903. *Hydrolysis of* N-*Acylphosphoramidic Acids. Part III.*[1] N-*Benzoylphosphoramidic Acid and Diphenyl* N-*Dihydroxyphosphinylphosphoramidate.*

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The hydrolysis of *N*-benzoylphosphoramidic acid, NHBz·PO(OH)$_2$, is acid-catalysed, and has a maximum rate at about pH 4·0. In slightly acid and in alkaline solutions the rate is small. Both at pH 4 and in strongly acid solutions, solvolysis is faster in D_2O than in water. At high concentrations of perchloric acid the rate is proportional to the concentration of H_3O^+ with a slope of 1.

The hydrolysis of diphenyl *N*-dihydroxyphosphinylphosphoramidate, (HO)$_2$PO·NH·PO(OPh)$_2$, is very slow in acid and is negligible in alkaline solution. Around pH 4 the rate of hydrolysis passes through a pronounced maximum. This rate is much less than that of other *N*-acylphosphoramidic acids.

Hydrolysis of N-*Benzoylphosphoramidic Acid.*—*N*-Benzoylphosphoramidic acid,[2] being stable, crystalline, and easily purified, is convenient for a study of the hydrolysis of the phosphorus–nitrogen bond. It is now found to be hydrolysed quantitatively in aqueous solutions to benzamide and orthophosphate, the reaction being of the first order. The pH-dependence is described in Table 1 and in Fig. 1. The hydrolysis of *N*-benzoylphosphoramidic acid showed the characteristic maximum rate at pH 4·0, where most of

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[1] (*a*) Lapidot and Halmann, *J.*, 1958, 1713, and (*b*) Halmann and Lapidot, *J.*, 1960, 419, are to be considered as Parts I and II.

[2] Titherley and Worral, *J.*, 1909, **95**, 1143; Kirsanov and Makitra, *Zhur. obshchei Khim.*, 1956, **26**, 905, 907; 1957, **27**, 450.

the substrate exists as the monoanion. This is analogous to the results for *N*-phosphourethane,[1] *N*-arylphosphoramidates,[3] and various alkyl phosphates.[4] However, in contrast to results with *N*-phosphourethane, a minimum in the rate of hydrolysis occurs at about 1N-acid, and at higher concentrations of perchloric acid the rate increases steeply.

TABLE 1. *Dependence of rate of hydrolysis of* N-*benzoylphosphoramidic acid on* pH *at* 37·0°.

Solvent *	HCl 1·7N	HCl 0·117N	KHT 0·03M	KHP 0·05M	AB 0·1M	DB 0·05M	$Na_2B_4O_7$ 0·5M	NaOH 0·12N
pH		0·93	3·58	4·02	4·67	7·03	9·07	
10^4k (obs.) (sec.$^{-1}$) ...	0·314	0·89	1·78	1·85	1·67	0·13	0·0021	0·0015 †

* KHT = KH tartrate; KHP = KH phthalate; AB = acetate buffer; DB = Et_2 barbiturate.
† At 60·0°.

At the pH range 1—9 the rate data can be analysed [1b] to yield the specific rate constants of hydrolysis of the neutral acid k_0, of the monoanion k_1, and of the dianion k_2. Results at several temperatures are given in Table 2, together with energies and entropies of activation.

TABLE 2. *Specific rate constants of hydrolysis of* N-*benzoylphosphoramidate.*

Temp.	25·0°	37·0°	60·0:	ΔE (kcal./mole)	ΔS (e.u.)
10^4k_0 (sec.$^{-1}$)	0·165	0·865	17·1	26·4	+10
10^4k_1 (sec.$^{-1}$)	0·34	1·84	33·9	25·8	+7·54
10^4k_2 (sec.$^{-1}$)		0·0021			

In order to determine whether the amino-hydrogen atom of *N*-benzoylphosphoramidic acid has any function in the transition state the rates were measured in water and in D_2O. The rates of solvolysis of phosphourethane have been found [1b] to be the same in water and in D_2O within experimental error. However, as is shown in Table 3, there is a noticeable isotope effect in the hydrolysis of *N*-benzoylphosphoramidic acid at 37·0°. As is shown in the last column, both at pH 4 and in concentrated acid solution, the rate of hydrolysis increases by about 20% in D_2O. Further, the rate of solvolysis of *N*-benzoylphosphoramidic acid in which both the amino- and the hydroxyl-hydrogen atoms are replaced by deuterium is equal to that of the unsubstituted compounds. This is probably due to the fact that both the hydroxyl and the amino-hydrogen atoms are exchanged with the solvent hydrogen faster than the substrate is solvolysed. While the dissociation constants of protio- and deutero-phthalic acid certainly are not equal, the difference is probably similar to that between protio- and deutero-*N*-benzoylphosphoramidic acid, which is of a similar charge type. At about pH 4, a slight shift in the pH value of the maximum rate should be expected. The increase in rate in D_2O indicates a higher dissociation constant for *N*-benzoylphosphoramidic acid.

