

The Hydrolysis of *p*-Nitrophenylacetate in Water

Mechanism and Method of Measurement

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The hydrolysis of *p*-nitrophenylacetate has been studied between pH 6 and 9 in 3.3% (v/v) dioxane–0.1 M phosphate or Tris buffer. The results indicate that the hydrolysis is acid-catalyzed, base-catalyzed and has a pH-independent rate component. The observed rate constants for the hydrolysis were determined spectrophotometrically from the initial rate of appearance of *p*-nitrophenol (at 320 nm), *p*-nitrophenoxide (at 400 nm) and the initial rate of disappearance of *p*-nitrophenylacetate (at 270 nm). An equation has been derived permitting the calculation of first and second-order rate constants from the initial slopes of product appearance or reactant disappearance. The semilog method of first-order kinetic analyses could not be used since the hydrolysis of *p*-nitrophenylacetate did not go to completion.

The apparent incomplete reaction is attributed to the establishment of an equilibrium between *p*-nitrophenol and *p*-nitrophenyl esters. The latter probably includes *p*-nitrophenyl phosphate. The rate constant for the synthesis of *p*-nitrophenyl esters has been determined and it also has acid, base and pH-independent catalytic components.

The hydrolysis of an ester may occur through base-catalyzed, pH-independent and acid-catalyzed reaction pathways [1]. Hine [2] has demonstrated that these reaction pathways are independent of each other. Thus, the observed pseudo-first-order rate constant for the hydrolysis of the ester is made up of the sum of products of the catalyst concentration and a second-order rate constant. The latter rate constant represents the inherent ability of the catalyst to catalyze the reaction. In distilled water, the observed rate constant (k_0^{water}) may be expressed as [3]:

$$k_0^{\text{water}} = k_{\text{H}_2\text{O}} + k_{\text{H}^+} [\text{H}_3\text{O}^+] + k_{\text{OH}^-} [\text{OH}^-] \quad (1)$$

where $k_{\text{H}_2\text{O}}$ is the pH-independent rate constant [4], k_{H^+} is the second-order rate constant for the hydronium-ion-catalyzed reaction, k_{OH^-} is the second-order rate constant for the hydroxide-ion-catalyzed reaction and $[\text{H}_3\text{O}^+]$ and $[\text{OH}^-]$ are the hydronium ion and hydroxide ion concentrations, respectively. For the ester *p*-nitrophenylacetate at neutral pH the

Abbreviations. Tris, tris(hydroxymethyl)aminomethane; m.p., melting point.

observed pseudo-first-order rate constant may be described as [4–7]:

$$k_0^{\text{water}} = k_{\text{H}_2\text{O}} + k_{\text{OH}^-} [\text{OH}^-]. \quad (2)$$

Yates and McClelland [8] have shown that in 15% aqueous sulfuric acid, the mechanism of *p*-nitrophenylacetate hydrolysis is second order, acid-catalyzed with acyl-cleavage [9]. This may be expressed as:

$$k_0^{\text{water}} = k_{\text{H}_2\text{O}} + k_{\text{H}^+} [\text{H}_3\text{O}^+]. \quad (3)$$

We present evidence here that acid-catalyzed *p*-nitrophenylacetate hydrolysis may also occur at neutral pH.

The hydrolysis of *p*-nitrophenylacetate is generally followed spectrophotometrically at 270 nm [8] for *p*-nitrophenylacetate loss or 400 nm for *p*-nitrophenoxide ion appearance or 300 nm for *p*-nitrophenol appearance [10]. The spectroscopic data may yield rate constants [10,11]. To obtain k_0^{water} , the procedure has been to determine the hydrolytic rate constants, k , in the presence of varying concentrations of catalyst and then to extrapolate the k

data back to zero catalyst concentration [2,7,12–14] since:

$$k = k_0^{\text{water}} + k_c' \quad (4)$$

where k_c' is the pseudo-first-order rate constant for the catalyst-catalyzed *p*-nitrophenylacetate hydrolysis.

The assumptions that have been made regarding the determination of k spectrophotometrically are: (a) the reaction is irreversible, (b) the reaction is first order in *p*-nitrophenylacetate and (c) the relation between absorbance and concentration of product, *p*-nitrophenol, and/or reactant, *p*-nitrophenylacetate, is linear. Assumptions (a) and (c) have been rigorously demonstrated as correct for *p*-nitrophenylacetate hydrolysis in the presence of catalysts [10,13]. Assumption (b) also generally holds, since the concentration of the catalyst is usually far in excess of the ester. Studies of *p*-nitrophenylacetate hydrolysis using models where the ester concentration was greater than the catalyst (nucleophilic) concentration have, however, been reported [15]. We had reported [16] that assumption (c) was invalid in the *p*-nitrophenylacetate hydrolysis system. The results of the latter report have been re-examined and interpreted that *p*-nitrophenylacetate hydrolysis in phosphate buffer is reversible. An equation has, therefore, been derived which permits the determination of rate constants from the initial rate of appearance of product or disappearance of reactant regardless of whether assumptions (a) or (b) are met.

