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Structure of Corn Hull Hemicelluloses. Part IV. Partial Hydrolysis and Identification of 3-*O*-α-D-Xylopyranosyl-L-arabinose and 4-*O*-β-D-Galactopyranosyl-β-D-xylose[1,2]

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Mild acid hydrolysis of corn hull hemicellulose gives xylose, arabinose, galactose and a number of oligosaccharides, two of which have been shown to be 3-*O*-α-D-xylopyranosyl-L-arabinose and 4-*O*-β-D-galactopyranosyl-β-D-xylopyranose. The structure of the latter, a new disaccharide, has been established.

Previous studies[3] have shown that corn hull hemicellulose is composed of xylose, arabinose, galactose and uronic acid residues. The identity of the acid component has been established by the isolation of 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose.[4-6] Methylation studies[7-9] have shown that the corn hull hemicellulose is a branched xylan having D-xylose, L-arabinose, D- and L-galactose and D-glucuronic acid residues as the non-reducing ends.

Information has been obtained on the mutual union of the sugar residues in the polymer by graded hydrolysis with *N* sulfuric acid which yields 3-*O*-α-D-xylopyranosyl-L-arabinose and *O*-L-galactopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 2)-L-arabinose.[10]

Studies on the controlled depolymerization of the corn hull hemicellulose in this Laboratory have shown that hydrolysis of the polysaccharide with 0.01 *N* hydrochloric acid gives a number of oligosaccharides in addition to the monosaccharides, D-xylose, L-arabinose and D- and L-galactose. Thus far, two disaccharides have been obtained in crystalline form by successive fractionations of the hydrolyzate on columns of charcoal[11] and cellulose,[12] and they have been shown to be 3-*O*-α-D-xylopyranosyl-L-arabinose and 4-*O*-β-D-galactopyranosyl-β-D-xylopyranose (I).

The identity of the 3-*O*-α-D-xylopyranosyl-L-arabinose is based upon the following evidence. The sugar showed m.p. 117–119° and $[\alpha]_D$ +175° → +183° (H_2O), values that are in agreement with those quoted for 3-*O*-α-D-xylopyranosyl-L-arabinose.[10,13] Upon acid hydrolysis it afforded xylose and arabinose. When the disaccharide was oxidized with bromine to the corresponding acid and the latter hydrolyzed, only xylose was obtained as the free sugar; the disaccharide also afforded a phenylosazone,[13] which upon acid hydrolysis gave xylose but no arabinose. Both these observations indicated that the xylose moiety occupied the non-reducing end in the molecule. On treatment with sodium acetate and acetic anhydride, the disaccharide gave a good yield of crystalline hexa-*O*-acetyl-3-*O*-α-D-xylopyranosyl-L-arabinose, m.p. 168–170°, $[\alpha]^{28}_D$ +106° ($CHCl_3$), a derivative which is recommended for the identification of this sugar.

The structure of the second crystalline disaccharide, 4-*O*-β-D-galactopyranosyl-β-D-xylopyranose (I), m.p. 210–211°, $[\alpha]_D$ −1° → +15° (H_2O), is based upon the following evidence. A molecular weight determination by the alkaline hypoiodite method indicated that I was a disaccharide. Upon acid hydrolysis I gave D-galactose and D-xylose, whereas hydrolysis of I after bromine oxidation gave rise to D-galactose indicating that I was a D-galactosyl-D-xylosedisaccharide.

Treatment of I with 0.5% methanolic hydrogen chloride at room temperature furnished crystalline methyl 4-*O*-β-D-galactopyranosyl-D-xylopyranoside (II), m.p. 246–247°, $[\alpha]^{25}_D$ −18° (H_2O). Methylation of II with methyl iodide and silver oxide gave the fully methylated derivative which upon hydrolysis afforded equimolecular amounts of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-D-xylose, both of which were identified by converting them into their characteristic crystalline anilides. It was clear, therefore, that the D-galactosyl-D-xylosedisaccharide (I) contained a 1,4-linkage, and from the low rotation of I this linkage is probably of the β-type. Moreover, since I displays an upward mutarotation, the configuration at C_1 is of the β-type. This hitherto unknown disaccharide I is therefore designated 4-*O*-β-D-galactopyranosyl-β-D-xylopyranose.

