

Synthesis and kinetic perusal of Hydrolytic severing of a disubstituted-aryl phosphoric diamide having antimicrobial activity

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ABSTRACT

O-Nitro, p-chloro-phenyl Phosphoric diamide has been examined in pH range, 1.24 to 7.46 (using specific buffers to maintain each pH condition) in 12% DMSO-H₂O mixture at 40 (±0.5) °C. Bimolecular hydrolysis via (a) the neutral species (I) in the low pH range 1.24-4.17 and (b) via the Monoanion species (II) in the higher pH range 5.10 - 7.46 has been established. Effects of variables like temperature, solvent strongly support the above feature. P-N bond fission has been decided by testing during progress of hydrolysis. The substrate was synthesized by the phosphorylation technique in the presence of an alkaloidal catalyst. Its antimicrobial activity is attributed to the presence of o-NO₂ and p-Cl substituents in each aryl matrix.

Key words: Hydrolytic severing, antimicrobial activity.

INTRODUCTION

The susceptibility of organophosphate esters to hydrolysis depends largely on the electrophilic character¹ of the group attached to the phosphorus. A vast majority of phosphate esters reported so far consist of C-O-P linkage in them. The organic phosphoramidates, however, have significance recently and are quite useful synthetically² as well as industrially. Both types of esters undergo hydrolytic degradation under activated in vitro conditions.

EXPERIMENTAL

o-NO₂, p-Cl-Aniline with phosphorous oxychloride³ (4:1) in the presence of morpholine (0.01 ml) in C₆H₆ (dry) on refluxing for nearly seven hours gave a yellowish-brown sticky mass (on cooling). Purification with benzene gave a yellow solid which has a sharp melting point. It was quantitatively tested (Allen's modified method⁴) for phosphorus. The results are as.

1. M.p. (obsd) of the Phosphoro diamidate = 160°C

2. %age of phosphorus = 7.62 (Both Theo and Obsd).

o-NO₂-p-Cl-phenylphosphoric diamide was screened for its antimicrobial activity⁵, against *Candida albicans* and *Cryptococcus neoformans*, giving M.I.C. values 25 and 50µg/ml respectively.

RESULTS AND DISCUSSION

After synthesizing o-NO₂-p-Cl-phenylphosphoric diamide (for -o-NO₂, p-Cl-Phenylphosphorodiamidate), quantitative³ kinetic study of its hydrolysis has been carried out in the pH range 1.24 to 7.46, in 12% DMSO-H₂O mixture (for Solubility reasons) at 40 (±0.05)°C, under the physiological conditions nearly. Specific buffers⁶ have been employed to maintain each pH conditions, in the entire range of study. The observed rate data and the related functions of the reactive species compiled in Table 1 exhibit the highest rates at both PHs 1.24 and 5.60. Also a rate minimum is noticed at pH 6.70, beyond which the rates rise up to pH 5.60 only, after which they decrease up to pH 7.46.

The rates are known to be proportional to the concentration of the particular reactive species at the specific pH value. The following equations generally represent the overall rates via the expected Neutral (I) and the corresponding Monoanion (II) forms in the pH media.

$$K_e = K_N + K_M = K_{NO} \cdot N / (N+M) + K_{MO} \cdot M / (N+M) \quad \dots(1)$$

where K_e , K_N and K_M denote the calculated rate coefficients, the specific rate coefficients via the Neutral species and the species and the rate coefficients via the Monoanion species respectively. For the Neutral species, the value, of K_{NO} ($0.46 \text{ L mol}^{-1} \text{ min}^{-1}$) corresponds the minimum at pH 1.24, where mainly the Neutral species (95.8%) is present. Equation(I) can therefore be modified as follows for the Neutral species (II) prevailing between pH 1.24 to 4.17:

$$K_{e \text{ calcd}} = K_N = K_{NO} \cdot N / (N+M) \quad \dots(II)$$

$$K_{e \text{ calcd}} = 0.46 \times N / (N+M) \quad \dots(III)$$

Similarly, on the basis of the equation (i) in the higher pH region (4.17 to 7.46), the Monanion species is present and contributes solely, so that:

$$K_{e \text{ calcd}} = K_M = K_{MO} \cdot M / (N+M) \quad \dots(IV)$$

$$K_{e \text{ calcd}} = 0.197 \times M / (N+M) \quad \dots(V)$$

Here $K_{MO} = 0.197 \text{ L mol}^{-1} \text{ min}^{-1}$ and corresponds to the observed rate coefficients due to the presence of Monoanion (99.99%) species.

The fractional of both Neutral and the Monoanion forms have been derived from the sole pK value, 2.60 determined at the rate maximum i.e., pH 5.60. On the basis of these, the actual rate contributions purely via each reactive species have been calculated (Table 1) and are found to agree closely with the experimentally observed (Fig. 1) rate coefficients justifying the validity of equation (i), and its modified forms.

