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NON-CELLULOSIC β-D-GLUCANS FROM BAMBOO, AND INTERPRETATIVE PROBLEMS IN THE STUDY OF ALL HEMICELLULOSES*†

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ABSTRACT

D-Glucans from (1→3)- and (1→4)-β-D-linked glucans are present in the leaves and stems of the bamboos *Arundinaria japonica* and *A. anceps*. The ratios of the glucosidic linkages in the total hemicelluloses from leaves and stems from *A. japonica* and from the young and old leaves and young and old nodes of *A. anceps* were 1:2.5, 8.0, 2.6, 2.4, 19.5, and 15.6, respectively. It is suggested that the quantitative values obtained in studies of all hemicelluloses are subject to vagaries when it is implied that the results of methylation analyses are related directly to the material subjected to methylation. It is also suggested that, where interpretation is possible, quantitative values are more significant when total hemicelluloses, rather than fractionated hemicelluloses, are studied.

INTRODUCTION

The non-cellulosic polysaccharides, the hemicelluloses, of the non-endospermic tissues of grasses are principally composed of heteroxylan molecules of various degrees of complexity[1–3]. Low proportions of D-glucose residues have long been known to be extracted under the conditions that lead to the isolation of hemicelluloses. Until recently, the molecular location of these residues was a matter either of conjecture[4–6] or disregard, but it is now clear that they derive mainly, and in the case of barley[7], oats[8–11], maize[12], and wheat[13,14], apparently exclusively, from non-cellulosic D-homoglucans having (1→3)- and (1→4)-β-glucosidic links. Such glucans are also present in rye[14] and guinea grasses[15]. Similar, endospermic, cereal glucans[3] are well-known, and one such glucan is localized in the thin, primary, endospermic cell-walls of wheat[16]. There is clear evidence that there is a decrease in the ratio of (1→3) to (1→4) linkages in non-endospermic glucans as leaves, stems, and other organs age[17,18]. Possibly, these glucans play a role in cell-wall elongation or in some other form of cell-wall development[19,20]. The change in the proportion of the two

*Dedicated to the memory of Professor Edward J. Bourne.
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linkages may be due either to an alteration in the nature of molecules after deposition, or to an alteration in the nature of molecules later biosynthesized. β-D-Glucans have been synthesized by cell-free preparations from cultures of cells of *Lolium multiflorium* endosperm[21]; it was found that the proportion of (1→3) and (1→4) linkages was dependent on the concentration of UDPG.

Barley, oats, wheat, and rye are species of the *Festucoideae*, and maize and guinea grass of the *Panicoideae*, subfamilies of the Gramineae. Amongst the other subfamilies, there is one, the *Bambusoideae* (the bamboos), that includes grasses that are markedly different from those in all the other subfamilies. Bamboos are commonly massive, and their stems, unlike those of other grasses, are distinctly wood-like and perennial, and the deciduous leaves survive more than a year.

RESULTS AND DISCUSSION

The hemicelluloses have been isolated from the delignified[22] leaf and stem tissues of the bamboos *Arundinaria japonica* and *A. anceps* of well-established colonies cultivated outdoors in a temperate climate. The hemicelluloses of *A. japonica* have now been examined and the structural features of the various xylans in mature leaves established. In addition to xylans, both bamboos have β-D-glucans similar in the nature of their linkages to those mentioned above. The leaves of *A. japonica*, on delignification[22], yielded a holocellulose which corresponded to 66% of the dried plantstuff. Some hemicellulose was lost by dissolution when oat tissues were delignified[23]. By analogy, it is reasonable to assume that some loss will have taken place in the case of bamboo also. The study on oats showed that the low percentage of material lost did not seriously upset the determination of the amount of glucan nor would it be expected to upset the ratio of (1→3) to (1→4) linkages in the hemicelluloses isolated[23], compared to that in the bamboo.

