isolated from wheat-bran by Adams,[78] which contained 50% L-arabinose, 38.5% D-xylose, and 9% uronic acid. Methylation results appeared to suggest that the material was not a homogeneous xylan, but one with a highly branched araban as impurity, because a large amount of 2:5-di-O-methyl-L-arabinose, and a mono-O-methyl-L-arabinose was obtained. Adams was unable to reduce the arabinose content after several fractionations. A fraction containing acidic residues has been studied[50] in which two aldobiouronic acids were isolated and identified, namely glucuronic acid and its 4-methyl ether linked 1→2 to the xylan backbone. Recent studies have shown that graded hydrolysis of the polysaccharide removed the arabinose leaving an insoluble xylan which on methylation was found to consist of chains β 1:4 linked xylose units of approximately 7-8 units with 1 glucuronic acid group linked 1→2 to the

backbone.[79] The degraded polysaccharide was hydrolysed by an enzyme from Myrothecium Verrucaria, although the original polysaccharide was stable to the enzyme, which is specific for hydrolysis of 1:4 glycosidic links. Adams attributed this stability as being due to the presence of the arabinose.

Straw Type Hemicelluloses

Wheat straw hemicelluloses have been investigated by several workers. Aspinall and Meek[49] have pointed out that the products of different workers may not be comparable, for the compositions of products may be seriously affected by methods of preparation and it is often uncertain to what extent particular products are mixtures. Nevertheless there is a good measure of agreement that wheat straw xylan consists of 1:4 linked xylose residues with arabinosyl and glucuronosyl side chains linked 1:3 and 1:2, forming essential parts of the molecule.[49,78,34] Aspinall and Mahomed[80] isolated an arabinose free xylan of 40-45 unbranched xylose units with single D-glucuronic acid side chains mainly with a C_3 link. Arabinose rich fractions have been obtained, in which these units have been conclusively proved to be attached to the xylan, by Adams,[54] Roudier,[55] Smith,[76,77] Aspinall[49] and Bishop.[57] The polysaccharide isolated by Smith was non-acidic, which is strange as most workers have found uronic acid in the xylan. As in the neutral polysaccharide obtained from maize cobs he obtained a small quantity 2:6-di-O-methyl-D-glucose in the hydrolysate of the methylated derivative. The structure postulated is the same as that assigned to the

maize cob fraction, being two xylan units attached through the 3 and 4 positions of a bridging glucopyranose residue, but again it is not certain whether or not the glucose is an integral part of the xylan molecule.

Direct proof that at least some of the arabinose residues were directly linked to the backbone was obtained by Bishop and Whittaker(33) who obtained a series of arabinose containing oligosaccharides, see page 9.

Oat straw xylan(47) similarly has the xylan backbone chain with arabofuranosyl and methylglucuronosyl units linked (1→3) and (1→2).

Thus it appears that grain hemicelluloses are of two types with the typical husk type of hemicellulose characterised by high xylose content and always containing uronic acid, and the endospermic type characterised by a lower xylose content and relatively few uronic acid residues.

Molecular Weights

One of the most important and most difficult problems concerned with the elucidation of the structures of the polysaccharides is the determination of molecular size. The problem is complicated by the association of molecules to form supermolecular aggregates, but aggregation can be overcome in most cases by extrapolation of the observed results to infinite dilution where association is at a minimum. Another difficulty arises from the inhomogeneity of many polysaccharide products, thus giving a doubtful significance to many reported results.

Physical-chemical methods based on the counting of molecules, (osmotic pressure, freezing point depression), give number average molecular weights, which are affected principally by the smaller molecules present. On the other hand, the viscosity method gives a weight average molecular weight, mainly dependent on the larger molecules. Viscometric methods tend to be unreliable as the Staudinger constant has not been determined for xylan but the one used for cellulose is used instead, thus assuming the xylan to be a linear molecule.

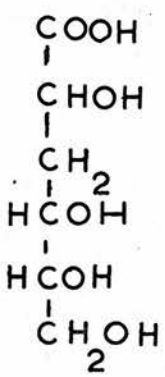
Molecular weight studies have not been made extensively in the xylan field. Husemann[81] reported viscosity and osmotic pressure measurements on xylan and xylan derivatives from straw and beechwood, while a D.P. of 70 was found for esparto xylan by osmotic pressure measurements.[16] Most determinations in the xylan series have been obtained using the isothermal distillation method on methylated derivatives in an organic solvent such as benzene. Unfortunately many methylated and acetylated xylans have a low solubility in this solvent and in other chemically pure stable solvents which are necessary for this type of determination. The D.P. of a number of xylans found to vary between 50 and 100 has been determined by this method.

Other properties which depend on the molecular weight have been employed for the determination of the molecular weights of polymers, such as the distribution of molecules in a gravitational field, and the light scattering method, but the osmotic and viscometric methods have been most widely used.

Alkaline Degradation

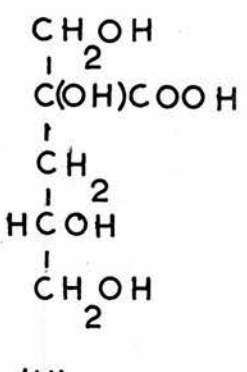
Much of the earlier work on polysaccharides, mainly starches and hemicelluloses, was carried out on materials which had been subject to vigorous alkaline treatments. Little is known of the extent to which polysaccharides may have undergone structural modification during their isolation by the use of alkaline solutions, by a process analogous to the alkaline degradation of reducing sugars.

The extensive work of Kiliani[82] and Nef[83] demonstrated the case of isomerisation of hexoses and pentoses to deoxyaldonic acids with the same elementary formulaes, $C_6H_{12}O_6$ and $C_5H_{10}O_5$ respectively. In the hexose series three types of saccharinic acids were found (IX - XI).



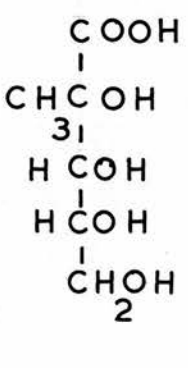
(IX)

Glucometasaccharinic acid (2:4:5:6-Tetrahydroxy hexanoic acid)



(X)

Glucisosaccharinic acid (2:4:5-trihydroxy-2-hydroxy-methyl pentanoic acid)

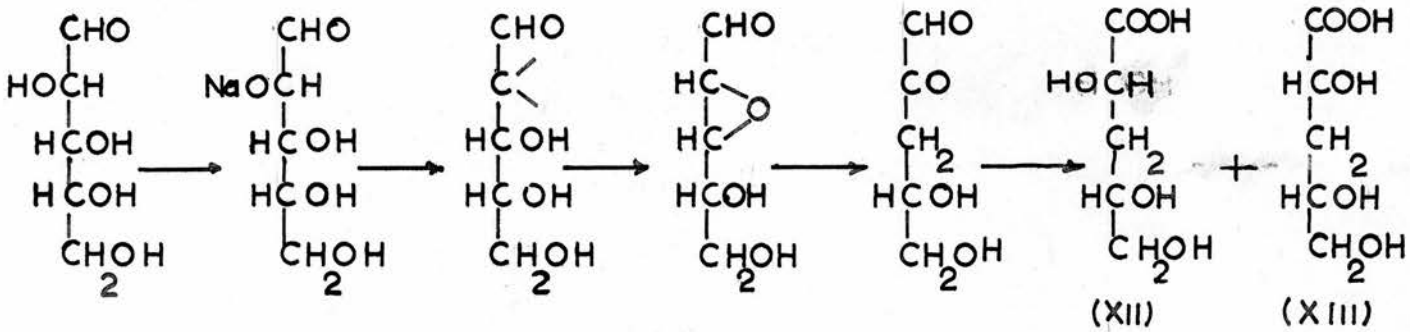


(XI)

Glucosaccharinic acid (2:3:4:5-tetra-hydroxy-2-methyl pentanoic acid)

These acids, whose lactones were termed saccharins were obtained from glucose or disaccharides with glucose as a reducing residue.

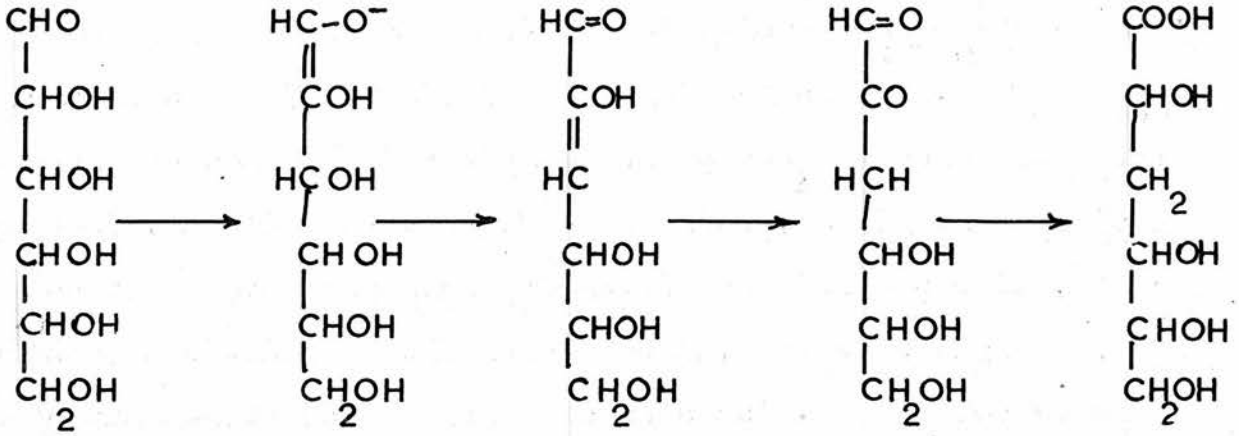
The mechanism of formation of the saccharinic acids was thought to be due to a fragment-recombination reaction[135] but Nef[83] replaced this by his isomerisation mechanism. He postulated progressive migration of the carbonyl group along the carbon chain, forming 1-2, 2-3, 3-4 enediols, as in the manner of the Lobry de Bruyn and Van Eckenstein enediol mechanism,[134] followed by splitting at the double bonds, producing fragments which rearranged to acids such as lactic acid. The formation of saccharinic acids was thought to take place by isomerisation of the sugar by means of an alkoxide intermediate to an α-dicarbonyl compound which underwent a benzilic acid type of rearrangement to the saccharinic acid. Thus xylose was converted to the mixture of D-threo and D-erythrometasaccharinic acids XII and XIII.



This mechanism was later superseded by Isbell's Ionic Mechanism,[136] a modification of which has been advanced by Kenner and Richards.[84]

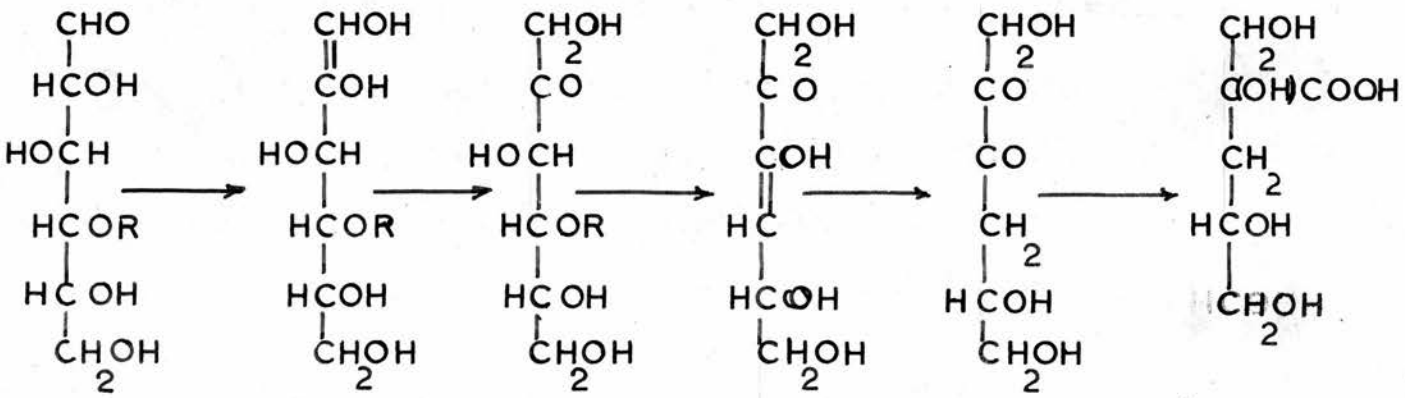
Isbell's Ionic Mechanism depended on the formation and ionisation of an enediol followed by the β-elimination of a hydroxyl or alkoxyl group, which on rearrangement to an α-dicarbonyl intermediate and a subsequent benzilic acid type

rearrangement produced the saccharinic acid.

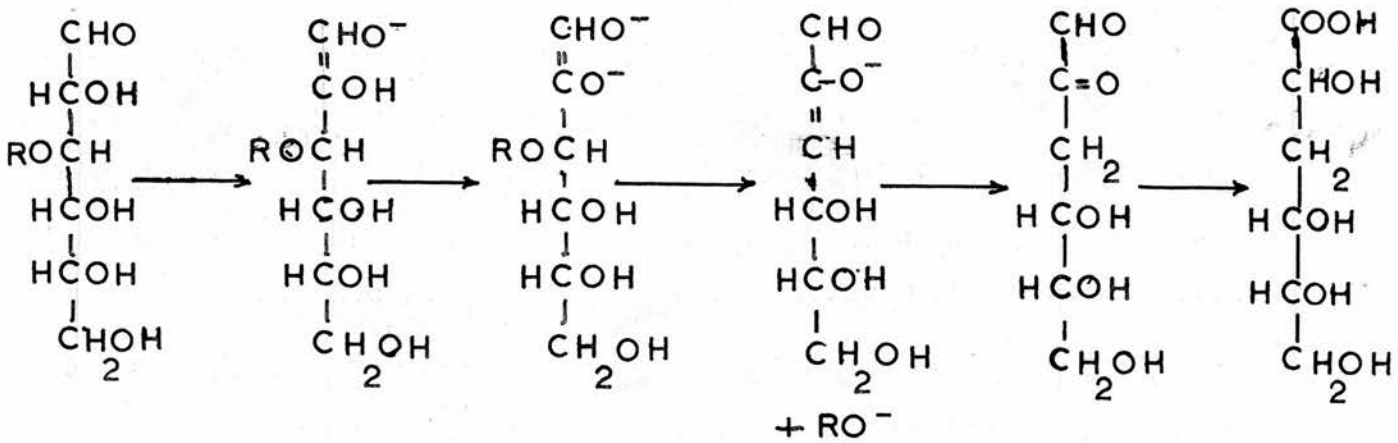


A similar mechanism was postulated for the production of isosaccharinic acids from the corresponding 2:3-enediols.

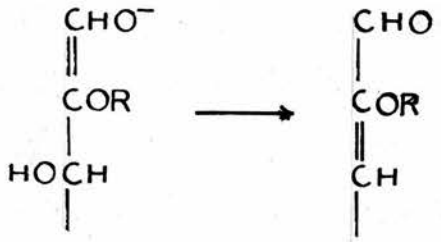
Kenner et al[84] have examined the production of saccharinic acids from substituted sugars and they concluded that the O-glycosyl or O-alkyl anion was more easily extruded from a sugar enediol anion than was a hydroxyl anion. They found that substitution in position 3 produced mainly metasaccharinic acids, whilst substitution in position 4 produced isosaccharinic acids. Thus D-glucoisosaccharinic acid was found as the principal product from the action of lime water on the following 4-O-substituted hexoses: lactose,[85] cellobiose, cellobiulose, cellotetraulose,[86] lactulose, maltose, maltulose,[87] 4-O-methyl-D-glucose and 4-O-methyl-D-fructose.[88] Kenner et al explain the following manner:



The corresponding D-glucometasaccharinic acids were obtained by the action of lime water on 3-O-methyl-D-glucose, 3-O-methyl-D-fructose,[84] laminaribiose, turanose,[90] and 3:6-anhydro-D-glucose,[91] the proposed degradation route being as follows.



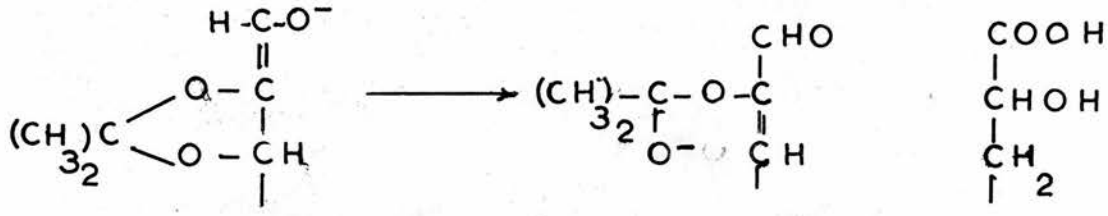
As a result of their work on the 3-O-substituted aldo- and keto-hexoses, the authors have suggested that the enediol di-ion is the reactive species in the formation of saccharinic acids, since the related 3-O-substituted aldo- and keto-hexoses were found to degrade at approximately equal rates. The common 1:2-enediol is reached either from the aldose or ketose by a one-step process, the metasaccharinic acid rearrangement then being initiated by ionisation of the enediol at C_1. Evidence for the necessity of the di-ion formation was cited by Whistler and Corbett[92] who found 2-O-D-xylopyranosyl-L-arabinose to be stable under mildly alkaline conditions. The stability was explained as being due to the inability of the glycosyl residue (R) at C_2 to ionise, thus blocking subsequent steps in the Isbell mechanism leading to saccharinic acids (XIV).



(XIV)

Similarly the action of lime water on 2:3-di-O-methyl-D-glucose[93] yielded no saccharinic acids, but a quantity of an $\alpha\beta$-unsaturated aldehyde (R = CH_3) was isolated.

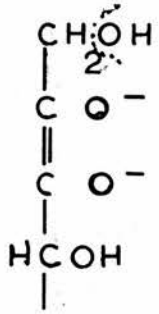
In contrast to this theory, it has been shown that the presence of an enediol di-ion is not essential for the initiation of the saccharinic acid rearrangement as it has been demonstrated that 2,3:5,6-di-O-isopropylidene-D-mannose can be converted to 5:6-O-isopropylidene-D-glucometasaccharinic acids (XVI) on treatment with lime water.[93] The reaction is thought to involve the intermediate (XV).



(XV)

Substitution at C_6 has been found to have only a minor effect on the course of saccharinic acid formation. Thus melibiose, 6-O-α-D-galactosyl-D-glucose was degraded with lime water to give lactic acid and a mixture of the corresponding galactosyl-metasaccharinic and isosaccharinic acids.[94] The di-ion was considered to be stabilised due to inactivation of the 6-hydroxyl group by substitution, and leading to metasaccharinic acid formation.

The effect of different alkalies and different strengths of alkali, have not been thoroughly investigated yet. Kiliani[82] found that glucose on treatment with lime water gave α-D-glucosaccharinic acid, while Nef found no trace of this acid on treatment of the same sugar with concentrated sodium hydroxide, but instead, he obtained metasaccharinic acids plus a smaller amount of the D-iso-saccharinic acid. Corbett and Kenner[90] suggest the difference in behaviour may be due to differences in the ionisation behaviour of the enediols at different pH values. They proposed that at low pH, the 2:3-enediol di-ion (XVII) was formed, and that a preferential elimination of the hydroxyl group at C_1 led to D-glucosaccharinic acid. At higher pH there was possibility of ionisation at C_1 of the 2:3-enediol thus restricting elimination of the hydroxyl group from the resulting tri-ion (XVIII) to C_4, and thus yielding the D-isosaccharinic acid.

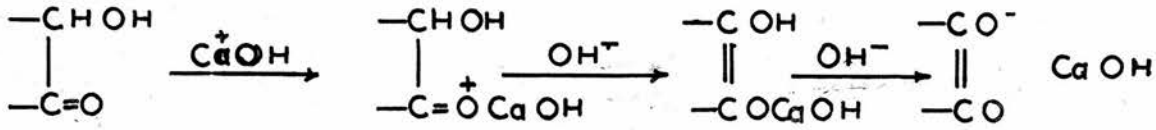


(XVII)

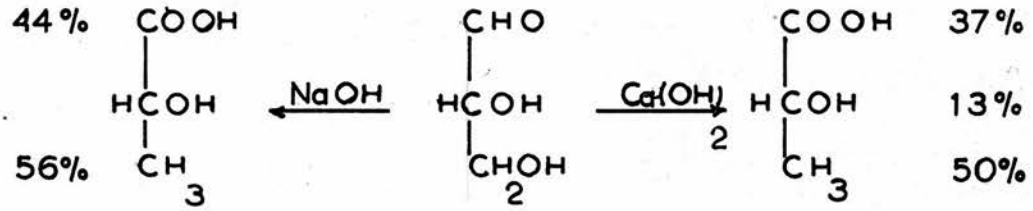
(XVIII)

Kenner and Richards[137] have studied the decomposition of a series of 3-O-alkyl glucoses in lime water in which they found the cation of the alkali employed to be important in the reactions, and that internal complex formation is a much more potent supplement in the reaction than is the basicity of the reagent.

