Anal. Calcd. for $C_7H_{14}O_7$: C, 40.0; H, 6.71. Found (dried sample): C, 39.88; H, 6.80.

D-*allo*-**Heptulose (IV).**—D-*allo*-Heptulose was obtained, in small yield, from a mixture of epimeric D-alloheptonolactones by buffered sodium amalgam reduction, followed by alkaline rearrangement of the resulting sirupy mixture of sugars.[15] The sugar appeared to be solvated and so was dried *in vacuo* at 100° for 1.5 hours whereupon it melted at 130–132° and showed $[\alpha]^{20}D$ in water (*c* 0.2) +52.8° (11 min.) with no mutarotation.

Anal. Calcd. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: 39.98; H, 6.67.

D-*glycero*-D-*altro*-**Heptitol (Synonym, D-*glycero*-L-*allo*-Heptitol) (VII).**—Sodium amalgam reduction of D-*glycero*-D-*altro*-heptono-γ-lactone (II) yielded a sirup (V) which failed to crystallize. Raney nickel-catalyzed high-pressure hydrogenation of this sirup produced 11.3 g. (56%) of white, extremely small needles which melted at 125–128° and showed $[\alpha]^{20}D$ −0.3 ± 0.4° in water (*c* 1.2); +53.2° in 5% ammonium molybdate (*c* 0.4); −17.3° in acidified molybdate (*c* 0.32).[11]

(15) W. C. Austin, THIS JOURNAL, **52**, 2106 (1930).

Anal. Calcd. for $C_7H_{16}O_7$: C, 39.62; H, 7.60. Found: C, 39.49; H, 7.62.

D-*talo*-**Heptulose (IX).**—By the procedure reported above for the preparation of L-*allo*-heptulose, 9.5 g. of D-*glycero*-D-*altro*-heptitol (VII) was oxidized with *A. suboxydans*. Estimation of reducing sugar showed a value of 79% of the theoretical maximum in 6 days (assuming the same reducing activity as D-*manno*-heptulose), and this was unchanged on further incubation for 3 days. After appropriate purification the reaction mixture yielded 5 g. (52%) of clusters of prismatic rods. On recrystallization from 90% ethanol the sugar melted at 135–137° and showed $[\alpha]^{20}D$ in water (*c* 1.6) +47.4° (2 min.) → +12.9° (6 hr.; constant).

Anal. Calcd. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: C, 40.02; H, 6.65.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Oligosaccharides from Partial Acid Hydrolysis of Corn Fiber Hemicellulose[1,2]

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Treatment of corn fiber hemicellulose with mild acid causes the preferential hydrolysis of side chains which consist essentially of L-arabofuranose units. Besides L-arabinose, the hydrolyzate contains 3-*O*-α-D-xylopyranosyl-L-arabinose and L-galactopyranosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinose which are possibly part of the side chains. These two oligosaccharides have been obtained crystalline.

In continuation of the researches of this Laboratory toward the elucidation of the structures of the hemicelluloses, corn fiber hemicellulose has now been examined. This hemicellulose, commercially termed corn fiber gum because of its physical properties, is industrially prepared by lime water extraction of corn hull residues obtained from the wet milling process. Since classical methylation studies only reveal in a general way the types of linkages connecting the various sugar units and not the order of sugar units in a heteroglycan, it is necessary to examine the partial acid hydrolysis products to obtain more complete information on polysaccharide structures.

Corn fiber hemicellulose can be hydrolyzed with normal sulfuric acid at 35°. The hydrolysis proceeds readily with the formation of xylose, arabinose and galactose together with a di- and a trisaccharide. From the rate of hydrolysis it is evident that the structure differs considerably from that of hemicellulose-B of corn cob[3] but is similar to that of the hemicellulose isolated from wheat bran.[4] Paper chromatographic examination of the partial hydrolyzate shows that the oligosaccharides are in highest concentration when the hydrolysis solution attains $[\alpha]^{25}D$ −25° after approximately 24 hr. The polysaccharide isolated in 65% yield from the neutralized hydrolyzate stopped at this stage has a lower arabinose:xylose ratio than the original hemicellulose. Separation of the hydrolyzate into its components is achieved by successive chromatography on columns of charcoal[5] and cellulose.[6] The main component isolated in this way is β-L-arabinose in 11% yield. This, with the recovery of the polysaccharide in high yield, indicated that the prime action of the acid is the hydrolysis of side chains which consist essentially of L-arabinose units, presumably in the furanose form because of their ease of hydrolysis. Further evidence for this is the fact that no uronic acids or oligosaccharides over three sugar units in length are detected by paper chromatography during the partial hydrolysis.

