[CONTRIBUTION FROM DEPT. OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Carbohydrates of Gramineae. II. The Constitution of the Hemicelluloses of Wheat Straw and Corn Cobs[1]

BY I. EHRENTHAL, R. MONTGOMERY AND F. SMITH

RECEIVED MARCH 19, 1954

The constitution of the hemicelluloses of wheat straw and corn cobs has been investigated by methylation studies. Results using the hitherto conventional method for separating the component methylated sugars, which necessitates relatively large amounts of material, and the more modern semi-microchromatographic techniques, are in agreement and show that methylated wheat straw hemicellulose gives upon hydrolysis 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 2,6-di-*O*-methyl-D-glucose, the respective molecular ratios being 5:1:48-50:10:2 approximately. By the same procedures the methyl derivative of corn cob hemicellulose afforded the same methylated cleavage fragments in the respective molecular ratios of 2:1:27–29:5:0.5 approximately. The constitutional significance of these findings is discussed.

Hemicelluloses composed largely of anhydro-D-xylose units are widely distributed in the plant kingdom as a universal type of cellular cement, being found in roots, stems, leaves and seeds. Early work was concerned with the elucidation of the structure of esparto grass hemicellulose[2] which led to its representation as a chain of D-xylopyranose units linked by 1,4-β-glycosidic bonds. A closer study of the cleavage fragments of the methylated esparto grass xylan showed that in addition to the large amount of 2,3-di-*O*-methyl-D-xylose there was also present about 6% of 2,3,5-tri-*O*-methyl-L-arabinose[3] and about 5% of 2-*O*-methyl-D-xylose.[4] It was therefore deduced that the xylan consisted of chains of about 17–18 D-xylopyranose units terminated by L-arabofuranose residues and linked to each other through a xylose unit. More recently it was found that, by repeated fractional precipitation as the copper hemicellulose complex under carefully controlled and very mild conditions, a xylan devoid of arabinose residues could be obtained[5] as well as an arabinose-rich fraction containing D-xylose, L-arabinose, D-glucose and D-galactose.[6] From methylation studies on this arabinose-rich fraction it was suggested that the hemicellulose fraction was a mixture of an araboxylan and a galactan. However, no methylated derivatives of glucose were isolated even though the fraction before methylation did contain glucose.

The question of the homogeneity of the hemicelluloses frequently has been raised in their study and the development of the structure of the esparto grass hemicellulose substantiates its pertinence.

This paper is concerned with the constitution of the hemicelluloses from wheat straw and corn cobs. The results of the methylation studies have been summarized in an earlier paper.[7] Both xylans were obtained by extraction with dilute sodium hydroxide followed by precipitation with ethanol. Impure wheat straw xylan showed a rotation of $[\alpha]^{20}D$ $-89.5°$ in 2% sodium hydroxide corresponding to $-103°$ on an ash-free basis. Corn cob xylan, which was obtained in a relatively pure state and free from inorganic impurity, showed a rotation of $[\alpha]^{20}D$ $-103°$ in 2% sodium hydroxide.

Both the xylan from wheat straw and that from corn cobs gave upon hydrolysis a mixture of D-glucose, L-arabinose and D-xylose, the composition of which was estimated by quantitative chromatographic analysis (see Tables II and III). Using a yeast especially selected to consume L-arabinose, workers in the Northern Utilization Research Branch have found similar amounts of this sugar in the xylan hydrolysates. Their work appears to indicate the presence of glucose.[8]

Attempts were made to purify wheat straw xylan by the Salkowski copper complex procedure[9] using Fehling solution followed by extraction of the insoluble complex with water. We confirmed, as had been previously reported,[5] that successive treatments of esparto grass xylan resulted in the isolation of a polysaccharide composed only of xylose residues. However, the same procedure when applied to wheat straw xylan resulted in little or no change in the composition of the polysaccharide (see Table III). It would appear, therefore, that the glucose residues are an integral part of the polysaccharides, further proof for which was afforded by methylation studies.

In order to determine the mode of union of the component sugars in these two xylans they were methylated with 45% potassium hydroxide and methyl sulfate. Fractional precipitation from chloroform solution with ether until no further precipitation occurred, followed by further precipitation with petroleum ether, gave a series of fractions, the properties of which indicated that the methylated polysaccharide from wheat straw and that from corn cobs were essentially homogeneous.

Methanolysis of the methylated xylans with 2% methanolic hydrogen chloride gave a mixture of glycosides which were first subjected to a preliminary separation by solvent extraction to remove fully methylated sugars.[10] The fractions were then distilled in high vacuum to complete the separation. Alternatively the mixture of glycosides was hydrolyzed with dilute hydrochloric acid, and the prod-

(1) Extracted from a thesis submitted by I. Ehrenthal to the University of Minnesota in partial fulfillment for the degree of Ph. D., 1950; Scientific Journal Series No. 3129, Agricultural Experiment Station, University of Minnesota.

(2) H. A. Hampton, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1739 (1929).

(3) W. N. Haworth, E. L. Hirst and E. Oliver, *ibid.*, 1917 (1934).

(4) R. A. S. Bywater, W. N. Haworth, E. L. Hirst and S. Peat, *ibid.*, 1983 (1937)

(5) S. K. Chanda, E. L. Hirst, J. K. N. Jones and E. G. V. Percival, *ibid.*, 1289 (1950).

(6) G. O. Aspinall, E. L. Hirst, R. W. Moody and E. G. V. Percival, *ibid.*, 1631 (1953).

(7) L. A. Boggs, L. S. Cuendet, M. Dubois and F. Smith, *Anal. Chem.*, **24**, 1148 (1952).

