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A HETEROXYLAN AND HEMICELLULOSIC MATERIALS FROM BAMBOO LEAVES, AND A RECONSIDERATION OF THE GENERAL NATURE OF COMMONLY OCCURRING XYLANS AND OTHER HEMICELLULOSES*†

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ABSTRACT

The molecular structures of water-soluble and -insoluble hemicelluloses of bamboo leaf, and of a hemicellulosic xylan from the former, have been examined. It is concluded that all the materials have the same structural features on their main xylan chain, namely, the side-chains or residues (*a*) L-arabinofuranose, (*b*) *O*-galactopyranosyl-(1→5)-L-arabinofuranose, (*c*) *O*-galactopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→2)-L-arabinofuranose, (*d*) *O*-D-xylopyranosyl-(1→2)-*O*-L-arabinofuranosyl-(1→2)-L-arabinofuranose, (*e*) *O*-(D-glucopyranosyluronic acid)-(1→4)-*O*-D-xylopyranosyl-(1→4)-galactopyranose, and (*f*) D-glucopyranuronic acid and its 4-methyl ether. Feature (*d*) is proposed for the first time, but it may have been present in xylans studied by others. The main xylose chain in the soluble, insoluble, and xylan materials have, respectively, 1 in every 2.8, 4.0, and 7.6 xylan residues substituted by side residues or chains (*a*)–(*e*). It is concluded that the polysaccharide product recovered after methylation of the three hemicellulosic materials does not fully represent the quantitative composition of the materials subjected to methylation, although structural features (*a*), (*d*), and (*e*) are not much altered. When the soluble material was treated with dilute acid under mild conditions, most of (*a*) was removed, while (*d*) and other substituents, including those with L-arabinofuranose residues, were not removed to the same extent.

INTRODUCTION

Xylans are the dominant hemicelluloses in plants of all species of the Gramineae (the grasses). Hirst and his colleagues, in early, and now classical, studies of the xylans from esparto grass, laid the modern foundation of hemicellulose chemistry by establishing that xylans have (1→4)-linked β-D-xylopyranosyl residues[1,2]. This is the

*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.
†The Hemicelluloses of the Bamboos *Arundinaria japonica* and *A. anceps*: Part II. For Part I, see Ref. 33.

ubiquitous and definitive structural feature in xylans of land plants. Non-endospermic heteroxylans, later isolated from grasses by Hirst and others, have non-reducing, terminal L-arabinofuranosyl groups[3], attached to a few of the C-3 positions of the xylose residues[4], and residues of D-glucopyranosyluronic acid, or the 4-*O*-methyl derivative, on a few C-2 positions[5,6]. Other structural features, some of which will be commented on below, have been discovered in xylans from different parts of grasses of various species[7–10].

Purity is a prerequisite of reliable structural studies of xylans; otherwise, it is difficult to decide which structural features are in xylans and which in other hemicelluloses. Pure xylans are obtained by fractionation of hemicellulosic materials, but such fractionations have a subjective aspect. The investigator must establish or accept criteria of purity that, in part, reflect expectations arising from what has been discovered and, in part, may be intuitive. Early criteria of purity were influenced by the attractive hypothesis that cellulose might be biosynthetically converted into xylan without alteration of the (1→4)-*β*-D-linked chain[11]. A homoxylan was sought, and isolated from esparto grass[12], but Hirst pointed out that the transformation of glucose to xylose residues could not take place at the polysaccharide level[7]. Fractionations designed to isolate homoxylans led to the isolation of xylans having low proportions of arabinose and uronic acid residues from the non-endospermic hemicellulosic materials of many grasses. These heteroxylans accounted for only part of each molecular population of hemicelluloses. Non-xylose residues in grass hemicellulosic materials were, and others still are, commonly alleged to be in arabinans or galactans, on little or no evidence. The literature on hemicelluloses is misleading and confused by the uncritical acceptance and repetitive usage of these terms. It has been established that, in several grasses, almost all of the hemicellulosic material is heteroxylan, and that part of it is composed of complex and highly substituted xylans that account for most of the arabinose, and many of the galactose, residues[13,14].

It is evident that xylans from different grasses have many structural features in common. The infrequent discovery of certain of these features, and the normal discovery of others, are both due to the tendency to concentrate on the study of pure xylans of already known structural type. Two points are clear from studies in recent years. Firstly, when a xylan having certain structural features is shown to be present in one grass, then, knowing its characteristics, it may be more readily identified in, and isolated from, other species of grass. Secondly, comparisons of the quantitative differences between pure xylans from different grass species, or from different parts of a plant, are of limited significance, because of the influence of plant maturation on hemicellulosic composition[15–21]. With discretion one can use the information on structural features in the hemicellulosic materials from various species of grass to interpret the structures of other grass hemicelluloses. Complex and highly substituted xylans are probably as common, although not necessarily as abundant, in grasses as are the less-substituted ones more often isolated and commented on.

There have been more studies of the hemicelluloses from temperate grasses and cereals than of those from tropical grasses. The Gramineae appear to have evolved

from bamboo or bamboo-like species existing at least as early as the Cretaceous period. The Bambusoideae sub-family of the Gramineae has ~76 genera, but some of the thousand species have synonymous names, and the sub-family is taxonomically ill-defined. The bamboos are different from most, but not all, other grasses, in that they are commonly massive perennials with woody-textured stems. Hemicellulosic material from *Dendrocalamus strictus* yielded not only the sugars typically found in temperate grasses but also a low proportion of mannose[22], a sugar so far rarely noted in aerial organs[23], although noted in low proportions in roots[24–27]. Acidic arabinoxylans containing low proportions of arabinose residues were obtained by fractionation of hemicelluloses from the Formosan bamboo *Sinocalamus latiflorus*[28]. Other species of bamboo are alleged to contain xylans and arabinans, but the evidence is equally indicative of arabinoxylans. The culms of the bamboo *Phyllostachys reticulata* C. Koch gave hemicellulosic material containing xylose, arabinose, galactose, 4-*O*-methylglucuronic acid, and glucose in the ratios 85.9:4.3:1.4:4.5:4.1. Methylation studies of this material indicated that one in every four of the glucose residues was terminal and non-reducing[30]. Shoots of *Phyllostachys reticulata* yielded an arabinogalactan having a β-(1→3)-galactan chain substituted on some C-6 positions by L-arabinofuranosyl residues[31]. Arabinan and galactan stated to be present in hemicellulosic material from the bamboo *Leleba oldhami*[32] may be arabinogalactan.