Inasmuch as the trisaccharide *O*-L-galactopyranosyl-(1 → 4)-*O*-D-xylopyranosyl-(1 → 2)-L-arabinose has already been isolated from corn hull hemicellulose by graded hydrolysis,[10] it appears that both D- and L-galactose residues are joined to D-xylose residues by 1,4-glycosidic bonds in the polysaccharide.

Experimental

The following solvents were used for the partition chromatography of sugars and their derivatives: (A) pyridine: ethyl acetate: water (1:2.5:3.5, upper layer)[14]; (B) 1-pro-

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(2) This research was done under contract with the United States 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.

(3) M. J. Wolf, M. M. MacMasters, J. A. Cannon, E. C. Rosewall and C. E. Rist, *Cereal Chem.*, **30**, 451 (1953).

(4) R. Montgomery, F. Smith and H. C. Srivastava, THIS JOURNAL, **78**, 2837 (1956).

(5) R. Montgomery, F. Smith and H. C. Srivastava, *ibid.*, **78**, 6169 (1956).

(6) R. Montgomery and F. Smith, *ibid.*, **79**, 695 (1957).

(7) R. L. Whistler and J. N. BeMiller, *ibid.*, **78**, 1163 (1956).

(8) R. Montgomery and F. Smith, *J. Agr. Food Chem.*, **4**, 716 (1956).

(9) I. J. Goldstein, R. Montgomery, F. Smith and H. C. Srivastava, to be published.

(10) R. L. Whistler and W. M. Corbett, THIS JOURNAL, **77**, 6328 (1955).

(11) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

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

(13) P. Andrews and J. K. N. Jones, *ibid.*, 4134 (1954).

(14) E. F. McFarren, Kathleen Brand and H. R. Rutkowski, *Anal. Chem.*, **23**, 1146 (1951).

panol:water azeotrope[15]; (C) 2-butanone:water azeotrope[16]; and (D) benzene:ethanol:water (200:47:15).[17] *p*-Anisidine trichloroacetate[18] was used for the detection of the sugars and their derivatives on the paper. R_x and R_{Mx} values represent the rates of movement on the paper relative to D-xylose and methyl D-xylopyranoside, respectively.

Hydrolysis of the Corn Hull Hemicellulose.—Corn hull hemicellulose (100 g.) was dissolved in 0.01 *N* hydrochloric acid (3000 ml.) and the solution heated on a boiling water-bath. The course of hydrolysis was followed by paper chromatographic analysis and by the change in optical rotation. After 62 hr. the solution showed $[\alpha]^{25}D$ −4° ($[\alpha]D$ not observable before this period). Since the hydrolysis proceeded slowly, the solution was heated under reflux (after 77 hr.) for another 50 hr., after which it showed $[\alpha]^{25}D$ +25°. Chromatographic analysis showed that L-arabinose residues were the first to be liberated, followed by D-xylose, (?D- and L-) galactose and oligosaccharides. The hydrolyzate was neutralized ($BaCO_3$) and centrifuged to remove insoluble material. The supernatant was concentrated *in vacuo* to about 200 ml. and poured with stirring into ethanol. The brown colored precipitate (P) which formed was centrifuged. The supernatant was evaporated *in vacuo* to give a sirup A (28 g.). The precipitate P was dissolved in water and reprecipitated (P_1) with methanol in the usual way. The precipitate P_1 was centrifuged and the supernatant evaporated *in vacuo* to give a sirup B (27 g.). The precipitate P_1 was dissolved in water and reprecipitated (P_2) by the addition of excess methanol; the precipitate P_2 was separated as before, washed successively with ethanol, diethyl ether, petroleum ether and dried to give a light brown powder (15.2 g.). To the methanolic supernatant solution absolute ethanol was added, when a yellowish-white precipitate was formed which was centrifuged off and dried by solvent exchange as before to give a yellowish-white powder (1.83 g.). The supernatant liquid from the above was evaporated to give a sirup C (2.2 g.).

