[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Synthesis of Maltose-1-phosphate and D-Xylose-1-phosphate

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The linkage between any pair of contiguous glucose units in the unbranched starch chain is the same as in the disaccharide maltose. Starch, which is a polymer of D-glucose, may therefore also be regarded as a polymer of maltose. Since starch is formed as the result of "de-phosphorylytic" condensation of a large number of D-glucose-1-phosphate units, it was thought that maltose-1-phosphate might be similarly converted by phosphorylase into a polysaccharide.

Inasmuch as D-xylose and D-glucose are structurally identical, except that D-glucose has an additional carbon atom attached to the six-membered ring, it was also of interest to examine the possibility of conversion of D-xylose-1-phosphate into a polysaccharide, xylan, by the same enzyme.

D-Glucose-1-phosphate produced through enzymatic breakdown of glycogen or starch is identical with the synthetically prepared ester[1,2,3] its structure being α-D-glucopyranose-1-phosphate. When acted upon by animal or plant phosphorylase this ester is converted by a reversible reaction into polysaccharide. However, neither β-D-glucose-1-phosphate[4] nor the α-forms of D-mannose-1-phosphate and D-galactose-1-phosphate are acted upon by phosphorylase to form polysaccharids.[5,6,7]

Maltose-1-phosphate and D-xylose-1-phosphate were prepared by treating α-acetobromomaltose and α-acetobromoxylose with freshly prepared trisilver phosphate and by partially hydrolyzing the acetylated trisugar phosphate derivatives in methanol solution with dilute acid. Since these esters were prepared through the reaction of the corresponding α-halogen sugar derivatives and trisilver phosphate,[6] the resulting maltose-1-phosphate and D-xylose-1-phosphate are most probably the α-forms.

When subjected to oxidation with sodium periodate, the maltose-1-phosphate consumed three moles of periodate and formed one mole of formic acid. The D-xylose-1-phosphate consumed two moles of periodate and gave rise to one mole of formic acid. These data are consistent with the view that the structure for maltose-1-phosphate is glucopyranosido-4-glucopyranose-1-phosphate and the structure of D-xylose-1-phosphate is D-xylopyranose-1-phosphate.

The maltose-1-phosphate and the xylose-1-phosphate when treated with potato phosphorylase do not undergo polysaccharide formation.

Experimental

Preparation of Maltose-1-(barium phosphate).—β-Octaacetyl maltose was prepared by the method of Ling and Baker[8] and converted to the α-bromoheptaacetylmaltose by the general procedure of Brauns,[9] slightly modified as follows: The reaction mixture, consisting of β-octaacetylmaltose, glacial acetic acid and hydrogen bromide at 0°, was poured into ice water and extracted with chloroform. The chloroform solution of the bromoacetyl maltose was washed with ice water and dried with calcium chloride, filtered and evaporated *in vacuo* to a thick sirup. After the addition of petroleum ether and repeated stirring, the sirup was converted into a white, amorphous mass. The product was dried in a vacuum desiccator over potassium hydroxide. A yield of 97% of α-bromoheptaacetyl maltose was obtained. Its specific rotation in chloroform (*c*, 2) was $[\alpha]_D$ + 178°, compared with Brauns'[9] value of $[\alpha]_D$ + 180.24°.

Twenty-five grams of the α-bromoheptaacetylmaltose was dissolved in 150 ml. of dry benzene in which was suspended a slight excess of the theoretical amount of freshly prepared trisilver phosphate needed to react with the bromoacetylmaltose. The mixture was refluxed for one hour with mechanical stirring, with a calcium chloride tube attached to the condenser. After cooling, the mixture was filtered with suction on a paper coated with diatomaceous silica, "Hyflo-Super-Cel" (Johns-Manville), decolorized with charcoal and again filtered with suction. The benzene was removed by evaporation under reduced pressure, the residue stirred with petroleum ether and the amorphous product thus obtained dried *in vacuo*. The specific rotation of this product in chloroform (*c*, 2) was $[\alpha]_D$ + 120°. No attempt was made to further purify this intermediate compound which is presumably tri-(heptaacetyl-maltose-1-)-phosphate. The yield was 91% of the theoretical.

