756. *The Reaction of Oximes with* iso*Propyl Methylphosphonofluoridate (Sarin).*

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In neutral or slightly alkaline aqueous solution, oximes react rapidly with *iso*propyl methylphosphonofluoridate (Sarin). The mechanism of the reaction and the effect of structure on the reactivity of different oximes are discussed.

The dissociation constants of thirteen oximes have been measured at 25° in 0·1M-aqueous potassium chloride.

Many organophosphorus compounds such as phosphorofluoridates, pyrophosphates, and *p*-nitrophenyl phosphates are potent inhibitors of the enzyme cholinesterase.[1] Inhibition by these compounds cannot be reversed by dilution or dialysis,[1] but hydroxylamine [2] and some of its derivatives, in particular hydroxamic acids [3] and oximes,[4] will restore the activity of the inhibited enzyme. Lately it has been shown [5] that hydroxamic acids react rapidly in neutral aqueous solution with di*iso*propyl phosphorofluoridate (DFP) and with *iso*propyl methylphosphonofluoridate (Sarin), two of the more powerful organophosphorus anticholinesterases.[6] The present paper describes the analogous reaction between oximes and Sarin.

[1] Evidence reviewed by Nachmansohn and Wilson, *Adv. Enzymology*, 1951, **12**, 259.
[2] Wilson, *J. Biol. Chem.*, 1951, **190**, 111.
[3] Wilson and Meislich, *J. Amer. Chem. Soc.*, 1953, **75**, 4628.
[4] Childs, Davies, Green, and Rutland, *Brit. J. Pharmacol.*, 1955, **10**, 462.
[5] Hackley, Plapinger, Stolberg, and Wagner-Jauregg, *J. Amer. Chem. Soc.*, 1955, **77**, 3651.
[6] Michel, *Fed. Proc.*, 1955, **14**, 255.

The compounds studied most extensively were 1 : 2-dione monoximes (I) since these were among the more active members of the group in nearly neutral solution. Evidence for the nature of the reaction between these oximes and Sarin was obtained in several ways.

(I) R·CO·CR′:N·OH (R = alkyl or aryl, R′ = H or alkyl)

For each mole of Sarin decomposed (as verified by Marsh and Neale's colorimetric method [7]) it was found that one mole of oxime (determined by its yellow colour in alkaline solution) was consumed. For each mole of oxime (I) consumed, one mole of the carboxylic acid $R{\cdot}CO_2H$ was formed (see Experimental for R = Ph, R′ = H). Hydrogen cyanide was also formed from 2-oxo-aldoximes (I; R′ = H) but not from the corresponding ketoximes (I; R′ = alkyl). The reactions were accompanied by the liberation of 3 mols. of acid titratable to pH 6—8 : one of these three is the carboxylic acid $R{\cdot}CO_2H$, and the other two must result from the splitting of the phosphonofluoridate.

The overall reaction may be expressed by the equation :

$$R{\cdot}CO{\cdot}CR'{:}N{\cdot}OH + Me{\cdot}PFO{\cdot}OPr^i + H_2O \longrightarrow R{\cdot}CO_2H + R'CN + HF + Me{\cdot}PO(OH){\cdot}OPr$$

In the presence of a large excess of oxime the rate of total acid production was found to obey a first-order law, the rate being proportional to the concentration of the oxime anion. It is therefore probable that the reaction involves a rate-determining *O*-phosphorylation of the oxime anion (step 1), followed by rapid decomposition of the oxime phosphonate to the observed products, by a step such as (2) :

(1) $R{\cdot}CO{\cdot}CR'{:}N{\cdot}O^- + Me{\cdot}PFO{\cdot}OPr^i \longrightarrow R{\cdot}CO{\cdot}CR'{:}N{\cdot}O{\cdot}PMeO{\cdot}OPr^i + F^-$ (slow)
(II)

(2) $HO^- + R{\cdot}CO{-}CR'{=}N{-}O{\cdot}PMeO{\cdot}OPr^i \longrightarrow R{\cdot}CO_2H + R'{\cdot}CN + Me{\cdot}PO(OPr^i){\cdot}O^-$ (fast)

The equally rapid formation of 3 mols. of acid from Sarin with either 2-oxo-aldoximes (I; R′ = H) or ketoximes (I; R′ = alkyl) makes it unlikely that 2-oxo-aldoxime phosphonates (II; R′ = H) decompose by initial elimination of *iso*propyl methylphosphonic acid to give an acyl cyanide.

