717. *The Reactions of Organic Phosphates. Part II.* The Hydrolysis of α-D-Glucose 1-(Dihydrogen Phosphate).*

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The rates of hydrolysis of α-D-glucose 1-phosphate in the range pH 1—8 can be interpreted in terms of two reactions, (*a*) involving the monoanion and proceeding with phosphorus–oxygen bond fission and (*b*) involving the neutral molecule and proceeding with carbon–oxygen bond fission. In strongly acidic media there is a rapid acid-catalysed reaction whose rate depends on Hammett's acidity function and which proceeds with carbon–oxygen bond fission. The mechanisms of these reactions are discussed.

Nomenclature. See Part I. Throughout this paper we use the name α-D-glucose 1-phosphate to denote species, ionic or non-ionic, in which one phosphate group is united to one α-D-glucose residue at the 1-position, *i.e.*, mono-α-D-glucose 1-phosphate.

α-D-Glucose 1-phosphate was chosen as the second substance for study in our investigation of the hydrolytic reactions of organic phosphates, first, because of the importance of sugar phosphates and, secondly, because it was hoped, in view of the known tendency for the pyranoside ring to separate as a carbonium ion, to observe S_N1 type reactions with carbon–oxygen bond fission.

From the literature [1] it is clear that α-D-glucose 1-phosphate is, typically of its class, rapidly hydrolysed in strongly acidic media. For example, Desjobert [2] studied its acid-catalysed hydrolysis at 1°, whereas with primary alkyl phosphates acid-catalysed hydrolysis can be conveniently studied only at much higher temperatures (cf. Part I). The reaction is, however, much slower in the range pH 1—8 than in strongly acid solutions and can, under these conditions, be studied at temperatures similar to those for simple alkyl phosphates.

The main features of the pH–rate profile have been established by Desjobert.[2] He found that the rate of hydrolysis increased slowly from pH 8 to pH 4 and then more rapidly as the pH decreased further. He concluded that between pH 4 and pH 1 the neutral species is the reactive entity. No systematic bond-fission studies have been reported, although Cohn,[3] using ^{18}O as tracer, showed that in the strongly acid region hydrolysis proceeds predominantly with carbon–oxygen bond fission.

The present paper reports an investigation by kinetic and isotopic techniques of the hydrolysis of α-D-glucose 1-phosphate over the range of acidities pH 8 to 3M-perchloric acid. Preliminary accounts of this work have been published.[4, 5]

Experimental

α-D-Glucose 1-(dipotassium phosphate) was kindly supplied to us by Dr. E. M. Crook of the Biochemistry Department, University College, London. It had $[\alpha]_D^{23}$ +76·2° (*c* 1·0 in H_2O). A sample was also made by McCready and Hassid's method,[6] by using potato phosphorylase and purified by passage through Zeo-Karb 215 (to remove unwanted cations) and then absorbed on Amberlite IR-4B, from which it was eluted with 2N-potassium hydroxide. After treatment with decolorising charcoal the salt was crystallised from methanol–water. It had $[\alpha]_D^{25}$ +78·9° (*c* 1·0 in H_2O) (Found: P, 8·3. Calc. for $C_6H_{11}O_6{\cdot}PO_3K_2,2H_2O$: P, 8·3%).

Kinetics.—Runs were followed colorimetrically, by Allen's method for the estimation of

* Part I, preceding paper.

[1] Leloir, " Progress in the Chemistry of Natural Products," Vol. 8, 1951, p. 47.
[2] Desjobert, *Bull. Soc. Chim. biol.*, 1951, **33**, 42.
[3] Cohn, *J. Biol. Chem.*, 1949, **180**, 771.
[4] Barnard, Bunton, Llewellyn, Oldham, Silver, and Vernon, *Chem. and Ind.*, 1955, 760.
[5] Vernon, *Chem. Soc. Special Publ.*, No. 8, 1957, 17.
[6] McCready and Hassid, *J. Amer. Chem. Soc.*, 1944, **66**, 560.

inorganic phosphate,[7] or polarimetrically. Good first-order rate coefficients were obtained in each case, and the observed and the calculated infinity values agreed closely.

