predicted. The observed ferromagnetic coupling thus suggests that the model is inadequate. The model, however, is consistent with the observed data if we consider that the acceptor ← donor charge-transfer excitation results from the next highest occupied molecular orbital (NHOMO), not the POMO of the cation, to the radical anion POMO, Figure 8. Thus, ferromagnetic coupling which may ultimately lead to bulk ferromagnetic behavior as noted for $[Fe(C_5Me_5)_2]^{+\bullet}[TCNE]^{-\bullet}$[5,8,9] is achievable for systems where both the donor and acceptor have ^2A_g ground states and may be useful for understanding the ferromagnetic coupling reported for galvinoxyl[37] and $[TTF]^{+\bullet}\{M[S_2C_2(CF_3)_2]_2\}^{-\bullet}$ (TTF = tetrathiofulvalene; M = Pt, Ni).[38] Computational studies involving the charge-transfer integral for various ^2A and ^2E ground-state donor and acceptors as a function of different geometries are in progress.

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Registry No. 1, 98397-77-4; **2**α, 122144-56-3; **2**β, 122144-58-5; **3**, 122144-57-4.

Supplementary Material Available: Tables of fractional coordinates/anisotropic thermal parameters, general temperature factors, and cation bond angles $[Cr^I(C_6Me_xH_{6-x})_2][TCNE]$ (x = 0, 3), figures of the atom labeling, stereoviews, and solid-state interactions, and tables of the bond distances, anion bond angles, and least-squares plane for **2**β (27 pages); listing of observed and calculated structure factors for $[Cr^I(C_6Me_xH_{6-x})_2][TCNE]$ (19 pages). Ordering information is given on any current masthead page.

Hydrolysis of Toxic Organophosphorus Compounds by *o*-Iodosobenzoic Acid and Its Derivatives

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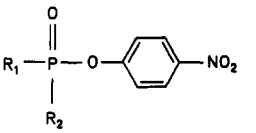
Abstract: The 1,2,2-trimethylpropyl and the 1-methylethyl esters of methylphosphonofluoridic acid (soman and sarin, respectively), ethyl *N*,*N*-dimethylphosphoramidocyanidate (tabun), and the diethyl 4-nitrophenyl ester of phosphoric acid (paraoxon) are hydrolyzed effectively in aqueous micellar cetyltrimethylammonium chloride (CTAC) solutions at pH 7.5 in the presence of the deprotonated form of *o*-iodosobenzoic acid (**5a**), 5-(*n*-octyloxy)-2-iodosobenzoate (**5b**), or 5-nitro-2-iodosobenzoate (**5d**). Aqueous CTAC solutions (1×10^{-2} M) containing 7.5×10^{-4} M **5a** hydrolyze 1×10^{-3} M soman with a half-life of approximately 15 s, $k_{obs} = 0.046$ s^{-1}, at 25 °C. Under similar conditions but with **5a** at 1×10^{-4} M, hydrolysis rates for both sarin and tabun were slower than that for soman, i.e., $k_{obs} = 2.35 \times 10^{-3}$ and 1.99×10^{-3} s^{-1}, respectively, while the k_{obs} for soman hydrolysis was equal to 8.08×10^{-3} s^{-1}. Reactions with soman, sarin, and paraoxon appear to be truly catalytic, while tabun hydrolysis may be more complicated. The 5-(*n*-octyloxy) derivative of *o*-iodosobenzoic acid (**5b**) gave rates with soman and sarin 2-3 times greater than those for the parent compound (**5a**). Derivative **5d** showed approximately 50% the activity of the parent compound for these same substrates. Similar rates of hydrolysis were found when either CTAC or cetylpyridinium chloride (CPC) was used as surfactants with **5a** as catalyst. Activity is not limited to a micellar environment. Both **5a** and **5b** promote the hydrolysis of organophosphorus compounds at pH 8.2 (bicarbonate buffer) in the absence of a surfactant and show even greater activity when ionically exchanged onto an ion exchange resin.

The facile hydrolysis of toxic organophosphorus compounds is of theoretical and practical interest since these compounds appear in day-to-day applications as pesticides and have been used as potent chemical warfare agents. Over the past decade, a number of approaches have been used to address the problem of hydrolysis/detoxification of such compounds. These include reactions of various organophosphorus esters with surface active oximes[1] or oximates in micellar solutions[2] and enzyme-catalyzed hydrolysis,[3] as well as reactions with aqueous sodium perborate,[4a] water-soluble *N*-bromooxazolidinones,[4b] and β-cyclodextrin (cycloheptaamylose) or related compounds.[5]

Many investigators have examined the reactivity of both carboxylic and organophosphorus esters in organized assemblies such as vesicles, micelles, and aggregates of various reagents.[6] For example, Menger examined the hydrolysis of *p*-nitrophenyl diphenyl phosphate (**1a**, PNPDPP) by a functionalized surfactant



1a (PNPDPP), $R_1 = R_2 = C_6H_5O$
1b (PNPDEP), $R_1 = R_2 = C_2H_5O$
1c (PNPIMP), $R_1 = i\text{-}C_4H_9O$; $R_2 = CH_3$
1d (PNPIPP), $R_1 = i\text{-}C_3H_7$; $R_2 = C_6H_5$

containing a hydrated aldehyde terminus (**2**) and has shown this reagent to provide catalytic turnover of that substrate.[7a] In more

†U.S. Army Medical Research Institute of Chemical Defense.
‡The Chemical Research, Development and Engineering Center.

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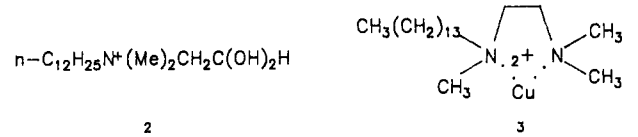
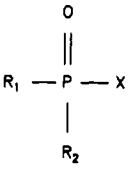
recent work, Menger has examined the activity of the $CuCl_2$ complex of *N,N,N'*-trimethyl-*N'*-tetradecylethylenediamine (**3**)



2 **3**

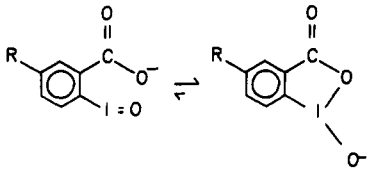
at pH 6–8 against this same substrate, as well as the activity of **3** against the chemical warfare agent 1,2,2-trimethylpropyl methylphosphonofluoridate (**4a**, soman).[7b] With PNPDPP (**1a**) as



4a (soman), $R_1 = (CH_3)_3CCH(CH_3)O$; $R_2 = CH_3$; X = F
4b (DFP), $R_1 = R_2 = i\text{-}C_3H_7O$; X = F
4c (sarin), $R_1 = i\text{-}C_3H_7O$; $R_2 = CH_3$; X = F
4d (tabun), $R_1 = (CH_3)_2N$; $R_2 = CH_3CH_2O$; X = CN
4e (VX), $R_1 = CH_3$; $R_2 = CH_3CH_2O$; X = $SCH_2CH_2N(i\text{-}C_3H_7)_2$

substrate, hydrolysis by **3** (as was the case with **2**) proceeded with catalytic turnover. Furthermore, at 25 °C and pH 7.0, the half-life of **4a** could be reduced to under 1 min, from 60 h at pH 10.

