JOC The Journal of Organic Chemistry

Article

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Dephosphorylation Reactions of Mono-, Di-, and Triesters of 2,4-Dinitrophenyl Phosphate with Deferoxamine and Benzohydroxamic Acid

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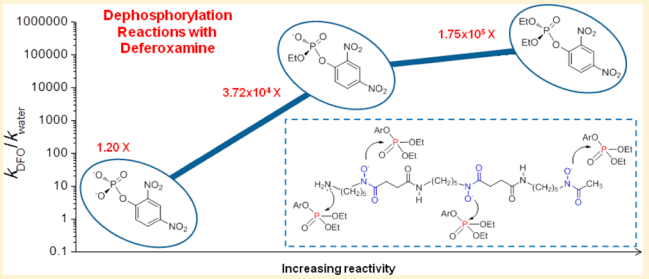
Supporting Information

ABSTRACT: This work presents a detailed kinetic and mechanistic study of biologically interesting dephosphorylation reactions involving the exceptionally reactive nucleophilic group, hydroxamate. We compare results for hydroxamate groups anchored on the simple molecular backbone of benzohydroxamate (BHA) and on the more complex structure of the widely used drug, deferoxamine (DFO). BHA shows extraordinary reactivity toward the triester diethyl 2,4-dinitrophenyl phosphate (DEDNPP) and the diester ethyl 2,4-dinitrophenyl phosphate (EDNPP) but reacts very slowly with the monoester 2,4-dinitrophenyl phosphate (DNPP). Nucleophilic attack on phosphorus is confirmed by the detection of the phosphorylated intermediates formed. These undergo Lossen-type rearrangements, resulting in the decomposition of the nucleophile. DFO, which is used therapeutically for the treatment of acute iron intoxication, carries three hydroxamate groups and shows correspondingly high nucleophilic activity toward both triester DEDNPP and diester EDNPP. This result suggests a potential use for DFO in cases of acute poisoning with phosphorus pesticides.



INTRODUCTION

The importance of phosphate esters in biological processes is the basis of the continuing interest in the study of phosphoryl transfer mechanisms. The hydrolysis and other nucleophilic substitution reactions of phosphoesters are studied in order to understand the mechanisms of these reactions.[1] α-Effect nucleophiles such as hydroxylamine,[2] hydrazine,[3] and hydroxamic acids[4,5] all act as effective dephosphorylating agents. We recently reported a detailed study of products and intermediates involved in the dephosphorylation of bis(2,4-dinitrophenyl) phosphate anion (BDNPP) by the benzohydroxamate anion (BHA^-), which showed competing nucleophilic attack on phosphorus and on the aromatic carbon.[6] Attack on carbon gives an intermediate, which was detected, but slowly decomposes to aniline and 2,4-dinitrophenol, whereas attack on phosphorus gives an unstable intermediate that undergoes a Lossen rearrangement to urea, amine, isocyanate, and carbamyl hydroxamate.[7,8] (Scheme 1)

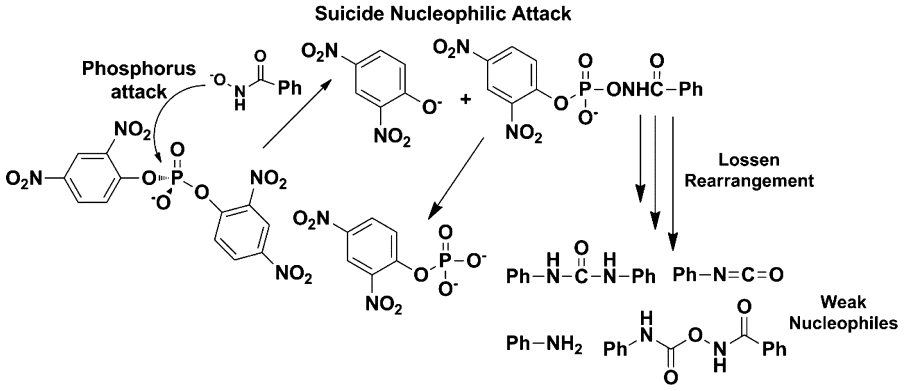
Thus, BHA behaves as a self-destructive molecular scissor, which loses its nucleophilicity upon reaction. The reaction with BHA (0.05 mol L^{-1}) is over 10^5 times faster than the spontaneous hydrolysis of BDNPP (1.9×10^{-7} s^{-1}).

