ml. of 12 *N* hydrochloric acid and 15 ml. of water was cooled to 0°. A total of 1.60 g. of solid sodium nitrite was added at such a rate that the temperature of the solution did not exceed 3°. After the sodium nitrite had been added the solution was kept at 0° for one-half hour.

To a suspension of 4.30 g. (0.018 mole) of N-(5'-desoxy-D-ribityl)-3,4-dimethylaniline in 40 ml. of water was added 6.1 ml of 12 *N* hydrochloric acid and 6.06 g. of sodium acetate. The mixture was cooled to −5° and the solution of diazotized aniline prepared above was added. The resulting solution was stirred at −5° for 1.5 hours and then at 0 to 5° for 1.5 hours. After warming to 20° a solution of 5.72 g. of sodium acetate in 50 ml. of water was added at such a rate that the *p*H remained at 3 to 3.5 and the temperature at 17 to 20°. After stirring the resultant mixture for 14 hours the insoluble product was collected and washed with water. The product was recrystallized from 200 ml. of 60% ethanol and dried over phosphorus pentoxide giving 5.04 g. (81%) of N-(5'-desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline melting at 174–176°.

Anal. Calcd. for $C_{19}H_{25}N_3O_3$: C, 66.45; H, 7.34; N, 12.24. Found: C, 66.32; H, 7.26; N, 11.71.

5'-Desoxyriboflavin (VIII).—N-(5'-Desoxy-D-ribityl)-2-phenylazo-4,5-dimethylaniline (4.98 g., 0.015 mole) and barbituric acid (3.05 g., 0.024 mole) were added to 43 ml. of butanol and 8 ml. of acetic acid. The resulting mixture was refluxed with stirring for 2.25 hours. After cooling in ice for one hour the insoluble material was collected and washed with butanol. The solid was then triturated with water at 80° for one-half hour, filtered and washed with methanol. The product was recrystallized by dissolving it in 25 ml. of hot 6 *N* hydrochloric acid, treating with decolorizing charcoal, filtering and diluting the filtrate with 50 ml. of hot water. On cooling, 5'-desoxyriboflavin (6,7-dimethyl-9-[1'-(5'-desoxy-D-ribityl)]-isoalloxazine), crystallized. The product (2.98 g., 57%) melted with decomposition at 282–283°. The analytical sample was recrystallized as above, m.p. 283–285° dec., $[\alpha]^{23}D$ 60° (*c* 1, 6 *N* hydrochloric acid). The compound has absorption maxima at 2230 mμ (*E* 33,400), 2670 mμ (*E* 33,800) and 3710 mμ (*E* 10,600).

Anal. Calcd. for $C_{17}H_{20}N_4O_5$: C, 56.66; H, 5.60; N, 15.56. Found: C, 56.83; H, 5.62; N, 15.27.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

An Aldotriouronic Acid from Hemicellulose-B of Corn Cob[1,2]

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Continued investigation of the acidic oligosaccharides isolated from a partial hydrolysate of corn cob hemicellulose-B suggests that one is *O*-α-D-glucopyranosyluronic acid-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose.

Absorption of the acidic oligosaccharides from a partial hydrolysate of corn cob hemicellulose-B on Amberlite IR-4B resin has led to the isolation of four aldobiouronic acids and one aldotriouronic acid. Three of the aldobiouronic acids have been characterized.[3,4] The aldotriouronic acid is described here.

Two additional procedures for the isolation of acidic oligosaccharides from polysaccharide partial hydrolysates have been investigated in an attempt to find the most rapid and convenient. Displacement from a carbon column[5] gave no clear-cut separation between acidic di- and trisaccharides nor could a satisfactory resolution of a mixture of neutral and acidic oligosaccharides be effected. The method now preferred involves precipitation of the acidic oligosaccharides as barium salts, followed by removal of barium ions on a column of IR-120 resin and separation of the free acids on a cellulose column.

