1207. *Hydrolysis of Hydroxyalkyl Phosphate Esters:*[1] *Effect of Changing Ester Group*

By D. M. Brown and D. A. Usher

The rates and products of alkaline hydrolysis of a series of esters of 2-hydroxypropylphosphate have been studied. Decomposition by an epoxide route is dominant in the cyclohexyl and absent in the phenyl ester, in which hydrolysis occurs through the cyclic phosphate only. A linear relationship between log k and the pK_a of the displaced alcohol is found to hold for the latter process, over a wide range of k and K_a values.

In the preceding Paper[1] a study of the base-catalysed hydrolysis of some cyclohexyl 2-hydroxyalkyl phosphates was described. Two modes of breakdown were discerned; one in which transesterification with ejection of the cyclohexyloxy-anion gave, initially, a cyclic phosphate, and another in which epoxide formation led to displacement of cyclohexyl phosphate. Substitution in the hydroxyalkyl group affected the rates of the two processes, and hence the ratios of products obtained.

We pointed out that the small percentage (8%) of reaction going by the epoxide route in, say, methyl 1-glycerol phosphate compared with the large amount (78%) in the cyclohexyl ester was probably to be related to the leaving character of the displaced groups,

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[1] D. M. Brown and D. A. Usher, preceding Paper.

and not to any peculiarity of the cyclohexyl ester as such. This general view has been confirmed by an investigation of the rates of hydrolysis of a series of esters of 2-hydroxypropyl phosphate (I). The esters were prepared by reaction between the alkyl or aryl phosphate and 1,2-epoxypropane in aqueous solution at about pH 8. The rates and product composition during hydrolysis of the alkyl esters were determined by the chromatographic method described earlier.[1,2] A spectrophotometric method was used for the aryl esters. The second-order rate constants and mole-percentage of products are given in Table 1. In the case of the alkyl esters the rate of cyclic phosphate formation is much slower than the rate of subsequent ring-opening,* and the concentration of cyclic phosphate during the hydrolysis remains negligibly small. Inspection of the Table shows that in the epoxide-forming process the alkyl phosphates are all essentially equivalent in ease of displacement. The differences in the product ratios are due to the very large differences in rate of the reaction leading to the alcohol, ROH. For the latter reaction we found that a linear free-energy relationship holds between the log of the second-order rate constant and the pK_a of the displaced alcohol (Figure).

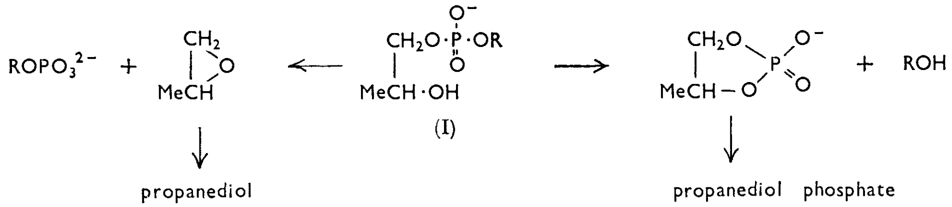

TABLE 1

Hydrolysis of esters of 2-hydroxypropyl phosphate

(Aqueous NaOH, 80°; k in l. mole^{-1} sec.$^{-1}$)

Ester R in (I)	Rate of formation of ROH (10^6k)	Mole (%) product	Rate of formation of $ROPO_3H_2$ (10^6k)	Mole (%) product
p-Nitrophenyl	260×10^{4} [a]	100	—	—
p-Chlorophenyl [b]	250×10^{3} [a]	100	—	—
Phenyl........................	740×10^{2} [a]	100	—	—
2-Methoxyethyl [c]	125	94	7·8	6
2-Hydroxyethyl [c]	235	63	137	37
Methyl [c]	127	93	9·8	7
Ethyl [b]........................	15·7	70	6·6	30
Isopropyl [c]	0·68	19	3·0	81
Cyclohexyl	0·55	16	3·0	84

[a] Extrapolated from data at 25° and 35°. [b] Rate measured in 0·05N-NaOH and assumed to be of first order in alkali. [c] Rate measured in N-NaOH and assumed to be of first order in alkali.

