Some Problems in the Chemistry of the Hemicelluloses.

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By E. L. HIRST.

IT is now generally accepted that in the cell walls of plants the fundamental skeletal substance consists mainly of α-cellulose, the long thread-like macromolecules of which are arranged in bundles. Within these bundles the free hydroxyl groups of the 1 : 4′-linked β-glucopyranose residues serve to align the cellulose molecules by hydrogen bonding into an organised structure which in parts is so well ordered that it displays crystallinity, whilst in others a looser and more amorphous arrangement is found. Few cells, however, possess a cell wall consisting, as is the case with the cotton hair, almost entirely of cellulose. In most cases, more especially with lignified material, the cellulose bundles are embedded in an amorphous mass of lignin and polysaccharide material, giving a strong and rigid structural element, for which a crude analogy can be found in reinforced concrete. In recent investigations by Rånby[1] and his collaborators in Sweden evidence to this effect has been obtained by the application of the electron microscope to cell-wall materials which had been disintegrated by ultrasonic vibrations. The exact nature of the association in the cell wall between the lignin, the amorphous mass of non-cellulosic carbohydrate material, and the true cellulose is not yet known, but many methods have been derived for the removal of the lignin from the carbohydrates. This can be effected, for instance, by mild treatment with chlorine or sodium chlorite which renders the lignin water-soluble and leaves a so-called holocellulose from which the non-cellulosic constituents can be extracted by aqueous alkali. These materials, for want of a better name, are referred to as hemicelluloses. Their molecular structure is the main theme of the present lecture. In general the hemicellulose fraction contains a number of different polysaccharides, and several kinds of sugar residues are concerned in their molecular architecture, notably D-xylose, L-arabinose, D-galactose, L-rhamnose, D-mannose, D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, and possibly D-galacturonic acid. Their function is probably to give strength and rigidity to the cell walls. They are therefore cell-wall polysaccharides, but it is not the case that all polysaccharides of this type associated with the cell wall have the same function. Many substances in this group, some of which will be mentioned later, appear rather to be concerned with metabolic processes.

Despite frequent attempts no satisfactory classification of the hemicellulose group has yet been proposed. This is due largely to the difficulties inherent in separating in a pure state the complex mixture of macromolecules of which the group is composed. Usually several molecular species occur in admixture with each other, and one of the major needs of this branch of carbohydrate chemistry is the development of new and powerful methods of fractionation. Advantage can sometimes be taken of special properties, such as the formation of an insoluble copper complex, but the ordinary methods of fractional precipitation or fractional dissolution are of little avail. It may well be that new developments in the technique of ionophoresis will be of assistance, and recently it has been shown by Preece and his collaborators[2] that for certain types of hemicellulose mixtures fractional precipitation by ammonium sulphate is effective.

It follows that much of the exploratory work in this field has been carried out on complex mixtures, examination of which can at best reveal only very general structural features. In this lecture, however, I propose to limit discussion essentially to the chemistry of those hemicellulosic materials which have been separated in a reasonably pure and homogeneous condition. The number of these is steadily increasing and from a study of their structure a general picture of structural relationships within the group is gradually emerging. These studies require the use of all the established methods employed in the polysaccharide field, and additional complications are encountered owing to the occurrence of several sugar residues in one and the same molecule. The question of the order in which these residues are arranged is now important and the problem becomes formally very similar to that of assigning a detailed molecular structure to a protein.

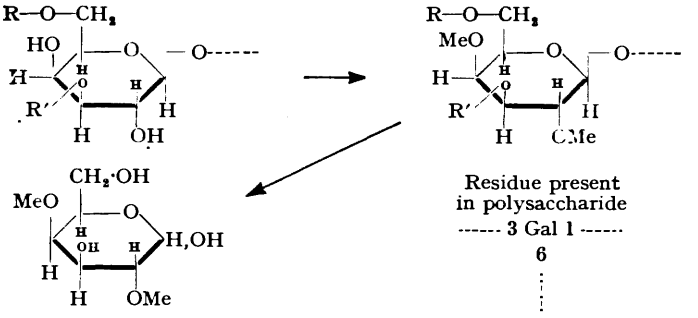
Hydrolysis with acid, followed by qualitative and quantitative chromatographic analysis,

[1] Rånby, Inaugural Dissertation, Inst. Phys. Chem., Uppsala, 1952.

[2] Preece and Mackenzie, *J. Inst. Brewing*, 1952, **58**, 353, 457; Preece and Hobkirk, *ibid.*, 1953, **59**, 385; 1954, **60**, 490.

gives the nature of the sugar residues present in the macromolecule and the proportions in which they are present. In passing, it may be emphasised that within the last few years the development of chromatographic techniques has opened up entirely new horizons in carbohydrate chemistry, and without these new methods the greater part of the work I am describing in this lecture would have been quite impossible. The methylation procedure, despite the 50 years which have elapsed since its first use by Purdie and Irvine, remains one of the most powerful