The intermediate compound was partially hydrolyzed with 0.2 *N* hydrochloric acid in methanol as described by Cori, Colowick and Cori[1] and the product isolated as a gelatinous barium salt. The salt was dissolved in a minimum amount of water, precipitated by the addition of 1.5 volumes of 95% ethanol, the mixture allowed to remain at 0° for two hours and centrifuged. This procedure was repeated and the precipitate washed with absolute alcohol, alcohol-ether mixture and ether and dried at 80° *in vacuo*. A yield of 2.5 g. was obtained. The white amorphous appearing product had a specific rotation in water (*c*, 2) of $[\alpha]_D$ + 107°. Analysis showed that the barium salt contained five molecules of water of crystallization.

Anal. Calcd. for $C_{12}H_{21}O_{14}PBa \cdot 5H_2O$: C, 22.25; P, 4.78; Ba, 21.22. Found: C, 22.20; P, 4.62; Ba, 20.90.

The substance did not reduce Fehling solution; it was stable to alkali, but was very acid-labile. On hydrolysis of the barium salt of maltose-1-phosphoric acid with 1 *N* sulfuric acid for ten minutes at 100°, neutralization of the solu-

(1) C. F. Cori, S. P. Colowick and Gerty T. Cori, *J. Biol. Chem.*, **121**, 465 (1937).

(2) (a) C. S. Hanes, *Proc. Roy. Soc.* (London), **B128**, 421 (1940); (b) **B129**, 174 (1940).

(3) M. L. Wolfrom and D. E. Pletcher, THIS JOURNAL, **63**, 1050 (1941).

(4) M. L. Wolfrom, C. S. Smith and A. E. Brown, *ibid.*, **65**, 255 (1943).

(5) S. P. Colowick, *J. Biol. Chem.*, **124**, 557 (1938).

(6) F. J. Reithel, THIS JOURNAL, **67**, 1056 (1945).

(7) Inasmuch as these galactose and mannose phosphate esters were prepared from the corresponding α-acetobromo-sugar derivatives and trisilver phosphate by a method similar to that used to prepare α-D-glucose-1-phosphate, they are probably the α-forms. Reithel[6] has shown that Colowick's[5] D-galactose-1-phosphate prepared in this way is the α-form and has pointed out that when monosilver phosphate is used the β-isomers of aldose-1-phosphates are obtained.

(8) A. R. Ling and J. L. Baker, *J. Chem. Soc.*, **67**, 212 (1895).

(9) D. H. Brauns, THIS JOURNAL, **51**, 1830 (1929).

tion and after removal of the barium sulfate precipitate, an osazone was obtained which was identified as maltosazone.

An attempt to prepare the potassium salt of maltose-1-phosphate was not successful, as the product obtained was extremely hygroscopic.

Rate of Hydrolysis and Dissociation Constants of Maltose-1-phosphate.—A quantity of barium salt of maltose-1-phosphoric acid was dissolved in 0.376 *N* hydrochloric acid to make 50 ml. of 0.1 *M* solution and placed in a glass cylinder. The cylinder and contents was immersed in a constant temperature bath at 36°. Samples were taken out at intervals, neutralized with sodium hydroxide, the barium removed with potassium sulfate and analyzed for inorganic phosphorus. The total amount of ester present was determined by hydrolyzing a sample for ten minutes with 1 *N* sulfuric acid at 100°. From the rate of hydrolysis the specific rate constant *K* at 36° for the maltose-1-phosphate was calculated. A straight line was obtained by plotting log $(a - x)$ against time, where a is equal to the total amount of phosphorus and x is equal to the amount of hydrolyzed phosphorus at time t. The rate constant was calculated from the slope of the curve, $K = 2.303\ \Delta \log (a - x)/\Delta t$, and was found to be 3.21×10^{-3}.

