exo-dicarboxylic acid (VIb) which was recrystallized from ethyl acetate–ether [mp 195–197°; $[\alpha]_D$ +60°; ORD $[\Phi]_{600}$ +186°, $[\Phi]_{386}$ +700°, $[\Phi]_{370}$ +339°, $[\Phi]_{363}$ +443°, $[\Phi]_{357}$ ±0°, $[\Phi]_{354}$ −41°, $[\Phi]_{353}$ ±0°, $[\Phi]_{346}$ +790°, $[\Phi]_{342}$ +690°, $[\Phi]_{250}$ +13,500°, $[\Phi]_{243}$ +11,200°, $[\Phi]_{227}$ +27,400°, $[\Phi]_{216}$ ±0°, $[\Phi]_{212}$ −14,800°; ν_{max}(KBr) 3600–2400, 1735, 1640 cm^{-1}; nmr 0.79 (18-H), 1.09 (19-H), 2.9–3.65 (multiple resonance, 6′-H, 7′-H), 5.72 ppm (broad singlet,[29] 4-H). *Anal.* Calcd for $C_{25}H_{32}O_7$: C, 67.55; H, 7.21; O, 25.22. Found: C, 67.62; H, 8.08; O, 25.21].

Photoaddition of Maleic Anhydride and I in Dioxane. The dienone (1.0 g) and maleic anhydride (305 mg) were dissolved in dry dioxane (50 ml) and irradiated for 40 min. Aqueous sodium bicarbonate (3%, 5 ml) was added to the solution, and the resulting mixture was stirred at room temperature for 3 hr. The solution was then acidified, and most of the dioxane was removed at the pump. Water and ethyl acetate were added, and the separated organic layer was dried and treated with excess ethereal diazomethane. An aliquot of the product was chromatographed as described in 1 above to give I (6%), VII (16%), VIa (33%), Va (42%), and 4% of a noncrystalline highly polar compound.

Oxidative Decarboxylation of Diacids Vb and VIb. The 6β,7β-diacid Vb (1.0 g) was dissolved in dry dioxane (60 ml), and finely powdered vacuum-dried lead tetraacetate (10 g) was added. The reaction mixture was heated to 80–90° and stirred vigorously under nitrogen for 90 min, by which time a drop of the solution when added to water did not produce a brown precipitate, indicating that all the lead tetraacetate had been consumed. Water and ethyl acetate were added and the separated organic layer was washed (2 *N* hydrochloric acid and water), dried, and evaporated. The semicrystalline residue (650 mg) was chromatographed (1.3-mm tlc plate, hexane–ethyl acetate, 2:1) to give 17β-acetoxy-6β,7β-vinyleneandrost-4-en-3-one (VIII) [290 mg, 36%; mp 112–115° dec (ether–hexane); $[\alpha]_D$ −61°; ORD $[\Phi]_{600}$ −177°, $[\Phi]_{394}$ ±0°, $[\Phi]_{378}$ +872°, $[\Phi]_{358}$ ±0°, $[\Phi]_{320}$ −6250°, $[\Phi]_{293}$ −8470° (shoulder), $[\Phi]_{260}$ −29,400°, $[\Phi]_{242}$ ±0°, $[\Phi]_{227}$ +56,000°, $[\Phi]_{211}$ +44,300°; nmr 0.85 (18-H), 1.22 (19-H), 2.74 (two doublets,[29] 7-H), 3.68 (doublet, 6-H), 5.78 (4-H), 6.1 ppm (narrow multiplet, 6′-H, 7′-H). *Anal.* Calcd for $C_{23}H_{30}O_3$: C, 77.95; H, 8.47; O, 13.56. Found: C, 77.37; H, 8.66; O, 13.93].