Moss and others have studied extensively the catalytic cleavage of carboxylate and phosphate esters by a series of *o*-iodoso- (Ar–I=O) and *o*-iodoxybenzoates ($Ar\text{–}IO_2$). In initial studies, they demonstrated that *o*-iodosobenzoate (**5a**), 5-(*n*-octyloxy)-



5 **6**

(a)R=H; (b)R=n-$C_8H_{17}O$; (c)R=OCH_3
(d)R=NO_2; (e)R=n-$C_{16}H_{33}N^+Me_2CH_2CH_2O$

2-iodosobenzoate (**5b**), and the corresponding *o*-iodoxybenzoate in solutions with micellar concentrations of a surfactant such as cetyltrimethylammonium chloride (CTAC) were true catalysts for the cleavage of these esters under mild (pH 8.0, 25 °C) conditions.[8]

The origin of catalytic activity for compound **5a** and its analogues has been suggested[8] to arise from its ability to exist in solution as the cyclized valence tautomer 1-hydroxy-1,2-benziodoxolin-3-one (**6a**).[9] This tautomeric form shows a pK_a of approximately 7.2 under CTAC micellar conditions[8] (other analogues have pK_a values ranging from 6.45 to 7.2[6c,10]) and is therefore at least partially deprotonated at or near neutral pH.[8] The deprotonated form may then act as an O-nucleophile with

Scheme I

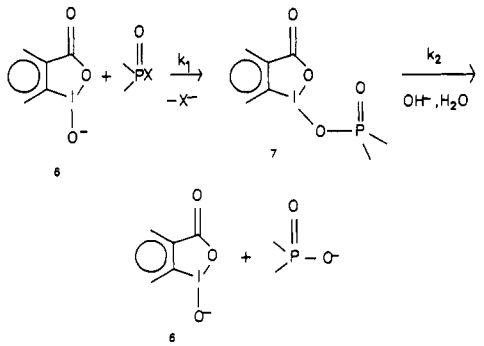


Table I. Comparison of Kinetic Rate Constants for **1a** Hydrolysis in Aqueous Buffers at pH 8 and 25.0 °C, for Various Catalysts[a]

cat.[b]	10^4[cat.], M	10^2k_{obs}, s^{-1} [c]	k_2, M^{-1} s^{-1} [d]	ref[e]
3	1.22	2.02 (0.0)	166	7b
6a	1.00	6.45 (1.0)	645	8b
6b	0.71	103.00 (0.2)	14 426	8b
6c	1.00	5.46 (1.0)	546	10
6d	1.00	6.16 (0.5)	616	10
6e	0.40	114.40 (0.2)	28 500	6c
6e	1.00	113.90 (0.5)	11 390	6c

[a] Conditions: For **3**, [**1a**] = 4.0×10^{-5} M, 0.01 M *N*-ethylmorpholine buffer, and μ = <0.01. For **6a–d**, 0.02 M phosphate buffer and μ = 0.08 (NaCl). For **6a–d**, 0.645 vol % DMF and 0.645 vol % CH_3CN. For **6c,d**, 1.0 vol % DMF and 0.33 vol. % CH_3CN. For **6e**, 0.01 M Tris buffer, μ = 0.01 (KCl), and 0.2 vol % CH_3CN. For **6a–e**, [**1a**] = 1.0×10^{-5}. [b] Catalyst for which pseudo-first-order rate constants were determined. [c] Pseudo-first-order rate constants for the hydrolysis of **1a**, chosen for comparable conditions; values in parentheses are concentrations of CTAC × 10^3 M at which k_{obs} was obtained; for **6a–d**, k_{obs} values represent the maximum observed rate constants for **1a** hydrolysis under these [CTAC]; for **6e**, k_{obs} measured at the beginning (first entry, [CTAC] = 0.2×10^{-3} M) as well as later (second entry, [CTAC] = 0.5×10^{-3} M) are in the plateau region found for **1a** hydrolysis; for **3**, k_{obs} chosen at [catalyst] comparable to that for **6a–e**. [d] Second-order rate constant calculated as $k_2 = k_{obs}/$[catalyst], uncorrected for extent ionization of the catalyst. [e] Data taken from references as indicated.

the hydrolysis reaction for organophosphorus compounds proceeding via the steps outlined in Scheme I.[10] In the case where k_1 is approximately the same as or less than k_2, turnover of the phosphylated[11] intermediate **7** will not be rate determining, and the behavior exhibited should be that of a true turnover catalyst.

For comparison purposes, a summary of the rate enhancements found for the hydrolysis of **1a** by **6a–e** and Menger's catalyst (**3**), under relatively comparable conditions, is shown in Table I. We see that these catalysts efficiently promote the hydrolysis of this substrate. For example, **6e** at 4.0×10^{-5} M provides a second-order rate constant, k_2, of 28 500 M^{-1} s^{-1} for the hydrolysis of PNPDPP. This represents a kinetic advantage of 14 700 over the cleavage rate of this compound in a nonfunctionalized, micelle-catalyzed reaction.[6c] It is of note that catalyst **3** gave results indicating approximately 1% the activity of compound **6b**, under comparable conditions.

On the basis of these studies with **1a** as substrate, the best catalysts for the rapid destruction of toxic organophosphorus agents appeared to be compounds **6b** and **6e**. These catalysts gave kinetic advantages of approximately 20:1 over compounds **6a**, **6c**, and **6d**. The assumption that the *o*-iodosobenzoates would promote enhanced hydrolysis of the more toxic organophosphorus agents, while seemingly reasonable, had not been examined directly. We have now demonstrated the activity of several of these compounds against those substrates.

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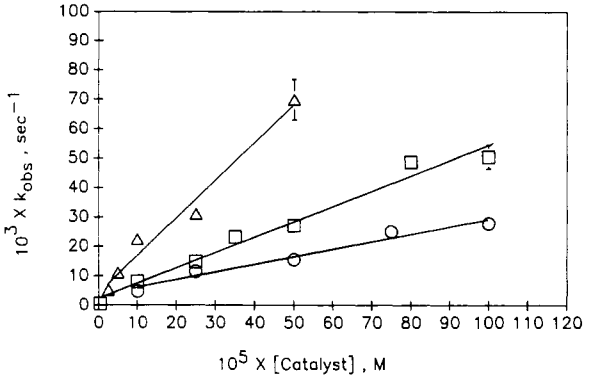

Figure 1. Soman (**4a**) hydrolysis. Pseudo-first-order rate constants (k_{obs} ± SEM, s^{-1}) as a function of [catalyst] for **6a** (□), **6b** (△), and **6d** (○) at 0.01 M CTAC, pH 7.5, 25 ± 0.1 °C. See text and Table II for reaction conditions.

