The relative constancy of specific activity of the streptomycin produced in fermentation III, in proceeding from the column eluate, through the helianthate crystals I–IV, to the regenerated trihydrochloride, is strong proof of the identity of the radioactivity with the streptomycin, *i.e.*, the radioactivity is associated with the streptomycin and not with incidental impurities.

The exact specific activity of the preparation is somewhat uncertain due to discrepancies between the various methods of determining streptomycin. Thus microbial assays of fermentation III helianthates III and IV, and of an authentic helianthate prepared from ordinary streptomycin, indicates 29.0, 25.4 and 28.0%, respectively, whereas the colorimetric (glucosamine assay) procedure showed more reasonable values such as 32.5, 31.5 and 33.5% streptomycin, respectively.

On the basis of the latter assay, the specific activity of the product from fermentation III is 5100 c.p.m./mg. streptomycin base, measured with a counting efficiency of ≃30%, or ≃0.008 μc. per mg. Fermentation IV product, after dilution with carrier, had a specific activity of ≃0.025 μc./mg., so that the original product must have had an activity of 0.2 μc./mg. It is obvious that the quantity of precursor C^{14} actually converted to streptomycin is ≃1% of that added. Higher specific activities would require precursors more efficiently utilized than glucose, or larger quantities of radioglucose.

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Crystalline Derivatives of Xylobiose[1,2]

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Partial hydrolysis of xylan leads to the isolation of a new disaccharide for which the name xylobiose is suggested. Four crystalline derivatives of xylobiose are described. Xylobiose is shown to consist of two xylopyranose units linked by a β-D-1,4′ bond.

By partial hydrolysis of xylan there has been obtained a disaccharide which is shown to be composed of two D-xylose units connected with a β-D-1,4′ linkage. Consequently the disaccharide is termed xylobiose to correspond with the disaccharide cellobiose similarly obtained from cellulose. By proper reactions xylobiose has been converted to four crystalline derivatives. This is the first time that crystalline products have been obtained from a disaccharide composed only of pentose units.

By acetolytic degradation of dimethylxylan of esparto grass Haworth and Percival[3] were able to recognize among the products a partly methylated, non-crystalline disaccharide which they showed to be composed of two xylose units linked by a β-D-1,4′ linkage.

In the present investigation xylan from corn cobs which is readily soluble in fuming hydrochloric acid was subjected to controlled partial hydrolysis. From the mixture of hydrolysis products the disaccharide was conveniently separated by the chromatographic technique of Whistler and Durso.[4] The disaccharide on acetylation yielded crystalline xylobiose hexaacetate. Deacetylation of xylobiose hexaacetate followed by hydrolytic cleavage of the amorphous disaccharide and reacetylation of the product yielded β-D-xylose tetraacetate in 85% yield.

The structure of xylobiose has been established by the procedure used by Haworth and Percival.[3] Crystalline xylobiose hexaacetate was deacetylated and oxidized to xylobionic acid which after repeated methylation with dimethyl sulfate and sodium hydroxide followed by methyl iodide and silver oxide yielded the methyl ester of hexamethylxylobionic acid. Hydrolytic cleavage of this product yielded crystalline 2,3,4-trimethyl-D-xylose in 86% of theoretical yield which was shown to be identical with an authentic specimen and a second component 2,3,5-trimethyl-γ-D-xylonolactone which distilled as a liquid in a yield of 80% of theoretical. This component was converted to the crystalline amide of the corresponding 2,3,5-trimethylxylonic acid and was shown to be identical with an authentic specimen.

Since xylobiose and its derivatives are strongly levorotatory and since this rotation decreases on hydrolysis, it is suggested that its two components are linked by a β-D-1,4′ bond.

Experimental

Hydrolysis of Xylan.—Xylan (1 g.) prepared as previously described[5,6] was dissolved in 100 ml. of fuming hydrochloric acid (sp. gr. 1.21) at −15°. This mixture was then placed in an ice–water-bath and 10-ml. samples were withdrawn at regular intervals. The samples were analyzed for reducing values.[7] When the reducing value data were plotted on a semi-logarithmic scale, a straight line was obtained. The rate constant during the initial 3–4 hours is approximately 3×10^{-4} sec.$^{-1}$; $[\alpha]^{25}D$ +40° at the time the hydrolysis is two-thirds complete.

Preparation of Xylobiose.—Xylan (30 g.) ground to pass a 60-mesh sieve was placed in a 2-l. flask and 1.5 l. of fuming hydrochloric acid (sp. gr. 1.21) previously cooled to −15° was added. Solution was complete within 30 minutes. The flask was then placed in an ice–water-bath. Hydroly-

(1) Journal Paper No. 526 of the Purdue University Agricultural Experiment Station.

