242. *The Mechanism of Hydrolysis of Phosphoramidic Acid.*

By M. HALMANN, A. LAPIDOT, and DAVID SAMUEL.

The rate of hydrolysis of phosphoramidic acid was measured at pH's from 0·09N-sodium hydroxide to 1·5N-perchloric acid by following the formation of ammonia. Phosphoramidic acid is not hydrolysed in basic solution. From pH 7 downwards the rate rises steadily and, in strongly acid solution, is proportional to the Hammett acidity function (H_0) with a slope of 1·0. The acid-catalysed reaction is faster in deuterium oxide than in water. The rate of hydrolysis at pH 4 is unchanged in deuterium oxide but is increased by added fluoride ion. The results indicate that both the neutral molecule (which probably exists as a zwitterion) and the conjugate acid, are hydrolysed by a unimolecular mechanism to form a transient metaphosphate intermediate, whereas it seems that reaction of the monoanion involves a bimolecular mechanism. The effect of substitution on nitrogen on the kinetics of hydrolysis of phosphoramidic acids is discussed.

N-SUBSTITUTED phosphoramidic acids $R{\cdot}NH{\cdot}PO_3H_2$ are hydrolysed in aqueous solution to the corresponding amide or amine $R{\cdot}NH_2$ and phosphoric acid. The rate of this reaction for various *N*-acyl-[1-3] and *N*-aryl-phosphoramidic acids[4] has been measured under different conditions of acidity. The hydrolysis of *N*-benzoyl- and *N*-aryl-phosphoramidic acids [1,4] was found to be acid-catalysed, the increase in rate being proportional to the stoicheiometric concentration of acid. This susceptibility to protonation in acid solution was accompanied by an increase in rate when deuterium oxide was used in place

[1] Halmann, Lapidot, and Samuel, *J.*, 1960, 4672.
[2] Halmann, Lapidot, and Samuel, *J.*, 1961, 3158.
[3] Halmann and Lapidot, *J.*, 1960, 419.
[4] Chanley and Feageson, *J. Amer. Chem. Soc.*, 1958, **80**, 2686.

of water as solvent. On the other hand, phosphourethane [3] $EtO_2C{\cdot}NH{\cdot}PO_3H_2$, *N*-diphenoxyphosphinylphosphoramidic acid $(PhO)_2PO{\cdot}NH{\cdot}PO_3H_2$ and *N*-benzenesulphonylphosphoramidic acid [2] were not susceptible to acid-catalysis.

We have now extended this study to the parent acid of this series, phosphoramidic acid, by measuring the rate of formation of ammonia under a wide range of conditions. The rate of hydrolysis of this compound has been only cursorily examined before, at low acidities,[5,6] being stated to be very fast in concentrated acid.

Phosphoramidic acid resists hydrolysis in the alkaline range. No reaction occurs in 14 days in 0·088N-sodium hydroxide at room temperature, and Rathler and Rosenberg [6] state that even in 0·5N-alkali at 100° only 1% is hydrolysed per hour. These results seem to indicate that the dianion of phosphoramidic acid, which is formed at this pH, is not readily attacked by either water or hydroxide ion. At higher acidities, from pH 7 to nearly 2N-perchloric acid, the rate of hydrolysis follows strict first-order kinetics and rises steadily with increasing acidity (Table 1; cf. Table 3). The rates are in good agreement

TABLE 1.

Solvolysis of phosphoramidic acid.

Temp.	pH	Buffer *	10^4k_{obs} (sec.$^{-1}$)
Room temp.	0·088M-NaOH	—	0 in 14 days
10°	7·4	0·05M-DB	0·0155
10	4·0	0·05M-KHP	0·0198
37	4·0	0·05M-KHP	0·835
37	4·0(D_2O)	0·05M-KHP	0·83
37	2·8	0·01M-HCl, 0·09M-KCl	1·22

* DB = diethylbarbiturate; KHP = K H phthalate.

with those obtained by Rathler and Rosenberg [6] at pH 7·2 and in the range pH 4—6·5 at 25°.

