[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic Acid)-D-xylose from Hemicellulose-B of Corn Cob[1,2]

BY ROY L. WHISTLER, H. E. CONRAD AND L. HOUGH

RECEIVED JUNE 1, 1953

Partial acid hydrolysis of hemicellulose-B produces a mixture of monosaccharides and oligosaccharides. The latter contain D-xylose units and uronic acid units. On separation of these acidic oligosaccharides there are obtained an aldotriouronic acid, three underivafied aldobiouronic acids and a fourth derivafied aldobiouronic acid which is shown to be a 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

Hemicelluloses on fractionation are found to be mixtures of polysaccharides.[3] To obtain further information on the hemicellulose-B portion of corn cobs,[4] it is fractionally precipitated from water solution by addition of acetone to produce 13 fractions which differ in uronic acid, arabinose and xylose contents (Table I). Acetylated hemicellulose-B is also fractionated from chloroform solution by addition of pentane. Here too, the fractions appear to be different on the basis of observed optical rotation, viscosity and uronic acid content (Table II). Hemicelluloses of wheat straw are likewise found[5,6] to be mixtures difficult to separate. After hydrolysis of hemicellulose-B qualitative paper chromatography indicates the presence of xylose, arabinose, galactose, glucose, glucuronic acid and oligosaccharides containing uronic acids.

Through the use of cellulose or charcoal columns it is possible to separate from the hydrolysate of hemicellulose-B identifiable quantities of four oligosaccharide fractions which contain xylose and uronic acid residues. Separation is facilitated if the crude hydrolysate is first passed through a column of an anion exchange resin to remove the acidic oligosaccharides which are later eluted by dilute sulfuric acid. Preliminary analysis of the four oligosaccharide fractions suggests that the first (A) is a mono-*O*-methylaldobiouronic acid, the second (B) is an aldobiouronic acid, the third (C) is a mixture of two aldobiouronic acids and the fourth (D) is an aldotriouronic acid.

This paper describes the structural characterization of the mono-*O*-methylaldobiouronic acid (A). Since the disaccharide hydrolyzes with considerable difficulty and concomitant destruction, it is expedient to convert it to the methyl ester methyl glucoside which is reduced with lithium aluminum hydride to an easily handled neutral disaccharide.[7] This mono-*O*-methylglucosylxylose is readily hydrolyzed to D-xylose and 4-*O*-methyl-D-glucose. Thus the methoxyl group of the aldobiouronic acid is linked to carbon C_4 of the D-glucuronic acid portion. 4-*O*-Methyl-D-glucuronic acid has been found as a component of mesquite gum,[8] gum myrrh[9] and aspen wood hemicellulose.[10] Mono-*O*-methylhexuronic acids have been detected in other hemicelluloses but have not been identified.[6,11-15]

Lithium aluminum hydride reduction of the fully methylated aldobiouronic acid followed by a second methylation produces a fully methylated glucosylxylose which on hydrolysis yields 3,4-di-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-

TABLE I

SOME PROPERTIES OF HEMICELLULOSE-B FRACTIONS

Fraction	Acetone added, l.	Yield, %	Uronic acid anhydro units, %	η_1	Moles xylose per mole arabinose
1	0	9.2	5.1	0.40	10.7
2	0.5	2.8	8.0	.53	...
3	1.5	3.3	4.5	.47	...
4	4	9.6	7.5	.52	10.3
5	6	7.0	8.1	.52	7.9
6	8.5	2.5	9.1	...	6.0
7	11	6.5	8.3	.51	6.3
8	12	7.0	8.5	.71	5.3
9	13	4.6	8.7	.65	...
10	14	1.7	5.2	.51	5.2
11	16	3.6			
12	18	4.7	7.0	.37	...
13	22.5	4.7			

TABLE II

SOME PROPERTIES OF ACETYLATED HEMICELLULOSE-B FRACTIONS

Fraction	Petroleum ether, l.	Yield, %	Uronic acid anhydro units, %	$[\alpha]^{25}D$	η_1
1	2.3	2.2	...	...	...
2	2.7	46.5	5.2	...	0.71
3	3.0	13.3	4.1	−100	.63
4	3.5	9.0	4.7	−101	.42
5	4.0	6.3	3.3	− 89	.38
6	4.7	6.1	2.8	− 83	.34
7	5.7	5.9	3.4	− 80	.25

(1) Journal Paper No. 722 of the Purdue Agricultural Experiment Station.