EXPERIMENTAL PROCEDURE

Materials

Monobasic and dibasic sodium phosphate and tris(hydroxymethyl)aminomethane (Tris) were of reagent grade and were used to make up the buffers according to Gomori [17]. The water was double-distilled and deionized. The organic solvents, ethanol and dioxane were from Fisher (certified grade). *p*-Nitrophenol (Fisher, reagent grade) and *p*-nitrophenylacetate (Eastman organic chemicals m.p. 77.7 °C) were used without further recrystallization. Some of the kinetic studies used *p*-nitrophenylacetate synthesized by the method of Spasov [18], Chattaway [19] or by a third method. In this third method *p*-nitrophenol was dissolved in glacial acetic acid to which one equivalent (to the alcohol) of dicyclohexylcarbodiimide was added. After 2 h at room temperature the acetic acid was removed *in vacuo*. The residue was mixed with warm ethyl acetate and after cooling to room temperature dicyclohexylurea was filtered off (yield 100%, m.p. 225 °C). The filtrate was dried over sodium sulphate and flash-evaporated to dryness to yield a residue which on several recrystallizations from warm ethanol yielded *p*-nitro-

phenylacetate (yield 20%, m.p. 77.8 °C: literature m.p. 83 °C [19], 77.8 °C [20], 79.5–80 °C [21], 77.5–78 °C [13], 78.9 °C [4]). Thin-layer chromatography [22] and non-aqueous titration [23] confirmed the identity of the product and the purity. The poor yield is probably due to formation of acetyldicyclohexylureide [24]. Melting points were taken on a Mettler FP-5 with FP51 heating block or on a *Schmelzpunktbestimmungsapparat*, W. Buchi (Flavil, Switzerland). The three spectrophotometers used in this study were the Beckman DK1, DU2 and Acta 3. The latter two spectrophotometers had the cell compartment thermostated *via* an external circulating water bath. A Radiometer (Copenhagen) pH meter (Type TTT2) with a combination electrode (GN 2321C) was used to measure the pH of the solutions.

Methods

Dioxane which had been stored over Linde molecular sieve (type 4A) was used to make up the *p*-nitrophenylacetate or *p*-nitrophenol solutions. The ester-dioxane solution stored at 4 °C was used within a two-week period. Hydrolysis in dioxane due to the condensation of water from the atmosphere was detectable after two weeks.

Spectra of *p*-nitrophenylacetate and *p*-nitrophenol were performed on freshly prepared samples (Fig. 1). The ester in dioxane was diluted with either dioxane or with phosphate buffer. The alcohol, *p*-nitrophenol, dissolved in distilled water or dioxane was diluted with various phosphate buffers. In each instance the reference cell contained the same solution as the sample cell except for ester or alcohol. The spectrum of *p*-nitrophenylacetate ($\lambda_{\text{max}} = 270 \text{ nm}$) shows a higher absorption coefficient when the ester is in phosphate buffer than when the ester is in dioxane (Fig. 1). The explanation for this appears to be that the ester is associating in dioxane, since further dilution of *p*-nitrophenylacetate with dioxane raises the absorption coefficient to that of the ester in phosphate buffer. This association is probably a stacking of the aromatic rings of *p*-nitrophenylacetate. Trace amounts of the conjugated acid of the ester will eliminate the association since 7% (v/v) 0.1 M aqueous HCl in dioxane will raise the coefficient of *p*-nitrophenylacetate in dioxane to that of the ester in phosphate buffer.

The Beer-Lambert relationship was demonstrated for *p*-nitrophenylacetate, *p*-nitrophenol and *p*-nitrophenoxide at 270 nm, 320 nm and 400 nm, respectively. Spectra of *p*-nitrophenol in phosphate buffers between pH 6 and 8 demonstrated that both the 320-nm absorbance and the 400-nm absorbance showed a sigmoidal relationship to pH. Assuming the pK_a of *p*-nitrophenol is 7.15 [25,26], a relationship of absorption coefficient (ϵ) to the degree of ionized

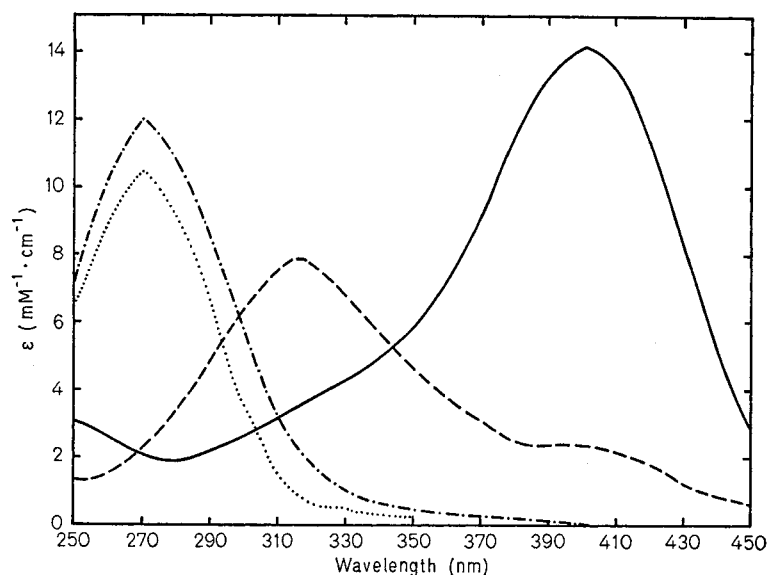


Fig.1. Spectrum of *p*-nitrophenylacetate in dioxane (.....); *p*-nitrophenylacetate in 3% (v/v) dioxane-0.1 M phosphate buffer pH 7.0 (-.-.-); *p*-nitrophenol in 1% (v/v) dioxane-0.1 M phosphate buffer pH 6.1 (----); *p*-nitrophenol in 0.1 M phosphate buffer pH 7.6 (—). All spectra were taken at 25 °C