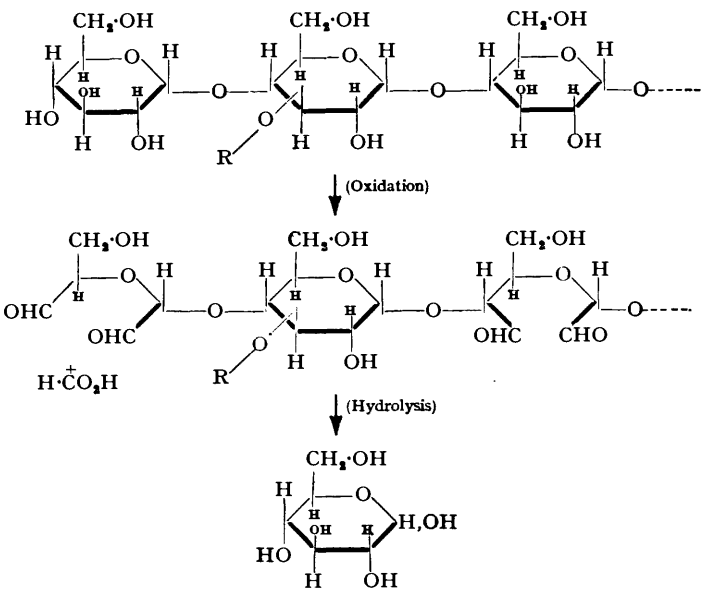
FIG. 1. *Methylation method.*



2 : 4-Dimethyl-D-galactose.

of weapons and by its use, as illustrated for one residue of a polysaccharide in Fig. 1, the mode of linkage of each residue in the molecule can be ascertained. But this method reveals neither the order of the residues nor the nature of the glycosidic links by which the individual sugar units are joined together.

FIG. 2. *Periodate oxidation.*

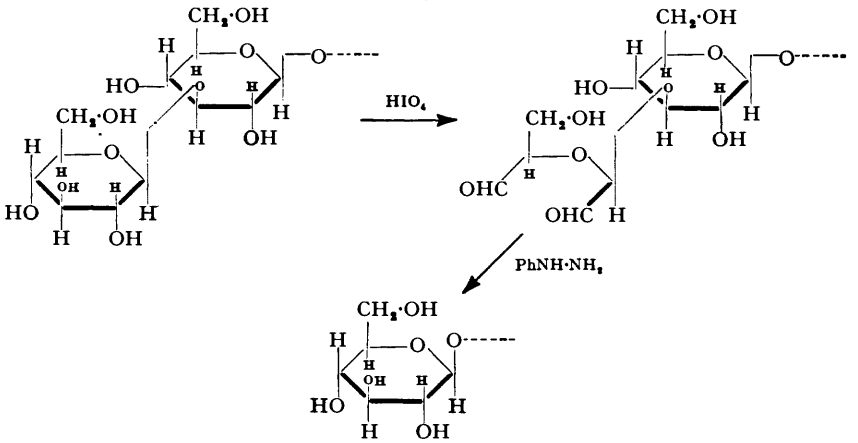


Insight into the order in which the residues occur is now being increasingly obtained by use of the method of partial hydrolysis. Fragments containing from two to eight or more sugar residues can be separated by chromatography on columns of charcoal–Celite, starch granules or other materials and the structures of these break-down products can be determined by standard methods.

Much useful information can be derived from a study of the action of periodate ions on the macromolecules. As indicated in Fig. 2, end-group determinations, the nature and the number of the residues so linked that they are not attacked by periodate, and the proportion of residues

giving rise to a dialdehyde can all be dealt with in this way. By the use of Barry's extension [3] of the procedure polysaccharides containing 1 : 3′-linked residues can sometimes be broken down one residue at a time (see Fig. 3), and the method has recently been still further developed by Barry who has found that insoluble derivatives of the polyaldehyde can be obtained by reaction with *iso*nicotinhydrazide or thiosemicarbazide. These precipitates are free from the unwanted by-products which often render interpretation of periodate oxidation results somewhat uncertain.

FIG. 3. *Barry's method.*



Enzymic methods of degradation have not yet been developed in the hemicellulose field to the remarkable degree of precision they have reached in starch chemistry, but there is no doubt that they are of rapidly increasing importance and, when methods have been worked out for the separation and purification of the appropriate enzymes, results of major importance can confidently be expected.

As with starch and cellulose physicochemical methods for determining the molecular shape and size are employed to complete the picture of molecular structure. These include *X*-ray analysis, viscosity determinations, and light-scattering and osmotic-pressure measurements. As regards the last-mentioned procedure difficulties often arise in the hemicellulose field in that molecular weights are often in the region 3000—20,000 for which suitable and reliable membranes are not yet available. It is hoped however that as a result of work in progress at the Chemical Research Laboratory, Teddington, membranes covering this important region will be developed but in the meantime it is often necessary to determine molecular weights by the tedious process of isothermal distillation.

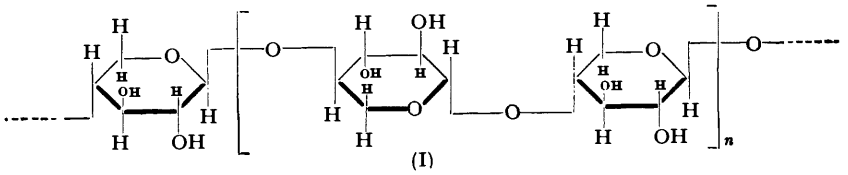
A 1 . . . Residue . . . 4 **X** 1 . . . Residue

(A = arabinose; X = xylose.)