Upon paper chromatographic analysis (solvent A), the two sirups A and B were found to contain besides D-xylose and L-arabinose, a number of other components. Sirup A contained sugars with R_x values of 0.05, 0.15, 0.30 and 0.48 while sirup B contained sugars with R_x values: 0.03, 0.09, 0.21, 0.26, 0.35 and 0.49.

The sirup B was deionized by passing a solution of it successively through Amberlite IR 4B[19] and Duolite A4[20] ion exchange resins, and the resulting solution evaporated to give a colorless sirup D (22.4 g.) which was used for the isolation of the oligosaccharides as described below.

Separation of the Mixture of Sugars on a Charcoal Column.—A solution of the sirup D (18 g.) in 2.5% aqueous ethanol (150 ml.) was put on a charcoal–Celite column (42 cm. × 6 cm., prepared by packing a 50:50 mixture of Darco G60 and Celite in the form of a slurry in 2.5% ethanol). The column was eluted first with 2.5% aqueous ethanol (2.5 liters) and then with 5% aqueous ethanol (2.5 liters), the fractions being collected in approximately 150-ml. portions. Almost all the monosaccharide components (D-xylose, L-arabinose and (?D- and L-) galactose) were eluted with 1200 ml. of 2.5% aqueous ethanol. The fractions containing the monosaccharides were combined and evaporated to give a sirup (7.6 g.). The fractions 1–7, from the 5% aqueous ethanolic eluate, were combined to give a sirup (0.540 g.) which on chromatographic analysis using solvent A gave spots with R_x values 0.07, 0.27, 0.36, 0.57 besides traces of D-xylose and L-arabinose. The fractions 8–18 when combined and evaporated afforded a sirup (3.02 g.) which upon chromatographic analysis (solvent A) gave only three spots with R_x values 0.23, 0.35 and 0.49.

Separation of the Oligosaccharides on a Cellulose Column.—The mixture of oligosaccharides (3.02 g.) obtained as above from the charcoal column was dissolved in 1-propanol:water azeotrope (20 ml.) to which a few drops of water were added and the solution put on a cellulose column, the latter being developed with 1-propanol:water azeotrope.[14] The fractions were collected at 30-minute intervals, the flow rate being 20 ml./hr. Only a partial separation of the oligosaccharides was achieved in the first experiment on the cellulose column, but on repeating the process with the appropriate fractions from the first experiment, two oligosaccharides (R_x 0.49 and 0.21) were obtained in a chromatographically pure state.

Identification of 3-O-α-D-Xylopyranosyl-L-arabinose.—This oligosaccharide, obtained as a sirup from the cellulose column (R_x 0.49), was crystallized by dissolving it in aqueous ethanol and allowing the solution to evaporate gradually in air. The crystals were separated and recrystallized from hot aqueous ethanol to give 3-O-α-D-xylopyranosyl-L-arabinose as the monohydrate, m.p. 117–119°, $[\alpha]^{25}D$ +175° changing in 1 hr. to +183° in water (*c* 1).

Anal. Calcd. for $C_{10}H_{18}O_9 \cdot H_2O$: C, 40.0; H, 6.7. Found: C, 40.3; H, 6.6.

Hydrolysis of 3-O-α-D-Xylopyranosyl-L-arabinose.—A solution of the sugar (10 mg.) in 0.7 *N* sulfuric acid (3 ml.) was heated (sealed tube) for 3 hr. in a boiling water-bath. The reaction mixture was neutralized ($BaCO_3$), filtered and evaporated *in vacuo* to a sirup. Paper chromatography of the sirup using solvent A showed the presence of D-xylose and L-arabinose.