RESULTS AND DISCUSSION

Studies have now been carried out on hemicellulosic materials from the leaves of *Arundinaria japonica* and *A. anceps* grown in a temperate climate. β-D-Glucans having (1→3) and (1→4) links have been isolated from the leaves and stems of plants of each species. The total hemicellulose (*T*) was extracted from a chlorite holocellulose of *A. japonica* and, from it, water-soluble (*S*) and water-insoluble (*I*) hemicellulosic fractions were isolated, as described[33] in Part I. An acidic galactoarabinoxylan (*X*) was obtained from an alkaline solution of *S* material which, typically, gave a gel on addition of Fehling's solution (Fig. 1, Part I). The various materials were hydrolysed and the neutral sugars determined as their glycitol acetates by g.l.c. Xylose, arabinose, galactose, and glucose residues were present in the *X* material in the molar ratios of 50:13.7:5.8:1.9, and in the *T*, *S*, and *I* materials in the ratios reported elsewhere[33] (Table I, Part I). Traces of rhamnose and glucuronic, galacturonic, and aldobiouronic acids were present in each hydrolysate. The methoxyl contents of *I*, *S*, and *X* materials were 0.31, 0.39, and 0.26%, respectively. D-Glucuronic acid residues and those of its 4-methyl ether, D-galacturonic acid, and a low proportion of rhamnose residues were present in the various materials. The *X* material accounted for 26% of the *S* material, and contained 5.8% of uronic anhydride as determined by decarboxylation. The *X* material was treated with the enzyme preparation from *Cytophaga* previously used[33]. On periodate oxidation, reduction, and hydrolysis, and conversion of the products into glycitol acetates, the material gave a low proportion of erythritol tetra-acetate and glucitol hexa-acetate in the molar ratio of 2.55 to 1. The presence of glucose residues

and of derivatives is noted later in this paper, but adequate comment has been made in Part I on their presence in separate hemicellulosic glucans. The *X* material was fractionated with ammonium sulphate in an effort to remove or diminish the proportion of galactose. The 0.7% of *X* material that precipitated was hydrolysed, and the sugars (estimated as glycitol acetates) released were xylose, arabinose, galactose, and glucose in the molar ratios of 50:13.2:3.3:11.0. There was a fall in the proportion of the galactose residues in the precipitated material, but no determinable alteration in the proportions of the sugars in the non-precipitated material. The *X* material was examined electrophoretically in borate buffer on glass paper, and a Procion-dyed derivative was also examined electrophoretically on cellulose acetate film and on paper The material in each case travelled as a discrete zone. A single, symmetrical Schlieren peak was obtained on ultracentrifugation of *X*. The *X* material was concluded to be a xylan contaminated by glucan—a view supported by methylation studies.

The *X* and *S* materials were partially hydrolysed by acid, and the neutral and acidic products were separated by a combination of anion-exchange chromatography and high-voltage electrophoresis on paper. The components from the two materials were indistinguishable qualitatively and similar quantitatively. Further studies of the components obtained on partial hydrolysis were carried out on the *S* material, which was expected to have all the structural features in the *X*, and probably in the *I*, materials, although in different proportions. Ten neutral components were isolated from a partial hydrolysate of the *S* material. In addition to xylose, arabinose, and galactose, four neutral disaccharides and two neutral trisaccharides were isolated, including the β-(1→4)-linked D-xylose di- and tri-saccharides. A xylosylarabinose isolated did not form a formazan derivative and was concluded to be *O*-β-D-xylosyl-(1→2)-L-arabinose, thereby accounting for the presence of some of the 2,3,4-tri-*O*-methyl-D-xylose and some of the 3,5-di-*O*-methyl-L-arabinose in hydrolysates of methylated *X*, *S*, and *I* materials. Such a disaccharide has been isolated from xylans of corn cobs[34], perennial ryegrass roots[35], esparto grass[36], and barley husk[37]. A galactosylarabinose in the partial hydrolysate gave a formazan derivative which indicated it to have a (1→3) or (1→5) glycosidic linkage. The latter linkage is more probable, as *O*-β-D-galactosyl-(1→5)-L-arabinose has been obtained from corn-hull[38] and oat-stem[14] xylans, and hydrolysates of the methylated *X*, *S*, and *I* materials contained 2,3-di-*O*-methyl-L-arabinose. The galactosylxylose in the partial hydrolysate also gave a formazan derivative, which indicated it to have a (1→3) or (1→4) linkage, but as no 2,4-di-*O*-methyl-D-xylose was detected during the various methylation analyses, the disaccharide was concluded to be (1→4)-linked. *O*-β-D-Galactopyranosyl-(1→4)-D-xylose has been isolated from corn hull[39]. A trisaccharide in the partial hydrolysate was either a galactosylxylosylarabinose or a xylosylgalactosylarabinose. *O*-Galactosyl-(1→4)-*O*-D-xylopyranosyl-(1→2)-L-arabinoses have been extracted from the roots of perennial ryegrass[35], oat stem[14], and corn fibre[40]. The galactose in hemicellulosic materials is commonly assumed to be the D enantiomer, but the L enantiomer has been noted in xylans of various grasses[25,40,41]. The enantiomeric nature of the galactose was not investigated in the present studies.