The acylating properties of the postulated oxime phosphonate (II) have been shown by performing the reaction between Sarin and hydroxyiminoacetone (I; R = Me, R′ = H) in the presence of aniline, acetanilide being formed, presumably as follows :

$$Ph{\cdot}NH_2 + Ac{\cdot}CH{:}N{\cdot}O{\cdot}PMeO{\cdot}OPr^i \longrightarrow Ph{\cdot}NHAc + HCN + Me{\cdot}PO(OH){\cdot}OPr^i$$

The reaction of Sarin with hydroxyiminoacetylacetone (I; R = Me, R′ = Ac), in which the oxime group is flanked by two acyl groups instead of one, was more complicated. Although at moderately high pH (8·5) the expected 3 mols. of acid were formed, in neutral and more acid solutions (pH 6·5) over 6 mols. of acid were formed and about 3 mols. of oxime were consumed. Hydroxyiminoacetylacetone itself is slowly decomposed in neutral aqueous solution (see Figure). Pyruvic acid was found among the products of both the spontaneous and the Sarin-accelerated decomposition but the mechanism is obscure and was not further investigated.

Rates of Reaction between Oximes and Sarin.—A preliminary study has been made of the reactivity of Sarin with a number of oximes by measurement of the rate of acid production by continuous titration to constant pH with sodium hydroxide.*

In the presence of a large excess of oxime, the observed rate of total-acid production was of the first order and proportional to the oxime concentration and to the degree of ionisation, *i*, of the oxime calculated from the Henderson–Hasselbalch equation (see Table

* A more detailed kinetic study of the reaction between oximes and organophosphorus anticholinesterases generally, in which the rate of disappearance of the anticholinesterase was measured directly, will be published later. The rates obtained by this method for the reaction of oximes with Sarin agree quite well with those obtained here by means of the acid production.

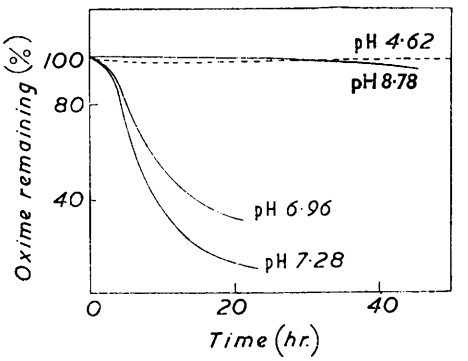
[7] Marsh and Neale, *Chem. and Ind.*, 1956, 494.

1), *i.e.*, $-d[Sarin]/dt = d[nH^+]/dt = k_2i[A][Sarin]$, where n is the number of moles of acid obtained per mole of Sarin decomposed and [A] is the oxime concentration.

TABLE 1. *Second-order rate constants (l. mole^{-1} min.$^{-1}$) for the reaction of hydroxyiminoacetone (pK_a 8·30) with Sarin at 25°.*

pH	7·4	7·6	7·8
Apparent rate constant (= ik_2)	27·8	45	57·9
Anion (%) (= 100i)	11·2	16·6	24
k_2	248	271	242

Table 2 shows the anion rate constants (k_2) for a number of 1 : 2-dione monoximes, salicylhydroxamic acid, and some substituted benzaldoximes. With simple aldoximes 1—2 mols. of acid are produced, again at a first-order rate, but the nature of the products was not investigated. The main general tendency, for all the compounds, is an increase of reactivity with increasing pK_a, *i.e.*, the greater the affinity of the anion for a proton, the



