Anal. Calcd. for $C_6H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.96; H, 7.27.

Oxidation of 1,5-Anhydro-D-altritol with Sodium Metaperiodate.—A solution containing 0.2001 g. of 1,5-anhydro-D-altritol and 6.0 ml. of 0.48 *M* aqueous sodium periodate in a total volume of 25 ml. reached the constant rotation $[\alpha]^{20}D$ −9.4° (calculated as the expected dialdehyde) sometime within a week at 20°. This rotation is in good agreement with the values −9.9 and −9.7° observed for the similar oxidations of 1,5-anhydro-D-glucitol and 1,5-anhydro-D-mannitol, respectively.[9] Titrations showed the consumption of 1.95 molar equivalents of oxidant and the liberation of 0.99 molar equivalent of formic acid; the dimedone test for formaldehyde was negative.

2,3,4,6-Tetra-*O*-acetyl-1,5-anhydro-D-altritol.—Acetylation of 1,5-anhydro-D-altritol with acetic anhydride and fused sodium acetate gave a 75% yield of the tetraacetate: prisms, from chloroform–pentane, with m.p. 104–105° and $[\alpha]^{20}D$ −22.7° in chloroform (*c* 1.3).

Anal. Calcd. for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07; CH_3CO, 51.8. Found: C, 50.54; H, 6.23; CH_3CO, 51.5.

1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-altritol.—To a solution of 0.6 g. of 1,5-anhydro-D-altritol in 10 ml. of dry pyridine was added 3 ml. of benzoyl chloride and the mixture was heated for 15 minutes on the steam-bath. A few drops of water were added and the solution was then poured onto cracked ice. The resulting sirup crystallized readily upon decantation of the aqueous layer and the addition of water. The product, filtered and washed with cold water and cold 50% ethanol, and dried in the air, weighed 2.1 g. (quantitative). The tetrabenzoate crystallized as small prisms from 95% ethanol and as thicker prisms from chloroform–pentane, melted at 176–177°, and showed $[\alpha]^{20}D$ −6.8° in chloroform (*c* 1.5).

Anal. Calcd. for $C_{34}H_{28}O_9$: C, 70.34; H, 4.86. Found: C, 70.34; H, 4.88.

1,5-Anhydro-2,3,4,6-tetra-*O*-benzoyl-D-altritol from α-D-Altropyranose Pentaacetate.—To 7.4 g. of α-D-altropyranose pentaacetate[4c] in 100 ml. of glacial acetic acid was added 100 ml. of a 30% solution of hydrogen bromide in glacial acetic acid. The solution quickly reached a constant rotation of about $[\alpha]^{20}D$ +150° (calculated as the expected acetobromo compound) and after 3 hours at room temperature it was poured onto cracked ice and the mixture extracted with chloroform. The chloroform solution was washed successively with water, aqueous bicarbonate, and water, dried with sodium sulfate, and concentrated *in vacuo*. The resulting sirup was dissolved in 400 ml. of anhydrous ether and added dropwise to a well-stirred suspension of 14 g. of lithium aluminum hydride in 600 ml. of ether. The reaction mixture was left overnight at 20° and then refluxed for 1 hour; excess lithium aluminum hydride was decomposed by the cautious addition of ethyl acetate, followed by water. The coagulated precipitate was filtered and washed with water, and the aqueous solution was deionized and concentrated to a sirup that weighed only 0.2 g. Benzoylation of the sirup produced 0.15 g. of a crystalline product that was identified by m.p., mixed m.p. and rotation as 1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-altritol.

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(9) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **65**, 64 (1943).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

Periodate Oxidation of Hexose Phosphates[1]

BY MARTIN MORRISON, GEORGE ROUSER[2] AND ELMER STOTZ

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The products of periodate oxidation of several sugar phosphates were investigated by paper chromatography. The results show that the oxidation occurs with the sugars in their ring forms. Those sugars with the phosphate on carbon 6 yield derivatives of the biologically important D-glyceraldehyde-3-phosphate, which have been shown to be active with triosephosphate dehydrogenase.