The acidic components in the partial hydrolysate of the *S* material were D-glucuronic acid and its 4-methyl ether, D-galacturonic acid, and acids that were indistinguishable from *O*-(D-glucopyranosyluronic acid)-(1→2)-D-xylose and *O*-(D-glucopyranosyluronic acid)-(1→2)-*O*-D-xylopyranosyl-(1→4)-D-xylose. The hydrolysates of methylated *S* and *X* materials contained low molar proportions of 2,3,6-tri-*O*-methylgalactose, which probably derived from *O*-(D-glucosyluronic acid)-(1→4)-*O*-D-xylosyl-(1→4)-galactosyl side-chains similar to those concluded to be present in various oat hemicelluloses[13,14,42]. The acidic components in the partial hydrolysate were esterified and converted into methyl glycosides, and the products reduced with sodium borohydride. The materials were hydrolysed with acid to give D-glucose, galactose, and 4-*O*-methyl-D-glucose (analysed as glycitol acetates), which were assumed to derive mainly from D-glucuronic, D-galacturonic, and 4-*O*-methyl-D-glucuronic acids in the diffusible acidic oligosaccharides, although some of the galactose would derive from the uronosylxylosylgalactose. There is evidence to indicate that xylans have both D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid residues attached to C-2 positions[6], but no recent support for early evidence that these residues are attached to C-3 positions[5,43]. It is well-known that, on acid hydrolysis of acidic xylans, *O*-D-(glucopyranosyluronic acid)-(1→2)-D-xylose and *O*-(D-glucopyranosyluronic acid)-(1→2)-*O*-D-xylopyranosyl-(1→4)-D-xylose and, presumably, their 4-*O*-methyl-substituted uronic acid analogues, are released[44,45]. The unusual stability of the glycosiduronic linkage is not explicable in terms of any inductive effect due to the carboxylic acid group[46]. D-Glucuronic acid and the 4-methyl ether noted in this and other xylan hydrolysates may, in part, derive from uronosylxylosylgalactosyl side-chains in which the glycosiduronic linkage is (1→4)[14,47]. The *S*, *I*, and *X* materials were separately methylated by a modification of the method of Hakomori[48,49], and each yielded products that did not absorb in the hydroxyl region of the i.r. The methylated materials were methanolysed in sealed tubes, care being taken to avoid losses due to volatilisation, and the methanolysates were examined by g.l.c. and g.l.c.–m.s. Other samples of the methylated materials were hydrolysed successively with 90% formic acid at 100°, and then with 0.25M sulphuric acid at 100°. The components in the neutralised hydrolysates were reduced with sodium borohydride, and then fully acetylated and analysed by g.l.c. The molar responses of the flame-ionisation detector of the g.l.c. apparatus to 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-D-xylitol, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol were 0.99, 1.00, and 1.19, respectively. The ratios correspond well with those (1–1.2) calculated[50] on the basis of "effective carbon number contributions". The molar values in Table V for the various methylated pentoses are the relative areas of the peaks given by the derived glycitol acetates, and the relative areas, divided by 1.2, given by the corresponding derivatives of the methylated hexoses. The nature of the components in the partial hydrolysate and the results of methylation analyses indicate the structural features in the various materials. The xylans in the *X*, *S*, and *I* materials appear to have the same structural features, but in different proportions. The molar proportions of the features were calculated as

follows. In the argument, the molar proportions of the parent methylated sugar in hydrolysates of methylated materials are referred to, instead of those of the derived glycitol acetates actually determined. Reports of the presence of an *O*-D-xylosyl-(1→2)-L-arabinosyl side-chain suggest that it is directly attached to the xylan chain, but a trace of trisaccharide isolated from a partial hydrolysate of an oat xylan was concluded to be either a xylosylarabinosylarabinose or an arabinosylxylosylarabinose[51]. Either of these structures as side-chains would yield xylosylarabinose on acid hydrolysis (Fig. 1). As will be noted later, the proportions of 2,3,4-tri-*O*-methyl-D-xylose and of 3,5-di-*O*-methyl-L-arabinose in the hydrolysates of the methylated *X*, *S*, and *I* materials accorded well with the presence of *O*-D-xylosyl-(1→2)-*O*-L-arabinofuranosyl-(1→2)-L-arabinofuranosyl side-chains. The corresponding trisaccharide was not isolated, but this is not surprising, as any such free trisaccharide would be expected normally to hydrolyse to give *O*-D-xylosyl-(1→2)-L-arabinose, while any xylosylarabinosylarabinosyl side-chains would give the disaccharide and temporarily leave arabinosyl side-residues on the main xylan chain. The molar proportion of *O*-galactosyl-(1→5)-L-arabinofuranosyl chains is equated with the proportion of 2,3-di-*O*-methyl-L-arabinose. The 2,3,4,6-tetra-*O*-methylgalactose not formed from such a disaccharide is assumed to be formed from *O*-galactosyl-(1→4)-*O*-D-xylosyl-(1→2)-L-arabinosyl chains. It is possible that the *O*-galactosyl-(1→4)-D-xylose found in the partial hydrolysate derives from galactosylxylosylarabinosyl side-chains of this type[14,35,40]. The proportion of 3,5-di-*O*-methyl-L-arabinose derived from methylated *O*-D-xylosyl-(1→2)-*O*-L-arabinofuranosyl-(1→2)-L-arabinofuranosyl chains is the proportion present minus that calculated to be present in the galactosylxylosyl-arabinosyl chains. The 2,3,4-tri-*O*-methyl-D-xylose not accounted for in terms of xylosylarabinosylarabinose derives from the single, non-reducing, terminal D-xylose residue in each xylan molecule. Although the methylated *S* and *X* materials were highly substituted, mainly on the C-3 position, there was no indication of the presence of any 2,4-di-*O*-methyl-D-xylose derivatives when methanolysates and glycitol acetates were examined by g.l.c. This indicates that terminal xylose residues do not carry either side-chains or single residues. The presence of 2,3,6-tri-*O*-methyl-galactose is equated with *O*-(D-glucosyluronic acid)-(1→4)-*O*-D-xylopyranosyl-(1→4)-galactosyl chains. The proportion of 2,3,5-tri-*O*-methyl-L-arabinose is equated with the proportions of L-arabinofuranosyl residues directly attached to the main chain. The 2,3-di-*O*-methyl-D-xylose residues not in the glucuronosylxylosylgalactosyl or galactosylxylosylarabinosyl chains derive from the main chain, as do the mono-*O*-methyl-D-xyloses and the single, non-reducing, terminal xylose residue. The g.l.c. conditions used to separate the various fully acetylated, partially methylated glycitols failed to separate those of 2-*O*- and 3-*O*-methyl-D-xyloses. The proportion of each mono-*O*-methylxylose in the methylated *X* material was established by peak-area measurements of derived methyl glycosides separated by g.l.c. The molar proportions of the various structural features in the methylated *S*, *I*, and *X* materials are given in Table I. The d.p. values are calculated by assuming the presence of one terminal, non-reducing xylose residue on each main xylan chain, discounting uronic acid

residues and attached xylose residues, which, apparently, were not liberated during acidic hydrolysis. It will be noted that, in all the methylated materials, the ratio of the side-chains to mono-*O*-methyl-D-xyloses is 1:1. It appears highly improbable that such an equality in these three separate calculations is due to coincidence. The values also indicate that the C-2 linked D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid residues are indeed stable under acidic conditions. G.l.c. showed that methyl (methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronate was present in the methanolysate of methylated *S* material; the peak area corresponded to 0.7% of the total area given by all components. Four methylated sugars were present in the various methanolysates and hydrolysates, for which no structural role has been assigned in the xylan. Three of these are 2,4,6-tri-*O*-methylgalactose, 2,4-di-*O*-methyl-D-galactose, and 2,5-di-*O*-methyl-L-arabinose. After this work was complete, the isolation of a (1→3)-linked arabinogalactan from bamboo shoots was reported[31], in which the arabinose residues were attached to C-6 positions. Such a hemicellulose could be the source of the two types of methylated galactose residue, and of 2,5-di-*O*-methyl-L-arabinose residues in some way substituted on the C-3 positions. A low proportion of 2,3,4-tri-*O*-methylrhamnose was noted in the methylated *S* material. Galacturonic acid was present in hydrolysates of the various hemicellulosic materials. There was no evidence of its attachment to xylan molecules, and it may be in an independent hemicellulose, possibly a galacturonan in the portion of the cell wall categorised as primary cell-wall[45].

