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Parallel Hydrolysis Paths for Nucleophilic Displacement on a Phosphonofluoridate

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Organophosphonofluoridates [$CH_3(OR)P(O)F$] are potent neurotoxins. In aqueous solutions these compounds hydrolyze to release fluoride and form the corresponding *O*-alkyl methylphosphonic acid. Recently we examined the hydrolysis of *O*-isopropyl *t*-butylphosphonofluoridate (**I**) which was reputed to be relatively nontoxic relative to *O*-isopropyl methylphosphonofluoridate (sarin). The hydrolysis of **I** was measured with ^{31}P NMR at 20°C in buffered solutions with pH values of 4.0, 7.2, 9.2, and 10.0, respectively. At pH 10, **I** hydrolyzed approximately 4000 times slower than sarin. In addition **I** hydrolyzed by parallel reaction paths producing both *O*-isopropyl *t*-butylphosphonic acid and *t*-butylphosphonofluoridic acid. Below pH 9, the latter product predominates. To our knowledge this is the first example of parallel hydrolysis paths above pH 7 for a phosphonofluoridate.

I. INTRODUCTION

Organophosphonofluoridates are potent neurotoxins (*1*). The mechanism of nucleophilic substitution of such compounds is of interest in order to elucidate how acetylcholinesterase is inactivated and how such intoxication can be treated, and to find ways to decontaminate these compounds before intoxication can occur.

O-Isopropyl *t*-butylphosphonofluoridate (**I**) has been reported to be relatively nontoxic (*2*), so it was of interest to determine if **I** could be of value as a stimulant for the potent toxins such as sarin, where the *t*-butyl group in **I** is replaced by a methyl group. One part of this study involved measuring the hydrolysis of **I**. This article summarizes the results of experiments in which **I** was monitored at pH 10.0, 9.2, 7.2, and 4.0 using ^{31}P NMR to monitor the disappearance of **I** and to identify hydrolysis products. It will been seen that **I** hydrolyzes by parallel reaction paths.

II. RESULTS AND DISCUSSION

Table 1 lists the amounts of **I** and its hydrolysis products after standing for 374 days at various pH values. It is evident that **I** hydrolyzes much slower than sarin which has a half-life of 5 min at pH 10 (*3*). Of more interest is the fact that **I**

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TABLE 1

Hydrolysis Products from **I** after 374 Days at Various pH[a]

pH	I	ITBPA[b]	TBPA[c]
10	14.4	57.2	28.4
9.2	11.5	58.0	32.5
7.2	26.2	18.5	55.3
4.0	41.4	1.9	56.8

[a] Concentrations expressed as mole percent.

[b] *O*-Isopropyl *t*-butylphosphonic acid.

[c] *t*-Butylphosphonofluoridic acid.

hydrolyzes by parallel reaction paths. *O*-alkyl alkylphosphonofluoridates normally hydrolyze exclusively with P–F cleavage to form the *O*-alkyl alkylphosphonic acid (*4*).

For **I**, one observes that, even at pH 10, one-third of the hydrolyzed product is due to the loss of the isopropyl group. The fraction of reaction involving loss of alkoxy increases as the pH is lowered until at pH 4.0, the isopropyl group is almost the exclusive reaction site.

To our knowledge, this is the first example of simultaneous hydrolysis of a phosphonofluoridate above neutral pH. The most closely related study would be that of Larrson (*4*), who measured the hydrolysis *O*-isopropyl alkylphosphonofluoridate using methyl, ethyl, and isopropyl groups, respectively. In all cases Larrson reported exclusive cleavage at the P–F bond using thin-layer chromatography to analyze products. In our laboratory we have seen some evidence of alkoxy cleavage below pH 7 with diisopropyl phosphonofluoridate. Above pH 7, only reaction at the P–F bond was observed.

Rate coefficients for the hydrolysis of **I** were computed by fitting the equation

$$[C]_t = [C]_\infty + ([C]_0 - [C]_\infty)e^{-kt}, \qquad [1]$$

where

$[C]_t$ = concentration at time, t
$[C]_0$ = concentration at time zero
$[C]_\infty$ = concentration at time infinity
k = first-order observed rate coefficient, t^{-1}, and
t = time.

Best-fit values of $[C]$, $[C]_\infty$, and k were obtained for each kinetic run (*5*). Calculations were performed to determine rate coefficients for the disappearance of **I** as well as for the rate of appearance of the major product of the reaction. If the hydrolysis of **I** is simply a parallel reaction to form two products, the rate coefficients for each calculation should be the same.

Table 2 summarizes the results of these calculations. Excellent fits were ob-

TABLE 2

Computed Rate Coefficients for the Hydrolysis of **I** Assuming First-Order Kinetics

pH	k^a(s^{-1} × 10^7)	k(s^{-1} × 10^7)
10	1.06 ± 0.07	1.20 ± 0.09^b
9.2	0.84 ± 0.03	0.93 ± 0.03^b
7.2	0.09 ± 0.06	0.34 ± 0.09^c
4.0	0.17 ± 0.02	0.22 ± 0.02^c

[a] Based on disappearance of **I**.

[b] Based on appearance of *O*-isopropyl *t*-butylphosphoric acid.

