pH - Rate Profile for the Hydrolysis of Diphenyl p-Nitrophenyl Phosphate

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Summary: The pH-rate profile for the hydrolysis of diphenyl p-nitrophenyl phosphate has been determined. The hydronium ion and hydroxide ion catalysed reactions account for the observed hydrolysis over almost the whole pH range and the pH-independent water reaction is observed only close to the minimum in the pH-rate profile near pH 4.

Introduction

The reactions of phosphoryl compounds with nucleophiles have been studied by numerous authors [1,2] and many data are available for the alkaline hydrolysis of various phsophoric acid derivatives. Reactions with other nucleophiles have been extensively studied by Kirby and co-workers [3-5] and then continued in this laboratory [6-8]. The systematic work on triesters of phosphoric acid by various workers have been limited only to a selective range of conditions, and the phosphate esters. The hydrolysis of various dialkyl aryl phosphates has been studied either in alkali [9] or at a single buffer concentration at lower pH [9,10]. Bunton and Vernon and their co-workers have measured the (weakly) acid-catalysed hydrolysis of a few triesters [11,12] and obtained a rate constant for the neutral hydrolysis of triphenyl phosphate at 100° apparently without pH control [13]. Kirby and Khan [5] for the first time studied the hydrolysis of diaklyl substituted-phenyl phosphate esters over the whole range of pH and their reactions with a large number of nucleophiles.

We have now initiated a detailed systematic study of the triesters of the series diphenyl substituted-aryl phosphates. A tris (aryl) phosphate was not considered a suitable phosphate triester for the present study since the hydrolysis of the triester is complicated by the subsequent hydrolysis of diaryl phosphate - one of the products. Therefore, diphenyl substituted-aryl phosphate was used as a substrate which has two advantages over the tris (aryl) phosphate. In most of the cases in the series of diphenyl substituted-aryl phosphate, the conjugate acid of the substituted phenolate anion has a pK_a less than that of phenolate itself and is thus a better leaving group. Thus, diphenyl phosphate, one of the hydrolysis product, is very stable not only because phenolate anion is a poor leaving group as compared to the substituted-phenolate anion but also because the anions of diesters are generally exceedingly unreactive compounds. Another advantage of this substrate is that since the reactions are followed spectrophotometrically and the substituted-phenolate anion absorbs at a wavelength different from that of unsubstituted phenolate anion, the measured absorbance is only due to the release of the substituted-pheno-

late anion. Diphenyl p-nitrophenyl phosphate with a leaving group of intermediate pK_a value in the series, has been selected for a detailed study in the beginning to determine the general pattern of the behaviour of this series of phosphate triesters.

Experimental and Results

Materials

Inorganic salts were of analytical grade, and were used without further purification. Absolute alcohol was prepared from rectified spirit which was refluxed with calcium oxide for 6 hours, allowed to stand overnight and then distilled under anhydrous condition. The commercial ether was allowed to stand over calcium chloride for 24 hours with occasional shaking, then filtered through a large fluted filter paper into a clean dry bottle to which fine sodium wire was introduced with the aid of sodium press. Benzene was dried with anhydrous calcium chloride, filtered and then placed over sodium wire. Dioxane was refluxed over sodium for 6 hours and then distilled from sodium. 2,6-Lutidine was distilled over $AlCl_3$. Phosphorus oxychloride was also distilled before use. Commercially available phenol was purified by distillation and p-nitrophenol by recrystallization. Diphenyl phosphorochloridate was supplied by Merck, West Germany. The phosphate esters, after preparation, were confirmed by analytical and spectroscopic data.

Preparation of Diphenyl p-nitrophenyl phosphate

Sodium salt of p-nitrophenol was prepared by gradually adding 60 ml of 2.0 N - sodium hydroxide solution to a suspension of 13.9 g (0.1 mole) of p-nitrophenol in 100 ml of boiling water in a 500 ml round-bottommed flask fitted with a reflux condenser and a dropping funnel, with constant stirring over a period of half an hour. The mixture was refluxed for two hours with continuous stirring until the solution became clear. It was concentrated by evaporation and then allowed to cool. On refrigeration it gave dark yellow crystals of sodium p-nitrophenoxide which were collected by filtration, washed with iced water and dried at 80° under vacuum.

Sodium p-nitrophenoxide (16.0 g, 0.1 mole) was placed in a 500 ml round-bottomed flask equipped with a reflux condenser and a dropping funnel, and containing 100 ml of dry benzene. A solution of diphenyl phos phorochloridate (26.8 g, 0.1 mole) in 100 ml of dry benzene was added to it slowly with the help of the dropping funnel under anhydrous conditions with constant stirring. After the addition was complete, the mixture was refluxed for about two hours and then allowed to cool. Sodium chloride, separated during the reaction, was filtered off. The filtrate was dried over anhydrous Na_2SO_4 and then evaporated leaving behind an oily mass which solidified on cooling to give 23.0 g of diphenyl p-nitrophenyl phosphate. It was recrystallised from ethanol to fine needles with melting point 48 - 49° (lit.[12] m.p. 49-50°).

