The third mother liquor in the above series of recrystallizations deposited, upon evaporation of the solvent, a nearly colorless crystalline residue (89 mg.), m.p. 142–144° and $[\alpha]^{25}D$ −17° in pyridine. This material was triturated with hot ethyl acetate, and the insoluble residue was recrystallized from water to a maximum m.p. 158.5–159° and $[\alpha]^{25}D$ −47° in pyridine. These constants are in agreement with those of L-*xylo*-hexose phenylosotriazole.[8] The melting point was depressed to 140–141° by admixture with the authentic D-isomer but was undepressed by the authentic L-isomer.

Resolution of Authentic DL-*xylo*-Hexose Phenylosotriazole by Seeding.—Amounts of 150 mg. each of the authentic D- and L-*xylo*-hexose phenylosotriazoles were dissolved together in acetone and the solvent evaporated. The melting point (140.5–141°) of the residue was unchanged by recrystallization from 3 ml. of water. The recovered material (290 mg.) was optically inactive.

A solution of 277 mg. of the racemate in 13 ml. of hot water was cooled slightly and seeded with the D-enantiomorph. Crystals formed first in the region around the seed crystals as the solution cooled slowly to room temperature. The crystals (163 mg.) removed after a short time showed m.p. 141–142.5° and $[\alpha]^{25}D$ 13° in pyridine (calculated composition: 64% D, 36% L). The mother liquor with an additional 1 ml. of wash water was seeded with the L-enantiomorph and cooled to 0°. The resulting crystals (67 mg.) showed m.p. 150.5–152° and $[\alpha]^{25}D$ −28° in pyridine (calculated composition: 20% D, 80% L).

When the compositions (calculated from optical rotations) and melting points from this and the preceding section were plotted aginst each other, it was apparent that the composition of minimum melting point was near to 50:50. However, that the racemate is a true compound was verified by a 2° depression of its melting point on admixture with small amounts of either enantiomorph.

Reduction to DL-Glucitol.—An amount of 500 mg. of (DL + D)-sorbose, $[\alpha]^{25}D$ 12°, was hydrogenated in solution in 50% ethanol for 9 hours at 90° and 1500 p.s.i. of hydrogen, with Adams platinum oxide catalyst. After filtration, concentration and addition of methanol, there was obtained 185 mg. of crystals showing m.p. 125–135°. Recrystallization from water with the addition of dioxane gave 117 mg. of product with m.p. 136–138°; X-ray powder diffraction data[18]: 9.01 (2),[19] 5.90 (3), 4.38 (1), 3.88 (4), 3.59 (5), 2.62 (6), 2.81, 2.74, 2.46, 2.37, 2.33, 2.10, 1.99, 1.94, 1.63. These properties are in agreement with those reported for DL-glucitol.[20] Acetylation of 40 mg. of the hexitol gave 77 mg. of DL-glucitol hexaacetate, m.p. 117–117.5°.[20]

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(18) We are indebted to Mr. Eugene McLaren of this Department for the X-ray powder diffraction data.

(19) CuK_α radiation; interplanar spacings, Å.; order of intensities estimated visually, (1) most intense.

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SAINT LOUIS, MISSOURI

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Carbohydrates of the Gramineae. VII. The Constitution of a Water-soluble Hemicellulose of the Endosperm of Wheat (*Triticum vulgare*)[1,2]

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The constitution of a water-soluble hemicellulose from wheat flour has been investigated by methylation studies. The methylated hemicellulose gives upon hydrolysis 2,3,5-tri-*O*-methyl-L-arabinose (13 moles), 2,3-di-*O*-methyl-D-xylose (19 moles), 2-*O*-methyl-D-xylose (6 moles) and D-xylose (4 moles). The highly branched structure of this hemicellulose is similar to that of the hemicellulose from the "squeegee" fraction of wheat flour and other hemicelluloses of the endosperm of the *Gramineae*.

Water-soluble pentosans from the endosperm of wheat have been variously described[3–7] but only in the cases of the polysaccharide associated with wheat β-amylase[6] and of the araboxylan of wheat flour[7] have constitutional studies been made.