The following details are typical of experiments in which the rate of production of inorganic phosphate was determined. A solution containing the dipotassium salt ($1{\cdot}56 \times 10^{-3}$M) and perchloric acid (1·01M) was put in a thermostat at 25°. At appropriate times aliquot parts (5 c.c.) were removed and added to ice-cold water (13 c.c.). Sufficient 60% perchloric acid was added to bring the final strength to 0·72M; the colorimetric reagents were then added and the volume made up to 25 c.c. The solution was kept at 10° for 5 min., after which its optical density was measured in the usual way on a Hilger Spekker absorptiometer with a red filter (Kodak 608). Calculation showed that continuing hydrolysis of the substrate under the conditions of measurement introduced a negligible error into the rate coefficients and, in fact, the colour intensity was found to be sensibly constant for about 10 min. First-order rate coefficients, k_0, calculated as in Part I are shown below.

Time (min.)	O.D.	10^5k_0 (sec.$^{-1}$)	Time (min.)	O.D.	10^5k_0 (sec.$^{-1}$)
0	0·028	—	210	0·415	4·00
30	0·099	4·20	240	0·453	3·97
60	0·160	4·03	270	0·491	3·97
90	0·218	4·00	300	0·528	3·98
120	0·275	4·03	330	0·554	3·90
150	0·327	4·05	360	0·588	3·93
180	0·369	3·97	∞ (expt.)	1·005	—
			∞ (calc.)	1·005	—

The following details are typical of experiments in which the reaction was followed polarimetrically. A solution of the dipotassium salt (1·1367 g. in 25·2 c.c.) containing sulphuric acid (2·39M) was allowed to come to equilibrium in a 10 cm. jacketed polarimeter tube at 25°. Readings of the optical rotatory power were made at appropriate time intervals. The first-order rate coefficients k_0 are given below.

Time (min.)	$[\alpha]_D^{25}$	10^5k_0 (sec.$^{-1}$)	Time (min.)	$[\alpha]_D^{25}$	10^5k_0 (sec.$^{-1}$)
0	3·306°	—	45	2·420°	19·7
8	3·110	19·8	90	1·895	19·7
16	2·920	20·5	∞ (expt.)	1·149	—
26	2·720	19·8	∞ (calc.)	1·162	—
37	2·532	20·0			

Table 1 gives the first-order rate coefficients obtained in the range pH 8—1 at 82·0°. The reactions were all followed colorimetrically. The compositions of the buffers were similar to those given in Part I; the values of the pH were found, as before, from the data of Stene.[8]

The variation of rate coefficients with temperature was studied at each end of the pH range covered by Table 1. The results are given in Table 2.

TABLE 1. *Hydrolysis of* α-D-*glucose* 1-*phosphate at* pH 8—1.

pH	7·54	6·33	5·53	4·74	4·12	3·69	3·33	2·48	2·20	1·61	1·23
10^5k_0 (sec.$^{-1}$)	0·0253	0·106	0·212	0·360	0·635	1·50	3·43	20·7	38·9	175	467

TABLE 2.

pH	2·5	2·2	2·2	7·46	7·54
Temp.	100·1°	82·0°	25·0°	100·1°	82·0°
10^5k_0 (sec.$^{-1}$)	161	38·9	0·00877	0·288	0·0253

Table 3 gives the results obtained at 25·0° in strongly acidic media. Some of the runs were followed by rate of appearance of inorganic phosphate (substrate concentration *ca.* M/1000) and others polarimetrically (substrate concentration *ca.* M/10). The two methods gave sensibly similar results.

Isotope Experiments.—The positions of bond fission were determined, under various conditions, from the results of isotopic analysis of inorganic phosphate isolated from reactions run in water enriched with ^{18}O. The methods of isolation and isotopic analysis have already been described (Part I). Analysis of the other product of hydrolysis, glucose, would, of course, give no useful information since its rate of isotopic exchange is very rapid under all the relevant

[7] Allen, *Biochem. J.*, 1940, **34**, 858.
[8] Stene, *Rec. Trav. chim.*, 1930, **49**, 1133.

conditions. The results are shown in Table 4. As before, k_0 and k_E^P are the first-order rate coefficients, appropriate to the stated conditions, for hydrolysis of the substrate and for the exchange reaction of inorganic phosphate respectively; the symbols N_S and N_P refer

TABLE 3. *Hydrolysis of α-D-glucose 1-phosphate in strongly acid solution.*

Acid (M)	10^5k_0 (sec.$^{-1}$)	Acid (M)	10^5k_0 (sec.$^{-1}$)	Acid (M)	10^5k_0 (sec.$^{-1}$)	Acid (M)	10^5k_0 (sec.$^{-1}$)
0·51	1·65	2·01	12·2	3·00	28·7	0·93 [c]	3·83 [a]
1·01	4·00	2·11	11·7 [a]	3·00 [b]	43·9	1·54 [c]	9·17 [a]
1·16	4·13 [a]	2·50	18·3	3·36	39·8	2·39 [c]	20·0 [a]
1·48	6·95						