(8) A. H. Auernheimer, L. J. Wickerham and L. E. Schniepp, *ibid.*, **20**, 876 (1948).

(9) E. Salkowski, *Z. physiol. Chem.*, **34**, 162 (1901).

(10) F. Brown and J. K. N. Jones, *J. Chem. Soc.*, 1344 (1947).

ucts of hydrolysis chromatographed on a cellulose column using methyl ethyl ketone–water azeotrope as the developing solvent. The presence of the following sugars was established by the isolation of crystalline derivatives: 2,3,4-tri-*O*-methyl-D-xylose, 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and 2,6-di-*O*-methyl-D-glucose, the latter sugar as the 1,3,4-tri-*O*-azobenzoyl derivative.[11]

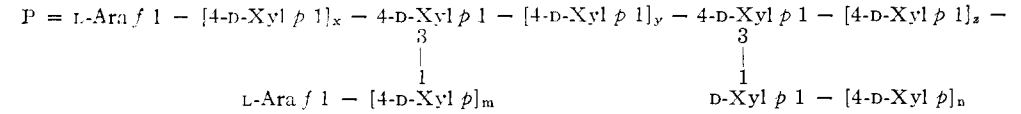
The composition of the methylated polysaccharides, whether determined by fractional distillation or by flowing partition chromatography was found to be approximately the same. The results obtained for the two xylans are shown in Table VII.

Although no unique structure can be assigned from these data to the xylans from wheat straw or corn cobs it is seen from the relatively large amount of 2,3-di-*O*-methyl xylose produced from both methyl polysaccharides that a backbone of D-xylopyranose units linked through positions 1 and 4 is present. As far as the wheat pentosan is concerned, this finding is in accord with the isolation of a crystalline xylan, which was essentially linear, from the autohydrolysis of wheat straw hemicellulose.[12] The presence of 2-*O*-methyl-D-xylose in both methylated xylans indicates that side chains of xylose units are attached to the xylose backbone through position 3, the side chains being terminated by either xylopyranose or arabofuranose residues.

The identification of 2,6-di-*O*-methyl-D-glucose as one of the cleavage fragments of both methylated xylans suggests that this residue is derived from either glucofuranose units joined to other units through positions 1, 3 and 5 or from glucopyranose units attached through positions 1, 3 and 4. However, treatment of wheat straw xylan with 30% aqueous formic acid resulted in the removal of only arabofuranose residues. Also periodate oxidation of both xylans followed by reduction[13] of the resulting polyaldehyde with hydrogen using a Raney nickel catalyst, yielded a polyalcohol which upon hydrolysis gave no evidence of glucose residues resistant to periodate oxidation. The cleavage fragments did, however, contain D-xylose, an observation also reported by Jayme.[14] One possible explanation for these results is that the glucose is linked through positions 3 and 4 and has position 1 free. The general formula shown in I explains the above experimental facts.

I

where



It is of interest to note that a methylated polysaccharide derived from the amounts of the cleavage fragments obtained by methylation studies gives a calculated methoxyl content of 38.2% which is in good agreement with the average value of 38.3% actually determined. Also, examination of the cleavage products obtained by hydrolysis of the methylated polysaccharide shows that the composition of the xylan polysaccharide resembles that obtained by chromatographic analysis of the mixture of sugars derived from the original polysaccharide (see Table I).

TABLE I

COMPOSITION OF XYLANS

Sugars	Wheat straw xylan, % Methylation	Wheat straw xylan, % Direct hydrolysis	Corn cob xylan, % Methylation	Corn cob xylan, % Direct hydrolysis
L-Arabinose	7.4	7.3	5.3	6.3
D-Xylose	89.3	87.2	93.3	90.5
D-Glucose	3.3	5.5	1.4	3.2

However, methylation of the polysaccharide apparently causes a reduction in the amount of glucose by 30 to 50%. In view of the fact that free sugars are sensitive to strong alkali especially in the presence of oxygen, this loss of glucose is not surprising and indeed would lend support to the theory advanced above that glucose exists in the complex as a terminal reducing group.

Consideration must be given to the possibility that the xylans examined in this work consist of a mixture of a true xylan (composed only of xylose units) as suggested by the work of Hirst, *et al.*,[5,6] and a glucoaraban and even an araban and a glucosan. Were this the case, however, it is still impossible to explain how the arabinose and glucose components of the original xylan could appear after methylation as only 2,3,5-tri-*O*-methyl-L-arabinose and 2,6-di-*O*-methyl-D-glucose, respectively. The available evidence therefore points to the fact that the three components, xylose, arabinose and glucose form an integral part of the xylan molecule.

Since this work was completed uronic acid residues have been identified in various wheat straw hemicelluloses.[15-18] The structural deductions cited above are based mainly upon a study of the methanolysis products of fractions of methylated hemicellulose having $[\alpha]_D$ −90° (approx.) ($CHCl_3$) which did not appear to contain any uronic acid components. It is not unlikely, however, that those fractions of methylated hemicellulose having a lower negative rotation $[\alpha]_D$ −58° ($CHCl_3$), which have not yet been examined in detail, may contain uronic acid residues. It is also possible that uronide-like substances were eliminated during the methylation step.

Experimental

Wheat Straw Xylan and Corn Cob Xylan.—The xylans

(11) K. Freudenberg and G. Hüll, *Ber.*, **74**, 237 (1941).

(12) C. T. Bishop, *Can. J. Chem.*, **31**, 793 (1953).

(13) M. A. Akher, J. K. Hamilton, R. Montgomery and F. Smith, THIS JOURNAL, **74**, 4970 (1952).

