[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE SCHOOL OF MEDICINE, STANFORD UNIVERSITY]

Periodate Oxidation of Sugar Phosphates in Neutral Solution. I. D-Ribose 5-Phosphate[1]

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The periodate oxidation of ribose 5-phosphate was studied under neutral *p*H conditions at room temperature and at 2°. In each case three moles of periodate was reduced with the liberation of three moles of formic acid and one mole of glycolaldehyde phosphate. The latter compound was identified by enzymic dephosphorylation to glycolaldehyde which was characterized (1) by its behavior upon periodate oxidation, (2) colorimetrically by a modified Dische–Borenfreund procedure, and (3) by conversion to the authentic dimedon derivative. Glycolaldehyde phosphate prepared either from ribose 5-phosphate or from α-glycerophosphate gave two components when examined by paper chromatography in *t*-butyl alcohol–picric acid. The significance of these results is discussed, and possible structures are presented for this compound.

It has been shown that the oxidation of certain sugars and sugar phosphates by sodium metaperiodate may give rise to intermediate formate esters of sufficient stability to resist further periodate action.[5,6] The oxidation of one mole of ribose 5-phosphate in furanose form similarly should yield 2-formylglyceraldehyde 3-phosphate with two moles of periodate consumed and one mole of formic acid produced.[7] However, in experiments conducted at room temperature and *p*H 5, Long has reported that approximately three moles of periodate is reduced per mole of D-ribose 5-phosphate oxidized.[8,9] It appears likely, therefore, either that the 2-*O*-formyl-D-glyceraldehyde 3-phosphate formed was hydrolyzed and further oxidized or that ribose 5-phosphate was oxidized in the acyclic or aldehyde structure.[10] In either case the expected end product of the reaction should be glycolaldehyde phosphate, which has been isolated previously as an amorphous barium salt by Fleury and associates after periodate oxidation of α-glycerophosphate[11] and has been detected by paper chromatography among the oxidation products of α-glycerophosphate,[6,12,13] of sphingosine phosphate[12] and of 6-phospho-D-gluconic acid and 2-keto-3-deoxy-6-phospho-D-gluconic acid.[13]

In the present investigation crystalline barium ribose 5-phosphate was oxidized with sodium metaperiodate under neutral or slightly acidic conditions, and the reaction products studied quantitatively. It was found that three moles of periodate was consumed and three moles of formic acid produced per mole of ribose 5-phosphate oxidized either at room temperature or at 2°. Glycolaldehyde phosphate was characterized as the chief phosphate containing end product of the oxidation by enzymatic hydrolysis to inorganic phosphate and glycolaldehyde. The latter was identified (1) by treatment with periodate and quantitative determination of the periodate reduced and formic acid and formaldehyde liberated, (2) colorimetrically by a modified procedure based on that of Dische and Borenfreund,[14] and (3) by conversion to the authentic dimedon derivative.

Examination of the periodate oxidation products of α-glycerophosphate and ribose 5-phosphate by paper chromatography in the *t*-butyl alcohol–picric acid–water system[15,16] showed the presence of at least two phosphorus-containing and reducing components with R_f values of 0.35, A, and 0.25, B. The last value mentioned was reported originally by Rouser, *et al.*,[12] for glycolaldehyde phosphate prepared both from α-glycerophosphate and from sphingosine phosphate. More recently, the higher value only was reported in work presented from the same laboratory.[6] The two fractions mentioned were separated by paper chromatography, eluted separately and hydrolyzed by semen phosphatase. It was demonstrated by subsequent periodate oxidation measurements and by the colorimetric test mentioned that glycolaldehyde was formed from each component.

Experimental

Materials.—Barium ribose 5-phosphate (Nutritional Biochemicals Corp.) was used without additional purifica-

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(2) Taken in part from a dissertation submitted by Luis Werner Levy in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Department of Chemistry, Stanford University.

(3) Monsanto Chemical Co. Fellow, 1954–1955; on leave of absence from Escuela Politécnica Nacional, Quito, Ecuador.

(4) Eli Lilly Predoctoral Research Fellow, 1955–1956.