The hydrolysis rate constant *K* for dipotassium glucose-1-phosphate (Cori ester) determined under the same conditions was 4.36×10^{-3}, as compared with Cori and co-workers'[11] value of 2.99×10^{-3} [10] determined at 37° and 0.25 *N* hydrochloric acid.

The first and second dissociation constants of maltose-1-phosphoric acid were determined by electrometric titration of the salt with 0.1 *N* hydrochloric acid using a glass electrode. The pK_1' was calculated by means of Van Slyke's[11] formula

$$K_a' = \frac{[H^+](B + [H^+])}{C - (B + [H^+])}$$

The pK_2' was calculated by means of the Henderson-Hasselbach equation

$$pK_a' = pH - \log B/(C - B)$$

The dissociation constants thus obtained for this ester are: $pK_1' = 1.52$; $pK_2' = 5.89$.

Oxidation of Maltose-1-phosphate with Sodium Periodate.—The technique for oxidizing carbohydrates with sodium periodate was described by Hudson and co-workers.[12] In oxidizing the maltose-1-phosphate with sodium periodate Wolfrom and Pletcher's[3] procedure for oxidation of glucose-1-phosphate was followed. Previous to oxidation with periodate the maltose-1-(barium phosphate) samples were treated with sodium sulfate to remove the barium. The results showed that 3.02 moles of periodate were consumed, giving rise to 1.1 moles of formic acid in the oxidation of one mole of barium pentahydrate maltose-1-phosphate. The theoretical requirement for oxidation of one mole of glucopyranosido-4-glucopyranose-1-phosphate is: consumption of three moles of periodate and the production of one mole of formic acid.

Preparation of Xylose-1-(barium phosphate). Tetra-acetyl-D-xylose and α-bromotriacetyl xylose were prepared by the method of Hudson and Johnson.[13]

Twenty-five grams of α-bromotriacetyl-D-xylose was treated with trisilver phosphate and the intermediate product was partially hydrolyzed with 0.2 *N* hydrochloric acid in methanol as described in the preparation of maltose-1-(barium phosphate). A white seemingly amorphous product was obtained in yield of 4.2 g., which had a specific rotation in water (c, 2) of $[\alpha]D + 65°$. Analysis showed that the barium salt of xylose-1-phosphoric acid contained 1.5 molecules of water of crystallization.

Anal. Calcd. for $C_5H_9O_8PBa \cdot 1\frac{1}{2}H_2O$: C, 15.30; P, 7.89; Ba, 35.01. Found: C, 15.25; P, 7.95; Ba, 35.32.

Preparation of D-Xylose-1-(dipotassium phosphate).—Two grams of the barium salt of xylose-1-phosphoric acid was dissolved in 30 ml. of warm water and treated with an equivalent amount of 10% potassium sulfate solution. The precipitated barium sulfate was removed through a precoated filter and absolute ethanol was added to the filtrate to incipient turbidity. The solution was allowed to remain at room temperature and absolute alcohol was added from time to time until a total of about 1.7 volumes of ethanol was reached. The mixture was then cooled, filtered, the crystals washed with dilute ethanol and recrystallized from water by addition of an equal volume of ethanol. A yield of 1.9 g. was obtained. The xylose-1-(dipotassium phosphate) thus prepared is a white, non-hygroscopic crystalline product, shown to contain two molecules of water of crystallization. Its specific rotation in water (c, 2) is $[\alpha]D + 76°$. The xylose-1-ester is readily hydrolyzed with dilute acid, but stable to alkali. It shows no Fehling reduction on prolonged boiling. The ester is completely hydrolyzed to xylose and inorganic phosphate when heated for ten minutes in 1 *N* sulfuric acid in a boiling water-bath. On hydrolysis of the solution, an osazone was obtained, identified as xylosazone.