The 5% ethanol eluate from the charcoal column gives in 0.6% yield a crystalline disaccharide which by its lability to alkali is readily differentiated from the 2-*O*-α-D-xylopyranosyl-L-arabinose isolated from hemicellulose-B of corn cob.[3,7] It is degraded by lime water at 25° to a saccharinic acid and xylose, indicating the presence of a 1 → 3 or 1 → 4 linkage. On hydrolysis it gives xylose and arabinose whereas hydrolysis of the bromine oxidized disaccharide gives only xylose. Thus, the disaccharide is a xylosyl-arabinose and analyzes for the monohydrate of such a sugar. The constants are similar to those reported for 3-*O*-α-D-xylopyranosyl-

(1) Journal Paper No. 891 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

(2) Paper presented before the joint Divisions of Carbohydrate and Cellulose Chemistry at the 128th Meeting of the American Chemical Society at Minneapolis, Minn., September, 1955.

(3) R. L. Whistler and D. I. McGilvray, THIS JOURNAL, **77**, 1884 (1955).

(4) G. A. Adams, *Canad. J. Chem.*, **33**, 56 (1955).

(5) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(6) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

(7) R. L. Whistler and W. M. Corbett, THIS JOURNAL, **77**, 3822 (1955).

L-arabinose isolated from golden apple gum.[8] By methylation and hydrolysis of the disaccharide, 2,3,4-tri-*O*-methyl-D-xylose and 2,4-di-*O*-methyl-L-arabinose are isolated suggesting that the glycosidic linkage is 1 → 3. The high positive optical rotation (+181.8°) of the disaccharide indicates an α-D character for the linkage. Thus, the structure of the disaccharide is 3-*O*-α-D-xylopyranosyl-L-arabinose since the probability of a 1-3 sugar ring is remote.

A crystalline trisaccharide is isolated in 0.51% yield from the 15% ethanol eluate of the charcoal column. On hydrolysis the trisaccharide gives galactose, xylose and arabinose whereas bromine oxidation followed by acid hydrolysis gives galactose and xylose as the only reducing components. Thus, arabinose is the reducing unit of the molecule. Since the trisaccharide is quite stable to lime water at 25°, substitution of the arabinose unit at C2 is indicated. Methylation of the bromine oxidized trisaccharide followed by acid hydrolysis gives 2,3,4,6-tetra-*O*-methyl-L-galactose, 2,3-di-*O*-methyl-D-xylose, 3,4,di-*O*-methyl-L-arabinose which probably arises from incomplete oxidation, and a tri-*O*-methyl-L-arabonic acid. Evidence that the latter is 3,4,5-tri-*O*-methyl-L-arabonic acid is obtained by measurement of the carbon dioxide produced by periodate oxidation, since only this tri-*O*-methyl-L-arabonic acid can give rise to carbon dioxide. Thus, the structure of the trisaccharide is L-galactopyranosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinose. This is the first time such a combination of sugars has been isolated from a natural product.

It is concluded that the di- and tri-saccharides are constituents of the side chains or branches of the corn fiber hemicellulose molecule. Because L-galactose has only been found to occur at the nonreducing ends of polysaccharides, it is assumed that some branches are terminated by the trisaccharide. The oligosaccharides isolated are not reversion products because they are not produced when a mixture of the respective monosaccharides are subjected to the hydrolytic conditions used in the depolymerization of the corn fiber hemicellulose.

Experimental

Paper Chromatography.—Chromatographic separations were made on strips of Whatman No. 1 filter paper and cellulose columns at room temperature, using one of the following solvents in the ratios indicated: (A) butanol-1–pyridine–water (6:4:3); (B) ethyl acetate–formic acid–acetic acid–water (18:1:3:4); (C) ethyl acetate–pyridine–water (10:4:3); (D) upper layer of butanol-1–ethanol–water (50:10:40) and (E) the azeotropic mixture of methyl ethyl ketone and water, b.p. 74–75°. Unless otherwise indicated, *p*-anisidine hydrochloride spray was used to detect the positions of the sugars.

