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A COMPARISON OF THE XYLANS FROM CORN LEAVES AND STALKS

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Abstract—The alkali soluble hemicelluloses of corn leaf and corn stalk have been shown to be arabinoglucuronoxylans of similar structure. Each has a xylan backbone with units joined 1–4 in the β-D-configuration. The degree of polymerization is about 55 for the leaf xylan and 70 for the stalk xylan. The structures were determined by methylation and periodate oxidation using gas liquid chromatography for separations and quantitative estimations and mass spectrometry for identifications.

INTRODUCTION

STUDIES on the biosynthesis of cellulose and hemicelluloses require plants which grow easily and rapidly under laboratory conditions. Such a plant is sweet corn (*Zea mays*) and is one of those being used by Professor F. Barnoud at Grenoble in his researches. In order to facilitate comparison of the hemicelluloses of mature plants with those of seedlings 7–10 cm high the principal xylans obtained from the stalks and the leaves of mature *Zea mays* (var. Golden Bantam) have been separately examined.

Many studies have been made of the xylans from corn cobs,[1,2] corn fibre[3,4] and corn hulls.[5,6] The nature of corn stalk hemicelluloses has been reported[7–9] but the leaves do not appear to have been examined. In the case of seedlings the leaves represent a high proportion of the plant material and in the context of the current investigations it was of interest to know whether the leaf xylan was significantly different from that in the stalk. The stalk has therefore been re-examined so that the xylans of the stalk and leaves of one variety, as determined by the same investigator, may be compared.

RESULTS AND DISCUSSION

Corn plants harvested in September 1966 were dried at about 40° in a current of air, separated into leaves and stalks and individually ground. Following preliminary extractions to remove lipids, water soluble and pectic substances the plant materials were delignified by treatment with sodium chlorite.[10] The delignified materials were extracted successively

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[2] R. L. WHISTLER and G. E. LAUTERBACH, *J. Am. Chem. Soc.* **80**, 1987 (1958).
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[6] H. C. SRIVASTAVA and F. SMITH, *J. Am. Chem. Soc.* **79**, 892 (1957).
[7] H. D. WEIHE and M. PHILIPS, *J. Agric. Res.* **64**, 401 (1942).
[8] E. BENNETT, *Arch. Biochem.* **27**, 99 (1950).
[9] R. E. GRAMERA and R. L. WHISTLER, *Arch. Biochem. Biophys.* **101**, 75 (1963).
[10] L. E. WISE, M. MURPHY and A. A. D'ADDIECO, *Paper Trade J.* **122**(2), 35 (1946).

TABLE 1. EXTRACTION OF CORN POLYSACCHARIDES

Fraction	Method	Yield* (g) L§	Yield* (g) S§	Uronic acid† (%) L	Uronic acid† (%) S	Rotation‡ L	Rotation‡ S	Methoxyl (%) L	Methoxyl (%) S
A	0·2 N KOH (H^+)	0·29‖	—	11·7	—	−51·2	—	0·65	—
B	(EtOH)	0·54	30·5	16·6	15·0	−47·9	−58·5	0·85	1·1
C	1·0 N KOH (H^+)	7·81	—	10·5	—	−49·9	—	0·47	—
D	(EtOH)	10·89	32·0	11·4	13·8	−64·1	−59·7	0·89	0·42
E	2·5 N KOH (H^+)	0·94	1·5	6·7	8·1	−45·7	−70·2	0·42	0·52
F	(EtOH)	9·54	21·0	8·8	10·9	−57·7	−64·3	0·54	0·92

* On ash-free basis. † Gravimetric estimation of CO_2.[37] ‡ $[\alpha]_D^{22}$ in 1 N NaOH. § L = leaves, S = stalk. ‖ Contained 1·6% N.

with 0·2, 1·0 and 2·5 N potassium hydroxide solutions. Each extract was acidified, any precipitate removed (conventionally hemicellulose A) and the filtrate poured into excess alcohol. Data on these fractions are given in Tables 1 and 2. Samples of most of these fractions were hydrolysed and the neutral sugars shown to consist mainly of xylose and arabinose although small amounts of glucose were also encountered, especially in those fractions obtained by alcohol precipitation. The acidic portion was identified as 2-*O*-(α-D-glucuronosyl)-D-xylose with a small amount of the corresponding 4-*O*-methyl-D-glucuronic acid derivative.

