KINETIC STUDY OF MONO-N-ETHYL-*O*-TOLUIDINE PHOSPHATE IN BUFFER MEDIUM

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ABSTRACT

The aim of present work is to study the hydrolytic reactions of mono-N-ethyl-*o*-toluidine phosphate in buffer medium at $50 \pm 0.5^{\circ}$C in the range of pH 0.00 to 7.80. The rate coefficients were determined from the rate of appearance of inorganic phosphate, spectrophotometrically by Allen's modified method. Pseudo first order rate coefficients were obtained. The rate of reaction increases with increase in pH upto 4.00. The maximum value at pH 4.00 is due to hydrolysis via mononegative and neutral species. After pH 4.00 the rate of reaction decreases due to dissociation of mononegative species in to dinegative species. The nature of dinegative species have been found to be inert. The theoretical rate determined from specific rate and fractions of the neutral species agree closely with the experimental rates. Bond fission and bimolecular nature of hydrolysis have been supported by Arrhenius parameters and study of solvent effect. The hydrolysis of monoester involves P-N bond fission, which is strengthened by isokinetic relationship.

Key words: Hydrolysis, Mono-N-ethyl-*o*-toluidine phosphate, Buffer medium, Mononegative species.

INTRODUCTION

Organophosphorus compounds have gained considerable attention in organic synthesis, as reactive intermediate particularly in nucleic acid synthesis and in the formation of C-C bonds in synthesis of terpenoids and steroids. Synthesis of new phosphate esters for applications in medicine and industry has attracted the attention of researchers in recent years[1]. Nucleoside phosphates and their phosphonate analogues have proven to be exceedingly important agents for anticancer, antiviral therapy[2] and act as HIV protease inhibitors[3]. Organophosphate, a group of pesticides commonly used for protecting crops, destroying insects such as fleas, lice, flies, mosquitoes[4] and mostly in sheep dips[5].

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Phosphorus compounds are also important in food stuffs-for example, phosphoric acid acidifies soft drinks[6].

The hydrolysis of phosphate esters is one of the most fundamental chemical and biochemical reaction[7]. Hydrolysis of these esters play a very important role in energy metabolism and in various cellular signal transduction pathways in biological systems[8]. Ester hydrolysis is one of the most frequent biotransformation takes place on such fundamental biomolecules as ATP, ADP and acetylcholine, as well as on acetylsalicylic acid the oldest and best known prodrug[9]. Owing to their widespread applications in various fields, synthesis and the hydrolytic study of a variety of organic phosphates has become the subject of research interest to chemists and biologist during the past few decades[10].

EXPERIMENTAL

Mono-N-ethyl-*o*-toluidine phosphate has been synthesized by the method described earlier[11]. Kinetic study of the hydrolysis of mono-N-ethyl-*o*-toluidine phosphate has been carried out at $50 \pm 0.5^{\circ}C$ employing 5×10^{-4} mol dm^{-3} solution of the monoester in aqueous medium. The buffer solutions were prepared using appropriate mixture of KCl, COOH.C$_6$H$_4$.COOK, NaOH and H$_3$BO$_3$. The inorganic phosphate produced during hydrolysis has been determined spectrophotometrically using Allen's modified method[12]. All the solutions have been prepared in triply distilled water and chemicals used were of A. R. grade.

RESULTS AND DISCUSSION

Hydrolysis of mono-N-ethyl-*o*-toluidine phosphate has been studied in the range of pH 0.00 to 7.80 at $50 \pm 0.5^{\circ}C$ using suitable buffer solutions. Pseudo-first-order rate constants obtained are shown in Table 1. From the results, it may be seen that the rate of reaction increases with the increase in pH upto 4.00. The maximum value at pH 4.00 is due to hydrolysis via mononegative species and dissociation of neutral species. After pH 4.00 the fall in rates is due to the inertness of the dinegative species[13].