When a solution of the heteroxylan was treated with 0.05M sulphuric acid at 45°, preliminary studies indicated that hydrolysis terminated after 200 h. Aliquots of the hydrolysate were withdrawn at intervals, diluted, and dialysed for three days against changes of water. The diffusates contained arabinose, galactose, xylose, and oligosaccharides that were not studied. The non-diffusible, stripped heteroxylans were recovered from each aliquot and were examined by g.l.c. after methylation and methanolysis. Determinations were made of the g.l.c. peak areas given by the methyl glycosides of 2- and 3-*O*-methylxyloses, 2,3-di-*O*-methylxylose, 2,3,4-tri-*O*-methylxylose, 2,3,5-tri-*O*-methylarabinose, 2,3,4,6-tetra-*O*-methylgalactose, and 3,5-di-*O*-methylarabinose. The molar ratios of these components had been established by the study of glycitol acetates of sugars derived from methylated *X* material (Table VI). Factors were used to convert the areas due to the methyl glycosides to correspond to the molar values obtained for glycitol acetates derived from the hydrolysate of the methylated xylan. The conversion factors thereby obtained were thereafter used to convert all other peak areas due to methyl glycosides to give molar ratios. Examination of the molar ratios (Tables II and VI) indicates that the d.p. of the main xylan chain is initially ~62; thereafter, it falls during hydrolysis to a value of ~53, if the end residues are unsubstituted, or to a higher value if the hydrolysis is random and some xylan chains have terminal, substituted xylose residues that do not yield 2,3,4-tri-*O*-methyl-D-xylose residues on methylation. Throughout the hydrolysis, the ratios of side-chains *a*–*e* (Table II) to branched main-chain xylose residues is within the range of 0.98 ±0.03. Again, this equimolar correspondence indicates that the

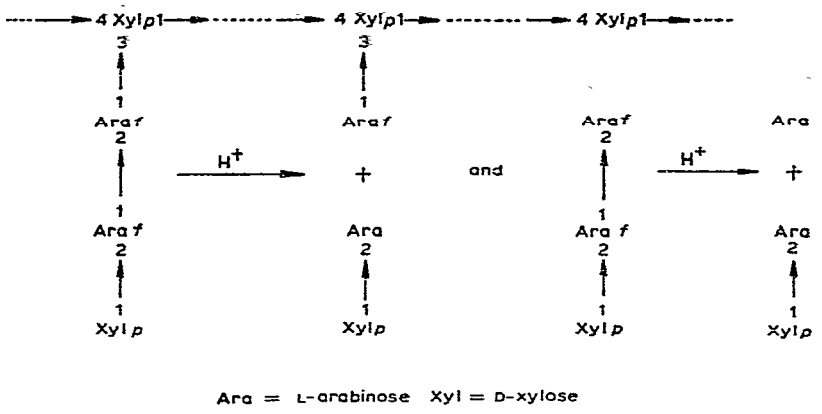

Fig. 1. Acidic hydrolysis of *O*-D-xylopyranosyl-(1→2)-*O*-L-arabinofuranosyl-(1→2)-L-arabinofuranosyl side-chains on bamboo-leaf xylans.

TABLE I

MOLAR PROPORTIONS OF STRUCTURAL FEATURES IN POLYSACCHARIDES CORRESPONDING TO THOSE ISOLATED ON METHYLATION OF A HETEROXYLAN AND THE WATER-SOLUBLE AND WATER-INSOLUBLE FRACTIONS OF TOTAL HEMICELLULOSES FROM THE LEAVES OF THE BAMBOO *Arundinaria japonica*[a]

Structural features and related data	*Insoluble total hemicellulose*	*Soluble total hemicellulose*	*Heteroxylan*
Side-chains			
a L-Ara*f*	9.6	22.0	12.2
b Gal*p*-(1→5)-L-Ara*f*	1.5	2.9	2.4
c Gal*p*-(1→4)-D-Xyl*p*-(1→2)-L-Ara*f*	0.9	1.4	1.3
d D-Xyl*p*-(1→2)-L-Ara*f*-(1→2)-L-Ara*f*	1.0	5.4	5.8
e D-UA*p*-(1→4)-D-Xyl*p*-(1→4)-Gal*p*[b]	0.0	3.0	3.3
Main-chain xylose			
Terminal	1.7	2.7	1.6
Unsubstituted	85.1	62.5	73.7
Substituted by above chains on C-3 and C-2	13.2	34.8	24.7
Ratios and values			
Ratio of side-chains *a*–*e* to substituted D-xylose	1.00	1.00	1.02
Ratio of unsubstituted to substituted residues in main chain	6.6	1.8	3.0

[a]Figures give comparative molar ratios of features, assuming each main chain to have 100 xylose residues and discounting the xylose residues to which uronic acid residues are directly attached. [b]UA is D-glucuronic acid or its 4-methyl derivative, or both.

TABLE II

MOLAR PROPORTIONS OF STRUCTURAL FEATURES IN BAMBOO HETEROXYLANS SUBJECTED TO PROGRESSIVE HYDROLYSIS WITH 0.05M SULPHURIC ACID AT 45°, FOLLOWED BY EXAMINATION OF METHANOLYSATES OF THE METHYLATED, PARTIALLY DEGRADED XYLANS[a]

Structural features and related data	*Period of hydrolysis (h)*								
	0	*17.5*	*41*	*66*	*89*	*112*	*137*	*162*	*190*
Side-chains									
a L-Ara*f*	12.2	9.6	6.7	4.6	3.5	2.7	2.2	1.8	1.8
b Gal*p*-(1→5)-L-Ara*f*	2.4	2.3	2.0	1.9	1.8	1.7	1.6	1.5	1.5
c Gal*p*-(1→4)-D-Xyl*p*-(1→2)-L-Ara*f*	1.3	1.2	1.2	1.0	1.0	0.9	0.8	0.8	0.8
d D-Xyl*p*-(1→2)-L-Ara*f*-(1→2)-L-Ara*f*	5.8	5.7	5.5	5.5	5.3	5.3	5.3	5.3	5.3
e UA*p*-(1→4)-D-Xyl*p*-(1→4)-Gal*p*[b]	3.3	3.3	3.5	3.6	3.7	3.7	3.7	3.7	3.7
Main-chain xylose									
Terminal	1.6	1.7	1.7	1.6	1.7	1.8	1.9	1.9	1.9
Unsubstituted	73.7	76.2	79.0	81.3	82.6	83.5	84.0	84.4	84.7
Substituted by above chains on C-2	5.3	4.9	4.5	4.2	4.0	4.0	3.9	3.7	3.7
Substituted by above chains on C-3	19.4	17.2	14.7	12.8	11.6	10.7	10.2	10.0	9.7
Ratios and values									
Ratio of side-chains *a*–*e* to substituted D-xylose	1.01	1.00	0.98	0.98	0.98	0.97	0.96	0.96	0.98
Ratio of unsubstituted to substituted residues in main chain	3.1	3.5	4.2	4.9	5.4	5.8	6.1	6.3	6.5