Recent studies have indicated that the hemiacetal carbon atoms of carbohydrates are oxidized by periodate to yield a formic acid ester.[3] The investigations to be reported were designed to determine whether this is a more general reaction that might be specifically applicable to the phosphate esters of carbohydrates. It has been shown that under the conditions employed, the carbohydrate moiety is oxidized in the ring form. The carbonyl carbon atom is then a hemi-acetal and is oxidized by periodate to yield an ester. The periodate oxidation of D-glucose-6-phosphate, fructose-6-phosphate and hexose diphosphate yield derivatives of D-glyceraldehyde-3-phosphate, the latter being an important intermediate of carbohydrate metabolism.

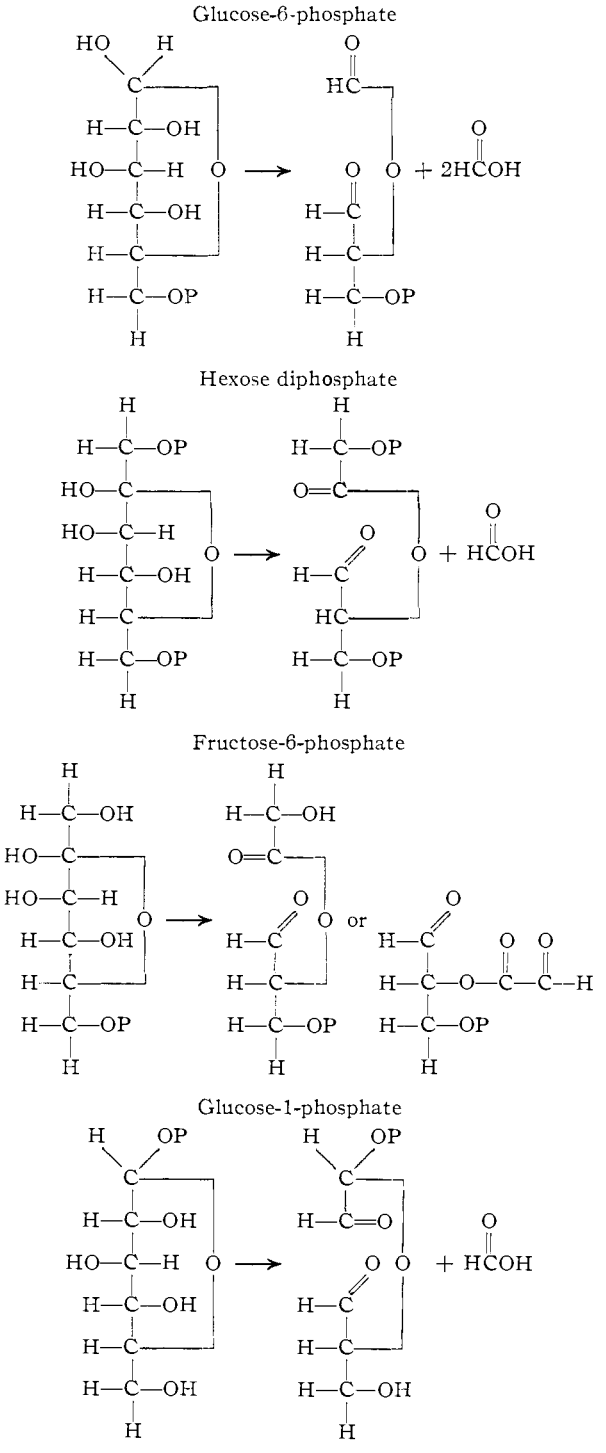
In order to follow the products of the periodate reaction, paper chromatographic techniques were employed. Figure 1 shows the paper chromatographic movement in the picric acid solvent[4] of α-glycerol phosphate (α-GP), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (HDP) and glucose-1-phosphate (G1P) and the products obtained from these compounds by periodate oxidation. The glycerol phosphate preparation contained some β-glycerol phosphate that moves just behind the α-glycerol phosphate and does not react with periodate. The product of α-glycerol phosphate oxidation is shown in Fig. 1 with an R_f value of about 0.36. It is evident that the hexose-6-phosphates do not produce the same glycolaldehyde phosphate as would be expected if the compounds reacted in the straight chain form. The sugar phosphates must, therefore, react in the ring form. The following products would be expected from the periodate oxidation of the four sugar phosphates tested. It will be noted that all four products are different, and that three of these compounds are car-

(1) This work was supported in part by the Damon Runyon Fund.