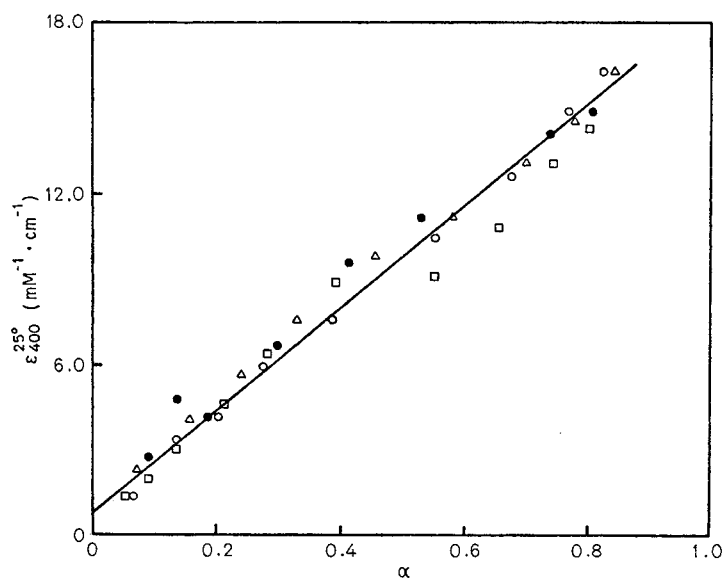


Fig.2. The absorption coefficient at 400 nm of *p*-nitrophenol at 25 °C versus the degree of ionization of the alcohol. Absorbance readings taken from the spectra (360 nm to 450 nm) of 36.8 μM *p*-nitrophenol in: 0.05 M (●), 0.1 M (□), 0.15 M (○) and 0.2 M (Δ) phosphate buffer

alcohol, α , may be determined (Fig.2 for ϵ_i^{400} ; a similar curve was used to determine ϵ_i^{320}):

$$\epsilon_i^{400} = 8.15 \times 10^2 + \alpha_i \cdot 1.81 \times 10^4 \quad (5)$$

$$\epsilon_i^{320} = 8.06 \times 10^3 - \alpha_i \cdot 6.63 \times 10^3 \quad (6)$$

where i refers to the pH of the analyzed solution. Eqns (5) and (6) have been shown to be independ-

ent of ionic strength (up to 0.2 M phosphate) and organic solvent up to 5% (v/v) dioxane and 10% (v/v) ethanol.

Kinetic Assay

0.1 ml *p*-nitrophenylacetate-dioxane solution was added to 2.9 ml 0.1 M phosphate buffer in a 1-cm cuvette. The cuvette was capped with parafilm, the

solution was mixed and absorbance readings were begun within 30 s. The reference cell contained a 3.3% (v/v) dioxane-phosphate buffer solution. The pH of the reaction mixture was taken after the kinetic assay and it agreed to within ± 0.05 pH units of the phosphate buffer alone.

Kinetic analyses were initially done by the "semilog" method [10] and then by the "initial slope" method [16]. In the latter method, the initial rate of appearance of product or disappearance of reactant is measured spectrophotometrically. The observed first-order rate constant, k , may be determined directly:

$$k = \frac{1}{x_0} \cdot \frac{1}{\varepsilon} \left(\frac{\Delta A}{\Delta t} \right)_{t \approx 0} \quad (7)$$

where x_0 is the initial concentration of *p*-nitrophenylacetate, and $(\Delta A/\Delta t)_{t \approx 0}$ is the initial slope as read off the spectrophotometer recorder chart. Following the reaction at 400 nm the absorption coefficient for Eqn (7) is determined from Eqn (5). Following the reaction at 270 nm, the disappearance of *p*-nitrophenylacetate, Eqn (7) simplifies to:

$$k_{270} = \frac{1.20}{(A)_{t=0}} \cdot \left(\frac{\Delta A}{\Delta t} \right)_{t \approx 0} \quad (8)$$

where $(A)_{t=0}$ is the extrapolated absorbance reading at zero time, $(A)_{t=0} = x_0 \cdot \varepsilon_{270}$. A correction factor, 1.20, has been incorporated into Eqn (8) since at 270 nm the spectrophotometer detects the appearance of *p*-nitrophenol (Fig. 1). The absorption coefficient at 270 nm of both *p*-nitrophenol and *p*-nitrophenoxide is one-sixth that of *p*-nitrophenylacetate.

Fig. 1 also illustrates that following the hydrolysis of *p*-nitrophenylacetate at 320 nm, one would need to correct for the simultaneous disappearance of the ester as the alcohol is being formed. The correction factor (c) at pH i is:

$$c_i = \frac{\varepsilon_i}{\Delta \varepsilon_i} \quad (9)$$

where ε_i is calculated from Eqn (6) and $\Delta \varepsilon_i$ is the difference between ε_i and the absorption coefficient for *p*-nitrophenylacetate at 320 nm, $1.6 \text{ mM}^{-1} \cdot \text{cm}^{-1}$. Thus, the observed first-order rate constant as determined by the initial slope method at 320 nm is:

$$k_{320} = \frac{1}{x_0} \cdot \frac{1}{\Delta \varepsilon_i} \cdot \left(\frac{\Delta A}{\Delta t} \right)_{t \approx 0} \quad (10)$$

RESULTS

The relationship of the observed first-order rate constant of *p*-nitrophenylacetate hydrolysis and pH in 0.1 M phosphate is illustrated in Fig. 3. The data appears to be widely scattered below pH 7.5. This scatter is due to the slowness of the reaction, that is, to the small amount of product being formed over