(14) G. Jayme and M. Satre, *Ber.*, **75**, 1840 (1942); **77**, 242, 248 (1944).

(15) C. T. Bishop and G. A. Adams, *Can. J. Research*, **28B**, 753 (1950).

(16) G. A. Adams and A. E. Castgane, *Can. J. Chem.*, **29**, 109 (1951), **30**, 515 (1952).

(17) G. A. Adams, *ibid.*, **30**, 698 (1952).

(18) C. T. Bishop, *ibid.*, **30**, 229 (1952).

used in these experiments were white powders which were insoluble in cold water. They dissolved slowly in boiling water to give solutions which when treated with iodine gave a blue to green color. The polysaccharides, like the mannan polysaccharides, gave insoluble complexes with Fehling solution but did not reduce it even on boiling. The xylans were soluble in dilute aqueous alkali: $[\alpha]^{20}D$ −103° in 2% NaOH (*c* 0.75) on an ash-free basis.

Purification of Wheat Straw Xylan.[2,5]—To a solution of xylan (10 g.) in 4% sodium hydroxide (650 ml.) was added an equal volume of freshly prepared Fehling solution. The precipitated copper complex was filtered and washed with water to remove the excess copper ions. A suspension of the precipitate in water was cooled in ice-water and cold 2 *N* hydrochloric acid was slowly added to decompose the copper complex. After filtering the resulting solution the polysaccharide was precipitated from the filtrate with acetone. The precipitate was washed successively with acetone–water (60:40 v./v.) containing 2% acetic acid to remove any remaining copper, acetone–water (60:40 v./v.), methanol and finally with ether. The polysaccharide was suspended in water (250 ml.) and mechanically shaken for 15 hours after which time the insoluble material obtained on centrifugation was dried by solvent exchange with methanol and ether.

This process was repeated six times. A suspension of the xylan in boiling water gave upon cooling a blue to green color with iodine, which has been reported[19,20] to indicate the presence of glucose.

Composition of the Xylans.—Hydrolysis of the crude and purified wheat straw xylan with *N* sulfuric acid at 90–95° for 12 hours gave a mixture of sugars ($[\alpha]^{23}D$ +28.6° in water) and an insoluble residue amounting to 15% of the polysaccharide by weight which remained even after prolonged heating at 90–95°. The final rotation, corrected for this insoluble residue, was $[\alpha]^{23}D$ +33.8°. Paper chromatographic examination of the mixture of sugars using phenol saturated with water as the developing solvent indicated the presence of D-xylose, L-arabinose and D-glucose.

The mixture of sugars from the hydrolysis of 0.1197 g. of xylan was separated on a column of cellulose using phenol saturated with water as the irrigating solvent and an automatic device to collect the fractions; the sugars were located and isolated in the manner described in a previous paper[7] (see Table II).

TABLE II

COMPOSITION OF WHEAT STRAW XYLAN BY COLUMN CHROMATOGRAPHY

Fraction	Sugar	Wt., mg.[a]	M.p., °C.	$[\alpha]^{21}D$ (H_2O) equilibrium value	Composition, %
1	L-Arabinose	7.36	157	+101.5°	6.7
2	D-Xylose	84.1	143	+ 18.1	76.4
3	D-Glucose	5.16	[b]	[b]	4.7

[a] An impurity accompanies each fraction in an amount which is proportional to the volume of phenol–water in which the fraction was distributed. This impurity which may be largely removed by extraction with ethanol[7] appears to be derived from an impurity in the cellulose. The weight of each sugar reported was calculated from the optical rotation. [b] Fraction 3 was shown by paper chromatography to be principally D-glucose with a trace of D-xylose.

A quantitative paper chromatographic analysis of the hydrolysates of the crude and purified wheat straw xylan by the methods described by Flood, *et al.*,[21] gave the results in Table III.

In a similar way, quantitative analyses of corn cob xylan by paper chromatography[21] gave the results in Table III. Analysis by column chromatography showed L-arabinose, 5.0%, D-xylose, 82%, and D-glucose, 2.0%.

Mild Hydrolysis of Wheat Straw Xylan.—Wheat straw xylan (0.10 g.) was treated with 30% aqueous formic acid (5 ml.) at 100° for 5–10 minutes. The solution, which then reduced boiling Fehling solution, was cooled, poured into 2–3 volumes of methanol and the precipitate washed with 70% aqueous methanol until the washings no longer reduced Fehling solution. The precipitate was then successively washed with methanol and ether and after drying it was hydrolyzed at 95° for 10 hours with *N* sulfuric acid (3 ml.). Examination of the resulting mixture of sugars by paper chromatography using butanol–ethanol–water as the developing solvent showed the presence of xylose and glucose. No arabinose was detected.

TABLE III

COMPOSITION OF THE XYLANS BY PAPER CHROMATOGRAPHY

Sugars	Composition, %[a] Crude wheat straw xylan	Purified wheat straw xylan	Corn cob xylan
L-Arabinose	7.3	7.1	5.9
D-Xylose	87.2	87.7	83.0
D-Glucose	5.5	5.2	1.7

[a] These values are calculated on the assumption that the polysaccharide gives only arabinose, xylose and glucose upon hydrolysis.

