Scientific Journal of Faculty of Education, Misurata University-Libya, Vol. 2, No. 6, Dec. 2016

Published on Web 01/12/2016

المجلة العلمية لكلية التربية، جامعة مصراتة،ليبيا، المجلد الثاني ـ العدد السادس، ديسمبر 2016م

# Enhancing The Hydrolysis of Phosphate Esters By Intramolecular Hydrogen Bonding

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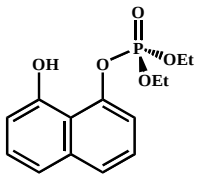
**Abstract:** The research project aim is to study the hydrolysis of 8-diethylphosphate-1-naphthalenoltriester, **1** with hydroxylamine in water. Compound **1** was successfully synthesized and its rate of reaction with hydroxylamine was monitored at 343 nm. Pseudo first order rate of P-O cleavage of **1** was measured to be $7.39\times 10^{-3}$ $M^{-1}s^{-1}$ at 60 °C, which is 178 fold and 7 fold slower than diethyl 8-dimethylamino-1-naphthyl phosphatetriester, **3** at 60 °C 1.32 $M^{-1}s^{-1}$ and diethyl 8-amino-1-naphthyl phosphatetriester, **2** at 90 °C $5.5 \times 10^{-2}$ $M^{-1}s^{-1}$ respectively. The rate of P-O cleavage of **1** with hydroxylamine was found to be faster than that of 4-nitrophenyl-1-cyclopropylphosphatetriester**4** and 4-chlorophenyl-1-cyclopropylphosphate triester**5**, where the reaction was too slow to detect at 60 °C.

***Keywords*:** The Hydrolysis of Phosphate, Intramolecular Hydrogen Bonding.

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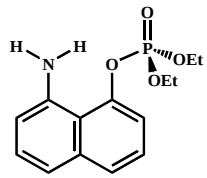


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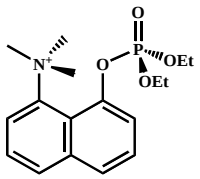


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## INTRODUCTION

Winmill and MOORE are recorded with first proposing the hydrogen bond in 1912 [3]. They used it to explain why trimethylammonium hydroxide is a weaker base than tetramethylammonium hydroxide. However the description of hydrogen bonding in arguably its most important setting, water, did not come until 1920. Latimer and Rodebush referenced the unpublished work of

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a Mr. Huggins in their own laboratory. He "used the idea of a hydrogen kernel held between two atoms as a theory. They described the phenomenon in water in terms of the Lewis theory of valence;"a free pair of electrons on one water molecule might be able to exert sufficient force on a hydrogen held by a pair of electrons on another water molecule to bind the two molecules together. Such combination need not be limited to the formation of double or triple molecules.

Indeed the liquid may be made up of large aggregates of molecules, continually breaking up and reforming under the influence of thermal agitation. Such an explanation amounts to saying that the hydrogen nucleus held between 2 octets constitutes a weak bond [4]. More recently an IUPAC team formally defined the hydrogen bond as: an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation [5]. A recent development in the area of hydrogen bonding is the proposal that low barrier hydrogen bonds (LBHBs), also known as short strong hydrogen bonds (SSHBs), play a role in enzyme catalysis [6]. The strength of a hydrogen bond is dependent upon its length, linearity, microenvironment and the degree to which the $pK_a$'s of the conjugate acids of the donor and acceptor [7].

In water, for example, the bound oxygen atoms are separated by ~ 2.8 Å and the weak $\Delta H$ of formation for the hydrogen bond is ~ 21 kJ $mol^{-1}$. The weakness of this bond comes from the poor $pK_a$ match between the oxygen atoms. The $pK_a$ of $H_3O^+$ is ~ -1.7 and the $pK_a$ of $H_2O$ is ~ 15.7. This means that the hydrogen atom is tightly associated with the water molecule. However, in organic solvents, strong hydrogen bonds between matched atoms are possible with a maximum $\Delta H$ of formation of ~ 85 kJ $mol^{-1}$. As a substrate bound to an enzyme active site is often perceived as no longer being in an aqueous environment, the properties of hydrogen bonds in organic solvent are very relevant [7].

The current thinking is that enzymes use LBHBs to catalyze reactions by converting a weak hydrogen bond in the substrate-enzyme complex into an

LBHB in the transition state. [6] - [8]. An enzyme does this by changing the $pK_a$ of the substrate (in a reaction) to match the enzyme residue that the substrate is hydrogen-bonded to. The thermodynamic benefit catalyses the reaction. This hypothesis is highly dependent upon how the enzyme active site microenvironment is perceived, as the ΔG of formation of hydrogen bonds dependence on $pK_a$ is reliant on the solvent conditions [9]. The dependence of ΔG on $\Delta pK_a$ in water is very small, but in organic solvents the dependence is much higher. Thus as an enzyme active site is perceived as a non-aqueous environment, we can assume that the ΔG of formation of hydrogen bonds' is highly dependent on the $\Delta pK_a$.

