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Binding of reactive organophosphate by oximes via hydrogen bond

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Abstract. In this contribution, the ability of simple oximes to bind a well-known nerve agent simulant (dimethylmethylphosphonate, DMMP) via hydrogen bond is reported. UV/Vis measurements indicate the formation of 1:1 complexes. ^{1}H-, ^{31}P-NMR titrations and T-ROESY experiments confirm that oximes bind the organophosphate via hydrogen bond.

Keywords. Nerve agents; oximes; hydrogen bond; organophosphate stimulant.

1. Introduction

Chemical warfare agents (CWAs) are classified into several groups; i.e., nerve agents, asphyxiant/blood agents, vesicant agents, pulmonary agents, lachrymatory agents, incapacitating agents and cytotoxic proteins.[1,2] All these nerve agents are among the most dangerous of chemical warfare species. In particular, nerve agents are a family of highly toxic phosphoric acid esters, structurally related to the larger family of organophosphate compounds (figure 1). Deadly nerve agents have rapid and severe effects on human and animal health, either in gas, aerosol or liquid forms.[3,4] Their effect is mainly due to their ability to inhibit the action of acetylcholineesterase (AChE), a critical central nervous system enzyme.

Ease of production and extreme toxicity of organophosphorous nerve agents underline the need to detect these odourless and colourless chemicals. As a consequence, intense research and many efforts have been directed towards developing sensitive and selective systems for detection of these harmful compounds.[5–10] A variety of detection methods for the CWAs has been developed, including enzymatic assays,[11] GC-MS (gas chromatography-mass spectrometry),[12–15] and electrochemical analysis.[16–18] However, all these methods present at least one of the following limitations: low sensitivity, limited selectivity, non-portability, difficulties in real-time monitoring and 'false positive' readings. The main antidote in poisoning by organophosphate is a very fast pharmacological treatment based on the use of certain oximes (e.g., pralidoxime and obidoxime chloride),[19,20] which due to the presence of the basic oxime group, promote the hydrolysis of phosphodiesteric bond. This mechanism is supported by 'basic activation', in particular by formation of 'supernucleophilic species' –NO–, that reacts with phosphodiesteric groups restoring the active site of AChE. This mechanism is irreversible and leads to wastage of oxime.

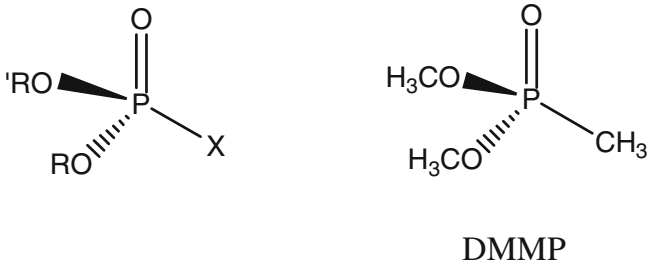

Figure 1. General formula of an organophosphate (R/R′ = alkyl groups, X = halogen) (left) and dimethylmethylphosphonate (DMMP) (right).

The ability of phosphoryl groups to accept hydrogen bond is well-known,[21,22] but to date the exploitation of this group for hydrogen bond-mediated recognition of CWAs remains under-exploited. Very recently, Gale reported on the complexation of Soman (an extremely toxic chemical warfare agent), by a series of diindolylurea-based receptors.[23] ^{1}H and ^{31}P NMR titrations have shown that these hydrogen-bond donating receptors bind Soman in organic solution. Here, we present the ability of three different oximes to bind a well-known nerve agents simulant (DMMP) in

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solution via hydrogen bond, without activation in basic condition.

2. Experimental

2.1 *General*

The NMR experiments were carried out at 27°C on a Varian UNITY Inova 500 MHz spectrometer (^{1}H NMR at 499.88 MHz, ^{13}C NMR at 125.7 MHz in $CDCl_3$) equipped with pulse field gradient module (Z axis) and a tunable 5 mm Varian inverse detection probe (ID-PFG). UV-VIS spectra were measured on Jasco-V630 spectrometer using solution in CH_3CN. All chemicals were of reagent grade and were used without further purification. Synthesis of oximes **1**[24] and **2**[25] and compound **5**[26–28] are in accordance with literature procedures.