Deferoxamine (DFO, Scheme 2) is a naturally occurring hydroxamate metal chelator,[9] therapeutically indicated in iron[10] and aluminum[11] overload disorders. It has for many years been a normal treatment for patients receiving blood transfusions.[12] Numerous side effects are known, including the inhibition of DNA synthesis in human lymphocytes.[13,14] DFO has been examined as an antiproliferative drug in cancer chemotherapy,[15] and studies have shown that DFO shows antitumor activity in the treatment of neuroblastoma, leukemia, bladder and hepatocellular carcinoma, and brain cancer.[16] Furthermore, this natural hydroxamate can also be used to suppress the oxidative degradation of DNA, via complexation of iron in a nonreactive form.[17] Thus, DFO is an extremely powerful bacterial siderophore, containing three bidentate oxygen-containing ligands, perfectly distributed and well-suited for the chelation of metal cations such as high-spin, octahedral ferric ion. However, these bidentate oxygen-containing ligands are hydroxamic acids (Scheme 2), with anions known to be exceptionally powerful nucleophiles, particularly toward the phosphorus centers of phosphate esters.[18]

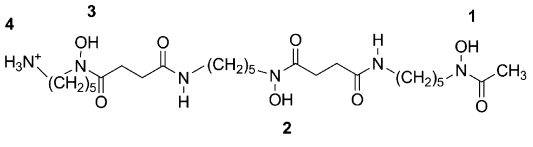
We have reported the reaction of DFO with the phosphate diester BDNPP, which involves nucleophilic attack by the hydroxamate groups of DFO specifically on phosphorus, as confirmed by the detection of two important phosphorylated intermediates. Furthermore, its reaction with plasmid DNA

Received: October 25, 2012
Published: November 20, 2012

Scheme 1. Nucleophilic Attack of BHA^- on Phosphorus

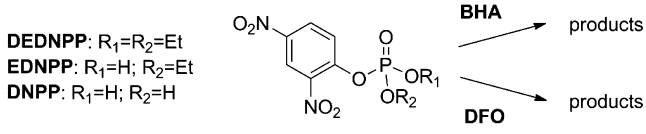


Scheme 2. DFO Structure and Acid Dissociation Constants of its Four Ionizing Groups[19]



confirmed the ability of DFO to cleave DNA strands.[20] It is known that the reactivity of phosphate di- and triesters depends strongly on the nature of the nucleophile and leaving group, with significant participation of the "spectator" groups in the reaction of triesters, where the two nonleaving groups are found to play important roles, accounting for the substantial difference in reactivity between triaryl and dialkyl aryl phosphate triesters.[21] In diesters, on the other hand, it seems that the nonleaving group has minimal effect on reactivity, because bis-2,4-dinitrophenyl phosphate is hydrolyzed at 39 °C less than 3 times faster than the methyl 2,4-dinitrophenyl ester, little more than the statistical factor of 2, despite the very large pK_a difference.[22] To better understand the effects of substituent groups on the kinetics and mechanisms of reaction of different phosphate esters with different nucleophiles, we have now extended the kinetic studies of the reactions of BHA and DFO to a complete series of 2,4-dinitrophenyl phosphate esters: the triester diethyl 2,4-dinitrophenyl phosphate (DEDNPP), the diester ethyl 2,4-dinitrophenyl phosphate (EDNPP), and the monoester 2,4-dinitrophenyl phosphate (DNPP) (Scheme 3).

Scheme 3. Reactions Studied: DFO and BHA with DEDNPP, EDNPP and DNPP



RESULTS AND DISCUSSION

Kinetic Studies. Reactions were followed spectrophotometrically, in water, by monitoring the appearance of 2,4-dinitrophenoxide at 400 nm. We compared the reactivities of the simple hydroxamic acid BHA (benzohydroxamic acid, 0.05 M) and the more complex DFO (0.01 M) as nucleophiles toward the three phosphate esters. pH–rate profiles for the reactions of BHA and DFO with DEDNPP and EDNPP (Figures 1 and 2)

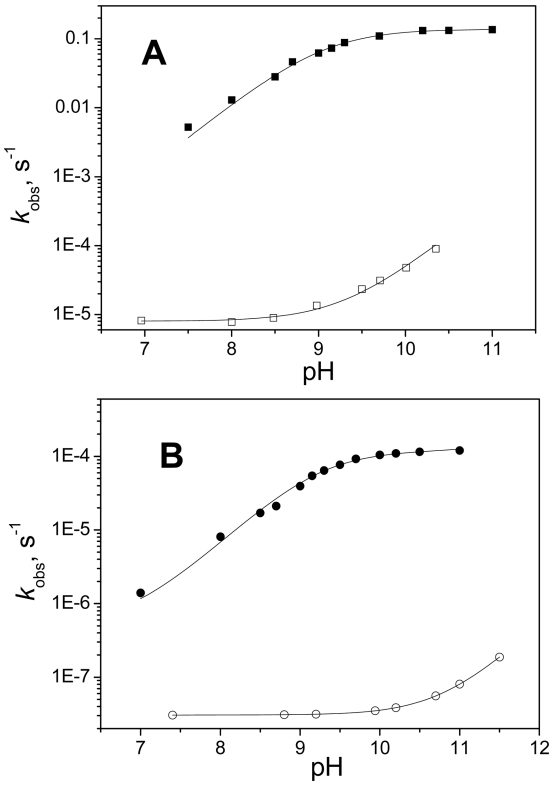


Figure 1. pH–rate profiles for the reactions of (A) DEDNPP (■) and (B) EDNPP (●) with 0.05 M BHA, μ = 1.0 M, 25 °C. The spontaneous hydrolysis reactions for the esters are shown for comparison: DEDNPP (□) and EDNPP (○).[22,23]

show that their reactions are accelerated many thousand-fold. In contrast, under conditions similar to those shown below for the di- and triester, the reaction of DNPP is accelerated only modestly, with a rate increase of about 20%. (Full data sets for these reactions are given in the Supporting Information (Tables S1 and S9).