(2) Paper presented before the Division of Sugar Chemistry and Technology at the 116th Meeting of the American Chemical Society, Atlantic City, N. J., 1949.

(3) W. N. Haworth and E. G. V. Percival, *J. Chem. Soc.*, 2850 (1931).

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

(5) R. L. Whistler, R. Bowman and J. Bachrach, *Arch. Biochem.*, **19**, 25 (1948).

(6) R. L. Whistler, E. Heyne and J. Bachrach, THIS JOURNAL, **71**, 1476 (1949).

(7) Method of P. A. Shaffer and A. F. Hartman, *J. Biol. Chem.*, **45**, 365 (1920).

sis was interrupted by pouring the mixture into 1.5 l. of ice and water when $[\alpha]^{25}D$ +40°, and the solution was neutralized with sodium bicarbonate as rapidly as possible. The neutral liquor was then chromatographed as described by Whistler and Durso.[4] To the top of a 52 × 260 mm. column of a mixture of equal parts (by weight) of charcoal (Darco G-60) and Celite (535) was added 1/3 of the hydrolysate. The column was then washed with distilled water until the eluate was free of salt and optically inactive. The column was next developed with 5% aqueous ethanol and the fraction showing a negative rotation was collected and evaporated *in vacuo*. The yield was 3 g. of sirup.

Xylobiose Hexaacetate.—Sirupy xylobiose (10 g.) was heated to 100–110° with a mixture of 90 ml. of acetic anhydride, 10 ml. of acetic acid and 10 g. of sodium acetate until dissolved. The solution was poured into a mixture of ice and water. The precipitated acetate crystallized spontaneously and was filtered after all of the acetic anhydride had been decomposed. The crude acetate was washed with dilute sodium bicarbonate and recrystallized from alcohol. The yield of recrystallized acetate was 10 g., $[\alpha]^{25}D$ −75° (*c* 10 in chloroform), m.p. 155.5–156°.

Anal. Calcd. for $C_{10}H_{12}O_5(CH_3CO)_6$: C, 49.44; H, 5.62; CH_3CO, 48.32; mol. wt., 534. Found: C, 49.3; H, 5.60; CH_3CO, 48.2; mol. wt. (Rast), 540, 541.

Hydrolysis of Xylobiose.—Recrystallized xylobiose hexaacetate (4 g.) was deacetylated according to the instruction of Isbell.[8] The resulting sirup was dissolved in 75 ml. of 1 *N* sulfuric acid and heated at 70°. The optical rotation changed from −0.65 to +0.60° in five hours. The optical rotation of an equivalent solution of xylose in 1 *N* sulfuric acid was +0.58°. The solution was cooled, neutralized and the product isolated in the usual way. After acetylation by means of the sodium acetate procedure described previously there was obtained in 85% yield crystalline β-D-xylose tetraacetate; m.p. 121–122°, $[\alpha]^{25}D$ −25° (*c* 10 in chloroform). When mixed with an authentic specimen no depression of the melting point occurred.

Methyl Pentaacetylxylobioside.—Xylobiose hexaacetate (2 g.) was dissolved in 20 ml. of ethanol-free chloroform, cooled to 0° and to it was added 5.2 ml. of 32% hydrogen bromide in acetic acid. The mixture was kept at 0° for two hours, poured into ice-water, extracted with chloroform and the chloroform extract was washed four times with water, dried over sodium sulfate and concentrated *in vacuo* to a volume of 40 ml. To the resulting solution were added 60 ml. of absolute methanol, 4 g. of powdered Drierite, 4 g. of silver carbonate and a crystal of iodine. The mixture was shaken overnight, filtered and evaporated *in vacuo*. The product crystallized readily from alcohol. The yield was 0.9 g.; m.p. 145–146°, $[\alpha]^{25}D$ −99.7° (*c* 5 in chloroform).

Anal. Calcd. for $C_{11}H_{15}O_9(CH_3CO)_5$: acetyl, 42.49; methoxyl, 6.12. Found: acetyl, 42.4; methoxyl, 6.1

Methyl Xylobioside.—Methyl pentaacetylxylobioside (1.75 g.) was dissolved in 35 ml. of absolute methanol and to it was added 2 ml. of 0.4 *N* barium methoxide and the mixture was kept at 0° for 24 hours. It was neutralized with 0.5 *N* sulfuric acid and the precipitated barium sulfate was removed. The filtrate was evaporated *in vacuo*. The yield was 1.5 g. The sirup was crystallized from alcohol and petroleum ether; m.p. 103–104°, $[\alpha]^{25}D$ −74.7° (*c* 3 in water).

