ml of 4% sodium sulfite was added to the solution to reduce the excess periodic acid and the iodic acid formed by its reduction. The solution was then neutralized with 10 *N* sodium hydroxide and analyzed for orthophosphate, as above.

Determination of the Position of Bond Breakage. Disodium glucose 6-phosphate (about 400 mg) was dissolved in ^{18}O-enriched water (about 10 ml), sealed in glass tubes, and kept at 100° to about 50% reaction. The solution was brought to room temperature; the resulting orthophosphate was precipitated as magnesium ammonium phosphate, separated by centrifugation, dissolved in hydrochloric acid, and reprecipitated with concentrated ammonia. A suspension of the precipitate in a small volume of water was shaken with an excess of Dowex 50 (ionic form H^+) ion exchanger. The resin was rinsed with ethanol, and the combined washings were brought to pH 4 (methyl orange) with 3 *N* potassium hydroxide, thus precipitating potassium dihydrogen phosphate (adding acetone to complete the precipitation). After drying at 100° under reduced pressure, the ^{18}O-content of the phosphate, as well as of the water at the completion of reaction, were determined by the method of Boyer, *et al.*,[24] with several duplicate runs being made for each sample.

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The Hydrolysis of Glucose 6-Phosphate[1,2]

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Abstract: At low pH the kinetic form of the hydrolysis of glucose 6-phosphate is very similar to those found for other simple monoalkyl phosphates. There is an acid-catalyzed hydrolysis, with an A2 mechanism, and halogen acids are very effective catalysts because of nucleophilic attack of the halide ion upon carbon. The reaction rate is constant in the region pH 3–5 where the monoanion is the bulk component, but at a high pH it increases as the dianion becomes the bulk component, and there is a second plateau at pH 7–8. This dianion reaction probably involves the β anomer and occurs with participation of the 1-OH group acting as an intramolecular general acid. At pH >9 two new reactions appear, one giving a 1,6-anhydro sugar, probably by nucleophilic attack of the 1-alkoxide ion upon C-6 with expulsion of phosphate trianion. The other (major) reaction involves either attack of hydroxide ion upon the dianion of glucose 6-phosphate assisted by intramolecular general acid catalysis by the 1-OH, or attack of water assisted by the 1-alkoxide group acting as a general base. This second mechanism appears to be the more probable.

The mechanisms of the hydrolysis of monoalkyl phosphates are generally well understood.[4] At pH 3–5 the predominant species is the monoanion (I), which probably decomposes by eliminating a metaphosphate ion,[5–8] although there is some question as to the intimate details of the reactions.[9,10]

The dianion II is generally unreactive,[4–7] and it has always been assumed that the monoalkyl phosphates are unreactive toward alkali,[11] simply because electrostatic repulsion hinders attack of hydroxide ion upon the dianion.

Attack of hydroxide upon the dianion can, however, be observed with aryl phosphates,[12] and powerful electron-attracting substituents change the mechanisms of hydrolysis so much that the dianion of 2,4-dinitrophenyl dihydrogen phosphate is very reactive both toward hydroxide ion and in its spontaneous hydrolysis.[13]

The undissociated acid (III) is generally less reactive than the monoanion (I), with exceptions for compounds in which the alkyl group can separate as a carbonium ion.[5,14,15]

There may also be an acid-catalyzed hydrolysis which involves the conjugate acid (IV)[5,6,14] and becomes important only at pH <1. Therefore, generally for hydrolysis of a simple monoalkyl phosphate, one observes a rate constant which decreases with decreasing acidity, with a minimum at pH ~1, followed by an increase to a maximum at pH 3–5, where I is the bulk component. The rate then decreases at high pH as the dianion becomes the bulk component.[4]

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$$\underset{\text{IV}}{\mathrm{ROPO_3\overset{+}{H}_3}} \rightleftarrows \underset{\text{III}}{\mathrm{ROPO_3H_2}} \rightleftarrows \underset{\text{I}}{\mathrm{ROPO_3\overset{-}{H}}} \rightleftarrows \underset{\text{II}}{\mathrm{ROPO_3^{2-}}}$$

No exceptions have been observed to the generalization that hydrolysis of the monoanion (I) involves phosphorus–oxygen fission,[4–8,16] but uni- and bimolecular mechanisms have been observed for hydrolyses of III and IV, with both carbon- and phosphorus–oxygen bond fission.[6,14,15]

The hydrolysis of glucose 1-phosphate is unusual, because it involves formation of the 1-glucosylcarbonium ion, which is stabilized by electron release from the ethereal oxygen atom.[14] Glucose 6-phosphate does not have this special structural feature, and one might expect its hydrolysis mechanisms to be similar to those of the simple monoalkyl phosphates. This expectation is fulfilled for hydrolyses at pH <5, but at higher pH new reaction mechanisms are observed which probably involve interactions between the 1-hydroxyl group and the phosphate residue, and new and unusual kinetic forms and reaction mechanisms are observed.

Experimental Section

Reagents. Glucose 6-phosphate was used as the disodium salt (Calbiochem reagent grade). It contained no free phosphate. Reagent solutions were made up using redistilled and deionized water. The following buffers were used: potassium hydrogen phthalate, pH 2.4–5.7; sodium succinate, pH 6.2–6.5; and sodium carbonate and hydrogen carbonate for pH >6.3 (all at 0.05 *M*). Tris buffers were unsatisfactory because of decomposition at 100°, borate complexes with the sugar residue, and phosphate buffers could not be used because we follow hydrolysis by formation of inorganic phosphate. The pH of the buffer was determined at 25°, and corrected to 100° using published data when available,[17] or by measuring the pH at 68.0 and 88.2° using a high-temperature cell and electrodes, and extrapolating the pH to 100°.[18] Solutions of pH <2 were made up with mineral acid, and for dilute acidic solutions pH was taken as $-\log C_{\mathrm{H^+}}$.