[a]Figures give comparative molar ratios of features, assuming each chain to have 100 xylose residues. The values at 0 h are equated by use of factors with the molar values in Table VI, and the other values determined have been altered by using the same factors. [b]UA is D-glucuronic acid or its 4-methyl derivative, or both.

structural features are correctly deduced. As the hydrolysis was mild, it is reasonable to assume that there was no significant hydrolytic loss of the directly attached uronosylxylosylgalactosyl side-chains. The xylan isolated after 162 h yielded a proportion of 3-*O*-methylxylose equimolar to the acidic trisaccharide chain originally present. It is concluded that these side chains are indeed stable under the acidic conditions used, and that they are attached to C-2 positions of the xylan. The ratios of methyl 2-*O*-methylxylosides to side chains calculated to be present in all the methanolysates show that the arabinosyl and xylosylarabinosylarabinosyl residues are attached to C-3 positions. Even after 190 h, a few arabinose residues remain attached to the xylan backbone. Some, but not all, of these may derive from xylosylarabinosylarabinosyl side-chains by the hydrolytic release of *O*-D-xylosyl-(1→2)-L-arabinose, as already described (Fig. 1). The chains directly attached by arabinosyl residues are obviously

much more stable than are the single arabinofuranosyl residues. The relative molar proportions of galactosylarabinosyl and of galactosylxylosylarabinosyl side-chains in the starting heteroxylan material are shown in Table II. The subsequent values of these are based on the molar proportion of methyl 2,3,4,6-tetra-*O*-methylgalactosides in each methanolysate, assuming that the original ratio of the disaccharide *a* to trisaccharide *c* chains is maintained during the hydrolysis. Initially, there is an excess of 2-branched xylose residues over that required by the acidic trisaccharide side-chain referred to above. The molar value of this excess indicates that the galactosylarabinosyl residues may account for the excess and be attached to C-2 positions. If this is so, the rate of hydrolytic release of galactosylarabinose will be more rapid than shown in Table II, while the rate of hydrolysis of xylosylarabinosylarabinosyl chains will be lower. Such alterations only alter other values to a negligible extent. The parent xylan contained 5.8% of uronic acid, of which the acidic trisaccharide side-chains account for 2.9%, so that, at most, only 2.9% of the uronic acid residues are directly attached to the main xylan chain. Some of the uronic acid determined by decarboxylation may be galacturonic acid not present in xylan. Glycosiduronic (1→2)-linkages of the type well known in methylated xylans are resistant to methanolysis, but methylated aldobiouronic acids were not sought in methanolysates. In Table VI, the molar ratios are calculated on the basis of a value of 100 for 2,3-di-*O*-methylxylose. The d.p. of the heteroxylan is initially ~93. During the period of hydrolysis, only 47% of the arabinose is removed. A marked discontinuity was also noted in the acidic release of arabinose from an oat xylan[14].

The proportions of the neutral sugars in hydrolysates of *S*, *I*, and *X* materials are shown in Table III for comparison with the proportions represented by the materials recovered after methylation. The *S*, *I*, and *X* materials were subjected successively to periodate oxidation for 28 days at 5°, reduction with borohydride, acid hydrolysis, and further reduction. The resultant glycitols were fully acetylated and the products determined by g.l.c. (Table VII). The values in Tables III and VII relate to the parent *S*, *X*, and *I* materials, the structural features of which are shown in Table II. These last values relate to the methylated materials, but during *any* methylation there may be selective losses. If there should be such losses, the structures established may not relate exactly to the structures under investigation; this problem is rarely commented on. The two sets of quantitative results obtained by acid hydrolysis and by the periodate-oxidation studies relate to the parent *S*, *X*, or *I* material. In the present studies, it was possible to deduce the structures of the *parent* hemicellulosic materials, assuming that the features present in them were all present in the methylated products. From the two sets of quantitative results by solution of numerous equations based on structural features, it was found that a very limited range of values was obtained. There was indeed little latitude in selecting "best fits"; the values are shown in Table IV. It is clear that the values for the material recovered after methylation fit well with the values for the materials subjected to methylation. The relative proportions of arabinosyl and xylosylarabinosylarabinosyl chains do not change, although the proportion of these chains on each xylan does change slightly.

TABLE III

PROPORTIONS OF NEUTRAL SUGARS IN HYDROLYSATES OF HEMICELLULOSIC MATERIALS FROM BAMBOO LEAF, AND THE CORRESPONDING PROPORTIONS OF THESE SUGARS IN THE PRODUCTS RECOVERED AFTER METHYLATION

Monosaccharide residues (xylose = 100)	*Insoluble hemicellulosic material*		*Soluble hemicellulosic material*		*Heteroxylan and associated hemicelluloses*		*Heteroxylan*
	Original	*Methylated*	*Original*	*Methylated*	*Original*	*Methylated*	*Methylated*
Arabinose	14.8	14.3	38.4	38.7	27.4	26.8	24.8
Galactose	2.2	2.4	14.8	8.6	11.6	7.8	7.7
Glucose	6.2	5.4	15.0	13.0	3.8	4.6	0

TABLE IV

CALCULATED MOLAR PROPORTIONS OF THE STRUCTURAL FEATURES DEDUCED PRESENT IN BAMBOO-LEAF HEMICELLULOSIC MATERIALS SUBJECTED TO METHYLATION, COMPARED TO THE PROPORTIONS, IN PARENTHESIS, OF THESE FEATURES IN THE MATERIALS RECOVERED AFTER METHYLATION