Anal. Calcd. for $C_5H_9O_8PK_2 \cdot 2H_2O$: C, 17.54; H, 3.83; P, 9.05. Found: C, 17.40; H, 3.69; P, 8.90.

Rate of Hydrolysis and Dissociation Constants of D-Xylose-1-phosphate.—The velocity constant for hydrolysis of the potassium salt of D-xylose-1-phosphoric acid in 0.376 *N* hydrochloric acid was determined at 36° as previously described. The value for *K* of this ester under these conditions is 6.21×10^{-3}.

The dissociation constants of the D-xylose-1-phosphate calculated by means of Van Slyke's formula and the Henderson-Hasselbach equation are: $pK_1' = 1.25$; $pK_2' = 6.15$.

Oxidation of D-Xylose-1-(dipotassium Phosphate) with Sodium Periodate.—The same procedure was employed for oxidizing the xylose-1-phosphate with sodium periodate as in the oxidation of the maltose-1-phosphate. In the oxidation of one mole of dipotassium dihydrate xylose-1-phosphate 2.05 moles of periodate were used with the production of 1.05 moles of formic acid, which closely agrees with the theoretical requirements of two moles of periodate and one mole of formic acid.

Action of Potato Phosphorylase on Maltose-1-phosphate and D-Xylose-1-phosphate.—Solutions of D-xylose-1-(dipotassium phosphate) and maltose-1-(barium phosphate), from which the barium was removed with potassium sulfate, were adjusted with acetic acid to *p*H 6.0 and treated with potato phosphorylase. In a control experiment D-glucose-1-phosphate was similarly treated. These mixtures were analyzed for inorganic phosphorus at several thirty-minute intervals. No hydrolysis of phosphate occurred under the influence of potato phosphorylase with maltose-1-phosphate or D-xylose-1-phosphate. Inorganic phosphorus appeared in the experiment with D-glucose-1-phosphate. The formation of starch was also observed when the solution was tested with iodine. In the case of maltose-1-phosphate and D-xylose-1-phosphate the starch-iodine test was negative.

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Summary

The barium salts of maltose-1-phosphoric acid and D-xylose-1-phosphoric acid have been synthesized and characterized. The D-xylose-1-phosphate has also been prepared as the crystalline potassium salt.

(10) The value for *K* reported by Cori, *et al.*,[9] is actually given as 1.30×10^{-3}, but it does not take into account the factor 2.303 necessary to convert the $\log_{10}$ to $\log_e$.

(11) D. D. Van Slyke, *J. Biol. Chem.*, **52**, 525 (1922).

(12) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937); **62**, 958 (1940); R. M. Hann, W. D. Maclay and C. S. Hudson, *ibid.*, **61**, 2432 (1939).

(13) C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 2748 (1915).

Data obtained from oxidation of these esters with sodium periodate show that the maltose ester is glucopyranosido-4-glucopyranose-1-phosphate and that the xylose ester is D-xylopyranose-1-phosphate. Both esters probably exist in the α-form.

The rate constants for hydrolysis of these esters in 0.376 N hydrochloric acid at 36° and also their dissociation constants were determined.

Maltose-1-phosphate and D-xylose-1-phosphate are not converted to polysaccharide by potato phosphorylase.

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[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

The Ultraviolet Absorption Spectra of Thiouracils[1]

BY GERTRUDE B. ELION, WALTER S. IDE AND GEORGE H. HITCHINGS

The ultraviolet absorption spectra of pyrimidines have received a good deal of attention in recent years. However, except for the recent paper of Miller, Roblin and Astwood[2] on 2-thiouracil and its oxidation product, no thiopyrimidine spectra have been reported in the literature.