Stability of hydroxyiminoacetylacetone in buffer solutions at various pH's.

greater its reactivity with a phosphoryl centre. For compounds with about the same pK_a, *e.g.*, hydroxyiminoacetylacetone, salicylhydroxamic acid, and 2-hydroxyiminomethylpyridine methiodide, even a large variation in structure of the substituent on the N·O$^-$ ion has surprisingly little effect on the reactivity. For the 2-oxo-ketoximes (I; R = Me, R′ = Me—Pr^i) the pK_a effect is modified by increase in the size of the alkyl radical which

TABLE 2. *Second-order rate constants (l. mole^{-1} min.$^{-1}$) for the reaction of oxime anions with Sarin at 25°.*

Compound	pK_a	k_2
Hydroxyiminoacetone (I; R = Me, R′ = H)	8·30	250
2-Oxo-*n*-butyraldoxime (I; R = Et, R′ = H)	8·37	240
Hydroxyiminoacetophenone (I; R = Ph, R′ = H)	8·25	140
Diacetyl monoxime (I; R = Me, R′ = Me)	9·30	410
3-Hydroxyiminopentan-2-one (I; R = Me, R′ = Et)	9·38	340
3-Hydroxyimino-4-methylpentan-2-one (I; R = Me, R′ = Pr^i)	9·50	310
Hydroxyiminoacetylacetone (I; R = Me, R′ = Ac)	7·38	80
Salicylhydroxamic acid	7·43	130
Salicylaldoxime	9·17	1490
p-Hydroxybenzaldoxime	8·93	30
2-Hydroxyiminomethylpyridine	10·10	1690
2-Hydroxyiminomethylpyridine methiodide	7·82	120
3-Hydroxyiminomethylpyridine methiodide	9·10	990
4-Hydroxyiminomethylpyridine methiodide	8·23	380

causes a drop in the reactivity despite a slight increase in pK_a. The very low reactivity of *p*-hydroxybenzaldoxime is almost certainly due to ionisation of the phenolic hydroxyl group rather than of the hydroxyimino-group. Acetylation of *p*-hydroxybenzaldoxime gives an aryl acetate whereas that of salicylaldoxime gives an oxime acetate owing to protection of the phenolic hydroxyl group by chelation with the nitrogen atom in the oxime.[8] Monophenolic compounds are known to react slowly with phosphonofluoridates.[9]

[8] Brady, *J.*, 1931, 105.
[9] Jandorf, Wagner-Jauregg, O'Neill, and Stolberg, *J. Amer. Chem. Soc.*, 1952, **74**, 1521.

Reaction of Oximes with Other Acid Anhydrides.—The organophosphorus anticholinesterases may be regarded as acid anhydrides, either simple, such as tetraethyl pyrophosphate which is the anhydride of *OO*-diethylphosphoric acid, or mixed, such as Sarin which may be looked upon as a mixed anhydride of *P*-methyl-*O*-*iso*propylphosphonic acid and hydrofluoric acid. The oximes are not specific nucleophilic reagents for phosphoric anhydrides but will also react rapidly with related compounds such as sulphonyl halides and acetic anhydride. Thus Darzens and Mentzer [10] have used the action of thionyl chloride on 2-oxo-alkyl phenyl ketoximes (I; R = Ph, R′ = alkyl) as a preparative method for alkyl cyanides (R′·CN), and Werner and Piguet [11] found that an alkaline aqueous solution of benzil α-monoxime (I; R = R′ = Ph) with benzenesulphonyl chloride gave benzoic acid and benzonitrile:

$$Ph\cdot CO\cdot CR'{:}N\cdot OH + SOCl_2 + H_2O \longrightarrow Ph\cdot CO_2H + R'\cdot CN + SO_2 + 2HCl$$

$$Ph\cdot CO\cdot CPh{:}N\cdot OH + Ph\cdot SO_2Cl + H_2O \longrightarrow Ph\cdot CO_2H + Ph\cdot CN + Ph\cdot SO_3H + HCl$$