Oxidation of 3-O-α-D-Xylopyranosyl-L-arabinose.—To a solution of the oligosaccharide (25 mg.) in water (3 ml.), bromine (0.5 ml.) was added. After addition of barium carbonate (50 mg.), the reaction mixture was kept at room temperature in the dark for 3.5 days after which time chromatography showed that the reaction was complete. After removing the excess of the bromine and the barium carbonate, the solution was treated with sulfuric acid to make the solution approximately *N* with respect to sulfuric acid. The solution was heated (sealed tube) in a boiling water-bath for 1.5 hr., neutralized ($BaCO_3$) and passed through Amberlite IR 120 cation exchange resin. The effluent was neutralized with silver carbonate, filtered before and after treatment with hydrogen sulfide, and concentrated to a sirup. Chromatographic analysis (solvent A) of the sirup revealed two spots, one corresponding to D-xylose and the other probably due to L-arabonic acid, which did not move on the paper.

The Phenylosazone of 3-O-α-D-Xylopyranosyl-L-arabinose.—The phenylosazone of the sugar prepared in the usual way[13] was obtained in the form of a gel which failed to crystallize satisfactorily. The osazone had m.p. 211–213°, literature value[13] m.p. 226°.

The phenylosazone (5 mg.) was dissolved in ethanol (1 ml.) and after the addition of 0.5 *N* sulfuric acid (5 ml.), the solution was heated on the steam-bath for 3 hr. Neutralization ($BaCO_3$) of the solution, followed by evaporation and chromatographic analysis (solvent A) of the resulting sirup showed the presence of D-xylose only.

Hexa-O-acetyl-3-O-α-D-xylopyranosyl-L-arabinose.—A mixture of the sugar (60 mg.), fused sodium acetate (0.5 g.) and acetic anhydride (8 ml.) was heated at 100–110° (bath temperature) for 4 hr. The reaction mixture was cooled and poured into ice-water. The acetate was extracted three times with chloroform and the combined chloroform extracts washed once with a solution of sodium bicarbonate and three times with water. The chloroform solution, after drying (Na_2SO_4), was evaporated *in vacuo* to a sirup which crystallized upon trituration with methanol. Recrystallization from hot methanol gave hexa-O-acetyl-3-O-α-D-xylopyranosyl-L-arabinose as colorless needles (yield 67 mg.) m.p. 168–170°, $[\alpha]^{28}D$ +106° in chloroform (*c* 1).

Anal. Calcd. for $C_{20}H_{30}O_{15}$: C, 49.4; H, 5.7. Found: C, 49.6; H, 5.7.

Characterization of 4-O-β-D-Galactopyranosyl-β-D-xylopyranose.—This disaccharide (I, R_x 0.21 in solvent A) was obtained chromatographically pure as a sirup (187 mg.). It separated from hot aqueous ethanol as a bulky gelatinous mass, but gradual evaporation of an aqueous solution of it in air provided crystals of the disaccharide in the form of rectangular plates. Recrystallization was effected by dissolving the product in the minimum quantity of water and adding ethanol to incipient turbidity. On standing, crystalline 4-O-β-D-galactopyranosyl-β-D-xylopyranose readily separated as the dihydrate and after washing (methanol) and drying, it showed m.p. 210–211° with previous sintering at

(15) F. Smith and H. C. Srivastava, THIS JOURNAL, **78**, 1404 (1956).

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

(17) G. A. Adams, *Can. J. Chem.*, **33**, 56 (1955).

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

(19) A product of the Rohm and Haas Chemical Co., Philadelphia, Pa.

(20) A product of the Chemical Process Co., Redwood City, Calif.

110–120°, $[\alpha]^{25}D$ −1° (approx.) (initial value) changing to +15° (equilibrium value) in water (*c* 1.5).