The 6α,7α-diacid VIb was decarboxylated in the same manner to give a 38% yield of 17β-acetoxy-6α,7α-vinyleneandrost-4-en-3-one (IX) [mp 138–139° (ether–hexane); $[\alpha]_D$ +73°; ORD $[\Phi]_{600}$ +180°, $[\Phi]_{445}$ +558°, $[\Phi]_{382}$ +1280°, $[\Phi]_{354}$ +256°, $[\Phi]_{294}$ +788°, $[\Phi]_{280}$ +13,700°, $[\Phi]_{257}$ +30,400°, $[\Phi]_{242}$ ±0°, $[\Phi]_{211}$ −55,900°; nmr 0.85 (18-H), 1.11 (19-H), 3.20 (two doublets,[29] 7-H), 3.67 (two doublets, 6-H), 5.74 (doublet, 4-H), 6.00 ppm (narrow multiplet, 6′-H and 7′-H). *Anal.* Calcd for $C_{23}H_{30}O_3$: C, 77.95; H, 8.47. Found: C, 77.24; H, 8.46].

Reduction of 17β-Acetoxy-6β,7β-vinyleneandrost-4-en-3-one (VIII). Tristriphenylphosphinerhodium chloride[10] (150 mg) was added to ethyl acetate (5 ml) and benzene (5 ml) and the mixture shaken in a hydrogen atmosphere for 15 min. The 6β,7β-vinylene compound VIII (60 mg) was added, and the shaking continued for 30 min. The solution was filtered and the filtrate evaporated. The residue was chromatographed (0.25-mm tlc plates, hexane–ethyl acetate, 2:1) to give starting material (27 mg) and 17β-acetoxy-6β,7β-ethyleneandrost-4-en-3-one [25 mg, 42%, mp 175–177° (ether–hexane); $[\alpha]_D$ +2°; ORD $[\Phi]_{600}$ ±0°, $[\Phi]_{450}$ +133°, $[\Phi]_{377}$ +187°, $[\Phi]_{367}$ +123°, $[\Phi]_{359}$ +123° (shoulder), $[\Phi]_{354}$ ±0°, $[\Phi]_{310}$ −4150°, $[\Phi]_{242}$ ±0°, $[\Phi]_{223}$ +23,400°, $[\Phi]_{206}$ +12,200°; nmr 0.88 (18-H), 1.41 (19-H), 5.67 ppm (4-H); mass spectrum *m/e* 356 (molecular ion) 328].

Alkaline Reactions of Glucose 6-Phosphate

Ch. Degani and M. Halmann

Contribution from the Isotope Department, The Weizmann Institute of Science, Rehovoth, Israel. *Received June 27, 1967*

Abstract: Glucose 6-phosphate in aqueous alkaline solutions, above pH 7 and up to 5 *N* sodium hydroxide, undergoes a series of consecutive and parallel reactions. The first step seems to involve an equilibrium of the dianion of the substrate with its conjugate base, which may be the 1,2-enediol. This in part rearranges irreversibly to the relatively stable 6-phosphoglucometasaccharinic acid, and in part undergoes reversible epimerization to fructose 6-phosphate. Fructose 6-phosphate splits by a reversed aldol condensation to glyceraldehyde 3-phosphate (and presumably dihydroxyacetone), which is hydrolyzed rapidly to lactic acid and orthophosphate. All the above phosphate esters, as well as lactic acid, were identified by paper chromatography and electrophoresis. The rate of disappearance of glucose 6-phosphate in alkaline media, measured by glucose 6-phosphate dehydrogenase, follows first-order kinetics. On the other hand, the rate of release of orthophosphate does not obey first-order kinetics. In the pH range 8.7–9.7, the observed first-order rate constant for the disappearance of glucose 6-phosphate is linearly related to the OH^- concentration. The bimolecular rate constant at 100.0° is 29.1 ± 0.7 l. mol^{-1} sec^{-1}. But in 1–5 *M* sodium hydroxide, the increase in rate levels off, possibly due to rapid conversion of the substrate into the 1,2-enediolate of glucose 6-phosphate. The alkaline reactions of glucose 6-phosphate are not a hydrolysis, because no glucose is formed. The partial similarity between the pathways for alkaline degradation of glucose 6-phosphate and the anaerobic metabolism of carbohydrate in muscle and in yeast is noted.