The dissociation constants of the alcohols and phenols were those obtained by Ballinger and Long,[3] and by Fernandez and Hepler.[4] No reliable measurements of the dissociation constants for propan-2-ol, cyclohexanol, and t-butyl alcohol were available, so the graph given by Bruice [5] of log(rate) *vs.* pK_a for the alkaline hydrolysis of a series of acetate esters † was extrapolated to include the esters of these alcohols; [6,7] in this way it was possible to estimate their " effective pK_a " values as 19·0, 19·4, and 23·8, respectively. These values

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* The first-order rate constant for hydrolysis of barium propane-1,2-diol cyclic phosphate in 0·1N-NaOH at 30° can be calculated as very roughly 3×10^{-5} sec.$^{-1}$ from the data of T. Ukita, K. Nazasawa, and M. Irie, *Pharm. Bull.* (*Tokyo*), 1957, **5**, 127.

† The equation of the line for alkaline hydrolysis of the acetates is $\log k_{OH} = -0{\cdot}26\ pK_a + 5{\cdot}0$ (cf. ref. 5).

[2] D. A. Usher, *J. Chromatog.*, 1963, **12**, 262.

[3] P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, 1960, **82**, 795.

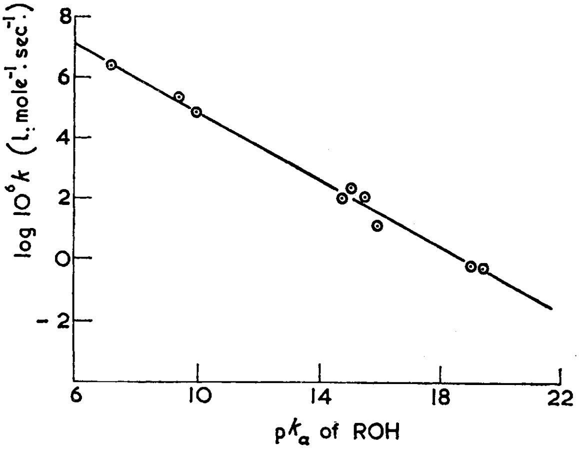
[4] L. P. Fernandez and L. G. Hepler, *J. Amer. Chem. Soc.*, 1959, **81**, 1783.

[5] T. C. Bruice, T. H. Fife, J. J. Bruno, and N. E. Brandon, *Biochemistry*, 1962, **1**, 7.

[6] L. P. Hammett, " Physical Organic Chemistry," McGraw-Hill, New York, 1940, p. 211.

[7] N. B. Chapman, R. E. Parker, and P. J. A. Smith, *J.*, 1960, 3634.

were inserted in the Figure connecting the pK_a of ROH the alcohol with the rate of loss of alkoxide ion from the corresponding 2-hydroxypropyl phosphate diester. The t-butyl ester was not sufficiently characterised, and appeared to break down by a different mechanism and is not included here. The rate constants were measured at, or extrapolated to 80°, and the pK_a were measured [3,4] at 25°. The temperature dependence of K_a for the alcohols has not been determined, and it is not known to what extent the linear relationship would be affected if the rates and dissociation constants were both measured at 80°.



Hydrolysis of 2-hydroxypropyl phosphate esters

The equation of the line in the Figure was determined by the method of least squares to be:

$$\log k = -0{\cdot}56 \text{ p}K_a + 4{\cdot}43 \ (k \text{ in l.mole}^{-1}\text{sec.}^{-1}).$$

Since the differences in the rate of hydrolysis between members of the series are largely due to differences in the activation energy (see below), the gradient would have been steeper if the rates at 25° had been plotted. This reaction is therefore more sensitive to the basicity of the leaving group than is the corresponding reaction of the acetates.[5]

The rates of formation of ethanediol and of 2-methoxyethanol from their respective 2-hydroxypropyl phosphate esters are not widely different. Since these two alcohols have about the same pK_a,[3] [the observed pK_a of ethanediol is 14·8; the statistical factor, log 2, has been *added* to this value in the Figure (cf. ref. 3)], this result shows that, in these compounds at least, there is no large increase in the hydrolysis rate due to a hydrogen bond from the vicinal hydroxyl group to the phosphoryl oxygen. If, in N-sodium hydroxide solution, the primary hydroxyl group of the 2-hydroxyethyl ester existed to a large extent as the alkoxide anion, the observed rate of formation of ethanediol would not represent the rate of loss of 2-hydroxyethoxide, for the expulsion of the dianion of ethanediol would probably be very slow. However, it has been shown [1] that the rate of hydrolysis of cyclohexyl 2-hydroxyethyl phosphate is approximately first-order in hydroxide ion up to an alkali concentration of 1N, and therefore the percentage existing in solution as the alkoxide anion must here be fairly small.[8]

The absence of a significant rate enhancement that could be ascribed to hydrogen-bonding does not mean that in other more rigid molecules such an effect will not be noticed, for the degree of assistance by neighbouring hydroxyl in the solvolysis of acetate esters [9] is quite sensitive to the relative stereochemistry of the groups concerned.[9,10] The point

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[8] See D. A. Usher, Ph.D. Dissertation, Cambridge, 1963, pp. 43—45 for a fuller discussion.