Isolation of Corn Fiber Hemicellulose.—A commercial preparation of corn fiber hemicellulose was used. It was isolated by lime water extraction of corn hull residues obtained from the wet milling process and had $[\alpha]^{25}D$ −92.2° (*c* 1.03, water). The behavior of the polysaccharide on fractional precipitation by alcohol from aqueous solutions and on electrophoresis indicated that the hemicellulose was homogeneous.[9]

Polysaccharide Hydrolysis.—One hundred grams of corn fiber gum was dissolved in 2 l. of *N* sulfuric acid and allowed to stand at 35°. The hydrolysis was followed by periodic observation of the optical rotation and chromatographic analysis of neutralized samples of the hydrolyzate. After 24 hours, at which time the oligosaccharide concentrations appeared to have reached a maximum, the solution which had $[\alpha]^{25}D$ −25.0°, was neutralized with barium hydroxide and filtered. The filtrate was poured with stirring into 8 l. of 95% ethanol and the polysaccharide collected in 65% yield by centrifugation. Chromatographic analysis of a solution of the precipitated polysaccharide after removal of any barium with Amberlite IR-120(H) resin indicated that no barium aldobiouronates had been precipitated with the polysaccharide. The polysaccharide had $[\alpha]^{25}D$ −69.0° (*c* 1.03, water) and complete hydrolysis with *N* sulfuric acid followed by chromatographic analysis of the neutralized hydrolyzate indicated the formation of xylose, arabinose, galactose and uronic acids. When the chromatogram was compared with one similarly prepared from the original polysaccharide, the intensity of the spots showed the arabinose:xylose ratio of the precipitated polysaccharide to be lower than that for the original polysaccharide.

Concentration of the alcoholic centrifugate from the partially hydrolyzed polysaccharide produced 24.0 g. of sirup, paper chromatography of which with solvent A indicated the following components[10]: Rx_2 1.89; 1.77; 1.58, xylose; 1.38, arabinose; 1.08, a xyloarabinose; 0.87; 0.65; 0.48 and 0.34. The sirup was chromatographed on a charcoal–Celite column in the usual way.[5] The aqueous eluate of 16-l. volume was concentrated to 13.64 g. of sirup which crystallized on trituration with methanol, and after one recrystallization from methanol gave 10.79 g. of β-L-arabinose, m.p. 157–158°, $[\alpha]^{25}D$ equil. +101.5° (*c* 2.15, water). Concentration of the 5% ethanol eluate of 16-l. volume gave 3.83 g. of sirup, and the 16 l. of 15% ethanol eluate 2.90 g. of sirup.

The partial hydrolysis was repeated on two further 100-g. samples of corn fiber gum to give a total of 10.95 g. of sirup eluted with 5% ethanol, and 10.32 g. sirup eluted with 15% ethanol.

Fractionation of Sirup Eluted with 5% Ethanol.—The sirup, 9.25 g., was chromatographed on a column of cellulose[6] (80 × 7 cm.), using butanol-1 saturated with water. The first components to be eluted were arabinose and xylose. Following these were a sirupy disaccharide, 4.78 g., and a sirupy trisaccharide, 0.94 g., as well as a trace component with Rx_2 0.88 in solvent A.

Characterization of the Disaccharide.—The sirupy disaccharide slowly deposited 1.50 g. of crystals which after recrystallization from aqueous alcohol had m.p. 117.5–119°, $[\alpha]^{25}D$ +166.0° → +181.8° (*c* 0.82, water), Rx_2 1.08 in solvent A, 1.21 in B and 1.07 in C.

Anal. Calcd. for $C_{10}H_{18}O_9 \cdot H_2O$: C, 40.00; H, 6.66. Found: C, 40.08; H, 6.69.

Andrews and Jones[8] report m.p. 123° for 3-*O*-α-D-xylopyranosyl-L-arabinose, and $[\alpha]D$ +173° for the sirupy form.

Hydrolysis of a 5-mg. sample with 5 ml. of *N* sulfuric acid at 100° for 3 hr. followed by neutralization with barium carbonate and subsequent paper chromatography with solvents A and C showed the presence of xylose and arabinose only. When a 5-mg. sample in 5 ml. of water was oxidized with bromine, the resulting aldobionic acid hydrolyzed with *N* sulfuric acid, and the neutralized hydrolyzate chromatographed, only xylose was detected. The disaccharide was readily degraded by lime water at 25° to xylose and a saccharinic acid, the lactone of which had R_f 0.79 in solvent A.