In contrast to sugar phosphates in which the reducing group is protected, sugar phosphates having a free aldehyde or ketonic group are labile to alkali. Thus, glucose 1-phosphate and ribose 1-phosphate are stable in alkali, while glucose 6-phosphate and ribose 5-phosphate undergo rapid degradation.[1] It has been proposed that an enediol is involved as an intermediate in the degradation of the alkali-labile sugar phosphates.[2] However, relatively little is known about the mechanism of these reactions, and several pieces of contradictory data appear in the literature. For instance, fructose 1,6-diphosphate is reported to liberate orthophosphate quantitatively in 0.2 *M* alkali at 100°,[1b] but a recent investigation has shown that only part of the orthophosphate is released and that alkali-resistant 6-phosphoglucosaccharinic acid is also formed.[3] Also, the hy-

(1) (a) R. L. Bieleski and R. E. Young, *Anal. Biochem.*, **6**, 54 (1963); (b) W. Kiessling, *Biochem. Z.*, **298**, 421 (1938); (c) J. X. Khym, D. G. Doherty, and W. E. Cohn, *J. Amer. Chem. Soc.*, **76**, 5523 (1954); (d) R. S. Wright and H. G. Khorana, *ibid.*, **78**, 811 (1956); (e) L. F. Leloir, *Progr. Chem. Org. Natur. Prod.*, **8**, 63 (1951).

(2) K. R. Farrar, *J. Chem. Soc.*, 3131 (1949); W. G. Overend and M. Stacey, *ibid.*, 987 (1951); D. M. Brown, F. Hayes, and A. R. Todd, *Chem. Ber.*, **90**, 936 (1957).

(3) J. B. Lee, *J. Org. Chem.*, **28**, 2473 (1963).

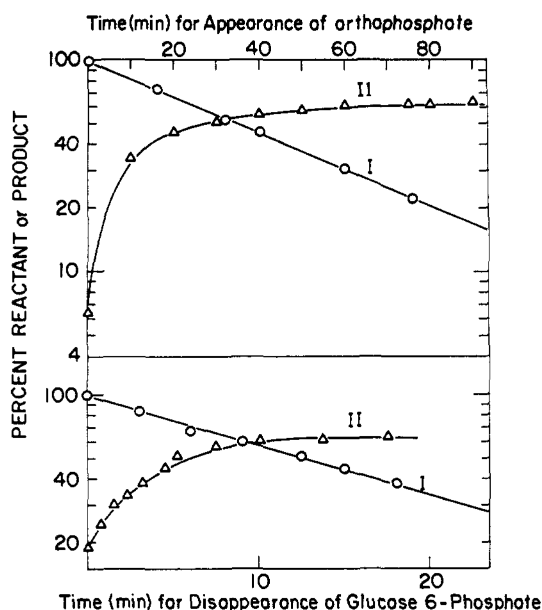


Figure 1. Rates of disappearance of glucose 6-phosphate (I) and of appearance of orthophosphate (II): (a, top) at 100° in pH 9.65; (b, bottom) at 55.1° in 4.78 M NaOH. The per cent reactant or product is represented on a log scale.

drolysis of glucose 6-phosphate in alkaline medium is stated to involve simple first-order kinetics and to proceed without the formation of intermediate phosphate esters;[4] however, the rate of appearance of orthophosphate appears to be slower than the rate of disappearance of glucose 6-phosphate.[5] It is with this latter system that the present paper deals, and it is the purpose of this report to present data in support of the existence of intermediate phosphate species in the alkaline degradation of glucose 6-phosphate.

Results

A. Rate Measurements. The rate of appearance of orthophosphate from glucose 6-phosphate in alkaline media does not obey first-order kinetics, if followed for several half-lives. On the other hand, the rate of disappearance of glucose 6-phosphate strictly follows first-order kinetics. Data from two runs (in pH 9.65 at 100° and in 4.78 N NaOH at 55.1°) are presented in Table I and in Figure 1. Only about two-thirds of the orthophosphate is released within seven half-lives. These experimental results suggest that glucose 6-phosphate in alkaline solutions undergoes at least two competitive consecutive reactions. Therefore, the first-order rate constants which we reported in our previous paper[5] for the *appearance* of orthophosphate in alkaline media (Table V and Figure 3 of that paper) should be treated with reserve.