(2) Paper presented before the Division of Carbohydrate Chemistry at the 123rd Meeting of the American Chemical Society at Los Angeles, Calif., March, 1953.

(3) R. L. Whistler and C. L. Smart, "Polysaccharide Chemistry," Academic Press, Inc., New York, N. Y., 1953.

(4) R. L. Whistler, J. Bachrach and D. R. Bowman, *Arch. Biochem.*, **19**, 25 (1948).

(5) V. D. Harwood, *Can. J. Chem.*, **29**, 974 (1951).

(6) G. A. Adams and A. E. Castagne, *ibid.*, **29**, 109 (1951); **30**, 515 (1952).

(7) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(8) E. V. White, THIS JOURNAL, **70**, 367 (1948).

(9) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 796 (1952)

(10) J. K. N. Jones and L. E. Wise, *ibid.*, 2750 (1952).

(11) L. Sands and W. Y. Gary, *J. Biol. Chem.*, **101**, 573 (1933).

(12) E. Anderson, J. Kesselman and E. C. Bennett, *ibid.*, **140**, 563 (1941).

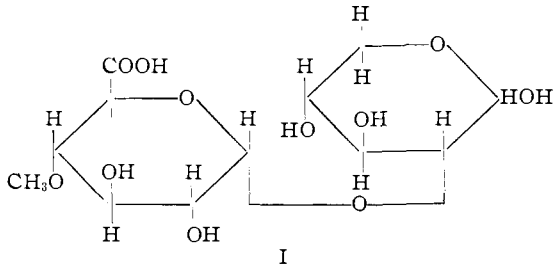
(13) E. Anderson, R. B. Kaster and M. G. Seeley, *ibid.*, **144**, 767 (1942).

(14) M. O'Dwyer, *Biochem. J.*, **33**, 713 (1939).

(15) P. B. Sarker, *Nature*, **167**, 357 (1951).

glucose which are identified by conversion to appropriate crystalline derivatives.

Since the disaccharide exhibits a high positive specific optical rotation (+95°), it is presumed that there is present an α-glycosidic linkage. If such is the case, the aldobiouronic acid is 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose (I) and is identified with that found in aspen wood hemicellulose.[16]



I

Experimental

Paper Chromatography.—Separations were performed on Whatman No. 1 filter paper at 25° by the descending method,[17] using one of the following solvents in the volume ratios as indicated: (A) butanol-1-ethanol-water (40:11:19), (B) ethyl acetate–acetic acid–water–formic acid (18:3:4:1), (C) benzene–ethanol–water (169:47:15; upper layer), (D) ethyl acetate–pyridine–water (2:1:2). *p*-Anisidine hydrochloride[18] was used to detect the sugars and their derivatives on paper chromatograms.

Hemicellulose-B.—The procedure of Whistler, Bachrach and Bowman[19] was used for the preparation of hemicellulose-B. Powdered corn cobs were delignified and the resultant holocellulose extracted with 10% potassium hydroxide solution. The extract was brought to *p*H 5.0 by adding 50% acetic acid and the precipitated hemicellulose-A (crude xylan) removed by centrifugation. Addition of two volumes of 95% ethanol to the supernatant liquor precipitated hemicellulose-B. The filtered product was dried by vigorous stirring with fresh ethanol four successive times and finally dried in a vacuum desiccator over calcium chloride. The product contained 8.9% uronic acid anhydro units and paper chromatography of the products obtained on complete hydrolysis suggested the presence of xylose, arabinose, galactose, glucose and uronic acid derivatives.

Fractionation of Hemicellulose-B.—Hemicellulose-B (76 g.), purified by solution in 0.5% potassium hydroxide and reprecipitation by neutralization with acetic acid and addition of alcohol was suspended in water (7.6 l.) and autoclaved at 15 lb. pressure for 0.5 hour. The suspension was cooled and the insoluble material (7 g.) removed by supercentrifugation. Acetone was slowly added with stirring to the supernatant solution until a cloudy suspension was obtained. After standing at least 6 hours at 5° the precipitate was removed by supercentrifugation. Subsequent fractions were obtained in the same manner by graded increases in the acetone concentration. Each fraction was redissolved in water and reprecipitated by addition of acetone to the neutralized solution. Precipitates were filtered, washed with acetone and ether and dried at 100° under reduced pressure. The fractions were examined for uronic acid anhydro unit content,[20] viscosity[21] and ratio of xylose to arabinose.[22] Results are shown in Table I. Paper chromatograms of hydrolysates of these fractions suggest that glucose and galactose appear in small amounts in fraction 5 and increase in quantity with increased solubility of the fraction. In fractions 5 to 8 glucose seems to predominate, whereas in the later fractions galactose predominates.