(5) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1427 (1947); K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **32**, 1102 (1949); F. Brown, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 1125 (1950); G. R. Barker and D. C. C. Smith, *Chem. and Industry*, 1035 (1952); G. Neumueller and E. Vasseur, *Arkiv Kemi*, **5**, 235 (1953); P. A. J. Gorin and J. K. N. Jones, *Nature*, **172**, 1051 (1953); G. R. Barker, T. M. Noone, D. C. C. Smith and J. W. Spoors, *J. Chem. Soc.*, 1327 (1955).

(6) M. Morrison, G. Rouser and E. Stotz, THIS JOURNAL, **77**, 5156 (1955).

(7) The fact that no formaldehyde is formed during periodate oxidation of ribose 5-phosphate was first demonstrated by H. von Euler, P. Karrer and B. Becker, *Helv. Chim. Acta*, **19**, 1060 (1936). This property has been used by several investigators to distinguish between ribose 5-phosphate and other ribose phosphates (W. Kiessling and O. Meyerhof, *Biochem. Z.*, **296**, 410 (1938); F. Schlenk, *J. Biol. Chem.*, **146**, 619 (1942); J. O. Lampen, *ibid.*, **204**, 999 (1953)).

(8) C. Long, *Biochem. J.*, **59**, 322 (1955).

(9) A compound isolated by Lampen ref. 7 and tentatively identified as ribose 5-phosphate has also been shown to consume 3 moles of periodate per mole at room temperature and *p*H 6.

(10) These conclusions regarding the stability of 2-formalglyceraldehyde 3-phosphate at *p*H 5 in acetate buffer may be placed in doubt by very recent work by G. V. Marinetti and G. Rouser, THIS JOURNAL, **77**, 5345 (1955). These authors, employing a spectrophotometric method of following periodate reduction, report an uptake value of 2.5 moles per mole of ribose 5-phosphate oxidized under the conditions mentioned.

(11) P. Fleury, J. Courtois and A. Desjobert, *Bull. soc. chim. France*, **19**, 458 (1952).

(12) G. Rouser, J. F. Berry, G. Marinetti and E. Stotz, THIS JOURNAL, **75**, 310 (1953).

(13) J. MacGee and M. Doudoroff, *J. Biol. Chem.*, **210**, 617 (1954).

(14) Z. Dische and E. Borenfreund, *ibid.*, **180**, 1297 (1949).

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

(16) H. S. Loring, L. W. Levy and L. K. Moss, *Anal. Chem.*, **28**, 539 (1956).

tion. The specific rotation of the crystalline hydrate was $[\alpha]^{24}D$ +13.1° (*c* 3.28 in 0.2 *N* HCl).

Anal. Calcd. for $C_5H_9O_8PBa \cdot 5H_2O$ (455.5): P, 6.79; H_2O, 19.7. Found: P, 6.75[17]; H_2O, 18.7 (heated *in vacuo* for a total of 6 hr. 32 min. at 100° over P_2O_5).

Disodium α-glycerophosphate was prepared by a procedure based on that of Verkade, *et al.*[18] Crystalline disodium β-glycerophosphate (40.0 g.) (Eastman Kodak Co.) was refluxed for 1 hour in approximately 1 liter of dilute sulfuric acid (*p*H 1.4). Sulfate ions were removed by the addition of barium hydroxide to *p*H 7.8 and filtration. The precipitated barium sulfate was washed, and the filtrate and washings on concentration to a sirup gave two crops of disodium α-glycerophosphate of 15.0 and 14.8 g. (99 and 84% purity, respectively, based on periodate reduction).

A. Determination of Acid Liberation and Periodate Reduction.—The quantity of ribose 5-phosphate oxidized varied from 2 to 25 μmoles in a concentration of from 1 to 20 μmoles per ml. The solution of the barium salt was brought to *p*H 6.2 with sulfuric acid at room temperature. For each micromole of ribose 5-phosphate used, an amount of 10 μmoles of sodium metaperiodate was added as a 0.1 *M* solution adjusted to *p*H 6.2. The mixture was stirred with a magnetic bar and sufficient 0.01 *N* sodium hydroxide added from a Gilmont Micropipet-Buret to maintain the *p*H as nearly as possible at 6.2. The progress of the oxidation was followed by noting the rate of alkali addition, and the amount of acid liberated was calculated from the volume of alkali required. After 10 to 15 minutes the *p*H remained constant at 6.2, and the reaction was considered complete. Ribose 5-phosphate was also oxidized under the same *p*H conditions at 2°. As judged by the liberation of acid, which was complete in 40 minutes, the rate of oxidation was slower at this temperature. The liberation of acid with time under both conditions is shown in Fig. 1.

