316. *The Reactivity of Some Active Nucleophilic Reagents with Organophosphorus Anticholinesterases.*

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Rate constants have been measured for the reaction of some hydroxamic acids and oximes with a number of organophosphorus anticholinesterases in neutral aqueous solution. The relation between dissociation constant and reactivity is examined for the above compounds and for some other active nucleophiles, including hydroxide ions.

The influence of structure on the reactivity of the organophosphorus compounds is also discussed.

During the last 20 years many organophosphorus compounds RR'P(O)X (I) have been synthesised, which, being extremely active inhibitors of the enzyme cholinesterase, are highly toxic to insect and animal life.[1] Attempts to counter the toxicity by substances which react with them under physiological conditions have only recently led to compounds with significant therapeutic effect in animals.[2] With the exception of some metal chelates,[3] the most active of these are the anions of hydroxamic acids,[4] oximes,[5] hypohalous acids,[6] and hydrogen peroxide,[7] which have in common an electronegative atom linked directly to a negatively charged oxygen atom. In an attempt to relate the reactivities of these compounds to this structural feature, we have carried out a comparative study of the reactivity of a range of compounds of this type with organophosphorus anticholinesterases (I) in which all three substituents on the phosphorus atom were varied.

Reactivity of Oximes.—In a previous paper [5] the reaction between αβ-diketone monoximes and *iso*propyl methylphosphonofluoridate (Sarin) was shown to occur by a rate-controlling attack of the oxime anion on the phosphorus compound, followed by rapid splitting of the oxime phosphonate into acidic products. Second-order rate constants were determined from the rate of acid liberation. In Table 1, these rate constants are compared with those for hydroxyiminoacetone, hydroxyiminoacetylacetone, and diacetyl monoxime, measured by colorimetric determination of the unchanged Sarin.[7b] The agreement is good and confirms the view that the rate-determining stage is the initial attack of the oxime on the Sarin. The rates obtained by the colorimetric method were slightly dependent on the nature of the buffer, those in the Table being for reaction in aqueous collidine. In phosphate buffer of the same molar concentration the rate constants were increased by about 20%. Rate constants are also given in Table 1 for the reaction of the above three oximes with other anticholinesterases in which the two groups R and R' and the leaving group X were varied independently. Both absolute and relative reactivities of the three oximes are a function of all three groups on the phosphorus atom.

Reactivity of Hydroxamic Acids.—For the reaction between Sarin and benzhydroxamic acid Swidler and Steinberg [8] have shown that the rate-determining stage is attack by the hydroxamic acid anion and is followed by rapid breakdown of the intermediate phosphorylated hydroxamic acid. Rate constants for the reaction of benzhydroxamic acid and salicylhydroxamic acid with six organophosphorus anticholinesterases are included in Table 1. That for benzhydroxamic acid with Sarin is consistent with that obtained by Swidler and Steinberg although no exact comparison can be made as the experiments were

[1] Brown, "Insect Control by Chemicals," Wiley, New York, 1951; Sartori, *Chem. Rev.*, 1951, **48**, 245; Holmstedt, *Acta Physiol. Scand.*, 1951, **25**, Suppl., 90.
[2] Epstein and Freeman, *Fed. Proc.*, 1955, **14**, 50; Askew, *Brit. J. Pharmacol.*, 1956, **11**, 417.
[3] Wagner-Jauregg, Hackley, Lies, Owens, and Proper, *J. Amer. Chem. Soc.*, 1955, **77**, 922.
[4] Hackley, Plapinger, Stolberg, and Wagner-Jauregg, *ibid.*, p. 3651.
[5] Green and Saville, *J.*, 1956, 3887.
[6] Epstein, Bauer, Saxe, and Demek, *J. Amer. Chem. Soc.*, 1956, **78**, 4068.
[7] (*a*) Epstein, Demek, and Rosenblatt, *J. Org. Chem.*, 1956, **21**, 796; (*b*) Marsh and Neale, *Chem. and Ind.*, 1956, 494.
[8] Swidler and Steinberg, *J. Amer. Chem. Soc.*, 1956, **78**, 3594.

performed at different temperatures in different media. The rates obtained by the colorimetric method were, as for oximes, affected by the buffer, the reactivity in phosphate being greater than in collidine.