The runs at pH 8.0 and 9.0 were done in unbuffered solutions. The concentration of glucose phosphate was 2×10^{-3} *M*, and the pH change for complete reaction was less than 0.1 unit. These reactions in unbuffered solutions gave good first-order rate plots for more than two half-lives of reaction.

The experimental details for a run at pH 10.13 (pH 10.90 at 25°) follow: buffer, 0.05 *M* sodium carbonate; glucose 6-phosphate, 2×10^{-3}*M*. Aliquots of 1.5 cc were sealed in glass tubes, placed in the temperature bath, shaken vigorously at 100°, and removed from the bath at convenient times; the inorganic phosphate was determined by the Fiske–Subbarow method.

Kinetics. The reaction was followed by the colorimetric determination of inorganic phosphate, either bytheFiske– Subbarow method, in which the molybdate complex is reduced with Eiconegen in 0.5 *M* sulfuric acid,[19] or by the Lowry–Lopez method, in which the molybdate complex is reduced by ascorbic acid in acetate buffer at pH 4.[20] The optical densities of the solutions were determined using a Spectronic 20 colorimeter.

Sealed tubes were used for all the kinetic runs, and portions of the solutions were taken so that the optical density was always in a convenient range for measurement.

time (min)	0	1	2	4	6	8	10	12	15	∞	
OD	0.150	0.255	0.385	0.530	0.650	0.720	0.790	0.852	0.900	0.980	0.980

$k_\psi = 2.58 \times 10^{-3}\ \mathrm{sec^{-1}}$, calcd graphically

For some experiments at high pH the solutions were deoxygenated with a stream of nitrogen and the tubes sealed under nitrogen. The rate constants were the same in the presence and absence of oxygen.

Products. The hydrolysis products were detected by paper chromatography. The reaction mixtures were deionized by concentration under vacuum followed by extraction with ethanol or pyridine,[21] and the extracts were spotted on Whatman No. 1 paper and the chromatogram developed with a mixture of 1-butanol–acetic acid–water (10:1:3).[22] The color was developed by spraying with silver nitrate and sodium hydroxide.[23]

It was found, in agreement with other work,[24] that glucose is unstable in concentrated alkali and in moderately concentrated acid at 100°. However 1,6-anhydro-(1,5)-glucopyranose, which is a minor product of reaction at high pH, is stable under the reaction conditions; *e.g.*, there was no decomposition within 9 hr in 1.3 *M* KOH at 100°.[25]

The formation of 1,6-anhydro-(1,5)-glucopyranose at high pH was demonstrated by the identity of paper chromatograms of the reaction mixture with those made with added authentic material.[26] It had R_f 0.45 using the butanol–acetic acid–water solvent.[22]

Dissociation Constants. The classical dissociation constants of glucose 6-phosphate were determined at 25° by titration of an 0.02 *M* solution of the sodium salt with 1 *M* HCl, using a glass electrode. At 25° our value of $\mathrm{p}K_2$ (6.11) is the same as that determined by Meyerhoff and Lohman,[27] but our value of $\mathrm{p}K_1$ (1.65) is very different from their value of 0.94. However it is similar to those for other simple monoalkyl phosphates.[6,28] At 70° we used a similar method; however, because of the reactivity of glucose 6-phosphate at high pH, we converted it into the free acid by addition of 1 *M* HCl to the sodium salt, and then titrated the solution with 1 *M* NaOH (at 70° $\mathrm{p}K_1 = 2.1$ and $\mathrm{p}K_2 = 6.2$). As is generally found for phosphate esters, the value of $\mathrm{p}K_1$ is less precise than that of $\mathrm{p}K_2$, but the values of $\mathrm{p}K_1$ are larger and those of $\mathrm{p}K_2$ are smaller than the corresponding values for methyl phosphate.[6] On the assumption that a change from 70 to 100° will affect the p*K* values of glucose 6- and methyl phosphate similarly, we took the values $\mathrm{p}K_1 = 2.1$ and $\mathrm{p}K_2 = 6.35$ for the ionizations of glucose 6-phosphate at 100°.

The dissociation constant of glucose was determined conductometrically at 25°, by the method of Ballinger and Long,[29] using an Industrial Instruments bridge RC-18, or by comparison of the pH of mixtures of glucose and sodium hydroxide with those of sodium hydroxide. The glucose concentrations were 0.2, 0.4, and 0.8 *M*, and this second method was used for temperatures greater than 25°. Our values of $10^{13}K$ were, at 25°, 4.14 (conductometric measurement), 4.6 (pH measurement), and, at 45°, 15.8 (pH measurement), and accord with values in the literature.[30]

Results

Kinetics. Acid Hydrolysis. The kinetic form of the acid hydrolysis is similar to that observed for the hydrolysis of methyl phosphate in respect to the reaction rates and their variation with acidity and the fact that halogen acids are better catalysts than perchloric or sulfuric acids.[6,31] The acid-catalyzed hydrolysis is superimposed upon a spontaneous hydrolysis of the undissociated alkyl phosphate (III), but for perchloric and sulfuric acid concentrations >2.5 *M* this spon-

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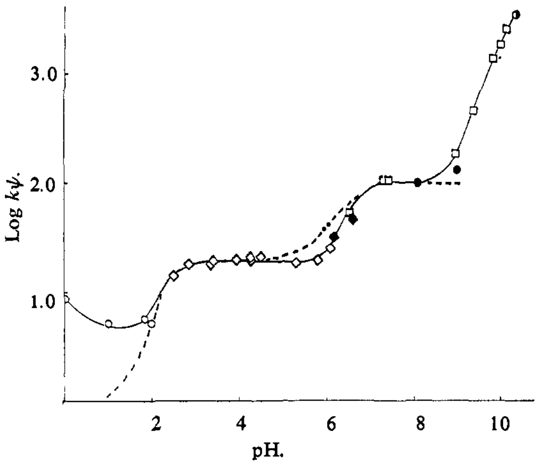


Figure 1. Plot of log k_ψ against pH at 100°: solid line, experimental; broken line, calculated. Buffers: ◇, phthalate; ◆, succinate; □, carbonate-bicarbonate; ◑, hydroxide ion; ○, hydrogen ion; ●, unbuffered.

taneous hydrolysis of III becomes relatively unimportant (Table I).