They consider the internal complex formation occurring from the ketose, thus:



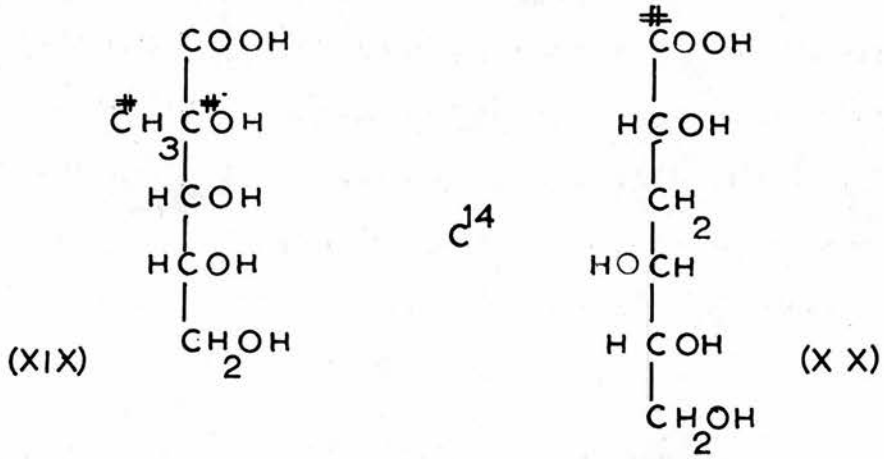
The use of radioactive tracer methods has shown interesting differences in the saccharinic acid formation by different bases. D-Glycerose-3-C^{14} has been isomerised by sodium hydroxide and lime water.(95) The lactic acid produced was found to have the radioactivity distributed between C_1 and C_3, after treatment with sodium hydroxide, but the lactic acid produced on treatment with lime water was labelled on all three carbon atoms.



The authors considered the acid formation to occur through the triose enediol, which will be labelled equally at C_1 and C_3, being transformed to pyruvaldehyde as the α-dicarbonyl intermediate. This is subject either to migration of either the methyl group or the hydrogen atom in the rearrangement step. Sodium hydroxide causes the latter migration to take place almost exclusively, producing the acid labelled at C_1 and C_3. With lime water, this migration preponderates but some migration of the methyl group also occurs producing the acid labelled at C_2 and C_3.

Work has been carried out on the mechanism of saccharinic acid

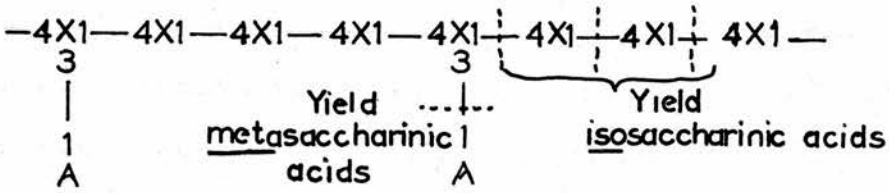
formation, using mannose, 1-C^{14}, and galactose, 1-C^{14}.(103) The D-glucosaccharinic acid produced from mannose (XIX) contained its radioactivity in carbon 2, and the methyl carbon attached to carbon 2 which is in contrast to the Isbell mechanism which predicts the appearance of the radioactivity entirely at the methyl carbon atom. The metasaccharinic acid (XX) produced from galactose contained its radioactivity in carbon 1, as would be expected from the Isbell mechanism.



Sowden and Keunne therefore suggest that the intermolecular recombination of fission fragments should be considered, but the nature of the fragments involved in the recombination step is not yet known with certainty.

As a result of much of the work on the degradation of substituted sugars, suggestions have been made as to a possible application in the determination of polysaccharide structures by means of alkaline degradation studies. For example, the nature of the saccharinic acid product is dependent on the position of substitution, that is, the position of the polysaccharide linkage, and the effect of branch points on the alkaline degradation should be specific and to some extent predictable. Therefore

polysaccharides with 1:3 linkages should give metasaccharinic acids, and those with 1:4 linkages should give isosaccharinic acids.



Much of the earlier work on the structure of polysaccharides was carried out on materials which had been subject to strong alkaline treatments both at the isolation and methylation stage. Although occurrence of degradation would not invalidate the structures assigned to the polysaccharides, it may have a significant effect on the fine structure of these polymers.

The presence of molecular oxygen in alkaline solutions of polysaccharides has long been known to have a degradative effect on these polysaccharides. Rapid depolymerisation of cellulose in cuprammonium solutions, for example, has necessitated in elaborate precautions in the utilisation of this medium for the determination of cellulose viscosities. In alkaline solutions, an acidic type of oxidised cellulose is formed, sometimes referred to as the "methylene blue" type, since it exhibits a high affinity for methylene blue.(89) Large decreases in D.P. of amylose solutions in butanol, in the presence of oxygen have also been reported.(96) Thus in all alkaline degradative work, strict precautions are taken, by carrying out the degradations in an atmosphere of nitrogen with the rigorous exclusion of oxygen.

It has been recognised for a long time that the action of alkali on simple sugars in the presence of air or other oxidants leads to the formation of aldonic acids, such as D-arabonic acid from D-glucose.(133)

The study of the degradation of polysaccharides has been tackled by a preliminary study of the action of alkali on model compounds. Thus in the pentose series, xylobiose and xylotriose, oligosaccharides containing the 1:4 linkages typical of the xylans, were investigated by Aspinall et al,(97) and also by Whistler and Corbett.(98) Xyloisosaccharinolactone (2:4-dihydroxy-2-hydroxymethylbutanoic acid) was isolated and found to be identical to the synthetic material, together with small amounts of the two C_5 metasaccharinic acids and 2:4-dihydroxybutyrolactone. The last three products were present in the products of the alkaline degradation of xylose, but only the isolactone was observed on the treatment of an acid degraded esparto xylan with alkali. Degradation was considered to have commenced at the reducing end of the molecule and continuing as a "peeling" reaction of the type, xylotriose → xylobiose → xylose, in which each successive reducing sugar gave rise to acidic products with the exposure of a new reducing group. In the hexose series, Kenner and his co-workers have studied the effect of alkali on 1:4-linked compounds such as cellobiulose, cellobiose, cellotetraose(86) and 4-O-methyl-D-glucose.(88) Complete degradation to the isosaccharinic acid was found to take place, which in the case of cellotetraose can be illustrated thus:

G-G-G-G ⟶ G-G-G-F ⟶ G-G-G + S ⟶ G-G-F + S

⟶ G-G + 2S ⟶ G-F + 2S ⟶ G + 3S ⟶ A+3S

(G = glucose; F = fructose; S = isosaccharinic acid;
A = acids from degradation of glucose.)

Glucose, fructose, cellobiose and cellobiulose were detected in the degradation products of the cellotetraose experiments.

Degradation of Polysaccharides

Some of the earlier work on the degradation of polysaccharides with alkalies was carried out by Prey and his co-workers who investigated the hemicellulose and holocellulose fractions from sprucewood and straw after heating with aqueous sodium hydroxide in an atmosphere of nitrogen at temperatures up to 190°.(99) Changes in D.P. and acid formation were observed, there being a large drop in D.P. at 190° with a corresponding rise in acidity. Richtzenhain et al(100) treated cellulose with boiling aqueous sodium hydroxide and obtained an alkali stable polysaccharide, and they showed that the extent of degradation before stability was related to the number of reducing end-groups at which degradation was known to commence. Samuelson and Wennerblom(101) found in the hot cooking of cellulose, an average of 50 units were broken off before the reaction stopped.

Much experimental support has been given to Davidson's original suggestion that attack by alkali occurs only at the reducing end of the cellulose molecule.(102) Thus it has been shown that modification of the reducing end-group by oxidation,(127)

reduction[128] or glycosidation[129] can render cellulose stable to alkali. Lindberg[130] has shown that glycosides are degraded by alkali under drastic conditions (10% NaOH/170°). For example, methyl-β-D-glucopyranoside and cellobitol are degraded under these conditions, the reaction mechanism being analogous to that of alkaline hydrolysis of phenylglycosides. The author stresses the importance of this type of reaction during the pulping of wood under alkaline conditions if there are linkages of a glycosidic nature between lignin and hemicellose in wood. The possibility of degradation occurring even under mild conditions by a chopping of the polysaccharide chains must also be considered.

A xylan obtained by Whistler and Corbett[98] after delignigication of maize cobs was found to be stable to alkali, a fact which was attributed to the oxidation of the aldehyde group to a carboxylic acid during the delignification process.

Two types of reaction are known to occur in the treatment of polysaccharides with alkali:

(1) "Degradation", consisting of rearrangement and fragmentation reactions.

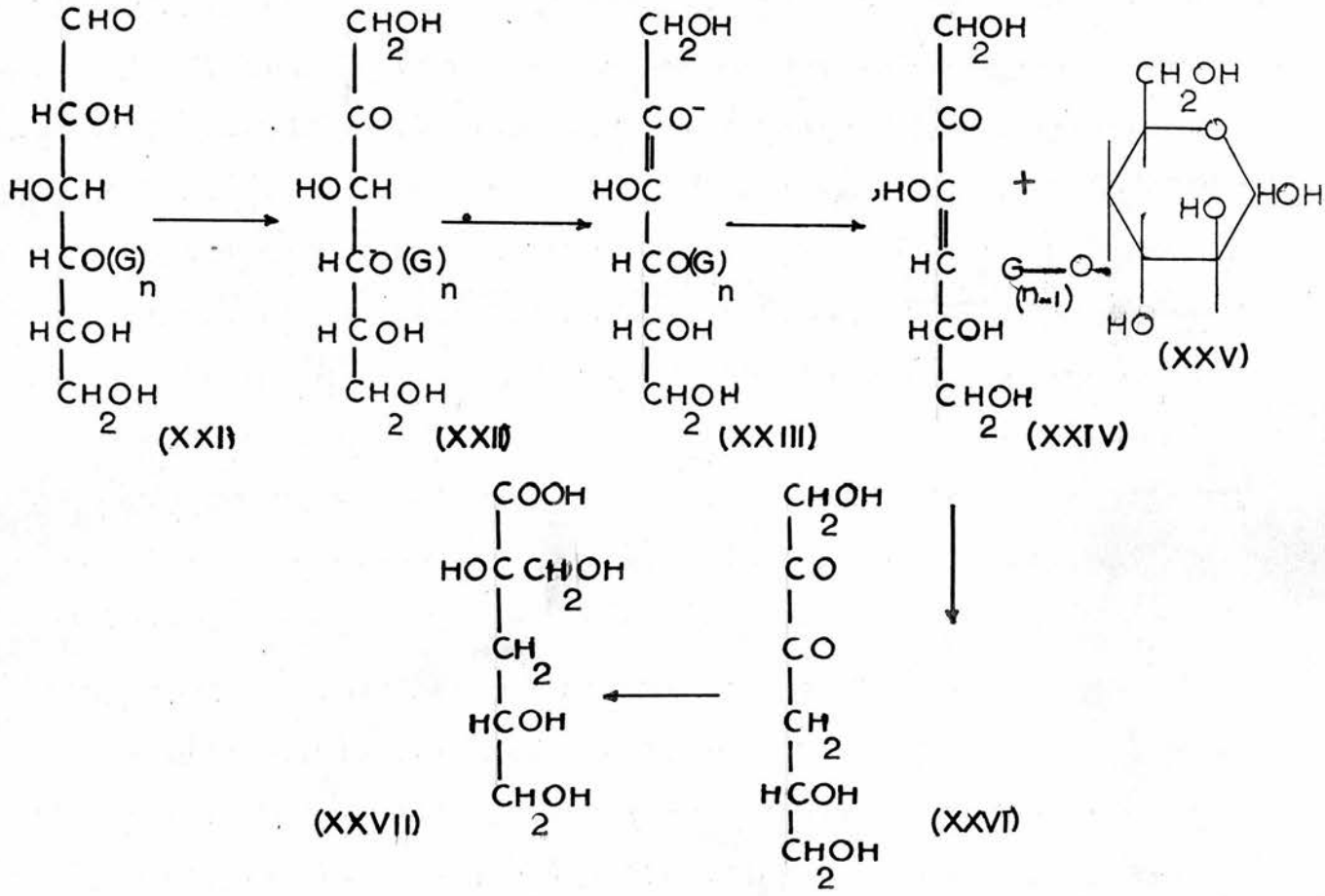
(2) Stopping reaction, in which a terminal sugar unit rearranges to a carboxylic acid.

The degradation reaction is in effect a "peeling' reaction in which each successive reducing sugar rearranges to an acidic product and giving rise to a fresh reducing group. This type of reaction is accompanied by a low change in D.P. There is also the possibility of scission of the polysaccharide chains, due to the presence of traces of molecular oxygen, at places remote from

the reducing ends, and consequently a large drop in D.P. is expected.

As would be expected, D-glucoisosaccharinic acid (XXVII) was obtained as a major product in the degradation of unmodified cotton cellulose (XXI) by Richards and Sephton.(131)

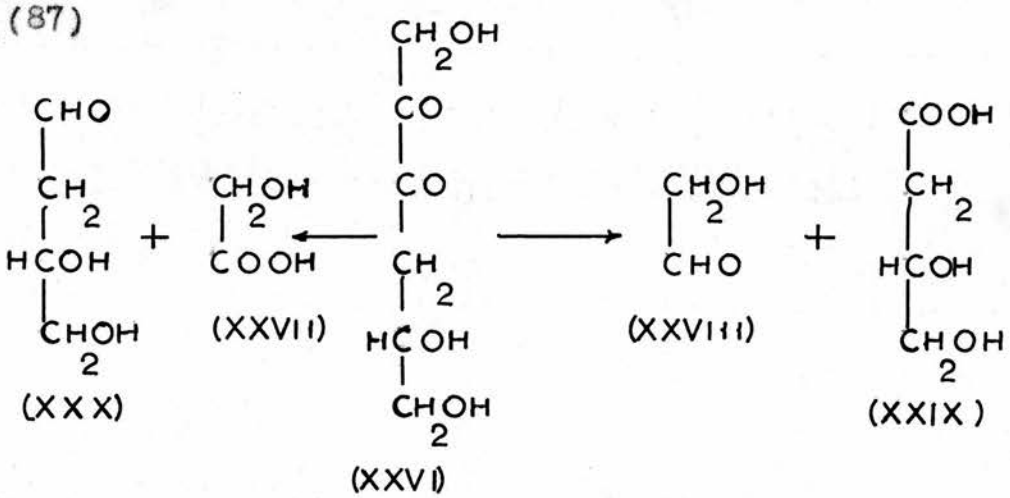
The mechanism proposed was as follows:



Appreciative quantities of other acids, mainly formic, acetic, lactic, glycollic, and 2:4-dihydroxybutyric acids together with a small quantity of D-glucometasaccharinic acid, were identified. The nature and proportions of the acidic degradation products of amylose were found to be similar to the analogous products from cellulose.(142) Their occurrence is attributed to degradation

and rearrangement of the diketone (XXVI), and fragmentation of the reducing end group (XXI), but other types of degradation are likely to take place.

The amount of formic acid observed both in relation to the total acid and to acetic acid, was quite different from that obtained from a similar degradation of glucose. Previously a fragmentation reaction was suggested for the breakdown of the diketone (XXVI), based on the results of the breakdown of maltose.(87)

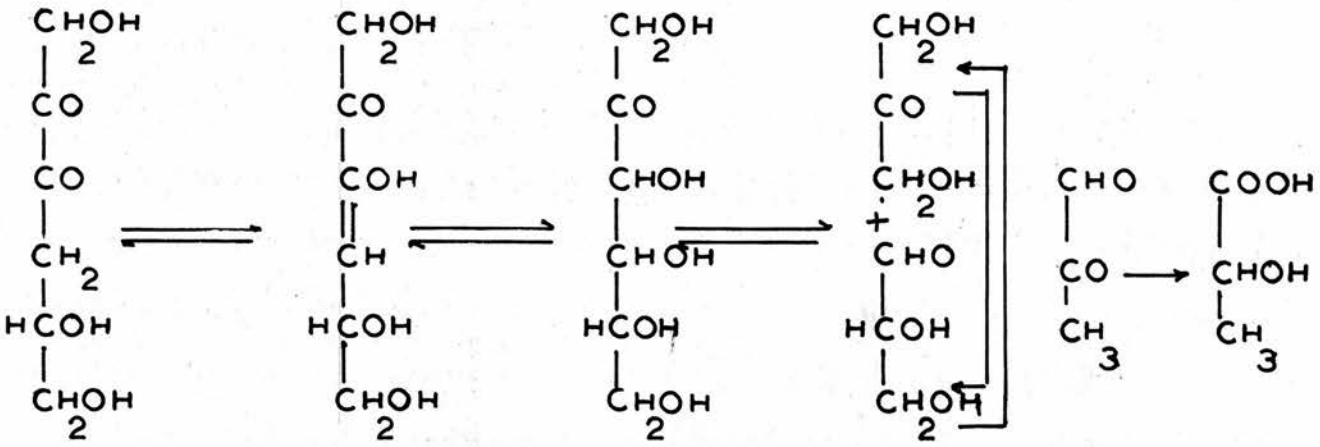


Evidence was obtained for the presence of glycolaldehyde (XXVIII) and the aldehyde (XXX), by oxidation with bromine and subsequent detection of the corresponding acids, and also for the presence of glycollic acid (XXVII) and βγ-dihydroxybutyric acid (XXIX).

The presence of large quantities of lactic acid cannot be explained by Nef's idea of a reverse aldolization of the enediols to give two fragments on scission between C_3 and C_4, due to the hydroxyl group at C_4 being blocked by another glucose unit.

Again the diketone (XXVI) is thought to be the precursor of

lactic acid, in which there is addition of a hydroxyl anion to the ketone, followed by fragmentation at C_{3-4}.

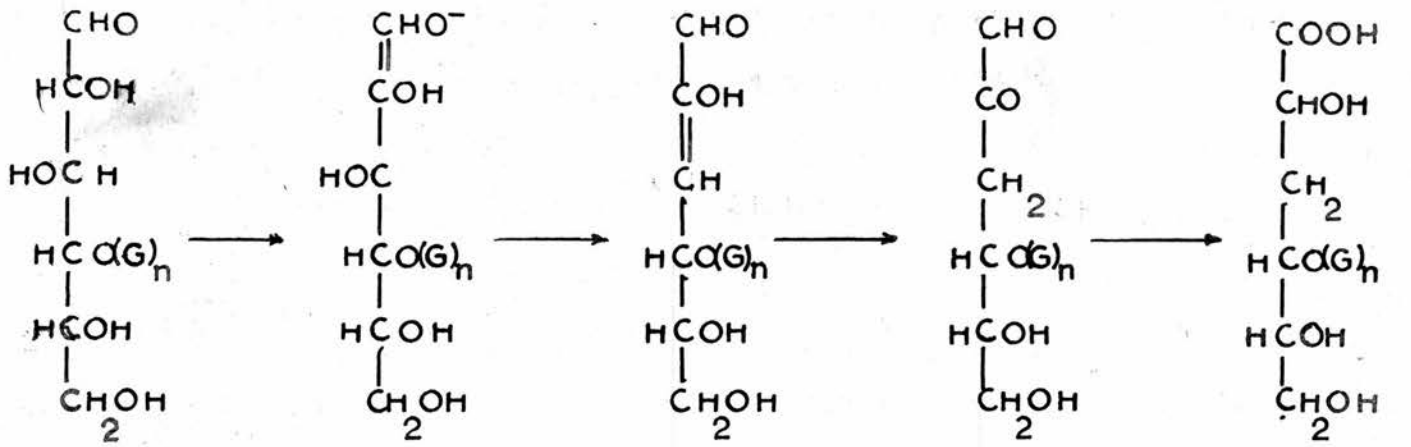


(XXV)

The question of fragmentation reactions can only be settled completely on the synthesis or isolation of the α-diketone (XXV), and studying the effect of alkali on it.