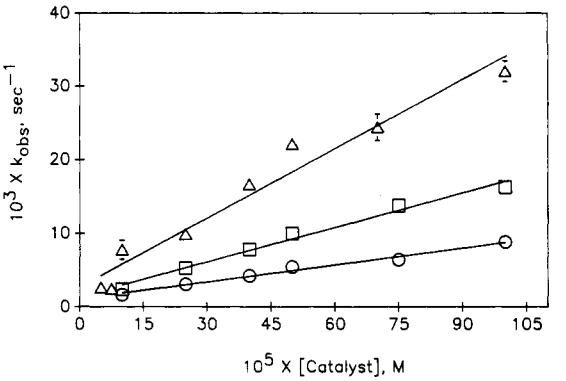

Figure 2. Sarin (**4c**) hydrolysis. Pseudo-first-order rate constants (k_{obs} ± SEM, s^{-1}) as a function of [catalyst] for **6a** (□), **6b** (△), and **6d** (○) at 0.01 M CTAC, pH 7.5, 25 ± 0.1 °C. See text and Table II for reaction conditions.

We have reported previously[12] on the ability of *o*-iodosobenzoate to catalyze the hydrolysis of bis(1-methylethyl)phosphonofluoridate (**4b**, DFP) as well as of the toxic, chemical warfare agents **4a** 1-methylethyl methylphosphonofluoridate (**4c**, sarin), ethyl *N,N*-dimethylphosphoramidocyanidate (**4d**, tabun), and *O*-ethyl *S*-[2-[bis(1-methylethyl)amino]ethyl]methylphosphonothioate (**4e**, VX) in aqueous, micellar solutions at pH 7.5.[13] In this paper, we discuss the activity of catalysts **6a**, **6b**, and **6d** in aqueous micellar media against these substrates, as well as their activity against *p*-nitrophenyl diethylphosphate (paraoxon, **1b**); examine the effect of surfactant concentrations on the hydrolytic activity for several substrate–catalyst combinations and provide surfactant/catalytic activity profiles; and describe preliminary work carried out in the absence of surfactant with an ion exchange resin providing a pseudomicellar environment for the hydrolysis of **4a** by catalysts **6a** and **6b**. We hope these investigations will ultimately lead to useful decontamination or prophylactic applications.

Results

Substrates. The chemical warfare agents **4a**, **4c**, **4d**, and **4e** were of greater than 95% purity (see Experimental Section). Paraoxon (**1b**) was obtained from Sigma Chemical Co. and used as received.

Kinetic Studies. Hydrolysis reactions for the organophosphonofluoridates **4a** and **4c** were followed with an ion-specific electrode to assess release of fluoride over the course of the reaction. Prior to each kinetic experiment, electrodes were standardized with known concentrations of NaF. Following standardization, hydrolysis reactions were initiated by the addition of aliquots of catalyst (prepared fresh daily in dimethylformamide) to a reaction mixture containing substrate and CTAC in pH 7.5 phosphate buffer. The mixture was typically thermostated at 25.0 ± 0.1 °C. Production of fluoride was followed by automatic data collection. Pseudo-first-order rate constants were derived from the data with a nonlinear exponential fitting program[14] or from plots of ln ($[F^-_{inf}] - [F^-_t]$). Hydrolysis reactions for these substrates and for **4d** and **4e** were also followed with a pH-stat technique. In these cases production of proton during the reaction was automatically titrated, with the resulting data used to derive the pseudo-first-order rate constants. Hydrolysis of **1b** was followed spectrophotometrically.

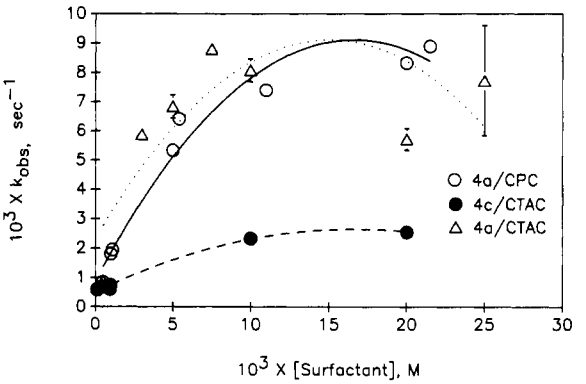

Figure 3. Pseudo-first-order rate constants (k_{obs} ± SEM, s^{-1}) for the cleavage of soman (**4a**) and sarin (**4c**) by 1×10^{-4} M **6a** as a function of [surfactant]: cetylpyridinium chloride (CPC) with soman (○), CTAC with soman (△), or sarin with CTAC (●) at pH 7.5. See text for reaction conditions.

Compounds **6a**, **6b**, and **6d** all promoted substantial increases in the observed hydrolysis rates for **4a** and **4c** over the background reaction rate, and all appeared to be truly catalytic against these substrates. The catalytic results for these compounds with **4a** and **4c** are shown in Figures 1 and 2. Figure 1 summarizes data showing the dependence of the pseudo-first-order rate constant for **4a** hydrolysis on catalyst concentration in aqueous solutions of **6a**, **6b**, and **6d** containing micellar CTAC in pH 7.5 phosphate buffer at 25 °C. Derivative **6b** demonstrated enhanced catalysis against **4a** with approximately 2-fold higher activity (at 1×10^{-4} M) than the parent compound. The 5-nitro derivative **6d** was found to show approximately 2-fold lower activity than the parent compound, perhaps due to the electron-withdrawing effect of the *p*-nitro group.

Selected examples of these reactions were also carried out with either a titrametric procedure, which followed proton production, or an enzymatic assay technique, which assessed loss of acetylcholinesterase inhibitory activity with reaction time. These techniques are described under Experimental Section. Pseudo-first-order rate constants for the hydrolysis of **4a**, determined either by following the production of fluoride ion or by monitoring proton release during reaction, were in good agreement. Results showed no statistically significant differences in the k_{obs} values at the $p < 0.05$ level. Enzymatic evaluation of the amounts of active inhibitor remaining at various times during the reaction of **4a** confirmed the loss of inhibition activity as hydrolysis proceeded.

The rate of **4a** hydrolysis by **6a** was dependent on the ionic strength of the reaction mixture. Thus, at pH 7.5, with ionic

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(13) Other workers have examined the activity of **6d** against soman and VX in microemulsions. In this medium, however, only minimal rate enhancements were found, with approximately a factor of 2 increase in the rate of soman hydrolysis occurring in the presence of **6d** compared to the background microemulsion rate. See: Hovanec, J. W.; Durst, H. D.; Ward, J. R. The Catalysis of the Hydrolysis of GD and VX by ortho-Iodobenzoates in a Strongly Cationic Microemulsion; In *Proceedings of the 1986 U.S. Army Chemical Research, Development and Engineering Center Scientific Conference on Chemical Defense Research*; Rousa, M. D., Ed.; CRDEC-TR-87008; June 1987; pp 111–115.

