80. *Kinetic and Tracer Studies of the Hydrolysis of Phosphourethane.*

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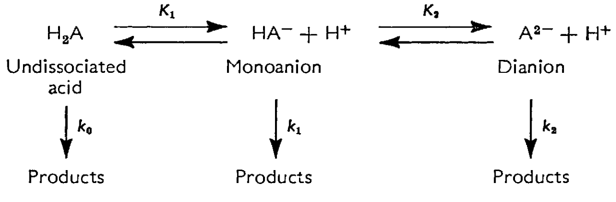
The hydrolysis of phosphourethane in the pH range 1—6 is due mainly to reaction of the monoanion. The effect of temperature was determined. Various nucleophilic and electrophilic reagents did not affect the rate of hydrolysis. In aqueous dioxan hydrolysis was faster than in water. No oxygen exchange occurred between water and the phosphoryl group of phosphourethane during hydrolysis. The rate of solvolysis in D_2O was similar to that in water.

Phosphourethane, $CO_2Et{\cdot}NH{\cdot}PO(OH)_2$, has been synthesised.[1] Its rate of hydrolysis shows a pronounced maximum at pH 4, at which practically all the phosphourethane is in the monoionised form. In acid and in basic solutions, the rate decreases rapidly. The rate of hydrolysis of aromatic phosphoramidates [2] in aqueous dioxan shows a similar maximum at about pH 5—6, and a strong decrease in rate at a higher pH. In strongly acid solution, acid catalysis was reported, and there was a slight minimum at pH 2. The kinetics of hydrolysis of phosphoric esters shows similar features.[3,4]

It has been suggested [1-4] that the pH dependence of rate of hydrolysis of monoalkyl phosphates and of phosphoramidates, outside the highly acid range, can be explained as a unimolecular decomposition of the monoanion in the rate-determining step. It was possible to calculate [4,5] the expected rate at various pH, since the concentration of the monoanion can be derived from the experimentally observed dissociation constants of phosphourethane. The following equilibria and reactions are assumed to occur:



The equilibrium constants are $K_1 = [HA^-][H^+]/[H_2A]$ and $K_2 = [A^{2-}][H^+]/[HA^-]$. The total stoicheiometric molar concentration of phosphourethane at any moment is

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[1] Lapidot and Halmann, *J.*, 1958, 1713.

[2] Chanley and Feageson, *J. Amer. Chem. Soc.*, 1958, **80**, 2686.

[3] Vernon, *Chem. Soc. Spec. Publ.* No. 8, 1957, p. 17 giving previous references; Herr and Koshland, *Biochim. Biophys. Acta*, 1957, **25**, 219.

[4] Bunton, Llewellyn, Oldham, and Vernon, *J.*, 1958, 3574, 3588.

[5] Chanley, Gindler, and Sobotka, *J. Amer. Chem. Soc.*, 1952, **74**, 4347.

$C = [H_2A] + [HA^-] + [A^{2-}]$. From these three equations, the concentrations of the undissociated phosphourethane and of the mono- and of the di-anion can be calculated: $[H_2A] = [H^+]^2C/\{K_1(K_2 + [H^+] + [H^+]^2/K_1)\}$; the mole fraction of H_2A is $[H_2A]/C = [H^+]^2/K_1F$ where $F = (K_2 + [H^+] + [H^+]^2/K_1)$. Similarly, $[HA^-]/C = (H^+)/F$ and $[A^{2-}]/C = K_2/F$.

In Table 1 are given the concentrations of these three species in mole fractions, *e.g.*, $[HA^-]/C$, as well as the individual first-order rate constants of the three species. The sum of the calculated rate constants, $k_{calc.} = k_0 + k_1 + k_2$, is compared with the observed value $k_{obs.}$. The equilibrium constants are[1] $K_1 = 2·17 \times 10^{-3}$ and $K_2 = 5·01 \times 10^{-7}$ at 29°.

