ORGANIC LETTERS

1999
Vol. 1, No. 11
1791−1793

Loci of Ceric Cation Mediated Hydrolyses of Dimethyl Phosphate and Methyl Methylphosphonate

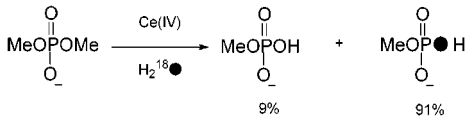
Robert A. Moss* and Hugo Morales-Rojas

Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903

moss@rutchem.rutgers.edu

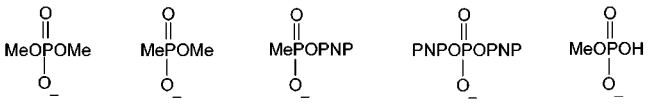
Received September 14, 1999

ABSTRACT



Dimethyl phosphate and methyl methylphosphonate are cleaved by Ce(IV)-mediated hydrolysis with 91% and 88% P–O scission, respectively, and rate accelerations of ≥ 10^{10} relative to pH 7 P–O hydrolysis.

Both dimethyl phosphate (**1**, DMP) and methyl methylphosphonate (**2**, MMP) are highly resistant to hydrolysis: $k_{hydrol} = 1.6 \times 10^{-13}$ s^{-1} for DMP (pH 7, 25 °C)[1] and, although analogous data are unavailable for MMP, k_{hydrol} must be considerably less than 2×10^{-9} s^{-1}, the rate constant for the hydrolysis of **3**, the *p*-nitrophenyl (PNP) analogue of MMP.[2] The centrality of the phosphate moiety to nucleic acid backbones and the desire to construct artificial phosphatases have driven many efforts to catalyze the hydrolysis of model esters, for example, bis(*p*-nitrophenyl)phosphate (BNPP, **4**).[3] Hydrolyses of MMP and PNPMP are also of interest because of their relation to the primary hydrolytic products of the chemical warfare agents sarin, soman, and VX.[4]



1 (DMP) **2 (MMP)** **3 (PNPMP)** **4 (BNPP)** **5 (MP)**

Lanthanide cations are particularly effective mediators of phosphodiester hydrolysis.[3,5] Among the lanthanide cations, Ce(IV) is the most reactive species for the mediated hydrolysis of, for example, BNPP.[6] Moreover, Ce(IV) affords large rate accelerations in the hydrolyses of both DMP and **3**: at 60 °C, k_{hydrol}(DMP)[7] $= 5.3 \times 10^{-4}$ s^{-1}, compared to 5.2×10^{-8} s^{-1} without Ce(IV) at pH 1.24,[8] and 2.6×10^{-12} s^{-1} at pH 7.[1] (Note that in the absence of Ce(IV) at pH 2.2 and 60 °C, DMP shows no measurable hydrolysis over several weeks.) For **3**, k_{hydrol} (pH 4.0, 37 °C) increases[9] to

(1) Wolfenden, R.; Ridgeway, C.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 833.

(2) Behrman, E. J.; Biallas, M. J.; Brass, H. J.; Edwards, J. O.; Isaks, M. *J. Org. Chem.* **1970**, *35*, 3063.

(3) (a) Schneider, H.-J.; Rammo, J.; Hettich, R. *Angew. Chem. Int. Ed.* **1993**, *32*, 1716. (b) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 9337. (c) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1994**, *116*, 7893. (d) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 8582. (e) Morrow, J. R.; Aures, K.; Epstein, D. *Chem. Commun.* **1995**, 2431. (f) Rammo, J.; Schneider, H.-J. *Liebigs Ann.* **1996**, 1757. (g) Kövari, E.; Krämer, R. *J. Am. Chem. Soc.* **1996**, *118*, 12704. (h) Roigk, A.; Hettich, R.; Schneider, H.-J. *Inorg. Chem.* **1998**, *37*, 751. (i) Ott, R.; Krämer, R. *Angew. Chem. Int. Ed.* **1998**, *37*, 1957. (j) Oh, S. J.; Choi, Y.-S.; Hwangbo, S.; Bae, S. C.; Ku, J. K.; Park, J. W. *Chem. Commun.* **1998**, 2189. (k) Gomez-Tagle, P.; Yatsimirsky, A. K. *J. Chem. Soc., Dalton Trans.* **1998**, 2957. (l) Stulz, E.; Leumann, C. *Chem. Commun.* **1999**, 239.

