Comparison of Reaction Rates of Various Diazo Compounds with Sulfamic Acid.—Tenth normal solutions of diazo compounds were prepared by known procedures and 50-ml. aliquots (0.005 mole) employed in each experiment. For each diazo compound the 50-ml. aliquots were treated with solutions (50 ml.) containing 0, 1, 10, or 20 equivalent amounts of sulfamic acid and the nitrogen gas evolved measured continuously by means of a rate nitrometer.[2] In all cases the *p*H was <1 and the temperature 25° except in the case of diazotetrazole which was run at 0–5°.

The original amine was isolated and identified as the primary reaction product except in the case of 2-chloro-4-nitroaniline. In this latter case, the diazoamino compound was isolated in good yield with the expected (50%) amount of N_2 liberated. Typical reaction rate curves are shown in Fig. 1.

2,5-Dichloroaniline and *p*-Nitroaniline.—The diazo compounds of these two amines were not sufficiently reactive for study with the rate nitrometer. Consequently, 0.05 *N* solutions of these diazo compounds were treated with 0, 1 and 10 equivalent amounts of sulfamic acid and stored at 5° for ten days. At the end of this time the solutions were filtered and the amounts of solid diazoamino compounds determined. Yields are given in Table I.

TABLE I

YIELD OF DIAZOAMINO COMPOUNDS

Sulfamic acid	% Yield from diazotized *p*-nitroaniline	% Yield from diazotized 2,5-dichloroaniline
None	..	..
1 equiv.	3.6	6.2
10 equiv.	12.0	19.0

Summary

1. Certain diazo compounds were shown to react with sulfamic acid in acid solution to yield the original amine from which the diazo compound was derived together with nitrogen and sulfuric acid.

2. A mechanism has been proposed to explain the reaction.

3. Relative reaction rates of various diazo compounds with sulfamic acid were compared by means of a rate nitrometer.

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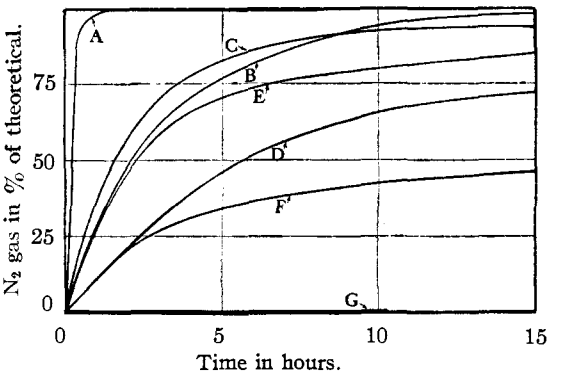

Fig. 1.—Rates of reaction of the diazo compounds of the following amines with sulfamic acid at the indicated concentration: A, 5-aminotetrazole, 20 equivalents of sulfamic acid; B, 2,4-dinitroaniline, 10 equivalents of sulfamic acid; C, 2,6-dichloro-4-nitroaniline, 20 equivalents of sulfamic acid; D, 2,6-dichloro-4-nitroaniline, 1 equivalent of sulfamic acid; E, 2-amino-5-nitro-N-ethylbenzenesulfonanilide, 20 equivalents of sulfamic acid; F, 2-chloro-4-nitroaniline, 20 equivalents of sulfamic acid; G, blank.

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[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION, COLLEGE OF AGRICULTURE, THE DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF CALIFORNIA, AND THE DEPARTMENT OF CHEMISTRY, BANTING INSTITUTE, UNIVERSITY OF TORONTO]

