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aldehydo-D-Galactose Heptaacetate and *aldehydo*-D-Xylose Hexaacetate from Acetolysis of Guaran and Xylan[1]

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Acetolysis offers a means for the structural analysis of polysaccharides. This process often yields, in addition to acetates of the constituent monosaccharides, various oligosaccharide acetates and particularly disaccharide acetates, in which glycosidic linkages characteristic of the parent polysaccharide molecule are preserved unchanged and therefore available for further and more convenient study. An examination of the acetolysis products from guaran and xylan is in progress in this Laboratory. Separation of the various sugar acetates has been made by the excellent chromatographic technique of Wolfrom and co-workers.[2] Among the acetolysis products there is always present a considerable quantity of usual monosaccharide acetates. However, in addition there has been found among the acetolysis products of guaran and xylan, acetates of the monosaccharide aldehydrols. Thus, crystalline *aldehydo*-D-galactose heptaacetate and *aldehydo*-D-xylose hexaacetate have been isolated from guaran and xylan, respectively.

Guaran is the principal polysaccharide[3] of the endosperm of guar seed. It consists of galactose and mannose units in the ratio of 1:2, respectively. Present evidence suggests that its structure may be represented as a chain of mannose units with attached galactosidic side chains.[4,5] Acetolysis of guaran triacetate produces a mixture of products which can be separated by chromatography. The carbohydrate acetate occurring in the second zone from the bottom crystallizes and is identified as *aldehydo*-D-galactose heptaacetate. This compound has been synthesized previously from *aldehydo*-D-galactose pentaacetate and acetic anhydride,[6,7] and the DL-compound has been obtained by acetolysis of agar.[8] So far the corresponding acetylated aldehydrol of mannose has not been isolated from guaran acetolysate.

Corn cob xylan or its diacetate readily undergoes acetolysis and the products are easily separated by chromatography. From the bottom zone *aldehydo*-D-xylose hexaacetate may be obtained readily in crystalline condition. This compound was synthesized previously by Pirie[7] who isolated it as a sirup but was unable to obtain it in crystalline form. By employing chromatographic purification, however, the present authors have been able to obtain in crystalline condition the synthetic compound prepared by acetylation of *aldehydo*-D-xylose tetraacetate. The synthetic compound is identical with the crystalline specimen derived from acetolysis of xylan.

The possibility of obtaining acetates of the aldehydrol form of sugars from acetolysis of glycosides has been demonstrated by Freudenberg and Soff[9] who obtained *aldehydo*-D-glucose heptaacetate by acetolysis of the methyl tetraacetyl-D-glucosides. The inference is, therefore, that sugar aldehydrol acetates may be expected as normal products of acetolysis of polysaccharides. The present work tends to substantiate this view.

Experimental

Acetolysis of Guaran Acetate.—Guaran triacetate was prepared as described previously.[3] Acetolysis was performed in a manner similar to that described by Nishida and Hashima for Konjak glucomannan.[10] A mixture of acetic anhydride (330 g.), glacial acetic acid (330 g.) and concentrated sulfuric acid (64 g.) in a 2 liter round-bottom flask was cooled to 0° in an ice-bath. Powdered guaran triacetate (32 g.) was slowly added with stirring. When all of the acetate had been added, the temperature was allowed to rise slowly to 25°. Any undissolved acetate went into solution during this period. The mixture was then allowed to stand at room temperature for thirty-one days. The acetolysis mixture was slowly poured into 3–5 liters of ice water and the solution neutralized with sodium bicarbonate. The acetates were extracted from the water solution with 5 portions of chloroform (total volume 1 liter). The chloroform extracts were evaporated to dryness, weighed, and extracted with 320 ml. of benzene to make a solution containing approximately 1 g. of solid per 10 ml.

Chromatographic Separation of the Dissolved Acetates.—The technique of McNeely, Binkley and Wolfrom[2] was followed. To the top of a 330 × 55 mm. (diam.) column of a mixture of 5 parts (by wt.) of "Magnesol" and 1 part of "Celite" was added 20 ml. of the benzene solution described above. The chromatogram was developed with 3500 ml. of benzene–ethanol (150:1 volume ratio). The column was extruded and streaked with a solution of 1% potassium permanganate in 2.5 *N* sodium hydroxide. The permanganate solution was applied in a thin stream to the column by ejection from a hypodermic syringe. Five zones quickly developed. The zone measurements from the top of the column were as follows: I, 0–15 mm.; II, 25–35 mm.; III, 100–120 mm.; IV, 230–255 mm.; and V, 285–315 mm. The zones were eluted with acetone, evaporated to dryness, and dissolved in 95% ethanol. Rectangular prism-like crystals developed in the solution from zone IV, m. p. 103°. The yield was approximately 3% of the original guaran triacetates. After several recrystallizations from ethanol, the crystals showed a melting point of 105–106° unchanged by further recrystalliza-

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tion, $[\alpha]^{2}D$ 4.0 (c, 3 in $CHCl_3$). These values agree with those obtained by Pirie.[8] Oxidation of the crystals in 25% nitric acid gave mucic acid, m. p. and mixed m. p. 213–214°.

Anal. Calculated for $C_6H_7O_7(CH_3CO)_7$: C, 48.80; H, 5.73; CH_3CO, 61.2; mol. wt., 492.4. Found: C, 48.85; H, 5.87; CH_3CO (Kunz and Hudson method[11]), 60.9; mol. wt. (Rast), 487.

