crystallization from 50% alcohol–water mixture; m.p. 115–117°.

Anal. Calcd. for $C_{23}H_{20}N_{10}O_{14}$: N, 21.21. Found: N, 21.34.

The dihydrochloride was obtained from the picrate in the manner described above for 3-β-isopropylaminoethyl-1,2,4-triazole dihydrochloride; m.p. 220° (capillary).

Anal. Calcd. for $C_{11}H_{14}N_4 \cdot 2HCl$: N, 20.36; Cl, 25.77. Found: N, 20.61; Cl, 25.51.

3-β-Ureidoethyl-1,2,4-triazole (XII).—A solution of 5.6 g. (0.03 mole) of 3-β-aminoethyl-1,2,4-triazole dihydrochloride, 2.4 g. (0.03 mole) of potassium cyanate and 2.5 g. (0.03 mole) of sodium bicarbonate in 100 ml. of water was evaporated on the steam-bath. The resulting solid was extracted with 50 ml. of ethanol from which 3-β-ureidoethyl-1,2,4-triazole crystallized after the addition of 500 ml. of dry ether; m.p. 188–190°.

Anal. Calcd. for $C_5H_9N_5O$: C, 38.70; H, 5.85. Found: C, 38.49; H, 5.88.

3-β-Benzamidoethyl-1,2,4-triazole (XIII).—To a solution of 5.5 g. (0.03 mole) of 3-β-aminoethyl-1,2,4-triazole dihydrochloride and 100 ml. of 2 *N* sodium hydroxide, cooled to 0°, was added with stirring 2.8 g. (0.02 mole) of benzoyl chloride. After two hours, 25 g. of ice was added and then concentrated hydrochloric acid to *p*H 5. The solid which formed was collected, washed with sodium bicarbonate solution and recollected by filtration. Recrystallized from water the solid gave 3.8 g. (55% yield) of 3-β-benzamidoethyl-1,2,4-triazole as feathery plates; m.p. 189–190°.

Anal. Calcd. for $C_{11}H_{12}N_4O$: C, 61.09; H, 5.59; N, 25.91. Found: C, 60.99; H, 5.45; N, 25.71.

3-β-Acetamidoethyl-1,2,4-triazole Hydrochloride (XIV).—A solution of 5.5 g. (0.03 mole) of 3-β-aminoethyl-1,2,4-triazole dihydrochloride and 50 ml. of 2 *N* sodium hydroxide solution, cooled to 0°, was treated with 2 ml. (0.02 mole) of acetic anhydride. After 30 minutes the solution was acidified with 6 *N* hydrochloric acid and was evaporated to dryness under reduced pressure. The organic hydrochloride was extracted with 100 ml. of warm absolute ethanol and to this was added 400 ml. of ether. 3-β-Acetamidoethyl-1,2,4-triazole hydrochloride precipitated on cooling as a white solid; m.p. 160°.

Anal. Calcd. for $C_6H_{10}N_4O \cdot HCl$: C, 37.80; H, 5.82; Cl, 18.60. Found: C, 37.94; H, 5.77; Cl, 18.94.

Condensation of 3-β-Aminoethyl-1,2,4-triazole and Acetic Anhydride.—A solution of 1.7 g. (0.015 mole) of 3-β-aminoethyl-1,2,4-triazole and 2 g. (0.020 mole) of acetic anhydride in 50 ml. of glacial acetic acid was heated on the steam-bath for three hours. Water (25 ml.) was added, and after standing for 15 minutes, the solution was concentrated to dryness under reduced pressure. The resulting solid was recrystallized from ethanol and obtained as needles; m.p. 215–216°.

Anal. Calcd. for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04; mol. wt., 254.2. Found: C, 61.31; H, 4.28; N, 22.08; mol. wt. (Rast), 255.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Two Further Aldobiouronic Acids from Hemicellulose-B of Corn Cob[1,2]

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Further investigation of the uronic acid-coutaining oligosaccharides produced by partial hydrolysis of corn cobs has shown that two of the four aldobiouronic acids present are 2-O-(α-D-glucopyranosyluronic acid)-D-xylose and 4-O-(α-D-glucopyranosyluronic acid)-D-xylose.