Periodate uptake was determined after the solutions had stood for periods of time varying from 15 to 75 minutes by treatment with arsenite and titration with standard iodine solution as given by Fleury and Lange.[19] The resulting solutions in some instances were distilled, and the distillates examined for formaldehyde by the method of MacFadyen.[20] The acid liberated and the periodate reduced in the above-mentioned experiments ranged from 2.8 to 3.3 moles per mole of ribose 5-phosphate oxidized with average values in six experiments of 2.9 and 3.1, respectively. Only small amounts of formaldehyde, *e.g.*, 0.03 mole per mole, were produced, in agreement with previous results.

B. Identification of Glycolaldehyde Phosphate. Periodate Oxidation After Enzymic Dephosphorylation.—Ribose 5-phosphate (41.2 μmoles, 2 ml.) was oxidized at 2° and *p*H 6.2 with periodate (626 μmoles in 10 ml. previously adjusted to *p*H 6.2). After standing in the cold for 25 minutes, during which the *p*H was maintained constant as described before, the excess periodate and the iodate formed were precipitated by treatment with excess barium acetate (2 mmoles, 2 ml.). The insoluble barium salts were removed by filtration after the solution had stood in the refrigerator overnight. The solution was adjusted to *p*H 5 with sulfuric acid, the barium sulfate separated by filtration, and the remaining barium ions removed by treatment with excess Dowex 50 sodium followed by filtration. The filtrate was diluted to 50 ml. and used for subsequent experiments. Analyses for total[17] and inorganic[21] phosphate showed that the solution contained 21.5 μmoles of organic phosphate corresponding to a yield of 52% of theoretical as glycolaldehyde phosphate.

Aliquots of 2 ml. of the glycolaldehyde phosphate solution were incubated with 100 μl. (1 mg.) of a solution of purified semen phosphatase[22] for 2 hours at 40°, the *p*H adjusted to 6.2 at room temp., and the solution treated with 200 μl. of periodate (0.1 *M*). The *p*H was maintained at 6.2 by the addition of standard sodium hydroxide, and the amount of acid liberated determined from the volume of base used. The reaction was essentially complete in 6 min. as judged by acid liberation. After 20 min. the resulting solution was used for the measurement of periodate uptake and formaldehyde content, as mentioned above for ribose 5-phosphate. The respective values found represented periodate reduction, acid liberation and formaldehyde production for the glycolaldehyde formed after enzymic hydrolysis as well as contributions to each of these by the enzyme itself and by the glycolaldehyde produced during the previous treatments. To obtain satisfactory blank values the glycolaldehyde phosphate and the enzyme solutions were treated with periodate, and uptake, acid and formaldehyde production determined under similar conditions to those used after enzyme hydrolysis. The net periodate uptake, acid and formaldehyde production for the glycolaldehyde formed after dephosphorylation were each 1.0 mole per mole of organic phosphate in agreement with the theory. The purified phosphatase gave an apparent periodate uptake and formaldehyde production of 0.60 and 0.53 μmole per mg., respectively, with a negligible liberation of acid.

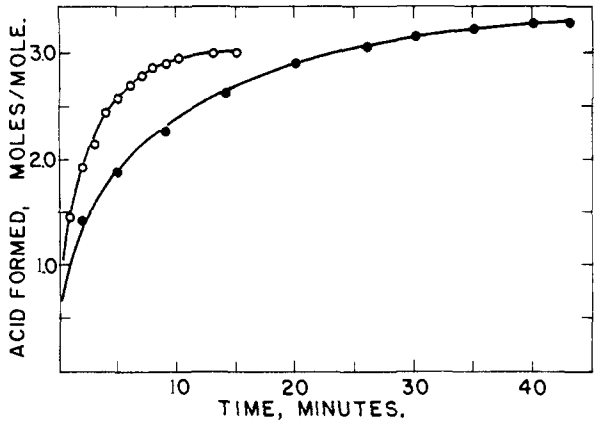


Fig. 1.—Rate of liberation of acid on oxidation of ribose 5-phosphate with periodate at *p*H 6.2; O, at room temperature; ●, at 2°.