(14) Nonlinear exponential regression program written by John R. Lowe, Copyright 1984. Lieske, C. N.; Clark, J. H.; Lowe, J. R.; Horton, G. L.; Jewell, D. K.; Dceasch, L. W.; Edgewood Arsenal Technical Report EB-TR-76113, Aberdeen Proving Ground, MD, Dec 1976; DTIC AD A035231.

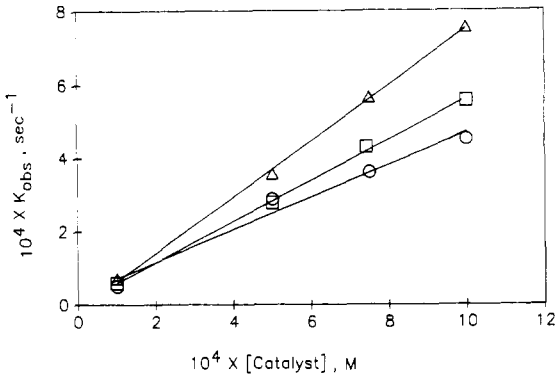


Figure 4. Paraoxon (**1b**) hydrolysis. Pseudo-first-order rate constants ($k_{obs} \pm$ SEM, s^{-1}) as a function of [catalyst] for **6a** (□), **6b** (△), and **6d** (○) at 0.01 M CTAC, pH 7.5. See text and Table II for reaction conditions.

strength = 0.092 and 25 °C, with CTAC and **6a** concentrations of 1×10^{-2} and 1×10^{-5} M, respectively, $k_{obs} = 6.19 \times 10^{-4}$ s^{-1}. Under identical conditions but with $\mu = 0.01$, a k_{obs} of 2.83×10^{-3} s^{-1} was found, a 4.6-fold rate enhancement.

Figure 2 shows results for the hydrolysis of **4c** by these same catalysts. While the same order of catalytic activity was found for **6a**, **6b**, and **6d** with both **4a** and **4c**, i.e., k_{obs} values for these reactions varied in the order **6b** > **6a** > **6d**, **4a** hydrolyzed approximately 2-fold faster than **4c**. This was somewhat unexpected, on the basis of steric considerations, but may arise from the increased lipophilicity of **4a** vs **4c**, thus allowing for a more favorable partitioning of **4a** into the micelle.

The dependence of k_{obs} on surfactant concentration was also examined, as shown in Figure 3. In these k_{obs} vs [surfactant] experiments, conditions were typically as follows: reactions were carried out in 0.1 M, pH 7.5 phosphate buffer, ionic strength 0.083–0.13, 2.4% (v/v) DMF, 25.0 ± 0.1 °C, [substrate] = 1×10^{-3} M, [**6a**] = 1×10^{-4} M; and [surfactant] as indicated. For the hydrolysis of **4a**, the choice of cationic surfactant does not appear to make a critical difference. (Thus, comparable curves were found for the observed first-order rate constants determined for the hydrolysis of this substrate using either CTAC or CPC as the surfactant.) For the hydrolysis of **4c** in CTAC micellar solutions, the rates of catalytic turnover appear to plateau at levels approximately half that of the comparable rate for **4a** hydrolysis. Higher concentrations of CPC (2-fold, data not shown) slightly decreased the k_{obs} for **4a** hydrolysis.

Paraoxon (**1b**) was also examined as a substrate for compounds **6a**, **6b**, and **6d**. The results for the observed first-order rate constants for this substrate, plotted as a function of catalyst concentration, are shown in Figure 4.

While the relative order of catalytic efficiency remains the same, the change in magnitude between the rate constants observed for **6a**, **6b**, and **6d** at [catalyst] of 1×10^{-4} M is less than that found for the phosphonates **4a** and **4c**. In addition, under comparable conditions, the k_{obs} value for **4a** hydrolysis was found to be approximately 138 times greater than that for **1b** hydrolysis. Thus, while simulants may provide useful information on the relative activity of a decontaminant, corroboration of the simulant results with data on the actual target compounds is critical. Many factors are likely to influence these differences in the rate of hydrolysis from one substrate to the next. For example, substrate hydrophobicity and the electronic characteristics of the central phosphorus atom in a substrate undoubtedly play a role. These issues will be discussed in future papers.

Tabun (**4d**) and VX (**4e**) have also been examined as substrates for these catalytic compounds. For these reactions, hydrolysis was followed titrametrically. At pH 7.5, $\mu = 0.1$, and 25 ± 0.1 °C, with **6a** and CTAC concentrations of 1×10^{-4} and 1×10^{-2} M, respectively, 1×10^{-3} M **4d** was hydrolyzed with a k_{obs} of 1.99 (SD = 0.33) $\times 10^{-3}$ s^{-1}. Under the same conditions, but with **6b** as the catalyst, the pseudo-first-order rate constant was determined to be 7.72 (SD = 0.23) $\times 10^{-3}$ s^{-1}.

Table II. Comparison of Pseudo-First-Order Rate Constants for the Hydrolysis of Various Substrates by **6a**, **6b**, and **6d** at pH 7.5 and 25 °C[a]

cat.	substrate	$10^3 k_{obs}$, s^{-1} [b]	k_o, s^{-1} [c]	k_{obs}/k_o
6a	soman (**4a**)	8.08 (0.39)	3.07×10^{-4}	26.3
6a	soman	7.46 (0.27)[d]	3.07×10^{-4}	24.3
6b	soman	22.35 (1.1)	3.07×10^{-4}	72.8
6d	soman	4.77 (0.73)	3.07×10^{-4}	15.5
6a	sarin (**4c**)	2.35 (0.05)	1.04×10^{-4}	22.6
6b	sarin	7.73 (1.3)	1.04×10^{-4}	74.3
6d	sarin	1.61 (0.12)	1.04×10^{-4}	15.5
6a	tabun (**4d**)	1.99 (0.08)[d]	2.37×10^{-4}	8.4
6b	tabun	7.72 (0.17)[d]	2.37×10^{-4}	32.6
6a	paraoxon (**1b**)	0.059 (0.00)	4.53×10^{-6}	13.1
6b	paraoxon	0.071 (0.00)	4.53×10^{-6}	15.7
6d	paraoxon	0.050 (0.00)	4.53×10^{-6}	11.1

[a] Conditions: 0.1 M phosphate buffer (except as noted for runs in which proton production was followed), pH 7.5, μ = 0.092–0.12, 2.4 vol % DMF, 25 ± 0.1 °C, [substrate] = 1×10^{-3} M except for [paraoxon] = 6.5×10^{-5} M, [catalyst] = 1×10^{-4} M, and [CTAC] = 1×10^{-2} M. [b] k_{obs} values under these standard conditions; values are uncorrected for the background hydrolysis rate. Reactions followed by ion-specific electrode (soman and sarin, except as noted), by titration of proton produced during hydrolysis (tabun and soman), or by spectrophotometric means (paraoxon). Procedures are described in detail under Experimental Section. Numbers in parentheses are the SEM for each value. [c] Observed first-order rate constants for hydrolysis of substrate in CTAC but in the absence of catalyst. [d] Reactions followed by titration of proton produced during hydrolysis of substrate.