This paper is concerned with the constitutional study of a hemicellulose extracted from wheat flour with water at room temperature. The wheat flour was first treated with boiling 82% ethanol in order to inactivate any enzymes, a procedure which has not always been applied to studies of this type.[7] Following an extensive extraction of the resulting wheat flour with 70% ethanol to remove the lower molecular weight sugars and glucofructosans, which have been studied separately,[8,9] the flour was extracted with water in a Waring blendor at room temperature and precipitated from the aqueous extract with ethanol. In the light of present knowledge such a vigorous extraction procedure is not to be recommended since water-soluble polyglucosans are produced by the action of the Waring blendor on the wheat starch,[10] and these make it difficult to isolate the pure hemicellulose component. It has been shown previously[2] that the water-soluble components of wheat flour cannot be separated by fractional precipitation from an aqueous solution with ethanol. Consequently, the acetate of the mixture of water-soluble polysaccharides was subjected to fractional precipitation from a solution in pyridine and acetone with ether and petroleum ether. Even after repeated fractional precipitation in this manner a complete separation of the hexosans from the pentosans was not achieved (Tables I and II). However, the principal product from the fractionation of the acetates, $[\alpha]^{23}D$ −91° in pyridine, upon deacetylation by heating an acetone solution with 15% sodium hydroxide, was found to give a polysaccharide, $[\alpha]^{22}D$ −94° in 2% sodium hydroxide, which was largely pentosan in character.

(1) This paper, No. 3303, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, is part of a report of research done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service.

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(7) A. S. Perlin, *Cereal Chem.*, **28**, 382 (1951).

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(9) R. Montgomery and F. Smith, unpublished work.

(10) T. J. Schoch, *Tappi*, **35**, 22A (1952).

Upon hydrolysis with acid the hemicellulose gave D-xylose (60%), L-arabinose (32%) and D-glucose (8%), the quantitative analysis being carried out by the phenol–sulfuric acid procedure.[11] In periodate oxidation studies, 0.67 mole of periodate was consumed per anhydropentose residue. The resulting hemicellulose polyaldehyde after reduction with hydrogen using a Raney nickel catalyst followed by acid hydrolysis of the polyalcohol gave a hydrolysate which was shown by chromatographic analysis to contain D-xylose; no L-arabinose or D-glucose was detected.[12]

In order to purify further the hemicellulose and to determine the mode of union of its component sugars, the hemicellulose acetate was methylated with methyl sulfate and 45% potassium hydroxide. Controlled precipitation of the methylated hemicellulose from an acetone solution with ether and petroleum ether gave a series of fractions; the major components were found by hydrolysis to be composed only of the methyl derivatives of D-xylose and L-arabinose.

Methanolysis of the methylated hemicellulose with 5% methanolic hydrogen chloride gave a mixture of glycosides, an aqueous solution of which was continuously extracted with petroleum ether (b.p. 30–60°) for 30 hours, the extract being concomitantly back-extracted with water. However, since no exclusive extraction of the fully methylated sugars was achieved,[13] the petroleum ether extract being found to contain the glycosides of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3-di-*O*-methyl-D-xylose, this fractionation procedure was not applied in subsequent experiments. The mixture of glycosides from the methylated hemicellulose was hydrolyzed with dilute hydrochloric acid to give the corresponding methylated reducing sugars. Chromatography on a cellulose–hydrocellulose column[14] using methyl ethyl ketone–water azeotrope as the developing solvent, followed by the isolation of crystalline derivatives showed that this mixture of methylated sugars consisted of 2,3,5-tri-*O*-methyl-L-arabinose (13 moles), 2,3-di-*O*-methyl-D-xylose (19 moles), 2-*O*-methyl-D-xylose (6 moles) and D-xylose (4 moles); the quantitative analyses were carried out separately using the phenol–sulfuric acid procedure.[11] Paper chromatography and polarimetric data indicated the presence of a very small amount of 3-*O*-methyl-D-xylose.

These results indicate that the hemicellulose is similar to that reported earlier[7] from a similar source and also to the hemicellulose isolated from the "squeegee" fraction of wheat flour.[15] Although the data do not permit a definite structure to be assigned to the polysaccharide, when taken in conjunction with earlier work on the graded hydrolysis of a water-soluble hemicellulose from wheat flour[7] it is seen that the general structure of the pentosan polysaccharide consists of a linear framework of D-xylopyranose units linked through positions 1 and 4, with L-arabofuranose side chain residues attached to D-xylose units of the framework principally through position 3 and to a small extent possibly through position 2. The rotation of the polysaccharide would indicate that the linkages are principally of the β-type though the isolation in small yield of a methylated oligosaccharide (unknown A, Table IV) with a high positive rotation, $[\alpha]^{28}D$ +83° (methanol), shows that some α-type linkages may be present also, provided that this oligosaccharide does not arise from reversion.