It is clear from Table 3 that many of the fractions contained a contaminating glucan. Attempts to remove this by successive reprecipitation into ethanol were unsuccessful (Table 2). In certain cases fractionation of polysaccharide mixtures may be achieved by gradual addition of ethanol and removal of the precipitates formed. Using this method Gramera and Whistler[9] claimed to have isolated from the hemicelluloses of corn stalk an acidic xylan free from glucan. The effectiveness of such separations may be increased by carrying out the precipitation in the presence of cations. Calcium ion has been successfully used in this connection.[11] Attempts were therefore made to fractionate both the leaf and the stalk xylans in the presence and absence of calcium ion. The results are recorded in Table 2

TABLE 2. FRACTIONATION OF CORN POLYSACCHARIDES

Fraction	Leaf: Ethanol added (%)	Leaf: Hemicellulose Pptd (%) With Ca^{2+}	Leaf: Hemicellulose Pptd (%) Without Ca^{2+}	Stalk: Ethanol added (%)	Stalk: Hemicellulose Pptd (%) With Ca^{2+}	Stalk: Hemicellulose Pptd (%) Without Ca^{2+}
1	28	12	16	30	3	4
2	37	50	36	37	49	50
3	45	5	4	42	7	7
4	50	16	18	46	16	13
5	55	6	12	50	4	8
6	65	6	4	53	13	9
7				60	3	1

[11] O. SMIDSROD and A. HAUG, *J. Polymer. Sci.* **16C**, 1587 (1967).

TABLE 3. ANALYSIS OF CORN POLYSACCHARIDES (mole %)

Fraction*	Arabinose L	Arabinose S	Xylose L	Xylose S	Glucose L	Glucose S
A	—	—	—	—	—	—
B	25·0	14·1	72·5	81·9	2·5	4·0
C	15·3	—	84·7	—	—	—
D	17·6	16·4	75·7	78·6	6·7	5·2
E	19·6	12·8	80·4	82·6	—	4·6
F	13·8	15·7	79·5	80·2	6·7	4·1

* As Table 1.

and it is clear that the addition of calcium has no significant effect. Paper chromatographic examination of hydrolyzates of each fraction revealed the presence of glucose in all cases.

Subsequent structural investigations were carried out on fraction D (Table 1) for both the corn leaf and corn stalk xylans. The overall structure of the xylans was shown by examination of the methylated polysaccharides. Methylation was accomplished in one step using the procedure of Hakomori[12] and the products fractionated by sequential extraction with chloroform–light petroleum (Table 4).

It is worth noting that the Hakomori method of methylation has now been applied to a wide variety of polysaccharides and there is only one specific instance recording failure with a xylan.[13] Experience suggests that success with this methylation technique, particularly for acidic polysaccharides, is largely dependent on using a thoroughly deionized sample and on allowing sufficient time for complete reaction between the polysaccharide and the methylsulphinyl anion before the addition of methyl iodide.