Structural characterization of hemicelluloses can be approached through an examination of oligosaccharide fragments obtained on partial hydrolysis. This approach has been used successfully with other polysaccharides and is particularly suitable since charcoal[3] and cellulose chromatography[4,5] allow clean-cut separations of oligosaccharides from complex sugar mixtures. In an attempt to apply the procedure to the soluble hemicelluloses of corn cob (B-fraction) there have been obtained five components containing uronic acids. One of these has been shown[5] to be 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose. Further work has been done on a second chromatographically separated fraction which is shown to be a mixture of two different unmethylated aldobiouronic acids. These two aldobiouronic acids are found together as one spot on paper chromatography and no combination of eluants have been found to separate them. Consequently the mixture was investigated to determine whether structural information could be elicited without separation of the individual disaccharides. The mixture was methylated and the fully methylated products reduced with lithium aluminum hydride. Cleavage of the resultant methylated glucosylxyloses gave a mixture of three products, 2,3,4,6-tetra-O-methyl-D-glucose, 3,4-di-O-methyl-D-xylose and 2,3-di-O-methyl-D-xylose which were separated by paper chromatography and converted into crystalline derivatives. This evidence conclusively identifies one of the components as 2-O-(α-D-glucopyranosyluronic acid)-D-xylose, the unmethylated derivative of the mono-O-methylaldobiouronic acid found previously[5] in corn cob hemicellulose-B. This aldobiouronic acid is thus identified in nature for the first time. The other component is probably 4-O-(α-D-glucopyranosyluronic acid)-D-xylose which has been previously characterized in part.[6] The remote possibility that this aldobiouronic acid contains the 1 → 5 linkage is not eliminated.

3-O-(α-D-Glucopyranosyluronic acid)-D-xylose has been found in wheat straw hemicellulose[7,8] and pear cell wall xylan.[9]

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[1] Journal Paper No. 721 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, California, March, 1953.

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[6] Paper presented by R. L. Whistler and R. F. Serro before the Division of Sugar Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Ill., 1950.

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Experimental

The aldobiouronic acids were isolated as described previously[5] by chromatography on cellulose. Fraction C was separated and used as the subject of the present investigation. On paper chromatography the fraction moved as one spot ($R^{25}\mathrm{x}$ 0.32, where x = xylose) in ethyl acetate–acetic acid–formic acid–water (18:3:1:4, v./v.) and gave a red color with *p*-anisidine hydrochloride spray reagent. It had $[\alpha]^{25}\mathrm{D}$ +59 (*c* 6.83, water).

Anal. Calcd. for $C_{11}H_{18}O_{11}$: equiv. wt., 326; OMe, nil. Found: equiv. wt., 352; OMe, 0.2%.

Hydrolysis of the fraction in 1 *N* sulfuric acid at 100° for 18 hours was accompanied by much decomposition. After neutralization with barium carbonate followed by removal of barium ions on Amberlite IR-120 resin, the hydrolysate was examined on paper chromatograms which revealed the presence of xylose, glucuronic acid, glucurone and unchanged aldobiouronic acid.

Methylation.—The mixture of aldobiouronic acids (0.68 g.) was dissolved in water (5 ml.) containing dimethyl sulfate (2 ml.) and 30% sodium hydroxide solution (5 ml.) was added dropwise over 5 hours with vigorous stirring and external ice-cooling. After stirring for a further 16 hours methyl glycoside formation was complete as indicated by a negative Fehling test. Methylation was continued by addition of 30% sodium hydroxide solution (10 ml.) to the reaction mixture and dimethyl sulfate (5 ml.) was added dropwise over 8 hours with vigorous stirring which was continued for a further 16 hours. Acidification of the solution with 2 *N* sulfuric acid, followed by continuous extraction with chloroform for 16 hours removed the methylated aldobiouronic acids which on evaporation of the chloroform were obtained as a thick sirup (0.49 g.). Two treatments with silver oxide (2 g.) and methyl iodide (5 ml.) gave the fully methylated aldobiouronic acid (0.45 g.) which had $[\alpha]^{25}\mathrm{D}$ +114° (*c* 2.2, chloroform).