Table I. Acid Hydrolysis

C_{H^+}, M	10^5k_ψ, sec^{-1} HClO$_4$	H$_2$SO$_4$	HCl	HBr
	At 100°			
0.10	0.53			
1.00	0.91			
2.41			2.61	
2.70	2.08			
3.60	3.25			
4.76	5.80			
4.86			10.0	
5.00		5.30		10.6
5.50				16.6
5.85	8.00			
6.00		7.90		34.5
6.10			19.0	
	At Lower Temperatures			
1.60	0.0093^a			
4.61	0.39^b			

^{a} 45.0°. ^{b} 72.6°.

Hydrolysis of the Mono- and Dianions. The increase of k_ψ at pH >1 (Table II and Figure 1) is caused by formation of the monoanion (I), and the rate constant of 2×10^{-5} sec^{-1} at pH 4, where the monoanion is the bulk component, is very similar to those found for hydrolyses of other alkyl phosphate monoanions,[4,6,14] *e.g.*, for hydrolysis of the monoanion of methyl phosphate at 100° and pH 4, $k_\psi = 0.8 \times 10^{-5}$ sec^{-1}.

The sharp increase of k_ψ at pH >5.8 could arise because of the incursion of either of two reactions: (i) the dianion could be the reactive species, or (ii) the monoanion could react with hydroxide ion.

With a further increase of pH, a new reaction path appears (Table III). For pH >7, the bulk component is the dianion, and the rate increase for pH >9 must arise either from reaction of the dianion with hydroxide ion, or a spontaneous decomposition of a trianion of glucose 6-phosphate. It is not possible to differentiate between these various reactions simply in terms of the dependence of rate upon pH, and other criteria must be used.

Table II. Hydrolysis at pH 2.5–9 at 100° Unless Specified

Buffer	pH, 25°	pH, cor	10^5k_ψ, sec^{-1}
Strong acid	...	1.90	0.58^a
	...	2.00	0.43^b
Phthalate	2.40	2.51	1.39
	2.75	2.85	1.86
	3.25	3.35	1.89
	3.30	3.40	1.96
	3.70	3.95	2.07
	4.00	4.25	2.01
	4.00	4.25	2.00
	4.00	4.13	0.079^c
	4.25	4.51	2.13
	4.95	5.28	1.89
	5.50	5.80	1.98
	5.70	6.10	2.59
Succinate	6.00	6.19	3.30
Bicarbonate	6.25	6.54	4.70
Succinate	6.50	6.65	4.65
Bicarbonate	6.90	7.28	10.9
	7.40	7.40	10.7
	7.40	7.40	0.307^c
None	8.0	8.0	10.0
	9.0	9.0	13.0
Bicarbonate	9.20	8.94	19.0

^{a} HCl. ^{b} HClO$_4$. ^{c} 72.6°.

Table III. Hydrolysis at High pH

Temp, °C	pH^a	pH, cor^b	$10^3C_{OH^-}$,^{c} M	10^5k_ψ, sec^{-1}
45	9.65	9.60	0.16	0.020
	10.30	10.26	0.73	0.048
	10.50	10.45	1.13	0.070
100	9.20	8.94	0.42	19.0
	9.65	9.36	1.09	46.0
	10.30	9.87	3.55	144
	10.50	9.98	4.57	190
	10.90	10.13	6.38	258
	12.00^d	10.32	10.0	360

^{a} At 25°. ^{b} Corrected to reaction temperature. ^{c} Calculated from K_W. ^{d} 0.01 M NaOH.

Hydrolysis of the Undissociated Alkyl Phosphate. Hydrolysis of the undissociated acid (III) apparently makes little contribution to the over-all reactivity, except at pH ~1 where III is the bulk component.

Arrhenius Parameters. Table IV gives the values of the activation energy E, the Arrhenius preexponential term, log A, and the entropy of activation. The entropy of activation is calculated taking 1 M reactants at 25° as the standard state. Because of uncertainties in the mechanism, the values of these parameters have not been tabulated for hydrolysis at high pH but are discussed in the text.

Table IV. Arrhenius Parameters for Hydrolysis

Reactive species	E, kcal mole^{-1}	Log A	ΔS^*, eu
Conjugate acid ($C_{H^+} = 4.6 M$)	24	9.8	−19
($C_{H^+} = 1.6 M$)	21	7.4	...
Monoanion (pH 4)	30.5	13.2	0
Dianion (pH 7.4)	33	15.3	+9

Acid Dissociation of Glucose. A plot of log K against $1/T_A$ for the dissociation of glucose is linear, and pK = 10.8 extrapolated to 100°. For the acid dissociation of glucose, ΔH = 10 kcal mole^{-1} and ΔS = -24 eu, calculated from our values at 25 and 45° and those of Thamsen, and Michaelis and Rona at lower temperatures.[30]

Products. The formation of glucose from its 6-phosphate is to be expected, but 1,6-anhydro-(1,5)-glucopyranose which is one of the products at high pH must arise by cyclization involving the 1 and 6 positions. Glucose is decomposed at high pH, whereas the anhydro sugar is stable (Experimental Section),[24] and therefore it is difficult to determine quantitatively the direct products of the decomposition of glucose 6-phosphate, but inspection of the paper chromatograms showed that the anhydro sugar is a minor product at pH 10.9, and the main spots relate to glucose and its alkaline decomposition products.[24] No anhydro sugar was found in the reactions at pH 7.4 at 100° where the dianion is the reactive species.