After alkaline extraction, 41% of the holocellulose was recovered as hemicellulose and 43% as α-cellulose. The total hemicellulose (*T*) accounted for 26% of the dried plantstuff. The *T*-material was separated into water-soluble (*S*, 61.5% of *T*) and water-insoluble (*I*, 34% of *T*) materials. Samples of the various materials were hydrolysed and the neutral sugars converted into glycitol acetates. Quantitative values determined by g.l.c. were corrected to take account of the molar responses of the flame-ionisation detector to each glycitol acetate; the response of each acetate had been determined by Black[13] under the conditions employed. On hydrolysis, all the materials also yielded uronic acid components and traces of rhamnose. As will be noted shortly, a homoglucan was concluded to be present; hence, the distinction in Table I. Attempts to fractionate *S* to isolate a homoglucan were unsuccessful, although fractions enriched in glucose residues were isolated by use, separately, of Cetavlon, ethanol, and ammonium sulphate. A gelatinous precipitate, typical of xylans, separated on the addition of Fehling's solution to *S* in 3% sodium hydroxide, and a homoglucan was isolated from the supernatant solution by the graded addition of ethanol (Fig. 1). The glucan accounted for ~2.6% of *S*, *i.e.*, for 36% of the glucose

residues in *T*. It had ν_{max} at 895 cm^{-1}, compatible with the presence of β-linked glucopyranosyl residues, and as it failed to give any colour with iodine, there was no evidence of the presence of starch. It had $[\alpha]_D$ −6.02°, which is a value similar to the values reported for β-D-glucans from other grasses.

TABLE I

MOLAR PERCENTAGES OF NEUTRAL SUGARS[a] IN HYDROLYSATES OF HEMICELLULOSIC MATERIALS FROM LEAVES OF *Arundinaria japonica*

Hemicellulosic material	*Xylose*	*Arabinose*	*Galactose*	*Glucose*
Total hemicellulose (*T*)	74.5	20.1	5.4	5.0
Water-soluble *T* (*S*)	65.0	25.3	9.7	9.7
Water-insoluble *T* (*I*)	85.4	12.7	1.9	5.4

[a]Xylose + arabinose + galactose = 100.

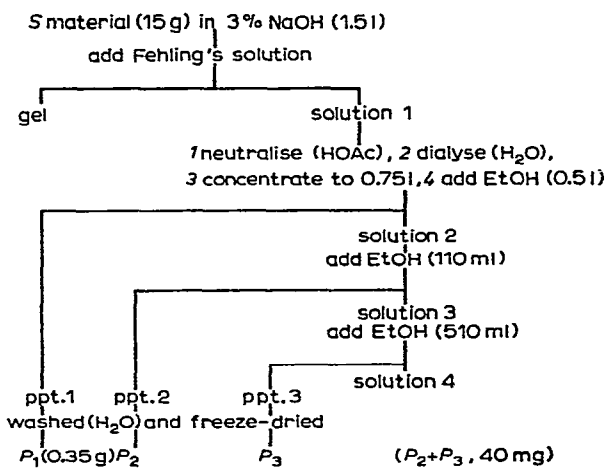

Fig. 1. Isolation of a glucan from the leaves of the bamboo *Arundinaria japonica*.

The glucan was partially hydrolysed with 0.05M oxalic acid at 100°. The products obtained were indistinguishable by p.c. from D-glucose, cellobiose, cellotriose, laminaribiose, and 3-*O*-β-cellobiosyl-D-glucose. The presence of the last compound is compatible with (1→3)- and (1→4)-β-D links in heterolinked glucans. There was no evidence for the presence of oligosaccharides derived from, and indicative of the presence of, starch.