Fractionation of Acetylated Hemicellulose-B.—Hemicellulose-B was acetylated in formamide, pyridine and acetic anhydride mixture.[23] The acetate (49 g.) was dissolved in a mixture of chloroform (4 l.) and 95% ethanol (1 l.). Fractionation by graded addition of pentane (b.p. 30–60°) gave 7 fractions which were removed by filtration, washed with pentane and dried at 70° under reduced pressure. Analyses[20,21] are given in Table II.

Partial Hydrolysis.—Hemicellulose-B (50 g.) was suspended in water (2 l.) and stirred at 80° overnight. A 20% solution of sulfuric acid (500 ml.) was then added and the course of the reaction followed by polarimetric observations: $[\alpha]^{25}D$ +5.5° (1.5 hours) → +33° (2.5 hours) → +40° (3.5 hours) → +43° (5.5 hours) → +43° (6 hours). After six hours the hydrolysis was stopped since at this point the change in optical rotation and reducing power, as determined iodometrically, was negligible; suggesting the presence of acid-stable aldobiouronic acid(s). Paper chromatography of aliquots taken during the course of the hydrolysis also suggested that a maximum yield of aldobiouronic acids occurred six hours after hydrolysis began. This mixture was brought to *p*H 6.5 by careful addition of a hot solution saturated with barium hydroxide. Barium sulfate was removed by filtration through Celite and the clear yellow filtrate passed through a column of Amberlite IR-120 (4 × 47 cm.) to remove cations and then through a column (5 × 54 cm.) of IR-4B where anions, including uronic acid derivatives, were absorbed. The IR-4B resin was washed with about 20 l. of water to remove pentoses which were detected by the furfural–aniline acetate method.[24] The uronic acid derivatives were then displaced from the resin by stirring it for one hour with an excess of 2 *N* sulfuric acid. After filtration the resin was washed twice with water. The combined filtrate and washings were brought to *p*H 6.5 with saturated barium hydroxide solution and filtered. The filtrate was passed through a column of Amberlite IR-120 to remove barium ions. The acidic effluent was concentrated at 30° under reduced pressure to a thick sirup (3 g.). A paper chromatogram using solvent B showed at least four components with R_X values[25] of 0.79, 0.46, 0.32 and 0.14. All gave bright red colors with *p*-anisidine hydrochloride spray reagents.

Separation on a Cellulose Column.—The above sirup was separated on a column of cellulose[18] (4.5 cm. diameter, 45 cm. long) using ethyl acetate–acetic acid–water (9:2:2) as mobile phase. The cellulose column was prepared from Whatman ashless filter tablets by homogenizing with acetone in a Waring blendor. The resultant sludge was poured into a glass column and packed under pressure. The effluent was collected on an automatic fraction collector and after examination of paper chromatograms was grouped into four separate fractions. Each fraction was extracted with an equal volume of water, the aqueous phase separated and the organic phase discarded. The aqueous solution was extracted four times with an equal volume of ether to remove acetic acid and ethyl acetate, and then evaporated to about 100 ml. at 30–35° under reduced pressure. The solution was extracted continuously with ether for five hours to remove any remaining acetic acid; it was then evaporated to dryness. Equivalent weights were determined by conversion to the barium salt and measurement of the organically-bound barium. Table III shows the yields and properties of the four fractions. These results suggest that fractions

TABLE III

ALDOBIOURONIC FRACTIONS FROM HYDROLYSIS OF HEMICELLULOSE-B

Fraction	Yield (%) from Hemicellulose-B	$[\alpha]^{25}D$	Equivalent weight	$R_X^{25°}$ (solvent B)
A	0.9	+95°	330	0.79
B	0.1	+53	321	.46
C	1.4	+59	352	.32
D	0.5	+39	478	.14

(16) J. K. N. Jones and L. E. Wise, *J. Chem. Soc.*, 3389 (1952).

(17) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(18) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(19) See reference 4.