The aldotriouronic acid thus isolated reduces boiling Fehling solution and is very resistant to complete hydrolysis. In the hydrolysate from the trisaccharide are found xylose, glucuronic acid and an aldobiouronic acid, which corresponds in chromatographic position to the mixture of 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose and 4-*O*-(α-D-glucopyranosyluronic acid)-D-xylose previously characterized.[4] The neutral trisaccharide produced by reduction of the methyl glycoside methyl ester of the aldotriouronic acid by lithium aluminum hydride[6–8] hydrolyzes to D-glucose and D-xylose only. Methylation of the neutral trisaccharide followed by hydrolysis yields two sugar derivatives which on paper chromatography correspond to 2,3-di-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-glucose. On bromine oxidation of the aldotriouronic acid with subsequent methylation and hydrolysis, 2,3-di-*O*-methyl-D-xylose and a methylated uronic acid derivative are detected as the only reducing units on paper chromatography. Methylation of the trisaccharide, first with dimethyl sulfate and sodium hydroxide and later with methyl iodide and silver oxide, gives a fully methylated derivative which hydrolyzes very readily with formic acid to 2,3-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucuronic acid. These compounds are separated by paper chromatography and converted to crystalline derivatives.

This evidence characterizes the aldotriuronic acid as a linear molecule in which D-glucuronic acid is the non-reducing end-group. The stability of the molecule during the initial depolymerization of hemicellulose-B and its resistance to hydrolysis when isolated suggest that the central D-xylosyl unit exists in the pyranose form. The remote possibility of a 1 → 5 linkage between the central and reducing xylosyl units is not eliminated.

On the basis of optical rotatory considerations, the α-D configuration was assigned to the glycosidic linkage in the 4-*O*-(D-glucopyranosyluronic acid)-

(1) Journal Paper No. 820 of the Purdue Agricultural Experiment Station.

(2) Paper presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society in New York, N. Y., September, 1954.

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D-xylose previously described.[4] On similar grounds the β-D configuration was given to the glycosidic linkage in 1 → 4 xylobiose.[9] It is not unreasonable to assume that these two linkages are present in this aldotriouronic acid and thus the structure would seem to be *O*-α-D-glucopyranosyluronic acid-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose.

Experimental

Paper Chromatography.—The following solvents were used with Whatman No. 1 filter paper; A, ethyl acetate–pyridine–water (8:2:1); B, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); C, methyl ethyl ketone, 90% saturated with water; D, butanol-1–ethanol–water (40:11:19). *p*-Anisidine hydrochloride[10] was used as spray to detect the sugars on the chromatograms.

Partial Hydrolysis and Isolation of the Aldotriouronic Acid.—Hemicellulose-B[11] (200 g.) was hydrolyzed as previously described for 4 hours, neutralized to *p*H 7.5 with a hot saturated solution of barium hydroxide and filtered. The filtrate was poured with stirring into 95% ethanol (5 vol.) and the precipitated barium salts collected on the centrifuge. These salts were taken up in water (500 ml.) and the solution passed through a column of Amberlite IR-120 resin. The acidic oligosaccharides which were obtained on evaporation of the column eluate were separated on a column of powdered cellulose using a solvent mixture of ethyl acetate–acetic acid–water (9:2:2). The aldobiouronic acids previously characterized[3,4] were eluted first followed by the aldotriouronic acid (1.5 g.), $\alpha^{25}D$ + 38.3° (*c*, 1.37 in water); equivalent weight 460, calcd. 441.

Characterization of the Aldotriouronic Acid.—This trisaccharide readily reduces boiling Fehling solution. Hydrolysis at 100° with *N* sulfuric acid was accompanied by much decomposition. After neutralization with barium carbonate, examination of the paper chromatogram (solvent B) revealed the presence of xylose, glucuronic acid, glucurone and an aldobiouronic acid corresponding in position to Fraction C of Whistler, Conrad and Hough.[3]