We may turn now to consider the results achieved when these methods are applied to typical members of the hemicellulose group. The sugar residue most frequently encountered is D-xylose and we can conveniently begin therefore with a consideration of the xylan series of polysaccharides. These are found in all lignified plant tissues and form a very considerable part of the dry weight of all woods, cereal straws, seed coats, and similar materials. The esparto grass used for paper making gives a holocellulose of which some 30% is a xylan readily extractable by alkali. Owing to its accessibility and the widespread importance of the raw material in which it occurs, this xylan has been extensively investigated. As normally obtained it consists mainly of xylose residues but the hydrolysis products usually contain in addition some

[3] Barry, *Nature*, 1943, **152**, 537; Barry, McCormick, and Mitchell, *J.*, 1954, 3692.

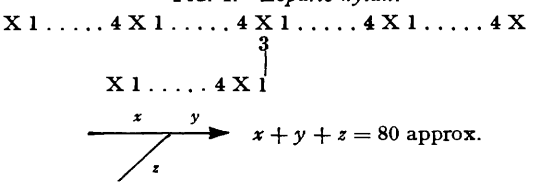
L-arabinose. After methylation and hydrolysis of the fully methylated derivative of the xylan the principal products obtained in the early work in this field were 2 : 3-di-*O*-methyl-D-xylose and 2 : 3 : 5-tri-*O*-methyl-L-arabinose, roughly in the proportions 20 : 1 respectively.[4] The polysaccharide must therefore be built up largely of a series of β-linked ... 4 X 1 ... residues (see above), the structure in this respect being very similar to that of cellulose (I). For a long time the rôle of the terminal A 1 ... residues remained uncertain, and, indeed, this problem has been solved only very recently. The arabinose could be removed easily by mild hydrolysis, leaving a polysaccharide containing xylose residues only and possessing approximately one



(I)

xylose end group for every 20 xylose residues. But the simple interpretation of this in the sense of a chain of ... 4 X 1 ... residues terminated at the non-reducing end by A 1 ..., *i.e.*, the structure A 1—4 X 1 ..... 4 X, was not acceptable since further investigation revealed that the proportion of arabinose residues in esparto xylan was not constant, but, on the contrary, varied widely with the methods used to purify the crude xylan. It remained a possibility therefore that the arabinose residues were not combined in the true xylan molecule but were associated with other polysaccharides present as impurities. Steps were then taken to carry out a rigorous fractionation of esparto xylan by methods which would be gentle enough chemically not to disturb the labile arabofuranoside linkages should they be present in the molecule. To do this advantage was taken of the insoluble copper complex which xylan gives in alkaline copper solutions of the Fehling's type. After several such precipitations esparto xylan which originally contained several per cent of arabinose gave fractions which analysed as true xylans containing no detectable amount of arabinose. Investigation of this arabinose-free material by the standard methylation procedure showed that the methylated derivative yielded on hydrolysis 2 : 3-di-*O*-methyl-D-xylose (over 90%) and 2 : 3 : 4-tri-*O*-methyl-D-xylose (2·5%), together with some 2-*O*-methyl-D-xylose and small amounts of other methylated xyloses. After full account had been taken of possible errors due to demethylation during hydrolysis and to other causes it was clear that this particular xylan had a molecular structure composed essentially of chains of 1 : 4′-β-linked xylopyranose residues, with one xylose end group per 40 xylose residues. Molecular-weight determinations indicated, however, that the degree of polymerisation of the methylated xylan was approximately 80, from which it appears that the molecule must contain a single branch. The position of the branch point along the chain is not known, but the isolation of 2-*O*-methylxylose on hydrolysis of the methylated xylan points to a linkage of the 1 : 3′-type at the branch point. Diagrammatically it is possible therefore to represent the main structural features of this fraction of esparto xylan by the formula shown in Fig. 4.[5]

FIG. 4. *Esparto xylan.*



The presence of chains of 1 : 4′-β-linked xylopyranose residues appears to be a general structural feature throughout most of the xylan group of polysaccharides. For maize-cob xylan this has been demonstrated in a particularly elegant way by Whistler and his colleagues at Purdue University.[6] Using the method of partial hydrolysis, they have succeeded in breaking down the xylan extracted from maize cobs into a series of oligosaccharides, ranging from

[4] Haworth, Hampton, and Hirst, *J.*, 1929, 1739; Haworth, Hirst, and Oliver, *J.*, 1934, 1917
[5] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[6] Whistler and Tu, *J. Amer. Chem. Soc.*, 1951, **73**, 1389; 1952, **74**, 3609; 1953, **75**, 645

xylobiose upwards to the heptasaccharide. Separation of these has been effected chromatographically on charcoal–Celite columns, and the individual crystalline sugars have been studied in detail. From the results it is clear that they form a series (Fig. 5), each sugar in which contains one additional xylose residue, the linkages throughout being of the 1 : 4′-β-type. The findings cannot be accounted for on the basis of reversion products and there is clear evidence therefore of the general structural arrangement of the xylose residues in maize-cob xylan.

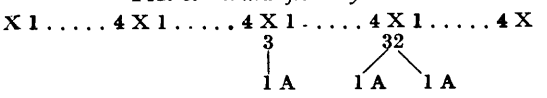
It is necessary, however, to revert at this stage to the subject of arabinose residues in xylans. At the time when the fractionation of esparto xylan was being studied in this country, A. S. Perlin was investigating in the Ottawa laboratories a very remarkable polysaccharide which he had obtained by careful fractionation of the hemicellulosic constituents of wheat flour.[7] One of the fractions from this was a water-soluble substance which gave xylose (63%) and arabinose

FIG. 5. *Structure of xylose oligosaccharides obtained from maize-cob xylan* (n = 0—6).