Stopping Reaction

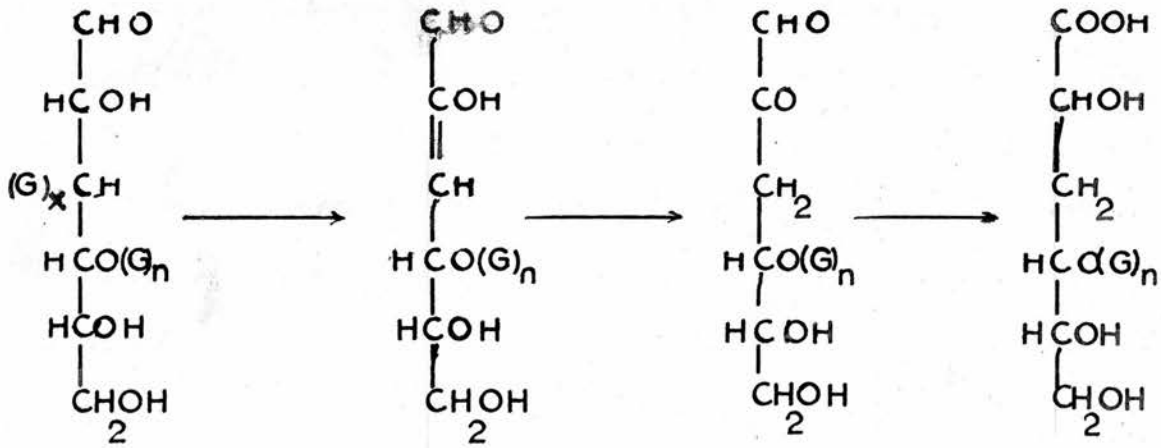
As has been previously mentioned (page 33), the degradation of cellulose by means of the peeling reaction was found to come to an end after about 50 units had been broken off the chain. Samuelson attributed this to the oxidation of the aldehyde group either by traces of oxygen, or by a "crossed" Cannizzaro reaction between the end-group and some degradation product. It is now apparent that at certain stages in the degradation the reducing end-group rearranges to a metasaccharinic acid which remains attached to the polysaccharide backbone, thus preventing further degradation. In this way, hydrolysis of the alkaline stable polysaccharide obtained after treatment of cotton hydrocellulose with NaOH, yielded D-glucometasaccharinic acid[132] by a mechanism which is considered to be as follows:



(XXXI) (XXXII) (XXXIII) (XXXIV) (XXXV)

The stopping reaction (XXI) - (XXXV) occurs at a much slower overall rate than the degradation reaction (XXI) - (XXVII) owing to the mass law effect in reactions (XXXII) - (XXIII) and (XXIII) - (XXIV), thus there is a considerable degradation of a given molecule before the stopping reaction renders it stable.

There is also the possibility of "stopping" occurring at branch points, such as 1:3 branches, thus involving rearrangement to a non-reducing end-unit (XXXVI - XXXIX).



(XXXVI) (XXXVII) (XXXVIII) (XXXIX)

Aim of the Present Work

In recent years there have been significant contributions to cereal carbohydrate chemistry, in which the water soluble gum contents of the grains have been studied. Since most hemicelluloses are extracted from the plant sources by means of alkali, cereal gums are useful in the study of alkaline degradation of polysaccharides.

The aim of this investigation has been to make a structural study on a water soluble gum from rye and to investigate the action of alkali on this and other cereal hemicelluloses.

SECTION 1

D I S C U S S I O N

Discussion

A Water Soluble Hemicellulose from Rye Flour

A quantity of hemicellulose from the water soluble gum fraction of rye flour was obtained from Professor I.A. Preece for this structural study. The material had been extracted from the flour with water at 40° and had been isolated as the pentosan-rich fraction by ammonium sulphate precipitations.[18]

Hydrolysis of the hemicellulose followed by quantitative chromatographic examination of the hydrolysate showed that it contained xylose (60%), arabinose (29%) and glucose (5%).

The polysaccharide was methylated using the standard procedures and the product was fractionally dissolved from petroleum ether with chloroform, giving a major fraction, (OCH_3, 38.7%, $[\alpha]_D$, -113°), which was used in subsequent work. This was hydrolysed in 1 N hydrochloric acid at 35° for 10 days, followed by heating on a water bath until its rotation was constant. A small insoluble residue was hydrolysed with methanolic hydrogen chloride followed by aqueous hydrochloric acid. The combined hydrolysate yielded a syrup which was placed on a cellulose column and the methyl ethers were eluted with butan-1-ol:petroleum ether (30:70) saturated with water; butan-1-ol:petroleum ether (1:1) saturated with water, and butan-1-ol half saturated with water, yielding fractions corresponding to trimethyl arabinose, dimethyl xylose, monomethyl xylose, and xylose.

The trimethyl arabinose fraction was shown by chromatography

to contain a small quantity of 2:3:4-tri-O-methyl xylose, the bulk of the fraction being identified at 2:3:5-tri-O-methyl-L-arabinose by conversion to the amide of the corresponding arabonolactone having m.p. and mixed m.p. 130-32° with 2:3:5-tri-O-methyl-L-arabonamide.

Fraction 3 was shown by chromatography to consist of 2:3-di-O-methyl-D-xylose, and a trace of tri-O-methyl-D-glucose. Conversion of the material to the corresponding anilide gave crystals which had m.p. and mixed m.p. 121-122° with 2:3-di-O-methyl-N-phenyl-D-xylopyranosylamine. A chromatographically pure dimethyl xylose fraction crystallised on seeding with authenic 2:3-di-O-methyl-β-D-xylose and was identified by conversion to the corresponding 2:3-di-O-methyl-N-phenyl-D-xylopyranosylamine.

The monomethyl xylose fraction which was crystalline, was found to be 2-O-methyl-D-xylose with a trace of 3-O-methyl-D-xylose, having m.p. and mixed m.p. (with 2-O-methyl-β-D-xylose) 130°. The unmethylated fraction was shown to consist of D-xylose by conversion to the di-O-benzylidene dimethyl acetal which had m.p. and mixed m.p. 208-209°.

The molar percentages of the identified sugars are as follows:

2:3-5-tri-O-methyl-L-arabinose	30%
2:3-di-O-methyl-D-xylose	36%
2-O-methyl-D-xylose	31%
D-xylose	2.5%

No estimation was made on the small amounts of 2:3:4-tri-

O-methyl-D-xylose, 3-O-methyl-D-xylose and tri-O-methylglucose which were obtained in the fractions.

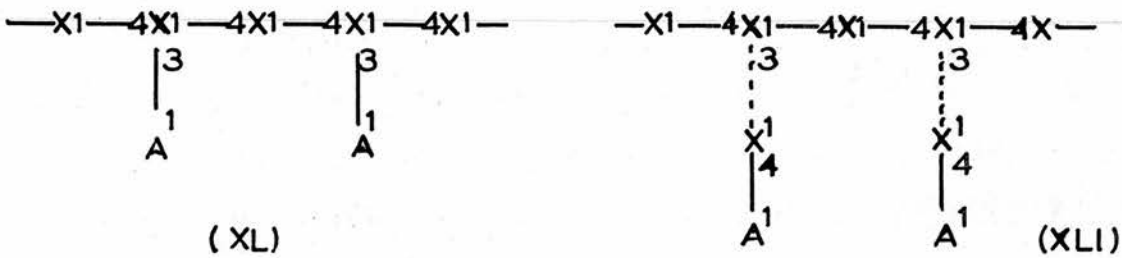
The molar proportions of methylated sugars can be approximated to tri-O-methylarabinose, 5, di-O-methylxylose, 6, monomethylxylose, 5. These results indicate the presence in the polysaccharide of chains of 1:4-linked D-xylose residues with branching mainly through position 3. All the side chains are terminated with L-arbofuranose residues, this being the sole mode of linkage of the arabinose residues. It is not certain whether the small amount of D-xylose isolated from the hydrolysis of the methylated polysaccharide represents some double branching points or whether the sugar arises from incomplete methylation of the polysaccharide or demethylation during hydrolysis. The presence of tri-O-methylglucose isolated on hydrolysis of the methylated polysaccharide and the glucose residues in the original polysaccharide probably arise from a contaminating glucan since no methyl ethers of glucose could be detected in the hydrolysate of another fraction of methylated polysaccharide.

Periodate oxidation of the rye araboxylan indicated an uptake of 0.67 moles of periodate per anhydro pentose unit, which is in agreement with the value of 0.69 moles per anhydro pentose unit for a 1:4-linked xylan containing 30% terminal arabinose units.

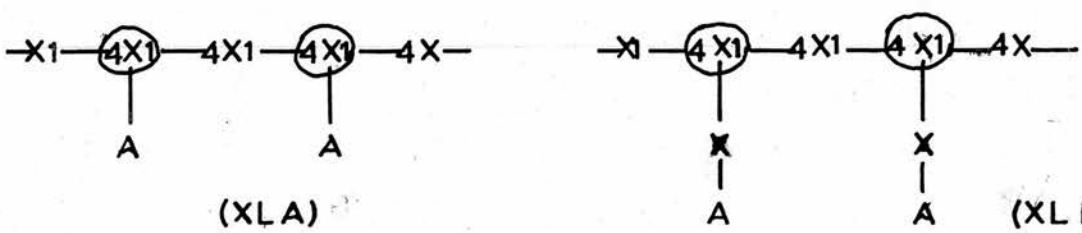
Estimation of the quantity of formic acid produced during periodate oxidations indicated the liberation of 1 mole of formic acid per 29 anhydropentose units.

It is clear from these results that the rye flour araboxylan is similar to other polysaccharides of the xylan group, in

particular to those obtained from wheat[14] and barley[65] flours, in containing 1:4-linked β-D-xylopyranose residues to which are attached L-arabofuranose residues as side chains to the backbone at C_3. Methylation studies alone cannot determine the lengths of any of the side chains and therefore two possible structures ((XL) and (XLI)) may be put forward for the repeating unit of the polysaccharide.



The dotted lines represent an unknown number of 1:4-linked xylose units. Thus XL represents the branch points in the molecule with the chain terminating units directly attached to the xylan backbone, and in XLI with those units not directly attached to the backbone.

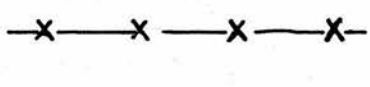


In a polysaccharide as illustrated in XL A and XLI B, all the sugar units except those marked by circling are susceptible to attack by the periodate ion, and the unattacked sugar units can be estimated on hydrolysis of the periodate oxidised polysaccharide.

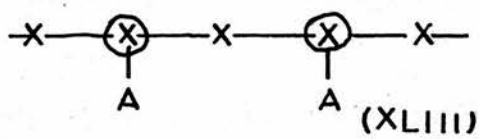
The following results provide evidence in favour of structure (XL).

The rye xylan was oxidised with sodium metaperiodate and the oxidised polysaccharide was obtained in 56% yield. Hydrolysis of this material liberated xylose (24-25%), a value which is slightly lower than would be expected if all the arabinose residues were attached to singly branched xylose residues (ca. 29%). The analysis was carried out in the usual way using galactose as the reference sugar.

Complete removal of the terminal arabofuranose residues would leave degraded polysaccharides as represented by XLII and XLIII causing an increase in the number of xylose units susceptible to periodate oxidation in the first case and no increase in the second.



(XLII)



(XLIII)

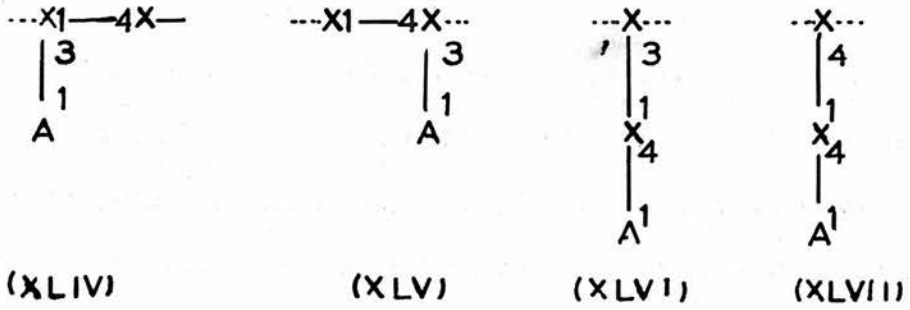
Optimum conditions for removal of the terminal arabofuranose residues without breaking the xylose backbone were found, and the degraded material was found to contain xylose (60%) and arabinose (10%) on hydrolysis. These and subsequent values are expressed as percentages of the original polysaccharide.

Oxidation of this material with periodate followed by hydrolysis, indicated the presence of xylose (8%) unattacked by periodate. The reduction (ca. 16-17%) in xylose residues unattacked by periodate accompanying the controlled degradation of the polysaccharide corresponds approximately to the decrease (ca. 19%) in arabinose residues. This result would be expected on the basis of structure (XL) only, and shows that the majority at least of the L-arabofuranosyl residues must be attached directly

to position 3 of the β-D-xylopyranose residues present in the essentially linear backbone of the molecule.

Additional evidence as to the presence of single unit arabinose side chains was obtained in the isolation of a trisaccharide which was obtained from the enzymic hydrolysis products of the polysaccharide. It has been shown by Conchie and Levvy[21] that selective inhibition of β-glycosidases may be obtained with aldonolactones, derived from the same parent sugars, as substrates. The polysaccharide was hydrolysed by means of hemicellulase, in the presence of L-arabonolactone as an inhibitor, in a sodium acetate buffer at pH 5. The mixture of oligosaccharides thus obtained was separated on a charcoal-celite column. After removal of the inorganic material and monosaccharides by elution with water, oligosaccharide fractions were obtained by continuing the elution with water containing increasing concentrations of ethanol. Fractions were obtained having R_{xylose} values of 0.40, 0.66, 0.30 (xylotriose) and 0.14 (xylotetraose). The fraction having an R_{xylose} value of 0.40 was obtained chromatographically pure and had $[\alpha]_D$, -15.5°, and on hydrolysis of a small quantity, followed by quantitative chromatographic examination, was found to contain xylose (65%) and arabinose (32%).

On the basis of the types of linkage known to occur in the polysaccharide, there are four possible structures for a trisaccharide of this type, viz:



(XLIV) (XLV) (XLVI) (XLVII)

Differentiation can be obtained by studies on partial hydrolysis, effect of periodate oxidation, and effect of alkali.

By preferential removal of the arabinose units by mild acid hydrolysis, 4-O-β-D-xylopyranosyl-D-xylopyranose would be expected from structures XLIV, XLV and XLVII, and 3-O-β-D-xylopyranosyl-D-xylopyranose (rhodymenabiose) from structure XLVI.

Degradation with alkali would produce xyloisosaccharinic acid from XLVII and XLIV initially, D-xylometasaccharinic acids from XLVI and 4-O-β-D-xylopyranosyl-D-xylometasaccharinic acids from XLV. These theoretical results together with those for periodate oxidation are summarised in Table IV.

Table IV

Structure	Partial Hydrolysis	Effect of Alkali	Periodate Uptake (moles)	Formic Acid Release (moles)	Formalde-hyde (moles)	Hydrolysis of oxypoly-saccharide
XLIV	xylobiose	X_{iso}	3	2	-	xylose (1 mole)
XLV	xylobiose	$X\text{-}X_{meta}$	4	2	-	-
XLVI	rhodymena-biose	X_{meta}	3 - 4	1	1	-
XLVII	xylobiose	X_{iso}	4	2	-	-
Observed Results	xylobiose	X_{iso}	3		-	xylose (1 mole)

X_{iso} = xylo*iso*saccharinic acid.

X_{meta} = D-xylo*meta*saccharinic acids.

$X\text{-}X_{meta}$ = 4-O-β-D-xylopyranosyl-D-xylo*meta*saccharinic acids.

That the trisaccharide is of structure XLIV is shown as follows.

Mild acid hydrolysis followed by paper chromatographic examination showed the presence of xylobiose (1→4). No evidence could be found for the presence of rhodymenabiose. On treatment of the trisaccharide with alkali over an atmosphere of nitrogen, xylo*iso*saccharinic acid was found to be present on deionisation and chromatography of the material.

Oxidation with sodium metaperiodate indicated an uptake of 3.1 moles of periodate per mole of sugar. Hydrolysis of a sample showed the presence of xylose, which on quantitative chromatographic estimation using rhamnose as reference sugar was found to be equivalent to 0.90 mole of xylose per mole of sugar. No evidence

was obtained for the production of formaldehyde during the periodate oxidation.

In a second experiment the hydrolysis of the polysaccharide was carried out with the "Hemicellulase" in the absence of the L-arabonolactone. The results showed the presence of xylobiose and xylotriose but no arabinose containing oligosaccharides could be found, indicating an inhibition of the enzyme by the aldonolactone. No evidence was found for any transglycosylation taking place during the hydrolysis of the polysaccharide in the presence of the enzyme and aldonolactone.

Thus the rye araboxylan has been shown to have a structure similar to those assigned to wheat[14] and barley[65] flours. It is concluded that this highly branched araboxylan contains chains of 1:4 linked β-D-xylopyranose residues with approximately every second xylose residue carrying a terminal L-arabofuranose residue linked through position 3.

E X P E R I M E N T A L

General Procedures

Chromatographic Solvents

a) benzene : butan-1-ol : pyridine : water (1:5:3:3)

b) butan-1-ol : ethanol : water (4:1:5)

c) benzene : ethanol : water (169:47:15)

d) ethyl acetate : pyridine : water (10:4:3)

e) methyl ethyl ketone : water (sat.)

f) amyl alcohol : ethyl acetate : formic acid : water (4:2:1:3)

g) propan-1-ol : ammonia (60:40)

When the solvent systems were two-phase, the upper layer was used.

Chromatographic Sprays

a) Saturated aqueous aniline oxalate

b) Hydroxylamine - ferric chloride (108)

c) Potassium iodate : potassium iodide : starch (109)

d) Bromcresol green (110)

e) Mercuric chloride (111)

Demethylations were carried out by heating the sugar (ca. 5 mg.) with hydrobromic acid (1 ml., 40%) in a sealed tube for 15 minutes at 100°. The dark coloured solutions were diluted with water and neutralised with silver carbonate. Any remaining silver was eliminated by precipitation with hydrogen sulphide. On evaporation of the filtrates to dryness, the organic material was extracted from any remaining inorganic material by dissolution in hot methanol.

Hydrolyses were carried out in N sulphuric acid which was removed by neutralisation with barium carbonate. In the case of hydrolysis with hydrochloric acid, the chloride ions were removed by means of silver carbonate, as before.

Ionophoresis. The technique and apparatus was similar to that described by Consden and Stanier.[112] The papers were run for four hours using borax (0.05 M) as the electrolyte. Aqueous aniline oxalate containing 5% glacial acetic acid was used to indicate the positions of the sugars on the papers.

Methoxyl Determinations were carried out by means of the micro Zeisel method.[113]

Acetyl Determinations were carried out according to the method described by Belcher and Godbert.[114]

Specific Rotations were measured in a 1 dm. tube unless otherwise stated, between 15 and 20°.

Refractive Indices were measured on an Abbe Refractometer at 20°.

Aniline Derivatives of Sugars were prepared by refluxing about 20 mg. of sugar with an equimolecular amount of freshly distilled aniline in ethanol (5 ml.) over a period of two hours. During the heating a steady stream of carbon dioxide was passed

through the reaction mixture and light was excluded as much as possible. The syrup obtained on removal of the solvent crystallised, and was recrystallised from ethyl acetate.

Preparation of Aldonolactones. The sugar was oxidised with an excess of bromine water over a period of four days at room temperature. The excess bromine was removed by aeration, and the solution was neutralised with silver carbonate, the last traces of silver being removed by treatment with hydrogen sulphide. After evaporation of the filtrate, the resulting syrup was heated on a boiling water bath at 15 mm. pressure for two hours.

Preparation of the Aldonamides. The lactone was treated at 4°C for 16 hours with a small volume of dry methanolic ammonia. On removal of the solvent at reduced pressure, the crystalline material was recrystallised from ethyl acetate.

Preparation of 4-Bromophenacyl esters. The acid (ca. 20 mg.) was neutralised with NaOH to a phenolphthalein end-point, and made faintly acid with very dilute HCl. 4-Bromophenacyl bromide (20 mg.), in ethanol (5 ml.), was added, and the mixture was refluxed for an hour. On cooling, the crystalline product was separated, and recrystallised from ethanol.