α-L-Glucose-1-phosphate

BY A. L. POTTER, JOHN C. SOWDEN, W. Z. HASSID AND M. DOUDOROFF

An enzyme obtained from the bacterium *Pseudomonas saccharophila* has been named sucrose phosphorylase because it catalyzes the reversible reaction between fructose and α-D-glucose-1-phosphate to form sucrose. This enzyme is also capable of catalyzing the reaction between other monosaccharides and the same ester, thus forming a number of disaccharides, namely, D-glucosido-L-sorboside, D-glucosido-D-xyloketoside, D-glucosido-L-araboketoside, and D-glucosido-L-arabinose.[1] The formation of these disaccharides demonstrates the versatility of the enzyme with regard to the non-glucose substrates which act as "glucose acceptors" in the synthetic reactions. However, the enzyme appears to be specific toward the glucose portion of its substrate. It has been found that the sucrose phosphorylase will not form compound sugars when α-maltose-1-phosphate, α-D-galactose-1-phosphate, or α-D-xylose-1-phosphate is substituted for α-D-glucose-1-phosphate. Similarly, potato and muscle phosphorylases will not form polysaccharides when these phosphorylated sugars are substituted for α-D-glucose-1-phosphate.

In this connection, it was of interest to test whether or not α-L-glucose-1-phosphate could be substituted for its optical isomer, α-D-glucose-1-phosphate in the enzymatic reaction with potato phosphorylase for polysaccharide synthesis or with sucrose phosphorylase for disaccharide formation.

In the present work the preparation of α-L-glucose-1-phosphate from L-glucose is described and

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its behavior as a substrate in these enzymatic reactions is determined.

Experimental

Preparation of α-L-Glucose-1-(barium phosphate).—L-Glucose was synthesized by the method previously described.[2] β-Pentaacetyl-L-glucose was prepared by heating 15 g. of L-glucose with 75 g. of acetic anhydride and 7.2 g. of powdered anhydrous sodium acetate according to the method of Fischer.[3] The yield of the acetylated derivative was 23.4 g. or 72%.

α-Bromotetraacetyl-L-glucose[4] was prepared as follows: fourteen grams of β-pentaacetyl-L-glucose was treated with 9.1 ml. of 30 to 32% solution of hydrogen bromide in glacial acetic acid and the mixture allowed to stand at room temperature for two hours. The solution was diluted with 60 ml. of chloroform, poured into 200 ml. of ice water and stirred rapidly. The chloroform layer was separated and the aqueous phase extracted once more with 15 ml. of chloroform. The chloroform extracts were washed twice with ice water, dried with calcium chloride and evaporated *in vacuo* at 40° to a thick sirup. The sirup was taken up with 35 ml. of anhydrous ether and petroleum ether was added until a second liquid phase began to appear. Crystallization was then allowed to take place. The yield of the crystalline acetobromo-L-glucose was 13.5 g. (91.6%).

α-L-Glucose-1-(barium phosphate) was prepared by treating 13.5 g. of α-bromotetraacetyl-L-glucose with trisilver phosphate, then partially hydrolyzing the intermediate product, presumably tri-(tetraacetyl-L-glucose-1)-phosphate, for twelve hours in 0.2 *N* hydrochloric acid in methanol at 23°, and neutralization with barium hydroxide.[5] A yield of 1.09 g. of the barium salt was obtained (22.2%). Analysis of the salt shows that it contains three molecules of water of crystallization. It is an amorphous, non-hygroscopic white powder, which is easily soluble in water and insoluble in 50% alcohol.

Anal. Calcd. for $C_6H_{11}O_5 \cdot O \cdot PO_3Ba \cdot 3H_2O$: P, 6.9. Found: P, 7.1; specific rotation, $[\alpha]_D$ −73.2° (*c*, 1.01, anhydrous barium salt, in water). Cori, Colowick and Cori's[5] value for the D-form of the barium salt, $[\alpha]_D$ +75°.

Preparation of α-L-Glucose-1-(dipotassium phosphate).—A portion of the barium salt (0.8 g.) was dissolved in 12 ml. of warm water and treated with an equivalent amount (0.31 g.) of potassium sulfate. The precipitated barium sulfate was removed through a precoated diatomaceous silica filter and absolute ethanol was added to the filtrate until a slight cloudiness appeared. The solution was allowed to remain at room temperature, and 1.7 volumes of absolute alcohol was added gradually. Crystallization was then allowed to take place. The crystals were filtered, washed with 65% ethanol and recrystallized from water by addition of an equal volume of ethanol. A yield of 0.48 g. was obtained (73%).