C. Colorimetric Estimation of Glycolaldehyde.—Ribose 5-phosphate (13.5 μmoles, 5 ml.) was oxidized at 2° and *p*H 6.36 with sodium metaperiodate (54 μmoles, 0.54 ml.). After 39 minutes the acid liberation was constant (2.92 moles per mole), and the solution was filtered from a large precipitate, presumably of sodium iodate, that had separated. Sodium sulfite was used in equivalent amount to reduce the excess periodate present, the solution was adjusted to *p*H 5 with acetic acid[23] and treated with phosphatase. The progress of the hydrolysis was followed by the determination of periodate uptake on suitable aliquots removed at various time intervals. When periodate uptake became constant, the hydrolysis was considered complete, and the solution, after treatment with excess Dowex 50 hydrogen and Dowex 2 hydroxide, used for quantitative estimation of glycolaldehyde. If no loss of glycolaldehyde phosphate or of glycolaldehyde occurred during the various treatments, the resulting solution was estimated to contain 0.37 μmoles per ml.

The method used was based on that of Dische and Borenfreund.[14] It was adopted when difficulty was encountered in attempts to use the quantitative procedure described by these authors, possibly because of impurities present in the sample. The modified procedure gave a "grass-green color" with an identical absorption spectrum as described by the authors mentioned, but was approximately four times as sensitive. The procedure was as follows: The sample containing from 0.1 to 0.5 μmole of glycolaldehyde in 1 ml. was mixed with 0.5 ml. of concentrated sulfuric acid, 2 ml. of 1% solution of diphenylamine in acetic acid,[23] and heated for 30 minutes in a boiling water-bath. The optical density

(17) H. S. Loring, H. W. Bortner, L. W. Levy and M. L. Hammell, *J. Biol. Chem.*, **196**, 807 (1952).

(18) P. E. Verkade, J. C. Stoppelenburg and W. D. Cohen, *Rec. trav. chim.*, **59**, 886 (1940).

(19) P. Fleury and J. Lange, *J. pharm. chim.*, **17**, 107 (1933).

(20) D. A. MacFadyen, *J. Biol. Chem.*, **158**, 107 (1945).

(21) O. H. Lowry and J. A. Lopez, *ibid.*, **162**, 421 (1946).

(22) Prepared in this Laboratory by Forrest H. Riordan, III. The activity of the enzyme preparation may be judged from the following: 75% of sodium β-glycerophosphate (17.1 μmoles in 1 ml. of 0.01 *N* acetate at *p*H 5.3) was hydrolyzed in 20 min. at 37° on treatment with 0.1 mg. (10 μl.) of the purified phosphatase.

(23) Acetic acid, Special for Shellac Analysis, which is required in the Dische–Borenfreund colorimetric method for glycolaldehyde was not available. Acetic acid prepared from equivalent amounts of acetic anhydride, J. T. Baker C.P., and water proved satisfactory.

of the cooled solution was determined at 660 mμ in comparison with a standard solution containing 0.21 μmole of crystalline glycolaldehyde[24] that had been subjected to the same treatment. The average optical density of the standard solution at 660 mμ was 1.23.

When the glycolaldehyde solution prepared from ribose 5-phosphate was assayed by the procedure described, it was found to contain 0.32 μmole per ml., representing a recovery of 86% of theoretical. The same solution by quantitative measurement of periodate uptake and acid and formaldehyde production by the procedures described above gave values of 0.87, 0.67 and 1.0 moles per mole, respectively, based on the quantity of ribose 5-phosphate oxidized.