Reaction of the Monoanion and the Neutral Species.—The dissociation constants of phosphoramidic acid at 10° are $K_1 = 2{\cdot}5 \times 10^{-5}$ and $K_2 = 2{\cdot}0 \times 10^{-8}$, corresponding to $pK_1 = 4{\cdot}6$ and $pK_2 = 7{\cdot}7$ which are somewhat higher than the p*K*'s measured [1-4] for other derivatives of phosphoric acid. Rathler and Rosenberg [6] give $pK_2 = 8{\cdot}2$ at 25°.

At pH 4 the reacting species is predominantly the monoanion which is not affected by changing the solvent from water to deuterium oxide (see Table 1) but is susceptible to added fluoride ion, as is shown in Table 2. At pH 4 the predominant species is the

TABLE 2.

Effect of added fluoride ions on rate of hydrolysis of phosphoramidic acid at pH 4 (0·25M-phthalate buffer) at 37·0.

[KF] (M)	0	0·1	0·2	1·0
10^4k (sec.$^{-1}$)	0·8	0·8	1·53	3·3

monoanion ion which appears to be sensitive to added nucleophilic reagents such as fluoride. This may indicate the possibility of nucleophilic attack on phosphorus of either a water molecule or a fluoride ion, or may be due to a salt effect—it is difficult to distinguish between these effects on the basis of the kinetics. Nevertheless a general salt effect is excluded by the observation that the rate of hydrolysis in 0·05M- and 0·25M-phthalate is the same (Tables 1 and 2). However, unlike the situation with most phosphoramidic acid derivatives [1-3] and many esters of phosphoric acid,[7] the rate of hydrolysis of phosphoramidic acid itself does not appear to go through a maximum at pH 4 (see Tables 1 and 3).

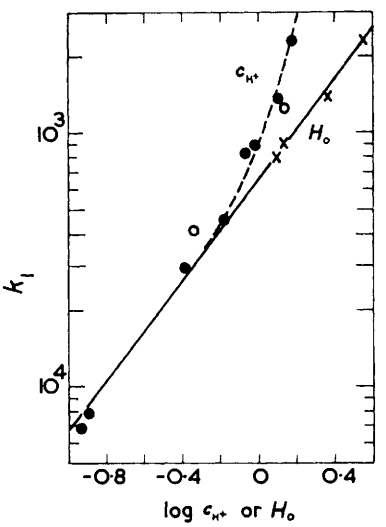
[5] Moller, *Biochim. Biophys. Acta*, 1955, 162.
[6] Rathler and Rosenberg, *Arch. Biochem. Biophys.*, 1956, **65**, 319.
[7] Westheimer, *Chem. Soc. Special Publ. No.* 8, 1957, p. 1; Vernon, *ibid.*, p. 17.

The steady rise in rate with increasing acidity suggests that the neutral species of phosphoramidic acid is more reactive than the monoanion. This situation has also been found in the hydrolysis of α-D-glucose 1-(dihydrogen phosphate)[8] and *N*-arylphosphoramidic acids.[4] The difference in the dependence of the rate may be due to the fact that the neutral species can exist as a zwitterion (I) in solution. In the solid state, the sodium hydrogen phosphoramidate has been found to have a short P–N bond,[9] which was considered to be due to such a dipolar structure. In aqueous solutions this zwitterion ion may break down by forming a kinetic intermediate (II) (in a unimolecular step) which then rapidly reacts with water to give the products:

$$H_2N{\cdot}PO_3H_2 \rightleftharpoons {}^{+}H_3N{\cdot}P(O^{-})O_2H\ (I) \xrightarrow{\text{Slow}} H_3N + PO_2{\cdot}OH\ (II) \xrightarrow{\text{Fast}} \text{Products}$$

The intermediate (II) here is the metaphosphate entity often suggested[7] as occurring in the hydrolysis of derivatives of phosphoric acid. A very similar mechanism was recently suggested[10] for the hydrolysis of sulphamic acid, which is also considered to have a zwitterionic structure in aqueous solution.