[9] H. B. Henbest and B. J. Lovell, *Chem. and Ind.*, 1956, 278; *J.*, 1957, 1965. S. M. Kupchan and W. S. Johnson, *J. Amer. Chem. Soc.*, 1956, **78**, 3864. T. C. Bruice and T. H. Fife, *ibid.*, 1962, **84**, 1973.

[10] T. C. Bruice, *J. Polymer. Sci.*, 1961, **49**, 100.

is of some importance, since a considerable number of naturally-occurring phosphate diesters have a neighbouring hydroxyl group on both ester functions.[11]

The *p*-nitrophenyl ester hydrolyses so rapidly that it was found necessary to carry out the rate measurements at room temperature in buffers at about pH 11. For a comparison with the other compounds in the Figure it was necessary to ascertain, in addition to the temperature and pH dependency of the rate, whether general base catalysis was involved. Under the conditions used none was observed, since the rate, measured at constant pH and ionic strength in piperidine–piperidine hydrochloride buffers 0·1 and 0·2M in base, was the same. The absence of general base catalysis would be consistent with an initial equilibrium, involving proton transfer,[12] as discussed in the preceding Paper,[1] though it is still possible that the use of a higher ratio of concentration of general base to hydroxide ion would allow the observation of general base catalysis. The breakdown of ribonucleic acids by ribonuclease is known to involve cyclic phosphate formation, and in these more rigid systems, where the neighbouring hydroxyl group is held in close proximity to the phosphate diester link, general base catalysis may again become important.[13] In the case of the *p*-nitrophenyl ester, formation of the cyclic phosphate was several hundred times faster than the subsequent cyclic phosphate ring opening.

The rate difference (about 10^6) between the fastest and the slowest hydrolysing ester is largely due to the difference in activation energy. The Arrhenius frequency factors, and energies of activation, for the formation of ROH in five of the 2-hydroxypropyl esters are given in Table 2. The corresponding values for the formation of epoxide plus alkyl phosphate for the methyl and cyclohexyl esters are given in parentheses.

TABLE 2

Ester	log pZ	E_a (kcal./mole)
p-Nitrophenyl	6·89 ± 0·3	10·5 ± 0·4
p-Chlorophenyl	8·15 ,,	14·2 ,,
Phenyl	7·36 ,,	13·7 ,,
Methyl	6·15 ± 0·7(8·76)	16·2 ± 1·2(22·2)
Cyclohexyl	6·20 ± 0·7(8·36)	20·1 ± 1·3(22·4)

The apparently anomalous behaviour of the *p*-chlorophenyl and phenyl esters, where the former hydrolyses the faster (at 25°) by virtue of the more favourable frequency term, has been noticed previously in the hydrolysis of some substituted phenyl acylates.[14] Indeed, as pointed out elsewhere,[4,14] the dissociation constants of the phenols seem to be governed more by entropy than by enthalpy differences, and it is thus not surprising that these differences are found in the rate activation parameters. As expected, formation of the epoxide three-membered ring has the higher frequency factor and activation energy (which is nearly the same for the methyl and cyclohexyl esters).

Although a comparison of the hydrolysis-rate activation parameters within this series of 2-hydroxypropyl phosphate diesters is of interest, direct comparison with other series of esters is not at present possible. At the concentration of alkali used here the observed rate consants are composite,[1] and thus the observed activation parameters are the sum of the true activation parameters for the displacement process and the enthalpy or entropy of ionisation of the neighbouring hydroxyl group. It is assumed that the latter terms will remain roughly constant for the members of the series here investigated, but their absolute magnitudes have not been determined.