Data for the reactions with BHA were analyzed using eq 1, which is consistent with Scheme 4, as described previously.[6]

$$k_{obs} = k_0 + k_{OH}[OH] + k_N[BHA]\chi_{BHA^-} \quad (1)$$

The rate constants k_0, k_{OH}, and k_N correspond to the first-order rate constant for the spontaneous hydrolysis and the second-order rate constants for the reaction of the di- and triesters with hydroxide ion and the hydroxamate nucleophile, respectively. The term χ_{BHA^-} represents the molar fraction of benzohydrox-

dx.doi.org/10.1021/jo302374q | J. Org. Chem. 2012, 77, 10907–10913

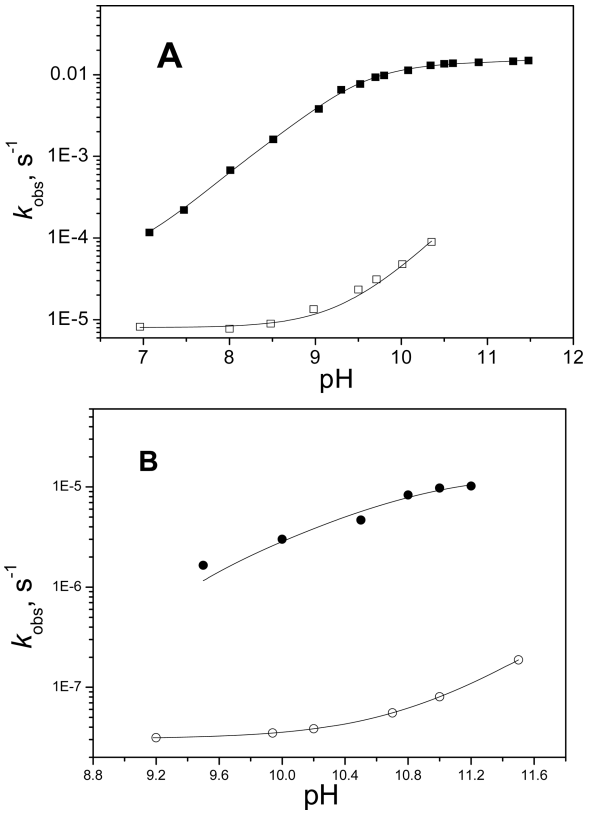
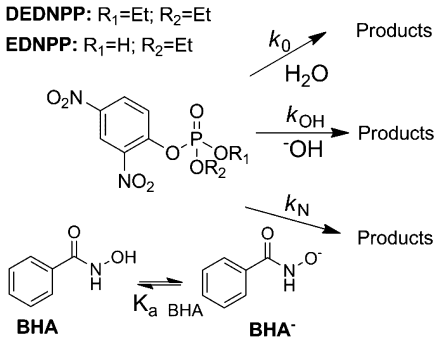


Figure 2. pH−rate profiles for the reactions of (A) DEDNPP (■)and (B) EDNPP (●) with 0.01 M DFO, μ = 1.0 M, 25 °C. The spontaneous hydrolysis reactions for the esters are shown for comparison: DEDNPP (□) and EDNPP (○).[22,23]

Scheme 4. Reactions of BHA with DEDNPP and EDNPP



amate ion.[6] The reaction showed a first-order dependence in relation to BHA, as shown by the linear plot of k_{obs} versus [BHA] (see Supporting Information). The pH−rate profiles for the formation of DNP shown in Figure 1A and 1B were fitted by using eq 1, considering initially the reaction in the absence of BHA, which allowed us to estimate rate constants for the spontaneous hydrolysis of the esters (k_0), and the reactions (k_{OH}) with OH^- to generate dinitrophenoxide ion. These reactions are relatively unimportant at pH 9−10, where the products for the reaction of BHA were examined, and their contribution to the observed rate constant are negligible: allowing a convenient fitting of the data with eq 1 and a confident determination of the rate constant k_N. The kinetic parameters obtained for the reactions of BHA with DEDNPP and EDNPP are given in Table 1.

Table 1. Kinetic Parameters, Activation Parameters, and Kinetic Solvent Isotope Effects for the Reactions of DEDNPP and EDNPP with BHA, pH 10.5, μ = 1.0 M, and 25 °C

	DEDNPP	EDNPP
k_0,[a] s^{-1}	(8.00 ± 0.50) × 10^{-6}	(3.60 ± 0.60) × 10^{-8}
k_{OH},[a] M^{-1} s^{-1}	(4.20 ± 0.20) × 10^{-1}	(4.90 ± 0.50) × 10^{-5}
k_N, M^{-1} s^{-1}	2.74 ± 0.03	(2.70 ± 0.04) × 10^{-3}
pK_a (BHA)[a]	9.16	
$\Delta H^{\ddagger}$, kcal/mol[b]	+8.9 ± 0.2	+16.8 ± 0.8
$\Delta S^{\ddagger}$, cal[b]	−33.1 ± 0.7	−20.5 ± 2.6
$\Delta G^{\ddagger}$, kcal/mol[b]	18.75	22.78
k^H/k^D	1.14	1.08

[a]Consistent with literature values.[22,23] [b]Calculated at pH 10.5 with eqs. S4−S6 and $\Delta G^{\ddagger}$ at 25 °C.