The rates of neutral and mononegative species are calculated from the equation (1) and (2):

$$k_N = k_{N_o} \frac{N}{N + M} \qquad \qquad …(1)$$

$$k_M = k_{M_o} \frac{M}{M + N} \qquad \qquad …(2)$$

Where k_{No} is specific neutral rate, k_{Mo} is specific mononegative rate at pH 4.00 and N/(N+M) and M/(M+N) are the fraction of neutral and mononegative species, respectively. The results summarized in Table 1 show that there is close agreement between calculated and experimental rates. The value of specific neutral rates (k_{No}) was determined from the equation:

$$k = k_{M_o} \frac{M}{M+N} + k_{N_o} \frac{N}{N+M} + k_H{}^+C_H{}^+ \qquad \ldots(3)$$

Where, k is experimental rate. There is good agreement of specific neutral rate (k_{No}) values obtained by equation (3) and ionic strength data. The value of k_{No} determined by equation (3) is 3.90 x 10^{-3} min^{-1} at different pH from pH 0.00 to 1.00 and the value of k_{No} obtained from ionic strength data is 3.16 x 10^{-3} min^{-1}. It is clear from Table 1 that in the region pH 0.00 to 1.00; the hydrolysis is governed by neutral, conjugate and mononegative species. In the region pH 1.00 to 1.20, the reactions proceed *via* neutral and mono negative species. In the region pH 1.20 to 7.80, only mononegative species are reactive.

Table 1: Estimated & Experimental rates of the hydrolysis of mono-N-ethyl-*o*-toluidine phosphate via neutral and mononegative species at different pH values at 50°C

pH	M/ M+N	N/ N+M	k_M x 10^3 (min^{-1})	k_N x 10^3 (min^{-1})	k_H+.C_H+ 10^3 (min^{-1})	k x 10^3 (min^{-1}) (Estd.)	k x 10^3 (min^{-1}) (Expt.)	3+log$k$ (Estd.)	3+log$k$ (Expt.)
0.00	0.06	0.94	0.47	3.67	14.79	18.93	21.10	1.28	1.32
0.30	0.12	0.88	0.95	3.43	7.08	11.46	12.87	1.06	1.11
0.70	0.25	0.75	1.97	2.93	2.75	7.65	6.28	0.88	0.80
1.00	0.40	0.60	3.16	2.34	1.35	6.85	5.60	0.84	0.75
1.20	0.54	0.45	4.27	1.76	-	6.03	4.61	0.78	0.66
2.20	0.92	0.09	7.27	0.35	-	7.62	5.49	0.88	0.74
3.20	0.99	0.01	7.82	0.04	-	7.86	6.54	0.93	0.82
4.00	1.00	0.00	7.90	0.00	-	7.90	7.90	0.90	0.90
5.20	0.98	-	7.74	-	-	7.74	6.62	0.89	0.82
6.00	0.85	-	6.72	-	-	6.72	5.27	0.83	0.72
7.80	0.34	-	2.69	-	-	2.69	3.58	0.43	0.55

Kinetic rate laws for the hydrolysis of mono-N-ethyl-*o*-toluidine phosphate may be represented as:

In the region pH 0.00 to 1.00

$$k = k_{H^+} \, C_{H^+} + 7.90 \times 10^{-3}.M/(M+N) + 3.16 \times 10^{-3}.N/(N+M)$$

In the region pH 1.00 to 1.20

$$k = 7.90 \times 10^{-3}.M/(M+N) + 3.16 \times 10^{-3}.N/(N+M)$$

In the region pH 1.20 to 7.80

$$k = 7.90 \times 10^{-3}.M/(M+N)$$

Effect of solvent

Table 2 shows a significant rise in rate constant values with increase in dioxane percentage. This may be due to better proton donating capacity of dioxane than the water. According to Chanley's observation[14], effect of solvent on the rate of hydrolysis may therefore, be taken to imply a bimolecular nucleophilic reaction with the formation of a transition state in which the charge is dispersed.

Table 2: Rate of the hydrolysis of mono-N-ethyl-*o*-toluidine phosphate in buffer media at 50°C

pH	% of dioxane	$k \times 10^3$ (min^{-1})	pH	% of dioxane	$k \times 10^3$ (min^{-1})
	0.0	4.61		0.0	7.90
	10.0	5.41		10.0	8.57
1.20	20.0	6.22	4.00	20.0	9.44
	30.0	7.33		30.0	10.03
	40.0	8.00		40.0	10.97

Effect of temperature

The effect of temperature gives valuable information about the energy requirements of reaction. In order to determine the associated Arrhenius parameter, kinetic runs were carried out at different temperature at pH 1.20 and pH 4.00. Arrhenius parameter for hydrolysis via neutral and mononegative species summarized in Table 3 are in favour of a bimolecular reaction.