EXPERIMENTAL

Paper Chromatography.—Details of the method of paper chromatography are given in the preceding Paper.[1] The following solvent systems were used: (A) propan-2-ol–ammonia

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[11] J. Baddiley, *J. Roy. Inst. Chem.*, 1962, 366; C. E. Ballou, E. Vilkas, and E. Lederer, *J. Biol. Chem.*, 1963, **238**, 69; J. LeCocq and C. E. Ballou, *Biochemistry*, 1964, **3**, 976.

[12] A. A. Frost and R. G. Pearson, " Kinetics and Mechanism," Wiley and Sons, New York, 2nd edn., 1961, p. 216.

[13] D. Findlay, D. G. Herries, A. P. Mathias, B. R. Rabin, and C. A. Ross, *Nature*, 1961, **190**, 791; C. A. Dekker, *Ann. Rev. Biochem.*, 1960, **29**, 454; J. P. Hummel and G. Kalnitzky, *ibid.*, 1964, **33**, 15.

[14] T. C. Bruice and S. J. Benkovic, *J. Amer. Chem. Soc.*, 1963, **85**, 1.

(d 0·880)–water (7 : 1 : 2 v/v); (B) propan-1-ol–ammonia (*d* 0·880)–water (10 : 1 : 1 v/v); (C) trichloroacetic acid–water–acetone–0·1M-Versene (aq) (6 g./25 ml. : 124 ml. : 1 ml.); (D) trichloroacetic acid–water–acetone–diethyl ketone–0·1M-Versene (5 g./20 ml. : 100 ml. : 2 ml.).

Monophosphate Esters.—Disodium *p*-nitrophenyl phosphate was the commercial product (British Drug Houses Ltd.), phenyl phosphate and *p*-chlorophenyl phosphate were prepared (by Drs. S. G. Warren and J. Flint) from the phenol and phosphoryl chloride, ethyl phosphate was prepared from ethyl bromide;[15] 2-hydroxyethyl phosphate was prepared from epoxyethane.[16]

The following monoesters were prepared from the alcohol and phosphoryl chloride: methyl phosphate;[17] the barium salt was purified by fractional precipitation from aqueous ethanol: 2-propyl phosphate[18] was purified in the same way (Found C, 12·2; H, 2·8. Calc. for $C_3H_7BaO_4P,H_2O$: C, 12·3; H, 3·1%): 2-methoxyethyl phosphate;[18] (Found C, 12·4; H, 2·9. Calc. for $C_3H_7BaO_5P$: C, 12·4; H, 2·4%): t-butyl phosphate; the dicyclohexylammonium salt was prepared by the method of Cramer *et al.*[19] (Found: C, 51·4; H, 10·4; N, 7·5. Calc. for $C_{16}H_{37}N_2O_4P,H_2O$: C, 51·8; H, 10·6; N, 7·6%). The n.m.r. spectrum of the lithium salt at 40 Mc./sec. showed a single peak, 3·38 p.p.m. up-field from the HOD peak.

2-Hydroxypropyl Phosphate Esters.—The diesters were prepared by the reaction of the ammonium salt of the alkyl or aryl phosphate with 1,2-epoxypropane in aqueous solution at about pH 8. The isomeric purity of the product (about 90%) was estimated from the n.m.r. spectrum in D_2O, except in the cases of the ethyl and isopropyl esters, where the useful part of the spectrum was obscured. The cyclohexyl ester was obtained isomerically pure as previously described.[1] Analytical data are given in Table 3. The preparation is illustrated for the *p*-nitrophenyl ester.

TABLE 3

		Found (%)		Calc. (%)	
2-Hydroxypropyl phosphate ester	Formula	C	H	C	H
p-Nitrophenyl	$C_9H_{11}Ba_{\frac{1}{2}}NO_7P$	31·3	3·0	31·3	3·2
p-Chlorophenyl	$C_9H_{11}Ba_{\frac{1}{2}}ClO_5P$	32·6	3·4	32·3	3·3
Phenyl	$C_9H_{12}Ba_{\frac{1}{2}}O_5P$	35·6	4·1	36·0	4·0
Methyl	$C_4H_{10}Ba_{\frac{1}{2}}O_5P$	20·4	4·4	20·2	4·2
2-Methoxyethyl	$C_6H_{14}Ba_{\frac{1}{2}}O_6P$	25·5	4·8	25·6	5·0
2-Hydroxyethyl	$C_5H_{12}Ba_{\frac{1}{2}}O_6P$	22·1	4·4	22·4	4·5
Ethyl	$C_5H_{12}Ba_{\frac{1}{2}}O_5P$	23·9	4·5	23·85	4·8
Isopropyl	$C_6H_{14}LiO_5P$	34·9	7·0	35·3	6·9
Cyclohexyl	$C_9H_{18}LiO_5P$	44·7	7·6	44·3	7·4