2.1a *Synthesis of 4-phtalimidododecyloxy-benzaldhyde (**6**)*: A solution in dry acetonitrile (10 mL) containing 200 mg (1.64 mmol) of **4**, 2.3 g (16.91 mmol) of anhydrous K_2CO_3 and 625 mg (1.8 mmol) of **5** was stirred at reflux for 36 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water (thrice), and the solvent was evaporated to afford an oil. Crystallization by CH_2Cl_2/hexane yielded the pure compound **6** (isolated yield 62%). ^{1}H NMR (500 MHz, $CDCl_3$), δ = 9.87 (s, 1H), 7.83 (dd, 2H), 7.81 (d, J = 8.5 Hz, 2H), 7.69 (m, 2H), 6.98 (d, J = 8.5 Hz, 2H), 4.02 (t, J = 6.5 Hz, 2H), 3.66 (t, J = 7.0 Hz, 2H), 1.80 (m, 2H), 1.66 (m, 2H), 1.44 (m, 2H), 1.28 (m, 14H). ^{13}C NMR (DMSO 125 MHz) δ 26.3, 27.1, 28.2, 29.3, 29.5 (×2), 29.7, 30.0 (×3), 38.1, 69.6, 115.8, 123.9, 130.5, 132.0, 132.2, 135.0, 164.4, 168.5, 192.1 ppm. Elemental Analysis Calcd. (%) for $C_{27}H_{33}NO_4$: C, 74.45; H, 7.64. Found: C, 74.43; H, 7.61.

2.1b *Synthesis of 4-phtalimidododecyloxy-benzaldoxime (**3**)*: To a solution of 100 mg (0.23 mmol) of aldehyde **6** in 10 mL of absolute ethanol, a solution of hydroxylamine chloride (32 mg, 0.46 mmol) and NaOH (18.3 mg, 0.46 mmol) in 5 mL of H_2O was added. The mixture was stirred at room temperature for 7 h. Acetic acid was added to the reaction mixture to pH 6 cooling with an ice bath for 1 h. The white solid formed was filtered and washed with a mixture of water/acetic acid (10:1) to afford the desired compound **3** (isolated yield 77%). ^{1}H NMR (500 MHz,$CDCl_3$), δ = 8.07 (s, 1H), 7.84 (dd, 2H), 7.71 (dd, 2H), 7.49 (d, J = 8.5 Hz, 2H), 7.04 (s br, 1H), 6.89 (d, J = 8.5 Hz, 2H), 3.98 (t, J = 6.5 Hz, 2H), 3.68 (t, J = 7.0 Hz, 2H), 1.78 (m, 2H), 1.66 (m, 2H), 1.44 (m, 2H), 1.28 (m, 14H). Elemental Analysis Calcd. (%) for $C_{27}H_{34}N_2O_4$: C, 71.97; H, 7.61. Found: C, 71.91; H, 7.64.

3. Results and discussion

Oximes **1** and **2** reported in figure 2 were synthesized following the literature procedures,[24,25] while **3** was prepared according to the reactions showen in scheme 1.

Introduction of a dodecylphtalimidic arm[26–28] in the aromatic ring of **1** leads to oxime **3**. This functionalization can be exploited to anchor the oxime **3** onto a solid support,[29] (conversion of the phthalimido moiety into an amino group by treatment with hydrazine, under standard Gabriel conditions), achieving a 'device' for the organophosphate detection. The target 4-phtalimidododecyloxy-benzaldoxime **3** (scheme 1) was synthesized in two steps starting from 4-hydroxybenzaldehyde **4**.

Compound **4** was treated in acetonitrile using an excess of potassium carbonate with *N*-(12-bromododecyl)phthalimide **5**[26–28] to yield the phtalimidododecyloxy derivative **6** (isolated yield 62%). Conversion of aldehyde into an oxime group by treatment with hydoxylamine hydrochloride and sodium hydroxide yielded the oxime **3** (isolated yield 77%). Compounds **3** and **6** were characterized by ^{1}H- and ^{13}C NMR spectroscopy, as well as by elementary analysis (see Electronic supplementary information).