For reactions in which **4d** was the substrate and **6a** was the catalyst, the amount of proton produced was typically lower than the theoretical amount. With 1×10^{-4} M catalyst, the average amount of proton produced during the entire reaction was only 67%, on average, of the theoretically predicted amount. This was not the case when this same catalyst was used with **4a** as the substrate. It is possible that with catalyst **6a** the hydrolysis of **4d** proceeds by two or more pathways, at least one of which does not produce an equivalent of proton. This would lead to production of less than an equivalent of proton over the course of the reaction. Further work is required to thoroughly investigate this possibility. Compound **6a** was not an effective catalyst for the hydrolysis of **4e**. At pH 7.5, 25 °C, with 2×10^{-3} M **6a** and 1×10^{-2} M CTAC, hydrolysis of this agent was very slow.

Table II shows a comparison of the pseudo-first-order rate constants for the hydrolysis of **4a**, **4c**, **4d**, and **1b** under similar conditions, i.e., 1×10^{-4} M catalyst and 1×10^{-2} M CTAC, in aqueous buffer or under pH-stat conditions at 25 °C and pH 7.5.

Even in the absence of surfactant, catalyst **6a** was found to enhance the hydrolysis of **4a** over the background rate found in bicarbonate buffer, pH 8.2. Furthermore, both **6a** and **6b** show even greater activity against this substrate when ionically exchanged onto a quaternary ammonium hydroxide polymeric resin.[15] Compounds **6a** and **6b**, functioning as the counterion for the polymer-bound quaternary ammonium moiety in the relatively hydrophobic resin environment, appear to maintain a significant portion of the enhanced activity found in a micellar environment. Typical results for studies in which **6a** was exchanged for approximately 10% of the theoretically available base sites on a polystyrene-based, quaternary trimethylammonium derived ion exchange resin are shown in Figure 5. In this experiment, the **6a**-loaded resin was suspended in 0.1 N $NaHCO_3$ with the hydrolysis of **4a** followed by periodic assays (ion specific electrode) for both total and free fluoride. Results for several control ex-

(15) Our laboratory has extensively examined a variety of polymeric quaternary ammonium hydroxide derived (Amberlite resins and others), as well as other counterion-derived, resins as potential decontaminants for toxic chemicals. These materials, available from the Rohm and Haas Co., Philadelphia, PA, have been shown to act as effective decontaminants of several of the substrates discussed in this paper (unpublished results). In addition, carbonaceous sorbent resins such as Ambersorb XE348F are effective at the rapid sorption of these same hydrophobic organophosphorus materials from aqueous mixtures. Resins were typically used as provided or were derivatized as indicated under Experimental Section.

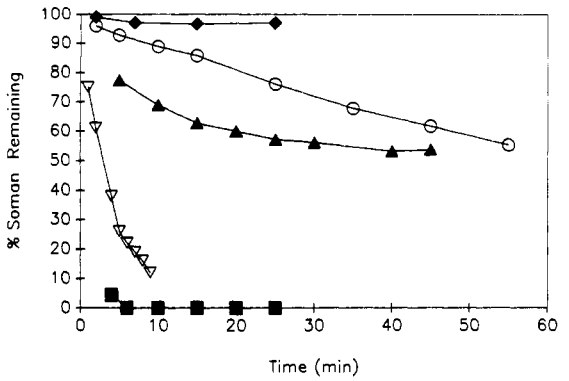


Figure 5. Percentage of originally available soman (**4a**) vs time under various nonmicellar conditions, that is, 0.01 N $NaHCO_3$ buffer, pH 8.2, and room temperature (25 ± 2 °C); initial [soman], and other conditions, as follows: (○) 8.8×10^{-4} M **4a** with no catalyst; (▽) 9.2×10^{-4} M **4a** with 1.7×10^{-3} M **6a**; (◆) 8.2×10^{-4} M **4a** with 1.7×10^{-3} M *iodo*benzoic acid and 0.5 g of ion exchange resin (IER); (■) 8.2×10^{-4} M **4a** with 1.67×10^{-3} M **6a** and 0.5 g of IER; (▲) 8.2×10^{-4} M **4a** with 1.67×10^{-3} M **6a**, 0.5 g of IER, and 0.5 g of XE348F sorbent resin. All reactions were followed with an ion-specific electrode to monitor release of fluoride ion, and all resin reactions were carried out in 60-mL total volume.

periments are also summarized in Figure 5.

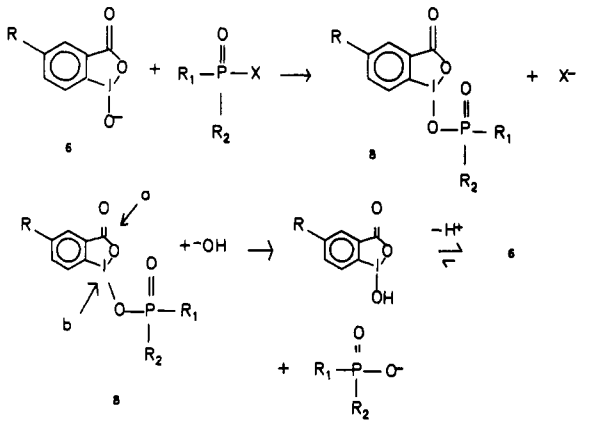
Compound **4a** was hydrolyzed even in the absence of surfactant, at an appreciable rate in 0.1 N $NaHCO_3$ (○); only about 55% of the initial **4a** concentration remained at 60 min following mixing. The addition of **6a** (▽), at 1.7×10^{-3} M, increased the rate of hydrolysis 22.5-fold over the uncatalyzed rate, with $k_{obs} = 4.43 \times 10^{-3}$ (SD = 0.07) s^{-1}. This corresponds to a second-order rate constant of 2.6 M^{-1} s^{-1} vs 80.8 M^{-1} s^{-1} for the CTAC-mediated, micellar reaction at pH 7.5. A significant increase in the hydrolysis rate was found in the presence of a **6a**-loaded ion exchange resin, with approximately 10% of the available ion exchange sites occupied by the counterion **6a**. Under these conditions, complete hydrolysis (■) of **4a** occurred in 3 min or less (earliest time point taken). As a control, resin with 10% of the available ion exchange positions occupied by 2-*iodo*benzoic acid (◆) gave little hydrolysis of **4a** over a 25-min period. Interestingly, the addition of 0.5 g of a carbonaceous sorbent resin to the **6a**-derived ion exchange resin (▲) slows hydrolysis of the agent markedly. This could occur if substrate partitions onto the more hydrophobic, but unreactive sorbent resin.