First-order rate constants for the disappearance of glucose 6-phosphate as a function of OH^- concentration are shown in Table II. In these measurements, the concentration of the unreacted glucose 6-phosphate was determined enzymatically, using glucose 6-phosphate dehydrogenase.[5] In moderately alkaline solutions (pH 8.7–9.6), the rate is proportional to the OH^- concentration, as shown by the constant value of $k_{obsd}/(OH^-)$ (last column of Table II).

(4) C. A. Bunton and H. Chaimovich, *J. Amer. Chem. Soc.*, **88**, 4082 (1966).
(5) Ch. Degani and M. Halmann, *ibid.*, **88**, 4075 (1966).

Table I. Rates of Disappearance of Glucose 6-Phosphate and Appearance of Orthophosphate in Alkaline Hydrolysis (Optical Density = OD)

At 100° in pH 9.65				At 55.1° in 4.78 M NaOH			
a		*b*		*a*		*b*	
Min	OD	Min	OD	Min	OD	Min	OD
0	0.033	0	0.304	0	0.079	0	0.255
10	0.177	4	0.217	3	0.103	3	0.215
20	0.234	8	0.160	6	0.128	6	0.174
30	0.260	10	0.137	9	0.140	9	0.155
40	0.285	15	0.093	12.5	0.159	12.5	0.131
50	0.299	19	0.067	18	0.189	15	0.113
70	0.314			21	0.213	18	0.097
75	0.316			30	0.236		
80	0.322			40	0.248		
90	0.324			55	0.254		
∞	0.516			70	0.262		
				∞	0.416		

[a] Colorimetric determination of orthophosphate by the molybdate method. [b] Enzymatic assay of glucose 6-phosphate.

B. Identification of Intermediate Reaction Products. In order to detect the intermediate products during alkaline treatment of glucose 6-phosphate, the reaction was interrupted after about one half-life of disappearance of the substrate. A large variety of chromatographic methods was applied in order to make definite identification of the intermediates. The same chromatographic pattern was obtained after partial hydrolysis at pH 9.65 and 1 N and 2 N sodium hydroxide. As shown in Table III, the following phosphate esters were observed: glucose 6-phosphate, fructose 6-phosphate, 6-phosphoglucometasaccharinic acid, and glyceraldehyde 3-phosphate, as well as the final products, orthophosphate and lactic acid. No appreciable spots due to the branched-chain acid 6-phosphoglucoisosaccharinic acid could be detected. All observed spots of phosphorus compounds could be accounted for by the above identifications. No glucose could be detected, after reaction of glucose 6-phosphate in either 2 N sodium hydroxide at 55.1° (during 5, 12, and 20 min) or pH 9.65 at 100° (during 4 and 8 min). The above reaction periods correspond to disappearance of glucose 6-phosphate up to one half-life. This result is in disagreement with the previous report that "considerable amounts of glucose are formed directly in the alkaline hydrolysis of glucose 6-phosphate."[4]

In order to find out if our failure to detect glucose may be due to the rapid decomposition of glucose in the alkaline medium, experiments were also made on the stability of glucose. Under the same alkaline conditions (during 5, 12, and 20 min in 2 N sodium hydroxide at 55.1° or during 4 and 8 min in pH 9.65 at 100°), glucose was found to be only slightly decomposed, and most of the glucose could be recovered after paper chromatography. Therefore, if an appreciable amount of glucose were initially formed in the alkaline degradation of glucose 6-phosphate, we would have detected it.

Lactic and 6-phosphoglucometasaccharinic acid yields, after 12 half-lives of reaction of glucose-1-C^{14} 6-phosphate in 2 N sodium hydroxide, were 75 and 25%, respectively (determined by paper electrophoresis, as in Table III).