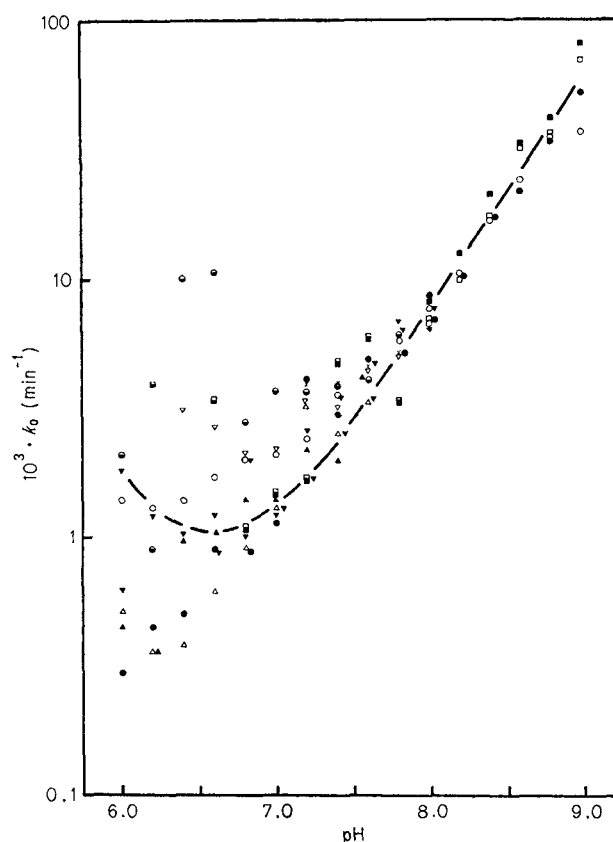


Fig. 3. The observed first-order rate constant for the hydrolysis of *p*-nitrophenylacetate at 25°C in 3.3% (v/v) dioxane-0.1 M phosphate buffers below pH 8.0 and in 3.3% (v/v) dioxane-0.1 M Tris buffer above pH 8.0. All rate constants were determined by the initial slope method (see text for detail): (a) at 400 nm and *p*-nitrophenylacetate concentration of: 84 μM (\circ), 0.84 mM (Δ), 48 μM (\times), 88 μM (\bullet), 87 μM (\blacksquare), 0.78 mM (∇), and 33 μM (\square); (b) at 270 nm and *p*-nitrophenylacetate concentration of: 84 μM (\bullet), 33 μM (\blacksquare) and 0.78 mM (\blacktriangledown); (c) at 320 nm and *p*-nitrophenylacetate concentration of 84 μM (\blacktriangle)

a relatively long period of time. This slowness of reaction results in great variability in the initial slope measurement. Due to the large difference observed, a mean value is of little significance and therefore Fig. 3 is shown with the solid line representing the data in a way consistent with the model for ester hydrolysis [3].

Phosphate ions do not appear to contribute to the observed rate constant since the pH profile of k (Fig. 3) is independent of whether the buffer is phosphate or Tris, or on the concentration of the phosphate buffer (Table 1). Thus all the observations described above and below are due to water and its ionic forms, that is k equals k_0^{water} [Eqn (4); hereafter k_0^{water} will be simplified to k_0].

The observed rate constant was plotted against the hydroxide ion concentration (Fig. 4). The

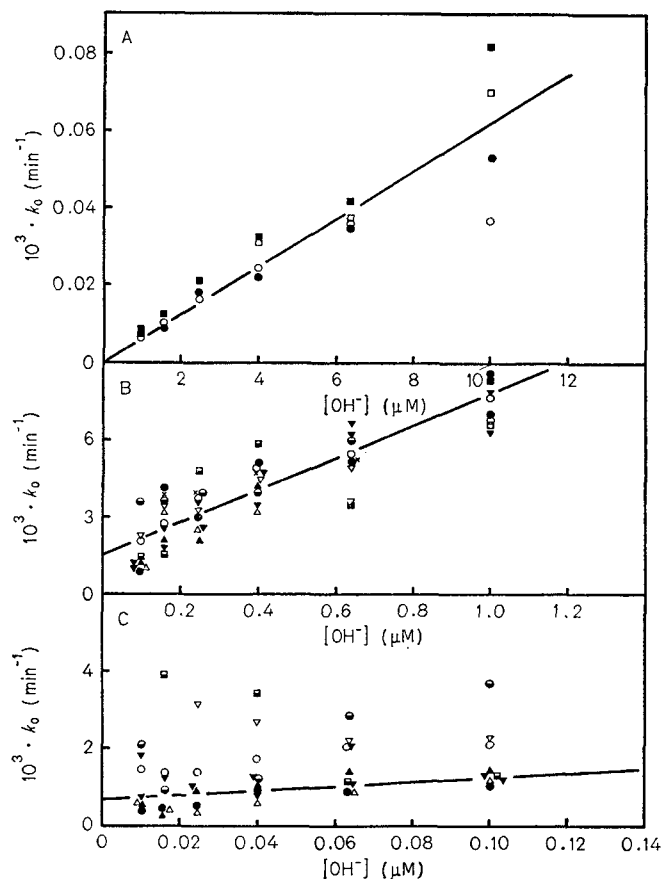


Fig. 4. The data of Fig. 3 replotted against the hydroxide ion concentration: (A) pH 8.0 to 9.0; (B) pH 7.0 to 8.0; (C) pH 6.0 to 7.0. The symbols are those of Fig. 3

Table 1. Effect of phosphate buffer concentration on the observed first-order rate constant of *p*-nitrophenylacetate hydrolysis. The observed first-order rate constant was determined spectrophotometrically at 400 nm at 25 °C using 3.3% (v/v) dioxane-phosphate buffer containing 78 μM *p*-nitrophenylacetate. The initial slope method was used to determine k_0

pH	phosphate concentration			
	0.05 M	0.1 M	0.15 M	0.2 M
	$10^3 \times k_0 \text{ min}^{-1}$			
7.00	—	1.4	2.1	2.1
7.10	2.3	1.5	—	—
7.58	3.1	3.3	3.5	3.7
8.01	—	8.0	8.2	—
8.06	—	8.6	—	8.7
8.10	9.9	9.4	—	—

hydroxide ion concentration was calculated from the relationship $K_w = [H^+] \cdot [OH^-]$, where $[H^+]$ is the hydrogen ion concentration and was assumed to be the antilog of the negative of the pH and K_w was assumed to be equal to $0.01 \mu\text{M}^2$. Although the data

in Fig. 4 are scattered, Eqn (2) seems to be an adequate description. The slopes of the lines in Fig. 4 are the k_{OH^-} and each one is equal to $6.25 \text{ mM}^{-1} \cdot \text{min}^{-1}$ (literature k_{OH^-} : $30.6 \text{ mM}^{-1} \cdot \text{min}^{-1}$ [7], $0.89 \text{ mM}^{-1} \cdot \text{min}^{-1}$ [4], $0.99 \text{ mM}^{-1} \cdot \text{min}^{-1}$ [5] and $1.3 \text{ mM}^{-1} \cdot \text{min}^{-1}$ [6]).