The glucan was fully methylated by a modification of the method of Hakomori[24,25] and the product, on methanolysis, yielded the methyl D-glucosides of the 2,3,4,6-tetra-, 2,3,6-tri-, and 2,4,6-tri-methyl ethers, identified by g.l.c. The derived, acetylated glycitols of the methylated glucan were determined by g.l.c. Assuming, as is reasonable, that the molar responses of the flame-ionisation detector are the same for 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol and for 1,4,5-tri-*O*-

acetyl-2,3,6-tri-*O*-methyl-D-glucitol, then the ratio of (1→3) to (1→4) linkages in the methylated material was 1:2.03 and, extending the assumption a little less certainly, to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, the d.p. was ~56. During the methylation of the glucan, 57% of the material was not accounted for by the product recovered. Such losses are typical of those encountered by other workers on all types of polysaccharide. By contrast, Smith-degradation studies avoid dangers of losses and, provided they yield results that are structurally and quantitatively interpretable, such studies are more reliable. The glucan was accordingly subjected to Smith degradation and the derived glycitol acetates were determined by g.l.c. The ratio of (1→3) to (1→4) linkages was 1:2.2. During the methylation of the glucan, there had evidently been a selective loss of material relatively slightly richer in (1→4) linkages. The glucan derived *from S* accounted for 36% of the glucose residues, and so was not necessarily representative of that *in S*. In concurrent studies on the non-glucan hemicelluloses in *S*, it was concluded that there was no evidence for the presence of glucose residues in the various xylans. Methylation studies on these xylans indicated that they contained no residues that would yield erythritol on Smith degradation. Accordingly, Smith-degradation studies were carried out on *S*, and the ratio of glucitol and erythritol was determined. These studies showed that the glucan *in S* had the above linkages in the ratio of 1:2.5. During the isolation of the glucan from *S*, molecules richer in (1→4) linkages had again been lost. Methylation studies on *S* yielded a product accounting for only ~14% of the material subjected to methylation G.l.c. of the two derived tri-*O*-acetyl-tri-*O*-methyl-D-glucitols referred to earlier indicated the linkages to be in the ratio 1:2.8. In this case, there was an increase in the ratio of the (1→4) linkages in the material recovered after methylation.

It is concluded that in this, as in methylation analyses by other workers, care should be taken to avoid attaching what may be an unwarranted degree of significance to quantitative results insofar as these refer to the material subjected to methylation rather than to that recovered after it. Similarly, caution must be exercised when interpreting analyses of hemicellulosic materials derived from material that may have undergone intentional or inadvertent fractionation involving selective losses. Only when methylated or fractionated materials account for most of the original material are dangers of misinterpretation minimised. This point should be borne in mind when assessing literature data or planning work. The emphasis in the past has largely been on the study of hemicellulosic fractions. Such studies are invaluable in establishing the types of molecules present, but it is contended that studies of compositional changes, for example during maturation, cannot easily be carried out on fractionated material unless allied to studies of unfractionated material.

Enzymic studies on *S* supported the view expressed above that the glucose residues were apparently all in heterolinked β-D-glucans. The *S* material was treated with an enzyme preparation from *Cytophaga* which, under the conditions used, had been demonstrated to hydrolyse the glucan isolated but to have no detectable action on cellulose, cellulose oligosaccharides, laminaribiose, cellobiose, cellotriose, or cellotetraose, or on either oat[17] or bamboo-leaf galactoarabinoxylans. It hydrolysed

laminarin and oat and bamboo-leaf glucans, and so evidently possessed marked (1→3)-β-D-glucanohydrolase activity. It has been shown that (1→3)-β-D-glucanohydrolase may act on a substrate having a sequence of adjacent (1→3)-linked residues[26]. The product (S_E) of enzymically treated *S* gave a much lower proportion of glucose on hydrolysis than did untreated *S*. The resistant glucose residues in S_E were in molecules that were still so large that they did not diffuse through a dialysis membrane. It seems probable that the resistant parts of glucans in S_E did not have the distribution of linkages needed for them to act as substrates. Methylation analysis of S_E material revealed very little change in the proportion of the methylated non-glucose residues, but there was a decrease in the proportion of the total of 2,3,6-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose. The fact that methylation analysis of *S* and S_E materials gave quantitatively and qualitatively similar results for the non-glucosidic residues indicates that the non-glucan material had not been significantly attacked by the enzyme; had it been, a variation in the results would be expected because of differences in the types of selective loss during methylation of modified and unmodified xylans. The S_E glucan, as represented by the methylated material, was still heterolinked and had (1→3)- and (1→4)-glucosidic linkages in the ratio of 1:2.8.