D. Preparation of the "Anhydride" of Glycolaldehyde Dimedon Derivative, 2,2-Di-(2,6-diketo-4,4-dimethylcyclohexyl)-ethanol.—Barium ribose 5-phosphate (1 mmole) was dissolved in water (5 ml.) and treated with Dowex 50 sodium (3 g.). The mixture was shaken for 15 minutes, and the Dowex 50 removed by filtration. The filtrate and washings (10 ml. total volume) were cooled in ice and mixed with the periodate solution (3 mmoles in 15 ml.). Sodium hydroxide was added as necessary to maintain the *p*H at 6.2 (0.75 ml. of 4 *N*). After 30 minutes in the cold, the solution was concentrated by freeze drying to 10 ml. and mixed with 20 ml. of cold absolute ethanol. The precipitate of sodium iodate was removed by centrifugation and washed in the centrifuge with two 5-ml. portions of cold 67% ethanol. The alcoholic solution was concentrated *in vacuo* to 6 ml., the resulting concentrate incubated overnight at 37° with 5 mg. of purified phosphatase and heated in a boiling water-bath for 10 minutes with 250 mg. of dimedon (dimethyldihydroresorcinol), essentially as described by Rigby[25] for the preparation of the anhydride of 2,2-di-(2,6-diketo-4,4-dimethylcyclohexyl)-ethanol from glycolaldehyde prepared from glycerol. On cooling in the refrigerator overnight 137 mg. of crystals separated, m.p. 190–210°. A single recrystallization from 70% ethanol gave a product melting at 210–212°. The sample was then treated with a small amount of boiling benzene as recommended by Rigby to convert the glycolaldehyde dimedon derivative to the anhydride. The material insoluble in benzene was recrystallized twice from 70% ethanol and gave a m.p. 230–231° (cor.). When a solution of glycolaldehyde was prepared from α-glycerophosphate and converted to the anhydride of the dimedon derivative by the procedures described for ribose 5-phosphate, a product was obtained with the same melting point. An equal mixture of the two showed no depression of the melting point. The same compound was likewise recovered when crystalline glycolaldehyde[24] was treated with dimedon. Rigby has reported a value of 228–229° for the melting point of the anhydride prepared with glycerol as the starting material.

E. Paper Chromatography of the Periodate Oxidation Products of α-Glycerophosphate and Ribose 5-Phosphate.—Barium glycolaldehyde phosphate was prepared by a procedure analogous to that given by Fleury, *et al.*,[11] except that the oxidation was performed near *p*H 5. Disodium α-glycerophosphate (1 mmole) was added to 5 ml. of a solution containing sodium metaperiodate (2 mmoles). After 73 minutes a white precipitate, presumably sodium iodate, separated and was filtered off. The *p*H of the solution was 5.5. Barium acetate (1.5 mmoles) was added, and the precipitate that formed was removed by filtration through celite, Hyflo Super-Cel. The solution was freed of cations by treatment with excess Dowex 50 hydrogen (*p*H 1.5), and traces of iodate and periodate were reduced by the addition of a drop of 1% sodium sulfite. Solid barium hydroxide (1 mmole) was added, the solution filtered from a slight cloudiness and concentrated to dryness by freeze-drying. A fine, white powder was obtained (325 mg., 93% yield).

Anal. Calcd. for $C_2H_3O_5PBa \cdot 4H_2O$ (347.5): P, 8.93. Found: P, 8.93.

A solution of the above described product was examined by paper chromatography[26] in the *t*-butyl alcohol–picric acid system.[15] Aliquots containing approximately 1 μmole were chromatographed over a 15 to 24 hr. period at room temperature. The areas containing phosphate were generally developed with the perchloric acid–molybdate spray,[27,28] but similar results were found with ethanolic sodium hydroxide.[16] In every instance two components were found with average R_f values of 0.40, A, and 0.27, B, and in an apparent ratio of about 3 to 1. It was observed that the A component behaved differently from the B when sprayed either with the perchloric acid–molybdate or the alcoholic sodium hydroxide spray. Whereas the A component gave a blue color with the molybdate spray only after ultraviolet irradiation, the B component developed a blue color without this treatment. The latter also developed the reddish-brown picramic acid color as soon as the chromatogram was sprayed with alcoholic sodium hydroxide whereas a period of heating was necessary with the A component.