The intercept of Fig. 4 yields the water-catalyzed hydrolysis (k_{H_2O}) of *p*-nitrophenylacetate, $7 \times 10^{-4} \text{ min}^{-1}$ (literature k_{H_2O} : $3 \times 10^{-3} \text{ min}^{-1}$ [7], $3.3 \times 10^{-5} \text{ min}^{-1}$ [4]). This value for k_{H_2O} is an approximation because Eqn (2) was assumed to be operative in Fig. 10C. It appears, however, that below pH 7 Eqn (1) is a better description of the catalytic hydrolysis of *p*-nitrophenylacetate. In Eqn (1) there is an acid-catalyzed component to k_0 and this manifests itself by a rise in k_0 with a fall in pH (Fig. 3).

To determine the catalytic rate constant for acid-catalyzed *p*-nitrophenylacetate hydrolysis (k_{H^+}), the Eqn (11) of Bell [3] may be used:

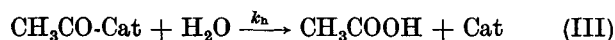
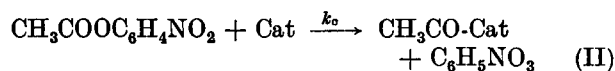
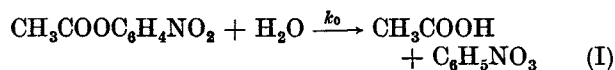
$$k_{\min} = k_{H_2O} + 2 \cdot \sqrt{K_w \cdot k_{H^+} \cdot k_{OH^-}} \quad (11)$$

where k_{\min} is the minimal observed rate constant and K_w , k_{H^+} , k_{OH^-} and k_{H_2O} are as described above. Assuming k_{\min} is $1 \times 10^{-3} \text{ min}^{-1}$ (Fig. 3) and using k_{H_2O} as $7 \times 10^{-4} \text{ min}^{-1}$ and k_{OH^-} as $6.25 \text{ mM}^{-1} \cdot \text{min}^{-1}$, k_{H^+} may be approximated as $0.36 \text{ mM}^{-1} \cdot \text{min}^{-1}$. This value for k_{H^+} is much greater than would be expected from the studies of Yates and McClelland [8]. The latter authors, however, studied the hydrolysis of *p*-nitrophenylacetate in sulfuric acid-water mixture.

DISCUSSION

Kinetic Analysis by the Initial Slope Method

A complete description of the hydrolysis of *p*-nitrophenylacetate in the presence of a nucleophilic catalyst would have the following three reactions occurring:



where k_0 and k_c have been defined above and k_h is the rate constant for the hydrolysis of the acetyl-catalyst ($\text{CH}_3\text{CO-Cat}$) to acetic acid and catalyst (Cat). If a is the concentration of *p*-nitrophenol formed in time t from reaction I and b is the concentration of *p*-nitrophenol formed in time t from reaction II then x , the sum of a and b , is the concentration of *p*-nitrophenol in the reaction mixture at that time. At any time t , Eqn (12) describes a :

$$1 - \frac{a}{x_0} = e^{-k_0 \cdot t} \quad (12)$$

Table 2. Amount of p-nitrophenol formed due to nucleophilic catalysis of p-nitrophenylacetate hydrolysis

x_0 is the initial concentration of p-nitrophenylacetate, c is the initial concentration of catalyst, k_c is the second-order rate constant for the hydrolysis of p-nitrophenylacetate by the nucleophilic catalyst and b is the concentration of p-nitrophenol at time t formed from the catalyst-catalyzed portion of the hydrolysis

Reaction conditions	Limiting factors	b	Definition
Excess catalyst	$t < 0.1$ (unit of time) of k_c' $c \leq \frac{1}{k_c}$	$x_0 \cdot k_c' \cdot t$	$k_c' = k_c \cdot c$
Excess ester	$t < 0.1$ (unit of time) of k_c'' $x_0 \leq \frac{1}{k_c}$	$c \cdot k_c'' \cdot t$	$k_c'' = k_c \cdot x_0$
$x_0 \approx c$	$t < 1$ (unit of time) of k_c x_0 or $c \leq \frac{1}{k_c}$	$\frac{x_0 \cdot c \cdot k_c \cdot t}{1 + x_0 \cdot k_c \cdot t}$
$x_0 = c$	$\frac{x_0^2 \cdot k_c \cdot t}{1 + x_0 \cdot k_c \cdot t}$

Assuming $k_0 \cdot t$ is much smaller than 1, the Taylor expansion of Eqn (12) yields:

$$a = x_0 \cdot k_0 \cdot t. \quad (13)$$

The value of b (Table 2) also may be obtained from the Taylor expansion of the various equations describing reaction II. In each instance the assumption is made that the numerical value of the product of k_c , t , and the concentration of the reactant which is in greater amount be much less than 1.