The Hemicellulose of Rye Flour

Preliminary extraction of the grain

The raw grain was ground to a fine flour by passage through a hammer mill, and the material was subjected to two successive half-hour extractions with boiling 80% (V/V) ethanol (250 ml./100 g.) in order to deactivate enzymes and remove fats, waxes, colouring materials, sugars, amino acids and other low molecular weight materials. After hot filtration, the extracts were discarded, and the grain residue was air dried.

The inactivated rye was subjected to three successive half-hour extractions with water at 40°. Stirring was applied throughout each extraction period and the amount of water used for each extraction was 2½ times the original weight of rye. Centrifugation of the extracts removed the insoluble grain residues and large quantities of starch. The cloudy solutions were clarified by allowing them to stand overnight in centrifuge bottles, when more starch was deposited. Further centrifugation gave a bright extract which was then concentrated to about $\frac{1}{15}$th of its original volume. Acetone (4 vols.) was added slowly with stirring, and the resulting precipitated polysaccharide was centrifuged off, washed with acetone, and finally with ether. The polysaccharide, after mechanical powdering, was dried over phosphorus pentoxide at 15 mm. pressure. In this way 85 g. of polysaccharide was isolated from 10 kg. of grain, which represents 0.85% of the rye. A sample of the extracted material was examined by paper chromatography after hydrolysis and was found

to contain sugars which ran at identical rates to standard xylose, arabinose, glucose and galactose. The presence of galactose could only be detected if the papers were spotted heavily, confirmation being obtained by the yellow fluorescence under ultra-violet light.

Attempted fractionation of the polysaccharide

Ammonium sulphate precipitation[18]

Polysaccharide (10 g.) was stirred into water (1 l.) and warmed to 40° for four hours. Much of the material remained undissolved, so the solution was placed in a stoppered bottle and the contents were shaken overnight on a mechanical shaker. The solution was centrifuged and the insoluble material (0.5 g.) was washed with 50% aqueous acetone and dried by solvent exchange. Hydrolysis of a small sample of this material and examination of the products by paper chromatography showed the presence of glucose with small amounts of xylose and arabinose. This material was rejected. Solid ammonium sulphate (100 g.) was stirred into the centrifugate until dissolved. No precipitation occurred at this point, and so a further 100 g. of salt was added as before. The precipitate which formed was removed by centrifugation, dissolved in a small volume of water, precipitated by addition of an equal volume of acetone, and dried by solvent exchange. The salt concentration was increased by amounts of 10% at a time up to 70% concentration. Precipitates were isolated in the usual manner. After removing the precipitate resulting from the 70% salt solution, the solution was concentrated to

remove as much of the ammonium sulphate as possible. The remaining inorganic material was removed by dialysis of the solution through cellophane against running water for three days. Addition of acetone (2 vols.) and a few drops of aqueous potassium chloride to coagulate the colloidal material, precipitated the polysaccharide, which was centrifuged off, and dried by solvent exchange. Samples (10 mg.) of each of the fractions were hydrolysed with $\bar{N}$ sulphuric acid, and the products examined by means of paper chromatography. For results see Table V.

Table V

Fraction	Salt conc. %	Weight of Fraction (g.)	Products of Hydrolysis			
			Galactose	Glucose	Xylose	Arabinose
1	-	0.50		++++	+	+
2	20	1.00	+	++	+++	+++
3	30	1.80	+	++	++++	+++
4	40	1.00	+	++	++++	+++
5	50	4.00	-	+	++++	+++
6	60	0.20	-	+	++++	+++
7	70	0.10	-	-	++++	+++
8	over 70	0.10	-	-	++++	+++

Key: ++++ , major component
++ , minor component
+ , trace
- , not detected

The separated precipitates were independently redissolved in water and to each was added ammonium sulphate equivalent to 20%. Any precipitate formed by these fractions was collected as a 20% fraction. Only the 20, 30 and 40% fractions gave precipitates at this stage. After removal of the precipitates, the concentrations of the individual solutions were raised by increments of 10% and the hemicellulose material was isolated from each one in the usual way. A further two series of re-precipitations was carried out on fractions 3 - 5. Examination of the hydrolysis products of each of those fractions indicated the absence of galactose but the presence of glucose to about 10%, the remainder of the polysaccharide consisting of xylose and arabinose.

A quantity of the polysaccharide (20 mg.) from fraction 3 was dissolved in water (2 ml.) and added to a solution of salivary α-amylase (1 ml.) in sodium acetate buffer (pH 5). After incubation at 35° for 48 hours, the polysaccharide was precipitated with acetone (2 vols.) and separated off. Examination of the filtrate by paper chromatography showed the presence of glucose and a small amount of maltose, thus indicating the presence of starch in the polysaccharide. Hydrolysis of the polysaccharide itself, followed by examination of the sugars, showed the presence of about 5% glucose, together with xylose and arabinose.

Acetone Precipitation

Polysaccharide (5 g.) obtained by combining fractions 3 - 5

from the ammonium sulphate precipitation, was stirred into water (500 ml.) and warmed to 40° for 6 hours, by which time all the material had dissolved. Acetone was added with vigorous stirring in increasing concentrations. No precipitate was formed until a concentration of 40% acetone was reached. The material was centrifuged off and isolated in the usual manner (0.1 g.). Further additions of acetone produced precipitates at 50% (2.5 g.) and 60% (1 g.) concentrations. Samples from the three hemicellulose fractions were examined chromatographically. The glucose content as far as could be seen from the paper, had diminished considerably to about 5% in the 40% and 50% fractions, and in the 60% fraction, the presence could only be shown by the yellow fluorescence under ultra-violet light. In this manner 10 g. of the pentosan was isolated, which in this thesis will be referred to as Rye xylan 'A'. A similar pentosan Rye xylan 'B' obtained from rye flour by ammonium sulphate precipitations was supplied by Prof. I.A. Preece, and it is this material which was used in the following experimental work unless otherwise stated.

Analysis of Polysaccharide

Specific Rotation

The specific rotation of the polysaccharide was measured in sodium hydroxide (1 $\bar{N}$) at a concentration of 1.17%

$$[\alpha]_D = -107^\circ$$

Ash

The polysaccharide (ca. 200 mg.) was accurately weighed out and ignited to constant weight in a platinum crucible. % Ash = 0.14%.

Quantitative Chromatographic Analysis[115]

A known weight of polysaccharide (ca. 30 mg.) was heated in a sealed tube with sulphuric acid (1 ml., $\bar{N}$), at 100° for four hours. Rhamnose (15 mg.) was weighed in, and the solution was neutralised with barium carbonate, filtered and deionised through a solution of Amberlite I.R. 120(H). The sugars were separated chromatographically on papers in solvent (a) over a period of three days and determined by the method of Somogyi.[116]

Results

Sugar	mg.	%
Glucose	0.13	5.5
Xylose	1.29	59.6
Arabinose	0.63	28.9
Rhamnose	1.25	-

Methylation

The polysaccharide was methylated, first by the method of Haworth and Hirst[117] using sodium hydroxide and dimethyl sulphate, and then by Purdie's method using silver oxide and methyl iodide.[118]

Polysaccharide (2.5 g.) was dissolved in water (16 ml.),

with the aid of mechanical stirring in a 2 l., 3-necked flask. The flask was swept out with a continual stream of nitrogen, from which oxygen had been removed, by bubbling through alkaline pyrogallol. Sodium hydroxide solution (34 ml., 60%) was added over a period of 15 minutes. The first methylation was then commenced by simultaneous additions of sodium hydroxide solution (40 ml., 40%) and dimethyl sulphate (20 ml.) over a period of 5 hours. During the addition, the flask was cooled in an ice bath to prevent frothing. The mixture was then left stirring overnight, and the methylation was continued with the addition of alkali (40 ml., 40%) and dimethyl sulphate (20 ml.) dropwise as before. The third and fourth methylations were carried out in the same manner as the second, the mixture being stirred overnight between each set of additions. At the end of the fourth methylation it was noticed that the methylated polysaccharide appeared to be coming out of solution. To counteract this, acetone (40 ml.) was added, and the fifth methylation was continued as before.

The pH of the solution was reduced to 6 - 7 by the addition of glacial acetic acid, and the acetone was removed under reduced pressure. On heating the mixture on a boiling water bath, the methylated material separated out on the surface and was separated off, washed with a small volume of water to remove inorganic material, and then dissolved in a small quantity of acetone. Repeated evaporation and dissolution in this solvent removed further small quantities of inorganic material. The aqueous solution was shaken with chloroform (3 x 50 ml.) and the

organic layers were combined, and evaporated to a syrup. The dried syrup was dissolved in chloroform (10 ml.) and poured into petrol-ether (200 ml.) to precipitate the methylated material, which was filtered off and dried in a vacuum desiccator containing pieces of paraffin wax. The weight of methylated polysaccharide after five methylations was 2.54 g. (OCH_3 = 32% - the theoretical methoxyl content for dimethyl pentose is 38.35%).

Purdie Methylations

The partially methylated material (2.5 g.) was dissolved in boiling methyl iodide (75 ml.), which had been freshly distilled over dry silver oxide. Dry methanol (5 ml.) was added to aid dissolution. Silver oxide (10 g.) was added in small portions over a period of six hours and heating was continued for a further 3 hours. The solvent was decanted and the inorganic residues were filtered off, washed with dry chloroform (4 x 20 ml.) and extracted in a Soxholet with dry chloroform. The filtrates were combined, and the solvents removed under reduced pressure, leaving a golden brown syrup (OCH_3, 38%). A second methylation was carried out using the same reagents. Methyl iodide, (75 ml.), silver oxide, (10 g.).

Yield, 1.80 g. OCH_3, 39.2%.

Fractionation of the Methylated Polysaccharide

The methylated polysaccharide (1.70 g.) was suspended in light petroleum (60-80°) (95 ml.) and chloroform (5 ml.), and refluxed for half an hour. The undissolved material was

centrifuged off and the filtrate was evaporated under reduced pressure. Fractionation was continued in this way, using increasing concentrations of chloroform (see Table VI).

Table VI

Fraction	Petrol ml.	Chloroform ml.	Weight of Fraction (g.)	Specific Rotation	Hydrolysis Products
1	95	5	0.007	-	Me pentoses + hexoses
2	90	10	0.085	-	" " + trace hexoses
3	85	15	0.013	-	" " + " "
4	80	20	0.013	-	" " + " "
5	75	25	0.901	-110°	" " + " "
6	70	30	0.434	-115°	" " + " "
7	65	35	0.237	-121°	Methylated pentoses
8	60	40	0.042	-	" "

Samples (ca. 5 mg.) from each fraction were hydrolysed by heating with methanolic HCl (1 ml. 4%) for 6 hours in a boiling water bath. After removal of the methanol the samples were heated with aqueous HCl (1 $\bar{N}$) for a further three hours. After removal of the inorganic material, the resulting syrups were chromatographed in solvent (b).

Hydrolysis of the Methylated Polysaccharide

The fully methylated polysaccharide (1.33 g.), obtained by combining fractions 5 and 6, was added to aqueous HCl (1 $\bar{N}$, 250 ml.), and allowed to stand in an incubator for six days, at 30°. A small

amount of material remained undissolved by this time, and was filtered off. The filtrate was heated on a boiling water bath for six hours to complete the hydrolysis (see Table VII). The insoluble material was dissolved in methanolic HCl (50 ml., 3.5%) and heated under reflux for five hours, by which time the methanolysis was complete (see Table VIII). Evaporation of the solvent, addition of water (50 ml.) and further evaporation were carried out. The solution was then adjusted to 0.5 $\bar{N}$ with hydrochloric acid, and heated at 100° for six hours, at the end of which time the hydrolysis of the glycosides was complete (see Table VIII).

Table VII

Time (hr.)	α	Time (hr.)	α
1	+ 2	4	+ 2
2	+11	5	+11
3	+ 4	6	+11

Table VIII

Methanolysis		Hydrolysis of Glycosides	
Time (hr.)	α	Time (hr.)	α
1	+0.27	1	+0.12
2	+0.22	2	+0.14
3	+0.18	3	+0.08
4	+0.12	4	+0.07
5	+0.18	5	+0.06
		6	+0.06

The aqueous acid hydrolysates were then combined and neutralised with silver carbonate, and the insoluble silver

salts were filtered off and washed with water and methanol. The colloidal silver in the filtrate was precipitated as the sulphide, and a small amount of silver carbonate was added to neutralise the amount of acid formed. The solution was then taken to dryness, redissolved in methanol, filtered to remove further traces of silver salts, and re-evaporated, yielding a golden syrup (1.066 g.).

Separation of the Methylated Sugars on a cellulose column

A column (65 x 3 cm.) was packed with dry cellulose after the method of Wadman and Jones.(119) The cellulose was then washed with water, butan-1-ol half-saturated with water, and then with petroleum ether (100-120°) : butan-1-ol (7:3) saturated with water.

The methylated sugars (1.06 g.) were dissolved in a small quantity of butan-1-ol and introduced on to the top of the column, and irrigation was commenced from a constant level reservoir with light petroleum-butan-1-ol (7:3). An automatic fraction cutter was used to collect the eluate, and the contents of every tenth tube were evaporated and examined chromatographically using solvent (b). Tubes containing the same sugars were combined, and the solvents removed under reduced pressure. The syrups were redissolved in water, filtered to remove fine cellulose particles, and waxy impurities, and finally filtered through a charcoal bed. After removal of the water the syrups were finally dried and weighed. The column solvent was changed to butan-1-ol : petrol (1:1) after the trimethyl pentose had

been collected, and to butan-1-ol, half saturated with water, on elution of the dimethyl pentose. Finally the unmethylated sugars were washed off with water.

Results of Column

Fraction	Tube No.	Contents	Weight (g.)	$[\alpha]_D$	(OCH_3)
1	200 - 290	M_3A + M_3X	0.273	-36.3°	46.4
2	870 - 970	M_3G	0.010	-	-
3	1020 - 1210	M_3G + M_3X	0.105	+24.2	35.5
4	1220 - 1810	M_2X	0.199	+22.6	34.7
5	2130 - 2240	MX	0.235	+35	18.7
6	2260 - 2360	X	0.017	+18	-

M_3X = 2:3:4-Tri-O-methyl-D-xylose

M_3A = 2:3:5-Tri-O-methyl-L-arabinose

M_3G = Tri-O-methyl-D-glucose

M_2X = 2:3-di-O-methyl-D-xylose

MX = 2-O-methyl-D-xylose

X = D-xylose

Examination of the Fractions

Fraction 1 had $[\alpha]_D$-36.3° and on this evidence was calculated to contain 93% tri-O-methyl arabinose and 7% tri-O-methyl xylose. { $[\alpha]_D$ 2:3:5-Tri-O-methyl-L-arabinose = -39.5°;(120) $[\alpha]_D$ 2:3:4-tri-O-methyl-D-xylose = +20°.(121) } Fraction 3 had $[\alpha]_D$ +24.2° and on this evidence it was calculated to contain 97% di-O-methyl xylose and 3% tri-O-methyl glucose. { $[\alpha]_D$ 2:3-di-O-methyl-D-

xylose = +23°,[28] 2:3:6-tri-O-methyl-D-glucose = +70°.[122]
There was also a small amount of D-glucose (ca. 5 mg.) obtained from the water washings of the column. This fraction, together with the trimethyl glucose fraction was thought to have arisen from a contaminating glucan since no methyl ethers of glucose could be detected in the hydrolysate of another fraction of the methylated polysaccharide (see Table VI, fraction 7).

Fraction 1

The syrup (273 mg.) had $[\alpha]_D$ -36.3° (C, 0.45 in water) and OCH_3, 46.4% (calculated for $C_8H_{16}O_5$: OCH_3, 48.4%). Chromatography in solvent (c) gave two spots on spraying with aniline oxalate, one, grey-black and travelling at the same rate as standard 2:3:5-tri-O-methyl-L-arabinose, the other pink and travelling at the same rate as standard 2:3:4-tri-O-methyl-D-xylose. Demethylation gave arabinose and a trace of xylose. The aldonolactone did not crystallise but the derived 2:3:5-tri-O-methyl-L-arabonamide crystallised, and after three recrystallisations from ethyl acetate had m.p. and mixed m.p. 130-132°. The sugar is therefore 2:3:5-tri-O-methyl-L-arabinose.

Fraction 3

The syrup (105 mg.) had $[\alpha]_D$ +24.2° (C, 0.44 in water) and OCH_3 -35.5% (calculated for $C_7H_{14}O_5$: OCH_3, 34.8%). Chromatography in solvent (b) showed the presence of 2:3-di-O-methyl-D-xylose and a small quantity of tri-O-methyl glucose. Demethylation gave xylose and a trace of glucose. The syrup crystallised

several weeks after seeding with 2:3-di-O-methyl-β-D-xylose, the crystals being tiled on a porous plate to remove some contaminating syrup, and had m.p. and mixed m.p. 76-78°. The derived 2:3-di-O-methyl-N-phenyl-D-xylosylamine had m.p. and mixed m.p. 121-122°. The sugar is therefore 2:3-di-O-methyl-β-D-xylose contaminated with a small amount of tri-O-methyl-D-glucose.

Fraction 4

Chromatography in solvent (b) showed only the presence of 2:3-di-O-methyl-β-D-xylose. The syrup crystallised on seeding with an authentic sample of that sugar and had m.p. and mixed m.p. 80-81°, and had $[\alpha]_D$ -20.1° ⟶ +22.6° (equil.) (C, 0.35 in water). (Found: OCH_3, 34.7% calculated for $C_7H_{14}O_5$: OCH_3, 34.7%.) The derived 2:3-di-O-methyl-N-phenyl-D-xylosylamine was recrystallised from ethyl acetate and had m.p. and mixed m.p. 121-123°. Fraction 4 was therefore 2:3-di-O-methyl-β-D-xylose.

Fraction 5

The sugar, which was chromatographically identical to 2-O-methyl-β-D-xylose in solvent (e), crystallised within an hour of isolation. Recrystallisation from aqueous methanol yielded which had m.p. and mixed m.p. (with 2-O-methyl-β-D-xylose) 130°, and $[\alpha]_D$ -9.5° ⟶ +35° (equil.) (C, 0.75 in water). (Found: OCH_3, 18.7%. Calculated for $C_6H_{12}O_5$, OCH_3, 18.8%.) Ionophonetic examination of the mother-liquors showed the presence of a small amount of the 3-O-monomethyl ether. The colours of the respective

sugars were obtained by spraying the paper with aniline oxalate solution, containing 5% glacial acetic acid, the 2-methyl ether being orange and the 3-methyl ether purple. The sugar was further characterised by its X-ray powder photograph, but the aniline derivative was obtained as a syrup. Fraction 5 was therefore 2-O-methyl-β-D-xylose contaminated with a small amount of 3-O-methyl-β-D-xylose.

Fraction 6

The syrup (16 mg.) travelled on the chromatogram in the solvents (b) and (d) at the same rate as D-xylose and had +18°, (C, 0.75 in water). Crystallisation could not be induced by seeding with D-xylose crystals. The material was characterised by conversion to the di-O-benzylidene dimethyl acetal which had m.p. and mixed m.p. 208-209°.(104)

Periodate Oxidation of the Polysaccharide(123)

Periodate Uptake

Dried polysaccharide (35.4 mg.) was warmed in water (5 ml.) and the mixture was shaken overnight. Sodium metaperiodate (94.7 m.g.) was added and the volume made up to 10 ml. The flask was shaken in the dark at 15° and at intervals samples (2 ml.) were withdrawn, and additions of saturated sodium bicarbonate (10 ml.), sodium arsenite (5 ml., 0.1015 $\bar{N}$) and aqueous potassium iodide (1 ml., 20%) were made. After shaking in the dark for 15 minutes the solutions were titrated with standard iodine (0.0525 $\bar{N}$). The results are given in Table IX.