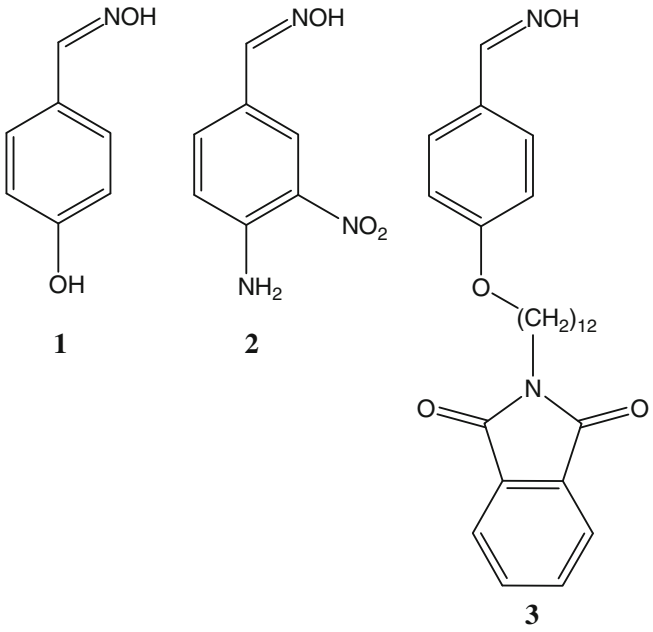
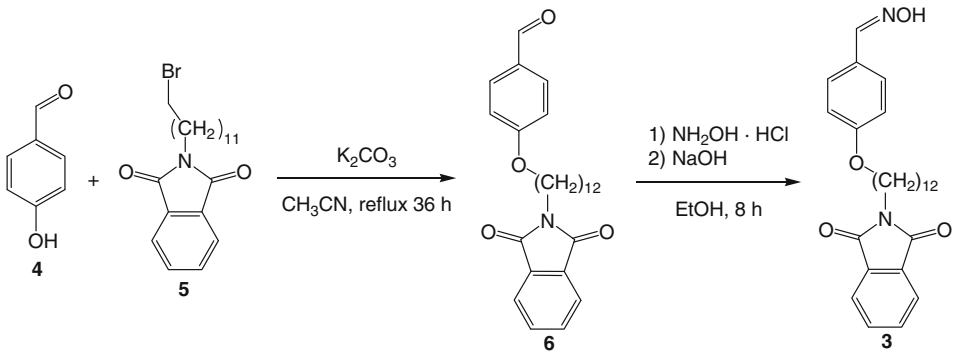


Figure 2. Chemical structure of oximes **1–3**.



Scheme 1. Synthesis of 4-phtalimido-dodecyloxy-benzaldoxime **3**.

The ability of oximes **1-3** to recognize and bind organophosphorous derivatives was studied by UV-Vis spectroscopy using DMMP as simulant of nerve agents.[30–32] Figure 3 shows a typical UV/Vis titration experiment between oxime **1** and DMMP.

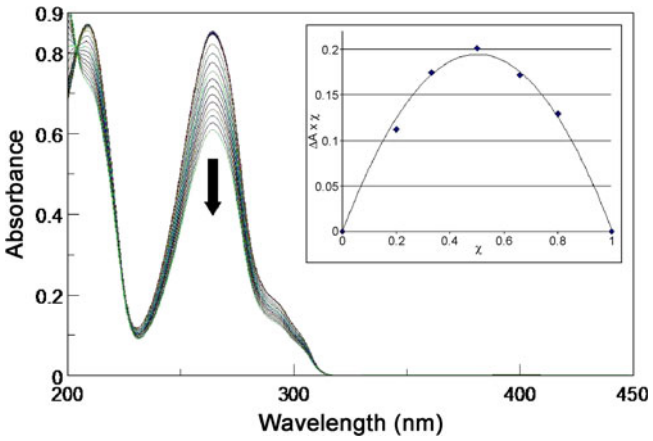


Figure 3. UV/Vis titration experiment of oxime **1** upon addition of increasing amounts of DMMP ($1.5{\cdot}10^{-5}$ to $1.7{\cdot}10^{-3}$ M) in CH_3CN at 25°C. The inset displays the corresponding job's plot.