C. Reactivities of Intermediate Reaction Products. In order to determine the precursor–product relationship between the various intermediate phosphate esters in the alkaline degradation of glucose 6-phosphate,

Table II. First-Order Rates as a Function of (OH^-) for Disappearance of Glucose 6-Phosphate (measured by enzymatic assay)[a]

Buffer soln, M	pH	(OH^-), M	Temp, °C	$10^4 k_{obsd}$, sec^{-1}	$10^4 k_{obsd}/(OH^-)$
Barbitone, 0.0974–HCl, 0.0015	8.70	0.50×10^{-5}	100	1.58 ± 0.20	31.6
$NaHCO_3$, 0.05–NaOH, 0.010	9.35	2.23×10^{-5}	100	6.63 ± 0.20	29.7
$NaHCO_3$, 0.05–NaOH, 0.015	9.50	3.16×10^{-5}	100	9.62 ± 0.40	30.4
$NaHCO_3$, 0.05–NaOH, 0.0214	9.65	4.46×10^{-5}	100	13.16 ± 0.10	29.5
NaOH, 0.956			55.1	5.77 ± 0.2	
NaOH, 1.912			55.1	6.98 ± 0.1	
NaOH, 2.868			55.1	8.30 ± 0.2	
NaOH, 3.824			55.1	8.59 ± 0.2	
NaOH, 4.78			55.1	8.81 ± 0.2	

[a] Initial concentrations of glucose 6-phosphate were about 0.003 M.

Table III. Paper Chromatography and Paper Electrophoresis of the Intermediates in Alkaline Degradation of Glucose 6-Phosphate

				R_p					R_f	
Solvent system[h]	V/cm	Paper treatment	Time for chromatog, hr	Glucose 6-phosphate	Fructose 6-phosphate	6-Phosphoglucometasaccharinic acid	6-Phosphoglucoisosaccharinic acid[i]	Glyceraldehyde 3-phosphate	Lactic acid	D-Glucose[i]
				Paper Chromatography						
I		*a*	18.5[f]	1.47	1.71					
I		*b*	19.5[f]	1.40	1.67					
I		*c*	19.5[f]	1.47	1.70					
II		*b*	23[f]	0.58	0.72	0.87	(0.91)			
III		*a*	17[f]	0.40	0.53	0.71	(0.79)			
III		*c*	14.5[f]	0.39	0.49	0.71	(0.84)	0.38		
IV		*e*	16[g]						0.59	
V		*e*	11[f]							(0.30)
VI		*e*	11[f]							(0.38)
				Paper Electrophoresis						
VII	53	*d*	0.83	0.68	0.68	0.72	(0.72)	0.79		
VII	42	*d*	1.00	0.68	0.68	0.72	(0.72)	0.79		

[a] Whatman No. 1, washed with 0.2% Versene (pH 8.5). [b] Whatman No. 1, washed with 0.01 M Versene (pH 8) and with 2 N acetic acid. [c] Whatman No. 1, washed with 1 N formic acid. [d] Whatman No. 1, paper, either unwashed, or washed as in *a*, *b*, and *c* above, gave similar results. [e] Whatman No. 1 paper, unwashed. [f] Descending chromatography. [g] Ascending chromatography. [h] Solvent systems: I, methyl Cellosolve–methyl ethyl ketone–3 N ammonia (7:2:3); II, *t*-butyl alcohol–water–picric acid (80:20:2 g); III, propyl acetate–90% formic acid–water (11:5:3); IV, ethanol–ammonia–water (80:5:15); V, *n*-butyl alcohol–acetic acid–water (10:1:3); VI, *n*-butyl alcohol–pyridine–water (6:4:3); VII, pyridine–acetate buffer, pH 3.5. [i] All values in parentheses refer to results for marker compounds. These compounds were not detected after alkaline degradation of glucose 6-phosphate.

these esters were submitted to the same alkaline treatment (2 N NaOH at 55.1°) and were then analyzed both by paper chromatography (solvent system III of Table III) and by paper electrophoresis (53 V cm^{-1}, as in Table III). 6-Phosphoglucometasaccharinic acid was found to be quite stable in alkaline media (reaction time 45 min). Its formation from glucose 6-phosphate must therefore be irreversible. On the other hand, fructose 6-phosphate under such conditions (during 20 min) forms both glucose 6-phosphate, 6-phosphoglucometasaccharinic acid, glyceraldehyde 3-phosphate, and orthophosphate. Thus, fructose 6-phosphate is in equilibrium with glucose 6-phosphate in alkaline solution.