Oxidation of Wheat Straw Xylan with Sodium Periodate.—Wheat straw xylan (0.598 g.) was suspended in water (155 ml.) to which was added 0.44 *N* sodium periodate (45 ml.) in this order. The suspension was kept in the dark at 5° and as the oxidation proceeded the material went into solution. At suitable intervals the periodate consumption was determined by the usual method[22] and the formation of formic acid was followed by titration (after the destruction of the excess periodate with ethylene glycol) of a suitable aliquot with 0.01 *N* barium hydroxide using methyl red as the indicator. The reaction was complete after 27 days; 0.04 mole of formic acid was produced and 1.08 moles of periodate was consumed per anhydropentose unit. In two other experiments, the periodate consumption was 1.03 and 1.07 moles and the formic acid was 0.04 mole per anhydropentose unit. The amount of formic acid liberated was constant after about 170 hours.

In another experiment, the xylan was oxidized with sodium periodate as before and the resulting solution was centrifuged. The supernatant liquid was dialyzed against distilled water until free from iodate and periodate ions. The aqueous solution was then concentrated *in vacuo* at 40° to a small volume and the polyaldehyde was precipitated with a mixture of alcohol and ether. The isolated polyaldehyde, $[\alpha]^{25}D$ +63.5° in water (*c* 0.4) was hygroscopic, strongly reduced Fehling solution and gave a positive Schiff test.

The polyaldehydic material (0.127 g.) was hydrolyzed with *N* sulfuric acid (3 ml.) by heating in a sealed tube at 95° for 12 hours. The acid was neutralized with "Duolite A-4" anion-exchange resin and the aqueous solution evaporated to dryness *in vacuo*. Partition chromatography of the residue using either butanol–ethanol–water or phenol saturated with water as the developing liquid indicated the presence of only D-xylose.

The polyaldehyde, without previous isolation, was also reduced with a pressure of hydrogen at room temperature using Raney nickel catalyst. The resulting solution no longer gave a positive Schiff test and the polyalcohol, which was not isolated, was hydrolyzed as described above for the polyaldehyde. Partition chromatography of the hydrolysate indicated the presence of D-xylose and a trace of L-arabinose; the presence of the latter is not understood. The fact that the arabinose is detected in the hydrolysate of the polyalcohol, and not in that of the polyaldehyde is probably due to the fact that during the acid hydrolysis of the polyaldehyde, decomposition occurs accompanied by the formation of interfering colored compounds.[13]

Oxidation of Corn Cob Xylan with Sodium Periodate.—In duplicate experiments, corn cob xylan consumed 1.09 and 1.05 moles of periodate per mole of anhydropentose, constant after 29 days, with the liberation of 0.06 mole of formic acid per mole of anhydropentose, constant after 170 hours.

The corn cob xylan polyaldehyde showed $[\alpha]^{25}D$ +69° in water (*c* 0.75) and had properties similar to wheat straw polyaldehyde. When hydrolyzed with *N* sulfuric acid in a sealed tube at 95°, the hydrolysate of the polyaldehyde showed the presence of only one sugar, xylose, by paper

(19) E. Anderson, M. Seeler, W. Stewart, J. Redd and D. Weaterbecke, *J. Biol. Chem.*, **135**, 189 (1940).

(20) M. H. O'Dwyer, *Biochem. J.*, **28**, 2116 (1934); **31**, 254 (1937); **33**, 712 (1939).

(21) A. E. Flood, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1679 (1948).

(22) P. Fleury and J. Lange, *J. Pharm. Chem.*, [8] **17**, 107 (1933).

chromatography. Acid hydrolysis of the corresponding corn cob polyalcohol followed by paper partition chromatography of the concentrated hydrolysate indicated the presence of D-xylose and a trace of L-arabinose.

Treatment of the hydrolysate of corn cob xylan polyalcohol with phenylhydrazine and aqueous acetic acid in the usual way afforded D-xylosazone, m.p. and mixed m.p. 158–160°, $[\alpha]^{22}D$ −36.2° in ethanol (*c* 0.3). The hydrolysate, when treated with alcoholic diphenylhydrazine, gave L-arabinose diphenylhydrazone, m.p. and mixed m.p. 196–197°.

Methylation of Wheat Straw Xylan.—Xylan (40 g.) was dissolved in 45% potassium hydroxide (700 ml.). Methyl sulfate (200 ml.) was added with vigorous stirring during a period of 3–4 hours. The methylation was completed by heating for one hour at 95–100°. The partially methylated xylan separated as insoluble particles from the reaction mixture and was removed by filtration through cloth. The material was resuspended in 45% potassium hydroxide and remethylated.

After six methylations, the insoluble methylated xylan was dissolved in 1,4-dioxan, diluted with water and subjected to dialysis against distilled water until free from sulfate ions. Evaporation of the solution gave the methyl xylan (22.5 g.); (found: OCH_3, 36.1).

Fractional precipitation of the methylxylan from chloroform with diethyl ether and petroleum ether (b.p. 30–60°) in the usual way afforded five fractions, the properties of which are summarized in Table IV.

TABLE IV

FRACTIONATION OF METHYL WHEAT STRAW XYLAN

Fraction	Wt., g.	$[\alpha]^{24}_{5461}$ (*c* 0.5 in $CHCl_3$)	OCH_3, %
1	4.5	−58	36.9
2	2.1	−86	38.1
3	9.2	−88	38.3
4	3.1	−90	38.8
5	1.5	−36	37.9

The viscosity of fraction 3 in *m*-cresol (*c* 0.5) was found to be $\eta^{22}_{sp}/c = 1.40$.

Treatment of Methyl Wheat Straw Xylan with Methyl Iodide in Pyridine.[23]—A specimen (0.5 g.) of the above methylated xylan (OCH_3, 38.3) in anhydrous pyridine (20 ml.) was treated with methyl iodide (0.5 ml.) at 150° for 2 hours. The product showed $[\alpha]^{20}D$ −74.5° in chloroform (*c* 0.5) (found: OCH_3, 37.2) indicating that little is gained by the application of this additional procedure.