Structural features and related data	*Insoluble total hemicellulose*		*Soluble total hemicellulose*		*Heteroxylans and associated hemicelluloses*	
Side-chains in xylan						
a L-Ara*f*	9.6	(9.6)	22.0	(22.0)	12.2	(12.2)
b Gal*p*-(1→5)-L-Ara*f*	−0.4	(1.5)	10.8	(2.9)	4.5	(2.4)
c Gal*p*-(1→4)-D-Xyl*p*-(1→2)-L-Ara*f*	0.1	(0.9)	1.9	(1.4)	2.4	(1.3)
d D-Xyl*p*-(1→2)-L-Ara*f*-(1→2)-L-Ara*f*	1.0	(1.0)	5.4	(5.4)	5.8	(5.8)
e D-UA*p*-(1→4)-D-Xyl*p*-(1→4)-Gal*p*[b]	0.0	(0.0)	1.6	(3.0)	3.2	(3.3)
Main-chain xylose						
Substituted xylose	10.3[a]	(13.2)	41.7[a]	(34.8)	28.1[a]	(24.7)
Unsubstituted xylose	86.8	(86.8)	74.1	(65.2)	73.8	(75.3)
Ratio of unsubstituted to substituted xylose residues	8.1	(6.6)	1.8	(1.8)	2.6	(3.0)
Other structural features						
3,6-Linked Gal	—	(0.0)	22.5	(3.7)	26.7	(2.1)
3-Linked Gal		(trace)		(2.5)		(1.9)
3-Linked L-Ara*f*	3.7	(0.4)	3.2	(5.3)	3.2	(2.1)

[a]The sum of the values for the xylan side-chains above. [b]UA is D-glucuronic acid or its 4-methyl derivative, or both.

TABLE V

MOLAR PROPORTIONS OF METHYLATED SUGARS IN HYDROLYSATES OF A METHYLATED HETEROXYLAN AND OF METHYLATED WATER-SOLUBLE AND WATER-INSOLUBLE FRACTIONS FROM THE TOTAL HEMICELLULOSE OF LEAVES OF *Arundinaria japonica*

Methylated sugar in hydrolysate[a]	*Insoluble total hemicellulose*	*Soluble total hemicellulose*	*Heteroxylan and associated material*
2,3,4-Tri-*O*-methylrhamnose	0.0	3.3	0.0
2,3,5-Tri-*O*-methyl-L-arabinose	11.2	32.9	15.5
2,3,4-Tri-*O*-methyl-D-xylose	3.2	12.2	9.4
3,5-Di-*O*-methyl-L-arabinose	3.5	18.3	16.5
2,5-Di-*O*-methyl-L-arabinose	0.5	8.0	2.7
2,3-Di-*O*-methyl-L-arabinose	1.8	4.4	3.0
2,3-Di-*O*-methyl-D-xylose	100.0	100.0	100.0
2,3,4,6-Tetra-*O*-methylgalactose	2.9	6.5	4.7
2,4,6-Tri-*O*-methylgalactose	trace	13.2	2.0
2-*O*-, and 3-*O*- Methyl-D-xyloses	15.3	52.0	31.3
2,3,6-Tri-*O*-methylgalactose	0.0	4.5	4.2
2,4,6-Tri-*O*-methyl-D-glucose	1.1	5.7	4.5
2,3,6-Tri-*O*-methyl-D-glucose	5.3	15.7	2.0
2,4-Di-*O*-methylgalactose	0.0	5.5	2.7
2,6-Di-*O*-methylglucose[b,56]	0.0	3.5	2.8

[a]Estimated by peak-area measurement in g.l.c. of derived glycitol acetates. All values corrected for flame-ionisation detector response. [b]Identity not proven.

TABLE VI

MOLAR PROPORTIONS OF METHYL GLYCOSIDES OBTAINED ON METHYLATION AND METHANOLYSIS OF BAMBOO-LEAF HETEROXYLAN AND PARTIALLY DEGRADED HETEROXYLANS OBTAINED FROM IT BY HYDROLYSIS WITH 0.05M SULPHURIC ACID AT 45°

Parent methylated sugar[a]	*Period of hydrolysis (h)*								
	0	*17.5*	*41*	*66*	*89*	*112*	*137*	*162*	*190*
2,3,4-Tri-*O*-methyl-D-xylose	9.4	9.1	8.7	8.3	8.1	8.1	8.1	8.1	8.1
2,3,5-Tri-*O*-methyl-L-arabinose	15.5	11.9	8.0	5.4	4.0	3.1	2.5	2.0	2.0
2,3,4,6-Tetra-*O*-methylgalactose	4.7	4.3	3.8	3.4	3.2	2.9	2.7	2.6	2.6
2-*O*-Methyl-D-xylose	24.6	21.3	17.6	14.9	13.3	12.2	11.5	11.2	10.9
3-*O*-Methyl-D-xylose	6.7	6.1	5.4	4.9	4.6	4.5	4.4	4.2	4.2
3,5-Di-*O*-methyl-L-arabinose	16.5	15.5	14.7	14.0	13.2	13.0	13.0	13.0	13.0

[a]2,3-Di-*O*-methyl-D-xylose = 100.

TABLE VII

MOLAR PROPORTIONS OF GLYCITOLS[a] OBTAINED ON TREATMENT OF BAMBOO-LEAF HEMICELLULOSIC MATERIALS SUCCESSIVELY BY PERIODATE OXIDATION, REDUCTION WITH BORCHYDRIDE, ACID HYDROLYSIS, AND FURTHER REDUCTION

Hemicellulosic material	*Glycerol*	*Threitol*	*Arabinitol*	*Xylitol*	*Galactitol*	*Erythritol*	*Glucitol*	*Rhamnitol*
Soluble hemicellulosic material (*S*)	251.4	4.0	39.1	100.0	12.0	24.3	9.8	0.4
Insoluble hemicellulosic material (*I*)	326.7	11.1	51.1	100.0	11.1	17.8	5.6	trace
Heteroxylan and associated material (*X*)	695.0	6.5	29.0	100.0	4.5	35.5	8.0	4.0

[a]Determined by g.l.c. of the acetates.