By Smith-degradation studies, the ratios of (1→3) to (1→4) linkages in the *T* and *I* leaf-materials were found to be 1:3.3 and 1:4.4, respectively; the methylated glucan derived from *I* had these linkages in the ratio of 1:5.0. The *S* and *I* materials together account for ~95% of the *T* material, and the calculated ratio of the linkages in the *T* material, from that in the *S* and *T* materials, is 1:3.2, which accords well with the determined value of 1:3.3.

The woody stems of *A. japonica* were delignified, and yielded a holocellulose accounting for 70% of the dried plantstuff, 20% of which was extractable hemicellulose (*t*). The water-soluble (*s*) and water-insoluble (*i*) materials were separated; from the former, a glucan was obtained by procedures analagous to those already described. The neutral sugars in Table II were determined as before; acidic sugars and traces of rhamnose were also present.

TABLE II

MOLAR PERCENTAGES OF NEUTRAL SUGARS[a] IN HYDROLYSATES OF HEMICELLULOSIC MATERIALS FROM STEMS OF *Arundinaria japonica*

Hemicellulosic material	*Xylose*	*Arabinose*	*Galactose*	*Glucose*
Total hemicellulose (*t*)	88.4	9.3	2.3	9.3
Water-soluble *t* (*s*)	64.6	26.7	8.6	27.1
Water-insoluble *t* (*i*)	89.6	6.0	4.4	5.2

[a]Xylose + arabinose + galactose = 100.

The glucan isolated from the stem had an unusually low value (−34°) of $[\alpha]_D$. It had ν_{max} at 895 cm^{-1} and gave no colour with iodine. The glucan from the

leaf was soluble in 2.5M sodium hydroxide, but that from the stem was incompletely soluble in 5M, but totally soluble in 10M, sodium hydroxide. Smith degradation of the glucan revealed the ratio of (1→3) and (1→4) linkages to be 1:7.7. The glucan in *t* was similarly found to have these linkages in the ratio of 1:8.0. Such high values have not been found for the glucans in other grasses, but it should be noted that the bamboos are not annuals and the stems are accordingly older than tissues hitherto studied. The trend is as expected.

Fresh plants of the bamboo *A. anceps* were separated into old-leaf, new-leaf, old-node, and new-node materials, and the total hemicelluloses (T) extracted. The T materials accounted for 31, 32, 28.5, and 17.7%, respectively, of the dry weights of the above plantstuffs, and the glucans *in* each corresponded to 6.5, 6.8, 8.9, and 9.9% of the weight of the respective materials. The ratio of the residues in each T material was determined by g.l.c. of the derived glycitol acetates (Table III). The ratios of (1→3) and (1→4) linkages for each of the glucans *in* each of the materials, determined by the Smith-degradation procedure, were 1:2.4, 2.6, 15.6, and 19.5, respectively. Once more, the stem glucans are seen to be very different from those in the annual grasses.

TABLE III

MOLAR PROPORTIONS OF NEUTRAL SUGARS[a] IN HYDROLYSATES OF TOTAL HEMICELLULOSES FROM THE LEAVES AND NODES OF *Arundinaria anceps*

Source of total hemicellulose	*Xylose*	*Arabinose*	*Galactose*	*Glucose*
New leaves	75	18.2	7.2	8.5
Old leaves	66	25.8	8.6	8.9
New nodes	90	8.0	2.4	12.1
Old nodes	91	7.0	1.9	13.6

[a]Xylose + arabinose + galactose = 100.