Progressive decreasing of absorbance at 264 nm, upon addition of increasing amounts of DMMP, is relative to π–π^* transition suggesting an interaction between oxime group and DMMP.[33] Free energy ΔG° calculated from the binding constant of the complex **1** $\subset$ DMMP ($K = 2.0 \times 10^3\ M^{-1}$)[34–39] is −4.49 Kcal/mol, in good agreement with an interaction based on the formation of an hydrogen bond (see ESI 4–6). A similar behaviour was found with oximes **2** and **3**; UV/Vis titration experiments between oximes **2** and **3** with DMMP are reported in figure 4.

Binding constant value for the complex **2** $\subset$ DMMP is $K = 1.0 \times 10^2\ M^{-1}$ and the associated ΔG° is −2.72 Kcal/mol, while affinity value of the complex **3** $\subset$ DMMP is $K = 2.9 \times 10^3\ M^{-1}$ with a related ΔG° of −4.71 Kcal/mol. Also, in these cases, the energy values clearly indicate that a weak interaction is involved. Differences between the binding constant values deserve a comment: it is clear that the presence of the strong electron-acceptor group NO_2 in **2** reduces the binding energy with DMMP, while oximes **1** and **3** show a comparable energy probably due to a similar electronic effect.

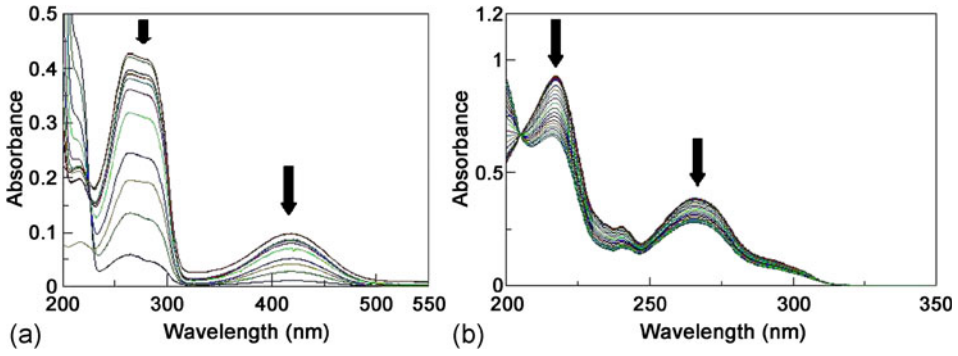


Figure 4. UV/Vis titration experiments of (**a**) oxime **2** and (**b**) oxime **3** upon addition of increasing amounts of DMMP in CH_3CN at 25°C.

Hence, to confirm this hypothesis, ^{1}H- and ^{31}P-NMR titration experiments were performed. Figure 5 shows the downfield shift of the proton of –NOH group of the oxime **2** upon the addition of 1 equivalent of DMMP in CD_3CN.

A similar experiment was carried out in DMSO-d_6 but no chemical shift changes were observed after the addition of 1 equivalent of DMMP (see ESI 10), indicating that hydrogen bonding between **2** and DMMP in DMSO is precluded by the competition of the strong polar solvent.

Furthermore, ^{31}P NMR titration of DMMP with different aliquots of oxime **2** (figure 6) shows a downfield shift of the phosphorous signal due to the interaction with the hydroxyl group of the oxime.

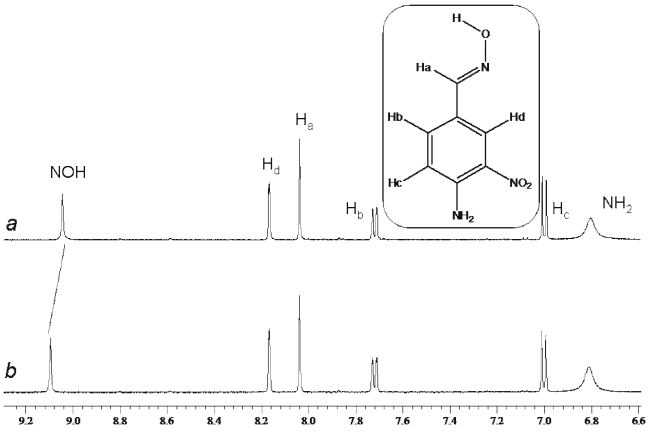


Figure 5. (**a**) ^{1}H NMR spectra of **2** (1×10^{-3} M in CD_3CN), (**b**) ^{1}H NMR spectra of **2** with 1 equivalent of DMMP in CD_3CN.