The highly branched structure of this hemicellulose conforms to the general type of structure found for the hemicelluloses of the endosperm of the Gramineae.[7,16]

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Experimental

Isolation of Pentosan from Wheat Four.—Unbleached Southwest bakers patent flour, 13.0% moisture, 10.8% protein (5,840 g.) was extracted in batches of approximately 600 g. as follows. A suspension of the flour in 90% ethanol (1300 ml.) was heated at 80° for 1.5 hours in order to inactivate any enzymes. The mixture was centrifuged and the insoluble residue was extracted three times with 70% ethanol (1500 ml.) at reflux for 2–3 hours; these extracts provided the lower molecular weight sugars.[8,9] The alcohol-insoluble residue was extracted further with 1.5 volumes of distilled water in a Waring blender for 10–15 minutes. After centrifugation and filtration, a second extraction with water was made in the same way and this extract was used for the first extraction of the next batch of material. Each extract was added to three volumes of 95% ethanol or acetone. The precipitate was separated by centrifugation, washed successively with 95% ethanol, absolute ethanol and ether and freed from solvent by heating at 50° *in vacuo*. The resulting white amorphous powder represented 1.3% by weight of the wheat flour on a dry basis and showed a specific rotation which varied from $[\alpha]^{23}D$ −19° to $[\alpha]^{23}D$ +10° in 4% sodium hydroxide in the different extractions.

Acetylation of the Crude Pentosan.—The crude pentosan (32 g.) was added to formamide (300 ml.) and the mixture kept at room temperature for a week. To the resulting very viscous solution, pyridine (410 ml.) was added in three portions with shaking. After cooling the resulting solution in an ice-water bath, acetic anhydride (200 ml.) was added dropwise with vigorous stirring. The reaction mixture was allowed to stand at room temperature for 48 hours and the crude acetate isolated as a string-like mass by pouring into ice-water (4000 ml.). The acetate was washed successively with water, methanol and ether and dried at 50° *in vacuo;* yield 46 g., $[\alpha]^{23}D$ +6° in chloroform.

Fractionation of Crude Pentosan Acetate.—The crude acetate (45 g.) was dissolved in pyridine–acetone (1:1 v./v., 900 ml.) and fractionally precipitated by the addition of one volume of ether followed by increasing amounts of petroleum ether (b.p. 30–60°). Five components were obtained in this way and each of them was again subjected to the same procedure. Those fractions with similar optical rotations were combined and two further refractionations in the same manner gave the results which are summarized in Table I.

TABLE I

FRACTIONAL PRECIPITATION OF ACETYLATED POLYSACCHARIDES FROM AQUEOUS EXTRACTION OF WHEAT FLOUR

Fraction	Wt. (g.)	$[\alpha]^{23}D$ (pyridine)	Fraction	Wt. (g.)	$[\alpha]^{23}D$ (pyridine)
1	1.07	+ 7°	9	0.66	+ 97°
2	1.94	− 1°	10	0.80	+108°
3	3.26	−49°	11	0.23	+123°
4	13.00	−91°	12	6.00	+126°
5	2.16	−98°	13	1.55	+109°
6	1.25	−75°	14	0.10	+ 86°
7	2.41	+46°	15	1.91	+ 65°
8	4.80	+69°			

(16) K. A. Gilles, W. O. S. Meredith and F. Smith, *Cereal Chem.*, **29**, 314 (1952).

Composition of Acetate Fractions 1, 4, 8 and 12, Table I.—The acetates (1 g.) of fractions 4, 8 and 12 were each dissolved in acetone (50 ml.) and an equal volume of 15% sodium hydroxide was added. The mixture was heated at 50–60° until the upper acetone layer became clear, the polysaccharide then having passed into the lower alkali layer. The latter was separated and after cooling in an ice-bath, the solution was acidified with acetic acid and treated with methanol (4 volumes). The precipitate was purified by precipitation from aqueous solution with ethanol and washed successively with absolute ethanol, petroleum ether and dried *in vacuo*.

