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Kinetics of the Micellar Nucleophilic Cleavage of Diastereomeric Phosphotriesters

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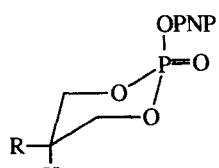
Summary. Diastereomeric phosphotriesters **1** and **2** are rapidly cleaved by micellar iodosobenzoate, iodosonaphthoate, and cetyltrimethylammonium hydroperoxide; diastereoselectivity is modest. © 1997, Elsevier Science Ltd. All rights reserved.

The decontamination of toxic phosphonates and phosphates remains topical,[1] with urgency engendered by the March 1995 Sarin attack in Tokyo.[2] Among the many nucleophiles surveyed for phosphorolytic properties against either the actual toxins or less dangerous simulant, or model substrates, micellar *o*-iodosobenzoate (IBA)[3] stands out as a potent catalyst, active at moderate pH.[3,4] In parallel to this "physical organic" approach to decontamination, biological methods for the neutralization of such phosphorous toxins as sarin, soman, paraoxon, and parathion focus on phosphotriesterase, an enzyme capable of mediating their rapid hydrolysis.[5] Not surprisingly, the related strategy of generating catalytic antibodies,[6] designed to perform the same task, has recently been demonstrated.[7]

Antibody Tx1-4C6, raised against an appropriate hapten, was shown to catalyze the hydrolyses of the diastereomeric phosphotriester substrates **1** and **2**, with rate enhancements of 364 or 21, respectively, compared to the analogous uncatalyzed reactions at pH 8.5.[7]



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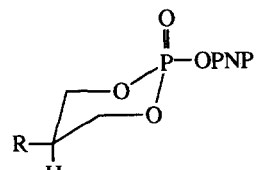

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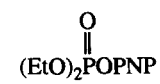

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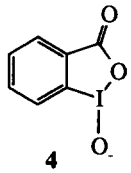
(R = $PhMe_2C$; PNP = *p*-nitrophenyl)

In that they are dialkyl *p*-nitrophenyl phosphates, substrates **1** and **2** are related to paraoxon, **3**, a persistent insecticide and a target of IBA.[8,9] Our continuing interest in the reactivities of toxic phosphates and their simulants[9] has now led us to determine the phosphorolytic efficacy toward **1** and **2** of (micellar) IBA (**4**), 2,3-iodosonaphthoate (INA, **5**),[10] and cetyltrimethylammonium hydroperoxide (CTAOOH, **6**).[11] The latter reagent was recently found to be highly reactive toward paraoxon.[11] The results show that the micellar



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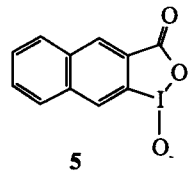

5

n-$C_{16}H_{33}N^{+}Me_3$ OOH^{-}

6

reagents are more efficient than the antibody from the standpoint of practical decontamination, although their diastereoselectivity remains inferior.

Substrates **1** and **2** were prepared as described, and characterized by TLC and NMR.[7] The catalysts were either obtained commercially (**4**) or prepared according to literature methods.[10,11] Hydrolyses of 1 x 10^{-5} M **1** or **2** were monitored spectrophotometrically, following released *p*-nitrophenylate ion at 400 nm. Pseudo-first-order rate constants were determined with standard computational methods; average deviations of duplicate runs were within ± 4%. Catalysts were used in excess, and cetyltrimethylammonium chloride (CTACl), when present, was maintained at 1 x 10^{-3} M. Kinetic results, together with reaction conditions, are collected in Table 1, which also contains data for antibody Tx1-4C6.[7]

Table 1. Kinetics of Phosphorolytic Cleavage of Phosphate Diastereomers[a]