TABLE 1. *Partial and overall rate constants for hydrolysis of phosphourethane at* 37·0°.

pH	F	$[H_2A]/C$	$[HA^-]/C$	$[A^{2-}]/C$	k_0	k_1	k_2	$10^4k_{calc.}$ (sec.$^{-1}$)	$10^4k_{obs.}$ (sec.$^{-1}$)
0·96	5·66	0·981	0·019	—	0·825				0·845
1·96	$6·6 \times 10^{-2}$	0·833	0·167	—				1·05	1·28
3·58	$29·5 \times 10^{-5}$	0·108	0·892	—				1·98	1·98
4·02	$10·02 \times 10^{-5}$	0·042	0·953	0·005				2·05	2·02
4·67	$2·30 \times 10^{-5}$	—	0·975	0·025		2·12			2·07
5·7	$2·50 \times 10^{-6}$	—	0·800	0·200				1·69	1·57
6·15	$1·21 \times 10^{-6}$	—	0·585	0·415				1·18	1·16
7·5	$5·42 \times 10^{-7}$	—	0·060	0·94				0·015	0·03
9·0	$5·02 \times 10^{-7}$			1·00			0·0024		0·0024

The partial rate constants were derived as follows: At pH 9·0 practically all the phosphourethane is doubly ionised, and the observed rate is thus equal to k_2. At pH 4·67, 97·5% of the substrate appears as the monoanion, while 2·5% is doubly ionised. From the equation $k_{obs.} = k_1[HA^-]/C + k_2[A^{2-}]/C$, k_1 is calculated. This value being used, the measurement at pH 0·96 is used to derive k_0, since in this case $k_{obs.} = k_0[H_2A]/C + k_1[HA^-]/C$. A check on the partial rate constants is made by calculating the total rate constants at other values of pH, and comparing them with the observed rates, as shown in the last column of Table 1.

The temperature-dependence of the hydrolysis of phosphourethane was determined at several values of pH. Results are given in Table 2.

TABLE 2. *Hydrolysis of phosphourethane at several temperatures. First-order rate constants* $\times 10^4$ (*sec.*$^{-1}$).

Temp.	HCl 0·100N pH 1·0	K H tartrate 0·03M pH 3·58	K H phthalate 0·05M pH 4·02	Acetate 0·2M pH 5·7
26·7°	0·193	0·403	0·435	0·326
37·0	0·843	1·98	2·02	1·53
51·4	5·4	12·4	14·5	10·5

The partial rate constants were similarly determined at other temperatures. Results are given in Table 3, together with heats and entropies of activation.

TABLE 3.

	Temp. 26·7°	37·0°	51·4°	ΔE^* (kcal. mole^{-1})	ΔS^* (e.u.)
k_0	0·189	0·825	5·4	26·4	+7·7
k_1	0·443	2·12	15·0	27·8	+14·4

For the hydrolysis of alkyl phosphates, somewhat higher activation energies of around 30 kcal. mole^{-1} have been reported;[3] for aromatic phosphoramidates[2] the activation energy was about 20 kcal. mole^{-1}.

In the hydrolysis of triphenylmethyl chloride, it was shown that the reaction proceeds by two stages—a rate-determining unimolecular heterolysis of the carbon–chlorine bond, and a fast combination of the intermediate carbonium ion with some nucleophilic reagent such as water or some ions in solution. Several ions, *e.g.*, azide, were very effective in competing [5] with water. Upon change of the ratio of the nucleophilic reagents in solution, the ratio of products was changed, but there was no appreciable influence on the rate-determining step.

We attempted to find out if addition of various reagents would affect the rate or products ratio in the hydrolysis of phosphourethane in water (W) or water–dioxan (4 : 1) (WD). Results are summarised in Table 4.

TABLE 4. *Effect of various reagents on the solvolysis of phosphourethane.*

Temp.	Phosphourethane (mM)	Solvent	pH	Reagent (mM)	10^4k_1 (sec.$^{-1}$
51·4°	9	W	4·0	—	14·7
51·4	9	W	4·0	Urethane (25)	14·3
37·0	9	W	4·0	—	2·0
36·6	5·5	W	4·0	NaF (25)	1·6
37·0	5·5	WD	4·0	—	1·8
37·0	4·5	WD	4·0	Bu^n_2NH (13·7)	1·75
37·0	5·5	WD	4·0	Bu^nSH (5)	—
37·0	3·7	W	4·0	$AgNO_3$ (10)	1·7
37·0	8	W	4·0	$CuSO_4$ (8)	1·87
37·0	6	W	4·0	$La(NO_3)_3$ (10)	0·6 to 0·8
37·0	6	W	4·0	$Ce(NO_3)_3$ (8)	0·6
37·0	6	W	2	$Ce(NO_3)_3$ (8)	1·0