In hydroxamic acids R·CO·NH·OH (II) the substituent R is further from the acidic hydroxyl group than are the substituents R′ and R″ in oximes HO·N:CR′R″, so that steric effects of this group are likely to be less important. It seems reasonable that the affinity of

TABLE 1. *Second-order rate constants (l. mole^{-1} min.$^{-1}$) for the reaction of some oximes and hydroxamic acids at 25° with anticholinesterases* RR′P(O)X.

			Rate constant * for anion derived from					
R	R′	X	Hydroxyimino-acetylacetone pK_a ‡: 7·38	Hydroxyimino-acetone 8·30	Diacetyl monoxime 9·30	Benzhydroxamic acid 8·75	Salicylhydroxamic acid 7·43	Rate constant for hydroxide ion † —
Et	Et	F	—	(530)	—	—	—	50,000
EtO	Et	F	—	(160)	—	—	—	1200
EtO	EtO	F	18	62	—	380	33	110
Pr^{i}O	Pr^{i}O	F [a]	3·4	12	—	36	4·9	50
Pr^{i}O	Me	F [b]	73	240	380	1020	114	2000
			(80)	(250)	(410)		(130)	
EtO	EtO	$(EtO)_2PO_2$ [c]	35	59	16	160	19	21
EtO	EtO	O·C_6H_4·NO_2 [d]	—	0·20	0·30	1·00	0·071	0·52
EtO	Me_2N	CN [e]	95	180	60	600	90	750

[a] DFP. [b] Sarin. [c] TEPP. [d] E.600. [e] Tabun.

* The rates in parentheses were obtained by the acid-production method, while those for E.600 were obtained from the *p*-nitrophenol liberation. The rates for TEPP and those for the two oximes with DFP are for reaction in phosphate buffer; all other rates are for collidine buffer.

† The hydroxide-ion rates for DFP, E.600, and Tabun are taken from Kilpatrick and Kilpatrick (*J. Phys. Chem.*, 1949, **53**, 1371), Topley (*Chem. and Ind.*, 1950, S.859), and D. J. Marsh (personal communication). The other values were obtained from the acid production.

‡ log (reciprocal of the dissociation constant in 0·1M-KCl).

the anion for a positively charged phosphorus atom in a polarised phosphoryl group would be related to its affinity for a proton by an equation of the Brønsted or Hammett type, namely $k = c(1/K_a)^b$. The relation between log k and pK_a for the reaction of a number of hydroxamic acids with four anticholinesterases is depicted in the Figure. There is some scatter, but the relation holds moderately satisfactorily. From this type of Brønsted relation, the most reactive hydroxamic acid at any pH, including that (7·4) desired physiologically, can be readily predicted.[9]

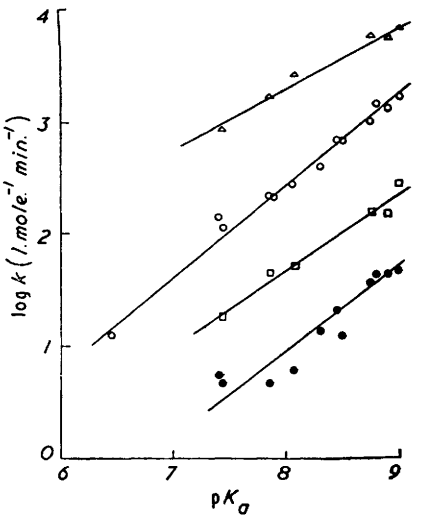
Since aldoximes [5] (III) and catechols [10] (IV) react rapidly with organophosphorus compounds, the even higher reactivity of hydroxamic acids has been attributed [8] to the hydroximic acid tautomer (V) which is related to oximes and to catechols. To decide whether the ability to adopt the hydroximic form (V) is essential, we have examined the reactivity with Sarin of *N*-hydroxyphthalimide (VI), whose anion cannot tautomerise. This compound is bright red in neutral solution so that the peroxide–dianisidine reagent could not be used, but the rate constant (5 l. mole^{-1} min.$^{-1}$) was obtained from the acid production. The pK_a of *N*-hydroxyphthalimide is 6·1 so that the above rate is not greatly below that expected from the pK_a–log k graph (Figure). It therefore seems unnecessary to invoke as an explanation that the reaction proceeds *via* the hydroximic tautomer rather than the predominant hydroxamic form.[11] The cyclic hydroxamic acids (VII) and (VIII), which also cannot adopt a hydroximic form analogous to (V), are relatively unreactive towards di*iso*propyl phosphorofluoridate (DFP),[4] but probably owing to their higher acidity.