(2) U. S. Public Health Post-Doctoral Fellow.

(3) M. Morrison, A. C. Kuyper and J. M. Orten, THIS JOURNAL, **75**, 1502 (1953).

(4) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).



boxylic acid esters of glyceraldehyde-3-phosphate. The fourth compound contains a complete acetal linkage to glyceraldehyde. Figure 1 shows that the periodate products from the two glucose phosphates can be distinguished in the picric acid solvent. Figure 2 shows that the periodate products of the two naturally occurring fructose phosphates are distinguishable in the isobutyric acid system. The chromatographic results demonstrate that the four phosphate esters yield four different products on periodate oxidation as predicted from the above formulations. The mechanism of oxidation is supported by the periodate consumption data of Marinetti and Rouser.[5]

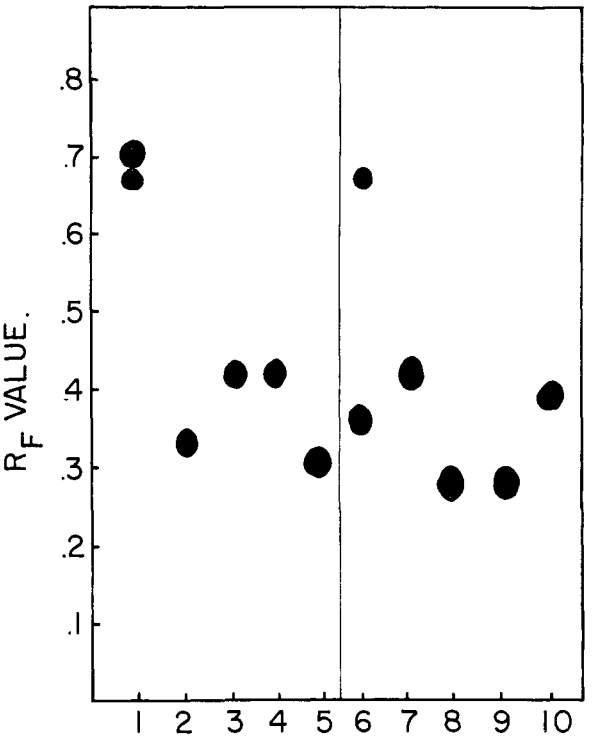


Fig. 1.—Relative chromatographic movements of phosphate esters and products of their periodate oxidation (picric acid solvent system): 1, α-glycerol phosphate; 2, glucose-6-phosphate; 3, fructose-6-phosphate; 4, fructose-1,6-diphosphate; 5, glucose-1-phosphate; 6–10-products of periodate oxidation of 1–5 in the same order.

If the phosphate were attached to other carbon atoms on the carbohydrate, the periodate products would vary according to its position. Since the products of periodate oxidation can easily be distinguished by paper chromatography, this offers a general method for locating the phosphate group in sugar phosphates.

The products of the periodate reaction of G6P, F6P and HDP were shown to be esters by use of the hydroxamic acid spray. This reagent is specific for carboxyl esters. The hydroxamate of the acid moiety is responsible for the color. The products of the periodate oxidation of α-GP and G1P do not give a color with this spray reagent as would be expected since they are not carboxyl esters. The periodate oxidation products were eluted from paper chromatograms after development in the formic acid solvent[4] and the hydroxamic acid test repeated in the test-tube. Strongly positive tests were obtained with G6P, F6P and HDP periodate products, but negative tests were obtained with G1P and α-GP periodate products, again as would be predicted by the previous formulations.

The Dische diphenylamine test[6] was also performed on the periodate products eluted from chromatograms. Glycoaldehyde phosphate pro-

(5) G. Marinetti and G. Rouser, THIS JOURNAL, Oct. 20 (1955).
(6) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **180**, 1297 (1949).