Methylation of Corn Cob Xylan.—Corn cob xylan (40 g.) was dissolved in 45% potassium hydroxide (700 ml.) and methylated with methyl sulfate (200 ml.) in a manner already described. After five such treatments the methylated xylan was dissolved in 1,4-dioxan, diluted with an equal volume of water and dialyzed against distilled water for 3 days. Evaporation of the solution to dryness *in vacuo* gave crude methylated xylan (31 g.); (found: OCH_3, 36.8). Fractional precipitation from chloroform solution with ether and petroleum ether (b.p. 30–60°) showed the methylxylan to be essentially homogeneous, $[\alpha]^{27}D$ −88° in chloroform (*c* 0.4), η^{23}_{sp}/c, 1.5 in *m*-cresol (*c* 0.5) (found: OCH_3, 38.3).

Methanolysis of Methylated Wheat Straw Xylan.—When the methylated xylan (15.95 g.) was subjected to methanolysis with boiling 2% methanolic hydrogen chloride (250 ml.) for 12 hours, it reached a constant specific rotation of $[\alpha]^{21}D$, +65°. A specimen (140 mg.) of the mixture of glycosides, after being freed from hydrogen chloride (silver carbonate) was hydrolyzed with *N* sulfuric acid for 15 hours on a boiling water-bath. The solution was neutralized with barium carbonate, filtered and the filtrate evaporated *in vacuo* at 35–40°. Examination of the resulting mixture of reducing methylated sugars by paper chromatography using butanol–ethanol–water as the developing solvent and ammoniacal silver nitrate as the spray reagent indicated a trimethylpentose spot, 2,3-di-*O*-methyl-D-xylose, 2,6-di-*O*-methyl-D-glucose, 2-*O*-methyl-D-xylose and a very faint spot corresponding to D-xylose. When another chromatogram, prepared in the same manner, was sprayed with N,N-dimethyl-*p*-aminoaniline,[24] the trimethylpentose spot was shown to consist of 2,3,4-tri-*O*-methyl-D-xylose in the slower moving part of the spot, giving a pinkish red color, and 2,3,5-tri-*O*-methyl-L-arabinose in the faster moving part of the spot, giving a greyish black color.

The glycoside mixture (18.71 g.) was dissolved in water (25–30 ml.) and extracted continuously with petroleum ether (b.p. 30–60°) for 5 hours. The petroleum ether fraction (1.755 g.) gave fractions 1 and 2 (Table V) upon fractional distillation.

The aqueous solution was evaporated *in vacuo*, the residue added to the still residue from the petroleum ether extract, and the distillation resumed giving fractions 3, 4 and 5 (Table V). There was an undistillable residue of 0.549 g.

The composition of each fraction was qualitatively determined by paper chromatography using methyl ethyl ketone–water azeotrope as the developing solvent[7,24] and the N,N-dimethyl-*p*-aminoaniline hydrochloride reagent[24] to detect the sugars on the chromatogram. The quantitative composition of each fraction was calculated from the refractive index of the glycoside mixtures or the specific rotations of the corresponding mixtures of reducing sugars.

Since the refractive indices of methyl 2,3,4-tri-*O*-methyl-D-xyloside and methyl 2,3,5-tri-*O*-methyl-L-arabinoside were essentially the same the percentage composition of mixtures of these glycosides was determined from the specific rotation of the mixture of reducing sugars obtained on hydrolysis with *N* sulfuric acid in the usual way. The same applies to mixtures of methyl 2-*O*-methyl-D-xyloside and

TABLE V

COMPOSITION OF GLYCOSIDIC FRACTIONS FROM METHANOLYZED METHYLATED WHEAT STRAW XYLAN

Fraction	B.p. (bath temp.) °C.	Mm.	Wt., g.	$n^{25}D$	$[\alpha]^{24}D$	OCH_3, %	Composition (approx.) Sugars	Wt., g.
1	70–73	0.15	1.156	1.4343	−63° (H_2O; *c* 0.5)	60.1	2,3,4-Tri-*O*-methyl-D-xylose	0.198
							2,3,5-Tri-*O*-methyl-L-arabinose	.958
2	80–90	.15	0.485	1.4471		50.5	2,3,4-Tri-*O*-methyl-D-xylose	.026
							2,3,5-Tri-*O*-methyl-L-arabinose	.127
							2,3-Di-*O*-methyl-D-xylose	.332
3	90–105	.05	2.058	1.4502		49.4	2,3,4-Tri-*O*-methyl-D-xylose	.052
							2,3,5-Tri-*O*-methyl-L-arabinose	.256
							2,3-Di-*O*-methyl-D-xylose	1.750
4	105–108	.05	8.733	1.4530	+58° (MeOH: *c* 0.8)	48.4	2,3-Di-*O*-methyl-D-xylose	8.733
5	110–130	.05	3.467	1.4660		39.1	2,3-Di-*O*-methyl-D-xylose	1.382
							2-*O*-Methyl-D-xylose	1.605
							2,6-Di-*O*-methyl-D-glucose	0.480
6	135–170	.05	0.698	1.4746	+109° (H_2O: *c* 0.4)	36.1	2-*O*-Methyl-D-xylose	0.538
							2,6-Di-*O*-methyl-D-glucose	0.160

(23) K. H. Meyer and P. Gürtler, *Helv. Chim. Acta*, **31**, 100 (1948).

(24) L. Boggs, L. S. Cuendet, I. Ehrenthal, R. Koch and F. Smith, *Nature*, **166**, 520 (1950).