[9] Steinberg, Swidler, and Seltzer, *Science*, 1957, **125**, 336.

[10] Jandorf, Wagner-Jauregg, O'Neill, and Stolberg, *J. Amer. Chem. Soc.*, 1951, **73**, 5202; Berry, Fellowes, Fraser, Rutland, and Todrick, *Biochem. J.*, 1955, **59**, 1.

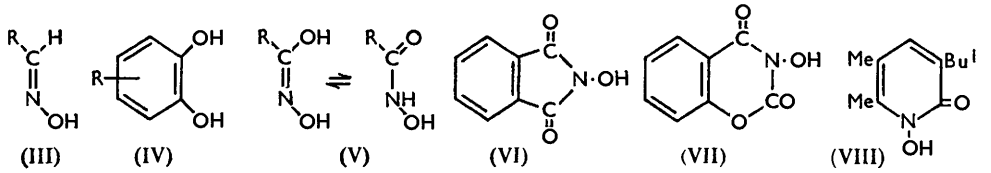
[11] Mathis, *Compt. rend.*, 1951, **232**, 505.

Discussion.—The two main factors influencing the nucleophilicity of a reagent are its electron density and polarisability, whose relative importance depends on the substrate.[12] Thus, for anions attacking a " saturated " substrate such as an alkyl halide, the repulsion between the partial negative charges on the entering and the leaving group in the transition



Reaction of hydroxamic acids at 25° in phosphate buffer with TEPP (□), and in collidine buffer with Tabun (△) (1 + log k), Sarin (○) or DFP (●). The hydroxamic acids used are listed in Table 4. pK_a = log (reciprocal of the dissociation constant in 0·1M-KCl).

state could be offset by high polarisability of the entering group. For " unsaturated " substrates such as acyl halides, this repulsion can be offset by polarisation of the substrate itself, so that polarisability is less important in the entering ion.



The mechanism of substitution in reactive phosphoryl compounds has not been established with certainty, although present evidence [13] favours direct nucleophilic replacement without the formation of an intermediate addition compound as occurs in some carbonyl substitution reactions; [14] but whether or not such intermediate formation occurs, the polarisability both of the reagent and of the phosphoryl bond appear to influence the reactivity of the phosphoryl compound with different reagents. Thus for TEPP and a single type of anion (such as those of hydroxamic acids) the rate increases with increasing basicity, but for a wider range of nucleophilic reagents the effect is less significant. On the other hand, for Sarin, basicity is much more important (cf. Table 2). It might be expected that the ability of the phosphoryl group to be polarised would decrease as conjugative substituents are attached to the phosphorus atom, in which case basicity should become less important as such substituents are added. This may explain the increased slope of the log k–pK_a curves (Figure) as the substituents on the phosphorus atom are changed from alkoxy-alkylamino to alkoxy-alkyl, and also the ratio of the reactivities of the hydroxide and the hydroxyiminoacetone ion shown in Table 3.

[12] Swain and Scott, *J. Amer. Chem. Soc.*, 1953, **75**, 141.
[13] Dostrovsky and Halmann, *J.*, 1956, 1004.
[14] Bender, *J. Amer. Chem. Soc.*, 1951, **73**, 1626.

The leaving group also has an effect on this ratio as can be seen for the diethoxyphosphoryl compounds in Table 1.

The reason for the high reactivity with organophosphorus anticholinesterases of compounds with the general structure R–Z(Y)·O⁻ remains obscure. The reactivity is far

TABLE 2. *Influence of basicity on the reactivity of nucleophiles with Sarin and TEPP.*

Reagent	pK_a *	k_{TEPP}	k_{Sarin}	k_{TEPP}/k_{Sarin}
H_2O	0	0·0017 [a]	0·0001 [b]	17
NH_2·OH	6	26 [b]	2·6	10
ClO^-	7·4	267 [b]	600 [d]	0·45
$Ac_2C{:}N{\cdot}O^-$	7·4	35	73	0·48
Ac·CH:N·O⁻	8·3	59	240	0·24
Bz·NH·O⁻	8·8	160	1020	0·16
Ac·CMe:N·O⁻	9·3	16	380	0·043
HO_2^-	11·8	2180 [b]	94,000 [b]	0·023
HO^-	14	21	2000	0·011

Values not in Table 1 are taken from: [a] Hall and Jacobson, *Ind. Eng. Chem.*, 1948, **40**, 694. [b] Unpublished results from this Laboratory. [c] Jandorf, *J. Amer. Chem. Soc.*, 1956, **78**, 3686. [d] Epstein, Bauer, Saxe, and Demek, *J. Amer. Chem. Soc.*, 1956, **78**, 4068.