X 1[4 X 1]$_n$ 4 X X = β-D-xylopyranose.

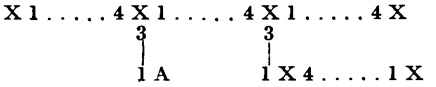
(37%) on hydrolysis. The high proportion of arabinose could not be altered by fractionation. The methylated derivative gave on hydrolysis equal amounts of 2 : 3 : 5-tri-*O*-methyl-L-arabofuranose and 2 : 3-di-*O*-methyl-D-xylose, together with 2-*O*-methyl-D-xylose and free D-xylose. The residues of which the polysaccharide is composed must therefore be A 1 . . . ., . . . 4 X 1 . . ., $\cdots \overset{4}{\underset{3}{}}$X 1 . . . and $\cdots \overset{3}{\underset{4}{}}$X $\overset{1}{\underset{2}{}}\cdots$ It appears certain from these results that the arabinose residues must be attached to xylose residues in the parent polysaccharide. They could however be removed completely by mild hydrolysis, leaving a true xylan the main structural feature of which was again a chain of 1 : 4′-β-linked xylopyranose residues. Perlin's xylan may therefore be pictured (Fig. 6) as a chain of xylose residues, similar to those in esparto and maize-cob xylans, but with numerous single terminal arabofuranose residues attached as side chains.

FIG. 6. *Wheat-flour xylan.*



Examination of the arabinose-rich, as distinct from the arabinose-free, fractions of esparto xylan revealed a similar state of affairs. Investigations by Aspinall and Moody[8] showed that after methylation and hydrolysis these fractions yielded 2 : 3-di-*O*-methyl-D-xylose (3 parts) and 2 : 3 : 5-tri-*O*-methyl-L-arabinose (1 part), together with 2-*O*-methyl-D-xylose (1 part) and small amounts of tri-*O*-methylxylose and other substances. The relative proportions of these sugars and the absence of lower methylated derivatives of L-arabinose make it certain that the molecule must contain side chains of arabofuranose residues. It follows that much of the xylan present in esparto must conform to the general structure shown in Fig. 7. Whether or not all the molecules are branched is still uncertain, and, as will appear later, there may be further complications in the way of combined uronic acid residues.

FIG. 7. *Arabo-xylan from esparto.*



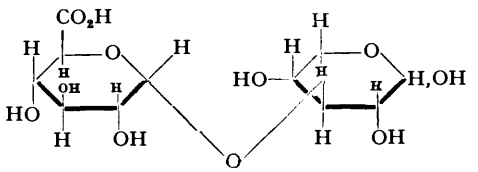
Still greater complexity of structure has been observed amongst the xylans present in cereal straws. Wheat-straw xylan, in particular, has been the subject for detailed study and evidence concerning its structure has been contributed by several groups of workers, including Roudier,[9] Adams[10] and Bishop,[10] F. Smith,[11] and Aspinall.[12] Once again it is found that fractions of different arabinose contents can be separated and, although in this instance it has not yet been possible to isolate material completely free from arabinose residues, some fractions containing only insignificant proportions of arabinose have been isolated.[11] In addition to arabinose

[7] Perlin, *Cereal Chem.*, 1951, **28**, 370, 382.
[8] Aspinall, Hirst, Moody, and Percival, *J.*, 1953, 1631.
[9] Roudier, *Compt. rend.*, 1953, **237**, 840; *Assoc. tech. ind. papetiere Bull.*, 1954, 53.
[10] Adams, *Canad. J. Chem.*, 1952, **30**, 698; Bishop, *ibid.*, 1953, **31**, 134.
[11] Ehrenthal, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 5509.
[12] Aspinall and Mahomed, *J.*, 1954, 1731.

this xylan gives rise on hydrolysis to D-glucuronic acid (*ca.* 4%) and possibly to some 4-*O*-methyl-D-glucuronic acid also. There is decisive evidence that the uronic acid residues are combined directly with D-xylose in the xylan macromolecule. In general the glycosidic link of a uronosyl compound is strongly resistant to hydrolysis and on partial hydrolysis of wheat-straw xylan an aldobiuronic acid can be obtained, consisting of a glucuronic acid linked through its reducing group to a xylose residue. On methylation, followed by hydrolysis of the product, a partially methylated xylose containing methyl groups in the 2- and the 4-position is obtained.[10] In other experiments a partially methylated aldobiuronic acid was isolated on hydrolysis of the methylated xylan and this gave on further hydrolysis 2 : 3 : 4-tri-*O*-methylglucuronic acid and 2-*O*-methylxylose.[12] The linkage in the aldobiuronic acid must therefore be of the 1 : 3′-type and its structure must be (II).