TABLE VI

COMPOSITION OF GLYCOSIDIC FRACTIONS FROM METHANOLYZED METHYLATED CORN COB XYLAN

Fraction	B.p. (bath temp.) °C.	Mm.	Wt., g.	$n^{25}D$	$[\alpha]^{21}D$	OCH_3, %	Composition (approx.) Sugars	Wt., g.
1	70–74	0.15	0.657	1.4348	−34.8° (H_2O; *c* 0.5)	59.3	2,3,4-Tri-*O*-methyl-D-xylose	0.230
							2,3,5-Tri-*O*-methyl-L-arabinose	.427
2	74–85	.15	.083	1.4500		49.4	2,3,4-Tri-*O*-methyl-D-xylose	.005
							2,3,5-Tri-*O*-methyl-L-arabinose	.009
							2,3-Di-*O*-methyl-D-xylose	.069
3	85–95	.15	.144	1.4505		49.5	2,3,4-Tri-*O*-methyl-D-xylose	.007
							2,3,5-Tri-*O*-methyl-L-arabinose	.015
							2,3-Di-*O*-methyl-D-xylose	.122
4	95–105	.1	1.907	1.4515		48.7	2,3,4-Tri-*O*-methyl-D-xylose	.058
							2,3,5-Tri-*O*-methyl-L-arabinose	.108
							2,3-Di-*O*-methyl-D-xylose	1.741
5	105	.07	0.323	1.4528		48.4	2,3-Di-*O*-methyl-D-xylose	0.323
6	108–112	.07	5.318	1.4531	+55 (MeOH, *c* 0.8)	48.5	2,3-Di-*O*-methyl-D-xylose	5.318
7	115–135	.07	1.868	1.4668		40.2	2,3-Di-*O*-methyl-D-xylose	0.637
							2-*O*-Methyl-D-xylose	1.108
							2,6-Di-*O*-methyl-D-glucose	0.123
8	135–170	.07	0.723	1.4739	+104.5 (H_2O; *c* 0.9)	35.5	2-*O*-Methyl-D-xylose	.651
							2,6-Di-*O*-methyl-D-glucose	.072

methyl 2,6-di-*O*-methyl-D-glucoside. It was found that the weights of glycosides in fractions 1 to 6 were: methyl 2,3,5-tri-*O*-methyl-L-arabinoside, 1.34 g. (4.9 mol. props.); methyl 2,3,4-tri-*O*-methyl-D-xyloside, 0.28 g. (1.0 mol. prop.); methyl 2,3-di-*O*-methyl-D-xyloside, 12.20 g. (48.0 mol. props.); methyl 2-*O*-methyl-D-xyloside, 2.14 g. (9.0 mol. props.) and methyl 2,6-di-*O*-methyl-D-glucoside 0.64 g. (2.18 mol. props.).

In a further experiment methylated wheat straw xylan (4.72 g.) gave results indicating that the molecular ratios of the methyl tri-*O*-methyl-L-arabinoside, methyl tri-*O*-, methyl di-*O*- and methyl mono-*O*-methyl-D-xyloside and methyl di-*O*-methyl-D-glucoside were 4.9:1.0:49.5:10.0:2.4, respectively. These results correspond to a composition of 7.4% L-arabinose, 89.3% D-xylose and 3.3% glucose for wheat straw xylan.

Methanolysis of Methylated Corn Cob Xylan.—Methylated corn cob xylan (11.074 g.) was boiled under reflux with 2% methanolic hydrogen chloride (250 ml.) for 12 hours. The solution reached a constant specific rotation of $[\alpha]^{24}D$ +67°. Fractional distillation of the mixture of glycosides (11.58 g.) thus produced yielded eight fractions, the compositions of which were determined as described above for the methylated glycoside fractions derived from methylated wheat straw xylan. The results are summarized in Table VI. The molecular ratios of the methyl glycosides of tri-*O*-methyl L-arabinose, tri-*O*-, di-*O*- and mono-*O*-methyl-D-xylose and di-*O*-methyl-D-glucose were 1.9:1.0:29.0:6.9:0.6, respectively, and in two other experiments the results were 1.8:1.0:27.0:4.8:0.43 and 1.8:1.0:28.0:6.2:0.6, respectively. An average of these three results corresponds to a composition of 5.3% L-arabinose, 93.3% D-xylose and 1.4% D-glucose (approx.).

Identification of the Cleavage Fragments of the Methyl Derivatives of Wheat Straw and Corn Cob Xylan.—All of the cleavage fragments from both methylxylans cited in Tables V and VI were identified as described below. For the sake of brevity only one experiment is recorded in each case.

Identification of 2,3,5-Tri-*O*-methyl-L-arabinose and 2,3,-4-Tri-*O*-methyl-D-xylose.—Hydrolysis of the glycoside fraction 1 (Table VI) (0.45 g.) afforded a mixture of 2,3,4-tri-*O*-methyl-D-xylose and 2,3,5-tri-*O*-methyl-L-arabinose as indicated by paper partition chromatography (solvent, methyl ethyl ketone saturated with water; spray reagent, N,N-dimethyl-*p*-aminoaniline hydrochloride[24]). The mixture of reducing sugars was distilled, b.p. (bath temp.) 110–115°, 0.15 mm., $n^{23}D$ 1.4515, $[\alpha]^{22}D$ −20.3° in water (*c* 1.0). *Anal.* Calcd. for $C_8H_{16}O_5$: OCH_3, 48.4. Found: OCH_3, 48.3.