(4) (a) Yang, Y.-C. *Acc. Chem. Res.* **1999**, *32*, 109. (b) Yang, Y.-C. *Chem. Ind. (London)* **1995**, May, 334. (c) Yang, Y.-C.; Baker, J. A.; Ward, J. R. *Chem. Rev.* **1992**, *92*, 1729.

(5) (a) Breslow, R.; Huang, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080. (b) Morrow, J. R.; Buttrey, L. A.; Berback, K. A. *Inorg. Chem.* **1992**, *31*, 16. (c) Komiyama, M.; Matsumura, K.; Matsumoto, Y. *Chem. Commun.* **1992**, 640.

(6) Braken, K.; Moss, R. A.; Ragunathan, K. G. *J. Am. Chem. Soc.* **1997**, *119*, 9323.

(7) Moss, R. A.; Ragunathan, K. G. *Chem. Commun.* **1998**, 1871. The Ce(IV) concentration was 10 mM, with [DMP]= 1 mM at pD 2.2, 60 °C.

(8) Bunton, C. A.; Mhala, M. M.; Oldham K. G.; Vernon, A. *J. Chem. Soc.* **1960**, 3293.

10.1021/ol990285o CCC: $18.00
Published on Web 10/24/1999

3.6×10^{-2} s^{-1}, compared to 2.0×10^{-9} s^{-1} without Ce(IV) at pH 7.6, 30 °C.[2]

Despite this significant progress in the rate enhancement of phosphodiester hydrolysis, relatively little is known about the *site* of metal cation mediated hydrolysis. To propose any detailed mechanism for these metal cation mediated phosphodiester cleavages, it is absolutely essential to know whether the P−O or C−O bond is cleaved.

DMP is hydrolyzed with 99.5% O−Me cleavage in neutral or basic water,[1] and with 78% O−Me cleavage at pH 1.24,[8] but the cleavage site is unknown for Ce(IV) or Co(III)-cyclen[10] mediated hydrolyses. A very recent report indicates that bis(η^5-cyclopentadienyl)molybdenum(IV) effects P−O scission of DMP.[11] Additionally, Komiyama has demonstrated P−O cleavage in the heterogeneous Ce(IV) hydrolysis of thymidylyl(3′,5′)thymidine.[12a]

Here we report that the (acidic) Ce(IV) hydrolyses of DMP and MMP as well as methyl phosphate (**5**, MP) involve extensive (but not exclusive) P−O cleavage. These site-selectivity results help refine our mechanistic conception of these enormously accelerated Ce(IV)-mediated hydrolyses.

^{13}C-Labeled MMP (**2**, O-$^{13}CH_3$) was prepared from methyl phosphonic dichloride and $^{13}CH_3OH$ (Et_3N, Et_2O, 0 °C, 3 h; then 1:5 H_2O/acetone, 12h). ^{13}C-**2** (7.5 mM) and 25 mM Ce(IV) (as ceric ammonium nitrate) were heated to 60 °C at pH 2.2 for 2 h in 1 mL of 43.8% enriched ^{18}O-water. The substrate cleaved with $k_{hydrol} = 4.8 \times 10^{-4}$ s^{-1} ($t_{1/2} = 24$ min) as followed by the formation of MeOH in the ^{1}H NMR spectrum.[13]

After 2 h, the Ce(IV) was chelated with 25 mg of sodium EDTA, and the ^{31}P NMR spectrum (161.9 MHz) revealed the product, methylphosphonic acid, at δ 25.94 (vs external 85% H_3PO_4). The singlet resonance for ^{31}P−^{16}O was accompanied by a second singlet (^{31}P−^{18}O) 0.026 ppm upfield.[14] Deconvolution analysis (with Varian VNMR software, version 5.2) gave the ^{16}O/^{18}O ^{31}P integral ratio as 61.3/38.7, which, corrected for 43.8% ^{18}O enrichment, indicates 88.3 (±1.8)% ^{18}O incorporation as P−O cleavage during the Ce(IV) hydrolysis of MMP.[15]