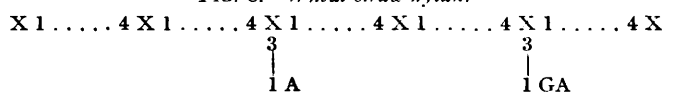
The main structural feature of the polysaccharide is again a chain of 1 : 4′-linked β-xylopyranose residues. Xylopyranose end groups (linkage X 1 . . .) are found to the extent of 2·5%. On the basis of this evidence the general type of structure present in the molecule of wheat-straw xylan is given in Fig. 8 which depicts a chain of xylose residues to which are attached at intervals short branches consisting respectively of single residues of D-glucuronic acid and

(II)



L-arabinose, the branch points being at $C_{(3)}$ of the xylose residue concerned. Very recently a direct proof has been given of the attachment of L-arabinose residues to the main chain of xylose residues. This has been achieved by enzymic breakdown of wheat-straw xylan by a pentosanase enzyme obtained from *Myrothecium verrucaria* which has been found by Bishop and Whittaker[13] to bring about the hydrolysis of . . . X 1–4 X 1 . . . links without a comparable effect on the A 1–3 X . . . links. In this way a series of oligosaccharides has been isolated from wheat-straw xylan, each of which has been shown to contain at least one arabinose residue directly linked to a xylose residue.

FIG. 8. *Wheat-straw xylan.*



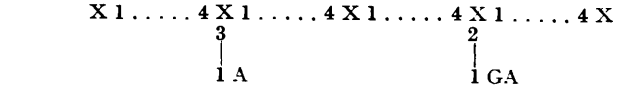
As in the case of the esparto xylans no more than a start has been made in the assignment of structural details in the wheat-straw group of xylans. Nevertheless it seems clear that here again we have to deal with a large group of polysaccharides with very similar main-chain structures but with very varied proportions of side chains. We do not yet know where in the main chain these side chains are attached and whether they occur in a regular pattern or are randomly located along the main chain. No clear evidence is yet available as to whether the main chain is linear or branched, and although there is concrete evidence in favour of $C_{(3)}$ branch points the possibility that $C_{(2)}$ branch points also occur cannot yet be ruled out. The degree of polymerisation of the various molecular species has yet to be determined in detail but preliminary evidence obtained by Greenwood for material examined in Edinburgh indicates a rather small molecular weight of the order of 50 xylose residues. It may well be, however, that the hemicelluloses as they exist in the plant have higher molecular weights. Evidence pointing in this direction is provided by Bishop[14] in his study of crystalline xylans obtained by autoclave treatment of hemicelluloses from wheat and other types of straw. A puzzling feature, recorded by Roudier, is that on removal of the arabofuranose residues by gentle hydrolysis, wheat-straw xylan gives a product containing one xylose end group per 18 residues. An exactly similar observation was made in the early days of the study of esparto xylan and the question naturally arises whether we have here something of special significance in xylan chemistry.

The study of xylans derived from other cereal strains has not yet reached the same stage of detail, but enough is known of the general properties of the xylans of barley straw and oat

[13] Bishop and Whittaker, *Chem. and Ind.*, 1955, 119.
[14] Bishop, *Canad. J. Chem.*, 1953, **31**, 793; cf. Yundt, *TAPPI*, 1951, **34**, 89.

straw,[15] to permit the conclusion that they are in general similar in constitution to wheat-straw xylan. In the case of the material from barley straw fractionation *via* the copper complex can be carried to a point where the arabinose content is probably not structurally significant. Such fractions still contain small but significant quantities of uronic acid residues. Fractions

FIG. 9. *Oat-straw xylan.*

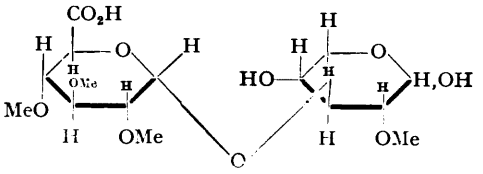


A = L-Arabofuranose;
X = β-D-xylopyranose;
GA = D-glucuronic acid.

of oat-straw xylan containing both arabinose and uronic acid residues have been examined and it appears that the general structure suggested for wheat-straw xylan may apply in these cases also (Fig. 9). There is some evidence that branch points of both the 1 : 3′-type and the 1 : 2′-type may be present in the same molecule. The glucuronic acid (which may be partly present as 4-*O*-methyl-D-glucuronic acid) is combined with xylose by a 1 : 2′-link of the kind found in beechwood xylan (see below). It is possible, but not certain, that the main xylose chain is branched.

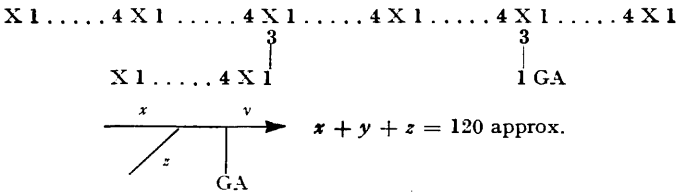
The xylans we have been discussing so far have been somewhat similar in respect of their botanical origin, being obtained from monocotyledonous plants of the grass type. For comparison we may turn now to two materials present in dicotyledonous plants. The first of these is the xylan isolated by Isherwood[16] from the cell-wall of the fruit of the pear (Constance). In this case there is little difficulty in obtaining a xylan free from arabinose, but containing a small proportion of uronic acid residues. These consist of D-glucuronic acid, possibly with some 4-*O*-methyl-D-glucuronic acid but this is not certain. The fully methylated derivative of the pear cell-wall xylan gives on hydrolysis 2 : 3-di-*O*-methyl-D-xylose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, some 2-*O*-methyl-D-xylose, and a partially methylated aldobiuronic acid (III). The

(III)



last substance in turn gives on hydrolysis 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid and a monomethylxylose. Xylose end groups are present in the macromolecule to the extent of 1 part in 60 whilst the percentage of uronic acid is rather less than one. The molecular weight of the methylated derivative corresponds to some 120 residues and on the basis of this evidence the structure of pear cell-wall xylan would appear to be of the type shown in Fig. 10.[17] The posi-

FIG. 10. *Pear cell-wall xylan.*



tions of the branch points at which the side chains are attached are not yet known, and it is to be remembered also that the identification of $C_{(3)}$ of a xylose residue as the point of attachment of the uronic acid side chains rests mainly on chromatographic differentiation between 2-*O*-methylxylose and the corresponding 3-derivative. In consequence it is not yet altogether ruled out that the linkage in the aldobiuronic acid may be GA 1 —2 X.