Discussion

A possible mechanism (Scheme II) for the activity of catalysts such as **5** (**6**) against organophosphorus esters has been suggested previously.[6c,16] It involves the nucleophilic attack of the oxyanion **6** on the central phosphorus atom of substrate to form the phosphylated intermediate **8**, with concomitant loss of leaving group X^-. The proposed intermediate may then hydrolyze by several pathways: (a) attack of OH^- (H_2O) on the carboxylic carbonyl group with subsequent ring opening and loss of $^-OP(O)R_1R_2$ to generate the *o*-iodosobenzoate form with later recyclization to **6**,[6c,17] or (b) direct attack of ^-OH (H_2O) on iodine with subsequent loss of $^-OP(O)R_1R_2$ to regenerate **6**. Data on reactions of these types of phosphylated intermediates, which would support either mechanism directly, are not available.[6c] Recent work on the acylated analogue of intermediate **8**[17] indicates the likelihood that pathway b is the predominant path.

We have shown *o*-iodosobenzoic acid and its derivatives, presumably acting in the cyclic valence tautomeric form **6**, to effectively promote the decomposition of several toxic organophosphorus compounds under mild conditions. Soman (**4a**), sarin (**4c**), tabun (**4d**), and paraoxon (**1b**) are all rapidly hydrolyzed in the presence of micellar concentrations of cationic surfactant.

Scheme II



The half-life of **4a** was reduced to approximately 86 s when its hydrolysis was followed at 1×10^{-4} M **6a** with 0.01 M CTAC at a pH of 7.5 and 25 °C. This represents a 26-fold increase over the surfactant-only hydrolysis rate (in the absence of **6a**), where the half-life was found to be 37.6 min. In the absence of both the micellar surfactant and **6a**, the half-life for soman at 27 °C is reported to be approximately 6.6 h in pH 7.4 (0.02 M) phosphate buffer.[18] Similar enhancements in the rate of hydrolysis for compounds **4c**, **4d**, and **1b** were also found.

As others have demonstrated, **6b** was a more efficient catalyst for the hydrolysis of all substrates examined than were either the parent compound **6a** or the 5-nitro derivative **6d**. In general, at 1×10^{-4} M catalyst concentrations, **6b** provides an approximately 2–4-fold rate enhancement over the parent compound **6a**, while **6d** shows about one-half to one-third the activity of **6a** against compounds **4a**, **4c**, and **4d**.

While the 26-fold rate enhancement over the uncatalyzed rate found for the hydrolysis of **4a** by **6a** represents a substantial increase, it is of note that this is a much smaller increase than that found for other substrates. For example, catalyst **6e** provides an apparent kinetic advantage for the hydrolysis of the phosphate **1a** of 14 700[6c] (from k_{obs}/k_o, where k_o is the uncatalyzed, micellar-mediated pseudo-first-order rate constant). Similarly, the kinetic advantage for the hydrolysis of **1b** by this same catalyst was found to be 43 600. In contrast, the comparable rate advantage for the hydrolysis of the methylphosphonate **1c** was found to be only 846 over the background turnover rate. This represents a substantial decrease in the ability of this catalyst to promote the hydrolysis of the phosphonate substrate when compared to that found for the phosphate substrates.

We have observed a similar phenomenon when comparing the relative activity of one catalyst to another. For example, with **1a**, catalysts **6a** and **6b** gave second-order rate constants of 645 and 14 426 M^{-1} s^{-1},[8b] respectively, representing an increase in the hydrolysis rate of 22.3-fold over the parent compound **6a** for catalyst **6b**. For catalysts **6a** and **6b** with **4a** as substrate, we found the second-order rate constants to be 80.8 and 223.5 M^{-1} s^{-1}, respectively. This represents both a more modest second-order rate constant and only a 2.8-fold increase in the hydrolysis rate effected by **6b** when compared to **6a**. For hydrolysis of paraoxon (**1b**), second-order rate constants were much smaller, with values of 0.59, 0.71, and 0.50 M^{-1} s^{-1} for the catalysts **6a**, **6b**, and **6d**, respectively. For this substrate, note that there was almost no difference in the rate constants for these three catalysts and that the values of the second-order rate constants were 2 orders of magnitude smaller than those found for **4a**, **4c**, and **4d**.

An additional issue is raised by data of Katritzky et al.[19] For a series of five 5-substituted derivatives of both *o*-iodoso and *o*-iodoxybenzoic acid, the second-order rate constants for the hydrolysis of substrates **1a** and **1d** were determined. For this series

(16) Mackay, R. A.; Longo, F. R.; Knier, B. L.; Durst, H. D. *J. Phys. Chem.* **1987**, *91*, 861.
(17) Moss, R. A.; Scrimin, P.; Rosen, R. T. *Tetrahedron Lett.* **1987**, *28*, 251.
(18) Broomfield, C. A.; Lenz, D. E.; MacIver, B. *Arch. Toxicol.* **1987**, *59*, 261–265.
(19) Katritzky, A. R.; Duell, B. L.; Durst, H. D.; Knier, B. *Tetrahedron Lett.* **1987**, *28*, 3899.

of catalysts, the relative order of catalytic efficiency was different for the different substrates. Thus, the substrate used to evaluate the effectiveness of a catalyst can both play a role in the magnitude of the kinetic advantage observed and influence the order of the apparent advantage from catalyst to catalyst. Clearly, choice of the simulant to be used plays a significant role in allowing one to accurately predict the activity against a more reactive substrate. These are important questions when attempting to choose a candidate catalyst for further development as a decontaminant of toxic organophosphorus compounds and emphasizes the need for work with the actual substrates of interest.

We found the *o*-iodosobenzoates to be catalytic in their activity. Under conditions where substrate was at least 10-fold in excess over the catalysts **6a**, **6b**, or **6d**, no deviation from normal first-order kinetics was demonstrated for **4a**, **4c**, **4d**, or **1d**. For the cleavage of **1a**, others have demonstrated[8] that compounds **6a** and **6b** show true turnover catalysis in CTAC micellar solutions. Furthermore, these catalysts did not demonstrate burst kinetics ($k_1 \gg k_2$, Scheme I) for the hydrolysis of this substrate. For **1b** at 1.0×10^{-4} M, we observed no evidence for burst kinetics when **6a** was used as the catalyst at a concentration of 4×10^{-5} M in the presence of 0.01 M CTAC. For reactions in which **4a** or **4c** was substrate, data fit to a first-order model and extrapolated to zero time showed no evidence of burst kinetics for release of fluoride with either of these compounds. It should be noted, however, that the response time of the ion-specific electrode limits its sensitivity in this type of kinetic application.