p-*Nitrophenyl 2-Hydroxypropyl Phosphate.* A solution of disodium *p*-nitrophenyl phosphate (1·32 g., 5 mmoles) in water (10 ml.) was passed down a column of IR-120 (H^+) resin, and the acid effluent brought to pH 8·0 with aqueous ammonia. 1,2-Epoxypropane (20 ml.) was added, and the solution kept at 35° for 40 hr. Unreacted expoxide was removed *in vacuo*, and the mixture passed down a column of IR-120 (H^+) resin. The solution was neutralised (pH no higher than 7) with barium hydroxide solution (carbonate-free) and concentrated to about 10 ml. *in vacuo* at room temperature. Ethanol (2 vol.) was added, and the precipitate of unchanged *p*-nitrophenyl phosphate was filtered off. The filtrate was concentrated to a small volume and added, with vigorous stirring, to 10% ethanol in acetone (300 ml.). The white precipitate of *barium* p-*nitrophenyl 2-hydroxypropyl phosphate* was filtered off. One similar precipitation gave the pure product (580 mg., 40%).

Hydrolysis Rates of 2-Hydroxypropyl Phosphate Esters.—The alkyl 2-hydroxypropyl phosphates were hydrolysed in aqueous sodium hydroxide in "Teflon" tubes[20] at 80° ± 0·1°. The rate constants were estimated by the chromatographic method described previously,[1] and are accurate to about ±5%.

In the case of the aryl esters, the rate of production of phenoxide ion was determined spectrophotometrically. The hydrolysis reaction mixture was kept in a thermostat (±0·1°) and

[15] A. Desjobert, *Bull. Soc. chim. France*, 1947, 809.

[16] F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J.*, 1945, 382.

[17] H. A. C. Montgomery and J. H. Turnbull, *J.*, 1958, 1963.

[18] W. W. Butcher and F. H. Westheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 2420.

[19] F. Cramer, W. Rittersdorf, and W. Böhm, *Annalen*, 1962, **654**, 180.

[20] J. Kumamoto, J. R. Cox, and F. H. Westheimer, *J. Amer. Chem. Soc.*, 1956, **78**, 4858.

aliquot samples were withdrawn at intervals. The optical density of the solution was measured using a Unicam S.P. 500 spectrophotometer. The delay between removing a sample and reading the optical density was <30 sec.; the error thereby introduced due to temperature change was found to be negligible.

The initial concentration of starting material (about 10^{-4}M) was calculated from the optical density of the solution after more than 10 half-lives and first-order rate constants were calculated from the gradient of the line obtained by plotting log (D_∞—D_t) *vs.* time. The reactions were followed for more than two half-lives. The rate constants are accurate to about $\pm 1\%$.

At the concentrations used, there was in all cases a negligible contribution by the starting material to the optical density of the solution, measured at the wavelength of maximum absorption of the phenoxide anion. It was found that Beer's Law held in the case of *p*-chlorophenol in 0·1N-sodium hydroxide over the range of concentration of interest, and it was assumed to hold for the other phenols also.

Details of Hydrolyses (Rate constants at 80° are collected in Table 1).—(*a*) *Methyl* 2-*hydroxypropyl phosphate.* The products of hydrolysis, methyl phosphate and 2-hydroxypropyl phosphate, were separated by descending front paper chromatography on Whatman No. 30 paper in solvent (B) for 7 days. (Distance moved by methyl phosphate = 1·5 in. and by 2-hydroxypropyl phosphate = 2·5 in.)

Rates and product ratios were run in N-sodium hydroxide at 60° and 80° and, ratios only, at 100°. The relative amounts of products at 100° (89·6% 2-hydroxypropyl phosphate; 10·4% methyl phosphate) agreed well with the amounts predicted by extrapolation from the first two temperatures (89·1% 2-hydroxypropyl phosphate; 10·9% methyl phosphate). The rate constants obtained at 60° were $31{\cdot}7 \times 10^{-6}$ sec.$^{-1}$ for the production of 2-hydroxypropyl phosphate, and $1{\cdot}46 \times 10^{-6}$ sec.$^{-1}$ for the production of methyl phosphate.