Discussion

Glucose 6-phosphate is unusually reactive at pH >6, and a major problem in explaining this high reactivity is that the sugar phosphate can exist in several structural modifications. With regard to cyclic structures the pyranose and furanose forms should be in equilibrium, with the former predominating, and there seems to be no reason for either of the furanosides being especially reactive relative to, *e.g.*, methyl phosphate. Somewhat similarly, the open-chain form of glucose 6-phosphate would not be expected to be especially reactive; *e.g.*, the glyceryl phosphates are not especially reactive in hydrolysis.[32] Therefore, in the following discussion we assume that glucose 6-phosphate has a pyranose structure, and that the α and β anomers will be in equilibrium, and that in principle either of them could be the reactive species. We do not know the rate of mutarotation of glucose 6-phosphate, but the mutarotation of glucose is very much faster than hydrolysis of its 6-phosphate,[33] suggesting that the anomeric equilibrium will be maintained.

Variation of Rate Constant with pH. For hydrolysis at pH 2.5–8 we can interpret the variations of k_ψ with pH in terms of eq 1[4,6] (where C is the concentration of

$$k_\psi = k_1C_1/C + k_2C_2/C \qquad (1)$$

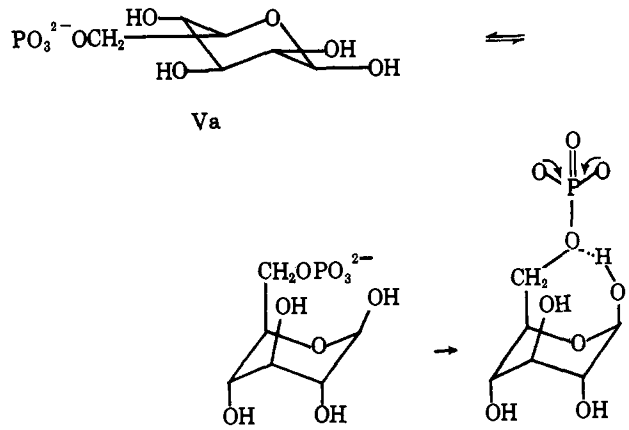
glucose phosphate, and the subscripts 1 and 2 refer to the mono- and dianion). The fraction of glucose 6-phosphate present as mono- and dianion can be calculated using pK_1 = 2.1 and pK_2 = 6.35 for glucose 6-phosphate, at 100°.

The broken line in Figure 1 gives the net contribution of the hydrolyses of the mono- and dianions, calculated using the mean values of k_1 = 2.00 × 10^{-5} sec^{-1}, and k_2 = 1.02 × 10^{-4} sec^{-1}, at 100° (Table II). The calculated and experimental values agree reasonably well within the pH range 2–9. The discrepancies at pH 5–6 arise because we are using either phthalate or succinate buffers at the extreme of their working range, or carbonate buffers which are unsatisfactory because of possible loss of carbon dioxide from the solution, with consequent variation of pH. We were unable to use phosphate, borate, or Tris buffers, and unfortunately our buffering is least satisfactory at the regions where we have a mechanistic change. The calculations of relative amounts of mono- and dianions neglected activity effects on the equilibria, and there is uncertainty in the pK values at 100°, but comparison of the calculated and experimental values shows that at high pH there is a reaction involving the dianion plus hydroxide ion, or stoichiometrically equivalent species, and that at low pH there is a spontaneous hydrolysis of the undissociated glucose 6-phosphate and an acid-catalyzed hydrolysis.

Reaction of the Monoanion. For hydrolysis of the monoanion of glucose 6-phosphate k_1 = 20 × 10^{-6} sec^{-1} at 100° (Table II and Figure 1); the corresponding value for methyl phosphate[6] is 8.2 × 10^{-6} sec^{-1}, and for glucose 1-phosphate[14] it is approximately 11 × 10^{-6} sec^{-1} (calculated at 100°). Inductively attracting substituents increase monoanion reactivities slightly,[4] *e.g.*, for glycerol 1-phosphate k_1 = 13.5 × 10^{-6} sec^{-1}, and for glycerol 2-phosphate it is 27.7 × 10^{-6} sec^{-1} at 100°. In none of these systems is there anchimeric assistance by the hydroxyl groups, and the entropy and energy of activation for hydrolysis of the monoanion of glucose 6-phosphate (Table IV) are very similar to those found for other monoalkyl phosphates.[4]

The similarity between these hydrolyses suggests that for glucose 6-phosphate breaking of the phosphorus–oxygen bond of the monoanion is assisted by transfer of one of the phosphate hydrogens to the alkoxy group either directly or more probably through water molecules.[4] Although it is generally assumed that metaphosphate ion is eliminated in these reactions,[4] experiments in aqueous alcohol mixtures show that it is sometimes not sufficiently free to be captured indiscriminantly by water and alcohol.[9,10]

Reaction of the Dianion. At 100° the dianion of glucose 6-phosphate is approximately five times more reactive than the monoanion. This unusual observation shows that some special structural feature of glucose 6-phosphate must be involved. The 1-hydroxyl group of glucose is relatively acidic, with pK = 10.8 at 100° (Experimental Section), and β-glucose 6-phosphate (Va,b) can take up an (unfavorable) conformation (Vb) in which the hydrogen atom of the 1-hydroxy group can interact with the phosphate group, and a plausible mechanism involves proton transfer to the 6-oxygen atom (VI) which would assist breaking of the phos-



Va

Vb

VI

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phorus–oxygen bond. At the same time the presence of two negative charges on the phosphate group provides great driving force for this process.

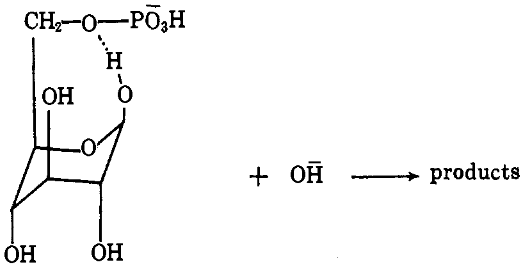
This suggested mechanism assumes that the phosphorus oxygen bond will be broken, and this point remains to be tested. We note that intramolecular hydrogen bonding to the 6-oxygen atom could also assist breaking of the carbon–oxygen bond, with nucleophilic attack upon carbon-6.