TABLE 3. *Solvolysis of* N-*benzoylphosphoramidic acid in water and* D_2O *at* 37°.

Buffer	pH	Substrate	Rate, 10^4k (sec.$^{-1}$) (obs.) In H_2O	In D_2O	k_D/k_H
K H phthalate, 0·05M......	4·0	$NHBz{\cdot}PO(OH)_2$	1·85 ± 0·01	2·26 ± 0·01	1·22
		$NDBz{\cdot}ND{\cdot}PO(OD)_2$	1·87 ± 0·01	2·16 ± 0·01	1·16
H_2SO_4, 1·7M		$NHBz{\cdot}PO(OH)_2$	0·34 ± 0·01	0·46 ± 0·01	1·35
		$NDBz{\cdot}PO(OD)_2$	0·34 ± 0·005		
H_2SO_4, 7·6M		$NHBz{\cdot}PO(OH)_2$	0·53 ± 0·005	0·65 ± 0·02	1·2

The effect of increasing acid concentration on the rate of solvolysis is given in Table 4. The plot of log *k* against log (stoicheiometric perchloric acid concentration) curves strongly upward. The plot against H_0 is linear, but with a slope of 0·14.

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

[4] Westheimer, *Chem. Soc. Spec. Publ.* No. 8, 1957, p. 1; Vernon, *ibid.*, p. 17, giving previous references.

The ionic-strength effect on acid-catalysis was investigated by a series of kinetic runs in the presence of varying concentrations of sodium perchlorate, the total perchlorate ion concentration being constant at 6·0N.[5] The results are given in Table 5. Here too, a plot of log k against H_0 is linear, with a slope of 0·2. From a plot of k against the concentration of perchloric acid, a value for the " neutral " rate of hydrolysis in strongly acid solution can be obtained by extrapolation to zero acid concentration. At 37° this is (0·41 ± 0·01) × 10^4 sec.$^{-1}$. A plot of log (k — 0·41) against log [$HClO_4$] fits a straight line with unit slope, within the experimental error. On the other hand, a plot of log (k — 0·41) against H_0 has a slope of 0·57. The rate of the acid-catalysed hydrolysis of N-benzoylphosphoramidic acid thus is not proportional to H_0, but is proportional to the concentration of $HClO_4$ (see Fig. 2).

TABLE 4.

$HClO_4$, M	1·02	4·05	5·9	6·1	7·8	8·75	9·4	11·3	11·5
$-H_0$	0·23	1·74	2·8	2·9	4·25	4·87	5·35		
10^4k (sec.$^{-1}$) (obs.)	0·372	0·50	0·80	0·98	1·09	1·7	2·25	5·85	7·2

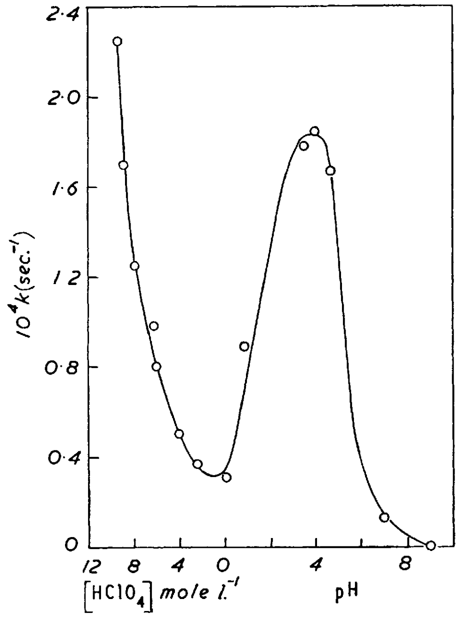

FIG. 1. pH-*dependence of hydrolysis of* N-*benzoylphosphoramidic acid in aqueous solution.*

TABLE 5. *Hydrolysis at constant perchlorate-ion concentration.*

$HClO_4$, M	1·0	2·0	3·0	5·0	6·0
$NaClO_4$, M	5·0	4·0	3·0	1·0	0
10^4k (sec.$^{-1}$).................	0·502	0·600	0·638	0·835	0·978
$-H_0$	1·46	1·84	2·13	2·77	3·06

Hydrolysis of $(HO)_2PO{\cdot}NH{\cdot}PO(OPh)_2$.—Kirsanov and Zhmurova [6] recently described the preparation of this asymmetric ester-acid. It is an amido-analogue of pyrophosphoric acid and its hydrolysis should show the effect of a phosphoryl group on the fission of a neighbouring N–P bond. Kirsanov and Zhmurova [6] claim that the compound is not hydrolysed in water during several minutes at 100°.