The acetate, fraction 1, was insoluble in acetone and was deacetylated by heating with 15% sodium hydroxide on a steam-bath for 8 hours. The resulting solution was centrifuged to remove a small amount of insoluble material and the polysaccharide isolated as described above.

The deacetylated polysaccharides were quantitatively analyzed using the phenol–sulfuric acid method.[11] The results are given in Table II.

TABLE II

COMPOSITION OF PRINCIPAL POLYSACCHARIDE FRACTIONS FROM AQUEOUS EXTRACT OF WHEAT FLOUR

Polysaccharide from acetate fraction	$[\alpha]_D$ in 2% NaOH	Component sugars, % Xylose	Arabinose	Glucose
1	+ 59°	13.8	9.6	71.7
4	− 94°	60.1	32.0	7.9
8	+ 89°	16.9	24.4	58.5
12	+144°	7.1	7.1	85.7

It would appear that even extensive fractional precipitation of the polysaccharide acetates did not achieve complete separation of the hexosans from the pentosans. However, the wheat pentosan was concentrated in fraction 4 upon which subsequent studies were made.

Wheat Pentosan from Acetate Fraction 4, Table I.—The pentosan obtained by deacetylation of the acetate fraction 4 (Table I) was a colorless amorphous powder which was precipitated by, but did not reduce, Fehling solution.

A sample of the pentosan was dissolved in 30% formic acid and the solution boiled for 6 minutes. The degraded polysaccharide, precipitated by adding the solution to 5 volumes of methanol, showed $[\alpha]^{24}D$ −89° in 2% sodium hydroxide (*c* 0.7). Upon complete hydrolysis with dilute mineral acid followed by quantitative analysis[11] the degraded pentosan was found to be composed of D-xylose (71%), L-arabinose (11.0%) and D-glucose (18%). The mother liquor from the formic acid hydrolysis contained L-arabinose, identified as the benzylphenylhydrazone, m.p. and mixed m.p. 166–167°.

In periodate oxidation studies 0.67 mole of sodium periodate was consumed per anhydropentose residue. The polyaldehyde so formed, without previous isolation, was reduced with a pressure (1500 lb. per sq. inch) of hydrogen at 80–90° for 7 hours using a Raney nickel catalyst.[12] The resulting solution was made 1 *N* with respect to sulfuric acid and heated under reflux for 15 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 1-butanol–ethanol–water (4:1:5) as the developing solvent indicated the presence of xylose; no glucose or arabinose was detected.

Methylation of Wheat Pentosan.—A portion (6 g.) of the acetate (fraction 4, Table I) was dissolved in acetone (300 ml.) to which solution was added 45% potassium hydroxide (45 ml.). Methyl sulfate (150 ml.) and 45% potassium hydroxide (450 ml.) were added in aliquot tenths over a period of 3 hours during which time the temperature was gradually increased during 2.5 hours from room temperature (for the first 30 min.) to 58°. The reaction mixture was then heated on a boiling water-bath for one hour when the product separated in a granular form. The product was filtered, washed with hot water and redissolved in acetone (250 ml.) and 1,4-dioxane (100 ml.). The second methylation was carried out at 50–60° and the material isolated as before. Four more methylations were applied in the same way using, however, acetone alone (400 ml.) as the solvent.

After the final methylation the product was dissolved in 50% aqueous 1,4-dioxane (400 ml.) and dialyzed against distilled water, during which procedure a yellow precipitate separated (0.5 g.) insoluble in chloroform but soluble in acetone, $[\alpha]^{25}D$ −132° in acetone (*c* 1.25). The mixture was extracted several times with chloroform and the combined extracts after drying over magnesium sulfate were concentrated. The methylated product was precipitated from the chloroform concentrate with petroleum ether: yield 2.77 g., $[\alpha]^{25}D$ −169° in acetone (*c* 0.29). A specific rotation of $[\alpha]D$ −165° in acetone has been quoted[15] for the methylated "squeegee" pentosan and one of −160° for the methylated barley pentosan.[16]

Fractional Precipitation of the Methylated Polysaccharide.—The material from the previous experiment was dissolved in acetone (200 ml.) and fractionally precipitated by adding ether (100 ml.) followed by increasing amounts of petroleum ether. Each precipitated fraction was dissolved in acetone and reprecipitated by pouring the solution into an excess of petroleum ether. The fractions obtained are shown in Table III.