* The pK_a values quoted for the oximes and hydroxamic acids are log (reciprocal of the dissociation constant in 0·1M-KCl).

TABLE 3. *Effect of conjugative substituents on the importance of basicity in phosphoryl substitution.*

Compound	$Et_2P(O)F$	EtO·PEt(O)F	$(EtO)_2P(O)F$
$k_{OH^-}/k_{Ac{\cdot}CH{:}N{\cdot}O^-}$	95	7·5	1·8

higher than expected from their basicity, yet is not primarily a function of polarisability since thiosulphate ions are almost unreactive with organophosphates.[7a] A feature that these anions have in common is a weak bond Z–O which breaks to give the cation $(R–Z–Y)^+$ once the oxygen atom has become attached to phosphorus. However, since this fission is not the rate-controlling step it is difficult to see how this potential bond-rupture can directly influence the rate-controlling nucleophilic attack.

EXPERIMENTAL

Materials.—Physical properties and references to methods of preparation of the oximes in Table 1 have been given earlier.[5] The hydroxamic acids used in the experiments summarised in the Figure are listed in Table 4. The organophosphorus compounds were provided by Dr. J. M. Wright of this Establishment.

TABLE 4.

Hydroxamic acid (and method)	Solvent	M. p.*	M. p. (lit.)	N (%) Calc.	N (%) Found	pK_a †
Benz- (*a*)	Ethyl acetate	127—128°	128° (*a*)	10·3	9·9	8·75
Furo- (*a*)	aq. EtOH	125—127	124 (*f*)	—	—	8·45
Glycine- (*b*)	H_2O	140—143	142—143 (*b*)	31·3	30·3	7·40
Hippuro- (*a*)	aq. EtOH	147—149	159 (*g*)	14·4	14·1	8·80
*iso*Nicotin- (*a*)	H_2O	151—153	155 (*c*)	20·3	20·1	7·85
p-Methylbenz- (*a*)	EtOH	142—144	148 (*h*)	—	—	8·90
Nicotin- (*a*)	H_2O	155—157	155 (*c*)	20·3	20·4	8·30
Nicotin- methiodide (*c*)	EtOH	177—179	187 (*c*)	—	—	6·46
m-Nitrobenz- (*a*)	aq. EtOH	140—141	151 (*i*)	15·4	15·4	8·07
Picolin- (*a*)	H_2O	119—120	120 (*c*)	20·3	20·5	8·50
Pyrimidine-2-carbox- (*a*)	H_2O	155—157	—	30·2	30·2	7·88
Salicyl- (*d*)	aq. MeOH	165	168—169 (*d*)	—	—	7·43
Tropo- (*a*)	H_2O	178—180	171—172 (*j*)	7·7	7·7	9·00
N-Hydroxyphthalimide (*e*)	aq. EtOH	225	230 (*e*)	8·6	8·5	6·10

* With decomp.; approx. † log (reciprocal of the dissociation constant in 0·1M-KCl). (*a*) Hauser and Renfrew, *Org. Synth.*, Coll. Vol. II, p. 67. (*b*) Safir and Williams, *J. Org. Chem.*, 1952, **17**, 1298. (*c*) Ref. 4. (*d*) Jeanrenaud, *Ber.*, 1889, **22**, 1273. (*e*) Lach, *Ber.*, 1883, **16**, 1781. (*f*) Pickard and Neville, *J.*, 1901, **79**, 847. (*g*) Wieland and Stimming, *Annalen*, 1953, **579**, 97. (*h*) Lossen, *ibid.*, 1895, **281**, 176. (*i*) Werner and Skiba, *Ber.*, 1899, **32**, 1662. (*j*) Steinberg and Bolger, *J. Org. Chem.*, 1956, **21**, 660,

Collidine–collidine hydrochloride buffer was prepared according to Gomori,[15] and sodium phosphate–citric acid buffer was prepared at half the concentration given by McIlvaine.[16]

Dissociation Constants.—These were determined by titration with 0·1N-sodium hydroxide of the oxime or hydroxamic acid (about 0·01M) in aqueous potassium chloride (0·1M) at 25°. The pK_a values were calculated by the Henderson–Hasselbalch equation from pH's around the half-neutralisation points. The potassium chloride was used to compensate approximately for the effect on the dissociation constants of the ionic strength of the buffers used in the kinetic experiments.