The C-N-P diester has been examined at 50°C and 60°C at pH 3.70 in 12% DMSO- H_2O mixture. The arhenius parameters⁷ have been

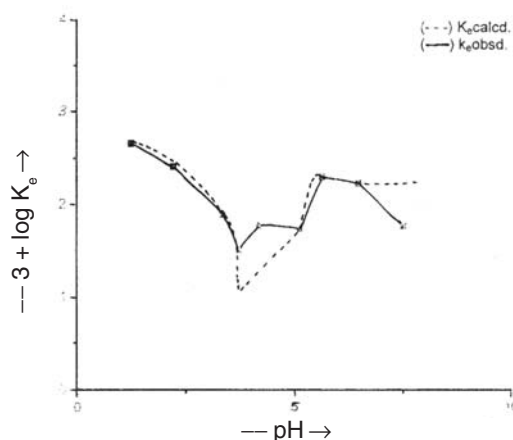


Fig. 1: Observed and calculated rates for the hydrolysis of $o\text{-NO}_2$, $p\text{-Cl}$ -Phenylphosphoric diamide in the pH region at $40 (\pm 0.5)^\circ\text{C}$ (12% DMSO - H_2O)

Table 1: Observed and calculated rate coefficients for the Hydrolysis of $o\text{-NO}_2$, $p\text{-Cl}$ -Phenylphosphoric diamide in the pH region at $40 (\pm 0.5)^\circ\text{C}$ (12% DMSO- H_2O)

S. No	pH	N/N+M	$k_N \text{ L mol}^{-1} \text{ min}^{-1}$	$k_{e \text{ calcd}} \text{ L mol}^{-1} \text{ min}^{-1}$	$k_{e \text{ obsd.}} \text{ L mol}^{-1} \text{ min}^{-1}$	$3 + \log k_e \text{ calcd}$
1.	1.24	0.958	0.441	0.441	0.460	2.64
2.	2.20	0.716	0.329	0.329	0.270	2.51
3.	3.33	0.167	0.076	0.076	0.080	1.88
4.	3.70	0.075	0.034	0.034	0.032	1.53
5.	4.17	0.038	0.018	0.018	0.061	1.25
		M/N+M	$k_N \text{ L mol}^{-1} \text{ min}^{-1}$			
6.	5.10	0.997	0.196	0.196	0.056	2.29
7.	5.60	0.999	0.197	0.197	0.197	2.29
8.	6.43	0.999	0.197	0.197	0.173	2.29
9.	7.46	0.999	0.197	0.197	0.060	2.29

$$K_{e \text{ calcd}} = K_N = K_M = K_{NO} \cdot N / (N+M) + K_{MO} \cdot M / (N+M)$$

$$K_{e \text{ calcd}} = 0.46 \times N / (N+M) + 0.197 \times M / (N+M)$$

calculated: the energy of activation ($E_a = 32.85$ KJ/mol) and the frequency factor ($A = 13.86 \times 10^2$ sec $^{-1}$) are both low and support bimolecular reaction. The term, ΔS^\ddagger (-6.24 e.u.), being negative facilitates the overall bimolecular reaction under observation. It therefore involves a compact type of the transition state (Chart 1) via the Neutral form (I). Both ΔS^\ddagger (30.27 KJ/mol) and ΔG^\ddagger (22.07 KJ/mol) also support the above aspect.

At pH 3.33 $K_{e_{\text{osbd}}}$ being $0.06 \text{ L mol}^{-1} \text{ min}^{-1}$, when DMSO $^{\text{a}}$ is increased to 36.0% (i.e., by nearly three times) the observed rates are only slightly lowered than those ($0.08 \text{ L mol}^{-1} \text{ min}^{-1}$) observed at 12% DMSO so that these changes in solvent composition do not influence the observed rate coefficients largely. At a much higher %age of 90.0 (DMSO) however, the rate ($K_{e_{\text{osbd}}} = 0.48 \text{ L mol}^{-1} \text{ min}^{-1}$) are elevated by nearly six times, so that DMSO itself dominates as a nucleophilic medium as compared to water (nearly 10% only). Such an elevation also suggests change in mechanism from substitution to an elimination type.