Since strictly first-order kinetics were always observed in the hydrolysis of phosphourethane, the reaction products do not affect the rate. This was further shown in a run in the presence of added urethane (Table 4). Thus if the hydrolysis were a two-stage reaction, the second stage would not be appreciably reversible. There is no analogy to the " mass law effect " in the hydrolysis of triphenylmethyl chloride.[6] Sodium fluoride, di-n-butylamine and butane-1-thiol were investigated as nucleophilic reagents. Neither was consumed in competition with water nor did they change the rate of hydrolysis. It is thus possible to conclude that the hydrolysis of phosphourethane is *not* analogous to the S_N2 reactions of primary alkyl halides and of organic phosphorus chlorides,[7] the rates of which are strongly susceptible to nucleophilic reagents, or to the S_N1 reactions of triarylmethyl chlorides, in which such reagents are able to compete with water.

The reactions with silver and copper salts were carried out to test for susceptibility of the hydrolysis of phosphourethane to electrophilic attack. No effect was observed. At pH 2, cerium nitrate had no effect on the rate of hydrolysis. At pH 4, insoluble salts of phosphourethane seemed to be formed with cerium and lanthanum salts.

Anilinium Salt of Phosphourethane.—In an attempt to find other solvolytic decompositions of phosphourethane, in addition to hydrolysis, the reaction with aniline was investigated. On addition of aniline to an ethanolic solution of phosphourethane, a white crystalline precipitate was formed immediately. This was shown to be the anilinium salt, $(EtO_2C{\cdot}NH{\cdot}PO_3H)^-(PhNH_3)^+$. No aminolysis of phosphourethane had taken place under the conditions used.

Hydrolysis in Aqueous Dioxan.—The pH dependence of the rate of hydrolysis of phosphourethane in aqueous dioxan (1 : 1 v/v) shows the same general pattern as in water (see Table 5). However, two maxima are observed, in buffer solution of potassium hydrogen phthalate with hydrochloric acid (measured pH: 4·7) and with sodium hydroxide (measured pH: 7·1), while there was a minimum between them in potassium hydrogen phthalate alone (measured pH: 5·0). The rate constants at the two maxima ($2{\cdot}35 \times 10^{-4}$ sec.$^{-1}$ at 36·7°) are larger than that in pure water at the maximum ($2{\cdot}07 \times 10^{-4}$ sec.$^{-1}$ at pH 4·67). No similar behaviour had been observed in the hydrolysis of aromatic phosphoramidates.[2] The maximum in the presence of hydrochloric acid seems to be due to catalysis by chloride ion; in the presence of sodium chloride a marked increase in rate was observed.

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[6] Swain, Scott, and Lohmann, *J. Amer. Chem. Soc.*, 1953, **75**, 136.

[7] Dostrovsky and Halmann, *J.*, 1953, 502, 516.

This is not a general salt effect, because added sodium perchlorate had no influence on the rate.

Test for Oxygen Exchange during Hydrolysis.—Phosphourethane was hydrolysed in ^{18}O-enriched water. After being boiled at 100° for 15 min., the phosphoric acid was neutralized with potassium hydroxide. Potassium dihydrogen phosphate was precipitated by addition of alcohol. Its ^{18}O content was determined by oxygen equilibration with carbon dioxide.[4] The following results were obtained:

TABLE 5. *Hydrolysis of phosphourethane in aqueous dioxan* (1 : 1) *at* 36·7°.

Buffer soln. (aqueous part)		pH of aq. part	pH of aq. dioxan	10^4k_1 (sec.$^{-1}$)	No. of runs
HCl	0·1N	1	1·3	0·25	2
HCl	0·01N	2	2·2	0·55	3
KHP * +HCl	0·05M	3·42	4·7	2·35	3
KHP * + $NaClO_4$	0·05M 0·1M	4·02	5·2	1·82	2
KHP *		4·02	5	1·75	5
KHP * + (0·1M) NaCl		4·02	5·5	2·8	2
KHP * + NaOH		4·7	7·1	2·35	3
KHP * + NaOH		6·15	8·5	1·1	2
Sodium veronal * + HCl		7·8	9·4	0·1	1
$Na_2B_4O_7$ + HCl		8·13	9·3	0·08	1

* KHP = potassium hydrogen phthalate mixtures with acid or base, according to W. M. Clark. Sodium veronal + HCl buffer solution according to L. Michaelis.