A series of experiments was next conducted with α-glycerophosphate and ribose 5-phosphate to determine whether two components were always formed regardless of the experimental conditions used for periodate oxidation. The respective procedures and the results obtained are summarized in Table I.

In each case where periodate was used in excess, the solution was treated with ethylene glycol before application to the paper. It may be seen that the A and B components were found in every instance except when sodium α-glycerophosphate was oxidized at 2° and its own *p*H (8 to 6) with a fivefold molar quantity of sodium metaperiodate. Under these conditions apparently the A component only was formed.

In an attempt to determine whether each component could be converted to glycolaldehyde after dephosphorylation, α-glycerophosphate was oxidized with an equimolar quantity of periodate at *p*H 4.7 and 2°. The two components were separated by paper chromatography into bands which were cut from the paper and freed from picric acid by extraction in a Soxhlet apparatus with acetone. The phosphate-containing compounds were eluted separately with water and their concentration determined by analysis for total phosphate. Approximately ten times as much of the A component as of the B was recovered. Each fraction was treated with phosphatase as described under Section B and examined for glycolaldehyde by the modified Dische–Borenfreund procedure. The results showed that approximately 50% of the phosphate in the A and 80% of that in the B component were accounted for as glycolaldehyde phosphate. The A component after enzymic dephosphorylation gave periodate uptake and acid liberation values of 1.1 and 0.5 mole, respectively, per mole of phosphate present.

It was of interest to determine whether the two components described would also be found in the absence of cations.[29] Chromatograms were prepared using a solution of barium glycolaldehyde phosphate freed of cations by treatment with excess Dowex 50 hydrogen. It was found that the slower migrating component was either eliminated or greatly reduced, under these conditions. This component reappeared, however, when the above acidic solution was neutralized with sodium hydroxide prior to application to the paper. The slower component was also eliminated when the solutions resulting from periodate oxidation of either α-glycerophosphate or ribose 5-phosphate were treated with excess Dowex 50 hydrogen before application to the paper.

α-Glycerophosphate was also oxidized to glycolaldehyde phosphate by shaking an aqueous solution (0.01 mole in 300 ml.) over a 24-hr. period at room temperature in the presence of an equimolar quantity of sodium bismuthate[25] and an excess of Dowex 50 hydrogen (10 g.). The filtrate from the Dowex 50 and an insoluble white precipitate which separated was used for paper chromatography. A single component with an average R_f value of 0.42 was found.

Discussion

The quantitative study of the periodate oxidation of D-ribose 5-phosphate at *p*H 6, which showed the formation of 2.9 moles of acid and a periodate

(24) H. O. L. Fischer and C. Taube, *Ber.*, **60**, 1704 (1927).

(25) W. Rigby, *J. Chem. Soc.*, 1907 (1950).

(26) Schleicher and Schuell, No. 589, White Ribbon paper was used in most cases. A few experiments with Whatman No. 1 paper gave similar results.

(27) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(28) When paper chromatograms are sprayed with perchloric acid–molybdate and developed by ultraviolet irradiation, the entire paper acquires a bluish cast. It was found that this could be largely eliminated if an air stream was directed at the paper during irradiation.

(29) A. S. Curry, *Nature*, **171**, 1026 (1953).

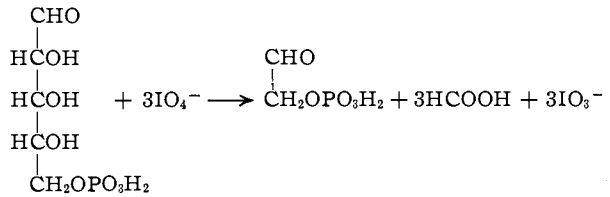
TABLE I

PAPER CHROMATOGRAPHIC BEHAVIOR OF PERIODATE OXIDATION PRODUCTS OF α-GLYCEROPHOSPHATE AND RIBOSE 5-PHOSPHATE IN THE PICRIC ACID SYSTEM