We have examined the action of acetic anhydride on hydroxyiminoacetone (I; R = Me, R′ = H), which in nearly neutral solution occurs almost instantaneously with liberation of a single mol. of acid. There is then a slower breakdown of the oxime acetate to give two more mols. of acid and hydrogen cyanide. The latter step occurs much more rapidly with increasing pH so that the reaction sequence is presumably:

$$Ac\cdot CH{:}N\cdot O^- + Ac_2O \longrightarrow Ac\cdot CH{:}N\cdot OAc + AcO^-$$

$$HO^- + Ac\cdot CH{:}N\cdot OAc \longrightarrow 2AcO^- + HCN + H^+$$

In weakly acid solution the oxime acetate is relatively stable but with aniline it gives acetanilide.

Experimental

Materials.—The oximes used are listed in Table 3. The aldoxime methiodides which have not previously been described were obtained by boiling the aldoximes in ethanol for 4 hr. with two mols. of methyl iodide.

Table 3.

Compound and method	Solvent *	M. p.	M. p. (lit.)	Calc. (%) N	Found (%) N
Hydroxyiminoacetone [12]	V.s.	65—66°	68° [12]	16·1	15·9
2-Oxo-*n*-butyraldoxime [13]	V.s.	55	55 [13]	13·9	14·5
Hydroxyiminoacetophenone [14]	$CHCl_3$	124—126	126—128 [14]	—	—
Diacetyl monoxide [15]	$CHCl_3$–Pet	73—75	75 [16]	—	—
3-Hydroxyiminopentan-2-one [15]	V.s.	52—54	53—55 [17]	12·2	12·7
3-Hydroxyimino-4-methylpentan-2-one [15]	Pet	78—80	75 [18]	10·9	11·2
Hydroxyiminoacetylacetone [19]	V.s.	73—75	75 [19]	—	—
Salicylhydroxamic acid [20]	MeOH	165	168—169 [21]	—	—
Salicylaldoxime [21]	$CHCl_3$–Pet	57	57 [22]	—	—
p-Hydroxybenzaldoxime [21]	C_6H_6	114—115	112 [23]	10·2	10·8
2-Hydroxyiminopyridine [21]	H_2O	111	113 [24]	22·9	22·8
				I	I
2-*Hydroxyiminomethylpyridine methiodide*	Aq. EtOH	218—220	—	48·0	47·7
3-*Hydroxyiminomethylpyridine methiodide*	EtOH	152—154	—	48·0	47·7
4-*Hydroxyiminomethylpyridine methiodide*	EtOH	171—173	—	48·0	47·7

* V.s. = purified by vacuum-sublimation; pet = light petroleum (b. p. 60—80°).

[10] Darzens and Mentzer, *Compt. rend.*, 1941, **213**, 268.
[11] Werner and Piguet, *Ber.*, 1904, **37**, 4295.
[12] Freon, *Ann. Chim.* (*France*), 1939, **11**, 460.
[13] Sharp and Spring, *J.*, 1948, 1862.
[14] Claisen and Manasse, *Ber.*, 1887, **20**, 2194.
[15] Diels and Jost, *Ber.*, 1902, **35**, 3292.
[16] Kalischer, *Ber.*, 1895, **28**, 1518.
[17] Meyer and Zublin, *Ber.*, 1878, **11**, 323.
[18] Westenberger, *Ber.*, 1883, **16**, 2991.
[19] Wolff, *Annalen*, 1902, **325**, 139.
[20] Jeanrenaud, *Ber.*, 1889, **22**, 1273.
[21] Hickinbottom, "Reactions of Organic Compounds," Longmans Green, London, 1945, p. **137**.
[22] Lach, *Ber.*, 1883, **16**, 1782.
[23] Dollfus, *Ber.*, 1892, **25**, 1925.
[24] Lenart, *Ber.*, 1914, **47**, 809.