EXPERIMENTAL

Solvents were removed either by rotary evaporation below 40° or by freeze-drying. Hydrolyses and methanolyses were effected in sealed tubes at 100°. A Perkin–Elmer 237 i.r. spectrophotometer and a 143 NPL Bendix automatic polarimeter were used. 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 OV-225; and *B*, 10% of polyphenyl ether [*m*-bis(*m*-phenoxyphenoxy)benzene]; each on AW-DMCS Chromosorb (100–200 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 *A* at 170°. The irrigants for p.c. were *A*, ethyl acetate–pyridine–water (10:4:3); and *B*, butan-1-ol–pyridine–benzene–water (5:3:1:3); sugars were detected with alkaline silver nitrate[27].

The molar proportions of neutral sugars in polysaccharides were determined by hydrolysing samples (1–10 mg) with 0.5M sulphuric acid (~3 ml) for 12–18 h,

neutralising the cold hydrolysates, and reducing the sugars in water (2 ml) with sodium borohydride (10 mg) for at least 12 h. The excess of borohydride was destroyed by acetic acid, and the borate removed by successive codistillations of the methyl borate produced by intermediate additions of methanol. The glycitols were treated with acetic anhydride (2 ml) at 110–117° for 3.5–4 h and the excess of anhydride was hydrolysed by water (3 ml). The solutions were extracted with chloroform, and the chloroform-soluble material was examined by g.l.c. at 170° on phase *A*. The molar responses of the flame-ionisation detector to the acetates of arabinitol, xylitol, glucitol, galactitol, threitol, erythritol, and glycerol were determined by Black[13]. The values reported throughout have been appropriately adjusted; Black had used the same apparatus and conditions.

Methylated hemicelluloses and glucans (3–10 mg) were methanolysed with 3 or 4% anhydrous methanolic hydrogen chloride (~0.5 ml) for 14–16 h. The cold solutions were immediately examined by g.l.c. on phase *A* at 125° and on phase *B* at 160°. Methylated polysaccharides (~3 mg) were partially hydrolysed with 90% formic acid (2 ml) at 100° for 1 h, and then hydrolysis was completed by heating with 0.25M sulphuric acid (3 ml) for ~15 h. After neutralisation with barium carbonate, the solutions were treated with Zeo-Karb 325 (H^+) resin, and the solvent was removed. The sugars were reduced and acetylated, and the products were examined by g.l.c. against standards and by g.l.c.–m.s.

Smith-degradation analyses involved dissolving, or suspending and shaking, each polysaccharide (10 mg) with 0.05M sodium metaperiodate (10 ml) in the dark at 5° for 21 days (glucan) or 28 days (*S*, *I*, and *T* materials). Each mixture or solution was then dialysed against running water for 4 days, sodium borohydride (20 mg) was added, and the excess of reductant was removed as before. The polyalcohols were hydrolysed with 3M trifluoroacetic acid for 1 day. Each hydrolysate was then taken to dryness, the components were dissolved in water (5 ml), and sodium borohydride (10 mg) was added. After 16 h, the excess of reductant was decomposed as before and the glycitol acetates were prepared. After removal of borate, dichloromethane solutions were examined by g.l.c. (phase *A* at 120°).

The plantstuffs and holocelluloses. — The bamboos *Arundinaria japonica* and *A. anceps* were collected in September 1971 in the Royal Botanic Gardens, Edinburgh. The leaves of the former were then mature and the stem was ~1 cm in diameter. The *A. anceps* was separated into old (previous years') and new (1971) leaves, and into old and new nodes. The various plantstuffs were treated with boiling benzene and ethanol (2:1), and the soluble materials were discarded. The dried plantstuffs, after reduction to fibrous powders using a Cassella mill, were delignified at 70° by the method of Wise[22], and the resulting holocelluloses were washed with water and acetone, and then air-dried.

Isolation of the total hemicellulose from Arundinaria japonica *leaves.* — The holocellulose (80 g) was extracted under nitrogen with 5%, and then with 24%, potassium hydroxide (2.4 l of each) and the residue was thoroughly washed with water. The solutions and washings were combined, and neutralised with acetic acid[28].