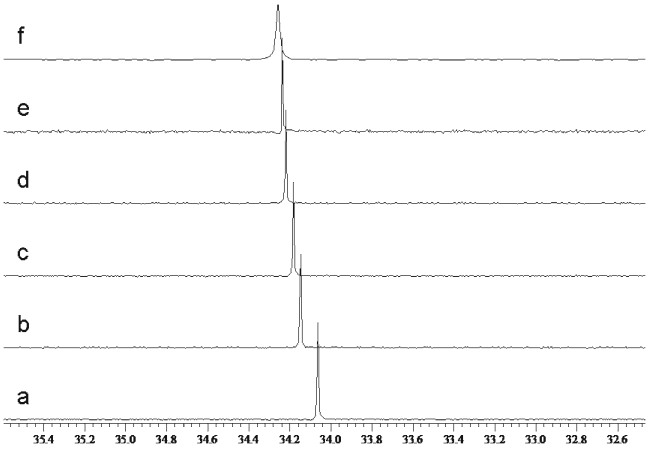


Figure 6. Selected region of the spectrum in a typical ^{31}P NMR titration of DMMP with oxime **2** in CD_3CN. Constant concentration of DMMP (1×10^{-3} M) with addition of various concentrations (0 to 2×10^{-3} M) of **2**. (**a**) DMMP; (**b**) [DMMP]/[**2**] 4:1; (**c**) [DMMP]/[**2**] 2:1; (**d**) [DMMP]/[**2**] 1:1 (**e**) [DMMP]/[**2**] 1:1.5; (**f**) [DMMP]/[**2**] 1:2.

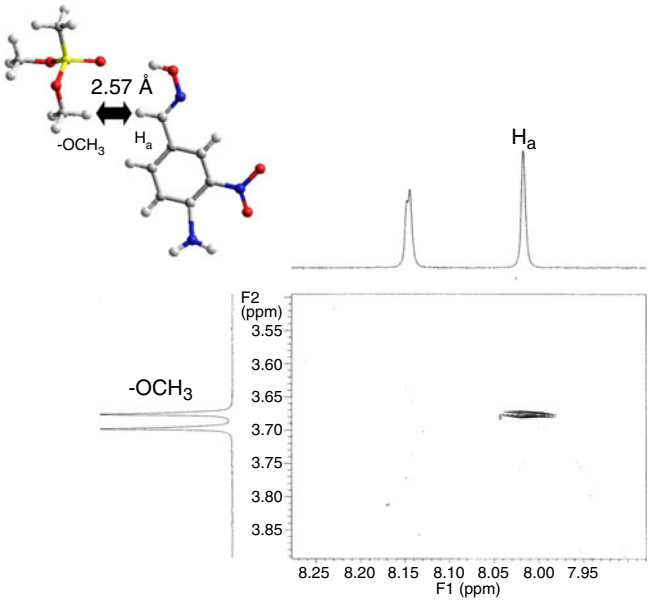


Figure 7. Details of T-ROESY of the complex **2** ⊂ DMMP (1×10^{-3} M in CD_3CN). (Optimized structure of the complex **2** ⊂ DMMP; HyperChem 7.5, Amber).

In addition, T-ROESY experiment of the complex **2** ⊂ DMMP reveals an Overhauser contact between the hydrogen of the imine group and the –OCH_3 group of the DMMP (figure 7); moreover, molecular modelling supports the hydrogen bonding interaction between the hydroxymethyl group of DMMP and the imine hydrogen of **2** (calculated distance is 2.57 Å, figure 7).

4. Conclusion

In summary, we reported the ability of three oximes to bind the simulant nerve agent DMMP in solution by hydrogen bond interaction. Pertinent binding constant values of the complexes of oximes **1–3** with DMMP were determined by UV-Vis titrations, while ^{1}H-, ^{31}P-NMR titrations and T-ROESY experiments provide good evidences of the hydrogen bond interaction. In oxime **3,** the phtalimido group can be converted into amino group, allowing to anchor the molecule onto a solid support via nucleophilic attack. The non-covalent interactions of this systems with DMMP are at the basis for the realization of reversible detectors of nerve agent simulants in solution and also on solid supports.

Supplementary Information

The electronic supporting information can be seen in www.ias.ac.in/chemsci.

Acknowledgement

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