Hydrolysis data for substrates **4a**, **4c**, and **1b** obeyed pseudo-first-order kinetics, as did the data for **4d** with catalyst **6a**. Other factors, however, indicate that the mechanism for the hydrolysis of this latter substrate by **6a** may be more complicated. At pH 7.5, hydrolysis of **4a** (or **4c**) should proceed with the liberation of 2 equiv of proton for every equivalent of substrate hydrolyzed (see Scheme II). For **4a**, this was found to be the case when the reaction was followed titrametrically. However, for **4d**, where release of a single proton is expected,[20] only about 67% of the theoretical equivalency was found to be liberated during the reaction with **6a**. This situation could arise if two parallel reactions were occurring simultaneously: the first following the expected reaction pathway (Scheme II) and the second proceeding via breakdown of intermediate **8** with loss of $(CH_3)_2NH$, leading ultimately to a new species of **8** where R_1 or R_2 is $-O^-$. The result for that side of the hypothetical reaction pathway would be the production of zero protons, since the amine liberated would be protonated at the reaction pH. A net production of less than 1 equiv of proton for the overall reaction would occur. We have no evidence to support this hypothesis but believe this to be an important consideration since this would represent a situation analogous to aging of a phosphylated enzyme.[21]

The ability of *o*-iodosobenzoate (**5a**) to hydrolyze **4a** in the absence of the micellar agent (see Figure 6) was of interest. But of even greater significance was the ability of **6a** and the 5-(*n*-octyloxy) derivative **6b** to promote enhanced hydrolytic activity when these are exchanged onto a cationic ion exchange resin. This observation raises interesting questions concerning the nature of the interaction of these catalysts with the relatively hydrophobic environment of an ion exchange resin. It also allows for considerable flexibility in the application of these compounds as decontaminants.

We have demonstrated that compounds **6a**, **6b**, and **6d** act as efficient catalysts for the hydrolysis of a variety of toxic organophosphorus substrates and that this activity is not limited to a micellar environment. In addition, we have shown that these compounds, under the conditions examined, appear to obey first-order kinetics, but with perhaps a complicated mechanism operating with substrate **4d**. The lack of significant hydrolytic activity for catalyst **6a** against **4e** was disappointing but not unexpected. Under most conditions, **4e** shows a much slower hydrolysis than any of the other organophosphorus agents, with rates approximately 240-fold slower than the rates for **4a** hydrolysis under alkaline conditions.[22] This result points out the need for synthetic modification of the parent compound to achieve catalysts with enhanced activity against these types of organophosphorus esters.

Experimental Section

Materials. Compounds **4a**, **4c**, **4d**, and **4e** were synthesized[23] by the Chemical Research, Development and Engineering Center's (CRDEC) Research Laboratory. The identity of each compound and purity were assayed by NMR spectroscopy. These compounds were also analyzed (GC) for content by the Analytical Chemistry Branch, USAMRICD, Aberdeen Proving Ground, MD. The compounds were found to be of greater than 95% purity. They were prepared as dilute solutions in water or saline and stored frozen prior to use. 5-Nitro and 5-(*n*-octyloxy) analogues of *o*-iodosobenzoic acid (**5d** and **5b**, respectively) were obtained from the CRDEC Laboratories, Aberdeen Proving Ground, MD. They were prepared by literature methods (**5d**[24] and **5b**[8b]) and were used as received. Acetylthiocholine iodide, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylcholinesterase (AChE), *o*-iodosobenzoic acid, and paraoxon (**1b**) were obtained from Sigma Chemical Co. Cetyltrimethylammonium chloride (CTAC, 1-hexadecyltrimethylammonium chloride) and cetylpyridinium chloride (CPC, 1-hexadecylpyridinium chloride) were obtained from Kodak Laboratory Chemicals. Ambersorb, XE348F (a carbonaceous sorbent resin), and Amberlite ion exchange resins were obtained from Rohm and Haas Co., Spring House, PA.

Cationic ion exchange resins in the hydroxide form were modified to contain either **5a** or **5b** through the following procedure: to a solution of 0.1 mmol of **5a** or **5b** in 36 mL of ethanol was added 0.50 g of ion exchange resin (approximately 2.4 mequiv of ^-OH/g of resin); the mixture was stirred for at least 1 h, followed by solvent removal in vacuo.

Aliquots of **1b** were hydrolyzed in 0.1 N NaOH with the *p*-nitrophenolate released measured spectrophotometrically to assess purity of the material. Three samples gave an average of 100.0 ± 0.1% of the theoretically available *p*-nitrophenolate. All kinetic studies were carried out in buffers or aqueous solutions prepared from doubly distilled water. All reagents and buffers were of reagent grade.

Kinetic Studies. Reactions were followed through measurement of fluoride release, through release of proton, or spectrophotometrically. For reactions in which fluoride release was followed, an Orion Model EA940 ion analyzer with an Orion combination fluoride electrode was used. For reactions monitored spectrophotometrically, a Varian DMS 90 spectrophotometer with a DS-15 data acquisition and analysis station was used, with data analysis carried out through Varian's first-order kinetics software package. Rate constants derived by this procedure were in good agreement with rate constants obtained from the same absorbance vs time data but analyzed with an in-house nonlinear exponential regression program or by linear regression of ln ($Abs_{inf} - Abs_t$) vs time plots of the data. For reactions in which proton release was followed, a Radiometer system was utilized, consisting of a TTT80 titrator, a PHM82 pH meter, an ABU80 autoburet, a REC servograph, a TTTA titrator assembly, and Radiometer pH and reference electrodes.

For reactions in which release of fluoride was measured, a standard curve was established with solutions of catalyst, buffer, and surfactant and a sodium fluoride standard solution. The concentrations of surfactant, buffer, and catalyst were equivalent to those in the actual reaction solutions. Final concentrations of NaF used for the standard solutions were 1.0×10^{-3} and 1.0×10^{-4} M. A typical kinetic run for the hydrolysis of **4a** or **4c** was carried out as follows: to a 5.0-mL reaction flask

(20) This is in contrast to the results for soman hydrolysis followed by this same method where, under the same conditions with 1×10^{-4} M **6a**, the hydrolysis reaction produced 94.7 ± 2.7% (SEM) of the theoretically expected two-proton equivalence. It was anticipated that the hydrolysis of tabun (**4d**) should produce 1 equiv of proton. This was based on the expectation that at pH 7.5, with CN^- as the leaving group, only 1 proton equiv should be produced for each equivalent of **4d** hydrolyzed, since CN^- would be protonated (pK_a 9.1) at this pH. pK_a value of CN^- taken from *The Chemist's Companion*; Gordon, A. J.; Ford, R. A., Eds.; Wiley: New York, 1972; p 61.

(21) Michel, H. O.; Hackley, B. E., Jr.; Berkowitz, L.; List, G.; Hackley, E. B.; Gillilan, W.; Pakau, M. *Arch. Biochem. Biophys.* **1967**, *121*, 29.

(22) See citation in ref 13 on the hydrolysis of **4a** and **4e**.

(23) Compounds **4a**, **4c**, **4d**, and **4e** were synthesized by procedures similar to those found in the following references: **4a**, Schrader, G. Die Entwicklung neuer Insektizide auf Grundlage organisher Fluor- und Phosphorverbindungen; Monograph No. 62; *Angewandte Chemie und Chemie-Ingenieur-Technik*; Verlag-Chemie: Weinheim, 1951 (See also *Protar* **1950**, *16*, 131). **4c**, Bryant, P. J. R.; Ford-Moore, A. H.; Perry, B. J.; Wardrop, A. W. H.; Watkins, T. F. *J. Chem. Soc.* **1960**, 1553. **4d**, Holmstedt, B. *Acta Physiol. Scand.* **1951**, *25* (*Suppl. 90*), 26. **4e**, Eckhaus, S. R.; Davis, J. C., Jr.; Zeffert, B. M.; Moore, T. R. U.S. Pat. 3,911,059, 1975. Authors' note—these compounds are **extremely toxic** and should not be prepared or handled by inexperienced personnel.