Therefore the strengthening of hydrogen bonds in an enzyme active site has the potential for a catalytic rate acceleration of many orders of magnitude [7]. A well characterized example of hydrogen bonding in enzymatic catalysis is the oxyanion hole. The oxyanion hole consists of two or three hydrogen bond donors orientated towards a central oxygen atom [10]. Upon nucleophilic attack of the carbonyl, electron density around the carbonyl oxygen increases. The carbonyl oxygen thus becomes a better hydrogen bond acceptor in the transition state. This means the hydrogen bond donors can have a catalytic effect on the reaction. Studies have shown that oxyanion holes in enzyme active sites are not optimized to bind the transition state, but instead are arranged to lower the activation energy of the reaction. They achieve this by orientating their hydrogen bond donors so that they stabilize the transition state in a way that is slightly less than optimal. This allows the oxyanion hole to stabilize the substrate much less well than is possible with the same number of hydrogen bonds, thus providing a driving force for the reaction [10]. It is of interest to apply the principle of hydrogen bond catalysis to other reactions, for example the hydrolysis of phosphate esters.

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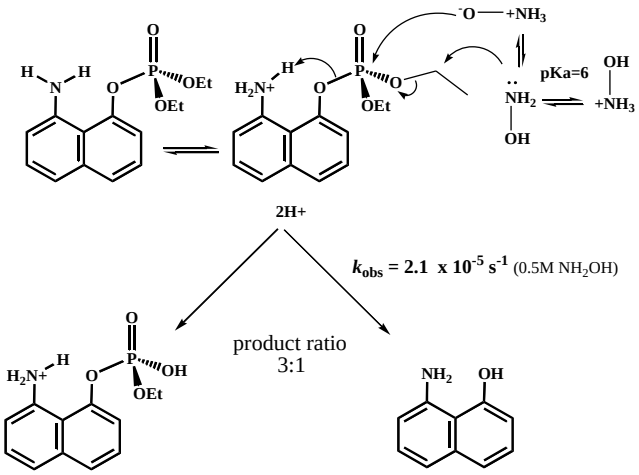



## MATERIALS AND METHODS

Preparation of 8-diethylphosphate-1-naphthalenol **1**: to a suspension of sodium hydride (0.036 g, 1.5 mmol) in DCM (10 ml) under nitrogen at 0 °C was added a solution of 1, 8-dihydroxynaphthalene (0.2 g, 1.25 mmol) in DCM (5 ml). Diethylchlorophosphate (0.259 g, 1.5 mmol) was added and the reaction stirred for 16 hours at room temperature. The reaction was quenched with HCl (1M) and extracted with DCM (2 x 15 ml), dried with $MgSO_4$ and the solvent removed. The brown residue was purified by column chromatography on silica gel (80:20 hexane: ethyl acetate) yielding a white solid. Mass obtained: 0.081 g. Yield 22%. MS (ES) m/z 297.1 $MH^+$. $^1$HNMR (250 MHz, $CDCl_3$): δ 1.35 (td, *J* = 7.5 Hz, 6H, *a*), δ 4.29 ($q_n$d, *J* = 7.5 Hz, 4H, *b*), δ 6.94-7.01 (m, 1H, *h*), δ 7.34 (s, 1H, *c*), δ 7.37 (d, *J* = 2.5 Hz, 1H, *g*), δ 7.39 (d, *J* = 5 Hz, 1H, *f*), δ 7.46 (t, *J* = 2.5 Hz, 1H, *d*), δ 7.63 (d, *J* = 7.5 Hz, 1H, *e*). $^{13}$CNMR (400 MHz, $CDCl_3$): (quaternary carbon peaks were not observed) δ 16.15 (down, $CH_3$, 2C, *a*), δ 65.37 (up, $CH_2$, 2C, *b*), δ 111.93 (down, CH, 1C, *h*), δ 114.06 (down, CH, 1C, *c*), δ 119.84 (down, CH, 1C, *f*), δ 125.41 (down, CH, 1C, *g*), δ 125.61 (down, CH, 1C, *d*), δ 127.74 (down, CH, 1C, *c*). $^{31}$PNMR (250 MHz, $CDCl_3$): δ – 6.62 (s, 1P).

## RESULTS AND DISCUSSION

Kinetic experiments were conducted for the reaction of compound**1** with Hydroxylamine as shown below at different pHs.