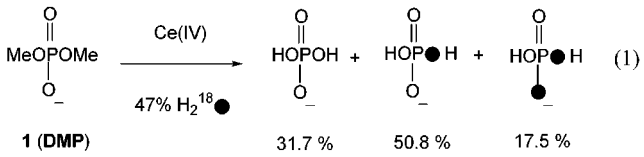
The ^{13}C spectrum (100.57 MHz) showed the product $^{13}CH_3OH$ singlet accompanied by a small (4.6%) satellite for $^{13}CH_3{}^{18}OH$ 0.019 ppm upfield.[14] After correction for the ^{18}O content of the water, this corresponds to 10.5 (±0.2)% water attack at the methyl group. The directly measured P−O (88%) and C−O (10.5%) cleavages of MMP are in good experimental balance. A control experiment revealed <1% ^{18}O exchange of the methylphosphonic acid product under our hydrolysis conditions.

For DMP, it was necessary to first determine the P−O/C−O selectivity of MP (**5**) hydrolysis because the Ce(IV)-mediated cleavage of MP is slightly faster than the DMP → MP reaction; MP does not sufficiently accumulate, and the site selectivity of the DMP cleavage must be extracted from the results of the overall (DMP → phosphate + 2MeOH) conversion.

MP (5 mM) was heated at pH 2.2, 60 °C for 5 h (>95% reaction) with 25 mM Ce(IV) in 47% ^{18}O-enriched water. The product phosphate singlet at δ 1.648 (59.3%) for ^{31}P−^{16}O was accompanied by a 0.019 ppm upfield singlet for ^{31}P−^{18}O (40.7%). Taking account of the ^{18}O enrichment, this corresponds to 86.6 (±1.7)% P−O cleavage of the MP. A control experiment demonstrated no ^{18}O exchange of phosphate or MP under the reaction conditions.

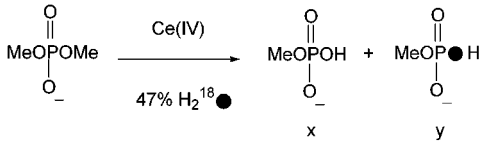
Next, 5 mM of DMP was cleaved by 25 mM Ce(IV) in 47% ^{18}O-enriched water at pH 2.2, 60 °C over 5 h. The reaction proceeded with $k = 1.8 \times 10^{-4}$ s^{-1} ($t_{1/2} = 1.07$ h). Hydrolysis occurs more slowly here, with a cation/substrate concentration ratio of Ce(IV)/DMP = 5, than it does with Ce(IV)/DMP = 10, where $k = 5.3 \times 10^{-4}$ s^{-1}.[7] After chelation of the Ce(IV), the ^{31}P NMR spectrum showed 87% of phosphate at δ 1.648, 6% of MP (δ 2.85, with a significant ^{31}P−^{18}O upfield satellite), and 7% of residual DMP (δ 4.01, *no* ^{18}O exchange).

The δ 1.648 ^{31}P−^{16}O singlet was accompanied by ^{31}P−^{18}O and ^{31}P−$^{18}O_2$ upfield satellites at 1.627 and 1.608 ppm. The distributions are shown in eq 1, where ^{18}O is represented



(1)

by a "dark" atom. The problem is to determine the ^{16}O/^{18}O product ratio of the DMP → MP cleavage, eq 2.



(2)

We assume that the 59.3/40.7 ^{16}O/^{18}O partition observed in the MP → phosphate hydrolysis (above) holds for the overall DMP → phosphate conversion. The source of unlabeled phosphate from DMP in eq 1 must be unlabeled MP (*x* in eq 2), while the source of doubly labeled phosphate in eq 1 must be ^{18}O-MP (*y* in eq 2). Therefore, the 31.7% of unlabeled phosphate in eq 1 must arise from *x*% of ^{16}O-MP, hydrolyzed without ^{18}O incorporation (59.3%, see above), determining *x* as 53.5%. Similarly, the 17.5% of doubly labeled phosphate, eq 1, reflects 40.7% of ^{18}O-incorporating cleavage of ^{18}O-MP (*y* in eq 2), fixing *y* as 43%. The deviation of the sum of *x* and *y* (96.5%) from 100% is

(9) Moss, R. A.; Ragunathan, K. G. *Langmuir* **1999**, *15*, 107. The Ce(IV) concentration was 1 mM, with [**3**] = 0.05 mM, in the presence of 2 mM Brij surfactant.