It appears that the two neutral side-chains that contain galactose residues are markedly diminished during methylation of both the *S* and *X* materials. The "fit" for these two chains is unsatisfactory in the case of the *I* material. This point will be discussed shortly. There is a markedly higher proportion of arabinosyl to xylosylarabinosyl-arabinosyl in the *I* material than in the others, and the *I* material is much less substituted. Were one to seek to isolate a pure xylan having a low proportion of arabinose residues, it is probable that it would also contain an even lower proportion of these trisaccharide side-chains and they would be difficult to detect. There may be no uronosyl residues directly attached to the xylan backbones of the *S* and *X* heteroxylan molecules. Such an absence would accord with the 5.8% of uronic acid noted in the *X* material, of which half is in uronosylxylosylgalactosyl residues; the remainder may be in galacturonan or elsewhere. The *S* material appears to be similar to the *X* material. In the *I* material, the unsatisfactory values for the galactosylarabinosyl and galactosylxylosylarabinosyl residues, compared to those in the methylated materials, could be due to xylitol derived from the *X*-material xylose residues to which an unknown proportion of uronosyl residues are directly attached. Such acidic arabinoxylans are, of course, amongst those most commonly studied. There is little in terms of purity to distinguish the *S* and *I* materials from the *X* material which, although a respectably pure heteroxylan, is contaminated by heterolinked β-D-glucan and other polysaccharide material. The structural features relate to average molecules. One must consider whether average molecules exist or whether, as seems more probable to the authors, there are various xylans that lack, or possess, certain of the side chains discussed. The complexity of the anatomy of any plant leaf and the variety of cells therein suggest that certain structural features in the molecules may be in particular types, or parts, of the various cells. Hemicelluloses may have unique structures obscured by the homogeniser on the first day of most work!

EXPERIMENTAL

Details of the plantstuffs, of the isolation of the *T*, *S*, and *I* materials, and of routine methods have been given[33]. G.l.c. was performed on a Perkin–Elmer F11 apparatus (flame-ionisation detector) fitted to a Hitachi chart-recorder. The phases used were *A*, 3% of silicone E301; *B*, 3% of OV 225; and *C*, 3% of OV17, each on AW-DMCS Chromosorb (100–120 mesh) supports. G.l.c.–m.s. was performed on a Pye 104 gas chromatograph linked to an AEI MS30 mass spectrometer, with g.l.c. on phase *B* at 170°. P.c. irrigants were *A*, ethyl acetate–pyridine–water (10:4:3); and *B*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4).

Hemicellulosic materials were methylated as previously described[48,49], and components identified by g.l.c. on phase *A* at 150° as derived methyl glycosides, and as derived acetylated glycitols by g.l.c. and by g.l.c.–m.s. on phase *B* at 170°[33]. A component having the same retention time as 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucose[57] was derived on reduction of the components in the hydrolysates of the methylated *S* and *X* materials. This component alone was not examined by g.l.c.–m.s.

Isolation of the acidic arabinogalactoxylan. — *S* material (15 g) was dissolved in 3% sodium hydroxide (1.5 l), and freshly prepared Fehling's solution was added dropwise until no further precipitation took place[53]. The gelatinous precipitate was washed with water (2 × 1 litre) and then dispersed an iced water (450 ml), and M hydrochloric acid added. The complex gave a clear solution which yielded a precipitate on addition to ethanol (1.8 l). The precipitate was dissolved in water (375 ml); after slight acidification with hydrochloric acid, the solution was added to ethanol (1.5 l), the precipitate recovered, and the dissolution and precipitation procedure repeated twice. The resulting white precipitate was dissolved in water, and after dialysis for 3 days, the non-dialysable solute (*X* material) was recovered by freeze-drying. When ammonium sulphate (4 g) was added to a 10% solution (20 ml) of *X* material, no precipitate formed. Addition of more sulphate (2 g) gave a precipitate that was recovered and dissolved in water; the solution was dialysed and the non-dialysable solute recovered by freeze-drying.

Examination of the acidic arabinogalactoxylan. — The xylan had $[\alpha]_D^{26}$ −111° (*c* 1.83, 2.5M sodium hydroxide). It formed a complex on treatment with iodine in aqueous calcium chloride[54]. A sample was hydrolysed with acid, and the hydrolysate examined by electrophoresis in 0.1M magnesium acetate and 0.1M calcium acetate. There were four acidic components, which were indistinguishable from glucuronic and galacturonic acids, *O*-(D-glucopyranosyluronic acid)-(1→2)-D-xylose and *O*-(D-glucopyranosyluronic acid)-(1→2)-*O*-D-xylopyranosyl-(1→4)-D-xylose. The xylan had 5.8% of uronic anhydride. A sample (5 mg) of *X* in water (0.5 ml) was dyed by adding Procion MG-S dye (5 mg) in water (0.5 ml), followed, after 5 min, by sodium chloride (10 mg) and, after 30 min, by sodium hydrogen carbonate (5 mg)[55]. After 4 h, the mixture was desalted on a column of Sephadex G-10, and the dyed material was examined in a Millipore Phoroslide microelectrophoresis cell on microporous cellulose acetate film at 14 $V.cm^{-1}$ The coloured zone migrated, but remained

discrete. The Procion-dyed *X* material was subjected to electrophoresis on Whatman No. 1 paper in a Miles Hivolt electrophoresis apparatus with 0.05M sodium borate at 11 $V.cm^{-1}$, and again the material migrated as a discrete zone. The undyed *X*-material was also subjected to electrophoresis on Whatman glass-fibre paper in 0.05M sodium borate at 15 $V.cm^{-1}$, and the polysaccharide detected with naphthol and sulphuric acid. Under these conditions, the *X* material and the Procion-dyed material both travelled as distinct and discrete zones. A 1.5% solution of the xylan was examined in a Beckmann Model E analytical ultracentrifuge at 22° and 56,000 r.p.m. The Schlieren photographs all displayed single, symmetrical peaks.