Experiment at pH 6.10:

A solution of hydroxylamine in water (200 µl, 1 M) and a potassium hydroxide solution (100 µl, 1 M) were added to water (1600 µl) in a 3 ml, 1 cm quartz cuvette. The solution was equilibrated to 60 °C in a Cary 300 Bio UV-visible spectrometer before a solution of **1** (100 µl, 1 mM) was added to the reaction mixture to initiate reaction. This yielded a 50 µM solution of **1**, a 0.1 M solution of hydroxylamine. The ionic strength of the solution was 1 M (KCl). The progress of reaction was monitored at 343 nm.

Experiment at pH 7.00:

A solution of hydroxylamine in water (200 µl, 1 M), a solution of EPPS in water (500 µl, 0.5 M) and a potassium hydroxide solution (250 µl, 1 M) were added to water (950 µl) in a 3 ml, 1 cm quartz cuvette. The solution was equilibrated to 60 °C in a Cary 300 Bio UV-visible spectrometer before a solution of **1** (100 µl, 1 mM) was added to the mixture to initiate reaction. This yielded a 50 µM solution of **1**, a 0.1 M solution of hydroxylamine and a buffer concentration of 125mM. The ionic strength of the solution was 1 M (KCl). The progress of reaction was monitored at 343 nm.

Experiment at pH 7.70:

A solution of hydroxylamine in water (200 µl, 1 M), a solution of EPPS in water (200 µl, 0.5 M) and a potassium hydroxide solution (250 µl, 1 M) were added to water (1250 µl) in a 3 ml, 1 cm quartz cuvette. The solution was equilibrated to 60 °C in a Cary 300 Bio UV-visible spectrometer before a solution of **1** (100 µl, 1 mM) was added to the mixture to initiate reaction. This yielded a 50 µM solution of **1**, a 0.1 M solution of hydroxylamine and a buffer concentration of 50 mM. The ionic strength of the solution was 1 M (KCl). The progress of reaction was monitored at 343 nm.

Experiment at pH 8.8:

A solution of hydroxylamine in water (200 µl, 1 M), a solution of EPPS in water (200 µl, 0.5 M) and a potassium hydroxide solution (290 µl, 1 M) were added to water (1210 µl) in a 3 ml, 1 cm quartz cuvette. The solution was

equilibrated to 60 °C in a Cary 300 Bio UV-visible spectrometer before a solution of **1** (100 µl, 1 mM) was added to the mixture to initiate reaction. This gives a 50 µM solution of **1**, a 0.1 M solution of hydroxylamine and a buffer concentration of 50 mM. The ionic strength of the solution was 1 M (KCl). The progress of reaction was monitored at 343 nm.

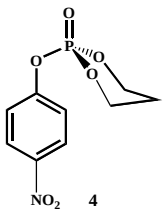
All observed pseudo-first order rate constantsfrom the experiments at pH 6.10, 7.00, 7.70 and 8.80 are summarized in table I as shown below.

**TABLE I**

THE CALCULATION OF THE SECOND ORDER RATE CONSTANT, $K_2$ FOR THE REACTION OF **1** WITH HYDROXYLAMINE

| pH at 60 °C | Observed pseudo-first order rate constant / $s^{-1}$ | $k_2$ / $M^{-1}s^{-1}$ |
|---|---|---|
| 6.10 ± 0.02 | $2.43 \pm 0.07 \times 10^{-4}$ | $2.43 \pm 0.07 \times 10^{-3}$ |
| 7.00 ± 0.02 | $5.61 \pm 0.01 \times 10^{-4}$ | $5.61 \pm 0.01 \times 10^{-3}$ |
| 7.70 ± 0.02 | $5.85 \pm 0.02 \times 10^{-4}$ | $5.85 \pm 0.02 \times 10^{-3}$ |
| 8.80 ± 0.02 | $7.39 \pm 0.02 \times 10^{-4}$ | $7.39 \pm 0.02 \times 10^{-3}$ |

The reactivity of similar compounds can be compared as here for the hydrolysis of similar phosphate triesters **1**, **2** and **3**. From the data in this work for compound**1**, and literature data for **2** and **3** we can find the relative rate constants as shown in table II.

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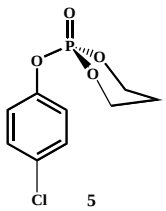


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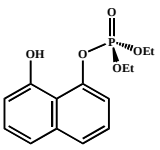
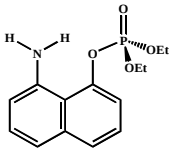
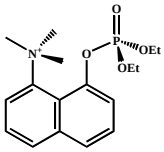
Kinetic Data for controlled compounds **4** and **5**:

Compounds **4** and **5** were selected for comparison with the reactivity of **1**. The p*K*a of their corresponding leaving groups is 7.14 and 9.38 respectively. Substrate **4** has a similar leaving group p$K_a$ to **1** and therefore provided a useful comparison compound for kinetic studies. Compound 5 has a significantly higher leaving group p$K_a$ than both **1** and **1**and therefore provided useful information on the sensitivity of the reaction to the $pK_a$ of the leaving group.