The initial rate of appearance of p-nitrophenol $(dx/dt)_{t \approx 0}$, that is to assure that the Taylor expansions described above are valid, may be described as:

$$\left(\frac{dx}{dt}\right)_{t \approx 0} = \left(\frac{da}{dt}\right)_{t \approx 0} + \left(\frac{db}{dt}\right)_{t \approx 0}. \quad (14)$$

Under pseudo-first-order reaction conditions (either in ester or in catalyst) the following equation is operative:

$$\left(\frac{dx}{dt}\right)_{t \approx 0} = x_0 \cdot k_0 + x_0 \cdot c \cdot k_c. \quad (15)$$

For second-order reaction conditions Eqn (14) becomes:

$$\left(\frac{dx}{dt}\right)_{t \approx 0} = x_0 \cdot k_0 + \frac{x_0 \cdot c \cdot k_c}{(1 + x_0 \cdot k_c \cdot t)^2}. \quad (16)$$

In the Taylor expansion of the second order equation, an assumption has already been made that $(x_0 - c) \cdot k_c \cdot t$ be much less than 1. If the concentration of p-nitrophenylacetate is such that $x_0 \cdot k_c \cdot t$ is much less than 1, then Eqn (16) becomes Eqn (15). This means that under conditions which permit the Taylor expansion to be made for both a and b , Eqn (15) describes the initial rate of appearance of product in terms of rate constants and the initial concentration of reactants, p-nitrophenylacetate and catalyst.

With the same assumptions, the same equation [Eqn (15)] may be derived for the disappearance of reactant, except the sign will be negative for the initial slope. If there are several nucleophilic catalysts then Eqn (15) may be modified by simply replacing $k_c \cdot c$, with $\sum k_{c_i} \cdot c_i$, where k_{c_i} and c_i have the same meaning as k_c and c respectively of the i th catalyst [2]. The derivation of Eqn (15), in addition to the limitations described above requires that there be no stable intermediate between reactant and product. It may be readily seen that with the same assumptions that have been made for p-nitrophenylacetate hydrolysis, Eqns (7) and (15) are applicable in general for analysis of all first and second-order reactions, respectively.

Mechanism of p-Nitrophenylacetate Hydrolysis

The hydrolysis of p-nitrophenylacetate in various buffers has been studied by several authors [4–7]. The mechanism of this ester hydrolysis includes in all instances the formation of a tetrahedral intermediate. The formation of this intermediate which is due to a water molecule attacking at the carbonyl-carbon atom is catalyzed by a second water molecule or a hydroxide ion in general-base catalysis. The tetrahedral intermediate rapidly decays to either p-nitrophenylacetate (starting material) or p-nitrophenol (product). This mechanism is very satisfactorily described by Eqn (2).

The data in Fig. 3, indicate that below pH 6.5 at 25 °C the rate of hydrolysis of p-nitrophenylacetate appears to rise. This type of curve is consistent with the curve for ester hydrolysis given by Bell [3]. The increase in rate of p-nitrophenylacetate hydrolysis below pH 6.5 is due to acid catalysis. Thus between pH 6.0 and 7.0, the observed rate constant for p-

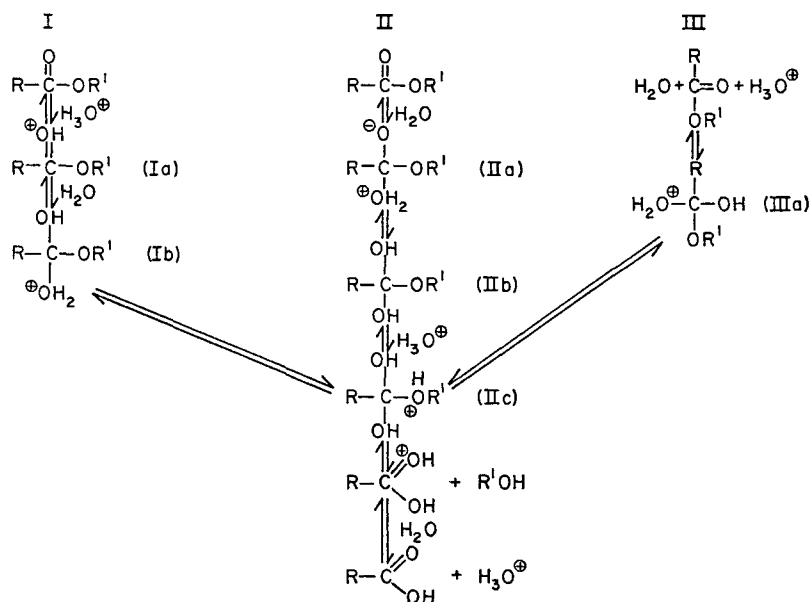


Fig.5. Reaction schemes for acid-catalyzed ester hydrolysis

nitrophenylacetate hydrolysis at 25 °C has an acid-catalyzed portion to it.

The mechanism for acid-catalyzed hydrolysis of esters, in general, is not well understood [1]. Fig.5 illustrates the three possible mechanisms for acid catalysis (for *p*-nitrophenylacetate R^- is CH_3^- , R' is $\text{C}_6\text{H}_4\text{O}_2\text{N}$). In mechanism I, the hydronium ion protonates the ester to form the conjugated ester. This may occur in strong acid solutions. A nucleophilic attack of water on the strongly electrophilic carbonyl-carbon atom (steps Ia to Ib) quickly follows the formation of the conjugated ester. Intramolecular proton shift (between Ib and IIc) determines whether the reaction goes to products or back to reactants [1, 2, 8, 31].

In dilute acid, scheme III, a concerted mechanism may be proposed for ester hydrolysis since the formation of either Ia or IIa is thermodynamically unfavourable [32]. At neutral pH, however, general-base catalysis by a second water molecule or a hydroxide ion, without a proton shift, may result in the formation of IIa. The latter intermediate may be stabilized by intramolecular proton shift to form IIb. The presence of hydronium ion, even trace amounts of them, will promote the formation of products *via* IIc, or reactants *via* Ib (*i.e.* protonation of hydroxy side chain rather than alcohol side chain).