A solution of 0.888 g. of the disaccharide in 3 ml. of water and 7 ml. of dimethyl sulfate was cooled in ice. To the vigorously stirred solution was added dropwise 12 ml. of 30% sodium hydroxide solution and then the stirring was continued at room temperature for 18 hours when a further 12 ml. of sodium hydroxide was added followed by the dropwise addition of 7 ml. of dimethyl sulfate. The stirring was maintained for 16 hours after which time further similar quantities of sodium hydroxide and dimethyl sulfate were added in like manner. After a further 16 hours stirring the solution was acidified to *p*H 6 by the addition of acetic acid and the solution was exhaustively extracted with chloroform. Concentration of the extract gave 0.678 g. of sirup. This was methylated three times with 10 ml. of methyl iodide and 2 g. of silver oxide. Hydrolysis of 0.347 g. of this sirup with 10 ml. of *N* sulfuric acid at 100° for 2.5 hours gave 0.266 g. of sirup which contained two components with R_g

(8) P. Andrews and J. K. N. Jones, *J. Chem. Soc.*, 4134 (1954).

(9) R. L. Whistler and G. E. Lauterbach, unpublished results.

(10) Rx_3 is the rate of movement relative to β-D-1→4 xylobiose.

0.99 and 0.48 in solvent E. These were separated on sheets of Whatman No. 1 filter paper. In this way were obtained 0.112 g. of 2,3,4-tri-*O*-methyl-D-xylose having m.p. 90° undepressed on admixture with an authentic sample, $[\alpha]^{25}D$ +14.0° (*c* 0.93, water), and 0.107 g. of 2,4-di-*O*-methyl-L-arabinose, $[\alpha]^{25}D$ +121.0° (*c* 0.75, water). On refluxing a methanolic solution of the latter with aniline, crystalline phenyl-L-arabopyranosylamine 2,4-dimethyl ether was obtained, m.p. 142–143°.

Characterization of the Sirupy Trisaccharide.—The sirup was rechromatographed on a cellulose column (50 × 2.5 cm.) to give 0.276 g. of sirup which moved as a single component with solvents A and C. The sirup had $[\alpha]^{25}D$ −38.0 (*c* 0.89, water) and when hydrolyzed it gave xylose, arabinose and galactose. Hydrolysis of the bromine oxidized trisaccharide gave only xylose and galactose. The sugar was stable toward lime water at 25° and was chromatographically identical to the original L-galactosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinose isolated from the 15% ethanol eluate. On long standing the sirup gave the above crystalline trisaccharide, m.p. 215°, undepressed on admixture.

Fractionation of the Sirup Eluted with 15% Ethanol.—The 10.32 g. of material eluted by 15% ethanol was fractionated on a 7 × 80 cm. cellulose column using butanol-1 saturated with water. The first component to be eluted was 0.63 g. of L-arabinose, m.p. 160°, and following this was 1.50 g. of sirup, $[\alpha]^{25}D$ −3.0 (*c* 1.08, water), Rx_2 1.01 in solvent A. From its chromatographic behavior before and after hydrolysis or bromine oxidation followed by hydrolysis, it appeared to be a mixture of L-galactose and 3-*O*-α-D-xylopyranosyl-L-arabinose. Methylation and hydrolysis of the sirup gave 2,3,4,6-tetra-*O*-methyl-L-galactose, 2,3,4-tri-*O*-methyl-D-xylose and 2,4-di-*O*-methyl-L-arabinose, identified by paper chromatography in solvent E.

The main component eluted from the column was 1.49 g. of sirup which crystallized on standing. After two recrystallizations from aqueous alcohol, the trisaccharide had m.p. 217–219°, $[\alpha]^{25}D$ −55.0° → −61.0° (*c* 1.0°, water), Rx_2 0.72 in solvent A and 0.64 in solvent C.

Anal. Calcd. for $C_{16}H_{28}O_{14}$: C, 43.24; H, 6.35. Found: C, 43.15; H, 6.55.

Trace components with Rx_2 0.90 and 0.54 in solvent A were also isolated.