TABLE III

FRACTIONAL PRECIPITATION OF METHYLATED WHEAT PENTOSAN

Fraction	Wt. (g.)	$[\alpha]^{22}D$ in acetone	OCH_3 (%)
1	0.6	−115°	34.6
2	0.1	−167°	..
3	0.7	−160°	36.4
4	1.0	−175°	38.0
5	0.6	−176°	38.8
6 (mother liquor)	0.1	−105°	..

Hydrolysis of the Methylated Wheat Pentosan.—A portion (0.46 g.) of fraction 5 (Table III) was heated under reflux with 5% methanolic hydrogen chloride (45 ml.) for 27 hours, ($[\alpha]^{23}D$ +9°, constant after 23 hours). The solution was neutralized with silver carbonate, filtered and evaporated under reduced pressure at 30° to a sirup (0.5 g.). The sirup was dissolved in water (50 ml.) and the solution was continuously extracted with petroleum ether (b.p. 30–60°) for 30 hours, the extract being continuously back-extracted with water. The petroleum ether extract, containing 0.195 g. of methyl glycosides, was hydrolyzed with 0.1 *N* sulfuric acid at 95–98° to constant optical rotation $[\alpha]^{26}D$ −54°. The hydrolysate was neutralized with barium carbonate, filtered and evaporated under reduced pressure at 35°. The residue was extracted with acetone, filtered and evaporated to a sirup (0.102 g.); $n^{26}D$ 1.4471, $[\alpha]^{26}D$ −25.0° in water (*c* 1.0), OCH_3, 43.2. Chromatographic analysis using methyl ethyl ketone–water azeotrope indicated the presence of a dimethylxylose and trimethylarabofuranose.

The mixture of glycosides not extracted by petroleum ether (0.231 g.) was hydrolyzed with *N* sulfuric acid at 95–100° for 12 hours, $[\alpha]D$ +23°, constant after 9 hours. The solution was neutralized with barium carbonate, filtered and evaporated under reduced pressure to a sirup which was extracted with acetone. The acetone extract contained a mixture of sugars (0.211 g.) composed of xylose, monomethyl- and dimethylxylose. The residue not extracted by acetone (0.033 g.) consisted principally of xylose with traces of monomethyl- and dimethylxylose.

Separation of the Cleavage Products from the Methylated Wheat Pentosan.—The mixtures of sugars obtained by the above extraction procedure were extracted with methyl ethyl ketone–water azeotrope (3 ml.) and the resulting solution chromatographed on a cellulose–hydrocellulose column in the usual way using methyl ethyl ketone–water azeotrope as the irrigating solvent.[14] The sugars which did not dissolve in the methyl ethyl ketone–water azeotrope (0.079 g.) were separated on Whatman No. 1 sheets.

The fractions resulting from the chromatographic separations and their properties are summarized in Tables IV and V. The weight of each fraction represents that amount of material obtained after acetone extraction of both the column and paper chromatographic fractions. This final purification step removed most of the impurities which are washed from the cellulose adsorbents.

Optical Rotations of Standard Sugars.—In connection with the purity of the fraction of sugars summarized in

TABLE IV

SEPARATION OF THE METHYL ETHYL KETONE–WATER AZEOTROPE EXTRACT OF THE CLEAVAGE PRODUCTS OF METHYLATED WHEAT PENTOSAN ON A CELLULOSE–HYDROCELLULOSE COLUMN

Sugar	Wt. (mg.)	$[\alpha]^{25}D$
2,3,5-Tri-*O*-methyl-L-arabofuranose	44.4	−37.8° in H_2O (*c* 1.5)
Unknown A	9.3	+82.8° in MeOH (*c* 1.9)
Unknown B	2.1	−72.8° in MeOH (*c* 0.2)
2,3-Di-*O*-methyl-D-xylose	93.3	+20.8° in H_2O (*c* 2.3)
3-*O*-Methyl-D-xylose	3.4	+14.7° in MeOH (*c* 0.7)
2-*O*-Methyl-D-xylose	26.3	+25.4° in MeOH (*c* 0.8)