Table IX

Time (hrs)	Titre (ml. 0.053 $\bar{N}$ iodine)	Moles periodate consumed per anhydro pentose unit
5	4.20	0.56
28	4.58	0.61
48	4.68	0.63
120	4.90	0.67

Liberation of formic acid

Dried hemicellulose (307.3 mg.) was dissolved in potassium chloride solution (50 ml., 0.56 M) by shaking for 24 hours. Sodium metaperiodate (20 ml., 0.2 M) was then added with stirring. Similar solutions were made up for a blank and the flasks were wrapped in black cloths and were shaken continuously at 15°. At intervals aliquots (10 ml.) were removed and the excess periodate was destroyed by the addition of freshly distilled ethylene glycol (1 ml.). The solutions were agitated in the dark for 15 minutes by passage of a continual stream of CO_2-free nitrogen. The liberated formic acid was titrated with carbonate-free sodium hydroxide (0.0151 $\bar{N}$) to pH 6.25, with a steady stream of nitrogen passing through the solution during the titration. The blank titres were found to be negligible during the experiment. The results are given in Table X; Fig. is the plot of formic acid released against the time. Extrapolation of the straight line part of the curve to zero time gives the correction for any acid produced by secondary reactions.

Table X

Time (hr.)	Titres (ml. 0.0151 $\bar{N}$ sodium hydroxide)	Moles formic acid released per anhydro pentose unit
24	0.510	0.0234
48	0.700	0.0322
122	0.705	0.0324
170	0.730	0.0335
230	0.740	0.0340

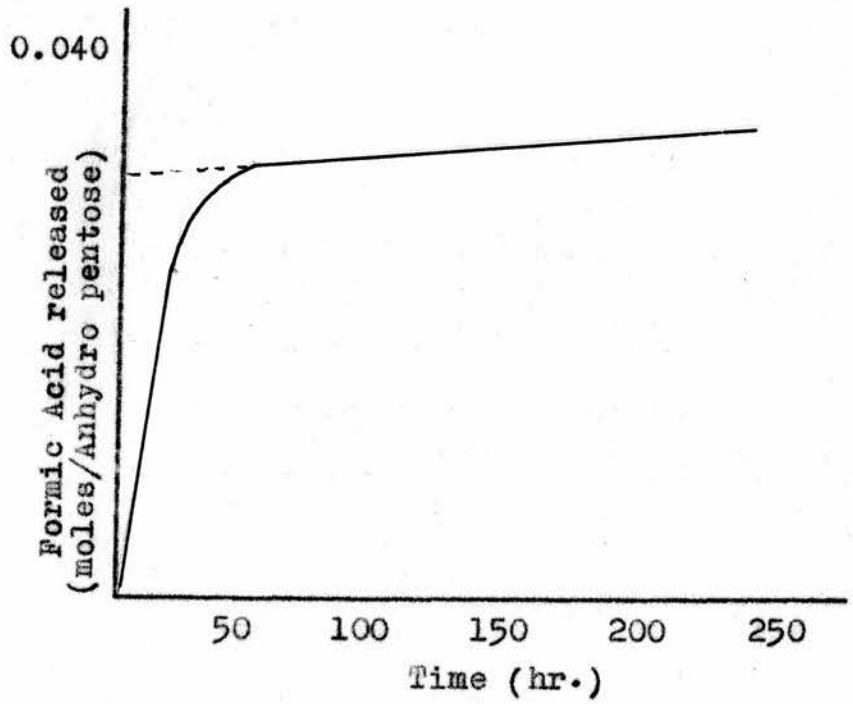


Fig.

Partial Hydrolysis of the Polysaccharide

Preliminary small scale experiments were carried out to obtain optimum conditions for the release of arabinose.

Hemicellulose (30 mg.) was heated on a boiling water bath with oxalic acid (12 ml., 0.01 $\overline{N}$). At 30 minute intervals, samples (2 ml.) were withdrawn and the degraded polysaccharide was precipitated with alcohol (5 vols.) and centrifuged off. The filtrates and hydrolysis products of the polysaccharides were examined chromatographically using solvent (c). It was shown that after 1½ hours heating, arabinose was liberated, but with longer periods of hydrolysis, xylose, xylobiose, and higher xylose oligosaccharides were formed.

Large scale hydrolysis of the polysaccharide (502 mg.) was carried out by dissolving the material in water (25 ml.), adding oxalic acid to a normality of 0.01 and heating on a boiling water bath for 1½ hours. After cooling, the acid was neutralised by shaking with an excess of calcium carbonate and the inorganic material was filtered off. The filtrate was evaporated to about 10 ml. and was poured into alcohol (40 ml.). The resulting degraded polysaccharide was washed with alcohol and ether and dried in a vacuum desiccator. Yield: 385 mg. Quantitative chromatographic analysis[115] gave xylose (77.5%) and arabinose (12.5%). The original polysaccharide contained 29% arabinose, and therefore the partial hydrolysis removed slightly more than half of this.

Preparation of the Periodate Oxidised Polysaccharides

The degraded polysaccharide (356 mg.) was dissolved in water (10 ml.) and shaken overnight. Sodium metaperiodate (801 mg.) was added and the samples were shaken in the dark for 4 days. Oxidation was then complete, the excess periodate and iodate being precipitated with a slight excess of barium chloride solution, followed by removal of the insoluble barium salts at the centrifuge. The centrifugates were dialysed against distilled water for 3 days, after which time chloride, iodate and periodate ions were absent. On evaporation to a small volume, acetone (10 vols.) was added, giving a colloid which coagulated on addition of a few drops of a dilute solution of potassium iodide in acetone. The precipitate was washed with ice water and dried by solvent exchange. Yield: 250 mg. (72%).

A sample of the undegraded hemicellulose (352 mg.) was treated in the same way with sodium metaperiodate (792 mg.) giving the corresponding periodate-oxidised polysaccharide (190 mg., 56%). The oxidised polysaccharides were hydrolysed with N sulphuric acid for 4 hours at 100°, and the hydrolysates analysed in the usual way, using galactose as the reference sugar.(115) (See Table XI.)

Table XI

	Before Oxidation		After Oxidation
	% Xylose	% Arabinose	% Xylose
A. Undegraded Polysaccharide	60	29	24.5
B. Degraded Polysaccharide	77.5	12.6	
B. Corrected*	60	10	8.6

* These values are expressed as percentages of the undegraded polysaccharide.

Isolation of Oligosaccharides by Enzymic Hydrolysis

Rye xylan 'A' (2 g.) was dissolved in water (150 ml.) by shaking overnight. Sodium acetate buffer (100 ml., pH 4.5) and an enzyme preparation,"Hemicellulase"(700 mg.) (marketed by Light's) in water (50 ml.), and L-arabonolactone (20 g.) was added to the solution. The digest was kept in an incubator at 35° for 3 hours, after which time the reaction was stopped by heating the solution on a boiling water bath for 5 minutes. Ethanol (1 vol.) was added and the mixture of degraded polysaccharide and protein material was centrifuged off. The centrifugate was concentrated to 100 ml., and poured on to a column of charcoal : celite (1:1, 20 g.). The column was washed with water until the eluate was free from monosaccharides (2 l.). Increasing concentrations of ethanol were used to wash the column (Table XII).

Table XII

Fraction	% Ethanol	Weight (mg.)	R_{xylose}	Sugars identified
1	-	-	1.0	xylose, arabinose
2	7	70	0.40, 0.66	
3	10	70	0.40	
4	15	48	0.14, 0.30, 0.40, 0.43	xylotriose, xylotetraose
5	17	21	0.14, 0.30	xylotriose, xylotetraose

Examination of Fraction 3

The sugar (70 mg.) had $[\alpha]_D$ -15.3° (C, 0.67 in water).

Chromatography in solvent (d) showed only one spot, R_{xylose} 0.40. Hydrolysis with $\overline{N}$ sulphuric acid showed the presence of xylose and arabinose on chromatography in solvents (a) and (d), and quantitative chromatographic analysis of the sugar (ca. 5 mg.) by the method of Somogyi[116] showed the presence of xylose (65%) and arabinose (32%).

Partial Hydrolysis

The sugar (ca. 5 mg.) was dissolved in aqueous oxalic acid (1 ml. 0.01 N) and heated on a boiling water bath. Samples (0.2 ml.) were withdrawn at intervals of 0.5 hour, and the resulting material was chromatographed in solvent (d). The results showed that after hydrolysis of one hour, arabinose was liberated and spots corresponding to arabinose, xylobiose and unchanged trisaccharide were obtained. After two hours the presence of xylose was also detected, and the concentration of unchanged trisaccharide had greatly decreased.

Alkaline Degradation

Sugar (ca. 5 mg.) was dissolved in oxygen-free sodium hydroxide (5 ml., 0.95 N). Samples (2 ml.) were removed at convenient intervals, deionised, and chromatographed in solvent (f) and papers were developed with spray (b). After a degradation period of 48 hours a lactone was detected chromatographically having $R_{xyloisosaccharinolactone}$ 1.0. A second slower moving lactone running at the same rate as xylometasaccharinic acids was observed in later chromatograms.

Periodate Oxidation[138]

Sugar (5 mg.) was oxidised with aqueous sodium metaperiodate (0.143 M, 10 ml.) at 35° for 24 hours. The uptake, measured spectrophotometrically was equivalent to 3.1 moles per mole of sugar.

Estimation of the residues unattacked by Periodate

Sugar (5 mg.) was oxidised with aqueous sodium metaperiodate (0.143 M, 10 ml.) for four days at room temperature. Sodium ions were then removed by means of Amberlite Resin I.R. 120 (H), and periodate ions were removed with excess barium hydroxide solution. The excess barium hydroxide was precipitated as the carbonate by passage of CO_2. The oxypolysaccharide was isolated as a syrup and hydrolysed with $\underline{N}$ H_2SO_4. Quantitative chromatographic examination of the hydrolysate[115] using rhamnose as the standard sugar showed the presence of xylose (0.90 moles).

Examination of Enzymic Activity in the absence of L-Arabonolactone

Polysaccharide (100 mg.) was dissolved in water (5 ml.) containing sodium acetate buffer (5 ml., pH 4.5), and "Hemicellulase" (30 mg. in water, 2 ml.). After being kept at 35° for 3 hours, the solution was boiled to destroy the enzyme, and poured onto a column of charcoal-celite (1:1). Elution with water and alcohol in increasing concentrations gave a series of fractions which on chromatographic examination were shown to travel at the same rate as xylose, arabinose, xylobiose and xylotriose. No evidence was found for the presence of any arabinose containing oligosaccharides.

Examination for the Possibility of Transglycosylation

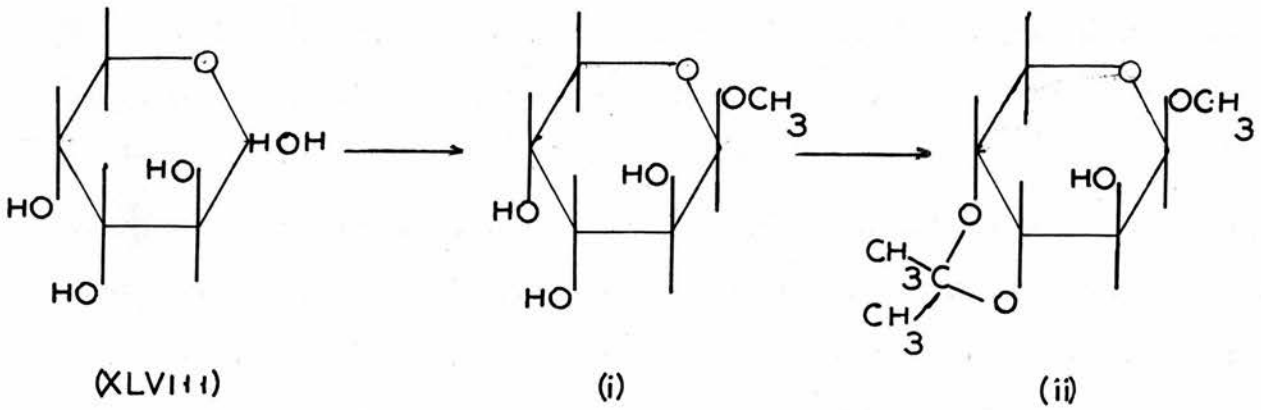
Xylobiose (100 mg.), xylotriose (100 mg.), arabinose (50 mg.) was dissolved in water (5 ml.) containing sodium acetate buffer (5 ml., pH 4.5), L-arabonolactone (2 g.) and "Hemicellulase" (40 mg., in water, 2 ml.). After storage at 35° for 3 hours, the solution was boiled and poured onto a charcoal-celite column and eluted with water and ethanol in the usual manner. Paper chromatography of the appropriate fractions showed the presence of xylose, arabinose, xylobiose and xylotriose. No evidence was found for the presence of any oligosaccharides containing arabinose residues.

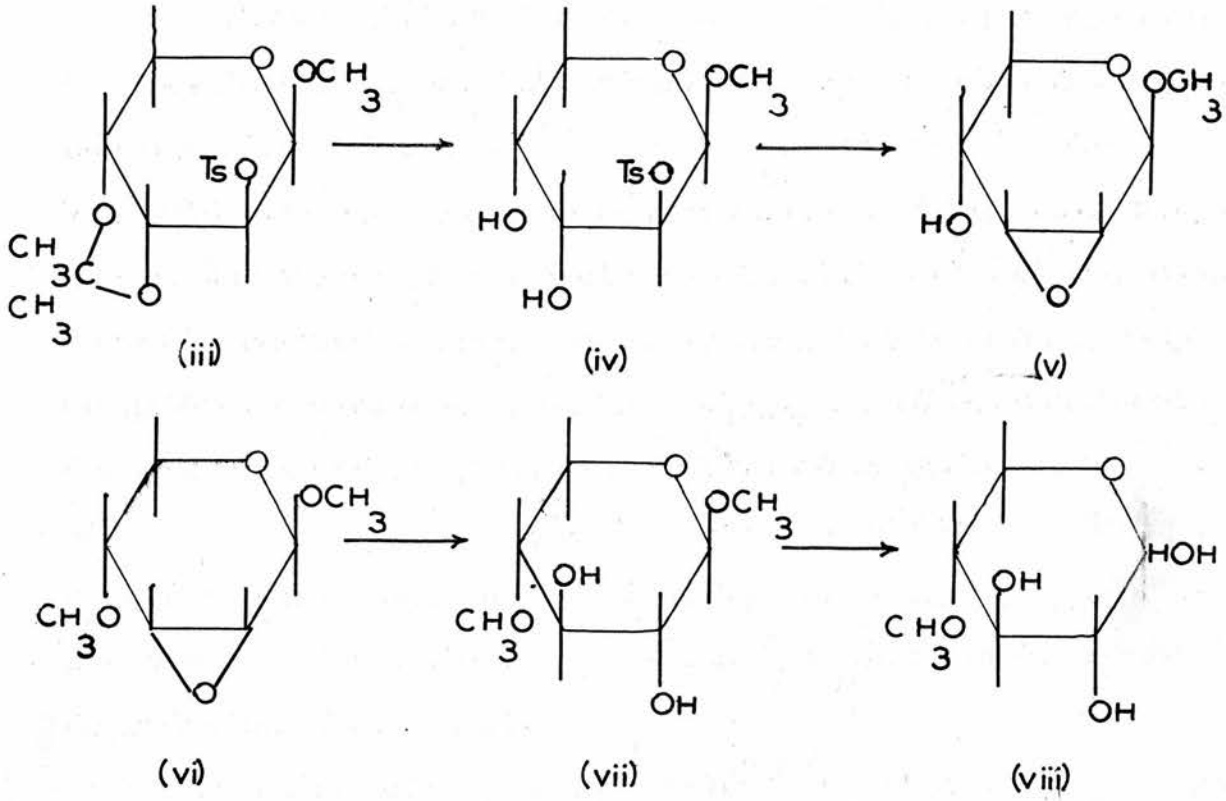
SECTION 11

Alkaline Degradation of 4-O-Methyl-α-D-Xylose

In the preliminary work on the alkaline degradation of 1:4-linked xylans, investigations were carried out on model compounds containing these 1:4 links, such as xylobiose and xylotriose.(97) Xyloisosaccharinolactone (0.4 mol./mol.) was formed together with appreciable quantities of other lactones probably occurring from the non-reducing xylose end group which is ultimately exposed to degradation, and non-lactonisable acids, probably occurring by an alternative degradation of the 1:4-linked sugar units. 4-O-Methyl-D-xylose which has the 1:4 type of linkage and would be expected to give only xyloisosaccharinolactone on degradation, was synthesised and submitted to degradation with alkali.

The method of Hough and Jones(143) was used in the synthesis of 4-O-methyl-D-xylose from D-arabinose (XLVIII) according to the following scheme.





D-Arabinose was converted to the corresponding β-methyl glycoside (i) which on condensation with acetone gave methyl 3:4-O-isopropylidene-β-D-arabopyranoside (iii). Treatment of this material with toluene-p-sulphonyl chloride in pyridene furnished the 2-tosyl derivative. Removal of the isopropylidene grouping was attempted with formic acid but very little hydrolysis was found to take place. The hydrolysis was continued with methanolic hydrogen chloride and the material was fractionated on an alumina column into two components which had $[\alpha]_D$, -102° and -85° respectively. Both components were treated with sodium methoxide to obtain the methyl 2:3-anhydro-D-ribopyranoside (v),

and then methylated with silver oxide and methyl iodide to give the 4-methyl ether. At this stage both fractions were found to have the same rotation and m.p., being in agreement with those quoted in the literature, and were combined before being treated with aqueous sodium hydroxide to produce the methyl-4-O-methyl-β-D-xylopyranoside (vii). The glycosidic group was removed by acid hydrolysis and the 4-O-methyl-D-xylose was obtained as a syrup. Some contaminating sugars were removed by cellulose column chromatography and the sugar was obtained crystalline in the α-form having m.p. 103-105° and $[\alpha]$ +44° → +6° at equilibrium. In the synthesis by Hough and Jones,(143) the sugar was obtained as a syrup with $[\alpha]$ +8°.

Small scale degradation of the sugar in sodium hydroxide over an atmosphere of nitrogen showed the presence of formic acid, identified as the 4-bromophenacyl ester, methanol, identified on oxidation to formaldehyde, and xyloisosaccharinic acid, together with smaller quantities of two other lactones having $R_{isosaccharinolactone}$ values of 0.76 and 1.57 respectively. The slower of the two lactones ran at the same rate as D-threo-2:4:5-trihydroxyvalerolactone, and the faster lactone was probably a four carbon fragment of the degradation. The xyloisosaccharinolactone was isolated in a pure form by separation on filter sheets, and on crystallisation the material had m.p. and mixed m.p. 94-96° with synthetic xyloisosaccharinolactone.

Quantitative estimations showed that 1 mole of the sugar produced a total of 1.12 moles of acid, of which 0.13 moles was formic acid. The total lactonisable acid, estimated by the

hydroxylamine-ferric chloride method was 0.75 moles per mole of sugar, of which 0.65 moles was isolated from filter sheets as xyloisosaccharinolactone.

Thus 4-O-methyl-D-xylose behaves as would be expected, by being degraded in alkali to xyloisosaccharinolactone, as has been previously shown in other 4-linked derivatives of D-xylose, xylobiose and xylotriose. Thus it is in accord with the predictions made by Corbett, Kenner and Richards[88] as a result of experiments on related oligosaccharides that 1:4-linked carbohydrates are degraded to an isosaccharinic acid with the same number of carbon atoms as the sugar unit from which it was derived.

The degradation of xylobiose and xylotriose with alkali[97] indicated the presence of 0.4 moles of xyloisosaccharinolactone per 1:4-linked anhydro sugar unit, together with quantities of other lactones and non-lactonisable acids. The non-lactonisable acids may occur by fragmentation of the reducing end-group during the peeling process before exposing another reducing end-group to degradation, or they may be formed by scission of the α-dicarbonyl intermediate (XLVII), which is considered an intermediate in the formation of xyloisosaccharinic acid.

(XLVII)

An attempt was made to synthesise this compound but it was unsuccessful.

Synthesis of 4-O-Methyl-D-Xylose

Methyl-β-D-arabopyranoside (i)

D-Arabinose (15 g.) was refluxed for seven hours with dry methanol (400 ml.) containing hydrogen chloride (1%). On removal of some of the solvent under reduced pressure, the -glycoside crystallised out and was filtered off and washed with dry methanol (3 x 25 ml.). The filtrates were then combined and refluxed for a further two hours. On concentration of the solution to 150 ml. a further crop of crystals was obtained and on removal was washed with dry methanol (3 x 25 ml.). The combined crops were then recrystallised from ethanol.

Found: m.p. 168-170°, $[\alpha]_D = -234°$ (c, 0.77 in water).