The low reactivity of the alkyl phosphate dianion has been explained by assuming that proton transfer to the alkoxy group was essential for reaction, and for a simple alkyl phosphate the proton had to come from the phosphate group.[4-7] Although glucose, and presumably the dianion of glucose 6-phosphate, is a much weaker acid than an alkyl phosphate monoanion, the 1-hydroxyl group as in Vb is in a very favorable position for transfer to the 6-oxygen atom, whereas the hydrogen atom of a phosphate monoanion is not in an especially favorable position for transfer. The conditions for this intramolecular proton transfer are that the hydroxyl group must be acidic and be oriented so that the proton can interact with the oxygen atom without distorting the structure of the molecule. Consistently the dianions of the glycerol phosphates are not especially reactive,[4,32] although the 2-OH group is relatively acidic, simply because the hydroxyl groups are not favorably oriented for proton transfer, but we find that the dianion of ribose 5-phosphate is much more reactive than the monoanion.[34]

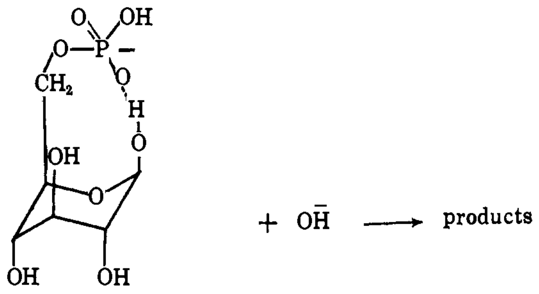
We assume that it is the 1-hydroxyl group which is involved in proton transfer, because the inductive effect of the ring oxygen atom makes it the most acidic of the alcoholic groups in glucose or glucose 6-phosphate, and it can interact very readily with the 6-O atom. However there is a report, based on qualitative observations only, that the rate of hydrolysis of glucose 2-phosphate is greatest at high pH,[35] and it is possible that here also the 1-hydroxyl group may anchimerically assist reaction. The 1-hydroxyl group must be very effective as an intramolecular general acid, because its participation requires that the sugar take up the β configuration (which is probably the more stable anomer) but in the unfavorable conformation in which all substituents are axial.

There are examples of phosphate esters whose hydrolyses are assisted by a neighboring group participation by a carboxyl (or phenolic hydroxy) group, which is functioning as an intramolecular general acid,[4,36,37] but so far as we know these sugar phosphates provide the only examples of this form of catalysis in which the neighboring group is as weakly acidic as are aliphatic hydroxy groups. Another special case is the hydrolysis of 2,4-dinitrophenyl dihydrogen phosphate, where the leaving phenoxide ion is sufficiently stable for the dianion to be a reactive species,[13] as it can be in hydrolyses of mixed carboxylic–phosphoric anhydrides,[38] or of pyrophosphoric acid,[39] which is a phosphoric anhydride.

There are other possible mechanistic models which would satisfy the observed relation between k_ψ and pH, but which can be eliminated on various grounds. (i) In reactions between monoanion and hydroxide ions, the 1-hydroxyl group might hydrogen bond with one of the phosphate oxygen atom, and the oxygen atom could then attack an intramolecularly hydrogen-bonded species (VIIa or b).



VIIa



VIIb

These reactions are improbable because for the reaction between trimethyl phosphate and hydroxide ion at 100° the estimated second-order rate constant $k_\psi = 3.3 \times 10^{-2}$ l. mole^{-1} sec^{-1}.[40] Hydrogen-bonded species such as VIIa,b should be considerably less reactive than trimethyl phosphate toward the hydroxide ion, but at 100°, at pH 8, $[\mathrm{OH^-}] \approx 10^{-5}$ *M*, and approximately 2% of the allyl phosphate is present as the monoanion. Therefore the observed value of $k_\psi = 1 \times 10^{-4}$ sec^{-1} requires that the second-order rate constant between hydroxide ion and the monoanion as in VIIa,b would have to be 1000 l. mole^{-1} sec^{-1}, and this value is so much greater than the second-order rate constant for reaction between trimethyl phosphate and hydroxide ion that we can reasonably exclude any mechanism involving reaction between hydroxide ion and monoanion, just as we can show that the decomposition of the monoanion does not arise from attack of hydroxide ion upon the undissociated acid $ROPO_3H_2$.[5,6]

(ii) One could suppose that the 1-hydroxyl group hydrogen bonds to one of the phosphoryl oxygen atoms of the dianion of glucose 6-phosphate, but it is hard to see how such a structure (VIII) could be *more* reactive than the monoanion because there would only be the driving force from one negative charge on a phosphoryl oxygen atom.

The activation energy for hydrolysis of the dianion is approximately 2.5 kcal mole^{-1} greater than for hydrolysis of the monoanion, but its contribution is more than offset by the entropy of activation which is approximately 9 eu more positive (Table IV). For the dianion

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(38) G. DiSabato and W. P. Jencks, *ibid.*, **83**, 4400 (1961).

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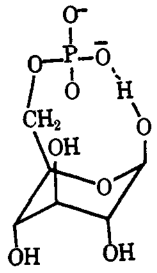
(40) P. W. C. Barnard, C. A. Bunton, D. R. Llewellyn, C. A. Vernon, and V. A. Welch, *J. Chem. Soc.*, 2670 (1961).