Phosphourethane (mM)	36	52	*B*, $H_2PO_4^-$ (atoms % excess of ^{18}O)	0·88	0·88
A, Water (atoms % excess of ^{18}O)	3·53	3·55	*B*/*A*	0·249	0·248

Thus within experimental error just one of the four oxygen atoms of phosphoric acid was derived from the water, and no measurable oxygen exchange with the phosphoryl group of phosphourethane took place during hydrolysis. The extent of oxygen exchange of phosphate ions is negligible under the conditions of the hydrolytic experiments.[8]

The hydrolyses of the monoanions of methyl[4] and phenyl[9] dihydrogen phosphates have similarly been shown to occur without appreciable oxygen exchange between the phosphoryl group and the solvent.

Effect of Deuterium.—The rate of solvolysis of phosphourethane in deuterium oxide was found to be equal to that in water. The following results were obtained at 37·0°:

	H_2O		D_2O	
Solvent	pH	10^4k_1 (sec.$^{-1}$)	pH	10^4k_1 (sec.$^{-1}$)
Potassium hydrogen phthalate	4·0	2·02	4	2·12
Hydrogen chloride	1·05	0·75	0·79	0·71

DISCUSSION

The following mechanisms can be postulated to account for the kinetic features of the hydrolysis of phosphourethane.

(*a*) One stage displacement (S_N2):

$$CO_2Et{\cdot}NH{\cdot}PO(OH){\cdot}O^- + H_2O \longrightarrow CO_2Et{\cdot}NH^- + H_3PO_4$$

(*b*) Reversible intermediate formation with quinquecovalent phosphorus:[3]

$$CO_2Et{\cdot}NH{\cdot}PO(OH)_2 + H_2O \overset{\text{Fast}}{\rightleftharpoons} CO_2Et{\cdot}NH{\cdot}P(OH)_4 \xrightarrow{\text{Slow}} CO_2Et{\cdot}NH_2 + H_3PO_4$$

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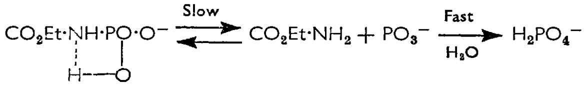
[8] Winter and Briscoe, *J.*, 1942, 631.

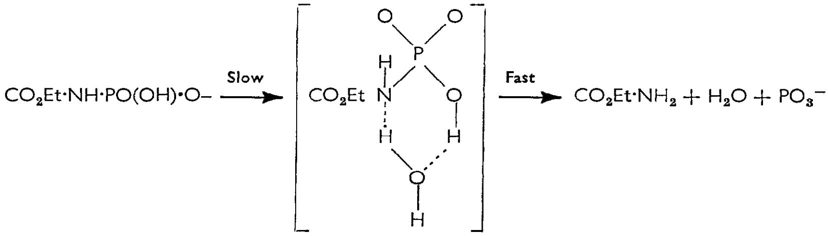
[9] Stein and Koshland, *Arch. Biochem. Biophys.*, 1952, **39**, 229.

(*c*) Unimolecular heterolysis:[3]



$$CO_2Et\cdot NH\cdot PO\cdot O^- \overset{Slow}{\rightleftharpoons} CO_2Et\cdot NH_2 + PO_3^- \xrightarrow[H_2O]{Fast} H_2PO_4^-$$

(*d*) Formation of a cyclic intermediate involving phosphorus and nitrogen:



The experimental results enabled elimination of some of the proposed mechanisms.

Mechanism (*a*), a one-stage nucleophilic displacement by water, cannot be excluded completely, but it seems unlikely because of the lack of effect of other nucleophilic reagents on the rate (Table 4).

Mechanism (*b*), fast reversible addition of water to the phosphoryl group, seems excluded because of the absence of oxygen exchange during hydrolysis. A variant of mechanism (*b*) is slow reversible formation of the same intermediate, which breaks up rapidly to the products. This is experimentally indistinguishable from mechanism (*a*).