Anal. Calcd. for $C_{18}H_{32}O_{11}$: OMe, 51.2. Found: OMe, 50.9.

Methanolysis of this methylated product proved very difficult; poor yields of dimethyl xyloside being obtained even after 24 hours at 100° in 4% methanolic hydrogen chloride. Consequently it was expedient to reduce[5] the methyl ester with lithium aluminum hydride in dry ethereal solution. After 0.5 hour at room temperature, excess lithium aluminum hydride was destroyed by addition of ethyl acetate. The mixture was then diluted with water (5 ml.) and organic solvents were evaporated under reduced pressure. The resultant solution was filtered and the filtrate deionized with Amberlite resins IR-4B and IR-120. Evaporation of the solution under reduced pressure, extraction of the residue with acetone, filtration and evaporation of the filtrate gave a neutral sirup which was methylated in the usual way with Purdie reagents. The product (0.35 g.) had $[\alpha]^{25}\mathrm{D}$ +120° (*c* 1.7, chloroform).

Anal. Calcd. for $C_{18}H_{34}O_{10}$: OMe, 52.9. Found: OMe, 52.1.

The methylated product was dissolved in a 4% solution of hydrogen chloride in methanol (25 ml.) and refluxed for 15 hours at which point the methanol was evaporated under reduced pressure at room temperature and the residue dissolved in 1 *N* sulfuric acid (10 ml.). After hydrolysis at 100° for 6 hours, the solution was neutralized by using Amberlite IR-4B resin and evaporated under reduced pressure. On paper chromatograms the sirup (0.34 g.) was observed to contain three components corresponding in R_g values[4] and color reactions with 2,3,4,6-tetra-O-methylglucose, 3,4-di-O-methylxylose and 2,3-di-O-methylxylose. The sugar derivatives were separated[10] on 4 sheets of Whatman No. 1 paper using benzene–ethanol–water (169:47:15, v./v., upper layer) as mobile phase. Crystalline 2,3,4,6-tetra-O-methyl-D-glucose was obtained from ether–petroleum ether. Its melting point alone and when mixed with the known derivative was 94–96°. The rotation was $[\alpha]^{25}\mathrm{D}$ +85° (*c* 0.7, water). An X-ray diffraction photograph of the crystals was identical with that of a known specimen.

Anal. Calcd. for $C_{10}H_{20}O_6$: OMe, 52.5. Found: OMe, 51.8.

The characteristic aniline derivative was prepared[11] and crystallized from light petroleum; m.p. 114° and $[\alpha]^{25}\mathrm{D}$ +212° (*c* 3.4, acetone).

2,3-Di-O-methyl-D-xylose was obtained as a sirup (25 mg.) which slowly crystallized on nucleation with an authentic specimen. The crystals melted at 77–79°, undepressed on admixture with a known sample,[12] and $[\alpha]^{25}\mathrm{D}$ $+62° \rightarrow +25°$ (24 hr., *c* 1.45, water).

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 34.1.

X-Ray diffraction photographs of the unknown and an authentic 2,3-di-O-methyl-D-xylose were identical.

3,4-Di-O-methyl-D-xylose was isolated as a sirup (55 mg.) which was oxidized with bromine water for 48 hours, aerated to remove excess bromine and neutralized by adding silver carbonate. After filtration, silver was precipitated as the sulfide, and filtered off. Evaporation under reduced pressure gave crystals (41 mg.) which recrystallized from ether; m.p. 68° and $[\alpha]^{25}\mathrm{D}$ $-55°$ (*c* 0.82, water) $\rightarrow -22°$ (44 hours).

Anal. Calcd. for $C_7H_{12}O_5$: OMe, 35.2; C, 47.7; H, 6.9. Found: OMe, 34.9; C, 47.4; H, 6.8.

The specimen and an authentic preparation of 3,4-di-O-methyl-D-xylonolactone had identical X-ray diffraction patterns.

Acknowledgment.—2,4-Di-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose and 3,4-di-O-methyl-D-xylonolactone were kindly supplied by Dr. J. K. N. Jones.

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