TABLE V

SEPARATION OF THE METHYL ETHYL KETONE–WATER AZEOTROPE INSOLUBLE FRACTION OF THE CLEAVAGE PRODUCTS OF METHYLATED WHEAT PENTOSAN ON FILTER PAPER (WHATMAN NO. 1)

Sugar	Wt. (mg.)	$[\alpha]^{25}D$
2,3,5-Tri-*O*-methyl-L-arabofuranose	4.0	−12.5° in H_2O (*c* 0.8)
2,3-Di-*O*-methyl-D-xylose	9.9	+20.8° in H_2O (*c* 2.3)
2-*O*-Methyl-D-xylose	17.3	+20.8° in MeOH (*c* 0.6)
D-Xylose	30.1	+18.1° in MeOH (*c* 0.6)

Tables IV and V, the optical rotations of the following sugars were determined in methanol

2,3,4-Tri-*O*-methyl-D-xylose	$[\alpha]^{27}D$ +68.0° (initial) → +18.5° (final); *c* 1.0
3-*O*-Methyl-D-xylose	$[\alpha]^{26}D$ +34.3° (initial) → +13.7° (final); *c* 1.2
2-*O*-Methyl-D-xylose	$[\alpha]^{26}D$ −28.5° (initial) → +28.5° (final); *c* 0.7
D-Xylose	$[\alpha]D$ +33.3° (final); *c* 1.0

Analysis of the Mixture of Methylated Sugars. (a) **By Paper Chromatography.**—The mixture of methylated sugars (0.80 g.) derived from the methylated polysaccharide (fraction 4, 0.80 g.) was dissolved in 20% aqueous methanol (25 ml.). An aliquot of this solution was quantitatively analyzed[11] and the relative amounts of the methyl sugars were found to be as follows: 2,3,5-tri-*O*-methyl-L-arabinose (12.9 moles), 2,3-di-*O*-methyl-D-xylose (18.7 moles), 2-*O*-methyl-D-xylose (6.0 moles), and D-xylose (4.0 moles). In additional experiments, the mole ratio of 2-*O*-methyl-D-xylose and D-xylose was checked further by developing the chromatograms until the monomethyl sugar was near to the bottom of the paper. This provided better separation of the two sugar bands and consequently the results were more reliable.

(b) **Column Chromatography.**—The solution of methylated sugars, remaining after the analyses by paper chromatography described in (a) had been carried out, was evaporated *in vacuo* to a sirup (0.7 g.) which was extracted with methyl ethyl ketone–water azeotrope (3 ml.) and the resulting solution chromatographed on a cellulose–hydrocellulose column[14] in the usual way with methyl ethyl ketone–water azeotrope as the irrigating solvent. Examination of the fractions furnished the results in Table VI.

TABLE VI

SEPARATION OF THE METHYL ETHYL KETONE–WATER AZEOTROPE EXTRACT OF THE CLEAVAGE PRODUCTS OF METHYLATED WHEAT PENTOSAN ON THE CELLULOSE HYDROCELLULOSE COLUMN WITH METHYL ETHYL KETONE–WATER AZEOTROPE

Sugar	Wt. (g.)	R_A[a]	$[\alpha]^{24}D$
2,3,5,-Tri-*O*-methyl-L-arabofuranose	0.2493	1.00	−28.7° (MeOH); *c* 2.2
Unknown C	.0038	0.90	+ 9.0° (MeOH); *c* 1.2
Unknown B	.0098	.77	+28.6° (MeOH); *c* 0.2
2,3-Di-*O*-methyl-D-xylose	.2973	.65	+26.9° (H_2O); *c* 3.0
Unknown E	.0109	.53	.....
2-*O*-Methyl-D-xylose	.0922	.21	+29.5° (MeOH); *c* 2.3

[a] The movement of the sugar, using methyl ethyl ketone–water azeotrope as the developing solvent is recorded relative to the movement of 2,3,5-tri-*O*-methyl-L-arabinose.