VIII

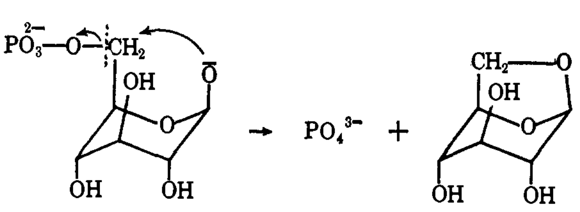
of glucose 6-phosphate to react, the β anomer (which by analogy with glucose is probably the main component[33]) must take up the energetically unfavorable conformation Vb in which all groups are axial, and the over-all energy of activation therefore includes the enthalpy of conversion of the mixture of α- and β-glucose 6-phosphate into the unfavorable conformation, followed by decomposition of Vb into products, and this kinetic step therefore has an activation energy which is much less than the experimentally measured value of 33 kcal $mole^{-1}$ and is probably less than that for hydrolysis of the monoanion. It is difficult to account for the contributions of these various equilibrium and rate steps to the over-all entropy of activation, but the positive value could arise because the initial state of the reaction, which is the equilibrium mixture of α- and β-glucose 6-phosphate dianions, will be heavily solvated by water molecules at the dinegatively charged phosphate group and at the 1-hydroxyl group, and at least some of these water molecules will be lost during formation of the transition state (VI).

Reactions at High pH. The high reactivity of glucose 6-phosphate at pH >10 is also unexpected. The dianions of monoalkyl phosphates are generally unreactive toward anionic nucleophiles because of electrostatic repulsions.[11] Glucose 3- and ribose 3-phosphates decompose in dilute alkali,[41] but these reactions are eliminations rather than hydrolyses, and the decomposition of glucose 2-phosphate in alkali is considerably slower than hydrolysis of the 6-phosphate.[35,42] Hydroxide ion reacts slowly with the dianion of *p*-nitrophenyl phosphate,[9] and this reaction is rapid only with the dinitrophenyl phosphates.[13]

A problem in elucidating the mechanism of the alkaline hydrolysis of glucose 6-phosphate is that glucose is unstable in alkali.[24] However, our evidence suggests that glucose 6-phosphate does not first decompose to some reactive organic phosphate which then generates inorganic phosphate. The observation of clean first-order kinetics of hydrolysis at high pH suggests that glucose 6-phosphate is not decomposing by a series of consecutive reactions involving organic phosphates of similar reactivities, or by reactions which involve more than one molecule of glucose 6-phosphate, or reactions between glucose and its phosphate. Therefore, we exclude Cannizaro reactions at C-1, and, because the rate is the same in the presence and absence of air, we assume that oxidation is not important.

Isomerization of glucose 6-phosphate to either α- or β-glucose 1-phosphate is not a reaction path for hydrolysis. At pH 10.1 at 100° the half-life for hydrolysis of α-glucose 1-phosphate is approximately 160 hr, and for the β anomer it is approximately 3 hr.[43] Under these conditions, hydrolysis of glucose 6-phosphate has a half-life of 4.4 min and is also considerably faster than the decompositions of glucose 2- and 3-phosphates.[35,41,42] Glucose 1-phosphate does not build up during hydrolysis of the 6-phosphate because the rate of hydrolysis of the 6-phosphate is the same whether the phosphate determination is carried in acidic solution (as in the Fiske–Subbarow method) which would decompose glucose 1-phosphate, or in less acidic solution (as in the Lowry–Lopez method) where glucose 1-phosphate would be stable.

At least one minor path for the alkaline decomposition of glucose 6-phosphate can be pin-pointed. This is the formation of 1,6-anhydro-1,5-glucopyranose (IX), which can occur by an intramolecular attack of the 1-alkoxide ion upon C-6 of glucose 6-phosphate with expulsion of the phosphate trianion. The β anomer of glucose 6-phosphate can take up the (unfavorable) conformation X whose geometry is favorable for participation of the neighboring 1-alkoxide group.



X IX

The phosphate trianion would not at first sight appear to be a particularly good leaving group, having p*K* = 12.32 at 25°, but its basicity is only about 10^2 times greater than those of many phenoxide ions,[44] and there are many alkaline decompositions of glycosides in which phenoxide is the leaving group and which go quite readily even when the phenol contains no electron-attracting groups.[25] Indeed the anhydro sugar IX can be prepared by the alkaline decomposition of phenyl glucosides, even though the participating hydroxyl group will not be extensively ionized, whereas in the glucose 6-phosphate ionization of the 1-hydroxyl group probably becomes extensive at pH >10.

We do not know the acidity of the 1-hydroxyl group in glucose 6-phosphate, but it should be similar to that of glucose itself, for which the extrapolated value of p*K* = 10.8 at 100°, and therefore the trianion X could be present in our reaction solutions at high pH.

Considerable amounts of glucose are formed directly in the alkaline hydrolysis of glucose 6-phosphate. Possible mechanisms in which a hydroxide ion attacks the glucose 6-phosphate dianion and in which bond breaking is assisted by hydrogen bonding from the 1-hydroxyl group are shown below. Path A involves transition states XIa or b.

An alternative reaction, path B, is an intramolecular general base catalyzed hydrolysis in which the negative charge on the 1-alkoxide group activates a water mole-

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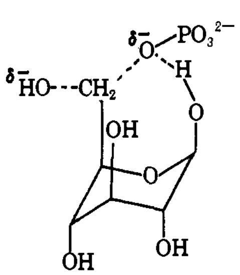
[41] D. Brown, F. Hayes, and A. R. Todd, *Chem. Ber.*, **90**, 936 (1957).

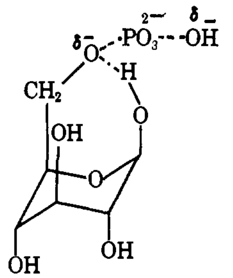
[42] K. R. Farrar, *J. Chem. Soc.*, 3131 (1949).

[43] H. Chaimovich, unpublished results.