During the present investigation of the absorption spectra of the mono- and dimercapto-analogs of uracil (2,4-dihydroxypyrimidine) and thymine (2,4-dihydroxy-5-methylpyrimidine) it was found that 2-hydroxy-4-mercaptopyrimidine (4-thiouracil) and 2-hydroxy-4-mercapto-5-methylpyrimidine (4-thiothymine) possess absorption bands so far removed from the usual absorption range of pyrimidines as to be unrecognizable as members of that group. It is interesting that the anomalous spectra of these 4-thio-derivatives are paralleled by their lack of antithyroid activity. Thus, Astwood[3] has reported that whereas 2-thiouracil and 2,4-dithiouracil have approximately the same antithyroid activity, 4-thiouracil is practically inactive.

Results and Discussion

The ultraviolet absorption spectra of 2-thiouracil, 2,4-dithiouracil, 4-thiouracil, 2-thiothymine, 2,4-dithiothymine and 4-thiothymine at pH values of 1.0, 7.0 and 11.0 are reported here.

A comparison of the spectra of these thiocompounds with the corresponding hydroxy-compounds reveals some expected as well as some unexpected differences. According to the data of Loofbourow, Stimson and Hart,[4] uracil at pH 7 shows an absorption maximum at 2580 Å. with a molecular extinction coefficient (ϵ) of 10,600. As might be anticipated from the increased mass of sulfur, the spectrum of 2-thiouracil at pH 7.0 (Fig. 1) shows a shift in the wave length of maximum absorption to 2740 Å., with an ϵ value only slightly higher than for uracil. The replacement of both oxygens by sulfur, as in 2,4-dithiouracil (Fig. 2) results in a shift of the wave length of maximum absorption still further toward the visible range, and also the introduction of a second, weaker band at 3600 Å. The molecular extinction coefficient of this compound for the main band is much higher than for 2-thiouracil. In contrast to these two compounds,

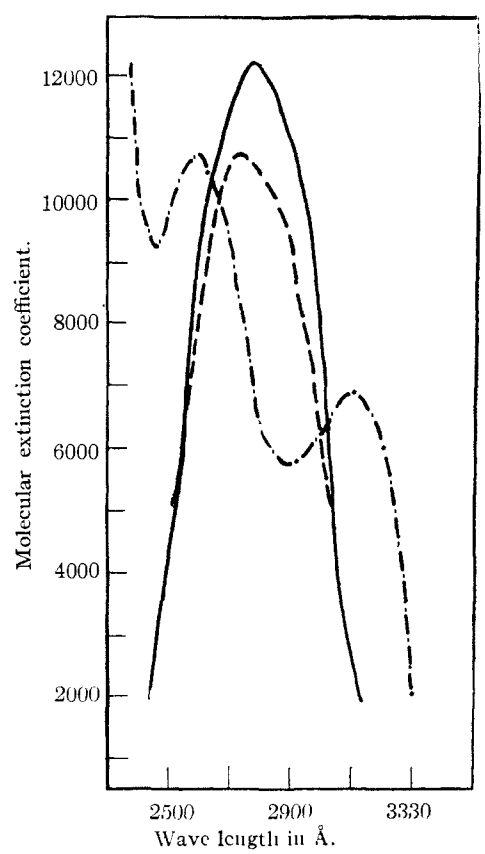

Fig. 1.—Absorption spectra of 2-thiouracil: —— at pH 1.0; - - - at pH 7.0; -·-·- at pH 11.0.

the spectrum of 4-thiouracil (Fig. 3) is quite unusual. The pyrimidine band has been shifted so far toward the visible that in the usual range of pyrimidine absorption (2600–2800 Å.) there occurs a minimum rather than a maximum in the spectral distribution curve. The peak occurs at

(1) Presented before the Division of Organic Chemistry at the 109th meeting of the American Chemical Society, Atlantic City, N. J., April, 1946.

(2) Miller, Roblin and Astwood, THIS JOURNAL, **67**, 2201 (1945).

(3) Astwood, Bissell and Hughes, *Endocrinology*, **37**, 456 (1945).

(4) Loofbourow, Stimson and Hart, THIS JOURNAL, **65**, 148 (1943).