(24) Morrison, G. F.; Hooz, J. *J. Org. Chem.* **1970**, *35*, 1196.

was added 0.208 mL (0.164 M) aqueous CTAC, 0.240 mL of aqueous substrate (1.425×10^{-2} M), and 2.891 mL of phosphate buffer. To initiate the reaction, a 0.081-mL aliquot of catalyst, freshly prepared in DMF, was then added to this solution, with rapid stirring. Final concentrations of substrate were typically 1×10^{-3} M. Final concentrations of the catalyst and surfactant used in each reaction were as noted in the text. Reaction temperatures were maintained at 25.0 ± 0.1 °C through the use of a constant-temperature water bath. Reactions were carried out with constant stirring and were allowed to run to 80% or greater hydrolysis while data were collected automatically. Rate constants, k_{obs}, were calculated either with an exponential fitting routine or through linear regression analysis of ln ($[\text{fluoride}]_{inf} - [\text{fluoride}]_t$) vs time. Infinity-point values, predicted by the exponential fitting routine, were in good agreement with observed final fluoride concentrations. Reproducibility for duplicate runs was good and is indicated by standard deviation values provided in the text.

Enzyme Inhibition Studies. To confirm the results obtained in the kinetic runs and to demonstrate loss of enzyme inhibition activity (decontamination) for the organophosphorus substrates, selected hydrolysis runs were tested for loss of AChE inhibition activity at various time points during the reaction. To carry out these studies, an assay procedure for enzymatic inhibitory activity was developed which made use of a Titertek Multiscan MCC plate reader (Flow Laboratories) and 96-well plates.[25] The assay is based on the hydrolysis of acetylthiocholine by uninhibited and inhibited AChE, with subsequent production of absorbance at 414 nm via reaction of the liberated thiocholine with DTNB. Plots of log (percent control enzyme activity) vs [inhibitor] provide standard curves from which unknown concentrations of inhibitor (substrate) could be determined at various times during an experiment.

For experiments in which the release of hydrogen ion was followed as a function of time, pH electrodes were standardized at pH 7.0 and 10.0. For each set of runs, standardized 0.01 M potassium hydroxide was used to maintain a constant pH during the course of the titration. KOH solutions were standardized with 0.01 M potassium acid phthalate, in triplicate. All reactions were carried out under a blanket of nitrogen to prevent adsorption of CO_2. In a typical reaction, an appropriate amount of sodium chloride solution at the required ionic strength was added to the reaction chamber (thermostated at 25.0 ± 0.1 °C) and titrated to pH 7.5. Surfactant (in aqueous solution) and catalyst in dimethylformamide (or substrate) were then added and titrated to pH 7.5, with the amount of base required for each step recorded. (In cases where substrate was added first, a background hydrolysis rate could be followed for several minutes prior to addition of the catalyst.) To initiate the catalyzed reaction, an aliquot of substrate in aqueous solution (or catalyst) was then added. The rate of base addition required to maintain a constant pH was recorded as a function of time. The amount of base required for any given reaction was less than 10% of the total volume, thus limiting the effects of dilution on the reaction rate. Reactions were typically allowed to proceed to at least 80% total theoretical proton production. Rate constants were derived through analysis, using a nonlinear regression routine,[14] of volume of base added (or the amount of proton titrated) as a function of time or were derived through linearization of the data (ln [substrate] vs time), followed by linear regression analysis.

Reactions on the ion exchange resins were carried out in a manner similar to the above studies and were followed with an ion specific electrode. Typically, 0.50 g of the modified resin was suspended in 55.5 mL of 0.1 M $NaCHO_3$ aqueous buffer, held in an ultrafiltration cell; to this rapidly stirred suspension was added 4.5 mL of 1.425×10^{-2} M substrate. Periodically, samples of filtrate and slurry were removed for fluoride analysis.

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Registry No. 1a, 330-13-2; **1b**, 311-45-5; **3**, 107556-72-9; **4a**, 96-64-0; **4c**, 107-44-8; **4d**, 77-81-6; **5a**, 304-91-6; **5b**, 89031-96-9; **5d**, 23330-00-9; **6a**, 84280-67-1; **6b**, 117203-79-9; **6c**, 104807-59-2; **6d**, 104807-60-5; **6e**, 107164-49-8.

(25) Accepted by *Anal. Biochem.* for publication. See also: Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88. Simpson, I. A., Sonne, O. *Anal. Biochem.* **1982**, *119*, 424. Brogdon, W. G., Dickinson, C. M. *Anal. Biochem.* **1982**, *131*, 499.

New Host Family Based on Small-Ring Compounds

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Abstract: Three- and four-membered ring compounds with functional groups and bulky substituents have proved to be a rewarding new source of inclusion hosts. These hosts form clathrates with a variety of uncharged organic molecules ranging from protic dipolar to apolar compounds (168 different inclusion species). Formation and selectivity depend in a systematic manner on structural parameters of the host, such as the nature, number, and position of functional groups, the substituents, and ring size. X-ray structure analyses of two inclusion compounds [**1**·*t*-BuOH (1:1): $P2_12_12_1$; $a = 9.782$ (1), $b = 11.376$ (1), $c = 17.603$ (1) Å; $Z = 4$. **17**·MeCN (1:1): *Pbcn*; $a = 12.314$ (1), $b = 16.074$ (1), $c = 12.938$ (1) Å; $Z = 4$] and of a free host molecule [**1**: $P2_1$; $a = 7.339$ (2), $b = 11.657$ (4), $c = 9.149$ (3) Å; $\beta = 110.07°$; $Z = 2$] are reported, revealing the building principles of the new clathrate family. The structures exhibit linear chains of inter-/intramolecular H bridges between carboxylic groups in the free host **1** and H-bridge aggregation of host and guest molecules in infinite helical chains for the **1**·*t*-BuOH (1:1) inclusion. In **17**·MeCN (1:1), the guest molecules are tightly enclosed by the host framework without further specific interactions.

Host–guest complexes[1] including clathrates[2] are expected to play an important role in the solution of theoretical and practical problems in chemistry and related fields.[3] The applicability may depend on designed host molecules becoming available in wide variety. We describe here the first examples **1–25** (see Table I) of a new host family possessing selective clathrate-forming properties. Their structures are based on a central small-ring

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(1) (a) *Host Guest Complex Chemistry—Macrocycles*; Vögtle, F., Weber, E., Eds.; Springer-Verlag: Berlin, Heidelberg, 1985. (b) Synthesis of Macrocycles: The Design of Selective Complexing Agents. *Progress in Macrocyclic Chemistry*; Izatt, R. M., Christensen, J. J., Eds.; Wiley-Interscience: New York, 1987; Vol. 3.

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