(10) Kim J. H.; Chin, J. *J. Am. Chem. Soc.* **1992**, *114*, 9792.

(11) Kuo, L. Y.; Barnes, L. A. *Inorg. Chem.* **1999**, *38*, 814.

(12) (a) Komiyama, M.; Takeda, N.; Takahashi, Y.; Uchida, H.; Shiiba, T.; Kodama, T.; Yashiro, M. *J. Chem. Soc., Perkin Trans. 2* **1995**, 269. (b) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443.

(13) With a higher Ce(IV)/MMP ratio (25/2.5, pD 2.2, 60 °C), $k_{hydrol} = 1.2 \times 10^{-3}$ s^{-1}.

(14) (a) Parente, J. E.; Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1984**, *106*, 8156. (b) Williams, N. H.; Cheung, W.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 8079.

(15) Error analysis indicates that the deconvolution procedure is the principal source of error; an error of 2% can be assigned to the deconvoluted integral values, which carries over to the ^{18}O enrichment results.

indicative of a relatively small composite experimental error. Corrected for the ^{18}O content of the water, the results indicate that DMP affords MP with (0.43/0.47) = 91.5% of P−O cleavage. Table 1 records the extent of P−O scission of DMP, MP, and MMP in Ce(IV) hydrolysis and under other conditions.

Table 1. P−O vs C−O Loci of Phosphate Ester Hydrolysis

		conditions		
substrate	Ce(IV)[a]	acidic	neutral	basic
MMP (**2**)	88% P−O			
DMP (**1**)	91% P−O	78% C−O[b]	>99% C−O[c]	>99% C−O[d]
MP (**5**)	87% P−O	>84% C−O[e]	>99% P−O[f]	>99% P−O[g]

[a] This work, pH 2.2, 60 °C. Errors are ±2%. [b] pH 1.24, 100 °C, neutral species, ref 8. [c] pH 6.8, 150 °C, monoanion, ref 1. [d] pH 7−13, 150 °C, monoanion, ref 1. [e] pH 1, 100 °C, neutral species (MeH_2PO_4), ref 16. [f] pH 4, 100 °C, monoanion ($MeHPO_4^-$), ref 16. [g] pH 10−11, 100 °C, dianion ($MePO_4^{2-}$), ref 1.

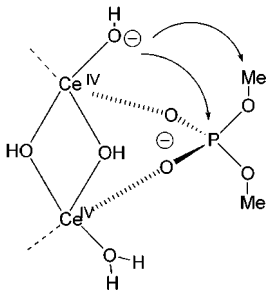
From the literature, it is clear that the cleavage site of MP depends on its degree of protonation, with the neutral species hydrolyzing at C−O, while the monoanion undergoes P−O scission.[16] DMP, however, affords mainly C−O cleavage at all common pH's.[1,8] The sites of the acid, neutral, or basic hydrolyses of MMP are not reported, although Chin's results imply P−O cleavage for the hydrolysis of methyl phenylphosphonate coordinated to a dinuclear Co(III) complex.[17]

We have shown here that the Ce(IV)-mediated hydrolyses of MMP, DMP, and MP all proceed with dominant P−O cleavage at pH 2.2. For DMP, this represents a *reversal* of the acidic hydrolysis locus from C−O to P−O; coordination[7] of DMP to Ce(IV) directs a rapid attack of Ce-bound OH at the substrate's P atom in preference to the much slower acid-catalyzed hydrolysis caused by H_2O attack at Me−O.[8]

Knowing the site of Ce(IV) hydrolysis of DMP, we can refine estimates of the hydrolytic acceleration of its P−O cleavage. In neutral hydrolysis of DMP, P−O cleavage is ≤ 0.5% of the total hydrolysis, setting an upper limit of (2.6×10^{-12} s^{-1} × 0.005) ~1.3×10^{-14} s^{-1} for k_{hydrol} of DMP by P−O cleavage at pH 7 (extrapolated to 60 °C from the measured activation parameters[1,7]). With 1 mM DMP and 10 mM Ce(IV) at pD 2.2, 60 °C, $k = 5.3 \times 10^{-4}$ s^{-1} with 91% P−O cleavage (Table 1), so that the P−O cleavage of DMP is accelerated by ($0.91 \times 5.3 \times 10^{-4} / 1.3 \times 10^{-14}$) ~$3.7 \times 10^{10}$.