Preparation of Triphenyl Phosphate

To a well-stirred solution of phenol (28.0 g, 0.3 mole) in 100 ml of 2.6-lutidine in a 500 ml round-bottom flask equipped with a reflux condenser and a dropping funnel, was added 15.3 g of phosphorus oxychloride (0.1 mole) gradually under anhydrous conditions at room temperature. After the addition was complete, the solution was warmed to about 50° for about 30 minutes and then allowed to cool. The reaction mixture was then poured into 100 ml of water. A precipitate of triphenyl phosphate separated out which was

collected by filtration, washed with water and dried (24.5 g). It was recrystallised from ethanol to melting point 47-48°.

Kinetic Methods

The rates of the reactions were measured by following the rate of release of the displaced phenolate anion concerned spectrophotometrically using Pye-Unicam SP-8400 spectrophotometer at the absorption maximum of the phenolate anion [6] at 39°. The ionic strength 1.0 was maintained with potassium chloride in water containing 5% dioxane for reasons of solubility, which does not affect the rates very much. pH values of the solutions were measured at the end of each run on a CORNING-EEL model 5 pH meter which was calibrated with solutions made from standard soloid buffer tablets at pH 4,7 and 9. Buffer solutions used for pH-rate profiles were those of HCl, CH_3COOH-CH_3COONa, NaH_2PO_4-Na_2HPO_4, $NaHCO_3$-Na_2CO_3 and NaOH. Fresh solutions were prepared each time and all solutions were filtered before use and incubated at 39° before mixing. The temperature control was generally better than $\pm$ 0.1°.

The experimental procedure was as follows. 10.0 ml of stock solution of known concentration of the phosphate triester was added to the buffer constituents and sufficient 2.0 M KCl was added to bring the final ionic strength to 1.0, in a flask; then distilled water was added to make the total volume of the solution to 100 ml. The flask was immersed in the thermostatted water bath maintained at 39°. Aliquots were taken out after regular intervals, put into a tube immersed in iced water to quench the reaction and diluted with tris (hydroxymethyl) aminomethane (tris buffer) to bring its pH to about two units above the pK_a of the released phenol to convert it into the anionic form. For reactions at pH already more than two units above the pK_a of the phenol, addition of tris buffer was omitted because the released phenol was already in the anionic form. The optical density was then measured at the wavelength of the absorption maximum of the phenolate anion concerned with the help of the spectrophotometer.

In the case of slow reactions, the first five percent of the reaction was followed according to the initial rate method. The end-points for each reaction, to determine the initial concentration, was obtained by determining the amount of the phenol released by separately hydrolysing an aliquot of the reaction mixture with 1.0 M sodium hydroxide to a constant optical density. The amount of p-nitrophenol determined spectrophotometrically after complete hydrolysis of diphenyl p-nitrophenyl phosphate in sodium hydroxide, was in good agreement with that calculated theoretically. The first-order rate constants were determined graphically using the integrated form of the first-order rate equation. A good straight line was obtained when absorbance (converted into concentration units using extinction coefficient for the liberated phenolate anion at their absorption maxima) was plotted against time. The slope of this linear plot divided by the initial concentration of the phosphate solution gave the first-order rate constant.

The fast reactions were followed to three half-lives and the end-point taken after at least ten half-lives. These reactions gave excellent pseudo-first-order semi-logarithmic plots when log ($O.D_\infty$ - $O.D_t$) was plotted against time. A few reactions were followed by both methods which gave identical results. Detailed results for

the hydrolysis of diphenyl p-nitrophenyl phosphate are given in Table 1 and the pH-rate profile for this phosphate ester is shown in Figure 1.

The formation of diphenyl phosphate as the other reaction product of the acid hydrolysis was shown by extracting the reaction mixture with ether, drying the ethereal solution with $MgSO_4$, evaporating ether and then identifying diphenyl phosphate by thin-layer chromatography [14] using silica gel GF 254 (type 60) chromatoplates, with chloroform:methanol (65:35) as an irrigating solvent system. The spots were detected by placing the irrigated and dried chromatographic plate directly under the UV light from Mineralight Short Wave UVS-12 and compared with that of the authentic sample [15].

Table-1: Frist-order Rate Constants for the Hydrolysis of Diphenyl p-Nitrophenyl Phosphate in Water:Dioxane (95:5, V/V)at 39° and μ = 1.0

Conditions	$k_{hyd}(min^{-1})$	Conditions	$k_{hyd}(min^{-1})$
HCl,1.0 M	$4.79x10^{-4}$	pH 7	$1.78x10^{-5}$
0.1 M	$2.82x10^{-4}$	8	$9.05x10^{-5}$
0.01 M	$5.01x10^{-5}$	9	$7.94x10^{-4}$
pH 3	$4.27x10^{-6}$	10	$3.31x10^{-3}$
4	$2.51x10^{-6}$	11	$3.98x10^{-2}$
5	$2.69x10^{-6}$	NaOH,0.01M	$3.55x10^{-1}$
6	$7.08x10^{-6}$		