Initial studies of both compounds were conducted with 50 µM of compound, 0.1 M hydroxylamine at pH 7.00, 60 °C and I = 1 M (KCl). The reaction of **5** was too slow to observe under these conditions. Studies of **4** indicated that P-O cleavage with hydroxylamine was slower than **1**.

**TABLE II**

THE SECOND ORDER RATE CONSTANTS, $K_2$ AND THE RELATIVE RATE CONSTANTS, $K_{REL}$ FOR THE REACTION OF PHOSPHATE TRIESTERS**1, 2** AND **3**

| substrate |  1 |  2 |  3 |
|---|---|---|---|
| $k_2$ / $M^{-1}s^{-1}$ | $7.39 \pm 0.02 \times 10^{-3}$ | $5.5 \pm 0.7 \times 10^{-2}$ [2] | 1.32[11] |
| $k_{rel}$ | 1 | 7 | 178 |

## Conclusion

It is known that in the presence of the dimethyl ammonium $NH^+$ in phosphate triester, **3** accelerates the rate of hydrolysis of the phosphate by $10^6$.This is significant rate acceleration. It is also known that the methylation of the amine is not important to the reactivity of amine. Rate of P-O cleavage in **1** with hydroxylamine is not significantly different to the rate of reaction of

phosphate triester, **2**. Although the hydroxyl compound, **1** is reacts 178 fold slower than the dimethyl ammonium compound, **3** with hydroxylamine, the hydroxyl group could be providing a significant catalytic effect. Initial kinetic studies of P-O cleavage of **4** and **5** with hydroxylamine seemed to indicate that the reactions proceeded more slowly than **1**. This again indicates that the hydroxyl group is having a significant catalytic effect on the reaction. However more data is required before a definitive conclusion can be drawn. It is also unclear as to whether the reaction involves proton transfer catalysis or solely the formation of a hydrogen bond.

## ACKNOWLEDGMENT

Financial support of this research by Misurata University, and Sheffield University is gratefully acknowledged. Thanks go to Prof. Williams, University of Sheffield, Misurata University is gratefully acknowledged for funding.

## REFERENCES

**[1]** A. J. Kirby, D. W. Tondo, M. Medeiros, B. S. Souza, J. P. Priebe, M. F. Lima, F. Nome, "Efficient intramolecular general-acid catalysis of the reactions of α-effect nucleophiles and ammonia oxide with a phosphate triester", *J. Am. Chem. Soc*., **2009**, 131, 5, 2023-2028.

**[2]** K. J. Tossell, "Catalysis of phosphate ester hydrolysis through hydrogen bonding", Doctoral Thesis, *University of Sheffield*, **2011**.

**[3]** T. S. Moore, T. F. Winmill, "The state of amines in aqueous solution", *J. Chem. Soc.*, **1912**, 101, 1635.

**[4]** W. M. Latimer, W. H. Rodebush, "Polarity and ionisation from the standpoint of Lewis Theory of valence", *J. Am. Chem. Soc.*, **1920**, 42, 1419-1433.

**[5]** E. Arunan, G. R. Desiraju, R. A. Klein, J. Sadlej, S. Scheiner, I. Alkorta, D. C. Clary, R. H. Crabtree, J. J. Dannenberg, P. Hobza, H. G. Kjaergaard, A. C. Legon, B. Mennucci and D. J. Nesbitt, "The Definition of the Hydrogen Bond", *IUPAC*, **2004.**

**[6]** J. A. Gerlt, P. G. Gassmann, "Proton affinity of the oxyanion in the active site of ketosteroidisomerise", *Biochemistry*, **1993**, 32, 11934-11952.

**[7]** W. W. Cleland, P. A. Frey, J. A. Gerlt, "The low barrier hydrogen bond in enzymatic catalysis", *J. Bio. Chem.*, **1998**, 273, 40, 25529-25532.

**[8]** P. A. Frey, S. A. Whitt, J. B. Tobin, "A low barrier hydrogen bond in the catalytic triad of serine proteases", *Science*, **1994**, 264, 1927-1930.

**[9]** S. Shan, S. Loh, D. Herschlag, "The energetics of hydrogen bonds in model systems. Implications for enzymatic catalysis", *Science*, **1996**, 272, 97-101.

**[10]** L. Simón, J. M. Goodman, "Enzyme Catalysis by Hydrogen Bonds: The Balance between Transition State Binding and Substrate Binding in Oxyanion Holes", *J. Org. Chem.*, **2010**, 75, 1831–1840.

**[11]** N. Asaad, A. J. Kirby, "Concurrent nucleophilic and general acid catalysis of the hydrolysis of a phosphate triester", *J. Chem. Soc., Perkin Trans. 2*, **2003**, 1708-1712.