Chin has noted that phosphonate monoesters are about as stable as phosphate diesters.[17,18] This suggests that the P−O hydrolysis of MMP at pH 7 (60 °C) should, like that of DMP, occur with $k \sim 10^{-14}$ s^{-1}. The 10-fold excess Ce(IV)−P−O hydrolysis of MMP occurs with $k \sim 1.2 \times 10^{-3}$ s^{-1},[13] pointing to an acceleration of ~10^{11}.

The P−O/C−O hydrolysis site data of Table 1 indicate that MMP, DMP, and MP all are cleaved in Ce(IV) hydrolyses with 90% P−O specificity. P−O cleavage is reasonable for a mechanism that involves initial binding of the susbtrate's P−O$^-$ to the Ce(IV), mitigating the former's negative charge and providing a metal-bound hydroxide nucleophile for subsequent attack on the P═O.[3,5,19] What is surprising, however, is the *persistence* of 10% C−O cleavage for the substrates of Table 1. The C−O hydrolysis cannot come from competitive, non-Ce(IV) hydrolysis, which, even in acidic solutions, is orders of magnitude slower[8] than the Ce(IV)-mediated process. The residual C−O hydrolysis must therefore also be Ce(IV)-mediated. An attractive rationale makes use of a dimeric Ce(IV)-phosphodiester intermediate (**6**), analogous to that proposed by Komiyama,[12b] in which attack of Ce(IV)-bound OH on the complexed DMP can occur either at P−O (90%) or C−O (10%).[20,21]



6

The enormous (P−O selective) Ce(IV)-induced rate accelerations of DMP and MMP hydrolyses are rather unique: with $k_{hydrol} \sim 10^{-4}$−10^{-3} s^{-1}, they are several orders of magnitude faster than the Co(III)-cyclen (2×10^{-7} s^{-1}) or Cp_2MoCl_2 (8×10^{-7} s^{-1}) mediated hydrolyses.[10,11] We are continuing to explore Ce(IV) and Zr(IV) hydrolyses of DMP and alkyl methylphosphonate esters; further kinetic and labeling results will appear in due course.

Acknowledgment. We thank Professor R. Wolfenden for helpful correspondence. H.M-R. acknowledges a Fulbright-CONACyT Fellowship. We are grateful to the U.S. Army Research Office for financial support.

OL990285O

(16) Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Vernon, C. A. *J. Chem. Soc.* **1958**, 3574.

(17) Seo, J. S.; Hynes, R. C.; Williams D.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 9943.

(18) Second-order rate constants for the OH$^-$-catalyzed hydrolyses of BNPP (**4**) and *p*-nitrophenyl methylphosphonate[2] (PNPMP, **3**) are comparable. The parity is not expected to change much with poorer leaving groups such as OMe.[17]

(19) We determined $\Delta H^{\ddagger} = 21.6 \pm 1.4$ kcal mol^{-1} and $\Delta S^{\ddagger} = -8.3 \pm 0.7$ eu for the Ce(IV) hydrolysis of DMP (30−70 °C; with [Ce(IV)] = 25 mM, [DMP] = 2.5 mM, pD 1.9 in D_2O). For comparison, the uncatalyzed hydrolysis of DMP at pH 6.8 displays $\Delta H^{\ddagger} = 25.9$ kcal mol^{-1} and $\Delta S^{\ddagger} = -34$ eu.[1] Remarkably, the major portion of the Ce(IV) hydrolytic rate acceleration results from the 25.7 eu (7.7 kcal mol^{-1} at 25 °C) favorable $\Delta\Delta S^{\ddagger}$ for the metal-mediated hydrolysis. This is in accord with the entropic advantage anticipated for prior association of the Ce(IV) and DMP, followed by rate-limiting attack of a Ce-bound hydroxyl ligand on the substrate's P atom.

(20) Attack at P by the bridging oxide of **6** is also possible; cf. Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485 and references therein.

(21) The binding constant of Ce(IV) and DMP is reported as ~95 M^{-1}.[7]