Reagent	pH	k_ψ(1), s^{-1}	k_ψ(2), s^{-1}	k(2)/k(1)	k_{rel}(1)[b]	k_{rel}(2)[b]
Uncatalyzed[c]	8.5	4.62 x 10^{-7}	1.46 x 10^{-6}	3.16	1	1
Tx1-4C6[c]	8.5	1.68 x 10^{-4}	3.08 x 10^{-5}	0.183	364	21
Uncatalyzed[d]	10.0	1.61 x 10^{-5}	3.52 x 10^{-5}	2.19	35	24
Uncatalyzed[e]	12.0	5.25 x 10^{-4}	2.06 x 10^{-3}	3.92	1140	1410
CTACl[f]	8.0	2.72 x 10^{-6}	1.26 x 10^{-5}	4.64	2	8
CTACl[d]	10.0	1.26 x 10^{-4}	4.93 x 10^{-4}	3.91	272	338
CTACl[e]	12.0	1.83 x 10^{-2}	8.94 x 10^{-2}	4.88	39600	61200
IBA/CTACl[g]	8.0	1.21 x 10^{-4}	3.84 x 10^{-4}	3.17	262	263
INA/CTACl[g]	8.0	6.59 x 10^{-4}	1.82 x 10^{-3}	2.76	1430	1250
CTAOOH[h]	8.0	1.79 x 10^{-3}	2.13 x 10^{-3}	1.19	3870	1460
CTAOOH[h]	9.0	5.43 x 10^{-3}	1.50 x 10^{-2}	2.76	11750	10300
CTAOOH[h]	10.0	4.19 x 10^{-2}	7.75 x 10^{-2}[i]	1.85	90700	53100

[a]At 25-26 °C, unless otherwise indicated; [substrate] = 1 x 10^{-5} M. [b]Rate constant relative to uncatalyzed reaction of this substrate. [c]50 mM Bicine, 30 °C; data from ref. 7. [d]0.02 M HCO_3^-/CO_3^{2-} buffer. [e]0.02 M NaOH. [f]Cetyltrimethylammonium chloride, 1 x 10^{-3} M, 0.02 M NaH_2PO_4 (0.08 M NaCl). [g]1 x 10^{-4} M catalyst, 1 x 10^{-3} M CTACl, 0.02 M NaH_2PO_4 (0.08 M NaCl). [h]1.5 x 10^{-3} M CTAOH, 0.02 M H_2O_2. [i]The reproducibility of this value was ± 10%.

The uncatalyzed hydrolyses of substrates **1** and **2** at pH 8.5 are very slow (Table 1), with half-lives ranging from 132 (**2**) to 417 (**1**) hrs. The equatorial-PNP isomer, **2**, reacts ~3 times more rapidly than its axial diastereomer, **1**, a difference that has been attributed to ground state stabilization of **1** by the generalized anomeric effect.[7,12] The kinetic disparity is small, however, and differential stereoelectronic effects in the transition states for in-line $S_N2(P)$ displacements might also contribute or even dominate.[12,13] Whatever the origins(s) of the innate kinetic advantage, the data reveal that it persists at higher pH, in CTACl micellar reactions, and in CTACl micellar nucleophilic cleavages mediated by IBA, INA, or hydroperoxide.[14]

Only in the case of Tx1-4C6 is **1** cleaved more rapidly than **2**. That is, although the antibody catalyzes the hydrolysis of both substrates, it enhances the cleavage of **1** ~17 times more than that of **2** (factors of 364 vs. 21), so that the antibody cleaves **1** ~5.5 times faster than **2**. This result can be traced to the "design" of the antibody, which was elicited by an amine oxide hapten that specifically modeled the scissile P-O bond of **1**.[7]

Kinetic specificity toward **1**, however, is the only advantage displayed by the antibody. The nucleophilic catalyst IBA/CTACl is nearly as reactive toward **1** at pH 8 as Tx1-4C6, and is 12.5 times more reactive than the antibody toward **2**, while the reactivity of INA/CTACl exceeds that of Tx1-4C6 toward both substrates (factors of ~4 and ~60). CTAOOH exhibits still greater reactivity, even at pH 8: the enhancements of 3870 and 1460 are 10.6 and 69.5 times greater, respectively, than the analogous antibody catalysis.

Moreover, both INA and CTAOOH exhibit modest kinetic selectivities toward axial substrate **1**, *i.e.*, $k_{rel}(\mathbf{1}) > k_{rel}(\mathbf{2})$. In neither case is this preference strong enough to make the overall cleavage of **1** faster than that of **2**. However, these micellar nucleophiles appear to be more practical as decontaminants, are more reactive toward **1** and **2** than the antibody, and express enhanced selectivity for the axial substrate in the same sense as the antibody. At higher pH (*e.g.*, pH 10), CTAOOH cleaves substrates **1** and **2** very rapidly, with half-lives <20 sec, and enhancements >2200 relative to the "uncatalyzed" hydrolyses at pH 10. The extraordinary reactivity of CTAOOH toward paraoxon[11] is thus also apparent with **1** and **2**.[16]

Improvement in the reactivity of the antibody will require second generation haptens designed to elicit properly disposed nucleophilic side chains on the protein backbone to complement the existing electrostatic features[7] built into the antibody. Enhancing the selectivity of the micellar nucleophiles will be difficult, given the constantly fluctuating aggregate-nucleophile assembly, but functionalized surfactants might be designed to afford at least modest recognition of the substrates.