(20) R. L. Whistler, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **24**, 13 (1940).

(21) M. A. Millet and A. T. Stamm, *J. Phys. Colloid Chem.*, **51**, 134 (1947).

(22) E. Borel, F. Hostettler and H. Deuel, *Helv. Chim. Acta*, **35**, 115 (1952).

(23) J. F. Carson and W. D. Maclay, THIS JOURNAL, **68**, 1015 (1946).

(24) "Polarimetry, Saccharimetry and the Sugars," U. S. Government Printing Office, Washington, D. C., 1942, p. 242.

(25) Rate of movement relative to xylose (R_X 1.0).

A, B and C are aldobiouronic acids, and fraction D an aldotriouronic acid.

Characterization of Fraction A.—Chromatography of this fraction on filter paper with solvent B showed that the fraction moved faster than glucuronic and galacturonic acids, which suggested[16] the presence of a methyl ether group.

Anal. Calcd. for $C_{12}H_{20}O_{11}$: OMe, 9.1; equiv. wt., 340. Found: OMe, 8.3; equiv. wt., 330.

After hydrolysis of the aldobiouronic acid with 1 *N* sulfuric acid for 24 hours, paper chromatography with solvent B indicated that the hydrolysate contained unchanged disaccharide, xylose and a small amount of monomethylglucuronic acid. Apparently most of the freed hexuronic acid was decomposed during the hydrolysis.

Another portion of monomethylaldobiouronic acid (1.2 g.) was refluxed with 2% methanolic hydrogen chloride (50 ml.) for 6 hours. After neutralization with silver carbonate, filtration and evaporation, the methyl ester methyl glycoside was obtained. It was dissolved in tetrahydrofuran and reduced with lithium aluminum hydride.[7,26,27] After one hour the excess lithium aluminum hydride was decomposed by addition of ethyl acetate and the mixture diluted with water (50 ml.). The organic solvents were evaporated, the solution filtered and the filtrate de-ionized with Amberlite resins IR-4B and IR-120. The neutral solution was evaporated to a sirup, which was hydrolyzed with 1 *N* sulfuric acid for 10 hours at 100°. After hydrolysis the solution was neutralized with Amberlite IR-4B and evaporated to a sirup (0.82 g.). On paper chromatograms it gave two spots corresponding to D-xylose and 4-*O*-methyl-D-glucose. The mixture was separated[28] on four sheets of Whatman No. 1 filter paper (18.5 × 22.5 in.) by irrigation with solvent A for 48 hours. Extraction of the appropriate strips of paper with methanol in a Soxhlet apparatus gave on evaporation crystalline D-xylose (0.22 g.), m.p. and mixed m.p. 143°, $[\alpha]^{25}D$ +58° (*c* 1.6, water), and sirupy 4-*O*-methyl-D-glucose.

Anal. Calcd. for $C_7H_{14}O_6$: OMe, 16.0. Found: OMe, 15.2.

The methyl-D-glucose on paper chromatograms gave the same color reactions as authentic 4-*O*-methyl-D-glucose and had the same R_x values of 1.80, 1.19 and 1.15 in solvents A, B and D, respectively. The methylglucose (78 mg.) was dissolved in water (2 ml.), acetic acid (0.2 ml.) and phenylhydrazine (0.2 ml.), heated at 80° for 3 hours and cooled. Crystals (57 mg.) which were filtered, washed with benzene and recrystallized from hot benzene, had m.p. 159° and $[\alpha]^{25}D$ +25° → +4° (final, *c* 1.21, ethanol).[29] Final evidence that this derivative was 4-*O*-methyl-D-glucosazone was obtained by comparison of the X-ray diffraction pattern with that of a known specimen and that the melting point (158°) was not lowered on mixture with an authentic specimen.

Anal. Calcd. for $C_{19}H_{26}O_4N_4$: OMe, 8.3; N, 15.0. Found: OMe, 8.2; N, 15.2.