(*b*) 2-*Hydroxyethyl* 2-*hydroxpropyl phosphate.* Approximate product ratios were obtained by precipitating the two phosphate monoesters as the barium salts, and estimating the relative amounts by measuring peak areas of the n.m.r. spectrum. Accurate ratios were obtained by paper chromatography in solvent (C):

	N.m.r.	Chromatography
2-Hydroxyethyl phosphate (R_F 0·74)	30%	36·7%
2-Hydroxypropyl phosphate (R_F 0·80)	70	63·3

(*c*) 2-*Methoxyethyl* 2′-*hydroxypropyl phosphate.* The products of the reaction, 2-methoxyethyl phosphate and 2-hydroxypropyl phosphate, were separated in 20 hr. using solvent (D). Fifteen other solvent systems gave insufficient separation for a product-ratio determination.

(*d*) 2-*Hydroxypropyl isopropyl phosphate.* The hydrolysis products were separated by descending paper chromatography on Whatman No. 30 paper during 5 days using solvent (B) (distance moved by isopropyl phosphate, 2·5 in.; distance moved by 2-hydroxypropyl phosphate, 1·5 in.).

(*e*) *Ethyl* 2-*hydroxypropyl phosphate.* The products of hydrolysis were ethyl phosphate and 2-hydroxypropyl phosphate, R_F values 0·87 and 0·80 in solvent (C).

(*f*) *Cyclohexyl* 2-*hydroxypropyl phosphate.* Hydrolysis products were separated in solvent (A). Hydrolyses were also carried out under the following conditions:

		10^6 Rate (sec.$^{-1}$) of formation of:	
Temp. ($\pm$0·1°)	Normality of alkali	Cyclohexyl phosphate	Hydroxypropyl phosphate
100°	0·5 (μ = 1, KCl)	8·25	1·35
100	1·0	16·6	2·6

(*g*) 2-*Hydroxypropyl phenyl phosphate.* The analytical wavelength used was 287 mμ. Hydrolyses were carried out under the following conditions (concentration of phosphate ester 2×10^{-4}M):

Temp. ($\pm$0·1°)	Normality of NaOH	Ionic strength	10^4k (sec.$^{-1}$)
24·6°	0·10	0·10	0·98
24·6	0·20	0·20	2·16
24·4	0·10	1·00 *	1·93
24·4	0·20	1·00 *	3·78
39·6	0·10	1·00 *	5·96

* With added NaCl.

(*h*) p-*Chlorophenyl 2-hydroxypropyl phosphate.* The analytical wavelength used was 298 mμ. Hydrolyses were carried out under the following conditions (concentration of phosphate ester 2×10^{-4}M):

Temp. (±0·1°)	Normality of NaOH	Ionic strength (with added NaCl)	$10^4 k$ (sec.$^{-1}$)
25·2°	0·05	1·0	2·98
34·9	0·05	1·0	6·35

(*i*) 2-*Hydroxypropyl* p-*nitrophenyl phosphate.* The analytical wavelength used was 402 mμ. Hydrolyses were carried out in piperidine–piperidine hydrochloride buffers containing potassium chloride (μ = 1·0). The buffers had negligible absorption at the analytical wavelength. The pH of the buffers was measured before and after each run with an E.I.L. 33B/c33B meter (Electronic Instruments Ltd., Richmond, Surrey, England), using a G.H.S. 23 glass electrode which was standardised at each temperature in 0·05M-phthalate and borate buffers.[21] Repeat determinations on the same buffer differed by less than 0·01 pH units. Errors due to specific salt effects, and the permeability of the glass electrode to potassium ions, have been neglected.

First-order rate constants were measured under the following conditions (concentration of phosphate ester 5×10^{-5}M):

Temp. (±0·1°)	Piperidine : piperidine HCl	Piperidine (M)	pH	$10^4 k$ (sec.$^{-1}$)
25°	1 : 1	0·1	11·37	3·94
25	1 : 1	0·2	11·37	4·09
25	3 : 1	0·3	11·82	9·93
35	1 : 1	0·1	11·09	7·65

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[21] A. Albert and E. P. Serjeant, " Ionization Constants of Acids and Bases," Methuen and Co. Ltd., London, 1962, p. 172.