The Acid-catalysed Reaction.—In the range 0·11—1·56M-perchloric acid the rate of hydrolysis of phosphoramidic acid increases rapidly with acidity; it is proportional to the Hammett acidity function (H_0) with a slope of 1·0 (see Figure and Table 3). The plot of



Dependence of rate of hydrolysis of phosphoramidic acid on the Hammett acidity function (H_0) and on acid concentration (log c_{H^+}). Open circles indicate rate in D_2O.

TABLE 3.

Solvolysis of phosphoramidic acid in acid solution at 10·0° at constant ionic strength (μ = 1·5 except where indicated).

[Acid] (M) ...	0·107	0·420	0·445 *	0·680	0·835 †	0·895	0·935 *	1·27	1·56
10^4k (sec.$^{-1}$) ...	0·69	2·92	4·17	4·62	8·15	9·05	12·7	14·1	23·5

* D_2O. † μ = 3.

log k against the stoicheiometric concentration of acid is markedly curved. The energy and entropy of activation for reaction in 0·127M-perchloric acid were calculated from the temperature-dependence of the rate of hydrolysis shown in Table 4. Long and Paul[11]

[8] Bunton, Llewellyn, Oldham, and Vernon, *J.*, 1958, 3588.
[9] Hobbs, Corbridge, and Raistrick, *Acta Cryst.*, 1953, **6**, 621.
[10] Candlin and Wilkins, *J.*, 1960, 4236.
[11] Long and Paul, *Chem. Rev.*, 1957, **57**, 935.

TABLE 4.

Hydrolysis of phosphoramidic acid in 0·217M-$HClO_4$ at various temperatures.

Temp.	10^4k (sec.$^{-1}$)	ΔE (kcal. mole^{-1})	ΔS (e.u.)
10°	0·79		
25	3·28	16·8	−14·06
35	8·35		

suggested, on the basis of the steep rise in the rate of hydrolysis of the dimethylphosphoramides [12] with increasing acidity, that the reaction of these compounds may follow the H_0 function. For unsubstituted phosphoramidic acid this is now confirmed. It should be noted that the increase in the rate of the acid-catalysed reaction of almost all *N*-substituted phosphoramidic acids and of phosphoric esters has been found to be proportional to the stoicheiometric concentration of acid (c_{H^+}).

At the same time, changing the solvent to deuterium oxide markedly increases the rate of hydrolysis, (k_D/k_H ~1·4) (see Table 3 and the Figure).

The effect of increasing acidity, as well as the deuterium solvent isotope effect, indicate that the conjugate acid of phosphoramidic present in equilibrium concentration undergoes a covalency change in the rate-determining step of the hydrolytic reaction without intervention of a solvent molecule:

$$H_2N{\cdot}PO_3H_2 + H_3O^+ \rightleftharpoons H_2O + {}^+H_3N{\cdot}PO_3H_2 \xrightarrow{\text{Slow}} H_4N^+ + [PO_3H] \xrightarrow[H_2O]{\text{Fast}} \text{Products}$$

Although there has been increasing doubt recently about the diagnostic value of the Zucker–Hammett hypothesis, we believe that in this case the results provide concrete evidence for a unimolecular mechanism. A similar interpretation [8] was given in the case of α-D-glucose 1-(dihydrogen phosphate) in which the rate of the acid-catalysed hydrolysis was also found to depend on the Hammett acidity function and to be markedly affected by changing to deuterium oxide as solvent.

Further evidence for the formation of a metaphosphate intermediate is the fact that significant quantities of pyrophosphate have been isolated [13] after hydrolysis of concentrated solutions of phosphoramidic acid in acid. In an attempt to trap this intermediate, the acid-catalysed reaction (at pH 1) was run in the presence of 2M-fluoride ion. After one hour, nearly 70% of fluorophosphate was formed. However, this product may be due to the direct attack of fluoride or of hydrofluoric acid on the amidophosphoric acid molecule, as was found at pH 4, and a bimolecular mechanism for the formation of fluorophosphoric acid in acid medium cannot be excluded. The nucleophilic attack of fluoride on phosphorus is well known (cf. the faster hydrolysis of acetyl phosphate in the presence of fluoride).