The methylated polysaccharides were hydrolysed and neutral and acidic sugars separated on ion exchange columns. The neutral sugars from corn leaf xylan were examined by paper chromatography and showed five components. The mixture was separated on a cellulose–hydrocellulose column and individual sugars were identified as 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose. A portion of the neutral hydrolyzate was converted to a mixture of alditol

TABLE 4. FRACTIONATION OF METHYLATED POLYSACCHARIDES

Fraction	Chloroform: petrol (v/v)	Weight (mg) L	Weight (mg) S	$[\alpha]_D^{22}$ ($CHCl_3$) L	$[\alpha]_D^{22}$ ($CHCl_3$) S	Methoxyl (%) L	Methoxyl (%) S
1	0:100	—	2·4	—	—	—	—
2	10:90	51·5	38·1	—	—	—	—
3	15:85	75·0	59·5	−52·6	−36·7	15·8	24·0
4	20:80	130·8	51·0	−51·2	−53·9	31·4	32·5
5	25:75	1745·0	892	−63·6	−57·8	40·0	39·5
6	30:70	105·4	91·3	−41·8	−68·8	37·3	37·6

[12] S. HAKOMORI, *J. Biochem.*, **55**, 205 (1964).
[13] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2053 (1969).

acetates which was separated by GLC[14] and the identity of the peaks confirmed by mass spectroscopy.[15] All the alditol acetates were clearly resolved except the two mono-*O*-methylxyloses which gave only one peak. When a second portion of the neutral hydrolyzate was trimethylsilylated and examined by GLC all components, including the mono-*O*-methylxyloses, were resolved. These trimethylsilyl (TMS) derivatives were also identified by mass spectroscopy.[16] The quantitative data are summarized in Table 5. The neutral sugars from methylated corn stalk xylan were similarly separated and identified as their alditol acetates and as their silyl derivatives with the results shown in Table 5.

TABLE 5. QUANTITATIVE ANALYSIS OF METHYLATED SUGARS

Compound	Wt., mg	Leaf xylan Mole ratio*	Mole ratio†	Stalk xylan Mole ratio†
2,3,5-Tri-*O*-methyl-L-arabinose	59·9	4·5	3·5	4
2,3,4-Tri-*O*-methyl-D-xylose	13·2	1·0	1	1
2,3-Di-*O*-methyl-D-xylose	491·9	40	40	55
3-*O*-Methyl-D-xylose	34·7	3	3	3·5
2-*O*-Methyl-D-xylose	48·3	4·6	3	5
2-*O*-(2,3,4-Tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xylose	109·0	6	—	7·6*

* Mole ratio from weights isolated.
† Mole ratio from GLC.

Other workers have reported that 2- and 3-*O*-methylxylose cannot be separated as their alditol acetates[17–19] and Sephton has studied the separation of methylated xyloses as their TMS derivatives.[20] Timell[18] has compared the results of separating methylxyloses as their methyl glycosides, as the TMS derivatives of the methyl glycosides and as the TMS derivatives of the free sugars. It is thus clear that these are all complementary methods some or all of which may be necessary in a particular situation. Lindberg has reported a model study on the identification of methylpentoses by the mass spectra of their alditol acetates[15] and Samuelson has similarly discussed their identification by studying the fragmentation pattern of their TMS derivatives.[16] The xylans from eucalypt and birch[21] as well as that from red cotton wood[22] have been examined using a combination of GC–MS techniques and thus the present investigation represents a further application of these methods to structural studies.

The acidic fraction from each methylated xylan was reduced and hydrolysed to give 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose thus showing the aldobiouronic acid to be 2-*O*-(2,3,4-tri-*O*-methyl-D-glucuronosyl)-3-*O*-methyl-D-xylose.

[14] H. BJORNDAL, B. LINDBERG and S. SVENSSON, *Acta Chem. Scand.* **21**, 1801 (1967).
[15] H. BJORNDAL, B. LINDBERG and S. SVENSSON, *Carbohydrate Res.* **5**, 433 (1967).
[16] G. PETERSSON and O. SAMUELSON, *Svensk Papperstidn.* **71**, 77 (1968).
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[19] J.-F. BOUHOURS and M. V. CHESHIRE, *Soil Biol. Biochem.* **1**, 185 (1969).
[20] H. H. SEPHTON, *J. Org. Chem.* **29**, 3415 (1964).
[21] M. HAN and B. SWAN, *Svensk Papperstidn.* **71**, 552 (1968).
[22] P. J. GAREGG and M. HAN, *Svensk Papperstidn.* **71**, 331 (1968).