Anal. Calcd. for $C_{11}H_{20}O_{10}\cdot 2H_2O$: C, 37.9; H, 6.9. Found: C, 37.9; H, 6.8.

A molecular weight determination of the sugar (dried *in vacuo* at 55° for 2 hr.) by the alkaline hypoiodite method[21] gave a value of 325 (calcd. for $C_{11}H_{20}O_{10}\cdot 2H_2O$: mol. wt., 348). The low value found is probably due to loss of water during drying.

Hydrolysis of 4-*O*-β-D-Galactopyranosyl-β-xylopyranose.—A solution of the sugar (10 mg.) in 0.5 *N* sulfuric acid (0.5 ml.) was heated (sealed tube) for 15 hr. in a boiling water-bath. The reaction mixture was neutralized ($BaCO_3$), filtered and evaporated *in vacuo*. Paper chromatographic analysis (solvent A) showed that the sirup contained two sugars which corresponded to D-galactose and D-xylose.

Bromine Oxidation of 4-*O*-β-D-Galactopyranosyl-β-D-xylopyranose.—A solution of the sugar (10 mg.) in water (2 ml.) was treated with bromine (0.5 ml.) in the presence of barium carbonate (20 mg.) at room temperature in the dark for 1.5 days, when chromatographic analysis of the mixture, after removal of excess bromine, showed that the oxidation was complete. To the reaction mixture *N* sulfuric acid (2 ml.) was added and the precipitated barium sulfate centrifuged off. The supernatant was then heated (sealed tube) in a boiling water-bath for 20 hr. The hydrolyzate, after neutralization ($BaCO_3$) and filtration, was passed through Amberlite IR 4B and the effluent neutralized with silver carbonate. The solution was filtered and passed successively through columns of Amberlite IR 4B and Duolite A4 resins. The effluent was concentrated *in vacuo* to a sirup which was chromatographed on paper. Only one component whose R_f value corresponded to D-galactose was detected.

Methyl 4-*O*-β-D-Galactopyranosyl-D-xylopyranoside (II).—The disaccharide I (81 mg., as the dihydrate) was shaken in a flask with 0.5% methanolic hydrogen chloride (10 ml.) at room temperature. The sugar dissolved completely in 1 hr., after which the reaction was followed polarimetrically: $[\alpha]D$ +16° (after 1 hr.), +19° (6 hr.), +21° (19 hr.), +23° (30 hr.) (constant value). The solution was neutralized with silver carbonate, filtered and evaporated *in vacuo* to a sirup (83 mg.) which crystallized in the form of fine needles on trituration with methanol. Recrystallization from methanol: ethanol (2:1) gave methyl 4-*O*-β-D-galactopyranosyl-D-xylopyranoside (25 mg.), m.p. 247–248°, $[\alpha]^{25}D$ −18° in water (*c* 1).

Anal. Calcd. for $C_{12}H_{22}O_{10}$: C, 44.2; H, 6.8. Found: C, 43.8; H, 6.6.

Whereas the crystalline glycoside gave only one spot (R_{Mx} 0.34) when chromatographed on paper using solvent A and the periodate–benzidine spray reagent,[22] the mother liquor gave additional weak spots corresponding to methyl D-galactoside (R_{Mx} 0.56) and methyl D-xyloside (R_{Mx} 1.0), thus showing that some cleavage of the disaccharide occurred during glycoside formation and that the disaccharide was composed of D-galactose and D-xylose.