Effect of Substitution on Nitrogen on the Mechanism of Reaction.—The present evidence suggests that hydrolysis of phosphoramidic acid involves unimolecular heterolysis of the P–N bond of the conjugate acid in the strongly acid region. In the range pH 1—7 the neutral molecule, which probably exists as a zwitterion, also appears to react by a unimolecular mechanism, whereas the monoanion is hydrolysed by a slow bimolecular reaction.

For *N*-arylphosphoramidic acids the tendency to zwitterion formation is somewhat reduced by the aromatic ring, so that a more pronounced " plateau " is found between pH 4 and 6 for reaction in water, this being transformed into a maximum when the dielectric constant is reduced by adding an equal volume of dioxan.[4]

In *N*-benzoylphosphoramidic acid,[1] the acyl group reduces the possibility of protonation on nitrogen still further, so that only in very strongly acid solution is the reaction acid-catalysed and then as a function of the stoicheiometric acid concentration. Finally, when all the basic properties of the amido-group are suppressed by replacement of hydrogen by

[12] Heath and Casapieri, *Trans. Faraday Soc.*, 1951, **47**, 1093.
[13] Quimby, Narath, and Lohman, *J. Amer. Chem. Soc.*, 1960, **82**, 1099.

such polar groups as ethoxycarbonyl,[3] diphenoxyphosphinyl, or benzenesulphonyl,[2] no catalysis by acid is observed, in the case of the last compound even in acidities of up to 8M-perchloric acid. At the other end of the scale, one may predict that *NN*-dimethylphosphoramidic acid is more sensitive to protonation than the parent acid and could be usefully applied to phosphorylation. Todd [14] has recently discussed sensitivity to protonation of monoesters of phosphoramidic acid in connexion with the different products formed on changing either the substituent on nitrogen or the solvent in phosphorylation.

Experimental.—Phosphoramidic acid was prepared by hydrolysis [15] of the diphenyl ester (Found: N, 14·5; P, 31·6. Calc. for H_4NO_3P: N, 14·4; P, 32%). The acid dissociation constants were determined at 10° in order to reduce decomposition by hydrolysis during measurement. The potentiometric method described previously was used.[1-3]

Kinetic experiments. The products of total hydrolysis were ammonia, determined by Nessler's reagent, and orthophosphate, measured as the phosphomolybdate complex.[16] In the dilute solutions used, no pyrophosphate was detected, as shown by the equivalence of yields of orthophosphate and ammonia. The rate of hydrolysis was followed in aqueous acid, buffer or fluoride solutions by withdrawing aliquot parts and determining the ammonia as above. The rate of formation of phosphate could not be measured, as the breakdown of phosphoramidic acid is very fast in acid and is catalysed by molybdate.[6] Rate constants were calculated for first-order reactions. Runs at higher acidities (0·1—1·56M-$HClO_4$) were made at constant ionic strength (μ = 1·5 or 3·0; see Table 3) by adding potassium perchlorate. Perchlorate ion was found to have no effect on the rate. Runs in deuterium oxide were carried out in solutions made up from D_2O (99·8%) and 70% perchloric acid. The amount of fluorophosphate formed was determined by measuring the difference in inorganic phosphate formed in the presence and absence of fluoride after 10 min. in M-hydrochloric acid at 100°.

This investigation was supported in part by a Research Grant from the U.S. Public Health Service.

THE ISOTOPE DEPARTMENT, THE WEIZMANN INSTITUTE OF SCIENCE,
REHOVOTH, ISRAEL. [*Received, September 24th*, 1962.]

[14] Todd, *Proc. Chem. Soc.*, 1962, 199.
[15] Bauer, " Handbuch der preparativen anorganischen Chemie," F. Enke, Stuttgart, 1954, p. 449.
[16] Fiske and Subbarow, *J. Biol. Chem.*, 1925, **66**, 375.