Table 3: Arrhenius parameter for the hydrolysis of mono-N-ethyl-*o*-toluidine phosphate *via* neutral & mononegative species at 50°C

pH	Slope	Parameters		
		E (Kcal mol^{-1})	A (sec^{-1})	$-\Delta S^{\neq}$ (e. u.)
1.20	-0.02	9.15	7.08×10^3	43.10
4.00	-0.03	13.73	1.55×10^7	27.82

A comparative kinetic rate data for the hydrolysis of some phosphate monoesters via neutral and mono-negative species shown in Table 4 and 5 also supports the bimolecular nature of hydrolysis involving P-N bond fission.

Table 4: Comparative kinetic rate data for the hydrolysis of some phosphate monoester *via* neutral species

S. No.	Phosphate monoesters	Medium pH	E (Kcal/mole)	$-\Delta S\neq$ e. u.	Molecularity	Bond fission
1.	2-Methyl-5-nitroaniline	1.20	11.44	35.29	2	P-N
2.	N-ethyl-*o*-toluidine	1.20	9.15	43.10	2*	Present work
3.	*p*-Toluidine	1.24	10.94	36.19	2	P-N
4.	2,5-Dichloroaniline	1.24	5.03	64.77	2	P-N
5.	2,5-Dimethylaniline	1.20	5.49	63.66	2	P-N

Table 5: Comparative kinetic rate data for the hydrolysis of some phosphate monoester *via* mononegative species

S. No.	Phosphate Monoesters	Medium pH	E (Kcal/mole)	$-\Delta S\neq$ e. u.	Molecularity	Bond fission
1.	Di-isopropyl aniline	4.17	8.69	54.74	2	P-N
2.	N-ethyl-*o*-toluidine	4.00	13.73	27.82	2*	Present work
3.	*p*-Toluidine	4.17	12.26	31.56	2	P-N
4.	2-Chloro-5-nitroaniline	4.17	9.56	49.90	2	P-N
5.	2-Chloroaniline	4.00	10.50	38.10	2	P-N

The probable reaction mechanism for the hydrolysis of mononegative species and neutral species of mono-N-ethyl-*o*-toluidine phosphate may be suggested as given below:
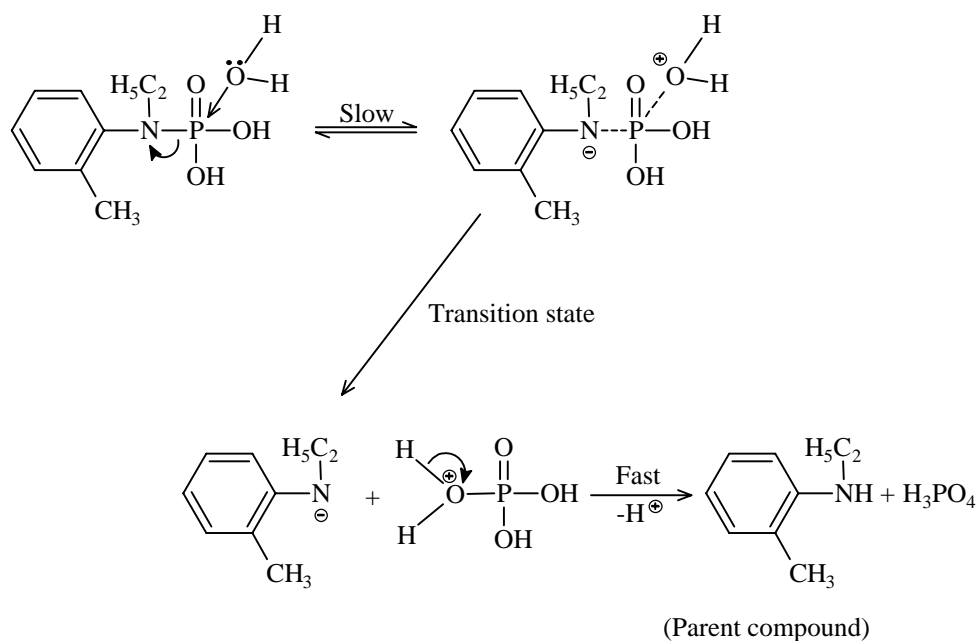
Mechanism via mononegative species