[44] I. M. Kolthoff and P. J. Elving, Ed., "Treatise on Analytical Chemistry," Part I, The Interscience Encyclopedia, Inc., New York, N. Y., 1959, Chapter 13; J. Bjerrum, G. Schwarzenbach, and L. G. Sillén, "Stability Constants," Part II, The Chemical Society, London, 1958.

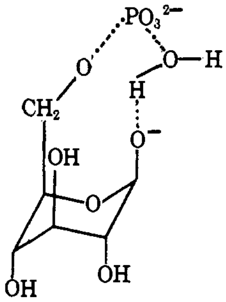


XIa

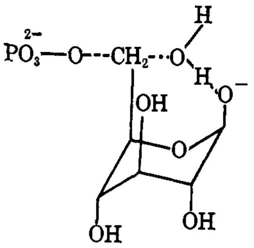


XIb

cule which attacks the phosphorus, or possibly the carbon atom as in XIIa or b.



XIIa



XIIb

At pH 11 the spontaneous hydrolysis of the dianion makes very little contribution to the over-all reaction rate, as does formation of the anhydro sugar IX (Experimental Section). Therefore k_ψ will not be much greater than the rate constant for the (hypothetical) attack of hydroxide ion upon the dianion of glucose 6-phosphate, as in XIa,b, or for the (hypothetical) spontaneous decomposition of the trianion. (These reactions will have the same dependence upon pH.)

Mechanisms similar to these have been discussed extensively in respect to other hydrolyses.[18,37,45,46] For carboxylic ester saponification, there is rate enhancement by a neighboring hydroxyl group which can be interpreted as involving attack of hydroxide ion upon an intramolecular hydrogen-bonded complex.[18,45] Similar behavior is found in amide hydrolysis.[46]

On the other hand, there can be a general base catalyzed attack of water upon the carbonyl group of an ester or amide, in which anionic oxygen acts as the general base,[18,45–47] and in some steroidal systems it is suggested that an amino nitrogen acts as the general base.[48] The present evidence suggests that when the neighboring group is an aliphatic hydroxyl, it acts as a general acid, and reaction involves attack of hydroxide ion upon an intramolecularly hydrogen-bonded complex; in alkaline solution, a phenolic hydroxyl group will be ionized, and the anionic oxygen will act as a general base and activate the incoming water molecule. Analogy between carboxyl and phosphate hydrolysis may be misleading, but we note that the acidity of the anomeric hydroxyl group in glucose (pK = 10.8 at 100°) is not much less than that of a phenolic hydroxyl group.

Attack of hydroxide ion upon the intramolecularly hydrogen-bonded form of the dianion of glucose 6-phosphate seems the less probable mechanism in the following grounds. From the slope of the initial part of the plot of k_ψ against $C_{\mathrm{OH^-}}$ (Figure 3) we can calculate a second-order rate constant of 3.8×10^{-1} l. sec^{-1} mole^{-1} for the (hypothetical) reaction between the dianion and hydroxide ion. For attack of hydroxide ion upon trimethyl phosphate at 100°, we estimated a value of 3.3×10^{-2} l. sec^{-1} mole^{-1} for the second-order rate constant,[40] and we expect a trialkyl phosphate to be much more reactive toward a hydroxide ion than a dianion of glucose 6-phosphate, even though decomposition of the latter might be assisted by intramolecular hydrogen bonding as in path A (XIa or b).

The alkaline decomposition of glucose 6-phosphate has a large temperature coefficient (Table III) and both hydrolysis and cyclization occur at high pH, although the latter is only a minor side reaction, and therefore the value of $k_\psi - k_2$ can be taken as a reasonable approximation to the actual value for the rate constant k_3 for hydrolysis at high pH. The possible reaction paths have the following kinetic forms (where C_3 is the concentration of trianion).

Path A

$$k_3 = k_3' C_{\mathrm{OH^-}} C_2/C$$

Path B

$$k_3 = k_3'' C_3/C$$

At 100° a plot of k_3 against $C_{\mathrm{OH^-}}$ is linear except at high pH (Figure 2), and $k_3' = 3.8 \times 10^{-1}$ at 100° and 5.2×10^{-4} l. sec^{-1} mole^{-1} at 45° (calculated taking $K_W = 4 \times 10^{-4}$ at 45° and 4.8×10^{-13} at 100°).[49] Therefore for path A we calculate an activation energy E = 28.5 kcal mole^{-1} and $\Delta S^* = 14$ eu.

It is difficult to make a similar calculation for path B, reaction between the trianion and water (XIIa,b), because we cannot determine the acid dissociation constant for the anomeric hydroxyl group of glucose 6-phosphate. As a first approximation, we assume that this dissociation constant will be the same as that of glucose itself, and calculate the concentration of the trianion on this basis, and at pH = 10.45 we estimate $C_3/C = 0.045$ at 45°, and at pH 9.98 $C_3/C = 0.15$ at 100°. Making these assumptions we estimate $k_3'' = 1.57 \times 10^{-5}$ sec^{-1} at 45.0° and 12.5×10^{-3} sec^{-1} at 100°. Therefore as a first approximation E = 29 kcal mole^{-1} and $\Delta S^* = 8$ eu, for hydrolysis by reaction path B. These two sets of parameters are very similar, because in path A we have to take into account the enthalpy and entropy of the autoprotolysis of water, whereas in path B we take into account the enthalpy and entropy for the acid dissociation of glucose.

It is difficult to compare effectiveness of the participation of the 1-hydroxyl or alkoxide group in this system with that in others, because the sugar phosphate has to take up the energetically costly conformation in which

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(45) H. B. Henbest and B. J. Lovell, *J. Chem. Soc.*, 1965 (1957); S. M. Kupchan and W. S. Johnson, *J. Am. Chem. Soc.*, **78**, 3864 (1956); S. M. Kupchan, P. Slade, and R. J. Young, *Tetrahedron Letters*, No. **24**, 22 (1960); T. C. Bruice and T. H. Fife, *J. Am. Chem. Soc.*, **84**, 1973 (1962).