Oxidation of a portion of the distillate (0.12 g.) with bromine (0.5 ml.) in water (3 ml.) gave a sirup (0.10 g.), b.p. (bath temp.) 115–120°, 0.08 mm., $[\alpha]^{22}D$ −30.0°, initial value in water (*c* 0.9). Crystallization from ether–petroleum ether followed by separation of the adhering sirup on a porous tile afforded 2,3,4-tri-*O*-methyl-D-xylono-δ-lactone, m.p. and mixed m.p. 50–53°, $[\alpha]^{21}D$ −8.6°, initial value in water (*c* 0.9), changing in 24 hours to $[\alpha]^{22}D$ +7.2° (equilibrium value).

The sirup extracted from the tile with acetone showed $[\alpha]^{22}D$ −30.9°, initial value in water (*c* 1.0), changing to $[\alpha]^{18}D$ −15.4° in 96 hours (mutarotation incomplete). When treated with methanolic ammonia this lactone gave 2,3,5-tri-*O*-methyl-L-arabonamide, m.p. and mixed m.p. 136°, $[\alpha]^{22}D$ +24° in ethanol (*c* 1.3). *Anal.* Calcd. for $C_8H_{17}O_5N$: OCH_3, 44.9. Found: OCH_3, 44.7.

Identification of 2,3-Di-*O*-methyl-D-xylose.—Hydrolysis of fraction 6, Table VI (0.522 g.) with *N* sulfuric acid gave 2,3-di-*O*-methyl-D-xylose as a sirup (0.44 g.). *Anal.* Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.6. The 2,3-di-*O*-methyl-D-xylose gave the corresponding anilide,[2,5,25] m.p. 145°, $[\alpha]^{22}D$ +183° (constant after 24 hours) in ethyl acetate (*c* 0.5). *Anal.* Calcd. for $C_{13}H_{19}O_4N$: OCH_3, 24.5. Found: OCH_3, 24.8. Methylation of 2,3-di-*O*-methyl-D-xylose anilide with methyl iodide and silver oxide[25] gave 2,3,4-tri-*O*-methyl-D-xylose anilide, m.p. and mixed m.p. 97–98°, $[\alpha]^{21}D$ −97°, initial value in methanol (*c* 1.4), changing in 24 hours to $[\alpha]^{21}D$ +32.8° (constant value). *Anal.* Calcd. for $C_{14}H_{21}O_4N$: OCH_3, 34.8. Found: OCH_3, 34.4.

Oxidation of 2,3-di-*O*-methyl-D-xylose (0.1 g.) with bromine in the usual way afforded 2,3-di-*O*-methyl-D-xylose-γ-lactone, b.p. (bath temp.) 135°, 0.05 mm., $[\alpha]^{23}D$ +87° initial value in water (*c* 0.8). *Anal.* Calcd. for $C_7H_{12}O_5$: OCH_3, 35.2. Found: OCH_3, 34.6. Treatment of the lactone with methanolic ammonia gave 2,3-di-*O*-methyl-D-xylonamide, m.p. 133°, $[\alpha]^{23}D$ +45.5° in water (*c* 1.0). *Anal.* Calcd. for $C_7H_{15}O_5N$: OCH_3, 32.1. Found: OCH_3, 32.4.

Identification of 2-*O*-Methyl-D-xylose.—Partial crystallization of fractions 5 (Table V) took place on standing and separation of the crystals on a porous tile in a desiccator followed by recrystallization from ethanol and ether–petroleum ether gave methyl 2-*O*-methyl-β-D-xylopyranoside, m.p. 111–112°, $[\alpha]^{24}D$ −70.5° in chloroform (*c* 0.23). *Anal.* Calcd. for $C_7H_{14}O_5$: C, 47.2; H, 7.3; OCH_3, 34.8. Found: C, 46.9; H, 7.8; OCH_3, 35.2.

Hydrolysis of the crystalline glycoside with *N* sulfuric acid gave 2-*O*-methyl-D-xylose, m.p. and mixed m.p. 131° (crystallized from ethanol), $[\alpha]^{25}D$ +34.5° in water (*c* 0.3). *Anal.* Calcd. for $C_6H_{12}O_5$: OCH_3, 18.9. Found: OCH_3, 19.3.

(25) I. Ehrenthal, M. C. Rafique and F. Smith, THIS JOURNAL, **74**, 1341 (1952).

TABLE VII

COMPOSITION OF METHYLATED XYLAN HYDROLYSATES BY FLOWING PARTITION CHROMATOGRAPHIC FRACTIONATION ON A CELLULOSE COLUMN

Fraction	Sugar	Methyl wheat straw xylan Wt., mg.	Mole ratio	Methyl corn cob xylan Wt., mg.	Mole ratio
1	2,3,4-Tri-*O*-methyl-D-xylose	7.06	1.0 (1.0)[a]	14.3	1.0 (1.0)[a]
	2,3,5-Tri-*O*-methyl-L-arabinose	32.14	4.6 (4.9)	26.5	1.9 (1.9)
2	2,3-Di-*O*-methyl-D-xylose	301.2	46.0 (49.0)	334.7	25.0 (28.0)
3	2-*O*-Methyl-D-xylose	57.5	9.5 (9.5)	61.1	5.0 (5.9)
	2,6-Di-*O*-methyl-D-glucose	12.6	1.7 (2.2)	6.79	0.4 (0.5)
	Total	410.5	(Recovery 97.0%)	443.4 mg.	(Recovery 93%)

[a] These figures in parentheses quoted for comparison are the values obtained by the classical distillation method.