Aldotriouronic acid (460 mg.) was refluxed with 2% methanolic hydrogen chloride for 6 hours. After neutralization with silver carbonate, filtration and evaporation, the methyl ester–methyl glycoside (468 mg.) was obtained. This was dissolved in tetrahydrofuran (20 ml.) and the solution added dropwise to a stirred slurry of lithium aluminum hydride (1.0 g.) in tetrahydrofuran. The mixture was stirred for 1 hour when the excess lithium aluminum hydride was decomposed by the cautious addition of water. Tetrahydrofuran was removed by evaporation and the filtered solution deionized with IR-120 and IR-4B resins, and evaporated to a sirup (350 mg.). A sample of the sirup was hydrolyzed with *N* sulfuric acid at 100° for 3 hours and the solution neutralized and examined on the paper chromatogram (solvents A, B and D). It contained two components, corresponding to D-glucose and D-xylose, the latter in much higher concentration than the former as evinced by the relative intensity and size of the spots on chromatograms. A partial hydrolysis of this neutral trisaccharide (*N* sulfuric acid, 100°, 15 min.) gave, in addition to D-glucose and D-xylose, a disaccharide which did not correspond in chromatographic mobility with β-D-1→4-xylobiose. The remainder of this sirup (280 mg.) was methylated by three treatments with sodium hydroxide and dimethyl sulfate, first at 0° and later at room temperature, followed by methyl iodide and silver oxide, as described elsewhere.[12] The fully methylated trisaccharide (150 mg.) was hydrolyzed with *N* sulfuric acid at 100° for 3 hours and the neutralized hydrolysate examined by paper chromatography (solvents C and D). Two components corresponding to 2,3-di-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-glucose were detected. Insufficient material was available for further characterization.

Aldotriouronic acid (100 mg.) was oxidized with bromine water for 4 days at room temperature. The excess bromine was removed by aeration and the solution neutralized with silver carbonate. After filtration the silver ions were removed by passage of hydrogen sulfide and the solution again filtered. Evaporation of the filtrate under reduced pressure gave an amorphous powder (90 mg.) which was methylated by the usual procedure.[12] The fully methylated material (66 mg.) was hydrolyzed by consecutive treatment with 90% formic acid (2 ml.) at 100° for 30 minutes and, after evaporation *in vacuo*, with *N* sulfuric acid (2 ml.) at 100° for 45 minutes. The hydrolysate was neutralized with barium carbonate and the only reducing sugar derivatives detected on the paper chromatogram (solvents B, C and D) were 2,3-di-*O*-methyl-D-xylose and a methylated uronic acid derivative.

Another portion of the aldotriouronic acid (500 mg.) was converted to the fully methylated derivative (420 mg.). Hydrolysis was effected by 90% formic acid (10 ml.) at 100° for 20 minutes (constant rotation) followed after evaporation of the formic acid *in vacuo* by *N* sulfuric acid (10 ml.) at 100° for 1 hour. After neutralization with barium carbonate the hydrolysate was shown to contain two sugar derivatives: 2,3-di-*O*-methyl-D-xylose and a methylated uronic acid. These two components were separated on sheets of filter paper using solvent C in which the barium salt of the methylated uronic acid does not move from the starting line of the chromatogram.

2,3-Di-*O*-methyl-D-xylose was obtained as a sirup (156 mg.), $[\alpha]^{25}D$ +25° (equilibrium; *c*, 1.4 in water). This sirup (100 mg.) was refluxed in methanol (5 ml.) with freshly distilled aniline (0.2 ml.) for 3 hours. The sirup produced on evaporation of the solvent readily crystallized and was purified by recrystallization from methanol, m.p. 124–125°. A mixed melting point with N-phenyl-D-xylosylamine 2,3-dimethyl ether was unchanged, and the X-ray diffraction pattern of the authentic material was identical with that derived from the isolated 2,3-di-*O*-methyl-D-xylose.

Anal. Calcd. for $C_{13}H_7O_4N$: C, 61.7; H, 7.51. Found: C, 61.36; H, 7.53.

A sample of methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid, m.p. 132°, was prepared by the method of Challinor, Haworth and Hirst.[13] This was converted to methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)-uronate by refluxing in methanol containing 2.5% hydrogen chloride for 6 hours. Treatment with methanol saturated with ammonia at 0° overnight gave crystalline methyl 2,3,4-tri-*O*-methyl-D-glucopyranosiduronamide, which was recrystallized from cyclohexane containing a little ethanol; m.p. 183°.[14]

The methylated uronic acid from above (135 mg.) was converted to the ester glycoside as described previously, from which the crystalline amide, m.p. 183°, was prepared. A mixed m.p. with the authentic methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronamide was unchanged and the X-ray diffraction pattern of the authentic material was identical with that of the unknown.

Anal. Calcd. for $C_{10}H_{19}O_6N$: C, 48.2; H, 7.63. Found: C, 47.96; H, 7.84.

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