The methylation results were confirmed by periodate oxidation of the xylans and hydrolysis of the polyalcohols obtained on borohydride reduction. Corn leaf xylan consumed 0·77 moles periodate per anhydropentose unit in 96 hr and yielded ethylene glycol, glycerol, erythritol and xylose in the ratio 1:34:8:18 as estimated by GLC of the trimethylsilyl derivatives. The corresponding figures for corn stalk xylan were a consumption of 0·66 moles of periodate in 144 hr and a ratio of 1:48·5:5·4:16·8. Since in each case no glucose derivatives were found in either of the hydrolyzates of the methylated xylans the small proportion of erythritol was assumed to arise from a contaminating glucan which could not be removed in the initial purification (Table 3). These periodate figures are in reasonable agreement with the methylation results.

It may thus be seen that the methylation and periodate data establish the close similarity of the xylans isolated from corn leaf and corn stalk. Each is an arabinoglucuronoxylan in which the xylose units are linked 1 → 4 in the β-D-configuration. There is on average one uronic acid for each nine xylose units with a slightly smaller amount of arabinose. The principal difference which emerges is the higher degree of polymerization of the stalk xylan which may well reflect the more mature nature of these cells. Although only one of each of the twelve fractions (Table 1) has been examined it is likely that the others differ principally in the proportion of arabinose and uronic acid rather than in basic structure.

EXPERIMENTAL

Paper chromatography was carried out by the descending technique using Whatman Nos. 1 and 3 MM papers with detection by *p*-anisidine trichloroacetate[23] and using the following solvent systems: A. butanone–H_2O azeotrope; B. EtOAc–HOAc–HCOOH–H_2O (18:3:1:4). All evaporations were carried out under reduced pressure at a bath temp. not exceeding 40°. The m.ps reported are uncorrected and the specific rotations quoted are equilibrium values. GLC was carried out on a F and M 720 dual column instrument fitted with thermal conductivity detectors. He was used as the carrier gas and peak areas were determined by a digital integrator (Infotronic model CRS 100). The MS were recorded on an A.E.I. M.S.9 mass spectrometer at an ionizing potential of 70 eV. Full experimental details together with tracings of the GLC results and summaries of the mass spectral fragmentations are available.[24]

Zea mays (var. Golden Bantam) grown during the summer of 1966 was harvested in the fall and air-dried at about 40°. Leaves and stalks were separated, ground and extracted with hot EtOH–benzene (1:2) in a Soxhlet apparatus. Dried leaves (396 g) were extracted with H_2O at room temp. for 96 hr and then 4× with 1·5 l. 2% EDTA, adjusted to pH 6·8, at 70° for 2 hr. The solvent and H_2O extracts were discarded and the EDTA extract, after concentration and precipitation into EtOH, gave 1·09 g of material which was not further examined. The residual plant material (100 g) was delignified by suspension in 3 l. H_2O at 75° and 3 additions of $NaClO_2$ (30 g) and HOAc (10 ml) at 1 hr intervals.

Delignified material was extracted successively with 0·2 N (3·2 l)., 1·0 N (2·0 l.) and 2·5 N (1·0 l.) KOH. Each extract was acidified with HOAc to about pH 4·2, precipitates removed and further precipitation obtained by pouring the filtrate into EtOH. Data are presented in Table 1. Corn stalks (460 g) were similarly treated and gave the fractions also shown in Table 1. Attempts to remove the contaminating glucan by reprecipitation into EtOH were not successful. Fractional precipitation with EtOH in the presence or absence of Ca^{2+} gave similar fractionation curves and the principal fractions (Table 2) still contained glucose and had a uronic acid content similar to the original material.