Methyl-3:4-O-isopropylidene-β-D-arabopyranoside (ii)

Methyl-β-D-arabinoside (10 g.) was suspended in dry acetone (500 ml.) and shaken vigorously with phosphorous pentoxide (8 g.) for five minutes. Water cooling was applied to the flask as considerable heat was evolved during the reaction. The acetone was decanted and the residue washed with acetone (3 x 20 ml.) and the combined extracts were shaken with solid potassium carbonate to remove traces of acid. After filtration and removal of the solvent, a pale yellow syrup was obtained, which contained small amounts of acetone condensation products. Distillation under high vacuum afforded a colourless syrup, 8 g., b.p. 82°/0.1 m.m. $[\alpha]_D = -201°$ (c, 0.39 in chloroform), OCH_3 = 15.4%.

(Isopropylidene methyl pentoside requires OCH_3 = 15.3%.)

Methyl-3:4-O-isopropylidene-2-O-toluene-p-sulphonyl-β-D-arabopyranoside (iii)

Methyl 3:4-O-isopropylidene-β-D-arabopyranoside (ii) was treated with freshly distilled pyridene (40 ml.) and toluene-p-sulphonyl chloride (10 g.). After 24 hours at room temperature, excess sodium bicarbonate solution (10%) was added and the mixture was extracted with chloroform (4 x 50 ml.) and the organic layer was dried over anhydrous sodium sulphate. On removal of the solvent under reduced pressure, several additions of water were made to remove final traces of pyridene. The crude methyl 3:4-isopropylidene-2-toluene-p-sulphonyl-β-D-arabinoside was insoluble in water and was recrystallised from aqueous ethanol (8 g.).

Found: m.p. 135°, $[\alpha]_D$ -177°, (c, 0.4 in chloroform), OCH_3 = 8.7%.

(Methyl 2-tosyl-3:4-isopropylidene pentoside requires OCH_3 = 8.7%.)

Methyl-2-O-toluene-p-sulphonyl-β-D-arabopyranoside (iv)

The methyl 2-tosyl-3:4-isopropylidene-β-D-arabopyranoside was dissolved in boiling acetone (25 ml.). Formic acid (50 ml., 0.1 N) was added slowly over a period of two hours. On cooling, unchanged starting material (6 g.) separated out and was filtered off. Evaporation of the solvent gave a further quantity of the starting material (1 g.) together with a pale yellow syrup (1 g.) which had OCH_3 = 9.6% ($C_{13}H_{18}O_7S$ requires OCH_3 = 9.8%) and $[\alpha]_D$ =

-85°. Methyl-2-O-toluene-p-sulphonyl-β-L-arabopyranoside was reported to have $[\alpha]_D = +110^{\circ}$.(124)

The unchanged material was dissolved in methanol (245 ml.) containing HCl (0.5 ml., 11 $\bar{N}$), and refluxed for 8 hours on a boiling water bath until a constant rotation was obtained. On cooling the reaction mixture, the acid was removed by addition of excess silver carbonate. Concentration of the filtrate afforded a pale yellow syrup (6 g.).

Found: $[\alpha]_D = -102^{\circ}$, $OCH_3 = 9.6\%$ ($C_{13}H_{18}O_7S$ requires $OCH_3 = 9.7\%$).

The syrup was placed on a column (30 x 3 cm.) containing alumina, which had previously been deactivated by washing with 10% acetic acid, and then with water until the eluate was free from acid, and finally heated overnight at 240°.

Elution with ether : chloroform (20:80) and then with chloroform, yielded two fractions:

a) 2.4 g. $[\alpha]_D$ -102° $OCH_3 = 9.6\%$

b) 1.6 g. $[\alpha]_D$ -85° $OCH_3 = 9.6\%$

Methyl 2:3-anhydro-β-D-ribopyranoside (v)

Each of the two fractions of the methyl 2-O-toluene-p-sulphonyl-β-D-arabinoside was dissolved in methanol containing sodium (1.5 g.) and set aside for 24 hours. The sodium toluene-p-sulphonate which crystallised out was removed by filtration. Each of the filtrates was diluted with water, neutralised with sulphuric acid (1 $\bar{N}$) to a phenolphthalein end-point and continuously extracted with chloroform for 48 hours. On removal

of the solvent the syrupy anhydro sugar was obtained.

Fraction a) (1.1 g.) $[\alpha]_D = -47^\circ$

b) (0.7 g.) $[\alpha]_D = -63^\circ$

Both fractions had OCH_3 = 21.1% ($C_6H_8O_4$ requires OCH_3 = 21.5%). The methyl 2:3-anhydro-β-L-ribopyranoside has been quoted as having $[\alpha]_D = +37^\circ$.(143)

Methyl 2:3-anhydro-4-O-methyl-β-D-ribopyranoside (vi)

Each of the methyl 2:3-anhydro-β-D-riboside fractions was independently dissolved in methyl iodide (25 ml.). Silver oxide (1 g.) was added to the boiling solution over a period of four hours, with vigorous agitation, and heating was continued for a further two hours. The insoluble silver salts were removed, washed with chloroform (3 x 5 ml.) and the combined filtrates were evaporated, leaving a pale yellow syrup which partially crystallised on standing.

A second methylation was carried out, using the same quantities as before. Each of the two fractions immediately crystallised on removal of the solvent, and was recrystallised from light petroleum (40-60°).

Fraction a) 1.0 g., m.p. 75-77° $[\alpha]_D = -8^\circ$, c, 0.63 in chloroform, OCH_3 = -38.2%

b) 0.5 g., m.p. 74-77° $[\alpha]_D = -8^\circ$, c, 0.5 in chloroform, OCH_3 = -38.0%

($C_7H_{10}O_4$ requires OCH_3 = 38.8%.)

The two fractions appeared to be identical compounds and gave no depression of melting point on admixture. They were

therefore combined for the next stage of the synthesis.

Methyl 4-O-methyl-β-D-xylopyranoside (vii)

Methyl-2:3-anhydro-4-O-methyl-D-riboside (1.5 g.) was heated on a boiling water bath with aqueous sodium hydroxide (5%, 150 ml.) for 48 hours. Initially a bright yellow colour was formed, which darkened on prolonged heating. The solution was cooled, neutralised with dilute N-sulphuric acid to a phenolphthalein end-point, and continuously extracted with chloroform for 48 hours. Removal of the solvent afforded a colourless syrup (0.85 g.) which crystallised on being stored in a vacuum desiccator for several weeks.

On recrystallisation from ether, the product had m.p. 92-94°.

Found: $[\alpha]_D$ -67° (c, 0.5 in water), and OCH_3 = 35% ($C_7H_{14}O_5$ requires 34.8%) (Hough and Jones[143] quote $[\alpha]_D$ = -69°, and m.p. 95°).

4-O-Methyl-D-xylose (viii)

Methyl 4-O-methyl-β-D-xyloside (0.85 g.) was hydrolysed with H_2SO_4 (50 cc., 1 $\bar{N}$) for six hours on a boiling water bath. The acid was neutralised with barium carbonate and the filtrate evaporated, yielding a colourless syrup (0.75 g.).

Samples of the sugar were chromatographed on papers using butanol : ethanol : water (40:19:11) as solvent. On development of the papers with aniline oxalate has an $R_{rhamnose}$ value of 1.13 compared with 1.18 for the corresponding 2-O-methyl ether. There were also traces of unmethylated material, together with some

higher methylated sugars. The syrup was therefore chromatographed on a cellulose column (50 x 2.5 cm.) using butan-1-ol : light petroleum (100-120°) (50:50) as the mobile phase. A chromatographically pure product was thus obtained (700 mg.), which crystallised on standing in vacuo. Recrystallisation from aqueous methanol yielded crystals with m.p. 103-105°, and $[\alpha]_D$, + 44° ⟶ + 6° (equib.), (c, = 1.01 in water) and OCH_3 = 18.5% ($C_6H_{12}O_5$ requires OCH_3 = 18.9%).

Chromatography in solvent (e) showed the sugar to have an $R_{rhamnose}$ value of 1.86 compared with 2.00 and 2.28 for the 2-O- and 3-O-methyl ethers respectively. It was also distinguishable from these two sugars by means of paper ionophoresis.

The corresponding 4-O-methyl-D-xylosazone was prepared by heating the sugar (10 mg.), phenylhydrazine hydrochloride (20 mg.) and sodium acetate (30 mg.) with water (1 ml.) on a boiling water bath for 1 hour. The yellow needles which separated out on cooling had m.p. 160°, on recrystallisation from aqueous methanol. Hough and Jones report m.p. of 161° for the derivative.[143]

Degradation of 4-O-Methyl-D-xylose by Alkali

Qualitative

4-O-Methyl-D-xylose (19 mg.) was dissolved in oxygen-free aqueous sodium hydroxide (10 ml., 0.88 N) and allowed to stand at room temperature for ten days. Sodium ions were removed by treatment with Amberlite resin I.R. 120(H) and then evaporated to a syrup under reduced pressure. The receiver was cooled with ice to trap any volatile products. Methanol was identified

in the first few drops of the distillate, by means of oxidation to formaldehyde which was in turn identified by the chromotropic acid test.(125)

The remaining solution was evaporated to a syrup at 60°, and the distillate was neutralised with sodium hydroxide (0.018 N). Evaporation of the distillate yielded the volatile acids which were chromatographed in solvent (g) and the papers were developed with spray (e), showing the presence of formic acid. No other volatile acids were observed on developing papers with sprays (d). Formic acid was identified by conversion to the 4-bromophenacyl ester, m.p. and mixed m.p. 140°.

The non-volatile acidic material was chromatographed in solvent (f). Sprays (c) and (d) gave no reaction for non-volatile acids, and spray (b) showed the presence of a lactone running at the same rate as xyloisosaccharinolactone, together with small quantities of two other lactones of R_{iso}saccharinolactone, values of 0.76 and 1.57. The slower moving of the two lactones travelled at the same rate as xylometasaccharinolactone.

From another sample of the sugar (50 mg.) which was degraded in alkali, the lactonisable acids obtained on deionisation, were separated on filter sheets (Whatman 3 MM), and the main fraction was eluted from the papers and concentrated to a syrup which crystallised and had m.p. and mixed m.p. with xyloisosaccharinolactone 94-96°.

Quantitative Estimation

An attempt was made to follow the degradation by means of

estimating the reducing power of the unchanged sugar in the solution but this was not possible due to interference by formic acid in the mixture.

Total Acid Produced

4-O-Methyl-D-xylose (200.7 mg.) was dissolved in oxygen-free sodium hydroxide (5 ml., 0.88 $\bar{N}$) and set aside for 14 days. Removal of the sodium ions with Amberlite resin I.R. - 120 (H) gave an acidic solution, which was made up to 50 ml. Samples (2 ml.) were withdrawn and titrated with carbonate-free sodium hydroxide (0.01876 $\bar{N}$) over an atmosphere of nitrogen.

Found: Total acid = 1.346 meq.

1 mole 4-O-methyl-D-xylose ≡ 1.12 moles acid.

Volatile Acid

Samples (10 ml.) were evaporated to dryness under reduced pressure at water bath temperature of 60°, the distillate being collected in an ice-cooled receiver. Water (2 x 5 ml.) was added and the contents of the flask again evaporated to dryness. The distillate was titrated with carbonate-free sodium hydroxide (0.00473 $\bar{N}$) in an atmosphere of nitrogen. The volatile acid content was 0.0314 meq. which represents a total volatile acid content of 0.157 meq., or 0.13 moles per mole of sugar.

Formic Acid

Aliquots (10 ml.) were evaporated as before and the distillate was boiled with an excess of freshly prepared yellow mercuric oxide

for 3 hours to ensure complete oxidation of the formic acid to carbon dioxide. On cooling, the inorganic salts were removed, washed with water (2 x 5 ml.) and the filtrate was titrated with sodium hydroxide (0.0047 N) indicating a formic acid content of 0.15 meq., or 0.13 moles per mole of sugar.

Estimation of Lactones(126)

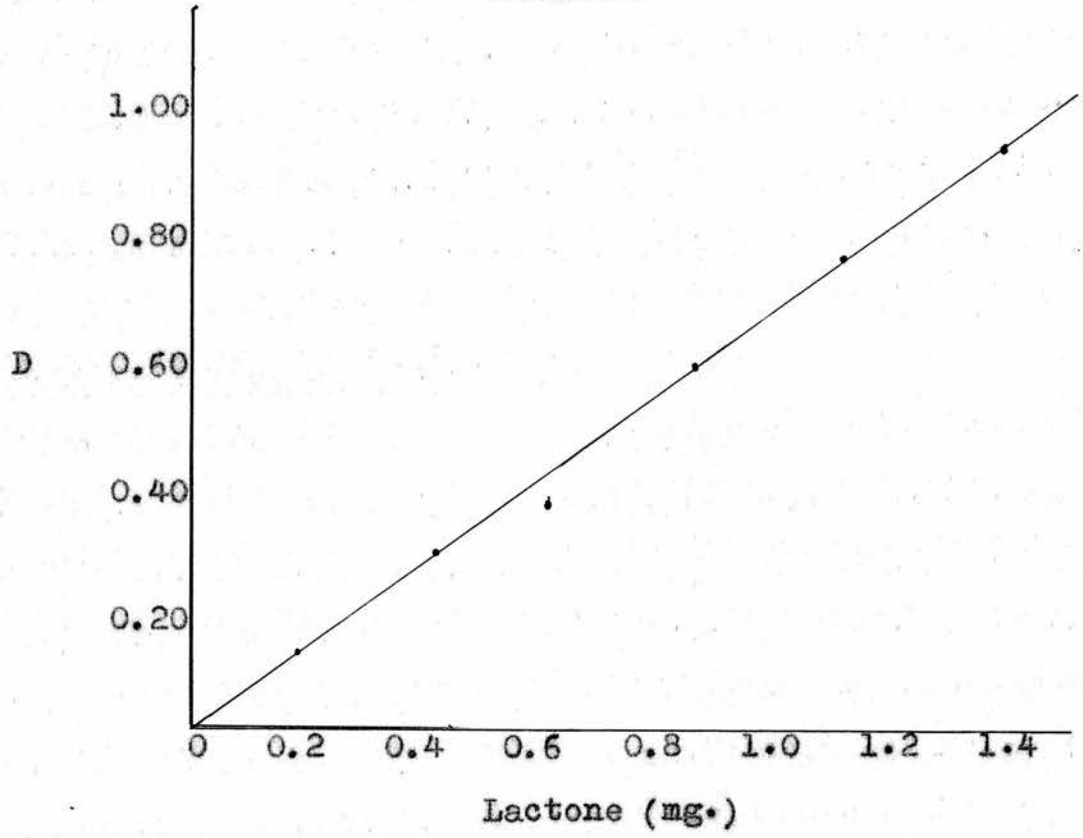
From a standard solution of xyloisosaccharinolactone (44.29 mg. in 10% aqueous methanol, 100 ml.), solutions containing 0.2 - 1.3 mg. of lactone in 10% aqueous methanol (2 ml.) were diluted with water (1 ml.). Freshly prepared hydroxylamine solution (2 ml.) obtained from equal volumes of sodium hydroxide (3.5 M) and hydroxylamine hydrochloride (2 M) was added and the solutions were allowed to stand for 20 minutes. Hydrochloric acid (1.0 ml., 3.34 M) and ferric chloride solution (1 ml., 0.37 M in 0.1 M hydrochloric acid) was added with thorough mixing. A reagent blank was prepared at the same time and the optical density of the solutions was measured on a Unicam Spectrophotometer at 505 m μ., see Table XIII and Fig.A

The solutions to be estimated were obtained from aliquots (0.05 ml.) of the degradation mixture which were evaporated to syrups to remove the volatile acids, and made up to 2 ml. with 10% aqueous methanol.

Table XIII

xyloisosaccharinolactone (mg.)	0.22	0.44	0.66	0.88	1.10	1.32
D	0.130	0.305	0.384	0.580	0.754	0.880

Fig. A



The solution resulting from the degradation of the 4-O-methyl-D-xylose was found to contain 1.21 mg. of lactonisable acid which represents 0.75 mole lactonisable acid per mole of sugar.

Sugar (100 mg.) was degraded in the usual way, and the lactones obtained were separated on filter sheets using solvent (f). The xyloisosaccharinolactone fraction was obtained as a syrup on extraction of the appropriate section of the paper (53.4 mg.) which represents 0.65 moles isosaccharinolactone per mole of sugar.

SECTION 111

Degradation of Xylans

The degradation of polysaccharides by alkali has come in for much detailed study in the last few years. In the hexosan series, cellulose[131,132] and amylose[142] have been thoroughly investigated but in the pentosan series only preliminary investigations have been reported[97,98] on an esparto xylan.

The xylans studied in this work were:

(1) A water soluble rye araboxylan 'A', isolated as described on page 56.

(2) Barley husk xylan, extracted with cold aqueous sodium hydroxide.

(3) Oat straw xylan I, extracted by cold aqueous sodium hydroxide.

(4) Oat straw xylan II, extracted by cold aqueous sodium hydroxide, and fractionated by means of the copper complex.

Small scale degradations carried out on the samples in oxygen-free aqueous sodium hydroxide (ca 0.8 N) over a period of 20-25 days, showed the presence of formic acid, identified as the 4-bromophenacyl ester, lactic acid, identified as the 4-bromophenacyl lactate, glycollic acid (R_{lactic} 0.68), xyloisosaccharinolactone (R_{lactic} 0.70), small amounts of xylometasaccharinolactones R_L (0.43 and 0.54) and two other unknown acids having R_{Lactic} 0.15 and 0.23 respectively. These acids were present in the degradation products of all the xylans studied but the proportions varied considerably from one xylan to the other.

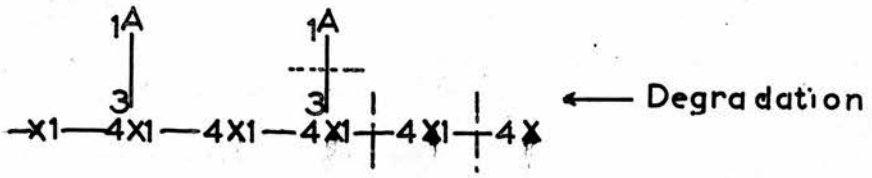
Quantitative estimations were carried out on the total acid

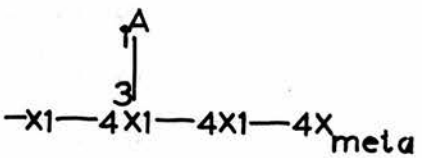
produced, formic acid and lactic acid, the results of which are tabulated below. As lactic acid interferes in the estimation of glycollic acid by colorimetric methods the estimation of that acid was not attempted.

Table XIV

Xylan	Total Acid		Formic Acid		Lactic Acid	
	meq.	meq./anhydro unit	meq.	meq./anhydro unit	meq.	meq./anhydro unit
Rye Xylan 'A'	1.29	170	0.134	17.6	0.44	57.7
Barley Husks	0.67	86	0.122	16.1	0.084	10.8
Oat Straw I	0.99	129	0.083	10.8	0.149	19.5
Oat Straw II	0.97	117	0.188	22.8	0.039	4.8

The alkaline stable polysaccharide obtained from the degradation of rye xylan 'A' was hydrolysed and examination of the hydrolysate showed the presence of xylometasaccharinic acids. This would be expected from an araboxylan containing a backbone of 1:4 linked xylose units with terminal arabinose side chains in position 3.





Alkali stable polysaccharide

Peeling of the backbone of 1:4 linked xylose units will produce xyloisosaccharinic acid. When a branch point is reached the sugar unit forming that branch is split off and rearranges to a metasaccharinic acid, while the unit in the backbone rearranges to a metasaccharinic acid and remains attached to the backbone.

Thus the degradations of the xylans by alkali appears to follow a similar path to the degradation of amylose and cellulose, in which two main types of reaction take place: "degradation" giving mainly xyloisosaccharinic acid, lactic acid and formic acid and the "stopping" reaction in which a xylose unit rearranges to xylometasaccharinic acid, due to either a 1:3-branch point or to a stopping mechanism as described on page 38. The xylometasaccharinic acid thus occupies the terminal position in the xylan chain.

The possibility of random scission of the polysaccharide with the possible production of new reducing groups, either by traces of oxygen or by some other degradation mechanism has been considered. In the peeling type of reaction in which successive 1:4-linked xylose units are split off until an side chain is encountered, or the degradation ceases due to some other cause, the difference in D.P. between the original polysaccharide and the degraded polysaccharide would be small. On the other hand, random scission of the xylan chains would be expected to produce a large drop in D.P.