Dissociation Constants.—These were determined by potentiometric titration with 0·1N-sodium hydroxide of the oxime (about 0·01M) in aqueous potassium chloride (0·1M) at 25°. The potassium chloride was used to maintain an approximately constant ionic strength. The pK_a values were calculated by the Henderson–Hasselbalch equation from pH's around the half-neutralisation points.

Kinetic Measurements.—The reactions were carried out at 25° in a small jacketed beaker fitted with a magnetic stirrer and containing a glass electrode. A calomel electrode immersed in saturated aqueous potassium chloride at 25° was connected to the reaction vessel by a glass salt bridge fitted with a Polythene capillary tube dipping into the reaction solution. A slow syphoning of liquid through the salt bridge was controlled by an ungreased glass tap. The two electrodes were connected to an E.E.L. Model 23 pH meter. A solution of Sarin (0·05M) in " AnalaR " propan-2-ol (0·1 ml.) was added to the oxime (0·01—0·1M) in aqueous potassium chloride (5 ml.; 0·1M), neutralised to the required pH with sodium hydroxide. The pH was maintained constant during the reaction by frequent addition of sodium hydroxide (0·1N) from an " Agla " micro-syringe fitted with a Polythene outlet tube dipping into the reaction mixture. Sufficient alkali was introduced at each addition to raise the pH slightly above the value required. The time was then recorded at which the pH-meter needle passed the required value. Generally the pH during the reaction was maintained within $\pm 0{\cdot}05$ unit.

The first-order rate constants (the oxime being in large excess) were calculated graphically from the relation $k_{obs.} = k_2 i[A] - k_{solv.} = (1/2{\cdot}303t) \log [a/(a - x)]$ where a is the total volume of alkali required to neutralise all the acid formed and x is the volume added after time t. [A] is the concentration of the oxime, and i the fraction ionised as calculated from the Henderson–Hasselbalch equation. The small spontaneous solvolysis rate, $k_{solv.}$, was determined independently over the pH range (6·5—9) used for the reactivity measurements. The slight dilution during the reaction due to the volume of the added alkali was neglected. A sample experiment is shown in Table 4.

TABLE 4. *Reaction of Sarin with hydroxyiminoacetone* (0·01M) *at* pH 7·80 *and* 25°.

t (sec.)	0	38	72	100	149	190	∞
x (μl.)	0	40	70	90	110	120	144
$a - x$	144	104	74	54	34	24	
log $(a - x)$	2·158	2·017	1·869	1·732	1·532	1·380	

Total volume of sodium hydroxide (0·1N) = 0·144 ml. Since this exactly neutralises the acid from 0·1 ml. of Sarin (0·05M), approx. 3 moles of acid are produced per mole of Sarin decomposed. From a graph of log $(a - x)$ against t, $k_{obs.} = 0{\cdot}582$ min.$^{-1}$. At pH 7·8, $k_{solv.} = 0{\cdot}003$ min.$^{-1}$, hence $k_2 i[A] = 0{\cdot}579$, and $k_2 i = 57{\cdot}9$ l. mole^{-1} min.$^{-1}$. Since $i = 1/\{1 + \text{antilog } (pK_a - pH)\} = 0{\cdot}24$, $k_2 = 242$ l. mole^{-1} min.$^{-1}$.

The reaction between hydroxyiminoacetone and acetic anhydride (in acetone) was studied similarly, but the rate of acid formation was too rapid to enable a rate constant to be obtained.

On completion of the reaction between Sarin and several of the 2-oxo-aldoximes the presence of a molar equivalent of cyanide ion was shown by conversion with bromine water into cyanogen bromide which was estimated by the intense red colour given with benzidine in pyridine.[23] No free cyanide ion could be detected by this method among the products of the reaction between Sarin and 2-oxo-ketoximes.