The mono-*O*-methylaldobiouronic acid (0.82 g.) was dissolved in water (5 ml.) containing dimethyl sulfate (2 ml.) and methylated by adding 30% sodium hydroxide solution (5 ml.) dropwise over 5 hours with stirring and cooling (ice-water). After stirring for a further 16 hours the solution no longer reduced Fehling solution, thus indicating that methyl glycoside formation was complete. An excess of 30% sodium hydroxide solution (10 ml.) was then added to the reaction mixture and methylation continued by dropwise addition of dimethyl sulfate (5 ml.) over 8 hours and stirring continued for a further 16 hours. The solution was acidified with 2 *N* sulfuric acid and extracted continuously for 16 hours with chloroform. Evaporation of the chloroform extract gave a sirup (0.61 g.) which was further methylated by two treatments with silver oxide (2 g.) and methyl iodide (5 ml.). The product (0.44 g.) had $[\alpha]^{25}D$ +90° (*c* 2.18, chloroform).

Anal. Calcd. for $C_{18}H_{32}O_{11}$: OMe, 51.2. Found: OMe, 51.3.

It was reduced with lithium aluminum hydride in ether as described previously and then methylated with silver oxide and methyl iodide to give a sirup (0.39 g.) with $[\alpha]^{25}D$ +94.5° (*c* 2.98, chloroform).

Anal. Calcd. for $C_{18}H_{34}O_{10}$: OMe, 52.9. Found: OMe, 52.2.

The fully methylated disaccharide (0.37 g.) was hydrolyzed by consecutive treatments with boiling 4% methanolic hydrogen chloride (25 ml.) for 15 hours and, after evaporation of the methanol under reduced pressure at room temperature, 1 *N* sulfuric acid at 100° for 6 hours. After cooling, the solution was neutralized by addition of Amberlite IR-4B, filtered and evaporated under reduced pressure. The residue was observed on a paper chromatogram to be a mixture of two components corresponding to 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. These sugars (0.30 g.) were separated[28] on four large sheets of paper (18.5 × 22.5 in.) using solvent A as mobile phase. 2,3,4,6-Tetra-*O*-methyl-D-glucose (102 mg.) was obtained crystalline from ether–pentane and it had m.p. and mixed m.p. 96°, $[\alpha]^{25}D$ +83° (*c* 1.02, water). An X-ray diffraction photograph of the crystals was identical with that of a known specimen.

Anal. Calcd. for $C_{10}H_{20}O_6$: C, 50.8; H, 8.5. Found: C, 51.0; H, 8.5.

The characteristic aniline derivative of this sugar was prepared[30] and crystallized from light petroleum with m.p. 114° and $[\alpha]^{25}D$ +207° (*c* 1.06, acetone). J. K. N. Jones and L. E. Wise[16] record a m.p. 114° and $[\alpha]D$ +200°.

3,4-Di-*O*-methyl-D-xylose was obtained as a sirup (72.2 mg.) with $[\alpha]^{25}D$ +12° (*c* 0.58, water). It was clearly distinguished from 2,4-di-*O*-methylxylose and 2,3-di-*O*-methylxylose by paper chromatography[16] as can be clearly seen in Table IV.

TABLE IV

Solvent	R_g^{25} value of di-*O*-methyl xylose derivatives		
	2,4-	2,3-	3,4-
A	0.775	0.84	0.77
B	.82	.855	.83
C	.23	.25	.33
D	.82	.86	.85

The reducing sugar (65 mg.) was oxidized with bromine water for 48 hours when bromine was removed by aeration, and the solution neutralized with silver carbonate. After filtration of silver bromide, silver ions were removed from the solution by passing hydrogen sulfide and filtering the insoluble silver sulfide. Evaporation of the filtrate under reduced pressure yielded crystals of 3,4-di-*O*-methyl-D-xylonolactone (56 mg.). After recrystallization from ether the m.p. was 68–69° and $[\alpha]^{25}D$ −53° (*c* 2.8, water) → −22° (40 hours). X-Ray diffraction pattern of the compound was identical with a known sample.

Anal. Calcd. for $C_7H_{12}O_5$: OMe, 35.2; C, 47.7; H, 6.9. Found: OMe, 34.7; C, 48.1; H, 7.4.

Acknowledgment.—We wish to thank Dr. J. K. N. Jones for samples of 2,4-di-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 3,4-di-*O*-methyl-D-xylonolactone.

LAFAYETTE, INDIANA

(26) M. Abdel Akher and F. Smith, *Nature*, **166**, 1037 (1950).

(27) S. Roseman, THIS JOURNAL, **74**, 4467 (1952).

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

(29) F. Smith, *ibid.*, 2646 (1951).

(30) M. L. Wolfrom and W. L. Lewis, THIS JOURNAL, **50**, 837 (1928).