In an attempt to settle this issue the molecular weights of the xylan acetates was measured by means of the isothermal

distillation method[144,145] (see Table XV). The molecular weights of xylan acetates do not appear to have been determined before by this method, presumably due to the lack of suitable solvent. In this work 1:3-dioxolane was found to be satisfactory for the acetates obtained from the rye and barley xylans but both the oat straw II acetates and the unmodified oat straw I acetate were insoluble in this solvent.

A quantity of rye xylan 'A' was reduced with potassium borohydride, and a sample was acetylated and the D.P. was found to be 70 ±3. The remaining material was treated with oxygen-free alkali for one month, and on isolation only a trace of lactic acid was found. Determination of the molecular weight of this material on acetylation gave a D.P. value of 70±3, indicating that degradation in the original polysaccharide is taking place at the reducing end group and not by random scission of the polysaccharide chains.

Table XV

	Molecular Weight	D.P.
Rye xylan 'A' acetate	15,700 ± 500	72± 3
Reduced Rye xylan 'A' acetate	15,100 ± 500	70± 3
Reduced Rye xylan 'A' acetate treated with alkali	15,000± 500	70± 3
Degraded Rye xylan 'A' acetate	12,500 ± 500	58± 3
Rye xylan 'A' methylate	10,800 ± 500	67± 3
Rye xylan 'A' methylate (via acetate)	10,400 ± 500	65± 3

Rye xylan 'B' acetate	13,200 ± 500	64 ± 3
Rye xylan 'B' methylate	9,100 ± 500	57 ± 3
Barley Husk acetate	18,200 ± 500	84 ± 3
Degraded Barley Husk acetate	14,500 ± 500	67 ± 3
Degraded Oat Straw I acetate	9,400 ± 500	43 ± 3

Both the rye xylan 'A' and the barley husk xylan have shown a decrease of about 20% in D.P. on being degraded with alkali. The D.P. of the methylated rye xylan 'A' (65 ± 3) for both the direct methylation and for the simultaneous deacetylation and methylation was found to be greater than the D.P. of the alkaline degraded polysaccharide, (58 ± 3) indicating little if any degradation to have taken place during the alkaline conditions of methylation, probably due to the glycosidation of the terminal reducing end-group.

Degradation of Xylans with Alkali

The xylans studied were:

(1) Rye xylan "A", isolated as described on page 56.

(2) Barley husk xylan, isolated by Aspinall and Ferrier,[32] containing about 4% glucuronic acid residues and a xylose arabinose ratio of about 6:1.

(3) Oat straw xylan I, isolated by sodium hydroxide extraction of oat straw.

(4) Oat straw xylan II, isolated by 4% sodium hydroxide extraction of the straw and fractionated by means of the copper complex. The material had xylose (93%), arabinose (3%) and glucuronic acid residues 3.5%.[47]

(5) Rye xylan "A" after reduction with potassium borohydride.

Reduction with Potassium Borohydride

Polysaccharide (500 mg.) was dissolved in water (30 ml.). Potassium borohydride (100 mg.) was added and the mixture was shaken overnight. Excess potassium borohydride was destroyed by the addition of glacial acetic acid to pH 4, and the polysaccharide was recovered by precipitation with alcohol (1 vol.). The reduced material was dissolved in water (5 ml.) and reprecipitated with ethanol (1 vol.) and finally washed with ethanol and ether.

Degradations

Xylan (ca. 1.0 g.) was stirred overnight with water (25 ml.)

and then made up to 50 ml. with oxygen-free sodium hydroxide giving a final normality of 0.88 N. Nitrogen was bubbled through the flask was sealed and kept at room temperature for 25 days. The solution was deionised with Amberlite Resin I.R. 120 (H) and the volume was adjusted to 250 ml.

Qualitative Examination

Volatile Acid Content

Samples (25 ml.) were evaporated to dryness under reduced pressure at a water bath temperature of 60°, and the distillate was collected in an ice-cooled receiver. Water (2 x 5 ml.) was added and the contents of the flask were again evaporated to dryness. The distillate was neutralised with sodium hydroxide (0.02 N) to a phenolphthalein end-point. On evaporation of the water, the contents of the flask were chromatographed in solvent (g) and the papers were developed with spray (e) showing the presence in all four samples of formic acid. The formic acid was identified by conversion of the sodium salts to the 4-bromo-phenacyl ester, m.p. and mixed m.p. 140°.

Non-Volatile Acid Content

Samples (25 ml.) were treated with ethanol (2 vols.) and the precipitated alkaline stable polysaccharide was filtered off, washed with ethanol and finally with ether. The filtrates were evaporated to syrups and again treated with ethanol (2 vols.) and additional polysaccharides were removed as before. On evaporation of the filtrates the syrups were examined

chromatographically in solvent (f), papers being sprayed with sprays (c) and (d) for non-lactonisable acids and spray (b) for lactonisable acids. The four polysaccharides were found to have produced the same lactonisable and non-lactonisable acids, but in different proportions.

	R Lactic	Relative Amounts of Acids				
		Rye 'A'	Barley	Oat I	Oat II	Reduced Rye 'A'
unknown acids	0.15, 0.23	+++	++	++	++	-
xylometasaccharino lactones (traces)	0.43, 0.54	+	+	+	+	-
xyloisosaccharino lactone	0.70	++	++	++	++	-
glycollic acid	0.68	++	++	++	++	-
lactic acid	1.0	++++	+++	+++	++	+
lactyl lactic acid	1.10	++	+	+	+	-

Treatment of the whole degradation mixture from the rye xylan in this way gave a syrup which was placed on a column of Amberlite Resin, I.R. 400 (Formate). The lactonisable acids were not absorbed and were washed out with water and elution with formic acid (15%) gave the non-lactionisable acids. On evaporation of the solvent from the latter portion, the syrup was converted to the 4-bromophenacyl ester which on recrystallisation gave crystals having m.p. and mixed m.p. 113° with 4-bromophenacyl lactate.

Quantitative Examination

Total Acid

Aliquots were titrated with oxygen-free 0.01876 N - NaOH in an atmosphere of nitrogen using phenolphthalein as indicator, giving the following results.

Xylan	Total Acid		Formic Acid	
	meq	meq/anhydro unit	meq	meq/anhydro unit
Rye	1.29	170	0.134	17.6
Barley Husks	0.67	86	0.122	16.1
Oat Straw I	0.99	129	0.083	10.8
Oat Straw II	0.97	117	0.188	22.8

Formic Acid

Aliquots were evaporated as before and the distillate was boiled with an excess of freshly prepared yellow mercuric oxide for 3 hours. On cooling the inorganic salts were removed at the pump, washed with water (2 x 5 ml.) and the filtrate was titrated with oxygen-free 0.0047 N sodium hydroxide over an atmosphere of nitrogen, see previous table.

Lactic Acid

The method used was essentially that of Barnett.(140)

Samples (25 ml.) except Oat Straw II (75 ml.) were treated as before to remove the alkaline stable polysaccharide. Copper sulphate solution (1 ml. 20% w/v) was added to each sample and the volumes were adjusted to 25 ml. with water.

A standard lactic acid solution was prepared by dissolving 0.2133 g. of pure dry lithium lactate in water (100 ml.) and conc. H_2SO_4 (1 ml.). The volume was made up to 1 l. with water, and the solution was stored at 4°. A solution of p-hydroxydiphenyl (1.5 g.) in 5% w/v sodium hydroxide was diluted to 100 ml. with water.

Samples (0.5 ml.) containing varying quantities of lactic acid from 4 to 16 µg. were measured out. Copper sulphate solution (0.05 ml.; 12% w/v) was added, together with concentrated H_2SO_4 (6 ml., nitrogen-free) dropwise from a burette into the ice-cooled solution. After complete addition, the tubes were stoppered, shaken and heated on a water bath at 90° for 5 minutes. The tubes were rapidly cooled to room temperature and p-hydroxydiphenyl reagent (0.1 ml.) was added to each one and dispersed in the sulphuric acid. After an incubation period of 20 minutes at 28-30°, the excess p-hydroxydiphenyl was destroyed by immersing the tubes for 90 seconds in a boiling water bath, and this was followed by cooling the tubes in ice-water. The optical density of the solution was measured on a "Unicam" Spectrophotometer at a wavelength of 560 mµ against a reagent blank.

Standardisation of Lactic Acid

Lactic Acid (μg.)	4	8	12	16
Optical Density	0.164	0.395	0.638	0.860

Xylan	Volume used (ml.)	Optical Density	Lactic Acid	
			μg.	meq/anhydro unit
Rye	0.4	0.730	13.6	57.7
Barley Husks	0.2	0.298	5.8	10.8
Oat Straw I	0.2	0.308	6.1	19.5
Oat Straw II	0.5	0.240	4.9	4.8

Examination of the Alkali Stable Degraded Polysaccharide from Rye Xylan

Degraded polysaccharide (200 mg.), previously obtained from ethanol precipitation of the deionised alkaline degradation mixture, was shaken overnight with water (10 ml.). Sodium acetate buffer (2 ml., pH 5), hemicellulase (20 mg.), as described on page 71, and toluene (2 ml.), were added and the mixture was set aside for 48 hours at 35°.

The enzyme was then deactivated by heating on a boiling water bath for 2 minutes, and sodium ions were removed by means of Amberlite Resin I.R. 120 (H). Evaporation of the water, followed by chromatography in solvent (d) using spray (1) showed the presence of only xylose and arabinose. The reducing sugars were separated from the acidic material by means of an anion exchange resin.[139] Amberlite Resin I.R. 400 (OH), (20 g.),

was ground to a fine powder, and then poured in a slurry into a column (20 x 1 cm.). The column was washed with formic acid (20 ml., 17%) to convert it to the formate form and then with water until free from acid. A solution of xylose (200 mg.) in water (5 ml.) was poured onto the column which was then washed with water until the eluate was free from reducing sugar (α-naphthol test).

The syrup obtained from the enzymic hydrolysis was neutralised with sodium hydroxide (0.02 N) to a phenolphthalein end point and the mixture of reducing sugars and sodium salts was placed on the top of the column. After complete removal of the reducing sugars by elution with water, the acidic material was removed from the column by washing with formic acid (100 ml., 10%). The eluate was concentrated to a small volume and the formic acid was removed by continual addition and evaporation of water. Chromatography of the resulting material in solvent (f) and developing with spray (b) indicated the presence of a small amount of xylometasaccharinic acids.

In a control experiment a synthetic mixture of D-xylose (200 mg.) and xyloisosaccharinic acid (5 mg.) was separated in this way. A recovery of xylose (90%), estimated by Somogyi's method, and lactone 90%, estimated by direct titration, was obtained.

Molecular Weights

Molecular weights were obtained by means of the isothermal distillation procedure[144,145] on methylated and acetylated xylans.

The acetylated xylans were prepared from the following polysaccharides:

(1) Rye xylan 'B'.

(2) Rye xylan 'A'.

Alkaline degraded Rye xylan 'A'.

(3) Rye xylan 'A', after potassium borohydride reduction.

Rye xylan 'A', after KBH_4 reduction and treatment with alkali.

(4) Barley Husks.

Alkaline degraded Barley Husks.

(5) Oat Straw I.

Alkaline degraded Oat Straw I.

(6) Oat Straw II.

Alkaline degraded Oat Straw II.

Alkaline Treated Reduced Rye Xylan 'A'

The reduced polysaccharide (200 mg.) was dissolved in oxygen-free NaOH (0.95 N, 10 ml.) and set aside for 25 days. Removal of the sodium ions with resin gave a neutral solution, from which the polysaccharide was precipitated with alcohol (2 vols.) and dried by solvent exchange.

Acetylation of the Polysaccharide[146]

Polysaccharide (160 mg.) was dissolved in formamide (3 ml.) at 50°. Freshly distilled pyridine (3 ml.) was added in small portions over 30 minutes at 15°, and freshly distilled acetic anhydride (2 ml.) was similarly added over 1 hour. After

shaking overnight, the mixture was poured into ice water (100 ml.). The precipitated material was washed with water (3 x 50 ml.), dissolved in chloroform (30 ml.) containing anhydrous sulphate. On concentration of the filtered chloroform solution and addition of petroleum ether (60-80°, 5 vols.), the precipitated material was filtered off, washed with petroleum ether and dried at 0.5 m.m. at 70°.

The following data was obtained:

Xylan		% Acetyl
Rye 'A'	- 80°	38.0
Rye 'B'	- 78°	38.2
Alkaline Degraded Rye 'A'	- 85°	38.7
Barley Husks	- 77°	38.9
Alkaline Degraded Barley	- 92°	38.8
Oat Straw I	- 90°	38.5
Alkaline Degraded Oat Straw I	-100°	38.1
Oat Straw II	- 85°	39.0
Alkaline Degraded Oat Straw II	- 87°	39.0

The methylated xylans were prepared from the following sources:

(1) Rye xylan 'B' (see page 63, fraction 7).

(2) Rye xylan 'A' (using the methods of Haworth and Purdie).

(3) Rye xylan 'A' (by simultaneous deacetylation and methylation of Rye xylan 'A' acetate).

Methylation of Rye Xylan 'A'

By the method of Haworth and Purdie

Polysaccharide (200 mg.) was given a series of five

methylations by simultaneous additions of sodium hydroxide (5 ml., 40%) and dimethyl sulphate (8 ml.) as previously described, followed by two methylations by Purdie's method using silver oxide (1g.) in methyl iodide (30 ml.). The methylated polysaccharide was isolated in the usual way and fractionated with chloroform - petrol ether (60-80°) giving a fraction (40 mg.) which was soluble in 35% chloroform, and had $[\alpha]_D$-118°, and OCH_3, 38.5%.

Deacetylation and Methylation of Rye Xylan 'A' Acetate

Acetylated polysaccharide (300 mg.) was methylated by successive treatments with sodium hydroxide and dimethyl sulphate, giving a product (200 mg.) which was given two further methylations with methyl iodide (30 ml.) and silver oxide (1 g.). The methylated polysaccharide (170 mg.) gave a fraction (40 mg.) which was soluble in chloroform - petroleum ether (60-80°), (35-65) and had $[\alpha]_D$-116°, and OCH_3 = 38.6%.

Purification of Solvents for Isothermal Distillation

a) Analar benzene (1.1). was refluxed with sodium (5g.) for 1 hour and distilled and stored over sodium wire.

b) 1:3 Dioxolane (1 l.) was refluxed with sodium(10g.) for 1 hour and distilled. It was found necessary to treat the distillate with lithium aluminium hydride (2g.) and to redistil the solvent into a brown bottle collecting the fraction boiling at 76°.

Calibration

The apparatus was calibrated using benzene and 1:3 dioxolane as solvents, and triolein (m.w. 885.4) and tristearin (m.w. 891.5) as the standard solutes. K, the appartus constant was calculated for both the change in solvent level and the change in solution level.

Calibration of Apparatus

Solvent	Solute	Solute Mole Fraction 10^{-4}	Apparatus Constant $\times 10^{-3}$	
			Solution Level	Solvent Level
Benzene	Triolein	4.32	2.4	2.3
		0.874	2.6	2.7
		Average value of K	2.5	2.5
1.3 Dioxolane	Triolein	2.23	0.59	0.57
		4.26	0.53	0.56
	Tristearin	2.63	Average value of K 0.56	0.58

Methylated Rye Xylan 'B'

Concentration 0.95 (g /100 g)	Time (hrs.)	(Mins.)	Change in Solution level (m.m.)	Change in Solvent level (m.m.)
Solvent:Benzene	0	0	0	0
	74	0	2.39	2.42
	80	0	2.50	2.68
	98	0	3.13	3.28

Mn. = 9,100

Mn. = 57

Acetylated Rye Xylan 'A'

Concentration 0.45g(100 g)	Time (hrs.	(Mins.	Change in Solution Level (m.m.)	Change in Solvent level (m.m.)
Solvent 1:3-dioxolane	0	0	0	0
	17	0	0.59	0.66
	24	0	0.84	0.96
	41	0	1.41	1.63

Mn. = 15,700

Mn. = 72

Acetylated Degraded Rye Xylan 'A'

Concentration 0.56g/100 g	Time (hrs.)	(Mins.)	Change in Solution level (m.m.)	Change in Solvent level (m.m.)
Solvent 1:3-dioxolane	0	0	0	0
	17	0	0.43	0.55
	21	30	0.98	1.43
	41	0	2.24	2.56

Mn. = 12,500

Mn. = 58

Acetylated Barley Husks

Concentration = 0.75g/100g.

Solvent: 1:3-dioxolane

Time (hrs)	(Mins)	Change in Solution level (mm.)	Change in Solvent level (mm.)
0	0	0	0
17	0	1.14	1.34
24	45	1.25	1.35
41	15	2.10	2.20
89	0	4.45	4.67

Mn = 18.200

D.P.= 84.

Acetylated Degraded Barley Husks

Concentration = 0.71/g/100g.

Solvent: 1:3-dioxolane

Time (hrs)	(Mins)	Change in Solution level m.m.	Change in Solevent level m.m.
0	0	0	0
17	30	1.02	1.12
24	30	1.47	1.59
41	0	2.47	2.61
48	15	3.02	3.22
60	0	3.66	3.86

Mn = 14,500

D.P.= 67

Acetylated Degraded Oat Straw

Concentration = 0.79g./100g.

Solvent :- 1:3-dioxolane

Time (hrs.)	(Mins)	Change in Solution level m.m.	Change in Solvent level m.m.
0	0	0	0
6	30	0.99	0.88
26	0	2.47	2.73
31	0	3.00	3.21
49	0	4.62	5.04

M n = 9,400

D.P. = 43

Rye Xylan 'A', reduced and acetylated

Concentration = 0.79g/100g.	Time (hrs)	(mins)	Change in Solution level mm.	Change in solvent level m.m.
solvent:- 1:3-dioxolane	0	0	0	0
	23	0	4.34	1.57
	71	0	4.30	4.50
	89	30	5.54	5.82

Mn = 15,100

D.P = 70

Rye xylan 'A', reduced, treated with alkali and acetylated

Concentration = 0.86g/100g.	Time (hrs)	(mins)	Change in Solution level m.m.	Change in solvent level m.m.
solvent:- 1:3-dioxolane	0	0	0	0
	17	15	1.27	1.09
	24	15	1.75	1.61
	41.15	15	2.88	3.08

Mn = 15,500

D.P. = 72

Rye xylan 'A' (Haworth and Purdie treatment)

Concentration = 0.84g/100g.	Time (hrs.)	(mins.)	Change in Solution level m.m.	Change in solvent level m.m.
solvent:- Benzene	0	0	0	0
	6	30	0.10	0.16
	30	0	0.78	0.80
	71	0	1.71	1.89
	86	0	2.10	2.26

Mn = 10,400

D.P. = 65

Rye Xylan 'A' (Methylated via the acetate).

Concentration = 0.93g/100g.	Time (hrs.)	(Mins)	Change in solution level (m.m.)	Change in solvent level (m.m.)
Solvent = benzene	0	0	0	0
	14	45	0.46	0.50
	21	30	0.72	0.85
	39	45	1.31	1.53
	106	45	2.99	3.19

Mn = 10,800

D.P.= 67

Rye xylan 'B' Acetate

Concentration = 0.51g/100g.	Time (hrs.)	(Mins)	Change in solution level (m.m.)	Change in solvent level (m.m.)
	0	0	0	0
solvent:- 1:3 dioxolane	17	15	0.81	0.97
	39	30	2.08	1.96
	46	0	2.11	2.25

Mn = 13,200

D.P.= 64

SECTION IV

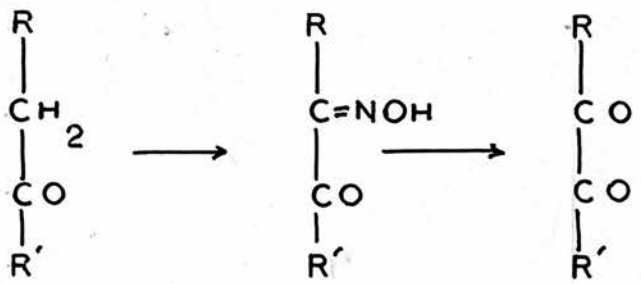
Attempted Synthesis of 1:5-Dihydroxypentan-2-3-dione

Discussion

As has been mentioned on page 78, the synthesis of the α-diketone, 1:5-dihydroxypentan-2:3-dione was attempted with a view to establishing the theory that it is an intermediate in the formation of xyloisosaccharinic acid and of acids from fragment reactions during the alkaline degradation of sugars.