The experimental data for DFO were fitted to an extended version (eq 2) of this equation, which takes account of the multiple acid dissociations of DFO, with the subscripts 1 to 4 referring to the neutral, monoanionic, dianionic, and trianionic species of DFO (Scheme 2).[20] The kinetic parameters obtained are given in Tables 1 and 2, for BHA and DFO, respectively.

$$k_{obs} = k_0 + k_{OH}[OH] + (k_1\chi_1 + k_2\chi_2 + k_3\chi_3 + k_4\chi_4) \times [DFO] \quad (2)$$

Table 2. Kinetic Results for the Reactions of DEDNPP and EDNPP with DFO at 25 °C[a]

	DEDNPP	EDNPP
k_1, M^{-1} s^{-1}	(1.65 ± 0.51) × 10^{-1}	–
k_2, M^{-1} s^{-1}	(3.48 ± 0.55) × 10^{-1}	–
k_3, M^{-1} s^{-1}	1.40 ± 0.03	(2.12 ± 0.82) × 10^{-4}
k_4, M^{-1} s^{-1}	1.38 ± 0.03	(1.37 ± 0.09) × 10^{-3}
DFO, pK_{a2}[b]	8.96	
DFO, pK_{a3}[b]	9.55	
DFO, pK_{a4}[b]	10.79	

[a]Values of k_0 and k_{OH} are given in Table 1. [b]Values taken from ref 19.

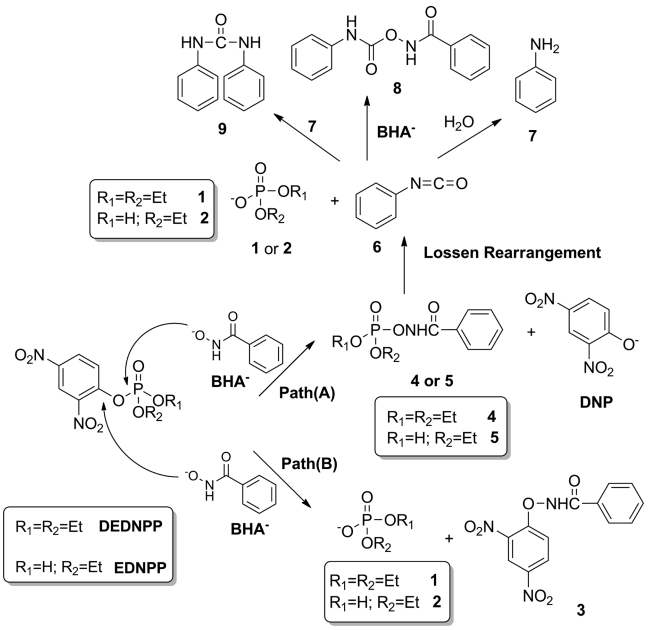
The results show BHA to be one of the most powerful known nucleophiles toward phosphate esters such as DEDNPP and EDNPP. Toward the model diester EDNPP only the more basic hydroperoxide and hydroxylamine anions are known to be more reactive.[24] The nucleophilic center of rigid benzohydroxamate is exposed, while those of DFO are surrounded by an extended conformationally mobile structure that could offer some steric hindrance to reaction, with the most basic center reacting fastest (see Tables 1 and 2). It is interesting to note that because DFO is already used as a drug, the high reactivity against triesters suggests a further potential use for DFO, in cases of acute poisoning with phosphorus pesticides such as paraoxon derivatives (see below for some kinetic results reinforcing this hypothesis). In terms of absolute reactivity the triester DEDNPP is the most reactive, as expected. However, the diester EDNPP is not the least reactive in the series, because (i) the reaction with the monoester DNPP involves the electrostatically unfavorable attack of the BHA monoanion on a dianion and (ii) α-effects are minimal for reactions of monoester dianions.[25] Table 1 includes thermodynamic activation parameters and kinetic solvent isotope effects for reaction with BHA. The large and negative entropies of activation and low solvent deuterium isotope effects k^H/k^D indicate that the reactions are at least primarily

dx.doi.org/10.1021/jo302374q | J. Org. Chem. 2012, 77, 10907−10913

nucleophilic. In the reaction of DNPP with BHA and DFO there is a small acceleration of about 20% in relation to the rate constant determined for the hydrolysis reaction: k_{obs}= 1.80 × 10^{-5} s^{-1} at 25 °C, pH 9, and ionic strength 1.0 M[26] (experimental data given in Tables S1 and S9 in the Supporting Information).