Substrate	Conditions of oxidation: Concn., μmole/ml.	IO_4^- to Substrate, moles/mole	Temp., °C	pH	Time, min.	R_1 values[a] A	R_1 values[a] B
α-Glycerophosphate	34	1	2	2.0	20	0.38, 0.39(+++)	0.18, 0.19(±)
	32	1	2	4.7	30	.33, 0.35(++)	.15, 0.19(+++)
	38	1	2	7.0	30	.35(+++)	.22(++)
	10	5	2	8–6	10	.38, 0.39	(−)
	10	5	20	8–6	4 days	.38, 0.38(+++)	.25(+)
	15	1.5	20	8–7	10	.38, 0.41(+++)	.25, 0.30(+)
	13	2.5	20	8–6	60	.41(+++)	.30(+)
	10	5	50	8–6	5	.38, 0.39(+++)	.25(+)
Ribose 5-phosphate	11	4	2	6.2	80	.42(++)	.28(±)
	6	7	20	8–6–8	47	.34(+++)	.19(+)

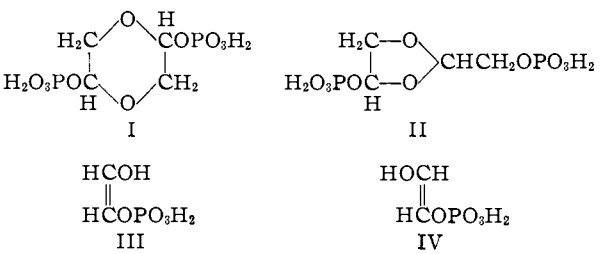
[a] R_1 values found on separate chromatograms in runs of 15 to 24 hr. at room temperature. The + signs indicate the relative intensities of the spots.

reduction of 3.1 moles indicated that the reaction proceeded according to the equation



It appears, therefore, either that ribose 5-phosphate reacted as an acyclic aldehyde under the conditions used or that 2-*O*-formyl-D-glyceraldehyde 3-phosphate, if formed as an intermediate, was hydrolyzed to D-glyceraldehyde 3-phosphate which was immediately further oxidized to glycolaldehyde phosphate. The identification of glycolaldehyde phosphate as the end product of the oxidation of ribose 5-phosphate involved removal of the excess periodate used and the iodate formed before treatment with phosphatase. It is likely, therefore, that some losses of glycolaldehyde phosphate or of glycolaldehyde occurred in the experiments in which an attempt was made to determine quantitatively the amount of glycolaldehyde phosphate formed. The high recoveries of glycolaldehyde, whether judged by the modified Dische–Borenfreund colorimetric procedure (86%) or by periodate reduction (87%) or by acid and formaldehyde production (67 and 100%, respectively), indicate that the reaction was essentially a quantitative one as carried out at *p*H 6.

The results of paper chromatography of glycolaldehyde phosphate prepared under a variety of conditions indicate that isomeric forms of this compound occur. It also appears from the results of Rouser, *et al.*,[12] Morrison, *et al.*,[6] and those described in Section E that one or the other of the two products may be formed preferentially depending on the conditions used. Because of the recognized occurrence of glycolaldehyde and glycolaldehyde acetate[30] as dimers of different chemical structure, it is logical to assume that a monomeric glycolaldehyde phosphate produced during periodate oxidation of either α-glycerophosphate or ribose 5-phosphate, may undergo dimerization similarly. Formulas I and II can be postulated as possible structures on this basis. Alternately, the apparent acid and alkali lability and particularly the difference in behavior of the A and B components, on treatment either with perchloric acid–molybdate or with alcoholic sodium hydroxide considered in conjunction with the postulated existence of enolic forms of glycolaldehyde[31] suggest enolic *cis* and *trans* structures such as III and IV as other possible isomers.



I II

III IV

STANFORD, CALIFORNIA

(30) R. K. Summerbell and L. K. Rochen, THIS JOURNAL, **63**, 3241 (1941); E. Spaeth and L. Raschik, *Monatsh.*, **76**, 65 (1947).

(31) H. von Euler and H. Hasselquist, "Reduktone," Verlag Ferdinand Enke, Stuttgart, 1950, p. 5.