Discussion

Simple alkyl dihydrogen phosphates are stable in alkaline solutions, presumably because the alkyl phosphate dianion is resistant toward nucleophilic reagents. The lability of sugar phosphates having a free reducing group toward alkaline hydrolysis must, therefore, be due to the particular effect of the carbonyl group. The alkaline decomposition of glucose 6-phosphate was studied by isotopic and kinetic methods and by identification of reaction products.

The ^{18}O-tracer experiments had shown that in alkaline solutions, the release of orthophosphate from glucose 6-phosphate occurs only with C–O bond fission.[5]

Kinetically, two phenomena characterize the behavior of glucose 6-phosphate in alkaline media.

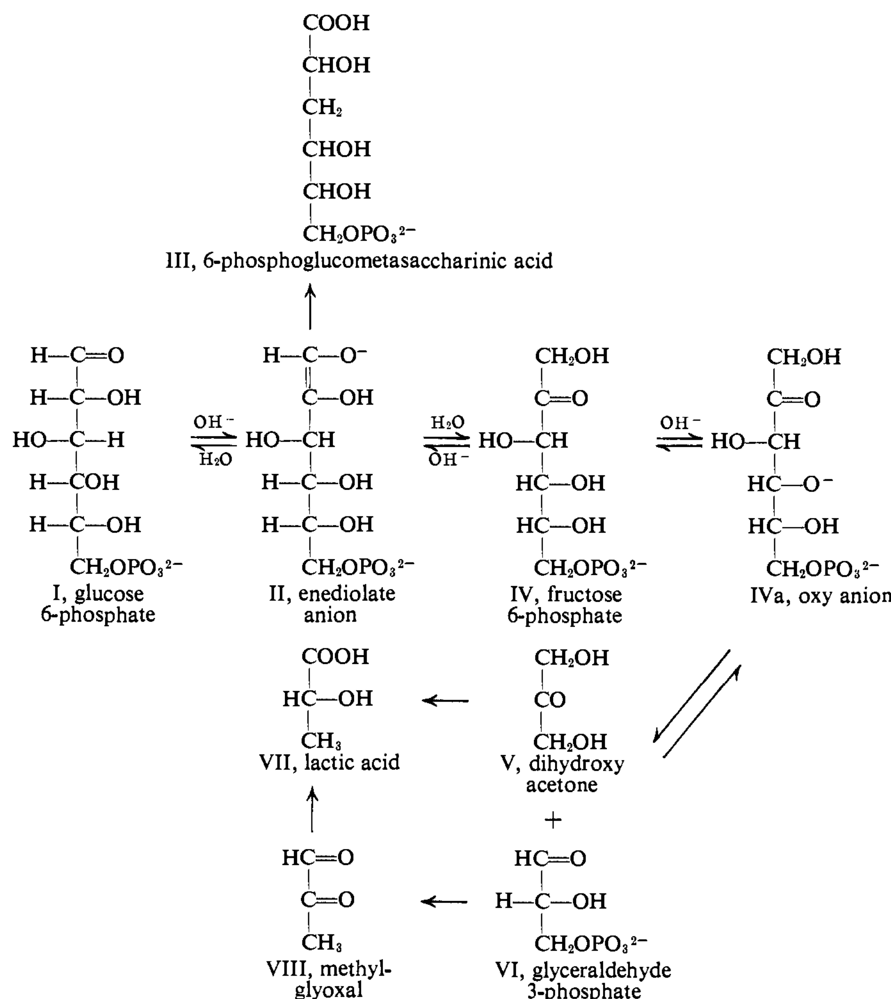
(a) The rate of disappearance of glucose 6-phosphate follows first-order kinetics, while the rate of appearance of orthophosphate does not.

(b) The release of the phosphate from glucose 6-phosphate is incomplete even after several half-lives of disappearance of the substrate (see Table I and Figure 1).

The experimental results suggest that the alkaline decomposition of glucose 6-phosphate occurs by at least two parallel paths, each proceeding through several consecutive reactions. In one path orthophosphate is formed, while in the other the final product is an alkali-stable phosphate ester, the formation of which explains the incomplete release of orthophosphate. In order to clarify this point, separation of phosphate esters formed during the decomposition of glucose 6-phosphate was carried out. As shown in Table III, three such esters could be identified: 6-phosphoglucometasaccharinic acid (III), fructose 6-phosphate (IV), and glyceraldehyde 3-phosphate (VI). The tests described also prove that 6-phosphoglucometasaccharinic acid is produced irreversibly (on the time scale of these experiments), and that fructose 6-phosphate is in equilibrium with glucose 6-phosphate.