(A) Formation of mononegative species-

Neutral species   **Mononegative species**
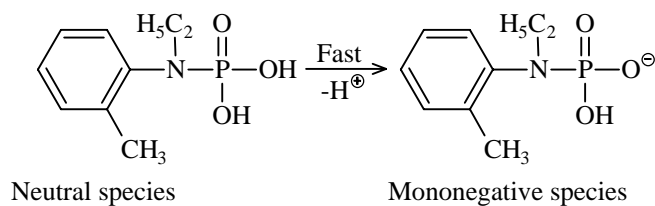
Scheme 1

(B) Bimolecular nucleophilic attack of water on phosphorus via mononegative species S_N^2 (P)-

Transition state

(Parent compound)
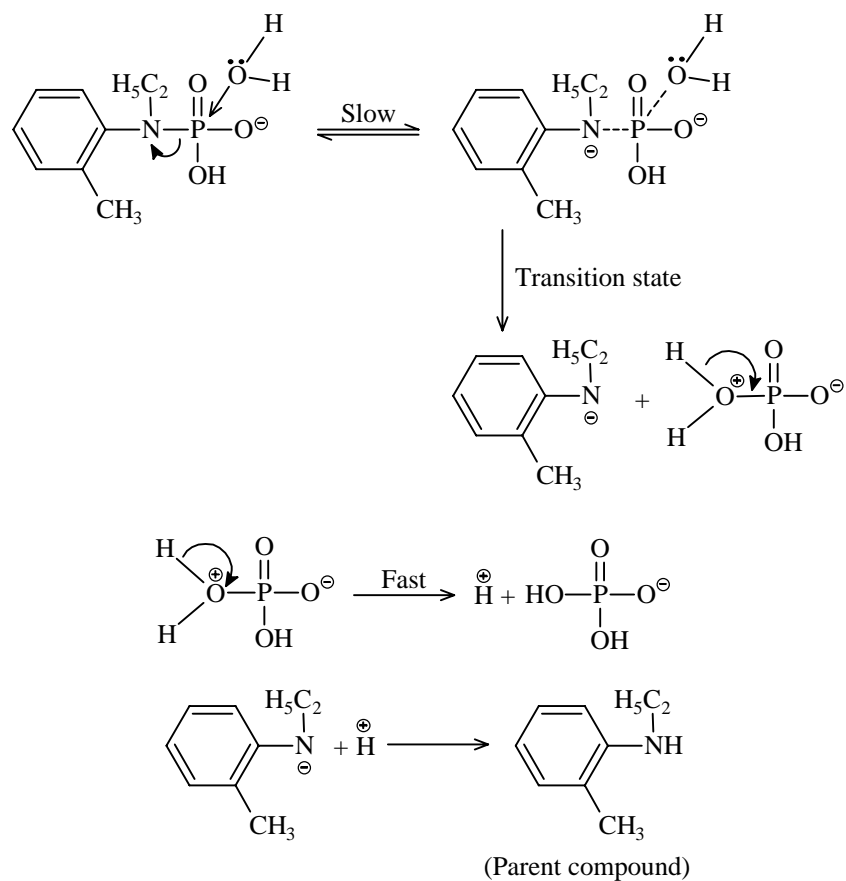
Scheme 2

Mechanism via neutral species

Bimolecular attack of water on phosphorus atom of the neutral species S_N^2 (P):

Scheme 3

CONCLUSION

Mono-N-ethyl-*o*-toluidine phosphate in the range of pH 1.00 to 7.80 was found to hydrolyze via neutral and mononegative species. The maximum value at pH 4.00 is due to hydrolysis via mononegative and neutral species. The estimated rate has been confirmed by those determined from specific rate and fractions of the neutral species agree closely with experimental rates. Bond fission, molecularity have been supported by Arrhenius parameters and solvent effect. The monoester involves P-N bond fission, which is strengthened by comparative kinetic rate data.

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