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(47) M. L. Bender, F. J. Kézdy, and B. Zerner, *J. Am. Chem. Soc.*, **85**, 3017 (1963).

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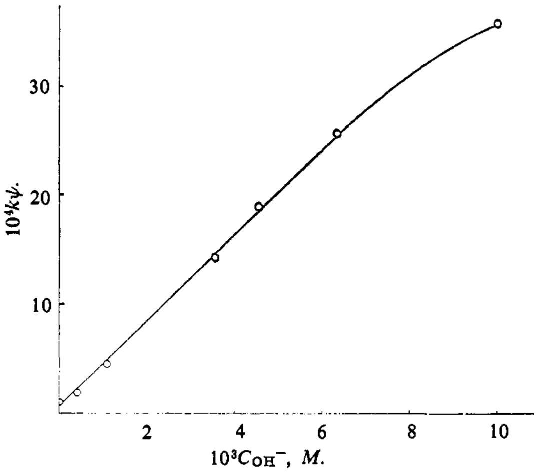

Figure 2. Plot of k_ψ (sec^{-1}) against hydroxide ion concentration at 100°.

all the groups are axial before the 1-hydroxyl group can participate in reaction.

There is some bending in the plot of $k_\psi - k_2$ against C_{OH^-} at the higher concentrations of hydroxide ion (Figure 2), which is understandable from either mechanism A or B. At high pH, appreciable amounts of the glucose 6-phosphate will be present as trianion. If hydrolysis follows path A the term C_2/C will decrease with increasing hydroxide ion concentration once the trianion concentration becomes appreciable, because the trianion will be unreactive toward hydroxide ion; on the other hand, if path B is followed the term C_3/C will be proportional to C_{OH^-} only so long as $C >> C_3$.

Acid Hydrolysis. The hydrolysis of glucose 6-phosphate in acid is qualitatively very similar to that of methyl phosphate.[6,31] Halogen acids are better catalysts than perchloric or sulfuric acid (Table I and Figure 3), and we assume that as for the hydrolysis of methyl phosphate they act by attack of halide ion upon the alkyl group of the conjugate acid.

$$\overset{-}{X} + RO\overset{+}{P}O_3H_3 \longrightarrow RX + H_3PO_4 \, (X = Cl, Br)$$

As is generally observed for reactions in water[31,50] (but not in aprotic solvents[51]), bromide appears to be more nucleophilic than chloride ion (Figure 3), because of differences in hydrogen bonding with water, although part of the different catalytic powers of the halogen acids could also arise because hydrobromic acid is a better protonating agent than hydrochloric acid.[52a,b]

The Arrhenius parameters for hydrolysis in 4.6 M perchloric acid (Table IV) are in the range expected for an A2 hydrolysis;[53] for example, the acid hydrolyses of both glucose 6-phosphate and pyrophosphoric acid[10] have negative entropies of activation.

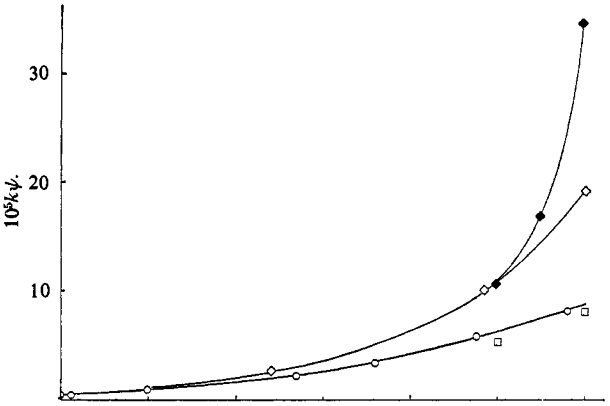

Figure 3. Plot of k_ψ (sec^{-1}) against acid concentration at 100°: ○, $HClO_4$; □, H_2SO_4; ◇, HCl; ◆, HBr.

The activation energy for the hydrolysis of glucose 6-phosphate is smaller in 1.6 M perchloric acid than in 4.6 M acid, and the log A factor is also appreciably smaller (Table IV), suggesting that there is some incursion of a spontaneous hydrolysis in the less concentrated acid, and that this spontaneous hydrolysis has relatively low values of E and A.

The presence of a spontaneous hydrolysis complicates the study of variations of reaction rate with acidity. The acid not only introduces a new reaction path in which the conjugate acid is the reactive species, but it may also have a salt effect upon the spontaneous hydrolysis.[6] The contribution of the spontaneous hydrolysis should be negligible in the more concentrated acid, and we assume that at pH 1 the monoanion- and the acid-catalyzed hydrolyses will be relatively unimportant, and therefore the first-order rate constant, k_0, for the spontaneous hydrolysis will be approximately 0.5 × 10^{-5} sec^{-1} (Figure 3). Neglecting any electrolyte effect upon k_0, the first-order rate constants, k_a, for the acid hydrolysis are given by $k_\psi - k_0$. Plots of log k_a against log C_{H^+} are approximately linear with slope = 1.8, and against $-H_0$ (where H_0 is Hammett's acidity function[52b]) are concave with slope 0.7 at low acidities and 0.3 at high. Bunnett's treatment in which log $k_a + H_0$ is plotted against log a_{H_2O} gives a straight line plot for perchloric acid, with a slope w of approximately 3.5. The values for sulfuric acid fall close to this line. These w values are in the general range expected for A2 hydrolyses,[54] and are similar to those found for A2 hydrolyses of other phosphates.[4,6,10] Despite uncertainties as to the contribution of the spontaneous hydrolysis this treatment appears to be meaningful, as applied to the hydrolysis of phosphate esters, because the acid hydrolysis of glucose 1-phosphate, which has an A1 mechanism of hydrolysis,[14] gives a plot of log k_a against $-H_0$ which has a slope close to unity and a w value close to zero.[54]

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(53) L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 1 (1963).

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