[c] Based on appearance of *t*-butylphosphonofluoridic acid.

tained at pH values of 10 and 9.2, respectively, along with good agreement between the rate of disappearance of **I** and the rate of appearance of *O*-isopropyl *t*-butylphosphonate. For the disappearance of **I** at pH 7.2, a poor fit was obtained as reflected in the large sample standard deviation for k. The calculation for appearance of *t*-butylphosphonofluoridate gave a reasonable fit considering the reaction was monitored for only two half-lives. The agreement between rate coefficients was also reasonable at pH 4.0, where the reaction was monitored for a single half-life.

The observed first-order rate coefficients were then divided into the rate coefficients for the two parallel reactions by means of the stoichiometry of the reaction. Table 3 summarizes the results of that calculation in which k_1 is the observed first-order rate coefficient for P–F cleavage and k_2 is the corresponding value for cleavage of the alkoxy group.

The next and final step in the manipulation of the rate data is to calculate the dependence, rate coefficient vs pH, to determine the apparent second-order rate coefficient.

TABLE 3

Computation of Observed First-Order Rate Coefficients from P–F and P–O–C Cleavage of **I**

pH	k(s^{-1} × 10^7)^a	$k_1/k_2{}^b$	k(s^{-1} × 10^7)	k_2(s^{-1} × 10^7)
10.0	1.20	57.2/28.4	0.80	0.40
9.2	0.93	58.0/30.5	0.60	0.33
4.0	0.22	1.9/56.8	0.01	0.21

[a] Rate coefficient for appearance of product from Table 2.

[b] Mole percent of product as measured at 374 days.

TABLE 4

Second-Order Rate Coefficients for Hydroxide-Ion Catalysis of *O*-Isopropyl Alkylphosphonofluoridate Hydrolysis at 25°C

Alkyl	$k(\mathrm{M}^{-1}\ \mathrm{s}^{-1})$	Ref.
Methyl	26	(*2*, *4*)
Ethyl	9	(*4*)
Isopropyl	2	(*4*)
t-Butyl	0.0002[a]	This work
t-Butyl (P–O–C)	0.0001[a]	This work

[a] Rate monitored at 20°C in buffered solution.

In the present set of experimentation in which buffers were used to maintain the pH constant, the second-order rate coefficients also have a contribution from the ionized buffer which acts as a general base catalyst. However, it is instructive to compare the apparent second-order rate coefficients for the two parallel paths and to compare these with other organophosphonofluoridates. Using the two values for k_0 at pH 10.0 and 9.2, respectively, one obtains the following, presuming a linear dependence with the hydroxide ion concentration:

$$k_1 = 6 \times 10^{-8} + 2.3 \times 10^{-4}[\mathrm{OH}^-] \qquad [2]$$

$$k_2 = 3 \times 10^{-8} + 0.9 \times 10^{-4}[\mathrm{OH}^-]. \qquad [3]$$

Finally, these rate coefficients are compared with those other isopropyl alkylphosphonofluoridates from Larsson's work (Table 4).

These experiments reinforce the need to monitor the products formed from hydrolysis of phosphorus esters and not simply assume that the reaction is a straightforward substitution. Recently, Breslow and co-workers (*6*) discovered parallel hydrolysis reaction pathways with diphenyl *p*-nitrophenylphosphate. A small, but measurable, degree of bond breaking involving phenoxide was observed as well as the anticipated release of *p*-nitrophenoxide.

III. EXPERIMENTAL

Materials

Isopropyl *t*-butylphosphonofluoridate (**I**) was prepared by a three step synthetic route described in the literature (*2*). This synthesis required chloride, phosphorus trichloride, and aluminum chloride. In the second step, the *t*-butylphosphonic dichloride was converted to the difluoride by means of antimony trifluoride. In the final step, *t*-butylphosphonic difluoride was treated with one molar equivalent of sodium isopropoxide to give the desired product. This product, was obtained as a colorless liquid, bp 57–59°C/7 mm, with a purity of 99.4 wt%, based on ^{1}H and ^{31}P NMR analysis.

A toxicity determination on **I** revealed a 24-h LD_{50} (iv mice) of 116 μg/kg. The accepted value for sarin under similar testing is 0.1 mg/kg.

The buffer solutions used were pH 10.0, 0.025 M $NaHCO_3$ + 0.025 M Na_2CO_3; pH 9.2, 0.05 M $Na_2B_4O_7$; pH 7.2, 0.05 M tris(hydroxymethyl)aminomethane; pH 4.0, 0.05 M $KHC_8H_4O_8$.

General Procedure

A weighed amount of **I** (3.2–4.6 mg) was placed into a 5-mm-o.d. Pyrex NMR tube, and 1.0 ml of the appropriate buffer was added. The tube was capped, wrapped with Parafilm, and shaken to ensure homogeneity of the sample. The samples were stored at ambient temperature, 20 ± 2°C, and monitored periodically using ^{31}P NMR spectroscopy.

Instrumentation

The ^{31}P NMR spectra were recorded at 81 MHz using a Varian XL-200 Superconducting FTNMR system. For each spectrum, 500–1000 transients were accumulated in the double precision mode using a pulse width of 3.0 μs (35°), a sweep width of 20 KHz, an acquisition time of 1.6 s, and a pulse delay of 4 s. Gated waltz decoupling was used, and quantitative data were obtained by digital integration of the peak areas.

IV. CONCLUSION

Substitution of *t*-butyl for methyl bonded to phosphorus on sarin slows the rate of alkaline hydrolysis as expected from inductive effects. However, unlike sarin, the *t*-butyl derivative hydrolyzes by parallel reaction paths at pH 10, 9.2, 7.2, and 4.0. At neutral pH and lower, the predominate hydrolysis path is the loss of the alkoxy group.

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