Anal. Calcd. for $C_{11}H_{20}O_9$: methoxyl, 10.47. Found: methoxyl, 10.5.

Methyl Pentamethylxylobioside.—Methyl pentaacetylxylobioside (1.5 g.) dissolved in 30 ml. of acetone was poured into a three-neck flask equipped with a stirrer and two separatory funnels. The flask was cooled in ice-water at the beginning of the reaction. A total of 20 ml. of dimethyl sulfate and 45 ml. of 30% sodium hydroxide was added. One-tenth of these amounts was added simultaneously with rapid stirring from the two separatory funnels at 15-minute intervals. During the last four additions the temperature was raised to 55°. After the final addition heating was continued for 15 minutes. The solution was neutralized with 10% sulfuric acid, concentrated *in vacuo* and the residue repeatedly extracted with chloroform. The extract was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The yield was 1 g. The sirup was dissolved in 10 ml. of absolute methanol and 20 ml. of methyl iodide. With rapid stirring there was added at 30-minute intervals 2 g. of silver oxide until a total of ten additions had been made. The temperature was maintained at 35° during the additions. The silver oxide was removed and the filtrate was evaporated *in vacuo*. The resulting sirup was remethylated and after the second Purdie treatment crystallized spontaneously on concentration. The yield was 0.5 g. Recrystallization was accomplished from low boiling petroleum ether (35–37°); m.p. 75.5–76°, $[\alpha]^{25}D$ −71° (*c* 3 in chloroform).

Anal. Calcd. for $C_{10}H_{12}O_5(CH_3O)_6$: methoxyl, 50.82. Found: methoxyl, 50.6.

Methyl Hexamethylxylobionate.—Sirupy xylobiose (10 g.) prepared by deacetylation of crystalline xylobiose hexaacetate according to the method of Isbell[8] was dissolved in 500 ml. of water and to it was added 10 g. of calcium benzoate and 2 ml. of bromine. The mixture was allowed to stand in the dark for 48 hours. Excess bromine was removed by aeration and the solution after filtration had no reducing action on Fehling solution. It was adsorbed on a carbon–Celite column as described previously. The aldobionic acid was eluted with 20% ethanol after the column had been washed free of salts, benzoic acid and possible monosaccharide fragments. The aldobionic acid was obtained as a sirup (5 g.) on evaporation of the eluate and was methylated with dimethyl sulfate and sodium hydroxide as previously described. The partly methylated aldobionic acid was extracted with chloroform and the unchanged aldobionic acid which did not dissolve in the chloroform phase was recovered by means of the previously described charcoal chromatogram. The recovered aldobionic acid was remethylated and by repeating this technique three times no optically active material remained in the extracted water layer. The partly methylated aldobionic acid was then methylated three times with methyl iodide and silver oxide. The yield was 3 g. of sirup.

Anal. Calcd. for $C_{10}H_{11}O_5(CH_3O)_7$: methoxyl, 59.62. Found: methoxyl, 59.3.

Hydrolysis of Methyl Hexamethylxylobionate.—Sirupy methyl hexamethylxylobionate (3 g.) was hydrolyzed with 100 ml. of 2% hydrochloric acid at 100°. Hydrolysis was complete after 15 hours. The solution was neutralized with barium carbonate, clarified, evaporated to dryness *in vacuo* and finally dried with ethanol and benzene. The residue was repeatedly extracted with dry ether. 2,3,4-Trimethyl-D-xylose crystallized from the ether extract on nucleation. The yield was 86% of theoretical, m.p. 90–92° alone or when mixed with an authentic specimen of 2,3,4-trimethyl-D-xylose.

The residue was redissolved in water, acidified with 10 ml. of 1 *N* hydrochloric acid and evaporated to dryness *in vacuo*. The residue was extracted with ether and the ether extract was distilled, b.p. 80–90°, 0.02 mm., $n^{15.5}D$ 1.4472. The yield was 80% of theoretical. The amide of the corresponding 2,3,5-trimethyl-D-xylonic acid was prepared by passing dried ammonia into this lactone solution in methanol. Crystallization was effected from ethanol; m.p. 84–85°, alone and when mixed with an authentic specimen. The X-ray diffraction patterns of the authentic amide and that isolated as described were identical.

The authentic specimen was prepared by passing dried ammonia into the methanol solution of the lactone of 2,3,5-trimethyl-D-xylonic acid, obtained by methylation of D-xylonic acid. The product crystallized from either ethanol or benzene. The amide was recrystallized from ethanol; m.p. 84–85°.

Anal. Calcd. for $C_5H_{17}O_5N$: C, 35.09; H, 9.94; N, 8.18. Found: C, 35.0; H, 9.9; N, 8.2.

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