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References and Notes

(1) Yang, Y-C. *Chem. Ind.(London)* **1995** (1 May), 334; Yang, Y-C.; Baker, J.A.; Ward, J.R. *Chem. Rev.* **1992**, *92*, 1729.

(2) Ember, L. *Chem. Eng. News* **1995** (27 March), *6*.

(3) Moss, R.A.; Alwis, K.W.; Bizzigotti, G.O. *J. Am. Chem. Soc.* **1983**, *105*, 681; Moss, R.A.; Alwis, K.W.; Shin, J-S. *Ibid.* **1984**, *106*, 2651.

(4) Hammond, P.S.; Forster, J.S.; Lieske, C.N.; Durst, H.D. *J. Am. Chem. Soc.* **1989**, *111*, 7860.

(5) Vanhooke, J.L.; Benning, M.M.; Raushel, F.M.; Holden, H.M. *Biochemistry* **1996**, *35*, 6020; Benning, M.M.; Kuo, J.M.; Raushel, F.M.; Holden, H.M. *Ibid.* **1994**, *33*, 15001; and references therein.

(6) For leading references to the production and properties of catalytic antibodies, see Yli-Kauhaluoma, J.T.; Ashley, J.A.; Lo, G-H.; Tucker, L.; Wolfe, M.M.; Janda, K.D. *J. Am. Chem. Soc.* **1995**, *117*, 7041; Koch, T.; Reymond, J-L.; Lerner, R.A. *Ibid.* **1995**, *117*, 9383; Hilvert, D. *Acc. Chem. Res.* **1993**, *26*, 552; Schultz, P.G.; Lerner, R.A. *Ibid.* **1993**, *26*, 391; Schultz, P.G. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1283.

(7) Rosenblum, J.S.; Lo, L-C.; Li, T.; Janda, K.D.; Lerner, R.A. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2275.

(8) Moss, R.A.; Kim, K-Y; Swarup, S. *J. Am. Chem. Soc.* **1986**, *108*, 788.

(9) Moss, R.A.; Kotchevar, A.T.; Park, B.D.; Scrimin, S. *Langmuir* **1996**, *12*, 2200.

(10) Moss, R.A.; Zhang, H.; Chatterjee, S.; Krogh-Jespersen, K. *Tetrahedron Lett.* **1993**, *34*, 1729.

(11) Toullec, J.; Moukawin, M. *Chem. Commun.* **1996**, 221.

(12) Kirby, A.J. "The Anomeric Effect and Related Stereoelectronic Effects at Oxygen," Springer-Verlag, Berlin, 1983, pp 78*f*, 126*f*.

(13) Thatcher, G.R.J.; Kluger, R. *Adv. Phys. Org. Chem.* **1989**, *25*, 99, esp. pp. 171*f*.

(14) The IBA and INA results allow comparisons with paraoxon (**3**) cleavage. Under reasonably comparable conditions to those in entries 8 and 9, IBA and INA cleave **3** with k_{ψ} = 1.4 x 10^{-4} and 2.4 x 10^{-4} s^{-1},[9] so that substrates **1** and **2** are (respectively) ~0.9 and 2.8 (IBA), or 2.7 and 7.6 times (INA) as reactive as **3.** These results probably reflect better binding of the more hydrophobic **1** and **2** to the CTACl micellar phase where the cleavage reactions occur.

(15) The high reactivity of hydroperoxide toward phosphotriesters is well known; see Bunton, C.A.; Foroudian, H.J. *Langmuir* **1993**, *9*, 2832, and references therein.

(16) Very recently, an antibody specific for the catalytic hydrolysis of paraoxon (**3**) has been described: Lavey, B.J.; Janda, K.D. *J. Org. Chem.* **1996**, *61*, 7633. This antibody enhances the hydrolysis of **3** by a factor of 373 at pH 8.25, but is 12 or 21 times less reactive toward **3** than IBA or INA, respectively, under conditions similar to those of Table 1.[9]

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