Separation of 2-*O*-Methyl-D-xylose from 2,6-Di-*O*-methyl-D-glucose by Flowing Partition Chromatography.—Hydrolysis of the glycoside fraction 6 (Table V), containing a mixture of methyl 2-*O*-methyl-D-xyloside and methyl 2,6-di-*O*-methyl-D-glucoside, gave a sirup (0.13 g.), $[\alpha]^{20}D$ +41.6° in water (*c* 1.0). *Anal.* Calcd. for $C_6H_{10}O_5$: OCH_3, 19.8 and for $C_8H_{16}O_6$: OCH_3, 29.8. Found: OCH_3, 22.4.

The mixture of sugars was separated on a cellulose column, using butanol–ethanol–water as the developing solvent, into three fractions as described in a previous paper.[7] Fraction A (35.7 mg.), $[\alpha]^{20}D$ +54.4° in ethanol (*c* 1.2), consisted of pure 2,6-di-*O*-methyl-D-glucose (for identification, see below); fraction B (10.5 mg.), $[\alpha]^{23}D$ +43.8° in ethanol (*c* 0.4), was found to be a mixture of 2-*O*-methyl-D-xylose and 2,6-di-*O*-methyl-D-glucose; fraction C (69.3 mg.), which crystallized upon nucleation, was 2-*O*-methyl-D-xylose, m.p. 133°, $[\alpha]^{20}D$ +35.2° equilibrium value in water (*c* 0.7); $[\alpha]^{23}D$ −21° initial value in ethanol (*c* 0.4) changing in 24 hours to $[\alpha]^{23}D$ +23.8° (constant value). *Anal.* Calcd. for $C_6H_{10}O_5$: OCH_3, 18.9. Found: OCH_3, 19.3. Treatment of the sugar with aniline in the usual way afforded 2-*O*-methyl-D-xylose anilide, m.p. 128°, $[\alpha]^{21}D$ +23.7° in ethyl acetate (*c* 0.7) (after recrystallization from ethyl acetate). *Anal.* Calcd. for $C_{12}H_{17}O_4N$: C, 60.2; H, 7.1; OCH_3, 13.0. Found: C, 60.1; H, 7.3; OCH_3, 13.2.

Identification of 2,6-Di-*O*-methyl-D-glucose.—The mixture of glycosides (0.121 g. fraction 6, Table V) was methylated with sodium and methyl iodide in liquid ammonia.[26] The mixture of completely methylated glycosides so formed was hydrolyzed with *N* sulfuric acid to give a sirup (0.049 g.) which was shown by paper chromatography (solvent: methyl ethyl ketone saturated with water) to consist of a mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose (R_f, 0.85) and 2,3,4-tri-*O*-methyl-D-xylose (R_f 0.82). The tetra-*O*-methyl-D-glucose gave a characteristic purple spot with N,N-dimethyl-*p*-aminoaniline reagent while the tri-*O*-methyl-D-xylose gave a pink spot. The mixture of reducing sugars (0.049 g.) when treated with aniline (0.03 g.) in ethanol (3 ml.) on a boiling water-bath for 2 hours gave, after removal of the solvent, a crystalline anilide which after recrystallization from benzene–light petroleum ether gave 2,3,4,6-tetra-*O*-methyl-D-glucose anilide, m.p. and mixed m.p. 126–128°.

(26) I. E. Muskat, THIS JOURNAL, **56**, 693 (1934).

Fraction A (0.03 g.) of the sugars separated on the column (see above) was dissolved in dry pyridine (3 ml.) and *p*-phenylazobenzoyl chloride (0.35 g.) was added to the solution. The reaction mixture was maintained at 40° for 2 days after which time water (0.5 ml.) was slowly added to decompose the excess acid chloride. After about one hour, the pyridine was removed by distillation and the residue was triturated with water to complete the decomposition of the excess acid chloride. The water was removed by distillation *in vacuo* and the residue dissolved in chloroform. The chloroform solution was passed through a column of zinc carbonate to remove the *p*-phenylazobenzoic acid, and the column washed with chloroform until the eluate was colorless. Evaporation of the eluate gave a residue which when recrystallized from ethyl acetate–petroleum ether afforded 1,3,4-tri-*O*-phenylazobenzoyl-2,6-di-*O*-methyl-D-glucose, m.p. and mixed m.p. 203–206°, $[\alpha]^{24}_{6300}$ −266° in chloroform (*c* 0.55). *Anal.* Calcd. for $C_{47}H_{40}O_9N_6$: OCH_3, 7.35. Found: OCH_3, 7.5. An authentic specimen of this compound prepared from 2,6-di-*O*-methyl-D-glucose had m.p. 205–208°, $[\alpha]^{22}_{6300}$ −273° in chloroform (*c* 0.6). Freudenberg[11] quotes m.p. 205–207°, $[\alpha]^{20}_{6250}$ −275° in chloroform (*c* 0.5).

Investigation of the Composition of Methylated Sugars Obtained from the Methylated Xylans by Flowing Column Partition Chromatography.—The mixture of glycosides obtained from the methanolysis of methylated wheat straw xylan (0.42 g.) was hydrolyzed with *N* sulfuric acid in the usual way to give a sirup (0.42 g.). The sirup was fractionated on a column of cellulose, using the methyl ethyl ketone–water azeotrope as the developing solvent, in the manner described previously.[7] Three fractions were obtained the compositions of which were determined as described above. The results, summarized in Table VII, agree with those obtained above by the conventional procedure.

The mixture of glycosides obtained from the methanolysis of methylated corn cob xylan (0.469 g.) was treated in a similar way and the results are summarized in Table VII.

Acknowledgment.—The authors wish to thank the Northern Utilization Research Branch for the wheat straw xylan and part of the corn cob xylan used in this work.

ST. PAUL, MINNESOTA