On the basis of the above results, it seems that in the alkaline decomposition of glucose 6-phosphate the

Scheme I



following steps are involved. Glucose 6-phosphate is in acid–base equilibrium with its 1,2-enediol (II), which can rearrange either irreversibly to form 6-phosphoglucometasaccharinic acid (III) or reversibly to form fructose 6-phosphate (IV) (Scheme I). Fructose 6-phosphate undergoes cleavage by a reverse aldol condensation to dihydroxyacetone (V) and glyceraldehyde 3-phosphate (VI). Both dihydroxyacetone and glyceraldehyde 3-phosphate in alkaline solution are rapidly converted into lactic acid (VII). In the case of glyceraldehyde 3-phosphate, this may be by elimination of orthophosphate to form an enediol CH_2=C(OH)CHO, which rearranges to form methylglyoxal (VIII) and finally lactic acid.

The formation of 6-phosphoglucometasaccharinic acid is thus similar to that suggested[6,7] for production of saccharinic acids from various sugars, and includes (a) proton abstraction to form the 1,2-enediol, (b) elimination of a hydroxyl group, (c) rearrangement to an α-dicarbonyl, and (d) a benzylic acid type of rearrangement to produce the metasaccharinic acid.

The hydrolysis of glyceraldehyde 3-phosphate is not rate determining under the conditions of our experiments. This compound was reported to lose its phosphate group on standing in 1 *N* alkali during 20 min at room temperature.[8]

(6) H. S. Isbell, *J. Res. Nat. Bur. Stand.*, 32, 45 (1944); J. C. Sowden, *Advan. Carbohydrate Chem.*, 12, 35 (1957); J. C. Speck, Jr., *ibid.*, 13, 63 (1958); R. L. Whistler and J. N. Bemiller, *ibid.*, 13, 289 (1958).

(7) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 2916 (1956).

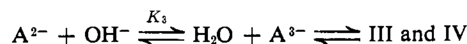
(8) K. Lohmann and O. Meyerhof, *Biochem. Z.*, 273, 413 (1934).

In moderately alkaline solutions (pH 8.7–9.7; see Table II), the first-order rate constants for the disappearance of glucose 6-phosphate are linearly related to the OH^- concentration. Therefore, the second-order (bimolecular) rate constant k_B is given by

$$k_{obsd} = k_B[OH^-] \quad (1)$$

From a least-squares fit of the linear plot of k_{obsd} against the OH^- concentration, we get for 100.0° the value k_B = (29.1 ± 0.7) l. mol^{-1} sec^{-1}. The rate of the bimolecular reaction, k_B, is remarkably high. Possibly the reaction step which is determined by this rate is the enolization of glucose 6-phosphate dianion (I) to its enediolate ion II. This rate is comparable with that reported for the glyceraldehyde–dihydroxyacetone interconversion.[9,10]

In highly alkaline solutions, above 1 *M* sodium hydroxide, the rate of disappearance does not increase linearly with the OH^- concentration, but instead levels off above about 3 *M* NaOH (see Table II). Such a kinetic behavior may be explained by a rapid proton-transfer equilibrium between the substrate and its conjugate base



$$A^{2-} + OH^- \overset{K_3}{\rightleftharpoons} H_2O + A^{3-} \rightleftharpoons \text{III and IV}$$

In the more strongly alkaline solutions used in the present work, most of the substrate should therefore be in a trianion form, A^{3-}. Since glucose 6-phosphate under-

(9) W. G. Berl and C. E. Feazel, *J. Amer. Chem. Soc.*, 73, 2054 (1951).