From the above discussion it follows that the acid-catalyzed hydrolysis of ester may follow three independent pathways, the pathways being determined by the acidity of the solution. In strong acid, mechanism I is operative. Mechanism III, which is described by the same equation [Eqn (3)] as

mechanism I is operative under mild acid conditions. Under neutral conditions, mechanism II is followed and therefore Eqn (1) describes the ester hydrolysis. Since, there are three pathways for the acid-catalyzed hydrolysis of ester, the k_{H^+} may have different values for the three mechanisms: in mechanism I, k_{H^+} describes the protonation of the ester; in mechanism II, k_{H^+} describes the protonation of the neutral hydrated tetrahedral intermediate (IIb); while in mechanism III, it describes the concerted attack of a hydrated proton and a water molecule on the ester. Thus, it is not surprising that the value determined for k_{H^+} in this study (see Results) is different from the value determined by Yates and McClelland [8].

Equilibrium Model of p-Nitrophenylacetate Hydrolysis in Phosphate Buffer

Since *p*-nitrophenoxide has been shown to rigorously follow the Beer-Lambert relationship under the conditions of hydrolysis, one would suspect that if *p*-nitrophenylacetate were totally hydrolyzed, the expected infinity absorbance value should be observed. The earlier report [16] and the data of Fig.5 demonstrate that the observed infinity absorbance value (A_0) and the expected absorbance value (A_e) are not the same. The difference ($A_e - A_0$) divided by A_e is dependent on the pH of the hydrolytic reaction (Fig.5) and appears to be relatively insensitive as to when the absorbance readings are made. The question, whether sufficient time was given to attain the expected infinity absorbance value, is answered by noting that for 85% completion of the

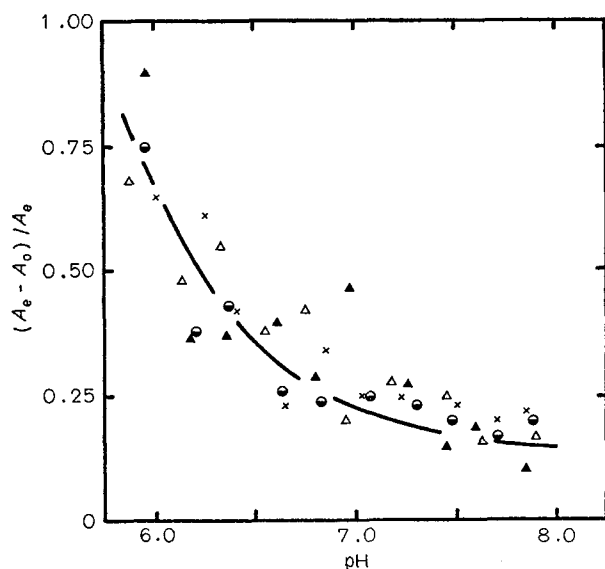
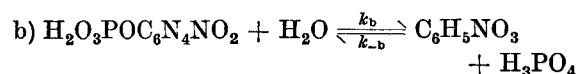
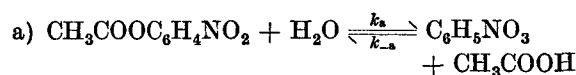


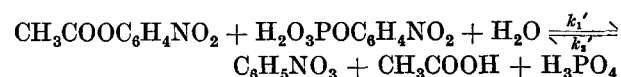
Fig. 6. The difference between the expected infinity absorbance value (A_e) and the observed infinity absorbance value (A_0) expressed as a fraction of A_e versus the pH of the hydrolytic reaction. The hydrolysis was at 25 °C and absorbance readings were at 400 nm; after one week of reaction of 48 μ M *p*-nitrophenylacetate in 0.1 M phosphate buffer (x), after three days (Δ) and four days (Δ) of reaction of 87.5 μ M *p*-nitrophenylacetate in 0.1 M phosphate buffer, after one day of reaction of 88 μ M *p*-nitrophenylacetate in 0.1 M phosphate buffer (\bullet)

hydrolytic reaction at k_0 of 10^{-2} min^{-1} and 10^{-3} min^{-1} , 3.2 h and 32 h are needed, respectively. This relationship of observed *versus* expected infinity absorbance value (Fig. 6) is also insensitive to the concentration of the ester used in the hydrolytic reaction.

The conclusion drawn on the latter results is that the hydrolysis of *p*-nitrophenylacetate in 0.1 M phosphate buffer does not go to completion but rather reaches an equilibrium level of *p*-nitrophenol and *p*-nitrophenyl ester. The *p*-nitrophenyl ester may not only be *p*-nitrophenylacetate but may quite possibly be *p*-nitrophenylphosphate [33]. The latter ester may be synthesized from *p*-nitrophenol produced on *p*-nitrophenylacetate hydrolysis and the phosphate present as a buffer. Thus, the reactions proceeding in phosphate buffer are:



or the overall overall reaction is:

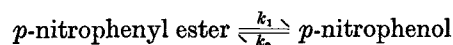


where k_a and k_b are the second-order rate constants for the hydrolysis of *p*-nitrophenylacetate and *p*-nitrophenylphosphate, respectively, k_{-a} and k_{-b} are the second-order rate constants for the synthesis of the acetate and phosphate esters, respectively, and

$$k_1' = k_a + k_b \quad (17)$$

$$k_2' = k_{-a} + k_{-b} \quad (18)$$

Since water and phosphate are in excess relative to the amount of *p*-nitrophenyl ester at any one time, then the latter equilibrium may be expressed as:



where $k_1 = k_1' \cdot [\text{H}_2\text{O}]$ and $k_2 = k_2' \cdot [\text{phosphate}]$. If the assumption is made that there are no stable intermediates in the above equilibrium then at any time, t , the concentration of *p*-nitrophenol, x_2 , may be determined from:

$$1 - \frac{k_1 + k_2}{k_1} \cdot \frac{x_2}{x_0} = e^{-(k_1 + k_2)t} \quad (19)$$

The Taylor expansion of Eqn (19), with the assumption that $(k_1 + k_2) \cdot t$ is much less than 1, yields:

$$x_2 = x_0 \cdot k_1 \cdot t \quad (20)$$

This means that k_0 described above is k_1 in the equilibrium model for *p*-nitrophenylacetate hydrolysis. In this model as t approaches infinity (equilibrium), Eqn (20) becomes:

$$x_0 = \frac{k_1 + k_2}{k_1} \cdot x_2^\infty \quad (21)$$

where x_2^∞ is the concentration of *p*-nitrophenol at equilibrium. If A_e equals x_0 , then A_0 equals x_2^∞ and therefore the plot in Fig. 6 is the fraction $k_2/(k_1 + k_2)$ *versus* pH. Using the solid line of Fig. 3 as the theoretical k_1 and the solid line of Fig. 6 as the theoretical fraction $k_2/(k_1 + k_2)$, a theoretical relationship of k_2 with pH may be determined (Fig. 7).