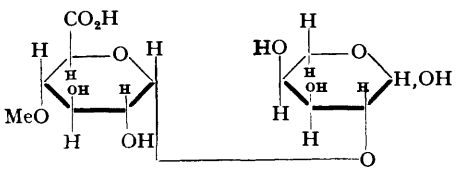
[15] Aspinall, unpublished results; Aspinall and Wilkie, unpublished results.
[16] Isherwood and Jermyn, unpublished results; see also Jermyn "Cellulose and Hemicelluloses" in "Modern Methods of Plant Analysis," Vol. II, Springer-Verlag.
[17] Chanda, Hirst, and Percival, *J.*, 1951, 1240.

From beechwood, by extraction with dilute alkali without previous delignification, Macdonald,[18] of the Forest Products Research Laboratory, isolated a xylan which on hydrolysis gave nearly 90% of D-xylose together with 10% of a uronic acid and a trace of L-rhamnose, which, however, is almost certainly present as an impurity. Structural investigations in Edinburgh by Aspinall and Mahomed[19] have shown that this is a most interesting substance possessing some quite unexpected structural features. The uronic acid turned out to be 4-*O*-methyl-D-glucuronic acid, which although of rare occurrence in Nature has been found also in mesquite gum, aspen wood, and cotton wood. Investigation by the methylation procedure revealed the presence of the following residues in the macromolecule; X 1 .... (1·3%), ... 4 X 1 .... (78%), $\begin{matrix}\cdots\cdot 4\\ \cdots\cdot 2\end{matrix}$ X 1 .... (9·5%). In addition aldobiuronic acid residues were present to the extent of 10·5%.

The tetra-*O*-methylaldobiuronic acid obtained by partial hydrolysis of fully methylated beechwood xylan gave after reduction by lithium aluminium hydride, followed by further hydrolysis, 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose. The fully methylated aldobiuronic acid, again after reduction and hydrolysis of the product, gave rise to 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose. The original xylan gave all its uronic acid as 4-*O*-methyl-D-glucuronic acid and there can be no doubt in this case that in the aldobiuronic acid the linkage is 1 : 2′ and that the aldobiuronic acid is 2-(4-*O*-methyl-α-D-glucuronsyl)-D-xylose (IV). We can therefore depict the main structural features of this xylan as a chain of β-1 : 4′-linked D-xylose residues, having one terminal xylose residue for every 80, with side chains of 4-*O*-methyl-D-glucuronic acid, attached on the average to every tenth xylose residue by a 1 : 2′-link (Fig. 11).

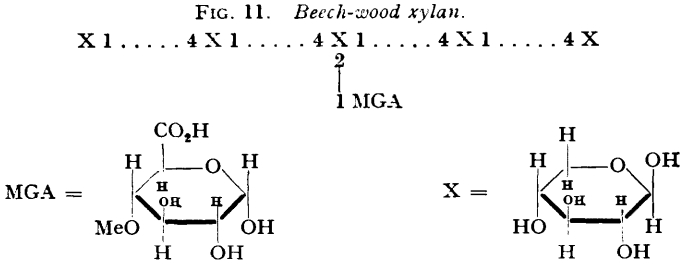
The presence in corn-cob hemicelluloses of 4-*O*-methyl-D-glucuronic acid residues in 1 : 2′-type linkage has been reported by Whistler, Conrad, and Hough[20] who isolated the

(IV)



aldobiuronic acid, 2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylose, after partial hydrolysis of the polysaccharide. Whistler and Hough[21] had previously isolated a mixture of 2-*O*-(α-D-glucuronosyl)-D-xylose and 4-*O*-(α-D-glucuronosyl)-D-xylose from the hydrolysis products of corn-cob xylan. Here also it appears that residues both of D-glucuronic acid and of 4-*O*-methyl-D-glucuronic acid occur in the same group of hemicellulosic materials. If the residue GA 1—4 X occurs in any part of the molecule other than the end of a chain it would point to a hitherto unknown type of linkage of main-chain xylose residues through positions other than 1 : 4′.

FIG. 11. *Beech-wood xylan.*



Amidst the variety of structural features described in the preceding sections one feature has remained constant, namely, the nature of the main chains of xylose residues. So versatile, however, is the xylose molecule in forming polymers that still further variations occur in natural

[18] McDonald, *J.*, 1952, 3183.
[19] Aspinall, Hirst, and Mahomed, *J.*, 1954, 1734.
[20] Whistler, Conrad, and Hough, *J. Amer. Chem. Soc.*, 1954, **76**, 1668.
[21] Whistler and Hough, *ibid.*, 1953, **75**, 4918.

products. One of the red seaweeds, *Rhodymenia palmata*, is remarkable in that it accumulates amongst its cell-wall polysaccharides large proportions of a xylan which can easily be leached away from the other components. It is a true xylan, giving only D-xylose on hydrolysis, but when examined by the methylation procedure it reveals a novel structural feature.[22] The methylated derivative gives on hydrolysis 2 : 3 : 4-tri-*O*-methyl-D-xylose (1 part), 2 : 3-di-*O*-methyl-D-xylose (12 parts), 2 : 4-di-*O*-methyl-D-xylose (3 parts), and less than 1 part of monomethylxyloses. All attempts to separate either the original or the methylated xylan into separate components have failed and the conclusion seems inescapable that in this substance we have a linear main chain containing both 1 : 4′- and 1 : 3′-linked xylose residues (Fig. 12),

FIG. 12. *Algal xylan from* Rhodymenia palmata.