[a] Polarimetric. [b] In 75% D_2O. [c] H_2SO_4 (all others $HClO_4$).

respectively to the excess abundance (atoms %) of tracer in the solvent and in the isolated inorganic phosphate; values of Q_P (the proportion of reaction proceeding by phosphorus–oxygen bond fission) have been calculated, except for the first two cases, by the equation given in Part I which allows for the subsequent exchange reaction of the inorganic phosphate.

TABLE 4. *Isotope results.*

Conditions	Time of heating (hr.)	10^5k_0 (sec.$^{-1}$)	$10^7k_E^P$ (sec.$^{-1}$)	N_S	N_P	Q_P (×100)
2M-$HClO_4$, 73·0°	1	—	—	0·72	0·00	0
pH 2·53, 82·0°	3·5	—	—	1·19	0·02	7
pH 4·18, ,, ...	57	0·635	1·17 *	0·91	0·069	25
pH 6·3, ,, ...	380	0·106	1·17 *	1·09	0·31	86

* Measured by Mr. V. A. Welch.

DISCUSSION

(a) *Reactions of the Monoanion and Neutral Species.*—Analysis of the kinetic data requires a knowledge of the dissociation constants of α-D-glucose 1-phosphate. The second dissociation constant has been accurately measured [9] as a function of temperature over the range 0—50°. Extrapolating these data gives the value pK_2 6·72 at 82·0°. The first dissociation constant is known less accurately; the value pK_1 1·23 is quoted [10] for 30°. It cannot, because of rapid hydrolysis of the substrate, be easily measured at higher temperatures. No great error will, however, be involved by using this value for the analysis of the present data.

The pH–rate profile for monomethyl phosphate in the range pH 2—8 can be accounted for, as shown in Part I, on the assumption that, in this range, reactions other than that involving the monoanion are unimportant. In the present case this assumption would clearly be wrong since from pH 5 to pH 1, where monoanion concentration must decrease, the rate increases in approximate proportion to the hydrogen-ion concentration.

Inspection of the shape of the profile (Fig. 1) suggests that the monoanion reaction occurs under the more alkaline conditions but is swamped, below pH 5, by some faster reaction. This view is supported by the isotope results which show that the predominating mechanisms at pH 6·3 and 2·5 must be different since they involve different modes of bond fission.

In solutions more acidic than pH 5 the dependence of rate on hydrogen-ion concentration suggests that the neutral species is, under these conditions, the reactive entity since its concentration, at least down to pH 2,* is approximately proportional to hydrogen-ion concentration and consequently proportionality between k_0 and c_{H^+} would be expected. The possibility that reaction proceeds *via* the conjugate acid of α-D-glucose 1-phosphate, *i.e.*, involves true acid-catalysis, can be excluded on the grounds that the

* The proportions of neutral species present at pH 5, 4, 3, and 2 are 0·00021, 0·0021, 0·021, and 0·176 respectively.

[9] Ashby, Clarke, Crook, and Datta, *Biochem. J.*, 1955, **59**, 203.
[10] Cori, Colowick, and Cori, *J. Biol. Chem.*, 1937, **121**, 465.

rate, in the region where the monoanion is the bulk component, would then be proportional to the square of the hydrogen-ion concentration.

If it is therefore accepted that the hydrolysis of α-D-glucose 1-phosphate in the range pH 2—8 can be represented as the sum of reactions proceeding *via* the neutral species (N) and the monoanion (M), the observed first-order rate coefficient at any given pH will be:

$$k_0 = \frac{C_N \cdot k_N}{C_P} + \frac{C_M \cdot k_M}{C_P} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where C_N and C_M are the concentrations of neutral and monoanion species respectively at that pH, C_P is the stoicheiometric concentration of organic phosphate, and k_N and k_M are the corresponding specific first-order rate coefficients. It remains to discover the best values of these coefficients. The simplest procedure is successive approximations as follows. At pH 2·2 the proportion of neutral species is 0·097; hence, assuming that at this relatively acid pH none of the rate is due to the monoanion gives an approximate value $3{\cdot}88 \times 10^{-4}/0{\cdot}097 = 4{\cdot}00 \times 10^{-3}$ sec.$^{-1}$ for the first-order rate coefficient (k_N) for the reaction of the neutral species. At pH 6·33 the proportion of neutral species present is very small (*ca.* 8×10^{-6}) and the rate coefficient arising from this would be $0{\cdot}32 \times 10^{-7}$ sec.$^{-1}$; the reaction at this pH mostly, therefore, involves the monoanion. The corresponding specific rate coefficient (k_M) can be obtained by subtracting from the observed value the small contribution due to the neutral species and dividing the result by the proportion of monoanion present: this gives $k_M = 1{\cdot}45 \times 10^{-6}$ sec.$^{-1}$. This " corrected " value can now be used to calculate values of k_N at several of the pH's studied. The results are given in Table 5. The constancy of the calculated values of k_N from pH 4·12 to 2·2

TABLE 5.

pH	4·12	3·69	3·33	2·48	2·20	1·61	1·23
$10^3 k_N$ (sec.$^{-1}$)	4·05	3·87	4·05	3·85	4·00	5·92	9·33

is good support for the view that in this region reaction is largely due to the neutral species. In Fig. 1 the rate profile calculated from equation 1 with the values k_N $3{\cdot}97 \times 10^{-3}$ sec.$^{-1}$ and k_M $1{\cdot}45 \times 10^{-6}$ sec.$^{-1}$ is seen to be in good agreement with the experimentally observed rate profile except below pH 2 where the observed rate is faster than predicted. The latter effect, which is reflected in the non-constancy of the values of k_N below pH 2 (Table 5), is clearly due to the contribution from a true acid-catalysed reaction.

The Arrhenius parameters for the two reactions can be calculated from the data in Table 2, after allowance has been made at the two pH's studied, for the amount of each reactant present. The results are: for the neutral species $E = 31{\cdot}0$ kcal. mole^{-1}, $A = 4{\cdot}30 \times 10^{16}$ sec.$^{-1}$; for the monoanion, $E = 30{\cdot}0$ kcal. mole^{-1}, $A = 5{\cdot}04 \times 10^{12}$ sec.$^{-1}$.

From equation (1) (and the assumption that the reactions of the monoanion and neutral species proceed with phosphorus–oxygen and carbon–oxygen bond fission respectively) the proportion of phosphorus–oxygen bond fission (Q_P) at any pH in the range pH 2—8 is given by:

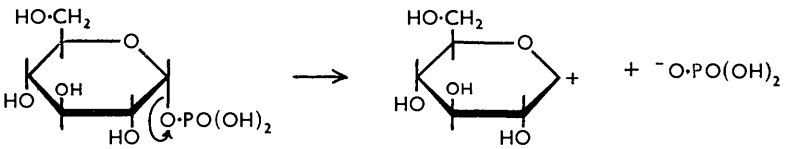
$$Q_P = C_M k_M / (C_M k_M + C_N k_N) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Using the values of k_M and k_N obtained above, the predicted values of Q_P at pH 2·53, 4·18, and 6·30 are respectively 0, 0·25, and 0·97. These are in fair agreement with the observed values of 0·07, 0·25, and 0·86 and it may be concluded that equations (1) and (2) together with the calculated values of k_N and k_M give an adequate account of the kinetic and isotope results for pH 2—8.

The specific rate coefficient and Arrhenius parameters for the hydrolysis of the monoanion of α-D-glucose 1-phosphate are not very different from the values of the corresponding quantities for monomethyl phosphate. As was pointed out in Part I, this is generally true; the kinetic parameters for hydrolysis of the monoanion do not vary much with the nature of the substituting group. Consequently, the mechanisms of hydrolysis of the

monoanions of monosubstituted phosphates are probably all very similar. A discussion for monomethyl phosphate has been given in Part I; the same discussion is relevant to α-D-glucose 1-phosphate, so will not be repeated.