Samples of all fractions (Table 1 and 2, *ca.* 50 mg) were hydrolysed with N H_2SO_4 (20 ml) in sealed tubes for 8 hr at 100°. Neutralization with $BaCO_3$ followed by passage through Amberlite IR 120 and Duolite A 4 columns gave the neutral sugars and elution of the latter column with 10% HCOOH gave the acidic components.

The neutral sugars were estimated by GLC as their trimethylsilyl derivatives (TMS) using a column (2·4 m × 6 mm) of 20% SF 96 on 60–80 mesh Diatoport S.[25] The column was held at 190° for 3 min after injection and then programmed at 3°/min to hold at 230°. The injection port was 270°, the detector block was 295° and the He flow 88 ml/min. The results for the fractions of Table 1 are given in Table 3.

In all further studies fraction D of both corn leaf and corn stalk (Table 1) was used. A larger sample of

[23] L. HOUGH, J. K. N. JONES and W. H. WADMAN, *J. Chem. Soc.* 1702 (1950).
[24] M. S. KABIR, M.Sc. Thesis, University of British Columbia (1970).
[25] G. G. S. DUTTON, K. B. GIBNEY, G. D. JENSEN and P. E. REID, *J. Chromatog.* **36**, 152 (1968).

fraction D (leaf) was hydrolysed and the acidic components isolated. Chromatography for 16 hr in solvent B showed spots corresponding to aldobiouronic acids (R_{xylose} 1·00 and 0·56), traces of free uronic acids (R_x 1·20, 0·65) and a higher oligouronide R_x 0·19). The component with R_x 0·56 was the most prominent. The acidic mixture (20 mg) was refluxed with 4% HCl in MeOH and the ester glycoside (24 mg) was reduced with $LiAlH_4$ in THF by refluxing for 4 hr. Hydrolysis with N H_2SO_4 and examination on paper in solvent B showed xylose, glucose and traces of 4-*O*-methylglycose.

Methylation

Each xylan was methylated by the method of Hakomori[12] following the detailed procedure of Sandford and Conrad.[26] Leaf xylan was passed through a 200 mesh sieve and dried for 6 hr at 60° under reduced pressure. A part (1·80 g) was added to 150 ml DMSO and the suspension stirred at 60° until all the polysaccharide was in solution (about 1 hr). Methylsulphinyl anion was prepared by washing 5 g of NaH (50% oil dispersion) several times with light petroleum (30–60°) and reacting the hydride at 50° under N_2 for 2 hr with DMSO (50 ml). Each solution was cooled to room temp. and on addition of the anion solution to the polysaccharide solution a gel formed but after stirring at room temp. for a further 6 hr the solution was judged to be homogeneous. MeI (15 ml) was added over 30 min to the stirred solution so that the temperature did not exceed 20°. Within a few minutes of starting the addition the solution cleared and the viscosity markedly decreased. After stirring for 6 hr, addition of H_2O precipitated the product and the suspension was dialysed overnight against running H_2O. Continuous $CHCl_3$ extraction of the remaining solution gave 2·11 g of product showing no OH band in the IR. Similarly, 1·00 g corn stalk xylan gave 1·23 g of product. Each methylated product was fractionally extracted with $CHCl_3$–light petroleum (30–60°) as shown in Table 4.

Fraction 5 (1·01 g) of the methylated leaf xylan was dissolved in cold H_2SO_4 (72%, 10 ml) and, after 1 hr, H_2O was added to reduce the acid concentration to 8% when the solution was boiled for 4 hr. After addition of $BaCO_3$ the neutral sugars (741 mg) were separated using Amberlite IR 120 and Duolite A4 and elution of the latter with 10% HCOOH gave the acidic components (109 mg).

Examination of the neutral sugars on paper in solvent A showed five components with R_f values 0·80, 0·78, 0·53, 0·23 and 0·12. The neutral sugars were separated on a cellulose-hydrocellulose column using solvent A with the following results.