Preparation of xylan.—Xylan was obtained by purification of hemicellulose-A (crude xylan) extracted from corn cob holocellulose with 10% potassium hydroxide as described previously.[12] The hemicellulose-A was redissolved in 4% potassium hydroxide and reprecipitated by addition of acetic acid to *p*H 4.5. The precipitate, after collection in a supercentrifuge, was freed from water by four passages through ethanol in a Waring Blendor and dried to a low moisture content (2–3%) over calcium chloride in a vacuum desiccator. On a dry basis it contained 3% uronic anhydride[13] and 94% xylan (from furfural determination[14]), $[\alpha]^{20}D$ −106° (c, 0.5 in 1 N sodium hydroxide).

Xylan Acetate.—Xylan acetate used in these acetolysis studies was prepared by an adaptation of the method of Hurd and Currie.[15] Approximately 10 g. of xylan which had not been dried previously was repeatedly washed with acetic acid in a Waring Blendor until free of water. It was then washed with acetic anhydride to remove most of the acetic acid, placed in a 500-ml. flask and treated with 200 ml. of acetic anhydride containing 0.5 ml. of nitric acid, thus giving a concentration of nitric acid of 0.25%. The mixture was kept at 70° for seven hours, filtered, if necessary, and poured into ice and water. A white fibrous precipitate was obtained which was washed free of acid in a Waring Blendor. The last traces of acid were removed by boiling in water for several minutes. The *p*H was maintained at about seven during this process. The yield was 10 to 14 g. *Anal.* Acetyl, 38.6%; nitrogen, 0.2 to 0.3%. The nitrogen content indicates that a small amount of nitration accompanies the acetylation.

Xylan acetate prepared by the above method was soluble in pyridine and strong films could be cast from such solutions.[16] Xylan acetate prepared by the method of Carson and Maclay[17] undergoes acetolysis in a similar manner but was found to be incompletely soluble in pyridine or other organic solvents and consequently was not cast into films.

Acetolysis of Xylan Acetate.—Dry xylan acetate (20 g.), ground in a Wiley mill to pass a 40-mesh sieve, was swelled in 250 ml. of acetic anhydride at 60° for twelve hours. The suspension was cooled to 0° and 20 ml. of a 1:1 mixture of acetic anhydride and sulfuric acid was gradually added with rapid stirring. The temperature was maintained at 0° for several hours and then permitted to come to room temperature. At this point an almost clear, slightly yellow solution was obtained which gradually darkened. Acetolysis was permitted to proceed for thirty to forty days. The dark brown solution was then poured into 3 liters of ice and water and placed in a refrigerator overnight, during which time most of the acetates settled out. The supernatant liquid was decanted and reserved. The precipitate was suspended in water, the suspension was neutralized with sodium bicarbonate and extracted several times with chloroform. The reserved supernatant liquid was neutralized and extracted similarly and the extracts combined. The chloroform was evaporated under reduced pressure and a dark sirup resulted. The yield was approximately 20 g. The sirup was dissolved in 500 ml. of benzene and 30 ml. of this solution was chromatographed at a time.

Acetolysis of Xylan.—Acetolysis of xylan was carried out in an identical manner except that it was unnecessary to grind the material in a Wiley mill.

Chromatography of Acetates.—The above method was used. Thirty ml. of the benzene solution was added to the top of a 48 × 350 mm. sorption column. The chromatogram was developed with 1 liter of benzene–ethanol (100:1 volume ratio). The column was extruded and streaked with potassium permanganate. Three zones developed. Positions of the zones, measured from the top of the column were as follows: I, 1–5 cm.; II, 6–10 cm.; III, 14–18 cm. Almost all of the dark colored material was held within the top 1 cm. of the column and was thus easily separated. The zones were cut apart, eluted with acetone, and evaporated to dryness. The sirup obtained from zone III was rechromatographed. On elution it crystallized from alcohol on standing at 0°. Yield of crude material was approximately 10% of the original acetate. After four recrystallizations, m. p. 54–55°, $[\alpha]^{25}D$ 4.1° (c, 10 in $CHCl_3$). *Anal.* Calcd. for $C_5H_6O_6(CH_3CO)_6$: acetyl, 61.44; C, 48.57; H, 5.75. Found: acetyl, 61.4; C, 48.69; H, 5.78.

Synthesis of aldehydo-D-Xylose Hexaacetate.—Crystalline aldehydo-D-xylosetetraacetate (2.3 g.), prepared according to the instructions of Wolfrom, *et al.*,[18] was added to a solution of 2.3 g. of zinc chloride in 100 ml. of acetic anhydride and the solution was allowed to stand overnight. The solution was poured into cracked ice and water, neutralized with sodium bicarbonate, and extracted with chloroform. The sirup obtained after evaporation of the chloroform was chromatographed as described above. Two zones were obtained, the lower one corresponding precisely in location to the third zone from the acetolysis experiment. This zone was eluted with acetone as before. The sirup obtained on evaporation of the solvent was crystallized from alcohol. The yield was 1 g. After three recrystallizations, the melting point and optical rotation were identical with the acetolysis product. A mixture of the material and acetolysis product showed no depression of the melting point.

Summary

By chromatographic methods *aldehydo*-D-galactose heptaacetate and *aldehydo*-D-xylose hexaacetate have been isolated in crystalline form from the acetolysis degradation products of guaran triacetate and xylan diacetate, respectively. This is the first reported occurrence of crystalline *aldehydo*-D-xylose hexaacetate. Isolation of acetates of these sugar aldehydrols lends further support to the view that aldehydrol acetates are to be expected as normal constituents in the acetolysis products from polysaccharide degradation.

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