The neutral species of α-D-glucose 1-phosphate is, on the other hand, much more reactive than that of monomethyl phosphate ($k_N = 3{\cdot}03 \times 10^{-2}$ sec.$^{-1}$ and $5{\cdot}0 \times 10^{-7}$ sec.$^{-1}$ respectively at 100·1°). *A priori*, the likely mechanisms are either a unimolecular [S_N1 (C)] or a bimolecular [S_N2 (C)] substitution at the $C_{(1)}$ atom of the pyranoside ring by a water molecule. The formal possibility that ring opening is involved is unlikely because such a process would require prior protonation of the ring oxygen atom (*i.e.*, would be acid-catalysed). There is, unfortunately, no direct kinetic criterion which is applicable for the determination of the molecularity. A strong argument, however, can be made for supposing the reaction to be unimolecular. It has been known for some time that aceto-halogeno-sugars readily undergo unimolecular replacement of halogen.[11] In a recent investigation, Rhind-Tutt [12] examined the conditions required for bimolecular substitution of a halogen atom at position 1 of the D-glucopyranoside ring. He found that 2 : 3 : 4 : 6-tetra-*O*-methyl-α-D-glucopyranosyl chloride underwent unimolecular substitution in methanol even in the presence of methoxide ions. The bimolecular process could be observed only in a solvent of lower dielectric constant (*e.g.*, propan-1-ol) and in the presence of a powerful nucleophile (*e.g.*, thiophenoxide ion). α-D-Glucose 1-phosphate, which has the same conformation as the compound studied by Rhind-Tutt, is therefore unlikely to undergo bimolecular substitution in water which has a high dielectric constant and a relatively low nucleophilic power. The situation is entirely analogous to that found for nucleophilic displacements of equatorial substituents in the *cyclo*hexane ring. The hydrolysis of the neutral species must, therefore, be interpreted as an S_N1(C) process, with slow heterolysis of the $C_{(1)}$–oxygen bond:



This type of process has not, so far as we are aware, previously been observed for any other organic phosphate. Its special facility in the present case is due to the large activation of the S_N1(C) process by the oxygen atom directly linked to the centre of substitution.[13]

(b) *The Reaction of the Conjugate Acid.*—In strongly acidic media the first-order rate coefficients (k_0) increase much more rapidly than stoicheiometric acidity (Table 3). However, log k_0 when plotted against Hammett's acidity function H_0 (values taken from Long and Paul [14]) for both sulphuric acid and perchloric acid gives a straight line of slope −0·94 (Fig. 2). This result taken in conjunction with the considerable increase in rate produced by change of solvent to deuterium oxide [15] means, on the Zucker–Hammett hypothesis, that the conjugate acid of α-D-glucose 1-phosphate, present in equilibrium concentration, undergoes covalency change in the rate-determining step of the reaction without the intervention of a solvent molecule.* The rate-determining step may be

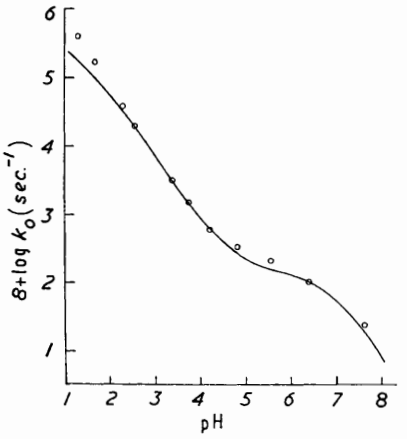
* The acid-catalysed reaction, as in the case of monomethyl phosphate, is accompanied by hydrolysis of the neutral species. However, the proportion of the latter reaction in M-perchloric acid is only *ca.* 2%, and since the correction would be much smaller than in the case of monomethyl phosphate and, because of the dependence of rate on H_0, more difficult to make, it has been neglected.

[11] Newth and Phillips, *J.*, 1953, 2896.
[12] Rhind-Tutt, Ph.D. Thesis, London, 1957.
[13] Ballinger, de la Mare, Kohnstam, and Prest, *J.*, 1955, 3641.
[14] Long and Paul, *Chem. Rev.*, 1957, **57**, 1.
[15] Bonhoeffer, *Trans. Faraday Soc.*, 1938, **34**, 252.

formulated, as in the case of the glycosides,[16] as the slow production of either a ring-closed ion (I) or a ring-opened ion (II), either of which would react rapidly to give the product.