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[5] Harbottle, *J. Amer. Chem. Soc.*, 1951, **73**, 4024; Paul and Long, *Chem. Rev.*, 1957, **57**, 1.

[6] Kirsanov and Zhmurova, *Zhur. obshchei Khim.*, 1958, **28**, 2478.

The products of the hydrolysis were phosphoric acid and diphenyl phosphoramidate (see p. 4677). Thus the hydrolytic fission occurs at the terminal phosphate group: $(PhO)_2PO{\cdot}NH{-}PO(OH)_2$.

The dependence of the rate of hydrolysis on pH was determined in various aqueous buffer solutions, and at several temperatures. The results are presented in Table 6 and Fig. 3. From the temperature-dependence of the rate of hydrolysis, energies and entropies of activation were calculated at pH 1 and 4:

TABLE 6. *Hydrolysis of* $(PhO)_2PO{\cdot}NH{\cdot}PO(OH)_2$. *First-order rate constants*, 10^4k (sec.$^{-1}$).

	$HClO_4$ 8·3M	$HClO_4$ 5·8M	HCl 1·25M	HCl 0·11N	KHT * 0·03M	KHP * 0·05M	DB * 0·05M	$Na_2B_4O_7$ 0·5M
pH:				0·93	3·58	4·02	7·03	9·07
Temp.								
37·0°			0·0099	0·021	0·0550	0·059		0·0068
60·0°		0·009		0·41	1·32	1·38	0·81	0·170
70·0°	0·0075			1·55	5·48	5·90		

In 10N-NaOH 14) there was no decomp. in 2 weeks at 60°.

* KHT = KH tartrate; KHP = KH phthalate; DB = Et_2 barbiturate.

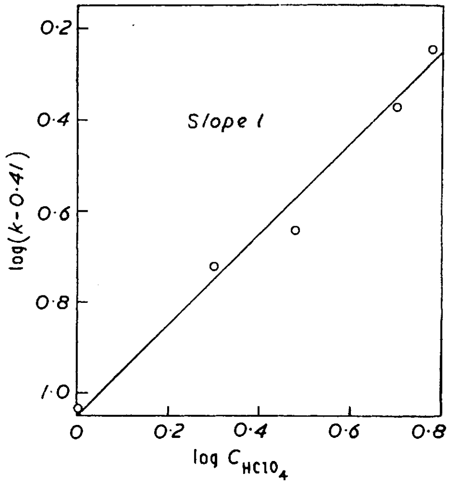

FIG. 2. *Dependence of rate constant for hydrolysis of* N-*benzoylphosphoramidic acid on acid concentration at* 37°.

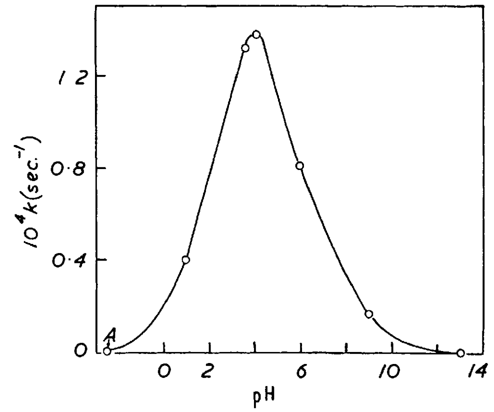

FIG. 3. pH-*dependence of hydrolysis of* N-*di-hydroxyphosphinylphosphoramidic acid in aqueous solution.*

Point A refers to 4M-*perchloric acid.*

$$\text{pH } 1: \Delta E^* = 28{\cdot}3 \text{ kcal./mole}; \quad \Delta S^* = +\,5{\cdot}8 \text{ e.u.}$$
$$\text{pH } 4: \Delta E^* = 30{\cdot}0 \text{ kcal./mole}; \quad \Delta S^* = +13{\cdot}2 \text{ e.u.}$$

The rate of hydrolysis in deuterium oxide was equal to that in water at 61·0° in presence of 0·05M-potassium hydrogen phthalate or 0·1M-sulphuric acid.

Discussion.—The rates of solvolysis of a number of *N*-substituted phosphoramidic acids are summarised in Table 7. Except for the last compound, the rates at pH 4 are of the same order of magnitude.