Products of the Reactions. To test if the reactions are nucleophilic and to elucidate mechanisms for all these reactions, we carried out two series of spectroscopic (^{1}H and ^{31}P NMR, ESI-MS) investigations. The results indicate that the reactions of BHA with phosphate diester EDNPP and triester DEDNPP follow parallel paths (Scheme 5), with hydroxamate attack on the

Scheme 5. Nucleophilic Attack of BHA^- by Two Reaction Paths: (A) at Phosphorus and (B) on the Aromatic Ring



aromatic ring (path B), giving an aromatic intermediate (**3**) and a less reactive phosphate ester (**1** or **2**). Hydroxamate attack on phosphorus (path A) generates dinitrophenol (DNP) and a phosphorylated intermediate (**4** or **5**). Intermediates **4** and **5** undergo rapid Lossen rearrangements to produce phenyl isocyanate (**6**), which is hydrolyzed to aniline (**7**). Phenyl isocyanate can then also react with BHA or with a molecule of the aniline produced, giving the carbamyl derivative **8** and diphenylurea (**9**), respectively. Thus, BHA reacts as a self-destructive molecular scissor, which loses its nucleophilicity upon reaction. The Lossen rearrangement has been observed previously for the reactions of hydroxamic acid with sulfonyl and phosphoryl halides,[7,27] and more recently for BDNPP,[6] as the first evidence of this type of reaction in dephosphorylation reactions.

Following Reactions by NMR. We followed the dephosphorylation reactions involving BHA and DFO by ^{1}H NMR. Because of the complex nature of the intermediates and products formed in the reactions of DFO,[20] we could make NMR assignments accurately only for BHA. The conclusions we reach for BHA can be extended with reasonable confidence to reactions with DFO. Sequential ^{1}H NMR spectra for the reactions of BHA with DEDNPP and EDNPP show the formation of important species of Scheme 5, as expected from previous results on the reaction of BHA with BDNPP.[6] (For details, see Figures S10 and S11 of the Supporting Information. Chemical shifts, multiplicities, and coupling constants for the species detected are shown in Table S12). Peaks were assigned by comparison with spectra of the pure compounds, and available literature data.[2,3] To confirm the structural assignment of product **3**, ^{1}H NMR spectra were recorded for the reaction of BHA with 1-chloro-2,4-dinitrobenzene (CDNB), which involves exclusive attack on the aromatic ring. NMR spectra obtained as a function of time for this reaction (Figure S13, Supporting Information) demonstrate the formation of **3** over the first 5 min: **3** subsequently breaks down to form phenyl isocyanate, aniline, and DNP. Thus, the aromatic intermediate **3**, aniline **7**, and DNP could all be detected by ^{1}H NMR for the reactions of BHA with DEDNPP and EDNPP, according to Scheme 5. In the case of the reaction of BHA with the monoester DNPP (Table S14, Supporting Information), the aromatic intermediate **3** is not observed, consistent with the rather small acceleration observed, which probably proceeds exclusively by attack on phosphorus.

Figure 3A and 3B shows the courses of the reactions of BHA with EDNPP and DEDNPP, respectively, according to Scheme

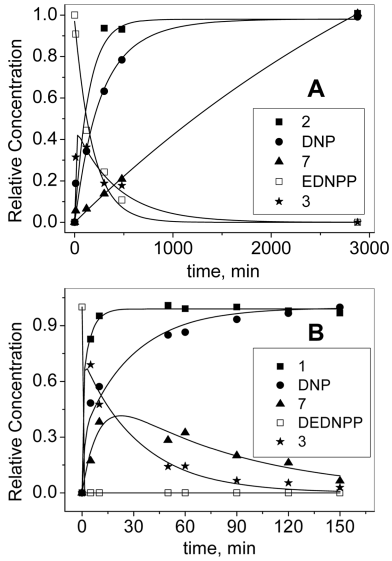


Figure 3. Relative concentration vs time profiles obtained by ^{1}H NMR for the reaction of BHA (0.105 M at pH = 10) with 5 × 10^{-3} M of (A) EDNPP and (B) DEDNPP, at 25 °C.

5, as followed continuously by ^{1}H NMR, in terms of relative concentration vs time profiles for the most important species, namely the rapid disappearance of EDNPP and DEDNPP and the appearance of DNP and the aromatic intermediate **3**. DNP forms from the start, and more is generated during the reaction, presumably from the breakdown of **3**. As expected, the phosphorylated intermediates are too unstable to be detected by this technique, but attack of BHA on phosphorus (path A, Scheme 5) is demonstrated by the initial formation of DNP, which must involve path A. The formation of aniline (**7**) is consistent with the mechanism proposed. For the reactions with DEDNPP, the substrate disappears after 10 min, with aniline reaching a concentration maximum at about 20 min, followed by a slow transformation to other Lossen degradation products, and the corresponding decrease in concentration. By contrast, aniline continues to appear throughout the much slower reaction with the diester EDNPP. It was possible to derive percentages of attack by BHA on phosphorus and the aromatic center (Table 3),

dx.doi.org/10.1021/jo302374q | J. Org. Chem. 2012, 77, 10907−10913

because the DNP detected is produced exclusively by attack at phosphorus.