Mechanism (*c*), with the first step reversible (analogous to the S_N1 reactions of alkyl halides), seems excluded by the observation that addition of urethane did not decrease the rate of hydrolysis of phosphourethane, nor was there any uptake of the nucleophilic reagents fluoride, di-n-butylamine or butane-1-thiol (Table 4). There was also no effect of such electrophilic reagents as silver or copper salts, which may have assisted a rate-determining liberation of the PO_3^- group. This seems to disfavour an irreversible first step. The rates of hydrolysis in H_2O and in D_2O were equal. A rate-determining hydrogen transfer from oxygen to nitrogen would probably be subject to an appreciable isotope effect and can thus be excluded.

Mechanism (*d*) involving a six-membered cyclic intermediate has been suggested to account for the kinetics of hydrolysis of monoalkyl phosphates.[3,10]

This mechanism seems to account for the slight difference in rate of hydrolysis of the monoanion of phosphourethane in aqueous dioxan compared with that in water (Table 5). Thus, the transition state seems to be less polar than the initial state of the reactants.[11] This is possible in formation of a cyclic intermediate, in which the negative charge would be distributed over several oxygen atoms.

EXPERIMENTAL

The preparation of urethane, and methods of following its hydrolytic decomposition have been described.[1]

^{18}O-Enriched water was obtained from the fractionating plant of this Institute. Deuterium oxide (Norsk Hydro-elektrisk Kvaelstofaktieselskab, 99·6% D) buffer solutions were prepared by dissolving anhydrous potassium hydrogen phthalate (48 mg.) or concentrated hydrochloric acid (0·05 ml.) in 5 ml. samples of D_2O.

^{18}O in orthophosphate [4] and in water [12] was analysed by equilibration with carbon dioxide, and determination of the ratio of masses 46 to 44, by use of a Consolidated Engineering Corporation Model 21—401 mass-spectrometer.

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[10] Butcher and Westheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 2423.

[11] Ingold, "Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, 1953, p. 346.

[12] Halmann, *J.*, 1959, 305.

Anilinium Hydrogen Phosphourethane.—Aniline (10 mmoles) was added to a solution of phosphourethane (2·1 mmoles) in dry ethanol (20 ml.). A white precipitate formed instantly; it was centrifuged off, washed several times with ethanol, and recrystallised from hot ethanol. The *phosphourethane* had m. p. 160—162° [Found: C, 41·6; H, 5·6; N, 10·7; P, 11·8%; equiv. potentiometrically with NaOH (infl. at pH 9), 256. $C_9H_{15}O_5N_2P$ requires C, 41·2; H, 5·7; N, 10·7; P, 11·8%; equiv. 262].

Hydrolysis of Phosphourethane in the Presence of Various Reagents.—(a) *Sodium fluoride.* The concentration of fluoride ion was determined by titration with 0·01N-thorium nitrate with sodium alizarinsulphonate as indicator. No decrease in the fluoride titre occurred during hydrolysis.

(b) *Silver nitrate.* The presence of silver nitrate interfered with the normal colorimetric phosphate determination. The silver ion was therefore precipitated by addition of a small excess of sodium chloride. In the filtrate phosphate could be assayed as usual.

(c) *n-Butane-*1*-thiol.* At various intervals aliquot portions of the solution were titrated with a standard solution of iodine in potassium iodide. The titre of the thiol solution did not change during a time sufficient to cause complete hydrolysis of phosphourethane. No direct determination of the rate of hydrolysis was carried out in the presence of the thiol.

(d) *Di-n-butylamine.* After completion of hydrolysis the amine (initially 20 mM) was recovered from the reaction mixture by addition of sodium hydroxide and extraction with ether. Its concentration in ether was determined by titration with aqueous perchloric acid (Found: 20 mM).

(e) Lanthanum nitrate and cerium nitrate. Owing to formation of precipitates in solutions of phosphourethane at pH 4·0 (phthalate buffer) in the presence of these salts, the procedure for kinetic runs was modified by placing aliquot parts of the reaction mixtures in separate flasks which were kept in thermostats as usually. At pH 2, in the presence of cerium nitrate, the reaction mixture remained clear.

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