At about pH 4 the rates of solvolysis are a maximum. It appears from the first dissociation constants (pK_1) listed in Table 7 that at this pH the compounds are mostly in the monoanion form. An acidic proton is apparently required for solvolysis in all these compounds.[4]

Replacing one hydrogen atom of the amido-group of phosphoramidic acid itself by phenyl,[3] phenoxy,[3] or benzoyl has little effect on the maximum rate of solvolysis. In more acidic solution, at pH 1, the differences in rate become more marked.

TABLE 7. *Hydrolysis of phosphoramidic acids; first-order rate constants,* 10^4k_1 (sec.$^{-1}$).

	Temp.	pK_1	10^4k (sec.$^{-1}$) pH 1	pH 4	Acid-catalysis	Ref.
$NH_2\cdot PO_3H_2$	25°		Very fast	0·15	Yes	8
$HO_2C\cdot CH_2\cdot NH\cdot PO_3H_2$	25		Very fast	9·8 (pH 3)	Yes	9
$Ph\cdot NH\cdot PO_3H_2$	20·0	1·6	4·25	0·99	Yes	3
p-$MeO\cdot C_6H_4\cdot NH\cdot PO_3H_2$	20·0 25		6·7	1·04 1·91	Yes	3
$Bz\cdot NH\cdot PO_3H_2$	25·0 37·0	2·7	0·168 0·89	0·36 1·85	Only in conc. acid	This paper
$EtO_2C\cdot NH\cdot PO_3H_2$	26·7 37·0	2·7 *	0·193 0·845	0·435 2·02	No	1
$(PhO)_2PO\cdot NH\cdot PO_3H_2$	37	3·8	0·02	0·059	No	This paper

* Not 2·4 as reported in ref. 1.

A steep rise in the rate of hydrolysis of dimethylamides of phosphoric acids with increasing hydrochloric acid concentration has been observed[7] which, it has been suggested,[5,7] may indicate a parallelism of rate and acidity function H_0. The rate of hydrolysis of *N*-benzoylphosphoramidic acid is proportional to the concentration of perchloric acid, at constant ionic strength, and a mechanism involving a water molecule in the transition state must be invoked.

Diphenyl *N*-dihydroxyphosphinylphosphoramidate also shows a maximum at pH 4 (see Fig. 3), though, as Kirsanov and Zhmurova have observed,[6] the rate is much smaller than for other amidophosphoric acids. They suggested that this is due to the stability of a hydrogen bond from the acidic hydrogen atom on the terminal phosphate group to the other phosphoryl group.[6] Molecular models indicate that this bond seems more likely to occur than in the analogous benzoylphosphoramidic acid which is hydrolysed much faster.

That only the terminal phosphate group is liberated in the hydrolysis of diphenyl *N*-dihydroxyphosphinylphosphoramidate indicates that only the terminal phosphorus atom is attacked and shows again the special reactivity of monoionised phosphates.

In Table 7, the most striking difference between the various phosphoramidic acids is that the hydrolysis of some of them is acid-catalysed. All those that are not susceptible to acid-catalysis have a carbonyl or phosphoryl group adjacent to the nitrogen atom. The phosphoryl bond is strongly polarised,[10] with the phosphorus atom as the positive centre. The neighbouring phosphoryl or carbonyl group seems thus to cause the withdrawal of negative charge from the nitrogen of the phosphoramidic acid. The nitrogen thus becomes less susceptible to protonation, and acid-catalysis of solvolysis is prevented. The effect of the P–O group is greater than that of the C–O group, which has only a partially polar bond. This is an alternative explanation of the low reactivity of $(PhO)_2PO\cdot NH\cdot PO_3H_2$, which seems of more general validity than that[6] based on internal hydrogen bond.

N-Benzoylphosphoramidic acid is in an intermediate position, as its hydrolysis shows an increase only in very strongly acid solutions (Table 4). Possibly, the phenyl group is able to reduce the electron-deficiency of the carbonyl-carbon atom.

The faster solvolysis of the monoanion in D_2O than in H_2O (Table 3) can be due to a preliminary stage of fast reversible proton-transfer from the solvent to nitrogen. If the

[7] Heath and Casapieri, *Trans. Faraday Soc.*, 1951, **47**, 1093.

[8] Moller, *Biochim. Biophys. Acta*, 1955, **16**, 162; Quimby, Narath, and Lohman, *J. Amer. Chem. Soc.*, 1960, **82**, 1100; Rathler and Rosenberg, *Arch. Biochem. Biophys.*, 1956, **65**, 319.

[9] Winnick and Scott, *Arch. Biochem. Biophys.*, 1947, **12**, 201.