Component 1. The sirup (73·1 mg), $[\alpha]_D^{22}$ −28·2° (*c* 1·5, MeOH) showed two overlapping components by paper chromatography, having R_f values of 0·80 and 0·78 respectively, consistent with the behaviour of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose. The rotations of these compounds are −38·5° and +18·5° respectively[27] which showed this fraction to be 82% tri-*O*-methyl-L-arabinose (59·9 mg) and 18% tri-*o*-methyl-D-xylose (13·2 mg). A portion of the sirup (30·1 mg) was converted to the methyl glycosides by refluxing with 3% MeOH–HCl and examined by GLC on a column of 5% butanediol succinate at 120°. Three peaks were observed and samples corresponding to each peak were collected. The contents of the first tube crystallized and had m.p. 48–50°. The reported value for methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside is 49–50°.[28] The second peak was due to a mixture of arabinosides and xylosides. The third peak was identified as an anomer of methyl 2,3,5-tri-*O*-methylarabinoside by mass spectrometry[29] and by hydrolysis to the free sugar identified by paper chromatography.

Component 2. This was shown to be chromatographically pure, R_f 0·53, and to have $[\alpha]_D^{22}$ +21·4° (*c* 1·0, H_2O). It was identified as 2,3-di-*O*-methyl-D-xylose by preparation of the *N*-phenylglycosylamine m.p 124–126° (from EtOAc–light petroleum). Lit.[30] 125–126°.

Component 3. This was obtained in small amount (15·5 mg), had R_f 0·40 and was not further examined.

Component 4. This fraction, R_f 0·23, had $[\alpha]_D^{22}$ +27·5° (*c* 1·5, H_2O) and was judged to contain 56% 2-*O*-methyl-D-xylose and 44% 3-*O*-methyl-D-xylose which have rotations of +35·9° and +17·0° respectively.[31–33]

Component 5. This sirup (12·1 mg) was chromatographically indistinguishable from xylose, R_f 0·12. Fraction 5 of the methylated corn stalk xylan (0·85 g) was similarly hydrolysed and examination by paper chromatography showed the same pattern as for the leaf xylan. The stalk polysaccharide was therefore not subjected to a column separation but only to the GLC procedures given below and the results are recorded in Table 5.

Gas–liquid chromatography of methylated neutral sugars. Partially methylated alditol acetates. Methylated neutral monosaccharides (20 mg) from each xylan were reduced in H_2O (100 ml) with $NaBH_4$ (400 mg)

[26] P. A. SANDFORD and H. E. CONRAD, *Biochem.* **5**, 1508 (1966).
[27] R. MONTGOMERY and F. SMITH, *J. Am. Chem. Soc.* **77**, 3325 (1955).
[28] O. WINTERSTEINER and A. KLINSBERG, *J. Am. Chem. Soc.* **71**, 939 (1949).
[29] N. K. KOCHETKOV and O. S. CHIZHOV, *Advan. Carbohydrate Chem.* **21**, 39 (1966).
[30] G. G. MAHLER, *Advan. in Carbohydrate Chem.* **10**, 257 (1955).
[31] G. J. ROBERTSON and T. H. SPEEDIE, *J. Chem. Soc.* 824 (1934).
[32] R. J. MCILROY, *J. Chem. Soc.* 100 (1946).
[33] P. A. LEVENE and A. L. RAYMOND, *J. Biol. Chem.* **102**, 331 (1933).

for 12 hr. After passage through Amberlite IR 120 and distillation with MeOH the product was acetylated by reaction for 1 hr at 100° with Ac_2O–pyridine (1:1, 40 ml). The mixture was diluted with H_2O, evaporated to dryness and dissolved in $CHCl_3$. A column (2·4 m × 6 mm.) containing 3% ECNSS-M on Gas Chrom Q (100–120 mesh)* was used. The column was held at 160° for 3 min after injection and then programmed at 2°/min to hold at 180°. Four peaks were obtained which by comparison with standards, were identified as corresponding to the alditol acetates derived from 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, all of which were clearly resolved, and unresolved monomethylxyloses. The assignment of the first three compounds was confirmed by mass spectrometry.[15]