A residue (35 g), the α-cellulose, remained. Most of the water was removed from the hemicellulosic solution, and ethanol (9 l) was added. The flocculs that formed were washed thoroughly with ethanol (4 × 3 l) and separated by centrifugation. The precipitate was suspended in water and freeze-dried to give a brown material, the total hemicellulose[28] (*T*, 32.5 g). A sample (32 g) of *T* was stirred vigorously with water (2.5 l) in a homogenizer for 2 h, and the indissoluble material was recovered by centrifugation, washed with water (2 × 1 l), dispersed in water, and freeze-dried to give fraction *I* (11 g). The centrifugate and washings were combined, dialysed (3 days), and freeze-dried to give *S* (20 g).

Attempted isolation of a glucan from the S *material.* — No fractionation was achieved either by polyacrylamide-gel electrophoresis with a buffer of Tris (52 g), acetic acid (14 ml), and water (1 l), or by use of columns of DEAE-cellulose (in the acetate form[2]) or porous-glass beads (CPG-10), each irrigated with water. A marked, but inadequate, enrichment of glucose residues was observed in the precipitate that formed on adding ethanol[29] slowly (to a concentration of 40%) to a solution of *S*; at higher concentrations of ethanol, the precipitates had lower proportions of glucose residues. Borate buffers were prepared from 25mM borax (*A* and *B*, 50 ml) and 0.1M hydrochloric acid (*A*, 20.5 ml; *B*, 18.3 ml) each diluted to 100 ml. Addition of 10% Cetavlon (30 ml) and borate buffer *A* (pH 8; 30 ml) to *S* (600 mg) in water (30 ml)[30] gave a precipitate impoverished in glucose residues. After its removal, addition of borate buffer *B* (pH 10; 90 ml) gave a further precipitate, the water-soluble part of which was enriched in glucose residues.

Attempted fractionation of *S* using ammonium sulphate[31,32] gave a fraction having xylose, arabinose, galactose, and glucose in the corrected molar ratios of 2.0, 0.5, trace, to 10, respectively (*cf.* Table I).

Isolation of a homoglucan from the S *material.* — Fehling's solution[33] was added dropwise to *S* material (2.5 g) in 3% sodium hydroxide (250 ml) until no further gel formed (the gel formed immediately). The gel was removed by centrifugation, and the supernatant liquor was neutralised with acetic acid and dialysed against water for 4 days. The dialysate was taken to 100 ml, ethanol (66 ml) was added, and 12 h later the flocculent precipitate was separated by centrifugation. A suspension of the product in water was freeze-dried, and the material thereafter soluble in water was separated from that which was insoluble. The latter material yielded only glucose on hydrolysis, whereas the former gave the above four sugars. The procedure outlined in Fig. 1 was used to isolate an adequate amount of homoglucan. The P_1 material yielded only glucose on acid hydrolysis. The combined P_2 and P_3 materials gave glucose and a trace of xylose. The P_1 material, which is referred to as the glucan, had $[\alpha]_D^{26}$ −6.02° (*c* 0.5, 2.5M sodium hydroxide). It did not give any colour with iodine.

Partial, acid hydrolysis of the leaf glucan. — A sample (10 mg) of the glucan was heated with 25mM oxalic acid (3 ml) at 100° for 18 h. The cold hydrolysate was neutralised with barium carbonate, and a centrifugate was examined by p.c. against standards. The components were indistinguishable from those named earlier.

Methylation of the leaf glucan[24,25]. — The methylsulphinyl anion was prepared under nitrogen from oil-free sodium hydride (0.7 g) and methyl sulphoxide (20 ml). Dried glucan (70 mg) was dissolved at 50–60° in methyl sulphoxide (20 ml) under nitrogen and, after cooling, the solution of methylsulphinyl anion was added. Methyl iodide (1.5 ml) was then added dropwise below 25°. After 6 h, the reaction mixture was added to water (300 ml), and the resulting suspension was dialysed against water and then extracted in a Soxhlet apparatus with chloroform. Addition of light petroleum (b.p. 60–80°) to the concentrated chloroform solution gave a precipitate that was then redissolved in chloroform. After filtration, the solvent was removed to give a product (35 mg) that had no i.r. absorption in the hydroxyl region.