Table 3. Percentages of Attack by BHA on Phosphorus and the Aromatic Center in the Reactions with DEDNPP and EDNPP

	DEDNPP	EDNPP
$\%_N^C$	45	34
$\%_N^P$	55	66

Fitting the data of Figure 3A and 3B to standard coupled differential equations for consecutive first-order reactions[28] (see the Supporting Information) gave a first approximation of the kinetic parameters for the various reactions the values are included in Table 4. Scheme 6 summarizes the full set of reaction

Table 4. Parameters Obtained for Fitting of Data of Figure 3A and 3B to Equations S20−S24 for Reaction of BHA with DEDNPP and EDNPP

	DEDNPP	EDNPP
$(k_1 + k_3)^a$, M^{-1} s^{-1}	2.74	2.70×10^{-3}
k_2, s^{-1}	$(3.5 \pm 1.8) \times 10^{-3}$	$(8.3 \pm 3.4) \times 10^{-4}$
k_4, s^{-1}	$(5.0 \pm 2.3) \times 10^{-4}$	$(4.0 \pm 1.8) \times 10^{-5}$
k_5, s^{-1}	$(1.8 \pm 0.9) \times 10^{-3}$	$(3.3 \pm 1.7) \times 10^{-6}$
k_6, s^{-1}	$(2.0 \pm 0.8) \times 10^{-4}$	

[a]Taken from Table 1.

paths considered in the fitting equations. The scheme includes the nucleophilic reactions at phosphorus and aromatic carbon, and the decomposition of the intermediates so formed.

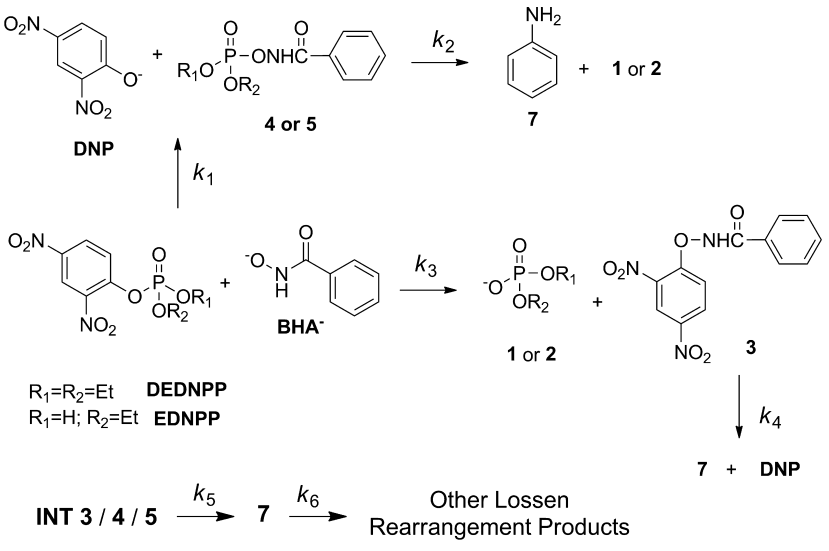
Mass Spectrometric Analysis. Hydroxamate attacks both the aromatic ring and the phosphorus centers of EDNPP and DEDNPP, as confirmed by ESI-MS in the negative mode, which monitors the course of reaction by collecting snapshots of its anionic composition. Reagents, intermediates, and products present as anions are expected to be transferred directly from the reaction solution to the gas phase and then detected by ESI-MS. Figure 4 shows a ESI-MS(−) spectrum recorded after 2 min for the reaction of BHA (0.05 M) with DEDNPP (8×10^{-3} M). In this spectrum, a series of key anions was detected and identified including (i) the (deprotonated) intermediate **3** of m/z 302, (ii) the BHA dimer of m/z 273, (iii) the (deprotonated) carbamyl derivative **8** of m/z 255, produced by a Lossen rearrangement, (iv) the phenoxide DNP of m/z 183, (v) the anion of the reagent BHA of m/z 136, (vi) and (deprotonated) aniline of m/z 92. The same species were detected for the reaction of BHA (0.05 M) with EDNPP.

ESI-MS/MS was then used to characterize some of these important species via collision-induced dissociation (CID). The resulting tandem ESI-MS/MS(−) for carbamyl derivative **8** (m/z 255) shows (Figure 5) that it dissociates to BHA (m/z 136) and the aniline anion (m/z 92). ESI-MS/MS spectra of other species detected in the reactions of BHA with DEDNPP and EDNPP appear in the Supporting Information (Figures S15 and S16). This ESI-MS study is crucial to the investigation because species such as the carbamyl derivative **8** were detected that could not be observed by NMR.

We also recorded ESI-MS spectra for the reaction of BHA with DNPP, where we observed very small amounts of (i) carbamyl derivative **8** (deprotonated) of m/z 255, (ii) the phenoxide DNP of m/z 183, (iii) the BHA anion of m/z 136, and (iv) aniline (deprotonated) of m/z 92. The absence of the deprotonated intermediate **3** (of m/z 302) indicates that the small contribution (rate increase of ≈20%) to the observed hydrolysis due to the reaction of BHA with DNPP probably proceeds by attack on phosphorus. ESI-MS spectra for species detected in the reactions of BHA with DNPP are shown in Figure S17.