Trimethylsilyl derivatives of methylated aldopentoses. Methylated neutral sugars (20 mg) from each xylan were converted to their TMS derivatives which were separated on a column (2·4 m × 6 mm) containing 8% SE 52 on 60–80 mesh Diatoport S.† The column was held isothermally at 110° for 3 min after injection and then programmed at 3°/min to hold at 140°. Five major peaks were obtained corresponding to the *O*-trimethylsilyl ethers of 2,3,5-tri-*O*-methylarabinose and 2,3,4-tri-*O*-methylxylose (incompletely resolved), 2,3-di-*O*-methylxylose, 3-*O*-methylxylose and 2-*O*-methylxylose (incompletely resolved but clearly distinguished). In the case of the monomethylxylose derivatives a small shoulder was observed with a shorter retention time than the 3-*O*-methylxylose derivative and a similar shoulder with a longer retention time than the 2-*O*-methylxylose derivative. These were attributed to the alternative anomeric forms. The nature of each component was established by comparison with standards, by peak enhancement and by mass spectrometry.[16]

Identification of the methylated aldobiouronic acid. The acidic fraction (109 mg) from the leaf xylan had $[\alpha]_D^{22}$ +86·5° (*c* 1·0, H_2O) and was converted to the ester glycoside, reduced with $LiAlH_4$ in THF, hydrolysed with 1 N H_2SO_4 and the sirup (95 mg) resolved into two components (R_f 0·51 and 0·19) by paper chromatography in solvent A.

Component 1. The sirup (38 mg) with R_f 0·51 had $[\alpha]_D^{23}$ +78·5° (*c* 1·0, H_2O) and was converted to the methyl glycosides separation of which by GLC on a column of 5% BDS at 120° gave methyl 2,3,4-tri-*O*-methyl-β-D-glucoside which crystallized spontaneously, m.p. 92–94°. Lit.[34] 93–94°. The mass spectrum was consistent with this assignment.[29]

Component 2. The sirup (29 mg) with R_f 0·19 was chromatographically identical to 3-*O*-methylxylose and crystallized on standing m.p. 91–93°. Lit.[35] 95–96°.

The methylated stalk xylan (0·85 g) similarly gave 100 mg of acidic component which after reduction and hydrolysis showed by paper chromatography the same two components as the leaf xylan.

Periodate oxidation. Corn leaf polysaccharide (2·74 g) was dissolved in 1 N NaOH (50 ml) and the solution was neutralized with HOAc. HIO_4 (0·5 M, 50 ml) was added and the final volume adjusted to 250 ml pH 4·5. The oxidation proceeded in the dark at 4° for 96 hr when 0·77 moles of HIO_4 per mole of sugar had been consumed as judged by the arsenite method.[36] To the filtrate obtained after adding a slurry of $BaCO_3$ $NaBH_4$ (1 g) was added and the solution left overnight. After deionization with IR 120 and distillation with 1% MeOH–HCl a portion (110 mg) of the product was hydrolysed with H_2SO_4 (1 N, 5 ml) at 100° for 8 hr. The solution was neutralized with $BaCO_3$ and resolved into neutral and acidic fractions by passage through ion exchange resins. A portion of the neutral hydrolyzate (10 mg) was silylated and separated on a stainless steel column (2·4 m × 6 mm) packed with 20% SF 96 on 60–80 mesh Diatoport S.† The column was held at 90° for 3 min after injection and then programmed at 3°/min to hold at 220°. Using molar response factors previously determined[25] the average molar ratio of ethylene glycol, glycerol, erythritol and xylose was 1:34:8:18. The stalk xylan consumed 0·65 moles of periodate after 7 days and on reduction and hydrolysis gave the same components in the ratio of 1:48·5:5·4:16·8.

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