Examination of the Cleavage Products of Methylated Wheat Pentosan. (1) **Identification of 2,3,5-Tri-*O*-methyl-L-arabinose.**—The trimethyl-L-arabinose (0.24 g.) was dissolved in water (15 ml.) and treated with bromine (0.5 ml.) at room temperature for 7 days. Chromatographic analysis on paper, using methyl ethyl ketone–water azeotrope as the developing solvent, indicated that unoxidized trimethyl-L-arabinose was still present. The reaction was therefore continued at 50–60° for 10 hours when the reaction was complete. The solution was freed from bromine by aeration, neutralized (Ag_2CO_3), filtered and passed through a column of "Amberlite IR 120" cation-exchange resin. The eluate was concentrated *in vacuo* and the sirupy residue extracted with ether. Evaporation of the ether extract gave the sirupy 2,3,5-tri-*O*-methyl-L-arabono-γ-lactone (0.17 g.) which distilled, b.p. (bath temp.) 120°, 0.01 mm., to give a partially crystalline product, $[\alpha]^{21}D$ −33° initial value in water (*c* 1.2), changing to $[\alpha]^{17}D$ −22° in 140 hours (mutarotation incomplete). The crystals, separated mechanically from the associated sirup, showed $[\alpha]^{21}D$ −41° initial value in water (*c* 1.1), changing to $[\alpha]^{21}D$ −38° in 20 hours (mutarotation incomplete).

When treated with methanolic ammonia in the usual way the lactone gave 2,3,5-tri-*O*-methyl-L-arabonamide, m.p. and mixed m.p. 138°, $[\alpha]^{22}D$ +17° in water (*c* 0.8) (after recrystallization from ethyl acetate–light petroleum ether).

(2) **Identification of 2,3-Di-*O*-methyl-D-xylose.**—Treatment of the di-*O*-methyl-D-xylose (0.10 g.) with aniline (0.09 ml.) in boiling ethanol (3 ml.) for 2 hours, followed by removal of the solvent *in vacuo* gave a crystalline residue. Two recrystallizations from ethyl acetate–petroleum ether gave 2,3-di-*O*-methyl-D-xylose anilide, m.p. and mixed m.p. 136°, $[\alpha]^{21}D$ +180° in ethyl acetate (*c* 0.7).

(3) **Identification of 2-*O*-Methyl-D-xylose.**—The crystalline material from the column separations was recrystallized from ethanol giving 2-*O*-methyl-D-xylose, m.p. and mixed m.p. 129–130°, $[\alpha]^{22}D$ −21° initial value in methanol (*c* 0.5) changing to $[\alpha]^{22}D$ +26° (constant value).

(4) **Identification of D-Xylose.**—The crystalline material from the column was recrystallized from methanol giving D-xylose, m.p. and mixed m.p. 149°, $[\alpha]^{22}D$ +40° after 10 minutes, changing to +28°, equilibrium value in methanol (*c* 1.7). The mother liquor from the recrystallization of the D-xylose was converted to the dibenzylidene dimethyl acetal of D-xylose, m.p. and mixed m.p. 208–209°, $[\alpha]^{24}D$ −8° in chloroform (*c* 0.9).[17]

Examination of the Unknown Components Obtained from the Methylated Wheat Pentosan.—The unknowns A and B, Table IV, and unknowns C, D and E, Table VI were each rehydrolyzed as follows. The unknown fraction was heated at 80–95° in a sealed tube with *N* sulfuric acid for 12 hours. After removing the acid with "Duolite A4" anion-exchange resin, the solution was evaporated *in vacuo*. The residue was subjected to paper chromatographic analysis using methyl ethyl ketone–water azeotrope as the developing liquid.

Unknown A, Table IV, R_A[18] 0.96, gave principally 2,3-di-*O*-methyl-D-xylose together with small amounts of 2-*O*-methyl-D-xylose, D-xylose and unknown E.

Unknown B, Table IV, R_A[18] 0.76, gave small amounts of 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose and D-xylose together with unchanged material.

Unknown C, Table VI, hydrolyzed to give principally 2-*O*-methyl-D-xylose together with smaller amounts of 2,3-di-*O*-methyl-D-xylose, D-xylose, unknown B and unchanged material.

Unknown D, Table VI, produced 2-*O*-methyl-D-xylose and unknown B.

Unknown E, Table VI, gave principally D-xylose, and 2-*O*-methyl-D-xylose together with 2,3-di-*O*-methyl-D-xylose and unchanged material.

It would appear that the above unknown components represent small amounts of partially degraded methylated polysaccharide.

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(17) L. J. Breddy and J. K. N. Jones, *J. Chem. Soc.*, 738 (1945).

(18) See footnote a, Table VI.