Characterization of the Crystalline Trisaccharide.—Hydrolysis of a 10-mg. sample with 2 ml. of *N* sulfuric acid at 100° for 3 hours, followed by neutralization with barium carbonate and subsequent paper chromatography in solvents A and C showed the presence of galactose, arabinose and xylose. A further 20 mg. dissolved in 5 ml. of water was oxidized with bromine at room temperature for 4 days. Hydrolysis and paper chromatography of the oxidized trisaccharide showed the presence of galactose and xylose only. The trisaccharide in saturated lime water was quite stable at 25°.

To a solution of 0.679 g. of the trisaccharide in 20 ml. of water was added 0.2 g. of calcium carbonate and 0.25 ml. of bromine. The oxidation solution was kept in the dark at room temperature for 7 days, after which time the solution failed to reduce Fehling solution. The excess bromine was removed by aeration, the calcium ions by Amberlite IR-120(H) resin, bromine ions by silver oxide, and the resulting silver ions by precipitation with hydrogen sulfide. The solution was neutralized with calcium carbonate and concentrated to 0.575 g. of amorphous calcium salt, $[\alpha]^{25}D$ −88.7° (*c* 5.75, water).

A solution of 0.575 g. of the calcium salt in 15 ml. of water and 12 ml. of 30% sodium hydroxide was vigorously stirred at room temperature while 7 ml. of dimethyl sulfate was added dropwise over a period of 6 hours. After the addition, the stirring was maintained for 16 hours at which time further similar quantities of the reagents were added in like manner. This procedure was repeated and after the last addition of dimethyl sulfate the reaction mixture was stirred at room temperature for 16 hours when acetic acid was added until the solution had a *p*H value of 5. The solution was exhaustively extracted with chloroform, concentration of the extract giving 0.304 g. of sirup. This was dissolved in water, stirred with Amberlite IR-120(H) resin, filtered, concentrated and dried.

The sirup was methylated three times with silver oxide and methyl iodide to yield 0.278 g. of the fully methylated aldotrionic acid which was hydrolyzed with 5 ml. of *N* sulfuric acid at 100° for 2 hours when the optical rotation was constant. The solution was neutralized with barium carbonate, filtered, stirred with Amberlite IR-120(H) resin, filtered and concentrated to 0.243 g. of sirup. Paper chromatography in solvent D showed the presence of three reducing components with R_g values 0.93, 0.80 and 0.56. A fourth component with R_g 0.07 was detected only with silver nitrate spray.[11] These components were separated on five sheets of filter paper using solvent D.

The first component to be isolated was 0.093 g. of sirup with $[\alpha]^{25}D$ −84.8° (*c* 0.92, water), −61.1° (*c* 0.90, ethanol). On refluxing it with an anhydrous methanol solution of aniline, N-phenyl-L-galactopyranosylamine 2,3,4,6-tetramethyl ether was obtained, m.p. 191–192° depressed on admixture with the D-form, $[\alpha]^{25}D$ +76.5° (*c* 1.1, acetone).

Anal. Calcd. for $C_{16}H_{24}O_5N$: N, 4.52. Found: N, 4.65.

The second component was 0.065 g. of sirup which was chromatographically identical to 2,3-di-*O*-methyl-D-xylose. It had $[\alpha]^{25}D$ +3.8° (*c* 1.3, water), the value being low due to traces of 2,3,4,6-tetra-*O*-methyl-L-galactose. On refluxing the sirup with a methanolic solution of aniline, N-phenyl-D-xylosylamine 2,3-dimethyl ether, m.p. 124–125°, was obtained.[12] The X-ray diffraction pattern of this material and an authentic sample were identical.

The third component was 0.016 g. of sirup which was chromatographically identical to 3,4-di-*O*-methyl-L-arabinose.

The fourth component was 0.048 g. of amorphous powder having $[\alpha]^{25}D$ −30.0° (*c* 0.4 water). It was 3,4,5-tri-*O*-methyl-L-arabonic acid. A 0.0092 *M* solution was prepared and 0.50 ml. was oxidized with 1 ml. of 0.5 *M* sodium metaperiodate. The carbon dioxide evolved was measured in a standard Warburg apparatus.[13] The number of moles of carbon dioxide produced per mole of 3,4,5-tri-*O*-methyl-L-arabonic acid was 0.922 after 0.5 hr.; 0.952, 1 hour; 0.975, 1.5 hours; 1.02, 2.5 hours; 1.04, 3.0 hours; 1.06, 4.0 hours; and 1.07, 4.5 hours.

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