X 1 . . . . . 4 X 1 . . . . . 3 X 1 X

Number of residues per molecule : X 1, 1; 4 X 1, 12; 3 X 1, 3.

X = β-D-Xylopyranose residue.

these being present in the proportions of 4 : 1, respectively. The chain containing these two types of residue is terminated by a xylopyranose unit and in the particular sample of methylated algal xylan we examined the chain was a short one containing only some 17 xylose residues. Nothing is yet known concerning the order in which the 1 : 4′- and 1 : 3′-links are arranged in the linear molecule.

The position here is somewhat reminiscent of that established for lichenin in which 1 : 4′- and 1 : 3′-linked β-D-glucose residues are present in the same main chain. Recently it has been established that precisely the same kind of structure, containing both 1 : 4′- and 1 : 3′-links, occurs in *iso*lichenin where the glucose residues are combined as α-glucosides.[23] It is interesting to find similar structural features in the hemicellulose designated barley gum, which has recently been isolated by Preece[2] and his collaborators from barley grains. It has been obtained in a pure condition, free from other polysaccharides, by fractional precipitation by aqueous ammonium sulphate. Structural investigations by Aspinall and Telfer[24] have shown that it gives only D-glucose on hydrolysis. Its rotation is such that β-glucosyl linkages must be present. Its methylated derivative yields on hydrolysis equal amounts of 2 : 3 : 6- and 2 : 4 : 6-tri-*O*-methyl-D-glucose, with a mere trace of tetramethylglucose (end group). The molecular weight determined by Greenwood was about 20,000 and it follows that the molecular structure of the substance must be in the form of a long unbranched chain containing equal numbers of β-1 : 4′- and β-1 : 3′-linked D-glucose residues (Fig. 13).

FIG. 13. *β-Glucosan from barley* (*barley gum*).

. . . . . 4 G β 1—3 G β 1 . . . . . G = D-Glucopyranose.

It was mentioned at the beginning of the Lecture that, in addition to residues of glucose, xylose, arabinose, and uronic acids, other sugars also, notably galactose and mannose, were encountered in the hemicellulose group. There is indeed a vast and almost unexplored field waiting for investigation, but progress is slow in the meantime owing primarily to difficulties of fractionation and separation. It would be unprofitable to deal with these in detail at the moment, and I propose to conclude with a brief reference to two further substances, detailed investigations of which have been possible. One of them has been chosen from the galactose series and the other is a mannose derivative. Amongst the many components of the hemicellulose group present in larch wood (*Larix europaea*) it is possible to separate with reasonable ease a polysaccharide, designated ε-galactan, which on hydrolysis gives rise to D-galactose and L-arabinose, approximately in the proportions of 6 : 1. This material has been extensively examined by White[25] in the U.S.A. and by my colleagues in this country.[26] There is general agreement as to the type of structure but it is still a very live problem whether the material isolated from larch wood is a single polysaccharide containing both galactose and arabinose residues or whether a portion of it consists of a true galactan, free from combined arabinose. In some early work on the methylated derivative of this galactan evidence was obtained that by careful fractionation it was possible to separate a portion which gave on hydrolysis solely

[22] Chanda and Percival, *Nature*, 1950, **166**, 787.
[23] Chanda and Hirst, unpublished results.
[24] Aspinall and Telfer, *J.*, 1954, 3519.
[25] White. *J. Amer. Chem. Soc.*, 1941, **63**, 2871; 1942, **64**, 302, 1507, 2838.
[26] Campbell, Hirst, and Jones, *J.*, 1948, 774; Aspinall, Hirst, and Ramstad, unpublished results.

D-galactose derivatives. The remainder gave an arabinose derivative (present as end group) and a mixture of methylated galactoses. The evidence at present available points to the presence of the following residues in the galactose skeleton : Gal 1 . . . ., ... 6 Gal 1 . . . . and $\begin{matrix}\cdot\cdot\cdot 3\\ \cdot\cdot\cdot 6\end{matrix}$ Gal 1 . . . .. The type of structure would, therefore, be that outlined in Fig. 14, and there are indications that in the arabogalactan portion—or in the whole polysaccharide if it is not separable into a galactan and an arabogalactan—arabinose chains are attached at $C_{(6)}$ of some

FIG. 14.

. . . . . Gal β 1—6 Gal β 1 . . . . .

$$\begin{matrix}3\\ |\\ 1\ \beta\ \text{Gal}\ 6\ *\end{matrix}$$

Gal = D galactopyranose.