Examination of the methylated, leaf glucan. — A solution of the components from a hydrolysate of the methylated glucan in dichloromethane was demethylated by boron trichloride. P.c. (irrigant *A*) showed the presence of glucose only. Glycitol acetates derived from the methylated glucan were examined by g.l.c. (phase *A*, 170°). Four components were noted with retention times of 1.00, 1.66, 1.8, and 2.23, and relative peak areas of 1.00, 3.5, 18.2, and 37.0. The second component was not identified, but the other three were indistinguishable from the acetates of 2,3,4,6-tetra-, 2,4,6-tri-, and 2,3,6-tri-*O*-methyl-D-glucitols. G.l.c.–m.s. confirmed the identity of the last two components. Methanolysates of the methylated glucan yielded components having retention times of 1.00, 1.36, 1.72, 2.27, and 2.38 on phase *A* at 170°, and 1.00, 1.54, 2.3, 2.51, and 3.51 on phase *B* at 125°. These values accord with those expected for the methyl glucosides of 2,3,4,6-tetra-, 2,4,6-tri-, and 2,3,6-trimethyl ethers.

Periodate-oxidation studies of leaf glucan. — A sample (10 mg) of the glucan was subjected to Smith-degradation studies. The corrected[13] molar ratio of glucitol hexa-acetate to erythritol tetra-acetate was 1:2.2.

The glucans in leaf hemicellulosic materials. — The *S* and *I* materials were methylated as described above, and the identity of the methyl 2,3,6- and 2,4,6-tri-*O*-methyl-D-glucosides were confirmed by g.l.c. and g.l.c.–m.s. The glucose derivatives in hydrolysates of the methylated *S* and *I* materials were determined as their glycitol acetates. The components indicated that the ratios of (1→3) to (1→4) linkages in the *S* and *I* materials were 1:2.8 and 1:5.0, respectively. Smith degradation of the *T*, *S*, and *I* materials showed the corrected ratios of (1→3) to (1→4) glucosidic linkages to be 1 to 3.3, 2.5, and 4.4, respectively.

The optimal pH conditions were established using *S* material to determine the (1→3)-β-D-glucanohydrolase activity of an enzyme preparation[17] from *Cytopagha* using phosphate–citrate buffers at 37°. The Nelson–Somogyi method[34,35] was used (absorbance at 520 nm) to monitor the reducing sugars released after 24 h. The optimal pH was at 5.6; at this pH, enzymic action on *S* material was complete after 170 h. A sample of the *S* material was hydrolysed under these conditions and, after inactivation of the enzymes, the mixture was centrifuged, and the centrifugate dialysed and concentrated. S_E material was precipitated by the addition of ethanol under conditions similar to those leading to the original precipitation of *S* material.

Hydrolysis and methylation studies showed a much lower proportion of the residues of glucose or of its derivatives in the S_E, than in the S, material. Smith-degradation studies of the S_E material showed that the ratio of (1→3) to (1→4) glucosidic linkages was 1:2.9.

The hemicelluloses from the stem of A. japonica. — A glucan was isolated from the holocellulose of stem material as described above. The gel formed very slowly after the addition of Fehling's solution. The glucan had $[\alpha]_D^{24}$ −34° (*c* 0.15, 10M sodium hydroxide). There was no colour with iodine. After methylation by the Hakomori method[24,25], the product showed no i.r. absorption for hydroxyl groups. A methanolysate of the methylated glucan contained the same methylated glucose derivatives as for the leaf glucan. Smith-degradation analysis showed the molar ratio of glucitol hexa-acetate to erythritol tetra-acetate to be 1:7.6; similar studies on the stem total-hemicellulosic material gave a ratio of 1:8.0. There was too little glucan for further studies.

The glucans in the leaves and nodes of A. anceps. — The total hemicelluloses were isolated, as described above, from new and old leaves and nodes of *A. anceps*. The molar ratios of the sugar residues were determined (Table III), and Smith-degradation studies carried out.

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