(10) C. D. Gutsche, D. Redmore, R. S. Buriks, K. Nowotny, H. Grassner, and C. W. Armbruster, *ibid.*, 89, 1235 (1967).

goes modification by two paths, the leveling off phenomenon must be due to that intermediate species which is responsible for the *disappearance* of glucose 6-phosphate. This species seems to be the enediolate ion II, which is the branching point for the two paths causing decomposition of glucose 6-phosphate. This enediolate ion is formed to an increasing extent in strongly basic media and thus the rate of disappearance of glucose 6-phosphate no longer depends on the alkali concentration.

The intermediate which is responsible for causing the retroaldol condensation is an oxyanion of fructose 6-phosphate obtained by ionization of an hydroxyl group at position 4 (IVa). A similar intermediate had been suggested for the alkaline decomposition of 6-O-methylglucose[7] and for the base-catalyzed triose condensation.[9,10]

The alkali lability of sugar phosphates having a free reducing group may be attributed to the particular effect of the carbonyl group. The presence of such a group permits the retroaldol reaction, as a result of which glyceraldehyde 3-phosphate is formed. The lability of this triose phosphate toward alkali is due to the location of a carbonyl function in the β position to the phosphate group, thus causing orthophosphate release by a β-elimination process.

Comparison with the Anaerobic Carbohydrate Metabolism. The alkaline degradation of glucose 6-phosphate, involving the intermediates fructose 6-phosphate and glyceraldehyde 3-phosphate, and leading finally to lactic acid and orthophosphate, has striking similarity to the enzymatic anaerobic metabolism of carbohydrates. Thus, glucose 6-phosphate in muscle isomerizes into fructose 6-phosphate (IV) (enzyme, glucose phosphate isomerase). This step is analogous to the reversible isomerization which we observe in alkaline solutions presumably through the enediolate ion II. However, in glycolysis, the splitting of fructose 6-phosphate to the three-carbon intermediates requires initially its phosphorylation by adenosine triphosphate to fructose 1,6-diphosphate (enzyme, phosphofructokinase), which then splits to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (VI). Several additional enzyme-catalyzed steps are involved until the final conversion into lactic acid. Almost analogous processes participate in the alcoholic fermentation of glucose.

Experimental Section

Materials. Disodium D-glucose 6-phosphate (Sigma), glucose-1-C^{14} 6-phosphate (Nuclear Science and Engineering), diethyl acetal glyceraldehyde 3-phosphate monobarium salt (Sigma), fructose 6-phosphate barium salt (Boehringer and Mannheim), 6-phosphoglucometasaccharinic acid, and 6-phosphoglucoisosaccharinic acid[11] were used without purification.

Methyl Cellosolve, methyl ethyl ketone, and propyl acetate were distilled before use as chromatographic solvents. Detection of phosphates after chromatography or electrophoresis was made by molybdate spray.[12-14] Lactic acid was detected by paper chromatography,[15] using either a bromocresol green spray[15] or a solution of glucose in aniline.[16]

Rate measurements were made as reported previously.[5] In attempts to detect glucose in the reaction mixture, paper chromatography was carried out either in butyl alcohol-acetic acid-water (10:1:3)[4] or in *n*-butyl alcohol-pyridine-water (6:4:3),[17] and development was affected by immersion in silver nitrate and sodium hydroxide.[18]

Lactic acid was determined by its conversion with concentrated sulfuric acid into acetaldehyde, and colorimetric assay of the purple color with *p*-hydroxydiphenyl.[19]

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(11) We are very much indebted to Professor L. Szabo for kindly providing us with samples of these two phosphosaccharinic acids.

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

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

(14) E. J. Wawszkiewicz, *Anal. Chem.*, **33**, 252 (1961).

(15) R. J. Block, E. L. Durrum, and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press Inc., New York, N. Y., 1958, p 233.

(16) E. Lederer, "Chromatographie," Vol. 1, Masson & Cie., Ed., Paris, 1959, p 474.

(17) I. M. Hais and K. Macek, "Paper Chromatography," Academic Press Inc., New York, N. Y., and Publishing House of the Czechoslovak Academy of Sciences, Prague, 1963.

(18) L. Hough and J. K. N. Jones, *Methods Carbohydrate Chem.*, **1**, 28 (1962).

(19) S. B. Barker, "Methods in Enzymology," Vol. III, S. P. Colowick and N. O. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1957, p 241.