* In the arabogalactan L-arabinose residues may be attached at some of these positions.

of the galactose side chains. It is possible that the situation here is not unlike that in the xylan group where, as we have seen, larger or smaller proportions of L-arabinose residues may be attached to a basal structure composed of xylose residues. There is however a further complication the implications of which are still under active investigation. J. K. N. Jones[27] has recently found that on mild hydrolysis of this ε-galactan some of the arabinose residues are removed and that one of the products of partial hydrolysis is a disaccharide 3-(L-arabopyranosyl)-L-arabinose in which arabinose occurs in the pyranose form. He has obtained this same disaccharide after partial hydrolysis of other naturally occurring polysaccharides under experimental conditions which render it unlikely that it is an artefact or reversion product. Only on rare occasions have arabopyranose residues been reported in natural products and this new finding introduces serious complexities into the problems of structural determination in the group which comprises the hemicelluloses, gums, and mucilages.

As an example of polysaccharides of this type containing mannose residues we may consider the mannan (guaran) obtained from the material designated guar. This is unique in that it is the only substance of its class to which it is possible to ascribe a reasonably definite and complete structural formula. It has been investigated in detail by Whistler and his colleagues[28] at Purdue University, who have made use of all the various methods for structural determination including partial hydrolysis, methylation, and *X*-ray investigations. The substance gave D-mannose and D-galatose on total hydrolysis. On partial hydrolysis several products were obtained, including two disaccharides (A) and (B) and two trisaccharides (C) and (D) (Fig. 15). These were separated and their identity proved by rigorous methods. The identification of these products, taken in conjunction with the results of the methylation and other experiments, enabled a unique structure to be assigned to the polysaccharide. In this the D-mannose residues are found as a chain of 1 : 4′-β-linked mannopyranose residues to which is attached at $C_{(6)}$ of

FIG. 15.

(A) Mβ 1—4 M
(B) Galα 1—6 M
Mβ 1—4 Mβ 1—6 M (C)
Galα 1—6 Mβ 1—4 M (D)

Gal = D-galactopyranose; M = D-mannopyranose.

every alternate mannose residue an α-linked D-galactopyranose residue as a side chain (see Fig. 16). It is of interest to recall in this connection that 1 : 4′-links are of common occurrence in mannose-containing polysaccharides, prominent features being, for instance, in the structure of the mannans of the ivory nut.

FIG. 16. *Guaran.*

$$\begin{matrix}\text{Gal}\alpha\ 1\\ |\\ 6\end{matrix}$$

. . . . . 4 Mβ 1—4 Mβ 1 . . . . .

These examples will, I hope, give some idea of the kind of problems encountered in the field of hemicellulose chemistry. It will be all too evident that there exist many gaps in our knowledge of this important group of natural products, and in indicating these deficiencies I can

[27] Jones, *J.*, 1953, 1672.
[28] Ahmed and Whistler, *J. Amer. Chem. Soc.*, 1950, **72**, 2524; Whistler and Durso, *ibid.*, 1951, **73**, 4189; 1952, **74**, 5140; Whistler and Smith, *ibid.*, 1952, **74**, 3795.

perhaps claim to have fulfilled some of the obligations imposed upon the Pedler Lecturer. He is required, however, to undertake a still more onerous task, namely, to indicate the general direction of future developments in the field of his Lecture. No prophecies concerning the outcome of scientific research are safe and this applies with particular force to structural investigations, where a discovery in another and apparently unrelated field may be seized upon to open up entirely new possibilities of exploration. Amongst the clamant needs at the moment are better methods of separating closely related polysaccharides. In the hemicellulose field this may be assisted in the not too distant future by the use of improved ionophoretic methods. It seems certain also that as our knowledge of pentosanase enzymes is increased much more use will be made of enzymic methods of degradation for structural determinations. Degradation by partial hydrolysis, whether by acids or enzymes, coupled with improvements in the chromatographic separation of oligosaccharides will go far to solve troublesome problems concerning the order in which the sugar residues occur in the macromolecule. But the determination of a structural formula is by no means the final goal of the worker interested in these problems. That is only one step towards the still more difficult problem of the phytochemical origin of the various sugar residues found combined in the hemicelluloses. A few years ago it was attractive to speculate on the possibility that the two pentoses encountered so frequently in this group, namely, D-xylose and L-arabinose, could be derived simply from D-glucose and D-galactose respectively, by simple oxidation at $C_{(6)}$ followed by decarboxylation. It is abundantly clear however from the structural determinations which have already been made, that the transformation of a hexosan into a pentosan is not effected at the polysaccharide level by simple changes of this kind. At the very least hydrolysis to monosaccharide units must be effected before transformation to a new sugar residue and rebuilding takes place, and some recent work by Hough and Jones [29] on the action of aldolase enzyme systems has indeed given hints that before the synthesis of pentose-containing polysaccharides can take place, further degradation of hexose units to triose and biose systems may be necessary. I venture to think that in the near future much attention will be paid to these problems concerning the synthesis of polysaccharides by cytoplasmic enzyme systems, and that in this difficult field all the resources of chemistry and biochemistry, including the extended use of radioactive tracer elements, will necessarily be called into play. The present gaps in knowledge are indeed large and formidable, but in assessing them it is perhaps not unfitting to recall that structural polysaccharide chemistry is a discipline of comparatively recent origin, dating back indeed less than 30 years. Much has been accomplished in that short time and with the aid of methods already available and in view we can confidently expect still more rapid progress in the future.

[29] Hough and Jones, *J.*, 1952, 4047, 4052; 1953, 342; Gorin and Jones, *J.*, 1953, 1537; Gorin, Hough, and Jones, *J.*, 1953, 2140.