Methyl 4-*O*-(2,3,4,6-Tetra-*O*-methyl-β-D-galactopyranosyl)-2,3-di-*O*-methyl-D-xylopyranoside.—A sirupy mixture (90 mg.) containing the methyl α- and β-glycosides of 4-*O*-β-D-galactopyranosyl-D-xylopyranose obtained as above was dissolved in methanol (10 ml.) and to the solution, methyl iodide (10 ml.) and silver oxide (2 g.) were added, after which the mixture was refluxed for 12 hr. The reaction mixture was filtered and the solution evaporated to a sirup which was remethylated by dissolving it in methanol (2 ml.) and treating the solution with methyl iodide and silver oxide in the previous manner. The product from the second methylation, now soluble in methyl iodide, was methylated three more times with methyl iodide (10 ml.) and silver oxide (4 g.). The sirupy methylated product (130 mg.), isolated in the usual manner, showed $[\alpha]^{23}D$ +20° in methanol (*c* 1.3). It was heated at 120–130° (bath temperature) (0.001 mm.) to distil off traces of methylated methyl glycosides of monosaccharides. The residual sirup was dissolved in methanol, decolorized with charcoal, filtered and the filtrate evaporated to give a pale yellow sirup (50 mg.), $[\alpha]^{25}D$ +20° in methanol (*c* 1).

Hydrolysis of Methyl 4-*O*-(2,3,4,6-Tetra-*O*-methyl-β-D-galactopyranosyl)-2,3-di-*O*-methyl-D-xylopyranoside.—The methylated disaccharide (50 mg.) was dissolved in *N* sulfuric acid (5 ml.) and the solution heated at 100–120° (bath temperature) for 6 hr., when the optical rotation had become constant ($[\alpha]^{25}D$ +68°). The solution was neutralized ($BaCO_3$) and evaporated *in vacuo* to give a sirup (46 mg.) which had $[\alpha]^{25}D$ +65° [calcd. for an equimolecular mixture of 2,3,4,6-tetra-*O*-methyl-D-galactose ($[\alpha]D$ +118°) and 2,3-di-*O*-methyl-D-xylose (+23°), +70°]. Paper chromatographic analysis of the hydrolyzate using solvents C and D revealed only two components (A and B) whose R_f values corresponded to those of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-D-xylose.

The mixture of methylated sugars was separated on Whatman No. 1 filter paper sheets in the usual way using solvent D. The areas of paper containing the methylated sugars were separately extracted with water, filtered and the filtrate evaporated *in vacuo*. Purification of the residual sirup by extraction with methanol gave 2,3-di-*O*-methyl-D-xylose (12.5 mg., 1 mole proportion) and 2,3,4,6-tetra-*O*-methyl-D-galactose (17.6 mg., 1.06 mole proportion).

Identification of 2,3-Di-*O*-methyl-D-xylose.—The 2,3-di-*O*-methyl-D-xylose fraction (12.5 mg.) which showed $[\alpha]^{30}D$ +24° in water (*c* 0.6) was boiled for 5 hr. with absolute ethanol (1 ml.) containing aniline (0.1 ml.). Removal of solvent *in vacuo* gave N-phenyl-D-xylopyranosylamine 2,3-dimethyl ether, m.p. and mixed m.p. 125–127°, $[\alpha]^{28}D$ +190° in ethyl acetate (*c* 1) (after recrystallization from ethyl acetate); literature values m.p. 126°,[23] $[\alpha]D$ +183° (ethyl acetate).[24]

Identification of 2,3,4,6-Tetra-*O*-methyl-D-galactose.—The 2,3,4,6-tetra-*O*-methyl-D-galactose fraction (17.6 mg.) showed $[\alpha]^{30}D$ +84° in water (*c* 0.9). Treatment of this fraction with ethanolic aniline as above gave crystalline N-phenyl-D-galactopyranosylamine 2,3,4,6-tetramethyl ether, m.p. and mixed m.p. 197–198°, $[\alpha]^{29}D$ −81° in acetone (*c* 1.0) (after recrystallization from ethyl acetate); literature values[25] m.p. 192°, $[\alpha]D$ −77°.

(21) J. L. Baker and H. F. E. Hulton, *Biochem. J.*, **14**, 754 (1920).

(22) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954).

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

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

(25) J. C. Irvine and D. McNicoll, *ibid.*, **97**, 1449 (1910).

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