Fig. 7 indicates that the hydrolysis of *p*-nitrophenylacetate is both acid and base-catalyzed and essentially follows Eqn (1), except k_0 becomes k_1 . The rate constants which make up k_0 have been described above. The first-order rate constant, k_2 , is for the loss of *p*-nitrophenol due to ester synthesis, either *p*-nitrophenylacetate or more likely *p*-nitrophenylphosphate. There is the possibility that the acetate ester is being resynthesized due to phosphate ion catalysis. The shape of the curve for k_2 indicates that this reaction is both acid- and base-catalyzed. The slope of the latter curve at pH 6.0 is approximately -2 , while at pH 8.0 it approaches $+1$. An equation which accounts for both the acid and base catalysis of k_2 is:

$$k_2 = k_2^{\text{H}_2\text{O}} + k_2^{\text{H}^+} \cdot [\text{H}_3\text{O}^+]^2 + k_2^{\text{OH}^-} \cdot [\text{OH}^-] \quad (22)$$

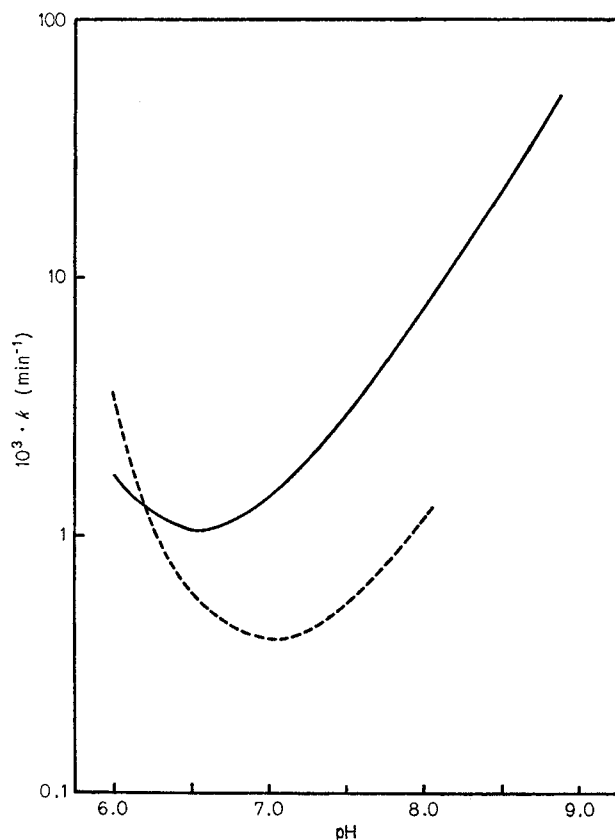


Fig. 7. Theoretical relationship of: k_1 (—), the first-order rate constant for the hydrolysis of *p*-nitrophenylacetate and k_2 (---), the first-order rate constant for the synthesis of *p*-nitrophenyl ester with pH

where $k_2^{\text{H}_2\text{O}}$ is the pH-independent portion, $k_2^{\text{H}^+}$ is the acid-catalyzed portion and $k_2^{\text{OH}^-}$ is the base-catalyzed portion of the ester synthesis. The rate constant $k_2^{\text{H}_2\text{O}}$ is first order, $k_2^{\text{H}^+}$ is second order and $k_2^{\text{OH}^-}$ is third order.

Fig. 8, scheme 1 is a possible mechanism for *p*-nitrophenylphosphate synthesis due to base catalysis. The base may be either a hydroxide ion (as illustrated) or a water molecule and its function is to increase the nucleophilicity of the phenolic oxygen. Scheme 2 (Fig. 8) illustrates why two hydrated protons may be needed in acid-catalyzed *p*-nitrophenylphosphate synthesis. Their function is to increase the electrophilicity of the phosphorus atom in the phosphate ion.

The hydrolysis of *p*-nitrophenylacetate and the synthesis of *p*-nitrophenyl esters are acid- and base-catalyzed. These reactions may be followed spectrophotometrically and their respective rate constants determined by the initial slope method. In the accompanying paper, the hydrolysis of *p*-nitrophenylacetate is investigated in the presence of imidazole, thiols and a synthetic heptapeptide.

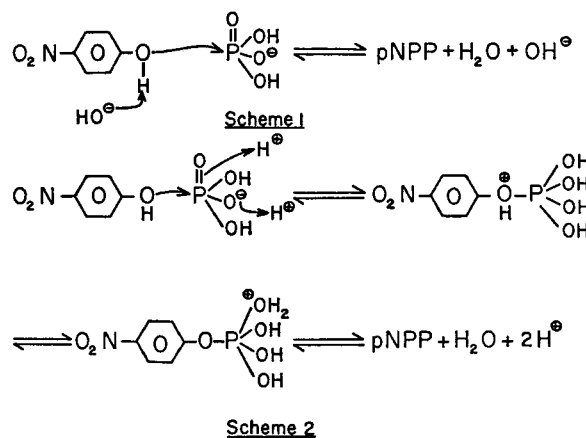


Fig. 8. *p*-Nitrophenylphosphate synthesis. Scheme 1 is base-catalyzed; Scheme 2 is acid-catalyzed

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