Disappearance of Oxime.—Solutions of 2-oxo-oximes in aqueous sodium hydroxide (0·01M) show a linear relation between concentration (up to at least 0·001M) and absorption density measured on a Hilger " Spekker " absorptiometer (601 Spectrum Violet filter). Colorimetric estimation of the oxime in samples taken at the beginning and end of a reaction at pH 7·4 between Sarin (0·002M) and hydroxyiminoacetophenone (0·01M) showed that one mole of oxime was consumed per mole of Sarin. However, in a corresponding reaction between Sarin and hydroxyiminoacetylacetone approximately 3 moles of oxime decomposed per mole of Sarin.

Decomposition of Hydroxyiminoacetylacetone.—The rate of spontaneous decomposition of this oxime at different pH's (see Figure) was determined colorimetrically as described above. For the more acidic buffer it was advantageous to separate the oxime from the buffer with ether and then to extract the oxime from the ether with 10% aqueous sodium carbonate. When the decomposition in the neutral buffers was complete, pyruvic acid (as its 2 : 4-dinitrophenylhydrazone) was isolated from the products in a way similar to that described below.

Formation of Pyruvic Acid from Hydroxyiminoacetylacetone.—A solution of the oxime (1·29 g., 0·01 mole) and Sarin (0·14 g., 0·001 mole) in aqueous disodium hydrogen phosphate (0·1M; 250 ml.) was set aside at 25° for 2 days. The mixture was made alkaline with 10%

aqueous sodium hydroxide and washed with chloroform to remove traces of non-acidic material. The aqueous solution was acidified (10% hydrochloric acid) to about pH 1 and washed with ether. Evaporation of the ether layer gave a residue containing no ketone but which smelt of acetic acid and gave an ester with ethanol and sulphuric acid. The residual acidic aqueous solution, after partial evaporation under reduced pressure to expel dissolved ether, was treated with 2 : 4-dinitrophenylhydrazine in hydrochloric acid (2N). A copious yellow precipitate was obtained, which after crystallisation from acetic acid melted at 214° alone or mixed with pyruvic acid 2 : 4-dinitrophenylhydrazone.

Formation of Benzoic Acid from Hydroxyiminoacetophenone.—A solution of Sarin (0·05M) in propan-2-ol (10 ml.) was added to the oxime (0·01M) in aqueous disodium hydrogen phosphate (0·1M; 50 ml.). After 18 hr. the solution was acidified with hydrochloric acid (1N; 10 ml.) and extracted with chloroform. The chloroform solution was washed with water, dried ($MgSO_4$), and evaporated. A slightly yellow crystalline solid (52 mg.; m .p. 100—104°) was obtained which crystallised from water to give benzoic acid in white needles, m. p. and mixed m. p. 119—121°.

Formation of Acetanilide.—(*a*) Acetic anhydride (2 ml.) was added to hydroxyiminoacetone (0·87 g.). The solid dissolved without generation of heat. After being warmed to 30—40° for 1 hr. the mixture was poured into water (100 ml.) and after a further hour aniline (3 ml.) was added to the solution. When shaken, the aniline dissolved, but the solution soon became cloudy with the formation of an oily emulsion. Hydrochloric acid was added, and the oil was extracted with ether. The ethereal solution was washed with aqueous sodium carbonate to remove residual oxime, dried ($MgSO_4$), and evaporated to pale brown crystals (0·55 g.), m. p. 100—104°. Recrystallisation from aqueous alcohol gave acetanilide as plates, m. p. and mixed m. p. 114°. Repetition of this experiment in the absence of the oxime gave no acetanilide, showing that this compound is not formed by reaction of aniline with incompletely hydrolysed acetic anhydride. Aniline does not react with this oxime alone. (*b*) Sarin (0·28 g.) was added to a solution of hydroxyiminoacetone (1·0 g.) and sodium hydroxide (0·4 g.) in water (100 ml.) previously saturated with aniline. After ½ hr. extraction with ether as described in (*a*) again gave a moderate yield of acetanilide.

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[25] Aldridge, *Analyst,* 1944, **69**, 262; 1945, **70**, 474.