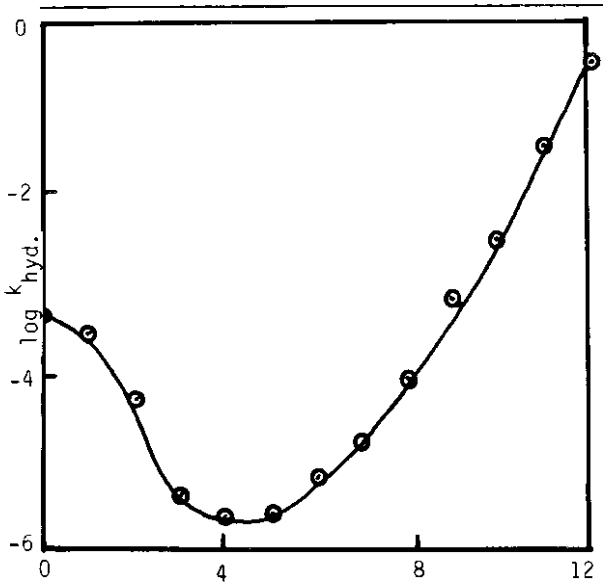

Fig.1: pH-rate profile for the hydrolysis of diphenyl p-nitro-phenyl phosphate at 39° and μ=1.0. Data from Table 1.

Second-order rate constants for the hydroxide ion catalysed hydrolysis of diphenyl p-nitrophenyl phosphate and triphenyl phosphate, were obtained from the slopes of the linear plots of the first-order rate constants at different hydroxide ion concentrations, against the concentration of hydroxide ion. The results are shown in Table II.

Table-2: Second-order Rate Constants for the Alkaline Hydrolysis of Phosphate Triesters in Water:Dioxane (95:5, V/V) at 39° and μ=1.0

Phosphate Triesters	pK_a of the leaving group	Range of [NaOH] (M)	No.of runs	K_2 ($l.mole^{-1}.min^{-1}$)
Diphenyl p-nitrophenyl phosphate	7.15	0.002-0.010	5	35.50
Triphenyl phosphate	10.0	0.02-0.10	5	1.80

Discussion

Simple trisubstituted phosphate esters are known to be relatively alkali-labile. The pH-rate profile for the hydrolysis of diphenyl p-nitrophenyl phosphate (Fig. 1) is qualitatively similar to that observed for the hydrolysis of methyl p-nitrophenyl phosphate anion [15]. The hydronium -and hydroxide-catalysed reactions account for the whole pH range, and the pH-independent water reaction is observed only close to the minimum in the pH-rate profile near pH 4.0. The spontaneous hydrolysis of the triester is unexpectedly slow. Triphenyl phosphate is not hydrolysed at a detectable rate at pH 4 and the half-life of diphenyl p-nitrophenyl phosphate is about 6 months at 39° and pH 4. This indicates that the water reaction is very sensitive to the nature of the leaving group and this spontaneous hydrolysis may be very fast for the more reactive compounds, with better leaving groups.

Khan and Kirby [5] have shown by isotopic labelling experiments on dialkyl aryl phosphates that the hydrolysis of the triesters involves quantitative phosphorus-oxygen bond cleavage over the entire pH range. This is generally true when at least one of the substituent of phosphoric acid is an aryl group. This fact can be explained in terms of the stability of the displaced phenolate anion. In view of the activation parameters determined by them it can be suggested that the mechanism of the spontaneous hydrolysis of a triester may involve a nucleophilic attack by a water molecule catalysed by a second water molecule acting as general base, on the electrophilic phosphorus centre of the triester.

The nature of the pH-rate profile towards the acidic end, shows that there may be a rate maximum in strong acidic solutions. This has actually been observed [12] in the acid-catalysed hydrolyses of diphenyl p-nitrophenyl phosphate which shows rate maxima at acid concentrations of 2-6 M, depending upon the nature of the catalysing acid. The rate maxima are not caused by complete protonation of the substrate since the pK_a of triphenyl phosphate is -2.8 and that of diphenyl p-nitrophenyl phosphate should be even more negative because of electron withdrawal by the nitro group. The electrolytic effects and the activation parameters for the acid catalysed hydrolysis suggest a strongly hydrated transition state.

It is well known [5] that these

compounds are highly reactive towards attack by hydroxide ion, and that even relatively poor leaving groups are readily displaced. The second-order rate constant for the alkaline hydrolysis of diphenyl p-nitrophenyl phosphate is only approximately twenty times faster than that of triphenyl phosphate and a two-point plot of the logarithms of the second-order rate constants for alkaline hydrolysis against the pK_a of the conjugate acid of the phenoxide leaving group, has a slope of ca. 0.45. The electrolytic and solvent effects [12] suggest that the transition state for the alkaline hydrolysis is also strongly hydrated which is consistent with the activation parameters for the alkaline hydrolysis of these phosphate triesters.

Strong interaction between water and the transition states for both the acid and the alkaline hydrolyses of these phosphate triesters are to be expected [1] in terms of the preferred model for bimolecular substitution at a phosphoryl phosphorus atom.

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