[10] Phillips, Hunter, and Sutton, *J.*, 1945, 146; Yaffé, *J. Chem. Phys.*, 1954, **22**, 1430.

returning step of breaking the nitrogen–hydrogen bond is slower for deuterium, the overall forward reaction should be faster in D_2O:

$$\mathrm{Bz{\cdot}ND{\cdot}PO(OD){\cdot}O^- + D_2O \underset{v.\ fast}{\overset{Fast}{\rightleftharpoons}} Bz{\cdot}\overset{+}{N}D_2{\cdot}PO(OD){\cdot}O^- + OD^- \xrightarrow{Slow} Products}$$

The rate-determining breakdown of the intermediate may involve a cyclic hydrated intermediate, as postulated by Westheimer.[4] The results available do not prove or disprove this. In strongly acid solutions, proportionality of rate to acid concentration indicates the following mechanism (*A*-2):

$$\mathrm{Bz{\cdot}NH{\cdot}PO_3H_2 + H_3O^+ \overset{Fast}{\rightleftharpoons} Bz{\cdot}\overset{+}{N}H_2{\cdot}PO_3H_2}$$

$$\mathrm{Bz{\cdot}\overset{+}{N}H_2{\cdot}PO_3H_2 + H_2O \xrightarrow{Slow} Products}$$

Again, the increase in rate in the deuterated solvent can best be explained by a smaller acidity constant of the deuterated conjugate acid, $[Bz{\cdot}ND_2{\cdot}PO_2D]^+$. It cannot be due to a difference in the acidity functions; H_0 and D_0 of similar concentrations of protio- and deutero-sulphuric acid have been shown to be equal.[12] For the hydrolysis of phosphourethane and $(HO)_2PO{\cdot}NH{\cdot}PO(OPh)_2$ protonation of the nitrogen does not take place at all—there is no acid-catalysis—and there is also no deuterium isotope effect.

Experimental.—*N*-Benzoylphosphoramidic acid, prepared according to Titherly and Worral's directions,[2] had m. p. 157—158°. Its acid dissociation constant was determined by the Henderson equation $pK = -\log [H^+][B + H^+]/(C - [B + H^+])$ from the curve of the titration with sodium hydroxide (Radiometer glass electrode pH-meter). The dissociation constants at 27° are $K_1 = 2{\cdot}14 \times 10^{-3}$ and $K_2 = 2{\cdot}14 \times 10^{-6}$. The products of its hydrolysis in water and in perchloric acid were benzamide, m. p. 130° (isolated by ether-extraction of the alkaline solution), and phosphoric acid (yield, determined colorimetrically, 100 ± 1%).

N-Benzoylphosphor[^{2}H]amidic [$^2H_2$]acid, $Bz{\cdot}ND{\cdot}PO(OD)_2$, was prepared analogously [2] (with D_2O instead of H_2O for the hydration and hydrolysis of the intermediate $BzNH{\cdot}PCl_2$) and had m. p. 157°.

Diphenyl *N*-dihydroxyphosphinylphosphoramidate, prepared as described [6] and recrystallized by adding ether to its solution in dioxan, had m. p. 170—172° (Found: C, 43·7; H, 4·0; N, 4·2; P, 19·0. Calc. for $C_{12}H_{13}NO_6P_2$: C, 43·7; H, 3·95; N, 4·2; P, 18·9%). The products of its hydrolysis were phosphoric acid and diphenyl phosphoramidate, which was isolated from cold water and had m. p. 145—146° (yield, 85%). The yield of phosphoric acid determined colorimetrically was 100 ± 1%. The acid dissociation constants at 25% are $K_1 = 1{\cdot}55 \times 10^{-4}$ and $K_2 = 2{\cdot}3 \times 10^{-7}$ (average of four calculations). D_2O containing sulphuric acid (7·6M) was prepared by diluting 5 g. of deuterosulphuric acid (1·92—1·96 D atoms; Fluka) with 10 g. of 99% deuterium oxide.

Kinetic experiments. The phosphoramidic acid (20—30 mg.) in aqueous buffer solution (25 ml.) was kept in a thermostat, and at intervals, aliquot parts were withdrawn for determination of orthophosphate by the method of Fiske and Subbarow.[13] Runs were made at least in duplicate, and rate constants calculated for first-order reactions. Rate constants of duplicate runs were within 1% of each other.

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[11] Long and Paul, *Chem. Rev.*, 1957, **57**, 940.
[12] Högfeldt and Bigeleisen, *J. Amer. Chem. Soc.*, 1960, **82**, 15.
[13] Fiske and Subbarow ,*J. Biol. Chem.*, 1925, **66**, 375.