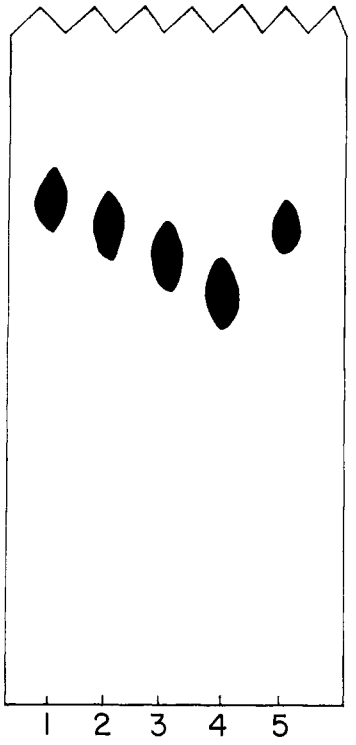


Fig. 2.—Relative chromatographic movements of periodate oxidation products (butyric acid solvent system). 1–5 are the oxidation prducts from: 1, α-glycerol phosphate; 2, glucose-1-phosphate; 3, fructose-6-phosphate; 4, fructose-1,6-diphosphate; 5, glucose-6-phosphate, respectively. The solvent was allowed to drip over the end of the chromagram for 50 hours.

duced by periodate oxidation of α-glycerophosphate gave the grass-green color characteristic for glycolaldehyde, but the periodate products from the hexose phosphates gave no color. The Schiff test, performed according to Feigl,[7] was very strong for glycolaldehyde phosphate, but was hardly detectable for the periodate products of the hexose phosphates at the same concentrations.

The periodate products were also tested as substrates for the enzyme triose phosphate dehydrogenase. Glycolaldehyde phosphate and the product from G1P oxidation reacted very slowly, while the periodate oxidation products from G6P, F6P and HDP reacted more rapidly than synthetic D,L-glyceraldehyde phosphate. It will be noted that the periodate oxidation products of the latter three compounds were predicted to be derivatives of D-glyceraldehyde-3-phosphate.

(7) F. Feigl, "Spot Tests," 3rd Ed., Elsevier Publishing Co., New York, N. Y., 1946.

Experimental

Periodate Oxidation Procedure.—The periodate reactions were conducted according to the procedure of Morrison, Kuyper and Orten[3] at 4° for 24 to 72 hours, using a calculated fourfold excess of sodium metaperiodate. The initial *p*H of the solutions was 4. Excess periodate was destroyed by the addition of ethylene glycol which was allowed to react for 30 minutes at room temperature in the dark. Iodate was then removed from the solution by addition of an equal volume of acetone. The aqueous acetone solution was allowed to stand at 4° until precipitation of iodate was complete and the iodate filtered off. Acetone was removed under vacuum and the aqueous solutions were then spotted on filter paper.

Chromatographic Techniques.—Glass battery jars closed with ground glass plates were used for the paper chromatography. Chromatograms were run in a descending manner at 25°.[8] The *t*-butyl alcohol/picric acid/H_2O and the isopropyl ether/formic acid/H_2O solvents of Hanes and Isherwood[4] and isobutyric acid saturated with H_2O were the principal solvent systems used. The picric acid solvent was allowed to drip over the end of an 18 inch length of paper so that the total running time for the chromatogram is about 50 hours.

The phosphate spray reagent consisted of 1 g. of ammonium molybdate, 10 ml. of 1 *N* HCl and 5 ml. of 60% perchloric acid, diluted to 100 ml. with water.

The hydroxamic acid test is conducted as follows. The dry chromatogram is first sprayed with a solution composed of equal volumes of 5% hydroxylamine hydrochloride and 12.5% sodium hydroxide, prepared immediately before use. The paper is allowed to dry and is then sprayed with a ferric chloride solution (50 g. $FeCl_3 \cdot 6H_2O$ per liter of 95% ethanol) and again dried. Finally, the paper is sprayed with a mixture of ethanol–concentrated hydrochloric acid (3/1). Transient pinkish-purple spots appear and then fade, but reappear usually within 30 minutes. The initial fading of the color is apparently due to removal of iron by the phosphate present. The color is quite stable once it is fully developed. The same reagents are used for the reaction in the test-tube.

Enzyme Assay.—The rate of reduction of diphosphopyridine nucleotide was followed spectrophotometrically using the crystalline enzyme triose phosphate dehydrogenase.[9]

ROCHESTER, N. Y.

(8) G. Rouser and M. Morrison, *Federation Proc.*, **12**, 261 (1953).

(9) G. T. Cori, M. W. Slein and C. F. Cori, *J. Biol. Chem.*, **173**, 605 (1948).