Partial hydrolysis of the S *and* X *materials.* — A sample of *S* material (1 g) was heated at 100° with 0.05M sulphuric acid (50 ml); a preliminary experiment had shown that these conditions led to the maximum yield of oligosaccharides. The cooled solution was neutralised with barium carbonate, and barium ions were removed from the solution with Zeo-Karb 325 (H^+) resin. The components in the partial hydrolysates were indistinguishable from those obtained in the same way from *X* material. The acidic and neutral components in the partial hydrolysate of the *S* material were separated on a column (30 × 2.5 cm) of Deacidite FF-IP (acetate form) irrigated with 30% acetic acid (1 litre). There were at least eleven neutral components, and ten of these were studied. P.c. (irrigants *A* and *B*) showed that all ten were homogeneous. Reductions were carried out with aqueous sodium borohydride, the excess was decomposed with Zeo-Karb FF-IP (H^+) resin, and borate was removed by the successive addition of methanol and volatilisation of methyl borate. *Components 1, 2, and 3* were indistinguishable (p.c.) from xylose, arabinose, and galactose, respectively; the derived trimethylsilyl ethers and glycitol acetates of these components had the same retention times on g.l.c. (column *A* for Me_3Si derivatives, and column *B* for glycitol acetates) as the corresponding derivatives of the sugars named. *Component 4* was homogeneous in p.c. [R_{XYL} 0.73 (*A*) and 0.41 (*B*)]. After reduction and hydrolysis, 4 gave equimolar amounts of two compounds indistinguishable (p.c.) from arabinitol and xylose. Examination of the derived glycitol acetates by g.l.c. (column *B*) confirmed these identities; the two glycitol acetates were almost equimolar. Component 4 did not react on treatment with alkaline 2,3,5-triphenyltetrazolium chloride, and its Me_3Si derivatives gave peaks with T_{SUC} 0.67, 0.71, and 0.93 in g.l.c. (column *C*). *Component 5* was indistinguishable from 4-*O*-β-D-xylopyranosyl-D-xylose (p.c., irrigants *A* and *B*) and its Me_3Si derivatives gave two peaks on g.l.c. (column *C*, T_{SUC} 1.11 and 1.17). On reduction and hydrolysis, component 4 gave xylose and xylitol (p.c.). The acetylated products were obtained in equimolar ratios and were indistinguishable in g.l.c. (column *B*) from those obtained from xylose and xylitol. Methylation of component 4 (Hakomori method[48,49]) gave a product that showed no i.r. absorption attributable to hydroxyl groups. The methylated product was hydrolysed at 100° for 16 h with 25mM sulphuric acid, and the products were reduced and acetylated to give glycitol derivatives that were indistinguishable in g.l.c. (column *B*) from 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-D-xylitol. They were in nearly equimolar proportions. *Component 6* had

R_{XYL} 0.53 and 0.26 (p.c., irrigants *A* and *B*), and, on reduction, hydrolysis, and acetylation, gave two compounds indistinguishable by g.l.c. (column *B*) from those obtained from D-galactose and L-arabinitol. Component 6 gave a formazan derivative with alkaline 2,3,5-triphenyltetrazolium chloride. *Component 7* had R_{XYL} 0.45 and 0.20 in the above irrigants. After reduction and hydrolysis, a sample gave equimolar amounts of galactose and xylitol identified by p.c. (irrigants *A* and *B*). On acetylation, the hydrolysis products gave derivatives indistinguishable by g.l.c. (column *B*) from those given by the named sugars. Component 7 gave a positive reaction with alkaline 2,3,5-triphenyltetrazolium chloride. *Component 8* was indistinguishable from cellobiose in p.c. (irrigants *A* and *B*), and gave only glucose on hydrolysis. After reduction, hydrolysis, and acetylation, derivatives indistinguishable by g.l.c. (column *B*) from those given by D-glucose and D-glucitol were obtained in equimolar proportions. *Component 9* was homogeneous in p.c. (irrigants *A* and *B*, R_{XYL} 0.40 and 0.16), and, on hydrolysis, gave galactose, xylose, and arabinose (p.c., irrigant *A*) in equimolar proportions. A reduced sample of component 9 gave xylose, galactose, and arabinitol in equimolar proportions on acid hydrolysis. *Component 10* was homogeneous in p.c. (irrigants *A* and *B*, R_{XYL} 0.35 and 0.11), and was indistinguishable from xylotriose. On hydrolysis, a reduced sample gave xylose and xylitol in the molar ratio of 2:1.

The acidic components were contaminated with some neutral monosaccharides and were further purified by electrophoresis at 40 $V.cm^{-1}$ for 1.5 h in 0.1M magnesium acetate (pH 7.3). The components that migrated more than 1 cm were extracted with water, and the extract was treated with Zeo-Karb 325 (H^+) resin, concentrated, and examined by p.c. (irrigants *A* and *B*). Arabinose, galactose, glucose, and xylose remained as contaminants, but there was too little material to permit further fractionation. The acidic components were indistinguishable (p.c., irrigant *B*) from glucuronic acid, galacturonic acid, *O*-(D-glucopyranosyluronic acid)-(1→2)-D-xylose and *O*-(D-glucopyranosyluronic acid)-(1→2)-*O*-D-xylopyranosyl-(1→4)-D-xylose. The components in a sample of the concentrate were converted into their methyl ester methyl glycosides and reduced. The products were treated at 100° for 18 h with 0.1M sulphuric acid, and the hydrolysate was neutralised with barium carbonate and then treated with Zeo-Karb 325 (H^+) resin. Glucose, galactose, 4-*O*-methylglucose, and xylose were concluded to be present on examination of derived glycitol acetates by g.l.c.

Progressive, acidic stripping of the heteroxylan. — A sample (100 mg) of the acidic heteroxylan was dissolved in 0.05M sulphuric acid (50 ml) and kept at 45°. Erythritol (40 mg) was added to the solution as a reference standard. Aliquots were withdrawn at intervals and neutralised with barium carbonate, and centrifugates were treated with Zeo-Karb (H^+) and then taken to dryness. Pyridine (0.2 ml), hexamethyldisilazane (0.2 ml), and chlorotrimethylsilane (0.2 ml) were added. After 40 min, the solutions were taken to dryness, and the material soluble in hexane was examined by g.l.c. (column *A*, 150°). After 200 h, the ratio of the Me_3Si derivatives of arabinose to that of erythritol was constant. Another sample (300 mg) of the heteroxylan was hydrolysed in acid (100 ml), as described, and aliquots (10 ml) were

withdrawn at intervals. After the addition of water (10 ml), the solutions were dialysed for 3 days against frequently changed distilled water. The combined, neutralised diffusates were found by p.c. (irrigants *A* and *B*) to contain arabinose, galactose, xylose, and other components which were not examined. The non-diffusible solutes were recovered by freeze-drying. A sample (2 mg) of each was hydrolysed, and the products were converted into glycitol acetates, and analysed by g.l.c. on column *B* at 170° (Table VIII). A sample of each of the above partially hydrolysed heteroxylans was methylated, as described, and the methanolysates were examined by g.l.c. on column *A* at 150° (Table VI).

TABLE VIII

MOLAR PROPORTIONS[a] OF GLYCITOL ACETATES DERIVED FROM SUGARS IN HYDROLYSATES OF A BAMBOO-LEAF HETEROXYLAN AND OF PARTIALLY DEGRADED HETEROXYLANS OBTAINED FROM IT ON HYDROLYSIS WITH 0.05M SULPHURIC ACID AT 45°

Parent sugar	*Period of hydrolysis (h)*								
	0	*17.5*	*41*	*66*	*89*	*112*	*137*	*162*	*190*
Arabinose	27.5	21.7	17.8	16.7	13.3	10.6	9.1	9.0	8.9
Galactose	11.6	7.6	8.2	7.9	—	6.1	6.2	6.7	6.7

[a]Xylose = 100. Glucose and a trace of rhamnose were present in all hydrolysates.

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