Kinetic Methods.—Three methods were used for determining the reaction rates. These were: (*a*) continuous titration with alkali of the acid produced during the reaction,[5] (*b*) colorimetric estimation of the rate of disappearance of the phosphorus compound, and (*c*) for diethyl *p*-nitrophenyl phosphate only, colorimetric measurement of the rate of liberation of *p*-nitrophenol.

Reactions (*b*) were carried out in a beaker at roughly controlled temperatures. The phosphorus compound (0·5 ml. of a 0·05M solution in " AnalaR " propan-2-ol) was added to a solution (50 ml.) of the hydroxamic acid or oxime (0·005—0·05M) in an appropriate buffer. The mixture was stirred and samples (1·0 ml.) were withdrawn at intervals and added to the peroxide–dianisidine reagent (24 ml.; see below) which was then set aside (5—30 min.) until the intensity (D_t) of the brown colour, measured on a Hilger " Spekker " photometer (601 Spectrum Violet filter), became constant. The " Spekker " readings were linearly proportional to the concentration of the phosphorus compound and were used directly in the graphical calculation of the first-order rate constants (the oxime or hydroxamic acid being in large excess) from the relation $k_{obs.} = kiA + k_{solv.} = (1/2{\cdot}303t) \log (D_0/D_t)$ where A is the concentration of the oxime or hydroxamic acid and i the fraction ionised, as calculated from the Henderson–Hasselbalch equation. The small spontaneous solvolysis rate, $k_{solv.}$, was determined independently for each organophosphate over the pH range (6·5—8) used for the reactivity measurements. A typical experiment is illustrated in Table 5. The pH of the

TABLE 5. *Reaction of* DFP *with* p-*methylbenzhydroxamic acid* (0·02M) *in collidine buffer* (pH 7·64) *at* 25°.

Time (t, min.)	0	3	6	9	12	15	18	21	25	29
" Spekker " reading (D_t)	0·628	0·551	0·468	0·416	0·368	0·311	0·272	0·237	0·194	0·161

From a graph of log D_t against t, $k_{obs.} = 0{\cdot}047$ min.$^{-1}$. At pH 7·6, $k_{solv.}$ for DFP is negligible, so that $ki = 2{\cdot}35$ l. mole^{-1} min.$^{-1}$. The pK_a of *p*-methylbenzhydroxamic acid is 8·90, so that $i = 1/\{1 + \text{antilog}\ (\text{p}K_a - \text{pH})\} = 0{\cdot}052$, and $k = 45$ l. mole^{-1} min.$^{-1}$.

reaction mixture was checked before addition of the phosphorus compound and at the end of the run.

The peroxide–dianisidine reagent contained the following solutions.[7b] Acetone–water buffer, 22—20 ml. of a solution containing potassium dihydrogen phosphate (8 g.) and potassium hydroxide (4·5 g.) in water (500 ml.) and acetone (500 ml.) (freed from aldehydes by contact with potassium permanganate for 1 week followed by distillation from 1% sodium hydroxide); *o*-dianisidine hydrochloride (1·0 ml. of a 1·3% aqueous solution); and 1% aqueous hydrogen peroxide (1—3 ml.).

Method (*c*): *p*-Nitrophenol in borate buffer gives an approximately linear relation between optical density [measured on a Hilger " Spekker " photometer (601 Spectrum Violet filter)] and concentration up to about 2×10^{-5}M. Above this concentration the colour intensity increased only slowly with concentration and is unsatisfactory for estimation of *p*-nitrophenol. Accordingly diethyl *p*-nitrophenyl phosphate was dissolved in an approximately half-neutralised solution of the oxime or hydroxamic acid (about 10^{-2}M) to give a concentration of at most 5×10^{-4}M. At suitable time intervals, samples (1 ml.) of this solution were diluted with 24 ml. of aqueous borax (0·05M), and the colour intensity was measured on the spectrophotometer. This dilution of the oxime or hydroxamic acid was sufficient effectively to stop the reaction. The rate constant was calculated from a graph of log $(X_\infty - X_t)$ against time, where X_t is the *p*-nitrophenol concentration at time t.

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[16] McIlvaine, *J. Biol. Chem.*, 1921, **49**, 183.