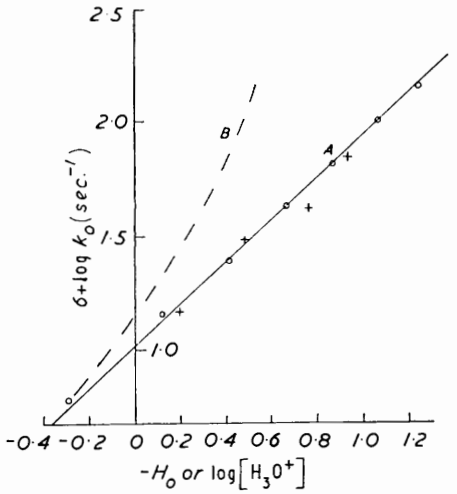
There is some reason for preferring the mechanisms involving (I); first, analogy with the mechanism of hydrolysis of the neutral species; and, secondly, a recent investigation by Rhind-Tutt [12] has shown that hydrolysis of glycosides is better interpreted as proceeding through intermediates of type (I) than of type (II). The reverse reaction (*i.e.*, combination

FIG. 1. *Hydrolysis of* α-D-*glucose* 1-*phosphate at* 82·0°.



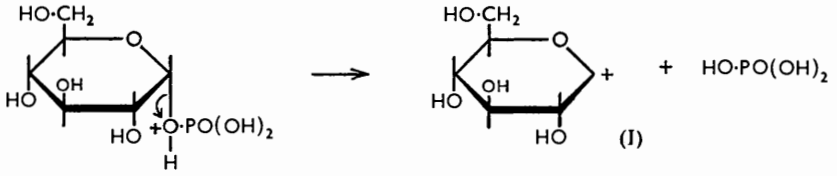
○, *Experimental values.* — *Calc. from equation* 1.

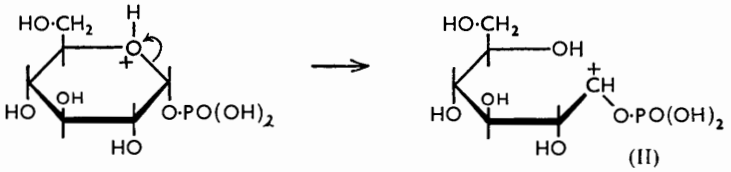
FIG. 2. *Acid-catalysed hydrolysis of* α-D-*glucose* 1-*phosphate at* 25·0°.



A, Plot against H_0, *gradient* −0·94. *B, Plot against* log $[H_3O]^+$. ○, *Colorimetric measurements.* +, *Polarimetric measurements.*

of phosphate and carbonium ion) is presumably unimportant since no difference in the rate of change of optical activity or appearance of inorganic phosphate was found.





(c) *Note on the Enzymic Hydrolysis of* α-D-*Glucose* 1-*Phosphate.*—Cohn,[3] in an important pioneer study of the enzymic hydrolysis of phosphates, first noticed that, whereas acidic hydrolysis of α-D-glucose 1-phosphate proceeds with carbon–oxygen bond fission, hydrolysis in the presence of prostatic acid phosphatase proceeds with phosphorus–oxygen bond fission. It is now known [17] that the result observed with the enzyme is a general one; whatever the substrate, hydrolysis with prostatic acid phosphatase always proceeds

[16] Bunton, Lewis, Llewellyn, and Vernon, *J.*, 1955, 4419.
[17] Bunton, Silver, and Vernon, *Proc. Chem. Soc.*, 1957, 348.

with phosphorus–oxygen bond fission. Cohn commented that her results were the first example in which it could be shown that an enzyme altered the reaction path. This statement is, perhaps, misleading. What the experiments actually show is that, for α-D-glucose 1-phosphate, the mechanisms of hydrolysis in acidic solution and in the presence of the enzyme are different. This is not surprising since the acidic mechanism arises from the structural features of the pyranoside ring whereas the enzymic mechanism involves the phosphate residue. Hydrolysis proceeding with phosphorus–oxygen bond fission in the absence of an enzyme can be realised, as shown above, with α-D-glucose 1-phosphate and reasons have been given elsewhere [17] for supposing that it is this reaction which is analogous to the enzymic one. In comparing enzymic and non-enzymic hydrolysis of phosphate esters it is important that, in the absence of the enzyme, reaction may proceed by a variety of reaction paths depending on conditions. In some instances the path which is specifically catalysed by the enzyme may not be experimentally realisable in its absence, for in certain circumstances, it will be masked by a readier reaction.

We thank Professor Sir Christopher Ingold, F.R.S., and Professor E. D. Hughes, F.R.S., for their continued interest and encouragement; Professor Sir Alexander Todd, F.R.S., and his colleagues for valuable advice and criticisms; Mr. P. Chaffe and Mr. V. A. Welch for assistance with the isotopic analysis; the Nuffield Foundation for a grant for equipment; and the Ministry of Education for a grant (to K. G. O.).

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[*Received, March 24th*, 1958.]