$K_{e_{\text{osbd}}} \text{ L mol}^{-1} \text{ min}^{-1}$ (DMSO % age) : 0.08 (12%) > 0.06 (36%) << 0.48 (90%)

Effect of changes (4.0×10^{-4} to 16.0×10^{-4}) in the concentration of parent amino o-NO $_2$, p-Cl-aniline, itself were also studied and the constancy of the rate data (at pH 3.70), shows that the present hydrolysis is independent of these concentration changes. When the concentration of the phosphoric diamide is halved, the rates are raised (at pH 3.70) by nearly one and a half times, while the rates are reduced to almost half, when the concentration is doubled. These rate coefficients (12% DMSO-H $_2$ O) clearly postulate second-order kinetics.

o-NO $_2$, p-Cl-phenylphosphoric diamide	8.0×10^{-4} (Half)	16.0×10^{-4} (Normal conc)	32.0×10^{-4} (Double)
$K_{e_{\text{osbd}}} \text{ L mol}^{-1} \text{ min}^{-1}$	0.49	0.032	0.019

Thus, for the present hydrolysis, the molecularity as well as the order of the reaction are both the same. However, the overall reaction $^{\text{a}}$ is pseudo-first order, due to the much excess of the reaction medium.

A decision regarding the bond rupture was made by a qualitative testing 10 , of one of the products

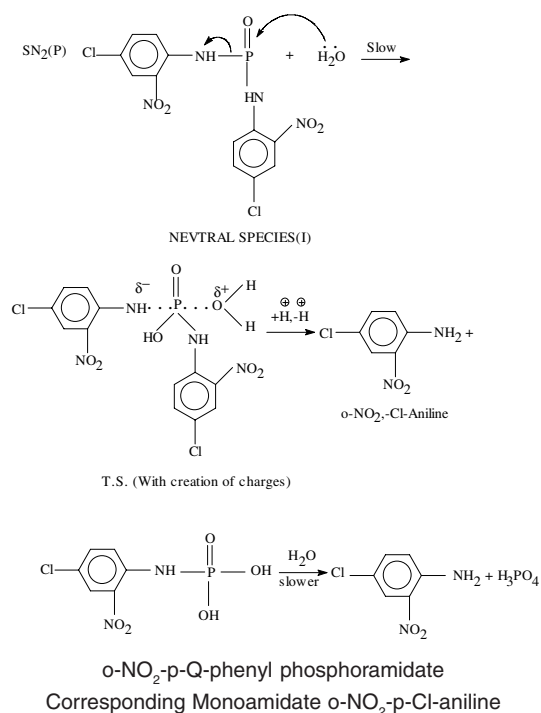


Chart 1: Bimolecular hydrolysis of o-NO $_2$, p-Cl-Phenylphosphoric diamide via the Neutral species (I) with P-N bond cleavage in the pH range 1.24 - 4.17

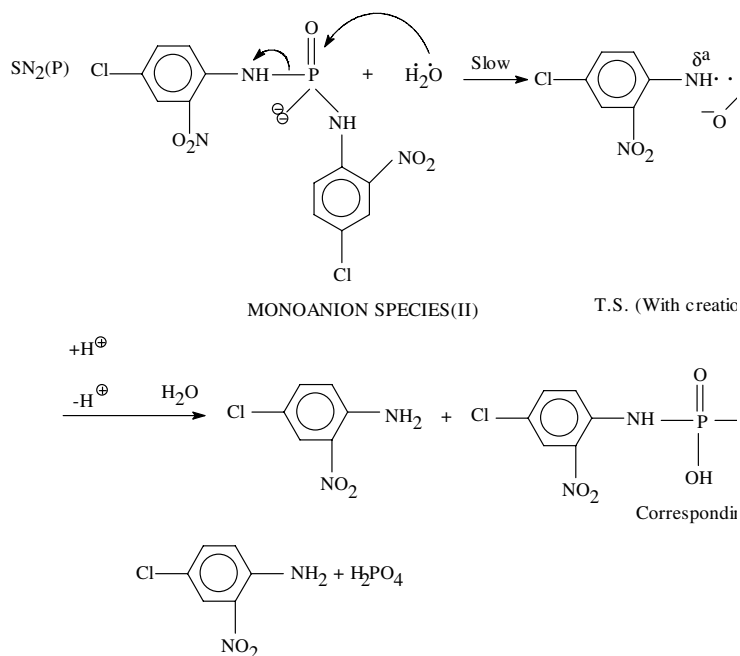


Chart 2: Bimolecular hydrolysis of o-NO₂, p-Cl-Phenylphosphoric diamide via the Neutral species (II) with P-N bond rupture in the pH range 5.10-7.46

during the progress of hydrolysis in the pH medium. An azo-dye test¹⁰ was therefore conducted and being positive, favoured the cleavage of the p-N bond in o-NO₂,p-Cl-phenylphosphoric diamide.

On the basis of results and discussion so

far, o-NO₂,p-Cl-phenylphosphoric diamide hydrolyses bimolecularly via two reactive (Neutral and Monoanion) species, with p-N bond rupture. Only two reaction mechanisms (Charts 1&2) have been arrived at in the range (1.24 - 7.46) examined at 40(±0.5)°C in 12% DMSO-H₂O mixture.

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