Reaction of DFO with Methyl Paraoxon. We performed our full kinetic study with the model triester DEDNPP because of its good leaving group (2,4-dinitrophenol, pK_a = 4.07). However, because DFO is already used as a drug, our results suggest a further potential use for DFO, in cases of acute poisoning with phosphorus pesticides. One such pesticide is paraoxon, with 4-nitrophenoxide as leaving group (pK_a = 7.14). To check this possibility, we followed the reaction of DFO with methyl paraoxon and once again observed a rate enhancement (Table 5), substantial enough to confirm that our conclusions about dephosphorylation of DEDNPP can be extended to organophosphorus pesticides.

Scheme 6. Reaction Paths Considered in Fitting the Data of the Figure 3A and 3B



dx.doi.org/10.1021/jo302374q | J. Org. Chem. 2012, 77, 10907−10913

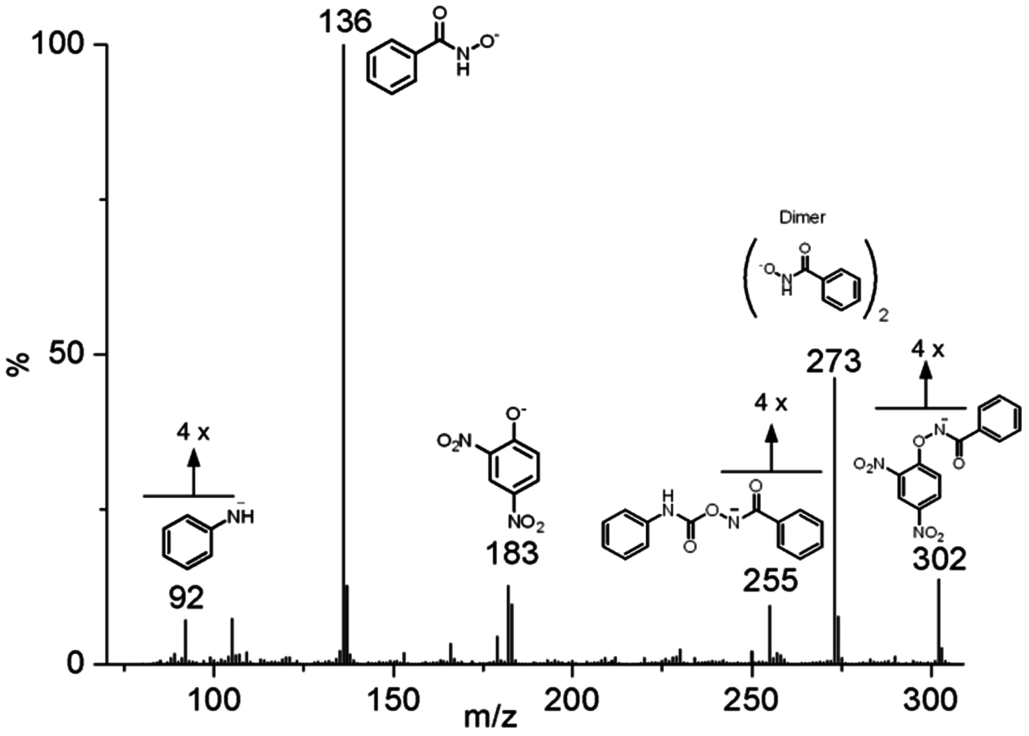


Figure 4. ESI-MS after 2 min for the reaction of BHA (0.05 M) with DEDNPP (8×10^{-3} M), pH 10.5, and 25 °C.

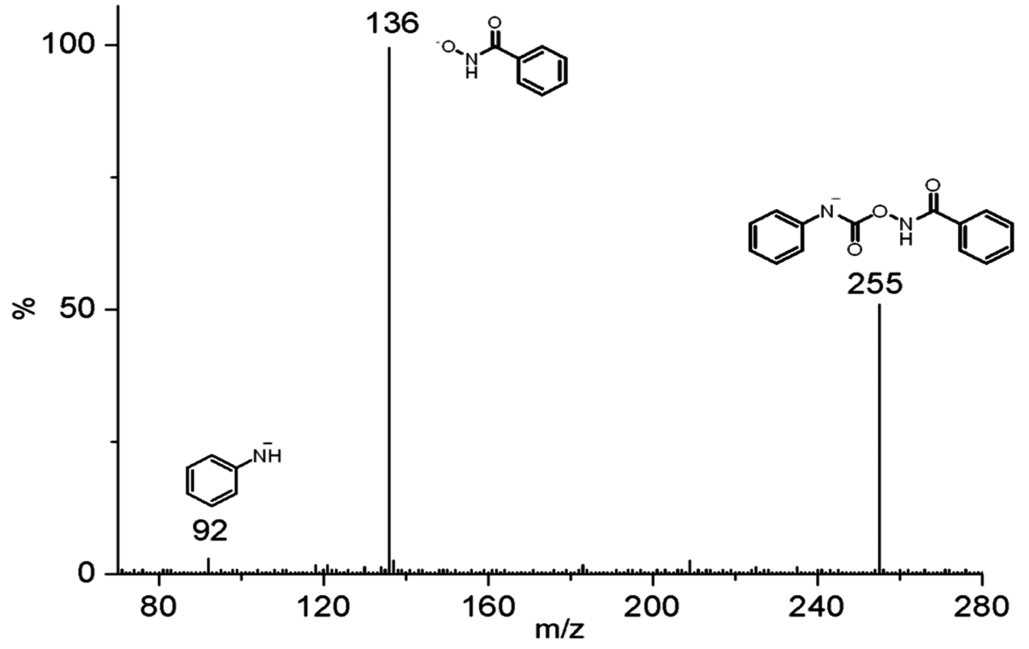
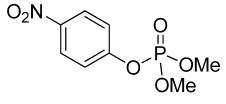


Figure 5. ESI-MS/MS(−) of the carbamyl derivative **8** of *m*/*z* 255.