The L-glucose-1-(dipotassium phosphate) thus prepared is a white non-hygroscopic crystalline product, containing two molecules of water of crystallization and, except for its negative rotation, is similar in its physical and chemical properties to the D-form of the hexosephosphate.

Anal. Calcd. for $C_6H_{11}O_5 \cdot O \cdot PO_3K_2 \cdot 2H_2O$: C, 19.35; H, 4.06; P, 8.33; aldose, 48.4. Found: C, 19.19; H, 4.03; P, 8.40; aldose, 48.7. Specific rotation, $[\alpha]_D$ −78.2° (in water, *c*, 1.01). Hanes'[6] value for the D-form of the dipotassium salt, $[\alpha]_D$ +78.5° (in water, *c*, 1.24).

The L-glucose-1-phosphate is readily hydrolyzed with dilute acid, is stable in alkali, and shows no Fehling reduction on prolonged boiling. The ester is completely hydrolyzed to glucose and inorganic phosphate when heated for seven minutes in 1 *N* hydrochloric acid in a boiling water-bath. Upon hydrolysis of the ester the reducing sugar produced was identified as glucose by the preparation of glucosazone.

Oxidation of α-L-Glucose-1-(dipotassium phosphate) with Sodium Periodate.—In oxidizing the L-glucose-1-phosphate Wolfrom and Pletcher's[7] procedure for oxidation of the D form of this ester was used. The results showed that in the oxidation of one mole of dipotassium dihydrate L-glucose-1-phosphate 2.0 moles of periodate were consumed with the production of 1.1 moles of formic acid. These data closely agree with the theoretical requirements of two moles of periodate and one mole of formic acid, assuming that the L-glucose of this ester exists in the pyranose configuration.

Action of Potato Phosphorylase and Sucrose Phosphorylase from *P. saccharophila* on α-L-Glucose-1-(dipotassium phosphate).—A solution of α-L-glucose-1-phosphate was adjusted with acetic acid to *p*H 6.0 and treated with potato phosphorylase. The mixture was analyzed for inorganic phosphorus at several thirty-minute intervals. No inorganic phosphate was liberated, except for a small amount which was attributed to hydrolytic decomposition of the ester. In a control experiment with D-glucose-1-phosphate, inorganic phosphate was rapidly liberated.

A similar experiment was performed with a mixture of α-L-glucose-1-phosphate, D-fructose and sucrose phosphorylase extracted from *P. saccharophila*. No liberation of inorganic phosphate could be observed. Neither was inorganic phosphate liberated when L-fructose[8] was substituted for D-fructose in the mixture. In a control experiment with D-glucose-1-phosphate, D-fructose and the same enzyme, inorganic phosphate was liberated under these conditions.

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Summary

The barium salt of α-L-glucose-1-phosphoric acid has been synthesized and converted into the dipotassium salt. An elementary analysis and data obtained from oxidation with sodium periodate of the potassium salt of this ester agree with the composition $C_6H_{11}O_5 \cdot O \cdot PO_3K_2 \cdot 2H_2O$. Except for the negative rotation, $[\alpha]_D$ −78.2°, of the α-L-glucose-1-phosphate, its physical and chemical properties agree with those of its optical isomer, α-D-glucose-1-phosphate.

α-L-Glucose-1-phosphate is not converted by potato phosphorylase to polysaccharide. Neither can it be used as substrate by sucrose phosphorylase from *P. saccharophila* with either D- or L-fructose to form a disaccharide.

BERKELEY 4, CALIFORNIA RECEIVED SEPTEMBER 29, 1947

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(8) The authors wish to thank Dr. M. L. Wolfrom for supplying a sample of L-fructose.