The synthesis was attempted by two separate routes, both of which failed to yield the desired product.

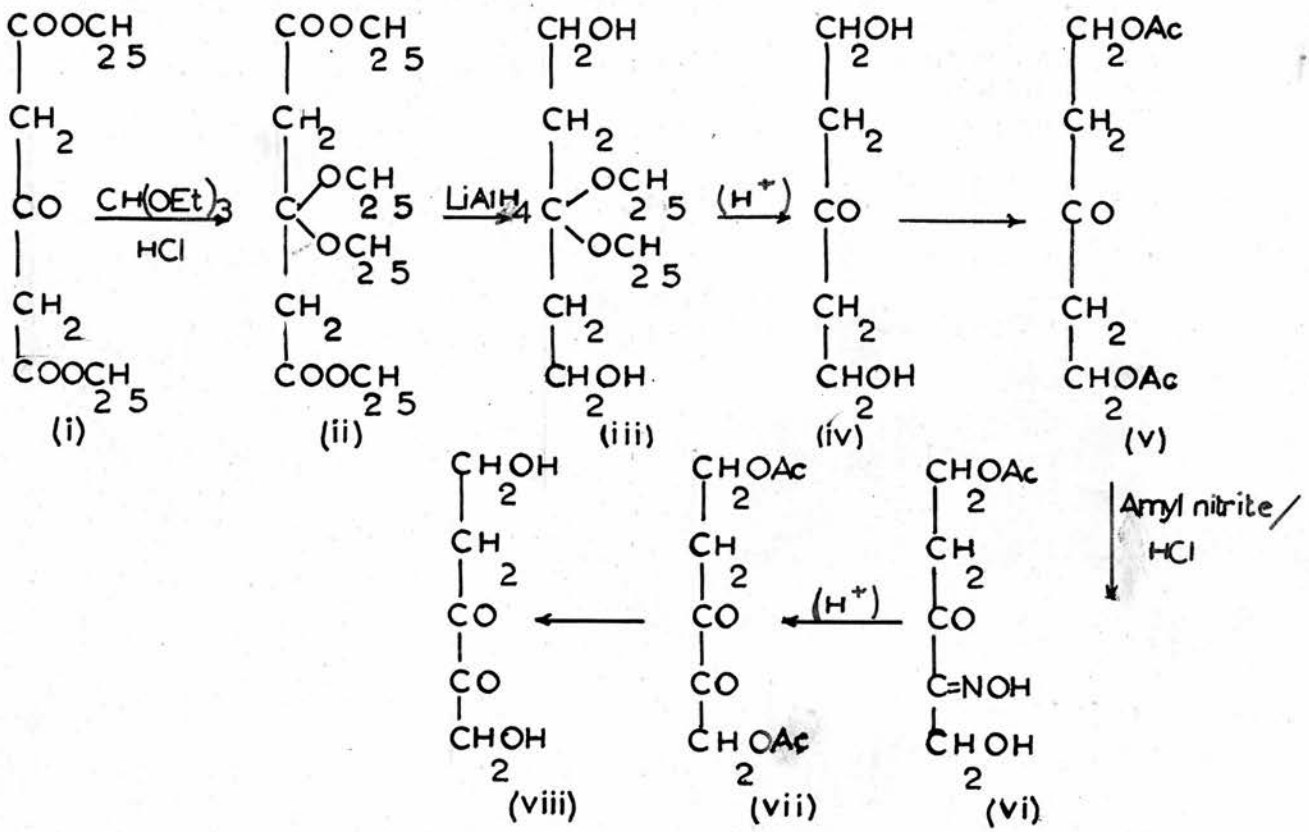
Acetylenic intermediates are often used in the synthesis of 1:2-diketones, thus Criegee[148] obtained 1:4-dibenzoxybutan-2:3-dione by ozonolysis of 1:4-dibenzoxybutyne, and Khan and Newman[149] have obtained 1:2-diketones by oxidation of acetylene compounds with potassium permanganate solutions at pH 7.5. Another common method is the formation of the isonitroso derivative from the monoketone followed by acid hydrolysis of the product.



Pent-2-yne-1:5-diol was synthesised by formylation of but-3-yne-1-ol. No ketonic material was obtained from the ozonisation or from the permanganate oxidation of the compound. The attempted hydration of pent-2-yne-1:5-diacetate to pentan-

2-one-1:5-diacetate was unsatisfactory owing to the production 5-acetoxy-pent-1-ene-4-one on the elimination of acetic acid.

A second route was attempted starting from acetone dicarboxylic ester, as follows:



Ethyl acetone dicarboxylate was treated with ethyl orthoformate in ethanolic hydrogen chloride to give the ketal (ii) which on reduction with lithium aluminium hydride and hydrolysis gave 1:5-dihydroxypentan-3-one (iv). The corresponding acetate (v) was treated with amyl nitrite and hydrogen chloride to give the isonitroso compound (vi). Hydrolysis of this material with hydrochloric acid gave a product which failed to condense with o-phenylene diamine.

Attempted Synthesis of 1:5-Dihydroxypentan-2:3-dione

Experimental

But-3-yne-1-ol[150]

Ferric nitrate (0.2 g.) together with freshly sliced sodium (1 g.) was added over a period of 10 minutes to liquid ammonia (2 l.). After the blue colour had disappeared, sodium (150 g.) was added as rapidly as possible (1½ hrs.).

Acetylene was passed through vigorously when the blue colour had completely disappeared. After 4 hours the acetylene was shut off and ethylene oxide (200 g.) in ether (200 ml.) was added from a dropping funnel over a period of 1 hour, and the mixture was allowed to stir overnight over an atmosphere of nitrogen. Solid ammonium chloride (300 g.) was added to destroy the sodamide, and then the liquid ammonia was allowed to evaporate (24 hours). The solid was removed from the flask and added in small portions to ice-water and the organic material was extracted with ether and dried over anhydrous sodium sulphate. On removal of the ether, the product was distilled through a Fenske column. But-3-yne-1-ol (100 g.) was collected at 125-129°.

Pent-2-yne-1:5-diol[151]

Cuprous chloride (24 g.), in hydrochloric acid (360 ml., 12%) was added to potassium hydroxide (360 ml., 40%) and the yellow-brown cuprous hydroxide was filtered, washed and transferred whilst moist to the but-3-yne-1-ol. Formaldehyde (160 g., 40%), water (48 cc.) and calcium carbonate (0.8 g.) were added and the

mixture was stirred on a steam bath for 4 days under an atmosphere of nitrogen. The solid material was filtered off, washed with 50% aqueous ethanol (500 cc.) and the filtrate was concentrated to give pent-2-yne-1:5-diol (70 g.) as an oil, b.p. 120° (0.5 mm.), n_{15}^{D} = 1.4885.

Pent-2-yne-1:5 diol (10 g.) in aceticanhydride (10 g.) and fused sodium acetate (2 g.) was heated on a boiling water bath for 1 hour. Water (50 ml.) was added and the acid produced was neutralised with sodium bicarbonate. The product was extracted with ether, dried with sodium sulphate and distilled to give a colourless oil (10.1 g.) having b.p. 63° (0.01 m.m.) an n_{15}^{D} = 1.4570.

Found: C, 58.3%, H, 6.72%, Calculated for $C_9H_{12}O_4$, C, 58.68%, H, 6.57%.

Attempted Synthesis of 1:5-Diacetoxypentane-2:3-dione(148)

1:5-Diacetoxypent-2-yne (2 g.) was dissolved in dry methanol (20 ml.) and cooled to -25°. Ozonised oxygen (5-6% ozone, 5 l./hr.) was passed through the solution for one hour and the solution was allowed to warm up to room temperature. Excess ozone was removed by passage of nitrogen through the solution, which was then hydrogenated over a palladium-calcium carbonate catalyst. The uptake of hydrogen indicated that much of the starting material was unattacked by the ozone and was thus being hydrogenated to a saturated compound. Removal of the solvent yielded a syrup (1.8 g.) which showed no ketonic properties (absence of 2:4-dinitrophenyl-hydrazone or quinoxaline).

Attempted Preparation of 1:5-Diacetoxypentan-2-one[152]

1:5-Diacetoxypent-2-yne (10 g.) was refluxed for two hours with a mixture of glacial acetic acid (30 g.), water (10 ml.), and mercuric acetate (2 g.). After removal of the inorganic material by filtration, the acetic acid and water was removed under reduced pressure, and the remaining syrup (7 g.) was distilled under high vacuum, giving a major fraction (3.9 g.), b.p. 60°/0.1 mm., which polymerised on the inside of the condenser, and a minor fraction (ca 500 mg.), b.p. 105°/0.1 mm. Owing to the small yield of the required product, the hydration of the acetylenic material was not further investigated.

Permanganate oxidation of Pent-2-yne-1:5-diol[149]

Pent-2-yne-1:5-diol (5 g.) in water (200 ml.) and magnesium sulphate buffer (pH 7.5) was cooled to 5° and the theoretical quantity of potassium permanganate was added with stirring over a period of 1 hour. The mixture was allowed to stand at room temperature for 2 hours, and was exhaustively extracted with ethyl acetate. Evaporation of the solvent yielded a colourless acidic syrup (50 mg.), b.p. 98°/0.1 m.m. which gave about 3 mg. of a 2:4-dinitrophenylhydrazine (m.p. 210°). Examination of the starting material and the oxidised products showed maxima at 229, 272, and 280 m in the U.V. spectrum.

Ethyl acetone dicarboxylate diethyl ketal

Ethyl acetone dicarboxylate (20 g.) in ethyl orthoformate (32 g.) and dry ethanol (25 ml.) containing 6% hydrogen chloride

was allowed to stand overnight. The alcohol was removed under reduced pressure and an excess of silver carbonate was added to neutralise the acid, and the product was distilled giving a colourless liquid (22 g.), b.p. 82°/0.1 m.m., n = 1.4350, OC_2H_5 = 58% ($C_{23}H_{24}O_6$ requires OC_2H_5 = 65.5%).

1:5-Dihydroxypentan-3-one diethyl ketal

Lithium aluminium hydride (5 g.) in anhydrous diethyl ether (50 ml.) was added with stirring to ethyl acetone dicarboxylate diethyl ketal (20 g.) in anhydrous ether (50 ml.) over a period of an hour. Excess lithium aluminium hydride was destroyed with ethyl acetate, and the product was extracted with ether giving a pale yellow liquid (A) (3 g.), b.p. 80°/14 m.m., OEt =44% The insoluble lithium and aluminium hydroxides were dissolved in hydrochloric acid (50 ml., 5 N) and the product was exhaustively extracted with ethyl acetate, producing a liquid (B) (2 g.) having b.p. 65°/14 m.m. (2:4-dinitrophenylhydrazone m.p.95°).

The ketal (fraction A) was hydrolysed with hydrochloric acid (1 N) by warming on a water bath for 15 mins. On removal of the chloride ions with silver carbonate, the product (2 g.) was combined with fraction (B) as 1:5-dihydroxypentan-3-one.

1:5-Diacetoxypentan-3-one

1:5-Dihydroxypentan-3-one (4 g.) was refluxed with acetic anhydride (2 ml.) and fused sodium acetate (1 g.) for 1 hour. The product was poured into water, neutralised with sodium carbonate, and extracted with ether, giving a product (3 g.),

b.p. 75°/14 m.m., 2:4-dinitrophenylhydrazone, m.p. 115°. (Found: C, 47.7%, H, 4.6%, N, 13.4%, $C_{15}H_{18}O_8N_4$ requires C, 47.1%, H, 4.7%, N, 14.7%.)

Nitrosation of 1:5-diacetoxypentan-3-one[154]

1:5-Diacetoxypentan-3-one (3 g.) in dry ether (50 ml.) was cooled to 0°. A steady stream of dry hydrogen chloride was bubbled through the solution with the simultaneous addition of amyl nitrite (3 g.) in dry ether (50 ml.) over a period of 2 hours. The mixture was stored at 4° for 24 hours, and on removal of the solvent the red brown liquid was heated on a boiling water-bath for 15 minutes with formaldehyde (10 g.) and hydrochloric acid (2 ml., 2 N). The acid and formaldehyde were removed by repeated distillation under reduced pressure, leaving material (1 g.) from which no quinoxaline derivative could be obtained.

Summary

The water soluble polysaccharide isolated from rye flour gave on hydrolysis, xylose (60%), arabinose (29%) and glucose (5%). After methylation of the hemicellulose, the sugars obtained on hydrolysis of the product were separated on a cellulose column, giving 2:3:5-tri-O-methyl-L-arabinose (5 parts), 2:3-di-O-methyl-D-xylose,(6) 2-O-methyl-D-xylose(5) and a small amount of D-xylose. The general structure of the xylan is therefore similar to other known araboxylans, having a backbone of 1:4-linked xylopyranose units with terminal arabinose units linked through position 3. In the isolation of the trisaccharide, O-L-arabofuranosyl (1⟶3)-O-D-xylopyranosyl (1⟶4)-D-xylopyranose, it has been shown that some of the arabinose units are in the form of single side chains.

4-O-Methyl-D-xylose has been synthesised from D-arabinose, and it has been shown to degrade in alkali giving mainly formic acid and xyloisosaccharinic acid, which is the expected product from sugars with 1:4-linked xylose residues.

The action of alkali on various cereal xylans has been studied. The degradation products have been shown to include formic, lactic acids together xyloisosaccharinolactone and small quantities of xylometasaccharinolactones. Xylometasaccharinolactone has been found to be present in the hydrolysis products of the alkali stable rye polysaccharide. The mechanisms for the degradation and stopping reactions are discussed.

Two unsuccessful attempts have been made to synthesise 1:5-dihydroxypentan-2:3-dione.

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900. *Cereal Gums. Part II.* The Constitution of an Araboxylan from Rye Flour.*

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A water-soluble polysaccharide isolated from rye flour gave on hydrolysis xylose (60%), arabinose (29%), and glucose (5%). From hydrolysis of the methylated polysaccharide, controlled acid hydrolysis of the polysaccharide, and quantitative estimation of xylose residues unattacked by periodate in the original and degraded polysaccharides it is concluded that this highly-branched araboxylan contains chains of 1 : 4-linked β-D-xylopyranose residues with approximately every second xylose residue carrying a terminal L-arabofuranose residue linked through position 3.

The water-soluble gums isolated from cereal grains contain residues of D-glucose, D-xylose, and L-arabinose in varying proportions. Hexosan-rich and pentosan-rich fractions may be obtained either by graded precipitation from aqueous solution by ammonium sulphate [1,2] or by fractional precipitation of the derived acetylated polysaccharides.[3,4] The β-glucans from barley [5] and oats [6] are similar to lichenin [7] in structure in containing chains of 1 : 3- and 1 : 4-linked D-glucopyranose residues. The pentosan from wheat flour has been studied by Perlin [3] and by Montgomery and Smith.[4] The L-arabinose residues are present exclusively as non-reducing end-groups in the furanose form, and it is probable that these units are directly attached to a backbone of 1 : 4-linked β-D-xylopyranose residues. It has been shown by Preece and Hobkirk [2] that the main component of the water-soluble gum fraction from rye flour is an araboxylan of similar composition to the polysaccharide from wheat flour. We are very grateful to Professor I. A. Preece for kindly placing at our disposal a quantity of the rye araboxylan for structural investigation, the results of which are described in this paper.

The polysaccharide had a high negative rotation ($[\alpha]_D$ −107° in N-NaOH) and yielded on hydrolysis xylose (60%), arabinose (29%), and glucose (5·5%). Hydrolysis of the derived methylated polysaccharide afforded the following sugars, characterised by crystalline derivatives: 2 : 3 : 5-tri-*O*-methyl-L-arabinose (30%), 2 : 3-di-*O*-methyl-D-xylose (36%), 2-*O*-methyl-D-xylose (31%), and D-xylose (2·5%). In addition, chromatography showed traces of 2 : 3 : 4-tri-*O*-methylxylose, tri-*O*-methylglucose, and 3-*O*-methylxylose. These results indicate the presence in the polysaccharide of chains of 1 : 4-linked D-xylose residues with branching mainly through position 3. All the side-chains are terminated by L-arabofuranose residues, this being the sole mode of linkage of the arabinose residues. It is not certain whether the small amount of D-xylose isolated from the hydrolysis of the methylated polysaccharide represents some double branching points or whether the sugar arises from incomplete methylation of the polysaccharide or demethylation during hydrolysis. It is probable that the glucose residues present in the polysaccharide and the tri-*O*-methylglucose isolated on hydrolysis of the methylated polysaccharide arise from a contaminating glucan since no methyl ethers of glucose could be detected in the hydrolysate of another fraction of methylated polysaccharide.

On the basis of the methylation results two probable structures (I and II) may be put forward for the repeating unit of the polysaccharide. The following results provide evidence in favour of structure (I). Hydrolysis of the periodate-oxidised polysaccharide indicated the presence in the polysaccharide of xylose (24—25%) residues unattacked by periodate. This value is slightly lower than would be expected if all the arabinose residues were attached to singly branched xylose residues (*ca.* 29%). Controlled hydrolysis of the polysaccharide caused selective cleavage of some of the arabofuranosyl linkages with the

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trace of xylose. The major component was identified as 2 : 3 : 5-tri-*O*-methyl-L-arabinose by conversion into 2 : 3 : 5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 130—132°.

Fraction 2. The syrup (105 mg.) had $[\alpha]_D^{18}$ +24·2° (*c* 0·44 in H_2O) (Found: OMe, 35·5. Calc. for $C_7H_{14}O_5$: OMe, 34·8%), and chromatography in solvent B showed 2 : 3-di-*O*-methylxylose and a small amount of tri-*O*-methylglucose. The syrup crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 76—78°. The derived 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 121—122°. Approximate calculation from optical rotation indicated the presence in the fraction of 102 mg. of di-*O*-methyl-D-xylose and 3 mg. of tri-*O*-methyl-D-glucose.

Fraction 3. The chromatographically pure sugar (199 mg.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose, and had m. p. and mixed m. p. 80—81° and $[\alpha]_D^{18}$ −20·1° ⟶ +22·6° (equil.) (*c* 0·35 in H_2O) (Found: OMe, 34·7. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The aniline derivative had m. p. and mixed m. p. 121—123°.

Fraction 4. The crystalline sugar (237 mg.), after recrystallisation from methanol–water, had m. p. and mixed m. p. (with 2-*O*-methyl-β-D-xylose) 130°, and $[\alpha]_D^{18}$ −9·5° ⟶ +35° (equil.) (*c* 0·75 in H_2O) (Found: OMe, 18·7. Calc. for $C_6H_{12}O_5$: OMe, 18·8%). Ionophoretic examination of the mother-liquors showed that a small amount of the 3-methyl ether was also present.

Fraction 5. The syrup (17 mg.) travelled on the chromatogram at the same rate as D-xylose, had $[\alpha]_D^{18}$ +18° (*c* 0·75 in H_2O), and was characterised by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 208—209°.

Estimation of Sugar Residues Unattacked by Periodate.—The polysaccharide (352 mg.) was dissolved in water (10 ml.), sodium metaperiodate (792 mg.) was added, and the solution set aside in the dark for 4 days. Excess of barium chloride solution was added, insoluble barium salts were filtered off, and the filtrate was dialysed for 3 days. Concentration of the solution to small volume and addition of acetone (10 vol.) precipitated the periodate-oxidised polysaccharide (190 mg.). Hydrolysis of this material with N-sulphuric acid for 4 hr. at 100° and chromatographic examination [9] of the hydrolysate, using galactose as reference sugar, showed the presence of xylose (24%).

The polysaccharide (502 mg.) was dissolved in 0·01N-oxalic acid (50 ml.) and heated on the boiling-water bath for 1·5 hr. Ethanol (5 vol.) was added to the cooled solution, and degraded polysaccharide (387 mg.) was precipitated. Chromatography of the supernatant liquor showed only arabinose. Hydrolysis of the degraded polysaccharide afforded xylose (60%) and arabinose (10%) (these and the subsequent value are expressed as percentages of the undegraded polysaccharide). The degraded polysaccharide was converted into the corresponding periodate-oxidised polysaccharide, hydrolysis of which with N-sulphuric acid for 4 hr. at 100°, followed by chromatography [9] of the hydrolysate, showed xylose (8%).

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