Table 5. Kinetic Results for the Reaction of DFO with Methyl Paraoxon at 25° C, pH 11, and μ = 1.0 M

	Second Order Rate Constants	k^{DFO}/k^{0}
H_2O	1.44×10^{-7} M^{-1} s^{-1}	8.3×10^{4}
DFO	1.20×10^{-2} $M^{-1}s^{-1}$	



Methyl Paraoxon

CONCLUSIONS

The results show that simple benzohydroxamate (BHA) and the more complex derivative, the widely used drug, deferoxamine (DFO), are extremely reactive toward phosphate esters. In general, BHA and DFO follow similar reactivity patterns, with reactions proceeding much faster with the triester diethyl 2,4-dinitrophenyl phosphate (DEDNPP) than with the diester ethyl

dx.doi.org/10.1021/jo302374q | J. Org. Chem. 2012, 77, 10907−10913

2,4-dinitrophenyl phosphate (EDNPP). The reactions with the monoester 2,4-dinitrophenyl phosphate (DNPP) are much slower and proceed almost exclusively by attack at phosphorus. For the reactions of DEDNPP and EDNPP, attack on phosphorus was confirmed by the detection of the phosphorylated intermediates, which undergo Lossen-type rearrangements, resulting in the decomposition of the nucleophile. DFO, which is used therapeutically for the treatment of acute iron intoxication, showed high nucleophilic activity toward the triester DEDNPP and the diester EDNPP, a result which suggested a potential use for DFO in cases of acute poisoning with phosphorus pesticides. The rapid detoxification of the pesticide methyl paraoxon supports this suggestion.

EXPERIMENTAL SECTION

Materials. Distilled water was used throughout these experiments. Deferoxamine (DFO), 2,4-dinitrophenol (DNP), and 1-chloro-2,4-dinitrobenzene (CDNB) were of the highest purity and were used as received. BHA,[6] DEDNPP,[29] EDNPP,[29] and DNPP[30] were prepared according to procedures described in the literature.

Kinetic Measurements. Reaction rates were followed by UV−vis spectrophotometry by monitoring the appearance of DNP at 400 nm in the thermostatted cell holder of a diode-array spectrophotometer maintained at 25.0 ± 0.1 °C. Reaction was initiated by injecting 30 μL of the substrate (1×10^{-3} M) from a stock solution into 3 mL of the reaction mixture, with a large excess (>0.01 M) of the nucleophile ensuring first-order-kinetics for the initial nucleophilic attacks upon the substrates. Ionic strength was maintained at 1.0 M with KCl. The pH was controlled using external Tris, Na_2CO_3, and phosphate buffers (0.01 M). Absorbance versus time data were stored directly on a microcomputer and first-order rate constants k_{obs} obtained from linear plots of $\ln(A_\infty - A_t)$ against time for at least 90% of reaction by using an iterative least-squares program; correlation coefficients were >0.999 for all kinetic runs.

^{1}H NMR Experiments. ^{1}H NMR spectra were recorded in D_2O with sodium 3-(trimethylsilyl)propionate (TMSP) as internal reference except for reactions with 1-chloro-2,4-dinitrobenzene, where 10% CD_3CN was used. Some aromatic ^{1}H signals were obscured by signals of the excess BHA^-. The pDs of the solutions were corrected considering $pD = pH_{read} + 0.4$ at 25 °C.

Mass Spectrometry. To identify intermediates and reaction products of phosphate esters with BHA, direct infusion electrospray ionization mass spectrometry analyses were performed using a hydrid triple quadrupole linear ion-trap mass spectrometer.[6] A microsyringe pump delivered the reagent solution into the ESI source at a flow rate of 10 μL/min. ESI and the QqQ (linear trap) mass spectrometer were operated in the negative-ion mode. Main conditions: curtain gas nitrogen flow = 20 mL min^{-1}; ion spray voltage = −4500 eV; declustering potential = −21 eV; entrance potential = −10 eV; collision cell exit potential = −12 eV. The carbamyl derivative **8** detected by ESI-MS was subjected to ESI-MS/MS by using collision-induced dissociation (CID), with nitrogen and collision energies ranging from 5 to 45 eV. Other MS analyses were performed to detect nonanionic products in the reaction medium.

ASSOCIATED CONTENT

Supporting Information

Tables and figures giving kinetic, ESI-MS(/MS), and ^{1}H and ^{31}P NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to INCT-Catálise, PRONEX, FAPESC, CNPq, and CAPES for support of this work and to FONDECYT 1100640.

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